
Image-Pro[®]
Version 10 for Windows[®]
Users Guide

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Preface

Before You Begin

Welcome to the *Image-Pro® User's Guide*. This product is designed to run under the Microsoft® Windows® 7.0 Professional or Ultimate (service pack 1) (32- and 64-bit), Windows® 8.0 or 8.1 Enterprise or Professional (32- and 64-bit), and Windows® 10 Enterprise or Professional (32- and 64-bit).

Product Features

Image-Pro 9.3 is a comprehensive application for image acquisition and measurement for industrial and physical scientists. It has been designed to automate and simplify routine image capture, inspection, and measurement tasks.

With *Image-Pro*, you can.....

Acquire and Open Images

- See when Live Preview is running with the new Live Preview Pane.
- Capture HDR images.
- Easily acquire single frame images and time-lapse experiments.
- Acquire and analyze high dynamic range (HDR) images.
- Stream multi-gigabyte movies directly to your hard drive.
- Use Live Tiling and Live Extended Depth of Field (EDF) to acquire large, beautifully focused images without an automated stage or z motor.
- Use the crosshair grid to align images while capturing.
- Compare live images with previously acquired images using the Image Compare workspace.

Automate and Customize

- Show and hide tabs or groups to display only the tools you need.
- Create and display mini-programs (Apps) and macros to automate procedures that you use frequently on the new Apps tab.
- Save and load your preferred workspace layout, and share your layout with others.
- Review your macros in an intuitive graphical macro editor or use the more advanced code-based editing tools to test, edit and debug your scripts.
- Download *Image-Pro* macros and Apps from the App Center website.
- Customize the user interface, so that you can set up *Image-Pro* in the most efficient way for your work, including dockable tool bars and the ability to show or

hide different toolbars and buttons. You can also reset your interface to “factory” or “default” settings if you don’t like what you’ve set up and want to start over.

- Never lose your work with *Image-Pro*’s AutoSave and AutoRecover.

Read and Write Image Data

- Read and write image data in all the standard image file formats including, TIFF, JPEG, AVI, and many others.
- Open and view multi-resolution image files, including Leica .scn, Aperio .svs and BigTiff, and extract out images to analyze.

Process and Analyze

- *Use Extended Depth of Field (EDF) test strips to preview EDF settings options to save time and ensure best results when creating in-focus images from a z-stack.*
- *Analyze colocalized channels. Perform parent/child analysis, analyze within specific regions and more.*
- Perform image enhancement using powerful color and contrast filters, including morphology, field flattening, background subtraction, Fast Fourier Transforms (FFT), and other spatial and geometric operations.
- Align, tile, or stitch multiple images together.
- Extract features with spatial tools that isolate a Region of Interest (ROI) from the rest of the image, or with segmentation tools that extract features by color or intensity value.
- Use 1-2-3 Batch Processing to apply your image processing steps to multiple opened images or folders of images.
- View and analyze very large images (64-bit systems only).

Measure and Count

- *Adjust the size and shape of irregular ROIs and Measurements with the Polygon Nudge Tool.*
- Use the Grow and Shrink tools to adjust the size of your counted objects .
- *Use the Close and Merge tools to combine neighboring objects manually when using automatic segmentation and counting tools.*

- *Automatically measure incremental distances between two irregular edges to quantify changes in surface size and shape.*
- Trace and count objects manually or automatically. Measure object attributes such as: area, angle, perimeter, diameter, roundness and aspect ratio. Calibrate your spatial scale to any unit of measure.
- Use Smart Segmentation to easily identify and segment challenging images with faintly-colored objects, textured objects and uneven backgrounds.
- View collected data numerically, statistically or in graphic form (histogram and scattergram). Save the measurement data to disk.
- Sort and classify your measurement data according to your predefined criteria. Color code your objects by class.
- Manually or automatically follow objects as they move over time using Object Tracking.
- Track changes in intensity over time.

3D Image Viewing and Rendering

- View 3D images from a variety of angles and views.

Report and Share

- Save images with all measurement overlays intact for future analysis.
- Apply annotations, labels, and overlays to your images.
- Export measurements and data graphs to Microsoft Excel™.
- Export all opened images into Microsoft PowerPoint™.
- Generate Adobe PDF™ reports that can include images, measurement data, text, and pictures.

Conventions Used in this Guide

The *Image-Pro User's Guide* uses the following documentation conventions:

- | | |
|---------------------------|--|
| 1. | This is a numbered step in a process. |
| <i>Bold Italic</i> | Indicates the name of a tab in the application ribbon |
| <i>Italics</i> | This indicates the name of a function group, dialog box or tab. |
| Bold | This indicates an object on a dialog box, such as a text box, button, spin box, list box, etc. |
| "quoted text" | This indicates information that you must type into the system exactly as shown. |
| <KEY> | This indicates a key to press in order to perform an action. |

A Note about this User Guide

This guide was designed to be a comprehensive overview of *Image-Pro* and *Image-Pro with 3D Module*. For that reason, some of the tab names, colors, screens, and images featured in this manual may be slightly different from those in your product.

For example, if you are using the versions of this product that do not include the ability to capture images, you will see the ***Home*** tab instead of the ***Capture*** tab. Also, if you are using *Image-Pro with 3D Module 9.3*, you will see some of the tabs labeled as ***2D Measure*** or ***2D Count/Size*** to distinguish them from similar features in the *Image-Pro with 3D Module* toolset.

Contacting Technical Support

Media Cybernetics provides technical support to registered users. We can be reached by phone, fax, or e-mail, as follows:

Telephone: 301- 495-3305, 800-263-2088 (North America only)

FAX: 301- 495-5964

E-mail: techsupport@mediacy.com

Web Page: <http://support.mediacy.com>

Operating Hours (Eastern Time)

Mon-Fri: 9:00 am - 5:00 pm

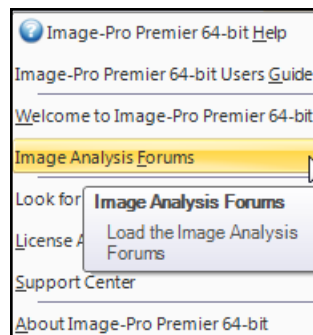
Sat-Sun: Closed

Holidays: Closed

Support for all **registered** users includes access to the latest information and downloads regarding updates, hot-fixes, and service packs. Technical support for all users is free for the first 30 days.

Support for users who have a valid *Image-Pro* maintenance agreement includes the following:

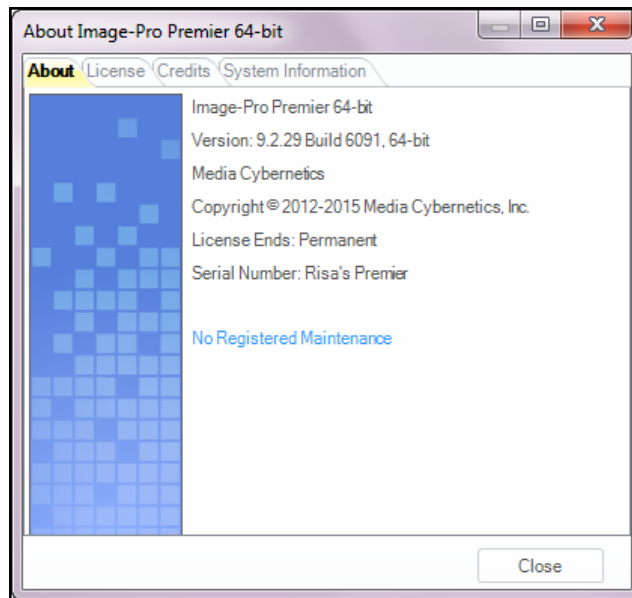
- Continuing access to the latest updates and fixes.
- Access to the *Image Analysis Forum Discussion Group*. *Image-Pro* Support Specialists and users from around the world participate and grow this Knowledge Base. It includes valuable information concerning the use and support of *Image-Pro*. To access this forum, click on the *Image Analysis* link on the **Help** menu:



- Access to *Image-Pro* Support Specialists. If you cannot find a solution in the Forum you can submit your problem to our *Image-Pro* Support Specialists. They will attempt to answer you as quickly as possible.

Before you contact *Media Cybernetics*' Technical Support for assistance, please be prepared to provide the following information:

- Your registration information: the product serial number, your name, phone number, organization name, mailing address, and e-mail address. The serial numbers are displayed on the **About Image-Pro** screen (open from the **Help** menu):



- The software version number and system information.
- A description of the problem, and any relevant information regarding prior technical support assistance.

Chapter 1

Installing *Image-Pro*

This chapter provides an overview of installation requirements and step-by-step instructions for installing and activating *Image-Pro/Image-Pro with 3D Module*.

Package Contents

Before installing *Image-Pro*, please check that you have received everything in your *Image-Pro* package:

- *Image-Pro* USB Installation Drive
- *Image-Pro* USB Licensing Dongle (if applicable)

System Requirements for Image-Pro 10


To install and work with *Image-Pro* you will need the following equipment and software:

Supported Operating Systems:

- Windows® 7 Professional and Ultimate (SP 1) – 32-bit and 64-bit
- Windows® 8.0 or 8.1 Enterprise and Professional – 32-bit and 64-bit
- Windows® 10 Enterprise and Professional – 32-bit and 64-bit

Minimum Requirements

- Dual core processor
- 4 GB of RAM or more
- 5 GB or more of available hard-disk space
- 1024 x 768 display with a 32-bit video card
- DVD/CD-ROM drive
- Microsoft .NET 4.0 Framework (automatically installed by application)
- USB port required for hard licenses and offline license activation
- Internet connection required for online services.

 *Note that Image-Pro will not install on computers running Windows® XP or Windows® Vista™. Nor will it run under Windows® N.*

Recommended Requirements

- OS: Windows 7, 8.1 or 10, 32-bit or 64-bit
- Processor: 2.8 GHz Intel quad-core 64-bit processor (Core i7 Series) or better
- RAM: 16 GB memory or higher
- Free Disk Space: Multiple High Speed SATA Hard Disks or SSDs; 8 GB free on installation drive + free space for images (500+GB)

- Graphics Card: nVIDIA GeForce GTX Cards with 4GB graphics memory & Open GL 4.2 or higher
- DVD-ROM drive
- USB port required for hard licenses and offline license activation
- Internet Explorer version 9 or higher
- Internet connection required for online services*

High Performance Requirements

- OS: Windows 7, 8.1 or 10, 64-bit
- Processor: 3.0 GHz Intel quad-core 64-bit processor (Core i7 Series) or better
- RAM: 48 GB memory or higher
- Free Disk Space: Multiple High Speed SATA Hard Disks or SSDs configured in RAID 5; 16 GB free on installation drive + free space for images (1 TB+)
- Graphics Card: nVIDIA GeForce GTX Cards with 4GB graphics memory & Open GL 4.2 or higher
- DVD-ROM drive
- USB port required for hard licenses and offline license activation
- Internet Explorer version 9 or higher
- Internet connection required for online services*

System Requirements for Image-Pro with 3D Module

Manufacturers change system specifications regularly so it is difficult to describe the exact configuration that is required however there are some important guidelines to consider before purchasing or upgrading your computer hardware for use with Image-Pro with 3D Module.

Rule of Thumb:

In short... More S Better. More GPU (Graphics Processing Unit) cores, more system RAM, more graphics RAM, more CPU cores, more speed for 3D viewing rates.

Image-Pro with 3D Module has no limits on the number of objects, the size of images, the number of images, or the number of frames. Everything depends on memory and speed. For users the best possible experience and product performance review the requirements below and follow-up with a review of the hardware priorities to understand how the whole system plays a part in the application's performance.

To install and work with Image-Pro with 3D Module you will need the following equipment and software:

Minimum Requirements (for small datasets only, performance will be low)

- OS: Windows 7, 8.1 or 10, 32-bit or 64-bit
- Processor: 2.4 GHz CPU Intel dual-core processor or better
- RAM: 4 GB memory
- Free Disk Space: 4 GB on installation drive + free space for images (20+GB)
- Graphics Card: nVIDIA GeForce GTX 560 (1GB & Open GL 4.2 or higher)
- DVD-ROM drive
- USB port required for hard licenses and offline license activation
- Internet Explorer version 9 or higher
- Internet connection required for online services*.

Recommended Requirements

- OS: Windows 7, 8.1 or 10, 64-bit

- Processor: 2.8 GHz CPU Intel quad-core processor or better
- RAM: 16 GB memory or higher
- Free Disk Space: Multiple High Speed SATA Hard Disks or SSDs; 8 GB free on installation drive + free space for images (20+GB)
- Graphics Card: nVIDIA GeForce GTX 580, 760 (2GB & Open GL 4.2 or higher) or AMD Radeon R9 290 (4.0 GB & Open GL 4.3 or higher)
- DVD-ROM drive
- USB port required for hard licenses and offline license activation
- Internet Explorer version 9 or higher
- Internet connection required for online services*.

High Performance Requirements

- OS: Windows 7, 8.1 or 10, 64-bit
- Processor: 3.0 GHz Intel quad-core processor or better
- RAM: 48 GB memory or higher
- Free Disk Space: Multiple High Speed SATA Hard Disks or SSDs configured in RAID 5; 16 GB free on installation drive + free space for images (1 TB+)
- Graphics Card: nVIDIA GeForce GTX 680 (4GB & Open GL 4.2 or higher) or AMD Radeon R9 290X (4GB and Open GL 4.3)
- DVD-ROM drive
- USB port required for hard licenses and offline license activation
- Internet Explorer version 9 or higher
- Internet connection required for online services*.

*This product may allow you to access certain features that are hosted online ("online services"), provided you have a high-speed Internet connection. The online services include but are not limited to, automatic updates, support links, access to video tutorials, and more.

Hardware Priorities

Graphics Card

Image-Pro with 3D Module is a 3D graphics-intensive product that requires a very capable graphics adapter. For this reason, purchase the most powerful graphics card possible because the single most important determinant of 3D viewing performance is the graphics card.

When choosing a graphics card for optimal visualization performance Open GL 4.2 is recommended along with at least 1GB of graphics memory, but ideally 2GB preferable. Securing the best possible graphics card is not buying the most expensive one, as many of these cards are designed for unique rendering applications, such as the nVidia Quadro series. These cards will not necessarily provide an Image-Pro with 3D Module user with the best price to performance ratio. NVidia GeForce GTX and AMD Radeon HD and ATI Radeon X cards typically are designed for video gaming, but deliver excellent performance for 3D image analysis as well. When looking into nVidia cards, be aware of their series of cards such as the 500, 600, and 700 have high and low ends mean a GTX 650 could be potentially worse than a GTX 580 because the first number denotes a series and not the level of performance. In this case the second number of the model explains how they match up.

If you are working with very large images or sets, then it is important to focus on the second priority, as much as you do the graphics card for visualization. Our suggestions regarding memory are in the next section.

- Users are discouraged from multi-video card (Crossfire and SLI) solutions for now, as this technology is known to decrease performance compared to single graphics card solutions.

System Memory

System memory is the second most important factor for users who need to inspect large data sets in particular, large volume data with direct volume rendering. This is why Image-Pro with 3D Module is a 64-bit ONLY software product (32-bit operating systems are limited to a maximum amount of about 3.2 GB of system memory. It is important to note that you may need much more memory than the actual size of the data you want to work with since some processing may require several times the memory required by the original data set.

For instance, if you want to load a 4GB data set in memory, you may need up to 16 or 20GB of additional memory for the intermediate results of your processing. More commonly you will need 2 or 3 times the memory footprint for data being processed.

Hard Drives

When working with large files that contain either a long sequence or many dimensions of data, reading from the disk can slow down your productivity.

Option 1 is a standard hard drive (e.g. 7200rpm SATA disk) that can only send data to your application at a continuous rate of about 60MB/second. That is the theoretical limit; your actual experience is likely to be closer to 40MB/second.

Option 2 is to configure multiple hard drives (3 or more) in a RAID5 configuration but please note that RAID configurations may require substantially more system administration.

Option 3 is to use solid state drives (SSD) instead of standard hard drives (HDD) in either a single-drive or RAID configuration. SSD's are becoming more popular as the prices continue to drop. The SSD can perform better than a HDD, generally having file reading/writing at speeds of 200-550 MB/second. The price of solid state drives is still higher and the capacity is not typically equivalent, but the performance can certainly be worth the investment.

CPU

The Image-Pro with 3D Module platform is designed to support multiple cores/processors using state-of-the-art background processing. This feature is on by default and enables the software to execute tasks in parallel. Since Image-Pro with 3D Module combines the best of 3D and 2D processing and analysis, additional CPU cores and/or processor speed will enhance the responsiveness of the Graphical User Interface (GUI), data tables, 2D outlines, and large image filters. If you are using the 3D rendering and measurements, they will be mostly exploiting GPU power, and little of Image-Pro's visualization processes are strongly affected by CPU performance. Some image processing routines are CPU-limited, so a better CPU can benefit performance on those routines.

Installing Image-Pro

To install *Image-Pro*, follow the steps below. Please close any other applications before starting the installation process. The setup screen will indicate which version of *Image-Pro* you are installing.

1. Insert the *Image-Pro* product into the correct drive.

If the program does not start automatically, you can install *Image-Pro* using the **Start** button on your desktop. Choose **Run** and then use the **Browse** button to find the *autorun.exe* file on the CD. Double-click *autorun.exe* to begin the installation process.

*Image-Pro requires the Microsoft *.NET framework. If this is not installed on your computer, the application will install it automatically.*


If you have restricted user access control, you may see a warning asking if you want the program to make changes to your system. Click **Yes**.

You will see the **Installation Start** screen. This screen has the following buttons:

- Install Image-Pro
- Documentation
- Install Demo Images
- Capture Support.

2. Click **Install Image-Pro**.

You will see a dialog extracting the *Image-Pro *.MSI* file. If you have restricted user access control, you may see a warning message asking if you want the **.MSI* file to make changes to your system. Click **Yes**.

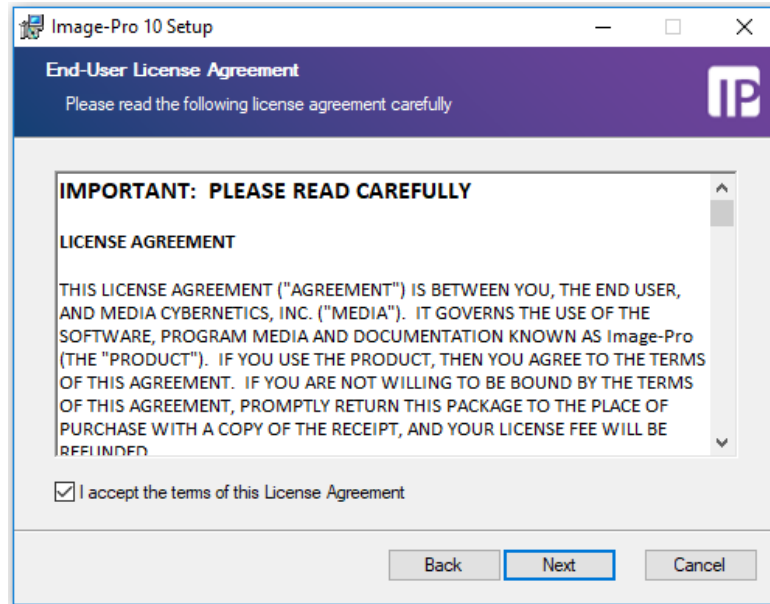
 *Image-Pro requires the Microsoft *.NET framework. If this is not installed on your computer, the application will install it automatically.*

Please wait.

In a few moments, you will see the *Image-Pro Setup* screen.

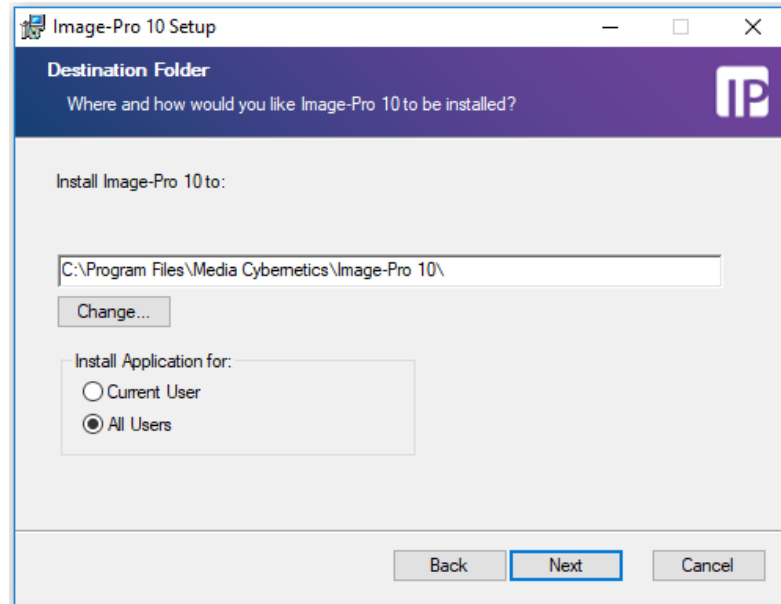
3. Click **Next**.

You will see the *Image-Pro License Agreement*.



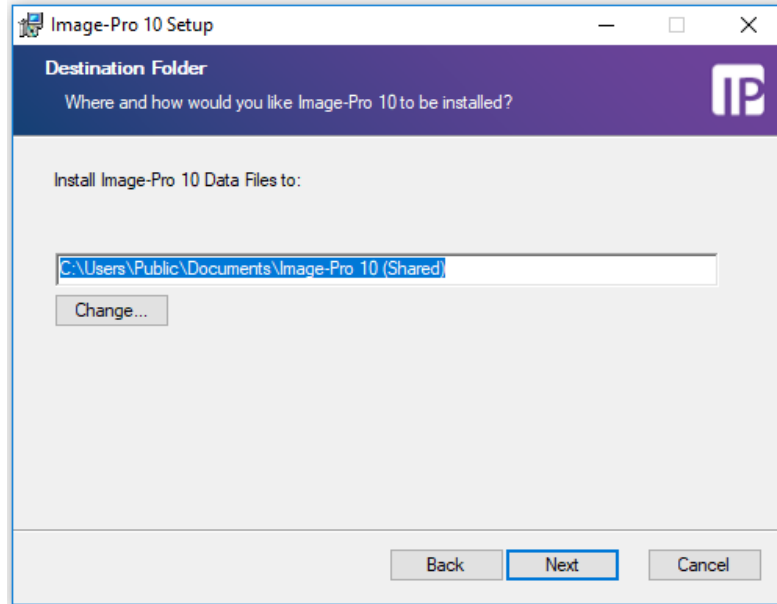
4. Check the **Accept** box, and click **Next**.

The *Select Destination Folder* location dialog box appears. This screen asks you for the destination for the *Image-Pro* program files and folders, and for who can access this installation.



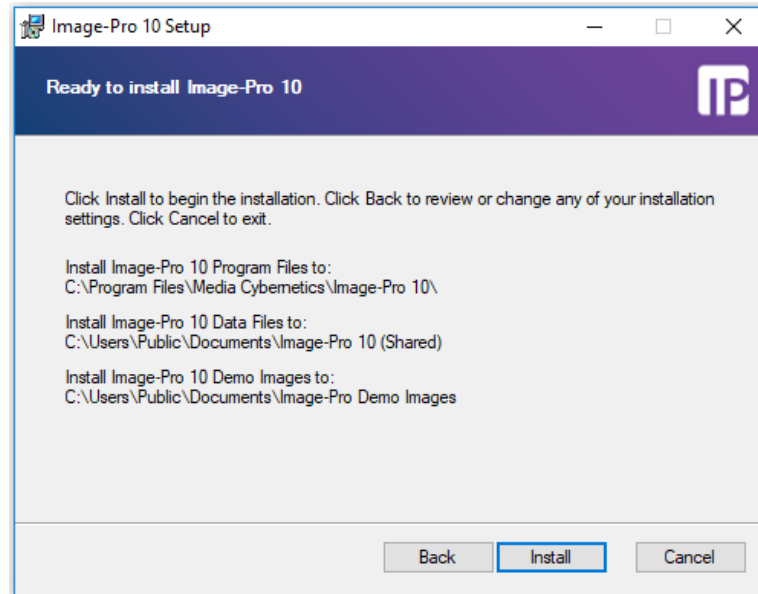
5. Click **Change** if you want to use a different location.
6. When the path is correct, click **Next** to continue.

The next screen verifies the destination folder for the *Image-Pro* data files:



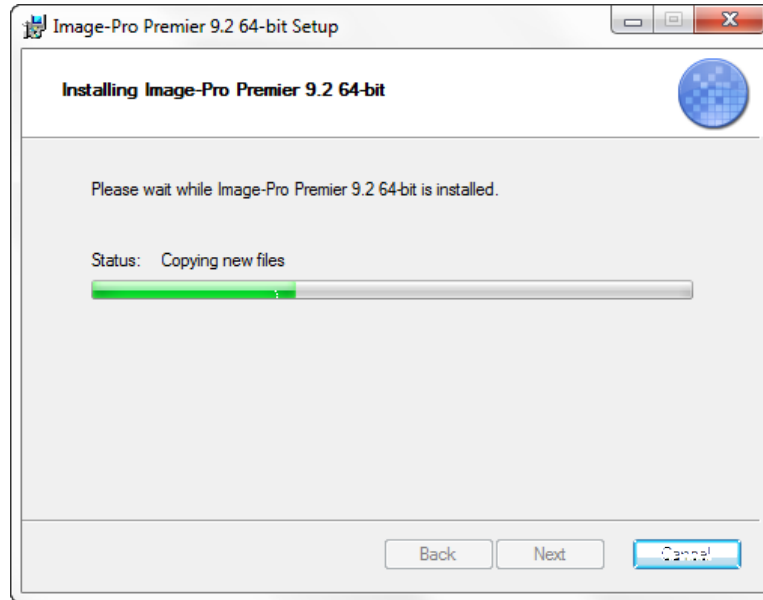
7. Confirm that the destination is correct. Click **Change** if you want to use a different location.
8. When the path is correct, click **Next** to continue.

The next screen indicates that your computer is ready to install *Image-Pro/Image-Pro with 3D Module*.

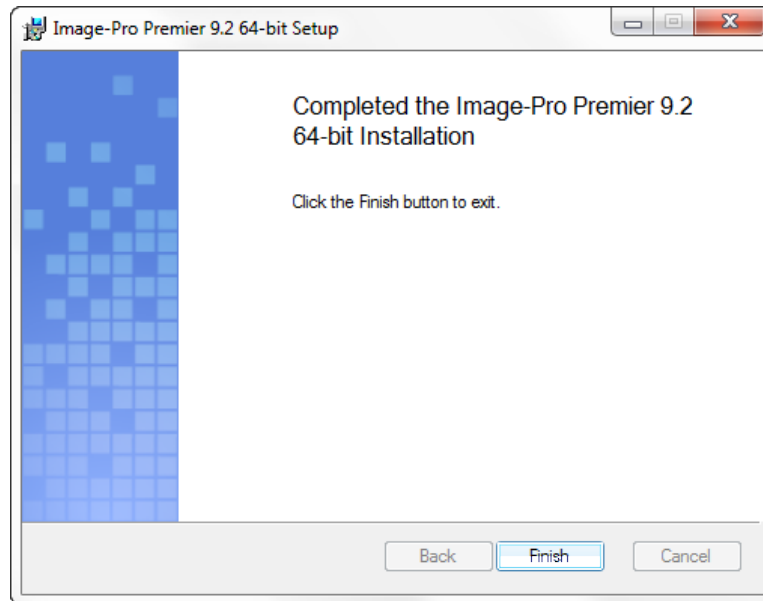


9. Click the **Install** button to begin the installation. This screen gives you a chance to verify your *Image-Pro* location and settings.

The *Installing* dialog box appears. The setup program will begin copying and installing files.

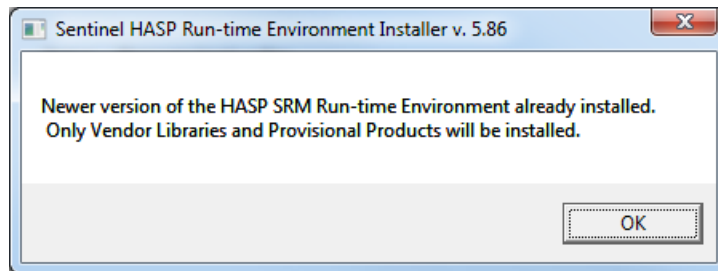


The final installation screen indicates that *Image-Pro* is now installed.



10. Click **Finish** to conclude the installation process.

Note: *During the installation process, you may see the following warning message:*



This warning can be ignored safely, and installation of your product will complete successfully.

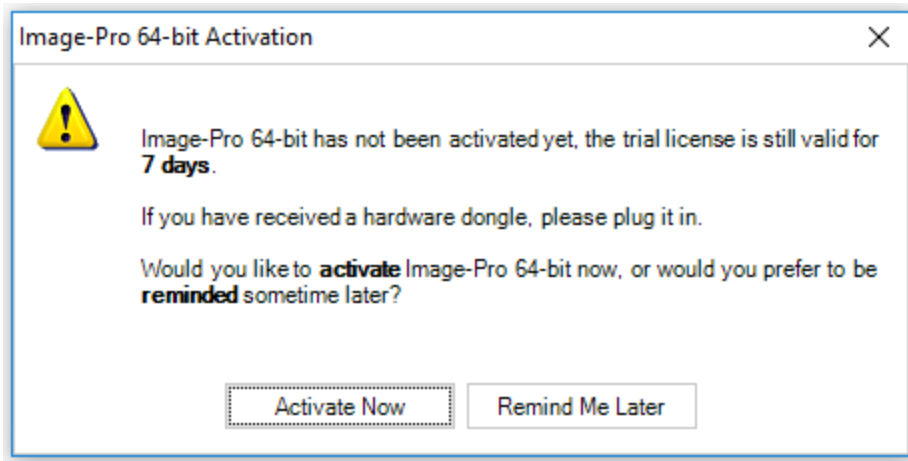
Activating Your Image-Pro License

USB license holders must activate their licenses after installing the software and plugging in the hardware key.

Activating the Image-Pro Program

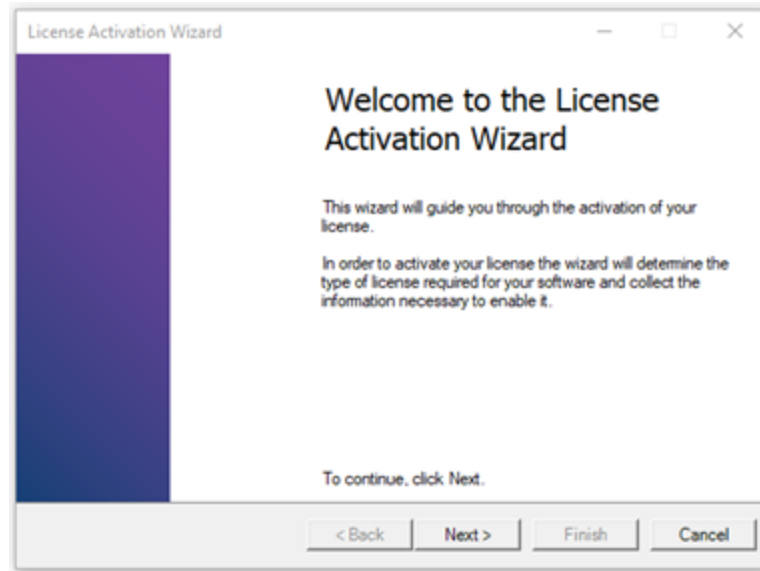
Before you can use *Image-Pro*, a valid license file must be obtained from the Media Cybernetics Web site. A 14-day trial license is included for evaluation purposes.

1. If you have an *Image-Pro* hardware key, attach it to a USB port now.
2. When you first start *Image-Pro*, you will see the following dialog:



3. Click Activate Now to activate your license.

This will load the License Activation Wizard which will guide you through the steps of activating your software.

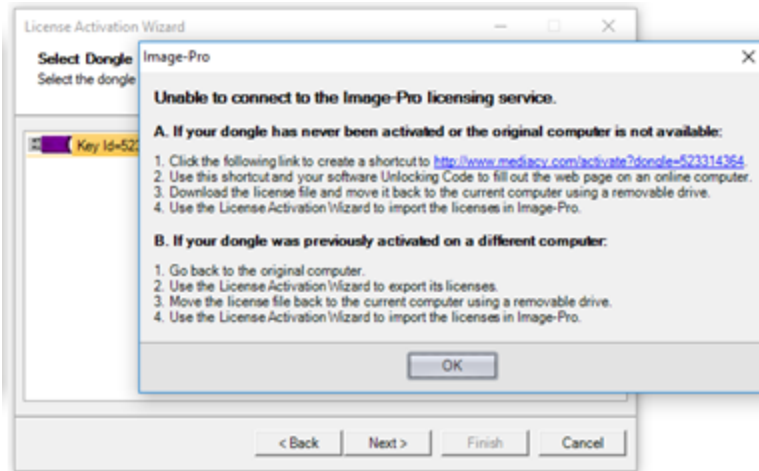


If your PC has Internet access

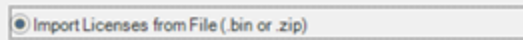
1. Confirm your license type and USB dongle.
2. Enter the Unlocking Code(s) that was sent with the product.
3. Provide standard registration information.
4. Once your information is received, all your licenses will be installed on your machine.

If your PC does not have Internet access

1. If you choose Activate Now, and your PC is not connected to the internet, the License Activation Wizard will bring you to the following prompt after the dongle selection:

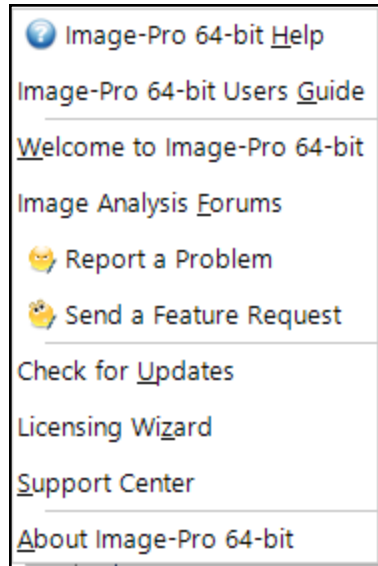


2. Get your license file(s).
 - a. If your dongle is not activated or you don't have access to the original computer, you can download your license(s) by visiting <http://www.-mediacy.com/activate> using the provided shortcut with your unlocking code(s).
 - b. If you have an already activated dongle you can obtain the license(s) using the License Activation Wizard to export them from the original computer.
3. In both cases, you can import the license(s) on the second page of the License Activation Wizard.

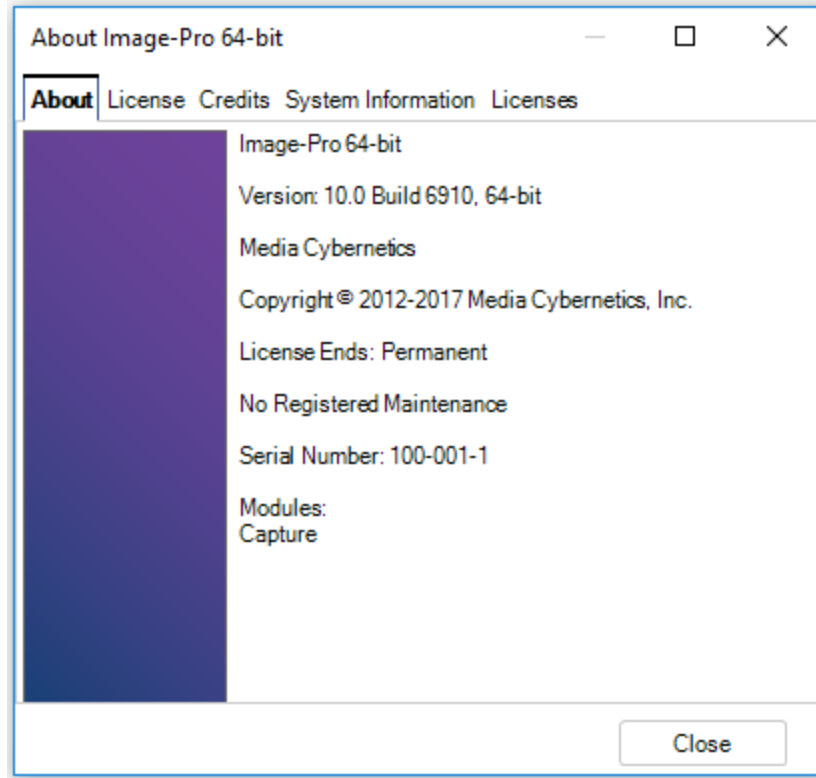


Verifying your Registration

1. (Optional) Verify your registration by going to the Help menu and choosing *About Image-Pro*:



The About page should show your (non-evaluation) license key:



This page will indicate if and when your license expires. It also provides information about your maintenance package, if any

Windows Security Alert

Depending on your Windows Firewall settings, you may see an alert when you start *Image-Pro* for the first time. This alert indicates that Windows may have blocked some aspects of the application.

Your software is checking on whether updates are available, and no personal information is being sent to *Media Cybernetics*. You will want to check the "Domain networks, such as a workplace network" option, and then click Allow access.

Product Versions and Updates

Most of the time, updates to *Image-Pro* happen automatically. If you have an Internet connection, **you can set your application software to check for updates when you start the product.** You must have an internet connection to update your product automatically.

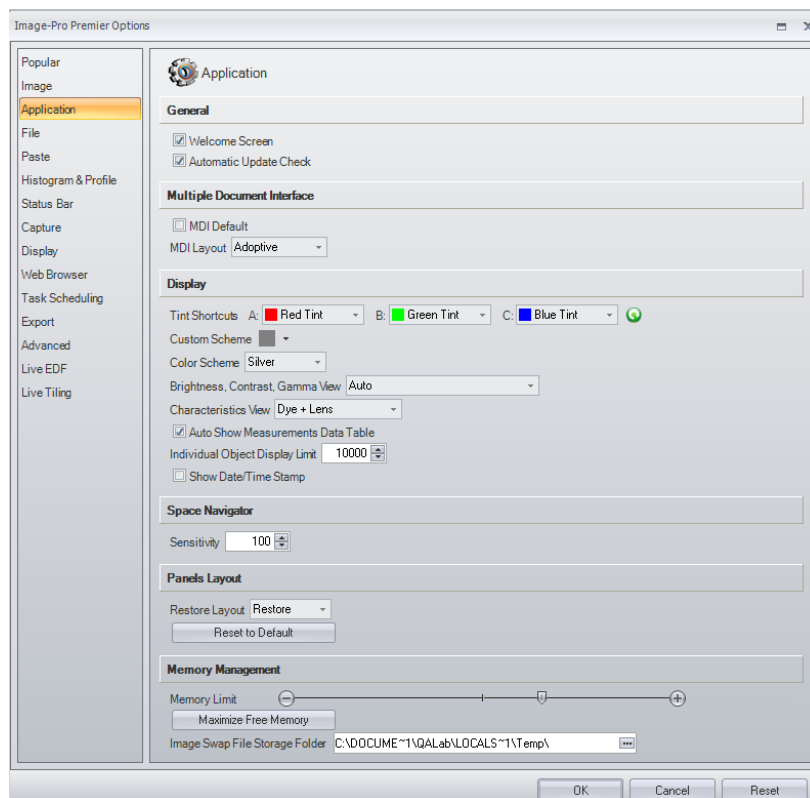
For information about upgrading your product and license to *Image-Pro 9.3*, please see the information about upgrading later in this chapter.

Updating Your Product

Image-Pro uses the Auto-Update feature to ensure that you are running the most current version of the software. Auto-Update is a small, unobtrusive feature that does not collect any personal information from you.

In order for the process to work, you must have an Internet connection available.

Select the Automatic Update Check option in the *Application* page of the *Options* dialog box, as shown here:



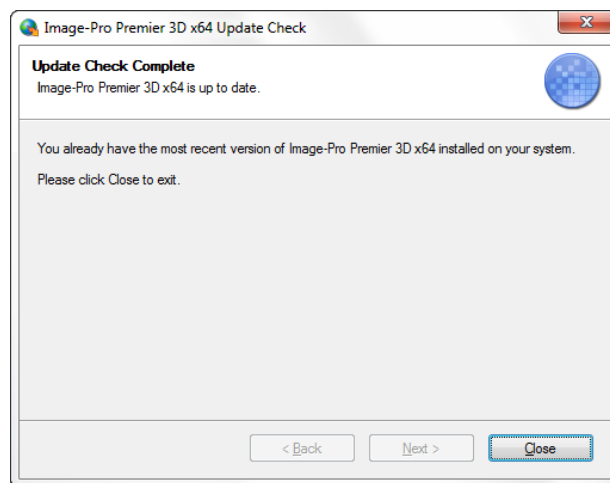
Checking this box ensures that software update checks will happen automatically. When an update is available, your application will offer to download the available updates. The downloads run in the background, and you may continue to use *Image-Pro* while they run. You can decide later when you want to install the downloaded updates. More information about *Application Options* appears in Chapter 2.

If Automatic Update Check is not selected, follow the steps below to look for updates to your software installation.

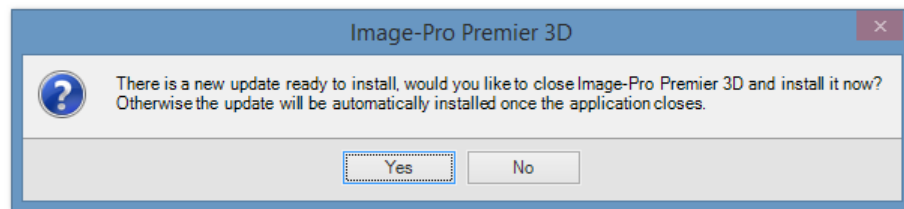
1. Click the small drop-down arrow at the upper right-hand corner of your application.



2. Click **Check For Updates**. If your product is current, you will see a message saying that you are running the most current version and no update is necessary.



3. If an update is found, you will see the Update Available dialog:



4. To install the update now, click Yes. To install the update later, click No.

If you click Yes the application closes (and prompts to save documents as appropriate), and the update is applied once the application has exited. If you click No, the update is installed after the end of the current session.

When the update process is complete, you may see a message, or a page of release notes pertaining to this upgrade.

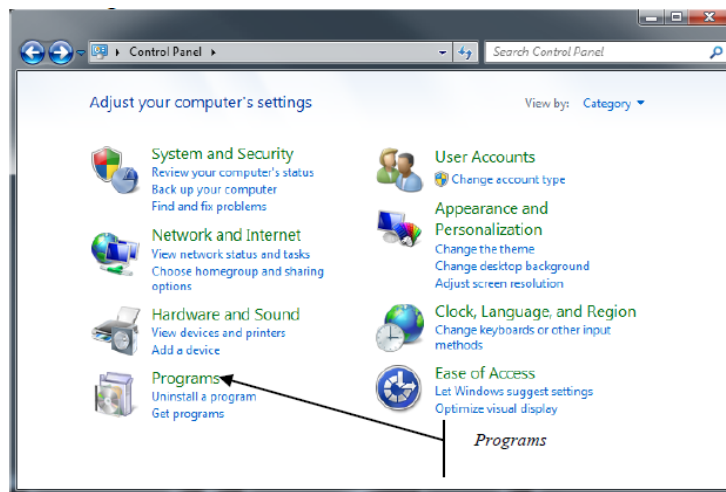
You may see a message reminding you to restart your computer before using *Image-Pro*. If you do, please restart your computer before using *Image-Pro*.

If you do not have an Internet connection, please contact Media Cybernetics' technical support for assistance.

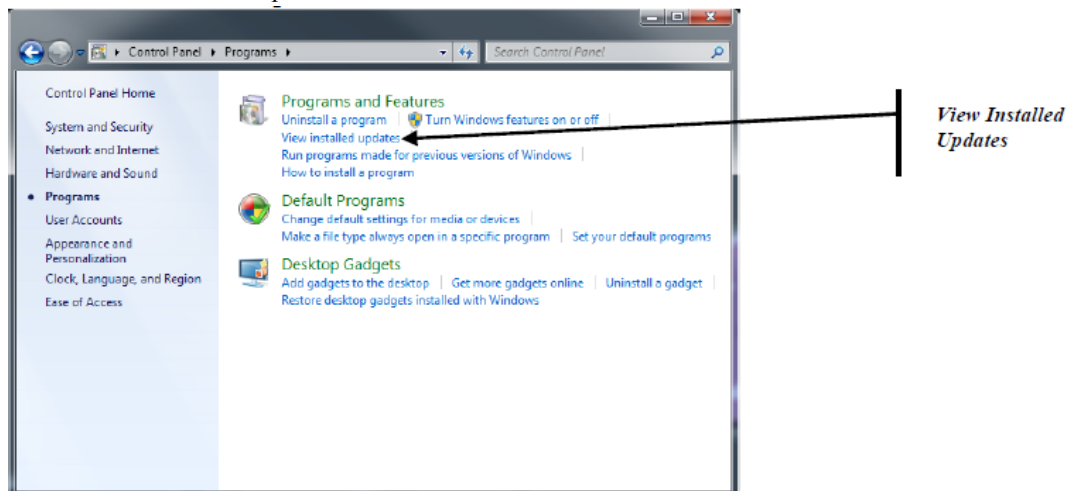
Verifying Updates

You can find out which updates of the application software have been applied by visiting the *Windows Control Panel*:

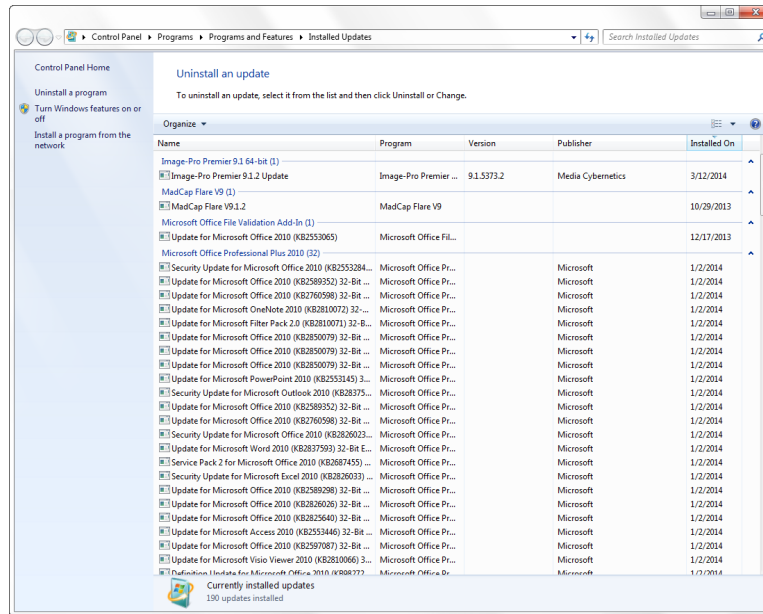
1. Go to Programs:



2. Select View Installed Updates:

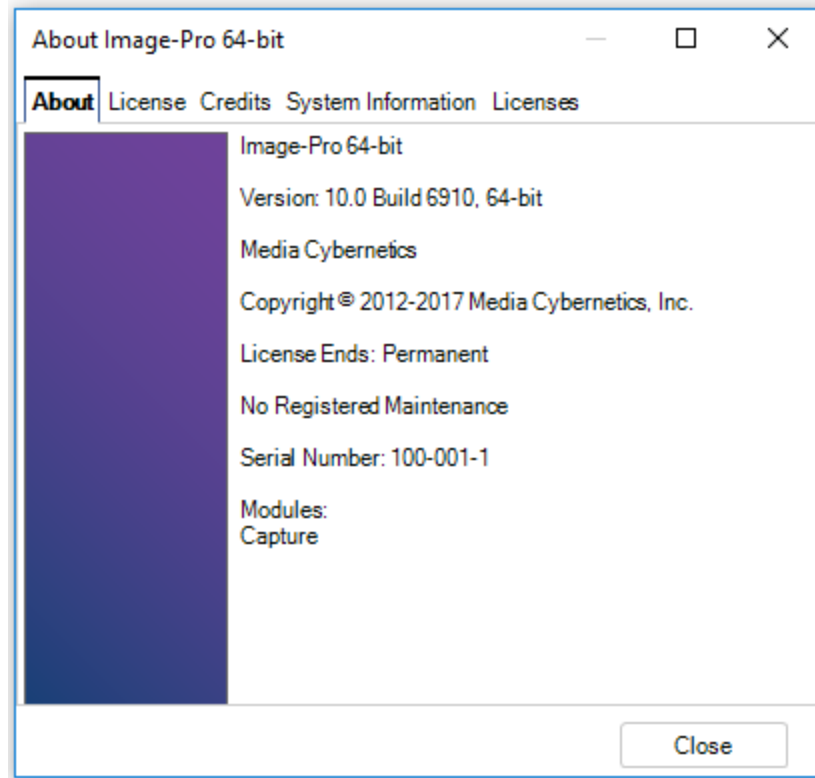


You will see which programs and updates have been installed on your machine.




- Once you have verified the update and version number, close the *Window Control Panel* dialog.

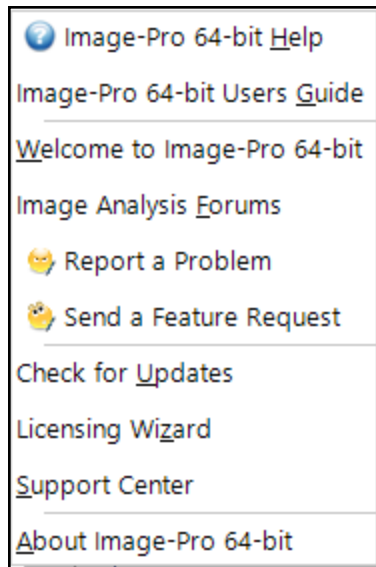
You can view the About box to verify the current software version and see additional information about your *Image-Pro* system.



Help and About Image-Pro

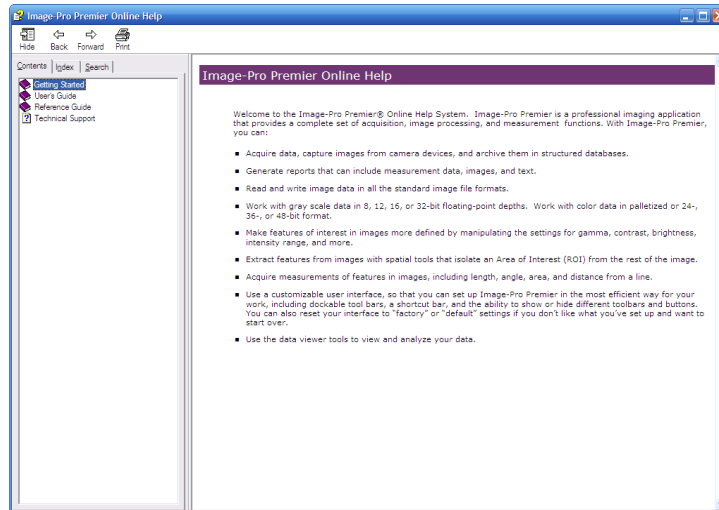
The *Help* menu, *User Guide*, *Support Center*, *About Image-Pro*, *Check for Updates*, and other useful features of *Image-Pro* can be accessed by clicking the drop-down arrow next

to the question mark in the upper right-hand corner of the application :



Once the drop-down menu appears, clicking anywhere on the menu item or its icon in the *Help* menu items will take you to that feature of *Image-Pro*.

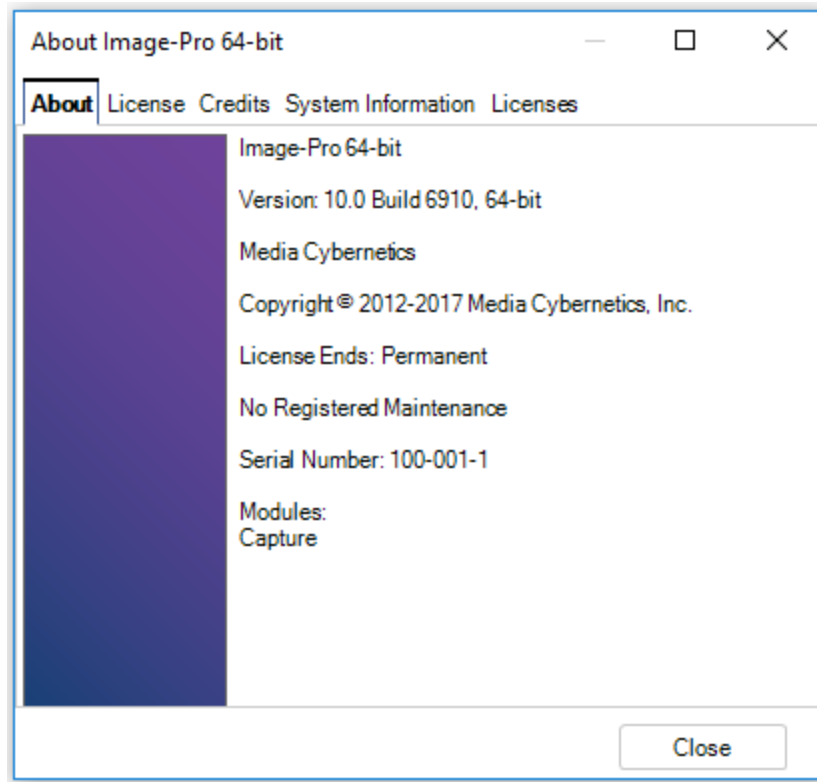
Clicking the Help question mark icon or H will open the first page of the in-product help file:



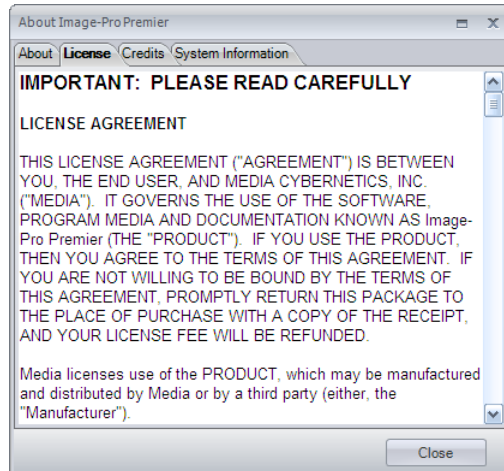
The Table of Contents, Index, and Search tabs in the left-side panel will help you navigate through the *Help* files.

- Clicking the *Image-Pro Users Guide* link or G opens the first page of the *Users Guide* in PDF. There are links and bookmarks to help you find the information you need in the *User's Guide*.
- Click the *Image Analysis Forum* or F sends you to the image analysis forum discussion group. This group includes links for you to send feedback, report a problem, or make a feature request.
- Clicking *Welcome to Image-Pro* or W opens the *Welcome* screen. Note that if you do not have an internet connection, the Welcome screen will display only offline content. Tutorials, news, and forum posts will be available only if you have an internet connection.
- Click *Look for Updates* or U to see if a new version of *Image-Pro* is available, as described earlier in this chapter.
- Click *SupportCenter* or S to open a support session with a Technical Support specialist:
- Click About Image-Pro or A to see important information about your *Image-Pro* system.

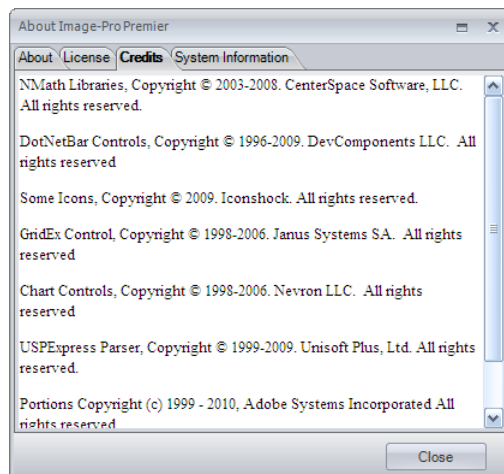
The About page displays the version, copyright, license, and maintenance information:



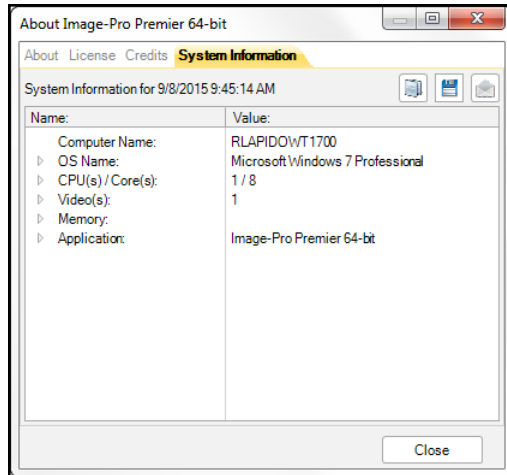
The License page displays the current license agreement.



The Credits page contains credits and acknowledgements:

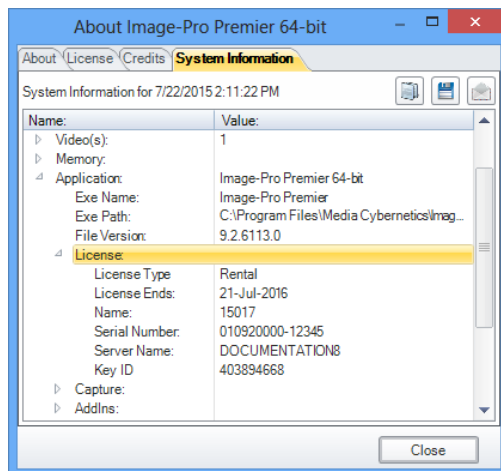


The System Information page displays information about your system:

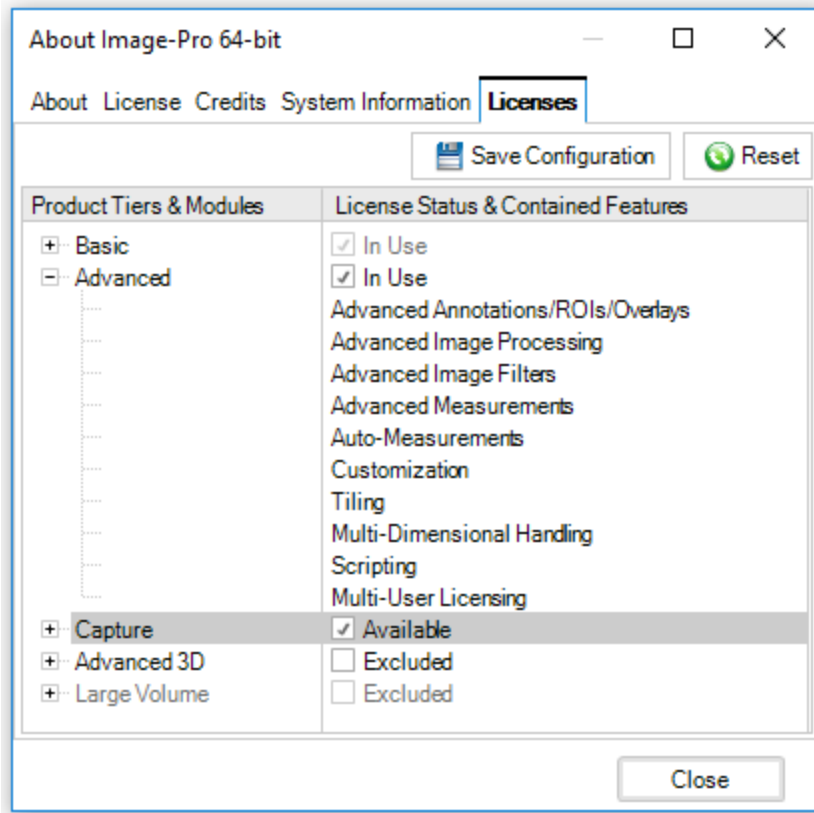


You can expand the system information folders to see the details of your installation. You may need this information when working with Technical Support.

The next screen shows the system information for a rental software license.



Finally, a Licenses tab is included in the *About* dialog to help some users configure their license use, see *Configuring License Use*.



Upgrading Image-Pro

There are two aspects to any upgrade:

- Upgrading the installed software from the previous product or version to the *Image-Pro 10* software, and
- Upgrading the license that authorizes *Image-Pro* to run.

You will want to review the next section, "Upgrading the Installed Software" on the next page, regardless of what type of upgrade you are doing.

If you are upgrading from a previous version of *Image-Pro*, then you will want to review the section "Upgrading your License" on page 39.

- If you are running a licensed and registered copy of *Image-Pro 9*, this will be a paid upgrade to version 10, and it *will not* be available via Auto-Update.

Upgrading the Installed Software

There are several ways that you can upgrade the software installed on your computer to *Image-Pro 10*:

- You may download the *Image-Pro 10* evaluation software and install it from the Media Cybernetics' website.
- If you have ordered an upgrade from *Image-Pro Plus* or *Image-Pro*, you will receive a USB stick that will install the software.

Regardless of how you obtain the *Image-Pro 10* installer, and regardless of which product and version you are upgrading from, *Image-Pro 10* can be installed alongside your other software. This allows you to evaluate *Image-Pro 10* for your purposes. When you are done with the older software and completely migrated to *Image-Pro 10*, then you can uninstall the older software from the Control Panel.

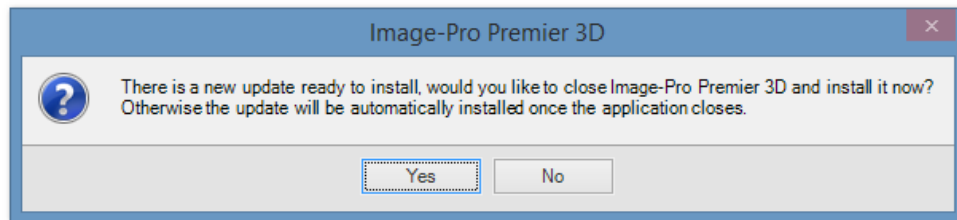
Upgrading Your Software

If you are running a licensed and registered copy of *Image-Pro* you will be offered free upgrades via Auto-Update. If you have Auto-Update enabled, you will be notified of the version upgrade when you start *Image-Pro*, and can download and install it as convenient. If you do not have Auto-Update enabled, you can follow the steps below.

1. Click the small drop-down arrow at the upper right-hand corner of your application



2. Choose Look For Updates. You will see the Update Available dialog:



3. Click Yes to continue.

Note that if you are upgrading to Image-Pro 10 from Image-Pro 9, you are required to update your product license as well as the software. The new version includes a

14-day temporary license. Follow the instructions regarding the License Upgrade Wizard later in this chapter to obtain a permanent license.

How to Return your Original Image-Pro Plus License

In order to receive a new *Image-Pro* license, you **MUST** return your original *Image-Pro* license dongle. If you do not return your dongle, you will be invoiced for the difference. Please contact Media Cybernetics' Customer Service for information about receiving a Return *Materials Authorization* (RMA) number and instructions on how to return this original *Image-Pro* license.

Here is a sample RMA packing slip:

Media Cybernetics Packing Slip

Cart # 93221 Customer No: 4676
 Cart Status: Shipped Order entered by: Maria Smith
 Order # 169388 Ship Order Status: Shipped

SOLD TO: SHIP TO:

F.O.B. Point ORIGIN	Customer Order No. DAVE SMITH	Ship Via FedEx-FEDEX_EXPRESS_SAVER (Partial Allowed)
Order Date 4/26/2012	Terms Credit Card	Sales Person House
Product	Product Code	Unit
Image-Pro Analyzer v7.0 - Upgrade from Plus 5.x and lower, Express, Insight and Optima - USB	15N7000-03 USB	Each
Date Shipped 5/2/2012	# of Boxes 1	Shipment Tracking No. 798350771 323

RMA: 2977

An item or items on this order requires that a previously shipped product or part be returned to Media Cybernetics. Your Return Materials Authorization (RMA) number for this return is 2977. Please return the replaced product to Media Cybernetics referencing RMA number 2977. All items not returned within 30 days will be invoiced. Keys not returned will be invoiced for an additional site license. See the notes of this order for any details regarding this RMA.

Serial Numbers:
15N7000-03 USB: 15N7000-32118

Unlocking Codes:
Product Activation Keys (PAKs):

Use the browser's PRINT command to print this page.

You have been issued an RMA.
 Please return your Media Product to the following address and referencing your RMA #:
 Media Cybernetics

If you don't have your original packing slip, please contact customerservice@mediacy.com

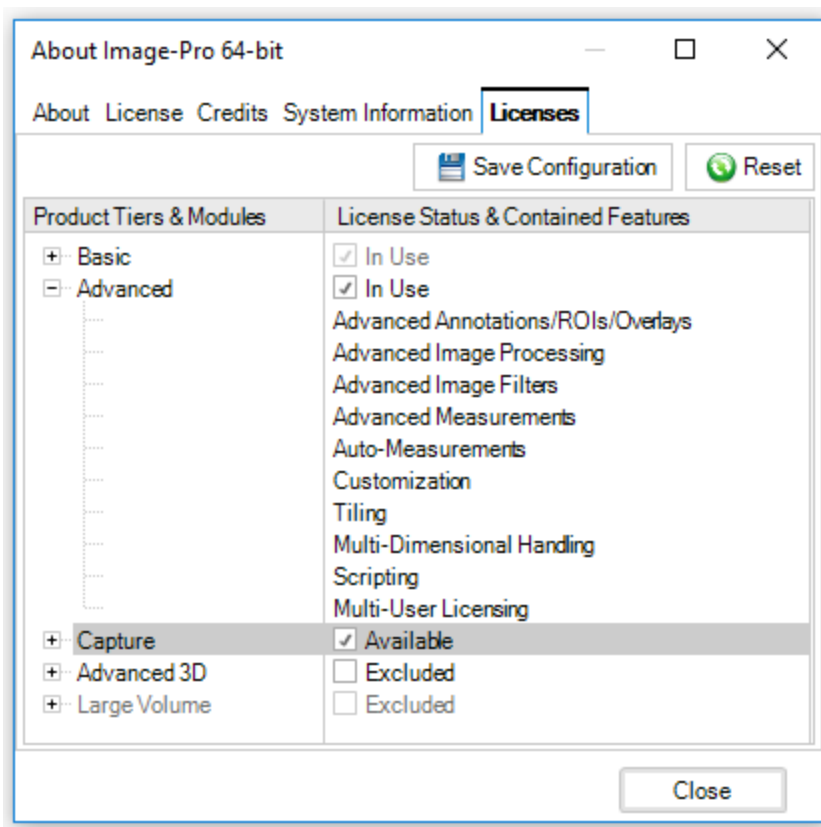
Upgrading your License

If you are upgrading from a previous version of *Image-Pro*, you may need to upgrade your license to authorize the new version you are trying to install. When you purchase your upgrade, you will receive an unlocking code. You will need to have this unlocking code handy during the upgrade process. *Image-Pro* includes a Licensing Wizard to step you through the process of upgrading your *Image-Pro* license to the latest version. Note that after the upgrade, the prior version license will still allow you to run previous versions.

In the *Image-Pro* application, open the Help menu by clicking the down arrow next to the Help icon, select **Licensing Wizard**.

Configuring License Use

Image-Pro uses a powerful licensing scheme allowing to provide the maximum flexibility when deploying the product. Although the user usually doesn't need to be involved with configuring its license use, there are a few cases where it may be convenient to do so. These include network configurations with shared licenses that may have to be assigned to specific workstations as well as demonstration or evaluation licenses which may be configured to select the adequate level of functionality. In order to help those users, a license management tab is included in the *About* dialog.




- Each available product tier and module is displayed in a hierarchical view with its contained tiers and features listed as child nodes.

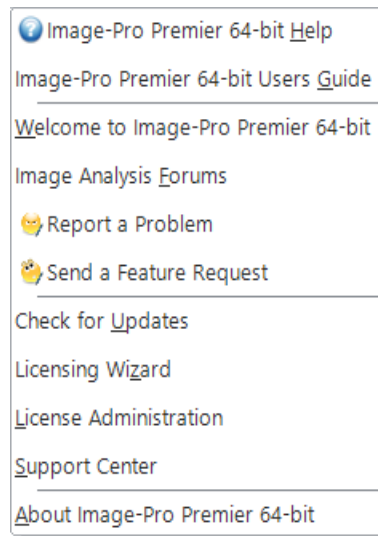
- Product tiers and modules can be excluded or left as available. Each selection gives visual feedback based on the tier hierarchy and dependencies so that selecting a tier will show exactly what features will be included or excluded. The three possible licensing states are as follows:
 - *In Use*: This tier or module is currently in use on this machine.
 - *Available*: This tier or module is currently available on this machine without being in use.
 - *Excluded*: This tier or module will not be used on this machine, leaving the license available for others to use.
- Once changes are made, the configuration can be saved using the *Save Configuration* button and will take effect in the next session. The information is saved in the identity so that different identities can use different licenses.

Sending Feedback

Media Cybernetics welcomes your feedback about our products. If you have a suggestion, question, or concern, please let us know. To send feedback, follow these steps:

1. Click the drop-down arrow in the upper right-hand corner of the application :

You will see this menu:



2. Choose **Report a Problem** or **Send a Feature Request** under the Image Analysis Forums.

You will see a form similar to the following according to your choice:

Send Your Feedback

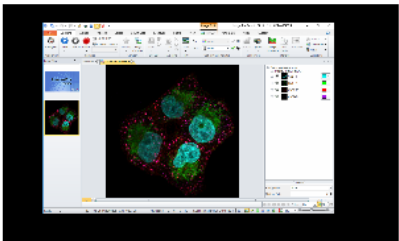
Report An Issue

We are sorry that you are experiencing difficulties with Image-Pro Premier 64-bit. Please select a category and describe the issue below.

Feedback Category
Issue Reports

Please add more details

If you agree, the snapshot below will be sent with your comments. If the picture is not current, [refresh it now](#). ☒ Send screenshot with comments



Tell us who you are (email address required)

[Media Cybernetics Feedback Tool Privacy Statement](#)

Submit

3. Choose a general comment, feature suggestion, or issue from the dropdown list.
4. Enter your comments, and remember to include your email address.
5. Press Submit.

Your feedback is always appreciated. You should hear from us shortly.


Chapter 2

Getting Started

This chapter introduces you to the *Image-Pro* and *Image-Pro with 3D Module* systems, including an overview of the interface components and features.

The *Image-Pro* Application Window

When *Image-Pro* is loaded, its application window becomes active.

The in-product Help contains detailed information about all the features and functions of *Image-Pro*. Click on the question mark  at the right side of the application window to open the help file. More information about using the Help appears at the end of this chapter.

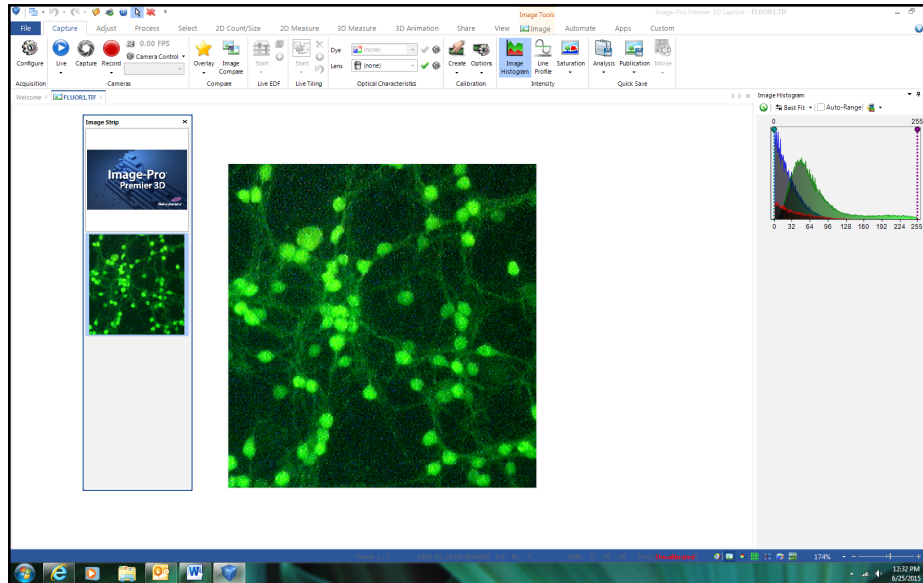
The center of the window contains a set of panels or panes. The far-left panel is the *Overview* panel, which contains the image strip and tools that provide an overview of the workspace contents. The center panel contains the active content, which can be an image, histogram, or other rendering. The far-right panel contains the image histogram as well as additional dialogs that enable you to modify the contents of the workspace.

The following sections explain these features of the *Image-Pro* application window in greater detail.

Floating Panels

One of the unique features of *Image-Pro* is the “floating” or “dockable” panels. The image strip, details panel, mini-histogram panel, or other workspace areas can be moved independently from the main image workspace. This may be particularly useful when working with a dual-monitor system, as you may want to display the image on one monitor and the details on the other. An example of floating or “undocked” panels appears below:

The Image-Pro Application Window



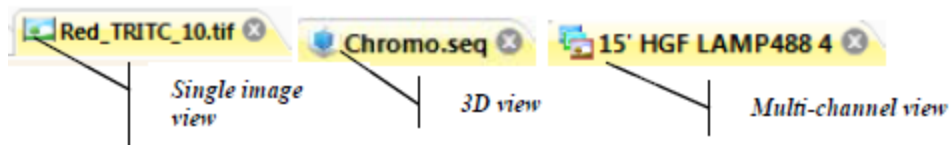
Workspace Tabs

Image-Pro and *Image-Pro with 3D Module* display your images in tabbed workspaces, as shown here:



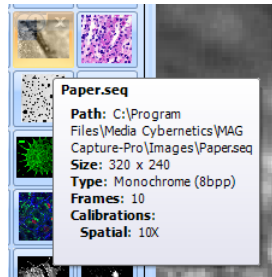
The name of the active image in the workspace is highlighted. You can switch back and forth among multiple images by clicking the image's tab.

The small icons next to the name of the image indicate what type of image is open. Some examples of common types are shown here:



Viewing Tooltips

Tooltips show the basic image information:

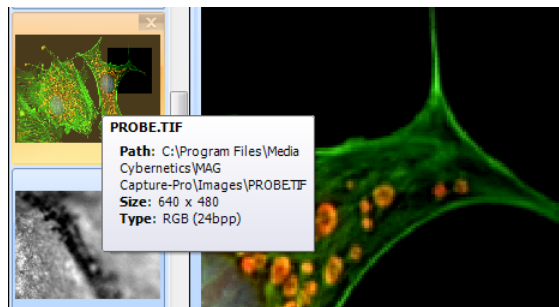


- To view an image tooltip, move your mouse cursor over the image thumbnail in the image strip.

The *zoom and pan controls* allow you to resize the image or a portion of the image.

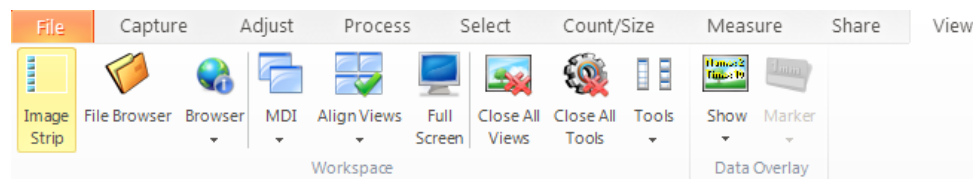
When the active image is zoomed and doesn't fit in the viewer, the viewing area is shown on the image strip thumbnail as a semitransparent orange overlay.

- Click and drag the center of the viewing area on the thumbnail to control pan and scroll of the image.



Tools and Layouts

The **View** tab contains specialized tools and graphs to display information about the active image or image set. Some of these tools allow you to see different aspects of the active image also.



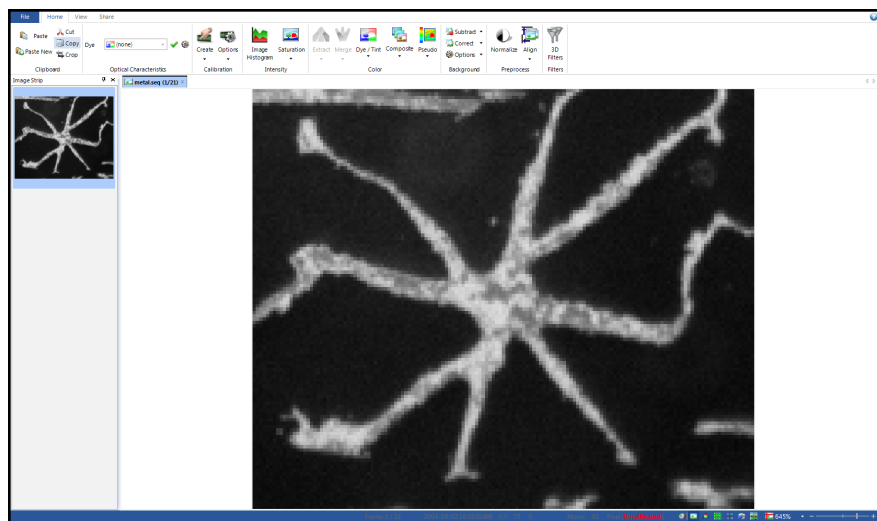
Using the Multiple Document Interface (MDI)

The *Switch to MDI* button will toggle the workspace windows to MDI windows or back to tabbed windows. This feature allows you to view more than one image at a time, and move the images and panels to different locations in the application.

This feature is located in the *Workspace* group on the *View* tab's ribbon:

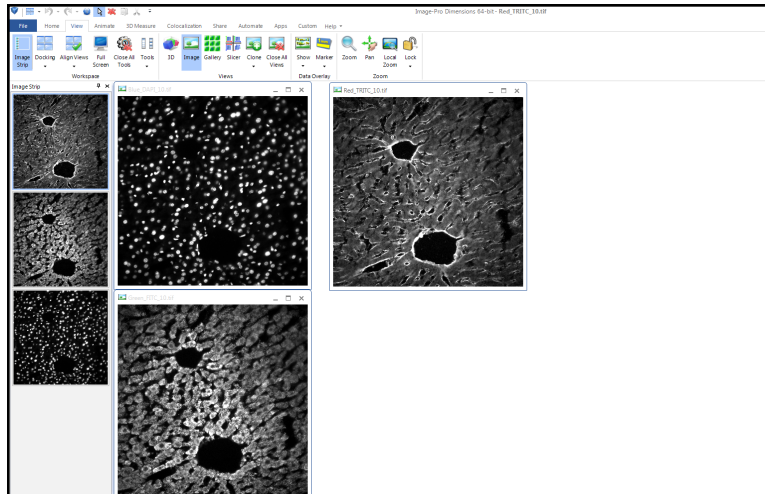


Normally, when the workspace windows are set to *Docking*, the image appears like this:

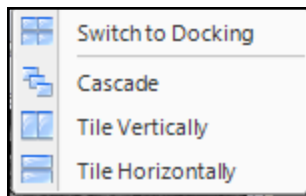


When you switch to the MDI view, several images can be viewed at once, as shown here. This example shows image windows tiled horizontally.

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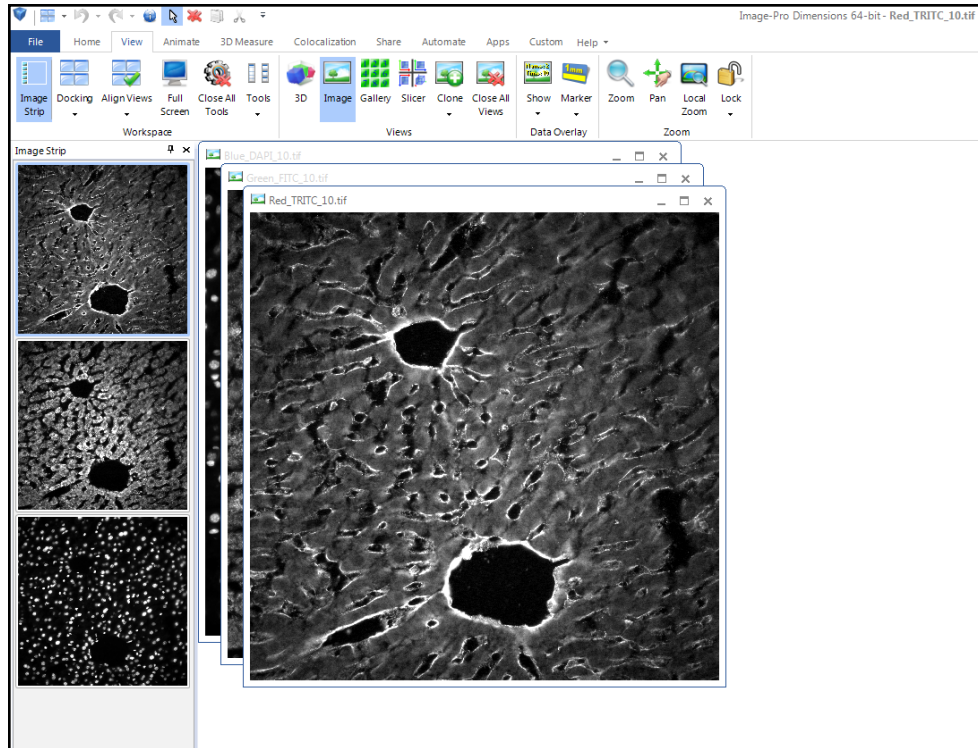


The pull-down MDI menu allows you to select a window display:

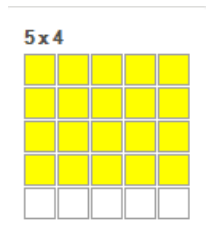


This example shows image windows in the *Cascade* layout:

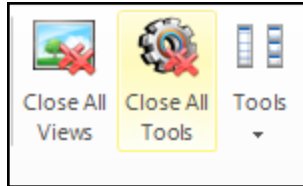
The Image-Pro Application Window



Align Views lets you organize your images in rows and columns, as shown here:



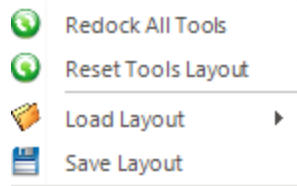
The *Tools* in the *Workspace* group allows you to load and save custom tool sets and layouts for your specific situation.



Close All Views closes all the images and windows in the workspace.

Close All Tools closes all open dialogs and panels.

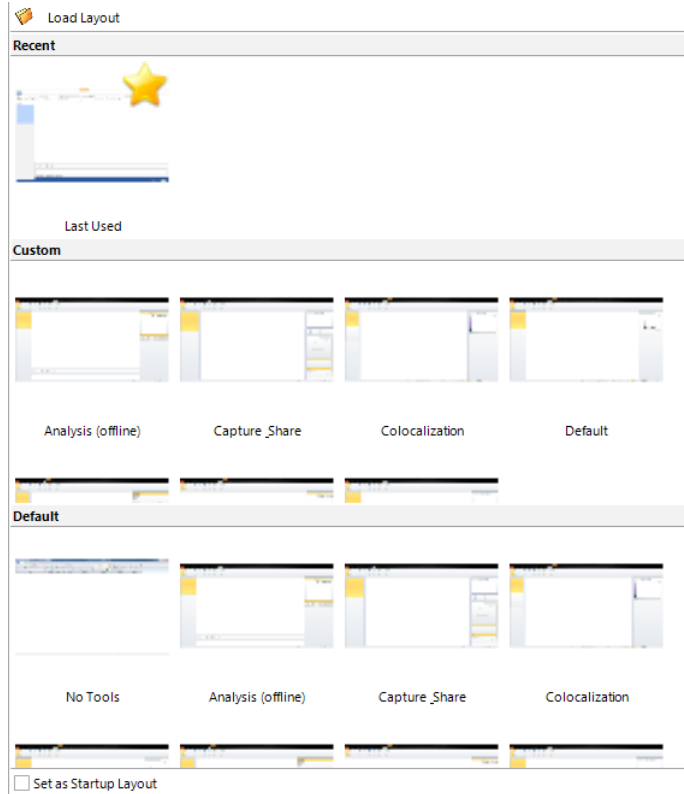
Tools redocks or resets any tools/ Click the drop-down arrow to see these features



Redock All Tools: Resets any open moveable panels that are undocked to their default docked position.

Reset Tools Layout: Resets the application to its factory default tools layout.

Load Layout: This tool allows you to load a previously-saved tools layout. When you click on this tool, the following dialog is displayed:












This dialog shows all available tool layouts to be loaded. The *Recent* area shows a list of the last loaded layouts. The *Custom* area shows a list of the custom layouts currently stored in the Configuration Files folder. The *Default* area shows a list of the available pre-defined layouts from installation directory **\Resources** folder.







SaveLayout: Click this tool to save the current layout of tools to a file that can be loaded at a later time to restore the layouts to its current configuration. When you click this option, you are prompted to give the layouts configuration file a name. Type a name for the layout configuration file, then click *Save*. You can use the *Load Layout* tool (described above) to restore the tools layout to its current configuration at a later time.

The Image Context Menu

Clicking the right mouse button on an image displays the following context menu:



- The *Zoom Scale Bar* allows you to adjust the size of the image by moving the slider to the right or left.

- The *Undo* and *Redo* buttons let you undo recent actions and reverse the effects of the Undo.

- The *Copy* button  will copy the part of the image under the ROI to the *Windows Clipboard*.
- The *Histogram* button  opens the image histogram.
- The *Image Information* button  displays information about the image in a table below the image.
- *Local Zoom*  opens or closes the *Local Zoom* feature.
 - The *Red, Green, Blue, and White* tint buttons  apply the appropriate color to the image.
- The *Remove Tint* and *Remove Tint All* buttons  remove tints from the active image or all open images.
- The *Toggle Nudge* polygon/polyline  editing mode button is enabled when the selection tool is running. Nudge editing mode becomes active only when a polygon or polyline is the selected object being edited. Pressing the <N> key has the same effect.


- The *Toggle Extended Vertex* polygon/polyline  editing mode button is enabled when the selection tool is running. *Extended Vertex* editing mode becomes active only when a polygon or polyline is the selected object being edited. Pressing the <E> key has the same effect.
- Clicking the *Convert* button  converts the selected graphic object(s) into poly form if possible.
- The *Toggle limiting graphic object*  button limits creation and editing to image bounds on the active overlay. By default, graphic object creation and editing is limited by the bounds of the underlying image on all overlays except for the Annotation overlay, where limiting is disabled by default.
- The *Selection tool*  enables selection and editing of displayed graphic objects.
- The *Dragging tool*  allows you to move the Pan/Scroll position by clicking and dragging on the image.
- The *Zoom tool*  allows you to zoom in or out on the image with mouse clicks or by drawing a box around the area to be zoomed. Only one tool may be active at any time, so starting one of these tools will stop any currently active one.

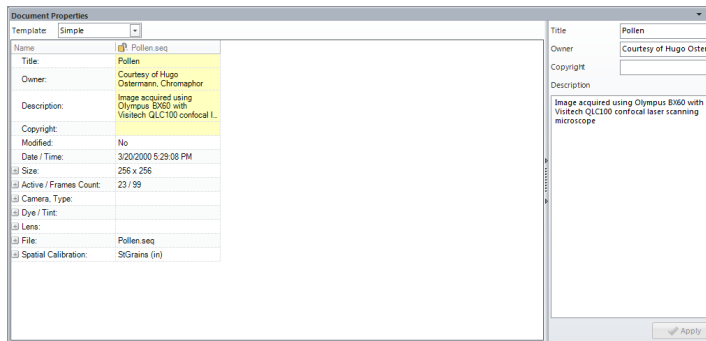
If your active workspace is an image set, you will see a different context menu.



More information about working with image sets appears in Chapter 5.

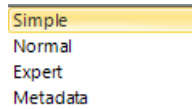
Viewing Image Document Information

Click the  symbol to see information about your active image. You will see the *DocumentProperties* window displayed at the bottom of the *Image-Pro* workspace, as shown here:



The image and document property information has been greatly expanded in from previous versions of *Image-Pro*. This expanded view allows you to find important information more easily. You can also edit the metadata.

The *Document Properties* feature allows you to view your data in four different layouts:

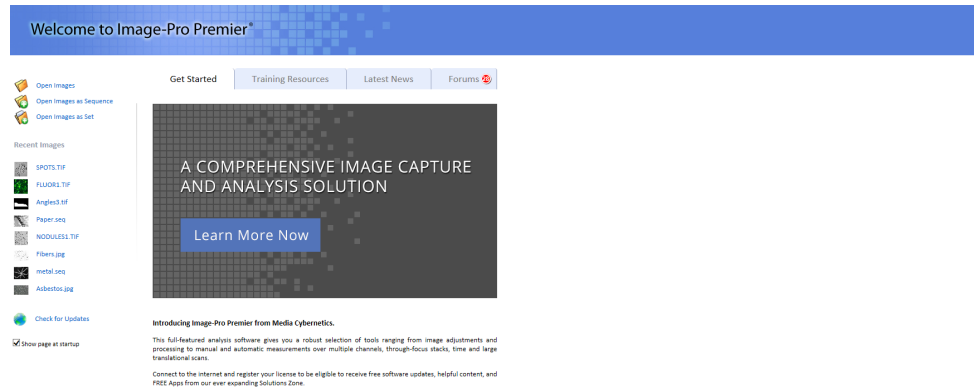


To compare information for more than one image, click the *Lock* icon.

The Welcome Screen

The first thing you see when you start *Image-Pro* is the *Welcome* screen. The updated and improved *Welcome* screen introduces a tabbed interface with 4 different tabs.

The **Get Started** tab displays a link to the *Apps Library* on the top part of the page. The bottom part of the page shows the last three posts from the Image Analysis Forum. Click any link to access the image analysis discussions and knowledge base.



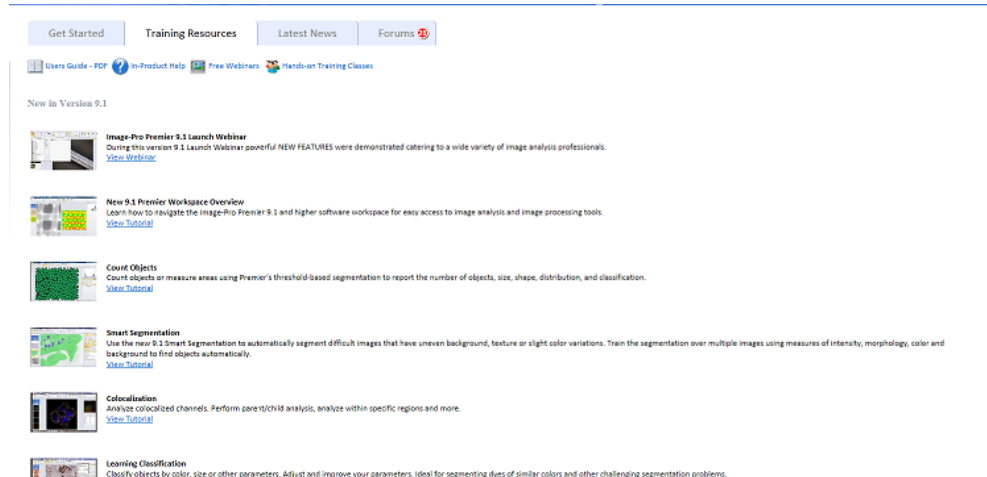
The information on the left side of the screen is always available. From here, you can open an image, sequence, or set. The list of *recentimages* displays a list of files that were recently opened.

Check for Updates: Click this button to see if any upgrades are available from *Media Cybernetics*.

Show page at startup: Check this box to see the *Welcome* screen each time that you start *Image-Pro*.

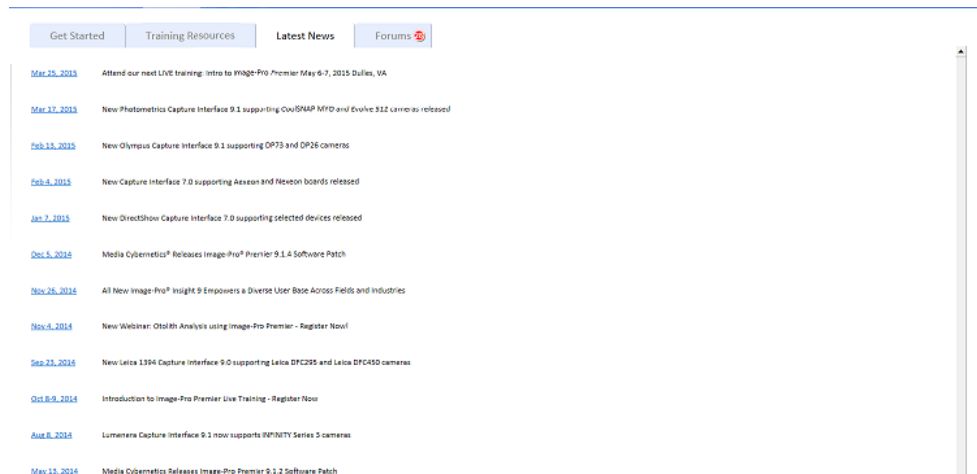
The **Training Resources** tab displays a copy of the contents of the video tutorials page on the website.

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The tutorials are in MP4 format. A media player is required to view these files.

The **Latest News** tab displays the latest news from Media Cybernetics.



A red notification displays the number of new articles and disappears when the tab is clicked.

The **Forums** tab displays the most recent discussions from the *Media Cybernetics Image Analysis Forum*.

The Welcome Screen

Get Started

Training Resources

Latest News

Forums

Image Analysis Forums: [Recent Discussions](#)

Update issue with POINTS from MANUAL TAG TOOL not RE-CLASSIFYING properly ... **NEW**

4 views 1 comment Most recent by Yurib September 03 5:17:38 AM Image-Pro Premier General Discussions

How do I record the code to execute the functions triggered by the CAPTURE BOX + LIVE BUTTON? **NEW**

3 views 3 comments Most recent by mborchelt September 03 Image-Pro Premier General Discussions

Creating an Image-Pro Premier Add-In **NEW**

41 views 2 comments Most recent by PierreD September 04 Image-Pro Premier Automation (Macros, Apps, Reports)

Is there a maximum number of frames to open and merge? **NEW**

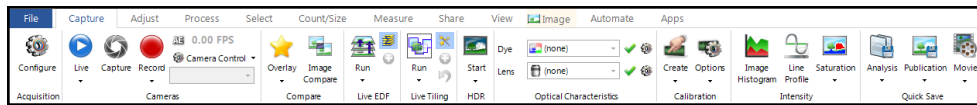
11 views 1 comment Most recent by Yurib September 02 Image-Pro Plus Automation with Macros

Extracting details of found blobs **NEW**

1 view 2 comments Most recent by richard bythway September 02 Image-Pro Premier Automation (Macros, Apps, Reports)

The Image-Pro Ribbon

The *Image-Pro Ribbon* contains commands that affect or change the content in the main workspace. The overwhelming majority of the commands in the Ribbon are buttons which either perform some command directly or open a dialog panel for setting options and performing related actions. The ribbon displays commands organized into tabs and groups. The ribbon displaying the **Capture** *tab* is shown below:



The different ribbons will be examined in more detail later in this chapter.

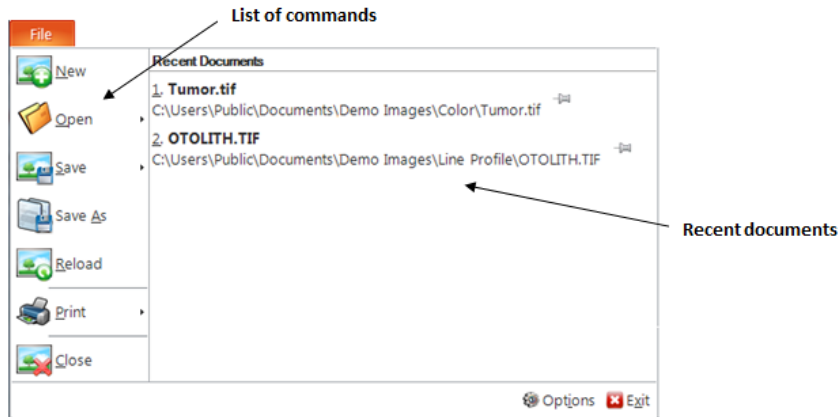
Note that if you have not licensed capture, or are running *Image-Pro with 3D Module*, some of the tabs and their associated ribbons will be different from this illustration.

```
All Readable Files (*.png;*.jpg;*.jpe;*.jpeg;*.jp2;*.jpf;*.fits;*.fit;*.fts;*.bmp;*.ipraw;*.mp4;*.mov;*.m4v;*.avi;*.seq;*.tif;*.tiff;*.btf;*.wmv;*.mcs;*.ips;*.deb;*.av)
All Image Files (*.png;*.jpg;*.jpe;*.jpeg;*.jp2;*.jpf;*.fits;*.fit;*.fts;*.bmp;*.ipraw;*.mp4;*.mov;*.m4v;*.avi;*.seq;*.tif;*.tiff;*.btf;*.wmv;*.mcs;*.ips;*.deb;*.av;*.s)
All Image Set Files (*.mcs;*.ips;*.deb;*.avz;*.czi;*.dm3;*.dm4;*.dv;*.oib;*.oif;*.ics;*.lei;*.lif;*.lsm;*.nd;*.nd2;*.ndpi;*.ndpr;*.ndpis;*.vms;*.vmu;*.scn;*.seq;*.st)
Aperio Virtual Slide (*.svs)
AutoQuant Legacy Sets (*.deb;*.avz)
Bitmap or Micro-Manager (*.btx)
Deltavision (*.dv)
FITS (*.fits;*.fit;*.fts)
Gatan Digital Micrograph (*.dm3;*.dm4)
Hamamatsu NanoZoomer Image (*.ndpi;*.ndpr;*.ndpis;*.vms;*.vmu)
Image Cytometry Standard (*.ics)
Image-Pro Plus Sets (*.ips)
Image-Pro raw (*.ipraw)
JPEG Image (*.jpg;*.jpe;*.jpeg)
JPEG2000 (*.jp2;*.jpf)
Leica LEI (*.lei)
Leica LIF (*.lif)
Leica SCN (*.scn)
Media Cybernetics Experiment (*.mce)
Media Cybernetics Sets (*.mcs)
Meta ND (*.nd)
Meta STK (*.stk)
Movie Sequence (*.seq)
MPEG 4 Files (*.mp4;*.mov;*.m4v)
Nikon Elements ND2 (*.nd2)
Objective Imaging Workspace (*.sws)
Olympus Fluoview 1000 (*.oib;*.oif)
PerkinElmer (*.tim)
Portable Network Graphic (PNG) (*.png)
TIFF (*.tif;*.tiff;*.btf)
```

Experiments are image files that contain different types of content stored in one, often very large, file.

The File Menu

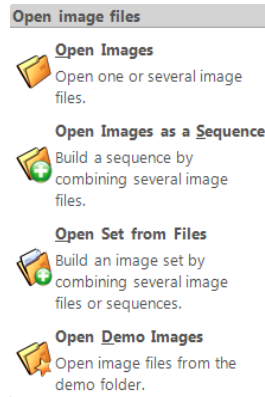
The *File* button is located in the upper-left corner of the application window. The *File* button menu contains commands that operate on the document as a whole, such as Print and Save, as well as other basic functions.



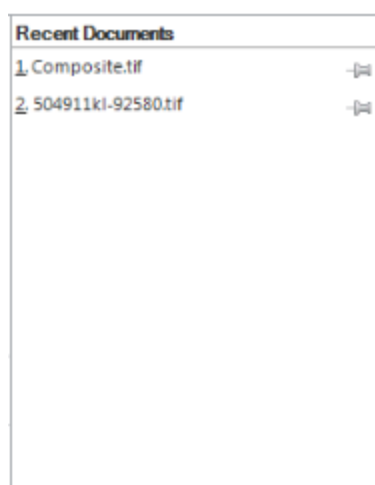
Clicking the *File* button displays functions that are always available:

- New
- Open
- Save
- Save As
- Reload
- Print
- Close

Some of the items in this list have small menu arrows next to them. Hover your mouse over the arrow to make the menu appear, as shown here:



The *Recent Documents* panel lists the files that you opened recently:



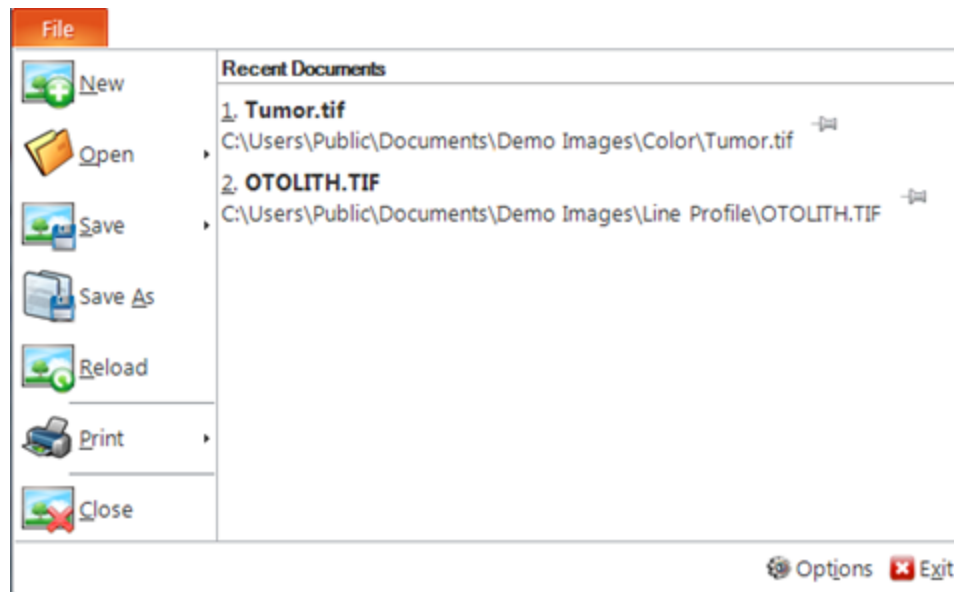
Click the name of the file to open it. You can use the push-pin icon to “pin” recently-opened files to keep them in the list. The drop-down arrow

Opening an Image

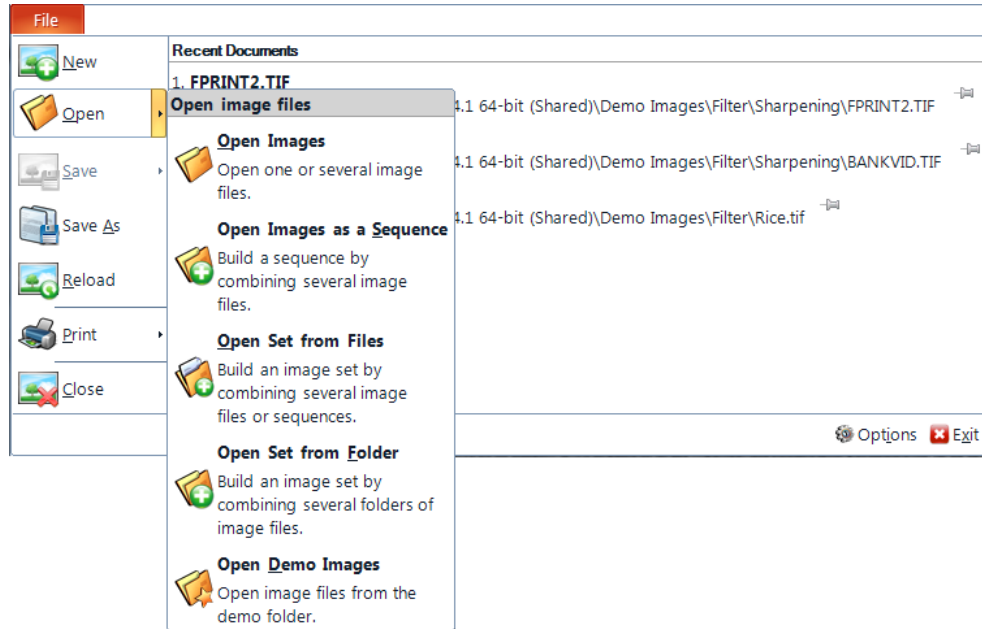
To open a saved image:

1. Click the *File* button.

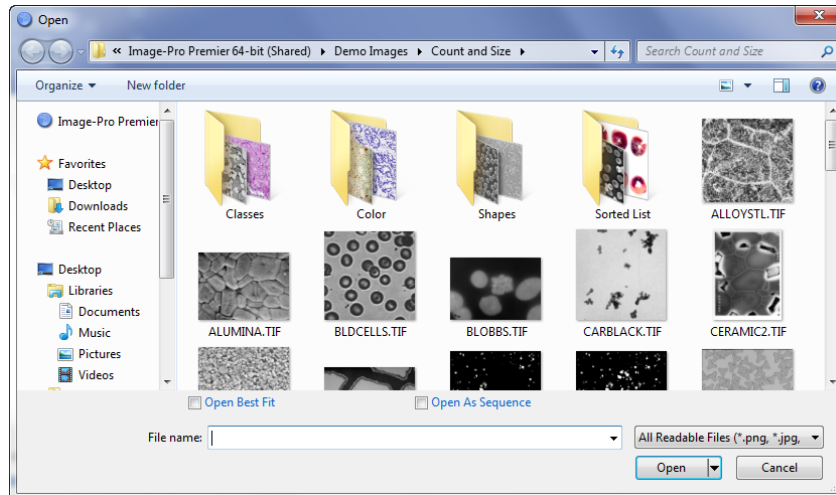
The pull-down application menu appears.



2. Choose *Open*. Click to go directly to the *Open Images* dialog, or hover your mouse over the menu arrow to see the menu of choices.



3. Select the location and type of image file that you want to open.
4. Select the image file that you want to open using the standard file open dialog as illustrated below.



To open more than one image, hold down the <Ctrl> key and use your cursor to select the images you want to open.

5. Click the *Open* button.

The selected image is opened in the *Image-Pro* workspace.

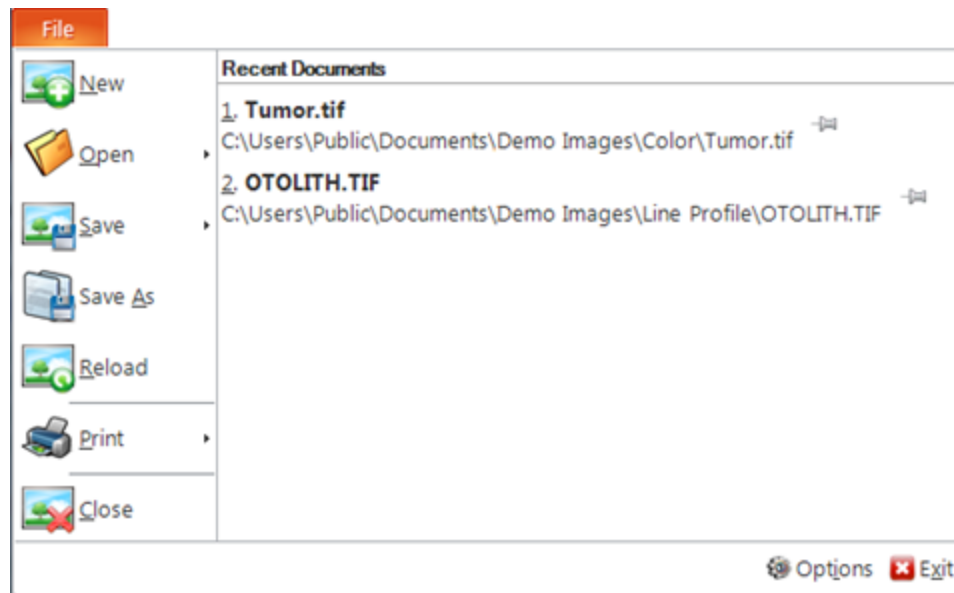
Opening an Image Sequence

Image sequences are like small movies. Image-Pro allows you to open, edit, and play image sequence files (*.seq).

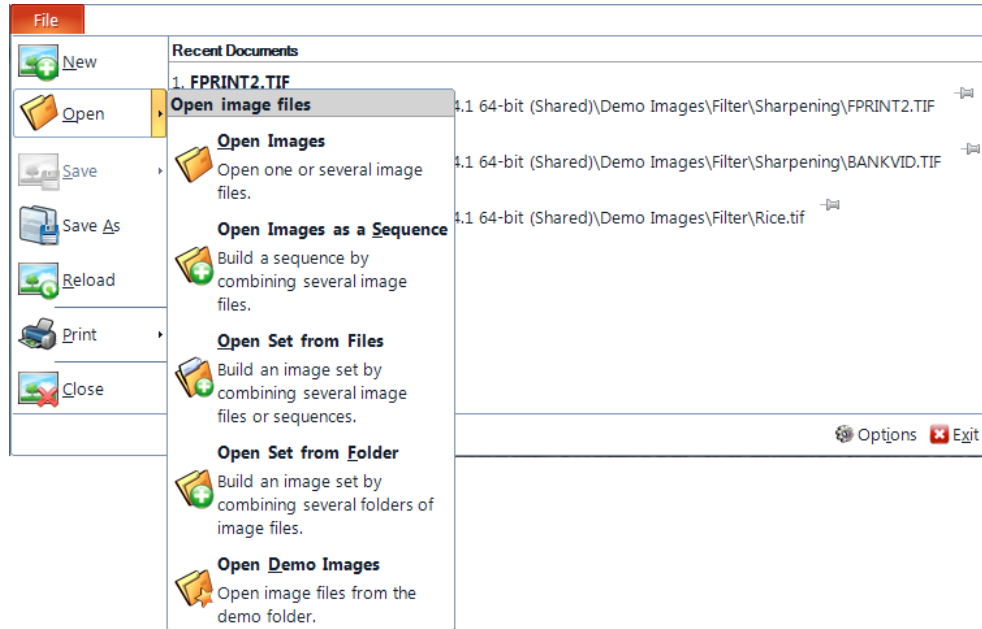
To open an image sequence:

1. Click the *File* button.

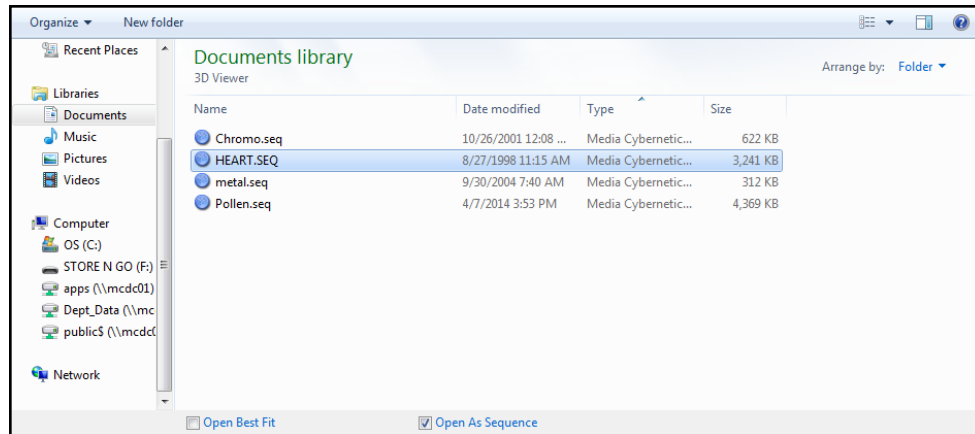
The pull-down application menu appears.



2. Choose *Open*. Click to go directly to the *Open Images* dialog, or hover your mouse over the menu arrow to see the menu of choices.

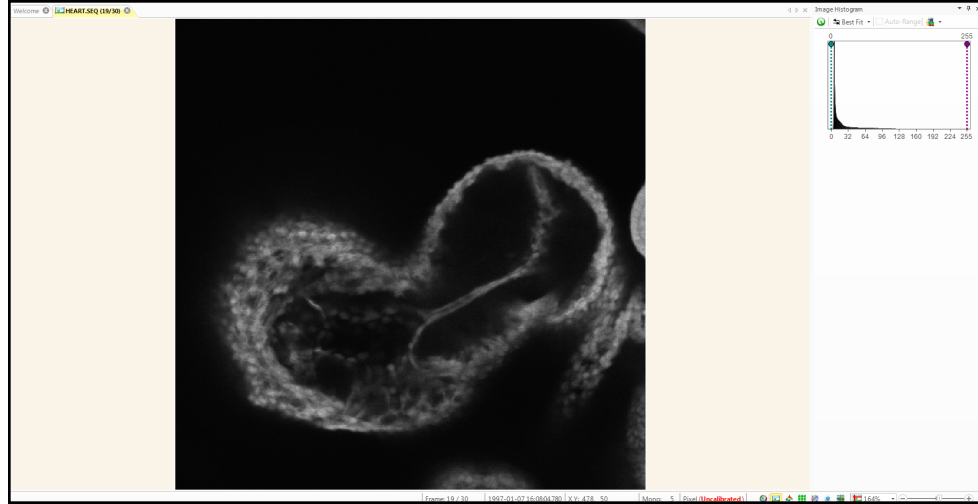


3. Select *Open Images as a Sequence*. Sequences have the extension **.seq*.
4. Select the image sequence that you want to open using the standard file open dialog as shown here.



6. Click the *Open* button.

The image sequence is opened in the *Image-Pro* workspace.



This example shows one frame of the *heart.seq*.

Opening an Image Set

An image set is a collection of images taken together to form a time lapse sequence, area scan, or a Z-stack, or any combination of these. Image sets are often complex groupings that include more than one dimension, such as both time and Z/focus dimensions, as well as channel specificity and more. This would mean that a single image member file of a given image set could, for example, represent a single channel at a single distance/focus and a single moment in time. The collection of images would therefore need to be organized and reconstituted to be viewable and analyzable in any meaningful way. is designed to work with the metadata and naming conventions found in many sets created by commonly used image acquisition tools and to organize the files according to the their significant dimensions (or let you organize them) so that they can be saved into a single image set file (with the extension *.mcs).

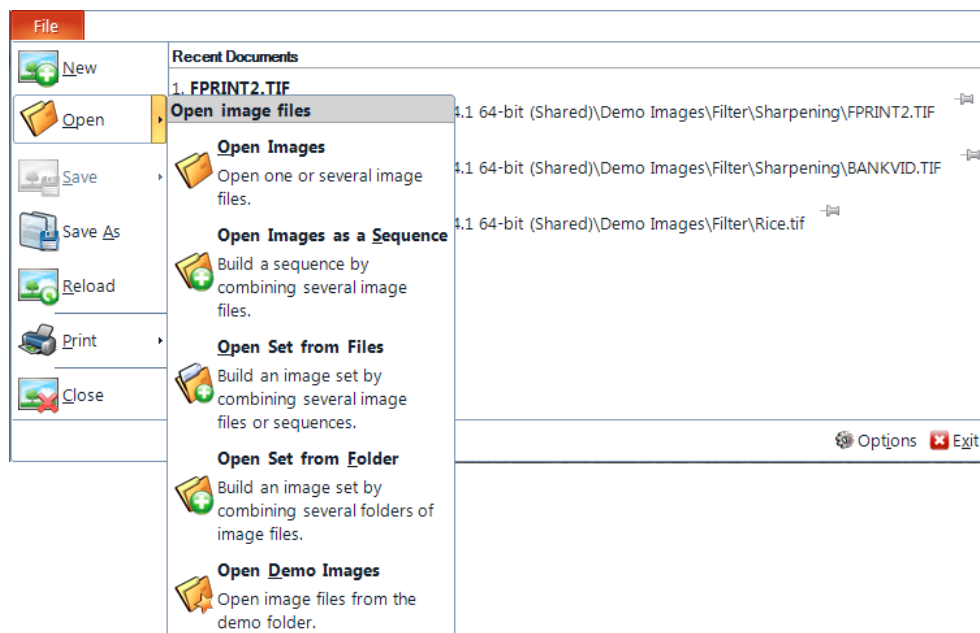
Note that there are five possible dimensions that can be used in the organization of files in a set:

- Channel: Index number for the color channel.
- Slice: Index number for the relative position in the Z stack or series of focal adjustments.

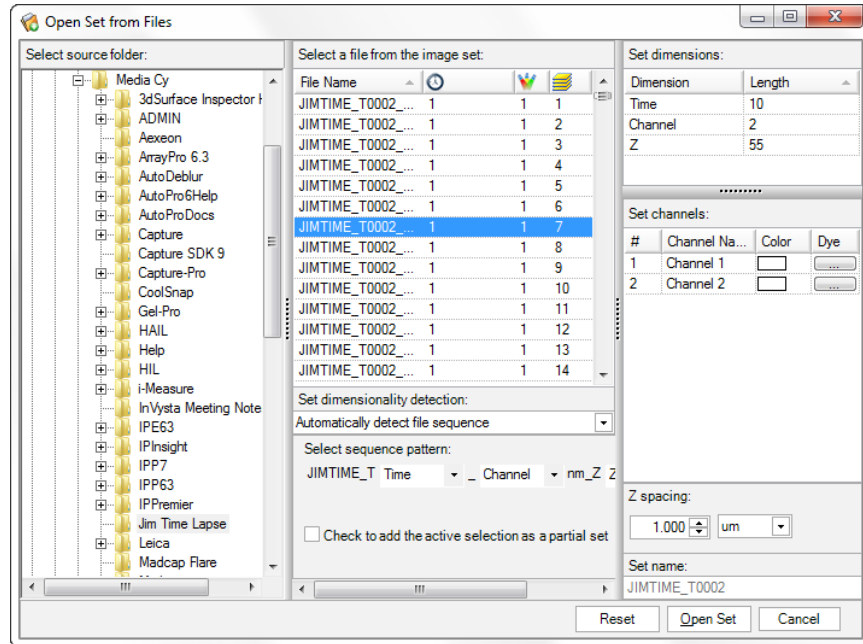
- Site: Index number for the site. Used for organizing image data per area of interest in a sample, for example.
- Time: Index number for the relative position in a time lapse sequence.
- XY Scan: Index number for the relative position in an XY scan (a series of images that are taken across the surface of a sample).

Image-Pro and *Image-Pro with 3D Module* feature the *SetBuilder*. You can use this feature to create an image set from a collection of individual image files. Follow the instructions below:

1. Click the *File* button.
2. Choose *Open Set from Files*.



You will see the following dialog:



3. Select the folder containing your image source files.

Use the *Select Source Folder* browser in the far-left panel to navigate to the folder where your image files are located. Your set files should be in numerical order if possible.

4. Select the method for determining the set. There are three modes of operation available:
 - *Automatically detect file sequence.* Select this option if the files in the sequence have matching internal dimensions and naming conventions that make their locations along each dimension distinctly identifiable. In such a case, *Image-Pro* can reconstitute the entire set instantly and automatically. If you select this option, you should:
 - a) Select a single file that is a member of the set you are trying to build. analyzes its name to tries to discern dimension information in the name. If any dimension information is detected, it prompts you to confirm the meaning the data in the name structure, as follows:

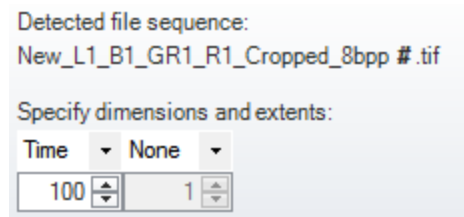
Image_T Time _S001_Z Z _C01.tif

b) Review the assigned dimensions. From the list boxes provided, select the correct dimension information if guessed wrongly.

c) For sequences that include numeric and non-numeric dimension information (such as when a channel name is included in the file name), use “Check to add the active selection as a partial set” to “lock in” the detected sequence to part of a larger set.

- *Split sequence into dimensions.* Select this option if the files in the sequence have naming conventions that mix location information for multiple dimensions into a single sequence. For example: a 50-slice, 2-channel set that includes files named “Image001.tif” through “Image100.tif”. In such a case, can automatically collect the relevant files into a table, and perhaps organize them according to one dimension, but it depends on you to specify the allocation of the specified sequence into the relevant dimensions. If you select this option, you should:

a) Select a single file that is a member of the set you are trying to build. Analyzes its name to try to locate sequence information in the name. If any dimension information is detected, it prompts you to confirm the meaning of the data in the name structure, as follows:



Detected file sequence:
New_L1_B1_GR1_R1_Cropped_8bpp #.tif

Specify dimensions and extents:

Time	▼	None	▼
100	▼	1	▼

b) Adjust the dimension allocation as appropriate using the list boxes provided. Note that additional dimension drop-downs appear as needed.

- *Manually add files to a dimension.* When you select this option, you are prompted to select a dimension and position within that dimension to which you want to add files, and then select the files to be added. Follow the steps below to manually organize your files:
 - a) In pull-down list box below the Add selected files to... button, select the dimension you want to define.
 - b) In the spin box to its right of the selected dimension, specify the position in the selected dimension to which you want to add files.

- c) In the file list, select all files belonging to the specified position of the selected dimension.
- d) Click the Add selected files to... button.
- e) Repeat steps a) through d) until all relevant dimensions and positions are defined.

Choosing a new operation mode will change the controls available in the control panel at the bottom of the center region of the Set Builder dialog.

- 5. Select a folder to add images to the *File Table* in the center panel. The file table in the center will list the supported image files that are present in that folder, whether or not they belong to a set.

The *Location* columns of the file table display the location index fields that have been assigned to that file. If the file has not been assigned to any location within the set, this column will be blank. Once the file has assigned to a location in the set, the *Location* column will be replaced with columns for each dimension that is currently active in the set.



Each dimension is represented by an icon: Color Composite Channel, Location in the Z –stack, and Time position. Each column header has a tool tip to indicate the dimension that the icon represents.

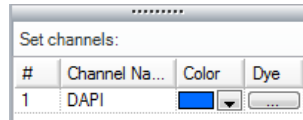
The dimensions of each set appear in a table on the right hand side of the dialog.

When you've selected a folder, the file table in the center will list the supported image files that are present in that folder, whether or not they belong to a set are in the panel on the right. This table indicates the extents of the set as currently constructed. In the cases of incomplete (jagged) sets, the extents in this table will represent the longest added set thus far:

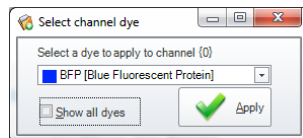
Set dimensions:	
Dimension	Length
Channel	2
Time	2
Z	3
Site	6

- 6. Add channel information for the set.

The channels table displays one row per channel. You can set the channel name, tint, or set them both by assigning a dye to the channel, as shown in the illustration below:

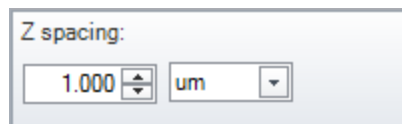


The channel name can be edited in-place. Use the *Color* drop-down arrow to see the standard color picker. Click the “...” button in the *Dye* column to select a dye from the favorites list, or from all available dyes:



- When *Show all dyes* is checked, all the dyes in the Dye Library will be included in the list. If this option is not checked, you will see only the dyes in the Favorites list. If the current channel name matches a dye in the list, that dye will be selected in the drop-down list.
 - Click *Apply* to set both the *Channel Name* and the *Color* for the selected dye. In addition, the dye will be associated with the channel and will later be associated with the image set channel when the set is imported.
7. Set the Z-spacing.

The Z- spacing panel on the dialog allows you to set the spacing between Z planes if the selected set has Z stacks:

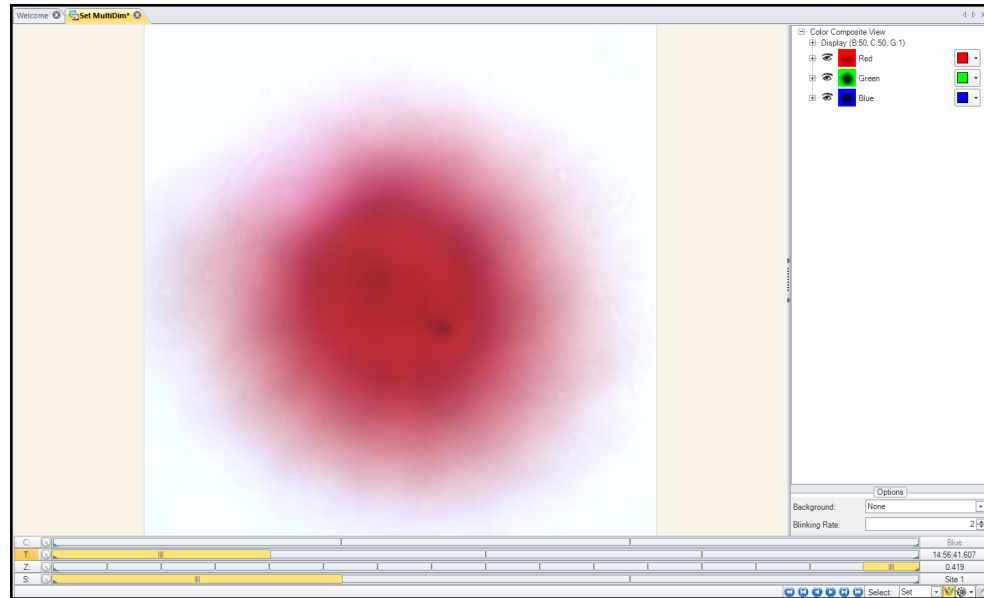


The selected spacing will be applied to both the set’s metadata and to the frames of the images

8. Open the set.

The *Open Set* button will be disabled until you have selected at least one file to define the set.

Here is an example of an image set open in the workspace:

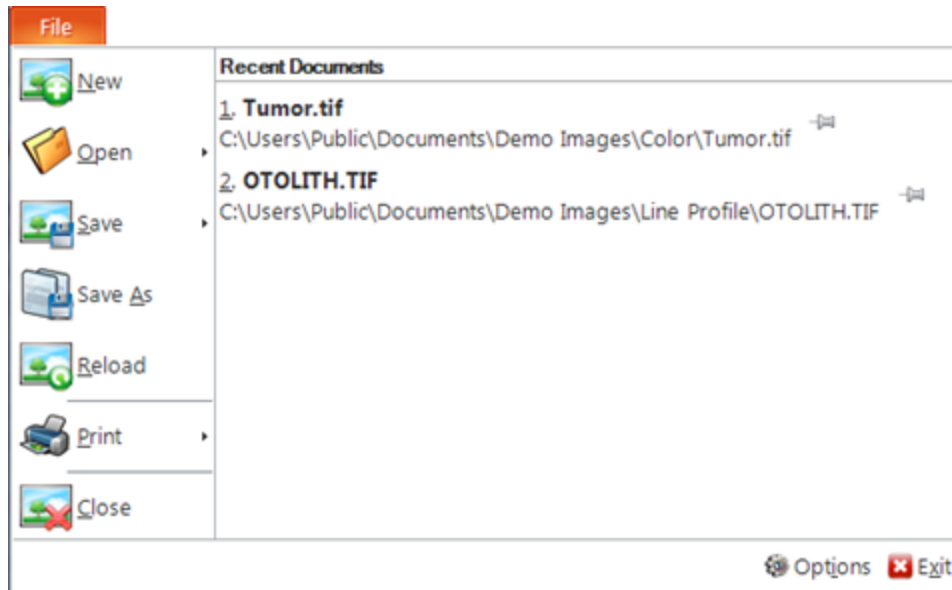


The slider controls and other features of an image set are discussed in Chapter 5, *Working with Images*.

Saving an Image

When you hover over the *Save* command in the application button drop-down menu, *Image-Pro* displays two options:

- Save
- Save All.



Save

Use the *Save* command to immediately store the contents of the current window to its file (the file listed on the window's title bar) while leaving the image active in its window. If the image is in an untitled window, *Image-Pro* will display the *Save File As* dialog box.

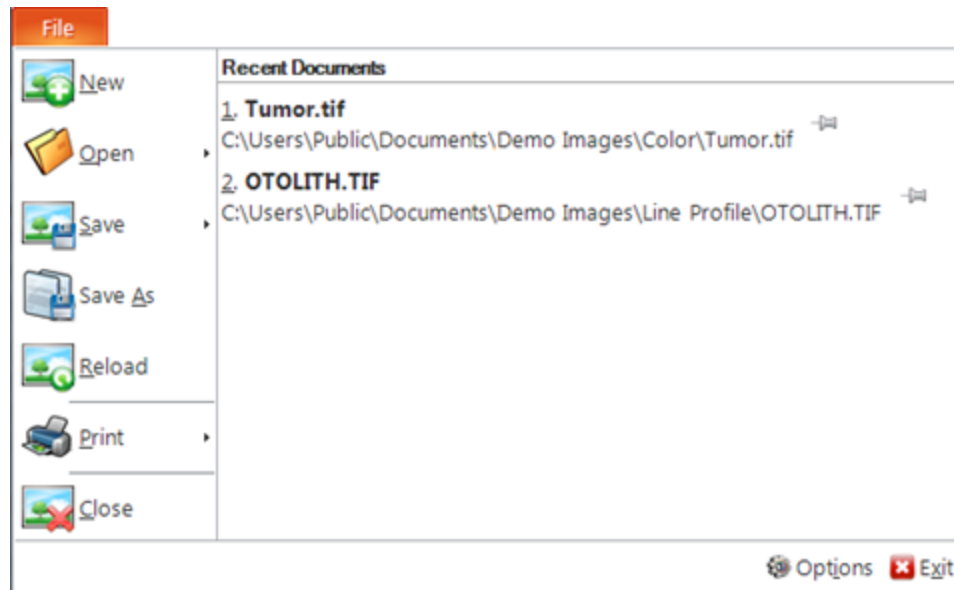
The *Save* command can be used to save your most recent changes to disk. It is often performed as a precautionary measure during lengthy or involved processes to reduce the amount of reprocessing that might be required in the event of a system failure or operational error. When you close an image and choose **not** to save its changes, *Image-Pro* discards all changes made since the last *Save* operation.

Save All

Use the *Save All* command to immediately store the contents of all images currently open in the *Image-Pro* workspace.

To save an image, follow the steps below:

1. Click the *File* button. The pull-down application menu appears.

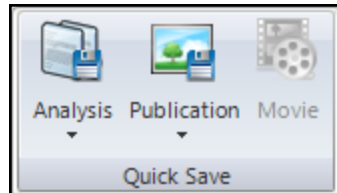


2. Choose *Save*. You have a choice of saving only the active document (image) or saving all modified documents or images. Your image is saved automatically.

Using Quick Save

In addition to using the *Save to File* feature of the image settings, *Image-Pro* offers you a quick and easy method of saving your images. Note that *Quick Save* does not work with image sets.

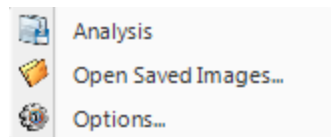
1. Go to the *Quick Save* group on the **Capture tab**.



2. Choose *Quick Save for Analysis* or *Quick Save for Publication*.

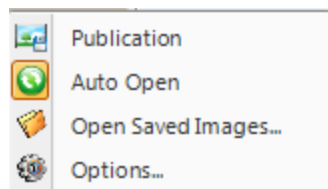
If you are saving a sequence of images, choose *Save as Movie*.

When you select *Analysis*, you will see the following drop-down menu:



- *Quick Save for Analysis* stores your images in *.TIF format so that they can be retrieved for analysis at another time.
- *Open Saved Images* goes to the *Open* dialog and lets you select a previously stored image.
- *Options* takes you to the *File Options* page where you can change your preferences.

When you select *Publication*, you will see the following drop-down menu:



- *Publication* stores your image in a *.JPG format that can be easily incorporated into a report or spreadsheet. It also burns-in any annotations, ROIs, or measurements and resizes your image.
- *Auto Open* will automatically open your saved image in the workspace.
- *Open Saved Images* goes to the *Open* dialog and lets you select a previously stored image.

- *Options* takes you to the *File Options* page where you can change your preferences.

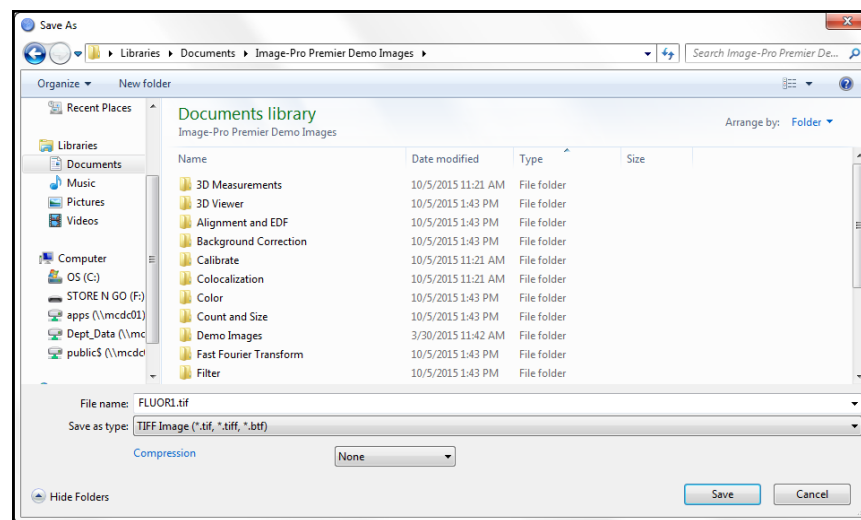
Save As

Use the *Save As* command to store the contents of the current window, the current ROI, or the active part of a sequence, to a file that you specify, in the format that you specify. The *Save As* command serves several important uses beyond simply storing an image to a new file name. It is also used to:

- Convert a single image from one format to another. For example, if you needed a TIFF file converted to PNG format, you would simply open the TIFF image, and then use *Save As* with the PNG format option to save it to a new file.
- Save an image with different compression or quality options from those it was originally created with. For example, if your original TIFF file was uncompressed, you can use *Save As* with the LZW compression option to store it in compressed form.
- Save an image to another disk or folder.

Note: Files containing multiple frames, or sequences, are supported only in the TIF, SEQ, IPW, and AVI formats. All other formats will save only the active frame.

The *Save As* dialog is shown here:



Save in: Find the folder into which you want to save the file. You may create a new folder using the *New Folder* button.

File name: Enter the name of the file you want the image saved to. To specify the file's location, you can either enter its entire path (disk and folder), or specify its location using the *Save in* list box.

Save as type: In this list box, select the format in which you want the image saved.

Compression: In this list box, select the form of compression you would like the file stored in. The available compression methods will vary from one file format to another. For example, LZW (Lempel-Zif encoding) compression is an option with TIFF files, but it is not available in JPG. Moreover, some formats have no compression options (*None* is the only choice).

Note: If you plan to use the image with another program, be sure it is compatible with the selected compression method. Not all programs support compressed and uncompressed format variants.

Reload

Use the *Reload* command to fill the active window with a new copy of the file it is linked to (the file listed on the window's title bar).

You might want to use this command if, after considerable editing, you wanted to restart with an untouched copy of the image. *Reload* is also very useful in automated procedures where a routine needs an image free from any effects of a preceding routine.

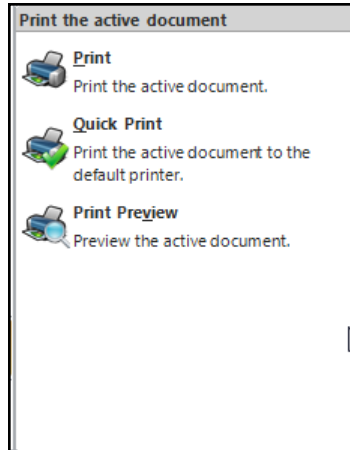
Note: *Image-Pro* loads the active window from the **current** copy of the file associated with it. If you have made changes to the stored file (whether from this window or another) after the image was opened, the changes will be reflected in the reloaded image.

Performing a *Reload* on an untitled image window has no effect.

Performing a *Reload* has no effect on the Lookup Table. If you have modified the Brightness, Contrast, Gamma settings, they will remain as you set them. If you want to return the LUT to the original settings, use the *Reset* button on the BCG tool bar.

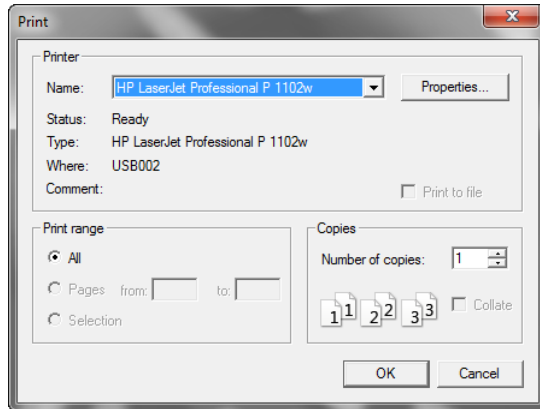
Printing an Image

Use the *Print* command to print one or more copies of the current image, or the current ROI, to the selected output device. When you hover over the *Print* command in the *File* menu, you are shown the following print options:



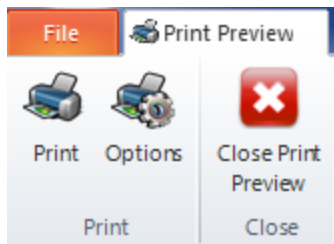
Quick Print: Click on this option to print the report to the default printer using the current printer settings.

Print: Click this option to display the *Print* dialog box prior to sending the report to the printer:



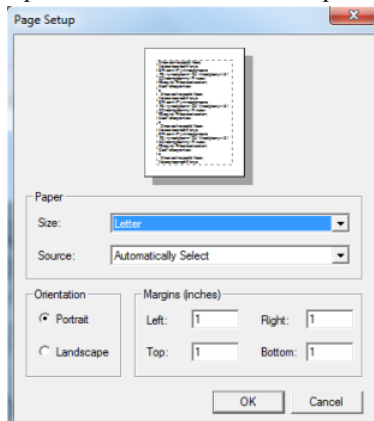
Through the *Print* dialog box, you can select the printer you want to print to, define any other print settings, such as number of copies. Click the *OK* button to send the page to the printer.

Print Preview: Click this option to display the report in a preview screen prior to printing. When you select this option, the preview is shown in a new image window with the following ribbon bar options:



Print: This is the same as clicking *Print* from the *File* menu (as described above).

Options: Click this tool to open the *Page Setup* dialog box:



Use the controls on this dialog box to specify the desired page layout options. Through this dialog box, you can specify the paper size, page orientation, and margins for the page containing the image you want to print. Click *OK* to apply your selections and return to the print preview.

Close Print Preview: Click this tool to exit the print preview window without printing.

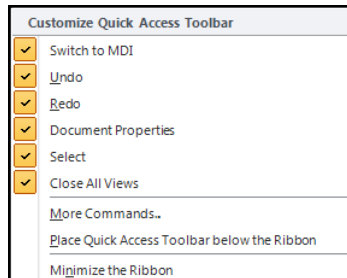
Quick Access Toolbar

Quick Access Toolbar drop-down arrows

This toolbar, located just above the ribbon bar tabs, contains controls which are usable regardless of which tab is active. You may add a copy of any almost ribbon bar button or menu item to the quick access toolbar. Added buttons will be retained from application session to application session.

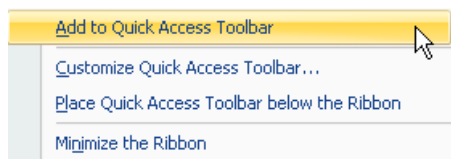


Clicking the drop-down arrows displays a drop-down menu that allows you to customize the selections that appear. A copy of the drop-down menu appears here:

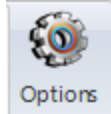


Use *More Commands* to add items to the *Quick Access Toolbar*. You can find information about adding commands in Chapter 9.

In addition, right-clicking on any of the buttons in a ribbon displays a menu that allows you to add that button to the quick access toolbar, as shown here:

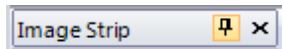


- Note that there may be some functions that cannot be mapped to the Quick Access Toolbar.



Options Buttons

Image-Pro includes *Options* buttons throughout the workspace. Click on these gear buttons to adjust colors, font sizes, and other feature settings.

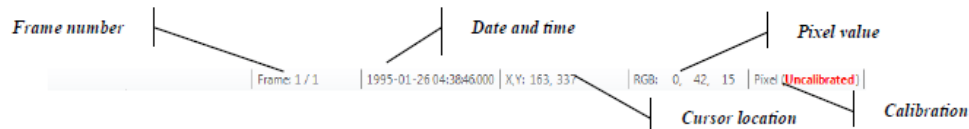


Close or Hide Dialogs for Extra Space

Open up your workspace by hiding open dialog boxes. If you are not using a panel to close the dialog, or may need it again later, collapse it by clicking thumbtack button on the top of each dialog. Hover over the dialog to expand and view it temporarily. When the dialog is expanded, you can click the thumbtack again to “pin” it open for future use.

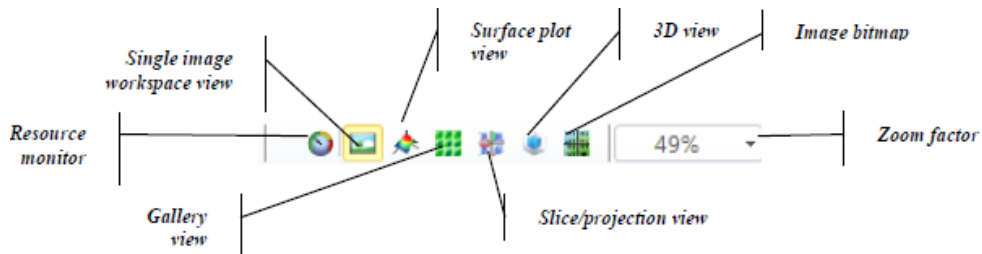
The Image-Pro Status Bar

The *Image-Pro* status bar provides context-sensitive information about the status of your activities.



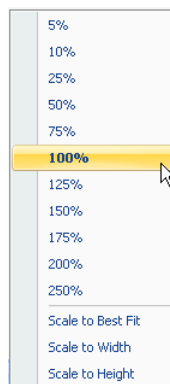
- Status area. This is where the progress bar and other indicators will appear.
- Frame number of the image in the workspace.
- Date and time that the image was captured.
- Pixel location of the cursor.
- Pixel value.
- Spatial calibration if known.

The image view icons allow you to view your image or sequence in different presentations:



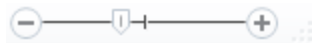
- Resource monitor. Clicking this icon opens the resource monitor to display important metrics about the management of images, and control over the application's use of image memory.
- Single image viewer. This icon changes depending upon if the image in the workspace is displayed as a single image or in a 3D view.
- Surface plot viewer. Click this icon to display the image as a surface plot.
- Gallery viewer. Click this icon to display an image sequence in gallery view.

- Slice viewer. Click this icon to display your image in slicer view.
- 3D viewer. Click this icon to display a 3D view of the image in the workspace. This will be different if you are using *Image-Pro with 3D Module*.
- Image bitmap. Click this icon to display an image bitmap of the image in the workspace.
- Zoom factor. Click this icon to adjust the size and scale of the image in the workspace. You will see the following dialog.



- Scale to best fit automatically adjusts the image display to show the best fit
- Scale to width automatically adjusts the image to the width of the workspace
- Scale to height automatically adjusts the image to the height of the *Image-Pro* allows you to enable or disable the status bar fields. Right-clicking on the status bar displays a menu that allows you to select what type of data you want to display in the status bar.

A zoom slider and a fixed-zoom control list allow you to control the size of the image in the workspace. The zoom slider is shown here:



When the magnification is known and the option active, the current zoom will be displayed as the effective magnification, as shown here:

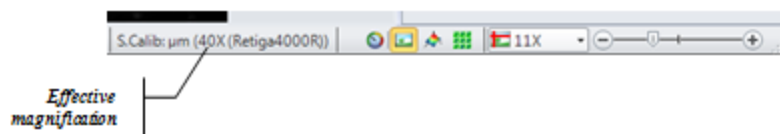
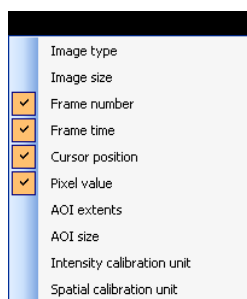


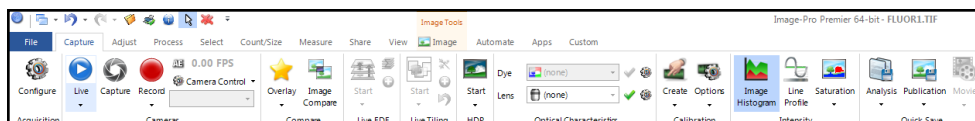
Image-Pro allows you to enable or disable the status bar fields. Right-clicking on the status bar displays a menu that allows you to select what type of data you want to display in the status bar.



Click the cursor next to the name of the field you wish to enable or disable. Enabled fields are checked. You can also enable or disable fields in the status bar using the options described earlier in this chapter.

Tabs and Ribbons

Tabs are used to display different groups of functions. The tabs are arranged in a progressive left to right order following a common workflow where a person using the application acquires an image from a source, adjusts it in some fashion, selects objects in the image for study, measures properties of those objects, visualizes those objects in multiple potential contexts, then shares this information with others. If you have licensed a copy of *Image-Pro* with capture, the default tabs are named **Capture**, **Adjust**, **Process**, **Select**, **Count/Size**, **Measure**, **Share**, **View**, **Image**, **Automate**, and **Apps**. The **Image** tab is only available when an image is open in the workspace. The **Extras** and **Custom** tabs are available only when certain features are enabled. Each tab opens a specific ribbon displaying groups of related tools.

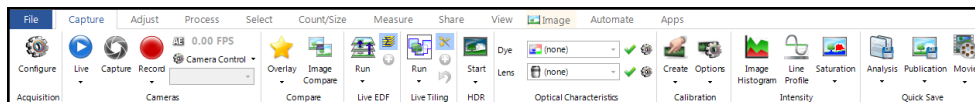


If capture is not available with your application, the **Capture** tab is replaced by the **Home** tab. If you are using *Image-Pro with 3D Module* (with or without capture) there are additional tabs: **3D View**, **3D Measure**, and **3D Animation**. More information about the 3D features appears in Chapter 12.

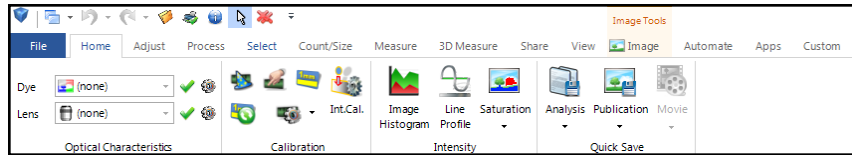
Some of the *Image-Pro* tabs are context-sensitive, which means that they are available only when you are using certain features, like Line Profile, or opening certain types of images, image sets, or Very Large Images (VLI).

Capture tab

The **Capture** tab contains groups for selecting dyes and lenses, calibration, and saving your images.



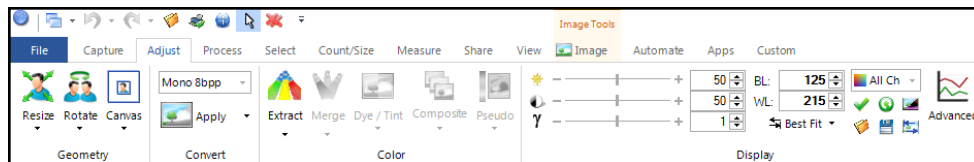
Note that this tab does not appear if you are using the non-acquisition versions of *Image-Pro* or *Image-Pro with 3D Module*. In this case, you will see the **Home** tab instead.



This tab contains groups for selecting dyes and lenses, calibration, and saving your images.

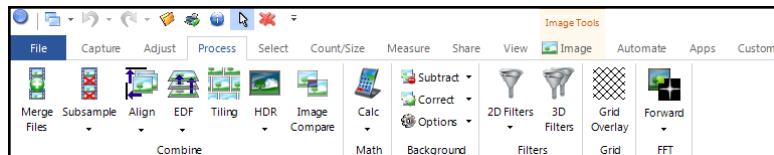
Adjust Tab

The **Adjust** tab contains all the functions that allows you to manipulate the image for best viewing and interpretation. Some functions may require making new or composite images from other images or changing the pixel values within an image.



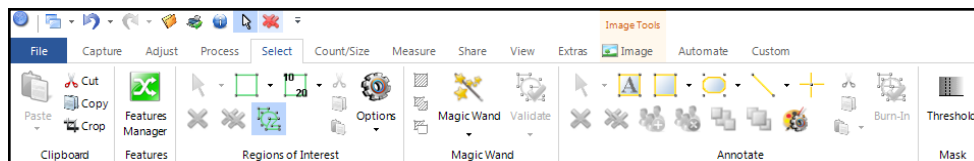
Process Tab

The **Process** tab contains the image processing tools that allow you to change an image or extract information from it.



Select Tab

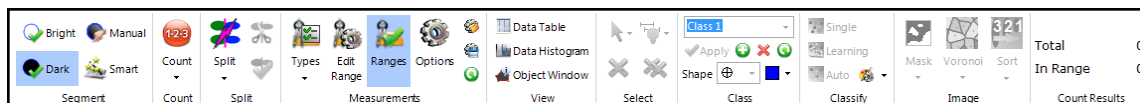
The **Select** tab contains the *Clipboard*, *Annotation*, *ROI*, and *Mask* features that enable you to examine and annotate an image or part of an image.



If you are using *Image-Pro with 3D Module*, this tab appears as **2D Selection**.

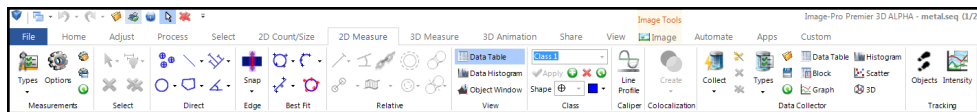
Count/Size Tab

The **Count/Size** tab contains tools that allow you to view, count, and measure objects in a two-dimensional image. It also contains the tools to display the resulting data in a variety of formats. If you are using *Image-Pro with 3D Module*, this tab appears as **2D Count/Size**.



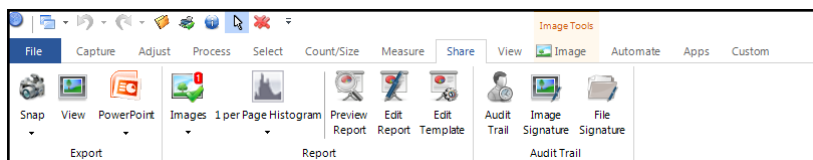
Measure Tab

The **Measure** tab contains features that enable you to measure and analyze features in all or part of your two-dimensional image. If you are using *Image-Pro with 3D Module*, this tab appears as **2D Measure**.



Share Tab

The **Share** tab contains features that allow you to share your images and data with others. You can also create an audit trail of all the recordable events during your image processing session. The features on the **Share** tab allow you to create reports and export your files.



View Tab

The **View** tab contains specialized tools and graphs to display information about the active image or image set. Some of these tools allow you to see different aspects of the active image also.

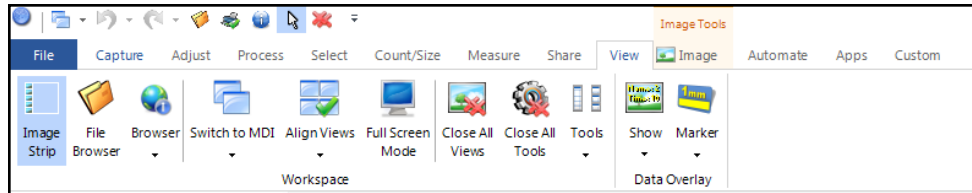
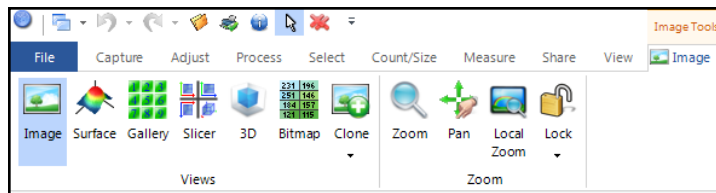


Image Tab

The **Image** tab is only visible when there is an image or image set open in the workspace. You can adjust the image display, show it in a gallery, or zoom/pan on a particular portion of the image.

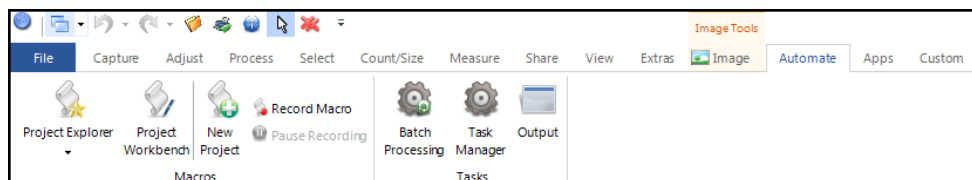


Optional Tabs

These tabs appear when you enable certain features using the Options described at the end of this chapter.

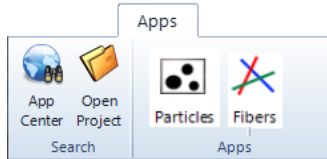
Automate Tab

The **Automate** tab contains the macro scripting features. This tab is visible by default, but you can turn it off by un-checking **Show Automate Tab** on the *Display* options page.



Apps Tab

The **Apps** tab contains any apps that you have loaded into your copy of *Image-Pro*. It contains a link to the *App Center*, where you can find and download the newest apps. It is empty until you have created or downloaded some apps.



Custom Tab

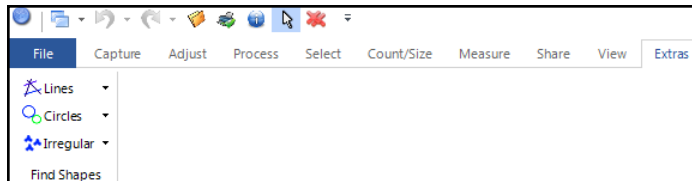
The **Custom** tab creates a ribbon for you to store the features that you use most frequently. The first time you open it, the **Custom** tab's ribbon will be empty. You can add any group or tool to it.



This tab does not appear until you check *Show Custom Tab* on the *Display* options page.

Extras Tab

The **Extras** tab contains advanced features, such as *Find Shapes*. This tab does not appear until you check *Show Extras Tab* on the *Display* options page.

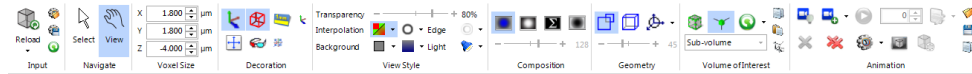


Context-Sensitive Tabs

These tabs are available when you have opened certain features.

3D View Tab

3D View lets you load, save, and examine your 3D image in a variety of ways:



This tab is available when you have a 3D image open in the *Image-Pro with 3D Module* workspace.

3D Measure Tab

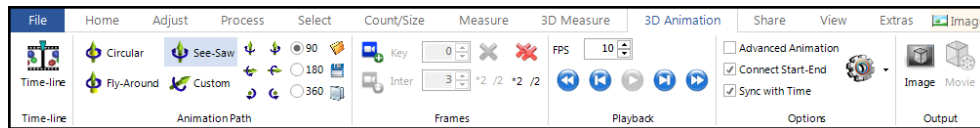
3D Measure contains the tools and features you will need to make volume measurements in a 3D image:



This tab is available when you have a 3D image open in the *Image-Pro with 3D Module* workspace.

3D Animation Tab

3D Animation lets you create animated images and movies from 3D images.



This tab is available when you have a 3D image open in the *Image-Pro with 3D Module* workspace.

Chapter 4

Viewing Intensity Measurements

This chapter explains how to:

- View an **Image Histogram**
- View a **Line Profile**
- View a **Data Table**
- Use the **Saturation Warning**

Image Histogram, Line Profile, and Saturation Warning

If you have a license for the *Capture* module, the **Image Histogram**, **Line Profile**, and **Saturation** features appear in the *Intensity* group on the *Capture* ribbon. If you do not have a *Capture* license, this group appears on the *Home* tab's ribbon.

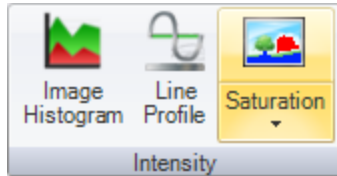


Image histograms measure, and illustrate in graph form, the distribution of pixel intensities in an image. Histogram data can be created and viewed for data gathering and analytical purposes or can be manipulated for image enhancement purposes.

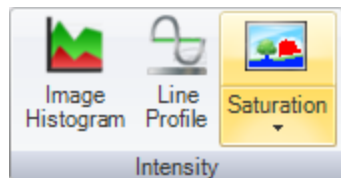
Line profile analysis allows you to collect the actual pixel intensities of the pixel values under the profile line. A profile plot shows the pixel positions of the line along the X-axis, and, along the Y-axis, indicates the pixel intensity for each position along the line. Either or both axes may use calibrated units if the image has a spatial calibration (for the X-axis) or intensity calibration (for the Y-axis). You can display a line profile on any type of image, whether it's the live image, a captured image, or an image opened from a file.

Saturation is used to show overexposed and underexposed areas with a given color. It can be used with the live preview to set proper exposure time and avoid over or under-saturated areas, so that intensity measurements on the image will be correct.

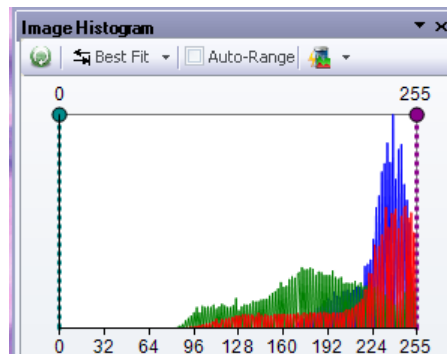
Viewing an Image Histogram

Image histograms measure, and illustrate in graph form, the distribution of pixel intensities in an image. Histogram data can be created and viewed for data gathering and analytical purposes or can be manipulated for image enhancement purposes. To create an Image Histogram:

1. Open the image that you want to measure if it is not already opened.
2. Select **Image Histogram** from the *Intensity* group.



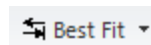
A histogram window appears in the *Image-Pro* workspace:



The histogram area is displayed with a gradient tint that ranges from black to white for mono images, and from black to the color of the channel for color images.

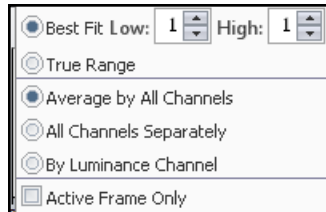
Histogram & Profile options control the display of an image histogram or line profile. More information about these options appears in Chapter 2.

The **Reset** button automatically resets the histogram display.




Best Fit applies the best fit display to the image .

If you click the drop-down arrow, you will see the available best-fit options:



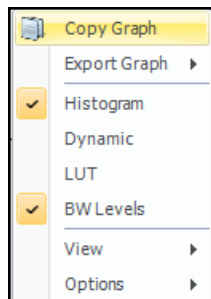
Auto-Range automatically adjusts the black and white levels on the image. This feature is available only if the current camera supports Auto-Range.

The **Data Collector**  feature lets you accumulate data to display in the data collector. Click the drop-down arrow to see these options:



Check the appropriate box to add or clear information for the data collector.

Right-click in the *Histogram* window to display the drop-down menu:

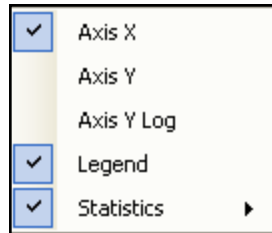


This menu lets you select a variety of options.

You can copy your graph, or export it to *Microsoft Word*, *Excel*, or *Powerpoint*. Other options let you show or hide the different features of the image histogram.

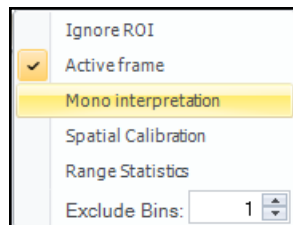
Note that you can zoom in on a part of the histogram by using your mouse to draw a region on the histogram graph.

The *View* sub-menu allows you to display the graph legend and statistics.



You can adjust the black and white levels by using the right and left sliders. As you move the sliders, you will see the changes in the black and white levels reflected in the image.

The *Options* sub-menu contains additional controls for displaying the graph.



- **Ignore ROI:** indicates that the histogram should reflect the entire image
- **Active Frame:** use only active frame for histogram data
- **Mono interpretation:** interpret color images as monochrome images
- **Spatial Calibration:** use spatial calibration to calculate histogram and statistics values
- **Range Statistics:** calculate statistics based on selected range only (selected range displayed on the chart as yellow bar – you can move or adjust left and right edges of the selected range with a mouse),
- **Exclude Bins:** exclude the number of bins to the left or right.

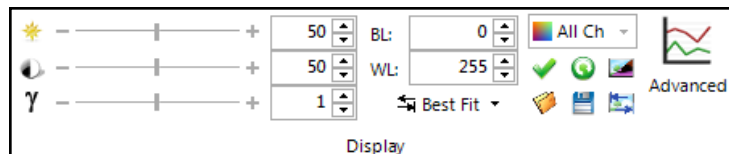
The *Statistics* option lets you hide or show the following information about the image histogram:

- **Mean:** the calibrated Mean of all the bin counts (or selected Range)
- **St. Dev.:** the calibrated standard deviation of all the bin counts (or selected Range)
- **Min:** the calibrated minimum of all bin x values (or selected Range) (calibrated intensity)

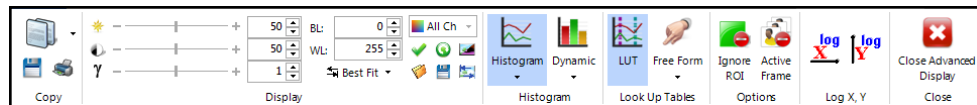
- **Max**: the calibrated maximum of all the bin x values (or selected Range) (calibrated intensity)
- **Sum**: the calibrated Sum of all the bin counts (or selected Range) where calibrated bin counts (Values) are multiplied by their x value (calibrated intensity), and accumulated
- **Area (...)**: the calibrated area of the pixels with intensity between Black and White Levels (or selected Range),
- **Area (%)**: the percentage of the pixels with intensity between Black and White Levels (or selected Range) to the total image area.

Advanced Display

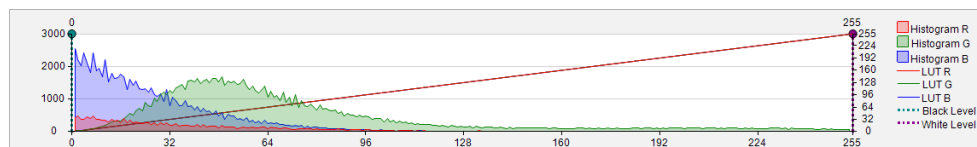
Additional histogram options are available when you select the **Advanced** button from the *Display* group on the *Adjust* tab:



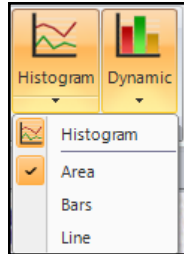
The *Advanced* context ribbon appears, with additional controls over image display and brightness/contrast adjustment.



When the **Histogram** button is activated, the Advanced Display panel opens to show a larger version of the image histogram appears in the bottom panel of the application workspace:



The drop-down menu of the *Histogram* group lets you control the appearance of the histogram display:

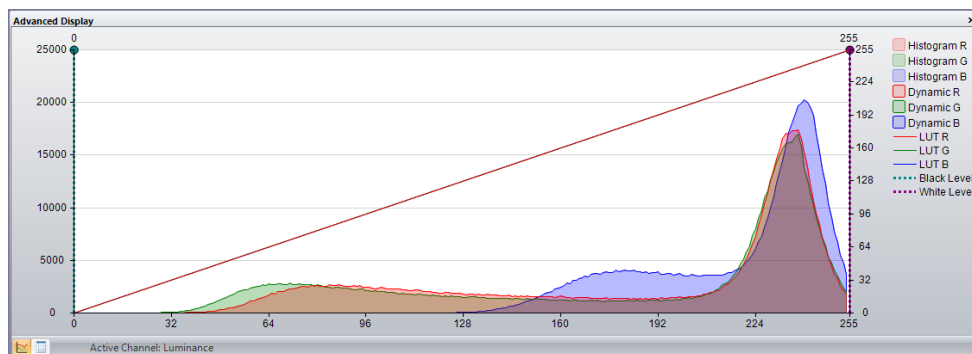


The example above shows an area histogram. The **Dynamic** button adds a display of the resulting or effective histogram after all of the current display adjustments (such as Brightness, Contrast, or changes in the Black/White levels) are applied.

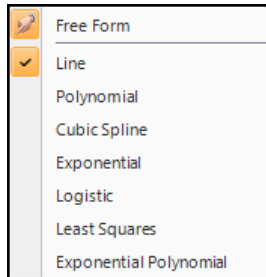
The **Look-Up Table (LUT)** is also displayed on the graph. The LUT determines how pixel intensities are displayed on your monitor. Changes to Brightness, Contrast, or the freeform LUT, will change the display of the image, but will not affect the underlying pixel values. You can turn it on or off using the **LUT** button in the *Look Up Tables* group.



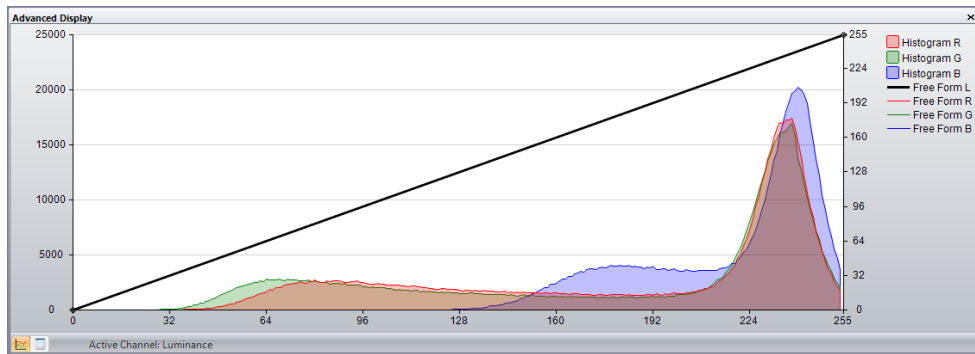
The LUT is indicated by a diagonal line on the histogram and by additional information in the right-hand panel:



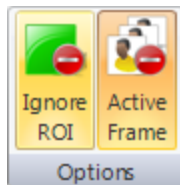
Clicking the **Freeform** button displays additional information on the histogram. Choose a display option from the drop-down list:



Here is a sample histogram with freeform and line information:



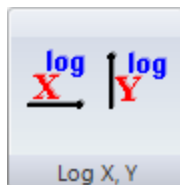
The *Options* group has the following features:




Ignore ROI creates a histogram using the entire image, even if an ROI is present.

Active Frame creates a histogram using only the active frame in a sequence.

The *Log X, Y* group uses logarithms to create the graph:



 **Log X** button is used to generate the graph's horizontal axis using a logarithmic scale.

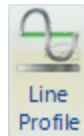
 **Log Y** button is used to generate the graph's vertical axis using a logarithmic scale.

Viewing a Line Profile

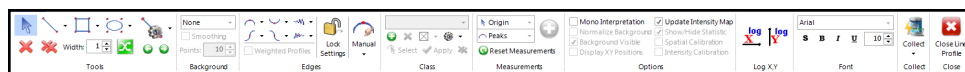
Line profile analysis allows you to collect the actual pixel intensities of the pixel values under the profile line. A profile plot shows the pixel positions of the line along the X-axis, and, along the Y-axis, indicates the pixel intensity for each position along the line. Either or both axes may use calibrated units if the image has a spatial calibration (for the X-axis) or intensity calibration (for the Y-axis). You can display a line profile on any type of image, whether it's the live image, a captured image, or an image opened from a file.

To view a line profile:

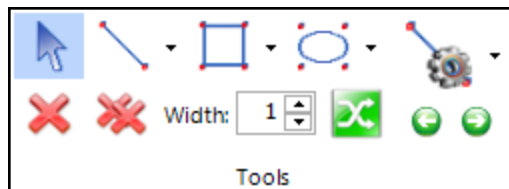
1. Open the image that you want to measure, if it isn't open already.
2. Select **Line Profile** from the *Capture* tab's ribbon.



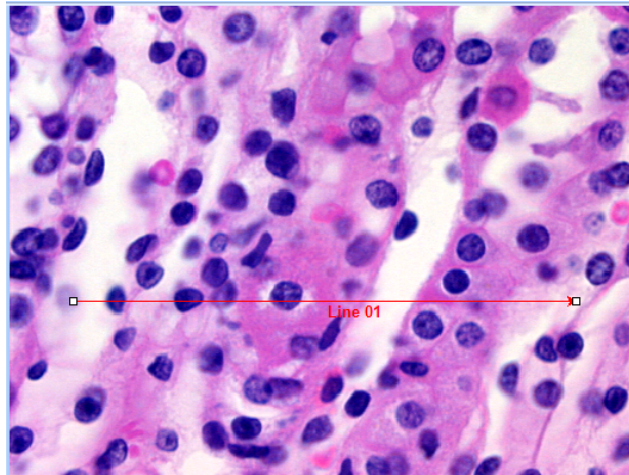
You will see the **Line Profile** ribbon:



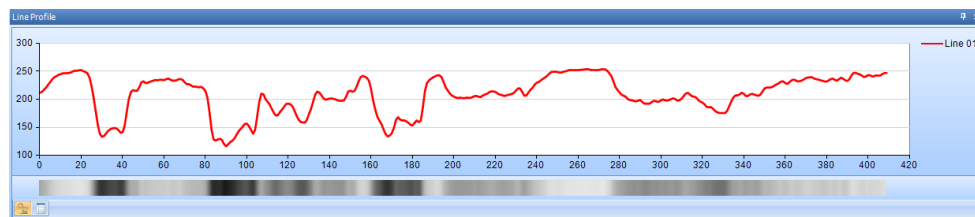
There are many different drawing tools in the **Line Profile** ribbon. They can be used to draw the sampling lines for the **Line Profile**.



3. When you select the **Line Profile**, *Image-Pro* will automatically draw an initial line profile on your image.



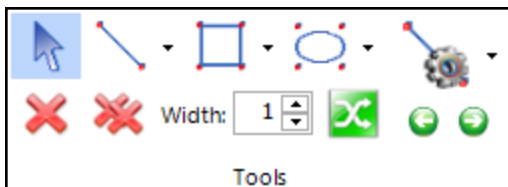
4. This profile is labeled “Line 1”. This is to help you get started with the Line Profile tools. You can adjust the *Initial Profile* in the **Histogram and Profile** panel in the **Application Options**. The **Line Profile** graph appears in a pane below the image:



As you draw more lines on the image, they will also appear in the profile pane. The plot scale along the X-axis will be adjusted to display the longest line profile that you’ve drawn. The currently selected Line Profile will appear on the plot and in the legend as a thicker trace. Under the line profile plot is an image display of the intensities of the pixels under the line profile at each position along the selected Line Profile. The image display will be as long as the selected profile, which may be shorter than the longest one that you’ve drawn.

Line Profile Tools

When the active image has one or more line profiles applied to it, the **Line Profile** context ribbon is displayed. It contains many useful tools for working with your line profiles.



There are many different drawing tools in the Line Profile ribbon that can be used to draw the sampling lines for the **Line Profile**. The table below describes each tool:



Select

The plot scale along the X axis will be adjusted to display the longest line profile that you've drawn. The currently selected Line Profile will appear on the plot and in the legend as a thicker trace. Under the line profile plot is an image display of the intensities of the pixels under the line profile at each position along the selected Line Profile. The image display will be as long as the selected profile, which may be shorter than the longest one that you've drawn. Use this tool to select a line profile feature or group of features.



Line

The Line button will display the currently selected line. This tool defaults to a simple line, but when you click the drop-down arrow on the line tool, you will see additional selections. Use the default Line tool to draw a sampling line on your image. Click on the first point and drag to the last point of the desired line.



Polyline

This tool allows you draw poly-vertices lines and polygons, or freehand lines and shapes.

To draw freeform lines and shapes

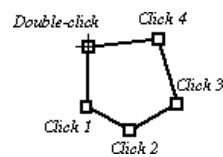
Use this technique to create lines and shapes that have smooth edges. Hold the left mouse down while you draw with the cursor to create the desired freeform shape. Double-click to complete the shape.



*Click, drag
around and
back, release
and double-
click*

To draw polyvertices lines and polygons

Use this technique to create lines and shapes that are made up of straight line segments. Click the left mouse button at each vertex (including the beginning point) of the polygon. Double-click to complete the polygon.



The auto-trace option for ploylines also appears when you click the drop-down arrow.



Rectangle

Click the left mouse button and hold it down to drag the rectangle to your desired size. Clicking the drop-down arrow will also display the **Polygon** tool.



Polygon

This tool allows you draw polygons, or freehand shapes. Click the left mouse button at each vertex (including the beginning point) of the polygon. Double-click to complete the polygon.

Auto-Trace Polygon. Use this tool to trace polyshapes automatically.



Circle or Ellipse

Position the crosshair cursor anywhere in the image. Click and drag the mouse from the insertion point to the desired destination. Hold down the **<Shift>** key while dragging the cursor to create a circle.



Delete Selected

Use this button to remove the selected profile feature from your image.



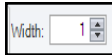
Delete All

Use this button to remove all the line profile features from your image. Note that if you delete all line profiles, the Line Profile pane will be empty until you draw another one.



Auto-Trace

Use this tool to trace the outline of an irregular object in the image automatically. Place your cursor on the object and click two points. *Image-Pro* automatically traces the object.



Width

Use the spin buttons to indicate the width of the profile line in pixel



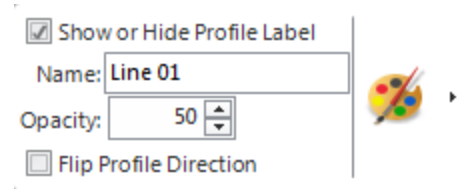
Features Manager

Use this button to manage feature collections. You will find more information about features and feature collections in Chapter 8 of this manual and in the in-product Help.



Line Profile Options

Use this button to display the options that control the appearance of the line profile labels in the line profile pane. You can control the size, opacity, direction, and color of the lines.

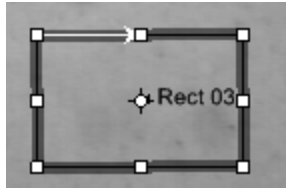


Line profile lines have a start and end point. Check the **Flip Profile Direction** box to reverse the direction of the profile lines.

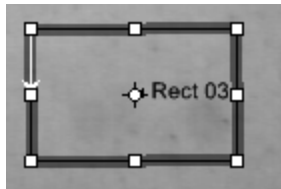
Use the right and left arrow buttons to move the profile points in either direction.

As noted above, each line profile has a beginning and an end. For example, lines start at the first point at the left, and end at the last point, on the right. The last

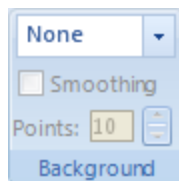
point gets an arrowhead by default. Closed figures are drawn in the clockwise direction, and an outline arrow indicates the direction, as shown here:



Checking the **Flip Profile Direction** box reverses the direction:




Background Tools

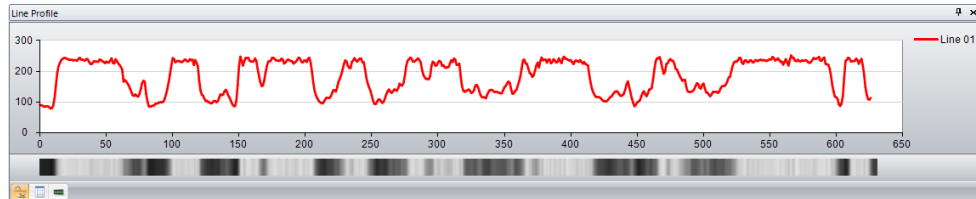


The *Background* combo box is used to select a background profile (values of background profile will be subtracted from all other profiles values). Background smoothing is used to select the type of smoothing for background profile values. The **Points** box indicates the number of points visible on the graph.

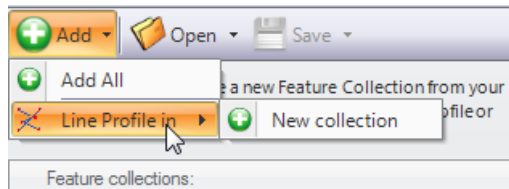
To Add a Line Profile to the Current Collection

Line Profile collections are created by adding defined Line Profiles (that is, Line Profiles defined in the active image) to the list of 'remembered' items through the *Features Manager* dialog box. You can add a single Line Profile definition, or you can add a group of Line Profile definitions under a single name.

1.  Open the Features Manager dialog.
2. Draw the Line Profile in the active image.

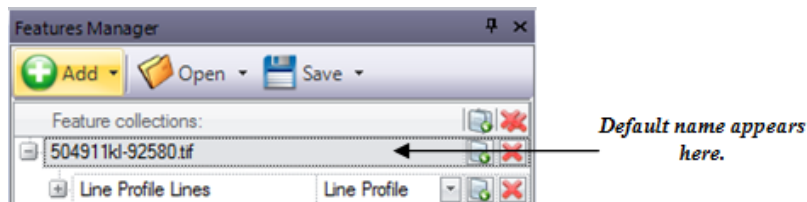


3. Click on the **Add** button.



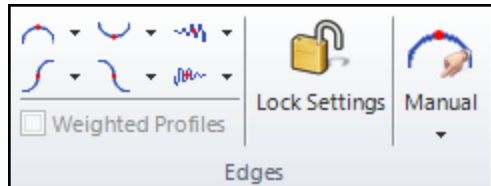
The collection definition is added to the list box. Listed names represent the members of the current collection.

When a single Line Profile is defined, a default name appears in the **Name** text box of the *Features Manager* dialog box.



If you define more than one Line Profile on the image, the features manager gives the entire group the default name, “Multiple.” Note that if multiple features are defined in the image, they will be added to the list as a group regardless of which feature among them is currently selected.

More information about the Features Manager appears in Chapter 8.

Edge Detection Tools

The **Edge Detection** tools find peaks, valleys, and patterns in the line profiles. The Edge detection tools are described below:



Peaks

Use this tool to find peaks on the profile.



Valleys

Use this tool to find valleys on the profile.



Rising Edges

Use this tool to determine rising edges in the profile



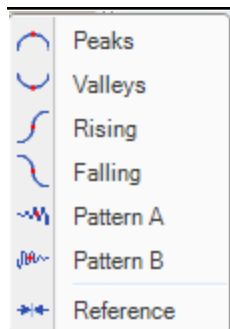
Falling Edges

Use this tool to detect falling edges in the profile.



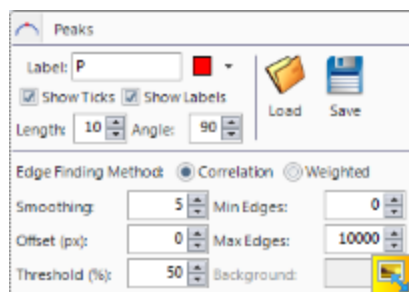
Manual

Click this button to activate the manual edit edge tool (used to add/remove edges on the image)



Edge Setting

Use the pull-down arrow next to the tools to open the **Settings** dialog.



Label lets you select the label style and color.

The **Color Selector** lets you change the color of the ticks and label text.

Checking the **Show Ticks** and/or **Show Labels** boxes will display the tick marks and labels on the image.

Smoothing: Use the spin buttons to apply the Gaussian smoothing filter to the luminance profile. The numbers indicate the degree of smoothing that the filter uses. **Smoothing** of 1 means no smoothing. If smoothing value is more than 1, a Gaussian smoothing kernel will be applied to the luminance profile.

Offset: Use the spin buttons to indicate how many pixels to offset the markers from the detected features.

Threshold defines threshold level for marking an “edge” on a weighted profile.

It uses the minimum difference between an extreme point and neighboring opposite extremes. If the difference between the extremes is smaller than the sensitivity threshold, the marker is ignored. The **Threshold** is defined in percentage from 0 to 100 from the dynamic range. The default value is 0, so that all peaks and valleys are detected, even when deviations of intensities are very small.

Min Edge indicates the minimum number of expected “edges” on each profile.

Max Edges indicates the maximum number of expected “edges” on each profile.

The pattern matching feature lets you select a pattern template or create your own pattern:



Pattern A

Use this button to select pattern template A



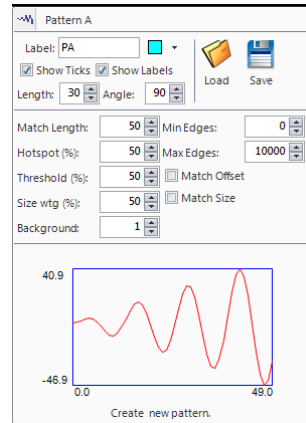
Pattern B

Use this button to select pattern template B.



Pattern Settings

Use the pull-down arrow next to the tools to open the Label Settings dialog.



Match length indicates the number of profile samples over which averaging or pattern matching is performed, i.e. this is the length of the segment where edge finding method is performed.

Hotspots (%) indicates the point in a weighted peak to mark as an edge, given as a percentage of the match length. This is an offset for the exact edge position as a percentage of the match length. 0% - beginning of match length segment, 100% - end of the segment.

Threshold defines threshold level for marking an “edge” on a weighted profile.

It uses the minimum difference between an extreme point and neighboring opposite extremes. If the difference between the extremes is smaller than the sensitivity threshold, the marker is ignored. The **Threshold** is defined in percentage from 0 to 100 from the dynamic range. The default value is 0, so that all peaks and valleys are detected, even when deviations of intensities are very small.

Min Edge indicates the minimum number of expected “edges” on each profile.


Max Edges indicates the maximum number of expected “edges” on each profile.


Match Offset is an additional setting to create a user-defined pattern. This edge finding method is based on the same mean lumin-



ance of a user defined pattern. You might use this option if you were looking for a “W” shaped pattern on top of bright sections of the profile, but you wanted to exclude such profile patterns where the image was dark.

Size Weighting (%) is an additional setting for creating a user-defined pattern. It defines the strength of the **Match Size** option.

Match Size is an additional setting for creating a user-defined pattern. This edge finding method is based on matching amplitudes in the user-defined pattern. The strength of the effect depends on the **Size Weighting** property (stronger with greater Size Weighting). You might use this option if you were looking for medium-sized luminance peaks, but you wanted to exclude very bright spots.

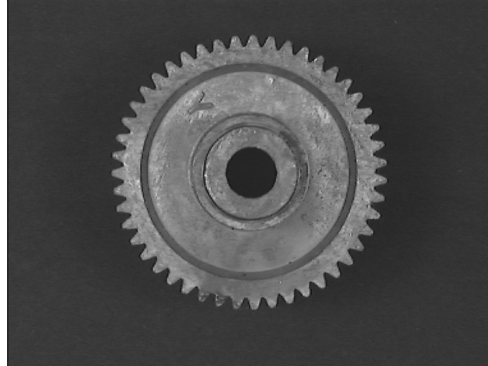
 **Lock Settings** . If this setting is on, then edge detection settings will be applied to the all profiles. This option could be used for profiles imported from Feature Manager and edge detection should be done on all imported profiles.

 **Manual**. This setting activates the manual edit edge tool (used to add/remove edges on the image)

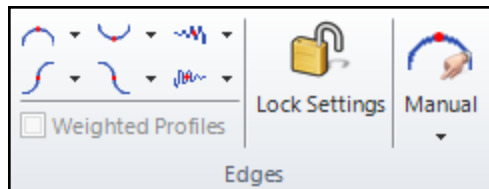
- Peaks – activates peaks manual tool,
- Valley – activates valleys manual tool,
- Rising – activates rising manual tool,
- Falling – activates falling manual tool,
-  Pattern A/B – activates pattern A/B manual tool,
-  Reference – activates reference manual tool

To use **Edge Detection**, follow these steps:

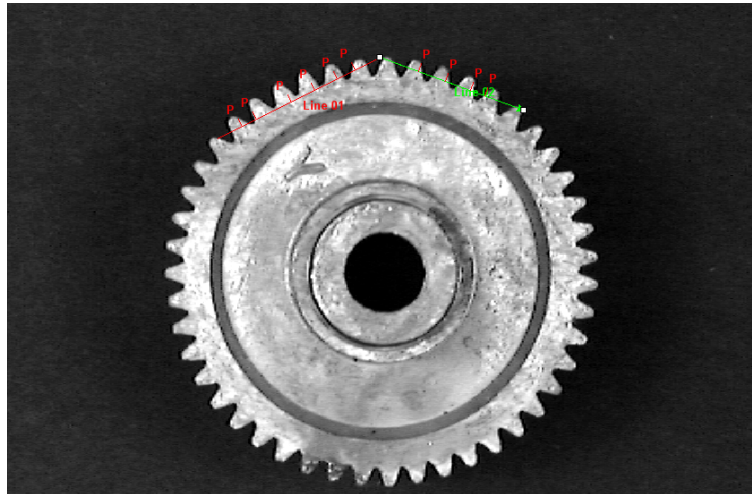
1. Open the image that you want to measure, if it isn't open already.



2. Select the *Edges* group from the Line Profile *ribbon*:



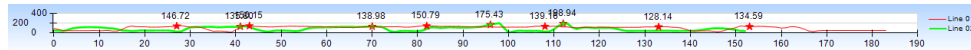
3. Draw a line or two on the image, as shown here. Line 1 is red and Line 2 is green.



4. Select the **Peaks** tool  from the *Edges* group.

Make sure that the tick marks option is turned on. The edges will be marked on the image, as shown in the previous image.

A line profile displaying the variations in the edges will appear in the line profile window at the bottom of the *Image-Pro* workspace.



The *Line Profile* window contains additional graphic display tools:



The **Graph** button displays the line profile as a graph, shown above.



The **Table** button displays the line profile information in a table, as shown here:

Index:	Position:	Line 01:	Line 02:
0	0	98.84	55.67
1	1	76.69	51.23
2	2	53.65	45.71



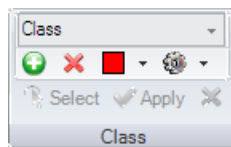
The **Measurements** button displays the line profile information with a picture strip below:

Index	Line 01 (P)	Line 01 (V)	Line 02 (P)	Line 02 (V)
1	27	41		
2	43	70		
3	62	96		

- Click the **Plus** button to add measurements to the table.

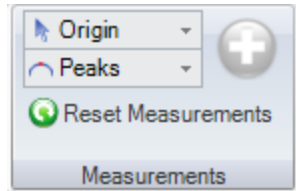
The *Line Profile* ribbon has other options available, which are described here:

The *Classes* group lets you apply labels to different features within the image.

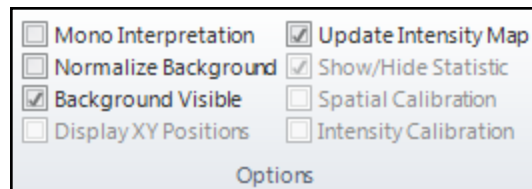


You can select the color for each class label, and have the labels appear on the image, graph, or data table.

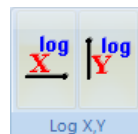
The *Measurements* group in the *Line Profile* ribbon contains features that allow you to add measurements to the Line Profile shown above.



The *Options* group contains controls for displaying the image.



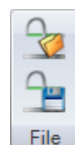
The *Log* group displays the X- and Y-axes in a logarithmic scale.



The *Font* group lets you select the font style, size and color for the class labels.



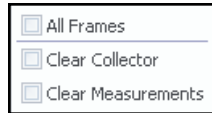
The *File* group allows you to save or load the current state of the line profile tools.



The *Collect* group allows you to accumulate data to display in the data collector.

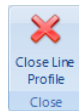


Click the drop-down arrow to see these options:



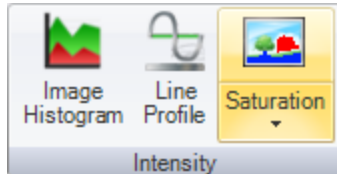
Check the appropriate box to add or clear information for the data collector.

To exit the *Line Profile* ribbon, click the **Close** button.

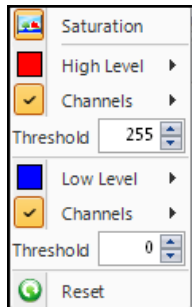


Using the Saturation Warning

Use **Saturation Warning** to show overexposed and underexposed areas with a given color. It can be used with the live preview to set proper exposure time and avoid saturated areas, so that intensity measurements on the image will be correct.



Click the **Saturation** button to see the drop-down menu:

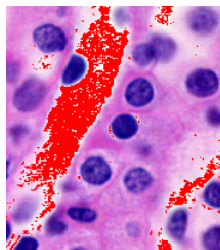


The colors are set using **High level** and **Low level** color pickers. Intensities that are greater than or equal to the High Level threshold will be colored with the designated high color.

Intensities that are less than or equal to Low Level threshold will be colored with the designated low color.

The channels used for saturation test can be selected using **Red/Green/Blue** checkboxes or by clicking the **Channels** button.

This is a sample of a partial image with the high saturation areas visible:



The **Reset** button sets the controls back to their default values.

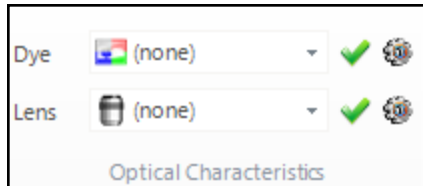
Chapter 5

Working with Images

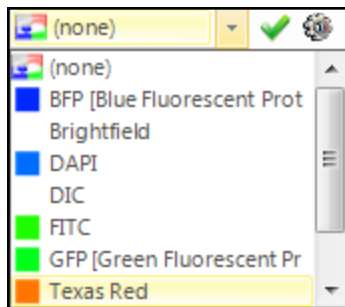
This chapter introduces you to image processing in *Image-Pro*. It provides instructions for opening, saving, and enhancing images through *Image-Pro*.

Viewing Optical Characteristics

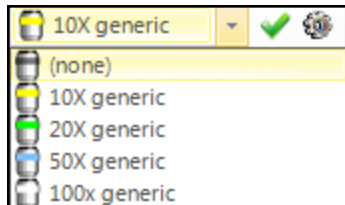
The *Characteristics* group on the **Capture** tab's ribbon lets you choose a dye, lens, and/or calibration to use while acquiring images.



Clicking the drop-down arrow next to the dye displays a list of available dyes:

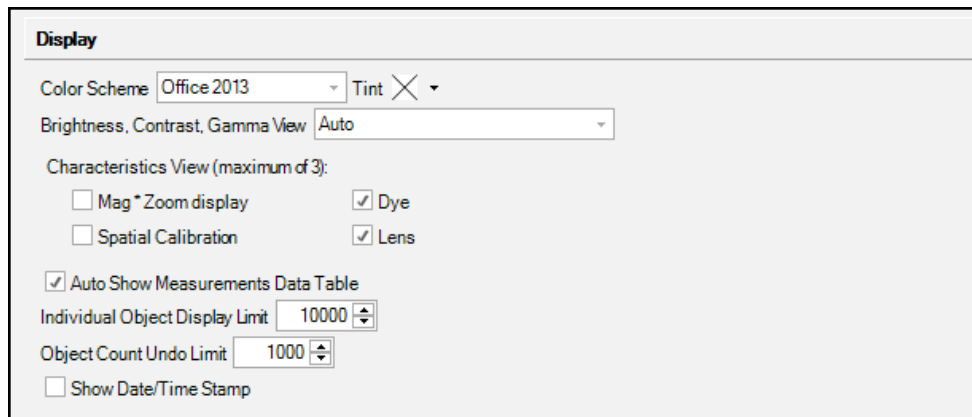


Clicking the drop-down arrow next to the lens displays a list of available lenses:



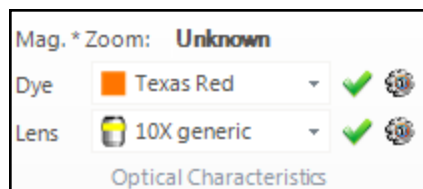
The Lens is particularly important, as you can associate a spatial calibration with each lens; in addition to the lens characteristics (such as Magnification) , a spatial calibration will be applied to each captured image, assuring accurate measurements. Please refer to the *Using Spatial Calibrations* section for more information.


This group can also display the magnification zoom level. You can select which characteristics to display in the *Display* area on the **Applications** page of the *Image-Pro* options dialog.



The **Mag. * Zoom** display option controls display of the zoom magnification level (which will always be at the top of the group, if displayed). As illustrated above, the group can only hold 3 sets of controls, so when three options are checked, the fourth will be disabled. To hide the *Characteristics* group, do not check any of the options.

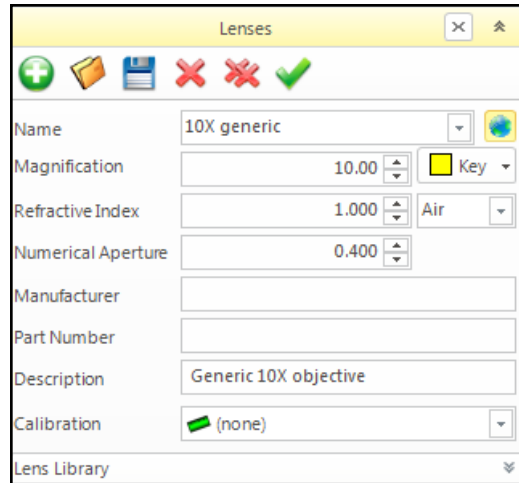
Your selections will appear in the *Characteristics* group on the **Capture** tab's ribbon:



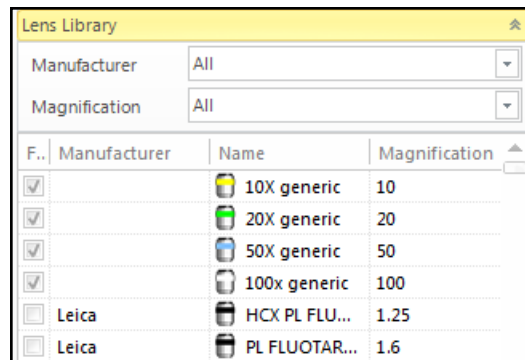
1. To select optical characteristics, click on the drop-down arrow and select an item from the drop-down list.
2. To edit one of the characteristics, click the edit button  at the far right.

You will see an editing panel at the right side of the workspace.

The **Lenses** panel is shown here as an example:



3. From this panel, you can edit or change the lens used to capture your images. The Lens Library displays a list of available lenses with their calibrations.

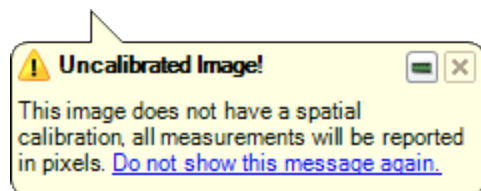


You can search for a specific lens by manufacturer or magnification.

Using Spatial Calibrations

Using a spatial calibration, *Image-Pro*'s pixel-level measurements can be converted to any unit of measurement. Spatial calibrations can also correct for irregularities in the image's vertical and horizontal spacing.

If you are working with an uncalibrated image, you will see this warning:



There are two primary components to a spatial calibration:

- Pixels per Unit
- Aspect Ratio

Pixels per Unit

A spatial calibration tells *Image-Pro* the size to which a given image is scaled, just as the key of a roadmap tells us how many millimeters of image length represent one mile. Likewise, the spatial calibration tells *Image-Pro* how many pixels of image length represent some more meaningful unit of length in the image, for example, one micron, millimeter, or inch. To create a new spatial calibration, you must either know the number of pixels per unit to specify for the image, or there must be a feature pictured in the image that has a known length value in terms of the units of interest. If a feature of known length is pictured, *Image-Pro* can calculate the number of pixels per unit by determining the number of pixels it takes to represent the known length. (Companies often 'plant' an object of known length into images for this purpose.)

Aspect Ratio

Aspect ratio refers to the ratio of vertical to horizontal lengths. For example, when a television screen's image appears flattened or squeezed, the ratio of horizontal length to vertical length is out of proportion. Similarly, cameras often inadvertently skew images in the process of capturing them and translating them to digital format.

Such skewing results in inaccurate lengths being ascribed to features that are measured in the image. When a skewed aspect ratio has a flattening effect, vertical lengths will be recorded as shorter than they actually are; when it has a squeezing effect, horizontal lengths will be recorded as shorter than they actually are.

If an image has a known aspect ratio problem, *Image-Pro* can compensate for the flattening or squeezing of vertical and horizontal lengths, and calculate the actual number of units that measurements of its features represent. To do this, you must be able to supply it with the value l/h (length divided by height) for a perfect square as represented in the image, or there must be a feature pictured in the image that represents a perfect square. If a square feature is pictured, *Image-Pro* can calculate the aspect ratio from that feature. (Companies often ‘plant’ a perfectly-square object into images for this purpose.) The selected image must have a reference feature of known size, which you will measure in step 5.

System and Reference Calibration

Image-Pro maintains a list of Reference Calibrations that will persist from session to session and can be applied to multiple images. *Image-Pro* also maintains a list of all of the calibrations that have been used in your current session, which will include all the reference calibrations, as well as any calibrations that have been loaded from any calibrated images that you have opened. One of the Reference Calibrations can be selected the System Calibration. The System Calibration is automatically attached to images that you Capture or Record using your camera, and may also be used as the default spatial calibration when loading a 3D volume from uncalibrated images.

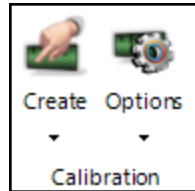
If you have configured your system using the Hardware Configuration Wizard (described in Chapter 3), then you will not need to be concerned with Reference or System calibrations. A list of Reference Calibrations will be created and associated with your Lenses, and when you select an active lens, the calibration associated with that lens will be made the System Calibration.

Creating a Spatial Calibration

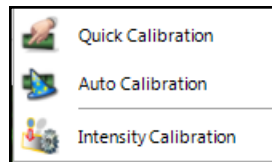
To create a new spatial calibration, you must either know the number of pixels per unit to specify for the image, or there must be a feature pictured in the image that has a known length value in terms of the units of interest (see “Pixels per Unit” under “About Spatial Calibrations” above)..


Quick Calibration Method

1. Open the image you want to calibrate or that you want to use to create the calibration definition.
2. On the **Capture** tab's ribbon, select the *Calibration* group.

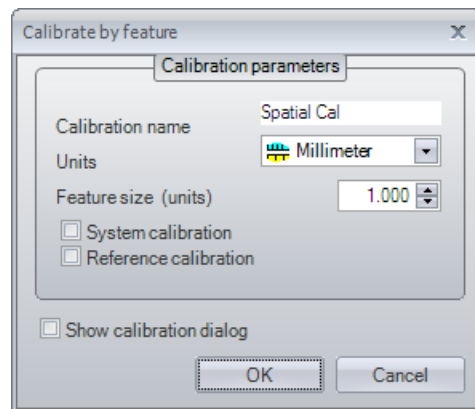


3. Click the **Create** drop-down arrow.



4. Click the **Quick Calibration**  button
5. Draw a line over a feature in the image.

You will see the **Calibrate by Feature** dialog:

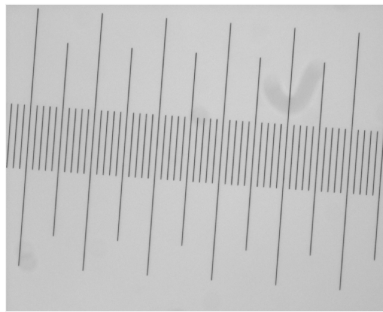


6. Select a calibration name in the drop-down list.
7. Select the units in the drop-down list, and indicate the number of units in the selected feature.
8. Indicate if this is a system or reference calibration.
9. Click **OK**.

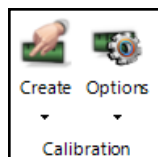
Auto-Calibration

To use Auto-Calibration, follow these steps:

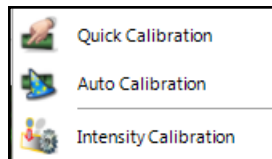
1. Open the image of the Stage Micrometer (included with the *Image-Pro* Demo Images).



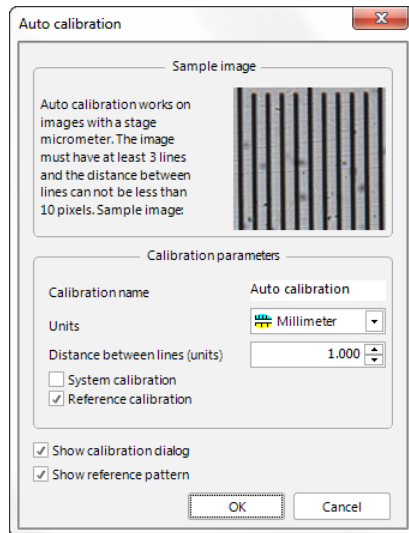
2. On the **Capture** tab's ribbon go to the *Calibration* group.



3. Click the **Create** drop-down arrow.

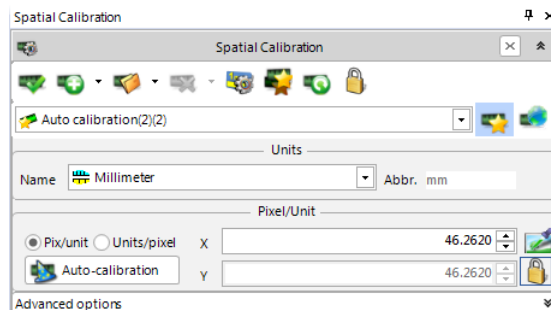


4. Select **Auto-Calibration**. You will see the *Auto-Calibration* dialog:



5. Click OK.

If the box is checked, you will see the Spatial Calibration dialog:



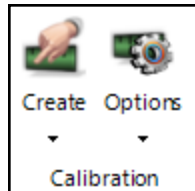
From here, you can rename your calibration and make further adjustments.


Calibrating by Aspect Ratio

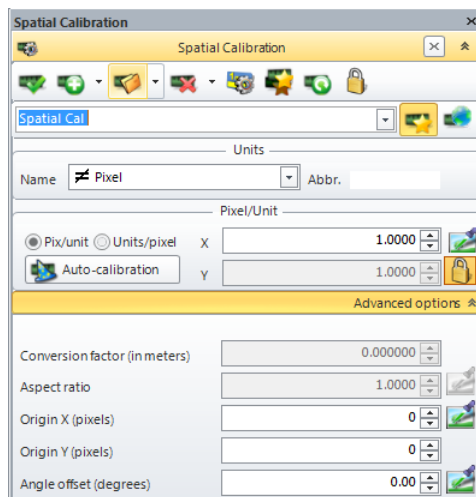
If your calibration must also correct for aspect ratio, you must either know the value l/h (length divided by height) for a perfect square as represented in the image, or there must be a feature pictured in the image that is known to be a perfect square (see “Aspect Ratio” under “About Spatial Calibrations” above).

To create a spatial calibration:

1. On the **Capture** tab's ribbon, select the *Calibration* group.




2. Click the **Calibration** options icon  to open the *Spatial Calibration* dialog box in the panel on the right.



The dialog box controls are enabled. This dialog has two view, **Basic** and **Advanced**.

In the list-box in the control window (**Calibration Name**) select the set of calibration values you want to apply to the image. If you want to use the default values, select **(none)**. Any calibration sets that were loaded or created since the application window was last opened, will be listed in this list box.

You will not be able to enter calibration values until a name other than **(none)** has been selected in the **Name** list box. If **(none)** is the only set listed, you can create a set

by clicking the **New**  button and specifying its calibration values.

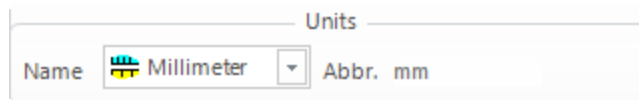
By default, the name "Spatial Cal 0" will be assigned to a new calibration set; however, you may change this to a more descriptive name if you'd like.

3. Type over the default name “Spatial Cal 0” with a name that is more meaningful.
4. In the **Unit** area, pull down the **Name** list box and select the units to which you want the pixel-level measurements converted, as shown below:

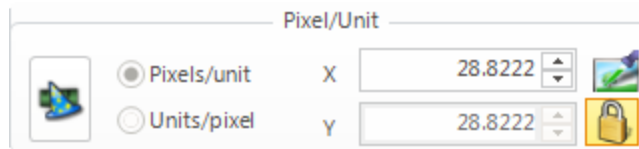
For example, if the size of objects in the image is best expressed in terms of micrometers, select “Micrometer” from the list.

5. In the **Abbreviation** text box, enter the text you want to use as the abbreviation for the selected units.

For example, a typical abbreviation for ‘Millimeter’ is “mm” as shown below. The specified abbreviation will appear on measurement reports.




6. In the **Pixel/Unit** group, do *one* of the following:



If you know the number of pixels per unit to specify:

1. In the **X** spin box, type the number of horizontal pixels it takes to represent one unit of length. For example, if you are converting to millimeters and 4 pixels equals one millimeter, type “4.”
2. Ignore the **Y** spin box.

If there is a feature pictured in the image that has a known length value:

1. Click the **From Image** button  in the **Pixel/Unit** area. A dialog box appears in the *Image-Pro* panel, and a horizontal annotation line appears in the image.
2. Click and drag the line over the reference object, and then adjust the line’s control points so that the beginning and ending of the line correspond to the beginning and ending of the reference object.

TIP: For greatest accuracy, it is often helpful to zoom in on the reference



object. To zoom in on the reference object, click on the **Zoom** tool from the *View* tab's ribbon. With the **Zoom** tool selected, position the cursor over the reference object, and click. Repeat these steps until the object is large enough to determine its starting and ending pixels.

3. In the **Reference length (units)** spin box, type the known length of the pictured object.
4. Click **OK**.

The *Spatial Calibration* dialog box is redisplayed and the calculated pixels/unit value appears in the **X** and **Y** spin boxes.

If you have an image of a micrometer, you can calibrate the image automatically:



1. Click the **Auto** button in the **Pixel/Unit** area.
2. A dialog box appears over the image of the micrometer.
3. Draw an ROI around the micrometer image.
4. Click **OK**, and the calibration will be calculated automatically.

7. Select the **Advanced Options** on the **Calibration** dialog.

Conversion factor (in meters)	0.000000	
Aspect ratio	1.0000	
Origin X (pixels)	0	
Origin Y (pixels)	0	
Angle offset (degrees)	0.00	

8. In the **Aspect Ratio** area, do one of the following:

If you know the aspect ratio value to specify, or if there is no aspect ratio problem to correct for:

1. In the Aspect Ratio spin box, type the aspect ratio value to be used. For example, if there is no aspect ratio problem to correct for, enter “1.” *Image-Pro* automatically adjusts the value for the **X** spin box to match the entered Aspect Ratio value.

If there is a feature pictured in the image that represents a perfect square:



1. Click the **From Image** button in the **Aspect Ratio** area. A dialog box appears in the *Image-Pro* workspace, and a diagonal annotation line appears in the image.
2. Click and drag the line over the reference object, and then adjust the line's control points so that the beginning of the line corresponds with the bottom-left corner of the reference object and the end of the line corresponds with the top-right corner of the reference object.

TIP: Again, for greatest accuracy, it is often helpful to zoom in on the reference object.

3. Click **OK**.
The *Spatial Calibration* dialog box is redisplayed and the calculated aspect ratio value appears in the **Aspect Ratio** spin box. Additionally, the **X** and **Y** spin box values are automatically adjusted to account for the aspect ratio.



9. Click **Apply to Active Image** in the spatial calibration toolbar.


The spatial calibration is applied and saved for the session. To make it available across sessions, you must save it to a file (see “Saving Calibrations” below).

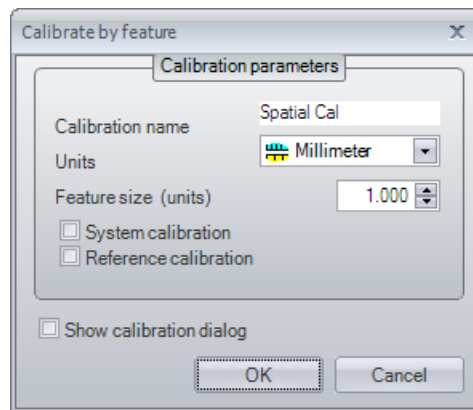
Calibrating by Feature

Calibrate by feature option allow you to calibrate an image by a feature with known size. Any line measurement can be used for image calibration.

Calibration by feature can be done using the following steps:

1. Create a measurement of an object of known size. For example, the length of a bracket can be measured.

2. Click the **Calibrate by feature length**  button (the same function can be called from the context menu in the Data Table).
3. Fill the **feature size** in calibration units and other calibration parameters, such as **Units** and **Calibration name** in the shown *Calibrate by feature* dialog:



4. (Optional) Use the **Show calibration** dialog to adjust the calibration measurements.
5. Click **Ok** to create a new calibration and apply it to the active image.

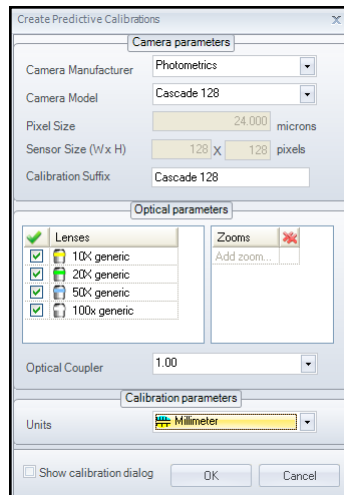
Predictive Calibration

Predictive calibration lets you create estimated spatial calibrations based on the optical characteristics of the camera and objectives. To use the predictive calibration feature, follow these steps:

1. From the *Calibration* group drop-down menu, select **Show Predictive Calibration**.



You will see the **Create Predictive Calibration** dialog:




Each camera is defined by the **Manufacturer** and **Camera Model** fields

2. Select your camera manufacturer and model from the drop-down lists. The pixel and sensor size is determined by the camera make and model. If you want to set the pixel and sensor size manually for the new calibration, choose **Other** in the list of cameras.

The **Calibration prefix** is also defined automatically the camera selection. You may type in a new prefix in the space provided.

3. Select the lenses from the calibration from the Lenses panel.

All active lenses are selected by default, so if some lenses should not be calibrated, uncheck them using the  button to select/deselect a lens.

4. Add additional **Zoom settings** in the *Zoom* panel. This list is empty by default.
5. Enter the calibration units in Units field under the Calibration parameters .
6. (Optional) Check the **Show Calibration Dialog** box if you want to edit the calibration after you've created it.
7. Click **OK** to create the predictive calibration.

Clicking the **OK** button will generated multiple calibrations for the list of selected lenses and zooms for the given camera. If multiple zooms are present, the calibration with zoom closest to 1 will be used as default lens calibration.

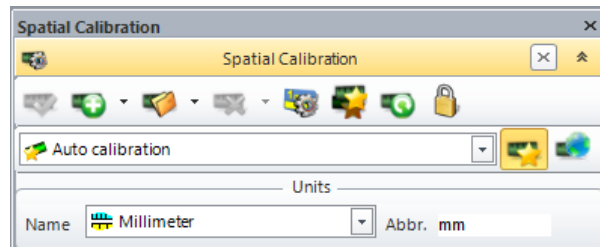
The list of camera manufacturers and camera models is loaded from "Camera list.cfg" file in the Resources folder of the application.

Saving Calibrations

Calibration definitions are saved per-session whenever they are applied to an image. If you want calibration definitions to be saved between sessions, however, you must mark them as Reference Calibrations or save them to a file. Once a calibration definition is saved to a file, you can load it whenever it is needed in the future.

To save calibration definitions:

1. Click the drop-down arrow next to the **Open Calibration** button on the calibration settings dialog.

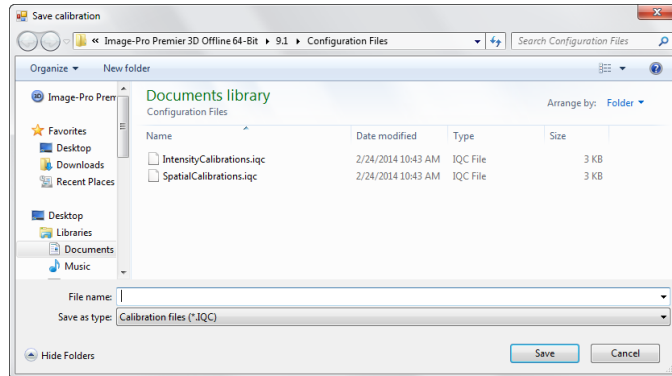


The drop-down list box appears. If an active image is displayed in the *Image-Pro* workspace that has a calibration definition assigned to it, that calibration definition appears in the *Spatial Calibration* dialog box.

2. From the list box at the top of the *Spatial Calibration* dialog box, select the calibration definition you want to save.
3. Click **Save** from the *Spatial Calibration* group.

*TIP: If you want to save all calibrations listed in the Spatial Calibrations list box, skip Step 2 and click on the **Save All** from the Spatial Calibration group.*

The *Save Calibration* dialog box appears.



4. In the **File Name** text box, type the name you want to give to the current calibration definition (or set of calibration definitions) being saved.
5. Click on the **Save** button.

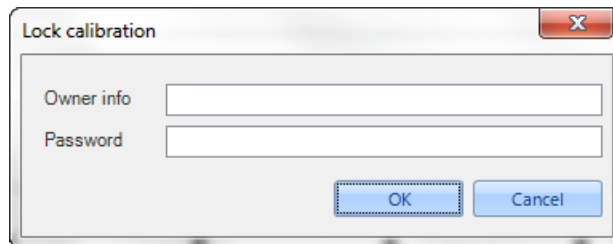
Locking Calibrations

To ensure that your calibration measurements are not deleted or changed accidentally, you can lock them. To lock a calibration, follow these steps:

1. Create a calibration using one of the methods described in this chapter.
2. Save your calibration.
3. Click the lock icon on the calibration ribbon:



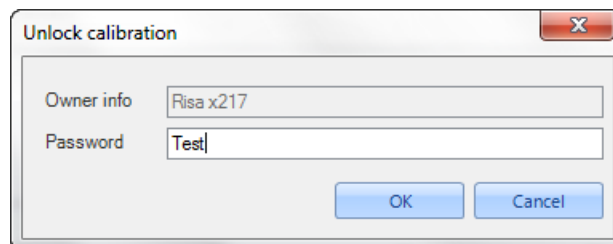
You will see the **Lock calibration** dialog:



4. Fill in your name and create a password.
5. Click **OK**. This calibration is now locked and protected against accidental changes. The lock icon changes to an open lock.



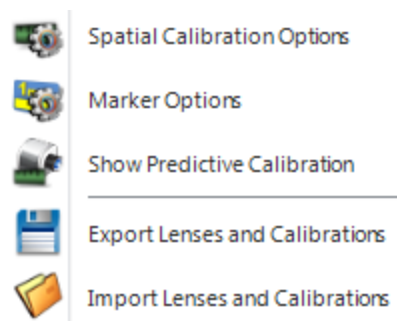
6. To unlock the calibration, click the open lock icon. You will see the **Unlock calibration** dialog:



7. Enter the owner name and password.
8. Click OK. The calibration is unlocked and may be changed.

Importing and Exporting Calibrations

Import Calibration and **Export Calibration** have been added to the *Calibration Options* drop-down menu, as shown here:



You can use these features to import/export the calibration and calibration/lens linkage as a package that can be imported into other copies of *Image-Pro*.

Applying a Calibration Marker to the Active Image

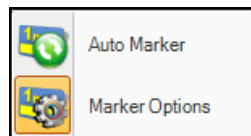
The calibration marker is like a distance key on a map – it shows a line and expresses what that line represents in terms of meaningful units. In *Image-Pro*, it is composed of two or three overlays, as shown below:




When you add a calibration marker, *Image-Pro* adds it to the currently active image based on the number of pixels in the image.

To set the calibration marker to appear in the active image:

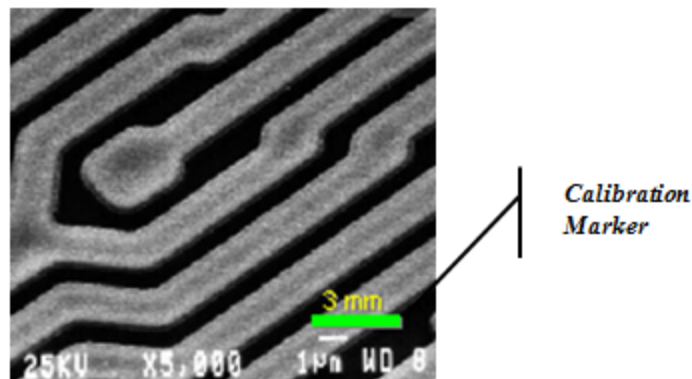
1. Choose the **Auto-Marker** button from the **Calibration Marker** drop-down menu:



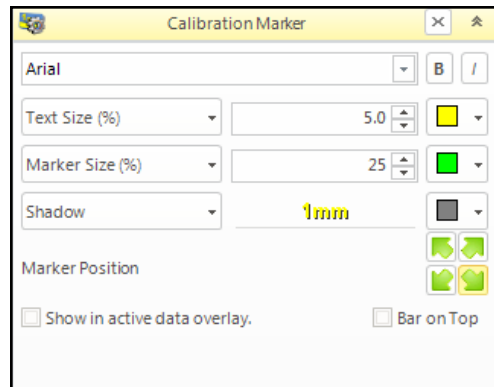
A marker will be placed on all open and active images automatically.

2. Otherwise, click the **Calibration Marker** button [] in the *Image-Pro* toolbar.

A calibration marker will appear in the image.



The *Marker Options* dialog box appears.



3. Use the **Position** buttons to place the marker on the image. You can resize the marker and the length and the label will be adjusted automatically based on the current size. The length is rounded to the nearest value from the list of 10 logarithmically equidistant values (1, 1.2, 1.5, 2, 2.5, 3, 4,...).
4. Set the **Control** options the way you want them. The **Length (units)** spin box controls how long the marker will be. *Image-Pro* renders a marker of the length you specify here. Adjust the **Length (units)** value so that the resulting marker line is long enough to give an indication of scale, but not so long that it takes up the entire image width. Also, use round numbers. Keys generally use ‘reference’ lengths, such as multiples of 25. The size of the calibration marker can be adjusted manually by resizing the marker on the image. Note that the minimum marker size is limited by the width of the calibration label text.
5. Set the **Font and Size** options the way you want them. Use the **Size** spin box to indicate the size, in points, to be used for the text. To hide the text and only display the marker, set the font size to zero. Use the **Color** button to change the text color.
6. Set the **Background** options the way you want them. Use the **Color** button to change the color of the background. Uncheck the **Show** button if you want to suppress the background.

*TIP: You may need to experiment with the **Text** and **Marker** settings to get the desired effect. Go with your best guess the first time you set the marker. Once the marker is added to the image, you can easily delete it and define a new one (see “Deleting an Annotation” in Chapter 7, “Annotating Images”). By refining the **Text** and **Marker** settings on subsequent iterations, you can achieve the desired effect.*

The length of the calibration marker can be adjusted manually by resizing the marker on the image. In this case the minimum marker size is limited by the width of the calibration label text.

7. Click **OK**.

The calibration marker is added to the image.

- To add a marker automatically to all active images, click the Auto Marker

button. 

When calibration markers are initially added to images, they are added as annotation overlays. This means that they can be moved and deleted the same as any annotation. This also means that they will only be saved with the image if that image is saved in a TIFF format.

Note that Image-Pro does not support calibration of the Z-axis. Where possible, the location of each Z plane along the Z-axis will be determined from Z stack sequences or image sets that you open.

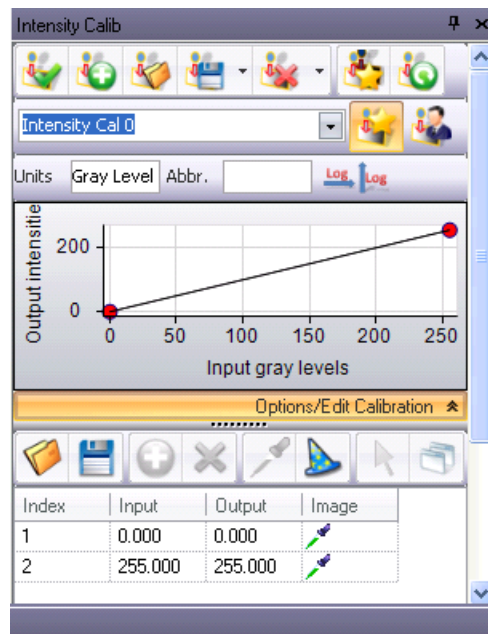
Using Intensity Calibration

The **Intensity Calibration** selections can be used to measure changes in intensity and pixel values in an image or set of images. You would use this command if you wanted to relate intensity to optical density, or relate intensity to temperature or relate intensity to protein content, for example.


1. To start using the *Intensity Calibration* feature, click the **Int. Cal** button in the *Calibration* group on the **Capture** ribbon.

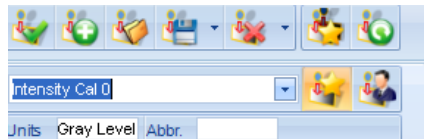


You will see the **Intensity Calibration** dialog:



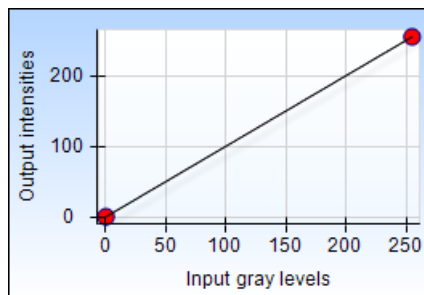
Index	Input	Output	Image
1	0.000	0.000	
2	255.000	255.000	

2. To create a new intensity calibration, click the **New**  button on the *Intensity Calibration* toolbar.
3. Choose a calibration name from the drop down list, as shown here:



The default is "Intensity Cal 0". You can rename it if you choose.

A new intensity calibration curve will be displayed in the window.



The main toolbar contains the following buttons:



Apply: Applies the selected calibration to the active image.



New: Click this button to create a new set of calibration values. When this button is clicked, the Application will place the "Intensity Cal 0" name in the Name list box (the 0 digit may be incremented to make the set name unique) and show the calibration curve. You can change this name to something more useful if you like.



Load calibration from file: Loads one or more saved calibration(s) from your files.



Save: Saves the current calibration to a file. Clicking the drop-down arrow will save all your calibrations.



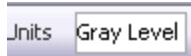
Delete: Deletes the current calibration. Clicking the drop-down arrow will delete all your calibrations.



Ref Only: Click this button to display only the reference calibrations.



Reset: Clicking this button resets the list of intensity calibrations.



Units: Type the name of your unit in this field (e.g., gray level, density, degrees, disbursement). This name will appear when the application reports intensity data.



Abbreviation: Enter the short name of the units (as *OD*, *ng* etc.).

Log scale X and Y turns the logarithmic scale along the corresponding axis on the chart on or off.



Toggle Reference Calibration: Click this button to mark or unmark the current calibration as a reference calibration.



Toggle System Calibration: Click this button to mark or unmark the current calibration as a system calibration.

Intensity calibrations are handled in the same way as system calibrations. To learn more about system and reference calibrations, please refer to the section on page 5-7 of this manual.

To use the edit mode, select the **Options/Edit calibration** button to see the tools for editing the intensity calibration:



Use these tools to add, delete, or edit points in the image for intensity calibration.

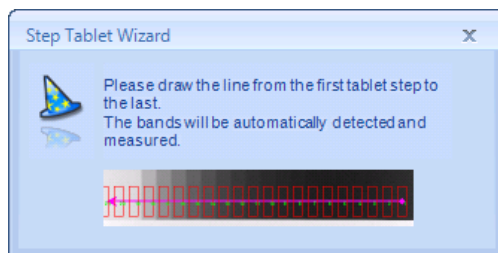
Step Tablet Wizard

Intensity calibration can be created from a step-tablet image with known optical density values using the *Step Tablet Wizard*.

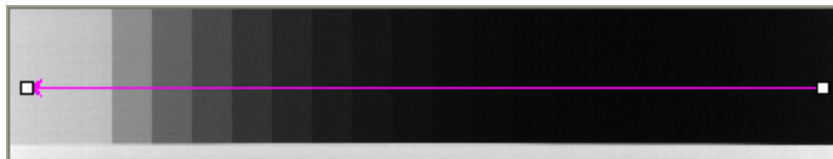
1. Open the image called `Steptab.tif`



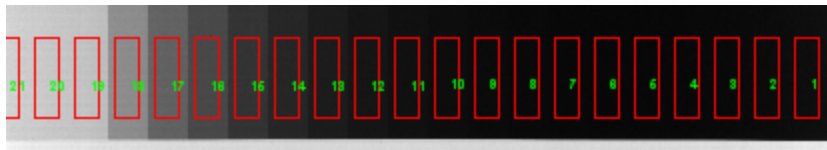
2. Click the *Step Tablet Wizard* button to display the following dialog:



3. Draw a line from center of the first to the center of the last step on the steptab image:



When the line is created, the bands on the step-tablet image are automatically detected and labeled:



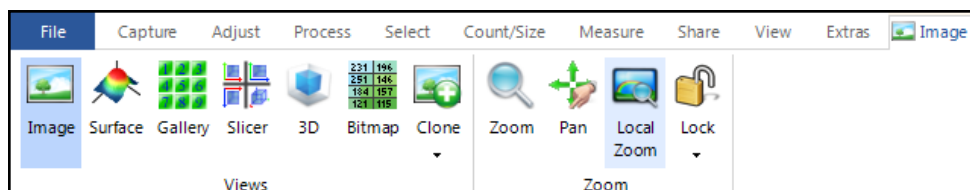
4. Add the correct **Output** values for every **Input** value in the table.

***Tip:** you can create a file with Output values for certain types of step-tablets and use it with multiple images using the **Load** and **Save** buttons.*

5. Once you are satisfied with your values, save the calibration.

The Image Tab

When you have an image active in the workspace, the **Image** tab appears in the application, as shown here:



From the *Views* group, you can select one of seven different ways to view your image:

- **Image** is the default view, with the active frame of the image or sequence displayed in the image workspace.
- **Surface** represents the **Surface Plot** view, which is an advanced view discussed in the next section of this chapter.
- **Gallery** displays all of the frames of the sequence in a grid, and allows you to quickly and easily change the active frame.
- **Slicer** displays X/Y, X/Z and Y/Z projections of a sequence in the slicer view.
- **3D** displays X/Y/Z sequences as a volume.
- **Bitmap** represents the **Bitmap Analysis** feature, which displays the pixel intensities of the active frame of the image, and is discussed in a subsequent section of this chapter.
- **Clone** allows you to open another view on the active image in any of the supported viewers.

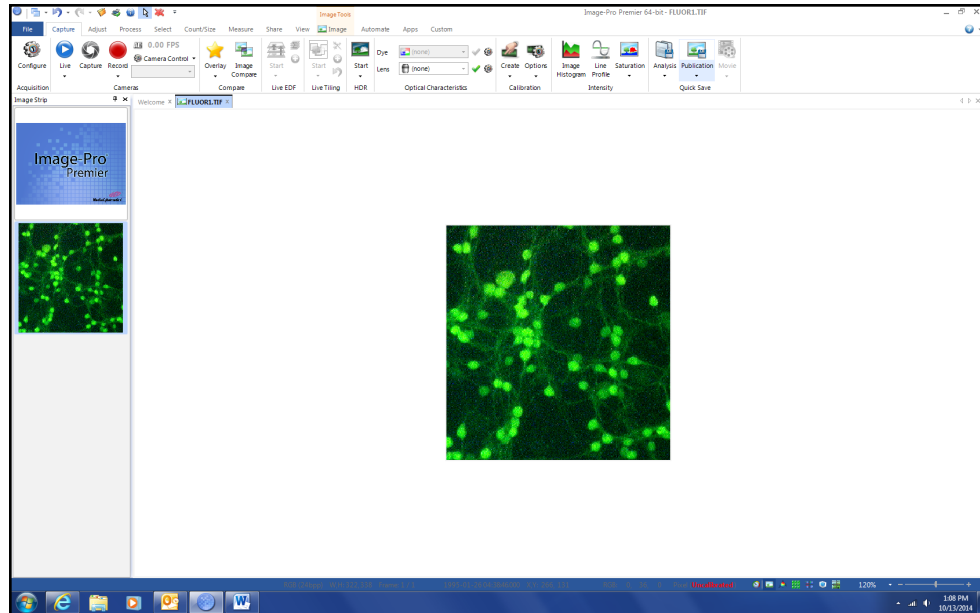
The *Zoom* group lets you focus on specific parts of an image using the **Zoom**, **Pan**, and **Local Zoom** features. The **Lock** button lets you synchronize two images or frames together.

Viewing a Surface Plot

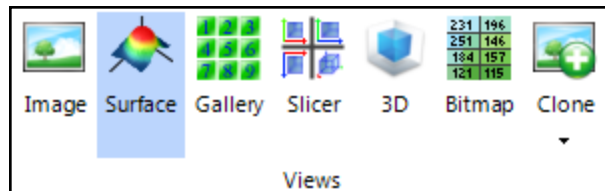
The *Surface Plot* (or 3-D Plot) tool creates a three-dimensional representation of the intensity of an image. When using the *Surface Plot* tool, keep in mind that **X** = length; **Y** = width; and **Z** = height. Learn more about *Surface Plot* by following these steps.

1. Open an image in the workspace.

Viewing a Surface Plot

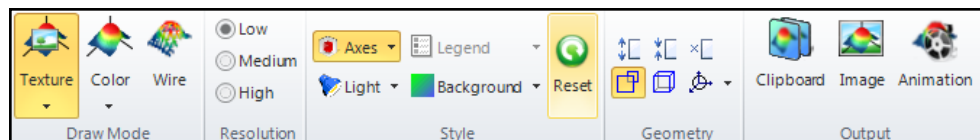


2. Go to the *Views* group on the **Image** tab.

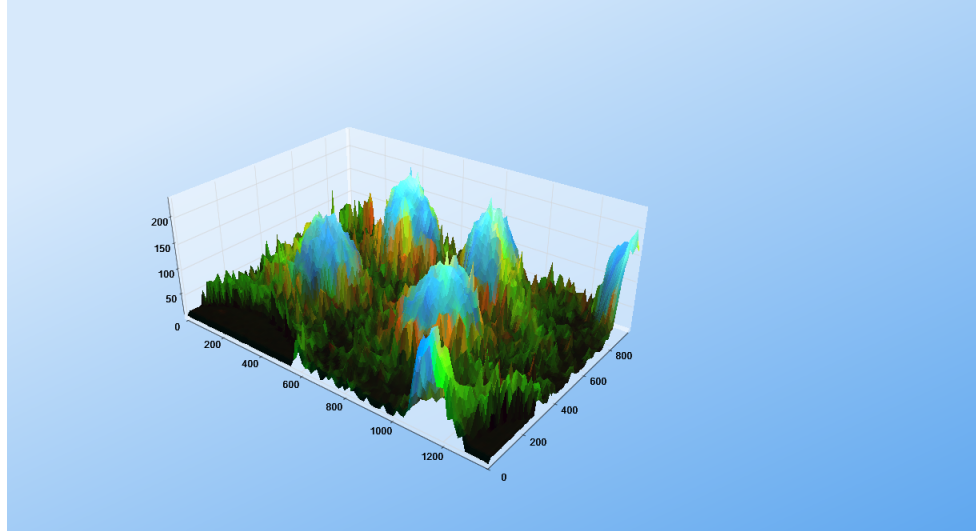


3. Choose the **Surface** icon.

You will see the **Surface Plot** context ribbon:



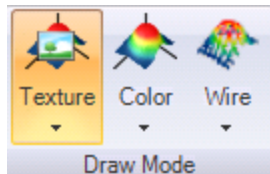
A three-dimensional representation of the image appears in the workspace:



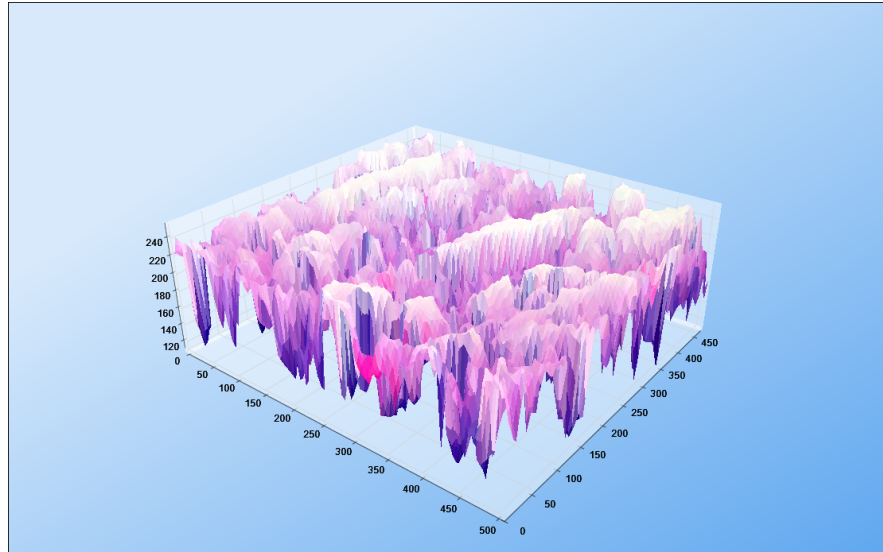
The lighter and taller areas of the plot represent areas of greater intensity on the image. Areas of lesser intensity are shorter and darker. You have many choices for adjusting the appearance and features of the surface plot diagram, as described here:

Draw Mode

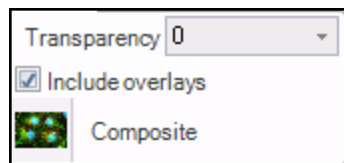
The options in the Draw Mode group control the appearance and display of the surface plot diagram. One of the three modes can be selected: *Texture*, *Color* or *Wire*.



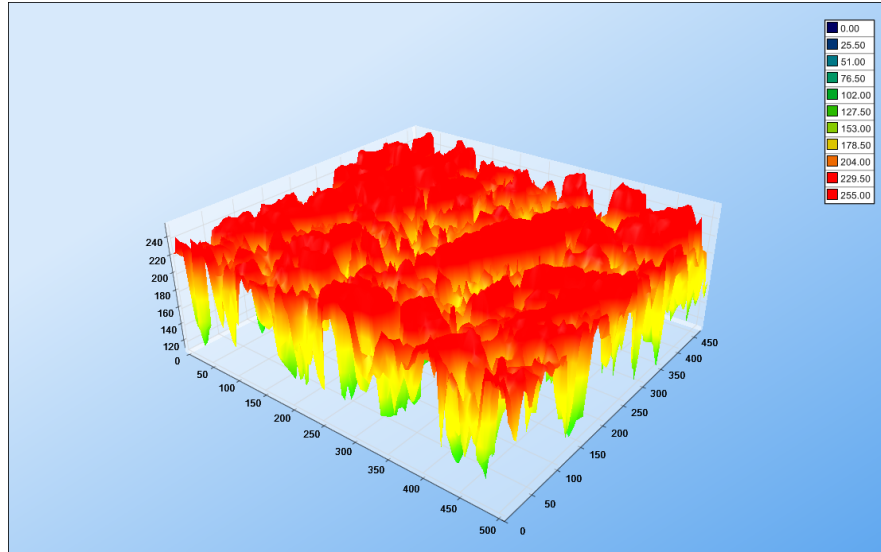
In **Texture** mode the surface uses an image for the surface plot texture.



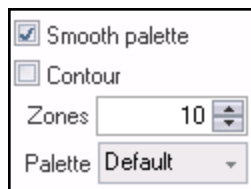
The **Texture** drop-down menu allows you to control the degree of transparency for the surface plot. Transparency set to 0 indicates no transparency.



The **Color** group lets you select the colors to map to the gray values found in the surface plot, as shown here.



In **Color** mode the surface plot is pseudo-colored. The color is assigned according to the height value. Click the drop-down arrow to see the context menu:

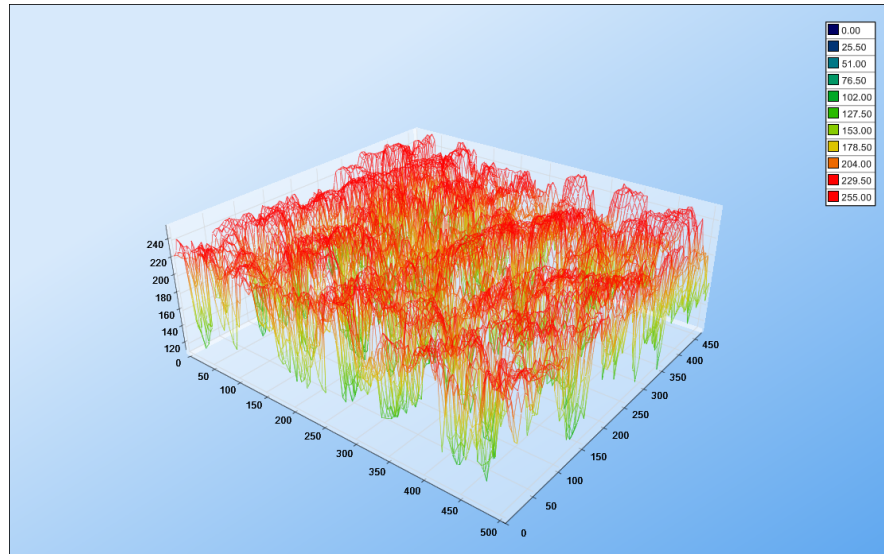



When **Smooth palette** is on the colors of palette entries are blended.

The **Contour** option adds black lines separating color bands.

The **Zones** control defines the maximum number of pseudo-color zones. Type a new value and press Enter key to change it. Note that zones are created automatically based on the total range of the height axis (see **Auto Range** option of **Axes**). The number of zones on the image corresponds to the number of major tick marks on the height axis and can be smaller than the **Zones** value.

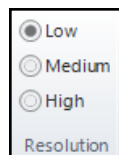
The **Wire** mode shows a wire frame version of the surface plot diagram.



The number of wires along the longest image direction is defined by the resolution option in the pull-down menu, shown here: 

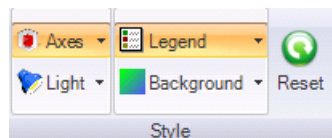
Resolution

The *Resolution* group enables you to adjust the display resolution of the surface plot:

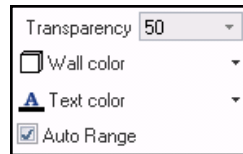


Style

This group controls the visual presentation of the surface plot diagram.



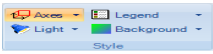
Clicking the **Axes** button toggles visibility of the axes and walls on or off. The drop-down contains the options:



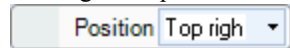
The **Text Color** and **Transparency** of the walls can be set using the pull-down controls.

***Tip:** To hide the walls but leave the axes visible set transparency to 100.*

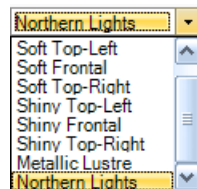
When **Auto Range** is on (default) the range of height axis is calculated based on the image data. When the option is off the height range is defined by the display range of the topology image (ActiveImage.RangeMin and ActiveImage.RangeMax properties).

The **Legend** button  turns the legend on or off.

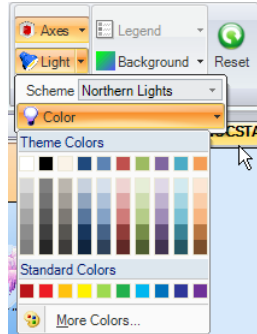
The legend's position can be selected from the drop-down menu



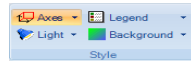
The **Light** button toggles lighting schemes in the order defined in the **Scheme** list. When the Light Scheme is set to **None**, the illumination is switched off. *Northern Lights* scheme uses three light sources, the rest of the schemes use only one.



The ambient color can be selected using the **Color** picker.

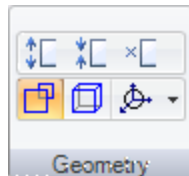


The **Background** control defines the style and color of the graph's background. **Start Color** and **End Color** define the colors of any background gradient.



Geometry

The *Geometry* group lets you view the surface plot from different angles.



Increase Height

Use this tool to increase the height of your surface plot image.



Decrease Height

Use this tool to decrease the height of your surface plot image.



Reset Height

Use this tool to reset the height of your surface plot image back to its original size.



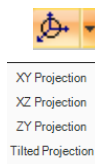
Perspective Projection

Use this tool to create a perspective projection of the surface plot image.



Orthogonal Projection

Use this tool to create an orthogonal projection of the surface plot image.

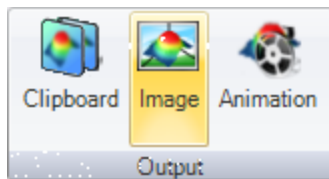


XYZ Perspective

Use this pull-down menu to select the X, Y, or Z perspective

Output

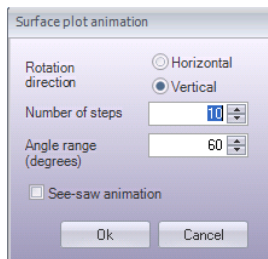
The **Output** options determine where your surface plot settings and information will be directed.



Clipboard: Clicking this button sends your surface plot information to the Windows clipboard, where it can be pasted into other applications that accept data from the clipboard.

Image: Clicking this button creates the surface plot in a new workspace. This function creates the surface plot in the same size as the original image. It is a good idea to create a new image when you want to save or print the surface plot, because in this case the surface plot has maximal resolution.

Animation: Clicking this button displays the **Surface Plot animation** dialog;



Rotation direction: Indicate the direction of the animation movement, either horizontal or vertical.

Number of steps: These spin buttons define the number of frames in the output animation.

Angle range: These controls define the rotation angle of the total animation around X, Y and Z axes in degrees.

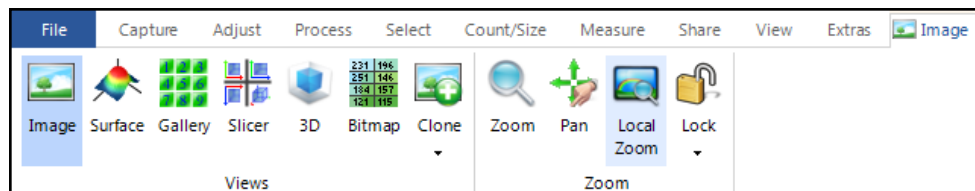
See-saw animation: Checking this box causes the animated image to move up and down, just as the name implies.

OK: Clicking the **Ok** button creates an animation sequence in the workspace. The new sequence then can be saved as a multi-frame *.TIF or *.AVI file.

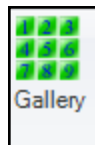
Using Gallery View

The **Image Gallery** feature creates a workspace from an active image sequence. The individual frames in the sequence are displayed in a gallery format. To see an image gallery, follow the steps below:

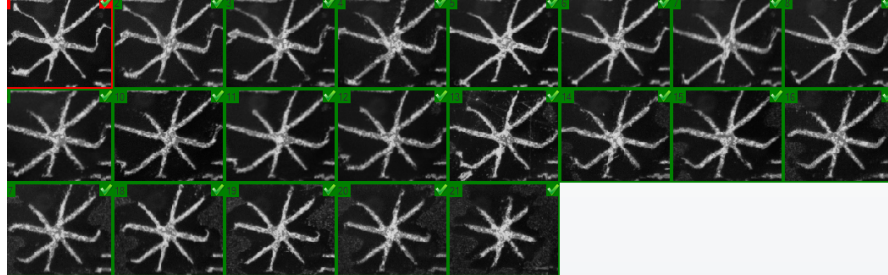
1. Open an image sequence in the *Image-Pro* workspace.
2. Go to the **Image** tab.



3. Click the **Gallery** button.



Clicking this button creates a image gallery workspace from the active workspace. This feature is disabled if there are no active images, or if the active image is a single-frame workspace. Here is a sample image gallery:



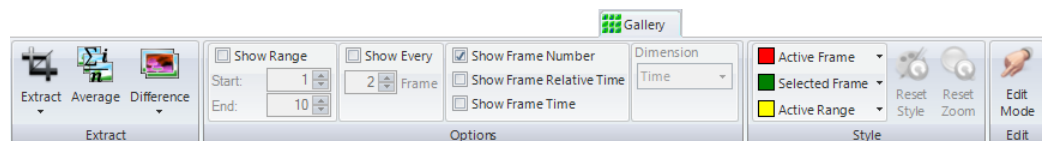
The red square in the gallery workspace indicates the active frame in the larger workspace. Use your cursor to click on different frames in the gallery, and the red square will move to the selected frame, and the active image in the workspace will change to the selected frame as well. You can also use the following keyboard shortcuts:

- <Ctrl> + <Click> will select or deselect more than one frame.
- <Ctrl> + <Mouse Wheel> zooms the view in or out.
- Clicking the right mouse button opens the context menu and also displays image information:





Gallery Ribbon


The gallery ribbon contains the following tools:




The *Extract* tools let you work with individual frames.

 *Extract*. Clicking this button extracts selected frames into a new image or set.

 *Extract Frame By Frame*. This button extracts selected frames into new individual images (a new image is created for each frame).

 *Average*. Clicking this button creates a new image by averaging the selected frames,






 **Difference**. This selection creates a new image as the difference between the selected frames.

- **Wrap**: Calculate the last frame as the difference between the last and first frames.
- **Diff Only**: Returns only the difference frames (the result will be one frame shorter than the original),
 - **Pad First** - pad the result with first frame of no difference,
 - **Pad Last** - pad the result with a last frame of no difference,

The *Options* let you specify how you will view the frames:

- ☒ **Show Range** will show frames only from the range Start/End,
- ☒ **Show Every Frame** will show each frame from the sequence,
- ☒ **Show Frame Number** show or hide the frame number in the top-left corner of the frame,
- ☒ **Show Frame Relative Time** shows or hides the frame relative time
- ☒ **Show Frame Time** shows or hides the frame date and time,

The *Style* tools let you customize the frame display:


-  **Active Frame** selects the active frame color
-  **Selected Frame** indicates the selected frames color
-  **Active Range** selects the active range color
-  **Reset Style** resets the colors to their default (Active frame – Red, Selected frames – green, Active Range – yellow)
-  **Reset Zoom** resets the zoom of the frames to the default size


Clicking the **Edit** button opens an additional ribbon:





Note: *Edit Mode enables “Drag and Drop” operations with frames. This allows you to (rearrange frames and add frames from the Image Strip. This feature is not usable with image sets.*


The *Clipboard* group contains the standard set of tools, applied to image frames:


 *Paste* Click this button to paste frames from the clipboard into the image next to the active frame.


 *Paste New* exports frames from the clipboard into the new image.

 *Paste Frame by Frame* exports frames from the clipboard into new images (new image created for each frame).

 *Cut* Click this button to cut selected frames from the image and place them onto the clipboard.

 *Copy* This selection copies the selected frames onto the clipboard.

 *Extract*. Click this button to extract selected frames into a new image or set.

 *Extract Frame By Frame*. Click this button to extract selected frames to new images frame by frame (a new image is created for each frame).


The *Frames* group contains additional frame editing tools:


Add Frames:


☒ *Add Active frame only*. When checked, only the active frame will be added from the source image


Location

- First adds frames to the beginning of the sequence.
- Current adds frames at the current location (active frame).
- Next Current adds frames to the next from the Current location.
- End adds frames at the end of the sequence.

 *Add All* adds all available frames to the sequence.

 *Add Blank Frame* adds a blank frame to the sequence.

 *Remove* is used to remove selected frames from the image.

 *Left* moves the selected frames on one frame to the left.



Right moves the selected frames on one frame to the right.

Close group:

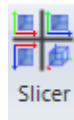
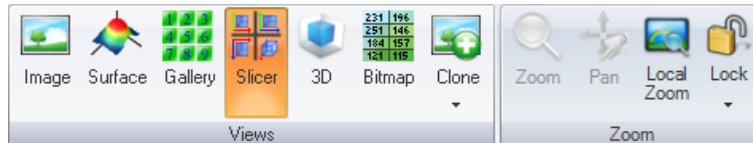


Close Edit Mode closes the **Edit** ribbon.

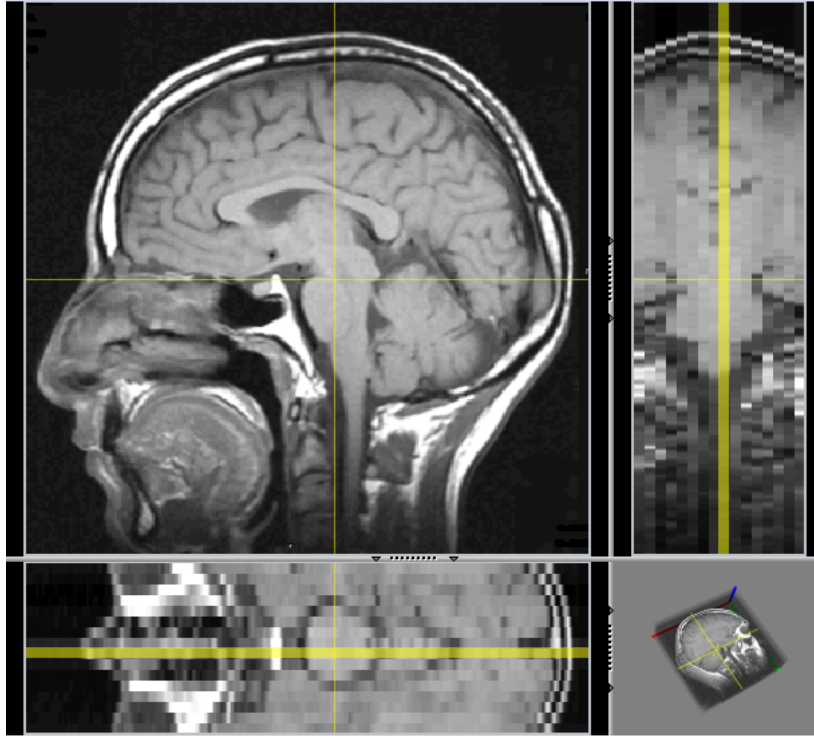
Using the Slicer Viewer

Note: The section documents the simple Slicer Viewer that is included with Image-Pro. Image-Pro with 3D Module includes a more advanced Slicer view, which is described in The 3D Slicer Ribbon section in Chapter 12 and in the in-product help.

The *Slicer Viewer* allows you to view and examine a Z-stack volume using cross sections of the XY,YZ , and XZ planes.The *Slicer* is located on the **Image** ribbon, as shown here.



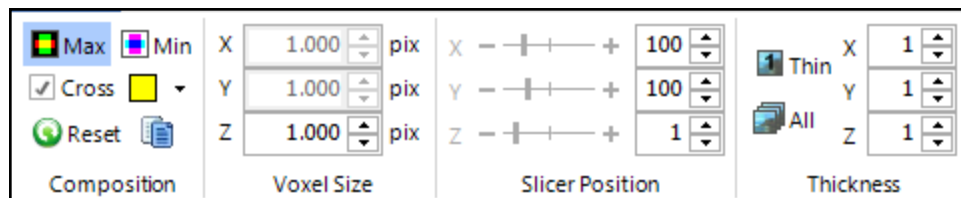
Clicking the **Slicer** button will create a slicer view of the current image:




The top left view is the XY plane. The top right view shows the ZY plane. The bottom left displays the XZ plane. The bottom-right displays a 3D view of the image.

The Slicer Viewer Ribbon

The *Slicer Viewer* has the following ribbon bar:



The *Composition* group defines the parameters of the viewer.

 **Max** Maximum intensity projection.




Minimum intensity projection.

*Note that the number of slides included in the projection is defined in the **Thickness** group.*


The **Cross** checkbox turns the crosshair marker on or off on all the views. The color of the crosshair marker can be selected using the color-picker drop-down menu.


The **Reset** button resets the view and resizes the viewers to display the best-fit image.

The **Duplicate**  button duplicates the active view in a new window.

The **Voxel Size** group defines the size of the voxel. *Note that the X- and Y- size are defined by the spatial calibration of the image. The Z-size is calculated from the distance between frames in Z direction. Only the Z- size is editable.*

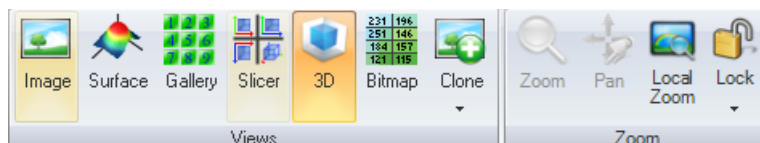
Slicer Position defines the X, Y, and Z coordinates of the cross-section.

Slice thickness is defined in the *Thickness* group. Clicking the **All**  button sets the thickness to the specified volume dimensions. This button can be used to create maximum

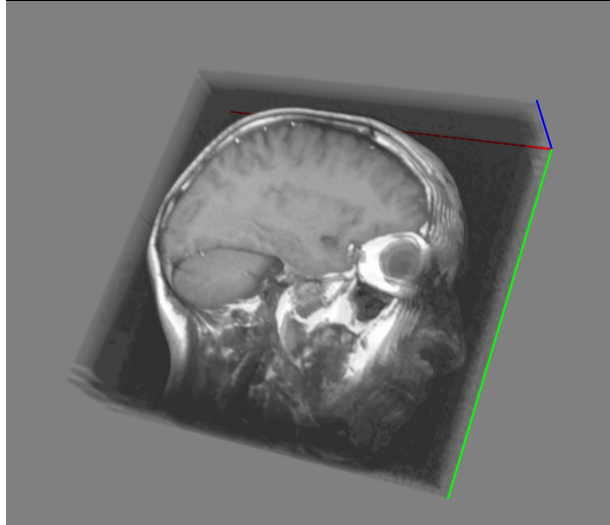
intensity projections for the whole volume. Clicking the **Thin**  button sets the thickness to 1.

Using the 3D Viewer

The *3D Viewer* window allows you to examine an image stack in a variety of different ways: by volume, in thick or thin slices, and as an animated display. In the *3D Viewer*, all geometric objects are shown in 3D space. The *3D Viewer* is located on the **Image** ribbon, as shown here.



Clicking the **3D** button creates a 3D view of the current image with the voxel size corresponding to the spatial calibration and the Z-distance between slices. Here is a sample 3D image:

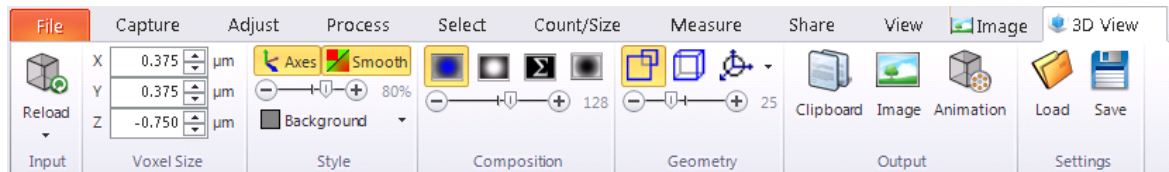


The image can be rotated by holding down the right mouse button and dragging the image in the workspace. Pressing <Shift> + <Click> and dragging will pan the view. Pressing <Ctrl> + <Click> and dragging the image up or down will change the zoom.

Note that if you are running Image-Pro with 3D Module, this feature is replaced by the features described in Chapter 12 of this manual.

The 3D Viewer Ribbon

The *3D Viewer* ribbon contains the following tools:

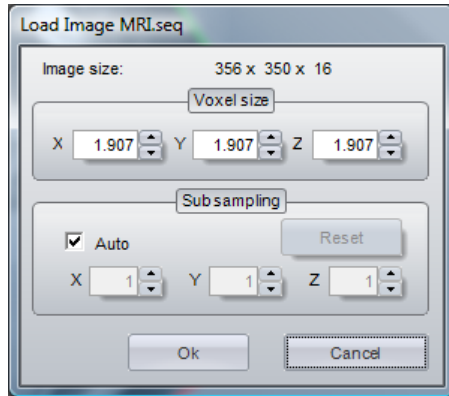


The **Reload** button allows you to reload the volume with different sub-sampling. Clicking the drop-down arrow displays these choices:

- Reload
- Reload when Image changed

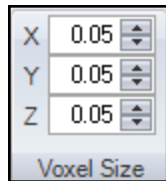
- Reload when LUT changed

Reload when image changed or **Reload when LUT changed** indicates if the image must be reloaded if the corresponding parameter changes.

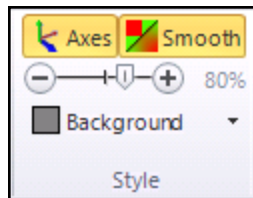


You can also define the voxel size and sub-sampling manually. When the **Auto** box is checked, the volume is subsampled automatically. Clicking the **Reset** button sets the sub-sampling to 1x1x1.

The X, Y, and Z voxel size can be edited interactively in the *Voxels Size* group.



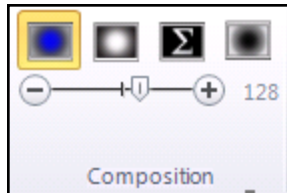
The *Style* group controls the display of the 3D image:



The **Axes** button turns the volume axes on or off. Activating the **Smooth** control enables sub-pixel approximation of the texture.

The **Transparency** slider of defines the volume transparency. The color of the background can be changed using the **Background** color picker.

The *Composition* group defines the options to create the view:



Blended composition



Maximum intensity projection



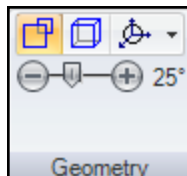
Sum projection



Minimum intensity projection

The slider defines the number of slices in volume presentation. The larger number may produce better quality volume rendering, but may slow down the processing.

The *Geometry* group defines the camera parameters:

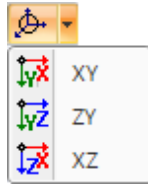


Activates the perspective view.



Activates the orthogonal view.

Clicking the **Projection** button activates following orthogonal views:



Sets the **XY** projection .



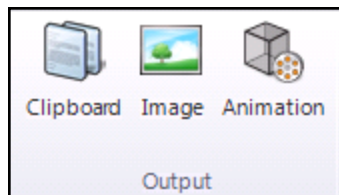
Sets the **ZY** projection.



Sets the **XZ** projection.

The slider controls the **Camera view angle** in degrees.

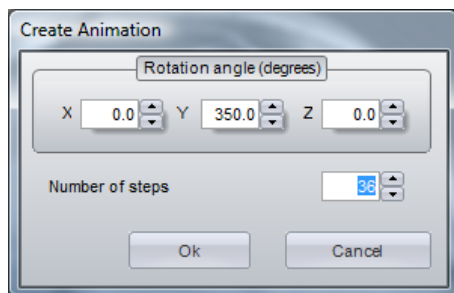
The *Output* group lets you decide how to display the 3D image results:



Clicking the **Clipboard** button copies the current view to the *WindowsClipboard*.

Clicking the **Image** button creates a new image in the workspace.

Clicking the **Animation** button opens a dialog where you can define parameters for simple animation.



Rotation angle controls define the rotation angle of the total animation around X, Y and Z axes in degrees. (X = horizontal, Y = vertical, and Z = perpendicular to the screen).

Number of steps defines the number of frames in the output animation.

Clicking the **Ok** button creates an animation as a new sequence in the application workspace. The new sequence then can be saved as multi-frame a TIF or AVI file.

The *Settings* group allows you to load or save your 3D viewer selections:



Load 3D viewer options and **Save 3D viewer options** allow you to load or save your 3D viewer selections to a file with V3O extension (the file is in XML text format). Loading saved options allows you to restore a particular view point and composition parameters.

Bitmap Analysis

The **Bitmap Analysis** command in the *View* group on the **Image** tab is used to view the pixel values of the active window (or a region of interest on that image) in numeric format. These values can be saved to an ASCII file for later use with an external program, or copied to the Clipboard and pasted into another application (a 3D plotting package, for example).

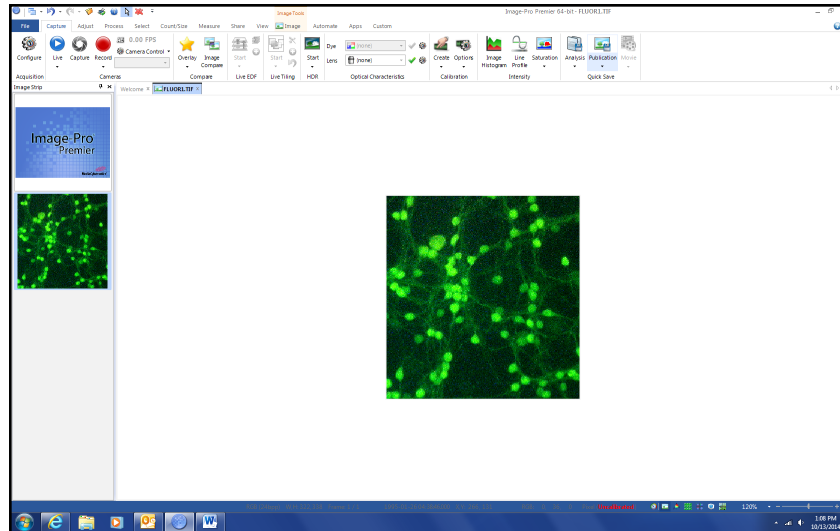
Pixel values can be displayed as they actually exist in the image or in their calibrated form. They are not interpreted through the display LUT however.

The **Bitmap Analysis** window imposes no limit on the number of pixels that can be processed. There is, however, a maximum of 30,000 pixels that can be saved, copied to the Clipboard or transferred to *Excel*™. If you need to save more than this number of pixels, you can define an AOI that is less than or equal to 30,000 pixels, and process the image in chunks (i.e., move the AOI systematically across the image and save the data at each interval). Or, you can use the **sub-sampling** option to read the pixel values at larger intervals.

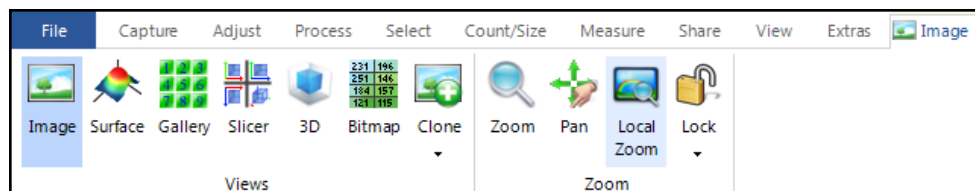
Note: Consider carefully how much data you actually need to save in ASCII format; a 100 x 100 segment of a Gray Scale image (10K in binary image form) will consume over 60K, and a True Color segment will take three times that amount.

To use the *Bitmap Analysis* feature, follow these steps:

1. Open an image in the *Image-Pro* workspace, as shown here:

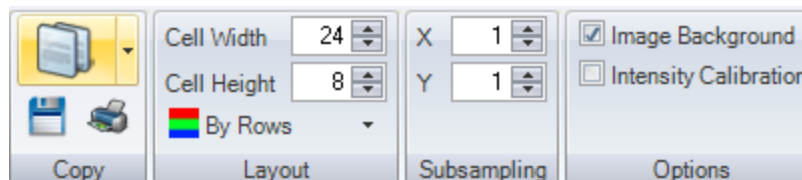


2. Go to the *Image* tab.



3. Click the **Bitmap** button.

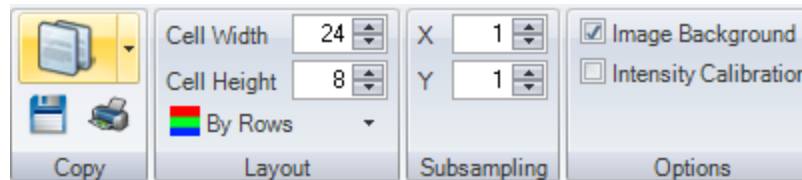
The button will be highlighted and you will see the hourglass icon and the *Bitmap* context ribbon:



After a few moments, a data table with the intensity values of all the pixels in the image or ROI will appear in the workspace.

The screenshot shows a software window titled 'Welcome' with tabs for 'Tumor.tif' and 'Tumort.tif2'. Below the tabs is a large data table. The columns are labeled 'Y1X' and the rows are labeled with coordinates like '352.1', '352.2', etc. The table contains numerical data, and the cells are colored based on their values, with a gradient from red (higher values) to blue (lower values).

The **Bitmap** context ribbon appears in the ribbon bar above the workspace:



The **Copy** group allows you to send the table data to the Windows Clipboard, Excel, Word, or save to disk in *.XLS or *.TXT (tab-delimited) formats for printing.

The **Layout** group defines the size of cells in the table (**Cell Width** and **Cell Height**) and the color channels layout (if you are workign with a color image). If the image has more than one channel, the channels can be reported in **Rows** or **Columns**:

Y \ X	196/1	196/2	196/3	197/1	197/2	197/3	198/1	198/2	198/3	199/1
222	80	61	10	254	252	173	254	250	143	143
223	82	60	0	251	233	142	254	251	136	136
224	174	155	46	254	241	118	251	235	94	94
225	232	223	87	254	254	125	246	238	94	94
226	236	243	94	242	249	111	164	172	46	46
227	254	254	123	243	254	124	131	146	34	34
228	251	254	112	235	244	118	138	149	48	48
229	250	250	119	245	250	138	147	155	69	69
230	247	242	133	242	244	151	123	127	59	59
231	235	236	123	234	241	146	127	132	65	65
232	226	243	102	250	254	153	85	101	19	19
233	228	240	97	249	254	142	118	124	41	41
234	236	222	104	254	254	158	100	75	9	9
235	254	210	142	253	199	146	151	89	57	57
236	140	42	50	229	126	139	143	34	56	56
237	153	30	67	171	43	83	164	32	73	73

In column format, the channel index is displayed with a colon to indicate the pixel positions. For example, 392:2 indicates the Y coordinate is 392, in channel 2. Switching to channels **by Rows** provides a pixel aspect ratio closer to the original image.

Sub-sampling is useful if you are working with a very large image. This feature lets you analyze pixels at various intervals on the image.

The *Options* group let you display the pixel values as they actually exist in the image or in their calibrated form.

Checking **Image Background** displays the image as a background for the bitmap data table. This option can help visually identify the objects of interest.

Y \ X	106	110	114	118	122
394:1	135	225	151	192	207
394:2	127	178	58	59	54
394:3	159	185	86	73	55
396:1	176	231	157	213	215
396:2	165	172	33	74	64
396:3	178	173	52	76	62
398:1	243	168	228	192	208
398:2	213	84	87	56	71
398:3	204	93	104	51	62
400:1	244	137	212	185	233
400:2	213	27	52	72	127
400:3	202	47	59	55	114
402:1	234	149	190	191	224

Y \ X	106	110	114	118	122
394:1	135	225	151	192	207
394:2	127	178	58	59	54
394:3	159	185	86	73	55
396:1	176	231	157	213	215
396:2	165	172	33	74	64
396:3	178	173	52	76	62
398:1	243	168	228	192	208
398:2	213	84	87	56	71
398:3	204	93	104	51	62
400:1	244	137	212	185	233
400:2	213	27	52	72	127
400:3	202	47	59	55	114
402:1	234	149	190	191	224
402:2	196	24	36	90	128
402:3	190	49	43	81	123
404:1	250	178	176	236	188
404:2	193	41	36	138	90
404:3	191	68	46	146	98
406:1	213	165	178	225	193

Image Background on Image Background off

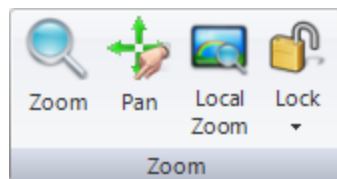
When **Intensity Calibration** is checked, the table shows intensity calibrated pixel values. (The number of decimals is defined in the application options described in Chapter 2).

Y \ X	106	110	114	118	122
394:1	105.882	176.471	118.431	150.588	162.353
394:2	99.608	139.608	45.490	46.275	42.353
394:3	124.706	145.098	67.451	57.255	43.137
396:1	138.039	181.176	123.137	167.059	168.627
396:2	129.412	134.902	25.882	58.039	50.196
396:3	139.608	135.686	40.784	59.608	48.627
398:1	190.588	131.765	178.824	150.588	163.137
398:2	167.059	65.882	68.235	43.922	55.686
398:3	160.000	72.941	81.569	40.000	48.627
400:1	191.373	107.451	166.275	145.098	182.745

Intensity calibration bitmap table

Zooming Images

The *Zoom* group contains the controls that allow you to resize the image or a portion of the image.



There are several ways to zoom in and out on an image, that is, to display it at different levels of magnification. You can:

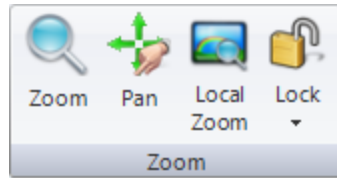
- Use the **Zoom** button in the *Zoom* group
- Use **Local Zoom**
- Use the wheel on your mouse (if available)
- Use the scale bar.


Zooming by Using the Zoom Button

This method of zooming allows you to zoom in on specific areas of the image.

1. Click the **Image** tab from the *Image-Pro* menu bar.


The *Zoom* group is displayed.



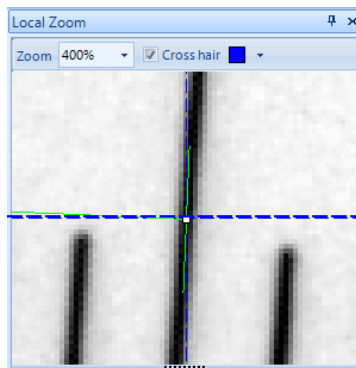
2. Click on the **Zoom** button [].
3. Do one of the following:
 - Position your mouse over the area of the image you want to magnify, and click the mouse.
 - Click and drag a rectangle around the area of interest on which you want to zoom.

The image is magnified. If needed, you can click or drag the mouse again to magnify the area of interest even further.

Zooming with Local Zoom

The *Local Zoom* feature provides a tool to show a zoomed area of an image in a separate window with a given zoom factor. It can be used with manual measurements, the calibration marker, manual tagging, etc. You can find it on the *Image* tab, or by clicking the  button on the context menu.

When you select **Local Zoom**, a new window opens on the right side of the application area.



The cross hairs in the **Local Zoom** window show the current cursor location. This makes placing accurate manual measurements very easy, even on large images:

Zoom: Choose the required zoom factor from the drop-down list.

Cross hairs: Checking this box turns the cross hairs on; un-checking the box turns the cross hairs off. The **color picker** button selects the color of the cross hairs.

Zooming by Using the Mouse Wheel

If your mouse has a wheel on top between the left and right mouse buttons, you can use this wheel to zoom in and out on the image. Simply roll the wheel while the image is active in the *Image-Pro* workspace. Roll the wheel toward you to zoom out on the image, and roll the wheel away from you to zoom in on the image.

Zooming by Using the Scale Bar

This method for zooming allows you to take advantage of zoom presets, or to put the image in 'fit to window' mode so that whenever you resize the image window, the image is enlarged or reduced to the size of the window. For example, you can zoom the image to a specific percentage of its actual size, such as 50% or 200%.

1. Position the cursor over the image and right-click the mouse.

You will see the scale bar.



2. Move the slider to the right to increase the size of the image, or to the left to decrease its size. You may also click the arrow to see the drop-down list of zoom presets. The scale bar also appears in the status bar at the bottom of the *Image-Pro* workspace.

Panning Images

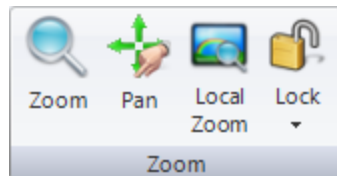
The **Pan** tool is used to position an image that does not fit entirely within the image window. You can:


- Use the **Pan** button in the *Zoom* group
- Pan in the *Image Strip*

Panning Using the Pan Button

1. Click the **Image** tab from the *Image-Pro* menu bar.

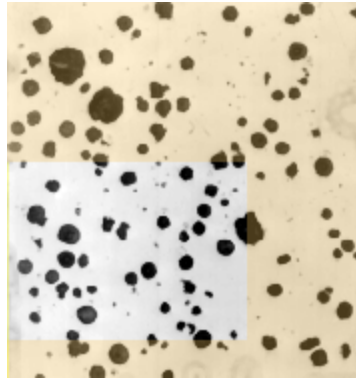
The *Zoom* group is displayed.



2. Click on the **Pan** button [].
3. Use your mouse to move the image around in the frame.

Panning in the Image Strip Using Image Navigator

The *Image Strip* displays a thumbnail of your entire image. When the active image is zoomed and doesn't fit in the viewer, the viewing area is shown on the image strip thumbnail as a semitransparent overlay.

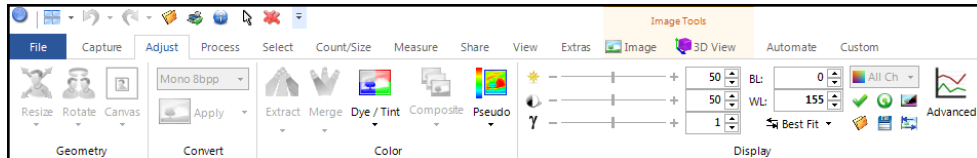


- Click and drag the center of the viewing area on the thumbnail to control pan and scroll of the image.

The *Image Navigator* feature allows you to use your mouse to move the white rectangle around in the image strip. The zoomed portion of the image in the workspace will move as you move the thumbnail in the image strip.

Enhancing an Image

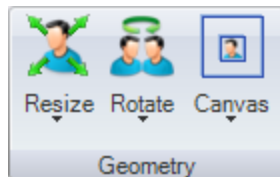
There are several tools available through *Image-Pro*'s **Adjust** tab for visually enhancing objects of interest in your images. The features on the **Adjust** tab include:



Resizing an Image

Image transformation allows you to rotate or resize the image in the *Image-Pro* workspace. These options allow you to examine the image in more detail. To resize an image, follow these instructions.

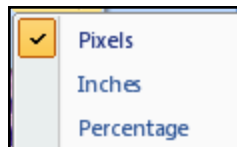
1. Open the `castiron.tif` image in the *Image-Pro* workspace, as described earlier in this chapter.
2. Select the **Geometry** group from the **Adjust** ribbon:



3. From the **Resize** pull-down menu, select an image size:

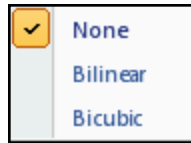


4. Click the lock icon and select a measurement unit from the drop-down menu.



This will maintain the current aspect ratio of the image. To unlock the aspect ratio, click the icon again. You can find more information about aspect ratios in *Chapter 3, Capturing Images*.

- From the second pull-down menu, select the type of smoothing you require in your resized image:

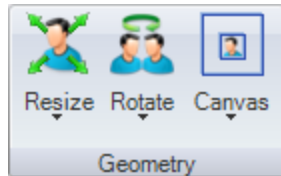


The resized image will appear in the workspace, with a name derived from the original image's name and a suffix indicating that the image has been resized, such as `castiron_100resz*`.

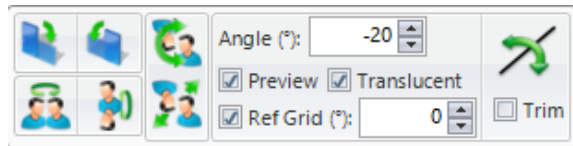
Rotating an Image

Rotating an image allows you many different views of the same image. For example, you can rotate it 90 degrees counterclockwise, or flip it left to right. To try some of the image rotation features, follow these steps:

- Open the image in the *Image-Pro* workspace, if it is not open already:
- Select **Rotate** from the *Geometry* group:




- Use the pull-down menu to see the **Rotate** tools:





The tools are:


- Rotate image 90° clockwise,

 Rotate image 90° counter-clockwise



 Flip image left to right

 Flip image top to bottom

 Rotate image 180°

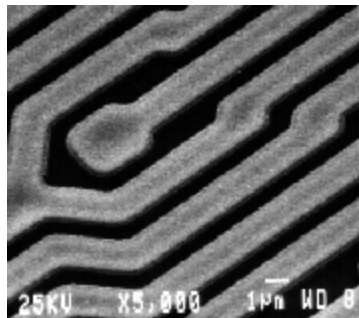
 Swap corners

Free angle rotation:

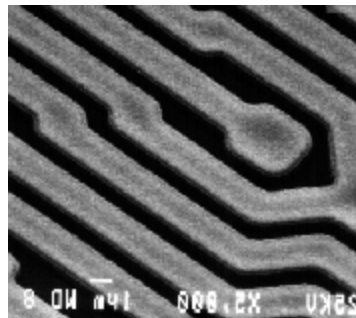
- **Angle** indicates the angle of rotation counterclockwise.
- **Preview** displays a preview of the rotated ROI.
- **Translucent** displays the rotated preview in a translucent format.
- **Ref. Grid** displays a reference grid with the preview on the image at the specified grid angle.
- **Trim** will trim the rotated ROI to the same size as the original ROI.
-  Rotate with trim into a new image
-  Rotate without trim into a new image.

4. Choose the flip left to right tool.

The image will flip automatically, as shown here:

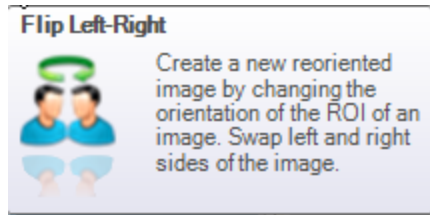


Original image



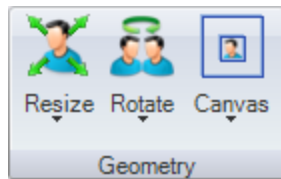
Flipped image

You may want to experiment with the other **Rotate** tools. The *Hint* windows and tooltips contain additional information about these tools. A sample hint window is shown here:



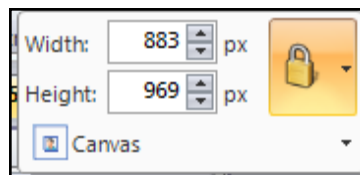
Using the Canvas

The drawing **Canvas** lets you add or remove space around an existing image.

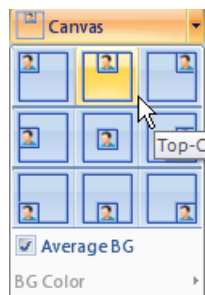


1. To use the canvas, click on the **Canvas** button on the *Adjust* tab's ribbon.

You will see the drop-down menu, as shown here:



2. Use the **Canvas** command from the drop-down arrow to position the image on the canvas, as shown here:

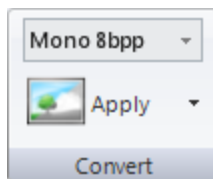


From here, you can add annotations and labels to the image on the canvas. You can also adjust the background and color. You can crop the image by removing space around it.

Converting Images

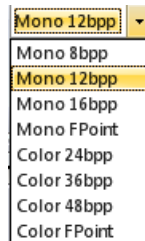
Use the *Convert* group commands to transform an image to another color model, to extract a specific color channel from a color image, or to merge an active, 8-bit gray scale image into a *True Color* image. You might do this to perform editing or analysis upon the image in another model or to save the image data in a different model for use with an external program.

The **Convert** group is shown here:

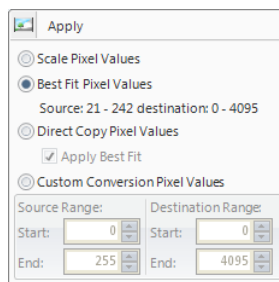


To convert an image:

1. Select the Destination image type from the drop-down list.



2. Click **Apply**.

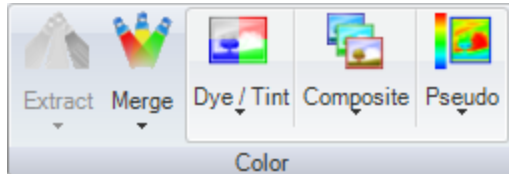


3. Select the type of conversion to perform.

Your image is automatically converted to the new model. Once you have set the conversion options the way that you want them, you can click the **Apply** button in the *Convert* group to convert the active image using the current settings without making further changes.

Using Color Group Commands

Use the *Color* group commands to transform an image to another color model, to extract a specific color channel from a color image, or to merge an active, 8-bit gray scale image into a *True Color* image. The *Color* group is shown here:

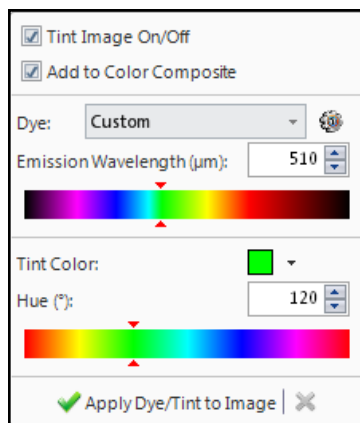


Use the *Extract* selections to extract a specific color channel from a color image, and view it as an 8-bit *Gray Scale* image. You might do this to reduce the saturation values in a HSI image, or boost just the Blue values in an RGB image.

Use the *Merge* command to merge an active, *Gray Scale* image into a *True Color* image. This is usually done to return a channel, extracted with the *Extract Channel* command, to an image after the channel data have been manipulated. However, you might use it to merge any *Gray Scale* image to a color channel in a *True Color* image.

Dye/Tint Images


The Dye/Tint feature lets you apply a color to your image(s) that corresponds to the emission color of your dye. If you are using color to explore the intensity distribution of your image, please refer to the Pseudo Color feature later in this manual and in the in-product Help. Click on the drop-down arrow to see the **Dye/Tint** dialog:



Check the **Tint Image On/Off** box to show or hide the tint on your image.

Check **Add to Color Composite** to automatically add your tinted image to the color composite image.

The **Dye** drop-down list displays a list of your current set of favorite dyes. You may select a different dye from the drop-down list, and all the remaining controls will be updated to show the characteristics of the new dye. If the dyes you want are not in the current set,

click the **settings** button  to display the **Dye Editor** dialog, where you can view a complete list of the available dyes. Here you can add select a new favorite dye, add a dye to the list, or delete old ones.

The **Emission Wavelength** drop-down displays the emission wavelength for the dye you have chosen. You can adjust the emission wavelength by clicking on the spectrum or entering a new value. The default **Tint Color** will change in response to changes in the emission wavelength.

The **Tint Color** box lets you choose a color to indicate a particular dye. Here you can change the color associated with the selected dye, or define a custom color. The default color is linked to the emission wavelength. When you change the emission wavelength, the tint color and hue change. The default **Tint Color** can be adjusted to a different hue by using the **Hue** slider or by entering a new **Hue** value. A completely custom **Tint Color** can be selected by clicking the down-arrow next to the Tint Color box, and selecting a standard or custom color.

The **Emission Wavelength** and **Hue** override a custom **Tint Color**, so to set a custom color, you should first set the wavelength, and then set the color using either the Hue controls or the Tint Color.

Click **Apply** to add your dye or tint color to the active image.

Using Color Composite

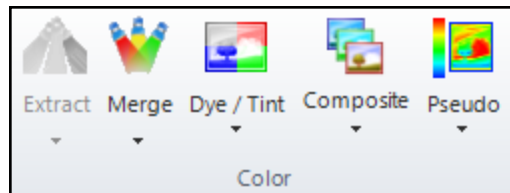
Color composite is a common operation for merging individual fluorescent images. For a variety of reasons it is not always possible to acquire the images simultaneously, so image processing tools are necessary to present a complete and accurate qualitative picture. The **Color Composite View** is used primarily to merge and register fluorescent images acquired as monochrome single wavelengths into a color composite image.

Use **Color Composite** to create and configure color composites using black and white source images. You can access the **Color Composite** dialog box through the **Color** group on the *Adjust* tab.

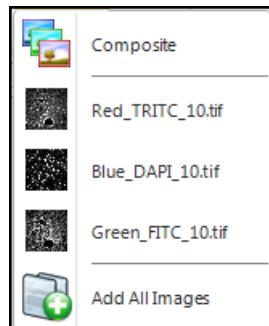
You can combine grayscale images into a color composite. Any group of grayscale images that are of the same size can be mixed in a color composite. Images of 8-, 12-, 16-bit integer or floating point format are combined into a 24-bit color composite. Each input channel will have individual LUT adjustments, as well as a registration offset to line it up with the rest of the images. You can also combine individual channels from a single image. Each channel will be listed separately.

To create a color composite, follow the steps below.

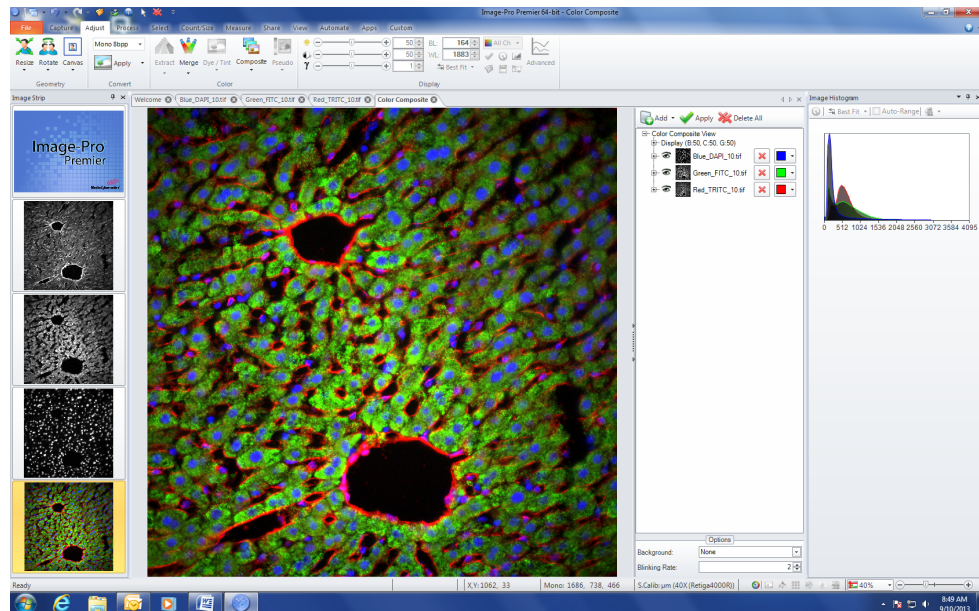
1. Open the images that you want to combine. In this example, we are using the `Blue_DAPI_10.tif`, `Green_FITC_10.tif`, and `Red_TRITC_10.tif`.
2. Click the drop-down arrow in the **Composite** area in the **Color Group** on the *Adjust* tab.



3. Select **Add All Images** from the drop-down menu.

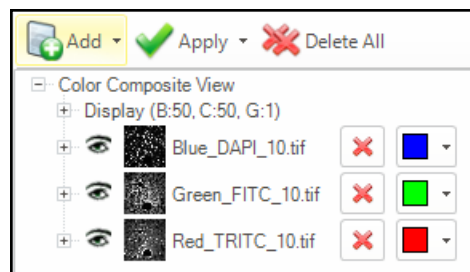


The three demo images are combined into a single color image in the *Image-Pro* workspace:



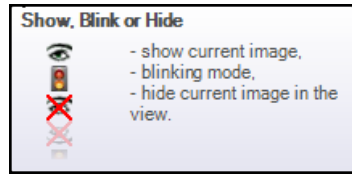
The three original images and the combined image appear as thumbnails in the image strip on the left side of the workspace.

The color composite dialog on the right side of the workspace contains information about each image. Each of the original images represents a single channel in the composite image:



Detailed information about each color channels can be displayed by clicking on the + sign to expand the image information. You can make changes to the image information, change the color display, or remove one image/channel entirely.

1. Click the eye button to show, blink, or hide the composite images, as shown here:



You can save your composite image as a single image or as an image set.

2. To save your composite, click the **Apply** button:



3. To save the composite as an image set, click the drop-down arrow and select image set:



You will see the **Save Image as Set** dialog.

4. Click **Save** to save the composite as a *.mcs set.

Pseudo-Coloring an Image

Use the *Pseudo-Color* command to “colorize” an active monochrome (*Gray Scale 8*, *Gray Scale 12*, *Gray Scale 16*, or *Floating Point*) image. You might do this to highlight certain features in a gray scale image. For example, you might want to display all densities above a certain point in red, or, if your imaging device recorded thermal information, all temperatures below a certain point in blue.

You might also use it to visually amplify specific intensities that are very difficult to distinguish from their surroundings. For example, features produced by pixels with values of 122 in a field of pixels with similar values would be impossible to see in a gray tone image, but would jump out if that value were to be rendered in color.

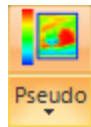
When you pseudo-color a monochrome image, you build a special palette through which your monochrome image is displayed. Pseudo-coloring an image does not modify the pixel values in your image bitmap in any way (it does not convert your image to *True Color* or *Palette*, for example). It simply associates a pseudo-color palette with the image, that *interprets* the gray-level values in the image as color.

Pseudo-colored images are very similar in structure to *Palette* class images, but they differ in a couple of important ways. First, the pixel values in a pseudo-colored image actually represent continuous-tone intensity information, whereas a *Palette* image's pixels carries no intensity significance. Secondly, a *Palette* image includes a palette table that is actually part of the image file.

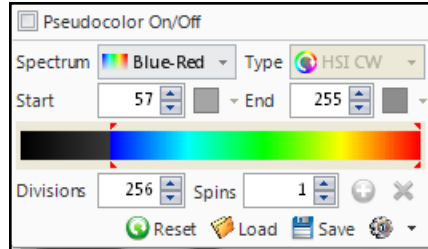
The pseudo-color palette associated with a pseudo-colored image is not a permanent part of the image. It is a palette that you assign to the image while you work with it in *Image-Pro*. Pseudo-color palettes can be saved to a disk file, and later loaded for subsequent use with any monochrome (*Gray Scale 8*, *Gray Scale 12*, *Gray Scale 16*, or *Floating Point*) image.

The basic steps involved in pseudo-coloring the active, monochrome image are to:

1. Select the **Pseudo** button from the *Color* group on the **Adjust** ribbon:



2. Click the drop-down arrow to see the **Pseudo-color** dialog:



3. Check the **Pseudocolor On/Off** to show (or hide) the pseudo-coloring on the image.
4. **Change the color assignments** and refine the intensity division widths. This is done using the **Spectrum** and **Type** drop-down menus or the color bar.

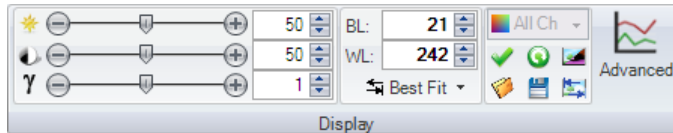
The **Spectrum** drop-down list lets you choose which colors will be used for coloring the images. Choices include Blue to Red, Red to Blue, Cyan, Magenta, and Yellow. You can also define a custom color spectrum.

Type lets you choose RGB, HSI CW, HSI CWW, or a custom type.

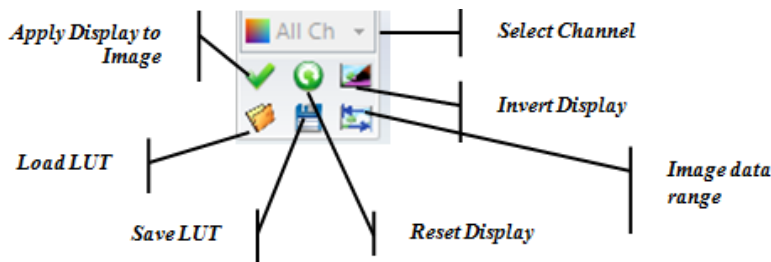
5. **Specify the range of intensities** you want to pseudo-color. The intensity range is specified using the **Start** and **End** spin buttons. You can load saved pseudo-color settings using the **Load** button.
6. **Specify the number of distinct colors** you want to associate with the selected intensity range. The number of colors is selected using the **Divisions** spin buttons.
7. **Indicate the number of spins.** If you want the spectrum to repeat over the intensity range of the image, you can set the Spins to the number of times to repeat the spectrum.
8. **Save the settings for future use** (with the current image, or with another). This step is optional. You do not need to save the pseudo-color assignments in order for them to be applied to the active image. If, however, you want to save it for continuing use or to exchange with a colleague, you need to save it. When you click the **Save** button, *Image-Pro* saves the current pseudo-color palette settings.

Adjusting Color Channels

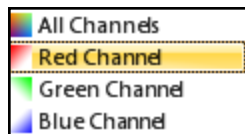
To adjust the color channels, or load a saved Look-Up Table (LUT) use the *Display* group on the *Adjust* ribbon.



This feature contains the following tools:



1. Click the drop-down menu arrow.
2. Select the channel you want to adjust. The default is *All channels*.



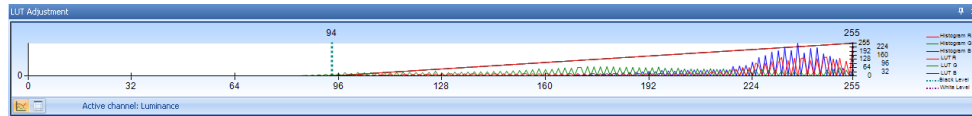
3. Click the **Advanced** button.

You will see the *Advanced* tools ribbon:



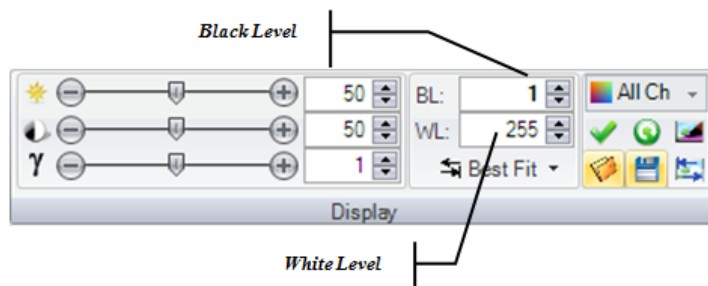
This tab contains all the features you may need to make and view changes to the **Lookup Table**.

As you make changes to the LUT, you will see them reflected in the LUT profile graph at the bottom of the workspace:



*Note: You will not get a dynamic LUT profile graph for *.AVI and *.SEQ files unless you select an active frame.*

If you are working with a black and white image, you can adjust the black and white levels by using the **BL:** and **WL:** spin buttons, or typing new values in those fields.



Changing the Display Range

The *Display Range* is the range of image intensities between the low and high values (inclusive) of the black and white levels. This feature allows you to improve the visualization of objects of interest in your images by:

- Excluding irrelevant image data
- Boosting the contrast between relevant intensities.

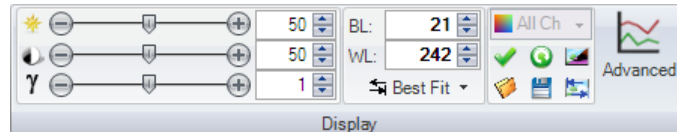
For example, the intensity values of an 8-bit gray scale image can range from zero to 255, and often the useful data covers only a small portion of that range.

With the *Display Range* feature, you can set high and low thresholds for intensity values such that all pixel intensities below the low level display as black and all pixel intensities above the high level display as white. *Image-Pro* scales all intensities within the active image to take advantage of the range of black and white values available on your monitor.

*Display range enhancements are implemented through a Lookup Table rather than by modifying the image bitmap. The original appearance of your image can be restored at any time. If you want to make the enhancements permanent, you must write them to the image with the **Apply Display to Image** button..*

To change the display range for an image:

1. Select the *Display* group in the *Adjust* ribbon.



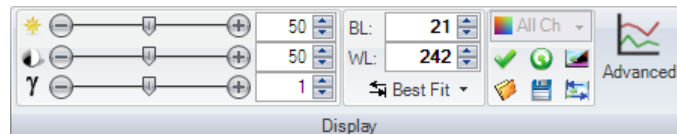
2. Click the **Best Fit** button to find the best display range automatically. You can use the BL and WL spin buttons to make further adjustments to the display range.

Adjusting Brightness, Contrast, and Gamma

The brightness, contrast, and gamma controls provide three ways to adjust pixel intensities in order to enhance objects of interest in the image. They allow you to adjust the display of image intensities between the black and white levels.

To adjust brightness, contrast, or gamma:

1. Click on the *Display* group in the *Adjust* tab's ribbon:



As you move the sliders, the image in the workspace displays the changes in intensity.

2. Use your cursor to select the color channel that you want to adjust.



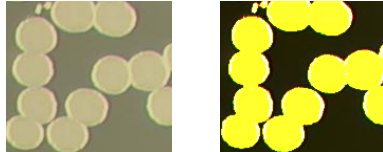
Brightness: Increases/decreases all intensity values uniformly. In the example below, brightness has been increased from the original.



Original After Brightness Increased



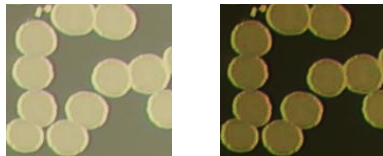
Contrast: Increases/decreases high and low intensity values reciprocally (that is, as the high values are increased, the corresponding low values are decreased, and vice versa). In the example below, contrast has been increased from the original.



Original After Contrast Increased



Gamma: Adjusts the contrast for the high and low intensity ranges simultaneously. In the example below, gamma has been decreased from the original.



Original After Gamma Decreased

Resetting the Image

Because the enhancement features (Equalize, Contrast Enhancement, Display Range, and Invert Contrast) are implemented through a Lookup Table, the changes made to the appearance of your images are not permanent. You can restore the image to its original appearance at any time. To do this:

- Click the **ResetDisplay** button  from the *Channels* group.

The Lookup Table is reset and the image appears as it did before any enhancement options were defined.

Making Enhancements Permanent

Because the enhancement features (Equalize, Contrast Enhancement, Display Range, and Invert Contrast) are implemented through a Lookup Table, the changes made to the appear-

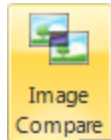
ance of your images are not permanent. This means they will not be saved with the image. To make the enhancements permanent:

- Click the **Apply Display to Image**  button.

All Lookup Table values are permanently applied to the image and the Lookup Table is reset to its default state.

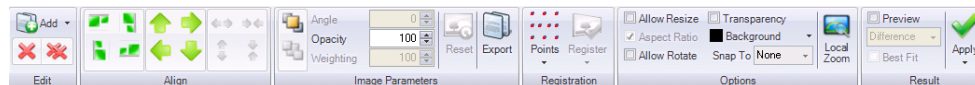
Comparing Images


The image comparison feature enables you to compare two images that may have changed over time. You will find the **Image Compare** feature in the *Compare* group on the **Capture** ribbon.



To use **Image Compare**, follow these steps:

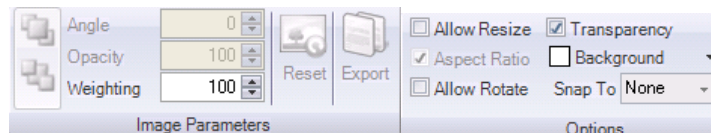
1. Open the two images that you want to compare.
2. Go to the *Compare* group on the **Capture** or **Home** tab's ribbon.
3. Click the **Image Compare** tool. You will see the **Comparison tools** ribbon.



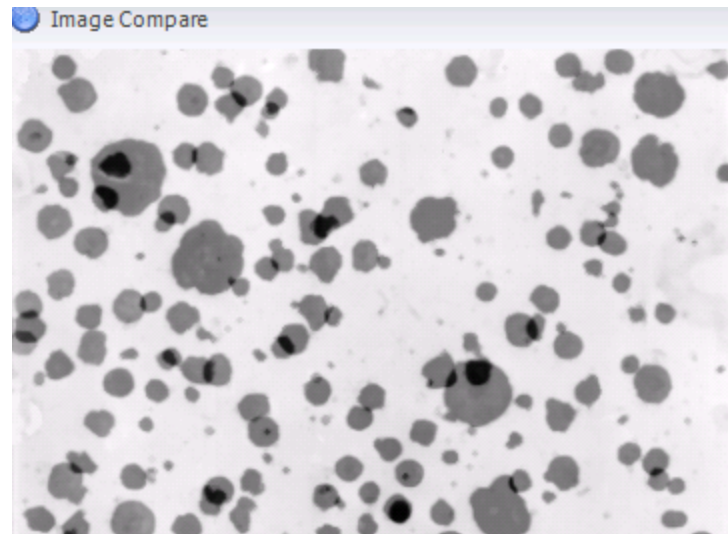
4. Click the **Add** button  to add images to the view.
5. Use the tools in the *Align* group to adjust the position of your images.



6. Use the *Parameters* and *Options* groups to make changes to the transparency, background, and other characteristics.

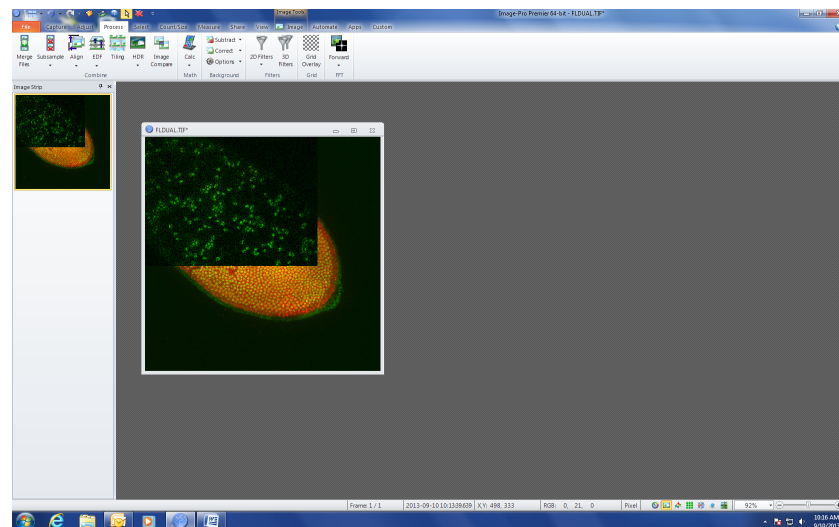


7. Click **Apply**. A new image will be created from the comparison of the two selected images.



The darker areas indicate where the image has changed.

The results appear in a combined image.



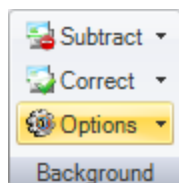
Correcting the Background

Use the *Background Correction* command to make adjustments to the background of your image. This tool is used to better distinguish image background from image objects, making it easier to extract the objects during a counting or measurement operation. The *Background Correction* command can correct uneven background intensities, and compensate for irregularities due to uneven lighting, nonuniform camera response or minor optic imperfections. It might be used to remove evidence of dust on the lens, or to correct for bright spots caused by the light beneath the microscope's stage.

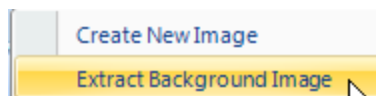
Two types of background correction are provided by *Image-Pro*:

- **Background Subtraction** should be used for all images except those of transmitted light experiments which will be used to measure optical density.
- **Background Corrections** should be used for optical density applications, as it accounts for the fact that optical density is not a linear function of the gray scale.

Image-Pro provides several tools that allow you to adjust the background of your image. They appear in the **Background** group on the *Process* ribbon:



The **Options** allow you to create a new image from the background image.

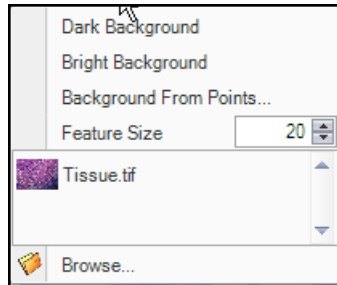


Extract background image displays the background image during black and white Calculation.

To use the **Background Subtraction** command, follow these steps:

1. Open the image that you want to correct.
2. Click the **Subtract** drop-down arrow .

You will see the following dialog:



3. Choose a dark or light background to subtract.

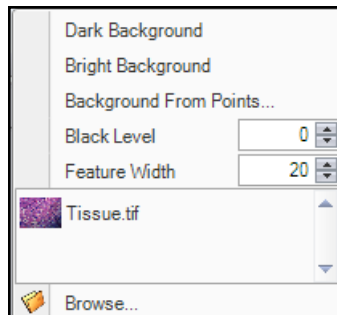
The subtracted background will appear in the workspace.

You may also use the browse button to choose a saved image to work with; or create a background image from existing points in the active image.

To use the **Background Correction** command, follow these steps:

1. Open the image that you want to correct.
2. Click the **Correct** drop-down arrow .

You will see the following dialog:



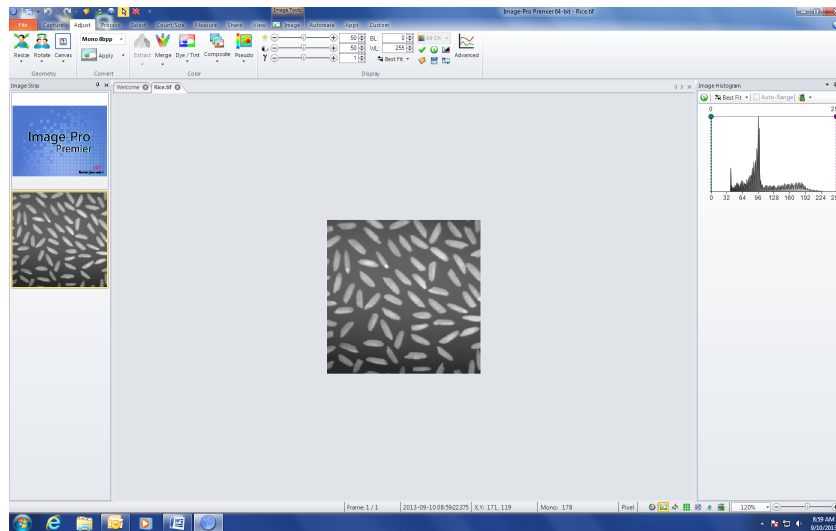
3. Choose a dark or light background for correction, and indicate the feature width or black level. Your corrected image will appear in the workspace.

You may also use the browse button to choose a saved image to work with; or create a background image from existing points in the active image.

Applying Filters

Image-Pro filters are used to emphasize features of an image. The instructions below demonstrate how to apply the **Sharpen** filter to accentuate the edges in the **castiron.tif** image.

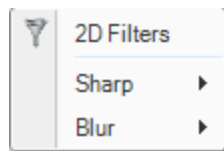
1. Open the **rice.tif** image in the *Image-Pro* workspace, as described earlier in this chapter.



2. Select the *Filters* group on the **Process** tab's ribbon:



3. Choose the **Sharpen** filter from the drop-down list of available 2D filters.



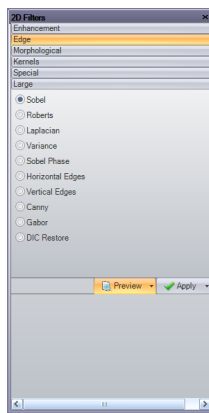
4. Select **Sharpen 50%** from the pop-out menu:



The filter is automatically applied to the image.

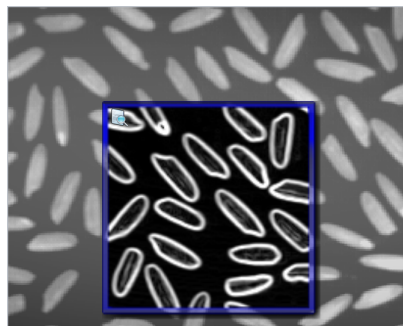
Advanced Filters

The **Sharpen** and **Blur** filters are only two of the 2D filters available in *Image-Pro*. To see the complete set of available filters, click the arrow for the drop-down menu:

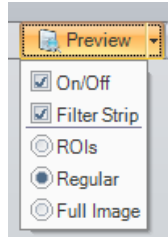


1. Click the radio button next to one of the filters.
2. Click **Preview** to see how it will look on a sample portion of your image.

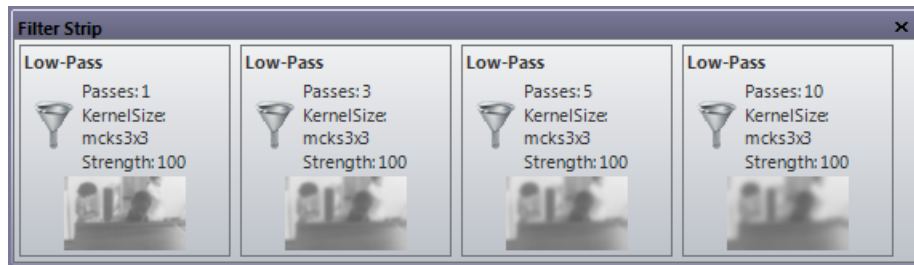
You may try this with different filters to see the various effects. The sample area appears in the center of the image.



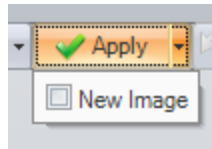
You can also preview the filter in a strip. Click the box filter strip box in the drop-down menu:



The filter strip shows how the image will appear after successive numbers of passes:



3. Click **Apply** to apply the filter to the whole image. To save your filtered image as a new image, check the **New Image** box.

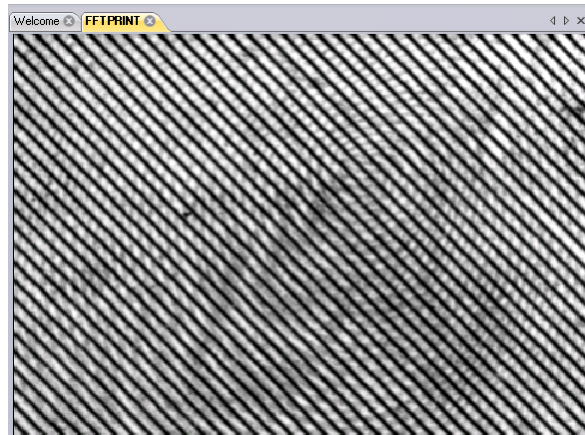


Filtering with Fast Fourier Transforms

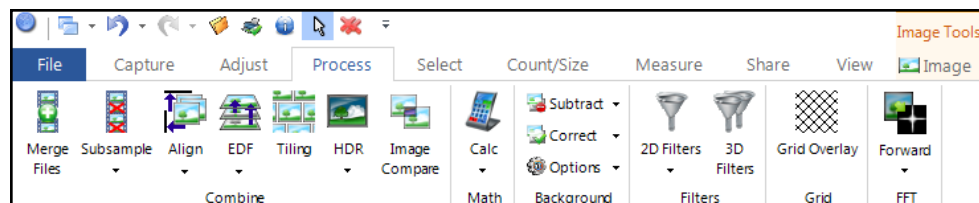
The *FFT* command transforms an image into a frequency spectrum. Image **noise** appears as a repetitive pattern which is difficult to remove using standard spatial-filtering tools. The *FFT* tool is especially designed to isolate and filter this noise, then transform and edit the noise pattern out of the image in easy steps.

1. Load the `Fftprint.tif` file from the *Fast Founier Transform* folder.

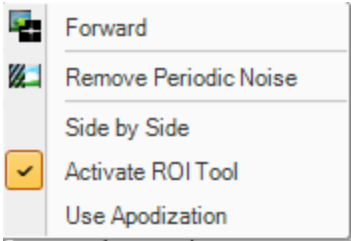
The **Fftprint.tif** image window appears. Notice the strong diagonal noise patterns.



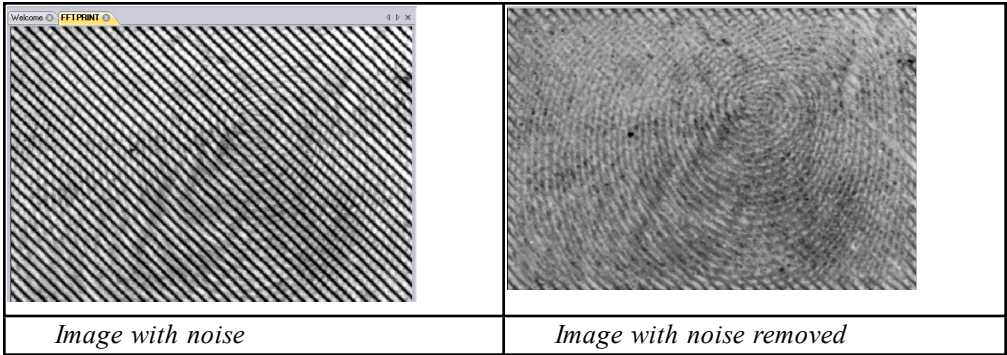
1. Go to the *Process* tab.



2. Choose the *FFT* group.
3. From the drop-down menu, choose **Remove Periodic Noise**.

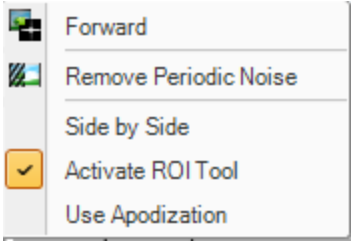


A copy of the original image with the noise removed appears in the workspace.

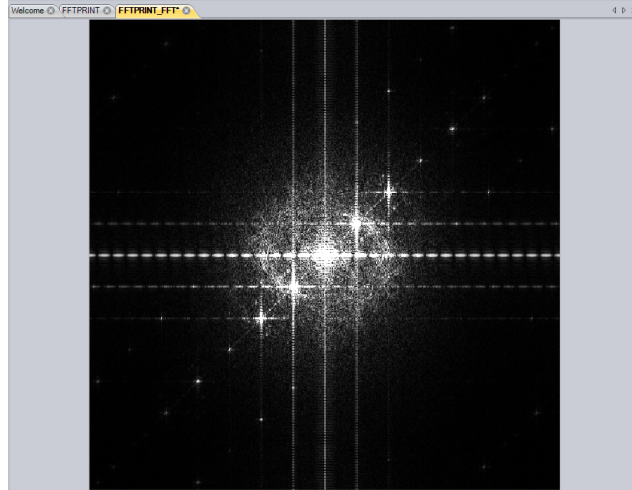


Viewing the Image Spectrum

- 1. To transform the image to its frequency spectrum, click the **Forward** button in the FFT drop-down menu.



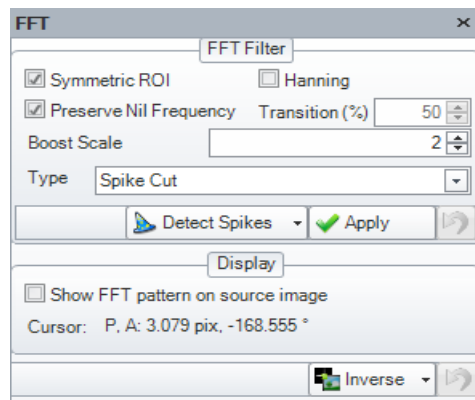
This process may take a few moments. When the transformation process is complete, the **fftprint-fft.tif** containing the spectrum appears in the workspace.



The spectrum appears as a centered cloud of points, with 4 bright points outside of the central point. These 4 points represent the pattern noise you see in the image.

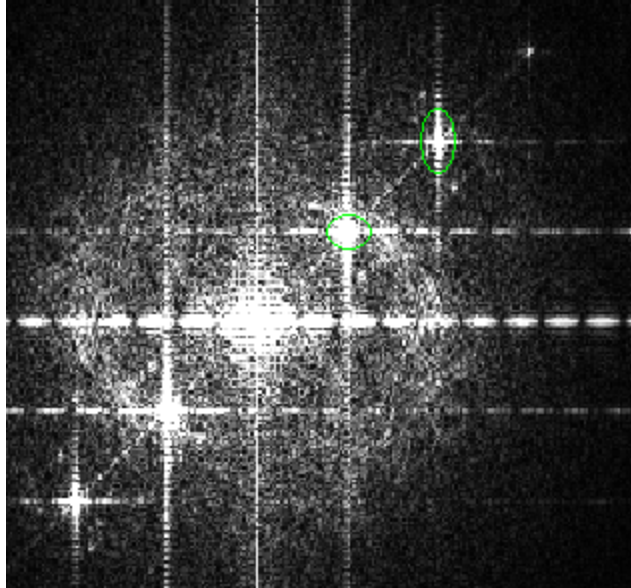
To eliminate the anomalous frequencies (the 4 outside bright points) you can defining an AOI around them and cutt the frequencies from the spectrum.

1. From the *FFT Filter Options* dialog, select **Spike Cut**.



2. Click the **Detect Spikes** button.

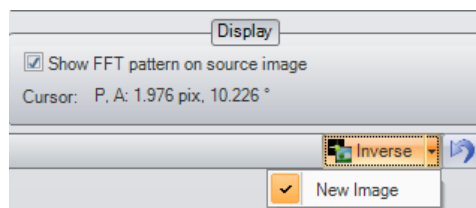
The frequency spikes will be selected in the filtered image:



3. Click the **Apply** button.

The frequency spikes will be removed from the image. The majority of the anomalous frequencies have now been removed from the image.

4. To return the image to spatial form, without the noise, select **New Image** from the **Inverse** drop-down menu:



A copy of the original image, without the noise, appears in the workspace.

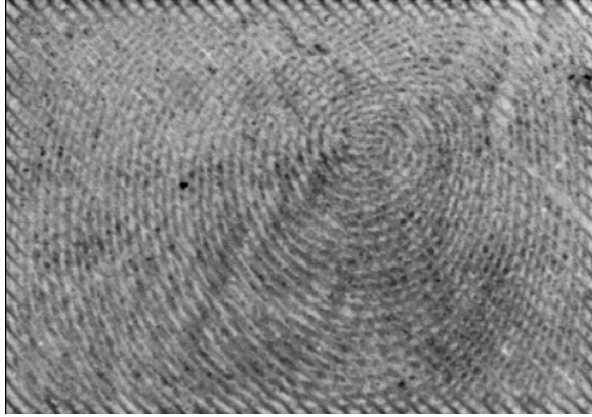
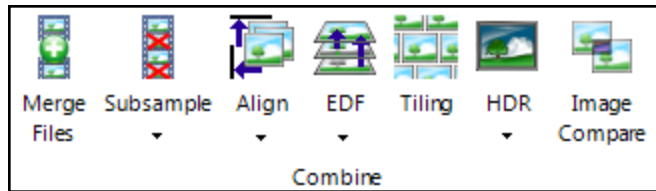
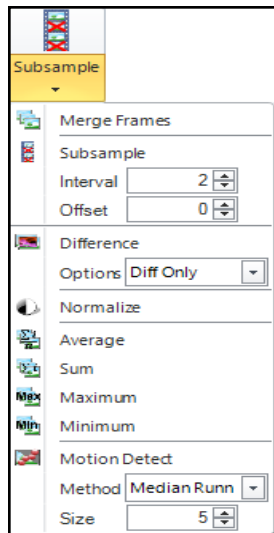


Image Sequences

Image-Pro allows you to open and navigate multi-frame images saved in the .seq format. The sequence viewer is automatically used for images that have more than one frame. The image sequence tools appear in the *Combine* group on the *Process* tab's menu:



To see the image sequence tools, click the drop-down arrow under **Subsample**:



Merge Frames merges the frames in all selected images into a sequence.

Subsample creates a subsampled sequence of selected images.

- The **Interval** control defines the sub-sampling frequency. The active frame range of the sequence is used; the operation always starts from the first frame in the active frame range. When Interval is less than 1, the input sequence will be oversampled.
- **Offset** defines the position of the first frame of subsampled sequence.

Note that all sequence operations work with selected images. Multiple images can be selected in image strip using <Ctrl> or <Shift> clicks. If no multiple images are selected, the active image sequence is used for processing.

Difference calculates frame-to-frame difference in selected images. The *Options* define how the last or first frames will be processed:

Wrap Around calculates the last frame as the difference between the last and first frames

Diff only return only difference frames (result will be one frame shorter than the original)

Pad First pad the result with first frame of no difference

Pad Last pad the result with a last frame of no difference

Normalize normalizes frame intensities on the whole sequence. The function uses the active frame as the reference and scales intensities on all other frames to match the mean intensity of the reference frame. If the image contains ROIs, the sequence is normalized by intensities within ROIs. The function can be used to normalize frame intensities on Z stacks before segmentation (including 3D) and measurements. When the option **By Gain and Offset** is active illumination is normalized by Gain and Offset, when the option is off, only normalization by Gain is used. When the option is On the Gain is calculated based on standard deviation of the intensity, when off - from the Mean value of histogram.

Average calculates the average of all frames in the selected images.

Sum calculates the sum of all frames in the selected images. Note that the output image will be a floating point type with the number of channels corresponding to the source image.

Maximum creates maximum intensity projection of all frames.

Minimum creates minimum intensity projection of all frames.

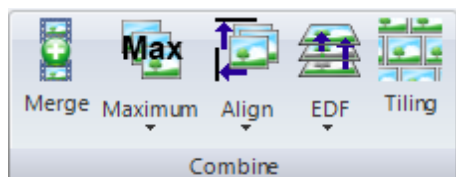
Motion Detect operation will detect only moving objects and extract them to new image, then this image can be used with tracking. There are 3 methods: **Average Global**, **Average Running** and **Median Running**. The operation subtracts filtered image from original, so all static objects get invisible and only moving objects get detected.

The **Size** parameter is used with *Running* algorithms and defines the number of neighboring frames that are used in filtration. When using **Median**, the size has to be bigger than the movement of the object through the given number of frames, basically the object has to cross any point for the given number of frames. For small and fast objects this value can be small, for big and slow the value should be bigger.

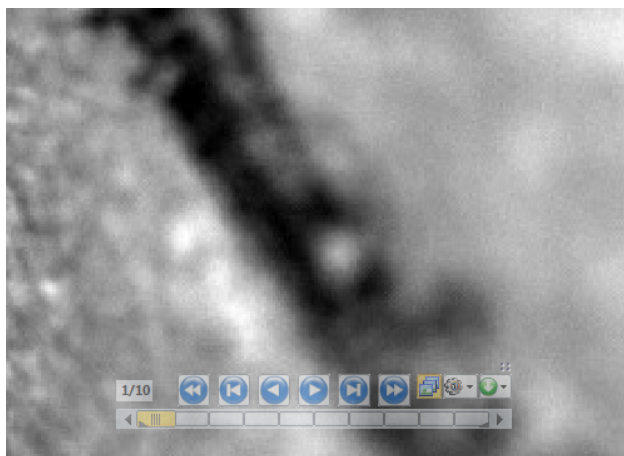
Flip to XZ: Flips sequence to XZ plane, so the new sequence will have Y axis corresponded to Z axis of the original sequence.

Flip to ZY: Flips sequence to ZY plane, so the new sequence will have X axis corresponded to Z axis of the original sequence.

The last selected operation will be set as default for Sequence operations button:



The sequence viewer is automatically used for images that have more than one frame. The sequence viewer has an on-screen toolbar, which appears only when you move your mouse over the image. The example below shows the sequence toolbar superimposed on the bottom of the image:



The toolbar becomes less transparent (more opaque) when the mouse is over the toolbar:



The toolbar has the following buttons:



Go to first frame



Go back one frame



Play sequence backward



Play sequence forward



Go forward one frame



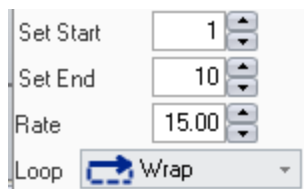
Go to last frame



Use active range applies filters and other Calculation to the active range of frames (on) or to the individual active frame only (off).



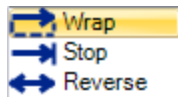
The playback options button opens the following options dialog:



Click **Set Start** or **Set End** to set the current frame as start or end of the active playback range of the sequence. By default the range is set to all frames.

The **Rate** control defines the playback speed in frames per second.

Loop combo-box determines the action taken during playback when the end or beginning of the sequence frame range is passed. Choose one of the following options:



Use this button to click and drag the sequence toolbar

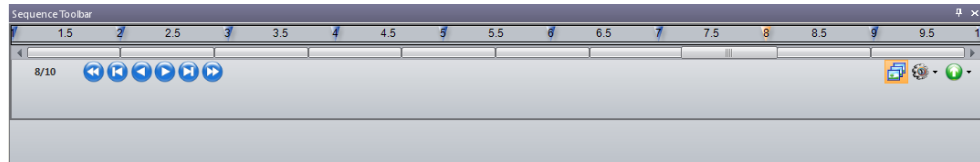


Click this button to move the toolbar to the area above the status bar.



Clicking the button will move the toolbar back to the image.

This button also displays the **Advanced Sequence Control** toolbar.



This version of the toolbar contains all the functions of the smaller version and displays the frame numbers also.

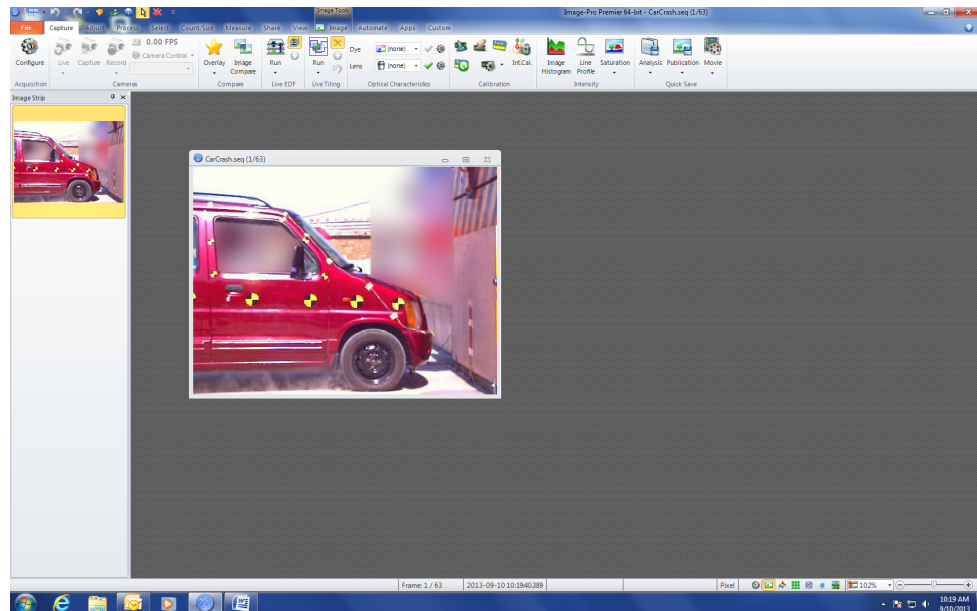
You can also use **<Ctrl>+<mouse click>** and **<Shift>+<mouse click>** to select active frames for processing.

Using the Active Data Overlay

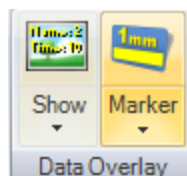
The **Active Data Overlay**, also known as a *heads-up display*, is a method of displaying changing numeric and graphic information over the active image so that you don't need to shift your eyes away from the primary view. The term, "heads-up display" was coined to describe information displayed on windshields, camera view-finders, medical imaging systems, video games, and other presentations where it would be inadvisable if not dangerous to distract the operator from the primary view.

To try out the active data overlay, follow these steps:

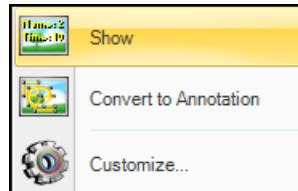
1. Open an image sequence in the *Image-Pro* workspace. Our example will use `car-crash.seq`.



2. Go to the *Data Overlay* group on the **View** ribbon:



3. Click the drop-down arrow under the **Show** button:

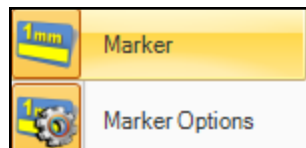


When you click **Show**, the data overlay will appear on the active image. In this example, the overlay data appear on the upper and lower left sides of the active image. A calibration marker appears at the lower right.

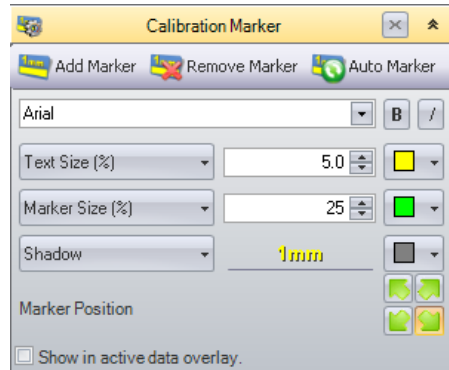


As you play the sequence, the overlay information updates the frame number and time information.

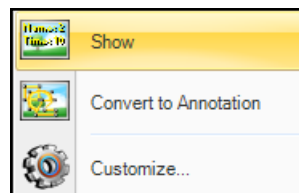
4. (Optional) Clicking **Marker** will turn the calibration marker at the lower right of the image on or off.



Marker Options let you adjust the position and appearance of the calibration marker:

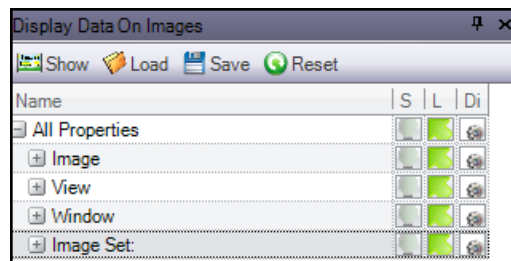


5. Click **Convert to Annotation** to convert the data overlay to an annotation that can be saved with the image.




Customizing the Data Overlay

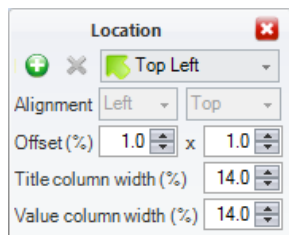
Click the **Customize** button to see the *Data Overlay Options* panel. Here you will find tools to display and adjust their location and style of the overlays.




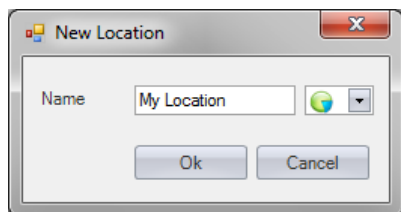
All of the properties can be expanded by clicking on the + sign at the left.

To show or hide an item in the overlay, click the button in the Show column. The button will turn yellow when the item and its sub-items are visible in the overlay. Hold down the <Ctrl> button when you click to prevent toggling of the sub-items.


The item location can be adjusted by clicking the **Location**  button.

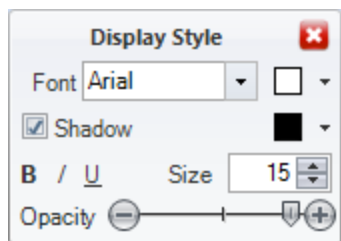


The **Location** drop-down menu contains predefined locations (Top Left, Top Right, Bottom Left, Bottom Right). You can create new locations using the **Add**  button and defining a name and an icon for the new location.






You can adjust the alignment of the location (Left or Right, Top or Bottom). Note that alignment of predefined locations cannot be changed and they cannot be deleted. Offset defines the space between window border and location anchor. Width of both columns (Title and Value) are defined in percents of window width.

Display style is adjusted using the **Display style** button .



You can modify font, shadow and opacity in this dialog. Note that all changes are applied to the current item and all its sub-items, as with the **Show** button..

The layout and attributes of the overlays are saved between sessions. These settings also can be saved and loaded using the **Save**  and **Load**  buttons.

Clicking the **Reset**  button loads defaults.

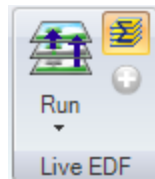
The format of numbers and dates can be set on the *Display* page of the **Application Options**, in the *Formats* group:

A note about measurement overlays: If you are making measurements on a Preview image, and copying these measurements in an overlay to the captured image, you will see the Live Preview Measurement Copy Warning if the captured image is a different size or resolution than the previewed image.

Using Live Extended Depth of Field

Live Extended Depth of Field(EDF) allows you to create real-time EDF images using a microscope with a manual focus control. It also supports stereo microscopes, where changing the focus position shifts the image.

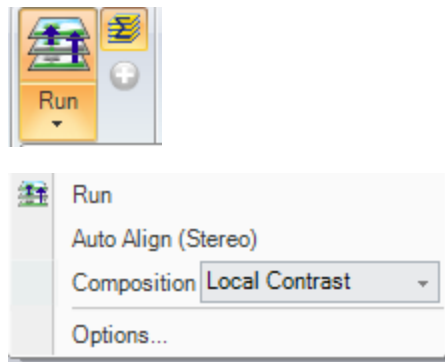
1. To use *Live EDF*, go to the **Capture** tab. You will see the *Live EDF* group:





The **Run** button will be enabled only when a live preview or image sequence is active. Clicking the button will start the EDF acquisition on a live image. live EDF process is active the button background turns orange and the text changes to **Stop**.

Clicking the button again will stop live EDF. If the active image is a sequence the text changes to **Run** and clicking the button will execute an EDF acquisition on all frames of the sequence.

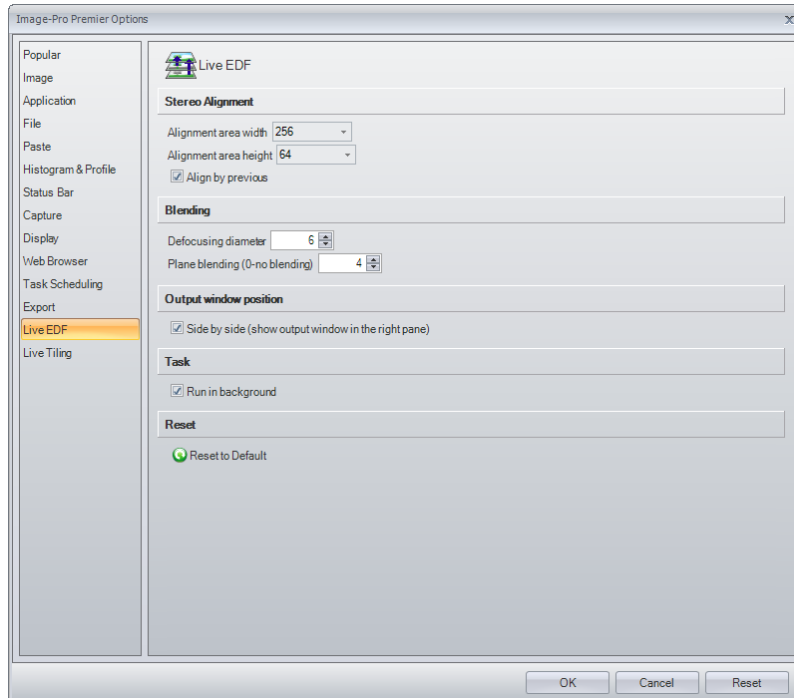
2. Clicking the drop-down arrow displays the Live EDF options:



3. Click the **Run** button to start the Live EDF acquisition
4. Check the **Auto Align (Stereo)** option to enable auto align for every acquired frame. Use this option on stereo microscopes where changing the focus position shifts the image.

When the **Accumulate**  option is on every new live image is added to the composition. This option is preferable for Local contrast composition. In other modes you can switch this option off and add images to a composite manually by clicking the **Add**  button.

5. **Composition Mode:** Make a selection from the pull-down combo box of composition options:
 - **Local contrast:** Choose this option for real time EDF using the Local Contrast algorithm. This is most useful for high contrast brightfield images.
 - **Maximum:** Choose this option for real-time EDF using the maximum composition mode for sample imaging on a dark background. For example, imaging of fluorescent samples.
 - **Minimum:** Choose the minimum composition mode for transparent mode illumination of brightfield images
 - **Difference:** Illustrates the difference between the base image and the current image (image with a grey background)
 - **AbsDifference:** Shows the absolute difference between the base image and the current image. Background is black, but you can also activate Pseudo-color on gray images, so you that can see changing objects in color on a black background.
6. Click the **Options...** button to see the **Live EDF Options** dialog:



Stereo Alignment:

- **Alignment area width/height:** Indicate width and height of the size of the area in the middle of the image, which is used for stereo alignment of images in EDF. If the images have a little texture the area can be extended to achieve better correlation
- **Align by Previous:** This option defines the alignment method, which is used with Auto Align (Stereo) mode. When the option is on, the new image is aligned by the last added to image, then the option is off, the start image is used to align all following images.

Blending:

- Defocusing diameter defines the size of the filter that detects in-focus areas on the image. The default value is 6.
- Plane blending sets the size of the blending area between in-focus zones from different planes. The default value is 4.

Output Window Position

- **Side by side:** When this option is on, the workspace is split into 2 panes, where the live preview window is on the left and the output window is on the right.

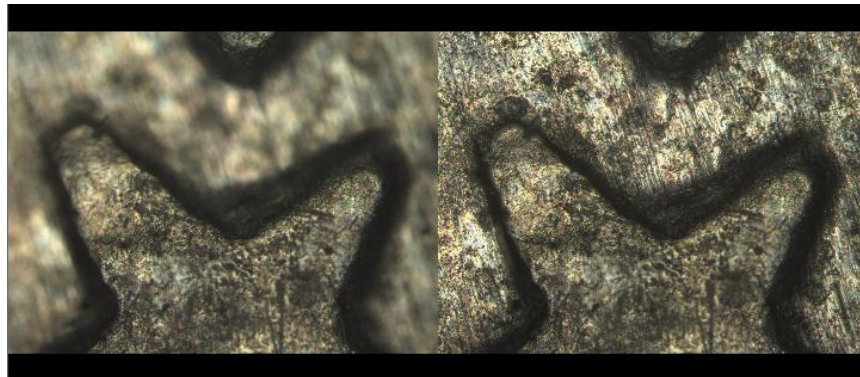
Task

- **Run in background thread:** When the option is on, the Live EDF is started in a separate thread. This option should be used with multi-core processors, so Live EDF will run in parallel to the live preview and not slow down preview frame rate.

Reset

- Click this button to reset the Live EDF settings to the default selections.
7. Click the **Reset** button to restore the defaults for all options.

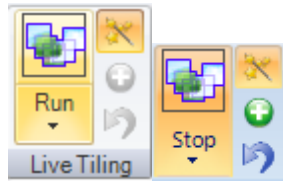
A sample dual-viewed, real-time EDF composite image is shown here:



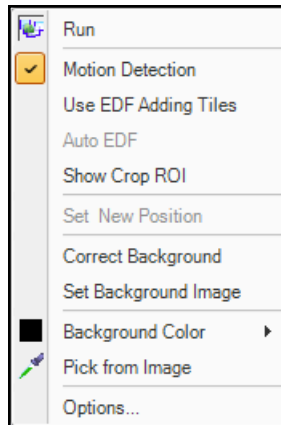
Using Live Tiling

The *Live Tiling* module for *Image-Pro* makes it easy to acquire a large tiled image without an automated microscope stage.

1. To use *Live Tiling*, go to the **Capture tab** on the *Image-Pro* ribbon. You will see the *Live Tiling* group:



2. The **Run** button will be enabled only when a live preview or image sequence is active. Click the button to start tiling on a live image. When the *Live Tiling* process is active, the button background turns orange and the text changes to **Stop**.
3. Click the button again to stop *Live Tiling*. If the active image is a sequence the text changes to **Run** and clicking the button will tile all the image frames in the sequence.
4. Click the drop-down arrow to display the *Live Tiling* options:

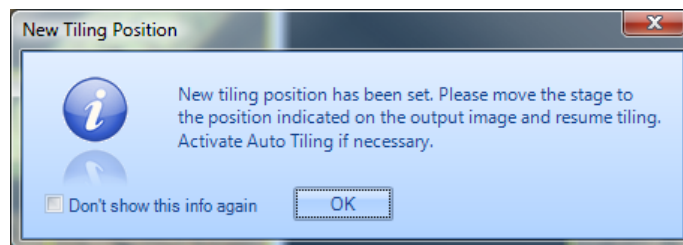


5. Click the **Run** button to start or stop the *Live Tiling*.


To start the tiling procedure, move the microscope to the area of the specimen where you want to start tiling and click the **Run** button. When you click **Run** the current image becomes the starting point for the tiled image. Yellow rectangles will show you the


outline of the original frame when you move objects under the microscope. The tiling uses cross-correlation to find the image shift. Use objects with texture for tiling. Note that empty fields cannot be aligned correctly automatically. When you see that the yellow frame follows the image correctly, you can start tiling. Note that the maximum image shift between slices cannot exceed 25-40%.


- **Motion Detection:** Motion blur is a common problem with capturing images of moving objects. This problem can be eliminated by activating the Motion detection option. Activating this option is recommended for long exposure times. When this option is active, a new tile in auto-tiling mode will be added only when the camera is still.
- **Use EDF Adding Tiles:** If the image contains out-of-focus areas, use the Use EDF Adding Tiles option. When this option is *on*, every new tile is added using the EDF algorithm. The borders of the EDF image are blended with BlendingWidth (see Tiling Options). When the option is *off*, the tiles are added with full overlap blending. The option can be activated temporary to merge several focus planes adding tiles using the Add button.
- **Auto EDF:** When the Auto EDF option is on, the new images are added to the output constantly and clicking the Add button is not necessary. You can simply adjust the focus, and during the adjustment the EDF image will be created. EDF images are not added when the stage is moving.
- **Show Crop ROI:** Shows the outline of any ROI on the image.
- **Set New Position:** Use this button if, in the middle of creating a tiled output, you want to continue from another side of the tiled image. Clicking the button will activate the position selection mode, where you must click on the output image where you want to continue tiling. You will see the following message;

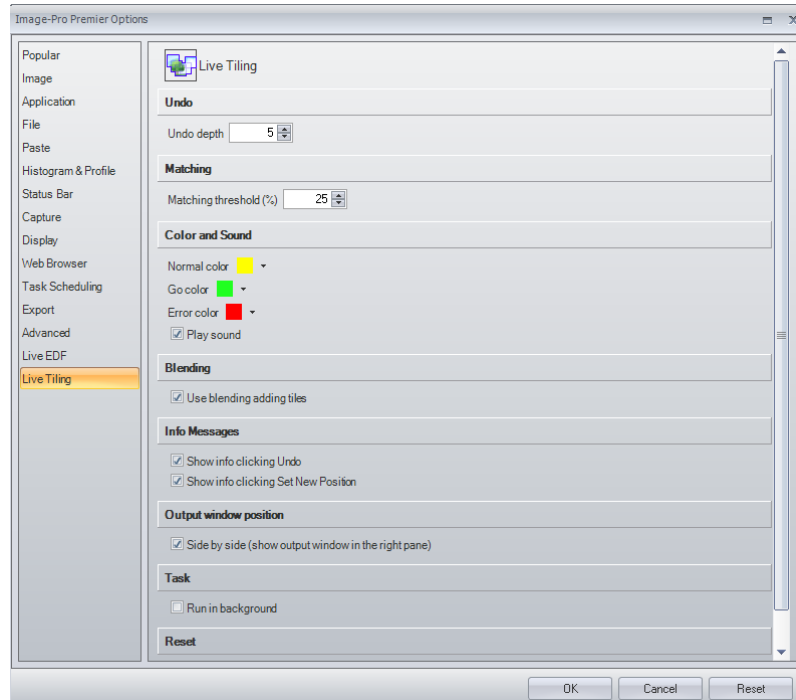


- **Correct Background:** If the microscope has uneven illumination, it can be corrected using this option. Use the empty area on slide as the background, move the slide under microscope, and click the Set backgroundImage button.

- **Background Color:** This option is used for empty areas outside the tiles on an output image. Clicking the Pick from Image button will activate the color picking tool, which you can use to select a color for the image.
 - The easiest way of tiling is with the Auto tiling  option. In that case the images will be added to the output automatically when the image shift exceeds 25% (when an internal yellow rectangle touches the image border). Move the stage slowly, and the images will be acquired and tiled automatically.
6. Move the object by 30-35% from the original position (when the inner rectangle will touch image border) and stop the stage. The image will be automatically captured when the object will stay still for 2 frames. It will eliminate motion blur and improve the quality of tiled image.
 7. Repeat step 2 (move-stop) until whole area is tiled.

The tiles can be also added manually using the Add  button. If the tiled position is not correct you can use the Undo button to remove the last tile and try other location. Note that if you use Auto tiling the Add button will be switched off.

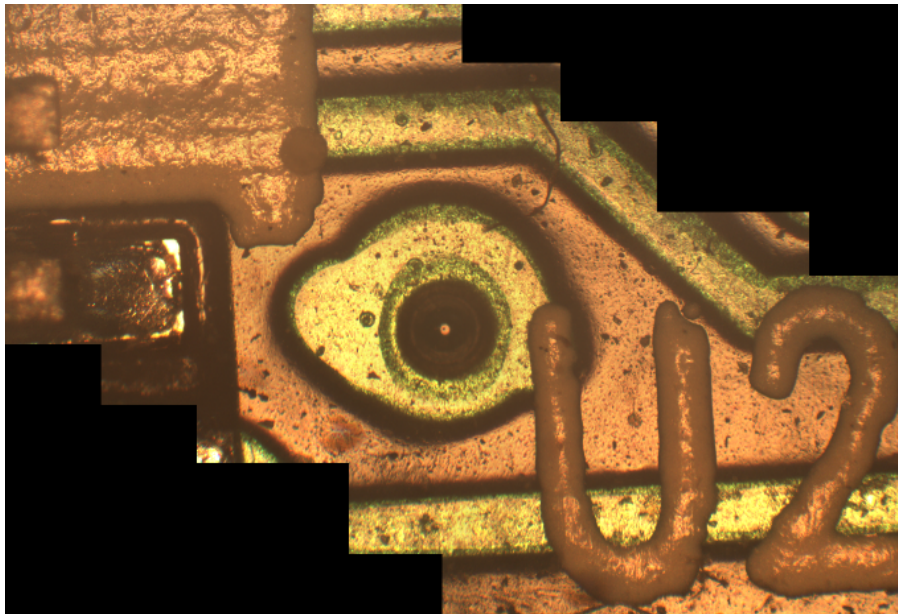
8. To use the Add feature, click Undo  and move microscope to the location when correlation is stable. Then activate Auto tiling option again.
9. When the tiling is done, click the **Stop** button. It will stop the output and any blank areas will be filled with the **background color**.
10. Click the **Options...** button to open the *Live Tiling Options* dialog:



- **Tiling Undo depth:** This option defines the number of undo buffers.
- **MatchingThreshold:** Use the matching threshold for new images. The matching threshold is within 0...1 range, and the default value is 0.25. If the image correlation is below the threshold, the tile is not automatically added, although it can be added using the **Add** button.
- **Color and Sound.** Select the **Normal color**, **Go color**, and **Error color**: These options define the colors use for the tracking box for normal, active, and error states. You may also indicate if you want to play a sound when an error is detected.
- **Blending:** When the **Use blending adding tiles** option is on (default) new tiles are added using gradient blending, so no edges between tiles is visible.
- **Info Messages:** Check these boxes to see helpful messages when clicking Undo or Set New Position.
- **Output Window Position:Side by side:** When this option is on, the workspace is split into 2 panes, where the live preview window is on the left and the output

window is on the right.

- Task: When Run in background thread is on, the *Live Tiling* is started in a separate thread. This option should be used with multi-core processors, so *Live Tiling* will run in parallel to *Live Preview* and not slow down the preview frame rate.
- Reset: Click the Reset button to restore the defaults to all options. Here is an example of a tiled image:



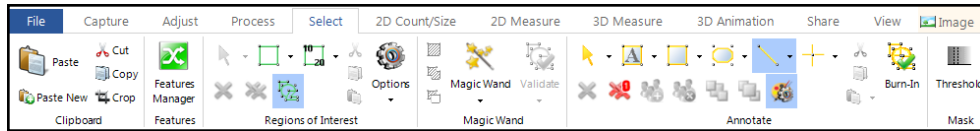
Chapter 6

Using Regions of Interest

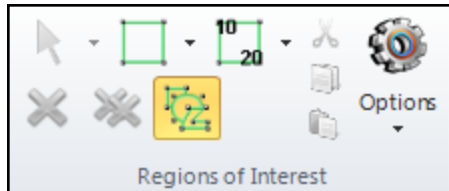
You can define one or more *Regions of Interest (ROIs)* in a variety of shapes, and you can control the positioning, size, appearance and display status of ROIs. This chapter provides step-by-step instructions for defining and working with ROIs to isolate areas of interest in your images.

The Select Tab

The **Select** tab's ribbon contains those functions that allow you to mark up an image with regions of interest or annotations, and share it with another person or work group.



You can find the ROI feature on the **Select** tab's ribbon:



If you are working with *Image-Pro with 3D Module*, this tab is called **2D Selection**.

What is an ROI?

An ROI is a *Region of Interest* that is isolated from the rest of the image. Certain *Image-Pro* commands can be constrained by an ROI. Once you have defined an ROI, the command applies to only the pixels within the ROI.

Examples of commands that are constrained by an ROI are: Save As, Cut, Copy, Duplicate, Rotate, and Resize.

For example, in Figure 6-1 below, a square-shaped ROI has been drawn around a particular object of interest in the image. When the Duplicate command is applied to the image, the ROI portion of the image is copied to a new image, as shown in Figure 6-2.

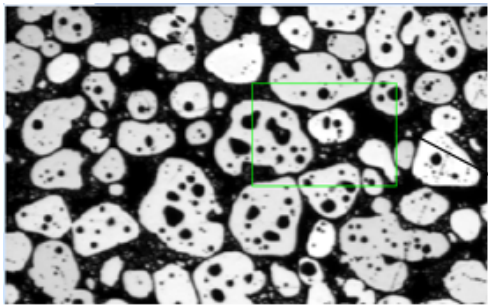


Figure 6-1

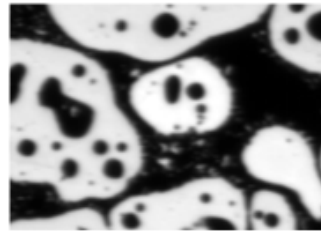


Figure 6-2

You can also apply a filter to an ROI. Figure 6-3 shows an image with an ROI drawn on it, and a Sharpen filter applied to the area defined by the ROI:

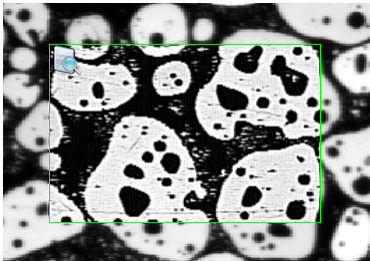


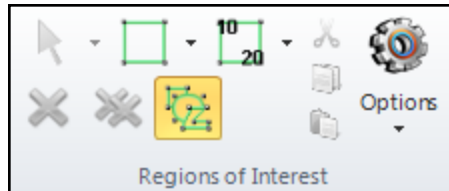
Figure 6-3

More information about applying filters appears in Chapter 5.

Defining an ROI

Before defining an ROI, be sure the image you want to work with is opened in the *Image-Pro* workspace. To define an ROI in the active image:

1. Go to the *ROI* group on the **Select** tab's ribbon:



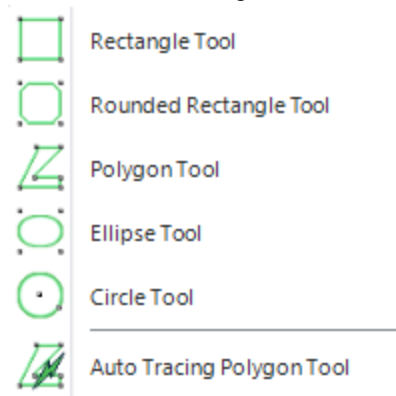
2. Click on the ROI tool you want to use. Clicking this button starts the drawing of this type of ROI. The different ROI tools and types are described below.



Selection Tool: Use this arrow to select an ROI on the image. Click the drop-down arrow and use the **Select All** tool to select more than one ROI.

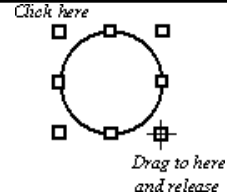


Rectangle ROI: This tool allows you to define a rectangular or square ROI. Click and drag the mouse from one corner of the Region of Interest to the opposite corner. To force a perfect square, hold down the <Shift> key as you click and drag the mouse. Click the drop-down arrow to see additional ROI shapes.





Circle or Ellipse: This tool allows you to define a circular or elliptical ROI in the image. To force a perfect circle, hold down the <Shift> key as you click and drag the mouse.



Rectangle by Numbers: This tool allows you to define a rectangular ROI by entering the left, right, top, and bottom locations.

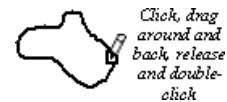
New ROI by Numbers	
Left:	50
Top:	10
Right:	51
Bottom:	100



Polygon: This tool allows you define a polygon-shaped or freeform ROI in the image.

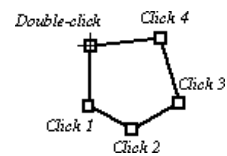
To define a freeform ROI

Use this technique to create smooth edges. Hold the left mouse down while you draw with the cursor to create the desired free-form shape. Double-click to complete the shape.



To define a polygon-shaped ROI

Use this technique to create polygons that are made up of straight line segments. Click the left mouse button at each vertex (including the beginning point) of the polygon. Double-click to complete the polygon.



Auto-Trace. Use this tool to trace the outline of an irregular object in the image automatically. Place your cursor on the object and click two

points. *Image-Pro* automatically traces the object.



Display Options

Use this button to see the *Display Options* panel.



Delete Selected/Delete All:

Use the buttons to remove one or more of the ROIs from your image.

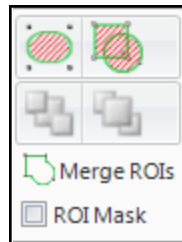


Multiple ROIs: Click this button to place multiple ROIs on your image. This option is always on by default.



Options

Use this button to see some additional ROI options:



Invert ROI: Click this button to invert the ROI; for example, a black ROI on a white background.



XOR Pixels: Click this button to include or exclude pixels in overlapping ROIs. This option is always *on* by default.





.Order: Use these buttons to arrange your ROIs from back to front, or front to back.

Arranging ROIs

After you have defined an ROI, you can move it, resize it, and delete it.




Moving an ROI

To move an ROI:

1. Click the **Selection** tool [] from the ROI group.
Click on the ROI you want to move. The ROI is selected.
2. Position the cursor over the selected ROI.
If the cursor is positioned correctly, the **Dragging** tool [] appears.
3. Click and drag the ROI to the desired location.

Resizing an ROI

ROIs can be resized by moving their control points. To resize an ROI:

1. Click the **Selection** tool [] from the ROI group.
2. Click on the ROI you want to resize.
The selected ROI is selected.
3. Position the cursor over the control point that you want to move.
If the cursor is positioned correctly, the **Resize** tool [ or ] appears over the control point.
4. Click and drag the control point to the desired location.

Using XOR Pixels

Use this feature to examine pixels in overlapping ROIs on your image. To apply a filter using XOR pixels, follow these steps:

1. Draw two or more ROIs on your image, as shown here:

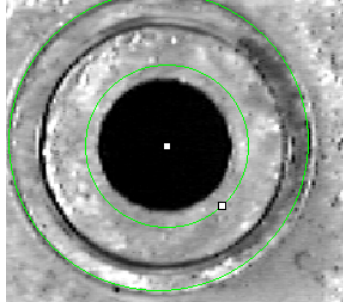
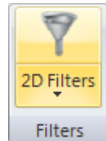
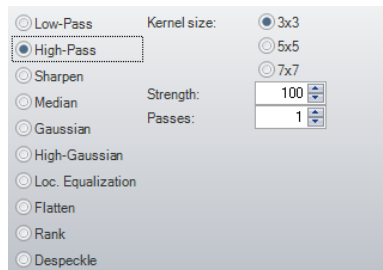


Figure 6-4

2. Go to the **Adjust** tab and select the *Filters* group. Click the drop-down arrow.



3. Choose a filter from the filters list:



4. Your filter will be applied to the area between the flags on the image.

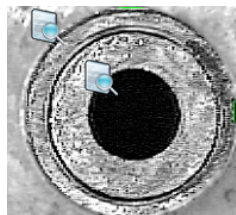
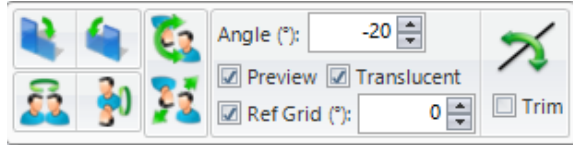



Figure 6-5



Rotating an ROI

You can rotate a selected ROI without

rotating the image. The Rotate tools are shown here:

1. Click the **Selection** tool [] from the ROI group.
2. Click on the ROI you want to resize.
The selected ROI is selected.
3. Position the cursor over the center point in the ROI that you want to rotate. You will see the rotation cursor.
4. Click and drag to rotate the ROI.

Improvements to the ROI controls allow you to rotate the ROI with or without trimming and place it a new image. When the **Trim** box is checked, the rotated size of the ROI is the same size as the original ROI bounds, which usually requires trimming the rotated ROI. The icons displayed are:

-  Rotate with trim into a new image
-  Rotate without trim into a new image.

An example appears here:

The below shows a translucent preview of a -20 degree rotation with the **Trim** option *unchecked*. The green rectangle is the ROI to be rotated. The red rectangle shows the rotated ROI. The yellow rectangle shows the bounds of the new, rotated result image.

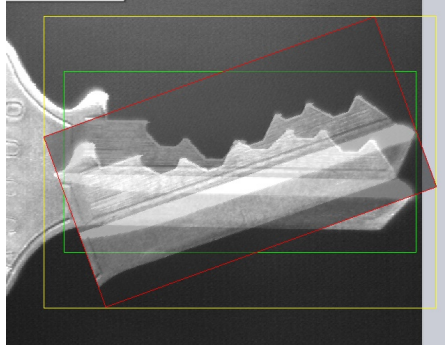


Figure 6-6

The rotated result is shown in the following image. Note that no part of the rotated ROI has been trimmed off, but as a consequence the new image is larger than the source ROI. Also note that areas outside the source ROI are shown as black in the new image.

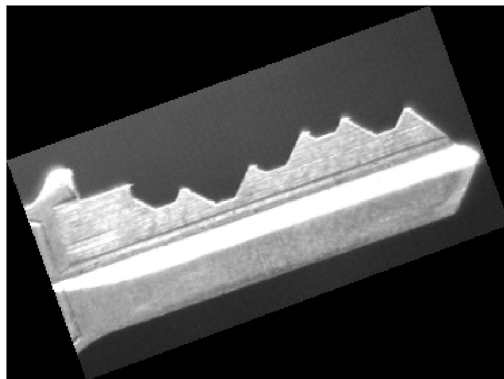


Figure 6-7

The next example shows a translucent preview of the same -20 degree rotation with the Trim option checked. The green ROI rectangle is not visible here, because the yellow rectangle showing the bounds of the new image lies exactly on top of it (this would not be case if the ROI were a non-rectangle, as in the next example). The red, cut-off rectangle shows the rotated ROI with its trim.

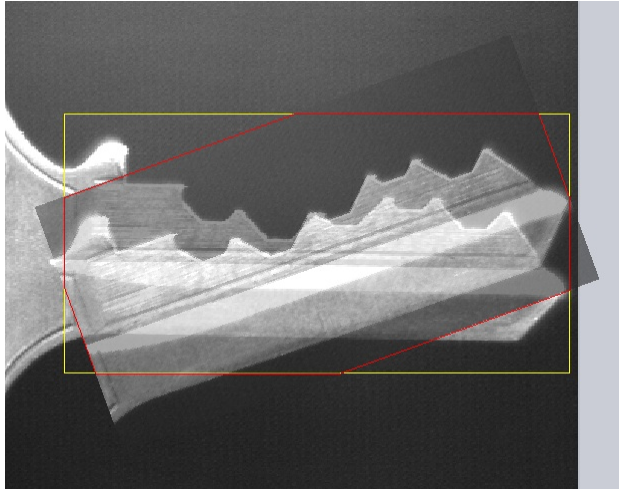


Figure 6-8

The rotated result is in the following image. Note that part of the rotated ROI has been trimmed off in order to keep the new image the same size as the bounds of the source ROI.

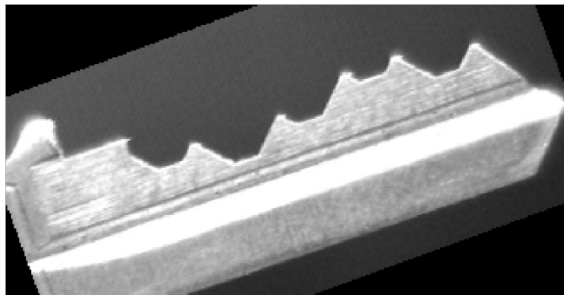




Figure 6-9

Deleting an ROI

To delete an ROI:

1. Click the **Selection** tool [] from the ROI group.
2. Click on the ROI you want to delete.
3. Click the **Delete** [] button or Press the <**Delete**> key.

Note that you can also use the delete button in the image context menu (click the right mouse button on the image when an ROI is selected) to delete the selected ROI.


TIP: You can use the Delete All tool in the ROI group to instantly remove all ROIs defined in the active image.

Setting the Appearance of ROIs


These instructions explain how to change the appearance of an existing ROI and how to set the default appearance attributes for the different ROI types.

Changing the Appearance of an ROI

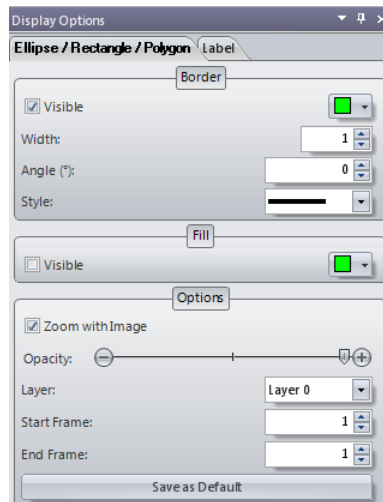
Sometimes you might want to change the border or fill color or size of your ROI to make it easier to see on your image. To change the appearance of an ROI:

1. Click the **Selection** tool [] from the *ROI* group.
2. Click on the ROI that you want to change.

The ROI is highlighted.

3. Click the **Display Options** button  in the *ROI* group.

You will see the *Display Options* dialog box in the panel along the right side of the workspace.



4. Use the controls available through this dialog box to change the appearance of the ROI.

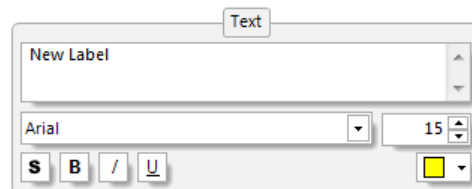
Drawing Properties




Some common controls available for changing the appearance of graphical annotations are described below.

- **Border:** Allows you to change the visibility, color, width, and line style of a border around your annotation.
- **Fill:** Allows you to choose the fill color for your annotation, including opacity and/or transparency.
- **Options:** These options allow you to change the size of the annotation when the size of the images changes, adjust the opacity/transparency of the annotation, and layer annotations on the image. If you are working with an image sequence, you can specify the first and last frames where the annotation will appear.
- **Color:** Click on the **Color** button to change the color of the bounding box border or fill, or to change the color of the text.
- **Zoom with Image:** Check this box to make the annotation larger or smaller as the image is resized.

Label Properties

Some common controls available for changing the appearance of label annotations are described below.




- **Text:** Click on the **Text** tab to change the text of the annotation.
- **Font** : Allows you to change the font to be used for rendering text.
- **Size** : Allows you to change the font size to be used for rendering text.
- **S** **B** **I** **U** : Allows you to make the text shadowed, bold, italics, underlined, or shadowed.

5. When you have made the desired changes, click the *ROI Display Options* panel.


The changes are applied to the selected ROI.

Setting the Default Appearance for ROIs

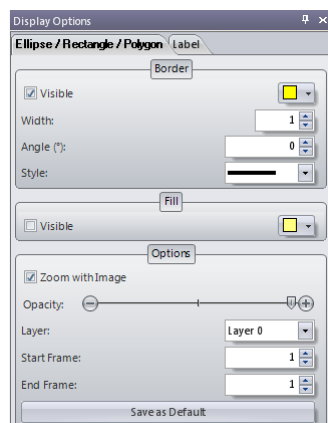
The default appearance attributes for ROIs are set by designating the appearance attributes of an existing ROI as the default for future ROIs of that type. Note that defaults exist independently for each type of ROI you can draw: rectangle, rounded rectangle, ellipse, polygon, point, and text. To set the default appearance for an ROI type:

1. Click the **Selection** tool  from the *ROI* group.
2. In the active image, click on an ROI of the type for which you want to set appearance defaults.

For example, if you want to set the default appearance for ellipse-type ROIs, click on an ellipse-type ROI in the active image. If one doesn't exist, draw one.

3. Click the **Display Options** button  in the ROI group.

You will see the **Display Options** panel along the right side of the workspace.

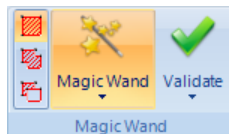


4. Click **Save as Default**

The default appearance values are set. Future ROIs of that type will appear as the selected ROI.


Using the Magic Wand


The **Magic Wand** tool allows you to select regions in an image based on the color of pixels in the image. You can use it to select irregular regions and convert them into ROIs, Measurements, or Annotations. The **Magic Wand** tool appears on the *Select* tab's ribbon:




The *Magic Wand* group includes the following features:

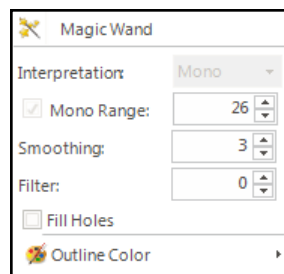
- **Options**

 *New Selection.* All previous selections will be replaced with a new selection. Hold down the <Alt> key to temporarily activate this option.

 *Add Selections.* A new selection will be added or merged with any previous selections. Hold down the <Ctrl> key to temporarily activate this option.

 *Subtract Selection.* A new selection will be subtracted from any previous selections. Hold down the <Shift> key to temporarily activate this option.

- **Magic Wand button**



Interpretation (Mono, RGB, HSL or HSI). Converts the image into a different interpretation before thresholding (not available for mono images).

Mono Ranges. Indicates the number of gray levels used for thresholding (combination of gray ranges depends from type of active image and selected interpretation).

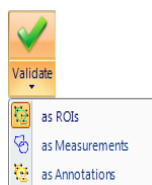
Filtering: Indicates the intensity of any filter to be applied to the selected outline.

Smoothing. Indicates the degree of post-filtering of the outline (0 – no filtering, 9 – high degree smoothing).

Fill Holes: Check this box to automatically fill any holes in the outline and include the hole area in your measurements.

Outline color. Allows you to change the color of the object's outline using the color selector.

- **Validate button**



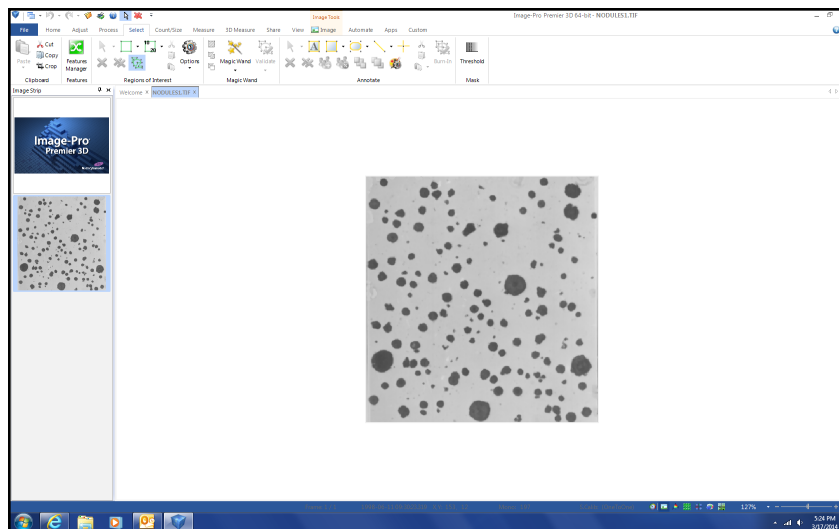
as ROI. Converts and validates the selections as ROIs.

as Measurement: Converts and validates the selections as measurements.

as Annotations: Converts and validates the selections as annotations.

To use the magic wand, follow these steps:

1. Open an image with irregular areas in the workspace:



2. Select the **Magic Wand** tool.
-

3. Place the wand cursor inside the feature containing the area(s) that you want to trace, and click the left mouse button once.

The magic wand will automatically trace the outline of the object(s) based on the color similarities or difference of intensity ranges between the pixel under your cursor, plus or minus a specified tolerance interval.

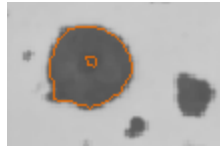


Figure 6-10

To outline a different region, place the cursor on that region and click with the left mouse button. To outline more than one region, hold the <Ctrl> key down while clicking on the region you want to outline.

4. To save the outline areas as irregular ROIs, click **Validate as ROI**.

The outline color changes, as shown here:

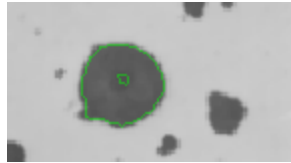
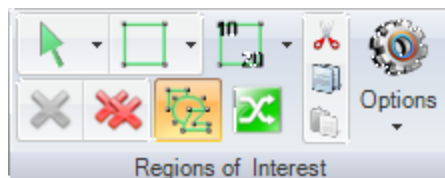


Figure 6-11

The *Regions of Interest* group becomes active, and you can now use all the ROI features to modify or save this ROI.




Polygon Nudge Tool

The **Polygon Nudge** tool allows you to adjust the size and shape of irregular ROIs and measurements.

The nudging technique for reshaping polygons and polylines allows you to push control points around, rather than clicking and dragging them. This technique makes it easy to contour curves in curvy polyforms by 'nudging' the control points into place.

To use nudging mode:

1. Enable nudging mode by right-clicking the mouse in the image window, and clicking on the [] tool in the popup context menu.



Initially the nudge tool will be a dashed circle and has no effect on the selected object.

2. Use the mouse wheel to adjust the size of the nudge tool

The size of the nudge tool determines how many controls points it can nudge at one time.

3. Press the mouse button and move the nudge tool into the control points you want to nudge.

Notice that, when you press the mouse button, the circle becomes solid and shows an arrow tracking the direction of mouse movement, and the nudge follows the direction of the mouse movement. The nudge tool is designed to operate only at slow speeds. So by moving quickly, you can skip the tool from one side of a polyline to the other or from the exterior to interior of a polygon without needing to let up the mouse button and without disturbing the boundary as it is quickly crossed. This may take some practice to master, but it is often necessary to nudge from both sides of a poly line section to get a boundary placed correctly, so using this technique can speed things up.

Holding down the <Ctrl> key while editing a polygon or polyline temporarily leaves nudge editing mode to allow you to insert (<Ctrl> double-click) or move (<Ctrl> drag) a single control point.

4. To turn nudge mode off, press the space bar.

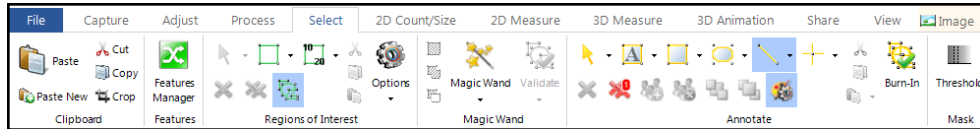
Chapter 7

Using Annotations

Annotations are used to add text or drawings to your images. The annotations are in a graphics overlay that is displayed over the image without modifying the image data itself. This chapter provides instructions for creating text and graphical annotations, arranging them, and saving them with your images.

The Select Tab

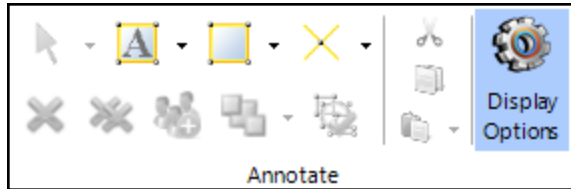
The **Select tab**'s ribbon contains those functions that allow you to annotate an image and share it with another person or work group. The functions in this chapter can be found in the **Select** ribbon's *Annotate* group.



If you are working with *Image-Pro with 3D Module*, this tab is called **2D Selection**.

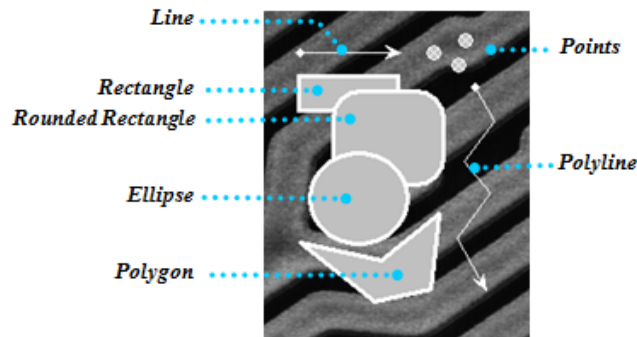
Creating an Annotation

Using the tools on the *Annotate* group, you can draw an array of graphical and textual annotations. This section provides instructions for drawing various shapes and for placing text overlays in images. The *Annotate* group looks like this:



Creating a Graphical Annotation

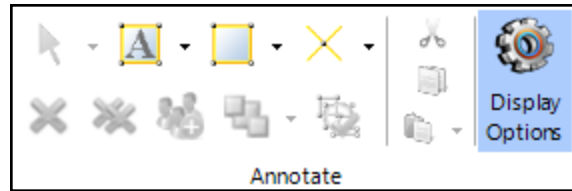
There are a number of options for annotating your images with graphical overlays. You can define points, lines, rectangles, rounded rectangles, ellipses, polygons, and polylines.



To define a graphical annotation:

1. Click on the *Annotate* group in the **Select** tab's ribbon.

The *Annotate* group contains these tools:

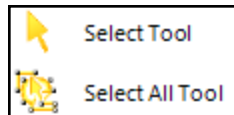


2. In the toolbar, click on the annotation tool you want to use, and draw the annotation in the image as described for that tool below.



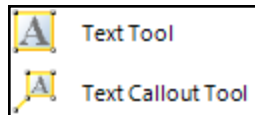
Select/ Select All

Use this tool to select an annotation object. Click the drop-down arrow to see the **Select All** tool to select a group of objects.



Text Box

Use this tool to add a text box annotation. Click the drop-down arrow to add a text call-out annotation:

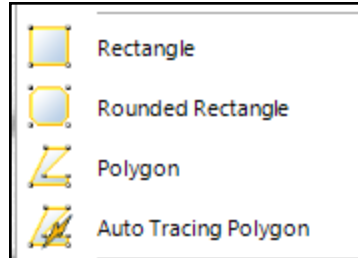


You can add a callout line using the callout tool.



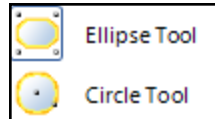
Rectangle or Rounded Rectangle

Click the left mouse button and hold it down to drag the rectangle to your desired size. Use the drop-down arrow to see the rounded rectangle, circle, and polygon tools.



Circle or Ellipse

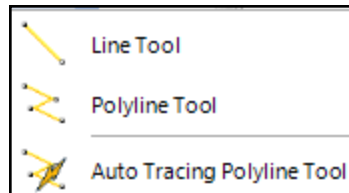
Position the crosshair cursor anywhere in the image. Use the drop-down arrow to see the other tools.



Click and drag the mouse from the insertion point to the desired destination.

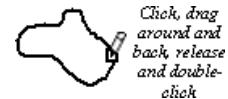


Line or Polyline: This tool allows you draw poly-vertices lines and polygons, or freehand lines and shapes.

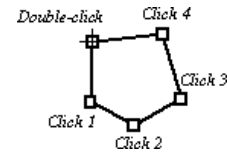


*Note: Whether this tool draws a polygon or a poly-line depends on the current setting for the **Connect Start/End** option. To change the status of this option, simply right-click the mouse over the object after it is drawn, and select Annotation|Object Properties.... The **Connect Start/End** option appears on the **Polygon** tab of the Graphic Object Properties dialog box.*

To draw freeform lines and shapes



Use this technique to create lines and shapes that have smooth edges. Hold the left mouse down while you draw with the cursor to create the desired freeform shape. Double-click to complete the shape.



To draw polyvertices lines and polygons

Use this technique to create lines and shapes that are made up of straight line segments. Click the left mouse button at each vertex (including the beginning point) of the polygon. Double-click to complete the polygon.



Auto-Trace. Use this tool to trace the outline of an irregular object in the image automatically. Place your cursor on the object and click two points. *Image-Pro* automatically traces the object.



Point Position the crosshair cursor in the image where you want to place the point marker. Click the mouse to place the point. The drop-down menu for the point tool displays a wide variety of point and arrow styles.



Delete Selected/Delete All



Use the buttons to remove one or more of the annotations from your image.



Group/Ungroup

Use these buttons to collect your annotations into a group or to ungroup them.



Order

Use these buttons to order your annotations front to back or back to front



Cut, Copy, Paste

Use these tools to cut, copy, or paste annotations.



Properties

Use this button to see the Display Options information.

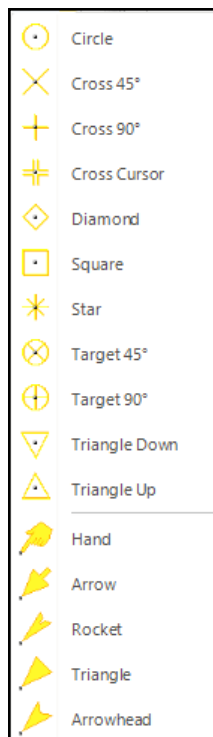


Burn In

Click this button to make your annotation a permanent part of the image.

Annotation Shapes

The drop-down menu for the point tool displays a wide variety of point and arrow styles:



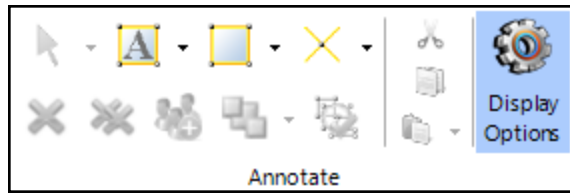
You can use your mouse wheel to adjust the size of your annotations.


Creating a Text Annotation

To create a text annotation:

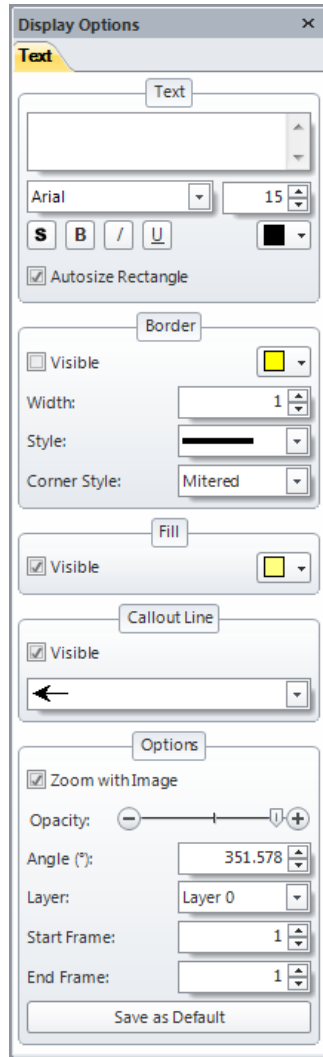
1. Click on **Annotate**

The *Annotate group* is displayed.



2. In the toolbar, click on the **Text** tool [].
3. Click and drag the mouse in the image to draw a bounding box for the text.

When you release the mouse, the **Text** tab of the *Display Options* dialog box is displayed. An examples appears on the next page.



4. Type the desired text in the **Text** tab's textbox.
5. Click **OK**.

The specified text appears as an overlay in the image. For instructions on changing the font, color, or bounding box attributes of the text annotation, please refer to “Setting the Appearance of Annotations” later in this chapter.

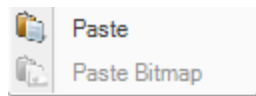
Inserting a Bitmap Annotation


To insert a bitmap annotation:

1. Open the desired bitmap image in the *Image-Pro* workspace, that is, open the bitmap image you want to use as an annotation. (See “Opening an Image” in Chapter 4, “Working with Images.”)

The image appears in the application workspace.

2. Choose **Paste Bitmap** from the drop-down menu in the Cut, Copy, and Paste group.



3. Right-click the mouse in the image to which you want the bitmap added as an annotation.
4. Click the **Paste** button [].


The bitmap is added as an annotation to the image. For instructions on moving the bitmap annotation, please refer to “Moving an Annotation” later in this chapter.

Arranging Annotations

After you have created a set of annotations, you can move them, resize them, layer them, and delete them.


Moving an Annotation

To move an annotation:

1. Click **Select** [- 2. Click on the annotation you want to move.

The annotation is selected.

3. Position the cursor over the selected annotation.


If the cursor is positioned correctly, the **Dragging** tool [

4. Click and drag the annotation to the desired location.

TIP: You can also move the annotation by pressing the shift key along with the arrow keys (up/down, right/left). For example, press the <Shift> and <Up Arrow> keys and notice the annotation moving upwards.

Rotating an Annotation


Annotations can be rotated by using the Select tool and the <Control> key.



1. Click **Select** [- 2. Click on the Annotation you want to rotate.
The Annotation is highlighted.
- 3. While the Annotation is selected, move your cursor to the center point of the Annotation. The rotate cursor (a double-ended arrow) will appear, enabling you to rotate the Annotation either clockwise or counter-clockwise. Holding down the <Shift> key while rotating an Annotation will move the Annotation in 15-degree increments.

You can also rotate an Annotation while creating or editing it by holding down the <Control> key as you move the Annotation.

Resizing/Reshaping an Annotation

Annotations can be resized and reshaped by moving their control points. To resize or reshape an annotation:


1. Click **Select** [- 2. Click on the Annotation you want to resize or reshape.
The Annotation is highlighted.
- 3. Position the cursor over the control point that you want to move.

If the cursor is positioned correctly, the **Resize** tool [ or ] appears over the control point.

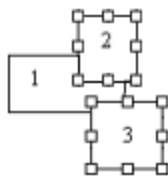
4. Click and drag the control point to the desired location.

Grouping Annotations

The Annotation feature allows you to group annotations in the image. Annotations that are grouped are fixed in positioning relative to each other and they are otherwise treated as one object. To group annotations:

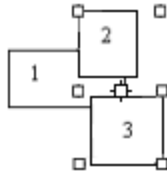
1. Click **Select** [- 2. While holding the <Ctrl> key down on the keyboard, click on the annotations you want to include in the group.

For example, annotations #2 and #3 have been selected below.




3. Click on the **Group** button [

The selected annotations are grouped.

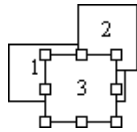


Ordering Annotations

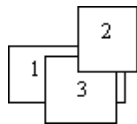
The Annotation feature allows you to control the order in which annotations appear. To change the order in which an annotation appears:

1. Click **Select** [] in the Annotate group.
2. Click on the annotation you want to relocate in the order.

For example, annotation #3 has been selected below.




3. Click the Order back to front  button in the Annotate group.



The annotations are arranged in the order specified.

Deleting an Annotation

To delete an annotation:

1. Click **Select** [] from the Annotate group.
2. Click on the annotation that you want to delete.

The annotation is highlighted.



3. Click **Delete** [Annotate group, or press the <Delete> key.

Setting the Appearance of Annotations

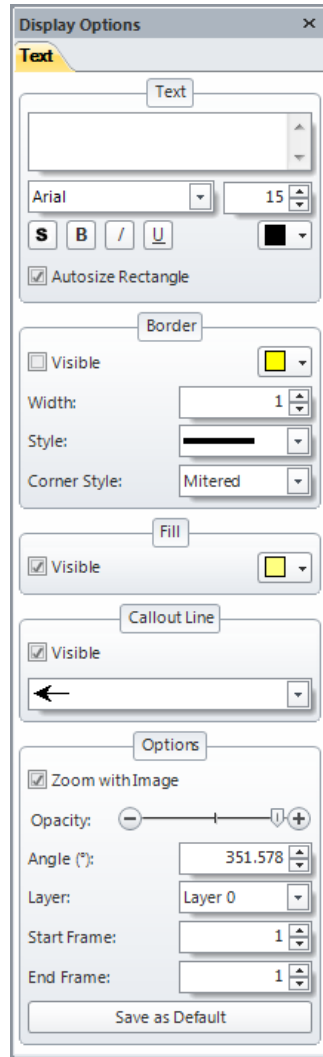
These instructions explain how to change the appearance of an existing annotation and how to set the default appearance attributes for the different annotation types.

Changing the Appearance of an Annotation

To change the appearance of an annotation:

1. Click **Select** [] from the Annotate group.
2. Click on the annotation that you want to change.
The annotation is highlighted.
3. Click the **Options** button  in the Annotate group.

You will see the *Display Options* dialog box in the panel along the right side of the workspace.



4. Use the controls available through the *Display Options* dialog box to change the appearance of the annotation.

Drawing Properties



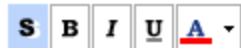
Some common controls available for changing the appearance of graphical annotations are described below.

NOTE: For complete descriptions of all available controls, refer to the “Graphic Object Properties (Dialog Box)” topic of the Image-Pro Application Help system.

- **Border:** Allows you to change the visibility, color, width, and line style of a border around your annotation.
- **Fill:** Allows you to choose the fill color for your annotation, including opacity and/or transparency.
- **Label position:** If you are including a label (different from the text box) in your annotation, you can adjust the position of the label here.
- **Callout line:** Allows you to show or hide the line attached to a callout box and to adjust the line thickness and end style.

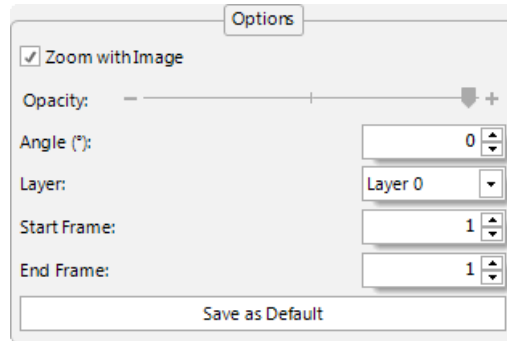
Label Properties

Some common controls available for changing the appearance of label annotations are described below.

- **Text:** Click on the **Text** tab to change the text of the annotation.
- : Allows you to change the font to be used for rendering text.
- : Allows you to change the font size to be used for rendering text.
- : Allows you to make the text shadowed, bold, italics, underlined or in a different color.
- **Autosize:** Allows you to have the box surrounding the text automatically resized to fit the text.
- **Fill:** Controls whether the box surrounding the text appears as opaque or transparent. This attribute does not apply to the text.
- **Color:** Click on the **Color** button to change the color of the bounding box border or fill, or to change the color of the text.
- **Zoom with Image:** Check this box to make the annotate larger or smaller as the image is resized.

Additional Options


Additional display options have been added to *Image-Pro*:



- **Zoom with image** automatically adjusts the size of your annotation as the size of the image changes.
 - **Opacity** adjusts the transparency of a line or shape as you move the slider.
 - **Angle** lets you specify angle of display for your annotation.
 - **Layer** lets you create overlay layers on your annotation. This can be very useful when displaying a 3D or Z-stack image.
 - **Start Frame/End Frame** lets you specify on which frames of a sequence the annotation will appear.
5. The changes are applied to the selected annotation.

Setting the Default Appearance for Annotations

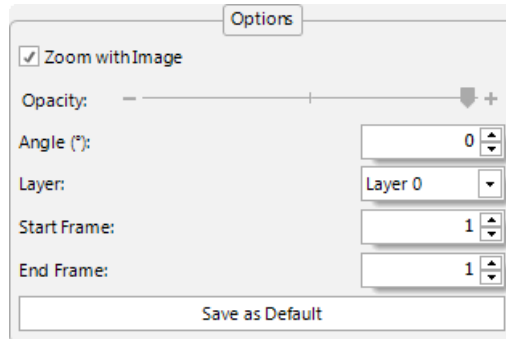
The default appearance attributes for annotations are set by designating the appearance attributes of an existing annotation as the default for future annotations of that type. Note that defaults exist independently for each type of annotation you can draw: rectangle, rounded rectangle, ellipse, polygon, point, and text. To set the default appearance for an annotation type:

1. Click **Select**  from the Annotate group.
2. In the active image, click on an annotation of the type for which you want to set appearance defaults.

For example, if you want to set the default appearance for ellipse-type annotations, click on an ellipse-type annotation in the active image. If one doesn't exist, draw one.

3. Click the **Options** button  in the Annotate group.

You will see the Display Options dialog box along the right side of the workspace.



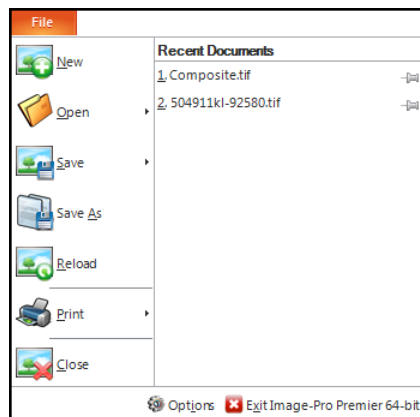
4. Click **Save as Default**.

The default appearance values are set. Future annotations of that type will appear as the selected annotation.

Saving Annotations

When annotations are originally placed on an image, they exist as graphical overlays and are not part of the image. If you want them to become part of the image, however, you can save them with the image.

1. Click **Save** from the **File** menu:



2. Answer **Yes** when the question, “Do you want to save the changes with [image name]” appears.

The annotations will be saved with the image.

Note: Annotations can be saved only with TIFF format images.

Note Regarding Zooming Annotations: Zoom with image is a per-view overlay display feature, but burn-in affects all views equally (since it changes the underlying image data). Thus, burn-in is always done at 100% zoom. This will make those graphic objects with "Zoom with image" off (such as labels) look larger (if the view is zoomed in) or smaller (if the view is zoomed out) after they are burned in.

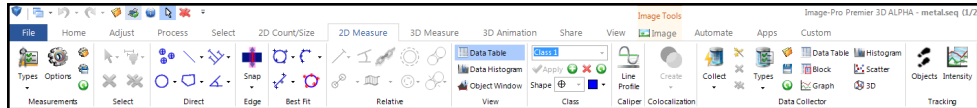
Chapter 8

Making Measurements

This chapter describes how to measure, count, and track features in images.

Overview of the Measurement Feature

Image-Pro provides tools for measuring features in images. For example, suppose you want to know the distance between two points in an image. Using the **Measurement** tools, you can simply draw a line between the two points and acquire an instant distance measurement. All of these tools are found on the *Measure ribbon*.



Measurement operations in *Image-Pro* are performed in terms of image pixel positions. For example, the length of a line feature is determined by the number of pixels along the line.

*Note: If you are running Image-Pro with 3D Module, some of the tab names may appear as **2D Count/Size** or **2D Measure**.*

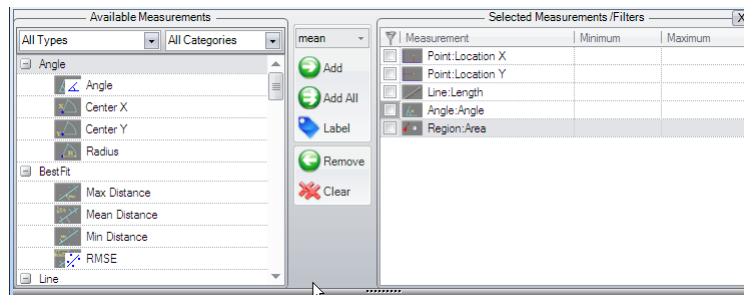
Data Measurements

Image-Pro offers you a variety of measurement types to analyze your images. These appear in the *Measurements* group on the **Count/Size ribbon** as well as on the **Measure ribbon**:



To select your measurements, follow the steps below.

1. Click the **Types** drop-down arrow. You will see the **Measurements** dialog:



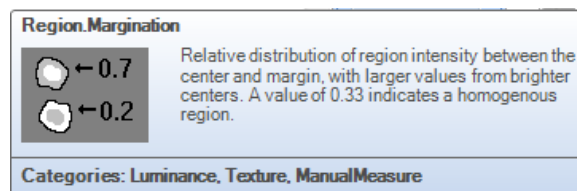
2. Using the **Add** or **Add All** buttons, select the measurements you want to make.


The **Available measurements** list contains the measurements that can be grouped by *Measurement type* (All Types, Regions, Lines, Points, Best-Fit, Angles) and *Measurement Category*:

- All Categories
- Basic
- Housekeeping
- Luminance
- Density - Optical density related measurement
- Histogram - A histogram or pixel-counting-based measurement
- Size - Size related measurement

- Position - Position related measurement
- Shape - Feature shape related measurement
- Reference - Measurement relating to a reference feature
- Nesting - Nesting relationship measurement
- Marker - Some marker or landmark measurement
- Holes - Measurement ignores holes
- Texture - A texture-related measurement
- Classified - A classification-related measurement
- Color - A color related measurement
- Manual Measure - Available as one of the Manual Measurements
- Polyline - The measurement is only relevant for lines with more than one segment
- User Defined - The measurement is a user-defined one
- Angle Result - The measurement results are angles (have units of Degrees).
- Uncalibrated Result - The measurement results do not use the Spatial Calibration or Intensity Calibration (length units are Pixels, area units are Pixels squared and intensity units are raw pixel luminance values).

Tooltips display additional information about each measurement, as in the example shown here:



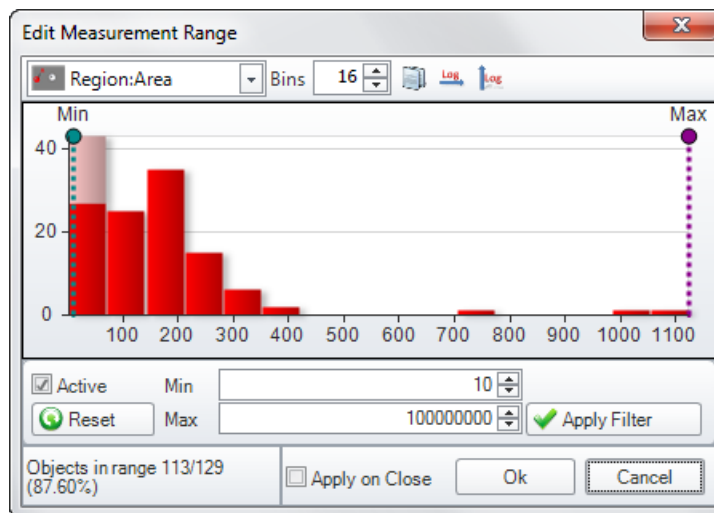
The **Selected Measurements** list contains the measurements that will be reported in the data windows (**Data Table**, **Histogram** and **ObjectWindow**). If the checkbox in the **Filter**  column is active, the measurement will be used as filter and only the objects with parameters within the defined limits will be counted. Additionally the filter limits can be set in the **Histogram** window, in the *Measurement filter* pane.

Note that the measurement filters are applied only when the **Ranges** button is checked:



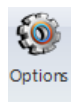
Edit Ranges

Adjusting filter ranges interactively can be done using the **Edit Range** button:



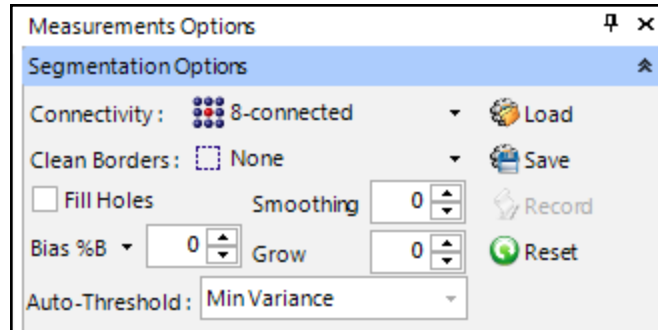
Min and **Max** filter values can be adjusted by dragging the corresponding markers on the histogram or editing their numerical values. The mask of objects in range is shown on the active image. The number of objects in range/total objects and percentage are shown below the histogram.

Measurement Options



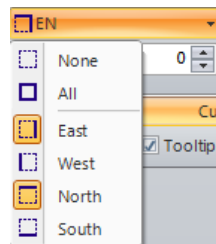
The **Options** button shows the measurements appearance options panel.

The first section contains the **Segmentation** options:



Connectivity, either *8-connected* or *4-connected*, defines the number of pixel neighbors segmenting the objects.

CleanBorders specifies if the objects touching the selected borders will be eliminated from the result.





Fill Holes option indicates if the holes inside objects should be included into the object area.

Smoothing defines the degree of smoothing of used for the object outlines. The default is zero when no smoothing is applied.

Segmentation bias automatically adjusts the calculated threshold values by the given bias value. More about segmentation bias appears later in this chapter.

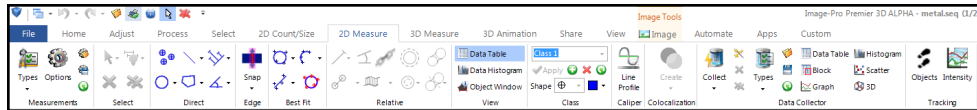
Grow defines the size increment (in pixels) of segmented objects that is applied after a Count operation using dilation or erosion of the segmented regions. More about growing or shrinking appears later in this chapter.

Auto Threshold allows you to define the method that is used to segment objects in Auto Bright/Dark segmentation modes. More about Auto-Threshold appears later in this chapter.

Load  and **Save**  buttons load and save threshold segmentation options. These buttons can be used to save and then restore counting environment in a new session. **Record** enables you to save your segmentation as a sequence or movie. **Reset** changes your settings back to their default state.

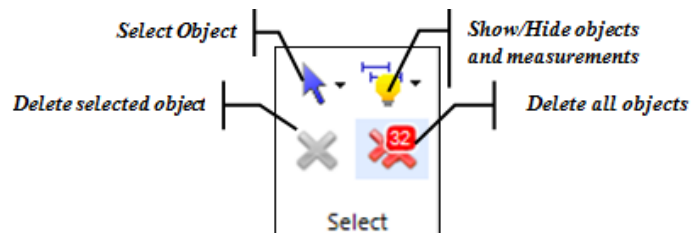
Manual Measurement Tools

This section describes how to use the measurement tools and view your measurement data. These tools appear on the *Measure* ribbon:



Selection Tools

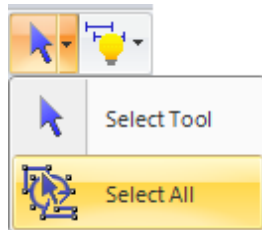
These tools allow you to select, hide, or show objects and measurements:



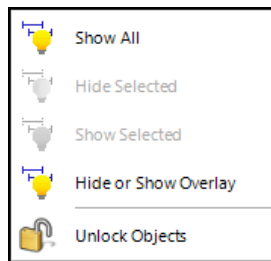
Clicking the **Select Object** button activates the selection tool. You can select measurement objects on the image and edit them. Additionally, when one or more objects are selected, a semi-transparent floating bar will appear on the image that allows you to perform some operations more conveniently (delete, hide, show, create relative measurements, calibrate).



The **Select All** tool lets you select all the measurements at once:



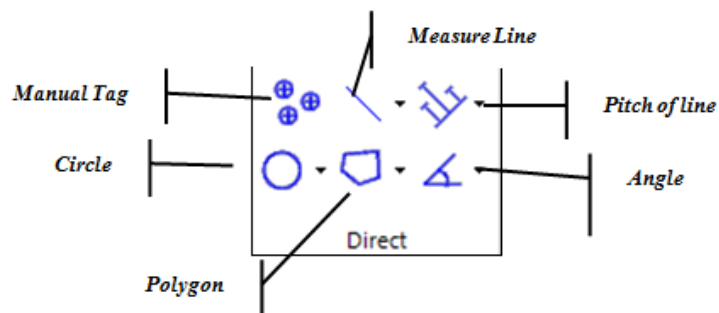
The **Show** button and its menu allow you to toggle hide or show the overlay and selected features on the image display. It also allows you to unlock the counted objects.



All objects created by a **Count** operation are locked by default and cannot be moved or adjusted. Clicking the **Unlock objects** button will unlock counted features and enable you to move or edit some of the objects.

Direct Measurements

This panel contains a “toolbox” of measuring instruments. These tools should be sufficient to take most measurements.



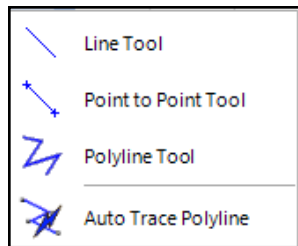
Manual Tag

Clicking this button activates the manual tag tool



Line

Activates the line tool, where you can draw a line by clicking and dragging. Activating any of the measurements from the drop-down makes it the default for the main button. Clicking the drop-down arrow displays additional line tools:



Point to point

Activates point to point tool. A line feature will be created after two clicks.



Polyline

Activates the polygonal line tool. As you click, new points will be added to the line. (You can stop adding points by double-click and by pressing the Enter button; pressing the Esc button cancels the line creation.) There is also an option to auto-trace the polyline.




Auto-trace Polyline activates the auto-trace polyline.



Pitch with line

Activates pitch with line tool. First two points define the reference line and every next point adds a perpendicular line from the point to the reference line. (You can stop adding points by double-click and by hitting the <Enter> button). The drop-down menu contains the

Show base line  option, which turns the base line (first 2 points) of the pitch group on or off.



Circle or Ellipse

Activates the Circle tool, where you can create a circle by clicking and dragging a rectangular area with the inscribed circle. Clicking the drop-down arrow displays the Ellipse and other circle tools.



Ellipse

Activates the Ellipse tool, where you can create an ellipse by clicking and dragging a rectangular area with the inscribed ellipse. There are the following options for creating circles:



Radial drawing – center and radius (only for circles) or



Box drawing - box diagonal points (for circles and ellipses).



Find circle – activates Snap to Circle mode (see Snap to Image).



Polygon

Activates the Polygon tool, where you can create an n-sided polygon by clicking n times. Clicking the drop-down arrow displays the Rect-

angle  and Auto-Trace Rectangle  tools.



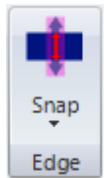
Angle (joined)

Activates angle tool, where you can create an angle measurements adding 3 points to the image. The angle range is defined in the drop-down menu :

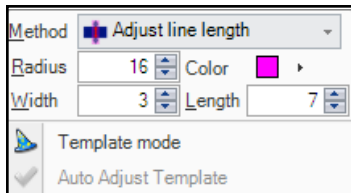


Edge Measurements

In most cases, manual measurements are done measuring the distances from one edge of object to another. Locating object edges manually is tedious work and the resulting measurements may have subjective factors. The **Snap to Edge** feature locates image edges within a cursor neighborhood automatically, and either moves the cursor to the calculated position or snaps the measurement features to the calculated position. It provides greater measurement accuracy, reduces the chance of subjective errors, and generally makes taking measurements easier.



When you select the **Snap to Edge** tool on the *Measurements* ribbon, you will see the following dialog:



There are a number of methods available to making the edge measurements:



Snap to straight edge

The closest straight edge is located within the cursor area defined by a radius. If the mouse stays still for a short interval (default is 0.4 sec), the cursor moves to the calculated position. This method is available with all manual measurement tools.

**Snap to any edge**

The closest edge is located within the cursor area defined by a radius. The option can be used on small objects or on objects with curved outlines. If the mouse stays still for a short interval, the cursor moves to the calculated position. This method is available with all manual measurement tools.

**Adjust line length**

The measurement feature is snapped to the sharpest rising and falling edges underneath the line. This tool can be used when the object is brighter or darker than background and there are no holes or other sharp edges in the middle of the object. The **Width** control defines the width of the caliper line and **Length** is the matched length of the edge detector. This method is available with the **Line** and **Point to Point** measurement tools

**Adjust line ends**

The created line is snapped to the object's edges along the line. The line ends are also adjusted by edges. The width of the snap area is defined by a radius. The **Width** control defines the width of the caliper line and **Length** is the matched length of the edge detector. On noisy images **Width** can be set to higher values to smooth the profile averaging pixels in perpendicular to the line direction (default is 3). This method is available with the **Line and Point to Point** measurement tools.

If **Adjust line length** or **Adjust line ends** option is active a line is drawn on image using *Line* or *Point to Point* tools will be automatically snapped to the object's edges as soon as it's created using the caliper functionality.

**Adjust along edge**

The created line is snapped to the object's edges in the neighborhood

of the line ends. The area length is defined by a radius. The **Width** control defines the width of the caliper line and **Length** is the matched length of the edge detector. The **Length** defines how sharp the detected edge can be. Low values (3 to 7) are recommended for high contrast images with uneven intensity of objects. If objects have even illumination and they are relatively wide, **Length** can be increased smoothing noise along the line. This method is available with the **Line** and **Point to Point** measurement tools.




Find circle

Finds a circle within the radius from the click position. Click inside the circular object and the position and radius will be calculated automatically, analyzing the object edges in the neighborhood.

This method is available with the **Circle** measurement tool.

The **template** button activates the mode, which can be used to create measurement templates. When **Template** mode is active the **Snap** functionality is *not* applied immediately after creating the line features,

but only when you click the **Auto Adjust**  button. Only **Adjust line ends**, **Adjust along edge** and **Find circle** can be used in **Template** mode.

Best-fit measurements



Best-fit measurements can be used to create features from a number of manually placed points.



Best-fit Circle

The **Best-fit Circle** tool allows you to add points to the image and a best-fit circle will be generated. Stop adding points by double-clicking or by pressing the **<Enter>** key.



Best-fit Arc

This tool allows you to add points to the image and a best-fit arc will be generated. You can stop adding points by double-clicking or pressing the <Enter> key. The angle of the created arc is defined by the positions of the first and the last points.



Best-fit Line

The **Best-fit Line** tool allows you to add points to the image and a best-fit line will be generated. You can stop adding points by double-clicking or pressing the <Enter> key.



Display points for best-fit features

When the **Display points for best-fit features** is *ON* the points, which define best-fit features are shown on the image and included in the data table. When the option is off the points are hidden.

The **Points** edit boxes in the drop-downs for **Line**, **Circle**, and **Arc** features are used to set the maximum number of points for best fit lines, circles, and arcs. Lines will allow from 2 to 1000 points, while circles and arcs will allow from 3 to 1000 points. When creating a best-fit feature, the feature will be automatically created when the current maximum is hit. This makes it possible to set feature creation to accept 2-point lines and 3-point circles and arcs (without requiring double-click on the last point) while still supporting multiple-point fits when desired.

Relative Measurements



The *Relative Measurements* group provides tools to create measurements between existing features. You have to create one or more objects first, and then create a derived measurement.

Distance between objects

Use these tools to create distance measurement between two objects.



Point group

These tools are used to create point features at the start of the

- **First point** creates a point feature at the start of the selected object.
- **Center point** creates a point feature at the center of the selected object.
- **Last point** creates a point feature at the end of the selected object.



Distance between centers

This tool creates distance measurements between the centers of two selected objects. Note, that if two lines (polygonal lines) are selected, the **Average gap between traces** will be measured.



Minimum distance

This tool measures the least distance between two selected objects. Note that if two lines (polygonal lines) are selected, the **Minimum gap between traces** will be measured.



Maximum distance

This tool measures the greatest distance between two2 selected objects. Note that if two lines (polygonal lines) are selected, the **Maximum gap between traces** will be measured.



Angle between lines

This tool creates an angle feature between two selected lines. When drawing lines, the order in which the lines are selected and the direction of the lines is taken into account. The shortest distance is calculated when the lines are drawn in the same direction.



Merge Objects

Clicking the **Merge** button will combine all selected objects into one by connecting all the objects by the shortest line. Note that the Merge function is available only for making region measurements of objects (polygon, ellipse, circle, count/size objects). The **Merge** button is also displayed in the on-image context bar.

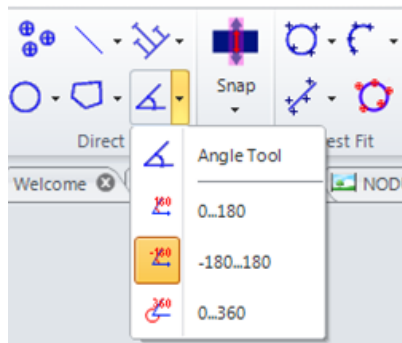


Incremental Distance

Clicking this button creates a set of vertices between two selected features (lines or regions). This feature is useful when measuring coating (between 2 poly-lines) or a doughnut (between external and internal regions) thickness distribution. It is also possible to create incremental distance measurements for a single object (closed polygon). If the object contains a hole, incremental distance will be calculated between the object's outline and the hole (doughnut thickness measurement). If more than one hole exists, the largest hole is used. If the object doesn't contain holes, the incremental distance will be calculated between 2 opposite sides of the object along the given angle. The step size offset on both sides of the object is used to avoid zero length lines. When auto-angle is on, the angle is calculated automatically.

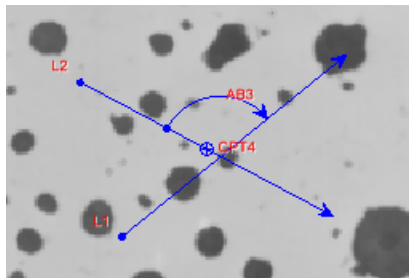
Additional information about the **Angle between Lines** measurement follows:

The angle range is determined by the option in the Angle tool dropdown and can be 0...180, -180...180 (default) or 0...360.



It is computed as the difference between the vector angles (measured counterclockwise from the positive, rightward, horizontal axis) of the first selected line, tail-to-head, minus the second selected line, head-to-tail, limited to ± 180 degrees. The measurement thus depends both on the order in which the lines are selected and the directions in which they are drawn (in an opposite sense).

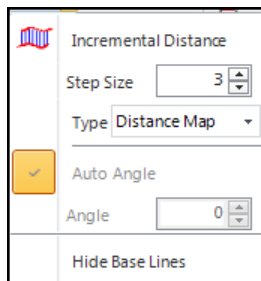
The arc is drawn from the center of the second line in most cases, unless the center is close to the intersection point. In that case the arc is drawn from the point with fixed offset from the center:



The arc is always drawn from the mid-point of the second selected line to the point where the arc intersects the first selected line or its projections. The arc is drawn in either a clockwise or counterclockwise direction to create an arc that covers an angle equal to the absolute value of the measurement (computed as described above). The arc starts at the midpoint of the second selected line and ends on the first selected line or its projection. The direction that the arc is drawn is not determined by the sine of the measurement value. Rather, it depends on the location of the midpoint of the second selected line relative to the trajectory of the first selected line.

Incremental Distance

Incremental Distance automatically measures distances between two irregular edges to quantify changes in surface size and shape. The drop-down contains the following options:



Step Size defines the distance between vertices in pixels.

Type selects the incremental distance measurement type that defines how vertices are created. It can be one of the following:

- **Shortest** measures the shortest distance between point and line. Points along the object line are equally distributed with the interval defined by the **step size**. The shortest distance to the reference line is created from every point on the object line.
- **Equidistant** measures the distance between points along the object line. Step Size defines the number of vertices. The interval between points on the reference line is calculated based on the number of points on the object line. Vertices connect a point on object line with the corresponded point on the reference line.
- **Directional** is used when all vertices are parallel. The distance between the vertices is equal to the steps Size.

When using the **Directional** type, the orientation of vertices is defined by an **angle** (in degrees from X axis counterclockwise). When **auto-angle** is on, the angle is calculated automatically based on the object and reference lines. Created vertices skip non-overlapping line ends.

- **Shortest bi-directional** measures the distances between points on the line and another line calculated as two sets of lines: from the object line to the reference line and from the reference line to the object line. This algorithm is used in *Image-Pro* to calculate distance between traces. The distances for object and reference lines are calculated in the same fashion as the **shortest** method.
- **Distance map** calculates thickness of the layer using a distance map. The source points are placed on the skeleton line between two traces created by the distance map. New lines perpendicular to the skeleton line and with a length corresponding to the thickness based on the distance map are created. The method can be used to measure thickness of bent profiles.

When **Hide Base Lines** is selected, the source base lines are hidden after the incremental distance lines are created, so that they don't affect statistics and histogram distribution of visible objects.

To measure incremental distances, follow these steps:

1. Go to the **Measure tab** and draw two polyline measurements on an image.
2. Select both polyline measurements
3. Select the **Incremental Distance** button in the *Relative Measurements* group.

Note that all relative features are adjusted when their base features are changed. Relative features cannot be moved manually; they will be moved by changing positions of the base features.

Note also that the order of selection also affects derived measurements.

Morphological Operations

The selected region-based features can be automatically modified using four morphological operations: **Grow**, **Shrink**, **Open**, and **Close**



Grow

Grows selected objects using a Dilate operation. When the "Use Restricted Grow" option is on, the Grow operations (with Count or Grow selected) do not merge objects (default is on).



Shrink

Shrinks selected objects using an Erode operation. The size **increment** for Grow and Shrink operations can be adjusted in the dropdown of the Shrink button. The increment size is defined in pixels.



Open

Applies morphological Open operations to selected regions. Use this operation to separate neighboring features if they are connected by a narrow path. The size of the morphological Open or Close operation is defined in the dropdown of the Open button. The size is in pixels.



Close

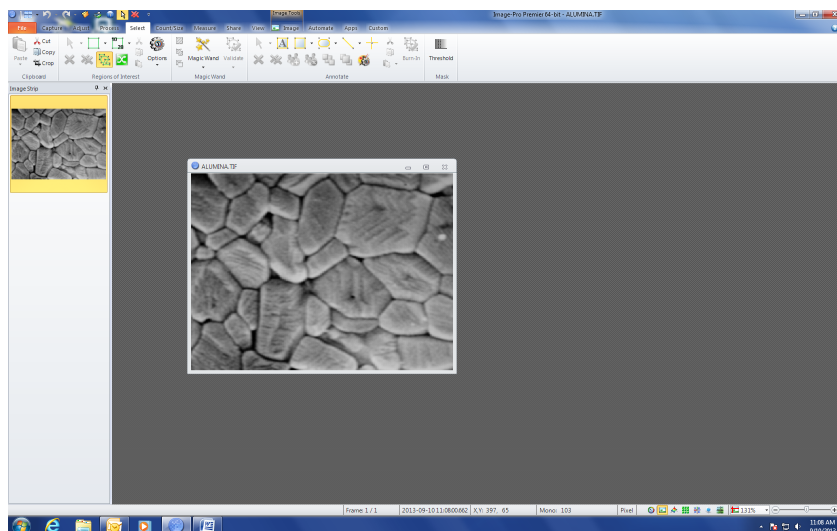
Applies morphological close operations to selected regions. Use this operation to merge neighboring features.

Note that all relative features are adjusted when their base features are changed. Relative features cannot be moved manually; they are moved by changing positions of the base features.

To make line measurements in an image:

1. Open the image that has features you want to measure.

The image appears in the *Image-Pro* workspace.



2. Select the **Direct** group on the *Measure* tab's ribbon.



3. Select the **measure line** tool.

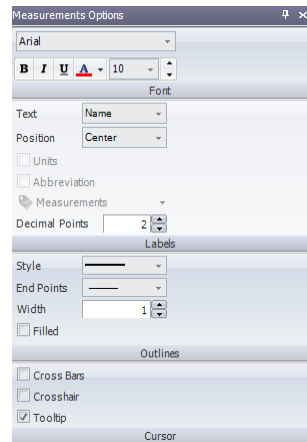
This tool allows you to make distance measurements directly on the image

4. Apply or define the spatial calibration that you want to use. See “Managing Calibrations” earlier in this chapter for more information.
5. Draw a line on the image.

The measurement information will appear in a label on the image. The label will appear near the center of the line without covering up the end symbol or much of the line itself. The measurement values will change as you drag the line.

You can make changes to the appearance of the label using the **Options** button.

6. Click the **Options** button. You will see the *Measurements Options* dialog:




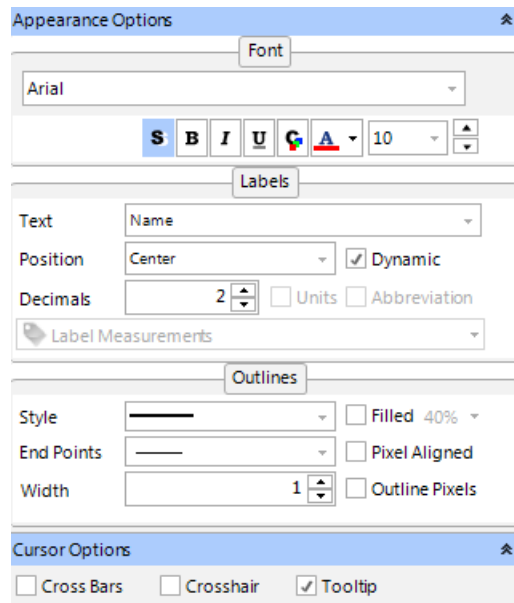
The text area of this dialog allows you to change the font and size of the text that will appear in the measurement label. The border and fill areas control the display of the border and background of the label. The options area lets you resize the label when you resize the image, and control the opacity/transparency of the label.

***Note:** Measurements and Manual Tagging can be applied to live preview images for quick measurement previews. However, these measurements are not saved with the captured image.*

Setting the Default Appearance for Measurement Labels

The default appearance attributes for measurement labels are set by designating the appearance attributes of an existing label as the default for future measurement labels. To set the default appearance for an annotation type:

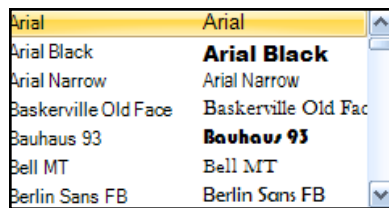
1. Click **Select** from the **Measurement group**.
2. In the active image, click on a label of the type for which you want to set appearance defaults. Most labels appear at the top-left or bottom-right positions near the marker.
3. Click the **Options**  button to display the **Measurement Options** panel.
4. Expand the **Appearance Options** section of the panel:



5. Go to the **Font** panel.



6. Choose a font from the drop down list:



You will see the measurement labels change to the font you select. Use the color, style, and size buttons to make additional changes to the measurement labels.

Shadow (S) lets you add a background shadow color to the label text.

Bold (B) displays the font in bold.

Italic (I) displays the italic font.

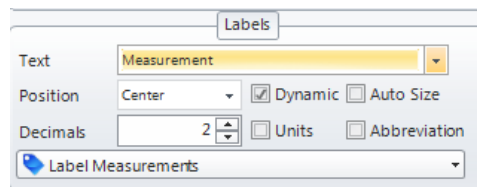
Underline (U) underlines the label text.

Class Color uses the class color for the label.

A lets you select the text color.

The number in the spin box indicates the font size.

7. In the **Labels** panel, select the text, position, and units of measure for the measurement labels:

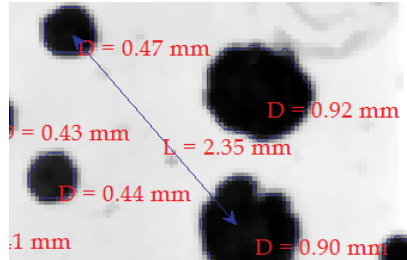


The choices for Text include:

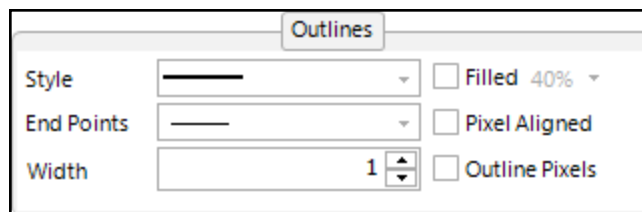
- *None* – no labels
- *Name* – measurement object name is displayed
- *Measurement* – the label measurement is displayed
- *Name And Measurement* – full name and measurement are displayed.

Units of measure and measurement **abbreviations** can be added to the measurement label by checking the appropriate boxes. You can also indicate the number of decimal points for each measurement. **Position** indicates the location of the measurement label. If the **Dynamic** box is checked, the label information will change as the information in the image changes. Measurement labels will also be resized automatically. When you select a feature, its associated label will be highlighted. If the **Auto Size** box is checked, the labels are scaled proportionally to object area.

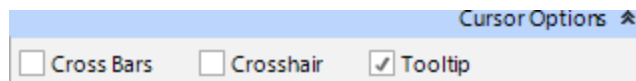
Here is an example of measurements with labels:



8. Use the **Outlines** panel to define the style of the each object class and shape:



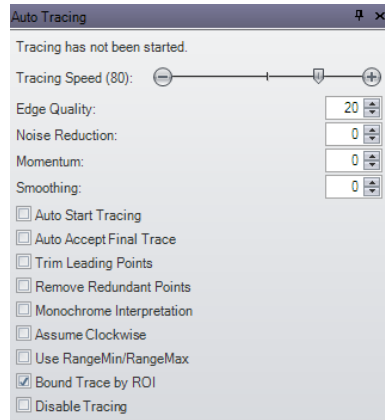
9. The **Cursor** panel lets you customize the measurements cursor:



- **Cross Bars** are useful adjusting line ends on the features. They are only active in the line editing mode.
- **Crosshair** helps you to center horizontal or vertical measurements.
- **Tooltips** show measurement data on the image when you are moving the mouse over an object on the image.

Using the Auto-Trace feature

Activating the **Auto-Trace** tool for an ROI or Annotation will cause the **Polyline** tool in **Line Profile** to behave the same as the **Auto-Trace** tool. When you click on the Polyline feature and activate **Auto-Trace**, you will see the following dialog:



The message at the top of the dialog indicates if auto-trace has started, or not.

Tracing speed: Use the slider to control the speed of the auto-trace.

Edge quality: Indicate the number of pixels to use for the edge trace.

Noise reduction: Use the spin buttons to indicate the degree of noise reduction in the traced object.

Momentum: Use the spin buttons to indicate the degree of momentum in the traced object.

Smoothing: Use the spin buttons to indicate the degree of smoothing in the traced object.

Check the box next to the auto trace feature that you want to use.

AutoStart Tracing: If this box is checked, the second mouse click made when creating a polygon or polyline will start the auto tracing. If unchecked, start auto trace by holding down the <Ctrl> key and clicking where you want the trace to start. The lightning bolt cursor shows you the auto trace path.

AutoAccept Final Trace: When this box is checked, the auto trace automatically returns to the starting point to finish tracing the object. If unchecked, you must press the <Enter> key or double-click the mouse to place the final point and complete the auto trace.

Trim Leading Points: Frequently, a trace will not return to its starting point but will intersect with itself some distance from the starting point. If this box is checked, the points before the intersection point are removed if the intersection is close to the start-

ing point. If this box is not checked, you must edit the trace manually to remove extraneous points.

Remove Redundant Points: If this box is checked, all horizontal, vertical, and diagonal runs leading to the auto trace are replaced by their end vertices in the final result. If this box is not checked, these points will be retained.

Monochrome Interpretation: When this box is checked, image color is ignored for edge detection; only average luminance is used. If unchecked, each color channel's luminance gradient is considered when doing edge tracking.

Assume Clockwise: When this box is checked, the trace will begin moving clockwise around the object. This information is used to determine which side of the trace is the interior color. This option is useful when working with colored images. If this box is not checked, no assumption is made about which side of the trace is the interior.

UseRangeMinimum/Range Maximum: When checked, the range for full-scale luminance swings is assumed to be the full minimum/maximum range of the entire image. If this box is not checked, range for full-scale luminance swings is assumed to be the displayed range of image luminance. This option is usually left clear.

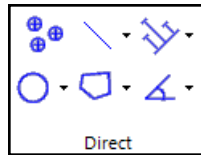
Bound Trace by ROI: When this box is checked, the trace object must stay within the bounds of the ROI. If this box is not checked, the boundaries of the ROI are ignored.

Disable Auto Trace: When this box is checked, auto-trace will be disabled and you cannot use it until you uncheck the box.

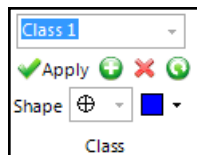
Manual Tagging

Use the *Manual Tag* tool to select individual objects or groups of objects in an image with your mouse. You can create different classes of objects for analysis, and “tag” each object with a specific marker. To use **Manual Tagging**:

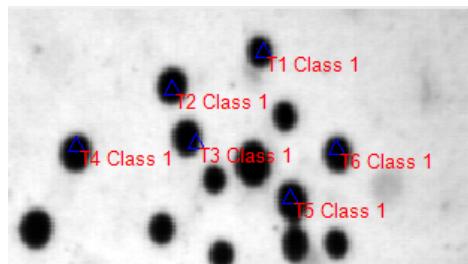
1. Open your image if it is not already open.
2. Select the **Manual Tag** tool from the *Direct* group.



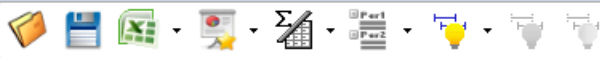
3. Select a shape and color from the *Class* group.



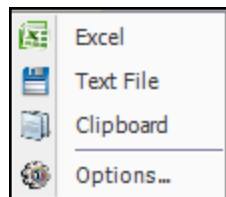
4. Click on the points in your image to tag them with a color and label:



Information about your tagged points appears in the **Data Table** below your image:

Measurement Table		
		
Feature Name	Area(pix^2)	Class Name
● P1R1	419.00	Class 1
● P1R2	221.00	Class 1
● P1R3	30.00	Class 1
● P1R4	918.00	Class 1
● P1R5	171.00	Class 1

5. Save your data to a file or export it to Excel.

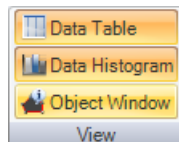


Viewing Measurement Information

Viewing a Data Table

The **Data Table** collects all the information from the measurements taken on your image. To view a data table:

1. Open the image if it is not already open.
2. Select the data type that you want to view.
3. Select the **Data Table** from the *View Data* group.



4. The data table will appear in a pane below your image:

 A screenshot of a window titled 'Measurement Table'. It has a toolbar with icons for file operations, editing, and viewing. Below the toolbar is a table with three columns: 'Feature Name', 'Area(pix^2)', and 'Class Name'. The table contains nine rows of data, all belonging to 'Class 1'.

Feature Name	Area(pix^2)	Class Name
P1R1	362.00	Class 1
P1R2	456.00	Class 1
P1R3	215.00	Class 1
P1R4	231.00	Class 1
P1R5	97.00	Class 1
P1R6	626.00	Class 1
P1R7	86.00	Class 1
P1R8	680.00	Class 1
P1R9	520.00	Class 1

The data table default names for measurements are:

Abbreviation	Measurement Name	Abbreviation	Measurement Name	Abbreviation	Measurement Name
AN	Angle	AB	Angle between Lines	BFA	Best-fit Arc
BFC	Best-fit Circle			BFL	Best-Fit Line
C	Circle	CCD	Center to Center dis-	CPT	Center Point

			tance		
E	Ellipse	FPT	First Point	L	Line
LPT	Last point	MMD	Minimum distance	MXD	Maximum Distance
P	Polygon	PL	Polygon Line	PR	Count/Size Polygon region
PPL	Point to Point distance	PWL	Pitch with Line		
R	Rectangle	T	Tag		

You can manually rename any features in the data table.

The **Data Table** provides the following tools:



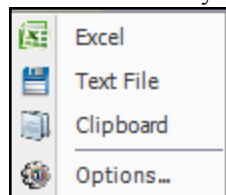
Load Data Table Measurements: This tool allows you to load measurements saved in a previous session from a *.ipm file.



Save Data Table Measurements: This tool allows you to save your measurements in a *.ipm file for use at another time.



Send Measurements to File: This tool allows you to save your measurement data to a file or export it to an *Excel* spreadsheet. The pull-down menu lets you choose one of the following:



Send to Report: This tool allows you to save your measurement data to *Image-Pro* report. The pull-down menu lets you create a new report or choose an existing one.



Show all Statistics: This tool allows you to hide or show the statistics panel for the data table measurements. A sample statistics panel is shown here:

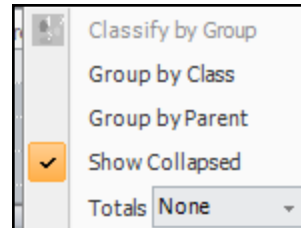
Mean value	0.00	233.99	67.11	15.35
Standard Deviat...	0.00	48.03	26.39	3.13
Minimum	0.00	151.95	23.43	12.87
Maximum	0.00	298.09	105.75	21.92
Range	0.00	146.15	82.32	9.05
Sum	0.00	1403.92	402.67	92.09
Index of Minimum	0.00	4.00	1.00	2.00

The drop-down option **Statistics per Group** defines what is displayed in the statistical pane. When this option is off, the statistics of all visible features is shown. When the option is on, the statistical values are shown per group. This option has affect only when **Grouping** is active

Setup Group Statistics display the statistics by group. It will activate grouping by class, add Percent Area Parent to the list of measurements and activate statistics per group



Grouping: Click this button to see options for grouping your data in the table. You can choose one of the following:



Show All, Show Selected Measurements, Hide Selected

Measurements: These tools let you choose to display or hide some of the measurement statistics.



3D Mask: Click this button to create a mask of volume objects.



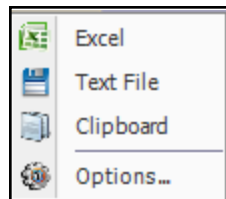
Load Data Table Measurements: This tool allows you to load measurements saved in a previous session from a *.ipm file.



Save Data Table Measurements: This tool allows you to save your measurements in a *.ipm file for use at another time.



Send Measurements to File: This tool allows you to save your measurement data to a file or export it to an *Excel* spreadsheet. The pull-down menu lets you choose one of the following:



Send to Report: This tool allows you to save your measurement data to *Image-Pro* report. The pull-down menu lets you create a new report or choose an existing one.



Show all Statistics: This tool allows you to hide or show the statistics panel for the data table measurements. A sample statistics panel is shown here:

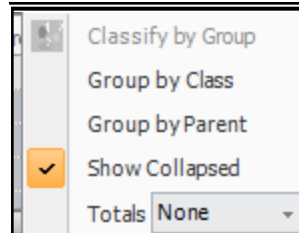
Mean value	0.00	233.99	67.11	15.35
Standard Deviat...	0.00	48.03	26.39	3.13
Minimum	0.00	151.95	23.43	12.87
Maximum	0.00	298.09	105.75	21.92
Range	0.00	146.15	82.32	9.05
Sum	0.00	1403.92	402.67	92.09
Index of Minimum	0.00	4.00	1.00	2.00

The drop-down option **Statistics per Group** defines what is displayed in the statistical pane. When this option is off, the statistics of all visible features is shown. When the option is on, the statistical values are shown per group. This option has affect only when **Grouping** is active

Setup Group Statistics display the statistics by group. It will activate grouping by class, add Percent Area Parent to the list of measurements and activate statistics per group



Grouping: Click this button to see options for grouping your data in the table. You can choose one of the following:

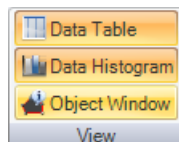


Show All, Show Selected Measurements, Hide Selected Measurements: These tools let you choose to display or hide some of the measurement statistics.

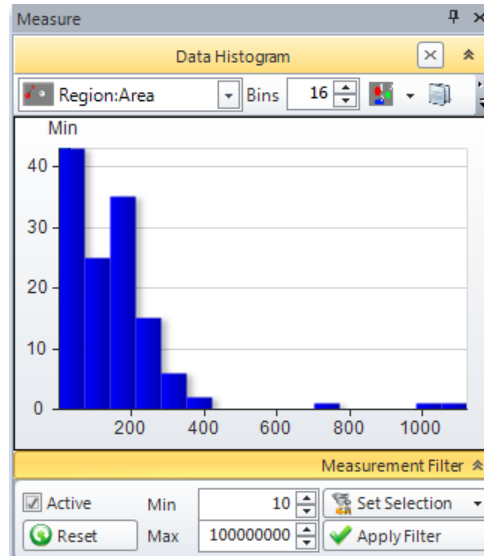
Viewing a Data Histogram

Histograms measure, and illustrate in graph form, the brightness and contrast characteristics of an image. Histogram data can be created and viewed for data gathering and analytical purposes or can be manipulated for image enhancement purposes. To create a data histogram:

1. Open the image that you want to measure if it is not already opened.
2. Select the type of measurement data to view. For example, point position X
3. Select **DataHistogram** from the *View* group.



A histogram of the data for the x position of the selected points appears in the right-side panel of the workspace:

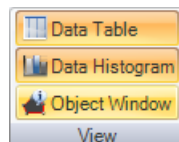


More information about histograms appears in *Chapter 4* of this manual.

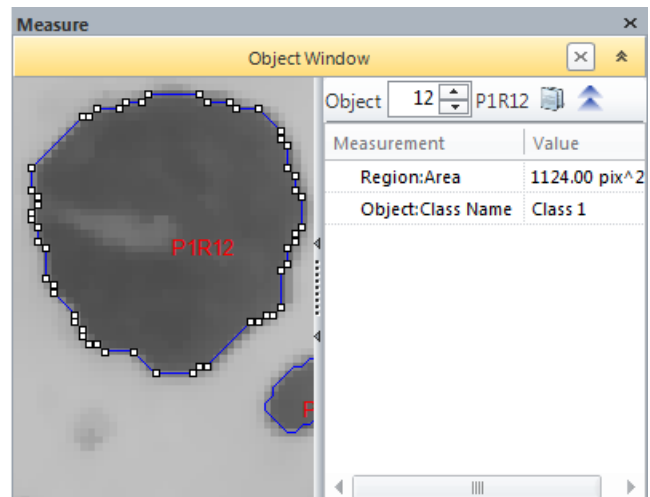
Viewing an Object Window

The **Object Window** allows you to view information about a specific object in an image. To view the object window, follow these steps:

1. Open the image that you want to measure if it is not already opened.
2. Select the type of object data to view. For example, point location T2.
3. Select **Object Window** from the *View Data* group on the **Measurements** ribbon.

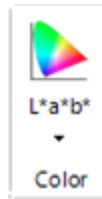


The **Object Window** containing data for point location P1R12 appears in the right-side panel of the workspace:



L*a*b* Color measurements and Color Correction

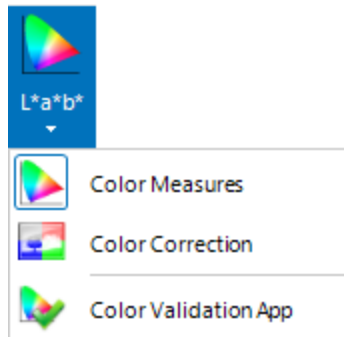
Color measurements and color correction tools are located on the Measure tab, in Color ribbon group:



The module is designed for absolute color measurements in CIE L*a*b* or XYZ color coordinates. This module also provides easy to use tools for color correction.

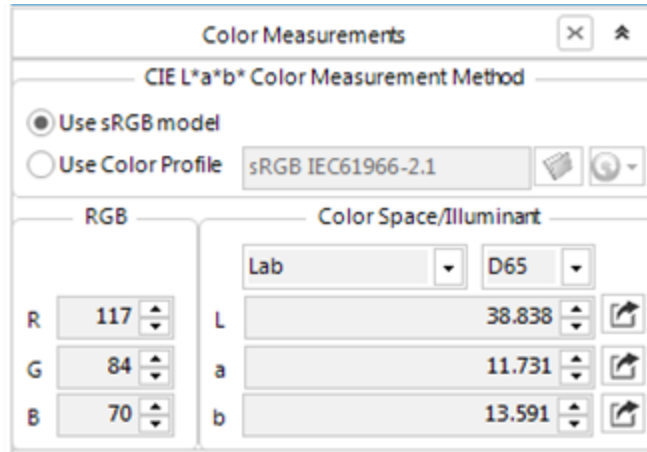
The module can be used in cosmetic, textile, pharmaceutical or food industries, those that require accurate measuring of colors or color differences.

The dropdown button contains 3 items that open dialogs with Color measurements and Color correction tools:



Color Measures

Color measures dialog allows measurement of colors under cursor and also extracting color channels as monochrome images.



CIE L*a*b* color measurements can be executed using 2 methods:

1. **sRGB color model**

2. **Color profile**

sRGB (standard Red Green Blue) is an RGB color space created cooperatively by HP and Microsoft in 1996 for use on monitors, printers and the Internet, and subsequently standardized by the IEC in 1999. It is often used as the "default" color space for images that do not contain any color space information, especially if the images are stored as 8-bit integers. sRGB color model assumes that RGB colors are captured with D65 illuminant. More info can be found [here](#).

sRGB color model can be used with images that do not have associated color profiles or when only measurements of color differences are required after executing of color correction to sRGB space.

If image has own color profile, the second option **Use color profile** should be used. In that case color profile associated with the image or the working color profile provide the correct XYZ and L*a*b* measurements.

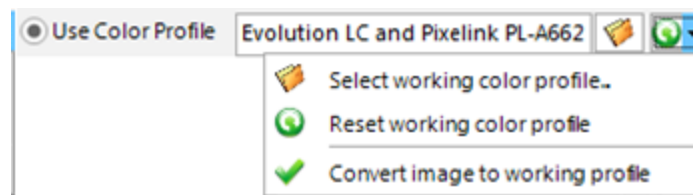
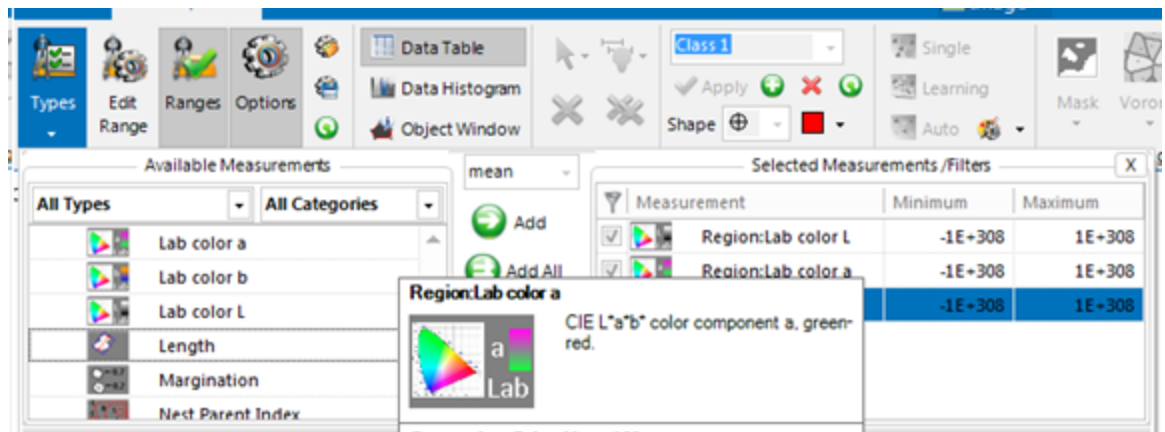


Image color profile can be loaded clicking the **Load** button and selecting [ICC or CMM color profile](#) file from the disk.

The **Reset** button will remove color profile from the image and activate the working color profile for measurements. The working color profile can be selected using the **Select working color profile** button. It can be reset to the default sRGB IEC61966-2.1 clicking the **Reset working color profile** button.

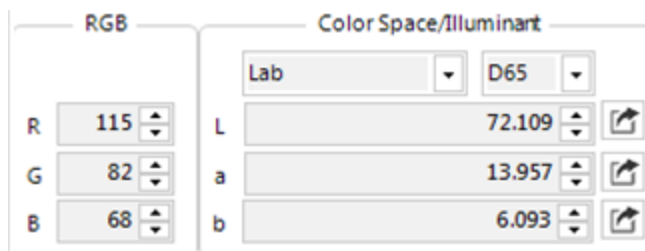
If image has own color profile, it can be converted to the working color profile using the **Convert image to working profile** button.

ICE L*a*b* measurements are also available for any Region objects using Count/Size or manual Measure operations:



Note, that L*a*b* color measurements generated by Count/Size use the color measurement method, color profile and Illuminant selected in the color measure dialog.

The bottom part of the dialog provides measurement values under cursor on the active image:



RGB group shows Red Green and Blue values under cursor and Color Space/Illuminant defines the active color space/illuminant and corresponded measurement values.

There are following color spaces available:


- **Lab** – [CIE Lab color space](#)
- **XYZ** – [intermediate color space](#) between RGB and Lab spaces
- **YIQ** - the color space used by the NTSC color TV system, employed mainly in North and Central America, and Japan. I stands for in-phase, while Q stands for quadrature, Y component represents the luma. (<https://en.wikipedia.org/wiki/YIQ>)
- **CMY** - subtractive color model, used in color printing, cyan, magenta, yellow. (https://en.wikipedia.org/wiki/CMYK_color_model).

Note, that YIQ and CMY are not absolute color measurement spaces and listed in the dialog for completeness of color measurements on the image.

The **Illuminant** defines the type of light source used when the image was taken. The following standard illuminants are listed:

- **D50** - CIE standard illuminant D50. Simulates warm daylight at sunrise or sunset with correlated color temperature of 5003 K. Also known as horizon light. (all color profiles use it as PCS color space)
- **D55** - CIE standard illuminant D55. Simulates mid-morning or mid-afternoon daylight with correlated color temperature of 5500 K.
- **D65** - CIE standard illuminant D65. Simulates noon daylight with correlated color temperature of 6504 K. (used with sRGB color space)
- **A** - CIE standard illuminant A. Simulates typical, domestic, tungsten-filament lighting with correlated color temperature of 2856 K.
- **C** - CIE standard illuminant C. Simulates average or north sky daylight with correlated color temperature of 6774 K.
- **E** - Equal-energy radiator. Useful as a theoretical reference.

The Illuminant affects XYZ and Lab color measurements. It's preferable to use the native Illuminant for specific color profiles, for example sRGB model used D65 illuminant, while Color profiles use D50 as intermediate white point. Conversion to other illuminates are done by using Bradford transform in form of [chromatic adaptation](#).

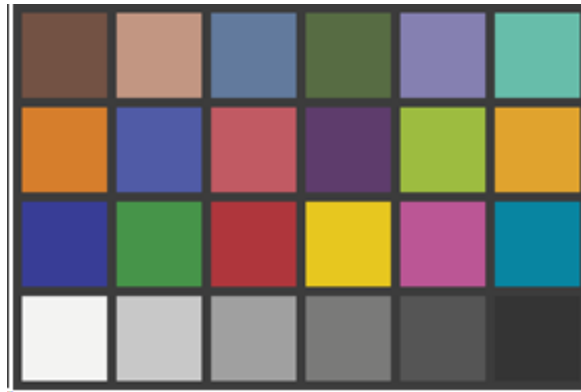
Every channel of a color image can be extracted to a monochrome image clicking the **Extract**  button.

Reference colors

Color measurements can be verified using some reference color images called color checkers, one of them is Gretag-Macbeth Color checker.

The patch has 24 colors covering visual gamut and has known colors published on the net.

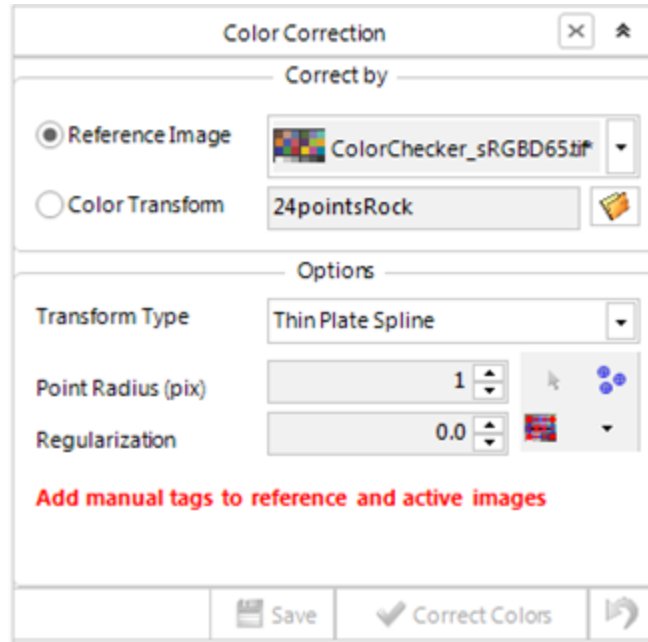
The application installs the color checker image as ColorChecker_sRGBD65.tif to the Colors folder of demo images:



The RGB values of every are generated with D65 illuminant. The list of patch values can be found in [wikipedia](https://en.wikipedia.org/wiki/List_of_colors) .

Color Correction

The Color Correction tool is closely related to the CIE L*a*b* measurements. The goal of the color correction is to adjust colors of one image to colors of the reference image with known Lab coordinates or just to some reference color patch, so the colors can be measured or compared.



Typically, the reference image can be a color checker (e.g. Macbeth ColorChecker) or just an image with color patches.

User should place manual Tags as reference points on corresponded color patched of the reference and active images and execute color correction.

There are 2 types of transforms are available:

- *Polynomial*
- *Thin Plate Spline*

With *Polynomial* type the images may have from 3 to 20 points for color correction. A polynomial transform is created matching colors on the active and reference images. The type of the polynomial depends on the number of tags:

- 3-point calibration corrects changes in color gain
- 4-point calibration corrects color shift and gain
- 7-point calibration corrects the linear color mix

- 10-point calibration corrects the color mix using polynomial approximation of power 2
- 20-point calibration corrects the color mix using polynomial approximation of power 3 .

Polynomial transform is recommended to use with small number of reference points (3-7) as high degree polynomials may produce unexpected color distortions for colors in between references.

Thin Plate Spline transform can be used with 3 or more reference points. This method produces smoother color space transforms comparing to Polynomial with large number of points, though reference point colors may not match exactly when Regularization is more than zero.

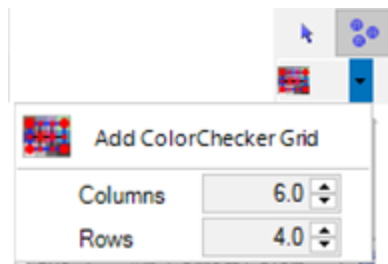
It is recommended to use **Thin Plate Spline** transform for large number of tags as in case of full 24-point matching of Macbeth ColorChecker.

Point Radius - radius of the measurement circle at the tag position in pixels. The average RGB values of the circle region are used for color matching. When the value is one - single pixel value is extracted.

Regularization – is a smoothing parameter used only with Thin Plate Spline transform method. Increasing the value will smooth color transform, though the reference values may not match exactly.

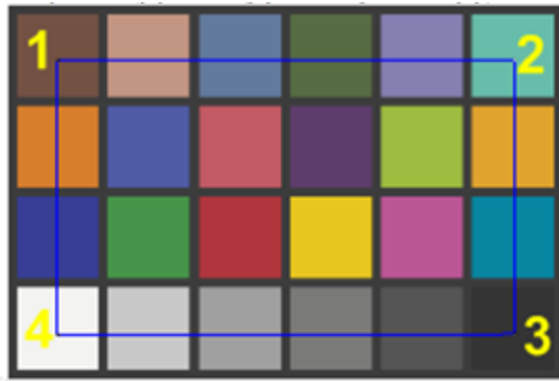
Typical color correction work-flow

Use image of Color Checker or image with color patches as a reference, add manual tags on the color patches using tag tools:



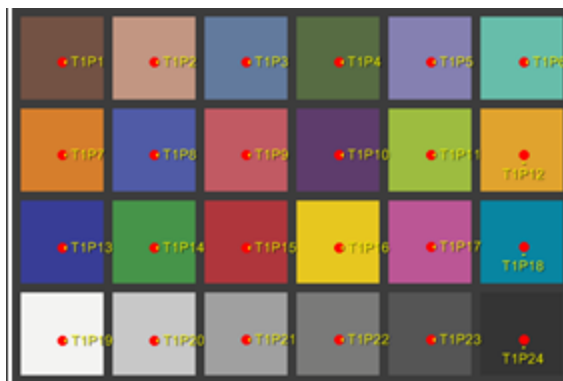
Tags can be added activating the **Manual Tag** tool and clicking at the color patches on the image. Tag positions can be modified activating the **Select** tool, selecting and moving or deleting them using the **Delete** key.

You can also add the whole grid of color tags clicking the **Add ColorChecker grid** button and drawing polygon clicking the centers of the corner color patches in the following order: top-left, top-right, bottom-right, bottom-left:

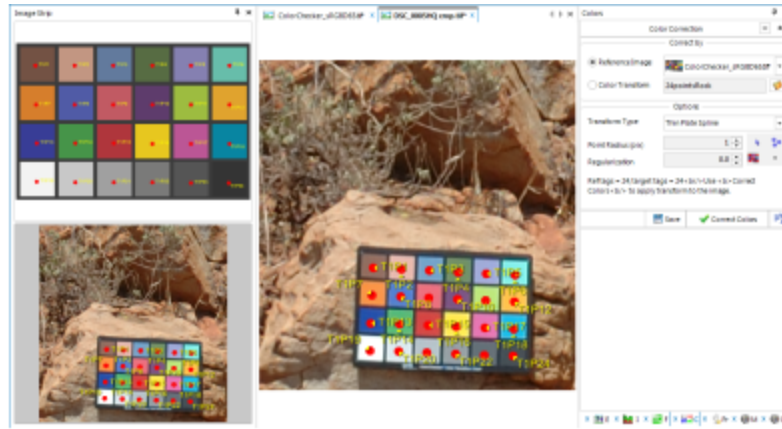


then close the polygon either by double click or hitting the Enter key.

The tag pattern will be created:



The tags on corresponded color patches on the active image should also be created and then color transform can be saved to a file or applied to the image.



The transform can be saved to a file using the Save button. When the transform is saved, it can be used correcting the current or other color image taken with the same conditions.

Clicking the **Correct Colors** button will apply transform to the active image.

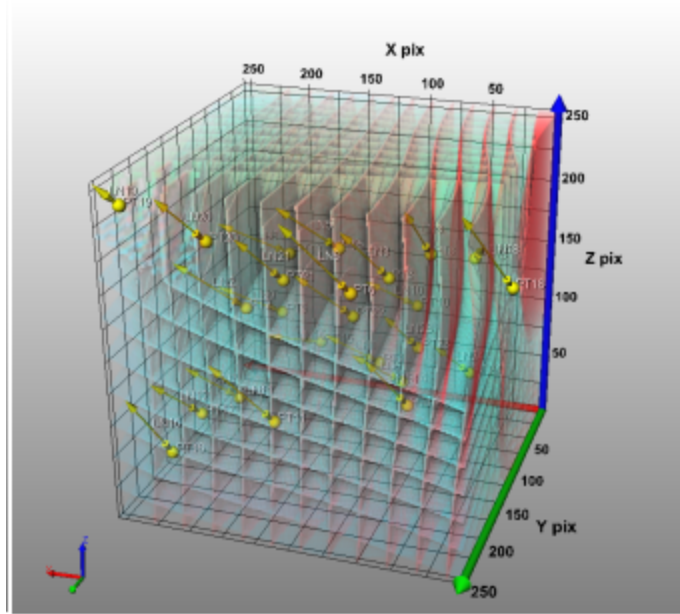


The correction can be undone using the **Undo** button.

After the correction the RGB values of the color patches at tag positions will be transformed to RGB values of the reference image and L*a*b* or XYZ measurements can be performed properly.

Color Validation App

Transform verification can be done using the pre-installed *Color Validation* app. The screenshot below shows the results of Visualize Color Transform operation:

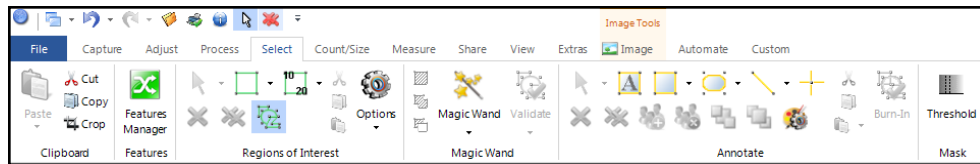


The app contains documentation describing the available tools, refer to the app's help file for more info.

Thresholding

Thresholding allows you to reduce your image to just two colors: black and white. This is done by specifying a range of intensities to be emphasized (set to white), and converting all others to black (0). Thresholding is often used to segment an image in order to extract its important features, or to reduce an image to two intensity levels in preparation for a watershed or thinning filtering operation.

Thresholding is performed using the *Mask* button on the *Select* tab.

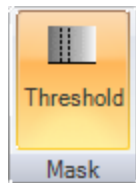


When the **Mask** command is selected, you will be asked to specify the range of values you want emphasized (set to white).

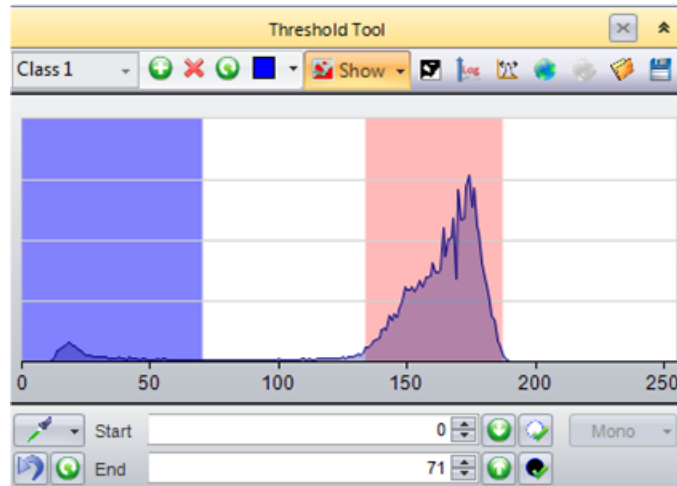
The *Threshold* command operates upon gray scale values, so if you are working with a *True Color* or *Palette* image, it must be converted to *Gray Scale* before it can be thresholded.

To use the *Threshold* tool, follow the steps below:

1. Open the image, if it is not already open.
2. Click the **Mask** button on the *Select* tab.







You will see the **Threshold Tool** panel.



The dialog shows the histogram of the active image and the colored area shows the range within thresholds. Using the mouse wheel will zoom in the histogram in and out.

3. Use the following tools to set your threshold range:

The **Class** combo-box shows the active segmentation class. You can add new segmentation classes by clicking the **Add**  button or delete classes using the **Delete**  button. All classes can be reset using the **Reset**  button. The class names that you defined here are assigned to thresholded objects created by a Count operation in Count/Size. Class color is defined by the color  button.

The visibility of the threshold overlay can be toggled using the **Show Overlay** button







The **Show All Classes** check box in the dropdown defines the preview overlay context, when the control is on, all classes are shown; when it is off, only the active class is visible. When **Allow Overlap** option is off, multi-range segmentation ranges are not allowed to overlap. You can enable this option to create overlapping ranges.

The **Mask** button creates a new binary image of thresholded areas for all classes of the active class depending on **Show All Classes** control.

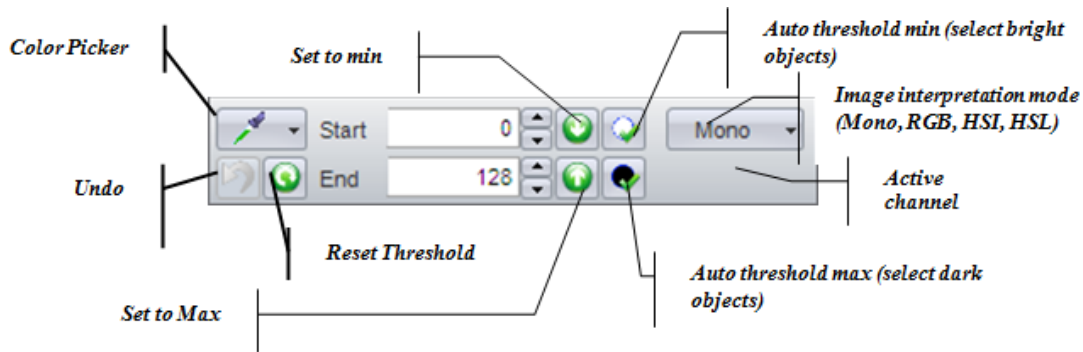
The **Log Y** button switches the Y axis to a logarithmic scale.

The **Reset zoom** button resets the histogram.

The **Set Global**  button sets threshold segmentation ranges to Global. These ranges can be applied to another image using the **Apply Global**  button.

The segmentation ranges can be saved to a file using the **Save**  button and loaded from a file using the **Load**  button.

The bottom part of the dialog displays these thresholding tools:



Color Picker (eyedropper)

Use this tool to pick intensities from the image. The pixel area that is used for threshold definition is shown in the cursor window, as in a magnifying glass. The area in the magnifying glass is defined by the **Size** parameter.



Start/End

The **Size** parameter determines the pixel used to sample the intensities.



Undo/Reset

Use the arrow to undo the most recent operation. Use the **Reset** button

to reset the threshold.



Minimum/Maximum

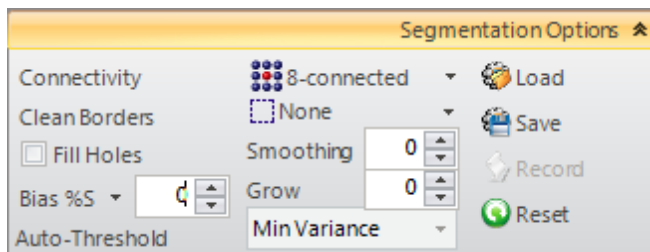
Use these buttons to set the minimum and maximum threshold values. Auto Threshold Min selects only bright objects, while Auto Threshold Max selects dark objects.

Auto-Threshold

Auto-Threshold allows you to define the method that is used to segment objects in **Auto Bright/Dark** segmentation modes. This feature appears when you click the **Options** button in the *Measurements* group on the **Count/Size** tab's ribbon.



You can find **Auto-Threshold** on the **Segmentation Options** ribbon, which appears on the panel on the right side of the workspace:



The auto-threshold methods include multi-phase auto segmentation methods and a histogram method that should be used with **Bias** to segment objects above or below the background. The methods are:

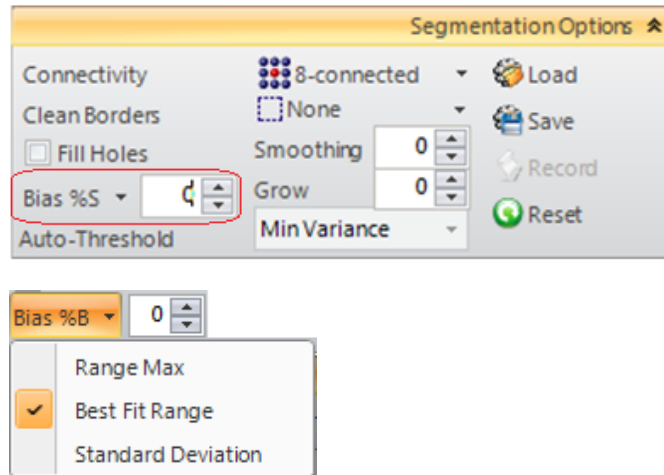
- *Min Variance* - The ratio of between-phase to total variances minimized (method of Otsu). This is the default method.

- *Legacy* method. A version of the Minimum Variance method with a bias towards low values. .
- *Mean* - Threshold is the Mean value of histogram. Use Bias to segment the objects above or below background. .
- *Mode* - Threshold is the Mode value of histogram. .
- *Median* - Threshold is the Median value of histogram. .
- *Minimum* - Threshold is the Minimum value of histogram. *Maximum* - Threshold is the Maximum value of histogram. .
- *Best Fit Min* - Threshold is the Minimum of best-fit range of histogram. .
- *Best Fit Max* -Threshold is the Maximum of best-fit range of histogram. .

The methods from *Mean* to *Best Fit Max* should be used with **Bias** to segment the objects above or below background. These methods can be used with batch macros to count and measure objects with intensities above or below threshold by fixed value.

Segmentation Bias

Segmentation Bias is defined by the **Bias** control:

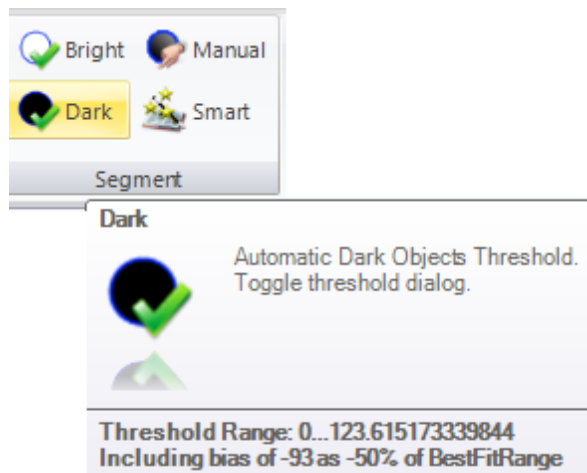


You can use this option to adjust automatically calculated threshold values by the given bias value. The bias is applied only to *AutoBright/Dark* segmentation methods. There are 3 bias methods:

- *Range Max* - where bias is calculated as a percentage of the maximum range of the given image type.
- *Best Fit Range* - bias is calculated as a percentage of the best-fit range. This is the default method.
- *Standard Deviation* - bias is calculated as percentage of standard deviation of the image intensity distribution.

Note that the **Bias** value, **Bias method** and **Auto-Threshold** methods are automatically reflected on the Threshold overlay when adjusted (when Threshold dialog is visible and segmentation method is set to Auto Bright or Auto-Dark).

The absolute Bias value is also reported in the tooltip over Bright or Dark segmentation buttons:



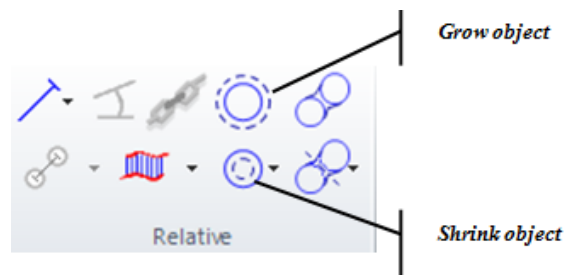
Grow or Shrink Object

The *Grow* or *Shrink* option can be used to grow or shrink segmented objects automatically. The option defines the size increment (in pixels) of segmented objects that is applied after a Count operation using dilation or erosion of the segmented regions. If the value is negative, the objects shrink. If the value is positive, the objects grow. To use this tool, follow the steps below:

1. Count the objects in your image.
2. Use the blue selection arrow to select multiple objects to grow or shrink.

Choose the drop down arrow next to the blue selection arrow to **Select All** if you want to grow or shrink all the objects.

3. Go to the **Measure tab** and choose the **Grow or Shrink** options from the *Relative Measurements* group.



The drop-down arrow next to the **Shrink** icon lets you specify the incremental amount to grow or shrink the object.

4. Adjust your image.

Open or Close Object

Use the **Open** and Close tools to manually combine neighboring objects when using automatic segmentation and counting tools. To use these tools, follow these steps:

1. Count the objects in your image.
2. Use the selection arrow to select multiple objects to merge or close.
3. Go to the **Measure tab** and choose the **Open** or **Close** options from the *Relative Measurements* group.



The **Open** tool will combine the two objects for your count results without changing the actual area of the two objects. The drop-down arrow next to the **Open** icon lets you specify the incremental amount to grow or shrink the object.

The **Close** option will use a morphological filter to close the space between the two objects, thus changing the total area of the counted object.

Chapter 9

Customizing the User Interface and Automating Processes

This chapter provides instructions for the many ways you can customize the *Image-Pro* application interface. You can use a Japanese version of the user interface. *Image-Pro* allows you to set up the interface in the most efficient way for your work. You can also use macros, apps, and batch processing to speed up various tasks.

Using the Quick Access Toolbar

The *Quick Access Toolbar* appears at the top left of the *Image-Pro* interface.

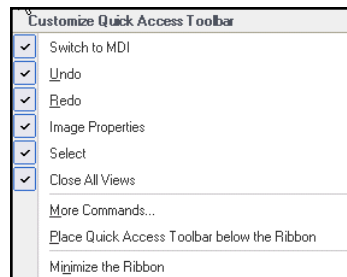


The *Quick Access Toolbar* allows you to display icons that, when clicked, execute a system-supplied or user-defined command. In this way, the *Quick Access Toolbar* provides a shortcut to commonly used functions.

To Add a Shortcut to the Quick Access Toolbar

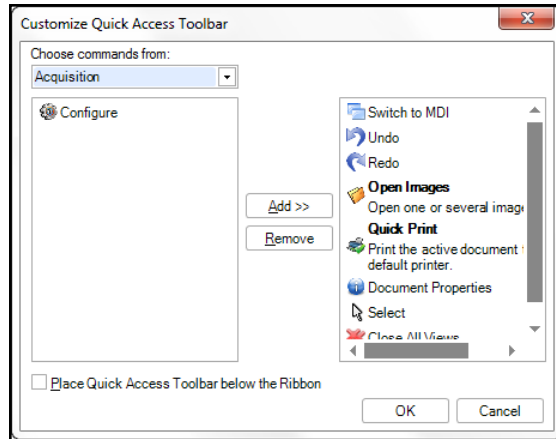
1. Click the drop-down button next to the Quick Access Toolbar.

The following menu appears.



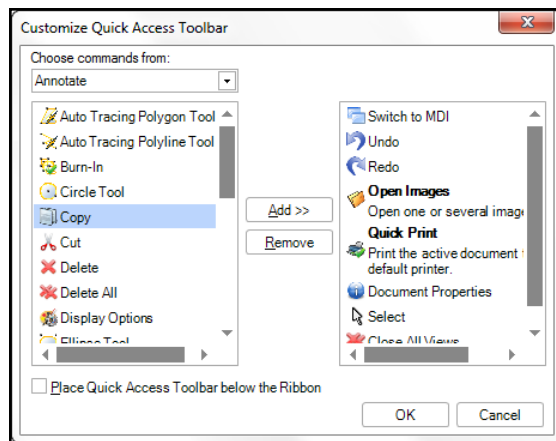
2. Click on **More Commands** in the drop-down menu.

The *Customize Quick Access* dialog box appears.



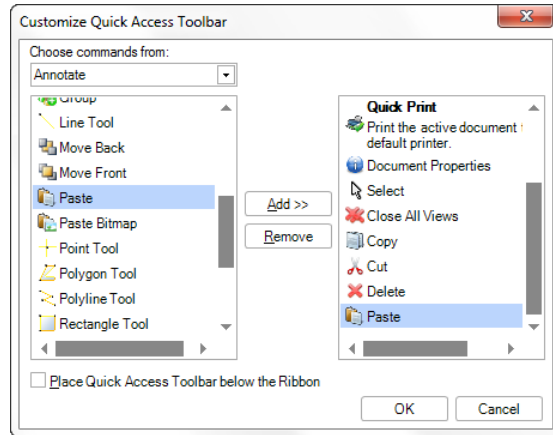
The list box on the left displays all the available commands and icons. The list box on the right displays those commands and icons currently displayed in the Quick Access Toolbar.

The **Choose commands from...** drop-down list displays different groups of available commands, as shown here:



3. Select a group of commands to display in the list box.
4. In the list box, highlight the command you want to add to the *Quick Access Toolbar*.

For example, if you regularly use the **Paste** command in *Image-Pro*, you can make a shortcut that performs a paste by adding a **Paste** command and icon to the *Quick Access Toolbar*.



5. Click the **Add** button and the command appears in the right hand list box.
6. Click on the **OK** button.

The shortcut is added to the *Quick Access Toolbar*.



Customizing the Image Strip

The **Image Strip** displays thumbnails of all opened images in the application and provides an easy way of activating and deleting images. The **Image Strip** controls are located in the *Workspace* group on the **View** tab's ribbon:

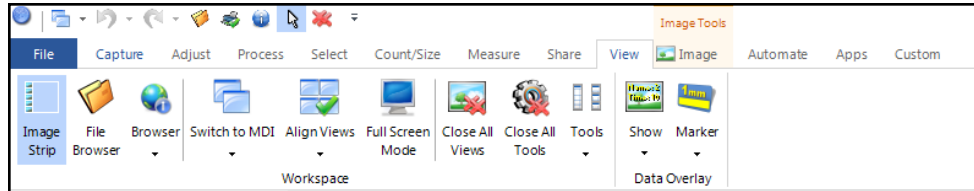
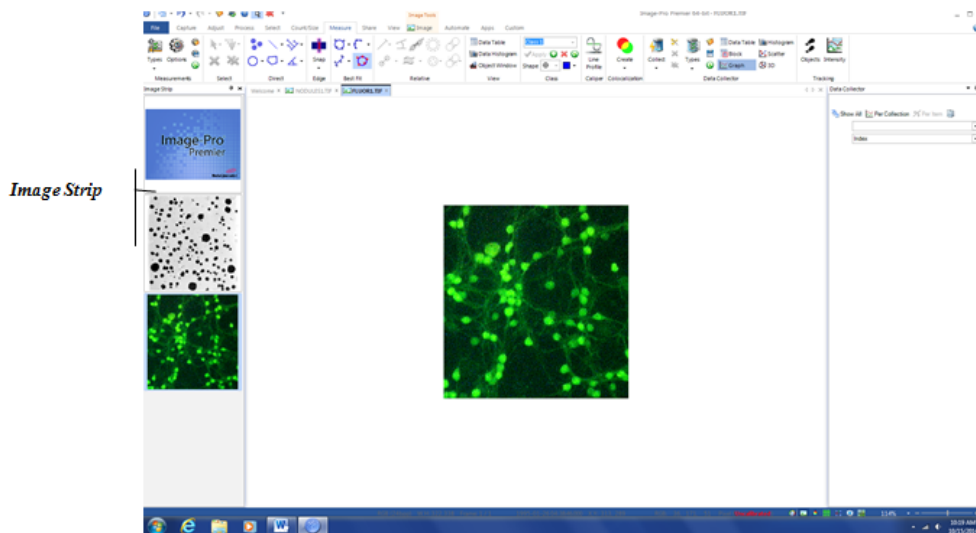
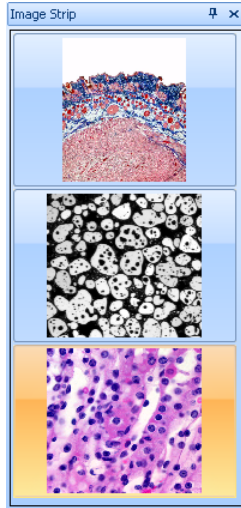


Image-Pro opens the image strip automatically when you launch the application. It appears on the left side of the application window.

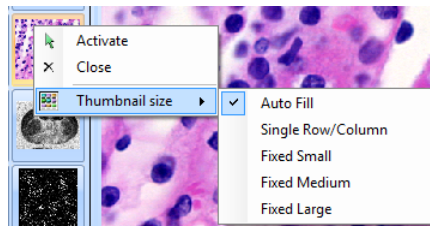


If you have several images open, the image strip will display thumbnails of all of them:

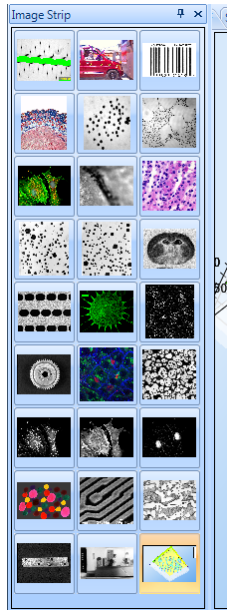


The layout of the images can be selected from the context menu.

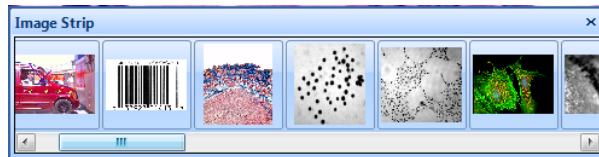
- Click the right mouse button on the image thumbnail to see the context menu.



- **Auto Fill:** all thumbnails are fitted to the strip window.

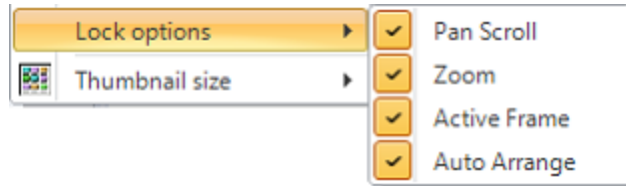


- **Single row/column:** when this mode is activated the thumbnails are resized to fit the width or height of the window. Scrollbars become visible if the number of images exceeds the size of the window.

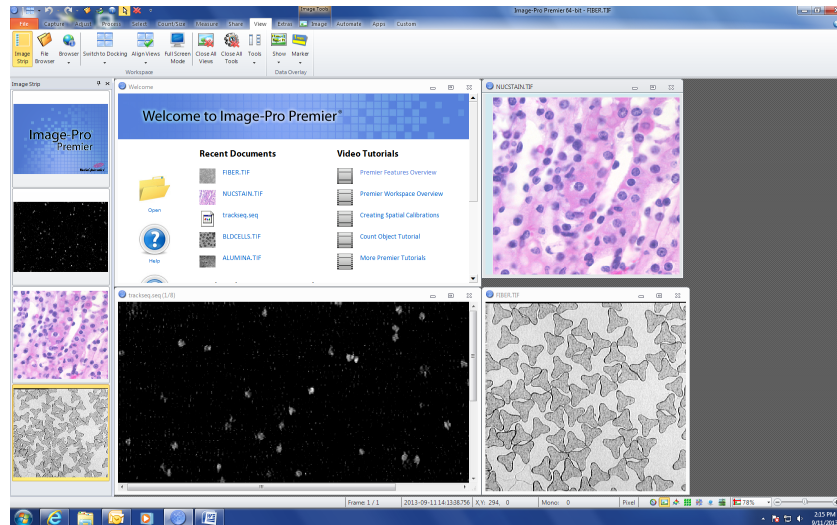


- **Fixed Small:** thumbnails are 64x64 pixels
- **Fixed Medium:** thumbnails are 128x128 pixels
- **Fixed Large:** thumbnails are 256x256 pixels
- **Lock/Unlock selected windows:** Select this option to lock the image windows for the currently selected thumbnail(s). The lock will disallow panning, zooming, or bringing other image windows to the foreground, depending on what is selected for Lock Options.

Lock options: Allows you to select what you want locked when the Lock/Unlock selected windows option is activated.

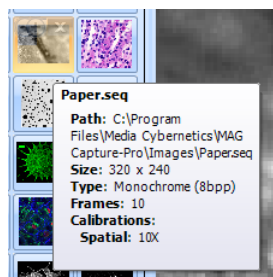


- **Pan Scroll** : Synchronizes image pan and scroll positions
- **Zoom**: Synchronizes magnification of images
- **Active Frame**: Synchronizes active frame index on all locked images
- **Auto Arrange**: When this option is on the locked images will be arranged on the in the application workspace to be visible. For example if you lock 4 images, they will be arranged in 2 rows and 2 columns. The option arranges only images to be locked. If the workspace contains more images, other images will be placed in the last pane below the last locked window, as shown here:



Viewing Tooltips

Tooltips show the basic image information:

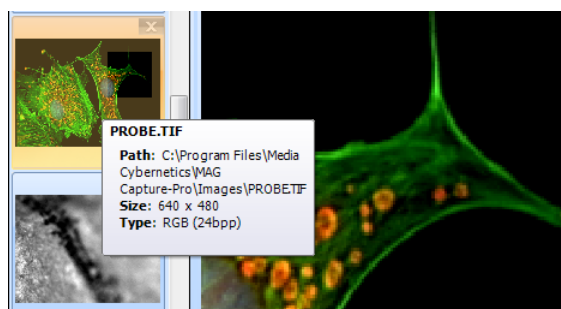


- To view an image tooltip, move your mouse cursor over the image thumbnail.

The **zoom and pan controls** allow you to resize the image or a portion of the image.

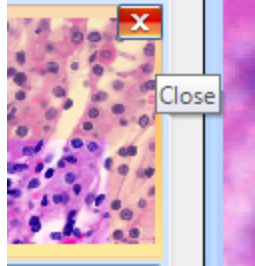
When the active image is zoomed and doesn't fit in the viewer, the viewing area is shown on the image strip thumbnail as a semitransparent orange overlay.

- Click and drag the center of the viewing area on the thumbnail to control pan and scroll of the image.



Activating and Closing

1. Click on a thumbnail in the image strip to activate the corresponding window.
If the thumbnail represent a surface plot or 3D view (or other viewer) clicking will also update the thumbnail.
2. To close the image, click the Close menu item from the context menu, or click the X button:



To remove an image from the image strip, select that image and click the **<Ctrl>** or **<Shift>** keys. More than one image can be removed at the same time using this method.

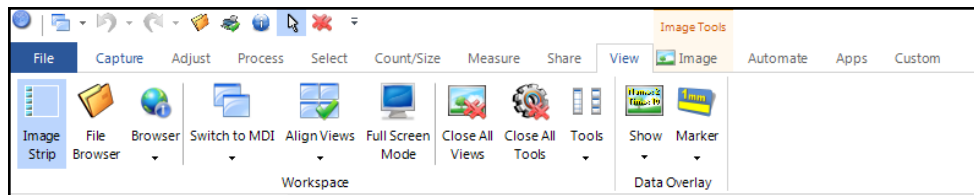
Using the interface customization capabilities provided by *Image-Pro*, you can customize the *Image-Pro* toolbars by:

- Moving, docking, and undocking toolbars
- Showing/hiding toolbars
- Creating new toolbars
- Adding/deleting buttons from existing toolbars.

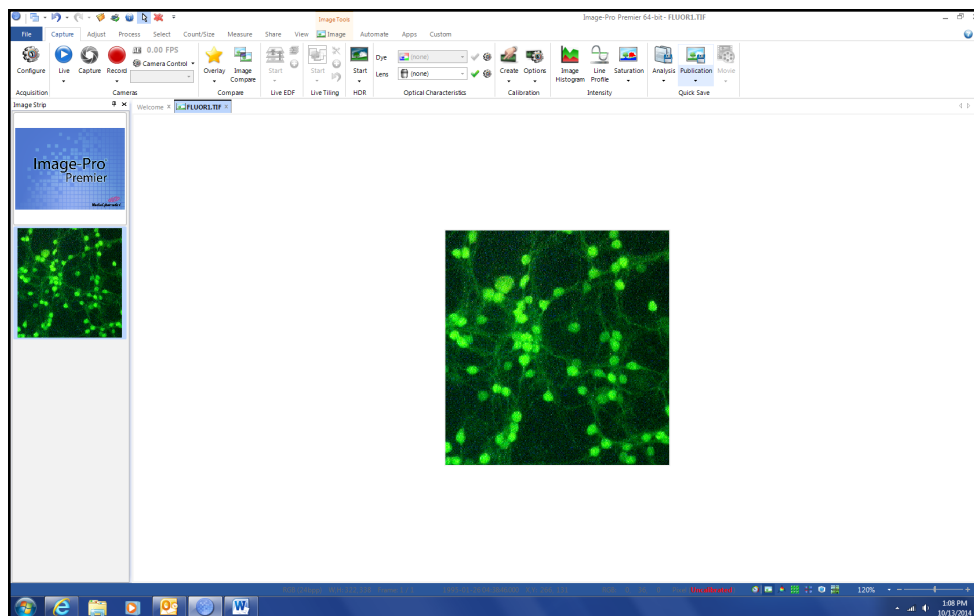
Using the Multiple Document Interface (MDI)

The **Switch to MDI** button will toggle the workspace windows to MDI windows or back to tabbed windows. This feature allows you to view more than one image at a time, and move the images and panels to different locations in the application.

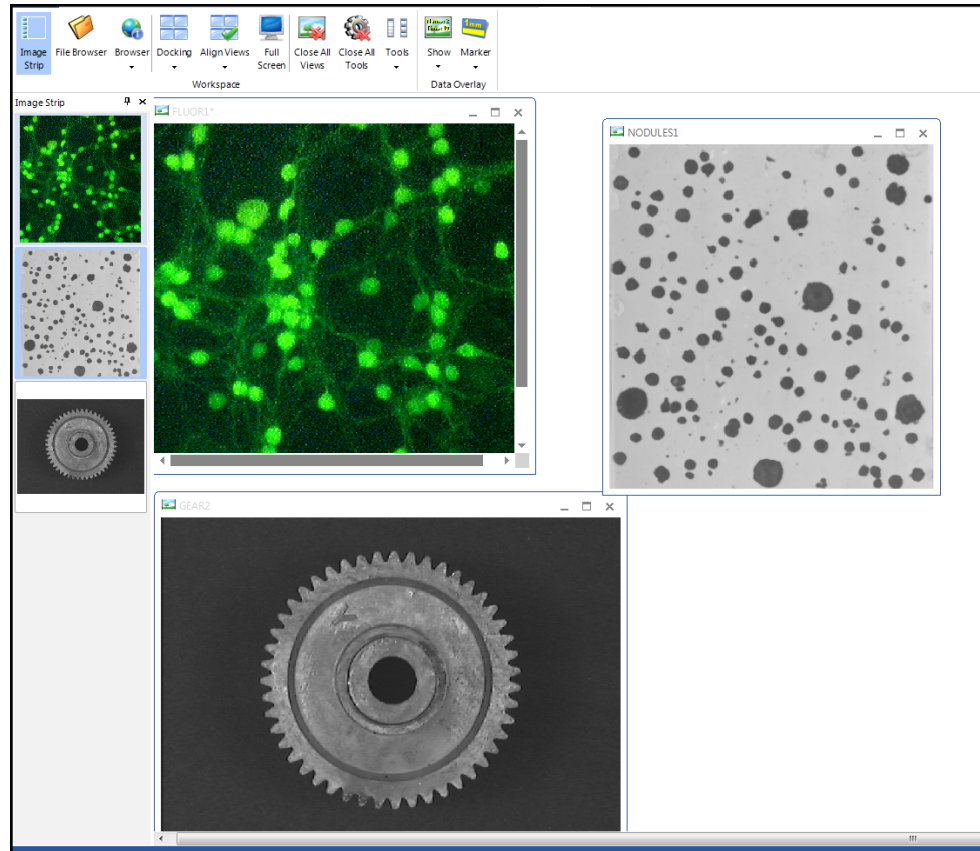
This feature is located in the *Workspace* group on the **View** tab's ribbon:



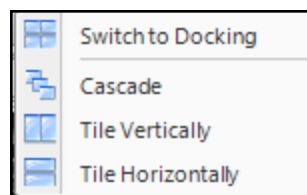
Normally, when the workspace windows are set to **Docking**, the image appears like this:



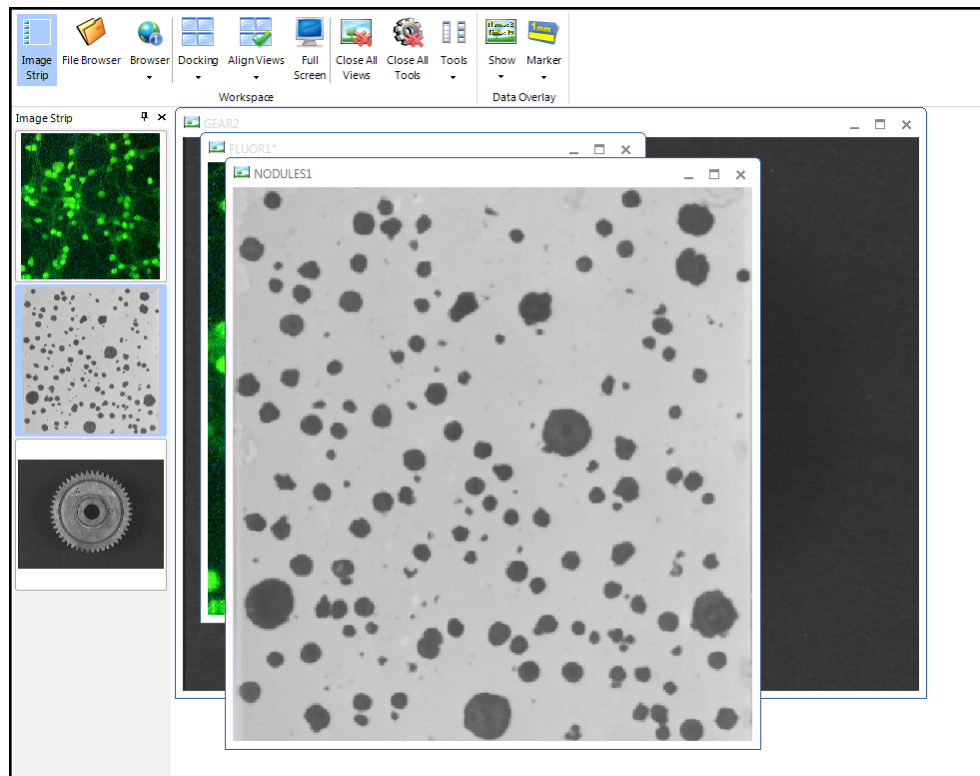
When you switch to the MDI view, several images can be viewed at once, as shown here (these are shown in the **Tiled** layout):



The pull-down MDI menu allows you to select window display:



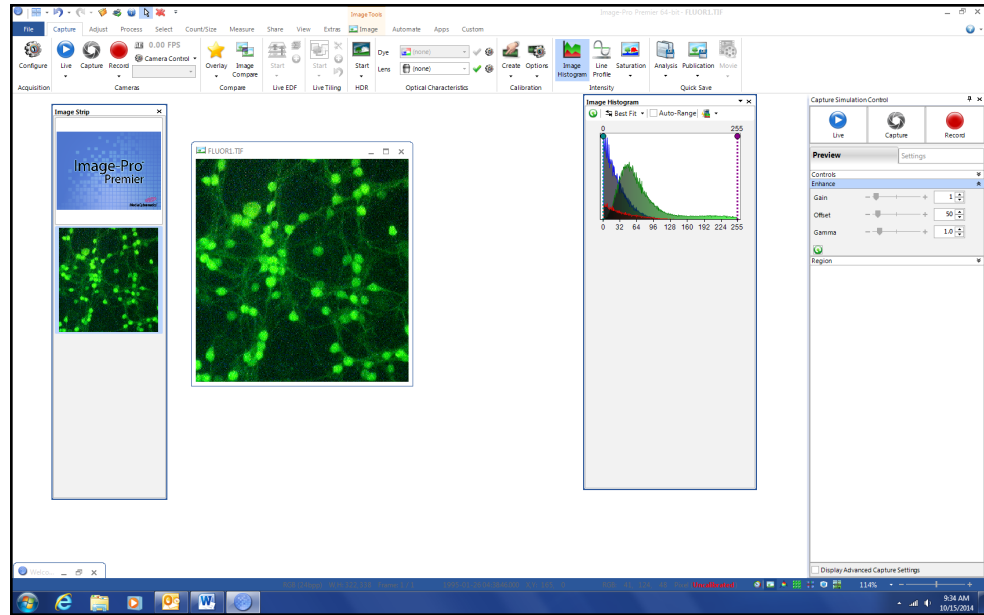
This version shows image windows in the **Cascade** layout:



Floating Panels

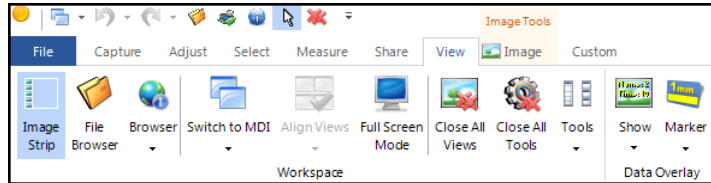
One of the unique features of *Image-Pro* is the “floating” or “dockable” panels. The image strip, details panel, mini-histogram panel, or other workspace areas can be moved independently from the main image workspace. This may be particularly useful when working with a dual-monitor system, as you may want to display the image on one monitor and the details on the other. An example of floating or “undocked” panels appears here:

Chapter 8



Tools and Layouts

The **View** tab contains specialized tools and graphs to display information about the active image or image set. Some of these tools allow you to see different aspects of the active image also.



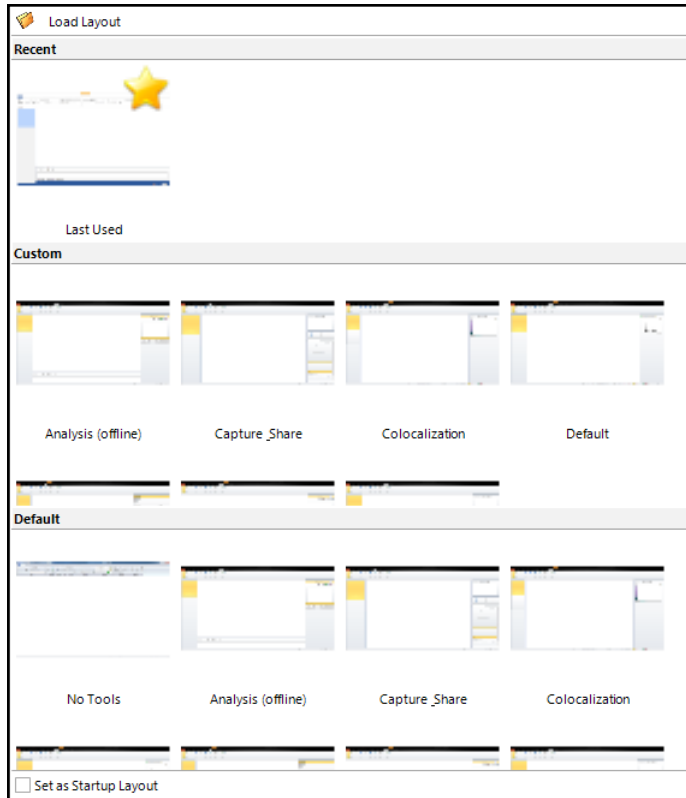
The **Tools** in the **Workspace** group allows you to load and save custom tool sets and layouts for your specific situation. Click the drop-down arrow to see these features



Redock All Tools: Resets any open moveable panels that are undocked to their default docked position.

Reset Tools Layout: Resets the application to its factory default tools layout.

Load Layout: This tool allows you to load a previously-saved tools layout. When you click on this tool, the following dialog is displayed:



This dialog shows all available tool layouts to be loaded. The **Recent** area shows a list of the last loaded layouts. The **Custom** area shows a list of the custom layouts currently stored in the Configuration Files folder. The **Default** area shows a list of the available pre-defined layouts from installation directory \Resources folder.

SaveLayout: Click this tool to save the current layout of tools to a file that can be loaded at a later time to restore the layouts to its current configuration. When you click this option, you are prompted to give the layouts configuration file a name. Type a name for the layout configuration file, then click **Save**. You can use the **Load Layout** tool (described above) to restore the tools layout to its current configuration at a later time.

Creating a Custom Tab

One useful feature of *Image-Pro* is the ability to create a **Custom** tab on the ribbon. It can contain any of the features you use most frequently, saved in one place.

By default, the **Custom** tab appears on the far right of the ribbon, but you can control its placement in the application **Options**. The default placement is shown here:

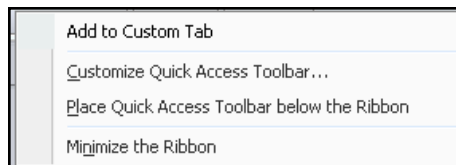


The first time you open it, the *Custom* tab's ribbon will be empty. You can add any group or tool to it, as described here:

1. Decide which tools or group you want to add to the **Custom** tab. For example, *Annotate*:

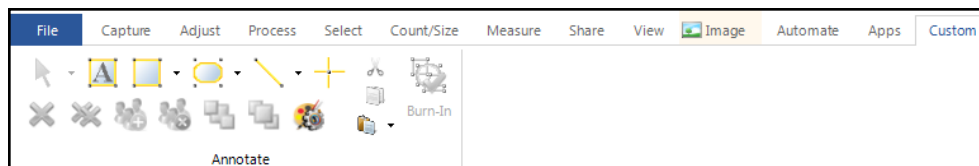


2. Right-click the mouse button in the *Annotate* group. You will see the context menu:

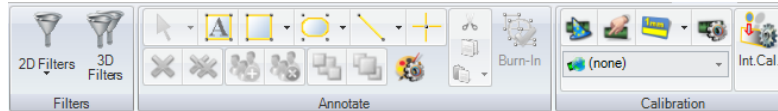


3. Choose **Add to Custom Tab**.

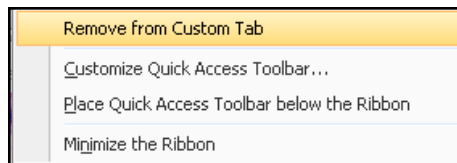
The *Annotate* group now appears in the **Custom** tab's ribbon:



You can drag the groups into different positions on the ribbon. The image below shows that the *Calibration* and *Filter* groups have changed position:



- To remove an item from the **Custom** tab, click **Remove from Custom tab** in the context menu:



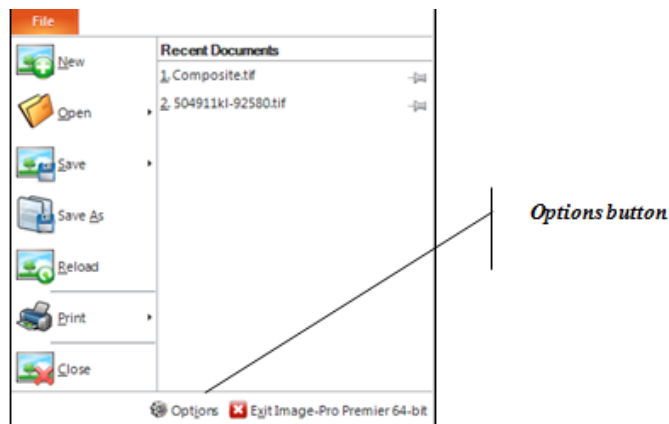
Rename and Make your Custom Tab the First Tab

If you want to give the **Custom** tab a new name, and make it the first tab that a user sees, use the following steps:

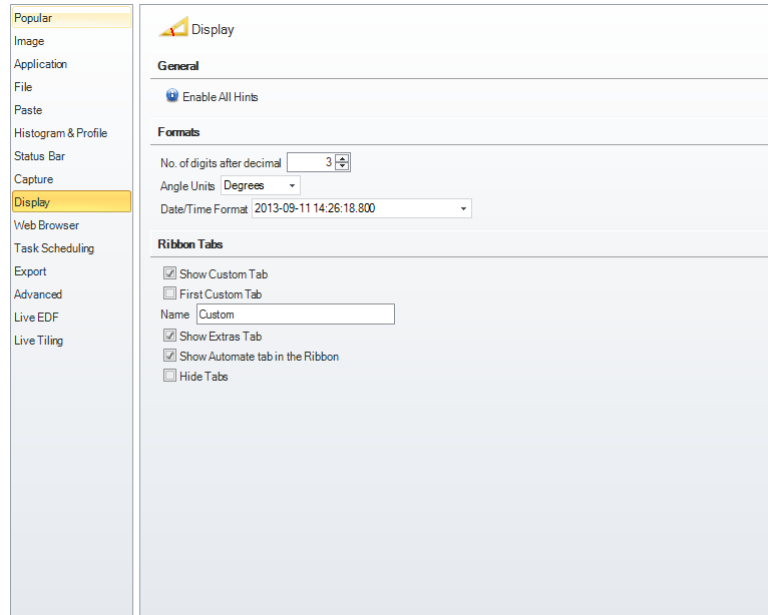
- Click on the File button in the top right corner of *Image-Pro*:



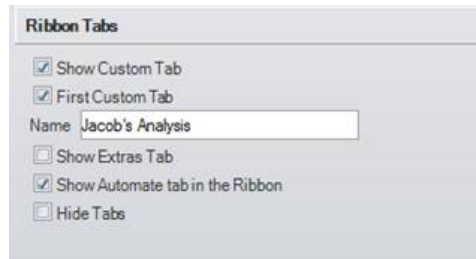
- Choose the **Options** button at the bottom of the menu.



- From the **Options** dialog, click on the *Display* section.



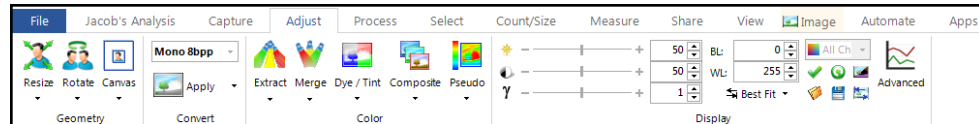
4. In the **Ribbon Tabs** area, select *First Custom Tab* to display your custom tab as the first tab.
5. Type in a new name for your tab in the *Name* text box



6. Press **OK** at the bottom of the **Options** dialog to apply the changes.

Your new tab should appear first in the tab list with its new, custom name.

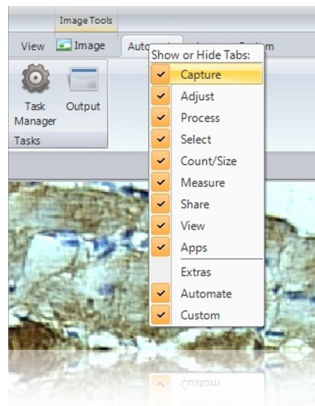
In the example below, we have created a new tab, named *Jacob's Analysis* which is displayed first in the ribbon.



Hide or Show Tabs

Image-Pro allows you to hide or show the tabs to display only the tools you need. To use this feature:

- Right click on any of the tabs to see the **Show or Hide Tabs** dialog.



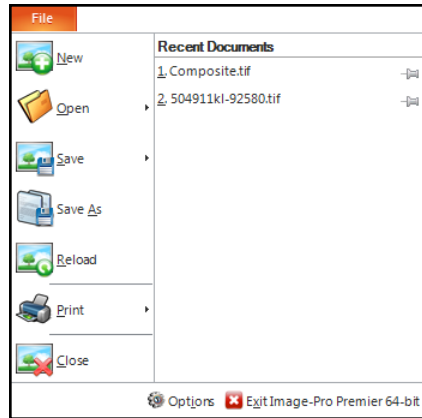
Tabs with an active check mark will be displayed. Those without will be hidden from view

For some situations, you may find it most effective to hide all of the other *Image-Pro* tabs and focus only on the **Custom** tab or a specific App. To hide the tabs, use the following steps:

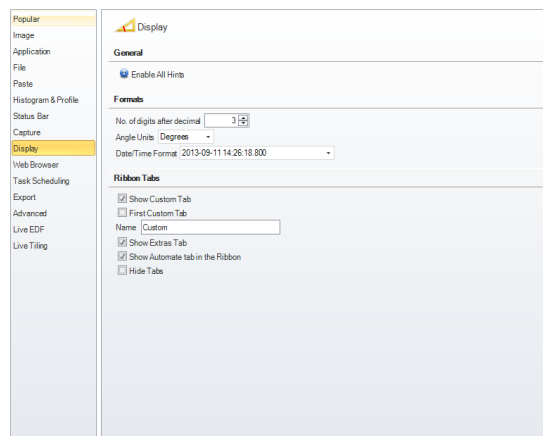
1. Click on the **File** button in the top right corner of *Image-Pro*.



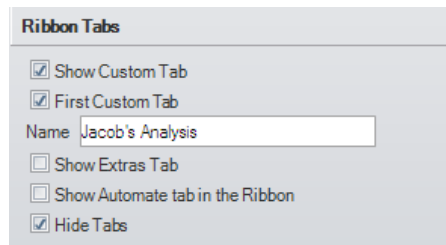
2. Choose the **Options** button at the bottom of the menu.



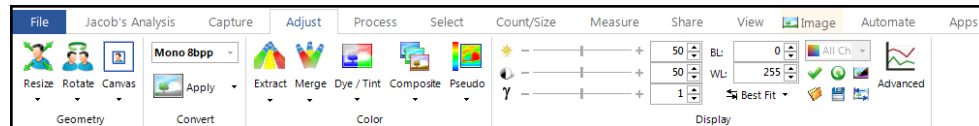
- From the **Options** dialog, click on the *Display* section.



- Under the **Ribbon Tabs** section, select the *Hide Tabs* option and make sure **Show Custom Tab** is checked.



- Now you will see that just the **Custom tab** is shown. If you happen to have an image opened, you will also see the contextual **Image** tab, which is displayed when an image is opened.

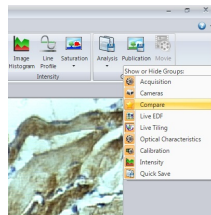


Showing or Hiding Groups

You can also hide or show *Image-Pro* tool groups to only display the tools that you need for your image analysis work. To use this feature, follow these steps:

- Right click on the open space in the ribbon bar.

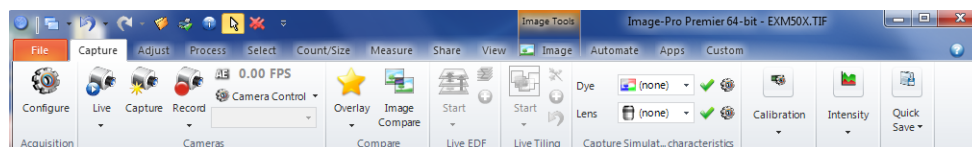
Tip: Click on the far right side of the workspace. In the example below, you would click in the grey area to the right of the “Movie” button.



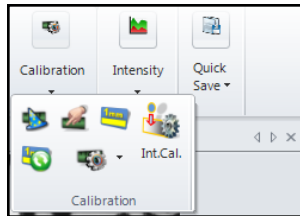
- You will see the **Show or Hide Groups** dialog. Groups that are highlighted will be displayed. Toggle this feature on or off to show/hide groups.

Expanding and Collapsing

Sometimes, you may need to resize the *Image-Pro* workspace to make it smaller. When you do, you will notice that the groups on the ribbon collapse and some of the tools are hidden. In the example below, the *Calibration*, *Intensity*, and *Quick Save* groups on the right side of the **Capture** tab's ribbon are collapsed:



To expand and see the group's controls, click the drop-down arrow, as shown here:



Users are encouraged to fully expand the *Image-Pro* workspace when at all possible, so that all the tools and features are available.

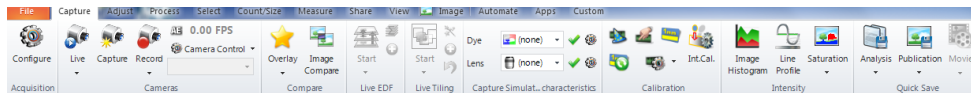
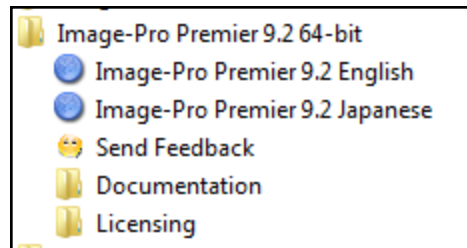


Image-Pro in Japanese

Image-Pro is also available with a Japanese-language user interface. It is installed along with the English-language version from your electronic download or product CD.

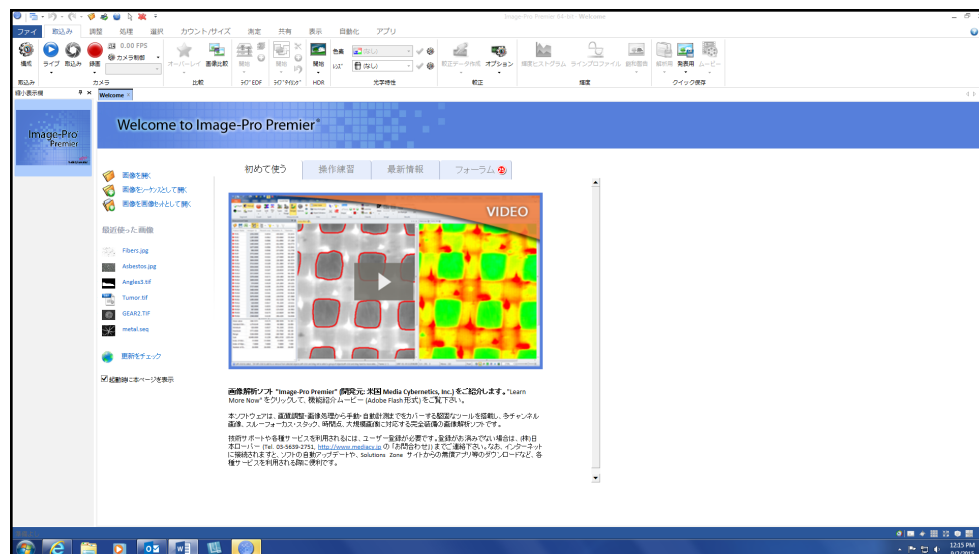
To use the Japanese version, follow these steps:

1. Go to your **Start** menu and select *Image-Pro*:



2. Choose *Image-Pro 9.3 Japanese*.

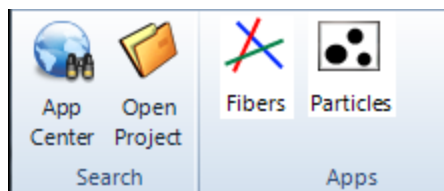
After a moment or two, you will see the Japanese version of the application.



All about Apps

Apps are little applications that run inside *Image-Pro*. Like mini-programs, they have their own user interfaces and they automate complex tasks in and through *Image-Pro*.

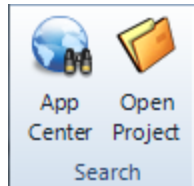
When you first open *Image-Pro*, the **Apps** group of the *Apps* ribbon will be empty. When an app has been loaded (as described in the next section), it appears on the **Apps** group, as shown here:



Loading an App

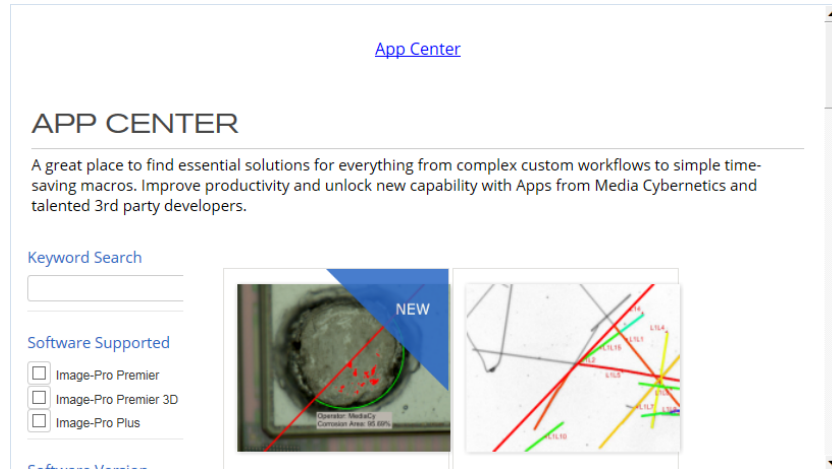
Image-Pro allows you to load pre-recorded apps, projects, and macros that you may find useful. To add an existing App (project or macro), follow these steps:

1. Go to the *Search* group on the **Apps** tab in Image-Pro.



2. If you want to load one of the pre-recorded apps provided by *Media Cybernetics*, click the **App Center** button.

The browser will take you directly to the *Apps* portion of the website:

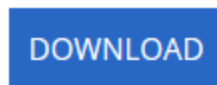


You will see a list of available apps.

3. Click the name of the app you want to use.

You will see a description of the app in the browser window.

4. Scroll to the bottom of the browser window, and click the **Download** button.



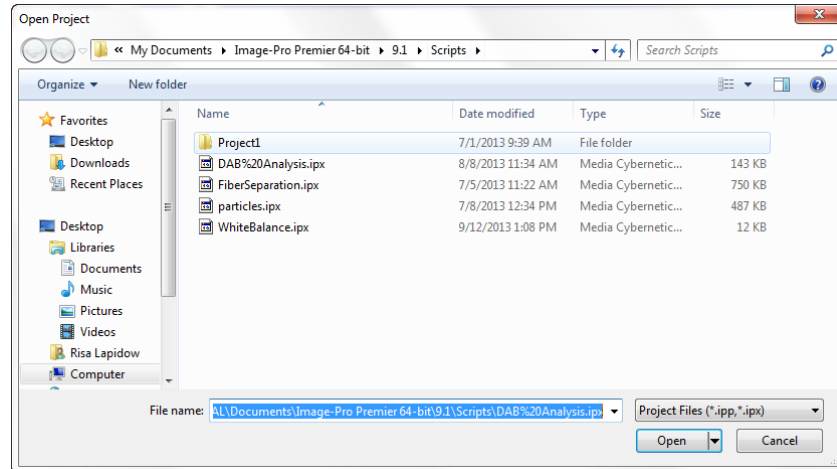
5. You will be asked to accept the license agreement. Click **I understand and accept the user agreement**.



Apps and projects are stored in the **Scripts** folder of *Image-Pro*. Apps have the extension *.ipx.

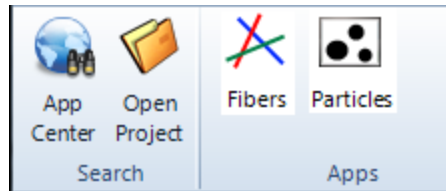
6. Click **Save**.

You will see the **Open Project** dialog.



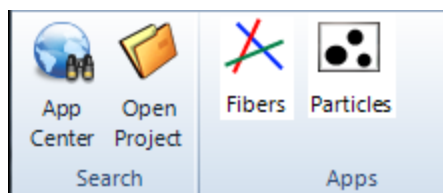
7. Select the app you want to load, and click **Open**.

Your app appears in the **Apps** tab:



Running Apps

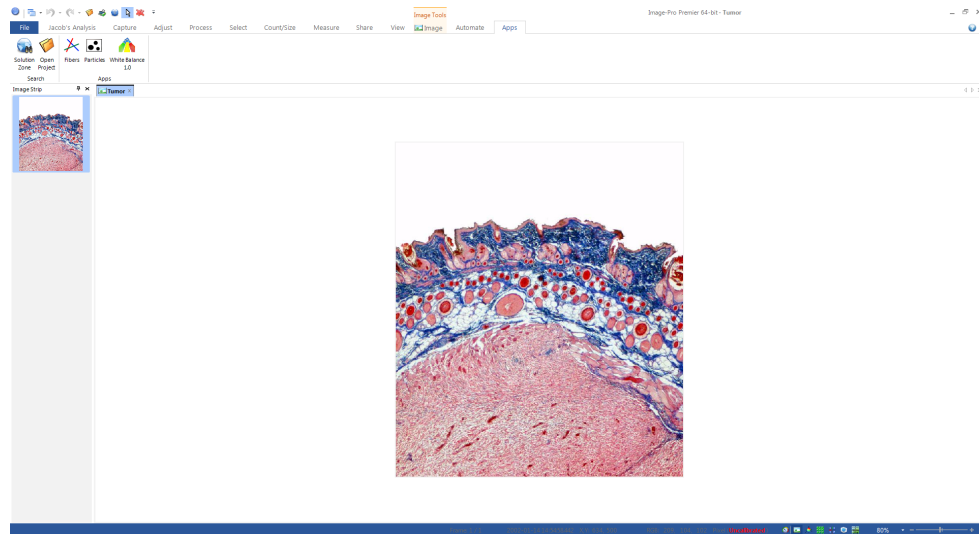
Once a project is loaded, its app appears in the **Apps** ribbon.



It can be started by clicking the appropriate button. To run the sample app, follow these steps.

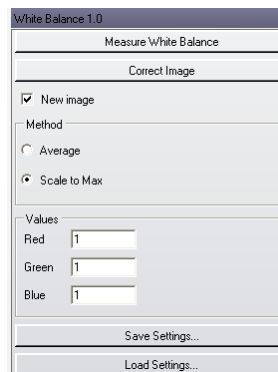
1. Open the image you want to process in the workspace. This example shows `tumor.tif`.

Chapter 8



2. Click the name of the app you want to run. This sample app is called *White Balance*.

The contents of the app appear in the right-side panel:

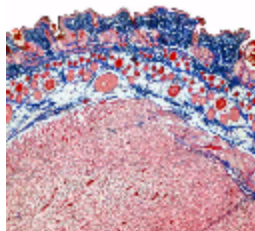


3. Click **Measure White Balance**.

The first step in the app appears.



4. Follow the instructions, and click **OK**.
 5. Click **Correct Image**.
- A new, corrected image appears in the workspace.

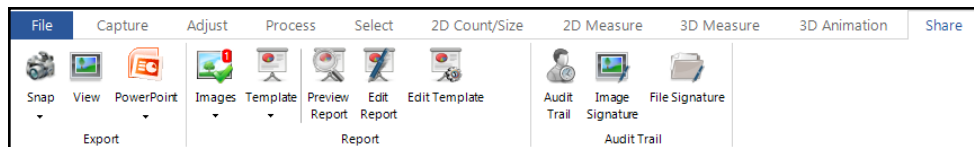


Chapter 10

Generating and Printing Reports

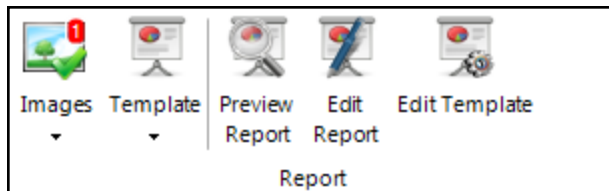
This chapter explains how to create and print reports in *Image-Pro*.

The *Report* tools are located on the **Share** tab's ribbon:



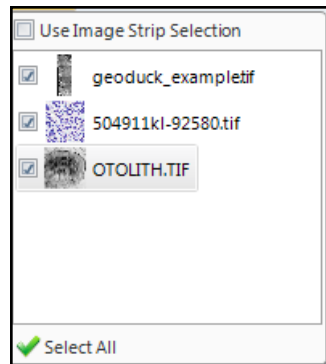
Creating and Printing Reports

Image-Pro allows you to create reports from images and data saved in your application. The *Reporting* feature uses an interface that is similar to *Microsoft Word*. You can display your data in any of several predefined layouts, and then send them to an output window, print device, or a file. To begin creating your report, go to the *Report* group on the **Share** tab's ribbon:



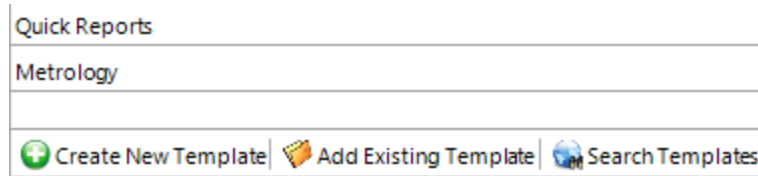
1. To begin creating a report, click the **Images** button.

The red number superimposed on the **Images** button indicates the number of active images. You will see the drop-down menu with all the available images on it. Instead limiting you to just the document open in the workspace, this feature allows you to select any document loaded in the software.

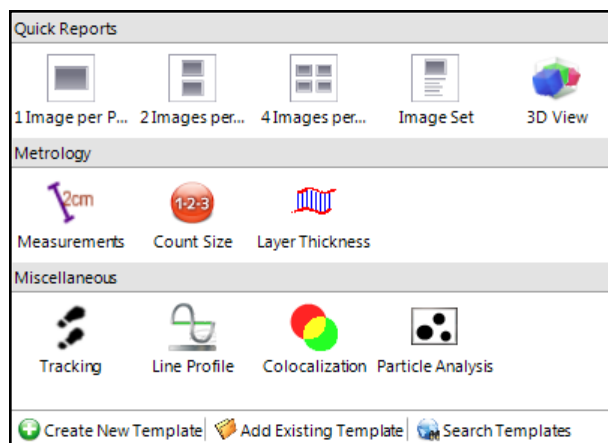


Images with a check next to them are available to include in a report. When the **Use Image StripSelection** option is checked, this selection will track and match the changes to the images in the image strip. The **Select All** option will ensure that all entries in this list are selected. Using the **Select All** option or adjusting the checkmark will disable the **Use Image Strip Selection** option and visa-versa.

2. Next, click the **Templates** drop-down arrow to select your layout .



3. Several predefined report layouts are included with *Image-Pro*:



4. Choose a format.

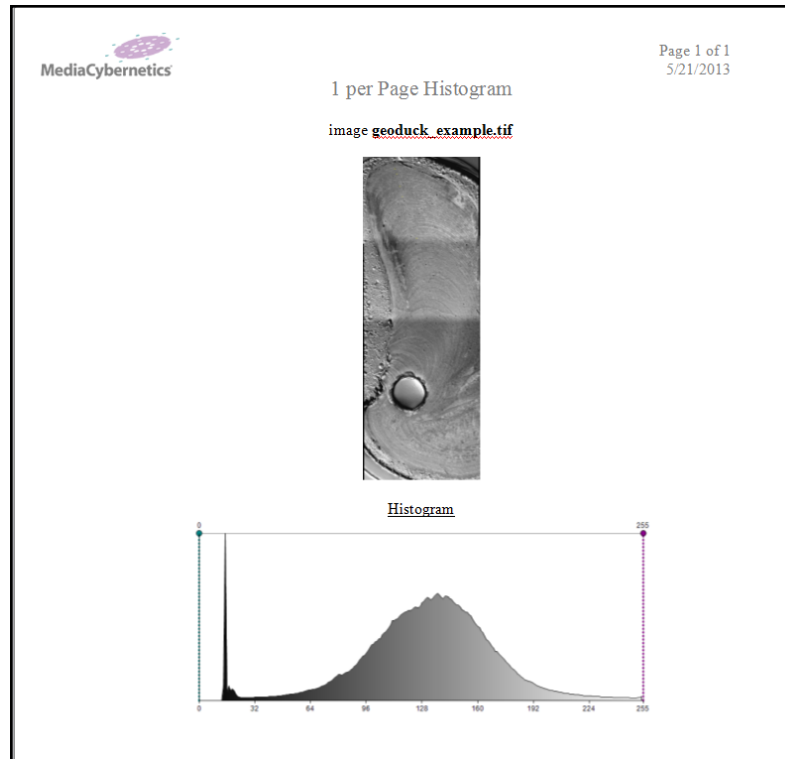
The *Image-Pro* reports are loosely designed for an A4 or letter paper output size. The sizing of the content and the naming of the reports are based on the output page size.

Once you have selected your documents and report template, you have the following options:

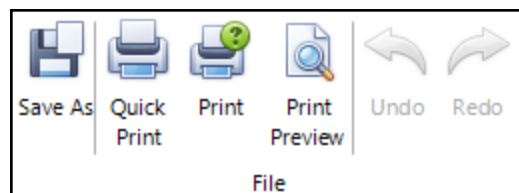
- **Preview the report.** In this case the resulting report is read-only and can only be saved (in Word, PDF or HTML format) or printed. The edit controls are disabled.
- **Edit the report.** The resulting document is loaded and contextual ribbons can be used to customize it further before saving or printing. The editing controls are similar to those available in Microsoft Word.
- **Edit the report template in the *Project Workbench*.** A copy of the report template itself is loaded for editing the report content.

5. Click **Preview Report**.

The hourglass icon will appear, indicating that your report is being created. When finished, your report will appear in the application's workspace:



6. If you are satisfied with the appearance of the report, go to the *File* group on the **Report** ribbon.



7. Select **Save As** to save your report as a PDF. Choose **Quick Print** or **Print** to print a paper copy.
8. To edit your report, close the sample report.

9. Click **Edit Report**.

You will return to the **Report** ribbon with the editing tools activated:

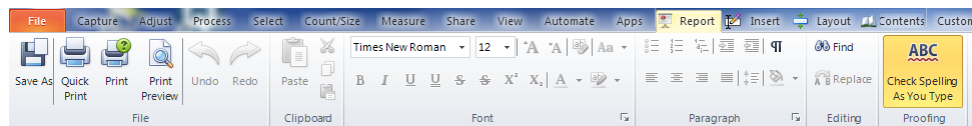


From here, you can use the **Report**, **Insert**, and **Layout** ribbons to adjust the content and appearance of your report.

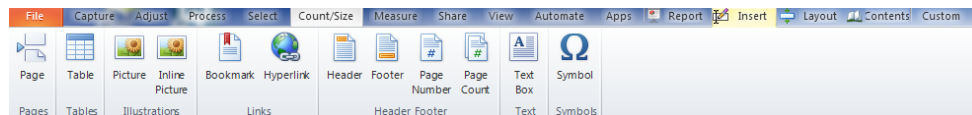
Report Tools

Once a report is created, you have the option to add specific content without leaving *Image-Pro*. The report tools use the familiar ribbon tabbed layout.

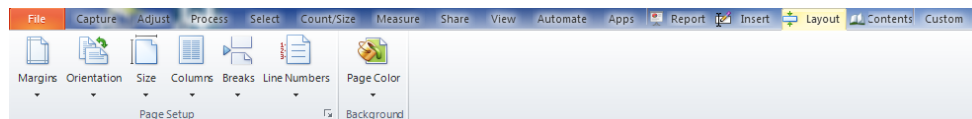
The **Report** tab's ribbon contains controls for saving, printing, and formatting text.



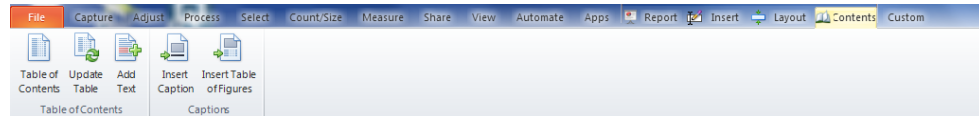
The **Insert** ribbon allows you to add pictures, illustrations, page numbers, and hyper links to the body of your report. You can also add and edit headers, footers, and text boxes using these controls.



The **Layout** tab's ribbon lets you adjust the page size, color, margins, and text orientation.

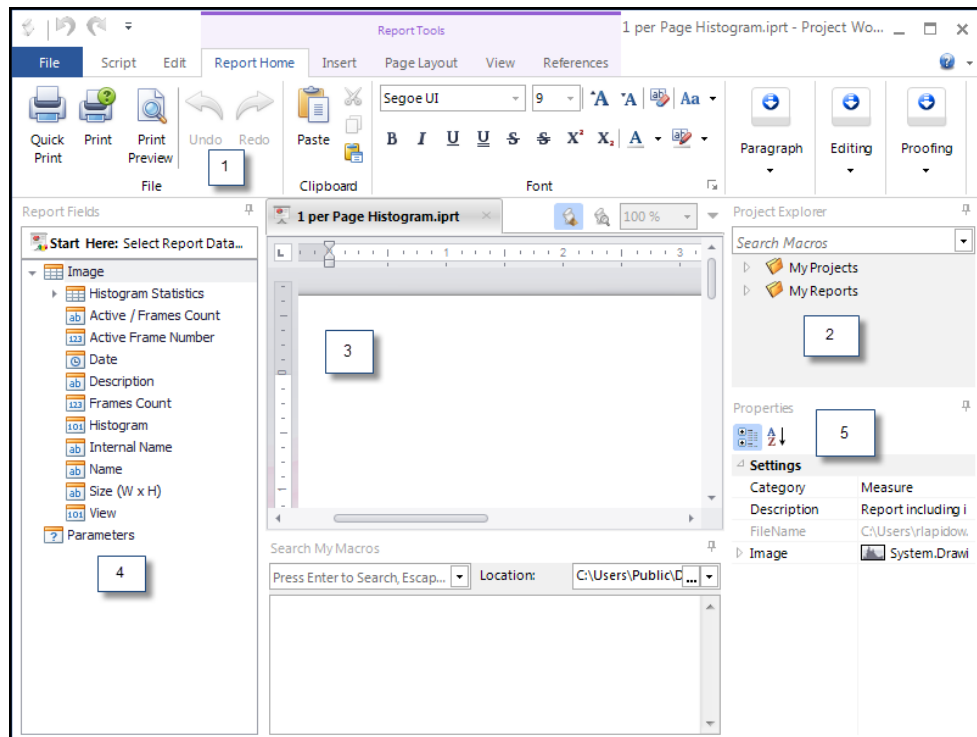


The **Contents** ribbon lets you add a table of contents to your report. You can also add captions to tables and figures using these controls.



Reporting in the Project Workbench

An alternative to the *Share* tab is the *Project Workbench* which appears on the *Automate* tab. You can use the image strip thumbnails to select images for your report. To create the report, double-click on the image or use the *Project Workbench* context menu, or select *Edit Template* on the *Share* tab.



The reporting features available in the *Project Workbench* are detailed below:

1. The *Report Tools* in the ribbon.
2. The *Project Explorer*, where reports can be loaded and created.

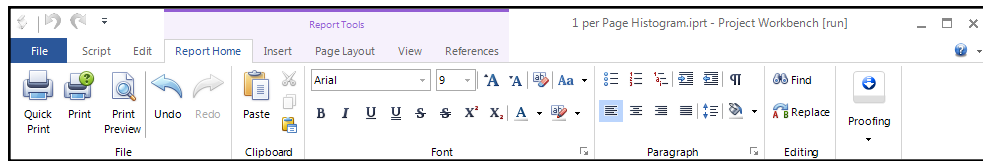
3. The *Report Designer*, to lay out the various report components as in an *MSWord* document. A context menu is also provided.
4. The *Report Data* and *Report Fields*, similar to the Toolbox allow selecting the report data and dragging data items to the report designer.
5. The *Properties Panel* is to configure reports and their components.

These features are explained in more detail in the next section.

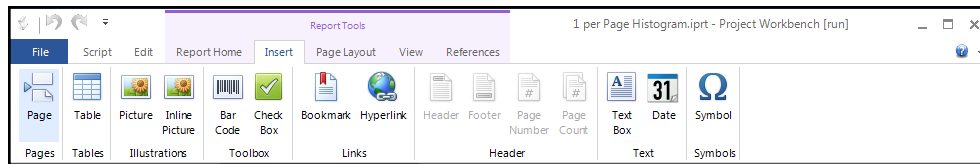
The Report Tools in the Project Workbench

The ribbon's *Report Tools* in the project workbench are similar to those in the main *Image-Pro* ribbon with the addition of a few ribbon tabs.

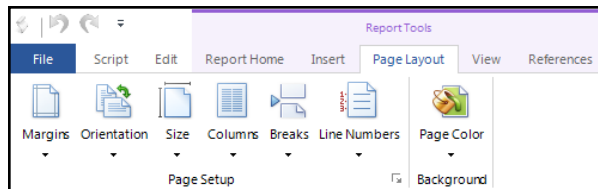
The **Home** tab contains the basic tools for creating and editing reports.



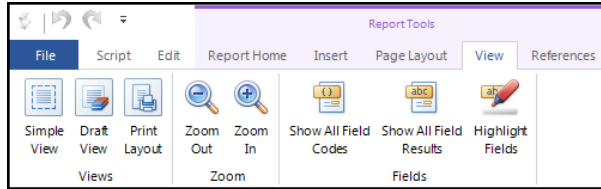
The **Insert** tab lets you add tables, symbols, dates and other specialized details to your report:



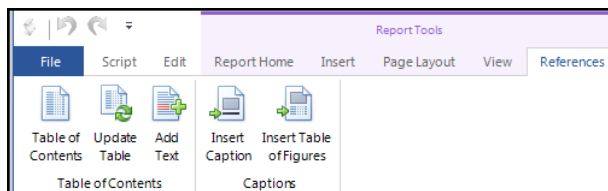
The **Layout** tab controls table cells, columns and rows. You can adjust the background color here also.



The **View** tab controls the aspect of the report template as well as the visibility of report fields.

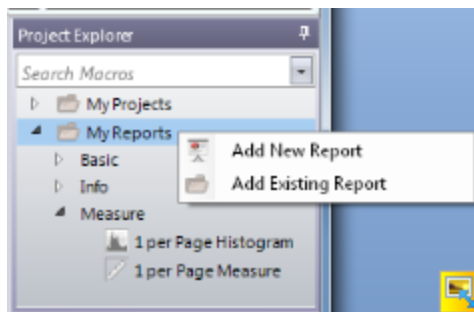


The **References** tab lets you add a table of contents, table of figures, or captions to your report.



Project Workbench

The *Project Workbench* now has a section called *My Reports* where known reports are listed (those appearing on the **Share** tab).

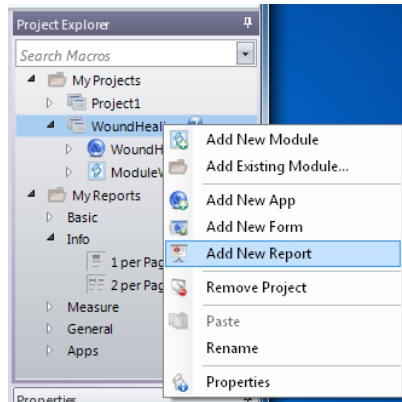


Right-click on the *My Reports* folder to create new reports or load existing ones.

When you double-click on one of the reports, a report designer is loaded in the workspace area together with Apps and macros.

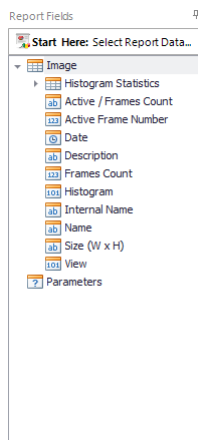
Reports as App/Project Components

In addition to being listed in the **My Reports** section, reports can also be added to projects. When projects are packaged, embedded reports become part of the package and are available for the App and macro code using simple commands.

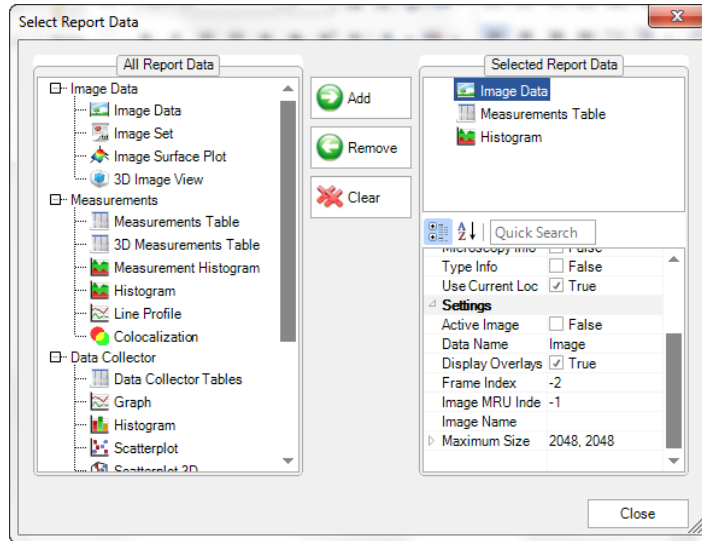


The Report Data and Report Fields panels

The **Report Fields** panel is displayed on the left-hand side of the Project Workbench, since it will be used most often. It reflects the actual content of existing reports.

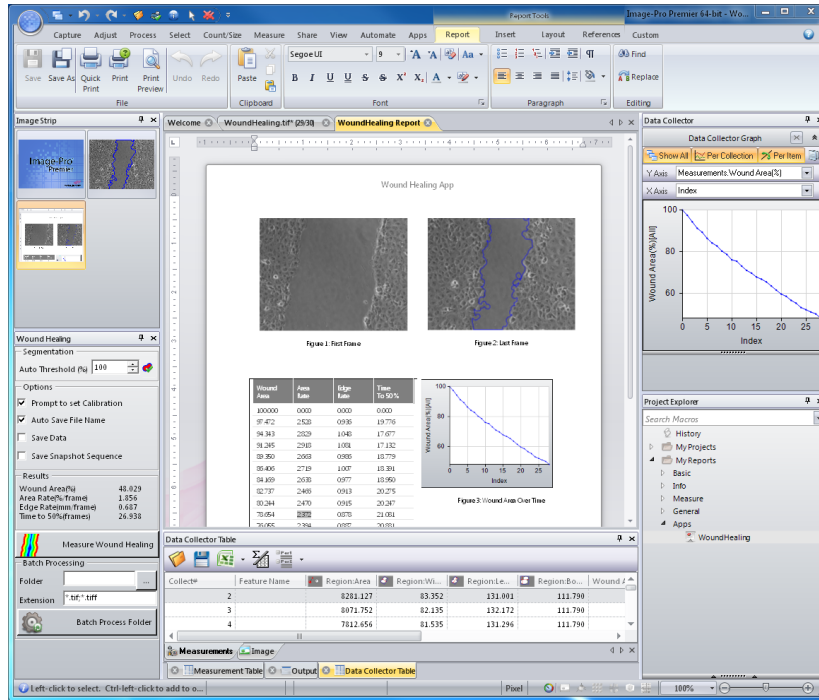


To add data to your report, click **Start Here**. You will see the **Select Report Data** dialog:



The **Report Data** panel is used to select and configure the data that will be available for the report and displayed in the **Report Fields** panel. You can select the individual fields using the **Add**, **Remove**, and **Clear** buttons.

The following example screenshot shows a sample report displayed at the end of the Wound Healing App:



Chapter 12

Using the 3D Features

Image-Pro with 3D Module includes the advanced **3D Viewer volume rendering**, **3D Measurements**, and **3D Animation** features. Volume rendering enables you to view an image stack, or a portion of an image stack, from any angle. You can also adjust the transparency and shading of the image. Animation allows you to create a moving image sequence. The measurement tools allow you to make manual and automatic measurements of object dimensions as 3D volumes.

In volume rendering, light emission and light absorption parameters are assigned to each point of the volume. Each of these points is called a *voxel*; that is, a 3-dimensional version of a pixel. Voxels have height, width, and depth, as well as intensity characteristics. Simulating the transmission of light through the volume makes it possible to display your data from any view in any direction.

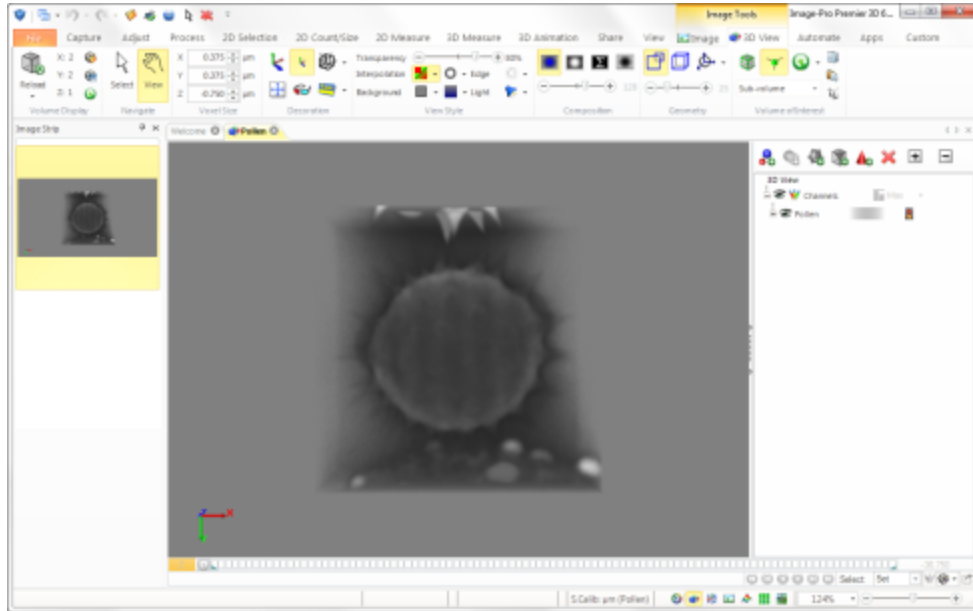
The *3D Viewer* window allows you to examine an image stack in a variety of different ways: by volume, in slices, and as an animated display. In the *3D Viewer*, all geometric objects are shown in 3D space. It can be regarded as a virtual camera which can be moved to an arbitrary position within or around the 3D scene.

Note: This chapter discusses the advanced 3D features of Image-Pro with 3D Module that are not included in the basic Image-Pro product. The 3D features of Image-Pro are discussed in Chapter 5, Working with Images.

Image-Pro with 3D Module requires a 64-bit computer and operating system, and also places greater demand on your operating system. Please see the Image-Pro with 3D Module system requirements in Chapter 1, Installing Image-Pro.

The Image-Pro with 3D Module Workspace

Once you have installed the *Image-Pro with 3D Module* software and opened an image sequence or image set containing on or more Z-stacks the workspace will look something like this:



If your image sequence does not include Z-position information, it may not load into the 3D Viewer automatically. In that case, you can use the 3D view button on the image context menu or in the status bar to switch to the 3D view.

When you open an image, image set, or image sequence in *Image-Pro with 3D Module*, an icon that indicates which view you have open appears next to the file name:



By default, a new volume is loaded in View mode, which allows you to click and drag anywhere in the 3D workspace to move the camera, effectively rotating the volume in the 3D space. The X/Y/Z axes that may be displayed in the bottom-left hand corner of the

workspace will show the current orientation of the camera with respect to the X, Y and Z axes of the volume. Use the controls described on the following pages to manipulate and analyze the image.


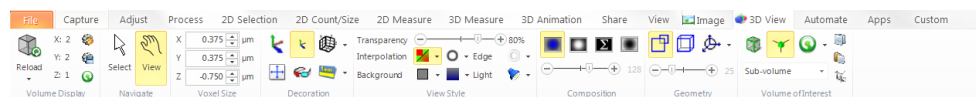
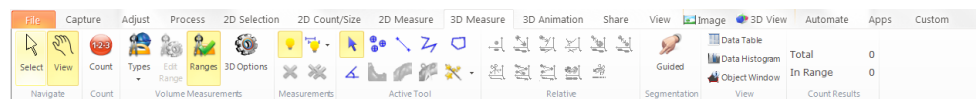
 Use the <F> key to check the performance of your 3D viewer on your graphics hardware. It may be difficult to manipulate the 3D view with less than 3-5 FPS (frames per second). Please refer to the system requirements in Chapter 1 for additional information.

Image-Pro with 3D Module includes three more tabs in addition to the tabs included in *Image-Pro*.

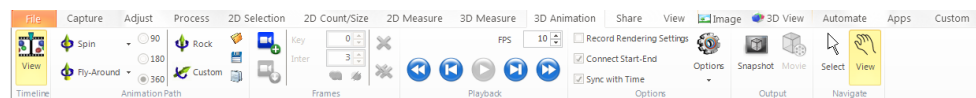
When the active view is using the **3D Viewer**, the **3D View** context tab will be displayed, which lets you load and save 3D rendering settings, and adjust a wide variety of 3D rendering settings that affect the display of your volumes:



3D Measure contains the tools and features you will need to make volume measurements:



3D Animation contains the tools for creating advanced animations and movies of your volume. The *Animation* feature allows you to create, record, and play animation files (*.anm) in the *Image-Pro with 3D Module* workspace



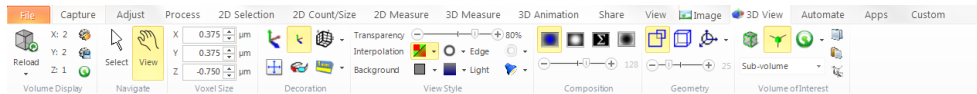
When you start *Image-Pro with 3D Module*, you will notice that the status bar has been rearranged:



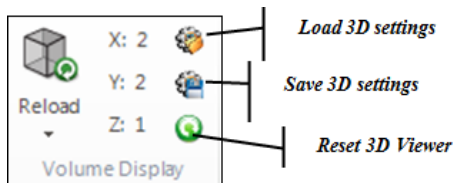
The icons for the 3D viewer and the Slicer views have moved to the start of the list of views on the image context ribbon, and in the tools portion of the status bar.

The 3D View Ribbon

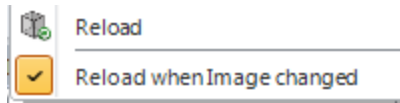
The **3D View** ribbon contains the following controls to view your image or sequence:



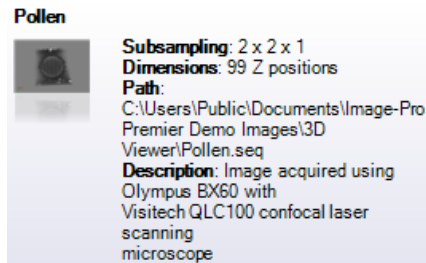
The *Volume Display* group lets you load, save, and refresh the settings for your 3D Viewer:



The **Reload** button allows you to reload the volume with different subsampling. Clicking the drop-down arrow displays these choices:



The X, Y, and Z indicators refer to the scaling factor use to subsample the display of the volume. If subsampling is used with this volume, you will see a tooltip similar to this:

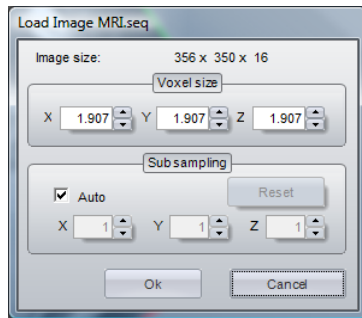


The **Load**, **Save**, and **Reset** buttons in the *Volume Display* group control the various rendering options. Rendering options include the current visual representation of all elements. The options can be saved/loaded using the REN file format.

The complete experiments including references to the source image and all rendering options can be saved to **Image-Pro with 3D ModuleExperiment (.s3d)** files.


These experiment files can be opened directly in the application's **File Open** dialog by selecting the *Image-Pro with 3D Module Experiment* type.


Reload opens the following dialog:




You can also define voxel size and subsampling manually. When the Auto checkbox is active, the volume is subsampled automatically. With typically large volumes, (larger than 128 x128 with 128 or fewer Z-planes) subsampling allows faster processing and smother volume handling, at a cost of reduced resolution in the display. Subsampling for display purposes does not affect the accuracy of any measurements taken.

Reload when Imagechanged indicates that the volume should be reloaded if the underlying image or image set is changed. For example, after filtering the image.

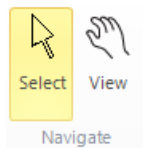
 **Load 3D** settings lets you open and load saved 3D Viewer settings files from a *.ren or *.S3D file. The *.ren file format saves only the 3D settings, which allows you to apply those settings to another volume. The *.S3D file format saves those settings and also saves a reference to the image or image set file to open. After saving an S3D file, you can use it to re-open the same image file with the same settings from the File:Open dialog

 **Save 3D** settings lets you save your current settings to a *.ren or *.SD3 file.

 Clicking the **Reset** button sets the subsampling to 1x1x1.

Navigate Group

The *Navigate* group allows you to select and view the 3D volume from different positions.




Select: Use this button to select object manipulation (and deselect *View* mode). The cursor shape will change to an arrow. In this mode, you are manipulating and holding objects in the *Viewer* window. Using the <Ctrl> key while clicking the mouse will allow normal multiple selection or deselection of objects.

View: Use this button to select camera or viewer mode (and deselect object manipulation mode). The cursor shape will change to a hand icon. In this mode, you are moving the camera in 3D space, which changes the perspective of the rendering in the *Viewer* window.

When **View** mode is selected, use the mouse to control the camera position of the viewer. In this mode, you can rotate, move (using <Shift>+drag), or zoom objects (using the mouse wheel).

The **View/Select** modes can also be activated from the image context menu by clicking the right mouse button:



The **AdvancedOptions**  button contains the advanced options for visualization and rendering. These features are discussed later in this chapter.

TIP: You can switch between *Select* and *View* modes by holding down the <V> key.

Voxel Size Group

The *Voxel Size* group controls the size and location of 3-dimensional pixels:

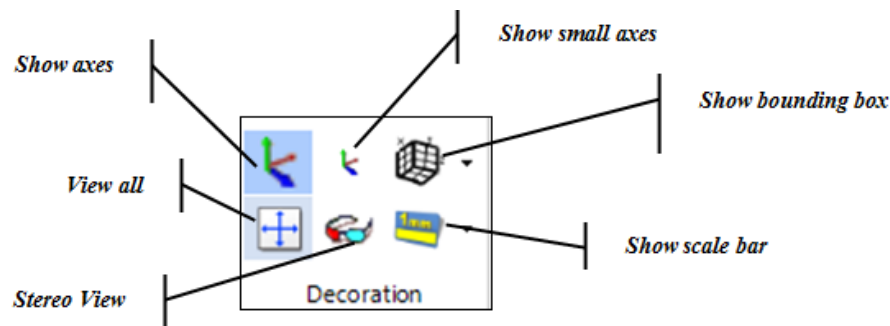
X	<input type="text" value="1.800"/>	<input type="button" value="↑"/> <input type="button" value="↓"/>	μm
Y	<input type="text" value="1.800"/>	<input type="button" value="↑"/> <input type="button" value="↓"/>	μm
Z	<input type="text" value="-4.000"/>	<input type="button" value="↑"/> <input type="button" value="↓"/>	μm


Voxel Size


Voxel size: This group box defines the dimensions of one voxel (a pixel in 3 dimensions). The values displayed are the last ones used. If the image does not have a spatial calibration, and no system calibration has been set, the values are 1, 1, and 1. If the image has a spatial calibration, the X and Y units per pixel values and the calibration units are taken from the calibration. If the active sequence or stack includes information about the Z frame position, the Z size is set from the distance between first and second frame in the sequence. If Z information is absent, Z voxel size is set to the average value of X and Y sizes. These values can be modified to achieve the desired volume aspect ratio, but doing so will affect all 3D measurements taken on the volume. For accuracy of your measurements, we strongly recommend that you do not modify calibrated values. Note that you can change the subsampling in the Load dialog on the previous page without affecting 3D measurements.


Decoration Group

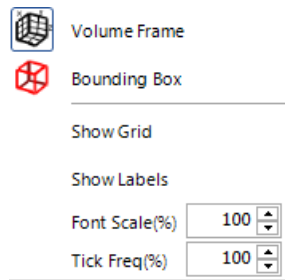
The *Decoration* group contains useful features for viewing and enhancing your 3D image:



The **Show Axes**  button hides or shows the volume axes. This option display axes that show the orientation of the volume (Red for the X axis, Green for the Y axis, Blue for the Z axis). The origin of the axes is at the 0, 0, 0 location.

Show Small Axes  hides or shows a pair of small axes in the bottom left corner of the view. **Show small axes** is the default selection.

The **Bounding box**  button hides or shows the bounding box. Select this button to display a bounding box that encloses the entire volume. Clicking the drop-down arrow lets you choose between the volume frame and bounding box views:



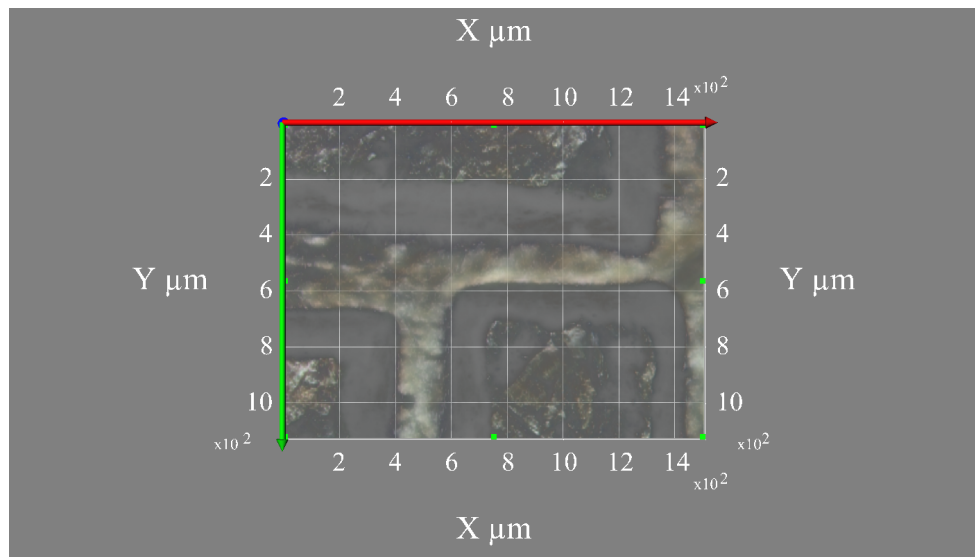
Show Grid superimposes a grid on top of the image.


Show Labels displays the volume labels around the outside of the image.

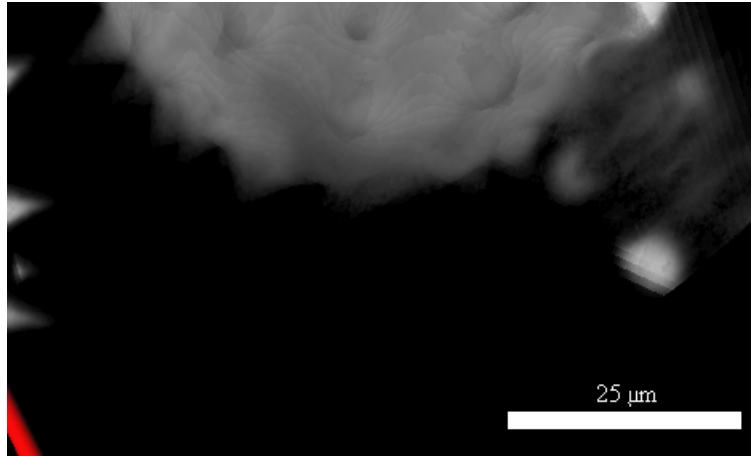
Font scale determines the size of the labels.

Tick Frequency controls the placement of tick marks.


The following example shows both grid and labels:




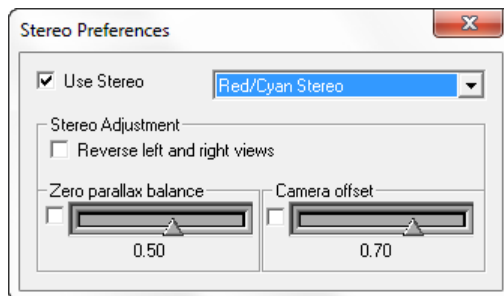
The **Scale bar**  button hides or shows the calculation markers on the view. Clicking the drop-down arrow lets you turn the marker on or off (Toggle Marker) or display the marker options. Calibration is discussed in greater detail in Chapter 5.



Note that the scale bar shows the size in the central part of the volume.

View All  sets the camera to view all visible objects and decorations in the scene.

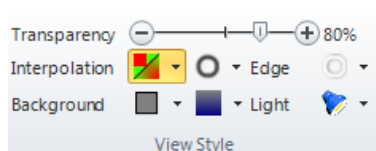
Stereo View  displays the Stereo Mode dialog:




From this dialog, you can turn the stereo mode on or off, and change the stereo type.

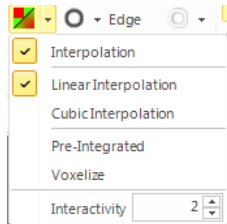
The View Style Group

The **ViewStyle** group contains controls for volume visualization.



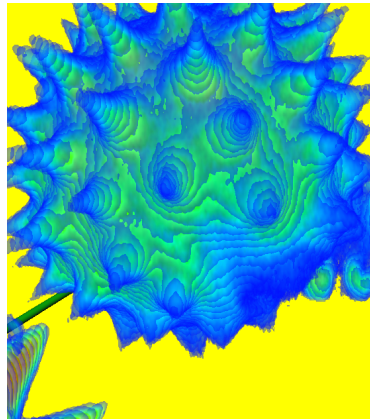
Transparency:  62% This slider controls the degree of transparency of the volume. Moving the slider to the left makes the entire volume progressively more transparent.

Interpolation activates voxel view smoothing. The pop-out menu displays your choices:

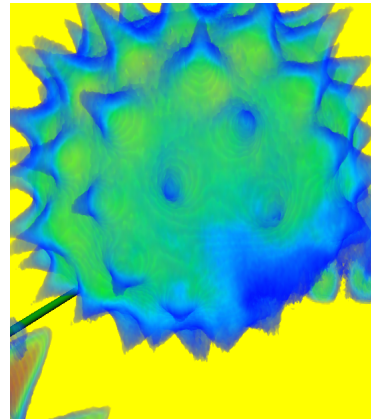


- **Linear interpolation** does bilinear approximation between voxels, which is fast but less accurate.
- When **Cubic interpolation** is activated, a cubic interpolation of neighboring voxels is used. This option produces smooth volumes, but is expensive in terms of computing resources.
- The **Pre-Integrated** option can be used to increase the quality of the volume view with a smaller number of slices. It is also useful when the transfer function has a lot of fast variations, i.e., the color changes very rapidly.

An example appears here:

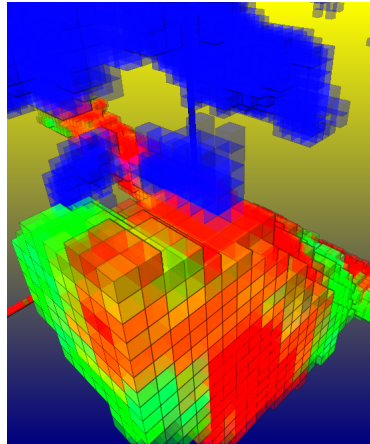


Pre-Integrated Off



Pre-Integrated On

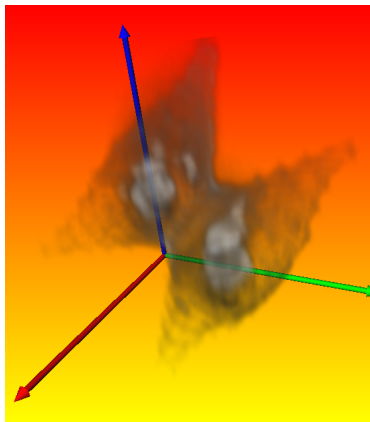
- The **Voxelize** options lets you visualize each non-transparent voxel by rendering each voxel as a cube of that voxel's volume and intensity. It can be used to look at the volumes at the voxel level. The picture below shows the volume with the voxelize option on:



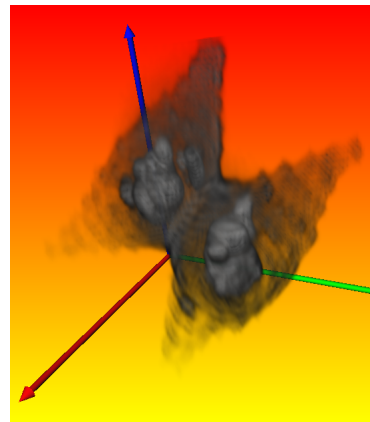
Voxelize on



Boundary Opacity increases or decrease the opacity of object edges, where gradients are high:

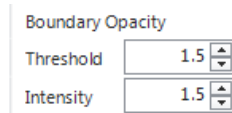


Boundary opacity off



Boundary opacity on (thr. =2.5, intens.= 8.5)

The pop-out menu lets you change the threshold or intensity:



- The **Threshold** parameter defines the sensitivity of boundary opacity. Gradients with a length less than the **Threshold** values are ignored during the lighting computation. This avoids doing lighting computations on noise in the volume while still lighting important data. In the screenshot, setting a threshold value of 2.5 removed lighting on the noise surrounding the pollen.
- The **Intensity** parameters define the darkness of the edges.

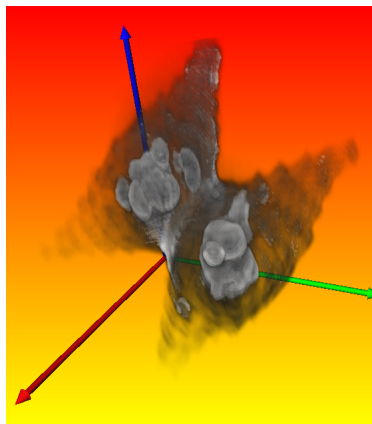
Note that using **Boundary Opacity** can significantly increase rendering time.





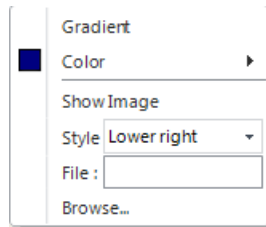
The **Edge Coloring** changes the color based on the gradient direction (normal). Areas where the normal direction (computed from the gradient) is facing the camera will have an unmodified color, whereas areas where the normal direction is more perpendicular to the view direction will tend towards **Edge Color**.


Edge Color lets you select a color from the palette. Edges are highlighted with the color specified in **Edge Color**.

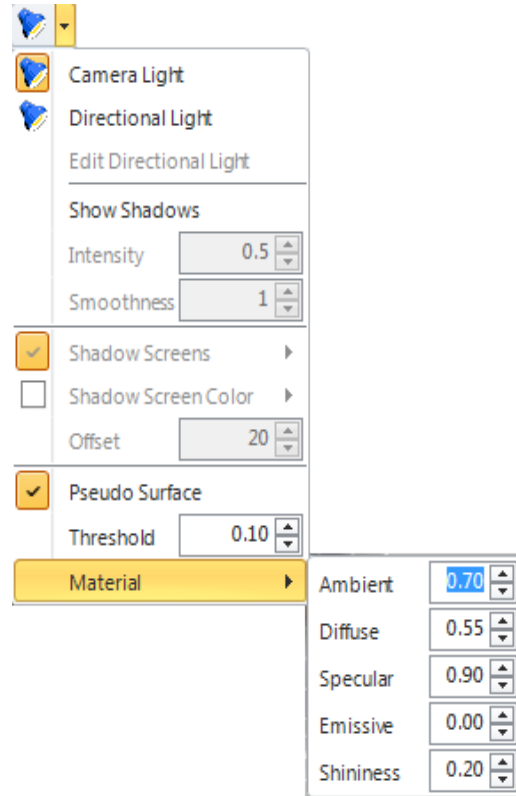
In the example below, the coloring is on and the color is white:



-  **Background**: Use the color picker to select a background color for your volume display.
-  When the **Gradient Light** option is *on*, the color gradient is drawn as a background for a scene. The **Background** color appears at the top of the window and the second color appears at the bottom of the window.

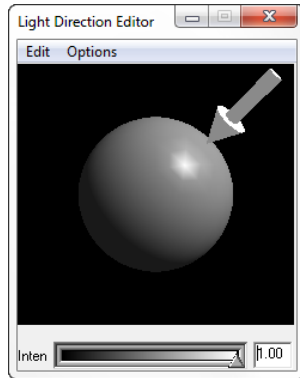


- When the **Show Image** option is *on*, an image will be displayed on the background. The position of the image is controlled in the **Style** drop-down box and the image file can be selected using the **Browse...** button. The following image formats are supported: BMP.
- The **Light**  button activates directional light and volume lighting. The drop-down menu contains additional lighting and volume appearance options:



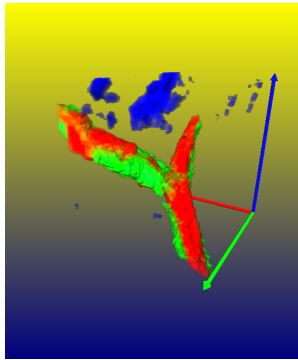
These options only affect volumes displayed in *Blended composition*.

- **Camera Light** turns a light coming from approximately the same location as your camera on or off.
 - **Directional Light** turns on or off a light that you can position relative to the volume itself. Use the **Edit Directional Light** menu item below to edit that light's position and intensity.
 - **Edit Directional Light**: Clicking this button activates the directional light editor. Note that this option is disabled when the Directional Light is off. Choosing this option displays the **Light Direction Editor**, shown here:

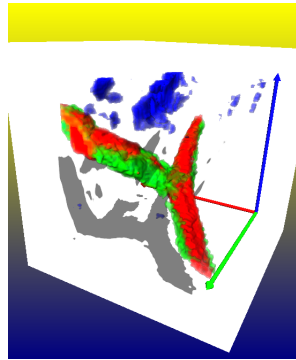


Use the handle to manipulate the light direction. The *Edit* menu allows you to change the color using a color editor. The options are set to the default, *always on top*.

- The **Show Shadows** option activates shadows on volume and scene elements. Note that when the light is off, the shadow is cast from the headlight, which follows the camera. When the directional light is on, the shadow is cast by the camera light, the position of which can be controlled using the **Edit Directional Light** dialog.



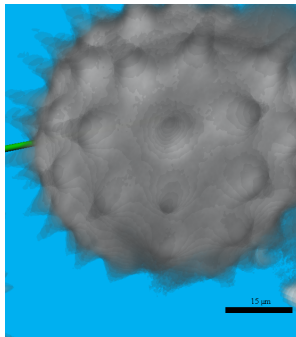
Shadow OFF



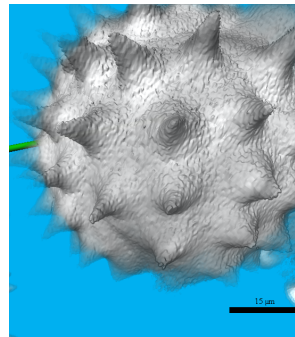
Shadows ON

- **Shadow intensity** and **smoothness** can be controlled using the corresponding controls. Intensity is given in range from 0 (no shadow) to 1 (dark shadow). Higher values of smoothness give smoother shadows.

- **Shadow screen** can be used to show dropped shadows. You can control the places where the shadow screens are shown (Y, Y, Z). You can also control the **color** and the screen **offset from the edges of the volume**. **Offset** is defined in percent of the volume size in the given direction.
- **Pseudo Surface** is a lighting effect that looks like a virtual surface created at the threshold level of volume opacity. It gives a better result when the volume data contain regional boundaries with relatively sharp gradients. **Threshold** defines the opacity level for the Pseudo Surface in a range from 0 to 1. The default is 0.1.
- **Material** groups defines material properties of pseudo surface. The color of the surface is calculated from a combination of 4 color components, each component is in range from 0 to 1:
- **Ambient** - the color of an object where it is in shadow. This color is what the object reflects when illuminated by ambient light rather than direct light.
- **Diffuse** - represents direct light hitting a surface. The Diffuse Light contribution is dependent on the incident angle.
- **Specular** - the white highlight reflection seen on smooth, shiny objects. Specular light is dependent on the direction of the light, the surface normal and the viewer location. Specular component makes the object look like metal or glass. The reflection property is also defined by **Shininess**, with values close to 1 the reflections will be very sharp as on polished metals, with lower values the radius of reflection increases to make it look as brushed metal.
- **Emissive** - the self-illumination component of the color, has neon effect.

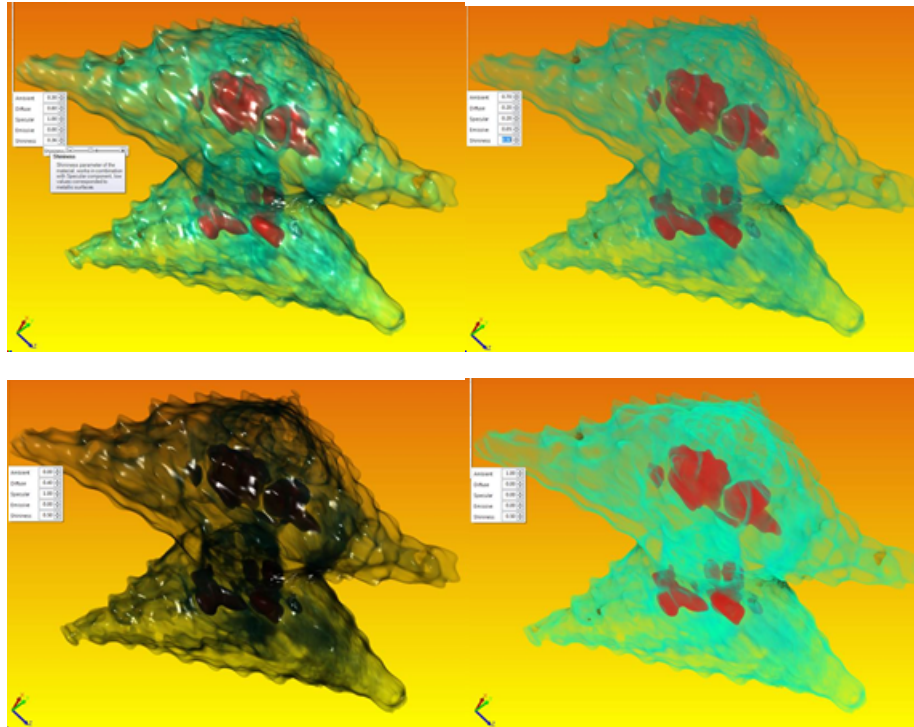


Pseudo Surface OFF



Pseudo Surface ON

Here are the examples of the **Pseudo Surface** mode with different **Material** properties configurations:

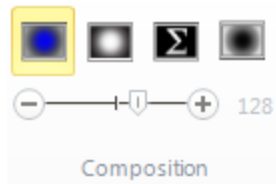


Use the <Q> key to switch between rendering modes. There are two rendering methods that support trade-offs between rendering speed and rendering quality:

- **Sorted Blended Transparency**
The default rendering mode, this features fast rendering, but may not accurately render semi-transparent surfaces.
- **Delayed Sorted Pixels Blended Transparency**
A more complex rendering mode for complex scenes with semi-transparent objects. Also more demanding of graphics resources.

Composition Group

The buttons in the *Composition* group define the image composition method.



Blend: Blending averages the values of the voxels in a straight line through the volume of the object.



Max: *Maximum Intensity Projection* takes the value of the brightest voxel in the same straight line as used by the Blend option

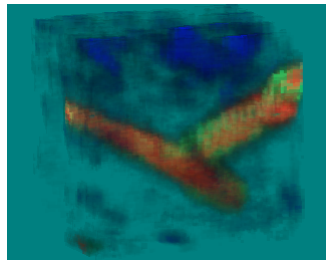


Sum: This method displays the sum of the value of the voxels in a straight line. It is most useful for dark images.



Min: *Minimum Intensity Projection* takes the value of the dimmest voxel in the same straight line as used by the Blend option.

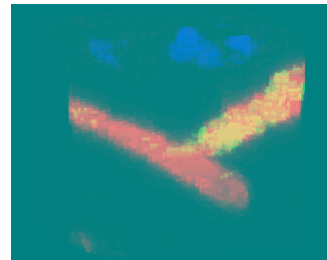
Some examples are shown here:




Blend



Sum

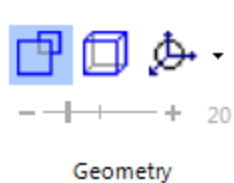


Max

Number of slices:  This slider controls the number of slices drawn for 3D and 2D multi-texture map rendering. Zero automatically selects the number of slices. Rendering fewer slices will generally improve performance (with reduced image quality).

Geometry Group

The *Geometry* group defines the camera parameters:



Activates the perspective view.



Activates the orthogonal view.

In *Perspective* projection view, the parts of the volume closest to the viewer will look bigger; those that are further away will appear smaller. Switch the projection type to *Orthogonal* to retain a cubic shape without perspective.



Clicking the **Projection** button sets the view to the last-selected projection, from one of the following:



Sets the camera perspective to an **XY** projection.

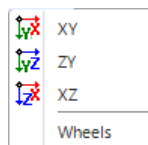


Sets the camera perspective to a **ZY** projection.



Sets the camera perspective to an **XZ** projection.

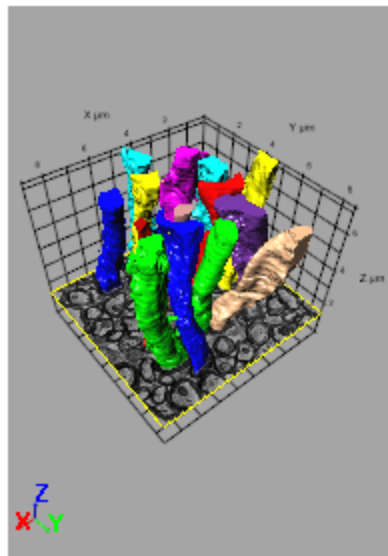
The drop-down menu also includes a new feature: **Wheels**. Wheels shows or hides the in-frame control for rotation wheels at the bottom and right side of the projection view. Use the wheels to rotate the display along a single axis.



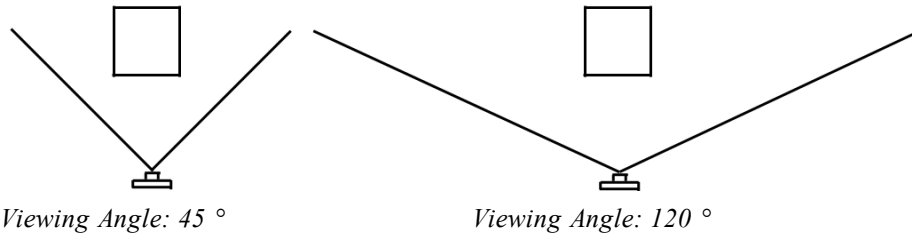
The bottom slider in the *Geometry* group is the **View Angle** slider:



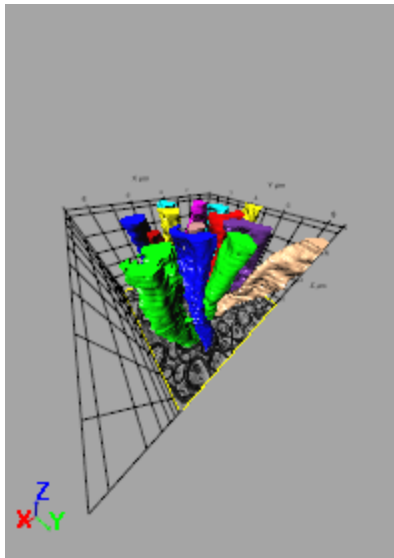
This slider adjusts characteristics of the camera's virtual lens, which changes the angle that defines the field of view of the camera. Changes to the field of view can indirectly make your scene look larger or smaller. Changes to the viewing angle can also cause lens effects like a "fish-eye" view of your scene if you are using the *Perspective* setting in the *Geometry* group. The following illustrations may help to understand how the view angle affects the rendering:



Note that the volume appears much smaller with the larger view angle. The following illustration may explain why this happens:

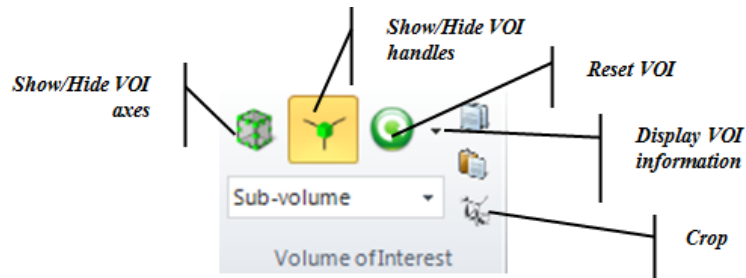



You can see with the wider field of view, from the greater viewing angle, that the same size box looks much smaller, even though it is the same distance from the camera. You can adjust for the change in the size of the scene by using the **Zoom** control to move the camera closer to the volume. In that case, you can clearly see the “fish-eye” effect of the wide angle lens:




Volume of Interest Group

The *Volume of Interest* group modifies the visible portion of the data volume in the *Viewer* window. It contains the following controls:

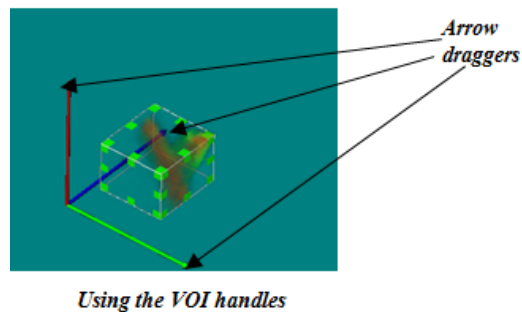


The **Show VOI Axes**  button turns the VOI axes on or off.

Show VOI handles  shows or hides the "dragger" associated with the VOI. The dragger is used to move and resize the VOI.

To use the dragger tool:

1. First make sure the *3D Viewer* window has focus (click in it if necessary).
2. Then make sure the *Viewer* is in selection mode (cursor is an arrow shape). If necessary, click the **Select** icon in the *Navigate* group to switch to selection mode.
3. Click on the **Show VOI handles** button to activate the VOI adjustment handles






To Resize the VOI:

1. Move the cursor onto one of the green handles
2. Press the left mouse button and drag. The VOI cannot be larger than the data volume.

To Move the VOI:

1. Move the cursor onto one of the sides of the dragger box. The side of the VOI that you click on will determine the axes along which the VOI can be moved. If you find that you cannot move the VOI in the direction that you want to move it, try clicking on an adjacent side of the VOI cube
2. Press the left mouse button and drag. The VOI cannot move outside the data volume.

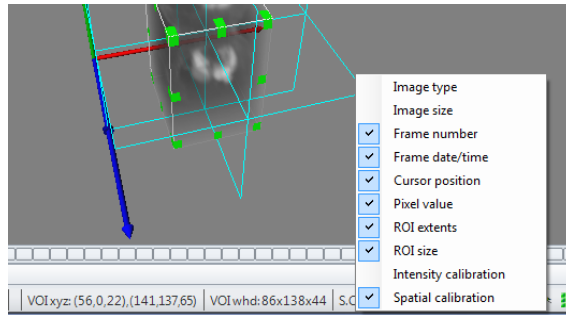
The size of the VOI (XYZ) and the volume are given in spatially calibrated units. Volume fraction shows the ratio between total volume of the image and the VOI.

You are able to copy and paste VOI extents from one 3D view to another using the **Copy**  and **Paste**  buttons. You can also crop the volume to the VOI extents using the **Crop**  button.

The **Reset**  button will reset the VOI to the full size of the data volume.

The sub-volume information is shown in the **Reset** button's drop-down panel which also has controls to set the VOI parameters by numbers. You can define start and size values for X, Y and Z axis and then click the Set by Numbers button. When the option In Calibrated Units is selected, the values are in calibrated units, otherwise in voxels.

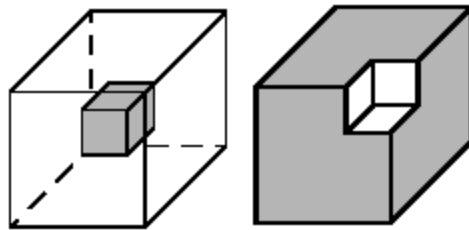
The position and size of a VOI can be displayed in the status bar when the *ROI extents* and *ROI size* are selected in the **Status Bar** options:



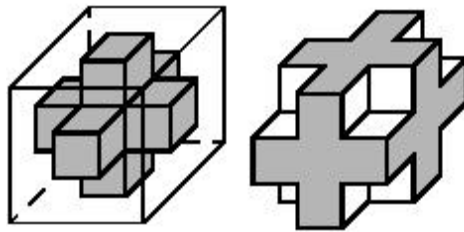
Sub-Volume: Choose a shape for the VOI (Volume of Interest) from the drop-down list.

- **Cross:** The VOI will be displayed as a cross-shaped volume.
- **Fence:** Displays a fence-shaped VOI.
- **Exclusion box:** The VOI can also be an exclusion box which specifies a region of the volume that will not be displayed. This is very useful for cutting away part of the volume.

Examples of the VOI shapes are shown here. The gray sections indicate which parts will be visible in the *Viewer*:



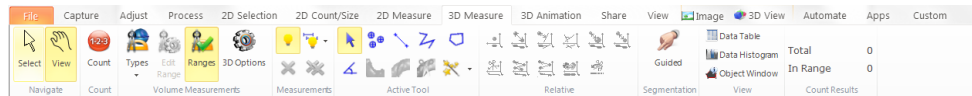
Sub -Volume Exclusion box



Cross Fence

The 3D Measure Ribbon

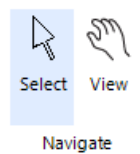
The **3D Measure** ribbon contains a variety of volume measurement tools for 3D analysis.



You may already be familiar with some of them.

Navigate Group

The *Navigate* group lets you choose between **Select** and **Viewing** mode.



When **View** mode is selected, the mouse controls the camera position of the viewer. In this mode, you can rotate, move, or zoom objects. In **Select** mode, you can select objects in 3D view (slices, measurements (volumetric and manual), draggers,) and create manual measurements. Using the <Ctrl> key while clicking the mouse will deselect any object.

TIP: You can switch between Select and View modes by holding down the <V> key.

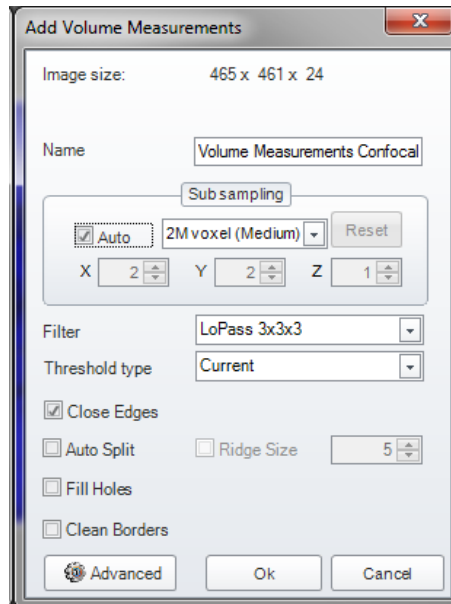
Count Group

The *Count* feature lets you count specified volumes within the 3D image. Clicking the **Count** Button executes volume segmentation and measurements on one of the volume channels.



If the **Count** control is active, the 3D objects in an isosurface are remeasured every time the threshold levels or isosurface parameters change.

When you click the count button, you will see the following dialog:



Add isosurface: Here you can select which channel of a color image will be used to create the isosurface. Your choices will depend on the volume that you have open:

- For monochrome volumes from a sequence and single-channel sets, your only choice is a Gray channel from the monochrome data.
- For color volumes, you will have Red, Green and Blue channels that correspond to the color components of the image.
- For single and multi-channel sets with channel information, there will be one volume channel per set channel, using whatever channel names and tinting information is available from the set metadata.

Sub sampling: The *Subsampling* group in this dialog is very important to understand, as it affects the isosurfaces that are created. You can use subsampling to smooth out the isosurface that is generated, which will allow it to render and animate more quickly. However, excessive subsampling can also result in removing smaller objects that you may wish to visualize or measure, and subsampling will always affect the volumes of the objects that may be measured later. For maximum accuracy, you will want to use **Reset** to make sure that there is no isosurface subsampling.

Auto: Selecting the **Auto** checkbox activates automatic subsampling mode. In this mode, the subsampling is calculated based on the size of the image. This provides faster loading and rendering of volume images. By default this option is on. Advanced users can switch this option off and set subsampling manually.

Reset: Clicking the **Reset** button clears all your volume settings and sets 1:1:1 subsampling. The **Reset** button is disabled when **Auto** is on.

Filters: Filters can be used to smooth the surface. Choose a filter from the drop-down list. Excessive smoothing using Filters can also affect the accuracy of any 3D measurements that you make, so use the minimum amount of filtering needed to get a representative isosurface.

Threshold Type: The initial threshold level can be selected automatically when **Threshold type** is defined as *Auto Bright* or *Auto Dark*.

Close edges: Select this check box close the surfaces of the objects where they touch the bounding box of the volume.

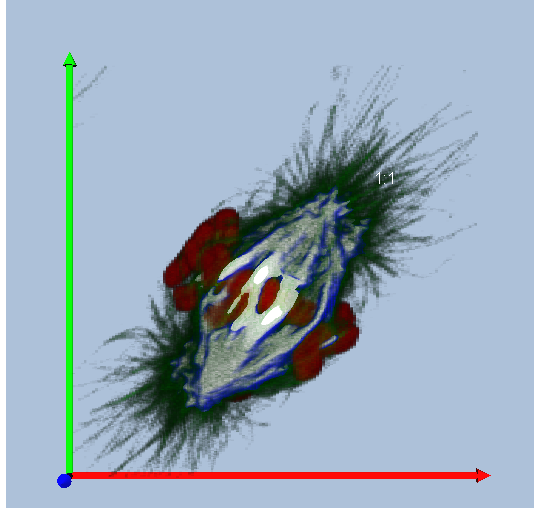
When **Close edges** is turned *on*, the surface is closed on the sides that touch edges regardless their intensity value. (The edges must be closed to measure volumes.)

Execute Count: Check this box to execute the object segmentation count when an isosurface is created.

Auto-Split: Check to this box to split objects that are touching in two.

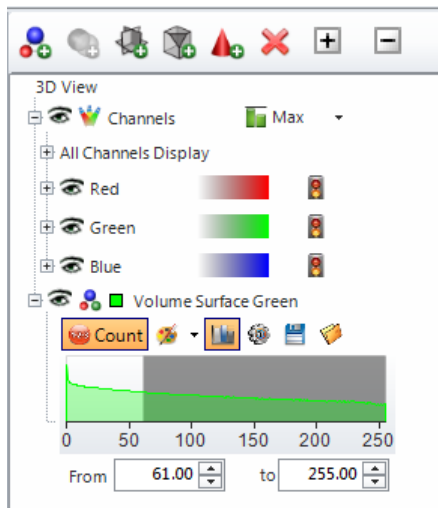
Ridge Size: When the *Ridge size* option is active, only narrow junctions with diameters below the given size are split. Note that the split is also applied after adjusting the threshold levels of the segmentation.

Your image will look something like this:



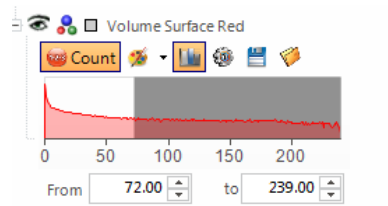
This example shows that the green channel was selected for the count.

Information about the isosurfaces created by the **Count** operation appear in the options panel on the right of the screen:



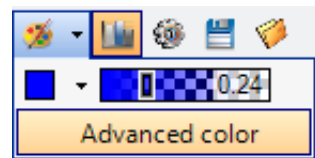
Counting the volume created an isosurface, which is represented by the Volume Surface Green section of the 3D View options panel. When **Execute Count** is turned off, only one

isosurface will be created. Then you can adjust the Threshold levels interactively, and execute the count by checking the **Count** check box:



The thresholds can be adjusted by clicking and/or dragging the start or end of the threshold range on the histogram. There are a number of controls here that you can use to fine-tune the segmentation of the isosurface, which are discussed in detail in the [Add Isosurface](#) section later in this chapter. You can also edit it in the **From** or **To** controls.

When the **Count** option is turned off, the color of isosurface can be adjusted in the **Advanced Color** drop-down:



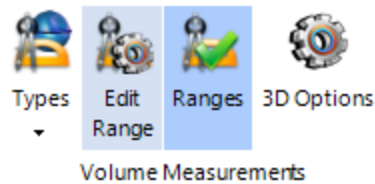
Note that when **Count** is on, the colors of individual objects are controlled by the **Coloring** parameter in the **3D Measurement options**. When **Coloring** is set to *Parent* the segmented objects have the color of the isosurface. When the **Coloring** is set to *Random*, then the color of every segmented object is selected randomly, but the transparency is taken from the isosurface.

The results of the count operation are displayed in the *Count Results* group:

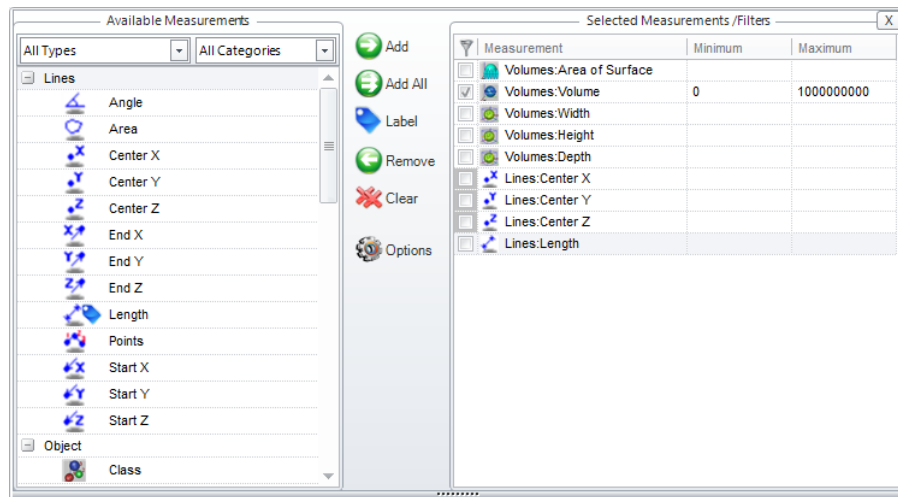
Total	2418
In Range	66
Count Results	

Volume Measurements Group

The *Volume Measurements* group provides a variety of measurements for volume analysis.



The measurement types can be defined in the **Types** drop-down list.



Available measurements are grouped by types:

- Lines, either manually created or derived line or point measurements.
- Volumes are automatically segmented volume objects
- Object class, which can be applied to both Lines and Volume measurement objects.

Clicking the **Add** button adds one of the available measurements to the **Selected Measurements / Filters** list.

Clicking the **Add All** button adds all of the available measurements to the **Selected Measurements / Filters** list.

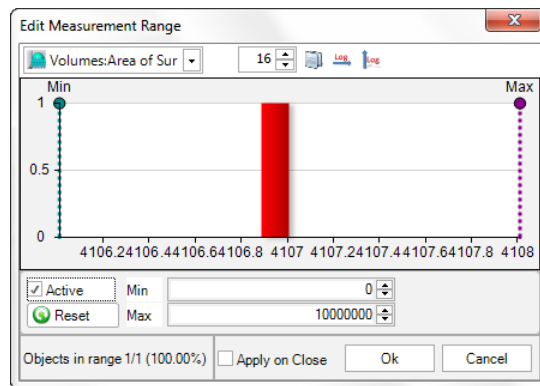
Clicking the **Label** button uses the name of the selected measurement as a label for a given group of objects.

Clicking the **Remove** button deletes a specific measurement from the list of **Selected Measurements/Filters**.

Clicking the **Clear** button deletes all the measurements from the list of **Selected Measurements/Filters**.

Clicking the **Options** button displays the **Orientation Options** dialog. Orientation measurements are described later in this chapter.

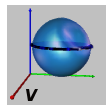
Edit Range lets you make adjustments to the measurement ranges:



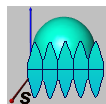
Min and **Max** filter values can be adjusted by dragging the corresponding markers on the histogram or editing their numerical values. The number of objects in range/total objects and percentage are shown below the histogram.

Ranges sets the filter range that can be applied to each measurement. If a filter range is set, objects with parameters below the minimum or above the maximum values are excluded from the view. The filter ranges are used only when the **Ranges** option is active.

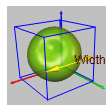
The **VolumeMeasurements** list includes the following measurements:



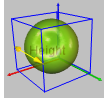
Volume indicates the volume of an object in calibrated units.



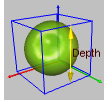
Surface area indicates the surface area of object in calibrated units.



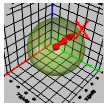
Width shows the size of a bounding box in the X direction.



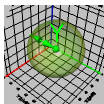
Height shows the size of a bounding box in the Y direction.



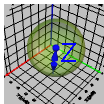
Depth displays the size of a bounding box in the Z direction.



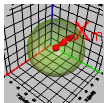
Center X indicates the X coordinate of the center of an object.



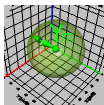
Center Y indicates the Y coordinate of the center of an object.



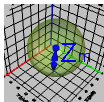
Center Z indicates the Z coordinate of the center of an object.



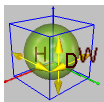
Center of mass X indicates the X coordinate of the center of mass of the object.



Center of mass Y indicates the Y coordinate of the center of mass of the object.

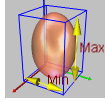


Center of mass Z indicates the Z coordinate of the center of mass of the object.

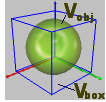


Box Volume displays the volume of an object's bounding box:

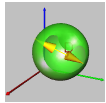
$$(V = W * H * D) .$$



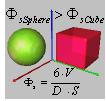
Box ratio displays the ratio between the maximum and minimum sizes of the bounding box: $R = \text{Max} / \text{Min}$.



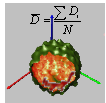
Volume fraction displays the ratio of an object's volume to the box volume. $R = V_{\text{obj}} / V_{\text{box}}$



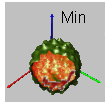
Diameter displays the equivalent diameter of an object.



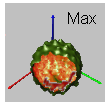
Sphericity displays the sphericity of object, calculated as 6 volumes of an object divided by the equivalent diameter and surface area of the object. For spherical objects, this parameter equals 1; for all other shapes it is less than 1.



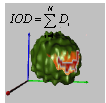
Density (mean) indicates the mean density of an object.



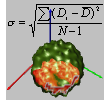
Density (min) indicates the minimum density of an object



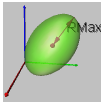
Density (max) indicates the maximum density of an object.



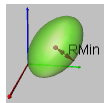
IOD displays the Integrated Optical Density of an object.



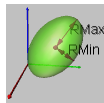
Density (st.dev.) displays the standard deviation of the optical density of an object.



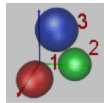
Radius (max) indicates the maximum distance between an object's centroid and surface.



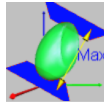
Radius (min) displays the minimum distance between an object's centroid and surface



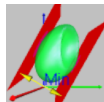
Radius Ratio displays the ratio between the minimum and maximum radii.



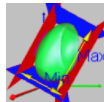
Class shows the class to which an object belongs. The classification can be done using the **Histogram** window.



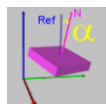
Feret (max) indicates the maximum distance between two parallel planes enclosing an object.



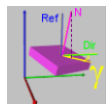
Feret (min) indicates the minimum distance between two parallel planes enclosing an object.



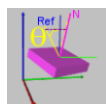
Feret Ratio displays the ratio between Feret (max) and Feret (min).



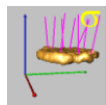
Alpha angle indicates the angle between the reference vector and surface normal (in degrees).



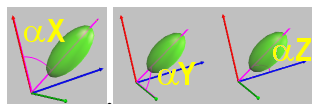
Gamma angle indicates the angle between the *Direction vector* (Dir) and the projection of the normal (Proj) to the plane created by the direction vector (Dir) and the vector (Perp) perpendicular to Ref-Dir plane. The angle can range from -180 to 180 degrees.



Theta angle indicates the angle between the *Reference vector* (Ref) and the projection of the normal (Proj2) to the plane perpendicular to the *Direction vector* (Dir). The angle can range from -180 to 180 degrees.



Surface deviation is calculated as the deviation of the end-points of triangular normal. The calculations are done as follows: all triangular normal vectors of the surface are normalized (length set to 1) and the average distance from the mean positions of the end-points to all other vectors is calculated. A uniform surface will have deviation of 0. The maximum deviation of 1.336 will have a sphere.



Angle X, Y and Z indicate the angle between longest object line (max feret) and the X, Y and Z axes in degrees.

The longest object line vectors can be visualized using the corresponding option in the **OrientationMeasurements Options** dialog.

Holes and Negative Volumes

The current version of *Image-Pro with 3D Module* handles holes as if they are independent objects. Holes are identified by *negativeVolume* measurement. By default, holes are

filtered out by the measurement filters when Volume limits are set from 0 to 1E9. You can disable or adjust the filters to display the holes.

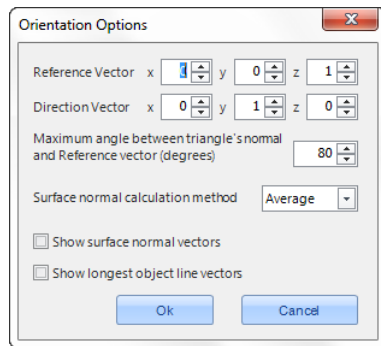
Object measurements ignore holes. It is equivalent to using the **Fill Holes** options in the 2D measurements. If it's important to exclude hole volumes from the parent objects, you

can do it by manually selecting the parent object and hole(s) and clicking the **Merge** button. This will merge the outlines of the parent object with those of the holes which will result in measurements that exclude the negative volumes of holes.



3D Measurement Type Options

Orientation measurement options can be set clicking the **Options** button on the **Measure Types** dialog:



Orientation in 3D space can be defined using at least 2 angles from perpendicular vectors. These angles are called *Alpha* and *Gamma*. *Alpha* is measured relatively to the *Reference vector* and *Gamma* relatively to *Direction vector*.

The *Reference vector* is defined by the X, Y and Z components. The default vector of 0, 0, 1 is parallel to the Z axis. The *alpha* angle is calculated between this vector and the surface normal.

The *Direction vector* is defined by the X, Y and Z components. The default vector of 0, 1, 0 is parallel to the Y axis. The *gamma* angle is calculated between this vector and the projection of the normal to the plane of the direction vector, and the vector perpendicular to the plane is defined by reference and direction vectors.

Maximum angle between triangle normal and reference vector defines which areas (triangles) of the object surface will be taken into account when calculating surface normal. Every surface consists of triangles; every triangle has its own normal vector. If the angle

between triangle's normal and the reference vector exceeds the maximum angle range, the triangle is excluded from the surface normal calculation. The default value is 80 degrees, which excludes triangles on the sides of the object (angles around 90 degrees) and triangles on the opposite side of the object (angles greater than 90 degrees). The maximum value for this parameter is 180.

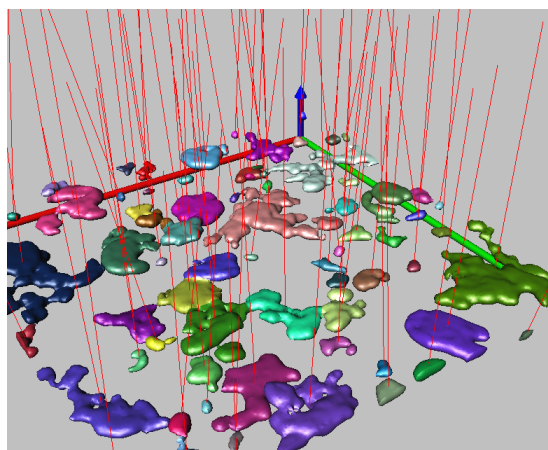
There are two methods provided to calculate the normal vector of surface:

- **Average vector**, which is calculated as area weighted average normal vector of all surface triangles (within *angle range*). This method is relatively fast in calculation, but sensitive to deviations on the surface orientation. Small bumps and pits on the surface can slightly affect the vector.
- **Median vector**, where median vector from all triangles are selected as surface normal. Median vector is the vector that has the minimum sum of distances to all other vectors. This method provides very stable results; as small bumps and pits on the surface do not affect the result. The vector represents the normal direction of the surface which covers most of the object. This method is very computation intensive, and can be much slower than the Average method for large objects (if each object contains more than 10000 triangles).

The **Show Surface Normal vectors** option hides or shows the surface normals on the image.

The **Show longest object line vectors** option hides or shows the longest object lines that are used to measure *Angle X,Y* and *Z*.

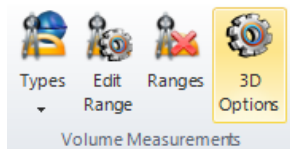
An example appears here:



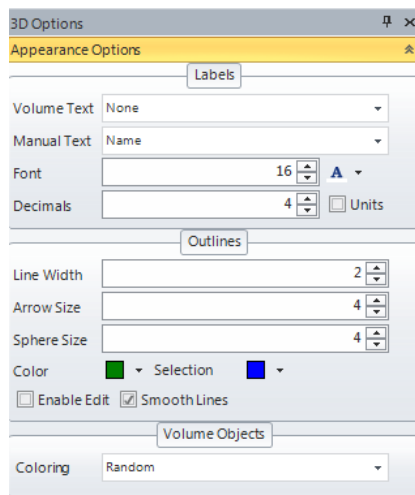
The colors of the normal vectors can be changed using the *Selection* color.

3D Options Panel

The **3D Options** panel can be opened by clicking the **3D Options** button in the *Volume Measurements* group:



The **3D Options** panel is shown here:



Appearance options

The *Appearance* options control **Labels** and **Outlines**.

Volume Text sets the appearance of measurement labels displayed on the 3d volume.

Manual Text sets the appearance of the manual measurement labels.

Both text combo-boxes indicates the type of labels. The labels can be:

- **None:** No labels are displayed.
- **Name:** The *measurement* name is displayed.
- **Measurement:** The value of the first selected measurement will be displayed as a label (you can drag any column in the data table to the first position to be displayed as a label).

Font sets the label font size.

Decimal defines the number of digits after the decimal point that will be displayed in the data table and labels.

The *Outlines* group contains display options for the measurement elements.



Line width defines the width of lines for the manual measurements

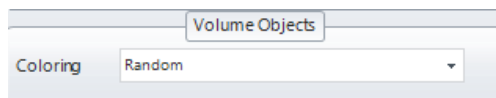
Arrow Size defines the size of arrow line ends.

Sphere Size defines the size of sphere line ends for point measurements. When a manual measurements feature (point, line, polyline) is selected, the points of the polyline are shown as spheres in the selected color with boxes around them. If the viewer is in the selection mode, and the selection mode of the manual measurements is active, the positions of the spheres can be edited by dragging the points to new positions.

Color and **Selection** define colors for normal and selected state of the elements.

The Spheres and Dragging boxes are switched off when the feature is deselected.

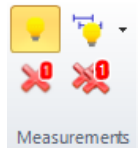
The *Volume Objects* group provides options for coloring.




The **Coloring** control defines what color the objects will have. Two choices are available: *Parent* and *Random*. If Parent is selected, the objects inherit the color from the parent

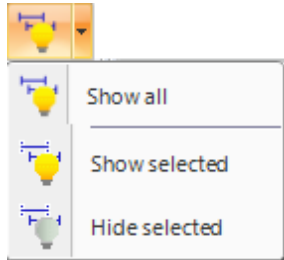
isosurface/volume measurements. If *Random* is selected, the colors for objects are generated randomly, so every object has different color.

Measurements Group



The *Measurements* group shows, hides, or deletes the various manual measurements on the image.

The **Show**  button shows or hides any manual measurement objects on image. The **Show All** group lets you show or hide selected measurement objects.



The object can be deleted using the **Delete selected**  or **Delete All**  buttons.

Active Tool Group

This group defines the active tool that can be used to create manual measurements:



The available tools are:



Select measurements allows you to select measurements on the image and also edit the positions of external objects.



Add point measurement activates the point tool.



Add line measurement activates the line tool. A line is added after 2 clicks.



Add polyline measurement activates the polyline tool. The line is added after you click the right mouse button.



Add polygon measurement activates the polygon tool. Click on several points to create the polygon. Click the right mouse button to close the shape.



Add angle measurements activates angle measurements. An angle is added after 3 clicks.



Add surface line along object surface connecting 2 points creates a poly-line feature that connects 2 points going along the surface in the plane that passes through the selected two points and the camera position.



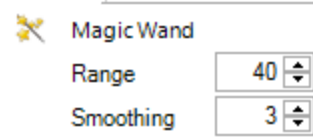
Add an outline of object crossed by a plane creates a closed poly-line feature that is an outline of a plane crossing an object. To add an outline, click twice on the imaginary line along which you want to create the outline of the object. The outline plane passes through the selected two points and through the position of viewing camera, so the outline is always created in the plane perpendicular to the screen plane.



Split objects activates the split mode. To split a 3D object into 2 objects, click twice on the imaginary splitting line of the object. The splitting plane passes through the selected two points and through the position of viewing camera, so the object is always split with a plane perpendicular to the screen plane.



The **Magic Wand** tool enables you to draw polygons on slices. Clicking the drop-down arrow displays this menu:



- **Range** indicates the number of gray levels used for thresholding (combination of gray ranges depends from type of active image and selected interpretation).
- **Smoothing** indicates the degree of post-filtering of the outline (0 – no filtering, 9 – high degree smoothing).

Relative Measurements Group

Various other measurements can be created based on the selected measurements



Getcenter of the object creates a point measurement of the center of selected objects.



Measure distance between centers creates a manual measurement line that connects centers of two selected objects.



Measure perpendicular distance between point and line creates a line measurement between a point and a line as a perpendicular line. Note that the function is active only when 1 line and 1 or more points are selected



Measure angle between lines creates an angle measurement between two lines. Note that the function is active only when 2 lines are selected.



Measure distance between point and surface of volume object creates a line between the selected point and a selected 3D object. The 3D object can be selected either

in the **Volume measurements data table** or on the image itself, using the **Select measurements/select objects** mode.



Measure distance between surfaces creates a manual measurement line between the surfaces of two selected objects. This line represents the shortest distance between two objects.



Split polyline into lines creates a new line measurement from every segment of the selected poly-lines/tracks. Analyzing the length of every line provides information related to velocity of the object moving along the track.



Create a polyline including points of all selected measurements creates a poly-line that includes all points of the selected measurements. For example, if 2 lines were selected the new poly-line would include 4 points.



Extract points from lines/polylines creates a new point measurement for every coordinate of the selected poly-lines/tracks. It can provide information about object coordinates on different time points (the same information can be obtained in the Volume measurements data table).



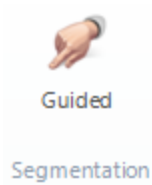
Merge selected objects merges selected objects into one. It can be used if segmentation does not allow connecting all the pieces that belong to one object.



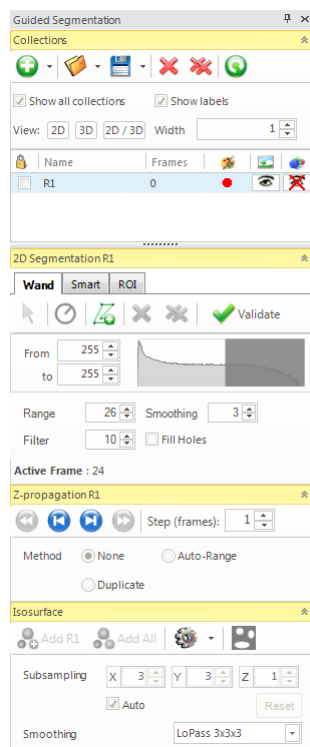
Undo split/merge undoes the last split or merge operation. The number of undo levels is practically unlimited.

Guided Segmentation

Guided Segmentation is designed to allow 3D segmentation of complex objects that cannot be easily segmented using threshold segmentation. Guided segmentation involves defining the object outlines on a 2D view and then generating 3D volume objects from those outlines. To activate this feature, click the **Guided Segmentation** button on the **3D Measure** ribbon:




The **Guided Segmentation** panel appears on the right hand side of the workspace:



The panel contains four sub-panels:

- Collections
- 2D Segmentation
- Z-propagation
- Isosurface

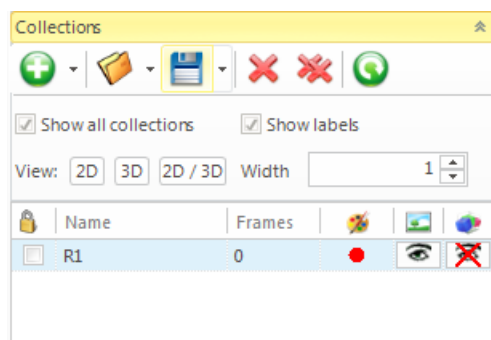
To use *Guided Segmentation*, follow these steps:

1. Open a sequence in the workspace.
2. On the **3D Measure** ribbon, press **Guided** to open the **Guided Segmentation** dialog. Note that in the **Collections** sub-panel, a first object collection will already have been created. If the volume that you want to segment opens in the 3D view, *Image-Pro* will switch to the Image (2D) view for the process of drawing object outlines. If you already had the *Guided Segmentation* panel open when you opened your sequence, use the **Image** button () in the status bar or **Image Context** menu, or the **2D** button in the **Collections** sub-panel to switch to the 2D image view.
3. Navigate to any frame (or Z-plane) in the volume where the first object of interest is visible. You can start at any plane in the Z-stack.
4. Use any tool in **2D Segmentation** sub-panel to create one or more object outlines for that object. See the documentation of the [2D Segmentation sub-panel](#) for more details about the tools that can be used to create the object outlines.
5. Once you have defined your outlines, use the **Validate** button to set those outlines as the objects' ROIs.
6. In the **Z-propagation** sub-panel, choose a method to copy your outlines to other frames in the z-stack.
7. Press the forward and/or back buttons in the **Z-propagation** sub-panel to extend the object ROIs through the stack.
8. Lock the object collection by checking the box next to its name in the **Collections sub-panel**. You may continue to add object collections by pressing the **Add** button in the **Collections** sub-panel. Note that adding a new collection will automatically lock the previous collection(s).
9. Repeat steps 4-9 for each collection to be added.
10. To view the collections in 3D, use one of the selections in the **Isosurface** sub-panel to turn your collections into isosurfaces in the *3D Viewer*.

More information about each of these steps appears in the following pages.






Collections Sub-Panel


The **Collections** sub-panel of the **Guided Segmentation** panel contains tools to control collections. It allows you to add and define certain properties, and to visualize segmented outlines interactively.



Segmentation collections are defined by a set of outlines on multiple frames. Multiple outlines can be defined for a single collection on one frame. A collection is identified by its name and color, which can be edited in the table shown above.

i Depending on your images, you may be able to define all your object outlines in a single collection. Image-Pro with 3D Module includes support for multiple collections to allow you to separate outlines that would otherwise cause incorrectly segmented objects. This can be a problem if separate objects touch or intersect anywhere in your volume. When the outlines are analyzed to create isosurfaces, separate outlines may be merged into a single isosurface if in any frame of the sequence, the outline for an object overlaps multiple outlines on an adjacent frame. This is necessary to be able to connect up Y-shaped or branching objects. However, if separate objects touch, they may both be overlapped by the outline in the adjacent frame, and incorrectly merged. If this occurs, then make a new collection and put the outlines for either of the objects in the new collection.

A new collection can be added clicking the **Add**  button. The collection can be deleted  or reset . It can also be saved  or loaded  from a file. You can

use the **Delete All**  button to remove all the existing collections and start over. Note that when all the collections are deleted, a new, default collection R1 is automatically added.

The **Lock** option applies only to the active collection. It allows you to review the segmentation without applying the active propagation method when scrolling through the image sequence. It also controls whether editing tools are available, or if the collections are locked. All collections except the active one will always show a locked state, and the active row can be locked or unlocked by clicking the check box. Checking the **Lock** check box freezes the selected collection and prevents additional editing until the collection is unlocked. Note that the **2D Segmentation** and **Z-propagation** sub-panels will include the name of the active collection.


Multiple collections can be displayed on the image when the **Show All Collections box** is checked. When this option is off, only the active object ROI will be displayed.

Display of the collections' labels is controlled by the **Show labels** check box. Note that the size and font of the labels can be controlled from the measurement options panel.

The **Frames** column shows the number of frames in the sequence.

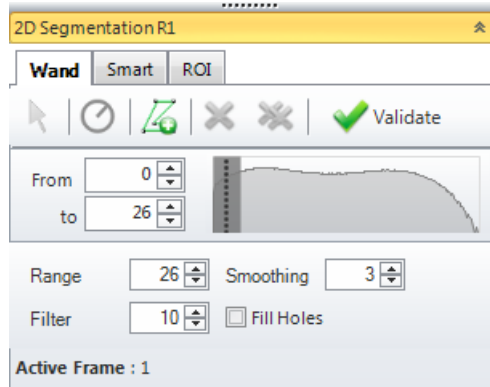
The **View** buttons are used to display the view(s) that you would like to have visible while defining the object outlines and isosurfaces. The **2D** button will open a new view of your volume in the Image view if there is not already an Image view open, and it will make that view the active workspace view. The **3D** button will open a new view of your volume in the 3D view if necessary, and it will make that view the active workspace view. The **2D / 3D** button will make sure that both views are open, and display them in a split workspace layout with the 2D view on the left and the 3D view on the right.

Outline color allows you to change the color of the object's outline using the color selector. When this option is off, only the outlines for the active collection will be displayed.

 *Volume objects can also be exported as outlines from the 3D Data Table to Guided Segmentation using the Export Outlines menu item from the context menu over the 3D Data Table object row.*

2D Segmentation Sub-panel

The **2D Segmentation** sub-panel offers 3 main modes of operation that enable different methods that you can use for defining the object outlines:



The three modes are:

- **Wand**, where you will primarily use the **Magic Wand** tool to define the outlines by outlining an area based on the color similarities or difference of intensity ranges between the pixel under your cursor, plus or minus a specified tolerance interval.
- **Smart**, where you will use the **Smart Segmentation** tools to define the outlines by defining reference areas for background versus objects.
- **ROI**, where you will primarily use manual tools to draw the object outlines.

These modes will be discussed in detail in upcoming sections of this chapter. In all three modes, there are tools available to define and refine the object outlines:



The 2D Segmentation sub-panel provides the following tools for all modes:



Use the **Selection Tool** arrow to select an ROI on the image.



The **Polygon Nudge** tool allows you to adjust the size and shape of irregular ROIs and measurements



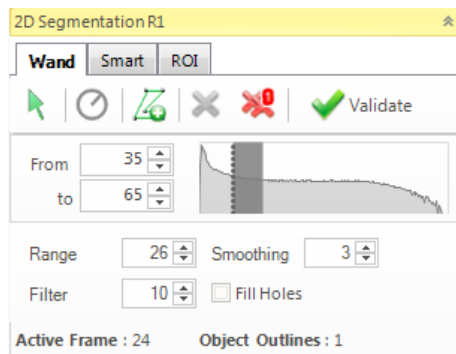
The **Polygon** tool allows you define a polygon-shaped or freeform ROI in the image.



Use the **Delete** buttons to delete the selected outline(s), or all outlines. Note that you can also use the Delete key to delete the selected outline(s).

Magic Wand Mode

You can define object outlines by selecting the **Wand** tab and clicking on the object to segment.



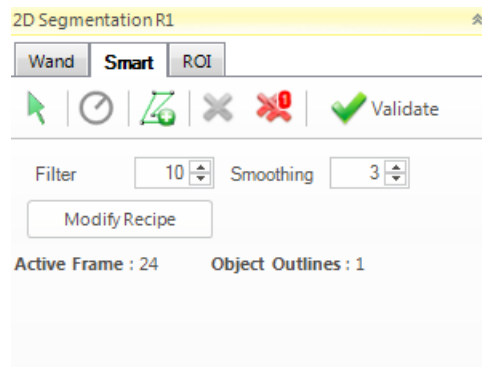
After the first click on the image, the threshold range will be displayed in the Histogram control. You can adjust intensity range of the **Magic Wand** using the **From** and **To** controls or by dragging the range indicators on the histogram. You can also use additional magic wand controls to adjust the automatic outlining: **Range**, **Filter**, **Smoothing** and **Fill Holes**.

- *Ranges*: Indicates the lower and upper levels used for thresholding.
- *Filter*: Defines the minimum object area in square pixels.
- *Smoothing*: Indicates the degree of smoothing that is applied to the object outline (0 = no smoothing, 9 = maximum smoothing).
- *Fill Holes*: Check this box to automatically fill any holes in the outline.

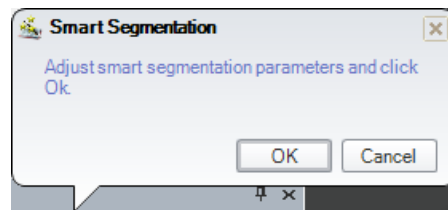
When you are satisfied with the outlines and the range, click the **Validate** button. That will convert the magic wand outline to an ROI and add it to the active object. Note that the defined intensity range will be then used to create outlines on other frames with the **Auto-Range** propagation method.

Smart Segmentation Mode

Use the **Smart Segmentation** tab to create objects. This method can be used on noisy objects where the **Magic Wand** has difficulties creating outlines.



Select the **Smart** tab to define the segmentation recipe parameters and draw reference objects and background areas. When you enter **Smart Segmentation** mode, the **Smart Segmentation** sub-panel will be displayed, along with a balloon message:



This message balloon will stay active while you set up the **Smart Segmentation** recipe in the **Smart Segmentation** panel. (More information about **Smart Segmentation** appears in *Chapter 8* of this manual and in the in-product Help.)

When you click **OK**, whichever objects have been identified by the **Select Object** tool will be added to the collection of outlines.

Filter defines the minimum object area in square pixels.

Smoothing is applied to the object outline (0 = no smoothing, 9 = maximum smoothing).

Note that if you want to modify the recipe going through frames, use the **Modify Recipe** button.

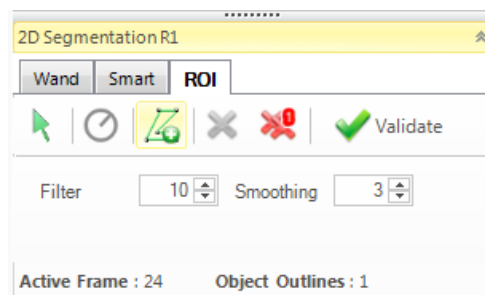
You can also add new outlines to the frame in *Smart* mode by following these steps:

1. Make sure that the **Auto-Smart** propagation mode is selected in the **Z-Propagation** sub-panel.

2. Select the **Polygon** drawing tool and draw one or more ROIs in areas that should be identified as objects. These ROIs can be very rough as they only need to overlap the area detected by the **Smart Segmentation**.
3. Click the **Validate** button. One new object will be created for each ROI that overlaps the smart segmentation mask.

ROI Mode

If you prefer creating object outlines manually, you can switch to the **ROI** tab. When you are in ROI mode, clicking the **Validate** button reads the intensity range within the region's outlines, which can be used for other frames when you choose the **Auto-Range propagation** method.

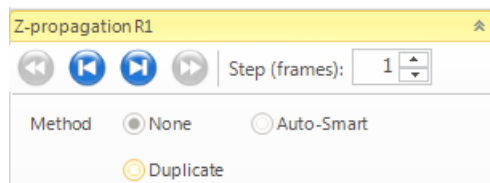


The following options will be applied when detecting regions using the **Auto-Range** propagation:

- Filter defines the minimum object area in square pixels.
- Smoothing is applied to the object outline (0 = no smoothing, 9 = maximum smoothing).

Z-Propagation



The **Z-Propagation sub-panel** offers you options for propagating the 2D objects from a particular frame across other frames in Z-space. The available options depend on the **2D segmentation** type. Options that are not available for a particular 2D segmentation type are shown as inactive:





Use the arrow buttons to navigate through the image stack. The spin controls indicate how many frames you advance each time. The following methods are used to create outlines on new frames:

- **None:** Advancing to a new frame will not add any new outlines to the next frame, or change any existing outlines. Use this method to review the outlines that you've defined so far, and also when the object outlines on neighboring frames are very different, and you want to draw outlines on every frame manually.
- **Duplicate:** Changing the frame will duplicate object outlines from the previously active frame.
- **Auto-Range:** Detects an object on a new frame that overlaps with the outline on the previous frame, using the same intensity threshold range. This propagation method is available when you are using the **Wand** or **ROI** modes.
- **Auto-Smart:** Detects an object on new frame using global smart segmentation recipe. It will identify any overlapping outlines. This propagation method is available when you are using **Smart** mode.

You can use any tool from the **2D Segmentation** sub-panel on any frame to redefine intensity ranges, smart segmentation recipe or modify the outlines. Any change of method will be repeated throughout the stack.

Use the **Forward**  and **Back**  button to go through the stack by one step at a time. If you use the **Auto-Range** or **Auto-Smart** method, and are going through the stack

to create correct outlines, you can automate this process using the **Detect Forward** 

and/or **Detect Back**  button to go through the all frames in the stack automatically. The detection will stop automatically when no more overlapping objects are detected. You can also stop the auto-detection process manually pressing the <Esc> key.

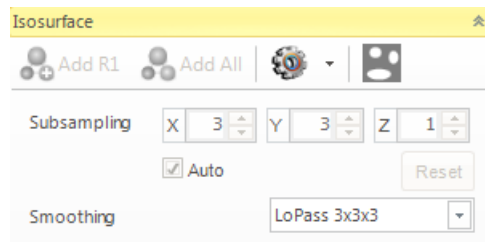
Object outlines will be automatically detected using the last segmentation recipe. If the object detection is not satisfactory on some frames, the outlines on those frames can be adjusted or re-drawn with the 2D Segmentation tools.

If you are using **Smart segmentation**, try adjusting the recipe by adding new reference or background objects and click **OK**.

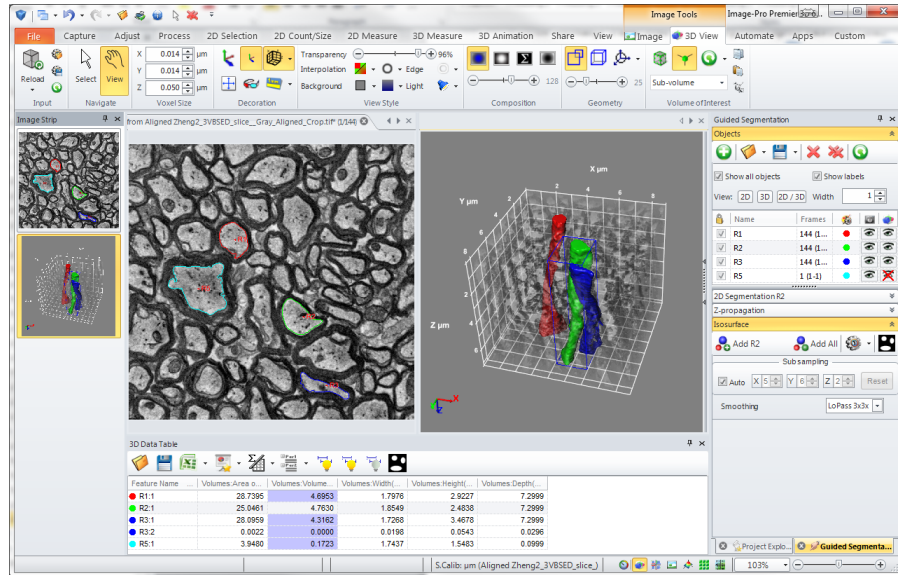
Note that when you draw outlines manually or edit them, the intensity range within the object's outlines will be used for other frames when you choose the **Auto-Range propagation** method. After adjusting the outline manually, click the **Validate** button to accept the changes. Note that the outline is automatically validated clicking by the **Forward** or **Back** button

Isosurface

The **Isosurface** sub-panel allows you to create isosurfaces on selected objects by connecting the 2D segmented areas across the Z-planes. When all object outlines are created, use the *Isosurface* group to add 3D objects as isosurfaces to the 3D view.



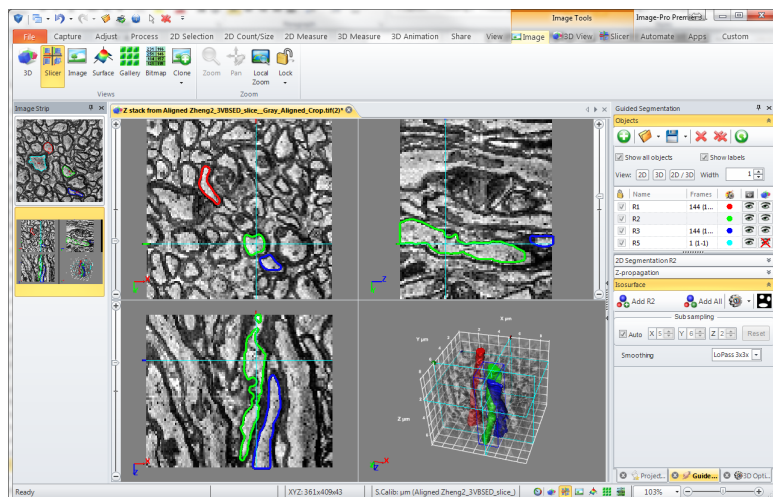
You can add only the active collection using the **Add Active** button (which contains the name of the active collection, for instance **Add R1** in the illustration above). Clicking the **Add All** button will add isosurfaces for all of the defined collections. You can set the sub-sampling and smoothing to use during isosurface creation (see the more detailed discussion of these settings in the [Add Isosurface](#) section later in this chapter).



Every object in each collection will be segmented and measured. The values appear in the data table.

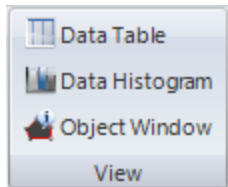
If you have an existing isosurface node and the **Create new isosurface** option is not checked, the new objects will be added to that existing isosurface. If there are no existing isosurface nodes, or the **Create new isosurface** option is checked, then a new isosurface named *Guided Segmentation* will be created with the new objects.

The outlines can be viewed in the **Slicer** by view activating the **Show Outlines** option:



View Group

The *View* group allows you to see the 3D data in different formats:



These tables contain the same features and functions as the tables and graphs described in Chapters 4 and 8 of this manual.

The **Data Table** collects all the information from the measurements taken on your image. The 3D data table shows measurement values for manual and segmented 3D objects.

3D Data Table					
Feature Name ...	Volumes:Area o...	Volumes:Volume...	Volumes:Width(...	Volumes:Height(...	Volumes:Depth(...
1:1	4107.0101	9431.2944	63.8400	64.3265	53.2438



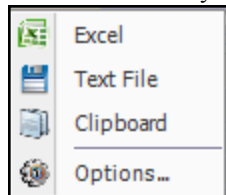
Load Data Table Measurements: This tool allows you to load measurements saved in a previous session from a *.ipm file.



Save Data Table Measurements: This tool allows you to save your measurements in a *.ipm file for use at another time.



Send Measurements to File: This tool allows you to save your measurement data to a file or export it to an *Excel* spreadsheet. The pull-down menu lets you choose one of the following:





Send to Report: This tool allows you to save your measurement data to *Image-Pro* report. The pull-down menu lets you create a new report or choose an existing one.



Show all Statistics: This tool allows you to hide or show the statistics panel for the data table measurements. A sample statistics panel is shown here:

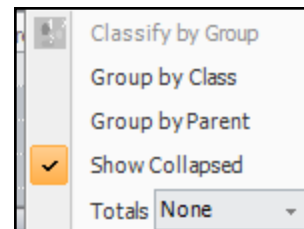
Mean value	0.00	233.99	67.11	15.35
Standard Deviat...	0.00	48.03	26.39	3.13
Minimum	0.00	151.95	23.43	12.87
Maximum	0.00	298.09	105.75	21.92
Range	0.00	146.15	82.32	9.05
Sum	0.00	1403.92	402.67	92.09
Index of Minimum	0.00	4.00	1.00	2.00

The drop-down option **Statistics per Group** defines what is displayed in the statistical pane. When this option is off, the statistics of all visible features is shown. When the option is on, the statistical values are shown per group. This option has affect only when **Grouping** is active

Setup Group Statistics display the statistics by group. It will activate grouping by class, add Percent Area Parent to the list of measurements and activate statistics per group



Grouping: Click this button to see options for grouping your data in the table. You can choose one of the following:



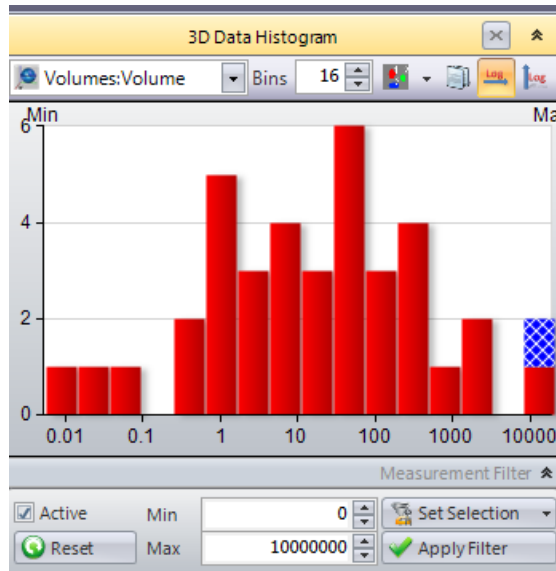
Show All, Show Selected Measurements, Hide Selected

Measurements: These tools let you choose to display or hide some of the measurement statistics.



3D Mask: Click this button to create a mask of volume objects.

The **3D Data Histogram** illustrates, in graph form, the distribution of 3D measurements for the selected measurement:



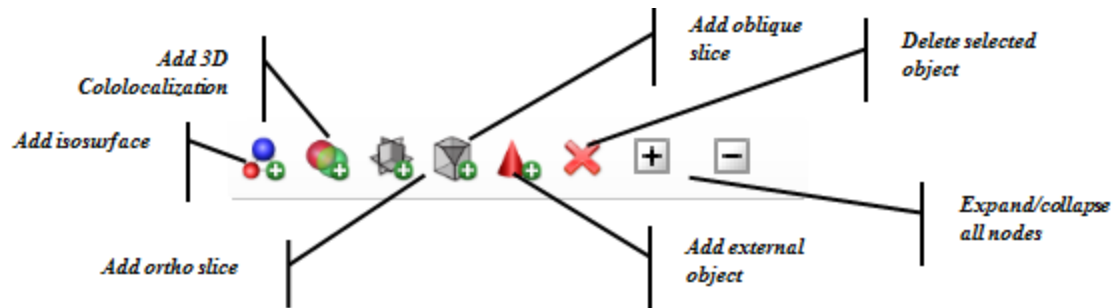
The **Object Window** allows you to view information about a specific object in an image. The example below shows complete data for one object:

The 3D Object Window window displays data for a specific object. The title bar is "3D Object Window". Below the title bar, there is a dropdown menu set to "Object 1" and a "1:1" zoom level. To the right of these is a small icon. The main area contains a table with two columns: "Measurement" and "Value".

Measurement	Value
Volumes:Area of Surface	4107.0101 pix ²
Volumes:Volume	9431.2944 pix ³
Volumes:Width	63.8400 pix
Volumes:Height	64.3265 pix
Volumes:Depth	53.2438 pix

3D Options

The **3D Options** panel on the right-hand side of the workspace contains the following controls:



All of the **Add** buttons add nodes to the 3D view that contribute to the rendering of your volume.

Add Isosurface/Volume measurement: Isosurfaces provide a very quick, yet often sufficient, method for reconstructing polygonal surface models. By correlating transparency with the local orientation of the surface relative to the viewing direction, complex spatial structures can be understood much more easily.

Delete: Click this button to remove the selected node.

Add Ortho Slice: Clicking this button adds individual ortho slices to the ND projection. Ortho Slices are used to display a slice through the X, Y, or Z axis orientation in the 3D Viewer. Voxels on the plane of the slice will appear as a different color.

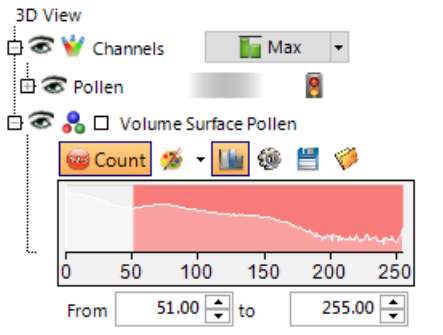
Each slice can have a different color outline. This can be useful for identifying slices on complex scenes with multiple elements (oblique, ortho slices). The visibility and the color of the outline can be set using the **Outline** control.

Add Oblique Slice: Oblique slices are used to display slices of the volume which are not precisely aligned with the X, Y, or Z axes.

Add External Object: External objects are isosurface coordinate links that can be imported into the *3D Viewer*.

Expand/Collapse all nodes: Click the plus sign to expand the channels and nodes. Click the minus sign to collapse them.

The rest of the **3D View** panel displays the rendering nodes that currently exist in your 3D visualization:

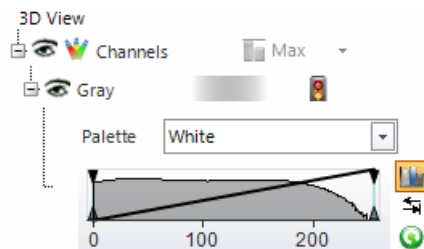



Color Image: All channels are loaded into an indexed color volume (The sequence is converted to a palette class using a palette containing the most popular colors in the sequence).

Channels: The channels node represents the visualization of the volume intensities. The drop-down box will only be enabled for multi-channel volumes, and controls the way that the channels are combined in the volume rendering. Clicking the drop-down arrow displays the following options:


- **Max:** *Maximum Intensity Projection* takes the value of the brightest voxel in the same straight line as used by the **Blend** option.
- **Blend:** Blending averages the values of the voxels in a straight line through the volume of the object.
- **Min:** *Minimum Intensity Projection* takes the value of the dimmest voxel in the same straight line as used by the **Blend** option.

Each channel of your volume will have a sub-node for the channel, with a name derived from the channel name (if the volume is a multi-channel set), the color component (of a color image), or “Gray” for monochrome images. Each channel or color component’s rendering can be adjusted with the following control panel:



The **Show**  button hides or shows the selected element. Every node in the **3D View** panel has this button, which lets you select which nodes are visible, and are included in the 3D rendering.

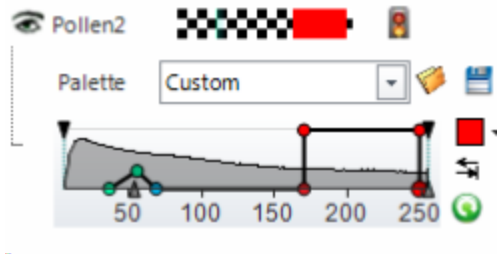
Every channel is shown using a palette that is scaled from 0 to 255 covering the whole dynamic range of the channel data. The **All Channel Display** controls will change **PaletteSpread** and **Opacity** range on all channels.

The **Blinking** icon  control activates the blinking mode of the channel.

The **Palette** can be one of the following:


- **Wavelength** allows you to display wavelength in nanometers.
- **Color** lets you select any color using the color picker.
- **Palette** allows you to select one of predefined palettes displaying the channel.
- **Custom**

Selecting **Custom** palette allows creating user-defined color combinations:

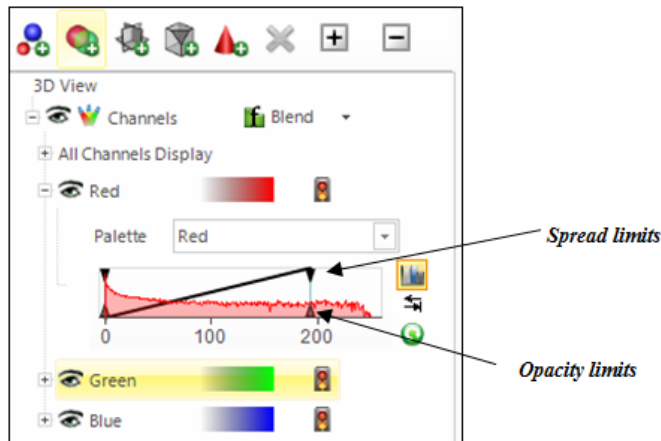


Palette points can be added by clicking on the histogram area. Clicking the existing point makes this point active and allows selecting the color using the *color-picker* button. Positions of palette points can be adjusted by mouse. The Y position of the point defines opacity of the color, higher position – higher opacity. *Ctrl-Click* on the existing point will delete the point. Custom palettes can be saved to a file (pal3D format) and loaded (iqpal, pal3D) using the *Save* and *Load* buttons.

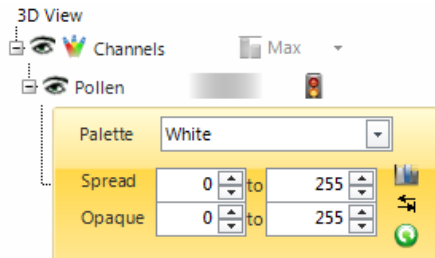
Histogram: Activating the Histogram option allows you to set the Spread and Opaque levels on a volume histogram. The Spread limits are controlled by the top controls and the Opaque limits are controlled by the bottom ones.


Activating the **Histogram**  option allows you to set the **Spread** and **Opaque** levels on a volume histogram. **Spread** defines the start and end positions for palette spread and the


Opaque control defines the range of intensities that are visible. The values outside the **Opaque** range are transparent. The **Spread** limits are controlled by the top controls and the **Opaque** limits are controlled by the bottom ones.



The **Histogram** view is the default view for the **Spread** and **Opacity** controls, but you can turn this view off to edit the limits directly,



The **Best-Fit** button () can be used to set the **Spread** and **Opacity** values to a best-fit display of the volume.

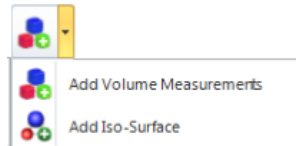
The **Reset** button () can be used to reset the **Spread** and **Opacity** values to the minimum and maximum intensities.

Add Isosurface

Isosurfaces/volume measurements provide a very quick, yet often sufficient, method for reconstructing polygonal surface models. By correlating transparency with the local

orientation of the surface relative to the viewing direction, complex spatial structures can be understood much more easily. If you are making volumetric measurements, you will need to define at least one isosurface/volume measurement.

Automatic segmentation of 3D objects can be done using 2 different tools:



- **Add Volume Measurements**

- **Add Iso-Surface**

The **Add Iso-Surface** segmentation is based on iso-surface, which is generated using the Marching cubes algorithm with consequent labeling of mesh triangles (we will call it ***Iso-Surface segmentation***). The **Add Volume Measurements** uses segmentation creating objects directly based on voxel connectivity data, without generation of iso-surfaces (we will call it ***Direct Volume Segmentation***).

Both methods have their advantages, which will be compared here:


Advantages of ***Direct Volume Segmentation*** over ***Iso-Surface segmentation***:


1. *Lower memory usage.* Since no iso-surface is generated, the memory requirements for direct segmentation is much lower than for the iso-surface segmentation, which allows segmenting larger volumes.
2. *Faster segmentation.* The segmentation is applied to a 3D volume directly, the algorithm uses a single pass labeling, which significantly speeds up the segmentation, up to 100 times on complex volumes.
3. *Holes handling.* Direct segmentation algorithm handles holes according to the **Fill Holes** option, which allows user to decide if they want to include or exclude the holes from segmented objects. Generated Hole measurements (*Hole Count*, *Hole Volume* and *Hole Volume Ratio*) are available only for directly segmented objects. The iso-surface segmentation handles holes differently, the holes are always included into the surface object measurements, no Hole measurements are generated.

Drawbacks of ***Direct Volume Segmentation*** over ***Iso-Surface segmentation***.

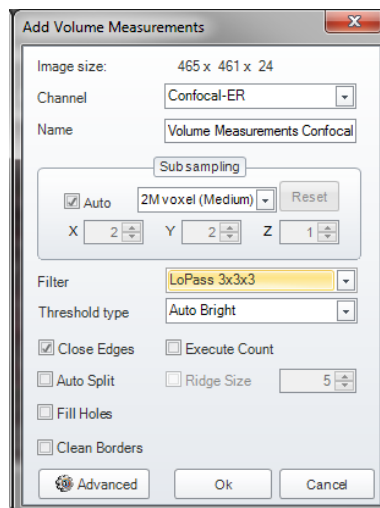
When Direct Volume Segmentation is used no iso-surface is generated, which leads to some drawbacks.

1. The segmented objects *cannot be saved as 3D models* to IV, WRL or STL files. If you need to have a 3D model file, you should use iso-surface segmentation.
2. Derived measurements that use volume objects, such as *Distance between surfaces*, *Point to Surface* are not available for Direct Volume Segmentation.
3. *Pixelated visualization*. Visualization of 3D objects are done using shader rendering on GPU, so it misses some smoothing effects of triangular mesh iso-surface rendering. You can change material appearance of segmented objects using **Pseudo Surface** tools, such as pseudo-surface material or using the Voxelize option in the Interpolation drop-down. If object visualization is important, you should use the iso-surface segmentation.
4. *Clipping planes* created by ortho and oblique slices can clip iso-surface objects and volume independently, depending on the position of the slice relatively to the iso-surface element in the options tree, so volume can be clipped, but the surface not. With Direct Volume Segmentation both Volume Channels and Segmented objects are clipped in the same manner.

Multiple isosurfaces can be added clicking the Add Isosurface  button in the options panel.

 Note that the same action can be performed from the 3D Measure tab by clicking the Count button.

When you add a volume measurement, you will see this dialog:



Here you can select which channel of the volume will be used to create the isosurface.

You can select the desired subsampling. The **Auto subsampling** is selected by default.

Filters can be used to smooth the surface. A filter can be selected from the following list:

- None
- LoPass 3x3x3
- LoPass 5x5x5,
- LoPass 7x7x7
- LoPass 9x9x9
- Gauss 5x5x5
- Gauss 7x7x7
- Gauss 9x9x9.

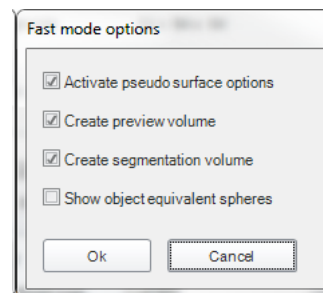
The initial threshold level can be selected automatically when the **Thresholdtype** is defined as *Auto Bright* or *Auto Dark*.

Close Edges will close surface edges on the volume boundary.

Fill Holes option is on, the holes are included into the measurements of the segmented objects.

If **Clean borders** is on selected the objects that touch the bounding box of the volume are excluded from the analyzing.

The segmentation with default options has advantages on memory consumption and speed over iso-surface segmentation, and if user wants to get even better performance measuring the volume, there are additional options available clicking the Advanced button.

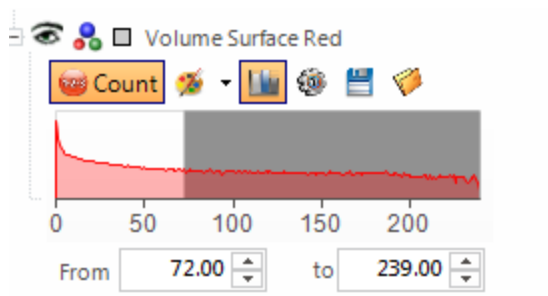


The **Activate pseudo surface** options check-box makes the best visualization options for direct segmentation surfaces active automatically. When this option is on, the volume visualization is automatically switches to the Pseudo Surface mode.

The option **Create Preview volume** defines creation of the visualization volume for threshold adjustment preview. You can switch this option off to save RAM, in that case threshold preview will be generated on the original volume channel and may look slightly different from the segmented volume as they may have different sub-sampling and filter options.

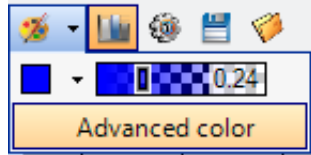
When the **Create segmentation volume** is on, the segmented objects are shown in different colors on the volume. If user just want to have measurements and the visualization is not important, this option can be switched off to save more RAM. Note, that all measurements will still be generated properly and object volume boxes will be displayed for selected objects. When this option is off user can show equivalent spheres instead of the proper outline by activating the **Show object equivalent spheres** option, spheres display takes less RAM then the segmentation volume and can be used to handle large volumes when Create preview volume and Create segmentation volume options are off.

Execute Count will automatically execute the object segmentation count when an isosurface is created. When **Execute Count** is off, only the isosurface will be created, so you can then adjust the Threshold levels interactively. Start the count by checking the **Count** button:




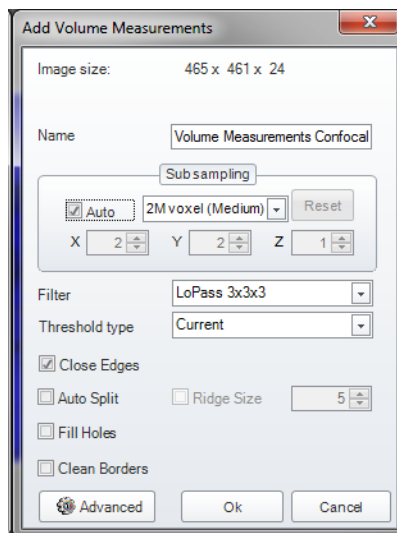
The thresholds can be adjusted by clicking and dragging at the start or end of the threshold range on the histogram, or editing it in the **From** or **To** controls.

When **Count** is turned off, the color of the isosurface can be adjusted in the **Color** dropdown:

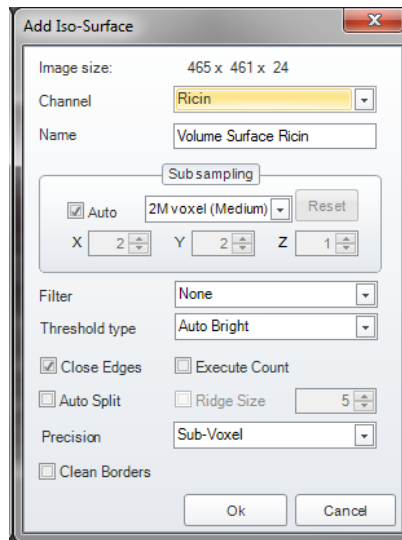


Note that when **Count** is on, the colors of individual objects are controlled by the **Coloring** parameter of the *3D Measurement* options. When **Coloring** is set to **Parent** the segmented objects are the same color as the isosurface. When the **Coloring** is set to **Random**, then the color of every segmented object is selected randomly, but the transparency is taken from the isosurface.

Clicking the **Options**  button allows you to edit the surface parameters: subsampling and filtering the auto-threshold:



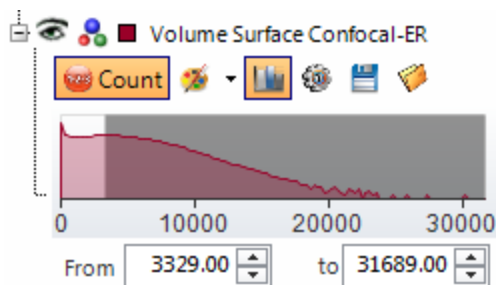
Using **Add Iso-Surface** button, the iso-surface based segmentation can be created.



The **Precision** control defines whether surface outlines of 3D object are created with *Sub-voxel* or *Voxel* granularity. The default option is *Sub-voxel*. This option allows creating smooth surface outlines that can be created in sub-voxel space and the position of this outline depends how close the threshold value is to the intensity value of the voxel.

When the *Voxel* option is active the surface outlines and all volumetric measurements are build from voxel cubes. Voxels with intensity above or equal the threshold level are completely included into the object.

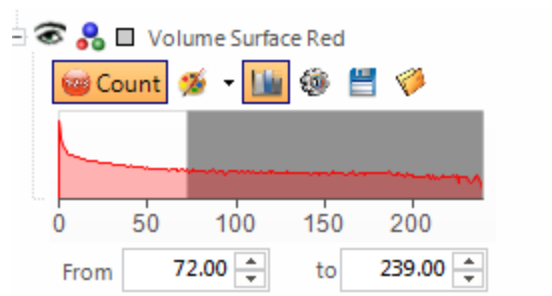
The following element added clicking the **Ok** button:




The visible volume measurement objects can be exported to an IV file by clicking the **Export** button.

It is possible to import volume objects using the **Import** button. This option can be used, for example, when you have segmented objects on one channel and want to measure intensities within these outlines on another channel.

When the outline is imported, the channel mode changes to outline where the **Count** option is always active:

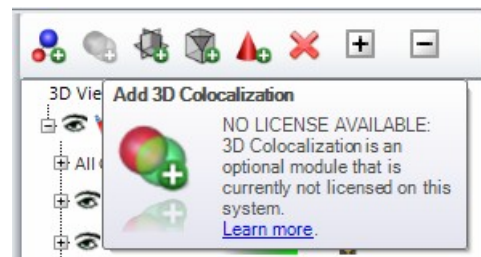


To return to the interactive segmentation mode, click the **Reset Outline**  button.

Add Colocalization

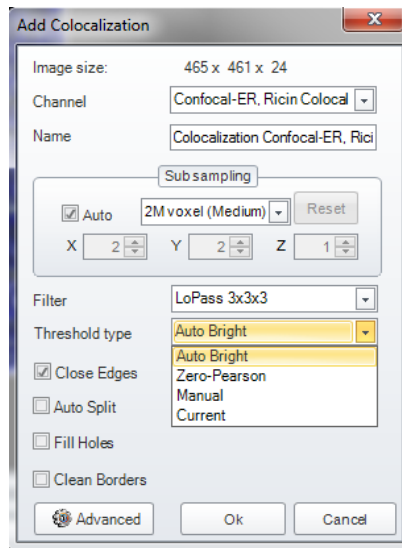
Colocalization measurements with independent threshold levels can be added using the **Add 3D Colocalization Measurements** and **Add 3D Colocalization Iso-Surface** buttons. Both actions will create colocalization measurements, the difference is the same as for volume measurements and iso-surface, the first one creates colocalization using direct volume segmentation and the second - using iso-surface segmentation.

3D Colocalization in Image-Pro with 3D Module requires a separate license. If you do not have a license for this option, you will see the following message:



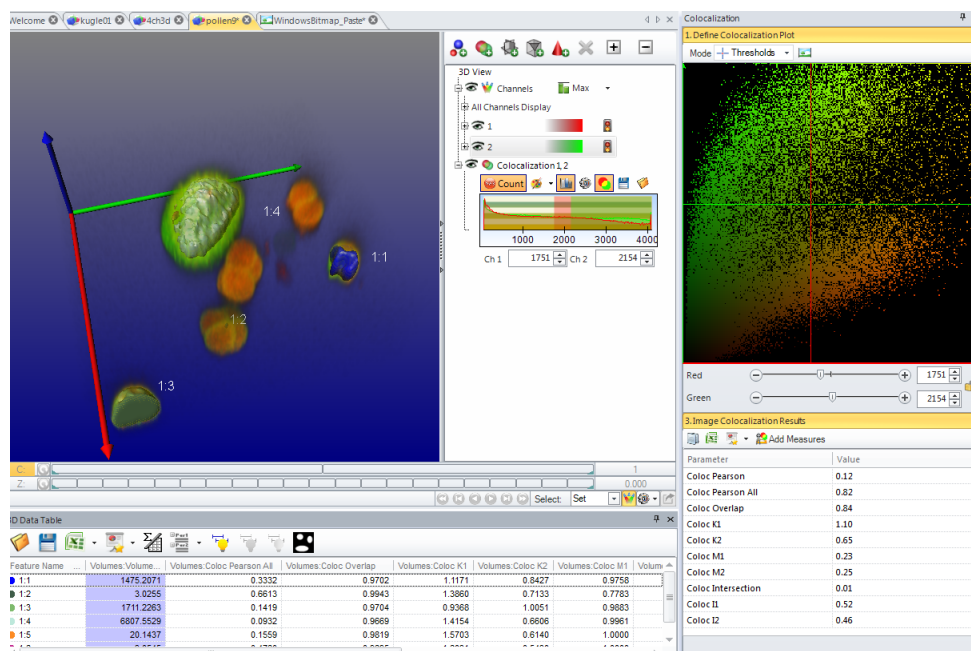
Please contact your Media Cybernetics representative for additional information.

Clicking the button shows the dialog where you can define the colocalization pair and also the initial auto-threshold method:



The default method is *Auto-Bright*.

Clicking the **Ok** button creates a new Colocalization measurement and opens the colocalization panel with colocalization scatterplot and the table with colocalization parameters. The histogram window shows the intensity distribution for two channels.



Colocalization thresholds can be adjusted for each channel independently using Channel 1 and Channel 2 numeric controls, dragging threshold levels on histogram or clicking on scatterplot. The colocalization objects and measures are updated automatically.

It is also possible to use Free ROI tools to define colocalization areas on the scatterplot, the colocalization objects get updated after creation or adjusting of ROIs. Note, that adjusting thresholds on histogram switches colocalization mode to “Thresholds”.

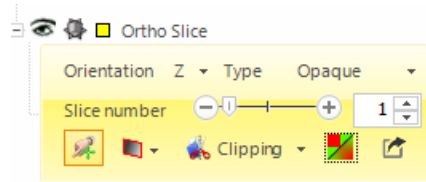
3D view may include multiple colocalization pairs. The colocalization panel shows results for the active colocalization pair. To activate the pair click the **Colocalization** button.


When the Count option is active, colocalization measurements can be reported for every object. To add colocalization to the list of selected measurements, click the **Add Measures** button. The measures will be added to the list and shown in the data table.

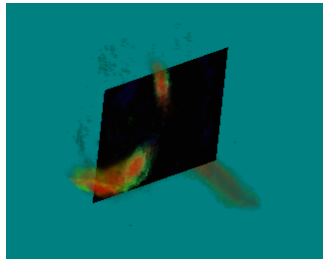
Add Ortho Slices

Ortho Slices are used to display a slice through the X, Y, or Z plane orientation in the *3D Viewer*. Voxels on the plane of the slice will appear as a different color.

You can add multiple ortho-slices by clicking the **Add Ortho Slice** button.



If the **Show**  control is on for **Ortho Slice**, an orthogonal slice is shown on the volume.




Orientation selects one of three ortho slices, aligned with the X, Y or Z axis.

Clipping enables the clipping plane associated with the slice. The clipping plane will clip off rendering of the volume channels on one side or the other of the clipping plane. The clipping side can be set to either *Front* or *Back*. The clipping plane has no effect if the slice is not visible.

The **Handles** controls visibility of the "dragger" associated with the ortho slice. The dragger is a 3D user interface gadget that can be used to drag the slice through the data volume. To use the dragger make sure the viewer is in selection mode (cursor is an arrow shape). Move the cursor onto the white cylinder, press the left mouse button, and drag to move the slice.

You can also drag slices just by clicking and dragging the slice itself.

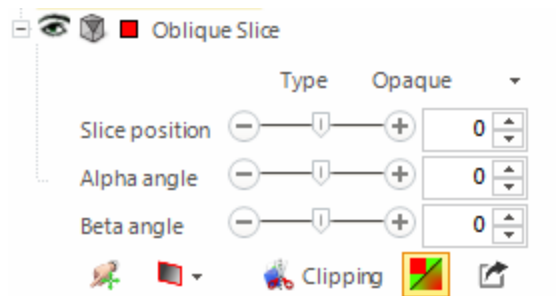
Slice composition is set by the dropdown **Type**  and can be *Opaque*, *Binary* or *Transparent*. *Binary* means that voxels with non-zero alpha values are completely opaque and voxels with zero-alpha values are completely transparent. In addition the drop-down contains control to define *Transparency* of the slice affects the slice display in *Opaque* and *Binary* modes. In addition the drop-down contains the **Transparency %** control to define the Transparency of the slice, which also affects the slice display in *Opaque* and *Binary* modes.

Slice Number shows the slice number where the slice is currently positioned. It can also be used to drag the slice through the data volume. The slice is moved dynamically as the slider moves. You may also type a slice number in the edit box.

Smooth switches on linear interpolation on the slice, thus increasing the visible resolution.


Add Oblique Slices

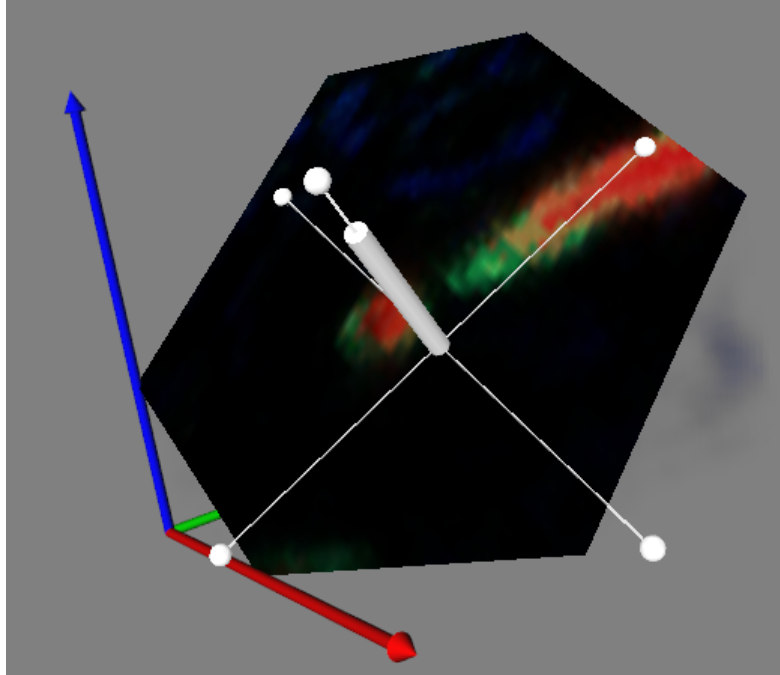
Oblique slices are used to display slices of the volume which are not precisely aligned with the X, Y, or Z plane. Multiple oblique slices can be added by clicking the **Add Oblique Slice** button in the panel.



The program can display an "oblique" (arbitrary orientation) slice through the data volume.

Slice position controls the slice position along the normal vector of the slice plane.

Alpha and **Beta** angles control orientation of the oblique slice plane, which can also be adjusted interactively using the slice handles  (see the following illustration):



The dragger is a 3D user interface feature that can be used to drag the slice through the data volume and change the orientation of the slice. To use the dragger make sure the viewer is in selection mode (cursor is an arrow shape). Move the cursor onto the white cylinder, press the left mouse button and drag to move the slice. Move the cursor onto one of the white lines, press the left mouse button, and drag to rotate the slice. You can also drag slices just by clicking *and dragging the slice itself*.

Clipping enables the clipping plane associated with the slice. The clipping plane has no effect if the slice is not visible.

Slice Number shows the slice number where the slice is currently positioned. It can also be used to drag the slice through the data volume. The slice is moved dynamically as the slider moves. You may also type a slice number in the edit box.

Smooth switches on linear interpolation on the slice, thus increasing the visible resolution.

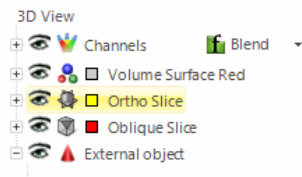
Slice composition is set by the dropdown Type Opaque and can be *Opaque*, *Binary* or *Transparent*. *Binary* means that voxels with non-zero alpha values are completely opaque and voxels with zero-alpha values are completely transparent. In addition the drop-

down contains control to define *Transparency* of the slice affects the slice display in *Opaque* and *Binary* modes. In addition the drop-down contains the **Transparency %** control to define the Transparency of the slice, which also affects the slice display in Opaque and Binary modes.

Each slice can have a different color outline. This is useful for identifying slices on complex scene with multiple elements (oblique, ortho slices). The visibility and the color of the outline can be set using the **Outline** control.

Add External Objects

External objects are isosurface coordinate links that can be imported into the *3D Viewer*. Multiple external objects can be loaded from files by selecting the **Add External Object** button in the panel.



The loaded external object can be edited by clicking on it in Select mode. Use the dragger to scale, rotate and move the object.

***Tip:** Holding down <Ctrl>+<Shift> allows you to adjust the dimensions independently.*

Clicking the object again will hide the draggers.

The 3D Slicer Ribbon

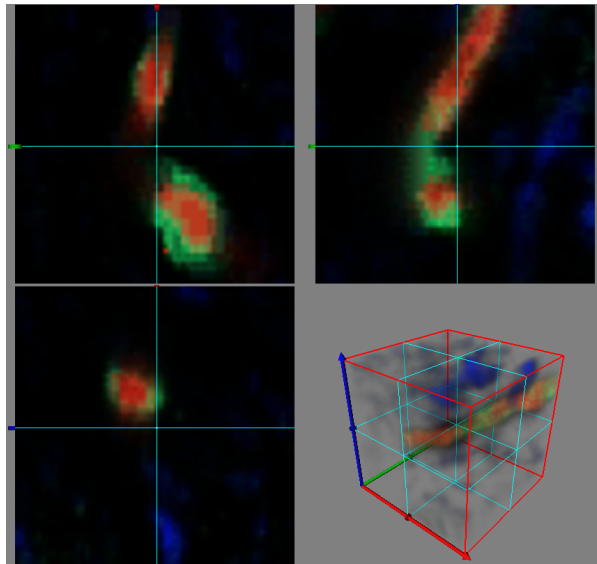
Image-Pro with 3D Module includes an advanced **Slicer** view in the available toolbars. To use the Slicer tools, click the **Slice** viewer button on the **Image** tab. The **Slice** viewer allows you to add up to 3 additional views of the volume from different orthogonal directions. A **Slicer** tab appears on the ribbon.

The **3D Slicer** ribbon looks like this:

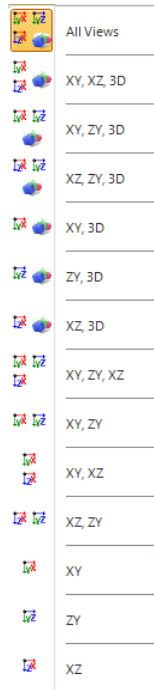


The *View* group shows the volume using three different modes (thin slices, thick slices or projections), volume object outlines, and manual measurements

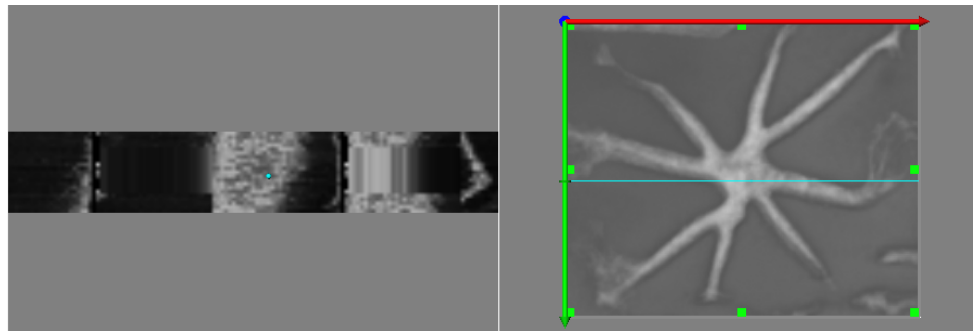
An example of the Slicer view with XY, YZ, XZ and 3D views all displayed appears on here:



You can control the layout of the **Slice Viewer** using the tools in the **Layout** drop-down menu in the *View* group:



The layout can include one, two, or three of the volumes and projections. For example, if you select the XZ, 3D and XYZ, 3D views, the workspace will look something like this:

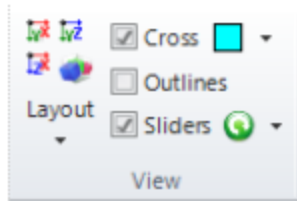


XZ 3D Projection


XYZ 3D Projection

The XZ3D projection is on the left, while the XYZ 3D projection is on the right.

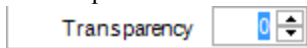
In addition to the layout selections, the *View* ribbon group contains other controls:



- **Cross** turns the crosshairs on the slicer views on or off. The color of the cross can be selected using the color-picker control.
- **Outlines** turns the *Volume Object* outlines on or off. The option can be used to see outlines of volume objects on 3D slices. Note that only the outlines for Counted objects are shown (execute Count to see the outlines). The thickness of the outlines is defined by *Line width* parameter of 3D measurement options.
- **Sliders** turns the slider controls for each of 3D slicer views on or off (the default is *on*). These controls appear on the sides of the panels in the workspace. The control can be used to adjust a displayed slice position in the corresponding view. Note that the slice position can also be adjusted by using the **Slicer Position** controls on the ***Slicer ribbon***, or by using the Navigate tools and clicking on any of the slicer views.

Slicer views can be zoomed using the mouse wheel or $\langle Alt \rangle + mousemove$ and translated using the $\langle Alt \rangle + \langle Shift \rangle + mousemove$ combination. The views can be reset to the original state (best-fit) using the **Reset views**  button.

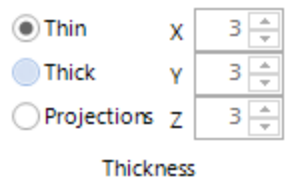
The drop-down menu of the **Reset** button contains controls that set the **slice transparency**.



Setting slice transparency to non-zero values allows the program to display manual measurement elements behind the slice in *thin* slice mode.

The *Slicer Position* group contains three **Extract**  buttons that allow you to extract the XY, ZY and XZ views into a new image.

The *Thickness* group defines the display mode for 3D slicer views:



- **Thin:** The view is displayed as a thin orthogonal slice. The slice can be semi-transparent based on the *Thin slice transparency* option.
- **Thick:** The view is displayed as thick slice including multiple slices. The thickness of the slices is defined by the corresponded numerical controls. The transparency of thick slices is defined by the main transparency control (on 3D view tab).
- **Projections:** Projections of all slices are shown. The view uses current composition and can be shown as maximum or minimum intensity projections (as well as blend and sum).
- **X, Y, Z:** You can set the thickness of the slices by using the spin buttons.

Use **<Ctrl>+ mouse click** to add manual measurements on the slicer views. Clicking the mouse button without the **<Ctrl>** key will change the slicer position.

Measurements created on a 3D view are also displayed on slicer views.

Note that all added 3D elements (slices, isosurfaces) can be rearranged by dragging and dropping them to a new position. It can be used to define proper clipping of one element by another, as slices can only clip isosurfaces and other slices located below the given slice in the scene.

The 3D Animation Ribbon

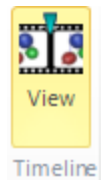
The **Animation** tab contains the tools for creating simple animations and movie files. The *Animation* feature allows you to create, record, and play animation files (*.anm) in the *Image-Pro3D* workspace.



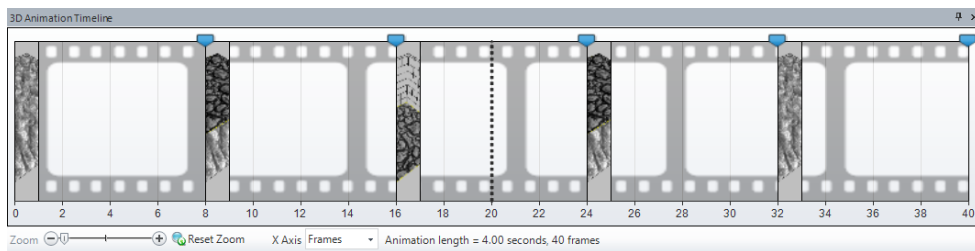
The animation tools are described in the following pages.

Timeline Group

The *Timeline* feature lets you synch your animation in time and space. The timeline panel shows the current animation with thumbnails of the key frames, and allows you to play and edit the animation sequence.



The timeline panel will open automatically at the bottom of the page when you activate the **3D Animation** ribbon.



The individual frames in your animated image are synched to the locations along the time line. The x-axis of the timeline represents the time of each key frame in the overall duration of the movie. The following controls are displayed in the status bar.

X Axis Time (s) Animation length = 26.78 seconds, 241 frames

The X axis can be labeled as **Frames** or **Time (s)**.

Clicking on any position in the timeline will change the current position in the timeline, and will activate the given camera position in 3D view. The current position is indicated by a dotted line, as you can see at frame 20 of the Animation timeline in the previous figure. Note that the current position is very important when adding key frames to the animation. The timeline can be zoomed in or out using the zoom controls or the mouse wheel. The number of key frames can be adjusted by dragging the key frame separators.

Timeline editing is done by adding or deleting key frames. Each key frame is indicated by a blue pointer at the top of the timeline. Multiple key frames can be selected by clicking on or before the first key frame, and then using **<Shift>+<Click>** on the last key frame. Multiple key frames can be selected using **<Shift> +<Click>**. The selected range can be then deleted by clicking the **Delete** button.

Animation Path Group

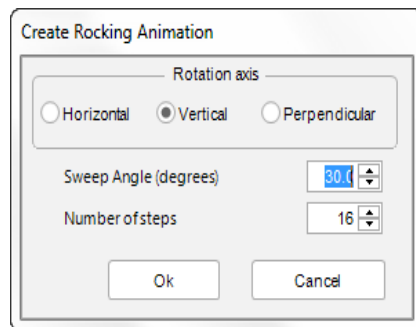
The *Animation Path* group lets you insert camera animations into your timeline with a single click.



- The **Spin** button allows you to add a sequence of frames rotating the camera around the *screen* axis (Vertical-Y, Horizontal-X, and Perpendicular to the screen – Z). The drop-down menu contains buttons to rotate the camera around 3 axes in both directions by the given angle defined by 90,180 and 360 radio-buttons. The number of intermediate frames for a 360-degree spin is defined by the **Circular Frames** option in the *Options* group.
- The **Fly-Around** button allows you to add a series of frames by moving the camera in an evenly spaced, circular path parallel to the *Viewer* window, and half-way between the two sides of the window. The **Fly Around** rotations are created around the *world* axis. *World axes* are X, Y and Z and can be visualized by activating the **Axes** button on **3D View** tab. **Fly Around** animation gives the effect of helicopter flight.
- The center options let you choose amount of rotation for both the **Spin** and **Fly-Around** selections. To go completely around your screen or volume one full turn, use 360 degrees, or 90 degrees to go only $\frac{1}{4}$ of the way around.

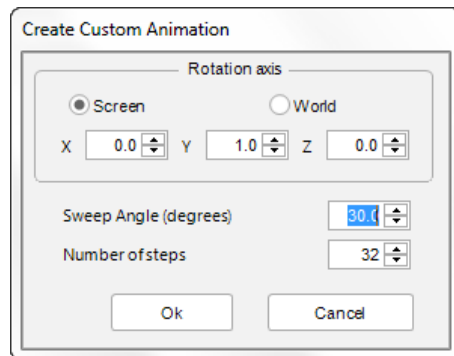
- ☒ 90
- ☐ 180
- ☐ 360

- **Rocking or “see-saw” animation.** This animation rocks the image around the vertical axis back and forth by approximately 30 degrees. Clicking this button displays the following dialog:



The **Rotation axis** group defines the *screen* rotation axis. The **Sweep angle** and **Number of steps** controls define the amplitude of rocking and the number of intermediate frames.

- **Custom** lets you design a custom animation by selecting time points and camera positions. You will see this dialog:

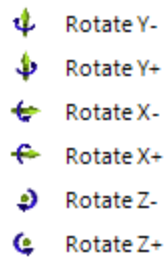


Clicking the **Screen** radio button allows you to create fast transitions by rotating the camera around the *screen* axis (Vertical-Y, Horizontal-X, and Perpendicular to the screen – Z).

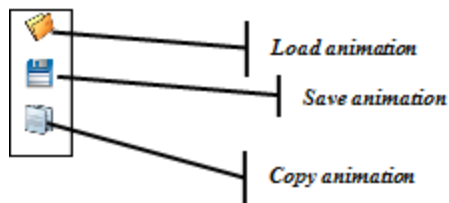
Clicking the **World** axis radio button let you create animation by activating the **Axes** button on **3D View** tab. World animation gives effect of helicopter flight.

The **Sweep** angle and **Number of steps** controls define the amplitude of the animation sweeps and the number of intermediate frames.

You can rotate your animation around the X, Y, or Z-axis, by clicking the drop-down menu button and displaying these items

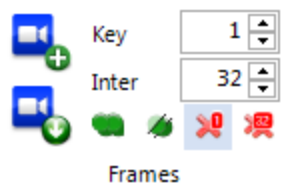



To save an animation path, click the **Save button** and select path and name for the animation file. Saved animations can be loaded using the **Load** button. The **Copy** button does not work directly with you animation timeline, but will copy the current 3D view of the volume to the clipboard as a picture that can be inserted into a presentation or document.




Frames Group

The *Frames* group gives you tools for managing the key frames in your animation, as well as spacing between the individual frames.



 Clicking the **Add Camera Position** button adds the current camera position (and any changes to the view) as a new key frame at the current position within the animation timeline.

 **Replace camera position** replaces the current key frame with the current camera position (and any changes to the view).

The **Key** spin box indicates the current key frame number.

The **Inter** spin button indicates the number of intermediate frames and camera positions in the key frame. You can adjust or edit the number of intermediate frames by using the spin button, or by dragging the key frame handle on the timeline.



Click the **Delete** button to delete the active key frame and its intermediate frames.



Click the **Clear** button to delete all key frames and clear the animation timeline.



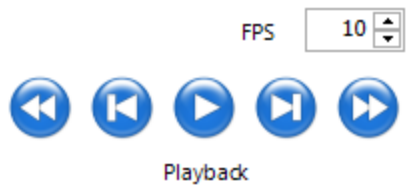
Click this button to double the number of intermediate frames at all camera positions.








Click this button to halve the number of intermediate frames at all camera positions.

Playback Group

The *Playback* group lets you play your animation forward and backward. It also controls the playback rate in *Frames Per Second (FPS)*.

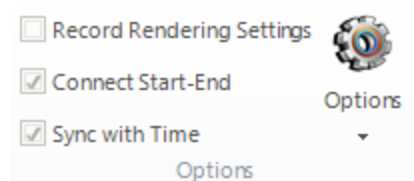


The toolbar has the following buttons:

-  Go to first frame
-  Go back one frame
-  Play animation forward
-  Go forward one frame
-  Go to last frame

Options Group

The *Animation options* group contains some additional controls:



The **Record Rendering Settings** option allows you to use different rendering settings for every camera position in the animation. You can change any rendering parameter, such as transparency or palette, and turn visibility of slices, channels, and volume measurements on or off. The changes will be applied when the corresponding camera position and following intermediate frames are rendered. Note that only existing rendering elements can be used here. You will need to add all necessary elements, such as ortho and oblique slices, isosurfaces, and volume measurements before starting to record an animation. The vis-

ibility and parameters of all rendering elements can be changed during recording. This option should be *on* unless you are animating only simple views of the basic volume.

Connect Start and End: Check this option to have the final intermediate frames interpolate between the last key frame of the animation, and the first one. Note that any option that has an on/off setting, for instance visibility of an element, cannot be interpolated. For this reason, the final key frame of any movie that should be looped should have the same on/off settings as the first frame.

Synch with Time: If the loaded image set has multiple time points, use this option to synchronize the time points with the camera positions.

Additional options are shown when you click the *Options* drop-down button.

Hide or Show Animation cameras.

Circular Frames 32

Inter Frames 8

Antialiasing

Passes 4

Use Fixed Image Size

Width 256

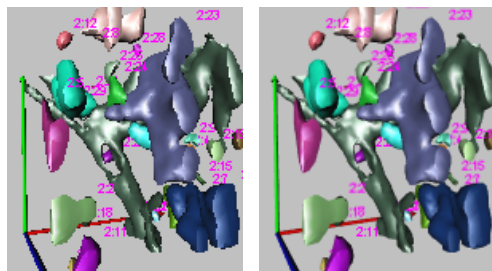
Height 256

Hide or Show Animation cameras: Click this button to turn the display of the camera positions on or off.

Circular Frames defines how many frames will be generated when you create a new circular animation path using the **Spin** or **Fly-Around** features in the *Animation Path* group.

The **Inter** control defines the default number of frames that will be added with each key frame. The actual number for any key frame can be adjusted later, as previously discussed.

Antialiasing increases the visibility of edges on created images. When the Antialiasing option is *on*, the multipass antialiasing is activated during creation of animation images. Every image is rendered x number of passes from slightly different camera positions, averaging the results.



a. b)

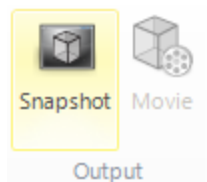
Image a) was created without antialiasing. Image b) was created with 8-pass antialiasing.

- **Passes:** The multi-pass antialiasing is activated during the creation of animation images. Every image is rendered according to the number of **passes** from slightly different camera positions, averaging the results.

Use fixed image size option allows you to define the size of the generated animation or image. Enter the values for **Width** and **Heights** (in pixels) to set the size. When this option is off, the current window size is used. If you will be making your movie for display on an HD monitor, you will usually want to use the **Use fixed image size** option, and set the **Width** and **Height** to either 1280 and 720 (for 720p HD display), or 1920 and 1080.

Output Group

The *Output* group lets you create snapshots of your current view, or to render your complete animation to a movie:



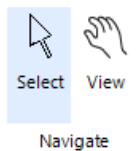
The **Snapshot** button will create a new image holding a snapshot of the view at the current position in the timeline. This image workspace can be saved to any file format supported by *Image-Pro*.

The **Movie** button will create a new image sequence of the complete animation. The new

image workspace will have as many frames as is currently specified in the timeline, and can be saved to any movie file format supported by *Image-Pro*.

Navigate Group

The *Navigate* group allows you to select and view the 3D animation from different positions.



Select Button: Use this button to select object manipulation (and deselect *View* mode). The cursor shape will change to an arrow. In this mode, you are manipulating and holding objects in the *Viewer* window. Using the <Ctrl> key while clicking the mouse will deselect any object.

View Button: Use this button to select camera or viewer mode (and deselect object manipulation mode). The cursor shape will change to a hand icon. In this mode, you are moving the camera in 3D space, which changes the perspective of the rendering in the *Viewer* window.

Adding a New Animation

To add a new animation, follow these steps:

1. Make that the current animation is empty (click the **Clear** button if necessary).
2. Set the camera mode (Perspective or Orthogonal) in the viewer. Note that if you want to create a Fly-through animation you have to select a perspective camera.
3. Using your mouse and controls for the viewer window, set the desired view of the volume and click the **Add** camera position button.

Clicking the **Add** button adds the current camera position and also the given number of intermediate frames between the previous and the current positions that produce smooth transition from one position to another.

4. Move camera to the next view position and click **Add** again.
5. Record all camera positions you want to have in the animation.

Note that to create a circular animation you have to record at least two camera positions first and then click the **Circular Animation** button.

A set of camera positions that go around the object pointing to the viewpoint of the first camera position, and in the plane defined by the first two camera positions and the center of view will be generated. The distance of the first camera to the focal point defines the radius of circular animation.

If the current animation was empty, the animation will be rotated around the vertical axis.

To create a seesaw animation, you should record the position of the first viewpoint and then the last viewpoint. Click the *Connect Start/End* control.

If the current animation was empty, the animation will be rotated around the vertical axis.

6. To play **your** animation, click the **Play** button. You can stop animation by clicking on the image in the viewer window or clicking the **Pause** button.

To create an animation sequence in the application workspace, follow these steps:

1. Resize the volume viewer window to the size you need, or check the **Use Fixed Size** option and specify the size.
2. Click the *Movie* button. This movie can then be saved to an AVI or WMV file.

The current view can be copied to the Windows clipboard using the **Clipboard** button or can be turned into a new image using the **Image** button.

Appendix A

File Format Specifications

Image-Pro supports the following file formats. A brief description of each format will list the name used by *Image-Pro* in the *File:Open* and *File:Save As* dialogs, the typical file extensions that identify the file format, and any limitations such as formats that are supported for *File:Open* only.

AVI (*.AVI)

Image-Pro supports many of the video varieties of the Microsoft™ *AVI* file format, which will usually have the *.AVI file extension. The varieties that will be available in your copy of *Image-Pro* depend on the *AVI* file compression support that has been installed by the operating system and other multi-media components. You cannot annotate *.AVI files in *Image-Pro*.

BMP (*.BMP, *.DIB, *.RLE)

Image-Pro supports all known varieties of the Microsoft™ *BMP* file format, which will usually have the *.BMP file extension but may also have a *.DIB or *.RLE extension. You cannot annotate *.BMP files in *Image-Pro*.

JPEG (*.JPG, *.JPEG, *.JP2, *.JPF)

JPEG files will have a *.JPG or JPEG file extension. *Image-Pro* supports all standard varieties of the JPEG file format, including JPEG 2000.

Flex Image Transport System (*.FTS, *.FIT, *.FITS)

Image-Pro supports all standard formats of the *Flex Image Transport System*.

Media Cybernetics Experiments (*.MCE)

Image-Pro allows you to read and write Media Cybernetics experiment files with the extension *.mce

Media Cybernetics Sets (*.MCS)

Image-Pro allows you to read and write Media Cybernetics set files with the extension *.mcs .

MPEG-4 (*.MP4, *.MOV, *.M4V)

Image-Pro allows you to read and write MPEG-4 files with the extensions *.MP4, *.MOV, and .M4V including H.264 .

Portable Internet Graphics (*.PNG)

Portable Internet Graphics are a common format for all types of graphics. The extension is *.PNG. *Image-Pro* supports all types of *.PNG files.

Picture (*.PCT, .PICT)

Image-Pro supports most types of picture files.

Sequence (*.SEQ)

Image-Pro supports multiple-image TIFF files with the .SEQ file extension. This format allows you to save annotations without burning them into the image.

TIFF (*.TIF, *.TIFF)

Image-Pro supports all known varieties of the TIFF (the *Tagged Image File*

Format) file format, including most multiple-image varieties and *PowerPoint* TIFF files. This format allows you to save annotations without burning them into the image.

Image-Pro can support TIFF files larger than 4GB to aid in the acquisition of large image sequences. The ability to read calibration data from the following EM files has been added:

- FEI
- Hitachi
- JEOL
- Zeiss

Image-Pro supports the following read-only file formats:

Aperio (*.svs)
AutoQuant (*.avz) (*.deb) (*.hdr) (*.xml)
BIGTIFF (*.btf)
DeltaVision (*.dv)
Gatan Digital Micrograph (*.dm3) (*.dm4)
Hamamatsu Nanozoomer
Image Cytometry Std (*.ics)
Image-Pro IQ Sets (*.iqs)
Leica (*.lei) (*.lif) (*.scn)
Micromanager (metadata.txt)
Molecular Devices Metamorph (*.nd) (*.stk)
Nikon Elements (*.nd2)
Olympus FV 1000 (*.oif) (*.oib)
OME-TIFF (.tiff)
PerkinElmer (*.tim)
Streampix (*.seq)
Windows Media Video (*.wmv)
Zeiss LSM (*.lsm)
Zeiss Widefield (*.zvi)
Zeiss CVI (*.cvi)

Flat file formats are now supported by *Image-Pro*. Please contact Media Cybernetics' Customer Support for assistance in setting up support for a specific flat file format.

Hitachi File Formats

There are a number of different types of image acquisitions that can be done with Hitachi TEM, SEM and table-top SEM microscopes. These include:

- Single-frame acquisitions (one or several saved to same folder, which can generally be saved in TIFF, BMP or JPEG file formats)
- Acquisitions of Z stacks or volumes
- Acquisitions of larger areas by moving the sample stage and taking additional images (tiling)
- Acquisition of TEM images where the stage is tilted through a range of +60 to -60 degrees.
- Acquisitions by third-party hardware/software vendors like Gatan

Image-Pro with 3D Module should be able to open and analyze these images.

Image-Pro with 3D Module will also open Hitachi mosaic (tiled) images directly in to the Tiling View. A mosaic image is one that contains multiple frames that are laid out in a regular X/Y grid in rows and columns.

Appendix B

Frequently Asked Questions

This section contains questions from our users, and answers from the *Media Cybernetics* support team. Please feel free to submit additional questions to us, using the email address info@mediacy.com.

Installation Questions

Q: What is the purpose of the `Update Log` in the product installation folder?

A: The `Update log.log` is a text file with trace information from the TrueUpdate program. It is created every time the product runs or looks for updates. It is not needed for ordinary daily use, but contains diagnostic information that will be helpful if there is an installation or update problem.

Q: What are the Application and Data folders for?

A: The Data folder usually contains sample data other than images. The Application folder is a system folder and is not visible by default in Windows Explorer. The Application folder for the product can be safely deleted.

Q: What does the `LastCapture.icq` file in Application data folder do? What happens if it is deleted?

A: This file saves the state of the capture settings when the application is closed. It is used if the ***None*** capture settings file selection is selected. If it is deleted, the capture settings will be set back to default and the file will be recreated when the application is shut down again.

Q: How often does TrueUpdate look for updates?

A: Every time you start the application.

Q: If one person installs the product into “All Users” will a second Windows user have access?

A: An “All Users” installation will install shortcuts, documents, and data to areas that are ordinarily accessible to everyone. However, it is always possible, by customizing Windows security to produce an environment where a second user may not have access.

Q: If the current Windows user installs the product will only that person be able to use the files?

A: In the common case (i.e. with default Windows security) the DEFAULT locations documents and data will be restricted to the installing user. If that user is concerned about access to their data by others, they need to be careful where they save their images and data.

Application Questions

Q: What does **Auto-Range** in the *Capture* settings do, and how do you set it?

A: **Auto-Range** sets the black and white levels using the standard display features, so the histogram B/W level bars, best-fit, reset, etc. are all able to set the auto-range functionality. Dynamic auto-range uses best-fit to set the display range on every preview frame. The settings for best fit apply, so you can set the low and high exclusion using the standard display controls on the ribbon. Also, reset and best-fit are now on the histogram window.

Q: In the *Capture* group, What does the **Lock** button do?

A: The **Lock** button locks the preview and capture controls together for that subpanel group. The other selections are Capture and Preview that unlock the controls and allow the user to specify them independently.

Q: If we have a 36-bit live preview and capture that image, then “save as” and choose JPEG, what happens? Does it down-convert to a 24-bit image? If so, there is a bug that the image has “36-bit” listed in the “info” table.

A: It down converts to 24-bits to save the file. The image, however, that is shown is the original captured image, not the copy that’s on the disk. That’s why the info is showing 36-bit. If you open the image file that was saved, the image will be 24-bit.

Q: On different operating systems, what will the default paths for *QuickSave* be?

A: The default path is My Documents\Image-Pro 64-bit\9.3\Images
It is a subfolder of “My Documents” that is always writable.

Q: What are the JPEG Compression differences? What is Quality or Quality Factor? Is this typical?

A: JPEG images are saved with Quality Factor = 95. This is the default, which produces small images without much visual artifacts. You can also use PNG or TIF to save a compressed file without losing quality (though the file size is bigger than JPEG).

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