ImageScope

User's Guide

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This document applies to software versions Release 11 and later.

User Resources

For the latest information on Aperio Technologies products and services, please visit the Aperio Technologies website at: http://www.aperio.com.

Disclaimers

Use normal care in maintaining and using the Spectrum servers. Interrupting network connections or turning off the Spectrum and DSR servers while they are processing data (such as when they are analyzing digital slides or generating an audit report) can result in data loss.

This manual is not a substitute for the detailed operator training provided by Aperio Technologies, Inc., or for other advanced instruction. Aperio Technologies Field Representatives should be contacted immediately for assistance in the event of any instrument malfunction. Installation of hardware should only be performed by a certified Aperio Technologies Service Engineer.

ImageServer is intended for use with the SVS file format (the native format for digital slides created by scanning glass slides with the ScanScope scanner). Educators will use Aperio software to view and modify digital slides in Composite WebSlide (CWS) format.

Aperio products are FDA cleared for specific clinical applications, and are intended for research use for other applications. For clearance updates, visit www.aperio.com

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Contents

1 Introduction

This chapter introduces you to ImageScope, discusses its features, and tells you where to go in this guide to find information on specific features.

ImageScope Features

Although ImageScope™'s basic function is to allow you to view digital slides created by a microscope slide scanner from glass tissue slides, it offers much more:

- View digital slides from any workstation on the network, eliminating the delay of physically transporting glass slides from one department to another.
- Share and discuss digital slides in real time in multiple remote locations by using digital slide conferencing.
- View multiple digital slides concurrently.
- Apply image adjustments in real time for contrast, brightness, and gamma.
- Analyze entire digital slides or selected regions using provided, purchased, or custom algorithms*.
- Interface directly to a ScanScope scanner through a network connection to view slides "live" and in different focal planes†.
- Rotate digital slide images and labels.
- Use Aperio Integrated Color Management to view digital slides to ensure the digital slides are displayed in accurate color.
- Use the Image Quality (IQ) feature to optimize viewing of a digital slide based on its stain.
- Annotate digital slides, making use of these features:
  - Multiple drawing tools
  - Ability to draw areas to be *excluded* from analysis*
  - Organize annotations by person or department by creating annotation layers
  - Link annotations or images to create a viewing sequence
  - Add text and descriptions to annotations
  - Export and import annotations

- Interface to Aperio's ImageServer™ and Spectrum™.
- Instantly pan and zoom to any region of the slide.
- Extract a region or selected regions of a digital slide to a file in a choice of formats.

Types of Files You Can View

You can use ImageScope to view:

- **ScanScope Virtual Slides –** These are .SVS files created when the ScanScope scanner scans glass microscope slides.
- **JPEG files –** Both .JPG and .JP2 files**.**
- **TIFF and TIF files**.
- **CWS files** – Composite WebSlides[1].
- **Hamamatsu NanoZoomer files** – NanoZoomer file formats supported are: NDPI, .VMS, and .VMU files. Slide label images are not available for these images in Spectrum, ImageScope, Digital Slide Studio, and WebScope, as these images do not contain labels.
- **Zeiss Mirax files –** Zeiss Mirax image file formats supported are .MRXS and SlideDat.ini. (Note that Mirax images are composite images that consist of a group of .DAT files. Either the .MRXS or SlideDat.ini file can be opened to open the composite image.)
- **ScanScope image set, .sis file –** The ImageScope *image view* is what you see when one or more digital slides are being viewed in the ImageScope window. When using ImageScope, you can save the image view as a ScanScope image set so that you can open all the slides at once in the future.

For More Information

Here is where to go in this guide for information on ImageScope features.

How do I…	Go to…
Install ImageScope?	Chapter 2, "Installing ImageScope" on page 5.
Register ImageScope?	Chapter 2, "Installing ImageScope" on page 5.
View digital slides?	Chapter 3, "Opening a Digital Slide" on page 11; Chapter 4, "Viewing a Digital Slide" on page 27.
View Pathology News and configure it?	Chapter 2, "Installing ImageScope" on page 5. Chapter 19, "ImageScope Options" on page 173.

[1]A Composite WebSlide, also known as a CWS slide, is a proprietary format created by Bacus Laboratories, Inc ("Bacus"). WebSlide® is a registered trademark of Bacus Laboratories Inc.

How do I…	Go to…
Enable or disable clinical viewing mode	"Clinical Viewing Mode" on page 28.
Make annotations and link annotations or digital slides to make a viewing sequence?	Chapter 9, "Annotating Digital Slides" on page 75; Chapter 10, "Using the Annotations Window" on page 81; Chapter 11, "Linking Annotations and Digital Slides" on page 99.
Use algorithms to analyze* digital slides?	Chapter 14, "Analyzing Digital Slides" on page 119.
Use the algorithm* tuning window to test algorithm parameters?	Chapter 15, "Registering Algorithm Macros on Spectrum" on page 133.
Create algorithm* macros?	Chapter 15, "Registering Algorithm Macros on Spectrum" on page 133.
Share slides with others in real time?	Chapter 16, "Digital Slide Conferencing" on page 145.
Adjust image color, brightness, contrast, and gamma?	Chapter 6, "Making Image Adjustments" on page 47. Chapter 7, "Working with Fluorescence Digital Slides" on page 55
Set and view image resolution?	Chapter 8, "Image Resolution" on page 71.
Track movements through a digital slide?	Chapter 12, "Tracking" on page 103.
Rotate images and digital slide labels?	Chapter 5, "Rotating Images and Slide Labels" on page 45.
Save image snapshots and extract part of a digital slide?	Chapter 13, "Saving Digital Slides and Regions" on page 109.
View a specimen in various focal planes directly on the ScanScope scanner†?	Chapter 17, "TelePath Live" on page 155.
View live video from the ScanScope scanner†?	Chapter 17, "TelePath Live" on page 155.
Fine-tune performance?	Chapter 19, "ImageScope Options" on page 173.
Debug and troubleshoot?	Chapter 18, "Utilities and Diagnostics" on page 167.
Use keyboard shortcuts for ImageScope commands?	Appendix A, "Keyboard Quick Reference" on page 189.
Use Aperio Integrated Color Management?	"Viewing with Color Management" on page 38. Appendix B, "Aperio Integrated Color Management" on page 193.

* Aperio's image analysis algorithms are FDA cleared for specific clinical applications, and are intended for research use for other applications.

† This application is not approved or cleared by the FDA for clinical use.

2 Installing ImageScope

This chapter contains information on installing the client software for ImageScope.

This guide covers client installation of ImageScope. If you are installing other Spectrum components, see the *Notes and News* for the latest release or obtain the user guide for the application in question for instructions.

Before You Start

Before installing ImageScope, make sure your workstation satisfies these requirements:

- **Operating System –** Windows 2000, Windows XP, or Windows 7
- **Memory –** At least 256MB
- **Hard Disk –** Must have at least 30MB of free hard disk space
- **CPU Speed –** 450 MHz or faster
- **Video Card –** Must support at least 24-bit color resolution
- **Monitor –** Resolution should be at least 1024X768

Security Alerts

If during installation you see popup messages from Microsoft or third-party firewall, VPN, or virus software telling you that the installation has been blocked, you should consult your network administrator for help resolving these issues before continuing.

Administrative Privileges

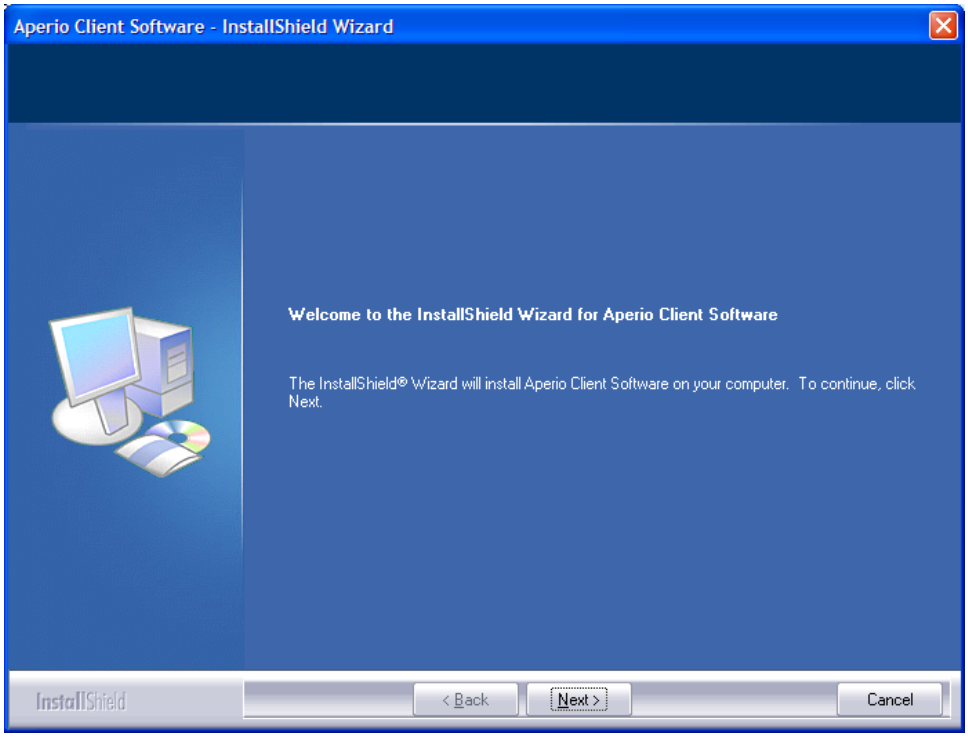
Before running the installer, make sure you are logged onto Windows as a user who has administrative privileges; otherwise, the installation will not be successful.

Installation

1. Double-click **My Computer** or open Windows Explorer and navigate to the ImageScope installer file. (This file may have been downloaded from the www.aperio.com web site, may have been provided on CD, or may reside on your network; contact your network administrator for help if you have trouble finding it.)

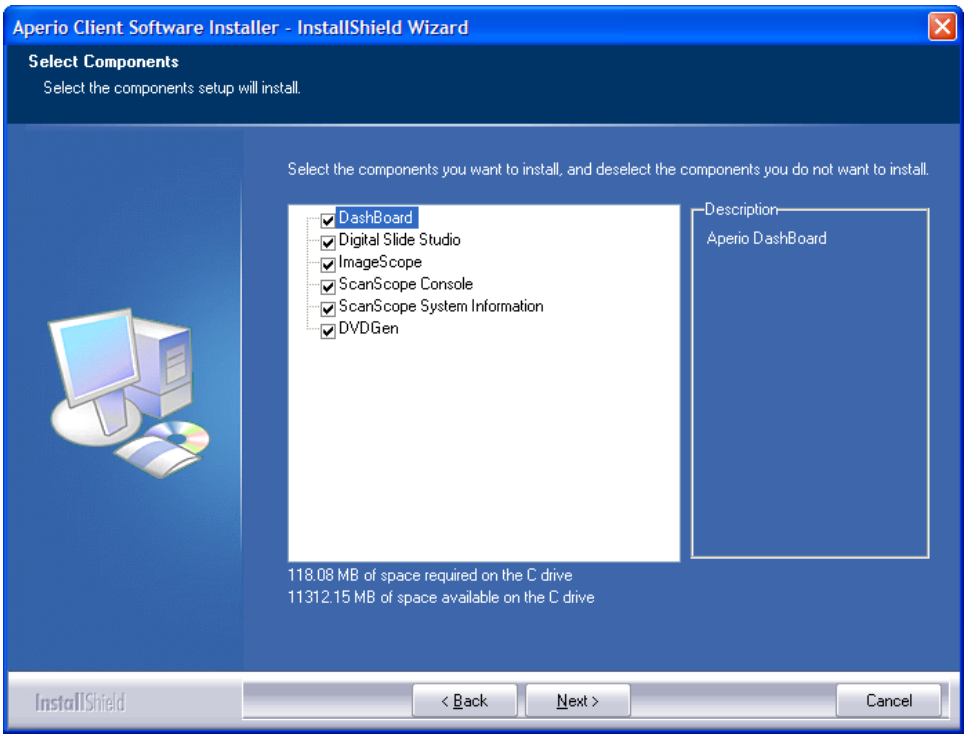
   If you are installing ImageScope on your DSR (Digital Slide Repository), use DSRInstall; if installing ImageScope on a user's workstation, use ClientInstall.

2. Double-click the .exe file to start the installer. When using the client installer, the following window appears:

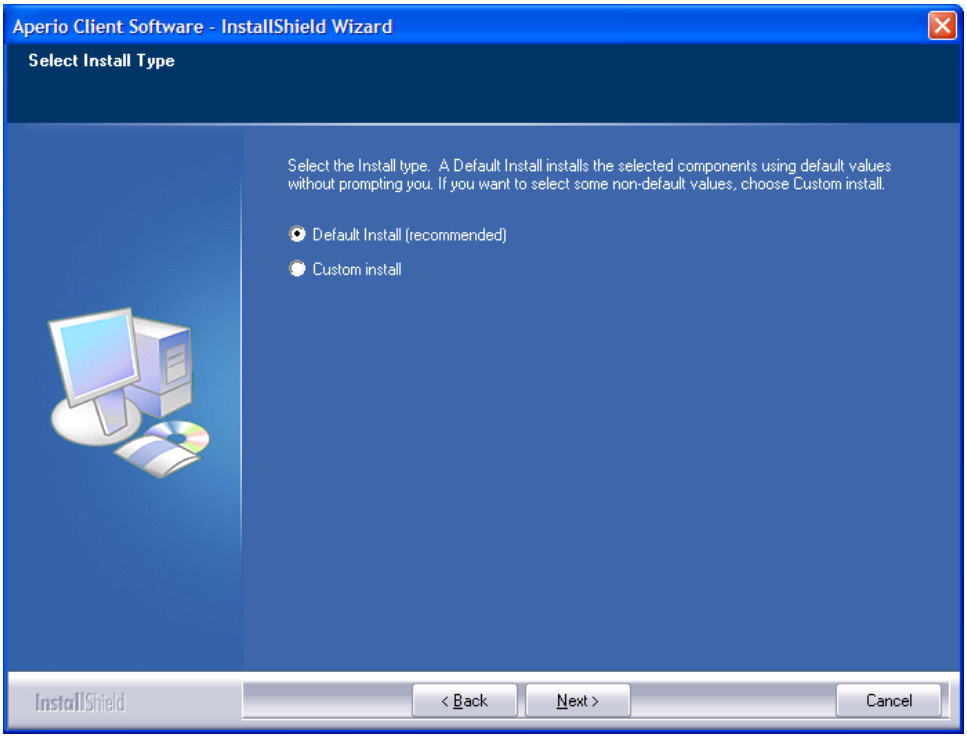


3. Click **Next** to continue. You now see the Aperio software license agreement.
4. Review the license and then select **I accept the terms of the license agreement**.

5. Click **Next**. The following window appears:

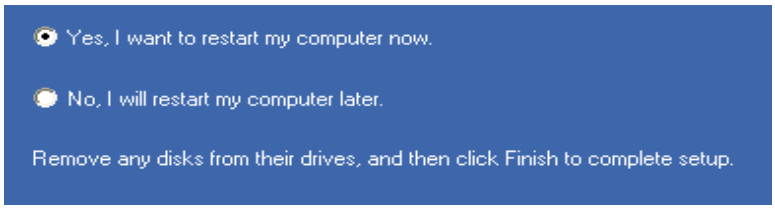


6. Click **Next** to continue or unselect any components you do not want to install and then click **Next**. The following window appears:



7. We recommend you select **Default Install**.

8. Click **Next**. A window appears that tells you the installer is ready to install ImageScope.
9. Click **Install** to continue. A status window informs you of the progress of the installation. When the installation is done, you see the completion window. If the installer needs to restart your workstation, you see two radio buttons:



If you see these choices, you won't be able to use ImageScope to view JPEG2000-compressed images until you restart your workstation; we recommend you select **Yes, I want to restart my computer now** and click **Finish** to exit the installer and restart; if you don't see these choices, simply click **Finish** to exit the installer.

Modifying or Removing the ImageScope Software

At any time after ImageScope is installed, you can run the installer again to modify, repair, or remove the ImageScope software. If ImageScope is already installed, running the installer displays the following window:

- Select **Modify** to change the ImageScope installation by adding or deleting components.
- Select **Repair** to reinstall all the components previously installed. This is the option to use if you are upgrading a previous installation to new software.

- Select **Remove** to uninstall the ImageScope software.

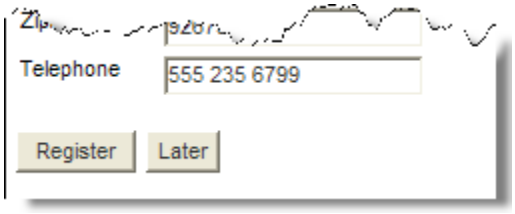
Starting ImageScope

To start ImageScope, click **Start** on the Windows taskbar, point to **All Programs > ScanScope**, and select **ImageScope**.

Registering ImageScope

The first time you start ImageScope after a new install, the Pathology News window opens with a registration form. Please type the requested information and click **Register**. By providing this information, you help Aperio to learn more about our customers.

If you do not wish to register at this time, click the **Later** button to defer registering ImageScope for two weeks. You can continue to defer registering ImageScope for another two weeks by clicking this button each time the registration window appears.



Viewing Pathology News

ImageScope periodically displays current digital pathology news in a pop-up window when you are opening an image. For example:

The Pathology News content is maintained by Aperio. If the workstation on which you are using ImageScope is not connected to the Internet, you will see

static content that is installed with ImageScope on your workstation. However, if your workstation has an Internet connection, the information in this window will be updated on a regular basis to bring you the latest news.

Any time this window is open, you can click **Close** on the window to close it. If you want to open the Pathology News window again, go to ImageScope's View menu and select **View News**. To see Pathology News in a separate Internet browser window, click **Open Page in a Browser** on the Pathology News window.

For information on configuring how often to display Pathology News or whether to view it at all, see "Pathology News Options" on page 187.

3 Opening a Digital Slide

This chapter contains information on opening digital slides in ImageScope, how to view information about the slide, and how to close digital slides.

Use ImageScope to view:

- *Local* digital slides; that is, images that reside on your workstation or your local network that are accessible via Microsoft file sharing (for example, by using Windows Explorer).
- *Remote* digital slides; that is, images that you open directly on an Aperio ImageServer or that you open using Spectrum.

Monitor Requirements

Because Aperio digital slides are by design high resolution and information rich, for best results you should use a high quality monitor to view them. Make sure the monitor is at the proper viewing height and in a room with appropriate lighting. We recommend any high quality LCD monitor meeting the following requirements:

Display Type:	CRT minimum, LCD (flat panel) recommended
Screen Resolution:	1024(h) x 768(v) pixels minimum 1920 x1050 or larger recommended
Screen Size:	15" minimum 19" or larger recommended
Color Depth:	24-bit
Brightness:	300 cd/m^2 minimum 500 or larger recommended
Contrast Ratio:	500:1 minimum 1000:1 recommended

About User Permissions

ImageScope makes use of Spectrum security to enforce user permissions when viewing images.

The Spectrum administrator uses data groups and user roles to define what data you can see and what you can do when you see it. Data groups organize data such as digital slides into different groups that can be seen and used by different

users. User roles define the commands you can use and the elements of a Spectrum page you can see.

What this means for ImageScope users is that when you open a remote image ImageScope may request that you log in so Spectrum can determine if you have the correct permissions to view the images you want to access. Type the same user name and password you use to log into Spectrum.

This also means that you may be restricted in what you can do with a digital slide. If, for example, you have read-only access to the data group that contains the digital slide you are viewing, you can use the ImageScope drawing tools to draw annotations but you won't be able to save them. If you have questions about your user permissions, contact your Spectrum administrator for assistance.

Some of the features of the Spectrum security system you should know about are:

- To keep user information secure, user credentials are encrypted and are never passed in clear text between the components of the Spectrum system.
- User credentials can time out, so if sufficient time has elapsed since you logged in, you may be asked to log in again.
- Data groups and user permissions are defined in Spectrum by the administrator.

Depending on how Spectrum is configured, you may be able to log in as Guest to see public images that do not require user authentication.

Opening a Digital Slide on Spectrum

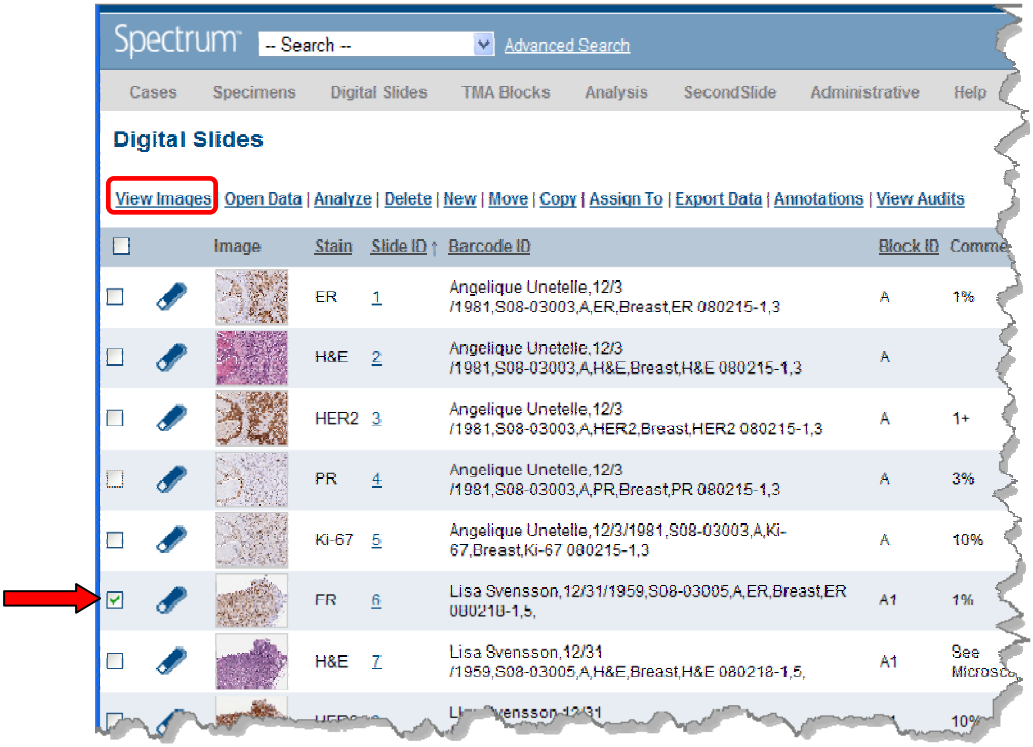
You can either open a remote digital slide from the Spectrum web interface, or you can open it directly from within ImageScope:

To open a digital slide from within Spectrum:

1. Start Spectrum in your Internet browser. (See the *Spectrum/Spectrum Plus Operator's Guide* for details.)
2. Log into Spectrum.
3. Use the Spectrum List commands to see the digital slides on your site or use the search feature to find the one you want.

Connection speeds may affect ImageScope performance when viewing remote images. For best viewing, we recommend a connection speed of 100mbps or greater.

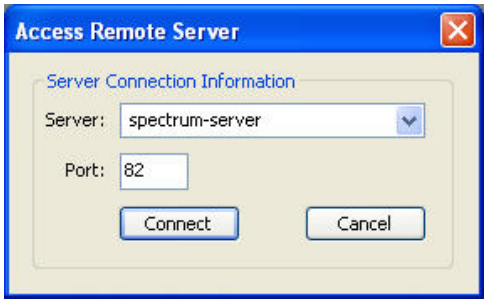
4. Select the digital slide you want to use by selecting the check box next to it and clicking **View Images**:



Or click on the image thumbnail. The digital slide opens in ImageScope.

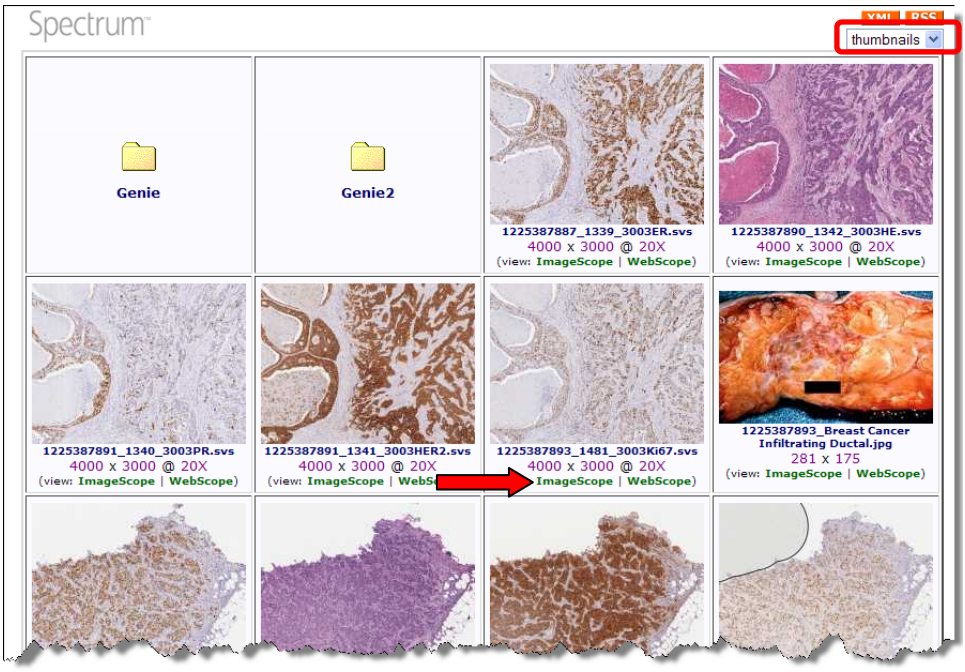
To open a Spectrum digital slide directly from within ImageScope:

1. Go to the ImageScope File menu and select **Access Remote Server** to connect to Spectrum. Enter the name of the server on which Spectrum resides:



2. Set the **Port** value to **82.**
3. Click **Connect**.
4. When asked for your user name and password, enter your Spectrum user name and password.

5. Now you see a page of digital slides on the Spectrum site. You can choose between two views: List and Thumbnail by selecting the view you want from the drop-down list at the upper right.



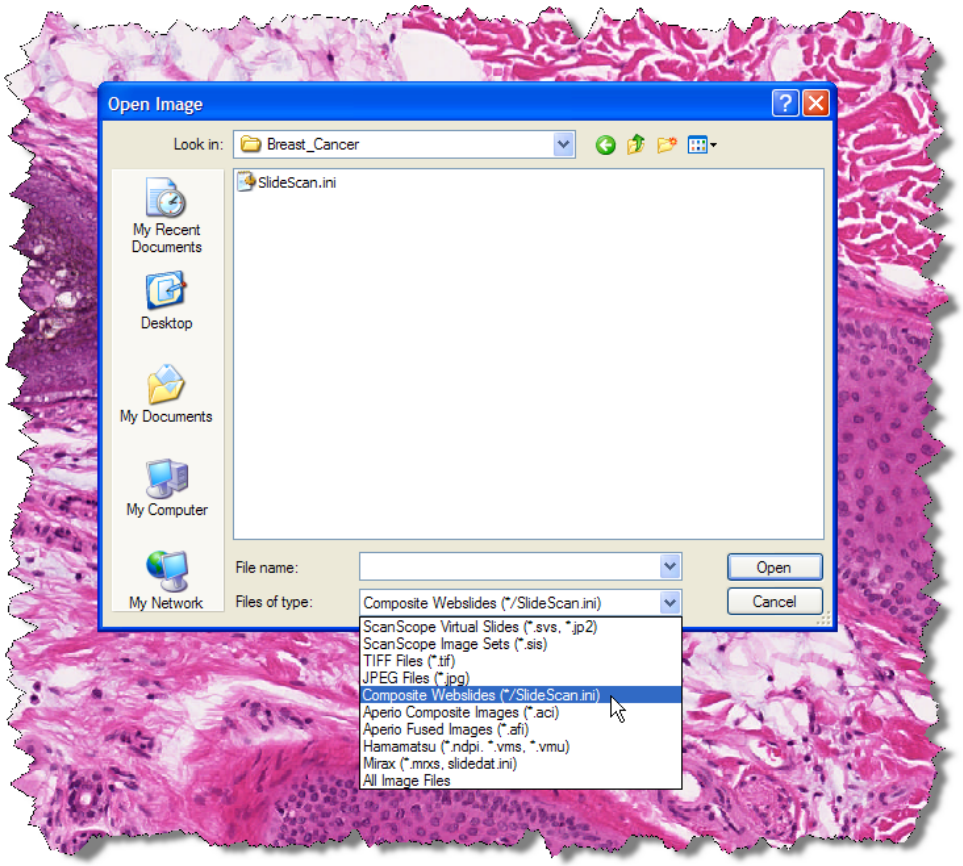
6. Select a digital slide by clicking **ImageScope** beneath an image.

Opening a Digital Slide on Your Workstation or LAN

To open a digital slide that resides on your workstation or local area network:

1. Start ImageScope by clicking **Start**, pointing to **All Programs > ScanScope**, and then selecting **ImageScope**.
2. Go to the **File** menu and select **Open Image** (or click on the ImageScope toolbar).
3. On the Open Image window, navigate to the location that contains the image you want to view.
4. Click the name of the digital slide you want to open and click **Open**.

You may need to change the file type in the Open Image window to see the type of image you want to view. For example, to view a CWS image, click the file type drop-down list and select **Composite WebSlides (*/SlideScan.ini)**.



Local Image Support

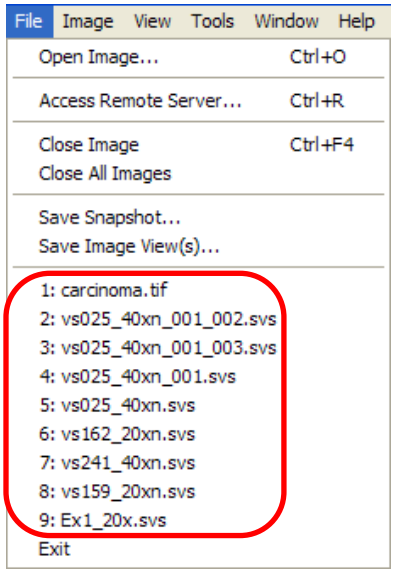
If you open a local image instead of an image in Spectrum, the following ImageScope features are not supported for that image:

- Smart sync
- Tracking
- IQ

Opening a Recently Viewed Local Digital Slide

ImageScope displays a list of the last few digital slides that were viewed on the **File** menu.

1. Go to the **File** menu.
2. Click on one of the digital slides listed at the bottom of the menu to open it.

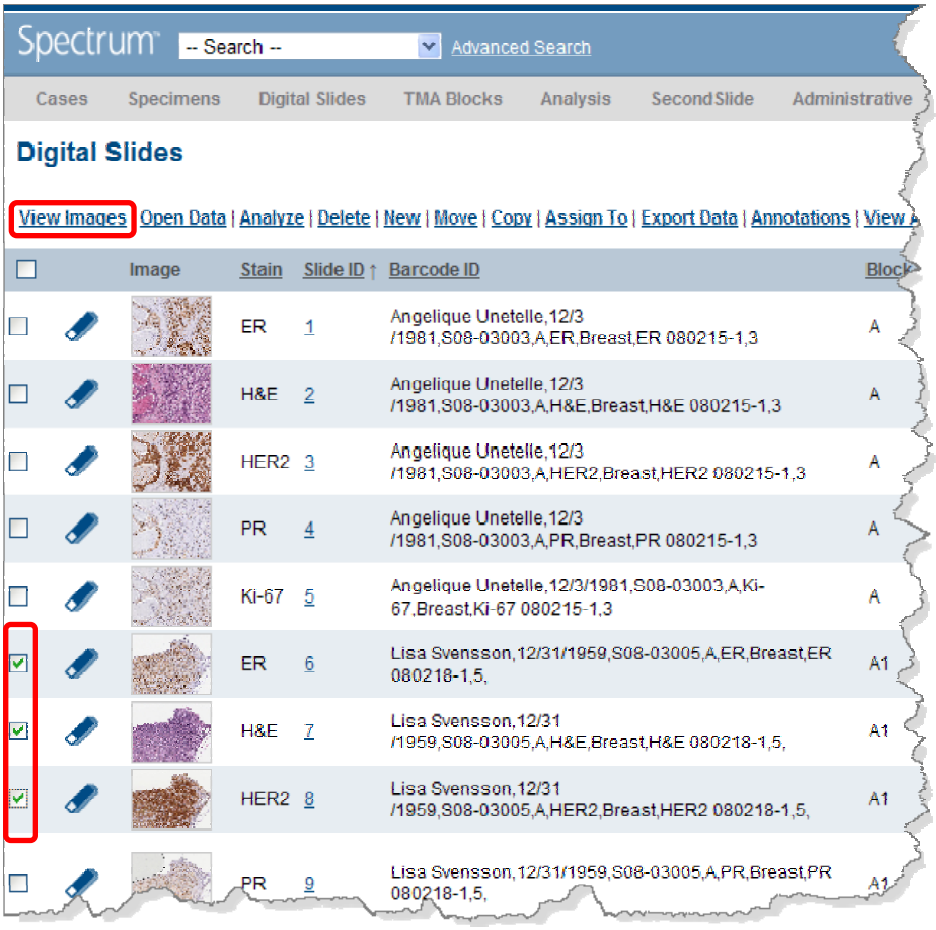


Opening and Viewing Multiple Digital Slides

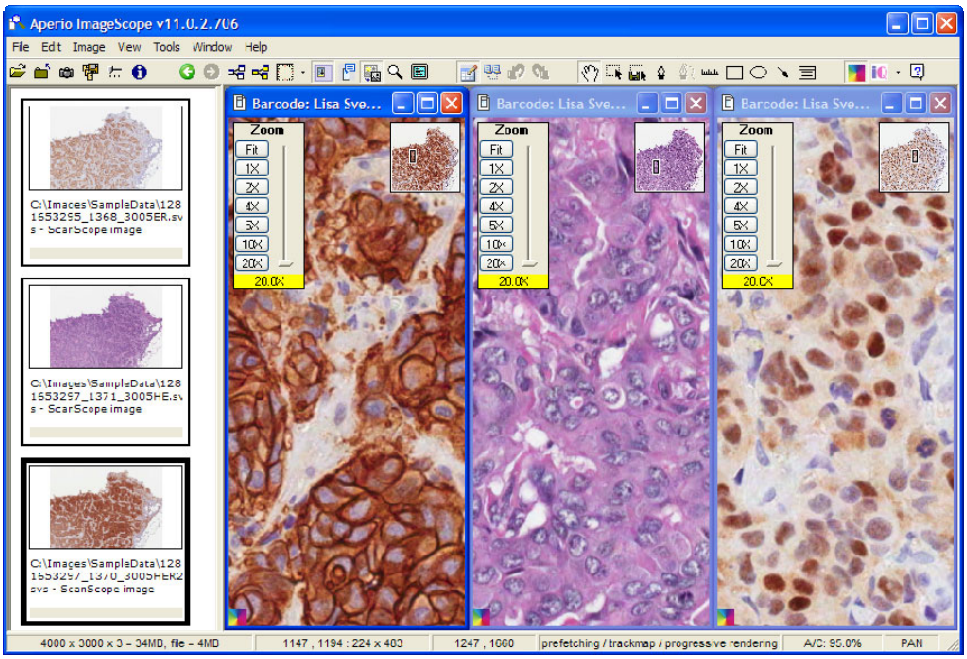
You can open multiple digital slides within ImageScope. To open multiple digital slides in Spectrum:

1. Log into Spectrum.
2. Use the Spectrum List command to see the digital slides on your site or use the search feature to find the ones you want.

3. Select the digital slides you want to use by selecting the check boxes next to them and clicking **View Images**:

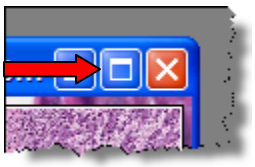


The digital slides open in ImageScope:

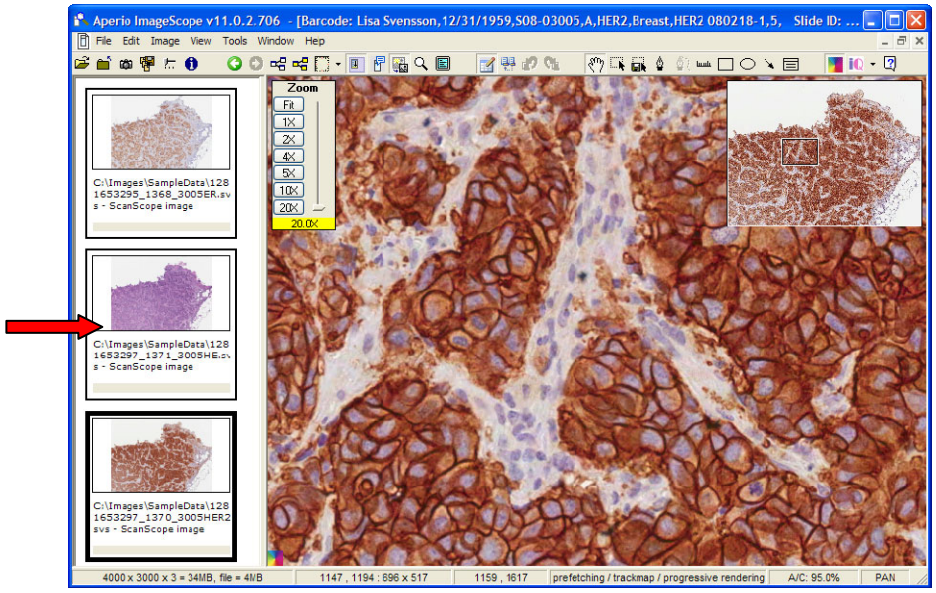


You can view all the slides at once or view them separately:

- To view all slides side by side vertically (as in the example above) go to the ImageScope Window menu and select **Tile Vertical**.
- To view all slides in a horizontal format, go to the ImageScope Window menu and select **Tile Horizontal**.
- To view the slides one at a time, go to the ImageScope Window menu and select **Cascade**. Then click the **Maximize** button at the top of the slide you want to view, which causes it to fill the ImageScope window:



- When multiple digital slides are open but only one is displayed, to move between the opened images, click on the image you want to view in the ImageScope filmstrip in the left pane of the window.

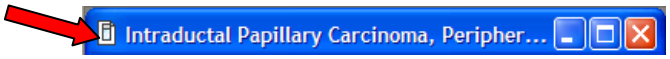


  If the ImageScope filmstrip is not visible, go to the **View** menu and select **Filmstrip**.

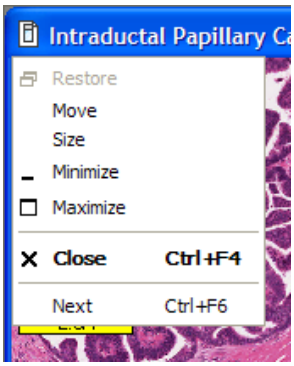
For details on how to move all images in step, see "Synchronizing Navigation of Multiple Digital Slides" on page 31.

Managing Digital Slide Windows

You can maximize, minimize/restore, or close the individual digital slide windows within the ImageScope main window by clicking the slide icon on the image's menu bar:



The following menu appears from which you can select an action to perform on this digital slide window:



The Keep Open Option

It may be convenient to keep a digital slide open in ImageScope as a reference image while you open and close other images to view side by side with it.

However, when you select one or more multiple images in Spectrum (by clicking the thumbnail or selecting multiple images and using the **View Images** command), any images already open in ImageScope are closed before displaying the new ones.

You can use the ImageScope Keep Open option to keep a specific image in ImageScope open when you select and view new images in Spectrum.

To keep an image open:

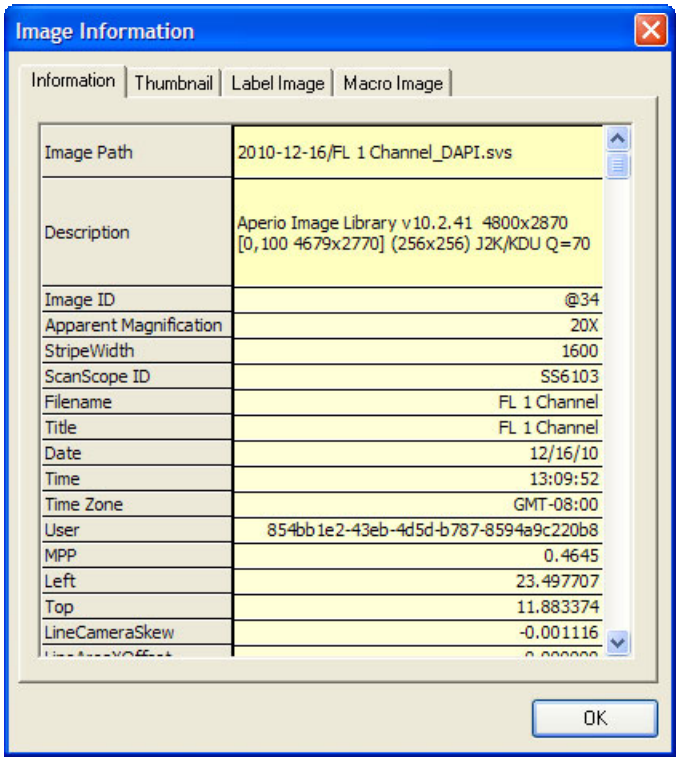
1. With the image open in ImageScope (and selected if there are more than one images displayed), go to the Image menu and select **Keep Open** or type Control K.
2. When you do want to close the image you have kept open, select the image, go to the ImageScope File menu and select **Close Image** or click the Close Image icon on the toolbar.

You can turn "keep open" on and off for the image by typing Control K or selecting the **Keep Open** command. To keep multiple images open, select each image in turn and type Control-K.

Viewing Digital Slide Information

To see information about the currently selected digital slide such as the image size, location, and compression ratio:

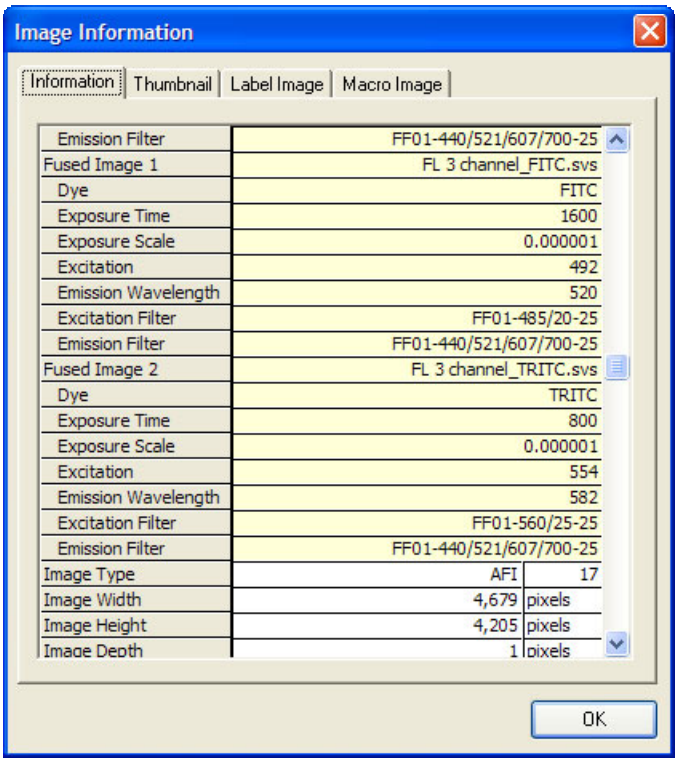
1. Go to the Image menu and select **Information** or click on the toolbar to see the Image Information window.



ICC Profile gives the name of the ICC profile if one is being used. (See Appendix B, "Aperio Integrated Color Management" on page 193 for information on ICC profiles and color management.)

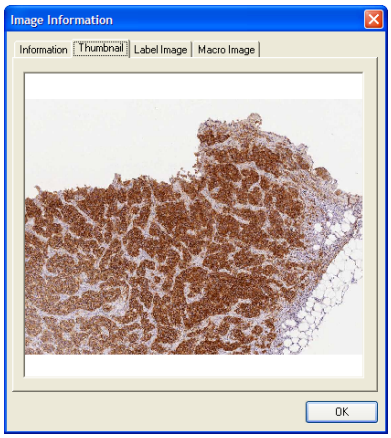
Note that if this digital slide was scanned on a ScanScope, the timezone of the scan location and time of the scan are also displayed. The Information tab is always shown. The other tabs may not appear if those elements have not been associated with the digital slide. (For example, if a label image does not exist for this digital slide, you do not see the Label Image tab.)

For an Aperio Fused Image (AFI), the Information window contains information on the separate channel images that make up the AFI image:

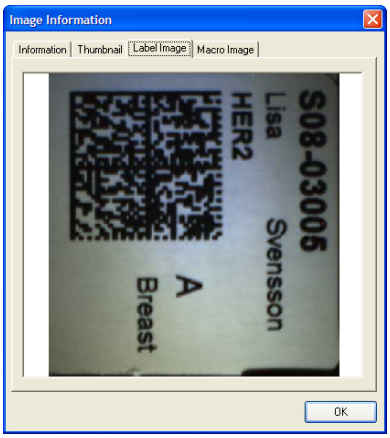


2. To see other information, click the:

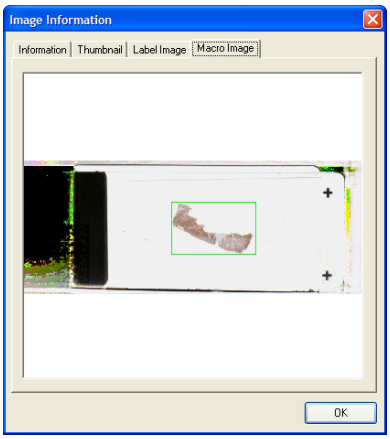
   - **Thumbnail** tab to see a thumbnail image of this digital slide, the area of the glass slide that was scanned:



- **Label Image** tab to see the slide's label:

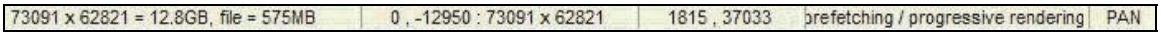


- **Macro Image** tab to see a macro camera image of the entire slide:



Status Bar

Information on the selected digital slide can also be seen in the status bar at the bottom of the ImageScope window. For example:



The sample status bar above shows the following information:

- **73091 x 62821 = 12.8GB, file = 575MB –** This means that the entire digital slide is 73,091 by 62,821 pixels in size. The digital slide's raw data is 12.8 gigabytes in size and the compressed size of the digital slide file is 575 megabytes.
- **0, -12950 : 73091 x 62821 –** The first two numbers indicate the pixel position of the top, left corner of the display. The second numbers indicate which part of the image is being viewed.
- **1815, 37033 –** This indicates the current pixel position of your cursor.
- **Prefetching/progressive rendering –** Indicates what performance options are in effect. For information on performance options such as

prefetching, interpolating, and progressive rendering, see "Performance Options" on page 183.

- **PAN –** This shows what navigation or annotation tool is selected. In this case, panning is selected.

You can turn the status bar off by going to the **View** menu and selecting **Status Bar**. (This command reverses the current state of the status bar display: if the status bar is showing, this command hides it; if it is hidden, this command shows it.)

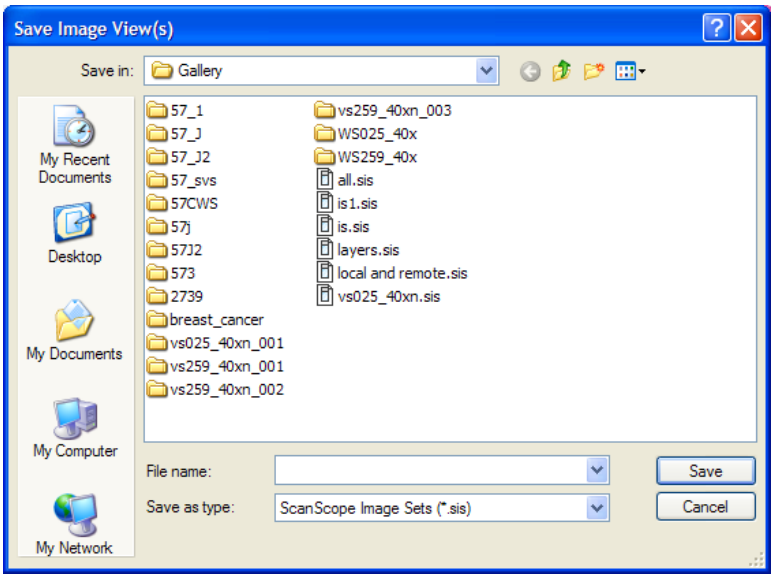
Saving and Opening an Image View

An ImageScope view is the entire set of slide images that are open at one time in ImageScope.

Saving an Image View

If you have a group of digital slides that you want to view together, open them all in ImageScope and zoom and pan until you get the view you want. Then:

1. Go to the **File** menu and select **Save Image View(s)**. You are asked to save the file as a ScanScope Image Set .sis file:

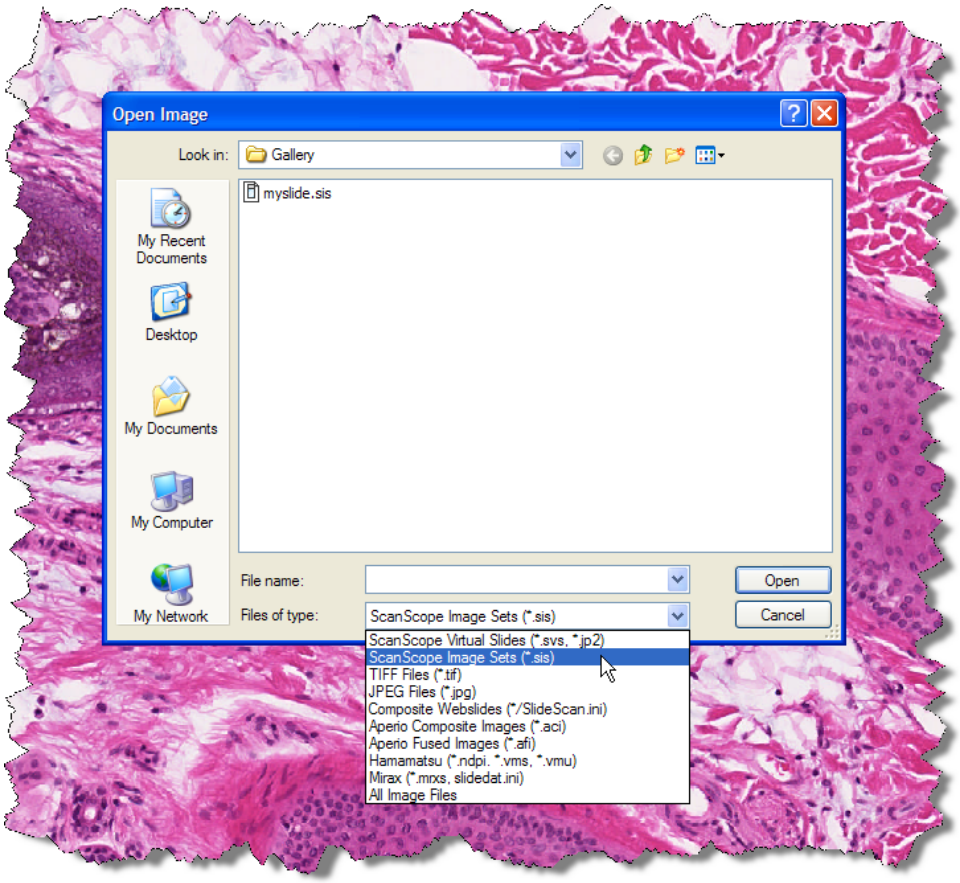


2. Type the name you want to use for the file and click **Save**.

Opening an Image View

To open the image view you previously created:

1. Go to the **File** menu and select **Open Image** to navigate on your network to the location of the .sis file you saved. You will need to select .sis from the **Files of type** drop-down list to see the file.



2. Select the .sis file you want to open and click **Open**. When ImageScope opens the .sis file, all digital slides that were in that image view will be open and in their former pan and zoom configuration.

Closing Digital Slides

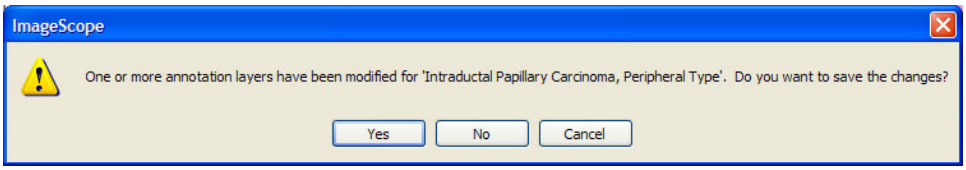
To close a single digital slide:

1. If you have multiple digital slides open, click the one you want to close in the filmstrip. If you only have one digital slide open, it is already selected.
2. Go to the **File** menu and select **Close Image**.

To close all digital slides:

1. Go to the **File** menu and select **Close All Images**.

If you made any changes to the digital slide (for example, you added or changed an annotation), you are asked if you want to save the changes before you close the slide.



Click:

- **Yes** to save your changes
- **No** to discard them, or
- **Cancel** to leave the image open.

You can configure ImageScope to always save annotations when you close an image without asking you for confirmation. See "Automatically Saving Annotations" on page 181 for instructions.

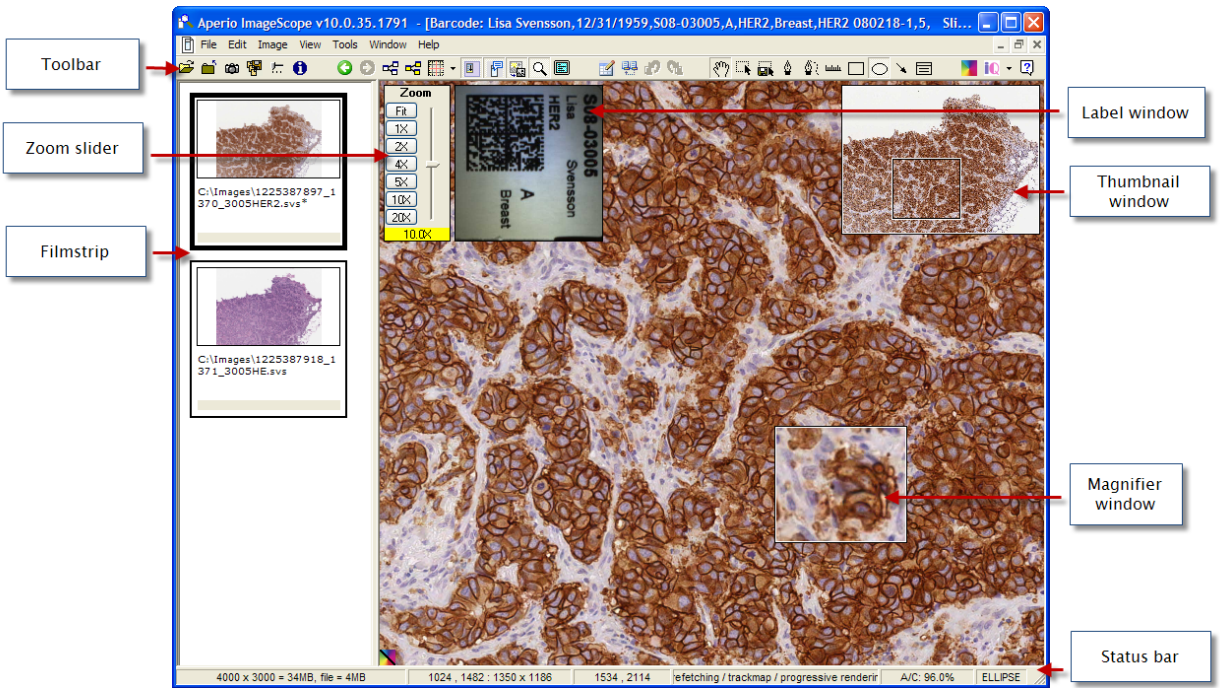
For More Information

- For a quick reference list of the ImageScope toolbar icons, see "ImageScope Toolbar Quick Reference" on page 29.
- To navigate and change magnification settings on a digital slide, see Chapter 4, "Viewing a Digital Slide," on page 27.
- To adjust image color and brightness, see Chapter 6, "Making Image Adjustments," on page 47.
- To annotate a digital slide, see Chapter 9, "Annotating Digital Slides," on page 75.
- To save a snapshot of a digital slide you have modified or to save a portion of a digital slide, see Chapter 13, "Saving Digital Slides and Regions," on page 109.

4
Viewing a Digital Slide

This chapter introduces you to using ImageScope by giving a tour of the ImageScope main window and showing you how to use the navigation and magnification tools.

The ImageScope Viewing Window



Throughout the rest of this guide, we will discuss these elements of the ImageScope viewing window.

- **Toolbar –** Many of the ImageScope commands and features are available on the toolbar. You can also access many of these features through the ImageScope menus. See the next section for a quick reference list of the ImageScope toolbar icons.
- **Zoom slider –** You can magnify or shrink the current view. See "Using the Zoom Slider" on page 37 for details.
- **Filmstrip –** The filmstrip shows what slides are open. You can move between digital slides by clicking the slide's image in the filmstrip.
- **Label window –** If an image of the slide label has been associated with the digital slide, you can see it in the slide label window.

- **Thumbnail window –** Digital slides are large and often you see only a portion of one in the ImageScope main window. The thumbnail is a view of the complete digital slide. See "Using the Thumbnail Window" on page 33.
- **Magnifier window–** Move this tool to the area you are interested in to see a magnified view. See "Using the Magnifier Window" on page 36.

Hiding and Showing ImageScope Window Features

To hide or show elements of the ImageScope window:

ImageScope Element	To turn on or off…
Filmstrip	Go to the **View** menu and select **Filmstrip** to show or hide the filmstrip.
Thumbnail	Go to the **View** menu and select **Thumbnail** or click on the toolbar to show or hide the thumbnail window.
Magnifier	Go to the **View** menu and select **Magnifier** or click on the toolbar to show or hide the magnifier window.
Status Bar	Go to the **View** menu and select **Status Bar** to show or hide the status bar.
Slide Label	Go to the **View** menu and select **Label Image** or click on the toolbar to show or hide the slide label window.
Zoom slider	Go to the **View** menu and select **Zoom Slider** or click on the toolbar to show or hide the zoom slider.

These commands reverse the current state of the element display: if the element is showing, the command hides it; if it is hidden, this command shows it.

Clinical Viewing Mode

Because ImageScope is so feature rich, the standard toolbar contains many different icons. A simplified version is available that contains just the tools used in a clinical environment.

- To see the clinical toolbar, go to the View menu and select **View Clinical Toolbar**.

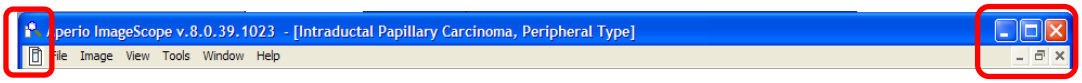
When clinical viewing mode is in effect, only the summary view of the Annotations window is available, to provide quick and easy digital slide analysis*.

- To see the full toolbar, go to the View menu and select **View Standard Toolbar**.

Adjusting the ImageScope Window

To adjust the ImageScope window to the size you prefer, grab the boundary of the window and drag it in or out.

You can maximize, minimize/restore, or close either the main ImageScope window or the individual digital slide window by using the appropriate controls:



- The controls on the right are standard Windows controls that allow you to minimize, maximize, restore, or close the window.
- The icons on the left perform the same functions by displaying a menu from which you can select the action to perform.

ImageScope Toolbar Quick Reference

Following chapters discuss using ImageScope features in detail—here is a quick list of the toolbar icons and what they do.

*These icons are shown in clinical viewing mode.

Icon	Action
	Go to the Open Image window where you can browse for a local digital slide to open for viewing.
	Close the digital slide that is currently being viewed in ImageScope.
	Create a snapshot image of the digital slide currently being viewed.
	Save an ImageScope view, the entire set of digital slides that is currently open in ImageScope, as a .sis file.
	Go to the Image Adjustment window where you can make color and other adjustments to the digital slide currently being viewed.
	Go to the Image Information window which displays information about the digital slide currently being viewed.
	Go to previous view of the digital slide.
	Go to the next view of the digital slide (only enabled if you first used the back arrow icon to go to a previous view).
	*Manually synchronize navigation for all digital slides currently being viewed. (Used when multiple digital slides are open in the ImageScope window.)
	*Use smart synchronization for multiple digital slides being viewed. Corresponding regions in the digital slide images are synchronized. (Same icon as for manual synchronization, but colored yellow.)
	Show or hide the magnifier window.
	Show or hide the digital slide label window.

Icon	Action
	Show or hide the thumbnail window.
	Show or hide the zoom slider.
	Display on the full monitor screen. (Or turn off if already in full-screen mode.)
	Show or hide axes or axes and grid.
	*Open the Annotations window where you can create multiple annotation layers and organize and add descriptions to annotations.
	Open the Annotation Link Manager window where you can link annotations or digital slides to create a viewing sequence.
	Go to the previous link (if a previous link exists).
	Go to the next link (if a next link exists).
	Pan the digital slide.
	Enable/disable Integrated Color Management. Only useful if the image contains an embedded ICC profile.
	*Turn Image Quality (IQ) mode on or off.
	Zoom the selected area of the digital slide.
	*Extract a region of a digital slide.
	*Draw a free-form annotation.
	Draw a free-form annotation to be excluded from analysis*. (This creates a *negative* annotation.)
	*Measure an object on a digital slide.
	*Draw a rectangular region (or a square if you hold down the Shift key while you draw).
	Draw an elliptical annotation (or a circle if you hold down the Shift key while you draw).
	*Draw an arrow pointing to an area of interest.
	*Select an image for a report. This feature is only useful if you have Spectrum Plus Reporting option installed and the report template you are using uses images.
	*See help information for ImageScope.

Full Screen Viewing

To view the digital slide with the maximum viewing area, switch to full screen viewing:

1. Go to the **View** menu and select **Full Screen** or click  on the ImageScope toolbar. The menu bar does not appear in this mode.

To switch back to regular viewing mode:

1. Click  on the ImageScope toolbar.

Synchronizing Navigation of Multiple Digital Slides

In some cases you may want all of the open slides to show the same navigation behavior when you are viewing them side by side. (For example, if you pan right in one slide, you may want every other digital slide to also pan right.) To synchronize navigation when multiple slides are open:

On the ImageScope toolbar click  . Now when you navigate around one of the digital slides, the others move in step.

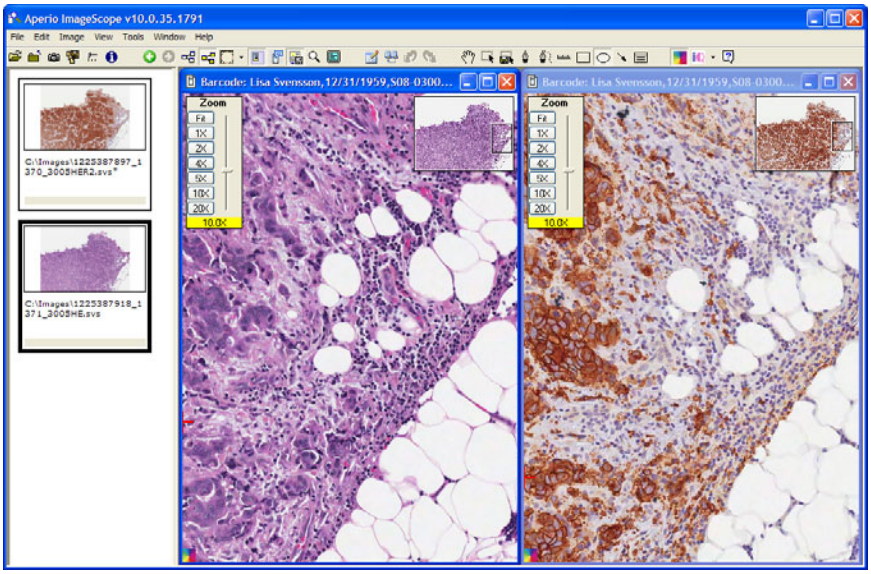
Smart Synchronization

Smart synchronization is able to compensate for rotation (non-flipped) but not for other factors such as stretched or missing tissue. In those cases, ImageScope will do its best to display the same tissue feature in all tiled images, but not necessarily in exactly the same location.

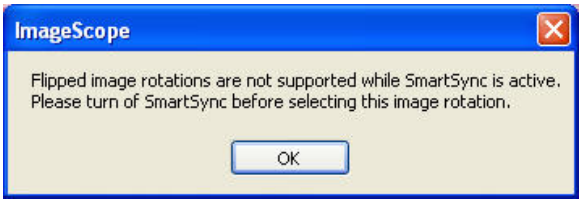
Smart synchronization is an extension of the manual synchronization feature discussed above.

Click the  smart synchronization icon on the ImageScope toolbar to use *smart synchronization*. (Notice that the smart synchronization icon is the same as the manual synchronization icon except that it is colored yellow.) Smart synchronization differs from manual synchronization—not only is navigation synchronized between the slides, but corresponding regions in the digital slide images are also synchronized.

A common use of this feature is when you have multiple digital slides scanned from microscope slides that were all prepared from the same tissue block but that are stained differently. For example, below are two images of slides made from the same block; with smart synchronization enabled, the main features of the slide stay locked in step as the operator moves through the slides.



Although smart synchronization is able to compensate for most of the rotation orientations made with the ImageScope rotation tool, it cannot compensate for "flipped" images, where you have used the rotation tool to flip the image vertically or horizontally on its axis; if you try to use smart synchronization on such images or try to "flip" images when smart synchronization is in effect, the following message appears:



Moving the Viewing Area

ImageScope offers many different options for moving around a digital slide.

Panning

The simplest method for moving the viewing area is to pan the image:

1. Click and hold the mouse button down while you drag the cursor across the digital slide. Your cursor turns into a closed fist  . Panning moves the slide in the direction you are dragging, allowing you to see more of the slide.

Panning Tips

- If you can't pan the image, you may have an annotation tool selected. Click the  icon on the ImageScope toolbar and try again.
- As you pan, the black rectangle in the thumbnail image also moves to indicate the section of the slide you are viewing.
- If you want to set ImageScope to pan in reverse (called "pathologist mode"), see "Panning Options" on page 179.

Autopanning

You can use autopanning to move at high speed over a digital slide:

1. Move the cursor to the center of the main viewing area.
2. If your mouse has a scroll wheel, click it to start autopanning or right-click and select **Autopan** from the context menu.

   A small navigation icon appears:  , and you also see a darker version showing your cursor position:  .
3. By moving the mouse in any direction, you can automatically pan in that direction very quickly. To stop autopanning, click any mouse button.

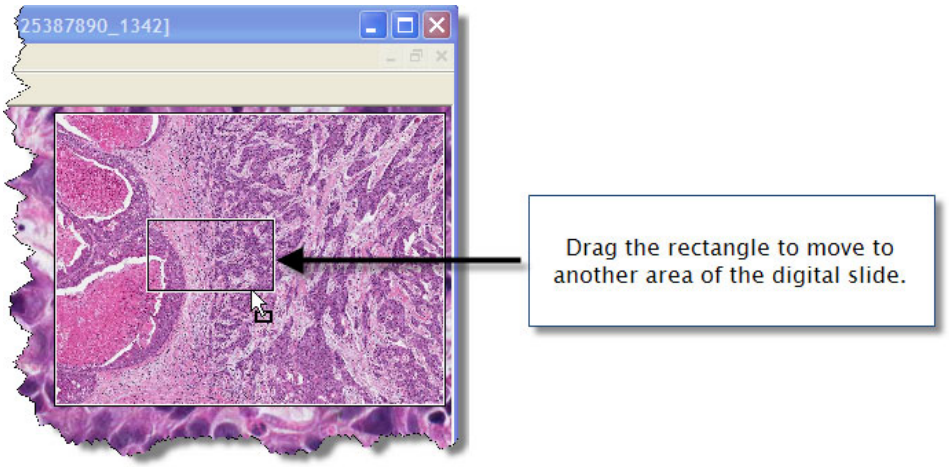
Scrolling

You can scroll across a digital slide to the right, left, up, or down. As you move your cursor toward the edge of the ImageScope main window, the cursor changes to an arrow:  ; click and hold the mouse button down to scroll in that direction. To stop scrolling, release the mouse button.

Using the Thumbnail Window

Depending on the magnification in use, the main ImageScope window may show only a portion of the slide. The thumbnail window shows the entire digital slide. The portion of the digital slide being displayed in the main window is enclosed in a black rectangle in the thumbnail window.

Click in the thumbnail window to move the main image to that part of the slide or drag the rectangle in the thumbnail window to move to another area of the digital slide.



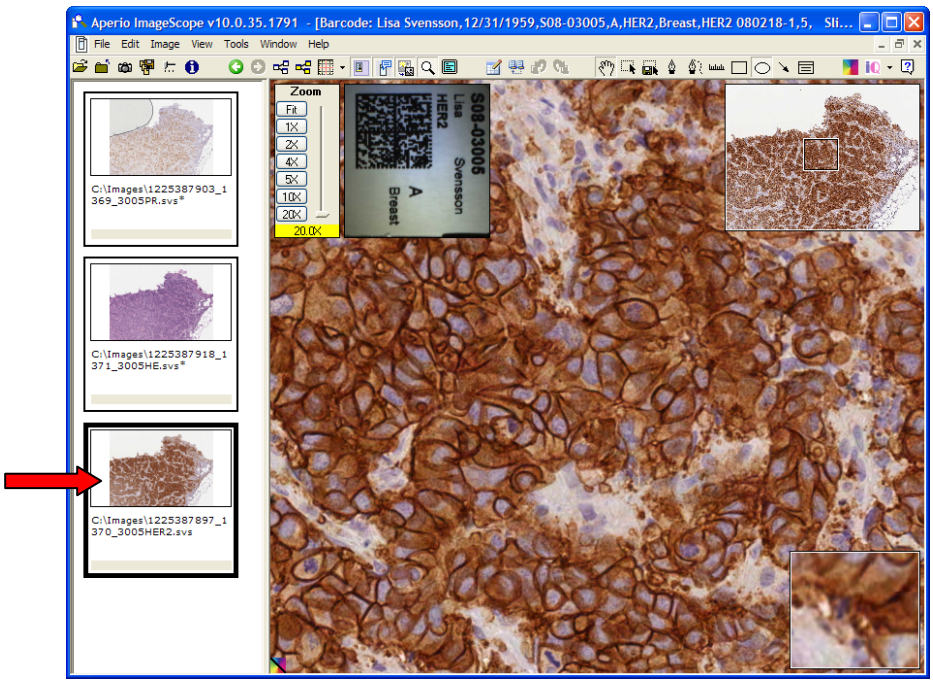
Re-sizing the Thumbnail Window

You can change the size of the thumbnail by moving your cursor on the lower left corner of the thumbnail until you see a double-ended arrow—then click and drag the corner in or out.

Using the Filmstrip

As discussed in Chapter 3, "Opening a Digital Slide," you can open multiple digital slides.

1. To move between multiple digital slides in ImageScope, click the image of the one you want to see in the filmstrip in the left pane of the window. For example:



Page Panning

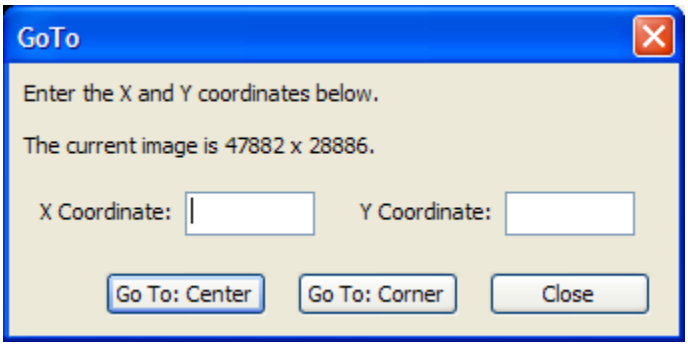
Use the keyboard arrow keys to move an entire screen-page at a time:

- **Shift+Right-arrow –** Move a page to the right
- **Shift+Left-arrow –** Move a page to the left
- **Shift+Up-arrow –** Move a page up
- **Shift+Down-arrow –** Move a page down

Moving to a Specific Point

To move to a specific spot on the digital slide:

1. Go to the **Image** menu and select **Go To**.



2. On the GoTo window, do one of the following:
   - Using the size of the image shown in pixels as a guide, type an X coordinate to select a point that is that number of pixels from the left edge of the image and a Y coordinate to select a point that is that number of pixels from the top of the image.
   - Click **Go To: Center** to position the point selected by those coordinates in the center of the current view.
   - Click **Go To: Corner** to position the point selected by those coordinates in the upper left corner of the current view.
3. Click **Close** to exit the GoTo window.

Using the Magnifier Window

Use the magnifier window to show a larger view of a particular portion of the digital slide.

Tips:

- Drag the magnifier window on the main window to the area you want to see in more detail. Or simply leave the magnifier window stationary and move the cursor on the main image to the area you want to see in more detail, and the area at the current cursor location will appear magnified in the magnifier window.
- You can resize the magnifier window by dragging its lower right corner.
- The magnifier window's default magnification is twice the resolution of the image in the main window. So if the main window is at 20x magnification, the magnifier window shows 40x. You can change the resolution of the magnifier window by going to the **Tools** menu and selecting **Options**. For details, see "Magnification" on page 174.

Changing Viewing Magnification

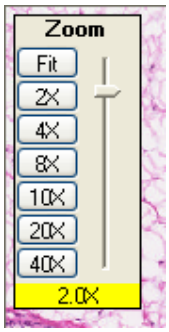
In addition to using the magnifier window to show a portion of the digital slide in greater resolution, you can also change the resolution of the entire main window image.

Immediate Maximum Zoom

Double-clicking on the image in the main window immediately zooms that image to the maximum magnification. Double-click again to return to the most recently used magnification that was not the maximum magnification.

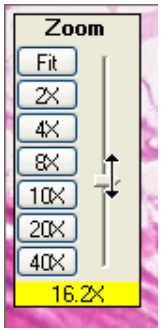
Using the Zoom Slider

You can adjust the magnification of the entire display by using the zoom slider.



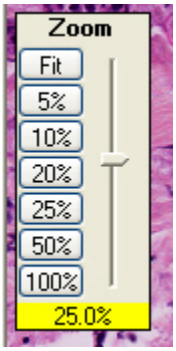
- Click **Fit** to set the magnification to 0x and fit the entire digital slide within the main viewing area.
- Click any other magnification setting to zoom in using that magnification.
- Click the slider and drag it up or down to change the magnification in small increments.
- If your mouse has a scroll wheel, click the image in the main window and roll the scroll wheel to move the slider.

For example, below we used the slider to select a magnification of 16.2:



To set magnification to percentages instead of 2x, 4x, and so on:

1. Go to the **Tools** menu and select **Options**.
2. Clear the **Use "X" magnification rather than "%"** check box and click **OK**. Now when you use the Zoom slider, it displays magnification percentages:



To turn the zoom slider on or off, click  on the toolbar or go to the View menu and select **Zoom Slider**.

Zoom Keyboard Shortcuts

Instead of using the Zoom slider, you can hold down the Control key and press:

- – key to zoom out
- + key to zoom in

Zoom Navigation

To zoom into a particular area of the digital slide:

1. Click  on the ImageScope toolbar.
2. Click in the main image window at the upper-left corner of the area you want to zoom into and, holding the mouse button down, draw a rectangle around the area you want to zoom into.
3. Let go of the mouse button and the main window will be zoomed to that area.

If you have already predefined a fixed size (see "Fixed Size Annotations" on page 180), to set the zoom rectangle to the predefined size, hold down the Control key while you click on the area you want to zoom into.

Viewing with Color Management

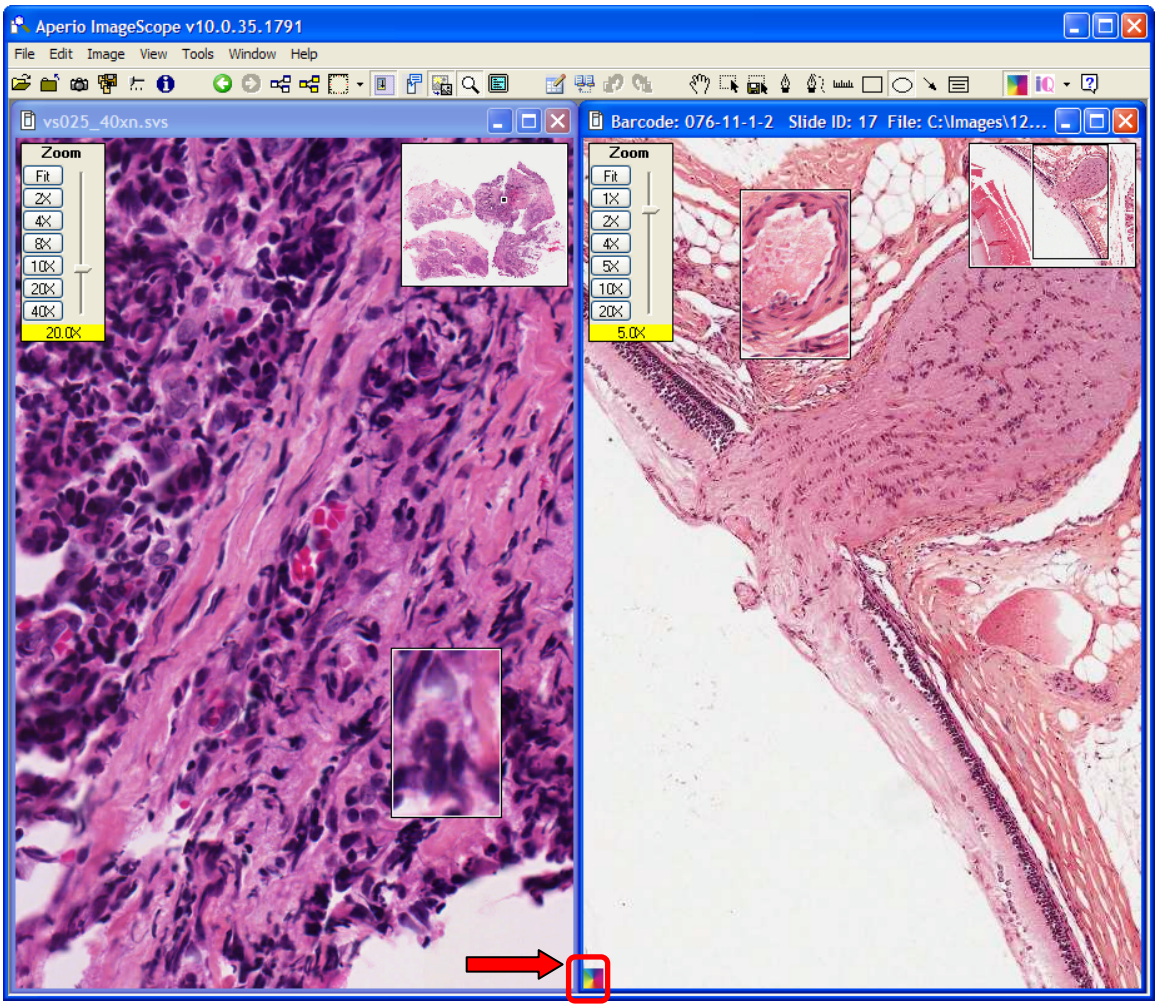
Aperio Integrated Color Management takes into account the optical characteristics of your ScanScope scanner and your display monitor to make sure that colors of the digital slides are displayed accurately. For information on Aperio Integrated Color Management, see Appendix B, "Aperio Integrated Color Management" on page 193.)

By default, ImageScope uses the ScanScope source ICC profile embedded in the digital slide and the target ICC profile for your monitor to make sure the image displays in accurate color. (The ICC profile is embedded in the digital slide image during scanning.)

The ImageScope toolbar allows you to turn Integrated Color Management on or off:

- Click the icon on the ImageScope toolbar to turn color management on or off. If color management is enabled, the icon looks like this: ; if it is disabled, it looks like this: .
- If an image has an embedded ICC profile, you see the symbol at the bottom of the image. If color management is turned off, the symbol on the image looks like this: .

For example, in the illustration below, color management is enabled. The image on the left does not have an embedded ICC profile, and the image on the right has an embedded ICC profile:



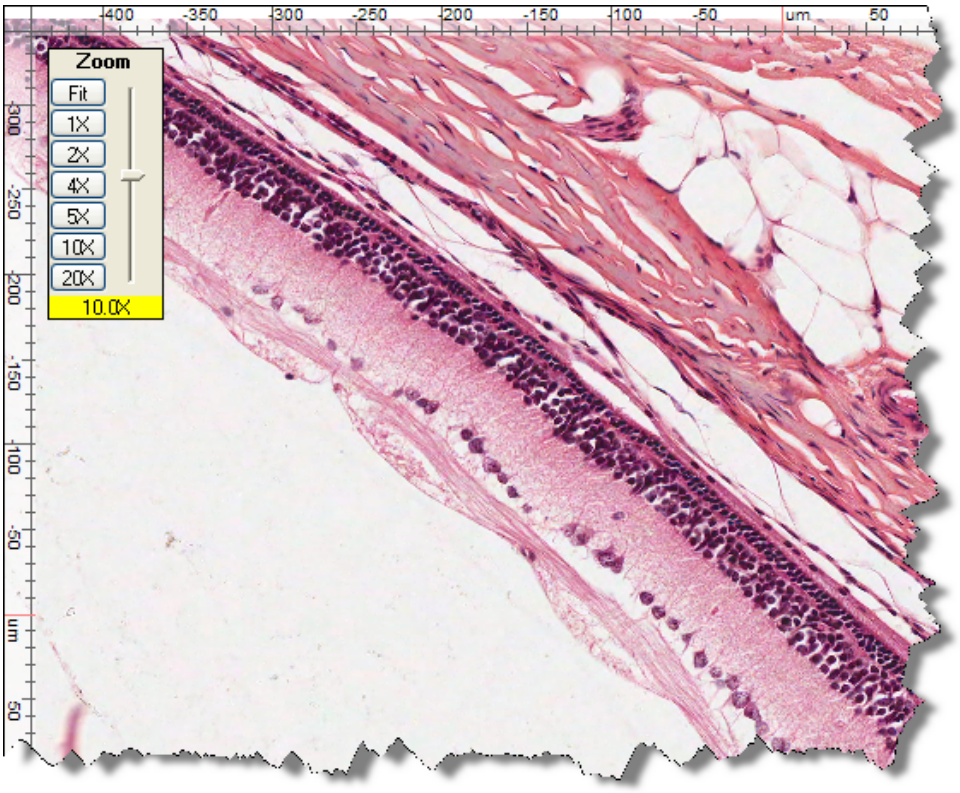
Viewing Scale Axes and Grid

You can view scale axes and a grid on an image in ImageScope. The units and spacing are adjusted to correspond to the resolution of the image and the current zoom level.

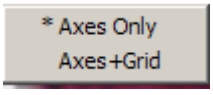
The zero point of the axes is in the center of the window; it is labeled with the current unit (for example, **um** for microns in the example below). If the resolution of the image is unknown, the units on the axes/grid will be **p** (pixels), **kp** (kilopixels), or **mp** (megapixels). This would be the case for photomicrographs and gross images before the resolution is set. The resolution on such images can be entered explicitly or by measuring a known item with a ruler (see Chapter 8 "Image Resolution" on page 71).

To enable the axes view:

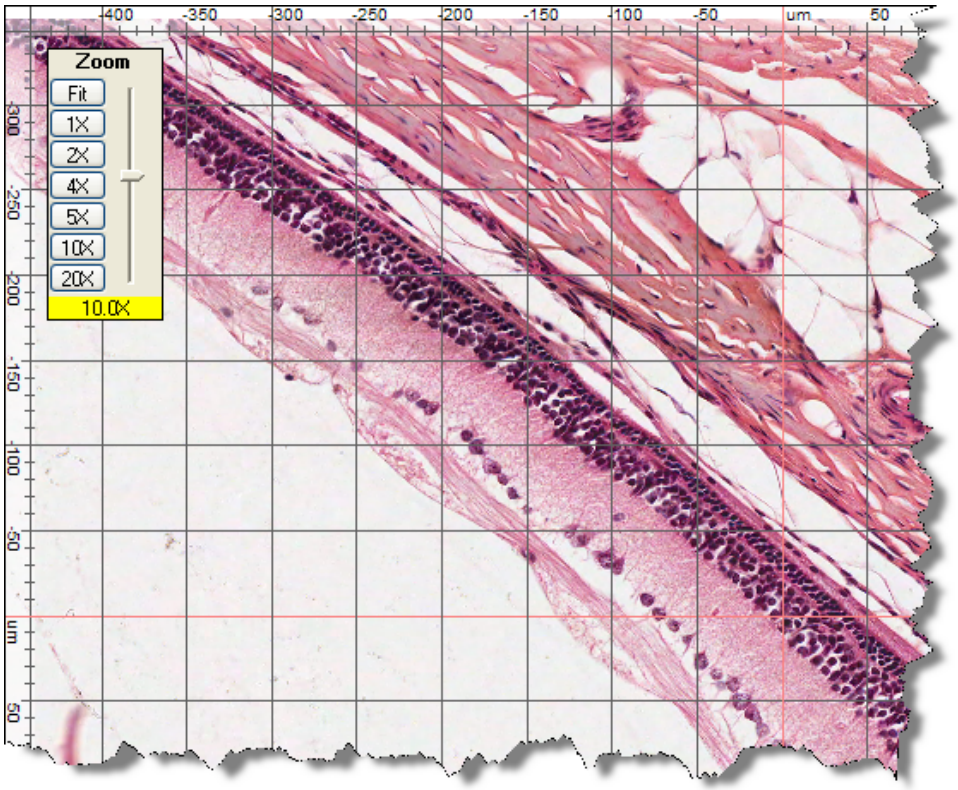
1. Click on the ImageScope toolbar or go to the View menu and select **Scale Axes/Grid**. The view in the main window appears with axes markers:



2. Click the down arrow next to the icon to select whether you want to see just the axes or the axes plus a grid:



The image with the scale axes and grid looks like this:



Note that the icon on the toolbar changes to reflect the fact that the grid is displayed:

 instead of  .

3. To turn the axes/grid off, click the axes/grid icon or go to the View menu and select **Scale Axes/Grid**.

Viewing Digital Slides with IQ

Aperio Image Quality (IQ) technology gives pathologists and other scientists who view digital slides the ability to customize the view of those slides to boost productivity and visual clarity by digitally adjusting the stain colors, viewing the individual stain images, and/or re-mixing the stains on the fly while they navigate the image.

IQ allows you to choose for yourself what view of the digital slide gives you the best results and makes it easier for you to identify the features of the slide you are most interested in.

IQ is available when you have opened a digital slide in ImageScope from Spectrum and if your site is licensed for Spectrum Plus.

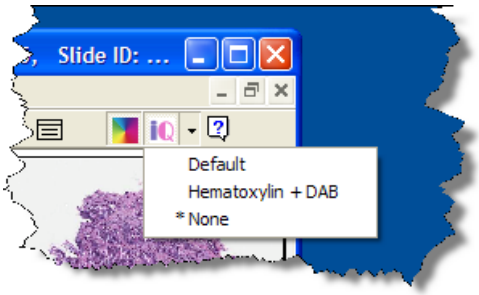
IQ Features

IQ uses color processing—analyzing each pixel of the digital slide image—to identify stains and modify their appearance on the digital slide. Some of its features include the ability to:

- View just a selected stain as you navigate the digital slide. IQ uses color deconvolution to separate the stains and present them as you pan or scroll about the image.
- Boost or dilute the displayed concentration (especially useful for overstained or understained slides, or to suit your personal preference).
- Enhance cellular detail such as nuclei.
- Digitally adjust individual stain colors for visual clarity and personal preferences (for example, darker/lighter, more or less vibrant, bluer/redder, and so on).

IQ Quick Reference

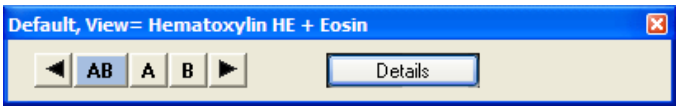
To turn IQ on for the image in the ImageScope window, click  on the toolbar. You can then select the stain set to use to view this digital slide by clicking the down-arrow next to the  icon:



The default stain set is optimized for Hematoxylin and Eosin stains.

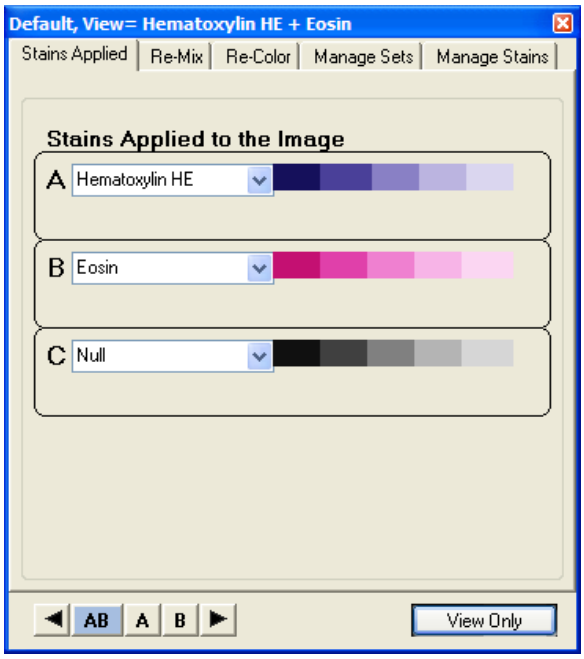
To use the IQ viewing toolbar and application:

1. With IQ turned on, go to the Image menu and select **Quality**. You see the IQ viewing toolbar:



2. Click the buttons to view the digital slide using all stains or individual stains.

3. To see the full IQ user interface, click the **Details** button on the viewing toolbar:



Now you can use the IQ tabs to define the stains applied to the digital slide, re-mix and re-color those stains, create your own stain sets, and measure the stains used by your lab. To return to just the viewing toolbar, click **View Only**.

For details on using IQ, see the *IQ Image Quality User's Guide*.

* Aperio's image analysis algorithms are FDA cleared for specific clinical application, and are intended for research use for other applications.

5 Rotating Images and Slide Labels

ImageScope rotation tools allow you to rotate an image. You can also rotate a digital slide label image.

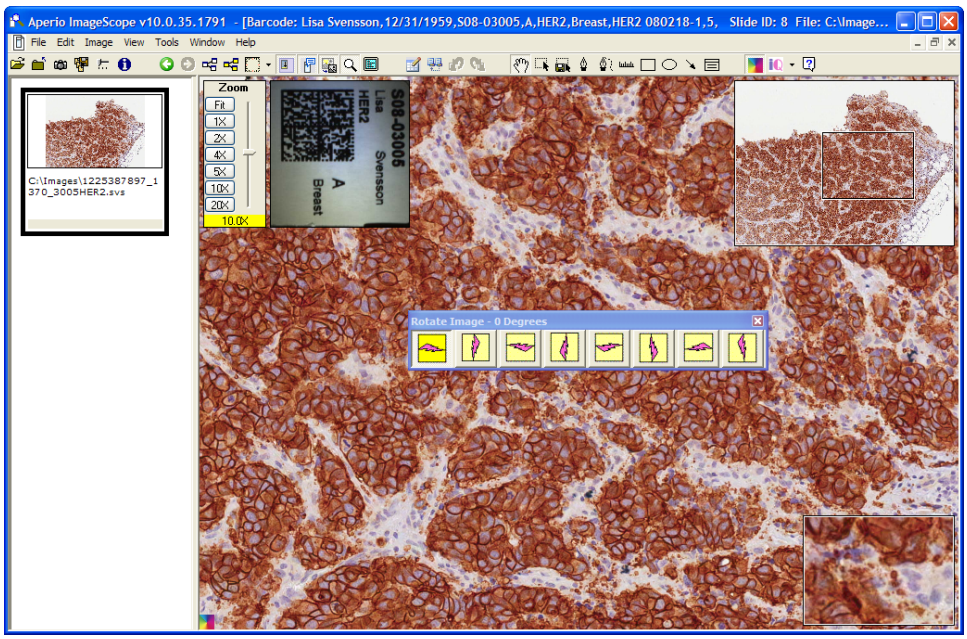
Rotating an Image

The rotation setting is in effect only for the current viewing session and is not saved with the image. However, when you create a new image by using the Snapshot or Extract Region commands, the new image will be saved in the current rotation. Saving an Image View also saves the current rotation settings so that opening the Image View displays the image with those rotation settings re-applied.

Image rotation is not enabled during a TelePath Live* session.

To use image rotation:

1. Go to the ImageScope Image menu and select **Rotate Image** (Control E). A new toolbar appears:



2. From the rotation toolbar, select the rotation setting you want to use:

Rotate Tool	Description
	Rotate zero degrees
	Rotate 90 degrees right
	Rotate 180 degrees
	Rotate 90 degrees left
	Flip vertically
	Rotate 90 degrees right and flip vertically
	Flip horizontally
	Rotate 90 degrees left and flip vertically

Label Rotation

You can rotate a digital slide label. Open a digital slide in ImageScope and position the cursor on one edge of the slide label. A small arrow appears:

Double-click an arrow on the side of the label you want on top. When you save the digital slide, the label rotation is saved.

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* This application is not approved or cleared by the FDA for clinical use.

6
Making Image Adjustments

It can be useful to modify the color settings of digital slides if particular colors do not show up well on your workstation monitor. This chapter discusses the different image adjustment settings.

> For information on adjusting fluorescence images, see Chapter 7, "Working with Fluorescence Digital Slides."

Image adjustments apply only to the current ImageScope session. They are not stored with the digital slide (so you don't have to worry that you have modified your original digital slide when making an image adjustment). You can save gamma settings to apply to the current digital slide or to apply to other digital slides later, and you can make a snapshot of the adjusted image if you want to save the adjusted digital slide image. (See Chapter 13, "Saving Digital Slides and Regions" on page 109 for information on making snapshots.)

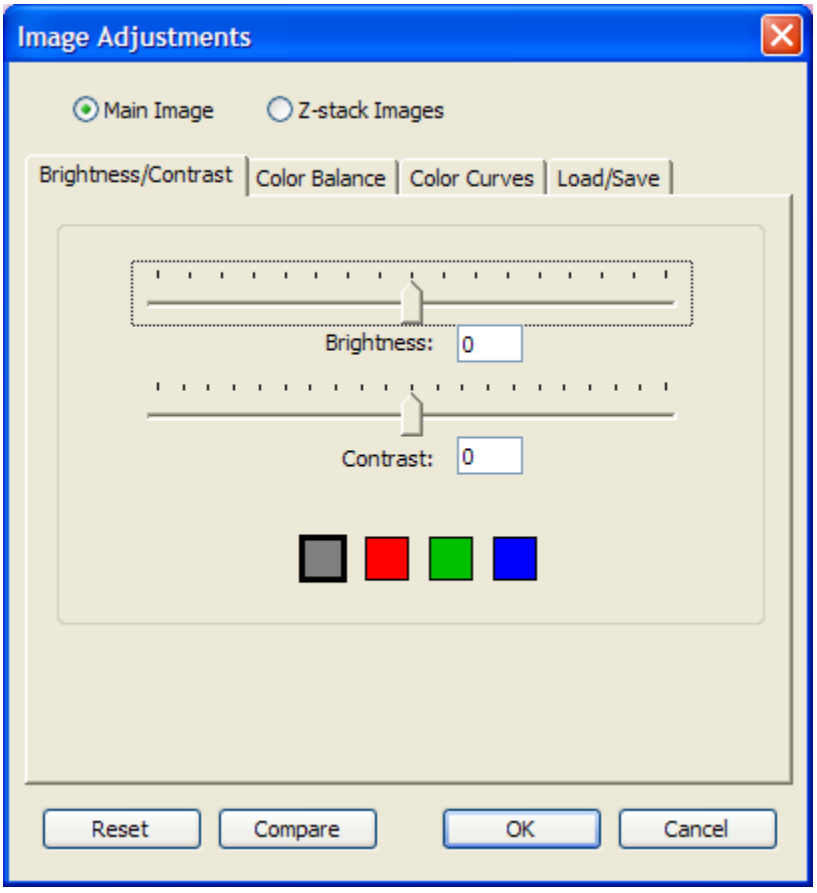
Use the image adjustment feature to:

- Adjust the brightness or contrast for all colors or for just the red, green, or blue channel.
- Modify the color balance (for example, make reds less red and more cyan).
- Adjust color curves for all colors or for just the red, green, or blue channel.
- Save the color adjustments you have made in a gamma table file so they can be re-applied to the same or other digital slides in future ImageScope sessions.
- Make image adjustments to the entire digital slide or to Z-stack* images.

Getting Started with Image Adjustments

To make image adjustments:

1. Go to the **Image** menu and select **Adjustments** or click on the ImageScope toolbar. The Image Adjustments window appears:



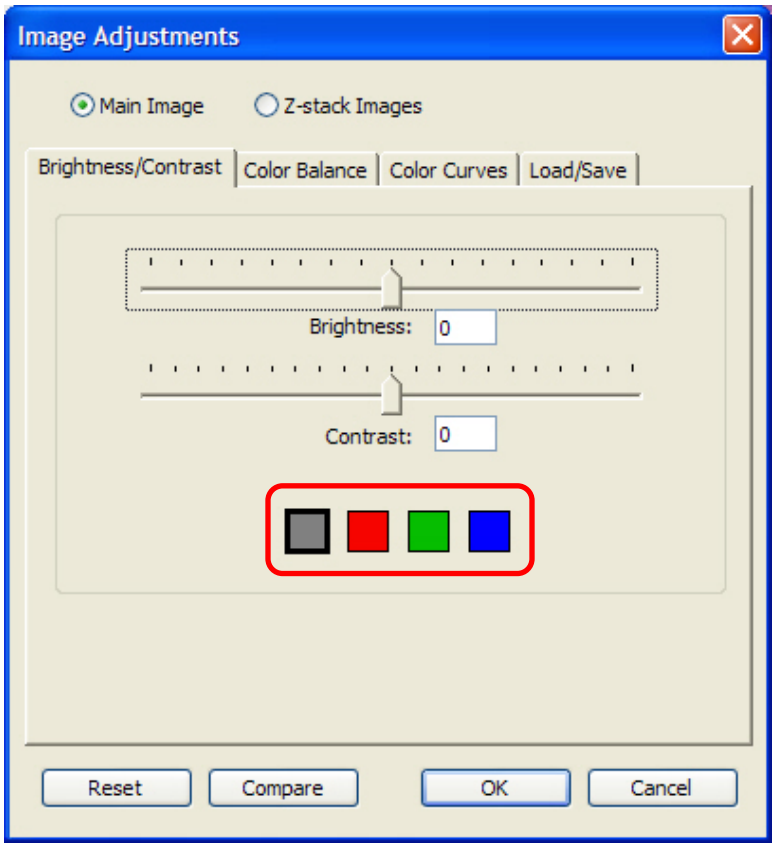
General Tips

- To modify the appearance of the entire digital slide, select **Main Image**; to modify just the current Z-stack* images, select **Z-stack Images**. (For information on 3-dimensional Z-stack images, see Chapter 17, "TelePath Live*," on page 155.)
- Click and hold the **Compare** button to temporarily change the image back to the original settings; release the button to revert back to the changed settings.
- Click the **Reset** button to change all colors back to the original default settings.

Modifying Brightness and Contrast

To modify brightness and contrast:

1. On the Image Adjustments window, click the **Brightness/Contrast** tab.

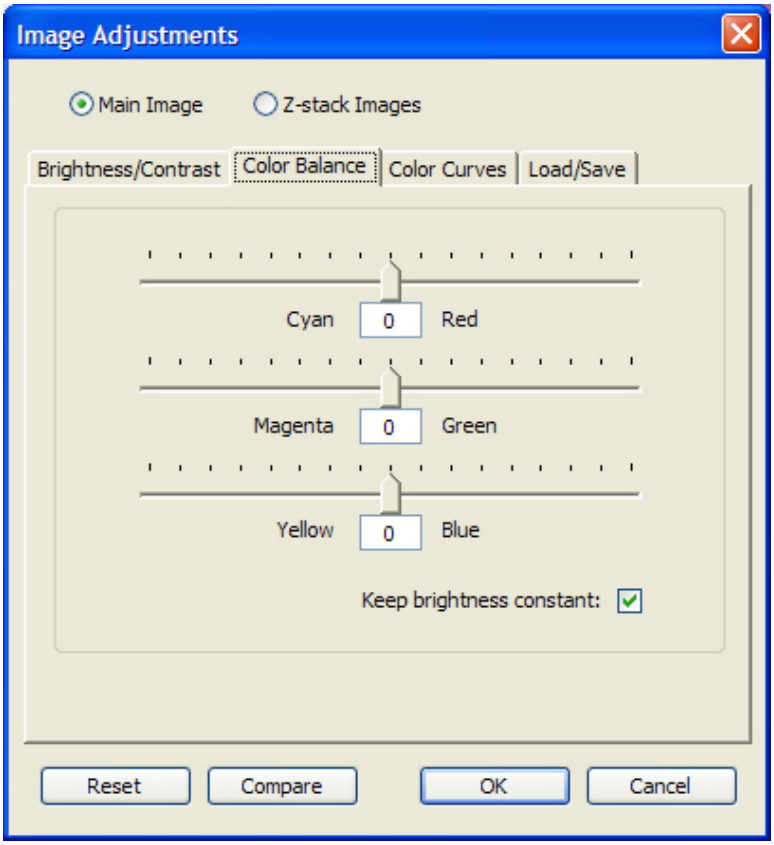


2. Select a colored square to adjust the brightness or contrast for a particular color channel:
   - **Gray –** All color channels
   - **Red –** Just the red channel
   - **Green –** Just the green channel
   - **Blue –** Just the blue channel
3. Click, hold, and drag the slider bars to adjust the brightness or contrast. Drag to the left to decrease brightness or contrast; drag to the right to increase brightness or contrast. Or type a number in the **Brightness** or **Contrast** box—a negative number to decrease brightness or contrast or a positive number to increase it.
4. When all color settings have been adjusted, click **OK**.

Modifying Color Balance

To adjust the color balance:

1. On the Image Adjustments window, click the **Color Balance** tab. The following Image Adjustment window appears:

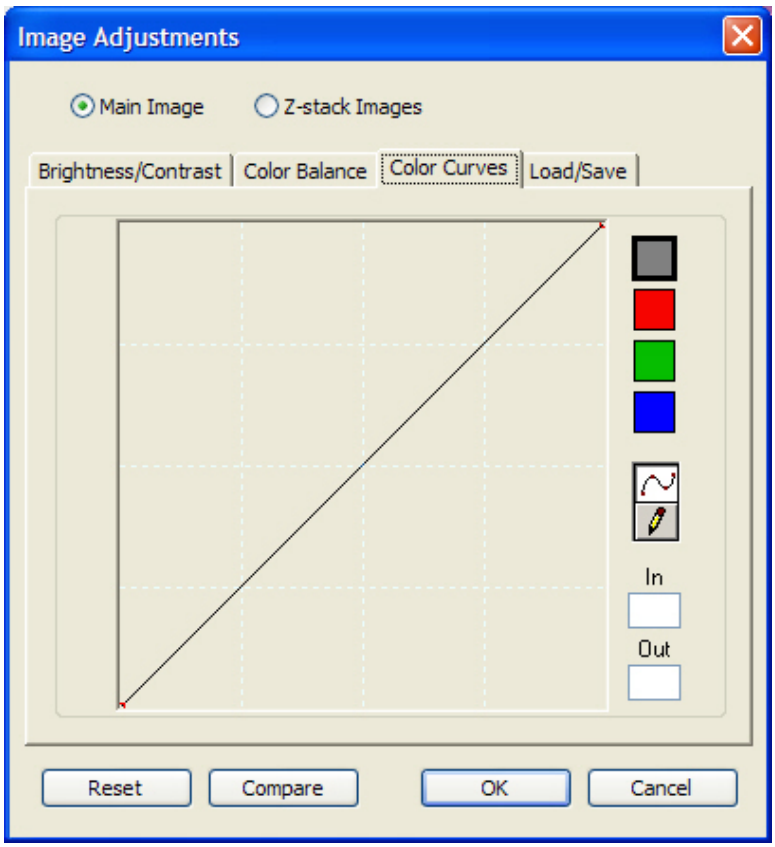


2. Click, hold, and drag the sliders to adjust the color balance in the red, green, and blue channels. Or type a number in the color boxes—a negative number to move the balance to the left or a positive number to move it to the right.
3. Select the **Keep brightness constant** check box to keep the overall brightness constant. When this is selected, as one channel's intensity is adjusted, the other channels' intensities are adjusted to balance the overall brightness. If you want to adjust the intensity of each color independently of the other channels, unselect this check box.
4. When all color settings have been adjusted, click **OK**.

Modifying Color Curves

To adjust the color curves:

1. On the Image Adjustments window, click the **Color Curves** tab. The following Image Adjustment window appears:



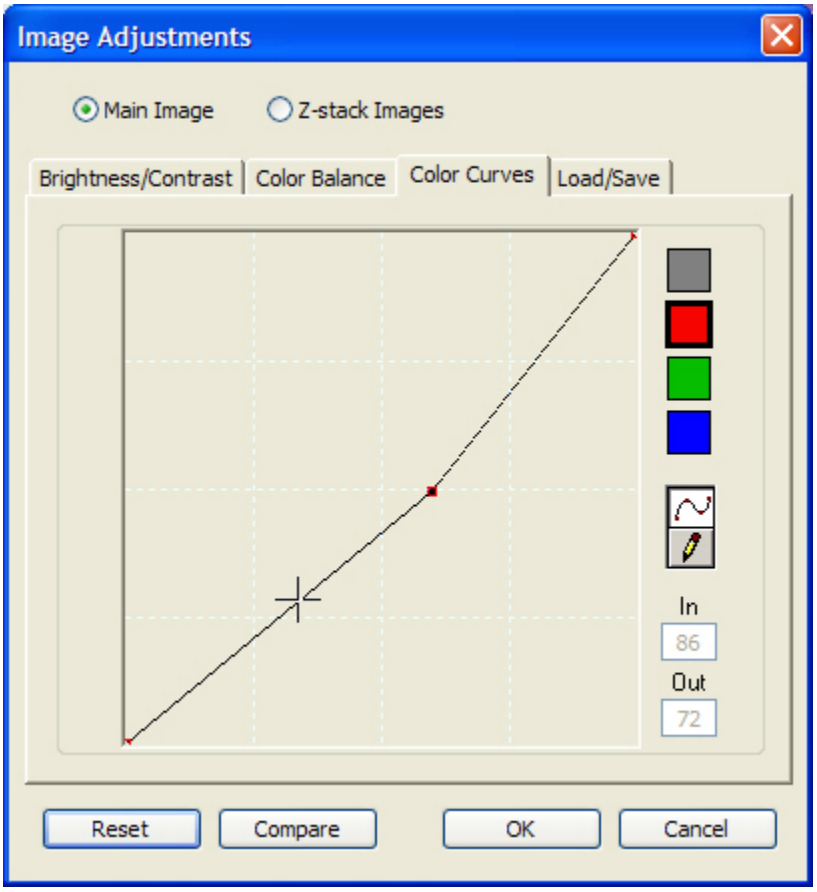
2. Select a colored square to adjust the brightness or contrast for a color channel:
   - **Gray –** All color channels
   - **Red –** Just the red channel
   - **Green –** Just the green channel
   - **Blue –** Just the blue channel

3. Select either the pencil tool, , or the points tool, .

   a) The pencil tool allows you to draw a free-form shape across the graph to define the color curve.

   b) The points tool allows you to edit the color curve by creating and dragging points that define the curve.

   In the example below, we selected the red channel, selected the points tool, clicked on the curve to create a point, and then dragged the point down to change the red channel curve. The In and Out boxes indicate the current cursor position on the curve.



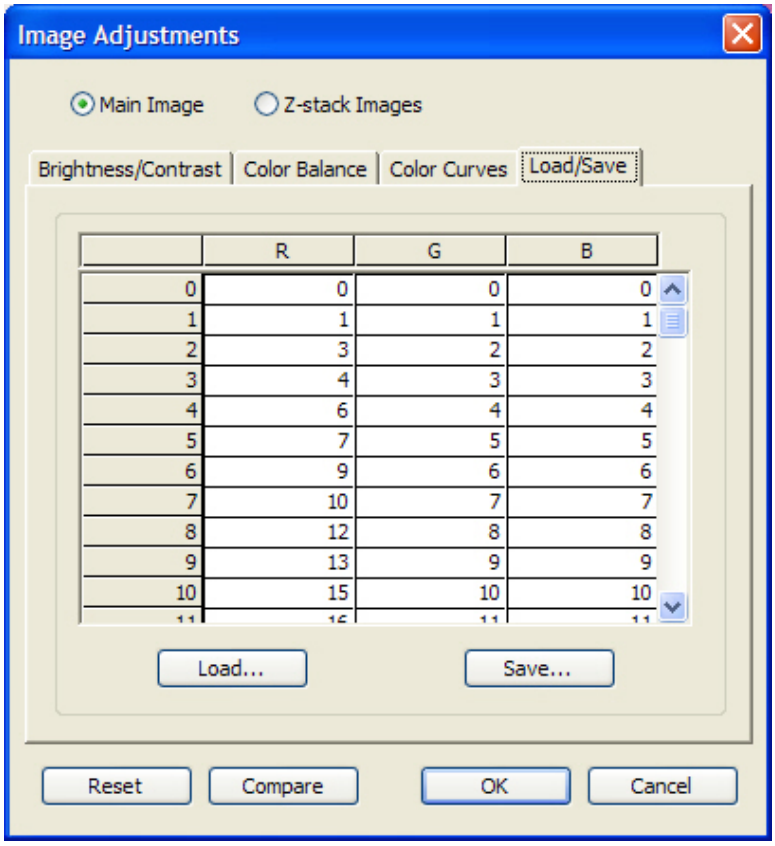
4. When you have adjusted all color curves, click **OK**.

Saving and Loading Color Settings

You can save all of the image adjustments you made on the other Image Adjustments window tabs by saving them on the Load/Save tab. You can also apply settings you previously saved to the current image by loading them.

For information on loading a *default* gamma table file to be used every time ImageScope opens a digital slide, see "Default Gamma Files" in the chapter "ImageScope Options."

1. On the Image Adjustments window, click the **Load/Save** tab. The following window appears:



Saving Color Adjustment Settings

To save the current color adjustment settings:

1. Click **Save**.
2. On the Save Gamma Tables window, navigate to the directory in which you want to save the gamma table file and type a file name.
3. Click **Save**.
4. Click **OK** to exit the Image Adjustments window.

Loading Color Adjustment Settings

To load previous settings

1. Click **Load**.
2. On the Load Gamma Tables window, navigate to the location of a previously saved gamma table file.
3. Select a file.
4. Click **Open**.
5. Click **OK** to exit the Image Adjustments window.

For More Information

- For information on Z-stacks, see Chapter 17, "TelePath Live*" on page 155.
- For information on loading color settings to be used every time ImageScope opens, see "Default Gamma Files" on page 175.

* This application is not approved or cleared by the FDA for clinical use.

7 Working with Fluorescence Digital Slides

This chapter discusses how to view and adjust fluorescence digital slide images.

Images from the ScanScope FL are grayscale images, pseudo-colored during the scanning process.

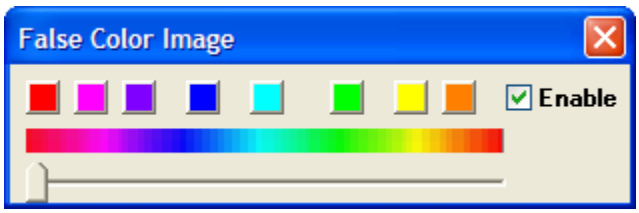
ImageScope offers a full range of fluorescence features:

- Temporarily apply a false color to a fluorescence image (this is not needed for fluorescence digital slides created by the ScanScope FL)
- For a fused image:
  - Change the display color for each channel image
  - Adjust brightness, contrast, and gamma (viewing the results on the image and on a histogram display)
  - Adjust registration between channels
- Fuse multiple fluorescence channel images into a fused image (automatically done for images acquired with the ScanScope FL)

Applying a Temporary False Color

If you are using a grayscale fluorescence image and want to display it in color:

1. Open the image in ImageScope.
2. Go to the Image menu and select **False Color**. The False Color window appears:



3. Select a color by clicking a color box or using the color slider.

4. Select the **Enable** check box to see the image in the color you have selected. To view the image without the false color, clear the **Enable** check box.

Applying a false color in this way does **not** permanently change the display color for the image—this adjustment applies only to the current ImageScope viewing session.

Adjusting Fluorescence Fused Images

Fluorescence images are displayed in ImageScope using the color, brightness, contrast, and registration settings made in the ScanScope Console when the scan was made.

Any changes you make on the Image Fusion Adjustments window are saved with the image so that they apply the next time you open the image in ImageScope.

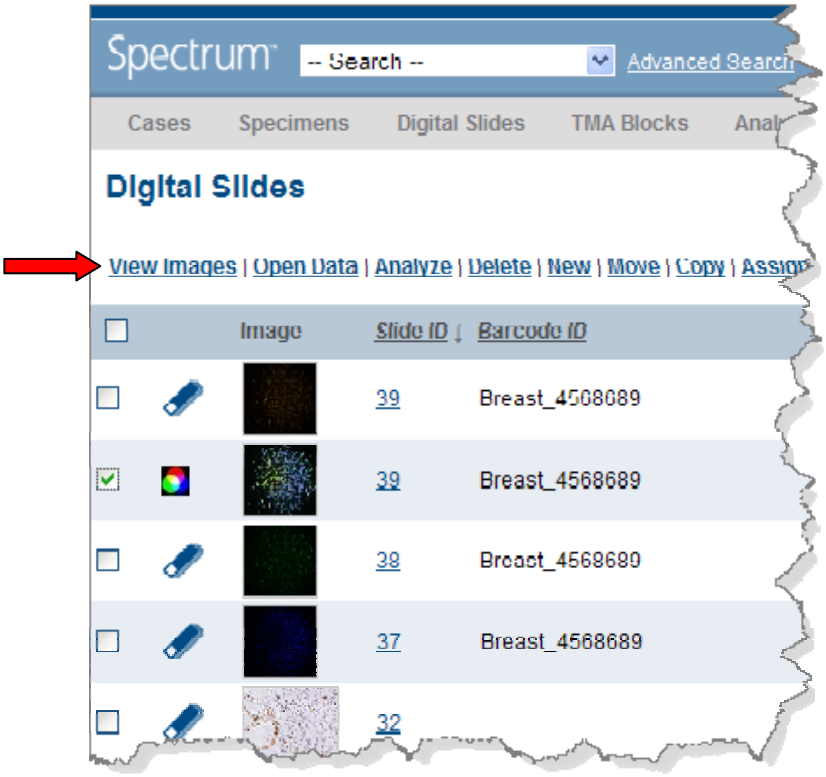
To use the Image Fusion Adjustments window:

1. Open an Aperio Fused Image (AFI) in ImageScope. The usual way you will do this is:

   a) Log into Spectrum.

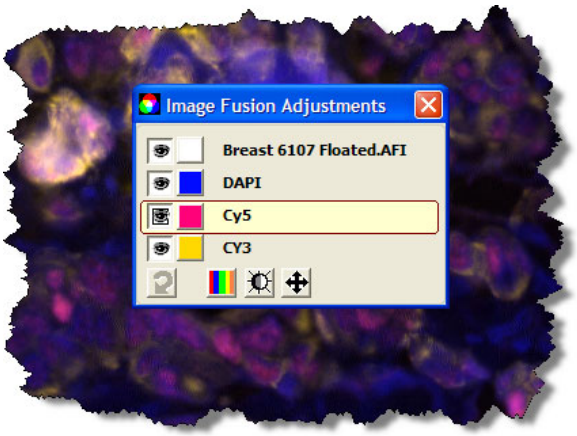
   b) Go to the Digital Slides menu and select **All Digital Slides (As List)**.

   c) Select the AFI and click **View Images** to open the image in ImageScope:



The AFI is indicated by the symbol.

2. Go to the ImageScope Image menu and select **Fusion Adjustments** (only available if viewing an AFI). You see the Image Fusion Adjustments window:



At the top is listed the fused image—beneath that are the individual channels that make up that image.

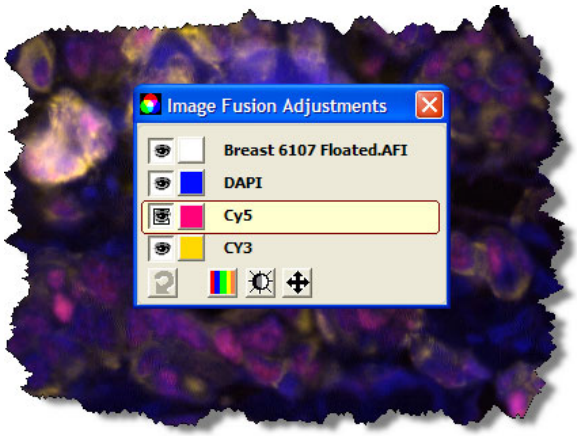
Using the Fusion Adjustment Window

You can enable features by clicking the symbols at the bottom of the Image Fusion Adjustments window (see the following sections for details on using each of these tools):

Tool	Function
	If at least one channel is hidden, this button cycles between the channels, showing different combinations.
	Show/hide color pane.
	Show/hide brightness, contrast, gamma adjustment pane.
	Show/hide registration pane.

On each secondary pane, click  to reset the image to the original image settings (at the time the image was scanned). If the fused image is selected, this button resets all channels.

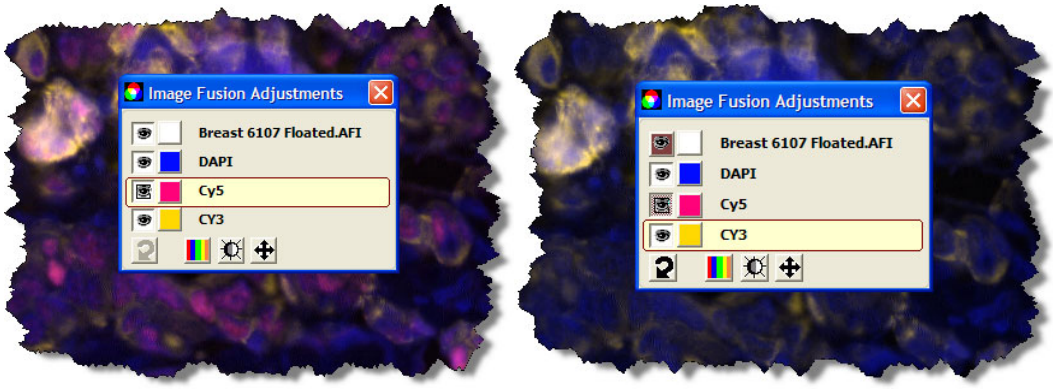
Before using one of the options at the bottom of the window, click a channel to select it so that the changes you make apply to that channel image. In this example, Cy5 is selected.



After opening a tool pane, click X to close the pane to exit back to the main Image Fusion Adjustments window.

Hiding a Channel

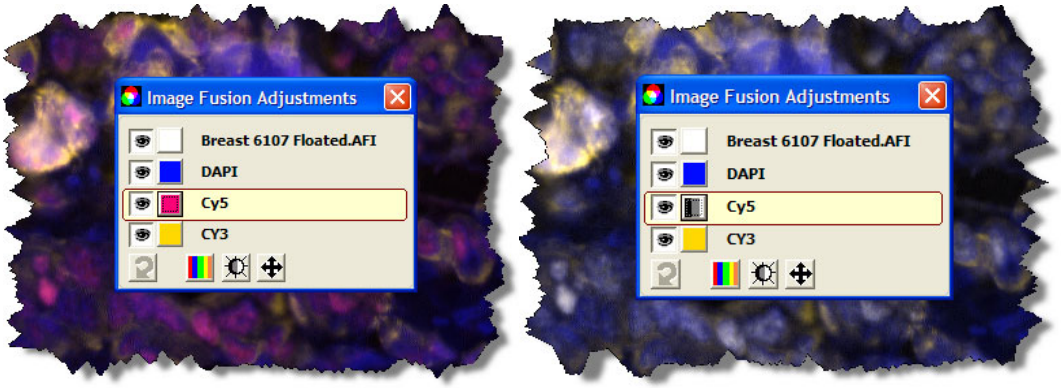
To hide a channel, click the eye symbol: next to it. (To add it back to the display, click the eye again.)



Notice the difference in the image with the Cy5 channel hidden (on the right).

Hiding a Channel Color

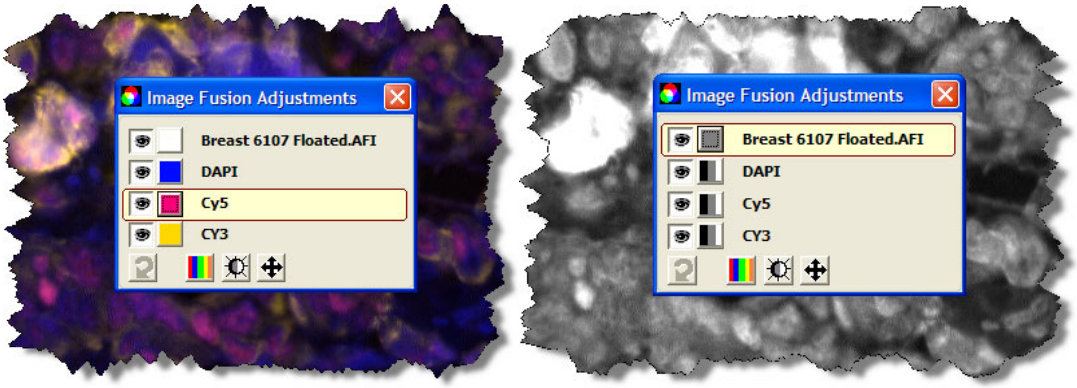
Click the color box next to a channel to turn off the false color for that channel (that is, to display it in grayscale). To turn the false color back on, click the color box again.



Notice the difference in the image with the Cy5 channel displayed in grayscale (on the right).

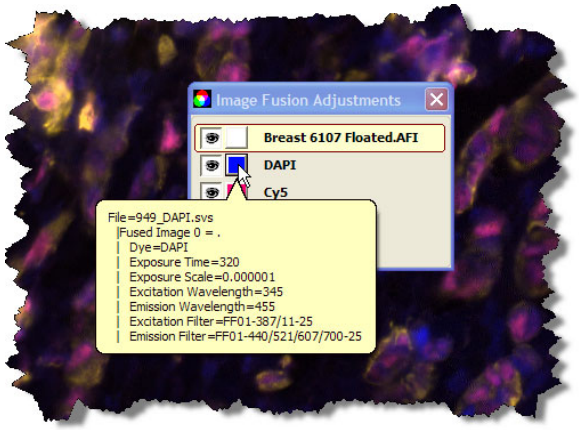
Hiding All Color

To remove all color, click the color box next to the AFI:



Seeing Channel Information

Place the cursor on a color box to see information about that channel.

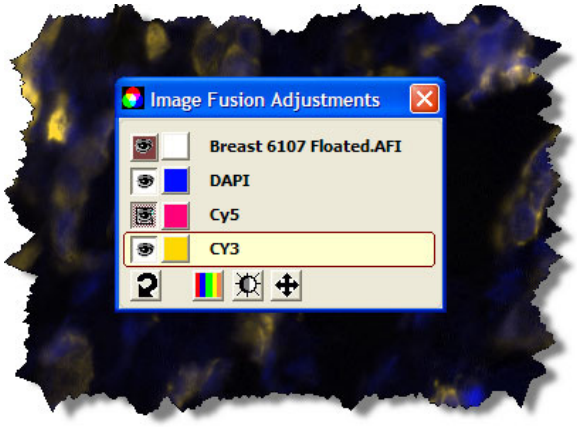


Cycling Channel Displays

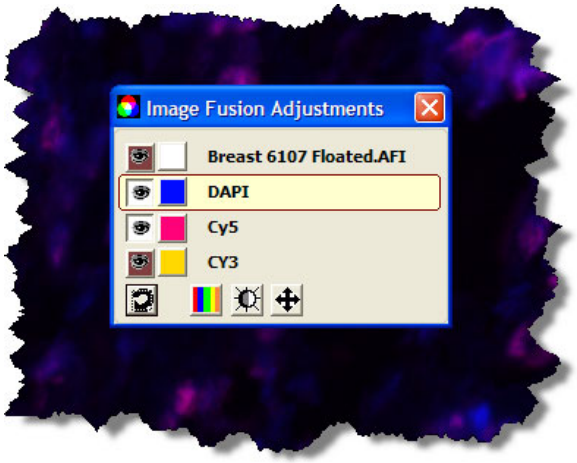
To cycle the display among the channels:

1. Hide a channel by clicking the eye symbol: next to it.
2. Click the button to cycle among different combinations of channels.

For example, below we have hidden the Cy5 channel (this also automatically turns off the AFI fused image as all channels must be on to see the fused image):



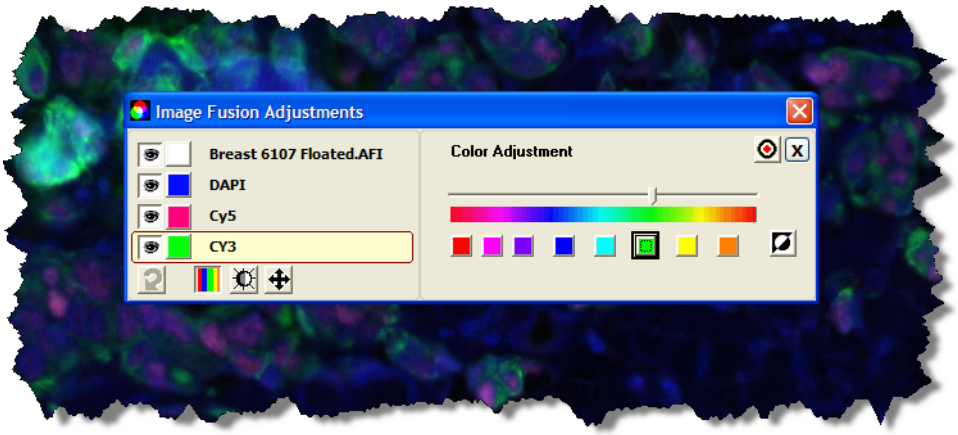
Now we click the button to cycle the display to a new combination of channels (DAPI and Cy5).



Clicking this button again cycles the display to another combination of channels. To turn off cycling, manually turn on all channels by clicking the next to hidden channels.

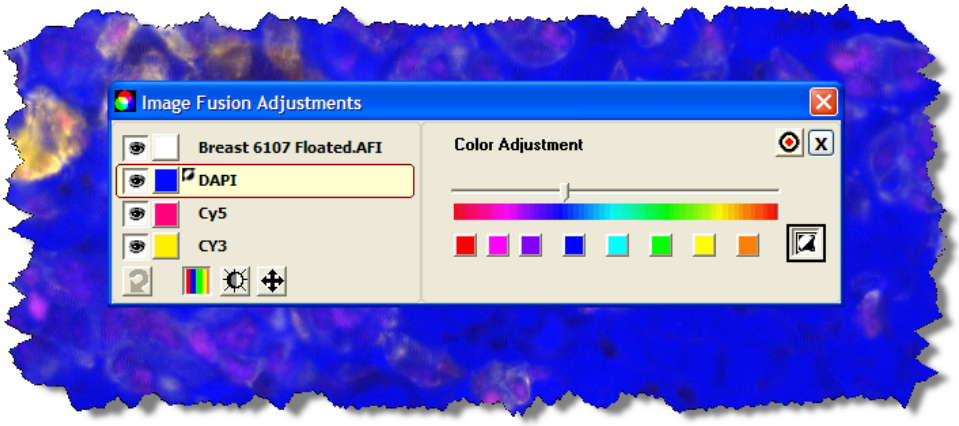
Adjusting Color

To change false colors, on the Image Fusion Adjustments window, click . On the color adjustment window, select a channel and select the color to be used to display that channel by clicking a color box on the Color Adjustment pane or using the color slider.



Click to invert color intensity in the display—the brightest pixels in the selected color become dark and the darkest pixels become bright. If a channel has been inverted, a small inversion symbol appears next to the color box for the channel.

For example:



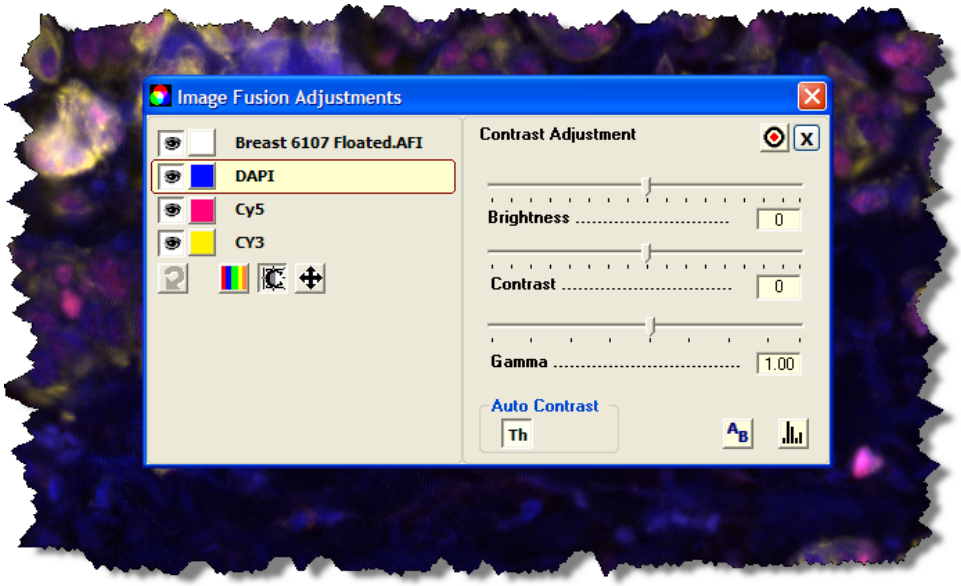
Adjusting Brightness, Contrast, and Gamma

To adjust brightness, contrast, and gamma, click  . You may want to change these settings to eliminate background noise, boost a weak signal, assign a histogram stretch, and so on. These settings affect the display of the fused image and channel images, but do not affect the actual pixel data in the image files.

- **Brightness –** This setting adjusts the overall intensity of every pixel. You may want to use this to brighten dark images or darken bright images.
- **Contrast –** This setting makes the dark pixels darker and the light pixels lighter.
- **Gamma –** This setting changes the midtones of your image. It is a nonlinear adjustment that can make faint objects more intense without saturating bright objects. At the same time, medium-intensity objects can be made fainter without dimming the bright objects.

A typical way of using these settings it to first adjust the contrast to stretch the intensity of the image and then, if the image is too bright or dark, adjust the gamma.

The best way to see the effect of the settings is just to try them on your image to see what improves the image.



Select a channel (or the fused image) and select the settings you want to use. You can make these changes with color turned on or off. Any adjustments you make are immediately visible in the image in the ImageScope window.

- To see an intensity histogram of the current view of the image, click the  symbol. (See "The Intensity Histogram" below.)
- To turn automatic contrast settings for the image thumbnail on or off, click ("Th" stands for "thumbnail"). Automatic contrast for

thumbnails is on by default to boost contrast, as thumbnails tend to be very dark.

If Auto Contrast is on, brightness, contrast, and gamma settings are not applied to the thumbnail; if it is off, they are.

If this setting is not beneficial to the visual quality of your particular image, turn it off. This setting is used not just by ImageScope, but also by Spectrum when displaying thumbnails, so turning it off in ImageScope also affects thumbnail display in Spectrum or any other Aperio application that displays thumbnail images. Once turned off, the setting stays off for this image until you turn it on again.

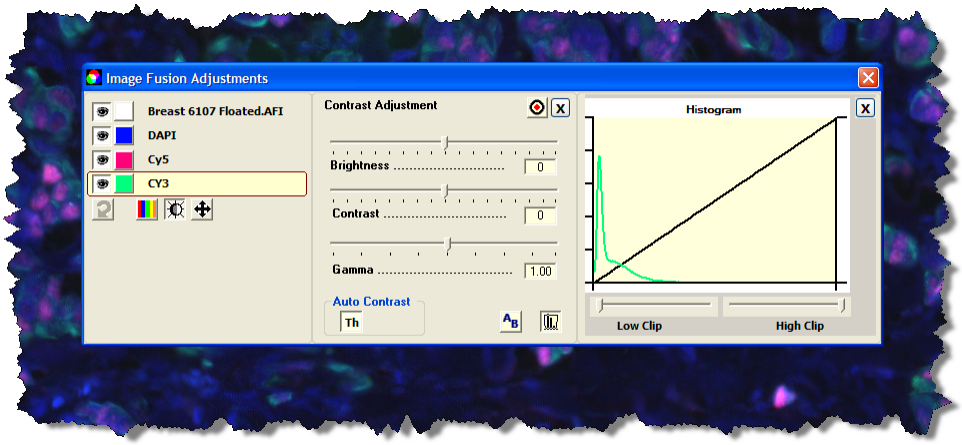
- To compare the new settings against the original, click  .

The Intensity Histogram

To see a histogram of the current image view, click the  button.

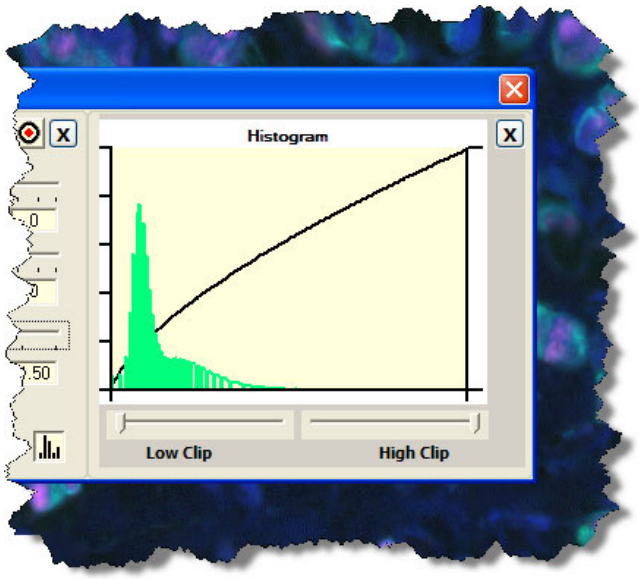
The histogram plots the number of pixels at each intensity. By looking at the left of the histogram you can see how many pixels are in the darkest intensity; the right of the histogram shows how many pixels are in the lightest intensity.

The black line shows a transfer function that relates input to output. You might also know this line as the *tone curve*. In an unadjusted image, this line is a straight line from zero to maximum intensity. As you change settings, the line changes to reflect your adjustments.

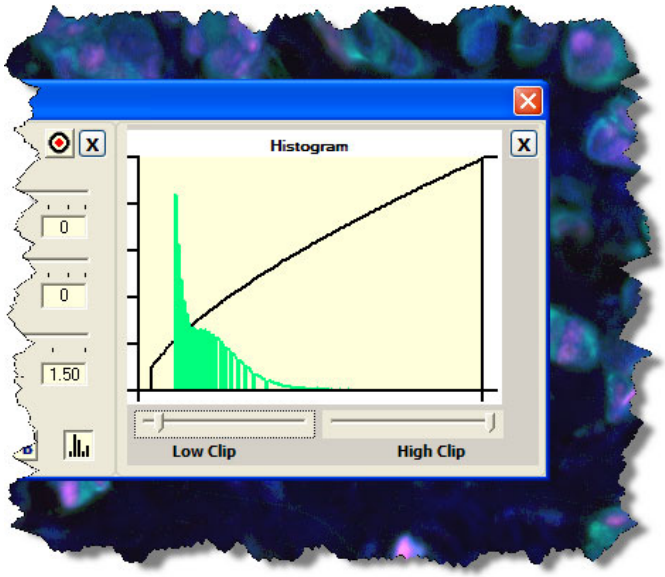


The **Low Clip** and **High Clip** sliders allow you to set thresholds. For example, the Low Clip slider sets a lower threshold point—if any pixels fall below that value they are forced to zero. This is often used to eliminate noise (the "noisy" pixels that do not convey real information are simply forced to black). The High Clip slider sets an upper threshold point—if any pixels fall above that value they are forced to the maximum value (white).

For example, in the image shown above, we adjust the gamma on the Cy3 channel to darken the midtones:



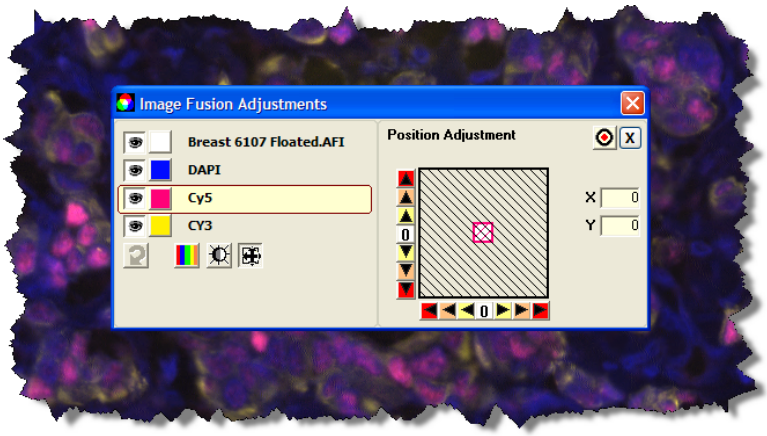
Now we move the Low Clip slider to eliminate noise in the lower intensities:



Adjusting Registration

Fluorescence images acquired on the ScanScope FL using the quad multi-bandpass filter cube should be perfectly registered. Images acquired using single-bandpass filter cubes or images imported from other sources may require registration changes so that the channels are aligned correctly.

To see the registration pane, click on the Image Fusion Adjustments window.



Select the channel you want to register. (In the example above, Cy5 is selected.)

- Use the arrows to move the image pixel by pixel in the X or Y axis. The arrows closest to the center of the arrow strip move the image by one pixel, the next arrows move it by 10, and the arrows at the end of the arrow strip move it by 100 pixels.
- Click the 0 at the center of the arrow strip to move the image back to its original position on that axis.
- The colored box in the middle of the display shows the position of the image relative to its original position. That is, the box starts in the center and moves as you use the arrows. The color of the border of the box tells you which channel you are working with. (In the example above, the red border is the same color used for the channel Cy5, so we know we are working with that channel.)
- The movement of the image in the X/Y axes is shown in the X and Y boxes.

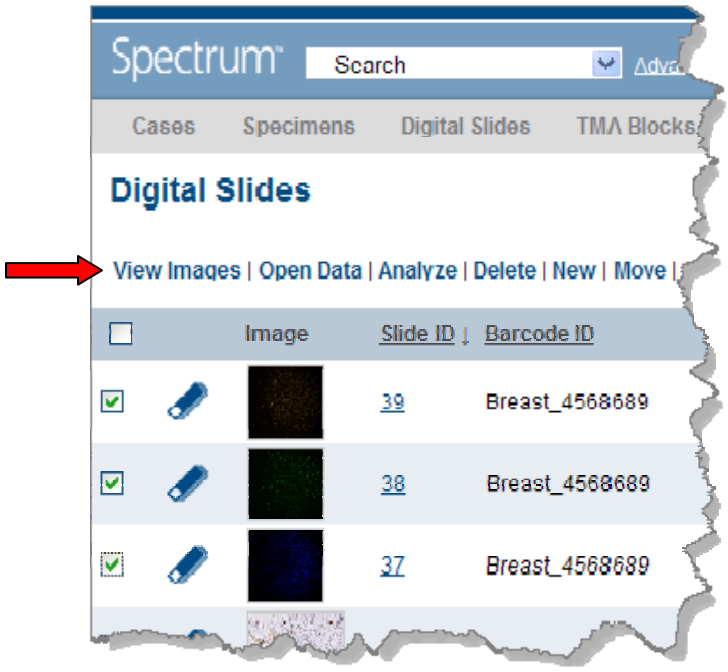
Fusing Fluorescence Images

The ScanScope creates a multi-layer "fused" image made up of the individual channel images. However, you can also use ImageScope to create your own fused image or create one from individual channel images you received from another source.

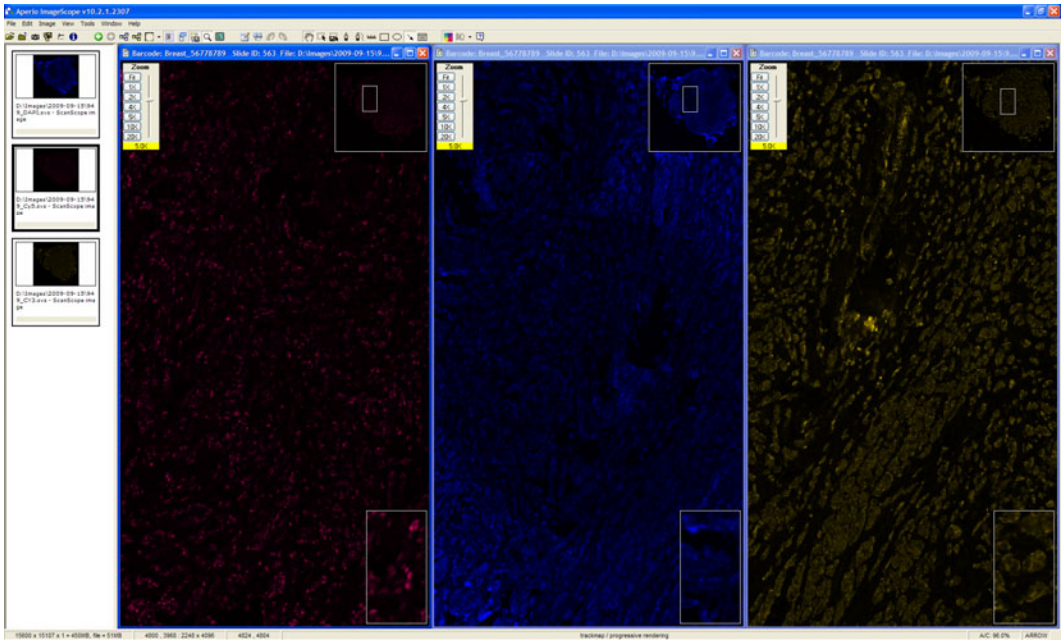
Our example shows opening images in Spectrum.

1. Log into Spectrum and select **All Digital Slides (As List)** from the Digital Slides menu.

2. Select the check boxes next to the channel images you want to use to create a fused image and click **View Images**:



ImageScope opens each of the selected images:

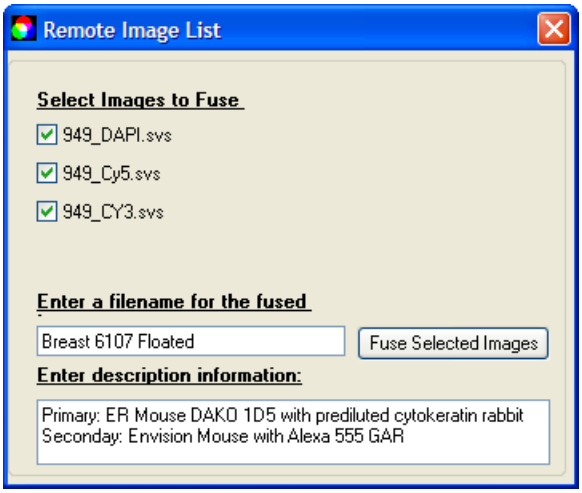


In this example, we have three images, one for each type of fluorochrome used to stain the slide: Cy5, DAPI, and Cy3.

If you want ImageScope to keep each of these images open after it creates the fused image, click the first image to select it, go to the Image

menu, and select **Keep Open** (or type Control K). Repeat for the other images.

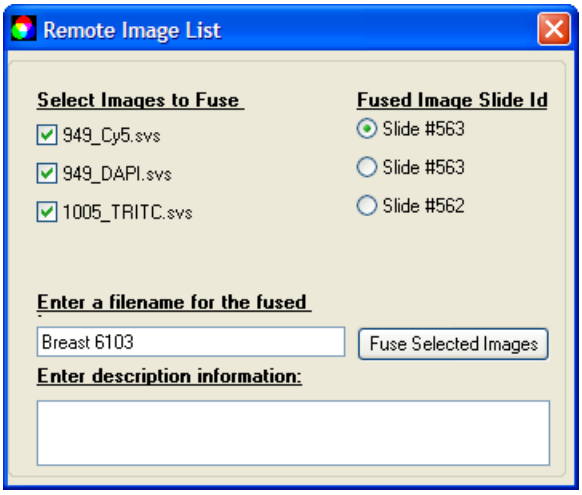
3. On the ImageScope menu bar, go to the Image menu and select **Fuse Images**.
4. On the Remote Image List Box, select all channel images that make up the fluorescence scan:



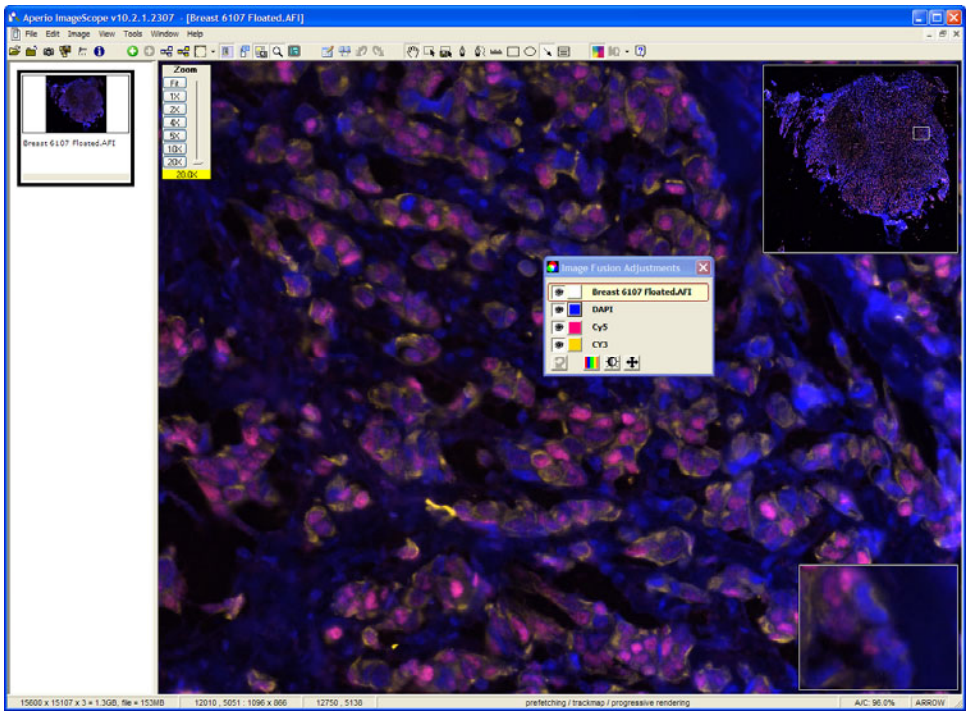
This window does not ask you for a file name or description if you opened the images directly from your local workstation or network location as ImageScope automatically assigns a file name based on the base file name of the channels when working with local images.

5. Type a file name to use for the AFI file and, optionally, a description.

   If the channel images belong to more than one digital slide, this window asks you to choose which digital slide to add the fused image to.



6. Click **Fuse Selected Images**. This creates an AFI. ImageScope opens the AFI and displays it in the main window:



When you close ImageScope and return to Spectrum, you see the AFI listed in the digital slide list:

The AFI is indicated by the  symbol.

8
Image Resolution

This chapter discusses how to see the resolution of an image and, if it is not known, how to set it.

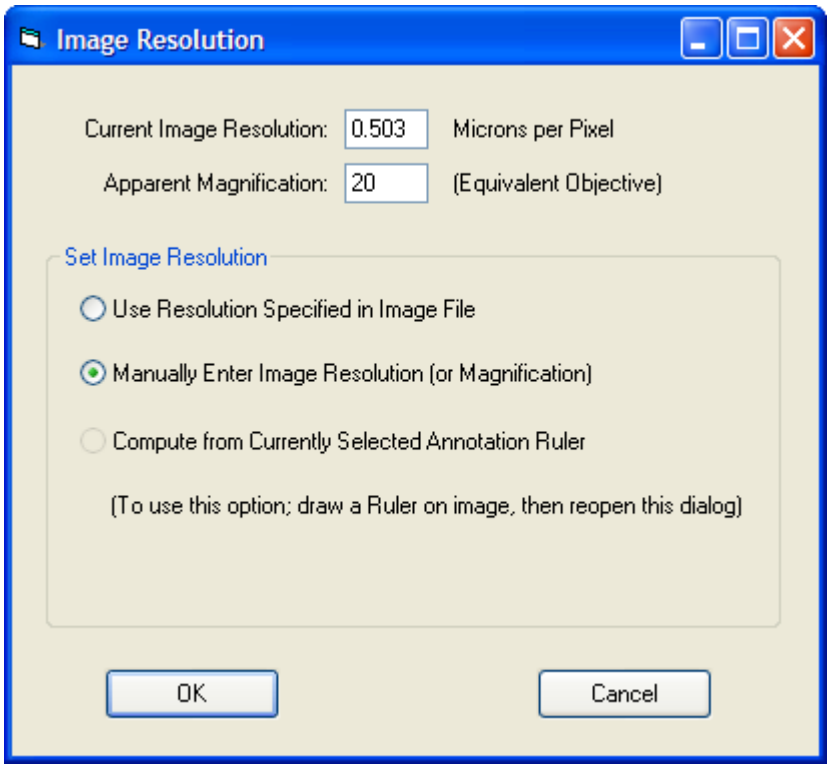
For digital slides created by scanning microscope slides on a ScanScope scanner, the resolution of the image is known and is stored in the image file.

The resolution may not be known for other types of images you are working with.

It is useful to know the resolution of an image because the resolution is used to display the magnification in the zoom slider, to compute ruler values, and to compute the length and area of annotation regions.

To view and set an image's resolution:

1. Go to the Image menu and select **Resolution.** The Image Resolution window appears:



If the resolution of the image is stored within the image file, **Current Image Resolution** and **Apparent Magnification** will contain values—if the resolution is not known, these boxes will be empty.

Setting or Changing Image Resolution

You can use the options on this window to change or set the image resolution:

- **Use Resolution Specified in Image File –** If the resolution is stored in the image file (for example, it was created by scanning a glass slide on a ScanScope scanner), select this option to reset the resolution to the value stored in the file.
- **Manually Enter Image Resolution (or Magnification) –** If you know the resolution for the image, select this option and type the image resolution and apparent magnification values in the boxes at the top of the window.
- **Compute from Currently Selected Annotation Ruler –** You can determine the resolution of the image from an object of known size in the scanned image. See the next section.

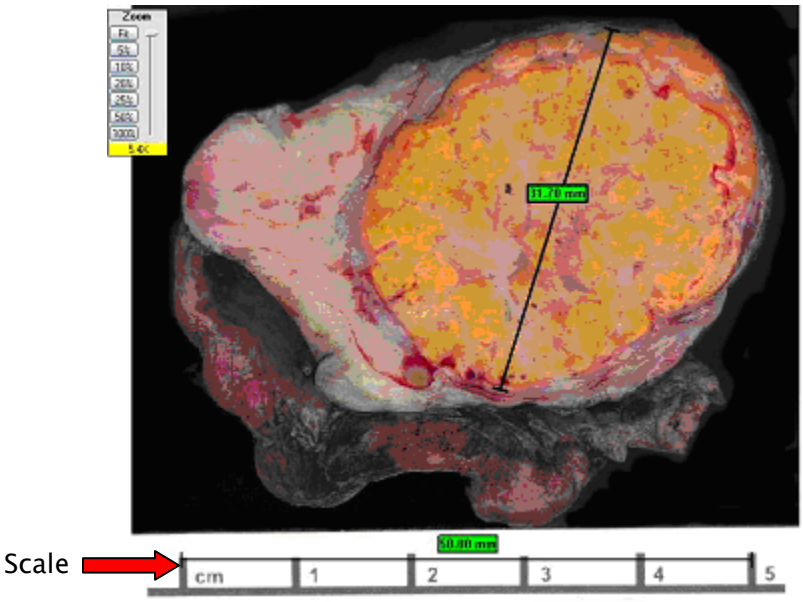
When you change the resolution, note that you only affect the way an image is viewed, but do not change the image file or information stored in it.

Because the resolution is used to display the magnification in the zoom slider, to compute ruler values, and to compute the length and width of annotations, these values will all change when you change the resolution.

Computing the Resolution from the Image

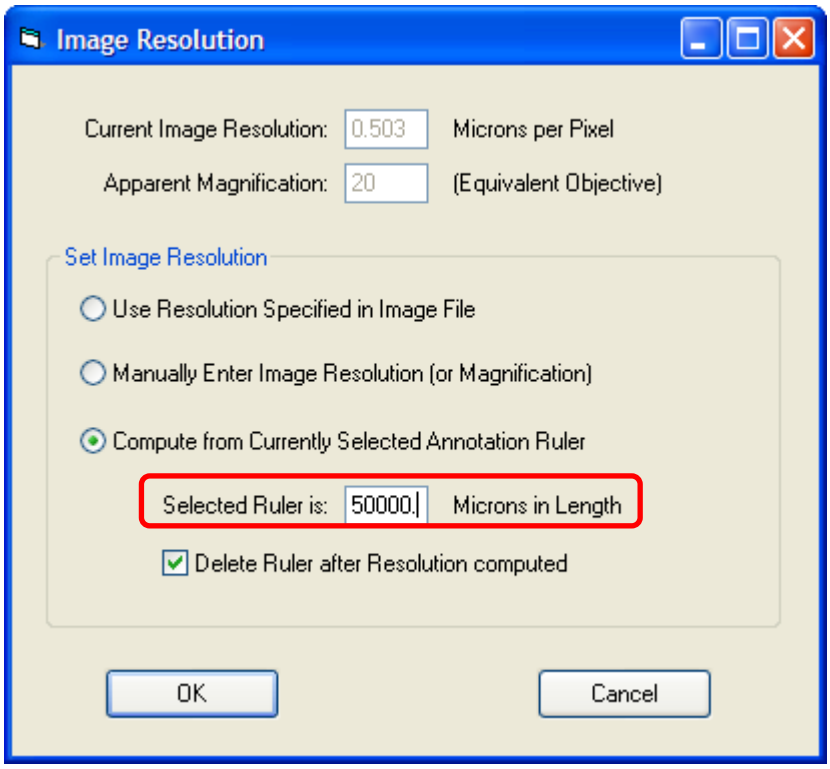
If you select the **Compute from Currently Selected Annotation Ruler** option in the Image Resolution window, you can use an object of known size in the image to calibrate the image resolution.

For example, in the following image a scale is part of the picture:



1. Draw an annotation ruler across the scale, which in this case shows a length of 5 centimeters.

2. Go to the Image menu and select **Resolution**. The Image Resolution window now has a place for you to enter the length of the ruler:



3. On the Image Resolution window, select **Compute from Currently Selected Annotation Ruler** and enter the length of the ruler in microns. In this case, enter a value of 50000 (5 cm = 50000 microns). If you want to delete the ruler after calibration, select the **Delete Ruler after Resolution Computed** check box.

Now all values measured by rulers will be accurately calibrated to this scale, such as the second ruler shown above which measures a length of 31mm across the specimen.

9
Annotating Digital Slides

Annotations direct viewers' attention to interesting elements of a digital slide. This chapter contains basic information on creating annotations. See the next chapter for advanced annotation information on moving and deleting annotations, using annotation layers to organize annotations, and storing algorithm analysis* results.

Annotations can define a region of the digital slide you are interested in, measure an object, or point to an interesting area. You also use annotations to define the areas of a digital slide on which to perform an algorithm analysis* or to define the areas on which *not* to perform an analysis*.

In the next chapter you will learn how to add text to an annotation so the text appears in the main window. To avoid having the text display on *top* of the annotation, when you create an annotation, finish drawing it at the upper right corner of the annotation.

To add an annotation to a digital slide, use the drawing tools on the ImageScope toolbar:

 Ellipsis (or circle if you hold down the Shift key while drawing)

 Rectangle (or square if you hold down the Shift key while drawing)

 Free-form shape

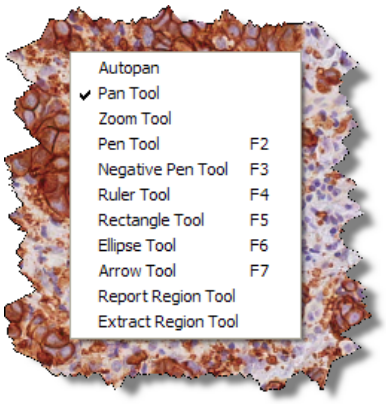
 Free-form shape to exclude from analysis* (negative pen)

 Arrow

 Measurement

 Select a Spectrum Plus report image

Note that you can also select these tools by right-clicking on the ImageScope main window and selecting the tool from the context menu:



Drawing Fixed Size Annotations

If you have predefined a fixed size, to draw an annotation of that size, hold down the Control key while you draw the annotation. (This works with the rectangle, ellipse, arrow, report image, and ruler annotations.)

See “Fixed Size Annotations” on page 180 and “Report Image Options” on page 185 for information on setting a fixed size for annotations.

Drawing Annotations with a Fixed Aspect Ratio

Drawing an image in a specific aspect ratio (or capturing an image in a fixed aspect ratio) can be useful when preparing an image for a publication.

To draw an image that uses the same aspect ratio as the fixed size you have defined for annotations, hold down both the Shift and the Control keys while you draw the annotation. For example, suppose you want to make sure all your annotations have a width/height ratio of 4:3. Specify a fixed annotation size of width of 400 pixels and height of 300 pixels. Then when you draw an annotation while holding down the Shift and Control keys, it will always have a ratio of 400 to 300 (4:3), regardless of its size.

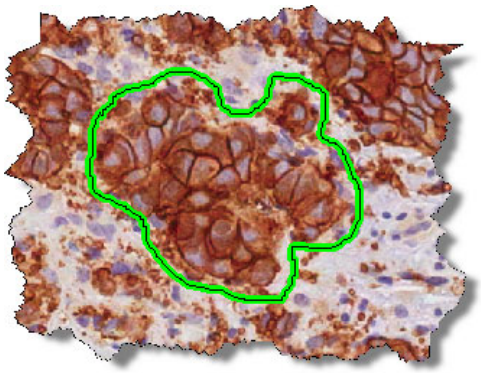
You can also use the Shift and Control keys to extract a region of a specific aspect ratio. See “Extracting an Image of a Predefined Size or Aspect Ratio” on page 114.

To set a fixed size for annotations (and thus a fixed aspect ratio), see “Fixed Size Annotations” on page 180.

Free-form Drawing

To draw a free-form shape on a digital slide:

1. Click on the toolbar.
2. Begin drawing in the main window by clicking and dragging. You see a colored line following the pen, as shown below. Release the pen when you have finished the shape.

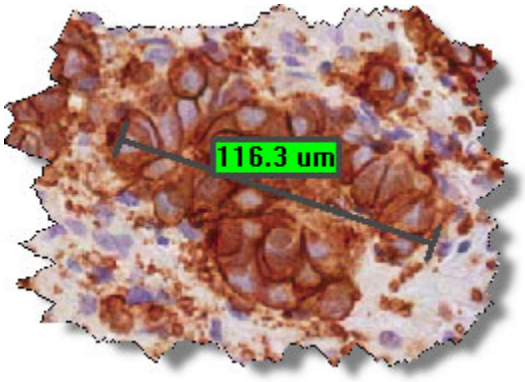


Measuring Objects

Use the ruler tool to measure an object on the digital slide.

The ruler tool may not work for all images you can view with ImageScope. Images that were not created by the ScanScope scanner may not have enough information included in them to allow the ruler tool to measure. (For instructions on setting resolution for images whose files do not contain resolution information, see Chapter 8, "Image Resolution," on page 71.)

1. Click on the toolbar.
2. Place your cursor on the object you want to measure in the main window and click while dragging the ruler over the object. Here is a sample display:



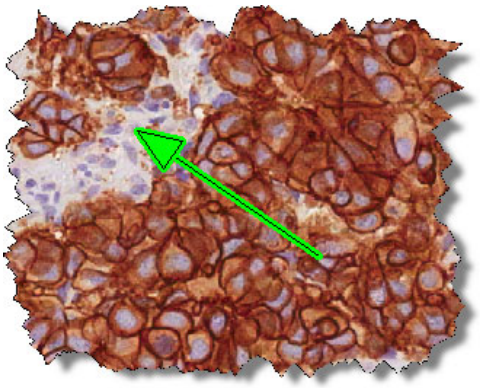
The ruler automatically adjusts for the current display resolution. If you change magnification, the ruler stays in the same location relative to the image when you zoom. When you measure with the image displayed at lower magnifications,

such as 2x, the ruler displays in millimeters rather than microns as it is measuring something in a larger scale.

Drawing an Arrow

To draw an arrow to direct someone's attention to an interesting feature of the digital slide:

1. Click on the toolbar.
2. Place your cursor on the image in the main window close to the object you want to point at, and click and drag away from it. The further you drag, the larger the arrow appears.



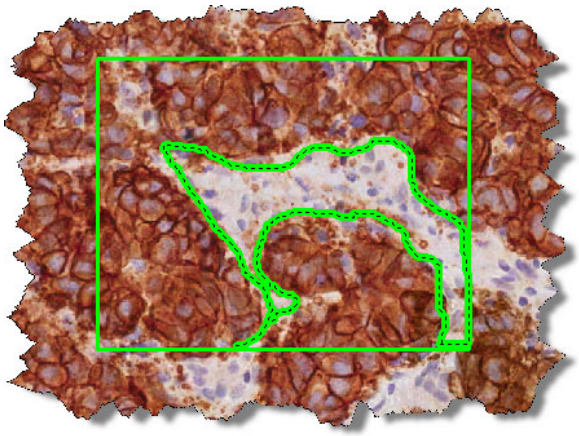
Drawing with the Negative Pen

The negative pen tool is used to draw negative free-form shapes. These are used in algorithm analysis* to designate areas *not* to analyze.

To draw a negative shape on the digital slide:

1. Click on the toolbar.

2. Place your cursor on the image in the main window and begin drawing by clicking and dragging. Release the mouse button when you have finished. A negative region is indicated on the display by a dashed line.



In this case, we used the rectangle tool to draw a boundary around the region we want to analyze, and used the negative pen to draw an area within the region that we want to exclude from the analysis.

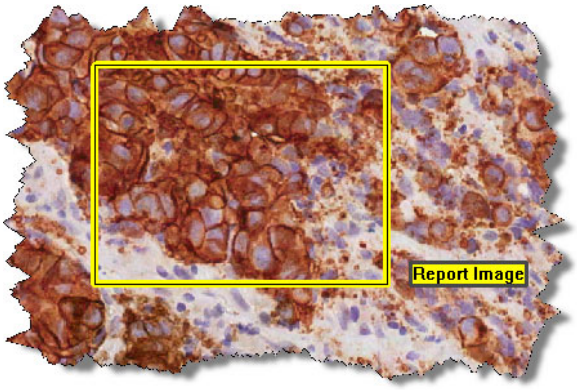
Selecting a Spectrum Plus Report Image

ImageScope allows you to select a specific image from a digital slide or specimen to be used in a report that includes that image. (This feature is used by the optional Spectrum Plus Reporting product.)

With a digital slide or specimen image open in ImageScope, click the  icon on the ImageScope toolbar and then click the area of the image that you want to appear in a report. The area selected is saved as an annotation with the text label "Report Image." Only one report image can be selected for a single image, and the report template you are using must contain images in order for this image to appear.

To draw an area of a fixed size, hold down the Control key while you draw. See "Report Image Options" on page 185 for information on setting the fixed size of a report image.

The report image appears on the image in the ImageScope window if you have annotation view enabled. As with all annotations, you can delete it or move it.



Panning While Annotating

If you need to draw an annotation that is larger than the current window in ImageScope, you can pan through the image while drawing, allowing you to draw the annotation on the entire image. Simply drag the annotation drawing tool—as you reach the edge of the image in the ImageScope window, the image will move in the direction you are moving your cursor to allow you to continue drawing.

For More Information

- See Chapter 10, "Using the Annotations Window," on page 81 for information on other things you can do with annotations:
  - Add text to an annotation
  - Delete or move an annotation
  - Export or import annotations
  - Define annotation attributes to add information to an annotation.
- For information on creating viewing sequences, see Chapter 11, "Linking Annotations and Digital Slides," on page 99.
- When analyzing* digital slides, you can use free-form or rectangle annotations to define what areas to analyze or use the free-form negative pen tool to define what areas *not* to analyze. See Chapter 14, "Analyzing Digital Slides," on page 119 for details.

* Aperio's image analysis algorithms are FDA cleared for specific clinical applications, and are intended for research use for other applications.

10 Using the Annotations Window

Annotation layers are useful for organizing annotations and for storing algorithm analysis* results. The ImageScope Annotations window shows you the annotations associated with the image.

ImageScope stores annotations in layers. You can use layers to organize annotations by reviewer or department so that each person's or group's annotations are on a different layer, and then:

- Hide or show specific layers for different uses of the digital slide.
- Delete only a specific annotation layer.

Annotation layers are also where algorithm analysis* results are stored as quantitative data (see Chapter 14, "Analyzing Slides," on page 119).

Annotations made on different layers are drawn in different colors. See below for information on changing annotation layer colors.

To open the Annotations window go to the View menu and select **Annotations** or type Control N. Now you can use the Annotations window in one of two modes:

- **Summary View** – Designed for use with the Digital IHC product to provide one-step annotation and analysis for IHC digital slides. However, this view can also be used for quick analysis of any type.
- **Detailed View** – Complete details on all annotations are available and you can add annotation attributes, text labels, and so on.

The Annotations Summary View Window – Quick IHC Analysis

The ImageScope detailed Annotations window provides a general solution for image analysis* and handling annotations. However, a more streamlined version of the Annotations window is also available—the Annotations *summary view*. The Annotations summary view was specifically developed for analyzing* IHC digital slides and makes the process quicker and simpler by fitting into a pathologist's or researcher's normal workflow.

For details on using the Digital IHC features and on Digital IHC setup steps for Spectrum, see:

- *Digital IHC User's Guide*
- *Digital IHC Guide to Spectrum Setup*
- The user's guide for the specific IHC application you are using.

Slide-Specific Processing

The key to the Digital IHC workflow is *slide-specific processing*, which defines how a digital slide will be processed based on its stain and type of tissue (body site). The slide-specific processing can define what algorithm* will be used to analyze* that type of slide, how analysis results will be displayed and how to interpret those results (alternatively, manual scoring can be set up for the slide), and what comments will be available to be used by the pathologist or researcher viewing the slide.

The slide-specific configuration for each stain/body site combination is defined by the Spectrum administrator. See the *Digital IHC Guide to Spectrum Setup* for details.

Once slide-specific processing is set up, viewing, annotating, and analyzing* a digital slide becomes a quick process that takes just a few mouse clicks.

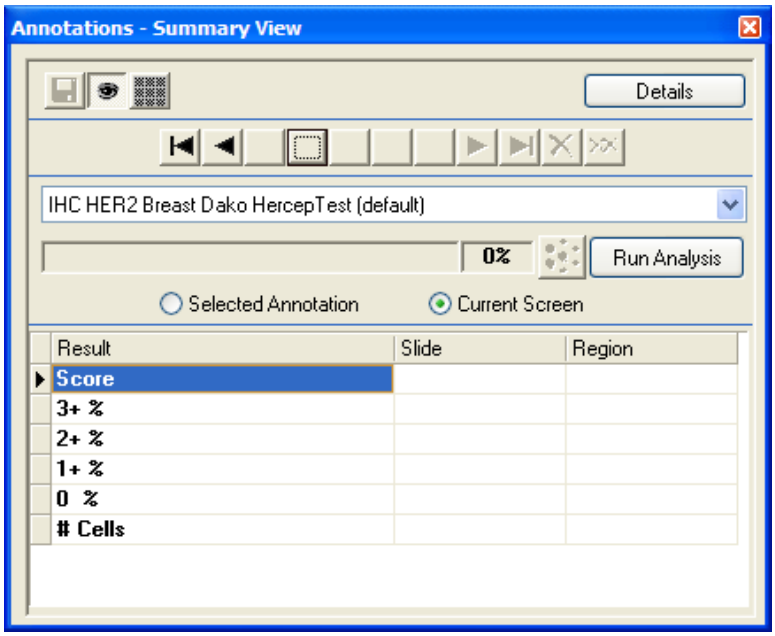
The summary view of the Annotations window is designed specifically for working with IHC digital slides to provide a quick way to mark tumor regions and analyze* them in one simple step.

Using the Annotations Summary View Window

To open the Annotations window in summary view:

1. Identify a digital slide in Spectrum for which stain/body site slide-specific processing has been defined.

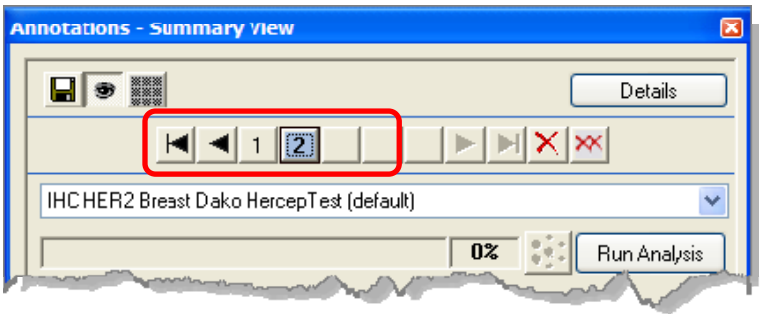
2. From the Spectrum page, open the digital slide in ImageScope by clicking its thumbnail. The Annotations window in summary view appears. (If the window does not look like this, click the **Summary** button to return the Annotations window to the summary view):



The algorithm* appropriate for this type of slide is listed in the drop-down box. You can select another algorithm* if you wish from that box. Note that if you are in clinical viewing mode, the summary view Annotations window is the only view you can see.

From this window you can draw annotations to identify areas to analyze* and run the analysis* all in one easy operation:

1. With the algorithm* that you want to use shown in the drop-down list, use the ImageScope pen or rectangle drawing tools to draw the areas of the digital slide you want to analyze*.
2. To navigate between annotations you have drawn, use the numbered buttons or arrow keys. (As you draw annotations with the analysis algorithm* shown in the drop-down box, the buttons at the top of the window display a number for each annotation.)



a) Click the numbered button that corresponds to the annotation you want to see. That annotation is centered on the ImageScope window.

b) Move between the annotations by using the arrow keys.

c) To delete the currently selected annotation click . To delete all annotations, click .

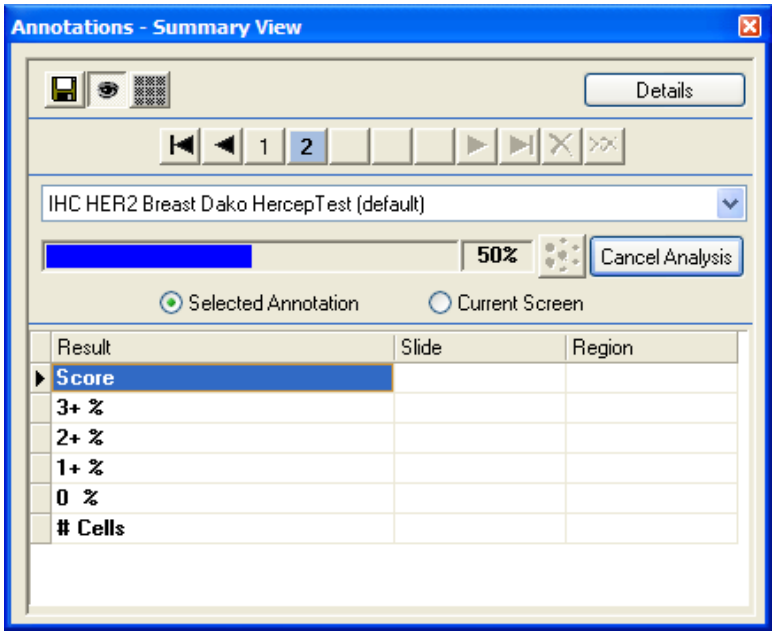
d) To hide the selected annotation on the ImageScope window, click .

e) To save the annotations to Spectrum, click .

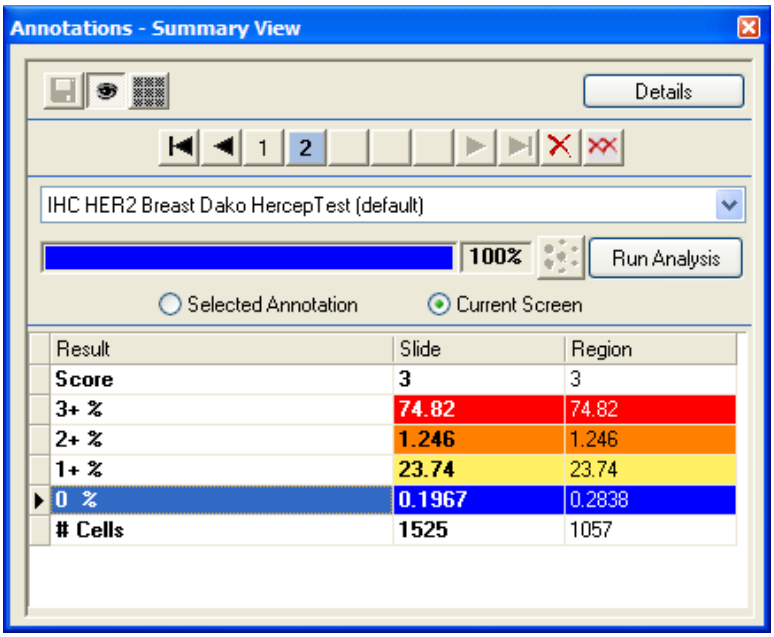
3. To analyze* the current digital slide with the algorithm* shown in the drop-down box:

a) Select the annotation drawn around the area you want to analyze* and select **Selected Annotation**. (Or, if you want to analyze* the entire area shown in the ImageScope window, select **Current Screen**.)

b) Click **Run Analysis**. As the analysis* runs, you see progress information:



When the analysis* is complete, you see the results:

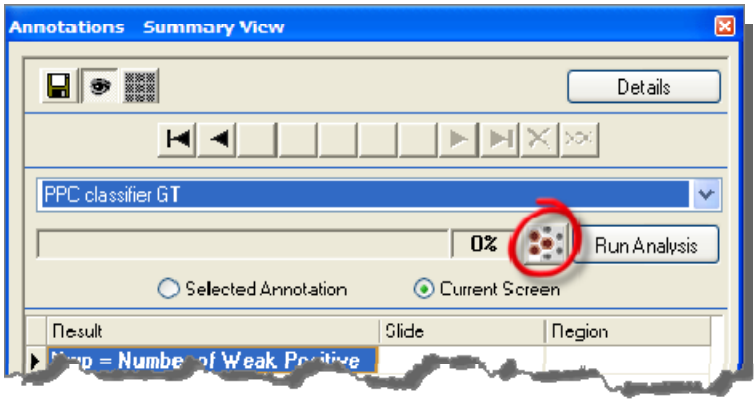


For more information on using the Annotations summary view window, see the *Digital IHC User's Guide*.

Enabling/Disabling Pre-processing

When using the Annotations window summary view, you may temporarily turn off pre-processing region finding for algorithms that support that feature (for example, if you want to select possible tumor areas yourself to analyze rather than allowing the algorithm to do it for you).

To disable pre-processing, open the Annotations window in summary view and click the pre-processing button:



To turn pre-processing on again, click the button again. Note that this button is only enabled when you are using an algorithm that supports pre-processing.

Incremental Processing

The IHC analysis applications are *incremental* algorithms, which means that as you add new regions and click **Run Analysis** on the Annotations window, only the new regions are analyzed, which can save a great deal of time. Any time you click **Run Analysis** again, all analysis results are updated.

Incremental processing is useful when a pathologist draws a single region and analyzes it, and after reviewing the analysis results wants to select additional regions and analyze them as well. The pathologist can add more annotation regions as needed or delete annotation regions and the analysis results will be updated accordingly.

If you delete a region, ImageScope will automatically re-run the analysis to update the summary analysis results that included that region. However, adding regions may make the analysis results incorrect until you re-run the analysis.

Other Options

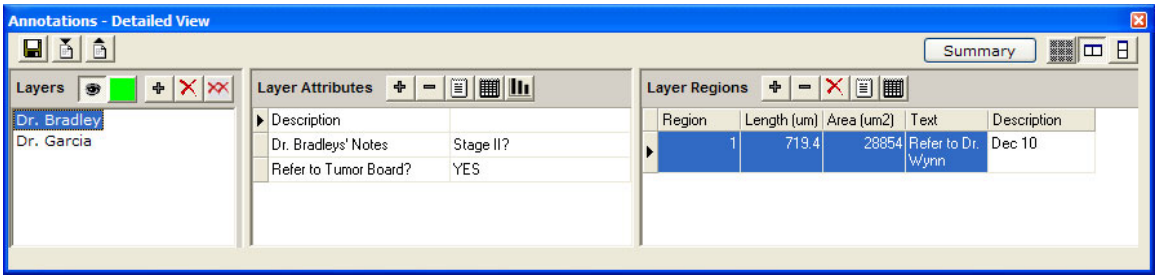
- To see a report image you have selected, select *Report Image* from the drop-down list.
- To create an annotation that will not be used for analysis* (for example, a ruler or arrow), select *Annotations* from the drop-down list before drawing.
- If the algorithm you are using support result plots and they are enabled, the summary view window automatically opens all plots in separate windows when the analysis is done.

The Annotations Detailed View Window

To see information on the annotation layers attached to a digital slide and to work with those layers:

1. Open the digital slide you want to work with.
2. Go to the **View** menu and select **Annotations**.

The following Annotations window shows a digital slide for which two layers have been created, "Dr. Bradley" and "Dr. Garcia". (If the window does not look like this, click the **Details** button to return the Annotations window to the detailed view)



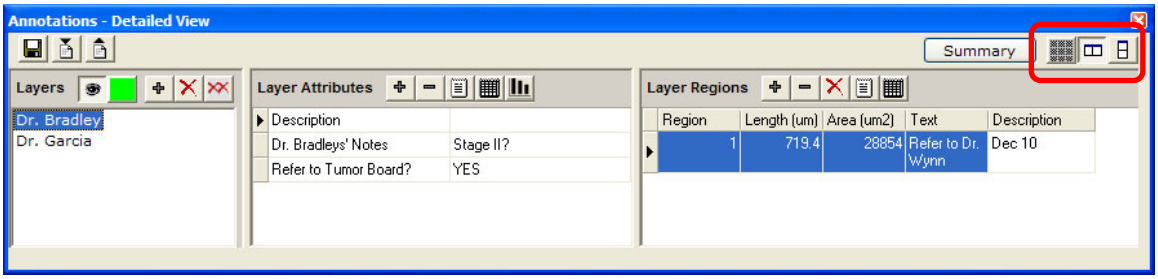
- The Layers pane on the left lists all defined layers for this digital slide. To select a layer to work with, click it in the list. See below for information on adding new layers, changing the annotation color for a layer, and deleting layers.
- The Layer Attributes pane in the middle is where you can add and delete attributes for a layer. See below for more information.
- The Layer Regions pane on the right is where you can add and delete attributes for an annotation. You can also select an annotation so you can delete or move it. See below for more information.

Here is a quick reference guide to the features of this window (we'll talk more about these features later in this section):

Icon	Location	Function
	Annotations window toolbar	Arrange the Annotations window panes side by side, horizontally.
	Annotations window toolbar	Arrange the Annotations window panes vertically.
	Annotations window toolbar	Make Annotations window transparent so digital slide shows through.
	Layers pane	Change the color of the annotations in the selected layer. (This box shows the current color for the layer annotations.)
	Layers pane	Hide or show the annotations on the selected layer in the ImageScope main window.
	Annotations window toolbar	Save all annotations with the digital slide.
	Annotations window toolbar	Import annotations from a previously exported annotation file.
	Annotations window toolbar	Export the current annotations to an annotation file. Saved as XML with the same name as the digital slide file.
	Layer Attributes pane Layer Regions pane	Export the contents of the pane to a text file.
	Layer Attributes Pane	Display algorithm result plots.
	Layer Attributes pane Layer Regions pane	Export the contents of the pane as an Excel spreadsheet.
	Layers pane Layer Attributes pane Layer Regions pane	Add: New layer (if on the Layers pane) New layer attribute (if on the Layer Attributes pane) New annotation attribute (if on the Layer Regions pane)
	Layer Attributes pane Layer Regions pane	Delete: Layer attribute (if on the Layer Attributes pane) Annotation attribute (if on the Layer Regions pane)
	Layers pane Layer Regions pane	Delete: Selected layer (if on the Layers pane) Selected annotation (if on the Layer Regions pane)
	Layers pane	Delete all layers.

Configuring the Annotations Window

There are several ways to configure the Annotations window to suit your preferences:



- Click  to arrange the panes side by side, horizontally.
- Click  to arrange the panes vertically.

You can also:

- Click the boundary between panes and drag it to expand or shrink the pane width.
- Within a pane, click the line between columns and drag it to expand or shrink the column.
- Drag any outside boundary of the Annotations window to expand or shrink the overall window.
- Click the transparent button  to make the Annotations window transparent so the digital slide shows through it.

Hiding or Showing Annotation Layers

To make an entire layer visible or invisible on the ImageScope main window:

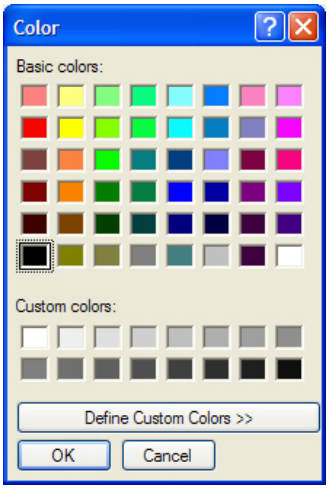
1. Click a layer in the Layers pane to select it.
2. Click  . (When this button is pressed down, the layer is visible; when it is raised, the layer is not visible).

Changing the Annotation Layer Color

All annotations created in a layer are displayed in the same color.

To change the default colors used for each layer:

1. Go to the ImageScope **Tools** menu and select **Options**.
2. Click the **Annotations** tab and click the color of the layer you want to change; a color selection window appears on which you can select the color you want to use.

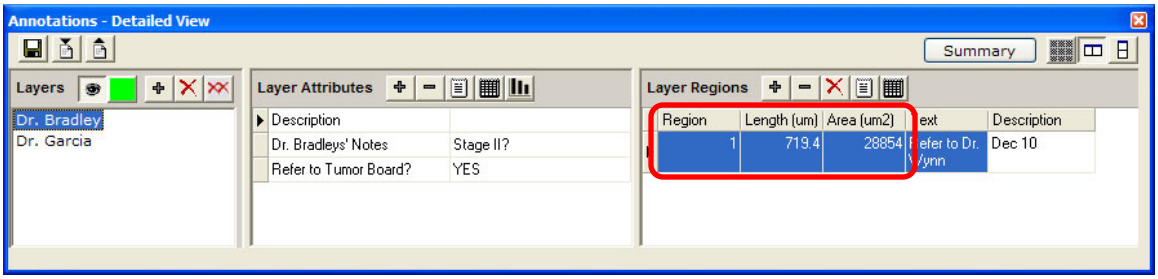


To change the color used for a specific layer:

1. In the Annotations window, click a layer in the Layers pane to select it.
2. Click  to open a color selection window from which you can select the color you want to use. All annotations in that layer immediately change to the new color.

Annotation Length and Area Display

ImageScope measures and displays length and area for annotation regions. If the resolution of the image is known, length and area are displayed in microns; if not, they are displayed in pixels.



It is possible to draw an annotation in such a way that text defined for it appears on top of the annotation. To avoid this, when you create annotations finish drawing the annotation at the upper right corner of the annotation so that text will be added outside the annotation.

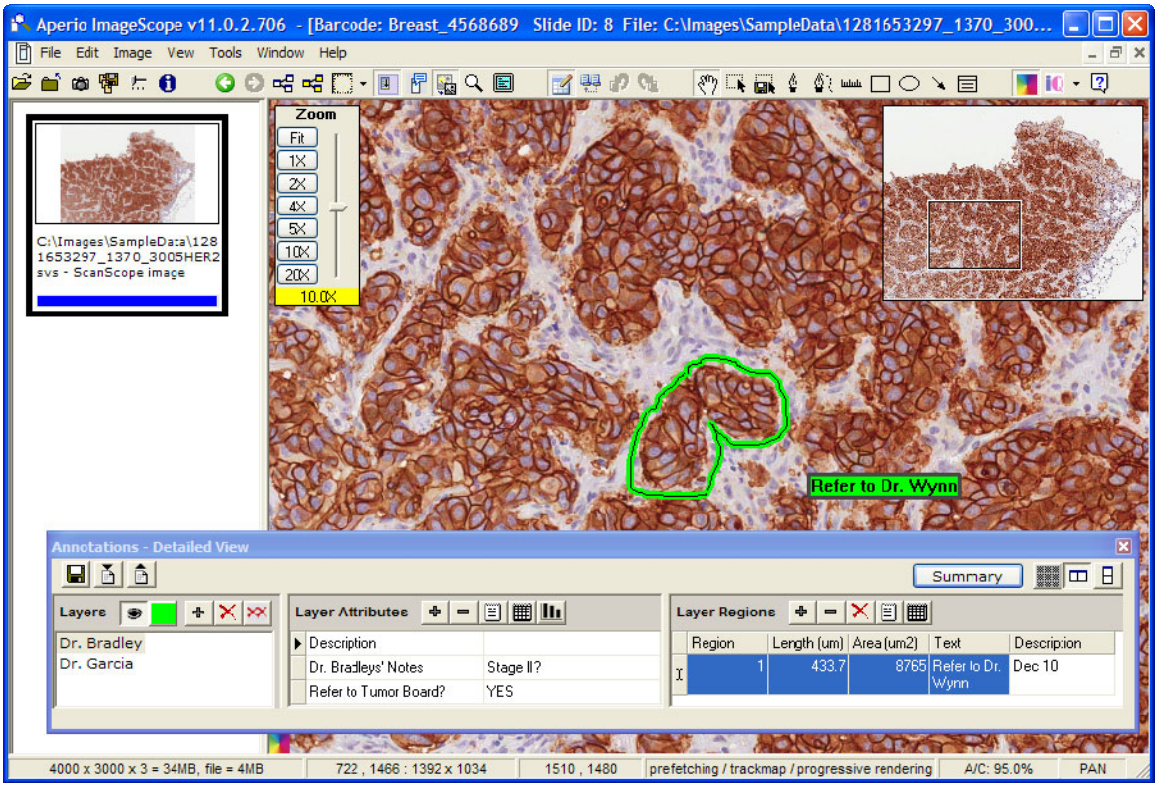
Adding Text to an Annotation

You can add a text note that will appear with an annotation on the ImageScope screen. The note will appear above and to the right of the point on the image where dragging stopped when the annotation was created.

To add a text note:

1. In the Layers pane, click the layer that contains the annotation for which you want to add a note.
2. Click the annotation in the Layer Regions pane to which you want to add text.

3. Type the note into the **Text** column. You can see the text note on the main window as well as in the Layer Regions pane of the Annotations window.



Adding and Deleting an Annotation Layer

To create a new layer in the current digital slide:

1. Click  in the Layers pane.
2. To change the name of the layer, click the name and type a new name.

To delete a layer in the current digital slide:

1. Click a layer in the Layers pane to select it.
2. Click  to delete the selected layer.

To delete all layers:

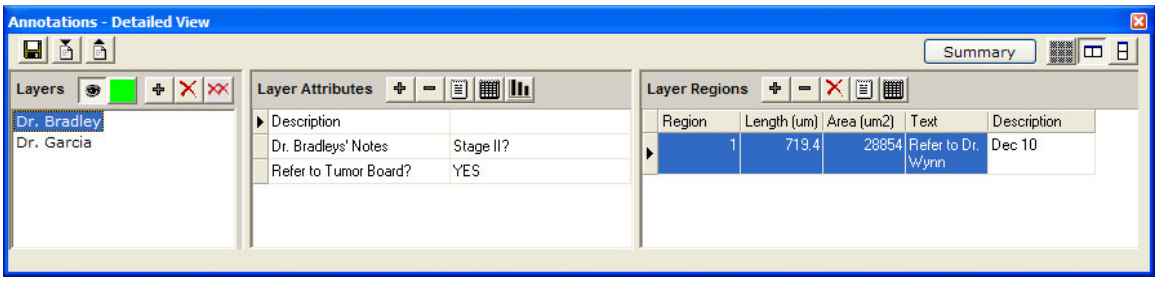
1. Click  in the Layers pane to delete all layers.

Deleting Annotations

To delete an annotation either click it in the main ImageScope window to select it and then press the Delete key on your keyboard, or:

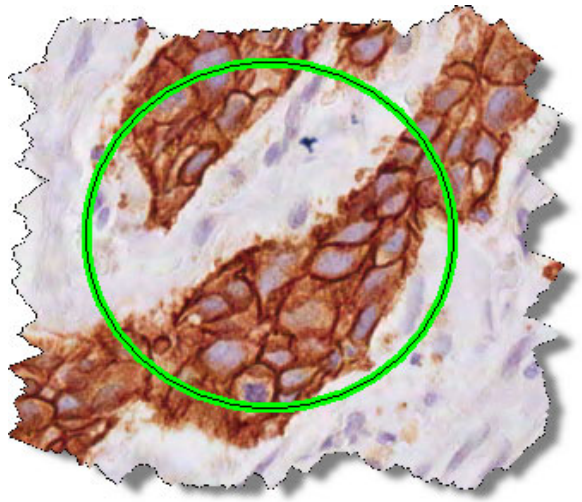
1. Go to the **View** menu and select **Annotations.**

2. Select the layer in the Layers pane that contains the annotation you want to delete by clicking it.
3. Then click the annotation in the Layer Regions pane that you want to delete to select it and click in the Layer Regions pane to delete it.

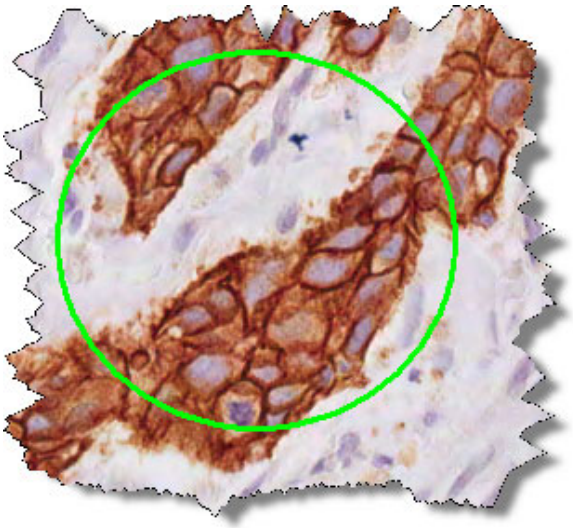


For example, to delete annotation 1 in the above example, we clicked **Dr. Bradley** in the Layers pane to select that layer, clicked **1** in the Layer Regions pane to select that annotation, and then clicked in the Layer Regions pane to delete it. Note the blue bar in the example above, which indicates the annotation has been selected.

On the ImageScope main window:



A selected annotation looks like this, with a dark line in the middle of the annotation boundary.



An *unselected* annotation looks like this.

Moving Annotations

You can move a single annotation or all annotations.

Moving a Single Annotation

To move a single annotation:

1. Click the annotation on the ImageScope window to select it. (See the section above for what a selected annotation looks like on the ImageScope main window.)
2. While holding down the Control key, grab the selected annotation and drag it to a new location.

Moving All Annotations at One Time

There are occasions when you want to move all annotations at the same time. For example, if you import annotations from another, similar digital slide, slight

variations in the new slide might require adjusting the position of the imported annotations. To move all annotations at one time:

On the ImageScope main window, while holding down the Control and Shift keys, drag an annotation to a new location—all other annotations will follow the motion of the one you are dragging.

All annotations in all layers will be moved except for Z-stacks† and markup images*.

Saving Annotation Layer Changes

To save any changes you made to the annotations, click  .

Exporting and Importing Annotation Layers

ImageScope provides several ways to export and import information and annotations.

Algorithm analysis* results are stored in an annotation layer so you may want to export that information into a text file to include it in a report or to chart the information in a spreadsheet program.

You can also export annotations to be used on other digital slides. For example, if working with several very similar digital slides, you may find the same annotations apply to all of them.

Importing and Exporting Annotations

To export all of the annotations for the current digital slide:

- On the Layers pane, click  . You will be asked to specify a name and location for the .xml file created.

To import an annotation file to the current digital slide:

- On the Layers pane click  . You will be asked to navigate to the location of a previously exported annotations file.

Exporting Text from Annotations Window Panes

To export the text of the Layer Attributes pane to a text file:

- On the Layer Attributes pane, click  . You will be asked to specify the name and location of the text file to be created. This text file is a tab-delimited file that can be imported into a spreadsheet program.

To export the text of the Regions Attributes pane to a text file:

- On the Regions Attributes pane, click  . You will be asked to specify the name and location of the text file to be created. This text file is a tab-delimited file that can be imported into a spreadsheet program.

Exporting Text from Annotations Window Panes to a Spreadsheet

Numeric data exported from the Annotations window to a spreadsheet is exported as text. Excel shows a warning note for each of these cells that the numbers are in text format—when you click on the note you can select an option to transform the text to numeric format.

To export the text of the Layer Attributes pane to a Microsoft Excel spreadsheet:

- On the Layer Attributes pane, click . You will be asked to specify the name and location of the spreadsheet .xls file to be created.

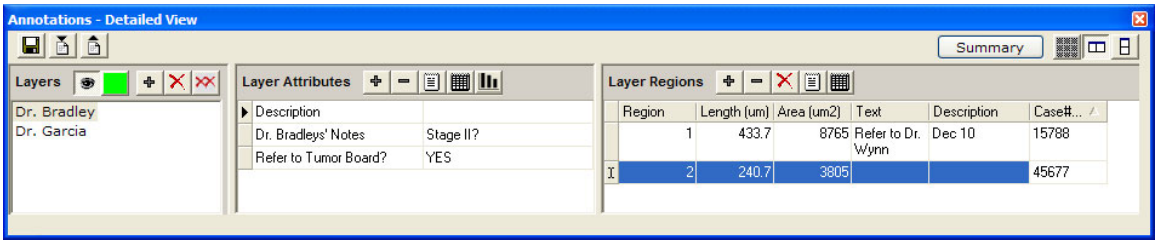
To export the text of the Regions Attributes pane to a Microsoft Excel spreadsheet:

- On the Regions Attributes pane, click . You will be asked to specify the name and location of the spreadsheet .xls file to be created.

Adding and Deleting Attributes

Attributes are text fields that describe the layer or annotation. Several attributes are already defined. For example, the Layers pane and the Layer Regions pane both contain a Description attribute. By typing text in this column, you can include comments or a description of the layer or annotation.

When multiple entries are defined for an attribute, you can sort the entries alphabetically by clicking the attribute title. In this example, we clicked **Case#** to sort the list of annotations by case number:

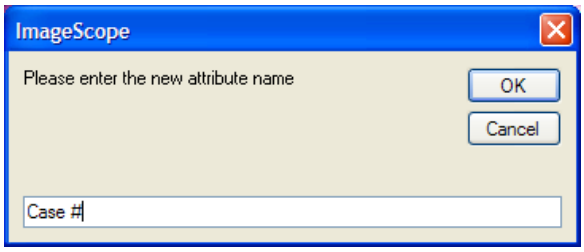


Adding Your Own Attributes

You can also add your own attributes (for example, slide routing information, test results, etc.). To add a new layer attribute:

1. On the Layers pane, click a layer to select it.

2. On the Layer Attributes pane, click [+] to add a new attribute. You will be asked to enter the name of the attribute. The new attribute is the title for a new column that contains a text field in which you can enter any data you wish.



To add a new annotation attribute:

1. On the Layers pane, click a layer to select it.
2. On the Layer Regions pane, click [+] to add a new attribute. You will be asked to enter the name of the attribute. The new attribute is the title for a new column containing a text field in which you can enter any data you wish.

Deleting Attributes

To delete a layer attribute:

1. On the Layers pane, click a layer to select it.
2. On the Layer Attributes pane, click an attribute to select it.
3. Click [–] to delete the selected attribute.

To delete an annotation attribute:

1. On the Layers pane, click a layer to select it.
2. On the Layer Regions pane, click an attribute to select it.
3. Click [–] to delete the selected attribute.

* Aperio's image analysis algorithms are FDA cleared for specific clinical applications, and are intended for research use for other applications.

† This application is not approved or cleared by the FDA for clinical use.

11 Linking Annotations and Digital Slides

Creating a viewing sequence by linking digital slides and/or annotated regions is a powerful way to organize and present information.

Here are two sample uses of linking annotations and digital slides:

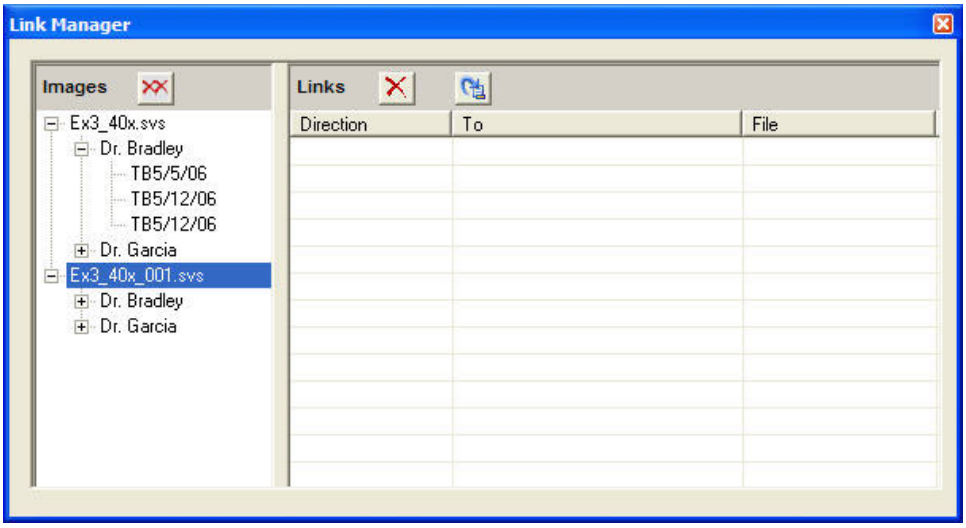
- Create a path of views through a slide or group of slides that takes the viewer on a tour of highlighted features.
- Create a hierarchy of images, perhaps from gross specimen down through blocks and then to slides.

Links have the following characteristics:

- Links can be associated with a region in a digital slide or with the entire image.
- Links have direction. If you traverse a link in the forward direction, you can follow the same link back.
- You can create any number of links into or out of a region or image.
- If there is more than one link to or from the currently selected annotation or slide, then the Link Manager window will open so that you can select a link.
- You cannot create links for layers, only for digital slides or annotations.

Working with the Link Manager

To start the Link Manager, go to the **View** menu and select **Annotation Links Manager**.



On the left side of the Link Manager window is a tree view of all digital slides that are open in ImageScope. The next level represents annotation layers, and the final level is annotations in that layer. For example, the first slide above, Ex3_40X.svs, contains two layers, "Dr. Bradley" and "Dr. Garcia." The "Dr. Bradley" layer contains three annotations, "TB5/5/06," "TB5/12/06," and "TB5/12/06."

Click the + symbols to expand the lists, and the – symbols to collapse them.

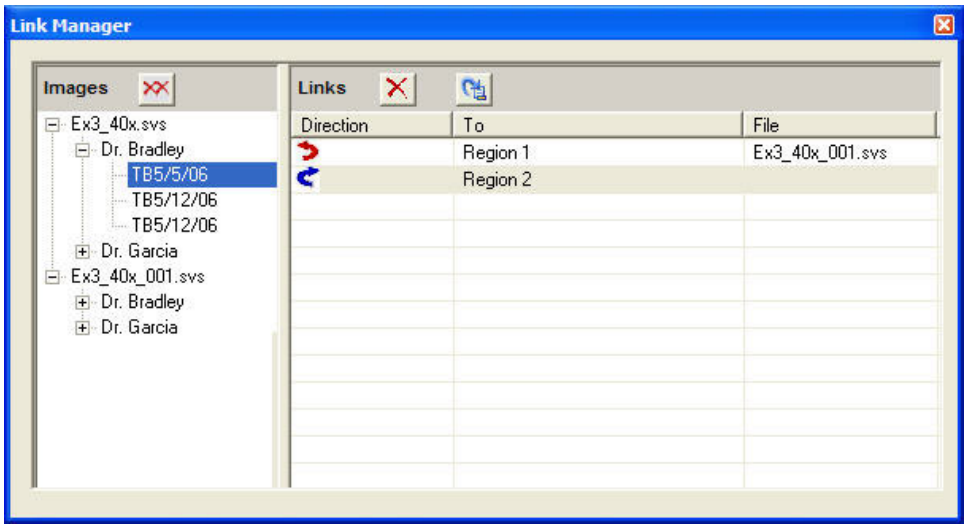
When you click a node in the tree, any links for that node will be displayed in the list on the right.

Creating a Link

To create a link:

1. In the left pane of the Link Manager window, drag a node and drop it onto another node.

For example, we dragged the first annotation on the Dr. Bradley layer onto the second annotation to create a link:



The  symbol indicates that the link is a forward link. The  symbol indicates the link is a backward link.

Viewing Links

The link navigation icons and commands are only enabled if a link exists. For example, if no previous link exists, you will not be able to use the **Previous Annotation Link** command.

You can follow links either on the main ImageScope window or from within the Link Manager window. (You do not need to have the Link Manager window open to follow links.)

If no annotation is currently selected, the viewing tools will follow slide-level links; if there is more than one link to or from the currently selected annotation or slide, then the Link Manager window will open so that you can select a link.

ImageScope displays the target of the link in its main window. If the target is a digital slide, then it will be fitted into the main window; if it is an annotation, then the annotation will be centered and zoomed.

To follow links in the main ImageScope window:

1. Open a digital slide that contains links.
2. To go to the next link, go to the **View** menu and select **Next Annotation Link**, or press Shift+F8, or click the  icon on the ImageScope toolbar.
3. To go to a previous link, go to the **View** menu and select **Previous Annotation Link**, or press Shift+F7, or click the  icon on the ImageScope toolbar.

To follow links in the Link Manager window:

1. Open a digital slide that contains links.
2. Go to the **View** menu and select **Annotation Link Manager** to open the Link Manager.

3. Click on the node for which links have been created and click  in the right pane of the Link Manager window to follow the link.

Deleting Links

To delete a single link:

1. In the Link Manager window, select a link in the right pane of the window and click  .

To delete all links:

1. In the Link Manager window, click  .

12 Tracking

A tracker tool provides a way to record your movements through a digital slide and to save that record (known as a *track*) with the image as an annotation layer.

Typical uses for the tracker tool are:

- Histologists and pathologists might want to use this tool to remind them what sections of the digital slide have been visited.
- The saved track might be useful for quality assurances purposes by providing permanent evidence of what sections of the digital slide were viewed.
- The saved track could be used for educational purposes to give students a tour of the digital slide.

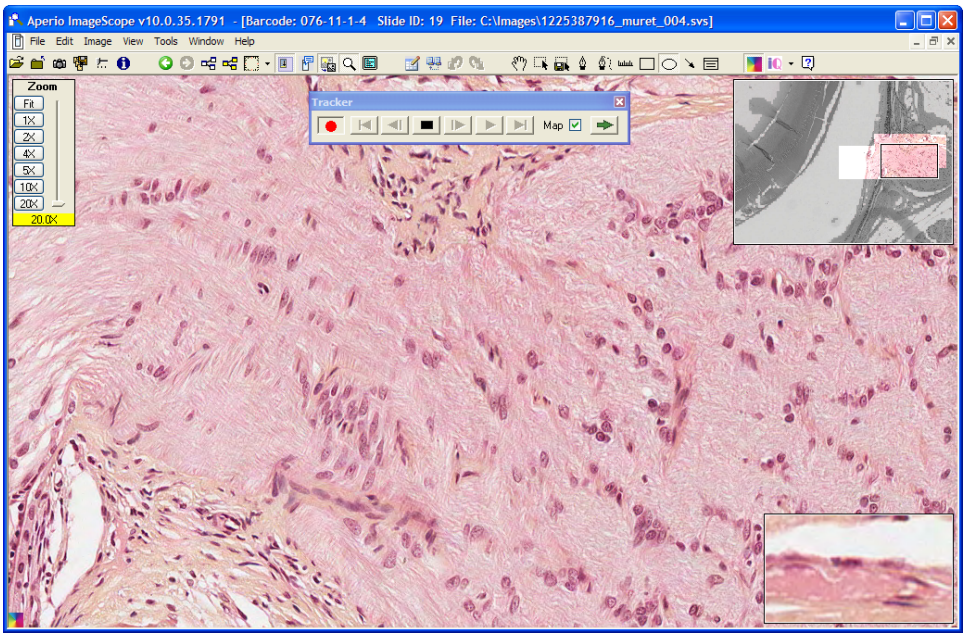
To turn on the tracker:

1. Go to the View menu and select **Tracker**. The tracker tool appears:

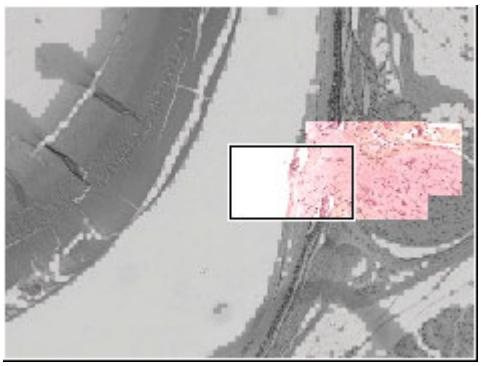
   You can move this tool anywhere on your monitor display, even off the ImageScope window, so it is out of the way.

2. To start recording your movements, click the red record button  :

When the recording begins, the thumbnail image turns gray. As you move, each section of the image you move to is highlighted in the thumbnail. The intensity of the highlight shows the resolution at which that part of the image was viewed.



For example, after moving through several portions of the digital slide shown above, the thumbnail looks like this:



If you want to change the size of the thumbnail to make viewing easier, hold and drag the lower left corner of the thumbnail.

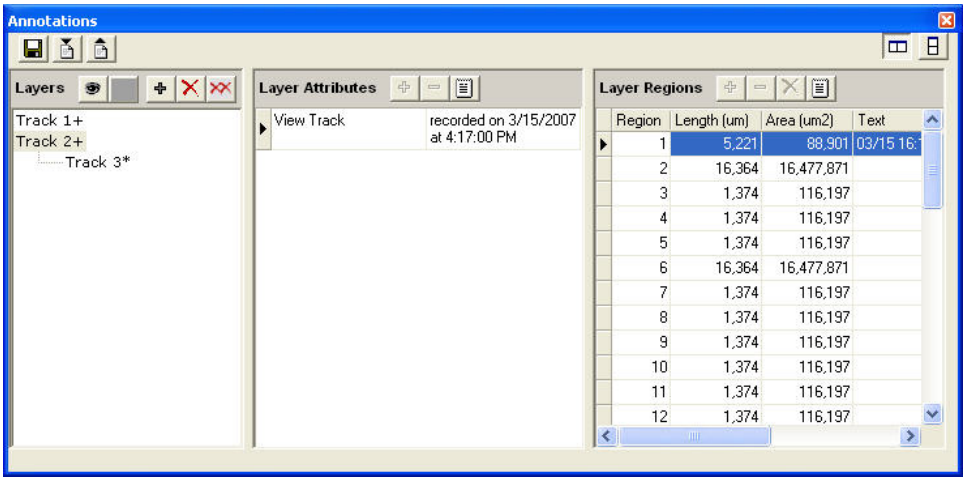
Tips:

- If you do not want to see your progress mapped on the thumbnail, clear the **Map** check box on the tracker tool. To show/hide mapping, type Control-M. (You can also set the default value of this check box. See "Tracking Options" on page 181.)
- To automatically move to the next unviewed section, click  on the tracker tool. This button moves through the image more or less in the way a person would, working left to right, top to bottom. Using this button, you can systematically view the entire slide.
- To stop recording, click  on the tracker tool.

Viewing a Track

To view a track:

1. Go to the View menu and select **Annotations**. The Annotations window appears:



Each track associated with this digital slide is listed in the Layers pane of the Annotations window. To see information on each track, click a Track in the Layers pane.

Tips:

- A + symbol after the Track name indicates the track recording is complete.
- An * after the Track name indicates the track recording is still in progress.
- An indented track (for example, Track 3 in the example above) indicates that the track has been appended to the track above it. (See "Appending to a Track" on page 107 for information on appending.)

- The information in the Layer Attributes pane tells when the selected track was recorded.
- The information in the Layer Regions pane gives information on each stage of the recording. The first region of the track is the path that connects the center of all the views in the session. The other regions in the track are rectangles corresponding to each view in the session.
- To see a track in the main ImageScope window, select the track in the Annotations window and click the eye icon at the top of the Layers pane.
- As with any annotation layer, you can change the name of the track (for example instead of "Track 1+" you can name the track "Dr. David") by clicking on it and typing in a new name.

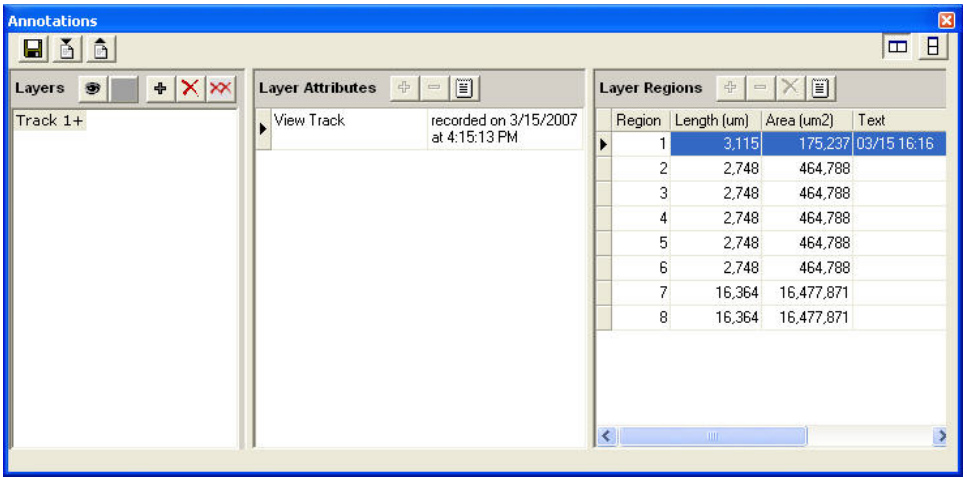
Playing a Track

To play the current track:

1. Click  on the tracker tool.

To select a track made in the past:

1. Open a digital slide.
2. Go to the **View** menu and select **Annotations.** The annotations window appears:



3. Select the track you want to play by clicking it in the Layers pane of the Annotations window.
4. Click  on the tracker tool.

Tips:

While playing a track, use the tracker tool buttons to affect the playback:

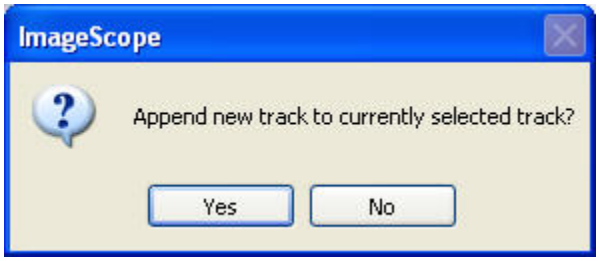
-  – Stop playing or recording the track.

-  – Go to first view in the track.
-  – Go to the last view in the track.
-  – Go to the next view in the track.
-  – Go to the previous view in the track.

As you play the track, the Annotations window updates to show your location in the Layer Regions pane.

Appending to a Track

You can either start a new recording or append to a previous one. If a track already exists for this digital slide, when you click the record button, the following message appears:



To start a new recording, click **No**; to append to the previous track, click **Yes**.

If more than one track has been created for the digital slide, to append to a specific track, open the Annotations window (as discussed above) and select that track by clicking on it in the Layers pane before clicking the record button.

When a track is appended, the recording starts off as if all the views in the parent track have already been viewed.

See “Viewing a Track,” on page 105 for an example of an appended track.

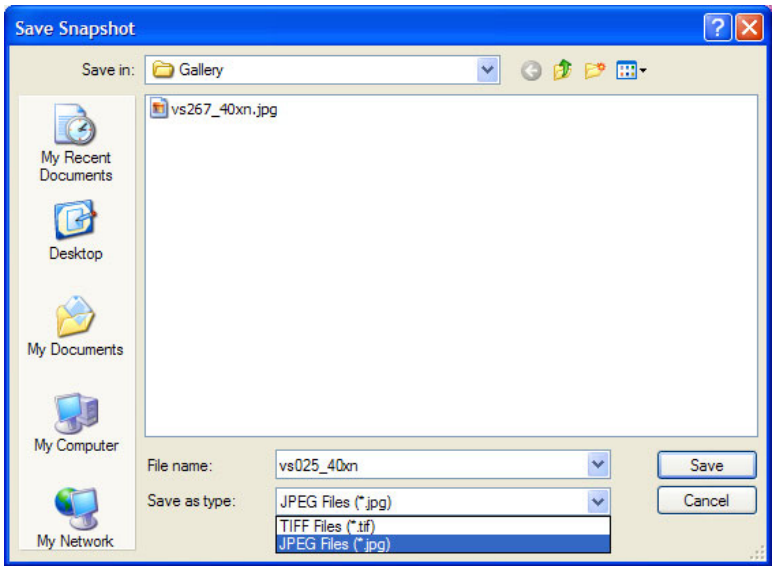
13 Saving Digital Slides and Regions

This chapter discusses several different ways to save images of digital slides.

Taking a Snapshot

You can capture a picture of the digital slide you are viewing by using the Save Snapshot command. The image is saved in JPEG or TIFF format.

1. Navigate to the area of the digital slide you want to capture so that it is displayed in the main ImageScope window.
2. Go to the File menu and select **Save Snapshot** or click  on the toolbar.
3. In the Save Snapshot window, browse to the location where you want to save the file.



4. Type a name for the file in the **File name** box.
5. In the **Save as Type** drop-down list, select **TIFF Files** or **JPEG Files**.
6. Click **Save**.

To view the file you saved, use Windows Explorer to browse to the folder location where you saved the file and double-click the file name. Or, you can

navigate to that location from within ImageScope and open the file with the ImageScope **Open** command.

Color Management

When you make a snapshot, and if the original image has an ICC profile embedded in it, ImageScope transforms the image using the monitor ICC profile if Integrated Color Management is turned on in ImageScope or embeds the ScanScope ICC profile if Integrated Color Management is turned off.

Saving an Image to the System Clipboard

You can save the image currently being displayed to the system clipboard by going to the Edit menu and selecting **Copy** (or type Control C). You can then paste the image from the clipboard into an image processing application like Microsoft Paint or Adobe Photoshop.

Extracting a Region

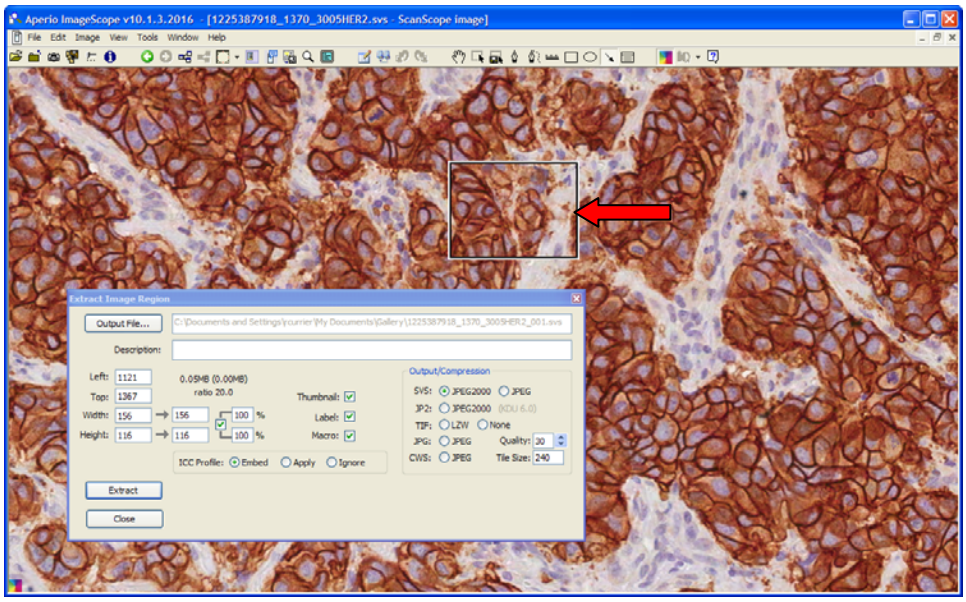
You can extract selected regions of a digital slide in different formats and open the extracted region in another application if required. When you extract a region, you can define the exact size of the new image or use a predefined fixed size, which can be very useful when preparing images for presentations or publication (see "Saving an Image of a Specific Size" on page 114).

Using the Extraction Tool

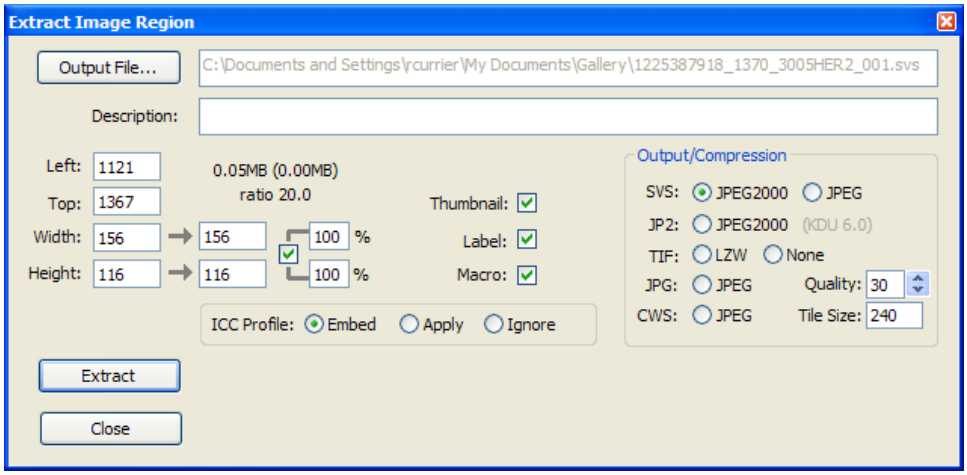
To extract a region:

1. Click  on the toolbar.
2. Place your cursor on the digital slide.

3. Click and drag a rectangle on the screen to capture the area:



When you release the mouse button, the following appears:



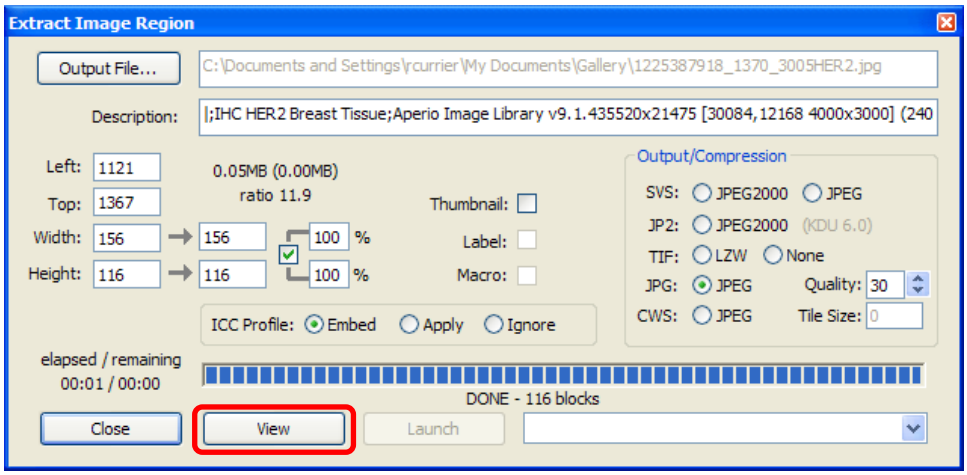
4. Select among the following options:

Option	Description
Output File	Location where you want the extracted region file to be saved and name to be used. If you do not use the Output File button, the new file will be created in the same location as the original digital slide and with the same name but with a number appended to it. For example, if the original digital slide file name is vs025_40Xn.svs, the extracted region is named vs025_40Xn_001.svs. (If a _001 file already exists, the extracted region file name ends in _002. If a _002 file already exists, the new file name ends in _003, and so on.)
Description	Your title for the extracted region.
Left	Left pixel co-ordinate of the original image.

Option	Description
Top	Top pixel co-ordinate of the original image.
Width	Width of the extracted image in pixels.
Height	Height of the extracted image in pixels.
Thumbnail	Attach the thumbnail of the exported image.
Label	Attach the label image from the original scan if it exists.
Macro	Attach the macro image from the original if it exists.
Tile Size	Determines the organization of the data within the extracted image. For large SVS and TIFF files, a tile size of 240 or 256 is optimal to enable fast access to the image. If you want to extract images for use with a third-party program which doesn't support blocked TIFF files, you can set the tile size to zero to create a "stripped" image. Stripped TIFF files are supported by all software which processes TIFF files, but there is a performance penalty for large images (it does not affect small images). JPEG files are always stripped (tile size is always zero).
Output/ Compression	The different file formats you can select for the saved image and their compression options: ■ SVS file format using JPEG2000 or JPEG compression ■ JP2 file format using JPEG2000 compression ■ TIF file format using LZW or no compression ■ JPG file format using JPEG compression ■ CWS file format using JPEG compression
ICC Profile	If an ICC profile has been embedded in this image, you can select how it will be managed in the extracted image (embedded, applied, ignored). For information on Aperio color management, see Appendix B, "Aperio Integrated Color Management" on page 193.

5. Choose the options you require and click **Extract**. You see a progress bar showing the status of the extraction.

   When the extraction is complete, you see additional buttons for viewing the image:

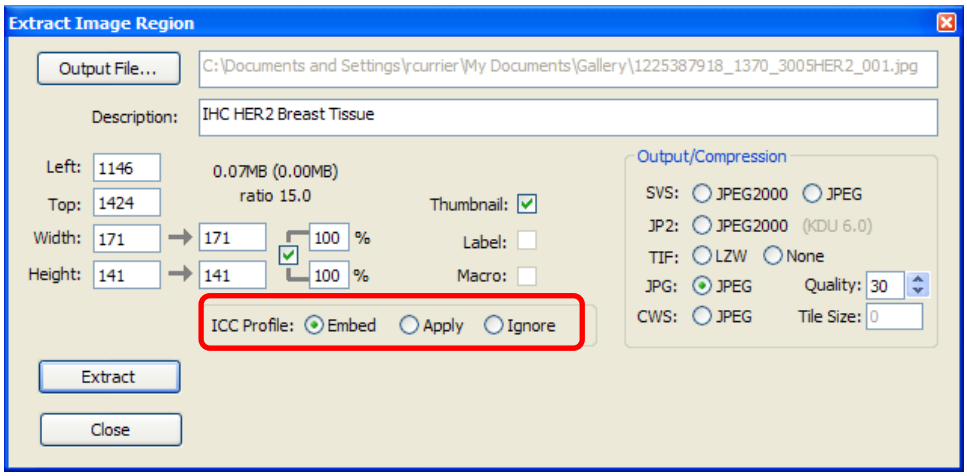


6. Click **View** to open the extracted image in ImageScope. (For information on the **Launch** button, see "Managing Viewing Applications" on page 115.)

Color Management Options

When you extract a region from ImageScope, you can specify one of the following:

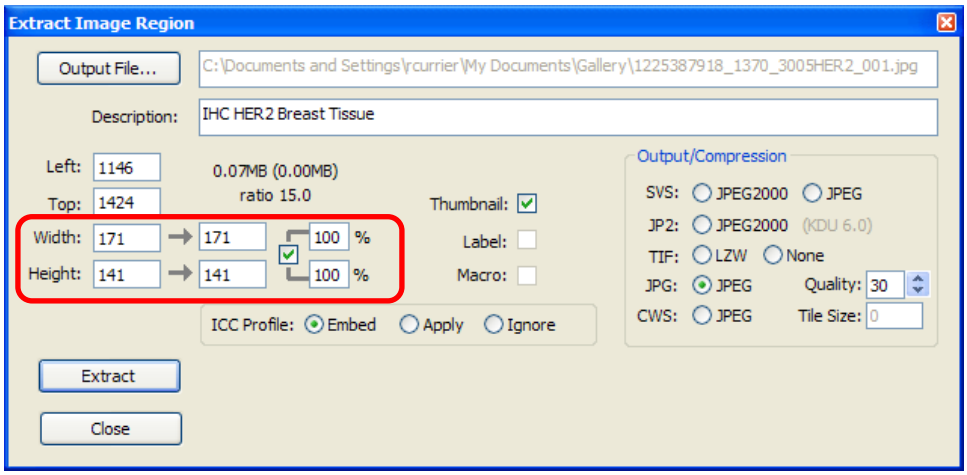
- **Embed –** The ScanScope ICC profile will be embedded in the image.
- **Apply –** The image will be transformed using the monitor ICC profile.
- **Ignore –** No color management will be done.



Saving an Image of a Specific Size

To create an image of a specific size:

1. Follow the steps above to capture the area you want to extract.
2. When the Extract Image Region window appears, adjust the size of the saved file by changing the settings in the **Width** and **Height** boxes.



Tips:

- The first column of numbers after **Width** and **Height** is the number of pixels of the original extracted image. The second column is the output dimensions, initially set the same as the original values.
- The percentages show the ratio between the original dimensions and the output dimensions. The check mark in the box causes both the width and height to be adjusted proportionally (thus preventing the image from being distorted). You will usually want to keep the box checked.
- You can change the percentages to make an image smaller. If you are extracting a number of images, using the same percentage for them all will ensure they will all have the same resolution. You can also adjust the percentage to greater than 100 to make the image larger.
- Instead of changing the percentages, you can define a specific output dimension by typing the exact number of pixels you want for the height and width in the second width and height columns.

Extracting an Image of a Predefined Size or Aspect Ratio

If you have predefined a fixed size (see "Fixed Size Annotations" on page 180), to extract an image in the predefined size, hold down the Control key while you click the extract tool on the toolbar.

To extract an image using the same aspect ratio of the predefined size (but not necessarily the same size), hold down the Shift and Control keys while you use the extract tool to draw the region to extract.

Managing Viewing Applications

You can define applications other than ImageScope to be used to view extracted regions. Doing so defines what viewing application ImageScope will launch from the Extract Image Region window, but of course you can also open the extracted file outside of ImageScope using any compatible image viewing software (see the next section).

Compatibility Notes

Be aware that the viewing application you define may not be able to open an image of the type you've extracted. For example, Internet Explorer cannot open TIFF files, but can open JPEG files. If you click **Launch** and the application is not able to open the extracted file, you will see an error message from the application similar to "Cannot open this type of file."

Also, the viewing application you define may not be able to handle a file as large as your extracted file. Some applications have a limit on the size of file they will open and work with.

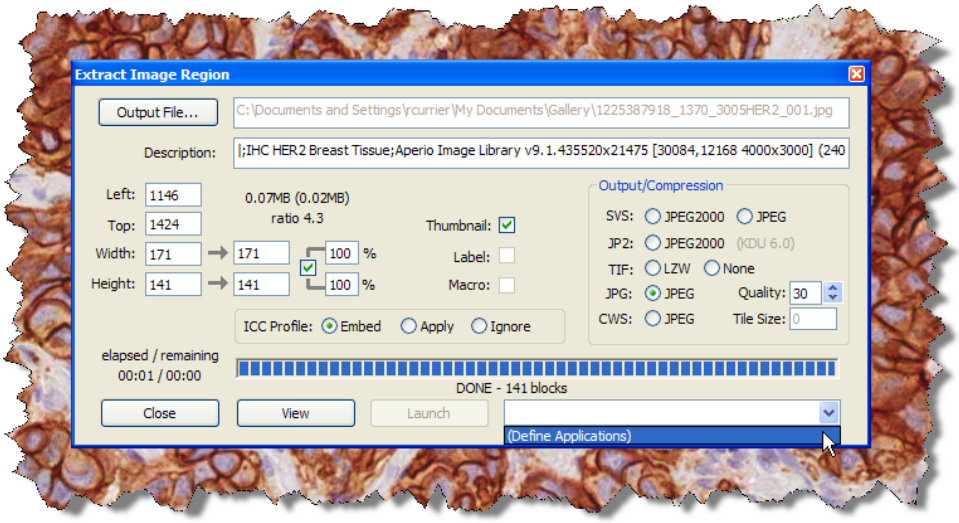
Some third-party programs cannot handle TIFF files which use any form of compression. When in doubt, you can always specify **None** for compression of TIFF files. However, if the region is large, using no compression will result in a very large file.

Defining a Viewing Application

To define a viewing application:

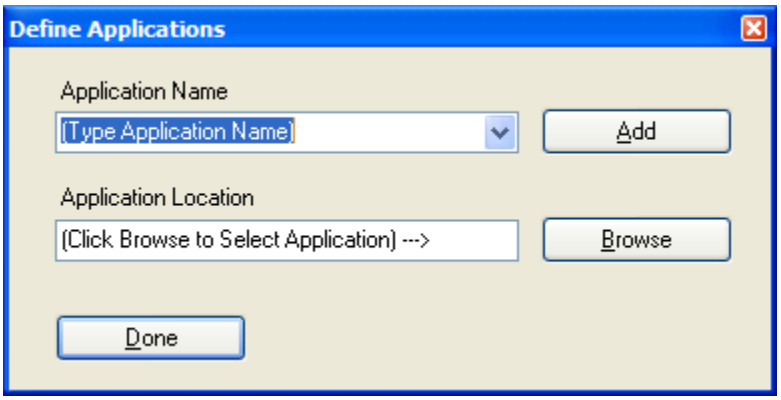
1. Follow the instructions above to capture and extract a region of a digital slide.

2. Click the drop-down box next to the **Launch** button and select **Define Application**.



If no applications have previously been defined, this list will be empty except for this selection. If applications have been defined, they will appear in alphabetical order.

The following window appears:



3. Type a description of the application (for example, **Photoshop**) in the **Application Name** text box. This can be any text you want to use.
4. Click **Browse** to navigate to the location of the application executable file and select that file.
5. Click **Add** to add this application to the list of ImageScope viewing applications.
6. Click **Done**.

Using the Viewing Application

To view an extracted region with a viewing application (after one has been defined):

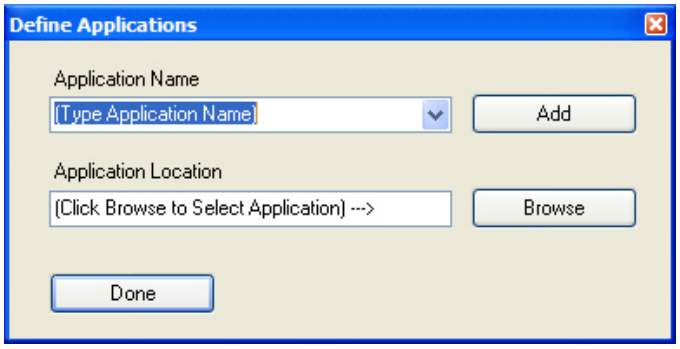
1. Follow the steps above to extract a region.
2. Click the drop-down box next to the **Launch** button and select a viewing application.
3. Click the **Launch** button**. (**If no applications have yet been defined, the **Launch** button is disabled.)

Deleting Viewing Applications

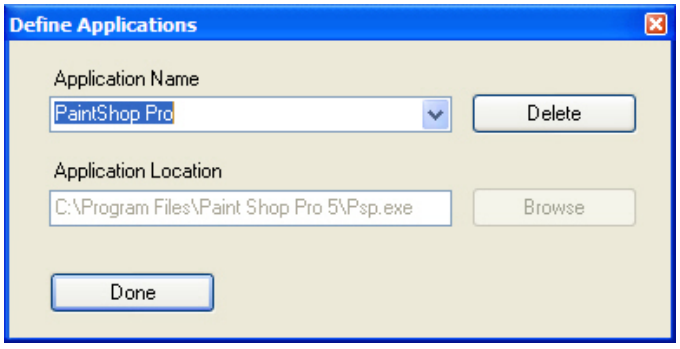
To delete applications previously defined:

1. Follow the steps above to extract a region.
2. Click the drop-down box next to the **Launch** button and select **Define Application.**

   The following window appears:



3. Click the **Application Name** drop-down box and select the application you want to delete from the list of ImageScope viewing applications. The **Add** button now changes to a **Delete** button.



4. Click **Delete** to delete the application from the list of ImageScope viewing applications. (This does not delete the application from your disk, but only from the list of ImageScope viewing applications.)
5. Click **Done**.

14
Analyzing Digital Slides

This chapter discusses using Aperio image analysis algorithms to analyze digital slides.

Aperio's image analysis algorithms are FDA cleared for specific clinical applications, and are intended for research use for other applications.

This chapter discusses how to use algorithms to analyze digital slides. We discuss the general-purpose analysis procedure that uses the Annotations detail View. For information on streamlined digital IHC analysis, see "The Annotations Window Summary View Window – Quick IHC Analysis" on page 81.

Analyzing digital slides helps you to examine the slide staining to find any unusual patterns. Using an algorithm to look for these patterns provides precise, quantitative data that is accurate and repeatable.

About Analyzing Digital Slides

There are three different types of analyses you can perform on digital slides:

1. You can use ImageScope to analyze a single, remote digital slide that resides on Spectrum. In this case, the analysis results are stored remotely with the image in the Aperio database.
2. You can use Spectrum to analyze a single digital slide or a batch of digital slides that reside on Spectrum. Results are stored in the Aperio database. For information on submitting batch jobs to analyze groups of digital slides, see the *Spectrum/Spectrum Plus Operator's Guide*.
3. You can use ImageScope to analyze a single, local digital slide that resides on your workstation or network location accessible by Microsoft file sharing. In this case, the algorithm analysis results are stored locally with the image as annotations.

Note that the ImageScope user interface is somewhat different depending on whether you are analyzing a local or a remote image. Please see the sections that follow for details:

- "Analyzing a Digital Slide in Spectrum" on page 120.
- "Analyzing a Local Digital Slide" on page 124.

Also note that before you can analyze a remote digital slide, you must fine-tune the algorithm parameters and save the settings as a *macro*. See Chapter 15,"Registering Algorithm Macros on Spectrum" on page 133.

Partial or Full Analysis

You can analyze an entire digital slide image or you can use the annotation tools to pick just an area to analyze. You can even draw an annotation that *exclude*s an area from analysis.

Results from the Analysis

You can save algorithm analysis results as a visual "markup image" and also as quantitative data that can be exported to be read by a spreadsheet program. The analysis results can also appear in Spectrum if slide-specific processing is set up.

In all cases, the original digital slide image is never modified. Rather, a new annotation layer with the markup image and quantitative data is created and linked to the image.

Algorithms

The process of analyzing a digital slide is done by applying algorithms directly to the digital slide or selected regions of the digital slide.

The Positive Pixel Count algorithm and Positive Pixel Count FL (for fluorescence images) are provided for free with Aperio software and installed with ImageScope. Other algorithms are available from Aperio for a fee. Algorithms have also been developed by third parties and tools are available from Aperio for creating your own algorithms. Contact Aperio for details.

Algorithms all have control parameters—for example, intensity and hue settings—that allow the algorithm to be tailored to your specific needs. See the algorithm documentation for information on specific algorithms.

Note that Aperio's image analysis algorithms are FDA cleared for specific clinical applications, and are intended for research use for other applications. See the documentation for the specific Aperio clinical application for details on the intended use of that application.

For general information on using Aperio algorithms, see the *Aperio Image Analysis User's Guide*.

Analyzing a Digital Slide in Spectrum

When you open a digital slide in Spectrum, the analysis runs on Spectrum, freeing your workstation to do other things. The results of the analysis are stored in Spectrum as an annotation layer of the digital slide.

Incremental Analysis

Some Aperio algorithms support incremental processing. Incremental processing allows the algorithm to analyze only regions added after the initial analysis without re-analyzing the previously analyzed regions. This can save a great deal of time and is useful when a pathologist draws a single region and analyzes it,

and after reviewing the analysis results wants to select additional regions and analyze them as well. The pathologist can add more annotation regions as needed or delete annotation regions and the analysis results will be updated accordingly.

If you delete a region, ImageScope will automatically re-run the analysis to update the summary analysis results. However, adding regions may make the analysis results incorrect until you re-run the analysis.

Opening a Remote Digital Slide

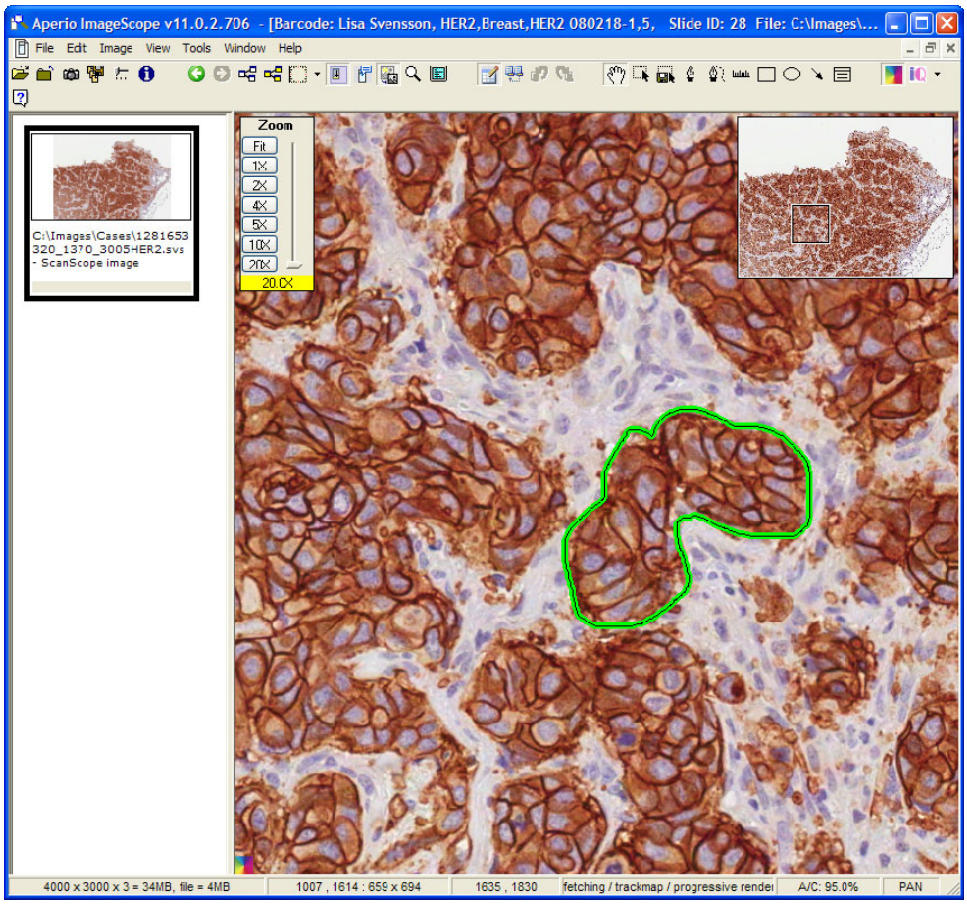
Log into Spectrum and select and open a digital slide in ImageScope. See "Opening a Digital Slide on Spectrum" on page 12 for instructions.

Selecting an Area of the Digital Slide to Analyze

Only the rectangle and free-form pen tools can be used to draw annotation regions to include in analysis. Only the free-form negative pen tool can be used to draw annotation regions to exclude from analysis.

If you want to analyze only part of the digital slide, use the pen or rectangle drawing tool to select the area to analyze, or use the negative pen to exclude an area from analysis. (See Chapter 9, "Annotating Digital Slides" on page 75 for details on the drawing tools.) If you do not draw areas to include or exclude from analysis, ImageScope will analyze the entire digital slide.

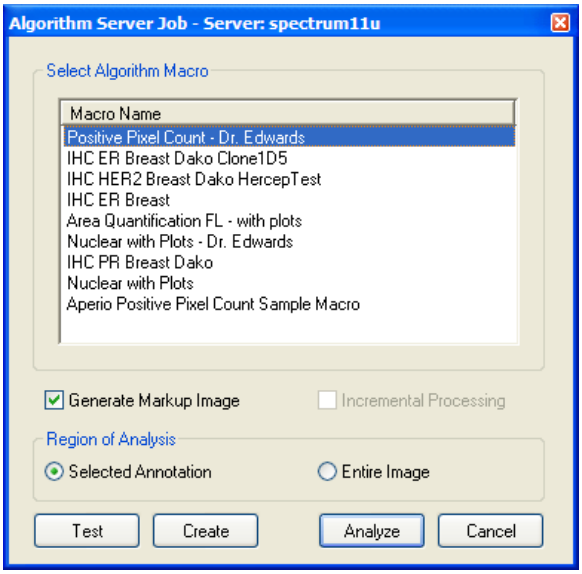
Here is an example of an area of analysis drawn by using the free-form pen tool:



Performing the Analysis

To analyze the digital slide:

1. Go to the **View** menu and select **Analysis**. You are asked to specify which algorithm you want to run.



   The list you see depends on which algorithm macros are installed on Spectrum. If you do not see any algorithms listed or do not see the one you want to use, see Chapter 15, "Registering Algorithm Macros on Spectrum" on page 133 for information on creating macros.

   To create a macro on Spectrum, you must be logged in as a Spectrum administrator—if you are, the **Test** and **Create** buttons on this window are enabled.

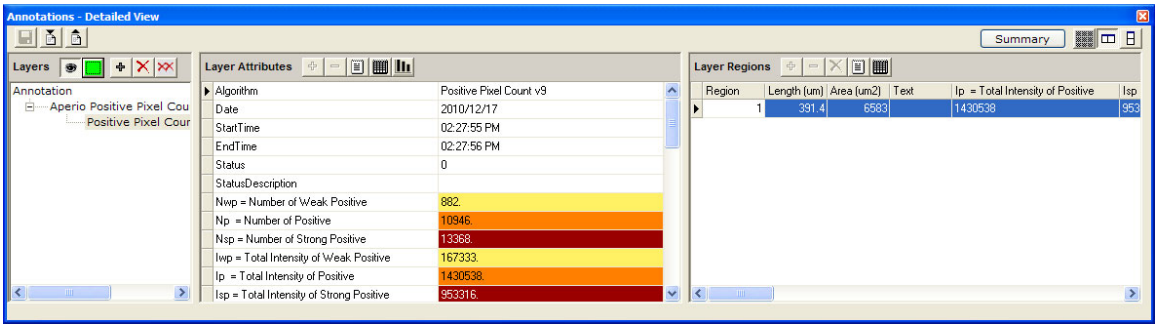
2. Select the algorithm you want to use. If you want to create a visual representation of the analysis as well as a quantitative one, select the **Generate Markup Image** check box.

3. If this algorithm supports incremental processing, you can select the **Incremental Processing** check box to use that feature (see "Incremental Analysis" on page 120). (If the check box is not enabled, this feature is not available with this algorithm.)

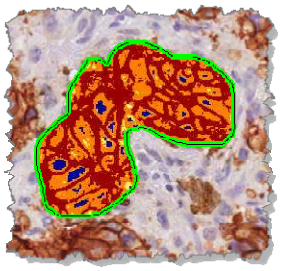
4. Click **Analyze** to start the analysis. A progress bar in the filmstrip shows the status of the analysis.

You can also check the status of the analysis by going to Spectrum and using the **Analysis > Jobs** command.

5. Open the Annotations window to see the quantitative results of the analysis, which are listed under the layer containing the annotations that define the area of analysis. Note that the quantitative results are color coded to match the mark-up image. (See "Algorithm Analysis Results" on page 130 for more information.)



If you selected the **Generate Markup Image** check box on the Algorithm Server Job window, the ImageScope main window also shows a visual representation of the analysis:



Saving Analysis Results

Both visual and quantitative results from a remote analysis are stored in the Spectrum database linked to the digital slide.

To save the quantitative results in a plain text form that can be imported into a spreadsheet program, click  on either the Layer Attributes pane to export the

combined results for all annotation regions on the selected layer or the Layer Regions pane to export the results in text format for each annotation region.

Or, click the  icon on the Layer Attributes or Layer Regions pane to save the data as an Excel spreadsheet. Numeric data exported to a spreadsheet is exported as text. Excel shows a warning note for each of these cells that the numbers are in text format—when you click on the note you can select an option to transform the text to numeric format.

See "Algorithm Analysis Results" on page 130 for more information.

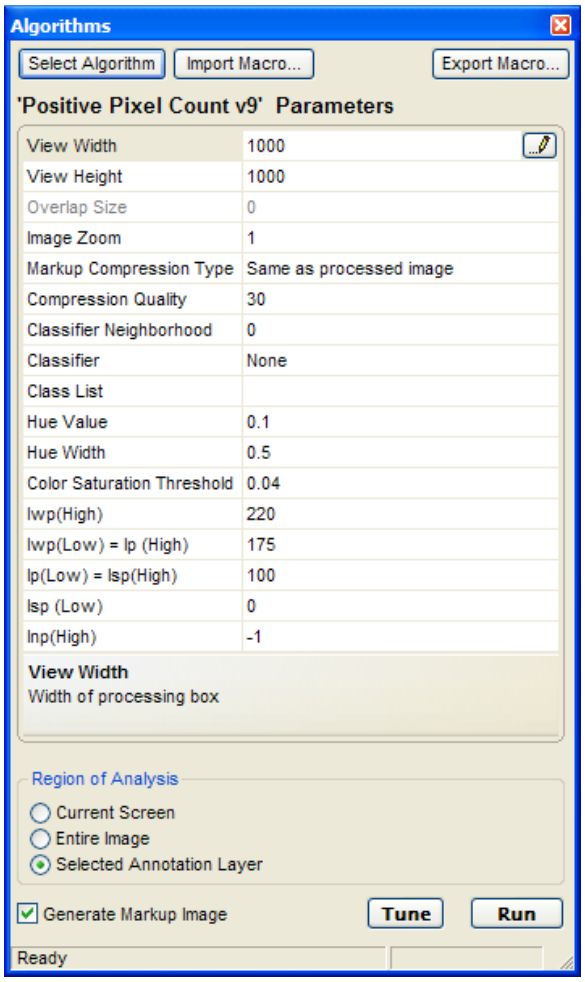
Analyzing a Local Digital Slide

1. Open a digital slide you want to analyze on your workstation or local network. (See Chapter 3, "Opening a Digital Slide" on page 11.)
2. If you are going to analyze only a portion of the digital slide, use the rectangle or free-form pen annotation drawing tools to:
   - Select one or more areas to analyze; or
   - Select one or more areas to *exclude* from the analysis.

   (See Chapter 9, "Annotating Digital Slides," on page 75 for information on using the annotation tools.)

Only the rectangle and free-form pen tools can be used to draw annotation regions to include in analysis. Only the free-form negative pen tool can be used to draw annotation regions to exclude from analysis.

3. Go to the **View** menu and select **Analysis**. The following window appears:



The last algorithm that was used is displayed in the Algorithms window. (Or if none has yet been used locally, this window is blank.)

Note that this window differs from the algorithm parameter window shown when you create an algorithm macro for a digital slide opened from Spectrum—there are no input/output buttons as you are not saving data on Spectrum and so do not need to specify which outputs to export to Spectrum.

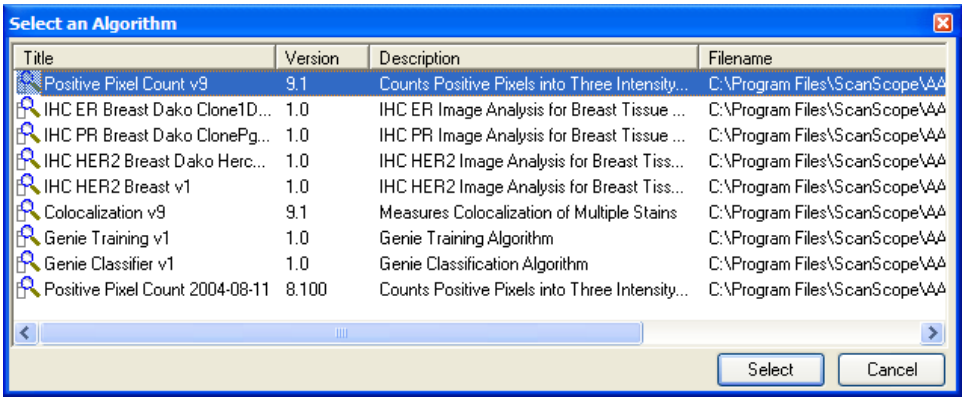
On this window you can:

- Select another algorithm to use
- Select whether to analyze the entire digital slide, just the portion of the digital slide on the ImageScope main window, or just a selected annotation.
- Import or export algorithm settings from or to your workstation.
- Modify algorithm settings

- Select whether to show the analysis results visually (*markup image*) as well as quantitatively.
- Click **Run** to run the analysis using the current parameter settings.
- Click **Tune** to open a tuning window to see instant feedback on parameter changes. See "Using the Tuning Window to Test Algorithm Parameters" on page 141 for details.

Selecting an Algorithm

If you want to use an algorithm other than the one displayed in the Algorithms window, click **Select Algorithm**. The following window appears:



The Positive Pixel Count algorithm is installed with ImageScope. If you have purchased and installed other algorithms, you will see them listed in this window also. Select the algorithm you want to use and click **Select**.

Note that the macros you create will work only with the version of the algorithm with which you created it. For example, if you previously created a macro to work with version 8.1 of the Positive Pixel Count Algorithm, you will see an error message if you try to use it with version 9 of the Positive Pixel Count Algorithm.

Selecting the Region of Analysis

You can choose to analyze:

- **Current Screen –** Analyzes just the portion of the digital slide on the ImageScope main window at the current zoom level. This is useful for testing the behavior of an algorithm and fine-tuning the algorithm parameters.
- **Entire Image –** Analyzes the entire digital slide. This can take a long time to complete depending on the size and complexity of the image.
- **Selected Annotation Layer –** Analyzes one or more annotations on the selected annotation layer or excludes from analysis one or more annotations (if they were drawn using the negative pen tool). Open the Annotations window and make sure the annotation layer containing the

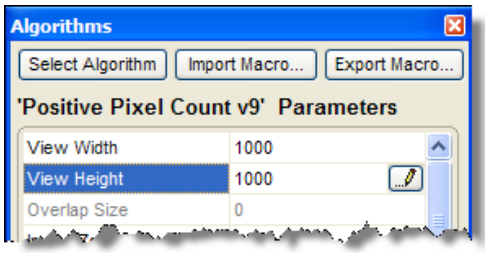
areas you have drawn is selected. (See Chapter 10, "Using the Annotations Window," on page 81 for information on annotation layers and the Annotations window.)

Modifying Algorithm Parameters

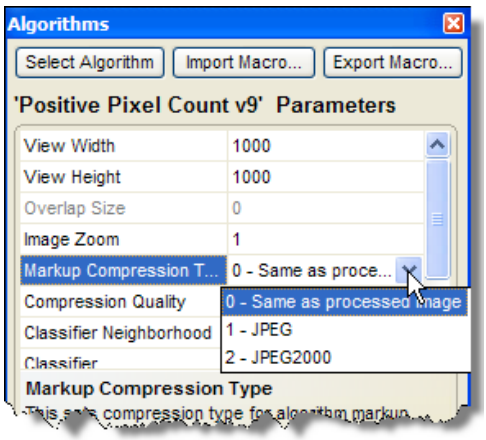
You can modify algorithm parameters to fine-tune the algorithm for your needs.

1. Click a parameter listed on the Algorithms window. If you can modify it, you either see:

   - The edit icon . Type a new value into the field or click the edit icon to select a new value using a slider control:



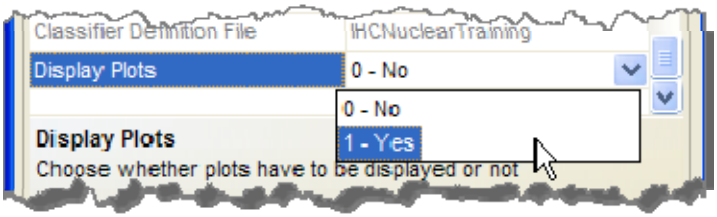
   - A down arrow, which means you can select from the drop-down list:



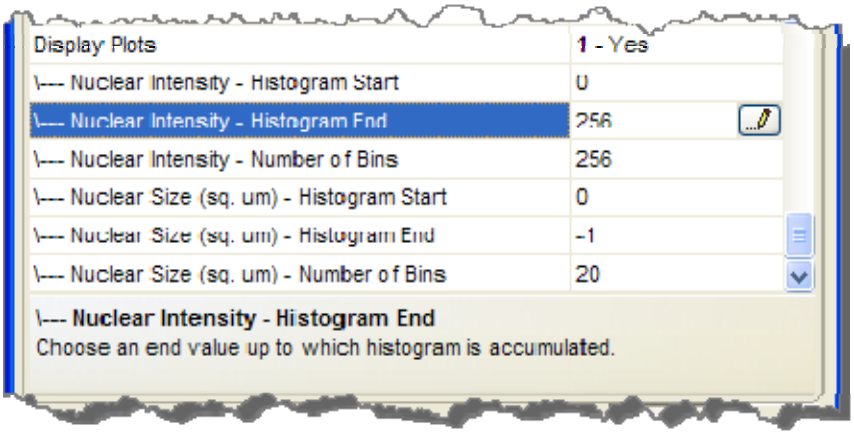
For information on the parameters for a particular algorithm, refer to the documentation that accompanies that algorithm.

Enabling Data Plots

When you are creating a macro for an algorithm that supports data plots, unless that algorithm enables plots by default, you will need to select plots in the algorithm input parameters. For example:



Depending on the algorithm, once plots are enabled, you may also be able to select details about the plots. For example, in this algorithm parameters list you can specify the beginning and end values of the different plots:



Importing/Exporting Macros

Once you have fine-tuned the algorithm parameters, you can save the algorithm and its changed parameters so you can use the modified algorithm again in the future. (The algorithm + modified parameters is known as a *macro*.)

1. Click **Export Macro**.
2. On the File Save window, type a descriptive file name in **File name** and click **Save**.

To import a previously saved macro, click **Import Macro** and select the macro file from the file selection window.

(To register the saved macro on Spectrum, log into Spectrum as an administrator and use the **Analysis > Macros > Add** command. See the *Spectrum/Spectrum Plus Administrator's Guide* for details.)

Instead of clicking **Run**, you can click the **Tune** button to open a tuning window in which you can see instant feedback on parameter changes. See "Using the Tuning Window to Test Algorithm Parameters" at the end of the next chapter for details.

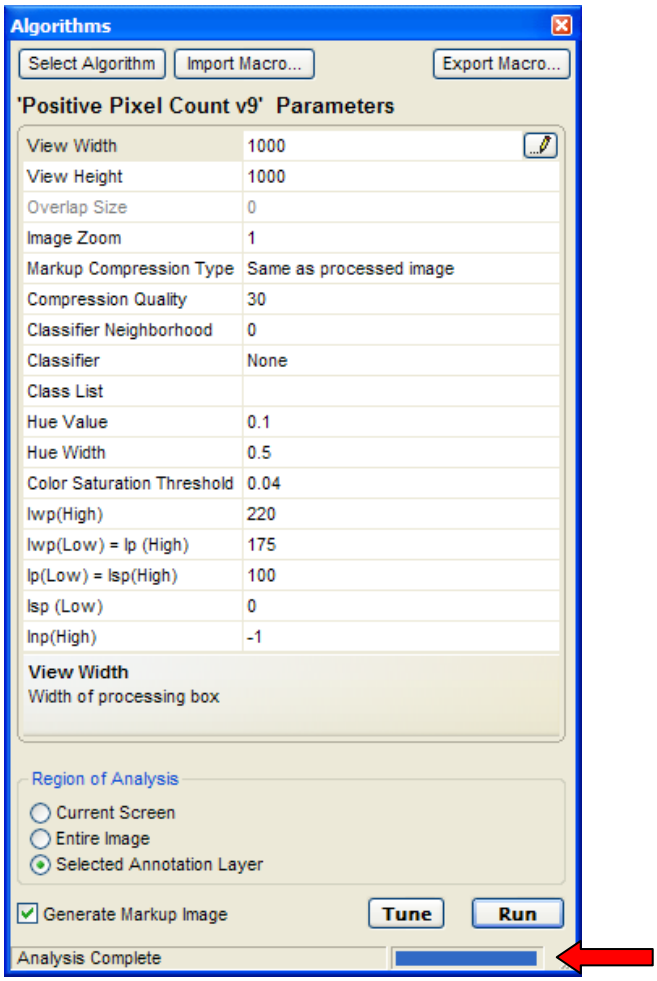
Running the Analysis

1. Go to the **View** menu and select **Annotations**. This opens the Annotations window where the quantitative portion of the analysis results will be displayed.

   If you drew annotations to include or exclude regions from the analysis, click on the annotation layer containing the annotations in the Annotations window to make sure the correct annotation layer is selected.

2. If you also want a visual representation of the analysis results, select the **Generate Markup Image** check box and you will see the results on the image in the ImageScope main window.

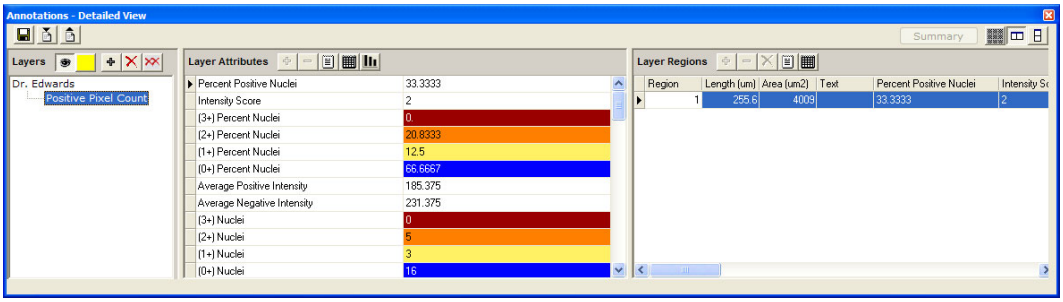
3. Click **Run**. As the analysis proceeds, you see status information displayed on the Algorithms window:



Algorithm Analysis Results

When the analysis is done, the Algorithms window displays "Analysis complete." If you analyzed this slide locally, the results are not saved in Spectrum, but are saved in an annotations file where the local file resides.

A new annotation layer appears in the Annotations window which contains the quantitative results of the analysis. For example:



The results for each annotation region are listed in the Layer Regions pane. The combined results for all annotation regions are listed in the Layer Attributes pane.

To save these results in a plain text form:

1. Click  on either the Layer Attributes pane (to export the combined results) or the Layer Regions pane (to export the results for each annotation region).
2. On the file save window, type a descriptive name in the **File name** text box and click **Save**.

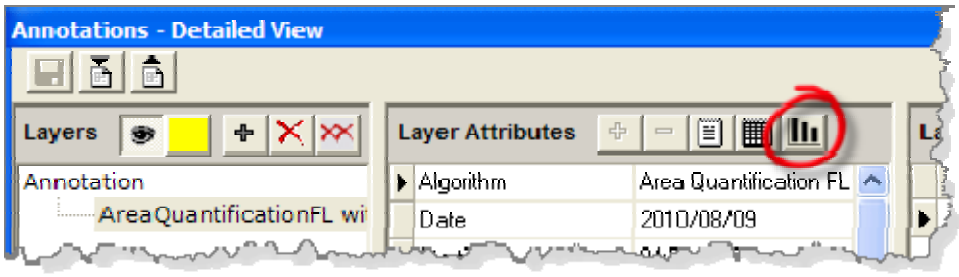
Or, click the  icon on the Layer Attributes or Layer Regions pane to save the data as an Excel spreadsheet. Numeric data exported to a spreadsheet is exported as text. Excel shows a warning note for each of these cells that the numbers are in text format—when you click on the note you can select an option to transform the text to numeric format.

If the Annotations window is in summary view, and you run an analysis using an algorithm macro in which data plots are enabled, all data plots will automatically open in individual windows when the analysis is complete.

Viewing Result Plots in ImageScope

After you run an algorithm for which plots are enabled, to see the resulting plots, go to the View menu in ImageScope and select **Annotations**.

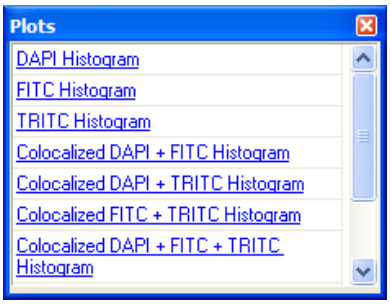
On the Annotations window in detailed view, click the Plots icon,  .



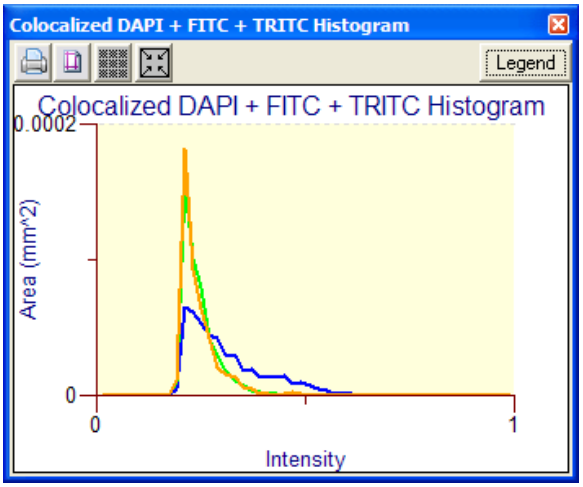
Location of Plots Icon on ImageScope Annotations Window

If you do not see the Plots icon, make sure the Annotations window is in detailed view, and not summary view. (If you only see summary view and there is no **Details** button to return to detailed view, you are in clinical viewing mode—go to the View menu and select **View Standard Toolbar**.)

After clicking the Plots icon, a plot window opens containing links to the various plots available for viewing.

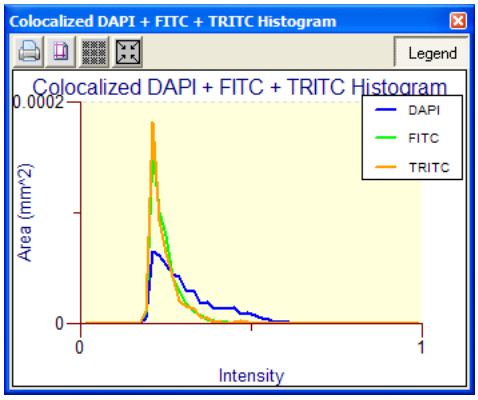


Click a link to see that histogram. The window appears for that plot. You can re-size this window by grabbing a corner of it and dragging.



Note the icons on this window:

Legend — Click to see the definition of each colored curve. For example:



 Set up printer so that you can print the plot. This opens the standard dialog window for your printer so you can choose paper size and other printer options.

 Print the plot.

 Make the plot window transparent so you can see the digital slide image through it.

 Restore window to original size.

15 Registering Algorithm Macros on Spectrum

This chapter discusses how to create and save an algorithm macro so it can be used on Spectrum. You must be logged into Spectrum as an administrator to perform this procedure.

Before you can use an algorithm to analyze a digital slide in Spectrum, a macro (an algorithm's settings) must first be registered on Spectrum.

To create an algorithm macro, you need to install the algorithm on both your local workstation and on Spectrum. (This consists of simply running the algorithm installer on both computers.)

Creating and Saving a Macro

To create a macro, you will:

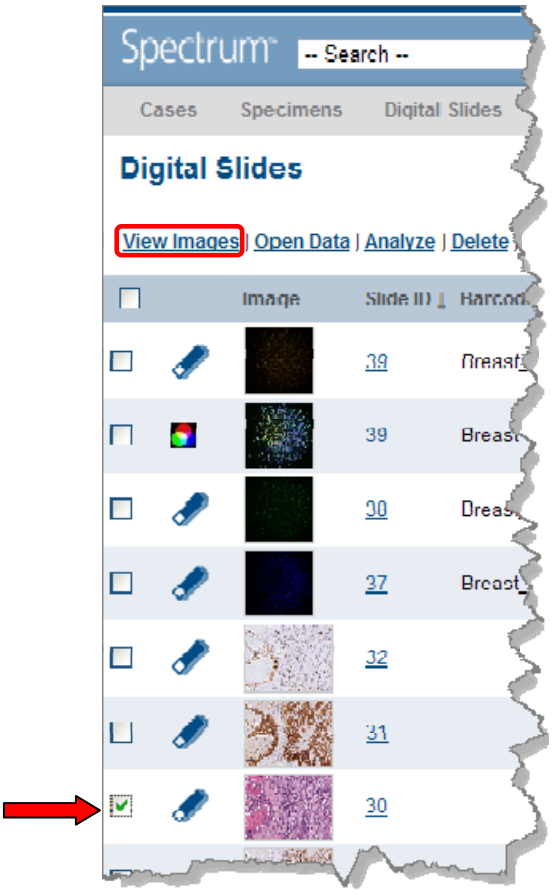
1. Open a digital slide in ImageScope from Spectrum.
2. Select an Algorithm from which to make a macro.
3. Create the macro.
4. Save the macro on Spectrum

Open a Digital Slide

To open a digital slide from within Spectrum:

1. Log into Spectrum as an administrator.
2. Use the Spectrum List commands to see the digital slides on your site or use the search feature to find the one you want.

3. Select the digital slide you want to use and click **View Images**:



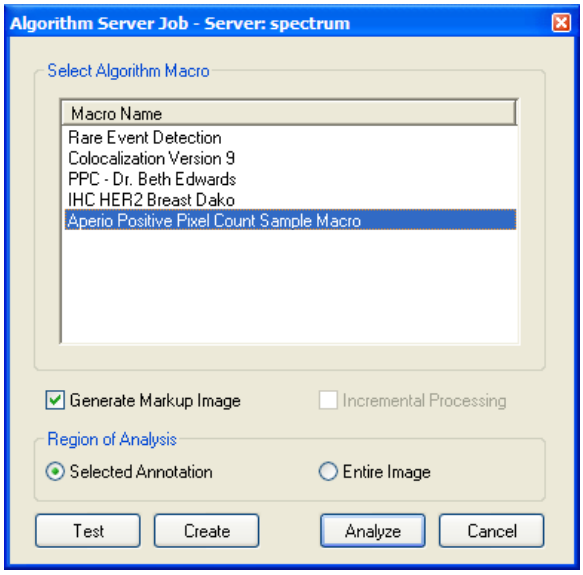
The digital slide opens in ImageScope.

Create a Macro

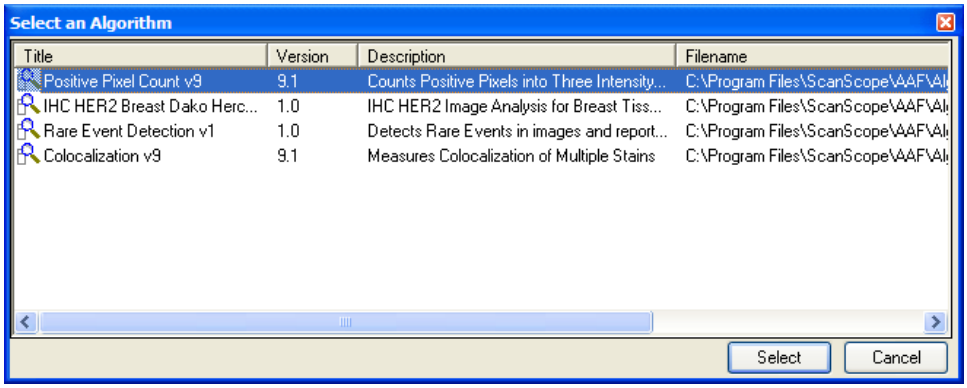
The **Test** and **Create** buttons are disabled if you are not logged in as a Spectrum administrator as only administrators can create and modify algorithm macros.

The **Analyze** button is disabled if your user permissions are not set to Full Control for the data group containing the digital slide image.

1. Go to the ImageScope View menu and select **Analysis** or type Control G. You see the Algorithm Server Job window:

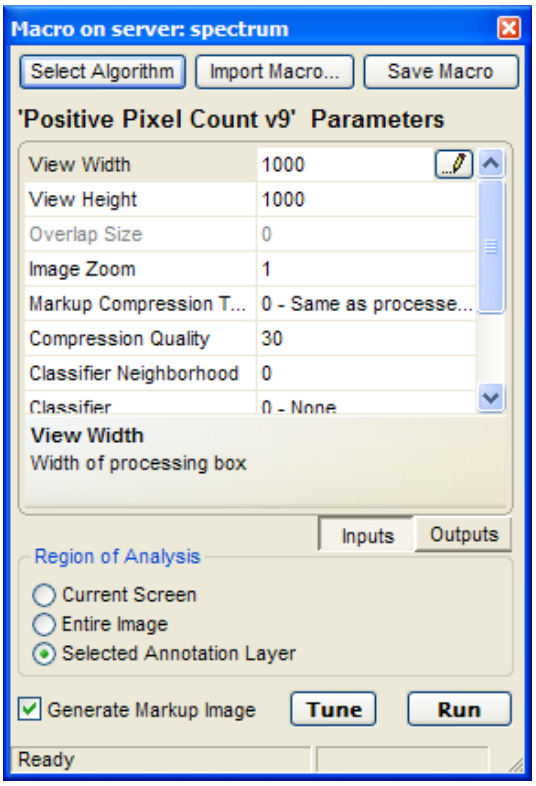


2. Now click **Create**. The Select an Algorithm window appears:



3. Select the algorithm you want to create a macro for and click **Select**.

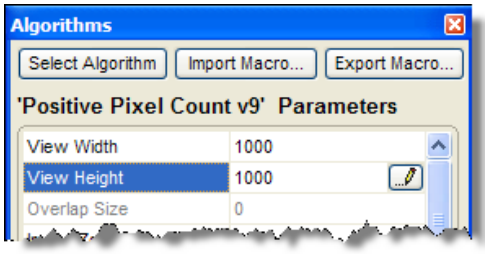
This loads the algorithm macro with its default parameters so you can see its unmodified parameters.



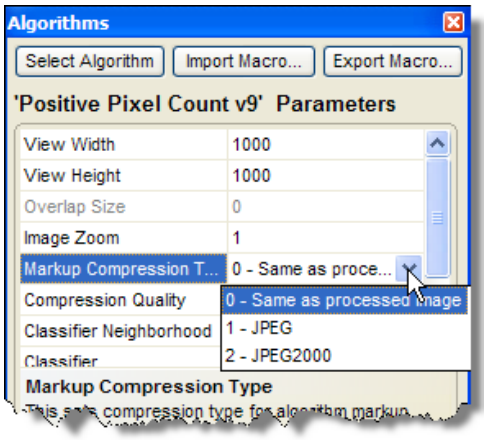
4. Modify the Input parameters to suit your application.

   Click a parameter listed on the Algorithms window. If you can modify it, you either see:

   - The edit icon  . Type a new value into the field or click the edit icon to select a new value using a slider control:

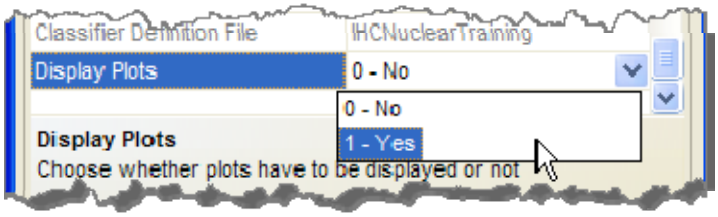


- A down arrow, which means you can select from the drop-down list:



For information on the parameters for a particular algorithm, refer to the documentation that accompanies that algorithm.

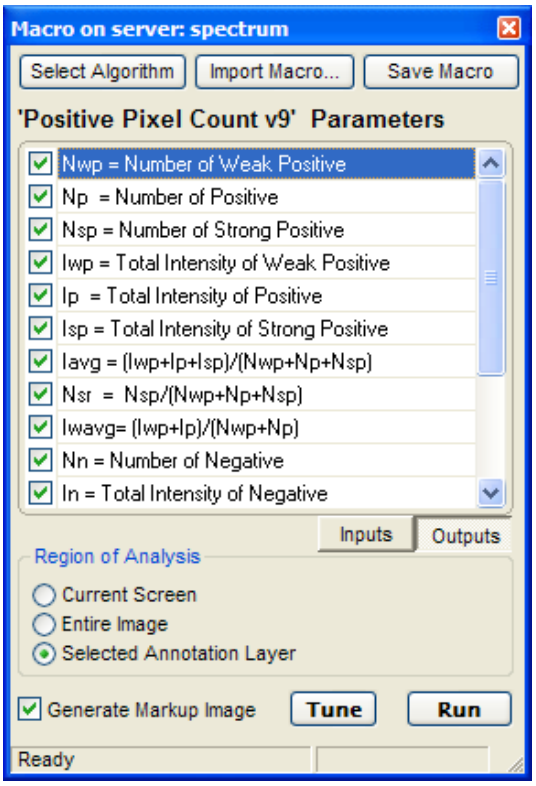
When you are creating a macro for an algorithm that supports data plots, unless that algorithm enables plots by default, you will need to select plots in the algorithm input parameters. For example:



For information on viewing result plots, see the previous chapter.

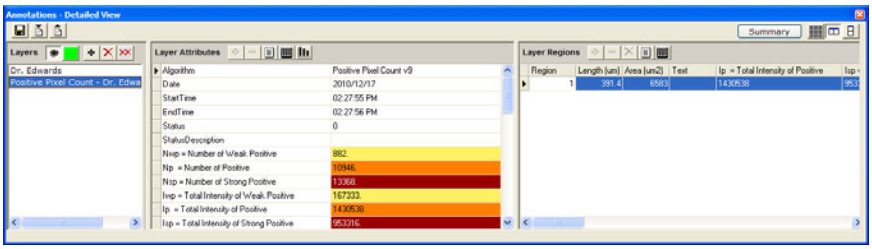
The *Classifier Neighborhood*, *Classifier*, and *Class List* parameters are only used if you are using Genie classifiers with this algorithm. Your Spectrum administrator can tell you if Genie classifiers are available on your Spectrum site. (Genie is a histology pattern recognition tool that works with Aperio algorithms to automatically identify tissue types for analysis. For more information, see the *Genie User's Guide*.)

5. Click **Outputs** to select what results you want to display in Spectrum. (If you are analyzing a local slide, you will not see the Input/Output buttons as the results won't go to Spectrum.)



Clear the check boxes next to the results you don't want to display in Spectrum.

6. Click **Run** to test the algorithm on the digital slide. You can see the results in the ImageScope Annotations window:



And in the mark-up images in the ImageScope window.

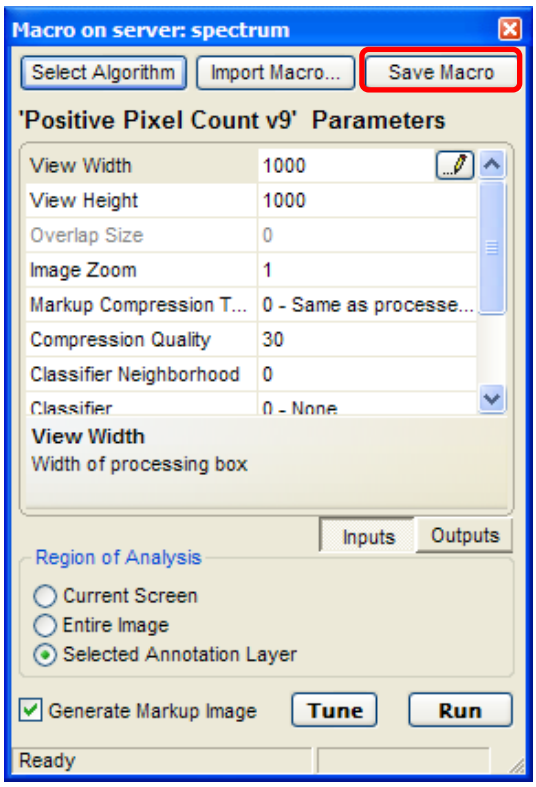
7. When you are satisfied with the results, save the macro on Spectrum (see the next section).

Instead of clicking **Run**, you can click the **Tune** button to open a tuning window in which you can see instant feedback on parameter changes. See "Using the Tuning Window to Test Algorithm Parameters" at the end of this chapter for details.

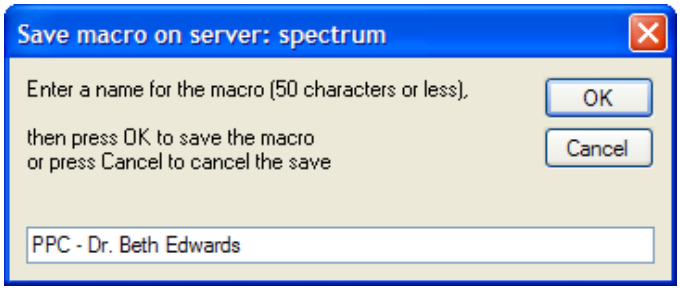
Save the Macro on Spectrum

After you have created the macro, save it to register it on Spectrum.

1. On the Analysis window, click **Save Macro** to save the macro and register it on Spectrum:

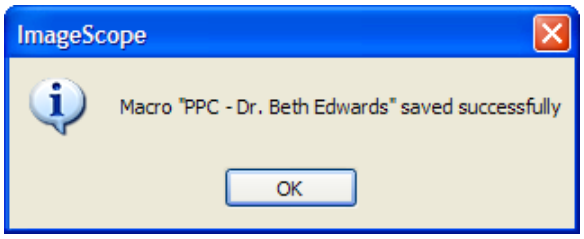


2. You are asked to enter a name for the macro:



Type a name that will help you identify the macro in the future and click **OK**.

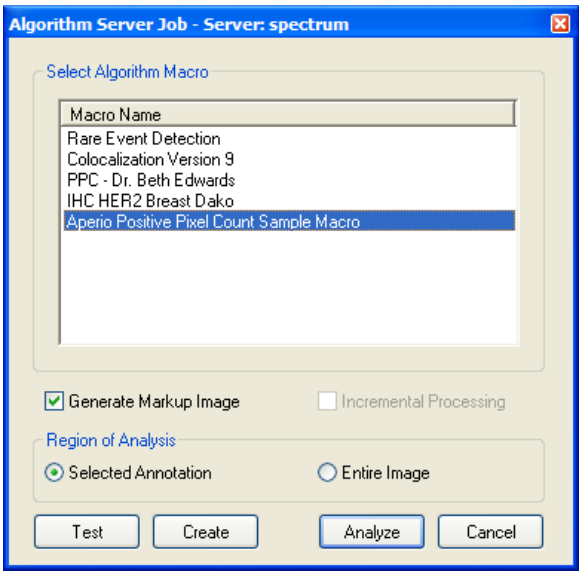
You now see a message letting you know that the macro is saved. It is now registered on Spectrum.



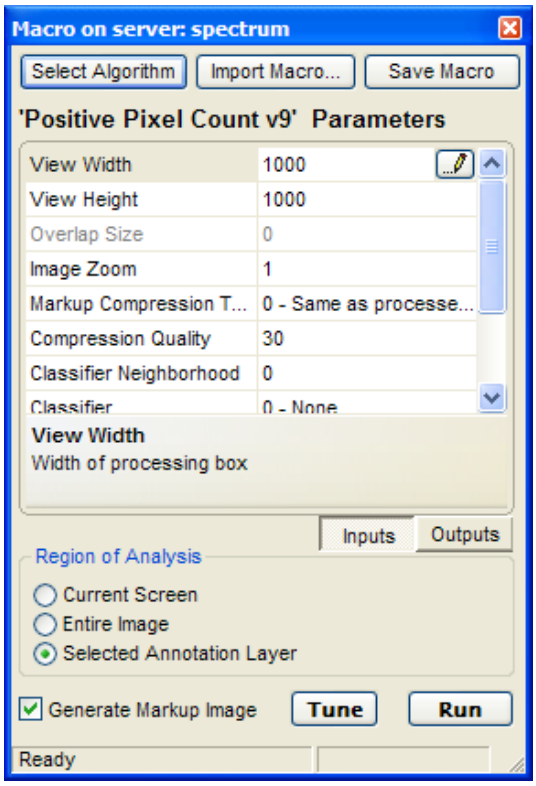
Testing and Modifying an Existing Macro

The **Test** button modifies an existing macro and tests it before saving it to Spectrum.

1. Open a digital slide on Spectrum.
2. Go to the View menu and select **Analysis** or type Control G.
3. Select a macro from the Algorithm Server Job window and click **Test**.



This loads the algorithm macro with its existing parameters.



4. Modify the parameters as discussed in the previous section and click **Run** to test the macro on the digital slide or click **Tune** to see immediate feedback on parameter changes (see the next section for details).
5. On the Analysis window, click **Save Macro** to save the macro and register it on Spectrum.
6. You are asked to enter a name for the macro. Type a name that will help you identify the macro in the future and click **OK**.

Using the Tuning Window to Test Algorithm Parameters

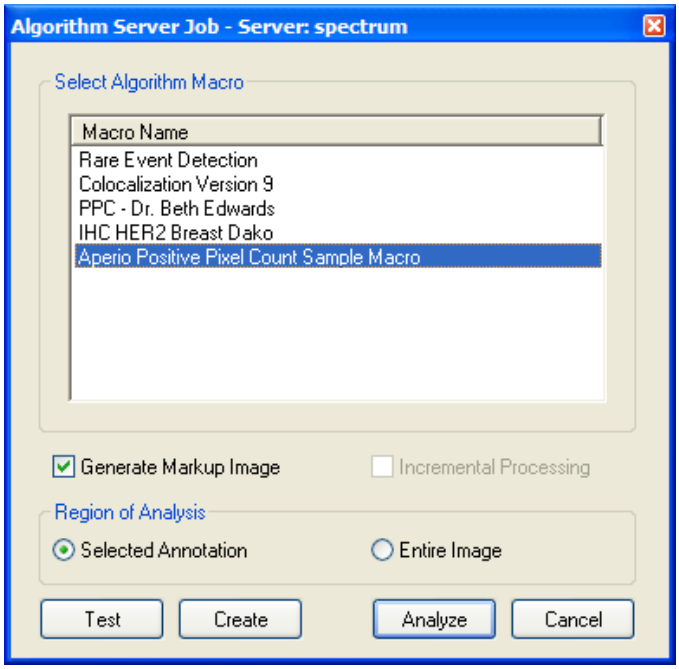
The purpose of the algorithm tuning window is to give you way to quickly see the results of analyzing a different area of an image or to test changes you make to the algorithm parameters.

To use the algorithm tuning window:

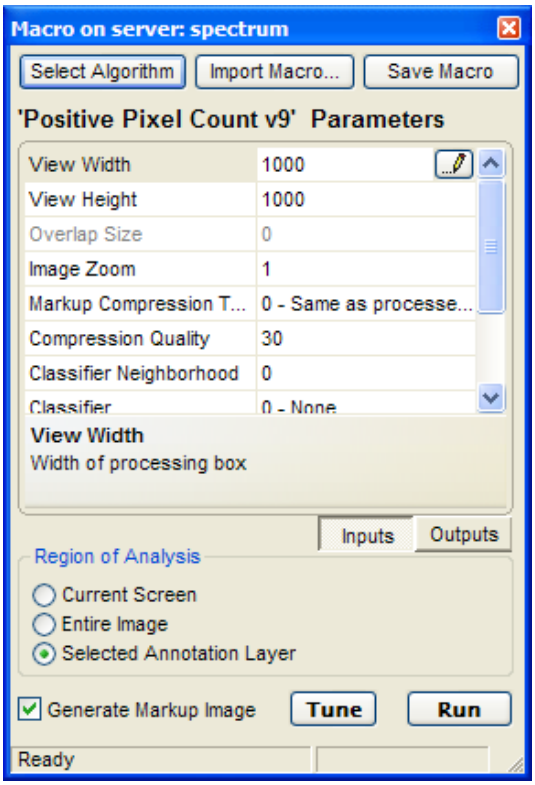
1. In Spectrum, open a digital slide in ImageScope.
2. In ImageScope, go to the View menu and select **Annotations** to open the Annotations window. This window is where your numeric algorithm analysis results appear.

3. Go to the View menu and select **Analysis**.

   You see the Algorithm Server Job where you can select the algorithm macro you want to use.



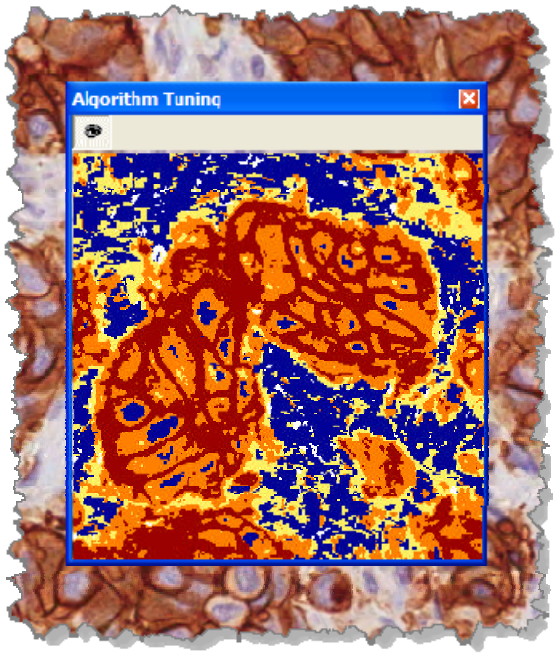
4. Select the macro you want to use and click **Test**. You see the algorithm parameter window.



These instructions discuss using the tuning window when opening an image from Spectrum.

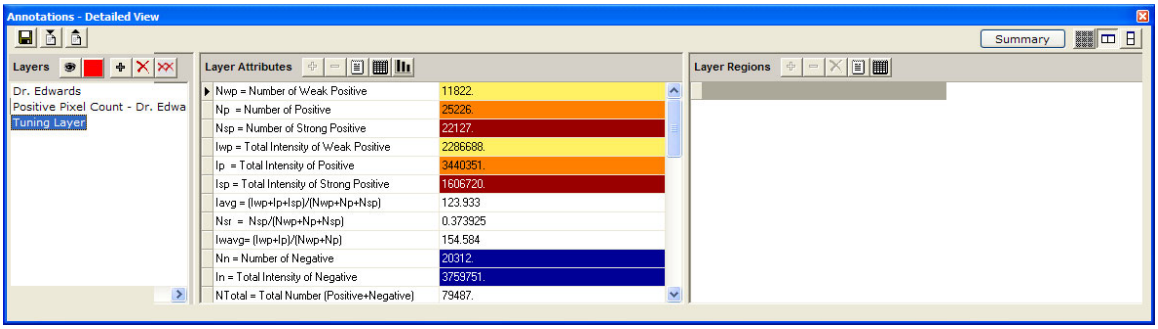
You can also use the algorithm tuning window when you open a local image—just remember that in this case analysis results will be saved locally, not in the Spectrum database. For details on analyzing local images, see the previous chapter.

5. On the algorithm parameter window, click **Tune**. On the ImageScope main window, you see a new Algorithm Tuning window with the mark-up image from the analysis using the current parameters.

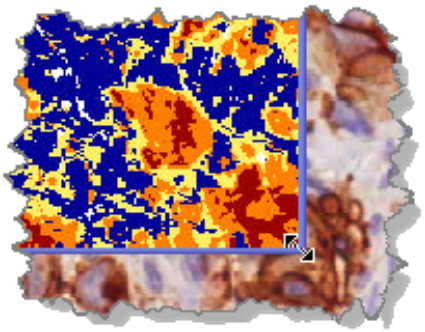


Click the eye icon to turn the mark-up display on or off.

You see the numeric results of the analysis in the Annotations window *Tuning Layer*:



6. To adjust the size of the Algorithm Tuning window, grab a corner until you see the double-headed arrow and pull the window to change the size.



Every time the Algorithm Tuning window updates the analysis, a new mark-up image appears in the window and the numeric data in the Annotations window changes to reflect the new analysis.

7. To see the analysis of another area of the digital slide, drag the Algorithm Tuning window to another area or move the digital slide under the window.

8. To see the results of the analysis when you change the parameters, simply change the parameters in the algorithm parameters window and the tuning window will update.

Note on the Algorithm Tuning Window

The purpose of the algorithm tuning window is to give you way to quickly see the results of analyzing a different area of an image or to test changes you make to the algorithm parameters. If you are viewing the digital slide in ImageScope at the same magnification as the one used to create the digital slide (for example, you scanned the glass slide at 20x and you are viewing the resulting digital slide at 20x), then the tuning window will give the same results as running the algorithm on the selected area. If you are viewing the digital slide at a different magnification than its original scan magnification, the tuning window results may differ slightly from those obtained by running the algorithm on the same area.

16
Digital Slide Conferencing

Digital slide conferencing makes it possible for several participants to view the same digital slide from multiple, remote locations. This section discusses how to use the Digital Slide Conferencing feature of ImageScope.

About Digital Slide Conferencing

ImageScope Digital Slide Conferencing (DSC) not only allows multiple people to see the same digital slide at the same time, but also provides the following features:

- Synchronized viewing so all participants see the same region of the digital slide at the same time
- Real-time annotation sharing
- Leader/follower roles and the ability to change those roles

All conference participants must have ImageScope installed on their workstations. (Download the latest free ImageScope software from www.aperio.com.)

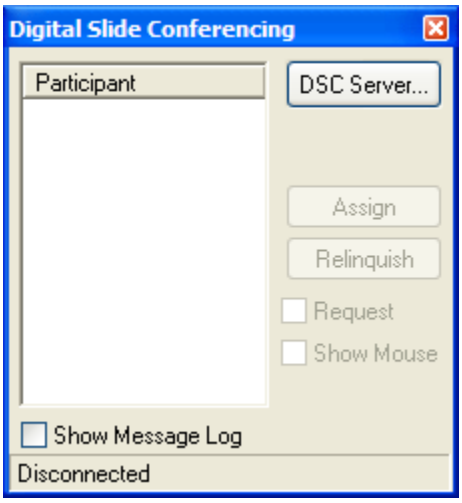
Concepts

- The person hosting the conference is called the *leader*. This is usually the person who created the conference.
- Participants who join the conference are called *followers*. Followers can see the digital slide the leader opens on the ImageScope viewer along with any annotations the leader makes.
- Conferencing requires that all parties have access to a common DSC server and a common ImageServer or network share where images are stored.

Starting a Digital Slide Conference

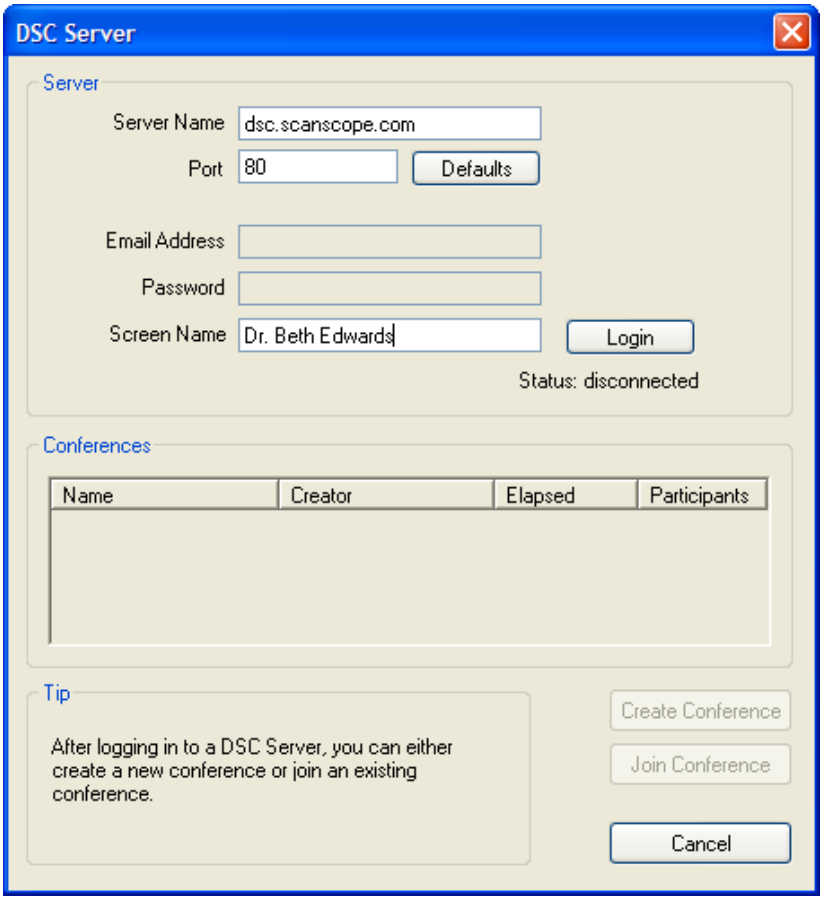
1. Log into Spectrum and open a digital slide by clicking a digital slide thumbnail on a Spectrum page.

2. Go to the **View** menu and click **Digital Slide Conferencing**. The following window appears:



Connecting to a Digital Slide Conferencing Server

1. On the **Digital Slide Conferencing** window, click **DSC Server**. The following window appears:

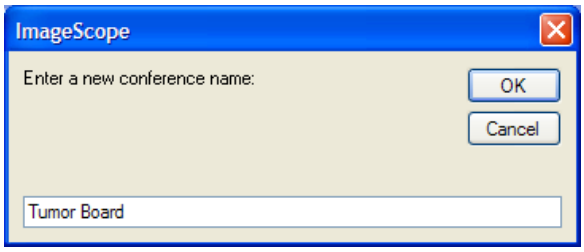


If you set up the DSC server on your workstation, make sure your workstation is accessible to others. See your network administrator if necessary to make sure the correct ports are open, firewall permissions are set appropriately, and so on.

2. Type the following information:
   - **Server Name –** The example shows a DSC server running at dsc.scanscope.com. Type in the name of the server on your network that is running the DSC service. Usually this is on your DSR, a dedicated server, or it may be on your workstation.
   - **Port –** Type the number of the port that has been configured for DSC. This is usually 80, but can be set to another port.
   - **Email Address –** This is not required.
   - **Password –** This is not required.
   - **Screen Name –** Type the name you want to use as the leader of the conference; the conference participants will see you identified by this name in the conference participant list.
3. Click **Login**. The status changes to **Connected** and the **Create Conference** button is enabled.

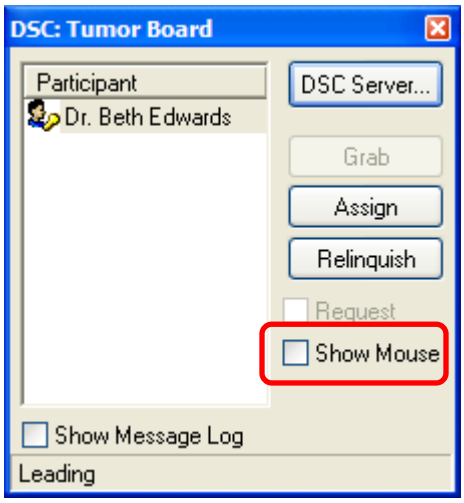
Creating a Conference

1. Click the **Create Conference** button and you are asked to enter the name of your conference:



2. Enter any name you wish and click **OK**. You are notified that you are the conference leader:

The baton next to your name indicates that you are the conference leader.



3. If you want your mouse cursor to appear on the participants' view, select the **Show Mouse** check box.

The conference has now been created.

Opening an Image to Share

The conference leader may open one or more digital slides to share in the conference. Because all participants must have access to the images too, the leader must open images on an ImageServer using the **Access Remote Server** command or directly from Spectrum. (You cannot share images in a digital slide conference if they are only on your workstation.)

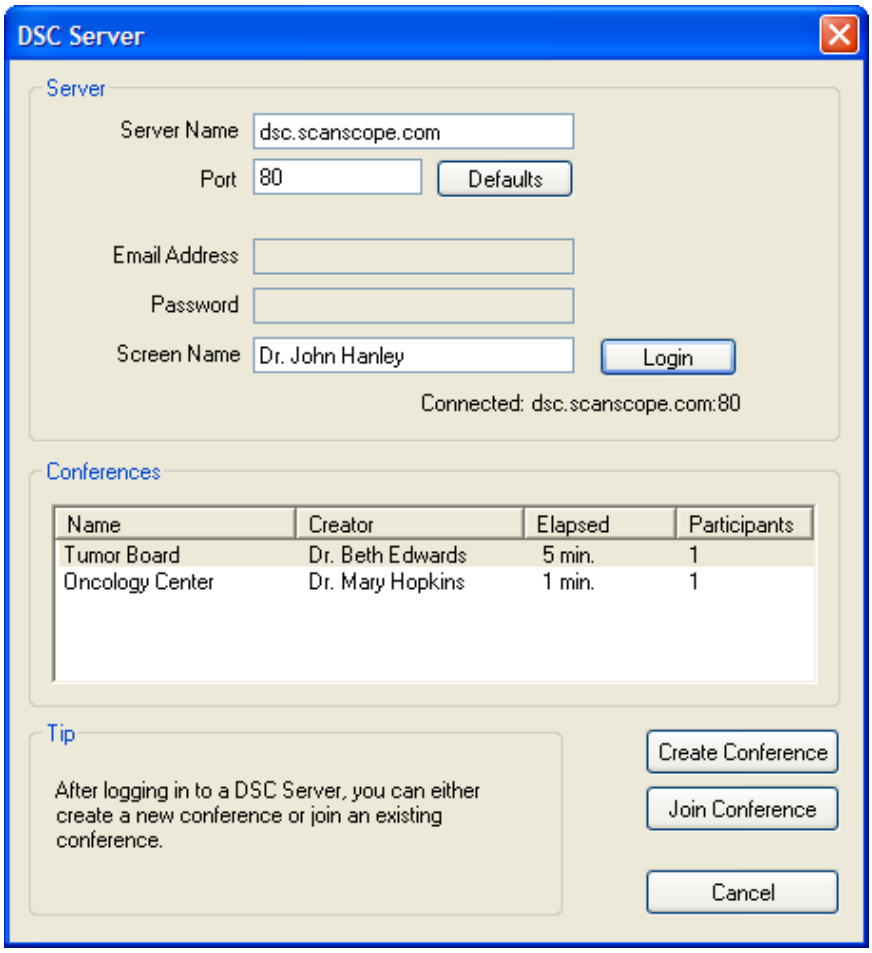
The leader can open multiple images and then move between them during the conference using the ImageScope filmstrip.

Joining a Conference

Once a conference has been created, participants may join.

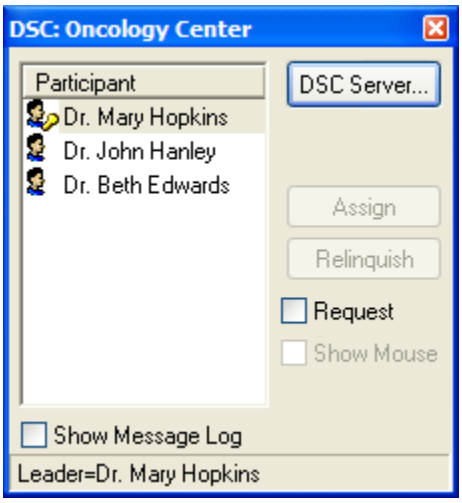
1. Log into Spectrum and open any digital slide by clicking on a digital slide thumbnail on a Spectrum page.

2. Follow the instructions in "Connecting to a Digital Slide Conference Server" on page 146 to connect to the DSC server. The following window appears listing any conferences running on that server:



Type the screen name you want to display to the other conference participants.

3. In the Conferences section select the conference you want to join and click **Join Conference**. The Digital Slide Conferencing window appears, listing you as a participant:



In the ImageScope window you now see the digital slide the conference leader has opened to share.

Viewing Slides in Conference

The conference leader is in charge of the conference:

- As the leader zooms and pans the digital slide, the view of the other participants changes to reflect what the leader is doing. The leader's mouse position is visible as a pointer on each participant's monitor if the leader selected the **Show Mouse** check box.
- Each participant may have a different screen size or window layout; each window is kept centered following the leader's actions, but the image is adapted to the participant's window configuration and monitor resolution.
- Only the leader can add annotations; these are seen by all participants.

Changing the Conference Leader

There may be times when either the leader or one of the followers wishes to change the conference leader. For example, one of the followers may wish to point to something on the digital slide for the other participants or the leader may leave the conference before it is finished.

If the leader of the conference exits the conference before it is finished, the next person who joined automatically becomes the leader.

When conference leadership changes, ImageScope lets you know:

Leader Initiates Change in Leadership

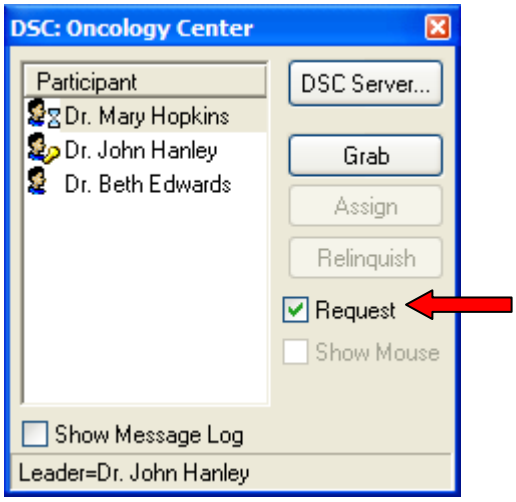
If the leader wishes to assign leadership to another participant:

1. Select a follower in the list of participants and click **Assign**. The new leader receives the message:

Follower Initiates Change in Leadership

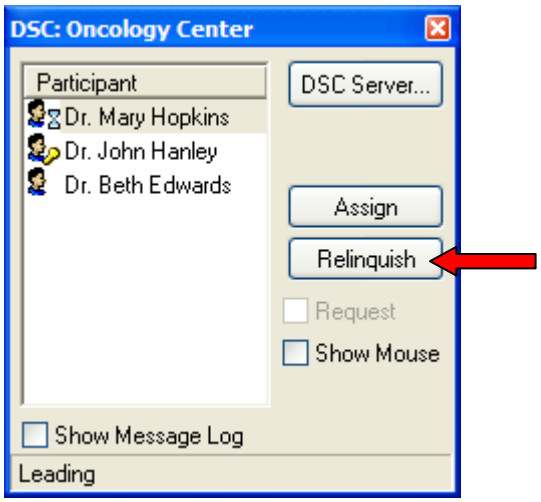
If a follower wishes to become the conference leader:

1. On the Digital Slide Conferencing window, select the **Request** check box.



   The DSC server maintains a list of all participants who want to become the leader. An hourglass appears next to the names of followers waiting to become the leader.

2. The current leader passes leadership to the next waiting participant by clicking **Relinquish**.

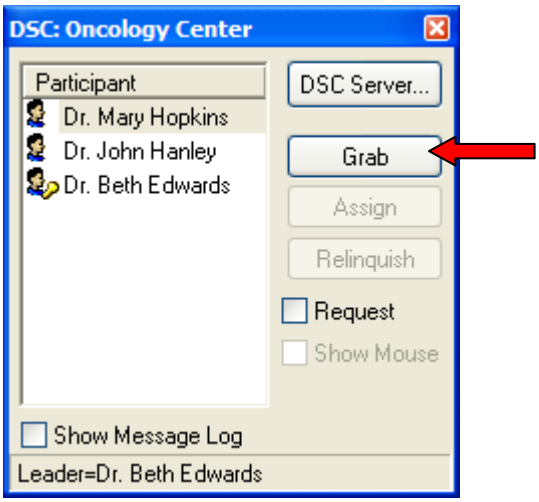


The requester is notified that she has leadership:

Conference Creator Re-asserts Leadership

If the person who created the conference wants to become leader again:

1. On the Digital Slide Conference window, click **Grab**.



17
TelePath Live

TelePath Live (Remote Revisit) is not approved by the FDA for clinical use.

TelePath Live, also known as Remote Revisit, provides a way to connect to your ScanScope remotely.

By using TelePath Live to directly connect to your ScanScope, you can:

- See a live video feed from the ScanScope from a remote location.
- Capture Z-stacks so you can view specimens in multiple focal planes.
- Perform a scan directly from ImageScope.

ScanScope Compatibility Notes

- For ScanScopes that contain an AutoLoader—to use the Z-stack feature a slide must be on the slide tray; you cannot capture Z-stacks from slides in an AutoLoader.
- Some older ScanScope models cannot use the TelePath Live feature; contact Aperio Technical Support if you have questions about whether your ScanScope can use TelePath Live.
- ScanScope CS scanners support TelePath Live from all five slide positions in the slide tray.

Before You Use TelePath Live

There are some things you should do and know before you use TelePath Live.

Calibration

Your ScanScope has been calibrated at the factory. However, if while using TelePath Live you find that the Live window does not line up with your cursor position in the ImageScope main window, you will want to calibrate it again.

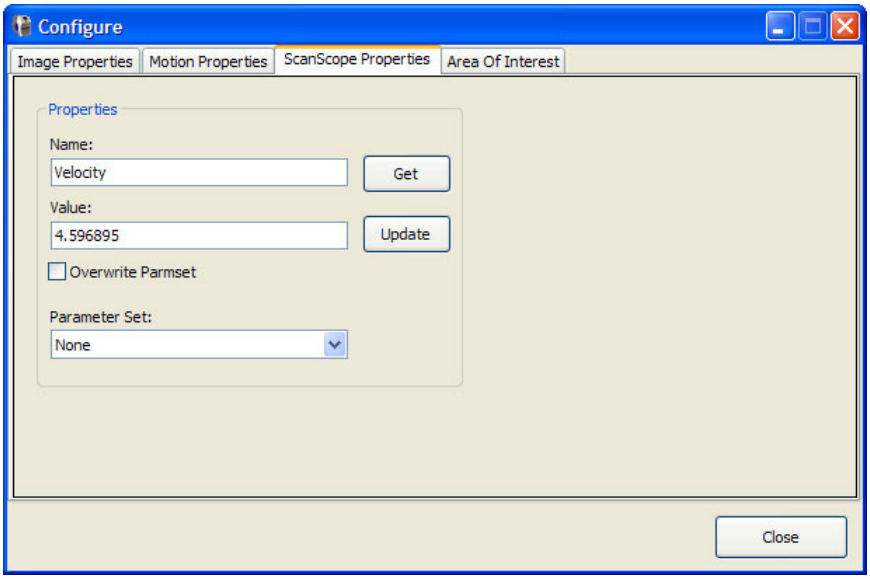
For information on calibrating the ScanScope in preparation for using TelePath Live, please see the *TelePath Live (Remote Revisit) Setup Application Note*.

Setting up the ImageServerURL

In order for the Console program **View Slide** button to bring up images in ImageScope that can be used for TelePath Live, the ImageServerURL parameter needs to be set up with your ImageServer name.

Here is how to set up ImageServerURL:

1. Start the ScanScope Console program by clicking **Start > All Programs > ScanScope > Console**.
2. Open the Configure window. (See your *Console User's Guide* for specific instructions.)
3. Click the **ScanScope Properties** tab.



4. In the **Name** field, type: **ImageServerURL** and click **Get**.
5. The name of your ImageServer appears in the **Value** field.
   a) If the ImageServer name is correct, you are done.
   b) If the ImageServer name is not correct or is blank, type the correct name of the ImageServer into the **Value** box and click **Update.**
6. Click **Close** to exit the Configure window. After you exit the Console, your change to ImageServerURL will take effect the next time you start the Console again.

Here is a little more information about why you need to set this parameter:

When ImageServerURL is set to a valid ImageServer machine name, ImageScope will open the digital slide by passing the imageID directly to ImageServer. ImageServer will then use the database to figure out where the digital slide is located. You can tell that you are in the *database mode* if you see a number beginning with the @ symbol in the title bar of ImageScope.

You must use ImageServer in this database mode in order to save any captured Z-stacks into the database. If ImageServerURL is not set, Z-stacks you capture in ImageScope will not be saved in the database when you open the digital slide using the **View Slide** button in the Console.

What Is a Z-Stack?

A Z-stack is a three-dimensional image that allows you to view a specimen by moving up and down in the focal planes. This is especially useful for viewing "thick" specimens.

Z-stack images are cataloged in the ImageServer database, and linked to the original image as an annotation. One of the reasons for using the TelePath Live feature is to capture and view Z-stack images. See "Capturing Z-stacks" on page 163 for more information.

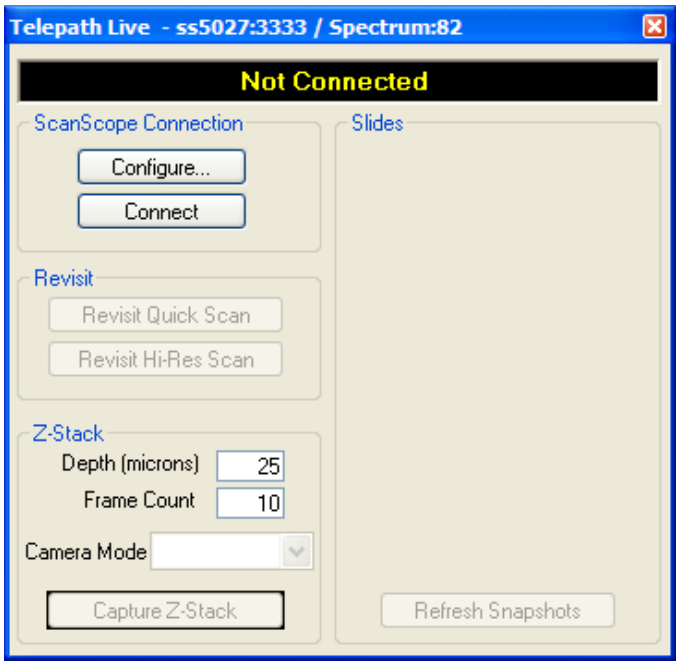
Troubleshooting Tips

If you are unable to connect to the ScanScope, contact your network administrator to verify the following conditions are true for your network:

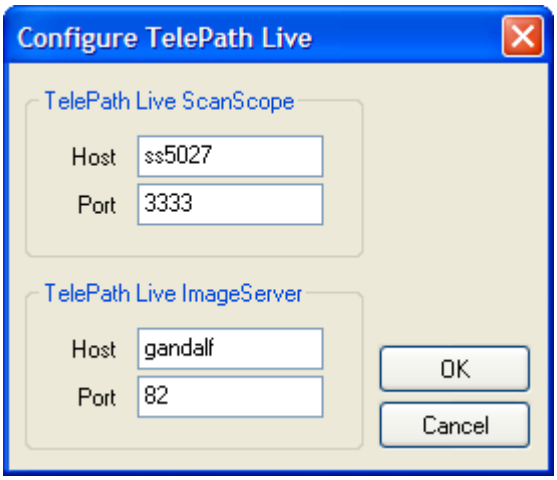
- Port 3333 is open and directed to the ScanScope controller.
- Port 82 is open and directed to the ImageServer.

Connecting to a ScanScope

1. Go to the ImageScope **View** menu and select **TelePath Live**. The following window appears:



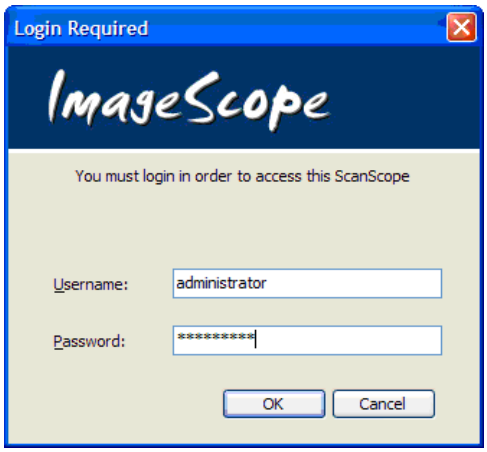
2. Click **Configure** to define which ScanScope to connect to. The following window appears:



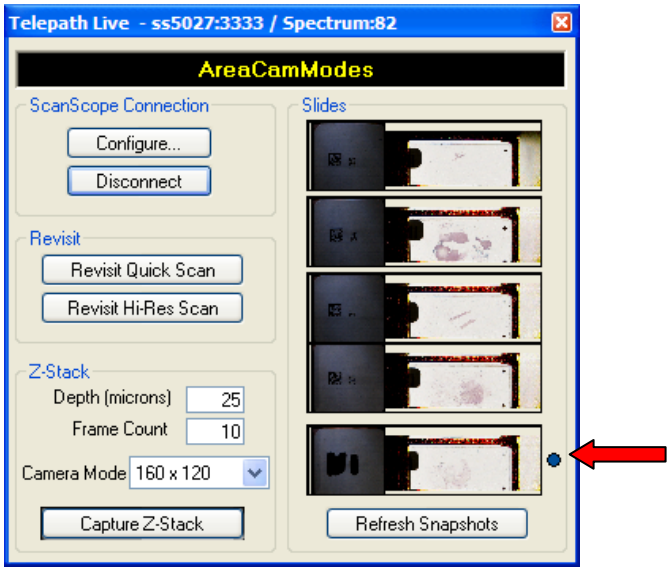
3. Type the name of the ScanScope and its port (usually 3333).
4. Define the ImageServer by typing the host name and port number. If you are using a ScanScope CS or T3, this will be the same name and port number as your ScanScope.

   Note that the name of your ImageServer host will normally be the same as the ImageServerURL setting (see "Setting up the ImageServerURL" on page 155) unless you access the ImageServer from outside a firewall, in which case it may be different—contact your network administrator for assistance if you think this may be the case.
5. Click **OK**.
6. On the TelePath Live window, click **Connect**.

7. You will be asked to log in. Enter your Spectrum user name and password.

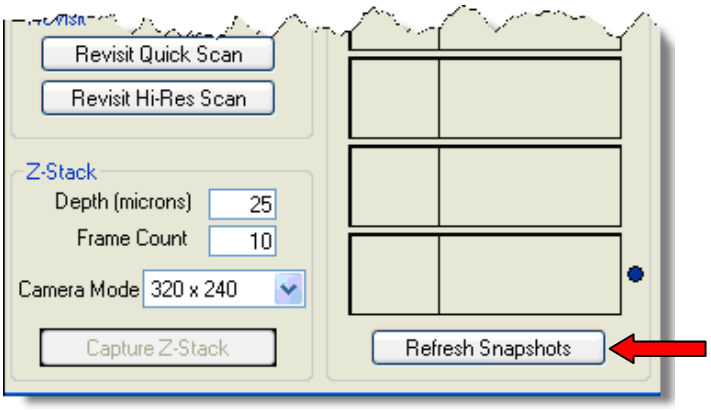


After a few seconds, you see a window that looks something like this, showing slides that are loaded in the tray. You are now connected to a ScanScope.



The blue dot indicates the slide you are working on. You can click on any slide displayed to work with that slide.

If instead the window has no slides displayed:



Click the **Refresh Snapshots** button to tell the ScanScope to load macro slide images.

Preparing a Slide for TelePath Live

There are two ways to prepare a slide to be used with the TelePath Live window:

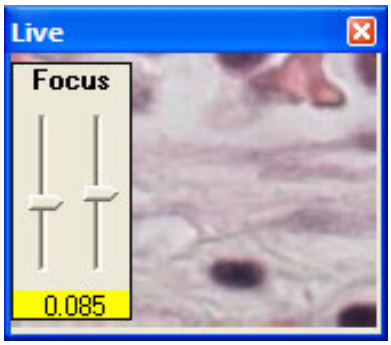
1. Scan a slide on the ScanScope and click the **View Slide** button in the Console. When ImageScope opens, it knows about the slide and you can begin placing Z-stack images on the digital slide from the ImageScope TelePath Live window.
2. Use the **Revisit Quick Scan** or **Revisit Hi-Res Scan** buttons on the ImageScope TelePath Live window. (See "Capturing Z-Stacks" on page 163.)

Viewing Live Video from the ScanScope

Connect to a ScanScope and open the ImageScope TelePath Live window as discussed in "Connecting to a ScanScope" on page 157.

After a scan is performed (see the previous section), click around on a digital slide in the TelePath Live window and see a live video feed from the ScanScope showing the tissue in the Live window.

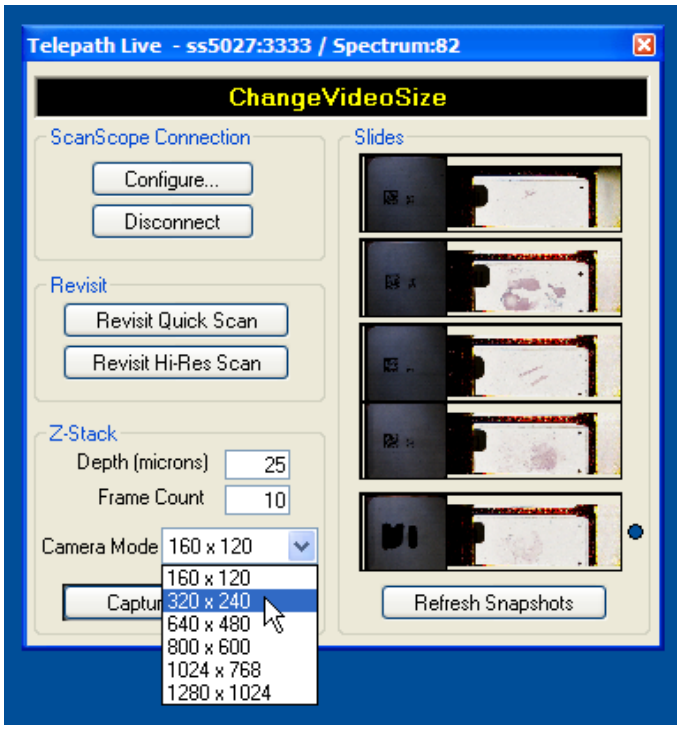
When using TelePath Live, coarse and fine focus sliders provide fine-resolution focusing, allowing you to fine-tune the focus:



The right slider's range is 10% of the entire focus range provided by the left slider, centered on the value of the left slider. For example, if the left slider is set at 50, the right slider's range is 50 – 5% of the focus range to 50 + 5% of the focus range.

Use the slider on the left to get the image roughly into focus. Then use the slider on the right to fine-tune the focus.

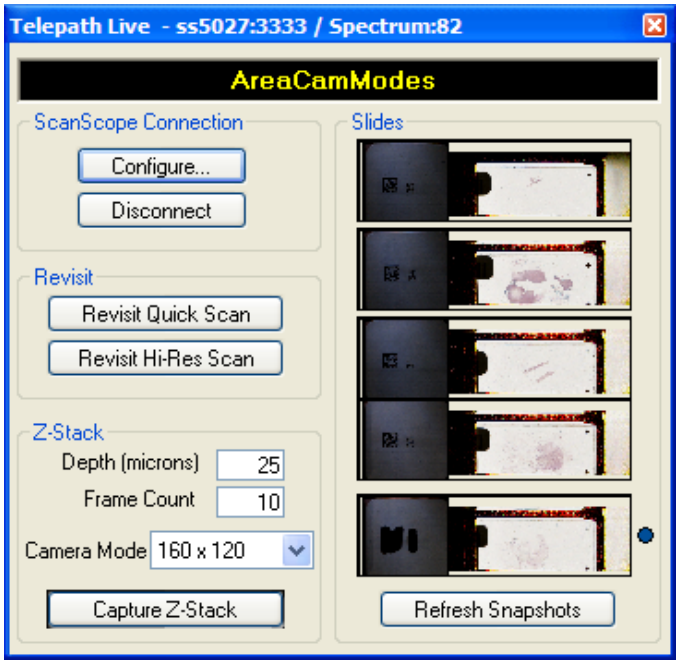
- Adjust the size of the Live window by using the Camera Mode drop-down list to select a window size:



- If you find an area of interest, click the **Capture Z-Stack** button on the TelePath Live window to capture a Z-stack (3-dimensional) image of that area. (See the next section.)

Capturing Z-stacks

1. Open the TelePath Live window and connect to a ScanScope as discussed in "Connecting to a ScanScope" on page 157.



   The blue dot indicates the currently selected slide. Click on any other slide if you want to use it instead.

2. Choose your Z-stack options:

   - **Depth –** The total number of microns in the Z-stack.
   - **Frame Count –** The number of individual snapshots taken. The limit is 50.
   - **Camera Mode –** The width and height of the Z-stack snapshots. These are limited by the available modes of the area camera.

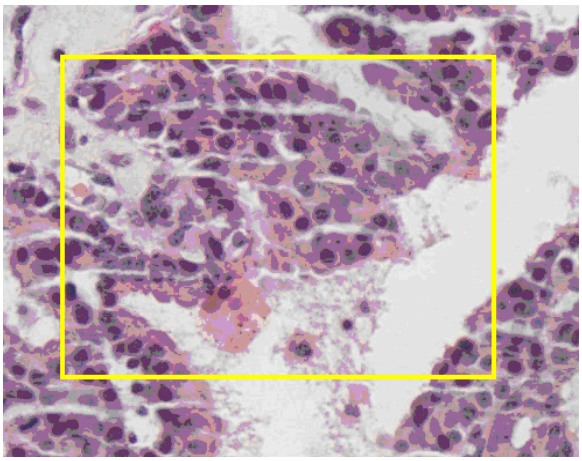
3. If you did not open ImageScope by clicking the **View Slide** button in the Console program, you may need to scan the slide:

   - Clicking the **Revisit Quick Scan** button on the TelePath Live window performs a high-resolution macro scan of the slide, generally used when time is short. The slide will be scanned at 5x.
   - Clicking the **Revisit Hi-Res Scan** button on the TelePath Live window is the same as pressing the green button on the ScanScope. Only the currently selected slide will be scanned, and it will be scanned at the currently selected magnification.

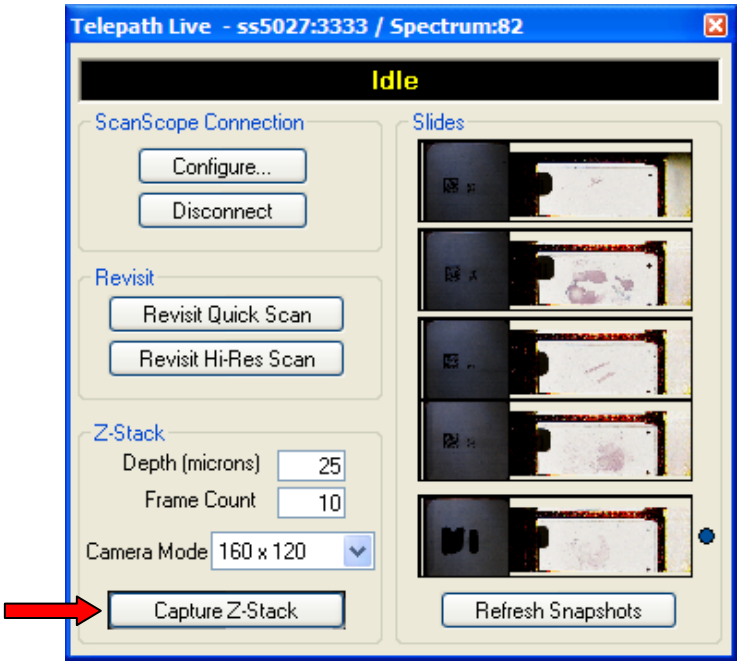
   Whether the slide was scanned from the Console or from the TelePath Live window, when the slide is scanned its image opens in the

ImageScope main window along with a Live window that shows the video feed from the ScanScope.

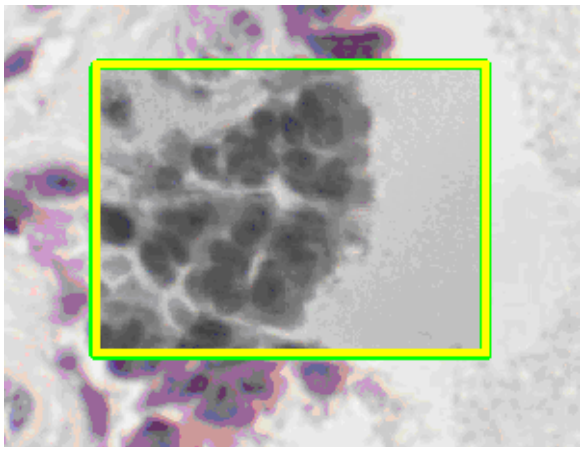
4. Click on the image in the ImageScope main window. A yellow rectangle appears showing what area of the image is displayed in the Live window. This also selects the area to perform a Z-stack capture. To move the selection rectangle, click another location in the main image.



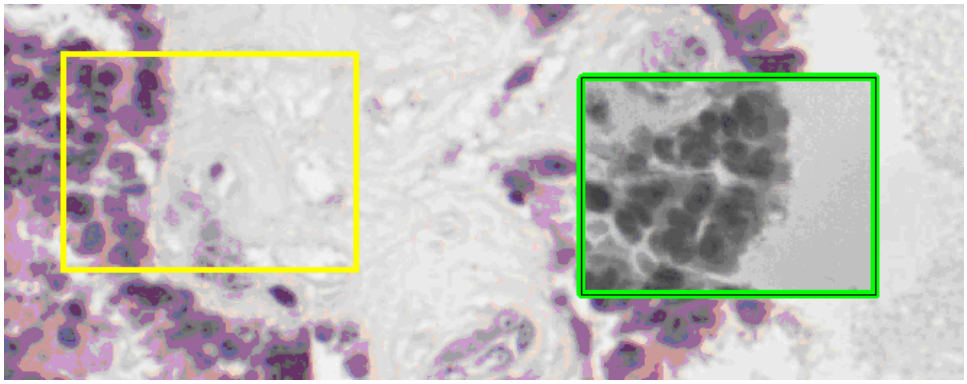
5. To capture a Z-stack from the selected slide, click the **Capture Z-Stack** button.



When you see the message "Opening Z-stack" on the TelePath Live window, the Z-stack has been captured and you see a green rectangle overlaid on top of the yellow rectangle in the ImageScope main window.

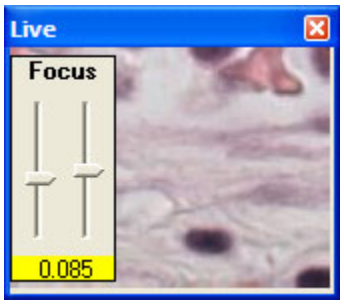


Click elsewhere in the image to move the yellow selection rectangle away from the Z-stack image, and you can see the green rectangle showing the location of the Z-stack.



Viewing Z-stacks

Once you have captured a Z-stack image, you will see that the Live window now contains a coarse and fine focus tool:



To focus through the stack, simply slide the focus bar up and down.

18
Utilities and Diagnostics

The utilities discussed in this section are primarily for troubleshooting problems, and you may be requested to use them by Aperio Technical Support if they are trying to pinpoint a problem.

Logging

If requested to do so by Aperio Technical Support, enable logging by going to the **Tools** menu and selecting **Logging**. This creates a file named viewport.log into which text messages on ImageScope actions are logged.

Typically, if you are having a problem Technical Support will ask you to:

1. Turn logging on.
2. Run until you encounter the problem again.
3. Then email Technical Support the viewport.log file.

You should then turn logging off (by again going to the **Tools** menu and selecting **Logging**) as logging will affect system performance.

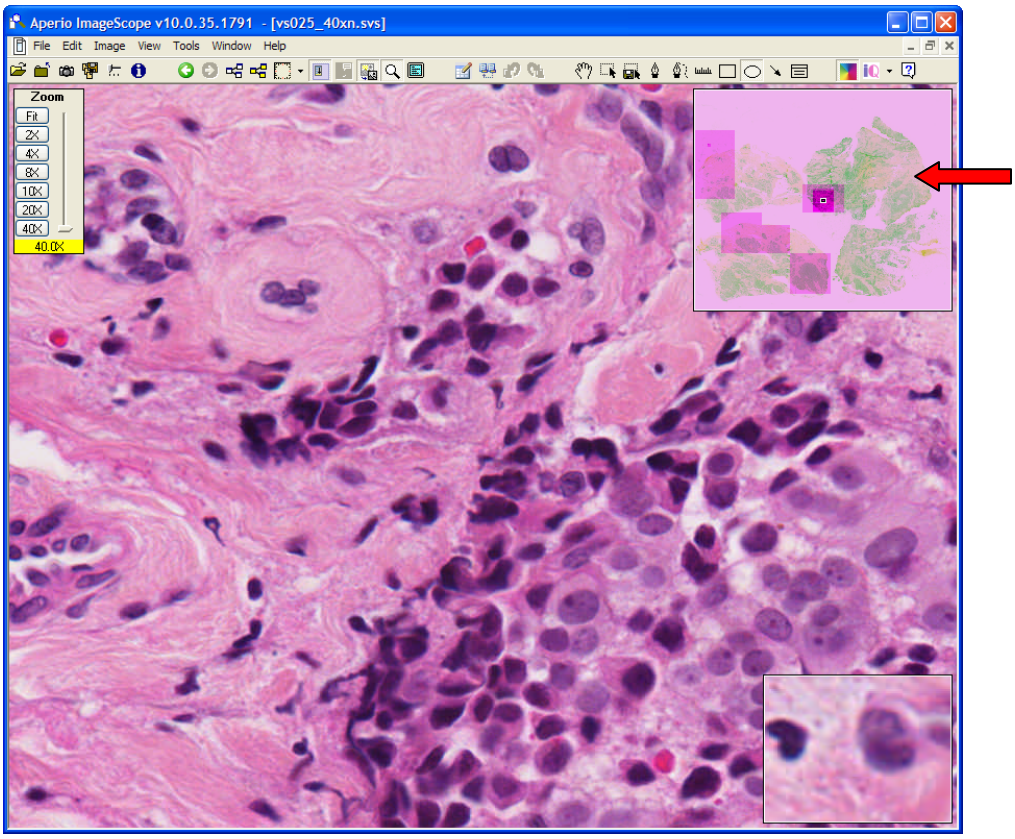
Cache Display

Because digital slides are typically much larger than can be loaded into memory all at once, ImageScope keeps portions of the digital slide in computer memory so it can display them quickly. It prefetches data for the digital slide from disk or from a remote server, determining which portions of the image to fetch by anticipating where the user is going to go next.

Enable the cache display by typing Shift Control M. (Type Shift Control M again to turn it off.)

The cache display shows which parts of the digital slide are loaded into ImageScope's cache memory. The darker the area, the higher the resolution of the part of the image that is loaded.

Below is an image of the ImageScope screen showing the cache display:



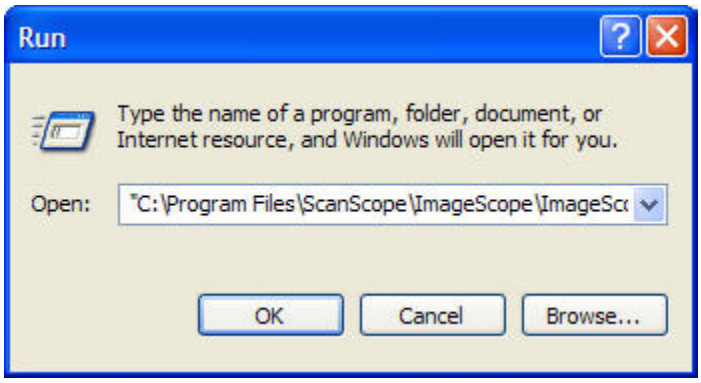
Running Multiple ImageScopes

ImageScope normally allows only one instance of itself to run. (If ImageScope is open and you try to start it again, the first copy of ImageScope closes.) There may be special occasions when troubleshooting a problem or testing Digital Slide Conferencing, for example, when you want to run more than one copy of ImageScope at a time.

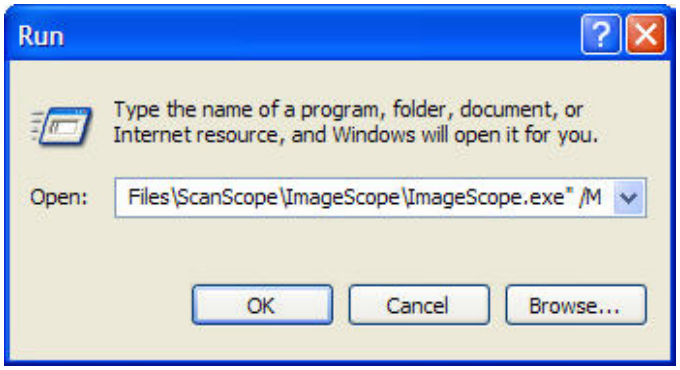
To run multiple copies of ImageScope:

1. Click **Start** and select **Run**.
2. Click **Browse** on the Browse window and navigate to the ImageScope.exe file on your workstation. (The default location is: C:\Program Files\ScanScope\ImageScope.)
3. Click **Open**.

The Run window now contains the path to the ImageScope.exe file.



4. At the end of the path specification, type a space and the characters **/M** (outside of the quotation marks) so it looks like this:

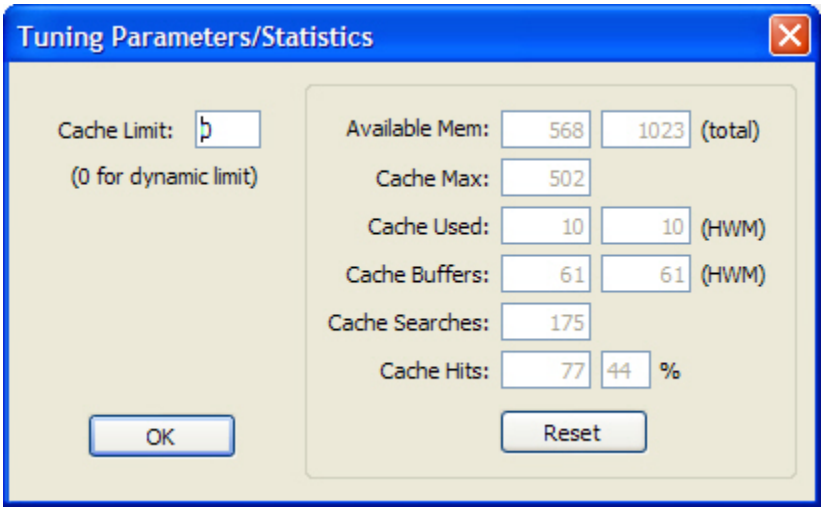


5. Click **OK**. One copy of ImageScope has now started. Repeat this procedure again to bring up a second copy of ImageScope.

Tuning Parameters/Statistics

To see how ImageScope is making use of memory and caching:

1. Go to the **Tools** menu and select **Advanced**. The following window appears:



Maximum Cache Size

- **Cache Limit –** Limits the amount of memory that will be allocated for image caching. If you set this to zero, then ImageScope will use up to 75% of your system's memory as needed. You will normally want to leave this set to zero. If you want to set the cache limit to a specific size, type the number of megabytes you want to use. For example, **128** sets the cache limit to 128 megabytes. This is the only value on this screen that you can change (except for resetting the statistics to zero).

Statistics

The following fields change as you view and pan images. ImageScope only reports these values if a digital slide is open. You can reset them to zero by clicking **Reset** (unless noted otherwise below).

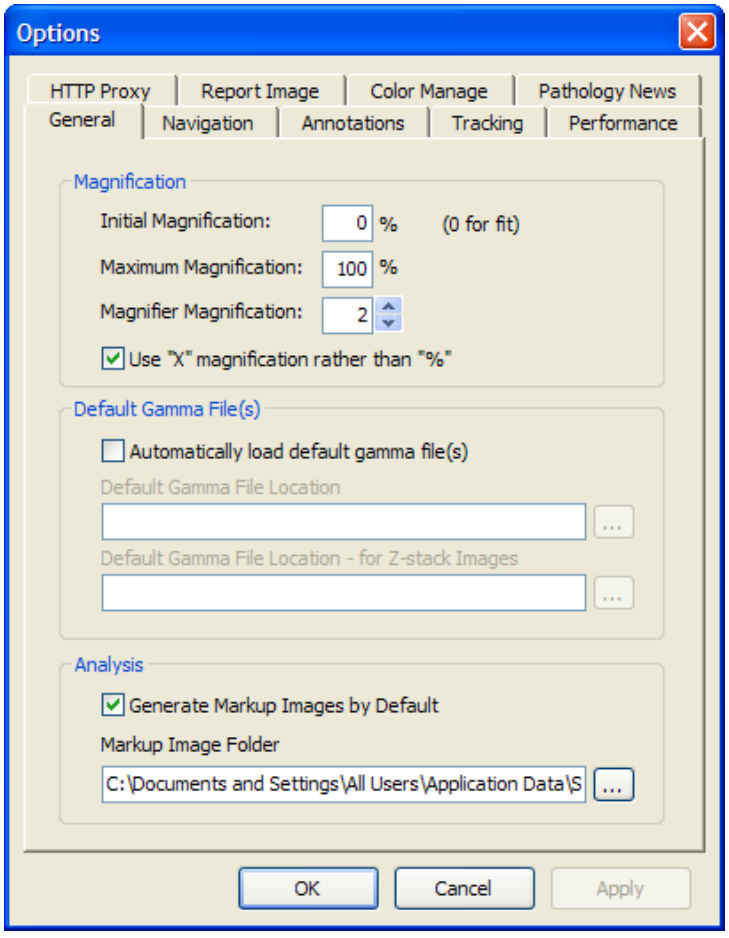
- **Available Mem –** Total amount of physical memory not in use on your system. The **Reset** button does not affect this value.
- **Total Mem –** Total amount of memory installed on your system. The **Reset** button does not affect this value.
- **Cache Max –** Amount of cache memory used in megabytes.
- **Cache Used –** Current amount of memory cache used in megabytes.
- **Cache Used HWM –** High water mark for memory cache in megabytes.
- **Cache Buffers –** Current number of buffers stored in cache.
- **Cache Buffers HWM –** High water mark for buffers stored in cache.
- **Cache Searches –** Number of cache searches.

- **Cache Hits –** Ratio of cache hits to cache searches.
- **Cache Hits % –** Ratio of cache hits to cache searches.

19 ImageScope Options

Use the Options command to set and review ImageScope settings and preferences.

To access ImageScope's settings, go to the **Tools** menu and select **Options.** The Options window appears:

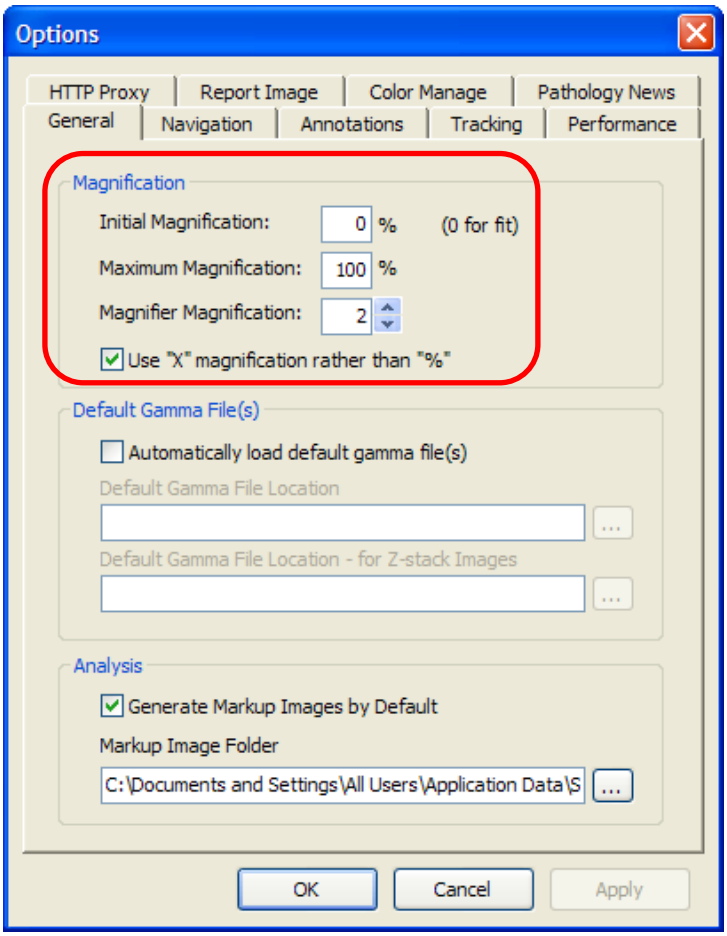


General Options

The general options are organized into three areas:

- Magnification
- Default Gamma Files
- Analysis

Magnification



The magnification options are:

- **Initial Magnification –** Specify the initial magnification for images as they are opened. This can be a percentage from 1 to 100 or you can specify 0 to fit the entire digital slide in the main window display.
- **Maximum Magnification –** Specifies how far you can zoom into the image. Note that setting this value to greater than 100% does not increase the resolution of the image—you are simply enlarging the pixels of the existing image.
- **Magnifier Magnification –** Specifies the ratio between the main window display and the magnifier window. For instance, if this value is set to 3, and you are viewing the main image at 50% zoom, then the magnifier window will display 150% zoom. See "Using the Magnifier Window" on page 36 for information on the magnifier window.
- **Use "X" magnification rather than "%" –** Adjusts the Zoom slider from displaying magnification in percentages to displaying them as "2x," 4x," and so on. See "Using the Zoom Slider" on page 37 for examples of the Zoom slider in both modes.

Default Gamma Files

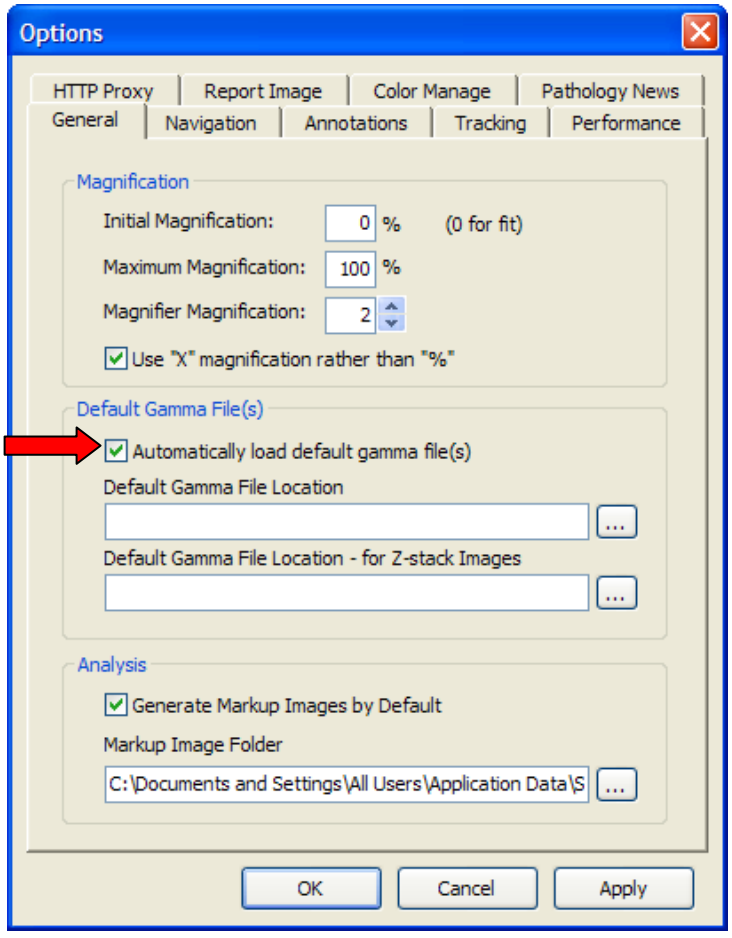
Adjusting the display of a digital slide does not affect the original digital slide, but only its appearance during the current ImageScope session. You can save these adjustments in a gamma table file to be re-applied in a later session to one or more digital slides. (See "Saving and Loading Color Settings" on page 53 for information on creating gamma table files.)

The Default Gamma File sections on the General tab allow you to load specific gamma table files every time you start up ImageScope. This can be useful if you know you need to compensate for a particular monitor, for example (although a better long-term solution is to calibrate the monitor so it does not have a color bias).

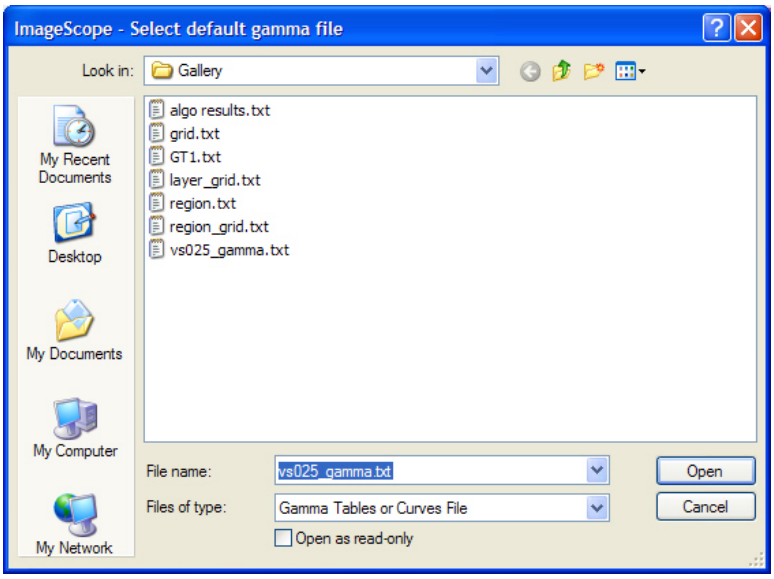
Loading a Default Gamma Table File for the Main Image

To load a default gamma table file to be applied to all images opened in the main ImageScope window:

1. Select the **Automatically load default gamma file(s)** check box.



2. Use the browse button in the **Default Gamma File Location** text box to search for the gamma table file you want to use. The Select default gamma file window appears.



3. To protect the selected gamma table file from being modified, select the **Open as read-only** check box.
4. Select the gamma table file you want to use and click **Open**.

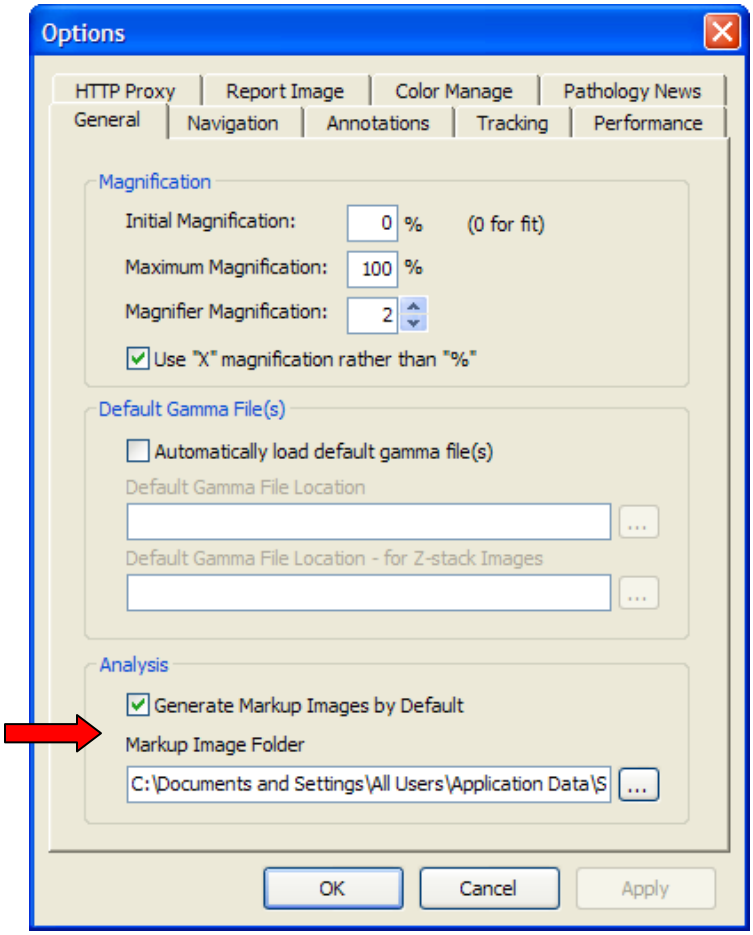
Loading a Default Gamma Table File for Z-stack Images

To load a default gamma table file to be applied only to Z-stack* images:

1. Select the **Automatically load default gamma file(s)** check box.
2. Use the browse button in the **Default Gamma File Location – for Z-stack Images** text box to search for the gamma table file you want to use.
3. To protect the selected gamma table file from being modified, select the **Open as read-only** check box.
4. Select the gamma table file you want to use and click **Open**.

Analysis

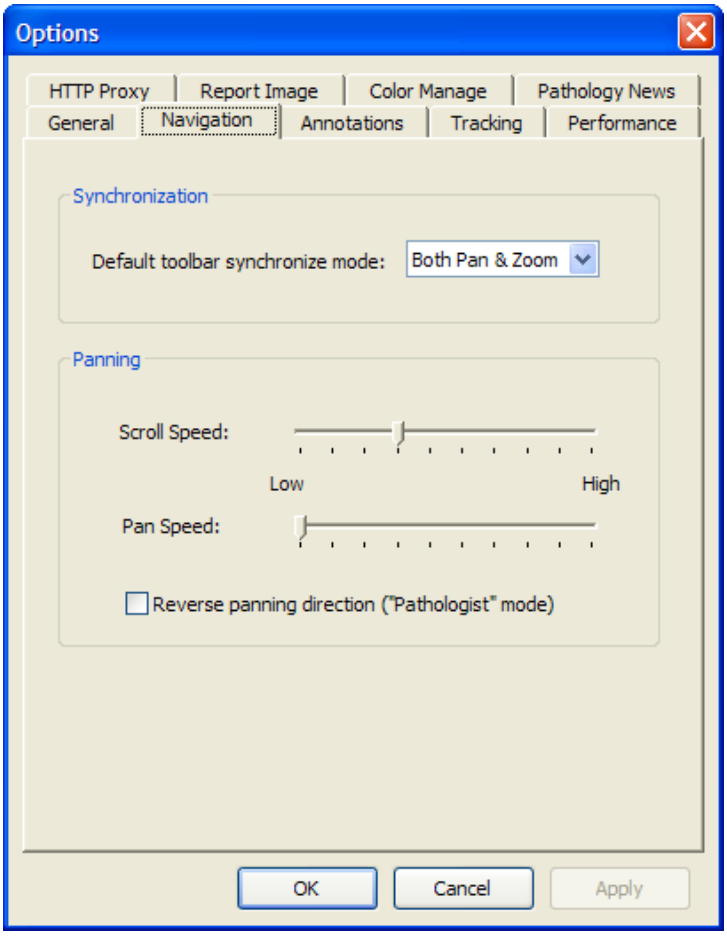
When you run an algorithm† to analyze a digital slide, you can optionally request that the analysis generate a visual representation of the analysis in addition to providing quantitative data. (See Chapter 14, "Analyzing Digital Slides" on page 119 for more.) This visual representation is called a *markup image*.



- To instruct ImageScope to always generate a markup image by default (this setting can be overridden when you actually perform the analysis), select the **Generate Markup Images** check box.
- To specify the location of the markup file, click the browse button and navigate to the location where you want to store this data. (This location is used for analyses performed on *local* digital slides. Remote analyses store the markup image *only* in the Spectrum database.)

Navigation Options

The ImageScope navigation options affect image panning and multiple image synchronization.



Synchronization Option

When you have multiple digital slides open in ImageScope and they are all visible at the same time, you can click or (yellow) on the toolbar to synchronize navigation among the open images. If the image navigation is synchronized and you pan or zoom the first image, the other images will show the same behavior. The synchronization option defines the behavior of that synchronization:

- **Both Pan & Zoom –** Synchronizes both panning and zooming.
- **Pan Only –** Synchronizes panning only.
- **Zoom Only –** Synchronizes zooming only.

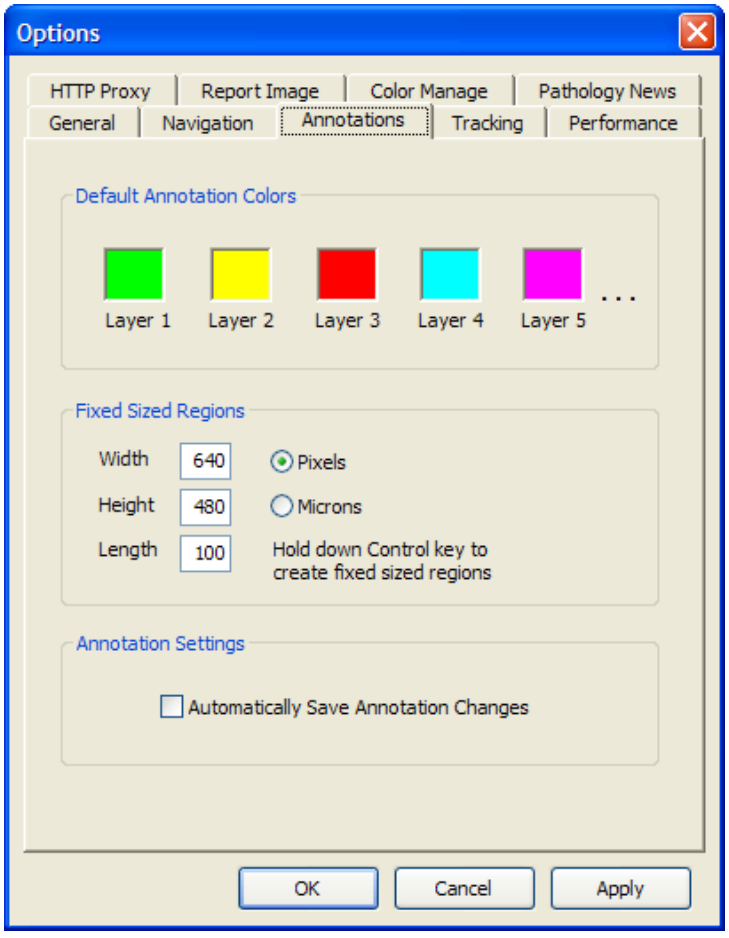
Panning Options

Several panning options are available:

- **Scroll Speed –** Specifies how fast the image scrolls when holding down the mouse button near the edge of an image.
- **Pan Speed –** Specifies how fast the image pans when clicking and dragging the image.
- **Reverse panning direction ("Pathologist" mode) –** Selecting this check box causes panning to move in reverse. With this option disabled, as you click and drag the image to the left, the image moves to the left. With this option enabled, as you click and drag to the left, the image moves to the right.

Annotation Options

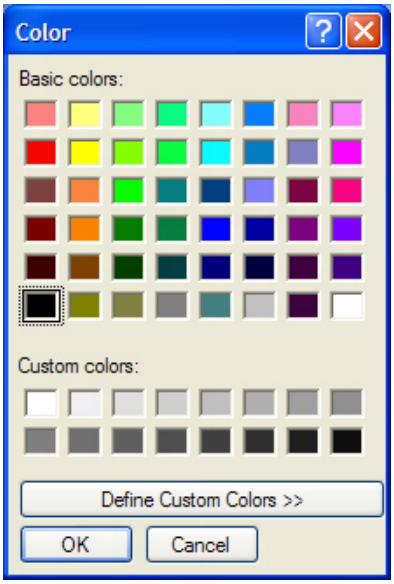
The Annotations tab of the Options window allows you to select various annotation options.



Annotation Color Options

Each annotation layer shows annotations in a different color. The Default Annotation Colors section allow you to select the color to be used for each layer.

1. Click the colored box above the annotation layer for which you want to change the color. You see a standard Windows color selection window:



2. Select a color from the palette shown or click **Define Custom Colors** to define your own color.
3. Click **OK**.

If you use more than five annotation layers, the new layers will re-use the colors defined for the first five layers. For example, layer 6 will use the color defined for layer 1, layer 7 will use the color defined for layer 2, and so on.

Fixed Size Annotations

The Fixed Size Regions options on the Annotations tab set a fixed size for drawn annotations that is used when you hold the Control key down while you draw. These settings are also used to extract a region of fixed size and to zoom to a region of fixed size.

- **Width and Height –** these values are used to define the size of rectangles or circles. For example, to always draw a square of 200 pixels by 200 pixels when you hold down the Control key while using the Rectangle tool, type **200** into the **Width** and **Height** boxes.
- **Length –** This value is used for the ruler and arrow annotations.

See “Report Image Options” on page 185 for information on setting the fixed size of a report image.

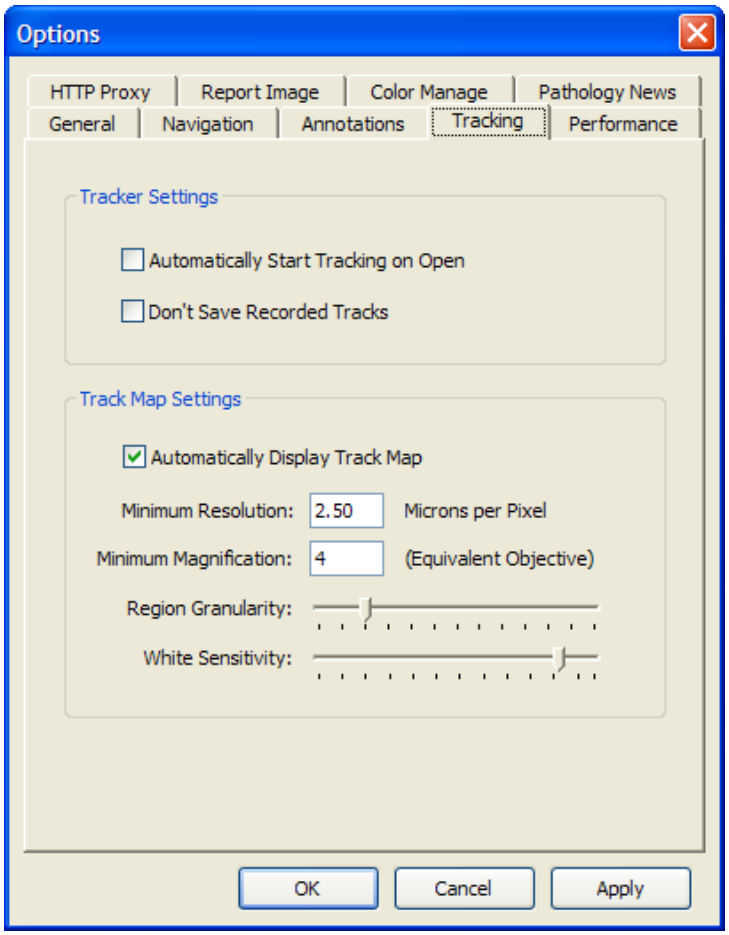
Automatically Saving Annotations

The **Automatically Save Annotation Changes** check box saves annotations and changes to annotations when you exit ImageScope without prompting for a confirmation.

If this option is enabled, but you have also selected **Don't Save Recorded Tracks** on the Tracking options tab, tracks will not be saved when you exit even though other annotations are. (See the next section for information on tracking options.)

Tracking Options

The Tracking tab contains various options that affect tracking, for example enabling tracking every time you open a digital slide in ImageScope. For information tracking ,see Chapter 12, "Tracking," on page 103.



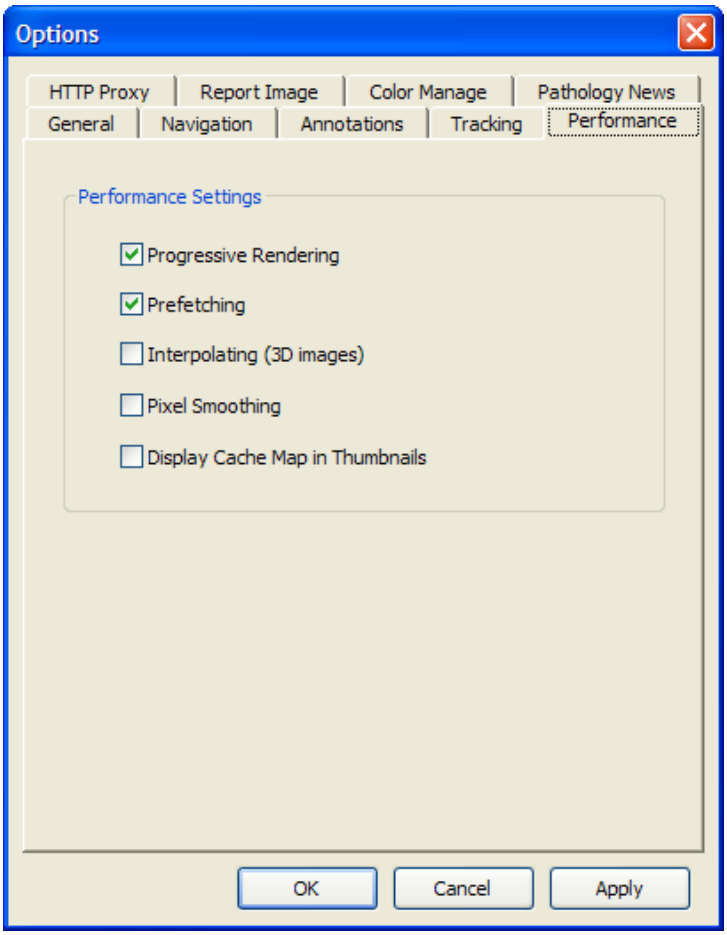
The options on this tab include:

- **Automatically Start Tracking on Open –** Select this check box to always begin recording when you open a digital slide. If a track already exists for the digital slide, opening the slide starts appending a track to the original track.

- **Don't Save Recorded Tracks** – Select this check box to disable saving the track with the digital slide.
- **Automatically Display Track Map –** Select this check box to enable track mapping by default. (This selects the Map check box on the Tracker tool.)
- **Minimum Resolution –** This option specifies the minimum resolution of a view which is mapped. This defines the lowest resolution at which a region of the image may be considered to have been viewed.
- **Minimum Magnification –** This option specifies the minimum magnification of a view which is mapped. This defines the lowest magnification at which a region of the image may be considered to have been viewed..
- **Region Granularity –** The tracker divides the image into granules when deciding what part of the image is glass and what is tissue. By changing the granularity, you affect the glass/tissue distinction. Slide the control to the left to decrease the granule size and to the right to increase granule size. Smaller granules generally mean that less of the image will be classified as glass, while larger granules mean more if it will be classified as glass. You can see its effect if a tracking map is shown in the thumbnail as you move this slider.
- **White Sensitivity –** The tracker shows your progress through tissue, not glass. This setting helps define what is tissue and what is glass. This slider adjusts the "whiteness" of detected glass. Dragging it to the left increases sensitivity, causing less of the slide to be classified as glass, and dragging it to the right decreases the sensitivity, causing more of the slide to be classified as glass. You can see its effect if a tracking map is shown in the thumbnail as you move this slider.

Performance Options

The Performance tab contains several settings that allow you to balance display speed with image quality. These settings are provided because digital slides are usually too large to fit into memory all at once; therefore, some decisions have to be made about how ImageScope will act when swapping data in and out of memory from disk.



- **Progressive Rendering –** When enabled, ImageScope renders image views in low resolution first, and then increasingly improves the image (by de-pixilating it) as it reads more image data from the disk or retrieves it from a remote server. With this feature disabled, ImageScope does not render an image view until the entire view has been loaded into memory.
- **Prefetching –** When enabled, ImageScope tries to anticipate your next view requests and loads those sections of the image into memory. By anticipating where you will move next in the image and loading that information into memory, ImageScope speeds up the image display.
- **Interpolating (3D images) –** Display a weighted average of the two neighboring Z-stack* views for any given Z-level.

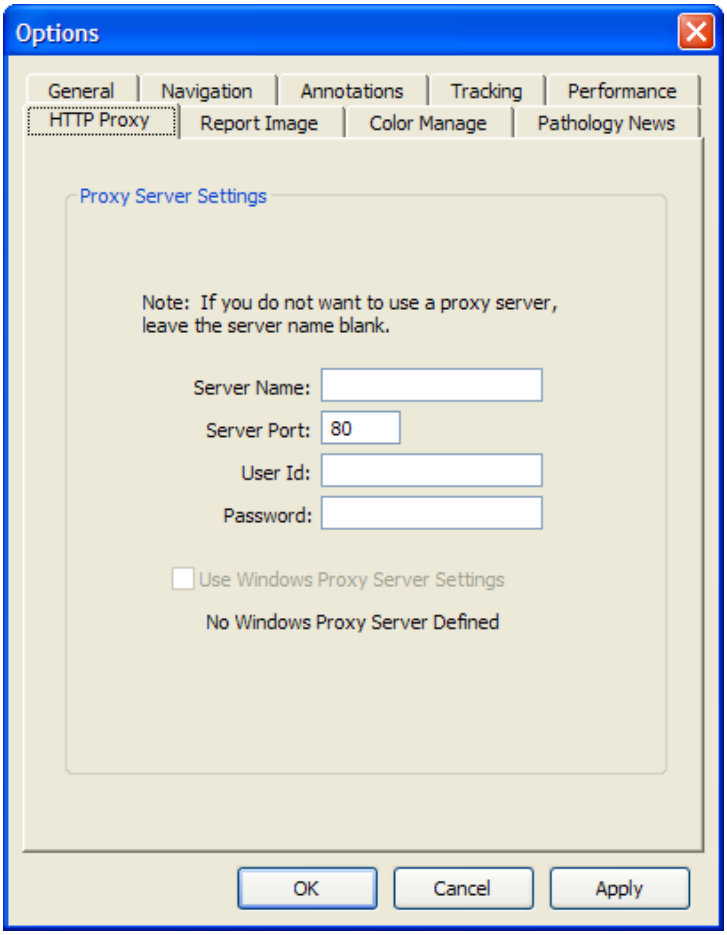
- **Pixel Smoothing –** Uses a high fidelity (but slower) scaling routine.

HTTP Proxy Option

Some networks require that all HTTP traffic be routed through an HTTP proxy server. If you are using ImageScope inside a network that uses an HTTP proxy server, you need to define the proxy server to ImageScope.

Your network administrator can provide the settings you need for the server name, server port, user name, and password. Note: Not all proxy servers require a user name and password.

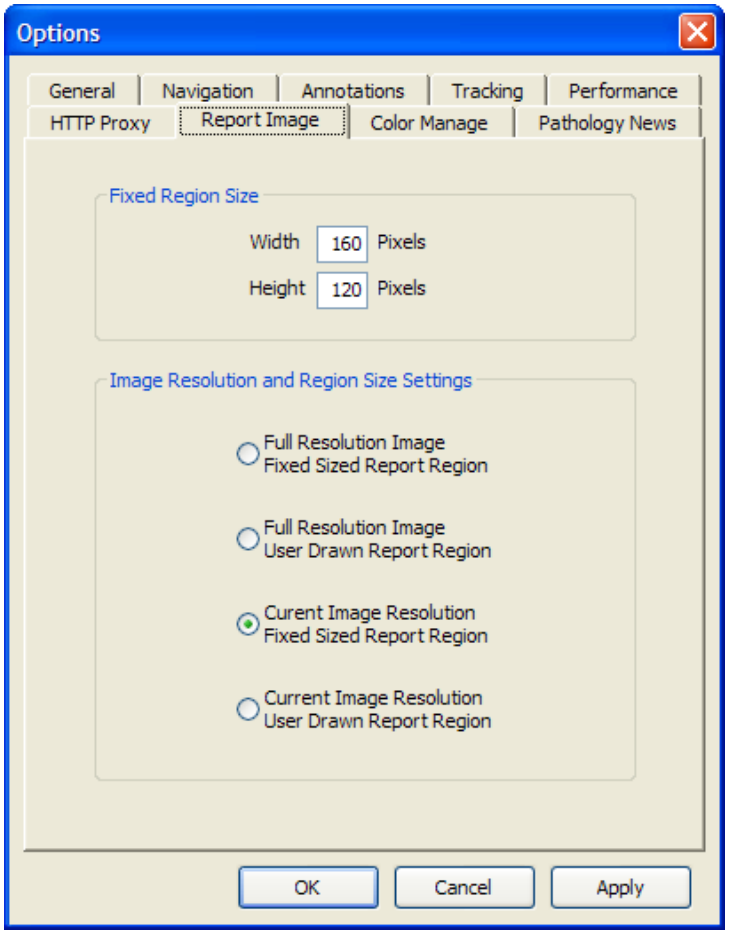
If you have already created Windows proxy settings on your workstation, then ImageScope will use those settings if you select the **Use Windows Proxy Server Settings** check box.



Spectrum Plus Reporting is an optional upgrade that lets you create attractive Spectrum Plus reports with a few clicks of the mouse. The report image feature is used with this product if you are using report templates that contain images.

Report Image Options

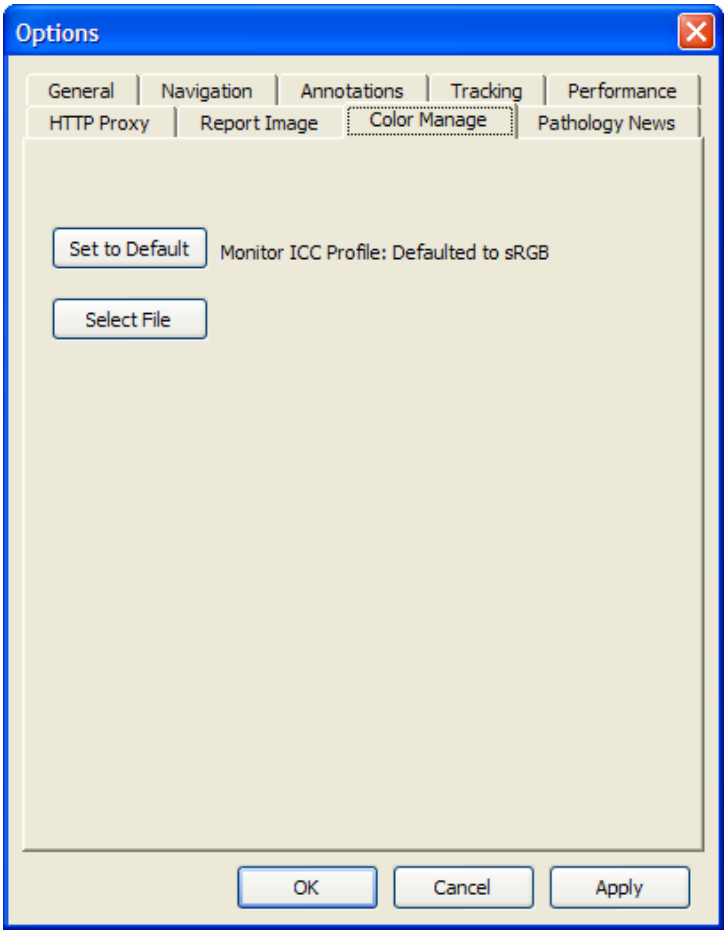
A Report Image tab on the ImageScope Tools Options window allows you to select a combination of fixed or user selectable sizes and resolutions. Note the Fixed Region Size parameters that define the size of the report image rectangle if the operator holds down the Control key while selecting the image area.



Color Management Options

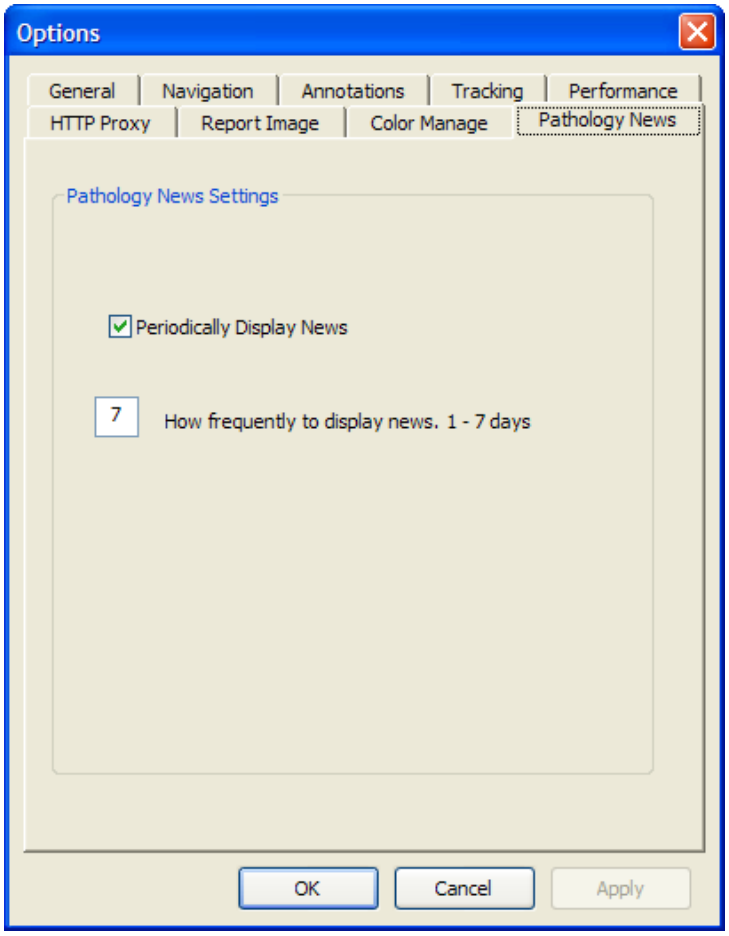
Aperio Integrated Color Management takes into account the optical characteristics of your ScanScope and your display monitor to ensure digital slide color is displayed accurately. For information on color management and ICC profiles, see Appendix B, “Aperio Integrated Color Management” on page 193.

The ImageScope Options window color management tab is where you can view the target monitor ICC profile or choose a new one:



Pathology News Options

The Pathology News tab is where you can configure how often the Pathology News window appears:



Enter a number from 1 to 7 in the **How frequently to display news. 1-7 days** box to select how often the Pathology News window appears (from every day up to once a week).

If you are connected to Spectrum (that is, you have opened an image in ImageScope from a Spectrum page) you can turn the Pathology News off and prevent it from opening unless you specifically use the View menu **View News** command:

- Either clear the **Periodically Display News** check box on the Pathology News window or clear the same check box on the Pathology News tab of the Options page (shown above).

For More Information

For more on ImageScope's settings, see:

- Chapter 18, "Utilities and Diagnostics" on page 167.

* This application is not approved or cleared by the FDA for clinical use.

† Aperio's image analysis algorithms are FDA cleared for specific clinical applications, and are intended for research use for other applications.

A
Keyboard Quick Reference

This section contains a quick reference list of keyboard shortcuts you can use with ImageScope.

ImageScope Keyboard Shortcuts

Key Sequence	Command	Toolbar Icon	Action
Arrow key	None		Nudge image.
Control +	None		Zoom in
Control -	None		Zoom out
Control			Hold down while drawing an annotation to draw the annotation in a predefined size.
Control A	Image > Adjustments		Go to the Image Adjustments window where you can make image adjustments to the main image or to the Z-stack image, as well as load and save adjustments.
Control C	None		Enable/disable integrated color management
Control D	View > Digital Slide Conferencing Window		Open the Digital Slide Conferencing window where you can create or join a digital slide conference.
Control E	Image > Rotate Image		Open image rotation toolbar
Control F	None		Turn image prefetching on/off.
Control F4	File > Close Image		Close the image currently open in ImageScope.
Control G	View > Analysis		Run an algorithm analysis on a local or remote digital slide.
Control I	Tools > Options > Performance		Turn interpolating on/off (used for viewing 3D images).
Control J	Tools > Options > Performance		Turn progressive rendering on/off.
Control K	Image > Keep Open		Turn Keep Open option on/off.
Control L	Tools > Logging		Turn logging on/off.

Key Sequence	Command	Toolbar Icon	Action
Control M	None		Show/hide track map
Control Shift M	None		Show/hide cache map.
Control N	View > Annotations Window		Open the Annotations window where you can work with annotation layers for the current image.
Control O	File > Open Image		Go to the Open Image window where you can browse for a local image file to open.
Control P	Tools > Options		Open the Options window to set general, navigation, annotation, performance, and HTTP proxy options.
Control Q	Image > Quality		Turn IQ on/off.
Control R	File > Access Remote Server		Connect to an Aperio ImageServer where you can select an image to view.
Control S	Tools > Options > Performance		Turn pixel smoothing on/off.
Control Shift			Move all annotations.
Control T	View > Thumbnail		Show/hide the thumbnail window.
F1	Help > Help		Open ImageScope help.
F2	None		Pen drawing tool.
F3	None		Negative pen drawing tool.
F4	None		Ruler drawing tool.
F5	None		Rectangle drawing tool.
F6	None		Ellipsis drawing tool.
F7	None		Arrow drawing tool.
F8	View > Annotation Link Manager		Open the Annotation Link Manager window where you can link slide annotations in a specific viewing order.
F11	View > Full Screen		View ImageScope on your entire monitor screen.
Shift	None		Hold down while drawing annotations: ellipse becomes a circle; rectangle becomes a square.
Shift Arrow	None		Move one screen at a time.

Key Sequence	Command	Toolbar Icon	Action
Shift Control	None		Hold down while drawing an annotation or using the extract region tool to create a region of the same aspect ratio as the predefined fixed annotation size.
Shift F7	View > Previous Annotation Link		If annotation links have been set up using the Annotation Link Manager, this moves to the previous annotation in the viewing sequence.
Shift F8	View > Next Annotation Link		If annotation links have been set up using the Annotation Link Manager, this moves to the next annotation in the viewing sequence.

B
Aperio Integrated Color Management

Aperio Integrated Color Management takes into account the optical characteristics of your ScanScope scanner and your display monitor to make sure that colors of the digital slides are displayed accurately.

A challenge for any provider of applications or devices that work with images is to make sure that the end result (in Aperio's case, viewing a digital slide created by scanning a microscope slide) gives a true representation of the color of the original glass slide from which the digital slide was made.

Every image capturing device will transform the image color due to the particular characteristics of that device.

Fortunately, there is a way to take those characteristics into account to make sure that all along the path the image travels, from creation to display, color accuracy is maintained. As of Release 9, Aperio provides Integrated Color Management that works with the international-standard ICC color management specification to maintain image color accuracy.

ICC Profiles

The ICC (International Color Consortium) is a body that maintains a specification defining a color management system that makes sure that the color of images moved among applications, devices, and operating systems is maintained accurately.

The ICC defines a format for ICC Profiles, which describe the color attributes of a particular device.

The ScanScope ICC Profile

In order to describe the behavior of image capture devices such as a ScanScope, the devices must be calibrated to a standard UUcolor space, which creates an ICC profile. At the Aperio factory, an ICC profile is created for your ScanScope. The ScanScope ICC profile is called the *source profile*. This profile is stored as a standalone file in \ScanScope\Profiles on the ScanScope workstation.

As you scan glass slides, the ICC profile for your scanner is also embedded in the resulting digital slide file.

The embedded profile tells ICC-aware applications (such as ImageScope and WebViewer) what color-transformation was done by the ScanScope so that the Aperio Integrated Color Management can transform the digital slide to its original color.

The Display Monitor ICC Profile

The ScanScope ICC profile is not the only component of the integrated color management equation. The monitor you use to view the digital slide also has characteristics that affect color display. Your monitor may have shipped with drivers that contain an ICC profile for the monitor. Or, you can create a new monitor profile by using an external calibration tool. As the monitor is the final device in the image transformation chain, its profile is called the *target profile*.

If a monitor ICC profile exists, the Aperio ICC-aware applications will use it along with the ScanScope's ICC profile to display the digital slide color accurately.

How ImageScope Uses Color Management

ImageScope is an ICC-aware application, which means that—for any digital slide that contains an embedded ICC profile—it is able to use the embedded ScanScope ICC profile and your monitor ICC profile to ensure digital slides are displayed with accurate color.

For information on how ImageScope uses color management with various ImageScope features, see:

- "Viewing with Color Management" on page 38.
- Chapter 13, "Saving Digital Slides and Regions" on page 109.
- "Color Management Options" on page 186.

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