

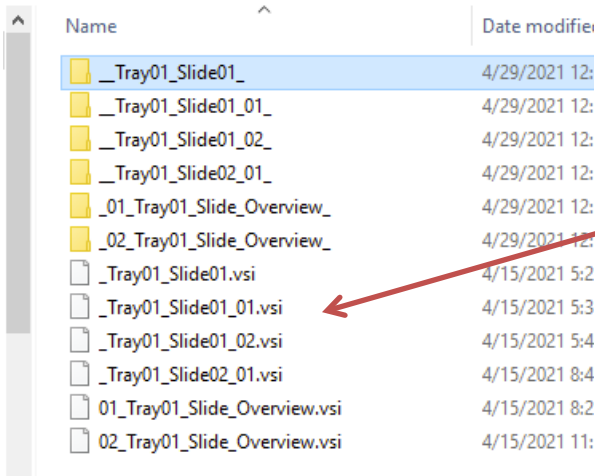
OlyVIA for VS200 .vsi files

This document will show you how to open, view and manage large format images created with the VS200 whole slide scanner using the Olympus OlyVIA software

About Olympus .vsi and overview .vsi files

There are two kinds of files created during Olympus VS200 scans: .vsi files and Overview.vsi files. Depending on how your scan was collected, there may be multiple .vsi files per overview .vsi file, with the overview.vsi file containing a low-resolution image of the entire slide plus an image of the slide label, and the .vsi(s) containing high resolution image(s) of the slide contents.

Each overview.vsi and .vsi file has a corresponding folder with the same name. **The .vsi file and corresponding folder MUST be downloaded and kept together**, and if you choose to rename, they MUST both be re-named with the same name, otherwise important information about the image is lost and the .vsi file cannot be opened.



Name	Date modified
__Tray01_Slide01_	4/29/2021 12:
__Tray01_Slide01_01_	4/29/2021 12:
__Tray01_Slide01_02_	4/29/2021 12:
__Tray01_Slide02_01_	4/29/2021 12:
_01_Tray01_Slide_Overview_	4/29/2021 12:
_02_Tray01_Slide_Overview_	4/29/2021 12:
_Tray01_Slide01.vsi	4/15/2021 5:2
_Tray01_Slide01_01.vsi	4/15/2021 5:3
_Tray01_Slide01_02.vsi	4/15/2021 5:4
_Tray01_Slide02_01.vsi	4/15/2021 8:4
01_Tray01_Slide_Overview.vsi	4/15/2021 8:2
02_Tray01_Slide_Overview.vsi	4/15/2021 11:

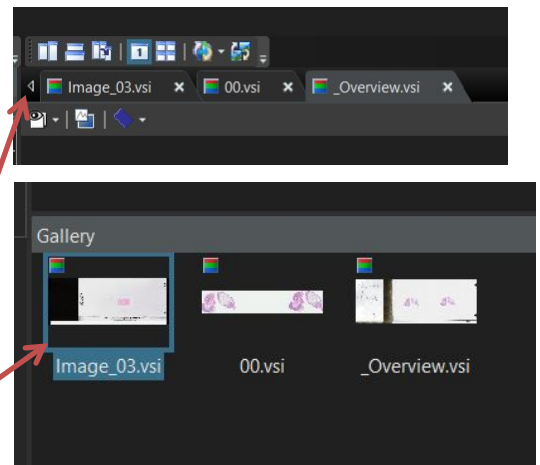
In this example, there are images from two slides. Tray01_Slide01 has an overview.vsi image and three .vsi images (blank, 01_ and 02_) each representing one piece of tissue from a multi-tissue slide. Tray01_Slide02 has an overview.vsi and only one .vsi image, meaning it only has one piece of tissue OR that all tissues on the slide were scanned into one large, high mag file.

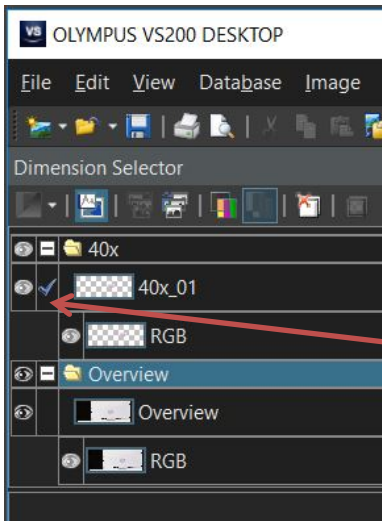
Depending on how the slide scan was set up, you may only see .vsi files in your folder. In this case, the overview and scan images are both contained in the .vsi file. These can be viewed as separate images when the file is opened (see Opening an Olympus .vsi or overview .vsi file, below).

Opening an Olympus .vsi or overview .vsi file

Files can be opened in several ways. The easiest way is to start OlyVIA, then find the .vsi file(s) you want to open and drag and drop onto the big, empty window in the middle. You can also use File -> Open -> File or Cntl + O.

You can open multiple .vsi files and each will appear as a tab at the top of the window. View -> Tool Windows -> Gallery will open a thumbnail gallery at the bottom of the window.



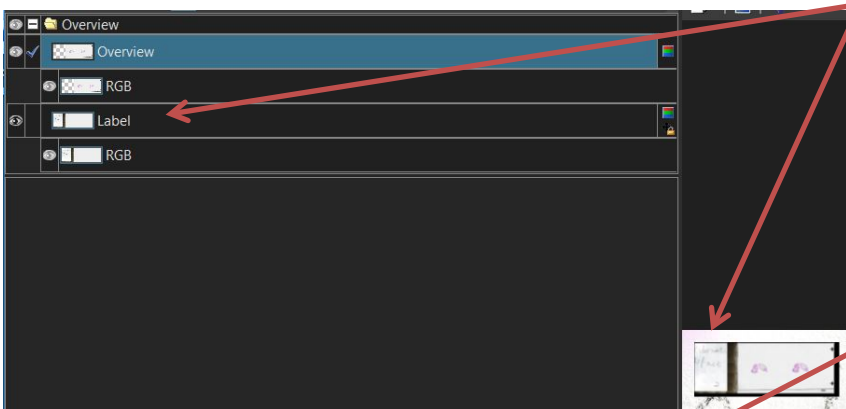


When you open a .vsi file with overview and scan images combined or a file with multiple magnifications of the same slide, the menu to the left of the image (titled Dimension Selector) will allow you to navigate through the different images.

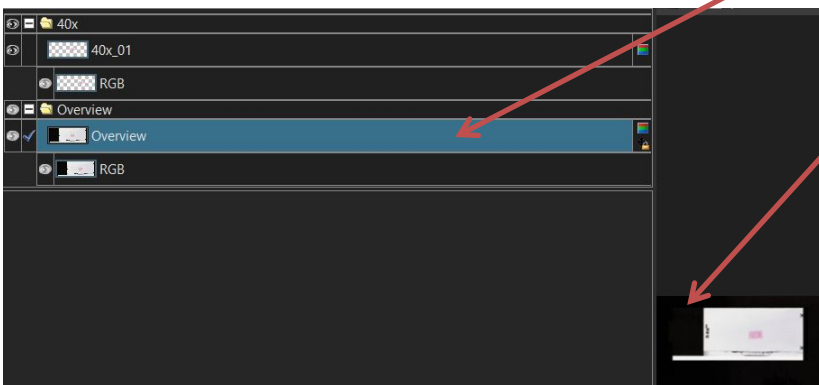
In this example, we have a 40x scan image with its corresponding lower magnification overview image together in one file. Clicking the eye icon on the lefthand side makes the corresponding image visible or not in the main window. You cannot turn all of the images off at once, one must be visible at all times. The blue check seems to indicate which image cannot be toggled on/off with the eye icon.

Slide label image in different .vsi file formats – CRITICAL INFORMATION

Note that when the overview is saved as a *separate* file, there is an image of the slide label as part of the .vsi file.



When the overview and scan files are saved in the *same* .vsi file, the label image is NOT part of the .vsi file.



We have asked Olympus if this is a feature or a bug.

If you want to be sure an image of the label is part of your scan, please specify when dropping off your slides that you would like SEPARATE overview and image .vsi files for your scan.

Image Information (metadata)

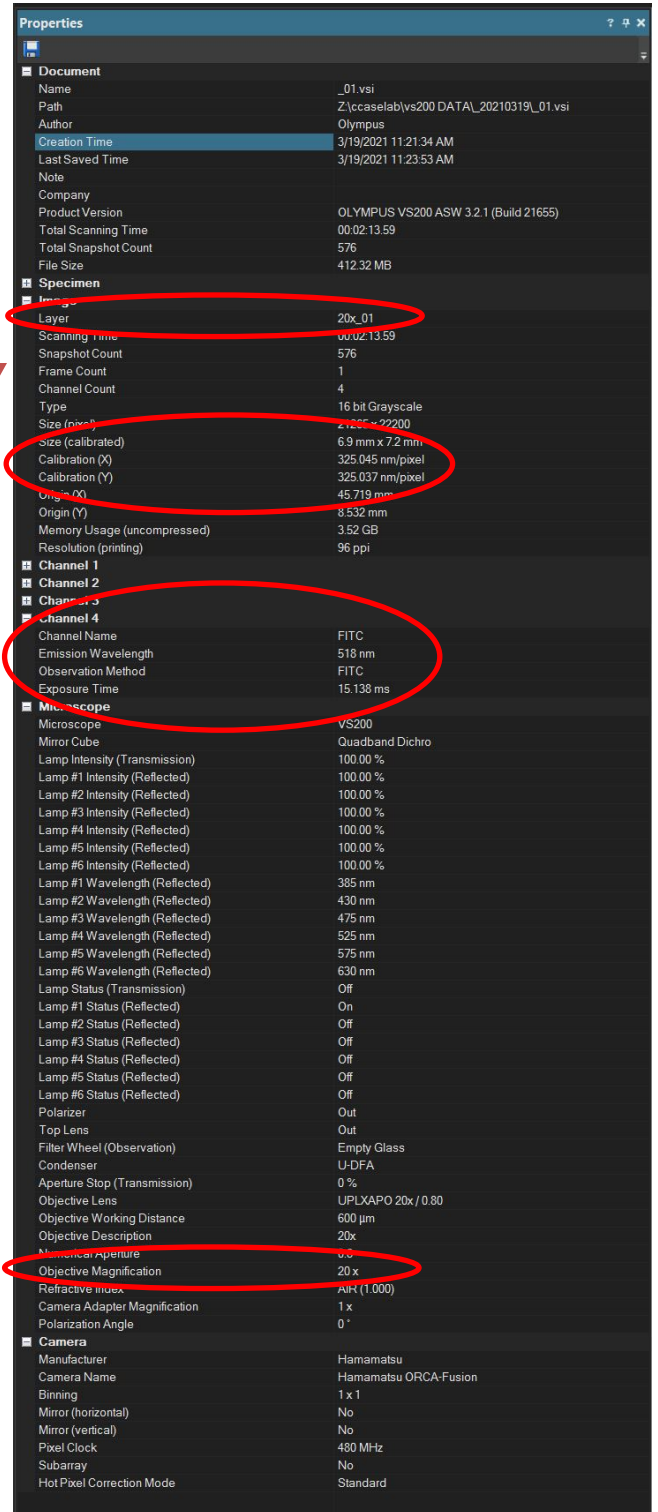
All of the information about how the image was collected, including the filename, date, time, objective used for the scan, pixel size and fluorescence filters (collectively known as the metadata) is displayed in the Properties box in the lower right corner of the screen. If this window is not visible, you can open it using View -> Tool Windows -> Properties or Alt+Enter.

If there is more than one image in the file, which metadata is displayed is determined by which image is highlighted with blue in the Dimension Selector menu in the upper left. The Layer name confirms which metadata is displayed.

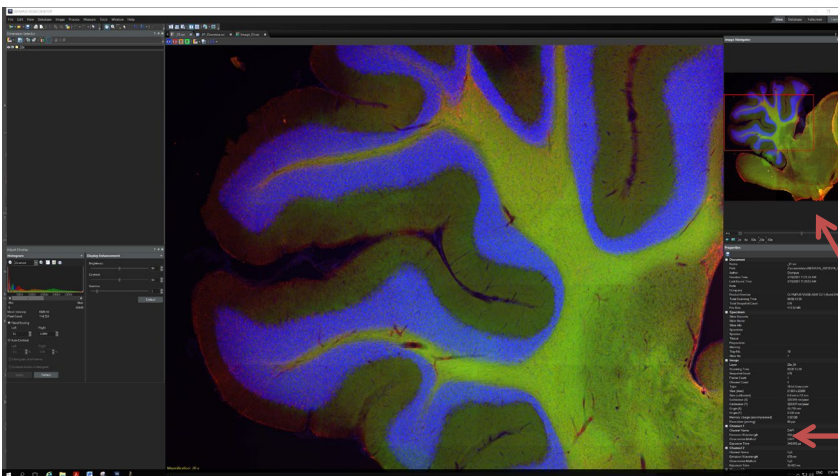
When You Open a .vsi File

When you open your file in the OlyVIA software, you will see several panels. These are customizable using the View -> Tool Windows menu, so feel free to customize your display to show the things you need.

In the default layout, the upper left will show the different images in the file as layers, allowing you to toggle different layers on and off (details of this are in the section on opening .vsi files above). Below that is the Adjust Display box, with the brightness/contrast histogram and gamma adjustment slider (details on this below).

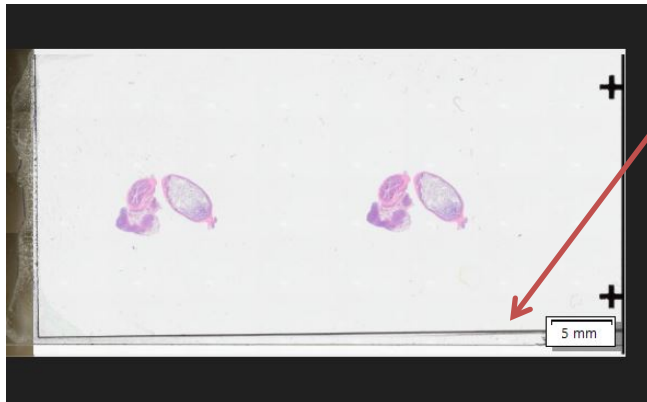


Document	
Name	_01.vsi
Path	Z:\ccaslab\vs200 DATA_20210319_01.vsi
Author	Olympus
Creation Time	3/19/2021 11:21:34 AM
Last Saved Time	3/19/2021 11:23:53 AM
Note	
Company	
Product Version	OLYMPUS VS200 ASW 3.2.1 (Build 21655)
Total Scanning Time	00:02:13.59
Total Snapshot Count	576
File Size	412.32 MB
Specimen	
Image	
Layer	20x_01
Scanning Time	00:02:13.59
Snapshot Count	576
Frame Count	1
Channel Count	4
Type	16 bit Grayscale
Size (pixel)	2125 x 2200
Size (calibrated)	6.9 mm x 7.2 mm
Calibration (X)	325.045 nm/pixel
Calibration (Y)	325.037 nm/pixel
Origin (X)	45.719 mm
Origin (Y)	8.532 mm
Memory Usage (uncompressed)	3.52 GB
Resolution (printing)	96 ppi
Channel 1	
Channel 2	
Channel 3	
Channel 4	
Channel Name	FITC
Emission Wavelength	518 nm
Observation Method	FITC
Exposure Time	15.138 ms
Microscope	
Microscope	VS200
Mirror Cube	Quadband Dichro
Lamp Intensity (Transmission)	100.00 %
Lamp #1 Intensity (Reflected)	100.00 %
Lamp #2 Intensity (Reflected)	100.00 %
Lamp #3 Intensity (Reflected)	100.00 %
Lamp #4 Intensity (Reflected)	100.00 %
Lamp #5 Intensity (Reflected)	100.00 %
Lamp #6 Intensity (Reflected)	100.00 %
Lamp #1 Wavelength (Reflected)	385 nm
Lamp #2 Wavelength (Reflected)	430 nm
Lamp #3 Wavelength (Reflected)	475 nm
Lamp #4 Wavelength (Reflected)	525 nm
Lamp #5 Wavelength (Reflected)	575 nm
Lamp #6 Wavelength (Reflected)	630 nm
Lamp Status (Transmission)	Off
Lamp #1 Status (Reflected)	On
Lamp #2 Status (Reflected)	Off
Lamp #3 Status (Reflected)	Off
Lamp #4 Status (Reflected)	Off
Lamp #5 Status (Reflected)	Off
Lamp #6 Status (Reflected)	Off
Polarizer	Out
Top Lens	Out
Filter Wheel (Observation)	Empty Glass
Condenser	U-DFA
Aperture Stop (Transmission)	0 %
Objective Lens	UPLXAPO 20x / 0.80
Objective Working Distance	600 µm
Objective Description	20x
Numerical Aperture	0.8
Objective Magnification	20 x
Refractive Index	AIRY (1.000)
Camera Adapter Magnification	1 x
Polarization Angle	0 °
Camera	
Manufacturer	Hamamatsu
Camera Name	Hamamatsu ORCA-Fusion
Binning	1 x 1
Mirror (horizontal)	No
Mirror (vertical)	No
Pixel Clock	480 MHz
Subarray	No
Hot Pixel Correction Mode	Standard



On the right, Image Navigator has a zoom slider and a red box showing the current FOV in the context of the whole scan. Below that the Properties section contains the image metadata.

Is this a low resolution Overview image or a high resolution Scan image?



The overview image will look like the scan of a whole slide, and may show slide markings, coverslip edges, writing, excess mounting media and other artifacts in addition to the tissue.

The tissue in the overview image is low mag / low res and will look blurry or pixelated when you try to zoom in. For detailed images, open the file which contains the scan image OR use

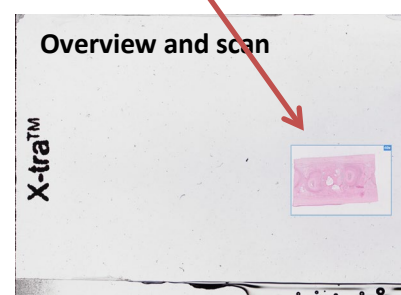
the eye icon in the Dimension Selector on the upper left hand side to turn on and look at the scan image (depending on how your images were scanned).



The scan image will be cropped close to the tissue(s) and may or may not show as many slide artifacts.

If you open a .vsi file that contains both the overview image and the scan image(s), you must be careful that you are looking at the higher magnification image when you want detail views, otherwise zooming in will just make the image look pixelated or blurry. The scan image will have a colored border with the scan magnification in the upper right hand corner.

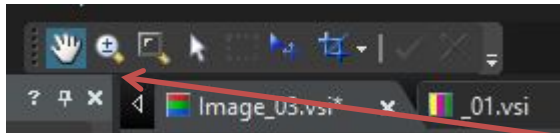
Use the eye icon in the Dimension Selector in the upper left corner to overlay the scan image on top of the overview (far right image below) or to turn off the image of the overview completely and just display the scan image (middle image below).



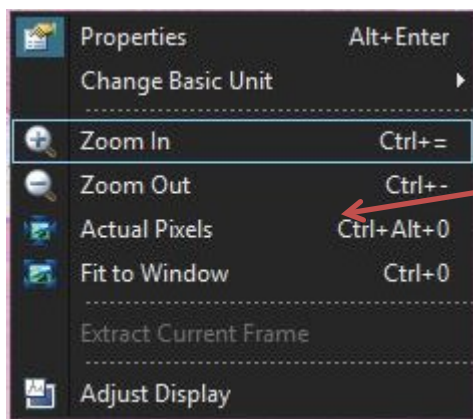
Zooming the Image

Before you zoom on an image, be sure you are looking at a high resolution scan image and not an overview image. If you're not sure, see the section just before this called "Is this a low resolution Overview image or a high resolution Scan image?"

There are a multitude of ways to zoom the image in OlyVIA:



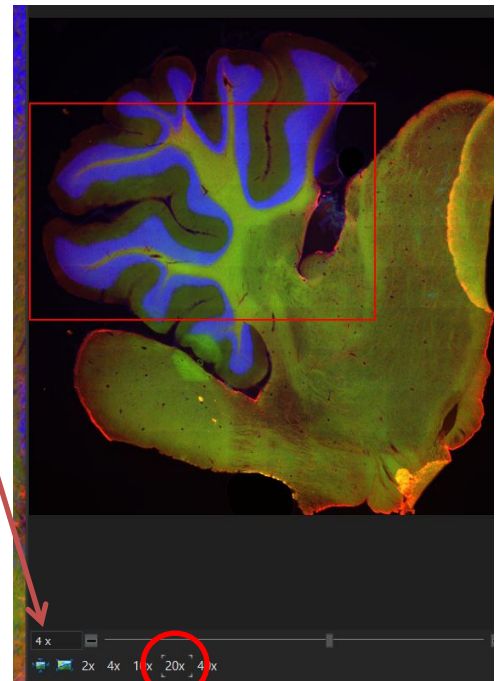
1) The easiest way to zoom is to click on the image and scroll with the mouse wheel.



2) Click the zoom icon in the toolbox bar, located just above the tabs at the top of the screen. Then use left and right mouse clicks to zoom in and out on the image.

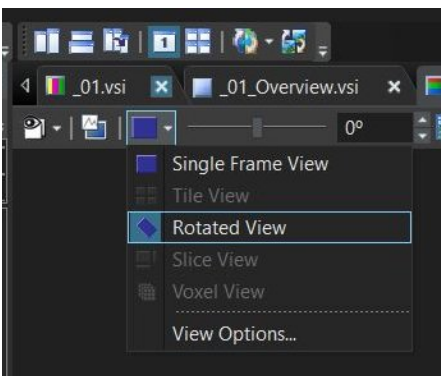
3) Right click on the image to pull up a submenu and use the commands or keyboard shortcuts found there.

4) Use the slider or buttons under the Image Navigator window in the upper right.



For any of these zoom methods, readout of your current zoom factor is always in the small box to the left of the zoom slider under the Image Navigator. Brackets (circled in red, right) indicate the magnification of the objective used to create the zoom and should match the magnification listed in the Properties window.

Rotating the Image

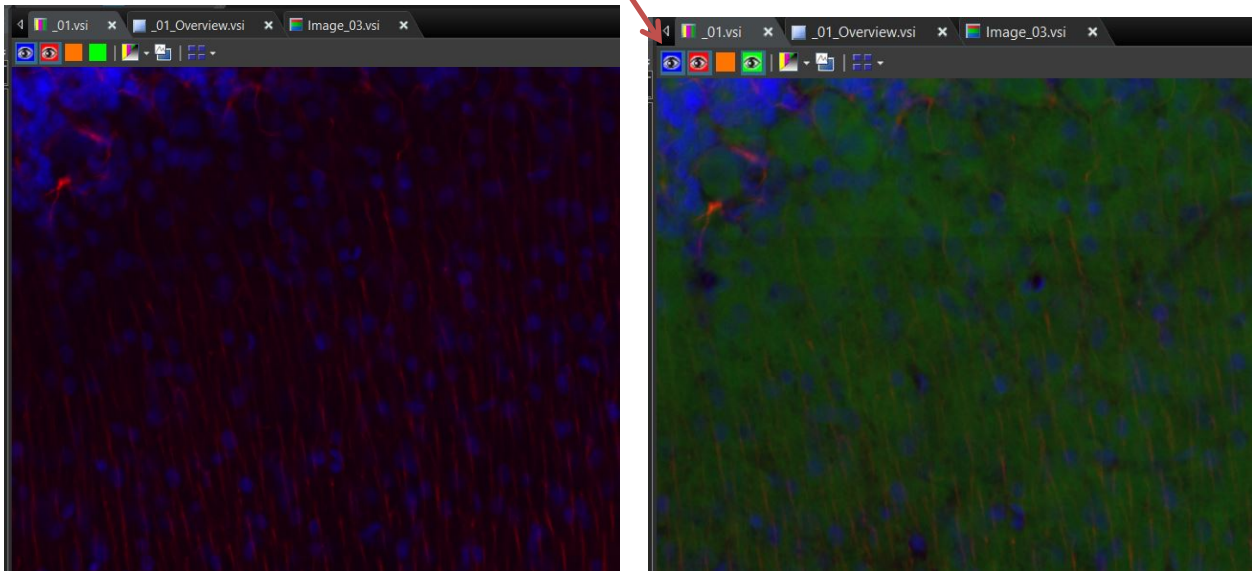


To rotate an image, click the blue box just under the image tabs at the top of the image window. Use the pulldown to select "Rotated View."

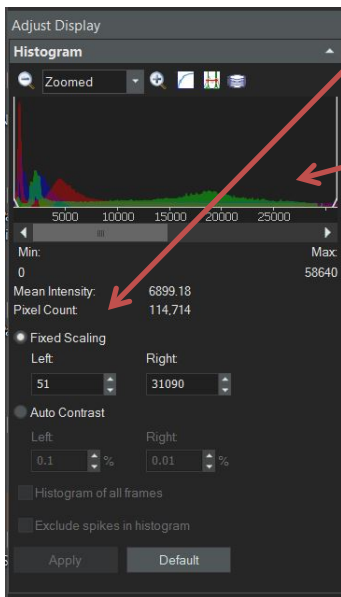
The whole slide rotates regardless of which image / layer is "active."

To overlay a grid for alignment, click the blue and green puzzle-looking button to the right of the rotation angle readout (I cut it off in this screensnap).

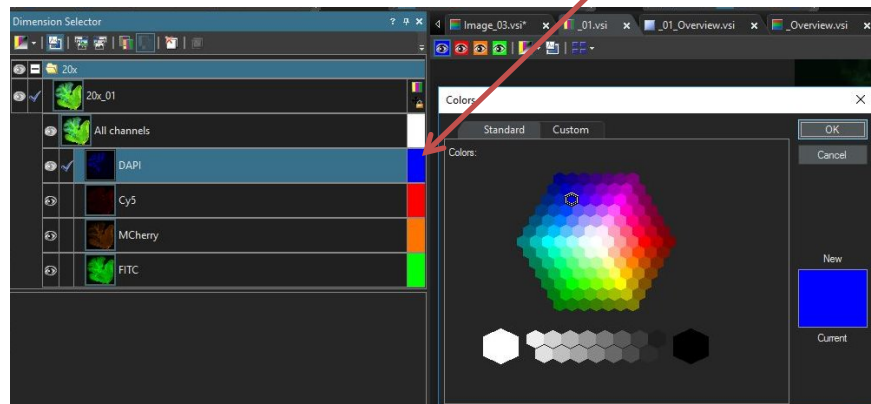
Adjusting Brightness & Contrast and Changing Fluorescent Channel Fluorescent images will have a series of boxes at the top left of the main window.



Toggle the eye icons in the boxes on and off to change which channel(s) are displayed. To adjust brightness and contrast, set the display to a single channel by leaving only one of these boxes with an eye icon. Then go to the Adjust Display menu on the left side and set the display to “Fixed Scaling.” Then you can either adjust the numbers in this section or use the sliders on the left and right sides of the histogram above to adjust the intensity of the current channel. Channels must be adjusted one by one, we haven’t found a way to adjust one channel while still displaying others in this software.



Changing Channel Color – Use the down arrow next to the image name in the Dimension Selector menu to reveal the channel selector. The names can be changed by double clicking the text and typing in new text. Channel false color can be changed by clicking the color bar to the right of the name and selecting a new color from the pop-up menu.



Display More Than One Image at a Time and Syncing Multiple Views)

To Save Your Image Workspace

File -> Export Functions to Use All of Part of the Image in Another Program