

# 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

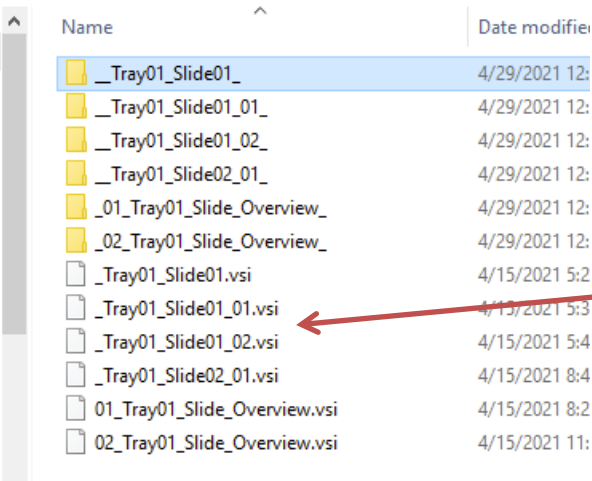
There are two kinds of files created during Olympus VS200 scans: .vsi files and Overview.vsi files. Depending on how your scan was collected, there may be multiple .vsi files per overview.vsi file, with the overview.vsi file containing a low-resolution/magnification image of the entire slide (called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

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 renamed with the same name, otherwise important information about the image is lost and the .vsi file cannot be opened.

In this example, there are images from two slides.

Tray01\_Slide01 has an Overview image and three Detail images (blank, 01 and 02) each representing one piece of tissue from a multi-tissue slide. Tray01\_Slide02 has an Overview and one Detail image, meaning it only has one piece of tissue OR that all tissues on the slide were scanned into one Detail image.



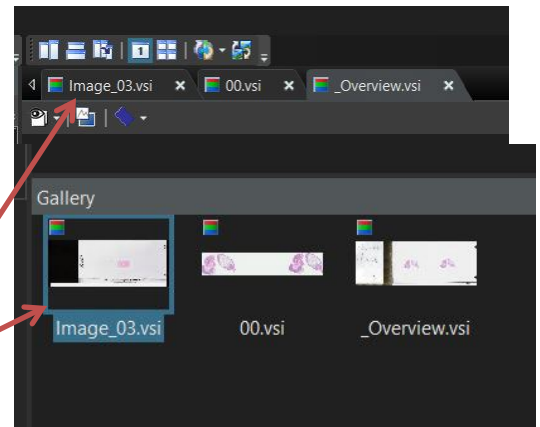
Name	Date modified
__Tray01_Slide01_	4/29/2021 12:
__Tray01_Slide01_01_	4/29/2021 12:
__Tray01_Slide01_02_	4/29/2021 12:
__Tray01_Slide02_01_	4/29/2021 12:
_01_Tray01_Slide_Overview_	4/29/2021 12:
_02_Tray01_Slide_Overview_	4/29/2021 12:
_Tray01_Slide01.vsi	4/15/2021 5:2
_Tray01_Slide01_01.vsi	4/15/2021 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:

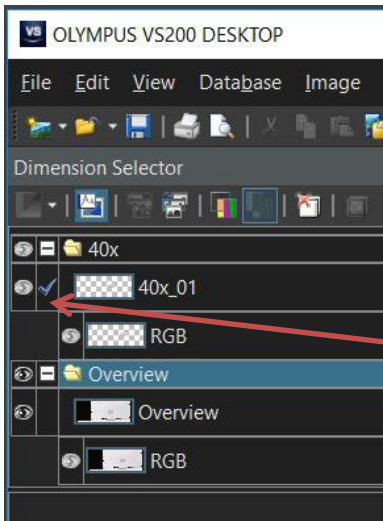
Depending on how the slide scan was set up, you may only see .vsi files in your folder. In this case, the Overview and Detail images are both contained in the .vsi file. These can be viewed as separate images when the file is opened (see Opening an Olympus .vsi or overview.vsi file, below).

## 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.





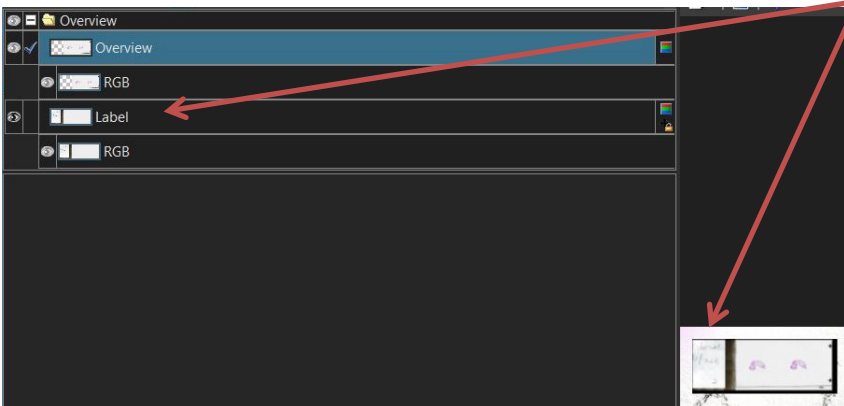
When you open a .vsi file with overview and scan images combined or a file with multiple magnifications of the same slide, use the Dimension Selector window (View -> Tool Windows -> Dimension Selector) to navigate through the different images.

In this example, we have a 40x Detail image with its corresponding lower magnification Overview image together in one file. Clicking the eye icon on the left 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 to the right on the eye indicates which image you are working with in the Adjust Display window (get there with View -> Tool Windows -> Adjust

Display or by clicking  in the Dimension Selector window).

### Where is the image of the slide label?

Whether the Overview and Detail images are contained in the same .vsi file or in separate



overview.vsi and .vsi files, the image of the slide label is ALWAYS grouped with the Overview image. The eye icon can be used to toggle the label image on and off in the Dimension Selector window.

## Image Information (metadata)

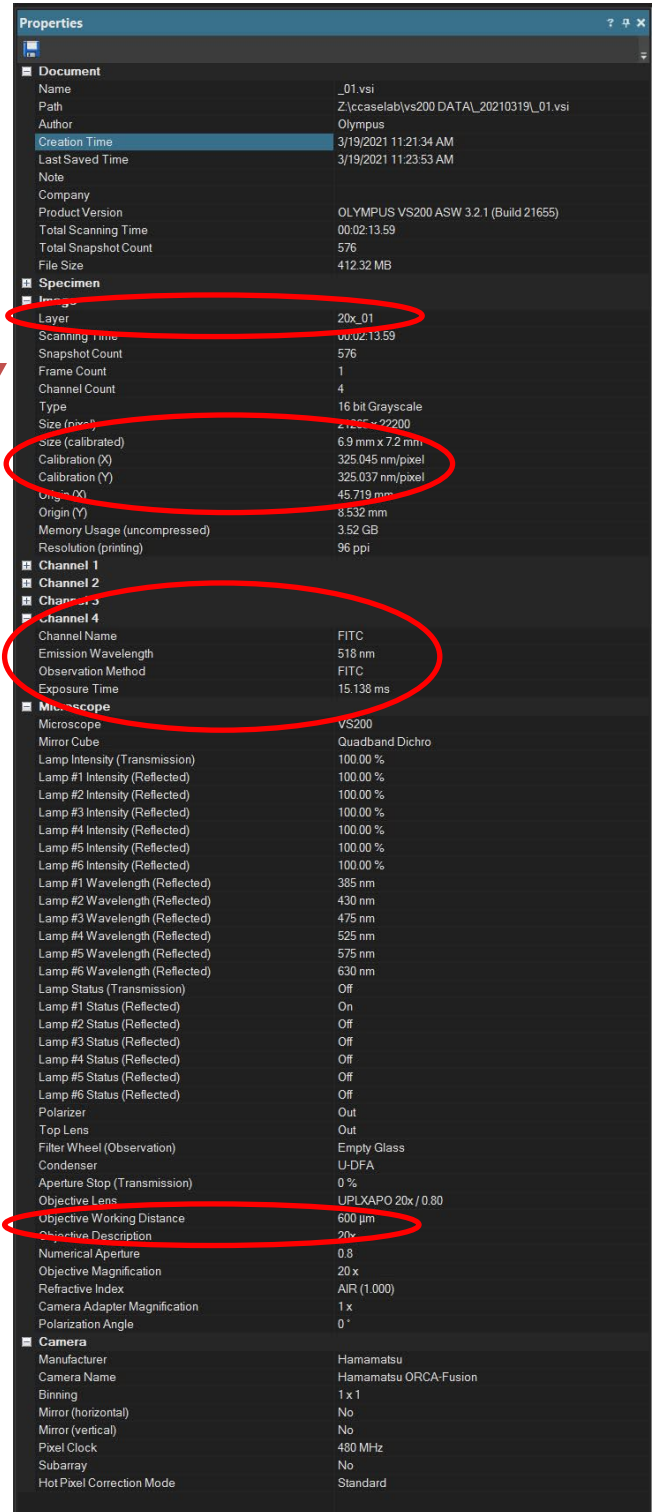
All of the information about how the image was collected, including the filename, date, time, objective used for the scan, pixel size and fluorescence filters (collectively known as the metadata) is displayed in the Properties box in the lower right corner of the screen. If this window is not visible, 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 window. The Layer name confirms which metadata is displayed.

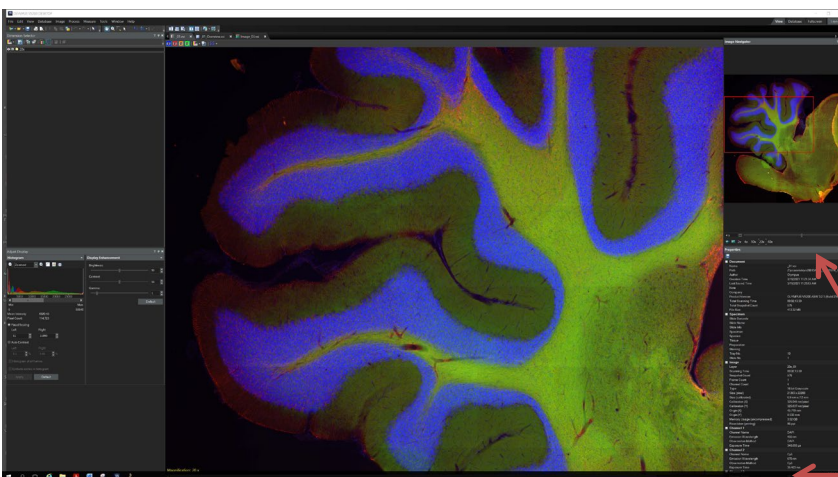
## When You Open a .vsi File

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).

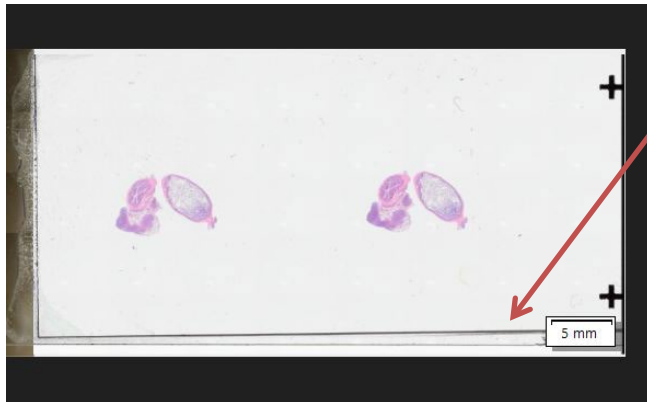


Document	
Name	_01.vsi
Path	Z:\ccsclab\vs200 DATA_20210319_01.vsi
Author	Olympus
Creation Time	3/19/2021 11:21:34 AM
Last Saved Time	3/19/2021 11:23:53 AM
Note	
Company	
Product Version	OLYMPUS VS200 ASW 3.2.1 (Build 21655)
Total Scanning Time	00:02:13.59
Total Snapshot Count	576
File Size	412.32 MB
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 2220
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	AIR (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 Detail 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.

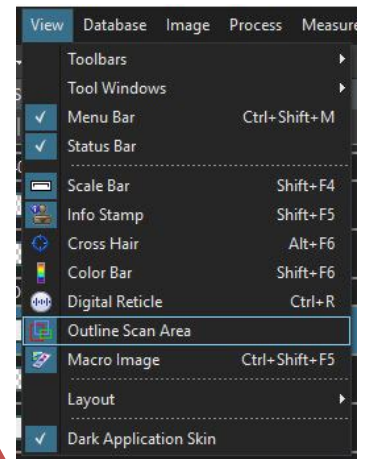
The tissue in the overview image is low mag / low res and will look blurry or pixelated when you try to zoom in. For detailed images, open the file which contains the scan image OR use

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).

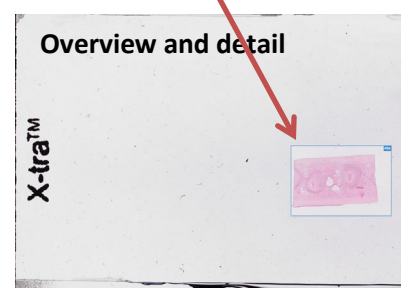


The detail image will be cropped close to the tissue(s) and may or may not show as many slide artifacts.

If you open a .vsi file that contains both the overview image and the detail image(s), you must be careful that you are looking at the higher magnification image when you want high magnification / high resolution views, otherwise zooming in will just make the image look pixelated or blurry. The detail image will have a colored boarder with the scan magnification in the upper right hand corner. This color outline around the Detail image view can be turned on and off under View -> Outline Scan Area.



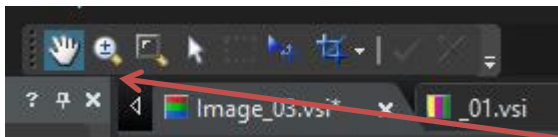
Use the eye icon in the Dimension Selector in the upper left corner to overlay the detail image on top of the overview (far right image below) or to turn off the image of the overview completely and just display the detail image (middle image below).



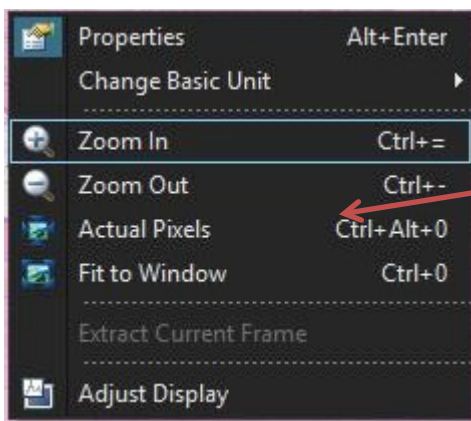
## Zooming the Image

Before you zoom on an image, be sure you are looking at a high resolution detail 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 Detail image?"

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



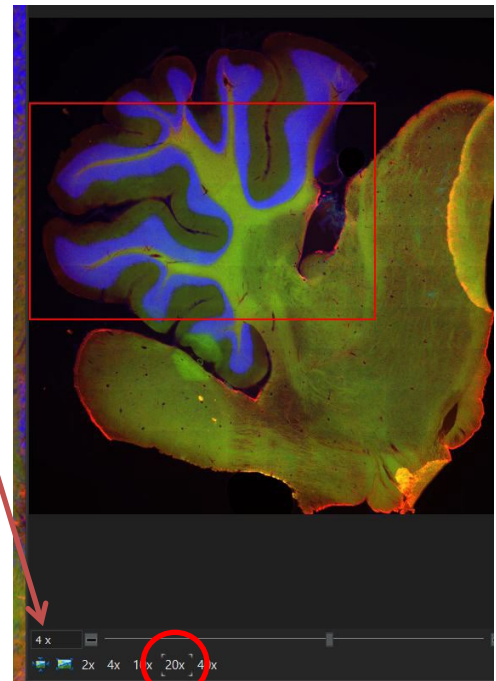
1) The easiest way to zoom is to click on the image and scroll with the mouse wheel.



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.

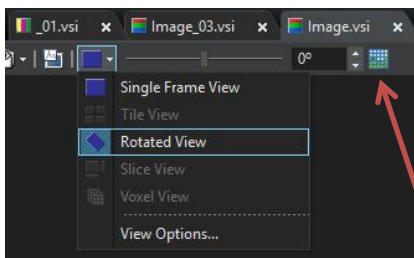
3) Right click on the image to pull up a submenu and use the commands or keyboard shortcuts found there.

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 metadata in the Properties window (View -> Tool Windows -> Properties).

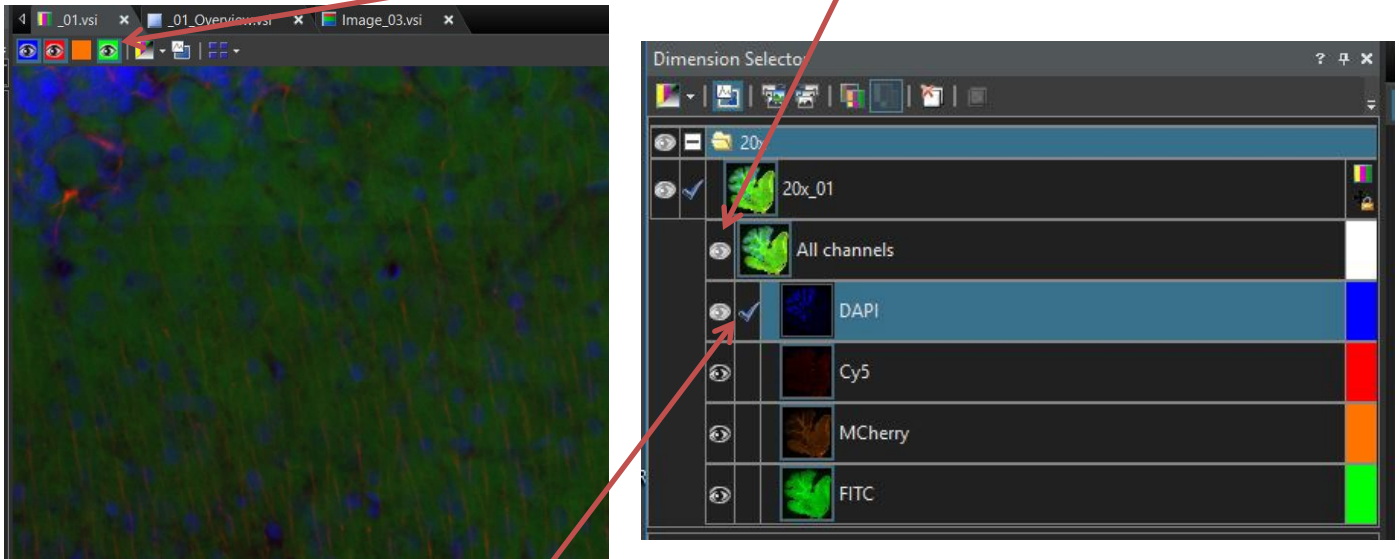
## Rotating the Image




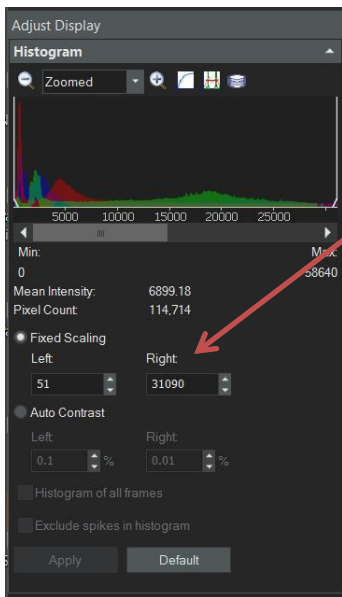
To rotate an image, click the blue box just under the image tabs at the top of the image window. Use the pulldown to select "Rotated View."

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.

**Adjusting Brightness & Contrast and Changing Fluorescent Channel** Fluorescent images will have a series of boxes at the top left of the main window. To change which channel(s) are displayed, you can toggle the eye icons in these boxes or in the Dimension Selector window.



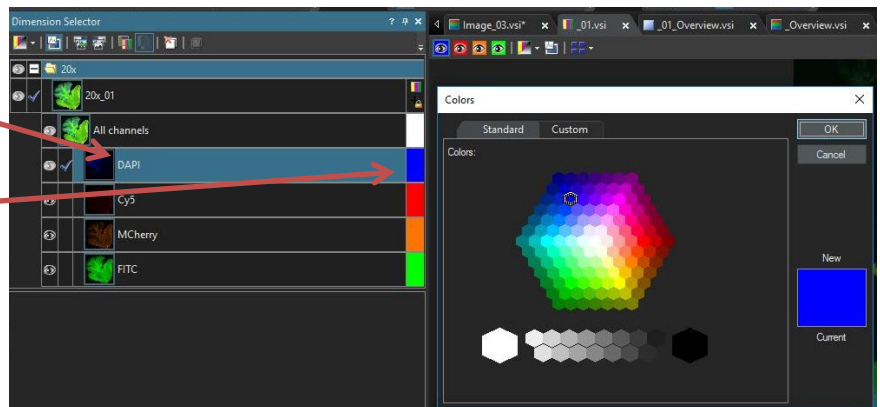
**To Adjust Brightness & Contrast** Choose the channel you want to adjust by moving the blue checkmark next to that channel. You will see the eye icon dim and you will not be able to turn off the display of that channel as long as the blue checkmark is present. Then go to the Adjust Display menu on the left side (if this window is not visible, open it with View -> Tool Windows -> Adjust Display or by clicking  in the Dimension Selector window).



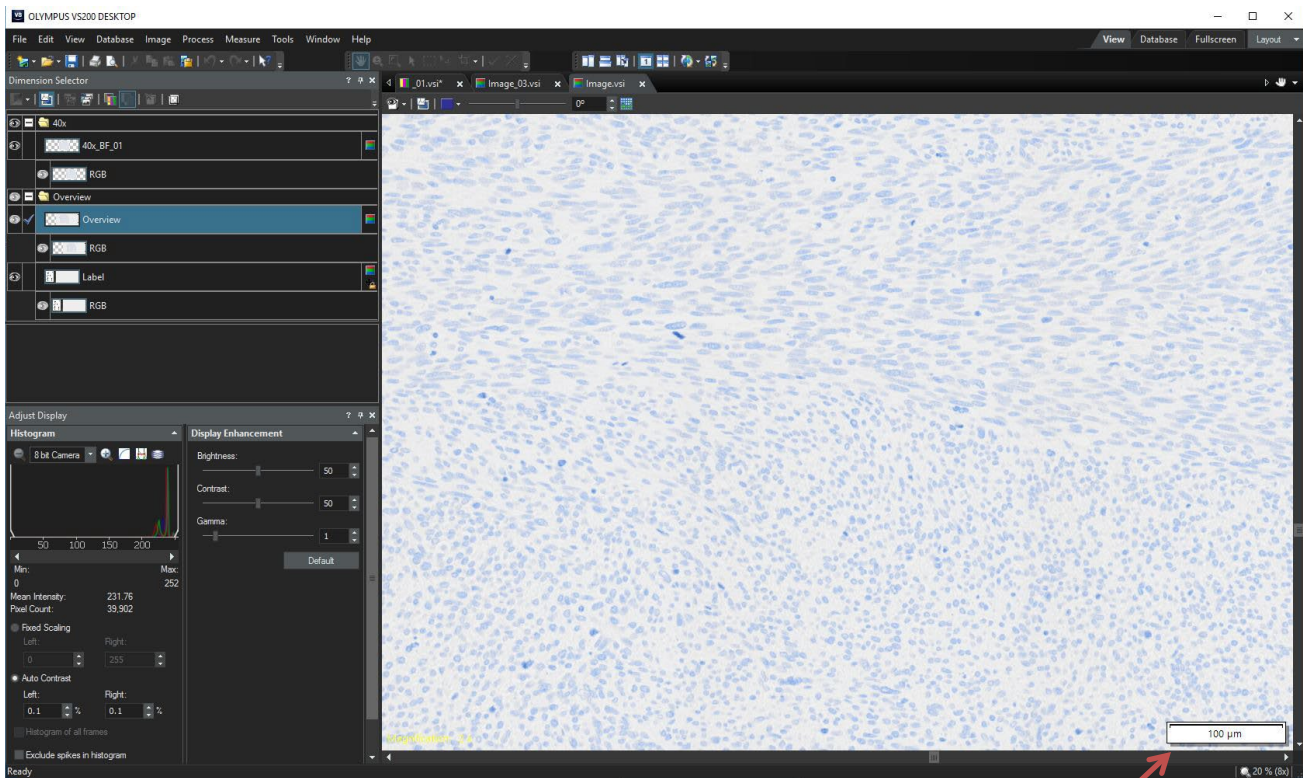
Set the display to “Fixed Scaling.” Then either adjust the numbers or use the sliders on the left and right sides of the histogram to adjust the intensity of the current channel. The Display Enhancement section of the Adjust Display window has sliders for Brightness, Contrast and Gamma (defaults are 50, 50 and 1 respectively).

**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.



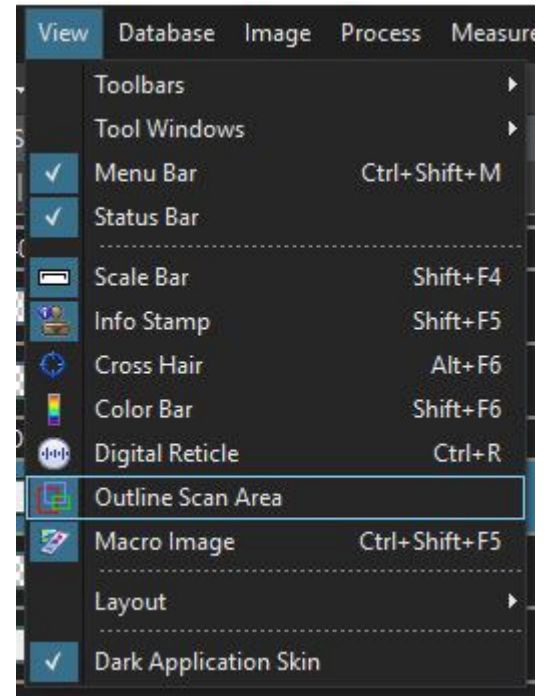
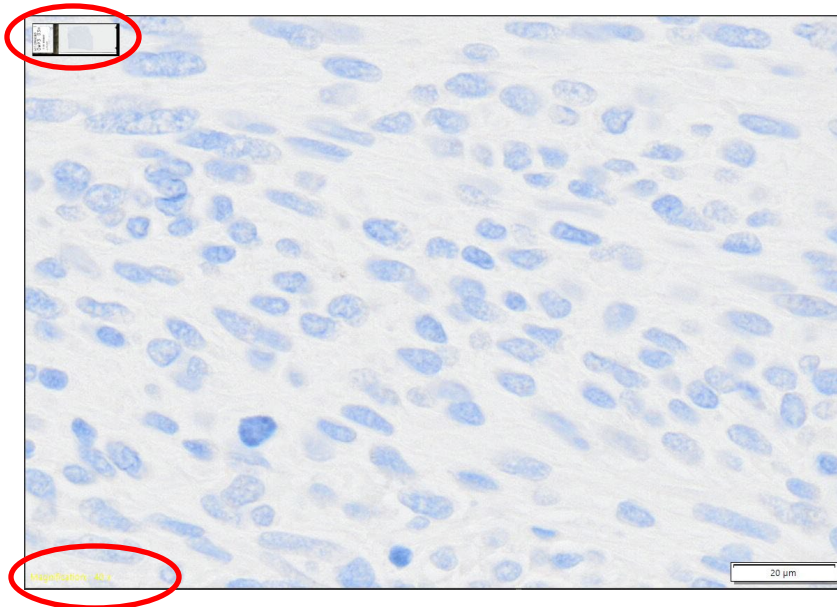
## Adding a Scale Bar



The scale bar is turned on under View -> Scale Bar and appears in the lower right hand corner of the image. The bar size changes as you zoom in and out on either the Overview or Detail image, and is accurate as long as the image was created on the Core VS200 scanner.

There does not seem to be a way to customize the scale bar in the OlyVIA software, the location and appearance are always the same. To save a .tif file of the current view EXACTLY AS SHOWN in the viewer window with the scale bar and possibly other annotations (see next section) printed on as a permanent part of the file, use File -> Save Display As and choose .tif or .jpg.

## Adding and Removing Other Annotations



There are other annotations available through the View menu, including the Info Stamp, which lists the original scan magnification (in this case 40x) in yellow in the lower left corner of the image, and Macro Image, a whole slide overview in the upper left corner. These annotations and others all become a permanent part of the image (i.e. they cannot be removed) with the File -> Save Display As function.

## Displaying More Than One Image at a Time and Syncing Multiple Views

### To Save Your Image Workspace (is this a thing in OlyVIA???)

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

The File -> Save Display As function has been discussed in the sections on adding a scale bar and adding and removing other annotations above. Briefly, it saves the image *exactly* as shown in the viewer window, with all annotations permanently burned in to the image (i.e they cannot be removed in OlyVIA or another program). File formats available here include .tif and .jpg.