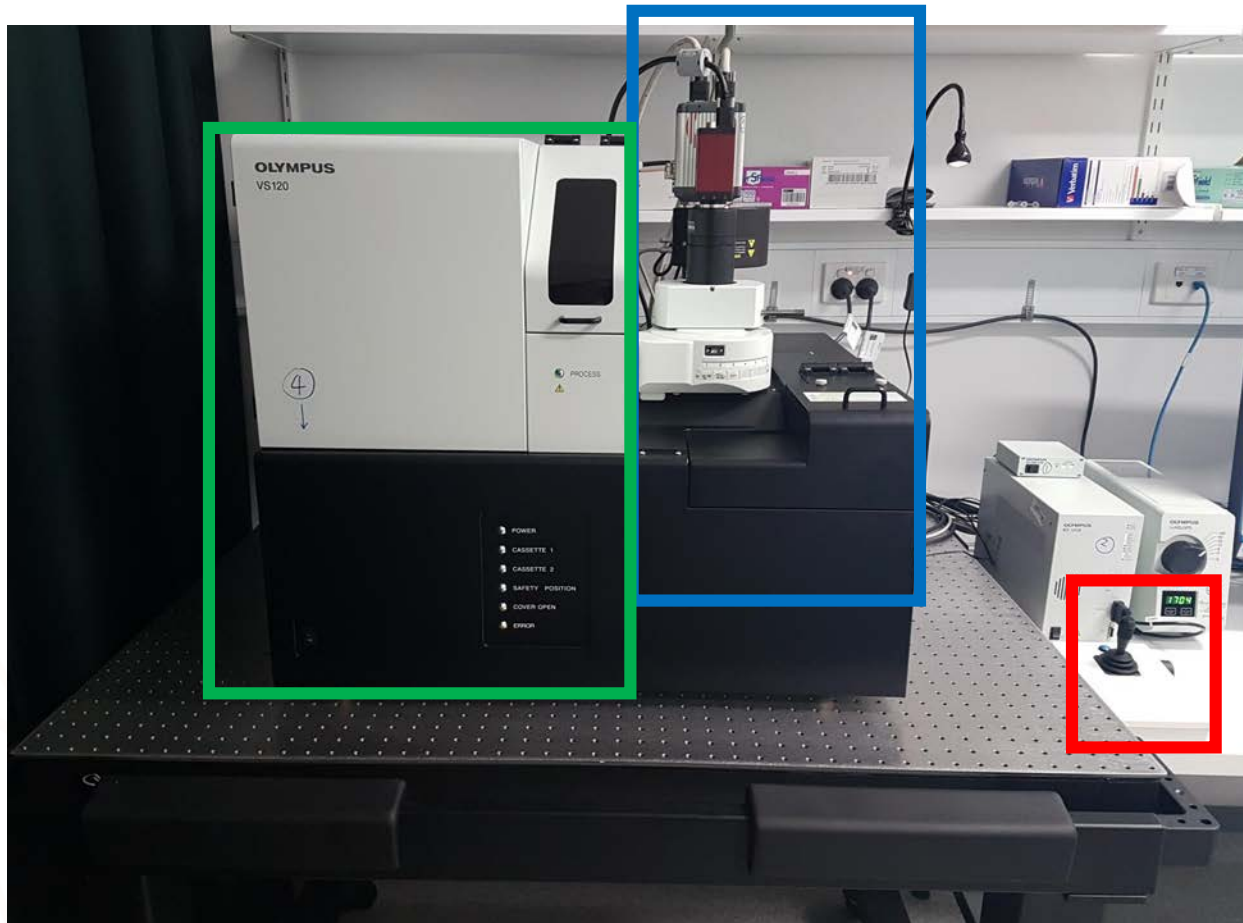


# Tricks & tips on image acquisition with Olympus VS 120 & handling large sized images

Hong Yu, Westmead Imaging Facility 25 July 2019

# What is Olympus VS 120? Lab J2.08

- Microscope based slide scanner—100 slides



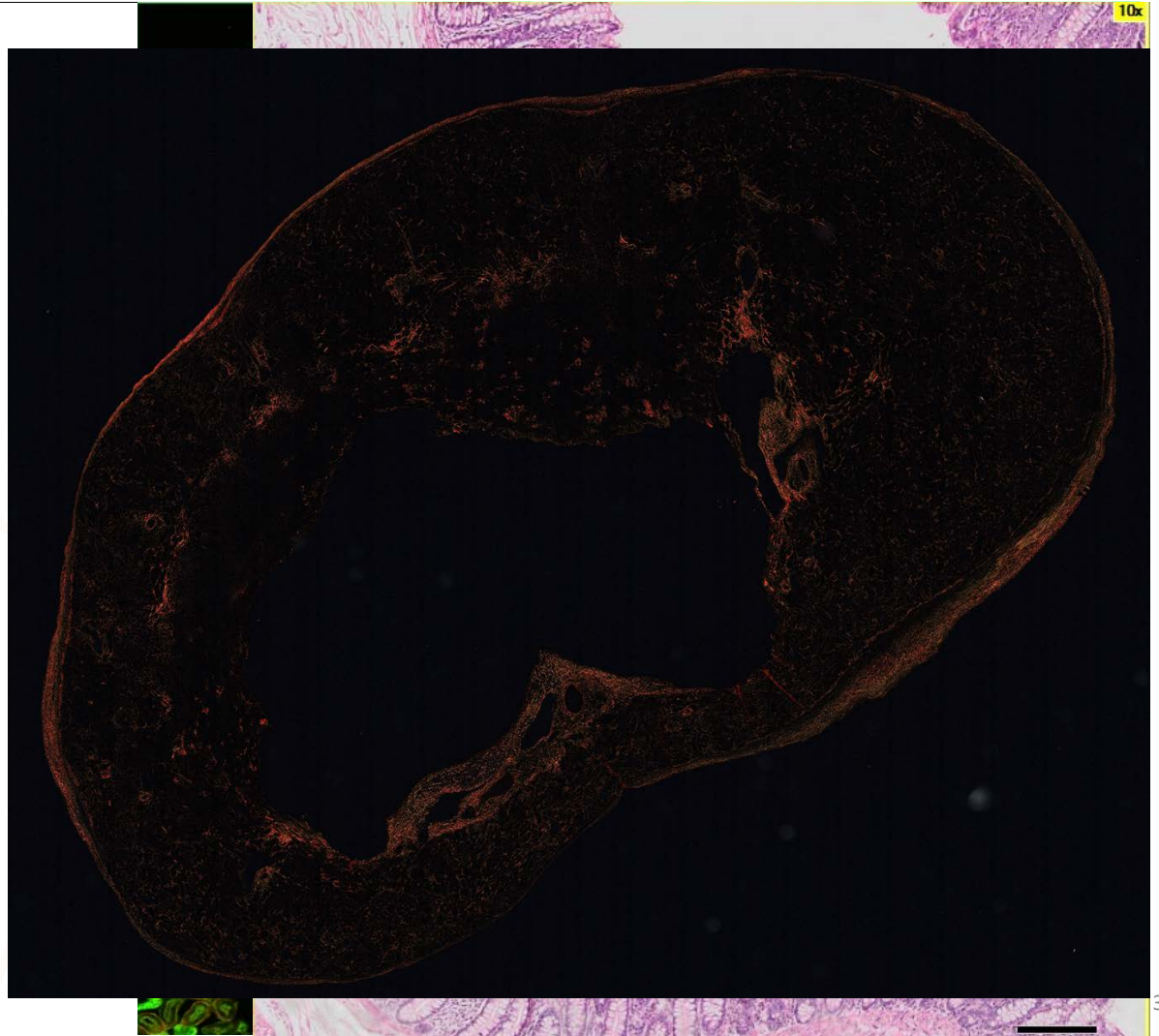
Slide loader

Microscope

Joystick & Focusing

# Typical applications

- Bright Field (BF)
- Fluorescence
- Polarisation



# Part 1: tips & tricks on image acquisition

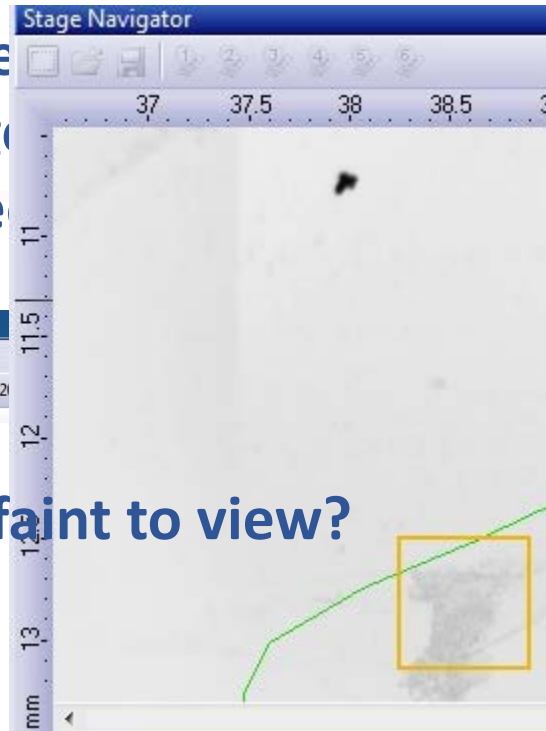
---

## 5 common issues & their resolutions

1. Unable to view your sample in live window
2. Faint signals
3. Out of focus image (blurry)
4. Shading issue
5. Photobleaching

# 1. Unable to view your sample in live window

- Is your fluorescence lamp turned on?
- Make sure your sample is positioned correctly
- Take advantage of microscope hardware: stage and software: objective



- If signal is too faint to view?



## 2. Faint signal

- Remap dynamic range
- Push up illumination intensity
- Extend exposure time
- MIP (maximum intensity projection)

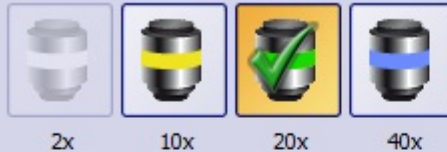
Exposure Time



6.676 ms



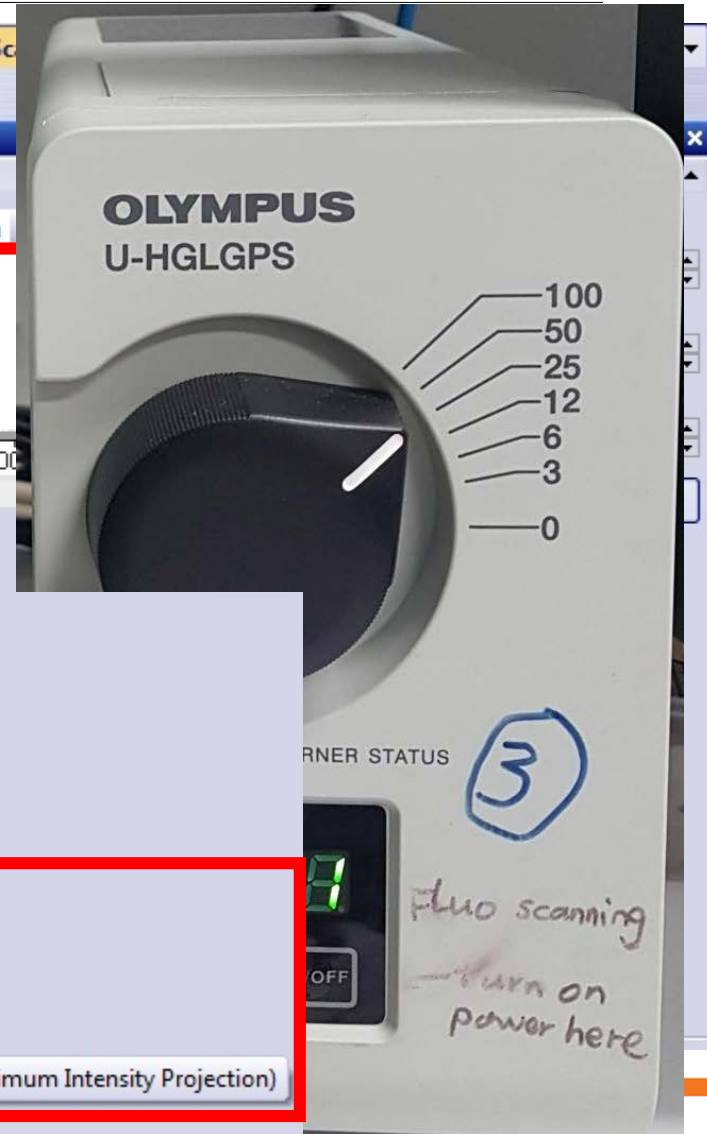
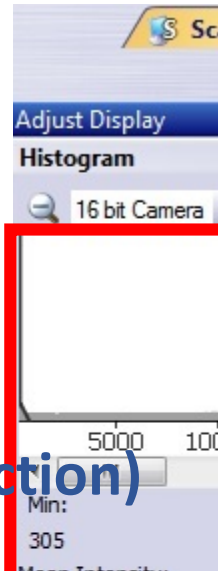
Display Limits:



Z-Scan mode:



Scan MIP (Maximum Intensity Projection)



### 3. Out of focus image (blurry)

- Sample placement
- Focus points
- EFI (extended focal image)
- Z-offset



→ Overview → Magnification → Scan Area → Illumination Settings → Focus Settings → Review Focus

Magnification

→ Overview → Magnification → Scan Area → Illumination Settings → Focus Sett

Channel Settings

--	--	--	--	--	--

Channel Name	Exposure Time	Z-Offset	Deblur

Z-Scan mode:

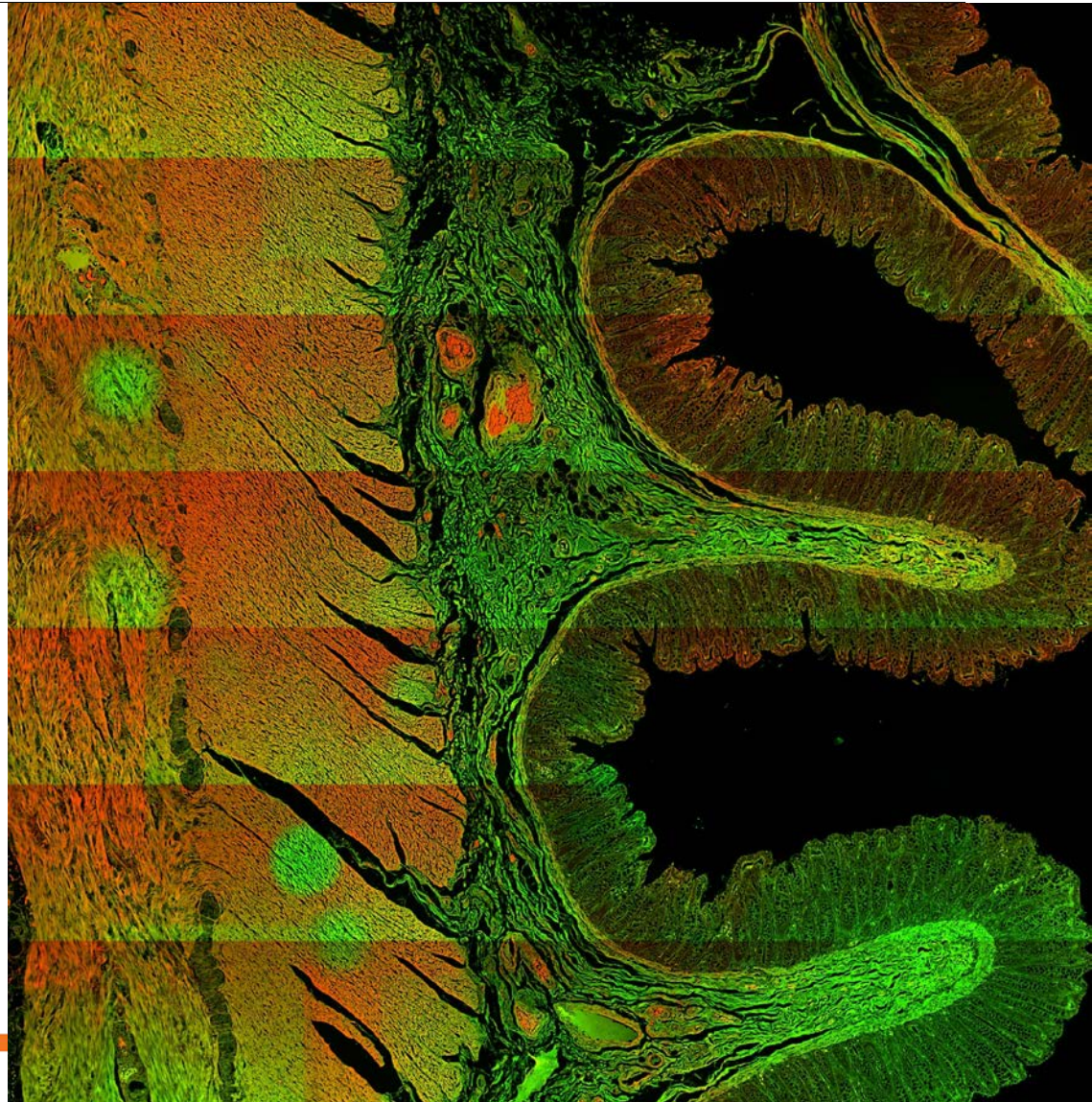
--	--	--	--

Scan EFI (Extended Focal Image)

## 4. Shading issue

---

- Calibrate it!





## 5. Photobleaching

---

- Decrease illumination
- Overview using BF
- Close proximity to sample ROI (free hand drawing)
- Multiple selection of ROIs


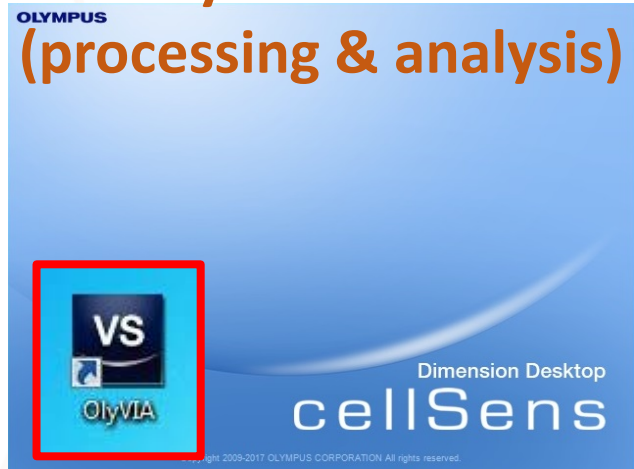





# Part 2: handling large-sized scanning images

## Introduction

- Creation of large files, i.e. BF 1.5CM X 1.5CM—2-3GB
- Always save in VSI format first
- Can be directly opened with Olyvia, CellSens, VS-ASW/Desktop, Fiji ImageJ, not Huygens yet

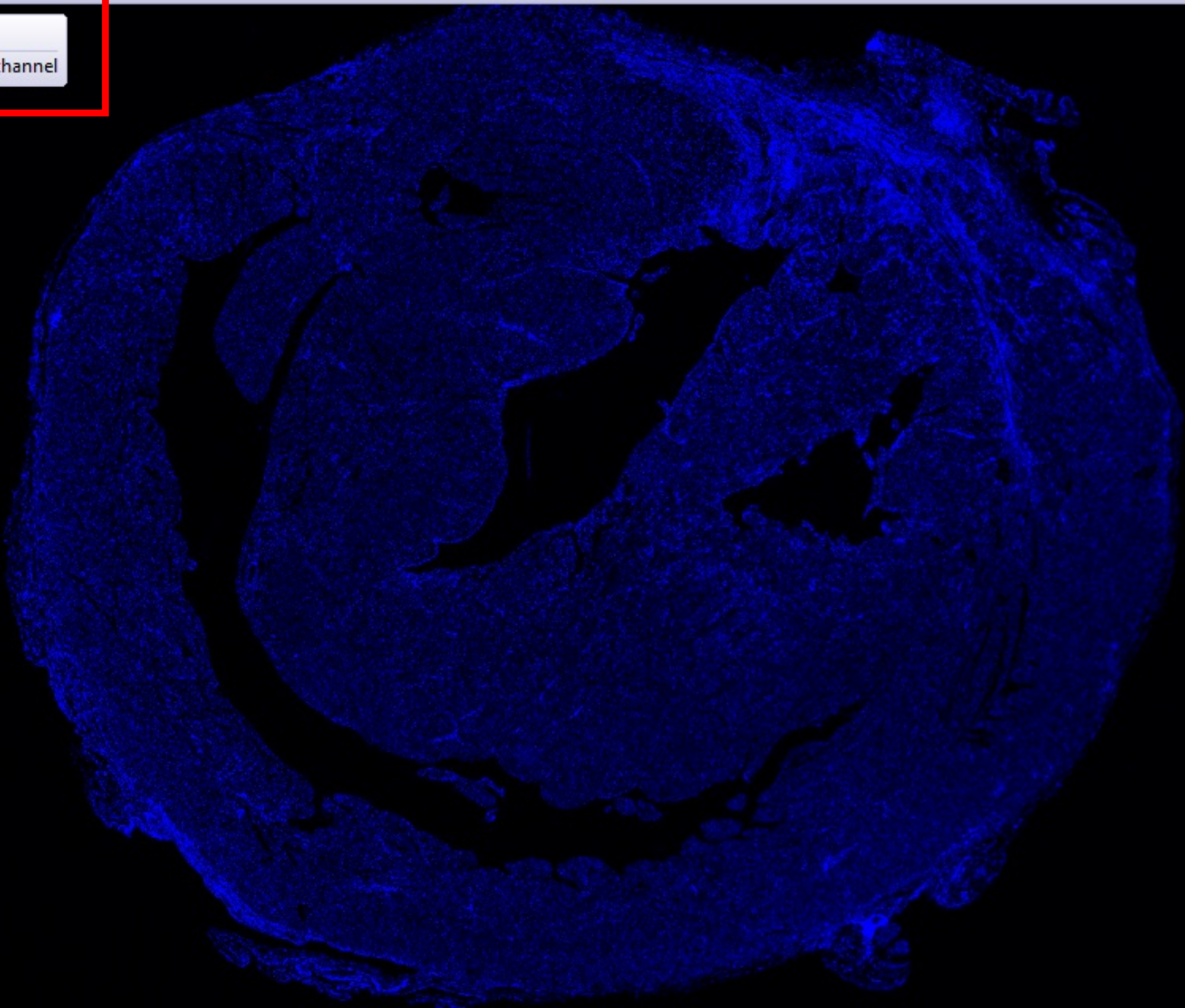
Any PC viewer	Analysis PC: C2.34 (processing & analysis)	Scanner PC: J2.08 viewer acquisition processing & analysis
		



Image\_01Smiley Face 40X AE.vsi x

Channel selection toolbar with icons for visibility, color, and zoom. A tooltip is displayed for the orange icon:

- TX Red-Tri**  
Shows/Hides the channel



# CellSens: save as, export, analyse .VSI data

The screenshot displays the OLYMPUS cellSens Dimension Desktop software interface. The main window shows a multi-channel fluorescence image of a smiley face, with different channels represented by different colors (green, blue, red). The image is displayed on a grid with coordinates from 21 to 37 mm on the x-axis and 7 to 18 mm on the y-axis. A 2 mm scale bar is visible in the bottom right corner. The software title bar reads "OLYMPUS cellSens Dimension Desktop". The menu bar includes File, Edit, View, Image, Process, Measure, Tools, Window, and Help. The toolbar contains various icons for image manipulation and analysis. The Properties panel on the left is expanded to show the "Image" section, with the "File Size" field circled in red, indicating a size of 6.05 GB. Other fields in the "Image" section include Layer (4), Frame Count (1), Channel Count (3), Type (16 bit Grayscale), Size (pixel) (104600 x 88338), Size (calibrated) (17.5 mm x 14.4 mm), Calibration (X) (162.5 nm/pixel), Calibration (Y) (162.5 nm/pixel), Origin (X) (0.00 mm), Origin (Y) (-67.74 mm), Total Magnification (40 x), and Memory Usage (uncompressed) (51.63 GB). The "Channel 1" section shows Channel Name (DAPI), Emission Wavelength (455 nm), Observation Method (DAPI), and Exposure Time (32.018 ms). The "Channel 2" section shows Channel Name (FITC), Emission Wavelength (518 nm), Observation Method (FITC), and Exposure Time (250 ms). The "Channel 3" section shows Channel Name (TX Red-Tri), Emission Wavelength (615 nm), Observation Method (TX Red-Tri), and Exposure Time (103.616 ms). The "Microscope" section shows Microscope (VS-BX), Filter Wheel (Reflected) (387/11-25), Mirror Cube (U-DM-DA/FI/TR/Cy5), Lamp Intensity (Transmission) (9.00 V), Lamp Status (Transmission) (Off), Top Lens (Out), Filter Wheel (Observation) (440/40-25), Condenser (U-ND6), Aperture Stop (0 %), and Objective Lens (UPLSAPO 2.40x / 0.95).

Section	Property	Value
Image	Name	Image_01Smiley Face 40X AE.vsi
	Path	G:\Cell Imaging\Olympus VS 120\...
	Author	Hong.Yu
	Creation Time	15/11/2017 10:30:28 AM
	Note	
	Company	Hewlett-Packard Company
	Product Version	OLYMPUS VS-ASW 2.9 (Build 13...
	File Size	6.05 GB
	Type	16 bit Grayscale
	Memory Usage (uncompressed)	51.63 GB
Channel 1	Channel Name	DAPI
	Emission Wavelength	455 nm
	Observation Method	DAPI
	Exposure Time	32.018 ms
Channel 2	Channel Name	FITC
	Emission Wavelength	518 nm
	Observation Method	FITC
	Exposure Time	250 ms
Channel 3	Channel Name	TX Red-Tri
	Emission Wavelength	615 nm
	Observation Method	TX Red-Tri
	Exposure Time	103.616 ms
Microscope	Microscope	VS-BX
	Filter Wheel (Reflected)	387/11-25
	Mirror Cube	U-DM-DA/FI/TR/Cy5
	Lamp Intensity (Transmission)	9.00 V
	Lamp Status (Transmission)	Off
	Top Lens	Out
	Filter Wheel (Observation)	440/40-25
	Condenser	U-ND6
	Aperture Stop	0 %
	Objective Lens	UPLSAPO 2.40x / 0.95

# CellSens: VSI to Tiff (1) < 2G

“save as” directly

The screenshot shows the OLYMPUS cellSens Dimension Desktop interface. A warning dialog box is displayed in the center, stating: "The image size exceeds the limit for the specified file format. If you continue you may get a corrupt file (depends on the compression rate). Proceed anyway?" with "Yes" and "No" buttons. The dialog box is highlighted with a red border.

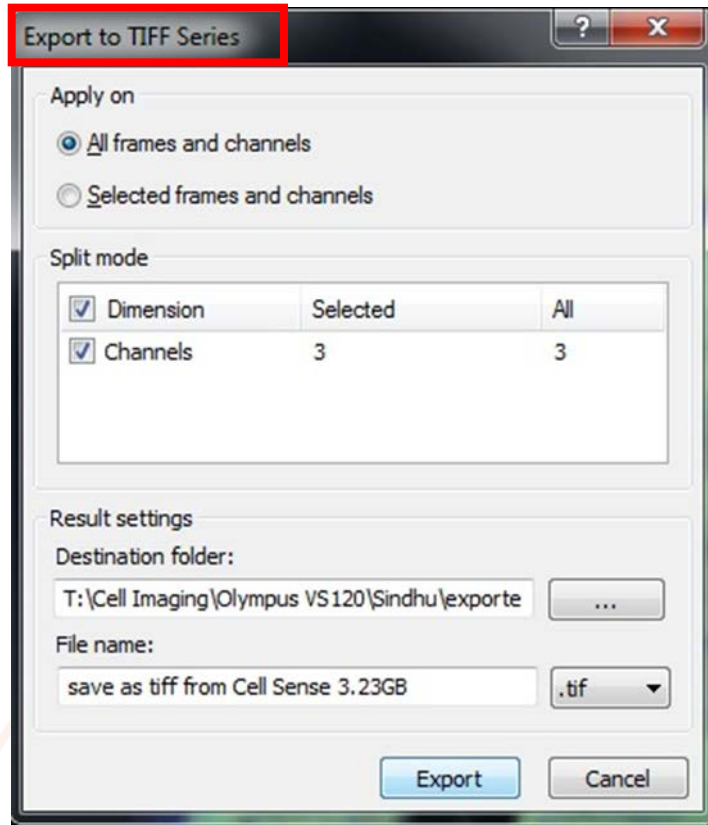
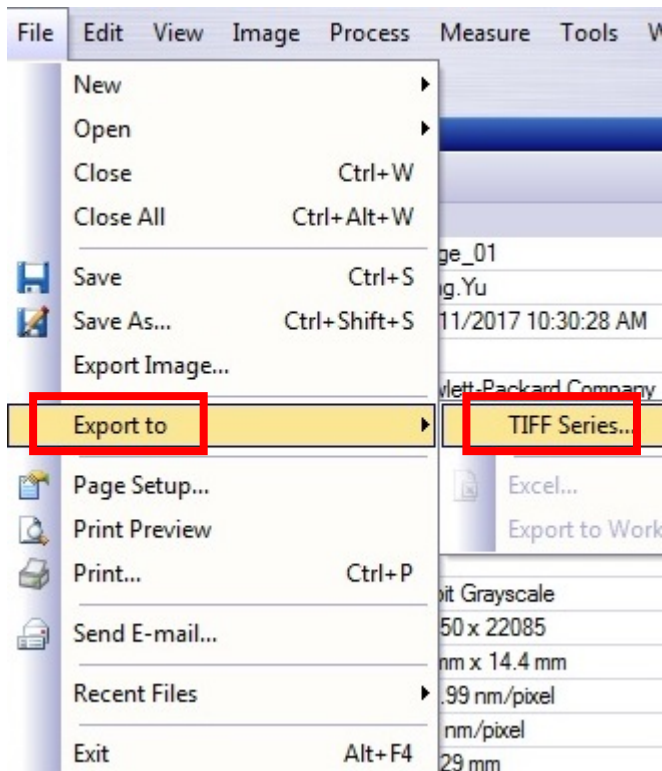
On the left, the Properties panel shows the following details:

Category	Property	Value	
Document	Name	Image_01Smiley Face 40X AE.vsi	
	Path	T:\Cell Imaging\Olympus VS120\...	
	Author	Hong.Yu	
	Creation Time	15/11/2017 10:30:28 AM	
	Note		
	Company	Hewlett-Packard Company	
	Product Version	OLYMPUS VS-ASW 2.9 (Build 13...	
	File Size	6.05 GB	
	Image	Layer	40x
		Frame Count	1
Channel Count		3	
Type		16 bit Grayscale	
Size (pixel)		104600 x 88338	
Size (calibrated)		162.5 mm x 14.4 mm	
Calibration (X)		162.5 nm/pixel	
Calibration (Y)		162.5 nm/pixel	
Origin (X)		-89.29 mm	
Origin (Y)		-67.74 mm	
Total Magnification		40x	
Memory Usage (uncompressed)		51.63 GB	
Channel 1	Channel Name	DAPI	
	Emission Wavelength	455 nm	
	Observation Method	DAPI	
	Exposure Time	32.018 ms	
Channel 2	Channel Name	FITC	
	Emission Wavelength	518 nm	
	Exposure Time	250 ms	
Channel 3	Channel Name	TX Red-Tr	
	Emission Wavelength	615 nm	
	Observation Method	TX Red-Tr	
	Exposure Time	103.616 ms	
Microscope	Microscope	VS-BX	
	Filter Wheel (Reflected)	387/11-25	
	Mirror Cube	LLDM.DA/EL/TR/Cv5	

The main image area shows a fluorescence micrograph of a cell with a magnification of 40x. The image is displayed on a grid with a vertical scale on the left ranging from 0 to 22.

# CellSens: VSI to Tiff (2) 2-5G

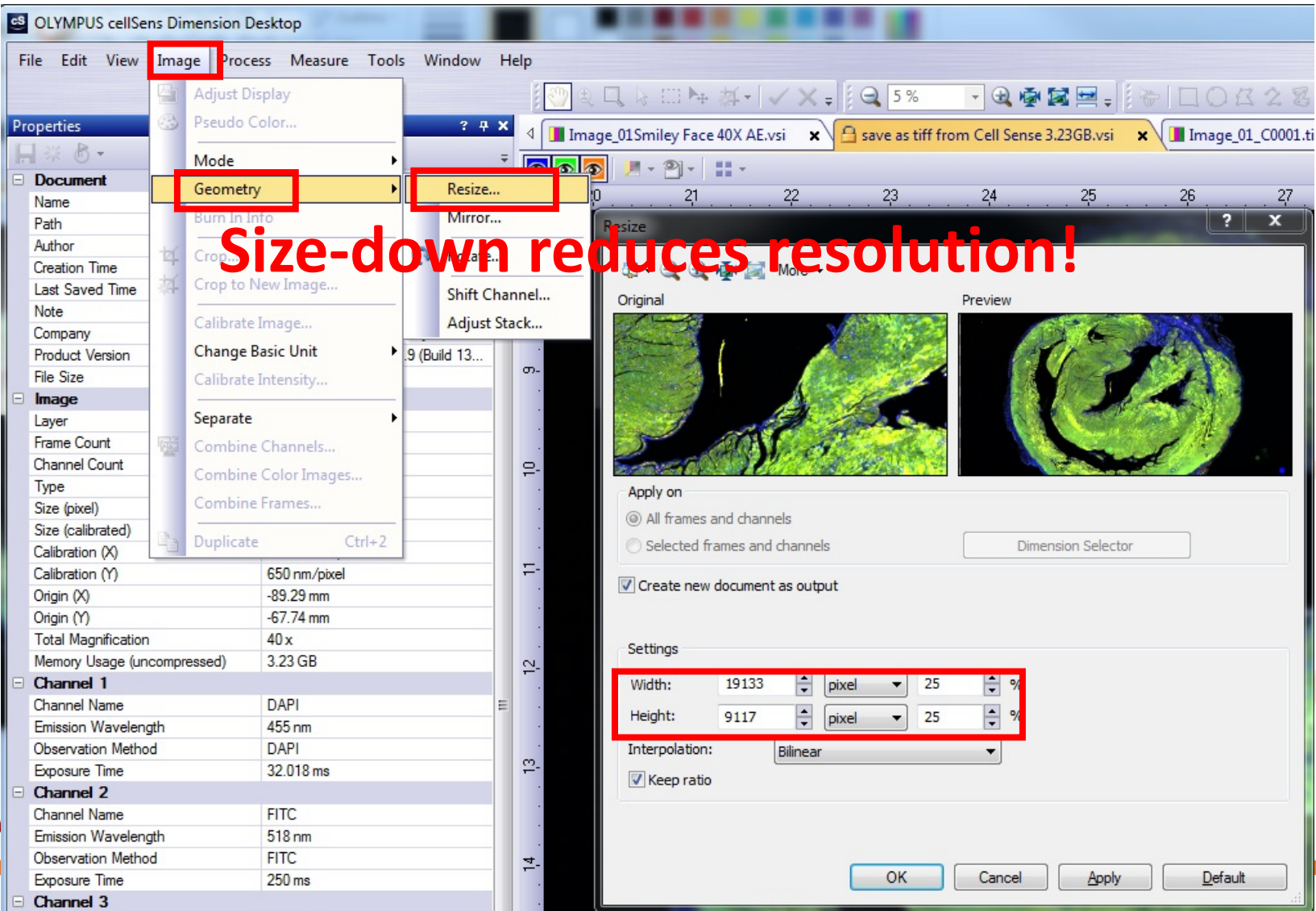
“export to tiff series”



Image_01_C0001.tif	11/06/2018 2:11 PM	TIF File	1,131,028
Image_01_C0002.tif	11/06/2018 2:12 PM	TIF File	1,131,028
Image_01_C0003.tif	11/06/2018 2:12 PM	TIF File	1,131,028

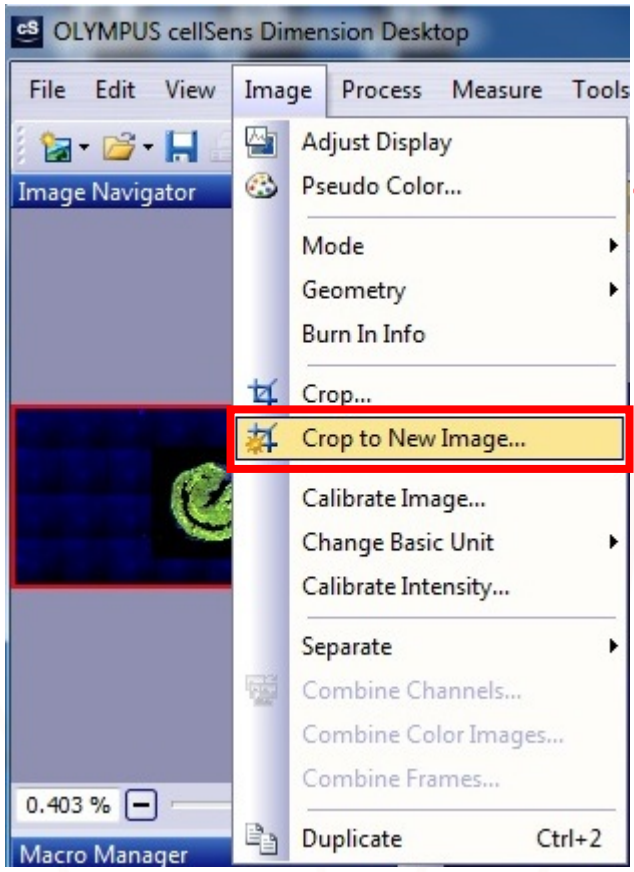
# CellSens: VSI to Tiff (3) >5G

“resize” down first

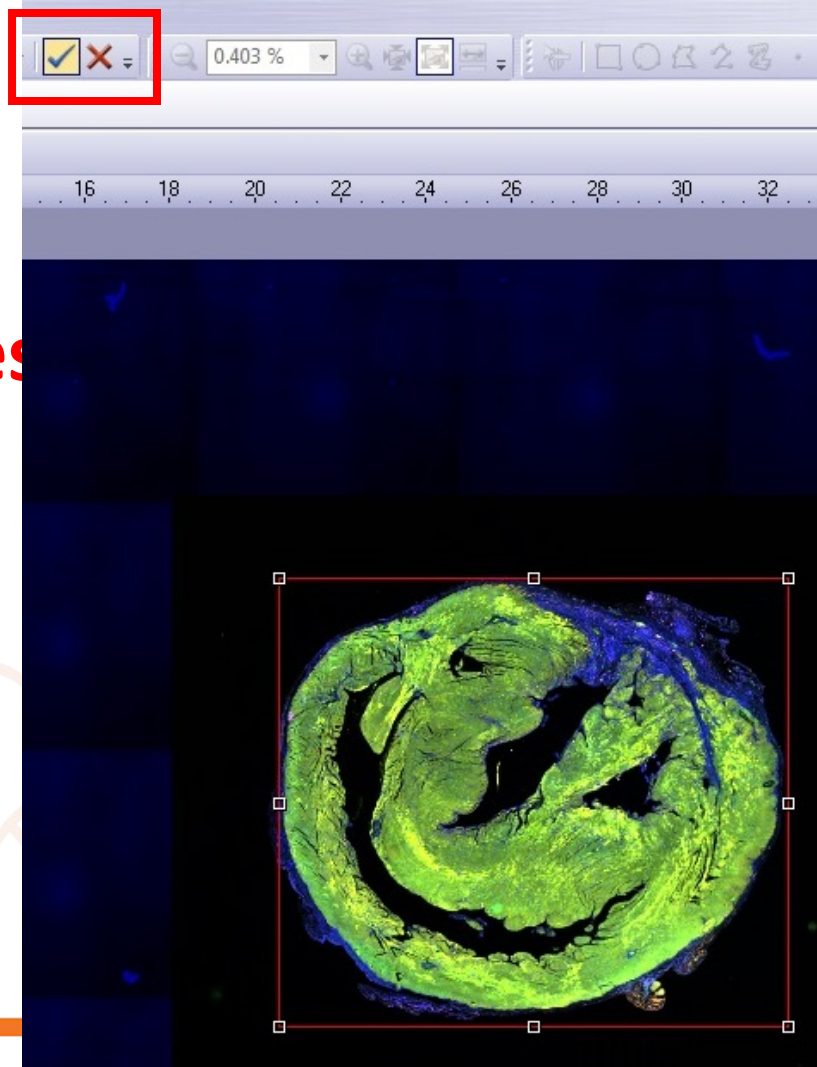


# CellSens: VSI to Tiff (3) >5G

or “crop” first...



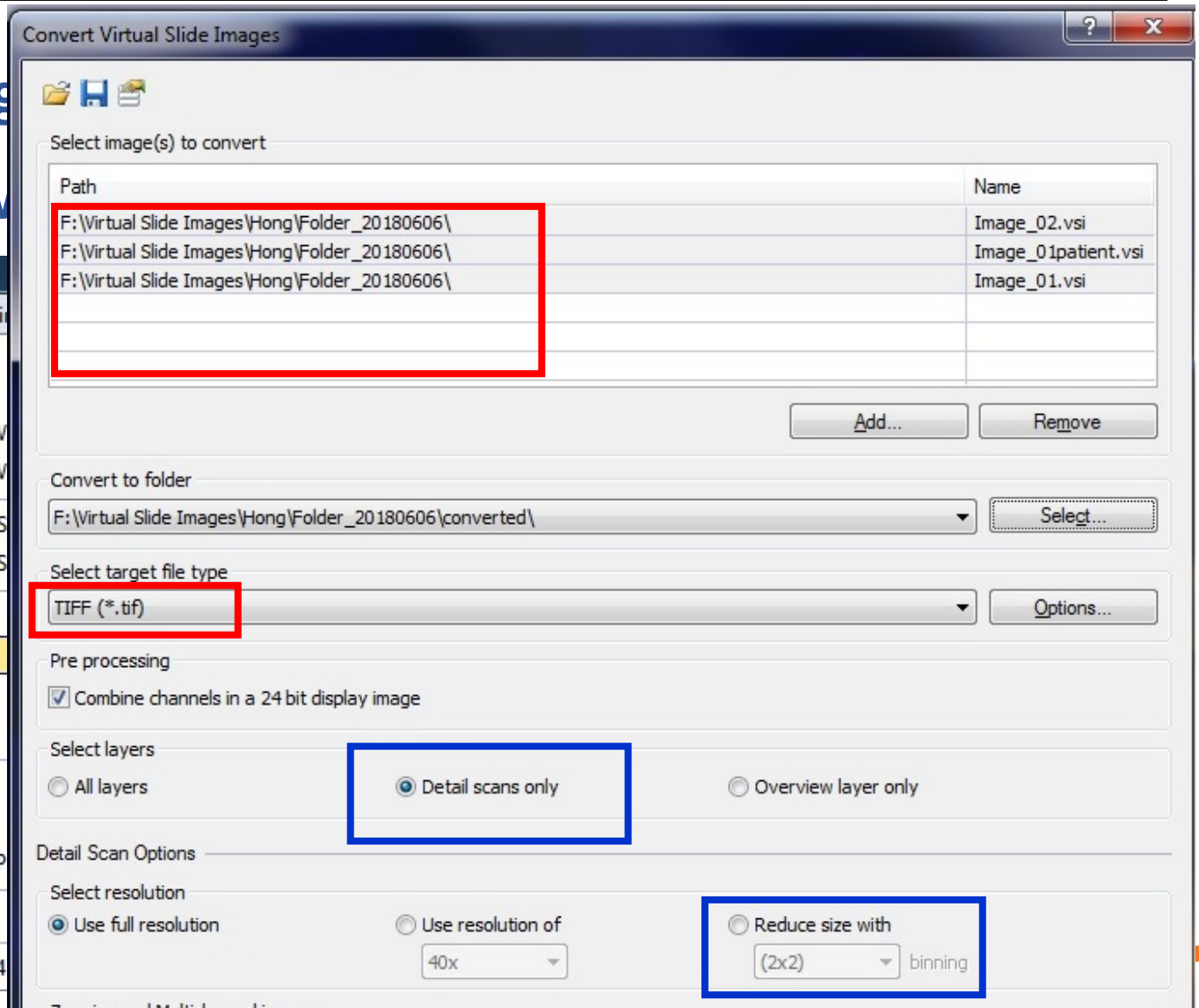
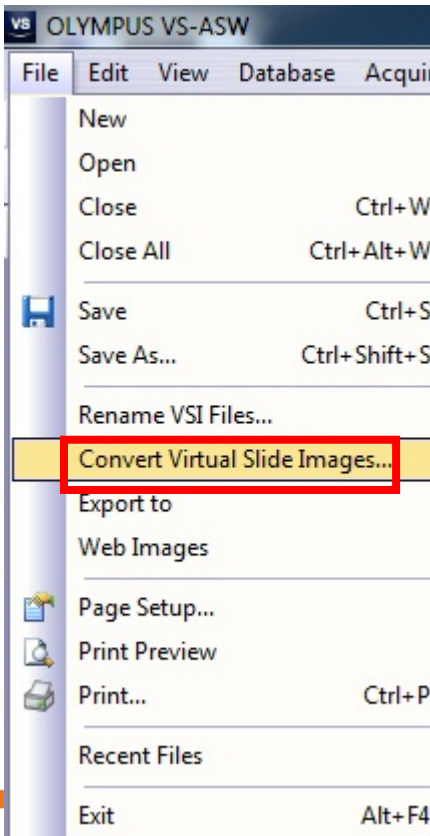
maintains res





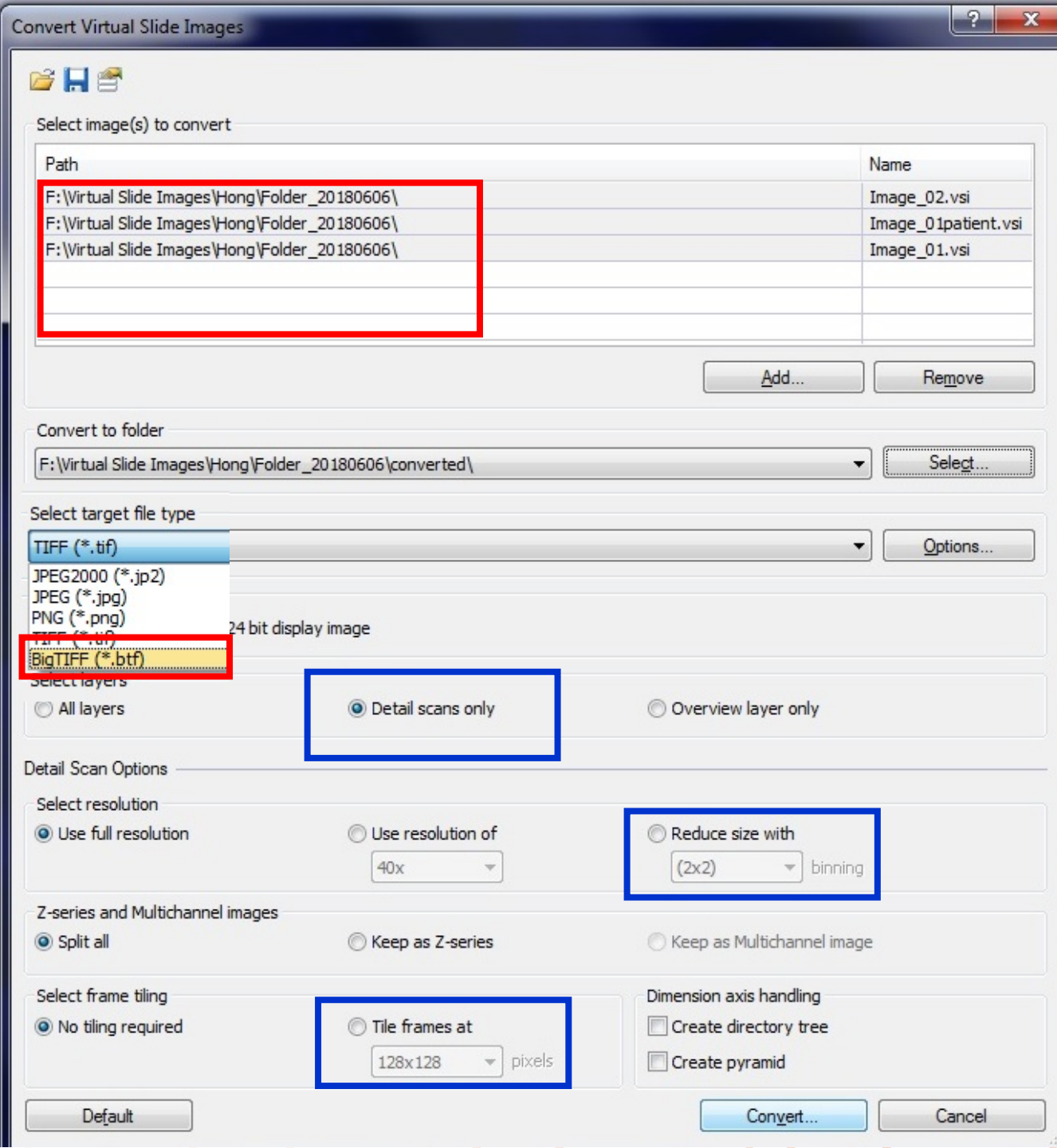
# VS Desktop: VSI to Tiff using “converter” < 4G

- Changing
- Built-in v



# VS Desktop: V

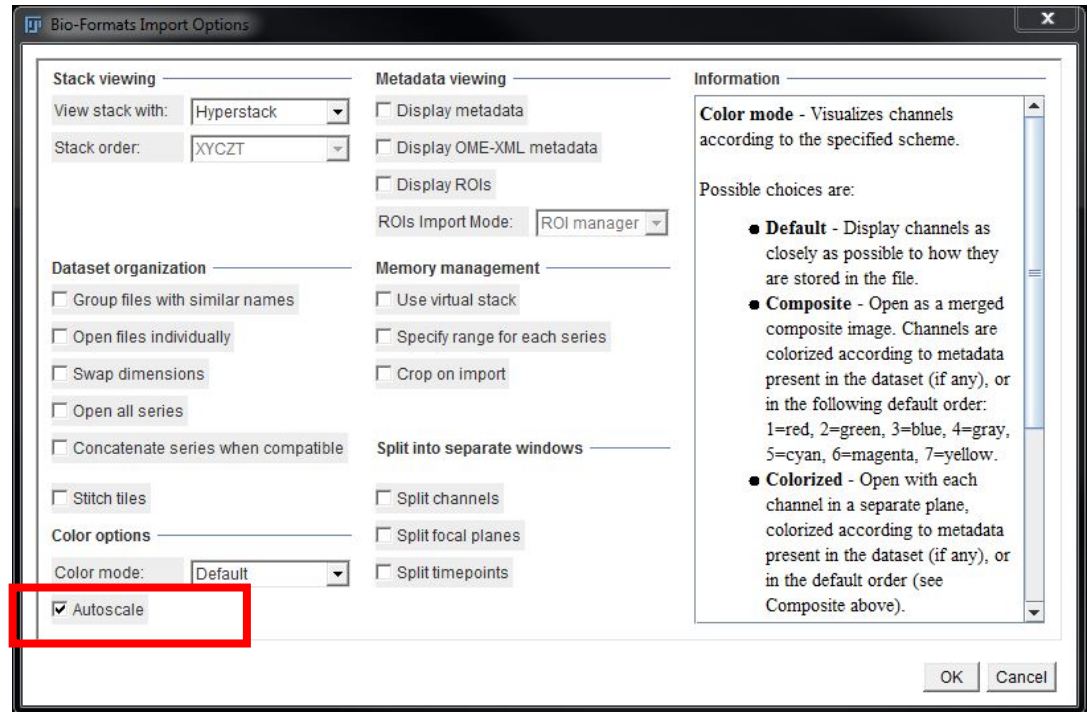
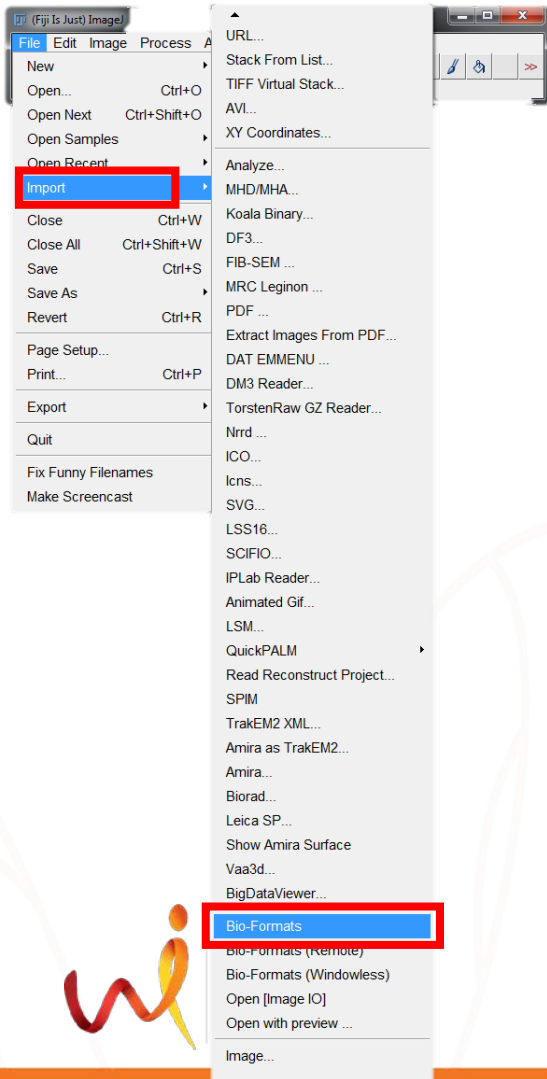
- Convert to BigTIFF!

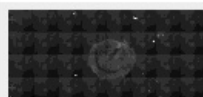
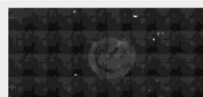
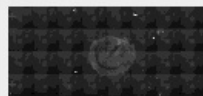
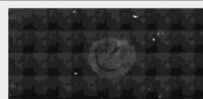


# Tip: extract data in CellSens & VS ASW/Desktop

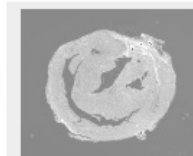


# FIJI ImageJ: open .VSI images directly



Series 1: overview: 15306 x 7293; 1 plane Series 2: Image 01Smiley Face 40X AE.vsi #2: 7653 x 3647; 1 plane Series 3: Image 01Smiley Face 40X AE.vsi #3: 3827 x 1824; 1 plane Series 4: Image 01Smiley Face 40X AE.vsi #4: 1914 x 912; 1 plane Series 5: Image 01Smiley Face 40X AE.vsi #5: 957 x 456; 1 plane Series 6: Image 01Smiley Face 40X AE.vsi #6: 479 x 228; 1 plane Series 7: 40x: 104600 x 88338; 3 planes Series 8: Image 01Smiley Face 40X AE.vsi #8: 52300 x 22085; 3 planes Series 9: Image 01Smiley Face 40X AE.vsi #9: 26150 x 22085; 3 planes Series 10: Image 01Smiley Face 40X AE.vsi #10: 13075 x 11043; 3 planes

**Original resolution  
file is too big!**

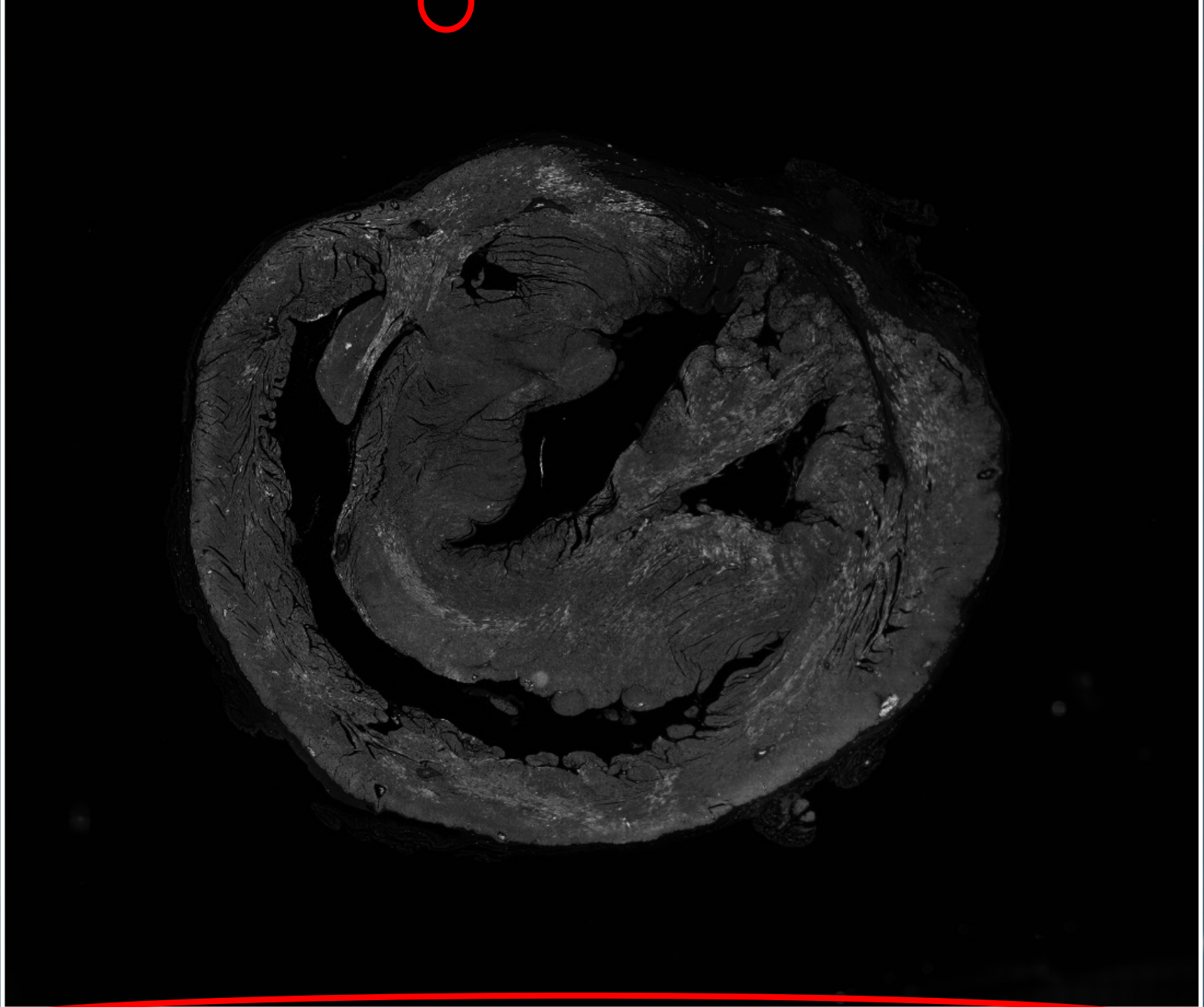
 Series 7: 40x: 104600 x 88338; 3 planes Series 8: Image 01Smiley Face 40X AE.vsi #8: 52300 x 44169; 3 planes Series 9: Image 01Smiley Face 40X AE.vsi #9: 26150 x 22085; 3 planes Series 10: Image 01Smiley Face 40X AE.vsi #10: 13075 x 11043; 3 planes Series 11: Image 01Smiley Face 40X AE.vsi #11: 6538 x 5522; 3 planes Series 12: Image 01Smiley Face 40X AE.vsi #12: 3269 x 2761; 3 planes Series 13: Image 01Smiley Face 40X AE.vsi #13: 1635 x 1381; 3 planes

OK

Cancel

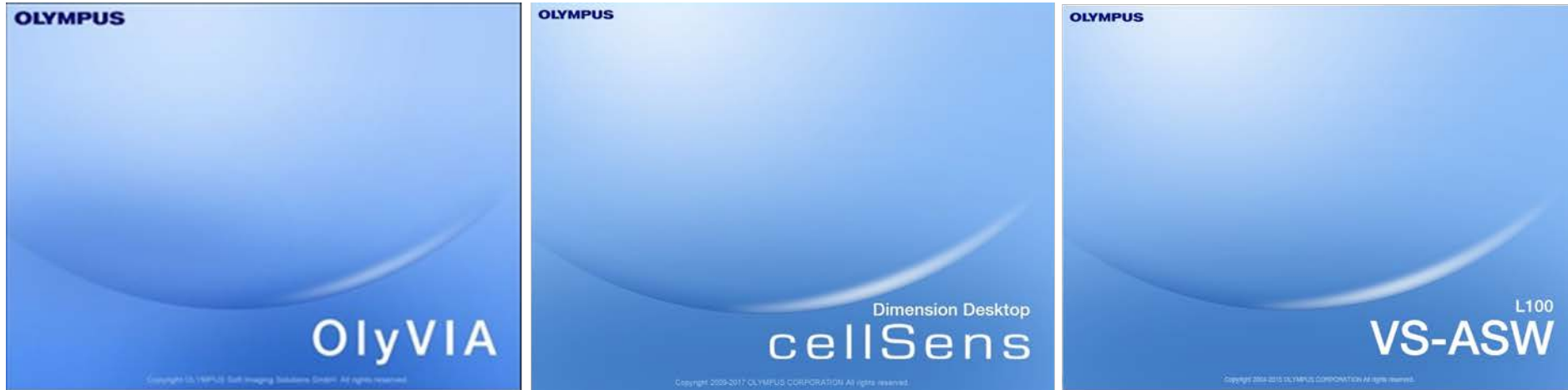
Select All

Deselect All



# Take-home messages

## 1. Available software and applications



## 2. How to reduce image size?

- CellSens: resize, crop
- VS Desktop: scan details only, binning, tiling
- FIJI ImageJ: import small sized series
- Olyvia: viewer only, cannot save images...



# Enquiries, questions, or review training?

---

- Contact me: [hong.yu@Sydney.edu.au](mailto:hong.yu@Sydney.edu.au); 8627 3211
- Visit our website: <https://sydneyuni.atlassian.net/wiki/spaces/WIF/>





**Thank you!**