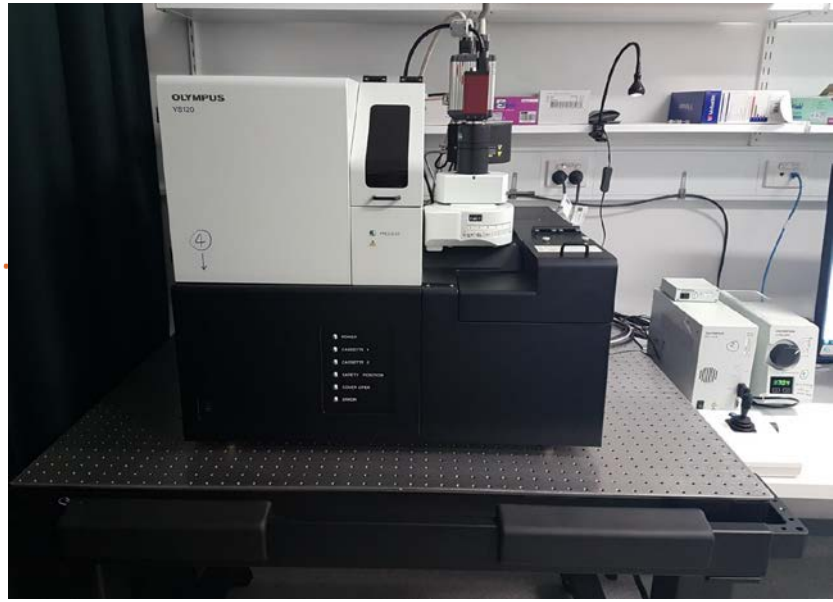
The Westmead Institute

Finding cures. Saving lives. Giving hope.

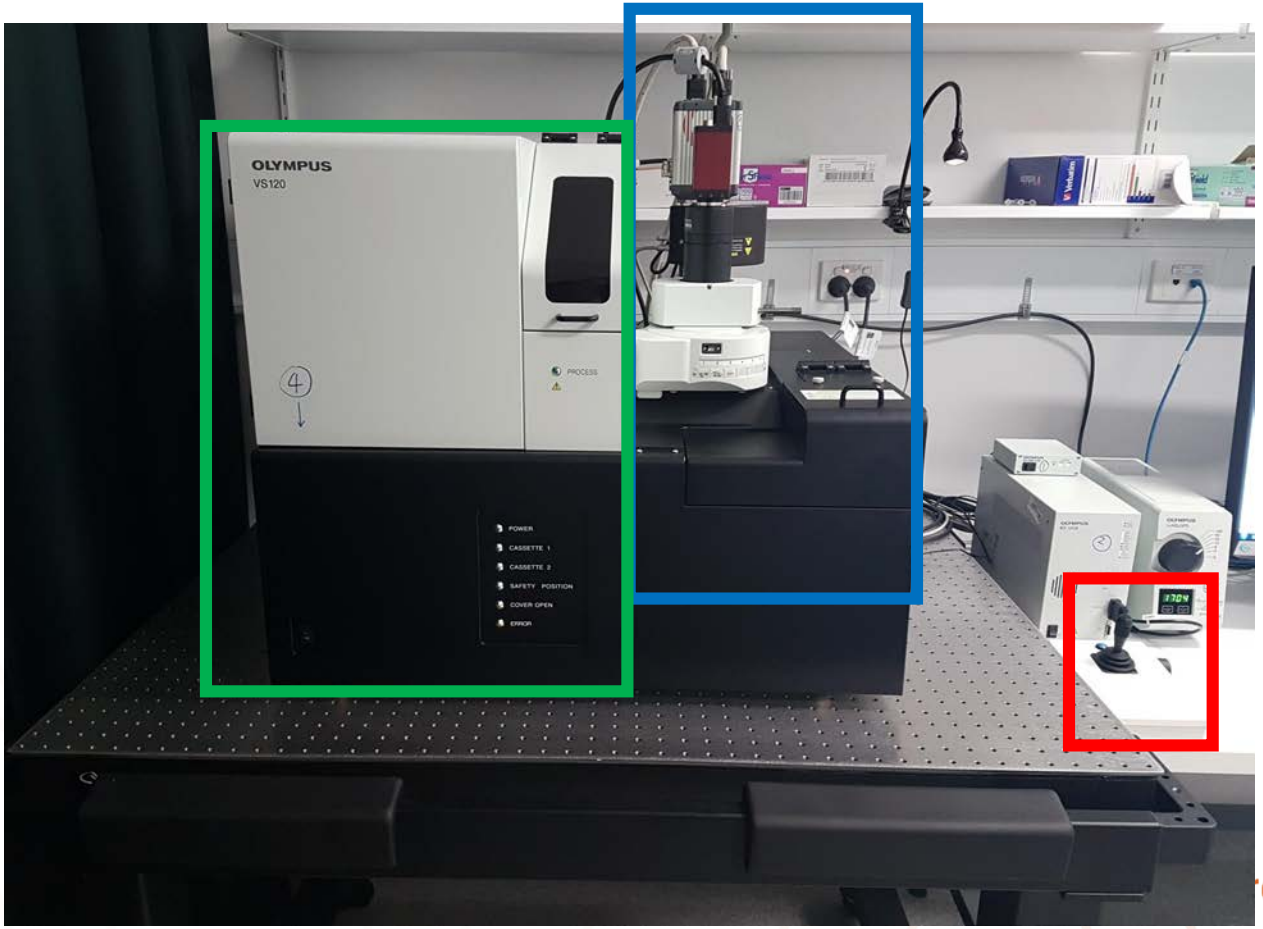


Tricks & tips on image acquisition with Olympus VS 120 & handling large sized images

Hong Yu, Westmead Imaging Facility 25 July 2019

What is Olympus VS 120? Lab J2.08

- Microscope based slide scanner—100 slides



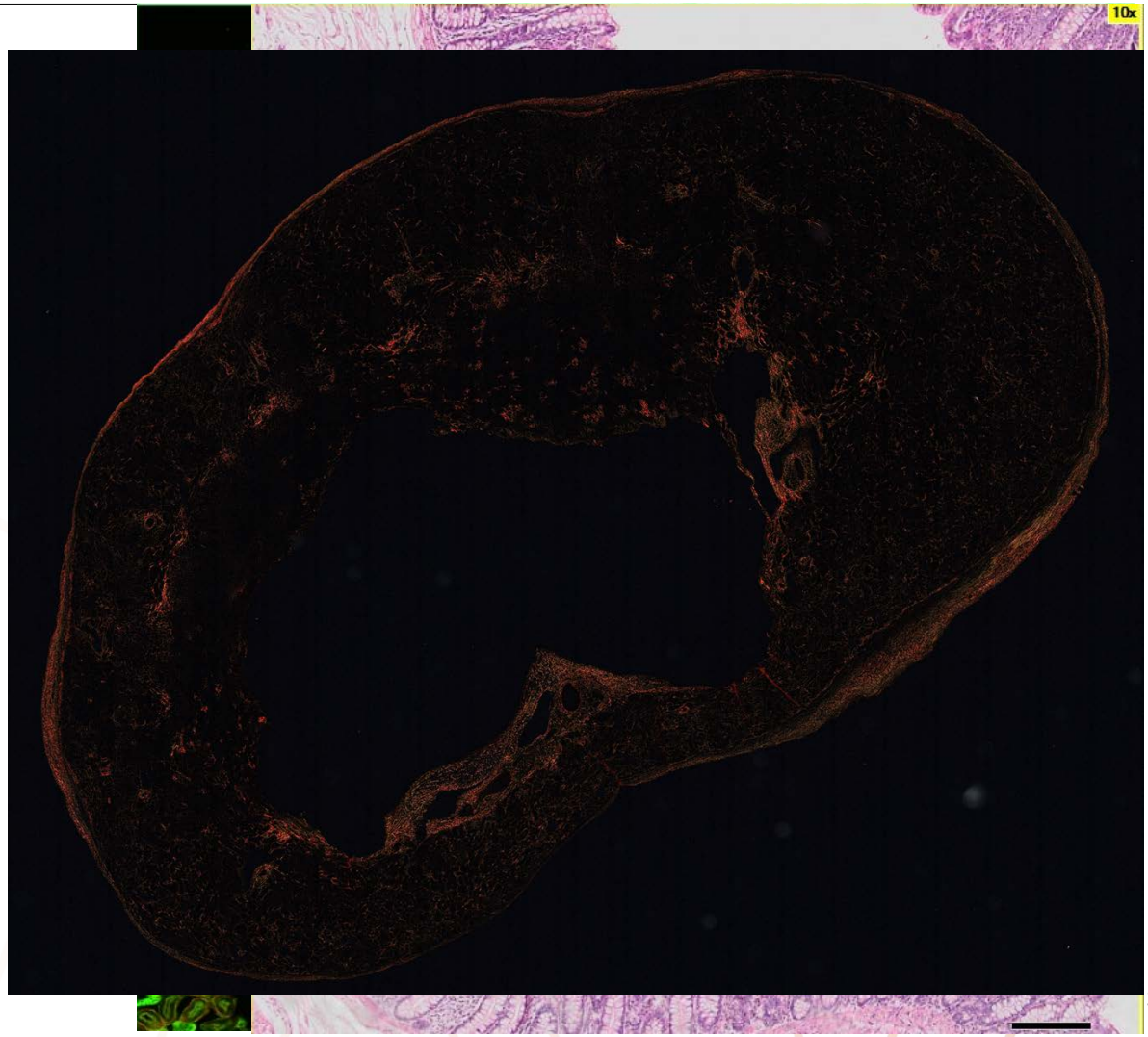
Slide loader

Microscope

Joystick & Focusing

Typical applications

- Bright Field (BF)
- Fluorescence
- Polarisation



The Westmead Institute

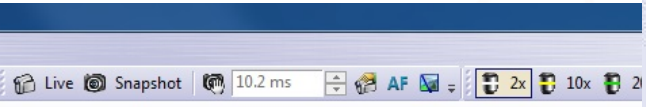
Part 1: tips & tricks on image acquisition

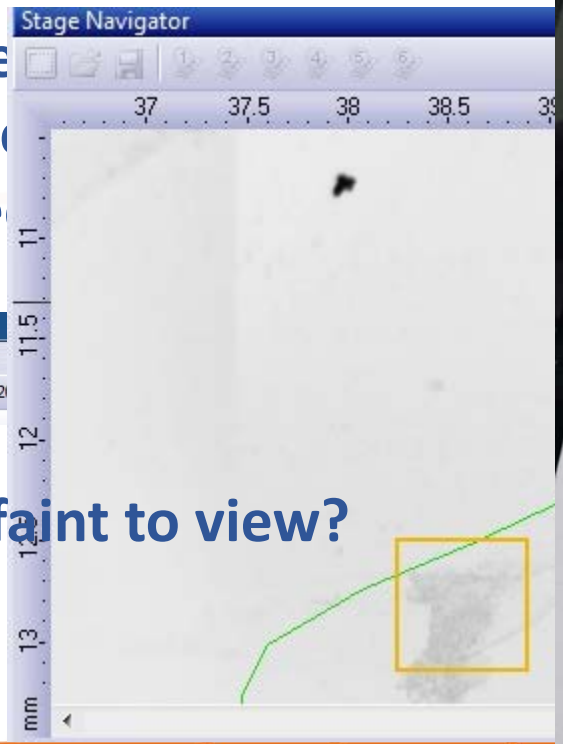
5 common issues & their resolutions

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 corr
- Take advantage
  hardware: stag
  software: obje
- If signal is too faint to view?

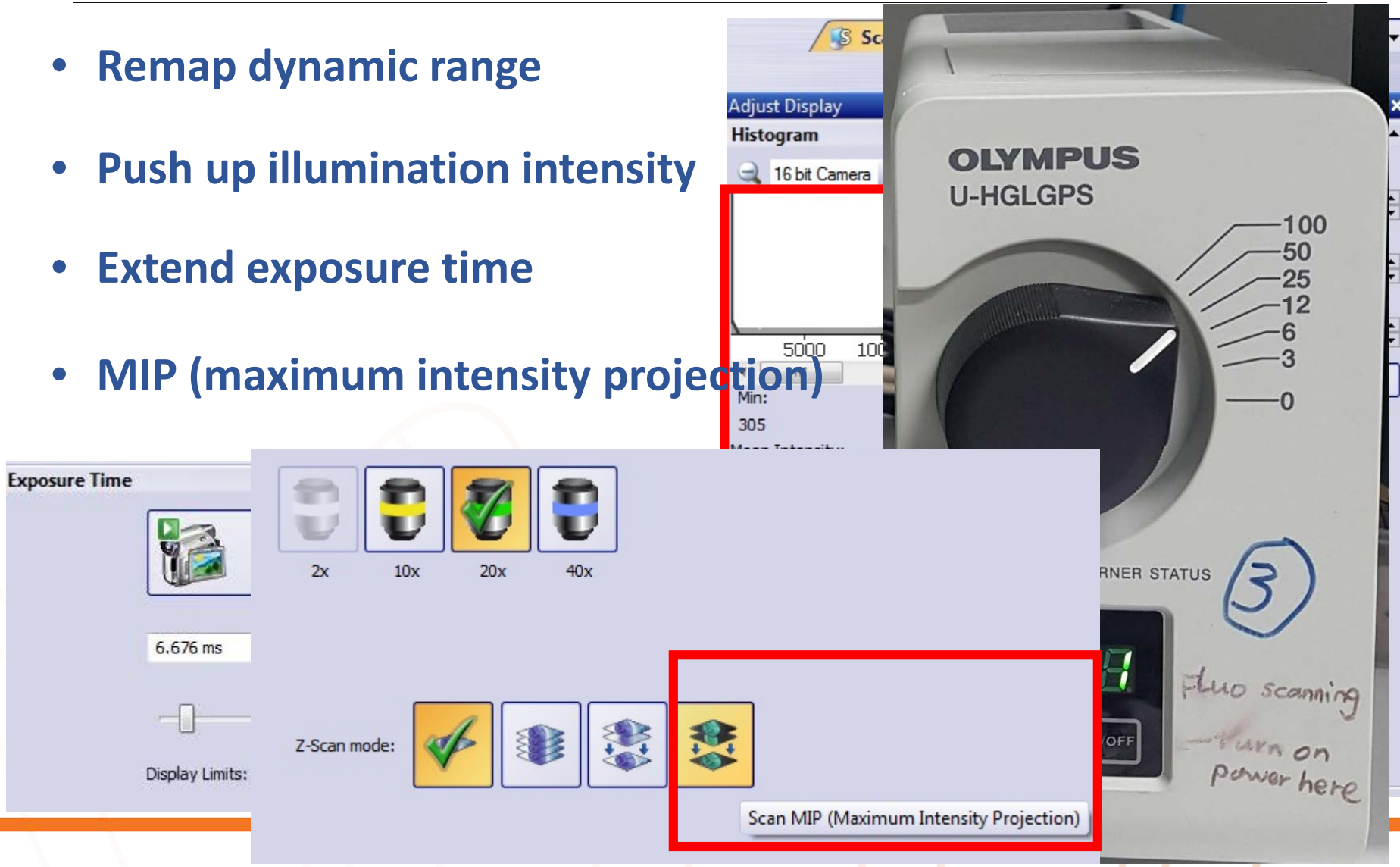




The Westmead Institute

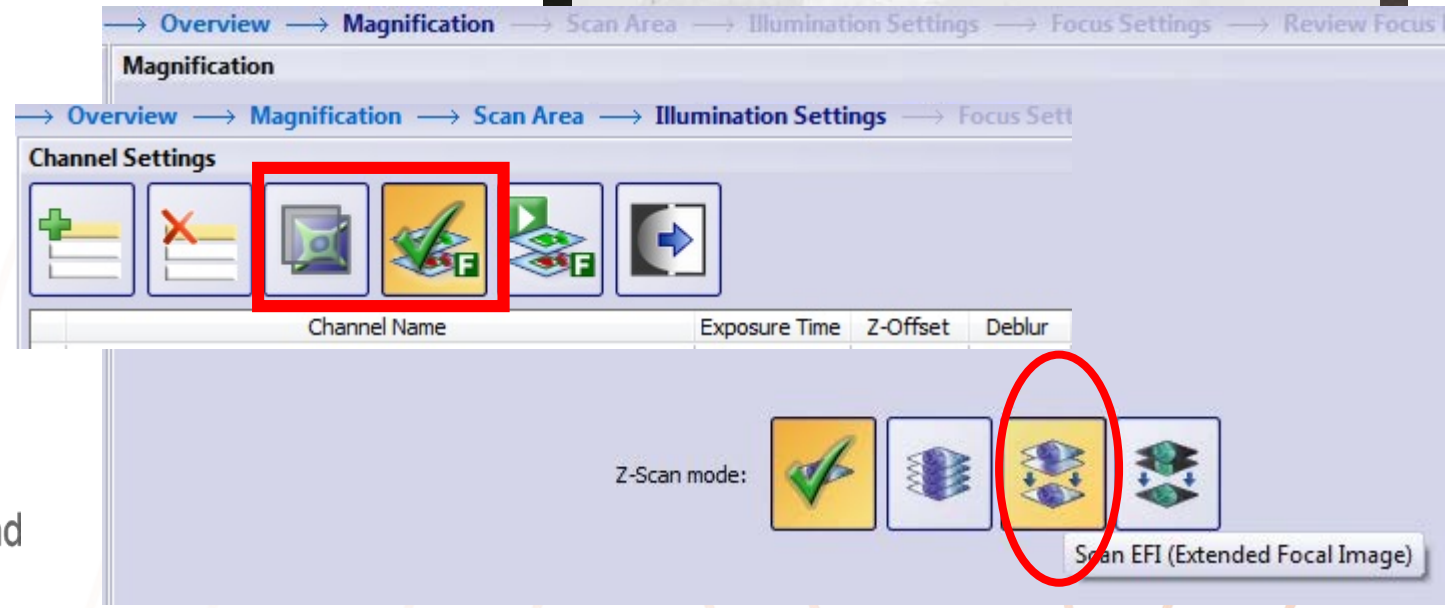
2. Faint signal

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



3. Out of focus image (blurry)

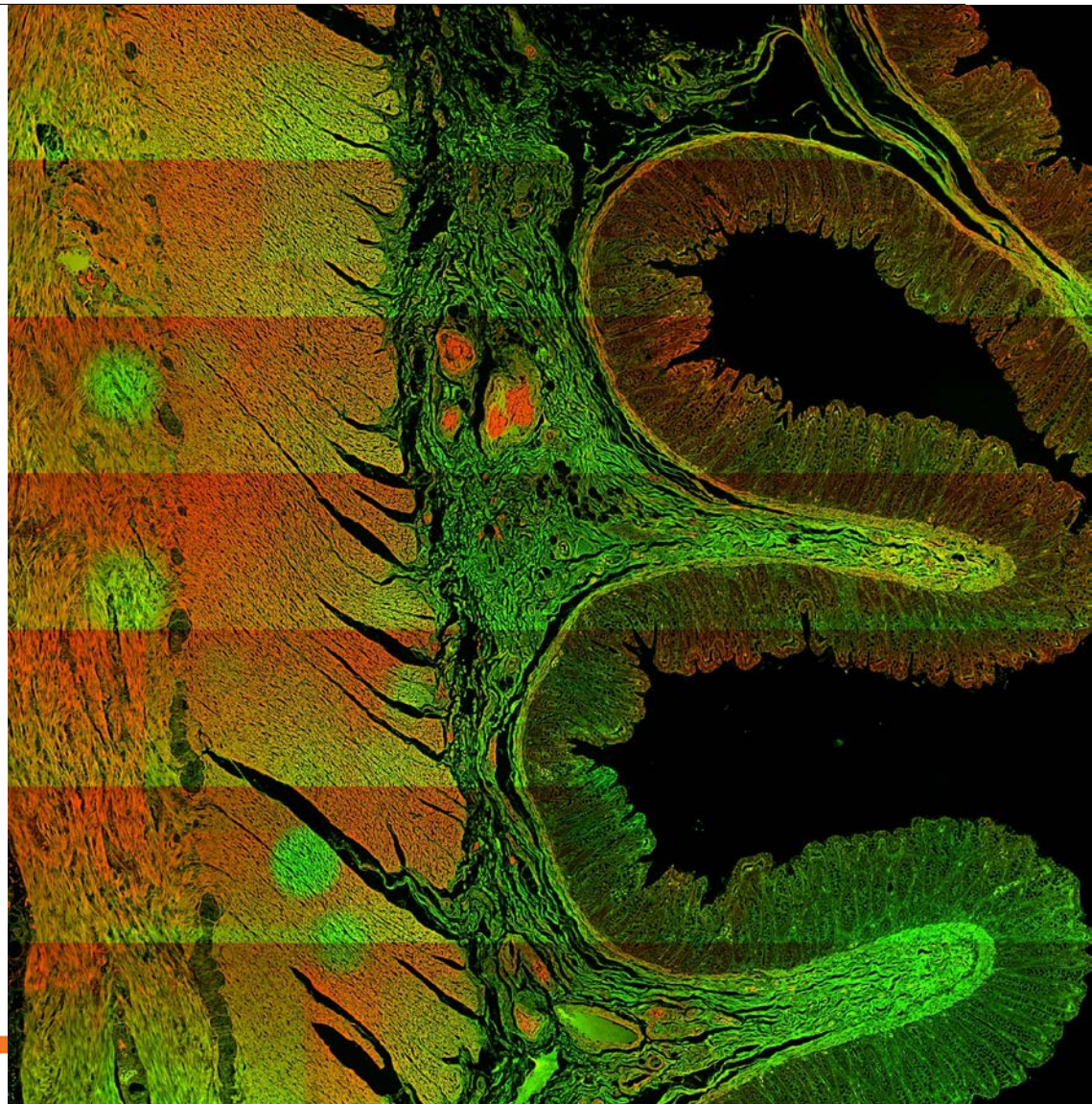
- Sample placement
- Focus points
- EFI (extended focal image)
- Z-offset



The Westmead Institute

4. Shading issue

- **Calibrate it!**



The Westmead Institute

5. Photobleaching

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

The Westmead Institute

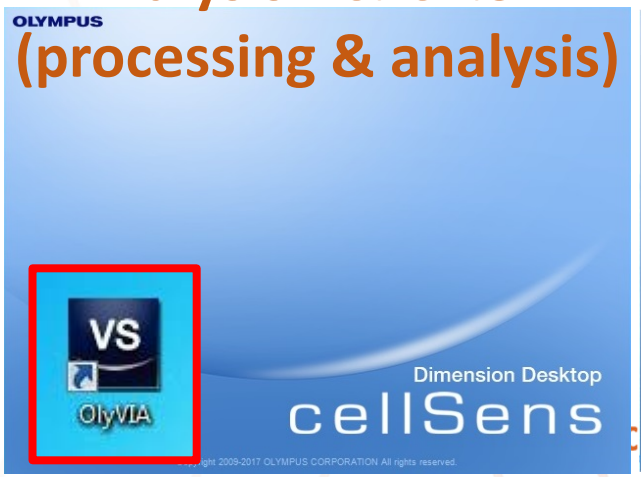
Part 2: handling large-sized scanning images

Introduction

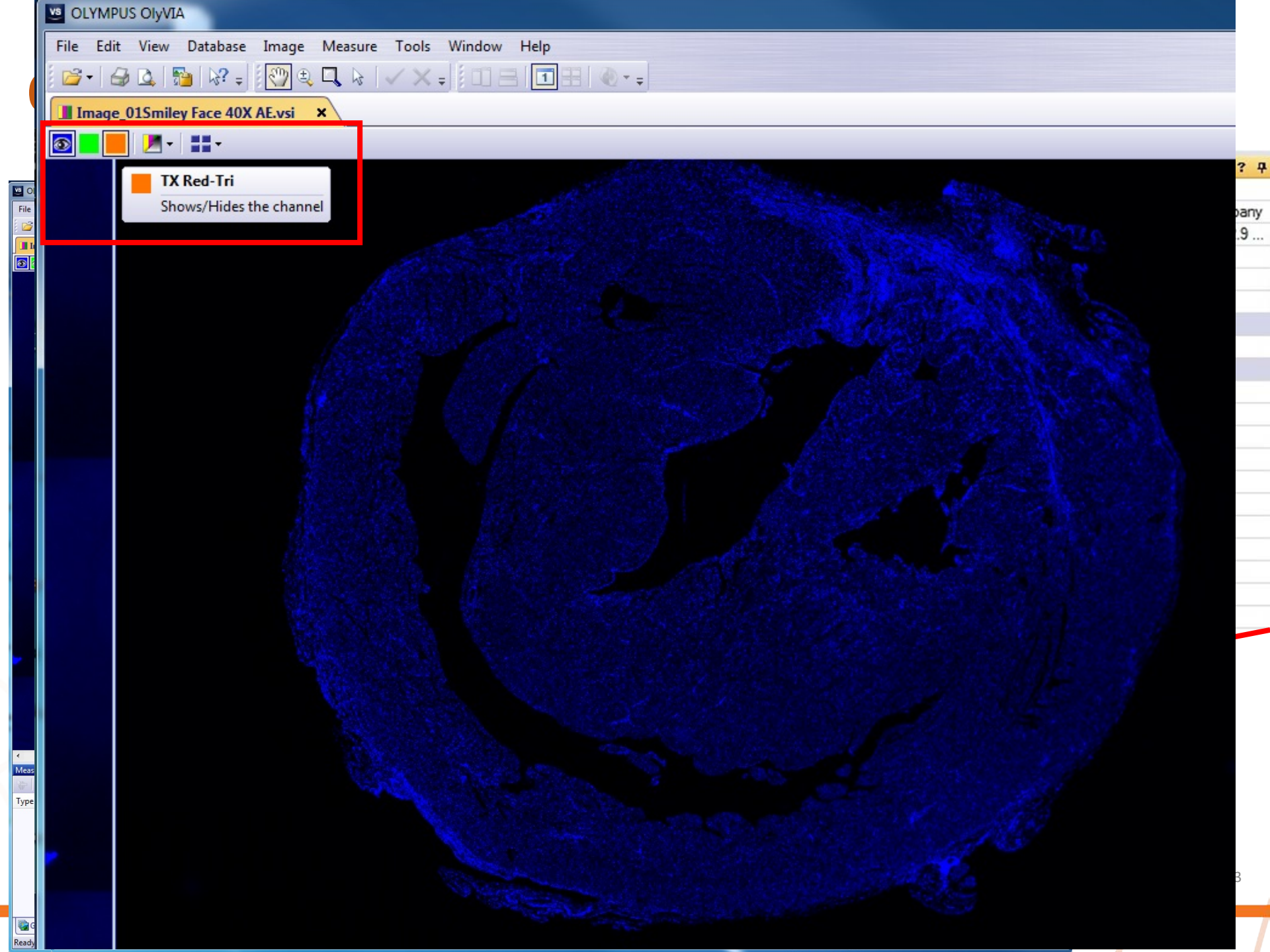
- Creation of large files, i.e. BF 1.5CM X 1.5CM—2-3GB
- Always save in VSI format first
- Can be directly opened with Olyvia, CellSens, VS-ASW/Desktop, Fiji ImageJ, not Huygens yet

Any PC
viewer

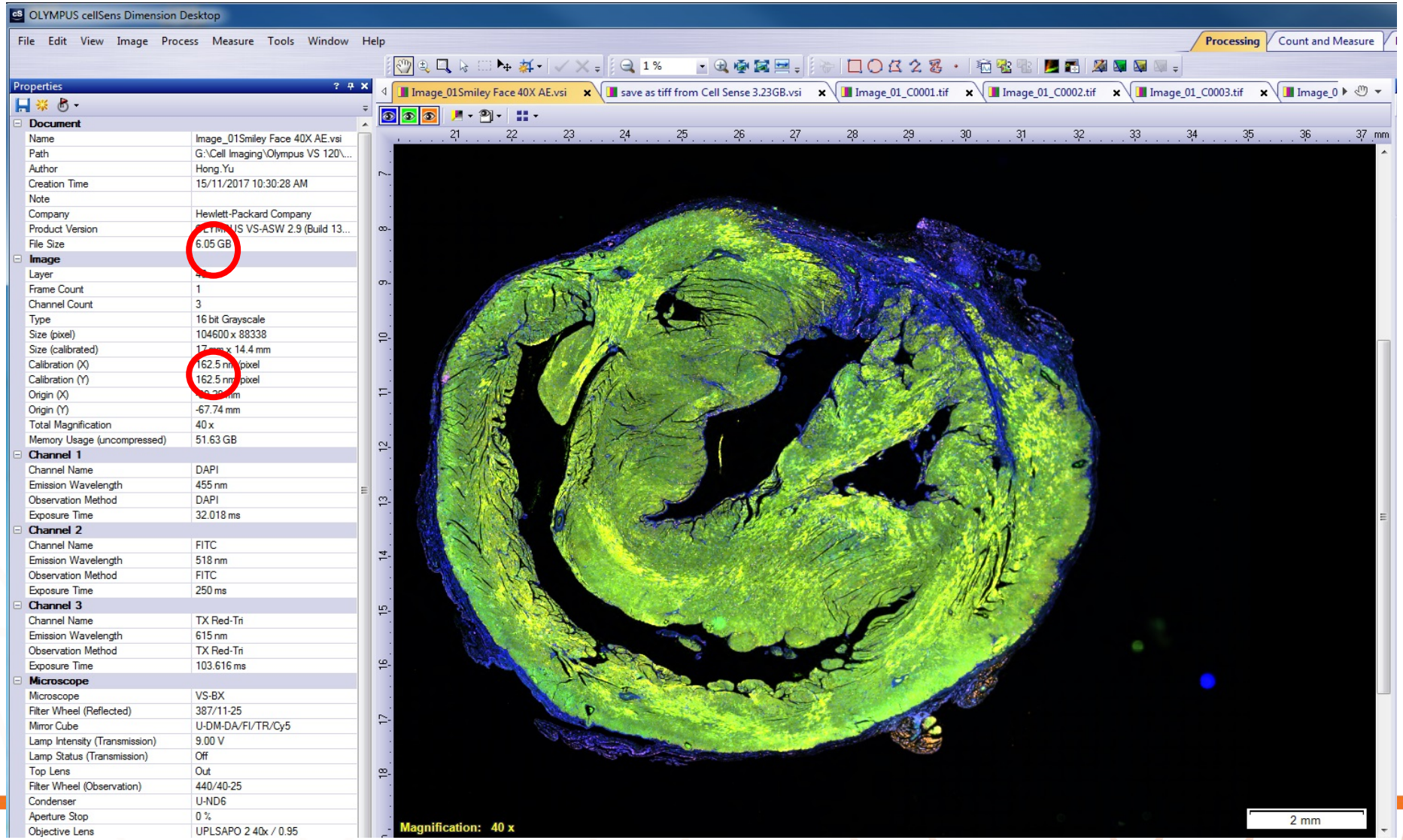
Analysis PC: C2.34
(processing & analysis)



Scanner PC: J2.08
viewer
acquisition
processing & analysis

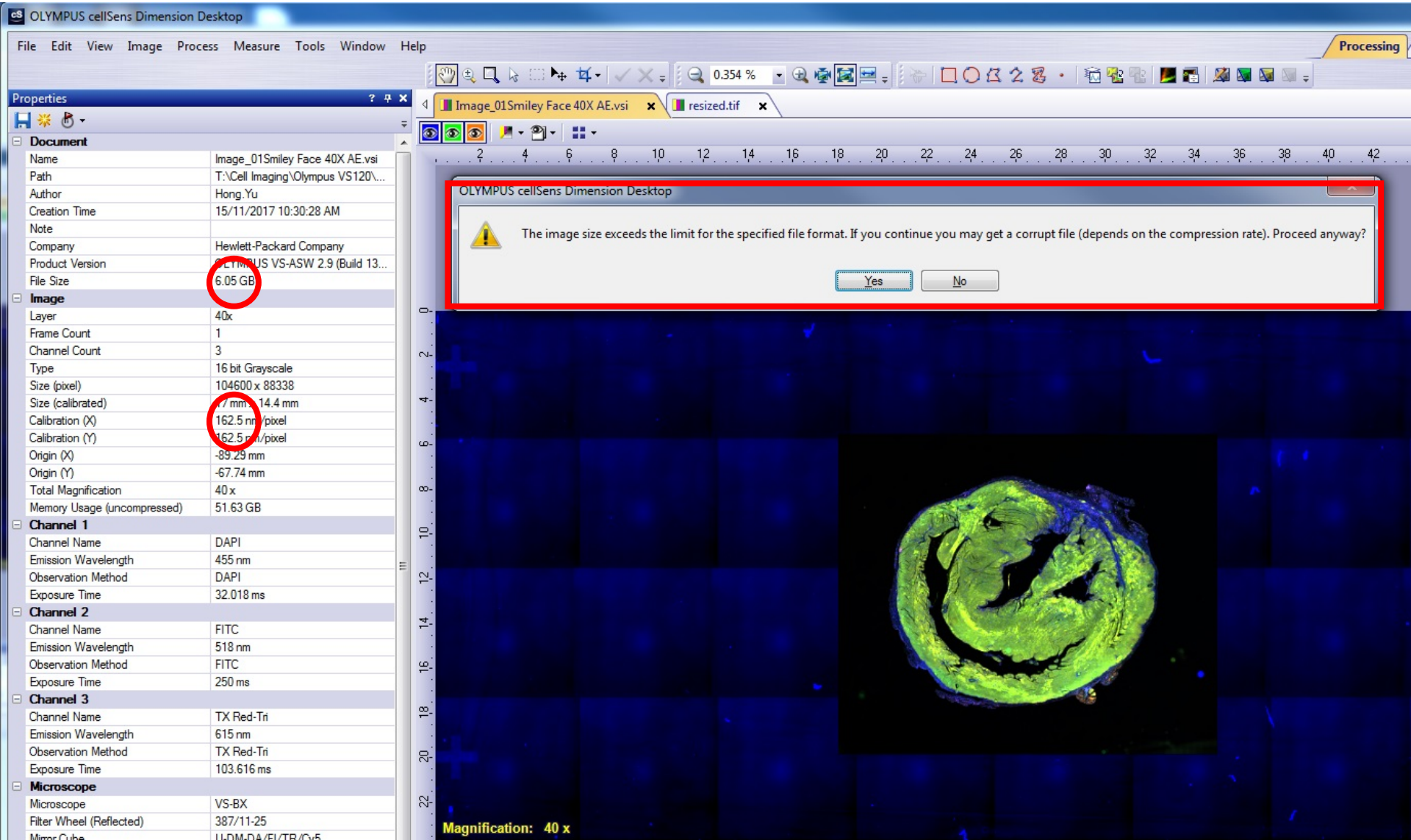

OLYMPUS OlyVIA
File Edit View Database Image Measure Tools Window Help
Image_01Smiley Face 40X AE.vsi
TX Red-Tri
Shows/Hides the channel

CellSens: save as, export, analyse .VSI data



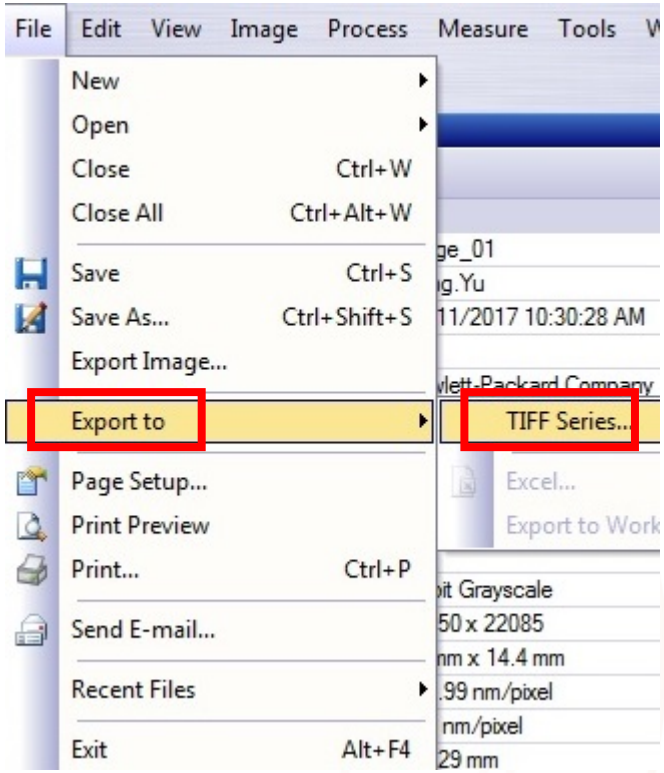
CellSens: VSI to Tiff (1) < 2G

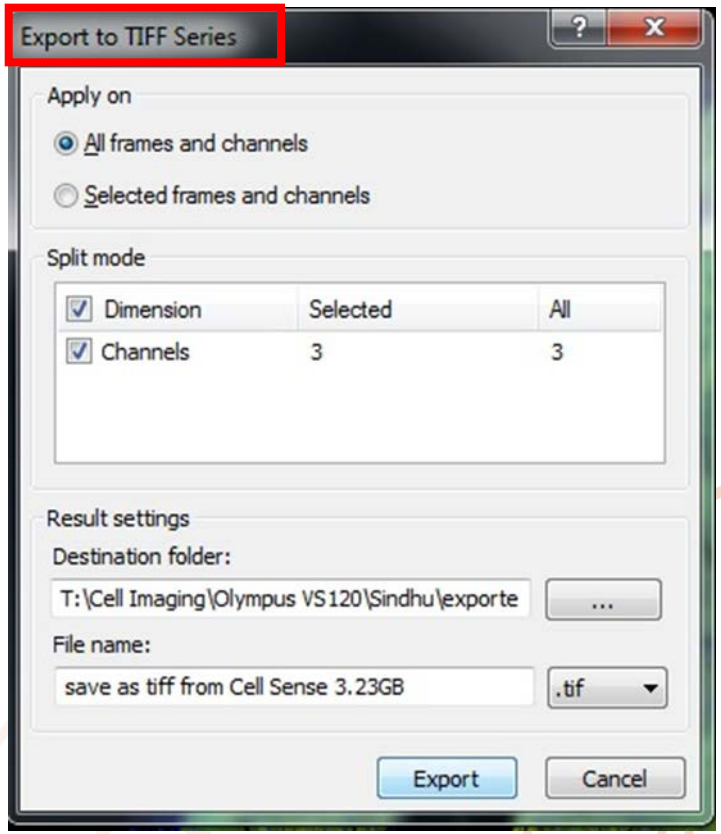
"save as" directly

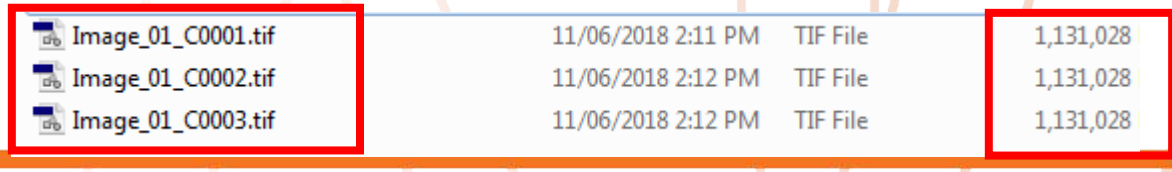


CellSens: VSI to Tiff (2) 2-5G

"export to tiff series"



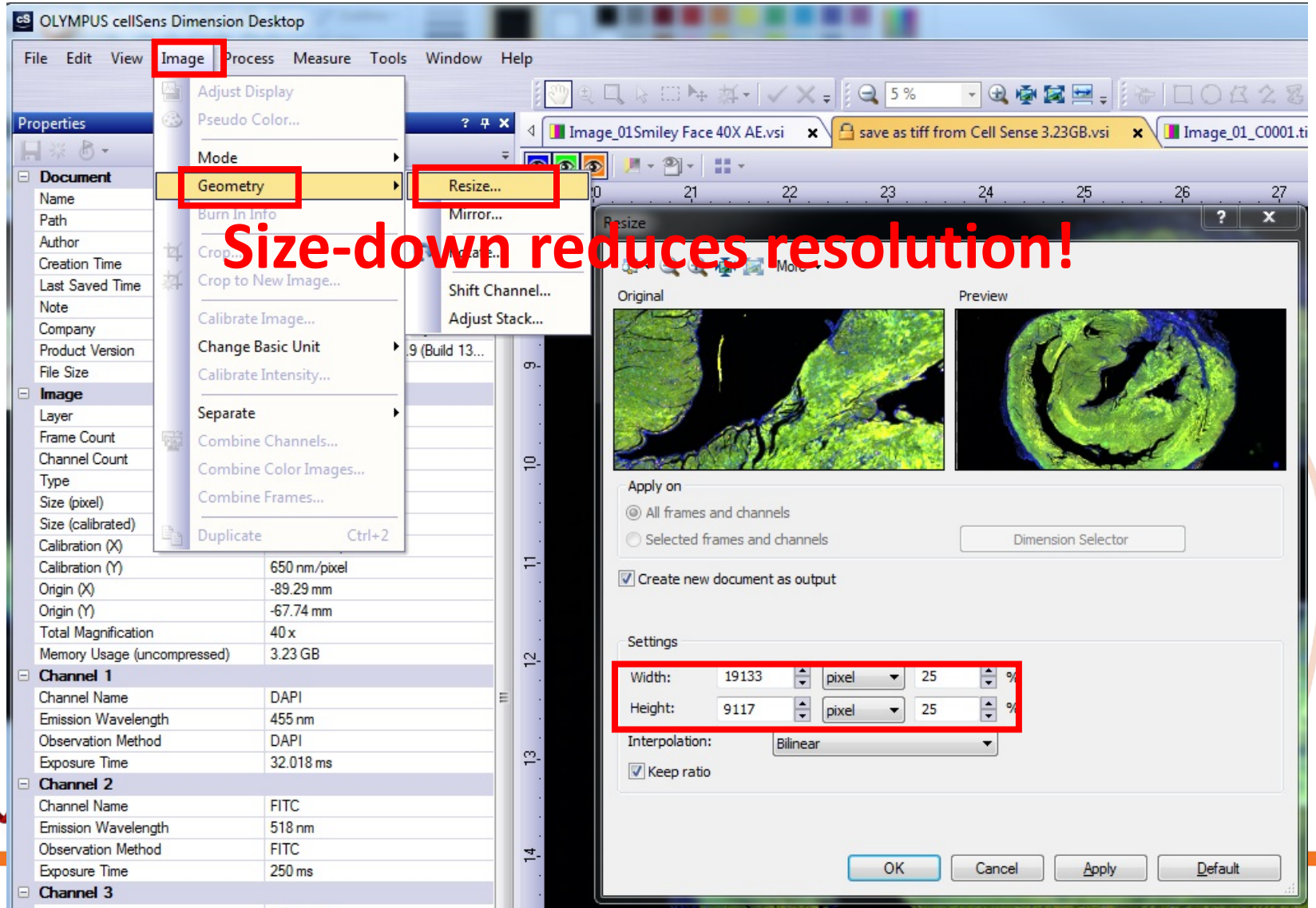




The Westmead Institute

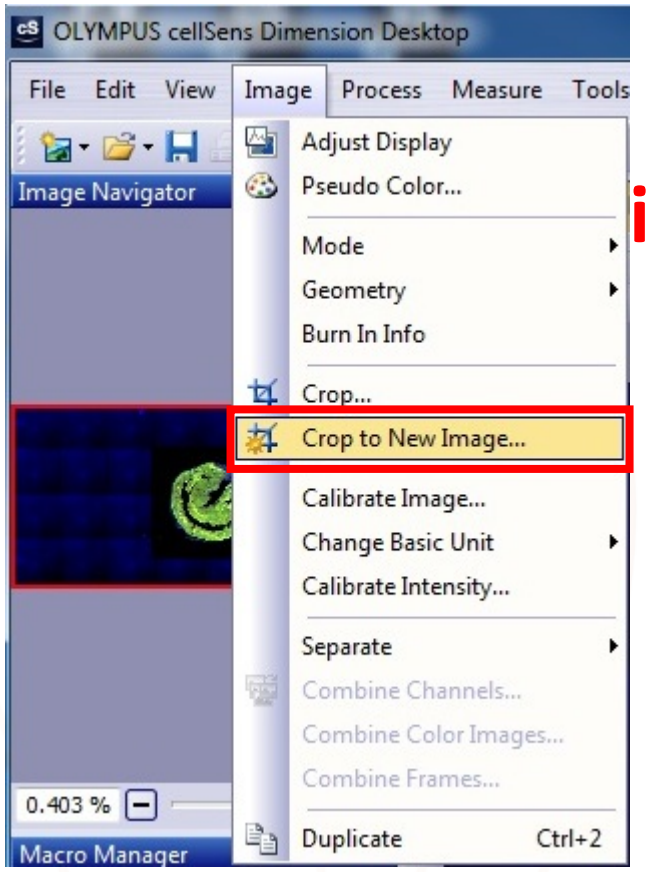
CellSens: VSI to Tiff (3) >5G

"resize" down first

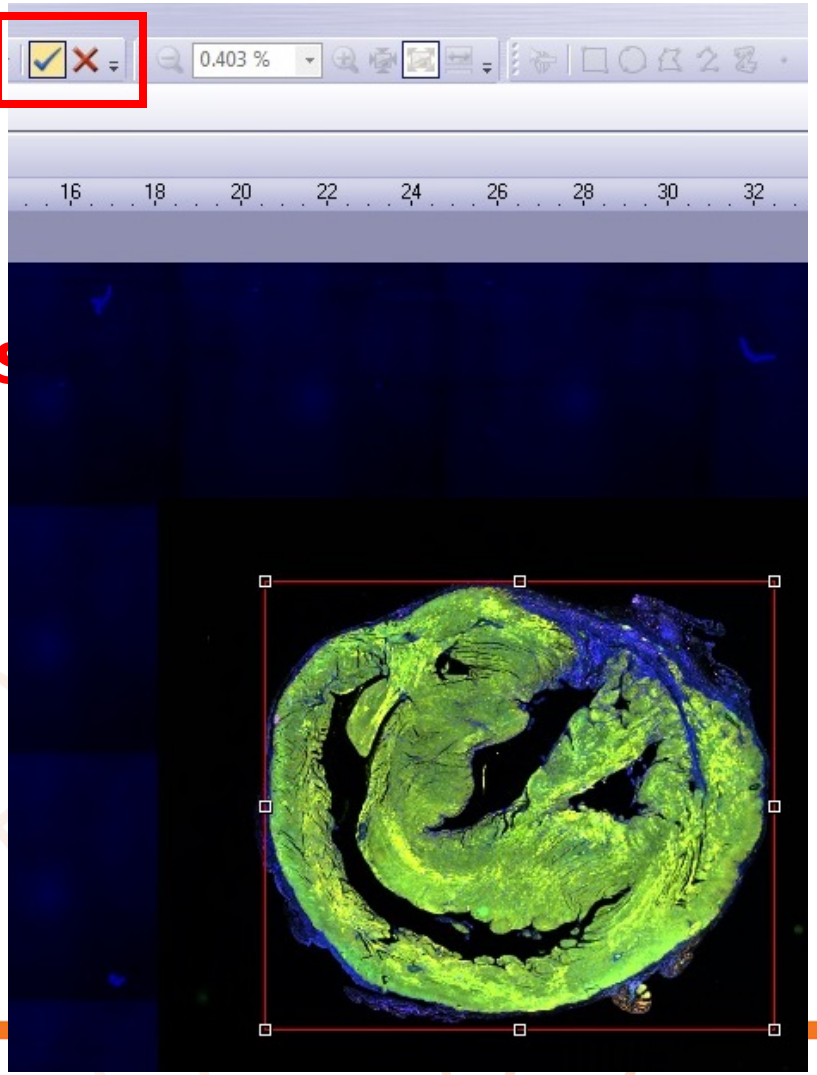


CellSens: VSI to Tiff (3) >5G

or “crop” first…

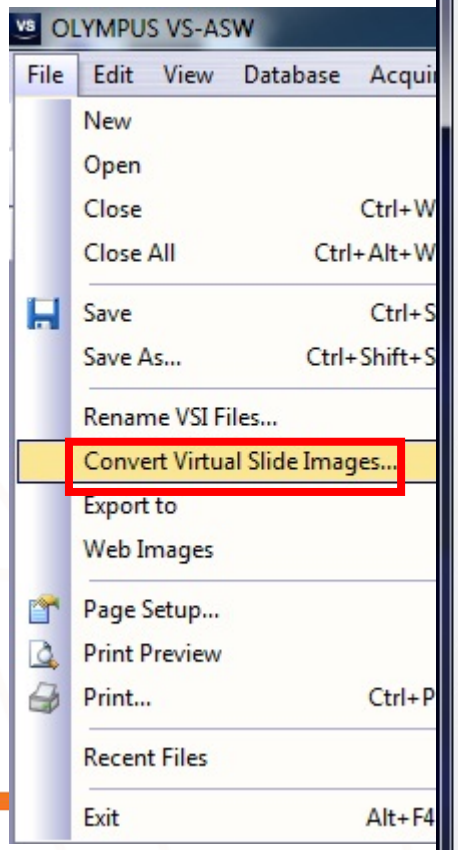


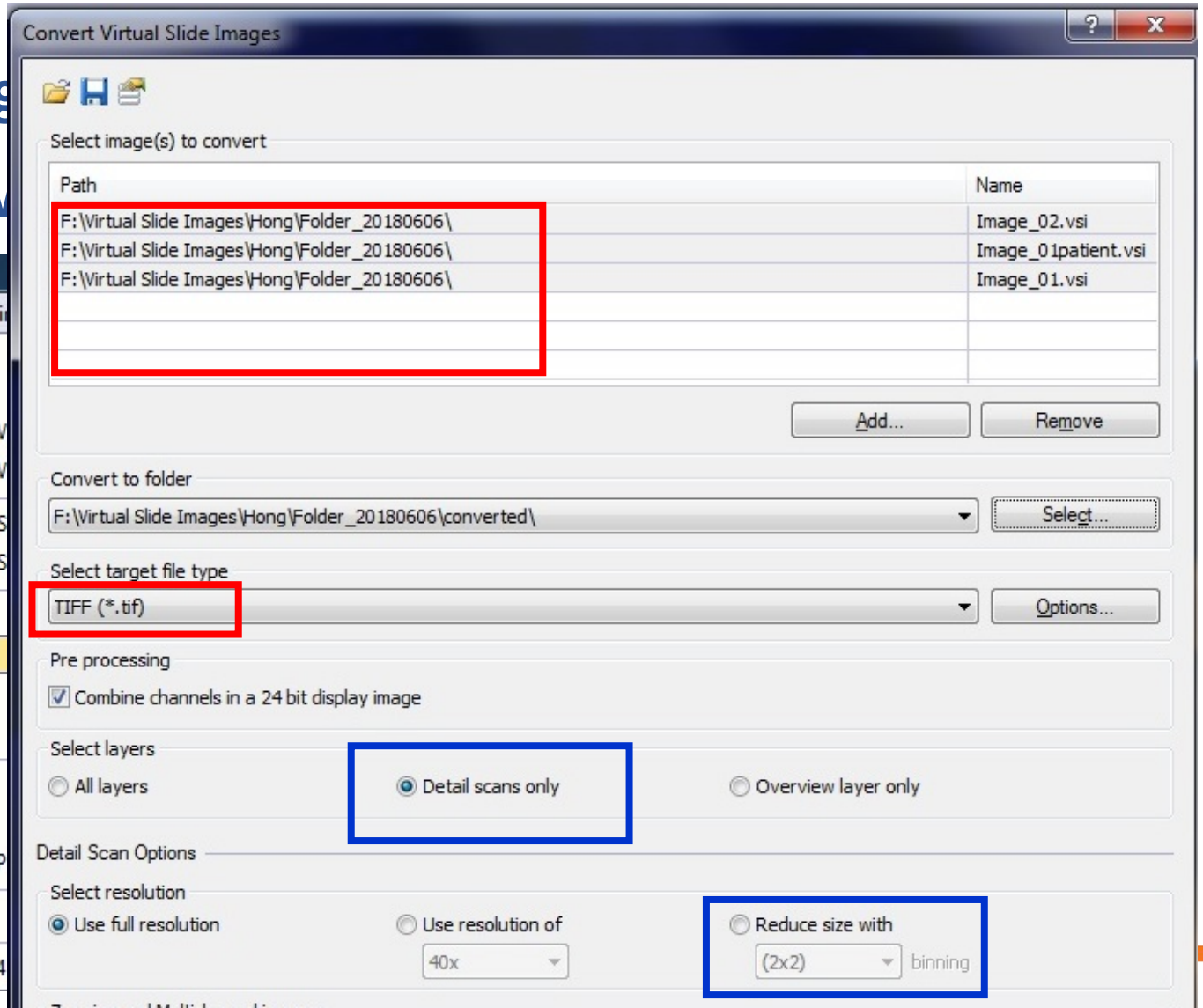
intains res



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

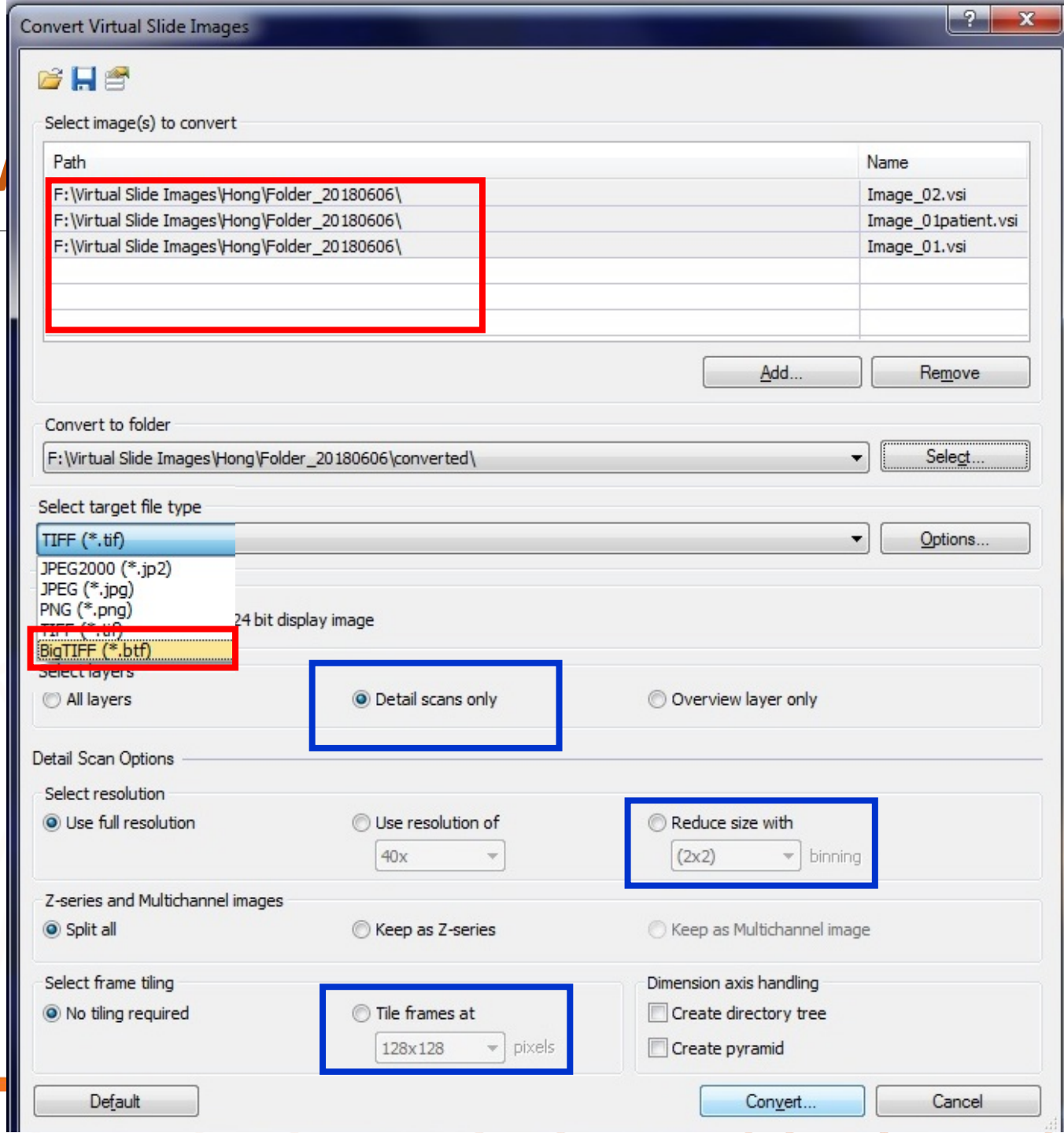
- Changing
- Built-in v





VS Desktop: V

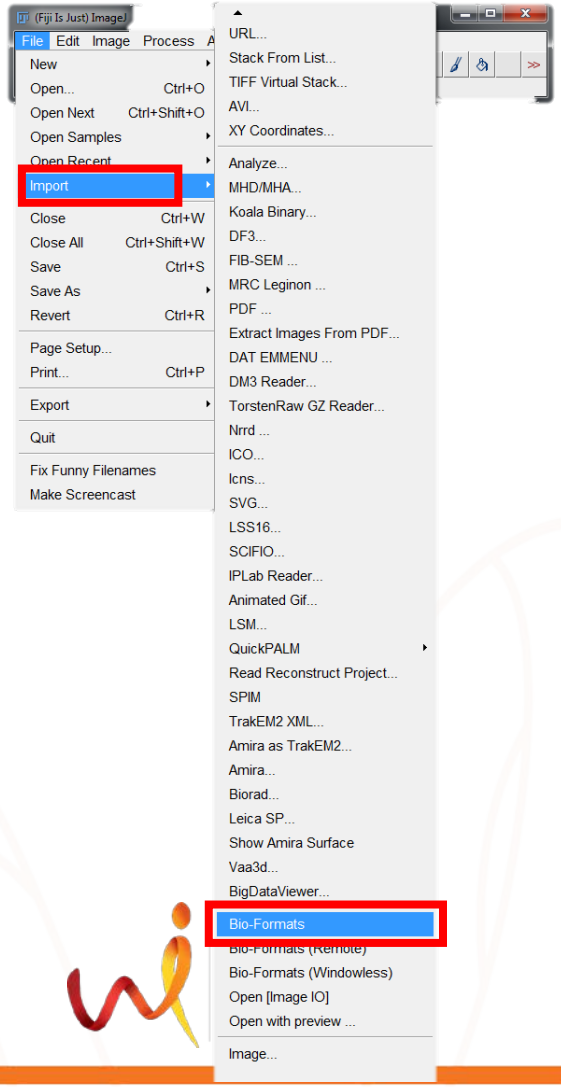
- **Convert to BigTIFF!**

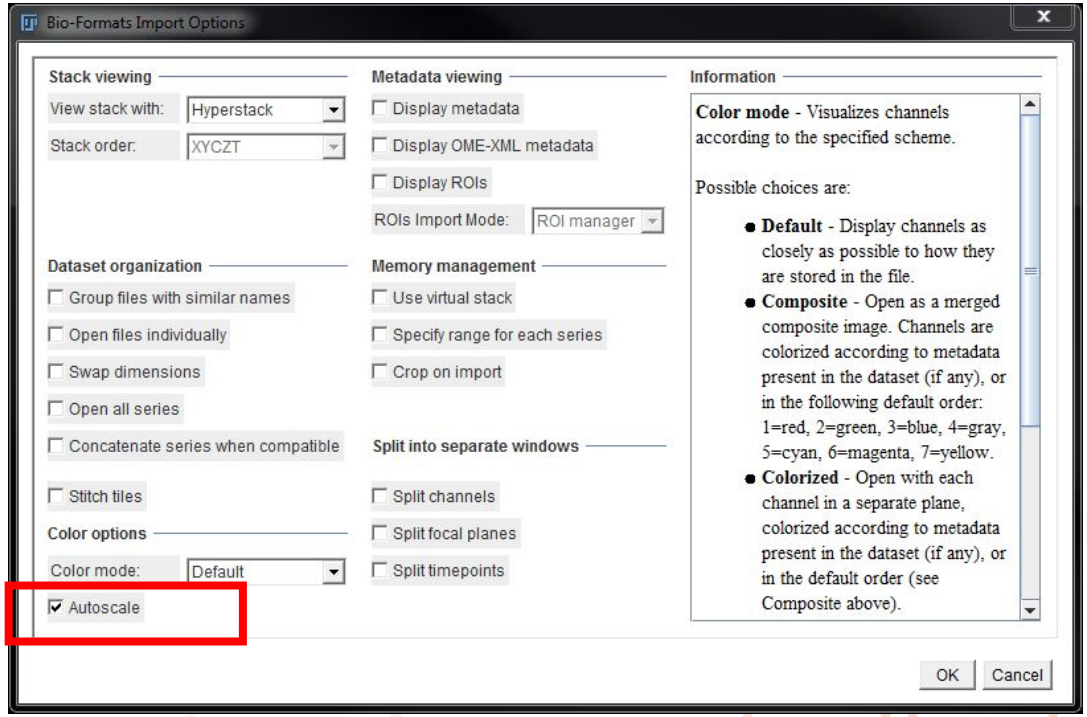


The Westmead Institute

Tip: extract data in CellSens & VS ASW/Desktop

FIJI ImageJ: open .VSI images directly





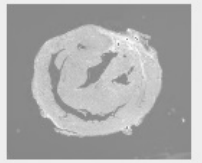
Bio-Formats Series Options

- [ ] 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
- [x] Series 7: 40x: 104600 x 88338; 3 planes
- [ ] Series 8: Image 01Smiley Face 40X AE.vsi #8: 5230
- [ ] 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!

Bio-Formats Series Options

- [ ] 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
- [x] 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

Image_01Smiley Face 40X AE.vsi - Image_01Smiley Face 40X AE.vsi #12 (33.3%)
2/3 (c:2/3 - Image_01Smiley Face 40X AE.vsi #12); 3269x2761 pixels;

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; 8627 3211**
- **Visit our website: https://sydneyuni.atlassian.net/wiki/spaces/WIF/**

The Westmead Institute

Thank you!