



Zen Blue for Histology Imaging (Colour cameras)

The histology scopes in room 310 (Scope 2-NDU and scope 5-MMU/NU) now have UCL windows 10. Log in with your UCL username and password. Unlock the grey screen (booking system) again with UCL username and password.

Software is located in the Application folder on the desktop.
2)NDU Applications or 5)MMU Applications.

These folders contain:

- Zen Software.  ZEN
- Fiji software.  ImageJ-Fiji
- Link to saves files on local C drive.
- Macros for setting scale bars in tif images
- Macros for adjusting gamma in czi images
- Instruction for Kohler illumination (microscope set up)
- Instructions for Zen software (capturing and saving images)



The software only controls the camera.

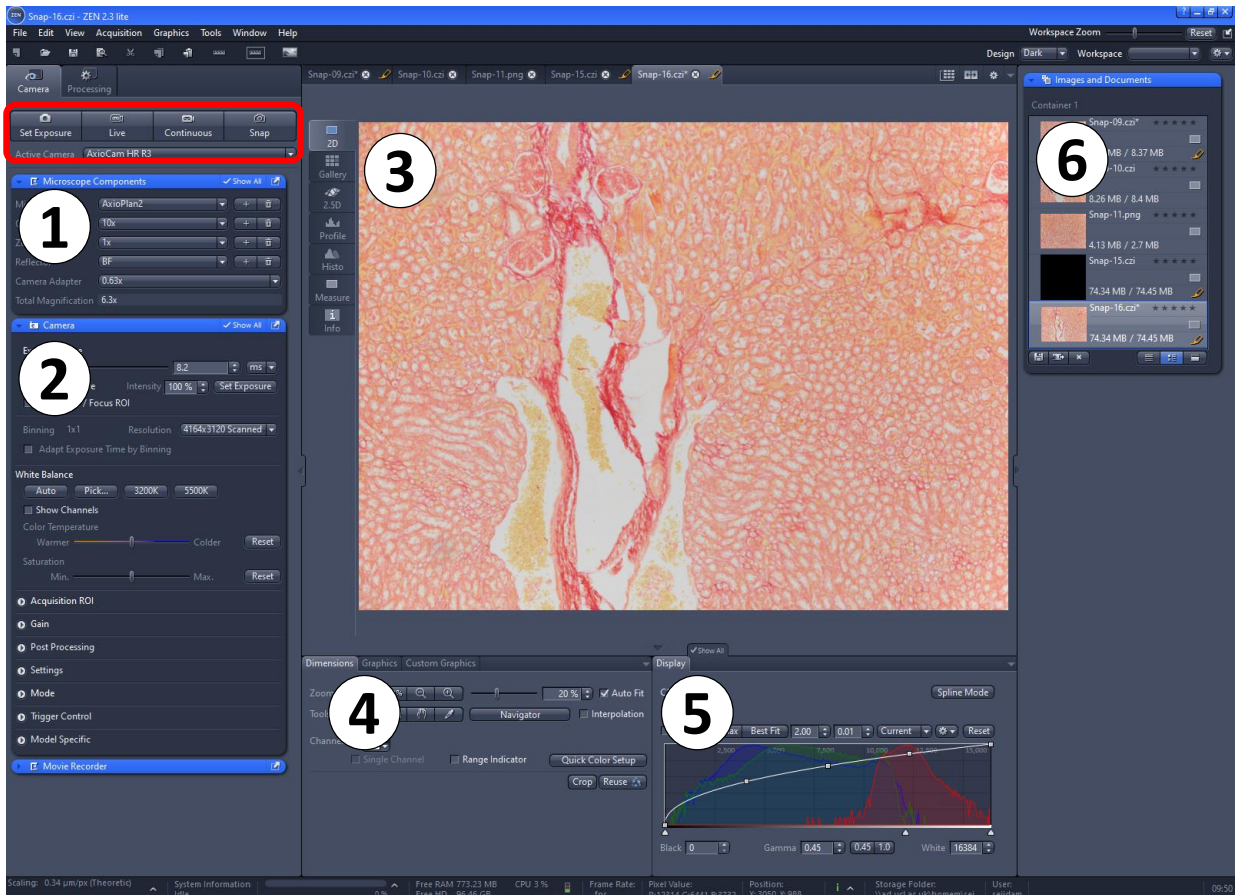
Change objectives using the buttons below the right side focus wheel.



Buttons to change objectives

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Software layout



1) **Microscope set up** – you can enter the magnification used here then the scale is automatically set during imaging and if you save as a CZI file. Ignore if saving as a TIF. See page 4 for details

2) **Camera Controls** – exposure time, resolution (number of pixels), white balance, shading correction. See page 5 & 6.

Use with the buttons: Set Exposure, Live (or red STOP when in use) and Snap. Buttons highlighted with **Red Rectangle** on image above.

3) **Current Image** – also the info tab for image detail (pixel size etc).

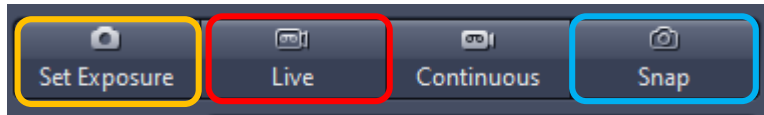
4) **Image zoom**


5) **Image brightness** – set gamma to 0.45 for all colour imaging. **Important!**

6) **All images in this session** – make sure to save each image.

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Quick Guide – How to Capture and save images



- 1) Press **Live** and Press **Set Exposure**
- 2) Set white balance and shading correction if not already done. (Details pages 5 & 6).
- 3) Choose your resolution (Scanned 1388x1040 or 2776x2080 or 4164x3120).
- 4) Stop **Live**  & Press **Snap**
- 5) All images captured are listed on the right of the screen.

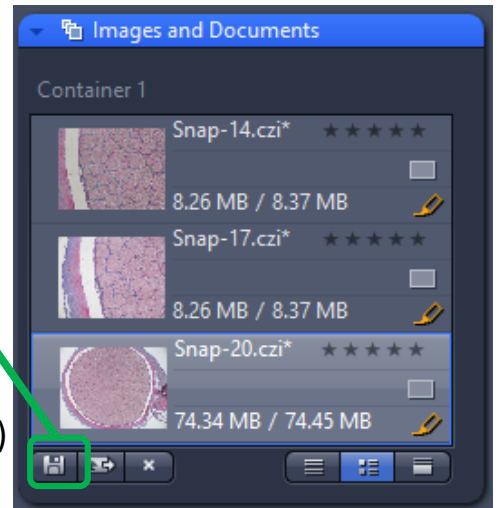
6) To save each image, highlight the image to save (double click) then press the Save icon.

Save as either:

1) .tif (colour/gamma is fine but scale needs setting – like the older axiovision software)

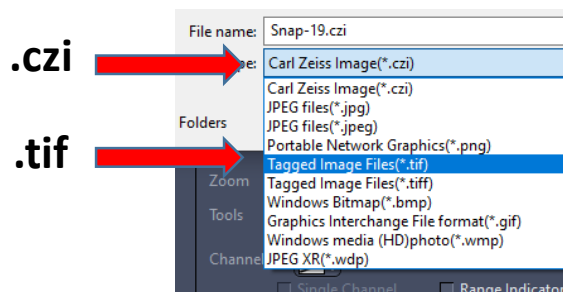
OR

2) .czi file (keeps scale but gamma needs correcting)
CZI files appear too dark, see page 8.



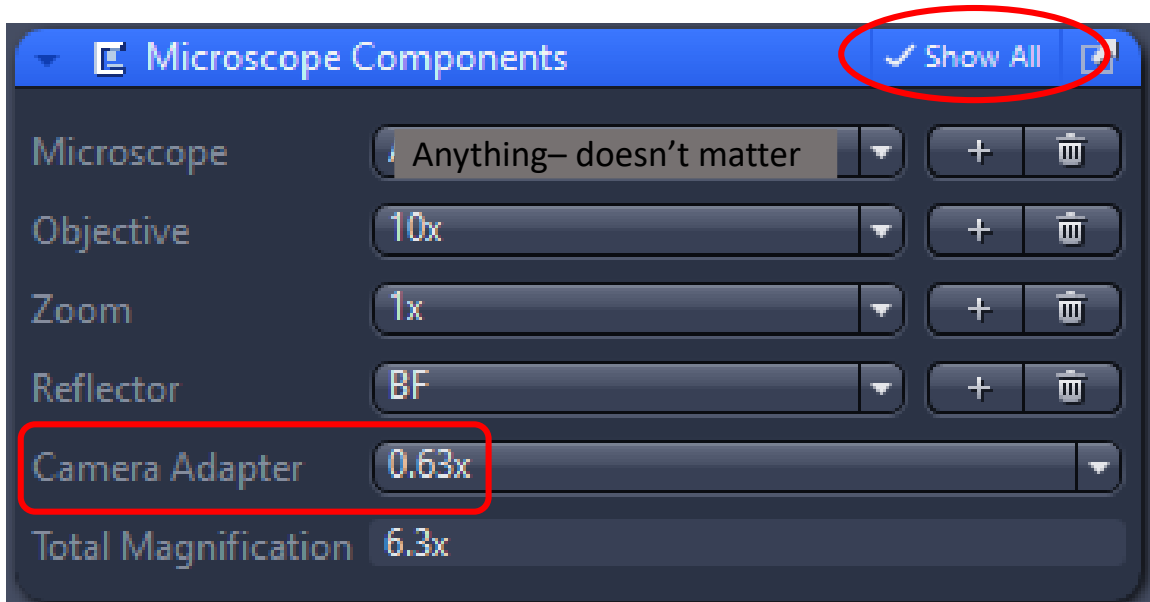
Recommended: save as a TIF. Remember to include the magnification in the image name to run the scalebar macro.

If saving as a CZI make sure you set the magnification in the software before you capture the image. See page 4. You can then run the CZItoRGB macro (either on a whole folder or individual files).



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**Set the current magnification in the software
(optional – only if saving as CZI files, not needed for TIF)**



In Microscope components you can (optionally) set the magnification. If you save files as .czi and you have set the magnification correctly then the files will have the scale set when you save them. If you save as .tif files the scale is not set, and you need to run the ICH scalebar macro.

.czi files: Scale is set but you need to adjust gamma in saved files.

.tif files: Scale is not set but gamma is correct (like older AxioVision software).

Make sure 'Show All' is selected at the top of the window.

Set the options as follows:

Microscope: anything, it doesn't matter, leave as it is.

Objective: set to match the current objective (2.5x, 5x, 10x, 20x, 40x or 63x)

Zoom: 1x

Reflector: BF

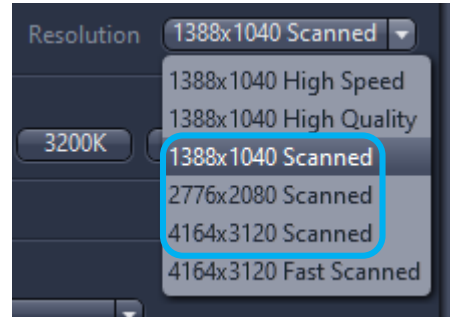
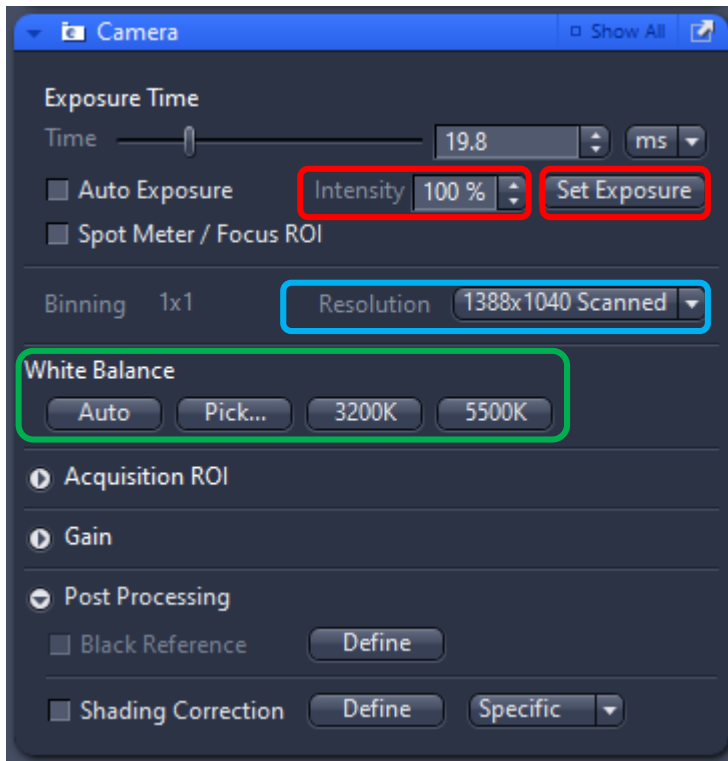
Camera Adapter: 0.63x

Important: Camera adapter must be set to 0.63x

Total Magnification: this is set for you by the software, according to details entered above.

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Imaging set up, Exposure, White Balance, Resolution



Exposure Time

Press **Set Exposure** with **100% intensity**. Your exposure time will now be optimal. Alternatively, tick Auto Exposure (although this can flicker when you move the slide / objectives before it settles down).

White balance: set this at the start of each session.

- 1) Focus your sample and set the correct exposure time.
- 2) Press Pick... then click on a white bit of your slide.

OR

Move your slide to a totally clear area, and press Auto

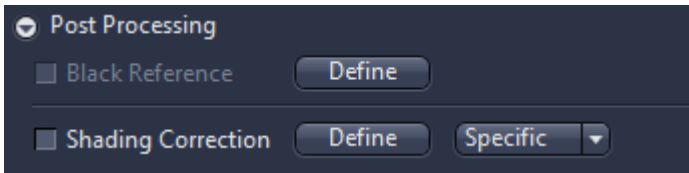
Shading correction: you can set shading correction here, see over page...

Resolution: Use a 'Scanned' setting. 1388x1040 is fine.

For smaller pixel sizes (so higher resolution and larger file sizes), you can use 2776x2080 or 4164x3120.

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Shading Correction



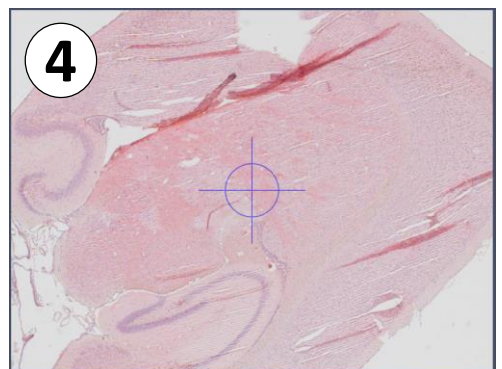
Shading correction controls in the Camera window

At low magnification (10x and less) you may find that the edges of the image aren't as bright as the centre. To compensate for this you can use 'Shading Correction'. This must be set up for each objective, every time you change magnification.

Initial image before correction

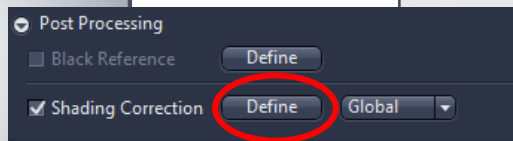
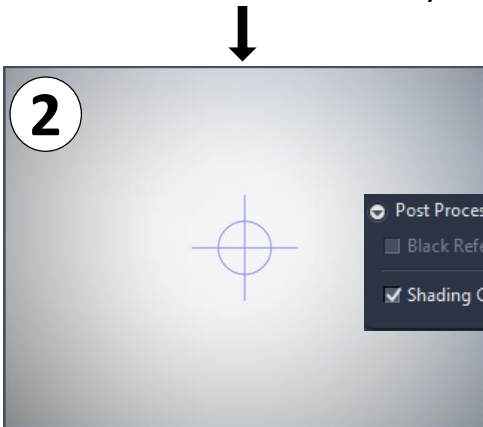


Shading correction enabled



↓
Move to a totally empty part of the slide (you may need to defocus) or remove the slide entirely

↑
Move your sample back into view

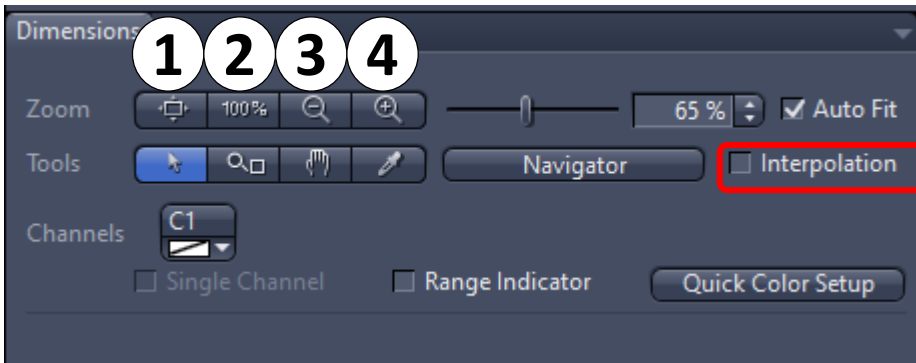


Press **Define**

The uneven background is corrected (while Shading correction is Ticked)

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Image display. Zoom in/out.



- 1) Fit image to screen
- 2) View Image at full size
- 3) Zoom out
- 4) Zoom in (you can zoom with the mouse wheel also)

Don't use **Interpolation**

Important! Set display properties (Gamma 0.45)



Select **'Show all'**

Set **Gamma to 0.45**

If you save as a TIF the gamma will change, but the file is saved as it looks on the screen. This is fine.

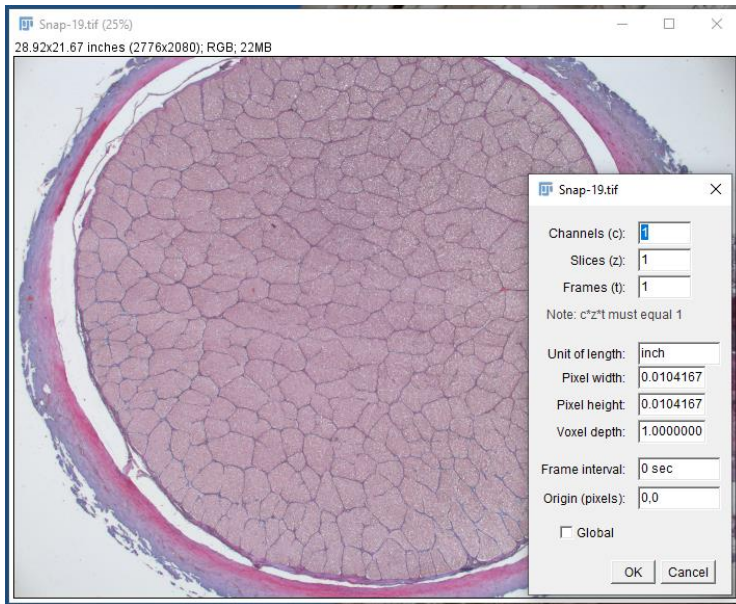
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Save as .tif or .czi

.tif file. Scale not set (its in inches!).

Remember: write the magnification in the filename.

Run the ICH scalebar macro on your files.



You will get an .xml file with each tif file.

The xml files can be deleted.

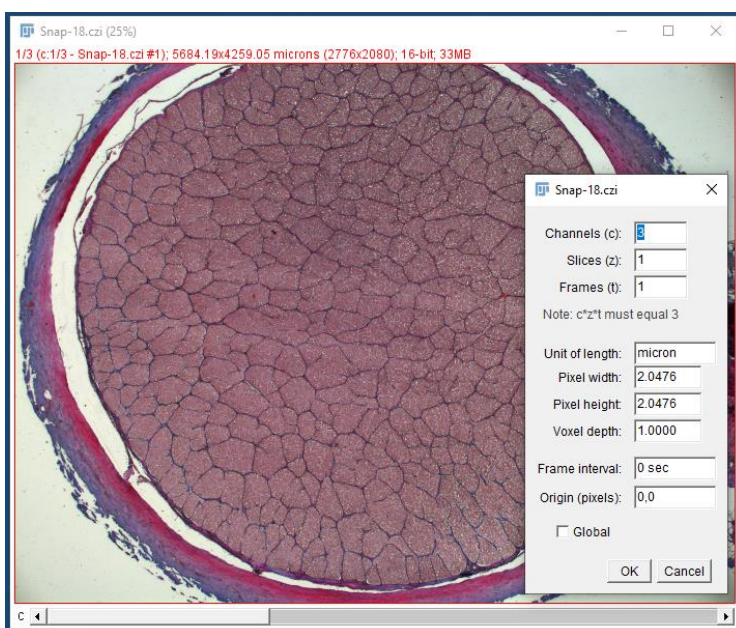
Use the ICH ScaleBar macros to set the scale in your images.

Remember to write the magnification in the file name.

.czi file. Image appears too dark (gamma) but scale correct.

Remember: set the magnification in the software (see page 4).

Easily fixed with the CZItoRGB macros.




Use the CZItoRGB.ijm macro to fix it.

It is in the 2) NDU UserImages Folder.

 CZItoRGB.ijm

Drag to Fiji and press RUN. It processes a single open image.

Alternatively run the folder version and it does a whole folder of files.

 CZItoRGB Folder.ijm

It adjusts the display to the full 14 bit range, makes it RGB then sets gamma to 0.45.