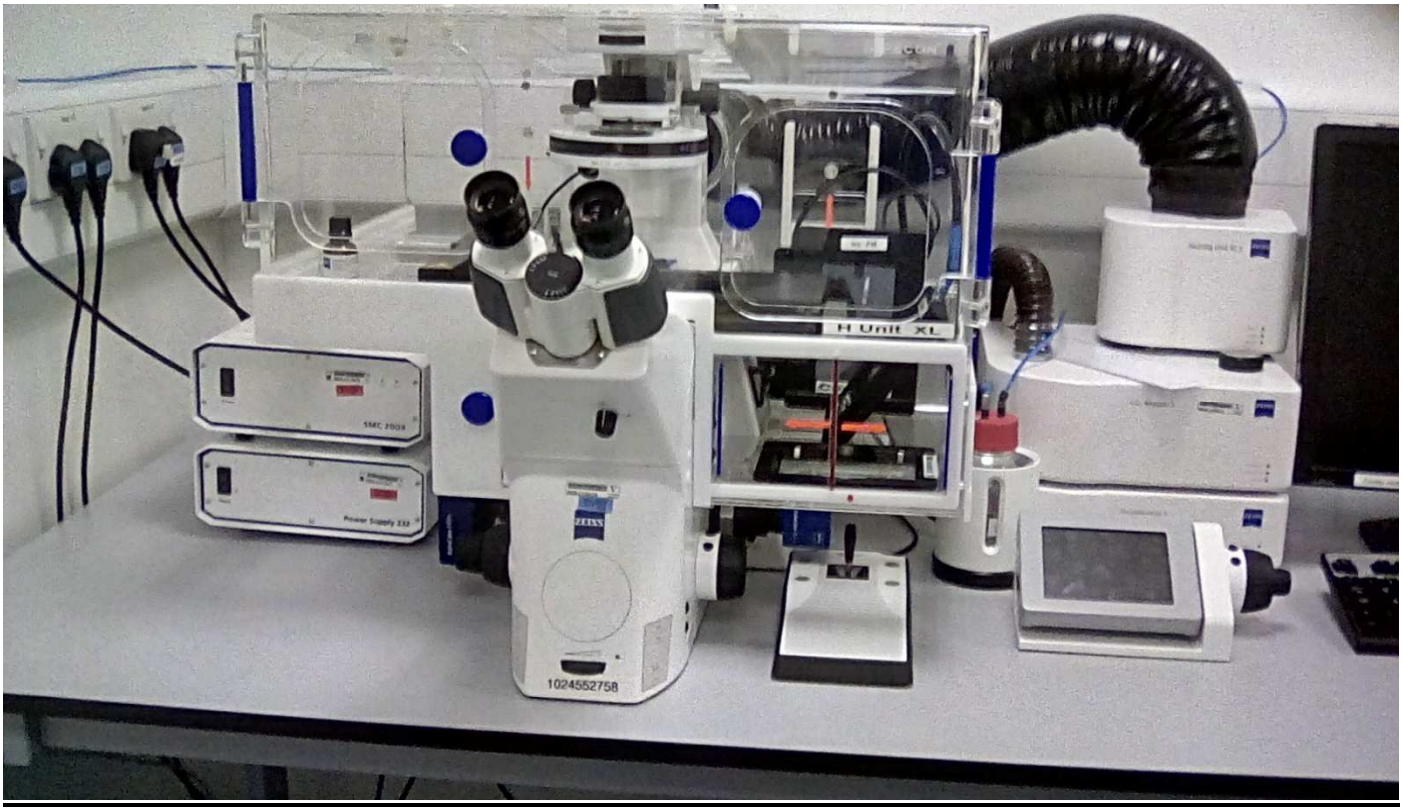
University of Glasgow | Institute of Cancer Sciences

College of Medical, Veterinary and Life Sciences, Garscube Estate.

ZEISS Axio Observer SOP



Note - If using the attached incubator see also – Axio Observer Live Cell Imaging SOP

START UP

Switch on sockets

1. SMC 2009 power suppy.
2. 232 power supply.
3. HXP 120V fluorescence lamp.
4. CO2 Module S and Temp Module S **(only required if using incubator chamber for live cell imaging).**
5. Heating unit XLS **(only required if using incubator chamber for live cell imaging).**

Switch on Power Supply 232

Switch on SMC 2009

SMC 2009

Power Supply 232

Switch on HXP 120V FL Lamp (this is only required for imaging fluorescence)

1. Switch the power switch on.
2. Leave the intensity level at 2/3 (you can turn this down intensity if sample is sensitive or bleaches easily).
3. Check the blue shutter open light is on, if not press the shutter open switch.

Switch on microscope (button switch on left hand side of microscope body)

Switch on PC

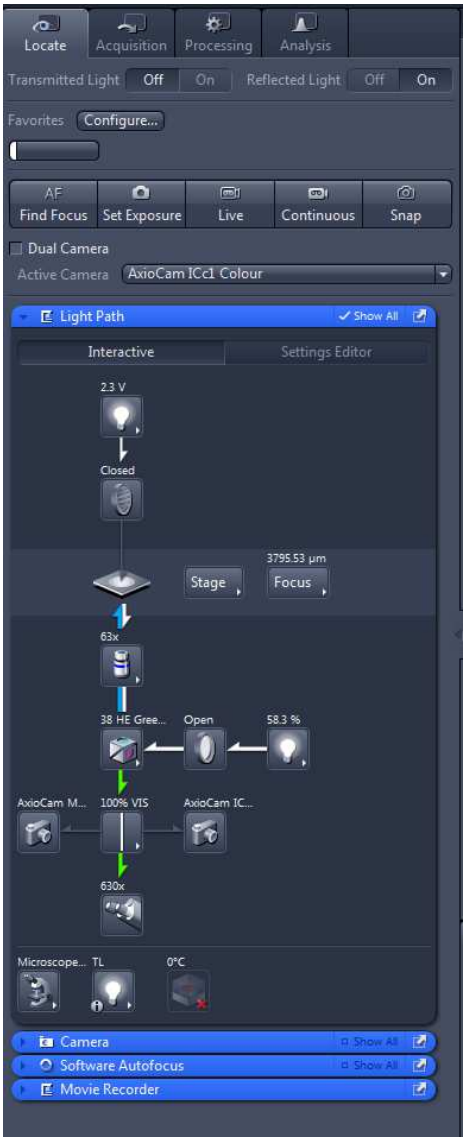
1. Switch on the PC and login using your UoG ID and password.
2. If you are going to be doing large tile scans in LCI, you may need to use the .\ZEISS login (no password) in order for the computer to be able to process and save large files.
3. Open the **ZEN blue** software to run the microscope. Select **ZEN Pro** for image acquisition.

Running software

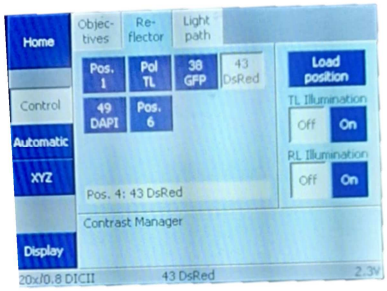
Locate

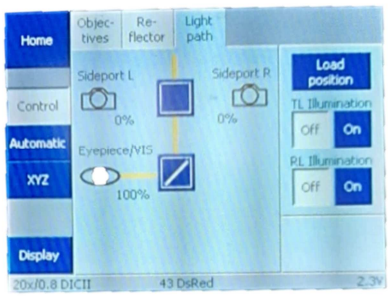
- Open the **locate** window.
- Either
    - Use the software
    - Or use the TFT on the microscope
- Select **ocular online** to be able to see the sample using the eyepiece.
- Adjust the knob on the microscope to divert the image to the eyepiece.
- Check the lightpath settings in ZEN are directing the light to the eyepiece.
- Select **transmitted light (TL) on** and **reflected light (RL) off** to look at **brightfield** images.
- Select **reflected light (RL) on** and **transmitted light (TL) off** and then select the appropriate filter to look at **fluorescence** images.
- Select the appropriate objective.
- All objectives are dry, except the X63 and X100 which need oil. A message will pop up to prompt you to change the media. Click ‘done’ when you have added/ removed oil and the objective will move back into position for viewing.

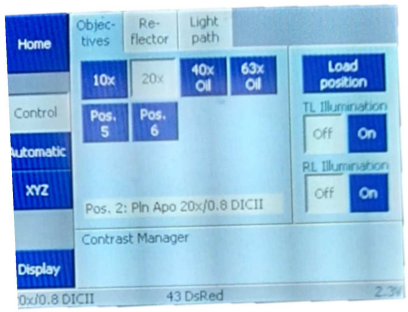
Software



TFT

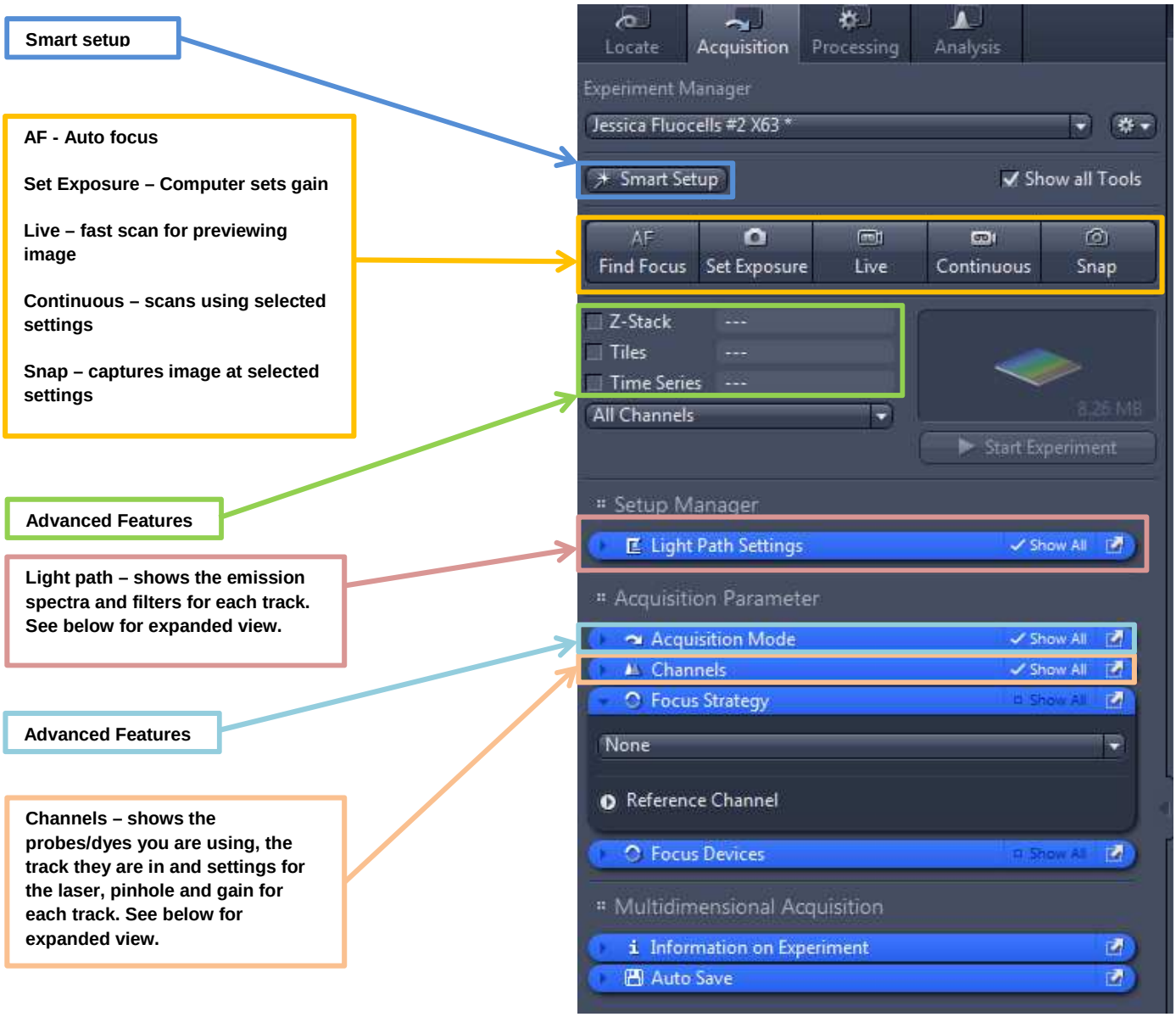




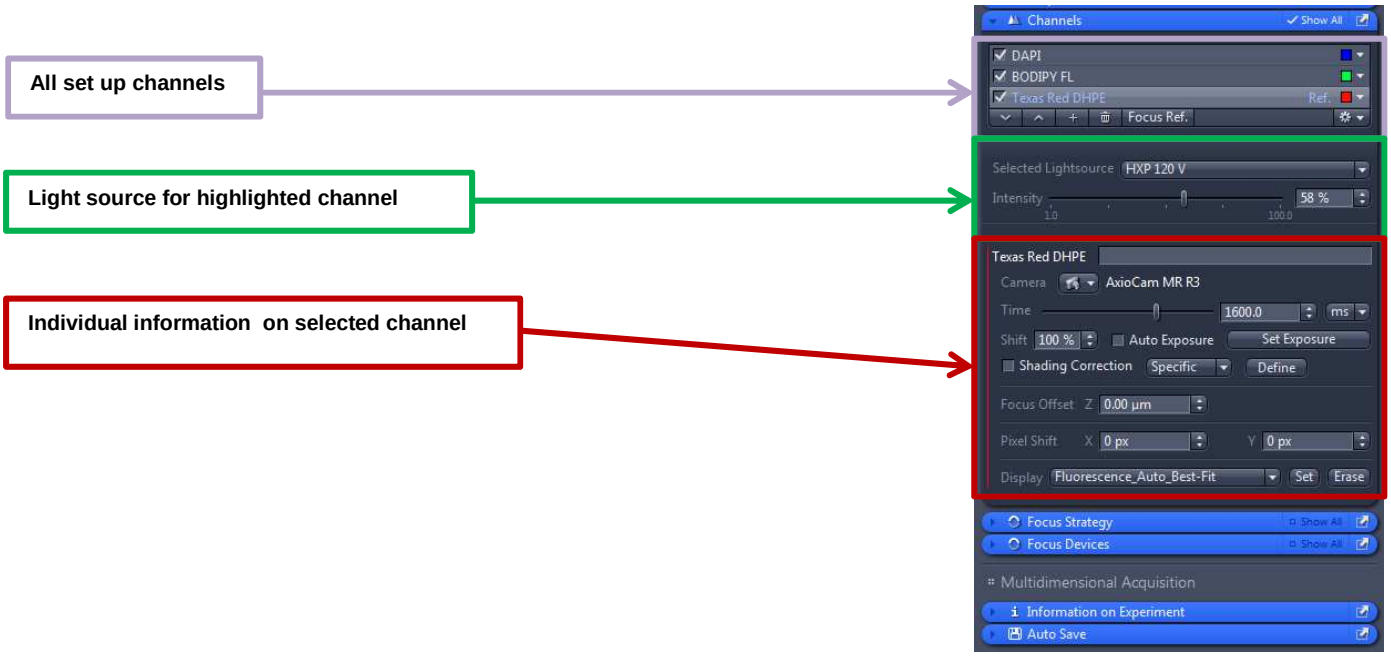


Acquire Images

Acquire - Open the **acquisition** window.



Channels (expanded)



Light Path (expanded)

Hardware set up sequence to capture all setup channels

Current light path set up in the microscope showing, light sources, filters , beam splitters and camera.

Transmitted light setup

If you are using transmitted light- brightfield, phase or DIC you need to check that the Koehler illumination is correct for the objective you are using for your images. By correctly setting up the correct Koehler illumination you are making sure that your sample is illuminated by a good even light source. (see separate SOP on light microscopy

- Select the appropriate objective, condenser, and slider (you only need this for DIC) for your requirements.

Objective	Condenser	Slider
X2.5	H	
X5	H/ Ph1	
X10	H/ Ph1	
X20	H/ Ph2/ Plas DIC*	DIC I/II
X40	H/ Ph2/ Plas DIC*	DIC I/II
X40	H/ Ph2	
X63 (oil)	H/ Ph3/ DIC II	DIC II/III
X100 (oil)	H	

***If using plasDIC (x20 & x40), adjust the correction collar on the objective to correspond to the thickness of the surface on which the sample is mounted: 1 = for use with thick plastic surfaces eg petri-dish/ cell culture plate; 0. = for use with thin coverslips.**

*If using DIC, you also need to move the polarising discs above the condenser into the light path.

*If you want to swap objectives over eg the X10 for the X5, ask the microscope facility manager to do this for you. **Changes to components have to be registered in the software.**

- Check the **Active Camera** is the colour camera.

- Alter the lightpath settings in ZEN, to divert the light to the camera.

- In **live** mode, set the **exposure** and the **white balance**, then select **snap** to capture an image.

- In **live** mode or following acquisition, go to the **Display** tab and select **min/max** (for best contrast) or **best fit** (for best spread of values) or adjust the arrows underneath the histogram to set the contrast (left arrow), gamma (middle arrow), and brightness (right arrow). Gamma correction (relationship between detected brightness and display brightness) may be adjusted. The default, optimum value is 0.45.

- The software automatically opens a new window for each snap (2D and 3D).

Kohler illumination - http://zeiss-campus.magnet.fsu.edu/articles/basics/kohler.html

Improving contrast - http://zeiss-campus.magnet.fsu.edu/articles/basics/contrast.html

Fluorescent light setup

Check that the condenser is in the H position.

For a single fluorophore select the FL reflector module with the appropriate fluorescence filter.

Filter	excitation nm BP, emission nm BP
DAPI (49)	ex BP 365, em BP 445/50
GFP (38 HE)	ex BP 470/40, em BP 525/50
DsRed (43 HE)	ex BP 550/25, em BP 605/70
Cy 5 (50)	ex 640/30, em 690/50

- Check the **Active Camera** is the monochrome camera.
- Open the **Smart Setup** and add your colour channels by clicking the '+' button/ double-click on a fluorophore or contrasting method from the list. Use the **Automatic 'P'** motif.
- Go to **Experiment Manager** and select **All Channels**.
- In **live** mode, adjust the focus and then **set exposure** for each channel in turn, then select **snap** to capture an image.

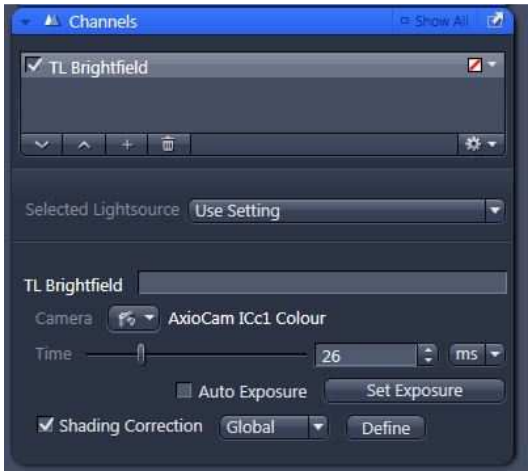
*When checking the exposure of a channel in live mode, only the highlighted channel will be shown (in grey scale) even when the other channels are ticked. To image all channels sequentially (and in colour) use the continuous button.

- As in the TL setup, images can be further enhanced by adjusting the settings in the display tab. You can also change the pseudocolours applied to each channel, as preferred.

Tile scan:

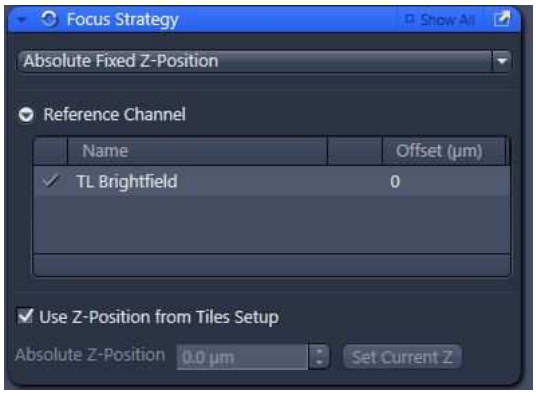
- Select **tile scan.**
- Check that you have the **Automatic Scaling** box ticked in the bottom left hand corner.

- In **live** mode, after setting the exposure and white balance, perform shading correction:
  In the camera tab, tick show all. Tick **shading correction** and select **global**. Go to a clean, blank area of the slide, make sure this is clear of dirt and debris. Click **define**. This should prevent shadowing occurring around the margins of tiles.



- In **live** mode, move the position of the specimen to select the central point of the tile scan.
- Select the number of tiles… or the size of the area you want to scan.
- Click the '+' button. This adds this tile region to your experiment. You can add multiple tile regions.

- Using the **position** tab you can choose your tile scan area by indicating 2 points of interest, clicking the '+' button to add the positions to a list. If the positions are then ticked before starting the experiment, the microscope will automatically scan these points and the area between them.
- To ensure that the image is focused correctly in a set of tile scans, you can use the **Focus Strategy** tool. If you are happy to acquire all tile scans at the same Z-position, you don't need to do this. In the focus strategy tool, you can select **Absolute Fixed Z-position** from the dropdown list and select **use Z-position from Tiles Setup**.



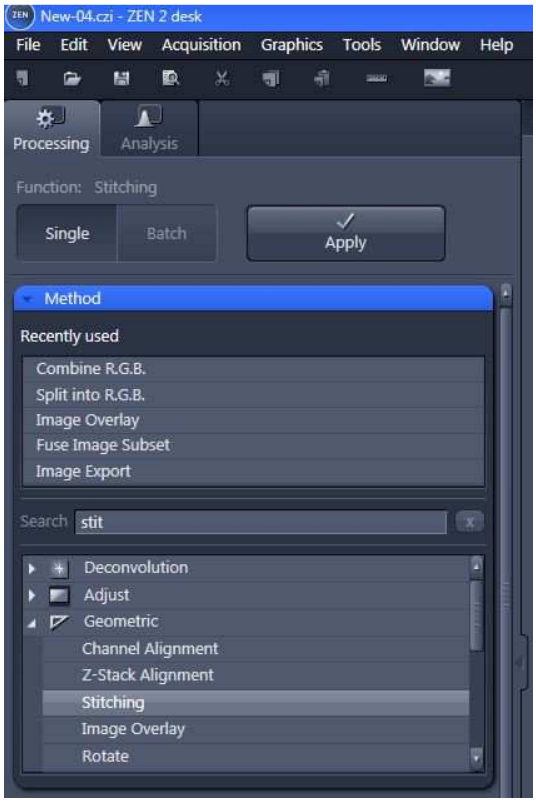
***For advanced tile scan setup please refer to the Axio Observer Advanced Tile Scan Setup guide.**

- In the **Options** section of the tiles tab, select **overlap 10%** if using X10 objective or above, and tick **stitching during acquisition** (this can also be applied after acquisition if not applied during).
- If using the X2.5 or X5 objective select **overlap 0%** and tick **stitching during acquisition**.

- Select **Start Experiment** to start the tile scan.

***If there is still a problem with shadowing and/ stitching of tiles, this can usually be resolved by processing images post-acquisition…**

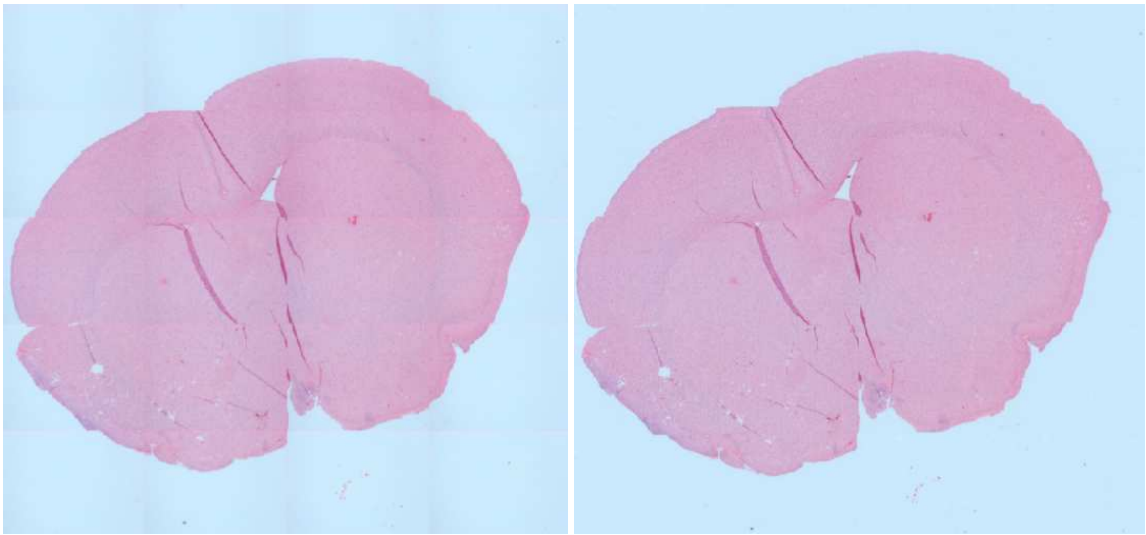
- To perform post-acquisition processing (may be required for tiling performed with X2.5 or X5 objectives):
  - Go to the **Processing** tab and select **stitching**.



- Select **New Output.**
- Tick **Fuse Tiles** and **Correct Shading.**
- Select **10% overlap.**

- Select your tiled image as the **Input image** and a snap of a clean, blank area of the slide as the **reference image** for shading correction.
- Click **Apply**.

eg Before Processing **After processing**



Z Stack:

- Select **Z stack**.
- In **live** mode, move through your sample, then click **set first** and **set last** in **first/last** mode OR click **set center** in **center** mode.
- Select **set optimal** to set the optimal number of slices and depth (there will be overlap between slices of ~50% so you can choose to reduce the number of slices; a higher number of slices may be required at higher objectives).
- Select **Start Experiment** to start recording the Z-stack.

To add annotations:

- Select the graphics tab, select the annotation you want, and then draw it onto the image.
- Right-click over annotations to change their format.

Acquisition with saved configurations

- Open the folder of saved configurations and load the appropriate file in the Experiment Manager tab…

Saving

Saving images

- Images can be saved as Zeiss files (.czi) to begin with, then re-opened using ZEN blue software and exported as TIFFs or JPEGs later as necessary.
- Zeiss files can also undergo processing using the ZEN blue software eg to burn in features and alter colour settings.

Saving configurations

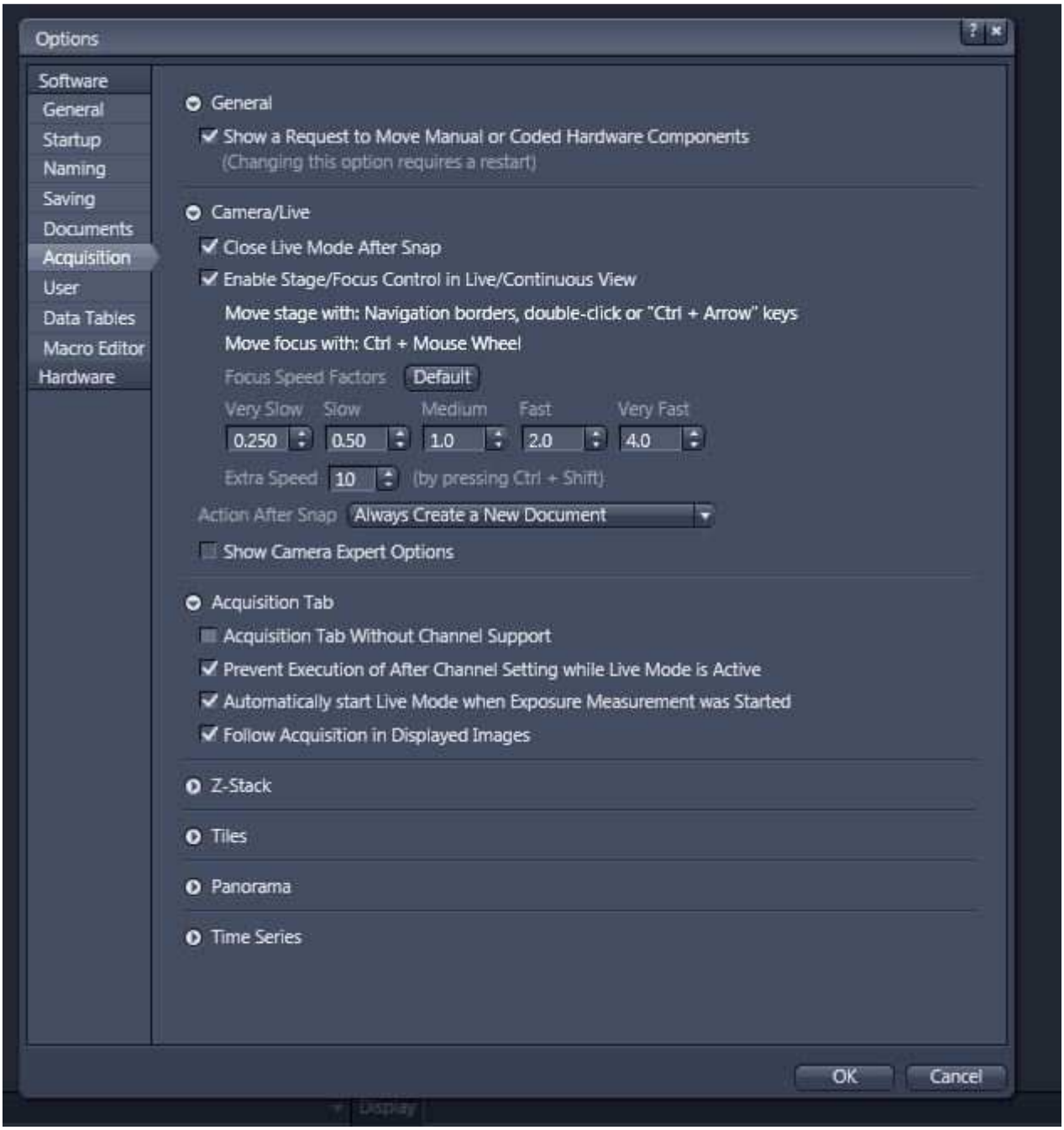
- Click the cog next to the Experiment Manager tab… and save as…

SHUT DOWN

= START UP in reverse

Troubleshooting

- Blurry edge on pictures at lower magnification – could indicate that the field aperture is out of alignment – ask the microscope facility manager to reset the Koehler illumination.
- Crashes during tile scanning – there may be insufficient memory on the computer – make sure you have saved your files onto a USB/ onto the intranet, and do not have files stored on the computer.
- Live mode ends after exposure has been set – go to **Tools**, then **Options** and make sure your **Acquisition** settings are as below:



- Not sure how to perform certain functions in ZEN - press F1 to bring up the online help window, or click the **?** icon in the top right hand corner of ZEN, and then point the cursor to the area of the software on which you need information.