Performing a Multi-well Experiment

This protocol describes how to re-use a pre-calibrated well plate for an experiment. For reference consult the Zen 2 Blue manual section 7.4.6 starting on page 79.

Materials needed

- Cellvis 96 well glass bottom plate (P96-1.5H-N) (glass is 0.17 +/- 0.005 mm thick)

Protocol

follow start up procedure protocol to turn microscope on

to start the ZEN blue software, make sure the microscope has completed its starting procedure and the microscope display is on without displaying any errors. At this point click on ZEN blue icon on windows desktop to start Zeiss software

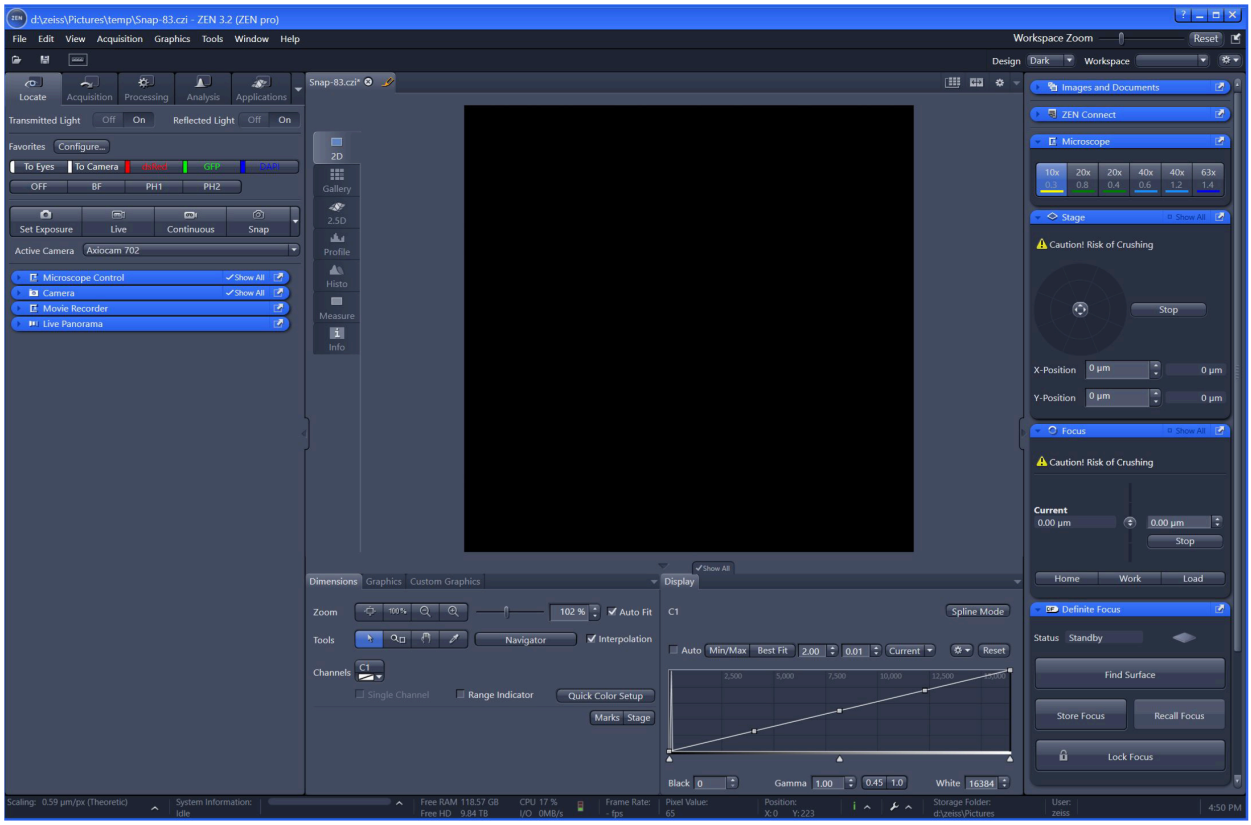
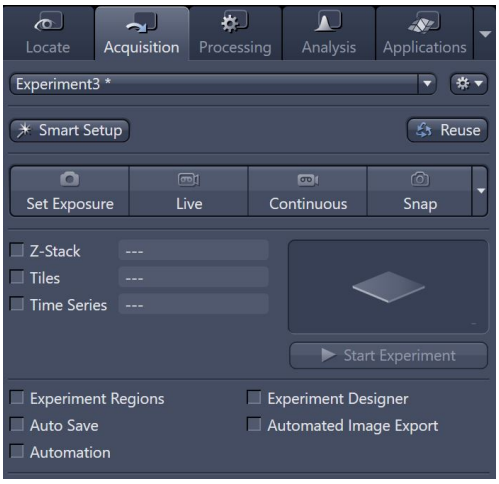


Figure 1. ZEN Blue program interface.

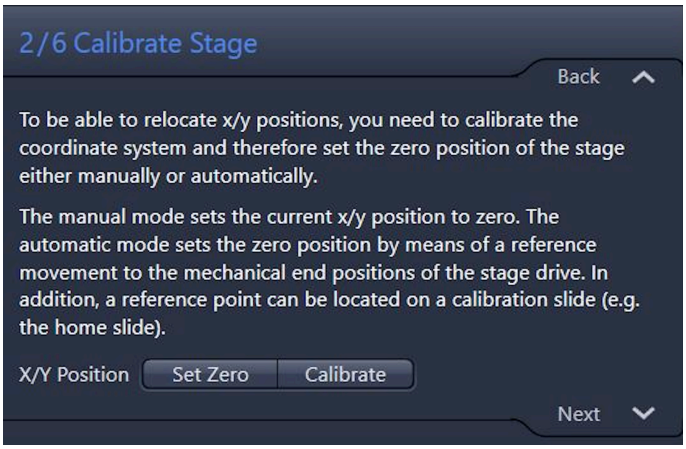
Zero XY Stage

Make sure the XY stage does not have anything mounted on it (well holders, well plates, and or petri dishes).



Navigate to the **Acquisition** tab and check the **Tiles** option and then navigate to **Multidimensional Acquisition**

Under **Tiles** navigate to **Sample Carrier**, click **Calibrate**, and click next on step 1 of the Sample Carrier Calibration Wizard



Click **Calibrate** to zeroed the XY stage.

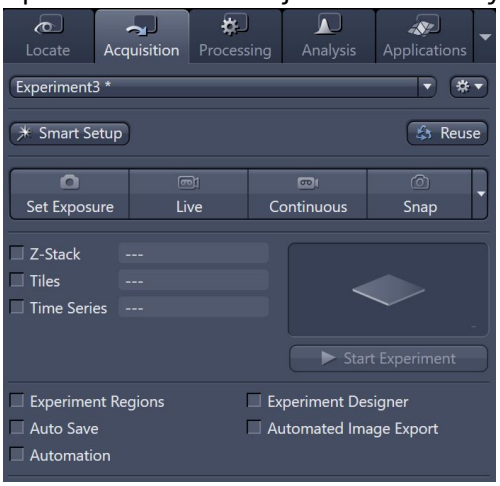
Now you can open a previously save experiment and use it as a template under the acquisition tab.

Locate Sample

Figure 2. Locate tab.

Under the locate tab you can set up the light path, power of light source, and filter cube to image your sample. First move the joystick to an approximate location of your sample then set the optical path by clicking to **To Eyes** button. At this point you should be able to use the eyepiece to locate your sample. (Move stage to well A1 if working with a 96 well plate)

tip - use the 10x objective to easily locate your well of interest



Select a previously save experimental set up where you can re use the calibration for your well plate.

Set experimental parameters of interes:

Z-Stack: click if you want a Z-stack
Tiles: use when setting a multi-well plate
Time Series: use for time series experiments.

Once your find your sample transition to the next tab **Acquisition** to start setting up for your experiment.
Tip: follow the order presented in the acquisition tab from top to bottom

First you must set up your imaging settings by navigating to the **imaging setup** submenu and clicking on it (see figure below). Here you can set up the optical path to image your sample. You have two options either brightfield (Phase contrast and dark ilumination) or fluorescence.

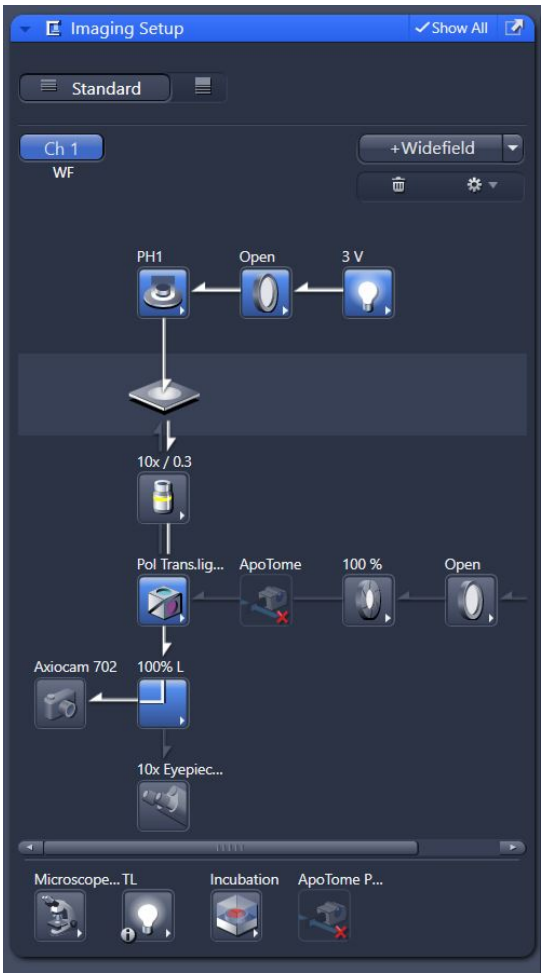
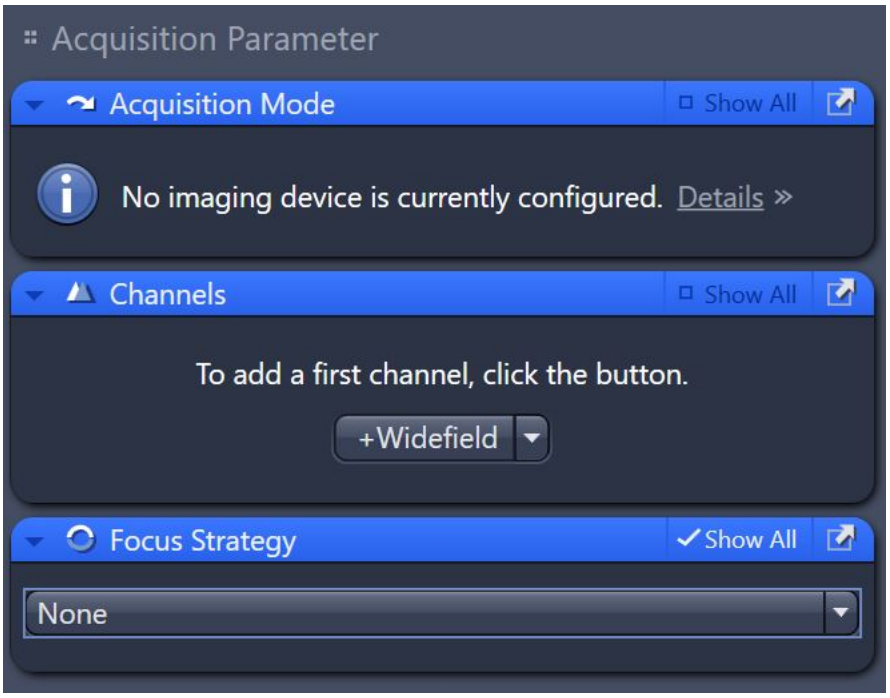


Figure 3. Imaging set up. It shows the optical path use to illuminate and image your sample. Make sure you select the right filters that match your excitation and emission.

Once the light path is set make sure the exposure and light intensity are correct for your sample. Note: to proceed with the calibration it is better if you use the 10x objective.



Under **Acquisition Parameter** click **+Widefield** to set the

Light source

Halogen Lamp for brightfield

LED for fluorescent

Filter sets see bellow

Position	Filter Set	Exitation (nm)	Emmision (nm)	Beamsplitter
1	38 HE GFP	BP 470/40	BP 525/50	FT 495
2	43 HE DsRED	BP 550/25	BP 605/70	FT 570
3	50 CY5	BP 640/30	BP 690/50	FT 660
4	90 HE D/G/C3	BP 385/30 BP 469/38 BP 555/30 BP 631/33	QBP 425/30 QBP 514/30 QBP 592/25 QBP 709/100	QBS 405 QBS 493 QBS 575 QBS 653
5	96 HE BFP	BP 390	BP 450	FT 420
6	POL TL			