Calibrate XY Stage for Multi-well Experiments

This protocol describes how to calibrate the XY stage of the microscope to locate a multi-well plate relative to the XY stages. 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 strting 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

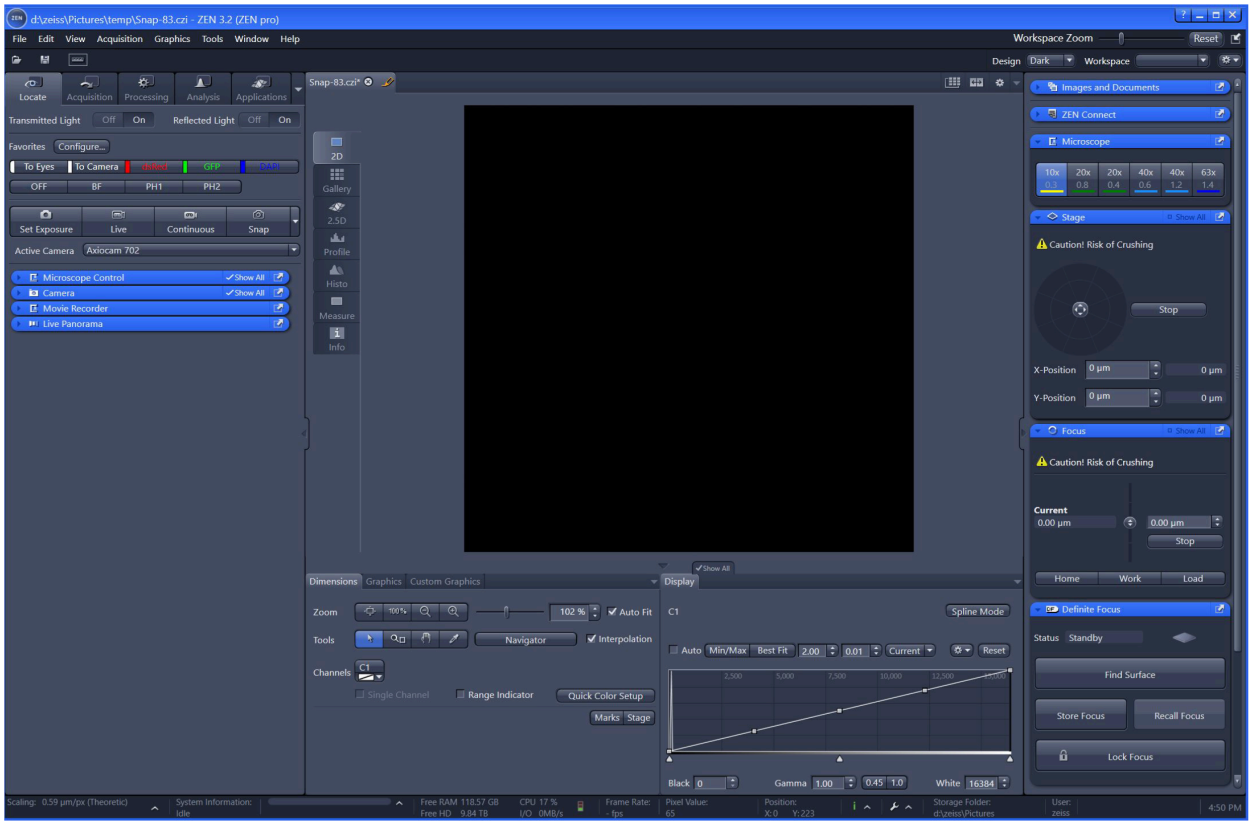


Figure 1. ZEN Blue program interface.

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

Once your find your sample locate well transition to the next tab "Acquisition" to start the calibration process, but 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) and fluorescence.

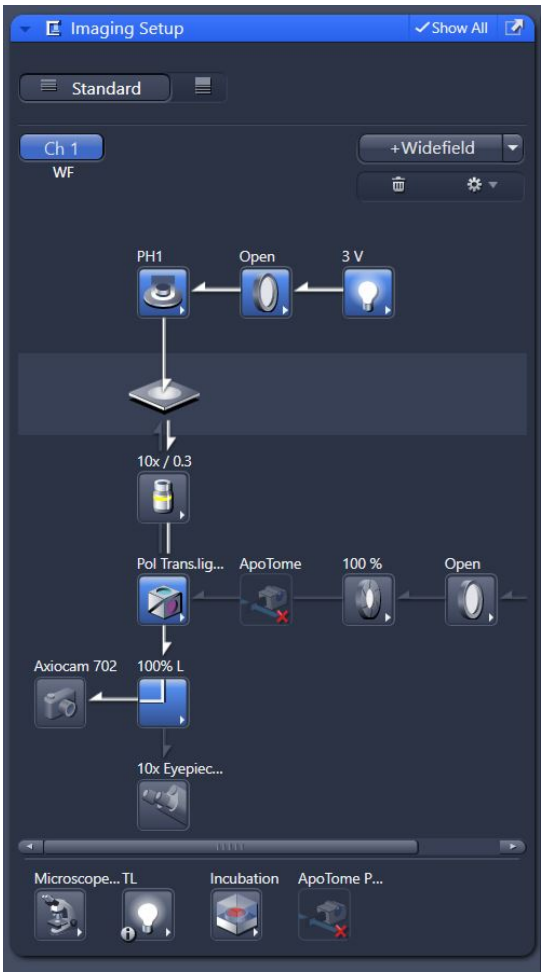


Figure 3. Imaging set up. It shows the optical path use to illuminate and image your sample. Make sure you select the right filters that match your excitation and emission.

Under imaging set up click widefield and select TL Phase (make sure you are using the 10x objective on light path diagram)
standard image settings:

- TL Halogen Lamp: 3V
- TL motorize condenser: Open
- condenser: contrast, PH1, N.A. 0.3
- objective: 10x Air |0.3
- 6x motorized Reflector Changer: Pol Trans.light
- 3x motorized side port turret: 100% L

If you want to use the camera make sure the port turret is set to L.

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.

The first step to start the calibration is to navigate to Sample Carrier menu under Multidimensional Acquisition in the Tiles sub menu. Here you can select from a list of templates the well that you plan to use for your experiments.

- Click select a well plate (multiwell 96) click Calibrate to start the Sample Carrier Calibration Wizard that will guide you through the set up (6 steps)

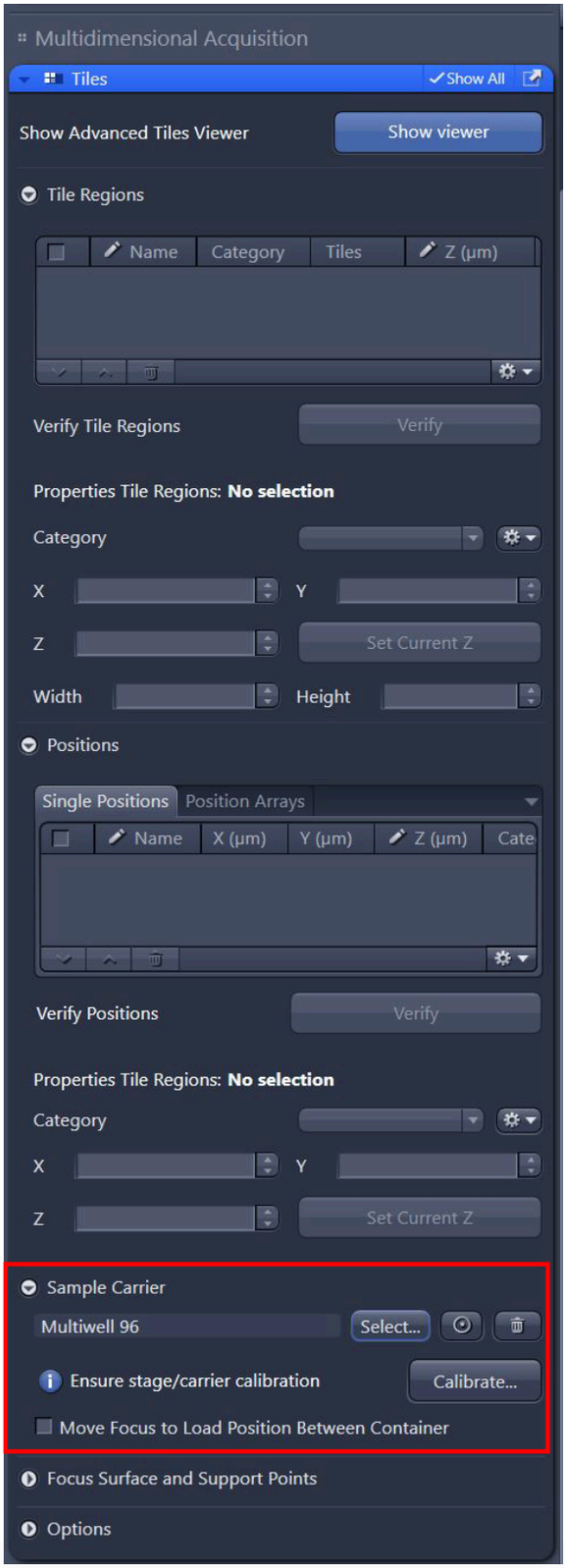


Figure 4. Multidimensional Acquisition. Make sure Tiles is selected under Acquisition menu.

Calibration Wizard

First set the illumination settings for the calibration set. To calibrate your multiwell plate you will need to locate pre-determine points on your multiwell plate depending on the type of calibration selected. It is recommended you use the 7-point calibration if using a 96 well plate.

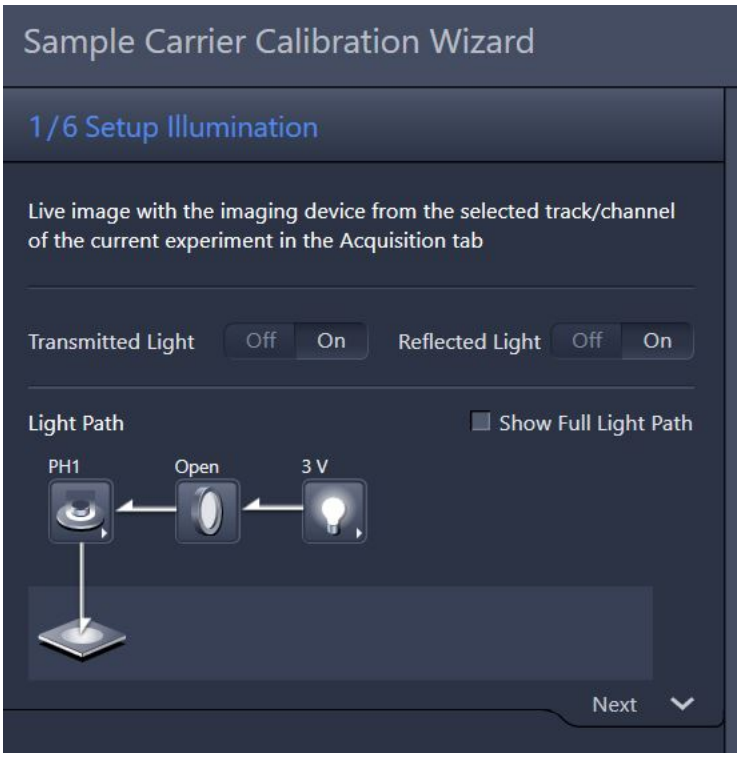


Figure 5. step 1 of calibration

step 1 of 6 Set up illumination (this should have been set previously (click next)

Note: The XY stages does not know its exact location (it only knows its location relative to a pre determine zero point activated with limit switches) At this point the XY stage does not know where it is relative to your well plate.

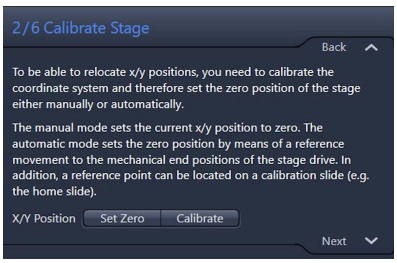
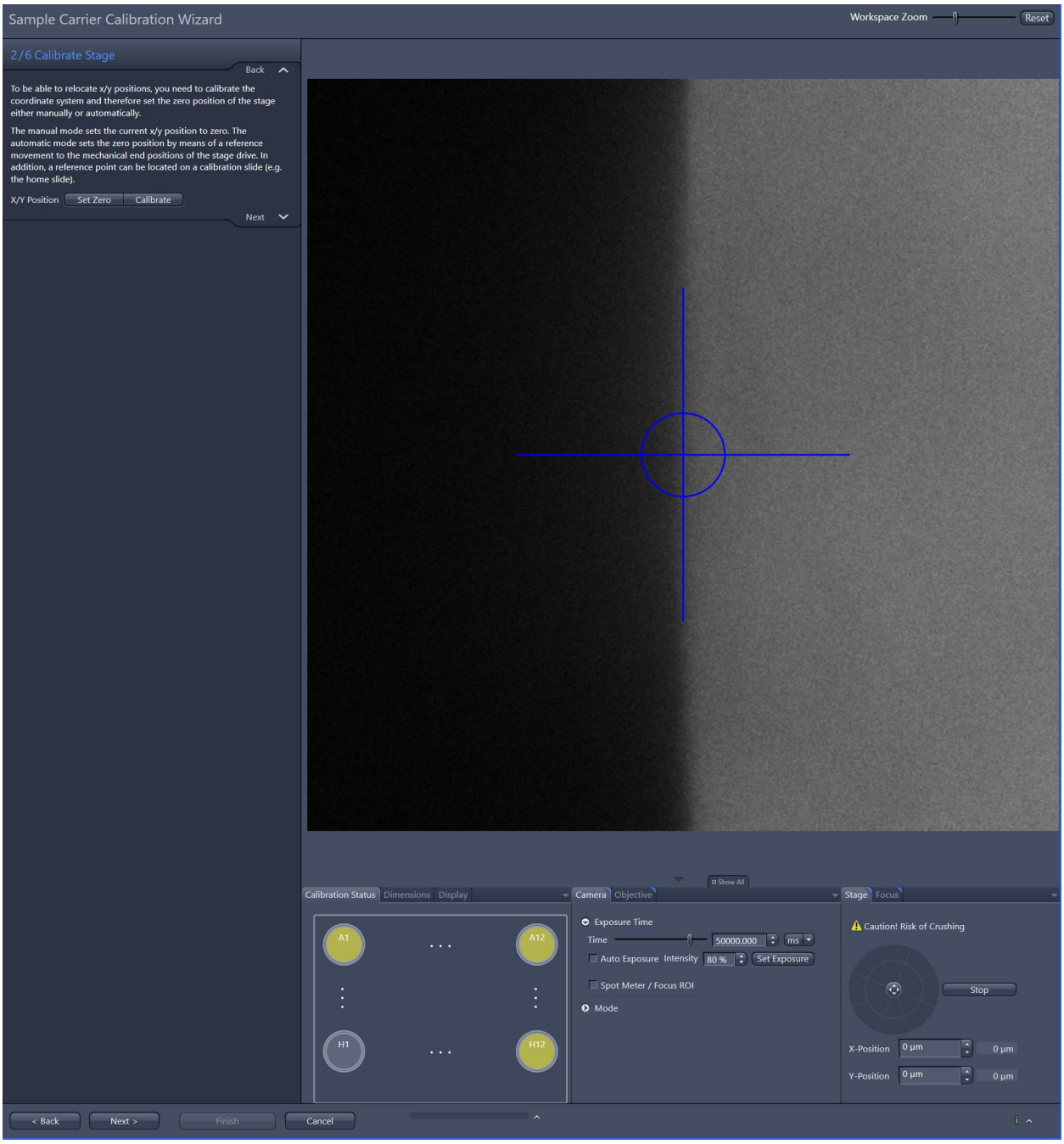


Figure 6. Step 2 of calibration

First locate the left corner of the A1 well and press Set Zero button to record the XY locations as the zero points of the XY stage. Then click Calibrate to find the limit switches



step 2 of 6 move to well A1 left corner

Set Zero (records XY point)

Calibrate (moves stages to mechanical end positions (reference location))

press continue under Caution message

click next

step 3 of 6 Select calibration method
for 96 well plate select Search Well Edges (7 points)
click next
step 4 of 6 Define Upper Left Well (A1)
find tune focus for every location
once in the desired location click set position
move to next location (in blue top edge)
follow procedure until all four points are located and set
click next
step 5 of 6 Define Upper Right Well (A12)
find tune focus for every location
Click Move Stage to move to well A12
located desired location in well
once the two locations are located and set click next
step 6 of 6 Define Lower Right Well (H12)
find tune focus
Click Move Stage to move to well H12
located desired location in well
once the two locations are located and set click next
click Finish

Sample Carrier is now Calibrated

Under Multidimensional Acquisition
Tiles
show viewer to select the wells that you desire to image