



Imager Z1 Instructions

For Multichannel Fluorescent Imaging

MICROSCOPE PARTS:

1. Power Supply 231
2. Microscope power switch
3. Fluorescent Lamp (not needed)
4. TFT Touchscreen
5. Condenser top lens control
6. Objectives
7. Stage Controls
8. Objective Focus
9. Fluorescent mMR3 camera
10. Colour mMRC5 Camera
11. Eye/Camera Slider Bar
12. Lighting Controls

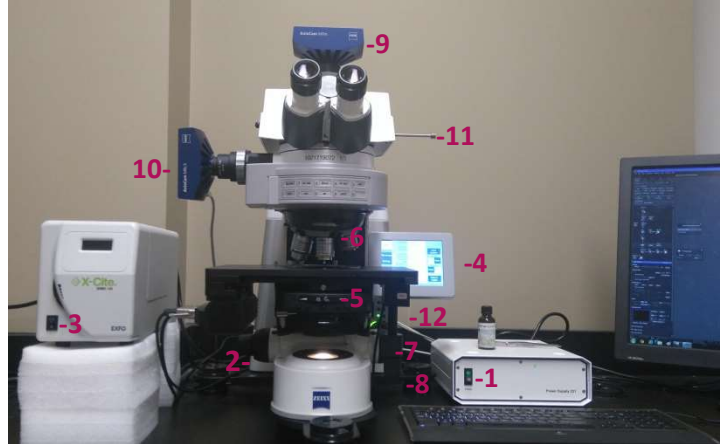


Figure 1, Axiomager Z1 Microscope Controls

STARTUP:

1. Sign in on the Calendar systems (BOTH online AND paper)
2. Computer start up: Log in = Zeiss user, Password= Imager_Z1
3. Turn on Power Supply 231 switch (Fig1-1)
4. Turn on microscope switch (Fig 1-2) on the left at bottom back of scope
5. Turn on fluorescent lamp (Fig 1-3)
6. Wait for the small TFT screen (Fig 1-4) attached to the scope to fully boot up to the "home" screen shown before launching the Zen software
7. Launch Zen software and start up software in "Zen Pro" mode
8. Ensure the objective (Fig 1-6) is on 10x -20x magnification to start.
9. Insert slide and focus on specimen.
10. If you don't see any light, check the following:
 - a. On Microscope, push in the Eye/Camera slider bar (Fig 1-11)
 - b. On TFT touchscreen (Fig 1-4):
 - o Go to Light Path and set the path to 100% Tube (Fig 2-1)
 - o On Reflector Tab set filter to required position (Fig 3-1)
 - o Set contrast manager to Fluorescence (FL) (Fig 3-2)
 - o Ensure that the Reflected light (RL) shutter is open and the (TL) is closed (Fig 3-3,4)
 - o Make sure the wheel on the lamp (Fig 1-3) is fully open to maximum

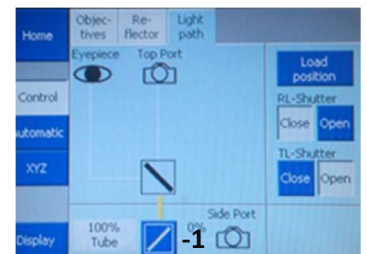


Figure 2, TFT Light Path Screen



Figure 3, TFT Reflector Screen

SET UP MULTICHANNEL ACQUISITION:

1. Proceed to desired objective and find region of interest by eye through oculars
2. Pull out Eye/Camera slider bar to right (Fig 1-11)
3. In ZEN software, Go to Acquisition Tab
4. Under "Experiment Manager" select your personal experiment (see staff) (Fig 4-A)
5. Ensure that you have the MR3 fluorescence camera selected (Fig 4-B)
6. On the "channels" window, select the fluorescence channels you wish to use (Fig 4-C) (checkboxes)
7. Select (highlight) one of these channels at a time and select "Live" to open the image onscreen
8. Use the camera slider to adjust the exposure time for that channel to yield a good image (Fig 4-D) Clicking "Set Exposure" will allow the software to guess for you.

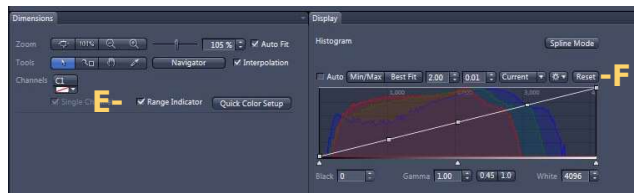


Figure 5, ZEN Dimensions and Display, Bottom of image screen

9. Use the "Range Indicator" (Fig 5-E) button at the bottom of the image screen to avoid overexposing your image. Overexposed areas will be shown in red.
10. Ensure that the histogram is reset to linear (Fig 5-F)
NOTE: Don't use auto display corrections for comparative or analytical imaging !
11. If you have difficulty with uneven lighting in the image, see staff for help to set up the "Post Processing" or "Shading" corrections (Fig 4-G,H)
12. Repeat with each desired channel (Fig 4-C)

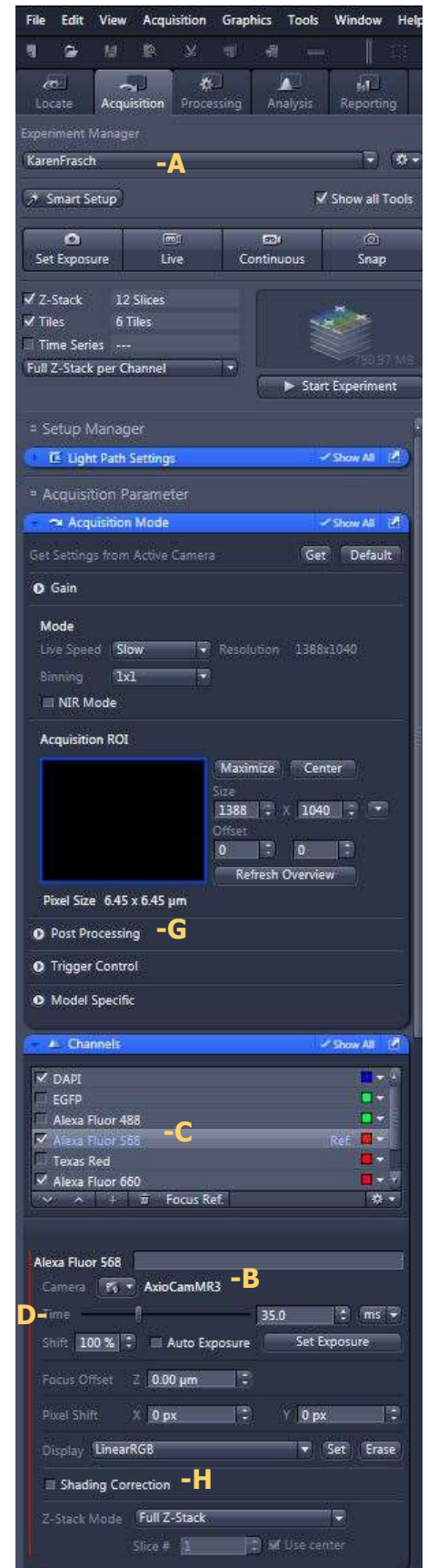


Figure 4, ZEN Acquisition Panel



CAPTURE AND SAVE:

1. On "Acquisition" Tab in top right of screen, click the "Snap" button (Fig 6-A) to capture image. For images involving z-stack, time series, and tiling, refer to separate instructions and use "Start Experiment" to capture. (Fig 6-C)
2. The onscreen image will freeze, and a thumbnail of the image will appear in the file handling area.
3. If you wish to add a scale bar to the image, you click on the ruler icon (Fig 6-B) under the main menu bar.
4. You can format the bar by right-clicking it and choosing "Format Graphical Elements." Set the desired format as your default to maintain your selections.
5. To save your image, make sure it is selected in the file handling list or active onscreen. Click the save icon below the thumbnails (Fig 7-D) or use the file menu or upper save icon (Fig 6-E)
6. We suggest saving as a Carl Zeiss .czi format image and batch exporting the files at the end of the session. The Zeiss format saves information about the calibration, graphics, scale bars, and camera information. It also saves the full 16-bit raw data for analysis.
7. To export images, refer to the document on exporting files from Zen

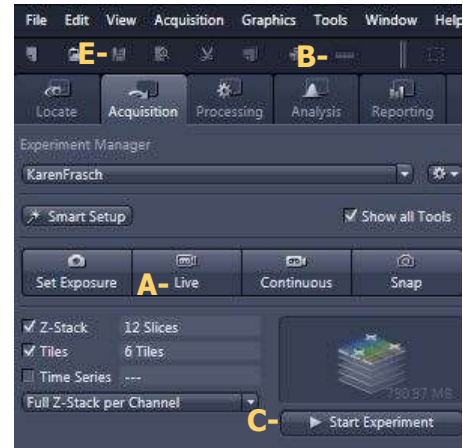


Figure 6, ZEN Acquisition Panel, Top

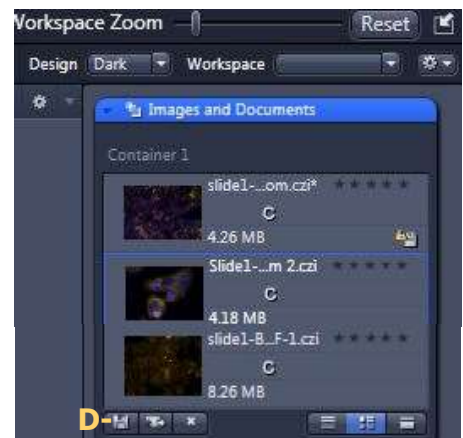


Figure 7, File Handling Area

TO SHUT DOWN-Check first to be sure no one is coming immediately!

1. Switch off all microscope switches and quit ZEN unless someone is coming. The software will ask you about any images you haven't saved
2. Leave the computer on.
3. Clean any oily lenses thoroughly with a dry wiper.
4. Leave the 10x Objective in place to prevent harm to the scope.
5. Clean the workspace and sign out on the calendar