User – Manual

Zeiss - Confocal

(10 – 31 - 2018)
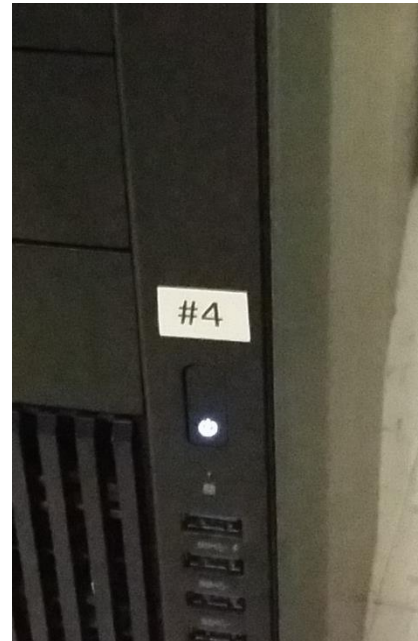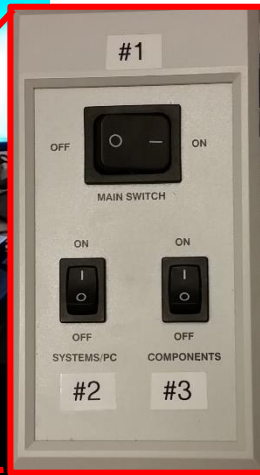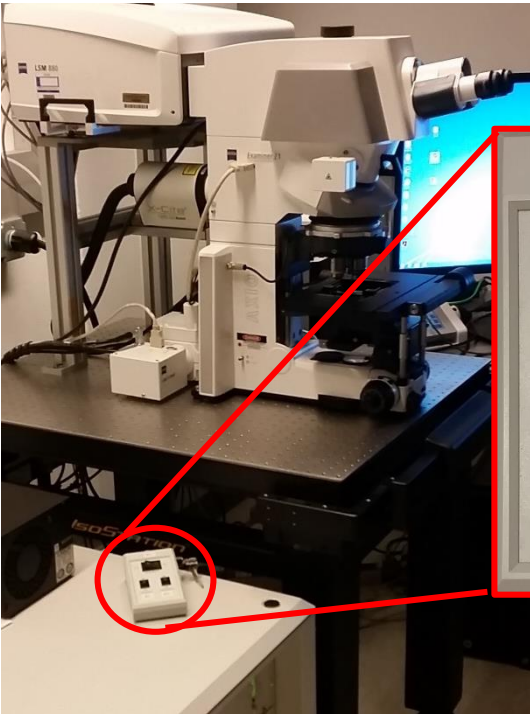
From: Karl Ferdinand Ziegler

Contents

1. Start/Stop the System
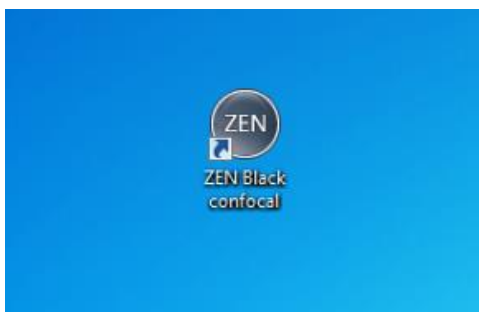
1.1. Hardware

1. Turn ON the Hardware in the order shown in the image above by flipping the switches	**2.** If the PC is not already ON, start the PC after the Hardware is running

1.2. Software

1. Prior to starting ZEN, move the objective turret to position #1

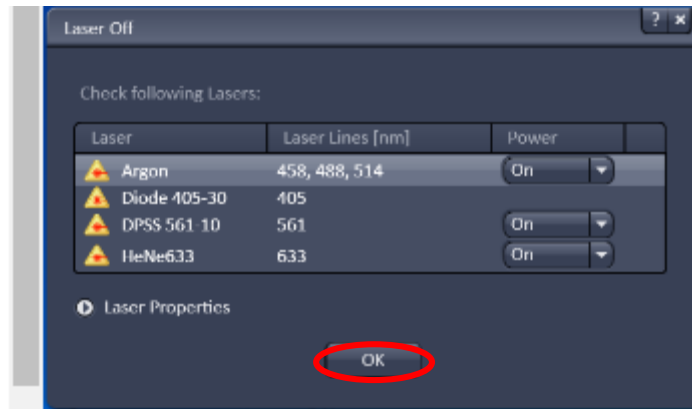**2.** After the Hardware is running and the objective turret is in position #1 you can start the Software

3. Double click on the icon "ZEN Black confocal"	**4.** Click on "Start System"

1.3. Turn the system OFF

1. Save/transfer your data

2. Click on the "X" on the top right corner

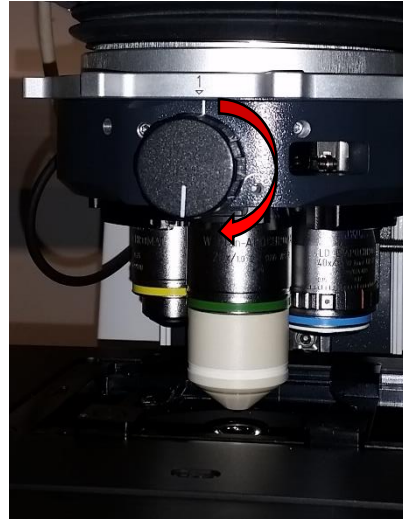**3.** Click on "OK" in the window shown below to turn off the Lasers

4. Now you can turn off the hardware in the opposite way as shown in Point 1.1 by flipping the switches. **DON'T TURN OFF THE PC.**

2. Find your sample

2.1. Place the sample and setup the objective

1. After the System is running, place your sample on the stage

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2. Place the sample on the sample stage and choose the objective for your experiment by rotating the Objective turret until the number are overlapping with the white lines (as shown above).	**3.** Move the Objective in working distance by rotating the knob as shown above. If necessary, put Oil/Water on top of the sample before you move the Objective down.

4. If you need a different objective on the microscope than contact Andy Schaber

2.2. Locate your sample

2.2.1. Transmitted light (Bright field/DIC)

1. After the sample and the Objective is in the right place you can use the software to find your sample.
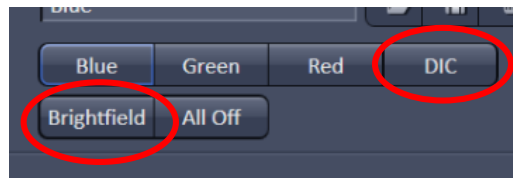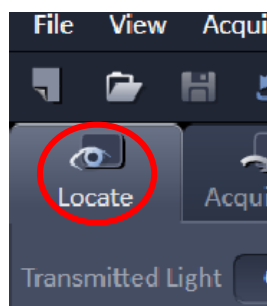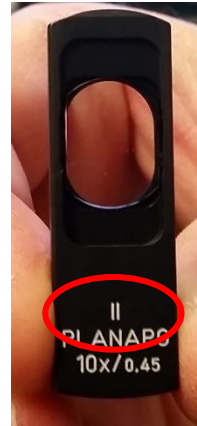

2. Click on the Tab "Locate" on the top left corner	**3.** Choose either DIC or Bright field

5. If you want to use DIC please make sure you follow the next steps, otherwise skip to step 10.
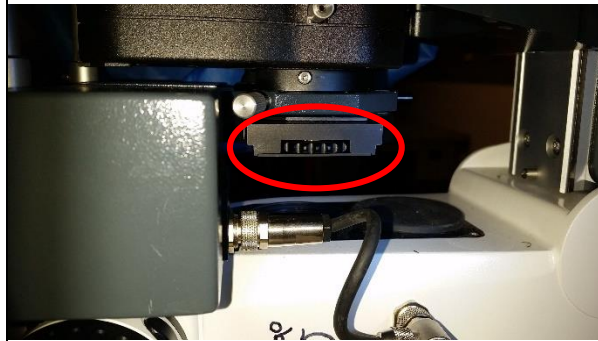
6. Make sure the DIC prism is in the Objective	**7.** Check if the prism matches the Objective with the list close to the microscope.
8. Rotate the condenser turret to match the roman numbers between the 2 prisms (objective and condenser)	**9.** Contrast can be adjusted by rotating the wheel on the polarizer under the condenser

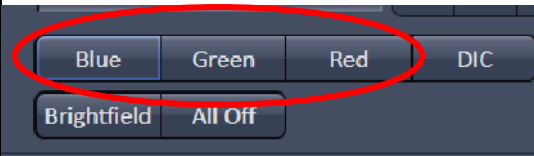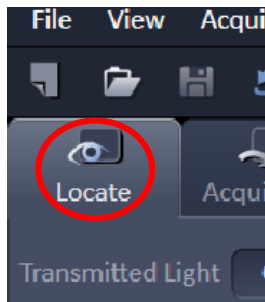**10.** Use the Eye piece to locate the sample, either with or without DIC

11. Change the focal position by rotating the knob.	**12.** Rotate the wheel to change the light intensity	**13.** Move the joystick to change the X,Y Position of the sample

2.2.2. Epi-fluorescence

1. After the sample and the Objective is in the right place you can use the software to find your sample.

|		
2. Click on the Tab "Locate" on the top left corner	**3.** Select a Filter set for your experiment	**4.** Press the wheel to turn ON/OFF the LED light source

|		
5. Change the focal position by rotating the knob.	**6.** Rotate the wheel to change the light intensity.	**7.** Move the joystick to change the X,Y Position of the sample

3. Pre-setup an experiment

1. After you found the sample through the eye piece you are ready to pre-setup the system with the right laser and detection combination

2. Click on "Acquisition" on the top left corner. Then pre-configure the system by one of the next methods.
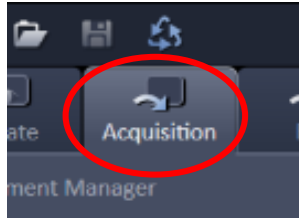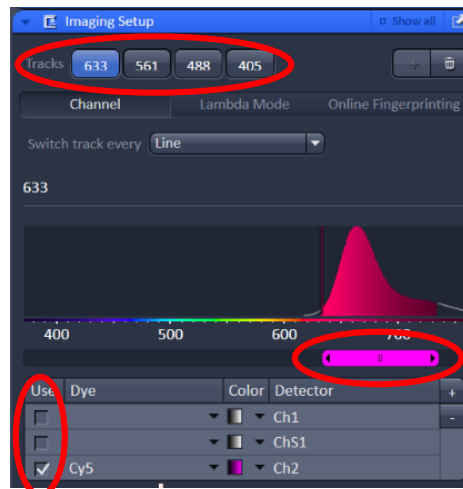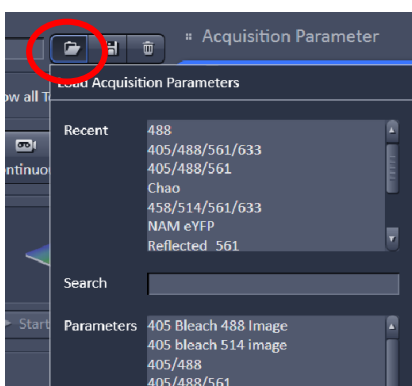

Method a:
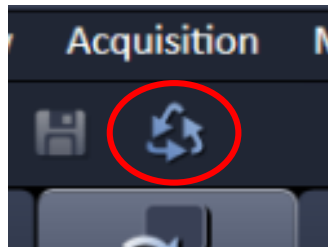
3a. Pre-configure the system by loading an old data set and press the "Recycle" button.
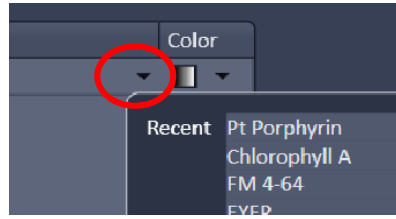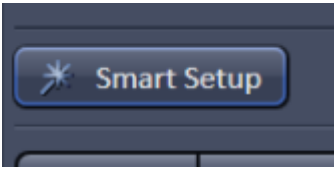
Method b:

3b. Click in the field "Experiment Manager" on the button shown above and choose one of the pre setups from the drop down menu.	**4b.** Click through each "Track" and make sure: - the right channel is active - detection spectrum is at the right position and has the right size

Method c:

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3c. Select "Smart Setup" in the "Experiment Manager".	**4c.** Select the dyes you will use in your experiment from the drop down menu after clicking on the arrow. Choose as much as you need.	**5c.** Then choose between three different modes: fastest, best signal or smartest and press apply.

4. Acquire a 2D image

1. After you found the sample through the eye pieces and you have setup the system you can acquire an image.

2. In the window "Acquisition Mode" you can setup your scan parameter.
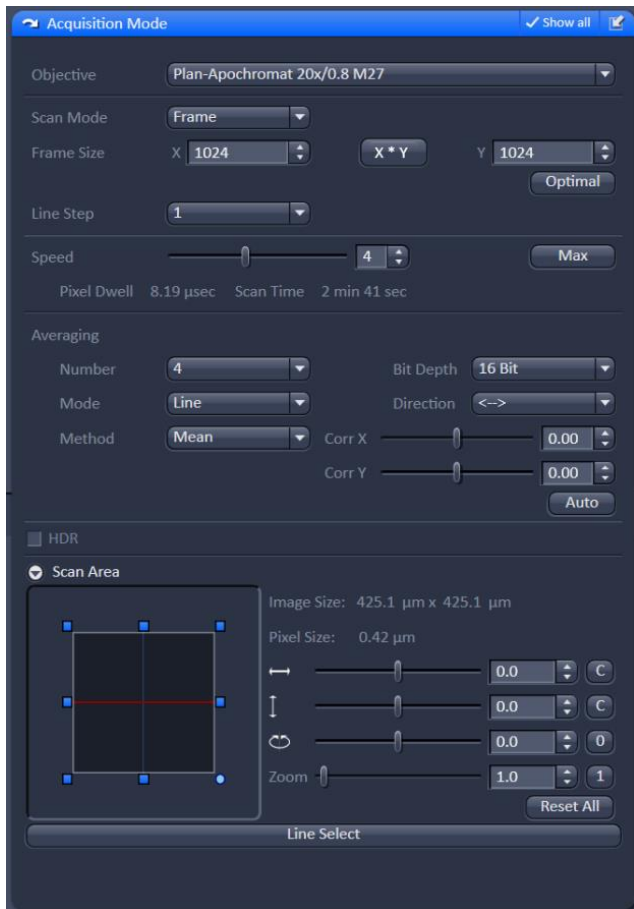
Frame Size: This option allows you to change the pixel density for your scanning area. Higher frame size led to longer acquisition times and more photo bleaching.

Speed: Changes the Pixel Dwell time or the frame per second (fps). Faster scanning reduces photo bleaching but decreases the signal to noise ratio (SNR).

Averaging: Allows you to increase the SNR by scanning the Frame/Lines multiple times. Increase photo bleaching as well.

Scan Area: Changes the size of your scanning area. The optical zoom increases the magnification and decreases the field of view.
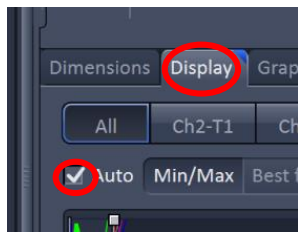
Scan Time: Indicate the time which is necessary to acquire an image.
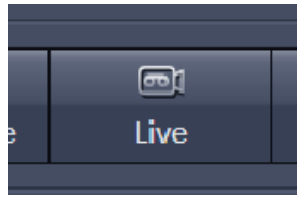
Recommended settings to start with:

- Frame size 512x512 or 1024x1024
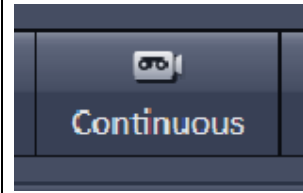- Speed   max
- Averaging   1
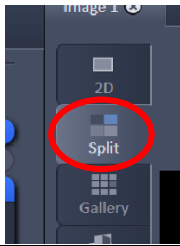
|			
3. If using multichannel, change the displayed view to Split	**4.** Activate the tab "Display" and check the box "Auto". That enable the auto-contrast function of the Look Up Table.	**5a.** Click in the "Experiment Manager" on "Live" to get an impression of your sample regardless of your acquisition mode.	**5b.** Click in the "Experiment Manager" on "Continuous" to get impression of your sample in regards to your acquisition mode.

6. Press "Expand All" to visualize all track settings

7. Set the Pinhole size by clicking one of the button (1) "AU". The active Pinhole size counts for all tracks/channels. You cannot set a different Pinhole size for each track.

8. Change the Laser Power (2) and the Master gain (3) for each tracks/channels that your signal reaches 2/3 of the scale shown in the "Display" tab.
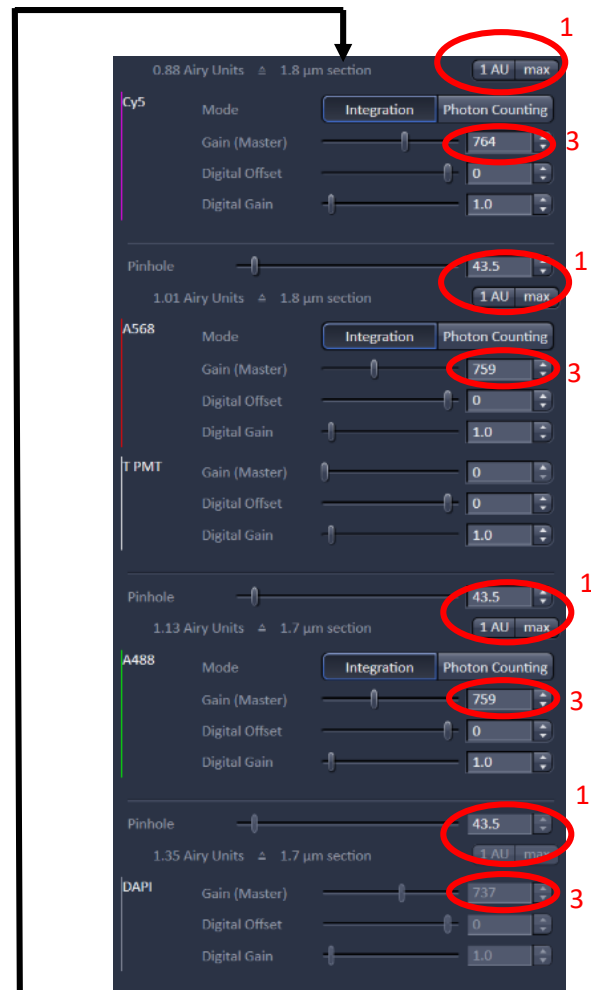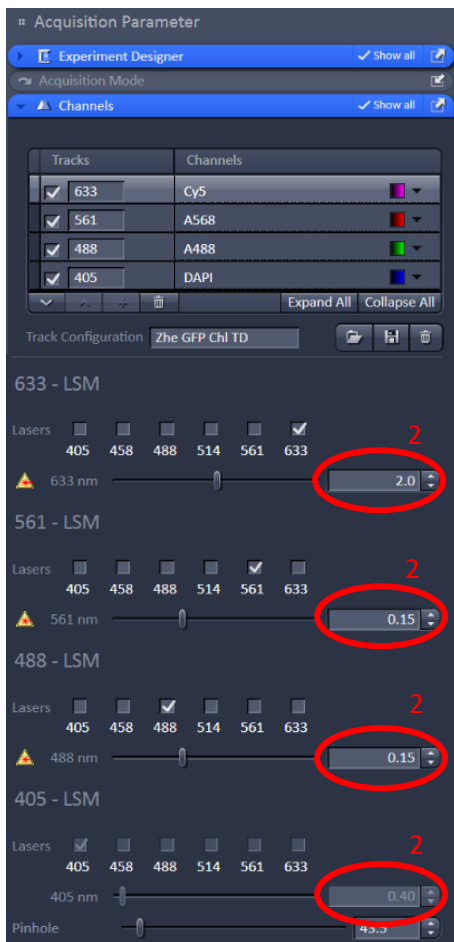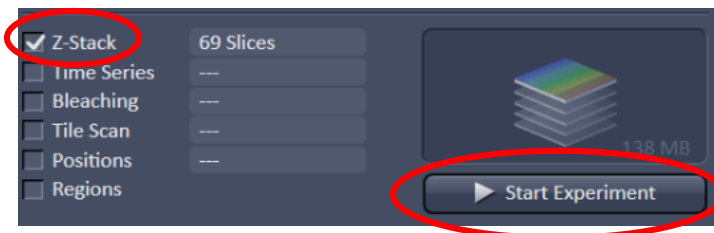
9. When everything is setup up and you are satisfied with the image then press the button "Snap" to acquire a single XY image.
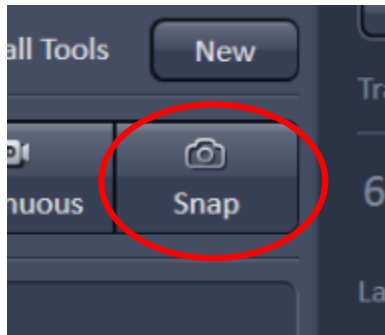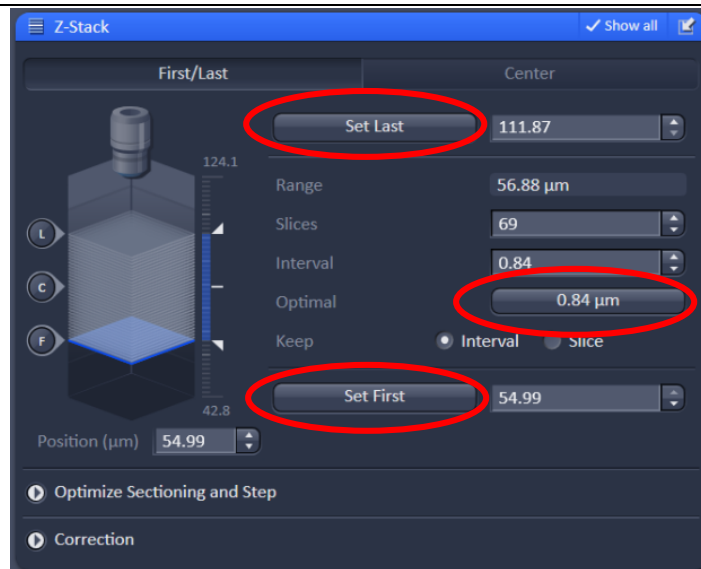

5. Acquire a Z-Stack image

1. After you configure the system and are able to acquire a 2D image you can also collect a Z-stack (3D) image

2. Activate the Z-Stack experiment manger by checking the box

3. Choose between two different modes: "First/Last" and "Center".
First/Last: Define the bottom and top boundary by moving the focus position up/down and click "Set Last", "Set First" respectively. Change the slice thickness to the optimum (Indicated by the button "Optimal"). After that you can press "Start Experiment".
Center: Move the focal position to the center of your sample. Set the top and bottom boundary by tipping the distance to the center position. Change the slice thickness to the optimum (Indicated by the button "Optimal"). After that you can press "Start Experiment".

6. Troubleshooting

If something doesn't work well, you need help or the microscope have malfunction, please contact Andy Schaber (Facility manager) by phone: **(765) 496-3148** and/or email: **schaber@purdue.edu**