Nano BioImaging SRiS 2.0 STORM Super-resolution Microscope Standard Operating Procedure

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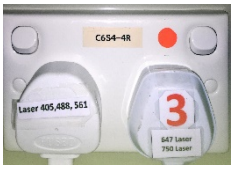
A. Basic Operation

1. Turning ON the system



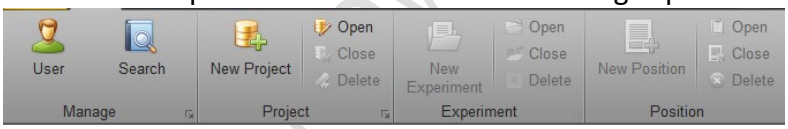
1.1. Switch on the power supply ① and ② mounted on the wall behind monitors.
1.2. Switch ON the 647 nm and 750 nm laser power supply ③.
1.3. [Optional] For back pumping please turn on wall port for "Laser 405,488,561"
1.4. Switch ON 647 and/or 750 nm laser module and turn emission key to ON (under air table).
1.5. Laser 405nm is the left most unit. Reach to the back of the controller for I/O rocker switch and then turnkey for emission.
1.6. Switch ON computer system ④ under bench.

> Warning: High power laser source. Do not look directly at the laser beam.

2. Rohdea 2.0 Software start up

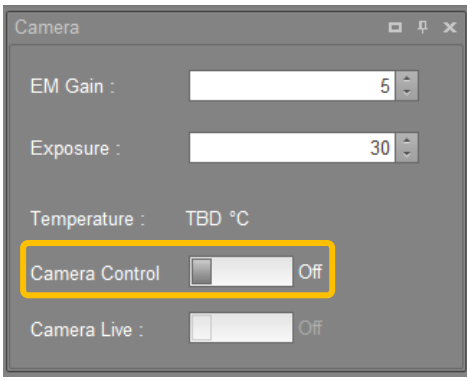
…A2.1

2.1. Launch the Rhodea 2.0 software on desktop.
2.2. Create a new Username (Format = "your PI's initials"_"your name") or search for an existing user name.
2.3. Create new Project or search for an existing Project.
2.4. Create new Experiment or search for an existing Experiment.



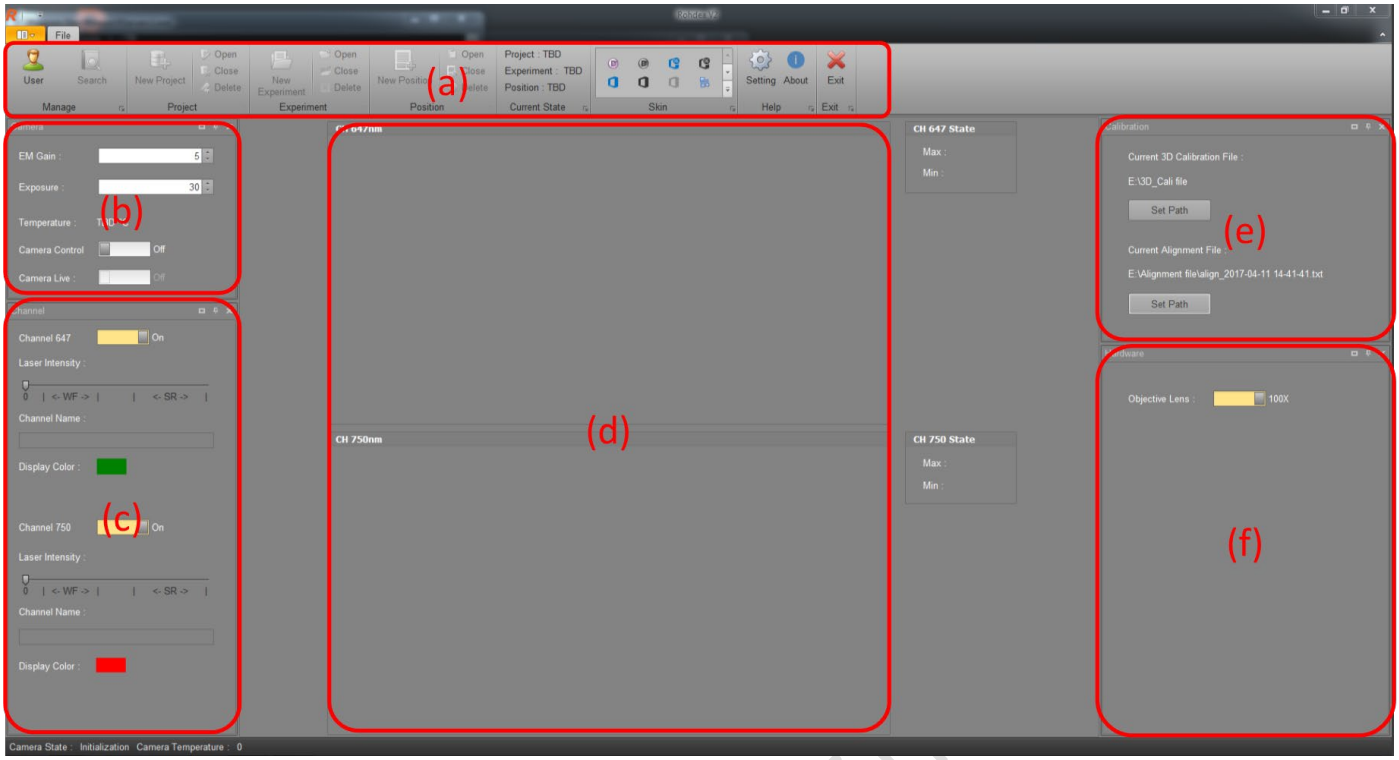
2.5. Your files will be created under Computer\Local Disk (E:)\Rohdea Workspace\(your PI's initials)
2.6. Click "New Position" to save a new image location for each field of view on the coverslip.

3. Turn on "camera control" slider switch.



3.1. The EM-CCD camera will be activated and start to cool to -70°C. **Camera Live will only be available after the EM-CCD has been cooled to -30°C

B. Rohdea V2.0 Software Interface



a) Operation Ribbon
b) Camera panel
c) Channel panel
d) Alignment and Calibration panel (administrator use only, DO NOT CHANGE)
e) Hardware panel
f) Live window

C. Mounting coverslip sample onto NBI SRiS super-resolution microscope

Prerequisite: your specimen (cell / tissue section / material) must be labelled and immobilized onto a 18 mm coverslip coated with microbeads.

1. **Make imaging buffer according to the following composition table:**

For imaging with Alexa Fluor 647 nm single channel:

Container	Reagent	Volume (1ml)	Final Conc.
15ml tube	Milli-Q H_2O	343ul	N/A
15ml tube	20% Glucose	500ul	10%
15ml tube	1M Tris-HCl 8.0	50ul	50mM
15ml tube	200mM NaCl	50ul	10mM
Eppendorf tube	200mM Cyclooctatetrane	10ul	2mM
Eppendorf tube	14.3M β-ME	10ul	143mM
Eppendorf tube	100X Glucose oxidase (24mg/ml)	27ul	1X
Eppendorf tube	100X Catalase (4mg/ml)	10ul	1X

For imaging with Alexa Fluor 647 and 750 dual channel:

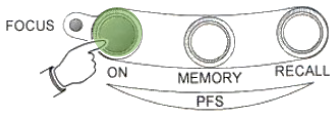
Container	Reagent	Volume (1ml)	Final Conc.
15ml tube	Milli-Q H_2O	333ul	N/A
15ml tube	20% Glucose	500ul	10%
15ml tube	1M Tris-HCl 8.0	50ul	50mM
Eppendorf tube	0.5M TCEP	50ul	25mM
Eppendorf tube	200mM Cyclooctatetrane	10ul	2mM
Eppendorf tube	100mM Ascorbic acid	10ul	1mM
Eppendorf tube	100mM Methyl Viologen	10ul	1mM
Eppendorf tube	100X Glucose oxidase (24mg/ml)	27ul	1X
Eppendorf tube	100X Catalase (4mg/ml)	10ul	1X

**Avoid vortexing the mixture once it is complete.

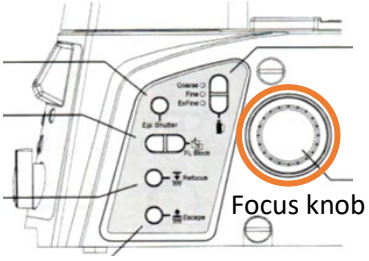
2. **Lift the coverslip containing your specimen from well plate with forceps.**
3. **Blot dry the underside of coverslip with Kimwipe™ paper.**
4. **Place the coverslip in provided imaging chamber.**
5. **Lock the coverslip in place. Screw tight the two halves of the imaging chamber. (Please use two fingers worth of force to prevent breaking the coverslip)**
6. **Add 380 µl of imaging buffer into imaging chamber and cover with a 18 mm x 18 mm square coverslip.**
7. **Store remaining imaging buffer at 4°C for later use. Freshly made imaging buffer is viable for 2 -3 hours.**
8. **Apply immersion oil to the lens.**
9. **Place the imaging chamber onto microscope stage.**
10. **To focus onto the sample, turn on Perfect Focusing System (PFS) on the Nikon microscope body by pressing "ON" button. PFS will blink green.**
11. **Turn focus knob until the PFS button is constantly on. The specimen is now in focus.**
12.

C5

C10

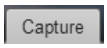


C11



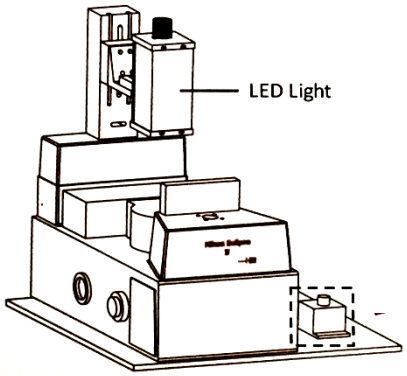
Right panel of the microscope

D. Finding sample on SRiS

1. **Switch light path to detector by pressing "L80" on the front panel of microscope body**
2. **Turn ON "camera live."**
3. **Switch Channel 647 and / or Channel 750 to "ON" and drag the laser intensity slider to "WF" (Widefield).**
4. **Search for cells in the live window.**
5. **You can activate "Luna" module.**
   **Go to "Capture" tab ** 

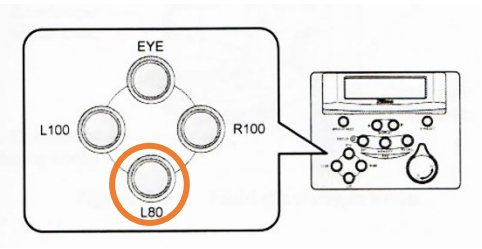
   "Luna" to aid finding sample under a wider view.
   DO NOT CLICK on the "play" button.

6. **Turn ON blue LED illumination with LED controller on air-table to the right of the microscope.**

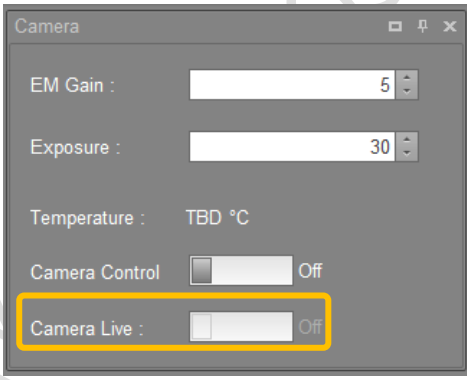


7. **Fine focus with Nikon PFS offset controller. Use the blue button to toggle between coarse and fine focus movement.**

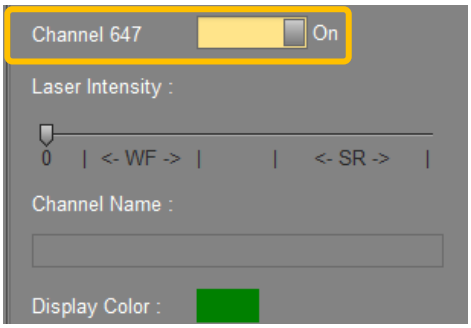
D1



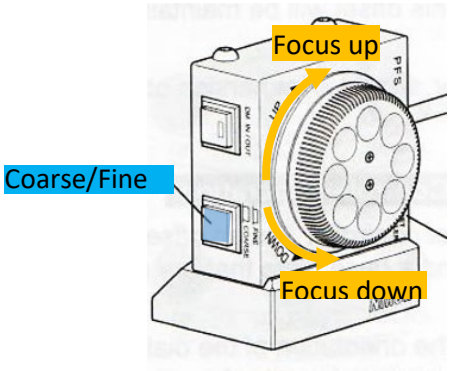
D2



D3



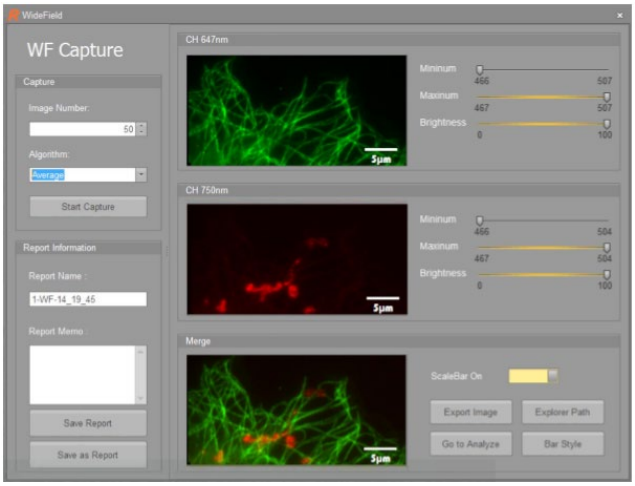
D7



E. Acquire Wide Field image

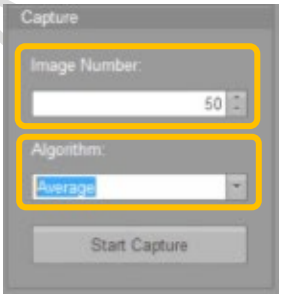
1. **Go to Capture tab.**
2. **Select "Wide Field" button to access wide field imaging options.**

3. **A "WF Capture" window should be seen as below:**



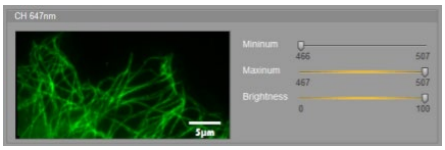
4. **Adjust imaging parameters:**
   - 4.1. "Image Number": 50 frames (recommended)
   - 4.2. "Algorithum": Average / maximum / Minimum (This will change the displayed image after certain number pof images has been captured)
5. **Input report name (or use the name as generated by the Rohdea software).**
6. **Press "Start Capture" Button to acquire image.**
7. **Image brightness can be adjusted for individual channels.**
8. **Press "Export" to save a TIFF file.**
9. **Your files will be saved to:**
   - 9.1. Computer\Local Disk (E:)\Rohdea Workspace\(your PI's initials)\(Experiment name)\(Position #)\WFReport

E4



E5

E7



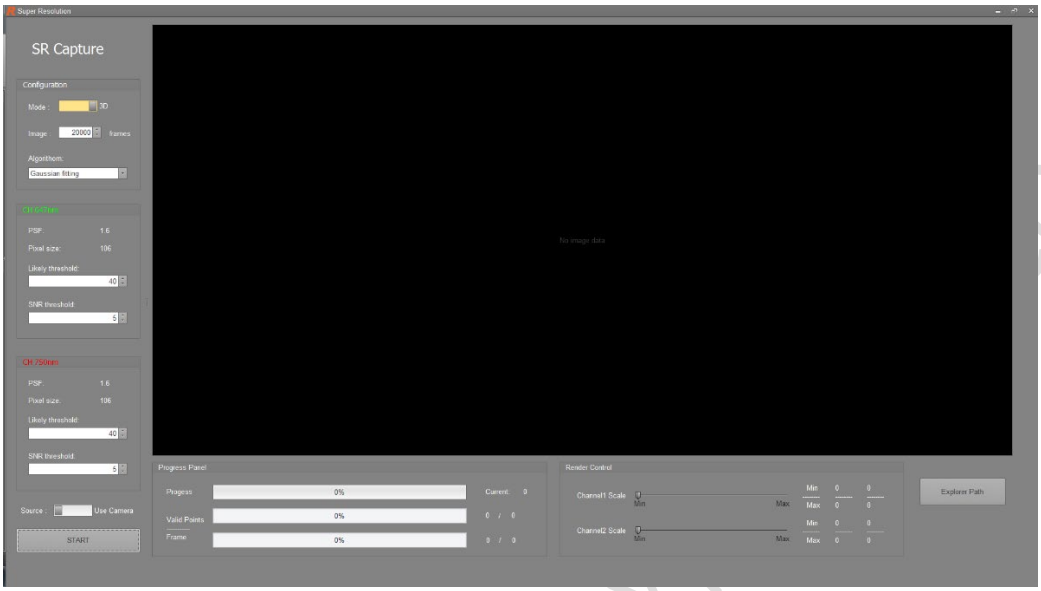
E8

F. Acquire Super-resolution Image

Prerequisite:
Your sample is on 18 mm coverslips seeded with locking beads and labelled with appropriate fluorescent dye.
Your sample can be seen in the live window and at desired focus level.

1. **Go to Capture tab.**
2. **Select "Super Resolution" to toggle the super resolution imaging options.**

3. **A "SR Capture" window should be seen as below:**

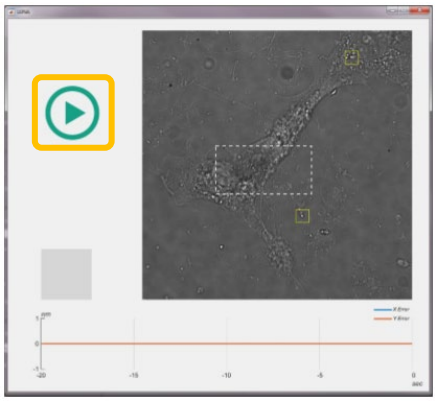


4. **Set imaging parameter**

4.1. [Image frames]: 5,000 - 20,000
(frame number varies depending on the complexity of the stained structure.)
4.2. [Algorithm]: Gaussian fitting.
4.3. [Source Switch]: Camera
4.4. [Likely Threshold]: adjust the size of light spot to be regarded as signal
4.5. [SNR Threshold]: (signal-to-noise ratio) adjust blink intensity to be regarded as signal.

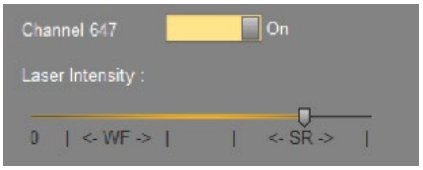
5. **Start Active lockng system. (DO NOT MOVE STAGE)**

F5



6. **Adjust the brightness of LED controller on air table.**
7. **Set "EM gain" in the camera panel as "0". Exposure time 30 ms = default. The fastest available is 17 ms per frame.**

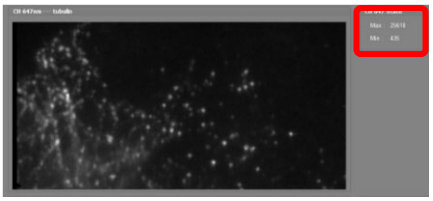
F8



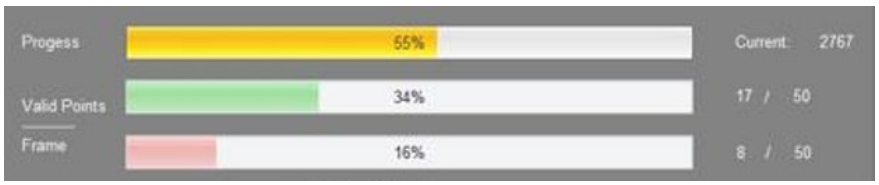
8. **Increase laser intensity stepwise to "SR" region. Some of the fluorescent dye is driven to dark state thus overall image feed appear as "blinking".**

9. **Increase "EM gain" in the camera panel stepwise with values from 5, 20, 50, 100. Please keep the "Max" maximum intensity value on the right side of live window below 50000 untis to protect the camera.**

F9



10. **Press "Start Capture" in "SR Capture" window to acquire image.**
11. **During image acquisition, the "Progress Panel" shows the following information:**

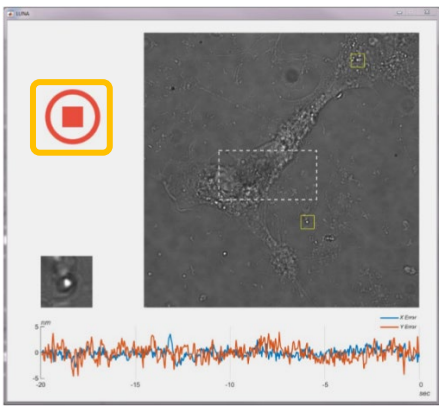


11.1. [**Progress**]: The progress of image acquisition, expressed as the percentage of images taken out of the total number of images to acquire.

11.2. [**Valid Points/ Frame**]: The count of valid points in the current frame (Green bar: Ch 647-nm; red bar: Ch 750nm). The initial count of valid points is also provided for reference regarding photo-bleaching effect.

12. **Once "Progress" bar reaches 100% the image acquisition is finished. Decrease laser intensity to "WF" range to prevent bleaching your sample.**
13. **Your files will be saved to:**
14. **Computer\Local Disk (E:)\Rohdea Workspace\(your PI's initials)\(Experiment name)\(Position #)\SRReport**
15. **Pause active locking in the "Lunar" window.**
16. **Close the SR Capture window.**
17. **If more images has to be taken, assign a new position number before moving the stage to find another cell.**

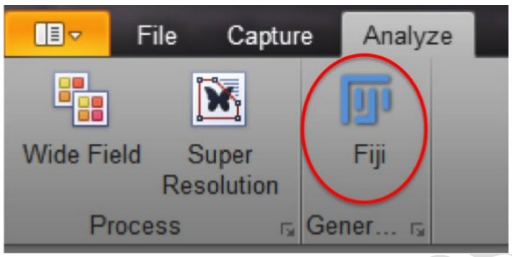
F14



G. Image Analysis

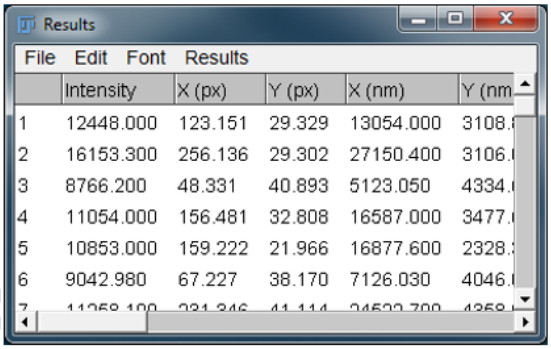
1. **Go to Analyze tab.**
2. **Select Fiji.**
3. **In File explorer, go to:**
4. **Computer\Local Disk (E:)\Rohdea Workspace\(your PI's initials)\(Experiment name)\(Position #)\SRReport**
5. **Find 647 or 750 .csv files and import into Fiji. Please process the files one by one.**

G2



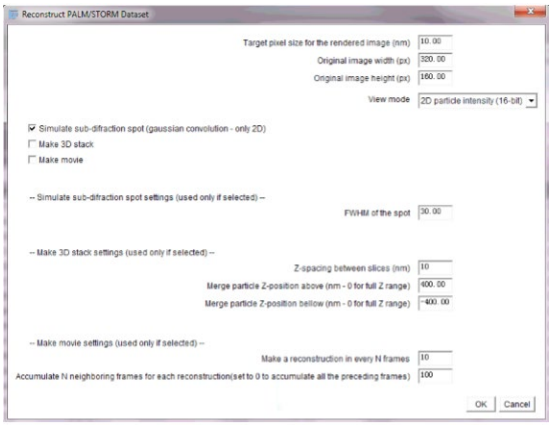
6. **You should be able to view imaging data in a new window.**
7. **Go to: Analyze → Quick PALM → Reconstruct Dataset.**

G5



8. **Select reconstruct parameter:**
   - 8.1. **[Original image width/ height (px)]**: Type in 320 x 160 camera pixels (ROI).
   - 8.2. **[View mode]**: Choose "2D particle intensity (16-bit) to build a 2D SR image.
   - 8.3. [FWHM of the spot]: 20 (nm)
   - 8.4. [Z-spacing between slices (nm)]: 30 (nm)
   - 8.5. [**Merge particle Z-position**]: "400, -400"; or "0, 0" for full range.

G7



H. SRiS System Power down

1. Turn off Lunar active locking system.
2. Close Rohdea V2.0 program.
3. Upload data to server according to instructions.
4. Turn OFF computer.
5. Turn laser emission key to “OFF”.
6. Switch OFF laser power.
7. Switch OFF laser power supply ③.
8. Turn off Nikon PFS.
9. Remove imaging chamber from microscope stage.
10. Clean microscope lens. (below is a brief summary from training. Note this is only applicable to Nikon systems not Carl Zeiss microscopes).
    **fold lens cleaning tissue into long strip; wipe first dry to remove most of the immersion oil by drawing the tissue across lens surface. 2nd wipe may use absolute ethanol to remove most immersion oil. Repeat wipes with absolute ethanol until there is no residue visually.
11. Lower lens to lowest Z-level by pressing escape button on right side panel.
12. Turn off LED light controller.
13. Switch OFF microscope and camera power supply ① and ②.
14. Replace curtain.