



## 1. Initial Set-up

- ~ Log in to NUCore and start a reservation.
- ~ Turn on power strip to turn on all components (Computer, Scanner power, Laser power, Laser key). **Note:** Do not turn off the laser power key by itself!!
- ~ Log into computer, if necessary (**password: user**).
- ~ Launch LAS software. Select Resonant Scanning, if applicable.
- ~ Ensure 10x objective is in place and say "OK" when dialog appears.
- ~ Navigate to Laser panel (**A**) and select "classic" or "express" view for your convenience.
- ~ Put slide on scope and set up image acquisition.



## 2. Mount sample on microscope

- ~ Place slide on stage 'coverslip down'.
- ~ For transmitted light imaging: Select BF button and press TL-Shutter on LED screen. Focus on a sample.
- ~ For fluorescent light imaging: Select FLUO, then filter cube from buttons on LED screen, open TL-Shutter.
- ~ Focus on sample and find cell/region of interest. Close shutter.

## 3. Confocal imaging

- ~ In software, navigate to "Acquire" tab (**B**).
- ~ To set Beam Path Settings, first activate the laser(s) needed by clicking "ON" and "+" (**C**). Set laser intensities at 0.5-2% to start.
- ~ Activate the HyD detector(s) or PMT(s) by selecting the "ON" button(s) (**D**). Choose a color for the detector display by clicking the color bar (**E**) and selecting a LUT from the window that opens.
- ~ To display emission peak to guide detector settings, choose from list of common fluorophores (**F**).



- ~ Place the detector bar **(G)** in correspondence with the fluorophore emission by sliding right or left and resizing. Repeat for each detector.
- ~ Click the “**Live**” Button to check settings. View image as intensity values by selecting the Glow (OAU) LUT with the QLUT button.
- ~ Adjust **Smart Gain** so that there are mostly orange and white pixels and minimal blue (saturated) pixels. If necessary, adjust laser intensity or detector bar positions.
- ~ Click “**Stop**” then click “**Capture Image**” to acquire a single image.



#### 4. Setting up a sequential scan.

- ~ If you are using the UV laser or have significant overlap between fluorophore emission, a **Sequential Scan** may be required. To get started, select the “Seq” button **(H)**.
- ~ In the Sequential Scan window, click the (+) button until you have the desired number of scans present. Choose “Between Lines” “...Frames” or “...Stacks.” “Between lines” can only be used when the same dichroic is used in all scans.
- ~ Set up the laser and detector bars for each scan as appropriate for each fluorophore, following directions from #3 above. Changes made in one “Scan” will not affect those made in others.
- ~ **Save settings** for use in the future, if desired. Click “**Save**” in the “Sequential Scan” window.

#### 5. Acquiring a Z-series

- ~ In the Acquisition tab **(B)**, be sure “xyz” is selected under Acquisition Mode **(I)**.
- ~ Click “Live”. Move to the top of your sample with the Z position knob in **Smart control bar** and set the position by clicking the “Begin” arrowhead **(J)**.  
**Note:** using focus knob on microscope or joystick will not adjust galvostage position for z-stack.
- ~ Move to the bottom of your sample with the Z position knob and set the position by clicking on the “End” **(J)** arrowhead. Click “Stop”.
- ~ Set the desired number/size of z steps.
- ~ Ensure imaging settings are correct then hit “Start” to collect z-stack.
- ~ The z-stack will be saved under the Open projects tab, as a Series00X file.

#### 6. Save images

- ~ At the bottom of the Experiment tab, click “**Save All**”. Select the appropriate directory and change the Experiment file name, if desired.
- ~ Click OK to save.

#### 7. Shut Down

- ~ Remove sample. Clean oil off objectives with lens paper. Return objective turret to 10x.
- ~ Transfer files to flash drive or network storage. Close software.
- ~ **If there is another user within a 2-hour time period, stop here, leaving microscope and computer on.** End reservation in NUCore. **If not, proceed with following steps for complete shutdown.**
- ~ Turn OFF power strip to turn off all components.
- ~ Cover microscope. **End reservation in NUCore.**