1. What we need to bring every time
    a. Animal
    b. Instruments and supplies
        i. Shaver
        ii. Tape
        iii. Betadine
        iv. Saline
        v. Sterile gloves
        vi. Sterile drape
        vii. 27g needle x2
        viii. 18g needle x2
        ix. 10cc syringe
        x. 5cc syringe
        xi. Scalpel blades -2
        xii. Adsons x2
        xiii. Tonsil/hemostat x2
        xiv. Freer
        xv. Sterile 2x2s
        xvi. Towel clamps x3
        xvii. Chucks x2
        xviii. Loupes
    c. Stimulator/control box for TDT…and anything for Ephis at LOCI
    d. Christy upstairs can order new O2 tanks if needed
2. LOCI has
    a. O2
    b. Iso
    c. Charcoal for iso
    d. Scale for weighing charcoal bins
3. Random set up notes
    a. Can take a photo through the eyepiece for a bright field image – especially because the camera is not perfectly aligned
    b. The body moves in xyz directions
    c. The stage moves in xy directions
    d. PMTs are damaged by light
        i. Be **extremely** cognizant of this throughout entire session
4. When you arrive / Setup
    a. Ultima user guide in a binder on the top shelf on your left after you enter
        i. Ok to keep surgery records and some supplies at LOCI
    b. Chiller for laser is always on
        i. Lights will be on (bottom left area of the room)
        ii. Never turn it off
    c. Dell laptop on top of laser on the left controls the laser
        i. Don't go in back corner of room bc laser is always on
        ii. Laser should always be aligned for us – if there is an issue contact someone
    d. Always image pollen grains as a test before and after imaging

i. Record info in the logbook which is to the right of the Dell laptop

e. Again, check to make sure chiller is on
f. Turn on laser using Dell laptop:
    i. Click "Insight" software
        1. Press and hold for 3s
        2. It will turn on and take a few minutes to warm up
    ii. Once the power button on computer turns orange, turn on the laser shutter
        1. Click "Main Shutter" (press and hold for 3s)
g. Turn on the master rack of electrons
    i. White on/off switch behind monitor/rack
    ii. Also turn on the desktop computer tower
h. Log into desktop computer
    i. "User"
    ii. password "nhojk4"
i. Double click "PrairieView Stage XY" on Desktop (the laser software)
j. While this software is warming up…
    i. Stage height adjusting – make sure to use the level to check
    ii. Metal bar to right of eyepiece is very important
        1. Push bar all the way IN for bright field
        2. Pull bar all the way to the RIGHT/OUT for laser scanning microscopy (LSM)
    iii. Then also adjust the wheel beneath the eyepieces
        1. Select "BF" or "LSM" depending on what you're doing. If the box is open/light is on you need to have it in BF
k. Filters (cubes)
    i. Little box to far left of scope
    ii. Dichroic A/B and ">495"
        1. The little boxes come stay in with magnets
    iii. Filters are stored in top drawer of side cabinet/bench
        1. 890 excitation
        2. 445 for 2nd harmonic generation
    iv. Rhodamine
        1. GFP or RFP
        2. "C" or "H"
    v. 2nd harmonic generation
        1. SHG 445/40
        2. "G" or "E"
    vi. Put the filter in with the mirror angling up to split the light up and to the left
        1. Again, clicks in with magnets
        2. Little notches align so that you know it's in correctly
l. Moving the microscope body
    i. Dial for moving up and down (z) on the left of body
        1. Has fine and coarse
    ii. Also can move stage in x and y as above (silver dials)

m. For imaging rats
    i. Use aluminum stand – more space and no central hole like you have with the slides
n. For attaching objectives
    i. See chart at back desk
    ii. Many have adapters
    iii. 20x is long and fat
    iv. 10x is a little smaller
        1. for the window study they used 4x and 10x
        2. can look at the LOCI website to see all the objectives
    v. can use adapters to increase the length
    vi. after you select an attach the appropriate adapters, slide the objective on and turn dial at right to secure

5. Getting started
    a. Pollen grain slide
        i. Pollen grains are used for checking whether everything works because they are autofluorescent in a wide range
        ii. Use the sign-in sheet next to the laptop to record the necessary info (this is done at the end of the day…see below)
            1. Record the PMT number for channel 1
    b. Bright field
        i. Again, push stick on right in and turn dial to BF
        ii. Use flashlight to help you focus using the eyepieces – really just need to use Z dial to go up and down
    c. Then to switch to LSM
        i. Turn dial back to LSM
        ii. Pull out bar on R
        iii. Close box securely
        iv. Turn light off
    d. Now you're ready to go to the computer software
    e. Turn PMT boxes ON (on/off switch on each PMT) – the lights are confusing bc the light will always be on…check the switch
        i. Top box controls channels 1 and 2
        ii. This always needs to be turned off every time you open the door to the scope/laser
    f. Open 2nd shutter
        i. "Pockel cell" at bottom left on computer software main screen
            1. Reflects %
            2. Start at 10 for pollen grains
                a. In general, start low
                b. Range 4-90 depending
            3. Note that higher mag needs less laser power
6. Using the imaging software
    a. Resolution
        i. 512x512 for live scanning

1. Higher resolution when you are doing a z-stack and recording/saving…it just takes longer

b. Dwell time
    i. How long the laser stays at each pixel
    ii. 4ms usually
    iii. thin tissue will burn faster and need decreased dwell time
    iv. better to start low and be gentle
    v. burns look like red spots that grow
        1. stop live scanning!
        2. move the sample
c. Optical zoom
    i. Usually you do want some optical zoom to avoid dark around the edges
    ii. Pollen grain: keep at 1
    iii. Samples usually 1.5 or 2
    iv. If you are at an optical zoom of 5 or more you need to go to a higher objective
d. Scan rotation
    i. Just keep it at 0
e. Middle column
    i. PMTs
        1. 4 are in the system
        2. go from 0-1200
        3. Pollen – start at 450
        4. Samples usually 500-700
f. Right column
    i. Objective lens
        1. Make sure to select the objective that you are using
    ii. "Live scan"
        1. click this after double checking everything
        2. In your window that pops up there's a colored bar – make sure it's on "range check"
        3. Ideally when you click live scan you see a few pixels of blue, red and mostly grayscale once you're in focus
        4. Increasing PMT will more likely give you more noisy background
        5. Increasing Pockel cells will be more likely to burn
        6. Opening the box will give you noisy background (like increasing the PMT)
        7. It's ok to leave it on live scan while you are adjusting
        8. But just watch out for laser burn or bleach
g. Record Images
    i. Set a save path using the "…" buttons

7. Cleanup/Pollen Grain
    a. Doing pollen grain at the end of the day
        i. turn the PMT down to where you only have a few pixels of red

1. you want mostly blue with grains in focus and a few dots of red
   ii. look using the laser
   iii. if you have a filter in it will cut out some of the laser
   iv. do this without a filter
      1. it will be ~450 to 550 gain range
      2. use channel 1 reading on pmt area on computer software and record it on clipboard

b. To take the stage apart
   i. use screwdriver top two screws on the device that holds the pollen grain slides
   ii. take off whole thing but leave the bars
   iii. remove spacers

c. Lens cleaning
   i. always handle base but don't pick up by the lid
   ii. clean with lens paper (drawer at left) and sparkle spray

8. Extra safety and learning points
   a. Remember to turn the PMT box off every time you're going to turn the lights on and open the laser box
   b. For imaging live tissue
      - pmts – for the nerve/animal we did 650 for each pmt
      - pockels at 18
      - suture did not burn, collagen wrap did not burn
   - 4x – has longer focal length so needs to be further away
      - need to have higher pockel cell aka higher laser energy compared to the 10x
   - 10x – needs to be closer to tissue – 1.5mm
9. 8/16/16 using the cadaver and Joey's holster
   a. 10x
      i. Nikon air
   b. 20x
      i. Need to fill the well with water
   c. Joey's device
      i. ?4 screws
      ii. more secure screws
      iii. shorter/smaller acrylic board for animal
   d. Corinne advice for trouble shooting
      i. First try playing with the PMT – that will give you noise but will not increase the laser power so won't burn your tissue
      ii. Check to make sure water is still there (when using 20x)
      iii.
10. Fiji – imaging a Z-stack all together
   a. Go to "Plugins"
      i. Bioformats
      ii. Bioformats importer
      iii. Find your metadata from the laser software
      iv. Use the xml file to open it (at the top)

v. Window that pops up – do 'composite color'
vi. Brings your z-stack to Fiji/ImageJ
vii. Image
    1. Stack
    2. Z-project
    3. Select 'max intensity'
    4. B&C
        a. Brightness and contrast
            i. Under image →adjust → brightness and color
            ii. Use left and right arrows to go between channel 1 and channel 2
        b. the histogram is heavily skewed to be saturated

b. Can save images in tif or jpg but jpg easier to work with on your computer