To start the instrument:
1) Make sure there is no slide present on the stage.
2) Fluorescence light source (if required): On the left of microscope, flip switch.
   - For the fluorescent bulb: Turn power on and wait 15 to 30 min for stability before use OR before to turning off again. If powered off too soon after ignition, the lifetime of the bulb decreases!
3) Microscope control box: Left side, near the back of the table, flip switch and allow the stage to calibrate prior to initializing the software before turning on any other unit.
4) Power up computer and monitor.
5) Laser: Turn the key to the on position. Key is located on the back of the laser box (sloping box connected to microscope); indicator light will be red above key. Allow laser to warm up for at least 5 minutes prior to use. Note: Laser will not power up if visor is off of microscope.

The software will default to LIVE mode in BRIGHTFIELD of the specimen.

You can select FREEZE to hold the field or FLUORESCENCE to use fluorescent settings (buttons on top of active window), or look at the collection cap, using COLLECTOR.

Remember that the cubes for light paths are manual. Position 1 is for brightfield, rotate for red, green, blue. A STOP shutter to block the fluorescent light path is located on the top right hand side of the microscope.

When viewing fluorescent mode, you can select the exposure time to increase/decrease the amount of time the camera will collect light from the specimen. Remember that low fluorescence will require longer exposures, but may cause a lag or delay in the camera refresh rate. To cut or save an image while in fluorescent mode, select the INTEGRAL option on the exposure time window

Once software is available, you can find the LASER CONTROL in a drop down menu under LASER.

2 adapters are available: 0.2 ml Eppendorf tubes and 0.5 Eppendorf tubes. Tubes should fit snugly into the proper adaptor, but should not need to be forced. Fill the holder with 4 adapters each time, even if you do not need 4 tubes – since the adapters are not mounted in, they may float or drift unless the collector device is filled with adapters when in use.
   - NOTE: for specimen observation, it is best to be in the NO CAP position because no tube will be in the light path – however, once you select a cap, you may need to adjust focus and/intensity when collecting imaging of cut area.

TO BEGIN:
To add or remove the specimen holder AND/OR the collector device use the UNLOAD button. Note that the collector should be in the NO CAP position (activated, shown by green dot) if you wish to remove it from the system.

Add specimen to the stage and collection caps. Remember that specimens on glass slides should be on the BOTTOM (close to the collection device), so that dissectates will drop into the caps after the laser cuts the membrane.
Focus and move to the desired location using the SmartMove controller knobs
- Top/bottom of the front part of the device allow you to move.
- Back cap is the focus knob.
- Buttons on the left side increase/decrease light intensity while in BRIGHTFIELD mode.
- Buttons on the right side select objectives.

Focused on your using your specimen using the desired level of magnification. Now, move away and find an area on the slide that you can sacrifice to calibrate the laser to the software’s cut lines.
1) Select CALIBRATE in the drop down under LASER.
2) Adjust your laser settings using the laser control panel. To begin, Aperture of 8-12 and default Intensity and Speed is a good place to start.

LASER SETTINGS
a) Aperture – the ‘size’ of the beam of laser, higher number indicate thicker laser lines.
b) Intensity – power of the laser, higher levels needed for thicker tissues and for the lower mag objectives.
c) Speed – speed of the cut, if set too fast, the laser may appear to ‘skip’ during the cut
d) Offset should be factory (40 for the 40X obj).
e) Bridge – only functional while in COMBINED MODE – causes a final laser ‘blast’ to cut through the dissectate (the ‘puff” at the end of the cut to knock it off and into the device). The higher the number, the larger the aperture used.

3) Click APPLY, or the settings are not actually altered.
4) Create the best cutting line for your purpose by testing and altering these parameters.
   - Move and Cut allows mouse control of the laser – this is a good way to test your laser settings.
   - Standard mode is the standard cutting protocol.
   - Standard/Combined Mode allows for the final Bridge blast.

5) Save your desired cutting conditions using the option in drop down under EDIT for SAVE APPLICATION CONFIGURATION

NOTE: 4x is NOT an accurate cutting objective. 10X will work, however 40X is your best objective for cutting. The 40X has a correction collar – adjust it to best focus (which will be different if using frame slides or glass slides. DO NOT unscrew the objective when adjusting the collar!

Use APPLY to activate change setting and allow the window to remain open. OK will close the window, but will apply the settings.

After cutting, check for the dissectate in the cap by using COLLECTOR option button. You can change the magnification. Once you find the dissectate, you can save the position/focal plane using OPTIONS ->SETTINGS->INSPECTION POSITION

If the laser does not fire after you select START CUT, make sure that the protective shield is in place and that the key is turned to the on position.
Leica AS LMD Shutdown Procedure:
1) Press “Unload” slice and remove any slides Click “Unload” collector and remove PCR tubes.
2) Close AS LMD Software.
3) Shut down computer.
4) Turn power off to:
   a. CTR MIC controller box
   b. Laser (turn key to off position)
   c. Fluorescence Power Supply (if utilized)
   d. Monitor

Slide Scanning
To create a slide scan use OPTIONS-> SPECIMEN OVERVIEW, then set the top left and bottom right corner of the area you are interested in seeing. Once the image is created, double click on box and move it to the area of interest.

Leica AS LMD Filter Sets
Position
1) Brightfield, no cube
2) Blue long-pass; BP355-425 / DM455 / LP470 (Leica Filter # D)
3) Green band-pass BP480/40 / DM405/ BP527/30 (Leica Filter # L5)
4) Red long-pass BP515-560 / DM580 / LP590 (Leica Filter # N2.1)

- 4x
- 10x
- 40x
- 337nm UV Laser

Notes:
- 40um is the max sample thickness.