

# Operation menu for the CLSM (Leica TCS SP2)

## 1. Turning on the system

- 1.1. Turn on the Mercury Lamp **first** (Fig 1.1), if you need fluorescent light, in most of cases, you do.
- 1.2. Turn on powers for LASER, SCANNER and PC (The 3 red buttons in Fig. 1.2).
- 1.2a. Turn on laser(s)—The 3 keys above the red buttons.
  - ➡ For the first one (Ar/ArKr) – You need to flip the key to ON, then to START and release- The yellow light should be on (Fig. 1.2)
  - ➡ For the other two (GreNe and HeNe), just flip the keys to ON. The yellow light should be on.
  - ➡ NO need to turn all the lasers on. ONLY turn on the laser(s) you need for your samples.
- 1.3. Turn on the microscope – It should be on once you turn the PC, if not, switch it ON (Fig. 1.3).
- 1.4. While wait for the computer to boot up, please logon the LOGbook on the desk.
- 1.5. Check the Objective, **MAKE SURE** that the objective is lower than the stage (Fig 1.4), then double click the Leica program icon on the computer desktop, to start the program.

## 2. Using the microscope

- 2.1. Changing objectives: Use the two black buttons on the left side of the microscope (Fig. 2.1-Objective switch) to change into different objectives.  
**CAUTION:** the 63X objective is a WATER IMMERSION objective; do not put oil on it.  
40X and 100X = oil immersion  
10X and 20X = dry

**You are NOT allowed to use your own immersion oil. If you have any problem with the oil provided, talk to Zhaojie Zhang at 766-3038**

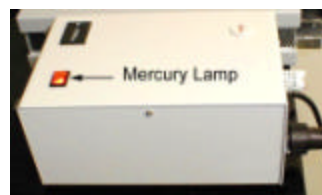


Fig. 1.1 Mercury lamp



Fig. 1.2 Power control



Fig. 1.3 Microscope, lower left



Fig. 1.4 Microscope, stage

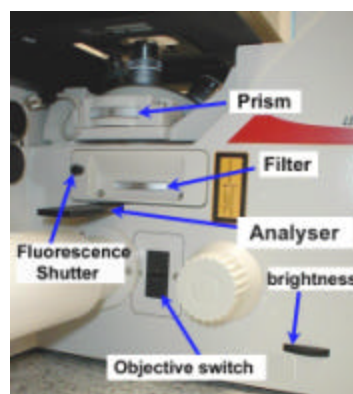


Fig. 2.1 Microscope, lower left

2.2. Putting your sample/slide on the stage – before putting your slide on the stage, make sure that the lens is all the way down (Fig. 2.2), by pressing the lower focus button (■)(Fig. 2.2). Press the upper focus button (■) will bring the objective to the up again.

2.3. Focusing your specimen – Try to focus your specimen first with transmitted light using the focus KNOB (Fig. 2.2). Press the two black focus BUTTONS (Fig. 2.2) simultaneously once to change from fine focus to coarse focus, press again will change it back from coarse to fine.

CAUTION: When high magnification objective is used (40X and up), use fine focus to prevent damaging the objective or your sample, or both.

2.4. Switching to fluorescent light (Mercury) – Once your sample is focused with transmitted light, you can switch to mercury lamp (fluorescent light).

(1). Select the correct filter (Fig 2.1). There are four filter settings as named 1,2,3,4.

- # 1 and # 4 – empty – used for scan mode
- # 2 – green excitation/red emission, e.g. TRITC
- # 3 – blue excitation/green emission, e.g. FIFC

(2). Switch off the transmitted light, by turning the brightness wheel (Fig. 2.1) away from you, till the lamp voltage display on the LCD showed as “0 V”).

(3). Pull the Fluorescent shutter (Fig. 2.1) all the way OUT.

### 3. Scanning an image

3.1. Push the fluorescent shutter (Fig 2.1) all the way IN.

This action will also prevent fading your sample.

3.2. Turn the filter (Fig. 2.1) to #1 or #4.

3.3. Turn the Vis/Scan Switch (Fig. 3.1) to SCAN.

3.4. Pull the Shutter for scanner (Fig 2.2) all the way out.

3.5. Scan an image using the confocal software.

### 4. Obtaining a DIC image for unstained specimen

4.1. Switch the prism on Figure 4.1 to “BF” position.

These positions on the prism are:

- BF—for Bright Field
- C—for 20X objective
- D—for DIC and 100X objective
- E—for 40X objective

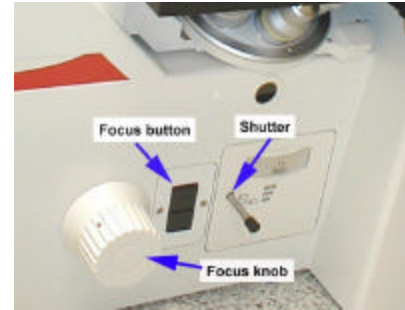


Fig. 2.2 Microscope, lower right

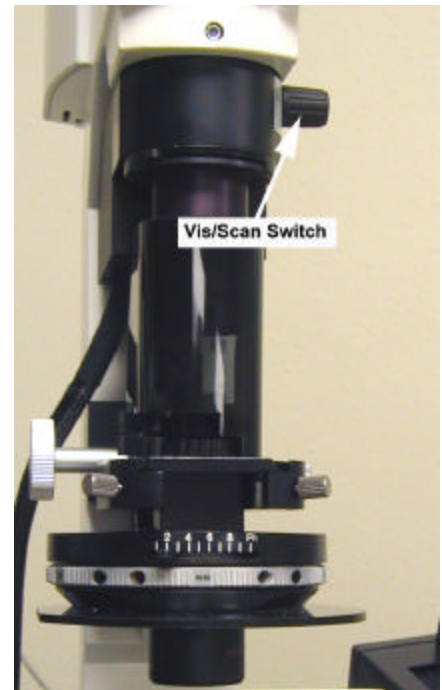


Fig. 3.1 Microscope, top

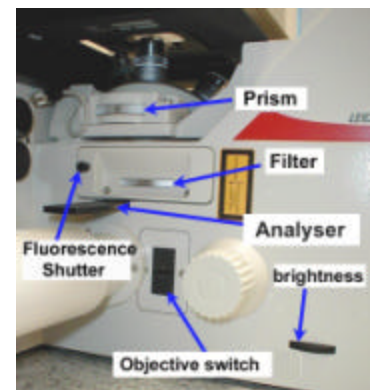


Fig. 4.1 Microscope, lower left

- 4.2. Insert the analyzer (Fig 4.1) in.
- 4.3. Insert the polarizer (Fig. 4.2)
- 4.4. Switch the prism in Figure 4.2 to a null position (a stop without any number on)
- 4.5. Slightly adjust (moving back and forth) the polarizer to get the darkest image (i.e. polarized to close 90 °).
- 4.6. Switch the prism on Figure 4.2 to a position that matches the objective you use.
- 4.6. Switch the prism on Figure 4.1 to “D” position and slightly moving back and forth the prism (as well as the two apertures on Figure 4.2) to get a nice DIC image.
- 4.8. Take the analyzer (Fig 4.1) out and you are ready to scan the DIC image with laser.

### 5. Shutting the system down

**You need to have at least 10 minutes to let the system (lasers, mercury and halogen lights) cool down.**

- 5.1. Turn off the laser(s) (turn the three keys on Figure 1.2 back to 9 o'clock).
- 5.2. Remove your sample from the microscope stage, then lower the objectives by pushing lower focus button (■)(Fig. 2.2).
- 5.3. Clean the oil or water on ALL OBJECTIVES. Be VERY careful and VERY gentle!! – This is the “eyes” of the microscope. Use lens cleaner if necessary.
- 5.4. If you want to work on the computer, you can do it now – Copying your images on CD, or transferring files through network....
- 5.5. Shut down the computer, then turn off the PC and SCANNER (the two red buttons on Figure 1.2)
- 5.6. Wait at least 5 minutes, from the time you turn off the lasers (the three keys on Fig 1.2), then turn of the power of the laser (the left, red button on Fig 1.2).
- 5.7. Turn off the Mercury lamp.
- 5.8. Sign the laser time on the logbook.
- 5.9. Put the cover on the microscope, WHEN LAMPS (TRANSMITTED AND MERCURY) ARE COOL.

If you are in hurry, you can either leave the microscope

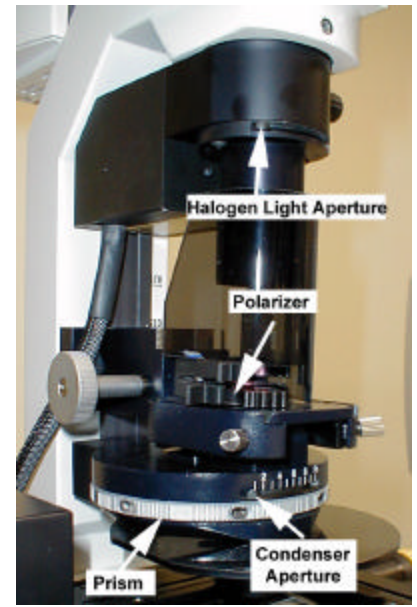


Fig. 4.2 Microscope, top

un-covered or put the cover on but avoid touching the lamps with the cover. A hot lamp house may burn the cover

5.10. Check if the bottles of the immersion oil, lens cleaner etc. are covered

9.1. Finally, you can go home!!!

**Golden rule #1**

**If you do not know how to fix it, do not break it.**

**Golden rule #2**

**If you do not know how to do it, ASK someone who knows.**