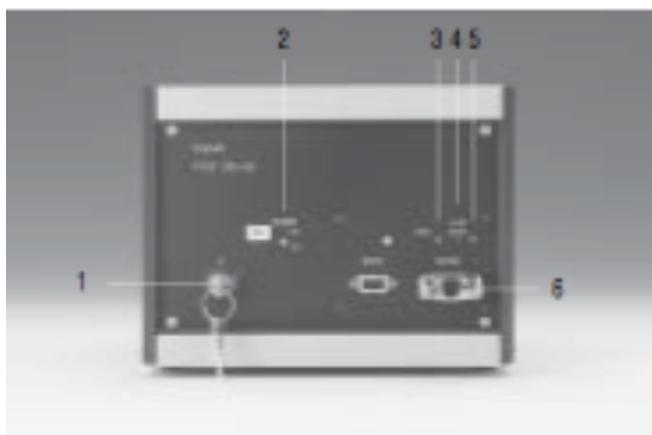


# USING LEICA AS LASER MICRODISSECTION (LMD6000) MICROSCOPE

*Written By Jungim Hur*



Front side of the laser box

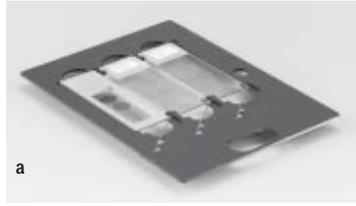
1. Key-operated switch
2. Switch for external trigger
3. Status LED: Operating status of laser
4. Push-button: Laser on/off
5. Status LED: Laser active/inactive
6. Service plug



LEICA CTR6500 electronics box  
1. Mains switch  
2. Pilot indicator

## Leica LMD6000 Operation Protocol

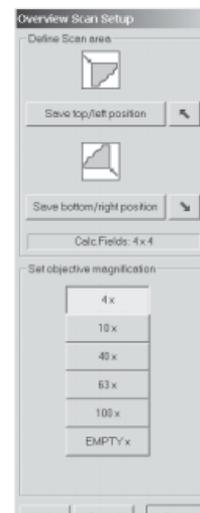
- 1) **Clean** the LMD station including slide holder, PCR tube tray sub-stage holder, the 3-D Satellite controls and keyboard with Kimwipes damped with **DEPC treated H<sub>2</sub>O** (500  $\mu$ l DEPC solution in 500 ml H<sub>2</sub>O).



- 2) Turn on power to:
  - a. **CTR-MIC control box** (microscope will go through self diagnostic tests, stage will move; requires about 30 seconds).
  - b. CPU/monitor the usual way by the on button on the computer.
  - c. **Laser** (with key). During warm-up, the laser power box will display **one green light (left)**. When the **second green light (right)** comes on, **the laser is ready**. Touch the red button between the two green lights on the laser power supply to activate the laser. Now there will be **one green light (left)** and **one red light (right)**. The laser is ready to fire. Requires about 5 minutes. **If you want to take a break more than 5 minute**, then press **red button** and the laser box will display two green lights. **It will be in the “stand-by” mode and will be re-activated by pressing the red button between the two green lights.**
- 3) Allow the microscope to fully initialize the stage before starting the LMD application software.
- 4) Check to be sure **the Laser Safety Shield** is properly in place. **Laser will not fire without the laser safety shield in place.**
- 5) Activate the LMD software from the Desktop by double clicking on the LMD icon. Click on the **“LOAD” slide (left) button** in the software menu. Slide holder will move forward; pull the slide holder forward and out of the microscope stage. Insert the special foil membrane slides with mounted tissue sections into the slide holder (be sure to have the proper orientation of **the specimen facing down in the microscope**). Return the slide holder back into the microscope stage by sliding the holder along the slide rails until it snaps into place. Click the **“Continue”** button on the dialog box to take the slide holder into the microscope.



Create **Specimen Overview** images by clicking “Options/Specimen Overview”. Use lower magnification (4X-10X) and define scanning area. Then, save scanned overview image.

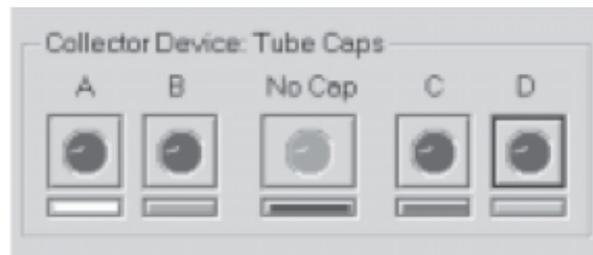


- 6) Click the “**Unload**” **Collector (right) button** in the menu bar to unload the collection device (moves forward), Load PCR tubes with caps into the collection device.



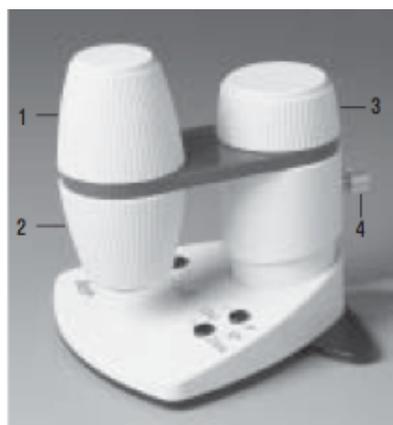
Inserting the cap into the holder  
1. Lid of the cap  
2. Holder

For **RNA isolation** from LCM samples, place **Extraction buffer** (20-30  $\mu$ l) from PicoPure RNA Isolation kit into the caps (**Avoid bubbles** when you place buffer into the caps). Return the collector to the sub-stage holder and click OK to return the collector into the sub-stage of the microscope. **Select a cap** to collect samples by clicking the corresponding circular red marking at the bottom left of the work window. The selected cap turns **green**.



**While collecting the cell types that you are interested, the buffer added into the tube cap might be dried.** You may add **10  $\mu$ l of H<sub>2</sub>O** into the tube cap **after 1-1.5 hour of cutting** if the light is getting dimmer because the buffer is dried and **should not collect samples more than 2.5 hours** into sample cap.

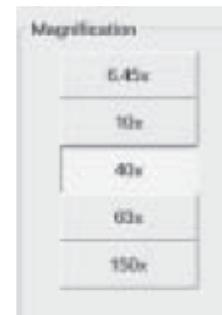
- 7) **The 3-D Satellite controls** all basic remote functions of the microscope.
- These include motorized X and Y stage movements and Z microscope focus functions.
  - The buttons on the right side switch between coarse or fine focus and stage motion.
  - The buttons on the left side control the up and down magnification by changing the objectives on the nosepiece, one position at a time.



**Smartmove control**

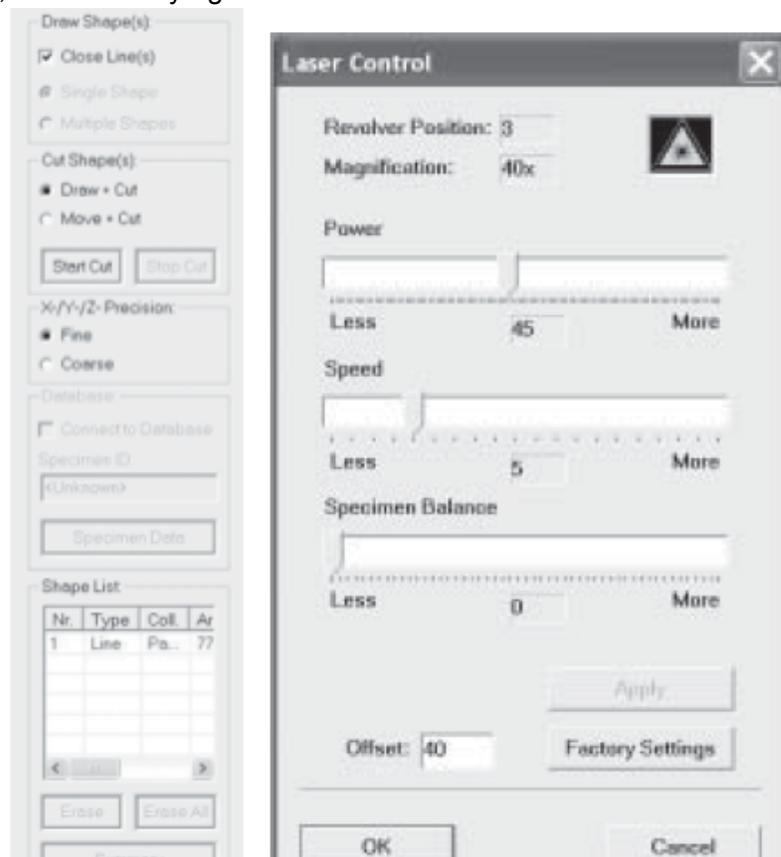
1. Movement in x direction
2. Movement in y direction
3. Setting the focus
4. Individual knob height position

- 8) **Focus the specimen** with a low power objective and **adjust for proper illumination levels** and binocular phototube prism setting for an on screen image as necessary. Move the stage to bring the area or cells of interest into the center of the field of view. **Switch to a higher power objective** by touching your selection on the front touch screen of the microscope, **for better detail and identification of the cells of interest for cutting**, and adjust **focus** and **light level** if necessary.



**If obtained sample image is not focused or it is hard to define the cell type that you are interested, you may align condenser adjust.** To align condenser, close the field diaphragm all the way (control the field diaphragm on the microscope control dialogue window) and **rotate the knob on the condenser** until the circle of light is **crisp and in focus**. Then open up closed field diaphragm and adjust the aperture diaphragm. Refer to Condenser Alignment Protocol provided by Leica microsystem.

- 9) **Perform Laser calibration** on set-up before cutting the region of interest.
- 10) **Test different laser settings (power and speed)** to obtain optimized condition for **the cell types that you are interested. Then, record the setting and proceed.** On the right side of the software interface, select the **“Draw and Cut” mode**. **Use the optional touch screen pen to draw the region of interest (ROI) directly on the monitor or** move the mouse cursor to the image area, and click and hold the left mouse button to draw a line or shape on the monitor around the cell or cells of interest. **If the drawing is not desirable**, remove the line from the shape list. **Click on the “Start Cut” button** in the software, or alternately right click and select “Cut”.



**It is recommended to save the image with drawn the region of interest before and after cut.**

- 11) **If the laser does not cut properly through the specimen**, optimize laser settings in “Laser Control” **by adjusting “Power”, and/or “Speed”**. **Sometimes, laser loses power after few hours of use**, then **increase laser power and re-draw the line and cut it again**. (Refer to suggested laser settings for a “normal” histological tissue sections by clicking “Factory Settings” in the Laser Control window. Also, you can refer to the settings previously used to cut the cells of interest.)
- 12) Cutting options include: free hand figures with close line option, circle and rectangle figures.



- 13) Cutting options also include: “Draw and Cut” or “Move and Cut”. If region of interest is not released after cut, re-draw the line and cut again or **increase laser power** and **click “Move and Cut” button to release cut**.

You can inspect the samples in the cap of collection tube by pressing the "Collector" key in the toolbar. Pressing the "Specimen" key restores the previous cutting position.



- 14) The “Close Line” feature can simply be checked on or off, but area measurements are only functional with the Close Line activated.
- 15) Laser Alignment and laser controls settings are specific to each objective and can be saved under “File” and “Save Configuration File As.” By naming this file and saving it to the folder “Profiles,” one is able to recall all laser settings saved during a cutting session.
- 16) To recall a user specific configuration files, go to “File” and “Restore Configuration File,” find and activate your specific file.
- 17) **When you finish collecting samples**, take out the tube from the collection device by unloading collector. **The samples on the tube cap should be collected into the tube by brief spin and stored at -80°C until use**. Also, **unload slide** by clicking on the **“LOAD” slide (left) button** in the software menu. Pull the slide holder out of the microscope stage and remove slide and store in the slide box.



- 18) Make sure to record the laser setting and the information of **the cell types** you have captured, **the objective** used to capture, **the volume of extraction buffer** and **H<sub>2</sub>O**, **the collection tube name** and **the date** of capture.
- 19) Close LMD Software
- 20) Shut down Windows (computer)
- 21) Turn power off to:
  - a. CTR MIC controller box
  - b. Laser (turn key to off position)
  - c. Fluorescence Power Supply (if utilized during your session)
  - d. Monitor

#### **Adjustment of CORR objective settings for the L20x, L40x and L63x objective**

- 1) Depending on whether you use the Glass PEN foil slide or Metal PET Frame foil slides or Metal POL Frame foil slides, one must properly set the correction collar of all CORR objectives in order to achieve the best image possible. The wrong correction collar setting will result in a poor or hazy image and a sub-optimal laser cutting. The correction collar settings are dramatically different between the glass PEN foil slides and the metal frame foil slides. Using the settings of one for the other type of slide will not give optimal imaging results.
- 2) For **Glass PEN foil slides**, the correction collar setting should be approximately **0.9** while for any **Metal frame foil slide**, the correction collar setting should be approximately **0.1**. **However, proper setting should be obtained by observing the image and turning the correction collar until optimum contrast and clarity in the image is achieved.**

**NOTE: Refer to Leica LMD6000 V6.3.1. Quick (Basic Operation) Start Up and comprehensive online “Help” files from the LMD software or consult the LMD printed instruction manual provided with the system.**