

Leica Laser Microdissection Scope (LMD 6500) Manual

Purpose: LMD is a UV-laser based microdissection system combines with an automated upright microscope. LMD uses laser ablation technology to cut out single cells or cell clusters directed along the contours of the area of interest. The area cut by the laser are transferred to PCR test tubes by gravity alone without contamination.

1. Startup procedure

Wear powder free nitrile gloves while using the LMD 6500.

- 1.1 Turn on microscope controller box then turn on computer.
- 1.2 Logon to computer by clicking the user icon, type in “user” as the password.
- 1.3 Click the desktop “Leica Laser Microdissection” icon to start the software.
- 1.4 If you plan to use fluorescence during your laser microdissection session, then turn on the fluorescence illuminator/power supply white box on the right of microscope.
- 1.5 Turn the key to the “On” position to start up the laser only when you are about ready to cut the sample. The Laser Power Supply (Silver Gray box labeled “CryLaS”) needs time to warm up from a cold start. After the power supply is turned on, a single orange LED light will be on. This shows that the laser is in the “warm up mode”. After a few minutes, you will see the two green LED lights when laser is in the “Standby Mode”. Make sure the Laser Safety Shield is properly in place.

2. Calibrate the Reference Point (RP) for the PCR tube holder tray (first time you use the unit).

Purpose: To align the collection holder with the stage. You should set the Reference Point.

- 2.1 Clicking the left “Unload” icon (controls specimen holder). The “Change Specimen” window will appear. Remove the specimen holder and insert the slide into the specimen holder. Do not put back into the microscope at this point. Put your specimen to the side. Click “Continue”.
- 2.2 Clicking the right “Unload” icon, the “Change Collector Device” window will appear. Place the collection holder on the stage and make sure it’s properly going straight back. Click on the “Move to Reference Point” button, in the “change Collector Device” window.
- 2.3 The microscope should automatically switch to 5x and the collection holder should move to the reference point (RP). The RP should be at the center of the field of view. You may need to focus the camera to clearly view the RP. If it is not centered, use the arrows in the window to move the RP to the center of your field view.
- 2.4 Once the RP has properly positioned, click “OK” to save the settings.
- 2.5 Make sure “No Cap” is selected under Collector holder: Tube Caps.

3. Loading specimen slide

- 3.1 To put a slide on the stage or exchange slides, press the left “Unload” button in the toolbar. The stage then move to a lower position for slide change. Withdraw specimen holder by pulling towards yourself.
- 3.2 [Optional] Remove the laser safety shield.
- 3.3 Slide is oriented with the tissue section facing downwards, tissue sections are below the PEN membrane foil. Make sure you put the slide label end to the right side (closer to the holding pin).
- 3.4 Insert the slide onto specimen holder, then slide the holder back into position on the stage.
- 3.5 Click “Continue”.
- 3.6 Replace the laser safety shield (2.2) – Laser will not fire unless the safety shield is correctly in place.

4. Loading specimen collector

- 4.1 Click on the right "Unload" button in the software toolbar, push the collector holder out.
- 4.2 Place PCR tube ("0.2" or "0.5" mL) into each lever (4 levers total) with caps facing upwards, there is only one way to insert it. Put 4 levers onto collector holder.
- 4.3 Click 'OK' in the loading window after push collector holder in.
- 4.4 The center position is kept empty for viewing the sample.

5 Setting laser parameters (optional)

- 5.1 To change laser setting, select the "Control" option in the "Laser" menu, or click the "Laser" icon.
- 5.2 **Power:** adjust the power of the laser to the cutting beam. Reduce the power for lower speed to give a finer cut. It's useful at higher magnification.
- 5.3 **Aperture:** adjust thickness of the laser (lower number = thin cut, higher number = thicker cut)
- 5.4 **Speed:** how quickly the line is cut
- 5.5 **Specimen Balance:** changes the current settings to provide more or less cutting. These values varies with selected objective lens. Value should be between 5 and 20.
- 5.6 **Factory Settings:** Returns the settings to the factory defaults for the selected objective lens.
- 5.7 **Offset:** focus offset for the lens, set at the factory for each lens.

6 Laser calibration (optional)

Purpose: To ensure the figure drawn by the mouse on the computer monitor is identical to the shape cut by the laser.

- 6.1 Open the "Laser" and "MicCtrl" windows. You can use these to calibrate, adjust laser settings, change objectives and adjust illumination.
- 6.2 Navigate using the joystick (y-axis is on the top, x-axis is on the bottom).
- 6.3 Switch to the microscope objective of your choice (5x, 10x, or 40x) using left buttons on the joystick, the touch pad (Objective icon), or the "MicCtrl" icon in the software.
- 6.4 Find and focus on an empty area of a slide using the joystick.
- 6.5 On the right hand panel of the software "draw shape(s)" choose "Single Shapes" mode, Select the "Calibrate" option in the "Laser" menu or using "Laser" icon and confirm message on the screen with "Yes."
- 6.6 The laser cuts a "cross" at each of the four corners of the current view. Follow the pop-up instruction; move the mouse cursor to the center of each cross and confirm by clicking on it. Select the "Start cut" button and to see if laser follows your drawing precisely.
- 6.7 Conclude calibration procedure by clicking "OK" in the dialog box.
- 6.8 To validate laser setting, make sure the "Close Line" option is not selected and draw S-shaped line using "Line" button in the toolbar.

7 Obtaining an overview of the specimen

- 7.1 Bring your specimen into focus with a low magnification objective.
- 7.2 Select Option > Specimen Overview from menu bar.
- 7.3 A window will open, select "Create Specimen Overview".
- 7.4 Position the upper left corner of the specimen in the eyepieces, click "Save top/left position".
- 7.5 Position the lower right corner of the specimen in the eyepieces, click "Save bottom/right position".
- 7.6 Press "Scan" at the bottom of setup window, the system starts to create a slide overview.

- 7.7 Once a sample has been scanned, you can activate the yellow box by double clicking on it and change the magnification. The activated box will then turn green and you have the ability to move.
- 7.8 If you click "Save Overview Image", the montage will be saved.

8 Cutting and collecting area of interest (Be careful not to bump the table while the laser is cutting)

- 8.1 You can use the specimen overview option to navigate to specific areas of your sample, detail See 7. **Obtaining an Overview of the Specimen.**
- 8.2 Locate your area of interest. Select a cap (A, B, C, D) by clicking the corresponding circular red mark at the **Collector Device: Tube Caps window**. The selected cap turns green. You can change the names of the caps by clicking on the letter. You can also assign each cap its own corresponding color by selecting the color field just below the cap icon to change the color.
- 8.3 Draw a shape around target area by selecting "Line", "Ellipse", or "Rect" icon in the left toolbar.
- 8.4 If you are using the "Line" tool, the "Close line" option can be activated if the drawn contour needs to be automatically closed. The drawing line is closed upon release of the mouse key.
- 8.5 Select "Draw + Cut" cutting mode and cut specimen by pressing the "Start Cut" button. The 2nd green light will become "orange" when the laser starts cutting.
- 8.6 You can choose "Move and Cut" to cut specimen manually, using mouse. If you select "Move and Cut", always go back to "Draw and Cut" when you are done.
- 8.7 The most common dissection mode: Draw+cut (select it in the cut shape(s) manual). While in "Single Shapes" mode, you can draw a new shape and the previous shape will be automatically erased. While in "Multiple Shapes" mode, every time a shape is created, it's saved in the Shape list. These shapes will be recut whenever "Start Cut" is selected.
- 8.8 The shapes and the shape list data can be saved by selecting "Save Shape list Data" in the "File" menu.
- 8.9 You can save image by selecting "Save Image As" in the "File" menu.

9 View the cut specimen in the collector cup

- 9.1 Click the "Collector" button in the toolbar
- 9.2 The stage will move and the collection cup will come into view.
- 9.3 Use the joystick to navigate and find the tissue that was collected in the cap.
- 9.4 Return to the specimen image of previous cutting position by pressing "Specimen" on the toolbar.

10. Shut down procedure

- 10.1 Click the left "Unload" button. Carefully remove the specimen tray and remove your slide. Replace the specimen tray and select "Continue".
- 10.2 Click the right "Unload" button. Carefully remove the collector holder and the PCR Tubes. Replace the holder and choose "Continue".
- 10.3 Select the 5x lens in microscope control
- 10.4 Quit software by "File -> Exit"
- 10.5 Turn off Laser by turning key switch to vertical position.
- 10.6 Shut down computer
- 10.7 Turn off fluorescence power, if fluorescence is used.
- 10.8 Turn off microscope controller box.