Laser Capture Microdissection using Leica AS LMD (Leica Microsystems, Germany)

The Leica LMD is a UV-laser based microdissection system developed in 2001 which combines automated upright microscope architecture, three-dimensional optical control of the dissecting laser beam and the dissected area, non-contact tissue sampling and motorized post-dissection handling. Leica LMD based on technology of laser ablation to cut out single cells or cell clusters with a focused pulsed UV laser beam (class 1 nitrogen laser) directed along the contours of the area of interest. The areas cut by the laser are transferred to PCR test tubes by gravity alone, i.e. without any mechanical contact and without the application of additional physical forces. This technology guarantees extremely gentle specimen handling.

1. Starting the Microscope and Software

- Log in and turn on the electronic controller box.

**Note:** Use your CNet ID to log in. If you do not know your CNet ID, go to this website [http://nsit.uchicago.edu/services/cnetid](http://nsit.uchicago.edu/services/cnetid).

- Wait for the initialization of the microscope, the collection device and the laser indent light axis to finish.
- Insert the slide (Leica Membrane Slide) into the specimen holder with section face down.
- Insert 0.5 ml microcentrifuge tube caps into collection device.
- Fill tube caps with 75 μL of lysis buffer.
- Start the software by double-clicking the “Leica Laser Microdissection” icon on the desktop.
- Turn on the laser by turning key switch into a vertical position.

2. Calibrating and Setting Laser Parameters

Calibration of the laser is necessary for the conversion of the coordinates of the mouse cursor into the coordinates of the laser beam. The aim is to ensure that the figure drawn by the mouse on the screen is identical to the shape cut by the laser.

**Note:** The laser calibration may be repeated at any time and is performed and stored for each objective separately.

- Make sure the microscope is in “Brightfield” mode, located at the top of the screen.
- Switch to the cutting objective of your choice (4x, 6.3x, 10x, 20x, or 40x) using right buttons on the joystick and move relevant tissue detail into the field of view.
- Focus on an empty area of a slide using joystick.
• Adjust the microscope white light level using left buttons on the joystick (if necessary).

• Select the “Calibrate” option in the “Laser” menu and confirm message on the screen with “Yes”.
• The laser cuts a cross in the corner of the specimen. Move the mouse cursor to the center of the cross and confirm by clicking on it.

• Repeat this process for the other two crosses.

• Conclude calibration procedure by clicking “OK” in the dialog box.

• To validate the calibration, deselect the “Close Line” option and draw S-shaped line using “Line” key in the toolbar and select the “Start cut” key to ensure that laser precisely follows drawing. If laser is slightly misaligned re-calibrate laser.

Note: To highlight the Draw Shape(s)/Cut Shape(s) options in the right column, choose the “Select” key.

[Optional]: To soften the image, select any cap. The cap softens the sharp image (due to the absence of a cover slip).

• Check laser settings by drawing a straight line using “Line” and select “Start cut”.

• To adjust the laser beam settings, select the “Control” option in the “Laser” menu. Start from “Factory settings”. If factory setting is sub-optimal change parameters to achieve effective laser cutting. The power/intensity, and speed parameters can be changed here. Click “Apply” and close window by pressing “OK”.

Note: Reduce the power for lower speeds to give a finer cut. This is useful for higher magnifications since the cutting distance is shorter.

• Adjust the “Offset” for thicker samples. This adjusts the integrated offset lens which moves the laser focus into the image plane. The pre-set values are optimized for each objective.

• For fluorescence, skip to step 4.

3. Cutting and Collecting Areas of Interest

• Select a cap (A, B, C, or D) by clicking the corresponding circular red marking at the cap placement window. The selected cap turns green.

• Draw a shape around target area by pressing “Line” key in the left toolbar.

• 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.

• In “Single shape” mode, if your drawing result is not a satisfactory, you can draw a new shape and the previous shape will be automatically erased. To draw multiple shapes, select the “Multiple shapes” mode. To delete shapes in “Multiple shapes” mode, choose the region (i.e. 1, 2, 3 etc.) under the “Shape list” and select Delete on the keyboard.

Note: Multiple lines can be drawn to corresponding caps A, B, C, and D. Select all necessary areas, then “cut” the specimen. The samples will fall into the proper cap, chosen by the investigator. Use the color option located below each cap to designate tissue type with a cap.

• Select “Draw and Cut” cutting mode and cut specimen by pressing the “Start cut” key.

Note: Select “Move and Cut” to cut specimen manually, using the mouse. If you use “Move and Cut”, always go back to “Draw and Cut” when you are done. The software limits your options when “Move and Cut” is selected.
- **Inspect results** by pressing the “Collector” key in the menu toolbar. Change the objective to 10x and focus the image and inspect the result of the laser microdissection.

  **Note:** If you are unable to see your results, go to the “options” menu in the toolbar and select “settings”. Choose the ‘Inspection position’ tab and click on Learn Inspection Settings. Select “Ok”.

- Choose the 10x objective and focus the image so you can see the edge of the cap clearly
- Use the joystick to navigate and find the tissue that was collected
- Return to the specimen image of previous cutting position by pressing the “Specimen” key.

  **Note:** The current image can be saved at any time by pressing “Freeze” key followed by “Save as” key in the toolbar. Please save all images on a flash drive or CD.

![Before.jpg](image1)  ![During.jpg](image2)  ![After.jpg](image3)

4. **Fluorescence**

- The mercury lamp needs 30 minutes to warm up. Let the lamp run for at least 60 min before turning it off.
- After calibrating, select “Fluo” mode and choose a filter. Position 2 is an FITC filter (red), position 3 is a Rhodamine filter (green), and position 4 is a yellow filter.
- While in “Live” mode, choose an exposure time (start with ¼ or ½).
- Find the area of interest and draw around it. Before cutting select “Freeze” mode. This closes the shutter, blocking the UV laser. The selected area will be cut and fall into the corresponding cap.

  **Note:** You will not see the laser cutting the tissue because the program is in “freeze” mode.

5. **End of Session, Shutdown**

- Close Leica software.
- Turn off the laser by turning the key switch to horizontal position.
- **Turn off the electronic controller box.**
- Log off computer (DO NOT SHUT DOWN!)
- Remove consumables and **cover microscope**.

For specific questions regarding RNA isolation/extraction, please contact the Functional Genomics Core Facility [http://fgf.uchicago.edu/](http://fgf.uchicago.edu/).

For specific questions regarding protein isolation/extraction, please contact the Proteomics Core Facility [http://proteomics.bsd.uchicago.edu/contacts.php](http://proteomics.bsd.uchicago.edu/contacts.php)

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For questions please contact: Lei-Ann Arceneaux, larceneaux@bsd.uchicago.edu
[http://pathcore.bsd.uchicago.edu](http://pathcore.bsd.uchicago.edu)
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