THE UNIVERSITY OF CHICAGO
HUMAN TISSUE RESOURCE CENTER

Laser Capture Microdissection using Leica LMD 6500

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 is based on the 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

- Wear nitrile gloves while using the LMD.
- Logon to the computer.

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.

- **REMOVE COLLECTION DEVICE PRIOR TO TURNING ON MICROSCOPE**.
- Turn on the controller, and wait for the initialization of the microscope (stage, illumination, and collection device). Turn the key to the "On" position to start up the laser.

Note: *The Laser will need approximately 10 minutes to warm up.*

- Open the software and wait for the initialization.
- **REPLACE COLLECTION DEVICE**.

Note: *Make sure the collection device is placed in properly going straight back.*

2. Calibrating Collection Device and Preparation of Caps

Note: *Calibration of the collection device should be done every time you use the LMD.*

- Remove the slide tray (aka the specimen holder) by clicking on the ***first*** "Unload" icon. The "Change Specimen" window will appear. Select "Continue".
  - The ***first*** "Unload" icon controls the specimen holder. Use this whenever you want to remove or replace the slide you're working with.
- Insert the slide (***Leica*** Membrane Slide) into the specimen holder with the section face down with the label pointing to the right. Do not put back into the microscope at this point. Put your specimen to the side.

- Remove the collection device by clicking on the ***second*** "Unload" icon. The "Change Collector Device" window will appear. DO **NOT** CLOSE "Change Collector Device" WINDOW.
    - The ***second*** "Unload" icon controls the collection device. Use this whenever you want to remove or replace the PCR tubes.

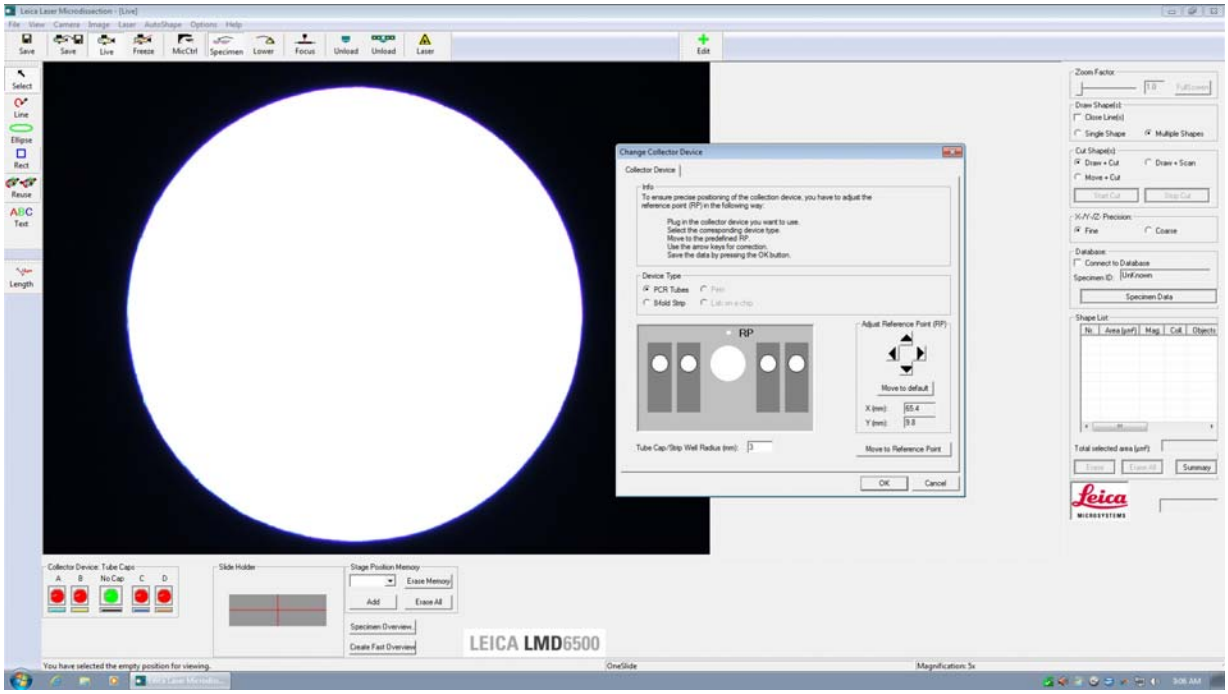
- Carefully remove the collection device and insert the cap/s of a 0.5 mL PCR tube into the collection device.

- Fill tube caps with lysis buffer.

- Insert the collection device back into the microscope.

- Click on the "Move to Reference Point" button, in the "Change Collector Device" window.

- The microscope should automatically switch to 5x (if it does not, manually change) and the collection device should move to the reference point (RP). The RP should be in the center of the field of view. You may need to focus the camera to clearly view the RP. If it does not, use the arrows in the window to move the RP to the center of your field of view.



- Once the RP has properly positioned, click "OK" to save the settings.

- Insert specimen tray into microscope. Remember to use the ***first*** "Unload" icon to insert the specimen try into the microscope. Make sure that "No Cap" is selected under Collector Device: Tube Caps.

3. 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.*

- Open the Laser and MicCtrl windows. You can use these to calibrate, adjust laser settings, change objectives, and adjust illumination.

- Navigate using the joystick (y-axis is on the top, x-axis is on the bottom).

- Switch to the cutting objective of your choice (5x, 10x, 20x, 40x, or 63x) using left buttons on the joystick, the touch pad (Objective icon), or the MicCtrl icon in the software.

- Find and focus on an empty area of a slide using the joystick.

- To correct for white balance, select "Camera Setup Window" in the "Camera" toolbar menu. Make sure you're focused on a clear glass area. Select the "White Balance" button. "Close" when finished.

***Note**: You can use the touch pad to adjust illumination (Microscope icon) intensity; however, this may not be necessary.*

- Select the "Calibrate" option in the "Laser" menu and confirm message on the screen with "Yes."
    - *You can also access the "Calibrate" option using the "Laser" icon.*

- The laser cuts a cross in all 4 corners of the current view. Move the mouse cursor to the center of each cross and confirm by clicking on it. **Please take the time to make sure you are accurately calibrating the system.**

- Conclude calibration procedure by clicking "OK" in the dialog box.

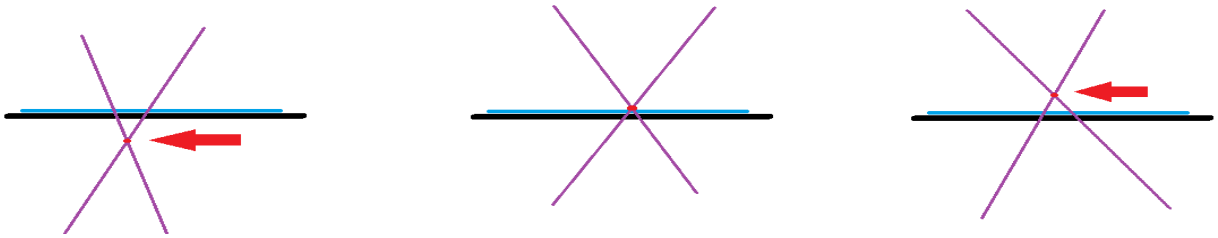
***Note**: During calibration and optimization of laser settings, it may be beneficial to be in the "Single Shapes" mode. While in "Single Shapes" mode, you can draw a new shape and the previous shape will be automatically erased. While in "Multiple Shapes" mode, every time a shape is created, it is saved in the Shape list. These shapes will be recut whenever "Start Cut" is selected. To save time, either select "Single Shapes" mode or delete unwanted shapes as you go along.*

- To validate the calibration, make sure the "Close Line" option is not selected and draw S-shaped line using "Line" key in the toolbar. Select the "Start cut" key and ensure that laser precisely follows your drawing. When prompted, select "No Cap." This is telling the computer where (what cap) you want the tissue to fall into. If laser is slightly misaligned re-calibrate laser. Perform this step in a blank area of the slide or an area of tissue you will **NOT** be dissecting.

- To check laser settings, draw a straight line using "Line" and select "Start cut." When prompted, select "No Cap." You will want to test this on an area of tissue you will **NOT** be dissecting.

- If the laser's default setting is sub-optimal, you can change parameters to achieve effective laser cutting. To adjust the laser beam settings, select the "Control" option in the "Laser" menu. Alternately, click the "Laser" icon.

    - **Power** adjusts the power of the laser.
    - **Aperture** adjusts the thickness of the laser (lower number = thin cut, higher number = thicker cut).
    - **Speed** adjusts how quickly the laser cuts.
    - **Specimen Balance** will increase the aperture at the end of the cut. This value should be between 5 and 20. Please note that these values will vary between objectives.
    - The **Offset** value adjusts the integrated offset lens and changes the laser focus along the image plane. This value can be between 0 and 449. Once you have optimized the laser settings, click "OK". *See image below.*

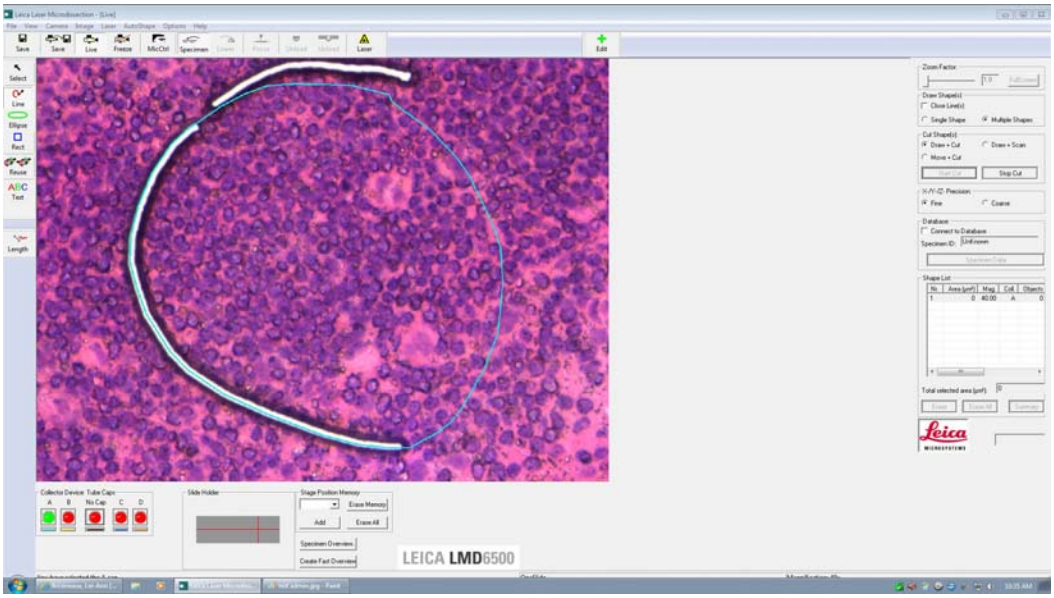


LOWER OFFSET VALUE HIGHER OFFSET VALUE

TIP! *Reduce the power for lower speeds to give a finer cut. This is useful for higher magnifications since the cutting distance is shorter. The pre-set values are optimized for each objective. For a thicker section, the offset may need to be adjusted.*

- It is recommended you save your laser settings by going to the "File" menu and select "Save Application Configuration As." Give your configuration a name (include your name) and click save. Your configuration settings will be automatically saved the following location: ***[your file name]/AppData/Local/Leica Microsystems/LMD/Profile***
  - To access your settings in the future, just go to the "File" menu, click on "Restore Application Configuration," and select your file.
  - If you want to upload the default configuration, go to the "File" menu, click on "Restore Application Configuration," and choose the following file: ***OS(C:)/Users/Default/Appdata/Local/LeicaMicrosystems/LMC/Profile/AsInstalled 11Nov13***

Note: *Be VERY CAREFUL not to bump the bench while the laser is cutting. See image below.*



4. Cutting and Collecting Areas of Interest

- Locate your area 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.
    - 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 to change the color.

- **Draw a shape** around target area by pressing "Line", "Circle", or "Rectangle" icon in the left toolbar.
- 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.

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.

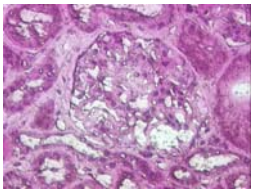
Note: *Select "Move and Cut" to cut specimen manually, using the mouse.* ***If you use "Move and Cut", always go back to "Draw and Cut" when you are done.*** *The software limits your options when "Move and Cut" is selected.*

- You can save your shapes as well as the shape list data. To save the shape list information, go to "File" and select "Save Shape list Data". Please include your name or initials in the file name. To save and reuse a shape, select "Save Last Shape For Reuse".

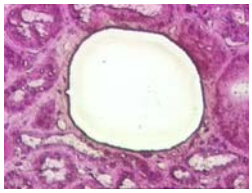
- Go to the "File" menu to restore your shapes or shape list. Restoring will **not** overwrite existing shapes/shape list.

- OPTIONAL: You can use the specimen overview option to easily navigate specific areas of your sample.
    - Click on the "Specimen Overview" button. Click "Create Specimen Overview" to scan your image.
    - Navigate around the slide and choose your and find the "top left" position and then click "Save top/left position". Repeat this step for the "bottom right" position and click "Save bottom/right position".
    - Click "Scan".
    - Once your image has been scanned, you can activate the yellow box by double clicking on it. Once activated the box will turn green and you have the ability to move around and change the magnification.
        - Note you will not be able to zoom lower than the scanned magnification.

- OPTIONAL: You can save a before and after image by selecting "Save Image As" in the "File" menu.
    - Please save all images (and exports) to your designated FTP site folder, located on the Z: drive.

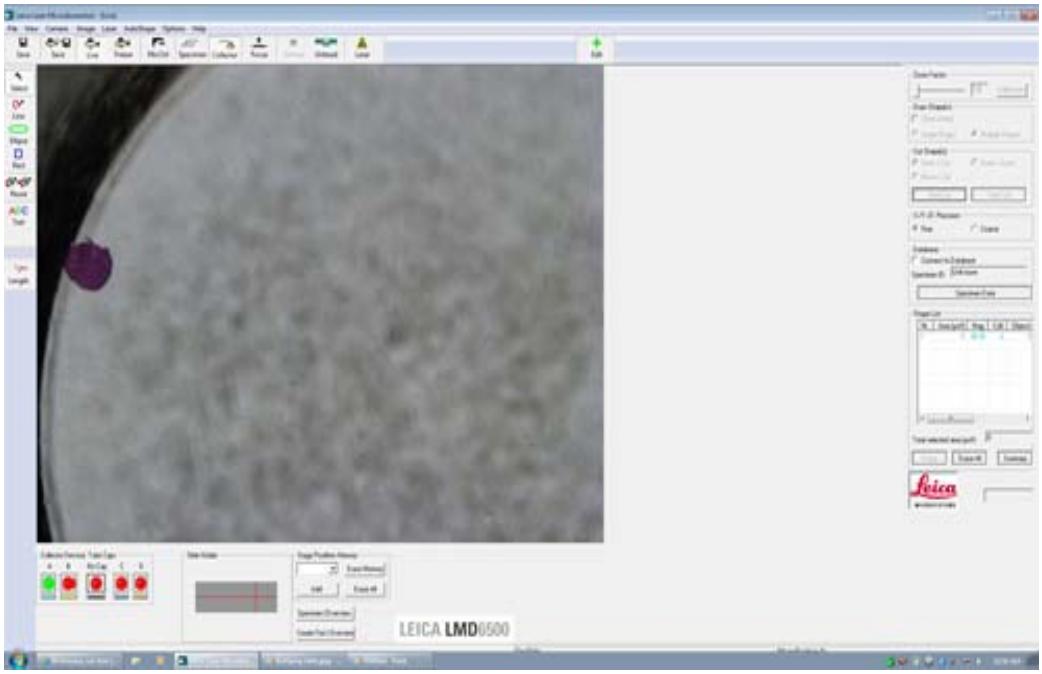


Before.jpg



After.jpg

- OPTIONAL: To view inside the cap, unload the slide by selecting the ***first*** "Unload" icon. Gently remove the specimen tray. Select the "Lower"/ "Collector" icon in the menu toolbar. Choose a cap and focus the image and inspect the result(s) of the laser microdissection.
    - Use the joystick to navigate and find the tissue that was collected in the cap
        - See image below.
    - Return to the specimen image of previous cutting position by pressing the "Specimen" icon.
    - Carefully insert specimen tray.
    - See image below for an example.



5. Text, Measurements, and Exports

- To add text to your image, select the "Text" icon and enter the information you want to include. Double-click on the actual text to change the font settings. To delete the text, click on the "Text" icon twice.

- To add measurements, select the "Length" icon. Click and drag to measure your area of interest. To delete your measurement, right click and choose "**Erase Measures.**"

- You can also export shape list data by selecting "Summary" and then "Export." Save your file in your designated FTP site folder.

6. End of Session, Shutdown

- Save all your configuration settings!
- To remove the slide tray, click on the ***first*** "Unload" icon. Carefully remove specimen tray and remove your slide. Replace the specimen tray and select "Continue".
- To remove the collection device, click on the ***second*** "Upload" icon. Carefully remove the collection device and the caps. Replace the collection device and choose "Cancel".
- Close Leica software.
- Turn off the laser by turning the key switch to vertical position.
- Turn off controller.
- Log off computer (Please DO NOT shutdown).
- Remove consumables and **cover microscope**.

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

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

For specific questions regarding DNA isolation/extraction, please contact the DNA Sequencing and Genotyping Facility http://cancer-seqbase.uchicago.edu/