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Laser Capture Microdissection

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Laser Capture Microdissection (LCM) is used to excise specific tissue regions for in-solution analysis. Tissue sections are mounted on membrane-coated slides, and a combination of brightfield and/or fluorescent microscopy can be used to select regions of interest. A cutting laser excises a border around the region, and laser propulsion is used to propel the excised region into a sample tube.

Angela Kruse, Carrie Romer, Jamie Allen, Danielle Gutierrez, Jeff Spraggins 2022. Laser Capture Microdissection . **protocols.io** https://protocols.io/view/laser-capture-microdissection-brtgm6jw

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Turning on the instrument and software

1 Turn on the computer and microscope in the following order.

1.1 Turn on the two power supply strips.

1.2 Turn key to ON position.

1.3 Turn on computer.

1.4 Turn on microscope with On/OFF button on the left side of the microscope.

1.5 Log into Windows with your MSRC account or the Research account.

1.6 Turn on side monitor.

1.7 Open PALMRobo software.

1.8 Check the laser indicators on the PALM microbeam. If both are green, the laser is ready to use.

Loading a Sample

2 Load a sample mounted on a suitable membrane-coated slide.

2.1 It is critical to ensure that the objective is at the lowest possible position before loading your sample to avoid collisions with the stage. Select the lowest objective and make sure the objective is in the “Load” Position.

2.2 Move stage to load position (microscope slide button). 

2.3 Load slide in slide holder (SlideHolder 3x). Click “Return to the working area”.

2.4 Open navigation window by clicking on compass star button. 

2.5 Click mouse on area where you want the stage to move to view your tissue section

2.6 On the left side of the software screen choose which objective you would like to use by clicking on the corresponding button.

2.7 Bring your sample into focus using the knobs on the right of the microscope.

2.8 There are several ways to navigate around the slide:
1) Selecting the crosshairs icon from the toolbar at the bottom of the screen allows one to click the screen and center the view on the crosshairs.
2) Use the arrow keys on the keyboard.
3) Use the scroll bar on the right and bottom of the microscope image.

Fluorescence

3 Turn on the XCite unit (top of computer) and let it warm up ~5 minutes. The filter wheel on the XCite may be used to adjust intensity. When lamp has been turned on, leave it on for at least a half an hour to stabilize. Rapidly turning on and off the lamp greatly decreases lamp life. The lamp has a 1000 hr life.

3.1 On the top of the instrument, where the stack of lenses are, there are two black buttons on the right hand side. These may be pressed to physically adjust contrast.

3.2 Turn on the brightfield light using the iris icon under the Common/Light Tab.

3.3 Find your sample area of interest.

3.4 Adjust the light level using the sliding bar until the sample is focused within the field of view.

3.5 Click “Measure” to optimize exposure time (brightfield).

3.6 Click “Balance” to white balance.

3.7 Adjust objectives and viewing until view is optimized for your area of interest. You will be switching back and forth from brightfield and fluorescence to cut the tissue.

4 5.2.3. Optimizing the laser cutting energy and Laser Catapult
5.2.3.1. In the bottom left of the screen select the laser setting you want to optimize in the drop down menu (Cut, RoboLPC, etc.).
5.2.3.2. Move to an area of the sample that will not be used for analysis. Draw a long switchback pattern with the freehand tool that you can select from the bottom central drawing icons.
(Draw the line by selecting this feature)
5.2.3.3. Activate the laser to cut the selected area (Click continue to ignore the capture warning since you do not intend to collect anything at this point).
5.2.3.4. As the laser moves through the pattern, adjust the laser energy using the right or left arrow underneath the laser energy bar. An optimized cutting laser will have a thin clear path.
5.2.3.5. To optimize laser catapult, select the RoboLPC in a similar manner but draw a circle on your sample instead of a line.
5.2.3.6. Activate the cutting and catapult using the laser icon. A message will prompt that the collection device is not in position. Click continue to ignore this message.
5.2.3.7. With this setting you should neatly clear away the selected tissue. You want to blast but not burn your sample. Too high of an energy results in thick burn marks with charred tissue on either side of the laser cutting space.
5.2.3.8. If catapulted area shows thick burn marks, LPC energy and focus must be optimized. The dot tool may be used for optimization.
5.2.3.9. Laser settings need to be set for each objective used. They can be saved in your folder and pulled up for later use.

5 5.2.4. Selecting regions of interest
5.2.4.1. Select regions you would like to capture by drawing objects around them on the screen.
5.2.4.2. View all elements drawn and total area of regions in the Element List (button along

top portion of screen).
5.2.5. Loading capture device
5.2.5.1. Once all elements are drawn insert capture caps and add ~20-40 uL liquid to the cap. Make sure the liquid spans the entire cap.

6 Loading Capture Device
5.2.5.2. Click the “Show Capture Device” button along the top portion of the screen.
5.2.5.3. Click “Change Collector” and load caps into the capture device holder.
5.2.5.4. Click cap 1 to move the collector into position.
5.2.5.5. In RoboMover adjust the z height to bring the cap close to the slide (about the spacing of a business card).
5.2.5.6. Click “set” to save this working position for the height.
5.2.5.7. Click the check cap position button.
5.2.5.8. Turn up the light on the microscope so you can see ring effect when the cap is centered. This is the position that you want to use while capturing. In the RoboMover screen click “set as default” for the x and y position and this will save that position. Exit “check cap”.

7 5.2.6. Collecting/capturing your sample
5.2.6.1. Select the regions you want collected into cap 1 in the Element List.
5.2.6.2. Start laser. Stage will automatically move to each region and dissect areas selected with the laser settings you have chosen.
5.2.6.3. When the collection is finished click the “Check Cap” button along the top portion of the screen. Change the objective to the 5x. Focus the microscope to the top of the liquid surface in your cap. You should be able to see the material that you captured.
5.2.6.4. Uncheck cap to move back to position.
5.2.6.5. Open the capture device window and click “Change Collector” in the RoboMover window.
5.2.6.6. Remove the capture cap.
5.2.6.7. Unload the sample by clicking on the microscope slide button to move stage to load position.
***Refer to the following two sections if you will be using the fluorescence features of the microscope. If not, continue on to section 5.2.9.

8 Fluorescence
5.2.7.9. Close the iris under the Common tab. Go to reflected light under microscope tab. There are several buttons under fluorescence with filter names. Laser refers to the cutting laser, and cutting is only performed in brightfield.
5.2.7.10. Click on a filter name to open the reflected light iris and use filter light.
5.2.7.11. Hit measure to automatically adjust exposure time. Do not over expose your sample as this will decrease fluorescence signal. Limit the time that the sample is exposed to light. Analog gain is 3-4 dB, exposure depends on filter selection.
5.2.7.12. Click on advanced settings. Live Image window opens showing a histogram. 0-255 refers to intensity, RGB are binned.
5.2.7.13. For saturation in histogram beyond 255, adjust intensity using by XCite wheel, exposure time, reducing gain.
5.2.7.14. To optimize histogram, drag the line by grabbing at the top and placing where light

peak ends or bottom and placing were light path of overlapping light ends. Increase exposure time until optimized. Save settings under live image.

5.2.7.15. Changing cameras to black and white may increase sensitivity. Histograms are optimized the same way.

5.2.7.16. Adjusting the gain: XCite wheel, reduce gain under microscope tab, or reduce exposure time.

9 Cutting fluorescent samples

5.2.8.1. Click on the snowflake on top menu bar to freeze image and select areas of interest to cut. NOTE: This must be performed using the objective you are intending to cut from.

5.2.8.2. Unfreeze image.

5.2.8.3. Click on the Laser button under the Reflected light tab.

5.2.8.4. Go to Common Tab. Exposure time and white balance may be readjusted if needed.

5.2.8.5. Click the cutting icon to cut and catapult the tissue into the capture tube.

5.2.8.6. Once tissue is cut, the bright field iris is closed and the user may go back to fluorescence under the Reflected light tab to repeat the process.

5.2.8.7. Turn XCite unit off immediately when finished.

10 5.2.9. Turning off the LCM

5.2.9.1. Close the software.

5.2.9.2. Shutdown the computer.

5.2.9.3. Turn off microscope.

5.2.9.4. Turn key to OFF position.

5.2.9.5. Turn off the two power supply strips.

5.2.9.6. Cover the instrument.