



Laser Capture Microdissection Condensed User Guide

The Zeiss PALM MicroBeam laser capture microdissection system uses pressure catapulting technology to collect material (including whole cells) from thin (<20um) fixed or frozen tissue for subsequent DNA, RNA, or protein analysis.

Turn On Sequence

1. If you plan to work under fluorescence mode, turn on the power of the HXP Illuminator located in front of the PALM Microbeam tower.
2. Flip on the power of the two power strips located underneath the table. This turns on the whole system, including the halogen lamp, microscope, and the UV (355 nm) laser.
3. Turn on the computer (if not already on) and login with your Cornell net ID and password.
4. On the desktop, open the software – PALM RoboSoftware v 4.6. Login using credentials displayed below monitor.
5. Wait till system is completely initialized (includes objective focus position adjustment and stage movement).
6. In the main menu of the software, click "Settings" and choose Load Settings from the dropdown menu (if you had previously saved settings). If no previously established settings have been saved and loaded, load newest default settings saved under the Users folder.
7. Put sample on stage (tissue facing upward) and proceed to locating areas to be cut and collected.

Turn Off Sequence

1. Exit the PALM RoboSoftware. When the program is closed, a prompt appears as to whether you want to save your settings or your elements for later use.
2. If you saved images, copy your files to the Imaging fileshare so that you can access your data from your lab/office.
3. On regular office hours weekdays, if somebody is signed up in the next hour please leave the whole system on (just need to log off the computer). If no one else is signed up to use the system, exit Windows and shut down the computer.
4. Turn off HXP Illuminator (if turned on) and turn off power strips; cover microscope
5. Log number of slides/caps taken from the facility in the Materials Inventory sheet.



PALM Microbeam

Mechanism for Laser Pressure Catapult (LPC)

Objective Lenses

Magnification	Type	Immersion	N.A.	Working Distance
5x	Fluar	---	0.25	12.5 mm
20x	LD Plan-Neofluar	—	0.4	7.9 mm at coverglass 0.75
40x	LD Plan-Neofluar	---	0.6	2.9 mm at coverglass 0.75
63x	LD Plan-Neofluar	---	0.75	1.7 mm at coverglass 0.75
150x	Plan-Apochromat	glycerin	1.35	0.17 mm at coverglass 0.16

Filter Positions (Fluorescence/Reflected light)

Designation	Excitation	Emission
LASER	Transmitted light	brightfield (cutting only)
AF430	390 – 420 nm	450 - ∞
FITC	455 – 495 nm	505 – 555 nm
AF546	510 – 560 nm	590 - ∞

Laser Functions

Tool	Function
Cut	Laser cuts sample along pre-drawn lines so that a clearly split line between required and non-required material is generated
Close & Cut	Laser cuts sample along defined lines; the start and end dot of a line element are connected by a straight cut line
Joint Cut	Similar to Close & Cut, but start and end dot remains a joint. Cut area remains fixed within the tissue and can be catapulted later by a single laser pulse.
LPC	Only cells from sample upon which a "Dot" element is drawn are catapulted into cap.
Line Auto LPC	A line-shaped structure is catapulted into collection vessel; line is not catapulted in one piece, but with several laser pulses.
Auto LPC	Contents of a defined area of glass-mounted samples are catapulted into cap with several laser pulses.
Robo LPC	Defined structures are cut out and catapulted intact into the cap. This can only be used when there is an LPC membrane between your sample and the slide.
CenterRobo LPC	Defined structures are cut out and catapulted intact into the cap in a single operation. Laser pulse is placed in the center of figure for catapulting. It can only be used with membrane-coated samples.