



Zeiss PALM (Position Ablation Laser Microbeam) – Standard Operating Procedure

Turn ON the system

- a. Power bars
- b. Power supply (white box)
- c. Apotome (NOT for PALM, for SIM)
- d. HXP lamp (for epi, 10 min warm up)
- e. Controller laser key (30 min warm up)
- f. Computer/PALM Robot

Turn OFF the system

- a. PALM Robot/Computer
 - b. Controller Laser key
 - c. HXP lamp (if used)
 - d. Apotome (if used)
 - e. Power supply (white box)
 - f. Power bars
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Illumination: Halogen lamp, Epi lamp, and Laser

- General: Do not toggle on/off! If off, wait 10/30 min (epi/laser) before turning back on.
- Next user: If there is a user within 1 hour after you, leave on. Otherwise, turn off and write the time.
- Out-of-hours: Always wait for the next user to show or turn off and write the time.

****MAKE SURE APOTOME IS REMOVED FROM LIGHT PATH (slider out!)****

****LOADING SLIDES: FIRST MAKE SURE CAPTURE DEVICE IS IN HOME POSITION****

Steps

1. Load slides/dishes: “slide” icon, use appropriate holder, insert slides/dishes facing up
2. Load PCR tubes: tube horizontal, cap faces down
3. Select objective, Adjust collar, Find & Focus on specimen, Adjust for Kohler illumination
4. Insert diffuser/cap (capture device) in light path: “plate” icon, adjust height as close as possible without touching
5. Adjust camera image: measure & white balance in empty area, typical exposure 7-10 ms
6. Test laser cut and catapulting & Calibrate if needed:
 - a. Go to empty area & Test/Adjust laser cut:
 - i. Draw squiggly line & Select cut mode; Pull out elements list for reference or “go to”
 - ii. Adjust cut speed to slowest
 - iii. Start laser (FTSS 355 nm pulsed at 100Hz) with “laser” icon
 - iv. Reduce laser energy until no laser cut visible & Adjust laser focus to sharp/narrow cut
 1. Guide - 5x: energy 40 / focus 80; 20x: energy 34 / focus 70; 40x: energy 31 / focus 57
 - b. Test/Adjust laser position
 - i. Find a recognizable spot
 - ii. Turn to stage mode: “arrow & hand” icon
 - iii. Hit pedal for laser pulse & Escape button to escape stage mode
 - iv. Calibration/Position Cut-Laser & Select your spot/hole
 - c. Test/Adjust laser catapulting



- i. Draw close loop ROI & Select cut mode
 - ii. Select cap for catapulting
 - iii. Start laser to cut
 - iv. Adjust laser energy and focus deltas for LPC (laser pressure catapulting)
 1. Guide - energy 10 / focus 3
 - v. Draw dot near ROI perimeter & Select LPC mode
 - vi. Start laser to catapult
 - vii. Verify if ROI is in the cap: “cap” icon
7. Automatic/Batch Cut & Catapulting
- a. Draw all ROIs in RobotLPC mode
 - b. Pull out elements list to verify & adjust all parameters: assign tubes, colors, slides ...
 - c. Activate autofocus (AF) for multiple slides or high topography
 - d. Select operations & Start laser

Tips

- For sample prep, see Zeiss PALM website
<https://www.zeiss.com/microscopy/int/products/laser-microdissection/microbeam.html>
- For multiple slides: use “compass” icon to navigate
- For thick tissue (>20um): use cycles (instead of increasing laser energy) to avoid tissue damage
- To image: use “freeze” or camera icons and save as tiff
- To save elements and images: go to Settings/Preferences
 - /Elements: direct to D/Users/UserElements/ create your name
 - /Images: direct to D/Users/UserElements/ create your name
 - /Laser and check before after cut or LPC