

Confocal protocol

1. turn on mercury lamp first
2. turn on remote control in back right side of scope
3. turn on computer on desk
4. log on
5. "LSM510"
 - "scan new images" to collect – start "expert mode"
 - "laser"
 - turn on argon laser, output 75%, tube current 3.3
 - turn on other lasers as necessary
 - "configuration"
 - click "config" disk to pick fluorescent option (i.e. FITC)
 - "single track"
 - "scan"
 - mode (adjust- frame size 1024, scan speed 7 [6 for slower speed], number 1 [2 for better quality], can also set zoom and rotation later)
 - channel (adjust confocal aspect- pinhole 1 airy unit)
6. find sample
 - use 10X lens to find sample and then increase magnification. Inverted scope- cover slip is down. Make sure in visual mode (bottom knob on right side of scope). Press button on left side of scope, near focusing knob) for focus between coarse and fine.
 - lens: 63 and 100X oil; 40X water; 25 water or oil
 - put back in laser scanning mode (LSM) by pressing in knob

 - "scan"
 - find (selects exposure time that computer thinks is most appropriate)
 - fast xy (for continuous scanning and making adjustments to image)
 - xy scan (for better quality scan)

 - to change brightness, adjust:
 - detector gain- makes brights brighter (as you increase to right)
 - amplifier offset- makes darks brighter (as you increase to right) or darks darker (as you decrease to left)
 - amplifier gain- brightens all equally
 - z settings (to mark first and last scanning during fast xy)
 - z slice (to set optical slice interval)
 - start
7. make projection- 3D view

8. save data- create new database (in LSM format). Put data on enigma to access later.
9. if no one signed up, turn off
-if signed up, leave on but log out
10. clean up lens: rub around and then blot
11. shut down: turn laser off, wait 5 minutes to turn off rest, then computer, remote box, mercury lamp last

Microscope manipulation

To move base:

Upper knob: clockwise for right, counterclockwise for left

Lower knob: clockwise for up, counterclockwise for down

To move focal plane:

Top of knob away from me, to go down (towards yolk, ventral)

Bottom of knob away from me, to go up (towards dorsal)