Microscopy Clinic

Oct 22nd 2010

Mechanobiology Institute Singapore

DeltaVision

- CO2/humidification is not sufficient:
  - New cover is being designed and fabricated, for the time being 6-well plates cannot be used on the system
  - Please seal the stage openings if there is any doubt about the leakage. Use tissue paper soaked with miliQ water for humidification
  - Usage of the sample holder insert

DeltaVision

- Granulated background with a fringe in the edge of image:
  - Check if the deconvolution is on

Spinning Disk

- Immersion water for long term imaging
- Maximum frame rate depends on:
  - CCD readout speed
  - Number of channels
  - Number of z positions and intervals, piezo or microscope
  - Shutter management
  - Exposure time

Spinning Disk

- Save the settings to each channel if changes are made
- Save the designed experiment to prevent loss of settings in case volocity crashes or hangs
- If volocity hangs during the experiment setups, try ‘freezing’ the video preview

Spinning Disk

- Synchronization unit
  - Turn on and off only by the power button in the back (please do not turn the keys to turn off the unit)
  - The green light for ‘usb’ is on whenever the computer is powered. If that is the only light on, the synchronization unit is not yet on

Nikon A1r Si

- When switching from Camera mode to Eye mode, TD in the NIS-element software should be unchecked to enable transmitted light to go through for Eye mode viewing
- 2 types of immersion oil available
  - Room temperature
  - 37 degrees
- PFS: unable to trigger control
  - Insert all ND filters into designated pockets
  - Infrared dichroic mirror swing => ‘IN’ position
  - Coverslip is clean

Nikon A1r Si

- When switching between Camera mode - Eye mode - Camera mode, laser interlocked tab from the NIS-element software is not working

  Solution: Try clicking on other mode, e.g. GFP or mCherry, click on Confocal tab again, then click on the Laser Interlocked tab. By doing this the laser interlocked should be able to remove

Nikon A1r Si

- For live imaging, the camera has to reach a temperature of -85 degrees Celsius to minimise the noise level in 'Live' mode.

  (typical waiting time: 5-10 mins)
- 35 mm dish holder: not flat at the moment
- EVERYTIME switching from eye to confocal:

  need to check the ‘confocal’ from the software, otherwise software might hang and sometimes you will need to restart the computer

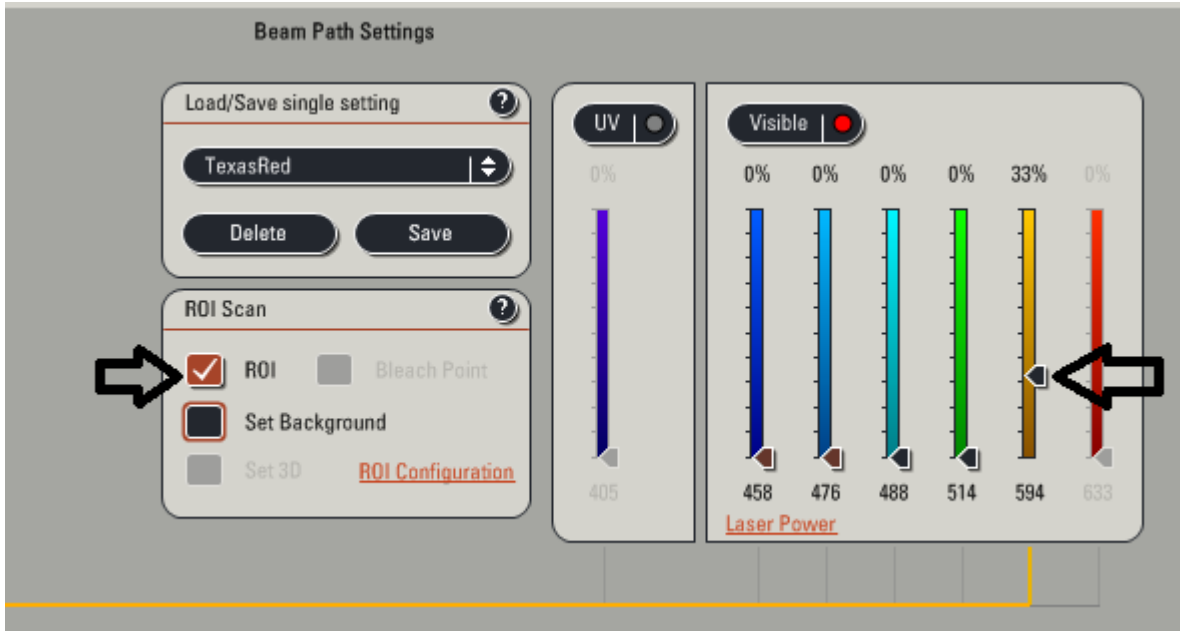
Nikon A1r Si

- PFS does not work with plastic dishes (regardless of the thickness of the plastic bottom)

  alternative: do not use PFS, allow the stage to stabilize itself before starting to image

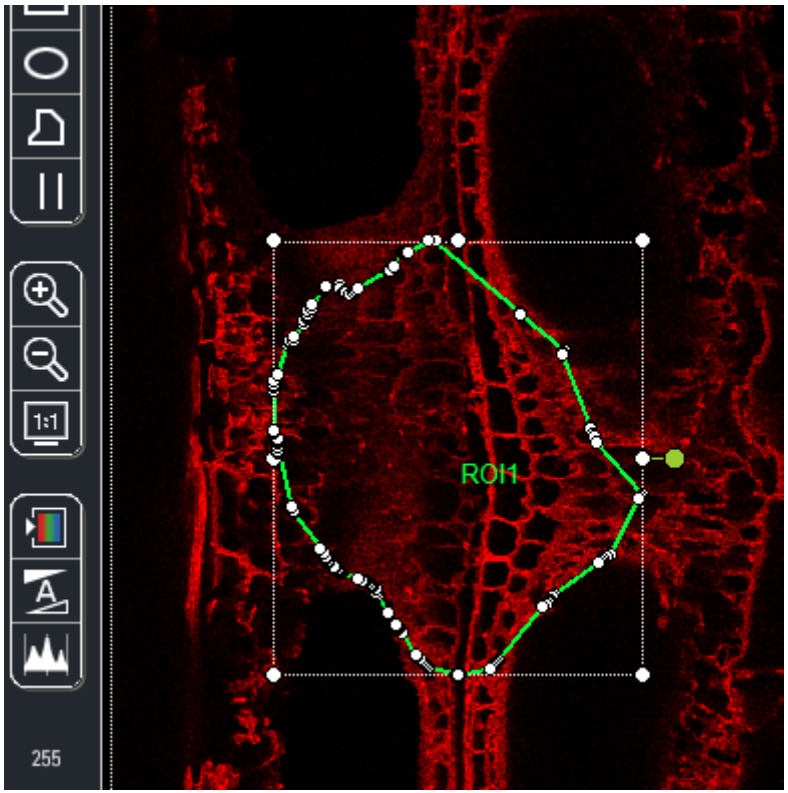
Leica SP5

- Activate ROI button at ROI scan window



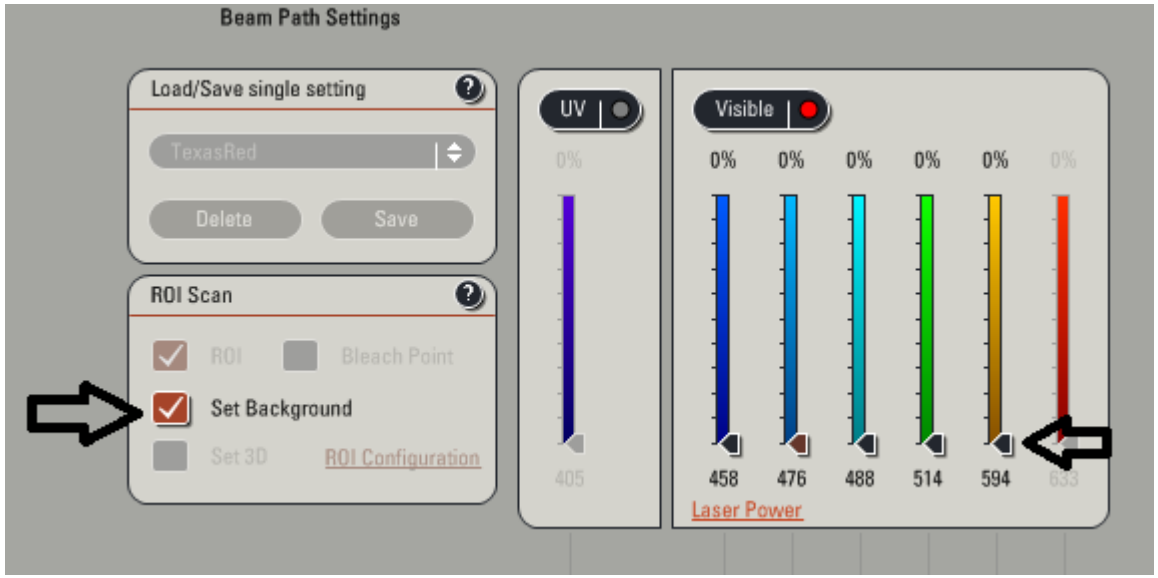
Leica SP5

- Draw any shape of ROI on your image



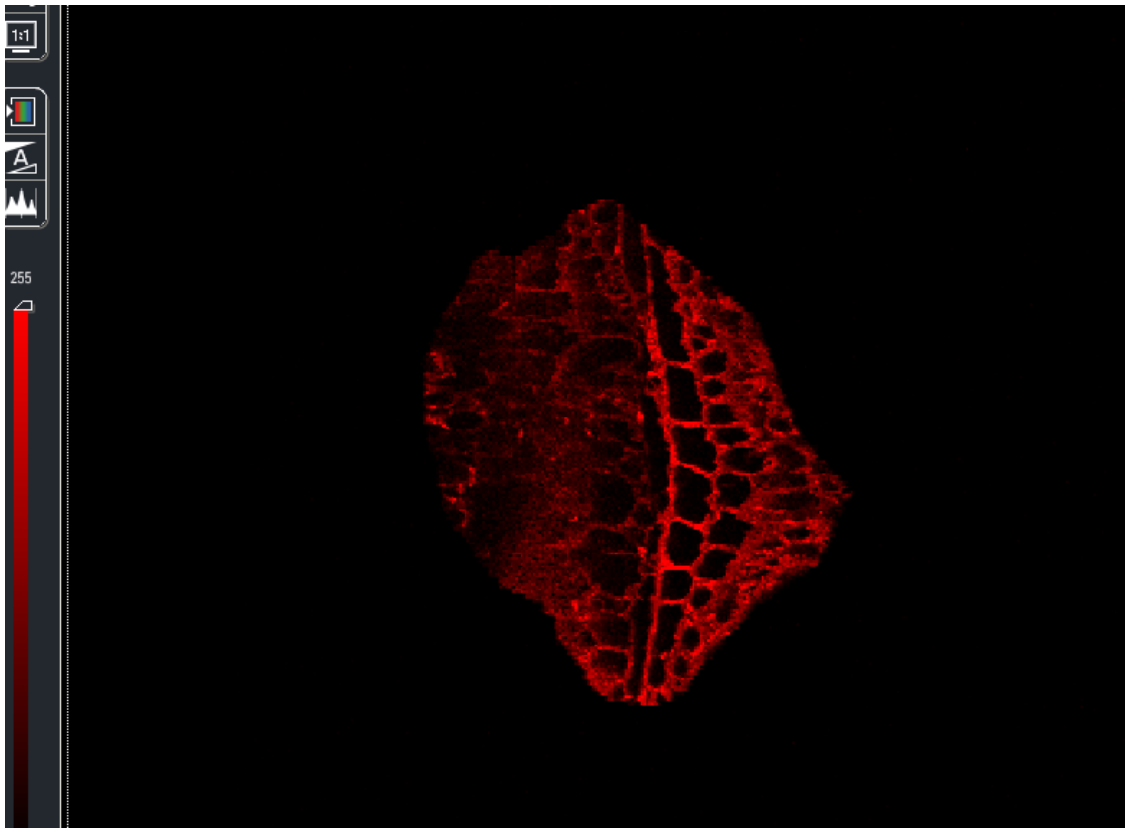
Leica SP5

- Activate Set Background button at ROI scan window and drag laser power to 0%



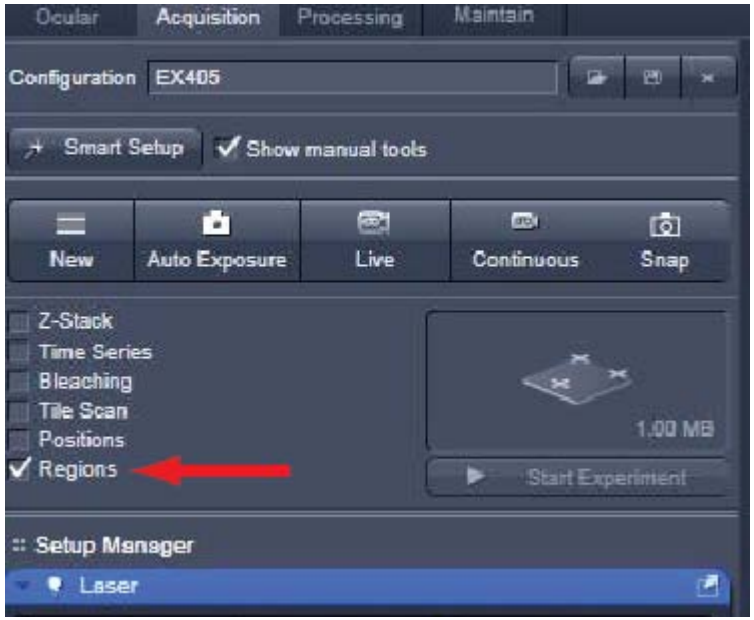
Leica SP5

- ROI scan is achieved



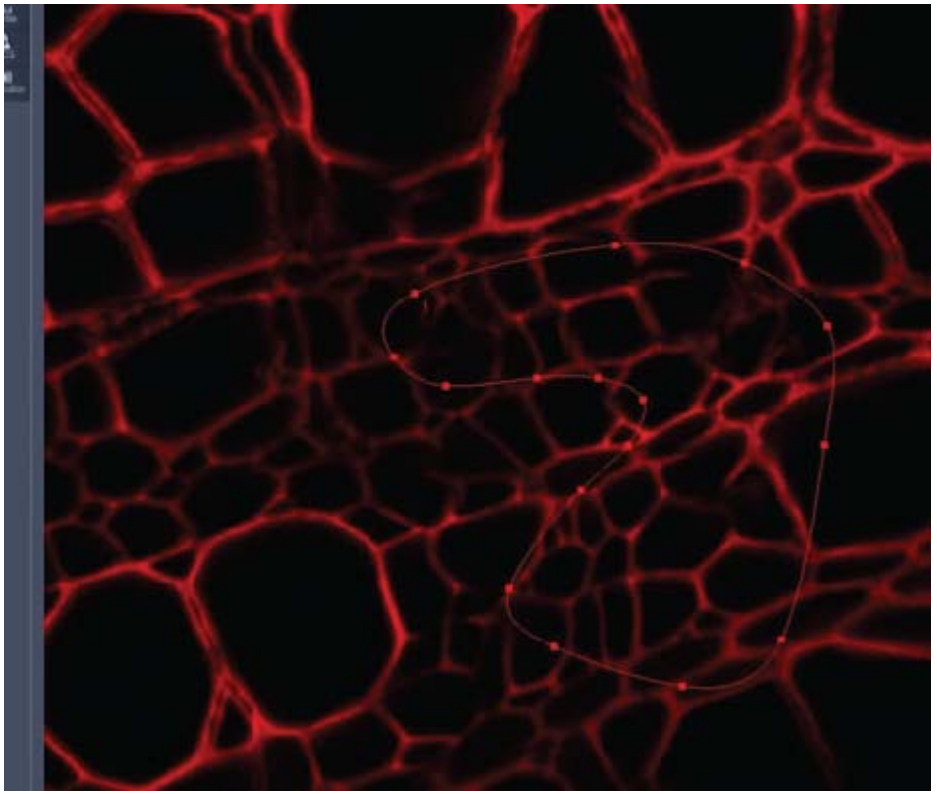
Zeiss LSM 710/confocor III

- Activate the “Regions” function.



Zeiss LSM 710/confocor III

- Use any of the drawing tools to draw your ROI in your preview image.

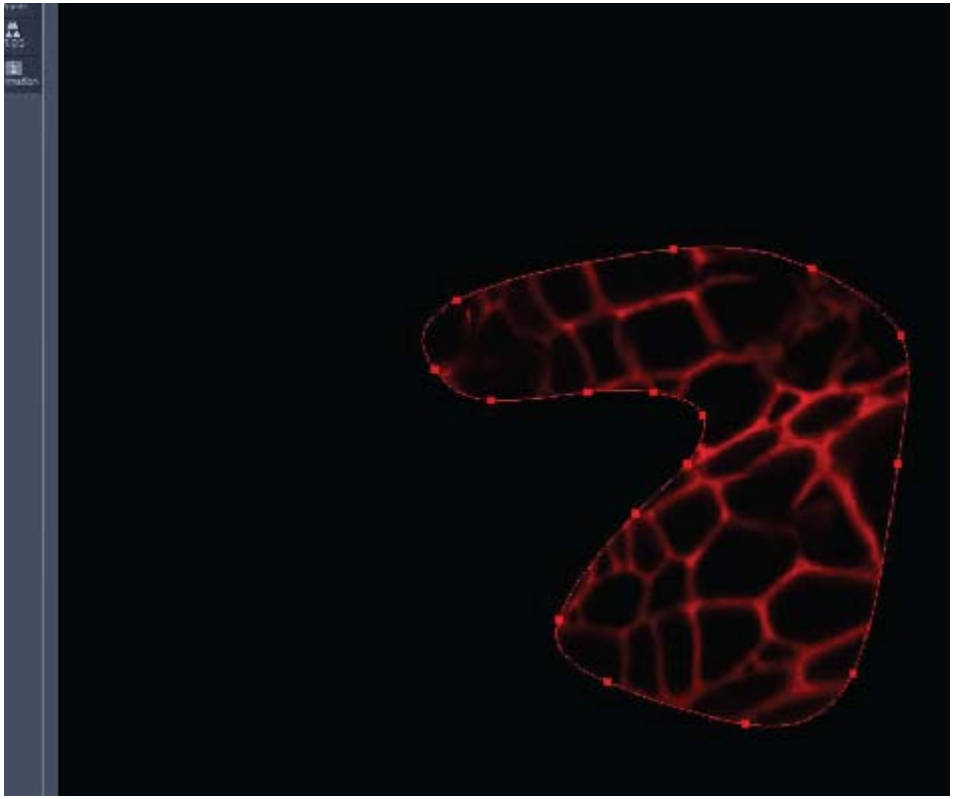


Zeiss LSM 710/confocor III

- Activate only “Acquisition” and deactivate “Bleach” and “Analysis”.

Zeiss LSM 710/confocor III

- ROI scan is achieved



Olympus TIRF

- Turning on the lasers:

  a. 405 nm, switch on power buttom and turn interlock key

  b. 488/514 nm, switch on power buttom and turn interlock key

  c. 559 nm, switch on power buttom, wait until both green light lights up and then turn interlock key.

  d. 633 nm, turn interlock key.

Olympus TIRF

- Things to check when there is no laser coming out:
  - Objective is pointing straight upwards (sometimes this does not block the laser entirely but instead the laser appears very week)
  - Laser emission key is turned to ‘on’
  - Laser safety slider pushed in
  - The switch bar at the back of the microscope is pushed in
  - Changing the laser fibre position (touching, turning, pushing or pulling it) does not help and will almost certainly causes misalignment of the laser.

Olympus Live

- Metamorph might crash if file transfer is done when image acquisition is ongoing
- Another suspected reason for Metamorph crashing: saving image files to F: drive (please write all your data in C: drive)

Difference between Spinning Disk and Laser Scanning Confocal: Strengths

Spinning Disk	Laser Scanning Confocal
-Optimized for use where transmission, or speed, is a higher priority than ultra-thin sectioning. -Since this method applies low dose of multiple excitation beams to the specimen, fluorescence bleaching is very slow and the damage to sample (phototoxicity) is very low.	-Offers the highest level of confocality and the ability to do extremely thin optical sections of specimens. -Near-total control of where and how a very bright illumination source strikes the specimen

Difference between Spinning Disk and Laser Scanning Confocal: Weakness

Spinning Disk	Laser Scanning Confocal
-With their larger pinhole, these systems cannot deliver the same thinness of optical sectioning as their laser-based cousins, and offer thus less confocality. The head reduces the light throughput and weak signals are hard to detect.	-Emission from the specimen must be very bright, and the objective used of the highest numerical aperture, or light gathering ability. -The intensity of the laser light causes photobleaching in fluorescent probes, and phototoxicity in the specimen itself. -The point-by-point image acquisition is time consuming, making the system ineffective for recording short-time-period events. -Specimen movement during the raster scan results in jagged edges in the image and poor definition of intracellular details.