Laser Scanning Confocal Microscope @ BSBE

PROTOCOL 1

Switching ON the instrument:

1. Switch the key for Multiphoton Laser from standby to active mode.
2. Remote Control:
   Turn ON Key
   Main switch ON
   System and Component switch ON
3. Turn on the shutter on the metal halide lamp (HXP120) for whitefield imaging
4. Argon Ion laser:
   a. Turn ON key
   b. Turn the switch from idle to run mode
5. Switch ON the Secondary CPU and click on LSM user account
6. Click ZEN (Black) software tab on the moniter
7. Select the boot system and click on Start system.
8. Switch on the lasers in the acquisition mode (software).

PROTOCOL 2

Switching OFF the instrument:

1. Save images. Close all the windows by clicking on **Windows** tab and select close all.
2. Turn OFF the lasers (Software).
3. Close the Zen (Black) software.
4. Shut down the system.
5. Argon Ion laser:
   a. Turn the switch from run mode to idle mode.
   b. Turn key OFF
6. Remote Control:
   a. System and Component switch OFF
   b. Main switch OFF
   c. Turn OFF the Key

Switch the key for Multiphoton Laser from active to standby mode.

PROTOCOL 3

Protocol for Single/ Multi-colour image acquisition :

Sample focusing using eyepiece

1. Select the objective in TFT display and click load mode. Add immersion oil for oil immersion objective. Place the sample upside down with the coverslip facing the objective on the stage. Use coarse adjustment knob to bring the immersion objective in contact with the sample.
2. Go to locate mode. Open the reflector shutter and select the reflector (green/red) for locating the sample
3. Focus the image using fine adjustment knob.
4. Turn off the reflector shutter on the locate mode

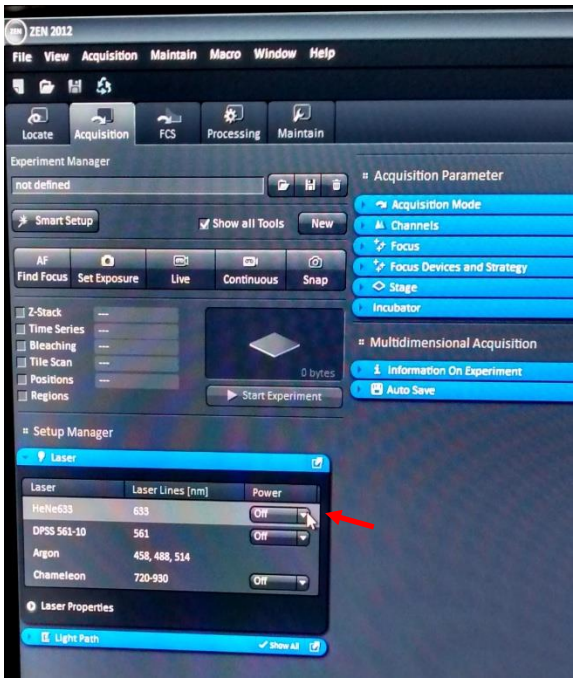
Image acquisition using confocal mode

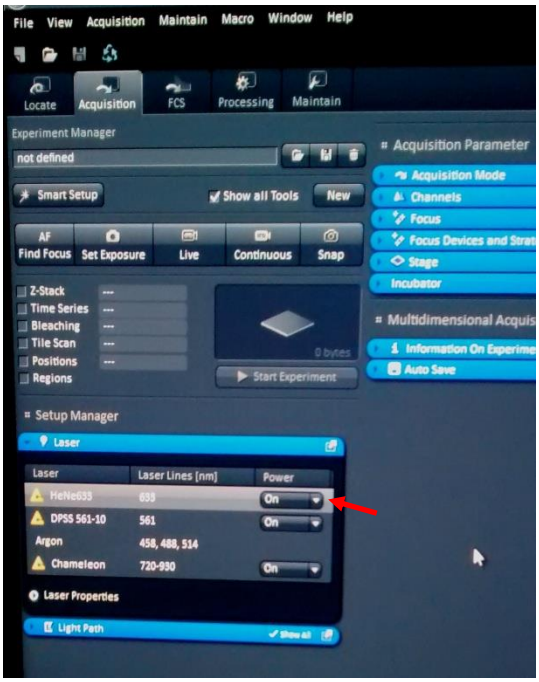
1. Click on the acquisition tab
2. Go to light path tab, click on LSM tab and select channel.
3. Go to channel tab and define the tracks.
    a. Add track
    b. Name the track and select the colour
    c. Select the laser, laser power (start with minimum value), Gain (Master)-PMT (650) and GaAsP (750)
    d. Select pinhole 1AU for all other lasers and maximum for multiphoton laser

4. Go to light path
    a. Select the appropriate detector (Ch-1 and Ch-2 [PMT detector] and ChS1 [GaAsP detector]), name the dye, select the colour and specify the range.
    b. Select the light path (Set appropriate mean beam splitters (MBS))

MBS/Lasers	**Single lasers**	**Multiphoton lasers for internal detector**
MBS for visible light	Wavelength specific appropriate MBS	Plate
MBS for invisible light	Plate	Wavelength specific appropriate MBS
Reflector revolver	Rear	Rear

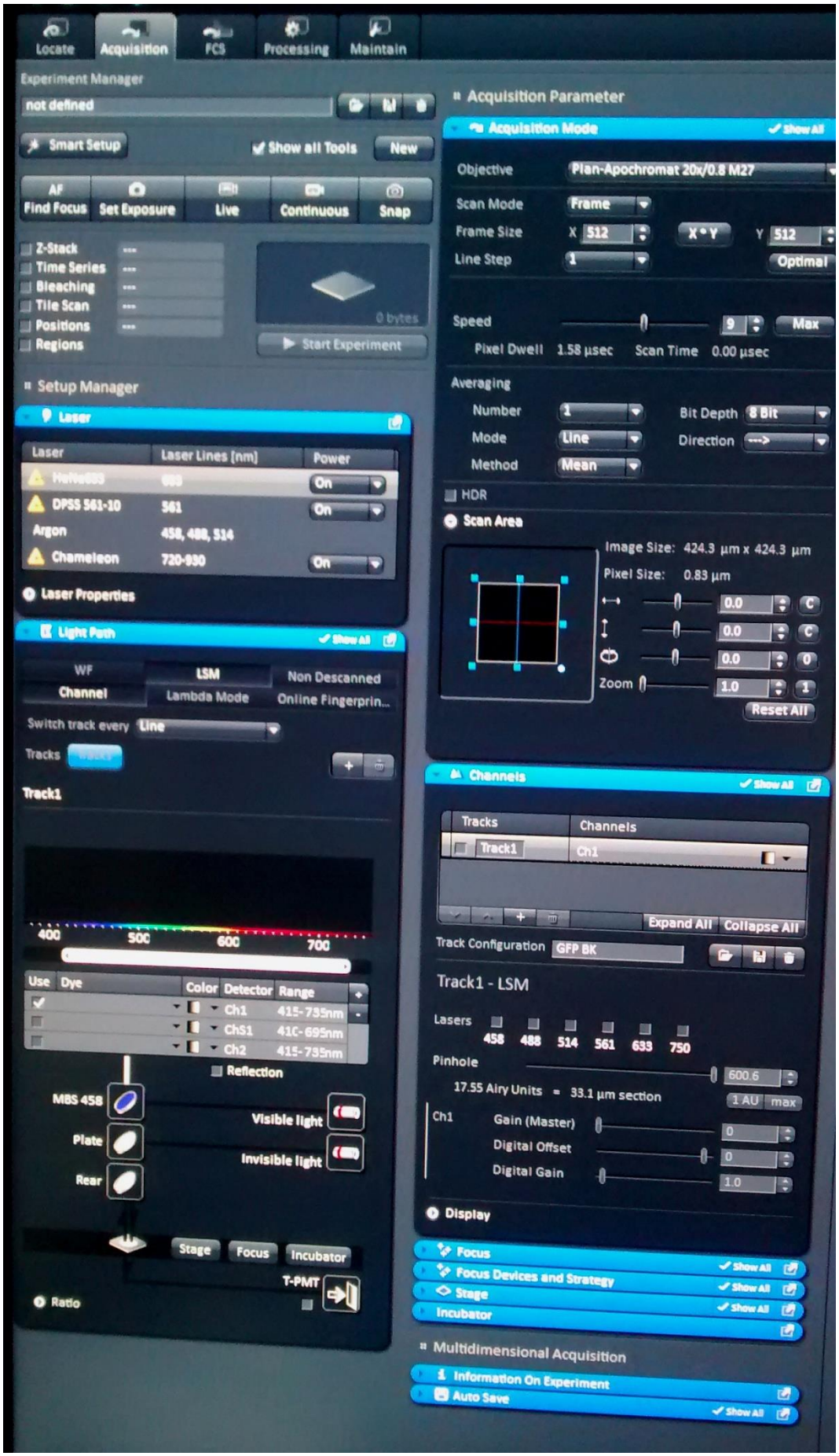
5. Select **T-PMT** for DIC imaging with excitation laser 488.
6. Go to acquisition mode:
   Select the desired scan mode (Line/Frame), frame size, scan speed.
7. Open a **New** window and then click **Continuous** tab. Adjust the Gain and power accordingly.
8. Tick the Range indicator and correct for undersaturation (adjusting the digital offset) and oversaturation (adjusting Gain and laser power). Click on Stop.
9. Click **Snap** to get final image.





Do's and Don't's

1. While doing imaging in sequential mode for multicolor confocal microscopy, set tracks in decreasing order of wavelengths to avoid blead-through.
2. Use Chs1 (GaAsP) detector for samples with weak signal.
3. Reduce the scan speed for better image acquisition.
4. Adjust the polarizer to 0 degree (Unpolarized) for Brightfield imaging and to 90 degree (polarized) for DIC + confocal imaging.
5. Switch off the room lights during image acquisition.


Locate
Acquisition
FCS
Processing
Maintain
Experiment Manager
not defined
Smart Setup
Show all Tools
New
AF
Find Focus
Set Exposure
Live
Continuous
Snap
Z-Stack
Time Series
Bleaching
Tile Scan
Positions
Regions
0 bytes
Start Experiment
Setup Manager
Laser
Laser
Laser Lines (nm)
Power
DPSS 561-10
561
On
Argon
458, 488, 514
Chameleon
720-930
On
Laser Properties
Light Path
Show All
WF
LSM
Non Descanned
Channel
Lambda Mode
Online Fingerprin...
Switch track every
Line
Tracks
Track1
400
500
600
700
Use
Dye
Color
Detector
Range
Ch1
415-735nm
Ch2
415-735nm
Reflection
MBS 458
Plate
Rear
Visible light
Invisible light
Stage
Focus
Incubator
T-PMT
Ratio
Acquisition Parameter
Acquisition Mode
Show All
Objective
Plan-Apochromat 20x/0.8 M27
Scan Mode
Frame
Frame Size
X
512
X • Y
Y
512
Line Step
1
Optimal
Speed
9
Max
Pixel Dwell
1.58 µsec
Scan Time
0.00 µsec
Averaging
Number
1
Bit Depth
8 Bit
Mode
Line
Direction
Method
Mean
HDR
Scan Area
Image Size: 424.3 µm x 424.3 µm
Pixel Size: 0.83 µm
0.0
C
0.0
C
0.0
0
Zoom
1.0
1
Reset All
Channels
Show All
Tracks
Channels
Track1
Ch1
Expand All
Collapse All
Track Configuration
GFP BK
Track1 - LSM
Lasers
458
488
514
561
633
750
Pinhole
600.6
17.55 Airy Units = 33.1 µm section
1 AU
max
Ch1
Gain (Master)
0
Digital Offset
0
Digital Gain
1.0
Display
Focus
Show All
Focus Devices and Strategy
Show All
Stage
Show All
Incubator
Show All
Multidimensional Acquisition
Information On Experiment
Auto Save
Show All

PROTOCOL 4

Protocol for Z-Stack:

1. Follow the **Protocol 3** for initial image acquisition.
2. Tick the z-stack option.
3. Select the highest wavelength laser under channels tab.
4. Click on Continuous tab
5. Under z-stack tab, define the thickness by selecting the lower and upper limit using fine adjustment knob.
6. Click on **Start Experiment**.

Note: When using Multiphoton laser, manually select the interval between stacks as pinhole aperture is maximum.

PROTOCOL 5

Protocol for Time Lapse:

1. Follow the **Protocol 3** for initial image acquisition.
2. Go to Time Series.
3. Define number of cycles and time interval (between two imaging).
4. Click on **Start Experiment**.

Note: For Z stacking with Time lapse combine **Protocol 4** and **Protocol 5.**

PROTOCOL 6

Protocol for Fluorescence Recovery After Photo-bleaching (FRAP):

1. Follow the **Protocol 3** for initial image acquisition.
2. Select **Bleaching** (Time Series and regions will automatically be selected)
3. Click the **Regions tab** and define the Region of interest (ROI) to be bleached. Tick Bleach and Analysis.
4. Click on Bleaching tab and set bleaching parameters.
    a. Define the number of scans for pre-bleaching
    b. Define number of iterations (number of times laser falls on the sample for bleaching).
    c. Select the laser and set it to maximum power (100%).
5. Click on **Time Series** tab
    a. Define the number of cycles (includes total number of pre-bleach and post-bleach scans).
    b. Set the Time interval (time between two scans).
9. Click on **Start Experiment**.

PROTOCOL 7

Protocol for Fluorescence Resonance Energy Transfer (FRET):

1. Define the parameters for the Donor and Acceptor fluorophores by following steps of **Protocol 3** for initial image acquisition.
2. Select **Bleaching** (Time Series and regions will automatically be selected)
3. Click the **Regions tab** and define the Region of interest (ROI) to be bleached. Tick Bleach and Analysis.
4. Click on Bleaching tab and set bleaching parameters.
    d. Define the number of scans for pre-bleaching
    e. Define number of iterations (number of times laser falls on the sample for bleaching).
    f. Select the laser for the **Acceptor fluorophore** and set it to maximum power (100%).
5. Click on **Time Series** tab
    a. Define the number of cycles (includes total number of pre-bleach and post-bleach scans).
    b. Set the Time interval (time between two scans).
6. Click on **Start Experiment**.