# Zeiss LSM 710 Guide

## Getting Started

1. Make sure the ***System/PC*** and ***Components*** switches are on (located left of the microscope).
2. Make sure the Argon laser is on (green LED on laser control).
    - If the Argon laser is off:
        i. Switch on the cooling unit by turning on the key
        ii. Switch the argon laser on (toggle switch upwards)
3. Switch on the computer and log in to PPMS.
4. Start the **Zen Black** software. On the start-up window select "**Start System**".


## Shutting Down

1. Lower the stage and remove your sample and *gently wipe* any oil objectives you have used with *lens tissue*
   **do not use kim wipes to clean objectives - use only lens tissue**
2. Turn off the lasers within Zen.
3. Exit the software and copy your files to the server
4. Shut down the computer.

**If you are *the last user for the day*:**

1. Switch off the ***System/PC*** and ***Components*** *switches*
2. Switch off the argon laser (toggle switch downwards)
3. Switch off the cooling unit by turning the key off

**If you notice the objectives are not clean or you are concerned you may have spilt oil on an air objective please contact the facility staff immediately**

# Visualising a Sample Through the Oculars

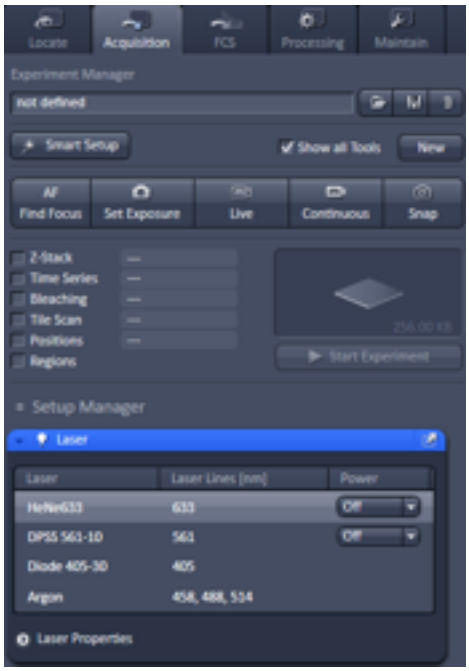
1. Switch on the HBO power supply and open the shutter.
2. Use the **Locate** tab in Zen to visualise the sample.
3. Press the **Oculars Online** button to switch the microscope light-path 100% eyes.
4. Either:
    a. use the short-cut buttons labelled **DAPI**, **GFP**, **DsRed** to configure the microscope for the respective channels, or
    b. use the **Ocular** tool to configure the light-path for your channel of interest
        i. Choose a reflector filter cube
        ii. Open the reflected light shutter

# A note about the LSM710

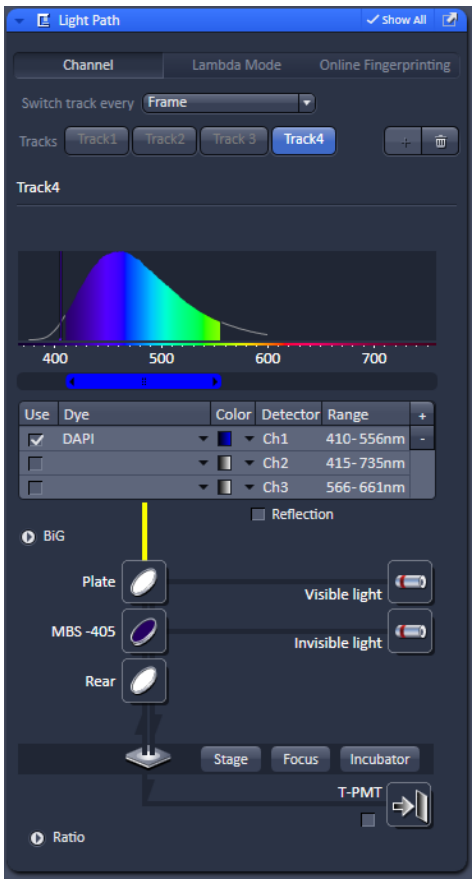
The LSM710 is equipped with the latest detection technology for confocal microscopes. Instead of two standard photo-multiplier tube (PMT) detectors and a spectral detector (META) the LSM710 is equipped with the Zeiss Quasar spectral head and a GaAsP detector (BiG) allowing measurement of very low light levels enabling the confocal to be used for fluorescence correlation spectroscopy (FCS) in addition to confocal imaging. Channels that in the past would be imaged using the standard PMTs are now imaged using the Quasar spectral detector.

# Zen 2012 - Setup Manager

1. **Lasers**
   - Here you need to switch on the following lasers:
     - **DPSS 561-10** – typically for **RFP/Cy3/Alexa546**
     - **HeNe633** – typically for **Alexa633/647/Cy5**
   - The other two lasers are activated on startup:
     - **Diode 405-30** – typically for **DAPI**
     - **Argon/2** – typically for **GFP/YFP**

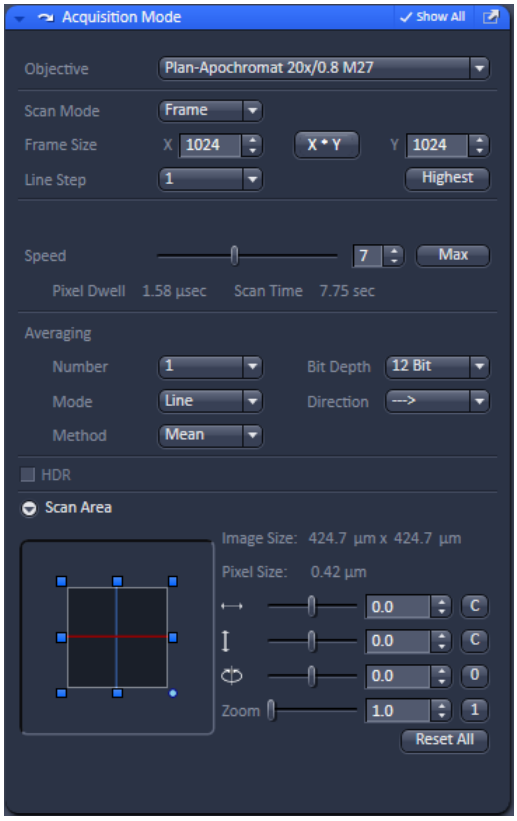



2. **Light Path**
   - Here you need setup each light path you will be using to capture your images.
   - Each light path configuration is known as a **track** (the detector settings appear under **Channels**).
   - You can capture multiple channels (using different detectors) within the same track OR
   - You can capture multiple channels (using the same or different detectors) with different light paths, using separate tracks.
   - Configure a track under the **Channel** tab:
     i. Choose the required laser line and primary dichroic. The laser line will appear on the track definition as a vertical line at its emission wavelength.
     ii. Check one or more detection channels and choose the fluorophore being imaged from the drop-down menu. The emission spectrum for the fluorophore (from PubSpectra) is displayed on the track definition.
     iii. Choose the detection bandwidth using the slider covering the required emission wavelengths.
     iv. Assign a colour map to the track.



# Zen 2012 - Acquisition Parameter

## 1. Acquisition Mode




- Here you select the resolution and quality of imaging – essentially you need to find a balance between image quality and speed while considering how much your sample will bleach

- **Frame size**
  - 512x512 will give you a fast image but a resolution of at least 1024x1024 is recommended

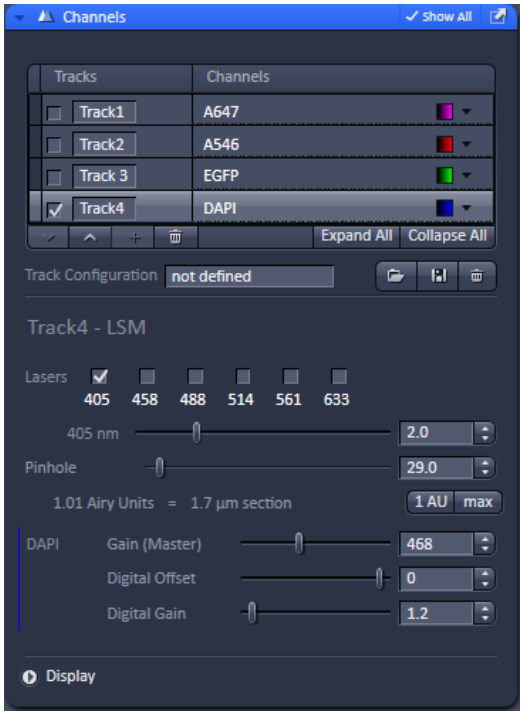
- **Speed**
  - A slower speed will give you a better image but will take more time
  - A scan speed of 7 or less is recommended

- **Averaging**
  - Averaging will remove noise from the image
  - A slower scan speed will often require less averaging
  - Generally 2 – 4 x averaging will produce a good image

- **Bit Depth**
  - 8 Bit uses 0-256 levels of grey - standard
  - 12 Bit uses 0-4096 levels of grey – producing a smoother image with more information for intensity analysis but will generate a larger file

- **Direction**
  - Choose between --> and <--> for mono- and bi-directional scanning.
  - Bi-directional scanning will significantly reduce the acquisition time for each frame

## 2. Channels



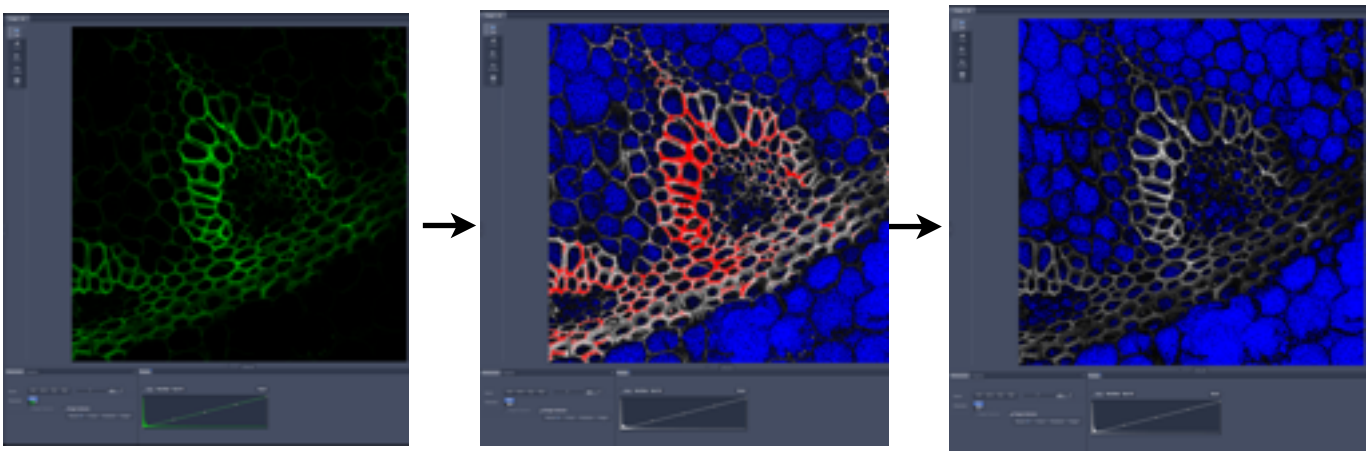

- This is the control window for adjusting the detector sensitivity and pinhole size and needs to be set *for each channel.*

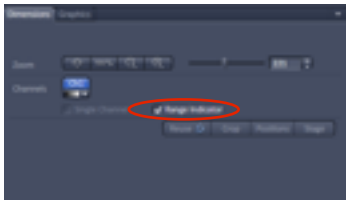
- **Pinhole**
  - The pinhole setting always defaults to wide open. So for each channel you need to press the **1 AU button** (1 airy unit) to ensure you are taking a confocal slice

- **Gain**
  - Controls the sensitivity of the detector
  - The higher the setting the brighter the white
  - Drag the controller to about half way – any higher than this and you begin to introduce noise. If it is too bright when you take an image you can drag it back towards zero – if the image is still too dim you may need to increase your laser power.

# Optimising the dynamic range

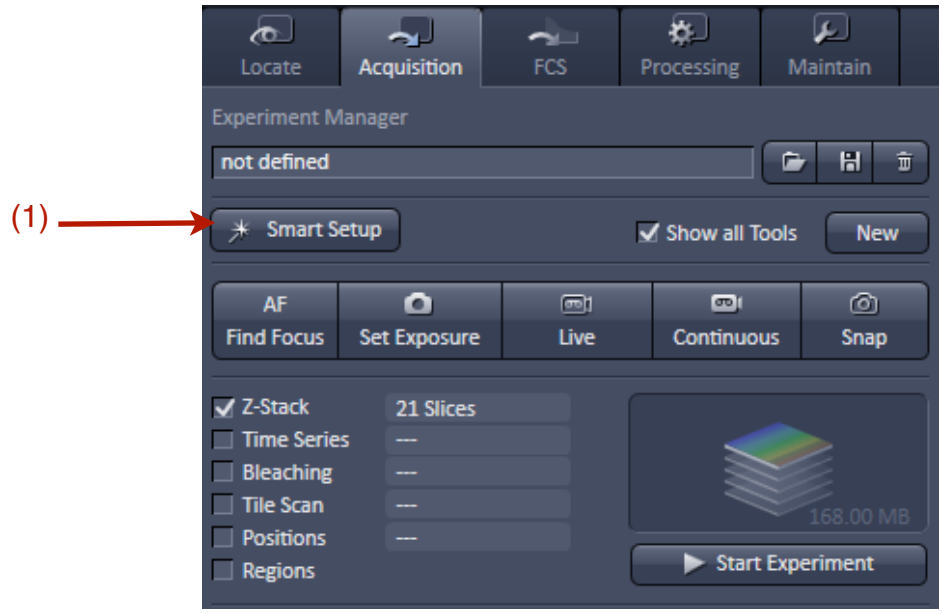
1. Use **continuous** or **live** imaging so the image is constantly updated**. Live** mode will configure the scanner to capture images as fast as possible whereas **continuous** uses the scanner settings from **Acquisition mode**.
2. In the bottom left, below the image, check the "range indicator" box.
   - The range indicator shows over-saturated pixels as **red** and true black pixels as **blue**
3. Adjust the ***master gain*** until no/little red is seen and the ***digital offset*** until small amount of blue is visible (usually 0.0).
4. Use **Snap** to record images for all selected tracks.





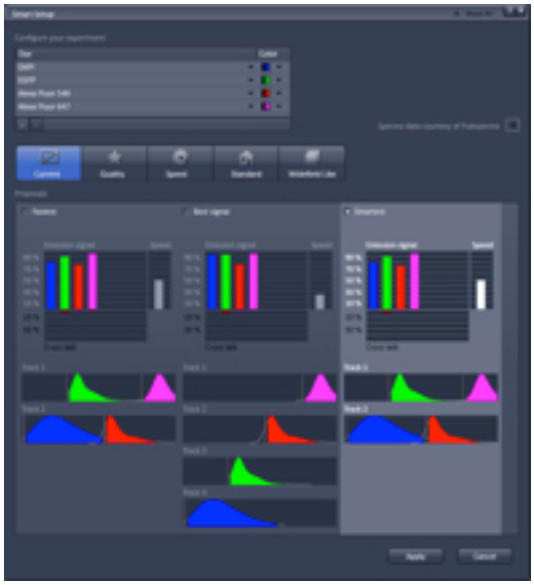
# Smart setup

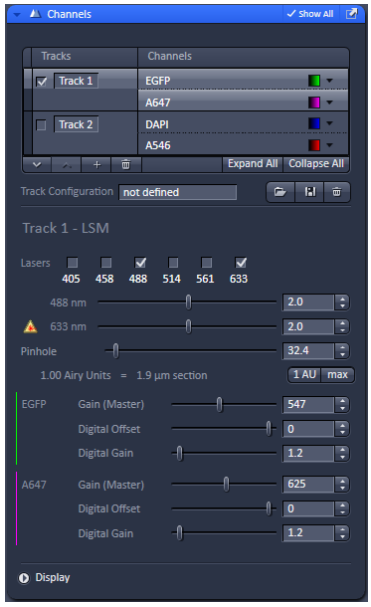
Zen 2012 includes a **smart setup** feature which can be very useful in quickly configuring the microscope for multiple channels and for obtaining the optimal configuration for a particular experiment. (1)

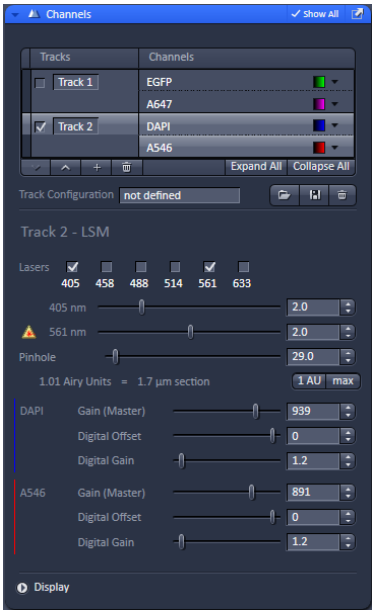



Define the fluorophore(s) under **Configure you experiment**. As before, the emission spectrum for each dye is stored in a database with information collected from PubSpectra.

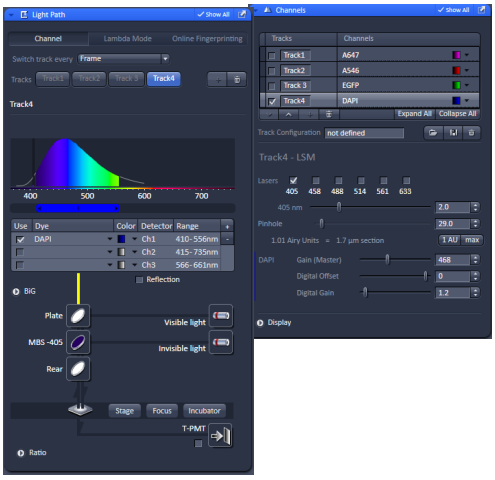
1. There are five motifs to choose from to optimise the scan settings (frame size, speed, pin-hole, gain):
   i. **Current**: this uses the settings currently applied
   ii. **Quality**: this optimises the scan settings based on image quality (sampling, optical sectioning)
   iii. **Speed**: this favours speed of acquisition
   iv. **Standard**: is a compromise between quality and speed
   v. **Widefield** Like: uses a wide pin-hole to optimise light efficiency
2. Choose a **Proposal** to setup the light-path selecting between **Fastest**, **Best signal** and **Smartest.**
   i. **Fastest**: favours speed over cross-talk and uses frame-wise switching
   ii. **Best Signal**: attempts to eliminate cross-talk; uses frame-wise switching
   iii. **Smartest**: attempts to combine spectrally distinct channels into the same track in order to optimise speed and minimise cross-talk.
3. Pressing **Apply** uses the selected proposal and if the motif is not current will optimise the scan settings and start recording data.

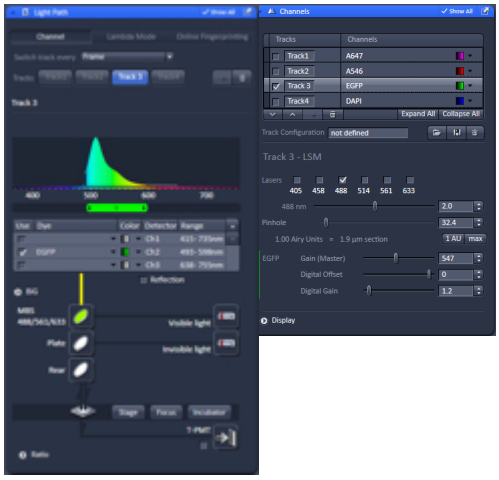


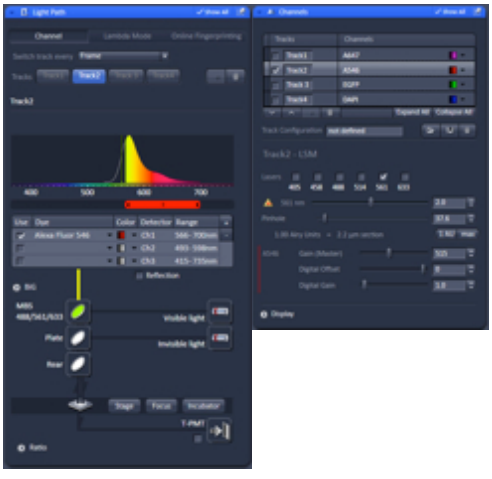


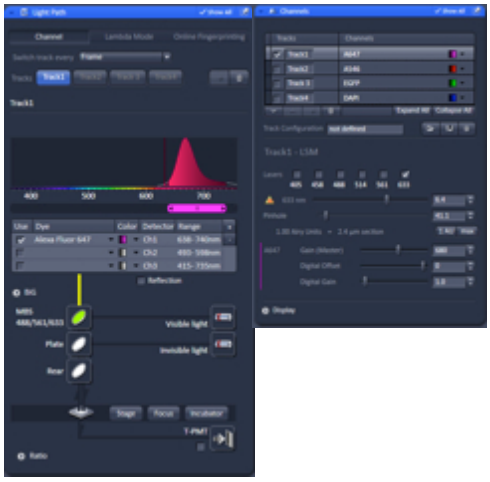




The following shows an example of the lightpaths and channel setup for 4 tracks configured for a sequential acqusition (to minimise cross-talk).



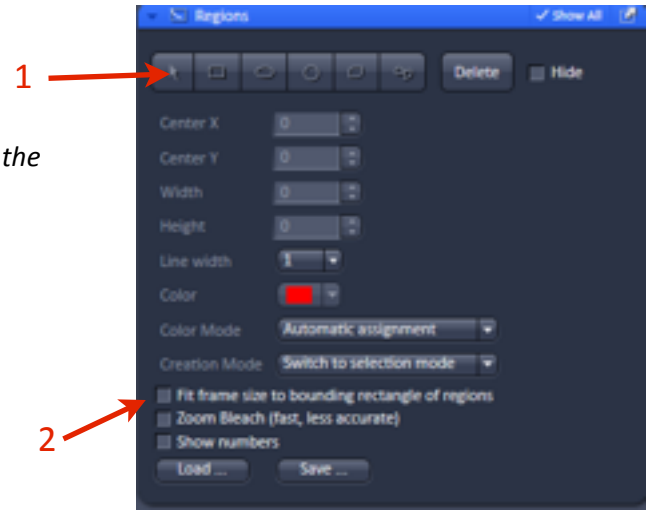



# Regions of Interest / ROIs

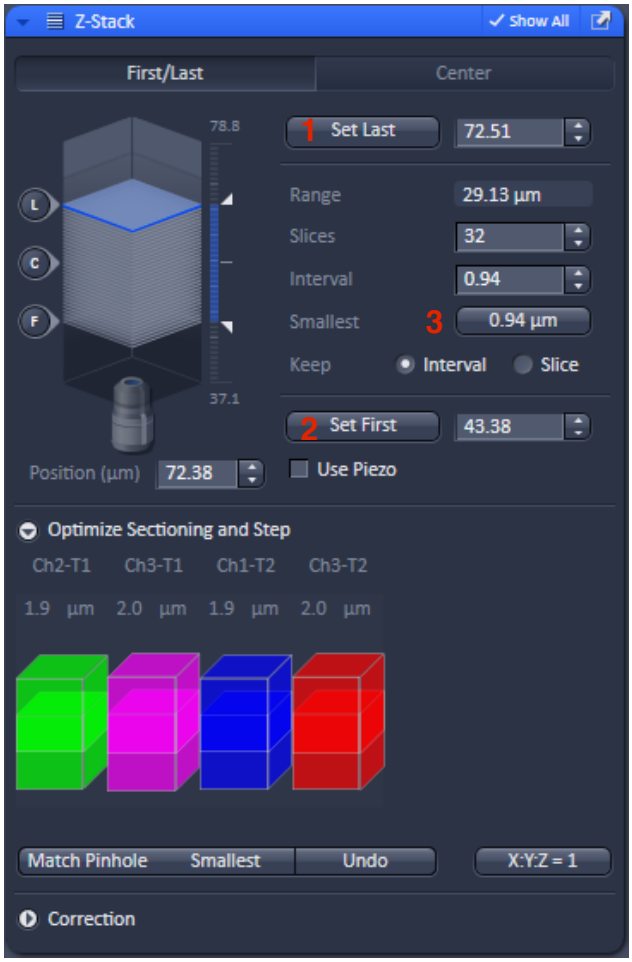
Can be used to image a small section of the image frame or for bleaching experiments.
**The the smaller the region in the Y axis (height) the faster the confocal can scan the image.*
**If using small regions for imaging Z-stacks ensure you enable the piezo Z-drive*




1. Open the **regions** toolbox.
2. Chose the region shape. **(1)**
3. Draw the region over the image.
4. Tick the "fit frame size to bounding rectangle of regions" box. **(2)**
5. If you are using the region tool image ensure you tick on the acquisition check box
6. Click single/continuous to image the region of interest.

# Z-Stacks

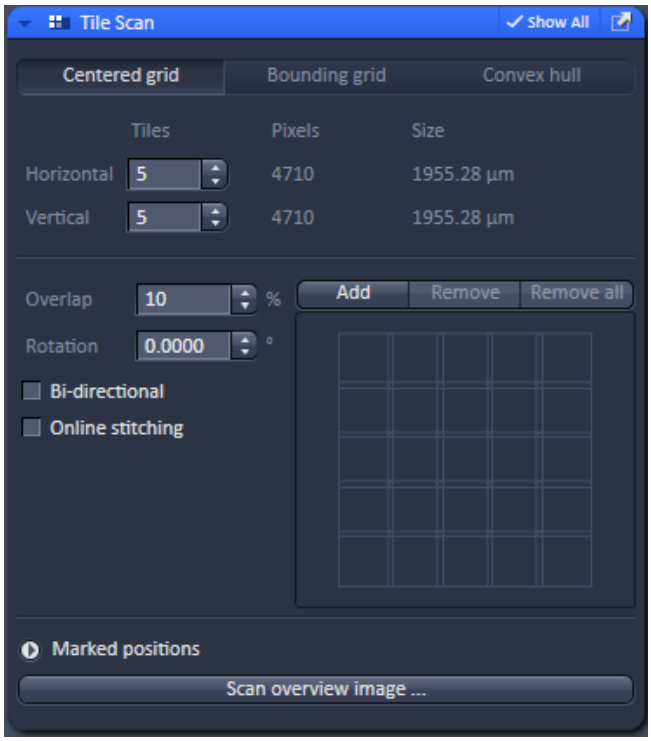
1. Open the **Z-Stack** toolbox (ensure **Show All** is enabled).
2. Select **First/Last** imaging Mode.
3. While fast scanning **using Live,** move the focus to the top of your sample and click **Set Last.** **(1)**
4. Now move the focus to the bottom of your sample and click **Set First.** **(2)**
5. Click **Smallest Interval.** **(3)**
   - To achieve Nyquist sampling, the optimal interval is half the slice thickness (determined by the pinhole settings and objective).
   - For best results you should check each channel in the **Channels** tool and ensure they all have the same *optical section thickness.* This will mean adjusting the pinhole settings slightly for each channel (remember to recheck the gain settings after doing this).
6. Check **Z Stack** under the **Experiment Manager**.
7. Click **Start** record the Z-stack .



# Tile Scan

To create mosiac images of large areas of your sample select **Tile Scan** from the **Experiment Manager**.

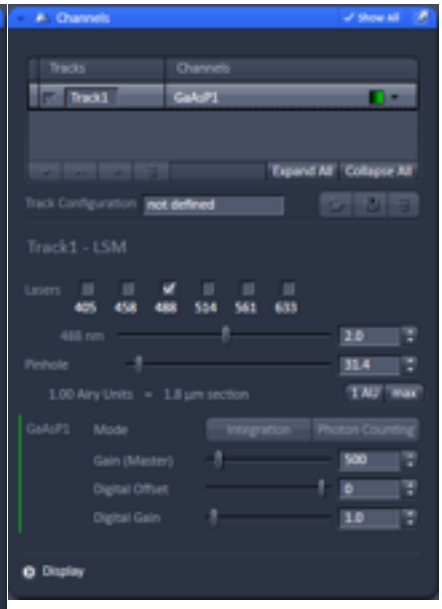
1. Define the number of tiles in the image using the **Horizontal** and **Vertical** spin dials.
2. Define an overlap (typically 10%) between tiles. This is an important step for stitching the tiles in a post-processing step.
3. After acquisition click the **Processing** and using the **stiching** tool to merge the image tiles together into a single image.




# BiG detection

For low light levels the GaAsP detectors can be used.

1. Click the BiG drop-down menu to make the light-path visible.
2. Select **Plate** from secondary dichroics if you are not using recording a track using the Quasar detector.
3. Select between **GaAsP1** for emission between 500-550nm, and **GaAsP2** for emission between 575-610nm.
4. Use **Channels** to configure the detector gain, pin hole size and laser power. Note that **Mode** will be set to **Integration** when imaging, **Photon Counting** is used when recording FCS data.
5. Note that the **Gain (master)** starts at a value of 500 for these detectors.


* The BiG detectors are very sensitive to light - ensure you turn down laser powers to ~1% before acquiring an image

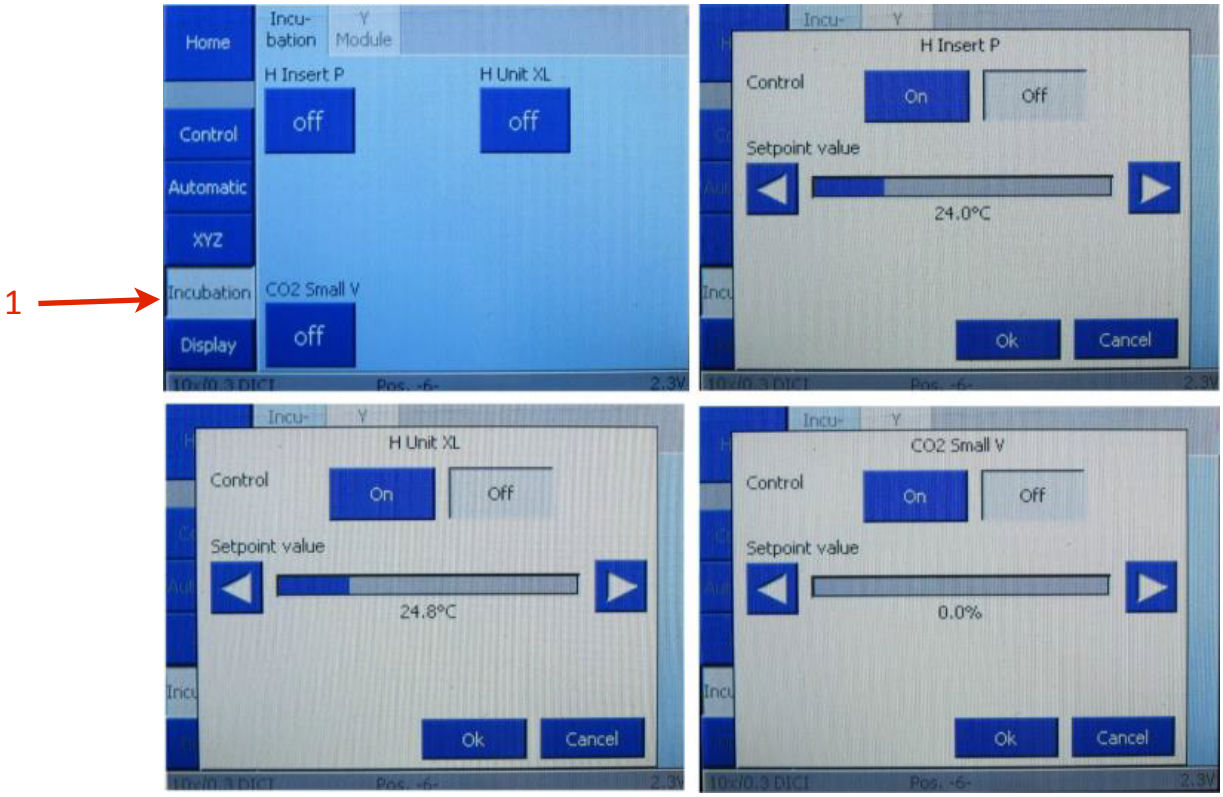
# Heating and CO2 units

The heating & CO2 units can be controlled either by the TFT touchpad or through the software.
It is recommended to pre-heat the chamber for at least 30 minutes prior to a booking which is automatically added when incubation is selected in the PPMS booking system.

**Ensure the power switches for the Heating and CO2 units are ON before turning on the microscope power otherwise the microscope will not recognize these components on start-up.*

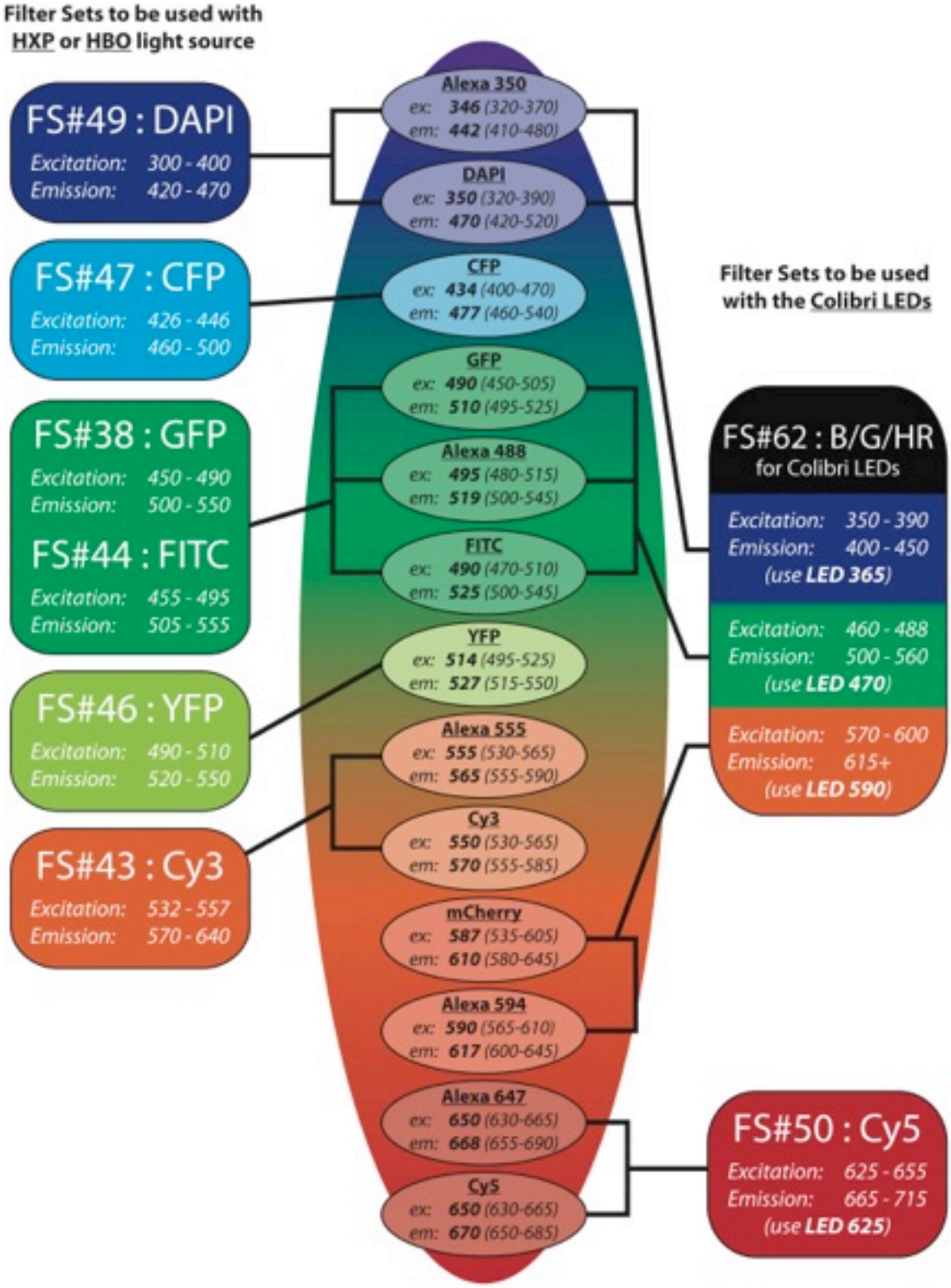
1. On the TFT Touchpad
   a. Select "Incubation" **(1)**
   b. Select unit to control
      i. H Insert controls temperature of the heated stage insert (if present)
      ii. H Unit XL controls temperature of the large incubation chamber (if present)
      iii. CO2 Small V controls the concentration of CO2 pumped through the CO2 cover plate (if present)
2. Set required environmental conditions
3. Set to "On"
4. Select "Ok"

5. Set all Incubation modules to "Off" when finished your imaging session and shut down normally.




- If using software, start PC, login, run software, select Incubator tab and set conditions (Channel 1 is the stage insert, Channel 2 is the CO2, Channel 3 is the chamber). Logout and shutdown as above.

# QBI Fluorescent Marker Guide



# WORKING WITH CONFOCAL AND TIRF MICROSCOPES

**Ergonomics: Use of mouse and keyboard / viewing computer screen** – Prolonged use of the microscope and microscope computer without breaks can increase the risk of muscular strain.

**Eye strain and fatigue** – Viewing samples through microscope eye piece or computer monitor over lengthy periods of time can result in eyestrain and headaches.

**Exposure to sharps** – Exposure to razor blades, scalpels, forceps, cover slips, glass slides could result in cuts or puncture wounds to hands or other areas of the body. Any microscope slide shards or glass debris must be disposed of in the appropriate shapes disposable bin in accordance with PC2 regulations.

**Exposure to intense fluorescent and laser light** – Lasers and a xenon light source are attached to this microscope and are the source of intense and potentially dangerous light. Under no circumstances should any optical elements be removed from the microscope light path or fail-safe switches be circumvented. Do not attempt to adjust the lasers, laser light path, or laser modules in any way. Avoid direct exposure to the light.

## Scope

This procedure details the method for using the microscopes equipped with laser light sources.

## Safety Considerations

### Personal Protective Equipment (PPE):

**Laboratory coat**, **latex gloves** and **closed in shoes** should be worn to prevent injury.

### Ergonomics and Risk Exposure:

Appropriate ergonomics, including adjustment of the seat, computer screen and microscope oculars should be undertaken to reduce risk of strain injuries.

### Emergency Procedures:

**First aid may be required for:**

**Exposure to sharps** – Contact the nearest first aid officer from the list that is beside all first aid kits and on safety notice board.

**Exposure to intense fluorescent and laser light** – Seek immediate medical assistance if you have been exposed to intense direct light or laser light.

**In the event of a laser accident, do the following:**

1. **Shut down the laser system.**
2. **Provide for the safety of personnel (first aid, evacuation, etc). If needed, provide further medical assistance for Eye Injuries by:**
   **Proceed directly to:** **Royal Brisbane and Women's Hospital at**
   **Cnr Butterfield St and Bowen Bridge Rd**
   **HERSTON, QUEENSLAND AUSTRALIA 4029**
   **(07) 3636 8222**

   **Note: If a laser eye injury is suspected, have the injured person keep still and looking straight up to restrict bleeding in the eye. Laser eye injuries should be evaluated by a physician as soon as possible.**
3. **Contact UQ Security Emergency on 336 5333.**
4. **Inform QBI's Laser Safety Officer, Rumelo Amor on 04 4907 8485, of the accident as soon as possible.**
5. **A UQ online incident report must be completed as soon as possible after the incident.**

All incidents must be reported to the OH&S Manager and on UQs online incident reporting system.

**Contacts:** Security x53333 or OH&S Manager Ross Dixon 0401 673 654