Multiphoton system user guide

Start up:

1) Unlock the computer by logging in with your PPMS username and password
2) If required, login to the computer with the local username (see note at bottom of monitor)
3) Switch on fluorescence lamp
4) Switch on scan electronics button
5) Switch on power for lasers
6) Turn laser key to 12 o'clock position
7) Double click the LASX software icon
8) Check the configuration is correct:
   Configuration = machine_MP-laser_ON (if using the MP lasers)
   Microscope = DM6000

If using the resonant scanner, it needs to be switched on at this point
Leave the Customised User Settings off.
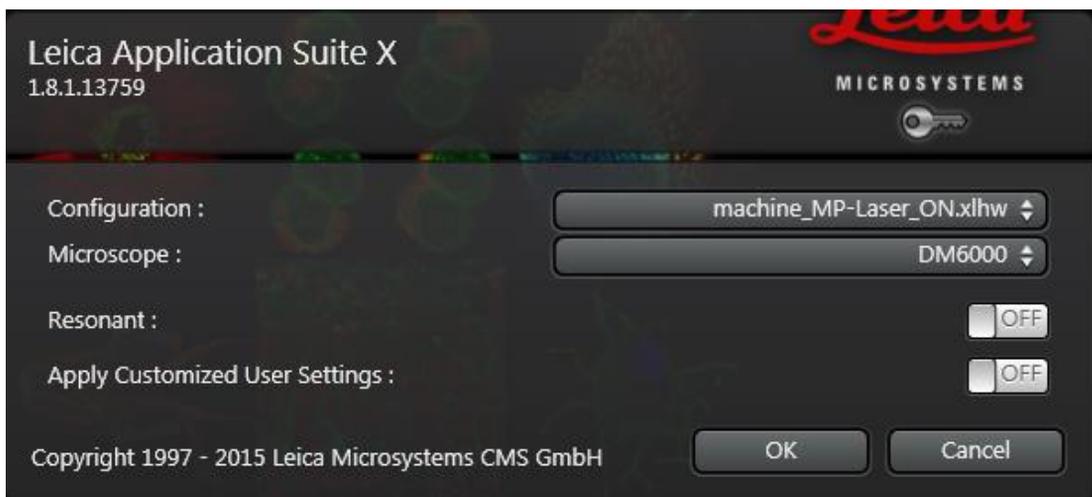
Notes:

- The fluorescence lamp has a shutter button that should always be left on its "remote setting".
- The software will take 1 or 2 minutes to load. It is best to wait until this has finished before trying to use the microscope as this can interfere with the initialisation
- There is a power switch for the microscope touchscreen on its back. This is generally left on.
- Power to the microscope is always left on. The control box is behind the table. Be aware that when power to the microscope is switched on, the microscope stage will initialise by going through its full range of movement - the moving stage could potentially damage objective and condenser lens if they have not been moved out of the path of the moving stage. If a sample is on the stage, this could also get knocked off.
- Power to the cooler for the external detectors (box under the table) is always left on.

Microscope touchpad and controls:

XY stage movement

Focus (black dial = course, white dial = fine)
Note turning the black dial in long slow movements gives faster z movement than turning it rapidly

First menu option

Functions:
- Changing lamp intensity of brightfield (BF) or FLUO lamps
- Changing field diaphragm of transmitted light path (useful for setting up condenser for SHG or BF)
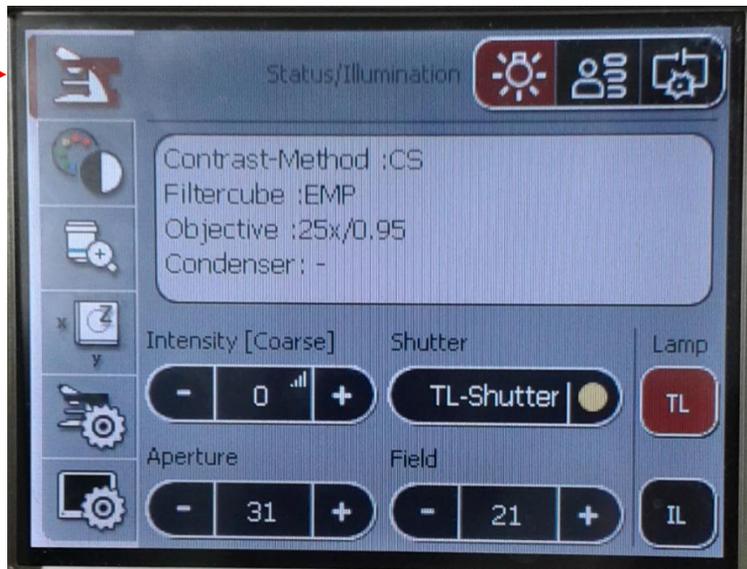
Second menu option

Functions:
- Switching from scanning mode (CS) to FLUO or BF mode.
- Changing FLUO filter cubes

Forth menu option

Functions:
Change the coarseness of XY stage movement

Initial steps in software:

- Go to "Configuration" page, select laser(s) and turn on the MP laser.
- Go back to "Acquire" page and select the lens you want to use (you can also do this by rotating the lens turret by hand).

Note that we have 2 water dipping lenses (10x and 25x with ceramic endpieces) which are suitable for dipping into aqueous media. The other water lenses (20x and 40x) must not be dipped into aqueous media but can be used with a drop of water on a coverslip on top of your sample.

It is best to select the lens before putting your sample on the stage to avoid knocking the sample when the lens turret rotates.

Putting sample on the stage:

- Open front door panels and lift up the viewing window on the front of the microscope.
- Turn on the internal light switch (beneath viewing window).
- Check that a suitable sample holder is on the swinging arm.
- Before moving the arm under the objective lens, ensure there is clearance between it and the condenser and objective lenses:

  - Move the objective lens fully up (using microscope control unit)
  - Check that the condenser lens is fully lowered (using dial directly behind condenser)
  - Use lever to unlock position of arm, then supporting the weight of the arm, lift or lower height as appropriate. Then move underneath the objective lens **very slowly**
  - Note that the arm may not move up and down smoothly – rotate the arm left and right whilst moving it up or down to aid smooth movement

- Place the sample on the stage

Objective lens

Condenser lens

Lever to lock/release position of rotatable arm

Swinging arm

Position lock used to fix height of swinging arm

Adjust height of condenser
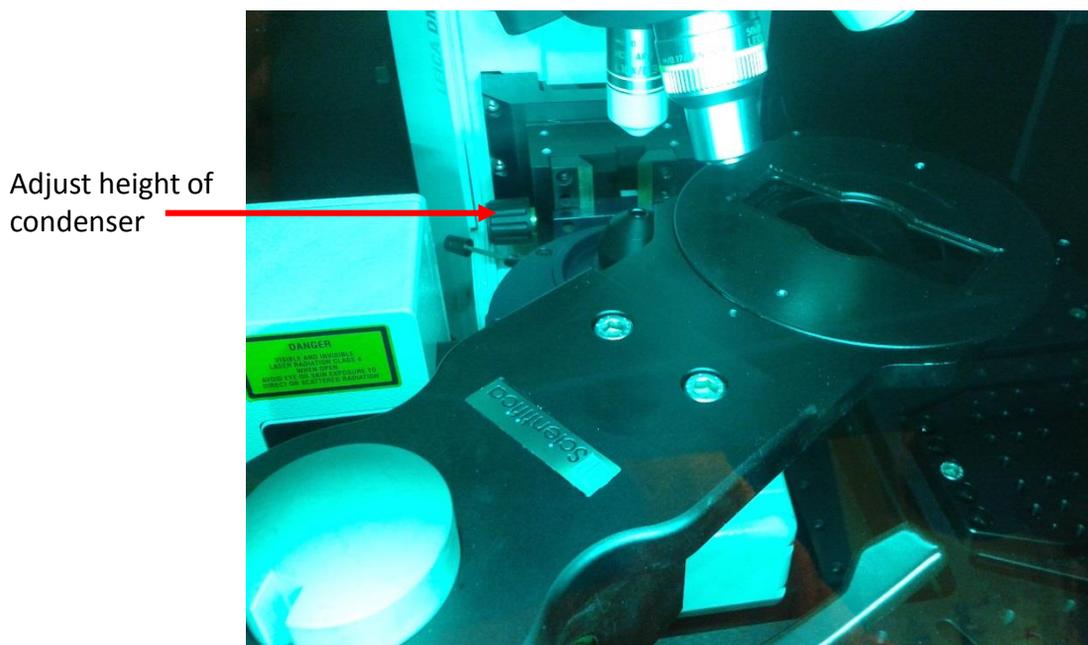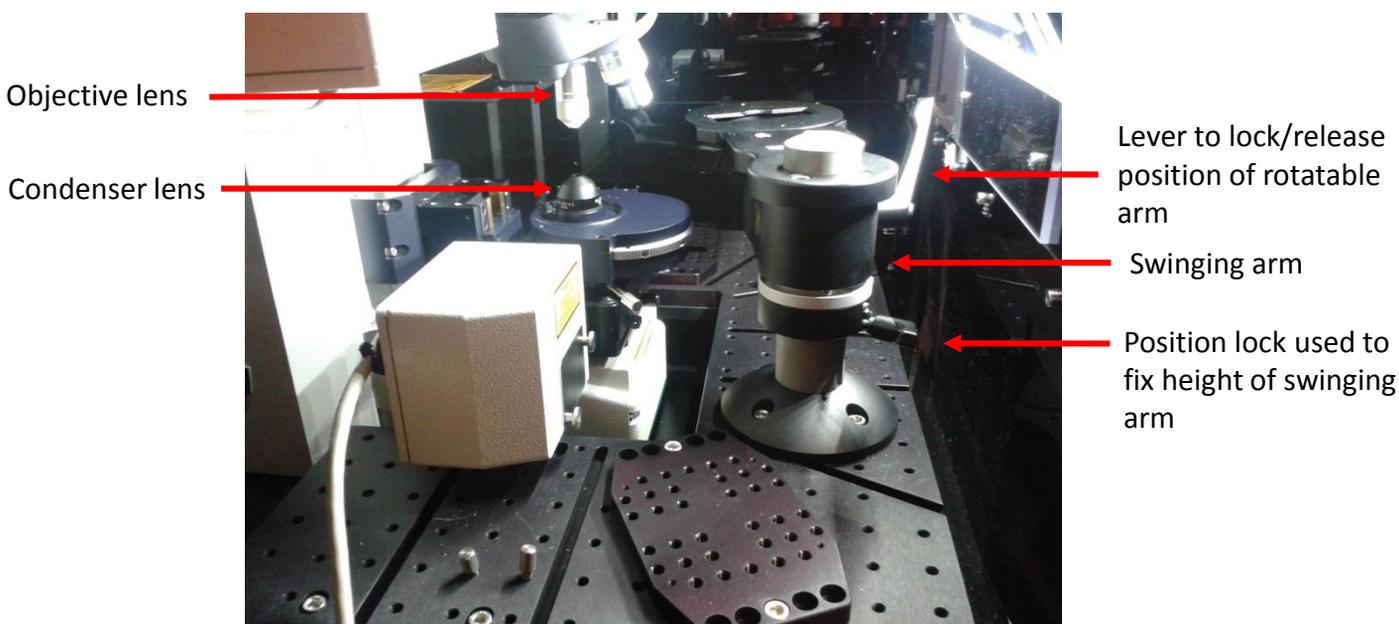
**N.B.
An alternative arm is also available.**

Focusing on sample:

- Adjust the rotatable arm to roughly position the sample beneath the objective lens.
- Finely adjust sample position using the XY control dials on the control pad.
- If using a water dipping lens, lower the lens into the aqueous media in the dish using course focus dial.
- Looking down the eyepieces, use either fluorescence or brightfield to focus on your sample.

Notes:

- The dipping lenses (10x and 25x) are suitable for dipping into aqueous media such as water, PBS and cell culture media. There needs to be sufficient fluid above the sample to allow the lens to dip into it. (approx. 2 - 5mm above top of sample).
Depending on the amount of fluid above your sample, the dipping lenses may need to be dipped into fluid and then withdrawn slightly to find the focus position.

- If your sample is in a petri dish it must be positioned far enough away from the edge of the dish to allow the lens to approach it from directly above.
The photo below shows the 25x lens dipping in to a 35mm dish. The lens is quite wide relative to the dish diameter and this restricts movements of the stage in XY without hitting the sides of the dish.
The sample would have to be placed absolutely central in the dish to allow it to be focussed on. A larger dish may be preferable.
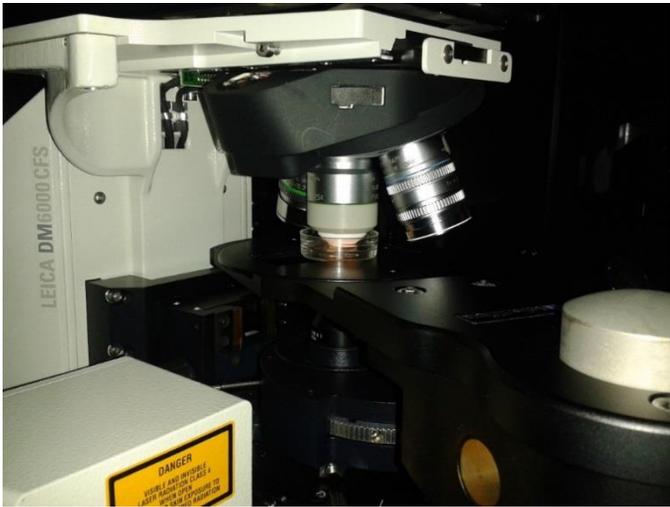
Approx. 1cm for small samples in dishes

- In order to focus on your sample using brightfield, the condenser lens (beneath the stage) will need to be positioned correctly (see next page). Also the slider beneath the condenser needs to be moved to the right.
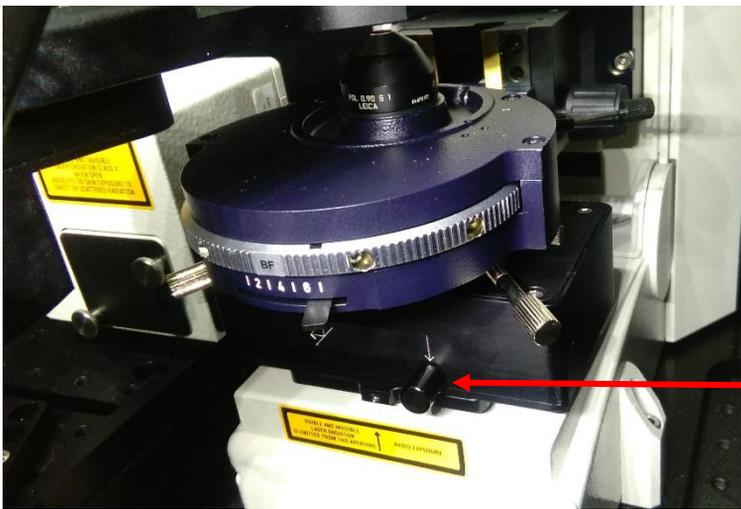
Slider in current position allows you to view brightfield images down the eyepieces.
Move to the left to allow capture of brightfield images

Stage position:

The total range of movement of the objective lens turret in Z is 12.5mm. If the sample is positioned too low then it will be out of the range of movement of the objective lens. If this happens, move the lens fully up, adjust and lock the height of the sample arm higher, then refocus. As a rough guide, for samples in imaging dishes or slides the sample arm can be fixed about 1cm from its top position.

If you are using the transmitted light detector for brightfield or forward direction SHG, it is important also not to position the stage too high as this will move the sample too far away from the condenser lens beneath the stage (see next page)

Software window showing focus range of objective lens:

As you focus the lens down towards the sample, the bar will move upwards.

Ideally you'll have a more than adequate range of movement both up and down from your central focus position

Bar indicating current focus position

Range of Z movement of lens
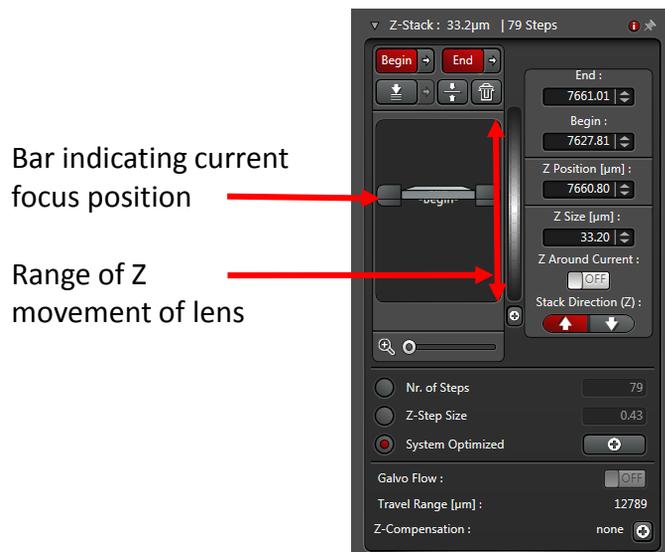
If the stage position has been set too high then the bar will be close to the bottom of the Z range in the above software graphic. If the stage has been set too low then the bar will be close to the top position. In either case the lens will be near the end of its range of movement and you'll need to adjust the stage position and refocus - focus the lens away from the sample first, then adjust stage height and lock, then refocus and check the position of the bar.

Setting up the condenser for transmitted light detection:

For the transmitted light detector to be used effectively for forward direction SHG, it's important to optimise the height and alignment of the condenser lens (this is less important for brightfield imaging but still worth doing):

- Focus sample using fluorescence and switch to BF mode.
- Move the slider under the condenser to the right to view BF down eyepieces.
- Close BF field diaphragm (using touchpad).
- Looking down the eyepieces, move the condenser up slowly towards the bottom of the sample.
- Adjust until you can see the edges of the field diaphragm in focus (or the best focus possible). Generally the octagon of light will be in the centre of the field of view but if necessary there are adjustment screws at the front of the condenser which can be used to align towards the centre.
- Open field diaphragm.
- The slider under the condenser must be pushed to the left to send light to the PMT when capturing images.

Notes:

- **Important** - the condenser lens must be lowered at the end of a session or before moving the rotatable arm from under the objective lens, otherwise the stage will hit it.

- The default condenser lens is an air lens (NA =0.9). Alternatively an oil condenser lens (NA =1.4) can be used (with Type F immersion oil). This should give transmitted light images approx. 2x brighter.

- To get a brightfield image in the transmitted light detector, the filter block in front of it should be carefully removed and placed into the adjacent container. Please replace this filter when you finish your session.

- To get an SHG image, the filter cube needs to be in position (the cube holds a BP 440/20 filter by default for SHG using 880nm laser light).

Software overview:

Configuration tab. Use to switch on lasers

Fixed 1040nm MP laser

Tuneable MP laser

Visible light lasers

Acquisition modes (see below)

Image acquisition parameters

Z-stack parameters
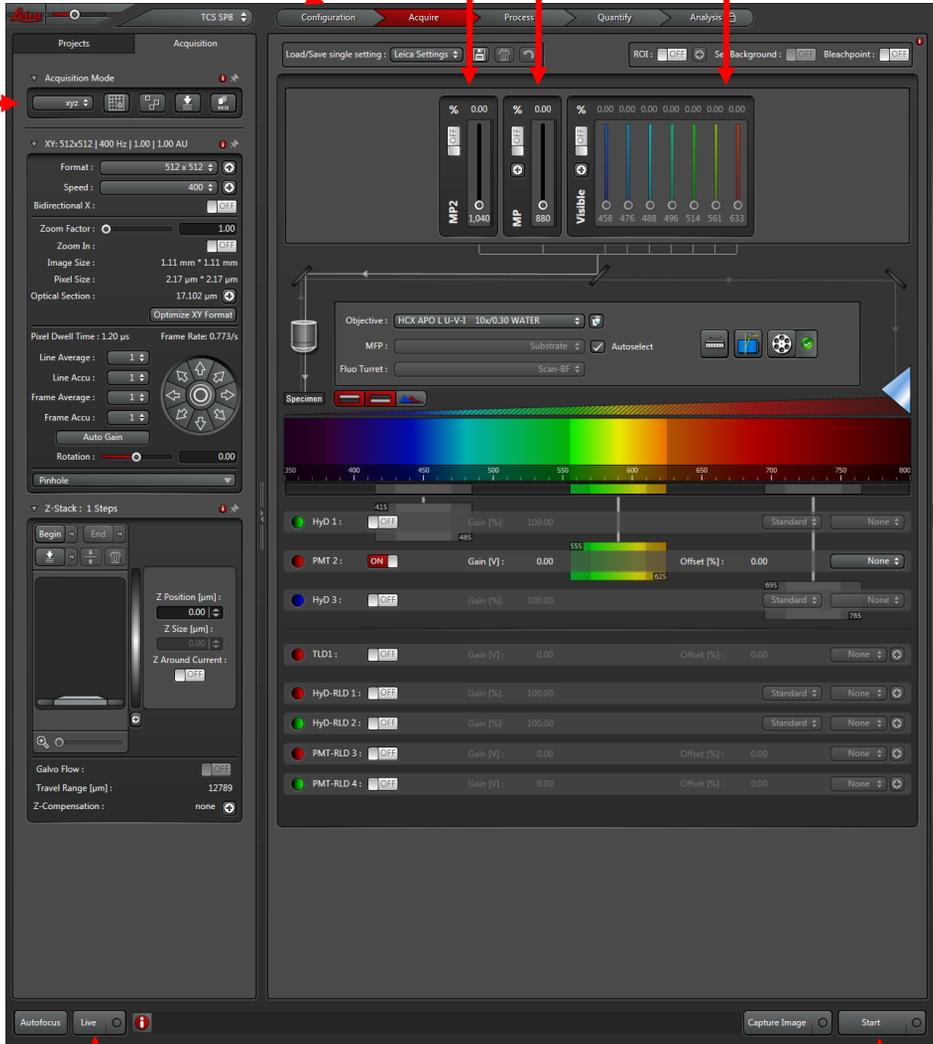
Internal detectors

External detectors

Live – for continuous scanning to set brightness, focus, zoom etc..

To capture a single image

To capture multiple images – Z stack, timecourse etc…

Acquisition modes :
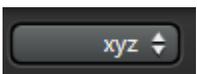
| Icon	Description
xyz	Drop down list for other options (e.g. XYZT, XT).
(tile icon)	Tile scans – for capturing adjacent fields of view and then automatically stitching them together .
(mark and find icon)	Mark and Find – for capturing images at multiple discrete positions over time.
(autofocus icon)	Autofocus – note that there is no AFC (adaptive focus control) system on this microscope, only software based best focus.
SEQ.	Sequential scanning – note that "between lines" sequential scans are not possible with the MP lasers. "Between frames" or "between stacks" can be used instead.

Setting up the software

Laser Excitation:

- Go to Configuration window and switch on the MP laser

- Click the ON buttons of the lasers you want to use

- Click the plus sign under the MP laser (this will bring up this window)

Configuration | Acquire | Process | Quantify | Analysis

Load/Save single setting : Leica Settings

ROI : OFF Set

% 0.00   % 8.06   % 0.00 0.00 0.00 0.00 0.00 0.00 0.00

ON   ON   OFF

MP2 1,040   MP 800   Visible 458 476 488 496 514 561 633
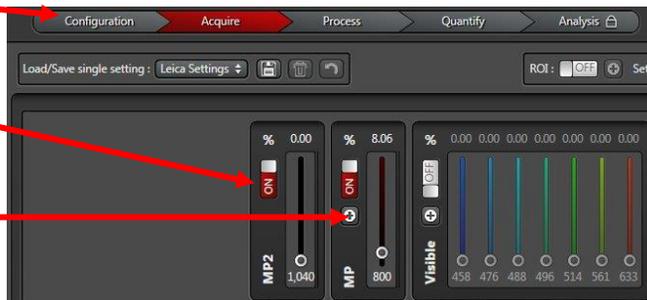
1040nm laser   Tuneable laser

- Click on "Shutter tuneable" and/or "Shutter :1040" and **hold for 3 seconds** to enable these lasers.

- To adjust the wavelength of the tuneable laser, type in required value or adjust slider and click "Tune" (may need to click twice) It will take a few seconds for the laser to tune to the new wavelength (see bottom of the screen for progress bar).

- **NEVER** click the Laser Shutdown button (we keep the MP laser on standby).

- GDD (Group Delay Dispersion) curve - select the lens you are using from the drop down list.

▽ Current Laser Values
Wavelength   800 nm Output Power :   1.27 W
▽ Adjust Laser
680 nm   1300 nm
                          800 nm Tune
▽ Status :
Shutter Tuneable ●
Shutter : 1040 nm ●
Laser Shutdown ○
Power :   on
Mode :   pulsing
Shutter :   Open
▽ GDD
GDD Curve :   25x/0.95 Water
GDD Fine Tuning : - ◎ +   ready
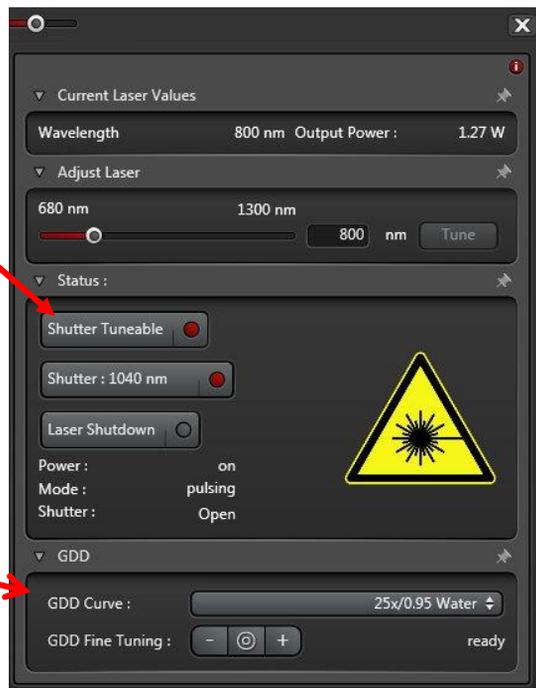
Emission detection:

Important:
- Before selecting an external detector for your emitted light check which emission filters are in place (see overleaf).
- The external HyD detectors can be damaged if hit with too much light -before switching them on you must make sure there is no light inside the black box (from the internal viewing lamp, brightfield or fluorescence light) and that the main room lights are off.

Internal detectors (generally for confocal imaging rather than MP imaging)

Transmitted light detector for brightfield or SHG (Second Harmonic Generation) imaging

External detectors
Note that the light that is detected depends upon which filters are positioned in front of them and which beamsplitter is in place (see diagram overleaf).

HyDs

PMTs

350 400 450 500 550 600 650 700 750 800

HyD 1 : OFF   Gain [%]   100.00   Standard   None
415
485
PMT 2 : OFF   Gain [V]   0.00   Offset [%] :   0.00   None
555
625
HyD 3 : OFF   Gain [%]   100.00   Standard   None
695
765
TLD1 : OFF   Gain [V]   0.00   Offset [%] :   0.00   None
HyD-RLD 1 : OFF   Gain [%]   100.00   Standard   None
HyD-RLD 2 : OFF   Gain [%]   100.00   Standard   None
PMT-RLD 3 : OFF   Gain [V]   0.00   Offset [%] :   0.00   None
PMT-RLD 4 : OFF   Gain [V]   0.00   Offset [%] :   0.00   None
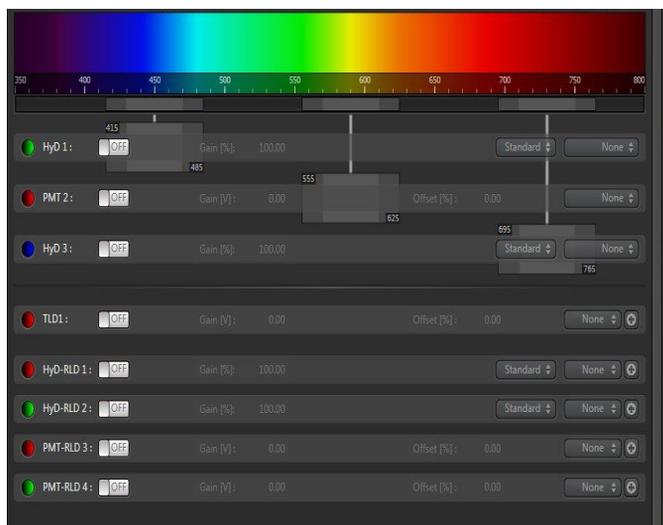
Emission detection continued:

Checking which emission filters are in front of the external HyD detectors

To check which emission filters are in front of the HyD detectors, remove the panel on the top of the environmental chamber.

Use the Allen keys to unscrew the protective lid to the HyD filter chamber.

Look inside. The standard setup has a filter cube with an mCherry (BP 630/75) and GFP (BP 525/50) emission filter.

If these are the filters you want to use just screw the protective lid back on and replace the panel on the top of the environmental chamber

If you want to use a different set of emission filters with the HyD / PMT detectors please call down to the office (x12142) and ask one of the facility staff to change the filters and the beamsplitter mirror
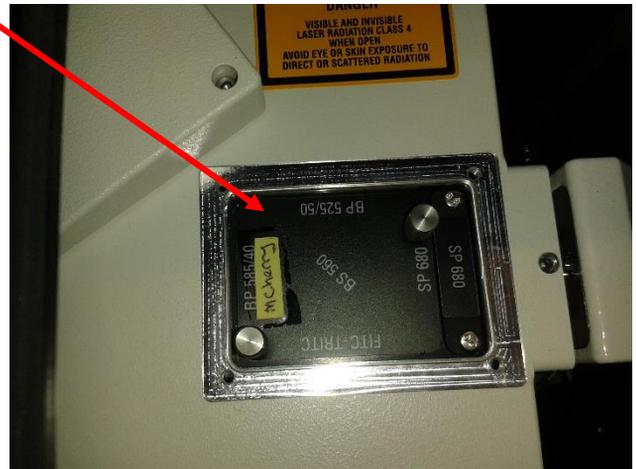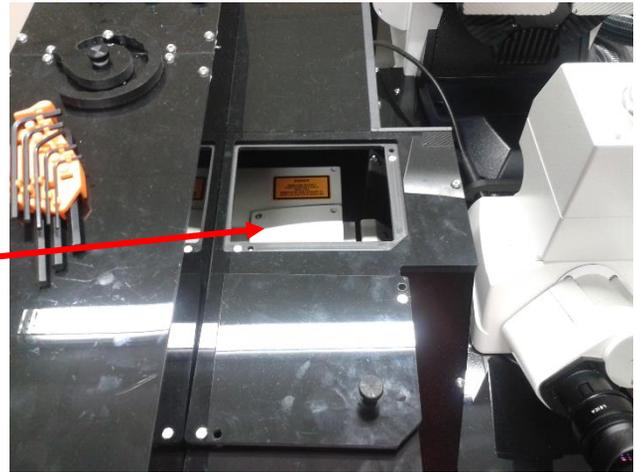
Standard configuration - with SP 500 beamsplitter in place

Detector	Emission Filter	Example fluorophore
HyD 1	BP 630/75	mCherry
HyD 2	BP 525/50	GFP
PMT 3	BP 483/32	CFP
PMT 4	BP 440/20	DAPI, SHG with 880nm laser (reflected)
Transmitted light PMT *	BP 440/20	SHG with 880nm laser (forward)

Alternative configuration - with SP 565 beamsplitter in place

Detector	Emission Filter	Example fluorophore
HyD 1	BP 650/50	Alexa 647
HyD 2	BP 585/40	TRITC
PMT 3	BP 525/50	GFP
PMT 4	BP 460/50	DAPI, SHG with ~ 920 nm laser (reflected)
Transmitted light PMT *	BP 440/20	SHG with 880nm laser (forward)

SP = shortpass
BP = bandpass

- BP 525/50 filter also available.
- Brightfield images can be obtained by removal of the 440/20 emission filter from the lightpath – if you require this please ask one of facility staff to do it safely.

Scan settings:

In the XY window:

- Select the image format (in pixels). For custom formats click on the adjacent plus sign.
- Select scan speed (400 Hz is the default). For custom formats click on the adjacent plus sign.
- Select bidirectional to capture information on forward and backward sweeps of the laser (Phase X alignment should be on -33.72 if bidirectional scanning is active).
- Normally best to start with a low zoom to get a large field of view (lowest zoom factor is 0.75).
- To zoom in – turn on the "Zoom In" option and draw a ROI on your image.

Notes:

○ Increasing the scan format will decrease the pixel size but the images will become noisier due to lower pixel dwell time. This can be countered by using more averaging, lower gain or slower scan speeds.

○ 600Hz is the maximum scan speed that can be achieved at zoom of 1 (unless the resonance scanner option is active). If you select a speed higher than 600Hz then the software will automatically fix a higher minimum zoom

○ Selecting "Optimize XY format" will increase the number of pixels (format) to give the optimal pixel size for the lens and zoom factor you are on.

○ The optical section
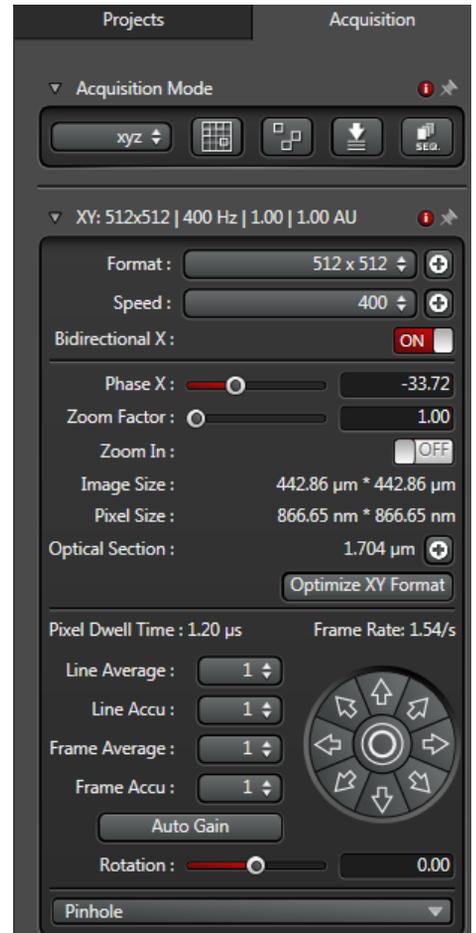
- Line or frame average can be used to average pixel intensities of successive scans.
- Line or frame accumulations can be used to add the pixel intensity of successive scans.
- The direction arrows allow you to pan around your sample (only if zoomed in).
- The rotation slider allows you to rotate the image on screen.

Notes:

○ By default, averaging takes place only when capturing images and not on the live scans.
○ Often 2 – 4 averages should give clean images if capturing at 512 x 512 or 1024 x 1024 and if detector gain setting are not very high (below 100% on HyD detectors). More averaging could be advantageous if the format is set to higher pixel number or if a higher gain is being used or if using high scan speeds (especially the resonant scanner).
○ Line averaging can be combined with frame accumulation but not line accumulation.
○ Note that the pinhole is not used when using the MP lasers and the external detectors

Imaging 2 or more fluorophores / channels:

Simultaneous excitation

It's possible to excite 2 fluorophores simultaneously using a single wavelength laser excitation due to the broad 2 photon excitation peaks of many fluorophores. Simultaneous excitation of 2 fluorophores can also be achieved using both the tuneable and fixed 1040nm laser
In both cases, the emission filters in front of the detectors may provide sufficient separation of the 2 fluorophores
Advantage = faster acquisition
Disadvantage = bleed through of one fluorophore's emission into the other's channel

Sequential excitation

Alternatively 2 fluorophores can be excited one at a time using sequential scanning which will give better separation of fluorescence signals at the cost of speed of acquisition:

Setting up sequential scanning for multiple channels:

- Select the sequential scan acquisition mode and the sequential scan window will open.

- "Seq 1" (channel 1) will be highlighted. Define your channel 1 (i.e. which laser and which detector is active).

- Click on the plus sign to bring up "Seq 2" (channel 2). With this highlighted, define channel 2.

- Select either "Between Frames" or "Between Stacks" option (see below). Note that "between lines" sequential scanning is not possible with the multiphoton lasers.

Further channels can be added and defined in the same way.

Optimising images:

- When "Seq 1" is selected, click live and optimise the images (laser power, detector gain, averaging etc.).
- Change to "Seq 2" and optimise this channel.
- Click "Capture Image" to capture both channels or "Start" to capture both channels with any other acquisition dimensions (e.g. z stack or timecourse).

Between frames / Between stacks:

- Either option is fine for capturing images at a single focus position, however for capturing z stacks there can be a significant difference in time:

- Capturing a 2 channel sequential scan "Between Frames" will capture channel 1 then channel 2 before moving to the next Z position. There will be significant time delays (approx. 1.5s) switching the detectors on/off each time the software switches channels.
  If using the tunable laser for both channels then this causes approx. 5s delay each time it tunes.

- "Between Stacks" will capture the entire z stack in channel 1, then change to channel 2 and capture the z stack again. So less time delays overall but a time mismatch between when corresponding z positions in each channel were captured. This is likely to be undesirable if imaging live specimens.

- If imaging live samples using the tunable laser for 1 channel and 1040nm laser for the 2nd channel, a "fast" alternative is:

o Capture "Between Frames" but leave both the detectors used active on both Channels.
o Set the laser powers for both lasers to be the same on both channels but use the laser shutter to switch off the undesired laser
o This will remove the delays of switching the detectors on/off. The only delay will be the laser shutter times
o Post acquisition you can crop out the 2 undesired images (where the detectors were left on)
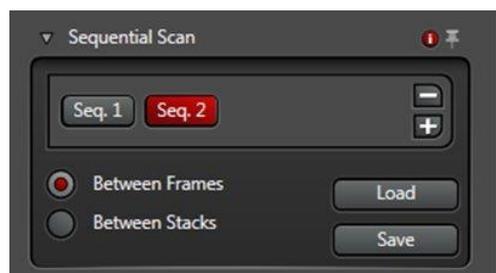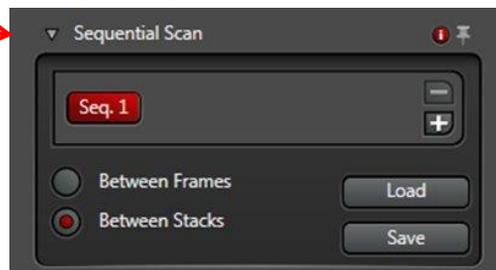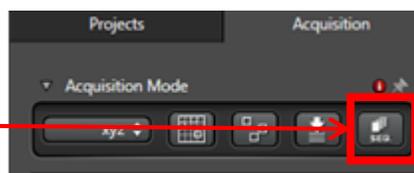
Z stacks:

To set up:

- Start a live scan, adjust focus and move to the top or bottom of you sample – click begin.
- Focus into your sample until you come to the other end of your area of interest – click end.
- Stop the live scan.
- Note the "Z size" - the distance between the begin and end positions you have set.
- Define "Z-step size" (distance in z between images in the stack) or alternatively the total number of steps you want to take.
- For optimal z resolution click the (+) button next to "System Optimised". Tick the multiphoton option and input the emission wavelength of one of the fluorophores you're using.
- The magnifying glass slider allows you to zoom in on the z-stack you have set. You can then interactively adjust the beginning and end of the z-stack whilst scanning.

Note that it is not always necessary/desirable to capture at the optimized Z resolution settings (due to the time taken, photo bleaching etc.).

Formula of Optical Section Thickness

$$dz\ (AU)\ [\mu m] : 1.271 \qquad \sqrt{(\lambda * n / NA^2)^2 + (AU * n * \sqrt{2} * 1.22 * \lambda / NA^2)^2}$$

Emission λ [nm] : 580
Refractive Index (n) : 1.33
Numerical Aperture (NA) : 1.1
Airy Units [AU]: 1

Multiphoton
Use from System
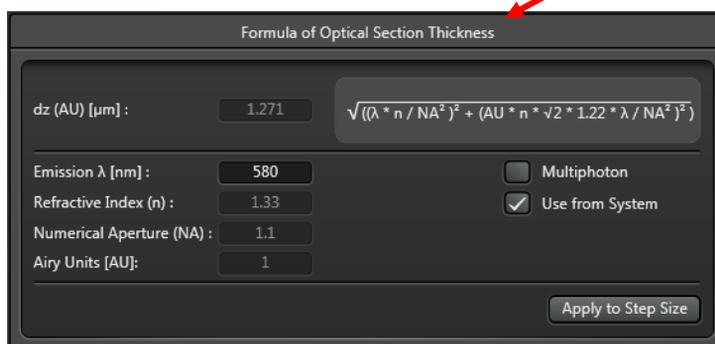
Notes:

- The z movement on the control panel is set to 1 micron per turn of the dial by default. You can change the sensitivity of this dial by clicking the control panel button.

- Z stacks can alternatively be defined by specifying a z range around the current focus position.

- The Z compensation option can be used to adjust for decreasing brightness of fluorescence as you focus further into the sample – you can set different laser powers and/or detector gains for different positions in the z-stack (see page 16).

- The z motor on this system is relatively slow compared to the z-galvo motor on our other confocals. Generally focus movements between z positions take about 100ms.

The "Galvo Flow" option allows you to capture images in the z stack without the z-galvo stopping at each focal plane.

Timecourses:

Select an acquisition mode with a time dimension from the drop down list (e.g. xyzt).

You can define your timecourse in the corresponding dialogue box :
Time interval = time between timepoints
The length of the timecourse can be defined by:

- acquiring until you click stop, or
- the duration in days/hours/minutes/seconds, or
- total number of timepoints ("stacks")
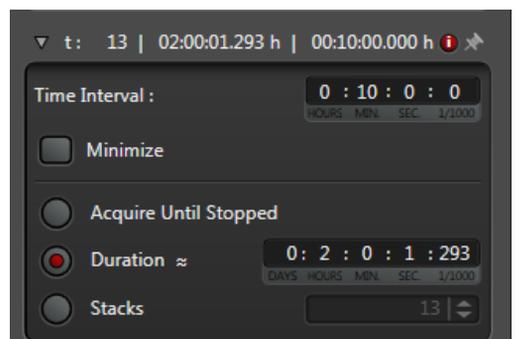
Notes:

- Selecting minimise should show you the minimum time that the software will take to capture a timepoint. However this is not always accurate when you doing "between frames" sequential scans or if capturing multiple fields of view (mark and find).

- Setting up a timecourse in the current version of the software is a bit buggy! When you enter a time interval and duration, hit enter and check that the number of "stacks" adjusts to an appropriate figure (this seems to not update sometimes).

Mark and Find:

This module allows you to define multiple discrete positions on your sample. It is useful primarily in combination with the timecourse mode so that images at each position are taken over time.

To set up:

Select the tile Mark and Find option in the acquisition settings.
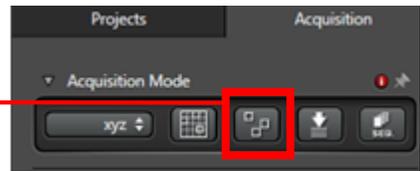
- Click Live" and adjust imaging settings as usual.
- Click "Mark Position" (this will record the XY and Z positions)
- Move XY stage position and adjust focus if necessary. Click "Mark Position" again.
- You can define as many positions as you require, however the limiting factor will be the time interval between timepoints.

Mark Position   Clear Position   Clear All   Redefine Position
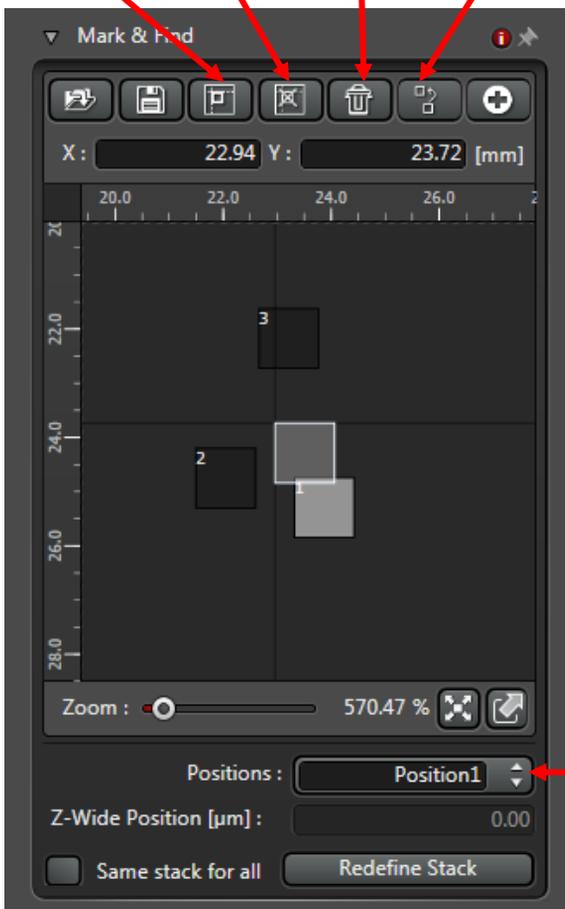
After marking a position, it will be added to a list of saved positions. These can be revisited by selecting them from the drop down list here.

You can capture a z stack at each of the positions you have defined.
By default the software has the option "Same z stack for all" selected.
If you want to define a different z stack for each of your positions, follow the procedure below:

1) Deselect the "Same stack for all" option
2) Move to position 1. Set up your z-stack as normal, then in the mark and find dialogue box click "Redefine Stack"
3) Move to position 2. Repeat the above procedure.
4) After defining z stacks for all of your positions, it's worth checking them. Selecting a position in the mark and find panel will change the Z stack panel accordingly.

Note that you cannot use different imaging parameters (laser power, gain, zoom etc.) at different positions using the "Mark and Find" module. If you want to do this you can use the "Live data mode" Module (see page 17).

Tile Scan:

This module allows you to capture images of adjacent fields of view and then stitch them together. This enables you to capture large fields of view at high resolution

To set up:

- Select the tile scan option in the acquisition settings.

- Move the stage to one corner (e.g. top left) of your area of interest and click "Mark Position".
- Move to the opposite corner (e.g. bottom right) of your area of interest and click "Mark Position" again.
- The software will create a grid of adjacent images positions encompassing the area bounded by these two positions.
- Click "Start" to capture the images. By default the software will stitch together the captured images.

In the screenshot to the right, position 1 was marked at the bottom right and position 2 at the top left of the sample. The software has calculated a 4x4 grid of images to encompass the area bounded by these 2 positions.
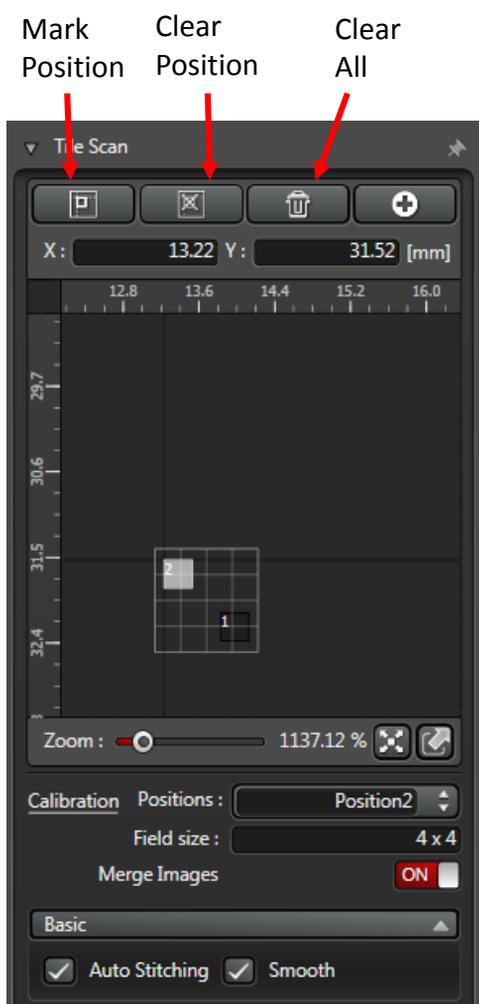
Notes:

- Tile scans can be captured with multiple channels, Z stacks and timecourses.

- Large tile scans can be time consuming. It may be worthwhile having a quick test run of your image grid in order to verify that it covers the entire area of interest in X, Y and Z. For the test scan you can capture at low resolution (e.g. 64x64 pixels), faster scan speed (600Hz), use no averaging and with larger z spacing (if capturing z stacks).

- Image brightness and Z stack positions can be different in the centre of you sample that at the edges (positions 1 and 2). Check the settings by moving the stage with the xy dials on the microscope controller until the highlighted square is somewhere within the centre of the grid. Avoid clicking on the tile scan graphic to do this! Use the dials on the microscope controller. Clicking outside the boxed area will cause the stage to move off to that position (it's then possible to knock the sample off the stage or damage the lenses)

Using the Resonant Scanner:

The resonant scanner allows you to scan your sample at 8000Hz, allowing faster acquisition rates.
High scan speed with the resonant scanner can be achieved lower zoom factors (minimum 1.25x) than faster scan rates without it, i.e. using the default galvo scanners where fast scan speeds are not compatible with low zooms:

e.g. when the 1000hz scan speed is selected with the normal scanner, the minimum zoom permitted is xx

The resonance scanner may be advantageous for imaging live cells as the low pixel dwell times can help reduce photo damage. (have we shown this/can we test this again?)

In order to use the resonant scanner, it must be switched on during the software initialisation.

The scan speed of the resonant scanner is 8000Hz.

The Phase X for bidirectional scanning with the resonant scanner should be set to -3.14.

The minimum zoom factor is 1.25x.
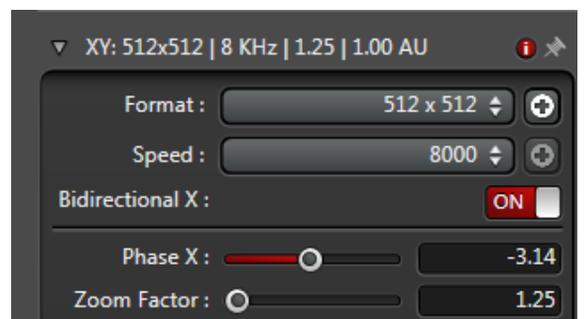
The images acquired with the resonant scanner are much noisier than at lower scan speeds and therefore it can be hard to see what's going on without using line averaging.
It can therefore be useful to use activate line averaging on the live scans. You can do this under in the hardware settings in the configuration menu.
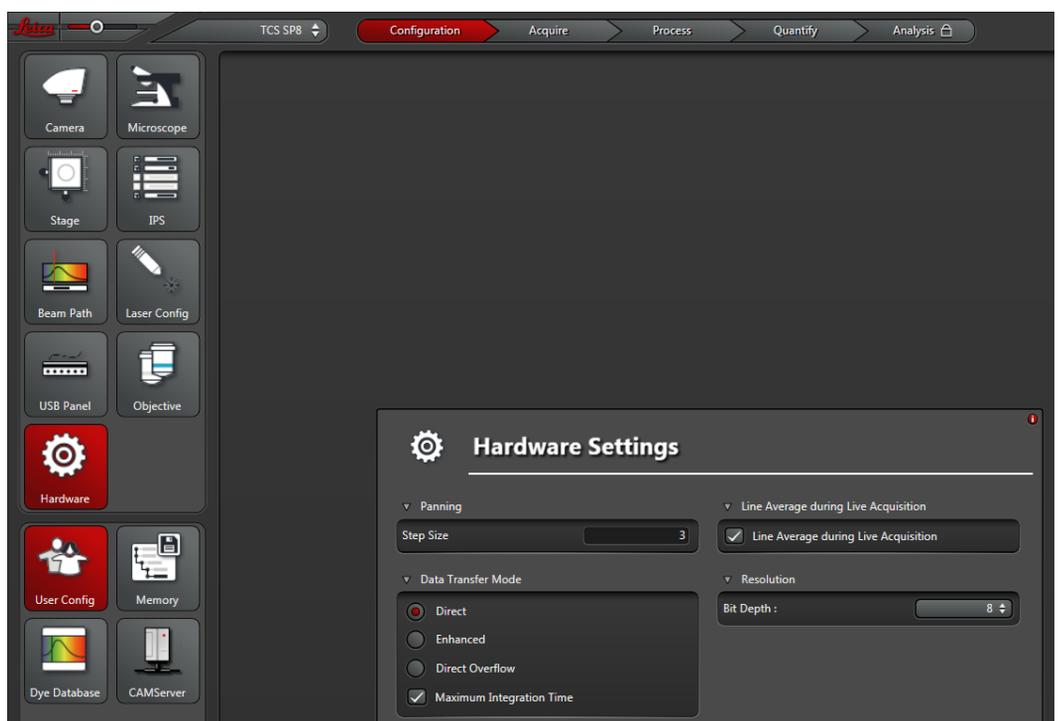
Z compensation:

Z compensation can be used to correct for the possible restriction of emitted light reaching the detector as sample depth increases.

- Define the begin and end position of your z stack.
- Click the (+) sign next to Z compensation to bring up the dialogue box below.

- Select whether you want to compensate by changing laser power, gain or both. In the example below, just the MP laser power attenuation is selected:


- A window will appear showing the laser powers for the begin and end positions (the same power at this stage).
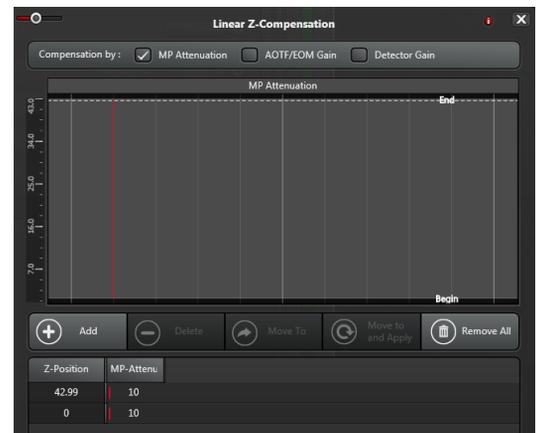
- Click on the end position (here 42.99) and click "move to". The focus will move to this position. Use live scan and adjust the laser power for this end position.
- Click add.
- The software will linearly change the laser power from its level at the begin position to the level at the end position.

- You could alternatively focus back through your z stack (position shown by the horizontal white line) and add other z–positions at which to specify laser powers.
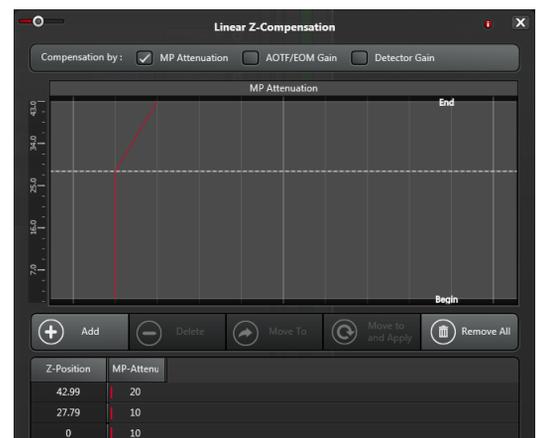
Note that you can also use z compensation with sequential scans using different adjustments of laser power on the different channels (see next page).

Z compensation on sequential scans

- After setting up z compensation on your 1st channel, switch to Seq 2 in the sequential scan window

- The z compensation window will now appear blank and ready to set up your laser powers for Seq 2

- To set the laser power for the begin position of the stack, click to "move to begin" arrow in the z stack window.

- Use a live scan to adjust the laser power and then click Add in the z compensation window

- Click the "move to end" arrow in the z stack window. Then use a live scan to adjust the laser power for this position in the z stack and click add in the Z compensation window.

- Additional positions / laser powers can be added between the begin and end positions

Live data mode:

This mode allows more flexibility in image capture settings than the regular software interface. Discrete confocal "jobs" are defined and a "pattern" created for the image acquisition.

For example:
- Capturing timecourses at phased acquisition rates.
- Capturing images at multiple positions using different laser powers/gains/zooms etc.

For demonstration, the setup below captures z stacks at 2 different XYZ positions using different laser powers at each position. An initial fast capture rate is followed by a longer slow capture rate:

1) Select "Live Data Mode" from the drop down menu.
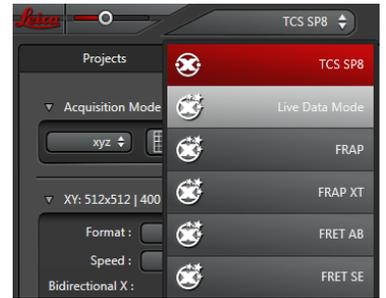
2) Add a new confocal job and define z stack and laser power for position 1.
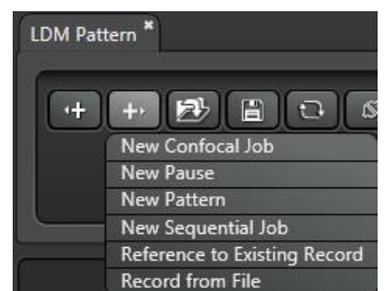
3) Add a second new confocal job, move to second XYZ position and Define the z-stack and laser power for this position.

4) Add a pause.
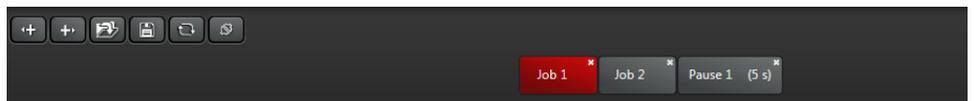
5) Right click on the pause to define its length.
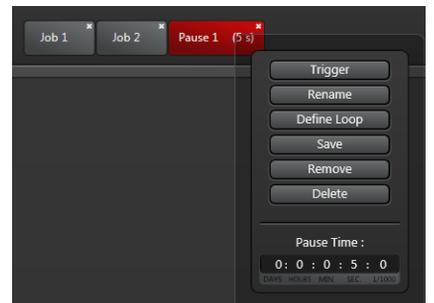
6) Use the shift key to select both jobs and pause then right click to define the loop. Enter how many times to loop.

7) For the second phase of the timecourse:
Duplicate jobs 1 and 2 adding "Reference to Existing Record" from the dropdown list of add options and select the jobs from the list.

Add another pause and define the second loop.

8) "Start Pattern" will begin acquisition of all the jobs.

Note: Be very careful switching between Live Data Mode and the SP8 mode – the last used focus and stage positions changes in those modes are restored! If in doubt, it may be safest to remove sample when doing this and moving stage out of the way of the condenser/objective lenses.

Finishing a session

Always:

- Save and close your files.
- Switch off external hybrid detectors (if on) in the software.
- Move objective lens up to its top position and lower condenser lens to its bottom position.
- Remove your sample from the stage - you may need to lower the stage slightly and swing the arm out (slowly) to do this.
- Samples and liquid waste must be taken back to your own lab for disposal.
- Put some distilled water on a piece of lens tissue and wipe objective lens.
- If you have removed the filter cube in front of the transmitted light detector, put this back into its slot.
- Check on PPMS to see if anyone is booked on after you.

If nobody is booked on immediately after you:

- **Important** - Switch off the MP laser - go to configuration > laser > click the MP laser off button (you may need to click this twice).
- Close software.
- Log out of PPMS.
- Turn laser key off, switch off fan for lasers and scan electronics button.
- Switch off fluorescence lamp.

If somebody is booked on immediately after you:

- Leave the MP laser on, fluorescence lamp on, software on. Also leave laser key and other switches on.
- Log out of PPMS.