Olympus FW1200 MPE User Guide

Table of Contents

Starting

1. Start the PC (screen and box)
   Username: Olympus
   Password: BX61WI
2. Turn two red switches on (**1**)
   Do not touch other switches
3. Turn the fluorescence lamp on (**2**)
4. Turn the laser switches on (**3**)
5. Wait until control diodes stop flashing (**4**)
6. Turn the laser keys to the ON position (**5**)
7. Start FV software (there are no credentials)
   Wait for sw booting (ca 1 min)

End of the work

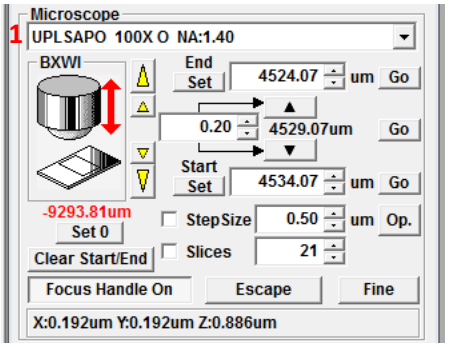
If there is another user after you:

1. Save and close images
2. Turn the fl. lamp off (**2**)
3. Do not turn other switches off
4. Save and close images
5. Do not close the FV software
6. Clean everything
7. Write your name into logbook

If you are the last person using microscope:

1. Save and close images
2. Switch the software and PC off
3. Switch laser keys off (**5**)
4. Switch the fl lamp off (**2**)
5. Wait for timer (300s (5 min) on fl lamp display) (**6**)
6. Turn the laser switches (**3**), fl. lamp (**2**) and two red switches off (**1**)
7. Clean everything
8. Write your name into logbook

Objective change & sample inserting



1. Remove the frontal part of the microscope box
2. Chose objective (manually):

   Objective revolver (10x, 20x dry, 63x, 100x oil)

   Water immersion objectives with long working distance (25x, 40x, 60x)

   Call technician for objective change (2718, room 3.08A)

3. Set the objective in the FW software (*Acquisition Setting* window) (**1**)
4. Insert sample into holder, place holder into microscope

Sample focusing

Microscope is in bright field or epifluorescence mode (using *Trans lamp* or *Epi lamp* and eyepiece)

1. Go to the Image *Acquisition Control* window (on the left side)
2. Chose irradiation: *Trans Lamp* (for BF) (**2**) or *Epi Lamp* (fluorescence) (**3**)
   In case of fluorescence select the filter set on microscope control panel
   DAPI (blue), GFP (green), RFP (red)
3. Focus sample

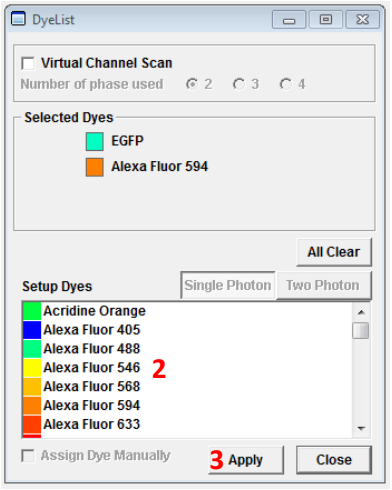
   Recommended way of focusing:

   Carefully place the objective as close to the cover glas as you can (check the distance by your eye while you are turning the focus knob)

   Than look into eyepiece and slowly move the objective up (away from cover glass). You should find the focus plane in a while.

Sample Scanning

Microscope is in confocal mode (using lasers)



Microscope Setting (Lasers, Detectors, filters)

Automatic Setting

1.Click on *Define Dyes* button (*Image Acquisition Control*) (**1**)
2.Select the dyes from list (double click) (**2**)
3.Use *Apply* button – sets lasers, filters and detectors (**3**)

Manual settings (advanced users)

1. Use *Light path & Dyes* button (*Image Acquisition Control*) (**4**)
   Light path & Dyes window will appear

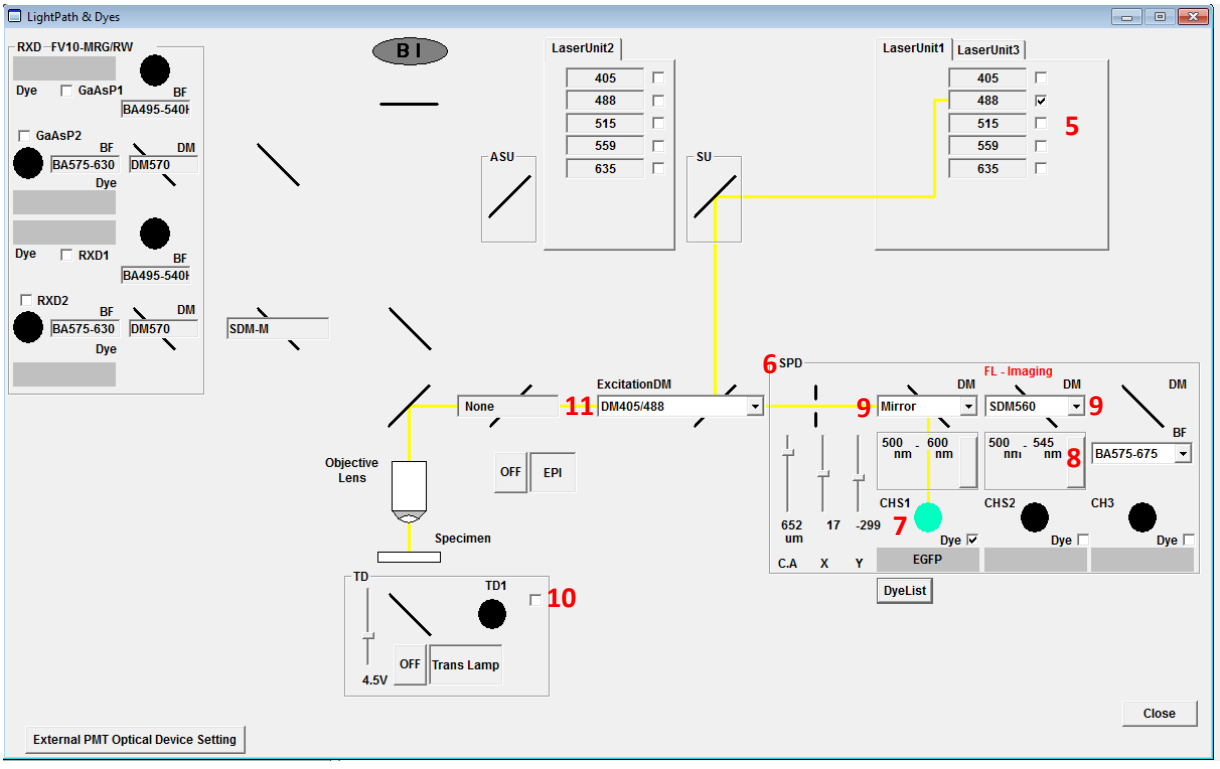
2.Select Excitation Lasers (**5**)

3.Set detectors (**6**) (Dyes (**7**), Range (**8**), Dichroic mirrors (*DM*) (**9**))

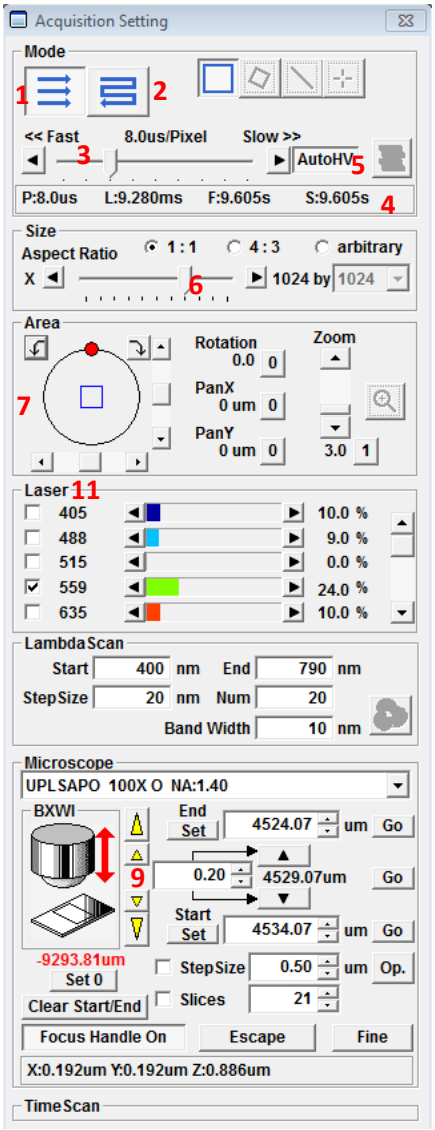
4.You can select the Transmission light detector (*TD1*) (**10**)

5.Set Excitation dichroic mirror (*ExcitationDM*) (**11**)

Call for support if you need an advice or help



Acquisition Settings



1. Set Scanning *Direction*:

   One-directional (default) (**1**): more precise, slower

   Bi-directional (**2**): less precise, faster

2. Set Scan speed (**3**) - the lower speed, the better image quality

   Scan time for one pixel (P), line (L), frame (F), serie (S) (**4**)

   AutoHV – automatical correction of HV with the Scan speed change (**5**)

3. Set Resolution (**6**)
4. *Scan Area* (**7**)

   Allows using *Zoom* or view field shifting or rotation

Sample Scanning

Focusing

1. Go to *Image Acquisition Control* window
2. Start fast scan (*Focus x4* buton) (**8**)
3. Focus (focus knob or arrows in *Acquisition Setting* window (**9**))
4. stop fast scan (**10**)

If there is nothing to see in the Fast scan:

1. Focus
2. Rise *HV* value
3. Check detector range, laser intensity (2 – 10%) (**11**), filters

   Be careful of high laser intensities. Bleaching, autofluorescence or sample degradation is possible in case of high laser power (laser power >15%)
4. Call support

Fast scans (*Focus x2*, *Focus x4*) are only for focusing, quality of images from fast scans is low due to high scan speed and low resolution.

Detector sensitivity setting

This setting must be performed for every channel (dye)

1. Start *XY Repeat* (**1**)
2. Display oversaturated pixels (ctrl H) - oversaturated pixels are red
3. Set *HV* value (**2**) to see only few red dots (not areas), *HV* is generally among 500 and 800.

   HV value is voltage on detector, more *HV* = higher detector sensitivity = better signal = higher noise

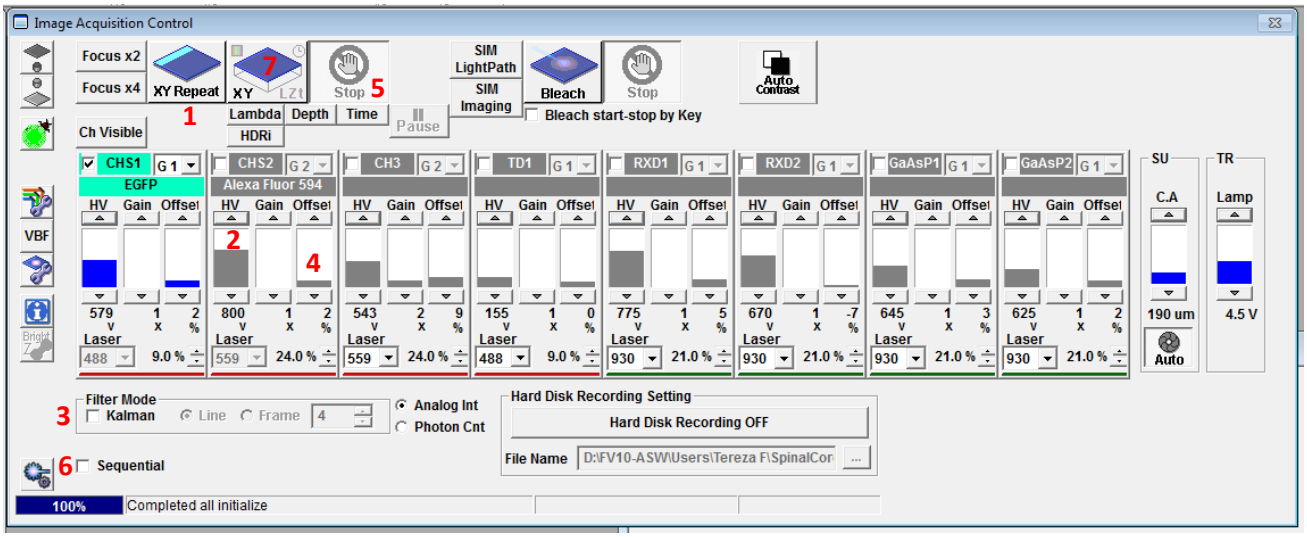
   In case of high noise (high *HV* values) use averaging (*Kalman* box) (**3**) right before final snap

   HV will be corrected automatically with the scan speed, if *AutoHV* button is ON

4. I case of weak samples, you can rise the Laser Intensity (*Acquisition Setting* window)

   Be careful of high laser intensities. Bleaching, autofluorescence or sample degradation is possible in case of high laser power (laser power >15%)

5. Set the *Offset* value if it is necessary (**4**) - zero pixels are blue
6. Hide oversaturated pixels (ctrl H)
7. Repeat this procedure for every channel
8. Stop *XY Repeat* (**5**)



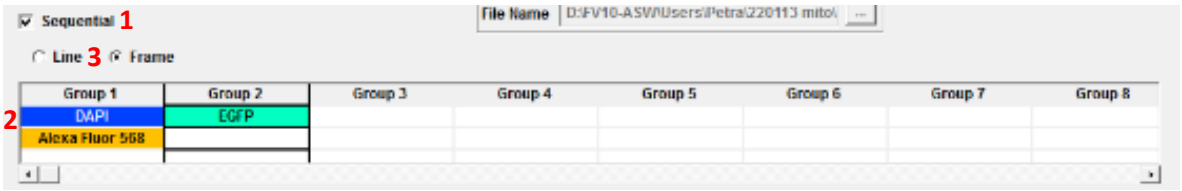
Final image

1. Use averaging in case of high *HV* value (*Kalman* box) (**3**)

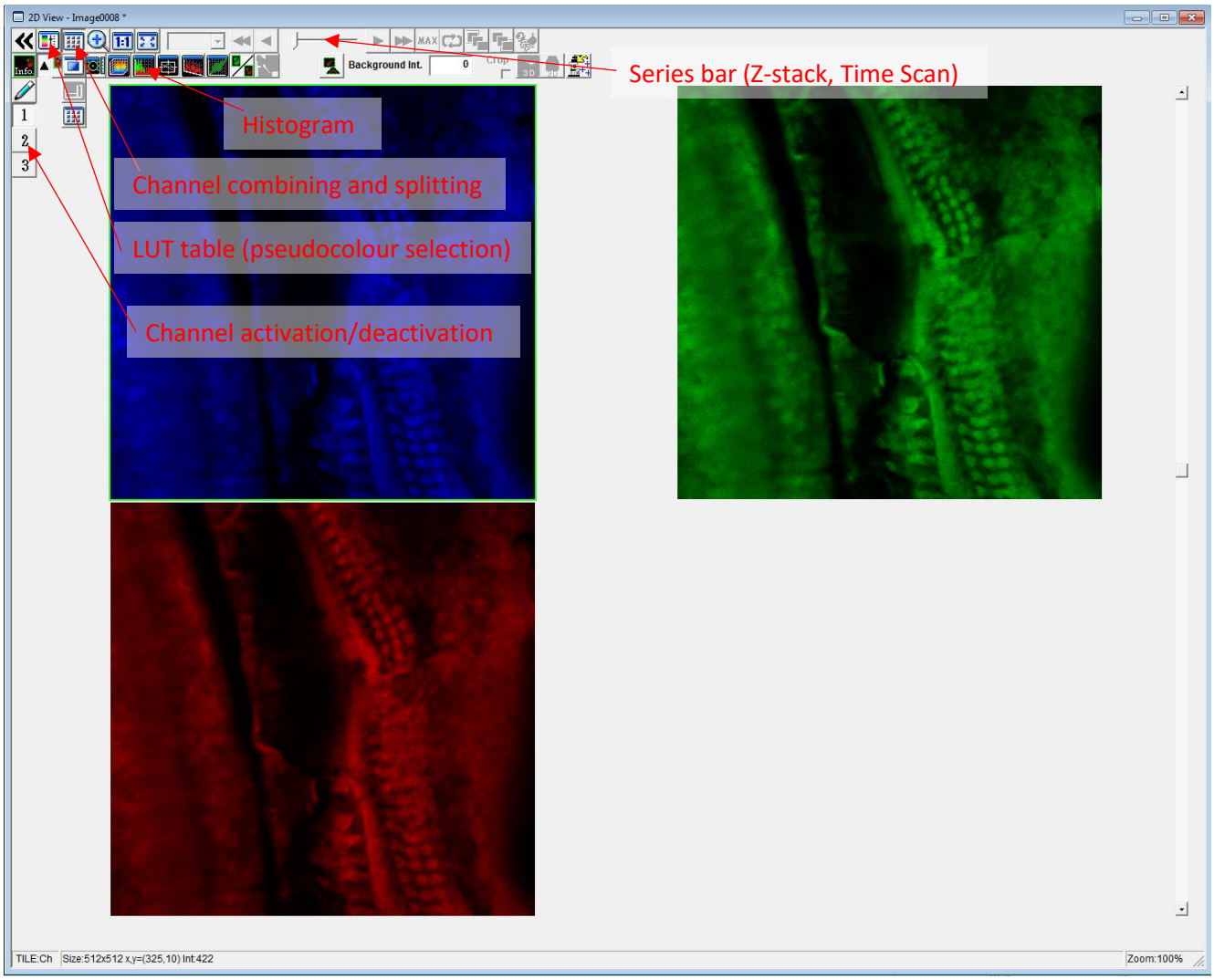
   Use 2 or 4, higher value significantly prolongs acquisition time
2. In case of sequential scanning mark *Sequential* box (**6**)
3. Start *XY* scan (*XY* button) (**7**)

Sequential scanning

1. Mark *Sequential* box (**1**, under *Kalman* averaging)
2. Split the channels into groups (drag it by mouse) (**2**)
3. Set the *HV* value and laser power for every channel separately
4. Channels can be shifted after every *Line* or *Frame* (**3**)
5. Acquire an image (*XY* button)



2D View (final image window)



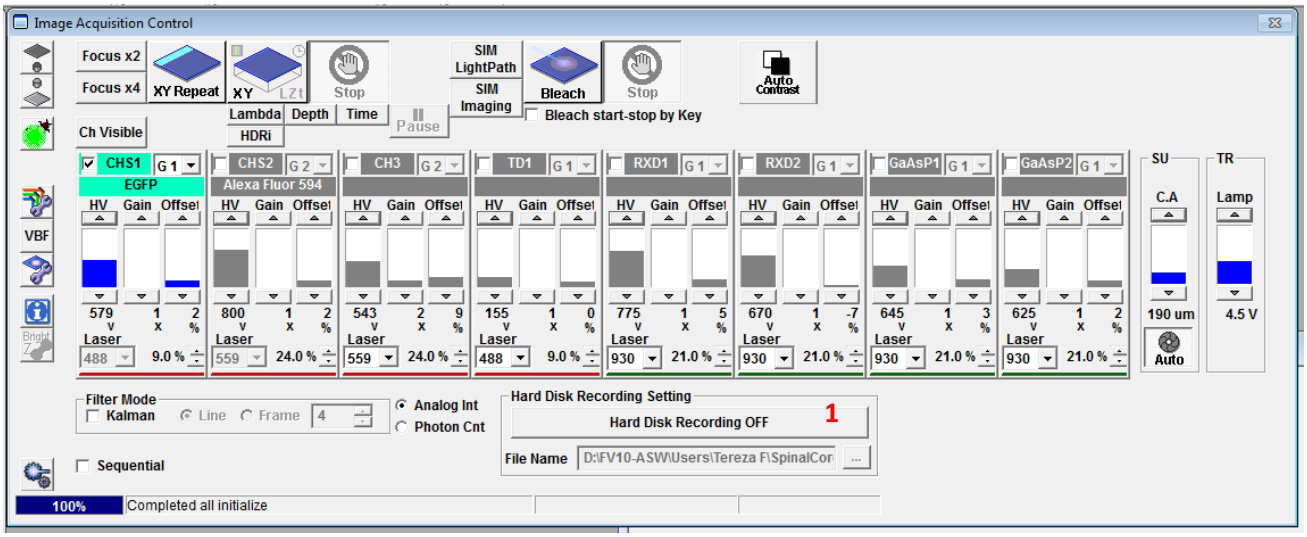
Save Image

Folder: D:FV10/Users/name

a) Manually: File – save or right click on the imege - save

b) Autosave: Hard Disk Recording ON/OFF (**1**)

Automatic save of every image



Z-Stack

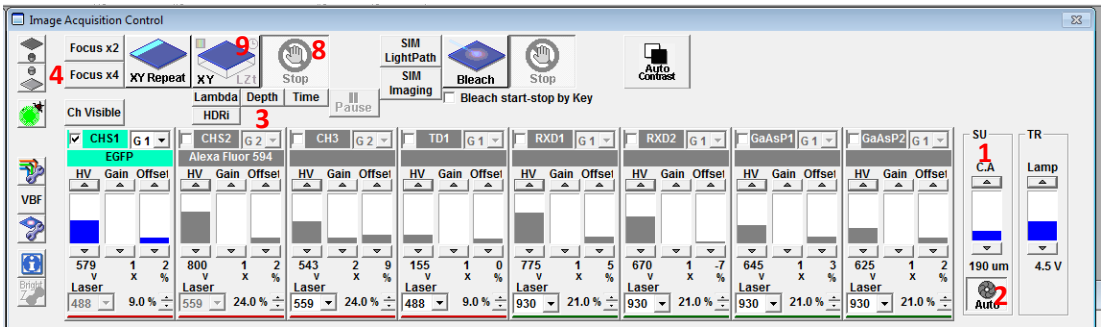
Snaps series of scans (slices) along z – axis

The thickness of the slice is determined by pinhole (Confocal Aperture, *CA*) (**1**) diameter (pinhole is thin aperture in front of the detector). The thinner pinhole = the thinner slice. Pinhole diameter (slice thickness) influent the overall resolution in the z-axis. The thinner slice = more slices contained in Z - stack = better resolution.

The suitable pinhole diameter for reasonable z-axis resolution can be set by *Auto* button (**2**) (Image Acquisition Control). Better resolution can be achieved using lower pinhole diameter, but the signal intensity is remarkably reduced. Lesser pinhole diameters are useful only for bright samples.

If you are not interested in z-axis resolution, wider pinhole diameter can be utilized. The overall acquisition time is then reduced.

The slices in Z-stack must overlap in order to include whole area of interest. The recommended overlap is 50%.



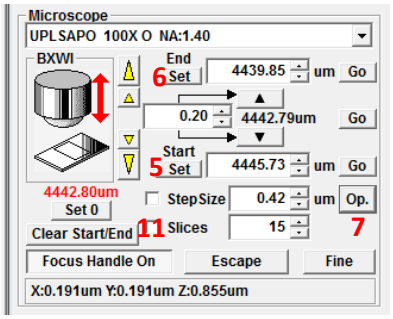
1) Make all settings as for single Image
   Detector sensitivity (*HV*) setting should be performed on the brightest slice
2) Activate the *Depth* button (*Image Acquisition Control* window) (**3**)
3) Activate fast scanning mode (*Focus x4* button) (**4**)
4) Set the Z-Stack Range (*Acquisition Setting* window)

   Define the first position using focus knob

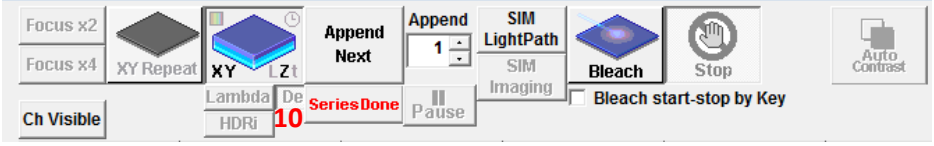
   Use *Set Start* button (**5**)

   Define last position using the focus knob

   Use *Set End* button – Z-Stack range is then defined (**6**)



5) Click on *Op.* button (**7**) – sets the slice number (slice overlap is 50%)
6) Stop fast scanning mode (**8**)
7) Start Experiment using *XYZ* button (**9**)
8) The acquired Z-Stack can be displayed using *SeriesDone* button (**10**) (Image Acquisition Control window)

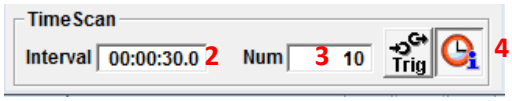


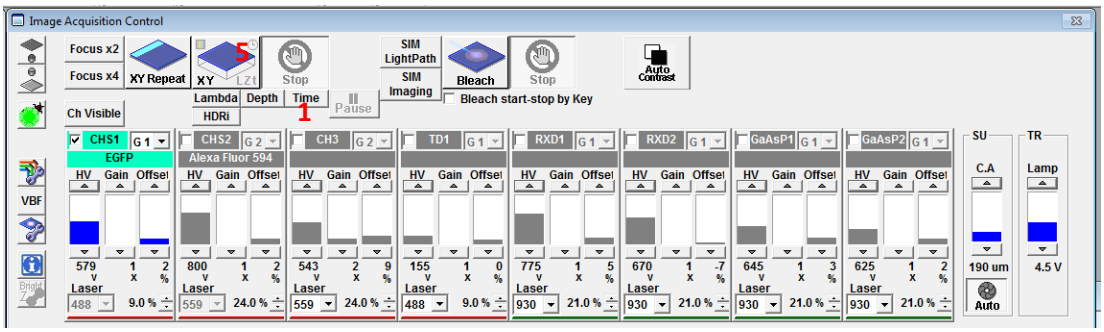
Slice number setting

You can set the number of slices manually (*Slices* box) (**11**). *Op.* button then correct Z-Stack range for 50% overlap)

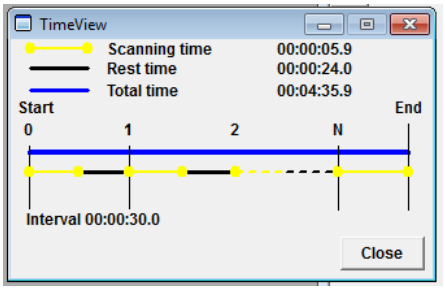
Time scan

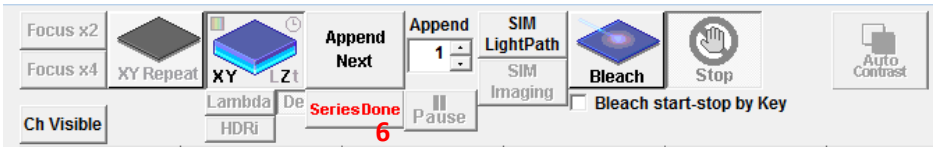
Snap a series of images during the time, you can define the interval between snaps and the overall number of cycles



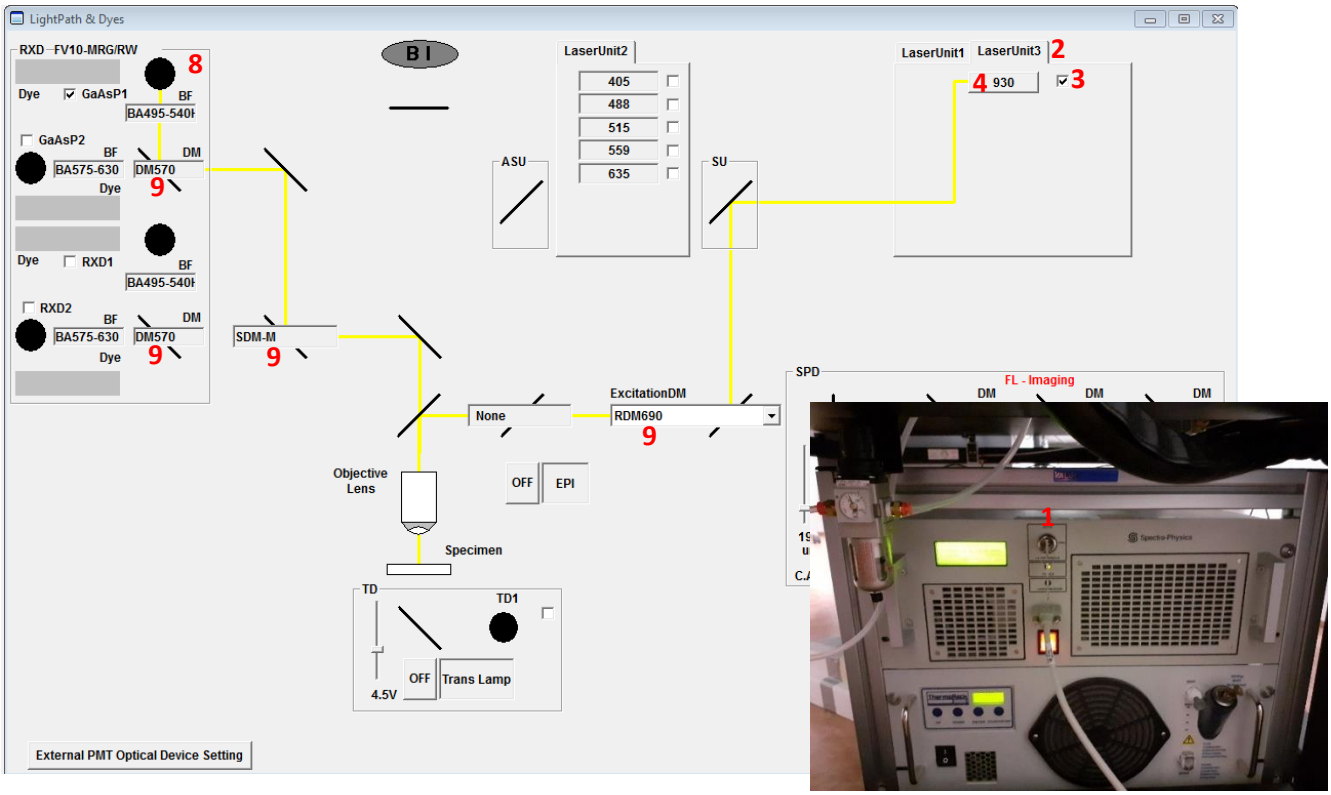


1. Make all settings as for single Image
2. Activate *Time* button (*Image Acquisition Control* window) (**1**)
3. Go to *Image Acquisition Control* window (*TimeScan* section)
4. Set *Interval* (hh.mm.ss.ms) (**2**)
5. Set number of cycles (**3**)
6. Clock button displays the experiment scheme (*TimeView*) (**4**)
7. Start experiment using *XYt* button (**5**)
8. The acquired Time scan can be displayed using *SeriesDone* button (**6**) (*Image Acquisition Control* window)



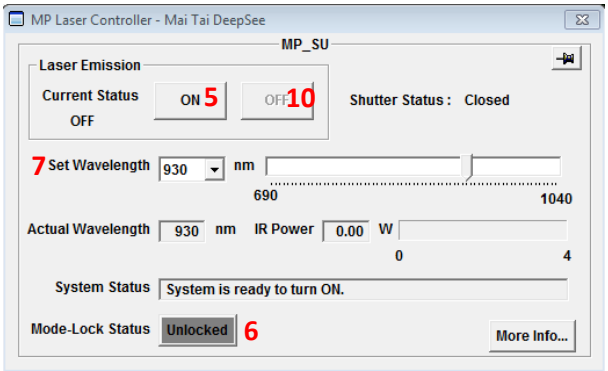


Two-photon microscopy



1. Turn the pulse IR laser ON
    a. Switch the key on laser box (**1**)
    b. Go to the *LaserUnit3* bookmark (**2**) and mark the IR laser (**3**) (*Light Path & Dyes* window)
    c. Click on the laser button (**4**) and Switch the laser ON (**5**)
    d. Wait until the laser is locked (ca 15 min) (*Mode-Lock Status* must be **Locked**) (**6**)

2. Set the wavelength (**7**)
   Set it slowly using 20nm steps
3. Choose detectors (mark proper box) (**8**)
4. Select the proper dichroic mirrors (*DM*) (**9**)
5. Pull the stick on the microscope body inside
6. Start scanning



At the end of the experiment, switch IR laser of:

1. Use OFF button in *MP Laser Controller* (**10**)
2. Switch the laser key off (**1**)
3. Unmark the MP detectors (**8**) and IR laser (**3**)

Non - descanned detectors for two-photon microscopy are very sensitive. Remember to close the microscope box and lit all lights off (in the microscope and in the room) during an image acquisition. The detectors will not work if they detect high light intensity.