# Olympus FW1200 MPE User Guide

# Table of Contents

Starting	2
End of the work	2
Objective change & sample inserting	3
Sample focusing	3
Sample Scanning	4
Microscope Setting (Lasers, Detectors, filters)	4
Automatic Setting	4
Manual settings (advanced users)	4
Acquisition Settings	5
Sample Scanning	5
Focusing	5
Detector sensitivity setting	6
Final image	6
Sequential scanning	7
Save Image	8
Z-Stack	9
Time scan1	0
Two-photon microscopy1	1

# Starting



- Start the PC (screen and box) Username: Olympus Password: BX61WI
- 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)
- 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)
- 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)
- 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



Click on *Define Dyes* button (*Image Acquisition Control*) (1)
Select the dyes from list (double click) (2)
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



Number of phase use	an d 672	C 3	C 4
number of pridae dae	au 18 2	0.0	5.7
Selected Dyes			
EGFP			
Alexa F	Fluor 594		
			All Clea
Setup Dyes	Single	Photon	All Clea
Setup Dyes	Single	Photon	All Clea
Setup Dyes Acridine Orange Alexa Fluor 405	Single	Photon	All Clea Two Photo
Setup Dyes Acridine Orange Alexa Fluor 405 Alexa Fluor 488	Single	Photon	All Clea
Setup Dyes Acridine Orange Alexa Fluor 405 Alexa Fluor 488 Alexa Fluor 546	Single	Photon	All Clea
Setup Dyes Acridine Orange Alexa Fluor 405 Alexa Fluor 568 Alexa Fluor 568	Single	Photon	All Clea
Setup Dyes Acridine Orange Alexa Fluor 405 Alexa Fluor 488 Alexa Fluor 586 Alexa Fluor 568 Alexa Fluor 594	Single	Photon	All Clea
Setup Dyes Acridine Orange Alexa Fluor 405 Alexa Fluor 488 Alexa Fluor 584 Alexa Fluor 554 Alexa Fluor 594 Alexa Fluor 533	Single	Photon	All Clea

#### 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)
- Focus (focus knob or arrows in *Acquisition Setting* window (9))
- 4. stop fast scan (10)

Image Acquisition Control	
Focus x2 B Focus x4 XY Repeat	XY LZ1 Stop 0 Stop 0
	Lambda Depth Time III Imaging Bleach start-stop by Key
Ch Visible	HDRi Fause

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.

Acquisition Setting
Mode
<
P:8.0us L:9.280ms F:9.605s S:9.605s 4
Size Aspect Ratio • 1:1 • 4:3 • arbitrary X   1024 by 1024
Rotation Zoom
7 PanX 0 um 0 PanY 0 um 0 3.0 1
▲ 405 ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲
▲ 488 ▲ ● 9.0 %
515 ◀ ▶ 0.0 %
✓     559     ▲     ▶     24.0 %       ✓     525     ✓     ▲ <td< td=""></td<>
Lambda Scan
Start 400 nm End 790 nm
StepSize 20 nm Num 20
Microscope
UPLSAPO 100X O NA:1.40
BAVI Set 4524.07 ÷ um Go ▲ 9 0.20 ÷ 4529.07um Go
Start Start 4534.07 ÷ um Go
-9293.81um □ StepSize 0.50 ÷ um Op.
Clear Start/End Slices 21 ÷
Focus Handle On Escape Fine
X:0.192um Y:0.192um Z:0.886um
TimeScan

#### 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

- 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)

Sequential 1 File Name DSFV10-ASW/Users/Petral/220113 mito/					(220113 mito)		
Group 1 DMPI	Group 2 EGFP	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
Alexa Fluor 568							

#### 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%.

🗖 Image	e Acquisition Control	٤	3
<b>●</b> ● ● ●	Focus x2 Focus x4 XY Repeat XY L2t Lambda Depth	Stop SM Stop   Stop Bleach Stop   Time Pattee Bleach start-stop by Key	
	CHVISIBLE HDRI 3	CH3 G2 T01 G1 RXD1 G1 RXD2 G1 GaAsp1 G1 GaAsp2 G1 SU	
VBF	HV Gain Offset	1 HV Gain Offset A A A A A A A A A A A A A A A A A A A	
2			
Bright Z	579     1     2     800     1     2       v     x     %     v     x     %       Laser     488     9.0 % ÷     559     24.0 % ÷	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	

- 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)



UPLSAPO 100X O NA:1.40 -		
BXWI 6 Set 4439.85 um Go		
▲ ▲ 0.20 ÷ 4442.79um Go		
Start 4445.73 ÷ um Go		
4442.80um Set 0 Step Size 0.42 ÷ um Op.		
Clear Start/End Sices		
Focus Handle On Escape Fine		
X:0.191um Y:0.191um Z:0.855um		

- Microscope

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)
- 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)

MP Laser Controller - Mai Tai DeepSee		×
	MP_SU	M
Laser Emission		
Current Status ON 5	OFF <b>10</b> Shutter Status :	Closed
7 Set Wavelength 930 ▼ nm		
69	90	1040
Actual Wavelength 930 nm IR	Power 0.00 W	
	0	4
System Status System is ready	y to turn ON.	
Mode-Lock Status Unlocked 6		More Info

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.