

Olympus FV1000 MPE Imaging Protocol

- 1) System Startup
 - Please note our sign-up policy. You must inform the facility at least 24 hours beforehand if you can't come; otherwise, you will receive a charge for unused time. The facility will allow for extenuating circumstances (cells dying, sick day, etc.) if you inform us in a timely fashion.
 - **Follow each step** of the startup poster.
 - *Computer with your user account.*



- [©] Open the FluoView software from the desktop: FV10-A5W 3.1
- The following screen, enter your unique user name and password, and click OK.

Welcome to "F¥10-ASW"
OLYMPUS
FV10-ASW
Ver.03.01
User ID: orourl01
Password: *****
Password OK Cancel

2) Lens Cleaning

- Please clean all of the lenses you intend to use before and after your session. Refer to the lens cleaning poster if you need any help recalling the rules and steps.
- 3) Software control
 - 🕗 Upon initialization of the software, click on the Dye List menu 🛄



One Gustave L. Levy Place | Annenberg 18-250 | New York, NY 10029 MicroscopySRF@mssm.edu | 212-241-0400 Controller.^(a) Ensure the MP laser is set to 800 nm, and turn

In the Acquisition Setting panel, click 800 to open the MP Laser

🔜 MP Laser Controller - Chameleon MP_SU -14 Laser Emission Current Status ON Shutter Status : Closed Set Wavelength 800 $\overline{}$ 1080 IR Power Actual Wavelength 800 nm 0.00 W System Status System is ready to turn ON. Mode-Lock Status Unlocked More Info..

ON

the laser emission

- The laser's Mode-Lock Status is shown at the bottom of the controller menu. When the MP laser is ready for imaging, the status will change
 - from **Unlocked** to **Locked**. This could take up to 5 minutes.
- Once the MP laser is locked, tune to your desired wavelength.
- In the Acquisition Setting menu (right) ensure the correct lens is selected from the Microscope dropdown. If the desired lens does not appear in the dropdown, see facility staff.







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Within the Dye List, select Two Photon and click Close



- 4) Microscope control
 - Coad your slide upside down and take care for the following issues:
 - Select and inspect each slide or dish.
 - If it is dirty, gently clean with a KimWipe and/or cotton swab. You should do this with all your samples before you come.
 - Carefully rest the slide or dish onto the stage, and adjust the stage adapter to fit accordingly.
 - *^{ce}* Use the stage movement control joystick to position your specimen under the lens.
 - To focus your sample:
 - Pull both sliders out on the top left of the microscope. These sliders have three configurations:



Light to oculars

Light to detectors, SDM570 disengaged

Light to detectors, SDM570 engaged

DAPI FITC TRITC

- Move the filterwheel to view the desired fluorescent signal.
 - 3 DAPI
 - 4 FITC
 - 5 TRITC
- Turn on the EPI lamp from the Image Acquisition Control menu.

🔳 Imag	e Acquisition Control			×
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• Open the reflected light shutter on the lower right side of the filterwheel.



- The Focus on your sample and center your region of interest.
 - Note the following controls in this diagram.



- Check all of your fluorophores in your specimen. You need to have an idea of what to expect before you begin.
- *Close the reflected light shutter and turn the EPI lamp* off to prevent photobleaching of your sample.



5) Setting up your experiment

© Examine the following schematic to determine the appropriate configuration for your samples:

4CH NDD FILTER: FV10-MRCYR/XR



SDM570 engaged
> Ch1(CFP), Ch2(YFP), Ch4(R)



SDM570 disengaged > Ch3(wide band DAPI or G) , Ch4 (R)



Light to detectors, SDM570 engaged RXD1 (CFP), RXD2 (YFP), and RXD4 (RFP) available.



Light to detectors, SDM570 disengaged RXD3 (wide DAPI-GFP) and RXD4 (RFP) available.

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📃 Imag	e Acquisition Control	
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6) The imaging process

- ***** Rotate the filterwheel to position 2, **R690**.
- The Push in one or both sliders on the top left of the microscope, depending on the configuration chosen above.
- The open the reflected light shutter.
- The Ensure the microscope is covered and room lights turned off to minimize ambient light exposure to the detectors.
- Click Focus x2 to view a fast scan of your sample.



Convallaria rhizome test slide, Leica Microsystems



☞ In the Live View menu, click 🚺 to show the full image frame.

📃 Live View			
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Click the LUT button to change the color display options.



- Select the Hi-Lo setting for each active channel, ie. select Ch1 and click Hi-Lo, select Ch2 and click Hi-Lo...
- *Close the LUT menu.*



Slowly increase the MP laser power until your signal is visible. Note: increasing laser power too high can damage your sample!



- ⁽²⁷⁾ Use the focus control knob to find the brightest focal plane in your specimen of interest.
- Click Repeation at the top of the Image Acquisition Control menu to stop the fast scan.
- Click XY Repeat to view a slow scan of your sample. This scan will be brighter, and is representative of what your final image will look like.



Adjust the HV (high voltage) on each individual channel to maximize the intensity of your signal while avoiding saturation (red pixels). There should be only a few red pixels in each channel.



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Click RepeatStop at the top of the Image Acquisition Control menu to stop the slow scan.

Click **EXAMPLE** to acquire your image.



To save the acquired images, right click within the image window, and select 'Save As...'



Save all your files as type '.oif' – this will save both the meta data as well as a folder of tif images.





7) Returning to the microscope

- To return to the microscope to find another field:
 - Pull both sliders out on the top left of the microscope.
 - Move the filterwheel to position 3, 4, or 5 to view the desired fluorescent signal.
 - Turn on the EPI lamp 🛃 from the Image Acquisition Control menu.
 - Open the reflected light shutter on the lower right side of the filterwheel.
- Focus on your sample and center your region of interest.

8) Taking a Z-Stack

- The Rotate the filterwheel to position 2, **R690**.
- Push in one or both sliders on the top left of the microscope, depending on the configuration chosen above.
- The open the reflected light shutter.
- The Ensure the microscope is covered and room lights turned off to minimize ambient light exposure to the detectors.
- Click Focus x2 to view a fast scan of your sample.
- Use the focus knob to search for the brightest focal plane in your specimen. Set the laser power and HV for all your channels.
- Set your Z-stack limits:
 - Use the focal knob to set the focal plane towards the bottom of your specimen (rotate down) and click **Set**
 - Use the focal knob to set the focal plane towards the top of your specimen (rotate up) and click **Set**



Start



- Click RepeatStop
- Note the stack size (z-Volume) and number of steps. You may select an optimized Z-interval by clicking Op. This step is critical for post processing techniques like deconvolution or 3D reconstruction.
- The second secon
 - **StepSize** of number of **Slices** in the selections below the set positions.
- The Enable Depth below the Enable below the Enable Depth below the E

The frame **xy** will now turn into a volume **xy**



- Click XY II to acquire your Z-stack.
- When acquisition is complete, you will have the option to either append additional optical slices, or complete the series.
 - To append additional optical slices to the completed Z-stack, enter the number of slices in



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To complete the series, click

🔳 Image	Acquisition	Control			and a second second second
	Focus x2		-	Append	Append
	Focus x4	XY Repeat	XY LZt	Next	
	Ch Visible		N <mark>, De</mark> HDRI	SeriesDone	

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- To save the acquired images, right click within the image window, and select 'Save As...' P
- You can view the maximum intensity projection of the Z-stack by clicking the **maximum** icon within the 2D View P



Microscopy Shared Resource Facility

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9) System Shutdown

- The end of every session, turn off the MP laser.
 - In the Acquisition Setting panel, click 800 to open the MP Laser Controller.
 - Return the MP laser to 800 nm, and turn

the laser emission to

MP Laser Controlle			
Laser Emission – Current Status OFF	ON OFF	Shutter Status :	니피.
Set Wavelength			1080
Actual Wavelength	800 nm IR Power	0.00 W	
System Status	System is ready to turn O	N.	
Mode-Lock Status	Unlocked		More Info

- Before you proceed with the shutdown sequence, please remember to back up all of your data and to clean the dry and oil immersion lenses.
- Sign onto the facility calendar and check for users after your session. If you don't see anything scheduled for less than 2 hours, proceed with the full shutdown sequence shown on the Shutdown poster. Otherwise, close the software and log off your account.
- If you do have to perform a full shutdown, please follow the shutdown poster steps in order.

Acquisition Setting
<< Fast 4.0us/Pixel Slow >>
P:4.0us L:5.184ms F:2.706s S:2.706s
Size Aspect Ratio ● 1:1 ● 4:3 ● arbitrary X ▲ ▲ ▲ ▶ 1024 by 1024 ▼
Area Area Area PanX PanX PanY PanY Oum 0 1.0 1
■ 473 ■ 0.0 % ■ 800 ■ 11.0 %
Microscope XLPLN 25XW NA:1.05
BXWI A End -157.26 🚽 um Go ▲ 10406.83 🛨 -132.26um Go
Start -107.26 - um Go
.54.02um StepSize 10.00 um Op. Set 0 Stices 6 -
Focus Handle On Escape Coarse
TimeScan
Interval FreeRun Num 10 🎲 😋