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.
   - Log into the computer with your user account.

   - Open the FluoView software from the desktop: FV10-ASW 3.1
   - On the following screen, enter your unique user name and password, and click OK.

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.
Within the Dye List, select **Two Photon** and click **Close**.

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

Ensure the MP laser is set to 800 nm, and turn the laser emission **ON**.

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.
4) **Microscope control**

**Load 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.

- 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:

  - 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.
- Open the reflected light shutter on the lower right side of the filterwheel.

![Image of filterwheel]

- Focus on your sample and center your region of interest.

- Note the following controls in this diagram.

  - **Fine/Course toggle button**
  - **Fast raise lens**
  - **Fast lower lens**
  - **Focus knob**

- 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**

![Schematic diagram]

- **SDM570 engaged**
  - Ch1 (CFP), Ch2 (YFP), Ch4 (RFP) available.

- **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.

Enable the corresponding channels within the Image Acquisition control menu:
6) **The imaging process**

- 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.
- Open the reflected light shutter.
- 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.

Click the LUT button to change the color display options.

Select the setting for each active channel, i.e. 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!

Use the focus control knob to find the brightest focal plane in your specimen of interest.

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

Click 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.

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

- 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.
- Open the reflected light shutter.
- Ensure the microscope is covered and room lights turned off to minimize ambient light exposure to the detectors.
- Click 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.
  - Use the focal knob to set the focal plane towards the top of your specimen (rotate up) and click.
Click \( \text{StepSize} \).

Note the stack size (\( z \)-Volume) and number of steps. You may select an optimized \( z \)-interval by clicking \( \text{Op.} \). This step is critical for post processing techniques like deconvolution or 3D reconstruction.

If you don’t need that many slices or to have such small \( z \) slice intervals, you may manually input either the number of \( z \) slices in the selections below the set positions.

Enable \( \text{Depth} \) below the \( \text{XY Lz} \) button within the Image Acquisition Control menu.

The frame \( \text{XY Lz} \) will now turn into a volume \( \text{XY Lz} \).

Click \( \text{XY Lz} \) 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 \( \text{Append} \) and click \( \text{Next} \).

- To complete the series, click \( \text{Complete} \).
To save the acquired images, right click within the image window, and select ‘Save As...’

You can view the maximum intensity projection of the Z-stack by clicking the icon within the 2D View window, and selecting button.
9) **System Shutdown**

- At 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 **off**.

- 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.