Confocal Microscopy Training Policy
(Olympus FV1000 Confocal Microscope)

All users of the laser scanning confocal microscope **MUST** take the online Laser Safety course. It is located at: https://ehsdtraining.tamu.edu. **After taking the Laser Safety course, save the confirmation email/training certificate to a file. You will be asked to upload the training confirmation when you request training.**

If you plan on imaging samples with Biosafety Level 1 (BSL-1) classification, have your PI amend his/her Biosafety permit to include the Building and Room number in the permit, so that when the training is completed, you are able to image these samples in the MIC. The room is not approved for BSL-2 samples.

The basic training is done in two sessions, 2-3hr each. It is recommended that for the training you bring a hard-copy of the confocal microscope user guide, available from the MIC web-site: **Olympus_confocal_user_guide.pdf**. In the first session MIC supplies the specimen. Users are encouraged to bring their own specimen for the second session.

Session 1: Facility-specific safety instructions; Start-up and Shut-Down procedures; visual inspection of samples, Kohler illumination, setting up confocal imaging parameters, multi-channel fluorescence imaging, sequential scanning, pixel size and resolution, z-stacks

Session 2: Practice skills from session 1; using water and oil immersion objectives; using High Sensitivity Detectors, time lapse imaging, ROI and line scanning, bidirectional scanning. Dealing with noise and weak signals. The effect of pinhole size on resolution and signal strength. Optimization of imaging parameters for user-supplied samples

When the basic confocal training is completed, you may use the microscope independently during normal business hours. Once you gain proficiency in using the instrument, you will undergo a practical check-out test to demonstrate that you can operate the microscope without endangering yourself or the microscope. The requirements for successful check-out on the Olympus confocal microscope are listed below. After successful checkout, you will get access to the online scheduling system, get building access using your university ID card, and a key to the facility for a deposit ($10.00).

Even after the basic training, the MIC will provide help, troubleshooting and guidance and suggestions how to optimize imaging and improve image quality. This advice is typically free of charge, unless extensive consultation is required.

Additional specialized LM training: the basic confocal microscopy training only covers the most common imaging techniques. You can get additional training, help and assistance with advanced methods, such as photoconversion, FRAP, FLIP, FRET, Raster Image Correlation Spectroscopy (RICS), multi-area time lapse, as well as with image processing and analysis.

Checkout requirements for the Olympus confocal microscope

For a successful checkout test, the trainee should demonstrate that he/she is able to operate the microscope independently and safely for both the operator and the instrument. The trainee can consult written notes and the provided startup/shutdown instructions, as well as the short user guide. The trainee should be able to perform the following:

1) Start the system (the choice of lasers needed for imaging will be provided by MIC staff if necessary)
2) Visually (looking through the oculars) focus on the specimen using transmitted light using the lowest magnification objective and set up Köhler illumination
3) Switch to the desired higher-magnification objective (dry or immersion), and re-check that the Köhler illumination is set up correctly.
4) Adjust DIC (Nomarski) prism for desired image appearance, if DIC is to be used
5) Visually (looking through the oculars) check fluorescence of the specimen using the mercury lamp illumination
6) Switch to the confocal mode and select the appropriate dye from the Dye List (if the user does not have his/her own sample, the name of the dye will be provided by MIC staff for the test sample)
7) Show how the detection bandwidth can be adjusted for Channels 1 and 2 (VBF – variable bandpass filter)
8) Start Live View and adjust detector HV and offset for best contrast, avoiding saturation, for both the fluorescence and the transmitted-light detector (use the saturation warning lookup table, keyboard shortcut Ctrl-H).
9) Set the scan size and zoom to achieve maximum XY resolution (satisfy the Nyquist criterion).
10) Explain how the scan speed affects image noise. Show three ways how to reduce image noise (scan speed, Kalman filtering, laser power)
11) Explain how the size of the Confocal Aperture (C.A.) affects axial resolution and signal intensity.
12) Record a single XY image and save it to disk.
13) Put a scale bar on the image and save the display as bitmap (.BMP) file that can be imported into common presentation software (e.g., PowerPoint®).
14) Set up z-stack acquisition: set the z-step to achieve maximum axial resolution (Nyquist criterion), then set the start and end positions.
15) Acquire z-stack and save to disc. Display Maximum Intensity projection.
16) Remove the specimen from the stage, clean the objective if immersion was used, and follow the standard shutdown procedure.

In addition to these requirements, it will be beneficial to most users to know how to minimize cross-talk in multiple-stained fluorescent specimens by using sequential scanning, and how to use the photon-counting mode for very weak signals (however, this knowledge is not required for successful checkout).