

## Checkout requirements for the Olympus confocal microscope

*Date modified: October 6, 2006 by Stan Vitha (vitha@mic.tamu.edu)*

For 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 (also provided). 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
- 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) Record a single XY image and save it to disk.
- 12) 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®).
- 13) Set up z-stack acquisition: set the start and end positions and set the z-step to achieve maximum axial resolution (Nyquist criterion).
- 14) Acquire z-stack and save to disc.
- 15) 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 (however, this knowledge is not required for successful checkout).