Standard Operating Procedure:

Olympus Spinning Disc Fluorescent Confocal Microscope

Purpose of This Instrument: To obtain magnified optical images, fluorescent-enhanced optical images, 3D confocal fluorescent-enhanced images and videos of samples.

Location: White Hall Room B-20B

Primary Staff Contact:
Dr. Weiqiang Ding
304-685-1938 (cell)
Office: ESB G75D
weding@mail.wvu.edu

The Shared Research Facilities are operated for the benefit of all researchers. If you encounter any problems with this piece of equipment, please contact the staff member listed above immediately. There is never a penalty for asking questions. If the equipment is not behaving exactly the way it should, contact a staff member.

Note: The purpose of this manual is for general observation with the confocal microscope. For more advanced measurements or measurements with accessories, please contact MFCF staff.

Start Up

1. Log in your session on the FOM. Sign in on the logbook located on the corner bench.
2. Find the microscope electronics on the black wire rack at the back of the acoustic enclosure (Figure 1).
3. Power up the microscope electronics following the sequence below:
   - Lambda LS Xenon light source (Figure 1a)
   - Digital camera cooling fan (Rofer Scientific) (Figure 1b)
   - Lambda 10-3 shutter controller (Figure 1d)
   - Olympus IX2-UCB microscope controller (Figure 1e)
4. Connect the loose end of the cooling pipe to the vent port on top of the CCD camera (Figure 1c)
5. The two monitors and the keyboard/mouse on the top shelf of the computer cart belong to the microscope computer. Wake up the computer by pressing any key on the keyboard. (Figure 2).
   Note: The PC at the bottom of the computer cart and the monitors/keybord/mouse on the middle shelf belong to the Asylum AFM system.
6. If the computer is off, start it by pressing the power button at the front panel. The computer for the microscope is located at the bottom shelf of the black wire rack behind the acoustic enclosure. Log into SRF user account (no password needed) when it restarts.
Figure 1. (a) Lambda LS Xenon light source; (b) Cooling fan for CCD camera; (c) Cooling pipe connected to the CCD camera vent port; (d) Lambda 10-3 shutter controller; (e) Olympus IX2-UCB microscope controller.

Figure 2. (a) Desktop computer for the microscope located at the bottom of the wire rack behind the acoustic enclosure; (b) Monitors and keyboard/mouse for PC on the top shelf of the computer cart.

7. On the microscope computer desktop, start the IX2-BSW 1.6 microscope controller program. The program will communicate with the microscope and display the microscope operating parameters (Objective Lens etc.) (Figure 3). Contact SRF staff for assistance if the program does not communicate with the microscope.
8. Close the IX2-BSW program.

9. Start the SlideBook program on computer desktop for imaging.

SAMPLE LOADING

1. Lift up the condenser unit (Figure 4a) to vertical position to expose the specimen stage.

2. Load sample slide on the microscope specimen stage over the objective lenses.

3. Lower down the condenser unit to horizontal position.

4. The 20x and 40x objective lenses have adjustable correction rings and numbers marked on the lenses for aberration compensation (Figure 4b). Turn the correction ring to 0.17 if you use cover slip glass or to 1.5 if you use a petri dish.

(a) (b)

Figure 4. (a) Lifting up condenser unit for sample loading; (b) Objective lenses with aberration compensation rings.
FOCUSING

1. On the SlideBook program, click on the Focus icon to open the Focus Controls window (Figure 5).

2. Click on the “Scope” tab on top left corner. Select the proper objective lens in Objectives section. Start from 4X or 20X lens to find your sample.

3. Turn on the white light by clicking on the “Open Bright” button on lower right side of the program window (Figure 5).

4. In “Filter Set” section at lower part of the program window, select “Live” in the drop down menu (Figure 6).

5. Select the “DIC-VI” filter for visual observation to locate area of interest on the sample through microscope eye piece or select “DIC-CA” to observe sample through digital camera.

6. Adjust the light intensity if necessary with the “Lamp” slide bar at top right corner of the program window (Figure 5).
7. Adjust the focusing knob on the right side of the microscope base to focus on the sample (Figure 7a). Use the “F/C” (Fine/Coarse) button below the focus knob to adjust focusing step.

8. Set Z focus as follow (Figure 7b):
   - Click on the Z tab on the top left corner of Focus Controls window.
   - Adjust focusing knob to focus on the sample surface. Turn the focusing knob counter clockwise to focus below the sample until image goes out of focus.
   - Click on the “Set Bottom” button on the Z tab to record the bottom position.
   - Turn the focusing knob clockwise to focus above the sample until image goes out of focus.
   - Click on the “Set Top” button on the Z tab to record the top position.
   - Click and drag the vertical slide bar to bring the image back in focus.

Figure 6. Live filter selection in Filter Set section.

Figure 7. (a) Focusing knob at the side of the microscope base; (b) Z focus setup interface.
9. Increase the magnification by selecting the desired objective lens in the Objective section of the Focus Controls window (Figure 5). For using 100x oil lens, please follow the procedure below:

- Use the stage X-Y adjustment knob (on the right side of the stage) to move the sample slide forward to expose the 100x objective lens.
- Find the lens oil squeeze bottle next to the microscope.
- Check the lens oil bottle to ensure that no air bubble trapped in the pipe. If there is air bubble trapped inside, squeeze it out first.
- Put a drop of lens oil on top of the 100x objective lens.
- Move the slide back.
- Use the Z-focus control (Figure 7b) to bring the image to focus.

**WARNING:** Do NOT go back to low magnification observation after using the 100x oil lens. The residual oil on the back of the sample slide will contaminate other objective lens.

10. Adjust the Exposure time (Figure 5) to get the desired image quality. Click on the “Snap” button to take a bright field image of the sample.

11. To observe sample with fluorescence filters, click on “Close Bright” button on lower right side of the program window to turn off the bright light. Then click on “Open Fluor” button to open the fluorescent light source (Figure 8).

12. In Filter Set drop box, select “Fixed” from the drop down menu (Figure 8).

13. Select the desired filter from the filter list (Figure 8). Filters labeled “-C”, “-CA”, “TRC-” are for non-confocal imaging with fluorescence filters under the objective lens. Filters labeled “-D”, “-DS” and “TRD-” are for confocal imaging with DSU spinning disc unit and fluorescence filters in front of the CCD camera.

![Figure 8. Fixed fluorescence filters selections.](image)

14. Adjust the Expose time to get better image quality.

15. If necessary, click on the “Camera” tab and adjust the gain and intensify to improve image quality.

16. Click on “Snap” button to take a picture with the selected individual filter in Filter Set setting. Taking images with multiple filters will be discussed in next section.
1. Click on the Capture icon \[\text{Capture icon} \] on top menu to open the Capture window (Figure 9).

2. Set the Image size to 512 by 512 in pixels.

3. Select proper “Capture Type” from the list: 3D, Timelapse, Stereology. For 3D capture, please follow the procedure at the end of this section.

![Figure 9. Capture controller interface.](image)

4. Select “Fixed” in the “Filter Set” pull-down menu.

5. Pick desired filter or filter combinations. Select filters labeled as “-CAM” or “TRC-” for regular fluorescent imaging without confocal unit. Select filters labeled as “-DSU” or “TRD-” for confocal imaging with DSU spinning disc unit.

6. Set proper Expose time. Adjust “Gain” and “Intensify” if necessary to improve image quality.

7. Provide image information (Name, Comments) at the bottom of the program window.

8. Click on the “Start” button to start image acquisition process.
3D Capture:

- Follow the procedure in the “Focusing” section to set the Z-focus range if you haven’t already done so (Figure 10a).
- Select “3D” in Capture Type section.
- Select “Use top and bottom positions” in 3D Capture section (Figure 10b).
- Type in the desired Step Size. The software will then calculate the number of planes to cover the depth range.
- Follow the steps 4-8 above to select proper filter sets, exposure time and then acquire images.

![Focus Controls](image1)

![3D Capture](image2)

Figure 10. (a) Z-focus setting; (b) 3D capture setup.

FINISHING UP

1. Lift up the condenser unit. Remove sample slides from the microscope specimen stage. Lower down the condenser unit.

2. If you’ve used the 100x oil lens, clean the lens oil off the objective lens by sliding a lens paper across the surface. Lens paper can be found on the workbench beside the computer cart. **NOTE:** The oil lens will be contaminated if the oil is not cleaned up promptly after the user session.

3. Turn off the electronics on the wire rack following the sequence below:
   - Olympus IX2-UCB microscope controller
   - Lambda 10-3 shutter controller
   - Digital camera cooling fan power
   - Lambda LS Xenon light source

4. Disconnect the cooling pipe from the vent port of the CCD camera.

5. Close the doors of the acoustic enclosure.

6. Sign out on the FOM and the logbook. Report any problem on the logbook and the sign off window on FOM.
EMERGENCY PROCEDURES

If a user ever has a problem or an uncertainty should ASK someone who knows and can help. There are no penalties for asking for help but there may be for not reporting damage to the equipment that may delay or prevent others from working.

If, at any time, the user needs to contact someone for help, call or locate the following staff of the Materials Fabrication and Characterization Facility (MFCF):

<table>
<thead>
<tr>
<th>Name</th>
<th>Office</th>
<th>Phone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weiqiang Ding</td>
<td>Office: ESB G75D</td>
<td>Phone: (304) 685-1938 cell</td>
</tr>
<tr>
<td>Harley Hart</td>
<td>Office: White Hall 409</td>
<td>Phone: (412) 443-1514 cell</td>
</tr>
</tbody>
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If no one is available and the optical microscope is not acting as expected, the user should do the following:

- Shut down the software
- Shut down the computer

Then, if possible, the user should stay with the microscope while trying to contact the above individuals. If it becomes necessary to leave the instrument then the user should leave a large, legible note on both the AFM and at least one of the above individuals’ offices, stating:

- The problem, describing what happened and steps taken
- When it occurred date and time
- User name and phone number

If a dangerous situation is evident (smoke, fire, sparks, etc.), the user should press the power button on the power strip behind the microscope enclosure box or unplug the power strip to turn OFF power to the entire microscope system and notify the proper emergency personnel. If turning off the power would be unsafe in the user’s estimation, the user should leave the facility and contact emergency personnel immediately.