Part A: System Start-up

System Components:

- BX61 Upright Motorized Research Microscope
- FV500 Laser Scan Head
- Laser Combiner and Argon, Gr HeNe and Red HeNe lasers
- Fiber Optic Delivery System
- Transmitted Light Detector
- BX61 Handswitch, Prior Motorized Stage Control Joystick, Transmitted Light Source
- Microscope Control Unit
- Argon, Green HeNe and Red HeNe Laser Power Supplies
- Prior Motorized Stage Controller
- Mercury Burner Power Supply
- FV500 Control Unit and Power Supply
- FV500 Computer
- Computer Monitor and Surge Protected Power Outlet

System Start-Up

1. Turn on the Surge Protected Power Outlet (Computer, monitor, FV500 Control Unit & LG-PS2). Press the [POWER] button on the tower to turn on the computer.

2. Turn on Laser Power Supplies (Argon, Gr HeNe, Red HeNe) **ONLY as NEEDED**. The recommended warm-up is 10 min for the Argon and 30 min for the Gr HeNe.

3. Turn on the Mercury Burner.

4. Turn on the BX-UCB (Microscope Control Unit) [located on the top shelf].

5. Turn on the Prior Stage Controller Power Supply.

6. Logon to system using USER NAME and PASSWORD.

7. Wait at least 2 min for the microscope systems to initialize then double-click the Fluoview Icon (right) to launch the confocal program. Note: it takes about 2 minutes to launch.

8. Sign in on the log sheet.

9. Turn the red lever on the nitrogen tank 90° counter clockwise to power the air table.
Part B: Microscope Control

After the system has initialized, the BX61 microscope is ready to use. Microscope control is accessed through the keypad or Fluoview Software.

Motorized Features of the Microscope:

1. Objective Nosepiece
2. Fluorescent Mirror Cube Turret
3. Fluorescent Light Shutter
4. Condenser Turret
5. Condenser Top Lens
6. Reflected Light Aperture
7. Reflected Light Field Diaphragm

Transmitted Light Source is external from the microscope and is either controlled manually or through Fluoview Software.

Hand Switch Function when running Fluoview software

<table>
<thead>
<tr>
<th>Reflected light shutter OPEN/CLOSE</th>
<th>Stage escape</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSHT</td>
<td>TL</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Transmitted light ON/OFF</th>
<th>esc</th>
</tr>
</thead>
<tbody>
<tr>
<td>BF</td>
<td>FITC</td>
</tr>
<tr>
<td>TRITC</td>
<td>DAPI</td>
</tr>
<tr>
<td>CY5</td>
<td>DIC</td>
</tr>
</tbody>
</table>

Dichroic Mirror Setting [cube positions 1-6]

<table>
<thead>
<tr>
<th>4x</th>
<th>10x</th>
<th>20x</th>
<th>40x</th>
</tr>
</thead>
<tbody>
<tr>
<td>not used</td>
<td>100x</td>
<td>60x</td>
<td></td>
</tr>
</tbody>
</table>

Objectives

Microscope Frame Controls when running Fluoview Software

The ONLY button not found elsewhere is 2 or 8. This is the switch (toggle) between fine and coarse focus.

The stage escape, buttons 1 or 7, is also useful, but is duplicated on the handswitch.

Buttons 3 thru 6 are duplicated elsewhere.
Part C: Software Control

After entering the Fluoview software this window will open.

This software uses panel-type windows. A software function can be executed easily by selecting the panel page tab of the function to be executed.

The FLUOVIEW software is organized by two kinds of panels, the function panels and display panel. The function panels include the [Acquire], [File I/O], [Tile], [Process], [Analyze] and [Visualize] panels. The display panel shows either the [Live] panel or the panel image loaded from a file ([filename] panel).

The BX61 Microscope can be controlled via the Fluoview Software by clicking the scope control button on the settings tab. The Graphic User Interface (GUI) will appear:

All functions of the microscope can be controlled here from the condenser to the filter cubes to the light sources. Just click on the diagram and the software will configure the microscope.

Position and focus the specimen using the appropriate filter cube. Now you are ready to scan.
Part D: Scan Control

Select the <LSM> button in the [Light Path] group box. The <LSM> button looks pushed in to indicate that it is selected. (When scanning is started while the <BI> button is selected, the LSM light path is selected automatically. It is switched back to the visual observation automatically when scanning completes.)

Selecting the Dyeing Method

1. From the page tabs on the bottom right of the [Acquire] panel, select the [Dyes] sub-panel.

   ![Selected Dyes](image)

   - Select the specimen dyeing method by dragging [FITC] and [TRITC] in the [Available Dyes] list box in the [Selected Dyes] group box to the field immediately above the list box.

2. Click the <Apply> button to apply the selected dyeing method to the [Ch] group box on the upper part of the [Acquire] panel.

   ![Selected Dyes](image)

Setting the XY Observation Mode

1. In the [Mode] group box in the [Acquire] panel, select the [Surface] option button.

2. In the [Mode] group box in the [Acquire] panel, select [800 by 600] from the [Size] drop-down list.

3. In the [Acquire] panel, select the XY observation mode option button (top left).

   ![Acquire Panel](image)

Select the <XY Repeat> button. The acquired image will be displayed in the [Live] panel. Note: this is your preview. The <stop scan> button will replace the <XY Repeat> button.

![XY Repeat Button](image)
Setting the Cross-section to be Observed

After the preview image appears, move the Z stage to select the cross-section to be observed.

1. From the panel page tabs shown on the bottom right of the [Acquire] panel, select the [Z Stage] sub-panel

2. Check the [Locked] check box in the [Z Stage] sub-panel. You have now transferred control of the z-stage to the computer. **Do not turn the fine focus adjustment knob while the [Locked] check box is checked, for this may damage the Z motor.**

3. While observing the image in the [Live] panel, locate the plane to be observed by displacing the stage using the <Z stage coarse adjustment> and <Z stage fine adjustment> buttons in the [Z Stage] sub-panel [not on the microscope].

Stopping Repeated Scanning

After the brightness and gain have been adjusted, select the <STOP SCAN> button in the [Acquire] panel to stop scanning temporarily

Acquiring Images

Select the <Once> button. The acquired image will be displayed in the [Live] panel.
Part E: Saving Images

1. Display the [File I/O] panel.

2. When saving images acquired with more than one channel, it is possible to select whether images from more than one image are saved simultaneously or only one of the images is saved. Use the <Display channel switch> buttons to select the images to be saved. The selected images will be saved under the conditions set for each channel. Example) When only the image of Channel 1 is displayed, only the image of Channel 1 will be saved.

3. Click <Experiment> button in the [Save] group box. The [Save Experiment As] dialog box will open.

4. Enter the file name in the [File name:] text box.

5. Select “FLUOVIEW MultiTif” from [Save as type:]

6. Click the <Save> button
Saving, Opening and Shredding Images

Use the [File I/O] panel (below) to save, open or shred an image.

Display the [File I/O] panel at the front

Shredding Images

Shredding an image refers to removing it from the objects of processing including display. **Shredding does not actually delete the image saved in the disk.**

1. Click the <Experiment List> button in the toolbar at the bottom of the [File I/O] panel. The [Experiments in Memory] dialog box appears as shown below.

2. In the [Experiments in Memory] dialog box, select the file name of the image to be shredded and click the <Shredder> button. The file can also be shredded by placing the mouse pointer on it and dragging it to the <Shredder> button. The mouse pointer transforms to an image icon during dragging.

3. Click the <Done> button in the [Experiments in Memory] dialog box to close it. Multiple images displayed can be shredded simultaneously.
Displaying an Image in Simulated Colors

1. Display the [Display] panel of the image to be colored at the front.
2. Click the <LUT> button in the toolbar at the bottom left of the screen. The [Color Tool] dialog box appears.
3. When the image was acquired from more than one channel, select the channels to be colored using the [Ch1], [Ch2], [Ch3] option buttons. (The [Ch1], [Ch2] and [Ch3] option buttons are displayed only when an image acquired from more than one channel mode is displayed (selected).
4. From the [Standard Color LUTs] group box, select the desired color button. The selected LUT will be applied immediately to the image in the [Display] panel.
5. Click the <OK> button.

Image Annotation

Images can be annotated by clicking the Annotation button in the toolbar
Part F: System Shutdown

System Shutdown

1. Make sure objectives are clean of any residual oil using lens paper.
2. Return microscope stage to non-escaped position and objectives to 4x.
3. Exit from the FV program. This may take awhile, however if you turn off the microscope before exiting, a system error will occur.
4. Transfer your files to removable media. If you have 1 GB or more in your folder on the hard drive, consider removing older files permanently (only 1.5 GB allowed).
5. If someone is signed up after you, do not turn things off. Log off (skip to #10), but do not shut down the computer (#11) and sign out on the log sheet (skip to #14). Otherwise:
6. Turn off the Prior Stage Controller Power Supply.
7. Turn off the BX-UCB (Microscope Control Unit) [located on the top shelf].
8. Turn off the Mercury Burner.
9. Turn off Laser Power Supplies (Argon, Gr HeNe, Red HeNe)
10. Select <shut down> on the computer and select LOG OFF from the drop-down menu. The computer will log your name out and return to the login menu box.
11. Select the shut down button to shut down the computer. Note: power will go off.
12. Turn off the Surge Protected Power Outlet (Computer, monitor, FV 500 Control Unit and LG-PS2). Do not turn the monitor, FV 500 Control Unit or LG-PS2 off.
13. Turn red lever on tank 90° clockwise to stop flow of gas to table.
15. Report any problems. In the log sheet if minor. Call Steve or Ken if major hardware problem. Let Margaret know if any service is needed or supplies run out.

Dispose of waste (e.g. used lens paper) in wastebasket and take your samples away to be disposed of according to hazardous waste/glass requirements. Cover microscope and turn off lights.
Resources

People

- Steve Ricchio, Digital Imaging Specialist, Leeds Precision Instruments, Inc. Office 763-546-8575 • cel 612-209-1086 • sricchio@leedsmicro.com
- Ken Kilby, Sales Representative (Microscopes), Leeds Precision Instruments, Inc. Office 763-546-8575 • cel 651-270-9379 • kkilby@leedsmicro.com
- Angela Goodacre, Marketing Manager – Imaging Systems Applications, Olympus America, Inc. Voicemail 800-645-8100 ext 6004 • angela.goodacre@olympus.com
- Jerry Sedgewick, Program Director, Biomedical Image Processing Lab (BIPL, www.bipl.ahc.umn.edu), 612-624-6607 • sedge001@umn.edu
- Margaret Ramnaraine, Scientist, Confocal Administrator, 612-626-3672 • ramna001@umn.edu

User Manuals

- Olympus Fluoview FV500 Training, Presented by Leeds Precision Instruments, Inc. [version 1, 4/7/03] Powerpoint presentation
- Olympus Fluoview FV500 Laser Scanning Confocal System Operating Instructions [version 1.1, 6/19/03] PDF file
- Olympus Fluoview FV500 Multi Point Time Lapse Software [Version 4.3a, 12/01/03] full version of the stage control manual, PDF file
- Shared Confocal Microscope USER HANDBOOK: POLICIES AND PRACTICES [version 1, 4/30/04] PDF file

References