

## **Biology Shared Instrumentation Facility Olympus IX70 Inverted Microscope and Simple PCI Imaging System**

### **Use Protocols:**

- A. Reservation and Starting Up**
- B. Sample Observation (No Image Capture) – Transmitted Light**
- C. Sample Observation (No Image Capture) – Fluorescent Light**
- D. Image Capture – Transmitted Light**
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- F. Shutting Down**
- G. Notes on Simple PCI Software Usage**
- H. Notes on IX70 Microscope Usage**

### **A. Reservation and Starting Up**

**1. Reservation: Log on to the Reservation Calendar in Groupwise. You can access Groupwise via the icon on the front page of the Biology Website.**

**Username: OlympusIX**

**Password: SIF(followed by the door code number- no spaces)**

**Once in Groupwise, click on the calendar icon and then click to create an APPOINTMENT. In the SUBJECT box, enter name, lab and contact number. Set the duration of the experiment from the start time. Click SEND. Exit Groupwise.**

**2. Starting Up the System: As a general rule, always turn on external devices (lamps, camera, etc.) before activating computers. Thus, Start Up follows this sequence- Power strip, then Lamp Power Supplies, then other external devices, and finally the PC Power.**

**Always sign up ahead of time for scope use, log in and log out (on notebook log in room)**

### **B. Sample Observation (IX70 Only, No Image Capture) – Transmitted Light**

- 1. Microscope Power Strip (on the floor under scope): ON**
- 2. Microscope Power (rear, right side of scope body): ON**
- 3. If required for observing live samples (cell cultures, etc), Stage Warmer: ON**
  - a. Small box to left of scope**
  - b. Allow 10 minutes for temperature to stabilize**
  - c. Temperature may vary +/- 1° C, if slide stage adapter is used**
- 4. Hardware Power Strip (on table, behind Olympus/DG4 burners): ON**
- 5. Prior Proscan shutter control box (along back wall, top unit): ON**

6. Shutter S2 on Prior remote control (left side of scope): ON
7. Observe samples
8. Shut down according to protocol F (below)

#### **C. Sample Observation (No Image Capture) – Fluorescent Light**

1. Microscope Power Strip (on the floor under scope): ON
2. Microscope Power (rear, right side of scope body): ON
3. If required for observing live samples (cell cultures, etc), Stage Warmer: ON
  - a. Small box to left of scope
  - b. Allow 10 minutes for temperature to stabilize
  - c. Temperature may vary +/- 1° C, if slide stage adapter is used
4. Hardware Power Strip (on table, behind Olympus/DG4 burners): ON
5. Olympus Fluorescence Lamp Burner power supply: ON
  - a. Smaller power supply on right side of hardware table
  - b. Lamp must remain on for at least 30 minutes before turning off
6. Prior Proscan shutter control box (along back wall, top unit): ON
7. Shutter control box (left of scope)
  - a. S1 fluorescent shutter: ON for FLUORESCENCE
  - b. S2 transmitted light shutter: ON for TRANSMITTED LIGHT
  - c. S2 should be OFF for fluorescence microscopy
8. Observe samples
9. Shut down according to protocol F (below)

#### **D. Image Capture with Simple PCI – Transmitted Light**

1. Microscope Power Strip (on the floor under scope): ON
2. Microscope Power (rear, right side of scope body): ON
3. If required for observing live samples (cell cultures, etc), Stage Warmer: ON
  - a. Small box to left of scope
  - b. Allow 10 minutes for temperature to stabilize
  - c. Temperature may vary +/- 1° C, if slide stage adapter is used
4. Hardware Power Strip (on table, behind Olympus/DG4 burners): ON
5. Prior Proscan shutter control box (along back wall, top unit): ON
6. Sutter Lambda 10-2 emission filter wheel (unit below Prior Proscan): ON
7. Hamamatsu camera controller box (above PC): ON
8. PC: ON (Always turn computer ON LAST)
  - a. Activation button on front of computer
  - b. See notes on Simple PCI Software (Section H, below)
9. Shutter S2 on Prior remote control (left side of scope): ON
10. Observe/Image Samples
  - a. Be sure Light Path Selector knob is on the SP (side port) position
  - b. Be sure computer software has the 10-2 filter wheel in open position (see Simple PCI software notes)

**11. Shut down according to protocol F (below)**

**E. Simple PCI Image Capture – Fluorescent Light**

- 1. Microscope Power Strip (on the floor under scope): ON**
- 2. Microscope Power (rear, right side of scope body): ON**
- 3. If required for observing live samples (cell cultures, etc), Stage Warmer: ON**
  - a. Small box to left of scope**
  - b. Allow 10 minutes for temperature to stabilize**
  - c. Temperature may vary +/- 1° C, if slide stage adapter is used**
- 4. Hardware Power Strip (on table, behind Olympus/DG4 burners): ON**
- 5. Olympus Fluorescence Lamp Burner power supply: ON**
  - a. Smaller power supply on right side of hardware table**
  - b. Lamp must remain on for at least 30 minutes before turning off**
- 6. Prior Proscan shutter control box (along back wall, top unit): ON**
- 7. Shutter control box (left of scope)**
  - a. S1 fluorescent shutter: ON for FLUORESCENCE**
  - b. S2 transmitted light shutter: ON for TRANSMITTED LIGHT**
  - c. S2 should be OFF for fluorescence microscopy**
- 8. Sutter Lambda 10-2 emission filter wheel (unit below Prior Proscan): ON**
- 9. Hamamatsu camera controller box (above PC): ON**
- 10. PC: ON (Always turn computer ON LAST)**
  - a. Activation button on front of computer**
  - b. See notes on Simple PCI Software (Section G, below)**
- 11. Observe/Image Samples**
  - a. Be sure Light Path Selector knob is on the SP (side port) position**
  - b. Be sure computer software has the 10-2 filter wheel in open position  
(see Simple PCI software notes)**
- 12. Shut down according to protocol F (below)**

**F. Shutting Down**

**In general, these steps should be followed regardless of hardware used.**

- 1. Exit the Simple PCI Software, if image capture has been used.**
  - a. If prompted to save changes, click NO.**
- 2. PC: OFF (PC Always turned OFF FIRST)**
  - a. Wait for message on Screen prompting it is safe to turn PC OFF**
- 3. Prior Shutter Control Box: OFF**
- 4. Sutter Lambda 10-2: OFF**
- 5. Hamamatsu Camera Controller: OFF**
- 6. Olympus Burner Power Supply: OFF**
  - a. Burner Power Supply always turned OFF LAST**
- 7. Hardware Power Strip: OFF**
- 8. Stage Warmer: OFF**

9. **Microscope power: OFF**
10. **Return IX70 to default status, as listed on sheet at upper left of cage**
11. **Replace Dust Cover on Microscope**
12. **Pull down cage screen in front of scope**
13. **Pull black plastic shroud down the over the scope front**
14. **SIGN OUT**

#### **G. Note on Simple PCI Software Usage**

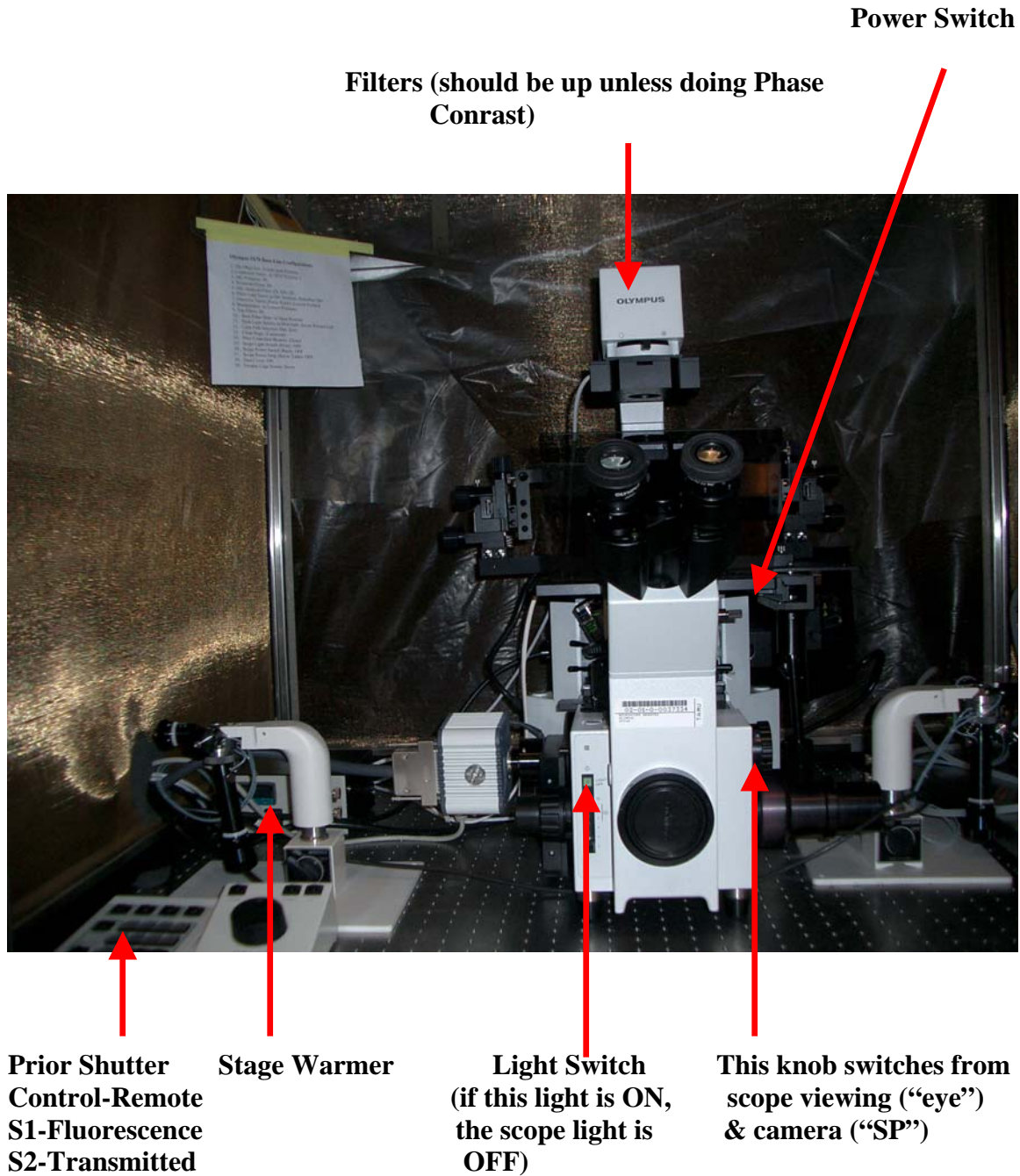
1. **Once PC has booted, log on as follows:**
  - a. **Press Ctrl + Alt + Delete**
  - b. **Leave password blank – Press Enter**
  - c. **Click OK on Service Control Manager window**
  - d. **Double Click SimplePCI Icon on lower left of Desktop**
2. **For Image Acquisition**
  - a. **Click Camera Icon at Upper Left-Hand Corner**
    - i. **Capture and Image Display Windows should appear**
3. **Capture Window – DO USE SENSOR TAB UNLESS AUTHORIZED**
  - a. **Upper Right hand Corner – default setting to Orca 100 No Binning Monochrome**
  - b. **Keep this setting for normal Image Capture**
  - c. **Select 2x2 Binning for Low Light or Fluorescence Imaging**
  - d. **Select 4x4 Binning for EXTREME Low Light/Signal Imaging**
  - e. **Monochrome yields black and white images**
  - f. **2 color and 3 color settings use computerized pseudocolor to achieve colored image**
    - i. **ONLY USE IF YOU HAVE BEEN TRAINED IN THEIR USW AND KNOW WHAT THEY MEAN**
  - g. **Default Idle Filter Setup (Default) should be used for most applications, unless advanced camera/shutter control is needed**
    - i. **DO NOT ALTER OTHER FILTER SETUPS – SOME ARE CUSTOMIZED FOR SPECIFI LABS' USAGE**
  - h. **Control Enhance Offset: should always be set to ZERO**
  - i. **Control Enhance Gain: May be increased for fluorescence imaging**
    - i. **Only if exposure time too long, to prevent bleaching**
    - ii. **Higher Gain = Lower Resolution**
  - j. **Exposure Time: Can be increased and decreased as needed**
4. **Display Window**
  - a. **Can be made larger or smaller using zoom (magnifying glass) icons**
  - b. **SAVE Icon has different options**
    - i. **Original Image (8 bit) – Saves current field being sent to camera – WHAT YOU SEE ON MICROSCOPE**

- ii. **Display Image (8 bit) – Saves current image on DISPLAY SCREEN**
- iii. **Display + All Layers (24 bit) – Saves any editing (scale bars, etc)**

**5. IMAGE CAPTURE**

- a. **Rotate selector knob on microscope to SP**
  - i. **Setting of “eye” is for observation only**
  - ii. **SP setting will not allow for observation through oculars**
- b. **Click FOCUS on Capture Window**
  - i. **Adjust Exposure Time as needed**
  - ii. **Adjust Focus on Microscope as needed**
- c. **Click STOP**
- d. **If image displayed is satisfactory, Click Capture 1**
- e. **Save as desired.**

# Notes on Microscope



## Hardware Map

