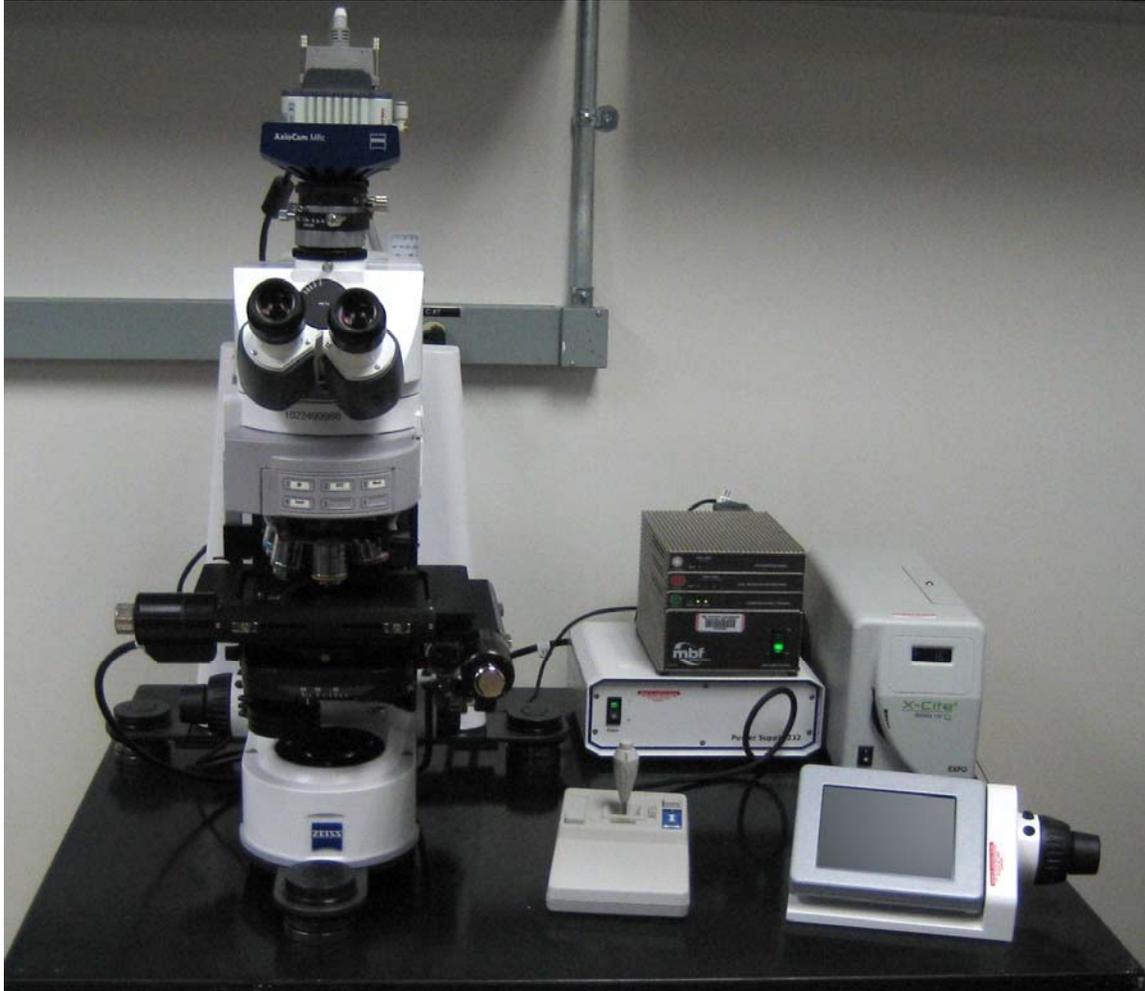


# Operation of the Stereology System



The stereology system is composed of a Zeiss AxioImager.M2 microscope and MicroBrightField's Stereo Investigator software package. This guide is not meant to be an exhaustive help resource nor is it intended to teach those who have not received proper training how to use the system. It is intended only as a quick reference to help trained users. If you are interested in training or if you need more details on the use of the system, please contact Dennis McDaniel.

## Turning on the system:

1. If you are collecting fluorescent images, turn on the X-Cite fluorescent lamp power supply (fig 1) and the Hamamatsu fluorescent camera power supply (fig 2). The X-Cite fluorescent lamp requires about 2 minutes to warm up before use, and should not be turned off if it has been on for less than 10 minutes. Once it is turned off, it must remain off for at least 10 minutes before it is turned back on.

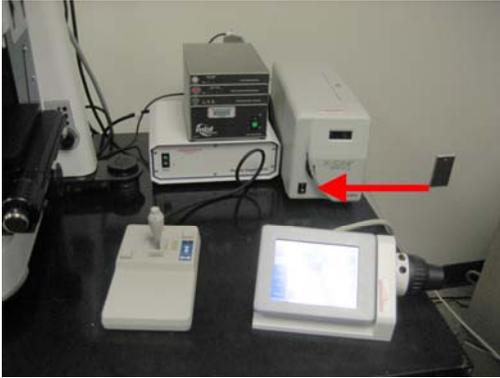


Fig 1



Fig 2

2. Next, turn on the stage controller (fig 3) and the microscope. The microscope has two power switches, one on the external power supply (fig 3) and one on the left side of the base of the microscope (fig 4). Note: If you are collecting brightfield images, the brightfield camera will turn on when the computer is turned on.

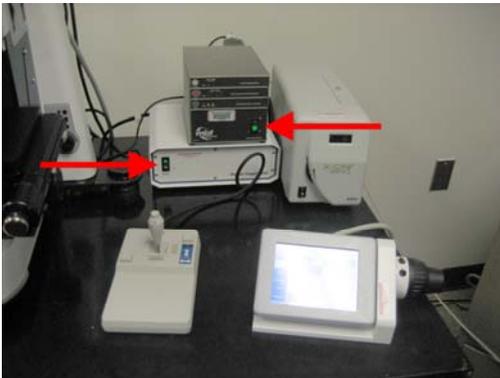


Fig 3

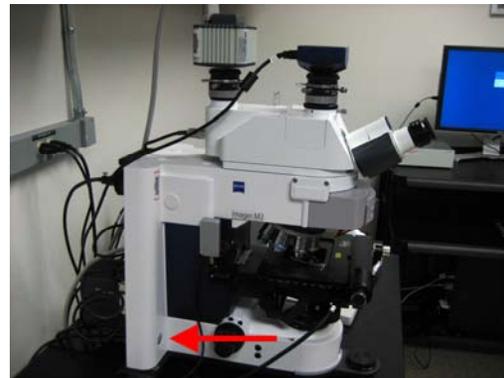


Fig 4

3. Turn on the computer (under the desk) and logon to the system. The user name for the system is “MBF stereology”. No password is required.

## Controlling the microscope:

The Zeiss AxioImager.M2 may be controlled by the buttons and switches on the microscope itself as well as the touch-screen satellite control panel. It does not matter which of these methods you use to configure the microscope. The Stereo Investigator software is unable to control the microscope components with the exception of the fluorescent filter cube revolver.

Most components of the microscope are electronically controlled and may be configured through the touch-screen satellite panel. The biggest exception to this is the condenser. All condenser controls (including height, centering, aperture, and top lens position) must be configured manually. The condenser plays no role in fluorescent imaging, so it is not critical to ensure that these condenser components are optimized if you are only collecting fluorescent images. For brightfield imaging, however, it is critical that optimal specimen illumination is achieved (Koehler illumination). The condenser that will remain on the microscope for general use is a standard condenser with an adjustable numerical aperture (NA). The NA of the condenser should always be matched to the objective lens in use. For critical imaging using the 63x, 1.4NA objective, we have an oil immersion condenser. Please see Dennis McDaniel for help in setting up Koehler illumination or using the oil immersion condenser.

### Getting Started - Brightfield Observations:

When the microscope is first powered up, the satellite control panel will show the following screen:

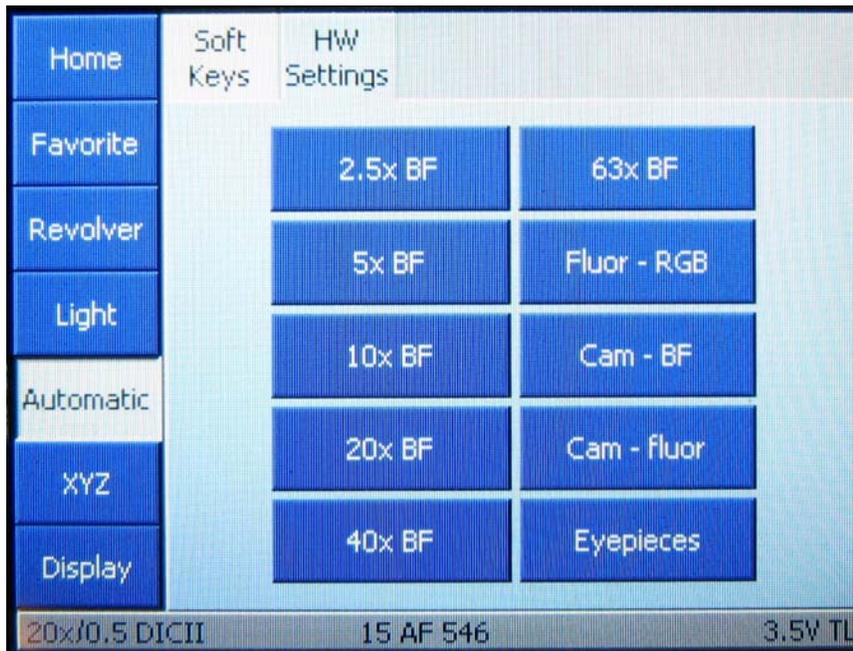


Fig 5

If you press any of the softkeys labeled with a magnification (2.5x BF, 5x BF, 10x BF, 20x BF, 40x BF and 63x BF), the microscope will rotate the objective turret to the desired lens, open up the transmitted light path shutter, and send all of the light to the eyepieces. This makes it easy to find your specimen and get an initial focus.

### Stage Controls:

The X and Y positions of the stage are controlled with the joystick. Pressing the button on top of the joystick causes the stage to move at a faster rate. **Never push the stage or attempt to turn the knobs on the stage manually – the stage will be damaged (fig 6)!!!** Focus may be adjusted with the coarse and fine focus knobs located on either side of the base of the microscope or with the same knobs located on the side of the satellite control panel.



Fig 6

### Fluorescent Observations:

This microscope is not equipped with any contrast enhancement optics, so it may be necessary to use fluorescent illumination to find fluorescently labeled specimens. Pressing the softkey labeled “Fluor – RGB” (fig 5) closes the transmitted light shutter and opens the fluorescent light shutter with all of the light still going to the eyepieces. There are three ways to select which filter cube you are using for fluorescent illumination. You can press the two rear buttons located on the left-hand focus knob (fig 7, red arrows), or you can press the same two buttons on the satellite control panel focus knob. These buttons will cycle through the installed filter cubes.

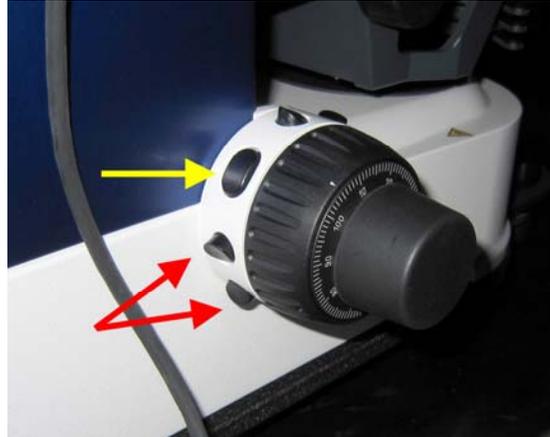


Fig 7

You can also change filter cubes by pressing the “Revolver” softkey followed by the “Reflector” tab on the satellite control panel which will take you to the following screen:



Fig 8

On this screen, press “10 FITC” to illuminate with blue light (green fluorescence), press “15 AF 546” to illuminate with green light (red fluorescence), and press “49 DAPI” to illuminate with UV light (blue fluorescence). Positions 1, 5 and 6 currently have no filter cubes installed.

To stop illuminating your specimen with fluorescent light, press the middle button on the left hand focus knob (fig 7, yellow arrow) or the same button on the satellite control panel focus knob. Alternately, you can also press the “RL Illumination Off” softkey (fig 8).

### Changing Objective Lenses:

To change objective lenses, press the “Revolver” softkey followed by the “Objectives” tab. This will take you to the following screen:

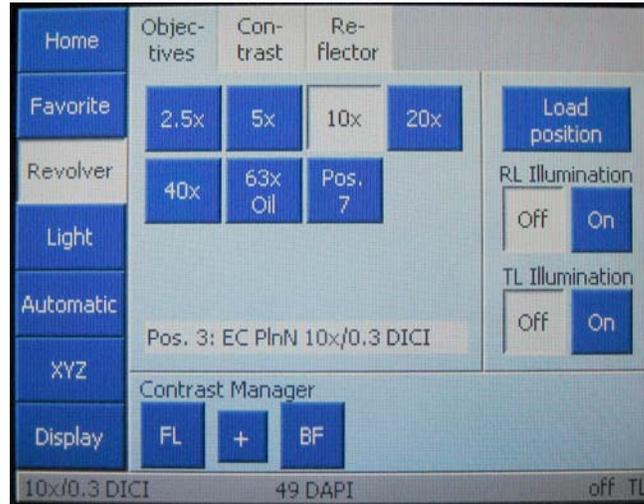


Fig 9

To change objectives, simply press the softkey corresponding to the objective you want to use. If you switch to the 63x oil immersion objective, the stage will lower and a message will appear reminding you to add a drop of immersion oil. If you then switch back to a lower power objective, the stage will again lower and a message will appear to remind you to remove the oil from your slide so that it does not get on the dry objectives.

### Sending Light to the Cameras:

To send light to the brightfield camera, press the “Favorite” button on the satellite control panel followed by the “Cam – BF” softkey (fig 5). To send light to the fluorescence camera, press the “Favorite” button on the satellite control panel followed by the “Cam – fluor” softkey (fig 5). These two buttons change the light path to send light to the cameras, but do not affect what viewing mode you are currently using (brightfield or fluorescence) and also have no effect on the objective lens turret.

### Sending Light to the Eyepieces:

To send light to the eyepieces without changing any other imaging configurations, press the “Favorite” button on the satellite control panel followed by the “Eyepieces” softkey (fig 5).

## **Shutting down the system:**

1. When you are finished with the system, shut down the software and the computer. It is necessary to shut down the computer since the brightfield camera is on whenever there is power to the computer.
2. Lower the microscope stage and remove your slide. Clean any oil off of the 63x objective if you used it by gently wiping a piece of lens tissue across the lens surface until the tissue comes away dry.
3. Press the “Favorites” softkey followed by the “10x BF” softkey. This ensures that the microscope is ready for the next user.
4. Turn off the Hamamatsu fluorescent camera power supply (fig 2) and microscope external power supply and stage controller (fig 3).
5. Turn off the X-Cite power supply (fig 1).
6. Place the dust cover back over the microscope.
7. Fill in details in the log book.