

Everest System / Slidebook Operating Procedures

NOTICE: This guide is meant to supplement training, not replace it. All users must be trained first hand by a core employee. Training of others in your lab is not allowed and will result in revocation of your privileges.

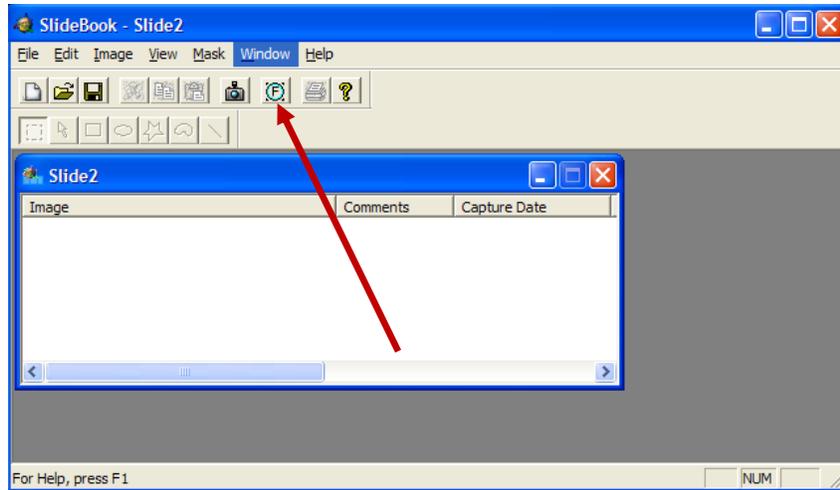
Startup:

1. **Sign In** the log book.
 - a. ***we bill you for lamp hours, not for the time you spend taking pictures. Therefore, log your time at the time you turn on the lamp and the time when you turn off the lamp.
2. Turn on Xenon Lamp and write the number on the meter in the sign in book.
3. Turn on the scope
 - a. To do this, turn on the surge protector behind the scope.
4. Turn on the computer and enter your sign in information.

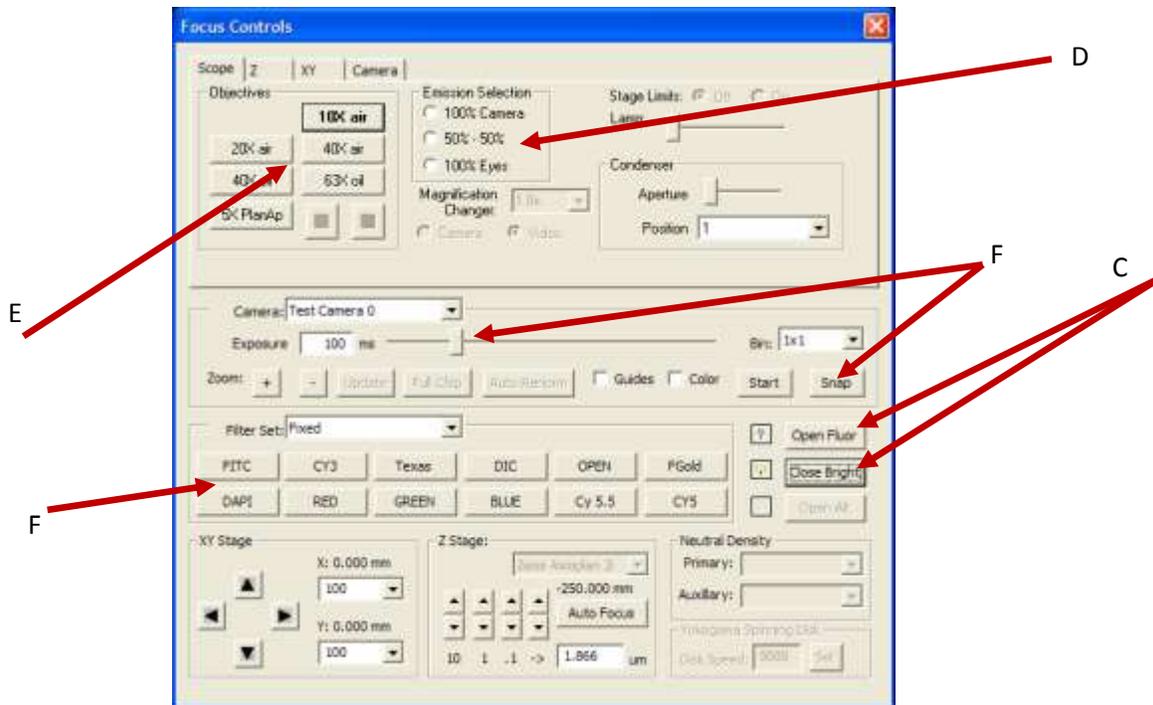
MAKE SURE THAT YOUR SLIDES ARE COMPLETELY DRY AND FREE FROM EXCESS MOUNTING MEDIA OR NAIL POLISH! THESE MATERIALS CAN DAMAGE THE OBJECTIVES IF THEY CONTACT THEM.

Basic Imaging:

1. Open the Slidebook program
2. Load Specimen on the stage
 - a. Use the stage control buttons on the bottom left side of the scope to change the stage height between the load and work positions.
Do not touch the focus knob until you have raised the stage to the work position. If you do, you will have to manually move the stage back to the work position.
3. Open the Focus Window:
 - a. Click the FOCUS button (red arrow points to it below)



b. This window will open:

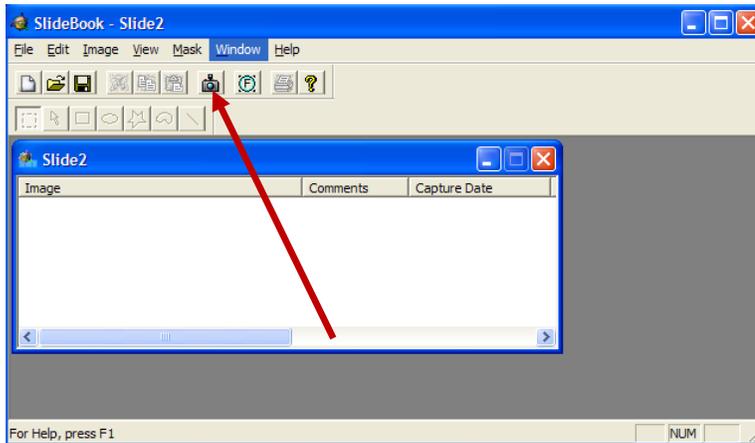


- c. Click OPEN BRIGHT or OPEN FLUOR to turn on either the brightfield (white) light or the fluorescent light. Click them again to turn them off.
- d. Use the controls in this box to change the light to go to the camera (computer) or to the scope (to focus your specimen)
- e. Use these buttons to change between objectives
 - i. *Be careful not to get oil on the dry objectives. BE SURE to CLEAN any oil off of objectives after use!*
- f. Use these buttons to change to the correct filter cubes
 - i. When using brightfield, usually select DIC cube.
- g. Focus on your area of interest

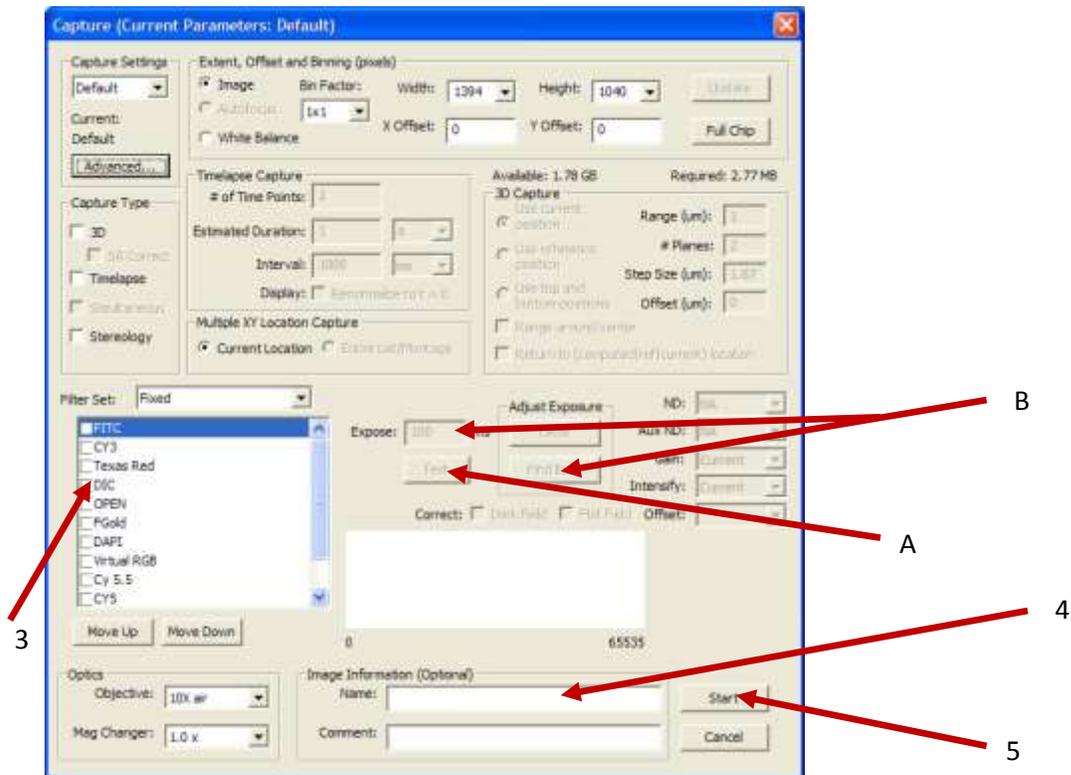
- h. Turn off the lights using the buttons by arrow C when not taking pictures or focusing to avoid bleaching your samples.
- i. You can take a quick picture here by clicking the SNAP button. Adjust Exposure slider to avoid saturated pixels.

Basic Image Capture:

1. After you have focused and found your area of interest, click the Camera button:



2. This window will open:



3. Pick one of the Fluorophores that you want to image (such as FITC).

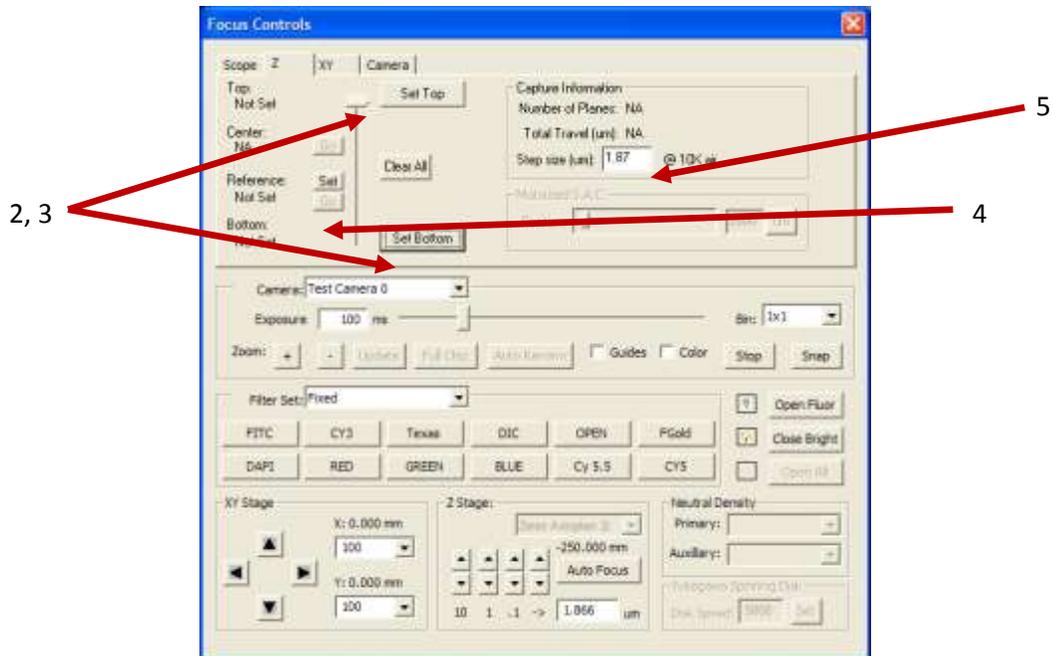
- a. Click the TEST button and look at the peak. The peak should cover about half of the graph window with a maximum value of approximately 3000.
 - b. Adjust Exposure:
 - i. Hit FIND BEST and wait or use the ONCE button. You will see the exposure time change.
 - ii. Or, you may adjust the exposure manually by typing how many milliseconds you want the shutter open.
 - c. Repeat this for each fluorophore you want to image, one at a time.
4. You may name your image at this point if you desire.
 5. Click START to take the image.

Saving Images:

1. Make sure you save the image and the slide. We recommend doing this after every 2-3 pictures, to avoid losing your work.
2. Save Picture:
 - a. On the slide, double click the picture you want to save.
 - i. For SINGLE 8-bit images:
 1. View > Export > Tiff OR
 2. View > Export > Default Views of all Images as TIFFs
 - ii. For 3D images in 8-bit format:
 1. View > Export > Tiff Series
 - iii. For 16-bit TIFF images to be analyzed in other software:
 1. Image > Export > 16-Bit Tiff
3. SAVE YOUR SLIDE!
 - a. File > Save Slide As
 - b. Update your slide regularly, usually each time you save a single picture by using File > Save Slide

3-D Imaging:

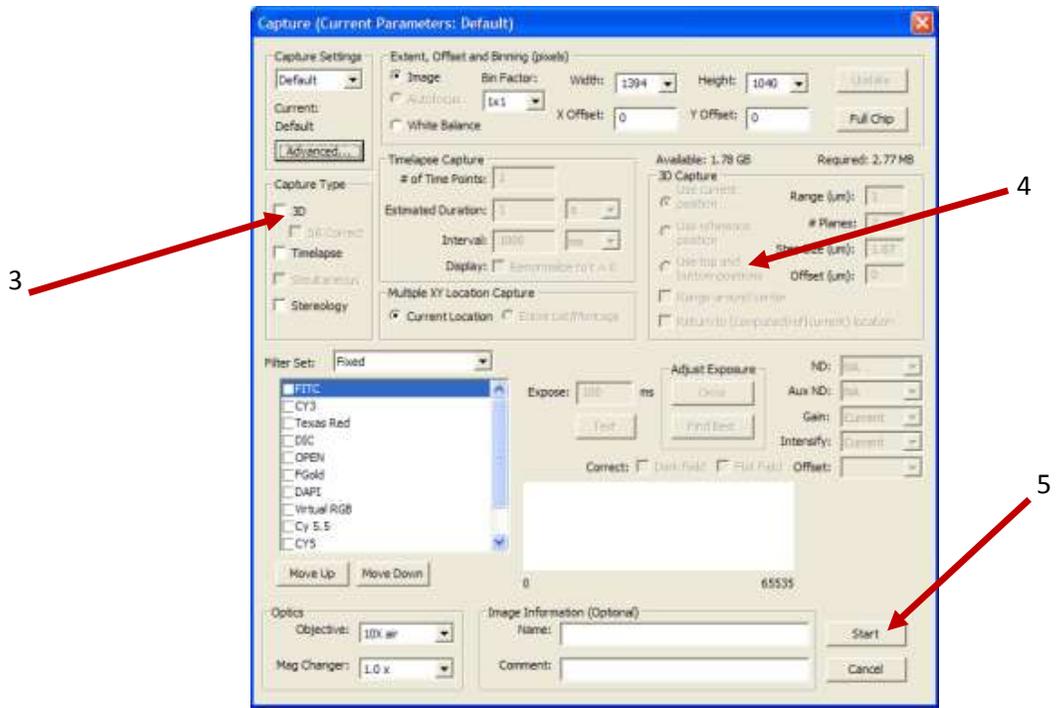
1. In the Focus Window, click the "Z" tab:



2. While viewing your image live (with 100% light to camera and the bright or fluor on), use the focus knob to defocus your image in one direction. Turn the focus knob until you can barely see your specimen and click SET TOP.
3. Repeat step 2, turning the knob the other direction and click SET BOTTOM.
4. Determine what step size you want to use and then enter that into the box.

3-D Image Capture:

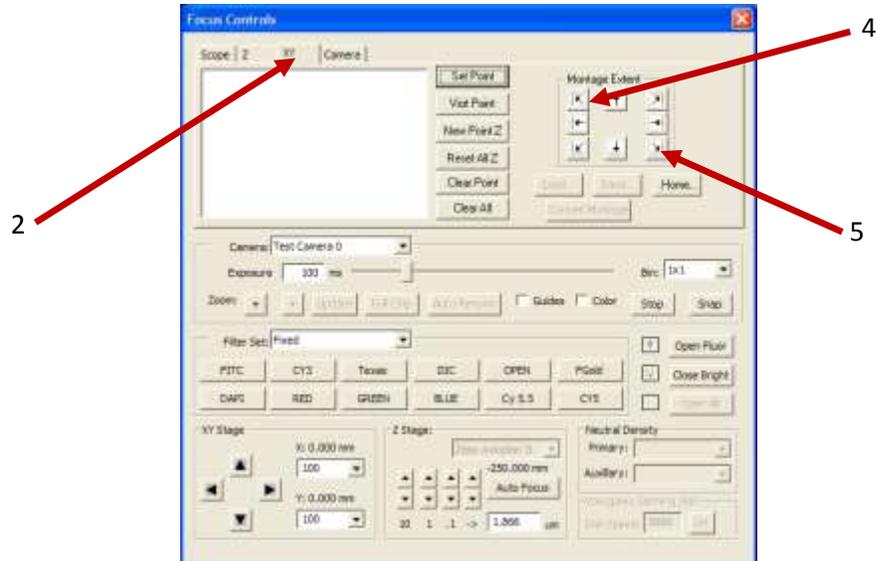
1. Do steps 1-4 from Basic Image Capture section if you haven't done so already.
2. Select "3D" under capture type



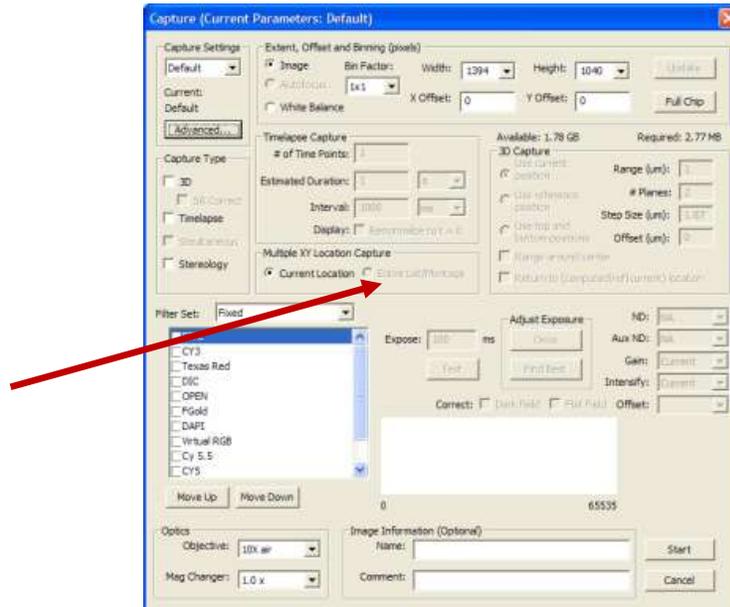
3. Select "Use Top and Bottom Positions"
4. Click START to take the stack.
5. Once the 3D stack has been collected, you may scroll through the different planes of the stack using the middle mouse wheel.

Montaging/ImageStitching:

1. Do the steps in the Basic Imaging Section and steps 1-4 in the Basic Image Capture Section.
2. Click the “XY” tab in the Focus Window.
3. While looking at the image live (with 100% light to camera and the bright or fluor on), find the top left corner of your area of interest using the joystick. (The button on top of the joystick will make the stage move slower after it is clicked.)
4. Click the top left arrow button in the “Montage Extent” area (4).
5. Find the bottom right side of your area of interest and click the bottom right button.



6. In the Camera window, select “Entire List/Montage”



7. As before, you may name your picture here, and then click start to begin the image capture.
8. When working with large montages, we recommend that you don't put more than 1-2 montages on a slide, but save each slide separately and open a new one for a new montage.