# Directions for Zeiss Axiovert 200M

START UP
1. Turn on mercury lamp controller (#1). Wait a few seconds to make certain lamp is on.
2. Turn on the x-y stage controller (#2)
3. Turn on the camera controller (#3)
4. Turn on the microscope (#4) (green button on the right side of the microscope.
5. Login to the computer with your CNET ID and password. Double click on the Openlab program on the computer.

IMAGING
1. Choose the correct stage for slide or dish. Make sure the stage is properly inserted (there is a spring in the corner). The stage should be laying level: Don't have one side higher than the other- it should not wobble. If your sample is on a slide, make sure the coverslip is facing down. Choose the objective you want by moving it in to place or choose the objective on the Microscope palette. The dry objectives are 10x, 20x, and the first 40x. If you are using an oil objective (the second 40x, 63x or 100x) put 1 drop of oil directly onto the objective. When focusing with the oil objective you should have a space of oil between the objective and the cover slip. The objective and cover slip should NOT be touching.
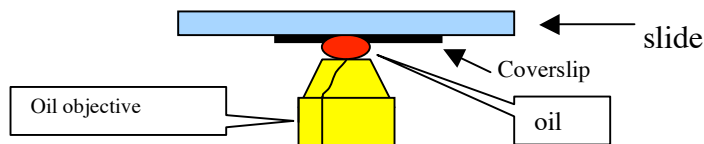
2. Check the fluorescent palette in the program: if the filter you need is not in place, use the red- handled Zeiss tool and unscrew the screw that holds the reflector turret in place with 2 turns. (DON'T unscrew until the screw falls out). Gently push the reflector turret out and exchange filter cubes. (Do not touch the actual filters with your fingers). The cubes should all be level to each other if properly inserted in the turret. Carefully replace the reflector turret (it should be smooth and easy to insert- do not force it! If you are forcing it, then you are inserting it incorrectly, and it will get jammed!) Use the Zeiss tool to lock in the reflector turret after changing your filter. Then change the label for the filter in the reflector pallet in the program.
3. If possible start by imaging your sample using **transmitted light** to prevent bleaching. Click on Transmitted Light or the dic (#5 position) in the Zeiss reflector palette and focus using the focusing dial on the right side of the microscope. **(Do not open the fluorescent shutter at any time while on transmitted light!)** Use Koehler illumination to focus the condenser for transmitted light every time you switch to a new objective. For transmitted light, choose the camera shutter that sends 50% of the light to the camera and 50% of the light to the ocular.
   a. Koehler Illumination: After focusing on your sample with transmitted light, close the diaphragm, and use the condenser-focusing knob on the right side of the transmitted lamp to focus the condenser unit so that the diaphragm is sharply focused. You should now see your sample and the

diaphragm – both in focus. Now center the diaphragm using the centering screws. Choose your image and place it in the center of the field. Then reopen the diaphragm just until the camera no longer sees it. Look at the computer screen and click V on the keyboard for viewing the live image.

b. Exposure: click on AE on the Video controls palette to get an automatic exposure. You can adjust it manually as well by double clicking on the time and entering it from the keyboard or clicking on the up or down button. Make sure that the image on the screen is focused and tap the Space Bar on the keyboard to take a picture. You will see the picture you just took on the layers palette. To label your picture, double click on the word "captured layer" and type in your label.

4. Set up your State Saver for transmitted light using brightfield, phase, or DIC and matching the lens to the objective that you are using (i.e. phase2 for 40x dry). Save your settings with the edit *pencil* on the State Saver palette by deselecting previous settings and selecting your new settings. Thereafter, just hit RESTORE when you want to bring the particular filter settings up again.

5. Set up your State Savers for fluorescence as well. To colorize your fluorescent image using the State Saver, highlight the camera layer and choose a color from the color table on the left of your image. Send 100% of the image to the camera and set the exposure. Increase the gain if the exposure is too long (i.e. 5 seconds) to shorten the exposure. Make sure your images are focused on the computer screen and take the picture using the space bar. Initially save your working file as a LIFF file. As you take pictures, update your working LIFF file with APPLE-S. When you are finished select all of your images and save as MULTIPLE TIFF FOR PUBLICATION (For Photoshop- millions of colors) or MULTIPLE TIFF (For ImageJ- 12 bit grey scale). Save onto a CD or your memory stick or onto your server folder. If you save onto the server, be aware that the files get automatically deleted every Sunday after 1 month. If you have old files (older than 1 month), they will get deleted on the first Sunday!*@!>!

If someone that needs to use the microscope after you, log off of your account, and leave the microscope on for them. BUT if they are not physically there and if the technical director is not in the facility, then TURN OFF the microscope.

SHUT DOWN
1. When you are finished, quit the Openlab program (Apple-Q)
2. Turn off the microscope (#4)
3. Turn off #3
4. Turn off #2
5. And lastly, turn off the mercury lamp (#4)