Instructions for using Heilshorn group microscope

1. Always sign up on the microscope sheet.

2. Turn on the microscope.

3. Click on Axiovision software on the desktop to open the imaging screen. If the software is opened before turning on the microscope, the computer will crash.

4. Place specimen on the stage, and turn on the halogen light.

4. Focus the specimen using the focus knob.

5. Completely close the Field Diaphragm (Fig. 2A) using the slider shown in Fig 1.

6. Focus the edges of the field diaphragm by cranking the condenser up or down using the condenser focus knob. You should see the sharp edges of the hexagonal field diaphragm (Fig. 2B).

7. If the Field Diaphragm is off center, use the two adjusting screws to center the condenser so that the diaphragm image is exactly centered in the field of view (Fig. 2C).

8. Open the Field Diaphragm until its shadow just disappears from the field of view. Opening the Field Diaphragm more than that causes extra glare and decreases image contrast.

When turning off the microscope, the software should be closed first to avoid crashing the computer.

Fluorescent Imaging:

1. Use brightfield or phase contrast to locate your specimen.

2. Turn on the Mercury lamp as the light source. <u>The mercury</u> <u>lamp should be turned off</u> after fluorescence imaging unless you will perform more fluorescence imaging within one hour.

3. Select the proper filter cube for your fluorochrome using the reflector buttons at the right side of the microscope.

4. Change the fluorescence light intensity using the halogen lamp adjustment wheel. Choosing lower light intensity and a longer exposure time generally results in better images.

5. If you want to have a better resolution and a smaller z-focal plane, open the aperture and luminous field diaphragm sliders marked with "A" and "L", (Fig. 3). To gain contrast (but lose resolution) close "A" and "L" sliders to increase the z-focal plane.

6. When not viewing the specimen, close the fluorescent light shutter to avoid photobleaching your sample.

Other Important Rules:

1. If oil immersion was used, clean the objectives using clean lens paper in the microscope room to gently blot off the oil from around the lens. <u>Never rub directly on the lens, as this may</u> <u>change the lens coating. Because the dry 40x objective has a</u> <u>very small working distance, it will get contaminated with</u> <u>immersion oil if there is some on the slide.</u> Therefore, if you are using the 40x oil immersion objective and want to switch to the dry 20x, you must turn the objective turret in such a direction to avoid swinging the 40x dry lens through the oil. You cannot use the 40x objective for observation at all, unless the slide has been cleaned with 70% ethanol and all oil removed from the coverslip. If a dry objective becomes contaminated with oil, use lens paper to clean the area around the lens, blot the lens with lens paper (no rubbing!), and use a cotton swab immersed in Kodak lens cleaner to place a drop of solvent on the lens and let air dry.

2. If you want to borrow one of the **fluorescence filters**, you should **use gloves** for removing them and be very careful not to contaminate the filters by touching them. When returning the filters back, it is critical to correctly put them in their positions an avoiding any misplacements (Fig. 4).

3. Do not move the microscope! If you must change its location, only touch the base of the stand. Pushing or moving the other parts of the microscope will cause physical and visual damage.



Fig. 1. The microscope top part



Fig. 2. Adjusting the field diaphragm



Fig. 3. Field diaphragm slider Fig. 4. Fluorescent filters