Microscopy procedures.11-19-15.docx

Students in the lab have found my notes on light microscopy to be helpful. I've written them up and Dr. Caprette and I have edited them into a document that I will post on Owl-Space. I will also make hard copies available in lab.

Good luck. Charlie Stewart

A. Basic procedures:

1. Place a slide on the microscope stage, held in place by, but not under, the movable clamp. Adjust the position of the slide so the light path goes through the sample to be observed. To do this, use the knobs on the post descending from the right rear of the stage.

2. The lamp is turned on and off with a wheel at the lower left. Turning that wheel counterclockwise from the off position turns the lamp on, and continuing to turn it counterclockwise increases the intensity of illumination, up to a maximum at the 6 setting on that wheel. Always start with maximum setting.

3. Vertical positioning of the whole condenser is adjusted by the knob at the back left side of the condenser. The "full up" position just means the highest position that you can get the condenser to with that knob. This position is usually suitable for all of our uses of the microscope. *If you encounter resistance when trying to raise the condenser then stop and ask for assistance. Trying to force the knob to turn will damage the mechanism.*

4. In the manual on the web site, the "condenser position" usually refers to the setting of the condenser turret, which is a black wheel whose front edge sticks out from the condenser. The label for each setting can be seen on top of that edge that sticks out. The settings on the condenser turret are 0, DF, Ph1, Ph2, Ph3, Ph4. To see these clearly, you have to look from straight above.

For bright field, the condenser position is always at 0.

For dark field optics, setting ph4 is used with the 4X and 10X objectives; setting DF is used with the 40X objective; dark field is not available with the 100X objective.

5. To adjust the aperture diaphragm, use the black rectangle at the front, just below condenser turret, which is only visible when the condenser turret is set to 0. Move to the right to increase contrast; to the left to decrease.

6. The objectives are 4X, 10X, 40X, and 100X. The 40X objective is sometimes referred to as the high dry objective. The 100X objective is used only with oil immersion, regardless of whether it is used for phase contrast or bright field. With the 100X objective, if it has a red band, it can be used for phase contrast, with the ph4 condenser setting. On some microscopes, the 100X objective has a white band. Those can only be used for bright field; not for phase contrast.

7. The eyepieces (oculars) provide another 10X magnification (eg for a total of 1000X if using the 100X objective). To start, each eyepiece should be removed from the microscope and adjusted so its edge is centered at the white line.

8. To start, be sure the lamp intensity is set to 6, the condenser turret to 0, and each eyepiece adjusted so its edge is centered at the white line. Put the 4X objective in place, and bring the specimen into focus, using the crude and fine knobs at the back of the microscope (either those on the right or the left, which are equivalent).

Go up to the 10X objective, and refocus there. Go up to the 40X objective and refocus there. Notice which direction you have to turn the focus knob to bring it into focus.

To go to the 100X objective, you must use immersion oil. Rotate the objectives so you get halfway between the 40X and 100X objective (i.e. get the 40X objective out of the way, so you can get the oil in there). Coming in from the back side with an eyedropper, put a healthy drop of oil so it covers the

light path. Then move the 100X objective into place, watching it as you do to be sure it doesn't actually contact the slide. Now bring the image into focus. Be careful not to focus so far down that you touch the lens to the glass slide. Use only the fine knob. Try turning it upward first (counterclockwise with the right knob). Only if that moves it further out of focus should you try turning it the other way.

9. When finished with the 100X objective, turn it away from the sample, in the direction so the 4X objective comes into position (ie avoid turning the 40X into position, because it might get contaminated with oil). Using lens paper, dab the 100X objective until all oil has been removed.

10. For phase contrast:

To align the condenser and 40x objective, set the condenser turret to Ph3. Use a phase telescope in place of the right hand eyepiece. Focus on the bright white ring seen with that ocular. There is also a black ring, nearly concentric with the white ring. To bring the two rings into focus, you may also have to focus the phase telescope, by rotating it in the same way that you do with the regular oculars. Use the phase centering screws to adjust the 2 rings so they are as close to concentric as possible. These are the 2 flat silver colored knobs at the front of the microscope, just below the condenser turret.

If a 100X objective has a red band, it can be used for phase contrast, with the ph4 condenser setting. You can align condenser and 100x objective using a phase telescope the same as for a 40x objective.

On some microscopes, the 100X objective has a white band. Those can only be used for bright field; not for phase contrast.

11. When finished, use lens paper (<u>only</u> lens paper) to blot the oil off of any 100x lens that you used and check the 40x lens just in case it was rotated through a drop of oil. Stop blotting when the lens paper comes away dry. Turn the light off and cover the microscope with its dust jacket.

B. Wet mount.procedures.

1. Put a very thin ridge of Vaseline along the 4 edges of one side of a cover slip. Put the Vaseline on your thumb and scrape it off onto each edge. Set down the cover slip with Vaseline side up.

2. Put several loopfuls of water on a slide as a single large drop.

3. Inoculate a visible amount of bacteria into one edge of that drop.

4. Lay cover slip on top of the drop, Vaseline side down. As the drop spreads as it's compressed by cover slip, a gradient of bacterial density forms, decreasing from the side where bacteria were inoculated. *The coverslip must contact and spread out the sample – some bubbles are okay but there must not be an air gap.*

5. To observe microscopically:

a. Place condenser turret in Ph 4 setting. Obtain a good focus with 4X objective. This gives dark field. You can see bacteria as white specks. Go up to 10X, focus, still dark field. Can see bacteria clearly, but fairly small.

b. Move condenser turret setting to DF and objective to 40X. Still dark field. Can see bacteria very well in white, and can see movement clearly if it is taking place.

c. Still at 40X, turn to Ph3. This now gives phase contrast. (See above for details of phase contrast alignment.) If there are endospores present, they can be seen as bright (highly refractile) circles within part of a cell, or as separate bright structures outside of cells, especially in older cultures. *Note: You will only find spores in Gram positive cultures. You need not look for them in Gram negative cultures.*

C. Notes on cleaning and storage

1. For cleaning optical surfaces, including objective lens, condenser lens, field lens, and eyepiece: *Use only lens paper or a cotton tip applicator on any optical surface. Never use a paper towel, kimwipe or any other material for cleaning.*

First blot the surface with lens paper to absorb any liquid or solid material. Using lens paper or cotton-tipped applicator stick, dab distilled water onto the surface, then dab the surface dry. If that isn't sufficient, dip a cotton-tipped applicator stick in 10% acetic acid (if not available then ask an instructor). Wipe that tip *gently* across the surface. Dab the acetic acid off with lens paper.

2. We have been keeping your microscopes in the working position, with the binocular eyepiece tube rotated so as to place the oculars forward. In storage, the upper portion (with the oculars) is rotated 180 degrees, so the oculars point backwards. You may leave them in the working position for the duration of the lab course. If you are asked to store a microscope, loosen the set screw at the right hand side. Holding the upper portion with both hands, rotate it 180 degrees, so the oculars are pointing backward. Re-tighten the set screw.

3. Both the condenser and the stage are removable for cleaning. There is a set screw at the front of the stage that must be loosened to slide the stage out, and must be tightened after the stage slides back in; otherwise, the stage is free to rotate. There is also a set screw for the condenser, straight left on the condenser mount.