PROTOCOL: MICROSCOPE SETUP – OLYMPUS BX51

The following procedure should help you set up your microscope and achieve Köhler illumination. You should run through this procedure each lab period to optimize the alignment. Try the tutorial on Microscopy U to make sure you understand the procedure.

In this procedure, you will adjust two diaphragms to optimize the image: a field diaphragm and a condenser diaphragm. These create the field and aperture stops shown in Fig. 3 in the MM labscript.

- The *field diaphragm*, which creates the field stop, is located on the base of the microscope, and its adjustment ring shows its open and closed position. It controls the area of the sample that is illuminated.
- The *condenser diaphragm*, which creates the aperture stop, is mounted under the stage. The size of the aperture is controlled by a small lever under the condenser between the two centering screws. The condenser diaphragm controls the angle of the cone of rays reaching the objective and the depth of field.

A diagram of the apparatus and flow chart of this procedure follow the instructions. Review these as you follow the steps below.

1. Turn on the illumination lamp. The brightness of the lamp can be changed by rotating the illumination adjustment knob.

2. Start with the *condenser diaphragm* largely closed (lever to the right) and the *field diaphragm* open. Make sure the neutral density filter, located just below the centering screws, is rotated out of the light path.

3. Place the calibration slide on the stage. Use the condenser *height adjustment knob* at the back of, and under, the stage to raise the condenser lens to its highest position. Select either the 10x or 20x objective lens by rotating the lens turret. Then, use the *fine and coarse focus knobs* to raise/lower the stage and bring the slide into focus. DO NOT change the height of the stage any further in the following steps of this procedure. If you cannot see anything through the eye piece, make sure that the microscope is set in *viewing* and not in *camera* mode.

4. Close the field diaphragm to reduce the amount of light entering the sample until the illuminated spot seen through the eyepiece is smaller than the field of view and you can see the edges of the diaphragm (may be blurry). Figure 1 shows what you should see through the eyepiece.

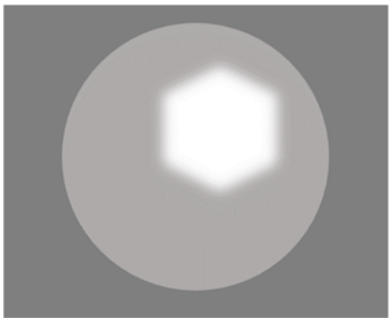

Figure 1. Image you may see after closing the field diaphragm in Step 4.

5. Adjust the height of the condenser (NOT THE STAGE!) to optimize the focus of the field diaphragm. The diaphragm edges should be sharp, not blurry. Use the small *condenser height adjustment knob* attached to the condenser for this adjustment, not the big focussing knob that changes the stage height. Because the condenser lens has chromatic aberrations, the image goes from a blue to a red halo as you move through the plane of sharpest focus. (The different colours are in focus at slightly different condenser heights.)

6. Use the two *condenser centering screws* to centre the illuminated spot (which tracks the condenser lens) relative to the circular microscope field of view. (Look directly through the eyepiece rather than using the camera for this adjustment.) The focal region can be moved in two directions using two centering screws, each at a 45° angle with respect to the principal axes of the rectangular stage. Start with the field diaphragm opening fairly small; then gradually open it as you perform the adjustment, making the offset from the centre more obvious. After this adjustment, the image of the diaphragm should look like a polygon inscribed and centred within the circular viewing region of the eyepiece. If the microscope is badly out of alignment, you may need to refocus and adjust the height of the condenser iteratively.

7. Open the field diaphragm more, but just enough to fully illuminate the sample. Now, the circular viewing region inscribes the polygon, as seen below.

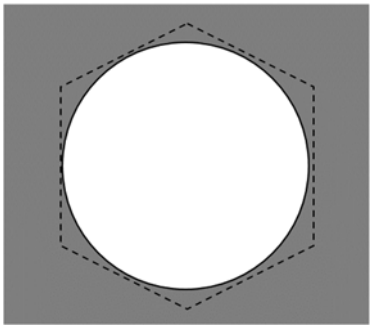

Figure2. Image you should see after the field is opened after adjustment

If the image is not bright enough, increase the lamp brightness rather than open the diaphragm any further.

8. Adjust the condenser aperture so that its NA (the angle of the rays focussed on the sample) approximately matches the NA of your objective lens. (See Fig. 2 in the MM labscript or the Nikon MicroscopyU website.) Thus, for a 100x objective, the condenser diaphragm should be fully open (lever to the left) to collect the largest amount of light; for a 10x objective, the diaphragm should be closed down more.
9. Repeat this procedure with the high magnification (100x) objective. In general, if you want to work with a high magnification objective lens, it is better to first centre the polygon using a lower magnification lens (e.g. 10x) as described above, and then carefully rotate the objective turret to the 100x lens.
    - Note that the 100x objective is an *oil-immersion* objective, which means that the medium between lens and sample is oil, with $n{\sim}1.5$ (the n in $NA = n\ sin\ \theta$; recall that using $n > 1$ increases the microscope's resolution). In practice, you need to put a drop of microscope oil between the sample chamber and the lens in order to focus properly into an aqueous sample. There are several ways of doing this. We recommend that you find the object using a lower magnification objective, swing the lens turret so that you are between that objective and the high magnification objective, add the oil, and then swing the turret so that the high magnification objective enters the oil. %Alternately, you can lower the sample chamber away from the objective, place a small drop of oil on the sample chamber and then raise it again. But it can be hard to find the focal plane again!
    - After you have finished experiments for the day, be sure to gently wipe the oil off the objective with a tissue, and then again with a tissue wet with ethanol.

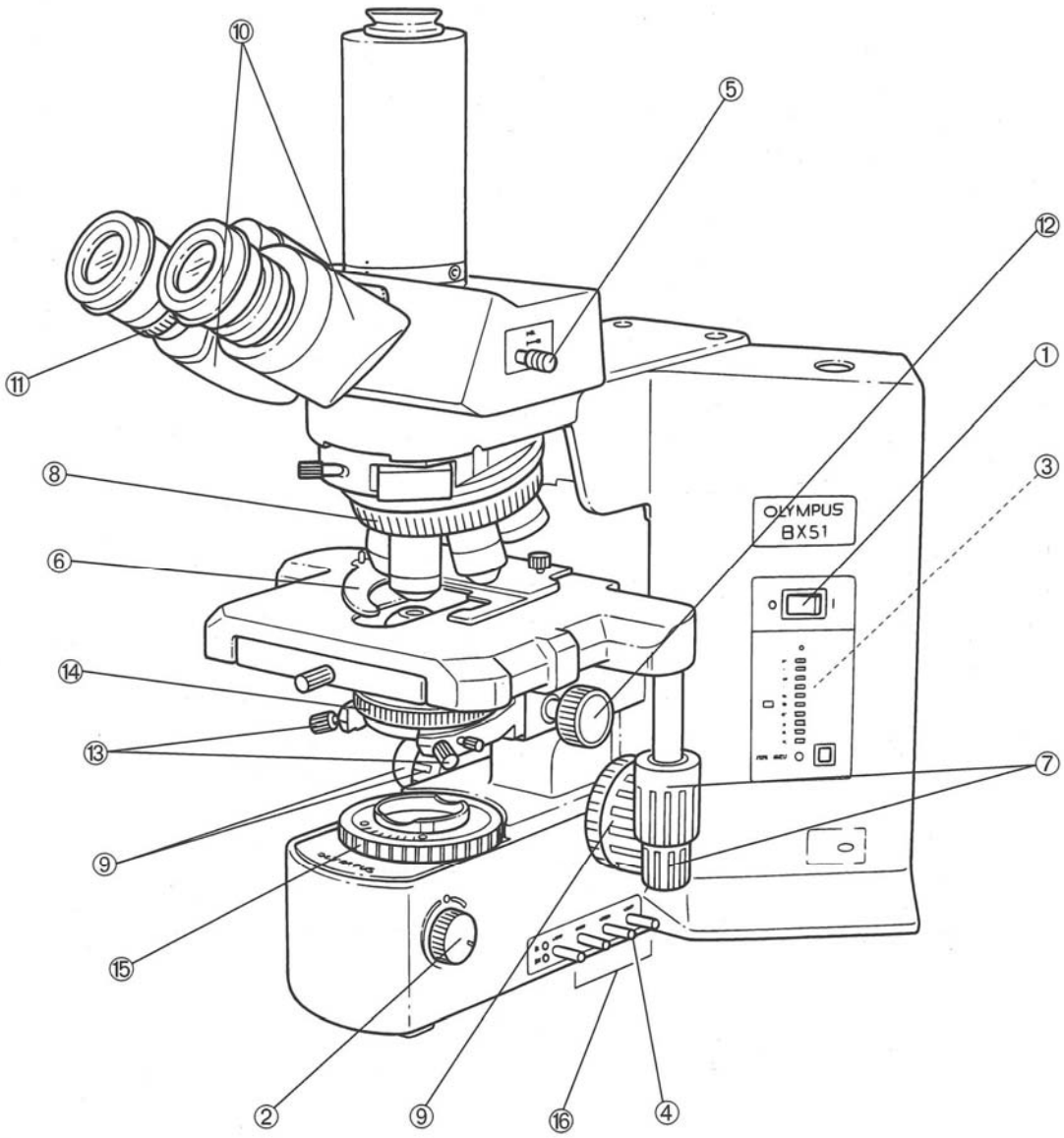

Figure 1. Schematic of the Olympus CX33 microscope (Olympus CX33 manual).

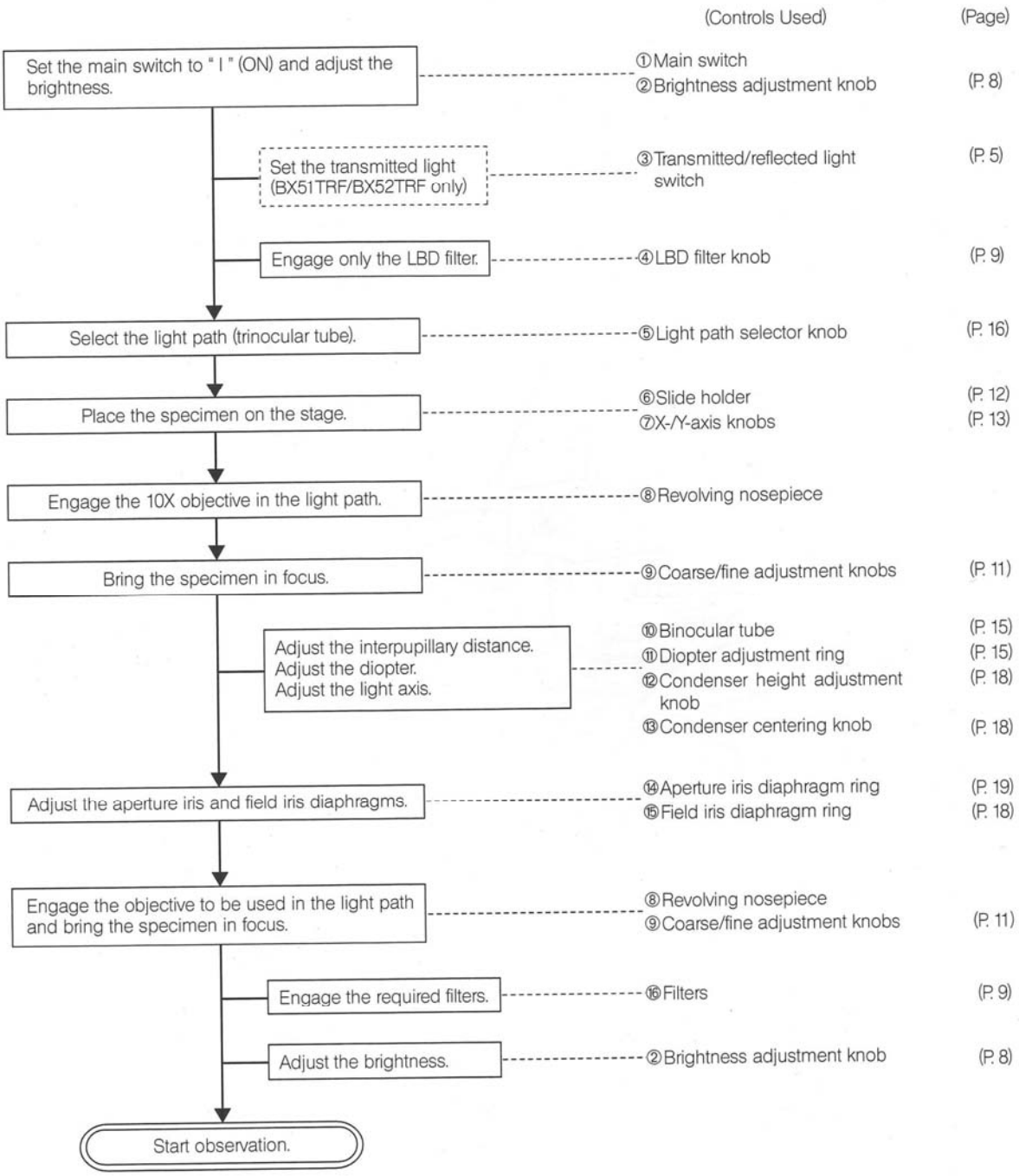

Figure 2. Flowchart describing setup procedure (Olympus CX33 manual).