Setting up Köhler Illumination

1. Turn on the light source and swing in a 10x objective. This is of sufficiently low magnification not to hit the slide while initial adjustments are being made.
2. Raise the condenser to the top of its travel.
3. Open fully the illuminated field (lamp) diaphragm (IFD) control in the base of the microscope, and the illuminating aperture (condenser) diaphragm (IAD) control on the condenser.
4. Place a well-stained slide on the stage, checking that the coverslip is facing the objective.
5. Use the coarse focus, to reduce the distance between slide and objective to a minimum, closer than the focal point of the objective. Look side-on at the gap between the objective and slide whilst doing this, to ensure that they do not collide. Now, whilst looking into the eyepiece(s), increase the distance between slide and objective using the coarse focus control, and stop when the image of the specimen comes into focus. Adjust as necessary with the fine focus control. This sets the specimen in correct relation to the objective.
6. Close down the IFD almost to a pinhole. This diaphragm is usually situated in the base of the microscope, underneath the condenser and substage assembly. Rack the condenser down slowly until the image of this diaphragm is sharply in focus at the specimen plane, superimposed upon the image of the object.
7. Open up the IFD until its image is almost reaches the edge of the field of view.
8. Centre the condenser with its adjusting screws, if provided. Once done, open the field diaphragm further by a small amount until the image of the diaphragm lies just outside the field of view. Do not open it too much, otherwise stray light will reduce contrast in the image.
9. The correct height of the condenser has now been set. The condenser IAD can now be adjusted so that the aperture of the variable cone of light supplied by the condenser can be correctly matched to the (generally) fixed numerical aperture (NA) of the objective.
10. Remove an eyepiece and look into the microscope body tube to inspect the back focal plane of the objective, which is seen as a disc of light at the base of the tube. Adjust the condenser IAD until the image of this iris is just a little smaller (about 80%) than the diameter of the disc of light, which represents the full aperture of the objective.
11. Replace the eyepiece.

In theory, the aperture of the condenser should equal that of the objective. However, in this case stray light refracted from the extreme edges of the objective lens elements would cause an appreciable loss in contrast. It is worse, however, to close down the aperture diaphragm too far: this will cause serious degradation in image quality, and loss of resolving power. This diaphragm is not be used to control brightness in the image; rather, use the rheostat control on the lamp transformer, or (where the intensity of the lamp must remain constant, as for colour photomicrography for example) use neutral density filters. Closing down the aperture diaphragm from its optimum position will increase contrast at the expense of severe loss of resolving power. Decreasing the aperture of the condenser will also increase its depth of field, and bring into focus dust and other contamination from the surfaces of the specimen preparation normally invisible in the properly adjusted microscope.

12. If the microscope is fitted with a binocular head, the eyepieces can now be adjusted for comfortable viewing. One or both eyepiece tubes may have adjustable dioptre focusing controls capable of adjusting the tubelength of the microscope. If only one eyepiece tube of the binocular has a variable control, set up Köhler illumination with the fixed eyepiece and adjust the variable control for the other eye until the image is in focus while the eyes are relaxed.

If the binocular head has two adjustable focusing controls, first focus the microscope using a high magnification objective (e.g.40x). Change to the 10x (or preferably a lower magnification objective if it is part of a parfocal set), and focus the eye adjustments separately without refocusing the objective.

When changing to another objective of different magnification, this will have a different field of view (requiring a change in the diameter of the illuminated field) or numerical aperture (requiring a different illuminating aperture), and both field and condenser diaphragms must be adjusted. With objectives below 10x, the field of view may not be fully illuminated even with the field diaphragm fully open. In this case, the top lens of the condenser should be either swung out or unscrewed from the condenser assembly. For objective magnifications higher than 10x, do not leave the condenser top lens swung out of the optical path, otherwise the objective will not be fully illuminated, and severe loss of resolving power will be the result.