Köhler illumination adjustment for the microscope

There are four steps to follow in order successfully to adjust a microscope so that it gives a good image. These are:

- **Focus the specimen upon the microscope stage**
- **Illuminate the specimen using the illuminated field diaphragm control**
- **Adjust the illumination using the condenser control**
- **Fine-tune the eyepiece adjustments to suit your eyes for relaxed viewing**

Köhler illumination provides a large and uniform, intense, structureless light source from a small, filament structure. The illuminating filament in the aperture set of planes does not disturb the image which lies in the field set of conjugate planes. Köhler illumination allows independent control of the illuminating and imaging ray paths. Stray non image-forming light can (largely) be excluded from the microscope, leading to an improvement in contrast. The illuminated field diaphragm acts as the control for *area* of illumination. The illuminating aperture diaphragm (condenser diaphragm) controls the included *angle* of illumination focused by the condenser onto the object.

A. Focus the specimen

1. Open the illuminated field diaphragm and the condenser diaphragm fully.

2. Turn on the light source and swing in a 10x objective. This will have sufficient working distance (the clearance between the front of the objective and the slide) not to hit the slide while initial adjustments are being made.

3. When using the microscope, your eyes should be relaxed. Before using the microscope, first look into the distance i.e. at 'infinity' then check that the inter-ocular distance between the two eyepieces via the folding (or sliding) bridge on the binocular head is set correctly for you to see one circle of light instead of two.

4. Raise the condenser to the top of its travel.

5. Place a slide on the stage, checking that the coverslip is uppermost (or on an inverted microscope downwards) facing towards the objective – we all forget this at one time or another!

6. 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. Remind yourself which way to turn the coarse focus to focus away from the objective. Looking down the eyepieces, you should see the image of the specimen very much out of focus.

7. 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.

B. <u>Set the height of the condenser</u>: Illuminate the specimen using the illuminated field diaphragm (IFD) control

8. The correct height of the condenser must now be set. Raise the condenser up to its limit.

9. Close down the illuminated field diaphragm almost to a pinhole, or at least to its smallest extent. This diaphragm is usually situated in the base of the microscope, underneath the condenser and substage assembly. If the lamp is an external one, the field diaphragm will be the iris built into the lamp at the front, after the lamp collector lens. 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.

10. Open up the field diaphragm until its image is almost reaches the edge of the field of view. This way, the it is easy to operate the two centring screws such that the narrow penumbral shadow just inside the field of view and surrounding the illuminated part of the image of the specimen, can be made of parallel width.

11. Open the 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. The IFD should confine the area illuminated to that under observation by a particular objective lens. (For contrast enhancement methods such as phase-contrast and dark-field, which employ their own annular diaphragms in the condenser, the IFD must be left fully open).

C. <u>Adjust the condenser control</u>

12. The correct height of the condenser has now been set. The condenser diaphragm 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.

13. Remove an eyepiece[1] and look into the microscope body tube to inspect the back focal plane of the objective, which should be seen as a disc of light at the base of the tube. The rays of light are brought to a focus in the back focal plane of the objective, where a (usually fixed) diaphragm is located. This *aperture diaphragm of the objective* limits the effective numerical aperture of the objective.

14. Adjust the condenser aperture diaphragm until the image of this iris is just a little smaller than the diameter of the disc of light (about 80% diameter), which represents the full aperture of the objective. Replace the eyepiece.

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[1] In addition to removing an eyepiece, the back focal plane of the objective may be observed by two other means. Insert a phase-contrast centring telescope, or screw a low-power objective to the bottom of the drawtube (if fitted). These methods provide a magnified image of the back focal plane of the objective for inspection. If a magnification changer is included, this often includes a supplementary lens system - a Bertrand lens - for the same purpose. A final method is to place an inverted eyepiece over the exit pupil of the eyepiece to view the exit pupil of the microscope.

In theory, the aperture of the condenser should equal that of the objective (see the image below). 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 condenser diaphragm is *not* be used to control brightness in the image; rather, use the rheostat control on the lamp transformer. Closing down the aperture diaphragm from its optimum position will increase contrast, and may sometimes be the only method of introducing sufficient contrast to visualise the image at all, but this contrast is gained at the expense of resolution of fine details and good image quality. The rule that the IAD is set open at approximately 80% of the numerical aperture of the objective is for guidance and good practice; by all means break it if you know what you are doing! Closing the condenser diaphragm too far will cause the image gradually to become a less faithful representation of the object: outlines of all features will appear thickened, and adjacent features merge. 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.

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 diaphragms be adjusted. With objectives below ten times, 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. Do not defocus the condenser to enlarge the illuminated area, since uneven illumination and a loss of resolving power and image quality will result.

D. <u>Adjust the eyepieces</u>

15. This is done to avoid eye-strain under conditions of prolonged visual use (e.g. when then image is viewed directly by eye, and not projected onto a computer monitor) for comfortable viewing.

16. One or both eyepiece tubes on the microscope binocular head may have adjustable 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. Try not to close the opposing eye while focusing, nor screw the eyes up, but rather relax them – you should be aiming to 'look through' the microscope to infinity. Fatigued eyes result if the eyepieces are not correctly adjusted for relaxed viewing of an image at infinity - which is how the microscope is designed to be used.

17. If the binocular has two adjustable focusing controls, first focus the microscope using a high magnification objective (forty times; a 'high-dry' objective – a non-immersion one with high aperture, but ideally *without* a correction collar). Change to the ten times (or preferably a lower magnification objective if it is part of a parfocal set), and adjust the eyepiece focusing adjustments separately *without* refocusing the objective so that the specimen is in focus.

Now the contrast-enhancement methods (e.g. phase contrast and DIC may be adjusted if provision has been made for these techniques in the configuration of the microscope.