SETTING UP THE MICROSCOPE part 1

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Once a microscope is available it is important to get the best images that it can offer. Real life microscopy is usually a matter of compromise but it is essential to understand the nature of the compromise. This article is based on the use of bright field microscopy and assumes that the microscope is equipped with a condenser. Although the condenser is below the stage and may not have the dominant position of the objective and eyepiece lenses, it is an important part of the light path and an understanding of how it functions and how it can be manipulated is essential.

The condenser is usually held in place by a single screw and it is worth plucking up courage and taking it out for close inspection so that all the component parts can be examined (Fig. 1). There are usually two lenses and the top one can often be unscrewed from the rest of the condenser body. An important component is the iris diaphragm and it is well worth opening and closing it to see the full range of apertures available and to appreciate what a clever mechanical device it is. Below the iris diaphragm there will be a carrier which can be used for coloured filters and simple dark ground stops.

The condenser illustrated also has a third lens below the filter carrier which may be required for low power objectives but is swivelled out of the light path for the higher power objectives. If the condenser is removed, and this can often be the most convenient way of cleaning it, it is necessary to ensure that it is put back properly. It fits very snugly into its outer sleeve and it is possible to get it jammed while pushing it up into its highest position before tightening the retaining screw. Although it may feel a tight fit it is essential to tighten the retaining screw so that it does not slide out again. Some models have a small screw in the side of the condenser which locates in a groove in the outer sleeve. It is essential that the screw locates into the groove so that the condenser can be pushed properly home.

Failure to put the condenser back properly makes it impossible to carry out some of the operations needed for critical illumination.

General principles for bright field microscopy

There are three desirable goals:-

1. Resolution – the ability to resolve fine detail and have confidence in the validity of the image. That is, to be confident that the microscope is telling you the truth.

2. Contrast – the ability to see an object against its background – a special problem when both are colourless. We can see colourless objects in a colourless medium if they have sufficiently different refractive indices.

3. Depth of focus – this is often a matter of aesthetics, especially in photomicrography. Indeed, the process of focusing through an object can often provide valuable information about its third dimension.

Goal number one is incompatible with goals two and three using an ordinary bright field microscope. To achieve contrast (and depth of focus) is usually to introduce artefacts and hence compromise the confidence in the validity of the image. On the other hand there is no point in having the best resolution in the world if you cannot detect the image! It is (in my view) best to set up the microscope to provide optimum resolution and IF NECESSARY increase contrast by moving away from optimum resolution as little as possible.

Setting up for Critical or Nelsonian illumination

1. It is important that the light source, condenser, objective and eyepiece are all aligned along the same optical path (although some valuable tricks can be played by deliberately illuminating the object at an angle!). If the condenser has centering screws:-

- Select a low power objective and x10 eyepiece and focus on a simple object on a prepared slide. Close the condenser iris diaphragm and bring it into focus by racking the condenser up or down. Check that the image of the iris diaphragm is in

the centre of the field of view (see Fig. 2). As you carefully open the iris diaphragm while viewing through the eyepiece it should go out of the field of view uniformly. Once this adjustment is made it should not be nessesary to do it again unless the microscope is violently handled or fiddled with. Many 'modern' microscopes do not have readily accessible centering screws for the condenser which is set up by the manufacturer.

2. It is important that the light source is focused on the specimen (see Fig. 3):-

- Open the condenser iris diaphragm, put a good contrast specimen on the stage and bring it into focus. Carefully place a dissecting needle or a pencil point in the centre of the exit aperture of the light source and raise the condenser until this is in focus with the specimen. The focus will never be very sharp but as you go through the focus point a halo outlining the dissecting needle will change from reds to blues. This adjustment does not need to be changed for different powers of objectives.

3. It is important that the cone of light from the specimen just fills about 4/5 of the back lens of the objective:-

- Remove the eyepiece from the microscope and look down the tube. Close the iris diaphragm of the condenser to leave a field of view of about 4/5 of the diameter of the objective lens (see Fig. 4). If the object on the slide is obscuring the view then move it to a clear part without altering the focus. It is worth opening and closing the iris diaphragm several times to become familiar with where the edge of the back lens of the objective is. As one opens the condenser there will come a point at which the circle of light will not get any larger and that is the edge of the lens. Close it again so that about 4/5 remains illuminated. Replace the eyepiece.

4. The system is now set up for optimum resolution for the low power objective. This final step does need to be carried out for each objective thus, when it is required to use a higher power objective, swing it into the light path, refocus on the specimen and repeat stage 3.

Setting up for Kohler illumination

If the microscope is also equipped with a field diaphragm (i.e. an iris diaphragm at the light source) then a more refined lighting system can be achieved which will provide a uniform field of light which is especially useful for photomicrography. That the brightness of the background is not uniform from the centre to the edge of the field of view does not seem to worry the human eye/brain combination but it can be detected by the film in a camera.

1. Remove any diffusing screen from the light path and open all the iris diaphragms. Focus the condenser close to the specimen slide and focus the microscope on a specimen. Close the field iris diaphragm, focus and centre it in the specimen plane using the condenser focus and centering controls. Reopen the field iris diaphragm to just beyond the field of view.

2. Remove the eyepiece and slide the specimen out of the field of view without altering the focus. Observe the lamp filament and focus it using the lamp condenser lens. Replace the diffusing screen. Close the condenser iris diaphragm to leave a field of view of about 4/5. Replace the eyepiece.

Kohler illumination is especially useful for ensuring that the field of view is uniformly illuminated as well as providing optimal resolution for photomicrography.

Increasing contrast (and depth of focus).

Having adjusted the microscope for optimum resolution, both closing the condenser iris diaphragm and lowering the condenser will increase contrast and increase depth of focus but note that image integrity will be compromised. It is often the case that, unless material has been strongly stained or is naturally deeply coloured, the contrast of fungal materials mounted in the most commonly used mountants will be low. However, by starting from a position of optimum resolution it should be possible to achieve sufficient contrast without compromising resolution too much. It is worth experimenting by opening and closing the condenser iris diaphragm slowly while looking at the image and notice the manner in which it changes. When the iris diaphragm is opened too wide there will be considerable glare and the image will be very difficult to see. When the iris is correctly set and time allowed for the eye to get used to the low contrast, it will be possible to see fine detail. As the iris is closed, it will be evident that darker outlines are appearing around objects and the depth of focus increases but those darker outlines may give a false impression of the thickness of walls or membranes surrounding the structures under study. Beware of artefacts!

Alternative ways to increase contrast without compromising resolution are phase contrast, Nomarsky optics and dark field illumination.

Articles on microscopy in *Mycologist* and *Mycologia*:-

Cunningham, J.L. (1972). A miracle mounting fluid for permanent whole-mounts of microfungi. *Mycologia* 64, 906-911.

Volkmann-Kohlmeyer, B. & Kohlmeyer, J. (1996). How to prepare truly permanent microscope slides. *Mycologist* 10, 107-108.

Jensen, C., Neumeister-Kemp, H. & Lysek, G. (1998). Fluorescence Microscopy for the observation of nematophagous fungi inside soil. *Mycologist* 12, 107-111.

Useful sources of advice:-

Bradbury, S. & Bracegirdle, B. (1998). *Introduction to light microscopy*. Bios Scientific Publishers, Oxford.

Bradbury, S. & Evennett, P.J. (1996). *Contrast techniques in light microscopy*. Bios Scientific Publishers, Oxford.

Bracegirdle, B. & Bradbury, S. (1995). *Modern PhotoMicrography*. Bios Scientific Publishers, Oxford.

Gams, W., van der Aa, H.A., van der Plaats-Niterink, A.J., Samson, R.A. & Stalpers, J.A. (1987). *CBS Course of Mycology (3rd Ed.)* Centraalbureau voor Schimmelcultures, Baarn. Pp. 16-21.

Rost, F. & Oldfield, R. (2000). *Photography with a microscope*. Cambridge University Press, Cambridge.

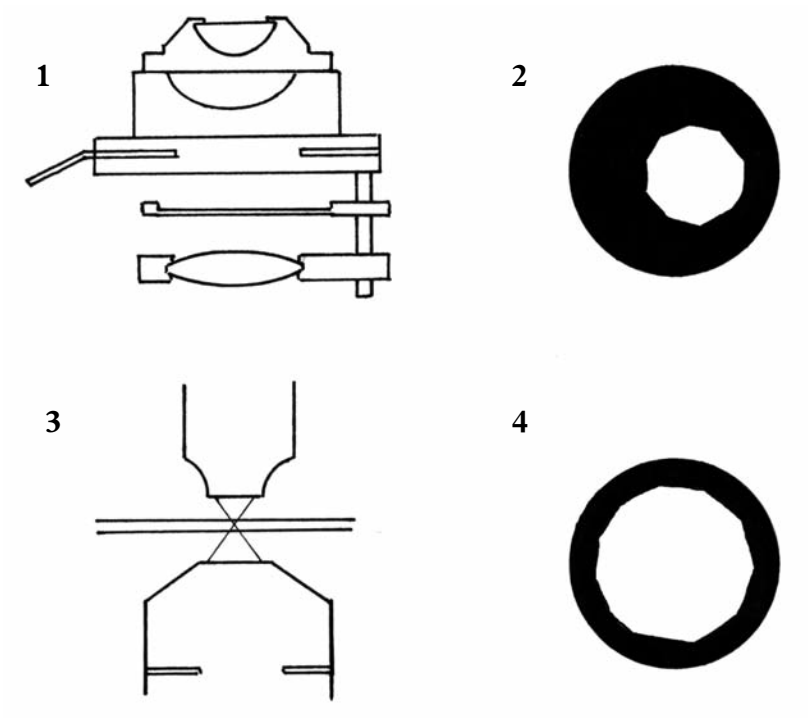

Fig. 1. Cross section of a simple condenser. Fig. 2. View of condenser iris diaphragm fully closed when viewed with a low power objective with the eyepiece in place. In this instance it needs to be centred! Fig. 3. Condenser set to focus light on the object being studied. Fig. 4. Correct setting for the iris diaphragm viewed down the barrel of the microscope with the eyepiece removed.