GETTING STARTED WITH A MICROSCOPE

Your microscope and equipment – some *very basic* advice on care and practice (PC - July 2022)

Buying a compound scope. If money allows, get a binocular or - if you're likely to want to use it for photography – a trinocular scope. If the latter you are likely to need to buy the camera specifically designed for that scope, also the accompanying software etc to link it to your computer. If 'techy stuff' is not for you / your pocket will not stretch this far, it is quite possible to take reasonable manual photos down the eyepiece using a small digital camera. For mycological study you need a scope with a minimum capability of x 400 magnification – without this spores cannot be measured accurately enough – and ideally one with x 1000 capability using oil immersion. However, a cheaper student-type model with less powerful magnification will allow you to see basic spore and cystidia shapes etc. and provide a good introduction to the world of fungal microscopy. You can always progress to a more sophisticated set-up at a later stage.

An eyepiece graticule. This is a small glass disc which fits inside one of the two eyepieces and enables you to make accurate measurements in microns (indicated thus: µm). A micron = a 1000th of a millimetre, a 10,000th of a centimetre. If you're going to want to measure spores etc, make sure your scope can accommodate a graticule and preferably ask for one to be fitted for you before you receive it. The graticule has 100 divisions etched onto it like a ruler, marked off in 10s. The measurement of each division depends upon which objective you are viewing through:

- At x 1000 (the white objective using oil immersion) each division = 1 micron
- At x 400 (the blue objective) each division = 2.5 microns (or thereabouts)
- At x 100 (yellow objective) each division = 10 microns (or thereabouts)
- At x 40 (red objective) each division = 25 microns (or thereabouts)

NB: Individual scopes will vary in accuracy though most have been set up to give exact measuring at x 1000, ie 1 division = 1 micron. For the other objectives it is necessary to check the exact calibration for your particular scope using a stage micrometer. These can be bought (or borrowed if you know someone who has one – you will only need to use it once to check your scope) and directions how to make the calculations are available online. If in doubt, ask for this to be done for you if possible before taking possession of it.

- It is useful to keep a calculator to hand beside your scope for measuring at x 400. Spores are mostly small enough to make the necessary calculation (the number of divisions x 2.5 or thereabouts) in your head, but for cystidia and other longer cells it is handy to be able to multiply by 2.5. A calculator is also useful for working out the average spore size if required, measuring a minimum of 10 spores selected at random. (For more on this go to 'Microscope Workshop – some general information / tips' under 'Measuring spores'.)

More sophisticated scopes having cameras which are linked to a computer will have high tech measuring devices built into their software, possibly making a graticule superfluous. However, being entirely reliant on such systems can have obvious disadvantages; it is advisable to have an eyepiece graticule as back up and for speedier calculations.

A disecting scope. This is a much simpler type of scope having lower magnification for viewing an object directly. It is extremely useful (*in fact pretty well essential*) for preparing slides ready for viewing with the compound. If your pocket will stretch to it, one having understage lighting as well as top lighting, also a zoom stereo capability, is money well spent. Even the cheaper more basic models will enable you to make much more accurate slide preparations and therefore get far better images and information from your compound scope. (For instruction on preparing slides etc, see 'Microscope Workshop – some general information / tips'.)

Where to buy from. There are a myriad of suppliers online. Two companies which come highly recommended are Brunel Microscopes (www.brunelmicroscopes.co.uk) and MicroScience (https://micro-science.co.uk). Both provide friendly service, are used to mycologists' requirements and will give help and advice.

A few tips about looking after your scope.

- If possible keep your scope permanently set up and ready for use. One which is packed away in its box is much more likely to remain there and not get used.
- When not in use always cover your scope to keep it clean and free from dust. As with cameras, binoculars, etc, dust is the enemy of such equipment. A plastic bag, carrier bag or old pillowcase will do the job if a proper cover is not supplied with the scope.
- When working with your scope, turn the light down whilst preparing slides, making notes, using reference books etc, to avoid it overheating and to prolong the life of the bulb.
- When turning your scope light off after use, *turn it right down first*. This helps to prolong the life of the bulb: if left turned up high, when you next use the scope the power surge may cause the bulb to blow. It's worth buying a spare bulb to have to hand – it will be needed at some stage!
- If you are likely to be transporting your scope, it is well worth buying the carrying case designed specifically for the purpose.

- The more your scope is moved around the more likely it is that the bulb will blow or that something may get out of alignment. Treat it with great respect and always transport it with care in its carrying case.
- If you suspect your scope is not performing as it should, it is worth getting it serviced / cleaned by a specialist. This is unlikely to be required more than once every few years at most, depending on how much you use it. (Many mycologists have never had their scope serviced.) A recommended fairly local company is Optech Microscope Services Ltd in Thame, Oxfordshire (details online). They provide an efficient and reasonably cheap service and will also check the calibration for your scope for you.

USING THE OBJECTIVES

Most modern compound scopes have four objective lenses as standard: x 40 (marked with a red ring), x 100 (yellow), x 400 (blue), x 1000 (white, needing oil immersion).

Step 1: Having placed your prepared slide on the stage, start off using either x 40 (red) or x 100 (yellow). With practice you can usually locate your sample on the slide at x 100, but if not it's far easier to search around using x 40, focussing up and down with the coarse focus knob and using the mechanical stage controls (back and forth / sideways) till your sample is nicely in the middle of your view and in focus.

Step 2: Move up to the next objective, ie from red to yellow, or yellow to blue. *NB make sure you turn the correct way and not from red to white!* Your scope should be set up so that your sample, once in focus using the red objective, should remain more or less in focus when you move up from one objective to the next. A small adjustment to improve focus may be needed: when using the yellow objective adjust the focus using the coarse focus knob, then move up to the blue objective and adjust again if necessary *BUT now using the fine focus knob*.

Step 3: It's at x 400 (blue) that you'll be carrying out most of your observations. You'll need to move around your sample using the stage controls and adjusting the fine focus up and down as you go: different cells will come into and out of focus according to their position and the thickness of your sample. Take your time, making notes if necessary. You can always go back down to lower magnification at any stage to locate different bits of your sample if needs be, then return to x 400 for more study.

- Remember, using the graticule for measuring at x 400 (blue), you should multiply by 2.5 (or thereabouts) to give you microns.
- Trouble-shooting. You may find after time that you've run out of fine focus, ie the knob will no longer turn clockwise. Don't try to force it. The remedy is to wind your fine focus knob back (anticlockwise) a considerable distance and as you do so you'll see the stage gradually lowering. You'll find this action will have solved the problem.

Step 4 using oil immersion: There are instances when insufficient detail is visible even at x 400, or when precise measurements are necessary. This is when your x 1000 objective (white) using oil immersion comes into play.

Procedure: Whilst viewing at x 400, move the stage controls to place the bit of your sample you wish to study at x 1000 into the centre of view (ie under the 50 division on your eyepiece graticule). Remember that at x 1000 your field of view will be extremely limited and moving around to search for different parts becomes much harder. Now you need to do two things before adding your oil: with the coarse focus knob wind the mechanical stage well down, also move the objectives to *half way between* x 400 (blue) and x 1000 (white). You will now have enough space to add a small drop of immersion oil to the centre of your cover slip (it must be immersion oil – no other oil should be used). Add your drop, then fully engage the x 1000 objective ready for use. Now *instead of using the eyepieces keep your eye on the oil droplet on your slide* and carefully wind the stage back up again. Slow right down as it approaches the objective, watching for the exact moment of contact between droplet and objective – this will be obvious, like a 'pop', and at that moment *stop winding*. Now, using the eyepieces, move to the fine focus knob and cautiously and very slowly continue to wind until your sample starts to come slowly into focus. Make the tiniest movements possible, pausing to check each time: if you proceed too fast it is all too easy to pass the moment of focus at this high power without realising.

- Remember, using the graticule for measuring at x 1000 (white), each division = 1 micron.
- Before going 'up to oil' make sure you've made all the study you want at lower magnification first. Once oil has been added to a slide *you cannot return to lower magnification*.
- If you have too much liquid or too much tissue on your slide, also too much oil on your cover slip, you are less likely to have success. This process takes practice but is well worth the effort!
- If your scope has an immobile stage, proceed as above omitting the winding down then up steps. The moment of contact between droplet and objective will occur as you swing the x 1000 objective into place.
- TAKE CARE not to get oil on any other objectives! This is easily done if you move the objectives round the wrong way in error. Should this happen, unscrew the affected objective and carefully clean the tip with a lens cleaning cloth or a drop of lens cleaner on a cotton wool bud. This also applies to any stains / chemicals used in slide preparation. Permanent damage to the tip of your objective may be the result.

LIGHTING. The higher the magnification you are using, the more light is needed. It's good practice (and better for your eyes) to start off with your light source turned down when using the less powerful objectives, gradually increasing it to maximum for x 1000. Depending on what you are viewing, you can increase the light still further using the

condenser diaphragm located immediately under your light source. There is a condenser focus knob, on the right when turned down, which can be moved round to increase the intensity of light – useful for seeing details of spore ornamentation. Some cells are better seen with the condenser turned down, however. Trial and error is needed.

RE-USING SLIDES AND COVER SLIPS

Apart from cover slips which have been used for oil immersion, you can clean off slides and cover slips for re-use. Keep a small bottle of water and some kitchen roll / tissues handy. After use, separate the cover slip from the slide, add some water to both in turn and wipe clean then dry with your tissue. Cover slips will probably last only a few times before breaking – they are very fragile. Slides will last much longer but will eventually get scratched.

- Make yourself a 'sharps Pot' – a plastic container with a slot cut in the lid large enough to accommodate a slide. This is handy for quick disposal of oily or broken cover slips etc.

ESSENTIAL TOOLS AND STAINS ETC

For preparing slides to view with your compound scope, you will need the following tools apart from slides and cover slips:

Fine forceps - either straight or curved - or tweezers; razor blades or a scalpel; a needle; possibly a small glass rod for transferring stains from a bottle to your slide.

- A child's paint brush with a needle fixed onto the opposite end with sticky tape is very useful.
- Recommended razor blades: Derby Professional single edge blades available cheaply online in packs of 100.

Useful basic stains and chemicals for microscopy:

Congo red; 10% ammonia in water; Melzers reagent; KOH.

- Both Brunel Microscopes and MicroScience (mentioned earlier under 'Where to buy from') can supply these apart from the ammonia which is available from HomeBase in bottles labelled Ammonia Max Strength.

There is plenty more information and instructions about how and when to use stains / chemicals available in other BFG Microscopy pages. See under 'Microscope Workshop – some general tips / information', also 'Microscope Workshop – details of common genera'.