OCR (A) Biology A-level

Module 1: Development of practical skills in Biology

PAG 1: Microscopy

Please note: You only need to do one from each PAG, and you don't need to do the PAGs listed here, as long as you show the same skills that these are testing (see 5f of the specification for more information). However, you need to at least be able to design your own method for most of these experiments in the exam.

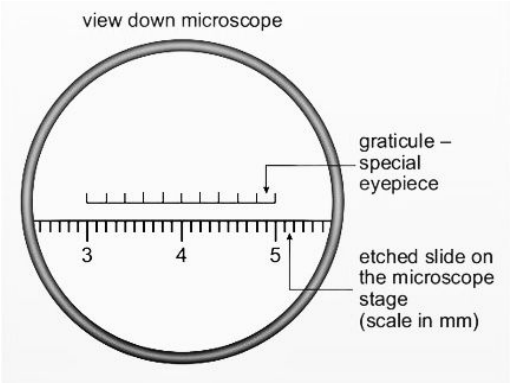
Observing structures using a light microscope

Calibrating the graticule:

An **eyepiece graticule** can be fitted to a microscope - this acts like a ruler, allowing structures to be measured under the microscope. A **stage graticule** (aka **stage micrometer**) is a microscope slide with an accurate measuring scale – this is used to calibrate the value of the eyepiece divisions at different magnifications.

The following process is used to calibrate an eyepiece graticule:

1. Set up the microscope to the required magnification to view the sample.
2. Place a stage graticule on the stage.
3. Line up the two scales (the stage and eyepiece graticules) similar to the diagram.
4. Count the number of divisions on the eyepiece graticule equivalent to each division on the stage micrometre.
5. As the length equivalent to each division on the stage micrometer are known, it is possible to calculate the length of one eyepiece division.



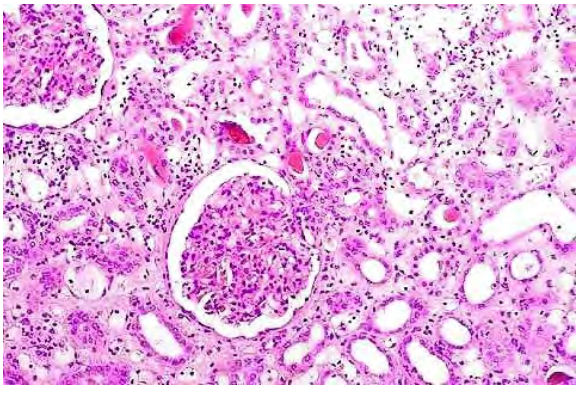
Note that if you change the magnification of the microscope, the eyepiece graticule must be re-calibrated! The process should be repeated for each objective lens, then you will have a calibration factor for each lens.

Preparing slides:

1. **Staining**: stain the sample using the appropriate dye.

   This provides contrast to distinguish between different structures in the sample. **Differential staining** is when multiple stains are used, and each stain binds to a specific cell structure, staining each structure differently so the structures can be easily identified.

   - **Acetic orcein** binds to DNA and stains chromosomes dark red
   - **Eosin** stains cytoplasm dark red or pink
   - **Iodine** stains starch blue-black (appears violet under the microscope)
   - **Iodine in potassium iodide solution** stains cellulose yellow



- **Haematoxylin** stains RNA/DNA a purple/blue colour
- **Methylene blue** is an all-purpose stain, used often to stain DNA blue

2. Mount the sample using one of two techniques:

Wet mount – this is used for a variety of live specimens, such as aquatic animals

a) Use a pipette to put a drop of water on the slide.

b) Use tweezers to place the specimen in the water.

c) Put the cover slip on by standing it upright on the slide, next to the water droplet, then carefully tilt it down onto the specimen. Be careful to not add bubbles – these will obstruct the view of the image.

d) Add a stain. Put a drop on one edge of the cover slip. Put a paper towel on the opposite edge. The paper towel will absorb the stain, drawing it under the coverslip, staining the specimen. (You want to make sure the stain used is not toxic to the live specimen).

Dry mount – this is used for specimens such as hairs, parts of insects, pollen, parts of flowers etc.

a) Slice the specimen into a thin piece so light can pass through

b) Use tweezers to pick it up and put it in the middle of the slide

c) Put a cover slip on top of it.

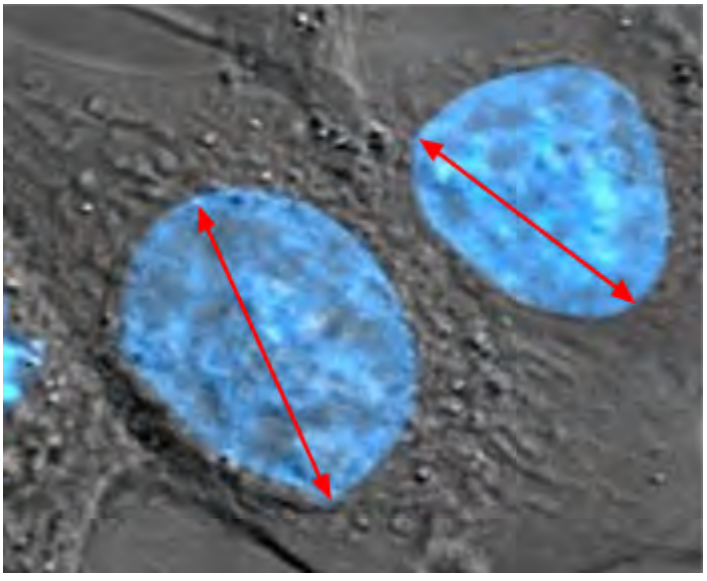
Using a Light Microscope:

1. Clip the slide onto the stage
2. Select the **lowest-powered objective lens**
3. Use the coarse adjustment knob to move the objective lens to just above the slide.
4. Look down the eyepiece and adjust the focus by moving the lens away from the slide using the fine adjustment knob, until a clear image appears. Note: always adjust the focus by moving the lens away from the slide – this prevents you from moving the lens too close to the slide and breaking it
5. If a higher magnification is needed, swap to a higher-powered objective lens and refocus (repeat steps 3-4).

Measuring size with a microscope:

For this an eyepiece graticule should be used. Once the eyepiece graticule is calibrated (see above: calibrating the graticule), structures can be measured by counting the number of eyepiece divisions.

To find the actual length, the number of divisions should be multiplied by the length of one division (you calculated this when you calibrated the graticule).



To measure the diameter of an irregularly shaped object (such as a cell), the length should be measured of the greatest distance from one side to the other.

Drawings:

They should be consistent with the following:

- No shading. Areas that should be shaded should be labelled instead.
- The drawing should take up at least half of the page.
- Label lines must be completely horizontal, drawn with a ruler, exactly touch the object that they're labelling and must not overlap each other.
- Drawing lines should completely connect and should not be hairy.
- A scale should be given e.g. for the magnification of the image size.
- They should be drawn in pencil and look like the actual image.