Royal Microscopical Society

A Teacher's Guide to the RMS Secondary School Microscope: Suggested Microscopy Activities

These activities are a small selection of common microscopy related and microscopy educational/instruction activities appropriate for a variety of ages at secondary school and accessible to the use of the RMS secondary microscope. Here you will find some instruction and limited theory of bright and dark field imaging, polarised light microscopy as well as the basics of magnification. The activities are more about the application of microscopy techniques rather than further understanding of the theory behind image formation and contrast. However by following the links to the various resources further theoretical information can be found.

This document is a compilation from around the world. Sources have been referenced as accurately as possible and should be appropriately acknowledged. This is a list of suggested activities - further details for each can be found by visiting the referenced websites (selected as online resources - correct 2022).

You may also be interested in www.microscopy-uk.org.uk, which is full of great light microscopy resources.

Safety and risks

Teachers must assess each activity for local health and safety considerations. It may not be possible to use cheek cells/ blood in all centres. Please ensure your own health and safety policy is adhered to. This document is for suggested activities not a prescriptive list of instructions.

Recommended equipment

- Plastic tweezers
- Fine tweezers
- Glass slides & cover slips
- Needle/wire loop
- Scalpel/craft knife
- Desk lamp/detail light
- Stain (refer to the references in section 5 for specific stains that you may wish to try)
- Mobile phone (for light and to attempt to take some pictures if you don't have a ccd camera)

Activities

	Subjects	Ages
1. Using the RMS Microscope	Biology/Chemistry/Physics	11 - 19 (yr 7 - 13)
2. Magnification and use of Graticule	Biology/Chemistry/Physics	11 - 19
3. Bright and Dark Field Microscopy	Biology/Chemistry/Physics	14 - 19 (yr 9 - 13)
4. Polarised Light Microscopy	Biology/Chemistry/Physics	14 - 19
5. Contrast and Staining	Biology	11 - 19
6. Fracture Sections	Physics	14 - 19
7. Brownian Motion	Physics	11 - 16 (yr 7 - 11)
8. Fibre Detective	Biology/Chemistry/Physics	11 - 16

This document has been produced by Dr James Perkins, Queen Elizabeth's Grammar School, Faversham, Kent in conjunction with the Royal Microscopical Society

1. Using the RMS Microscope

Biology/Chemistry/Physics | 11 - 19 (yr 7 - 13)

What is special about the RMS secondary microscope? There are two key things which set this microscope apart, one is the ability to image in dark field mode and the other is the polarising filter; two elements that are not normally found on school microscopes. Within each activity a brief explanation as well as links to useful resources and information are provided for you and your students to understand more.

Background

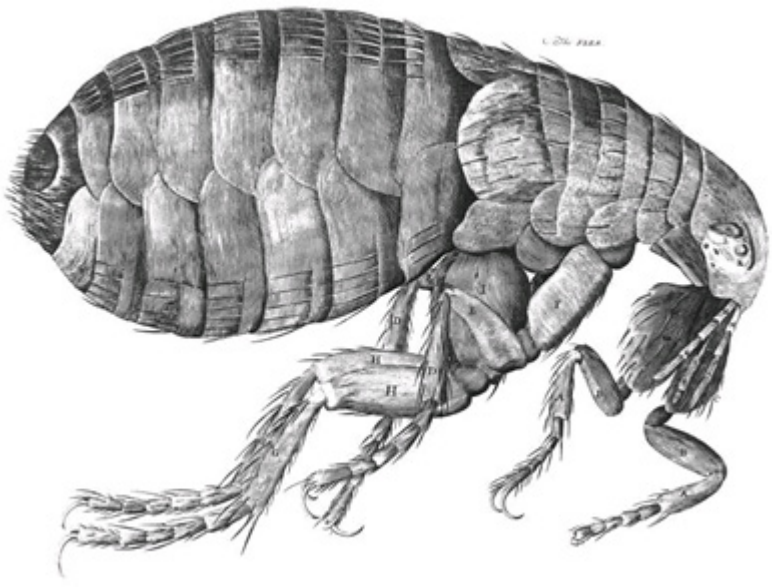
The microscope was one of the single most important inventions in the history of biology. Before microscopes we had no idea about the existence of really tiny things. We thought diseases were caused by noxious vapours in the night, or evil spirits, or curses because we couldn't see bacteria. So never take microscopes for granted, they offer a window into a miraculous new world.

The Greeks and Romans experimented with simple lenses following the invention of glass. Improvements in lens making led to the manufacture of the first spectacles during the 13th Century, but it was not until the late Renaissance period that serious progress was made. Antonie van Leeuwenhoek was a cloth merchant who developed simple microscopes for examining cloth in the 16th Century. They could achieve magnifications up to x270 but had only one lens, so were really just powerful magnifying glasses. Nevertheless he saw bacteria, red blood cells and single celled Protoctists like Amoeba. Zacharias Jansen is credited with inventing the first two-lens ("compound") microscope in 1595. It wasn't very powerful, magnifying up to x9. Still, it was good enough for the king of Holland to order as an expensive toy!

Robert Hooke was an English scientist who developed telescopes and microscopes of very high quality. In 1665 he published Micrographia, a book which Samuel Pepys said was "the most ingenious he ever read in his life". It contained many famous pictures and in it Hooke used the word "cell" for the first time.[4]

Modern microscopes are the result of continuous improvements on 17th Century instruments. The lenses are ground by machine instead of by hand, and the glass is of higher quality, giving a clearer image. A good light microscope can magnify up to x1,800 and see an object 0.0002 mm (200 nm) across. This is approaching the limit of what is possible using light to view an image.





Hooke's flea. Illustration from his famous work; 'Micrographia'.

The RMS secondary microscope has a number of features you may not find on your own school microscopes:

Eyepiece lens (with or without graticule)

The lens through which you look. 10x lens (ten 'times' magnification), 18mm internal diameter so that the eyepiece can be replaced with a suitable digital camera (linked to a computer or projector). A mobile phone camera can be placed on the top of the eyepiece lens to very simply record images.

Graticule - a 'ruler' inside the microscope. This gives a scale for reading relative distances or linear sizes of objects. However it is not an absolute measurement and a calculation is needed for a given eyepiece/objective lens combination. (see Activity 2. Magnification and use of Graticule)

Polarising filter slider (in out slider below the eyepiece lens tube and prism)

This is used in conjunction with the polarising filter below the stage (see activity 4)

Objective lens turret containing three lenses

4x/0.1 NA (*magnification/numerical aperture size - determines resolution of image*); 10x/0.25 NA; 20x/0.40 NA

This set provides a good range of resolution and total magnification (40 – 200x) and field of view (approximately 4 mm – 1 mm).

Stage and stage clips

The stage is the flat area with the hole where a slide will sit. A standard glass slide will be held in place by the stage clips. Slides are easily released by pressing on the curved arm at the back of the clips.

Rotating aperture/disc filter

(a) 2 diaphragms of different sizes (to limit the angle of the cone of light from the condenser and to achieve, for each objective, near-optimum conditions for resolution and contrast).

(b) A central 'dark ground' stop which blocks the light which (in bright field) normally passes from the condenser directly into the objective, but which allows the light scattered from the specimen to enter the objective for dark field imaging.

(c) A polarising filter (polariser) to be used in conjunction with the analyser above the objective lens turret.

Focus adjustment and fine focus adjustment:

Coarse focus adjustment moves the stage with respect to the main body of the microscope in the case of the RMS microscope. The relative movement is important but this may be slightly different on different models.

Fine adjustment: Fine tunes the focus and increases the detail of the specimen.

Light source and condenser lens:

The light is a battery powered LED lamp with a dial to control the brightness.

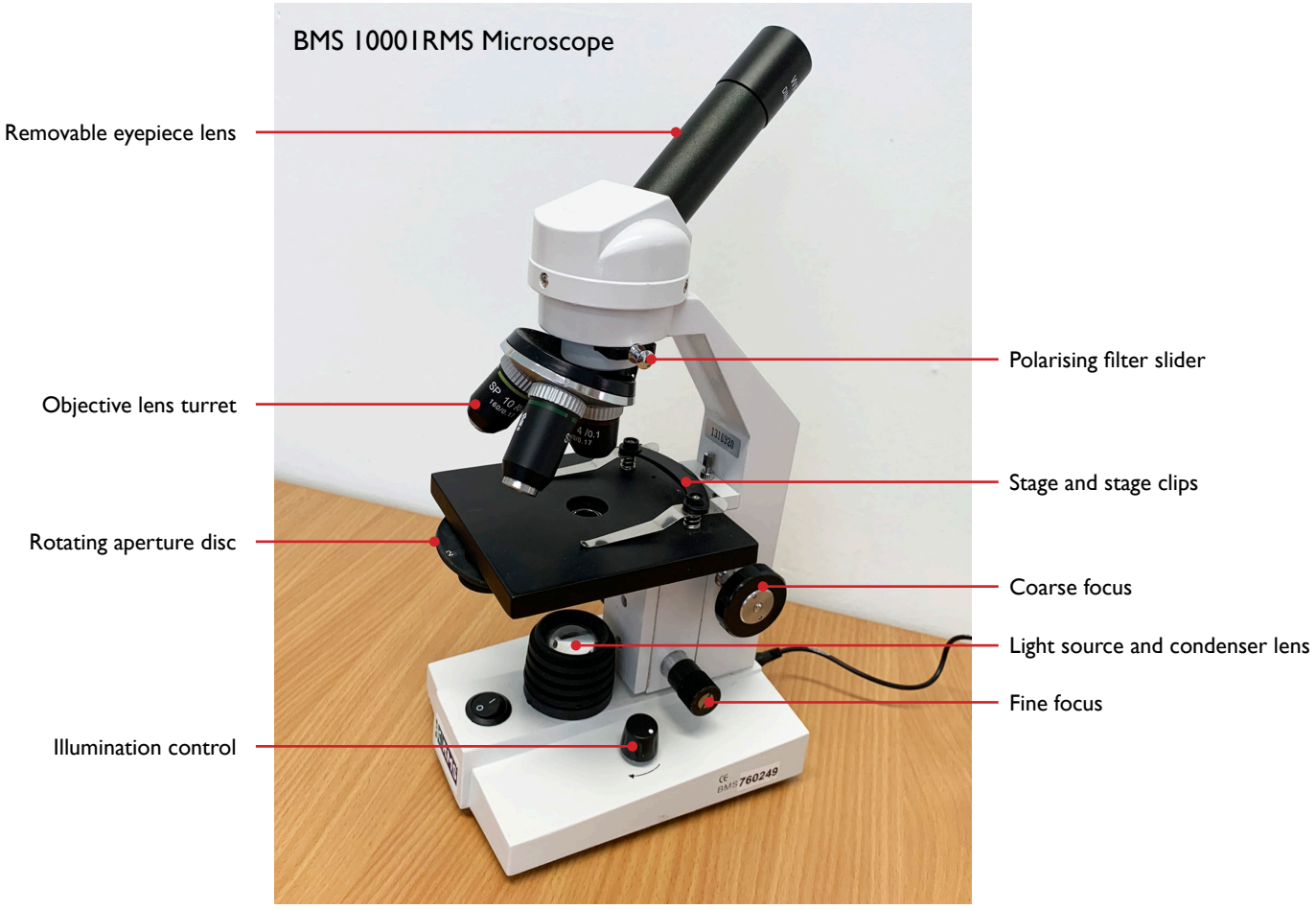
The condenser lens allows the light to be focused in such a way as to broadly illuminate the entire stage aperture such that the whole field of view is illuminated for the 4x lens and that enough light can pass through to the 20x lens.

In order to move your microscope safely, one hand should be under its base for support and the other at its arm. When finished, return the objective lens turret to the lowest magnification lens, turn the illumination to the lowest setting, then turn off the lamp.

Over the past few years, we have worked closely with Scientific & Chemical Supplies to develop a microscope that fulfils all of these requirements, the BMS 10001RMS Microscope is now available to buy from SciChem.
https://www.scichem.com/product/microscope-bms-10001rms

Instructions

1. Turn on the illuminator. The RMS microscope has a battery powered LED light. It may need recharging before first use. The light quality will be different to a standard lamp that you may be used to; the LED will be white compared to the off white/yellow of a filament lamp.
2. Place a slide (with cover slip) or specimen on the stage with the sample directly above the aperture. Ensure slides are fixed using the clips (press the curved connector at the back of the stage to raise the clips).



3. Ensure the circular diaphragm slider is set to position "3" - this will ensure the maximum illumination. If the image appears too bright use the dimmer to adjust.
4. Rotate the nosepiece so that the 4x objective lens is above the sample.
5. Turn the coarse adjustment knob until the specimen comes into broad focus.
6. Do not use the coarse focus with a high magnification objective - you may make contact with the slide, damaging both the sample and the lens.
7. Turn the fine adjustment knob until the specimen comes into sharp focus.
8. If you cannot find the focus position lower the stage and begin again with the coarse adjustment - be careful that you do not hit the sample with the objective lens!
9. There is a rack stop but thick samples or those without a cover slip because of their size may well still be too large for the 20x lens. Be careful!
10. Change to a higher power lens as necessary - you should only use fine adjustment once the low magnification focus position has been found.

Enjoy observing your specimens!

Different imaging conditions and experiments can be found elsewhere in the secondary microscope documents.

Alternatively, visit some of the referenced websites to find experiments you can do with the microscope

References

https://www.microscopemaster.com/use-and-adjust-a-compound-microscope.html
https://www.rms.org.uk/asset/6AAE0DC8-106E-4E72-A58D59E36E842282
Bennet. J. "introduction to microscopy", St Paul's school lesson resources, 2017
https://www.bl.uk/collection-items/micrographia-by-robert-hooke-1665
https://www.rms.org.uk/discover-engage/microscopes-for-schools.html
https://www.asm.org/index.php/educators/k-12-classroom-activities

2. Magnification and use of Graticule

Biology/Chemistry/Physics | 11 - 19 (yr 7 - 13)

To magnify is to make an object appear larger than it really is. This is the entire point of a microscope so that details are clear and visible to the observer. In a compound microscope, the eyepiece and objective lenses work together to produce a clear magnified image.

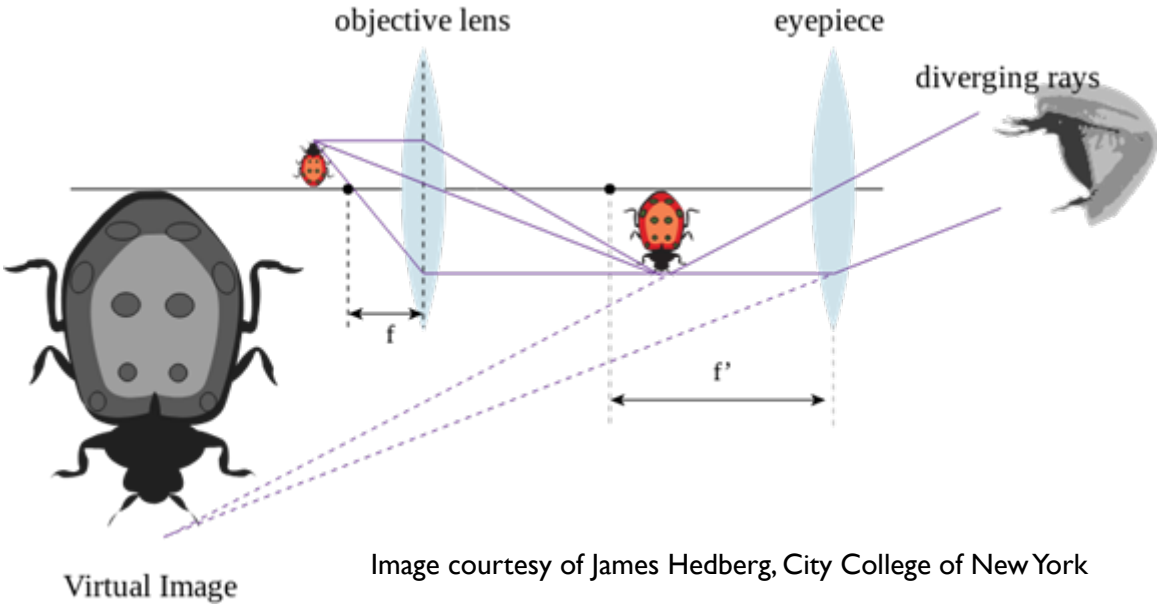


Image courtesy of James Hedberg, City College of New York

The image in a compound microscope (like the RMS one) is magnified twice, once by the objective lens and once by the eyepiece lens. The total magnification is the product of the two magnifications.

Power	Eyepiece	Objective	Magnification
Low	x10	x4	x40
Medium	x10	x10	x100
High	x10	x20	x200

We use image size, actual size of object, and magnification. To calculate the actual object size, divide the image size by the magnification. To calculate the magnification divide the image size by the object size.

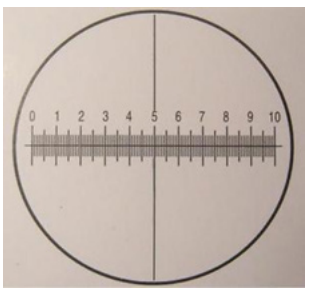
$$\text{Object size} = \frac{\text{Image size}}{\text{Magnification}} \quad (1)$$

$$\text{Magnification} = \frac{\text{Image size}}{\text{Object size}} \quad (2)$$

Graticule

The graticule is a series of etched lines within the eyepiece lens that allow measurement of features of an image. The graticule is calibrated so that each line corresponds to a particular distance depending on the power of the objective lens being used.

To calibrate the graticule you must use a stage graticule/stage micrometer - If you do not have one of these then try using a simple mm scale ruler from your pencil case. You'll soon see all the imperfections in the ruler and why it would be useful to have a more precise instrument! You may also use an object where you know the size already and compare this with the eyepiece graticule.



A graticule showing the overlay measurement scale which would be seen on top of an image in the eyepiece lens.

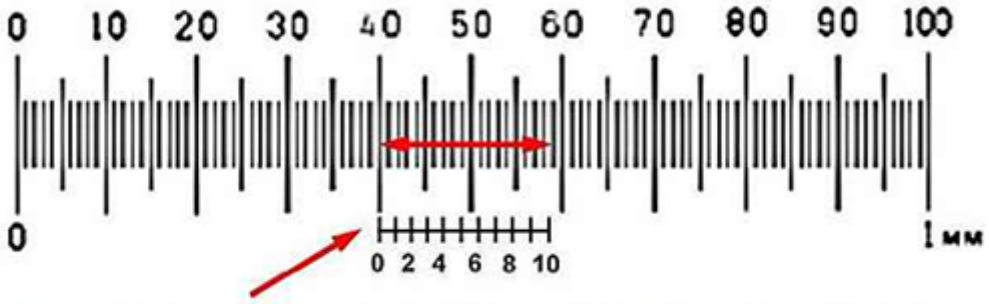
A stage micrometer consists of a microscope slide on which is engraved a fine and accurate scale. Because the scale has to be accurately produced to give reference dimensions, stage micrometers are much more expensive than eyepiece graticules.

When carrying out calibration, each objective lens has to be separately calibrated. You should check this for the RMS microscope (do the numbers make sense? I.e. does the scaling factor fit with the different power objective lenses?)

Start with the lowest power objective on the microscope. The scale on the stage micrometer is aligned with the scale of the eyepiece graticule and then a reading is taken from the scales. These readings are then used to calculate the calibration factor for the objective lens in use.

Take an example image viewed below:

- Reading from the two scales 10 divisions on the eyepiece graticule equals 19 divisions on the stage micrometer.
- If the stage micrometer we are using is: 100 divisions = 1 mm therefore each division is 1/100 mm (10μm).
- 100 eyepiece division = 19 x 10 μm
- 1 eyepiece div = 190/100 μm = 19 μm



Repeat the calibration for the remaining objectives.

Measurements

Now that you have your calibration for each objective you can measure some of the objects that you've looked at in the microscope!

References

https://www.bbc.com/bitesize/guides/z84jtv4/revision/9 (bbc has a great video for magnification/graticule use)

https://www.tecmicroscopes.co.uk/wp-content/uploads/2014/08/Microtec_Graticule_Calibration.pdf

https://www.npl.co.uk/products-services/dimensional/scales-micrometers

Magnification image courtesy of James Hedberg, City College of New York

3. Bright and Dark Field Microscopy

Biology/Chemistry/Physics | 14 - 19 (yr 9 - 13)

Bright field microscopy uses light from the lamp source under the microscope stage to illuminate the specimen. In order to view a specimen with bright field conditions, the light rays that pass through it must be affected by the sample itself. In dark field microscopy the entire field of view appears dark when there is no sample on the microscope stage. However, when a sample is placed on the stage it appears bright against a dark background. This is because the condenser is designed to form a hollow cone of light rather than illuminating the entire sample at the same time. The objective lens is placed within the dark region of the hollow cone and light travels around the objective lens.

Contrast arises because light is refracted or absorbed by different parts of the sample making some regions brighter and some darker. However, sometimes a specimen will have a refractive index (*a property that affects the speed of light through different transparent materials*) very similar to the surrounding medium (e.g. water droplets or air around the sample) between the microscope stage and the objective lens. When this happens, the image can not be seen - i.e. there is very little *contrast*.

In order to observe materials with similar optical properties we must alter the refractive index of different parts of the object. One way of doing this is to stain (see Activity 5. Contrast and Staining). Staining can kill some specimens but also may not enhance the features of interest thus dark field microscopy is used instead.

Also, because dust and other small contaminants are enhanced with darkfield microscopy, it is important to have very clean slide and liquid used for samples.

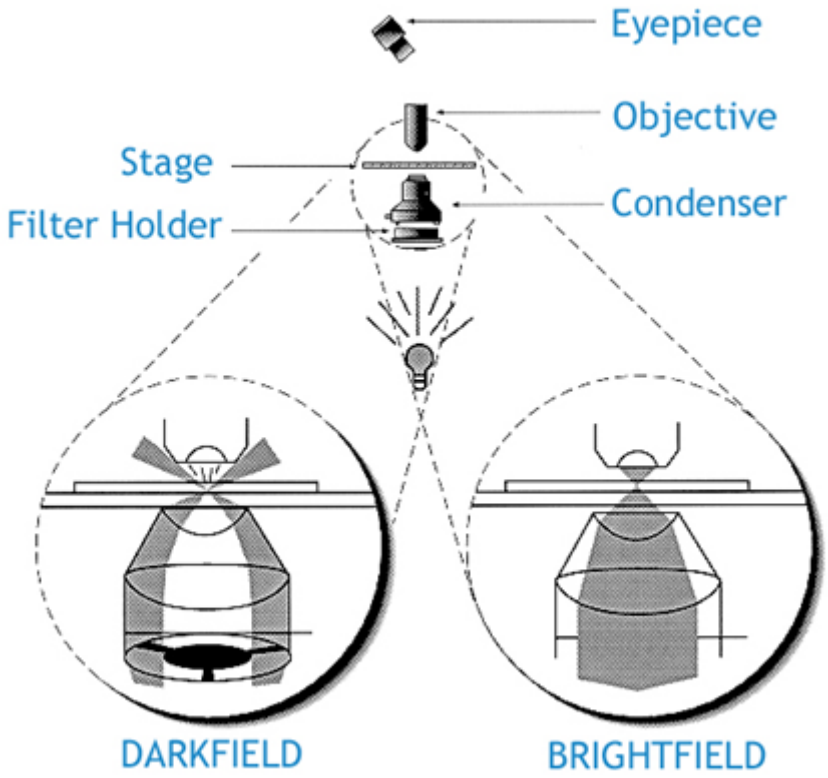


Illustration provided courtesy of Washington State University taken from The American Biology Teacher issue 61, October 1999, pg 622

Method

- Insert the slide and focus as best you can using the method from section 1.
- Turn the diaphragm selector until "DF" appears.
- Adjust illumination so that features of the sample can be seen clearly.

Activities

- Try different types of sample, which ones have better contrast in dark field compared with bright field?
- See if you can view the following: blood cells, bacteria, different types of algae, hairline metal fractures, diamonds and other precious stones, shrimp or other invertebrates.
- Ink dots on paper can appear as blobs - by using dark field microscopy carefully the ink mixing and boundaries between colours can be seen. Try altering the brightness while in bright and dark field imaging modes and see what happens.

Tasks

- Determine in which type of material the dark field image provides complementary information to the bright field image.
- Which elements of the image improve (i.e. become clearer or more understandable as a feature).



Complementary bright field and dark field images from infocus Magazine, issue 37, March 2015, pg 69

The head of a mosquito larva in bright field (a) and dark field (b). Notice that the fine hairs projecting from the head, which scatter light only weakly, are clearly visible in dark field against the black background. [Field of view 2mm]

References

https://www.microscopeworld.com/t-darkfield_microscopy.aspx

https://www.qualitydigest.com/june04/articles/02_article.shtml

Charlotte K. Omoto, Department of Genetics and Cell Biology, Washington State University

4. Polarised Light Microscopy

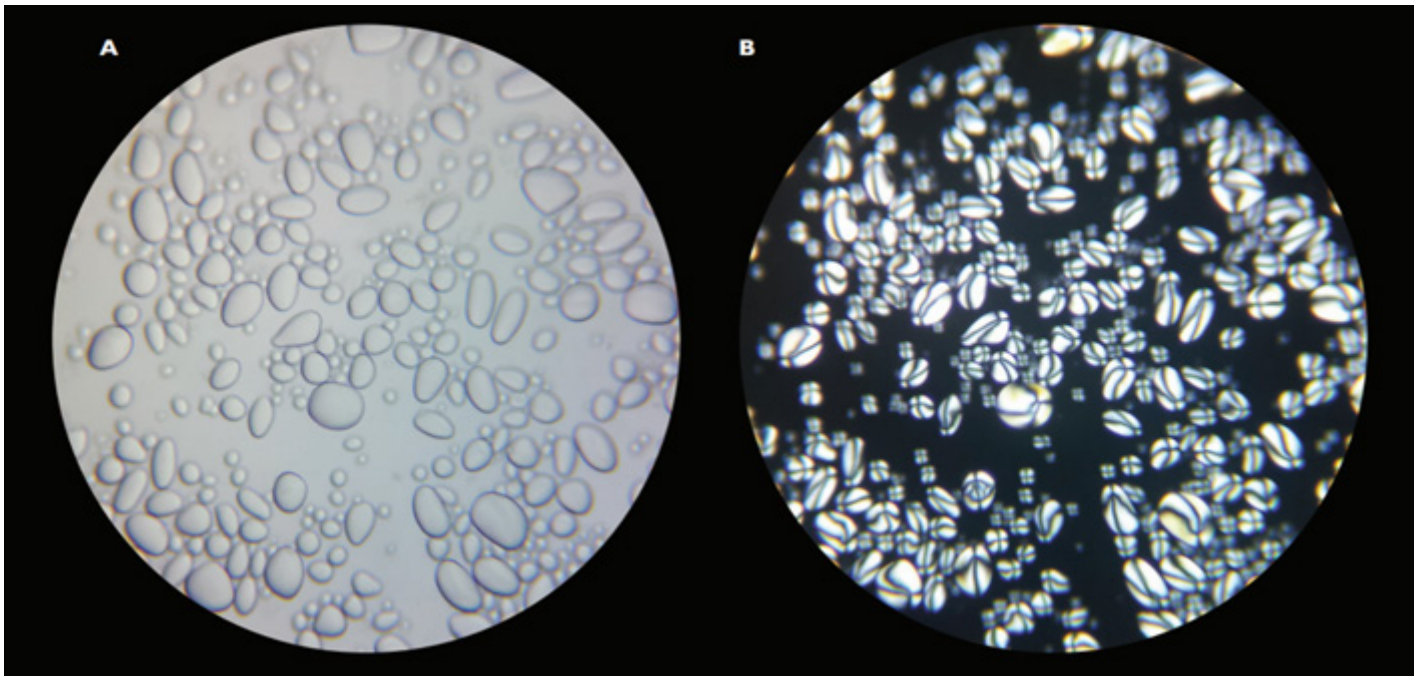
Biology/Chemistry/Physics | 14 - 19 (yr 9 - 13)

The physics of polarised light is beyond the scope of this activity sheet, however it is recommended that the student reads up a little on polarised light and topics such as birefringence in crystals before undertaking any experiment involving the polarising filters. Some good resources to understand polarised light are given in the references.

The RMS microscope has a polarising filter on the rotating diaphragm (spin the diaphragm until it reads "p"). This means polarised light is being sent through the specimen.

The analyser slider is placed in position by sliding it through the body of the microscope until it clicks into place (you should be able to 'feel' this position).

With no sample in place there should be no light reaching the eyepiece lens. A sample suitable for polarisation microscopy will affect the light passing through. This means that, depending on the properties of the chosen samples, contrast between different areas of a sample will appear.



Potato starch grains, (a) in bright field,showing little contrast, and in (b) the polarised-light image ('between crossed polars'), which reveals the optical properties of the arrangement of the starch molecules. [Field of view 1mm] - from infocus Magazine, issue 37, March 2015, pg 69

References

http://hyperphysics.phy-astr.gsu.edu/hbase/phyopt/polarcon.html

https://bitesizebio.com/13395/everything-you-ought-to-know-about-polarising-light-microscopy/

https://www.olympus-lifescience.com/en/microscope-resource/primer/virtual/polarizing/

5. Contrast and Staining

Biology | 11 - 19 (yr 7 - 13)

Contrast is defined as the difference in light intensity between the image and the adjacent background relative to the overall background intensity. Different techniques will improve contrast in images in different ways, for example; use of polarised light or dark field imaging. A common and useful technique is staining, use of an alternative chemical that reacts with or preferentially attaches to particular features of a sample in order to make a particular feature darker or appear as a colour. A barium meal is used for x-ray contrast where the 'meal' is greater density than the surrounding tissue a clear, high contrast, image can be produced.

Different stains can be used for different purposes - for biological imaging it is important to know if the stain will kill the process/cell you are wishing to image! "Vital stain" - stain of living cells, "non vital stain" - stain of dead cells. A list of different types of stain can be found in reference 2.

A note for ordering of slide samples; "st" is a stained preparation; "wm" whole mount (the complete organism); "cs" cross section; "ls" longitudinal section, a section cut lengthwise; "sq" is a squash preparation; and, "sm" is a smear, such as a blood smear.

Cell

The first use of the word "cell" to describe biological structures was by Robert Hooke when describing the structure of cork in his book, Micrographia. Over 350 years later you will see the features that led Hooke to describe the structure in such a way.

1. Take some cork from an old noticeboard or (empty!) wine bottle and carefully shave off a few small pieces. The cork dust will be the most appropriate size for this experiment.

Or

1. If you have access to a microtome or are able to very carefully slice a very thin layer of cork you could use that instead.
2. Place the cork dust or thin slice on a drop of water on a slide and add a cover slip.
3. Place the slide on the microscope and carefully bring to focus in low power mode initially.

Observations

Why might Hooke have described what he saw as cells?

A mature cork cell is a dead cell with cell walls made up of a waxy substance called suberin. This substance is highly impermeable to water and gases. Depending upon the species of woody plant you have, the cork cells may contain traces of tannins, lignin, or fatty acids or the cork cells may just be filled with air and the thickness from one cell to the other may be different.

At higher powers the separation between cells can be observed. This separation is believed to be caused by structures that arise from the cork cambium, known as lenticels. These lenticels or pore-like structures as can be seen in higher powers allowing for the exchange of gases between the outside environment and the stem of the plant.

Flora or Fauna?

If there is one experiment that you should do (or may have seen done at school) then this is the staining of onion cells to show cellular structure of plants compared with animals.

1. Remove a thin membrane from the inside of an onion (Do not use the outer leafy section). Place the thin membrane at the centre of the microscopic slide on a drop of water.
2. Add a drop of iodine solution or methylene blue to the side of the sample.
3. Place the cover slip carefully on the membrane and press it down until air bubbles have been removed. If necessary or to keep the slide, wipe some petroleum jelly around the outer edge of the cover slip.
4. Touch some tissue paper on one side of the slide to remove any excess liquid.
5. Start observing as per Activity 1 instructions, at low power.

Iodine is used as a stain for this experiment because iodine binds to starch. Onions contain simple sugars (carbohydrates) of which some are stored as starch granules. The stain makes the starch visible. You are not likely to see chloroplasts in the onion slide as the chloroplasts exist in the outer leafy layers (where photosynthesis occurs) that see daylight!

Observations

- Sketch the cells. Are they random or regularly shaped?
- Practice drawing (to scale) what you see on the slide.
- Why might onion cells have a regular structure, why are the cell walls strong enough to maintain this shape?

Cheeky!

For this activity you will need methylene blue stain. Check your health and safety policy for using human cheek cells.

1. Extract cheek cells from inside the mouth using a cotton bud swab.
2. Drop physiological saline on a clean microscopic slide and smear the swab on to the centre.
3. Drop the methylene blue solution on to the smear and add a cover slip.
4. Remove excess liquid using a tissue, touch the side of the cover slip.
5. Image the cells at low magnification first.

Methylene blue has an affinity for DNA and RNA and produces a dark stain when in contact with either.

As the cell nucleus contains DNA it will appear dark blue compared with a light blue colour for the rest of the cell and other structures. It should then be possible to make observations clearl.

Observations

- How does the cell structure compare with the onion and cork cells?
- What is the shape, are all the cell contents clearly visible?

Tasks

Now try imaging the different cell structures using polarised or dark field imaging techniques. Do they work? Do they provide complementary information?

References

https://micro.magnet.fsu.edu/primer/techniques/contrast.html

https://www.microscopeworld.com/t-microscope_slide_staining.aspx

https://microscope-microscope.org/microscope-info/preparing-microscope-slides/

https://www.microscopemaster.com/onion-cells-microscope.html

https://www.microscopemaster.com/cork-cells-under-the-microscope.html

6. Fracture Sections

Physics | 14 - 19 (yr 9 - 13)

Fractography in material science is vital to understand how different materials fail under different circumstances. Samples of an appropriate size can be imaged in the microscope.

Suggested fracture sections

- Small wire samples can be cut or snapped under tension.
- Hair samples both cut and snapped can also be included.
- Paper cut, torn or punctured can be used.

7. Brownian Motion

Physics | 11 - 19 (yr 7 - 13)

In 1827 the botanist Robert Brown noticed that if you looked at pollen grains in water through a microscope, the pollen jiggles about. He called this jiggling 'Brownian motion', but Brown couldn't understand what was causing it. He thought at first the pollen must be alive, but after testing the phenomenon with fine dust particles, he confirmed that the movement was not due to any living organism.

In 1905, Einstein became interested in the phenomenon of Brownian Motion, and in the same year he published three papers which finally came up with an explanation. Einstein realised that the jiggling of the pollen grains seen in Brownian motion was due to molecules of water hitting the tiny pollen grains. The pollen grains were visible but the water molecules were not, which was why it looked like the pollen was bouncing around on its own.

Einstein also showed that it was possible to work out how many molecules were hitting a single pollen grain and how fast the water molecules were moving - all by looking at the pollen grains.

In this experiment you will be able to see Brownian motion for yourself while practicing different imaging modes of the optical microscope:

Milk

Place a very small droplet of milk on a slide using a needle (or end of a paperclip) then add a larger droplet of distilled water to ensure a dilute concentration. Add a cover slip and remove excess liquid with tissue paper.

Carefully focus on the larger fat droplets, you should see the smaller droplets come in and out of focus. Any movement of all the droplets in a particular direction is NOT Brownian motion, if this happens wait a while for the liquid to settle.

Tasks

- What do you notice about the movement?
- Is there a difference between the larger and smaller droplets?
- Using the graticule, can you estimate the size of the particles?

Pollen

As per the original experiment find different types (and ages) of pollen. Carefully tap a small number of grains on to a slide and then place a droplet of distilled water on top. Add the cover slide carefully.

Smoke

If you have access to a smoke cell (see the Institute of Physics link for further details). Again, bring to focus the larger particles first then observe how the smaller ones come in and out of focus.

References

http://www.microscopy-uk.org.uk/dww/home/hombrown.htm

https://nrich.maths.org/6127

https://spark.iop.org/brownian-motion-smoke-cell

www.microscopy-uk.org.uk/mag/artjun09/dw-brown2.html

https://www.youtube.com/watch?v=UDj7BXA1CHU

8. Fibre Detective

Biology/Chemistry/Physics | 11 - 19 (yr 7 - 13)

Hair from people with different colours of hair, dyed and natural. Hair from different parts of the body (within reason!.)

Fibres from a cotton bag, fibres from clothes, spider web etc.

Method

1. The hair you collect can be removed from a hairbrush, or collected by cutting or pulling. Make a note of the type of hair (macroscopic properties - dyed, bleached, conditioned, gelled etc.)
2. With tweezers, place a few short pieces of fibre in a drop of water on a slide, and cover with a cover slip.
3. Observe the fibre - look at the thickness (use the graticule to accurately measure) and look at the surface quality. Try to use different imaging modes if you are having difficulty with the level of contrast.

Tasks

- Determine common similarities and differences between different fibres.
- Which are easier to image than others (why?)
- Is there anything obvious about hair (animal) that could be used to in an unknown sample? Forensic science often uses 'fingerprint' techniques that explain particular features - e.g. presence of hair gel, hair spray, dye etc.
- Look at the end that came from the head of a pulled hair (the root).
- Look at the difference in width of a white hair and a colored hair from a person whose hair is turning gray.
- Are there any differences in freshly washed or 'dirty' hair (that may not have been washed for a few days?

Challenge

Take some lint from a clothes dryer - use a tiny amount on a glass slide with a drop of water before adding a cover slide. How many of the fibres can you identify now that you've seen some known examples?

References

http://www.greatscopes.com/act006.htm (Marcia Williams, Homeschool Grandmother)

http://www.greatscopes.com/act007.htm



Royal Microscopical Society

www.rms.org.uk