MiSAC*methods* 1
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Looking at Microbes

Searching for, finding and identifying microbes using a microscope are hugely exciting experiences but can be disappointing when the view down a microscope is either blindingly bright or completely dark. However, it takes only a small amount of care to set up a microscope correctly, allowing you to see an amount of detail which enhances your understanding and appreciation of some of the major principles of biology. Care is particularly necessary when studying microbes with low-power magnification because their small size approaches the limit of the amount of detail that the microscope can reveal, ie, the *resolving power* of the instrument. Information on setting up your microscope correctly can be found below.

Excitement at the possibilities of using light microscopy for advancing knowledge is no better exemplified than by the award of one of the 2014 Nobel Prizes for work which shattered the principle held for some 150 years that the limit of resolution of the light microscope is 0.2 micrometres. This means that scientists can now visualise the pathways of individual molecules in living cells. www.nobelprize.org/nobel_prizes/chemistry/laureates/2014/press.html

There are also several organisations that cater for the interest of the enthusiast. One such is the Quekett Microscopical Club (www.quekett.org). Founded in 1865, it is second in seniority only to the Royal Microscopical Society and is called a 'Club' rather than a society to emphasize the nature of its membership of both amateurs and professionals dedicated to optical microscopy.

Suitable materials

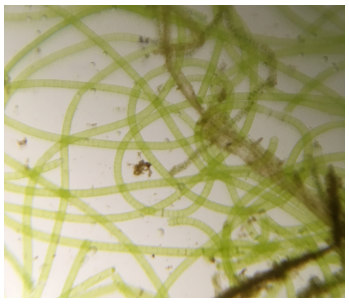
Observing microbes in natural sources is both extremely fascinating and rewarding. This is because of the diversity of types of microbe likely to be present and the influence on their growth of both the nature of a particular source and different niches within the source. Part of the fascination is that the variety of microbes and the habitats within which they live is such that the results of their relationships cannot be predicted with the same precision as in an investigation with a pure culture in a culture medium of known composition. Therefore, the outcomes and time scales given below must be viewed as guidelines only.

Hay infusions and flower vase water

Hay infusions (www.misac.org.uk/PDFs/MiSAC_helps.pdf) and old, very cloudy flower vase water (preferably *without* the sachet of additive) are convenient sources of a rich variety of microbial life. To see the wide range of organisms present, it is wise either to stir the liquid before taking a sample for making a microscopical preparation or to make several preparations, each taken from a different part of the material. Different parts include the inside surface of a vessel and debris in its depths, and the water/air interface of flower stems in a vase. It is also worth emphasising that no two hay infusions or vases will yield the same range of microbes in either a 'one-off' observation or a series when following successions of populations over time. Alternative sources of microbes are the ready-made mixtures of protists available from suppliers of microbial cultures (see *Links and publications*, *Suppliers*) www.misac.org.uk/links.html.

Some types of protozoa become visible in a hay infusion after about a week and others appear later but it will be a month or so before algae develop in large numbers. It is also likely that rotifers and, more rarely, nematodes will develop. They are small invertebrate animals which, although not microbes, are worth recording for comparison of size. '*Pond Life Identification Kit*' is a simple identification guide to protozoa and algae (http://www.microscopy-uk.org.uk/index.html?http://www.microscopy-uk.org.uk/pond/index.html). Bacteria develop quickly and often in large numbers but, because of their small size, are difficult to see under low-power magnification unless the microscope is set up correctly (see *Microscopy and recording results* below, for guidance). Those present are mostly motile and rod-shaped but spiral forms may also be seen. They have a grey appearance against the pale background of the surrounding water. Few fungi are found in this type of environment.

Hay infusion in medical flat bottle closed with a non-absorbent cotton wool plug.



Filamentous algae found in a hay infusion. Image taken with a smart phone placed up against the eye piece of a microscope.

Pond water

Unless pond water is green, the direct examination of samples is unlikely to be rewarding. An alternative approach is to immerse microscope slides or cover slips in pond water

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at room temperature to allow a variety of microbes to become attached to the surfaces over time and form *biofilms*. Biofilms develop slowly and therefore it is wise to set up several slides, one for examination after one week and the others at up to 3 or more weekly intervals. There is a variety of ways of applying this simple technique such as by inserting a slide into a slit cut in a large cork and suspending the slide below the floating cork.

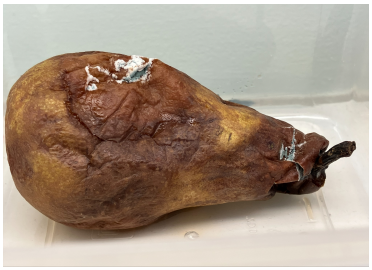
Alternatively, make a chamber by placing 2 cover slips flat on a slide about 1 cm apart, placing another slide on top and securing them together by elastic bands - or devise a system of your own. Slides can be suspended at a number of different depths including among material that settles at the bottom of a vessel.

Spoiled fruit and bread

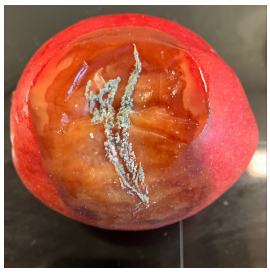
Products that have gone 'mouldy' are suitable for the direct examination of fungi by the 'sticky tape method' although care has to be taken to prevent the release of spores (see *Health & Safety* below). The procedure involves pressing a portion of clear transparent adhesive tape, eg, *Sellotape*, onto the mould growth, thereby transferring hyphae and spores for examination on a microscope slide. The specimen can be stained by placing the impregnated tape onto a thin line of stain on a microscope slide. Suitable stains include lactophenol cotton blue but they might not be readily available and some have associated hazards. However, acceptable results are obtained with blue ink.

Alternatively, unstained preparations mounted in water may be used; improved contrast is achieved by adding a little washing-up liquid. For a web site that gives a description of the method and provides comments on identification of fungi, see www.microscopy-uk.org.uk/mag/artjan99/mmould.html.

Spoiled bread in a plastic bag showing moulds. *Image John Schollar*



Mould growing on a decaying pear in a plastic box. *Image John Schollar*



Mould on rotting apple. *Image John Schollar*

Examination of specimens

Label the preparation for recording purposes. Careful and patient examination of a preparation is essential. However, a loopful of a sample on a slide or a wet mount under a cover slip, quickly dries out because of evaporation which is accelerated by the heat of the microscope lamp. The period of observation can nevertheless be readily extended by sealing the edges of the cover slip with a thin film of petroleum jelly, eg, *Vaseline*. (For the procedure, see the 'hay infusion' entry at www.misac.org.uk/PDFs/MiSAC_helps.pdf). If necessary, movement of fast-moving organisms can be reduced by adding carboxymethyl cellulose, sold as 'Protoslow', eg, from www.blades-bio.co.uk.

Microscopy and recording results

Refer to guides on the correct use of the microscope, eg, http://www.davidmoore.org.uk/assets/fungi4schools/Microscopy_page.htm or CLEAPSS Guide GL372 *Setting up and using a microscope* (accessible only for schools that are members of CLEAPSS). Many models of school microscope are fitted with a substage condenser which has a key role in achieving the best balance between glare and contrast. A main function of the substage condenser is to focus the source of illumination on the specimen. For this purpose, the condenser is either fixed in position close to the stage of the microscope or, if adjustable, should be moved to that position. Altering the position of the condenser should not be used to control the brightness of the field of view; this is done by adjusting the iris diaphragm of the condenser and/or using the brightness control which is fitted to some models. Spend several minutes searching over the field of view while constantly adjusting the focus slightly to view the full depth of the preparation.

Bacteria are the smallest microbes that can be observed under low-power magnification and then only if the illumination is very carefully set to provide ideal lighting. They are more clearly seen with the better resolution provided by a high-power, dry, objective lens (x40) for which it is necessary to examine a wet mount prepared under a cover slip and to increase the level of illumination.

For measurement of size, a graticule eyepiece pre-fitted into an eyepiece lens and a stage micrometer, both made of glass, are very expensive. However, a set of 10 eyepiece graticules, printed onto a strip of plastic, is much cheaper. The graticule is cut out with scissors and placed into an eyepiece lens or mounted onto a microscope slide to serve as a stage micrometer. Available from www.scichem.com (specify item VTT12300678) or www.timstar.co.uk (specify MI84115).

There is a variety of ways to make a photographic record of a preparation including the use of a digital camera positioned at the eyepiece lens. See also CLEAPSS Guide GL144 *Using a mobile phone to take pictures of the very small* (only for CLEAPSS members) and MiSAC*matters* Article 31 *DIY smartphone microscope* http://misac.org.uk/article-downloads/31.Schollar.pdf

Health & Safety

There are no specific hazards inherent in this activity that cannot be contained by good microbiological laboratory practice (GMLP); see *Safety Guidelines* at www.misac.org.uk/healthandsafety.html). Take particular care to avoid hand-to-mouth or eye operations and wiping eyes - and to wash your hands after touching any sample. It is important that, when handling 'mouldy' fruit or bread, the release of spores into the atmosphere is minimised. This can be achieved by keeping samples in a container and accessing them for only the minimum amount of time necessary for completing a manipulation. Suitable transparent containers include a Petri dish, a sandwich box with a lid or a plastic bag.

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