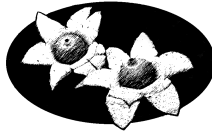
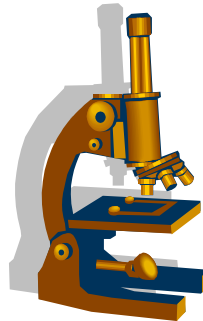


**Queensland Mycological Society**



**BASICS OF MICROSCOPY**



**Compiled by Diana Leemon**

## Why do a Workshop on Microscopy?

- Although many features of Macro-fungi can be seen with the naked eye, key diagnostic features used in identification such as spore size and shape require a microscope .....pillipelli cylosystidia etc.
- Advanced levels of identification of some fungi require an examination of spore ornamentation using stains, spore bearing structures and hyphal arrangements in the cap, where good microscopy is essential.
- What you get out of a microscope depends on how well you use it. It is easy to use a microscope without much knowledge but to use it well it helps to:
  - Understand how it works
  - Understand how to adjust the lighting and lenses to best view your sections
  - Know how to make a good slide

This workshop will cover:

- Definition of Microscopy
- History of Microscopy
- Types of Microscopes
- Compound Microscope
  - Parts
  - How it works
  - How to set it up
  - Magnification
  - Basic care and maintenance
  - Slide preparation

## **MICROSCOPY**

Microscopy covers any technique for producing visible structures, or details too small to otherwise be seen by the naked eye, by using a microscope or other magnification tool; specifically the technique of using a microscope.

Microscopy has evolved with the development of the microscope, which has underpinned the development of the field of biology. What once could not be seen was unknown. An ability to see smaller and smaller details has led to an understanding of the diversity of living organisms as well as their structure and physiology.

Today biology relies on light and electron microscopy. However electron microscopy is a specialist field and the instruments are only affordable to well funded research organisations.

Light microscopy uses visible light and glass magnifying lenses

### **Brief History of Microscopy**

Light microscopes date to at least 1595, when Zacharias Jansen (1580 – 1638) of Holland invented a compound light microscope, one that used two lenses, with the second lens further magnifying the image produce by the first. His microscopes were collapsing tubes used like a telescope in reverse, and produced magnifications up to nine times ( $\times 9$ ).

Antony van Leeuwenhoek (1632 – 1723) invented a simple (one lens) microscope around 1670 that magnified up to  $\times 200$  and achieved twice the resolution of the best compound microscopes of his day, mainly because he crafted better lenses. Others made lenses by squashing molten glass between pieces of wood, Leeuwenhoek made his by grinding and polishing solid glass. He became the first to see individual cells, including bacteria, protozoans, muscle cells and sperm.

Robert Hooke (1635 – 1703) further refined the compound microscope, adding such features as a stage to hold the specimen, an illuminator, and coarse and fine focus controls. Until 1800, compound microscopes designed by Hooke and others were limited to magnifications of  $\times 30$  to  $\times 50$ , and their images had blurry edges (spherical aberration) and rainbow like distortions (chromatic aberrations). The most significant improvement in microscope optics was achieved in the nineteenth century, when Carl Zeiss (1816 – 1888) and Ernst Abbe (1840 – 1905) added the substage condenser and developed superior lenses that greatly reduced chromatic and spherical aberration, while permitting vastly improved resolution and higher magnification.

Today the light microscope has developed to its full potential in that you are limited in what is visible with today's light microscopes by the properties of light, specifically the wavelength of light. To view even smaller objects requires an electron microscope, which uses a beam of electrons instead of light and magnetic fields instead glass lenses for magnification.

### **Types of microscopes**

There are two basic types of light microscope : The compound microscope (with double eyepieces can be called a binocular microscope) and the stereo/dissecting microscope. Both are useful in mycology, but the compound microscope requires a bit more skill to use.

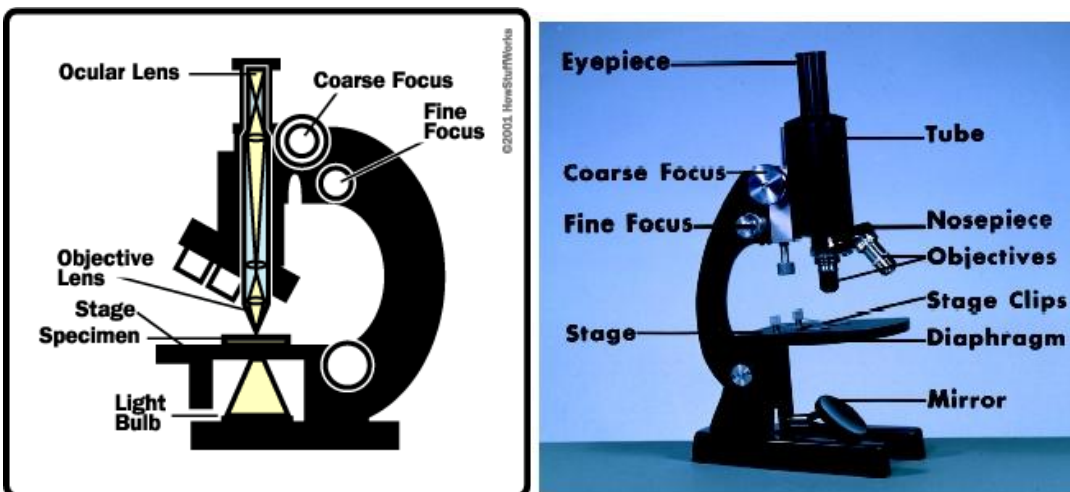


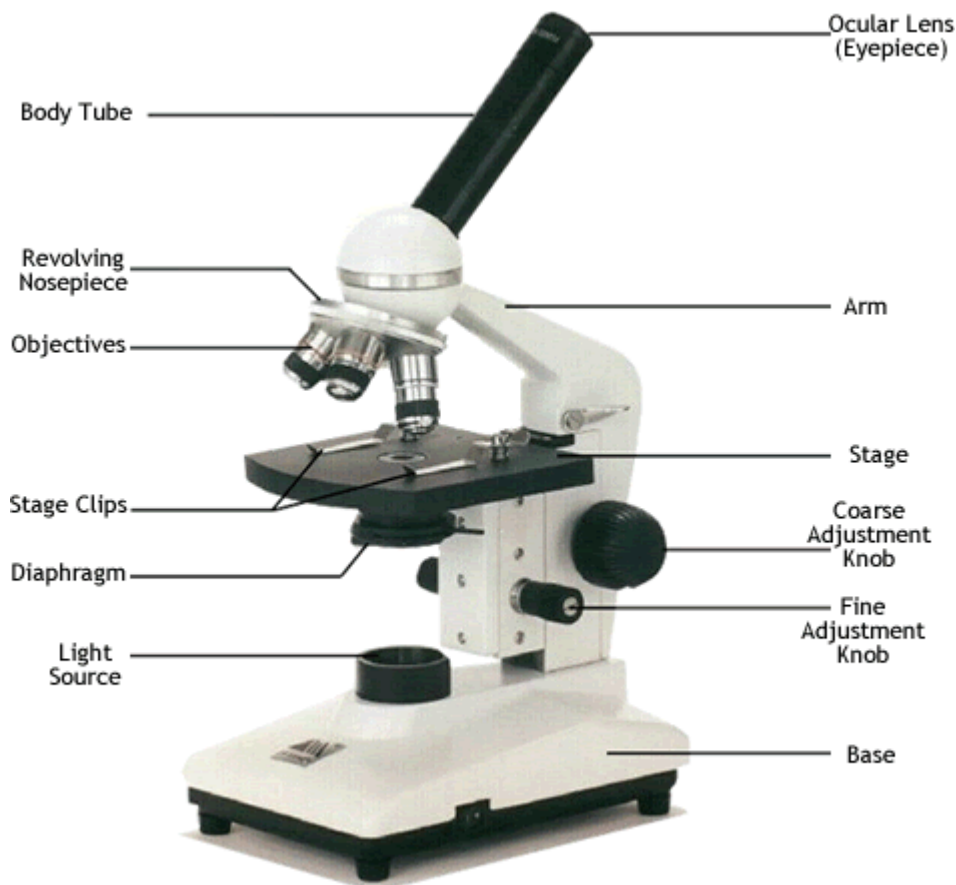
### Types of Stereo Microscopes

The Stereo microscope provides a three dimensional (3-D) image of the sample being examined (it uses 2 separate optical paths with 2 objectives and 2 eyepieces to provide slightly different viewing angles to the left and right eyes). The Stereo microscope is often used to study the surfaces of solid specimens or carry out close work such as sorting, dissection, microsurgery, watch making etc. In mycology it is useful for examining small fruit bodies or the gill arrangements etc. in larger fruit bodies. A Stereo microscope has a useful magnification up to  $\times 100$ .

(The Stereo should not be confused with a compound microscope equipped with binocular eyepieces. In such a microscope both eyes see the same image, but the binocular eyepieces provide greater viewing comfort. However the image in such a microscope is no different from that obtained with a single monocular eyepiece.)

### Compound Microscope





## ❖ Parts

### **Eyepiece or ocular**

- cylinder containing 2 or more lenses to bring the image to focus for the eye
- Eyepiece is inserted into top end of body tube
- Eyepieces are interchangeable & many different eyepieces can be inserted with different magnifications
- Typical eyepieces -  $\times 5$ ,  $\times 10$  and  $\times 20$

### **Objective lens**

- Cylinder containing 1 or more lenses to collect light from the sample at the lower end of the microscope
- Objective lenses are screwed into a circular nose piece which may be rotated to select the required lens
- Typical objectives -  $\times 4$ ,  $\times 10$ ,  $\times 20$ ,  $\times 40$ ,  $\times 60$ ,  $\times 80$  and  $\times 100$
- To work correctly the  $\times 100$  must be used with immersion oil to correct for the refraction of light through the glass and air at this magnification.

### **Stage**

- A platform below the objective, supports the specimen being viewed.
- In the centre is a circular hole through which the light shines to illuminate the specimen.
- The stage usually has arms/clips to hold the slide being viewed

### **Illumination source**

- Below the stage, a light provided and controlled in various ways (simplest microscopes use a mirror to focus daylight)

- Most have a built in controllable light source that is focussed through an optical device called a condenser with diaphragms and filters available to manage the quality and intensity of the light.

(Some modern microscopes may have many more features, including transmission/reflection illumination, filters, apparatus for phase contrast microscopy and differential interference contrast microscopy, digital cameras etc.)

## Magnification

The actual power of magnification of an optical microscope is the product of the powers of the ocular (eyepiece) – usually  $\times 10$ , and the objective lens being used.

Special scales (graticules) for measuring objects in view can be purchased and added to one of the ocular lenses. They need to be calibrated before use.

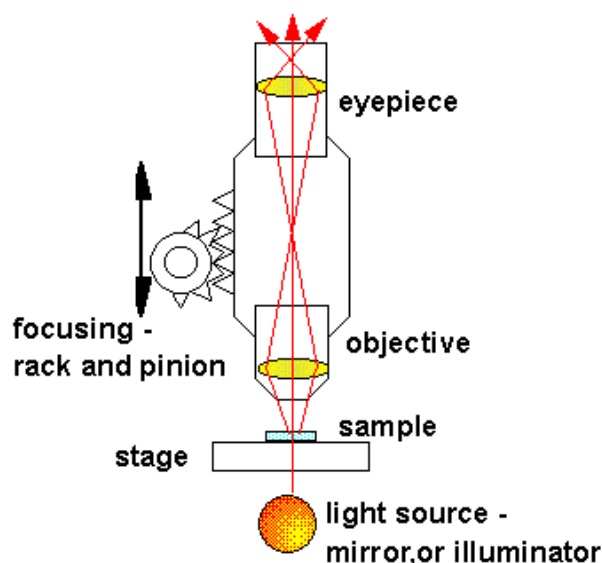
## ❖ How the compound microscope works

Compound optical microscopes are typically used to examine a smear/ or thinly sectioned slice of some material. With few exceptions they utilize light passing through the sample from below.

The objective lens, at its simplest is a very high powered magnifying glass i.e. a lens with a very short focal length. This is bought close to the specimen being examined so that the light from the specimen comes to focus about 160 mm inside the microscope tube. This creates an enlarged image of the subject. The image is inverted and can be seen by removing the eyepiece and placing a piece of tracing paper over the end of the tube. It is this real image that is viewed by the eyepiece that provides further enlargement. In most microscopes the eyepiece is a compound lens, which is made of two lenses one near the front and one near the back of the eyepiece tube forming an air separated couplet. The virtual image comes to a focus between the two lenses of the eyepiece, the first lens bringing the real image to a focus and the second lens enabling the eye to focus on the now virtual image.

In all microscopes the image is viewed with the eyes focussed at infinity.

Headaches and tired eyes after using a microscope are usually signs that the eye is being forced to focus at a close distance rather than at infinity.



## ❖ How to set up the compound microscope

Many beginners may think that the challenge of viewing small objects lies in getting enough magnification. However when looking at living things the biggest challenges in order are,

- Obtaining sufficient contrast
- Finding the focal plane
- Obtaining good resolution
- Recognising the subject when one sees it (how often does the humble air bubble get elevated to the status of living thing!)

Image quality is determined by:

1. **Brightness**, this can be changed by adjusting:
  - Voltage of light source = amount of light
  - Condenser - focus of light
  - Diaphragm/pinhole aperture – amount of light coming through

Brightness is also related to the numerical aperture of the objective lens, the larger the numerical aperture the brighter the image.

2. **Focus**, whether image is blurry or well defined and sharp. Control with the focus knob.

The thickness of the specimen (& thickness of the coverslip) can affect this.

NEVER TOUCH THE COARSE FOCUS WHILE LOOKING DOWN THE MICROSCOPE.

3. **Resolution**, how close 2 points in an image can be before they are no longer seen as 2 separate points. This is innate to the lenses and properties of light.
4. **Contrast**, Difference in lighting between adjacent areas of the specimen – related to the illumination system and can be adjusted by changing the intensity of the light and the diaphragm/pinhole aperture. Chemical stains can be added to the specimen to enhance contrast.

## SETTING UP FOR VIEWING

1. Mount the specimen on the stage, position specimen under lenses
2. Optimise the lighting (field bright without hurting eyes)
3. Adjust Condenser
4. Think about what you are looking for – start with lowest magnification
5. Focus, locate and centre specimen
6. Adjust eyepiece separation, and compensate focus in eyepieces if your eyes are different
7. Select objective lens for viewing - move up the magnification by steps – good lenses should be parfocal (remember that bigger is not always better – unless a specimen is extremely thin you will be unable to focus well with a high magnification objective A moving specimen is harder to “chase” at high magnification)
8. Adjust illumination for the selected objective lens

## ❖ Basic care and maintenance

- Everything on a good quality microscope is expensive, so be careful, look after it.

- Hold a microscope firmly by the stand only. Never grab it by the eyepiece holder, for example.
- Since bulbs are expensive and have a limited life turn down the light after using then turn off. This means that next time the light is turned on it will not be turned on at its highest setting, move to the highest setting gradually as you need to. Turn light off when not using the microscope.
- Always check that the stage and lenses are clean before putting away the microscope.
- NEVER use paper towel, your shirt, or other material other than good quality lens tissue or a cotton swab (100% natural cotton only) to clean an optical surface. Be gentle! You must use an appropriate lens cleaner or distilled water to help remove dried material. Organic solvents may separate or damage the lens elements or coatings.
- Cover the instrument with a dust jacket when not in use.
- Focus smoothly, don't try to speed through the focussing process or force anything. You should NEVER be using the coarse focus knob while looking down the eyepieces at high magnification. This is a sure way to crunch your lens surface into the slide (whereas at high Magnification it is good practice to have your hand on the fine focus to gently focus up down through the field, especially if the slide is not of a very thin section)

### ❖ Slide preparation

- Most specimens are mounted in liquids, called a wet mount, dry mounts are rarely used.
  - The liquid may be water, saline or a stain to highlight structures.
  - When making a wet mount use the maxim: "less is more".
1. Place a drop of liquid on the slide
  2. place object in the drop of liquid
  3. Lower the coverslip carefully to avoid air pockets/bubbles
  4. Absorb excess liquid with paper towel
  5. The slide material & stain can be heat fixed by gently heating in a flame, but not allowing the liquid to boil. This can be quite an art. Best not to practise this on limited material.
  6. Cover slip can be sealed with nail polish if you wish to keep the slide for some time (not permanently though)

### ACTIVITY: Practise slide making

1. Slide of cheek cells mounted in saline, then mounted in methylene blue stain for contrast.
2. Slide of onion cells mounted in water, then mounted in iodine
3. Slide of mushroom spores



# TEST YOURSELF

