Equipment Packet: Microscopes

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Equipment Packet Contents:

This packet contains information about the operation, maintenance, and repair of laboratory microscopes.

Part I: External From the Packet:

1. **An Introduction to Microscopes:** PowerPoint

Part II: Included in this Packet:

1. Operation and Use of Microscopes

Featured in this Section:

WHO. "Learning Unit 6: The Microscope." From the Publication: *Basic Malaria Microscopy: part 1. A Learner's Guide.* WHO: Switzerland (2010), p. 37-44. Retrieved from: http://apps.who.int/iris/bitstream/10665/44208/1/9789241547826_eng.pdfhttp://apps.who.int/iris/bitstream/10665/44208/1/9789241547826_eng.pdf

Optical microscope

The **optical microscope**, often referred to as the "**light microscope**", is a type of microscope which uses visible light and a system of lenses to magnify images of small samples. Optical microscopes are the oldest and simplest of the microscopes. Digital microscopes are now available which use a CCD camera to examine a sample, and the image is shown directly on a computer screen without the need for optics such as eye-pieces. Other microscopic methods which do not use visible light include scanning electron microscopy and transmission electron microscopy.

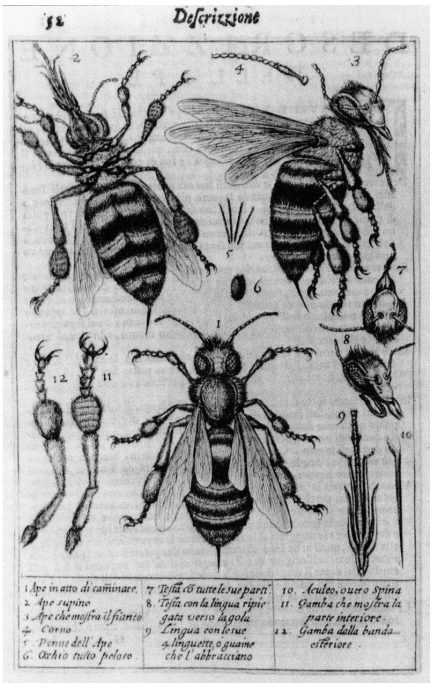
Optical configurations

There are two basic configurations of the conventional optical microscope in use, the simple (one lens) and compound (many lenses). Digital microscopes are based on an entirely different system of collecting the reflected light from a sample.

Light microscope

A *simple microscope* is a microscope that uses only one lens for magnification, and is the original light microscope. Van Leeuwenhoek's microscopes consisted of a small, single converging lens mounted on a brass plate, with a screw mechanism to hold the sample or specimen to be examined. Demonstrations [1] by British microscopist have images from such basic instruments. Though now considered primitive, the use of a single, convex lens for viewing is still found in simple magnification devices, such as the magnifying glass, and the loupe. Light microscopes are able to view specimens in color, an important advantage when compared with electron microscopes, especially for forensic analysis, where blood traces may be important, for example.

History



The oldest published image known to have been made with a microscope: bees by Francesco Stelluti, 1630[2]

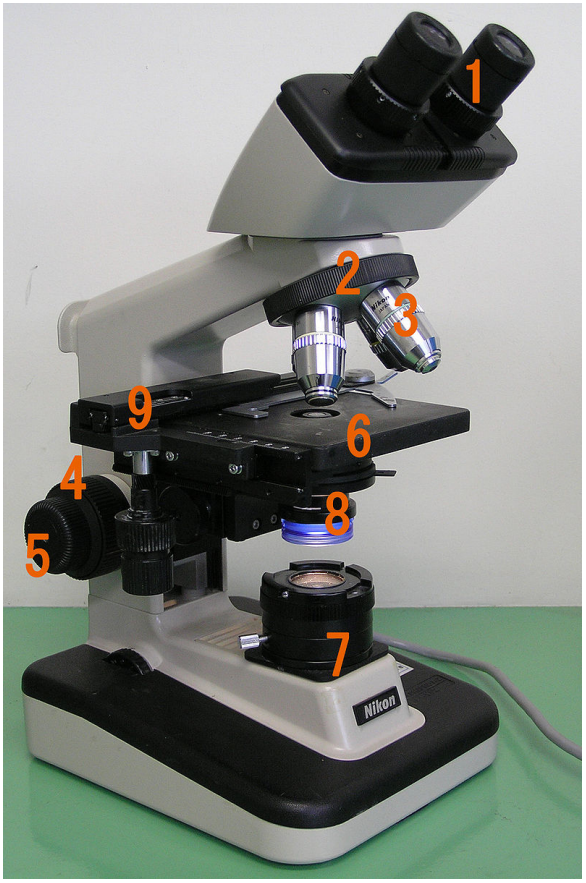
It is difficult to say who invented the compound microscope. Dutch spectacle-makers Hans Janssen and his son Zacharias Janssen are often said to have invented the first compound microscope in 1590, but this was a declaration made by Zacharias Janssen himself during the mid 1600s. The date is unlikely, as it has been shown that Zacharias Janssen actually was born around 1590. Another favorite for the title of 'inventor of the microscope' was Galileo Galilei. He developed an *occhiolino* or compound microscope with a convex and a concave lens in 1609. Galileo's microscope was celebrated in the Accademia dei Lincei in 1624 and was the first such device to be given the name "microscope" a year later by fellow Lincean Giovanni Faber. Faber coined the name from the Greek words *μικρόν* (micron) meaning "small", and *σκοπεῖν* (skopein) meaning "to look at", a name meant to be analogous with "telescope", another word coined by the Linceans.[3]

Christiaan Huygens, another Dutchman, developed a simple 2-lens ocular system in the late 1600s that was achromatically corrected, and therefore a huge step forward in microscope development. The Huygens ocular is still being produced to this day, but suffers from a small field size, and other minor problems.

Anton van Leeuwenhoek (1632-1723) is credited with bringing the microscope to the attention of biologists, even though simple magnifying lenses were already being produced in the 1500s. Van Leeuwenhoek's home-made microscopes were very small simple instruments, with a single, yet strong lens. They were awkward in use, but enabled van Leeuwenhoek to see detailed images. It took about 150 years of optical development before the compound microscope was able to provide the same quality image as van Leeuwenhoek's simple microscopes, due to timely difficulties of configuring multiple lenses. Still, despite widespread claims, van Leeuwenhoek is not the inventor of the microscope.

Components

1. ocular lens, or eyepiece
2. objective turret
3. objective lenses
4. coarse adjustment knob
5. fine adjustment knob
6. object holder or stage
7. mirror or light (illuminator)
8. diaphragm and condenser



Basic optical transmission microscope elements(1990's)

All optical microscopes share the same basic components:

- The eyepiece - A cylinder containing two or more lenses to bring the image to focus for the eye. The eyepiece is inserted into the top end of the body tube. Eyepieces are interchangeable and many different eyepieces can be inserted with different degrees of magnification. Typical magnification values for eyepieces include 5x, 10x and 2x. In some high performance microscopes, the optical configuration of the objective lens and eyepiece are matched to give the best possible optical performance. This occurs most commonly with apochromatic objectives.
- The objective lens - a cylinder containing one or more lenses, typically made of glass, to collect light from the sample. At the lower end of the microscope tube one or more objective lenses are screwed into a circular nose piece which may be rotated to select the required objective lens. Typical magnification values of objective lenses are 4x, 5x, 10x, 20x, 40x, 50x and 100x. Some high performance objective lenses may require matched eyepieces to deliver the best optical performance.
- The stage - a platform below the objective which supports the specimen being viewed. In the center of the stage is a hole through which light passes to illuminate the specimen. The stage usually has arms to hold *slides* (rectangular glass plates with typical dimensions of 25 mm by 75 mm, on which the specimen is mounted).
- The illumination source - below the stage, light is provided and controlled in a variety of ways. At its simplest, daylight is directed via a mirror. Most microscopes, however, have their own controllable light source that is focused through an optical device called a condenser, with diaphragms and filters available to manage the quality and intensity of the light.

The whole of the optical assembly is attached to a rigid arm which in turn is attached to a robust U shaped foot to provide the necessary rigidity. The arm is usually able to pivot on its joint with the foot to allow the viewing angle to be adjusted. Mounted on the arm are controls for focusing, typically a large knurled wheel to adjust coarse focus,

together with a smaller knurled wheel to control fine focus.

Updated microscopes may have many more features, including reflected light (incident) illumination, fluorescence microscopy, phase contrast microscopy and differential interference contrast microscopy, spectroscopy, automation, and digital imaging.

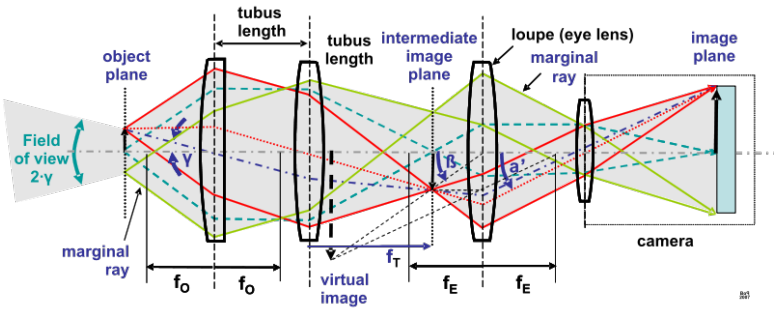
On a typical compound optical microscope, there are three objective lenses: a scanning lens (4×), low power lens (10×)and high power lens (ranging from 20 to 100×). Some microscopes have a fourth objective lens, called an oil immersion lens. To use this lens, a drop of immersion oil is placed on top of the cover slip, and the lens is very carefully lowered until the front objective element is immersed in the oil film. Such immersion lenses are designed so that the refractive index of the oil and of the cover slip are closely matched so that the light is transmitted from the specimen to the outer face of the objective lens with minimal refraction. An oil immersion lens usually has a magnification of 50 to 100×.

The actual power or magnification of an optical microscope is the product of the powers of the ocular (eyepiece), usually about 10×, and the objective lens being used.

Compound optical microscopes can produce a magnified image of a specimen up to 1000× and, at high magnifications, are used to study thin specimens as they have a very limited depth of field.

Operation

The optical components of a modern microscope are very complex and for a microscope to work well, the whole optical path has to be very accurately set up and controlled. Despite this, the basic operating principles of a microscope are quite simple.



Optical path in a typical microscope

The objective lens is, at its simplest, a very high powered magnifying glass *i.e.* a lens with a very short focal length. This is brought very close to the specimen being examined so that the light from the specimen comes to a focus about 160 mm inside the microscope tube. This creates an enlarged image of the subject. This image is inverted and can be seen by removing the eyepiece and placing a piece of tracing paper over the end of the tube. By carefully focusing a brightly lit specimen, a highly enlarged image can be seen. It is this real image that is viewed by the eyepiece lens that provides further enlargement.

In most microscopes, the eyepiece is a compound lens, with one component lens near the front and one near the back of the eyepiece tube. This forms an air-separated couplet. In many designs, 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 virtual image.

In all microscopes the image is viewed with the eyes focused at infinity (mind that the position of the eye in the above figure is determined by the eye's focus). 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.

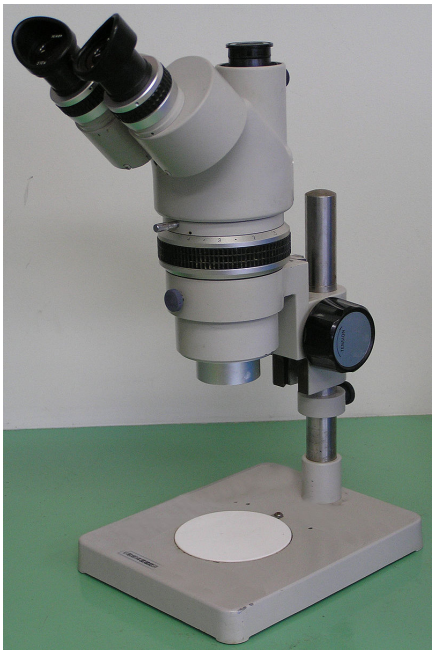
The essential principle of the microscope is that an objective lens with very short focal length (often a few mm) is used to form a highly magnified real image of the object. Here, the quantity of interest is linear magnification, and this number is generally inscribed on the objective lens casing. In practice, today, this magnification is carried out by means of two lenses: the objective lens which creates an image at infinity, and a second weak tube lens which then forms a real image in its focal plane.[4]

Applications

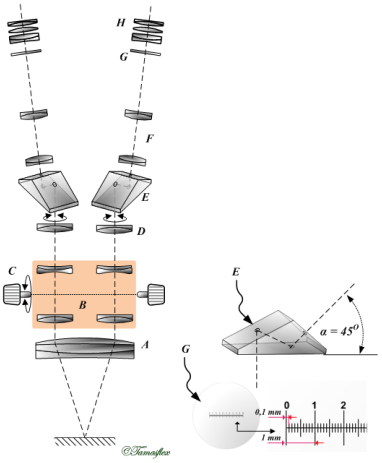
Optical microscopy is used extensively in microelectronics, nanophysics, biotechnology, pharmaceutic research and microbiology.[5]

Optical microscopy is used for medical diagnosis, the field being termed histopathology when dealing with tissues, or in smear tests on free cells or tissue fragments.

Stereo microscope



Stereo microscope



Modern stereomicroscope optical design.
A - Objective **B** - Galilean telescopes (*rotating objectives*) **C** - Zoom control **D** - Internal objective **E** - Prism **F** - Relay lens **G** - Reticle **H** - Eyepiece

The **stereo** or **dissecting microscope** is designed differently from the diagrams above, and serves a different purpose. It uses two separate optical paths with two objectives and two eyepieces to provide slightly different viewing angles to the left and right eyes. In this way it produces a three-dimensional visualization of the sample being examined.[6]

The stereo microscope is often used to study the surfaces of solid specimens or to carry out close work such as sorting, dissection, microsurgery, watch-making, small circuit board manufacture or inspection, and the like.

Unlike compound microscopes, illumination in a stereo microscope most often uses reflected (episcopic) illumination rather than transmitted (diascopic) illumination, that is, light reflected from the surface of an object rather than light transmitted through an object. Use of reflected light from the object allows examination of specimens that would be too thick or otherwise opaque for compound microscopy. However, stereo microscopes are also capable of transmitted light illumination as well, typically by having a bulb or mirror beneath a transparent stage underneath the object, though unlike a compound microscope, transmitted illumination is not focused through a condenser in most systems.[7] Stereoscopes with specially-equipped illuminators can be used for dark field microscopy, using either reflected or transmitted light.[8]

Great working distance and depth of field here are important qualities for this type of microscope. Both qualities are inversely correlated with resolution: the higher the resolution (*i.e.* the shorter the distance at which two adjacent points can be distinguished as separate), the smaller the depth of field and working distance. A stereo microscope has a useful magnification up to 100×. The resolution is maximally in the order of an average 10× objective in a compound microscope, and often much lower.

There are two major types of magnification systems in stereo microscopes. One is fixed magnification in which primary magnification is achieved by a paired set of objective lenses with a set degree of magnification. The other is zoom or pancratic magnification, which are capable of a continuously variable degree of magnification across a set range. Zoom systems can achieve further magnification through the use of auxiliary objectives that increase total magnification by a set factor. Also, total magnification in both fixed and zoom systems can be varied by changing eyepieces.[6]

Scientist using a stereo microscope outfitted with a digital imaging pick-up

Intermediate between fixed magnification and zoom magnification systems is a system attributed to Galileo as the "Galilean optical system" ; here an arrangement of fixed-focus convex lenses is used to provide a fixed magnification, but with the crucial distinction that the same optical components in the same spacing will, if physically inverted, result in a different, though still fixed, magnification. This allows one set of lenses to provide two different magnifications ; two sets of lenses to provide four magnifications on one turret ; three sets of lenses provide six magnifications and will still fit into one turret. Practical experience shows that such Galilean optics systems are as useful as a considerably more expensive zoom system, with the advantage of knowing the magnification in use as a set value without having to read analogue scales. (In remote locations, the robustness of the systems is also a non-trivial advantage.)

The stereo microscope should not be confused with a compound microscope equipped with double eyepieces and a binoviewer. 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.

Digital display with stereo microscopes

Recently various video dual CCD camera pickups have been fitted to stereo microscopes, allowing the images to be displayed on a high resolution LCD monitor. Software converts the two images to an integrated anaglyph 3D image, for viewing with plastic red/cyan glasses, or to the cross converged process for clear glasses and somewhat better color accuracy. The results are viewable by a group wearing the glasses.

Digital microscopes

A miniature digital microscope.

Low power microscopy is also possible with digital microscopes, with a camera attached directly to the USB port of a computer, so that the images are shown directly on the monitor. Often called "USB" microscopes, they offer high magnifications (up to about 200×) without the need to use eyepieces, and at very low cost. The precise magnification is determined by the working distance between the camera and the object, and good supports are needed to control the image. The images can be recorded and stored in the normal way on the computer. The camera is usually fitted with a light source, although extra sources (such as a fiber-optic light) can be used to highlight features of interest in the object. They also offer a large depth of field, a great advantage at high magnifications.

They are most useful when examining flat objects such as coins, printed circuit boards, or documents such as banknotes. However, they can be used for examining any object which can be studied in a standard stereo-microscope. Such microscopes offer the great advantage of being much less bulky than a conventional microscope, so can be used in the field, attached to a laptop computer. Although convenient, the magnifying abilities of these instruments are often overstated; typically offering 200x magnification, this claim is based usually on 25x to 30x actual magnification PLUS the expansion of the image facilitated by the size of the available screen- so for genuine 200x magnification a ten-foot screen would be required.

Special designs

Other types of optical microscope include:

- the inverted microscope for studying samples from below; useful for cell cultures in liquid;
- the student microscope designed for low cost, durability, and ease of use;
- the petrographic microscope whose design usually includes a polarizing filter, rotating stage and gypsum plate to facilitate the study of minerals or other crystalline materials whose optical properties can vary with orientation.
- the polarizing microscope
- the fluorescence microscope
- the phase contrast microscope

Limitations

At very high magnifications with transmitted light, point objects are seen as fuzzy discs surrounded by diffraction rings. These are called Airy disks. The *resolving power* of a microscope is taken as the ability to distinguish between two closely spaced Airy disks (or, in other words the ability of the microscope to reveal adjacent structural detail as distinct and separate). It is these impacts of diffraction that limit the ability to resolve fine details. The extent of and magnitude of the diffraction patterns are affected by both by the wavelength of light (λ), the refractive materials used to manufacture the objective lens and the numerical aperture (NA) of the objective lens. There is therefore a finite limit beyond which it is impossible to resolve separate points in the objective field, known as the diffraction limit. Assuming that optical aberrations in the whole optical set-up are negligible, the resolution *d*, is given by:

$$d = \frac{\lambda}{2NA}$$

Usually, a λ of 550 nm is assumed, corresponding to green light. With air as medium, the highest practical *NA* is 0.95, and with oil, up to 1.5. In practice the lowest value of *d* obtainable is about 200 nm.

Other optical microscope designs can offer an improved resolution. These include ultraviolet microscopes, which use shorter wavelengths of light so the diffraction limit is lower, Vertico SMI, near field scanning optical microscopy which uses evanescent waves, and Stimulated Emission Depletion (STED) microscopy which is used for observing self-luminous particles. In the latter, non-self-luminous particle is illuminated by an external source, and thus this microscope is not diffraction limited by the Abbe's theory. Stefan Hell of the Max Planck Institute for Biophysical Chemistry was awarded the 10th German Future Prize in 2006 for his development of the Stimulated Emission Depletion (STED) microscope.[9]

A modern microscope with a mercury bulb for fluorescence microscopy. The microscope has a digital camera, and is attached to a computer.

Several other optical microscopes have been able to see beyond the theoretical Abbe limit of 200 nm. In 2005, a microscope capable of detecting a single molecule was described as a teaching tool.[10] A holographic microscope described by Courjon and Bulabois in 1979 is also capable of breaking this magnification limit, although resolution was restricted in their experimental analysis.[11]

As a sensitivity improvement, a sarfus method was developed, which uses contrast-enhancing substrates and thereby allows to directly visualize films as thin as 0.3 nanometers.

Alternatives

In order to overcome the limitations set by the diffraction limit of visible light other microscopes have been designed which use other waves.

- Atomic Force Microscope (AFM)
- Scanning Electron Microscope (SEM)
- Scanning Ion-Conductance Microscope (SICM)
- Scanning Tunneling Microscope (STM)
- Transmission Electron Microscope (TEM)
- X-ray microscope

The use of electrons and x-rays in place of light allows much higher resolution - the wavelength of the radiation is shorter so the diffraction limit is lower. To make the short-wavelength probe non-destructive, the atomic beam imaging system (atomic nanoscope) has been proposed and widely discussed in the literature, but it is not yet competitive with conventional imaging systems.

STM and AFM are scanning probe techniques using a small probe which is scanned over the sample surface. Resolution in these cases is limited by the size of the probe; micromachining techniques can produce probes with tip radii of 5-10 nm.

Additionally, methods such as electron or X-ray microscopy use a vacuum or partial vacuum, which limits their use for live and biological samples (with the exception of ESEM). The specimen chambers needed for all such instruments also limits sample size, and sample manipulation is more difficult. Color cannot be seen in images made by these methods, so some information is lost. They are however, essential when investigating molecular or atomic effects, such as age hardening in aluminium alloys, or the microstructure of polymers.

See also

- Digital microscope
- Köhler illumination
- Microscope slide
- Objective

Further reading

- "Metallographic and Materialographic Specimen Preparation, Light Microscopy, Image Analysis and Hardness Testing", Kay Geels in collaboration with Struers A/S, ASTM International 2006.

External links

- A collection of early microscopes [12]
- Historical microscopes [13], an illustrated collection with more than 3000 photos of scientific microscopes by European makers **(German)**
- *Molecular Expressions* [14], concepts in optical microscopy
- Online tutorial of practical optical microscopy [15]
- OpenWetWare [16]
- Interactive Overview of a Light Microscope [17]

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Operation and Use of Microscopes

Learning unit 6
The microscope

Learning objectives

By the end of this unit, you will be able to:

- **demonstrate** the correct set-up and use of a binocular microscope with artificial and with natural light;
- **demonstrate** the correct use of the x10 paired oculars and x100 oil immersion objective;*
- **operate** the mechanical stage correctly;
- **name** correctly 10 component parts of the microscope;
- **describe** the correct way in which to maintain a microscope in good working order;
- **describe** two ways of storing a microscope correctly; and
- **demonstrate** the correct way of packing a microscope for long-distance transport.

* Or x7 oculars if they are used in the programme

For efficient malaria microscopy, learn to use the microscope correctly; know its limitations and how to keep it in good working condition.

Monocular microscopes have a single eyepiece (ocular). They are most useful when no power supply is available. Daylight provides a bright microscopic field for monocular microscopes. Binocular microscopes, with two eyepieces, have replaced monocular ones, as they are more comfortable to use, but daylight provides poor illumination for these microscopes.

The microscope you will use during training and back at your home base is called *a compound binocular microscope*. Optimal malaria microscopy is done with microscopes fitted with x10 paired eyepieces and an x100 oil immersion objective.[1]

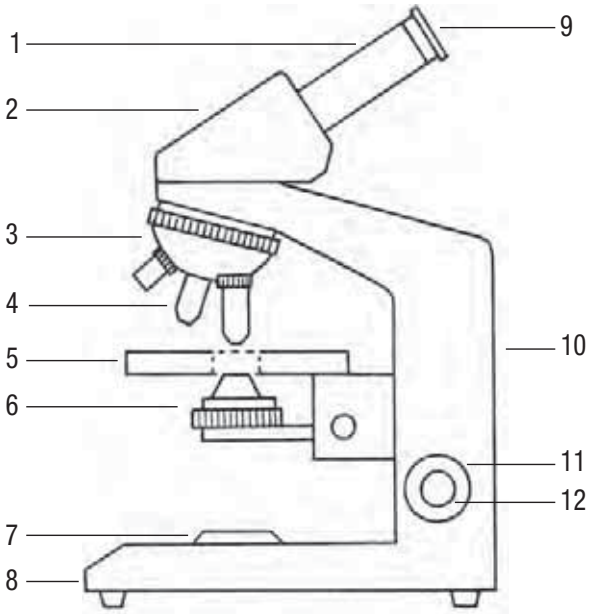
To ensure the high standards of illumination required for routine binocular malaria microscopy, it is essential to have a good, reliable source of artificial light. If a constant supply of electricity is not available, a generator can be used. Delivering

1 Some programmes prefer ×7-paired oculars but they are not easy to obtain. The ×7 ocular covers more blood per field and is therefore considered by some workers to be more sensitive.

WHO. "Learning Unit 6: The Microscope." From the Publication: Basic Malaria Microscopy: part 1. A Learner's Guide. WHO: Switzerland (2010), p. 37-44. Retrieved from: http://apps.who.int/iris/bitstream/10665/44208/1/9789241547826_eng.pdfhttp://apps.who.int/iris/bitstream/10665/44208/1/9789241547826_eng.pdf

even small generators and fuel to remote clinics can be difficult, however, and high running costs make this method unacceptable. Cheaper, easier sources of artificial light for microscopy are light-emitting diodes (LED), a form of electroluminescence that can be derived from small, low-voltage batteries. The batteries can be charged by a small solar panel mounted on a pole or the roof of the laboratory. A range of these products is available on the market. Most are affordable, easy to use and require minimal maintenance. Your facilitator will discuss this subject further, depending on how important it is to you and the programme.

The LED light illustrated here can run for a minimum of 200 hours on four standard 1.5-volt batteries.



Parts of the compound binocular microscope

The main parts of a typical compound binocular microscope are shown above.

1 and 2. Main tube and body tube

Collectively called the microscope head, the main tube and body tube are designed to slope towards the user and are called an 'inclined head'. Polished glass prisms

WHO. "Learning Unit 6: The Microscope." From the Publication: Basic Malaria Microscopy: part 1. A Learner's Guide. WHO: Switzerland (2010), p. 37-44. Retrieved from: http://apps.who.int/iris/bitstream/10665/44208/1/9789241547826_eng.pdfhttp://apps.who.int/iris/bitstream/10665/44208/1/9789241547826_eng.pdf

inside the body tube of the inclined head bend the light so that the image reaches the user's eyes through the paired oculars.

3. Revolving nosepiece

Three or four objective lenses of different magnifications screw into the nosepiece. The nosepiece revolves to place a different objective over the specimen, in line with the eyepieces, which increases or decreases magnification of the specimen.

4. Objective lenses

All the parts of the microscope are important, but the objective lenses must be treated with particular care. An objective consists of two or more lenses kept in place by a special glue or cement. Solvents such as alcohol, xylol and acetone can dissolve the cement holding the lens in place and should not be used to clean the objectives or any other part of the microscope.

An objective is referred to by its magnifying power, which is usually marked on the side of the body. Each microscope usually has a x10, a x40 and a x100 objective. The x100 is called the 'oil immersion objective' and can be identified by a distinctive black, red or white ring.

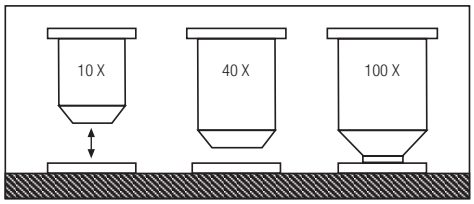
When you examine an objective lens, you will notice that the size of the front lens decreases with the magnifying power. The working distance between the front lens and the focused specimen on the stage changes with the magnification. Thus, the higher the objective's magnifying power, the shorter the working distance. Care must therefore be taken not to damage the specimen with the objective lens.

Although there may be small variations according to the manufacturer, the working distance for each objective is approximately:

x10 15.98 mm

x40 4.31 mm

x100 1.81 mm (oil immersion)



Objectives, showing working distance between front lens and specimen

The microscope must be used with care, as specimens, slides and even the objective lens can easily be damaged by rough manipulation or when objectives are changed.

5. Mechanical stage

The mechanical stage holds the slide secure while allowing specimens to be moved smoothly. A scale fitted to two sides shows the specimen's position and subsequent movement during examination. This scale is called the Vernier scale. You will use this scale to trace portions of the blood film that should be re-examined or shown to others. In modern binocular microscopes, the stage moves when the specimen is focused. In older microscopes, the body and tube move during focusing.

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6. Substage condenser, iris diaphragm and filter holder

The substage condenser consists of a number of lenses that centre the light from the source or mirror onto a central spot on the microscopic field. The substage condenser can be raised or lowered to give maximum or minimum illumination.

Inside the condenser is the iris diaphragm, which is used to control the amount of light passing through the condenser. It consists of a number of thin, interlocking metal leaves, which are adjusted by moving a small lever.

Beneath the iris diaphragm is the filter holder, in which a frosted blue-glass filter is placed when electricity is the light source. This makes the microscopic field appear white rather than yellow.

The procedure for setting the correct illumination of the microscope, i.e. Köhler illumination, is important for optimum resolution and contrast, ensuring an evenly illuminated field, removing glare and reducing heating of the specimen, as described in the enclosed CD-ROM.

7. Illuminator

Modern microscopes have a fixed illuminator, in which a built-in prism mirror brings light to the microscopic field. Others have a removable illuminator, which can be replaced by a mirror when electricity is not available.

The substage mirror is used to direct light from the light source to the microscope field. It has two sides: one plane (flat) and the other concave. The flat surface is used with the substage condenser. The concave side is used without the substage condenser, as the curved surface itself acts as a condenser.

8. Base or foot

To avoid movement or wobbling, the solid base, or foot, of the microscope must rest on a firm, flat surface. The shape of the foot may vary. Most have a threaded hole in the underside of the base to receive a securing screw that keeps the microscope rigid in the box during transport.

9. Ocular, or eyepiece

The top of the main tube of modern microscopes is fitted with a binocular head, i.e with two oculars, one for each eye. Monocular microscopes are seldom used today in national malaria control programmes.

The ocular fits into the upper end of the main tube, and the microscopist looks through it when using the microscope. The magnifying power of each ocular is marked on it. The 'magnifying power' is the number of times by which it will magnify the image produced by the objective. For example, with oculars of x10 and an oil immersion objective of x100, the total magnification of the specimen would be 10 x 100 = 1000 diameters. The magnification is actually a little more, but 1000 diameters is accurate enough for our purposes.

Oculars are available in a range of powers, from x5 to x25 or even x30. In malaria microscopy, a range of x6 to x10 is used routinely. One large programme has used x5 oculars for many years. Today, x10 is probably the most commonly used. Programmes are strongly advised to use oculars between x7 and x10 for routine malaria microscopy.

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Oculars fitted to binocular microscopes are called paired oculars. The marking 'x10P' on the rim of a x10 ocular indicates that it is one of a paired set of eyepieces.

10. Arm or limb

The arm forms a rigid support for the main tube and stage of the microscope. It is robust and can be used as a handle for carrying the microscope. When carrying a microscope in this way, always support the base of the microscope with the other hand.

11 and 12. Coarse and fine adjustments

The two adjustment systems, coarse and fine, are used to focus on the specimen being examined. The coarse adjustment is used for rapid, relatively large vertical focusing movements, while the fine adjustment is for the more precise focusing required with higher-powered objectives. In modern microscopes, the coarse and fine adjustments raise and lower the mechanical stage. In older microscopes, the main tube is raised to focus.

Usually, a specimen is first examined with the coarse adjustment and then examined in detail with the fine adjustment.

The coarse adjustment is used differently when the oil immersion objective is used, as will be explained in a later learning unit.

Use of the microscope

In the practical sessions, you will use and become familiar with all the features of the microscope. Early on, you will see the image of the specimen becoming larger as the magnification is increased. This takes place when you change objectives. You will also examine everyday objects and see how different they look under the microscope. These exercises are designed to help you learn to adjust the illumination correctly and to use the substage condenser and iris diaphragm. You will also practise using the mechanical stage and Vernier scale.

The light source

A good source of artificial light is needed to examine specimens properly. Light that is either too bright or too dim will interfere with malaria microscopy.

When the oil immersion objective of a binocular microscope is used routinely, electric light from a mains supply or a generator should be used. Battery-operated LED light sources are a useful alternative when electric light is not available and should be directed towards the mirror. Artificial LED light travels through the mirror on a path from the source as follows:

source → mirror → substage condenser and diaphragm → specimen → objective → oculars

When artificial light is used, a frosted blue filter must be placed between the source and the substage condenser. The flat side of the mirror is used.

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Daylight should be used only in an emergency. When daylight is the light source, the concave mirror should be used without the substage condenser. It is dangerous to point the mirror directly at the sun when obtaining illumination, as serious damage can be caused to the eyes.

Obtaining even illumination

Using x10 paired oculars and an x10 objective:

1. Place the slide on the mechanical stage, with the specimen over the central opening in the stage.
2. Focus on the specimen using the coarse adjustment.
3. Make sure that the iris diaphragm is wide open, and raise the substage condenser until the microscopic field is brightest.
4. Remove the eyepieces and, looking down the tube, adjust the mirror (if it is being used) until the objective lens is fully illuminated.
5. Replace the eyepieces. Use the fine adjustment to sharpen the focus on the specimen.
6. Remove the eyepieces again, and slowly close the iris diaphragm until the aperture of the objective is two-thirds visible. The specimen will appear clearer, with maximum resolution.
7. Replace the eyepieces, and revolve the nosepiece to select the objective you want to use. Each time you change the objective, you must refocus.
8. If the intensity of the light from the substage lamp is constant, the illumination can be adjusted by increasing or decreasing the aperture of the iris diaphragm. In some microscopes, it is possible to adjust the intensity of the light from the substage lamp.

Using the oil immersion objective

When preparing the microscope for oil immersion microscopy:

1. Arrange the illumination as described above, then observe the next steps from the side of the microscope.
2. Using the coarse adjustment, rack the stage down, away from the objective lens.
3. Place the slide on the microscope stage, with the blood film uppermost.
4. Making sure that there will be sufficient space between the stage and the x100 objective, revolve the nosepiece until the x100 objective is over the specimen.
5. Place one or two drops of immersion oil on the area of the blood film to be examined.
6. Using the coarse adjustment, move the stage until the objective lens is in contact with the immersion oil. Raise the stage slightly, making sure that the lens and oil remain in contact.
7. Looking down the eyepieces, focus on the specimen with the fine adjustment. Make sure that the lens does not touch the slide. Correct the illumination by adjusting the iris diaphragm.

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Immersion oil is used between the microscope slide and the objective lens to reduce scattering of transmitted light. The oil must reproduce the optical properties of the glass used for the lenses and must therefore have a refractive index of 1.515, which is approximately 1.5 times the refractive index of water.

Commercially available immersion oils can be cleaned off the objective lens with a soft cotton cloth. Do not use this cloth to clean other lenses. Immersion oil on blood films can be gently washed away with the solvent recommended by the manufacturers, or the slides can be placed face down for a while on clean, white absorbent tissue paper that soaks up the oil. Some workers wipe the oil off films with absorbent tissue, but this method is rough and is not recommended. Another method is to roll examined slides in white tissue paper (toilet paper will do), with one layer of tissue paper between each slide. After a few days, when the paper has absorbed the oil, the slides can be removed from the paper. Coloured tissue should not be used as it is often acidic and will de-stain blood films.

Care of the microscope

Provided normal care and common sense are exercised, your microscope will remain in good condition for many years.

Removing dust and grease

During the day, when the microscope is not in use, it should be kept covered with a clean cloth or plastic cover to protect the lenses from settling dust. Overnight, or if the microscope will remain unused for a long time, it should be placed inside its box, with the door tightly closed. To protect the objective lenses, the x10 objective should be rotated to line up with the ocular.

Oil from eyelashes, facial skin and fingers is easily deposited on lenses and oculars during use. These parts should be cleaned carefully with lens tissue or a soft cotton cloth.

Oil immersion objectives must be cleaned immediately after use. If not, the oil will thicken and harden over time, and the objective will become useless. To avoid further transfer of oils, never use contaminated cloths to clean other objectives, oculars or the mirror.

Preventing fungal growth

In warm, humid climates, fungal growths are easily established on lenses and prisms. Fungal growth causes problems and can become so bad that a microscope cannot be used. In such cases, the affected surfaces might have to be cleaned and repolished—a job usually done by the manufacturer, which takes time and can be expensive.

- Fungus cannot grow on glass surfaces when the atmosphere is dry. Therefore, it is important to store the microscope in dry conditions when not in use. One of the following methods should be used.
- Keep the microscope in a 'warm cupboard', which has a tightly fitting door and two or more, constantly burning 25-watt bulbs, depending on the size of the

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cupboard. The temperature inside the cupboard should be a constant 30–35 °C, with low humidity.

- Keep all lenses and prism heads in an airtight box or desiccator containing active silica gel, which is a 'desiccant' and absorbs water vapour from the air. Self-indicating silica gel is blue when active and becomes pink as it absorbs water vapour. When it is bright pink, it can be reactivated by heating; it is ready to use again (after cooling) when it has become bright blue.
- If possible, keep the microscope in a continuously air-conditioned room. Rooms that are air-conditioned only during the working day are not suitable.

Transporting the microscope

When transporting the microscope between laboratories or to the field, it is important to ensure that it is properly secured inside its box. The best way to do this is by screwing the securing device through the hole in the bottom of the box into the base or foot of the microscope. When this is done correctly, the microscope remains rigid in its box on even the roughest road.

Read Learning unit 7 in preparation for the next session.

Notes

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2. Diagrams and Schematics of Microscopes

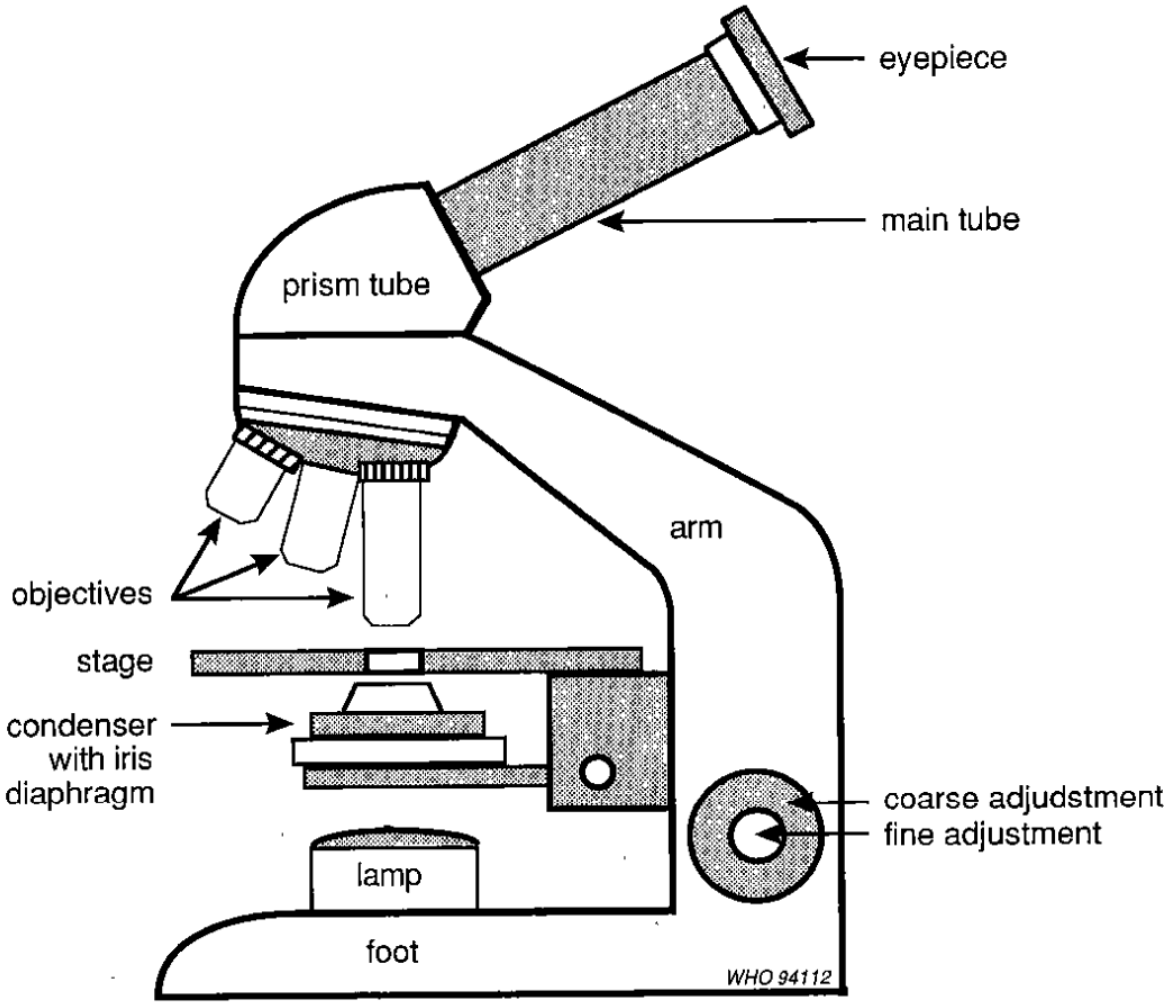
Featured in this Section:

Malkin, Robert. “Balances Use and Operation.” *Medical Instrumentation in the Developing World*. Engineering World Health, 2006.

WHO. *Maintenance and Repair of Laboratory, Diagnostic Imaging, and Hospital Equipment* (WHO: 1996).

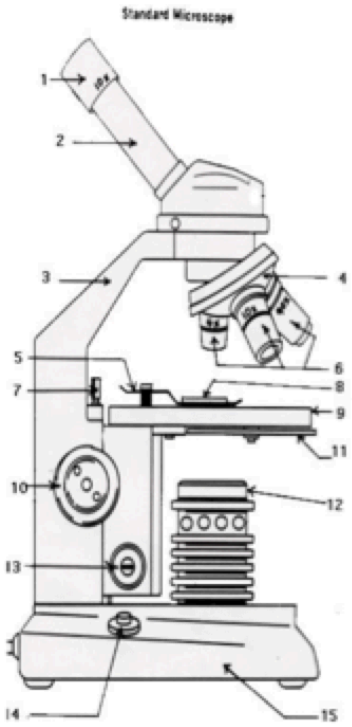
Figure 1: Diagram of a Light Microscope

Fig. 2.18. Light microscope.



WHO. Maintenance and Repair of Laboratory, Diagnostic Imaging, and Hospital Equipment (WHO: 1996).

Figure 2: Diagram of a Compound Light Microscope and Corresponding Parts



(1) eyepiece
(2) body tube
(3) arm
(4) nose piece
(5) stage clips
(6) objectives
(7) stage stops
(8) aperture
(9) stage
(10) coarse adjustment knob
(11) diaphragm
(12) light source
(13) fine adjustment knob
(14) power switch
(15) base

Figure 2: General layout of standard compound light microscope, labeled by part.

Malkin, Robert. "Microscopes: Use and Operation." Medical Instrumentation in the Developing World. Engineering World Health, 2006.

3. Microscopes Preventative Maintenance

Featured in this Section:

Cooper, Justin and Alex Dahinten for EWH. “Microscope Troubleshooting Flowchart.” From the publication: *Medical Equipment Troubleshooting Flowchart Handbook*. Durham, NC: Engineering World Health, 2013.

EQUIPMENT

Microscope Preventative Maintenance

Preventative Maintenance

Complete every six months.

- Clean machine with air brush or air blower, lint free cloth with lens cleaner. Start cleaning objective lens from center and spiral out with cotton swab or cloth. If lens cleaner is not available, ethyl ether, xylene, petrol can be used. However, alcohol, acetones or any other ketones should not be used, as they may dissolve the sealants around the lens.
- Check for and remove any present fungal growth.
- Ensure a specimen can be viewed clearly through all objectives. Do not scratch or damage the lens. Oil lens - X100 needs a drop of oil to confirm clear image. Clean all objectives and eyepieces first, and then check for image clarity.
- Check optical alignment.
- Lubricate adjustments.
- Ensure light source is working properly.
- Inspect for signs of damage, scratches, or dirt.
- Examine switches and controls for proper function.
- Replace uncooked rice before storage to prevent fungal growth.

Kohler Illumination: It is a method of illumination which involves optimizing a microscope's optical train to produce homogenously bright light free from artifacts and glare. In Kohler illumination, four separate planes combine to form conjugate planes in both the illumination and image-forming light pathways. The lamp filament, aperture diaphragm, back focal plane of the objective lens, and the eye point which is approximately one centimeter above the top lens of the ocular, form the illumination conjugate plane. The conjugate planes of the imaging light path are the field diaphragm, specimen, the fixed diaphragm of the ocular, and the retinal plane of the viewer. In Kohler illumination the collector lens or field diaphragm collects light from the illumination source and focuses it at the front focal plane of the sub-stage condenser's aperture diaphragm which, in essence, projects an image of the lamp filament onto the lens.

4. Troubleshooting and Repair of Microscopes

Featured in this Section:

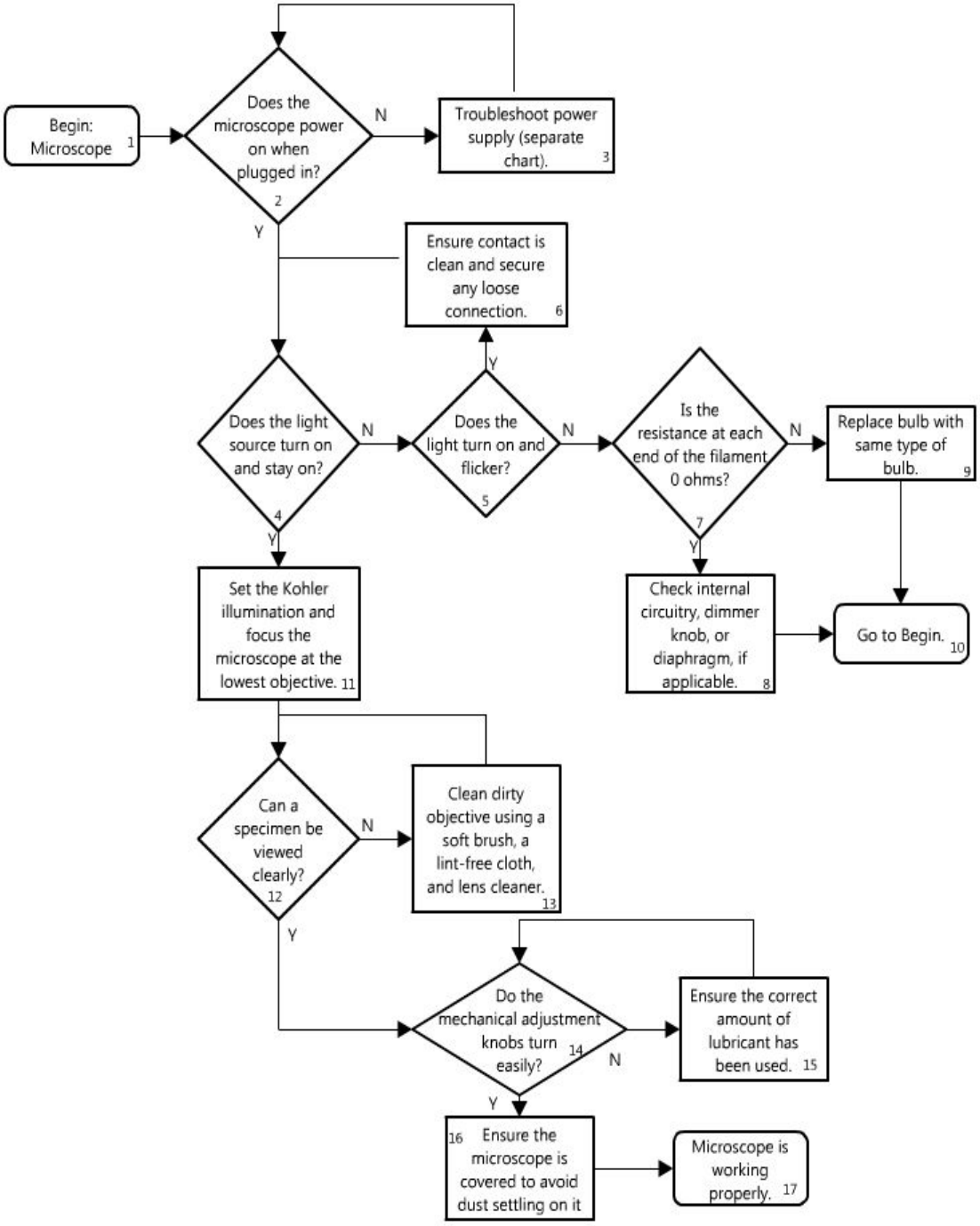
Cooper, Justin and Alex Dahinten for EWH. “Microscopes Troubleshooting Flowchart.” From the publication: *Medical Equipment Troubleshooting Flowchart Handbook*. Durham, NC: Engineering World Health, 2013.

Microscope Troubleshooting Flowchart

EQUIPMENT

Microscope Repair and Troubleshooting

Microscope Flowchart



Cooper, Justin and Alex Dahinten for EWH. "Microscopes Troubleshooting Flowchart." From the publication: Medical Equipment Troubleshooting Flowchart Handbook. Durham, NC: Engineering World Health, 2013.

Description

#	Text Box	Comments
1	Begin: Microscope	Begin diagnostic process for a work order for Microscope. Maintenance is generally requested on a microscope when a specimen cannot be viewed clearly or at all.
2	Does the microscope power on when plugged in?	When plugged in, the microscope should power on completely.
3	Troubleshoot power supply (separate chart).	If no power reaches the machine, there may be problems with the switch, fuse, power supply components, or wiring. See flowchart on Power Supply and BTA skills on Power Supply.
4	Does the light source turn on and stay on?	The light source should remain constant across the stage when on.
5	Does the light turn on and flicker?	If the light turns on but does not remain constant, there may be a minor problem that can be fixed without replacing the bulb completely.
6	Ensure contact is clean and secure any loose connection.	The inside of the lamp house or the connections may be dirty. The connections should also be secured firmly. See BTA skills for Connections.
7	Is resistance at each end of the filament 0 ohms?	This checks if the filament in the bulb is functioning.
8	Check internal circuitry, dimmer knob, or diaphragm, if applicable.	Ensure that the circuitry that connects to the light source is intact. In addition, ensure the dimmer knob is turned on and that the diaphragm is open.
9	Replace bulb with same type of bulb.	If the light source still does not turn on, replace the bulb with another of the same type. If the same type of bulb is not available, a new source can be wired in. See BTA skills on Replacement of Light Bulbs and Light Fixtures.
10	Go to begin.	Restart the diagnostic process.
11	Focus the microscope at the lowest objective.	Begin this portion of the diagnostic process at the lowest objective.
12	Can the specimen be viewed clearly?	If the specimen is out of focus or cannot be viewed at all, the objectives may need attention.
13	Clean dirty objective using a soft brush, a lint-free cloth, and lens cleaner.	Objectives can be unscrewed or removed for cleaning. Unscrew eyepiece to use as a magnifying glass if one is not available to inspect objective for scratches, nicks, cracks, deterioration of seal around lens or oil seepage into lens. Blow off any dust with canned air before cleaning. Start cleaning objective lens from

		center and spiral out with cotton swab or cloth. If lens cleaner is not available, ethyl ether, xylene, petrol can be used. Alcohol, acetones or any other ketones should not be used, as they may dissolve sealants.
14	Do the mechanical adjustment knobs turn easily?	The knobs and stage should be able to move freely and also maintain a steady position. The screws holding each in place may need some adjustment.
15	Ensure the correct amount of lubricant has been used.	Clean off excess lubricant (especially if it has dried and is clumping) using a soft cloth dampened with alcohol. Do not use solvents that leave residue or lint on the surface. Dust, clean (with solvent listed above), polish (with metal polish, if available) and lubricate adjustments if they are stuck or difficult to turn.
16	Ensure the microscope is covered to avoid dust settling on it.	When covering the microscope, put a small amount of uncooked rice to prevent fungal growth. Replace uncooked rice weekly.
17	Microscope is working properly.	Return the machine to the appropriate clinical personnel.

5. Resources for More Information about Microscopes

Featured in this Section:

Malkin, Robert. “Microscopes: Use and Operation.” *Medical Instrumentation in the Developing World*. Engineering World Health, 2006.

Resources for More Information:

Internal Resources at library.ewh.org: For More Information about Infusion pumps, please see this resource in the BMET Library!

1. Malkin, Robert. "Microscopes: Use and Operation." *Medical Instrumentation in the Developing World*. Engineering World Health, 2006.

Microscopes Bibliography:

Cooper, Justin and Alex Dahinten for EWH. “Microscope Preventative Maintenance.” From the publication: *Medical Equipment Troubleshooting Flowchart Handbook*. Durham, NC: Engineering World Health, 2013.

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WHO. *Maintenance and Repair of Laboratory, Diagnostic Imaging, and Hospital Equipment* (WHO: 1996).

Wikipedia. “Optical Microscope.” *Wikipedia*. Retrieved from: https://en.wikipedia.org/wiki/Optical_microscope