Physics of Nanotechnology

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Lecture content

Optical microscope

- The **optical microscope**, also referred to as a **light microscope**, is a type of microscope that commonly uses visible light and a system of lenses to generate magnified images of small objects. Optical microscopes are the oldest design of microscope and were possibly invented in their present compound form in the 17th century. Basic optical microscopes can be very simple, although many complex designs aim to improve resolution and sample contrast.
- The object is placed on a **stage** and may be directly viewed through one or two eyepieces on the microscope. In high-power microscopes, both eyepieces typically show the same image, but with a stereo microscope, slightly different images are used to create a 3-D effect. A camera is typically used to capture the image (micrograph).

A modern optical microscope with a mercury bulb for fluorescence microscopy. The microscope has a digital camera which is connected to a computer.

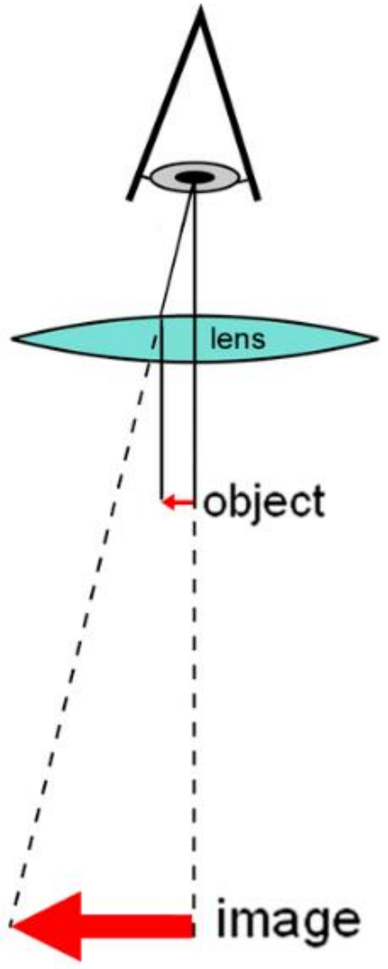
Types

There are two basic types of optical microscopes: simple microscopes and compound microscopes. A simple microscope uses the optical power of single lens or group of lenses for magnification. A compound microscope uses a system of lenses (one set enlarging the image produced by another) to achieve much higher magnification of an object. The vast majority of modern research microscopes are compound microscopes while some cheaper commercial digital microscopes are simple single lens microscopes. Compound microscopes can be further divided into a variety of other types of microscopes which differ in their optical configurations, cost, and intended purposes

Simple microscope

A simple microscope uses a lens or set of lenses to enlarge an object through angular magnification alone, giving the viewer an erect enlarged virtual image.

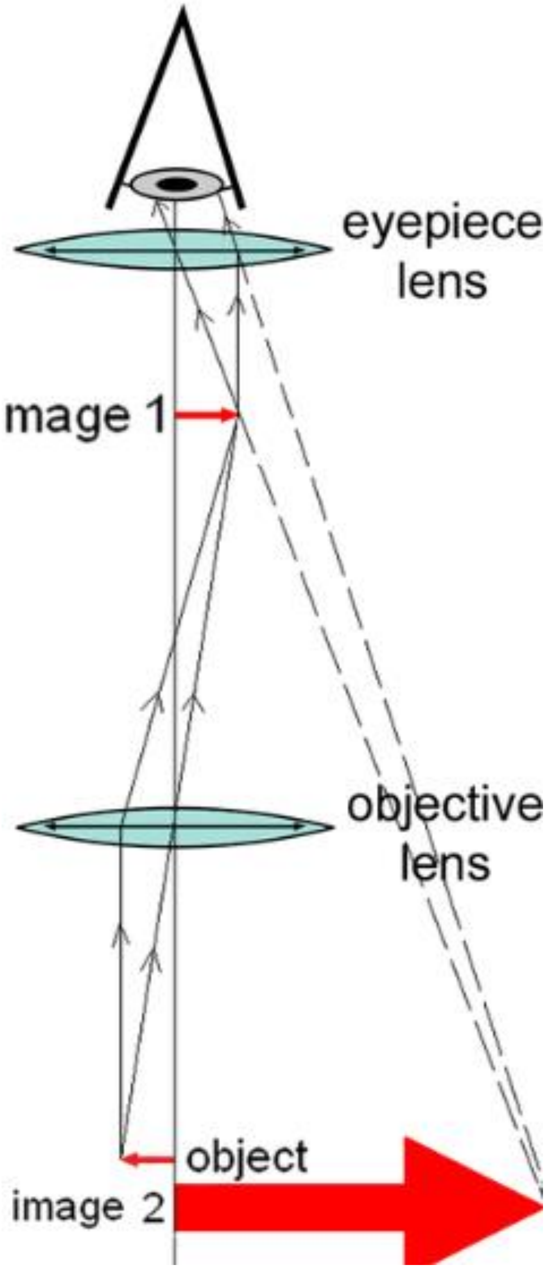
The use of a single convex lens or groups of lenses are found in simple magnification devices such as the magnifying glass, loupes, and eyepieces for telescopes and microscopes



Compound microscope

A compound microscope uses a lens close to the object being viewed to collect light (called the objective lens) which focuses a real image of the object inside the microscope (image 1). That image is then magnified by a second lens or group of lenses (called the eyepiece) that gives the viewer an enlarged inverted virtual image of the object (image 2). The use of a compound objective/eyepiece combination allows for much higher magnification. Common compound microscopes often feature exchangeable objective lenses, allowing the user to quickly adjust the magnification.[3] A compound microscope also enables more advanced illumination setups, such as phase contrast.

Image 2:
Diagram of a compound microscope



Other microscope variants

There are many variants of the compound optical microscope design for specialized purposes. Some of these are physical design differences allowing specialization for certain purposes:

- Stereo microscope, a low-powered microscope which provides a stereoscopic view of the sample, commonly used for dissection.
- Comparison microscope, which has two separate light paths allowing direct comparison of two samples via one image in each eye.
- Inverted microscope, for studying samples from below; useful for cell cultures in liquid, or for metallography.
- Fiber optic connector inspection microscope, designed for connector end-face inspection
- Traveling microscope, for studying samples of high optical resolution.

Other microscope variants are designed for different illumination techniques:

- 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.

Other microscope variants

- Phase-contrast microscope, which applies the phase contrast illumination method.
- Epifluorescence microscope, designed for analysis of samples which include fluorophores.
- Confocal microscope, a widely used variant of epifluorescent illumination which uses a scanning laser to illuminate a sample for fluorescence.
- Two-photon microscope, used to image fluorescence deeper in scattering media and reduce photobleaching, especially in living samples.
- Student microscope – an often low-power microscope with simplified controls and sometimes low quality optics designed for school use or as a starter instrument for children.[4]
- Ultramicroscope, an adapted light microscope that uses light scattering to allow viewing of tiny particles whose diameter is below or near the wavelength of visible light (around 500 nanometers); mostly obsolete since the advent of electron microscopes

Digital microscope

- A digital microscope is a microscope equipped with a digital camera allowing observation of a sample via a computer. Microscopes can also be partly or wholly computer-controlled with various levels of automation. Digital microscopy allows greater analysis of a microscope image, for example measurements of distances and areas and quantitaton of a fluorescent or histological stain.
- Low-powered digital microscopes, USB microscopes, are also commercially available. These are essentially webcams with a high-powered macro lens and generally do not use transillumination. The camera attached directly to the USB port of a computer, so that the images are shown directly on the monitor. They offer modest magnifications (up to about 200×) without the need to use eyepieces, and at very low cost. High power illumination is usually provided by an LED source or sources adjacent to the camera lens.

Components

- All modern optical microscopes designed for viewing samples by transmitted light share the same basic components of the light path. In addition, the vast majority of microscopes have the same 'structural' components[27] (numbered below according to the image on the right):
- Eyepiece (ocular lens) (1)
- Objective turret, revolver, or revolving nose piece (to hold multiple objective lenses) (2)
- Objective lenses (3)
- Focus knobs (to move the stage)
  - Coarse adjustment (4)
  - Fine adjustment (5)
- Stage (to hold the specimen) (6)
- Light source (a light or a mirror) (7)
- Diaphragm and condenser (8)
- Mechanical stage (9)

Basic optical transmission microscope elements (1990s)

Eyepiece (ocular lens)

- The eyepiece, or ocular lens, is a cylinder containing two or more lenses; its function is to bring the image into 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 5×, 10× (the most common), 15× and 20×. 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.

Objective Lens

- At the lower end of a typical compound optical microscope, there are one or more objective lenses that collect light from the sample. The objective is usually in a cylinder housing containing a glass single or multi-element compound lens. Typically there will be around three objective lenses screwed into a circular nose piece which may be rotated to select the required objective lens. These arrangements are designed to be parfocal, which means that when one changes from one lens to another on a microscope, the sample stays in focus. Microscope objectives are characterized by two parameters, namely, magnification and numerical aperture. The former typically ranges from 5× to 100× while the latter ranges from 0.14 to 0.7, corresponding to focal lengths of about 40 to 2 mm, respectively. Objective lenses with higher magnifications normally have a higher numerical aperture and a shorter depth of field in the resulting image. Some high performance objective lenses may require matched eyepieces to deliver the best optical performance.

Focus knobs

- Adjustment knobs move the stage up and down with separate adjustment for coarse and fine focusing. The same controls enable the microscope to adjust to specimens of different thickness. In older designs of microscopes, the focus adjustment wheels move the microscope tube up or down relative to the stand and had a fixed stage

Frame

- The whole of the optical assembly is traditionally attached to a rigid arm, which in turn is attached to a robust U-shaped foot to provide the necessary rigidity. The arm angle may be adjustable to allow the viewing angle to be adjusted.
- The frame provides a mounting point for various microscope controls. Normally this will include controls for focusing, typically a large knurled wheel to adjust coarse focus, together with a smaller knurled wheel to control fine focus. Other features may be lamp controls and/or controls for adjusting the condenser.

Stage

- The stage is a platform below the objective lens 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 25×75 mm, on which the specimen is mounted).
- At magnifications higher than 100× moving a slide by hand is not practical. A mechanical stage, typical of medium and higher priced microscopes, allows tiny movements of the slide via control knobs that reposition the sample/slide as desired. If a microscope did not originally have a mechanical stage it may be possible to add one.
- All stages move up and down for focus. With a mechanical stage slides move on two horizontal axes for positioning the specimen to examine specimen details.
- Focusing starts at lower magnification in order to center the specimen by the user on the stage. Moving to a higher magnification requires the stage to be moved higher vertically for re-focus at the higher magnification and may also require slight horizontal specimen position adjustment. Horizontal specimen position adjustments are the reason for having a mechanical stage.
- Due to the difficulty in preparing specimens and mounting them on slides, for children it's best to begin with prepared slides that are centered and focus easily regardless of the focus level used

Light source

- Many sources of light can be used. At its simplest, daylight is directed via a mirror. Most microscopes, however, have their own adjustable and controllable light source – often a halogen lamp, although illumination using LEDs and lasers are becoming a more common provision. Köhler illumination is often provided on more expensive instruments.

Condenser

- The condenser is a lens designed to focus light from the illumination source onto the sample. The condenser may also include other features, such as a diaphragm and/or filters, to manage the quality and intensity of the illumination. For illumination techniques like dark field, phase contrast and differential interference contrast microscopy additional optical components must be precisely aligned in the light path.

Magnification

- The actual power or magnification of a compound optical microscope is the product of the powers of the ocular (eyepiece) and the objective lens. The maximum normal magnifications of the ocular and objective are 10× and 100× respectively, giving a final magnification of 1,000×.