

DEPTH STUDY: ELECTRON MICROSCOPES



Shortly after the experimental confirmation of the wave properties of the electron, it was suggested that the electron could be used to examine objects in much greater detail than an optical microscope. The resolution (ability to resolve or distinguish two points close together) of a microscope is limited by the wavelength of the radiation. For an optical microscope, the best resolution is about 100 nm (wavelength \sim 200 nm). This corresponds to a magnification of 500x. An electron in an electron microscope can have a wavelength less than 1 nm thus increasing the magnification by a factor of more than 100x that of an optical microscope.

Resolution

The resolution of an optical microscope is defined as the shortest distance between two points on a specimen that can still be distinguished by the observer as separate entities. When light passes through a circular aperture a diffraction pattern is produced on an observation screen which is called an **Airy disc** (figure 1).

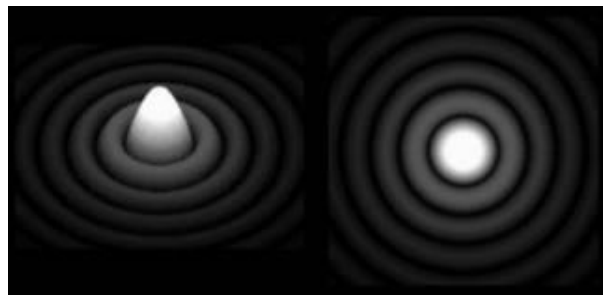


Fig. 1. Diffraction pattern (Airy disc or pattern) from a circular aperture.

Light passing through two circular apertures or from two point sources produces an interference pattern on an observing screen. If the two apertures or sources are too close together than it is not possible to distinguish the two entities (figure 2).



Fig. 2. Interference patterns: single entity; single or double entities; two entities?

Figure 3 shows where point sources of light from a specimen appear as Airy diffraction patterns at a microscope's intermediate image plane. The limit of resolution of a microscope objective refers to its ability to distinguish between two closely spaced Airy disks in the diffraction pattern. Three-dimensional representations of the diffraction pattern near the intermediate image plane are known as the point spread function, and are illustrated in the lower portion of figure 3. The specimen image is represented by a series of closely spaced point light sources that form Airy patterns and is illustrated in both two and three dimensions.

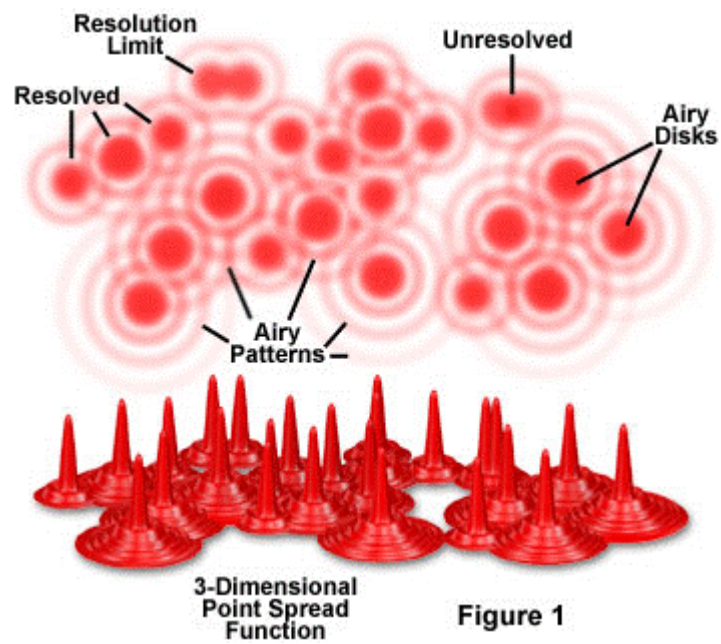


Fig. 3. Airy Patterns and the limit of resolution.

For more information and an animation, go to:

<http://www.microscopyu.com/articles/formulas/formulasresolution.html>

The smaller the wavelength than the smaller the airy disk (or point spread function) and the better the resolution.

The Light Microscope

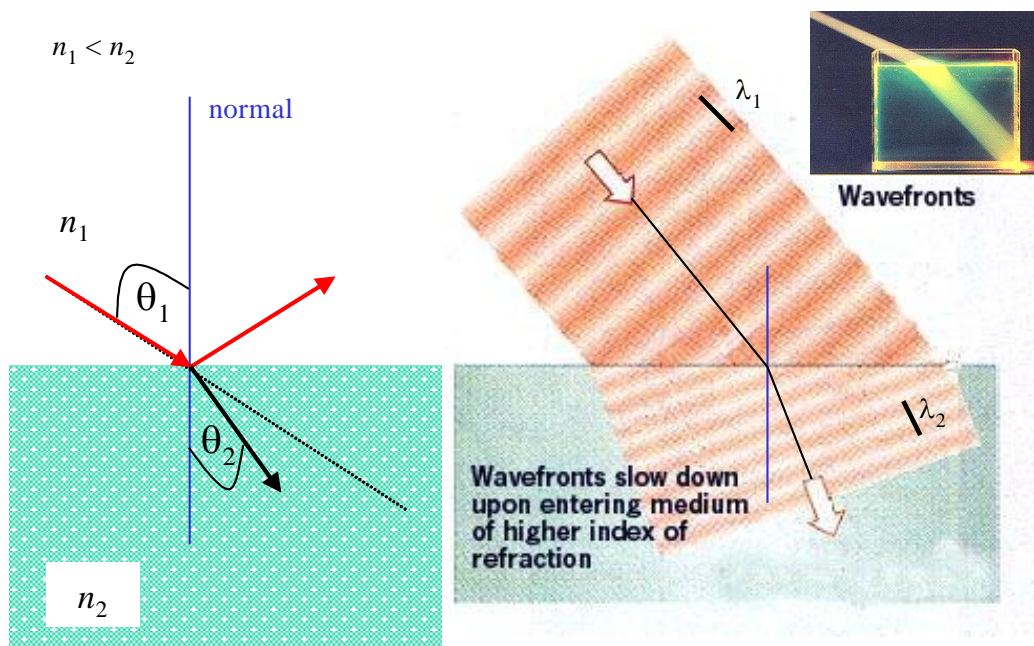
A microscope must gather light from a tiny area of a thin, well-illuminated specimen that is close-by. The **objective lens** of a microscope is small, spherical, and has a short focal length. It brings the image of the object into focus at a short distance within the microscope's tube. The image is then magnified by a second lens, called an **ocular lens** or **eyepiece**, as it is brought to your eye. The microscope has a light source and a condenser. The **condenser** is a lens system that focuses the light from the source onto a tiny, bright spot of the specimen, which is the same area that the objective lens examines. Typically, there are interchangeable objective lenses and fixed eyepieces. By changing the objective lenses (going from relatively flat, low-magnification objectives to rounder, high-magnification objectives), a microscope can bring increasingly smaller areas into view.

A **lens** can be considered as a transparent material. In transparent medium, the speed of propagation of light is $v = c / n$ where n is a property of the medium called the **refractive index**. When light enters the lens, it slows and is bent. This phenomenon is known as **refraction**. The amount of bending is described by **Snell's Law** (Law of Refraction).

How can we see?

What produces an image in an optical microscope?

What happens when light passes through a transparent material?



Snell's Law $n_1 \sin \theta_1 = n_2 \sin \theta_2$



When light enters the eye, most of the light is bent at the cornea and fine adjustments are made of the lens of the eye to focus the light onto the retina. Glasses correct for eye defects to produce a focussed image on the retina.

In a transmission optical microscope light passes through a sample and the path of the light is bent by the various lens. The image gives a "map" of the refractive index variation throughout the sample.

A converging (convex) lens focuses light from a point on an object to a point on the focal plane of the lens.

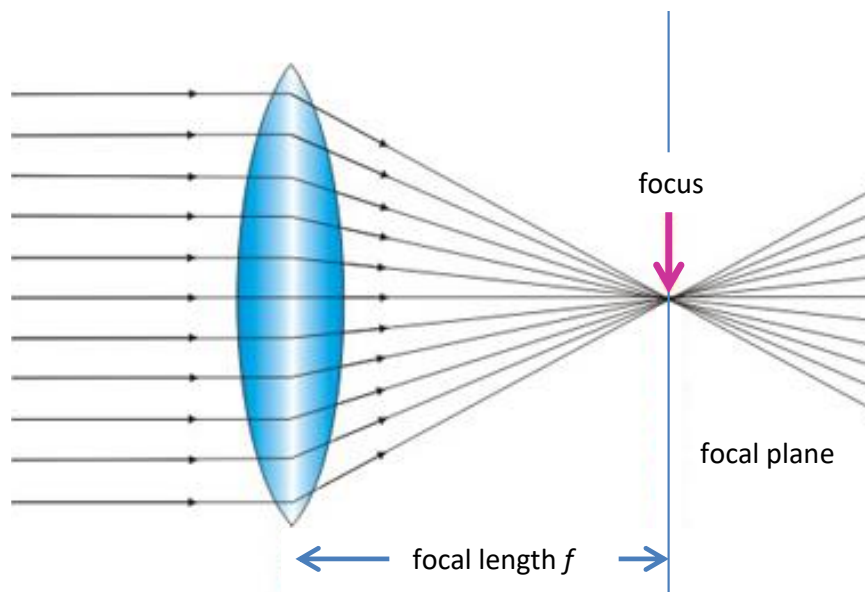


Fig. 4. Converging lens.

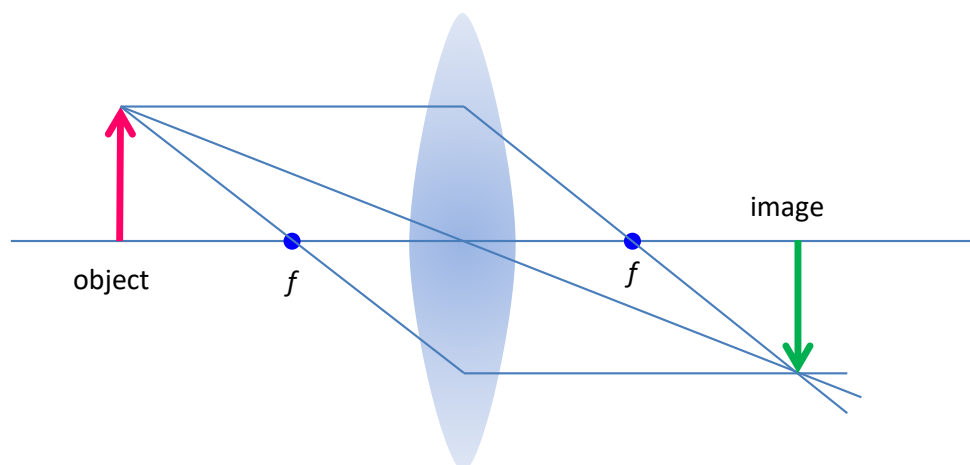


Fig. 5. Ray tracing diagram showing how a real image is formed with a thin converging lens.

The compound microscope invented around 1590 by Zacharias Janssen provides high magnification for nearby objects.

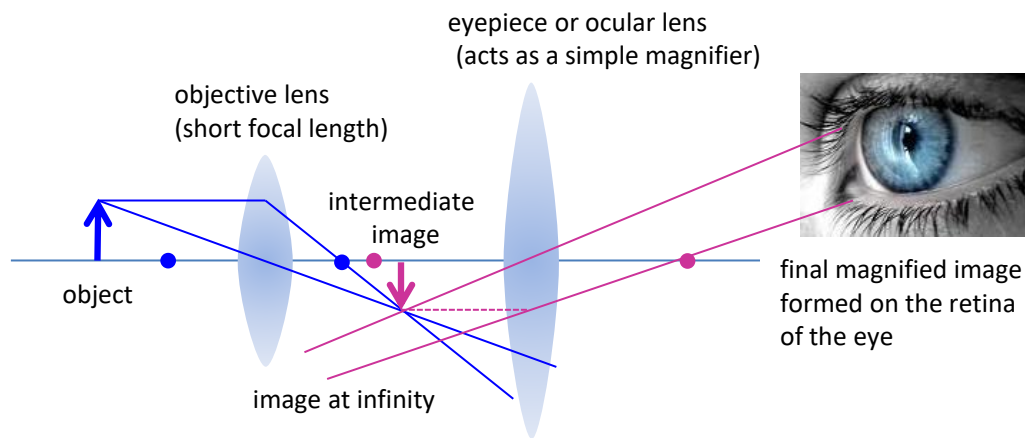


Fig. 6. A schematic diagram of a compound microscope.

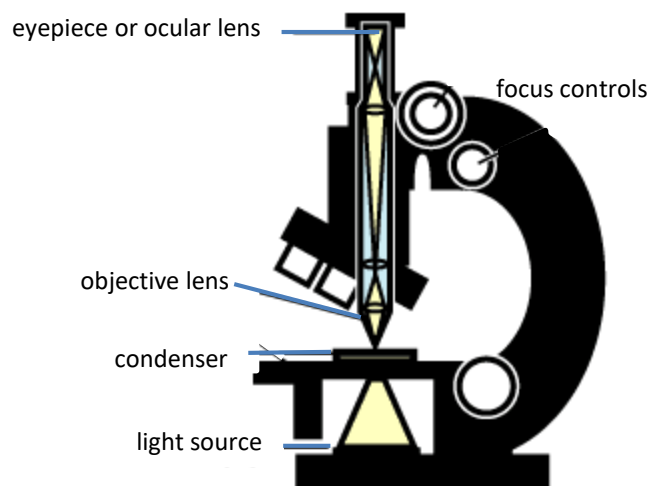


Fig.7. Schematic diagram of a light microscope.

Transmission electron microscope

The electrons because of their wave nature can be reflected, refracted and focussed to form an image. When an electron beam is directed through a very thin object it is partially absorbed, scattered and transmitted just as light.

Thus, when a beam of electrons passes through a very thin object, it carries an image of the density of the object. This beam is then magnified by means of magnetic lenses. The beam is then allowed to fall on a fluorescent screen for visual observation or onto a photographic plate, just as in a light microscope.



Scanning electron microscope

A scanning electron microscope can produce three-dimensional images of an object. A carefully directed beam of electrons is focussed onto a specimen. The beam is scattered across the specimen by means of a varying magnetic or electric field. At each point across the specimen, secondary electrons are emitted and it is these electrons that are detected and an image formed. The number of secondary electrons detected determines the brightness of the image. Contrast and the effect of depth results from the fact that the number of secondary electrons created is much greater when the primary beam strikes the surface of the specimen at a glancing angle. Two different pictures are taken at slightly different angles and when the image is viewed with a stereoscopic viewer a three-dimensional image is observed.

Typical specifications of a scanning tunnelling microscope:

Magnification	30x to 120 000x
Acceleration voltage	~ 15 kV
Specimen	80 mm diameter, 25 mm thick
Resolution	~ 10 nm
Angular resolution	0.0001° (optical microscope ~ 5°)

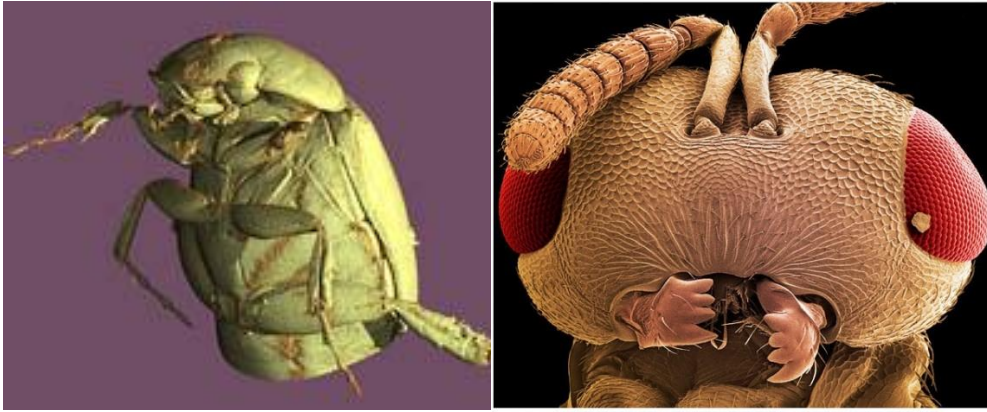


Fig. 8. Images of a beetle and an ant head.



Fig. 9. Images of a head of a maggot, cat flea and household fly.

<http://oddstuffmagazine.com/minature-photography-insects-and-fly-under-the-microscope.html>

Resolution

The **numerical aperture NA** of a microscope objective is a measure of its ability to gather light and resolve fine specimen detail at a fixed object distance. Image-forming light waves pass through the specimen and enter the objective lens in an inverted cone as shown in figure (10). The angle θ is one-half the angular aperture (2θ) and is related to the numerical aperture NA

$$(1) \quad \text{Numerical Aperture } NA = n \sin \theta$$

where n is the refractive index of the imaging medium between the front lens of the objective and the specimen cover glass, a value that ranges from 1.00 for air to 1.51 for specialized immersion oils.

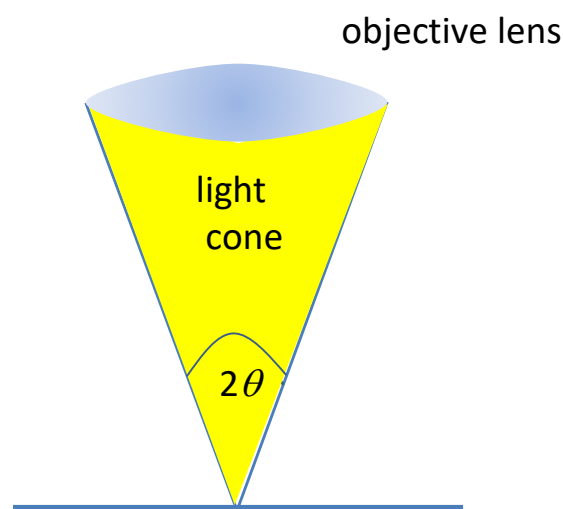


Fig. 10. Numerical aperture of a lens.

By examining the numerical aperture equation, it is apparent that refractive index is the limiting factor in achieving numerical apertures greater than 1.0. Therefore, in order to obtain higher working numerical apertures, the refractive index of the medium between the front lens of the objective and the specimen must be increased. Microscope objectives are now available that allow imaging in alternative media such as water (refractive index = 1.33), glycerin (refractive index = 1.47), and immersion oil (refractive index = 1.51).

Most objectives in the magnification range between 60x and 100x (and higher) are designed for use with immersion oil.

The resolution of a microscope objective is defined as the smallest distance between two points on a specimen that can still be distinguished as two separate entities. Resolution is a somewhat subjective value in microscopy because at high magnification, an image may appear un-sharp but still be resolved to the maximum ability of the objective. Numerical aperture determines the resolving power of an objective, but the total resolution of a microscope system is also dependent upon the numerical aperture of the substage condenser. The higher the numerical aperture of the total system, the better the resolution.

Correct alignment of the microscope optical system is also of paramount importance to ensure maximum resolution. The substage condenser must be matched to the objective with respect to numerical aperture and adjustment of the aperture iris diaphragm for accurate light cone formation. The wavelength spectrum of light used to image a specimen is also a determining factor in resolution. Shorter wavelengths are capable of resolving details to a greater degree than are the longer wavelengths. There are several equations that have been derived to express the relationship between numerical aperture, wavelength, and resolution:

$$(2) \quad \text{resolution } R = 0.61 \lambda / 2NA$$

$$\lambda \downarrow \Rightarrow R \uparrow \quad NA \uparrow \Rightarrow R \uparrow$$

The smaller the value of R , the better the resolution and points closer together can be identified.

It is well worth visiting the web site

<http://www.olympusmicro.com/index.html>

[VISUAL PHYSICS ONLINE](#)

If you have any feedback, comments, suggestions or corrections
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