I. THE MICROSCOPE

I.A. PRINCIPLES OF THE TRANSMISSION ELECTRON MICROSCOPE (TEM)

I.A.1. Origin of the Transmission Electron Microscope

DATE	NAME	EVENT
1897	J. J. Thompson	Discovers the electron
1924	Louis deBroglie (as a grad student)	Identifies a wavelength to moving electrons $\lambda = h/mv$ where λ = wavelength h = Planck's constant m = mass v = velocity (For an electron at 60kV λ = 0.005 nm)
1926	H. Busch	Magnetic or electric fields act as lenses for electrons
1929	E. Ruska	Ph. D thesis on magnetic lenses
1931	Knoll & Ruska	First electron microscope built
1931	Davisson & Calbrick	Properties of electrostatic lenses
1934	Driest & Muller	Surpass resolution of the LM
1938	von Borries & Ruska	First practical EM (Siemens) - 10 nm resolution
1940	RCA	Commercial EM with 2.4 nm resolution
1945		1.0 nm resolution

I.A.2. Comparison of Light (LM) and Electron Microscopes (Fig. I.1)

a. Similarities (Arrangement and function of components are similar)

1) Illumination system: produces required radiation and directs it onto the specimen. Consists of a source, which emits the radiation, and a condenser lens, which focuses the illuminating beam (allowing variations of intensity to be made) on the specimen.

2) Specimen stage: situated between the illumination and imaging systems.

3) Imaging system: Lenses which together produce the final magnified image of the specimen. Consists of i) an objective lens which focuses the beam after it passes through the specimen and forms an intermediate image of the specimen and ii) the projector lens(es) which magnifies a portion of the intermediate image to form the final image.

4) Image recording system: Converts the radiation into a permanent image (typically on a photographic emulsion) that can be viewed.

b. Differences

1) Optical lenses are generally made of glass with fixed focal lengths whereas magnetic lenses are constructed with ferromagnetic materials and windings of copper wire producing a focal length which can be changed by varying the current through the coil.

2) Magnification in the LM is generally changed by switching between different power objective lenses mounted on a rotating turret above the specimen. It can also be changed if oculars (eyepieces) of different power are used. In the TEM the magnification (focal length) of the objective remains fixed while the focal length of the projector lens is changed to vary magnification.

3) The LM has a small depth of field, thus different focal levels can be seen in the specimen. The large (relative) depth of field in the TEM means that the entire (thin) specimen is in focus simultaneously.

4) Mechanisms of image formation vary (phase and amplitude contrast).

5) TEMs are generally constructed with the radiation source at the top of the instrument: the source is generally situated at the bottom of LMs.

6) TEM is operated at high vacuum (since the mean free path of electrons in air is very small) so **most** specimens (biological) must be dehydrated (i.e. dead !!).

7) TEM specimens (biological) are rapidly damaged by the electron beam.

8) TEMs can achieve higher magnification and better resolution than LMs.

9) Price tag!!! (100x more than LM)

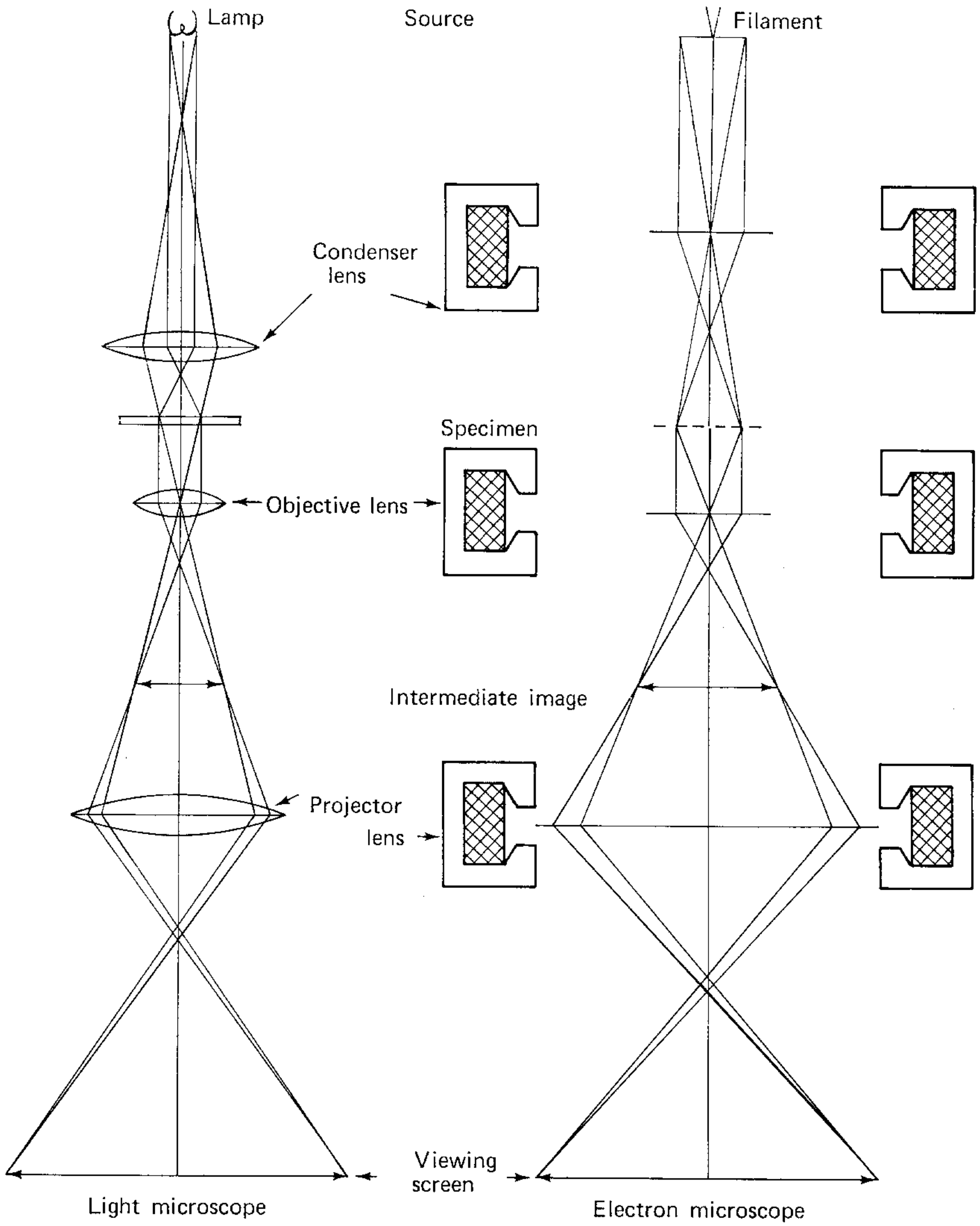


Fig. I.1 Comparison of light and electron microscopes. In each instrument, illumination from the source (lamp, filament in the electron gun) is focused by the condenser lens onto the specimen. A first magnified image is formed by the objective lens. This image is further magnified by the projector lens onto a ground glass screen (light) or fluorescent screen (electrons). (From Agar, p.8)

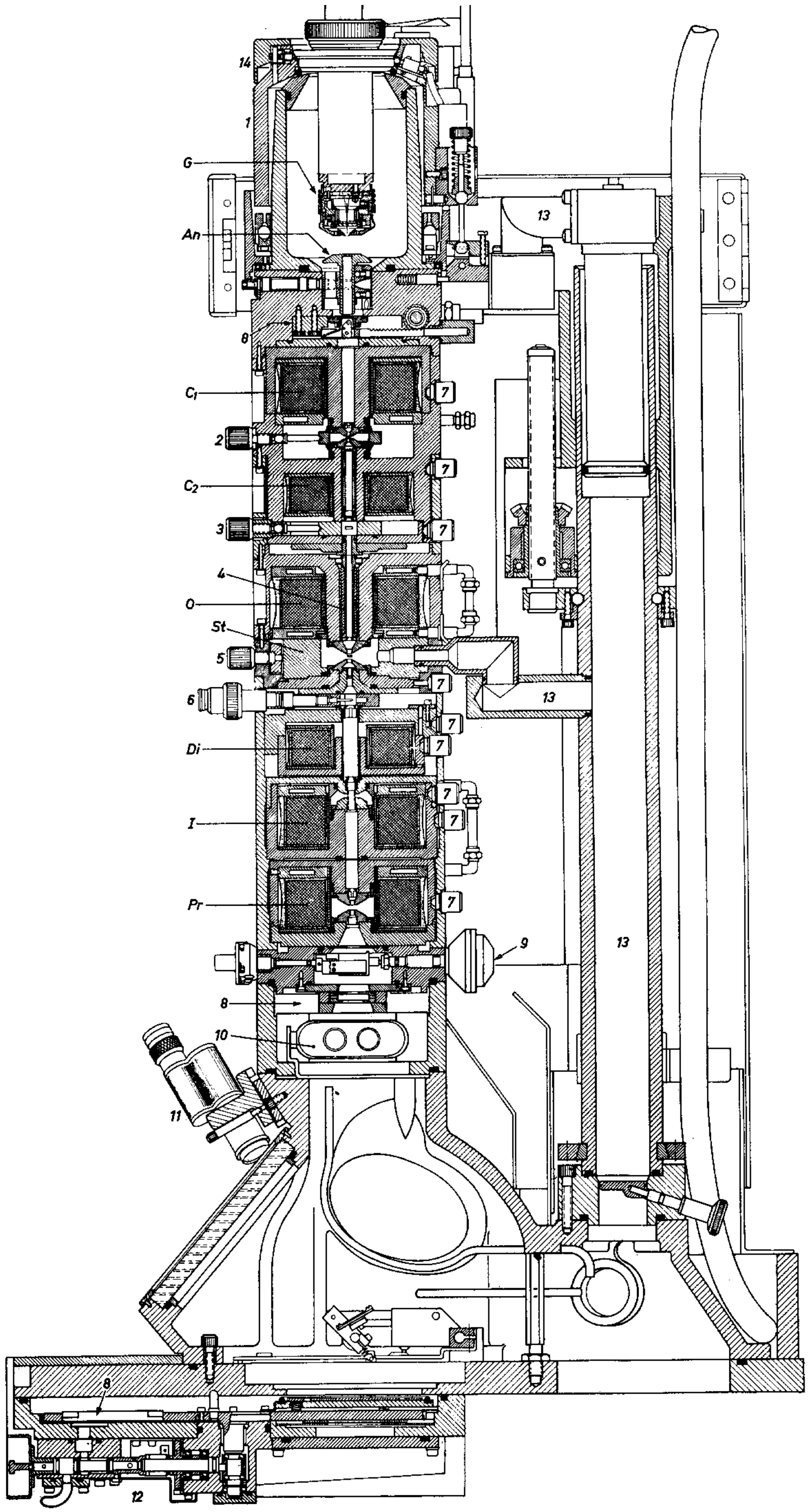


Fig. I.2 Cross-sectional view of the Philips EM 300. (From Agar, p.40)

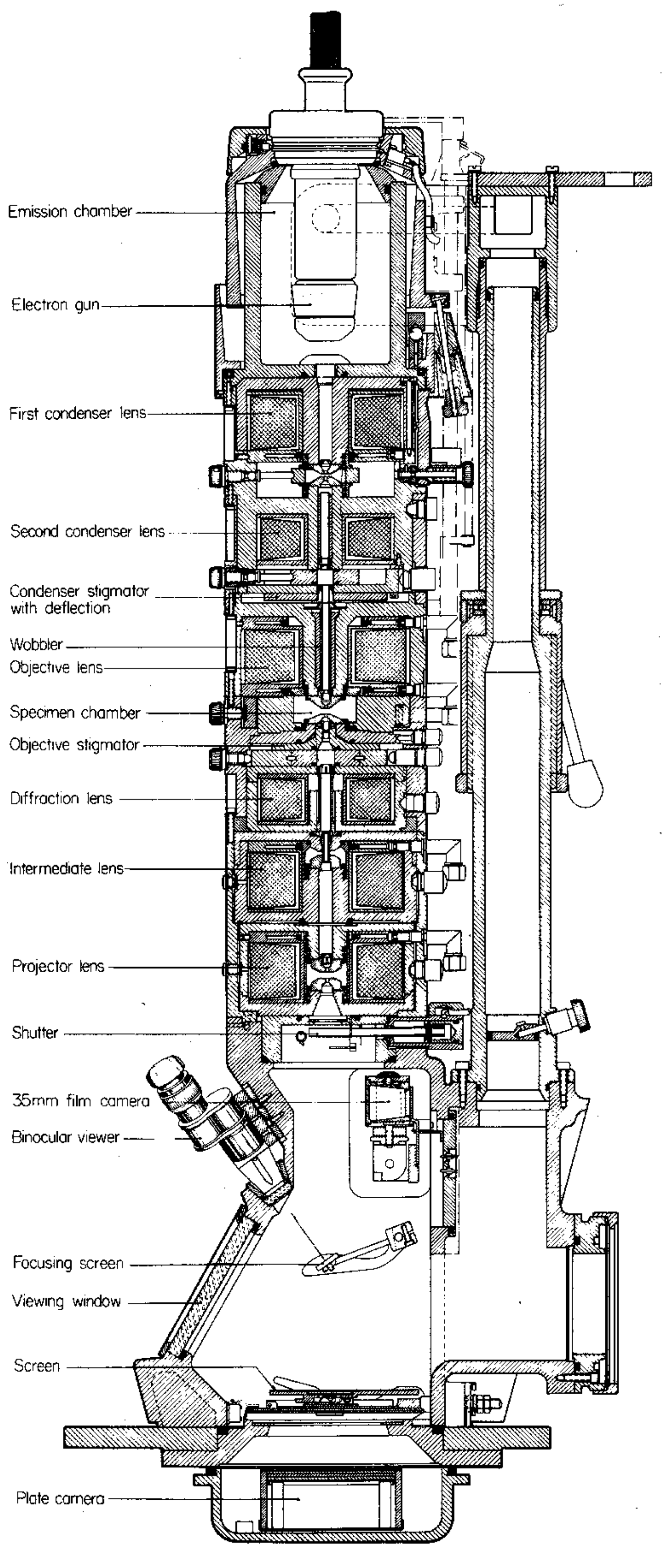


Fig. I.3 Section through a complex double-condenser 6-lens Philips EM200 electron microscope. (From Meek, p.99)

I.A.3. Photons/Electrons

a. Dual concept of wave and particle (Fig. I.4)

Light has properties both of a particle and a wave. This dual nature is required to satisfactorily explain the results of various physical experiments. The diffraction of light (bending around corners) illustrates the wave nature. The **WAVE THEORY** is based on the statistical nature of events and has little meaning with respect to the behavior of single particles. The wave theory was developed and expounded by Huygens (1629-1695) and Hooke (1638-1703). The **CORPUSCULAR THEORY** was proposed by Newton (1642-1727) and became the more accepted theory even after demonstration of diffraction by Young (1773-1829) and interference by Fresnel (1788-1827). At about 1850, the wave theory came back into favor until about 1900 when more evidence for the corpuscular theory was discovered.

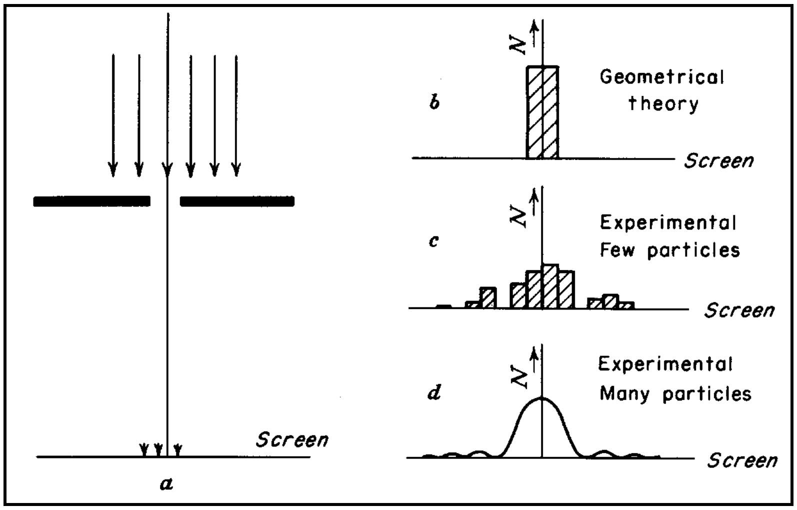


Fig. I.4 The statistical nature of diffraction patterns. (From Hall, p.13)

QUANTUM THEORY (Planck and Einstein) provides the basis for explaining the phenomena of **INTERFERENCE, DIFFRACTION** and the **PHOTOELECTRIC** effect (light falling on certain metals cause them to emit electrons) and thus forms a common basis for explaining the nature of action of light. The **transfer of energy between light and matter occurs only in discrete quantities proportional to the frequency of the light wave**.

$E = h\vartheta$

where E = energy of photon (joules)
h = Planck's constant (6.624×10^{-34} joule-sec)
ϑ = frequency (cycles/sec)

"The precise nature of 'electron waves' or 'matter waves' is very difficult to understand or describe in material terms. Electron waves are not electromagnetic radiation of the kind to which light, X-rays and radio waves belong. They constitute a sort of quantum or 'packet' of radiation which accompanies each individual electron, following its path and not radiating outwards from it." (Meek, 1976, pp. 48-49).

b. Electron velocity and wavelength

The relation between the wavelength (λ) of a particle of mass, m, moving at a velocity, v, is given by the DeBroglie wave equation:

$$\lambda = \frac{h}{mv} \qquad (1)$$

An electron of charge e(1.6×10^{-19} coulomb), and mass m (9.11×10^{-28} gm), when passing through a potential difference of V volts (expressed in joules/coulomb), has a kinetic energy:

$$\tfrac{1}{2} mv^2 = eV \qquad (2)$$

Solving for velocity:

$$v = \sqrt{\frac{2eV}{m}} \tag{3}$$

substituting into the DeBroglie equation (1):

$$\lambda = \left(\frac{h}{m}\right)\frac{1}{\sqrt{\frac{2eV}{m}}} = \sqrt{\frac{h^2}{2meV}} \tag{4}$$

Since 1 joule = 10^7 dyne·cm = 10^7 cm^2·gm/sec^2

$$\lambda = \sqrt{\frac{150}{V}}\ 10^{-8} cm = \frac{1.23}{\sqrt{V}} nm \tag{5}$$

Thus, for example, if V = 60,000 volts, λ = 0.005 nm.

From equation (3) the electron velocity can be calculated if the accelerating voltage, *V,* is known:

$$v = 0.593 \times 10^8 \sqrt{V} cm / \sec \tag{6}$$

The following table illustrates that, at high voltage, electron velocity is comparable to the speed of light in a vacuum (c = 3 x 10^{10} cm/sec).

V	λ (nm)	*v* (x10^{-10} cm/sec)	*v/c*
10,000	0.0123	0.593	0.198
50,000	0.0055	1.326	0.442
100,000	0.0039	1.875	0.625
1,000,000	0.0012	5.930	**1.977!**

The equation breaks down when the velocity of the electron approaches the speed of light because a relativistic correction must be made for the value of the mass where:

$$m_1 = \frac{m_0}{\sqrt{1 - \frac{v^2}{c^2}}} \tag{7}$$

The relation between λ and *V* is more correctly given by: (see pp.33-34 of Hall (1966))

$$\lambda = \frac{1.23}{\sqrt{V + 10^{-6} V^2}} nm \tag{8}$$

The following table is obtained when relativity effects are included:

V	λ (nm)	*v* (x10^{-10} cm/sec)	*v/c*
10,000	0.0122	0.585	0.195
50,000	0.0054	1.237	0.414
100,000	0.0037	1.644	0.548
1,000,000	0.0009	2.822	**0.941**

c. Interference/diffraction/coherence

An **ideal** lens system obtains an exact image of an object (each point faithfully reproduced). The phenomena of diffraction makes this unattainable (Figs. I.5, I.6).

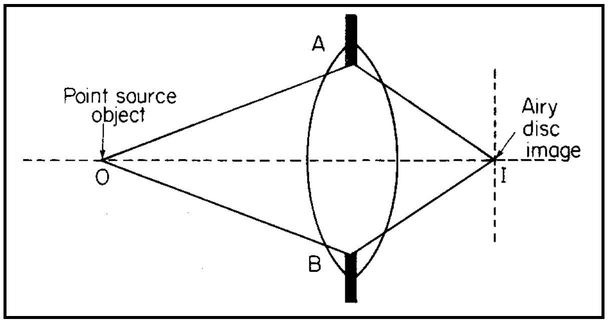


Fig I.5 A perfect point source can not be imaged by a lens as a perfect point image due to the presence of the aperture AB (which may be the edge of the lens). Diffraction at this aperture gives rise to a series of fringes, which surround the image formed of the point source. The pattern produced is called an 'Airy' disk. (From Meek 1st ed., p.35)

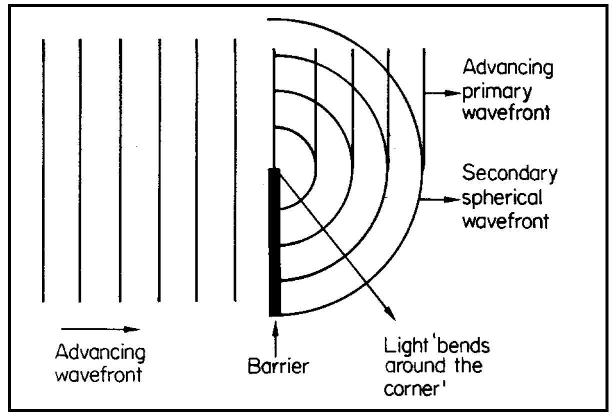


Fig. I.6 Whenever a wavefront strikes a barrier, it can bend around the corner' by giving rise to a secondary wavefront at the edge, since each point on the wavefront can give rise to a new source of waves. This phenomenon is called 'diffraction'. (From Meek 1st ed., p.22)

Diffraction phenomena involves the bending of the path of radiation passing close to an obstacle (Fig. I.6). This results in a spreading of the radiation into the region behind the obstruction that the waves passed. The diffraction at edges contributes to the contrast at which an edge can be observed. Diffraction also limits the resolving power of the microscope since the image point produced by a lens is a diffraction image of the opening of the lens or the aperture restricting the effective opening of the lens (Fig. I.5).

If the light source and the plane at which the diffraction pattern is observed are at finite distances from the edge, the phenomenon is called **Fresnel diffraction**. The pattern is described as due to **interference** between the non-diffracted light and a wave of light diffracted at the edge. The resulting superposition gives rise to a series of diffraction fringes oriented parallel to the edge and representing periodically varying brightness, maxima and minima. (Figs. I.7,1.8,I.9).

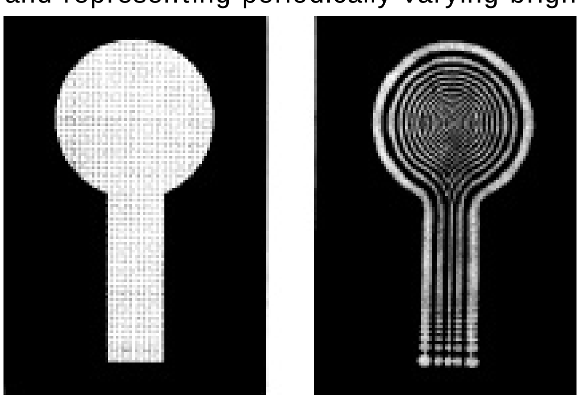


Fig. I.7 Diffraction pattern (right) formed by an irregularly shaped aperture (left). (From Young, p.95)

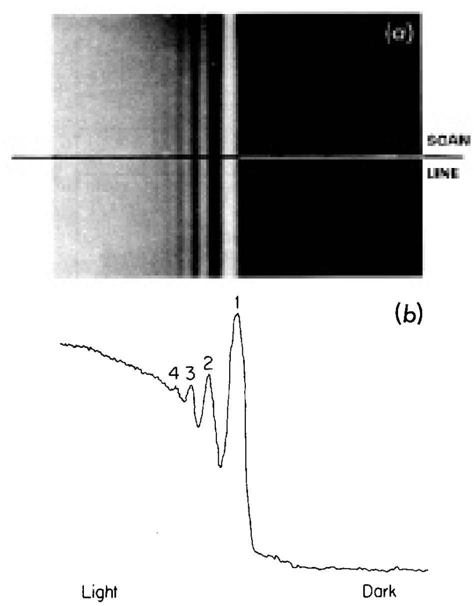
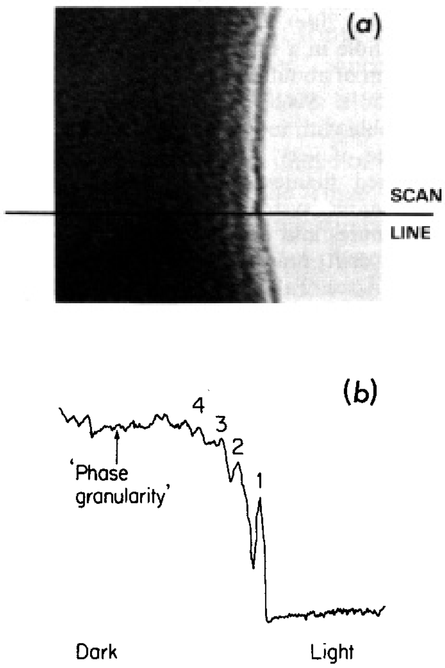


Fig. I.8 (a) Photograph of the edge of a razor blade illuminated by monochromatic (blue) light rendered coherent by passing through a narrow slit. (b) Microdensitometer tracing of the negative from which the photograph was made. Four Fresnel fringes can be distinguished. (From Meek 1st ed., p.27)



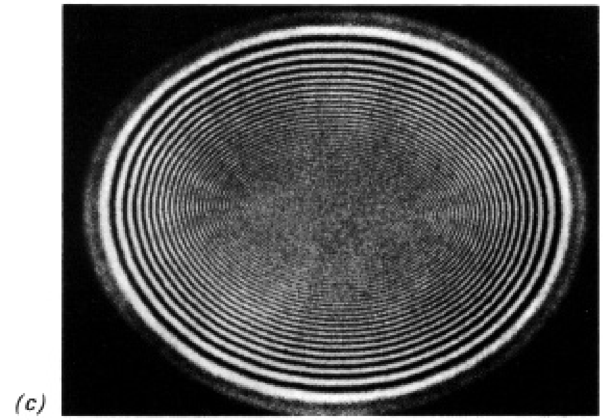


Fig. I.9 (a) Fresnel fringes formed by electrons. These fringes are formed outside the edge of a hole (white) in a carbon film (black).
(b) A microdensitometer tracing of the fringe system; the pattern is identical with the Fresnel fringe system formed by visible light (see Fig. I.8).
(c) An underfocused image of a hole in a film, showing a complete system of about 40 Fresnel fringes inside the hole. (From Meek 1st ed., pp.29-30)

Coherence: A prerequisite for interference is a superposition of wave systems whose **phase difference remains constant in time**. Two beams are **coherent** if, when combined, they produce an interference pattern. The beams are **incoherent** when they are incapable of producing an interference pattern. Two beams of light from self luminous sources are incoherent. If light from the two sources falls on a screen, the resultant intensity is simply the sum of the two intensities which would occur from each source separately (Figs. I.10,I.11). In practice an emitting source has finite extent and each point of the source can be considered to generate light. Each source gives rise to a system of Fresnel fringes at the edge. The superposition of these fringe systems is fairly good for the first maxima and minima but farther away from the edge shadow the overlap of the fringe patterns becomes sufficiently random to make the fringes disappear. The nature of waves, phase, amplitude, and interference are illustrated in Figs. I.12 and I.13.

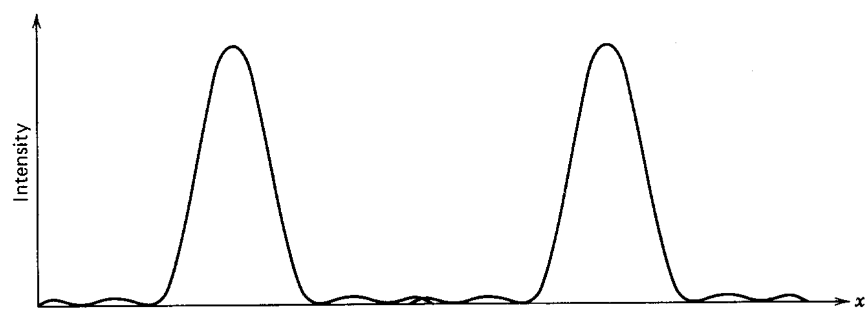


Fig. I.10 Diffraction images of two easily-resolved points. (From Slayter, p.243)

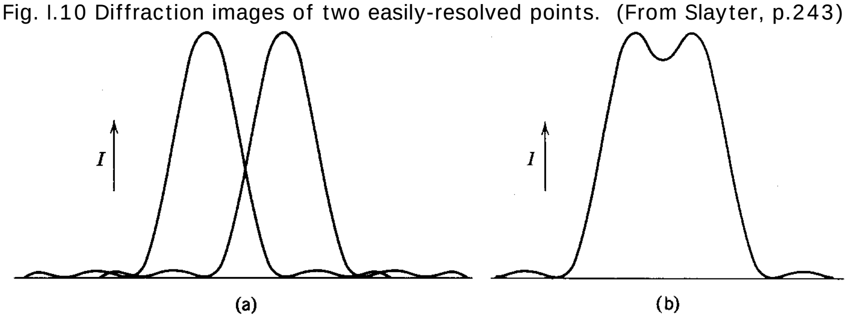


Fig. I.11 Images of two incoherently illuminated points at the limit of resolution.
(a) individual intensities; (b) summed intensities. (From Slayter, p.244)

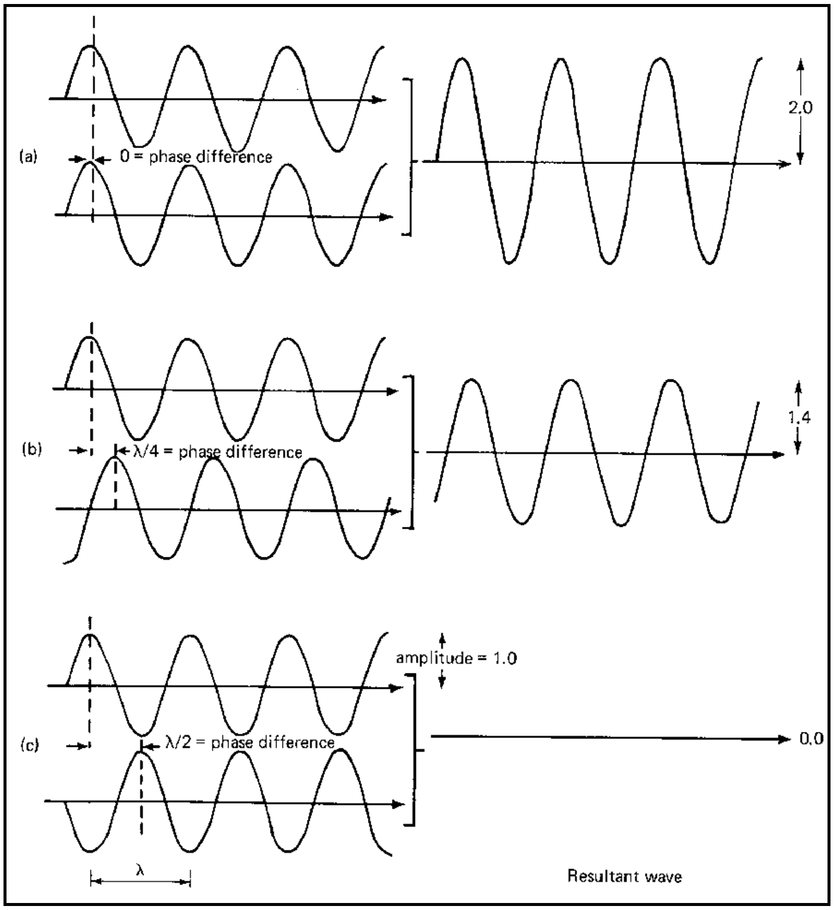


Fig. I.12 Examples of what occurs when two waves of the same wavelength and equal amplitude add. In each example, the two separate waves are shown on the left and their sum or resultant wave on the right. The different examples are characterized by varying phase differences. The phase of a wave (usually expressed as a fraction of the wavelength or in degrees) is the position of a crest relative to some arbitrary point. Although the phase of a given wave varies with time as the wave travels, the difference in phase of the two waves of the same wavelength with the same velocity, is independent of time. Such waves can interfere with one another. The resultant wave has the same wavelength, λ.

(a) Phase difference zero. The waves totally reinforce and are said to be "in phase" or to show constructive interference.

(b) Phase difference λ/4. Partial reinforcement, giving a wave of amplitude 1.4 (intensity 2.0).

(c) Phase difference λ/2. The waves are "out of phase" and there is total destructive interference to give no resultant wave (or a wave with amplitude 0 (intensity 0). (From Glusker and Trueblood, p.19)

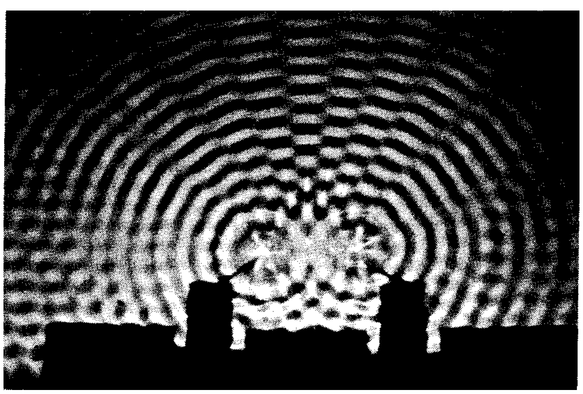


Fig. I.13 Photograph of an interference pattern in water waves formed by the superposition of waves from two sources oscillating in phase with the same frequency. (From Young, p.22)

d. Resolution

1) Definitions:

RESOLUTION: ability to distinguish closely spaced points as separate points.

RESOLUTION LIMIT: smallest separation of points which can be recognized as distinct.

RESOLVING POWER: resolution achieved by a particular instrument under optimum viewing conditions.

2) Distinction between resolution and resolving power:

Note the distinction between **resolution** and **resolving power**. **Resolving power** is a **property of the instrument** and is a quantity that may be estimated on theoretical grounds. **Resolution** is **equal to or poorer than the resolving power** and is the quantity observed under any given set of experimental conditions. In the TEM, especially with biological samples, the resolution achieved may be considerably inferior to the theoretical resolving power of the instrument.

Microscopy is the science of seeing the very small. Under ideal conditions, the eye resolves about 1 minute of arc (= 1/60 degree = 2.9×10^{-4} radian; recall there are 2π radians in 360°) and since it can focus down to about 250mm, the smallest object we can resolve is about 0.07mm (70µm) (Fig. I.14). This limit is related to the size of the receptors in the retina of the eye. The function of a microscope is to magnify the image falling on the retina (Fig. I.15). The advantage of light and electron microscopes is that they effectively get the object closer to the eye so a magnified image is obtained and more detail can be discerned.

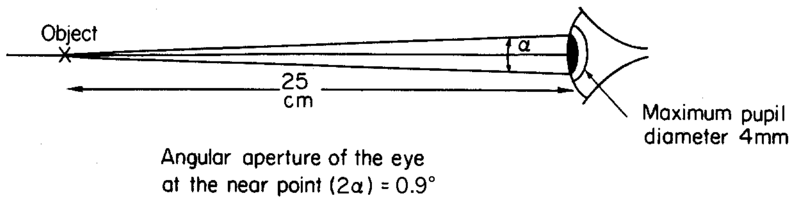


Fig. I.14 The angular aperture of the eye is relatively small. (From Meek, 1st ed., p. 13)

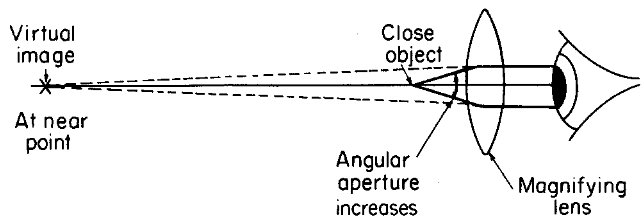


Fig. I.15 Increasing the angular aperture of the eye with a lens. The lens allows the object to be held closer to the eye, which is thereby enabled to gather more information. (From Meek 1st ed., p.14)

TENNIS BALL ANALOGY: Eye can resolve 3 cm at 100 meters, thus a tennis ball is clearly visible. But if the tennis ball is held up against a white background, the visibility decreases (because of the decrease in contrast).

3) Abbe simple criteria of resolution:

The fundamental nature of light poses limits on the detail that can be resolved (Fig. I.16). In 1893 Ernst Abbe (1840-1905) showed that the smallest resolvable distance is about 1/2 the wavelength of light used. Thus, 1/2 the wavelength of the radiation used is the ultimate resolving power of any instrument. This limits the usable magnification of optical microscopes to <1000X. At first it was thought that x-ray microscopes would be useful but the refractive index of substances for x-rays is nearly = 1, thus refracting lenses cannot be made for x-rays, and consequently, x-rays cannot be easily focused to form images.

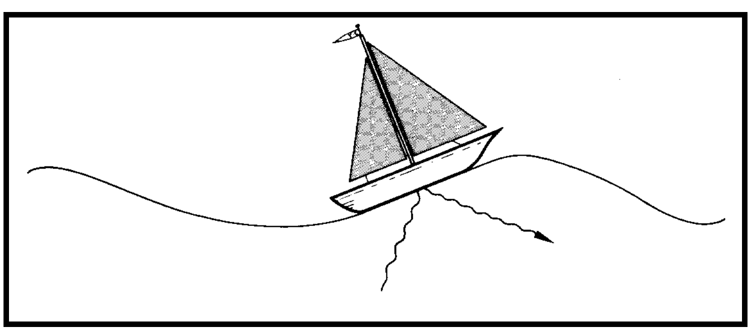


Fig. I.16 The interaction of waves with an obstacle. The boat rides the long wavelength ocean wave, but reflects the small wavelength surface ripple. An observer who wishes to detect the presence of the boat can do so only by observing waves which have wavelengths smaller than, or comparable to, the length of the boat. (From Sherwood, p.19)

4) Magnification limits:

The maximum magnification of an instrument is limited according to the following relationship:

$$\text{Maximum magnification} = \frac{\text{resolving power of the eye}}{\text{resolving power of the microscope}}$$

Thus, for the LM, with a resolving power of approximately 0.25µm, the maximum (useful) magnification is about 250µm/0.25µm = 1000X. The value used for the resolving power of the eye in this example (250µm) represents a more realistic viewing condition. Any magnification above the value given by the above formula represents **empty magnification**, since such magnification leads to no more useful information but rather a magnified blur.

According to Abbe's simple criteria of resolution, at 60,000 volts, the TEM should have a resolving power of about 0.0025 nm. This should allow a maximum useful magnification of about 100 million times!!! In practice, the maximum useful magnification of the TEM operated at 60 kV is limited to much less than 1,000,000X. Thus, although the LM nearly obeys the Abbe criteria, the TEM falls short by a considerable amount. The main limiting factor in the TEM, with respect to achieving the theoretical resolving power of the instrument, concerns the nature of the imaging lenses and the process of image formation.

5) Rayleigh criteria (practical but arbitrary): (Fig. I.17).

It is the ability of microscopes to make object points which are close together appear in the image as separate points. An **ideal lens** takes each object point and represents it exactly as a point in the image. A **real lens** takes each object point and spreads it out into a circular disk (**Airy disk**) in the image plane whose diameter depends on the angular aperture of the lens.

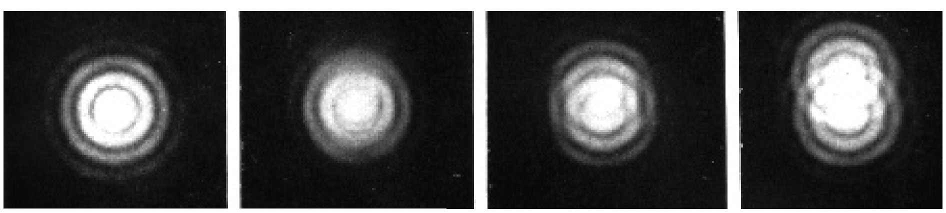
The shortest distance between 2 disks at which the two disks appear partially separated corresponds to about 1/2 the width of the disks. The distance, *d*, in object space is given by:

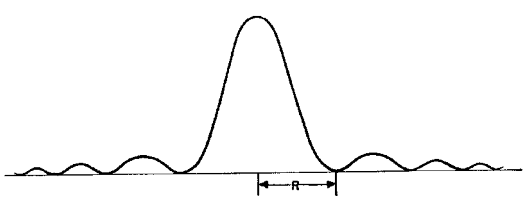
$$d = \frac{0.612\lambda}{n \sin \alpha}$$ (Abbe's Equation)

where λ = wavelength of the radiation
n = refractive index of the media
α = semi-angular aperture of the lens

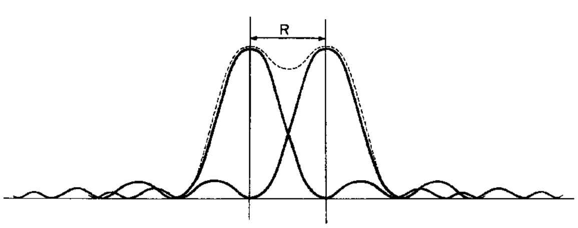
Note: $n \sin\alpha$ = the numerical aperture (N.A.) of the lens.

Thus, to maximize resolving power, λ must be decreased, n increased, or α increased. Recall that we are concerned at the moment with an **aberration free** optical system. The value for the constant (0.612) is controversial because it depends on the coherence of the radiation and a criteria of visibility.





(b)



(c)

Fig. I.17 (a) Two Airy disks representing two image points shown at increasing separation from left to right. In the picture at the extreme right the two disks can be distinguished as separate disks.

(b) The intensity distribution at an Airy disk. R, half width of the central maximum represented by a bell-shaped curve.

(c) The Rayleigh criteria for resolution. (From Sjostrand, p.115)

For the LM, using oil immersion optics (n = 1.5), sinα = 0.87, and violet light (λ = 400 nm), d = 0.2μm. The only way to improve the resolution is to use light of shorter wavelength since N.A. can not be increased beyond ~1.5. For the **TEM**, n = 1 (vacuum), sinα = 10^{-2} and λ = 0.005 nm for 60kV electrons, thus d = 0.3 nm.

I.A.4. Optics (Lens Theory)

a. Basic laws of classical geometrical optics

1) Rectilinear propagation of light when n (refractive index) is constant.

Recall that: $n = \frac{c}{v}$ where c = speed of light in a vacuum

v = speed of light in the medium

2) Law of reflection:

$i = r$

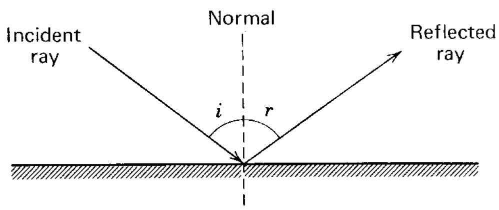


Fig. I.18 Reflection. (From Slayter, p.4)

3) Law of refraction (Snell's Law):

$$\frac{\sin(i)}{\sin(r)} = \frac{n_2}{n_1}$$

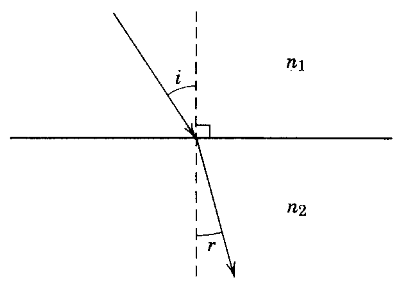


Fig. I.19 Refraction. (From Slayter, p.6)

4) Independence of rays. The assumption is made that light rays travel independently through space.

These laws hold for electrons, except #4, if the current density is too high when negative charged electrons can interfere.

b. Classical vs. electron optics

1) Classical optics: The refractive index changes abruptly at a surface and is constant between the surfaces. The refraction of light at surfaces separating media of different refractive indices makes it possible to construct imaging lenses. Glass surfaces can be shaped.

2) Electron optics: Here, changes in the refractive index are gradual so rays are continuous curves rather than broken straight lines. Refraction of electrons must be accomplished by fields in space around charged electrodes or solenoids, and these fields can assume only certain distributions consistent with field theory.

c. Geometrical and physical optics

The fundamental principles of optics govern the design and operation of both the light and electron microscopes. The basic optical principles involving the use of refractile elements or lenses in order to form magnified images are identical in both the LM and TEM. The TEM differs from the LM only in the radiation it uses and in the way in which the radiation is bent or refracted.

Geometrical optics deals with the study of the paths followed by 'rays' of light or electrons through lenses and apertures, and the geometrical constructions used to find the relative positions and sizes of objects and their images. A ray of light or electrons is defined as an infinitely thin pencil or beam. **Physical optics** shows that this an abstraction and cannot physically exist because of 'diffraction' which deals with the wave nature of light and electrons. All results obtained in geometrical optics can be derived from the principles of physical optics, along with other phenomena such as interference and diffraction which are not explicable in simple geometrical terms.

d. Ideal verses real lenses:

Lenses are used to bend rays of light or electrons so they are deflected in a predictable way from their original paths. The properties of an ideal lens, possessing an axis of rotational symmetry are:

1) Each ray of the bundle of rays which passes from an object point will be refracted by the ideal lens to meet in one image point.

2) Rays originating from points which lie on a plane perpendicular to the axis, must be imaged in a plane which is also perpendicular to the axis.

3) The image appears like the object irrespective of the magnification, so the relative linear dimensions of the object are preserved in the image.

In practice, the imaging by any real lens does not correspond to that of the ideal lens owing to the fact that an object point is represented by a diffraction image (**Airy disc**) of the lens opening or the aperture used for restricting the effective opening of the lens. This is a result of the wave-like properties of light. **Lens aberrations** also contribute to more or less pronounced deviations from the properties of the ideal lens.

The single refracting surface of spherical curvature is the fundamental unit of focusing action by glass lenses**.** Spherical refracting surfaces act as lenses for paraxial rays which are those rays that pass close to the principal axis of the lens. Rays with large angles will **NOT** obey ideal lens action.

A fundamental difference between light and magnetic lenses is that the electron beam does not change in forward velocity as it passes through the magnetic field (light rays slow down when passing into a medium of higher refractive index). Refraction is continuous with electrons when they are in the magnetic field: light is refracted only at the interface between media of differing refractive index. The electrons also follow spiral trajectories through the magnetic field (see also Sec. I.A.5.c).

e. Ray diagrams: (Figs. I.20-I.28)

The method of construction of ray diagrams is based on three simple principles:

1) All rays entering the lens parallel to the axis are brought to a common point on the axis, the focal point.

2) All rays passing through the geometrical center of the lens are undeviated and pass straight on, no matter from which direction they come.

3) Principle of reversibility: if the direction of a ray is reversed in any system the ray exactly retraces its path through the system. This applies only to the location of light paths and not to the intensity of the light.

The above principles are based on the assumptions that we are dealing with a thin lens and concerned with the paths of paraxial rays. The standard convention is to draw diagrams with rays that travel from left to right. The object is to the left (in front) of the lens and the image is to the right (behind) of the lens.

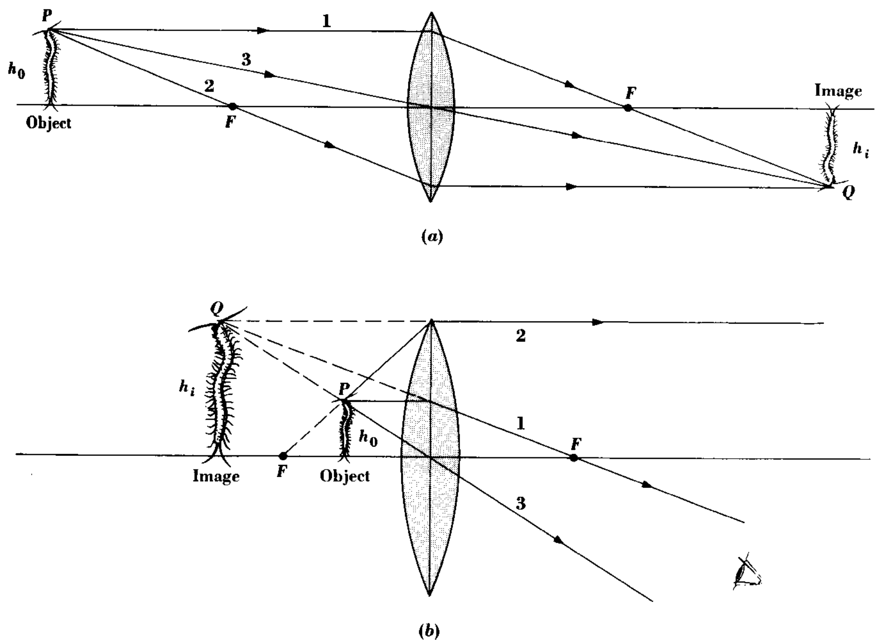


Fig. I.20 Principal ray diagrams showing image formation by a convex lens.

(a) When the object distance is greater than the focal length, a real, inverted image is formed.

(b) When the object distance is less than the focal length, a virtual, erect image is formed; its position is obtained by projecting the principal rays backward. The rays appear to come from point Q. (From Young, p.127)

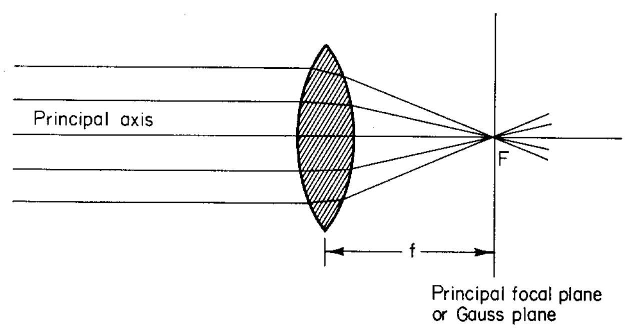


Fig. I.21 Definition of principal focus, F, in image space of a lens. (From Sjostrand, p.20)

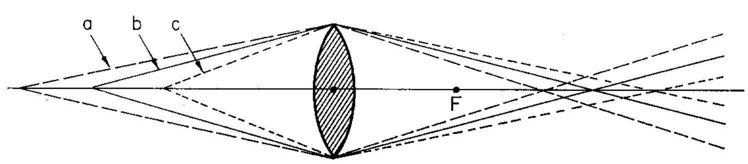


Fig. I.22 Focusing effect of lens on rays originating from points on principal axis located at different distances from the lens. (From Sjostrand, p.21)

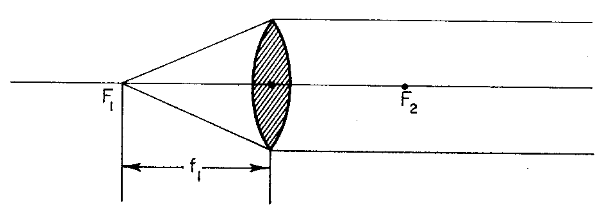


Fig. I.23 Definition of principal focus in object space F_1 of a lens. (From Sjostrand, p.21)

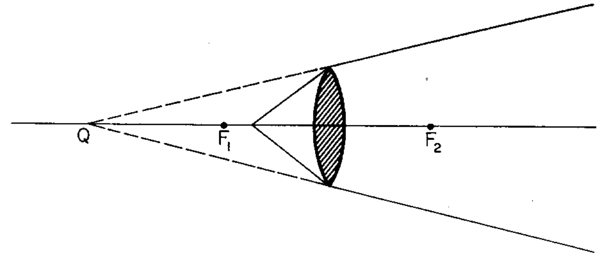


Fig. I.24 Virtual image Q of object point located between principal focus in object space and the lens. (From Sjostrand, p.21)

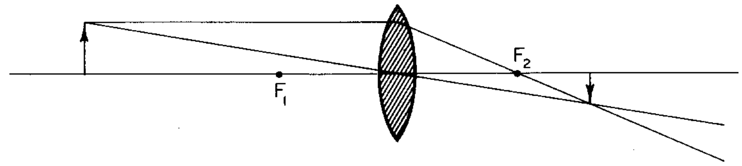


Fig. I.25 Construction of the image of an object by means of ray tracing. (From Sjostrand, p.22)

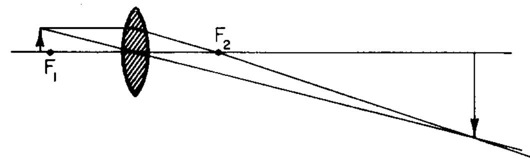


Fig. I.26 Magnifying effect of a positive lens. (From Sjostrand, p.22)

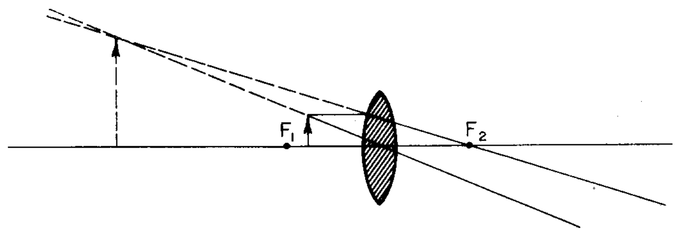


Fig. I.27 Virtual magnified image of object located between principal focus in object space and the lens. (From Sjostrand, p.22)

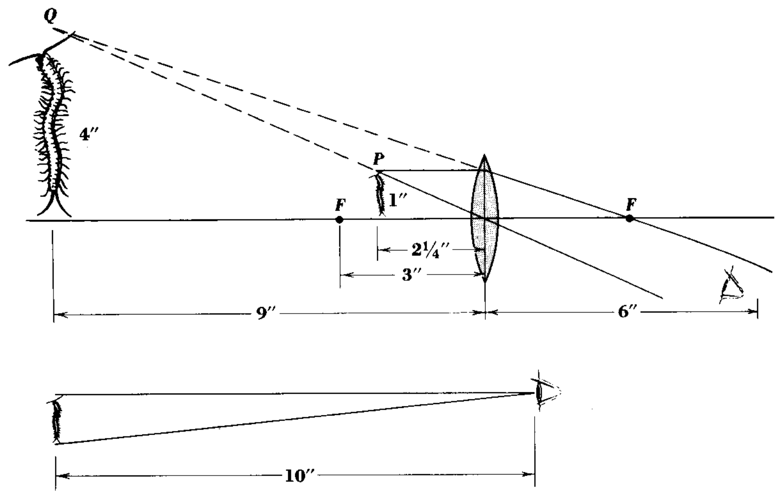


Fig. I.28 A magnifying glass forms an enlarged, erect virtual image. The angular size of this image is approximately 4"/15" or 4/15. The angular size of the object at the closest distance for comfortable viewing is 1"/10" or 1/10. The magnification in this situation is (4/15)/(1/10), or $2^{2}/_{3}$. (From Young, p.130)

f. Definitions:

Real image: one at which light rays physically reunite, so that a photographic plate placed at the position of a real image is exposed.

Virtual Image: one from which light rays appear to diverge; rays are not in fact concentrated at the position of a virtual image, so that a photographic plate placed at the position of the image is not exposed (by focused rays). Placing an optical system such as the eye behind the lens, will enable the divergent rays to be focused to form a real image. The intermediate lens of an TEM is sometimes used this way in order to reduce the final size of the real image formed by the projector lens(es).

Converging (positive) lens: bends rays toward the axis. It has a positive focal length. Forms a real inverted image of an object placed to the left of the first focal point and an erect virtual image of an object placed between the first focal point and the lens.

Diverging (negative) lens: bends the light rays away from the axis. It has a negative focal length. An object placed anywhere to the left of a diverging lens results in an erect virtual image. It is not possible to construct a negative magnetic lens although negative electrostatic lenses can be made.

g. Lens formula (thin lens equation): $1/f = (1/o) + (1/i)$

where f = focal length of the thin lens (same radius of curvature for both spherical surfaces)
o = distance of object from lens (positive to the left)
i = distance of image from the lens (positive to the right)

NOTE: For a virtual image, i has a negative value.

h. Magnification: $M = |i/o|$

For a converging lens, if the object is more than twice the focal length from the lens, then the image formed is **real, inverted,** and **smaller** in size than the object ($M < 1$). When the object is at a distance = $2f$, the image and object are the same size ($M = 1$); when it is between f and $2f$, the image is larger than the object ($M > 1$), and when it is $< f$, the image is **virtual, erect,** and **larger** than the object ($M > 1$).

i. Angular aperture of the lens (2α) (Fig. I.29)

The aperture determines the total amount of radiation arriving from the object which can be focused to form an image. The aperture thus controls the ability of the lens to gather information about the object. This depends on the angle of the cone of rays it is able to accept from the object. Bringing an object closer to the eye increases the angular aperture, but there is a limit to the closeness that the object can be brought to the eye (~25 cm corresponding to an angle of about 0.9° for a 4 mm exit pupil diameter of the eye lens; a typical LM with an oil immersion objective lens has 2α of ~175°).

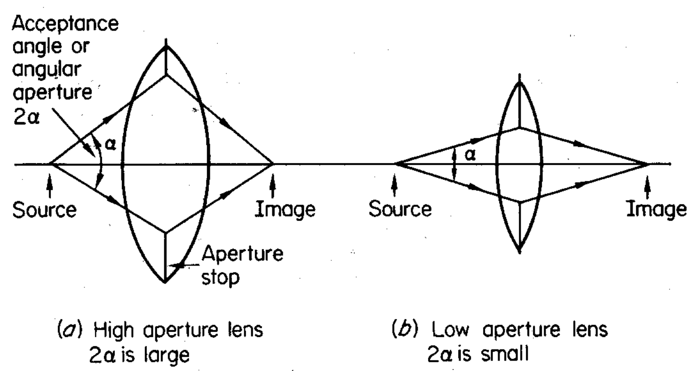


Fig. I.29 The angular aperture of a lens. The angle 2α is the acceptance angle of the lens, and the larger it can be made, the more information the lens can transmit. A large lens of high aperture can therefore tell us more about an object than a small lens of low power. (From Meek 1st ed., p.12)

j. Simple vs. compound microscope (Figs. I.30-I.32)

In principle, a real image of any desired magnification can be obtained from a single positive lens, but in practice this is cumbersome because of the long lens-image distance. One or more lenses can be used to magnify the image in stages (total magnification equaling the product of the magnifications of each lens). The image formed by one lens constitutes the object for the subsequent lens, whether or not a real intermediate image is formed.

Comparison of one verses two-stage magnification:

The following description illustrates how different path lengths are required to achieve a magnification of 10,000X using either one or two lenses with f = 2.0 cm.

One-Stage System:

$M = 10{,}000 = x_i / x_o$

recall that $1/f = (1/x_o) + (1/x_i)$, thus, $1/2 = (1/x_o) + (1/10{,}000\,x_o)$

$x_o = 2.0002$ cm
$x_i = 20002$ cm (= 200.02 meters !)

Two-Stage System:

Assume both lenses have f = 2.0 cm and are arranged so each gives 100X magnification. Thus, $M = 100 = x_i / x_o$ for **each** stage.

First Stage: $\frac{1}{2} = \left(\frac{1}{x_o}\right) + \left(\frac{1}{100x_o}\right)$

x_o = 2.02 cm

x_i = 202.00 cm

The real image formed in the first stage becomes the object for the second lens.

Second Stage: x_o, x_i same as in the first stage.

Total length of system = length of first stage + length of second stage

$= (x_o + x_i) + (x_o + x_i)$

$= 2(x_o + x_i)$

$= 2(2.02 + 202.00) = 408.04$ cm (4.08 meters)

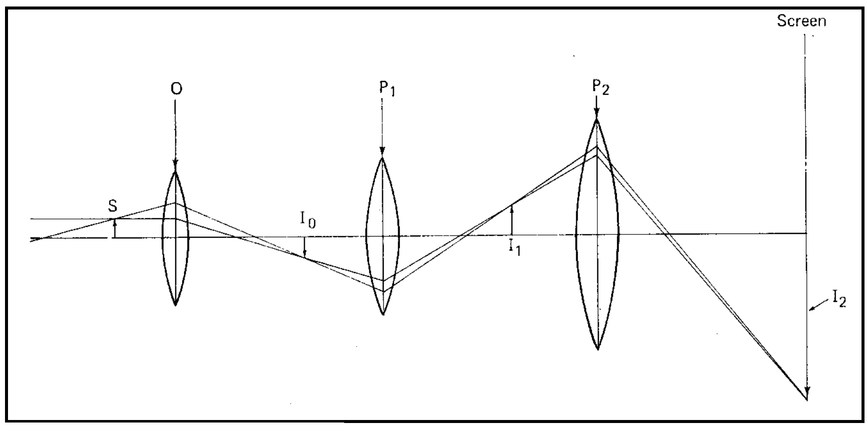


Fig. I.30 Ray diagram for high magnification mode of operation. Note that each lens forms a real image, with image inversion. I_0 is the image formed by the objective lens O, I_1 is formed by the first projector lens P_1 and I_2 by the second projector P_2, on the screen. (From Agar, p.30)

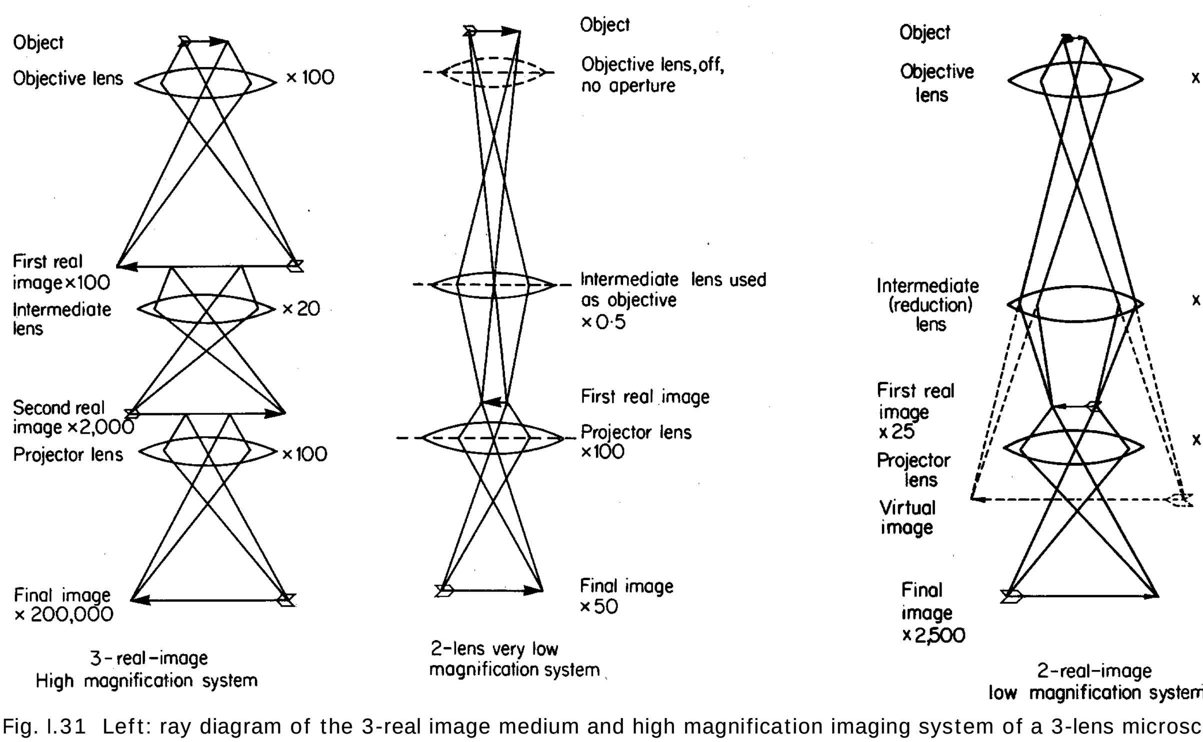


Fig. I.31 Left: ray diagram of the 3-real image medium and high magnification imaging system of a 3-lens microscope. Center: 2-real-image very low or 'scan' magnification range of a 3-lens microscope. Right: 2-real-image low magnification system of a 3-lens microscope. (From Meek 1st ed., pp.118,120-121)

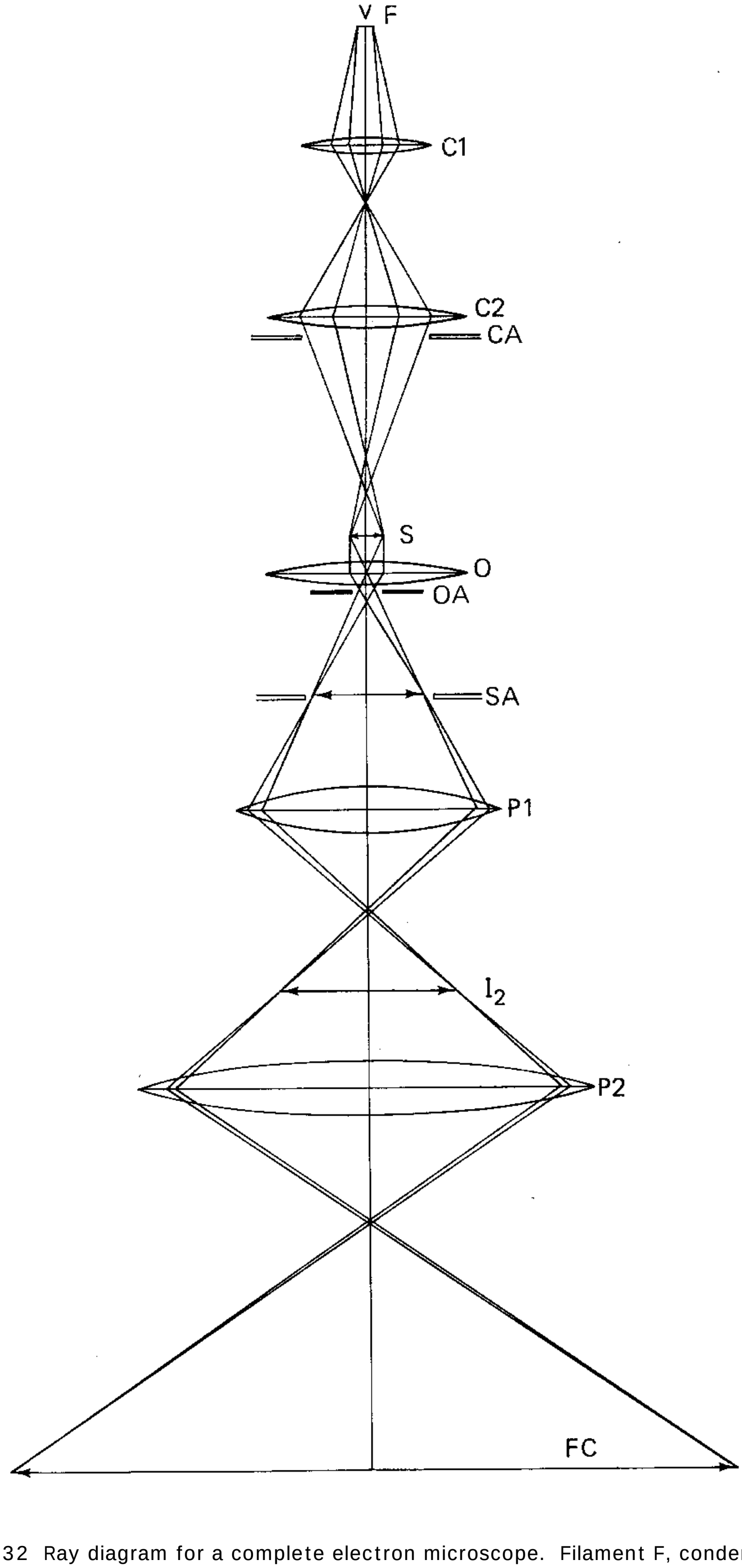


Fig. I.32 Ray diagram for a complete electron microscope. Filament F, condenser 1 lens C1, condenser 2 lens C2, condenser aperture CA, specimen S, objective lens O, objective aperture OA (in back focal plane). 1st intermediate image and selector aperture SA. Intermediate lens P1, second intermediate image I_2, projector lens P2 and final image on the fluorescent screen FC. (From Agar, p.35)

k. Problem set

The following problems are for your own edification to test how well you understand the basics of lens optics.

1. For each situation below, draw an accurate (use graph paper) ray diagram showing the path of (at least two) rays from the object to image. Indicate whether the image is **real** or **virtual** and specify the distance of the image from the lens and the magnification of the image with respect to the object. In each example, the lens is **converging**, with identical front and back focal points. Distances are in arbitrary units. Let f = lens focal length, x_o = distance of object in front of the lens, and x_i = distance of image behind the lens. The object may be any size.

a)	f = 2.0	x_o = 5.0	e)	f = 4.0	x_o = 3.0
b)	f = 10.5	x_o = 21.0	f)	f = 13.3	x_o = 13.3
c)	f = 3.5	x_o = 3.0	g)	f = 3.142	x_o = 0.0
d)	f = 5.0	x_o = 2.5			

2. In the following examples, there are **two converging lenses in succession**. Draw ray diagrams showing the formation of **both** the intermediate and final images and give appropriate information about the nature (**real/virtual**), positions, and sizes of the intermediate and final images. What is the magnification at each stage of image formation and what is the magnification of the final image? The distance from the center of lens 1 (**L1**) to the center of lens 2 (**L2**) equals **D**.

a) D = 8.0	L1: f = 2.0	x_o = 4.0
	L2: f = 3.0	
b) D = 8.0	L1: f = 2.0	x_o = 6.0
	L2: f = 3.0	
c) D = 4.0	L1: f = 2.0	x_o = 3.0
	L2: f = 2.0	

I.A.5. Electron Optics/Electron Lenses

a. Electron emission

Inner shell electrons in metal atoms shield the electric field of the nucleus through the repulsive forces they exert on the outer shell (valence) electrons. Thus the attraction between the valence electrons and the nucleus is reduced. Metal atoms are characterized as having two loosely-bound valence electrons which migrate freely (this is why metals are good electrical conductors) and can escape from the metal completely if sufficient additional energy is imparted to them. As the temperature of a metal is increased, the kinetic energy of the electrons increases because of increased thermal vibrations of the metal ions, which collide more frequently with the electrons. **Thermionic emission** is the term used to describe the process by which thermal energy is supplied to loosely bound electrons in order to form a source of electrons.

At room temperature electrons are effectively prevented from escaping the surface of the metal owing to the attractive force of the positively charged ions. As the temperature is increased some electrons acquire sufficient energy to overcome the attraction and leave the metal temporarily. Metal, shaped as a thin wire, can easily be heated by passing an electric current through it. Since the metal surface becomes positively charged, a certain level of energy (work function) must be supplied to allow electrons to escape from the surface. Each metal has a characteristic work function. Tungsten, with a low work function, emits more electrons than metals with higher work functions (see also pp.29-30 and Fig. I.59).

If a strong electrostatic field is applied in a vacuum between the wire (given a negative, **cathode**, potential) and an **anode,** the electric field will cause electrons to accelerate away from the wire towards the anode surface (Fig. I.33). The **speed** of the electrons depends on the strength of the electrostatic field (voltage) between the cathode and anode (equation (3), **Sec. I.A.3.b**). The **number** of electrons which leave the wire depends on the **temperature** to which the wire is heated, which depends on how much **filament current** passes through the wire.

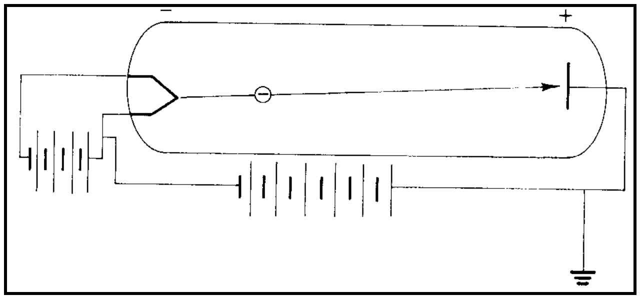


Fig I.33 Acceleration of electron in electric field. (From Sjostrand, p.26)

A "V" shaped wire will have the highest temperature at the tip. Electrons withdrawn from the filament tip carry electric charges to the anode. This electric current, which flows between the filament and the anode, is called the **beam current**.

1) Electric field / Equipotentials

An electrically-charged object has associated with it an electric field. Thus, an electrically-charged particle, when brought near a charged object, is influenced by an electrical force in the vicinity of the object. The force is directed toward the charged object if the charges are of opposite signs and away from the object if they are of similar sign. **DEFINITION:** The <u>direction of an electric field is defined as the direction of the force acting on a positive charge</u>**.** (Figs. I.34-35)

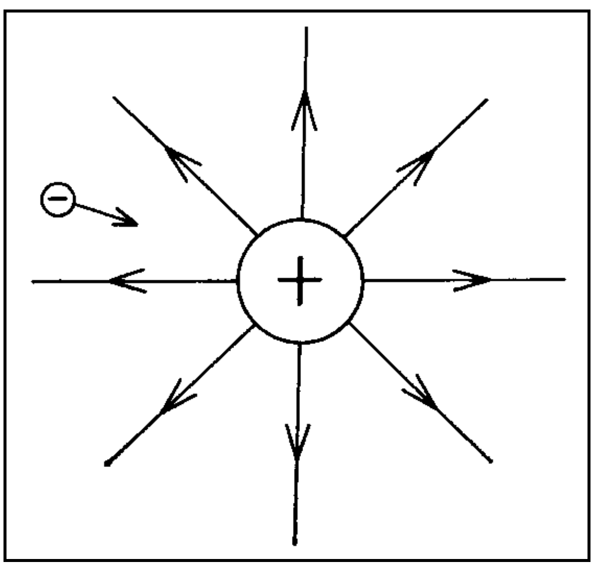


Fig. I.34 Lines of force at a positively charged spherical body. (From Sjostrand, p.32)

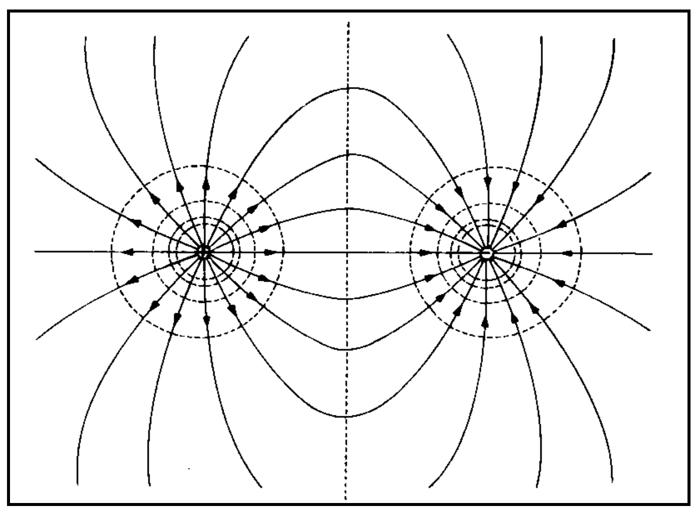


Fig. I.35 Lines of force and equipotential surfaces (stippled lines) associated with two equal charges of opposite sign. (From Sjostrand, p.32)

Along the lines of force connecting the two charges, the electric potential will change gradually between the extreme values represented by the two charges. **DEFINITION:** <u>Equipotential lines define the points along the lines of force with identical electrical potential.</u> These equipotential surfaces are always oriented perpendicular to the lines of force. The changes in the electric potential are gradual in space.

Electrons which enter a field between two parallel plates in a direction parallel to the plates are affected by the force directed **perpendicular** to the plates (Fig I.36). The electrons will be attracted toward the positive plate. The path changes in a series of gradual steps at the equipotential surfaces.

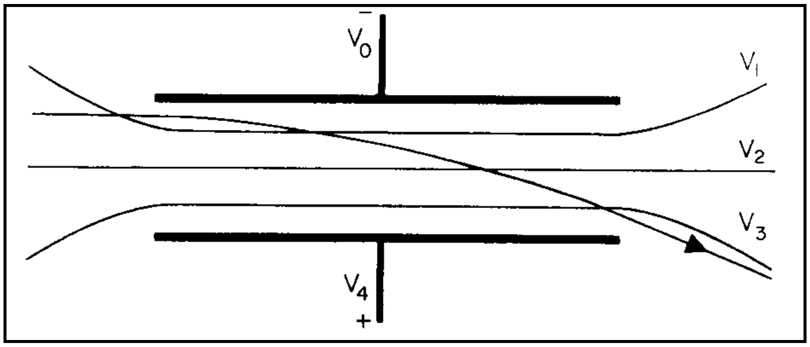


Fig. I.36 Equipotential surfaces at two parallel plates of opposite charges with the path of an electron indicated within the homogeneous part of the field. (From Sjostrand, p.33)

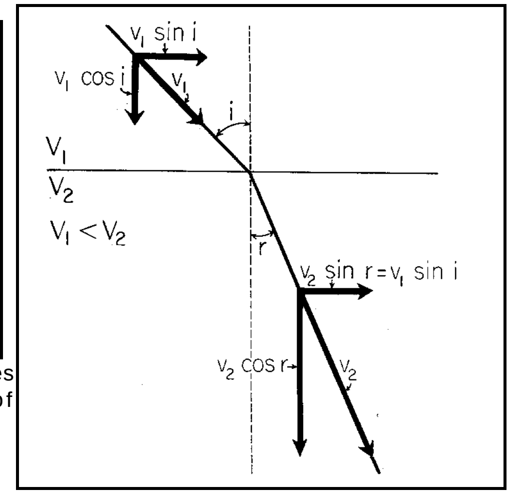


Fig. I.37 Refraction of electron at an equipotential surface. (From Sjostrand, p.33)

Figure I.37 shows how the electron path is "refracted" at the equipotential surface. The result is fundamentally the same as that given by Snell's Law of refraction in light optics. A consequence of this is that a spherically curved equipotential surface exhibits the properties of a lens**.**

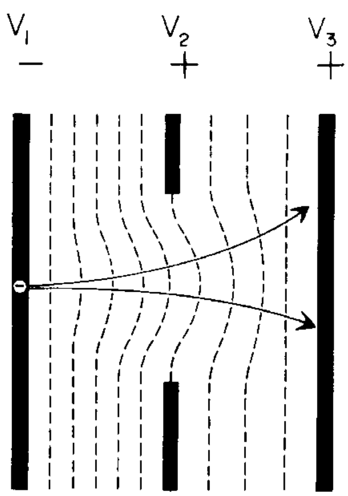


Fig. I.38. Negative lens action of electrostatic field at an aperture when $V_2 - V_1 > V_3 - V_2$. (From Sjostrand, p.34)

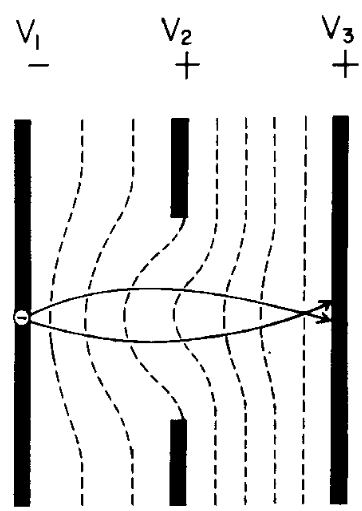


Fig. I.39. Positive lens action at an aperture when $V_2 - V_1 < V_3 - V_2$. (From Sjostrand, p.34)

Figures I.38 and I.39 show how both positive (converging) and negative (diverging) electrostatic lenses can be formed. This feature of electrostatic lenses differs from electromagnetic lenses which can only act as converging lenses.

2) Advantages/Disadvantages of electron lenses:

The fact that the refractive index does not change abruptly in electron lenses has one advantage in that there are no troublesome reflections at equipotentials as at glass interfaces. There is a serious disadvantage in that equipotentials cannot be shaped and combined in arbitrary fashion to correct for chromatic aberration and other errors.

Although electron microscopes which employ electrostatic lenses have been made, most microscopes use electromagnetic lenses. A major reason is that electrostatic lenses are more sensitive to the quality of the vacuum and cleanliness of the components than are electromagnetic lenses. Some lens aberrations are more severe for electrostatic lenses compared to electromagnetic lenses. Electrostatic lenses require very powerful electrostatic fields which can lead to electrical breakdown or "arcing-over" inside the column, especially under poor vacuum conditions. For this reason, electrostatic lenses cannot be made with focal lengths as short as magnetic lenses.

b. Electrostatic lens

A basic understanding of electrostatic lenses is important for two main reasons: i) the electron gun uses electrostatic lens action to form the primary beam source, and ii) it is quite common for a charge to develop on the non-conducting contamination which may accumulate on physical apertures (such as the objective aperture) and transform them into weak electrostatic lenses which can distort the electron image.

3) Properties of electrostatic lenses:

a) Any axially symmetrical electrostatic field has the properties of a lens for rays confined to the **paraxial** region. All the ideal lens formulas apply to electrostatic lenses.

b) For electron lenses, replace $\sqrt{(\varphi)}$ for refractive index in the lens equations (φ = value of the potential on the axis). In the image forming system of most TEMs, φ is the same on both sides of the lens (See Sec. I.A.5.c).

c) If bounded by regions where φ is constant, an electrostatic lens is always convergent.

c. Magnetic fields and magnetic lenses

1) Magnetic field

An electric current passing through a conductor gives rise to a magnetic field. The convention is that N is the direction in which the lines of the magnetic field point (Fig. I.40). The magnetic flux is the total number of lines and the flux density is the number of lines per unit area of a surface. Use the **RIGHT hand rule** to determine the direction of the field (thumb points toward current direction and fingers curl in direction of field. **NOTE**: By convention, the direction of electron flow is **opposite** to that of current flow.

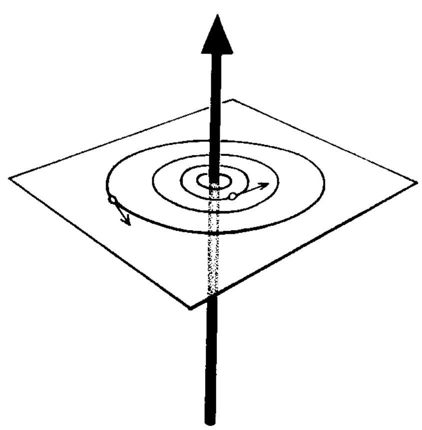


Fig. I.40 Magnetic field induced by current passing through a conductor. (From Sjostrand, p.35)

Flux density depends on the properties of the material surrounding the conductor. Iron induces a higher flux density than air or a vacuum. The property of the material which affects the flux density is called the **permeability**, μ, of the material. For air and vacuum, μ = 1.0. For ferromagnetic materials it can be as large as several hundred thousand.

If the conductor has the shape of a circular loop, the lines of force form circles around the loop. The flux density is greatest at the center of the loop. The magnet in the center of the loop is oriented perpendicular to the plane of the loop if the current through the loop is sufficiently strong to eliminate the influence of the earth's field. The side of the loop at which the lines of force leave the loop is the North (N) pole of the magnet (Figs. I.41,I.42).

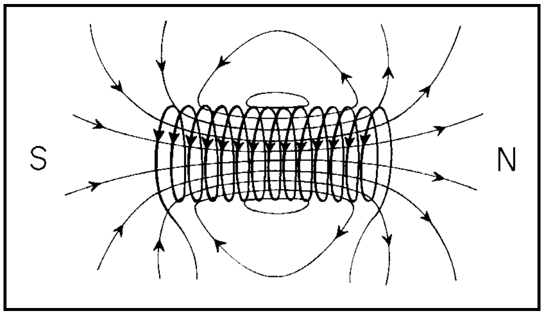


Fig. I.41 Magnetic field induced by current passing through a solenoid. (From Sjostrand, p.37)

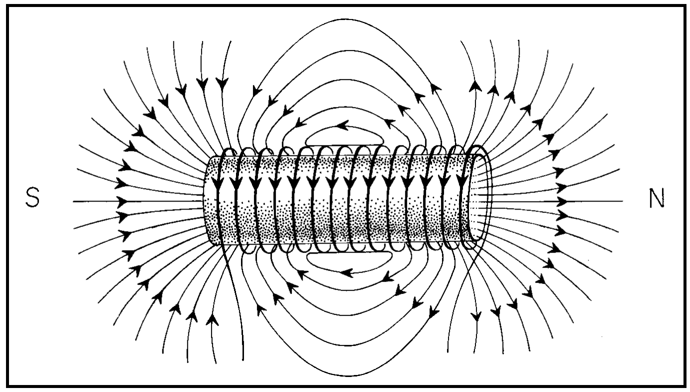


Fig. I.42 Solenoid with iron core. (From Sjostrand, p.40)

If the wire is wound in several turns around a cylindrical surface (**solenoid**), each turn will contribute to the induced magnetic field. The **flux density** in the **center** of the coil is given by:

$B = \mu(N \cdot I/l)$ where μ = permeability of surrounding material (webers/meter·amp)
N = number of turns of the wire
I = current strength (amps)
l = length of the solenoid (meters)
and $H = (N \cdot I/l)$ = magnetic field intensity (amps/meter)

Thus, $B = \mu H$ (webers/m^2), and $\mu = B/H$, so μ is the flux density per unit field strength. NOTE: One weber/m^2 = 10^4 gauss.

In the case of air and non-magnetic materials, $\mu = 1.0$ and $B = H$.

The permeability of iron depends on the field strength, H, and decreases to unity at high field strength or when the flux density, B = H (Fig. I.43). The high permeability of iron is due to the induced magnetic field orienting microscopic crystal regions acting as tiny magnets in the iron. All these tiny magnets add their magnetic fields to the induced field (Fig. I.44). When all micromagnets are nearly oriented, the iron will affect the flux density to a decreasing amount since the reorientation of micromagnets is nearing completion. Thus, the permeability of iron at high field strengths approaches that of empty space. At this point the iron reaches magnetic saturation.

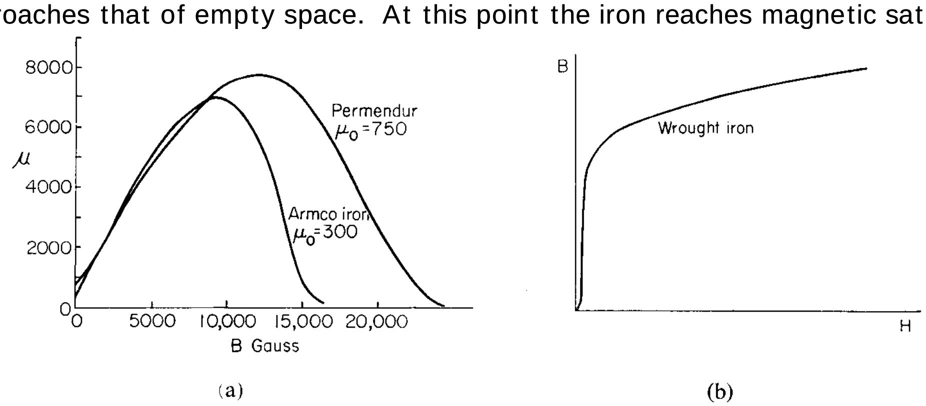


Fig. I.43 a. Dependence of permeability on flux density, B. b. Relationship between flux density B and field strength. (From Sjostrand, p.38).

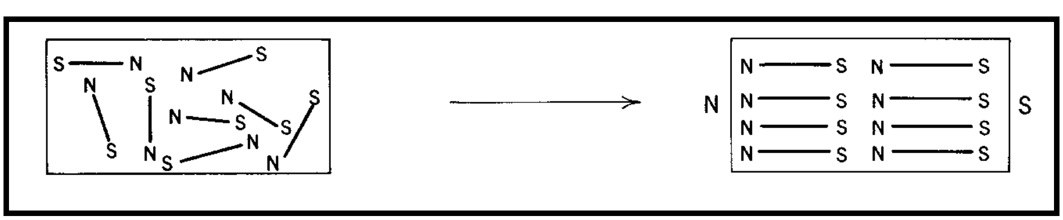


Fig. I.44 Magnetization. (From Slayter, p.361)

2) Hysteresis

The strength of the lens depends to some extent on the previous magnetic history of the lens. When the current in a lens is reduced, the decrease in magnetization does not retrace the same path obtained when the current was increased (Fig. I.45). Induction of magnetization involves a physical movement within the magnetized material, requiring the overcome of a certain degree of inertia. As a result, magnetization tends to lag behind the magnetizing force applied. Induced magnetic flux can only be returned to zero by application of a current in the opposite direction.

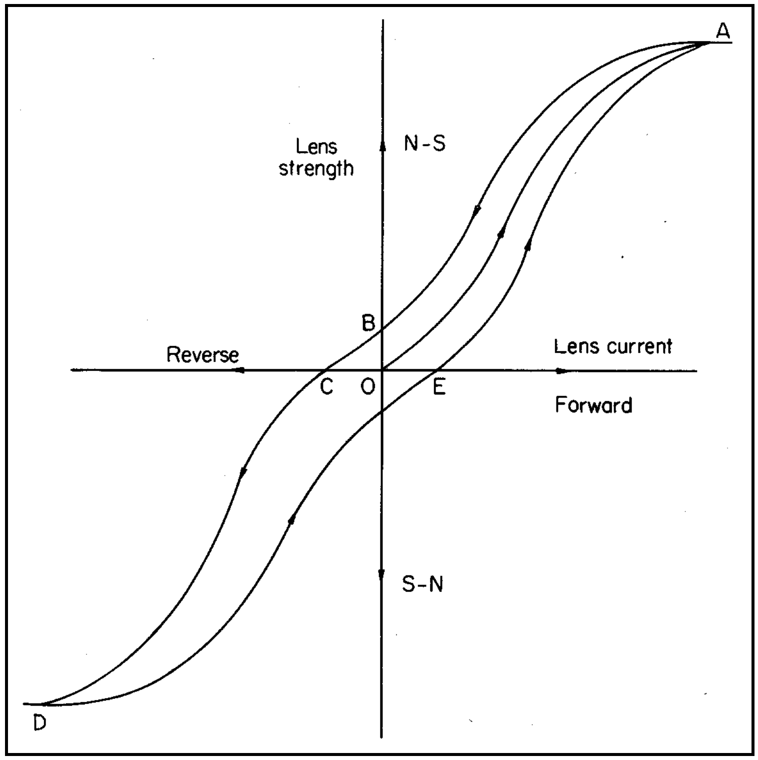


Fig. I.45 Curves showing how the magnetization of soft iron (lens strength) is related to the magnetizing force (lens current). An unmagnetized lens starts from the point O and follows the path OA as lens current increases. At the point A, further increase in lens current produces no further increase in lens strength; the lens is said to be 'saturated'. When lens current is reduced, the path OA is not retraced; a different path AB is followed. This displacement is called 'hystere-sis'. At zero current (point B), some residual lens strength remains; this is called 'remanence'. To bring the lens back to zero strength, a reverse current OC must be applied. Lens strength then increases with increasing reverse current, follow-ing the path CD. The polarity of the lens changes, but this does not affect its focusing power; only the spiral electron path is reversed. Because of hysteresis, it is not possible to cali-brate a lens current meter accurately in terms of lens strength or magnification.

A consequence of hysteresis is that the level of current used to energize a magnetic lens **DOES NOT** precisely specify the lens strength (*i.e.* focal length).

Normalization of TEM lenses is accomplished by reducing the lens current to zero some predetermined number of times. Hysteresis may also be minimized by taking a lens to saturation and then returning it to the working current without overshooting. When the field strength is reduced to zero, some magnetization still remains in the iron (**residual magnetization** or **remanence**). An advantage of soft iron is the fact that, when used in an electromagnet, hysteresis is low.

Introduction of pieces of iron in a magnetic field drastically affects the flux density. Magnetic material has a shielding effect, the effect being greater the greater the permeability. Permalloy (µ metal) has a maximum permeability of 80,000-140,000 compared to iron used in transformers (~7000). µ metal permeability is limited to lower field strengths than that at which iron still retains high permeability. This metal is therefore useful for shielding the TEM from external magnetic fields.

d. The electromagnetic lens

1) Lens design development (Figs. I.46-I.48)

The efficiency of the magnetic field produced by a short solenoid was first improved by encasing the energizing coil in a sheath of soft iron which has the property of concentrating the lines of force in a magnetic field and thus becoming magnetized by induction. In this way a much more powerful axial magnetic field is obtained for the same amount of current flowing through the solenoid. Further development involved encasing the entire coil with soft-iron except at a narrow annular gap in the inside of the coil. This produces a greater concentration of the magnetic field along a short axial distance. To achieve shorter focal length lenses (and obtain greater magnifications) a soft iron polepiece with an open axial bore was introduced at the position of the annular gap.

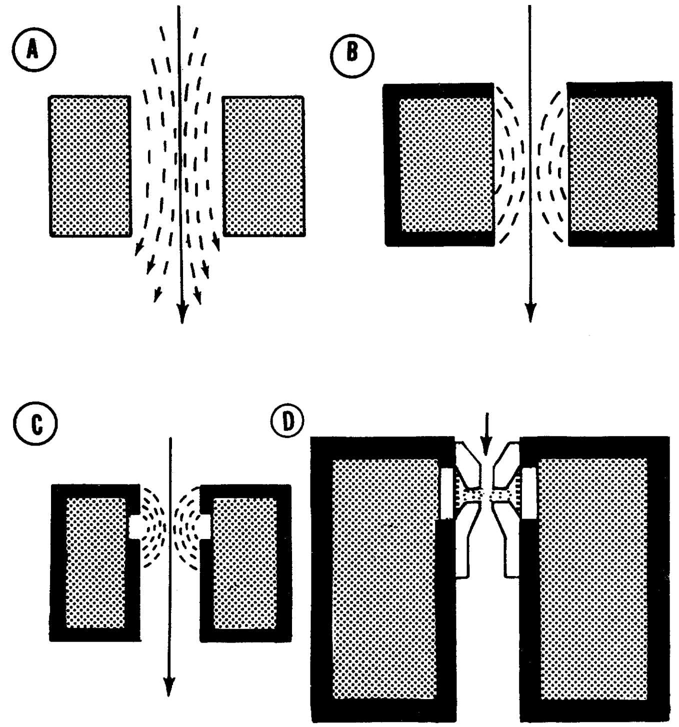


Fig. I.46 Evolution of magnetic electron lenses. (A) Short solenoid used as a magnetic lens. (B) Soft-iron casing enclosing outer surface of the solenoid, thus concentrating the field. (C) Soft-iron encasing the solenoid except at a narrow annular gap thereby reducing the magnetic field to a very short region along the lens axis. (D) Modern objective lens consisting of a soft-iron encased solenoid and soft-iron pole pieces so as to have an enormously concentrated field at the level of the annular gap. (From Wischnizter 2nd ed., p.33)

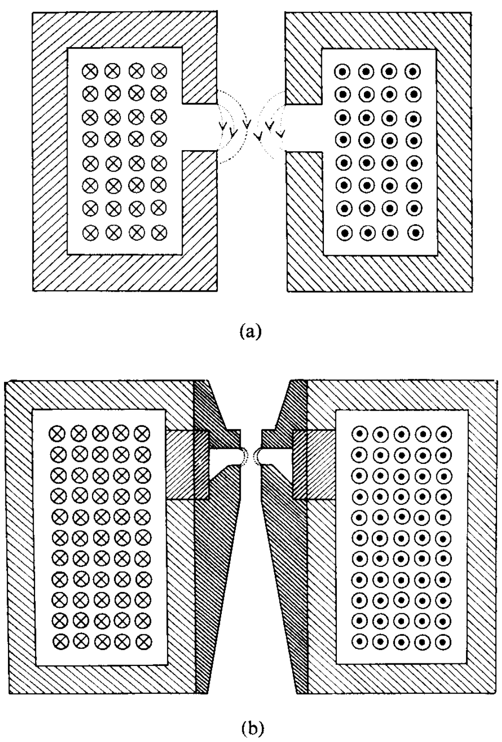


Fig. I.47 (a) A magnetic lens consisting of a tightly wound coil and a soft iron shroud surrounding the coil except for a small gap. The field is concentrated in that gap. (b) Short focal length electromagnetic lens with pole pieces. (From Sjostrand, p.50)

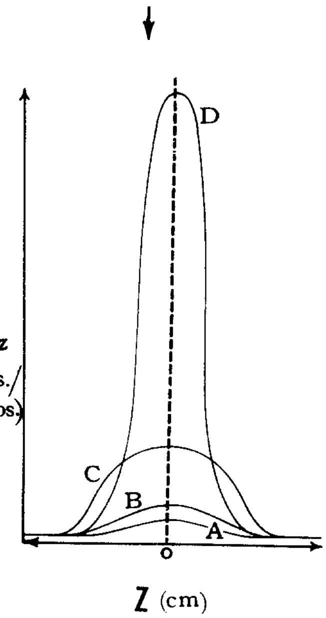


Fig. I.48 Field strength distribution curves. The curves A-D correspond to the respective lenses illustrated in Fig. I.46. Each represents the field strength along the long axis of the lens. The changes in the shape of the curves represent the shortening or concentration of the field over a shorter axial distance. H_Z = longitudinal magnetic field. Z = distance along the axis of symmetry. (From Wischnizter 2nd ed., p.33)

Magnetic lenses used in TEMs are always constructed with an iron circuit to produce a high field strength across a short gap. The magnetic fields for TEM lenses are in the range of 10,000-20,000 gauss.

2) Forces acting on a current in a magnetic field

The force on an electron in a magnetic field is always at right angles to the velocity and the direction of the field (Figs. I.49, I.50, and I.52). The field only acts on the velocity component which is directed perpendicular to the lines of force. Use the **left hand rule** (Fig. I.51: first finger for field direction, middle finger for current direction, and thumb for direction of force). Rays passing through the lens are turned through an angle which does **NOT** depend on the distance of the rays from the axis. All electrons contained in a given meridional plane before entering the field are contained in a rotating meridional plane as they pass through the lens, and then they leave the lens coplanar.

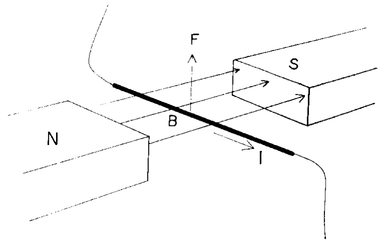


Fig. I.49 The force F acting on a straight conductor in a homogeneous magnetic field of flux density B when current I is passed through the conductor. (From Sjostrand, p.43)

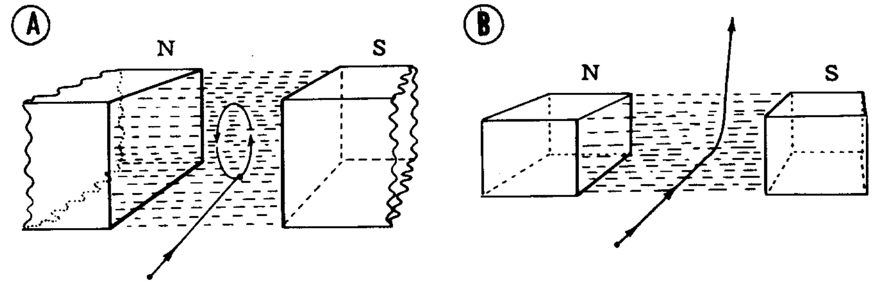


Fig. I.50 Path of an electron in a magnetic field. (A) In a large, uniform magnetic field. (B) In a small, uniform magnetic field. (From Wischnitzer 2nd ed., p.25)

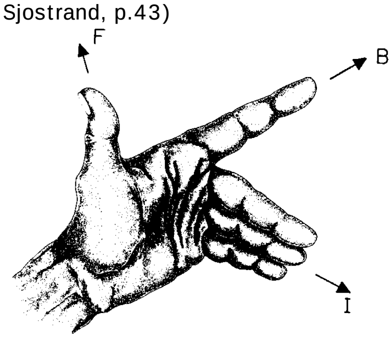


Fig. I.51 Left hand thumb rule. (From Sjostrand, p.43)

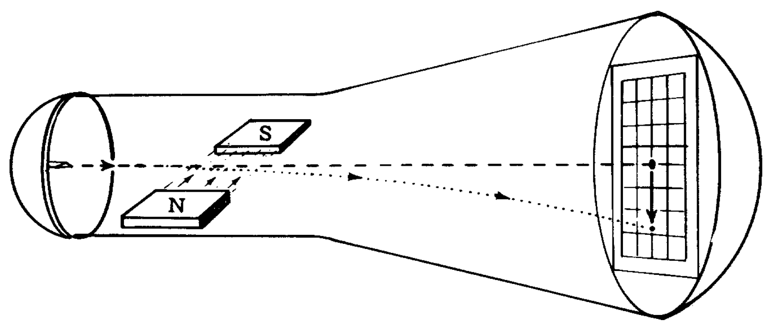


Fig. I.52 Thompson's experiment. A stream of electrons originating from a source and passing, *in vacuo*, through a magnetic field produced by a pair of magnets will be deflected. The direction of deflection demonstrates that electrons are negatively charged particles of matter. (From Wischnitzer 2nd ed., p.25)

When electrons enter the lens they encounter a **sideways force** which causes the electron to rotate as it con-tinues through the lens (Figs. I.53 - I.57). Since the radial component of the magnetic field reverses after the center of the lens, the rotational velocity set up in the first half of the lens is countered. The electron entered the lens without angular momentum about the axis and leaves without angular momentum. The net effect is a deflection toward the axis, which it must cross at the focal point f_2. The angle between the object vector and the image vector is 180° + θ, whereas, for glass lenses and electrostatic lenses, the angle between a real object and the image is exactly 180°. Since the radial force is directed toward the axis, the lens is convergent no matter what the direction of the field.

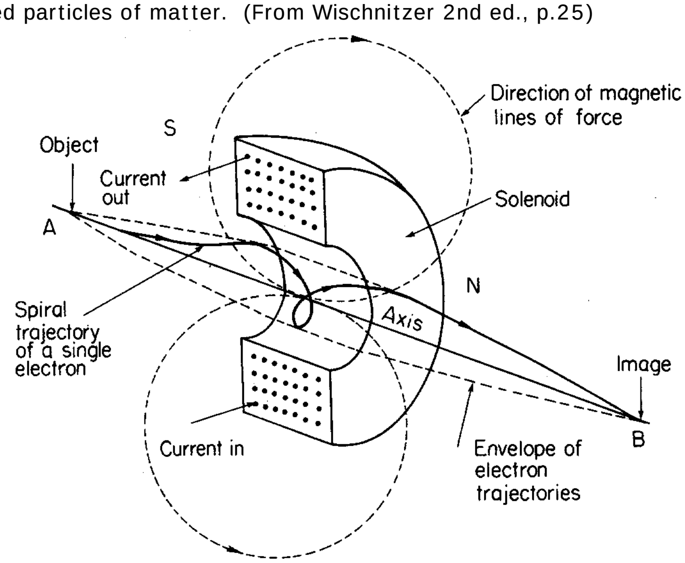


Fig. I.53 Action of a solenoid on an electron beam. An electric current passing through the coil produces an axial magnetic field. This is the refracting medium for the electrons. An electron starting at a point on the axis A and at an angle to it follows a spiral path, return-ing to the axis at the point B. The action is basically similar to that of the converging light lens shown in Fig. I.20. (From Meek 1st ed., p.8)

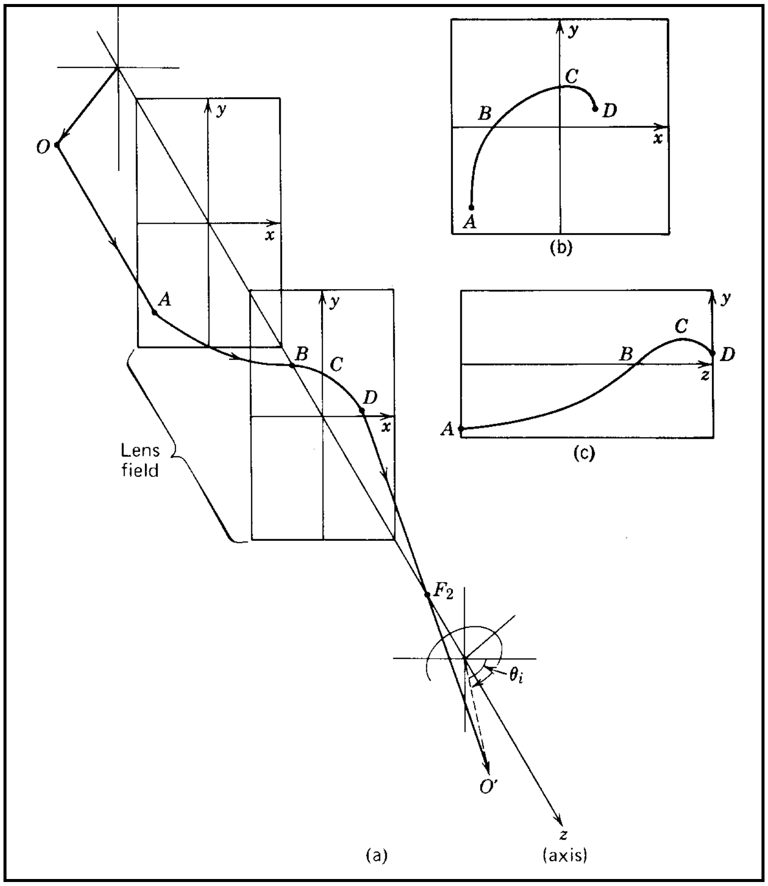


Fig. I.54 Action of the magnetic lens. (a) In perspective. (b) Electron trajectory in projection, along direction of propagation. (c) Electron trajectory in projection, side view. (From Slayter, p.358)

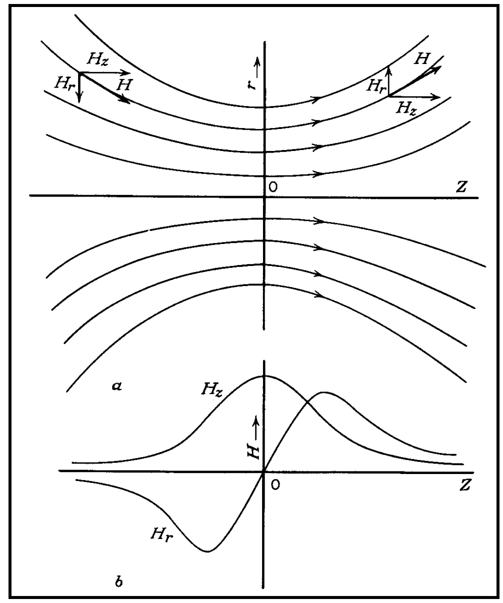


Fig. I.55 Components of the vector H near the axis of fields with axial symmetry. H is represented by two components, H_Z , the component in the *z* (axial) direction, and H_r, the compnent in the *r* (radial) direction. (From Hall, p.85)

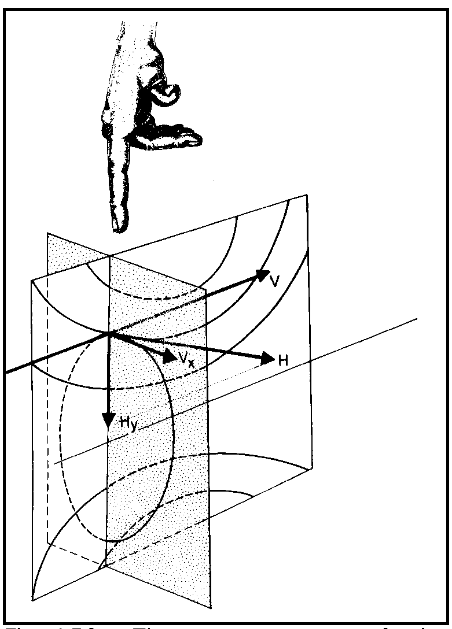


Fig. I.56 The *y* component of the magnetic field in a magnetic lens is oriented perpendicular to the direction of an electron entering the lens along a path parallel to the lens axis. This *y* component will affect the electron, deflecting it in the *x* direction as indicated by the arrow marked v_X. (From Sjostrand, p.48)

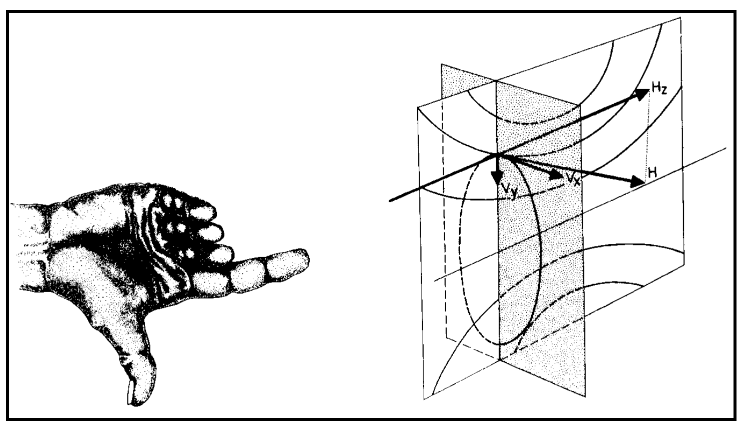


Fig. I.57 The *z* component of the magnetic field and the *x* velocity component of the electron in a magnetic lens interact, deflecting the electron in the *y* direction toward the lens axis. (From Sjostrand, p.49)

3) Properties of a magnetic lens:

Any axially-symmetric magnetic field has the properties of an ideal lens. All the formulas for the ideal lens may be applied.

Magnetic lenses are **always convergent**. The conventional, axially-symmetric lens is always bounded by regions which are field-free, the consequence being that the net action of electron lenses is inevitably **convergent**. Limited regions may be divergent but not the lens as a whole. The serious consequence of this is that neither spherical or chromatic aberrations can be corrected as is done in light optics by the use doublets of positive and negative lenses.

In the absence of electrostatic fields, the refractive index is the same in object and image space, therefore $f_1 = f_2$.

Electrons traveling through axially symmetric fields experience a spiral trajectory of diminishing radius. The image vector is at an angle 180° + θ to the object vector.

The deflection of the electron towards the axis means that an electron entering the lens parallel to its axis will cross the axis after having passed the lens. The deflection will increase with the distance from the axis. Thus, a beam of electrons in parallel paths parallel to the axis of the lens will be focused to an image point on the axis which represents the second (back) focal point of the lens (f_2). Note that magnetic lenses are highly inefficient in that only a minor portion of the total field strength is actually effective in focusing the electron.

4) Magnetic lens focal length

In a magnetic electron lens the focal length is determined by the field strength in the lens gap and by the speed of the electrons (determined by the accelerating voltage).

$$f = KV_r/(N \cdot I)^2$$

where f = the focal length of the lens
K = a constant
V_r = the accelerating voltage, relativistically corrected
$N \cdot I$ = the number of ampere turns in the excitation coils

For magnetic lenses, focusing is achieved by **varying the current** which passes through the electromagnet**.** This in turn changes the strength of the magnetic field and thereby alters the focal length of the lens and is equivalent to a combined change in both the "refractive index" and "curvature of surface". For a beam of more energetic electrons, the lens current has to be increased in order to keep the focal length constant. Focal length and current are **NOT** linearly related: strength increases in a sigmoid fashion (Fig. I.45) as current increases until a point is reached where the lens is saturated and no further increase in lens strength can be achieved.

Since the focal length of the lens is directly proportional to the accelerating voltage, a variation in the velocity of the electrons in the imaging beam affects image quality by eliminating perfect focus (chromatic aberration).

5) Magnetic lens design: (Figs. I.46, I.47).

Condenser lenses usually have a relatively large bore and spacing which results in a long field and long focal length .

Typical construction of the **objective lens** produces a strong field of short axial extent (i.e. short focal length between 1.5-3 mm) necessary for formation of images at high magnification. The specimen is placed within the magnetic field of the objective lens. Thus, any field introduced by contaminants in the specimen can distort the field of the lens. Note that this also means that part of the lens field ("pre-field") is on the front side of the object and affects the electron beam before it passes through the object.

Most of a typical magnetic lens lies outside the vacuum of the microscope. Only those regions through which the electron beam passes are in high vacuum. Magnetic lenses must be water-cooled to dissipate the large amounts of heat produced by the currents in the electromagnet coils.