I. THE MICROSCOPE

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

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

DATE	NAME or COMPANY	EVENT
1897	J. J. Thompson	Discovered the electron
1924	Louis deBroglie (as grad student)	Identifies wavelengths associated with moving electrons
1926	H. Busch	Magnetic or electric fields act as lenses for electrons
1929	E. Ruska	Ph. D thesis on magnetic lenses
1931	Davisson & Calbrick	Properties of electrostatic lenses
1932	M. Knoll & E. Ruska	First electron microscope built (prototype of modern microscopes)
1935	E. Driest & H. Muller	Surpass resolution of the LM
1938	B. von Borries & E. Ruska	Constructed TEM capable of resolving 10 nm (= 100 Å)
1939	Siemens	First practical TEM
1941	RCA	Commercial TEM with 2.5 nm resolution
1946	J. Hillier	1.0 nm resolution achieved

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**: holds and positions the specimen between the illumination and imaging systems.

3) **Imaging system**: Lenses that 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) one or more projector lenses, which magnify a portion of the intermediate image to form the final image.

4) **Image recording system**: Converts the radiation into a permanent image (recorded on a photographic emulsion or captured digitally by a CCD camera) that can be viewed.

b. Differences

1) Optical lenses are generally made of glass with fixed focal lengths. Magnetic lenses are constructed with ferromagnetic materials and windings of copper wire that produce a focal length that 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. Magnification can also be changed if oculars (eyepieces) of different power are used. In the TEM, the focal length and hence magnification of the objective lens remains fixed while the focal length of the projector lens is changed to vary magnification.

3) Depth of field is small in the LM, thus different focal levels can be seen in a 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. This, of course, is a trivial difference.

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

7) The electron beam rapidly damages biological TEM specimens.

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

9) Price tags are VERY different! (TEMs can be100 times more expensive than LMs)

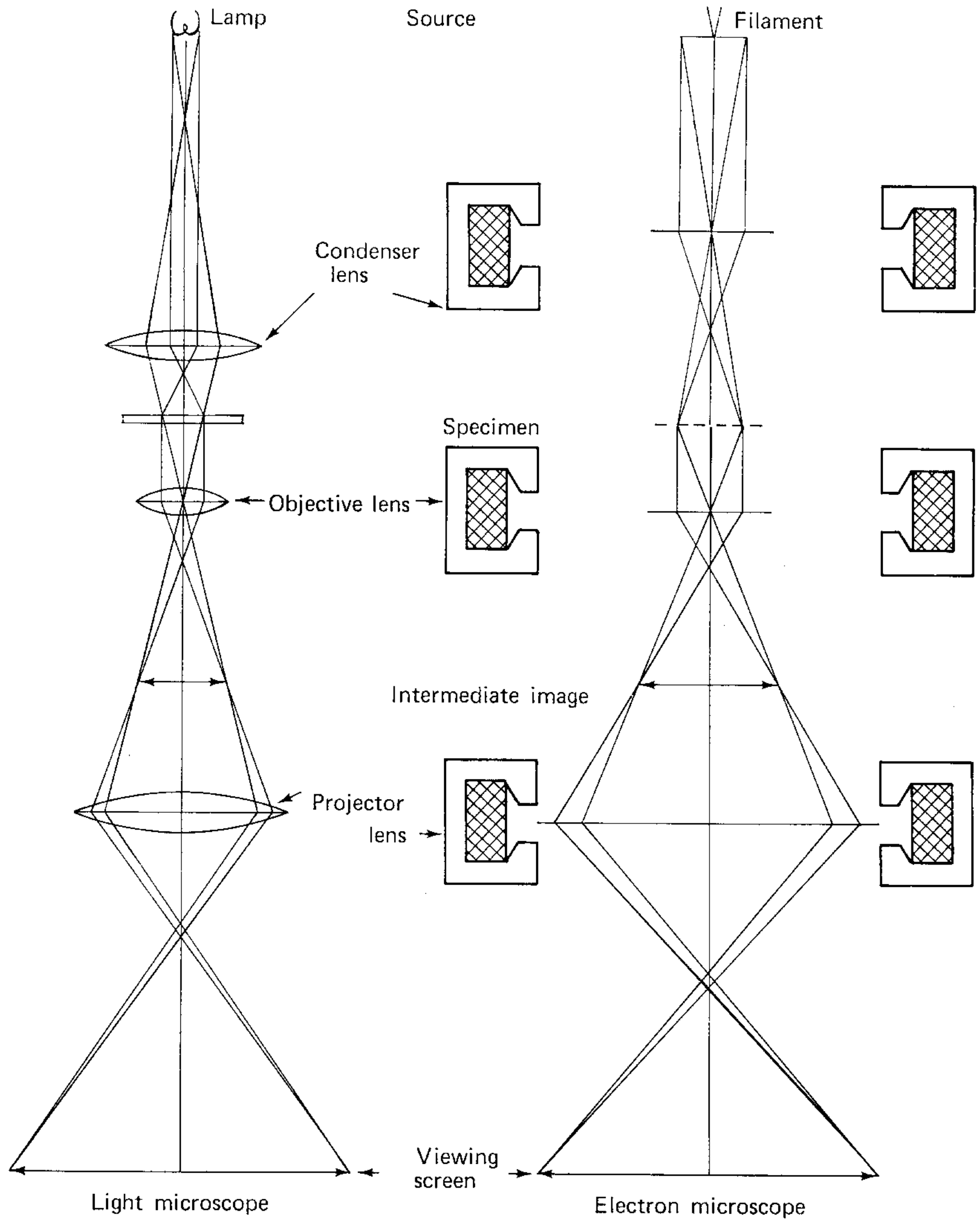


Fig. I.1 Comparison of light and electron microscopes. In each instrument, the condenser lens focuses illumination from the source (lamp, filament in the electron gun) onto the specimen. A first magnified image is formed by the objective lens. The projector lens further magnifies this image onto a ground glass screen (light) or fluorescent screen (electrons). (From Agar, 1974, p.8)

The basic design of transmission electron microscopes has remained fairly constant over the last 50 years as is illustrated in cutaway diagrams (Figs. I.2-I.5) and in photographs of modern instruments (Fig.I.6).

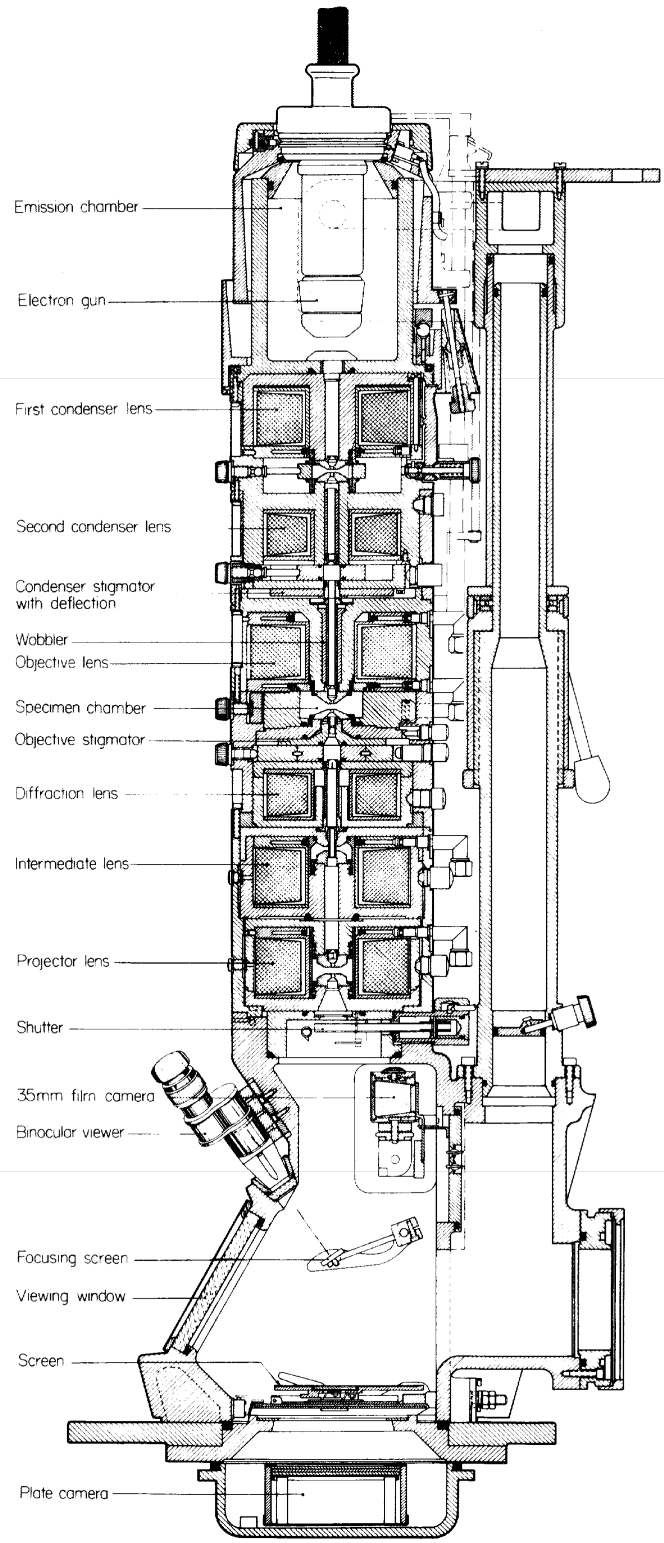


Fig. I.2 Diagrammatic cross-section through a double-condenser, 6-lens, Philips EM200 electron microscope. This microscope was first available in 1958. (From Meek, 1970, p.99)

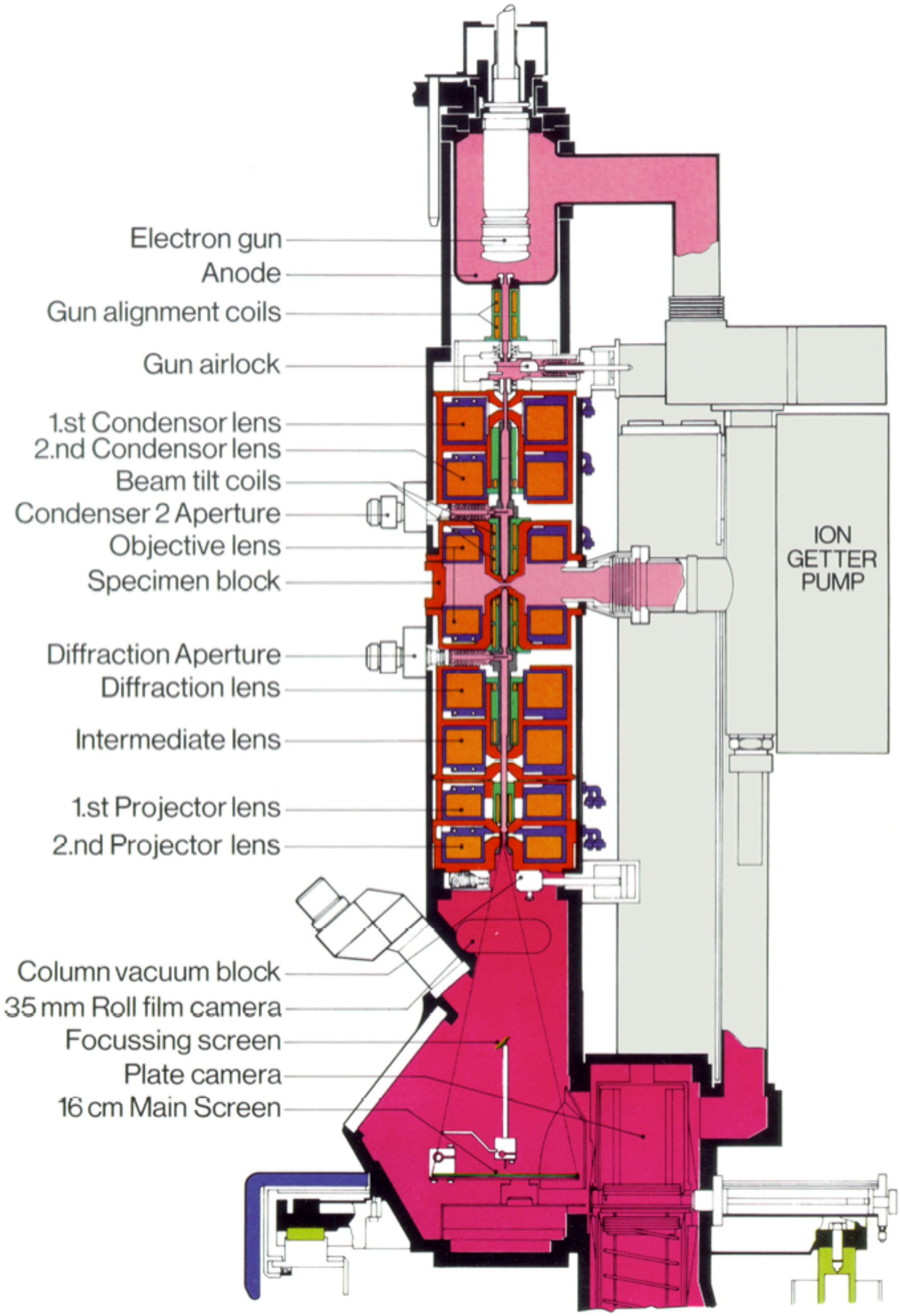


Fig. I.3 Diagrammatic cross-section through a Philips EM400 electron microscope. This microscope was first available in the late 1970s. (From Philips brochure)

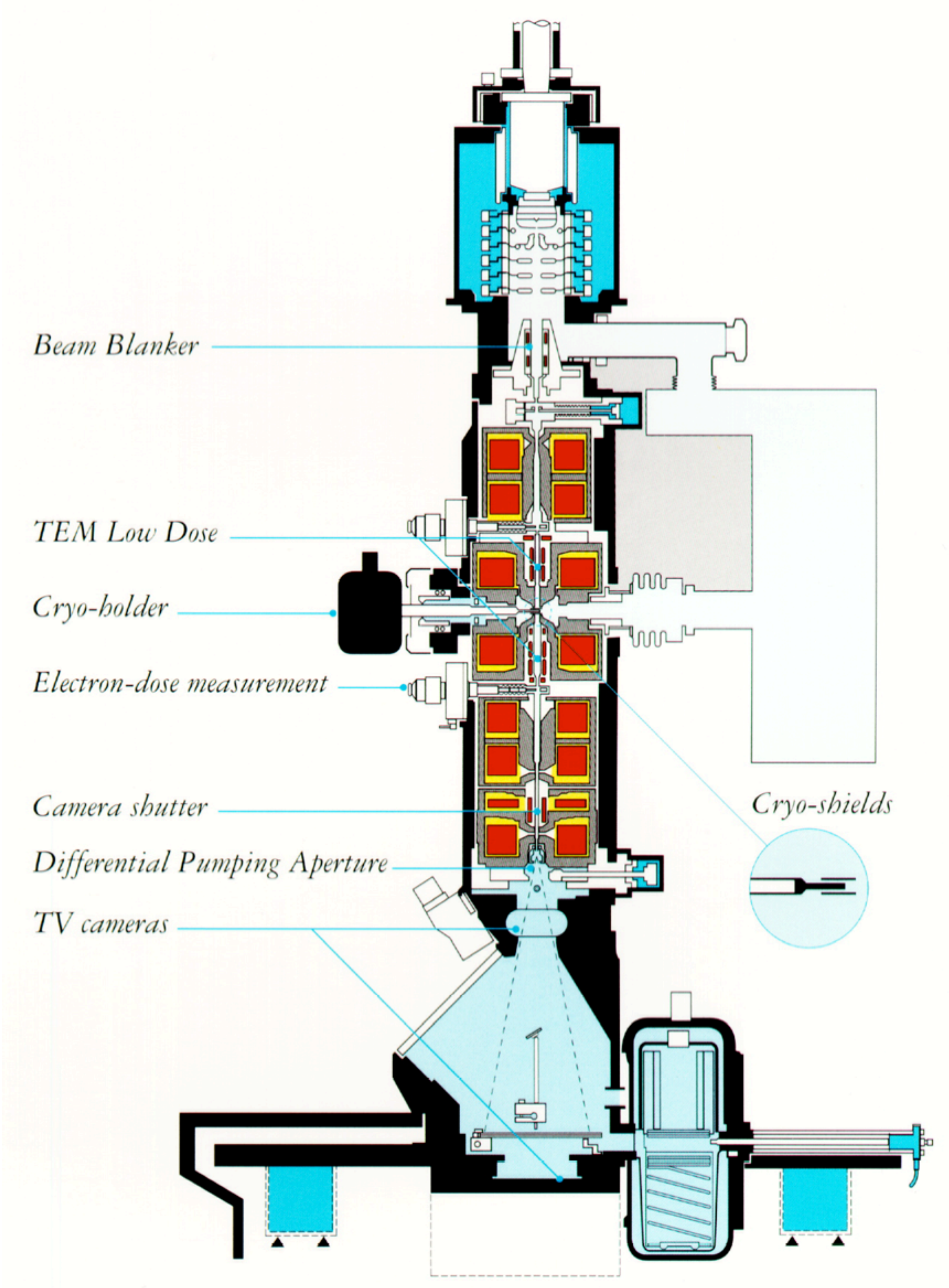


Fig. I.4 Diagrammatic cross-section through a Philips CM Cryo series electron microscope. The important features of this microscope include a cryo-holder for keeping specimens at low temperatures (~-175°C) in the microscope column, cryo-shields to minimize specimen contamination, and a low-dose device to minimize damage to the specimen caused by its exposure to the electron beam. (From Philips brochure)

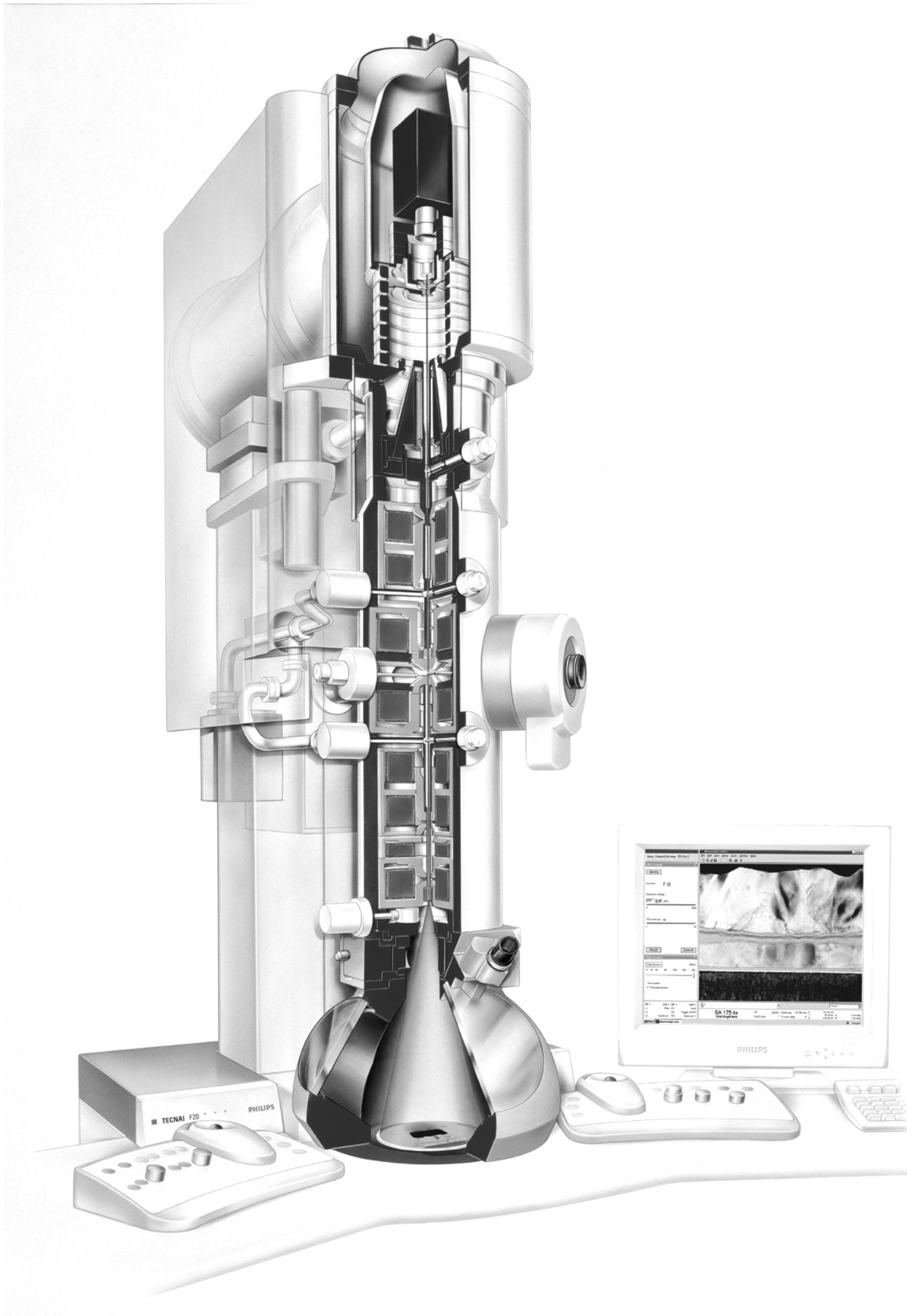


Fig. I.5 Cutaway view of a Philips Tecnai F20 electron microscope (first sold in 1998). The Tecnai operating system greatly expands the computer-controlled functions of the earlier CM series and allows each user to easily customize the microscope to their choosing. Microscope alignments are facilitated by an online help system that guides the user step-by-step through the procedures. Although this microscope still has a film camera, operation of a slow-scan CCD camera is integrated into the Tecnai operating system. (Image courtesy of Philips Electron Optics)

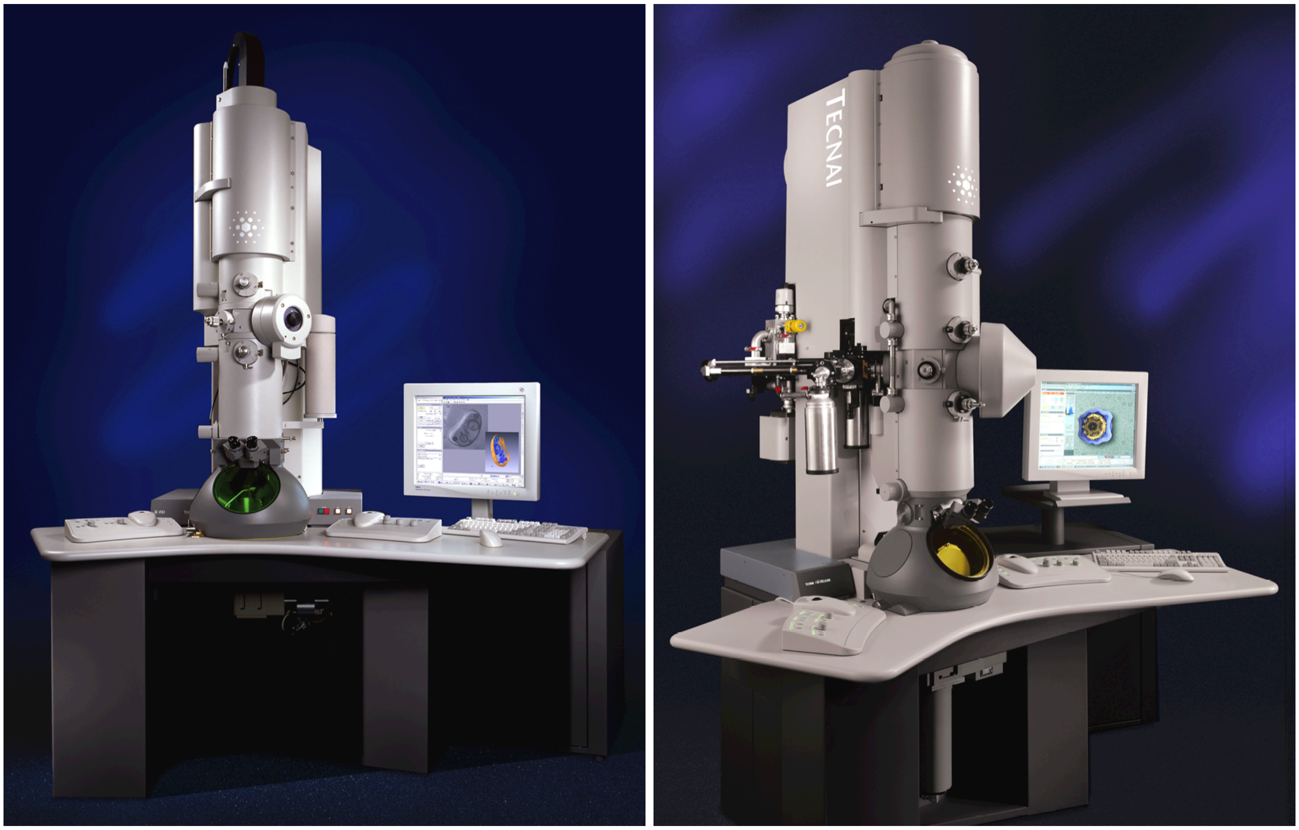


Fig. I.6 FEI/Philips 200keV Tecnai G^2 Sphera (left) and 300keV Tecnai G^2 Polara (right) microscopes. These two microscopes are part of the Cryo-EM facility in Bonner Hall on the UCSD campus. The Polara has a field emission gun and a specimen stage that can be cooled to liquid helium or liquid nitrogen temperatures to examine frozen-hydrated biological specimens. The Sphera has a LaB_6 filament and can examine specimens at liquid nitrogen temperature. (From FEI/Philips brochures)

I.A.3. Photons/Electrons

a. Dual concept of wave and particle properties of photons and electrons (Fig. I.7)

Light and electrons exhibit properties of both particles and waves. Most scientists probably realize that photons exhibit properties of waves and accept the fact that the rest mass of photons is zero. Scientists also understand that electrons are charged, sub-atomic particles, but many may not recognize the wave properties of moving electrons. This dual nature of light and electrons is required to satisfactorily explain the results of various physical experiments. Of course, long before our time, controversy arose over whether light was simply wavelike or a particle. The diffraction of light (bending around corners) illustrates the wave nature of light. Accordingly, Huygens (1629-1695) and Hooke (1638-1703) developed and expounded **WAVE THEORY**, which is based on the statistical nature of events and has little meaning with respect to the behavior of single particles. Newton (1642-1727) however proposed the **CORPUSCULAR THEORY**, which became the more accepted theory even after demonstrations of diffraction by Young (1773-1829) and interference by Fresnel (1788-1827). Wave theory returned to favor at about 1850 until more evidence for the corpuscular theory was discovered in about 1900. Then Einstein and Planck set the record straight.

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. According to quantum theory, the transfer of energy between light and matter occurs only in discrete quantities proportional to the frequency of the light wave.

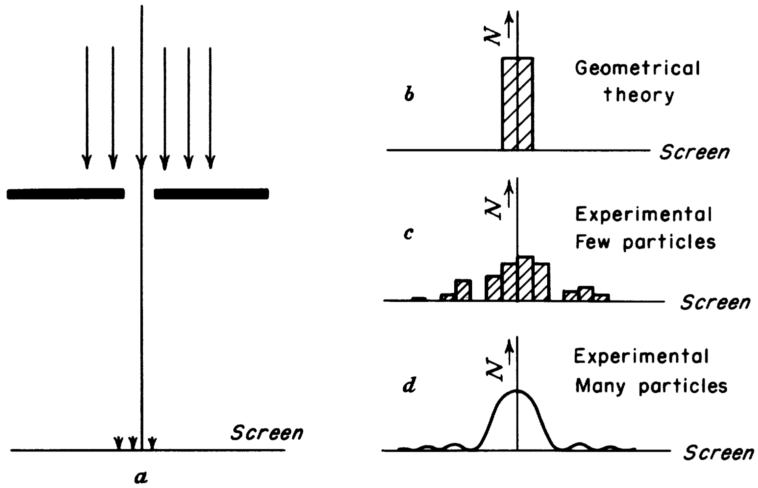


Fig. I.7 The statistical nature of diffraction patterns. (From Hall, 1966, p.13)

The quanta of light energy are given by the following relationship:

$E = h\vartheta$

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

Regarding electrons, it is somewhat harder to accept let alone understand their wave nature. In his basic textbook on transmission electron microscopy, Meek takes a stab at trying to describe the wave nature of electrons:

> *"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

DeBroglie showed many years ago that *any* particle of mass, *m*, moving at a velocity, *v*, has associated with it a wavelength (λ) given by the following relationship (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 equation (2) for velocity yields:

$$v = \sqrt{\frac{2eV}{m}} \qquad (3)$$

Substituting this into the DeBroglie equation (1) gives:

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

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). In fact, at an accelerating voltage of one million, the electron seems capable of moving at nearly twice the speed of light!

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

Table I.1. Relation between accelerating voltage, *V*, electron wavelength, λ, and electron velocity, *v*.

Naturally, nothing it seems is ever as simple as we'd like. Equation (5) is only an approximation and 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:

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

Hence, when the relativistic correction is used, 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 previous table now takes the following form when relativity effects are included (and Einstein can rest in peace):

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

Table I.2. Relation between accelerating voltage, *V*, electron wavelength, λ, and electron velocity, *v*, with relativity effects included.

c. Interference/diffraction/coherence

An <u>ideal</u> lens system obtains an <u>exact</u> image of an object (each point faithfully reproduced). However, the phenomenon of diffraction makes this unattainable (Figs. I.8, I.9).

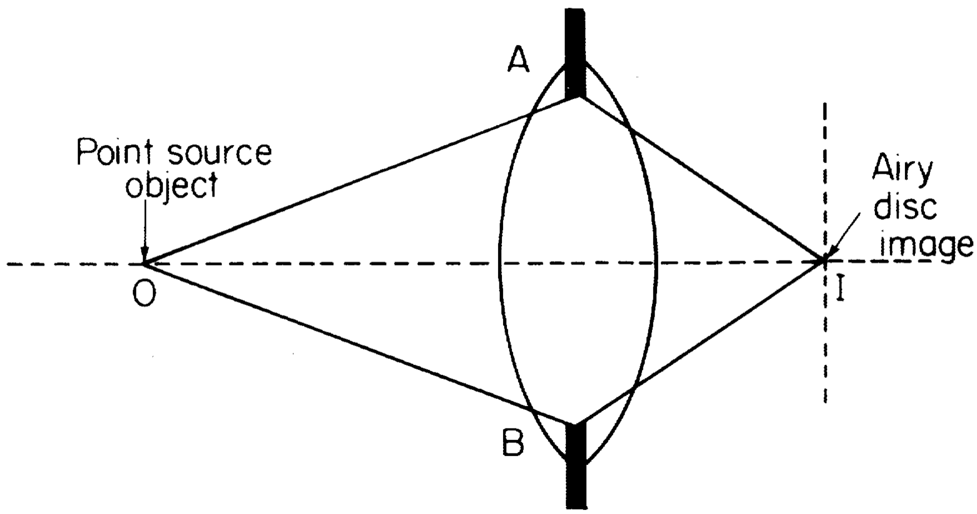


Fig. I.8 A perfect point source cannot be imaged by a lens as a perfect point image owing to the presence of the aperture AB (which may be the edge of the lens). Diffraction at this aperture gives rise to a series of fringes, which surround the image formed of the point source. The pattern produced is called an Airy disk. (From Meek, 1970, p.35)

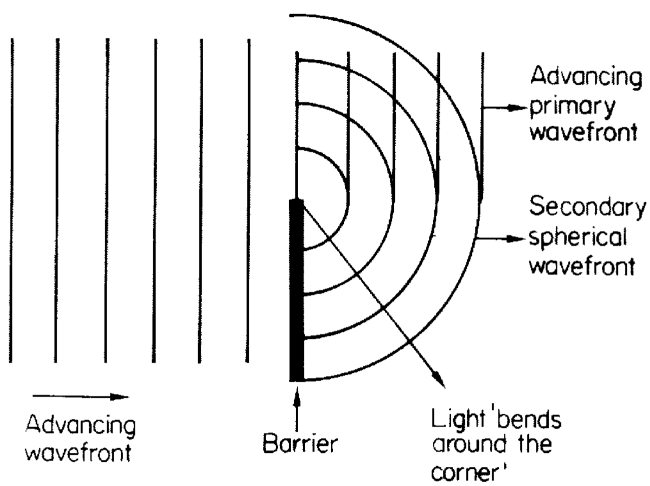


Fig. I.9 When a wavefront strikes a barrier, it can bend around the 'corner' and give rise to a secondary wavefront at the edge, since each point on the wave front can give rise to a new source of waves. This phenomenon is called diffraction. (From Meek, 1970, p.22)

Diffraction phenomena involve the <u>bending of the path of radiation passing close to an obstacle</u> (Fig. I.9). Diffraction results in a spreading of the radiation into the region behind the obstruction that the waves passed. Diffraction at edges contributes to the contrast at which an edge can be observed. Diffraction also <u>limits the resolving power</u> of the microscope since the image of each infinitely small point in an object produced by a lens is not a corresponding point but instead is a diffraction image of the opening of the lens or the aperture restricting the effective opening of the lens (Fig. I.8).

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.10 - I.12).

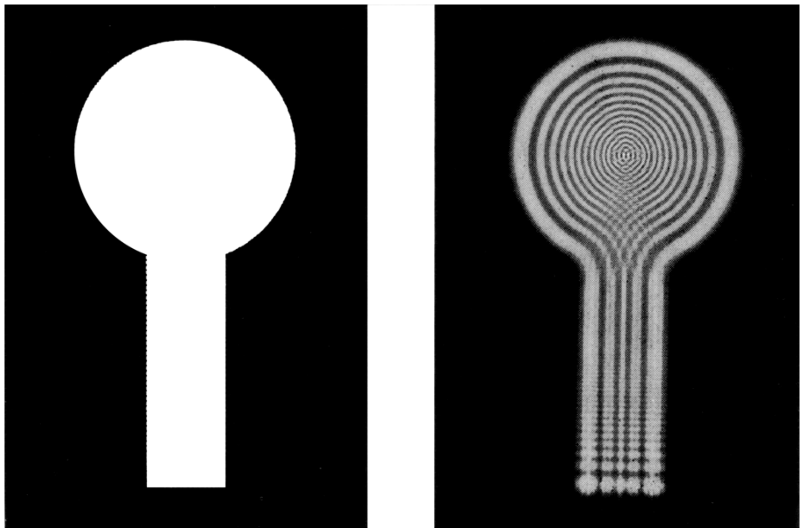


Fig. I.10 Diffraction pattern (right) formed by an irregularly shaped aperture (left). (From Young, 1968, p.95)

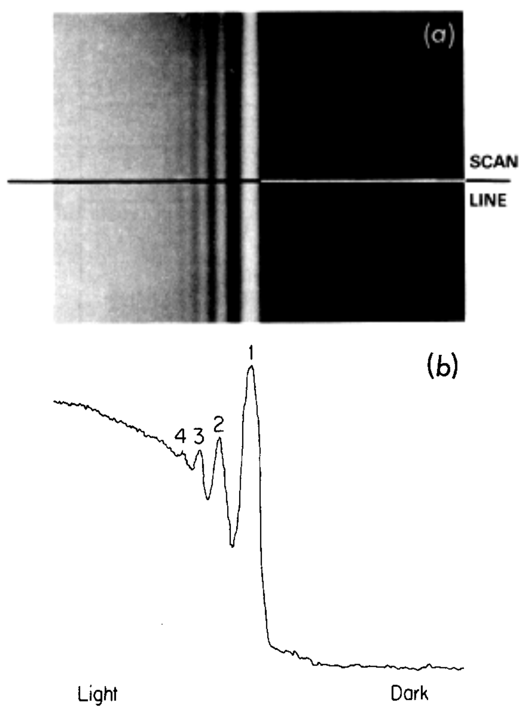


Fig. I.11 (a) Photograph of the edge of a razor blade illuminated by monochromatic (blue) light rendered coherent by passing through a narrow slit. (b) Microdensitometer tracing of the negative from which the photograph was made. Four Fresnel fringes can be distinguished. (From Meek, 1970, p.27)

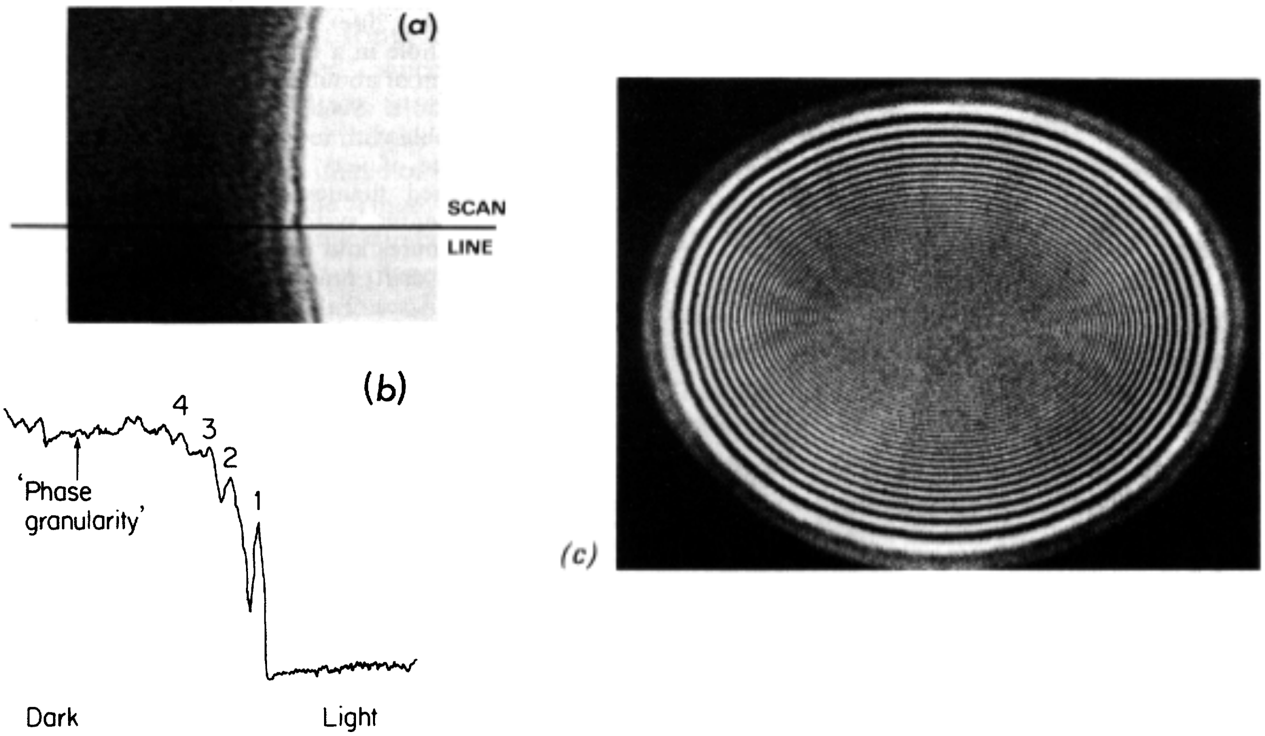


Fig. I.12 (a) Fresnel fringes formed by electrons. These fringes are formed outside the edge of a hole (white) in a carbon film (black).

(b) Microdensitometer tracing of the fringe system. The pattern is identical with the Fresnel fringe system formed by visible light (see Fig. I.11).

(c) Under focused image of a hole in a film, showing a complete system of about 40 Fresnel fringes inside the hole. (From Meek, 1970, 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 that would occur from each source separately (Figs. I.13, I.14). 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.15 and I.16.

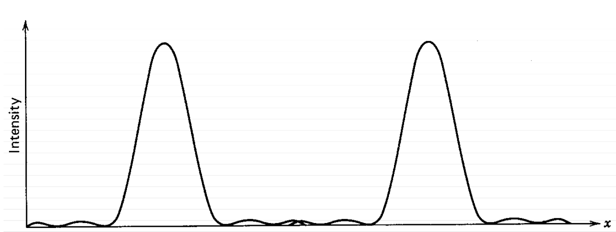


Fig. I.13 Diffraction images of two, easily resolved points. (From Slayter, 1970, p.243)

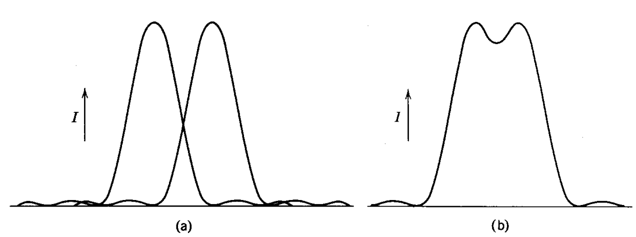


Fig. I.14 Images of two incoherently illuminated points at the limit of resolution. (a) Individual intensities (b) Summed intensities. (From Slayter, 1970, p.244)

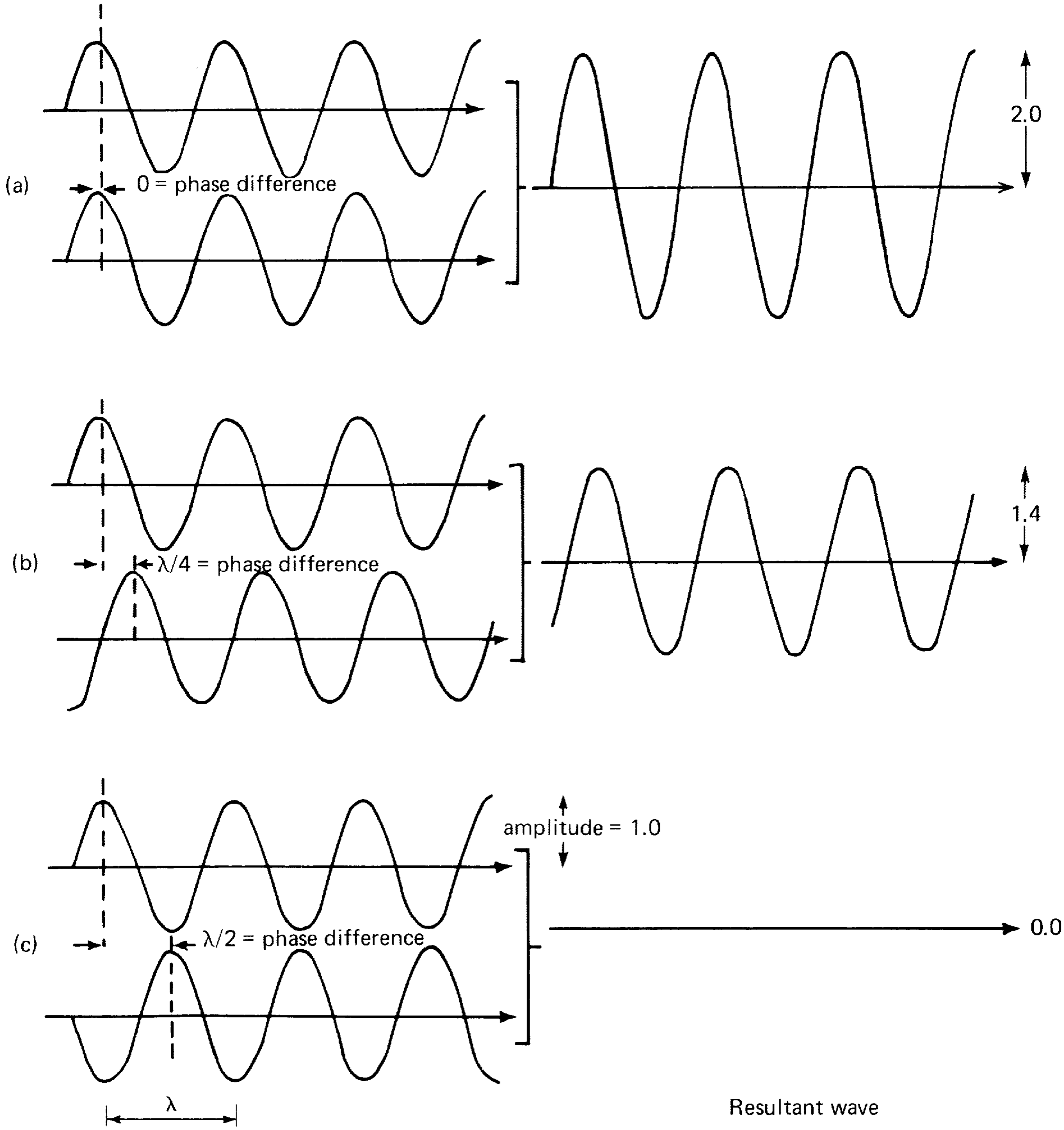


Fig. I.15 Effect of adding two waves of the same wavelength and equal amplitude. In each example, the two separate waves are shown on the left and their sum or resultant wave on the right. The different examples are characterized by varying phase differences. The phase of a wave (usually expressed as 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. The waves partially reinforce, resulting in 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, 1972, p.19)

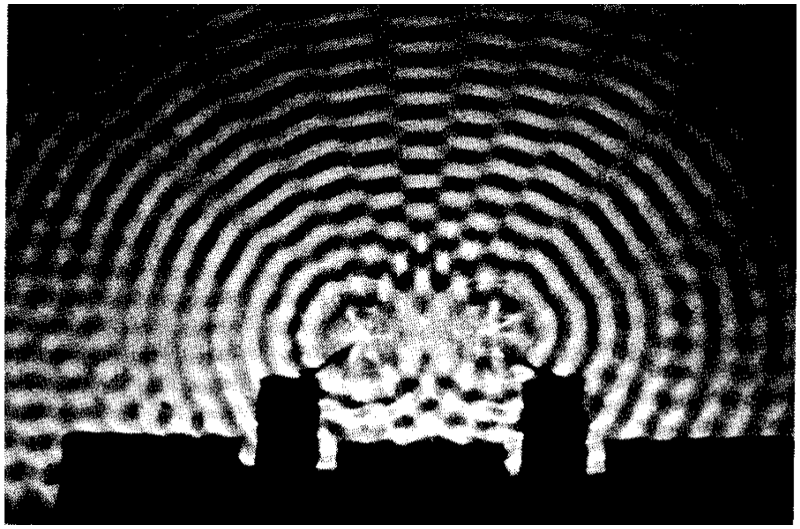


Fig. I.16 Photograph of an interference pattern in water waves formed by the superposition of waves from two sources that are oscillating in phase and with the same frequency. (From Young, 1968, p.22)

d. Resolution

The concept of "resolution" is central to every form of imaging. A goal of most imaging studies is to distinguish the finest details possible in the object under investigation, and to do this requires the formation and recording of images at the highest possible resolutions. The distinction between several resolution-related terms is important to understand.

1) Definitions:

RESOLUTION: ability to distinguish closely spaced points as separate points

RESOLUTION LIMIT: smallest separation of points that 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°). Since the eye can focus down to a distance of about 250 mm, the smallest object we can resolve is about 0.07mm (70μm) in size (Fig. I.17). 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.18). 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.

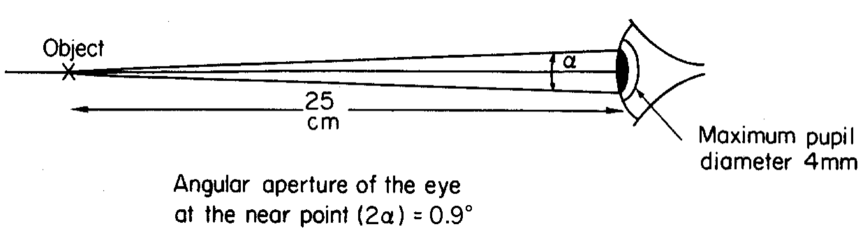


Fig. I.17 The eye has a relatively small angular aperture. (From Meek, 1970, p. 13)

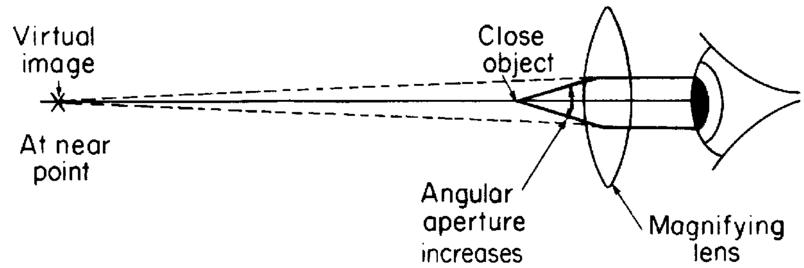


Fig. I.18 A lens increases the angular aperture of the eye. The lens allows the object to be held closer to the eye, which is thereby enabled to gather more information. (From Meek, 1970, p.14)

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 background of the same color as the ball, the visibility decreases (because of the decrease in contrast).

3) Abbe criteria of resolution:

The fundamental nature of light poses limits on the level of detail that can be resolved in a light microscope. In 1893 Ernst Abbe (1840-1905) showed that the smallest resolvable distance is about 1/2 the wavelength of light used. Thus, in this somewhat simplified description, the ultimate resolving power of any instrument is 1/2 the wavelength of the **radiation** used. This limits the usable magnification of optical microscopes to <1000X. The illustration in Sherwood's textbook provides a convenient way to recall that an object of a given size can only be "seen" with radiation whose wavelength is comparable or smaller than the object (Fig. I.19). As an aside, at first it was thought that X-ray microscopes would be useful owing to the much shorter wavelengths of X-rays compared to visible light, but the refractive index of substances for X-rays is nearly = 1, and this in turn means that refracting lenses cannot be made for X-rays, and X-rays cannot be easily focused to form images.

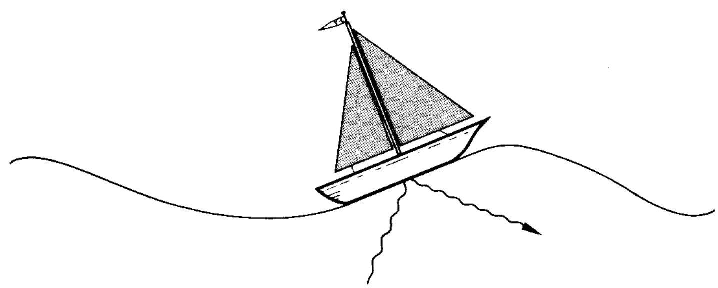


Fig. I.19 Interaction of waves with an obstacle. The boat rides the long wavelength ocean wave (and remains undetected), but reflects the small wavelength surface ripple. An observer who wishes to detect the presence of the boat can do so only by observing waves that have wavelengths smaller than, or comparable to, the length of the boat. (From Sherwood, 1976, 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 ordinary LM, with a resolving power of approximately 0.25 μm, the maximum (useful) magnification is about 250 μm / 0.25 μm = 1000X. The value used for the resolving power of the eye in this example (250 μm) 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. Note however, that it is generally not the eye but a piece of photograph film (or a digital camera) that captures and records the magnified image in a microscope. Thus, it is the resolving power of the film or camera that actually determines the maximum useful magnification of the instrument.

According to Abbe's simple criteria of resolution, at 60,000 volts, the TEM should have a resolving power of about 0.0025 nm. If you carefully consider this number, you would calculate that 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 simple 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.20).

Microscopes are particularly useful tools because they are able to make object points that 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 and whose diameter depends on the **angular aperture** of the lens.

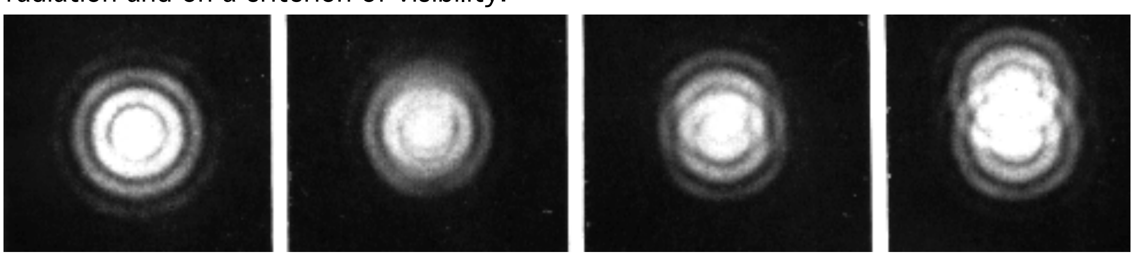
The shortest distance between two identical Airy disks at which the two disks appear partially separated corresponds to about 1/2 the width of one disk. The distance, *d*, in object space is given by:

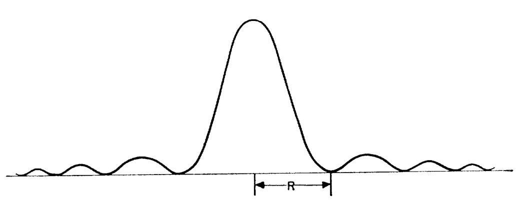
$$d = \frac{0.612\lambda}{n \sin\alpha} \qquad \text{Abbe Equation}$$

where λ = wavelength of the radiation used
n = refractive index of the media in which the radiation is refracted
α = semi-angular aperture of the lens used to form an image

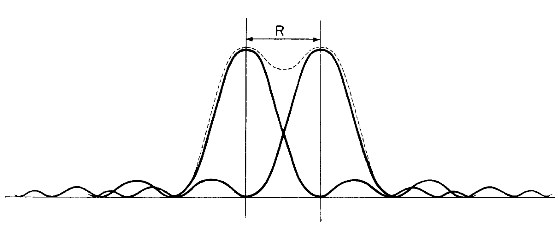
Note: $n \cdot \sin\alpha$ = the numerical aperture (N.A.) of the lens.

Thus, to maximize resolving power, λ must be decreased, n increased, or α increased. It is important to note that, in this treatment, we are concerned 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 on a criterion of visibility.





(b)



(c)

Fig. I.20 (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 one Airy disk. R, half width of the central maximum represented by a bell-shaped curve.

(c) Rayleigh criteria for resolution. Here, the separation of the two Airy disks is R, or one-half the width of one disk. (From Sjostrand, 1967, p.115)

For the LM, using oil immersion optics (n = 1.5), $\sin\alpha$ = 0.87, and violet light (λ = 400 nm), d = 0.2 μm. In the LM the only way to improve resolution is to use light of shorter wavelength since N.A. can't be increased beyond ~1.5. For the **TEM**, n = 1 (vacuum), $\sin\alpha = 10^{-2}$ and λ = 0.005 nm for 60kV electrons, thus d = 0.3 nm (*i.e.* much worse than predicted according to Abbe's simple criteria).

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:

$i = r$

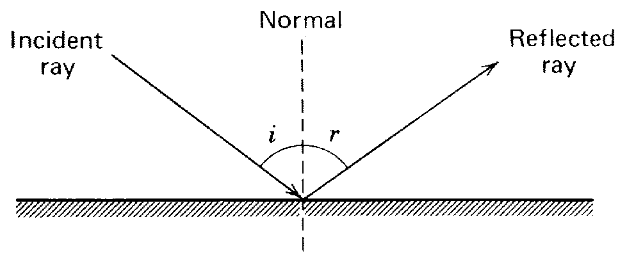


Fig. I.21 Reflection. (From Slayter, 1970, p.4)

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

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

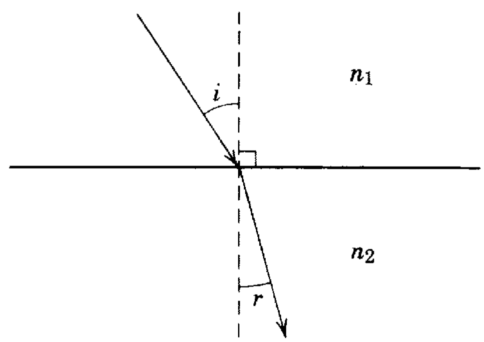


Fig. I.22 Refraction. (From Slayter, 1970, p.6)

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

All of these laws except #4 hold for electrons. When the current density is too high, electrons can repulsively interfere because of their negative charges.

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. As will be described in more detail later, fields in space around charged electrodes or solenoids is what causes electrons to be refracted.

c. Geometrical and physical optics

The fundamental principles of optics govern the design and operation of light and electron microscopes. The basic optical principles involving the use of refractive 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 is an abstraction and cannot physically exist because of the wave nature of light and electrons (*i.e.* because waves are diffracted). 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) An ideal lens refracts each ray of the bundle of rays that passes from an object point to meet in one image point.

2) Rays originating from points that lie on a plane perpendicular to the axis must be imaged in a plane that is also perpendicular to the axis. **Note**: this axis is commonly referred to as the **optical axis** of the lens.

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 because 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 properties of light. **Lens aberrations** in real lenses also contribute to various deviations from the properties of the ideal lens (discussed in more detail in § I.B.3).

The single refracting surface of spherical curvature is the <u>fundamental unit of focusing action by glass lenses</u>. Spherical refracting surfaces act as lenses for **paraxial rays**, which are those rays that pass close to the principal (optic) axis of the lens. Rays traveling at large angles and hence away from the optic axis of the lens do **NOT** obey ideal lens action.

One fundamental difference between light and magnetic lenses is that the <u>electron beam does not change in forward velocity</u> as it passes through the magnetic field (light rays slow down when passing into a medium of higher refractive index). Also, <u>refraction is continuous</u> for electrons when they are in the magnetic field. Conversely, light is refracted only at interfaces between media of differing refractive index. As an interesting footnote, electrons follow spiral rather than straight trajectories through the magnetic field (see also § I.A.5.c).

e. Ray diagrams: (Figs. I.23-I.29)

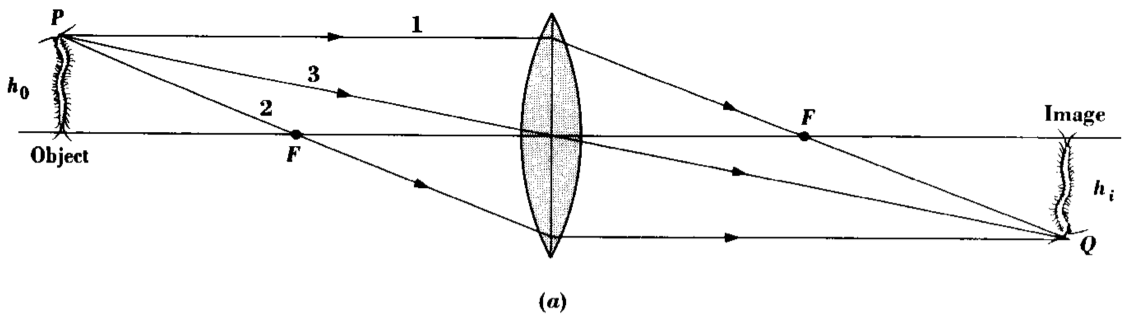
The method of construction of ray diagrams is based on three simple principles:

1) All rays entering a lens **parallel to its 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. Footnote: for light, this applies only to the location of light paths and not to its intensity.

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 of (behind) the lens.



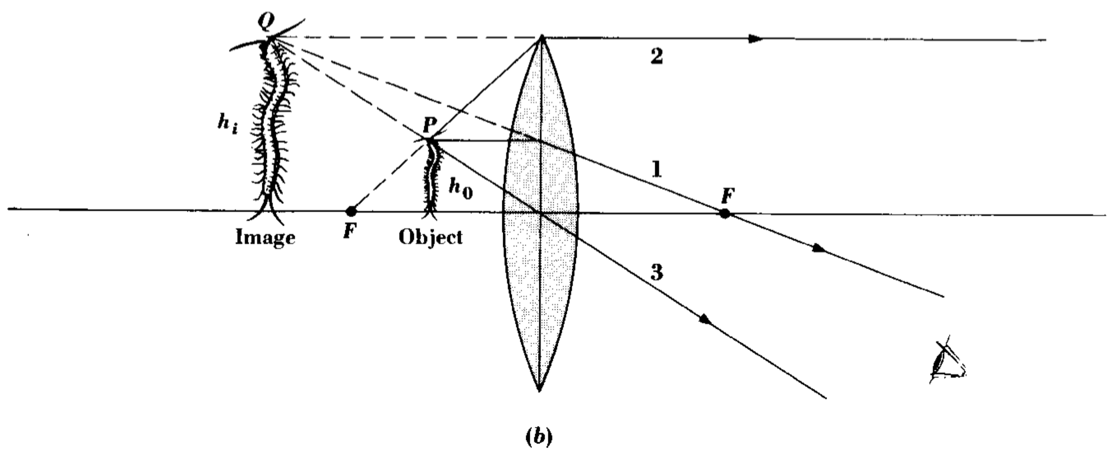


Fig. I.23 Principal ray diagrams illustrate image formation by a convex lens.
(a) When the object distance is greater than the focal length, a real, inverted image is formed.
(b) When the object distance is less than the focal length, a virtual, erect image is formed; its position is obtained by projecting the principal rays backward. The rays appear to come from point Q. (From Young, 1968, p.127)

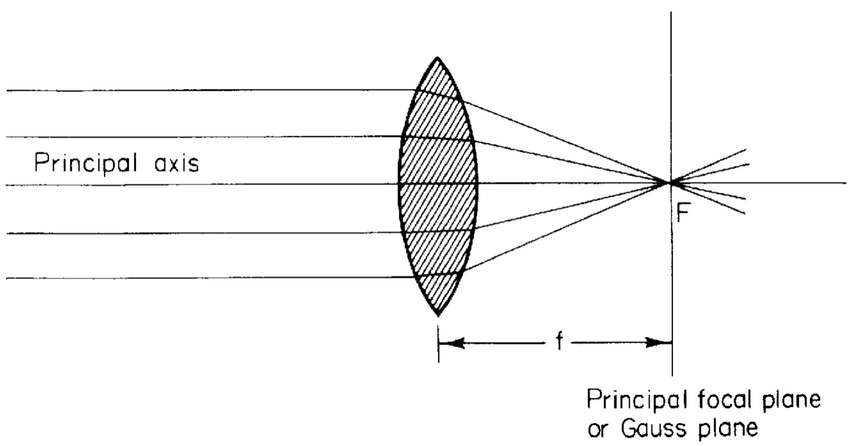


Fig. I.24 Definition of principal focus, F, in image space of a lens. (From Sjostrand, 1967, p.20)

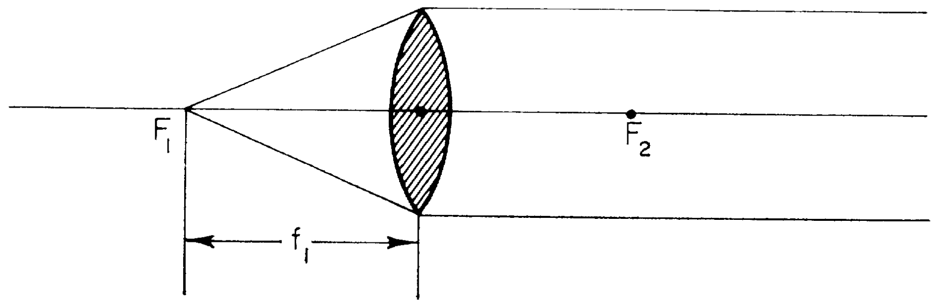


Fig. I.25 Definition of principal focus in object space F_1 of a lens. (From Sjostrand, p.21)

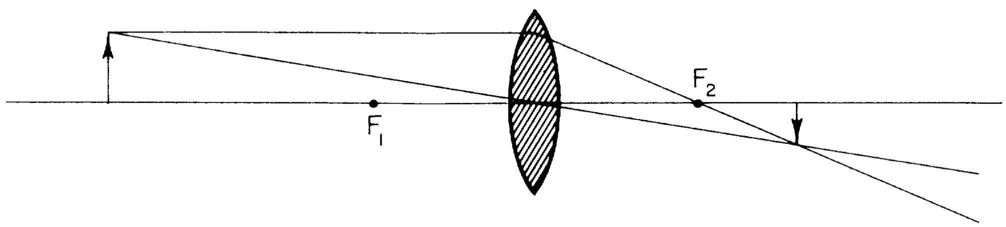


Fig. I.26 Construction of the image of an object by means of ray tracing. (From Sjostrand, 1967, p.22)

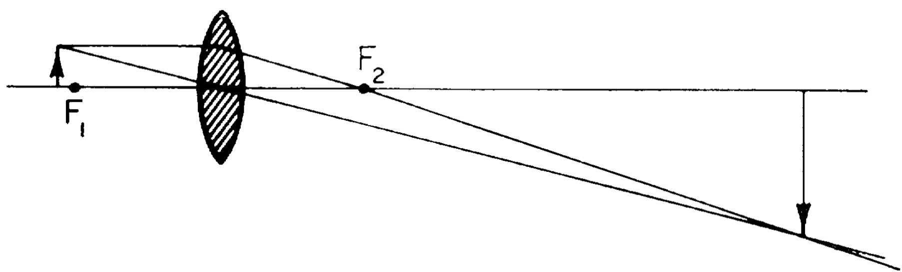


Fig. I.27 Magnifying effect of a positive lens. (From Sjostrand, 1967, p.22)

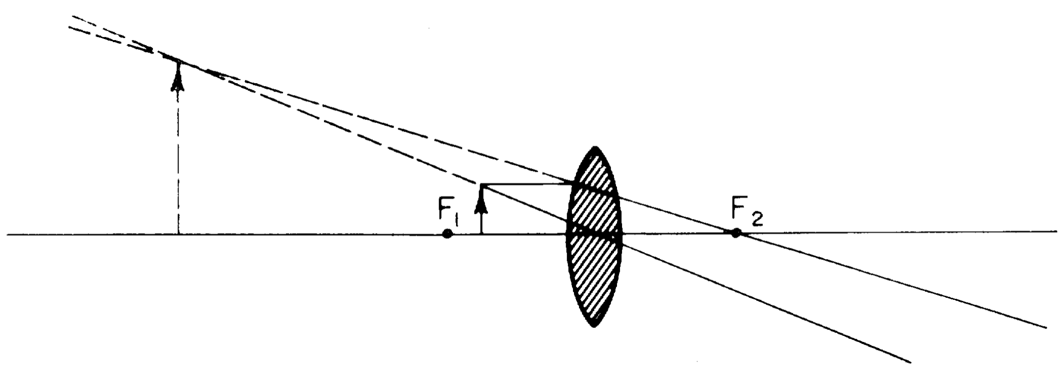


Fig. I.28 Virtual, magnified image of object located between principal focus in object space and the lens. (From Sjostrand, 1967, p.22)

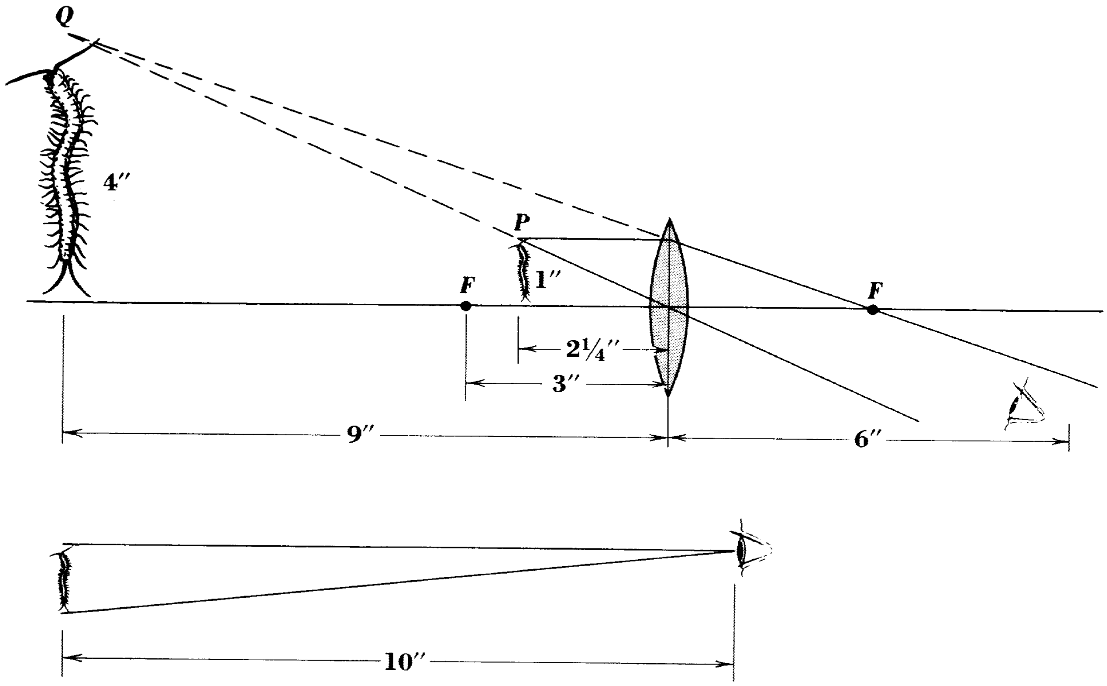


Fig. I.29 A magnifying glass forms an enlarged, erect virtual image. The **angular size** of this image is approximately 4"/15" or 4/15. The angular size of the object at the closest distance for comfortable viewing is 1"/10" or 1/10. The magnification in this situation is (4/15)/(1/10), or 2.67. (From Young, 1968, p.130)

f. Definitions:

REAL IMAGE: one at which light rays physically reunite and a photographic plate placed at the position of a real image will be 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 a TEM is sometimes used this way in order to reduce the final size of the real image formed by the projector lens or lenses.

CONVERGING (POSITIVE) LENS: bends rays **toward** the axis. It has a **positive focal length.** It 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.** Footnote: It is **NOT** possible to construct a negative **magnetic** lens, though 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 of the lens)
i = distance of image from the lens (positive to the right of the lens)

NOTE: For a <u>virtual</u> image, i has a <u>negative</u> value.

h. Magnification: $M = \left| \frac{i}{o} \right|$ (Figs. I.26-I.29)

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 exactly at a distance = $2f$, the image and object are the same size ($M = 1$); when the object lies between f and $2f$, the image is larger than the object ($M > 1$); when the object lies at a distance less than f, the image is **virtual, erect,** and **larger** than the object ($M > 1$).

i. Angular aperture of the lens (2α) (Fig. I.30)

The size (aperture) of the lens determines the total amount of radiation arriving from the object that 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 the lens is able to accept from the object. Placing 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, which corresponds to a 2α 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.17).

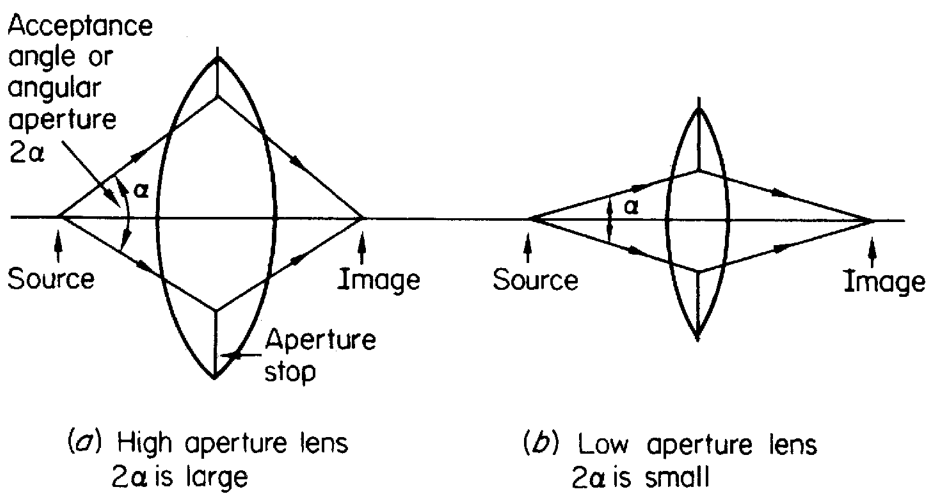


Fig. I.30 Angular aperture of a lens. The angle 2α is the acceptance angle of the lens, and the larger it can be made, the more information the lens can transmit from object to image. A large lens of high aperture can therefore tell us more about an object than a small lens of low aperture. (From Meek, 1970, p.12)

j. Simple vs. compound microscope (Figs. I.31-I.33)

In principle, a real image of any desired magnification can be obtained from a single positive lens. However, in practice this is cumbersome because of the long lens-image (*i*) 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:

$M = 10{,}000 = x_{image}/x_{object} = x_i/x_o$

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

Solving for x_o and x_i gives:

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

The obvious moral to this lesson is that a magnification on the order of 10,000X is not practical using a one-lens optical system.

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: $1/2 = (1/x_o) + (1/100x_o)$

$x_o = 2.02$ 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)

Hence, even a two-lens optical system is impractical for achieving magnifications of ~10,000X. TEMs must therefore use three or more lenses to produce images of sufficient magnification in the confines of a normal laboratory (*i.e.* within a space of 1-2 meters). (Figs. I.31-I.33)

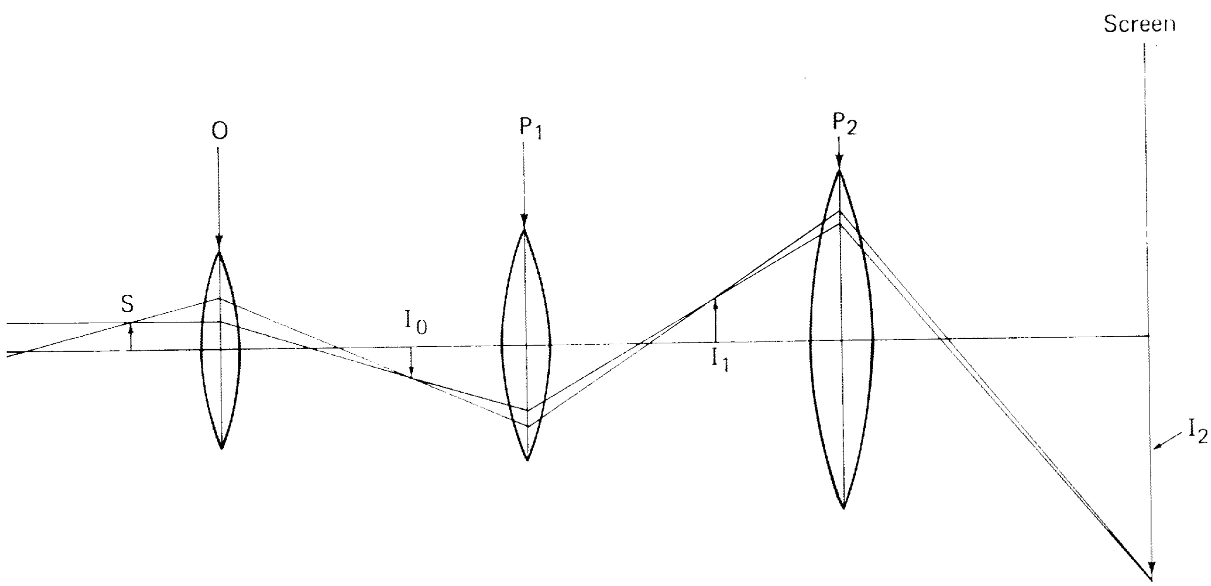


Fig. I.31 Ray diagram for high magnification mode of operation in a three lens TEM. Note that each lens forms a real, inverted image. I_0 is the image formed by the objective lens O; I_1 is formed by the first projector lens P_1 and I_2 by the second projector P_2, on the screen. (From Agar, 1974, p.30)

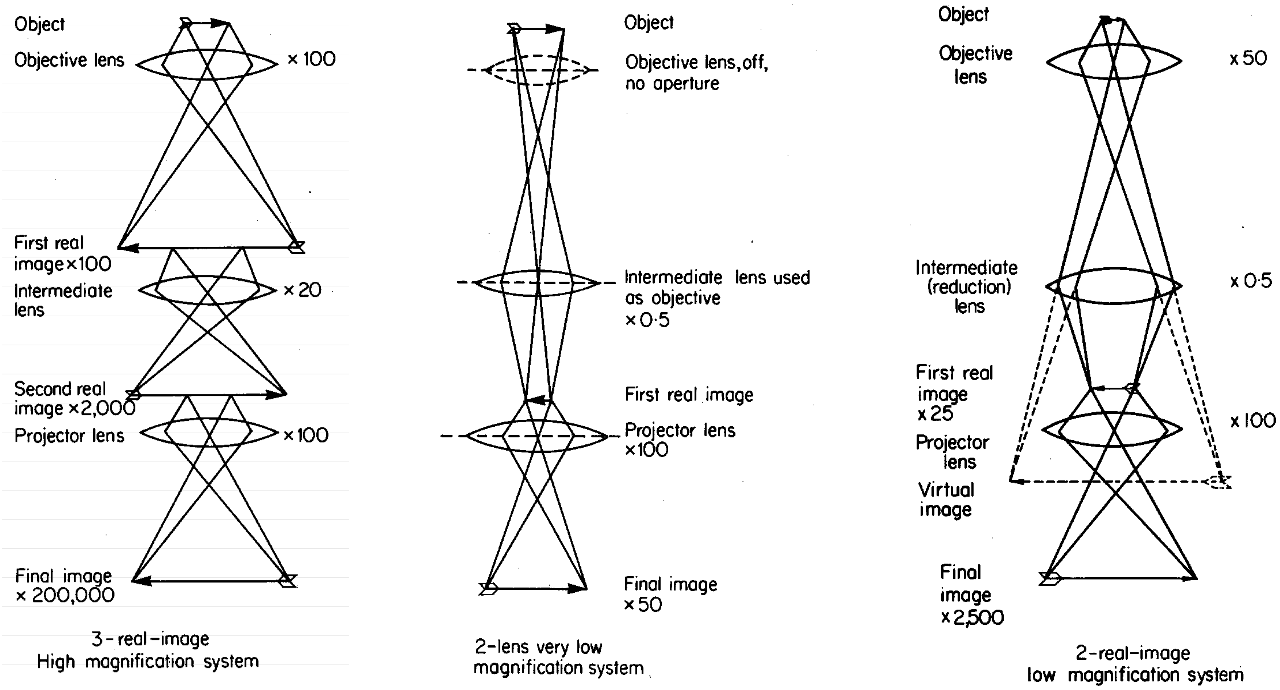


Fig. I.32 Left: ray diagram of the 3-real image, medium, and high magnification imaging system of a 3-lens microscope. Center: 2-real-image, very low or 'scan' magnification range of a 3-lens microscope. Right: 2-real-image low magnification system of a 3-lens microscope. (From Meek, 1970, pp.118 and 120-121)

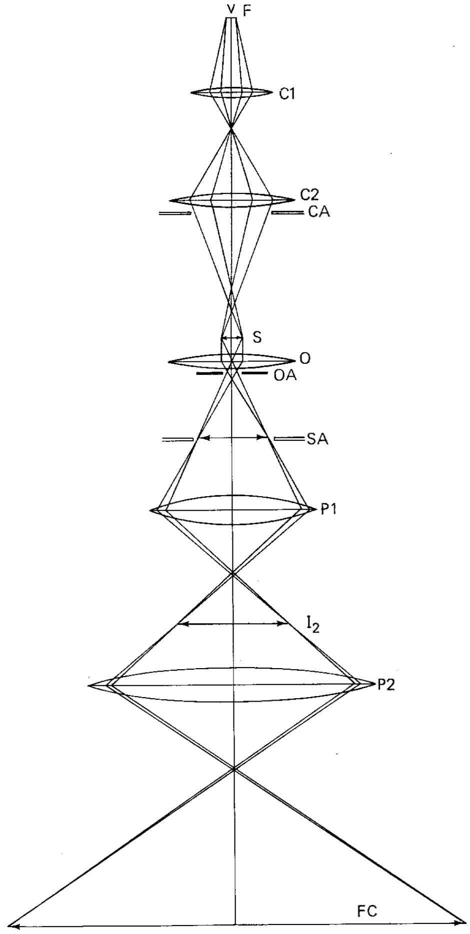


Fig. I.33 Ray diagram for a complete electron microscope. Filament (F), condenser 1 lens (C1), condenser 2 lens (C2), condenser 2 aperture (CA), specimen (S), objective lens (O), objective aperture (OA), 1st intermediate image and selector aperture (SA), intermediate or first projector lens (P1), second intermediate image (I_2), second projector lens (P2), and final image on the fluorescent screen (FC). (From Agar, 1974, 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**, and considered thin 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 <u>and</u> final images. What is the magnification at <u>each</u> stage of image formation and what is the magnification of the <u>final</u> image? **D** is the distance from the center of lens 1 (**L1**) to the center of lens 2 (**L2**). Distance values are in arbitrary units.

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

A basic understanding of the chemistry of metal atoms provides the foundation for explaining how a beam of imaging electrons is produced inside an electron microscope. 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. This shielding effect thus reduces the attraction between valence electrons and the nucleus. Metal atoms are characterized as having two, loosely bound, valence electrons that migrate freely (this is why metals are good electrical conductors) and can escape from the metal completely if sufficient additional energy is added to them. When the temperature of a metal is increased, the kinetic energy of the electrons increases because of increased thermal vibrations of the metal ions. **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 metal ions. With increasing temperature, some electrons acquire sufficient energy to overcome the attraction and leave the metal temporarily. When shaped in the form of a thin wire, metal 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 has a low work function and therefore it emits more electrons than metals with higher work functions (see also § I.B.1.b and Fig. I.57).

If a strong electrostatic field is applied in a vacuum between a **cathode** (a wire given a negative potential) and an **anode,** the electric field established between the cathode and anode will cause electrons that have escaped from the surface of the wire to accelerate away from the wire towards the anode surface (Fig. I.34). The strength of the electrostatic field (**voltage**) between the cathode and anode is what determines the **velocity** at which electrons travel from cathode to anode. Recall, the relation between the speed or velocity of electrons and voltage is given by equation (3) (see **§ I.A.3.b**). The **number** of electrons that leave the wire depends on the **temperature** to which the wire is heated, and this depends on how much **filament current** passes through the wire.

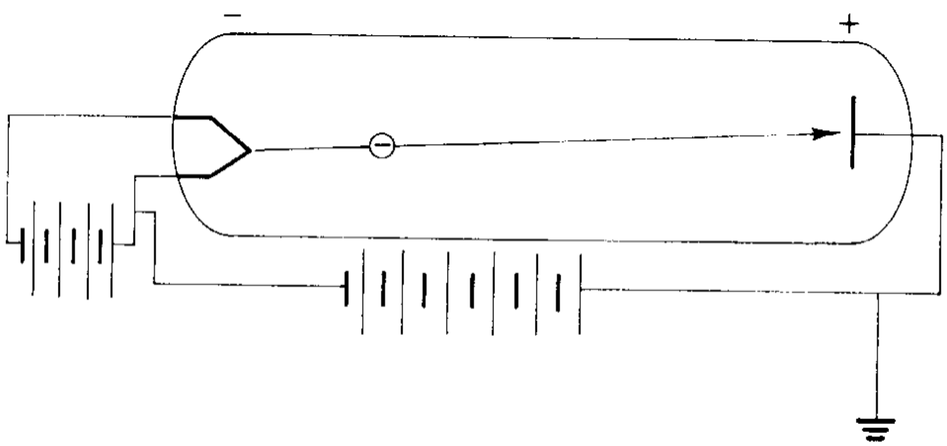


Fig. I.34 Acceleration of an electron in an electric field. (From Sjostrand, 1967, p.26)

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

b. Electric field / Equipotentials

Any 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.35-I.36)

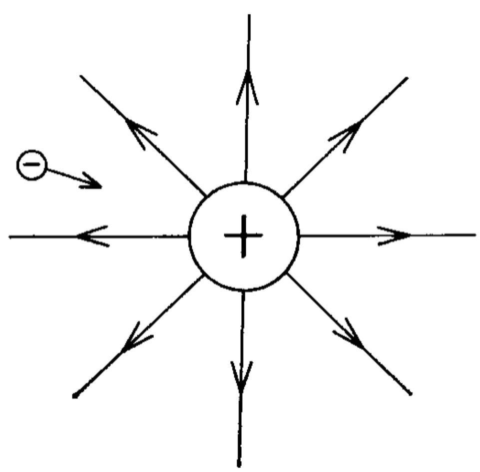


Fig. I.35 Lines of force at a positively charged spherical body. (From Sjostrand, 1967, p.32)

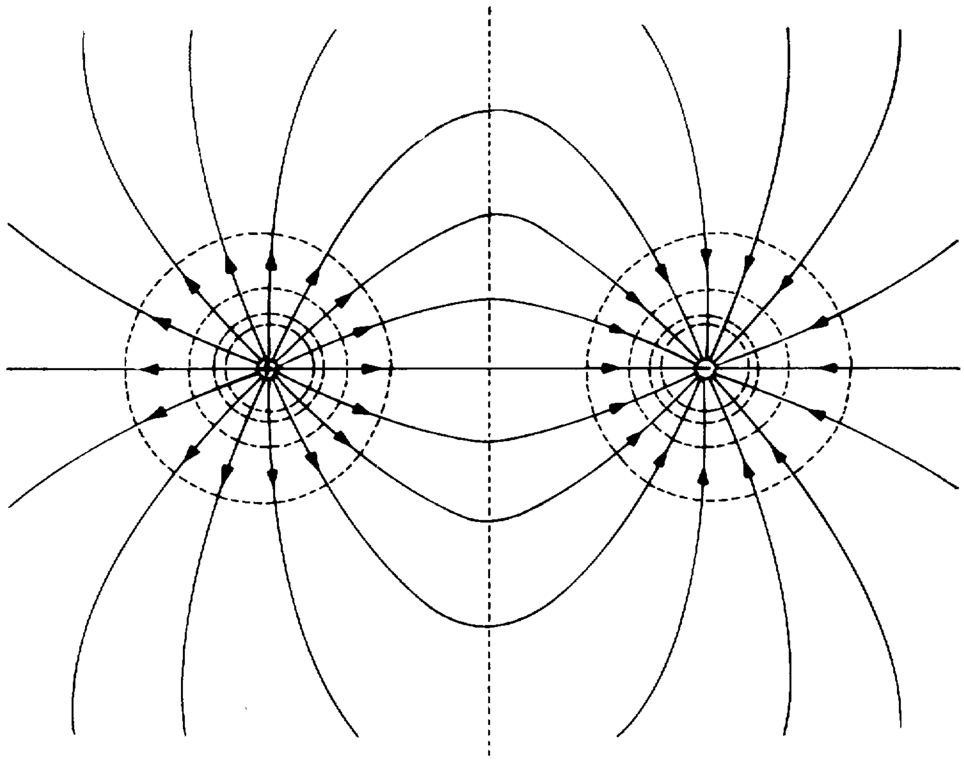


Fig. I.36 Lines of force and equipotential surfaces (stippled lines) associated with two equal charges of opposite sign. (From Sjostrand, 1967, p.32)

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:** Equipotential lines define the points along the lines of force with identical electrical potential. These equipotential surfaces are always oriented perpendicular to the lines of force. The changes in the electric potential are gradual in space.

Moving electrons that 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.37). These electrons are attracted toward the positive plate. The path changes in a series of gradual steps at the equipotential surfaces.

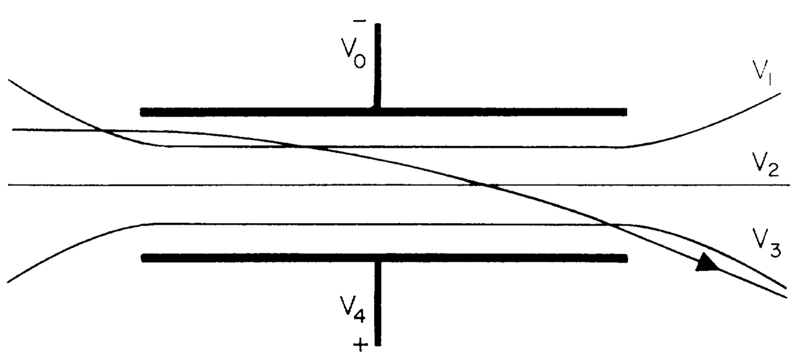


Fig. I.37 Equipotential surfaces at two parallel plates of opposite charges with the path of an electron indicated within the homogeneous part of the field. (From Sjostrand, 1967, p.33)

The electron path is "refracted" at the equipotential surface (Fig. I.38) and the result is fundamentally the same as that given by Snell's Law of refraction in light optics (§ I.A.4.a). A consequence of this is that a spherically curved equipotential surface exhibits the properties of a lens.

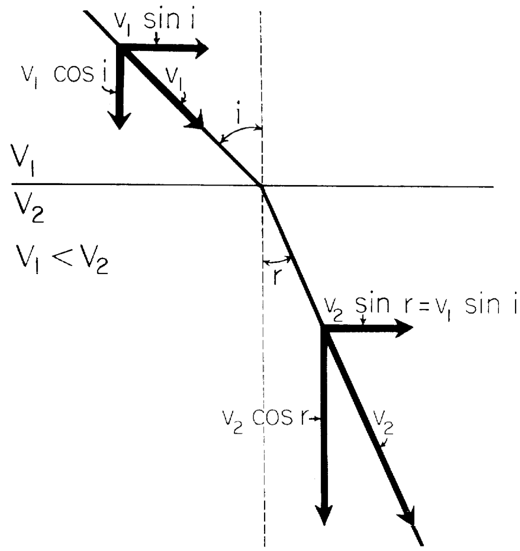


Fig. I.38 Refraction of electron at an equipotential surface. (From Sjostrand, 1967, p.33)

For **electrostatic** lenses, both positive (converging) and negative (diverging) lenses can be made (Figs. I.39 and I.40). This feature of electrostatic lenses differs from electromagnetic lenses since the latter can only act as converging lenses.

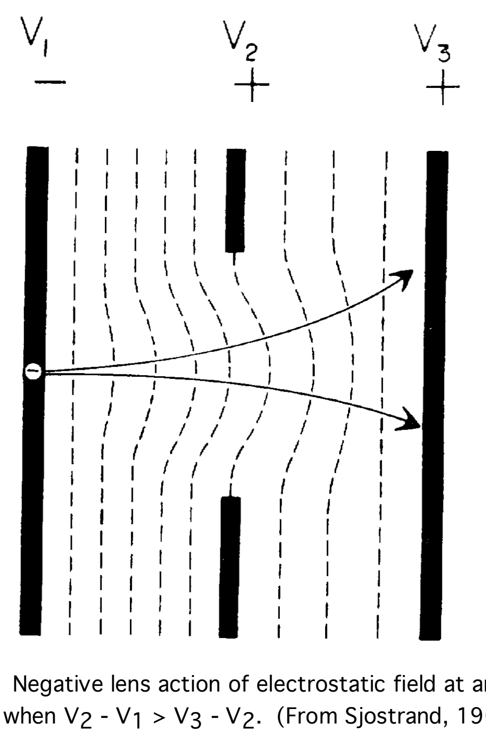


Fig. I.39. Negative lens action of electrostatic field at an aperture when $V_2 - V_1 > V_3 - V_2$. (From Sjostrand, 1967, p.34)

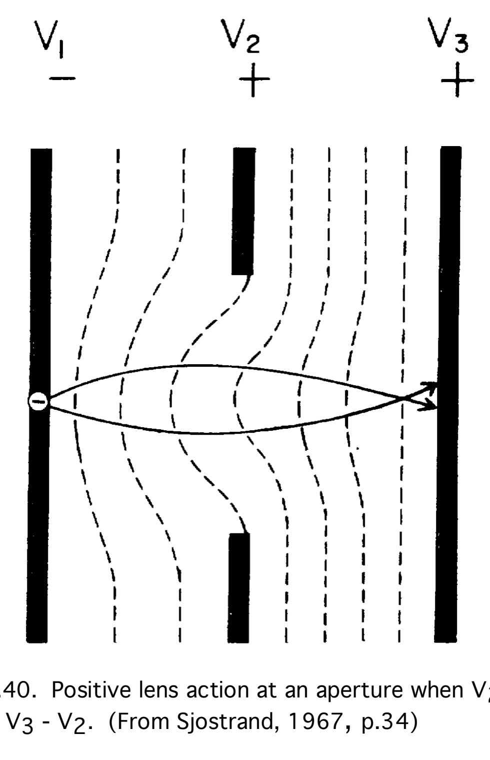


Fig. I.40. Positive lens action at an aperture when $V_2 - V_1 < V_3 - V_2$. (From Sjostrand, 1967, p.34)

c. 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 there are at abrupt air-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 as is possible with glass optics.

Although electron microscopes that use electrostatic lenses have been made, all modern, commercially available TEMs use electromagnetic lenses. A primary reason for this is that electrostatic lenses are more sensitive to the quality of the vacuum and cleanliness of the components than are electromagnetic lenses. Also, compared to electromagnetic lenses, some lens aberrations are more severe for electrostatic 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.

d. 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 that may accumulate on physical apertures (such as the objective aperture) and transform them into weak electrostatic lenses, which can distort the electron image.

Properties of electrostatic lenses:

- 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.
- For electron lenses, replace $\sqrt{(\phi)}$ 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 § I.A.5.c).
- If bounded by regions where ϕ is constant, an electrostatic lens is always convergent.

e. Magnetic fields and magnetic lenses

1) Magnetic field

An electric current passing through a conductor gives rise to a magnetic field. By convention, the lines of the magnetic field point North (N) (Fig. I.41). **Magnetic flux** is the total number of lines and the **flux density** is the number of lines per unit area of a surface. The **RIGHT hand rule** defines 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.

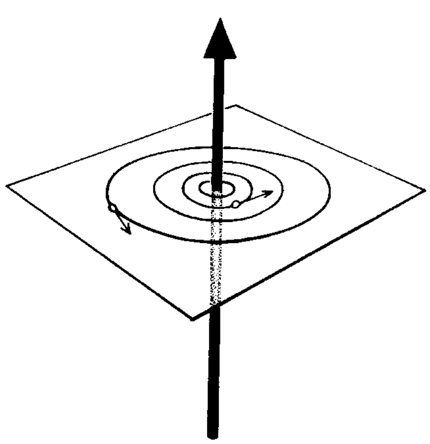


Fig. I.41 Magnetic field induced by current passing through a conductor. (From Sjostrand, 1967, p.35)

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 that affects the flux density is called the **permeability**, μ, of the material. For air and vacuum, $\mu = 1.0$. 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.42-I.43).

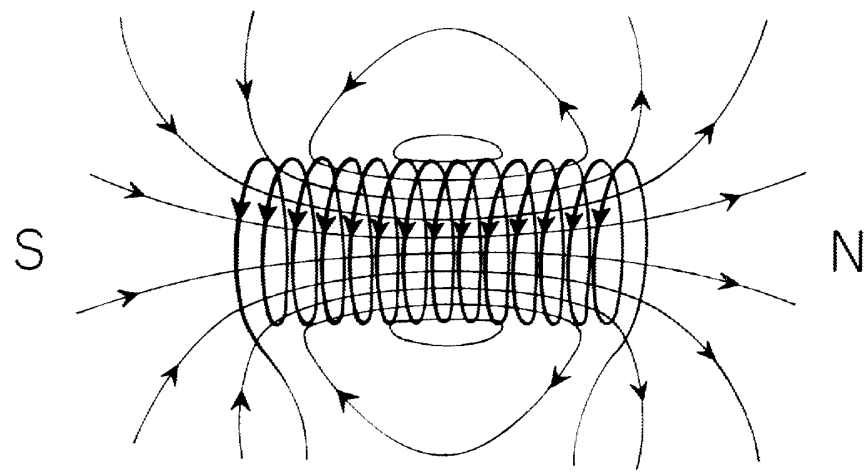


Fig. I.42 Magnetic field induced by current passing through a solenoid. (From Sjostrand, 1967, p.37)

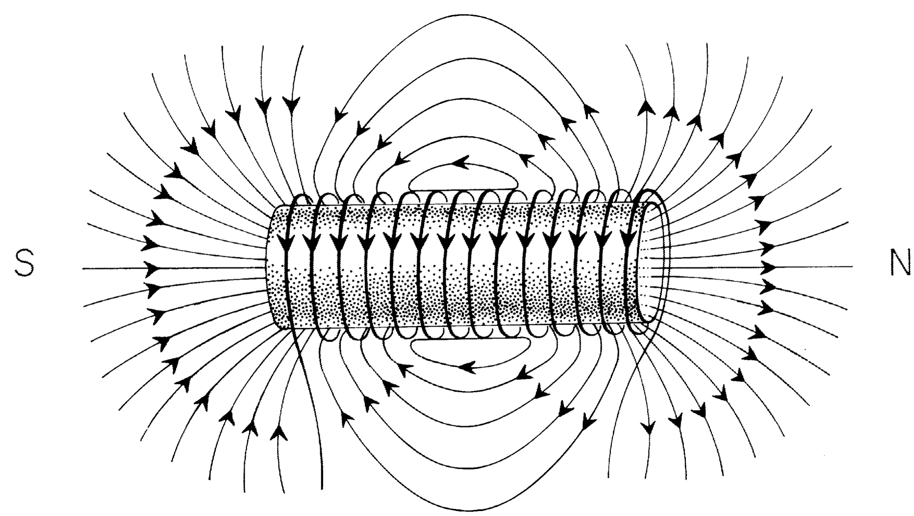


Fig. I.43 Solenoid with iron core. (From Sjostrand, 1967, p.40)

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·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. NOTES: one weber/m^2 = 10^4 gauss. For 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 for high values of H or when the flux density, B = H (Fig. I.44). Iron's high permeability is a consequence of the induced magnetic field that orients microscopic crystal regions in the iron, which act as tiny magnets. These tiny magnets add their magnetic fields to the induced field (Fig. I.45). 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.

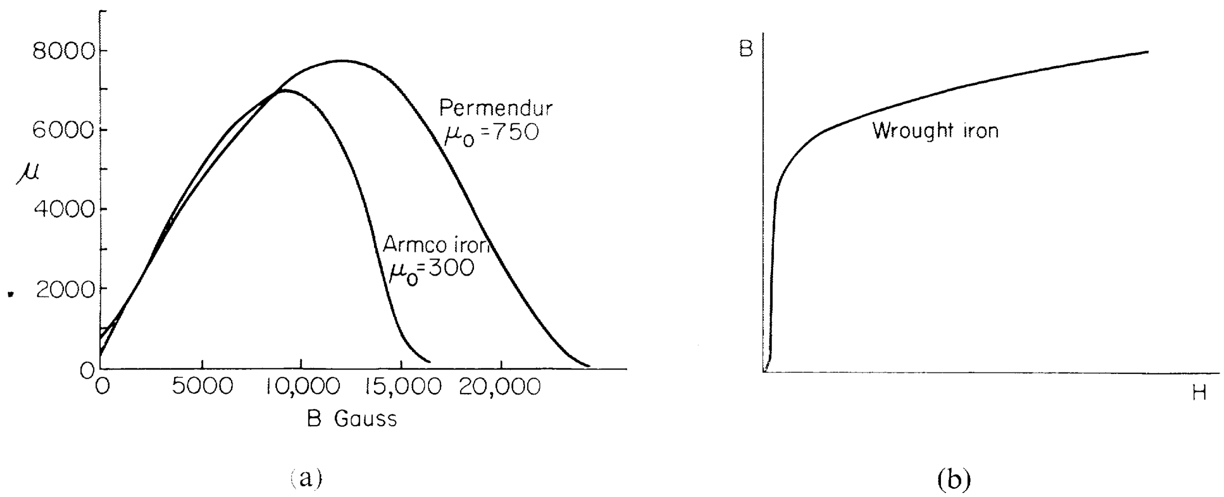


Fig. I.44 (a) Dependence of permeability on flux density, B. (b) Relationship between flux density B and field strength H. (From Sjostrand, 1967, p.38)

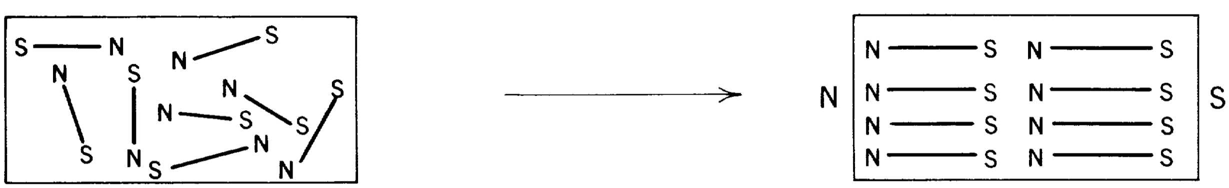


Fig. I.45 Magnetization of iron. On the left, the micromagnets are randomly oriented. On the right, the micromagnets line up under the influence of a field and the field strength increases. (From Slayter, 1970, p.361)

2) Hysteresis

Lens strength 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.46). 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.

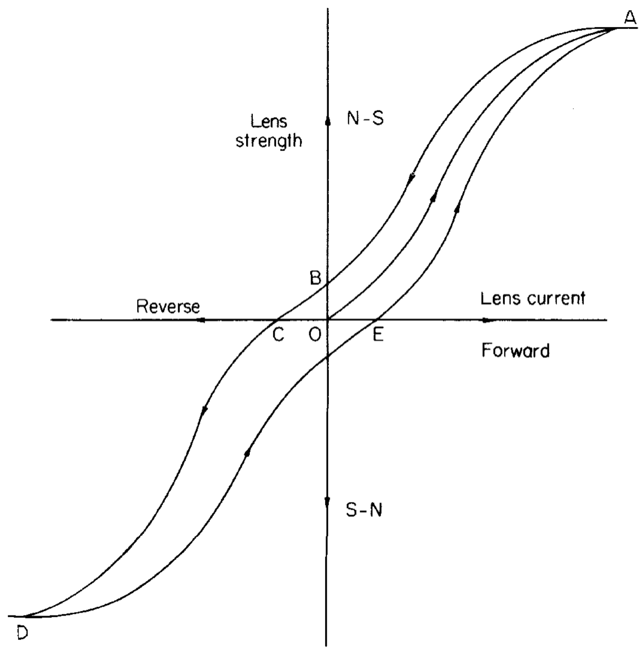


Fig. I.46 Curves showing how the magnetization of soft iron (lens strength) is related to the magnetizing force (lens current). An unmagnetized lens starts from the point O and follows the path OA as lens current increases. At point A, an additional increase in lens current produces no further increase in lens strength and 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 'hysteresis'. 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, following 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 calibrate a lens current meter accurately in terms of lens strength or magnification. (From Meek, 1970, p.68)

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

Normalization of a TEM lens is accomplished by reducing the lens current to zero some predetermined number of times. First saturating a lens and then returning it to the working current without overshooting can also minimize hysteresis. When the field strength is reduced to zero, some magnetization still remains in the iron (**residual magnetization** or **remanence**). Soft iron has the advantage that, when used in an electromagnet, hysteresis is low.

Introduction of pieces of iron into a magnetic field drastically affects the flux density. Magnetic material has a shielding effect, the effect being greater the greater the permeability of the material. Permalloy, or μ metal (20% iron, 80% nickel), has a maximum permeability of 80,000-140,000 compared to the ~7000 for iron used in transformers. μ metal permeability is limited to field strengths lower than those at which iron still retains high permeability. This metal is therefore useful for shielding the TEM from external magnetic fields.

f. The electromagnetic lens

1) Development of lens design (Figs. I.47-I.49)

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.

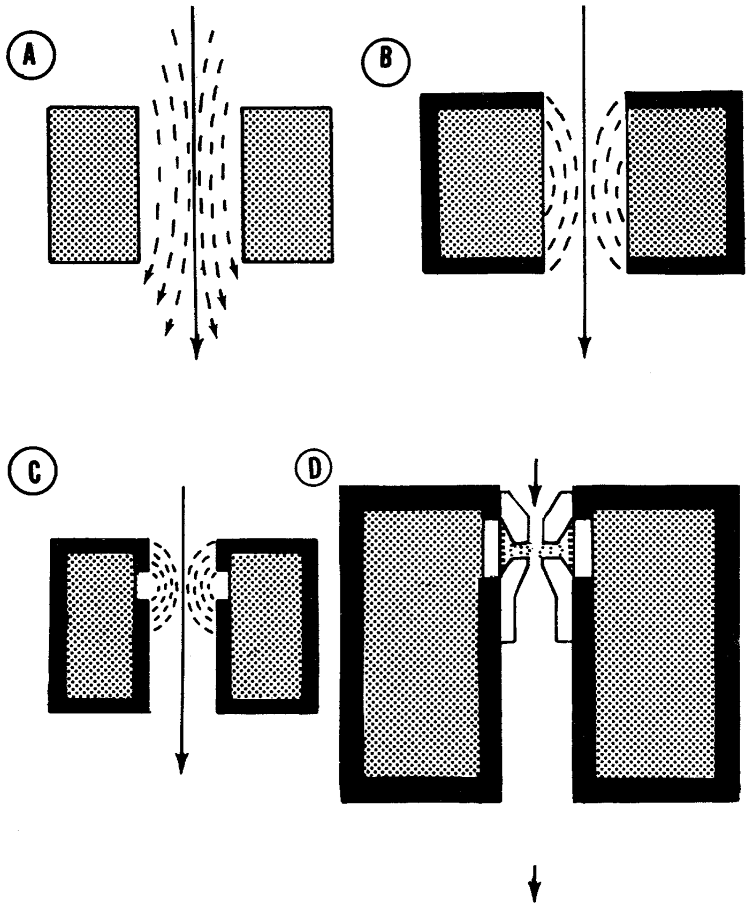


Fig. I.47 Evolution of magnetic electron lenses. (A) Short solenoid used as a magnetic lens. (B) Soft-iron casing enclosing outer surface of the solenoid, thus concentrating the field. (C) Soft-iron encasing the solenoid except at a narrow annular gap thereby reducing the magnetic field to a very short 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 Wischnitzer, 1970, p.33)

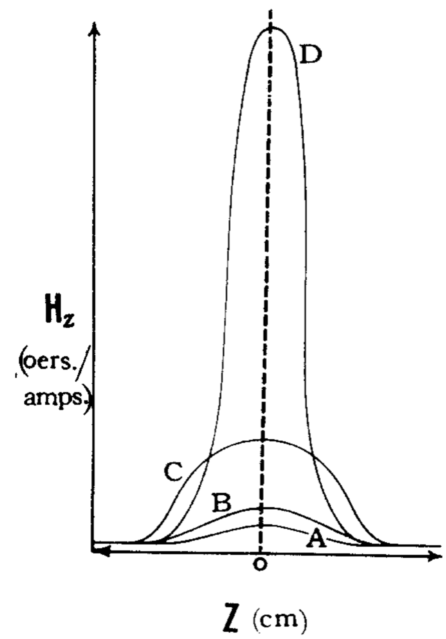


Fig. I.48 Field strength distribution curves. The curves A-D correspond to the respective lenses illustrated in Fig. I.47. Each represents the field strength along the long axis of the lens. The changes in the shape of the curves represent the shortening or concentration of the field over a shorter axial distance. H_Z = longitudinal magnetic field. Z = distance along the axis of symmetry. (From Wischnitzer, 1970, p.33)

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

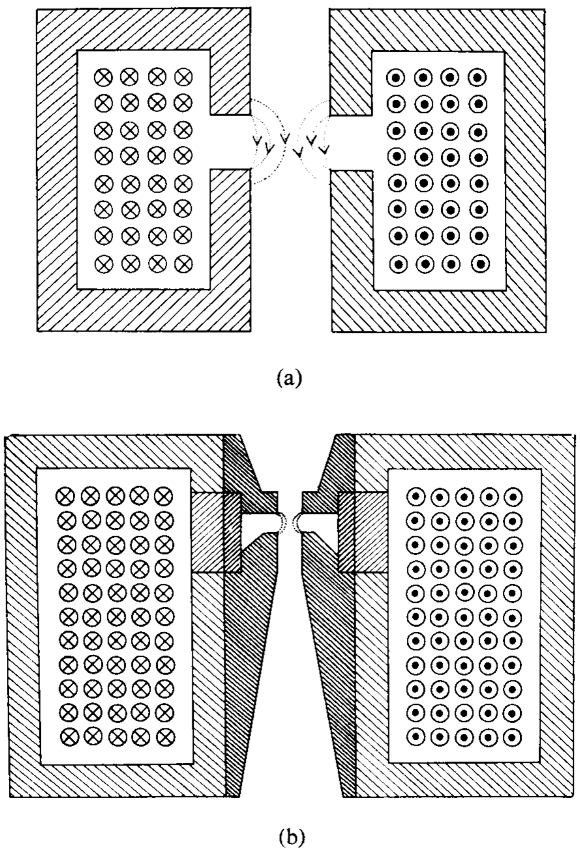


Fig. I.49 (a) Magnetic lens with a tightly wound coil and a soft iron shroud surrounding the coil except for a small gap, where the field is concentrated. (b) Electromagnetic lens with pole pieces and a short focal length. (From Sjostrand, 1967, p.50)

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.50 - I.52). An important but very subtle fact is that the field only acts on the velocity component that is directed perpendicular to the lines of force. The **left hand rule** is used to specify the directions of current, field and force on the electron (Fig. I.51: first finger points in direction of the field; middle finger the current direction; and thumb the 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.

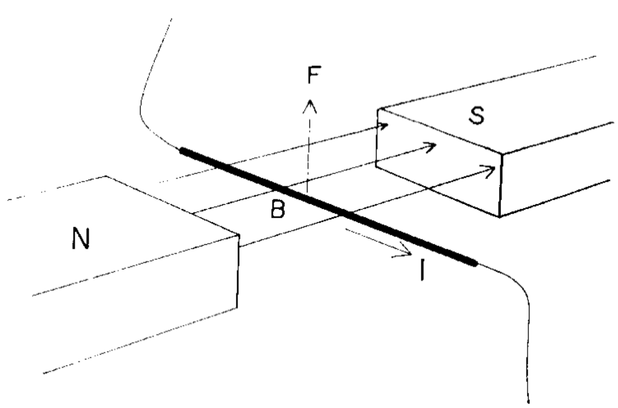


Fig. I.50 The force F acting on a straight conductor in a homogeneous magnetic field of flux density B when current I is passed through the conductor. (From Sjostrand, 1967, p.43)

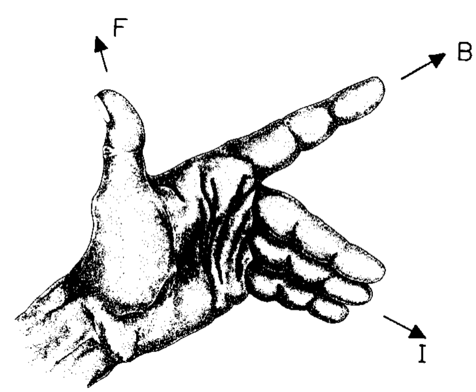


Fig. I.51 Left hand thumb rule. (From Sjostrand, 1967, p.43)

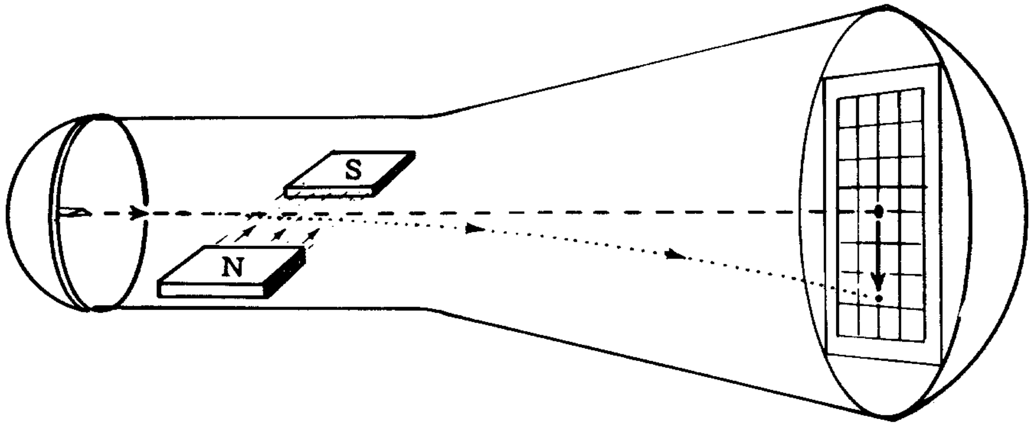


Fig. I.52 Thompson's experiment. A stream of electrons originating from a source and passing, *in vacuo*, through a magnetic field produced by a pair of magnets will be deflected. The direction of deflection demonstrates that electrons are negatively charged particles of matter. (From Wischnitzer, 1970, p.25)

When electrons enter the lens they encounter a **sideways force** that causes the path of the electron to curve as it passes through the lens (Figs. I.53 - I.55). 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. Hence, electrons enter an electromagnetic lens without angular momentum about the axis and leave without angular momentum. The net effect is a deflection of the electrons toward the axis, and those electrons that start at a point on the axis in front of the lens must also cross the axis at the back focal point behind the lens. The angle between any object vector and the corresponding 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 electromagnetic lens is convergent no matter what the direction of the field.

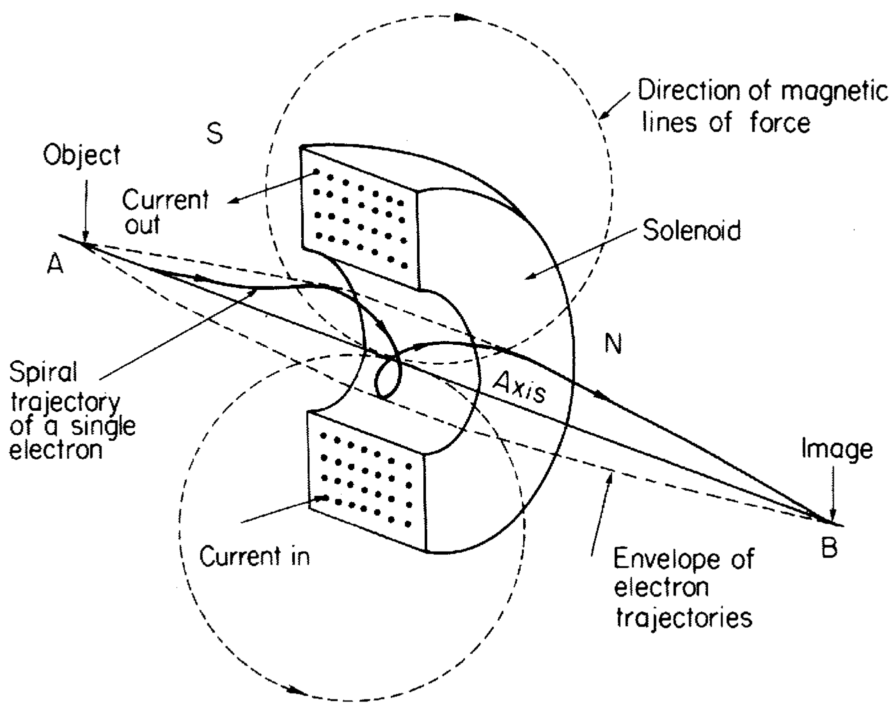


Fig. I.53 Action of a solenoid on an electron beam. An electric current passing through the coil produces an axial magnetic field. This is the refracting medium for the electrons. An electron starting at a point on the axis A and at an angle to it follows a spiral path, returning to the axis at the point B. The action is basically similar to that of the converging light lens shown in Fig. I.23. **NOTE:** The arrowheads on the dashed circles point in the wrong directions. (From Meek, 1970, p.8)

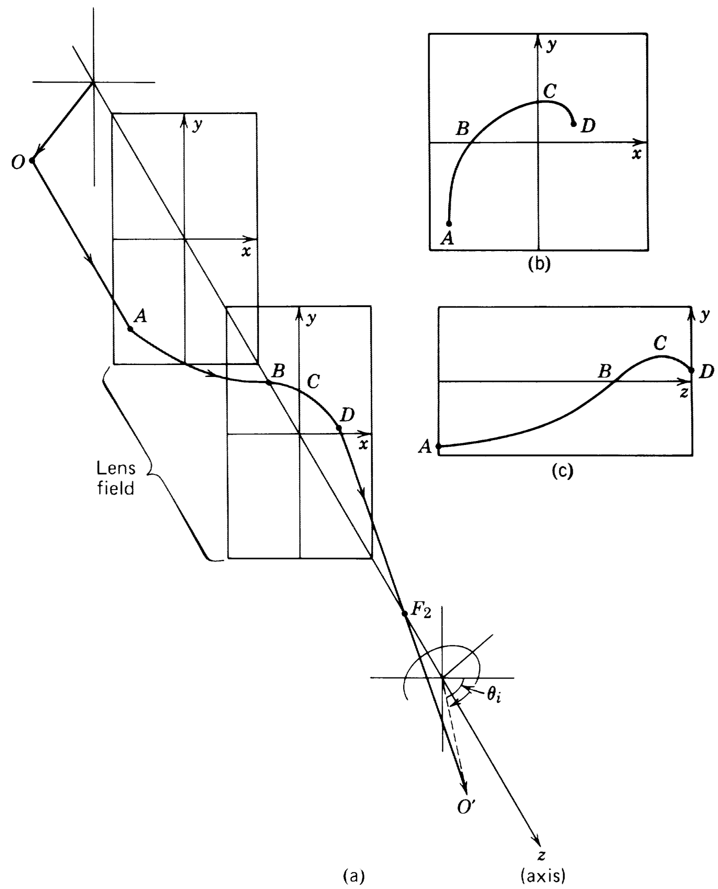


Fig. I.54 Action of the magnetic lens. (a) In perspective. (b) Electron trajectory seen in projection, along the direction of propagation. (c) Side view of electron trajectory in projection. (From Slayter, 1970, p.358)

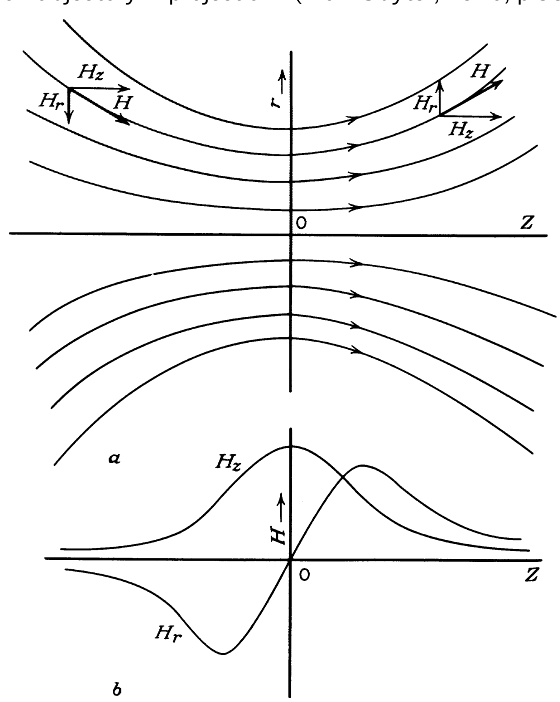


Fig. I.55 Components of the vector H near the axis of fields with axial symmetry. H is comprised of two components, H_z, the component in the *z* (axial) direction, and H_r, the component in the *r* (radial) direction. (From Hall, 1966, p.85)

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**. A conventional, axially symmetric lens is always bounded by regions that are field-free. As a consequence, the net action of any electron lens is inevitably **convergent**. Limited regions may be divergent but not the lens as a whole. The serious consequence of this is that neither spherical nor chromatic aberrations can be corrected as is done in light optics by combining 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 $180° + \theta$ 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 that 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, focal length, *f*, 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, corrected for relativistic effects
$N \cdot I$ = the number of ampere turns in the excitation coils

For magnetic lenses, focusing is achieved by **varying the current** that 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 as current increases until a point is reached where the lens is saturated and no further increase in lens strength can be achieved (Fig. I.46).

Since the focal length of the lens is directly proportional to accelerating voltage, if the imaging beam consists of electrons with different velocities, image quality is affected because there is no way to perfectly focus the electrons (chromatic aberration; see § I.B.3.d).

5) Magnetic lens design: (Figs. I.47, I.49).

Condenser lenses usually have a relatively large bore and spacing and these result 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 microscope vacuum. Only those regions through which the electron beam passes are subject to high vacuum. Magnetic lenses must be water-cooled to dissipate the large amounts of heat produced by the currents in the electromagnet coils.

I.A.6. References Cited in §I.A.

Agar, A. W., R. H. Alderson, and D. Chescoe (1974) Principles and Practice of Electron Microscope Operation, pp. 1-345. *In* A. M. Glauert, Ed., Practical Methods in Electron Microscopy. Vol. 2, North-Holland Pub. Co., Amsterdam.

Glusker, J. P., and K. N. Trueblood (1972) Crystal Structure Analysis: A Primer, p. 192. Oxford University Press, New York.

Hall, C. E. (1966) Introduction to Electron Microscopy, 2nd Ed., p.397. McGraw-Hill Book Co., New York.

Meek, G. A. (1970) Practical Electron Microscopy for Biologists, 1st Ed., p.498. Wiley-Interscience, London.

Sherwood, D. (1976) Crystals, X-rays and Proteins, p. 702. John Wiley & Sons, New York.

Slayter, E. M. (1970) Optical Methods in Biology) p.757. John Wiley & Sons, New York.

Sjöstrand, F. S. (1967) Electron Microscopy of Cells and Tissues, (Vol 1: Instrumentation and Techniques) p.462. Academic Press, New York.

Wischnitzer, S. (1970) Introduction to Electron Microscopy, 2nd Ed., p.292. Pergamon Press, New York.

Young, H. D. (1968) Fundamentals of Optics and Modern Physics, (McGraw-Hill Series in Fundamentals of Physics. Introductory Program) p. 510. McGraw-Hill, New York.