

I. THE MICROSCOPE (Continued)

I.B. DESIGN OF THE TRANSMISSION ELECTRON MICROSCOPE

Discussion of TEM instrumentation is subdivided into the following topics:

1. Electron gun
2. Condenser lens(es)
3. Lens aberrations and other non-ideal imaging properties
4. Objective lens and specimen stage
5. Projector lenses
6. Camera and viewing system
7. Vacuum system
8. Electrical system

I.B.1. The Electron Gun

a. Gun design

The gun in nearly all entry-level TEMs consists of a tungsten wire (**filament**; Fig. I.56a, I.57), which is bent into a hairpin ("V") shape and surrounded by a **shield** that has a circular aperture of 1-3 mm diameter centered just below the filament tip (Fig. I.56d). Electrons emitted by the filament travel with variable but low velocities (0.2 volt). Electrons in the gun are accelerated across a potential difference of the order of 100,000 volts between the **cathode** (at high negative potential) and **anode** (at ground potential).

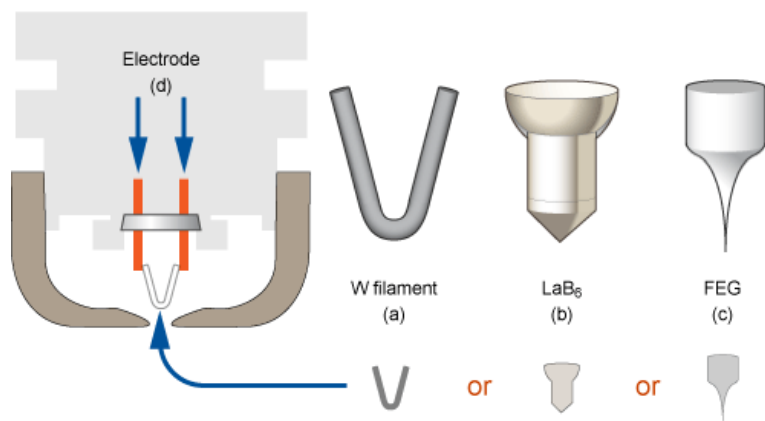


Fig. I.56. Electron gun filaments. (a) Standard tungsten filament. (b) Lanthanum hexaboride gun. (c) Tungsten crystal field emission gun. (d) Gun components. The filament (also called 'emitter') is surrounded by the Wehnelt cylinder that closes over the filament assembly and has a small hole in the center where the electrons exit. The electrode pins run to the filament through an insulator disc, and carry the current flow to the filament. (From www.ammr.org.au)

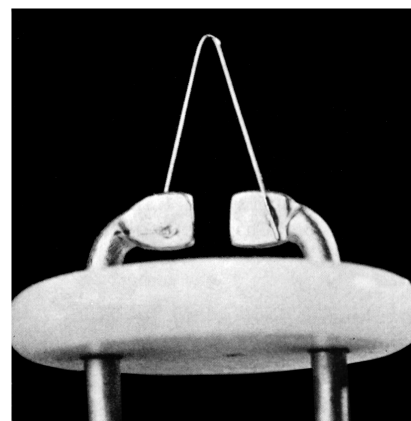


Fig. I.57. Standard hairpin tungsten filament. (From Agar, 1974, p.45)

Beam shaping and control of emission is affected by properties of the shield (gun cap; **wehnelt**) and acceleration by the anode. The functions of the three electrodes (cathode, shield, and anode) are analogous to the function of the three electrodes of a triode. In a **biased** gun the shield is maintained at a potential between 100-500V **negative** relative to the filament. This negative voltage serves to repel some emitted electrons back to the filament, and reduces total emission and brightness.

The negative potential of the shield with respect to the filament gives rise to a strong electrostatic field around the shield. This field acts as an electrostatic lens and focuses the true source (space charge of electrons that surround the filament tip) to form an image of the source below the anode. The image of the source (**gun-crossover**) is considered to be the actual source of electrons for the electron microscope. The shape of the crossover is elliptical, not circular, because the true source is a bent wire (a linear rather than point source).

b. Electron emission

Electron emission, i_s (amps/cm²), as a function of the absolute temperature, T , of a thermionic emitter, is given by Richardson's equation:

$$i_s = AT^2e^{-(b/T)}$$

where A and b are constants that are determined empirically.

Because of the exponential form of the above expression, emission is sensitive to T and also to the constant b , which is proportional to the **work function** (the excess energy an electron must have to escape from the surface of the emitter). Significant emission from tungsten occurs above about 2200°K; at higher temperatures the thermionic current increases rapidly but, as the melting temperature is approached (3410°K), evaporation of the atoms of the filament also increases and filament lifetime decreases. Filaments generally become thinner with use because of evaporation of tungsten and break near the "V" shaped tip where heating is greatest. Filament power is supplied through a transformer and the amount required to heat the filament is normally about 2 watts at 1.5 volts (AC or DC).

Lanthanum hexaboride (LaB₆) filaments (Fig. I.56b, I.58) have longer life and yield five to ten times higher brightness, but require better vacuum than tungsten filaments. Modern, high-end TEMs use field emission guns (FEG; Fig. I.56c), which produce an electron beam with much greater brightness (1000x more than the LaB₆) and coherence than conventional guns. The use of FEG microscopes is now essentially a requirement for those wishing to record images of biological specimens at the highest possible resolutions. However, the addition of a FEG alone to the TEM adds considerable cost to the instrument (> \$250,000 compared to tungsten filaments that are < \$50). Several literature citations concerning the principles and use of a FEG source are presented in the **Reading References List**.

c. Unbiased gun

In an unbiased gun the high tension is connected through a pair of balancing resistors to maintain the tip of the filament at the same potential as the shield at all times during the heater cycle. Total beam intensities from cathode to anode may be in the range 10-400 μ amp, but only a small fraction of this current passes through the anode and subsequent apertures to reach the specimen plane.

If the tip of the filament is close to the opening in the shield, the gun approaches conditions for a cathode lens and results in a beam, which diverges from a virtual image behind the plane of the shield. As the filament is drawn back into the shield (Fig. 1.59) the curvature of the equipotentials at the shield aperture produces a converging lens action, and at some position a crossover or image of the source will be formed below the shield. It is necessary to adjust the height of the filament while observing the emission pattern to obtain maximum intensity.

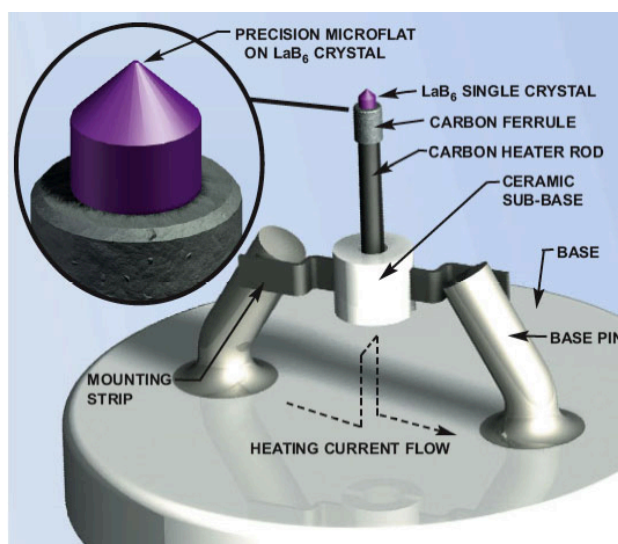


Fig. I.58. Schematic diagram of the Kimball Physics ES-423E LaB₆ cathode. This cathode source gives a greater brightness (higher current density), lower energy spread (better resolution), and a longer lifetime than standard tungsten filaments. Six or more months of continuous operation are regularly achieved in TEM's with a suitable gun vacuum (10⁻⁷ torr or better). The standard emitter is a 15 μm diameter, oriented, single crystal surface mounted on the end of a single piece, stress-free, carbon heater rod, held in place by a carbon ferrule. (From www.tedpella.com)

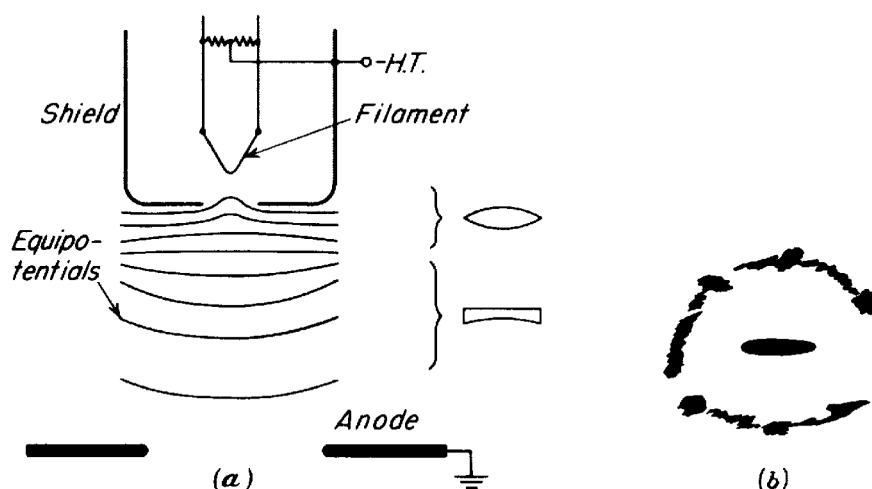


Fig. I.59. (a) Unbiased electron gun. (b) Typical emission pattern at a plane below the gun. (From Hall, 1966, p.147)

d. Biased/Self-biased gun (Fig. I.60)

A negative potential between shield and filament is produced by the flow of beam current through the bias resistor included in one of the supply leads to the filament. The bias resistor keeps the shield at a potential slightly negative with respect to that of the filament while the anode is at ground potential. For example, if the beam current equals $300 \mu\text{amp}$ and the bias resistor is $500,000 \text{ ohms}$, the bias potential would be -150 volts . Recall from basic physics (electricity) that $V = iR$, where V is the voltage (volts), i is the current (amps) and R is the resistance (ohms).

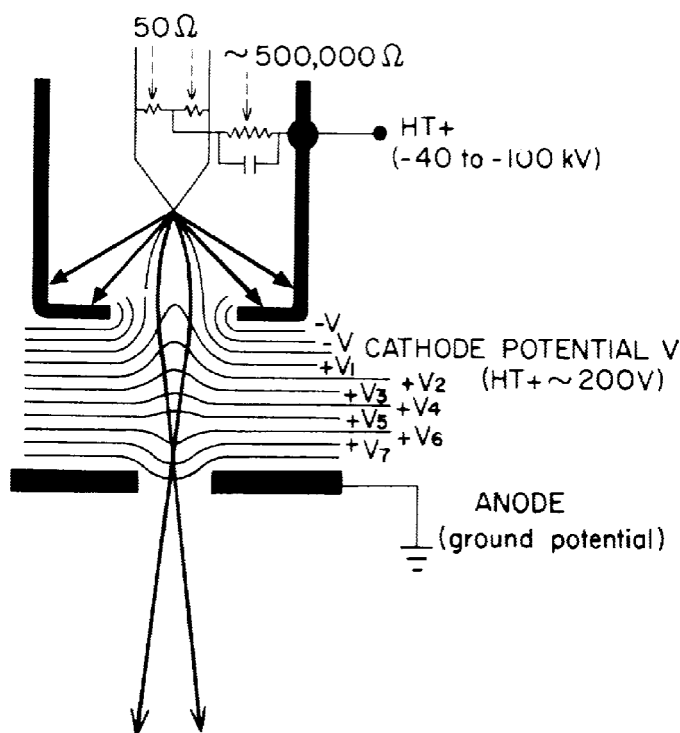


Fig. I.60. Self-biased gun. The filament is connected to the high voltage supply through a bias resistor and the shield is attached directly to the high voltage. The equipotentials of the field when the circuit is activated serve as a strongly converging lens. (From Sjostrand, 1967, p.74, modified from Hall, 1966, p.148)

The strong curvature of the equipotentials in the region of the shield aperture results in **strongly convergent lens action**. The negative potential also has the effect of funneling the electrons (by repulsion) through an area smaller than the shield opening since they cannot enter regions of negative potential.

With increasing filament current there is, beyond a certain T , a rapid rise in beam current to a flat maximum where the beam current is practically independent of filament current (Fig. I.61). In this region the two factors controlling the beam current, filament T and negative bias, are in balance. An increase in filament current tends to increase beam current, but an increase in beam current would also eventually increase the negative bias. The gun "**saturated**" when this condition occurs. Filament current is increased slowly until the beam current no longer changes. Going much beyond this point severely decreases filament life.

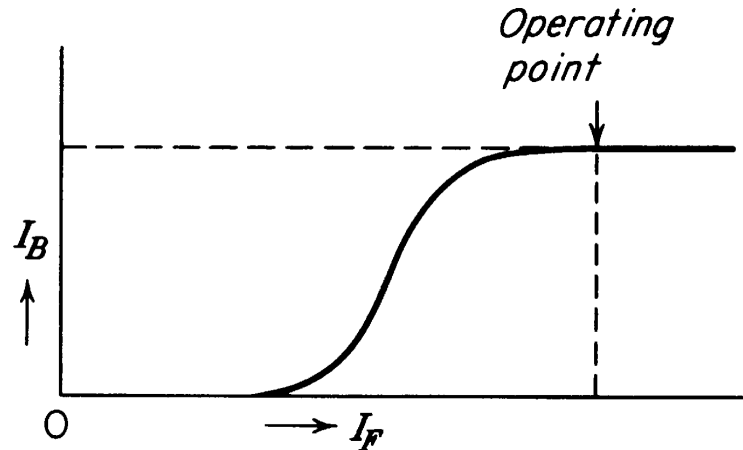


Fig. I.61. Emission characteristic of the self-biased electron gun. Beam current, I_B , versus filament current, I_F . (From Hall, 1966, p.148).

The shield plays a role in controlling the level of beam current. As filament current increases, at first there is no beam current, then the beam current increases up to a point where emission of electrons causes the surface of the filament to become positive with respect to the shield. The emitted electrons then tend to be repelled back onto the filament by the negative equipotential surfaces surrounding the shield. At saturation, increases in filament current cause the filament to become hotter, but produce no further net increase of emission. The levels of filament and beam current at saturation are determined by the value of the bias resistor and by the distance between the filament and the shield. The microscope should be operated just at saturation to maximize intensity and filament life. The level of intensity at saturation may be increased either by decreasing the value of the bias resistor or by moving the filament closer to the shield.

e. Self-biased versus unbiased gun

The chief advantages of a self-biased gun relative to an unbiased one are:

- Smaller source at crossover and absence of secondary sources
- Much higher intensity per unit solid angle for the same beam current
- Insensitivity to filament current fluctuations
- Relative insensitivity to variations in filament height and centering

I.B.2. Condenser Lens(es)

The condenser lens system is designed to focus the electron beam emerging from the electron gun onto the specimen to permit optimal illuminating conditions for visualizing and recording the image.

a. Single condenser system (Fig. 1.62a)

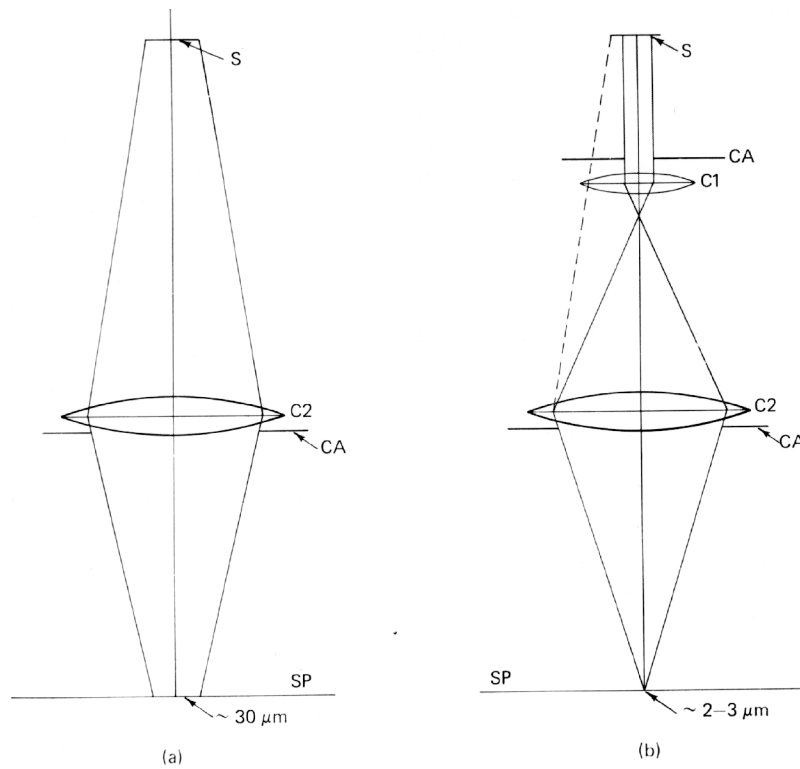


Fig. 1.62. Comparison of single and double condenser lens systems. (a) Single condenser system. (b) Double condenser system. The condenser 2 lens, C2, projects an image of about 30- μm diameter on the specimen plane, SP, from the source, S. When the condenser 1 lens, C1, is also used, the projected image is 2-3 μm in diameter. Condenser apertures, CA, limit the beam angle. (From Agar, 1974, p.22)

The condenser lens is located approximately halfway between the cathode and object plane. Thus, when the crossover is focused onto the object plane, the magnification of the crossover will be close to unity. The area illuminated is then approximately the same size as the source ($\sim 30\text{-}50 \mu\text{m}$). This is much larger than is needed for illumination at 10,000X and therefore leads to excessive heat dissipation in the specimen and irradiation of regions not yet examined.

b. Double condenser system: (Figs. 1.62b - 1.67)

A double condenser system adds considerable flexibility to the illuminating system by allowing a wider range of intensities with a given gun adjustment and making it possible to reduce the area of the object that is irradiated.

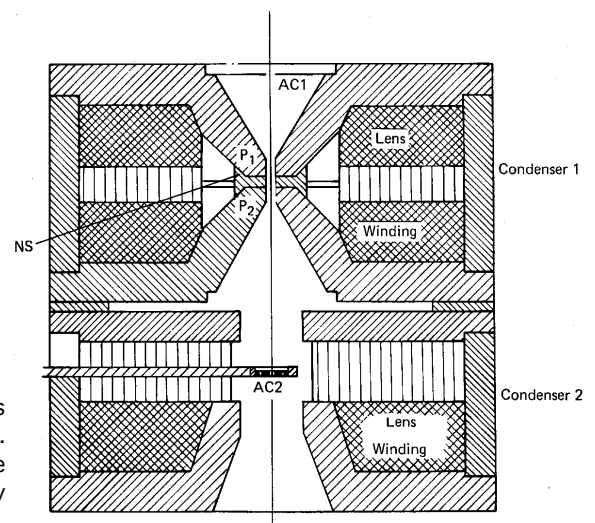


Fig. 1.63. Cross section through a double condenser lens assembly. The upper lens is condenser 1, with a fixed aperture. The lower lens is condenser 2 with an adjustable aperture. Pole pieces are indicated by P₁, P₂, and the nonmagnetic spacer by NS. (From Agar, 1974, p.47)

A strong first condenser lens (C1: short focal length) is used to produce a demagnified image ($\sim 1 \mu\text{m}$ diameter) of the electron source and a weaker, second condenser lens (C2: long focal length) projects this demagnified image onto the specimen plane producing a slight magnification so the final focused beam size is about $2\text{-}3 \mu\text{m}$. The focal length of C2 can be varied to spread the beam over a larger area of the specimen, for example, to record images at low magnification ($<10,000\times$).

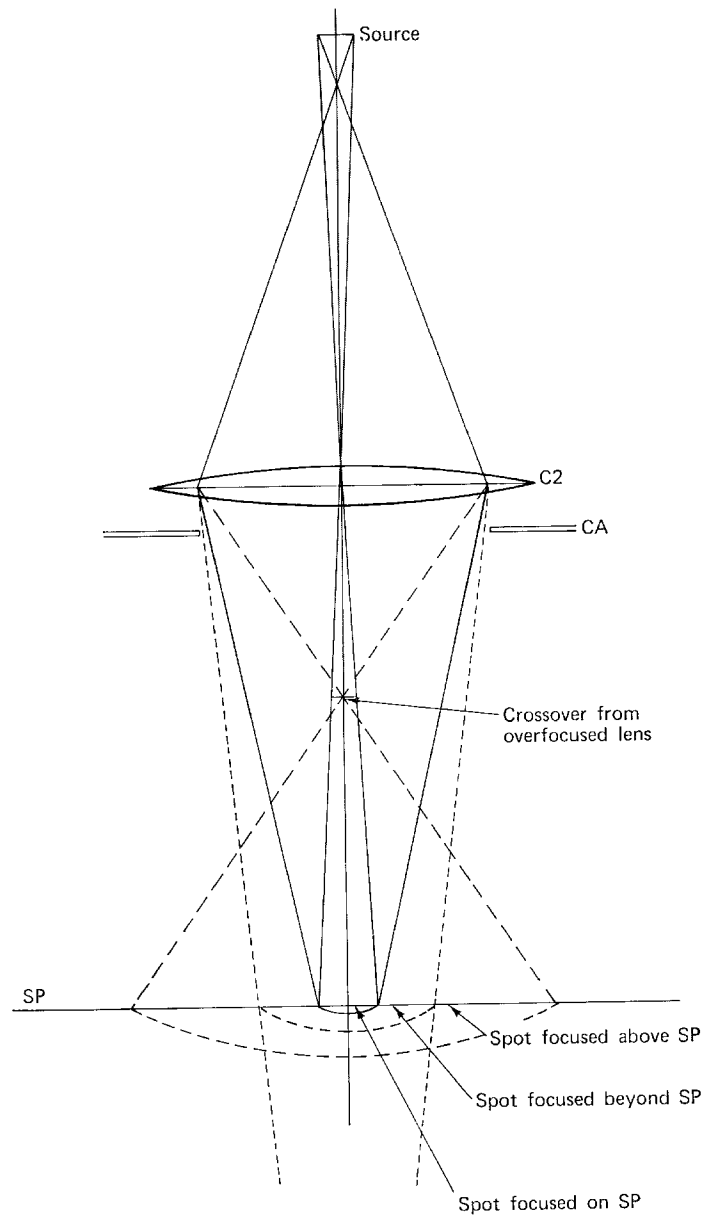


Fig. I.64. Variation of beam intensity at the specimen by condenser lens variation. C2 = second condenser lens, CA = condenser aperture. The spot diameter at the specimen plane, SP, is determined by the focus setting of C2. (From Agar, 1974, p.23)

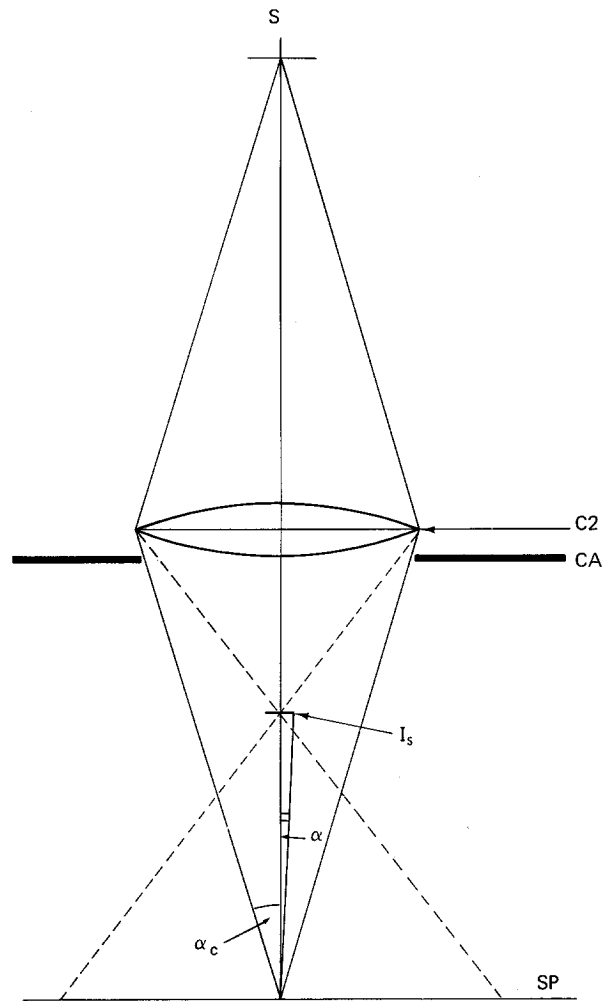


Fig. I.65. Variation of illumination semi-angular aperture with condenser excitation. When C2 is set so the beam is focused **at** the specimen plane, SP, the condenser aperture, CA, defines the limiting semi-angular aperture, α_c . When the lens is over-focused (image of electron source above SP), the source image, I_s , defines the semi-angle, α . (From Agar, 1974, p.24)

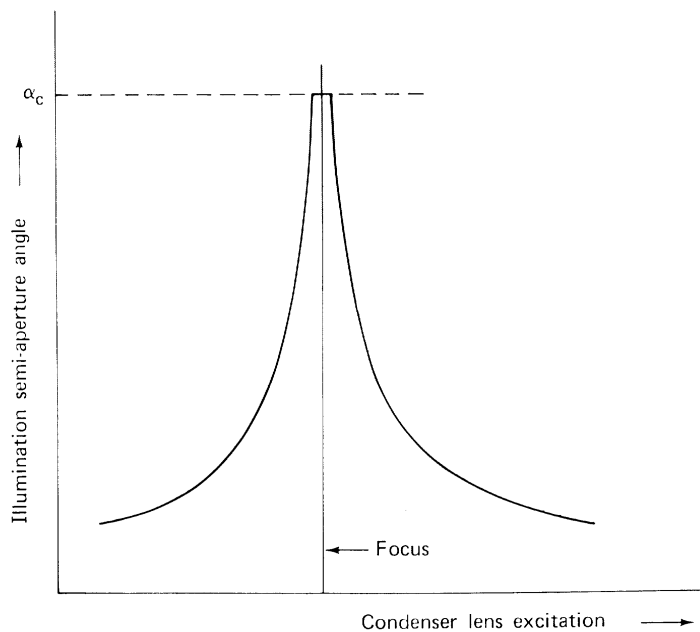


Fig. I.66. Plot of variation of illumination semi-angular aperture with condenser lens excitation. The limiting (*i.e.* largest) illumination aperture, α_c , is determined by the diameter of the physical condenser aperture. (From Agar, 1974, p.25)

c. Condenser apertures (Figs. I.62 - I.65)

An aperture in each condenser lens limits the amount of electrons that strike the specimen (protecting it from excessive irradiation) and limits the number of X-rays that could be generated from stray electrons hitting exposed parts of the microscope column. The size of the C2 aperture determines the maximum semi-angular aperture of the illumination, α_C , as viewed from the specimen (Fig. I.66). When crossover is focused by the condenser lens on or near the object plane, α_C is a maximum and decreases for smaller or larger condenser currents.

The larger the aperture angle, the greater the maximum illumination intensity will be. However (a most important 'however'), in general, image quality deteriorates when using larger apertures to achieve greater illumination intensity because beam coherence is at its lowest (i.e. worst) when the intensity is at its maximum. When C2 is defocused, the semi-angular angle is defined not by the size of the condenser aperture but by the size of the crossover image and its distance from the specimen. The variation in aperture angle with respect to the C2 lens excitation is plotted in Fig. I.66. The C1 aperture in most TEMs is a fixed aperture and the operator has little or no control over changing or adjusting it. The TEM operator generally can choose one of several different size C2 apertures and each one can be aligned along the optical axis of the instrument through use of centering controls. As the strength of C1 increases more electrons are lost outside the C2 aperture. In practice, the focal length of C1 is usually set to give a particular minimum spot size and the focal length of C2 is adjusted to vary the beam spread at the specimen.

d. Advantages of the double condenser: (Fig. I.67)

- Illumination of smaller areas reduces irradiation of specimen areas outside the field of view, which acts to cut down on the background scattering from such areas, and also reduces the total accumulation of contamination on the specimen.
- C1 is closer to the source than C2; therefore it has a larger acceptance (aperture) angle and collects more electrons from the source than C2 alone. The higher efficiency of the double condenser system means that the brightness of the gun can be reduced with consequent increase in filament life. If C1 is highly excited to produce a very small illuminating beam when C2 is focused, then a large proportion of the electrons focused by C1 fall outside the aperture of C2. This loss of illuminating beam intensity may consequently force the operator to increase the gun brightness (shortening filament lifetime) to achieve satisfactory working conditions.
- Image contrast is improved as a result of the increased coherence of the effectively smaller electron source.

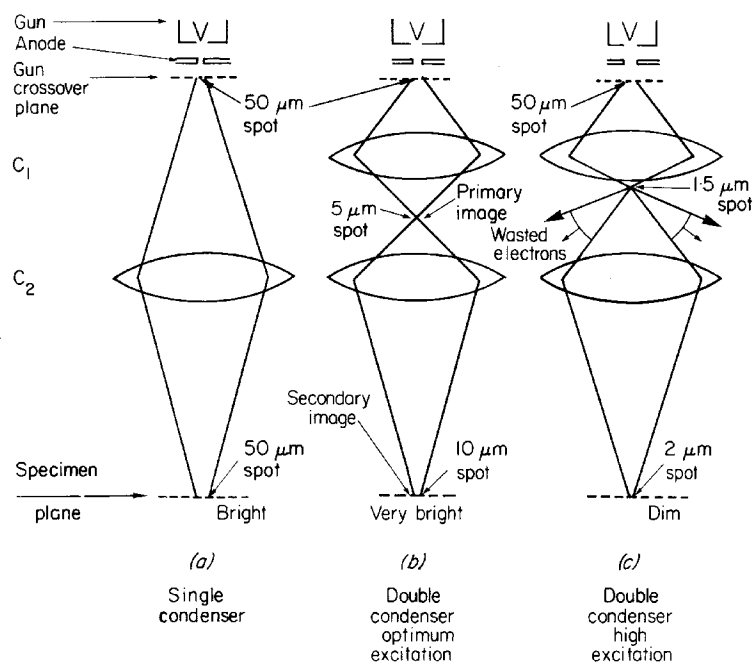


Fig. I.67 Diagram showing how the efficiency of the illuminating system and hence illuminating spot intensity change with the setting of C1 lens. (From Meek, 1970, p.110)

I.B.3. Lens Aberrations And Other Non-Ideal Imaging Properties

a. General description

Non-ideal imaging is caused both by imperfections in the geometry of the refracting fields and also by properties inherent in the radiation (electrons) used to form images.

Glass, electrostatic, and magnetic lenses **all suffer** to varying extents from the effects of five aberrations. These include spherical aberration, distortion, curvature of field, astigmatism, and coma. Because of the rotation effect of magnetic fields on electrons, magnetic lenses also suffer from anisotropic distortion, anisotropic astigmatism, and anisotropic coma. In addition, other factors that can result in image defects include chromatic aberration, rotational chromatic aberration, and space-charge distortion. In this course, only the most significant of these aberrations and distortions are discussed in any detail.

The above defects are predicted on theoretical grounds. In practice, **real** lenses also produce further defects in the image as a result of:

- Departure of the lens fields from perfect symmetry
- Imperfections in alignment of the lenses in the instrument
- Distortions due to stray fields (*e.g.* due to magnetic or electric fields)
- Many others

b. Spherical aberration (Fig. I.68)

This constitutes one of the principal factors limiting the resolution of the TEM. The power of the lens is greater for rays, the larger the distance from the optic axis at which they pass through the lens. The spherical aberration error is the same for all points in the image and thus is one aberration that does not disappear on the optic axis. Since electromagnetic lenses are always convergent, it is not possible to reduce the effect of the error in the image through combinations of positive and negative lenses with different refractive indices, as is possible for glass systems. Spherical aberration in electrostatic lenses is about 4-10 times more severe than in electromagnetic lenses.

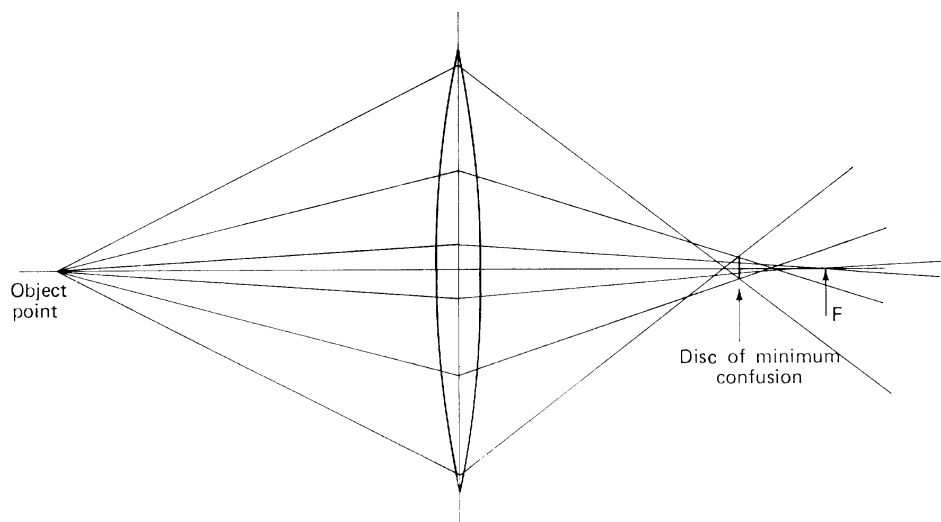


Fig. I.68. Spherical aberration in a lens. The rays close to the lens axis (paraxial rays) are focused at the Gaussian focus, F. Rays entering the lens at a larger angle are converged more strongly. The disc of minimum confusion is where the envelope of emergent rays has its smallest diameter. (From Agar, 1974, p.9)

Spherical aberration causes rays from one object point to **not** cross in a corresponding image point in the image space. Instead, the rays are distributed over a surface with a diameter d_{sa} in the image plane. The circle of least confusion is the point at which the envelope of the imaged rays has a minimum diameter. In the case of the TEM where the angular aperture of the primary imaging (objective) lens is small, the limiting disk (resolution limit) has a diameter given by:

$$d_{sa} = C_s \alpha^3 / 2$$

where C_s = spherical aberration coefficient
and α = semi-angular aperture of the lens

The C_s is a property of the lens and decreases as focal length decreases. The expected resolution (in nm) for various values of C_s and α (in radians) is:

C_s (mm)	$\alpha = 10^{-2}$	$\alpha = 5 \times 10^{-3}$	$\alpha = 10^{-3}$
1.0	0.500	0.063	0.00050
1.5	0.750	0.094	0.00075
2.0	1.000	0.125	0.00100
4.0	2.000	0.250	0.00200

Table I.3. Resolution limits due to spherical aberration, d_{sa} (nm), for objective lenses with different C_s constants and for a range of semi-angular aperture settings (α given in radians).

Thus, it would appear that, in the absence of other types of aberrations (which of course is never possible in the real world) or other factors such as diffraction effects, resolution could be improved significantly by reducing spherical aberration. This can be achieved by limiting the effective aperture of the objective lens either by using a small objective aperture or a highly collimated electron beam (small angular aperture).

In fact, we have already shown that decreasing α limits resolution as a consequence of diffraction effects (§ I.A.3.d). Using the same values of α in the above table and using the Rayleigh criteria for estimating resolution ($d_{di} = 0.61\lambda/n \sin\alpha$), we realize that spherical aberration and diffraction act in opposite fashion:

α	d_{di} (nm)
0.010	0.226
0.005	0.452
0.001	2.262

Table I.4. Diffraction limited resolution, d_{di} , according to the Rayleigh criteria (use $\lambda = 0.0037$ nm for 100kV electrons).

The aperture angle at which the resolution limits due to spherical aberration and diffraction (using the Rayleigh criteria) are equal is given by:

$$C_s \alpha^3 / 2 = 0.61\lambda / n \sin\alpha$$

Since $\sin\alpha = \alpha$ for small α , and $n = 1.0$ for a vacuum, the equation can be simplified and rearranged:

$$\alpha^4 = 1.22\lambda / C_s$$

Substituting for various values of C_s and using $\lambda = 0.0037$ nm for 100kV electrons, α , and thus d_{sa} (= d_{di}) can be determined.

C_s (mm)	α	d (nm)
1.0	8.2×10^{-3}	0.276
1.5	7.4×10^{-3}	0.306
2.0	6.9×10^{-3}	0.328
4.0	5.8×10^{-3}	0.390

Table I.5. Resolution, d (nm), at which the opposing effects of spherical aberration and diffraction are in balance for different objective lens C_s values.

c. Distortion (Figs. I.69 - I.72)

Distortion is simply another kind of spherical aberration. It mainly affects the projector lenses because their object is a magnified image (the intermediate image formed by the objective lens). Rays from each point in the "image" reunite at corresponding points in the image plane but the magnification varies throughout the plane. Three kinds of distortion include:

- **Pincushion:** magnification increases with distance from the axis and depends on the direction in which it is measured, being greater in the radial direction than in the circumferential direction.
- **Barrel:** magnification decreases with distance from the axis, being smaller in the radial direction than in the circumferential direction.
- **Anisotropic (spiral) distortion:** The rotation of a point about the axis depends on the distance of the point from the axis. When the effect of rotation is combined with that of distortion, a straight line is imaged as a sigmoid shape. This only occurs for magnetic lenses.

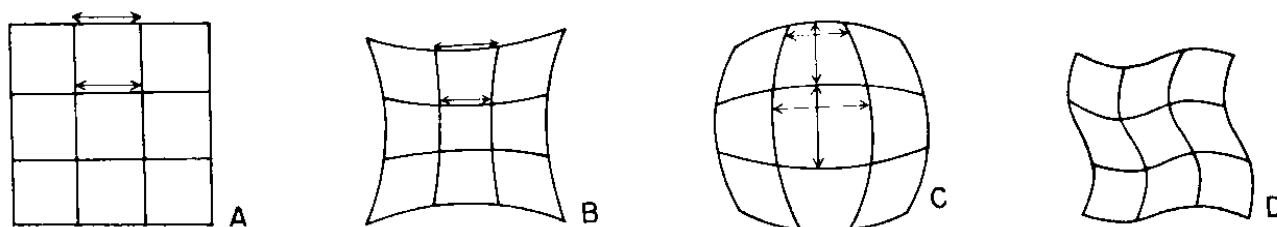


Fig. I.69. Distortion. (A) The undistorted image of an object. (B) Pincushion distortion. (C) Barrel distortion. (D) Spiral distortion. (From Sjostrand, 1967, p.58)

The effects of all three kinds of distortion are most noticeable at low magnifications. In this situation, the projector lens(es) accepts a large area of the intermediate image formed by the objective lens. Distortion can be reduced to almost negligible proportions by careful design of the two final image forming lenses such that barrel distortion in one lens is balanced against pincushion distortion in the other lens.

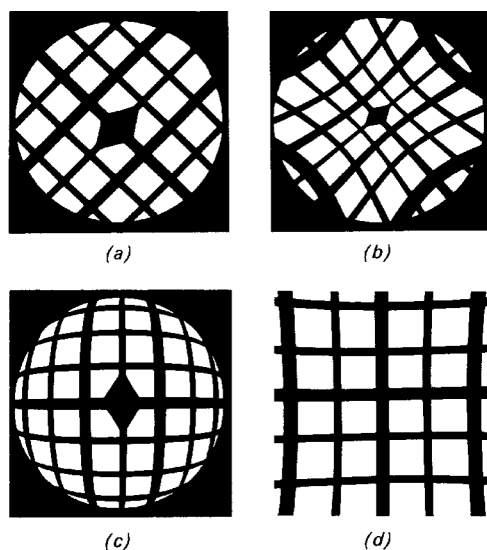


Fig. I.70. Four electron micrographs at very low magnification (approx. x50) showing: (a) negligible distortion; (b) pincushion distortion; (c) barrel distortion; and (d) sigmoid distortion. The distortions were introduced deliberately by altering the focus of the intermediate lens, which was being used as a long-focus objective. (From Meek, 1970, p.75)

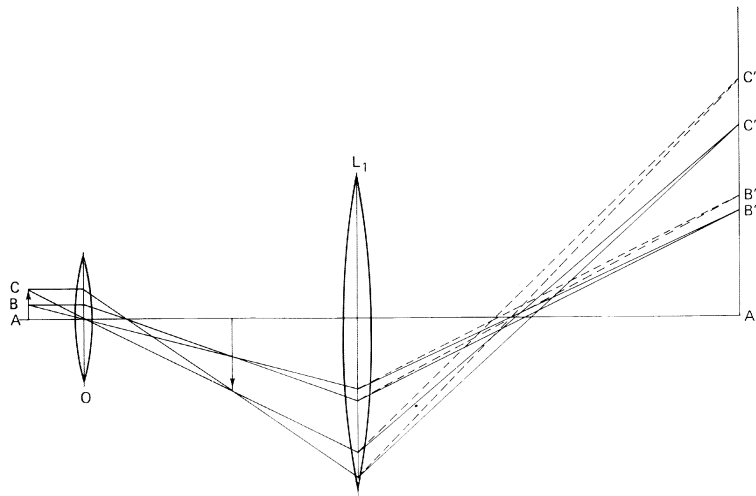


Fig. I.71. The origin of pincushion distortion in a projector (intermediate) lens forming a real image. An object ABC is imaged at A'B'C' in the intermediate image plane if the lens is perfect. The spherical aberration causes the actual image to be formed at A'B''C''. (From Agar, 1974, p.31)

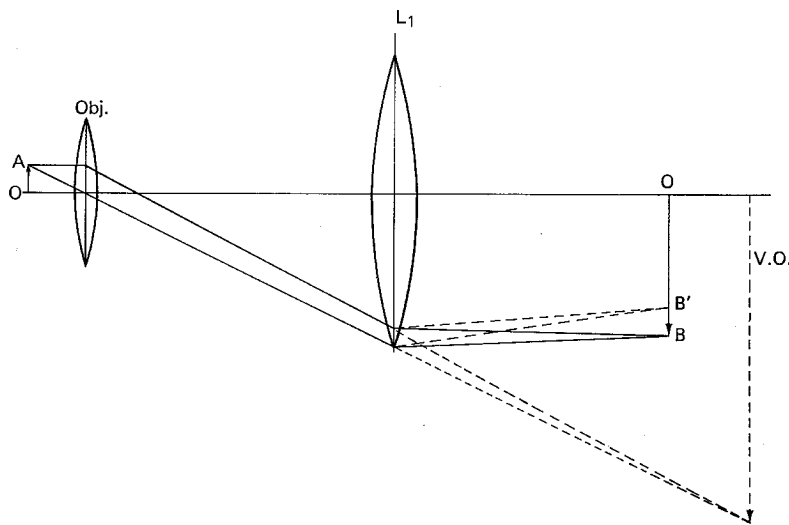


Fig. I.72. The origin of barrel distortion in a projector lens with a virtual object V.O. The specimen at OA would be imaged at OB by a perfect lens. The spherical aberration of L₁ causes the actual image to be formed at OB'. (From Agar, 1974, p.32)

d. Chromatic aberration (Figs. I.73 - I.76)

Electrons of different wavelength (velocity) leaving a point in object space will not be brought to a focus at the same point in image space. Variations in velocity arise from:

- Fluctuations in high tension supply (usually less than 1 part in 10^5 in stabilized circuits)
- Variation in speed at which electrons are emitted by the cathode (about ± 3.5 parts/million)
- Energy losses due to inelastic collisions in the specimen (minimized using thin specimens)

Since the focal lengths of magnetic lenses are proportional to the accelerating voltage (*i.e.* velocity of the electrons), then electrons of different velocity will effectively experience different focal points for the same lens. Rays converging to foci in front of and behind the ideal image plane will contribute to a disk of confusion at the image plane.

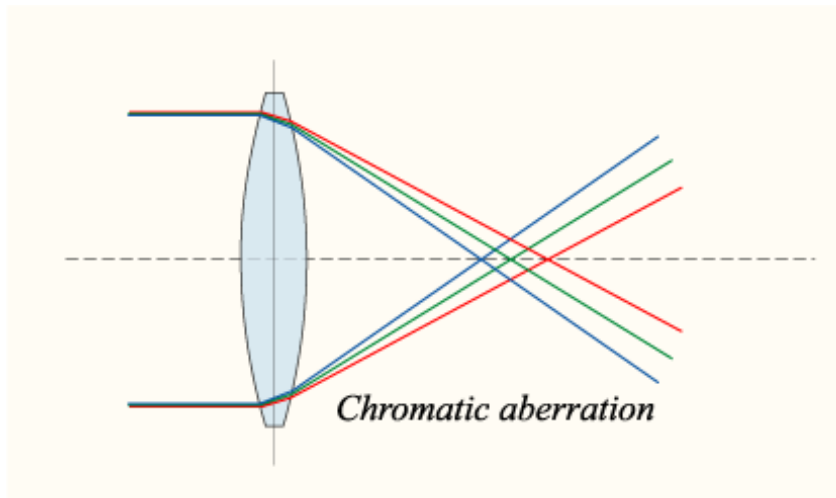


Fig. I.73. Longitudinal chromatic aberration in a glass lens. Here short wavelength (blue) photons are more strongly focused by the glass lens than longer wavelength (green or red) photons. (From <http://en.wikipedia.org/wiki/Image:Lens6a.png>).

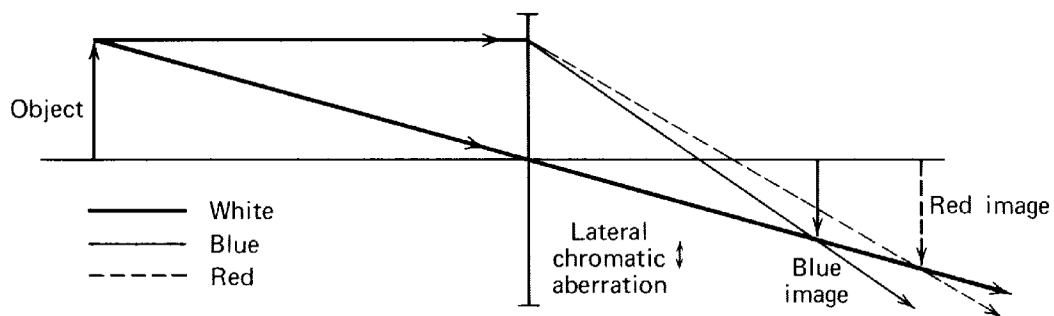


Fig. I.74. Lateral chromatic aberration in a **glass** lens. (From Slayter, 1970, p.216)

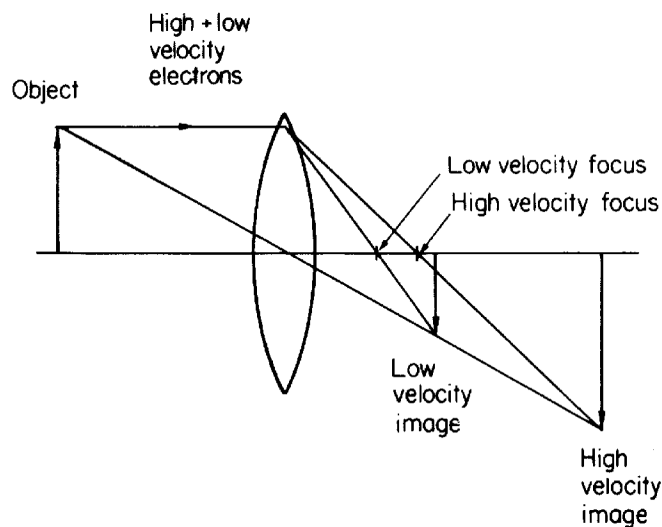


Fig. I.75. The analogue of chromatic aberration in an **electron** lens. Fast (short wavelength) electrons are brought to a focus at a point further from the lens than slow (long wavelength) electrons. The image formed by the high-velocity electrons will therefore be larger than that formed by the slow ones. An effect called 'chromatic change of magnification' is produced, which is aggravated by thick specimens and low accelerating voltages. (From Meek, 1970, p.78)

The limit to resolution strictly due to chromatic aberration can be estimated by either of the following formulas:

$$d_{CV} = C_C \cdot \alpha_O \cdot \Delta V / V$$

$$d_{Ci} = 2 C_C \cdot \alpha_O \cdot \Delta I / I$$

where d_{CV} = separation of two object points that are just resolved, considering voltage fluctuations

d_{Ci} = separation of two object points that are just resolved, considering current fluctuations

C_C = chromatic aberration coefficient of lens (usually 1-3 mm)

α_O = semi-angular aperture angle of objective lens

V = accelerating potential

ΔV = maximum departure from V of electrons contributing to the image

I = lens current

ΔI = maximum departure from I

For typical values of C_C (2 mm), α_O (5×10^{-3} radians), and $\Delta V/V$ (10^{-5}), $d_{CV} = 0.1$ nm. Thus, for **thin** specimens, chromatic aberration is not a major limit to resolution in electron images (but see § I.C.4).

Electron images that suffer from chromatic aberration may be thought of as being produced by the superposition of a series of images, each of which is formed by electrons of a different wavelength (Fig. I.76). Because lens focal length differs for each wavelength, the superimposed images are at different magnifications. Furthermore, since images are rotated through a different angle by the magnetic lens, for each level of magnification, the superimposed images are also rotated with respect to each other.

Fast, short wavelength electrons are deviated less by an electron lens than are slow electrons of longer wavelength. The reason this occurs is because the faster moving electrons spend less time in the magnetic field and hence are bent (refracted) less than electrons that move more slowly through the field and hence are more influenced by its effects. Chromatic aberration in an electron lens thus works in a direction opposite to that in the light microscope. The net effect is a similar blurring of the image. Note that chromatic aberration in electrostatic lenses is four to six times larger than that in magnetic lenses.

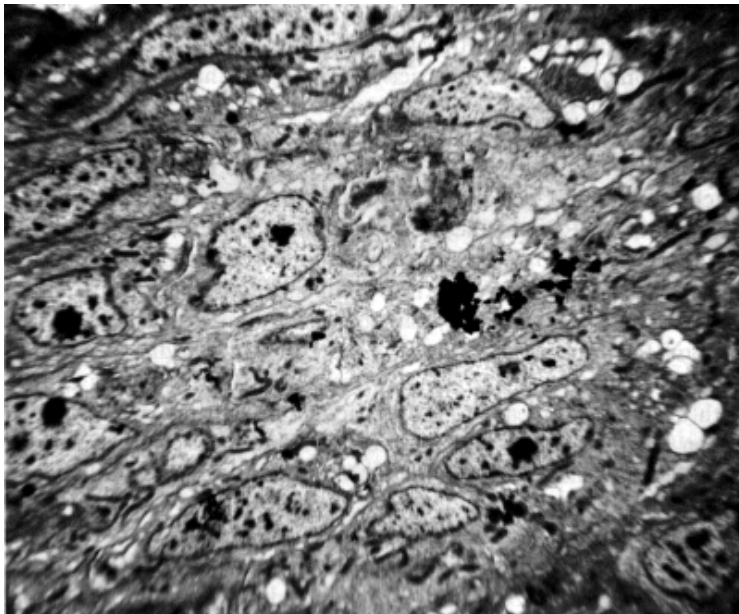


Fig. I.76 The effect of chromatic change of magnification. A 30kV beam was used to image a 1000 Å thick section of embedded tissue. The central part of the micrograph is sharp, but the out-of-focus effect becomes increasingly noticeable further from the axis. The effect is particularly noticeable at low magnifications. $\times 5,000$. (From Meek, 1970, p.79)

e. Lens asymmetry (Figs. I.77-I.78)

The homogeneity of available magnetic materials and the accuracy of machining these metals into lens pole pieces are inadequate for the direct production of lenses capable of displaying the theoretical resolving power established by the spherical aberration-diffraction limit. Asymmetry, resulting from lack of axial symmetry, has the effect of producing images in which the focal level varies with direction. The system is equivalent to the combination of a cylindrical lens with one of spherical curvature. The ray from an object point is brought to two mutually perpendicular line foci. There is no sharp image point but rather a circle of confusion at the image plane between the two line foci.

Correction of the defect is attained by imposing a second cylindrical lens field of the same magnitude as that already present, but oriented at right angles. A **stigmator** is the device used to compensate for asymmetrical lens fields.

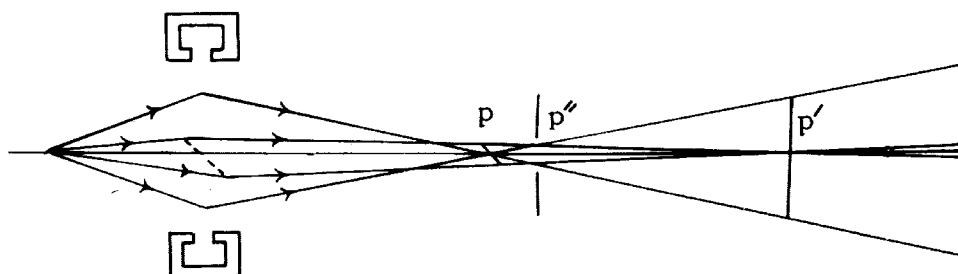


Fig. I.77. Astigmatism. Rays parallel to the axis in two mutually perpendicular planes that pass through an astigmatic lens are brought into focus at two different points, P and P'. The disc of least confusion would be located between P and P' and is designated as the "in-focus" position, P". (From Wischnitzer, 1970, p.89)

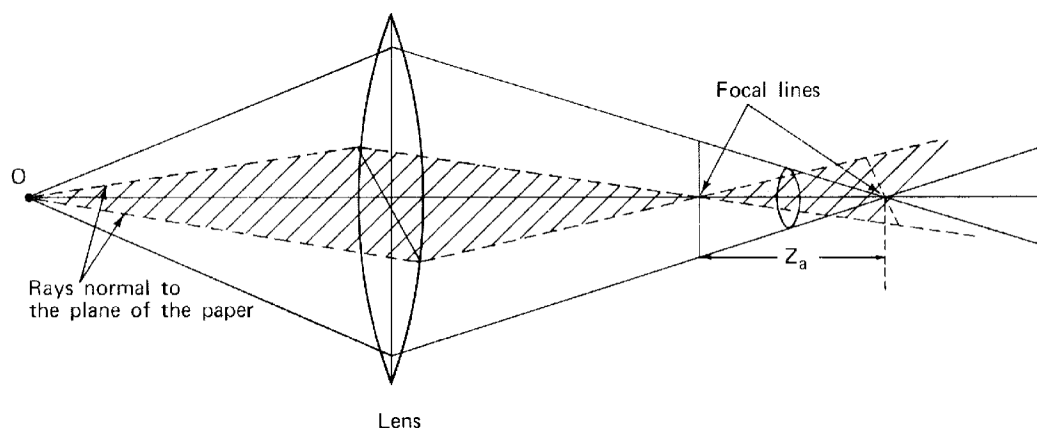


Fig. I.78. Image formation with an astigmatic lens. In this example, the lens is stronger in a plane perpendicular to the paper than in the plane of the paper, so that a point object O is imaged into two focal lines. Z_a , the distance between the focal lines, measures the astigmatism of the lens. A circular, blurred image is formed halfway between the lines. (From Agar, 1974, p.12)

f. Lens current fluctuations

Lens current levels, like accelerating potentials, vary at the level of about one part in 10^5 . Corresponding fluctuations in the lens focal length are induced. Since these fluctuations are rapid, images of different magnification are superimposed. The use of superconducting lenses (lenses cooled to liquid helium temperature) can be used to eliminate current fluctuations. This is because, in a super cooled lens, a single pulse of voltage causes current to flow for an indefinite time and is therefore insensitive to variations in the level of voltage supply.

g. Curvature of field

The image of a plane object is formed on a curved surface (See Fig. 6.6. p.122 of Hall). This is usually a negligible error in TEMs since only very small specimen areas are imaged and the object points are located close to the axis. In addition, the large depth of focus makes this defect of minor consequence.

h. Coma and anisotropic coma

These defects are of negligible significance in most TEM images of biological samples. (See Hall, pp. 134-135).

i. Space charge distortion

A concentrated beam of electrons will spread out owing to the mutual repulsion of electrons. This is generally not a significant error in conventional TEM under normal operating conditions.

I.B.4. The Objective Lens and Specimen Stage

a. General description

The optical enlarging system of an electron microscope consists of an objective lens followed by one or more projector lenses (Figs. I.31, I.33). The objective lens determines resolution and contrast in the image, and all subsequent lenses bring the final image to a convenient magnification for observation and recording.

The objective lens is most critical lens of the microscope since it determines the resolving power of the instrument and performs the first stage of imaging. Aberrations in the image formed by the objective lens are subject to further magnification by the projector system and by photographic processing of recorded images. The intermediate and projector lenses are used under conditions in which errors in these lenses do not interfere seriously with the imaging except at low magnifications. The reason for this is that the angular aperture of the electron beam entering the projector lenses is so small that spherical aberration is negligible. Note, however, that this may not be true for imaging performed at very low magnifications (<10,000X) where pincushion, barrel, and anisotropic distortions can occur (see § I.B.3.c) because a large portion of the intermediate image formed by the objective lens enters the projector lenses.

The objective forms, at relatively great distance, an image of an object placed close to the front focal point. Since the object is practically at the front focal plane, rays leaving the same object point are almost parallel on leaving the lens (neglecting spherical aberration) and unite at the image plane a relatively great distance below the lens. In the absence of an **objective aperture**, electrons scattered through angles much greater than the effective aperture of the lens reach the image plane so far from their proper image point that they constitute a background "fog". A region in the object with relatively high scattering power will therefore appear much darker than its surroundings in the image, even though there is negligible absorption. Intensity scattered outside the hole in the aperture and therefore removed from the electron image helps improve contrast in the image. (See also § I.B.4.e).

b. Lens construction (Fig. I.79)

The construction of the objective lens requires that specimens be placed in the bore of the first pole piece or in the pole piece gap. Hence, the specimen actually sits inside the magnetic field of the lens. Also, the size of the bore limits movement of the specimen. When the specimen is introduced in the gap between the two pole pieces, part of the objective lens acts as an additional condenser lens, which therefore slightly increases the angular aperture of the illuminating beam.

Requirements in the construction of the objective lens:

- Specimen must be situated close to the front focal plane of the objective to provide an initial magnification of 50-100X.

- Focal length should be as small as practical to insure minimum chromatic and spherical aberration since these aberrations decrease as the focal length decreases. The specimen has to be placed *inside* the lens field to obtain the necessary short focal length and this poses a problem of introducing a specimen into the confined space of the lens.
- There must be adequate clearance for insertion of specimen, aperture, and anticontaminator.
- There must be provision for inserting electrical or magnetic devices (**stigmators**) to correct for minute asymmetries in the lens field.

c. Lens asymmetry

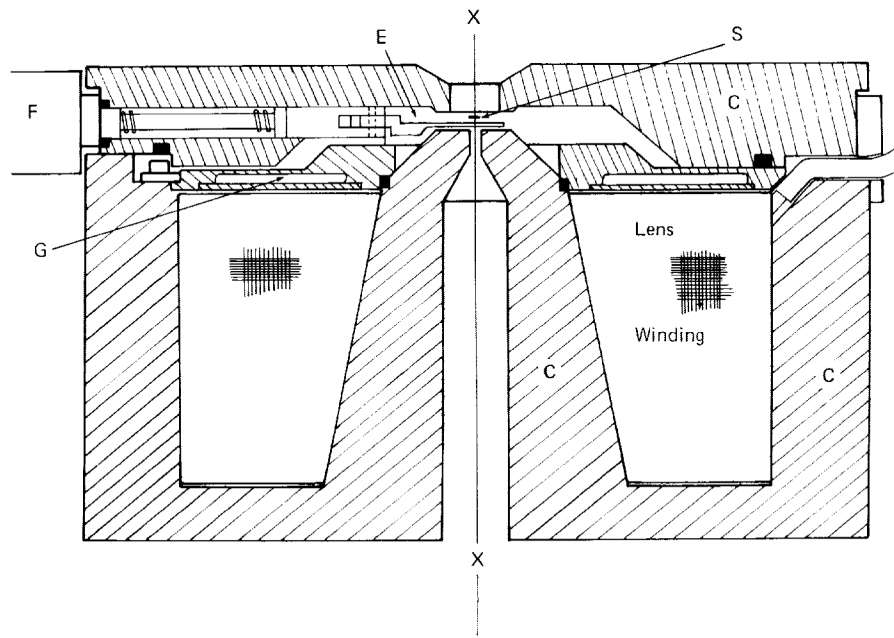


Fig. I.79. Cross section of a typical objective lens. The specimen is at S in a top-entry cartridge. The objective apertures are carried out on the rod E, and adjusted by control knob F. The water-cooling channels G are above the lens coil. Note the heavy iron circuit C. XX defines the optic axis of the lens. (From Agar, 1974, p.51)

Pole pieces usually cannot be produced completely free from mechanical and magnetic imperfections due to imperfect machining or to inhomogeneities of the iron. Such irregularities induce an asymmetry in the magnetic field, which must be eliminated in order to attain the maximum performance of the lens (§ I.B.3.e).

If the lens field is not perfectly symmetrical about the optic axis, the image will be **astigmatic** both on and off the axis. An asymmetric field is introduced perpendicular to the residual field to compensate for the astigmatism, in a way analogous to crossing two cylindrical lenses in glass optics. In older TEMs, **stigmators** generally consist of two iron pieces equally spaced on either side of the axis. Their distance from or along the axis may be varied to alter the strength of the applied asymmetry and the azimuth of the two pieces can be changed to set them perpendicular to the residual field. Electrostatic fields are used in modern microscopes to stigmatize the lens. Stigmators are also used with condenser lenses to give uniform, circularly symmetric illumination.

d. Focusing the image

The primary means of focusing the image of a specimen is by varying the objective lens strength. This important topic is discussed in greater detail in § I.E.5.

e. Objective aperture

The function of the objective aperture is to intercept electrons that have been scattered by the specimen through excessively large angles. The aperture may be positioned either in the gap between the two pole pieces (restricting the field of observation on the object to an area about the size of the aperture) or, most commonly, in the back focal plane of the objective lens, which lies a short distance behind the pole piece gap (Figs. I.80-I.82). The field of view of the specimen in this position is not restricted and widely scattered electrons are still prevented from reaching the image plane. Contamination effects are reduced in this position since only scattered electrons strike the periphery of the aperture opening.

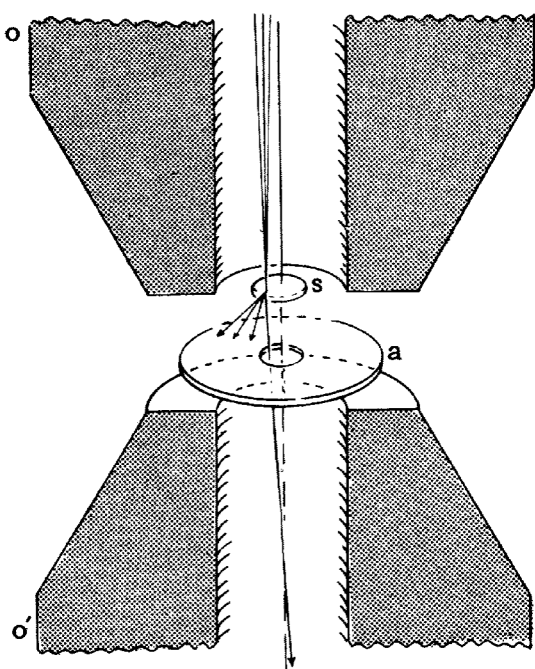


Fig. I.80. Interception of widely scattered electrons by the objective lens aperture. The diagram shows a lengthwise section through objective lens pole pieces, between which are seen the specimen, s, and physical aperture, a. The 'subtractive' action on a pencil of electrons, which images an individual image point, is demonstrated. (From Wischnitzer, 1970, p.60)

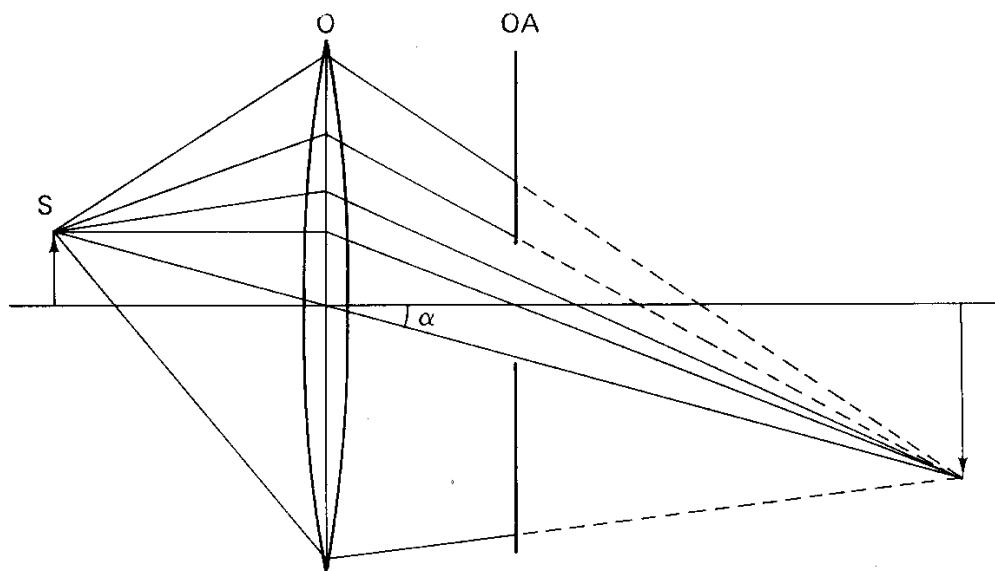


Fig. I.81. Function of the objective aperture (OA) in stopping widely scattered electrons from the specimen (S) in front of the objective lens (O); α is the semi-angular aperture of the lens. (From Agar, 1974, p.27)

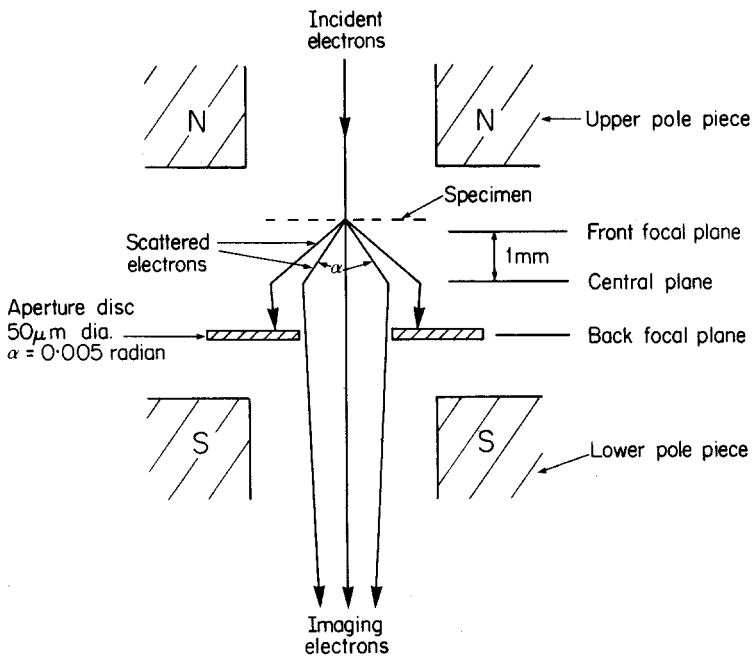


Fig. I.82. Action of the objective lens physical aperture in a short focal length immersion objective lens under high magnification conditions. The aperture intercepts widely scattered, high chromatic aberration electrons, which would degrade the image if not stopped by the aperture. The smaller the hole, the more scattered electrons are intercepted and the higher the image contrast. The hole also serves to define α , the semi-angular aperture of the lens. Note, however, that α should equal 0.0025 radians in this example. (From Meek, 1976, p.98)

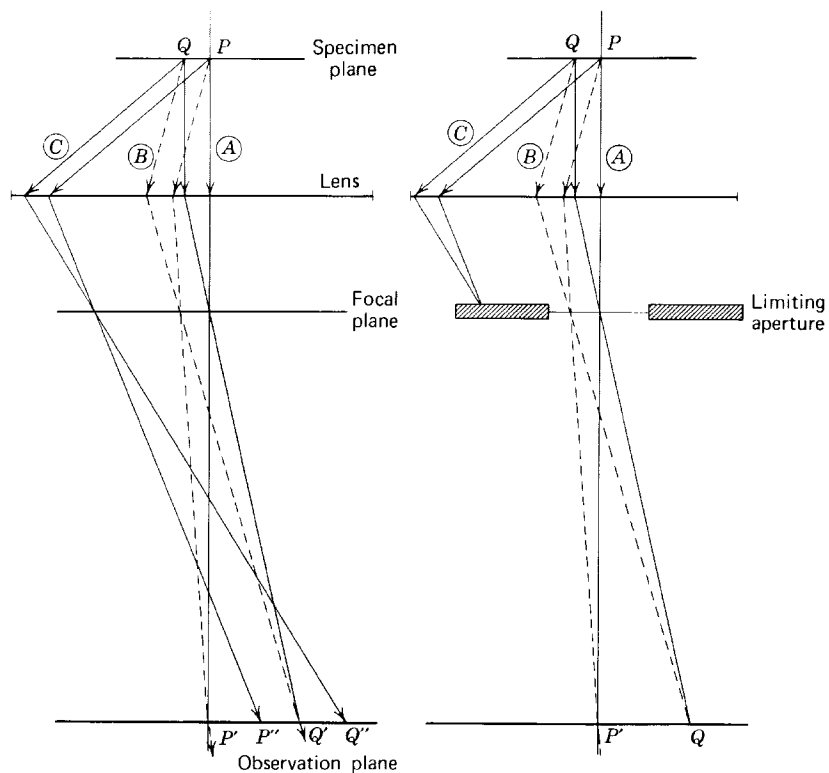


Fig. I.83. Action of the limiting objective aperture. When an aperture is placed in the back focal plane of the objective lens (b), widely scattered rays from the object (*i.e.* those that suffer most from spherical aberration of the lens) are blocked from contributing to the image. Hence, the image formed in (b) is superior to the one formed in (a) with no aperture in place. (From Slayter, 1970, p.428)

The aperture is easily centered if the **back focal plane** of the objective lens is imaged on the fluorescent screen (Fig. I.84). This method is the same way in which the TEM is used to form and image **electron diffraction** patterns (discussed in greater detail in § I.F.1). Note that, in order to observe the aperture, a specimen must be in the beam so electrons are scattered away from the optical axis onto the edge of the aperture. In the absence of a specimen and scattered electrons, the aperture is difficult or impossible to see.

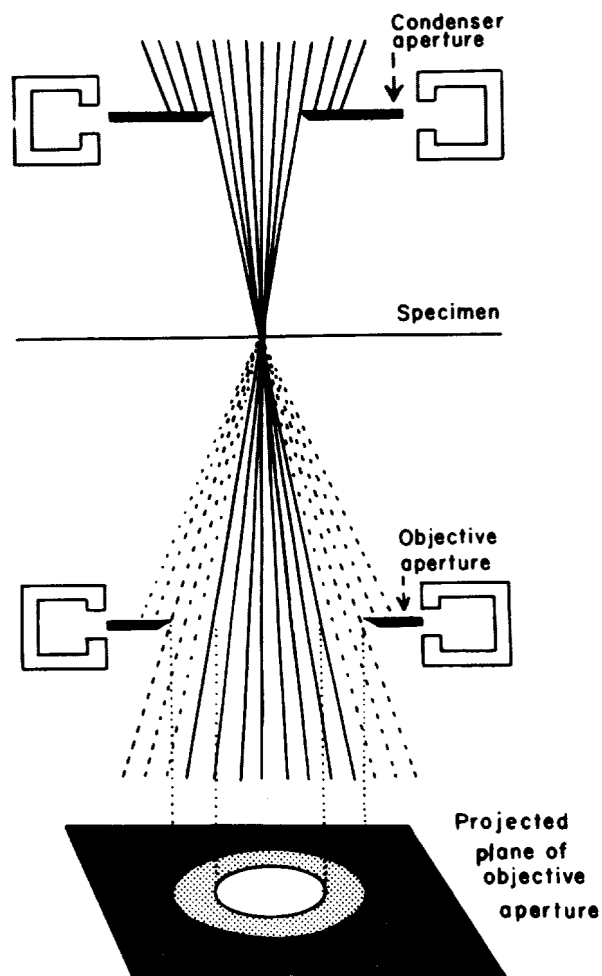


Fig. I.84. Objective aperture alignment with microscope operating in electron diffraction mode. (From Wischnitzer, 1970, p.88)

The central hole in objective apertures is generally 25-75 μm in diameter and should be perfectly circular and maintained scrupulously clean since contaminating deposits tend to become charged and distort the imaging field. The smaller the aperture the more image contrast is improved, but it is harder to manufacture small apertures with good circular symmetry and, with smaller apertures, the more serious are the effects of contaminants on the imaging beam. If the edge of the aperture becomes covered with a thin, electrically insulating layer of contamination, this becomes charged when subject to bombardment by the widely scattered electrons, and the charged aperture acts as a weak electrostatic lens and can deteriorate image quality. Also, astigmatism is introduced because the contamination buildup is not likely to be symmetrical. Even more important, if the size of the aperture is decreased excessively, image resolution will be limited owing to diffraction effects (§ I.A.3.d Abbe Equation and § I.B.3.b).

Most microscopes have a device that carries several apertures, any of which can easily be introduced into the column at the back focal plane of the objective lens (Fig. I.85). Ultrathin, self-cleaning metal apertures are most commonly used. Electrons that impinge on such apertures can be used to raise the temperature sufficiently to remove the contaminating layers while they are in

the microscope. Apertures in older TEMs were generally made of platinum or molybdenum, but these had to be regularly cleaned.

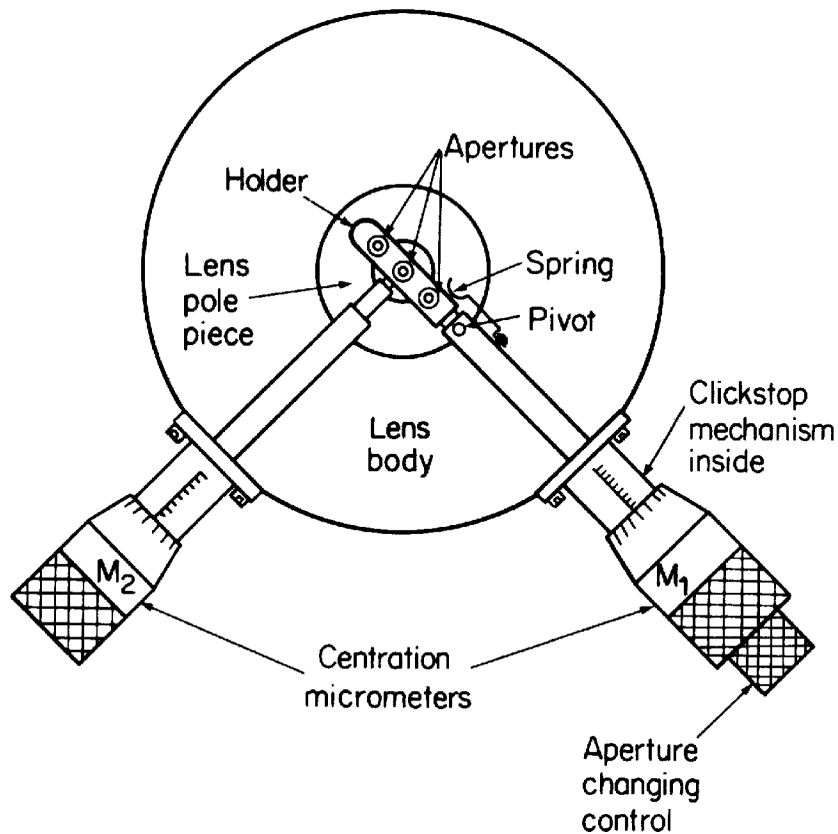


Fig. I.85. Top view (i.e. as if from the electron gun looking down the microscope column) of a movable lens aperture selector and centration mechanism. (From Meek, 1970, p.95)

f. Specimen stage (Figs. I.86-I.87)

A suitable specimen stage must meet the following requirements:

- The specimen carrier must be simply and rapidly exchangeable from outside the column. A minimum amount of air can be allowed into the column vacuum during specimen exchange so that the operating vacuum can be restored in a minimum time. This necessitates the provision of a specimen airlock so the column doesn't have to be brought to atmospheric pressure each time a new specimen is inserted into the column.
- The plane in which the specimen sits should be well defined with respect to its position along the axis of the optical system.
- There should be minimum backlash (<100 nm) in the specimen movements and there should be no drift after the operator finishes moving the specimen stage.
- Vibrations, thermal motions, mechanical drift, and movements of electrostatic origin must be reduced to such a level that the specimen moves through less than the minimum resolved distance during exposure of the image.
- The specimen holder must have good thermal contact with the specimen so that any heat dissipated in the specimen is quickly conducted away. The specimen stage is also required to make good thermal contact with the specimen holder in order to carry away the heat generated by the electron beam.
- The specimen holder has to be designed for entry through a vacuum lock and fit snugly into the specimen stage itself.

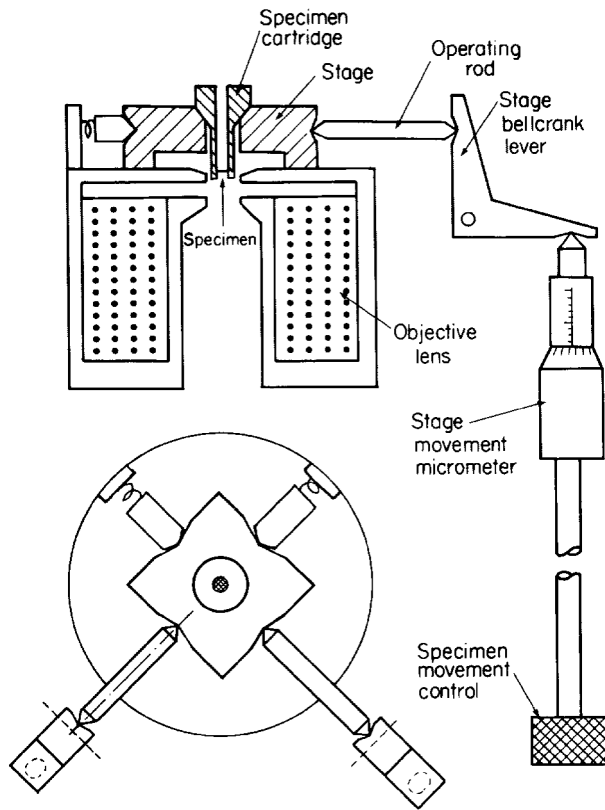


Fig. I.86. The top-specimen -entry, drop-in cartridge type of objective lens, stage, and stage motion. (From Meek, 1970, p.114)

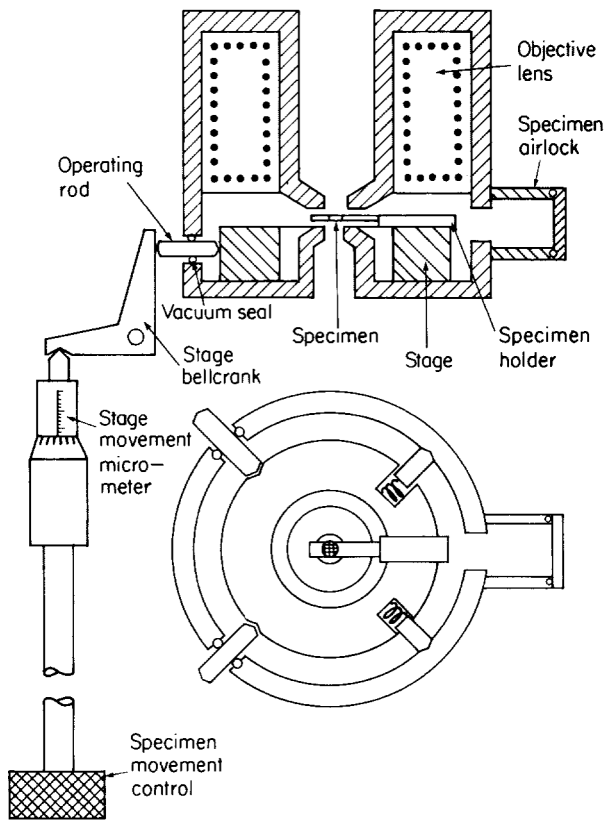


Fig. I.87. The side-specimen-entry, immersion type of objective lens, stage, and stage motion. (From Meek, 1970, p.115)

g. Special stages

- **Tilt stage:** Used to collect three-dimensional information about the specimen. In principle the specimen may be tilted to any angle, but in practice it is limited to $\pm 60\text{-}70^\circ$ because at large angles the specimen field of view narrows significantly as the openings between the grid bars contract in the plane perpendicular to the optic axis. The space required to accommodate the specimen is severely restricted if high resolution (and consequently short focal length) is required. Under normal conditions when the specimen is tilted, the image moves out of focus. A eucentric stage keeps the specimen area of interest near the tilt axis and minimizes changes in focus.
- **Multiple specimen stage:** Useful for observing several specimens in succession or, in special built holders, a set of serial sections.
- **Furnace heating stage:** Allows the specimen temperature to be increased in a controlled manner (up to $\sim 800^\circ\text{C}$). These stages generally take a long time (30-45 min) to stabilize before thermal drift is reduced to acceptable levels. This type of stage is primarily useful in metallurgical, not biological TEM.
- **Grid heater stage:** A heating current is passed directly through the grid mesh supporting the specimen. These stages have problems ensuring good thermal contact. Also, it is difficult to obtain an accurate estimate of the temperature, which can reach as high as 2000°C .
- **Cold stage:** Cooled by thermal contact with liquid nitrogen or liquid helium or by a constant flow of cold nitrogen gas. Temperatures in the range -130 to -170°C can be achieved. This type of stage has recently become quite popular and is useful for examining frozen-hydrated, biological samples (discussed in greater detail in § II.E).
- **Straining stages:** This stage provides a way to impose a stress on the specimen while it is being observed.
- **Gas reaction stage:** The specimen is enclosed in a cell that is sealed with two thin windows or with small apertures above and below the specimen to restrict gas flow into the microscope vacuum. The gas pressure around the specimen may then be raised to atmospheric pressure in order to examine gas reactions.
- **Hydration or "Wet" stage:** This is similar to the gas reaction stage wherein the specimen can be kept in a hydrated state by maintaining a relatively high humidity. Some early success was achieved with this type of stage in recording electron diffraction patterns at resolutions greater than 0.3 nm from hydrated, crystalline biological samples but currently available cold stages (as described above) seem to be better suited for examining "native" biological structures.
- There are **countless other special stages**, designed to examine specimens under a variety of conditions. The complexity and usefulness of these stages depends in large part on the ingenuity of the designer and demands made by the user.

h. Anticontaminator (Fig. I.88)

The anticontaminator is a **cooled surface** placed close to the specimen to trap residual gases in the column and prevent them from interacting unfavorably with the specimen. Most anticontaminators are cooled with liquid nitrogen. With frozen-hydrated specimens use of an anticontaminator is essential, otherwise the specimen itself would act as an anticontaminator.

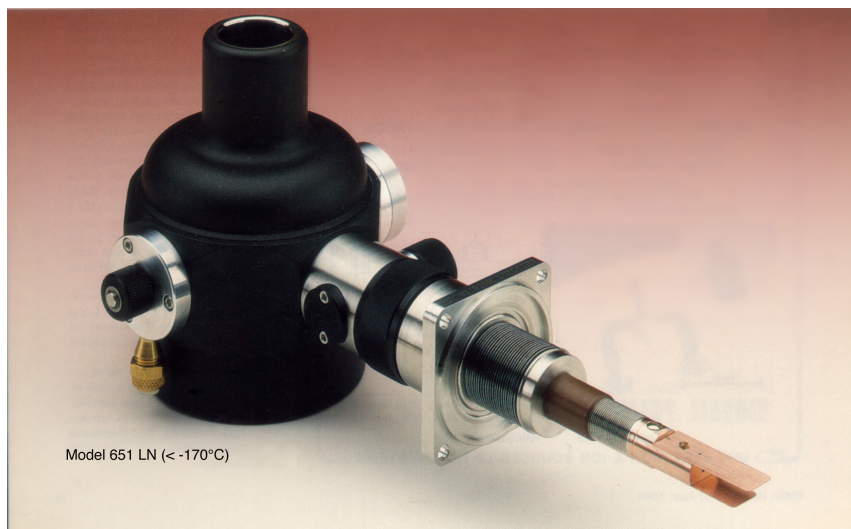


Fig. I.88. Twin copper-blade type anticontaminator made by Gatan, Inc. for use in FEI/Philips electron microscopes. The two blades form a 'sandwich' around frozen-hydrated specimens and prevent contamination from reaching the specimen surfaces from the microscope column above or below the specimen. Each blade has a small hole to allow unobstructed passage of the electron beam. (From Gatan, Inc. brochure).

I.B.5. Projector Lenses

a. General description

Projector systems produce images from relatively large areas (*i.e.* the magnified image produced by the objective lens) with a beam of electrons with a relatively small aperture angle. Lens aberrations of the projector do not influence final resolution but may produce distortion in the final image. The object of the projector lens is the intermediate image produced by the objective lens. The angular aperture of the imaging beam is α_c/M_o , where M_o is the magnification of the objective lens. The projector magnifies an area of the intermediate image, which may be several millimeters in diameter. Owing to the **large depth of focus** of the objective lens (§ I.B.5.c below), the strength of the projector can be varied over wide limits without noticeable alteration of focus in the final image. The **depth of field** of the projector (§ I.B.5.c below) exceeds that of the objective lens since the aperture angle of the electrons entering the projector lens is far smaller ($\sim 10^{-4}$ radians or less).

Most modern instruments employ a two to four projector lens system (diffraction, intermediate, and one or two projector lenses: Figs. I.31-I.33) to obtain a wide range of magnifications while, at the same time, keeping the overall length of the instrument reasonably short (~ 2 meters). A Philips EM420, for example, has four lenses arranged in the following order: diffraction, intermediate, projector 1, and projector 2. Each microscope has a different formula for producing a wide range of image magnifications (usually from 1000X up to 500,000X or more). In some instances, the intermediate lens is highly excited (high lens strength, short focal length), which demagnifies the image formed by the objective lens and gives a range of low magnification settings. At high magnification, the intermediate lens strength may be weakly excited or even turned completely off. In three- or four-lens projector systems, the addition of the diffraction lens means that the intermediate may be used in a different way than that just mentioned above. In any case, a **real image** is formed on the fluorescent screen or photographic emulsion or on a charge coupled device (CCD) camera or on a direct detector device (DDD) camera.

Since only slight variations in the objective lens current are used to focus the image, the magnification does not appreciably change as a result of adjustments in the objective lens strength.

b. Distortion (Figs. I.69-I.72)

The angular aperture of the electron beam **leaving** the objective lens is sufficiently small to make negligible the loss of resolution in the image due to spherical aberration in the projector lenses. The contribution of the projector system to magnification is sufficiently small to make unimportant the astigmatism introduced by these lenses caused by asymmetric lens fields. On the other hand, the intermediate image is considerably larger in extent than the original specimen imaged by the objective lens (by a factor equal to the magnification of the objective, M_o). Thus, some of the image points in the intermediate image that become object points for the projector lens system are

located a considerable distance off the optical axis. Therefore, the projector system can produce considerable distortion (**pincushion**, **barrel**, and some **spiral**), although resolution is unaffected. The distortion is negligible at high magnification since only the centermost portion of the image is observed (*i.e.* the paraxial portion of the image formed by the objective). At low magnifications, distortions can seriously affect the image.

The objective and projector lenses are normally at higher power than the intermediate lens, which is used as a weak lens of variable power to control overall magnification. As the magnification of the intermediate lens is reduced, its aperture increases and aberrations become noticeable in the final image. For **thick** specimens, **chromatic change of magnification** may give rise to an unacceptable, out-of-focus effect at the periphery of the image (Fig. I.76). Distortions arising in the intermediate and projector lenses can be arranged to be in opposite senses and thus cancel out. Thus, barrel distortion in the intermediate lens can be compensated by pincushion distortion in the projector, although exact compensation can only be achieved at one magnification.

Some projector systems include magnetic shielding (using μ metal) to reduce the effect of ambient stray magnetic fields on the final image.

c. Depth of field and depth of focus (Figs. I.89-I.90)

Recall that, any lens, however perfect, can only image a point object as an Airy disc, the diameter of which is the resolving power of the lens.

1) Depth of focus (Image Plane)

There is a finite distance along the axis, D_i , where the image appears equally sharp. This is called the **depth of focus**. If an image screen were placed anywhere within $D_i/2$ of the "exact" focal position, the image would still appear to be about as perfectly focused as is possible.

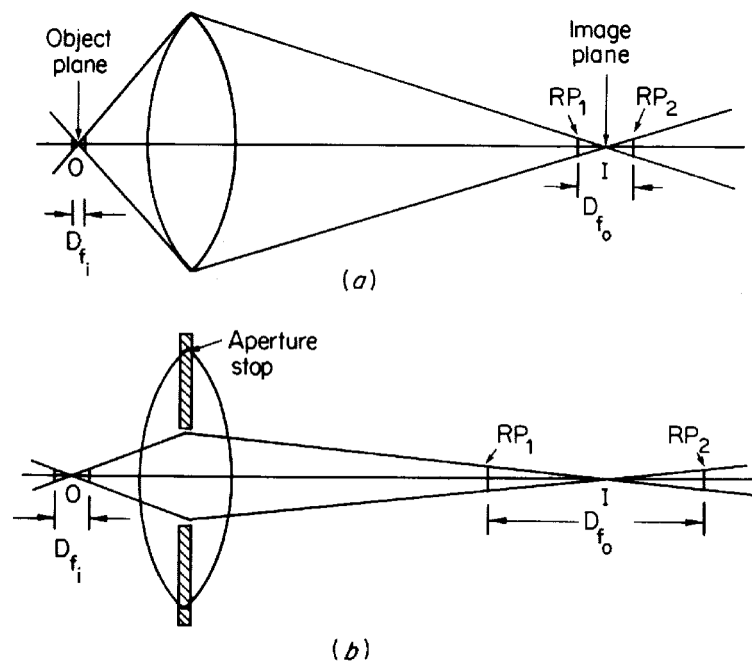


Fig. I.89. Depth of field and depth of focus, and the effect of lens aperture on them. As lens aperture is reduced, both depth of field and depth of focus are increased. (From Meek, 1970, p.81: Note the difference in convention - $D_{f_i} = D_o$ in text and $D_{f_o} = D_i$ in text)

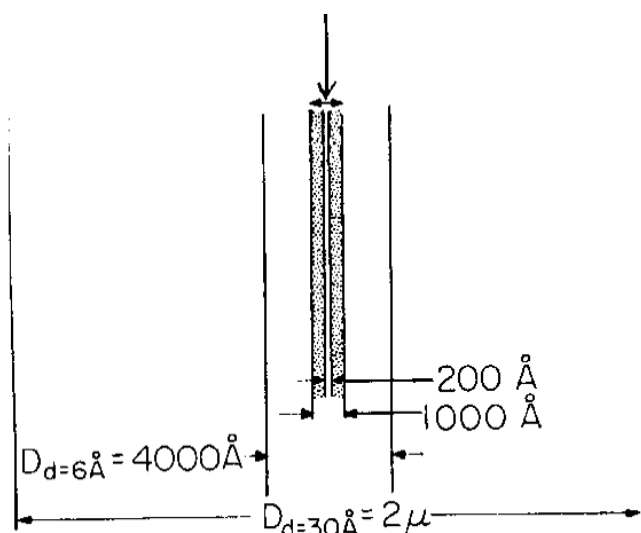


Fig. I.90. Depth of field at resolutions of 6 Å and 30 Å in relation to useful section thicknesses, below 1000Å. (From Sjostrand, 1967, p.119).

2) Depth of field (Object Plane)

Because of the existence of the depth of focus on the image side of the lens, there is an analogous distance along the axis on the object side over which the object could be moved and still give a maximally sharp image (at the position of the "exact" image plane). This distance is called the **depth of field**, D_o , and is related to the depth of focus by simple geometry.

The **depth of field** of the objective lens is given by: $D_o = 2d/\tan\alpha_o$

where d = minimum object spacing one hopes to resolve (given as the radius of the Airy diffraction disk corresponding to an object point, O (Fig. I.89)

and α_o = semi-angular aperture of the objective lens.

Thus, for $d = 1.0$ nm and $\alpha_o = 5 \times 10^{-3}$ radians, $D_o = 400$ nm, which is greater than the thickness of most specimens observed in a 100kV electron microscope at this resolution. Hence, a thin specimen appears equally sharp throughout its thickness. This characteristic of imaging in a TEM is why TEM images are generally called **projection** images. TEM images are, to a good approximation, simply projected views of the entire contents of the specimen in the direction of the electron beam.

The **depth of focus** (D_i) of the objective lens may be determined from the expression:

$$D_i = M^2 2d/\tan\alpha_o = D_o M^2$$

where M = total magnification of the compound magnifying system,

and α_o = semi-angular aperture of the objective lens.

If $M = 50,000X$, $d = 1.0$ nm, and $\alpha_o = 5 \times 10^{-3}$ radians, then $D_i = 1000$ meters!

Thus, the fluorescent screen, photographic plate or film can be placed **anywhere** beneath the projector lens and the final image will be in equally sharp focus although the magnification will differ. The magnification of the image that appears on the viewing screen may be as much as 20-50% lower than that on the final photographic emulsion.

Note that *decreasing* the aperture of the objective lens *increases* both D_o and D_i . Also note that the very large depth of field and focus **does NOT eliminate** the requirement for **VERY CAREFUL FOCUSING** of the image (by adjusting the objective lens strength). In light microscopy, depth of field and depth of focus are about the same magnitude. In TEM, depth of focus is many times greater than the depth of field (by the factor M^2).

I.B.6. Camera and Viewing System

a. Viewing the image

The electron optical image is typically projected onto a fluorescent screen where the kinetic energy of the electrons is transformed into light energy through fluorescence so the image can be observed directly. The fluorescent screen consists of a surface coated with a layer of activated zinc sulfide crystals. The resolution of the image on the screen is determined by the size of these crystals ($\sim 50\text{-}75\ \mu\text{m}$).

The fluorescent screen enables the operator to view the image to select an appropriate area and to focus the image before recording it on a photographic emulsion or in a CCD or DDD camera. Detail in the image on the fluorescent screen is conveniently viewed through an external binocular (usually 10X: Fig. I.91). The angular aperture of the binocular is large, which helps collect light at an appreciable solid angle from the screen. If large enough, the increased collection angle can balance the loss in illumination intensity due to the magnification of the system.

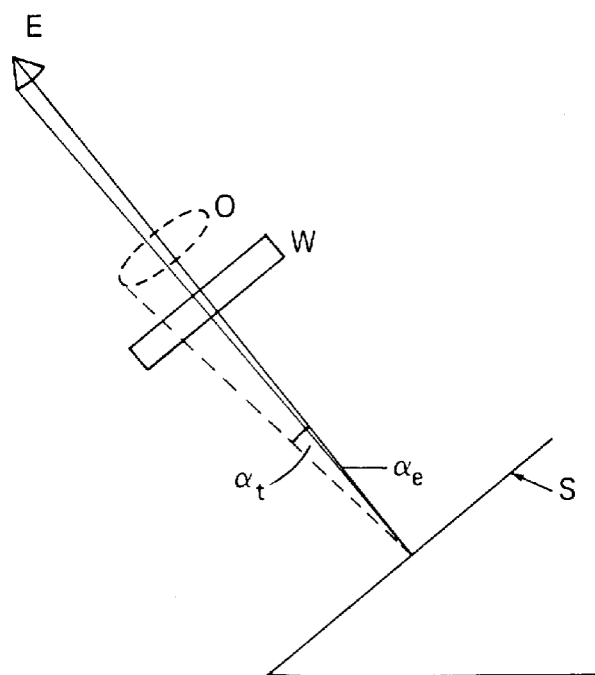


Fig. I.91. Diagram to illustrate how the use of the viewing binoculars (object lens O) increases the angular aperture of the viewing image formed on the screen S from α_e (by the unaided eye) to α_t . W is the lead glass viewing window. (From Agar, 1974, p.69)

b. Recording the image

Since the image on the screen lacks the resolution inherent in the electron image and does not provide a permanent record, the image is directly recorded on a photographic emulsion or in a CCD or DDD camera at a magnification sufficient such that the photographic or CCD or DDD record does not reduce the resolution captured in the electron image. For photographic emulsions, resolution is generally on the order of $5\text{-}10\ \mu\text{m}$, or at least five times better than the resolution of the fluorescent screen. For example, at a magnification of 50,000X, a detail 1.0 nm in size in the specimen will appear $50\ \mu\text{m}$ large in the recorded image. Although this is just beyond the limits of delectability to the eye on the fluorescent screen, the photographic emulsion easily resolves this level of detail. The pixel dimension of most modern CCD cameras is $15\ \mu\text{m}$ and ranges between 5 and $15\ \mu\text{m}$ for DDD cameras, and so these size pixels are sufficient to easily capture 1.0 nm details magnified at 50,000X. Hence, with any of these recording media, there is no need to form an unnecessarily magnified image, as this requires greater beam intensity and more sample irradiation, and leads to greater radiation damage especially in biological specimens. Also, the field of view of the specimen shrinks as magnification is increased.

The electron image has traditionally been made into a permanent record on a photographic film mounted either above or below the fluorescent screen. Because of their convenience relative to the inefficiencies of photographic processing, CCD and DDD cameras are increasingly being used in modern microscopes to record images in digital form. In fact, it may not be too long before microscopes will only be equipped with digital recording devices and photographic processing will cease to exist.

Owing to the great depth of focus of the projector system, the exact position of the photographic emulsion or CCD or DDD camera is not critical and the recorded image will appear to be in the same focus setting as on the fluorescent viewing screen. Thus, the $8 \times 10\ \text{cm}$ photographic film or CCD or DDD camera is usually located several centimeters below the fluorescent viewing screen normally used to focus the image. However, it is very important to note that the final magnification of the recorded image depends on the position of the emulsion or CCD or DDD in the TEM column. Because the CCD or DDD camera typically sits in a position several

centimeters below the film camera, image magnification at the CCD or DDD is often 30% or more greater than that at the film plane.

As was shown in the example in § I.B.5.c, the depth of focus, D_i , for a resolution in the range 1-2 nm, and magnifications in the range 1×10^4 - 5×10^4 , and assuming a relatively large objective aperture angle ($\alpha_0 = 10^{-2}$ radians), ranges between 10 and 1000 meters! This demonstrates how unimportant it is that the screen and camera lie in different planes since their separation is negligible compared to the depth of focus.

The proper **exposure time** may be determined from a reading taken by a photocell aimed at the fluorescent screen or by reading the current on the screen itself. The most common type of photographic material still used by microscopists is the 8x10 cm sheet film. Older microscopes also used 35 mm and 70 mm roll films.

c. Photographic emulsion

A detailed discussion of electron photomicrography is given in § I.E.10.a. Briefly, photographic recording material generally consists of a plastic base coated with an emulsion composed of a layer of gelatin in which is embedded a photosensitive silver halide. The electron beam acts by liberating free silver from the silver halide grains and produces, after conventional chemical development, a photographic negative of the final electron image. The fine-grained negative (electron micrograph) contains a more detailed and higher contrast image than that produced on the fluorescent screen. A photographic enlargement of the negative makes the detailed information of the final image visible to the eye. It is therefore much better to use photographic enlargement to make details visible to the eye as opposed to enlarging the electron image in the microscope (which requires more electrons to achieve a constant illumination intensity).

d. Charge Couple Device (CCD) and Direct Detector Device (DDD) Cameras

Details about digital microscopy appear in § I.E.11 and I.E.12.

I.B.7. Vacuum System

a. General description

In order to allow passage of the electron beam through the microscope without interference from gas molecules, the pressure within the instrument has to be reduced to the point where there is a very small probability that an electron will encounter a gas molecule. At atmospheric pressure, the mean free path for an electron is about 6.5×10^{-6} cm (= 65 nm), whereas at 10^{-6} Torr, the path is about 50 meters. At 10^{-6} Torr, a typical "high" vacuum for a conventional TEM, a 1 cm³ volume contains 3×10^{10} molecules. At atmospheric pressure an electron beam would be spread out over an angle of about 10° after passing a distance of only 0.2 mm.

The high voltage difference in the electron gun would cause discharges if the pressure were high enough to allow gas molecules present to become ionized. This causes instability or flickering of the electron beam. Residual gases not only erode the hot filament, shortening its life, but they also condense on the specimen and contaminate it.

The vacuum system of most conventional electron microscopes consists of a diffusion pump backed by a mechanical pump to maintain the column at a pressure $< 10^{-6}$ - 10^{-5} Torr (Fig. I.92). In most modern, and especially high-end microscopes, ion getter and turbomolecular pumps are used to create vacuums in the 10^{-10} - 10^{-4} Torr range.

b. Types of pumps

Mechanical pump

In this type of pump an eccentric rotor traps and compresses gas from the chamber to be evacuated subsequently allowing it to escape through a vent (Fig. I.93). Compression is confined to the outlet side of the pump through different types of valving mechanisms. A good mechanical pump can just achieve a pressure of 10^{-3} Torr and its pumping speed at this pressure is very low.

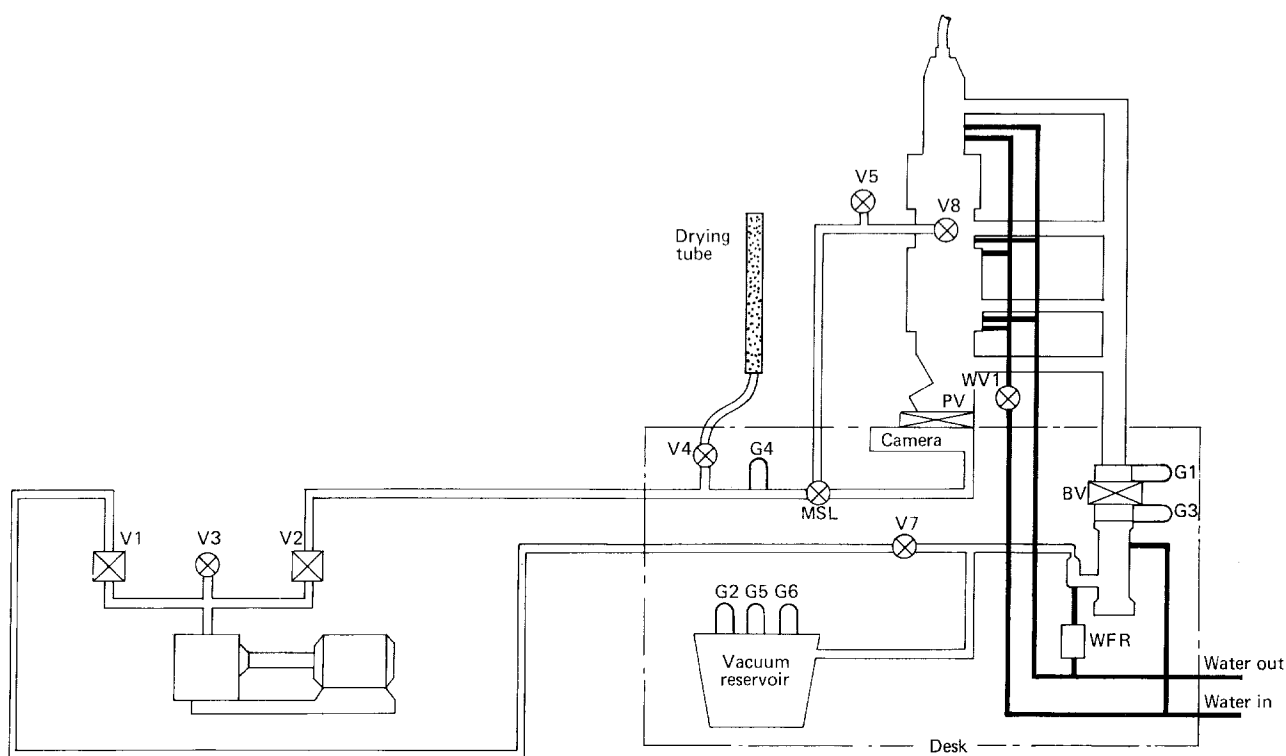


Fig. I.92. Schematic diagram of the vacuum and water systems in an electron microscope. The water circuit is indicated in thick black lines. A water flow relay WFR shuts off the electrical supplies to the diffusion pump and closes if the water flow rate is insufficient. (From Agar, 1974, p.76)

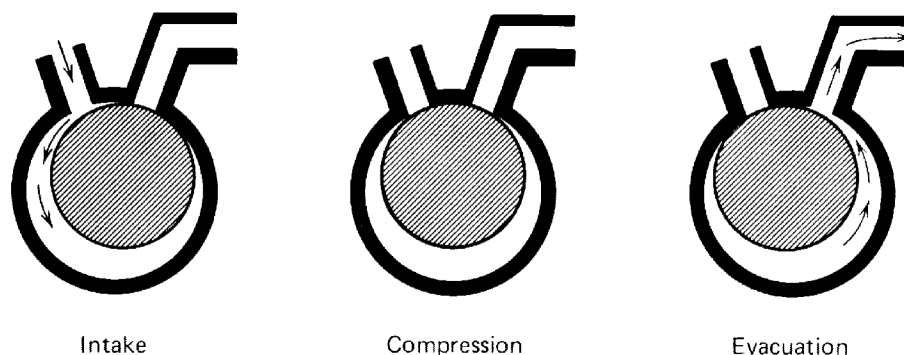


Fig. I.93. Action of a mechanical pump. (From Slayter, 1970, p.384)

Oil diffusion pump

The diffusion pump consists of i) an electrically heated reservoir of oil, ii) a chimney, and iii) a water-cooled casing (Fig. I.94). The diffusion pump operates by directing jets of vaporized oil molecules between the center column and the outer cooled wall (where condensation takes place). A gas molecule entering the jet from above the pump is trapped and forced downward into the body of the pump and eventually out through its exhaust. The rather high momentum of the oil vapor is transmitted by collision to the gas molecules in the path of the oil vapor jets and propels them to the bottom of the pump thus setting up a pressure gradient. The fore-pump removes the gas at the bottom (high pressure end) of the diffusion pump.

Gas molecules within the body of the pump are not able to penetrate back into the pumped space because of the dense jet of oil molecules. The pump continues to operate so long as the pumped gas pressure in the exhaust does not rise to too high a value. When this critical backing pressure is reached (usually about 10^{-1} Torr), there are enough gas molecules present to break through the oil

jet to stop the pumping action. The pumped gas normally exhausts into a vacuum reservoir of large volume and, when the pressure in the reservoir reaches a predetermined value, a mechanical backing pump periodically removes the gases.

The pressure in the system cannot be reduced indefinitely mainly due to small leaks of air into the vacuum past the seals and because of the vapor pressure of the oil. Thus, an equilibrium pressure of about 10^{-6} - 10^{-7} Torr is maintained by the pumping action of the diffusion pump. In addition, there are a considerable number of molecules trapped on the metal surfaces and within the metal itself of the microscope column and these take a long time to desorb. There may also be sources of relatively large number of organic molecules from the use of grease on gaskets or from careless handling of the parts of the vacuum system or the specimen holder. The use of Viton gaskets, which require no greasing, helps decrease the amount of organic molecules that deteriorate the vacuum inside the microscope. Also, water vapor from insufficiently prepumped photographic emulsions can enter the microscope and ruin its vacuum.

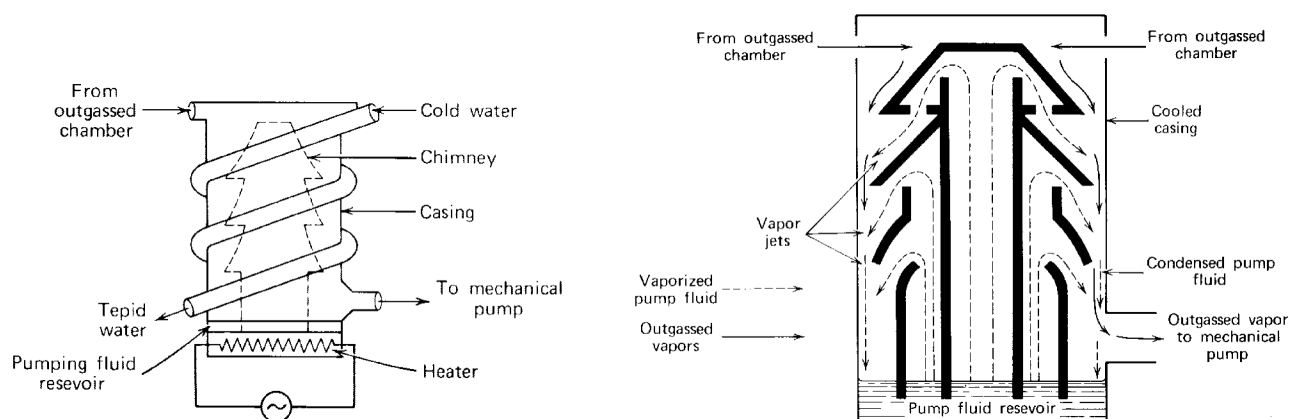


Fig. I.94. Diffusion pump: (Left) external view; (Right) cross section. (From Slayter, 1970, p.385)

Ion pump

Vacuums as high as 10^{-9} Torr can be obtained if the particles to be out gassed are ionized in a strong electric field that subsequently causes them to be trapped at a surface. The FEI Polara microscope, for example, uses a series of four **ion getter pumps** to reduce the vacuum in the gun and specimen area to less than 10^{-7} Torr, thus increasing lifetime of the expensive field emission gun and reducing specimen contamination.

Cryo pump

If a surface is cooled below the condensation temperature of a vapor, it acts as an effective pump, since vapor reaching the surface cannot escape again. A liquid nitrogen cooled surface is very effective for organic vapors, but not for lighter gases.

Turbomolecular pump

Turbomolecular pumps work on the principle that gas molecules can be given momentum in a desired direction by repeated collision with a moving solid surface. In the turbo pump (Fig. I.95), a rapidly spinning turbine rotor 'hits' gas molecules from the inlet side of the pump and directs them towards the exhaust side, which creates and maintains a vacuum. The exhaust is generally connected to a backing



Fig. I.95. Interior view of a turbomolecular pump. (From http://en.wikipedia.org/wiki/Turbomolecular_pump)

pump, which produces a pressure low enough (usually below 10^{-1} Torr) for the turbomolecular pump to work efficiently. Turbomolecular pumps are very versatile and can generate vacuums from intermediate ($\sim 10^{-4}$) up to ultra-high levels ($\sim 10^{-10}$ Torr).

To achieve extremely low pressures, the pump turbine reaches rotation rates of 20,000 to 90,000 revolutions per minute. The high rotor speed necessitates the use of very high-grade bearings, which increases costs. Because the compression ratio varies exponentially with the square root of the molecular weight of the gas, heavier molecules are pumped much more efficiently than light ones. Though most gases (including water vapor, which is introduced into the microscope column when biological specimens are examined and by photographic emulsions) are heavy enough to be well pumped, it is difficult to pump hydrogen and helium efficiently.

c. Design of the pumping system

During the normal operation of the microscope it is necessary to change the specimen, photographic film, the apertures, and the filament. Some or the entire column must be isolated from the rest of the vacuum system and brought up to atmospheric pressure. The specimen changing apparatus, for example, must be designed so only a minimal quantity of air is introduced into the column each time a new specimen is introduced. The camera is also isolated via a valve to allow changing the photographic film without disturbing the vacuum in the rest of the system.

d. Vacuum gauges

Vacuum gauges are an integral component of any modern microscope because they allow the operator or maintenance technician to monitor the quality of the vacuum obtained by each of the different pumps. The three most common types of gauges are described below.

Thermocouple gauge

A simple **thermocouple gauge** is suitable for pressures between atmospheric (760 Torr) and 10^{-1} Torr (Fig. I.96). It is used to monitor the vacuum produced by the mechanical rotary pump. This works on the principle by which a heated wire is cooled by gas molecules striking it. As pressure decreases, less heat is lost from the wire so that the temperature of the thermocouple rises. The rise in temperature is accompanied by an increased flow of current through the thermocouple circuit.

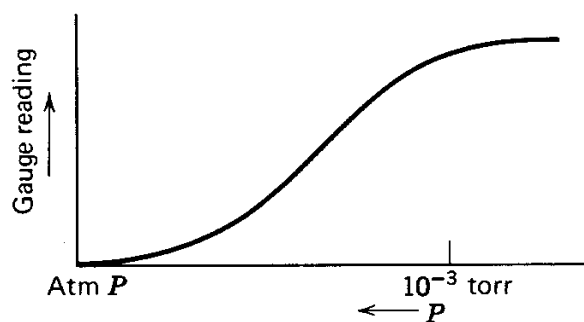


Fig. I.96. Thermocouple gauge characteristic. (From Slayter, 1970, p.386)

Pirani gauge

The **Pirani gauge** is another type of low vacuum meter (10^{-10} Torr) similar to the thermocouple except that the change in temperature of the heated wire leads to a change in the electrical resistance of the wire that can be monitored (Fig. I.97).

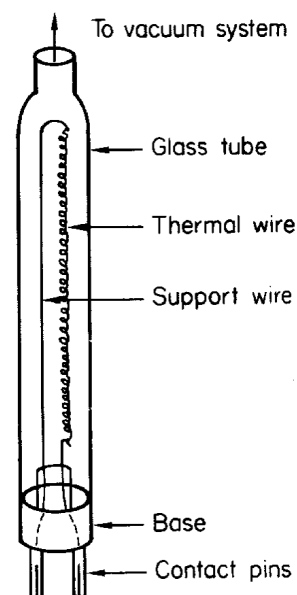


Fig. I.97. Schematic diagram of a Pirani gauge used to measure low vacuum. (From Meek, 1970, p.140)

Ion gauge

High vacuum (low pressure) is normally measured by a **penning-type gauge**, which depends on the ionization of the gas molecules in a high electrostatic field. The gauge is effective in the pressure range 10^{-3} - 10^{-6} Torr. In this type gauge (**ion gauge**) a stream of electrons, produced by thermal emission from a tungsten filament, is accelerated through about 150 volts toward a positively charged grid (Fig. I.98). Collisions between gas particles and the stream of electrons result in the formation of positively charged ions, which are attracted toward a negatively charged collector electrode, thus forming an ion current (Fig. I.99). At high pressure, the mean free path of the ion is small, so the intensity of the ion current is low. As pressure decreases, the mean free path increases and the ion current rises to a maximum at pressures of about 10^{-3} Torr. At still lower pressures, the current decreases owing to the unavailability of ionizable particles.

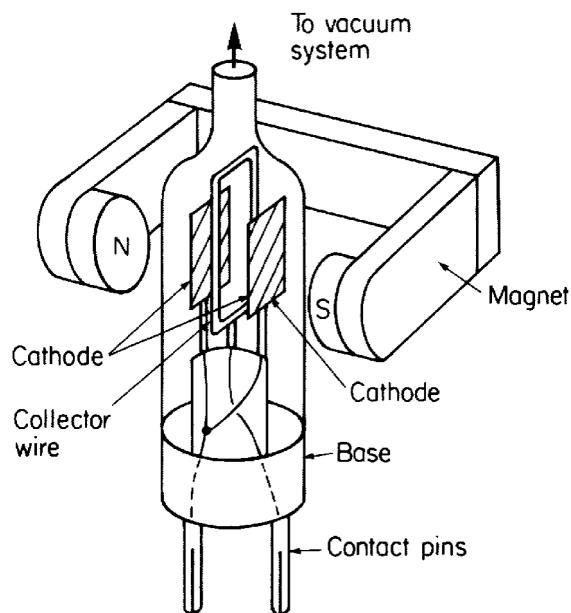


Fig. I.98. Schematic diagram of a Penning (Philips) gauge used to measure high vacuum. (From Meek, 1970, p.142)

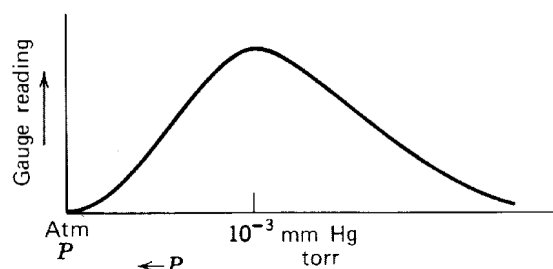


Fig. I.99. Ion gauge characteristic. (From Slayter, 1970, p.387)

e. Contamination of the column vacuum

The vacuum system can only pump down to a limiting pressure mainly determined by the vapor pressure of the fluid used in the diffusion pump (10^{-5} - 10^{-6} Torr). Since the pump fluid is generally a mineral oil, the vacuum in the column contains a large number of hydrocarbon molecules, which are also derived from the grease used to lubricate the movable rubber vacuum seals. The vacuum also contains residual gases, mainly water vapor and nitrogen and carbon monoxide.

Residual gases contaminate the specimen by condensing on it and thus reduce overall contrast by forming an amorphous layer over the specimen. Imaging electrons break up the hydrocarbon molecules into hydrogen and carbon atoms. Hydrogen is released but the carbon remains on the surface of the specimen. In a 'dirty' column, contamination can build up at a rate of 5 Å/sec.

The electron beam also ionizes residual water vapor, giving rise to hydroxyl ions (OH^-), which are very reactive and attack the carbon atoms in the specimen, causing release of carbon monoxide. This will act to burn away the contamination layer but it will also burn away the specimen.

Contamination affects all parts of the column that are struck by electrons. The most susceptible components are the movable apertures in the condenser and objective lenses. Contamination can also deposit on the inside of the apertures. Since the oily deposit is a bad conductor of electricity, it charges up and repels the electron beam passing through it, thus acting as an electrostatic lens. The layer is likely to build up unevenly, causing asymmetrical repulsion, which means the lens

behaves as though it were astigmatic.

Specimen contamination is reduced by using, i) a weaker electron beam, ii) shorter exposure times, and iii) an **anticontaminator**.

The anticontaminator is simply a cooled surface placed as close to the specimen as possible so hydrocarbons and other contaminants will preferentially condense on it (see § I.B.4.h and Fig. I.88). The usual design of this device consists of two copper blades placed above and below the grid carrier as close to it as possible. Holes in the blades allow the electron beam to pass through the specimen. The blades connect with a copper bar, which passes through a vacuum seal and out of the column into a dewar that is filled with liquid nitrogen.

I.B.8. Electrical System

a. General requirements

The electrical power supply of an EM must provide:

- Current to heat the filament and generate image-forming electrons.
- High voltage to accelerate the emitted electron beam.
- Current to each magnetic lens to provide the necessary focusing magnetic fields.
- Power to other circuits such as stigmators, beam deflectors, camera, and camera shutter, exposure meter, focus wobbler, safety devices, relay switches, heaters of the diffusion pumps, the rotary pump, etc.

b. Filament current supply

The filament requires between 2.5-3 amps as a heater current. The supply is usually D.C. to avoid ripples that would modulate the electron beam.

c. High voltage supply

The current supplied by the high voltage source must be on the order of magnitude of 0.1 milliamp or less. Thus, the accelerating potential supply is of the high voltage, low current type, delivering from 2-10 watts of power. The high voltage generated is negative with respect to earth since the anode is kept at ground potential.

d. Lens current supply

Individual current supplies are required for each lens with stabilities of a few parts per million. Most supplies are transistorized and operate at relatively low voltages. When high voltage to the gun is changed, a corresponding change is made in the lens currents to maintain focus and magnification (Recall that lens focal length is determined both by current in the lens coils **and** by the speed of the electrons in the imaging beam: § I.A.5.f). Most of the control of lens currents is programmed into the microscope so the correct lens combinations are obtained for minimizing distortion. The lens coils are cooled with water from a constant temperature source.

e. Stability requirements

To achieve maximum **high voltage stabilization**, stable high gain amplifiers with negative feedback from a resistive voltage divider between the cathode and ground are used. The gun potentials have to be generated in a tank containing filtered transformer oil, since air is not a sufficiently good insulator to prevent arching-over at the voltages employed. The whole high voltage generator is enclosed in the oil-filled tank. The stabilizer circuits, which are run at low potential for safety reasons, are isolated from the H.T. generator. The input to the generator is stabilized to the required degree and then the high potential is generated from the pre-stabilized input. This is generally performed in at least four stages to achieve a stabilization of the voltage to a few parts per million.

Lens current stabilization takes place in a fashion similar to that for the high voltage except that it is usual to generate the lens current first and to stabilize it afterwards. A high current primary generator is supplied from the mains regulator and provides sufficient current for all the lenses. A primary stabilizer then stabilizes this high current. Each individual lens then has a separate secondary stabilizer. The objective lens has the strictest stability requirement of all the lenses (one part in 10^5). The stigmators and beam deflectors require stabilities of the same order as the lenses and are generally fed from the lens circuits.

If the current through any of the imaging lenses varies, the image rotates about a point called the '**current rotation center**', which is coincident with the center of the screen when the optics of the microscope are properly aligned (see § I.D). A micrograph taken under these circumstances will be blurred at the edges and sharp at the center. If the accelerating voltage changes this also gives rise to an alteration in magnification: the image grows or contracts radially about a point called the '**voltage center**', which should also be coincident with the center of the screen.

Since the focal length of a magnetic lens is inversely related to the square of the current, the lens current stability requirement is higher than that of the accelerating voltage (by about a factor of two). Thus, if the accelerating voltage has to remain stable to one part in 5×10^4 , then the lens current must be stabilized to within one part in 10^5 over the period required for recording the image (1-5 seconds). The stability requirements for the other imaging lenses are less stringent (one part in 2×10^4) because the aberrations of these lenses are less highly magnified. Even larger instabilities in the condenser lenses can be tolerated. Auxiliary circuits that power the shutter, camera, exposure meter, alignment modulators and focus wobbler do not require as high a degree of stabilization as the lenses.

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