I. THE MICROSCOPE (Continued)

I.B. DESIGN OF THE TRANSMISSION ELECTRON MICROSCOPE

Discussion of the 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 usually consists of a tungsten wire (**filament**; Fig. I.58), which is bent into a hairpin ("V") shape and surrounded by a **shield** with a circular aperture (1-3 mm diameter) centered just below the filament tip. 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).

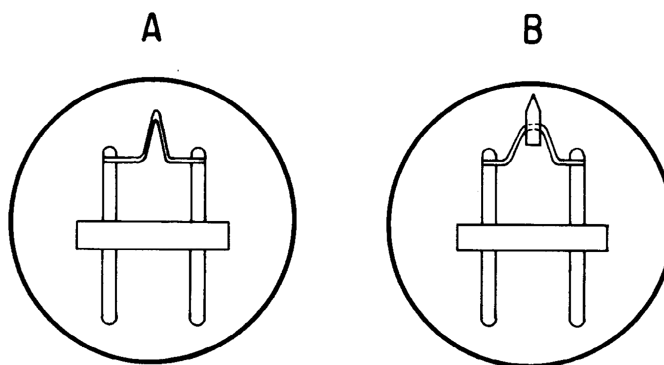

Fig. I.58. Electron gun filaments. (A) Standard tungsten filament. (B) A pointed filament. (From Wischnitzer 2nd ed., p.46)

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, which acts as an electrostatic lens focusing the true source (space charge of electrons which surround the filament tip) to form an image of the source below the anode. The **gun-crossover** is used as 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/cm^2), 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 (Fig. I.59).

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).

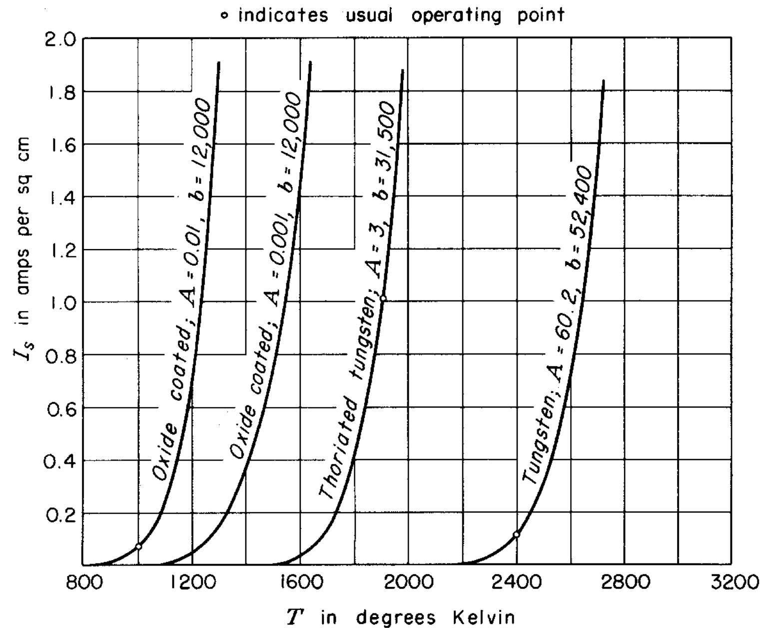

Fig. I.59. Dependence of electron emission on temperature for various thermionic emitters. (From Hall, p.146)

Lanthanum hexaboride (LaB_6) filaments have longer life and yield five to ten times higher brightness, but require better vacuum than tungsten filaments. Field emission guns (FEG) provide even greater brightness (1000x more than the LaB_6), but these add considerable cost to the instrument. Several literature citations concerning the principles and use of a FEG source are listed in the **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. 1.60) 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.

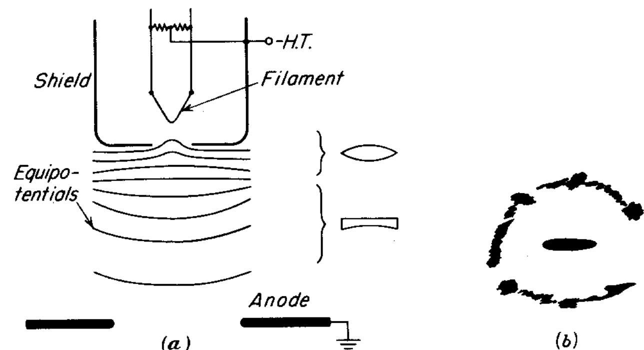

Fig. I.60. (a) Unbiased electron gun. (b) Typical emission pattern at a plane below the gun. (From Hall, p.147)

d. Biased/Self-biased gun (Figs. I.61-I.62)

A <u>negative potential between shield and filament</u> is produced by the flow of beam current through the bias resistor included in one of the supply leads to the filament. The shield is maintained by the bias resistor at a potential **slightly negative** with respect to that of the filament, while the anode

is at ground potential. For example, the beam current may be 300 μamp and the bias resistor 500,000 ohms, therefore the bias potential is -150 volts. Recall that $V = iR$, where V is the voltage (volts), i is the current (amps) and R is the resistance (ohms)

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.

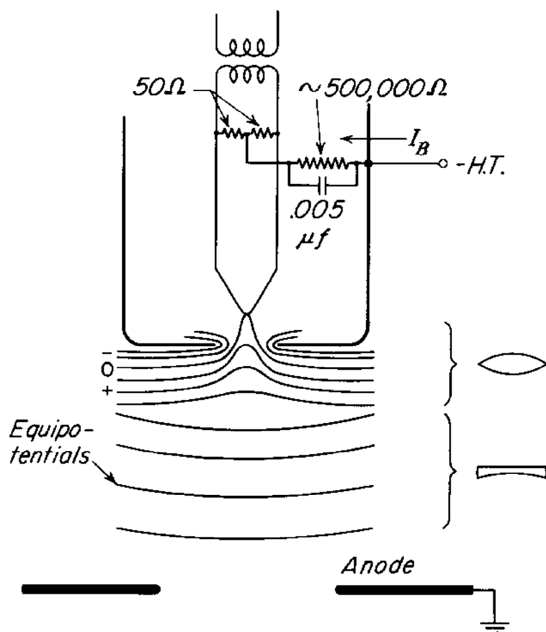

Fig. I.61. Self-biased electron gun. (from Hall, p.148)

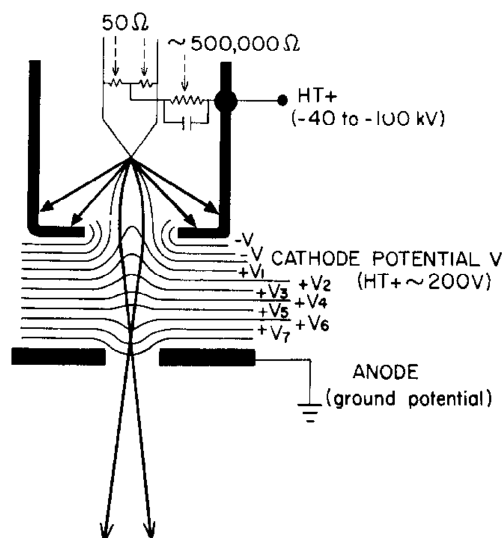

Fig. I.62. Self-biased gun. The filament is connected to the high voltage supply through a bias resistor and the shield is attached directly to the high voltage. The equipotentials of the field when the circuit is activated serve as a strongly converging lens. (From Sjostrand, p.74, modified from Hall, p.148)

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.63). 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. When this condition occurs, the gun is said to be **saturated.** Filament current is increased slowly until the beam current no longer changes. Going much beyond this point severely decreases filament life.

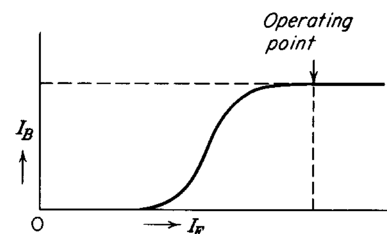

Fig. I.63. Emission characteristic of the self-biased electron gun. Beam current I_B verses filament current I_F. (From Hall, p.148).

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. Biased verses unbiased gun

The chief advantages of the biased gun over the unbiased gun 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 function of the condenser lens system is 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. 1.64a)

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 (~30-50μm). 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.

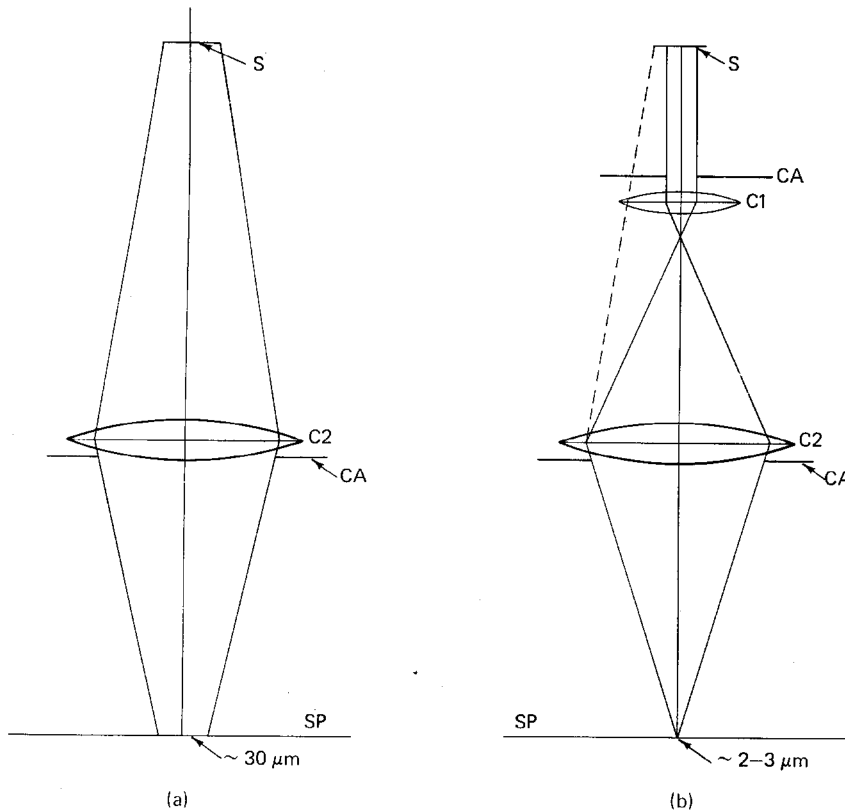

Fig. I.64. Comparison of single and double condenser lens systems. (a) Single condenser system. (b) Double condenser system. The condenser 2 lens C2 projects an image of about 30 μm diameter on the specimen plane SP from the source S. When the condenser 1 lens C1 is also used, the projected image is 2-3 μm in diameter. Apertures CA limit the beam angle. (From Agar, p.22)

b. Double condenser system: (Figs. I.64b-I.68)

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 which is irradiated.

A strong first lens (C1: short focal length) is used to produce a demagnified image (~1µm diameter) of the electron source and a weaker second 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 2-3µm. 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 (<10,000X).

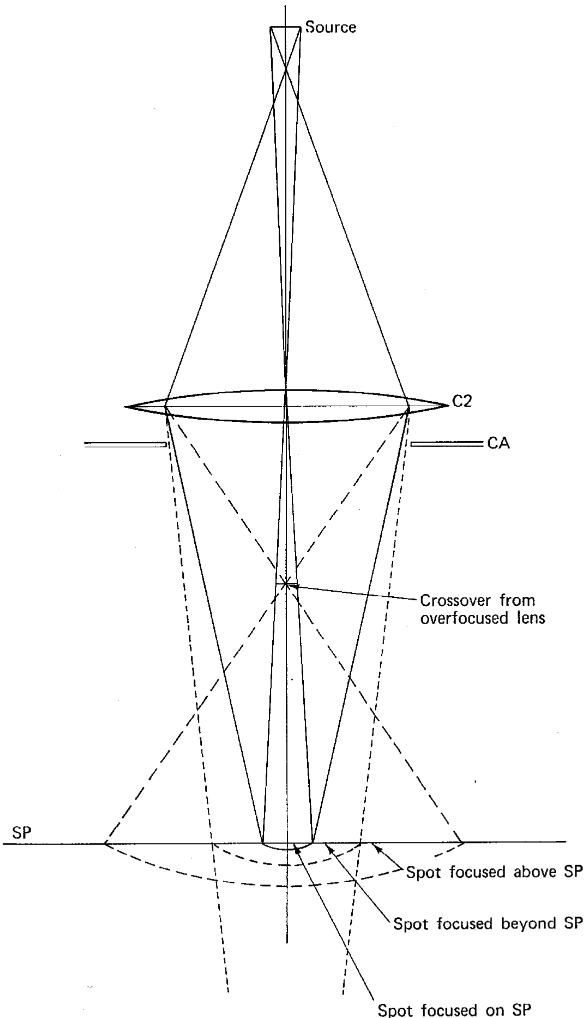

Fig. 1.65. Variation of beam intensity at the specimen by condenser lens variation. C2 is the condenser lens, CA is the condenser aperture. The spot diameter at the specimen plane SP is determined by the focus of C2. (From Agar, p.23)

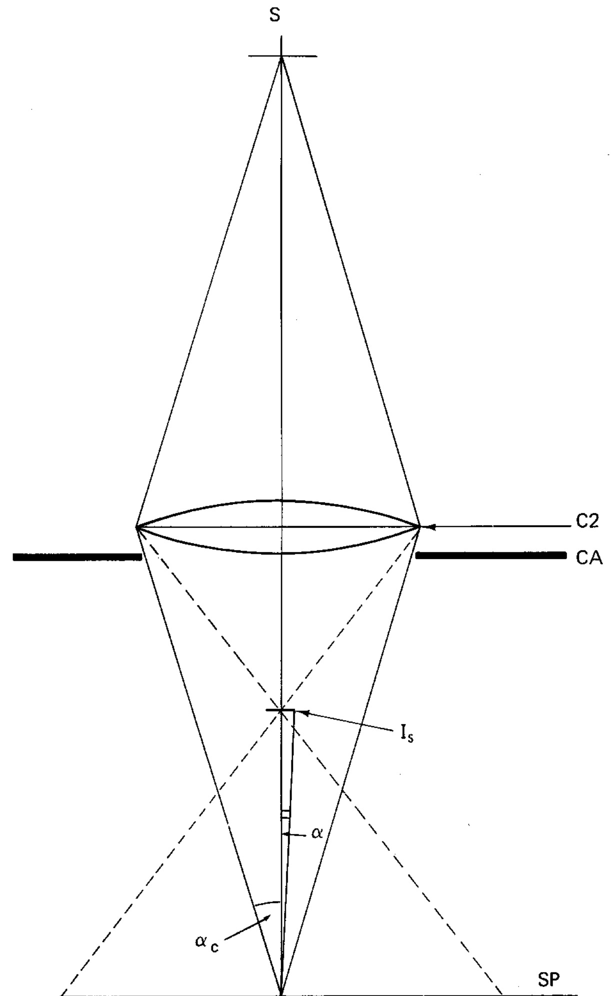

Fig. 1.66. Variation of illumination semi-angular aperture with condenser excitation. When C2 is focused on the specimen plane SP, the condenser aperture CA defines the limiting semi-angular aperture α_C. When the lens is overfocused, the source image Is defines the semi-angle α. (From Agar, p.24)

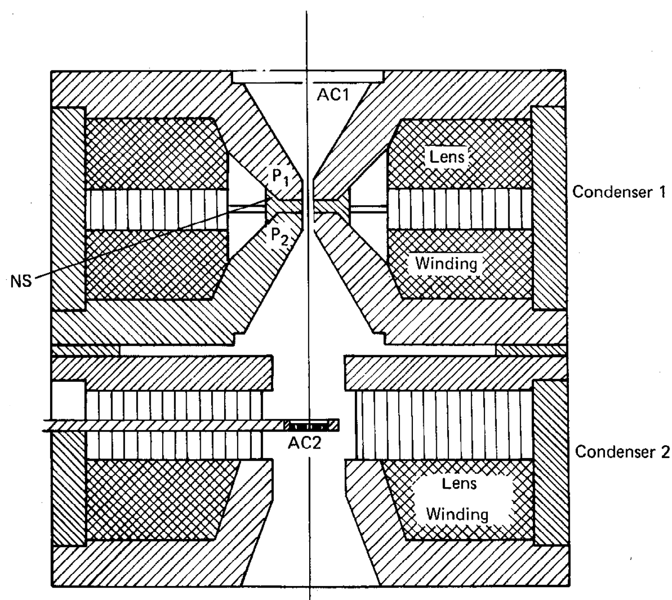

Fig. 1.67. Cross section through a double condenser lens assembly. The upper lens is condenser 1, with a fixed aperture. The lower lens is condenser 2 with an adjustable aperture. Pole pieces are indicated by P_1, P_2, and the nonmagnetic spacer by NS. (from Agar, p.47)

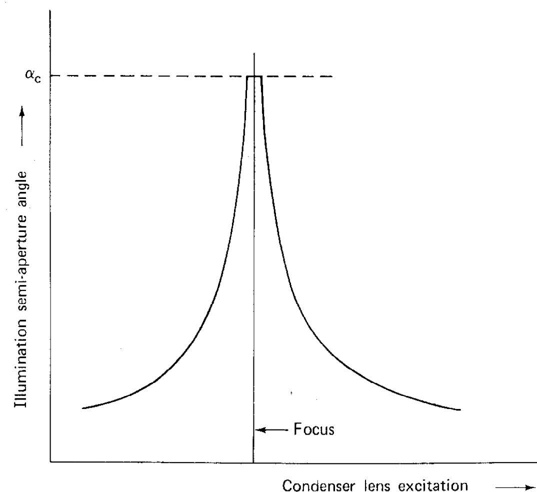

Fig. 1.68. Plot of variation of illumination semi-angular aperture with condenser lens excitation. The limiting illumination aperture α_C is determined by the diameter of the physical condenser aperture. (From Agar, p.25)

c. Condenser apertures (Figs. I.64-I.67)

Apertures in each lens limit the amount of electrons striking the specimen (protecting it from excessive irradiation) and limit the number of x-rays generated from electrons hitting 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. 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 is the maximum illumination intensity, but in general the poorer the image quality. 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. Fig. I.68 plots the variation in aperture angle with respect to the C2 lens excitation. C1 often has a fixed aperture and C2 a variable aperture (with 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.69)

- 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.
- Since C1 is closer to the source than C2 it has a larger acceptance angle (aperture) and therefore 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) than 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.

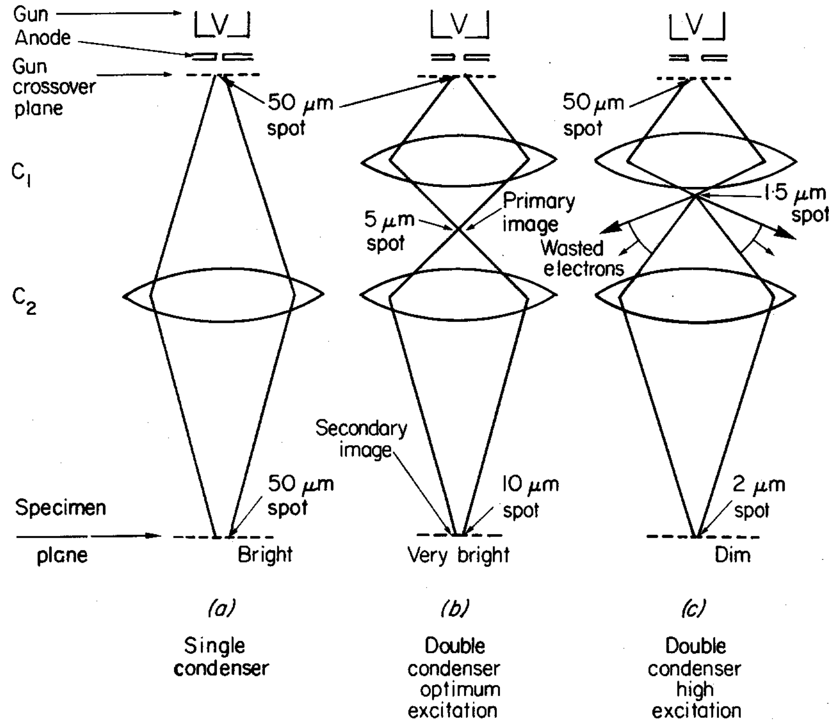

Fig. 1.69 Diagram showing how the efficiency of the illuminating system and hence illuminating spot intensity change with the setting of C1 lens. (From Meek 1st ed., p.110)

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.

The above defects are predicted on theoretical grounds. In practice, real lenses also suffer 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
- many others

b. Spherical aberration (Figs. I.70-I.72)

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 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 the case for glass systems. Spherical

aberration in electrostatic lenses is about 4-10 times more severe than in magnetic lenses.

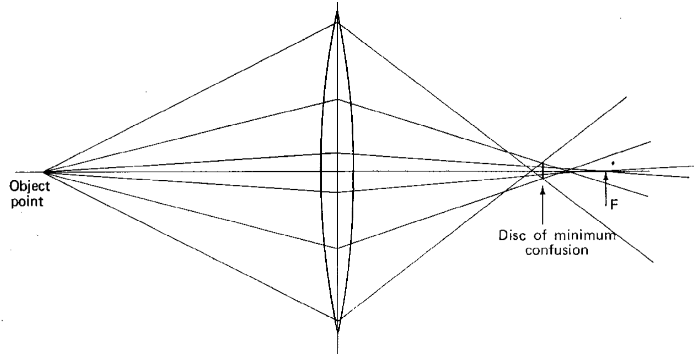

Fig. I.70. Spherical aberration in a lens. The rays close to the lens axis (paraxial rays) are focused at the Gaussian focus, F. Rays entering the lens at a larger angle are converged more strongly. The disc of minimum confusion is where the envelope of emergent rays has its smallest diameter. (From Agar, p.9)

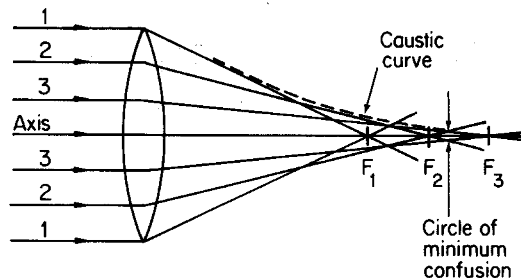

Fig. I.71. Spherical aberration arises because the peripheral rays are brought to a focus which is closer to the lens than rays which are nearer to the axis. (From Meek 1st ed., p.73)

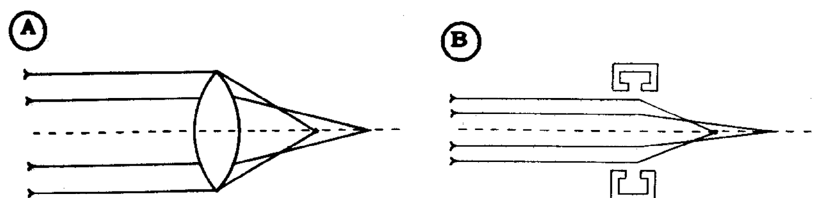

Fig. I.72. Spherical aberration for (A) a glass lens and (B) an electron lens. (From Wischnitzer 2nd ed., p.53)

Spherical aberration causes rays from one object point to not cross in a corresponding image point in the image space. Instead, they are distributed over a surface with a diameter d_{sa} in the image plane. At one point the envelope of the imaged rays has a minimum diameter known as the circle of least confusion. In the case of the TEM where the angular aperture 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 the focal length decreases.

The following table illustrates the expected resolution for various values of C_s and α (in radians):

Resolution limits due to spherical aberration, d_{sa} (nm)

C_s (mm)	$\alpha = 10^{-2}$	$\alpha = 5x10^{-3}$	$\alpha = 10^{-3}$
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

Thus, it would appear that in the absence of other types of aberrations or other factors (*i.e.* diffraction effects), resolution can be significantly improved 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). Slight underfocusing of the objective lens displaces the disk of least confusion to the image plane.

In fact, we have already shown that decreasing α limits resolution as a consequence of diffraction effects (Sec. I.A.3.d). Using the same values of α in the table on the previous page and using the Rayleigh criteria for estimating resolution ($d_{di} = 0.61\lambda/n \cdot \sin\alpha$, we realize that <u>spherical</u>

aberration and diffraction act in opposite fashion:

Diffraction limited resolution, d_{di} (Rayleigh criteria; use λ = 0.0037 nm for 100kV electrons)

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

The optimum aperture, α, at which the two major limits to resolution (spherical aberration and diffraction) are equal, can be computed. The aperture angle at which the resolution limits due to spherical aberration and diffraction (using the Rayleigh criteria) are equal is given by:

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

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 λ = 0.0037nm for 100kV electrons, α, and thus d_{sa} (or 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

c. Distortion (Figs. I.73-I.76)

Distortion is 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.

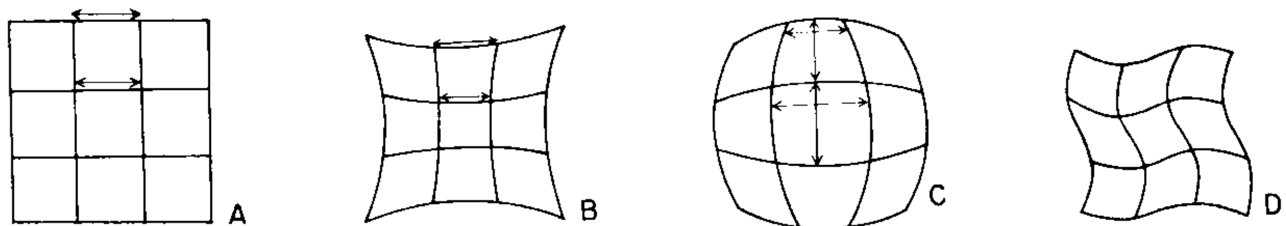

Fig. I.73. Distortion. (A) The undistorted image of the object. (B) Pincushion distortion. (C) Barrel distortion. (D) Spiral distortion. (From Sjostrand, p.58)

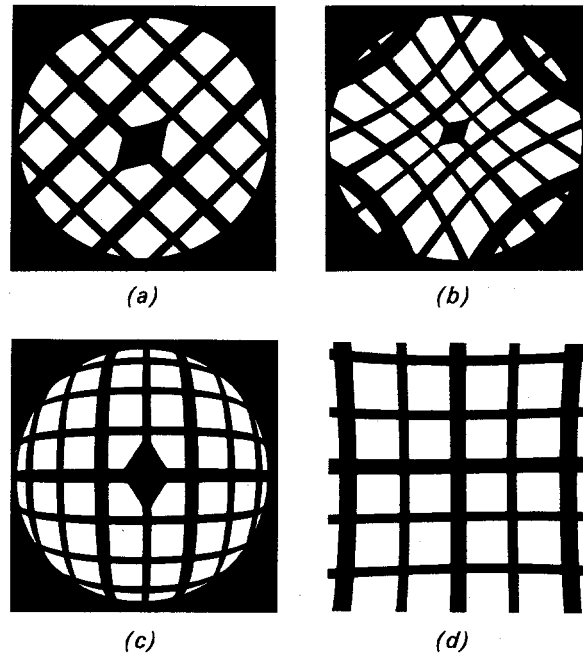

Fig. I.74. Four electron micrographs at very low magnification (approx. x50) showing: (a) negligible distortion; (b) pincushion distortion; (c) barrel distortion; and (d) sigmoid distortion. The distortions were introduced deliberately by altering the focus of the intermediate lens, which was being used as a long-focus objective. (From Meek 1st ed., p.75)

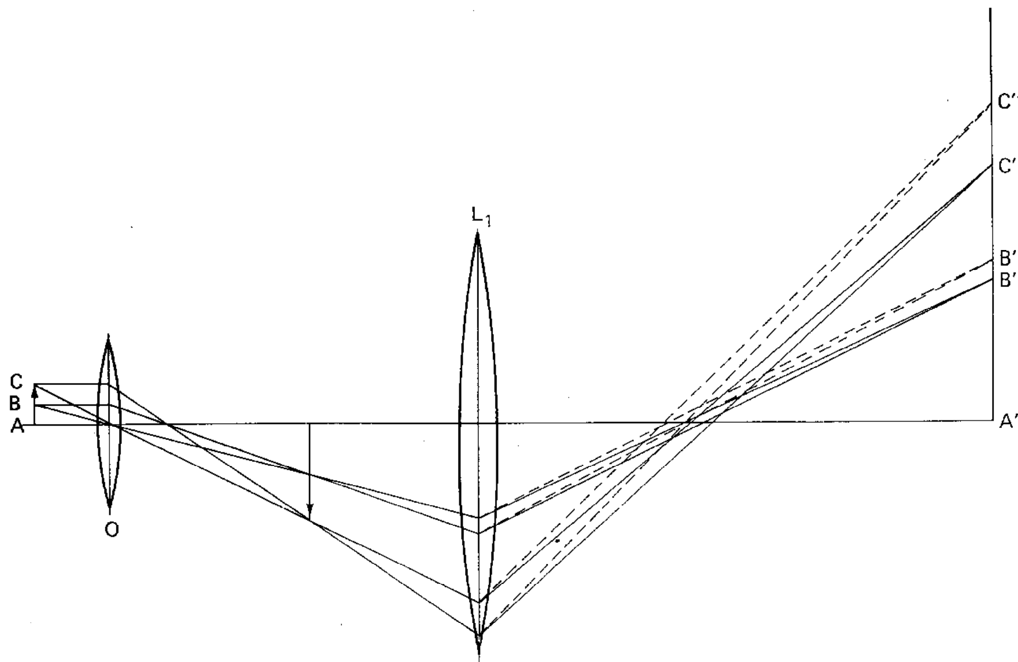

Fig. I.75. The origin of pincushion distortion in a projector (intermediate) lens forming a real image. An object ABC is imaged at A'B'C' in the intermediate image plane if the lens is perfect. The spherical aberration causes the actual image to be formed at A'B"C". (From Agar, p.31)

The effects of all three kinds of distortion are most noticeable at low magnifications since, in this situation, a large area of the intermediate image formed by the objective lens is accepted by the projector lens(es). 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.

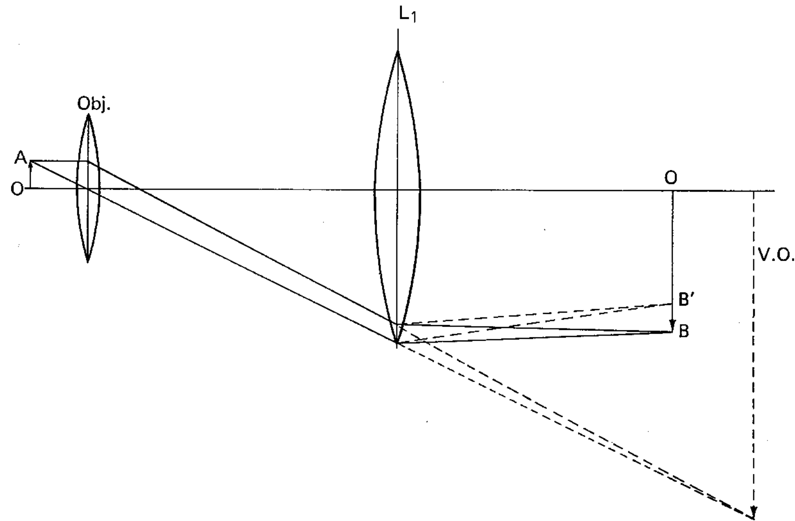

Fig. I.76. The origin of barrel distortion in a projector lens with a virtual object V.O. The specimen at OA would be imaged at OB by a perfect lens. The spherical aberration of L_1 causes the actual image to be formed at OB'. (From Agar, p.32)

d. Chromatic aberration (Figs. I.77-I.81)

Electrons of <u>different wavelength</u> (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 three causes:

- fluctuations in high tension supply (usually less than $1\text{x}10^{-5}$ in stabilized circuits)
- variation in velocity of electrons emitted by the cathode (about ±3.5 parts/million)
- energy losses due to inelastic collisions in the specimen (minimized using thin specimens).

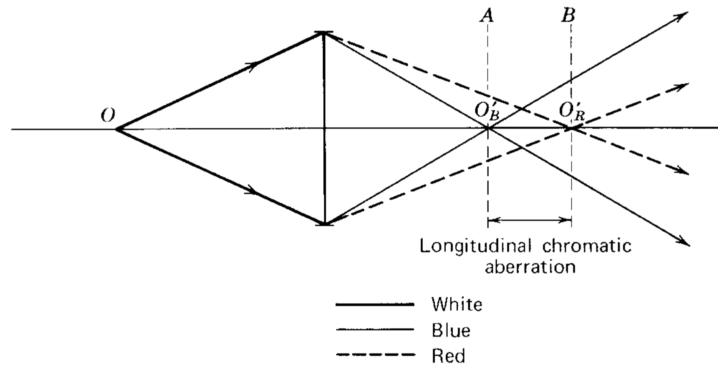

Fig. I.77. Longitudinal chromatic aberration. (From Slayter, p.215)

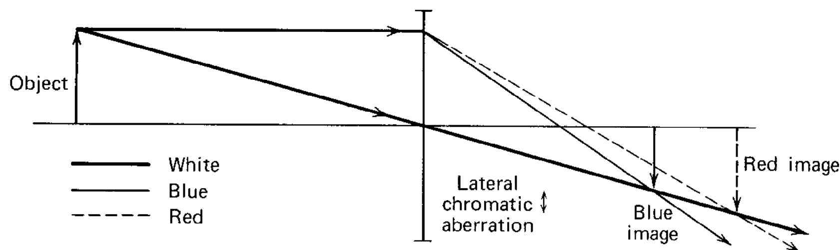

Fig. I.78. Lateral chromatic aberration. (From Slayter, p.216)

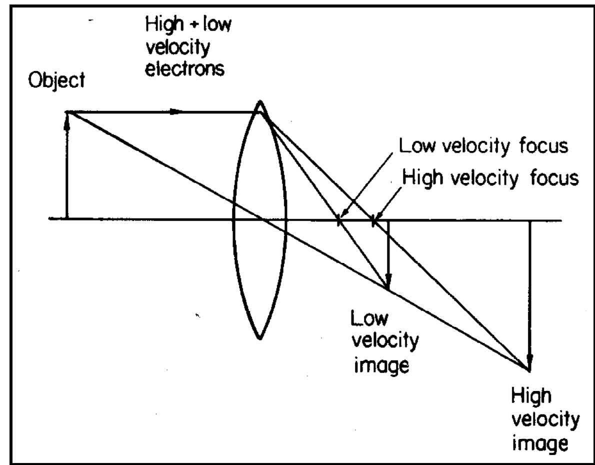

Fig. I.79. The analogue of chromatic aberration in an electron lens. Fast (short wavelength) electrons are brought to a focus at a point further from the lens than slow (long wavelength) electrons. The image formed by the high-velocity electrons will therefore be larger than that formed by the slow ones. An effect called 'chromatic change of magnification' is produced, which is aggravated by thick specimens and low accelerating voltages. (From Meek 1st ed., p.78)

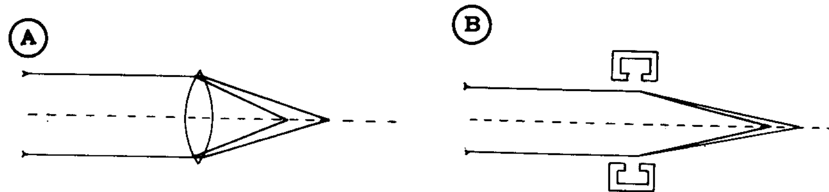

Fig. I.80. Chromatic aberration for (A) a glass lens and (B) an electron lens. (From Wischnitzer 2nd ed., p.54)

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.

The limit to resolution strictly due to chromatic aberration can be estimated by the following formula:

$$d_{cv} = C_c \cdot \alpha_o \cdot \Delta V / V$$

$$d_{ci} = 2 C_c \cdot \alpha_o \cdot \Delta I / I$$

where d_{cv} = separation of two object points which are just resolved, considering voltage
d_{ci} = separation of two object points which are just resolved, considering current
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 the images (but see Sec. I.C.4).

Electron images which suffer from chromatic aberration (Fig. I.81) 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. Since the focal length of the lens is different for each wavelength, the superimposed images are of 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. This effect thus works in a direction opposite to that in the light microscope. The net effect is a similar blurring of the image.

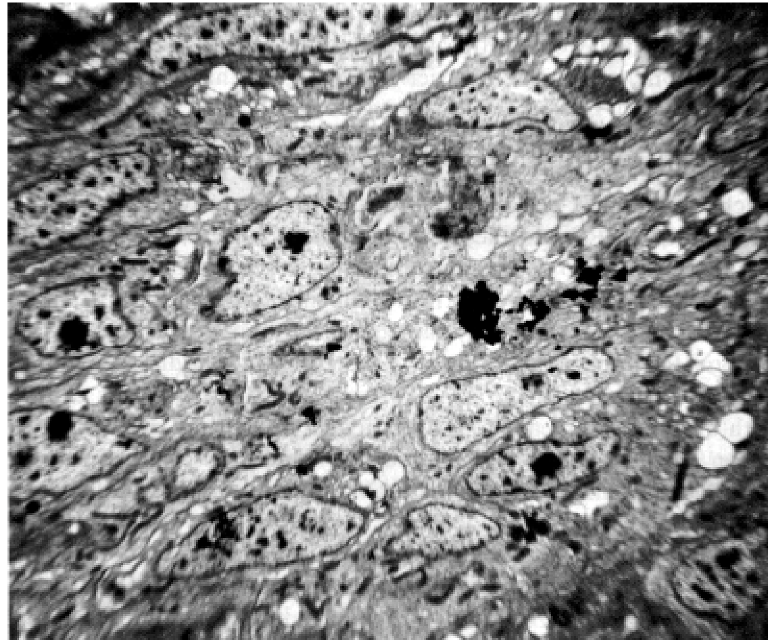

Fig. I.81 The effect of chromatic change of magnification. A 30kV beam was used to image a 1000 Å thick section of embedded tissue. The central part of the micrograph is sharp, but the out-of-focus effect becomes increasingly noticeable further from the axis. The effect is particularly noticeable at low magnifications. x5,000. (From Meek 1st ed., p.79)

The magnitude of chromatic aberration in electrostatic lenses is 4-6 times larger than for magnetic lenses.

e. Lens asymmetry (Figs. I.82-I.83)

Neither the homogeneity of available magnetic materials nor the accuracy of machining these metals into lens pole pieces is adequate 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. The device used to compensate for asymmetrical lens fields is called a **stigmator**.

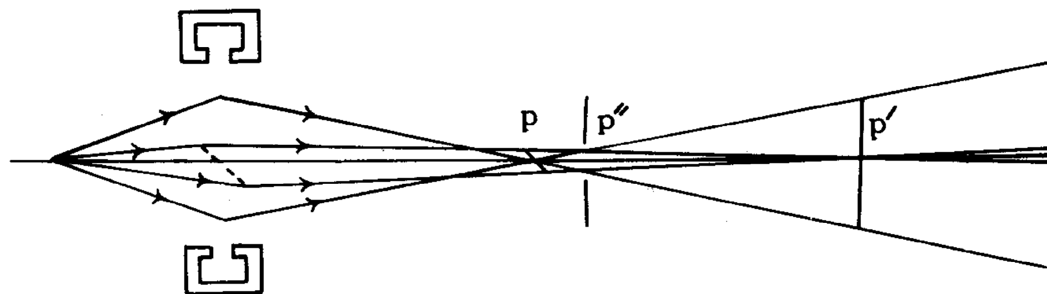

Fig. I.82. Astigmatism. Rays parallel to the axis in two mutually perpendicular planes which pass through an astigmatic lens are brought into focus at two different points, P and P'. The disc of least confusion would be located between P and P' and is designated as the "in-focus" position, P". (From Wischnitzer 2nd ed., p.89)

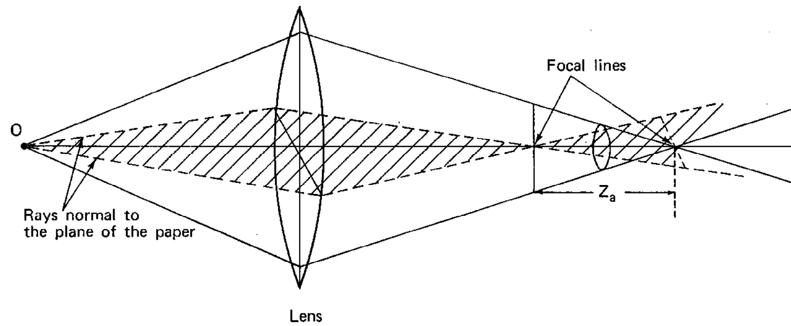

Fig. I.83. Image formation with an astigmatic lens. The lens is stronger in a plane perpendicular to the paper than in the plane of the paper, so that a point object O is imaged into two focal lines. Z_a, the distance between the focal lines, measures the astigmatism of the lens. A circular image is formed halfway between the lines. (From Agar., 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 supercooled 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. p122 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 TEM images. (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.30,I.32). The objective 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 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 photographically. 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 Sec. 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 first 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. Capturing intensity scattered outside the aperture helps improve contrast in the image. (See also Sec. I.B.4.e).

b. Lens construction (Fig. I.84)

The magnetic field is located eccentrically in relation to the axial extension of the lens coil in such a way that the field is close to the end of the coil at which the object is introduced. The specimen must be placed in the bore of the first pole piece or in the pole piece gap. The size of the bore limits the 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, increasing the angular aperture of the illuminating beam.

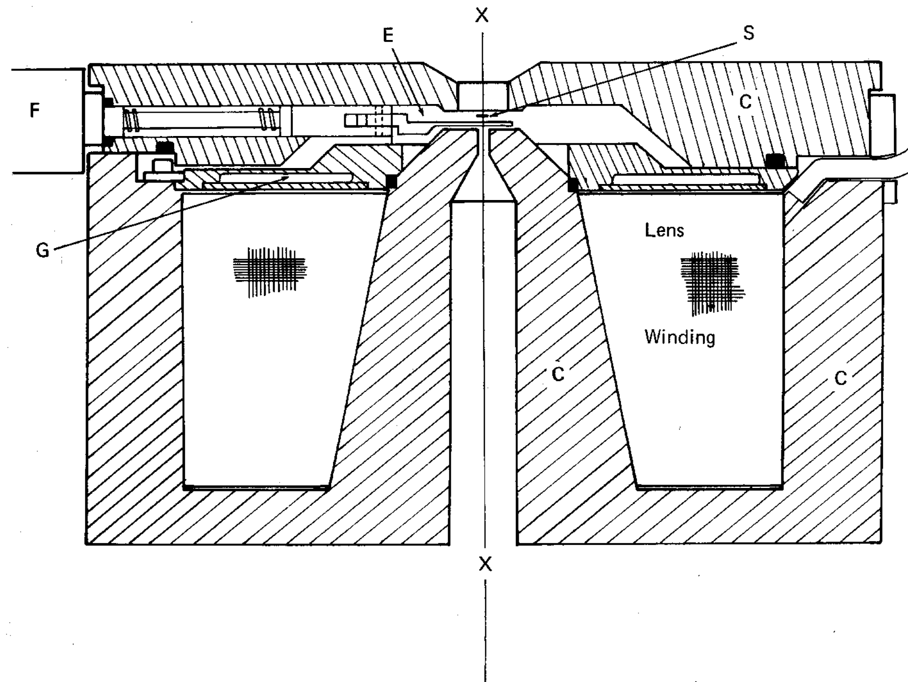

Fig. I.84. Cross section of a typical objective lens. The specimen is at S and in this lens is in a top-entry cartridge. The objective apertures are carried out on the rod E, and adjusted by control knob F. The water cooling channels G are above the lens coil. Note the heavy iron circuit C. XX is the lens axis. (From Agar, p.51)

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 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

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 (**Sec. I.B.3.e**).

If the lens field is not perfectly symmetrical about the optic axis, the image is **astigmatic** on the axis as well as off the axis. An asymmetric field perpendicular to the residual field is introduced to compensate for the astigmatism, in a way analogous to crossing two cylindrical lenses in glass optics. In older model microscopes, **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 stigmate the lens. Stigmators are also used with condenser lenses to give uniform, circularly-symmetric illumination.

d. Focusing the image

Variation of objective lens strength is the primary means of focusing the image of the specimen. This will be discussed in greater detail in Sec. 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, more commonly, in the back focal plane of the objective lens located a short distance behind the pole piece gap (Figs. I.85-I.88). In this position the field 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.

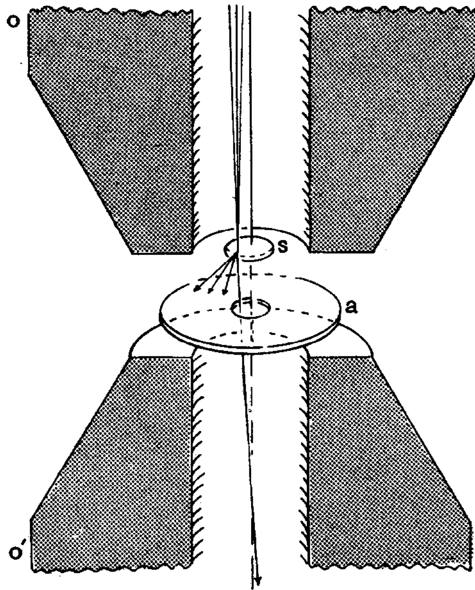

Fig. I.85. Image formation. The mechanism of image formation is diagrammatically illustrated. Shown is a lengthwise section through objective lens pole pieces, between which are seen the specimen, s, and physical aperture, a. The 'subtractive' action on a pencil of electrons, which images an individual image point, is demonstrated. (From Wischnitzer 2nd ed., p.60)

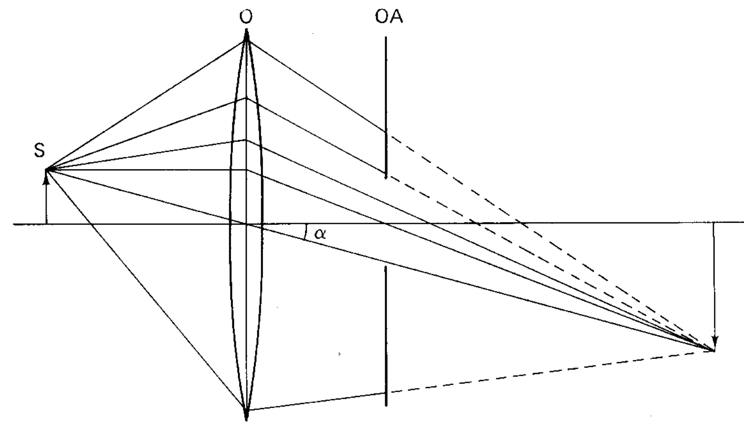

Fig. I.86. Function of the objective aperture OA in stopping widely scattered electrons from the specimen S in front of the objective lens O; α is the semi-angular aperture of the lens. (From Agar, p.27)

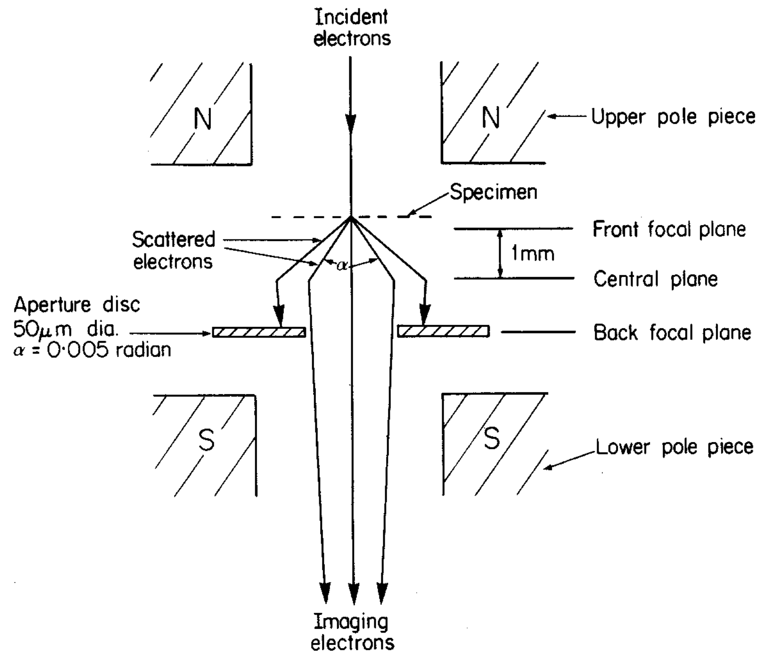

Fig. I.87. The action of the objective lens physical aperture in a short focal length immersion objective lens under high magnification conditions. Widely scattered, high chromatic aberration electrons, which degrade the image, are intercepted by the aperture disc. The smaller the hole, the more scattered electrons are intercepted and the higher the image contrast. The hole also serves to define the angular aperture of the lens α.. Note, however, that α should equal 0.05 radians. This unusually large angular aperture, is a consequence of the unusually small objective focal length. (From Meek 2nd ed., p.98)

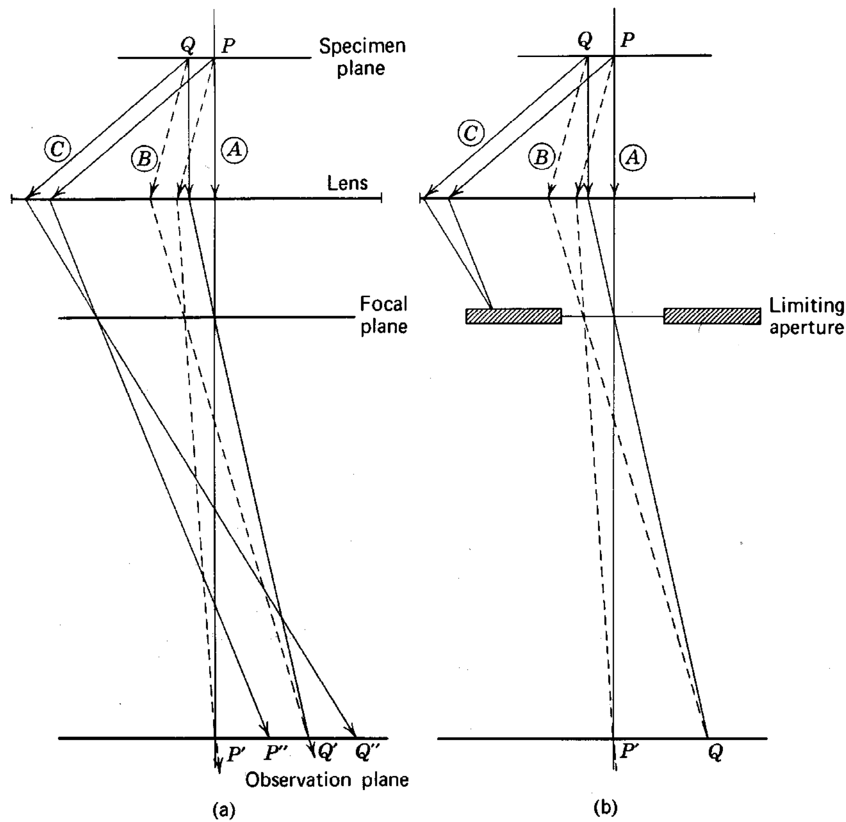

Fig. I.88. Action of the limiting objective aperture. (From Slayter, p.428)

Imaging the **back focal plane** of the objective lens on the fluorescent screen centers the aperture in this position (Fig. I.89). This method is the same way in which the microscope is used to image **electron diffraction** patterns (discussed in greater detail in Sec. I.F.1). 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.

Objective apertures are generally 25-75μm 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 better the improvement in image contrast, but the more difficult it is to manufacture with good circular symmetry and the more serious are effects of contaminants on the imaging beam. If the edge of the aperture becomes covered with a thin, electrically insulating layer of contamination, then, when subject to bombardment by the widely scattered electrons, it becomes charged and acts as a weak electrostatic lens capable of affecting image quality. Also, astigmatism is introduced because the contamination buildup is not likely to be symmetrical.

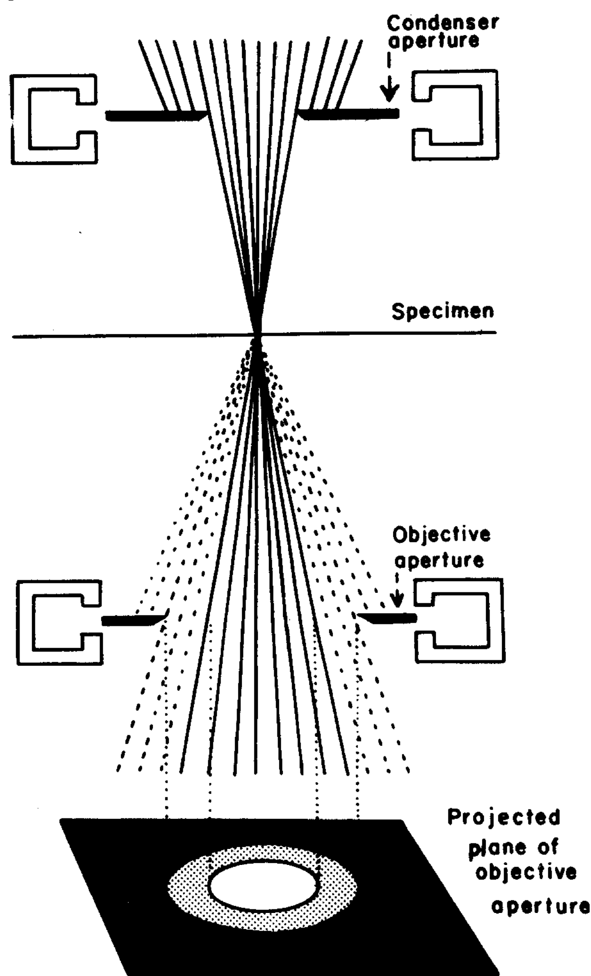

Fig. I.89. Objective aperture alignment. (From Wischnitzer 2nd ed., p.88)

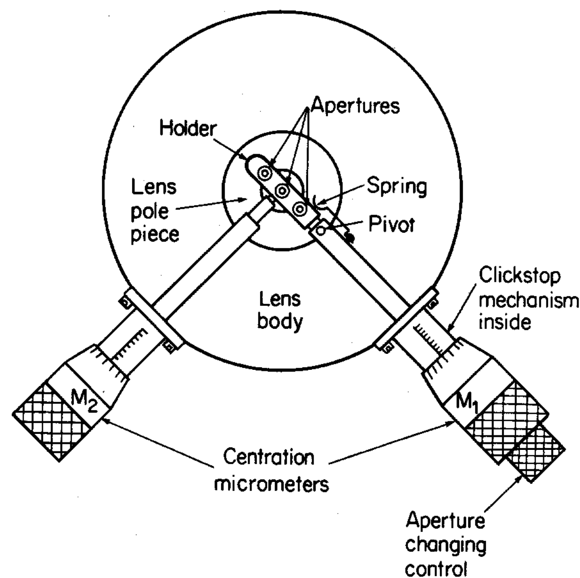

Fig. I.90. A plan view of a movable lens aperture selector and centration mechanism. (from Meek 1st ed., p.95)

Most microscopes have multiple aperture holders (Fig. I.90) and use ultrathin, self-cleaning metal apertures. Electrons impinging on such apertures can be used to raise the temperature sufficiently to remove the contaminating layers while in the microscope. In the past, apertures were generally made of platinum or molybdenum, but these had to be regularly cleaned.

When the aperture diameter is fixed, the focal length of the objective lens can be increased, thus decreasing α_0 and improving contrast but lowering resolving power (see also Sec.I.C.2). If the aperture used is too small, the resolution may be limited in the image due to diffraction effects.

f. Specimen stage (Figs. I.91-I.92)

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.

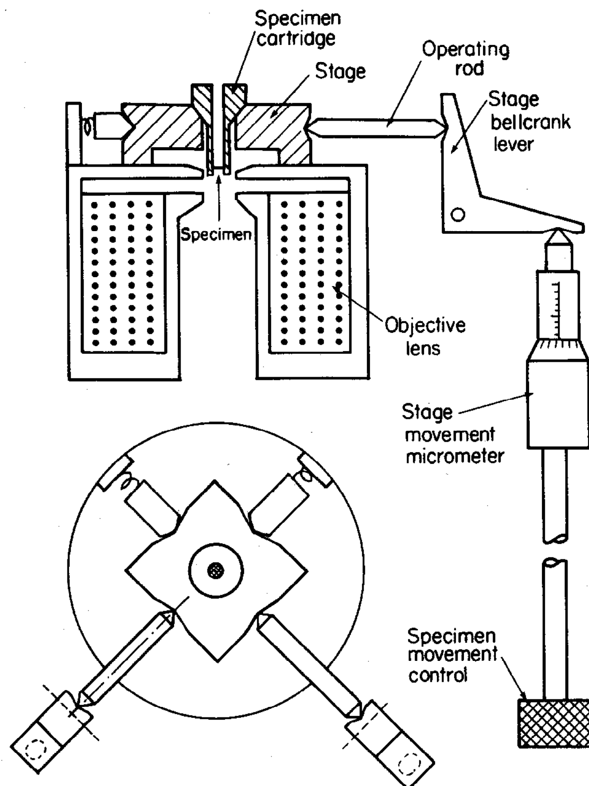

Fig. I.91. The top-specimen -entry, drop-in cartridge type of objective lens, stage, and stage motion. (From Meek 1st ed., p.114)

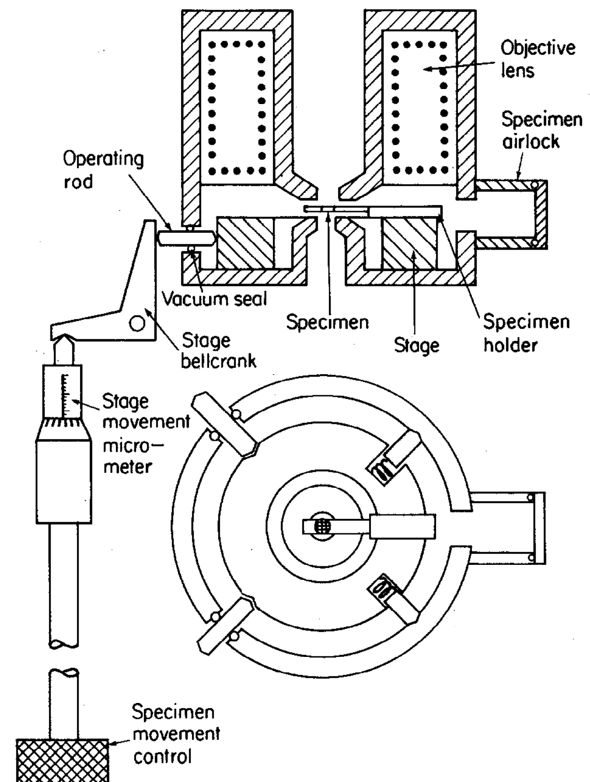

Fig. I.92. The side-specimen-entry, immersion type of objective lens, stage, and stage motion. (From Meek 1st ed., p.115)

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 ±60° because at large angles the electrons are no longer able to pass through the grid bars of the specimen support. 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 ~800°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 Sec. 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 success has been achieved with this type of stage in recording electron diffraction patterns at resolutions greater than 0.3 nm from hydrated, crystalline biological samples. Cold stages (as described above) seem to be more popular at present for examining "native" biological structure.
- 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

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.

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. 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 (Sec. 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 (Sec. 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.30-I.32) 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 cases the intermediate lens is highly excited (high lens strength, short focal length) to demagnify the image formed by the objective lens to give a range of settings at low magnification. 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.

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.73-I.76)

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 which 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. If the specimen is thick, **chromatic change of magnification** may give rise to an unacceptable, out-of-focus effect at the periphery of the image (Fig. I.81). 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.93-I.94)

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 in as perfect a focus as is possible to achieve.

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.

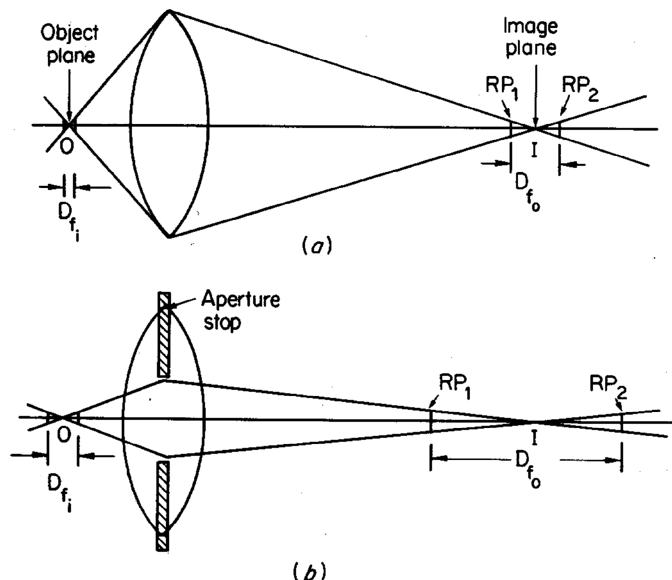

Fig. I.93. Depth of field and depth of focus, and the effect of lens aperture on them. As lens aperture is reduced, both depth of field and depth of focus are increased. (From Meek 1st ed., p.81: Note the difference in convention - D_{fi} = D_o in text and D_{fo} = D_i in text)

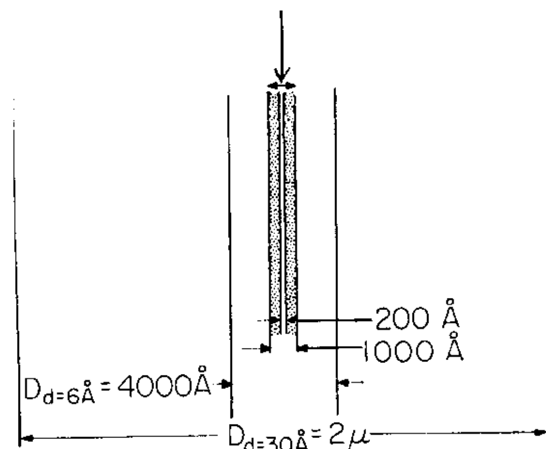

Fig. I.94. Depth of field at resolution 6 Å and 30 Å in relation to the useful section thickness, below 1000Å. (From Sjostrand, p.119).

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 diffraction disk corresponding to an object point, O (Fig.I.93)

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

Thus, for d = 1.0 nm and α_o = 5 x 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. *Decreasing* the aperture of the lens *increases* both D_o and D_i.

D_i may be determined from the following 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 α_o= 5x10^{-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 which appears on the viewing screen may be as much as 20-50% lower than that on the final photographic emulsion.

Note that the very large depth of field/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 projected onto the fluorescent screen where the kinetic energy of the electrons is transformed into light energy through fluorescence. 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 (~50-75! µm).

The fluorescent screen is needed both to enable the operator to view the image for selection of an appropriate area, and for focusing the image before recording it on a photographic emulsion. Detail in the image on the fluorescent screen is conveniently viewed through an external binocular (usually 10X: Fig. I.95). The objective aperture of the binocular is large in order to collect light from an appreciable 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.

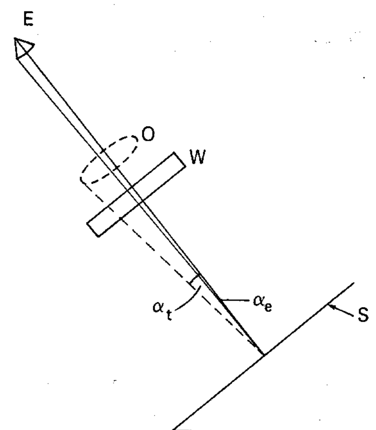

Fig. I.95. Diagram to illustrate how the use of the viewing binoculars (object lens O) increases the angular aperture of the viewing image formed on the screen S from α_e (by the unaided eye) to α_t. W is the lead glass viewing window. (From Agar, p.69)

b. Photographing the image

Since the image on the screen lacks the resolution inherent in the electron beam and does not provide a permanent record, the image is directly recorded on a photographic emulsion at a magnification sufficient such that the photographic record does not reduce the resolution. The resolution of photographic emulsions is generally about 4-5 times greater than the fluorescent screen. For example, at a magnification of 50,000X, a detail 1.0 nm in size will appear 50 µm large. Although this is just beyond the limits of delectability to the eye on the fluorescent screen, the photographic emulsion easily resolves it. There is no need to form an unnecessarily magnified image since this only requires greater beam intensity and leads to greater radiation damage of the specimen.

The electron image is usually made into a permanent record on a photographic film or plate mounted either above or below the fluorescent screen. Because of the great depth of focus of the projector system, the exact position of the photographic emulsion is not critical. Thus, the photographic material is usually (except for the 35mm camera) in a plane several centimeters below the viewing screen, where the image is focused. However, the final magnification is dependent on the position of the emulsion.

As was shown in the example in Sec. 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 ($\alpha_o = 10^{-2}$ 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 **exposure time** may be determined from a reading taken by a photo-cell looking at the fluorescent screen or by reading the current on the screen itself. The most common types of photographic materials are 3x4" sheets of film, 35mm film, and 70mm roll film.

c. Photographic emulsion

A 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 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. Thus, it is better to use photographic enlargement to make details visible to the eye than enlarging the electron image (which requires more electrons for the same

illuminating intensity). We will discuss important aspects of electron photomicrography in greater detail in Sec. I.E.10.

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 x 10^{-6} cm, 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 cm^3 volume contains 3 x 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 was 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.96).

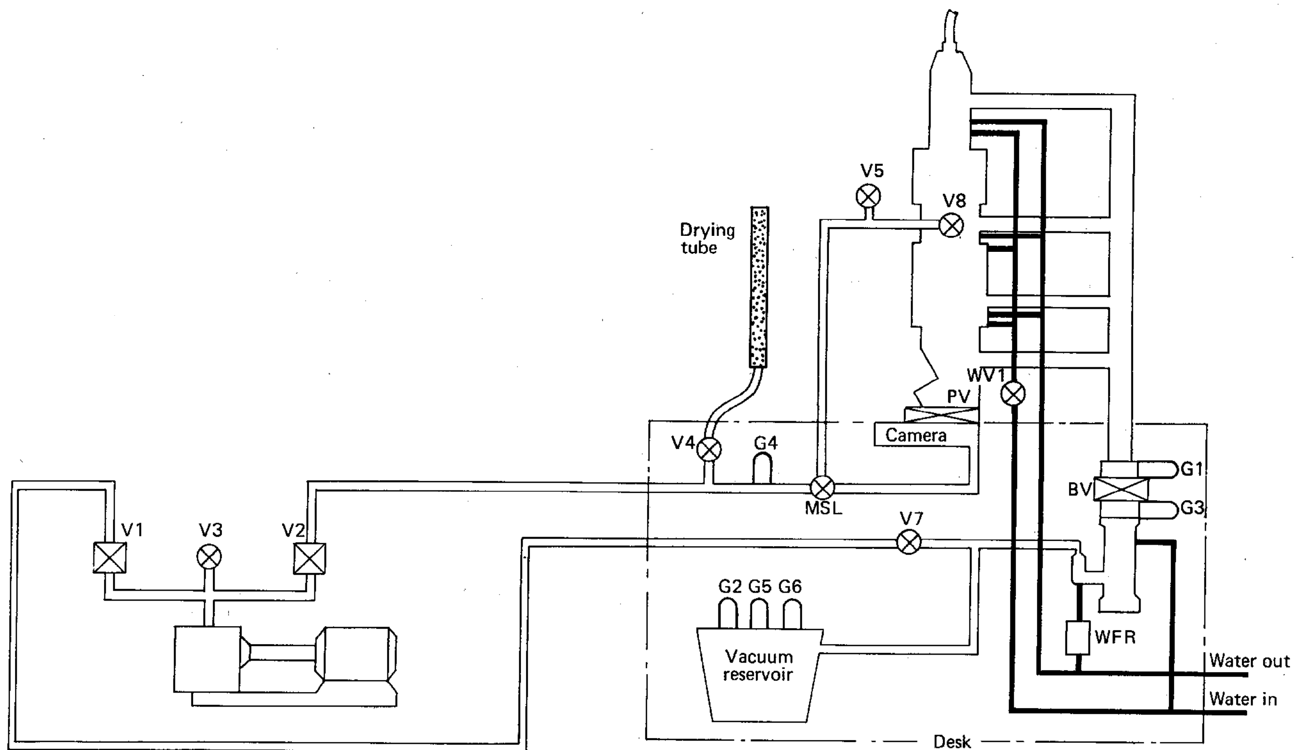

Fig. I.96. Schematic diagram of the vacuum and water systems for an electron microscope. The water circuit is indicated in thick black lines. A water flow relay WFR shuts off the electrical supplies to the diffusion pump and closes if the water flow rate is insufficient. (From Agar, p.76)

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.97). Compression is confined to the outlet side of the pump through valving mechanisms of different types. A good mechanical pump can just achieve a pressure of 10^{-3} torr and its pumping speed at this pressure is very low.

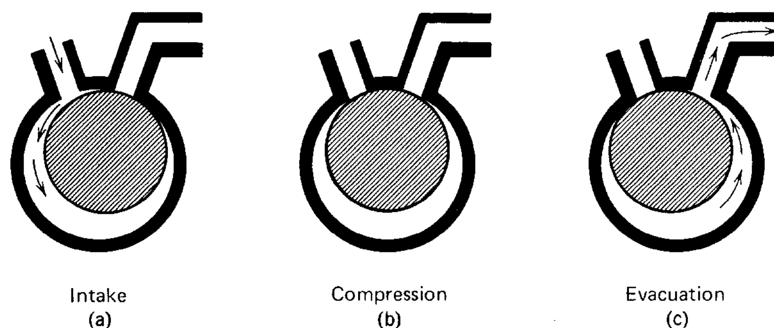

Fig. I.97. Action of a mechanical pump. (From Slayter, p.384)

Oil or mercury diffusion pump

The diffusion pump consists of i) an electrically heated reservoir of oil or mercury, ii) a chimney, and iii) a water-cooled casing (Fig. I.98). 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. Usually the pumped gas exhausts into a vacuum reservoir of large volume, and this gas is periodically removed by a mechanical backing pump when the pressure reaches a predetermined value in the reservoir.

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 (or mercury). 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.

Ion pump

Higher vacuums as high as 10^{-9} torr can be obtained by ionizing the particles to be outgassed in a strong electric field and subsequently causing them to be trapped at a surface. The Philips EM420, for example, uses an **ion getter pump** to reduce the vacuum in the gun and specimen area to less than 10^{-7} torr, thus increasing filament lifetime and reducing specimen contamination.

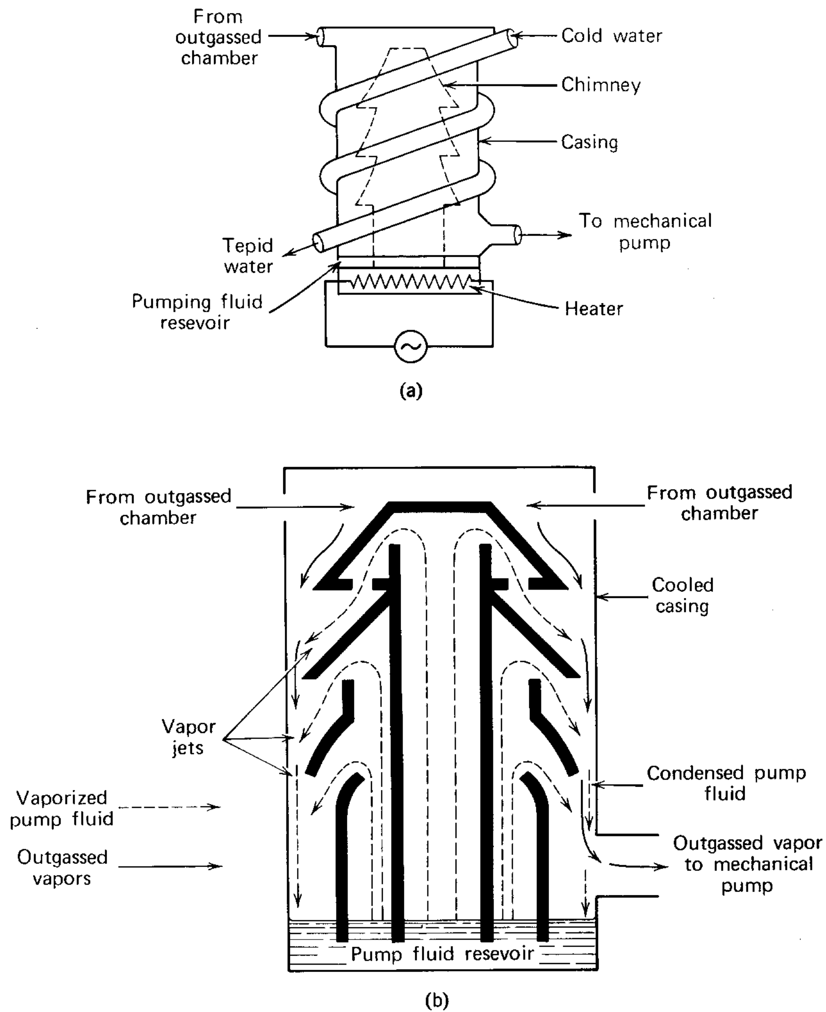

Fig. I98. Diffusion pump: (a) external view; (b) cross section. (From Slayter, p.385)

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.

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 all of the 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.99). 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.

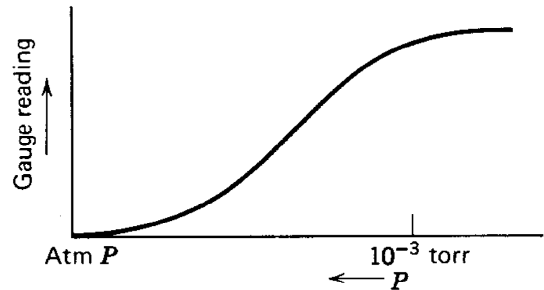

Fig. I.99. Thermocouple gauge characteristic. (From Slayter, p.386)

Pirani gauge

The **pirani gauge** is another type of low vacuum meter (10-10^{-3} 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 which can be monitored (Fig. I.100).

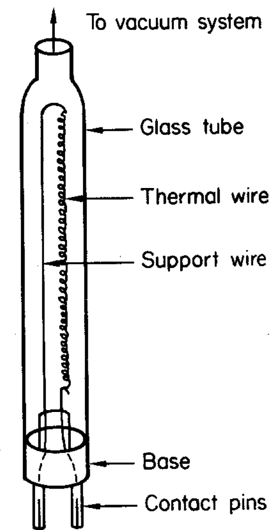

Fig. I.100. Diagram of a Pirani gauge used to measure low vacuum. (From Meek 1st ed., p.140)

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.101). 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.102). 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 the order of 10^{-3} torr. At still lower pressures, the current decreases, owing to the unavailability of ionizable particles.

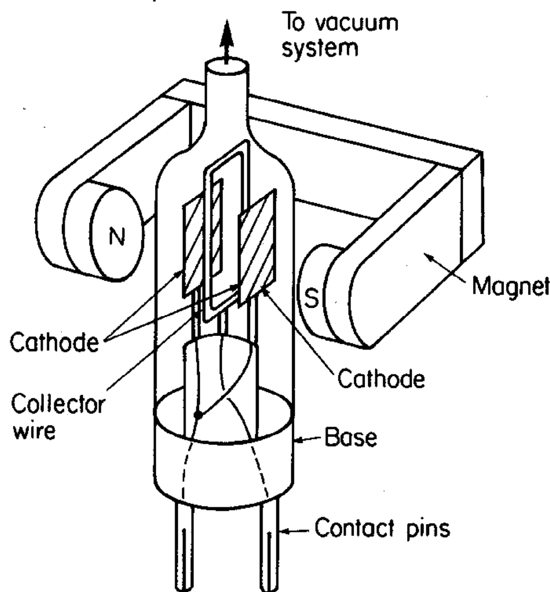

Fig. I.101. Diagram of a Penning (Philips) gauge used to measure high vacuum. (From Meek 1st ed., p.142)

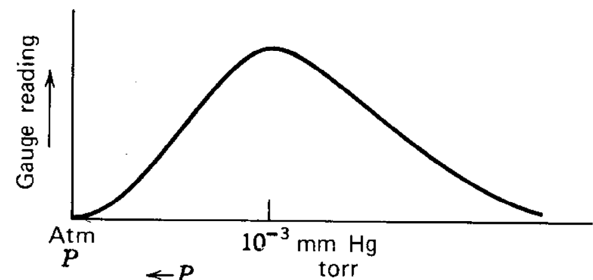

Fig. I.102. Ion gauge characteristic. (From Slayter, p.387)

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 the contamination can build up at the 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 that the hydrocarbons will preferentially condense on it. The usual design 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 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 milliampere 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. 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 prestabilized input. This is generally performed in at least 4 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 Sec.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 a function of 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 which power the shutter, camera, exposure meter, alignment modulators and focus wobbler do not require as high a degree of stabilization as the lenses.