ELECTRON MICROSCOPY

METROPOLITAN-VICKERS ELECTRICAL COMPANY LTD.
TRAFFORD PARK MANCHESTER 17, ENGLAND

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ELECTRON MICROSCOPY

Part I. Metrovick electron microscopes

by

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BEFORE describing the electron microscope and its applications it is of interest to consider the discoveries which led to the introduction of electronics into microscopy.

A microscope is a means of extending the resolving power of the eye. To illustrate this consider the appearance of two point sources of illumination spaced a distance d apart. The point source may be defined as a radiative source whose diameter is small compared with d.

It will be found that so long as the spacing d is greater than about 1 millimetre the points can easily be resolved as separate entities by the eye alone, but as d is reduced to less than 1 mm it becomes more difficult and requires greater effort until, at a value of $d = 0{\cdot}1$ mm, or thereabouts, it is no longer possible to define two separate point sources. The actual spacing at which this takes place is dependent on a number of factors such as brightness of the source and the state of the eye of the observer. The value $0{\cdot}1$ mm may be taken as an average figure expressing the smallest spacing or detail observable by the human eye. This is called the resolution limit of the eye.

If the two point sources are observed under a low power microscope they may be separated again. Upon a further reduction of d to $\frac{0{\cdot}1}{M}$ mm (M being the magnification) the images of the sources will merge together. Under greater magnification they again appear as entities ; but it will be found that this process cannot be continued indefinitely. A limit is reached beyond which no amount of extra magnification will separate the points ; it will produce merely an increasingly large blur of light formed by the merging together of the images of the two sources.

The reason for this effect can be understood by considering the image of a single point source produced by a lens system (see Fig. 1). By the laws of geometric or Gaussian optics a point object at O will be imaged at I in the conjugate plane, the position and size of the image being given by $\frac{1}{v} + \frac{1}{u} = \frac{1}{f}$ and $\frac{v}{u} = M$ . . . (I). These laws, however, only hold to first approximation and to obtain a more accurate picture one must consider the image formation in terms of the wave nature of the radiant energy. Consider the energy leaving O in the form of spherical wavefronts moving radially outwards. After traversing the lens (assumed perfect) a wave front is reversed, forming part of a circle, centre I. Now every point on the wavefront such as I can be considered as radiating Huygen wavelets radially from that point and in phase with all other points on the wavefront. On arrival at any point in the plane I off the axis OI it is clear that wavelets from some parts of the wavefront will have travelled a distance differing

from others, so that they arrive with a phase difference. The energy intensity at a point in the image plane is clearly the integration of all wavelets cutting the point. To obtain a true image this should be zero at all points except I.

In practice this condition is never satisfied, and the distribution of energy is as shown in Fig. 2a with a central maximum at I and a series of rings of diminishing intensity surrounding it. Such a distribution caused by interference between the light wavelets is called a *diffraction pattern*. Fig. 2b shows the distribution of energy in the image of two adjacent points which are still just separable. It is convenient for reference purposes to fix on an arbitrary distance, and this was taken by Abbe as that distance at which the maximum due to one point lies over the first minimum due to the other. This distance is called the *diffraction resolution limit*, and its magnitude is given by :—

$$\Delta d = \frac{K\lambda}{\mu \sin\alpha} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad \text{(II)}$$

where λ = the wavelength of the incident radiation ; μ = the refractive index of the medium in the object space ; α = the semi-angle of the beam entering the objective lens ; and K is a constant which may be taken as = 0·61.

Since the value of Δd gives the size of the smallest detail which can be seen with the microscope, it is of great practical interest. It can be minimized by making λ small and $\mu \sin\alpha$ large. With visible light λ equals about 5,000 Angstrom units* while no suitable material of greater refractive index than 1·6 is available ; $\sin\alpha$ cannot exceed the value of unity, and has a maximum value in practice of about 0·8. Thus the limiting resolving power of the optical microscope is about 2,000 Angstrom units. This value had been obtained before the year 1900 and the only improvement upon it, until the advent of the electron microscope, lay in the use of ultra-violet light and quartz lenses, which secured a resolving power of the order of 1,200 Angstrom units. The use of even shorter wavelengths in the electro-magnetic spectrum is prevented by the fact that there are no materials which can provide suitable refracting lenses.

In the 1920's two events gave hope of a new advance in this field by the use of electrons instead of electro-magnetic radiation to illuminate the object in the microscope.

In 1924 a young French physicist, Louis de Broglie, postulated that matter particles in motion might have wave trains associated with them in much the same way as light quanta. He suggested that the associated waves would have a wavelength λ given by equating the mechanical momentum, mv, with the quantum momentum, $\frac{h}{\lambda}$, (h being Planck's constant, m the particle mass and v its velocity).

*1 Angstrom unit = 1×10^{-8} cm.

$$\text{Thus } \lambda = \frac{h}{mv} \quad . \quad . \quad . \quad . \quad \text{(III)}$$

For v, in the case of the electron, the value given by $\frac{1}{2} mv^2 = Ve$ can be substituted, where V

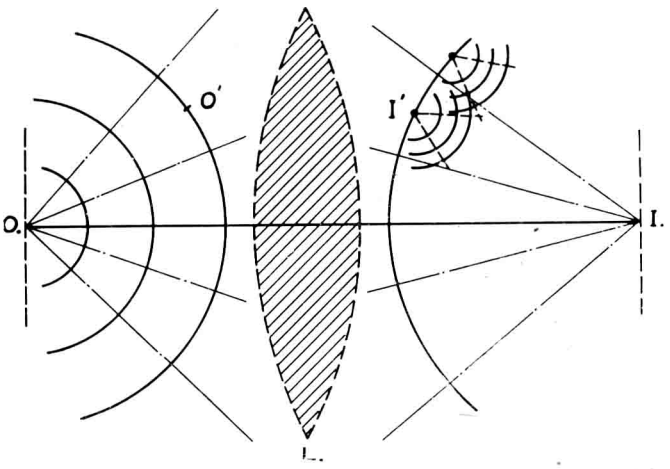

Fig. 1.—The formation of an image through a simple lens system. 79604

is the voltage accelerating the electron and e is the electronic charge. This gives :—

$$\lambda = \sqrt{\frac{150}{V}} \text{ Angstrom units} \quad . \quad . \quad . \quad \text{(IV)}$$

De Broglie's hypothesis was amply justified by the subsequent experiments of Davisson & Germer* in America, and G. P. Thomson in England, showing that crystal lattices produced diffraction effects with an electron beam, of magnitude compatible with the de Broglie wavelength. For 60,000-V electrons λ is equal to 0·05 Angstrom units. This is 100,000 times shorter than blue light, so that electron radiation has at least one property most suitable for use in a microscope, namely a very short wavelength.

The axially-symmetric magnetic field had been used to concentrate an electron beam since before 1900, but it was not until 1926 that Busch showed in an important mathematical paper that such fields formed true lenses for paraxial rays. He gave the focal length f of a magnetic electron lens as :

$$\frac{1}{f} = \frac{e}{8mV} \int_{-\infty}^{+\infty} H_z^2 \, dz \quad . \quad . \quad . \quad \text{(V)}$$

where H_z is the value of the axial magnetic field and the integration is taken along the axis.

Busch also showed similar properties to be true for axially-symmetric electric fields.

It was at once apparent that the essentials of an electron microscope lay in the combination of the de Broglie hypothesis and Busch's theory, and work on developing such an instrument was commenced immediately, in Germany. It was, however, ten years before results surpassed those of the optical microscope and before a satisfactory theory was evolved. The experimental technique was fraught with difficulties, some of which have still to be overcome.

*Wave mechanics of the free electron (London). *Phys. Rev.* 30, (1927) 707.

HISTORICAL DEVELOPMENT OF THE ELECTRON MICROSCOPE

A brief historical outline may be of interest at this stage. Early work from 1926 onwards was carried out by Knoll and Ruska at the technical school in Berlin, and by Bruche at the A.E.G. Co.

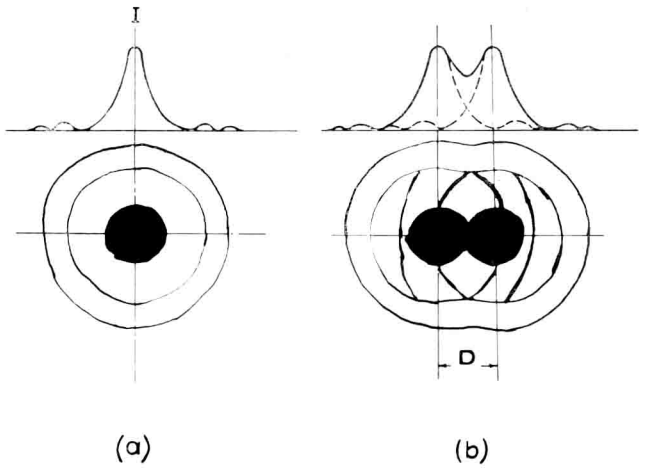

Fig. 2.—The distribution of energy about the image of : (*a*) a single point ; (*b*) two adjacent points. 79605

It was not until 1932 that any results were published. Then Knoll and Ruska described a two-stage instrument using magnetic lenses and having a resolving power still one order worse than the optical microscope. In 1934 Marton, in Brussels, was working on similar lines.

In 1935-6 an instrument was built in the M-V high voltage laboratory,* Trafford Park. The electron optical system of this instrument was designed by Professor L. C. Martin, of the Imperial College, London. On completion the instrument was sent to the Imperial College on loan, where further development work was carried out on it.

By 1937 the results in Germany had improved upon the optical resolution, and from then on progress was much more rapid. In 1938 Prebus and Hillier at Toronto, Canada, were working on a magnetic instrument and by 1939 they obtained a resolution better than 100 Angstrom units and comparable with contemporary German and Belgian results. In this year Siemens brought out a commercial instrument with a resolution of less than 100 Angstrom units.

In the war years a large amount of work was carried out in Germany. Mahl completed an instrument using electrostatic lenses, and obtained a resolution of less than 100 Angstrom units ; in Sweden, Siegbahn built a successful instrument ; in America, the R.C.A., obtaining the services of Marton from Brussels and Hillier from Toronto, commenced an intensive development programme, resulting in the production of a commercial instrument (R.C.A. Model B) in 1940-41 which was followed by the present model, type E.M.U.

*MARTIN, L. C., WHELPTON, R. V. and PARNUM, D. H. A new electron microscope. *Jnl. Sci. Inst.* 14, (1937) 14-24.

In the meantime, little progress had been made in this country. In 1940 the M-V instrument was still the only one in the country. It was transferred to the National Physical Laboratory early in the war years, and there some greatly improved results were obtained. Its design was very experimental, however, and in 1942 it was replaced at the National Physical Laboratory by one of the seven R.C.A. Model B instruments sent over on Lease-Lend. It was not possible for any development work to be done during the war years in this country, and it was not until the end of hostilities that any serious work was recommenced in the Company's own laboratory. The commercial model, type EM2 (shown in Fig. 11), was developed in 1944 and went into production in 1945. A later model (EM2.1) has since been developed with some slight constructional modification. Apart from an increase in total magnification, however, this instrument closely resembles the EM2 and a number of this type of instrument has now been installed in various laboratories and hospitals throughout the country.

The experience gained with the EM2 microscopes enabled a new design to be undertaken which incorporated many improvements and several new features. This is the type EM3* (Fig. 3) developed during 1946-47. This microscope, which is now in production, will be described in a later section.

LIMITATIONS OF THE ELECTRON MICROSCOPE

The best resolution so far obtained on any electron microscope is of the order of 10 Angstrom units. It is necessary to review the limitations of the instrument in order to understand why this value is not lower. In practice the theoretical resolution cannot be realised because of the limiting factors introduced by lens aberrations. These are similar to the aberrations in optical lenses but so far cannot be corrected as in the optical case. The most important are the chromatic aberration and the spherical aberration.

Dealing firstly with chromatic aberration it can be seen from the focal length equation (V) that the focal length, f, is a function of accelerating voltage V and the lens field H_z. Thus variations in H_z or V cause variations in focal length. If the accelerating voltage is stabilized to 1 part in 20,000 and the magnetic field to 1 part in 50,000, the loss of resolution due to chromatic aberration is about 10 Å. Spherical aberration may be expressed in terms of the diameter of the disc of confusion Δs formed at the image of a point object, by the relation $\Delta s = C_s f \alpha^3$ . . . (VI) where f is the focal length of the objective lens, α is the semi-angular aperture as before, and C_s a constant depending on the lens geometry. As stated earlier the diameter of the diffraction disc obtained is given by :—

$$\Delta d = \frac{K\lambda}{\mu \sin\alpha}$$

Clearly, if α is reduced the effect is increased. It

*HAINE, M. E., PAGE, R. S., GARFITT, R. G. A three-stage electron microscope with stereographic dark field, and electron diffraction capabilities *Jnl. Appl. Phys.* 21 (Feb. 1950) 173-182.

is to be expected that a minimum value of the total error would be obtained at some optimum value of α. This optimum value can be shown to be :

$$\alpha \text{ opt } = 0{\cdot}014\ (C_s f V^{\frac{1}{2}})^{-\frac{1}{4}} \quad . \ . \ . \ . \quad \text{(VII)}$$

and the minimum resolution (Δt) is then given by :

$$\Delta t = 750\ (C_s f)^{\frac{1}{4}}\ V^{-\frac{3}{4}} \quad . \ . \ . \ . \ . \ . \quad \text{(VIII)}$$

For a typical lens $C_s = 10$, $f = 5$ mm and for a 50-kV accelerating voltage the resultant resolving limit is given as 10 Angstrom units.

This theoretical limit agrees very closely with the best experimental results.

There are certain possibilities of improving the resolving power by further reducing spherical aberration ; but at present these are only suggestions, and involve great experimental difficulties.

CONSTRUCTIONAL DETAILS OF THE EM3 INSTRUMENT

Owing to the high absorption rate of electrons in air it is necessary to contain the whole electron optical ray path in a high vacuum. The system is outlined in the diagram (Fig. 4) and it will readily be seen that it closely follows that of an optical microscope. A source consisting of an electron gun produces a beam of monochromatic electrons which are focused on the specimen by a magnetic condenser lens. The transmitted electrons are focused by the objective lens to form a real intermediate image magnified 60-100 times. This image is further enlarged by the projector lens to form a final image magnified up to 20,000 times overall.

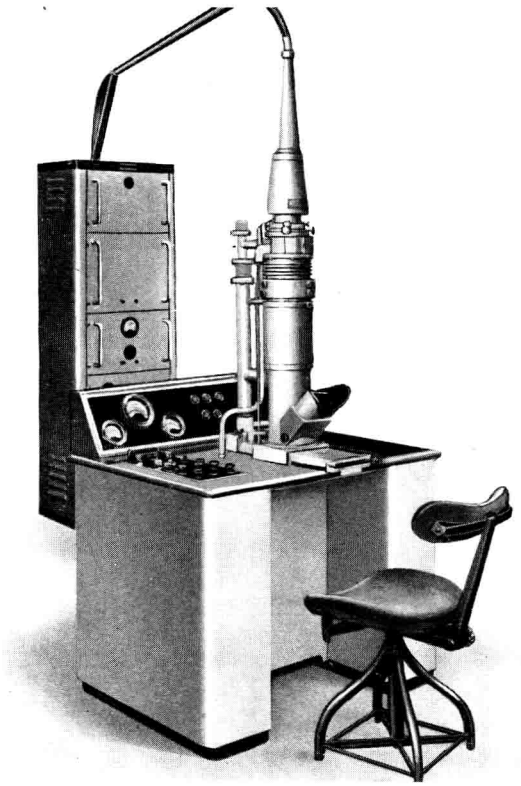

Fig. 3.—The EM3 electron microscope. A cross-section of the main stack is shown opposite. 87235B

This is the conventional system used in two-stage microscopes. The addition of an extra projector lens, as in the EM3, makes a three-stage system which proves to be far more flexible in operation, allowing a range of magnification from 1,000 to 100,000 times to be covered continuously. Furthermore, it is possible to obtain an electron diffraction pattern corresponding to a selected area of the specimen, without disturbing either specimen or lenses. This is accomplished by focusing the intermediate projector lens on to the back focal plane of the objective lens where the electron intensity distribution is a function of the angle through which electrons are scattered by the specimen.

The microscope column of the EM3, shown in Fig. 3 and in Fig. 5, is built up in eight sections : the electron gun, condenser lens, specimen chamber, objective lens, intermediate alignment section, twin projector lenses, viewing chamber, and camera. Each unit is supported directly by the section underneath and vacuum-sealed by rubber gaskets. Mechanical rigidity, a first consideration in electron microscope design, is maintained by the use of the type of rubber gasket seal used on the EM2 instrument* which allows the opposing flanges to be clamped together in metal-to-metal contact.

At the top of the column is the electron gun consisting of a hairpin tungsten filament surrounded by a self-biased cathode shield (111, Fig. 5). The high tension supply applied to the cathode through a cable (101), terminated in a completely screened bushing (103), is generated in a separate cubicle described later. The electron stream from the cathode is accelerated to the earthed anode (117) and directed through an aperture into the magnetic field of the condenser lens (200). This field, generated by an iron-shrouded solenoid, focuses the electron beam on to the specimen. The direction and lateral position of the beam can be adjusted by means of the controls 212 and 211 ; this is necessary in order to align the beam with the optical axis of the subsequent image-forming lenses. The flexible vacuum seal required between the condenser lens and the object chamber consists of a metal bellows.

The specimen chamber, situated between condenser lens and objective lens, is provided with a rubber-sealed door giving access to the mechanical stage in which the specimen to be observed is mounted. The fact that the specimen is to be observed at magnifications up to 50,000 times implies that the traversing mechanism must provide a very smooth and accurate motion together with positional stability. Any movement caused by thermal expansion and the like must be kept below a certain level fixed by the degree of resolution required and the length of time taken to record the image on a photographic plate. For the highest resolution possible movements must be kept below a level of about 50 Angstrom units (50×10^{-8} cm)

*HAINE, M. E. The design and construction of a new electron microscope. *J.I.E.E.* 94, Part I., No. 82, 447 (Oct. 1947).

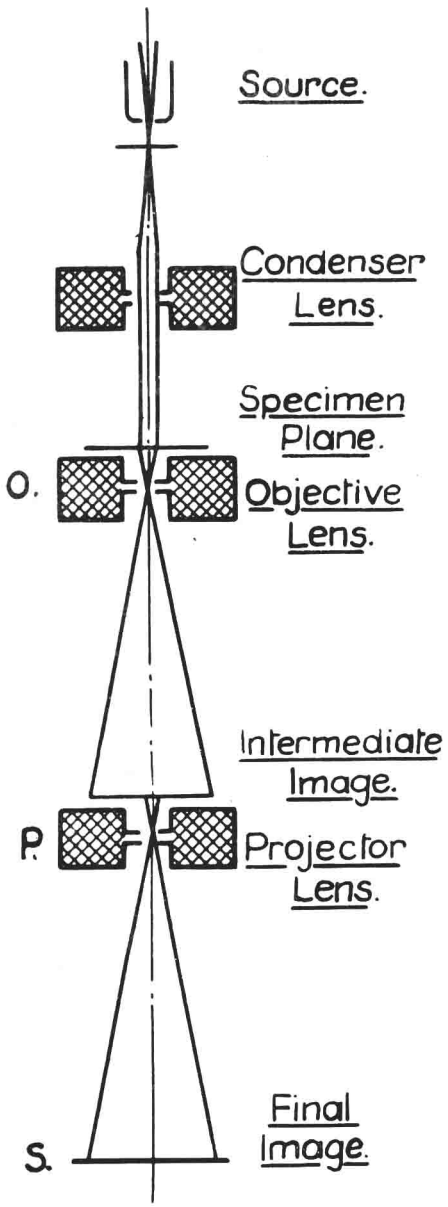

Fig. 4.—Electron optical ray path. 79606

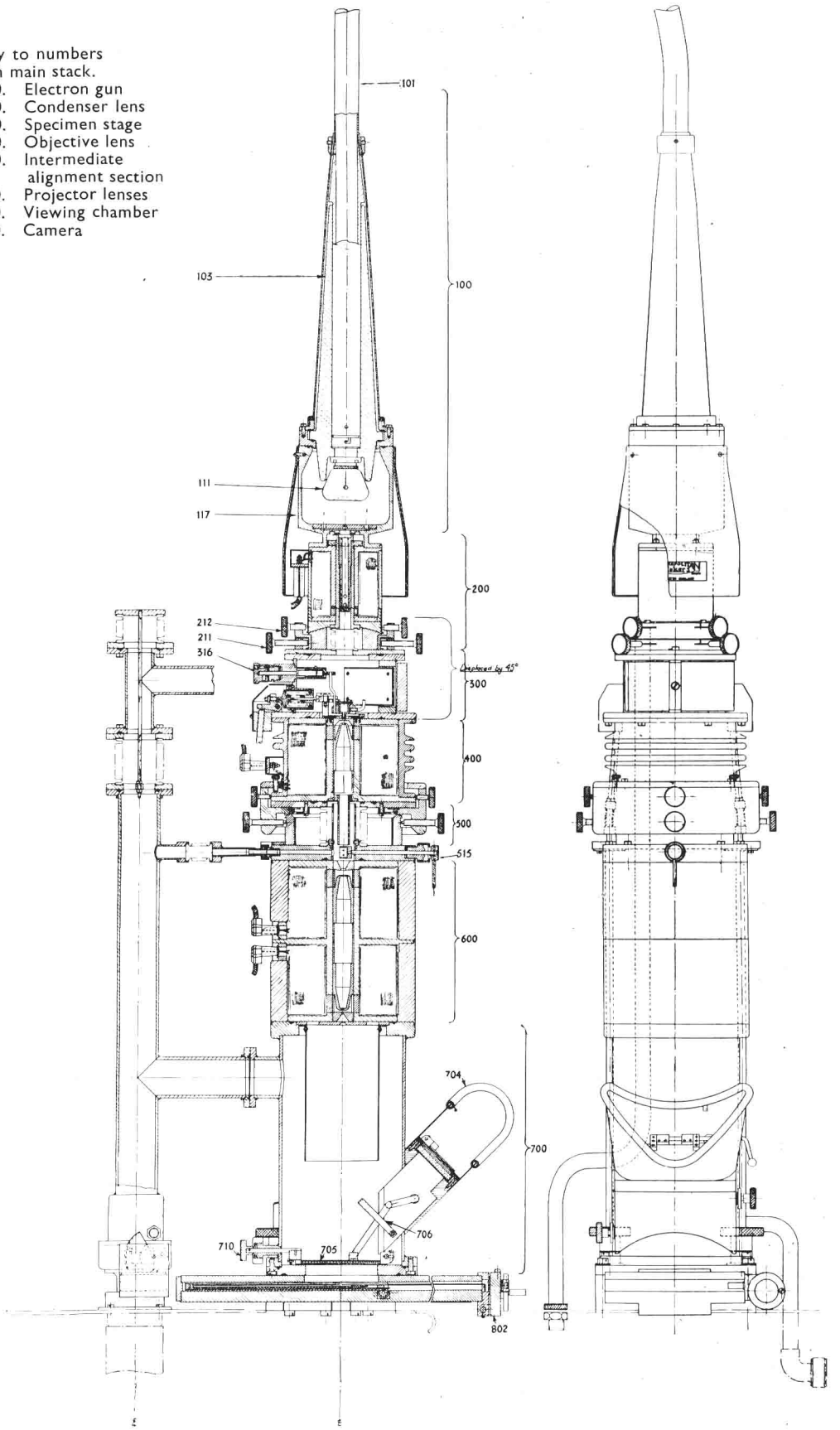

Fig. 5.—A cross-sectional drawing of the main stack of the EM3 electron microscope. 78618

per minute, assuming a ten second exposure for the photographic record.

These stringent requirements have mostly been met in the present design. All movements are heavily spring-loaded to prevent backlash and a certain amount of friction is introduced between the mechanical stage and objective lens to provide a "threshold" value for the force required to move the stage.

Sufficient mechanical reduction between the external manual controls and the stage itself is provided by the use of fine-threaded lead screws actuating bell cranks (322, Fig. 8) which thrust directly on to the push rods (318). These rods are flexibly sealed into the vacuum walls and make contact with V-blocks on the specimen stage. The stage consists of a flat-bottomed cup which slides directly on the surface of the objective lens ; it is spring-loaded against the push rods and against the lens surface. The inner part of the stage can be tilted by means of the external control (316), thus providing means for producing pairs of stereomicrographs.

Specimens are mounted on $\frac{1}{8}$-in. dia. 200 mesh gauzes held in the lower end of the removable cartridge (309). The plane of the specimen is slightly above the upper face of the objective lens.

The objective lens (Fig. 6) consists of an iron-shrouded solenoid with a non-magnetic gap in the cylindrical, vacuum-tight, bore. Accurately ground pole pieces fit inside the bore and concentrate the field across a narrow gap with a small axial bore. The field, fringing from the gap into the bore, constitutes the lens. The centre plane of this lens is about 0·6 cm from the specimen plane so that the focal length is approximately the same value. The axial symmetry of the small bore is most important because an eccentricity of 1 part in 10,000 is sufficient to cause bad astigmatism of the image.

The design of pole pieces is governed mainly by the necessity to obtain a short focal length and yet

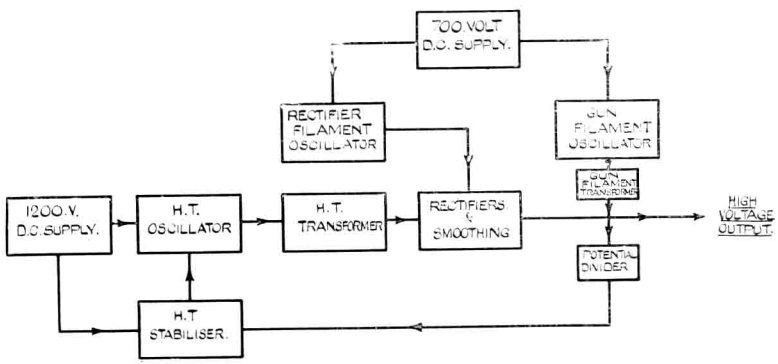

Fig. 7.—A block diagram of the h.t. set. 79338

have the specimen at a convenient position. With the present design a focal length of 0·4 cm can be obtained for 100 KeV electrons with the specimen plane just above the level of the lens face and a bore of 0·25 cm. Normally a larger bore is used because it is more favourable towards reducing aberrations. The objective lens is mounted on an alignment section providing lateral and tilting movements for the upper half of the column in order that the optical axes of the lenses may be

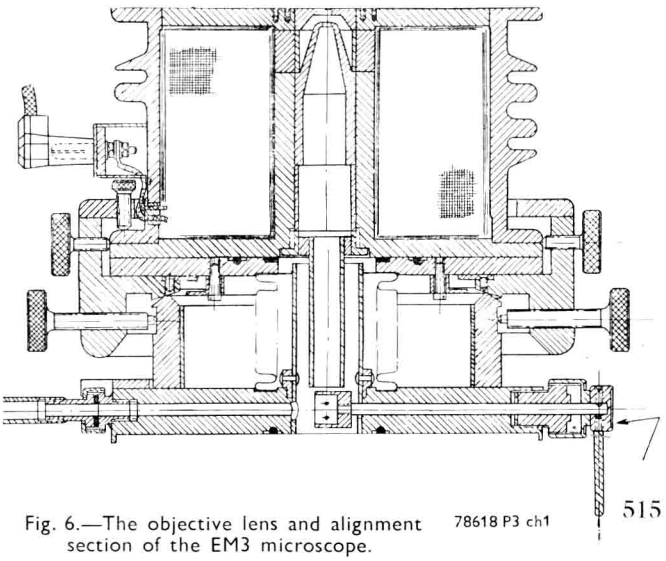

Fig. 6.—The objective lens and alignment section of the EM3 microscope. 78618 P3 ch1

aligned. This condition is necessary to reduce aberrations, particularly chromatic, to a minimum.

For many applications it is desirable that the electrons, scattered through large angles by the specimen, should not reach the final image because their effect is to produce an almost uniform fog that tends to obscure fine detail. To cut off these electrons, and hence improve image contrast, a small limiting aperture is introduced into the objective lens field.

Several types of aperture are provided for the microscope including one that is fully adjustable for lateral and axial position by external controls. This is assembled in a special section interchangeable with the standard alignment mechanism shown in Fig. 6.

The twin projector lenses are immediately below the alignment section and are constructed as one unit, the details of which are clear from Fig. 5. The actual lenses are similar to the objective lens. The double bobbin is wound with 30,000 turns to each winding, providing up to 3,000 ampere-turns excitation for each lens.

In use the lower projector provides a fixed magnification while the intermediate projector excitation is varied to control the overall magnification. This arrangement results in the lowest possible distortion of the image and allows the whole range from 1,000 to 100,000 times to be covered with one lens control.

A pair of small apertures selected by the control (515, Fig. 6) can be introduced successively into the electron beam just where the first image from the objective lens is formed. These two apertures

select areas of the specimen given by their diameters divided by the objective magnification, and allow only electrons from these areas to pass into the projector lens. Thus when the first projector is focused on the back focal plane of the objective instead of on the intermediate image, the diffraction pattern corresponding to the selected area is projected on to the screen.

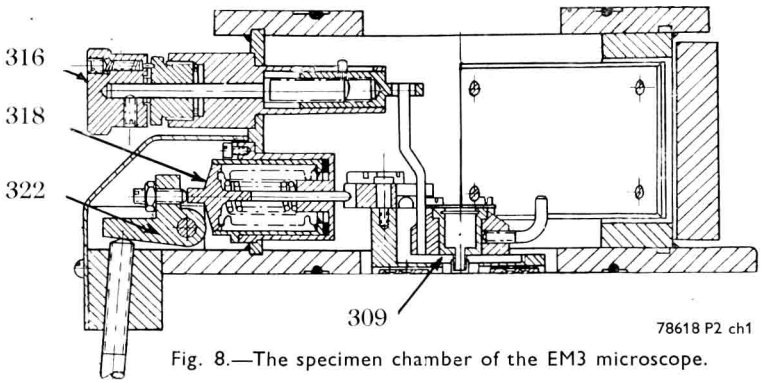

Fig. 8.—The specimen chamber of the EM3 microscope.

The lower section of the column is the viewing chamber beneath which is strapped the photographic camera. The fluorescent screen (705, Fig. 5) is viewed through a single wide-angle port provided with an eyeshield (704) and a magnifying glass (706) to aid fine focusing.

The screen can be raised to allow the image to pass through on to the photographic plate.

The camera carries two $3\frac{1}{4}$-in. square plates at one loading on which two, four or eight micrographs may be taken, the picture size being selected by masks controlled by the knob (710). The cassettes can be withdrawn through the door (802) at the front of the camera after the vacuum has been released.

Vacuum equipment and desk

The pumping equipment consists of a Metrovick type DR1 two-stage rotary pump backing a type 02 oil-diffusion pump. These are connected in a manually operated system provided with simple interlocks to prevent damage due to incorrect operation. The backing pump can be connected either direct to the microscope column for rough pumping, or to the diffusion pump for normal operation.

A fine side vacuum valve is incorporated between the diffusion pump and pumping manifold so that the microscope can be opened to the atmosphere without shutting down the pumps. The overall pumping time from atmospheric pressure is about three minutes.

The microscope desk is constructed from an angle-iron framework with sheet metal panelling, and is mounted on metalastic feet to insulate against vibration. A sloping panel at the rear of the desk displays beam current, lens current and vacuum gauge meters, indicating lights and the a.c. mains circuit switches and fuses. The microscope controls are all grouped on a hinged panel just to the left of the column, while the lens current stabilizers are built on sub-panels fastened to the control panel.

All internal wiring is by multiple cables or looms interconnecting each unit, terminated with multiple terminal blocks and spade-end coded leads.

The high-voltage power unit

The h.v. power supply is obtained from the high-frequency fed transformer-rectifier circuit developed so successfully by other workers for use with the electron microscope. Fig. 9 is a simplified circuit diagram and Fig. 7 is a block diagram of the set.

A tetrode valve V_1 (Fig. 9) oscillates in conjunction with the resonant air-cored transformer T_1, the grid drive being obtained via the small *capacity* C_c from the secondary winding. Rectification of the high alternating voltage across the transformer secondary winding is carried out by a voltage-doubling Greinacher circuit V_2, V_3, C_1 and C_2.

Stabilization of the high voltage to the required degree is achieved by a conventional degenerative feedback circuit through a resistive potential divider R_1R_2 and a high gain direct-coupled amplifier, which modulates the oscillator via its screen grid. A stable reference voltage is supplied by the battery B. For such a feedback arrangement, a well-known formula for the stabilization factor is :

$$S = 1 + An,$$

where S is the ratio of the voltage changes without stabilization to those with stabilization, A is the amplifier gain, and n is the potential divider ratio.

In fact, the gain of the amplifier used ($> 10^6$) is such that the output stability is limited by the inherent stability of the potential divider and batteries, which, however, is adequate over a period of a few minutes.

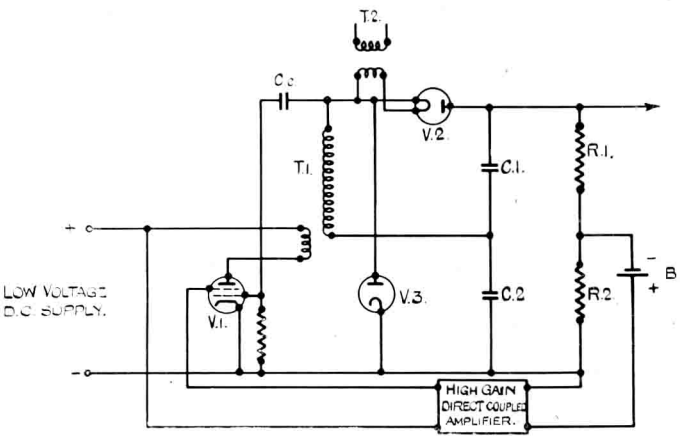

Fig. 9.—A simplified diagram of the h.t. set circuit. 79337

Three oscillators are used to supply power to the high voltage transformer, and to the filaments of the high voltage rectifier V_2 and electron gun. The filament of the rectifier V_3 is fed from a 50-c/s transformer.

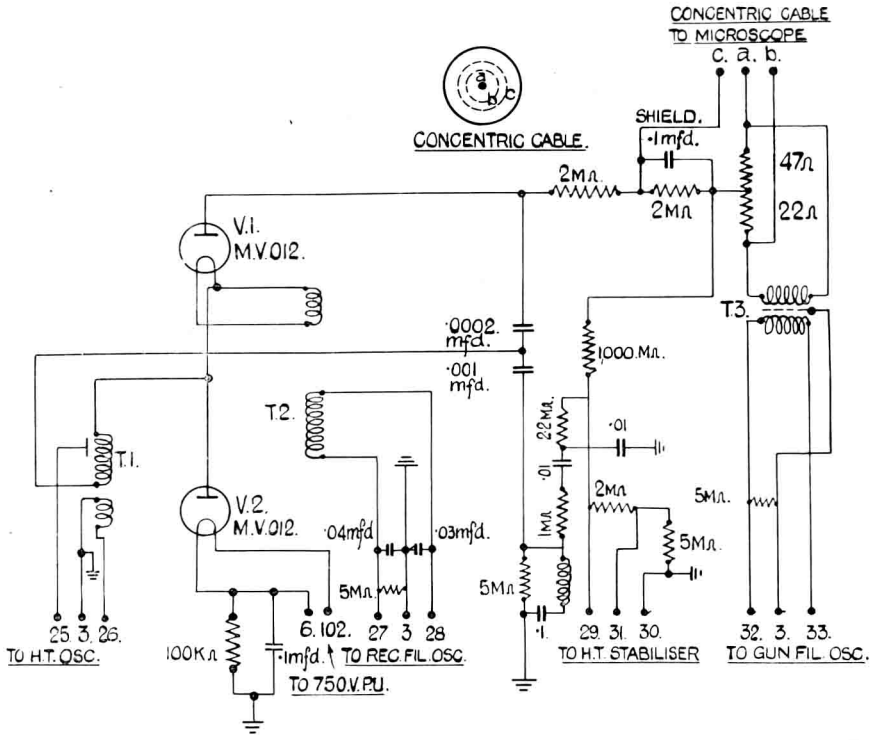

Fig. 10.—A circuit diagram of the oil tank assembly. 79339

Because the power loss in a resonant transformer is dependent on the circulating current in its winding, and this is dependent on the total capacity across the winding, it is important to keep stray capacities down to a minimum. This is achieved by careful design of such parts as the transformer secondary winding and the rectifier heating transformer.

The power unit is constructed with all the high voltage components in an oil tank and the oscillators and their supply circuits each on a separate rack-mounted chassis. The oil tank measures 25 in. × 17 in. × 11 in., and is constructed of sheet brass to eliminate hysteresis loss. The circuit diagram is shown in Fig. 10. The secondary winding of the h.v. transformer T_1 is 3 in. in diameter and 5 in. long ; it is subdivided into 25 sections, each containing 250 turns of 10/·0028 Litz wire. The primary winding of 600 turns is spaced 1 in. away from the end of the secondary coil to provide the necessary interwinding insulation. The secondary tuning capacity is of the order of 45 mm F. The transformer T_2 supplies the filament of the rectifier V_1 (approximately 1·9 V, 9A).

Smoothing is provided by the reservoir condenser (three metal plates sandwiched between Bakelite sheets), the 2MΩ resistor, and the self capacity of the concentric cable (about 1,000 mm F) feeding the high voltage and the filament supply to the electron gun. An appreciable ripple voltage is developed across the reservoir condenser by the current flowing in the resonant circuit ; this is cancelled out by the ripple developed across the inductance in series with the 0·001 mfd section.

The stabilizer potential divider is made up of one hundred 10-megohm 1-W units. Additional feedback at ripple frequencies is supplied by the filter network connecting Lead 29 to the reservoir condenser.

The voltage drop across the second 2MΩ (variable) resistor furnishes a negative bias for the electron gun cathode shield. The gun filament transformer T_3 is combined with the cable bushing on the oil tank.

The oscillator chassis

Fig. 12 is the circuit diagram of the oscillator unit. Four 12E1 tetrode valves feed the resonant high voltage transformer. The series condenser tuning the anode circuit affects the efficiency but not the operating frequency, which is about 30 kc/s. The direct-coupled amplifier forming the h.t. stabilizer comprises three valves, the last being a cathode-follower. Four 45-V batteries B_1, B_2, B_3, B_4, besides providing the stable bias voltage, also supply the h.t. for the first stabilizer valve ; the output voltage is adjusted in steps by means of the switch, which varies the number of reference batteries in circuit. 76732

Fig. 11.—The Metrovick 50-kV electron microscope, type EM2.

The rectifier filament oscillator consists of two Pen 46 valves in parallel, operating in a Colpitts circuit at a frequency of about 50 kc/s. Another Colpitts oscillator valve, also operating at about 50 kc/s, supplies the gun filament (about 2V, 2A); its anode voltage, and hence output, is controlled and stabilized by a simple two-valve stabilizer.

The power supply chassis

This contains two separate supplies: 700 V, 250 mA for the filament oscillators, and 750 V, 350 mA for the h.v. oscillator. The 700-V supply is continuously in operation, the 750-V supply being brought into operation by relays and push buttons. If an excessive load is taken from the h.v. circuit (i.e., owing to a bad vacuum in the gun), the h.v. stabilizer action, in attempting to maintain the voltage, causes the oscillator to draw an excessive anode current; this is arranged to trip the switching relay, and turn off the 750-V supply.

Output

The output voltage is variable in steps of 25 kV from 25 to 100 kV. The maximum output current is about 1 mA on the 25-kV, 50-kV and 75-kV ranges, and about 0·4 mA at 100 kV.

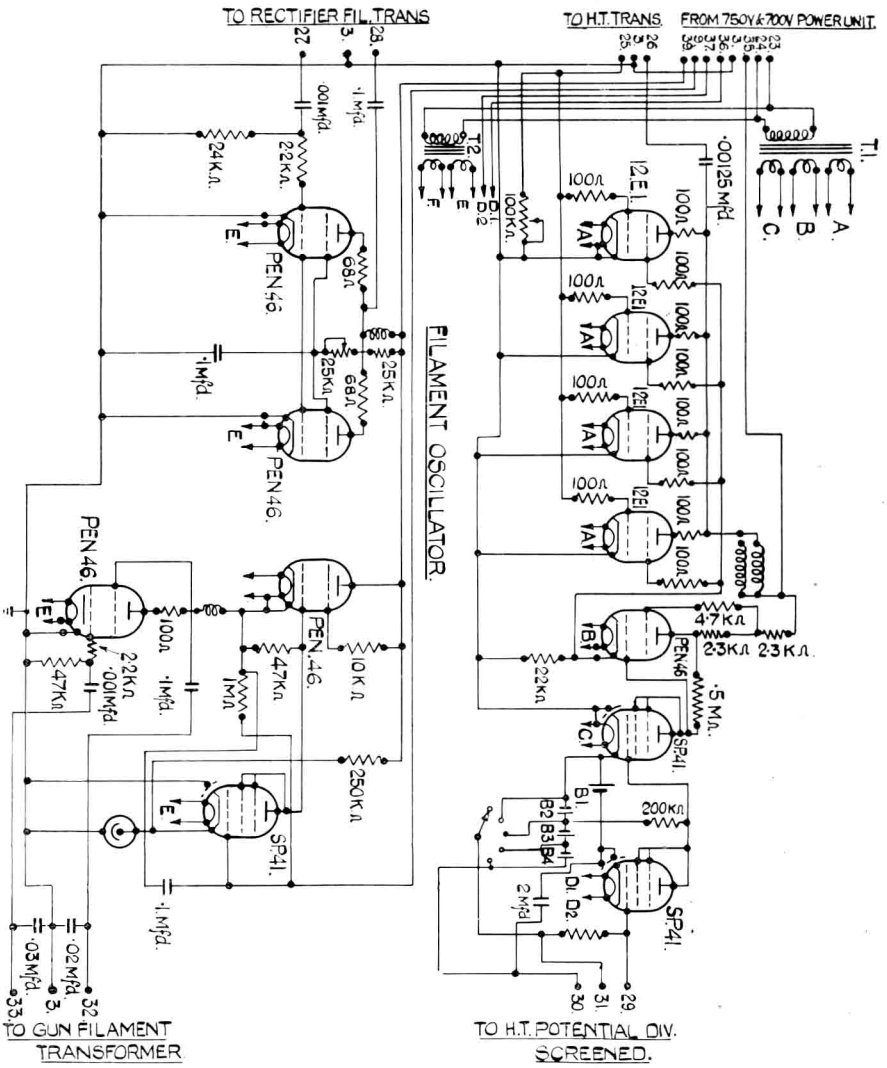

Fig. 12.—A circuit diagram of the oscillator unit. 79340A

The lens current stabilizers.

The lens stabilizers (see Fig. 13) are designed to supply a constant current to the lenses irrespective of changes of input voltage and lens resistance. The circuit follows a familiar principle in which the impedance of a series valve V_1 is controlled by an amplifying valve V_2 fed from a variable resistance r in series with the lens coil. Current changes in the coil create changes in bias which in turn vary the impedance of the series valve. Desired variations of current are achieved by varying the series resistance. Input voltage variations acquire additional correction by virtue of the action of the divider xy feeding the screen of valve V_2, so producing a stabilizing action in addition to that produced by the grid circuit of V_2.

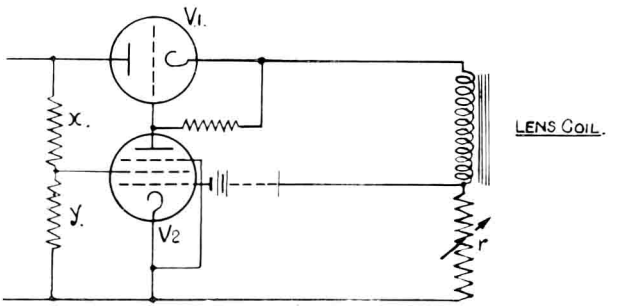

Fig. 13.—The lens current stabilizer circuit. 79607

Part II. Applications of the electron microscope

by

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SYNOPSIS

THE main techniques for the preparation of specimens for examination in the electron microscope are surveyed. Some of the problems investigated in the fields of industry, metallurgy and biology are described, and the prospects of the future applications of electron microscopy are discussed.

INTRODUCTION

The theory and constructional details of an electron microscope have been described by Haine, Tostevin, Page and Garfitt, and no further description will be added here.

Broadly speaking, the electron microscope can be used to examine any small particle or fine structure in the range between the limit of resolution of the optical microscope (about $\frac{1}{2}$ micron), and 20 Angstrom units. (1 micron (μ) = 1/25,000 inch ; 1 Angstrom unit (A.U.) = 1/250,000,000 inch).

LIMITATIONS OF THE ELECTRON MICROSCOPE

The electron beam, by its very nature, is easily stopped or scattered by atoms of even the lightest elements. This has a profound effect on the range of application of the electron microscope and its mode of use. The main results may be summarised as follows :—

(1) Since a 50-kV electron beam is completely stopped by only $\frac{1}{5}$ mm of air at atmospheric pressure, the microscope must be maintained at a high vacuum so that the air molecules do not interfere with the electron beam.

(2) Where a specimen support is required, it must be virtually transparent and structureless in the electron beam.

(3) It is not usually possible to examine the internal structure of specimens thicker than 0·5 micron in the case of organic specimens, even thinner specimens being necessary for materials of higher atomic weight.

(4) Any electrons stopped or scattered by the specimen will give up their energy to it, and will cause heating which may be sufficient to cause structural damage in some cases.

(5) Since the specimen is held in a high vacuum, it will be completely desiccated, with consequent danger of deformation.

(6) As a result of (4) and (5), it is not possible with existing techniques to examine living organisms.

Owing to the irregular fashion in which electrons are reflected from a surface, it is not possible to use the reflection techniques known in optical microscopy. In general, therefore, only direct transmission methods of observation are used, though some specialized techniques employ variants of the standard arrangement.

FIELDS OF APPLICATION OF THE ELECTRON MICROSCOPE

Bearing in mind the above limitations, the range of problems to which the electron microscope is applicable can now be reviewed. The specimens fall under three main headings :—

(*a*) Industrial powders, such as dusts, smokes, fillers, pigments, etc. where a knowledge of particle size and shape, or of size distribution, is of interest.

(*b*) Metallurgical specimens and surfaces of all types which have structural details below the resolving limit of the optical microscope. These can be examined by indirect methods.

(*c*) Biological specimens. Fine details of all types of bacteria, viruses, tissue sections, fibres, etc. are of great medical interest.

SPECIMEN AND OPERATION TECHNIQUES

Specimen Mounts

Electron microscope specimens can be divided into two main types ; one where the specimen is very small, e.g. smokes, powders in general, bacteria and viruses, and the other where it is of considerable extent either in length or area, but contains fine structure of interest (such as fibres, natural membranes or plastic replicas of metal or other surfaces). Specimens of the first category must be dispersed on a continuous mount which usually takes the form of a thin organic film. Such a film lacks the necessary strength for normal usage if it extends over an area greater than a square of about 100μ side and it therefore requires some form of additional support.

The support used usually consists of a copper grid of fine mesh (200 mesh/inch). This gives open squares of about 70 μ side, separated by grid bars about 50 μ wide. For most electron microscopes, the grids are $\frac{1}{8}$″ in diameter. Specimens of the second category mentioned above can be mounted direct on the grid. Fig. 1 shows a micrograph of the hairs on a fly's wing, a specimen which was mounted direct on the grid. Owing to the relative thickness of the wing, it was partially opaque to the electron beam, and the beam intensity had to

be raised in order to obtain the photograph. The resulting energy dissipation caused heating of the specimen and sagging of the hairs which can be seen in the figure.

Thin films suitable for specimen mounts are usually made from collodion (nitrocellulose) or formvar (polyvinyl formal). Such films, of a thickness of 200—300 A.U., are found to be strong enough to withstand the electron beam and to support a wide variety of specimens, while remaining substantially transparent to the electron beam. A film of collodion may conveniently be prepared by allowing a drop of a solution of the collodion in amyl acetate in a suitable concentration to fall on to a clean water surface. The solution instantly spreads over the surface and the solvent rapidly evaporates, leaving a film of plastic of suitable thickness on the water surface. The film can be picked up on the specimen grids and is ready for use when the water has dried off.

Fig. 1.—The hairs on a fly's wing. (× 10,000) 79433

Basic Preparation Techniques

Specimens of small over-all dimensions are usually deposited on film-covered grids from a suspension in a suitable liquid, or sometimes by airborne settling. The former method is used in much biological work ; either method can be used when smoke particles, dust or fine dispersions are to be examined. Fig. 2 shows a micrograph of carbon black particles, used as fillers in the rubber industry.

It will be apparent that such pictures will give two-dimensional information only, and that if an object is very thin, and of low atomic weight (and hence low electron scattering power), it will be difficult to distinguish its outlines or contour differences from the background film. In order to overcome these defects, Müller, and Williams and Wyckoff[1] introduced into electron microscopy a technique known as shadowcasting.

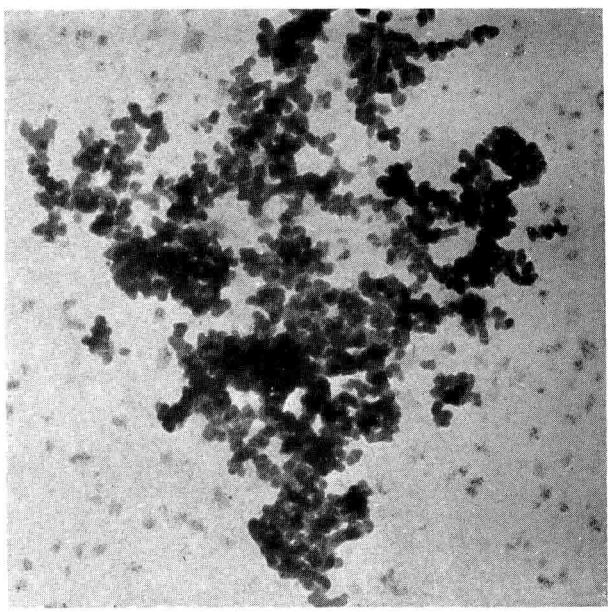

Fig. 2.—Carbon black particles. (× 30,000) 79434

Shadowcasting

A small amount of a heavy metal is evaporated in vacuo at an oblique angle on to the specimen. In a high vacuum the mean free path of the metal atoms will be larger than the distance from the evaporating filament to the specimen. If this distance is large compared with the dimensions of the specimen, the latter may be considered to be irradiated with a substantially parallel beam of heavy metal atoms. The metal will deposit in a thick layer over any protruding parts of the object, it will form a uniform thin layer over the supporting film, and in the geometrical shadow of the object there will be no metal deposited (Fig. 3).

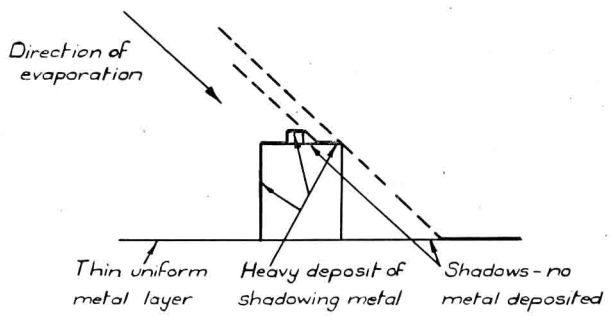

Fig. 3.—Heavy metal shadowcasting. 79608

If a specimen treated in this way is viewed in the electron microscope, it will appear with the small objects thrown into sharp relief, as a landscape illuminated by strong sunlight. This technique greatly increases the visibility of thin, uniform specimens and also gives an indication of their height dimension ; this can be obtained from a measurement of shadow length and a knowledge of the angle of incidence of the heavy metal.

The metal most frequently used for shadowcasting was gold, but it had a strong tendency to crystallize in fairly large particles, giving the background a pebbly appearance, and at the same time obscuring fine detail. More recently it has been found that uranium, chromium and a palladium alloy with platinum or gold give improved results.

If the amount of metal deposited is fairly thick, and the shadowing is not very oblique, a good picture of the gross detail is obtained. By reducing the metal thickness and the angle of shadowing, the fine detail can be brought out, and at angles of $\cot^{-1}4$ and more (i.e. shadow length $= 4 \times$ height) some structure in the supporting film becomes apparent. It can, therefore, be seen that the metal thickness and the shadowing angle must be adjusted in each individual case to bring out the maximum amount of useful information from a specimen.

Fig. 4 is a micrograph of magnesium oxide smoke shadowcast with gold, showing the smoke particles to be crystalline, and Fig. 5 a specimen of carbon black particles similar to those in Fig. 2, shadowcast with 40 per cent palladium gold alloy. The additional detail and enhanced contrast are strikingly apparent. Comparison of Fig. 4 and Fig. 5 shows the improved smoothness of the background obtained by using palladium gold alloy.

79435

Fig. 4.—Magnesium oxide crystals, shadowcast with gold at $\cot^{-1}2$. (× 31,000)

Three-dimensional information on suitable objects can also be obtained by stereoscopy. The electron microscope is particularly suited to such work by virtue of its great depth of focus.

Dark Field Illumination

As in optical microscopy, dark field illumination can be employed in the electron microscope. The main illumination is cut off by a suitable aperture and the scattered electrons only are viewed. Crystalline objects, therefore, give rise to local areas of high intensity due to Bragg reflections, and this can be useful in identifying crystals amongst amorphous material. Fig. 6 shows a comparison picture of crystals of zinc oxide smoke in light and dark field illumination.

Electron Micro-diffraction

A simple adjustment of the current in the first projector lens in a modern electron microscope produces on the final screen a diffraction pattern of a small area of the specimen. This is very useful for identifying any particular portions of the specimen under examination. Fig. 7 shows a micrograph of zinc oxide crystals and the diffraction pattern from this area.

PARTICLE INVESTIGATIONS

Most of these investigations can conveniently be made by using one of the simple preparation techniques mentioned earlier—that is deposition from liquid suspension or by settling from an airborne suspension. Starting from these basic techniques, each different type of particle to be investigated usually requires the development of a

79436

Fig. 5.—Carbon black particles, shadowcast with palladium gold alloy at $\cot^{-1}2$. (× 30,000)

specific technique differing in points of detail from the standard one.

Amongst the problems already investigated with the aid of the electron microscope may be mentioned the following :—

(*a*) The examination of airborne particles in mines. A study of the particle shape and size is being made as part of the general campaign against silicosis and similar diseases[2].

(*b*) Carbon black particles used as fillers in the rubber industry. Attempts are being made to relate the size and shape of the carbon particles to their effects on the process of vulcanization and the final properties of the rubber. See Figs. 2 and 5.

(*c*) Paint pigments. The size and shape of these particles have an effect on the quality of the paint and the ease with which it can be applied.

(*d*) Dyestuffs. The manner in which the particle shape and size change with temperature (Hamm and Van Norman[3]), and the way in which the dye adheres to fibres have been investigated.

METALLURGICAL EXAMINATIONS

Some efforts have been made to view metal surfaces by a reflected electron beam. The only condition in which a coherent beam can be obtained is that of grazing incidence. Some photographs have been obtained by this means, but they are, of course, violently foreshortened in the direction of the electron beam. The resolution obtained by these methods has not yet equalled that given by indirect replica methods (v. Borries[4], Semmler Alter[5]).

As these specimens are generally too thick to be examined by transmission in the electron microscope, the fine surface structure must be examined by a replica technique.

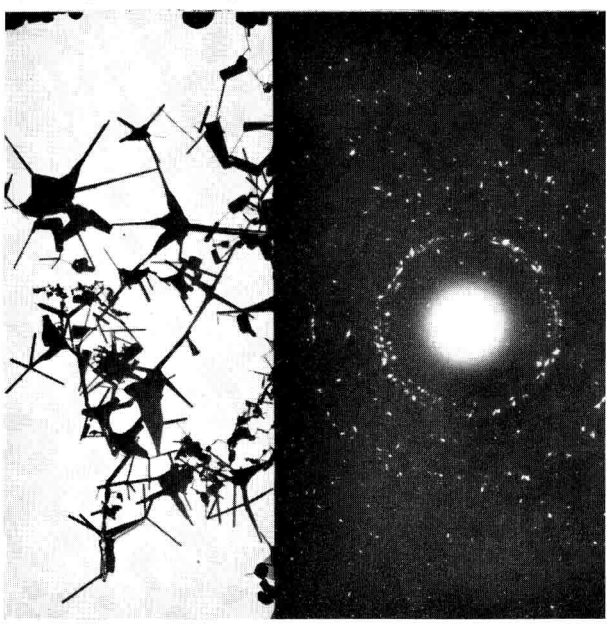

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Fig. 7.—Zinc oxide crystals. Micrograph and micro-diffraction pattern. (× 12,500).

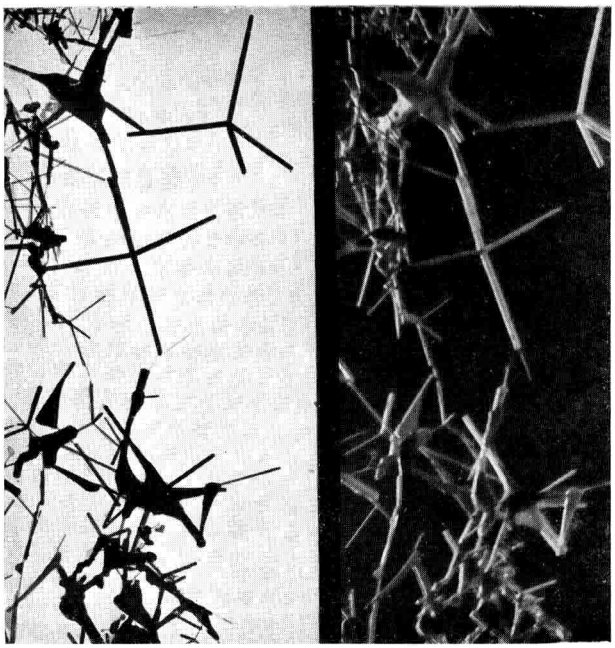

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Fig. 6.—Crystals of zinc oxide smoke in light and dark field illumination. (× 13,000).

Replica Techniques

The general principle of these is to obtain a replica film sufficiently thin to allow adequate electron penetration and which will reproduce accurately the configuration of the surface being investigated.

The simplest is the formvar replica (Fig. 8). A few drops of a solution of formvar in dioxane or chloroform is spread over the surface to be examined, and the solvent is allowed to evaporate. This leaves a thin film of formvar which takes up the contours of the specimen surface (in reverse) on its lower surface, its upper surface remaining theoretically plane. If this film be now stripped from the specimen (by flotation or by mechanical peeling off), and mounted on a specimen grid, it can be viewed in the electron microscope in the normal way. The image will show light and dark areas corresponding to the thin and thick parts of the formvar replica. It is thus possible to obtain information on the contours of the original specimen.

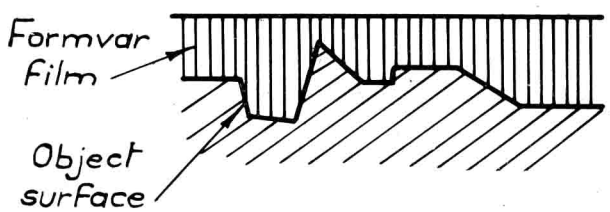

Fig. 8.—Negative formvar replica.

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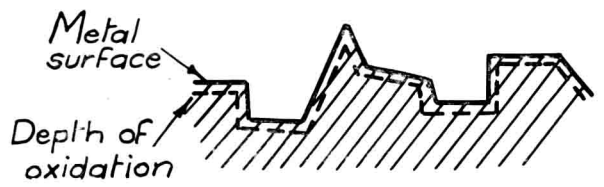

Fig. 9.—Aluminium oxide replica. 79609

More complicated processes have been devised, mostly two-stage processes giving a positive replica, which find useful application in some cases. Where aluminium surfaces are being examined, the surface may be oxidized, and the thin oxide layer removed and viewed in the microscope (Fig. 9). These replicas have high contrast and great clarity of detail, though their resolution is impaired by fine structure on the aluminium surface. The application of this type of replica has recently been extended by pressing electrolytically polished aluminium against steel fracture surfaces. When an oxide layer has been formed in the usual way, a replica of the fracture surface is obtained. This technique is very useful for examining surfaces containing deep pits where a formvar replica would key in and would be impossible to strip. The oxide replica technique has not yet been successful with any other metal.

Preshadowed Replicas

An extension of the replica technique has been developed by Wyckoff for use with very small biological specimens and for examining large organic molecules, and has been used with success on fine structure in metal surfaces. The ordinary formvar replica is limited in resolving power to about 200 A.U. by the intrinsic structure of the formvar. If, however, a specimen is shadowcast as previously described, the thin film of shadow metal forms a replica of the surface, and if is this backed with a formvar film it can be stripped from the surface.

Fig. 10 shows a preshadowed replica of a mild steel surface which has been cut with a carbide-tipped tool. This may be compared with the plain formvar replica of a similar surface shown in Fig. 11.

This technique also overcomes the objection to many plain formvar replicas that the upper surface of the film is not in fact plane, but may follow the gross structure of the under surface, giving rise to artefacts. (Trotter[6]).

Practical Applications of the Replica Technique

Investigations so far carried out have included the following :—

(*a*) Fine structures in carbon steel. Various transformation products of carbon steel have been examined in an attempt to supplement the information obtained by optical microscopy. Fig. 12 shows a negative formvar replica of the surface of pearlite steel. A grain boundary between pearlite and ferrite can be seen.

(*b*) Precipitation in aluminium copper alloys. Investigations are being carried out on the orientation and extent of the copper precipitation in these alloys. Some correlation with x-ray measurements has been obtained (Castaing[7]).

(*c*) Slip bands in aluminium and steel. A study of slip bands is being undertaken with a view to the clarification of the fracture process in metals. The work of Brown[8] on aluminium has caused him to advance a theory based on active and inactive slip planes to explain the slip band spacings found in practice.

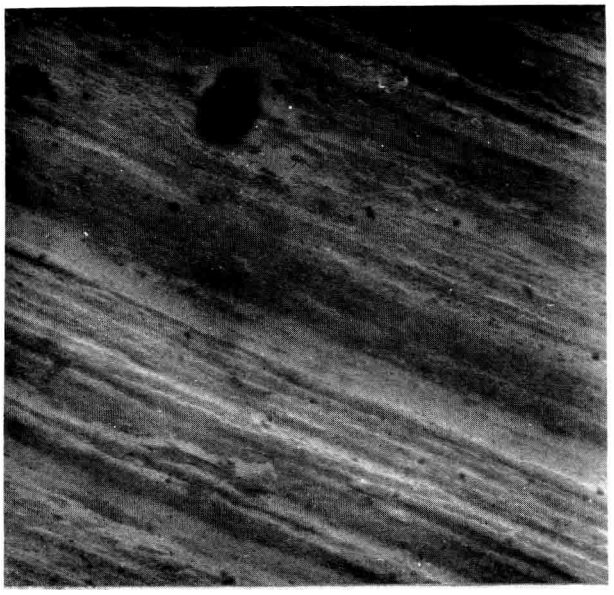

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Fig. 10.—Mild steel surface after cutting with a carbide-tipped tool. Pre-shadowed replica with palladium gold alloy at $\cot^{-1}3$. (× 24,000)

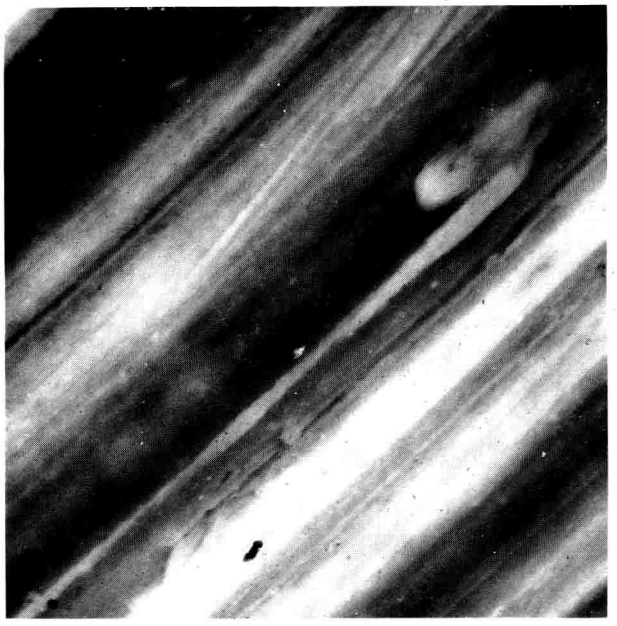

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Fig. 11.—Mild steel surface cut with a carbide-tipped tool. Negative formvar replica. (× 12,000)