# PHYSICS TODAY

# HIGH-VOLTAGE ELECTRON MICROSCOPY

British, French, Japanese and US teams added accelerators to electron microscopes. Now voltages ten times conventional levels permit viewing of thicker, more representative specimens.

# V. ELLIS COSSLETT

A SECOND GENERATION of electron microscopes, using ten times the voltage of conventional instruments, is beginning to fulfill designers' hopes of allowing study of thicker specimens with less radiation damage. Voltages in the 1-megavolt range have reduced the effect of chromatic aberration so that, a fixed value of resolution, usable specimen thickness can be increased roughly in the same ratio as voltage until it reaches a limit set by image visibility. Apart from a scaling up of the whole instrument, the only major difference in design is the insertion of an accelerator between the electron gun and the microscope itself. Highvoltage instruments are being used in metal studies by investigators who want a specimen thick enough to display bulk properties and, in biology, to probe living matter. A still larger machine is under construction in France.

#### Burst of activity

In the past few years operating voltages have jumped by a factor of ten and more. Ernst Ruska's original instrument, built in 1933 at the Technical University of Berlin, could work up to 70 kV, and the first Siemens production model had a maximal en-

ergy of 60 kV. Many years passed before commercially available microscopes offered 100 kV. Now much higher voltages have come into use; this started about 1960 with an instrument at Toulouse designed for 1.5 MV. At present 1-MV microscopes are operating in Britain, Japan and the US as well. A 3-MV instrument is being built in France, and one of 5 MV has been thoroughly discussed by Albert V. Crewe and a working party in the US. Why this sudden burst of activity?

The question could well be posed in reverse: Why have electron microscopists for so long been content with a limit of 100 kV? It was clear from early days that an electron beam of this voltage could only give an adequate image through a specimen a few hundred nanometers thick at maximum; this is due to the strong interaction of electrons with matter. This interaction decreases at higher accelerating voltages, however; so greater effective penetration can be attained. Two difficulties stood in the way of such a development. First, although the production of higher voltages was straightforward enough, the output could not be easily stabilized to the high degree (better than

1 part in 10 000) needed to offset the severe chromatic aberration of electron lenses. Second, the cost of an electron microscope rises at least in proportion to the operating voltage, and some estimates made it nearer to a square law. Most laboratories, whether metallurgical or biological, found \$25 000 or more a high enough price to pay for the conventional



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100-kV instrument. The main preoccupation of electron microscopists in the past 20 years or so has therefore been to adapt their specimenpreparation techniques to make the best of the situation. They cut tissue sections only a few tens of nanometers thick or etch metal foils down to this same limit.

# Circumstances change

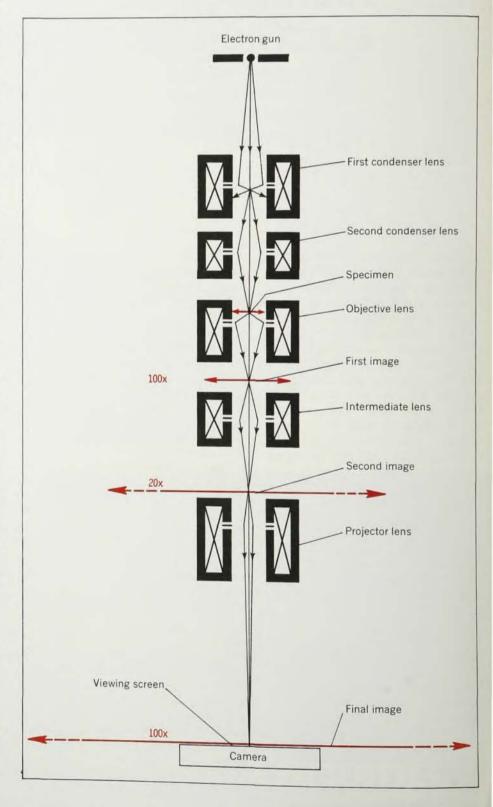
Technical improvements, new research requirements (especially in metal physics) and a more favorable assessment of the cost prompted the move to higher voltages. Advances in highvoltage engineering, using electronic feedback systems, made possible a stability of 1 part in 105 and better, even at 1 MV. Experience showed, too, that the effect on the electron image of microdischarges (corona) from electrodes at such voltages was very much less than had been feared, especially if the system was enclosed in a gas at high pressure. At the same time, metallurgists and metal physicists were demanding greater penetration because of understandable doubts about the relevance of observations made on foils 100 nm thick to the properties of bulk metal. Moreover the great contribution of the conventional electron microscope to our knowledge of the metallic state, particularly as regards the form and behavior of dislocations, made it easier to justify the cost of high-voltage microscopy. This cost need not rise much faster than linearly with voltage (figures 1 and 2).

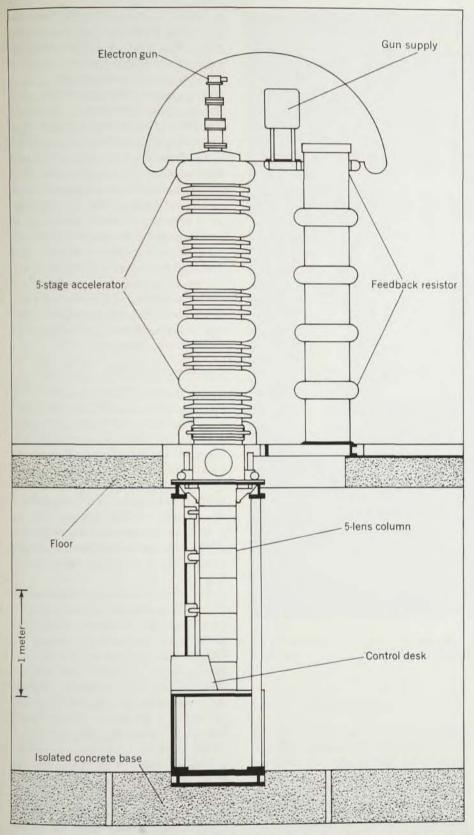
On this basis the great leap forward began in England and Japan, and later in the US (figure 3). But one caveat must be entered before beginning a more detailed discussion of the advantages of high-voltage microscopy. The Toulouse project was approached and justified on quite different grounds. From the early 1950's Gaston Dupouy of the University of Toulouse was convinced that the chief argument for very high voltages lay in the possibility of observing living matter. Greater penetration would allow a complete cell, within the plastic windows of a micro-chamber, to be imaged with reasonable definition, and also the damage caused by radiation would be less, according to accepted theory. As head, at that time, of the powerful Centre Nationale de la Recherche Scientifique Dupouy was able to obtain the funds necessary to design and construct a 1.5-MV electron microscope and a special laboratory to house it. In this project the overall cost may well have been in proportion to the square of the voltage. The instrument began operation at the end of 1960. Some of the biological results obtained with it remain in dispute; moving with the

pressure of research interests, the Toulouse microscope now is also being used for several applications in metal physics.

# High-voltage advantages

As we increase the accelerating voltage  $V_A$  in a microscope, the chief gain is in effective penetration, that is, in the specimen thickness that gives





CONVENTIONAL ELECTRON MICROSCOPE (left). Gun delivers electrons into a double-condenser system that controls intensity and size of illumination falling on specimen. A three-stage imaging system gives enough overall magnification to make resolved detail visible. The specimen is magnified 100 times by the objective lens, up to 20 times by the intermediate lens, and 100 times by the projector lens, giving a maximal total magnification of 200 000. Ten-power binoculars at the viewing screen give a final maximum of 2 million. Potentiometers that control current in the lens coils provide focusing and change of magnification.

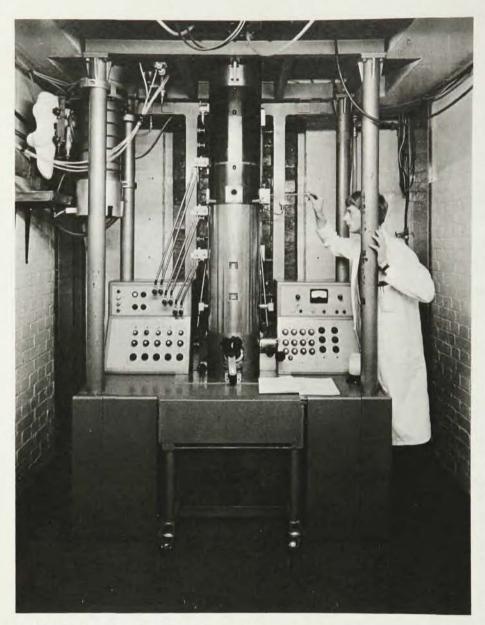
—FIG. 1

-FIG. 2

an image of acceptable quality. Radiation damage in most cases decreases. Image contrast also decreases, while image resolution improves (in a specimen of given thickness). So there will be an optimal voltage for studies in which the highest resolution of a thin specimen is the aim. For studies of internal structure there may be no limit to the useful voltage, but the maximal current a specimen can take without damage often sets a limit to the thickness through which an image can be obtained.

The ultimate resolution, as set by electron optics, depends on a compromise between spherical aberration (proportional to  $\alpha_0^3$ ) and diffraction (proportional to  $\alpha_0^{-1}$ ) which yields an optimal value for the angular semiaperture  $(\alpha_0)$  of the objective. The resulting value of the resolving power  $\delta_{\rm T}$ , in the classical sense of minimal point-to-point separation, is proportional to  $\lambda^{3/4}$  if  $\lambda$  is the wavelength of the incident electron beam. This proportionality can only be maintained as beam voltage is raised, however, if the magnetic field Bo in the objective can be increased by the same proportion so as to keep constant its focal length (and hence its spherical-aberration coefficient). In practice we have to assume that the maximal possible field already prevails at the starting voltage so that Bo is constant as VA rises. The value of  $\delta_T$  is then approximately proportional to  $\lambda^{1/2}$ .

The limit of resolution thus improves by a factor of about two between 0.1 and 1 MV, from 0.3 to 0.15 nm in a good objective, but this prospective gain is offset by the increasing difficulty of stabilizing the accelerating voltage and the magnetic field in the objective sufficiently to make chromatic aberration negligible. Image contrast deteriorates too, for the same reason that penetration increases-reduction in scattering cross section with voltage. Amplitude contrast is proportional to  $\beta^{-2}$ , for small values of  $\alpha_0$ , whereas phase contrast depends on  $\beta^{-1}$ . So the differential contrast for small crystallites on an amorphous supporting film would improve as VA is raised. But calculations of the absolute contrast1 in these conditions make it very doubtful whether single atoms could be visibly imaged at 1 MV. A resolving power of 0.15 nm is of little value if nothing can be seen with it. Projects aimed at extreme



COLUMN AND CONTROL DESK of 750-kV electron microscope at Cavendish Laboratory, Cambridge. An assistant operates the specimen airlock. —FIG. 3

resolution are therefore now using only moderate voltages, of the order of 200 kV (Ruska, now at the Max Planck Institute, Berlin-Dahlem, and Benjamin M. Siegel at Cornell).

#### Practical resolution

The practical resolution of an electron microscope, however, with all except the very thinnest specimens, is primarily limited by chromatic aberration. The beam loses energy by inelastic collisions in the specimen and is no longer monochromatic when focused by the objective. The latter has an appreciable chromatic coefficient  $C_c$ , so that the radius of the aberration disk is

$$\delta_{\rm c} = C_{\rm c} \; \alpha_{\rm o}' \; \Delta V/V_{\rm A}$$

where  $\Delta V$  is the mean energy loss (in eV). Even with the smaller objective aperture  $\alpha_{\rm o}'$  that can now be used before diffraction becomes important, the value of  $\delta_{\rm c}$  is much larger than that of  $\delta_{\rm T}$ , the limit set by spherical aberration. A film of carbon 100 nm thick gives a mean energy loss of 75 eV at 100 kV, leading to a chromatic aberration of 3 nm when  $C_{\rm c}=4$  mm and  $\alpha_{\rm o}'=10^{-3}$  radians. For thin metal films the resolution is worse still being approximately proportional to their physical density.

This picture changes radically with increase in voltage. Between 0.1 and 1 MV the mean energy loss  $\Delta V$  falls

by a factor between 2 and 2.5 according to the Bethe law, so that the quotient  $\Delta V/V_A$  falls to 1/20-1/25 (depending on atomic number Z). As  $V_A$  is raised,  $C_c$  increases but  $\alpha_o'$  falls; their product is roughly constant, and the chromatic aberration δ<sub>c</sub> falls nearly linearly with increasing voltage. The practical image resolution is about 20 to 25 times better at 1 than at 0.1 MV for a specimen of given thickness. For a fixed value of resolution the usable specimen thickness could, in principle, be increased in the same ratio as voltage, provided that enough of the beam came through to form a visible image. In practice, image visibility soon sets the limit (figure 4).

This reduction in the effect of chromatic aberration is the greatest advantage resulting from higher voltages in electron microscopy.

#### Specimen thickness

Effective penetration is linked with image brightness. The maximal useful thickness of specimen can be defined as that which allows enough electrons to be transmitted through it, within the cone angle  $(2\alpha_0)$  subtended by the objective aperture, to give an acceptable image. Thus it depends on the intensity of the incident beam  $I_0$  as well as on the scattering properties of the specimen (thickness t, atomic number Z) at given incident voltage  $V_A$ .

 $V_A$ . The minimal transmitted intensity I that can be tolerated should properly be assessed in relation to the minimal magnification required to bring out all the resolvable detail in the final image.<sup>2</sup> But for present purposes the magnification can be assumed constant as  $V_A$  is raised. I is set at the lowest value that will give an image bright enough for focusing. For a given incident intensity  $I_o$  the problem is then to find the relation between specimen thickness and incident voltage at constant transmittance  $(I/I_o)$ .

For thickness in the range usually studied,  $I/I_0$  is related to t by the exponential law

$$I/I_0 = \exp (-\mu t)$$

where  $\mu$  is an effective absorption coefficient, the value of which can be obtained from the atomic cross sections for elastic and inelastic collisions, provided plural scattering is taken into account.<sup>3,4</sup> Alternatively  $\mu$  can be taken as an empirical constant,

obtained by experiment. For amorphous and polycrystalline films the agreement of theory with experiment is almost within experimental error over a wide range of voltage and objective aperture. For single crystals the theory is not so well developed.

Up to an attenuation of  $e^{-2}$ , and possibly as far as  $e^{-4}$ , the "amorphous" theory predicts that t should be proportional to  $\beta^2$  as the accelerating voltage is raised (curve B, figure 4) if the aperture is negligibly small  $(\alpha_0 \to 0)$ . So, for given attenuation, t can be three times as great at 1 MV as at 0.1 MV. For an aperture of the size usually employed  $(3-5 \times 10^{-3} \text{ radians})$ the relationship becomes more nearly a straight line, and the more so the lower the atomic number of the scatterer (compare curves C and D in figure 4 for gold and carbon). We have recently made experiments on these elements5 that confirm this prediction of R. E. Burge and G. H. Smith.6

For a perfect single crystal the imaging conditions are quite different. Diffraction occurs, so well defined beams will emerge behind the specimen, the order and intensity of which depend on its lattice structure and its orientation to the incident beam. For the two-beam condition, in which only the direct (zero-order) beam and a single diffracted beam are considered, theory again predicts a  $\beta^2$  relation between thickness and electron energy.7 Inclusion of higher-order beams might increase the penetration at high voltages, particularly for elements of high Z,8 but no experimental evidence for this assumption has yet been found. On the other hand, the background intensity in the image, caused by inelastic and thermal diffuse scattering in the specimen, would be expected to depend on aperture and voltage. The background intensity is confined in smaller angles as VA rises so that a larger fraction passes through an aperture of given diameter. In fact the effective absorption coefficient for a given crystallographic orientation µg varies with voltage and aperture in much the same way as with amorphous or polycrystalline specimens.9

# Silicon, iron curves

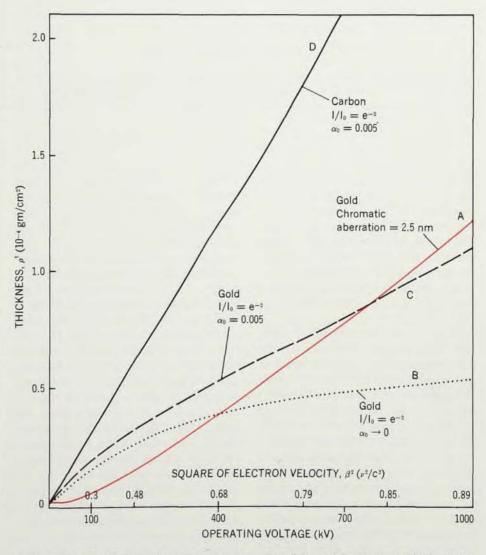
Gareth Thomas of the University of California at Berkeley recently confirmed these conclusions in experiments using the Cavendish and Toulouse high-voltage microscopes. His

penetration curve for silicon (Z=14) is much steeper than that for iron (Z=26) in the form of stainless steel, as figure 5 shows. The results of R. Uyeda and M. Nonoyama of Nagoya University, on molybdenum disulphide<sup>11</sup> are rather closer to a  $\beta^2$  curve, but they used a smaller objective aperture at high voltages than at 50 and 100 kV, depressing the upper end of the curve.

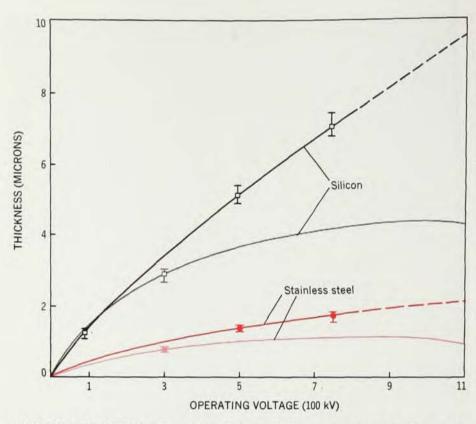
On present evidence, therefore, maximal specimen thickness increases almost linearly with voltage for elements of low Z if a relatively large objective aperture is used, that is, as long as low contrast can be tolerated. But the thickness-voltage relation becomes more nearly of  $\beta^2$  form as the aperture is reduced or the atomic number in-

creased. In optimal conditions adequate images of dislocations and fringes can be obtained at a voltage of 1 MV from aluminum foils up to 10 microns and iron up to 2 microns thick but in tungsten or uranium only up to about 0.5 microns.

Radiation damage sets a limit to usable thickness for many types of specimens, either directly or through rise in temperature. At least three effects can occur: ionization, ejection of atoms and heat absorption. Dissipation of energy by an electron beam, in a specimen of given thickness, falls by the factor of 2 to 2.5, mentioned above, when beam energy is increased from 0.1 to 1 MV. Ionization and bond breakage should, therefore, be less at higher voltage, thus enabling



THICKNESS LIMITS set by chromatic aberration (A) and by image visibility (B,C,D) as functions of voltage and square of electron velocity. For gold: Color curve A represents chromatic aberration of 2.5 nm in lens with angular aperture  $5 \times 10^{-3}$  and chromatic coefficient 4 mm. B and C show thicknesses giving constant transmission  $I/I_0 = e^{-2}$  for angular aperture approaching zero (B) and equal  $5 \times 10^{-3}$  (C). For carbon: D is the curve that corresponds to C.



SILICON AND STAINLESS STEEL thickness limits, as functions of voltage, determined from visibility of stacking-fault fringes. Theoretical curves are from two-beam theory, approximately fitted at 100 kV (Thomas<sup>10</sup>).

—FIG. 5

easier observation of polymers and living tissues. Ejection of atoms in knock-on collisions, however, becomes increasingly probable above a threshold voltage, which varies with atomic number; it is about 250 kV for aluminum and 500 kV for copper. M. J. Makin has seen radiation damage of this type in the Cavendish microscope. 12

Temperature rise also sets a limit, especially with specimens of low thermal conductivity. Present electron guns can deliver a current of the order of 1 microamp to a spot only 5 microns across on the specimen if a large condenser aperture is used. At 1 MV the energy flux is 5 MW/cm² of which the specimen will absorb a fraction determined by its mass thickness. Even with thin metal foils the temperature rise may be too great, and with nonconductors the incident beam must be reduced to the order of a nanoampere.

# Design and construction

All high-voltage microscopes so far constructed have been basically similar in design to ordinary 100-kV models. The difference is primarily one of scale; all dimensions are enlarged roughly in proportion to the square root of the maximal voltage. The only feature new in principle is the accelerator inserted between the electron gun and the electron microscope.

A magnetic lens has a focal length f that depends directly on the effective voltage Vr, where eVr is the relativistically corrected energy of electrons accelerated by voltage VA. As  $V_{\rm r}$  increases 18-fold when  $V_{\rm A}$  is raised from 0.1 to 1 MV, f would increase similarly in a lens of given physical dimensions at fixed excitation. But f also depends on the integral along the axis of the square of the magnetic field Bo2, so that it can be shortened again by increasing the gap between the pole pieces and increasing the excitation to keep Bo constant. In practice the maximal value of Bo is increased somewhat above the one usual in conventional microscopes by raising the current loading of the windings; this increase requires water cooling and a larger cross section of iron in the magnetic circuit to stave off saturation.

The focal length is kept short chiefly

to minimize spherical and chromatic aberrations (both are directly proportional to f), apart from the need to keep the microscope as a whole reasonably compact. The result is a lens, whether main condenser, objective or main projector, with pole-piece bore and gap both equal to 5–8 mm, focal length 5–6 mm, spherical-aberration coefficient about 6 mm and chromatic coefficient  $C_c$  about 4 mm. The maximal field is of the order of 20 kG at an excitation of 15 000–20 000 ampere turns.

# Half-ton lenses

Such a lens for 1 MV can have an adequate iron circuit within an overall diameter of 36 cm. The tendency in most microscopes, however, has been to include a much greater thickness of iron in the column, partly to reduce the danger of stray fields (the bane of an electron microscopist's life, when aligning the various lenses) and partly as built-in radiation protection. The engineer's love of a safety factor doubtless also plays a role. In consequence the lens diameter usually becomes 45 cm or even 55 cm, and its weight approaches half a ton, requiring a crane to erect or dismantle the column. By contrast, in the Cavendish 750-kV microscope (figure 3) the lenses have been kept as small and light as possible in the interests of flexibility. The column diameter is only 30 cm, and the largest lens weighs no more than 110 kg, so that the column can be rapidly dismantled with a simple forklift truck.13 This facility has especially proved its worth in allowing the normal specimen chamber to be readily replaced by a liquid-helium-cooled one. The RCA 1-MV microscope also has a small column diameter. The chief disadvantage is the need for remote operation from behind a radiation wall when a high beam current is required at high voltage. Toulouse microscope pays heavily for safety with both a massive column and remote control.14

The column of the Cavendish microscope is otherwise of conventional design. The objective is preceded by a double-condenser system and followed by a double projector. The magnification range is typically from 1000 to 150 000. An extra "diffraction" lens is becoming usual; it gives greater camera length and hence pattern magnification in electron-diffraction.

tion studies. Protective shields of lead or some other dense element are built around the main sources of x radiation, the aperture systems of the condenser lenses and the objective. For the same reason the viewing chamber is massive, and the windows are of lead glass 20–25 cm thick. Closed-circuit television provides remote viewing of the image on most high-voltage microscopes.

# Accelerators differ

Our accelerator and electron gun are also conventional. Although there is some variation in detail among different accelerators, particularly in the number of stages, they are all patterned on those used in nuclear physics. In the Toulouse, Cavendish and RCA microscopes the high figure of 150 kV per stage has been adopted by the makers of the accelerators, the Swiss firm of Emil Haefely. In the Japanese microscopes the gap voltage is usually no more than 50 kV, with a correspondingly greater number of stages. 150 kV per stage requires a much better vacuum, of course, which probably has the beneficial side effects of longer filament life and less carbonaceous contamination in the specimen region.

The high-voltage generator has not yet departed from the familiar Cockcroft-Walton (or Greinacher) system. Generation is at about 10 kHz to ease the problem of stabilization; a rectifier-capacitor stack of 10 or 12 stages steps up this input voltage. The very great stability demanded in the output voltage to provide a near-monochromatic electron beam is attained with a double feedback system: a fast loop for ripple and a slow loop for drift. A stability of 1 part in 105 over 3 min is attainable but calls for some electronic complication if it has to be met over a wide range of voltage, as in the Cavendish installation (from 75 to 750

#### Electron-optical problem

Such a wide range of operating voltage also poses an electron-optical problem: The overall focal length of the accelerator will vary widely so that the crossover in the beam delivered to the microscope will change in position and angle of convergence. This variation may be inconvenient in some microscopic investigations, and designers usually try to avoid it. One solution,

which is straightforward if the voltage range is not more than two- or threefold, as in the Japanese microscopes, is to feed the grid and anode of the electron gun from the same dividing resistor as the accelerator. With careful design of the first stage of the latter. which is a strong electrostatic lens, the position of the virtual source provided by the electron gun will vary in such a way as to keep the final crossover roughly stationary. However, the total beam current will then go up as the operating voltage is increased, unless some electronic compensating device is built in. A simpler alternative is to design the electron gun as an injector system completely separate from the accelerator so that it operates always at a fixed anode voltage, as in the Toulouse and Cavendish microscopes. A magnetic lens placed between gun and accelerator varies in step with the operating voltage so that the injected beam is focused by the amount needed to keep the final crossover fixed. disadvantage of this system is the need for a current supply at high voltage; a considerable advantage is that it provides a beam current that does not change with operating voltage.

The high-voltage supplies for the Toulouse, Cavendish and RCA microscopes are all air insulated. This is desirable in an experimental setup in which ready access to all components is essential (for experimenting with the electron injection system, for in-Most of the commercial stance). models understandably put both generator and accelerator into pressure tanks for compactness and safety. In the AEI and the Shimadzu microscopes they are in separate tanks joined by an enclosed busbar or cable, respectively, whereas Hitachi and Japan Electron Optical manage to put them into a single tank. This is the neater solution but must involve considerable difficulties in shielding the components from each other for the required degree of stability.

# High-voltage applications

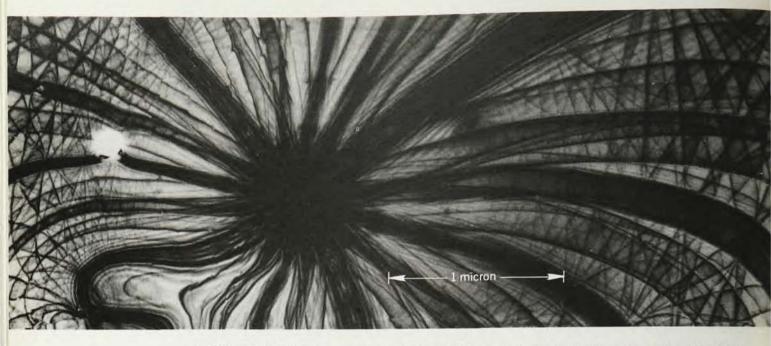
Although the Toulouse project was designed with biological applications in mind, most of the problems handled elsewhere so far have been in metal physics and metallurgy with smaller (but important) excursions into polymer science. (An example of metallurgical results is shown in figure 6.) As wide-ranging exploration is in full

swing, and much of the work is still unpublished, only an outline of the scope of high-voltage microscopy can be given.

Studies of the internal structure of metals make good use of the high-voltage machines' ability to penetrate foils much thicker than those that can be imaged at 100 kV. Precipitates of foreign inclusions larger than about 0.5 micron, which would be lost in preparation of thinner foils, are retained and can be examined by microscopy and diffraction. In this way we have established the nature of foreign particles that were affecting the finish of rolled aluminum sheet.

More quantitatively, it is important for practical metallurgy to find the thickness above which a foil shows the properties of the bulk metal. Fujita's first results15 show that this critical thickness is different for different types of structure and dynamic behavior. The bulk value for dislocation density does not appear at a thickness of less than 0.8 micron in aluminum although it is reached at 0.1 micron in iron, whereas for recrystallization phenomena the limit is about 1 micron in both metals. The dynamic behavior of dislocations in aluminum, which is under greater restraint from surface conditions, shows bulk properties only above 1.5 microns for cell formation and 3 microns for three-dimensional cell structure. Fujita puts maximum usable thickness at 500 kV as about 4 microns for direct observation in aluminum and 1 micron in iron, and about twice these values for dim images recorded with long exposure times.

These limiting thicknesses agree fairly closely with our own experience and with that of Dupouv and Frantz Perrier16 in the Toulouse microscope, which has been used mainly for studies of aluminum and its alloys with copper and silver. Little has so far been published on the heavier metals. In the Cavendish microscope we find that the effective penetration in tungsten and uranium increases somewhat faster with voltage than in aluminum: clear images of dislocations are obtained through a thickness of 0.5 microns at 500 kV (figure 7). There is also firm evidence that at liquid-helium temperatures penetration in lead is 2-3 times greater than at room temperature17; on the other hand, radiation damage is much less. An image intensifier, which allows either a



BEND CONTOURS in a tungsten foil about 100 nm thick stand out where the angle of the lattice to the incident beam satisfies Bragg diffraction conditions. This micrograph was taken with 750-kV operating voltage in the Cavendish microscope by M.S. Spring (Cambridge).

—FIG. 6

weaker illuminating beam or a thicker specimen to be used, gives further improvement in both penetration and damage reduction.

#### Magnetic thin films

The high-voltage microscope is also proving useful in studies of magnetic The domain structure thin films. changes radically with increasing thickness, and some new phenomena have been found18 (figure 8). In this and other fields of metal physics many experiments of a dynamic character, developed for the conventional electron microscope, now extend to the new instruments: heating, cooling, straining, etc. Study of the progressive thinning in situ of a stainless-steel foil by bombardment with a beam of argon ions represented a new departure.19

For polymer studies, higher voltages give the benefit of less radiation damage. K. Kobayashi of the Institute for Chemical Research, Kyoto University, and his collaborators made detailed experiments on a variety of polymer films. 20 They measured the time for which a clear diffraction pattern was observed, that is, until crystallinity was destroyed. They found that the total dose needed to do so was directly proportional to the operating voltage in each case although the absolute value of the critical dose differed by

an order of magnitude among the four polymers studied.

Other nonmetallic materials, such as cements and ceramics, which can not be prepared as films thin enough for penetration at 100 kV, are now open to examination at 500–1000 kV. There are now interesting results on the early stages of crystallization in Portland cement, with which darkfield techniques are especially useful. A wide area of research is being made accessible although radiation damage remains a problem, probably owing to local rise in temperature.

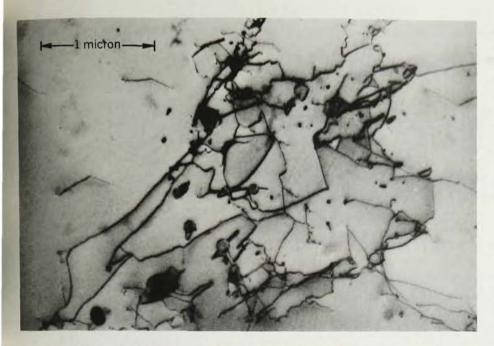
#### Living matter

As for biological applications the initial observations on bacteria made by Dupouy22 have not yet been repeated elsewhere. He claimed that various living microörganisms, examined by high-voltage microscopy in a microchamber with plastic windows, survived exposure and could be cultured again on removal from the specimen holder. The original work was done at 650 kV, but it has recently been repeated at 1 MV with the same result.23 On the basis of survival rates in macroscopic irradiation experiments, biologists have tended to reject these claims, especially as Dupouy has not so far taken a series of micrographs showing growth of the bacteria. But even if their development has been impaired by exposure to the electron beam, there may be great interest in the details of radiation damage, in tissue-cultured cells or viruses as well as in bacteria.

#### Future prospects

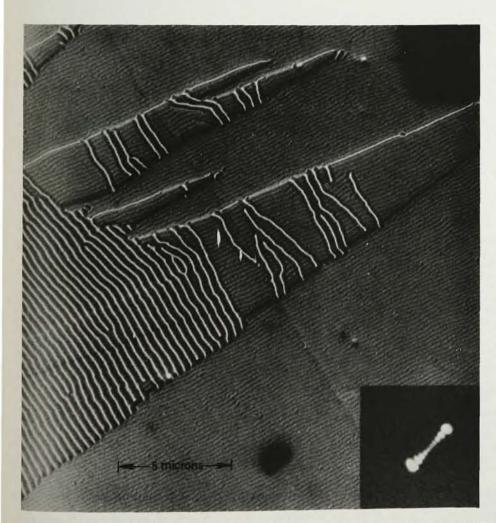
Several plans for microscopes of still higher voltage are in the air. A "workshop" at the Argonne Laboratory spent several weeks in the summer of 1966 studying the feasibility of a 5-MV instrument. The participants concluded that it would demand some major departures from present practice. For the high-voltage source a Cockcroft-Walton system no longer appears viable. The workshop considered the relative merits of Van de Graaff and linear accelerators; opinion largely favored the former if problems of mechanical and electrical stability can be solved. For lenses they agreed that the existing type was practicable only up to 3 MV, beyond which a superconducting objective appeared to be the best alternative, with quadrupole lenses for the first condenser and the final projector.

Although this project is hanging fire, for lack of money, experimental studies of these new generators and lenses are going forward in several laboratories. Meanwhile Dupouy has completed the design of a 3-MV microscope; construction has begun at Toulouse. It



DISLOCATION NETWORK in tungsten foil that is 0.5 microns thick and strained 1%. Picture was taken at 500-kV operating voltage in the Cavendish microscope (Hale and Henderson-Brown, National Physical Laboratory).

—FIG. 7



STRONG-STRIPE-DOMAIN structure (with 360-deg walls) starts to form at right angles to initial weak-stripe domains in a Permalloy film 0.25 micron thick, vacuum deposited at 45 deg to the substrate. Picture was taken at 500 kV in the Cavendish microscope. Inset: Low angle diffraction pattern obtained from the strong-stripe-domain structure (Puchalska and Ferrier<sup>18</sup>).

—FIG. 8

appears to be a scaled-up version of his 1.5-MV instrument but with a pressurized Cockcroft-Walton generator. Completion and performance are eagerly awaited. As the theory of penetration is in so uncertain a state, only experiment can decide whether there is any worthwhile bonus of information to be gained by going much above a voltage of 1 MV for microscopy.

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