

## Scanning Electron Microscopes: Is High Resolution Possible?

Use of a field-emission electron source may make it possible to overcome existing limitations on resolution.

Albert V. Crewe

The modern electron microscope is an extremely valuable and powerful instrument. Since its appearance some 30 years ago, numerous improvements and modifications have been made, particularly in the direction of increasing the resolving power. In the hands of skilled operators, resolutions of a very few angstroms have been achieved.

It is, however, doubtful whether any further substantial improvement can be effected without serious and perhaps radical changes. For example, the basic lens design has not changed during this period, and it is well known that such lenses have aberrations which cannot be eliminated. Ruska (1) has recently shown how to extract maximum performance from such lenses. He indicates that improvements in resolving power of only a factor of two or so can be expected.

This factor may make it possible to reach the most desirable goal of 1 Å, particularly at voltages of several hundred kilovolts. It should not be assumed, however, that we will then be able to see single atoms. To do this one needs to be able to see a single atom of one kind supported in or on a substrate of another kind. If, as seems likely, such a thing is possible only when the single visible atom is of gold

or osmium (typical contrast techniques), then such a feat may be of little scientific significance except for a few rare applications.

The reason for the existence of this state of affairs is that conventional electron microscopes are able to provide a picture by virtue of a single physical effect, namely, electron scattering (or diffraction).

Figure 1 indicates in a very schematic manner how a picture is formed in an electron microscope. Electrons passing through the specimen may be scattered (elastically or inelastically), and if the aperture is sufficiently small some of these electrons will be lost when they strike the aperture support. The absence of these electrons from the imaging system permits contrast to be obtained.

Fortunately, a small aperture is required in any case. The quality of even the best electron lens is rather poor, so a small aperture is essential. The value of this aperture is usually chosen to balance the effects of diffraction and spherical aberration. It is not often appreciated just how bad electron lenses really are. The very best lenses have a resolution of about 100 wavelengths. For example, at 100 kv the wavelength of electrons is about 0.03 Å, whereas the best obtainable resolution is about 3 Å. If we were to translate this situation to the optical range, we would not have any lenses

capable of resolving a single human hair.

From the point of view of successful microscopy, it is a most fortunate accident of nature that the requirements of contrast and resolution can be achieved with the same value for the lens aperture.

From a more general point of view, however, it may be said to be disadvantageous because it has produced a degree of complacency which the situation does not warrant.

As we have indicated, good resolution is just possible. By using heavy-atom staining excellent pictures can be obtained. However, the road to substantial improvement is fraught with difficulties.

The best lenses in use today operate at about  $f/1000$ . This value alone serves to indicate the major problem. If we had available to us an electron lens comparable to a good camera lens, say  $f/1$ , then it would be virtually useless because there would be no contrast in the resulting picture. Such a lens would have a resolution of the order of the electron wavelength, a small fraction of an angstrom, but, unfortunately, nothing would be visible.

This is perhaps the central enigma of electron microscopy. The greatest single need is for better lenses. However, if a very good lens were to appear tomorrow it could not be used to full advantage.

At first sight this situation has the appearance of a kind of uncertainty principle—the harder one looks the less one sees. Such a conclusion, however, would be erroneous because one of the necessary conditions for the existence of this state of affairs is the use of scattering processes to produce picture contrast.

If one agrees with these arguments, one is forced to conclude that any substantial improvement in electron microscopes requires two steps. First, one must abandon the scattering process as the contrast mechanism and instead use a mechanism that allows the use of better lenses. Second, one must develop better lenses.

In searching for a suitable alternative

The author is director of Argonne National Laboratory, Argonne, Illinois, and professor of physics at the University of Chicago and the Enrico Fermi Institute of Nuclear Studies, Chicago.

method for obtaining picture contrast, it is natural to explore the processes that can occur when an electron passes through a very thin specimen. These processes have been the object of numerous investigations (both experimental and theoretical) for several decades and are by now fairly well understood.

There are such things as the produc-

tion of ionization, production of x-rays, production of secondary electrons, emission of light, pair production (if the incident electron has high enough energy), bremsstrahlung, and loss of energy of the incident electron. There may also be many other useful phenomena.

Two of these phenomena have already been used in microscopy, name-

ly, x-ray and secondary-electron production. Commercial microprobe machines are available which utilize these effects (2).

X-ray microprobes are characterized by a contrast capability that is substantially infinite. That is, when the x-ray detector is adjusted to detect the characteristic radiation from some particular chemical element, a picture ele-

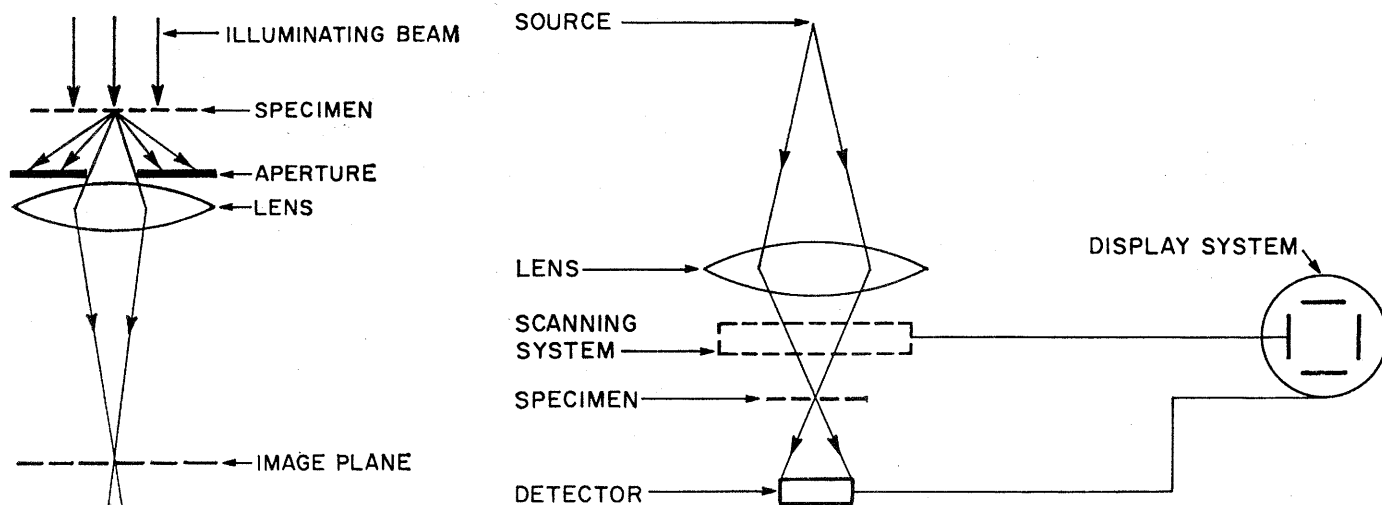


Fig. 1 (left). Operating principles of an electron microscope. Many scattered electrons are prevented from passing through the lens by the aperture support. The absence of these electrons at the image plane is the principal source of image contrast. In a practical microscope several lenses are used in order to provide the necessary magnification. Fig. 2 (right). Principles of operation of a scanning electron microscope. A focused spot of electrons is scanned across the specimen in the manner of a television raster. A synchronously scanned oscilloscope displays the output of a detector as variations in brightness.

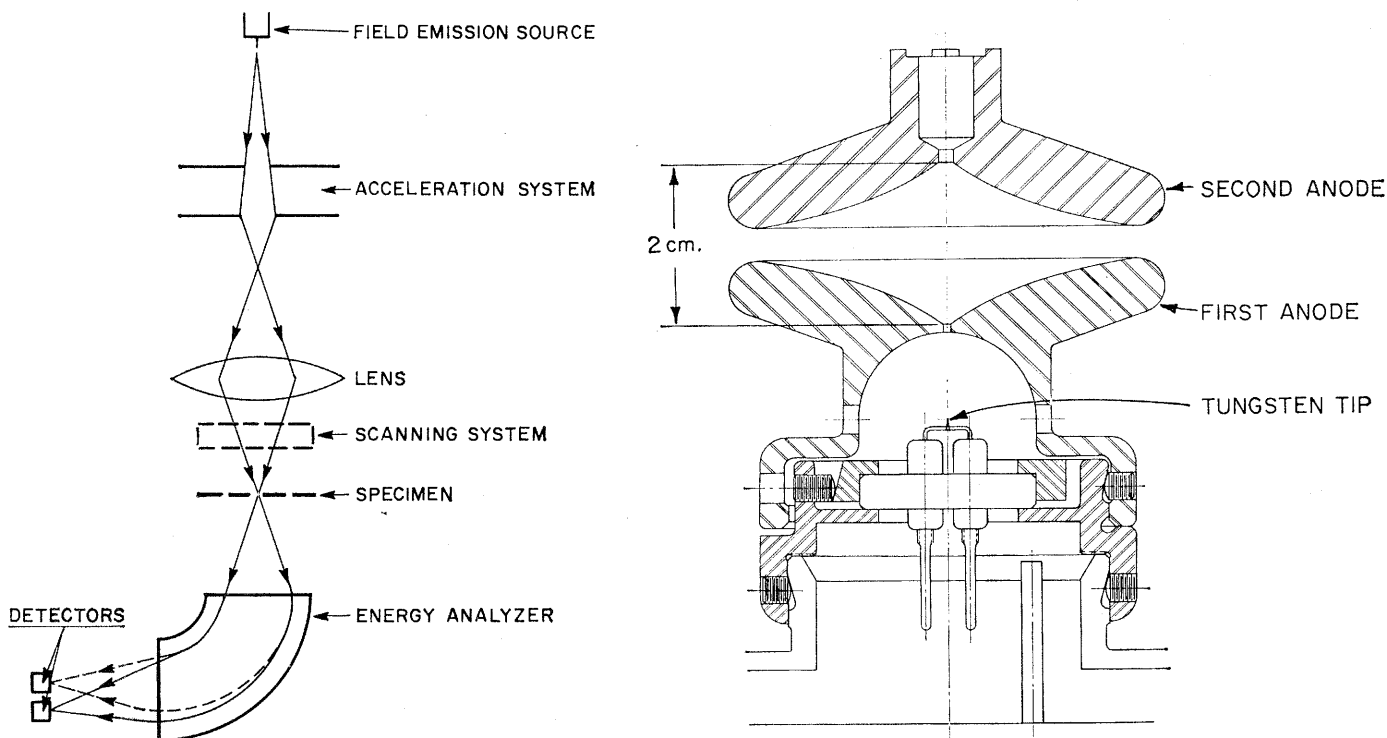


Fig. 3 (left). Scanning energy-loss microscope. Electrons from a cold field-emission tip are accelerated to normal microscope voltages and then focused onto a specimen with a single lens. An energy analyzer below the specimen allows the operator to select a particular energy of electrons for display on the output oscilloscope. Several detectors can be used simultaneously. Fig. 4 (right). Cross section of the electron gun. The gun is basically a triode. The voltage of the first anode defines the emission current from the field-emission tip; the voltage of the second anode defines the final energy of the electrons. The shape of the electrodes has been carefully chosen to reduce spherical aberration.

ment is white or black, depending on the presence or absence of that chemical element. Devices producing secondary emission are also capable of high contrast but do not have the powers of discrimination inherent in the x-ray microscope.

While it may be possible to develop these instruments further, they have some disadvantages at the moment. In particular, there are problems in obtaining adequate intensity, and the prospects for improving resolution appear limited.

In our survey of these effects it seemed that the most promising method for obtaining adequate contrast was by the use of energy-loss mechanisms. Many studies have been performed on mechanisms of energy loss (3). However, most of these studies have concentrated upon the effects in the medium involved or upon secondary effects. There is, however, a small body of knowledge on the behavior of the electron itself, and a few publications are concerned with electrons having energies in the range of interest (10 to 100 kv). The general picture is that there are several different types of energy loss that occur, depending on the particular material involved.

The most widely studied losses are the plasma losses that occur in metals. Electrons passing through thin metal foils can excite resonances whose excitation energy depends on the free electron density. Multiple losses are possible so that the energy distribution of the transmitted electrons consists of a number of equally spaced peaks with almost no background. The case of aluminum is very interesting because the peaks are almost 15 volts apart.

A nonmetallic specimen has less pronounced characteristics of energy loss. Many plastics, for example, show a broad energy-loss peak at around 20 volts. This peak appears to be of the same character as the plasma losses in metals because a thicker specimen can show a second, less distinct, peak at twice the energy loss. The case of polystyrene is of some interest because the existence of a benzene ring structure produces a pronounced energy-loss peak at 7 volts.

Another kind of energy loss that could be valuable in microscopy is the "characteristic" loss. This is caused by the excitation of x-rays in the material of the specimen. This generally shows sharp leading edges and a slower trailing edge as the electrons are ejected into the continuum.

In summary, then, the distribution of energy in a transmitted beam of electrons contains much information that is characteristic of the material of the specimen. Presumably this information can be used to produce picture contrast.

Intensity considerations should not be too severe because one can expect to be able to use *all* electrons that lose energy. The corresponding thing is not practicable at the moment with x-rays.

The use of such a contrast mechanism offers the intriguing possibility that heavy-atom staining of biological specimens can be dispensed with. Molecules with comparable densities and atomic composition are generally indistinguishable by the normal contrast mechanisms. However, if the detailed molecular structure produces different energy-loss characteristics, these characteristics may be separately visible. A good example of this is the pronounced 7-volt loss in materials with a benzene ring structure as opposed to a linear molecular chain.

Ability to eliminate heavy-atom and

other staining methods would increase our level of confidence in the accuracy of structural analysis at high resolution.

Most modern microscopes use cylindrical magnetic lenses. This type of lens has been studied extensively during the last 30 years or so. It is doubtful whether we can expect any substantial improvement in the future, although high-field superconducting lenses may have some advantages. The properties of these lenses have been calculated and measured, and it is now possible to design a lens for any desired performance (within the known limitations) and be confident that this performance will be achieved.

The fundamental limitations of this type of lens stem from two sources. The first one is the unfortunate fact that iron saturates when highly magnetized so that one cannot expect to achieve a magnetic field strength substantially greater than 30 kilogauss. The second problem area is that of machining tolerances. There is a limit to the precision with which pole pieces can be constructed, and this in turn limits the performance of the lens.

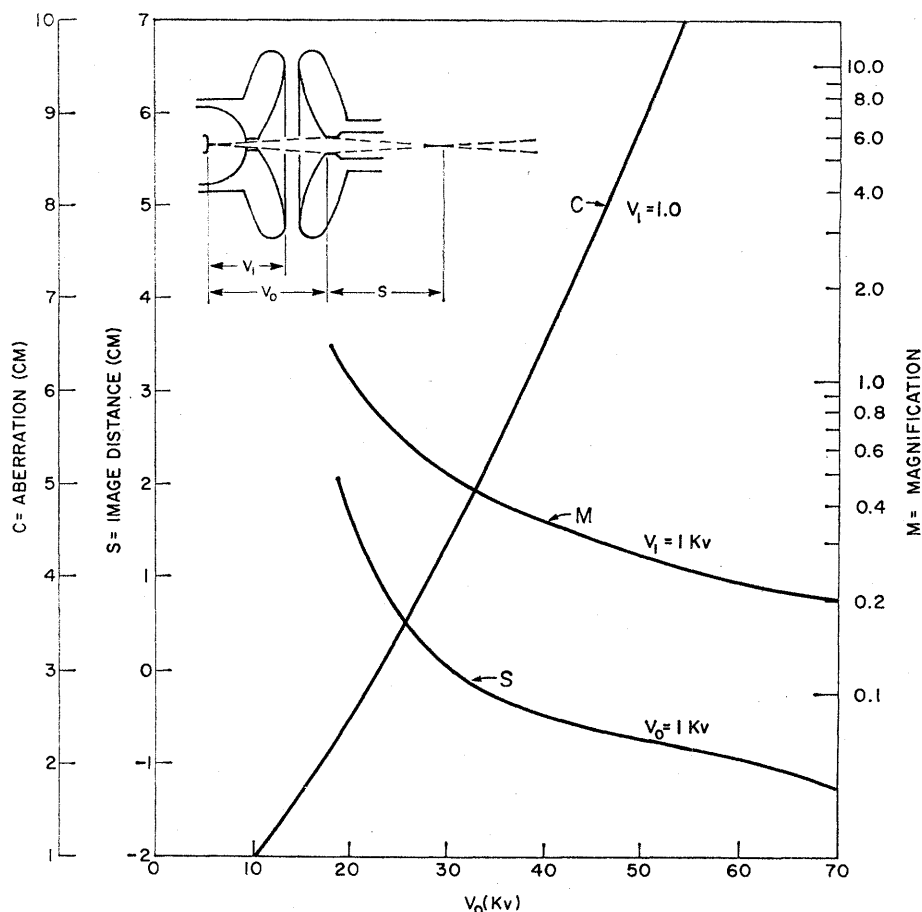


Fig. 5. The voltages applied to the two anodes define the first-order optical properties of the electron gun. The curves give the position of the image of the tip and its magnification as a function of second anode voltage for a first anode voltage of 1000. We also show the spherical aberration of the gun.

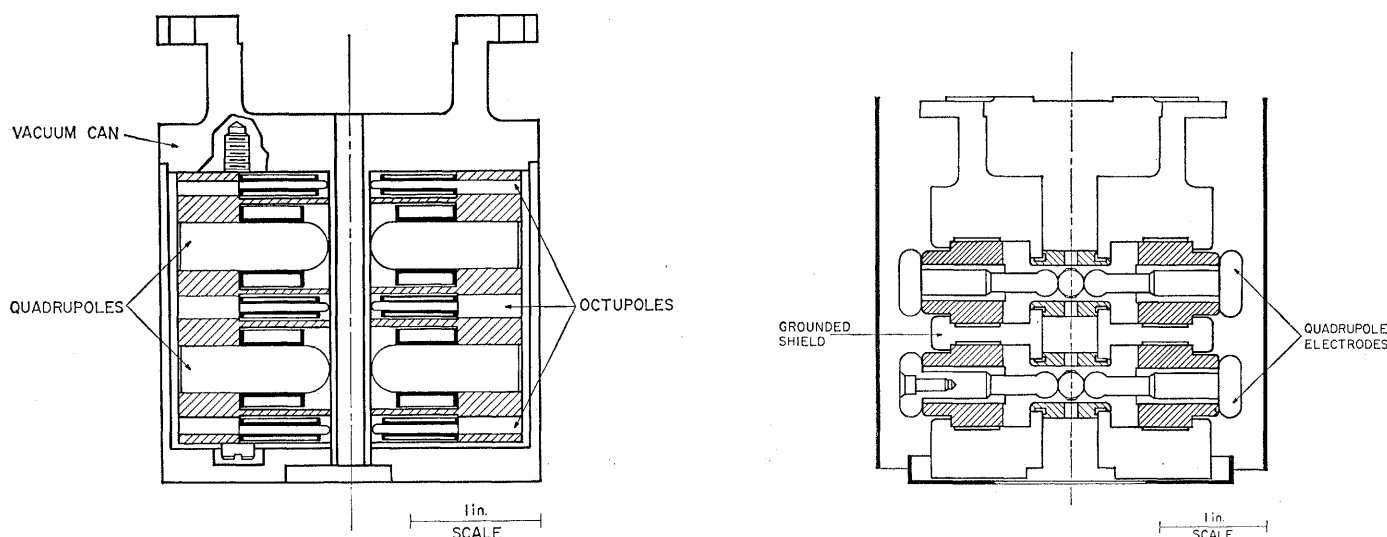


Fig. 6 (left). Cross section of the magnetic quadrupole lens. The two quadrupoles and three octupoles were encased in a metal can in order to improve the vacuum. The specimen was placed about 3 centimeters below the lens. Fig. 7 (right). Cross section of the electrostatic quadrupole lens. The dimensions of the system correspond closely to those of the magnetic quadrupole lens.

Electrostatic lenses have been used in some microscopes (4) but are not capable of a performance as high as that of magnetic lenses. It is not clear, however, that the potentials of this kind of lens have been adequately exploited. General statements in the literature indicate that electrostatic lenses have a spherical aberration coefficient about 100 times greater than magnetic lenses. This is a significant source of discouragement. Such lenses, however, are capable of being analyzed with the aid of modern computers. It appears that there has been no serious attempt to optimize the design of such lenses.

Quadrupole lenses have been somewhat neglected for microscope applications even though they are extensively used in high-energy physics (5). In general, a quadrupole lens system will produce an asymmetrical image of a source. Or rather, any such image will have two axes of symmetry but will not be radially symmetric. This is probably the reason these lenses have not been extensively used, because a real image of a specimen would be severely distorted. On the other hand, quadrupole symmetry presents a possible means for correcting aperture aberrations by the use of an octupole correction system (6). There are indications that it may be possible to design a quadrupole-octupole system with small aperture aberrations—perhaps small enough to compete favorably with cylindrical magnetic lenses.

Quadrupole systems can be either magnetic or electrostatic, although there are slight differences between the two. They have the added advantage

that the necessary field strengths are low and the axial distribution of the field could, in principle, be tailored for minimum aberrations.

It would appear at the moment that one should design a microscope that is capable of using quadrupole lenses in addition to conventional cylindrical lenses.

#### Design Considerations for a Microscope

If we are to design a microscope that is capable of using such phenomena as energy loss to provide contrast and unconventional lenses such as quadrupoles for resolving power, it is clear that the traditional design of electron microscopes must be abandoned. One is forced to conclude that the critical electron optics must operate on the electron beam *before* it strikes the specimen. The best way of accomplishing this objective is to use the principle of the scanning microscope.

The history of the scanning electron microscope can be traced back to about 1935, to an instrument built by Knoll (7); an excellent review of the field has been published by Oatley, Nixon, and Pease (8).

Many scanning microscopes are already in existence (9). Basically they consist of a source of electrons, an acceleration system, and a lens that images the source in the plane of the specimen. A scanning system scans the focused spot across the specimen in the manner of a television raster. A detector measures the quantity of interest (such as x-ray production), and

its output is applied to the intensity-control grid of a synchronously scanned television display tube (see Fig. 2).

The resolution of such a microscope depends entirely upon the size of the image of the source at the specimen (for a thin specimen). Contrast depends upon the detection system. It is clear that the lens system is only required to have good optical properties along the axis (except for the scanning action) and that the use of a quadrupole system would be possible because asymmetry in the focused spot does not affect the entire picture. It would mean, at the worst, that the resolution would be different in two orthogonal planes.

The utilization of energy loss (or any other phenomenon) for contrast is not very difficult because there is no need for any critical electron-optical system in the transmitted beam. The first scanning microscope to use energy loss for contrast mechanism was built by Hillier and Baker (10).

#### The Electron Source

Existing scanning microscopes are severely limited by source problems. The use of a hot filament is a common technique, but the brightness is strictly limited. It is not possible to demagnify such a source indefinitely in any usable fashion because the number of electrons in the focused spot decreases as the demagnification is increased. The optimum operating point corresponds to a usable resolution of about 100 Å (11). Further demagnification results in a loss of picture information that can

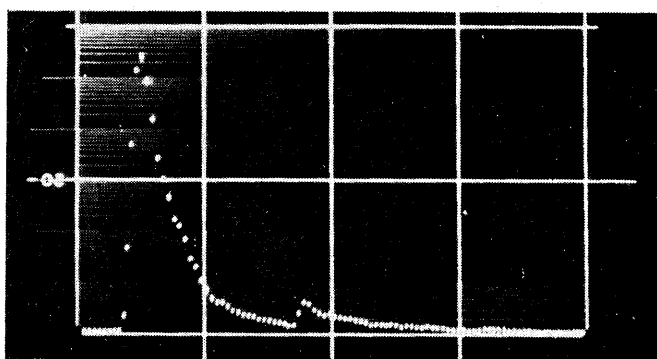
be compensated for only by increased scanning time. Increasing this time makes it difficult to focus the instrument and creates problems of stability.

Therefore we must either be content with such resolution or use a brighter source. Fortunately, such a source exists, although it carries with it other engineering problems. Field emission

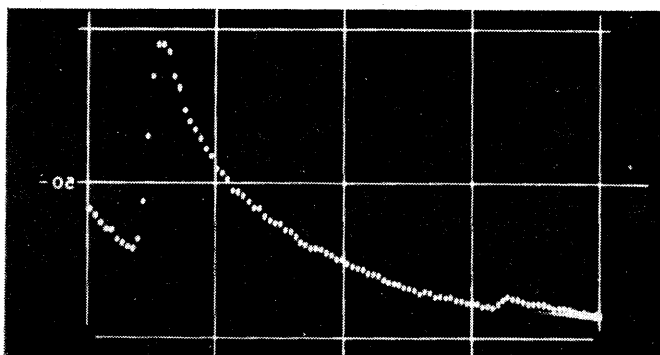
provides the new source. It has been known for some time; in particular, field emission from a small tungsten tip has been studied extensively (12).

When a negative potential is applied to a small tungsten tip with a rounded end, electrons are emitted. It is relatively easy to extract a substantial current with applied potentials of the order of

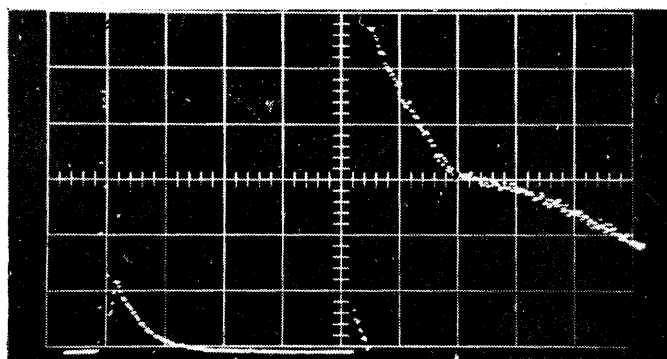
a few kilovolts. Steady emission of many microamperes can be obtained for periods of several hours if the ambient pressure is in the neighborhood of  $10^{-9}$  millimeter of mercury. The electrons from such a tip have the added advantage of appearing to emanate from a small region, possibly as small as 30 Å in diameter. This



(a) Complete Spectrum

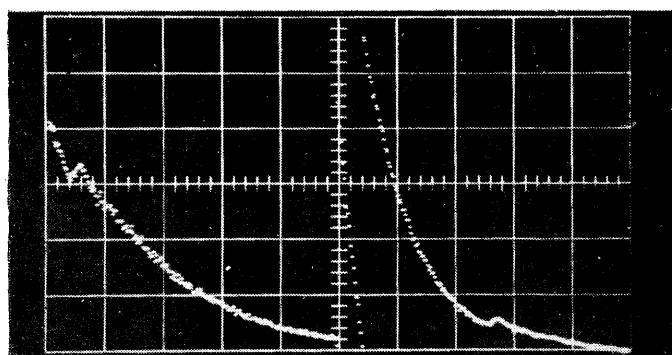


(b) Carbon and Oxygen Edges



(a) Complete Spectrum

(b) Fluorine Edge



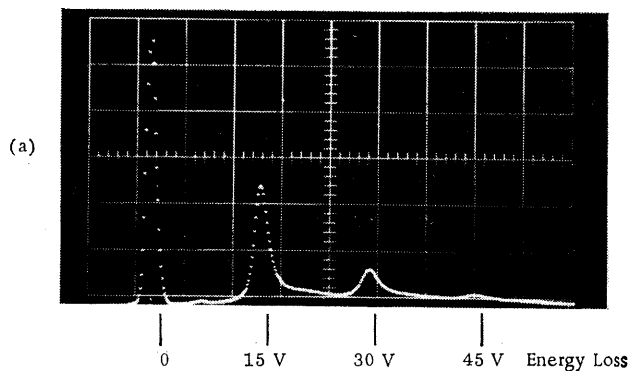
(c) Carbon

(d) Carbon

Fig. 8 (above). Energy-loss spectrum of collodion. The top curve shows the complete spectrum. The spectrum consists of a broad maximum with a pronounced "characteristic" loss at about 290 volts. This corresponds to the carbon k-absorption edge. The lower curve shows this carbon edge in more detail and also indicates another edge—this one corresponding to the oxygen k-line.

Fig. 9 (top right). The curve at the top left (a) shows the complete energy-loss spectrum of Teflon. The curve is uninteresting except for the change in slope which corresponds to the fluorine line. This can be seen easily in the enlargement of the tail of the spectrum which is given at the top right (b). The curves at the bottom show the carbon peak which occurs at 290 volts.

Fig. 10 (bottom right). Energy-loss spectrum of aluminum. These curves were taken by sweeping the electrons repeatedly across the detector slit of the analyzer and storing the information in a multichannel analyzer. The top curve shows the complete spectrum. The left-hand peak corresponds to electrons which have lost no energy. "Plasma" peaks can be seen at 15, 30, and 45 volts. The lower curve shows the detail that can be obtained by sweeping over a smaller energy interval. Here the first two large peaks are shown with a smaller peak (at 7 volts) between them.



(a) Complete Spectrum

(b) Fluorine Edge

(c) Carbon

(d) Carbon

(e) Carbon

(f) Carbon

(g) Carbon

(h) Carbon

(i) Carbon

(j) Carbon

(k) Carbon

(l) Carbon

(m) Carbon

(n) Carbon

(o) Carbon

(p) Carbon

(q) Carbon

(r) Carbon

(s) Carbon

(t) Carbon

(u) Carbon

(v) Carbon

(w) Carbon

(x) Carbon

(y) Carbon

(z) Carbon

source is therefore very much brighter than a hot filament. Furthermore, the demagnification that is required to reduce the size of the focused spot to usable dimensions is much less. Both these factors indicate that adequate intensity is available from a field-emission source.

The major engineering problem associated with use of such sources is the need for ultrahigh vacuum. Fortunately, equipment is readily available to reach pressures in the neighborhood of  $10^{-9}$  mm-Hg and if such a high vacuum is provided throughout the microscope there are other incidental benefits. Specimen contamination is normally a serious problem in high-resolution work and is apparently caused by decomposition of residual gases. Eliminating hydrocarbons substantially reduces the contamination rate, as does lowering the ambient pressure.

### Description of the Microscope

I now describe a microscope that has been designed according to these principles. This microscope was designed to be as flexible as possible so that the various aspects of the new approach could be studied independently. Experimental studies of the system have progressed to the point where it now appears possible to proceed with the design of a high-resolution instrument. The microscope is illustrated in Fig. 3. It consists of a field-emission source, an acceleration system, a focusing lens, a scanning system, and an energy analyzer. The whole microscope is maintained at a pressure of  $10^{-9}$  mm-Hg or better.

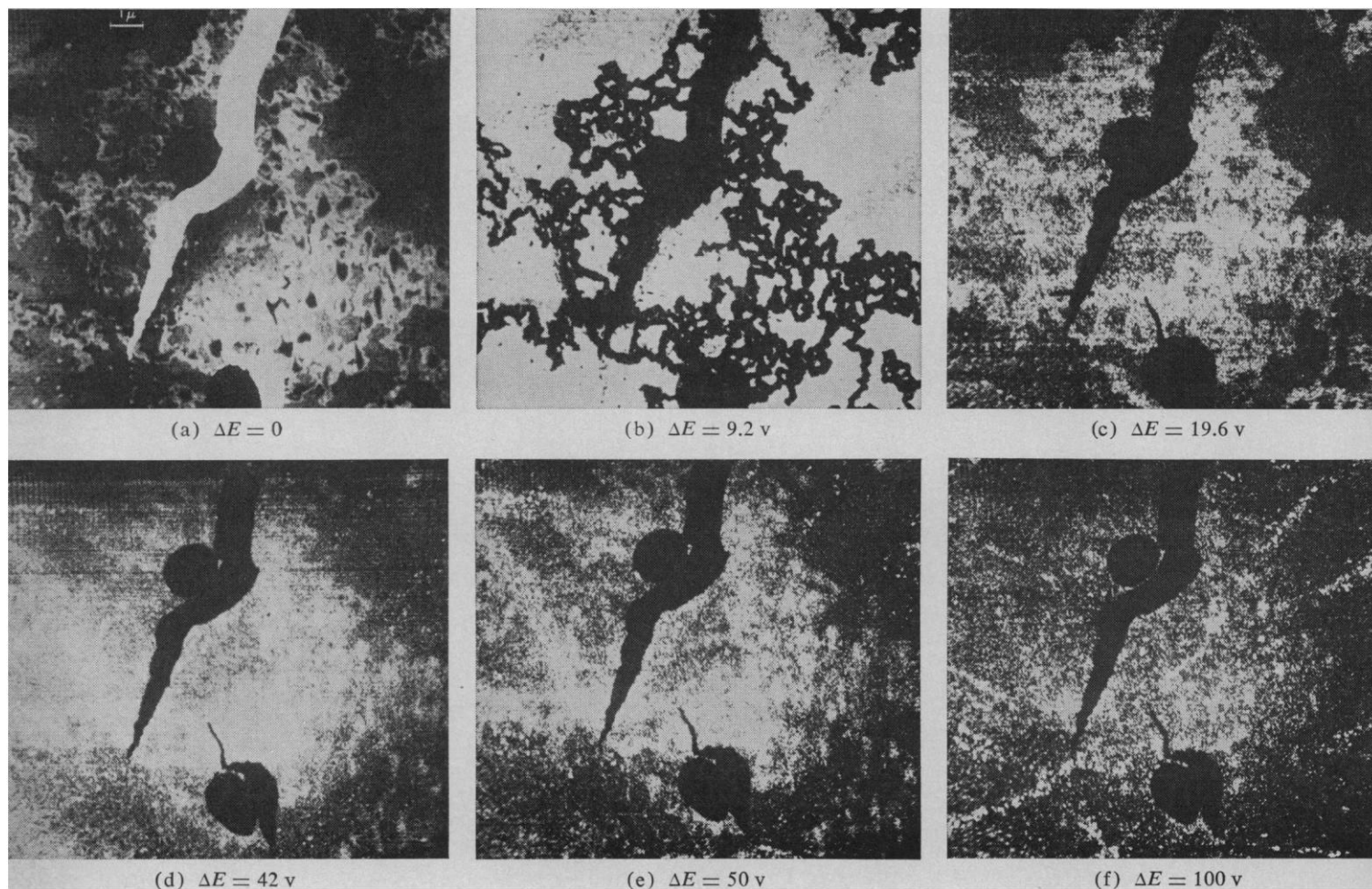
Very few of the parts of the microscope are conventional. Electrons are detected by a high-speed scintillator-photomultiplier system. Two such channels are provided. Each scintillator is

provided with a slit system that can be adjusted for optimum energy resolution. One of the slits can be physically moved to adjust the energy spacing between the two detectors. The scintillators are, of course, in the high-vacuum system. The light output is fed through quartz light-pipes to the photomultipliers, which are kept at atmospheric pressure.

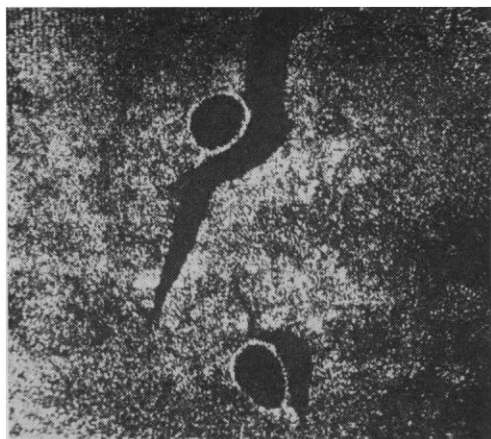
Details of the display system have already been published (13). The system is most interesting, perhaps because it uses a memory display tube. Usually a long-persistence phosphor is used for this kind of application, but we felt that operation of the microscope would be simpler with a display that did not fade, except upon demand. This system has been of enormous value and has a considerable advantage over previous methods.

The present system is a duplex one

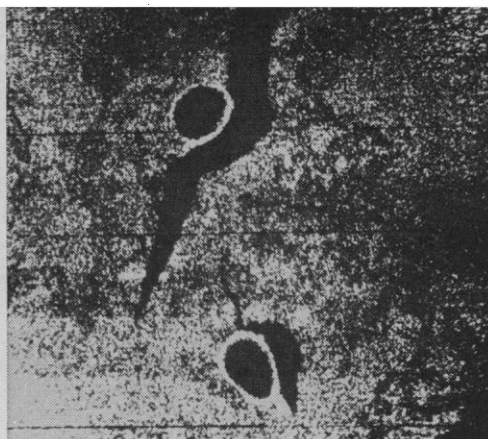
Fig. 11. Sequence of micrographs of a thin aluminum specimen taken with various energy losses ( $\Delta E$ ) from 0 (Fig. 11a) to 700 volts (Fig. 11r). Figure 11a corresponds to what would normally be visible in a conventional microscope. A large crack can be seen in the aluminum, and there is much contamination. The changes in the succeeding exposures indicate that there is a substantial amount of information in the transmitted beam of electrons—information which is not normally accessible. Particular features can be followed through the sequence as they change from white to black (or black to white) and appear or disappear. The two dense particles on the surface are particularly noticeable as they become more and more transparent. The pictures were taken with 23 kv electrons. The scan time is 10 seconds for most of the pictures. The intensity is so low in Fig. 11r that the picture is composed of white dots produced by single electrons.



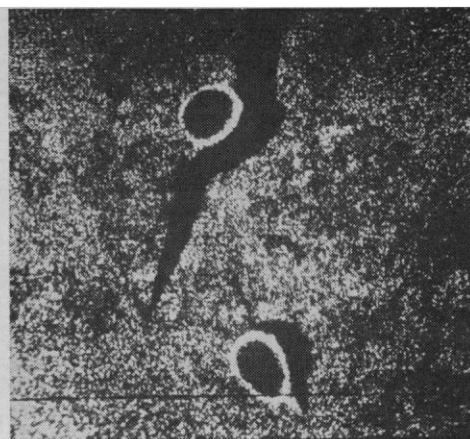




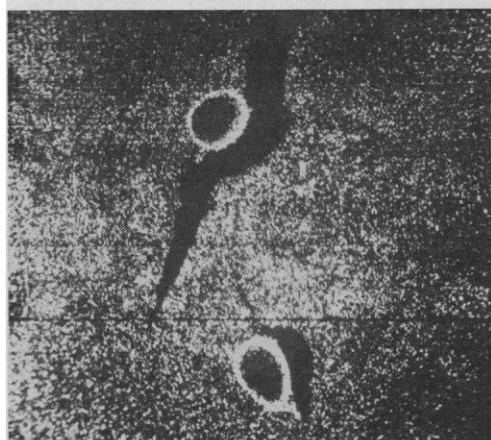
(g)  $\Delta E = 150$  v



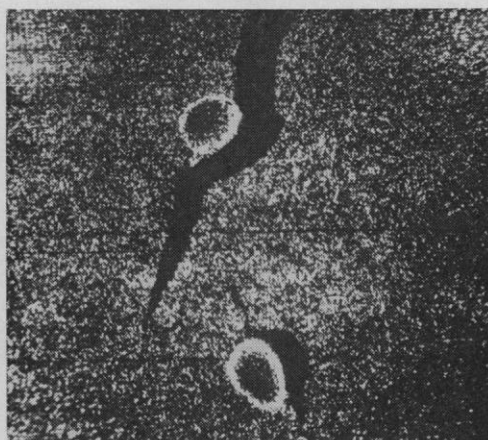
(h)  $\Delta E = 200$  v



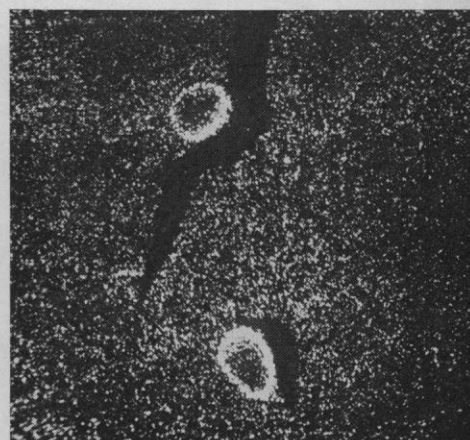
(i)  $\Delta E = 250$  v



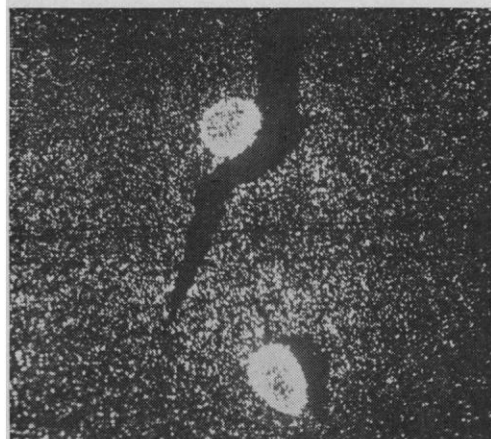
(j)  $\Delta E = 300$  v



(k)  $\Delta E = 350$  v



(l)  $\Delta E = 400$  v



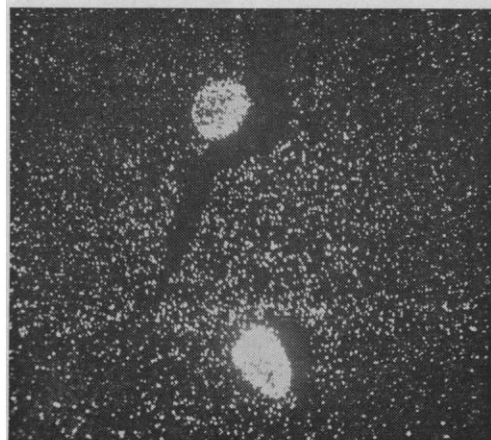
(m)  $\Delta E = 450$  v



(n)  $\Delta E = 500$  v



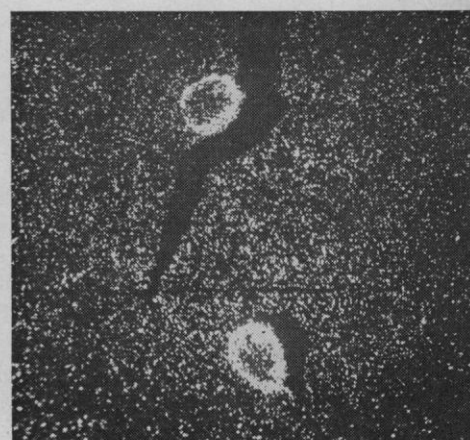
(o)  $\Delta E = 550$  v



(p)  $\Delta E = 600$  v



(q)  $\Delta E = 650$  v



(r)  $\Delta E = 700$  v

—that is, there are two detecting systems with independent displays. It allows simultaneous presentation of two pictures taken with different energy losses.

The electron gun is shown in Fig. 4. It consists of three electrodes: the field-emission tip itself, a first anode that controls the current emitted by the tip, and a second anode that controls the voltage of the final electron beam.

The tip itself needs little explanation, for there are many papers on the use of such tips. It may be sufficient to say that the application of about 1200 volts will generally produce a steady current of a few tenths of a microampere if the end of the tip has been previously rounded. Raising this voltage to, say, 3000 can produce as much as 1 milliamper. In the microscope itself we generally operate at a few tenths of a microampere, but in a test assembly we have run at 1 milliamper for as long as 170 hours. After an operating time of a few hours or so it is generally necessary to clean the tip by heating it briefly. The lifetime of such tips appears to be almost infinite.

The first anode has a hemispherical front surface in order to preserve the quality of the beam coming from the tip, and the shape of the other electrodes in the accelerating section was chosen to minimize the spherical aberration of the gun (14).

First- and third-order focal properties of the gun are shown in Fig. 5 for typical operating conditions. These curves are theoretical ones (see 14). Experimental data taken with this gun have shown no appreciable deviation from the calculated values, although these studies are not complete.

The operating currents mentioned previously are the total tip currents. The output beam current produced by the gun is smaller than this by a factor of about 1000. No attempt has been made to maximize this current because, even at total emission of a few tenths of a microampere, there is more than enough beam current to provide a good picture.

So far we have used three different lens systems, namely, magnetic quadrupoles, electrostatic quadrupoles, and a conventional cylindrical lens. The magnetic quadrupoles are shown in Fig. 6. There are two separate quadrupoles forming a simple doublet. If the excitation of the two sections of the doublet are allowed to be different, the system can produce a real image of a real ob-

ject and therefore both object and image can be outside the lens. The magnification of the lens in the two perpendicular planes of symmetry is different. As a result, we expect that in general the focused spot will be roughly elliptical and need not be circular even in the absence of lens aberrations.

At the time the lens was designed, the aperture aberrations were unknown but were expected to be of the same order as those of a cylindrical lens with similar focal length.

The magnetic field required in the lenses is small, of the order of 100 gauss, so that the power supplied to the lens is also small, about 0.1 watt.

Apertures are included in the system between the electron gun and the lens. They are in the form of two slits at right angles, oriented in the two planes of imaging symmetry of the quadrupole system. Several different slit sizes are available.

Extensive studies of the performance of the quadrupoles were made as a function of all readily available parameters. The best performance obtained corresponded to a spot size of about 200 by 500 Å.

Subsequent calculations by computer code (15) indicate that the resolution should have been 50 by 150 Å. The discrepancy has been shown to be due, in large measure, to astigmatism in the gun and perhaps to misalignment of the quadrupoles. The demonstrable experimental result of these effects was a coupling between the two imaging planes. In other words, changing the aperture in the *y*-direction caused a change of resolution in the *x*-direction and vice versa. We feel confident that these problems can be corrected and that the theoretical performance can be obtained. Such a performance is not impressive on an absolute scale, but if one bears in mind that the distance of the specimen from the lens is 3 centimeters it will be recognized that the performance of the quadrupole system probably exceeds that of a conventional lens.

Electrostatic quadrupoles were designed to be geometrically very similar to the magnetic quadrupoles. They are shown in Fig. 7. Operating voltages were around 4000 volts, and performance was comparable to that of magnetic quadrupoles.

This similarity of performance seems to demonstrate several encouraging conclusions. In the first place, the conclusion that the discrepancy between

experimental and theoretical resolution is caused by something external to the lens is strengthened. Second, the problem of machining errors in the construction of quadrupoles does not seem to be severe—the two different lenses have substantially the same performance, but the machining errors were presumably different. Finally, in spite of the low operating field of the quadrupoles, residual fields have a very small effect.

The use of a computer by Meads and Cohen to study this problem has proved to be of enormous value. Our experimental investigation of quadrupole systems occupied our attention for about a year. The computer provides the same answer in a few seconds. We have therefore suspended further investigations of quadrupole systems until an optimum design emerges from the computer studies.

Conventional microscope lenses have one decided advantage. Thirty years or so of experimental and theoretical work have resulted in lenses having calculable properties. It is therefore of great interest to use a conventional lens in this microscope and attempt to calculate its behavior under a wide range of conditions.

The lens we have used is an RCA intermediate lens. It was modified only to make it suitable for use in an ultra-high-vacuum system. Modification was accomplished simply by fitting the lens with an outside sleeve welded to the top and bottom plates. In addition, the pole piece was carefully machined to the highest standards of our machine shop in a successful attempt to reduce astigmatism.

With the specimen inserted in the lens (*f* about 7 mm) the best resolution obtained was about 50 Å. The calculated best resolution under the same conditions is essentially the same value.

In addition, the expected resolution has been calculated as a function of the operating parameters of the electron gun—in particular, the position of the intermediate image of the tip. Included in the calculation are apparent tip size (30 Å), gun magnification, spherical aberration of the gun, spherical aberration of the lens, and diffraction. All these terms are established theoretically and at some point in the range all have a significant effect. The experimental values for resolution agree very well with the theoretical values—within a factor of two or so.

As a result of all our measurements we are now confident that the essen-



tial parameters of the system are known. In particular, we believe that if a high-quality objective lens were to be substituted for the present lens, the microscope should have a theoretical resolution identical with that of a conventional microscope using the same objective lens.

Energy of the transmitted beam is analyzed by means of an electrostatic spectrometer. The two electrodes consist of metallized quartz pieces accurately ground to a spherical surface. The two pieces together represent a pie-shaped section of a spherical annulus.

With the addition of a single quadrupole between the spectrometer and the detector, energy resolutions of about 0.8 volt are readily obtained. The function of the quadrupole is to move the position of the focal point of the spectrometer until it coincides with the slit system of the detector.

With this system it is possible to take pictures of a specimen using electrons which have lost any (or no) energy at intervals as small as 1 volt. Of course, the quality of the picture decreases as the energy loss increases because the number of such electrons decreases. However, pictures have been obtained with energy losses up to 900 volts.

The spectrometer can also serve another purpose, and that is to measure the energy-loss spectrum. If a ramp voltage with a "saw-tooth" waveform is placed on the spectrometer electrodes, the energy-loss spectrum is swept repeatedly across the detector slit. This can be done with the picture scanning voltage removed so that the focused spot of electrons rests on a chosen point in the specimen. If the output of the detector is observed on an oscilloscope whose sweep uses the same waveform, the energy-loss spectrum can be displayed on the oscilloscope. An even more successful method is to accumulate this information in a multichannel pulse-height analyzer, reserving one channel for each energy.

A few examples of the use of this technique are shown in Figs. 8, 9, and 10. The energy-loss spectrum of colloidal (Fig. 8) shows a typical broad peak at about 20 volts, with no distinguishing features. However, careful inspection of the curve in the several-hundred-volt region shows the "characteristic" losses corresponding to the carbon and oxygen x-ray excitation energies.

Figure 9 shows an energy-loss spectrum taken from a Teflon specimen.

While the characteristic losses are less distinct here, the carbon line can be clearly seen, and there is a distinct change of slope at a position corresponding to the fluorine line. Figure 10 shows the energy-loss spectrum of aluminum. The plasma losses are clearly visible. The spacing between the peaks is 15 volts.

The amount of material used in these spectra can be estimated readily. The specimens are thought to be 100 to 200 Å thick. With a spot, say, 200 Å in diameter the volume of material is about  $6 \times 10^{-18}$  cubic centimeter, or, say,  $10^{-17}$  gram. There would not appear to be any difficulty in reducing this number if better resolution can be obtained.

These spectra demonstrate that there is a considerable amount of information in the transmitted beam of electrons—information which can be used for analytical purposes or simply to obtain contrast in a picture.

Figure 11 is a graphic example of the value of using energy-loss information for picture contrast. It shows a sequence of exposures taken at various values of energy loss. The specimen was aluminum. Such foils are prepared by evaporation onto a crystal of sodium chloride. The salt can be dissolved in water and the aluminum can be picked up on a specimen grid. Figure 11a shows a portion of the specimen using electrons which have lost no energy ( $\Delta E = 0$ ). It shows a basic aluminum substrate with a substantial crack, numerous holes, and much contamination. With  $\Delta E = 9.2$  volts (Fig. 11b) the picture has completely changed. The crack in the foil is black, the aluminum is white, and the filamentary nature of the contamination is clearly visible. At  $\Delta E = 19.6$  volts (Fig. 11c) the contamination is barely visible, although the resolution has not deteriorated if we judge by the sharpness of the edges of the crack.

Figure 11e ( $\Delta E = 50$  volts) shows that there are two egg-shaped particles on the surface. These particles are so thick that electrons which lose only 50 volts do not emerge. Figure 11f ( $\Delta E = 100$  volts) is interesting because of the appearance of structure which was previously invisible. The cause of these white lines is unknown. In Fig. 11g ( $\Delta E = 150$  volts) the two thick particles exhibit haloes. Presumably they do so because some electrons can pass through the edges of the particles and lose 150 volts.

The remainder of the sequence

(Fig. 11, h-r) confirms this interpretation, for the particles become more and more "transparent" until they appear to be self-luminous.

The change of intensity between the first and last pictures in the sequence is considerable—a factor of  $10^6$  or more. The final picture is composed of single-electron counts; each white dot corresponds to one electron.

It should be emphasized that there is no change in the specimen during this sequence. Returning to the conditions of Fig. 11a will produce the same picture. The contamination rate is so low that while working on resolution problems we used the same area of one aluminum specimen for 14 months with only barely visible contamination at the end of that time.

## Conclusion

Experiments with this scanning microscope have produced extremely encouraging results so that we feel confident in predicting high resolution and high contrast after some obvious modifications are made in the system, such as providing a good objective lens.

Experience with conventional lenses indicates that the instrument behaves in a predictable manner and there is no reason to doubt that the resolution can be as good as that of a conventional microscope.

The use of quadrupole lenses will depend on calculations now being performed. There is cause for optimism; high resolution may also be possible with this kind of lens.

Experience with field emission shows that the technology is not difficult and that there is more than enough current available for any conceivable use.

Energy-loss measurements have been made on a variety of materials. It is attractive to consider the possibility of chemical analysis of selected areas of a specimen. We believe that a very crude form of analysis may indeed be possible. The principal advantage of the use of energy-loss techniques, however, may be in the availability of another contrast mechanism. The ability to "see" small details may be considerably enhanced.

Finally, we are experimenting with the possibility of using transmitted electrons of different energy losses to produce different colors on a color television display. This should add an extra element to the picture contrast which may be of some value.

## References and Notes

1. E. Ruska, *J. Roy. Microscop. Soc.* **84**, 77 (1965).
2. R. Castaing, *Adv. Elect.* **13**, 317 (1960); V. E. Cosslett and W. C. Nixon, *X-Ray Microscopy* (Cambridge Univ. Press, London 1960).
3. G. Ruthemann, *Ann. Phys.* **2**, 113 (1948); D. Bohm and O. Pines, *Phys. Rev.* **72**, 609 (1953).
4. P. Grivet and G. Regenstreif, paper given at Int. Cong. Microbiol., Paris, 1950.
5. A. Septier, *Compt. Rend.* **243**, 132 (1956).
6. ———, *Adv. Elect.* **14**, 85 (1961).
7. C. W. Oatley, W. C. Nixon, R. F. W. Pease, *ibid.* **21**, 183 (1965).
8. ———, *ibid.*, p. 181.
9. J. Hillier and R. F. Baker, *J. Appl. Phys.* **15**, 664 (1944).
10. K. C. A. Smith, paper given at Int. Congr. Microbiol., Delft, 1960.
11. R. Gomer, *Field Emission and Field Ionization* (Oxford Univ. Press, New York, 1961).
12. W. K. Brookshier and J. Gilroy, *IEEE Trans. Nuc. Sci.* (Apr. 1965).
13. J. W. Butler, in preparation.
14. P. Meads, *Univ. Calif. Radiation Lab. Rept. UCRL 10807*; ——— and D. Cohen, private communication.
15. This work could never have been accomplished without the support of our Electronics Division under the leadership of J. Gilroy. In particular, I am indebted to D. E. Eggenberger, L. Welter, J. Wall, and R. Lill. Numerous others have given invaluable advice, but we all owe much to the skill of our Central Shops. Work performed under the auspices of the AEC.

# Visual and Nonvisual Auditory Systems in Mammals

Anatomical evidence indicates two kinds of auditory pathways and suggests two kinds of hearing in mammals.

J. M. Harrison and R. Irving

It is generally assumed by those interested in the study of the anatomy and physiology of mammalian hearing that the auditory system is essentially the same in all mammals (1). In work on the comparative anatomy of the auditory system of the brain stem (2) it has been shown that the various structures which comprise the system vary in size relative to other parts of the brain, but the underlying assumption appears to be that all the various structures are present in all mammals. For example, the auditory system is very large in many bats (3) and relatively small in many primates (4), but in both groups of animals it is assumed to consist of the same components. In this article we are concerned with variations in the size of the components of the auditory system of the brain stem in mammals and with evaluation of the idea that not all components of the auditory system are present in all mammalian species.

Anatomical work has shown that the cochlear nucleus (in cat and rat) contains groups of different classes of nerve cells and that the nerve fibers which arise from each of these cell groups terminate in different nuclei of the superior olivary complex (5, 6). That is, above the level of the cochlear nu-

cleus the auditory system consists of a number of separate pathways rather than a single pathway. One implication of this finding is that one or more of the pathways may be associated with behaviorally distinct aspects of hearing. This can be illustrated by a well-known analogous problem in vision. The retinas of many mammalian species contain primary light receptors which can be divided, on anatomical grounds, into two classes, rods and cones. Schultze was the first to note (7, 8) that the rods and cones are distributed differently in the retinas of different animals. He investigated the relation between the distribution of rods and cones in the retina and the nocturnal and diurnal habits of a large number of vertebrates and came to the conclusion that there were two kinds of vision rather than one. The idea of two kinds of vision was later elaborated by Ramon y Cajal's finding (9) that there were different kinds of bipolar cells associated with the rods and cones—in other words, that there were at least two visual pathways as well as two classes of receptors. It is now well established that there are two major classes of vision, scotopic and photopic. While most mammals have retinas containing both rods and cones and possess both photopic and scotopic vision, some have retinas containing only rods (many species of bats, hedgehogs, some pri-

mates, many rodents), while others have retinas containing only cones (many species of squirrels) (10).

This method of correlating structure with broad behavioral characteristics may be adopted in looking for particular behavioral characteristics of the several auditory pathways. One may examine the relation between the presence or absence (or changes in relative sizes) of any of these pathways and some aspect of the animal's normal behavioral environment (11). To this end we have examined the nuclei of the superior olivary complex in a number of mammalian species and have correlated variations in the relative sizes of the nuclei in different mammals with the behavior of the animal. The results of these observations are presented here.

## Superior Olivary Complex

The nuclei of the superior olivary complex receive nerve fibers from the cochlear nucleus, and the fibers which arise in the complex pass to higher levels in the auditory system. The nuclei of the superior olivary complex with which we are concerned may be seen in Fig. 1, a cross section of the medulla of the chinchilla. The material was prepared as follows. Each animal was anesthetized with nembutal and perfused through the aorta with normal saline followed by chilled fixing fluid containing alcohol, formalin, and acetic acid. After a further period of fixation the brain was dissected out of the skull and cut into serial sections 16 microns thick. Every other section was mounted and impregnated by the protargol silver method of Bodian (12).

The comparative study of groups of nerve cells (nuclei) of the nervous system imposes certain restrictions upon the way these nuclei can be defined, and in the interests of clarity it is necessary to make these restrictions explicit. The nuclei shown in Fig. 1 have names—lateral superior olivary nucleus and medial superior olivary

Mr. Harrison is professor of psychology and biology at Boston University, Boston, Massachusetts; Mr. Irving is a graduate student and research assistant in the Psychological Laboratory, Boston University.