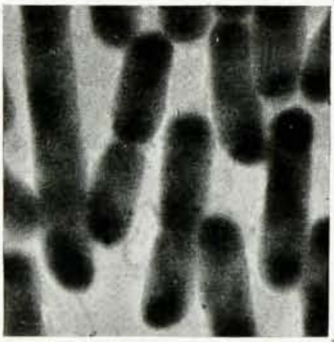
Asbestos (23,000 ×)

NEW WORLDS FOR SIGHT

Smoke particles (36,800 ×)



Bacteria (9,000 ×)

Magnesium Alloy (3,100 ×)

Gold Particles (91,000 ×)

Most of the electron micrographs used to illustrate this article have been made in collaboration with workers from other laboratories. It is through the kind permission of the following individuals and organizations that these micrographs are reproduced here: Stuart Mudd, University of Pennsylvania; C. H. Gerould, Dow Chemical Company; J. Turkevich, Princeton University; H. B. Woodruff, Merck & Co.; R. F. Baker, RCA Laboratories; Georges Knaysi, Cornell University.

by James Hillier

Several decades ago no one ever expected to see molecules, but electron microscopists are reaching down to expose them to the sight of man. A pioneer in this field tells how this new and rapidly improving instrument works, and what its potentialities are.

There is a scientific device that will measure the intensity, the wave-length, and the position of all of four or five million spots of light and do that every tenth of a second or less for many hours a day. This superb tool—a pair of eyes—also retains in itself a record of the measurements it has made to be recalled at any time for comparison with new measurements.

Much of science is seeing to sort, chart, compare, and understand, and the microscope, by extending the range of vision, has played a prominent part in scientific research. But the light microscope cannot produce accurate images of objects which are much smaller in length or breadth than the wave-length of the light used. For the ordinary microscope, using visible light, this sets a lower limit to the dimensions of observable objects in the neighborhood of two ten-thousandths of a millimeter. This limitation has prevailed for the past seventy years.

Meanwhile, research has been extended to the study of molecules, atoms, and even the smaller particles that constitute the atomic nucleus. But this has been done with simplified or purified systems in which all of the particles in the system are alike. It is then possible to study various properties of those particles by the statistical methods of chemistry or physics from which quite accurate measurements and deductions are made.

When the atoms and molecules are built into the larger solid structures that constitute our earth and all of the natural or manufactured objects on it, we find that the systems are rarely simple and pure. As a result, the indirect statistical methods of chemistry and physics cannot be used. Thus, unless there was some very special regularity such as in large crystals or in some parts of living organisms, the natural structures in the all-important range of dimensions between the molecules and the limit of the light microscope could be studied by no scientific method.

In the last ten years the scientist's sense of vision has been extended rather rapidly to cover this range.

Actinophage (115,000 ×)

Magnesium Alloy (4,160 ×)

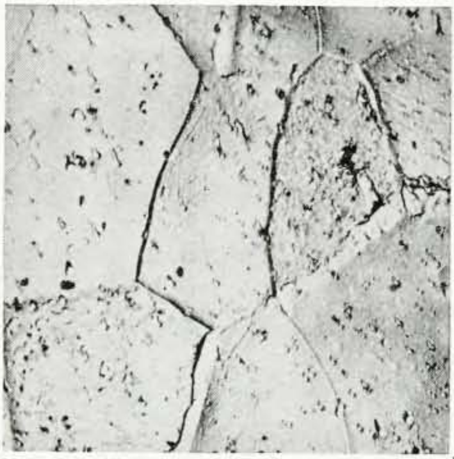
Diatom (6,000 ×)

The new instrument that makes this possible is the electron microscope.

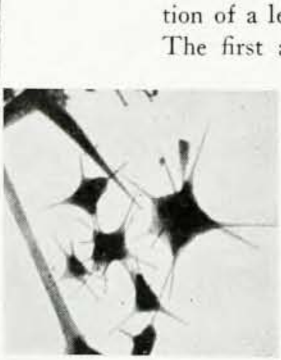
Finding Shorter Wave-Lengths

To the layman, the electron microscope appears to be a very complicated electronic device. Fundamentally, however, it is no more complex than a modern light microscope. Its apparent complexity lies in the auxiliary equipment which is necessary to make it function properly.

The electron microscope and, for that matter, all electron optical equipment, depend on the application of two fundamental principles. The first of these is the fact that electrons traveling freely in a vacuum are deflected by electric or magnetic fields. Beams of electrons can be controlled by electric or magnetic field lenses to magnify an object in their path much as light is controlled by glass lenses to magnify an object in its path. The second fundamental principle is that electrons have something periodic associated with them and act like waves of extremely short wave-length, thus pushing the limit of what can be discerned with modern electron lenses down to the size of large molecules.



Magnesium Alloy (3,100 ×)



← Smoke Particles (36,800 ×)

Germinating Bacterial Spore (49,000 ×)

Fundamentally, the electron microscope is accurately analogous to the light microscope. The light beam is replaced by an electron beam and each of the optical lenses is replaced by an electronic lens which performs exactly the same function. An incandescent tungsten wire acts as the electron source, while an applied negative potential of from thirty thousand volts to a few hundred thousand volts accelerates the electrons to produce a usable beam. The beam passes, in succession, through the condenser lens (which concentrates it), the specimen, the objective lens (the lens closest to the specimen, or 'object') where first magnification takes place, and the projection lens (corresponding to the eye lens of the light microscope) where a second magnification occurs. The electronic image is made visible by projecting it on a fluorescent screen or by using a photographic plate.

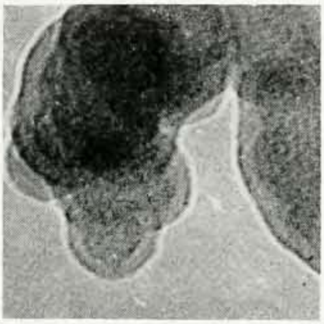
Limits of Vision

In modern electron microscopes, electrons are accelerated by fifty to one hundred thousand volts before streaming through the lens system. The wave-length associated with this voltage is approximately one hundred-thousandth of that of a visible light wave.

In electron optics, just as in light optics, the operation of a lens system can be described in two ways. The first and simpler way is by geometric optics which traces the path of an electron as it is affected by an electric or magnetic field. This is quite accurate for relatively large dimensions. It does not work when dimensions get very small for it is there that the wave nature of the electron must be taken into account to explain what happens. In physical optics the moving electron is considered only as a wave traveling through media which bend it. It is in this second approach that the existence of a fundamental limit to the resolving power becomes apparent. It is by this same wave analysis that the limits of light microscopy have been calculated and proven in observation.

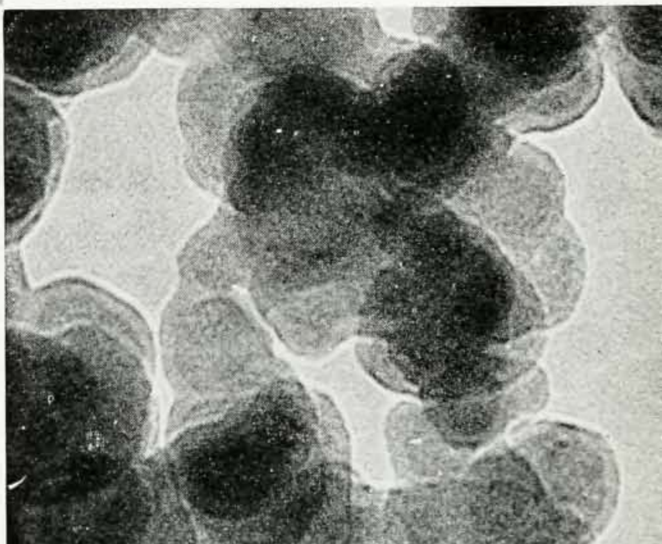
There is another thing which limits the magnification. Uncorrected magnetic and electron field lenses possess aberrations just as uncorrected glass lenses. This too limits the details which can be distinguished clearly. Taking the aberrations of present lenses into account, the minimum dimension which we can expect to distinguish is near five ten-millionths of a millimeter, corresponding to a useful magnification of over four hundred thousand. Actually this has not yet been achieved because it has not been possible to use the present lenses under the best conditions. However, useful magnifications of two hundred thousand have been achieved for suitable specimens, this value being very nearly the theoretical limit for the conditions under which the lens was used. These figures serve to indicate that now there are at least no unknown or unrecognized technical difficulties preventing the attainment of the theoretical limit.

Merely to produce sharp images of the details of an object is not sufficient to provide information. Some differentiation, that is contrast, must also be shown if details are to be distinguished. In the electron microscope contrast must come through variations in intensity. Such contrast is usually the result of differences in the way in which different



Asbestos (24,000 ×)

Carbon Black (340,000 ×)



points on the object scatter electrons. The electron microscope objective is arranged so that electrons which are scattered more than a certain amount are removed from the beam. In this way differences in the scattering power of different points in an object are translated into differences in the intensity of the corresponding points in the image. Absorption of electrons by the specimen can also introduce contrast but images produced in this way are simply silhouettes. It is a general rule in electron microscopy that, if the specimen or part of it is so thick that an observable number of the electrons are absorbed, the scattering which accompanies the absorption is sufficient to make the specimen appear completely opaque.

The limit of vision with the modern electron microscope is still a far cry from the magnification of one hundred million which the wave-length of the electron would seem to make possible. A nearer approach to this ultimate magnification will require the solution of two fundamental problems: the correction of electron lenses and the enhancement of contrast for objects of atomic dimensions. Both problems are exceedingly difficult and in a sense compete one with the other. To utilize the higher resolving power of which a corrected lens would be capable would require the use of larger apertures. This in turn would result in a reduction in what is already inadequate contrast.

The use of heavier particles with much shorter wave-lengths does not seem to offer an easier solution. While protons, accelerated by the same potentials as used in electron microscopes, would provide a forty fold reduction in wave-length, the expected improvement in resolving power with present lenses is, theoretically, but four- to sixfold and that only in the face of overwhelming and fundamental technical difficulties. By far the most important of these is the greater interaction of the heavy protons with the specimen so that, according to present day thinking here, the possibility of observing atomic arrangements by the use of protons is precluded.

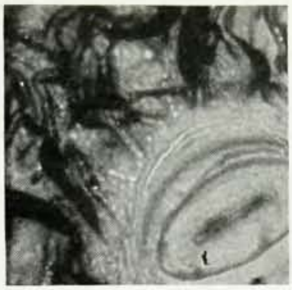
Thus, while an even further extension of our sense of vision is within the bounds of possibility the likelihood of its being achieved in the very near future seems quite remote.

Engineering

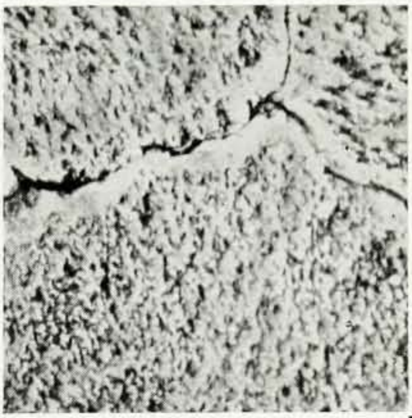
The physical structure of the electron microscope in no way resembles that of the light microscope. Mechanically, it is made quite complex by the fact that it is necessary to enclose the electron source and its entire path—including the lenses, the specimen, and the photographic plate—in a high vacuum chamber. In addition to this one must be able to make a number of adjustments inside the vacuum chamber while the instrument is in operation, such as moving the specimen, moving the photographic plate, or the shutter, etc. It must also be a simple operation to break the vacuum to change the specimen or the photographic plate and to regain that vacuum very rapidly. This requires a number of tricky mechanical features and high speed vacuum pumps which must run continuously during the operation of the instrument. In addition to the mechanical structure, there are the power supplies which consist at least of a high voltage supply and a means for heating the filament which is the source of electrons. In the magnetic type of instrument, it is also necessary to supply the electrical currents to the windings of the magnetic lenses. In this latter case, there is the added problem of regulating these power supplies so that there are no variations more than one or two parts in twenty thousand.

There are a number of other interesting points of difference between the structure of the electron and the light microscope. For instance, electron lenses do not necessarily have a fixed focal length. It is thus possible to focus the instrument without moving part of it mechanically, as is done with the light microscope. Similarly, it is possible to vary the magnification without changing objectives or eye lenses.

Vanadium Pentoxide Fibers about 12 atoms wide (153,000 ×)



← Leaf Stoma (1,520 ×)



Magnesium Alloy (4,160 ×)

Zinc Crystals (31,000 ×)

The magnification provided by the light microscope is usually the maximum useful value for the lenses used, while in the electron microscope the magnification obtained electronically may be several times less than the maximum useful value. In fact, in the electron microscope, because the useful limit of magnification is so high, it is much more practical to use low magnifications electronically, and supplement them by optical enlargement. It is customary to record images photographically at magnifications in the range of one thousand to thirty thousand. The finer details of the images at these magnifications are still too small to be resolved by the unaided human eye but they can be recorded faithfully on fine grain photographic emulsions. The fine details can then be made visible by optical enlargement.

In this way it is possible to obtain images of sufficiently large areas of the specimen at the highest magnifications without building mammoth cameras and without excessively long exposure times imposing prohibitive tolerances on the regulation of the power supplies. More recently it has become customary to use a low-powered light microscope to examine the final electronic image visually at total magnifications as high as two to three hundred thousand. However, such images are used almost exclusively for the adjustment and focusing of the instrument. Only rarely are studies of the specimen made visually since the contrast and definition is invariably better in the photographically recorded image. This is contrary to light microscope experience where the visually observed image is more informative than the photographically recorded image; a difference which is partly the result of the much greater depth of field of the electron microscope and partly the result of the contrasts provided by the photographic emulsion and the fluorescent screen.

At the present time the electron microscope in its best form can distinguish suitable specimens where dimensions are about one millionth of a millimeter. Thus its maximum *useful* magnification is two hundred thousand. This is one to two hundred times better than a light microscope and very nearly bridges the gap between the limit of the light microscope and the dimensions of molecules which can be studied by the indirect methods of physics and chemistry. We have, thus, in the electron microscope, an instrument which is potentially capable of providing for us a visual image of *all* of the small organized structures to be found in nature. This does not mean, however, that it is immediately possible to solve all the problems in this range with the electron microscope.

Limitations

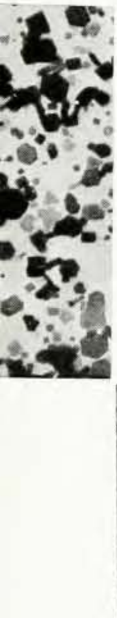
A large proportion of the forms seen through the electron microscope are totally unfamiliar to the observer. He has, therefore, no experience on which to base his interpretation of those images. What is even more difficult to realize is that he has no criteria by which to judge the accuracy of the representation with which he is provided. Since the electron microscope specimen must satisfy rather stringent conditions before it can even be examined with the instrument, it is not surprising that the problem of checking the accuracy of the representation can be a difficult one. In fact, the basic problem of the application of the electron microscope in any particular research program is precisely that. Once, by the accumulation of sufficient experience and by proper control of the specimen techniques, the electron microscopist has assured himself of the accuracy of the images he is obtaining, he can divorce electron microscopy *per se* from his problem and use the instrument merely as a high-powered pair of eyeglasses for examining the results of his experiments.

A specimen which is satisfactory for examination with the electron microscope must be thin, dry, and capable of withstanding a vacuum and considerable electronic bombardment. To get significant results, it must also be possible to prepare the specimen to satisfy these conditions without its having undergone any structural change and in such a way that the significant structures be visible.

In the early development of the electron microscope it seemed quite unlikely that many specimens could be prepared in ways which would satisfy all of these conditions simultaneously. At the present time, however, success has been achieved in so many of the seemingly impossible cases that the author is now of the belief that there are no fundamental limitations preventing the examination of any specimen in the useful range of the instrument.

The electron microscope can be used to examine solid materials only. This should not be considered a limitation in itself since any material which has heterogeneities on a microscopic scale above the molecules, and is susceptible to visual study, must be either a solid or have a solid phase. No difficulty is encountered in the study of true solids. Even those solids which evaporate in a vacuum, are unstable under the electron beam, or cannot be dispersed or cut into sufficiently thin layers can be studied without difficulty by various indirect techniques. Notable among these is the replica technique in which a mold or cast of the outer or an exposed inner surface is made in a form which is thin enough for electron microscope study. Nor is any great difficulty encountered in the study of truly dispersed colloids, at least as far as studying the solid phase is concerned.

Biological systems present a different situation, however. In general these are complex liquid-solid-gel combinations which undergo extensive changes on drying. The proper use of the electron microscope requires either that methods be discovered to prevent changes on drying or that these changes be understood thoroughly, allowing the original structure to be deduced. Fortunately it is only the initial drying out which introduces changes; introduction into the vacuum of the microscope and ordinary electron bombardment do not affect a sufficiently thin specimen.



Etched Copper (16,000 ×)

Use

The range of problems to which the electron microscope has already been applied is so great that it is possible to give only a few representative examples here.

For the chemist the electron microscope can provide information regarding the size, shape, and structure of any solid particles in which he may be interested. Electron micrographs of properly dispersed catalysts, pigments, filter material, textile fibers, dyes, etc. will enable him to correlate their physical form with their other properties. The earliest and most obvious use of the electron microscope in chemistry was to study the influence of the shape and size distributions of pigment particles on their hiding power, if used in paints, or their effect on rubber or plastics when used for reinforcing. More recently fundamental studies have been made on the growth of colloidal particles, the shape and size of various clay particles, the distribution of dye particles on synthetic fibers, and so on. It should be pointed out that in none of these problems was the electron microscope used exclusively, but it was used in conjunction with many other methods of physical chemistry such as x-ray and electron diffraction, spectroscopy, light scattering, and low angle x-ray scattering.

In metallurgy, the application of the electron microscope is a direct continuation of that of the metallographic microscope which provides images of the physical structure for correlation with properties and treatment. The surfaces of solid materials cannot be studied directly with the electron microscope. However, replica techniques have been developed which make it possible to observe the geometrical structure of almost any surface with a resolution of the order of ten millionths of a millimeter. These replica techniques have been so refined that at the present time the electronic image of a surface is identical with that obtained with a metallographic microscope, with the exception of course of the improved resolution. Thus, the application of the electron microscope to metallurgy does not pose any particular problem other than the reorientation of the metallographer in this range of improved resolution. With the electron microscope the metallographer has another dimension at his disposal in the preparation of his specimen.

The shallow depth of field of the high-powered metallographic microscope made it essential that the preparation have a very nearly plane surface. The great depth of field of the electron microscope has removed this limitation. Actually, it will take considerable time before the value of this third dimension will become apparent. Meanwhile, the electron microscope is being used to solve problems that have already been recognized. For instance, it is ideal for the study of age hardening, since it can make visible the very finest of precipitates very shortly after they begin to form. Similarly it is possible to use the electron microscope to study carefully the early stages of the corrosion of a metal surface.

While the initial use of the electron microscope has been mainly in connection with industrial problems of chemistry and metallurgy, ultimately its greatest benefit to mankind will undoubtedly come through its use in the field of biology. It is of course here that the preparation of the specimens for the electron microscope is most difficult and while a number of the problems have been solved, many still remain. In spite of this a number of definite advances have already been made.

For instance, most of the viruses which have been isolated and purified have been examined with the electron microscope and a number of related studies have been made. Preparation techniques in bacteriology have been improved to the point where it is now possible to follow, by a sampling technique, the germination of the spores in an organism, the changes in the cell that result in its division, the growth of flagella and even the action on the cells of bacteriophage particles. Studies have been made on the structure of muscle and nerve fibers and on cultured normal and tumor cells. Little work has been done on the study of cut sections of tissue because it is only recently that methods of preparing such sections have been developed. Even present techniques are primitive but the rate of improvement is already sufficiently high to lead us to anticipate that useful results will be forthcoming in the very near future.

The instruments and the techniques have now reached the point where few research workers studying solid materials of any kind can afford not to ask themselves the question, "Would an extension of my sense of vision into the range one thousandth to one millionth of a millimeter help me solve my problems?" If the answer is "Yes" he would do well to investigate the possibilities of using an electron microscope in his work.