

## THE ELECTRON MICROSCOPE DESCRIPTION AND APPLICATIONS

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The recent development of commercial electron microscopes which even the small research laboratories can afford is certain to contribute greatly to man's understanding of nature, particularly in those fields of science concerned directly with human welfare. The electron microscope, because of its tremendously high useful magnifying power, makes possible the direct observation of individual particles which lie in a previously unexplored domain, a domain which can be studied neither by direct optical methods nor by the indirect statistical methods utilizing X-ray and electron diffraction.

This paper gives a description of the electron microscope, including those basic principles which illustrate its possibilities and limitations as a tool for research and analysis. Since the same basic components are common to the familiar light microscope (L.M.) and the electron microscope (E.M.), the operation and special properties of the latter can be illustrated most conveniently by a comparison of the two types:

### Source of Illumination

- L. M.: Visible or ultraviolet light.
- E. M.: High velocity concentrated stream of electrons accelerated by 10,000 to 100,000 volts.

### Condenser Lens

- L. M.: Glass or quartz.
- E. M.: Electric or magnetic fields. The latter is more frequently used.

### Object

- L. M.: No limitation except size.
- E. M.: Must be thin enough to transmit large fraction of electrons, less than 10,000 A.U.\* In most cases the elec-

tron stream and high vacuum kills living specimens, although cell structure can still be studied.

### Objective and Projecting Lenses

- L. M.: Glass or Quartz.
- E. M.: Electric or magnetic fields.

### Observation of Final Image

- L. M.: Eye, photographic plate or ordinary screen.
- E. M.: Photographic plate or fluorescent screen.

### Changing Magnification

- L. M.: Change lens or change length of instrument.
- E. M.: Change current producing magnetic field.

### Support for Object

- L. M.: Glass slide.
- E. M.: Plastic film about 100 A.U. thick, or fine mesh metal screen.

### Effect of Object Upon Illuminating Beam

- L. M.: Selective absorption and reflection of light.
- E. M.: Scattering proportionate to density.

### "Optical Path" Through Microscope

- L. M.: Air and special oils.
- E. M.: Vacuum, 1/10,000 millimeter of mercury.

### Maximum Useful Magnification and Resolving Power

- L. M.: Magnification 2,000; Resolving power 1,000 A.U.
- E. M.: Magnification 200,000; Resolving power 10 A.U.

The measure of the performance of a microscope depends not primarily on its magnification (the ratio of the image size to the object size), but on its abili-

\* One Angstrom Unit (A.U.) equals 1/100,000,000 centimeter.

ty to distinguish between details of individual structures. As the magnification is increased beyond a certain value for any instrument, it is found that no more fine structure of the object appears, and the image becomes increasingly indistinct. The term *magnification* can have significance only when it is less than or equal to the *maximum useable magnification*. This is defined as the value of the magnification at which the fuzziness of the fine structure just begins to obscure the individual particles. This maximum depends on the *resolving power*, which is defined as the minimum possible separation of two individual lines or points of the object which can still be distinguished as two rather than one in the image.

This upper limit of magnification is caused by the failure of light to travel in straight lines. The divergence from rectilinear propagation is known as *diffraction*, and can be expected in any type of wave propagation whenever the apertures involved are of the same order of magnitude as the wave length of the radiation. The limitation in the light microscope is imposed by the nature of light, rather than by imperfections of the lens. From a mathematical study of diffraction phenomena, the following approximate relation for the resolving power (R.P.) of a lens can be derived:

$$R. P. = \text{wave length}/D$$

where  $D$  is the diameter of the objective aperture.

In order to make  $R.P.$  a minimum,  $D$  is made as large as practicable and the wave length as small as possible. The diameter cannot be increased by a large factor due to construction difficulties. The remaining term, the wave length, in light microscopy can be reduced by using ultraviolet light, for which a commonly used wave length is 2537 A.U., instead of visible light, for which 4,200 A.U. is the average wave length. At wave lengths below 2,000 A.U., a lens cannot be found which does not absorb a large fraction of the light. In ordinary practice, the most refined methods of light microscopy using ultraviolet light and oil immersion lenses, give a

resolving power of about 1,000 A.U. corresponding to a useful magnification of about 2,000.

Recalling that X-rays have wave lengths of about 1 A.U., one might propose an X-ray microscope. This must be excluded, because as yet, no medium has been found which will refract X-rays by a sufficient amount to provide a suitable lens system.

What about the wave length of a stream of high speed electrons? It may be 1/100,000 as large as the wave length of ordinary light. This concept of wave lengths being associated with a material stream of particles may be new to many, and it is certainly not an idea which can be seen from ordinary intuitive considerations. The de Broglie wave theory of matter is now a well-founded experimental fact, particularly for electrons. The length of electron waves is inversely proportional to the speed. It is given by the relations:

$$\text{Wave Length in A.U.} = 12.3/\sqrt{V}$$

where  $V$  is the accelerating voltage. In the particular case where  $V$  is 50,000 volts, the wave length is .055 A.U. The theoretical implication is that, if a lens system can be devised that is as free from aberrations as a light system, the possible resolving power will be about 1 A.U., which will be small enough to study the structure of individual atoms and crystalline structure. Thus far, neither electrostatic nor magnetic lens systems of this quality have been developed; and, moreover, practical considerations indicate that there is little hope of reaching the theoretical limit of resolution.

Aberrations inherent in the type of electron optical systems employed prescribe upper limits for the diameter of the effective apertures which may be used. These range between .01 and .001 radians. This leads to a practical limit of resolution for the electron microscope of the order of 10 A.U. Even this limitation signifies a gain by a factor of 100 in the resolving power and magnification as compared with the light microscope.

Since the depth of focus in a microscope system increases as the effective aperture decreases, the small aperture requirement for the electron microscope is not altogether disadvantageous. The best light microscope often has a depth of focus equal to or less than its resolving power. The electron microscope has, on the other hand, a depth of focus often as great as the object's diameter; thus portions of specimen lying outside the focal plane are still imaged sharply. Because of this the electron microscope can be easily adapted to stereomicroscopy.

Even though the electron microscope has been known and used for nearly sixteen years, only recently has it come into common use in industry. There, as in the university research laboratory, it is proving to be extremely valuable. Present commercial models have reduced the difficulties connected with high voltage and vacuum requirements to the extent that they can be operated by nontechnical personnel for routine analysis work. For nonroutine problems, an experienced operator with a good technical background is required. Operators in the latter category are rather scarce, but they can be trained within a reasonable length of time.

The standard commercial electron microscope can be easily modified for use in the electron diffraction investigations which are of such great value in the study of crystalline structure. When the microscope is used as an electron

diffraction camera, the lens system is inoperative.

The ability to observe details as small as 10 A.U. has opened new fields which offer practically unlimited possibilities. The type of objects which can now be studied by electron optical methods range between such things as the internal structure of bacteria to large organic molecules (molecular weight 400,000 or more). The electron microscope is rapidly becoming an essential tool in the fields of biology, medicine, chemistry, physics and metallurgy. The biologists are now able to study disease viruses and bacteria that are far beyond the range of the light microscope. Significant studies have already been made of such things as the role of blood plates and fibrin in the process of blood clotting, the degeneration of red corpuscles, the nature of organic cell structure, and the breathing system of the mosquito larva. The chemists can now predict quantitatively those physical properties of matter which depend upon particle size such as catalytic activity, covering capacity of paint pigments and wearing quality of synthetic rubber. Metallurgists are studying etched metallic surfaces by direct observation of replicas. Physicists are making rapid advances in surface physics by observing the nature of thin films of evaporated metals. These applications of the electron microscope are but a few of those which mark the beginning of a new era in microscopy.