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Recent Applications of High Voltage Electron Microscopy in Various Branches of Science[1]

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ABSTRACT

This review paper outlines some of the advantages of the high voltage electron microscope over conventional 100 kV microscopes and briefly describes some recent applications in various branches of science. In particular, penetration, resolution and radiation damage are considered, and some recent applications to biology metallurgy, mineralogy and solid state physics are discussed.

INTRODUCTION

The purpose of this article is to briefly review the use of the high voltage electron microscope (HVEM) in a wide variety of scientific disciplines. In particular, this paper will concentrate on work for which HVEM is an essential tool rather than just a useful extension of conventional 100 kV electron microscopes.

Throughout this paper the term high voltage electron microscope will be taken to refer to electron microscopes in which the accelerating potential is 1 million volts, unless otherwise stated. In sections 2, 3 and 4 some important features of the HVEM will be considered, namely penetration, resolution and radiation damage. In subsequent sections, some applications of the HVEM to zoology, metallurgy, botany, geology, solid state physics and to gas reactions with solids will be described.

PENETRATION

Probably the most important advantage of the HVEM relative to conventional 100 kV electron microscopes is the increase in the maximum usable specimen thickness. Some experimental results on the maximum usable specimen thickness as a function of accelerating voltage are shown in the table below. The experimental data are somewhat conflicting due mainly to different criteria for defining the maximum usable specimen thickness (1) (Table 1).

Taking an overall average of the results in the table indicates that the penetration increase in going from 100 kV to 1 MV is typically about a factor of five.

In an amorphous specimen the main reason for increased penetration is the decrease in inelastic scattering which leads to absorption of the electron beam. In a crystalline material the penetration is also a function of the orientation of the specimen relative to the incident beam. The variation of penetration with orientation is shown in Figure 1 for a bent gold foil (3).

The orientation which maximizes penetration is a function of the atomic weight of the specimen and also of the incident electron energy. This has been theoreti-

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Table 1. Maximum Specimen Thickness in μm

	100 kV	500 kV	1 MV
Al	1[2]	8[2]	6[3]
Al - 25% Ag			9[4]
Si	1.2[5]	5[5]	9[5]
MoS_2	1.5 - 2.0[6]	4 - 5[6]	5 - 7[6]
Fe	0.25[7], 0.4[2,5]	1[5,7], 2[2]	1.7[7], 2[5], 2.5[3]
Cu	0.4[2]	2[2]	
Au			1[3]

cally and experimentally investigated in some detail and tables giving the crystal orientations for best transmission have been published (3).

RESOLUTION

Unless the specimen is very thin, the main factor limiting resolution in an electron microscope is chromatic aberration due to energy losses of the incident electron beam within the specimen. The radius of the disc of least confusion in the Gaussian image plane due to chromatic aberration is given by the following relativistically correct expression.

$$r_c = \frac{2\alpha C_c (1 - \beta^2)^{1/2} \Delta E}{m_o \beta^2 c^2}$$

C_c is the chromatic aberration constant
α the angular aperture of the electron beam
ΔE the energy loss of the electrons
m_o the electron rest mass
$\beta = v/c$, the ratio of the electron velocity to the velocity of light.

Two cases can be distinguished:
1) If it is possible to focus the image accurately for thick regions of specimen then, in the above expression, ΔE should be set equal to the width (at half height) of the energy loss curve. 2) If the specimen is focused at thin edges then ΔE should be set equal to the value of the most probable electron energy loss for the given thickness of material.

Various theories have been used to calculate the energy loss distribution of electrons transmitted through the objective aperture as a function of incident electron energy and specimen thickness (8, 9, 10, 11). Although the various theories are quantitatively different, there is general agreement that if resolution is limited by chromatic aberration then, for a given specimen thickness, the resolution is considerably improved the higher the electron accelerating voltage. The theories also show that for thick specimens, accurate focusing is very important in order to maximize the resolution. Thus the HVEM not only gives increased

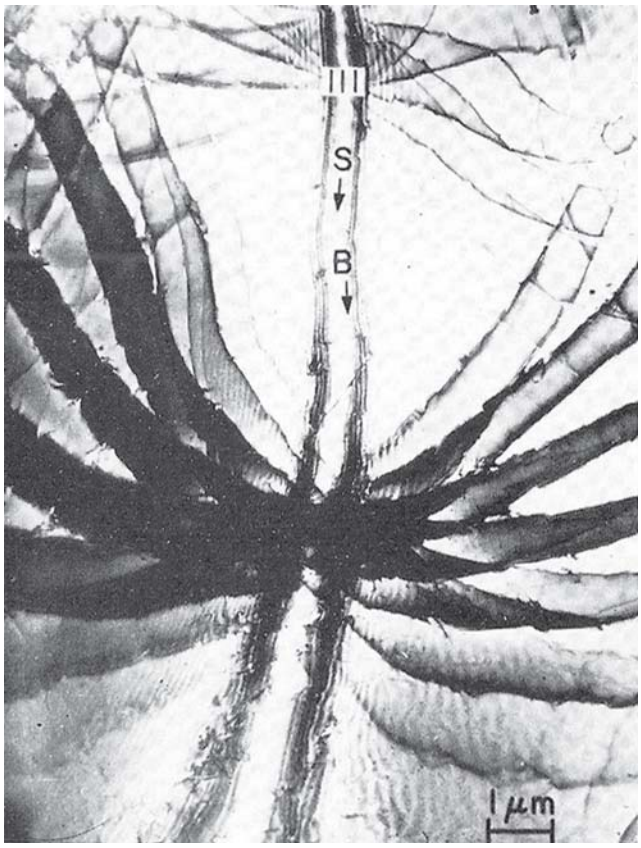

Figure 1. Brightfield 1 MV micrograph of a bent gold foil showing bend contours around a 123 pole. The symmetry and 111 Bragg positions are marked with the letters S and B respectively (3).

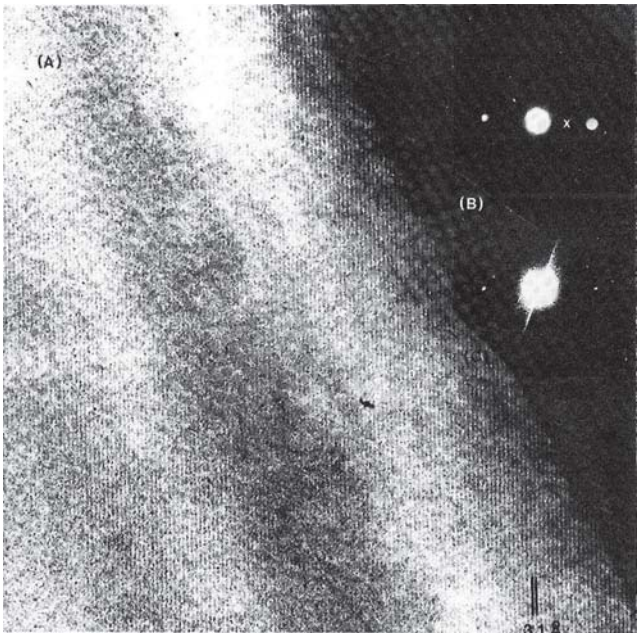

Figure 2. (a) Micrograph showing 111 lattice fringes in silicon imaged at 1 MV. The fringe spacing is 3.1 Å. (b) The corresponding electron diffraction pattern. The point X indicates the optic axis. (c) The optical diffraction pattern from the original negative of the micrograph with diffraction spots corresponding to a 3.1 Å grating (Goringe and Ray, unpublished).

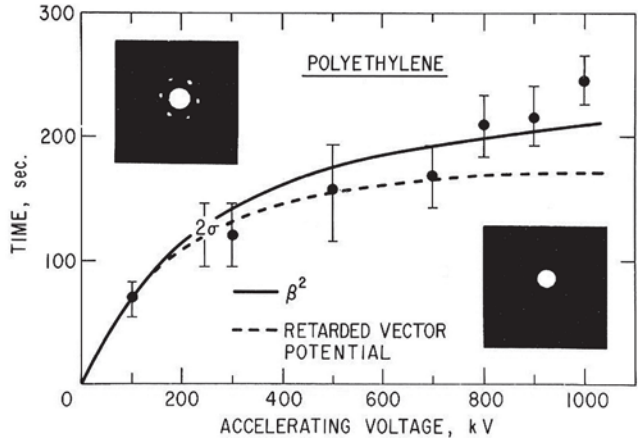

Figure 3. Voltage dependence of lifetime of polyethylene during electron irradiation. Electron intensity 1.0 A/m². Theoretical curves shown solid and dashed. Single crystal diffraction pattern top insert; amorphous diffraction pattern, after irradiation, bottom insert (13).

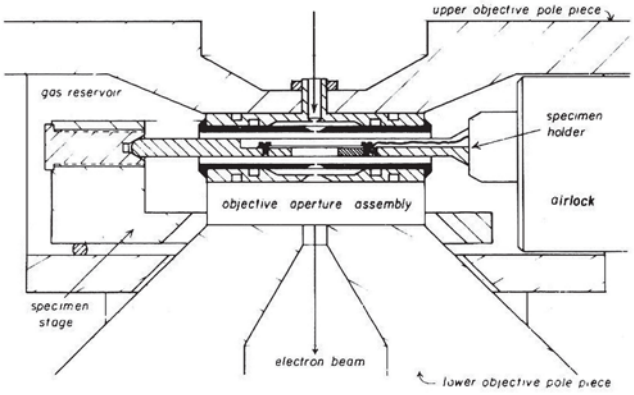

Figure 4. An aperture type environmental cell in position in a HVEM (15).

penetration of thick specimens, it also yields higher resolution.

Figure 2 is a lattice fringe image of the silicon lattice showing the 111 lattice planes (3.1 Å spacing) clearly resolved (Goringe and Ray, unpublished). The 3.1 Å fringes are resolved not only at the specimen edge, but also in thicker regions of the specimen.

RADIATION DAMAGE

The two main forms of radiation damage in a HVEM are radiation displacement damage and ionization damage. In radiation displacement damage, an incident fast electron transfers sufficient energy to an atom in the specimen to displace the atom from its lattice position into an interstitial position. The interstitial atom and vacancy thus formed may combine, or either or both types of point defect may migrate through the crystal and form clusters of point defects or, alternatively, they may be absorbed at point defect sinks, e.g., at dislocations or at the specimen surfaces. For a fuller account of displacement damage see, for example, reference 12.

Displacement damage is the principal type of radiation damage in a metal since if ionization occurs in a metal this does not produce structural damage. (If a metal atom is ionized the ejected electron is replaced by another flowing up from earth if there is a conducting path to earth, or if the specimen is insulated from earth then it will become charged. In neither case is any structural change produced.) On the other hand, if ionization occurs in a covalently bonded structure this may frequently produce a permanent change in the structure due to electron rearrangement.

Ionization damage is thus particularly important in biological materials. The nature of the electron rearrangement that results from ionization takes

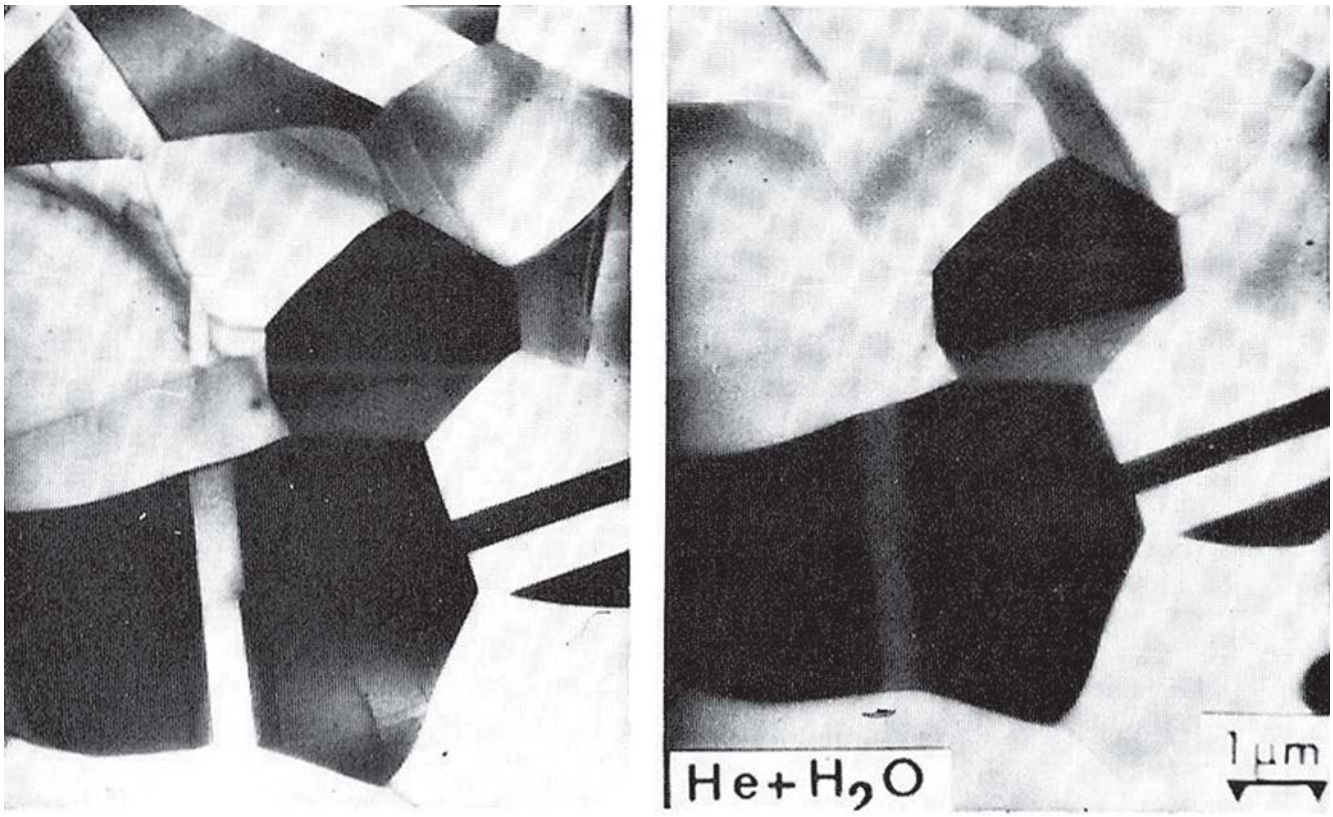

Figure 5. Brightfield 1 MV micrographs of a stainless steel specimen placed in the environmental cell of Figure 4. In the left-hand micrograph, the environment around the specimen was the column vacuum (about 10^{-4} torr at the specimen). In the right-hand micrograph, the environment was helium gas saturated with water vapor and the pressure was atmospheric pressure (760 torr) (15).

different forms in different structures. For example, the polymer polyethylene $(CH_2)_n$ crystallizes in an orthorhombic structure. The individual polymer chains are covalently bonded with Van-der-Waals bonding between neighboring chains. Ionization of main chain atoms produces electron rearrangement leading to crosslinking between neighboring chains. The resulting loss of crystalline order reduces image contrast and changes the electron diffraction pattern from a crystalline spot pattern to an amorphous ring pattern.

By observing the time taken for the spot pattern to decay as a function of the incident electron energy, Thomas et al. (13) found that the ionization damage is reduced by a factor of 3 at 1 MV relative to 100 kV which is in good agreement with theory. Some workers (14) claim that the factor is even greater than 3. Figure 3 plots the results obtained by Thomas et al. together with theoretical curves. This advantage of a HVEM is, to a certain extent, offset by the decreased efficiency of phosphor screens and photographic plates for 1 MV electrons.

Thus three of the main advantages of a HVEM relative to a conventional 100 kV electron microscope are increased penetration, increased resolution in thick specimens and reduced ionization damage. We will now briefly consider various applications of the HVEM in several different scientific fields. In particular, we will concentrate on areas in which the HVEM is an essential tool rather than merely a useful extension of conventional microscopes.

ENVIRONMENTAL CELLS

For a number of applications, a major disadvantage of 100 kV microscopes is that the specimen environment is the column vacuum. Hence, biological specimens, for example, rapidly become dehydrated. The greater penetration of a 1 MV microscope allows the specimen to be placed in an environmental cell and

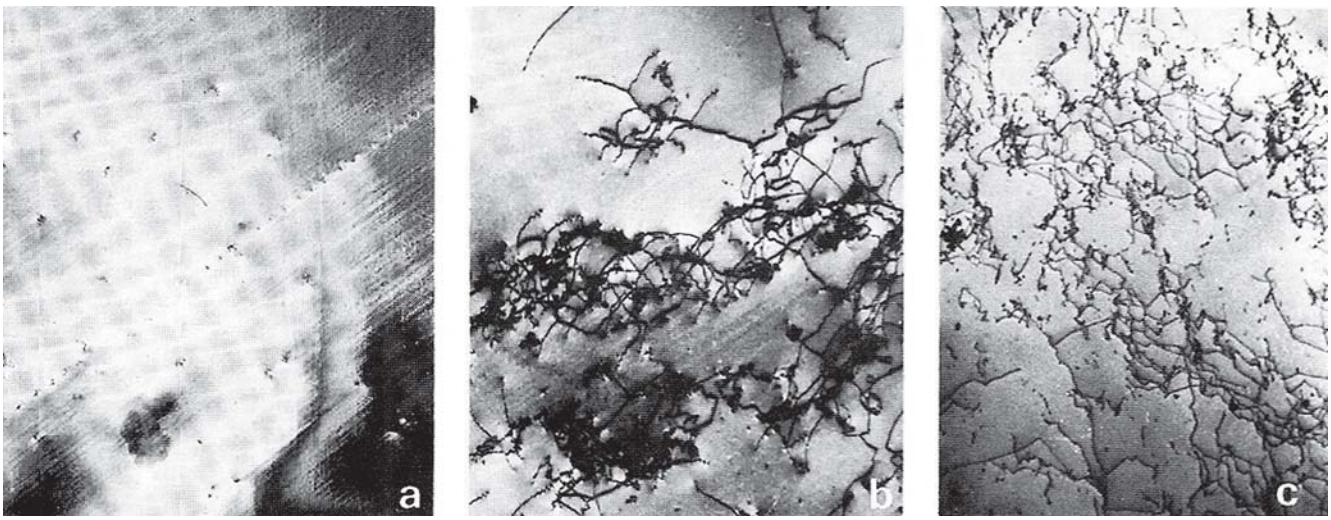

Figure 6. Dislocation structure in deformed molybdenum. (a) Thin specimen deformed in situ in 10 kV microscope; (b) thicker specimen deformed in situ in a HVEM; (c) specimen cut from bulk deformed material and examined in a HVEM (19).

surrounded by a chosen gaseous or liquid environment. Thus if the surrounding gas is hydrogen, reduction processes may be observed in situ in the microscope; in a similar manner in situ oxidation, or catalysis or corrosion may be studied. Of particular interest to biologists, the specimen may be surrounded by water or water vapor and hence studied in a wet state, at atmospheric pressure if desired.

Two types of environmental cell are being used in the HVEM. In the first type, the environment is contained between thin windows. This type of cell is suitable for containing liquids as well as gasses, but one disadvantage is the electron scattering caused by the window material. In the second type of cell, the electron beam passes through small apertures rather than through windows. The gaseous environment within the cell slowly diffuses out of the cell through the small apertures but is pumped away by an auxiliary pumping system so that the main column vacuum is not degraded. An example of the aperture type of cell is shown in Figure 4 (15).

Figure 5 shows a stainless steel specimen in vacuum and in an environment of helium saturated with water vapor at atmospheric pressure (15). The resolution is clearly acceptable for many purposes. Wet, unstained protein crystals have been observed (16, 17), and some preliminary wet state in situ biological experiments have been performed. For example, cancer cells have been observed to divide, and if a coagulant drug is introduced into the environment, red blood cells are observed to throw out processes and coagulate, this being stopped by the addition of an anticoagulant (18). Environmental cell work is at an early stage of development but the preliminary results are very promising.

METALLURGICAL APPLICATIONS

The HVEM is being used for a great variety of metallurgical work on account of the greater penetration of 1 MV electrons. One area of importance is the in situ deformation of materials in order to investigate the nature of dislocation sources and how they operate. Figure 6, due to Vesely (19), shows micrographs of deformed molybdenum. In situ deformation in a 100 kV microscope, Figure 6 (a), produces results that are markedly different from a bulk deformed specimen that has been subsequently sliced and thinned for observation, Figure 6 (c).

In situ deformation in a HVEM, however, produces dislocation structures, Figure 6 (b), similar to those observed in a bulk-deformed specimen. The reason is that at 100 kV the specimen thickness is so thin that surface effects dominate. At higher voltages, thicker specimens may be used so that their behavior is more representative of the bulk material, and hence the results are more meaningful.

BIOLOGICAL APPLICATIONS

Apart from the important unstained, wet state, environmental cell work mentioned above, the HVEM is also useful for observing stained biological specimens that are considerably thicker than 100 kV speci-

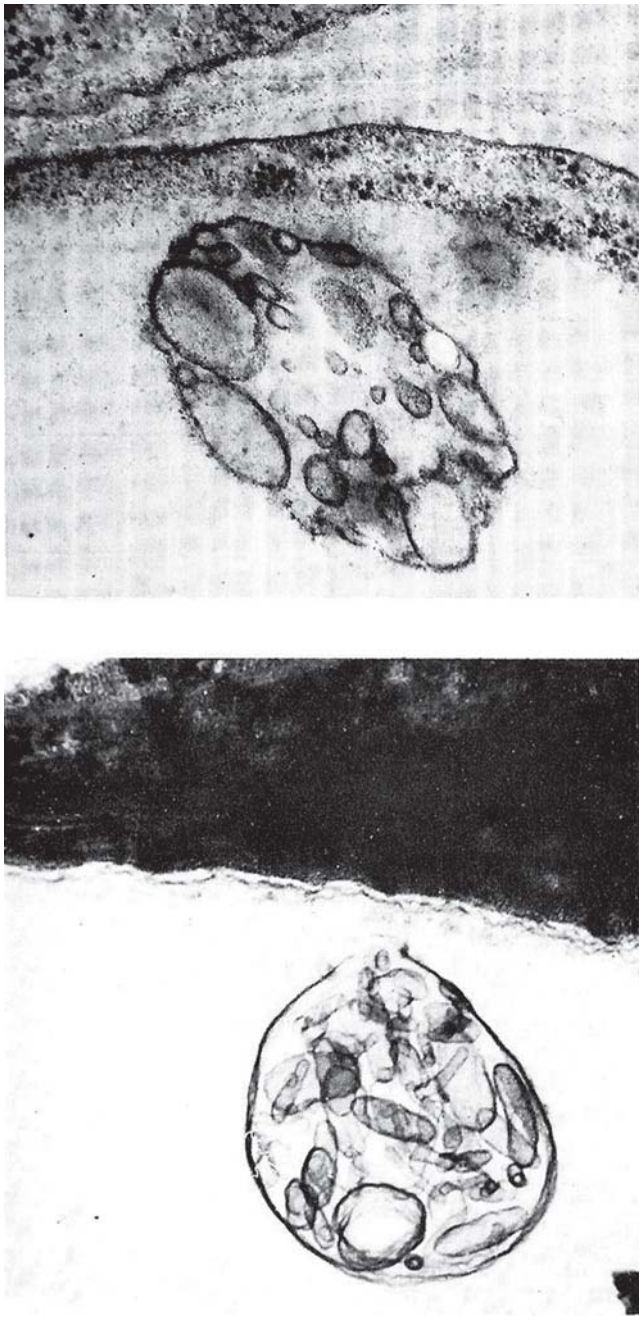

*Figure 7. Celery (*Apium graveolens*) collenchyma. (a) 100 kV; (b) 1 MV. Note the three-dimensional structure visible in the lomasome-like body in the 1 MV micrograph (20).*

mens. The use of thick sections and stereo microscopy enables the three dimensional distribution of material to be observed considerably more easily than at 100 kV. For example, plant cells are normally sliced into about 200 sections for observation at 100 kV. At 1 MV, only about 10 sections are necessary and a resolution of about 20 Å is achieved.

The greater specimen thickness is of considerable help in ascertaining the distribution of fibrous matter in cell walls, and in studying the numbers and distribution of the various constituents of the cells themselves (20). Figure 7 illustrates the greater penetration of a HVEM and the greater 3-D information available.

APPLICATIONS TO MINERALOGY

The examination of minerals in a 100 kV microscope is often extremely difficult. Thin specimens must be used, and since different phases thin at different rates (in an ion beam thinning machine) it is often difficult to retain all the phases of interest if the specimen is thin. The use of a HVEM enables thicker specimens to be examined and hence different phases can be more easily retained.

Figure 8 due to Boland (unpublished) is of a specimen of the orthoenstatite ($MgSiO_3$) from an earthquake region in Papua, New Guinea. The high pressures produced in an earthquake have induced a partial transformation from the orthorhombic form to the monoclinic form (clinoenstatite), with the monoclinic form existing in thin layers. From a knowledge of the pressures necessary for such a transformation to occur, it is possible to obtain information on the direction and magnitude of the forces involved in the earthquake.

APPLICATIONS TO SOLID STATE PHYSICS

Most of the above applications have been qualitative or semi-qualitative. However, the HVEM may also be used for precise quantitative work. One example of this is an effect called the critical voltage effect (21, 22). Since for many materials and reflections the critical voltage is in the range 100 kV to 1 MV, a HVEM is necessary for a study of the effect.

The critical voltage effect is a precise means of measuring electron densities. Hence it is useful for obtaining information on solid state bonding effects, on the degree of order of partially ordered alloys and on any effect which locally changes the electron density. Electron densities are conventionally measured by X-ray techniques. It has recently been established (Hewat and Humphreys, to be published) that for many materials the use of the critical voltage in a HVEM will be both more accurate and also simpler than X-ray methods. (For a more detailed description of this effect see Lally et al.) (23).

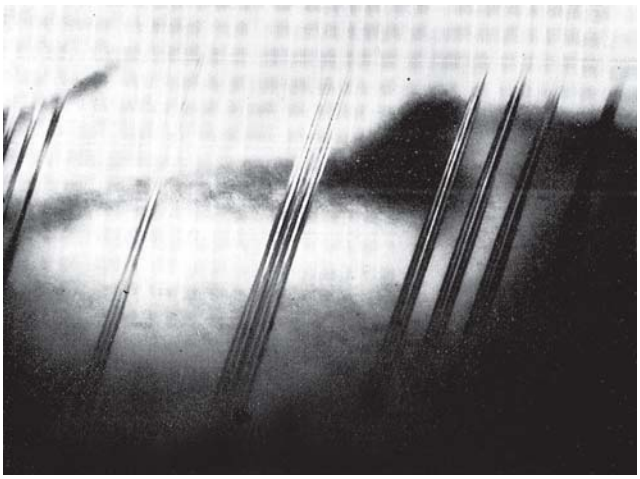

Figure 8. 1 MV brightfield micrograph of orthoenstatite ($MgSiO_3$) containing lamellae of the monoclinic form, clinoenstatite, giving rise to displacement fringes (Boland, unpublished).

CONCLUSIONS

This review has sought to briefly illustrate the versatility of the high voltage electron microscope, the reasons for its advantages over conventional 100 kV microscopes, and its use in various branches of science.

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REFERENCES

1. Humphreys, C.J. *Phil. Mag.* **25,** p 1459, 1972.

2. Fujita, H., Kawasaki, Y., Furubayashi, E., Kajiwara, S. and Taoka, T. *Jap. J. Appl. Phys.* **6,** p 214, 1967.

3. Humphreys, C.J., Thomas, L.E., Lally, J.S. and Fisher, R.M. *Phil. Mag.* **23,** p 87, 1971.

4. Dupouy, G. and Perrier, F. *C.r. hedb. Seanc. Acad. Sci., Paris.* **258,** p 4213, 1964.

5. Thomas, G. *Phil. Mag.* **17,** p 1097, 1968.

6. Uyeda, R. and Nonoyama, M. *Jap. J. Appl. Phys.* **7,** p 200, 1968.

7. Hale, K.F. and Henderson Brown, M. *Micron.* **1,** p 434, 1970.

8. Hirsch, P.B. and Humphreys, C.J. *Proc. Fourth Eur. Reg. Conf. on Electron Microscopy.* Roma, Tipographia Poliglotta Vaticana, **1,** p 49, 1968.

9. Dupouy, G. and Perrier, F. *Proc. Sixth Int. Congress of Electron Microscopy*. Tokyo, Maruzen, **1,** p 107, 1966.

10. Cosslett, V.E. *Optik.* **25,** p 383, 1969.

11. Cosslett, V.E. *Z. für Angew Physik.* **27**, p 138, 1969.

12. Makin, M.J. *Electron Microscopy in Material Science* (p 389). Academic Press: New York, 1971.

13. Thomas, L.E., Humphreys, C.J., Duff, W.R. and Grubb, D.T. *Rad. Effects.* **3,** p 89, 1970.

14. Kobayashi, K. and Ohara, M. *Proc. Sixth Int. Congress for Electron Microscopy.* Tokyo, Maruzen, 1, p 579, 1966.

15. Swann, P.R. and Tighe, N.J. *Jernkont. Ann.* **155,** p 497, 1971.

16. Matricardi, V.R., Moretz, R.C. and Parsons, D.F. *Science.* **177,** p 268, 1972.

17. Ward, P.R. and Mitchell, R.F. *J. Phys. E.* **5,** p 160, 1972.

18. Salisbury, A.J., Ward, P.R. and Clarke, J.A. *J. Microsc.* (in press).

19. Vesely, D. *ibid.* **97,** p 191, 1973.

20. Cox, G. and Juniper, B., *ibid.* **97,** p 29, 1973.

21. Nagata, F. and Fukuhara, A. *Japan J. Appl. Phys.* **6,** p 1233, 1967.

22. Uyeda, R. *Acta Crystallogr.* **A24,** p 175, 1968.

23. Lally, J.S., Humphreys, C.J., Metherell, A.J.F. and Fisher, R.M. *Phil. Mag.* **25,** p 321, 1972.