THE ELECTRON MICROSCOPE AND ITS ROLE IN DUST RESEARCH

PART II: TECHNIQUES

By J. H. Talbot*

Summary

The basic techniques of electron microscopy, viz. supporting film preparation, shadowing, stereoscopic micrography and selected area electron diffraction are described together with new techniques of surface replication and dark-field microscopy developed especially for dust research.

Introduction

I mentioned some of the fundamental principles of the microscope in Part I† and tried to explain why it is necessary to use electrons in place of light in order to see very fine detail. A short description of the electron microscope, its scope and limitations, was also given. The present part is devoted to a description of the techniques of electron microscopy. The dust sampling instruments and techniques are mentioned only briefly, although much time has been devoted to this aspect of the work. These may form the subject of a separate paper later.

A few words about the objects of dust studies with the electron microscope may elucidate the need for the techniques to be described. Perhaps the most important object is to find out whether particles too small to be seen with the light microscope are present in the air of the mines. In spite of the fact that the dust concentrations in our South African gold mines as measured with the light microscope are amongst the lowest in the world, many cases of silicosis still occur. If large numbers of such submicroscopic particles were to be found, this might be an explanation of this mystery.

Other objects are to study the mineralogical composition of the smaller dust particles with the object of discovering whether they are harmful; to make more accurate determinations of size distributions than is possible with the light microscope; to investigate the variation of shape factors with particle size of dust and of quartz fractions prepared by centrifuging; and to study the appearance and surface properties of airborne dust particles, ground quartz particles, and dust particles recovered from the lungs of deceased miners. In addition there are always the numerous *ad hoc* investigations which no one can foresee.

Supporting Films

It has already been mentioned that the glass slides and cover slips used in light microscopy are opaque to electrons. There is, unfortunately, no substance which is transparent to electrons in the sense that glass is transparent to light. To support the specimen it is necessary to use a film not more than about 0·05μ thick. Only certain materials are suitable, the main requirements being a low atomic number, good electrical and thermal conductivity and good mechanical strength. A low atomic number is necessary to ensure adequate electron penetration as the power to absorb electrons increases with atomic number. Poor electrical conductivity causes the specimen to charge up to a high negative potential tending to repel the incident electrons and leading to serious distortion of the image. Good thermal conductivity reduces the temperature rise in the specimen lessening the chances of it melting or decomposing.

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The most generally useful material for supporting films is carbon. The films are made by thermal evaporation of carbon in vacuo. Two pointed carbon rods are spring-loaded against each other with the points in contact. A large electric current passed through the rods causes an enormous rise in temperature at the junction. The carbon sublimes* at 2,400°C in a high vacuum. The carbon vapour condenses on the walls of the vacuum chamber and on a suitable substrate placed inside it (Fig. 1). Substrates commonly used include mica, a plastic known as Bedacryl (by Imperial Chemical Industries Ltd.), and sodium chloride either in the form of a cleaved rock salt crystal or as a layer previously deposited by evaporation and condensation. The film is removed from the substrate by floating off onto the surface of distilled water (mica), or by solution of the substrate in ether-acetone (Bedacryl) or water (sodium chloride).

Carbon films are a comparatively recent innovation, dating back only to 1953. Their introduction marked a big step forward. Unfortunately, carbon films are destroyed by oxidation when heated above 400°C in air, and are, therefore, useless for dust samples which have to be ignited. However, they have many uses in dust research and their preparation has been described in some detail for this reason and because of its similarity to many other techniques.

Silicon monoxide films are made in the same way except that the material is heated in a conical tungsten filament. These films oxidize to vitreous silicon dioxide at elevated temperatures, but are otherwise stable to well above 600°C. They have been used for more than nine-tenths of the dust samples taken underground.

Other materials used for film preparation by evaporation and condensation in vacuo are beryllium and aluminium. These are used for special purposes as will be described later.

Films are supported either on special copper grids with square apertures of about 80μ side, or on platinum holders with a single rectangular aperture 0·1 mm. × 1·0 mm. The latter are especially suitable for use in the thermal precipitator where they can be arranged to give an uninterrupted view of a complete traverse across the dust strip. Where copper grids are used for dust sampling it is first essential to coat all surfaces with aluminium to avoid destruction of the grid by oxidation of the copper during ignition.

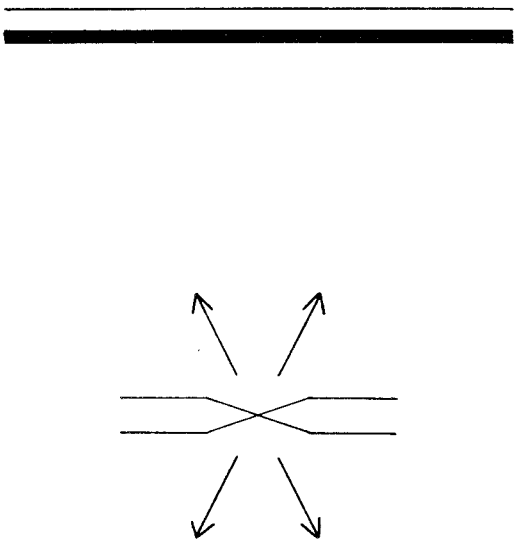

FIG. 1.—*Preparation of carbon supporting films. Arrows show directions of evaporation of carbon molecules from the two pointed spring-loaded electrodes. The carbon vapour condenses to form a solid layer (shown black) on the substrate (white).*

Shadowing

Shadowing is a very useful technique which gives the specimen an appearance similar to that obtained by oblique illumination of macroscopic objects with light. The technique is used to enhance contrast thus making visible fine detail which might otherwise be lost, to give additional information about the three dimensional shapes of objects, to measure the heights of projections above the supporting film, and to measure angles between faces of crystals.

*Changes from the solid to the gaseous phase without going through the liquid phase.

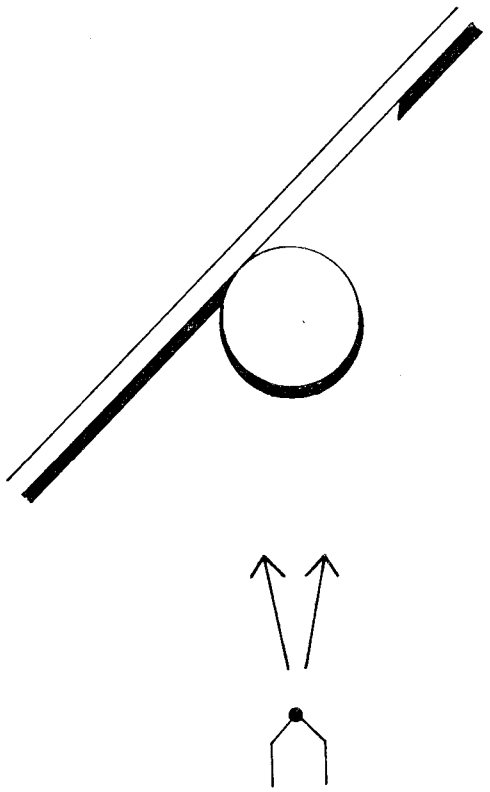

FIG. 2.—*Method of shadowing. The evaporating molecules travel outwards in straight lines from the tip of the filament.*

The effect is achieved by a method similar to that for preparing carbon films. The " shadowing agent " is usually evaporated from the tip of a hairpin filament. Being in a high vacuum (10^{-5} torr)* the evaporating molecules travel in straight lines outwards from the source. The plane of the film supporting the specimen is arranged to make a suitable angle (the " shadowing angle ") with the molecular beam. The vapour condenses where it strikes the specimen. Any projection above the surrounding parts of the specimen shields part of the specimen or supporting film on the leeward side from a deposit of the vapour (Fig. 2). Materials of high atomic number, i.e. of high electron optical density are used for shadowing. Parts which receive a deposit of the shadowing agent appear relatively opaque in the electron microscope, the shielded area appearing as a shadow in reverse. The shadowed effect is produced by making a negative photographic print of the image (Fig. 3).

The noble metals and their alloys are most commonly used as shadowing agents. These materials have very high electron optical densities, deposits only 0·002μ thick giving adequate contrast for most purposes. This thickness is below the resolving power of many microscopes so that little or no detail is lost by shadowing. In common with all metals, the noble metals are crystalline. Often the individual crystals are too small to be seen even in an electron microscope, but in the high vacuum and with the rise of temperature resulting from electron absorption the crystals grow in size and the shadowing deposit takes on a granular appearance. This granulation is a very serious drawback in high resolution microscopy and there has been a search for better shadowing agents. Recently D. E. Bradley, originator of the carbon evaporation technique, has devised a method of evaporating carbon and platinum simultaneously. The resulting deposit is amorphous and is extremely resistant to granulation in the microscope.

Palladium has been used almost exclusively for dust studies in this laboratory.

The most suitable shadowing angle for dust studies has been found to be 40°. When used for measuring the heights of particles an angle of 18½° is used. This gives a shadow of length three times the height of the particle.

Surface Replicas

The electron beam in an electron microscope will only penetrate quartz to a depth of about 0·1μ. Consequently any particles thicker than this appear as shadows or silhouettes (Fig. 3) and no surface or internal detail can be seen. Techniques for making the surface detail of thick specimens visible have been in use for some time. The surface to be studied is used as a substrate for the deposition of a film in the manner described under " Supporting Films." The

*1 Torr = 1 mm. mercury.

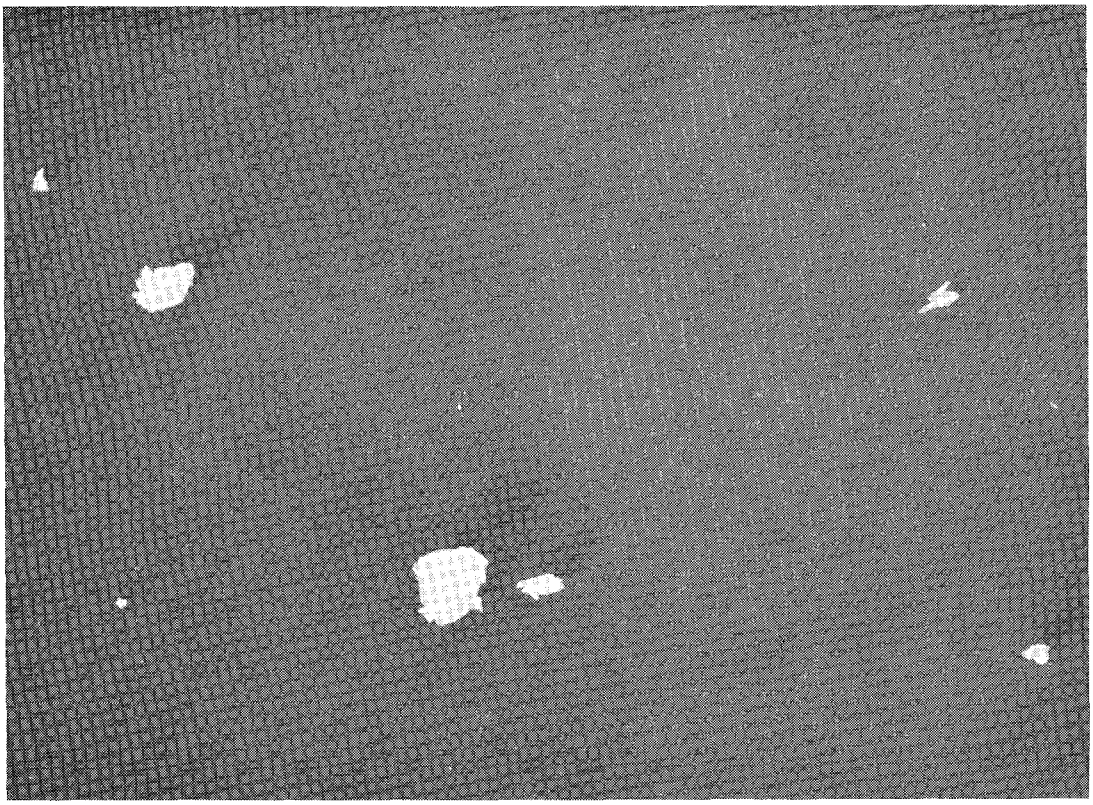

FIG. 3.—*Dust sample shadowed with palladium at 40°.*

film is then detached from the substrate and mounted on a grid for observation in the microscope. It has been found that such films faithfully reproduce the delineation of the substrate surface, hence the name *surface replica.*

Methods of replicating metal and other surfaces have been known for about 15 years, but it was not until the advent of the carbon evaporation techniques that it became possible to replicate dust samples and fine quartz particles. The first successful replication of the latter was achieved in the Chamber's laboratories in 1956.

Dust samples intended for replication are taken with the standard thermal precipitator (by Casella Ltd., London). The cover glasses are replaced by discs of mica $\frac{3}{4}$ in in dia. After the sample has been ignited it is shadowed with palladium followed by the deposition of a thin film of carbon (Fig. 4). The mica disk is then immersed slowly into a water bath. As it is immersed the carbon-palladium film, still containing

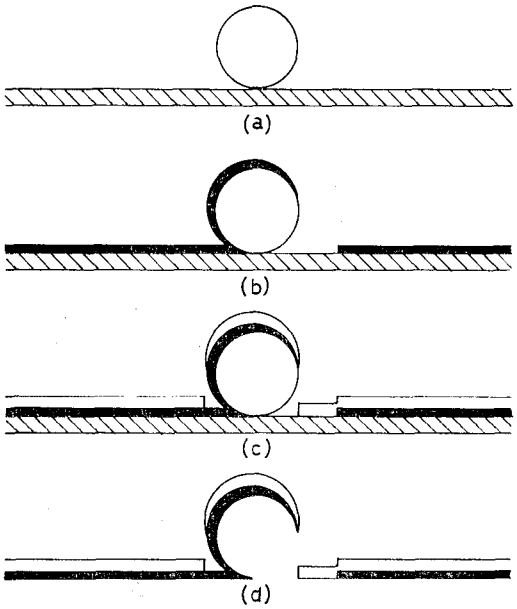

FIG. 4.—*Sequence of operations in making a surface replica: (a) particle on substrate, (b) after shadowing (black region), (c) after depositing a layer of carbon (white region), and (d) the finished replica after removal of the substrate and particle.*

the dust particles, parts company with the mica and floats onto the water surface. One or two hours on a bath of 48 per cent. hydrofluoric acid is usually sufficient to remove the dust particles from the replica. Where, as in the case described, the shadowing is done before deposition of the replicating film the replica is said to be *pre-shadowed*. An example of a pre-shadowed replica is given in Fig. 5.

Surface replicas have many applications in dust studies. Some of these will be described in Part III.

Stereoscopic Microscopy

Since the resolving power of the electron microscope is limited by lens defects and not by diffraction it is an advantage to use very small lens apertures.

This results in a very large depth of field. By depth of field is meant the distance at right angles to the specimen plane over which the specimen appears to be satisfactorily in focus. The high power light microscope has a depth of field of less than $\frac{1}{2}\mu$ so that when observing a particle a few microns thick it is impossible to have the whole particle in focus all at once. One can focus satisfactorily only on one section at a time. In the electron microscope it is possible to have the whole particle in focus at one setting of the focus control. Because of this remarkable feature the electron microscope lends itself to stereoscopic microscopy.

Stereoscopic observations are particularly useful on surface replicas where a complete three-dimensional image of the surface of a particle may be obtained. Stereoscopic views are obtained by taking two micrographs, the specimen being tilted through an angle of about 7° for the second. Observing the two with a conventional stereoscope yields the three-dimensional image.

This technique has been used for measuring the heights of particles. By a method similar to that used for making contour maps from aerial photographs, contours can be drawn through the particles, provided that the surface is not too irregular. It is hoped to develop this into a method of measuring the surface area of particles and hence the surface-diameter shape factor.

Electron Diffraction

Electron diffraction is a technique with numerous applications in many branches of science. In dust studies it is used mainly for the identification of substances in dust samples. This is the technique described here. Other applications will be mentioned in Part III.

Before proceeding to a discussion of electron diffraction it is necessary for us to learn just a little more about waves. Fig. 4 of Part I shows the cross section, or *profile*, at a particular instant, of a wave such as might form on the surface of a pond. Distance from some appropriate point is measured along the horizontal axis while displacement of the water surface from the undisturbed position is plotted along the vertical axis. Note that the wave is made up of a number of identical units, each consisting of one crest and one trough,

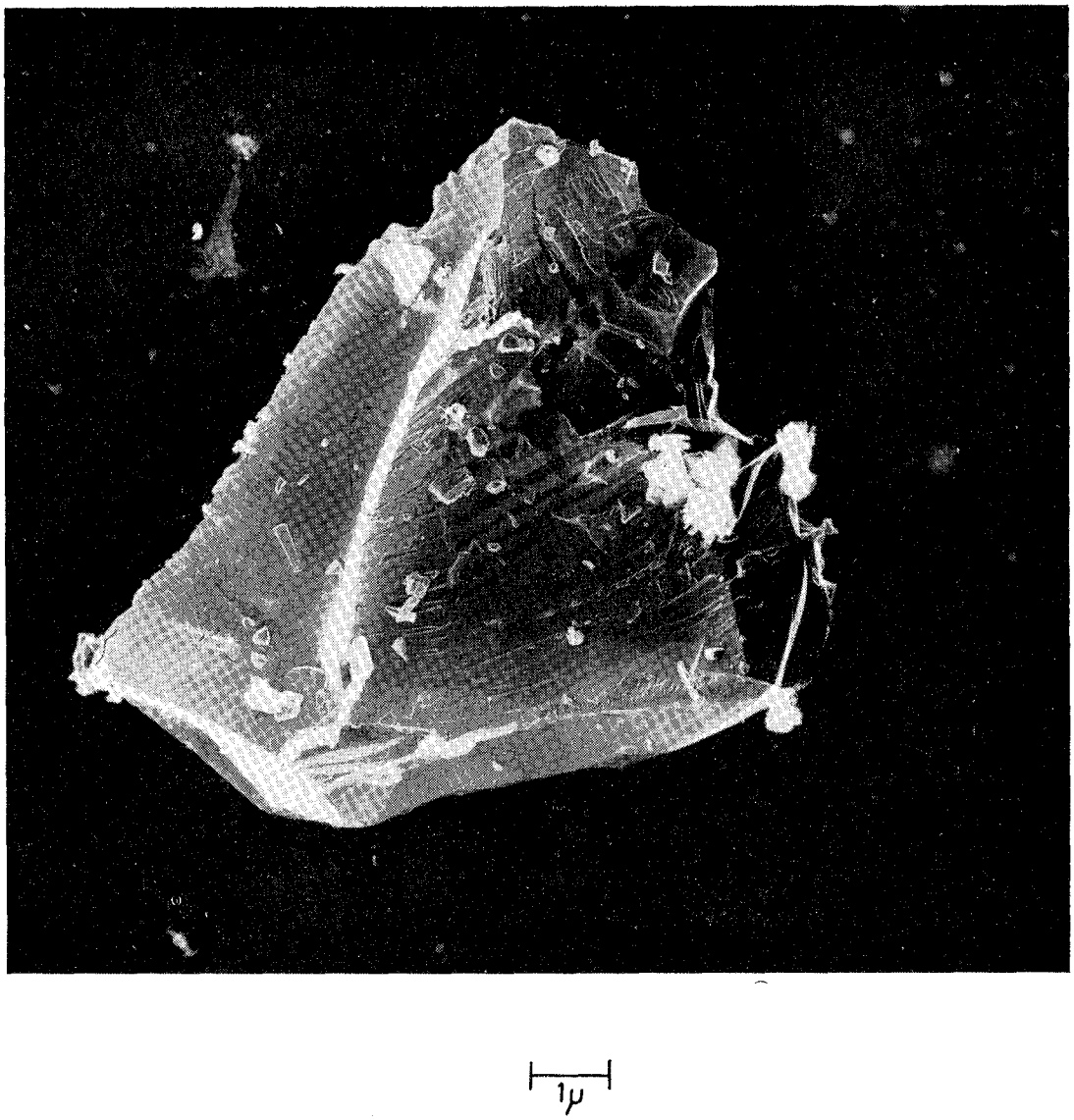

FIG. 5.—*Preshadowed replica of a dust particle.*

placed end to end. The length of each unit, or repeat distance, is called the wavelength. If two identical waves are superimposed so that their crests and troughs coincide, they combine to form a wave of the same wavelength, but with the heights of the crests and the depths of the troughs double those of the individual waves. If, however, the

crests of the one wave coincide with the troughs of the other, the two waves cancel each other, and their combined effect is the same as if there were no wave at all.

In Part I it was mentioned that much of the behaviour of an electron beam could be explained by regarding the electrons as a wave. Let us consider what happens when this wave strikes the specimen. We resort to an analogy, making use of the familiar concept of waves on the surface of a pond and their effect on a floating cork. The cork rises with the passage of a crest only to fall again as the next trough goes by. The resulting motion is a vertical oscillation of the cork. This causes small circular ripples of the same wavelength to radiate out in all directions from the cork. The observed effect is obtained by combining the individual effects of the original wave and the ripples in the way described above. At certain positions the crests and troughs of the ripples will coincide with those of the original wave producing an effect stronger than that of the original wave alone. At other positions the crests of the ripples will coincide with the troughs of the original wave, giving a weaker effect than the original wave alone.

Returning to a consideration of our specimen, each of its atoms is the analogue of a floating cork. The effect of the wave representing the incident electron beam is to produce a series of spherical ripples centred on each atom. The ripples from the different atoms combine with each other to produce an effect depending on the arrangement of the atoms. If the atoms are packed in a regular three-dimensional array forming a crystal, there will be certain directions in which the crests and troughs of the ripples from all the atoms coincide. The ripples combine to form strong waves propagating in these directions. In other directions the crests and troughs of different ripples will fail to coincide and the ripples combine to cancel the effects of each other.

Where the atoms are randomly arranged, as in an amorphous substance, there is no direction in which the crests and troughs of all the ripples will coincide. The result is merely a roughly uniform weak wave propagating over a large solid angle.

Therefore, when a specimen consisting of a single crystal is irradiated by a beam of electrons, the emerging electrons form a number of strong "diffracted" beams propagating in the directions in which the crests and troughs of the ripples from different atoms coincide. A screen or photographic plate placed so as to intercept these beams registers a pattern of regularly spaced spots (Fig. 6). If the specimen consists of numerous very small crystals of the same material, then each crystal produces its own pattern of spots and the patterns from the different crystals overlap to form concentric rings (Fig. 7). Each crystalline substance produces its own characteristic pattern of rings, a sort of fingerprint which can be used to identify it.

Specimen requirements for electron diffraction studies of mine dusts differ from those for electron microscopy. For this reason separate samples have always been taken for the two purposes. For electron diffraction there are some advantages in using a crystalline film. The electrons diffracted by the film are then concentrated into narrow rings which may also be used for calibrating the diffraction pattern. A disadvantage of a crystalline film for electron microscopy is that, under high resolution conditions, it exhibits a granular appearance which makes the detection of the very smallest dust particles difficult. With amorphous films the electrons diffracted by the film form a diffuse background in the diffraction pattern. This may be so strong as to obscure the diffraction pattern produced by the dust particles. Crystalline films also produce a diffuse background due to Compton or incoherent scattering, a phenomenon not yet considered. However, this is in general not as strong as the diffuse background due to an amorphous film.

To understand the Compton scattering it is necessary to consider the electrons and the atoms as hard billiard balls. When these collide the electron gives up part of its momentum to the atom. In the quantum theory this is equivalent to saying that the wavelength of the wave associated with the electron is increased. This increase may assume any value depending on the circum-

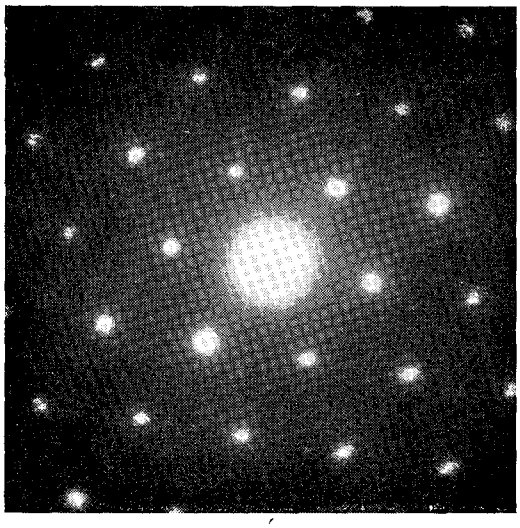

FIG. 6.—*Electron diffraction pattern of a single crystal of mica.*

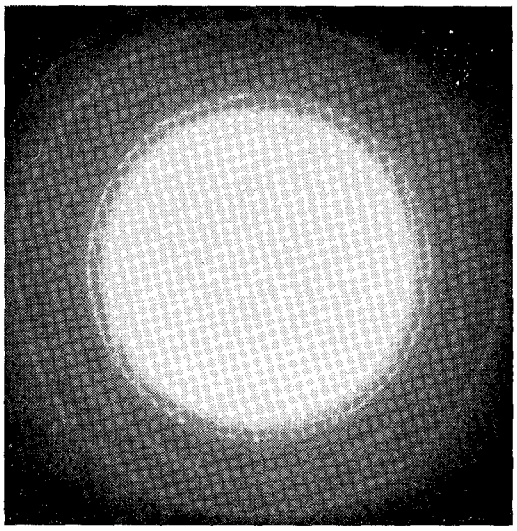

FIG. 7.—*Diffraction pattern of a powder specimen.*

stances of the collision. Electrons which have undergone this type of scattering all have different wavelengths so that in any particular direction the relationship between the positions of the crests and troughs of their waves is continually changing. The result is a diffuse background in the diffraction pattern. To minimize this background it is desirable to keep the film as thin as possible. Consequently, mechanical strength and thermal and electrical stability become very important. The material which best satisfies these requirements is beryllium. Films of this material, only 0·004μ thick, were used successfully in much of the earlier work. Owing to the extreme toxicity of beryllium, films of aluminium are now used for electron diffraction studies of mine dust. Aluminium films, 0·004μ thick, are made by evaporation in vacuo. Such films lack the extreme mechanical, thermal and electrical stability of beryllium, but are proving quite satisfactory.

Selected Reflexion Microscopy

This is a method which can greatly enhance the value of the electron diffraction observations.

It is, in a sense, the converse of *selected area electron diffraction* where the diffraction pattern of a small selected part of the specimen is obtained by means of an adjustable *field stop*. Before describing the method we shall consider how the contrast in an electron microscope image is produced.

In Part I it was mentioned that as the rays pass through the specimen in a microscope they undergo changes in direction, speed, strength or in other properties. All of these changes occur in the electron microscope, but one effect, the change in direction of the rays, is so predominant as to outweigh all the others in importance. A rough idea of the mechanism of image formation can be obtained by a consideration of this effect alone. In ordinary bright field operation those electrons which undergo an appreciable change in direction are *stopped out* by a metal aperture diaphragm as shown in Fig. 8 (a). Those electrons deflected through an angle less than α (called the *semi-angular aperture*), pass through the aperture and contribute to the image. Electrons which pass through a particular point in the object are focused

to the corresponding point in the image. Consequently, if a particular part of the object causes many electrons to be diffracted through angles greater than α these electrons will be stopped out by the diaphragm and only a few electrons will reach the corresponding part of the image; therefore this part of the image will appear dark. Those parts of the object where few electrons are deflected through angles greater than α will appear bright in the image as most of the electrons will pass through the aperture and be focused to the corresponding parts of the image. This is the principal cause of the intensity variations in the image.

Anything which limits the vertical angle (called the angular aperture) of the cone of electrons entering the imaging system of the microscope is called an *aperture stop*. In dark field operation a stop with a ring-shaped aperture is used [Fig. 8 (b)]. The central disk stops out the undeflected electrons. Electrons deflected through moderate angles pass through the ring-shaped aperture into the imaging system, while electrons deflected through large angles are again stopped out. The image is therefore formed by the deflected electrons. Consequently the intensity variations in the image will be the opposite of those in the bright field image. By increasing the size of the central opaque disk and reducing the outer radius of the aperture we obtain a narrow ring-shaped aperture as shown in Fig. 8 (c). By a suitable choice of the inner and outer radii of the ring-shaped aperture it can be made to pass only those electrons corresponding to one ring of the powder diffraction pattern.

Only those parts of the specimen diffracting electrons into that ring of the diffraction pattern will appear in the image. By making the position of the ring-shaped aperture stop adjustable along the optical axis, different rings can be selected with one aperture diaphragm and we have the system of *selected reflexion electron microscopy*

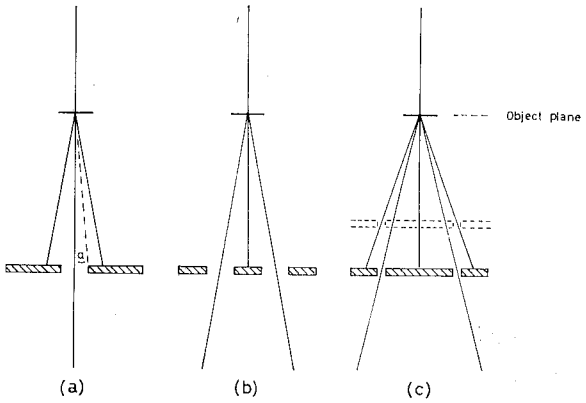

FIG. 8.—*Aperture systems for (a) bright field, (b) dark-field, and (c) selected reflexion. Dotted lines in (c) show position of the aperture for selecting the inner cone of electrons.*

devised by the author in 1953. Since each ring is characteristic of a particular substance the particles of different substances can be imaged separately; hence the method offers a useful means of mineralogical analysis.

Acknowledgements

The techniques described are based on developments by innumerable workers in many parts of the world. It would be impossible, therefore, to make adequate acknowledgement to them all. For this reason references are omitted. Especial acknowledgement is due to Dr. H. G. F. Wilsdorf, formerly of the National Physical Laboratory, Pretoria, and now director of the Franklin Institute, Philadelphia, who gave me my initial training in electron microscopy; to Mr. D. G. Beadle and to my co-workers at various times, viz. Mr. P. J. Jackson, Miss R. Cloete, Mr. A. H. Munro and Mr. E. B. Kempis. Thanks are due to the Transvaal and Orange Free State Chamber of Mines for permission to publish this article.