10

Coating Techniques for SEM and Microanalysis

10.1. Introduction

Nearly all nonconductive specimens examined in the scanning electron microscope or analyzed in an electron probe microanalyzer need to be coated with a thin film of conducting material. This coating is necessary to eliminate or reduce the electric charge which builds up rapidly in a nonconducting specimen when scanned by a beam of high-energy electrons. Figures 10.1a and 10.1b show examples of pronounced and minor charging as observed in the SEM. In the absence of a coating layer, nonconductive specimens examined at optimal instrumental parameters invariably exhibit charging phenomena which result in image distortion and thermal and radiation damage which can lead to a significant loss of material from the specimen. In extreme situations the specimen may acquire a sufficiently high charge to decelerate the primary beam and the specimen may act as an electron mirror. Numerous alternatives to coating have been proposed and some of these will be discussed in this chapter. Much of what will be discussed is directed towards biological material and organic samples simply because these types of specimens are invariably poor conductors and more readily damaged by the electron beam than most inorganic materials. However, it is safe to assume that the methods which will be described for organic samples will be equally effective for nonconducting inorganic specimens.

This chapter will concentrate on the practical aspects of some of the more commonly used vacuum evaporation and sputter coating techniques which are now standard procedures in most electron microscope and analytical laboratories. It is not proposed to enter into a detailed discussion of the theoretical aspects of thin film technology, but those readers inter-
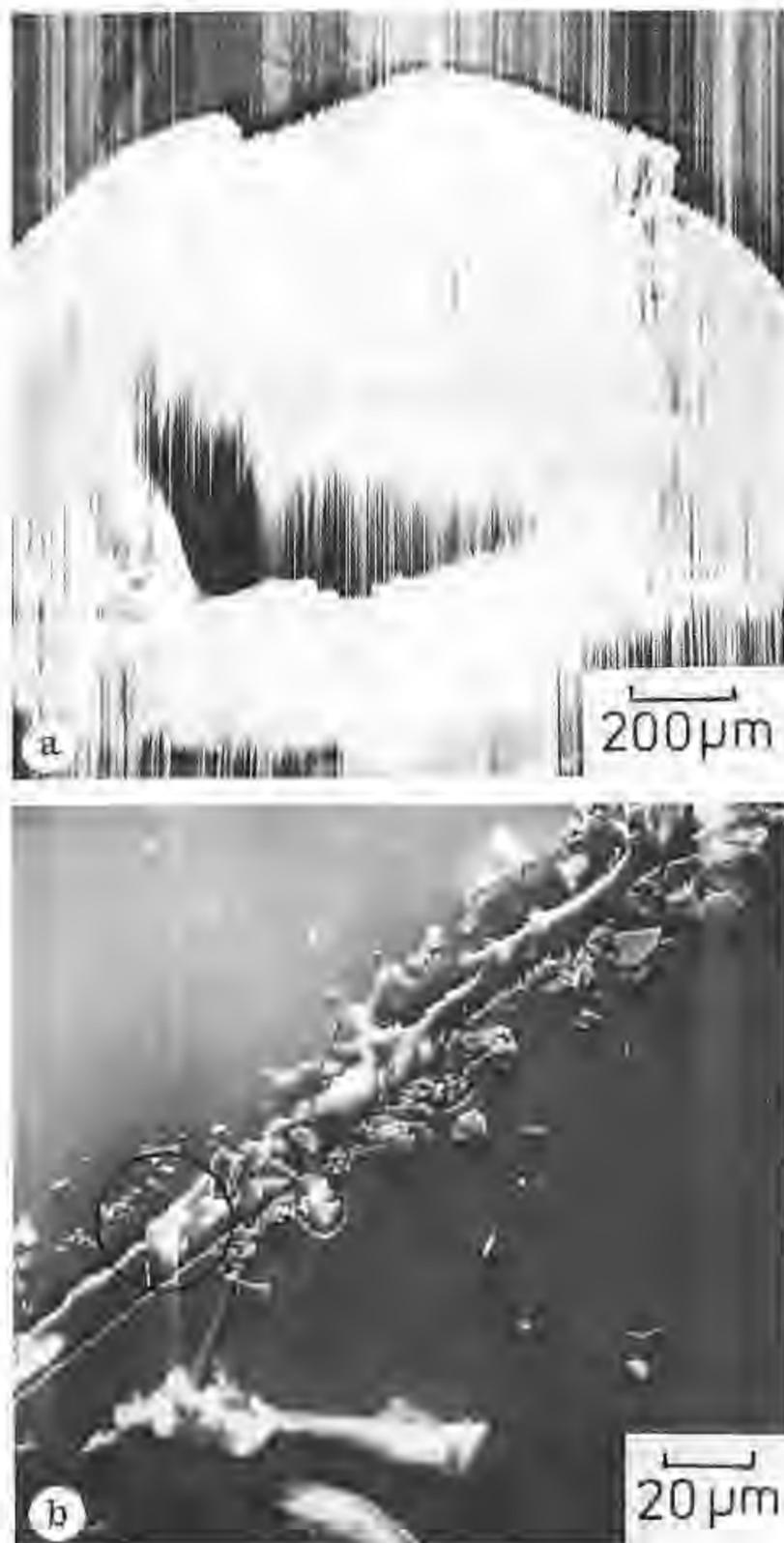
Figure 10.1. Charging effects in the examination of insulators. (a) Gross charging effects during examination of bare Teflon; beam: 30 keV. (b) Minor charging effects. The specimen is carbon-coated glass scratched through the conducting layer. Note local charging effects (circled) due to the exposure of bare glass by the scratch.

ested in this aspect of the subject are referred to the book by Maissel and Glang (1970).

10.1.1. Specimen Characteristics

10.1.1.1. Conductivity

The single most important reason for coating is to increase the electrical conductivity of the sample. Materials of high resistivity, i.e., exceeding 10^{10} Ω-m, will rapidly charge under the incident beam and may develop a potential sufficient to cause a dielectric breakdown in certain regions of the specimen. This leads to variations in the surface potential, giving rise to the complex image artifacts commonly referred to as "charging." These artifacts are manifest as deflection of low-energy secondary electrons, increased emission of secondaries within the crevices of a rough specimen, periodic bursts of secondary electron emission, and deflection of the electron beam, all of which degrade the resolving power and analytical capabilities of the system by introducing astigmatism instabilities, undue brightness, and spurious x-ray signals (Figure 10.2). This undesirable situation is frequently compounded because many of the adhesives used to attach the specimen to the substrate are themselves nonconductors and may prevent any electrical charge leaking away, even from conductive samples. A suitable conducting path may be established with silver or carbon paint. If one is concerned only about electrical conductivity, then a thin layer of gold, silver, or copper will suffice to eliminate the problems associated with charging. Even though metallic samples are usually conductive, there are situations where one may wish to examine nonconducting areas, e.g., inclusions, and in these cases it is necessary to apply a thin coating layer. The conductivity of the thin film should be sufficient to ensure that the specimen current is drained to ground without the development of a significant surface potential.

10.1.1.2. Thermal Damage

Specimen heating is not usually a problem in most samples examined in the SEM, because the probe current is usually in the picoampere range. Although higher currents are frequently used for TV scanning, these are unlikely to seriously degrade the specimen. Thermal effects are potentially more serious for cathodoluminescence and x-ray microanalysis, for which the probe currents are in the nanoampere and even the microampere range.
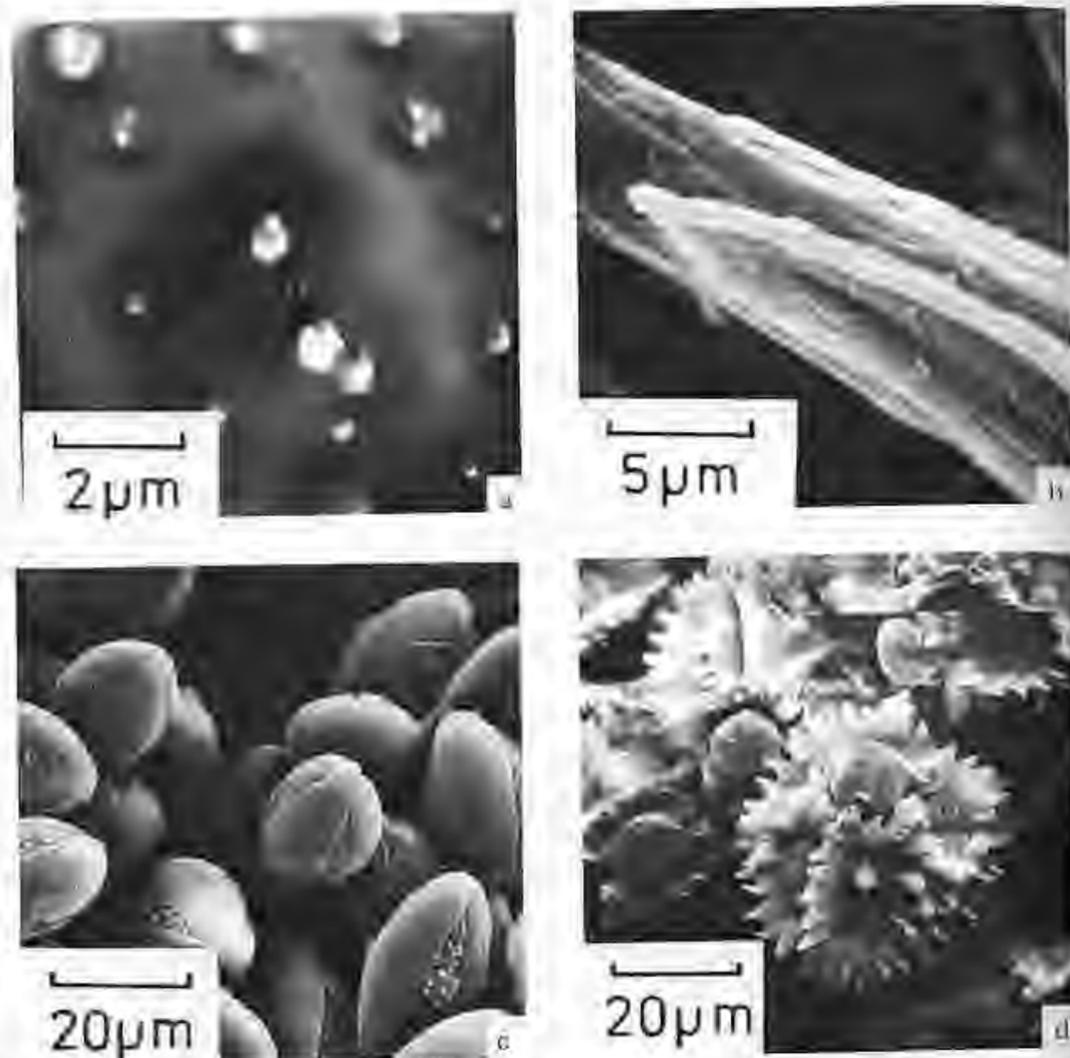
Figure 10.2. Artifacts during examination in the SEM (a) Beam damage causing cracks on the surface of pollen from *Ipomoea purpurea*. (b) Lines across image due to faulty scan generator. Wood fibers of *Quercus ilex*. (c) Charging causing small flecks on image of *Aesculus hippocastanum* pollen. (d) Charging causing image shift on image of *Lygodesmia grandiflora* pollen.

Excessive heating in the SEM can lead to specimen movement and instability and in extreme situations to breakdown and destruction. The phenomenon described as "beam damage" is most certainly a heating effect and is manifest as blisters, cracks, and even holes in and on the surface of the specimen (Figure 10.2a). In the electron probe microanalyzer the higher beam currents can cause a rapid loss of organic material from plastics, polymers, and biological samples and can even result in substantial elemental losses.

Thermal damage can be reduced by working at lower beam currents and using thin specimens which are in close contact with a good thermal conductor. Alternatively, the specimens may be coated with a thin film of a good heat conductor such as copper, aluminum, silver, or gold.

10.1.1.3. Secondary and Backscattered Electron Emission

The thin layer of metal which is usually applied to an insulator to make it electrically and thermally conductive is also the source of the bulk of the secondary electrons. A 10-nm layer of metal such as gold would certainly improve the coefficient of secondary-electron emission, δ, for an organic specimen examined at low kilovoltage, but might well diminish δ for a ceramic containing significant amounts of alkaline earth oxides.

Backscattered electrons have also been used in conjunction with standard cytological techniques to localize regions of physiological interest in biological tissue. Thus if one has gone to considerable effort to obtain deposits of lead or silver at specific sites in a piece of tissue it seems inappropriate to mask the atomic-number contrast these locations will give by covering them with a layer of heavy metal. The appropriate technique would be to apply a thin layer of a low-atomic-number conductor such as carbon which would not significantly scatter the incident beam, allowing it to reach the specimen.

For high-resolution, low-loss scanning electron microscopy where the image is dependent on the scattering of high-energy electrons from the specimen surface, it is necessary to coat the samples with a thin layer of a heavy metal which shows no structure at the 1-nm resolution level. Experimental evidence suggests that the refractory metals such as tantalum or tungsten yield such a coating.

10.1.1.4. Mechanical Stability

Particulate matter and fragile organic material are more firmly held in position on the specimen stub after coating with a thin layer of carbon. In many cases it is possible to place such material directly onto the specimen support and stabilize it with a very thin layer of carbon applied from two directions. This simple technique avoids the use of adhesives, most of which are highly nonconductive. Metal coatings, particulary those deposited by a sputtering process, are quite strong and contribute to the increased mechanical strength of otherwise fragile material.

10.1.1.5. Uncoated Specimens

Several methods can be used to examine uncoated specimens in the SEM, including operation at low beam energy, incorporating a second beam of electrons or ions to discharge the specimen, and examining the specimen in the presence of water.

Reduction of charging at low accelerating voltages is due to the characteristics of electron emission from solids, as indicated in the follow-
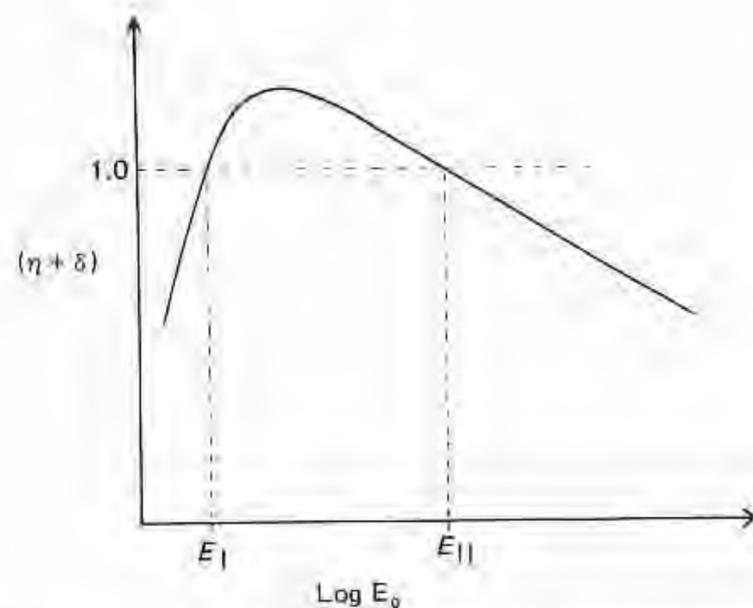
Figure 10.3. Total electron emission (backscattered and secondary electrons) as a function of beam incidence energy E_0. E_I and E_{II} are the first and second crossover points.

ing arguments due to Oatley (1972). If the electron emission coefficient, considering both backscattered primary electrons and secondary electrons, is plotted as a function of beam incident energy, E_0, the relationship shown in Figure 10.3 results. For insulators, a region exists for which the number of emitted electrons exceeds the number of incident electrons, i.e., $\delta + \eta > 1$. This region is defined by two values of the incident energy, E_I and E_{II} for which $\delta + \eta = 1$; these values are referred to as the first and second crossover points. E_I is of the order of several hundred electron volts and E_{II} ranges from 1 to 5 keV, depending on the material. If the incident beam energy is less than E_I, then $\delta + \eta < 1$ and fewer electrons leave the specimen than enter it, resulting in a buildup of negative charge. This charge lowers the effective energy of the incident beam, producing a further decrease in $\delta + \eta$. This situation continues until the specimen is charged to a sufficient level to totally deflect the beam. If the beam energy is between E_I and E_{II}, then more electrons leave the specimen than enter it, i.e., $\delta + \eta > 1$. This charges the specimen positively, and the positive charge acts to decrease the effective value of δ, since the low-energy secondary electrons are attracted back to the specimen. The effective value of $\delta + \eta$ becomes unity because of these processes, and a dynamic equilibrium is set up with the emitted current equal to the incident current at a small, positive, constant surface charge. Slight variations in the curve of Figure 10.3 at different places in the field of view are not significant, since these will result in slight differences in the equilibrium surface potential. Operation with the $E > E_{II}$ again results in negative surface charging, which decreases the effective value of E, raising $\delta + \eta$ until an equilibrium is

established with the effective E equal to E_{II}. Oatley (1972) points out that this equilibrium is unsatisfactory, since E_{II} can vary significantly about the surface, giving rise to large variations in the final surface potential from place to place. Slight leakage through surface conduction can greatly disturb such a situation, producing complicated image behavior as a function of time. It is thus desirable to operate with $E_I < E < E_{II}$, which is often achieved with E set to approximately 1 keV. Under such conditions, insulators can be examined uncoated. However, SEM performance is usually significantly poorer at such low accelerating potentials, since source brightness is greatly decreased under such conditions. With an SEM equipped with a field emission gun, the high brightness can be utilized to obtain good image resolution even at low beam energies (Welter and Coates, 1974).

Low beam energy prohibits x-ray microanalysis, since the overvoltage is inadequate except for x-rays of extremely low energies. To avoid this limitation several authors have reported methods which utilize a second beam of low-energy electrons or ions to discharge the sample during bombardment by the high-energy electron beam. Spivak et al. (1972) employed a pulsed beam of low-energy electrons to bombard the specimen during the scan line flybacks. The principle of the sample discharge is the same as that shown in Figure 10.3. Crawford (1979), described a charge neutralization method based on a low-energy beam of positive alkali ions which are attracted to the sites of negative surface charges.

If the specimen contains water, it will have sufficient conductivity to discharge. Considerable success has been obtained with examining and analyzing uncoated biological material at low termperatures (Echlin and Saubermann, 1977). In conditions where water is retained in the frozen state, so too are ions and electrolytes (Echlin, 1978) which provide conductivity. If specimens can be examined in an environmental cell, water can also be retained in the liquid state to discharge the primary beam (Robinson and Robinson, 1978).

10.1.2. Alternatives to Coating

One of the more useful techniques which has been devised is to increase the bulk conductivity of the sample as distinct from the *surface conductivity*. An increase in bulk conductivity may be achieved by metal impregnation from fixative solutions of osmium and manganese, with or without the use of organic metal ligands or mordants such as thiocarbohydrazide, galloglucose, paraphenylenediamine, by exposing specimens to OsO_4 vapor or by bulk staining the specimens after fixation with metallic salts. Kubotsu and Veda (1980) have devised a useful modification to the osmium vapor technique by also exposing the sample to hydrazine hydrate

vapors which result in the deposition of metallic osmium. The relative merits of these methods are discussed in the recent review papers by Munger (1977) and Murphey (1978, 1980).

Alternatively, specimen charging may be reduced by spraying or impregnating with organic antistatic agents derived from polyamines, i.e., Duron, Denkil, or sodium alkyl-benzene sulfonate, soaking in conducting colloids of noble metals or graphite, or covering the sample with a thin ($\sim$1.0–20.0-nm) polymer film such as Formvar or styrene-vinylpyridine (Pease and Bauley, 1975).

With the possible exception of the techniques which increase the bulk conductivity of the sample, none of these methods gives anything like the resolution and information content which may be obtained from properly coated samples. Indeed, these alternative methods have diminished in usefulness now that it has been shown (Panayi et al., 1977) that one can adequately coat even the most delicate and thermally sensitive specimens in the sputter coater. Further, it can be argued that if a sample cannot survive the moderate vacuum found in a sputter coater it is unlikely to survive the high vacuum of an electron beam instrument.

The techniques devised to increase bulk sample conductivity are useful especially when they are used in conjunction with coating techniques. Bulk sample conductivity methods are frequently used in connection with the examination of fractured surfaces of three-dimensional specimens whose internal morphology is being investigated. For example, the substructure of fractured surfaces may be revealed by atomic number contrast from osmium incorporation, rather than topographic contrast derived from surface irregularities.

10.1.3. Thin-Film Technology

Thin films can be produced in a variety of ways (Maissel and Glang, 1970), but of these methods only thermal evaporation and sputtering are useful for coating specimens for SEM and x-ray microanalysis. In discussing these methods below, it is important to consider the properties of the ideal film. Such a film should not exhibit any structural features above a scale of 3–4 nm resolution to avoid introducing unnecessary image artifacts. The ideal film should be of uniform thickness regardless of the specimen topography and should not contribute to the apparent chemical composition of the specimen or significantly modify the x-ray intensity emitted from the sample.

10.2. Thermal Evaporation

Many metals and some inorganic insulators when heated by one means or another in a vacuum begin to evaporate rapidly into a mono-

atomic state when their temperature has been raised sufficiently for the vapor pressure to reach a value in excess of 1.3 Pa (10^{-2} Torr). The high temperatures which are necessary to permit the evaporation of the materials can be achieved by three different methods.

In the resistive heating technique an electric current is used to heat a container made of a refractory material such as one of the metal oxides or a wire support made of a high-melting-point metal such as tungsten, molybdenum, or tantalum. The material to be evaporated is placed in or on the container, which is gradually heated until the substance melts and evaporates. In the electric arc method an arc is struck between two conductors separated by a few millimeters. Rapid evaporation of the conductor surface occurs. This is the usual way by which some of the high-melting-point metals are evaporated. For most high-melting-point materials such as tungsten, tantalum, and molybdenum, the most effective way of heating the substance is to use an electron beam. In this method the metal evaporant is the anode target and is heated by radiation from a cathode maintained at 2–3 keV. This is a very efficient means of heating, as the highest-temperature region is the vapor-emitting surface and not the evaporant source material. An advantage is that the metal evaporant is deposited with very small grain size. Electron gun evaporation can also be used for evaporating some of the lower-melting-point metals such as chromium and platinum which have a very small particle size.

We can conveniently consider evaporation methods under two headings, high- and low-vacuum techniques.

10.2.1. High-Vacuum Evaporation

In this context high-vacuum is considered to be the range between 10 μPa and 100 mPa ($\sim 10^{-7}$–10^{-3} Torr). High-vacuum evaporation techniques are commonly used in electron microscope laboratories.

The formation of a thin film is a complex process and proceeds through a series of well characterized steps: nucleation and coalescence to form a continuous film. The first atoms arriving at the surface of the specimen will only stay there if they can diffuse, collide, and adhere to each other on the surface to form nucleation sites of a critical size. The stronger the binding between the adsorbed atoms and the substrate, the higher the nucleation frequency and the smaller are critical nuclei. Most biological and organic samples are likely to have variable binding energy, which would result in variation of critical nuclei across the surface, producing uneven film deposition. For this reason precoating with carbon at low vacuum to cover the specimen with a homogeneous layer results in smaller, even-sized critical nuclei when subsequently coated with metal. For example the nucleation density of gold can be substantially increased by 5–10 nm carbon precoating. As deposition continues, the nucleation centers
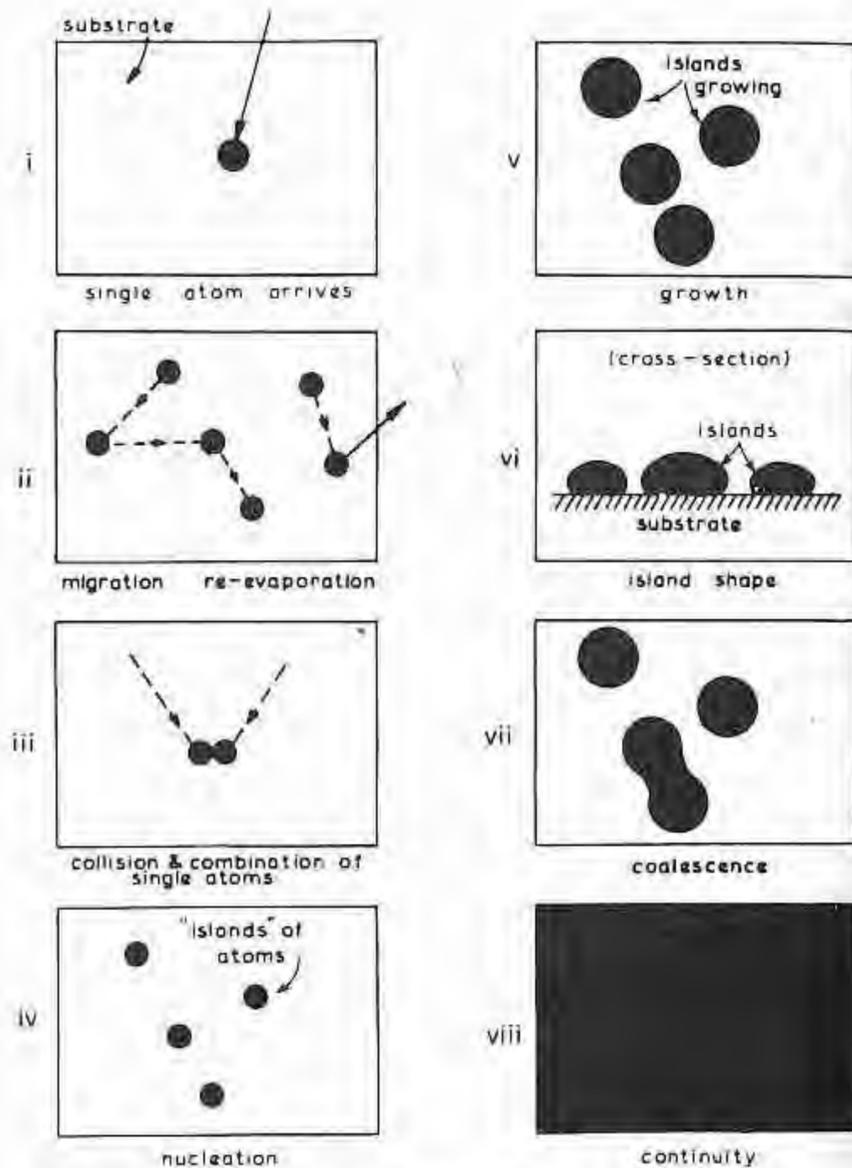
Figure 10.4a. Formation stages of a thin film (from Leaver and Chapman, 1971).

grow in three dimensions to form islands, which gradually coalesce to give a continuous film (Figures 10.4a–10.4e). The rate of continuous film formation and the average thickness at which a given film becomes continuous are influenced by a large number of variables. These include the nature of the evaporant and the substrate, the relative temperatures of the evaporant and substrates, the rate of deposition and final film thickness, and the surface topography of the specimen.

10.2.1.1. The Apparatus

There are four basic requirements for a high-vacuum evaporator. (1) It should have high pumping speeds at low gas pressure to ensure that there is
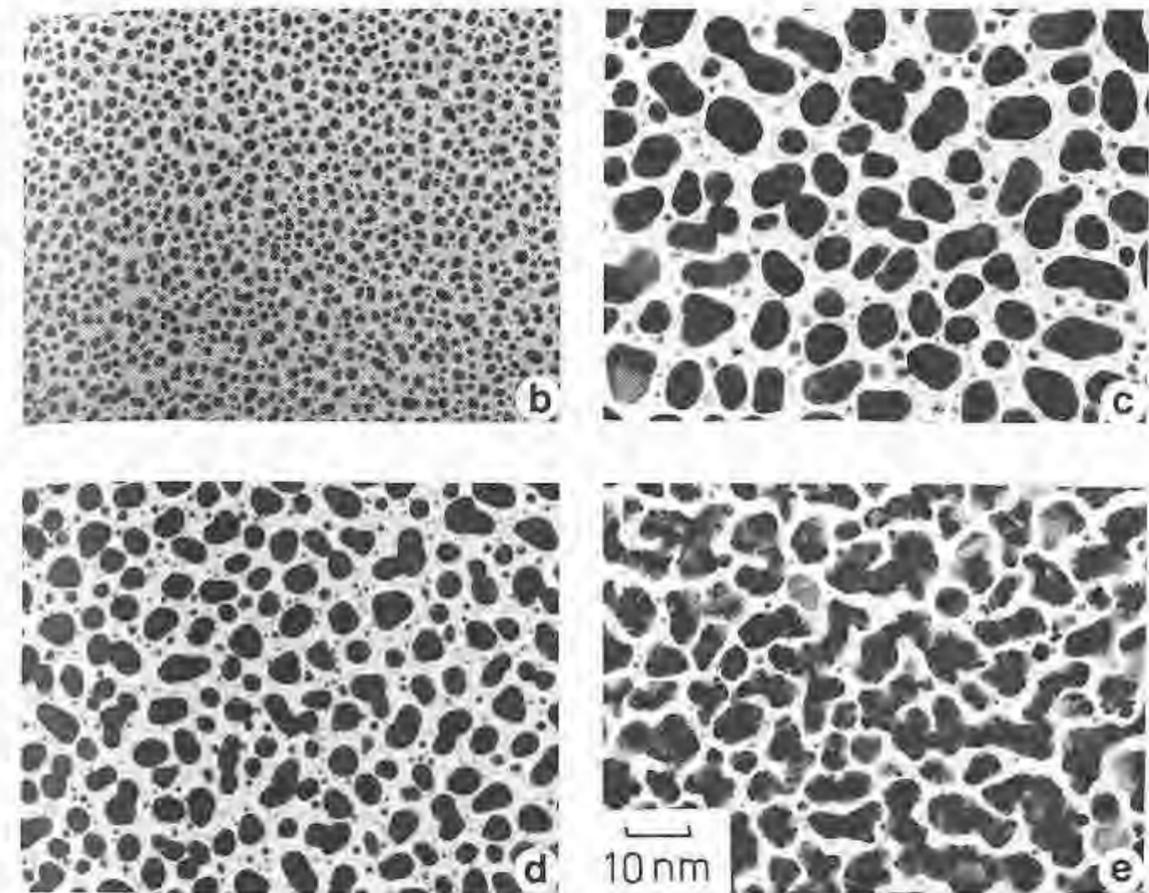
Figure 10.4b–e. Electron micrographs of ultrathin gold films evaporated at 0.05 nm/s. Thicknesses are (b) 1 nm, (c) 4 nm, (d) 6 nm, and (e) 15 nm. (From Kazmerski and Racine, 1975.)

a rapid removal of gases liberated from the evaporant source and specimen during coating. (2) There should be minimum backstreaming into the coating chamber of vapors from the pumps. (3) The system should be readily taken apart for cleaning and maintenance. (4) Adequate provision should be made for electrical connections for multiple evaporation schemes and sample manipulation.

Most units are pumped out using a diffusion pump backed by a rotary pump. The larger the throat size of the diffusion pump, i.e., 150 mm versus 75 mm, the faster the pump-down time of the evaporation chamber, although there will only be a marginal improvement in the ultimate pressure which is obtained. Some units are equipped with turbomolecular pumps backed by rotary pumps. Such units give faster pump-down times and a cleaner vacuum, although the ultimate pressure is no better than that obtained using a diffusion pump. The rotary pump should be a two-stage pump capable of being ballasted. Gas ballasting is one of the most effective means of preventing vapor condensation in a rotary pump. The method

consists of leaking a small quantity of air into the rotary pump during the compression cycle so that the exhausted vapor is mixed with a noncondensable gas. This decreases the compression necessary to raise the exhaust valve and prevents vapor condensation. Rotary pump vapor condensation can also be decreased by raising the pump temperature and by placing a desiccant such as P_2O_5 in the exhaust line. To avoid backstreaming, the system should not be pumped below 10 Pa (10^{-1} Torr) on the rotary pump alone. The rotary pump should not be exhausted into the laboratory but vented into a fume cupboard or to the outside. There seems little necessity to resort to ion pumps, sublimation pumps, or exotic cryopumping systems as ultrahigh vacuum (130 nPa to 130 pPa) is not required for the commonly used evaporation methods.

Whatever system is used it is necessary to ensure that the backing and roughing lines from the pumping unit to the chamber are fitted with baffles and/or an activated alumina foreline trap to minimize backstreaming of pump oil. Water-cooled baffles are quite effective, although liquid-nitrogen baffles are better. A liquid-nitrogen trap in the roughing line will minimize diffusion of forepump oil into the evaporation chamber during roughing operations and maintain the forepressure of a diffusion pump at approximately 10 mPa. It is also useful to have a liquid-nitrogen trap above the diffusion pump between it and the coating chamber. Care should be taken to note where the vacuum pressure is read in the system. If the pressure is read near the pumps it is likely to be ten times better than that found in the evaporation chamber. The evaporation chamber should be made of glass and as small as is convenient for the work to be carried out. A safety guard should be fitted over the evaporation chamber to minimize danger from implosion fragments. The chamber should contain at least four sets of electrical connections to allow evaporation of two different materials, specimen rotation and thin-film measurement. The electric power for the evaporation sources should be variable, and it is useful to have a pushbutton control to allow maximum power to be applied in short bursts. The unit should be brought up to atmospheric pressure by means of a controllable needle valve which can be connected to a dry inert gas.

10.2.1.2. Choice of Evaporant

The choice of material to be evaporated and the manner by which it is to be applied is very dependent on the particular application in hand. A tabulation of selected elements and their properties, which are useful for coatings, is listed in Table 14.11 of the data base, Chapter 14. For most SEM work, gold, gold–palladium, or platinum–carbon is used. Silver has a high secondary-electron coefficient and is one of best substances for faithfully following the surface contours. Unfortunately silver suffers from the disadvantages that it easily tarnishes and has a larger grain size than

other metals. Gold has a high secondary emission, is easily evaporated from tungsten wire, but has a tendency to graininess and agglomerates during coating, requiring a thicker coating layer to ensure a continuous film. A 60:40 gold–palladium alloy or palladium alone shows less granularity than gold and yields one of the thinnest continuous films. Unfortunately both metals easily alloy with the tungsten holder. Platinum–carbon when evaporated simultaneously produces a fine grain size but of rather low conductivity. Maeki and Benoki (1977) have found that low-angle ($\sim30°$) shadowing of specimens with evaporated chromium before carbon and gold–palladium coating improves the image of samples examined in the SEM. The finest granularity is obtained from high-melting-point metals, but they can only be evaporated with electron beam heaters. Figures 10.5 and 10.6
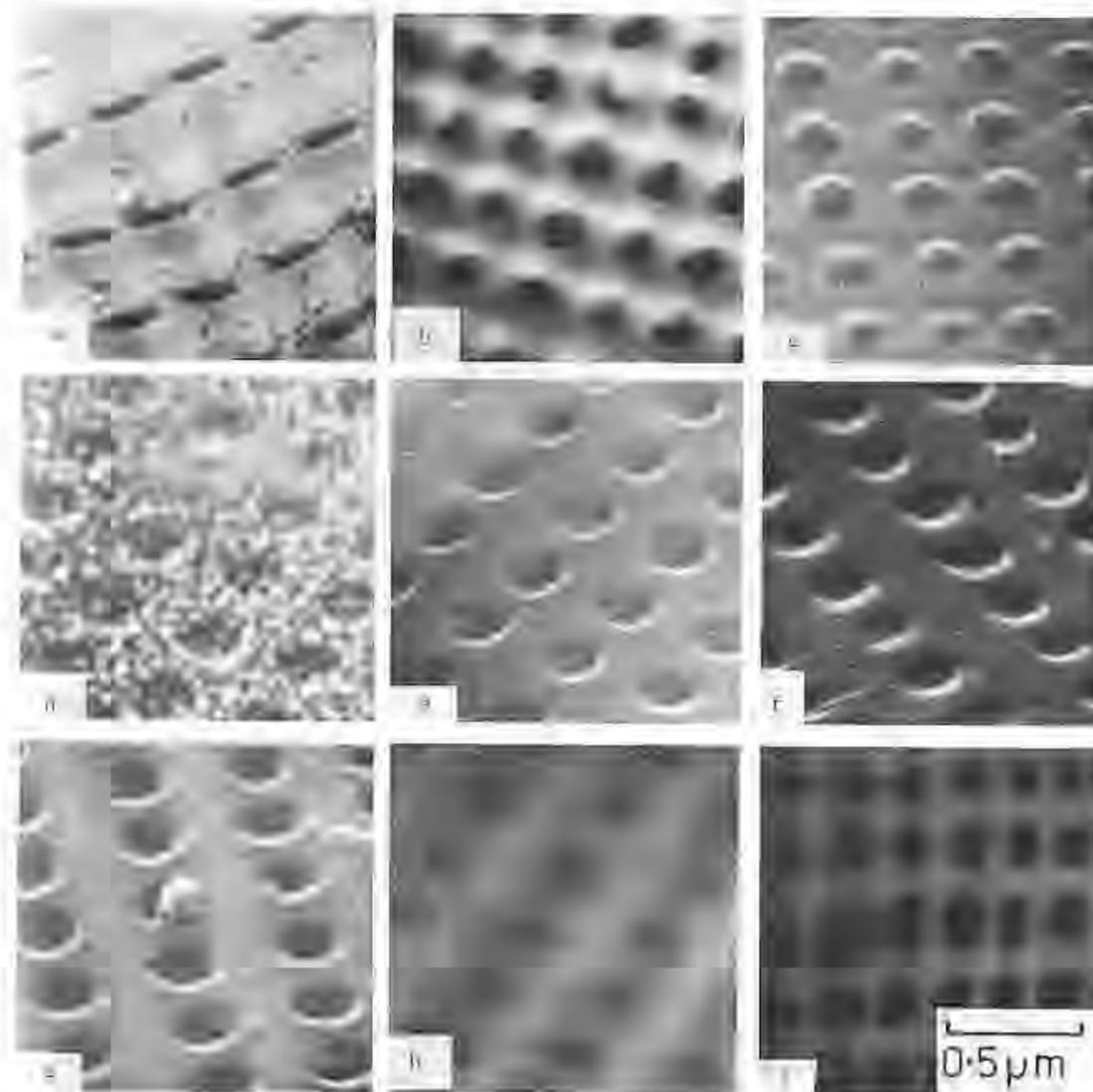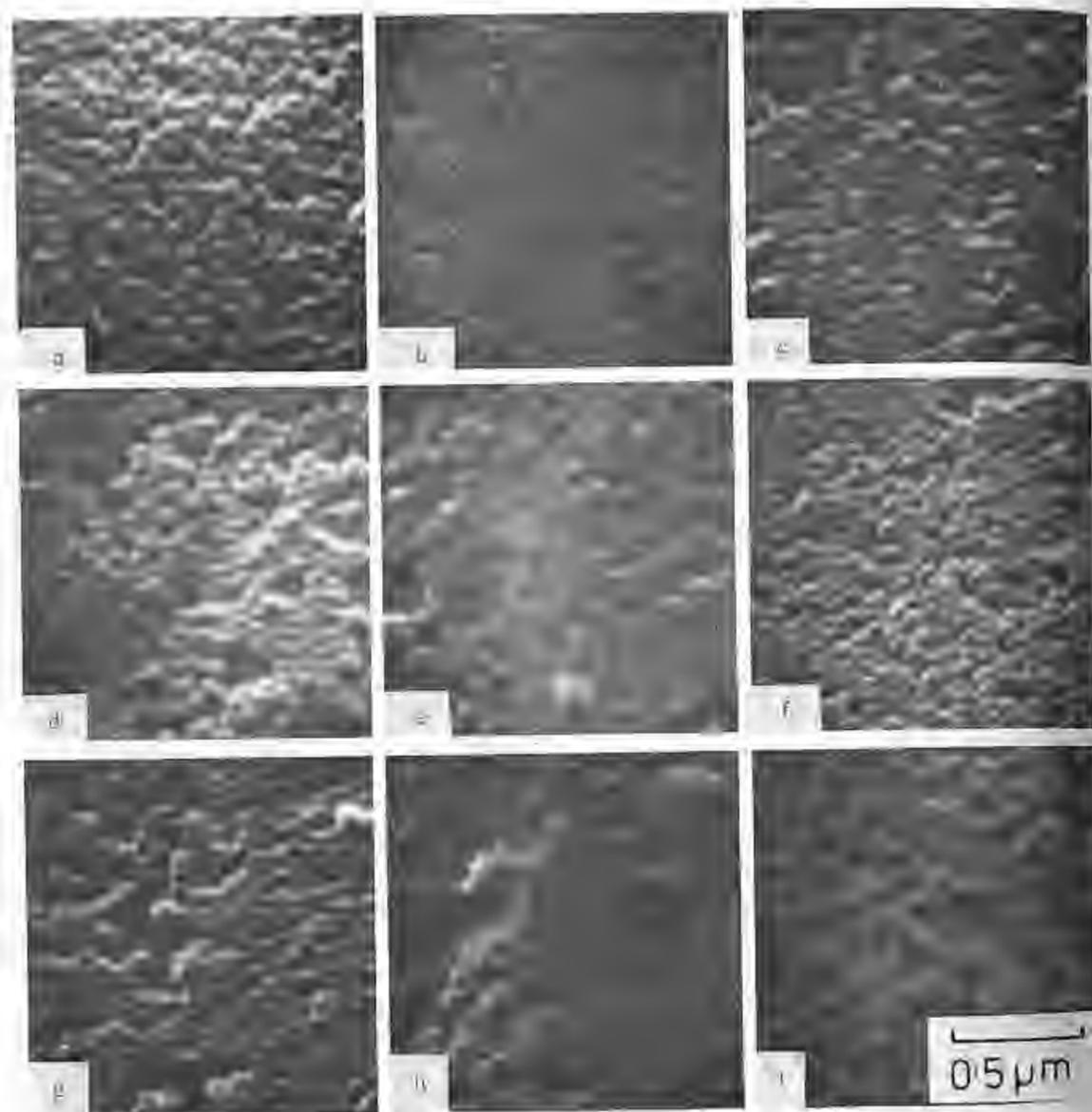

Figure 10.5. Cleaned diatom frustules coated with 10 nm of different metals and examined at 20 keV. (a) gold; (b) aluminum; (c) copper; (d) silver; (e) chromium; (f) gold–palladium; (g) titanium; (h) tin; (i) calcium.

Figure 10.6. Polystyrene latex spheres 0.109 μm in diameter coated with 10 nm of different metals and examined at 20 keV. (a) gold; (b) aluminum; (c) copper; (d) silver; (e) chromium; (f) gold–palladium; (g) titanium; (h) tin; (i) calcium.

show the differences in surface features of some of the metal thin films applied to nonconducting samples. The usual "rule of thumb" for x-ray microanalysis is that the thinnest coating yielding stable specimen current and x-ray flux is desired. This is true since the thinner the coating, the less x-ray absorption within it and the less the energy loss of the primary electron beam entering the specimen. Furthermore, the thinner the coating, the smaller will be the excitation of x-rays from the coating itself. For gold and gold–palladium coatings, which are often used in scanning electron microscopy to provide enough secondary electron flux, the characteristic and/or continuum radiation produced could interfere with the x-ray lines

of interest. Particular problems can occur if the element of interest is present in small or trace amounts. The usual thicknesses used range between 5 and 50 nm (50 and 500 Å). For 5–10-nm (50–100-Å) films of carbon, aluminum, gold, and gold–palladium, the energy loss of the primary beam appears to be of small consequence even at low nominal voltages. However, the beam and backscattered electrons obtained from the specimen could excite x-ray radiation from the film. This process may be particularly serious for gold and gold–palladium coatings on specimens with average atomic numbers greater than 10.

Examination of mass attenuation coefficients (μ/ρ) shows that for x-ray lines from 8 to 40 Å, aluminum is lowest of all four, followed in order by carbon, gold, and gold–palladium. In the region below 8 Å, the carbon (μ/ρ) value is lower than those for aluminum, gold, and gold–palladium. However, gold is best when electrical and thermal conductivities are considered, with aluminum about one-third as good, and carbon poor. It would seem that, for general purposes, aluminum is favored by its physical properties for use with x-ray lines of 0.8–4 nm (8–40 Å), while carbon is favored outside this region.

Most of the commonly used evaporants are available in the form of wire. It is recommended that thick wire, i.e., 0.5–1.0 mm is used, as short pieces can be easily looped over the appropriate refractory wire. Substances not available in wire form are available as powders or chips and can be conveniently evaporated from refractory crucibles or from boats made of high-melting-point metals. Care should be taken to ensure that the evaporant does not alloy or form compounds with the refractory. Most metals with a melting point below 2000 K can be evaporated from a support wire or boat made from a refractory metal such as tungsten, molybdenum, or tantalum. Such supports should be good electrical conductors, have a very low vapor pressure, and be mechanically stable.

10.2.1.3. Evaporation Techniques

When a pressure of about 10 mPa has been reached, the refractory support wire and, where appropriate, the carbon rods, can be heated to a dull red color. Gentle heating will cause a sharp rise in the vacuum pressure brought about by outgassing and removal of residual contamination. Once the outgassing is completed, the current is turned down and pumping continued. If a carbon coating is to be applied to the specimen before metal coating, this is best done at a pressure of about 10 mPa.

During coating the specimens are rotated and tilted to give an even coating on all surfaces. Following the deposition of the carbon layer the vacuum pressure is decreased to a pressure between 1.0 mPa and 100 μPa. An electric current may now be passed through the tungsten wire holding the metal to be evaporated. This should be done carefully and it is best to

gradually increase the current until the tungsten wire just begins to glow and then back off a small amount. This allows the metal evaporant to heat up slowly and melt as the tungsten wire current is slowly increased.

The metals commonly used in evaporation form a molten sphere in the V of the tungsten wire. This stage should be allowed to remain for a few moments to remove any residual contamination. The current should then be further increased until the sphere of molten metal appears to shimmer and "rotate." When this point is reached evaporation has started and the shutter may be opened to expose the rotating specimen to the evaporation source. In order to achieve a uniform coating on complexly sculptured specimens it is essential that they be rotated rapidly (6–8 rps) during coating. The ideal arrangement is to rotate the specimen in a planetary motion while continuously tilting it through 180°. This slow heating, melting, and evaporation of the metal evaporant is most important, and more particularly so with aluminum which alloys with tungsten. If the source is heated too rapidly the metal evaporant melts at its point of contact with the tungsten wire and falls off. However, the speed of coating is important in obtaining films of good quality, and the faster the speed of evaporation the finer the structure of the layer. This optimal high speed of coating must be balanced against the higher thermal output from the source, and the increased chance of the wire support alloying with the substrate and melting. The thickness of the evaporated film may be measured by a number of different techniques which will be described later, the most convenient of which is a quartz crystal thin-film monitor mounted inside the vacuum chamber. The thickness deposited also depends on the particular specimen being studied and the type of information required from the sample. It has usually been considered necessary to apply sufficient metal to give a continuous film since it has been assumed that only a continuous film would form a surface conductive layer on a nonconductive sample. Although the physical nature of charge transport in particulate films is not clearly understood, recent work suggests that particulate layers may be a useful method for SEM specimens because discontinuous metal films exhibit a significant dc conductivity. Paulson and Pierce (1972) have shown that discontinuous films can conduct a limited current by electron tunneling between evaporated island structures and suggest that such discontinuous films may be useful for the examination of nonconductive specimens viewed at very low current. Specimens have been successfully examined at 20 keV which have only been coated with 2.5 nm of gold (Figure 10.7).

The color of the layer on a white card or glass slide can give a quick practical estimate of thickness. For most specimens a carbon layer visible as a chocolate color and a gold layer which is a reddish-bronze color by reflected light and blue-green to transmitted light will be sufficient. For aluminum coating sufficient metal is deposited when the layer is a deep
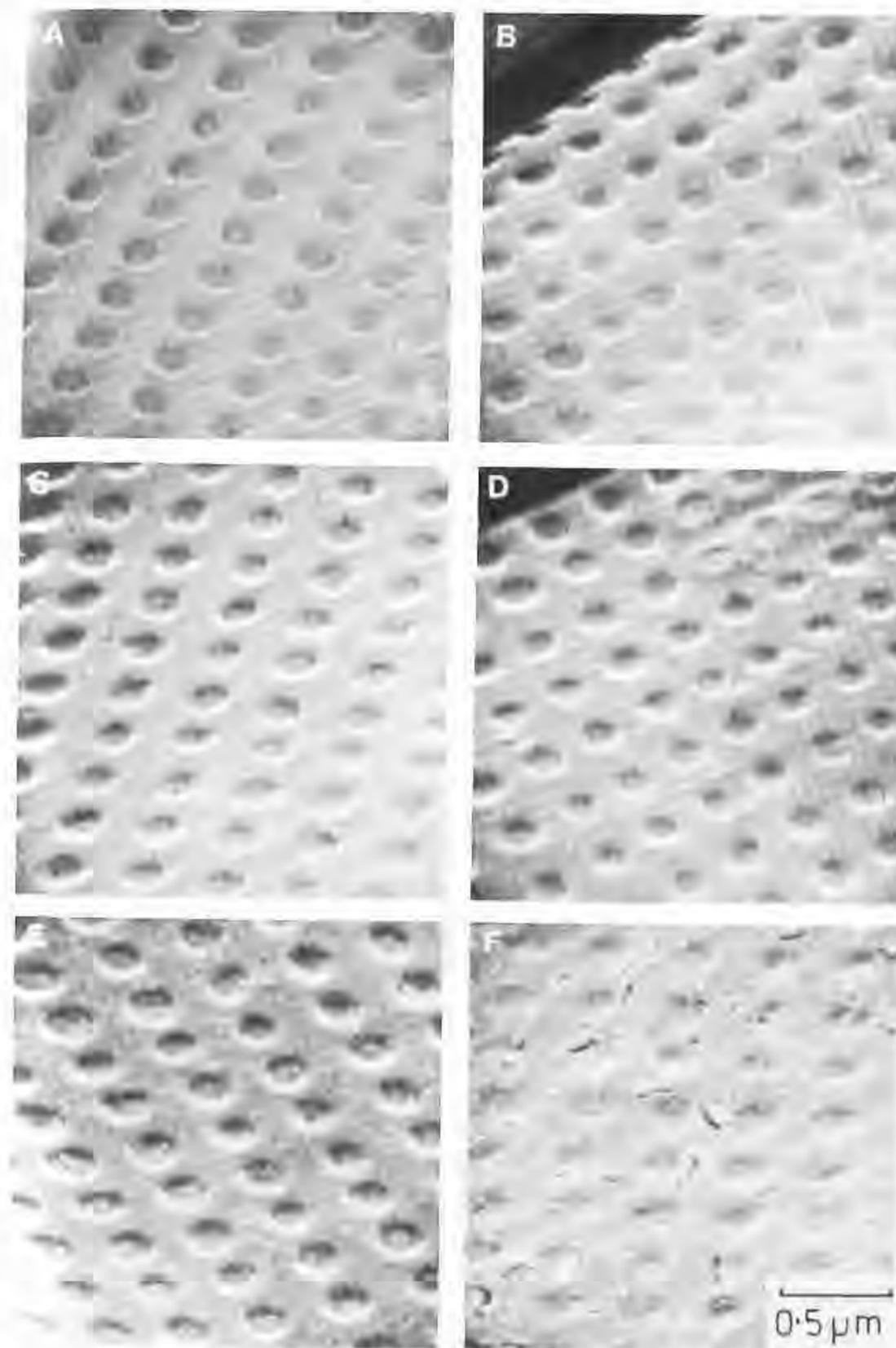
Figure 10.7. Cleaned diatom frustules coated by evaporation, with increasing thickness of gold. (a) 2.5 nm; (b) 5.0 nm; (c) 10 nm; (d) 20 nm; (e) 50 nm; (f) 100 nm and examined in the secondary mode of operation at 30 kV. Note that the very thin layers give optimal information, whereas the thicker layers obscure the specimen surface.