

blue to transmitted light. Once these parameters have been worked out for a particular evaporation unit with a fixed geometry of specimen and source, it is only necessary to cut off a standard length of evaporant metal wire for each coating.

#### 10.2.1.4. Coating Artifacts

If the coating procedure has been carried out properly, artifacts are rarely seen. Some of the possible causes of artifacts are discussed below.

(a) **Heat of Radiation.** The intensity of thermal radiation reaching the specimen depends on the source temperature and the source-to-specimen distance. The heat of radiation may be diminished by using a small source and/or moving the specimen further away. The best practical solution is to use a small source at high temperature and have at least 150 mm between the source and the sample. Provided the specimen is adequately shielded from the target, and the shutter only opened at the working temperature, little damage is likely to occur.

Thermal artifacts appear as smooth micromelted areas on inclined fracture faces of biological materials which are bombarded vertically, minute holes, and surface distortions. If specimens are being damaged by heat radiation, the damage can be diminished by placing a cold plate with an aperture over the specimen.

(b) **Contamination.** Contamination is due primarily to dirt and volatile substances in the vacuum system being deposited on the specimen, and it is for this reason that care must be taken to clean the system properly before use. The most effective way to reduce this problem is to surround the specimen with a cold surface. However, this is probably unnecessary except for very-high-resolution studies. Contamination may be recognized as uneven coating and hence charging, as small randomly arranged particulate matter, and in the most extreme situations as irregular dark areas on the specimen.

(c) **Decoration Effects.** Decoration or agglomeration of the evaporated material occurs to some extent with most metal coatings and is a result of uneven deposition of the evaporated metal. Agglomeration occurs if the cohesive forces of the film material are greater than the forces between the film molecules and the substrate. Because of geometrical effects, rough surfaces are particularly difficult to coat evenly, and it is inevitable that those parts which protrude will receive more coating than crevices and holes.

(d) **Film Adhesion.** Poor film adhesion is associated with hydrocarbon and water contamination, and, in the case of plastics, with the presence of a thin liquid layer of exuded plasticizer. Discontinuous and poorly adhesive films are recognized by a "crazed" appearance and have a

tendency to flake easily. In the microscope there are variations in the image brightness, and charging occurs on isolated "islands" of material which are not in contact with the rest of the film.

#### 10.2.2. Low-Vacuum Evaporation

In low-vacuum evaporation, carbon is evaporated in an atmosphere of argon at a pressure of about 1 Pa. The carbon atoms undergo multiple collisions and scatter in all directions. This technique is useful for preparing tough carbon films and for coating highly sculptured samples prior to analysis by x-rays, cathodoluminescence, and backscattered electrons. However, its general usefulness for SEM specimens is questionable, particularly as the yield of secondaries from carbon is very low. There is little doubt that the multiple scattering and surface diffusion of the carbon allows it to more effectively cover rough specimens, and for this reason would be a useful method to use if a sputter coater was not available.

#### 10.3. Sputter Coating

Although sputter coating has been known for a long time it is only recently that it has become more widely used for producing thin films. In the process of sputtering an energetic ion or neutral atom strikes the surface of a target and imparts momentum to the atoms over a range of a few nanometers. Some of the atoms receive enough energy in the collision to break the bonds with their neighbors and be dislodged. If the velocity imparted to them is sufficient, they are carried away from the solid (Wehner and Anderson, 1970).

The glow discharge normally associated with sputter coating is a result of electrons being ejected from the negatively charged target. Under the influence of an applied voltage the electron accelerates towards the positive anode and may collide with a gas molecule, leaving behind an ion and an extra free electron. The glow discharge is located some distance from the target. The positive ions are then accelerated towards the negatively biased target where they cause sputtering. At high accelerating voltages many electrons are ejected per impinging ion, and these electrons have sufficient energy to damage delicate targets.

A number of factors affect the deposition rate. The sputtering yield increases slowly with the energy of the bombarding gas ion (Figure 10.8). The current density has a greater effect than the voltage on the number of ions striking the target and thus is the more important parameter determining the deposition rate (Figure 10.9). Variations in the power input can have a dramatic effect on the properties and composition of sputtered

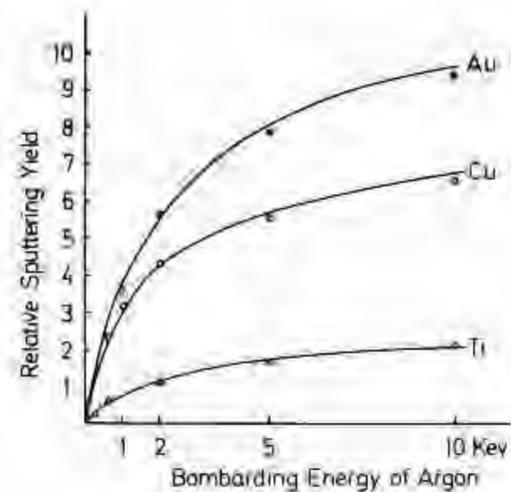


Figure 10.8. Relative sputtering yield of different metals at different bombarding energies of argon.

films; for example, with increasing power levels, aluminum films became smoother with fewer oxide particles. As the pressure of the sputtering system is increased, the ion density also increases. There is a linear increase in current, and hence sputtering rate, between pressures of 3 and 20 Pa. But because there is an increased tendency at higher pressures for the eroded material to return to the target, a compromise pressure of between 3 and 10 Pa is commonly used.

Impurities in the bombarding gas can appreciably reduce the deposition rate. Gases such as CO<sub>2</sub> and H<sub>2</sub>O are decomposed in the glow discharge to form O<sub>2</sub>, and the presence of this gas can halve the deposition rate. Deposition rate decreases with an increase in specimen temperature, although this phenomenon may not be peculiar to sputter coating. Finally, the deposition rate is higher the closer the target is to the specimen, but this also increases the heat load on the specimen. The ejected particles arrive at the substrate surface with high kinetic energy either as atoms or clusters of atoms, but not as vapor. There is some evidence that the sputtered atoms

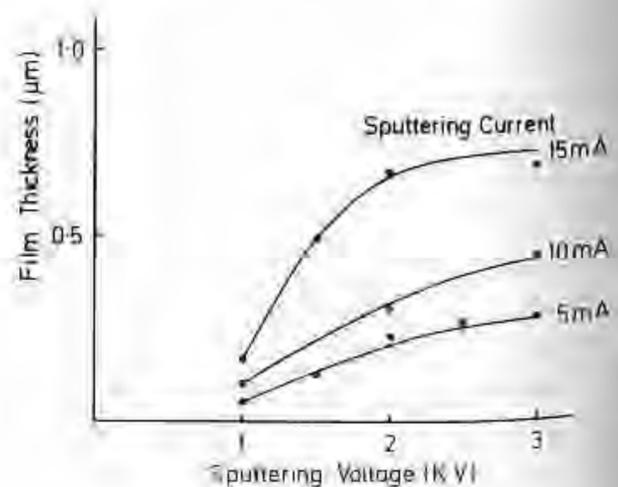


Figure 10.9. Variations in film thickness in relation to the sputtering voltage and current.

have sufficient energy to penetrate one or two atomic layers into the surface on which they land.

There are several different ways to produce the sputtering process, including ion beam sputtering, plasma sputtering, radiofrequency sputtering, triode sputtering, diode (DC) sputtering, and cool diode sputtering. As the technique has evolved for SEM and electron probe specimen coating, only ion beam, diode, and cool diode sputtering are now commonly employed.

10.3.1. Ion Beam Sputtering

The technique of ion beam sputtering is illustrated in Figure 10.10a. An inert gas such as argon is ionized in a cold cathode discharge, and the resulting ions from the ion gun are accelerated to an energy of 1-30 keV.

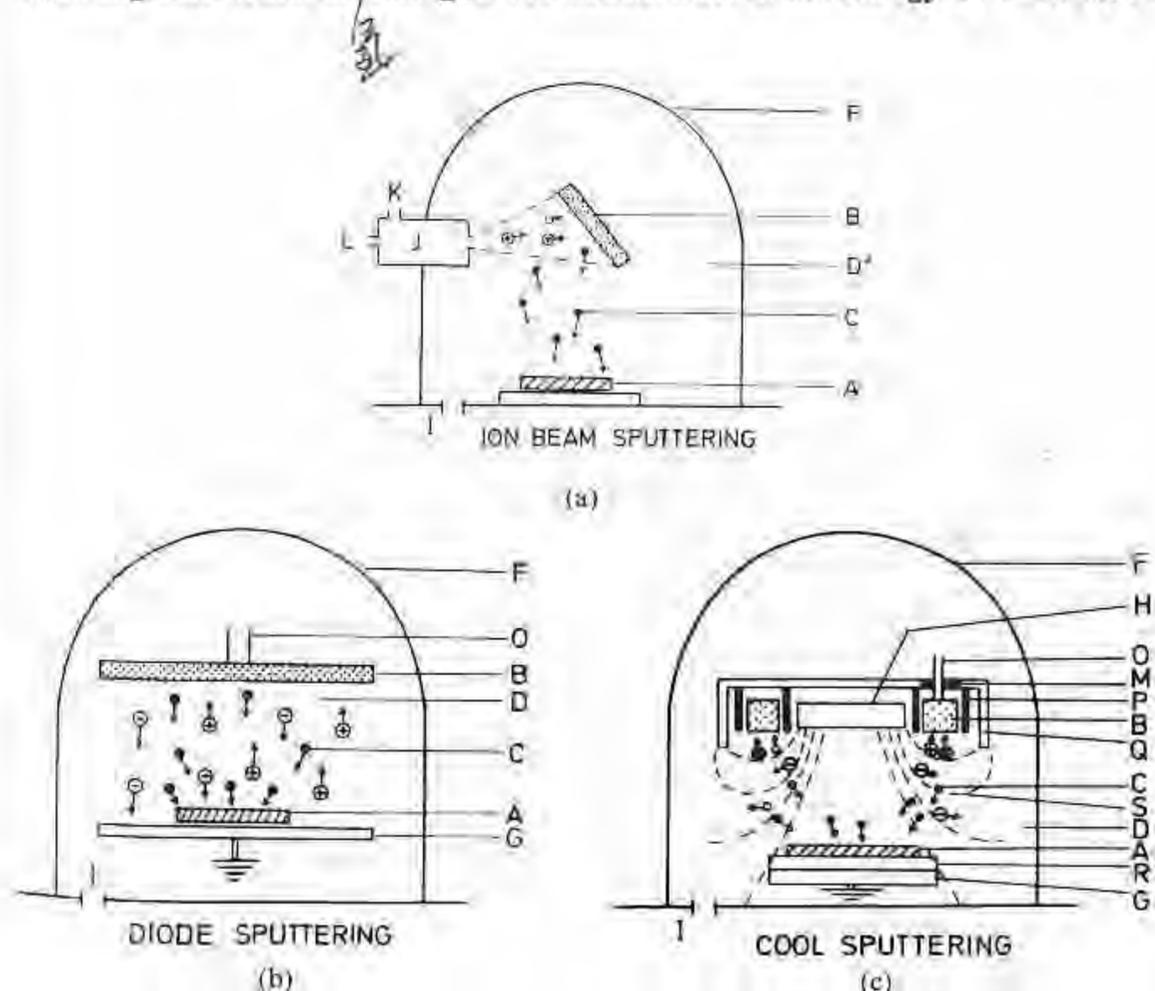


Figure 10.10. Sputter coating techniques: (a) ion beam sputtering; (b) diode sputtering; (c) cool diode sputtering. Key to symbols: A, specimen; B, target or evaporative source; C, target atoms; D, low-pressure argon discharge; D', high vacuum; E, electrode; F, glass vacuum vessel; G, anode; H, permanent magnet; I, line to vacuum pumps; J, ion gun; K, inert gas supply; L, high-voltage power supply; M, insulator; O, direct current power supply (1-3 keV); P, aluminum shield; Q, iron pole piece; R, cooling module; S, lines of magnetic flux.

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The ion beam may be formed by collimation or focused with a simple lens system to strike the target. The energetic ions strike the target atoms and impart momentum in elastic collisions; atoms lying near the surface of the target are ejected with energies in the range 0–100 eV. These sputtered atoms then deposit on the sample and all surfaces which have a line of sight to the target. An advantage of this arrangement is that a field-free region exists between the target and substrate so that negative ions and electrons are not accelerated toward the substrate. Multiple coatings can be applied from different targets, providing care is taken to prevent cross-contamination of the targets during sputtering. If a nonconducting target is used, the buildup of positive charge can be suppressed by an electron flood gun. Ion beam sputtering has been used by Adachi *et al.* (1976) and Hojou *et al.* (1977) for high-resolution shadowing. By using sputtered films of tungsten-tantalum, tungsten, and carbon, they were able to see details smaller than 1.0 nm.

### 10.3.2. Diode or Direct Current Sputtering

This is the simplest, most reliable, and most economical type of sputtering and is the basis of a number of commercially available dedicated instruments as well as sputtering attachments for thermal evaporators. These instruments, which operate between 1 and 3 keV, are sometimes referred to as diode sputtering units as well as DC sputtering units. DC sputter coating units consist of a small bell jar containing the cathode target and water-cooled specimen holder anode, which sits on top of a control module containing the vacuum gauge, high-voltage power supplies, leak valve, and a small timer (Figure 10.10b). The detailed mode of operation and application of this type of instrument has been previously described (Echlin, 1975a). One of the potential problems of this type of coater is that delicate specimens can be thermally damaged.

### 10.3.3. Cool Diode Sputtering

The heat problem of diode sputtering has been overcome by redesigning the diode coater to incorporate devices to keep the specimen cool throughout the coating procedure (Panayi *et al.*, 1977) as shown in Figure 10.10c. The electron bombardment of the specimens is significantly reduced by replacing the disk-shaped target of the diode coater with an annular target. Thermal damage to the specimen is further reduced by fitting a permanent magnet at the center of the target and an annular pole piece around the target. This arrangement deflects the electrons outside the periphery of the specimen holder. As a further precaution, the specimen holder is cooled by a small Peltier effect cooling module. Using this

improved equipment it has been possible to coat crystalline hydrocarbon waxes with melting points as low as 305 K, and thermolabile sensitive plastic films, neither of which had been previously coated successfully by any of the methods available.

### 10.3.4. Sputtering Techniques

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It is important to ensure that the coater is fitted to an adequate supply of clean, dry argon or, where appropriate, another noble gas. A small trace of nitrogen in the argon probably does not matter, but it is important that the gas is free of water, carbon dioxide, and oxygen. Traces of water vapor, which are recognizable by the blue tint in the glow discharge, can easily be removed by passing the gas through a column of desiccant. The final traces of volatile material may be removed from the target by bombarding it with a current of 20 mA at 2.5 kV at a pressure of between 2 and 8 pA. The target can be considered clean when there is no degradation of vacuum when the high-voltage discharge is switched on.

Specimens are placed on the specimen table and the unit pumped down to about 10–15 Pa ( $10^{-1}$  Torr) using a two-stage rotary pump fitted with an activated alumina foreline trap to prevent oil backstreaming. It is most important not to let the unit pump for a long time at the ultimate pressure which may be obtained by the rotary pump, because this will cause backstreaming, resulting in contamination. Because of this it is advisable to have the argon leak valve slightly open, to ensure a continual flow of inert gas through the system giving a pressure of about 6–7 Pa. If the unit is fitted with a water-cooled or, better still, a Peltier module cold stage, this should be turned on and the specimens cooled to the working temperature.

One of the commonest sources of poor pumping performance in both sputter and evaporative coaters is the continual outgassing of the specimen and the adhesive used to attach it to the support. It is recommended that biological and organic material, after it has been dried and attached to the specimen support, be placed in a 310 K vacuum oven overnight to ensure that all volatile substances are removed before coating.

Once the specimen has been cooled and an adequate pressure has been reached, the sputter coating may proceed. The system is pumped down to about 2 pA and the high voltage turned to 2.0 kV. The argon leak valve is opened until a plasma discharge current of 12–15 mA is recorded at a pressure of 6–7 Pa. The timer is set and coating continued until the desired thickness has been deposited on the specimen.

It is possible to use nitrogen as an alternative to argon. However, extended discharge times and/or higher plasma currents are necessary to give the same thickness of coating. Sputter coating in air should be avoided.

because the presence of water vapor, carbon dioxide, and oxygen gives rise to highly reactive ions which can quickly degrade the specimen.

### 10.3.5. Choice of Target

Platinum or the gold-palladium targets have been found to be satisfactory for the routine specimen preparation for the SEM. Targets are available in most of the other noble metals and their alloys as well as elements such as nickel, chromium, and copper. There are differences in the sputtering yield from different elements and these must be borne in mind when calculating the coating thickness. There are difficulties with a carbon target, for although it is possible to very slowly erode the target with argon, the sputtering rate falls off rather rapidly. This decrease is due either to the presence of forms of carbon which have a binding energy higher than the energy of the argon ions, or the poorer conductivity of the carbon gives rise to charging and a decreased erosion rate. The claims that carbon can be sputtered at low kilovoltage in a diode sputter coater are probably erroneous. The deposits of "carbon" which are achieved are more likely to be hydrocarbon contaminants degraded in the plasma than material eroded from the target. There seems to be little likelihood that a simple technique will be devised for sputter coating aluminum. The oxide layer which rapidly forms on the surface of aluminum is resistant to erosion at low keV, and the rather poor vacuum makes it difficult to deposit the metal. For details of other targets, particularly those made by nonconducting materials and bombarding gases, the reader is referred to the book by Maissel and Glang (1970).

### 10.3.6. Coating Thickness

For a given instrumental setting there is a linear relationship between the deposition rate and the power input. In practical terms this means the thickness of the coating layer is dependent on gas pressure, the plasma current, voltage, and duration of discharge. It is difficult to accurately deposit thin films, i.e., below 10 nm, and it is necessary to rely on evaporative techniques for such films. However, most biological material requires about 15–25 nm of metal coating, and once one has established the ideal film thickness for a given specimen it is easy to accurately reproduce this thickness on other samples.

### 10.3.7. Advantages of Sputter Coating

One of the main advantages of the technique is that it provides a continuous coating layer even on those parts of the specimen which are not in line of sight of the target. Figure 10.11 compares the major coating

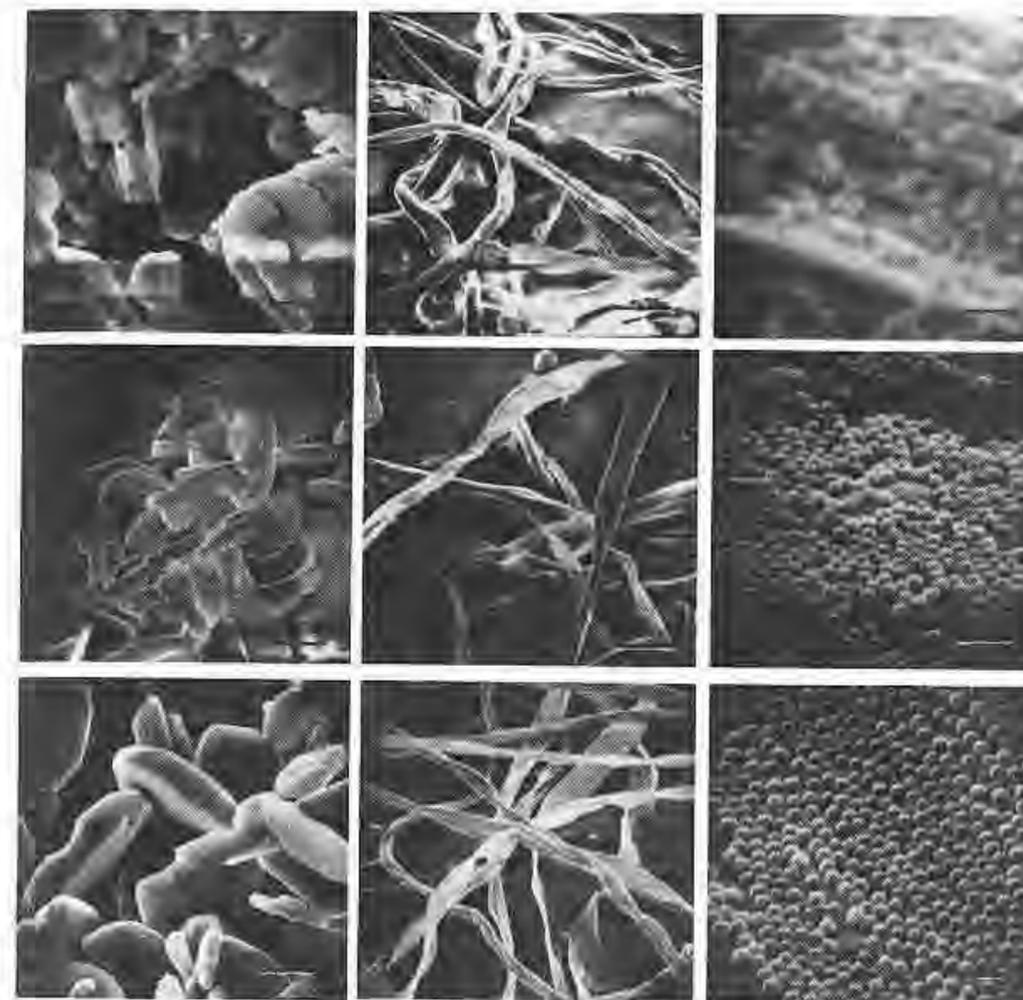


Figure 10.11. Comparison of uncoated, evaporative coated and sputter coated nonconductors. Left,  $\text{Al}_2\text{O}_3$ ; center, cotton wool; right, polystyrene latex spheres. Top row, uncoated; center row, evaporative coated with 10 nm of gold; bottom row, sputter coated with 10 nm of gold. Marker = 1  $\mu\text{m}$ .

processes. The continuous layer is achieved since sputtering is carried out at relatively high pressures and the target atoms suffer many collisions and are traveling in all directions as they arrive at the surface of the specimen. Highly sculptured structures or complexly reticulate surfaces are adequately coated. This ability of the target atoms to "go round corners" is particularly important in coating nonconductive biological materials, porous ceramics, and fibers. Complete coating is achieved without rotating or tilting the specimen, and using only a single source of coating material. Provided the accelerating voltage is sufficiently high it should be possible to sputter coat a number of nonconducting substances such as the alkaline halides and alkaline earth oxides, both of which have a high secondary-electron emission. Similarly, it should be possible to sputter substances which dissociate during evaporation. Film thickness control is relatively simple, and sputtering can be accomplished from large-area targets which

contain sufficient material for many deposition runs. There are no difficulties with large agglomerations of material landing on the specimen, and the specimens may more conveniently be coated from above. The surface of specimens can be easily cleaned before coating either by ion bombardment or by reversing the polarity of the electrodes. The plasma may be manipulated with magnetic fields, which gives greater film uniformity and reduces specimen heating.

### 10.3.8. Artifacts Associated with Sputter Coating

Sputter coating of SEM samples has attracted some unfavorable comments, because in the hands of certain users it has resulted in thermal damage to delicate specimens and to surface decoration artifacts. There is little doubt that the earlier diode sputter coaters could cause damage to heat-sensitive specimens, particularly if coating was carried out for a long time at high plasma currents on uncooled specimens. The thermal damage problem has been greatly reduced with the advent of the new series of cool sputter coaters. A careful examination of the examples of decoration artifacts which have been reported in the literature reveals that in most cases <sup>scan</sup> regard has been paid to cleanliness during coating, and that the artifacts were due to contamination from backstreaming oil vapors and/or the use of impure or inappropriate bombarding gases. Nevertheless it would be improper to suggest that artifacts never occur with sputter coated materials and some of the more common problems are discussed below.

#### 10.3.8.1. Thermal Damage

A significant rise in the temperature of the specimen can be obtained during sputter coating. The sources of heat are radiation from the target and electron bombardment of the specimen. There is an initial rapid rise in temperature which then begins to level out and, depending on the nature of the material being coated, may cause thermal damage. Depending on the accelerating voltage and the plasma current, the temperature rise can be as much as 40 K above ambient. However, as mentioned earlier heating effects can be entirely avoided by using the modified diode cool coater where the heat input due to electron bombardment is only 200 mW or somewhat reduced by operating a conventional diode coater intermittently at low power input.

When thermal damage occurs it is manifest as melting, pitting, and, in extreme cases, complete destruction of the specimen. While accepting that thermal damage can be a problem in sputter coating, in nearly all cases where this has been reported it is due to the specimen being subjected to inordinately high power fluxes.

#### 10.3.8.2. Surface Etching

This is a potential hazard in sputter coating and may be caused either by stray bombarding gas ions or, more likely, by metal particles hitting the surface with sufficient force to erode it away. It is possible to find very small holes in the surface of sputter coated specimens examined at high resolution. It is not clear whether these are the result of surface etching or simply thermal damage.

#### 10.3.8.3. Film Adhesion

Film adhesion is much less of a problem with sputter coated films than with evaporated films and is probably due to the fact that the metal particle penetrates the surface of the specimen, forming a strong bond. However, sputter coated samples should not be subject to wide excursions of temperature or humidity, both of which can give rise to expansion and contraction with a consequent rupture of the surface film.

#### 10.3.8.4. Contamination

Because of the low vacuum used in most sputter coaters, the problem of backstreaming from the rotary pumps, and the difficulty of placing effective cold traps in the backing lines, contamination can be a potentially serious problem, particularly if no foreline traps have been fitted. Many of the artifacts which have been described are probably due to contamination, and care should be taken in setting up and using the sputter coater.

## 10.4. Specialized Coating Methods

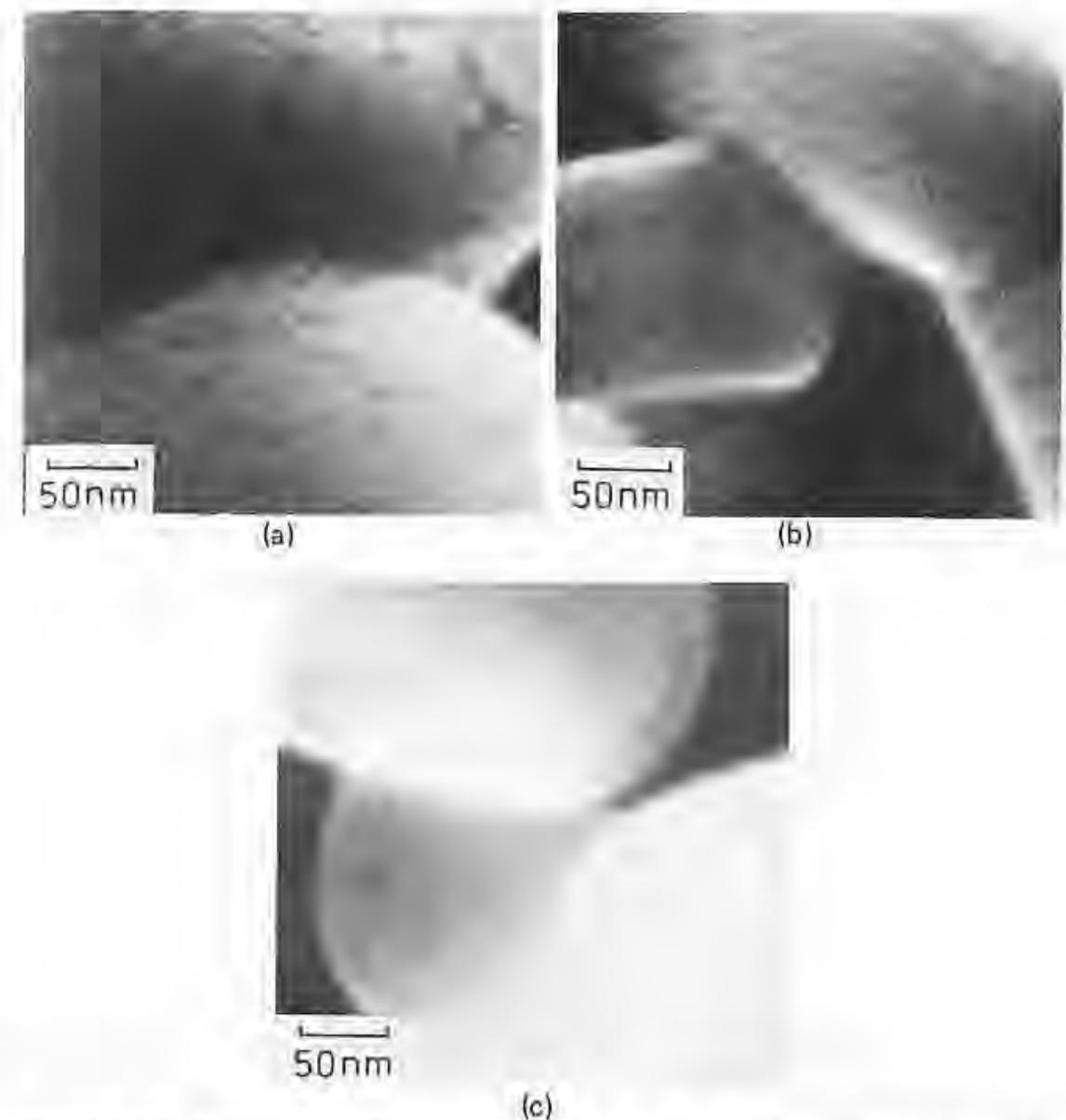
Although a description has been given of the general principles of evaporative and sputter coating, there are a number of specialized coating methods which should be discussed because they are applicable to both scanning electron microscopy and x-ray microanalysis.

### 10.4.1. High-Resolution Coating

As the resolution of scanning electron microscopes increases, greater attention must be given to the resolution limits of the coating layer. For many instruments which can resolve about 10 nm, the granularity of the film is of little consequence, with the possible exception of agglomeration of gold particles. A number of microscopes are routinely operating in the 5-10 nm range and the STEM instruments can give between 2- and 5-nm

resolution in the secondary mode. Transmission electron microscopists have long been concerned about high-resolution films, as they are necessary for specimen support, for shadowing, and for making replicas of frozen and ambient temperature fractured material. It is interesting to note that one of the favorite coating materials for scanning electron microscopists—gold—has a coarse granularity when examined at high resolution in the TEM. Chromium, gold-palladium, platinum, and zirconium have much finer grain size, and carbon, platinum-carbon, tungsten, and tantalum all have virtually no grain size when examined in the TEM. The graininess of the deposits generally decreases with an increase in the melting temperature. The studies of Adachi *et al.* (1976) and Hojou *et al.* (1977) show that sputter coated films of tantalum and tungsten have very small grain size and would be most suitable for high-resolution SEM studies.

Bräten (1978) found that thermally evaporated gold-palladium and carbon-gold-palladium gave the best result as far as specimen resolution and surface smoothness was concerned. Thermally evaporated and sputter coated gold gave a far more grainy appearance, and interweaving cracks could be seen on the surface of the specimen. The ultimate particle size is also dependent on the nature of the substrate. Echlin and Kaye (1979) and Echlin *et al.* (1980) found that for high-resolution (2–3 nm) SEM, electron beam evaporation of refractory metals (W, Ta) or carbon-platinum gave the best results. The most convenient means of preparing thin films for medium resolution (5–8 nm) SEM is to sputter coat films of platinum or gold-palladium onto specimens maintained below ambient temperature. Slower sputtering rates also result in smaller particle size. Advantage can also be made of the conductivity of discontinuous metal films which can provide an effective coating layer when only a few nanometers thick. Franks (1980) has used another sputtering technique based on a cold cathode saddle-field source to produce an ion beam. The high-energy beam was used to bombard a target producing coatings at a pressure of 10 mPa. The beam is directed onto the target with the substrate at a fixed or variable angle, and provides very fine grain deposits. Although the coating times are rather long (10–15 min) the films which are produced are ideal for high-resolution SEM studies. Figure 10.12 compares the results obtained with ion beam sputtering with those obtained with cold DC sputtering and evaporative coating. Whereas the sputter coated material and the evaporative coated material show artifacts at high resolution, only random electron noise is evident in the ion beam sputtered material. In a recent study, Clay and Peace (1981) have used ion beam sputtering to coat biological samples. They showed a significant reduction in surface artifacts compared to material which has been diode sputter coated (Figure 10.13). Peters (1980a) has used penning sputtering, which generates high-energy

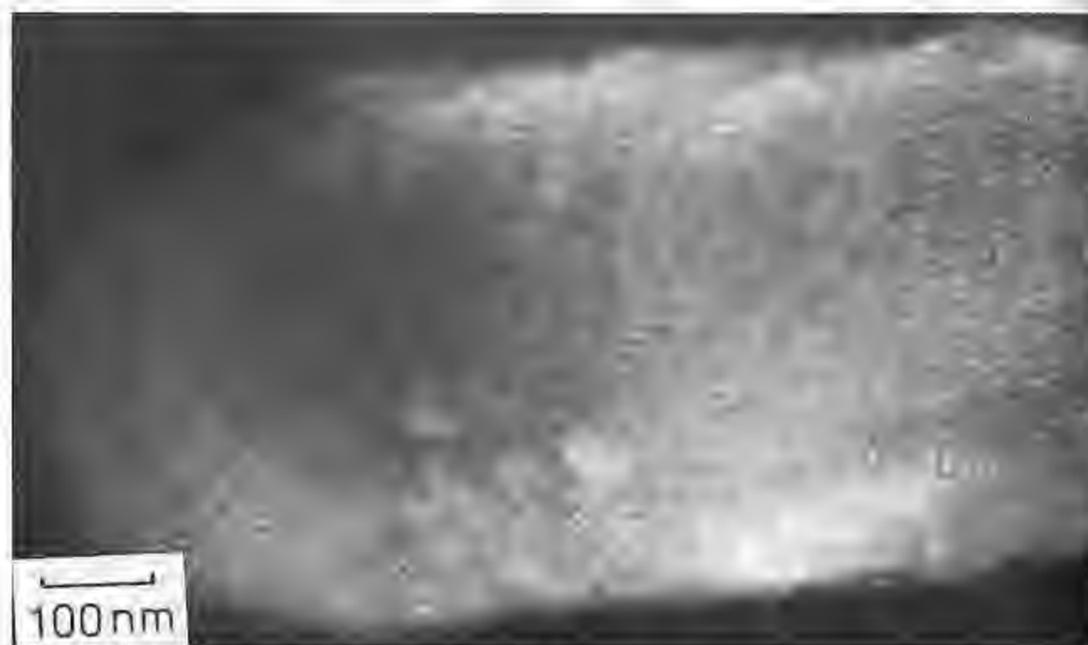


**Figure 10.12.** A comparison of (a) evaporative coating, (b) diode sputter coating, and (c) ion-beam sputter coating. Each sample has been coated with approximately the same amount of gold. The specimen is copy-paper crystals. Whereas the sputter coated and evaporative coated materials show artifacts at these high magnifications, only random electron noise is visible on the ion-beam sputtered material (Franks, 1980). Original micrographs taken by C. S. Clay, Unilever Research Laboratories, Sharnbrook, U.K.

target atoms resulting in very small crystallites. This technique allows very thin, i.e., 1–2-nm-thick films, to be produced with small particle size. In an earlier study, Peters (1979), has showed that provided great care is taken to minimize contamination, high-resolution films can also be obtained by thermal evaporation of high-melting-point metals.



(a)



(b)

**Figure 10.13.** The bacteria *Pseudomonas fluorescens*. (a) Cool diode sputter coated with gold-palladium; (b) ion-beam sputter coated with gold. Note the granularity on the diode sputter coated material, which is absent on the ion-sputtered sample (Clay and Peace, 1981).

#### 10.4.2. Low-Temperature Coating

The advantages of examining and analyzing frozen specimens in the scanning electron microscope and the x-ray microprobe have been discussed (Echlin and Saubermann, 1977; Echlin, 1978; Saubermann *et al.*,

1981). The main problems associated with low-temperature coating are contamination and maintaining the specimen below 143 K during the coating procedures. The main source of contamination is residual water vapor, which can readily interact with the metal during the coating process. Saubermann and Echlin (1975) found that unless great care was taken during the coating process, evaporated aluminum was deposited as a grey granular layer onto thin sections of frozen biological material held at below 123 K. A similar phenomenon was occasionally observed during gold coating of fractured, bulk frozen material. These effects are entirely eliminated if the coating is carried in a coating unit interfaced with the microscope via an airlock (Fuchs *et al.*, 1978; Pawley and Norton, 1978; Echlin *et al.*, 1979, 1980).

Low-temperature coating has only been successfully achieved using evaporative techniques, and care has to be taken not to melt the surface of the specimen during the coating procedure. Clark *et al.* (1976), using thin-film thermocouples, recorded an insignificant temperature rise on a thin-film substrate coated from a collimated and shuttered evaporative source. Although attempts have been made to sputter coat frozen samples (Robards and Crosby, 1979), the results bear no comparison with those which can be obtained using evaporative methods.

#### 10.5. Determination of Coating Thickness

There are a number of techniques which can be used to determine coating thickness, and a recent paper by Flood (1980) reviews the methods which are available. However, all the methods give an average value on a flat surface and it is necessary to briefly consider this type of measurement in relation to the coating thickness one might expect on rough surfaces. If the coating thickness is too thin it will be ineffective in eliminating the charge; if it is too thick it will obscure surface detail. A coating layer of a certain minimum thickness is necessary to form a continuous layer, and this thickness varies for different elements and for the relative roughness of the surface. On an absolutely flat surface a layer of carbon 0.5 nm thick will form a continuous coat. A layer ten times as thick is required to form a coat on an irregular surface. As a general rule, thicker coatings are required for irregular surfaces to ensure a continuous film over all edges, cavities, and protrusions.

It is clear that the film thickness we can measure on a flat surface bears little relation to the thickness we obtain on an irregular surface which may have been rotated and tilted during coating. For practical purposes, a given set of rough specimens should be coated with different thicknesses under standardized conditions, each film layer being measured and re-

corded from a reference flat surface such as a glass slide. The specimens should be examined in the microscope and the one which gives the optimum image in terms of resolution and information content can be considered to have the most suitable coating thickness.

### 10.5.1. Estimation of Coating Thickness

In high-vacuum thermal evaporation, one can assume that all the vapor molecules leave each part of the evaporant surface without preferred direction and pass to the substrate surface without colliding with residual gas molecules. By allowing for the vapor incident angle at the specimen, and assuming that all the incident vapor molecules have the same condensation coefficient, the coating thickness distribution may be calculated. The formula below may be applied to a flat, nonrotating surface, oriented at an angle  $\theta$  to the source direction and can be used to calculate the thickness from the quantity of material to be evaporated:

$$T = \frac{M}{4\pi R^2 \rho} \cos \theta \quad (10.1)$$

where  $T$  is the thickness in centimeters;  $M$  is the mass of the evaporant in grams;  $\rho$  is the density in  $\text{g cm}^{-3}$ ;  $R$  is the distance from source to specimen in centimeters; and  $\theta$  is the angle between the normal to the specimen and the evaporation direction. With Equation (10.1), the thickness can be calculated to within  $\pm 50\%$  if the geometry of the evaporation rig and substrate is known and if the material in question evaporates slowly and does not alloy with the heater. It is assumed that evaporation is from a point source and occurs uniformly in all directions. The uncertainty in  $T$  arises in part because of uncertainty in the sticking coefficient of the evaporant or the substrate. The efficiency is material dependent and is rarely better than 75%. The thickness calculated by Equation (10.1) must therefore be reduced by multiplying by the sticking efficiency (approximately 0.75).

The thickness of coating  $T$  in nanometers which may be obtained using a diode sputter coater is given by

$$T = \frac{CVtk}{10} \quad (10.2)$$

where  $C$  is the plasma discharge in milliamperes,  $V$  the accelerating voltage in kilovolts,  $t$  the time in minutes, and  $k$  a constant depending on the bombarding gas and the target material. The constant  $k$  must be calculated for each sputter coater, target material, and bombarding gas from experimental measurements.

### 10.5.2. Measurement during Coating

A number of different methods are available which vary in their sensitivity and accuracy. The devices are placed in the coating chamber close to the specimen and measure the thickness during the coating process. It is possible to measure the density of the evaporant vapor stream either by measuring the ionization which occurs when vapor molecules collide with electrons, or by measuring the force which impinging particles exert on a surface. Mass-sensing devices may be used for all evaporant materials; these operate by determining the weight of a deposit on a microbalance or by detecting the change in oscillating frequency of a small quartz crystal on which the evaporant is deposited. The quartz crystal thin-film monitor resonates at a frequency dependent on the mass of material deposited on its surface. The frequency of a "loaded" crystal is compared to the frequency of a clean crystal and the decrease in frequency gives a measure of the film thickness. A typical sensitivity value for a crystal monitor is a 1-Hz frequency change for an equivalent film of 0.1 nm C, 0.13 nm Al, and 0.9 nm Au. The uncertainty of these devices is of the order  $\pm 0.1 \mu\text{g cm}^{-2}$ . By multiplying this value of uncertainty by the evaporant density, the uncertainty in the film thickness can be determined.

It is also possible to monitor specific film properties such as light absorption, transmittance, reflection, and interference effects by various optical monitors. Film thickness of conductive materials can be measured by *in situ* resistance measurements and the thickness of dielectric materials measured by capacitance monitors. A further sophistication of most of these *in situ* techniques is that they can be used to control the coating process.

### 10.5.3. Measurement after Coating

The most accurate measurements of film thickness are made on the films themselves. These methods are based on optical techniques, gravimetric measurements, and x-ray absorption and emission. Multiple-beam interferometry is the most precise and depending on the method used can be as accurate to within one or two nanometers. The Fizeau method can be used to check film thickness and involves placing a reflective coating on top of the deposited film step and measuring a series of interference fringes. The film thickness can also be measured by sectioning flat pieces of resin on which a coating has been deposited and measuring the thickness of the metal deposit in the transmission electron microscope. The accuracy of this method is dependent on being able to section the resin and photograph the section at right angles to the metal deposit. A simple method for accurately determining film thickness and grain size has been recently described by

Roli and Flood (1978). They found that linear aggregates of latex spheres only accumulate coating material along their free surface. Transverse to the linear aggregate, the sphere diameter will increase in thickness by twice the film thickness, while parallel to the aggregate the thickness increase will correspond to the film thickness. Using this technique they have measured the film thickness derived from different coating procedures to within  $\pm 2$  nm. Film thickness may be estimated by interference colors or in the case of carbon by the density of the deposit on a white tile.

#### 10.5.4. Removing Coating Layers

Having taken all the trouble to deposit a coating layer on a sample it is sometimes necessary to remove it. Provided care is taken, hard specimens can be restored to their pristine uncoated state.

If the sample is a polished section, the coating can be easily removed by returning to one of the final polishing operations ( $6 \mu\text{m}$  diamond or  $1 \mu\text{m}$   $\text{Al}_2\text{O}_3$ ). If the sample is rough or a flat sample cannot be repolished, chemical means must be used to remove the coating.

If it is known that the coating layer is to be removed after examination of a rough sample, it is preferable to use aluminum as the coating material because of its ease of removal. Sylvester-Bradley (1969) removed aluminum from geological samples by immersing them in a freshly prepared solution of dilute alkali for a few minutes. Sela and Boyde (1977) describe a technique for removing gold films from mineralized samples by immersing the specimen in a cyanide solution. Gold-palladium may be removed using a 10%  $\text{FeCl}_3$  in ethanol for 1–8 h (Crissman and McCann 1979).

Carbon layers can be removed by reversing the polarity in the sputter coater and allowing the argon ions to strike the specimen. Care must be taken only to remove the carbon layer and not to damage the underlying specimen. Carbon layers on inorganic samples such as rocks, powders, and particulates can be efficiently removed in an oxygen-rich radiofrequency plasma.

## Preparation of Biological Samples for Scanning Electron Microscopy

### 11.1. Introduction

In this and the next chapter we will consider the practical aspects of specimen preparation for both the scanning electron microscope and the x-ray microanalyzer. Although the two types of instrument are very similar and in many respects can be used interchangeably, it is useful, from the biologist's point of view, to consider the preparative techniques separately. The scanning electron microscope gives morphological information, whereas the x-ray microanalyzer gives analytical information about the specimen. It is important for the user to appreciate fully these differences as they have a significant bearing on the rationale behind the specimen preparation techniques. The methods and techniques which are given in these two chapters will provide the optimal specimen preparation conditions for scanning microscopy or x-ray microanalysis. It must be realized that anything less than the optimal conditions will result in a diminished information transfer from the specimen. It will also become apparent that it will be frequently necessary to make some sort of compromise between the two approaches, which must result in less information from the specimen.

The practical, do-it-yourself aspects of specimen preparation will be emphasized, and while it is appreciated that a certain understanding of the theoretical aspects might be useful, such considerations will be kept to a minimum and readers will be directed to key review papers on the subject. It is not our intention to provide the biologists with a series of recipes. These already abound in the literature and very few of them can result in optimal specimen preparation for anything other than the specimens for