# 掃描式電子顯微鏡生物樣品實務操作研習班

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- 主要教材：
  **1.** 生物電子顯微鏡學。陳家全，**1999**，國家科學委員會。
  **2.** 清晰的奈米世界**~**初探電子顯微鏡。章效鋒**(**中國**)**，**2006**，五南文化事業。
- 參考書目：
- 1. Electron Microscopy: Principles and Techniqes.
- Hayat,2000, Cambridge University Press.
- 2. Electron Microscopy. Bozzola and Russell, 1998,
- Jones and Bartlett Publishers.
- 3. in situ Hybridization in Electron Microscopy. Morel etc.
- 2001, CRC.

# LM生物試樣前處理

(Specimen preparation for light microscopy)

〈石蠟切片〉

〈 Paraffin section 〉

採樣(sampling→)
固定(fixation)→
脫水
(dehydration)→
滲透(infiltration)
→包埋
(embedding) →
切片(sectioning)
→染色(staining)

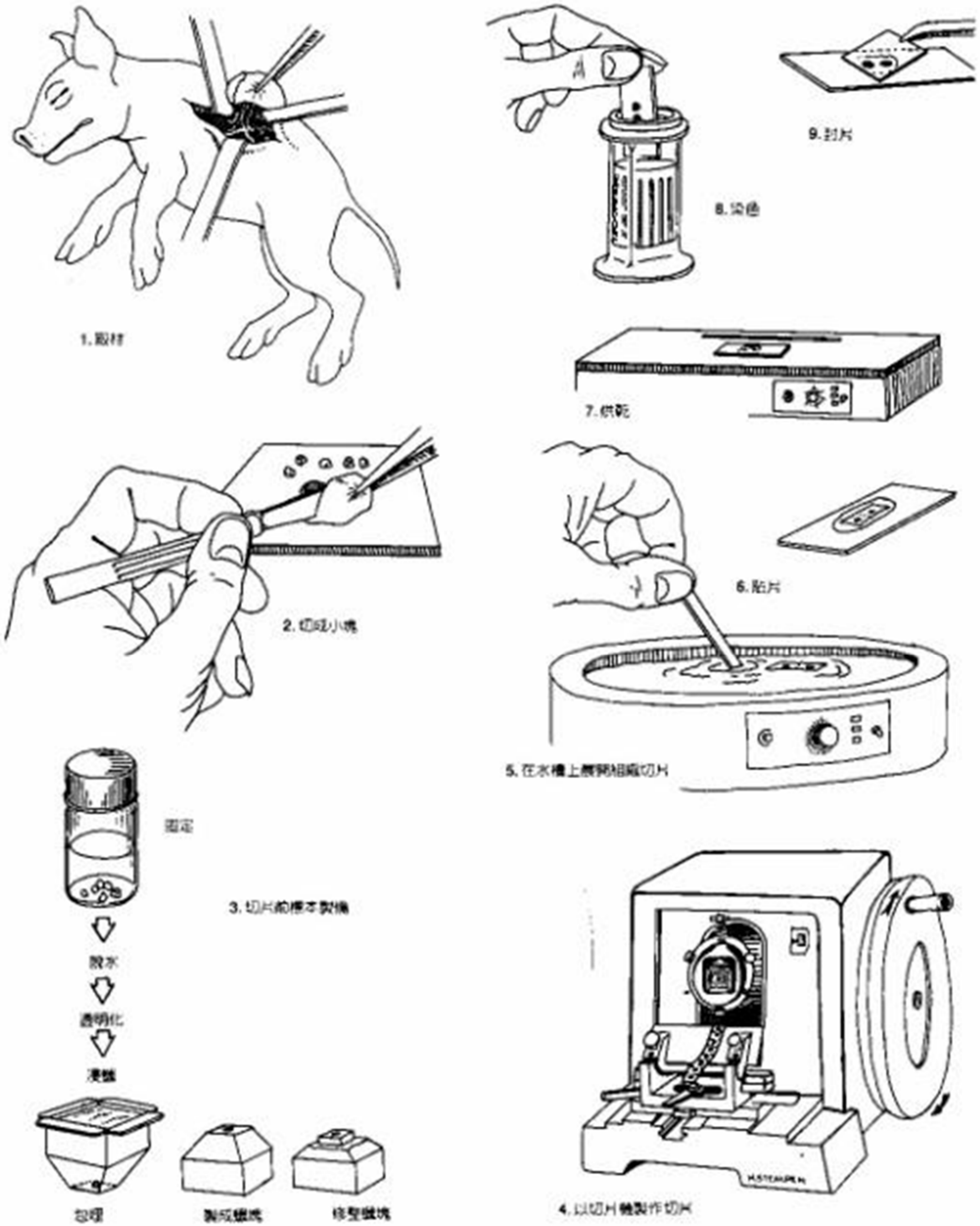


圖 1–1 石蠟切片製作的主要步驟

# TEM生物試樣前處理

(Specimen preparation for transmission electron microscopy)

## Table 2-1 General Tissue Preparation Scheme for Electron Microscopy

| Activity | Chemical | Time Involved* |
|---|---|---|
| Primary Fixation | tissue is fixed with 2-4% glutaraldehyde in buffer | 1-2 hr |
| Washing | buffer (3 changes, 1 of which may be overnight) | 1-12 hr |
| Secondary Fixation | osmium tetroxide (1-2%: usually buffered) | 1-2 hr |
| Dehydration | 30% ethanol | 5 min |
| | 50% ethanol | 5-15 min |
| | 70% ethanol | 5-15 min |
| | 95% ethanol (2 changes) | 5-15 min |
| | absolute ethanol (2 changes) | 20 min ea |
| Transitional Solvent | propylene oxide (3 changes) | 10 min ea |
| Infiltration of Resin | propylene oxide: resin mixtures gradually increasing concentration of resin | overnight-3 d |
| Embedding | pure resin mixture | 2-4 hr |
| Curing (at 60-70° C) | | 1-3 d |

* The specified times do not include the time involved in preparation of chemicals.

SEM

初固定(戊二醛，主要固定蛋白質)→緩衝液浸洗→後固定(四氧化鋨 ,主要與不飽和脂肪酸結合)(胞膜固定兼有染色功能, 惟毒性強 )→系列酒精脫水→轉換液→樹酯浸潤→包埋→固化

圖2.7　配製包埋劑應在抽氣罩中進行，並應帶手套以避免包埋劑直接接觸皮膚或造成污染。

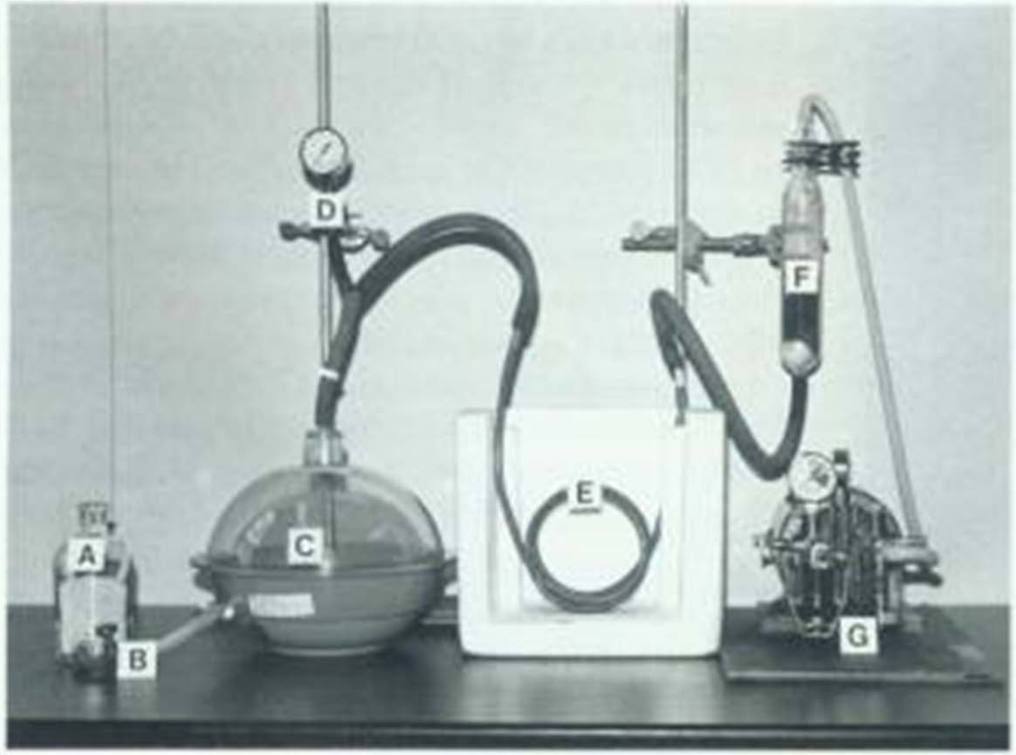


**Figure 3-8(A)** Sublimation apparatus used to dry specimens that have been dehydrated and treated with Peldri II fluorocarbon. A = container of $CaSO_4$ dessicant to maintain dryness of air, B = valve, C = vacuum desiccator, D = vacuum gauge, E = condensing coil to prevent reagents from going into vacuum pump, F = gas purifier column of activated charcoal and desiccant for further purification of gas being evacuated by vacuum pump, G = vacuum pump. (Courtesy of J. L. Pauly and J. Electron Microscopy Technique.)

- 固定時加裝抽氣裝置可得更佳效果

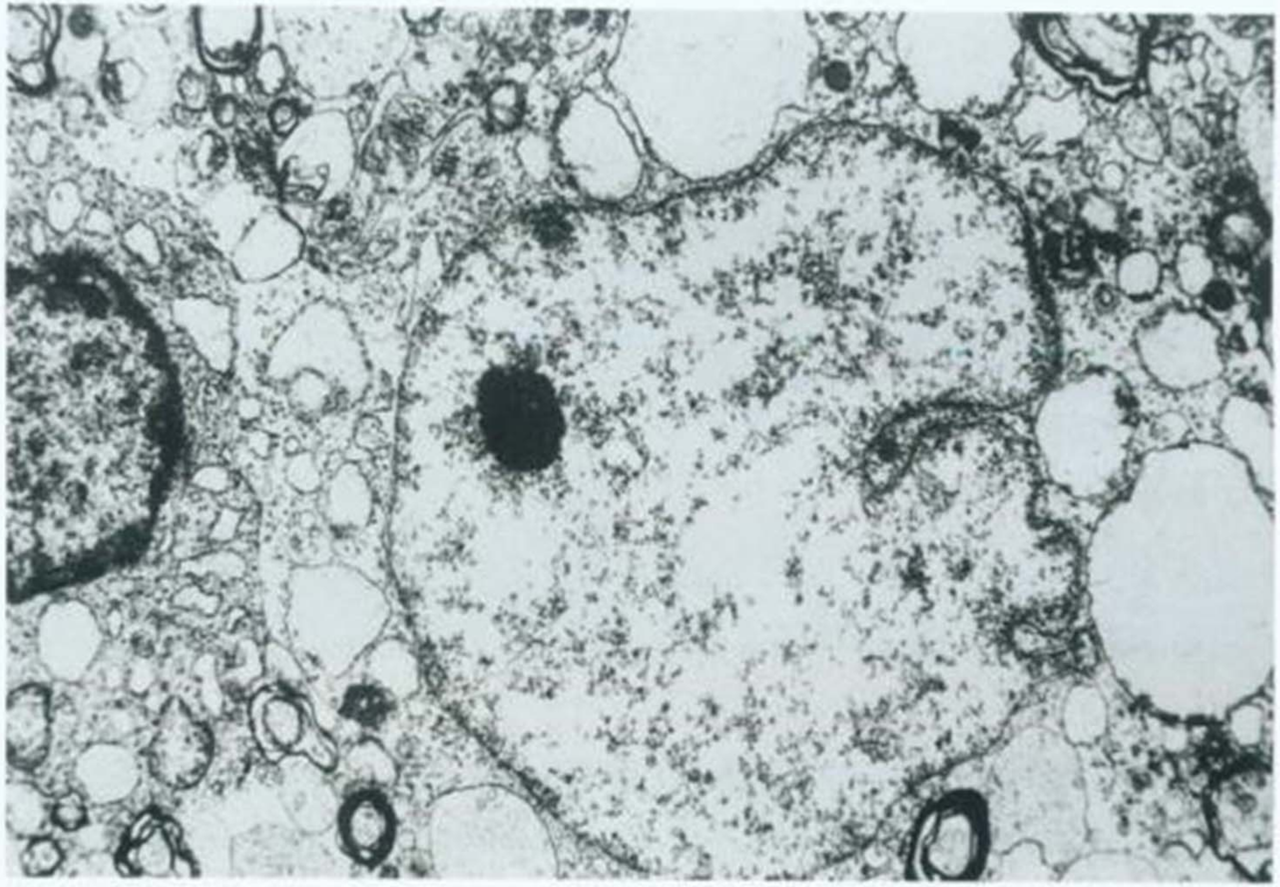

圖2.1 老鼠的腦部細胞以一般浸泡方式固定，由於取組織的時間過長，造成嚴重的自溶現象，細胞核與細胞質中物質均已分解流失，因而出現許多空泡。

圖2-1

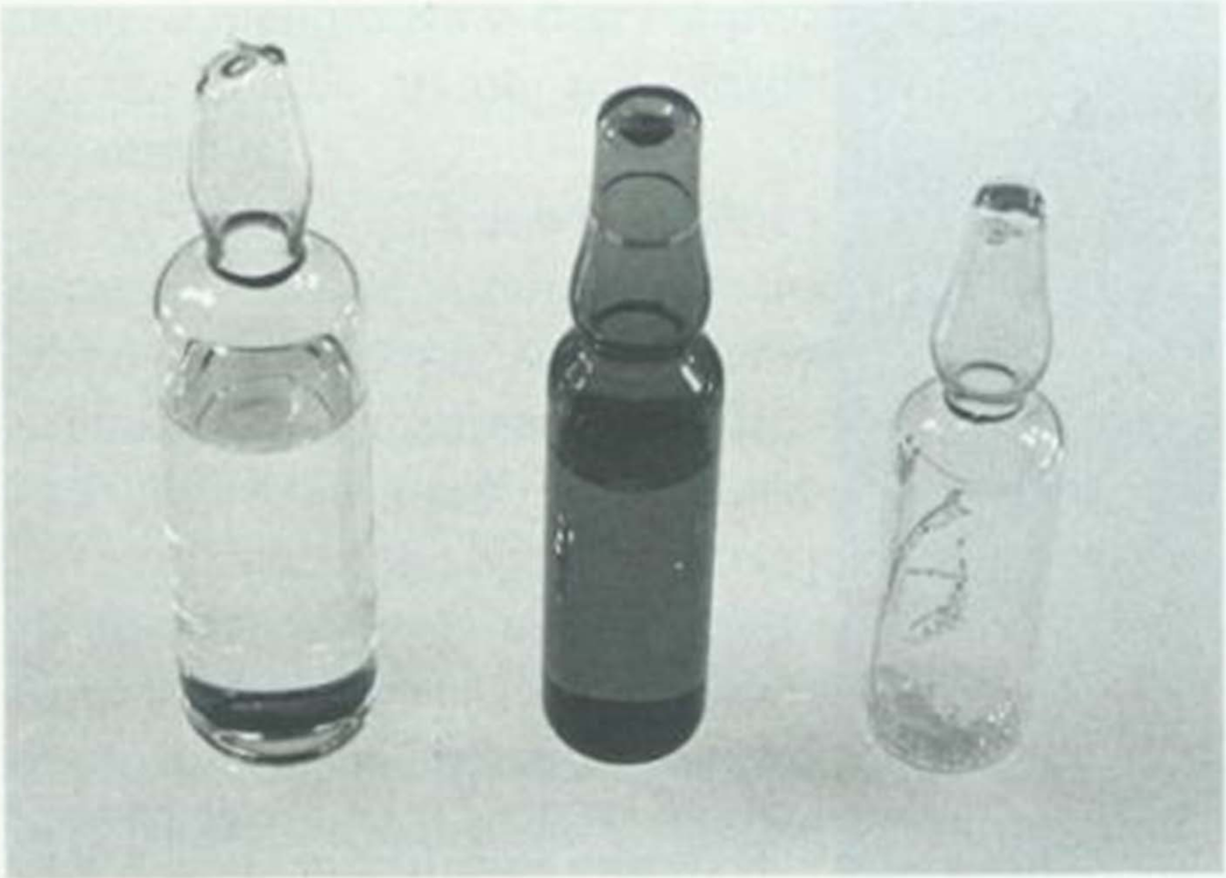

**Figure 2-2** Sealed ampoules of glutaraldehyde, 4% osmium tetroxide and, crystalline osmium tetroxide (left to right).

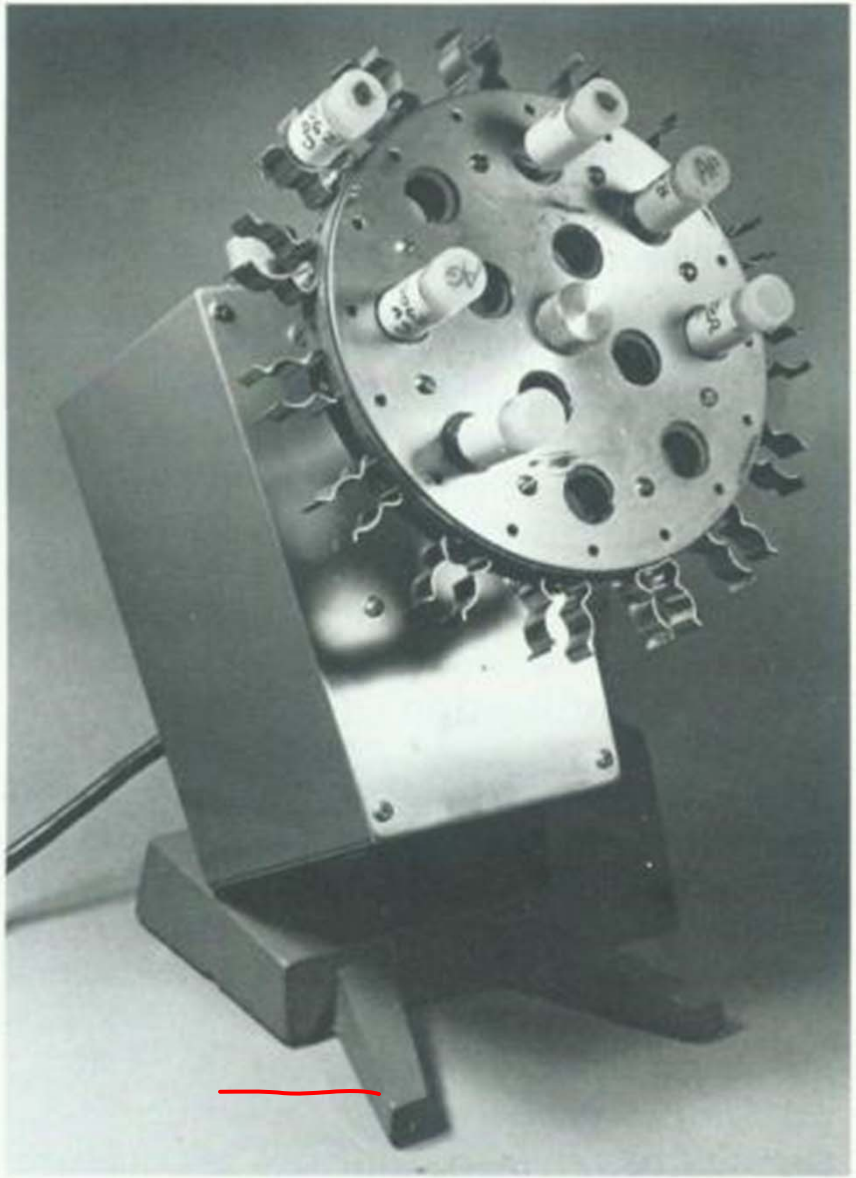

**Figure 2-6** Rotary mixer used to infiltrate tissues. Vials containing tissue are situated in holes of the mixer.

- X ml 配合 Table 2-3 可調整pH

Table 2-2 Preparation of Common Buffers Utilized in Electron Microscopy

| Buffer | pH Range | Stock Solutions A | Stock Solutions B | Formula |
|---|---|---|---|---|
| Plumel's cacodylate | 5.0-7.4 | 0.2 M sodium cacodylate (42.8 g $Na[CH_3]_2AsO_2 \cdot 3H_2O$) per liter of distilled $H_2O$ | 0.2 N HCl | 25 ml A + x ml B made. Bring volume up to 100 ml with distilled $H_2O$. |
| Sorenson's phosphate | 5.0-8.2 | 0.67 M monosodium phosphate (9.08 g $NaH_2PO_4$) per liter of distilled $H_2O$ | 0.67 M disodium phosphate (11.88 g $Na_2HPO_4 \cdot 4H_2O$) | x ml A plus (100-x) ml B |
| Gomori's tris-maleate | 5.2-8.6 | 0.2 M tris acid maleate (24.2 g tris-[hydroxymethyl] amino-methane + 23.2 g maleic acid 19.6 g maleic anhydride per liter) | 0.2 N NaOH | 25 ml A + x ml B made up to 100 ml |
| s-collidine | 6.0-8.0 | 2.67 ml pure s-collidine in 50.0 ml of distilled $H_2O$ | ~9.0 ml of 1.0 M HCl | A + B made up to 100 ml |

Table 2-3 pH Adjustment

| pH | Plumel's cacodylate | Sorenson's phosphate | Gomori's tris-maleate |
|---|---|---|---|
| 5.0 | 23.5 | 98.8 | |
| 5.2 | 22.5 | 98.0 | 3.5 |
| 5.4 | 21.5 | 96.7 | 5.4 |
| 5.6 | 19.6 | 94.8 | 7.8 |
| 5.8 | 17.4 | 91.9 | 10.3 |
| 6.0 | 14.8 | 87.7 | 13.0 |
| 6.2 | 11.9 | 81.5 | 15.8 |
| 6.4 | 9.2 | 73.2 | 18.5 |
| 6.6 | 6.7 | 62.7 | 21.3 |
| 6.8 | 4.7 | 50.8 | 22.5 |
| 7.0 | 3.3 | 39.2 | 24.0 |
| 7.2 | 2.1 | 28.5 | 25.5 |
| 7.4 | 1.4 | 19.6 | 27.0 |
| 7.6 | | 13.2 | 29.0 |
| 7.8 | | 8.6 | 31.8 |
| 8.0 | | 5.5 | 34.5 |
| 8.2 | | 3.3 | 37.5 |
| 8.4 | | | 40.5 |
| 8.6 | | | 43.3 |

- pH值：動物細胞 7.0至7.4
  植物細胞 6.8至7.2
  原生動物、無脊椎動物、
  胚胎組織　8.0左右

有時加入蔗糖(sucrose)作為維持滲透壓（osmolarity）之用，以避免固定時造成細胞皺縮或脹大

表2-3

# SEM生物試樣前處理

(Specimen preparation for scanning electron microscopy)

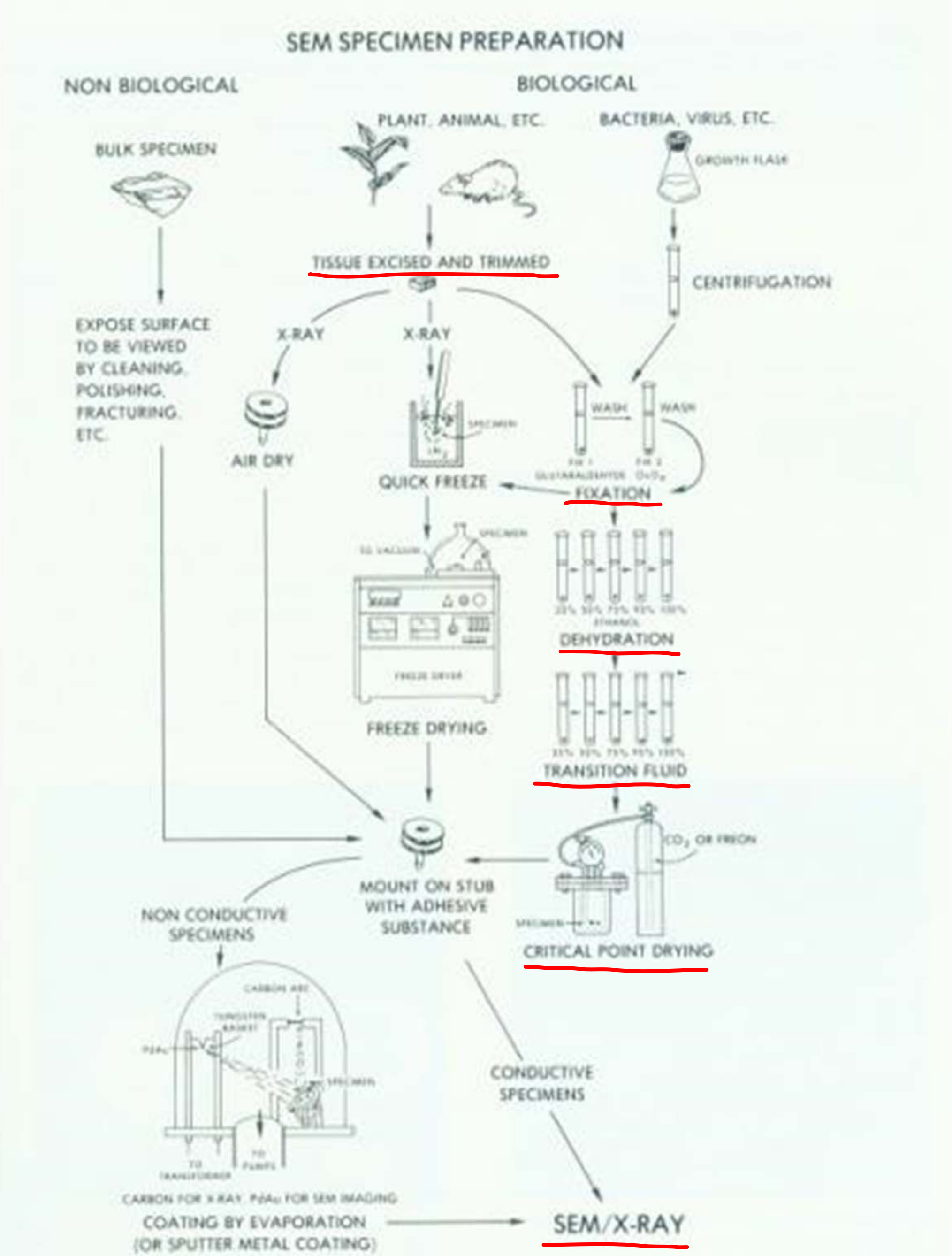


**Figure 3-1** Schematic showing sequence of events for processing biological specimens for SEM. (Courtesy of Judy Murphy.)

**Table 3-1 Fixatives Commonly Used in SEM**

| Specimen | Fixative | Buffer System | Reference |
|---|---|---|---|
| Procaryotes | glutaraldehyde | cacodylate, phosphate | Watson et al. 1984 |
| | osmium tetroxide | veronal-acetate | |
| | FAA (10% formalin, 85% ethanol, 5% glacial acetic acid) | | |
| Fungi | glutaraldehyde/$OsO_4$ followed by $OsO_4$ | cacodylate, phosphate | Watson et al. 1984 |
| | $OsO_4$ vapors | none | |
| | glutaraldehyde followed by aqueous uranyl acetate | cacodylate | |
| Aquatic Organisms (protozoa, sponges, metazoa) | glutaraldehyde/ formaldehyde | cacodylate, collidine | Maugel et al. 1980 |
| | Parducz (6 parts of 2% aqueous $OsO_4$ plus 1 part saturated aqueous $HgCl_2$—freshly prepared) | none | |
| | glutaraldehyde followed by $OsO_4$ | phosphate, cacodylate sea or pond water | |
| Higher Plants | glutaraldehyde followed by $OsO_4$ | phosphate buffer | Falk, 1980 |
| | FAA alone or followed by $OsO_4$ | phosphate or s-collidine | |
| | formaldehyde followed by freeze drying | none | |
| | osmium vapors | none | |
| Zoologicals | glutaraldehyde followed by $OsO_4$ | cacodylate or phosphate | Nowell and Pawley, 1980 |
| | $OsO_4$ | cacodylate or phosphate | |
| | glutaraldehyde/ formaldehyde | various | |
| | glutaraldehyde or glutaraldehyde/formaldehyde followed by $OsO_4$ | cacodylate or phosphate | |
| | FAA | none | |

表3-1

Table 2-1 General Tissue Preparation Scheme for Electron Microscopy

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SEM ↑

初固定(戊二醛，主要固定蛋白質)→緩衝液浸洗→後固定(四氧化鋨 ,主要與不飽和脂肪酸結合)(胞膜固定兼有染色功能, 惟毒性強 )→系列酒精脫水→轉換液→樹酯浸潤→包埋→固化

# 掃描式電子顯微鏡（SEM）操作步驟

將取得之樣本前處理進行脫水後，再利用臨界點乾燥機及鍍金機進行臨界點乾燥及鍍金，最後上機觀察。

SEM 前處理操作步驟

| | |
|---|---|
| 1.戊二醛(0~4℃) | 1~2 小時 |
| 2.BUFFER(0~4℃) | 15 分鐘 |
| 3.BUFFER(0~4℃) | 15 分鐘 |
| 4.鋨酸(OSNIUM) | 1 小時 |
| 5.BUFFER | 15 分鐘 |
| 6.BUFFER | 15 分鐘 |
| 7.30%酒精 | 10 分鐘 |
| 8.50%酒精 | 10 分鐘 |
| 9.70%酒精 | 10 分鐘 |
| 10.80%酒精 | 10 分鐘 |
| 11.90%酒精 | 10 分鐘 |
| 12.100%酒精 | 15 分鐘 |
| 13.100%酒精 | 15 分鐘 |
| 14.丙酮 | 15 分鐘 |
| 15.丙酮 | 15 分鐘 |

16.臨界點乾燥

17.鍍金

18.上機觀察

## 三、臨界點乾燥 Critical point drying(CPD)

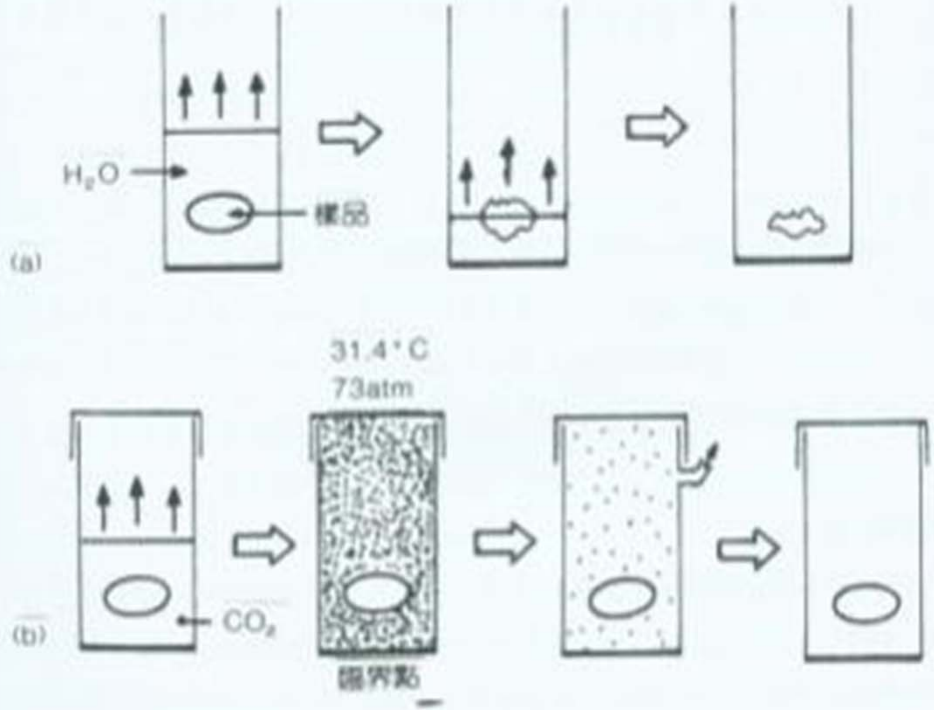



圖6.9 (a)自然乾燥時，水分子不斷脫離液面，表面張力的作用將使物體變形。(b)以 $CO_2$ 作為轉換液在臨界點時有液氣相並存之狀態，液體與氣體之間的界面消失，乾燥時可保持物體原形。

液氣相並存→兩相的界面消失→
表面張力不變→微細構造得以保存

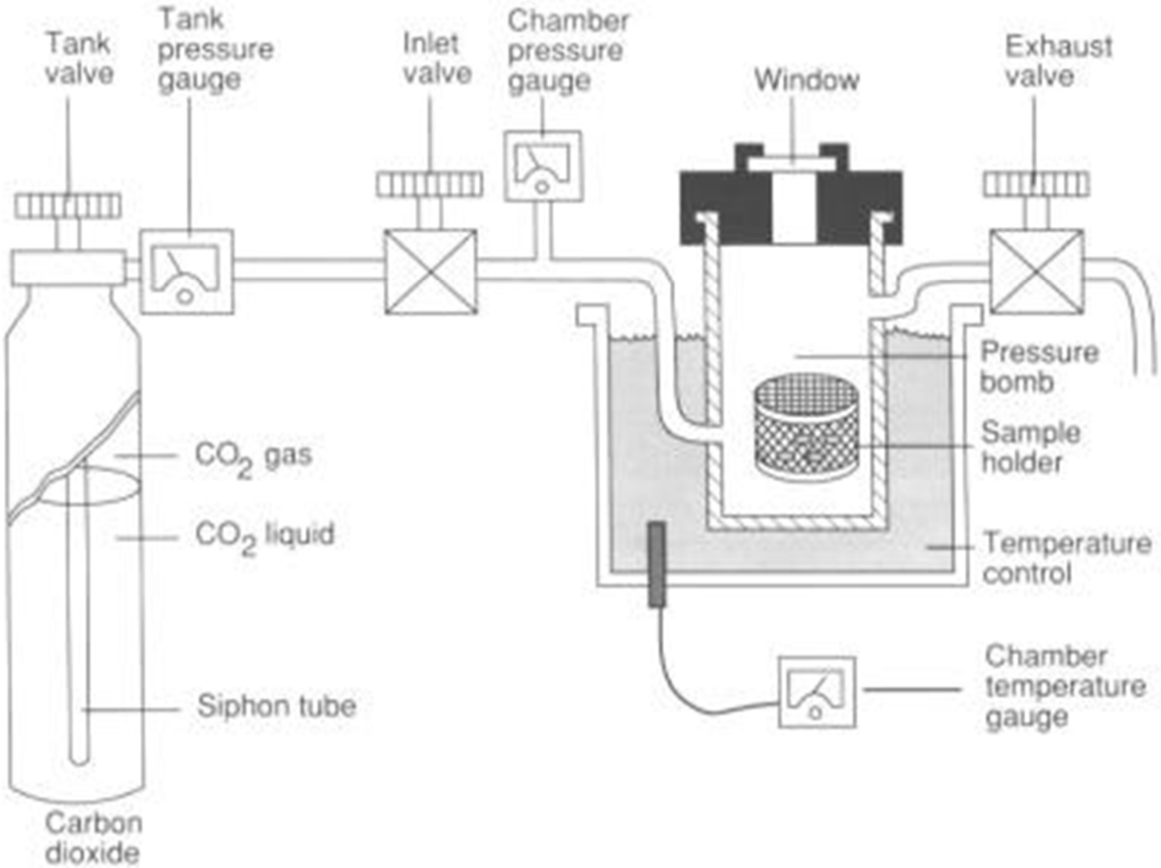



**Figure 3-3** Diagram of critical point drying apparatus. The temperature of the pressure bomb may be regulated by a water bath or an electric heating element.

臨界點乾燥機


圖6.13 臨界點乾燥機的外形構造圖。

圖6-13

## Table 3-2 Dehydrants and Transitional Fluids Used in Critical Point Drying

| Dehydrant | Transitional Fluid | Critical Temp °C | Critical Pressure PSI |
|---|---|---|---|
| Ethanol, Amyl Acetate | Liquid $CO_2$ | 31.1 | 1,073 |
| Acetone | Freon 116 | 19.7 | 432 |
| Ethanol | Freon 23 | 25.9 | 701 |
| Ethanol/Freon | Freon 13 | 28.9 | 561 |

表3-2


圖6.8　菊花花瓣表面之微細構造經過自然乾燥(A)與臨界點乾燥(B)處理後之比較，自然乾燥由於表面張力的影響，細胞產生極嚴重的皺縮現象。


**Figure 3-13** Adhesive transfer tabs are used to deposit a small amount of adhesive onto SEM stubs. The adhesive is adequate to hold most small specimens on the stub.

圖6.10 離子覆膜機的外形構造

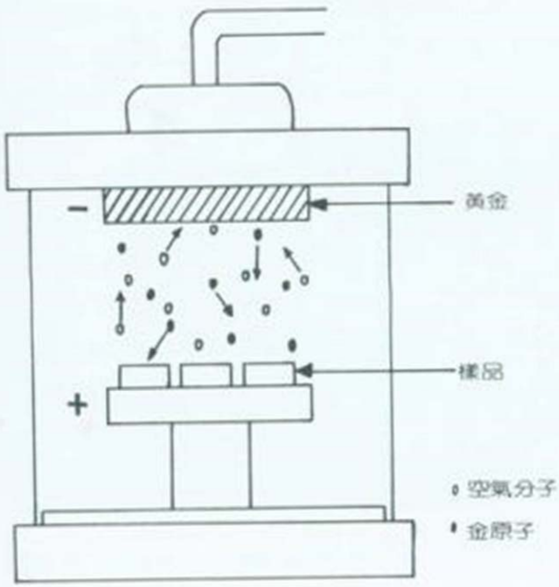



圖6.11 Sputter coater(iron coater)

離子覆膜機原理為殘留在操作室中的空氣分子被離子化後會撞擊陰極上的金塊，使金原子散落於標本表面上。

覆膜的目的：

1.使sample表面更能傳熱、導電→否則充電現象（charging）會影響觀察

2.較易產生二次電子（secondary electron）

3.保護sample不被電子束（electron beam）的高熱破壞樣品表面的微細構造

圖6-11

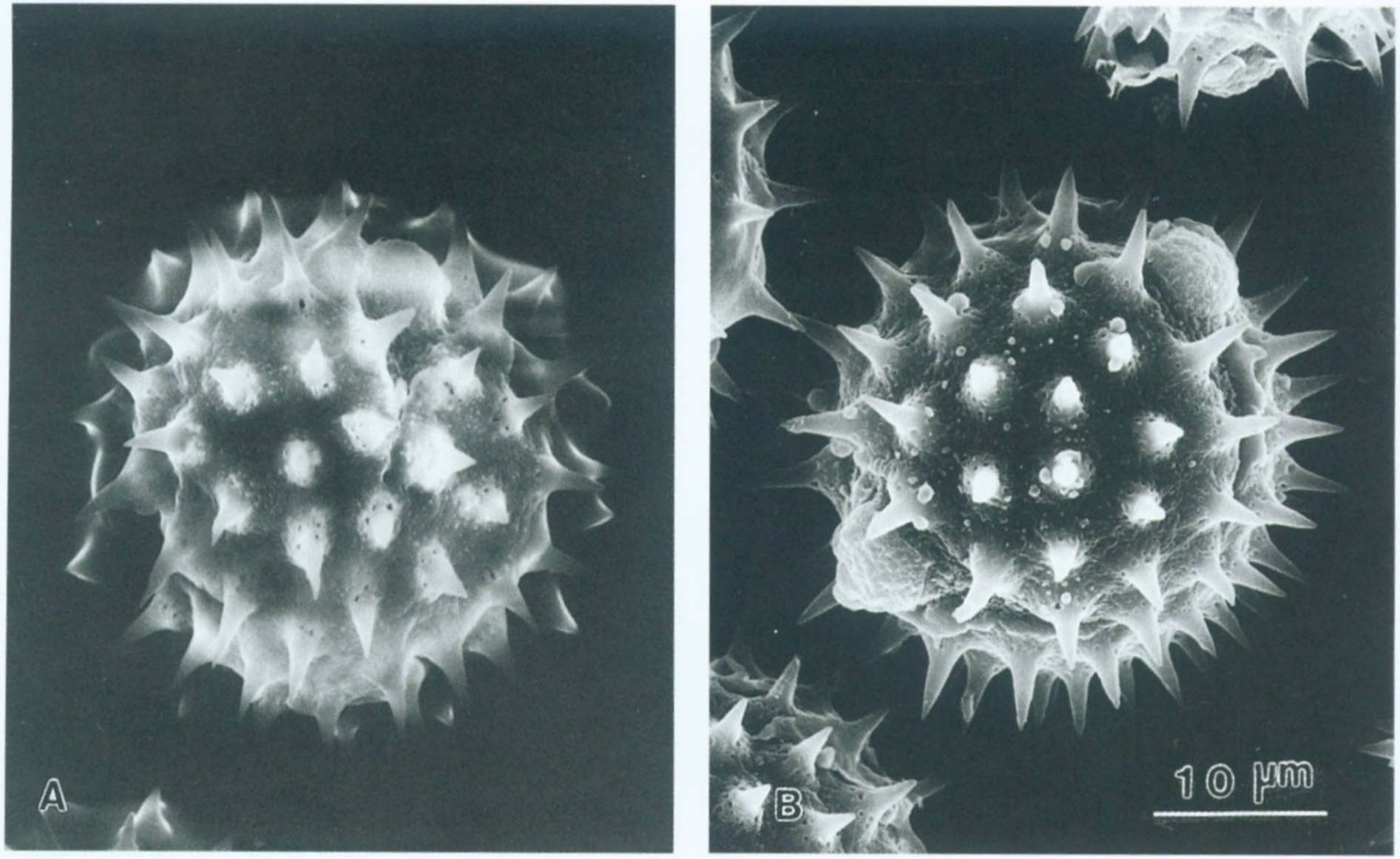



圖6.12　向日葵花粉未經覆膜步驟在顯微鏡下因導電效果不佳而造成影像品質不良(A)，若經覆膜後可使影像清晰穩定(B)。

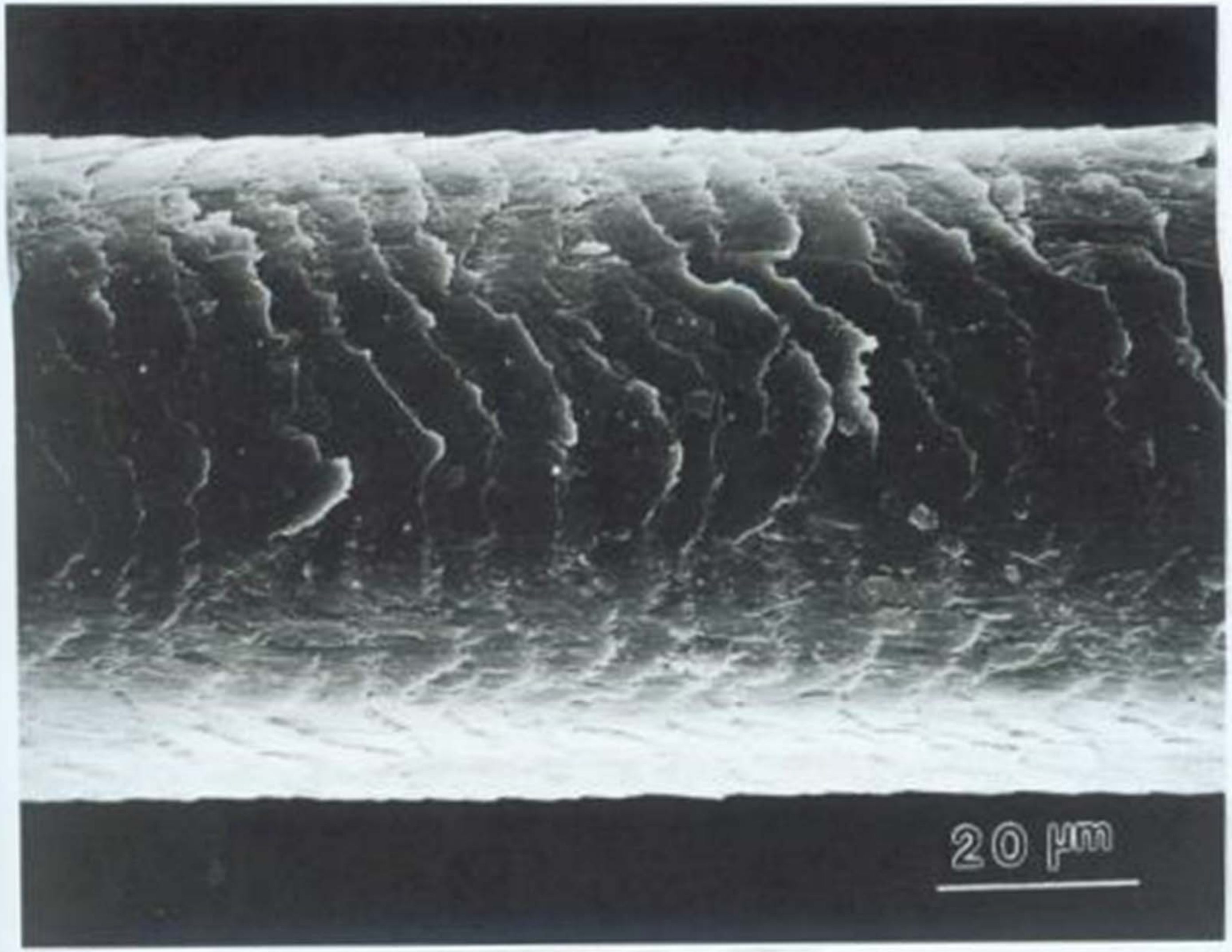



圖6.7　乾燥如頭髮之物體可直接經過覆膜後置 SEM 中觀察。

A B

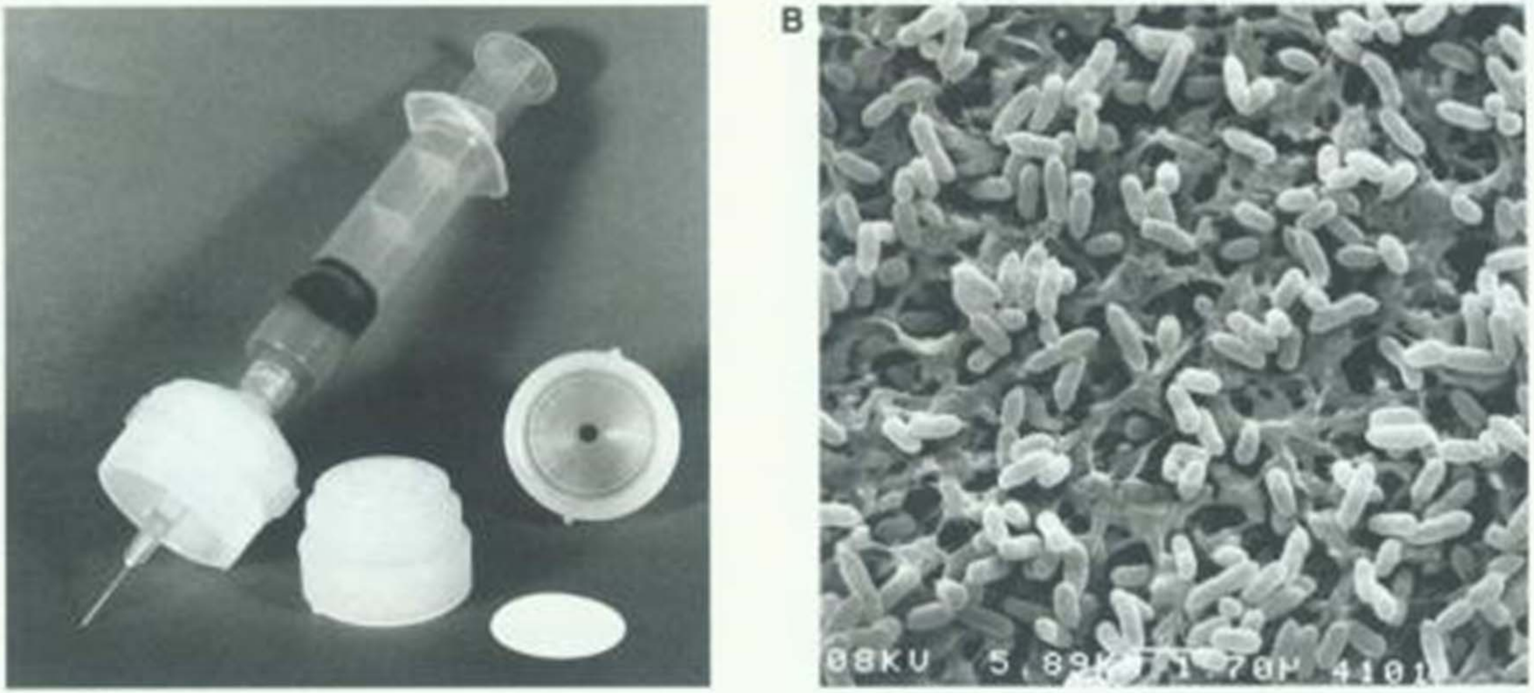


**Figure 3-2** (A) Individual cells or tiny specimens suspended in a buffer system may be deposited onto microporous filters by passage through a filtering device, as shown. Fixatives are then syringed over the cells, followed by ethanolic dehydration. The filter holder is then opened, and the filter is removed, dried, and mounted onto a specimen stub. After coating for conductivity the filter surface is then examined as shown in Figure B. (B) Bacterial cells trapped on a microporous membrane filter and subsequently processed for SEM. Marker bar = 1.7 μm. (Courtesy of R. de la Parra, Millipore Corp.)

細小試樣可經由濾膜過濾後再前處理

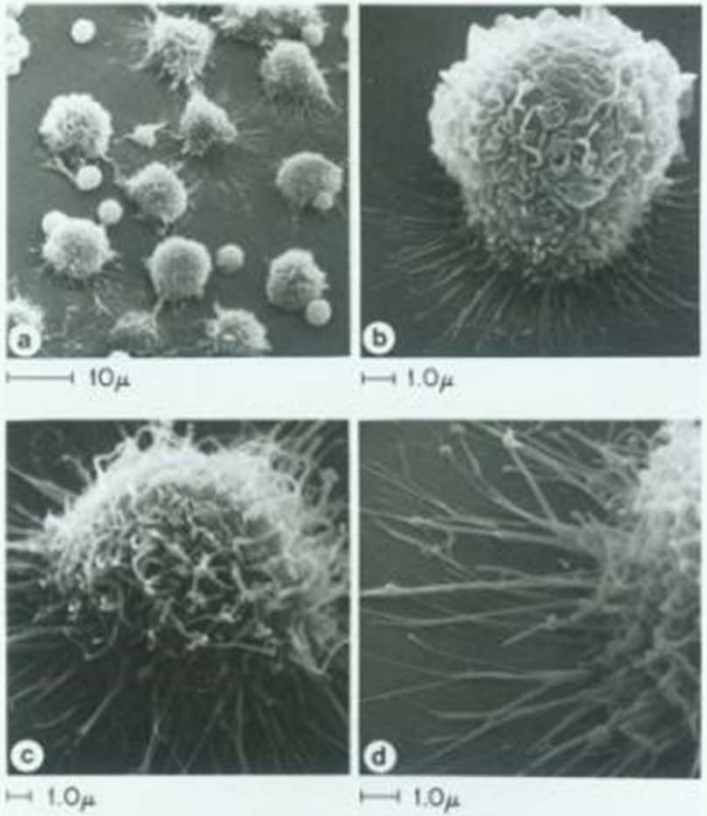



**Figure 3-8(B)** Morphology of human monocyte-derived macrophages that were cultivated for 11 days before harvesting and processing for SEM. (a) Low magnification of large adherent macrophages and small spherical lymphocytes. (b) Macrophages displaying typical ruffled membrane architecture. Note the filopodia that anchor the phagocytic cell to the substrate. (c) Pleomorphic nature of the surface membrane of macrophages is illustrated by this cell that displays numerous microvilli and a membrane architecture. (d) High magnification of filopodia illustrating the preservation of these delicate membrane structures obtained using Peldri II fluorocarbon procedure. (Courtesy of J. L. Pauly and J. Electron Microscopy Technique.)

- 組織培養細胞的固定液要和原培養溫度一致

# 掃描式電子顯微鏡

## (The scanning electron microscope )

## (SEM)


掃描式電鏡(SEM):

含可在接近大氣真空度下操作的BSE系統及可做元素分析的EDX分析儀

100KV穿透式電鏡(TEM):
最高放大倍率60萬倍(含傳統及數位化二套影像系統)

**Figure 7-1(A)** One of the first commercially produced SEMs, the Cambridge Mark II Stereoscan. (Courtesy of Leica.)

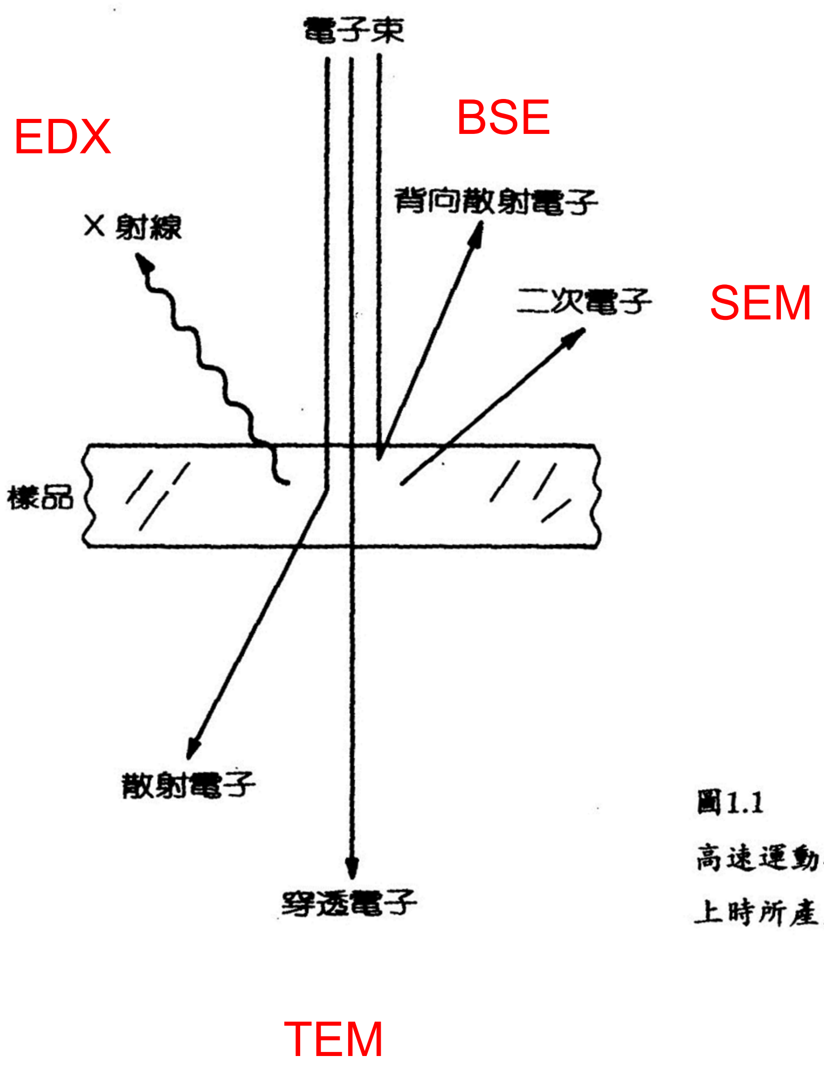



圖1.1
高速運動之電子撞擊在樣品
上時所產生的各種可能狀況。

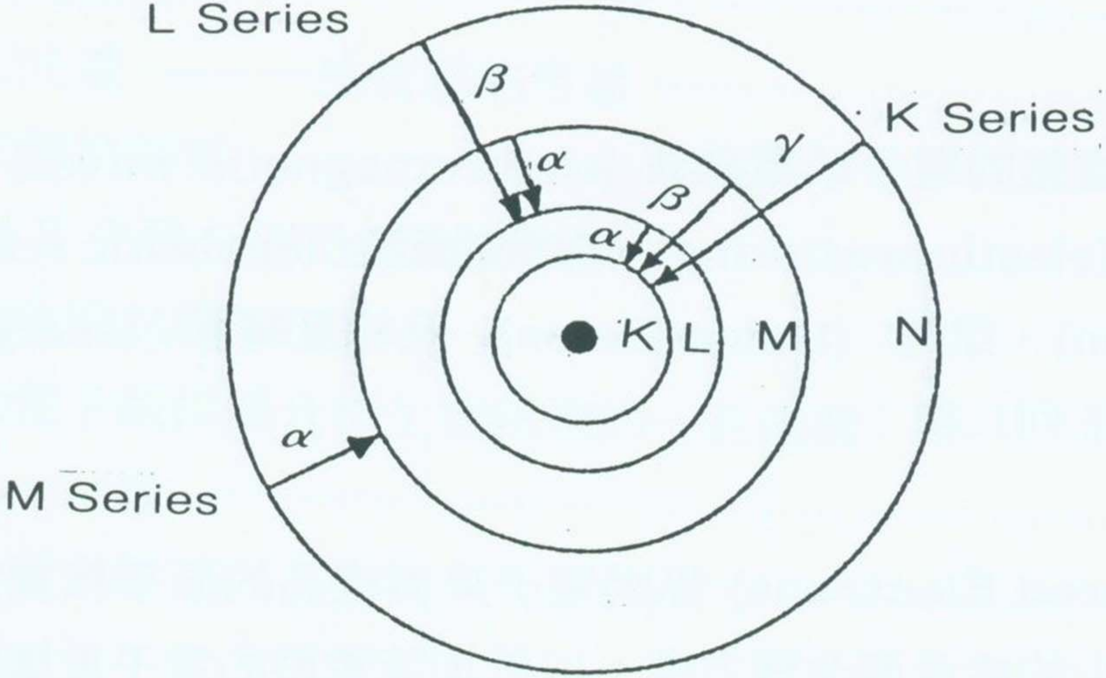
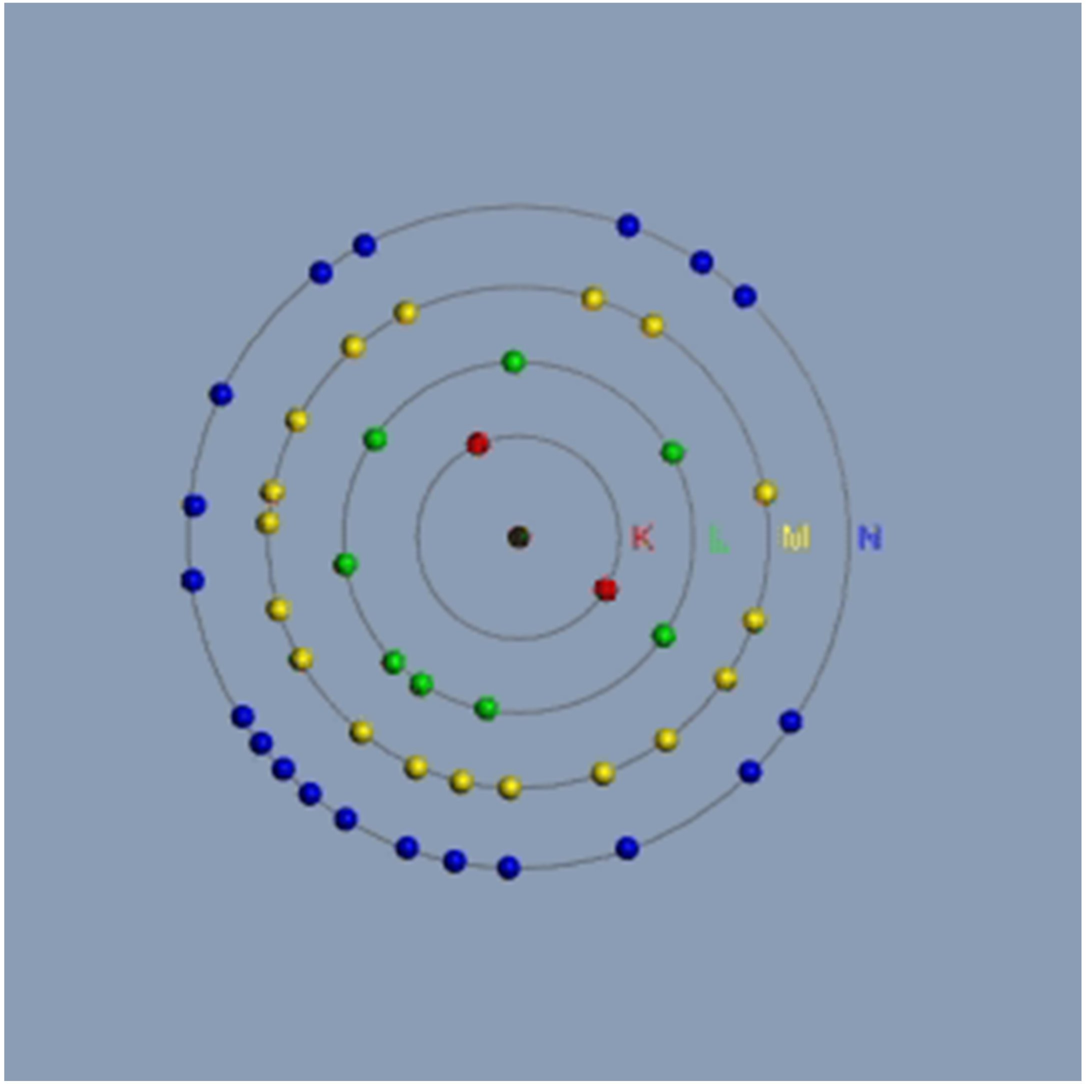



圖12.1

X 光光譜發生圖 (Postek et al. 1980)。

圖12-1


K
L
M
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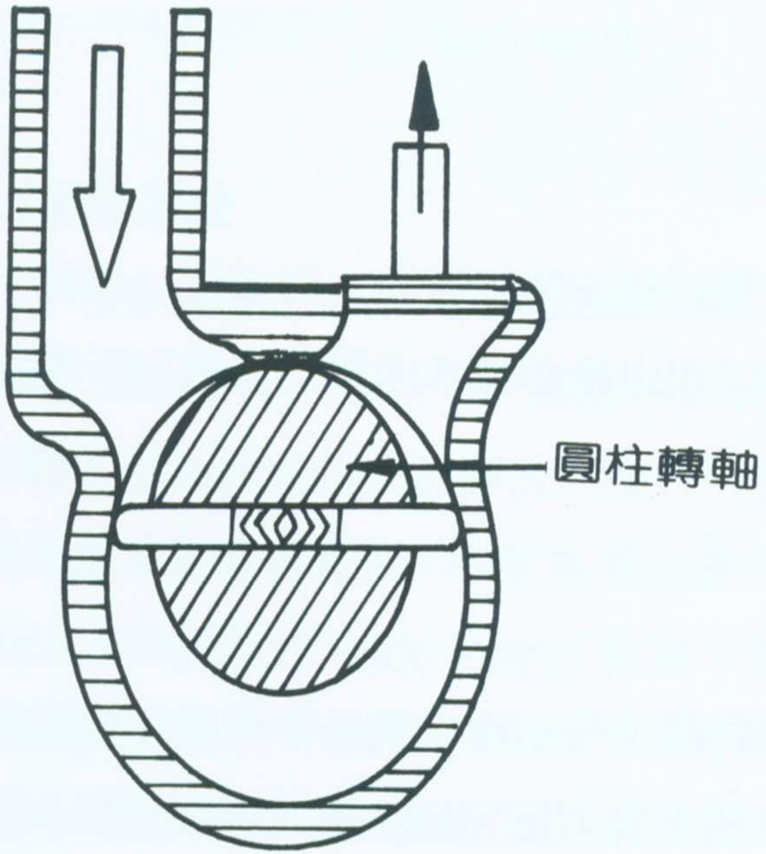


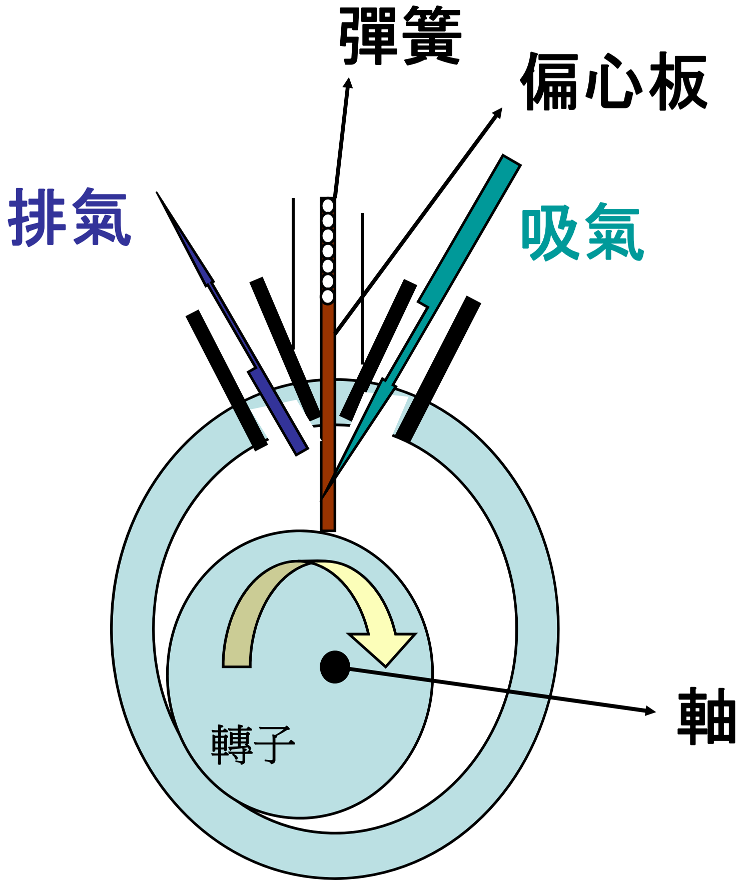

圖1.5

迴轉式唧筒之構造及其作用。利用偏離中心的圓柱形轉軸沿著內壁運轉形成三個腔室，並以壓縮的方式將空氣排出。

迴轉式唧筒(rotary pump)：真空度約為$10^{-2}$ torr

圖1-5

# Theory of Scanning Electron Microscope



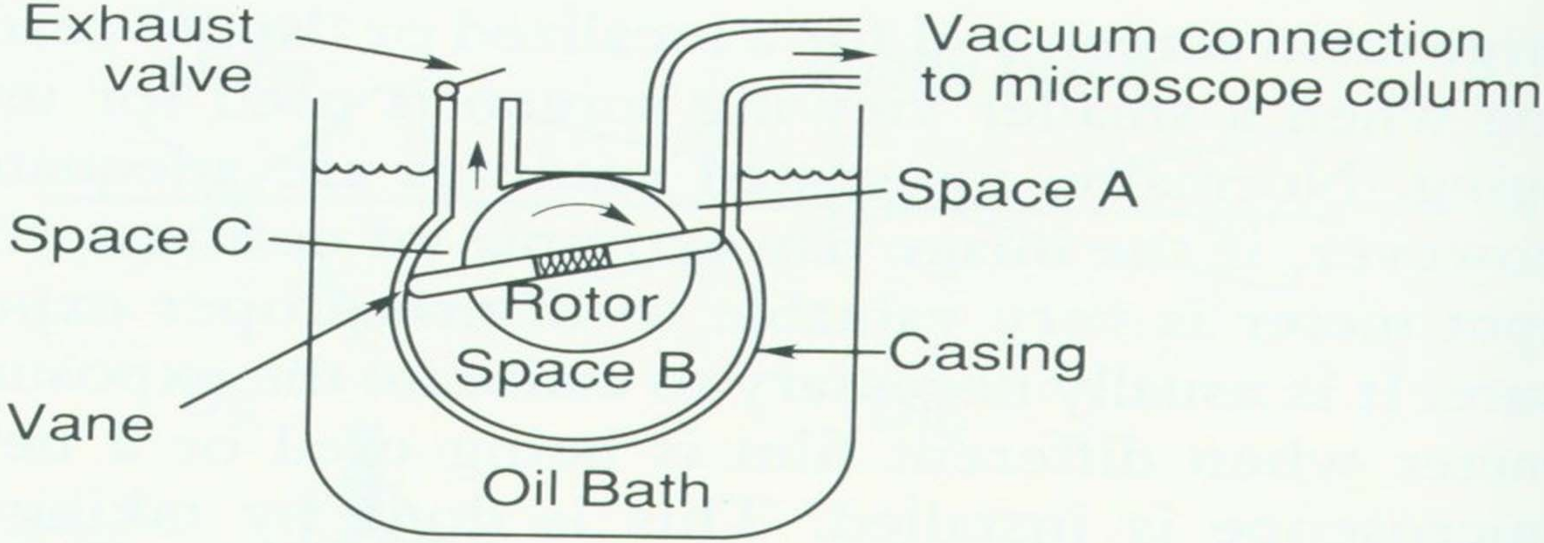




**Figure 6-37(A)** Rotary pump. Illustration of principle of operation of pump module. As the rotor turns, space A becomes enlarged, creating a vacuum that sucks air into the space. When the rotor rotates further and seals off the space by means of the spring-loaded vane, a large volume of air has been removed from the TEM and into the pump. Upon further rotation of the rotor, the sealed space designated B becomes smaller and the air is compressed. Eventually, the compressed air is moved over to space C by the rotor and a spring-loaded valve opens to exhaust the air to the outside of the system. The oil is used to lubricate the moving rotor and vanes and to carry frictional heat to the outside of the case.

## Table 6-5 Relationships Between Commonly Used Units of Pressure

| | Pascal | Torr | Millibar |
|---|---|---|---|
| 1 Torr = 1 mm Hg | 133 | 1 | 1.33 |
| 1 Millibar | 100 | 0.75 | 1 |
| 1 Atmosphere | $1.01 \times 10^5$ | 760 | $1.01 \times 10^3$ |

表6-5

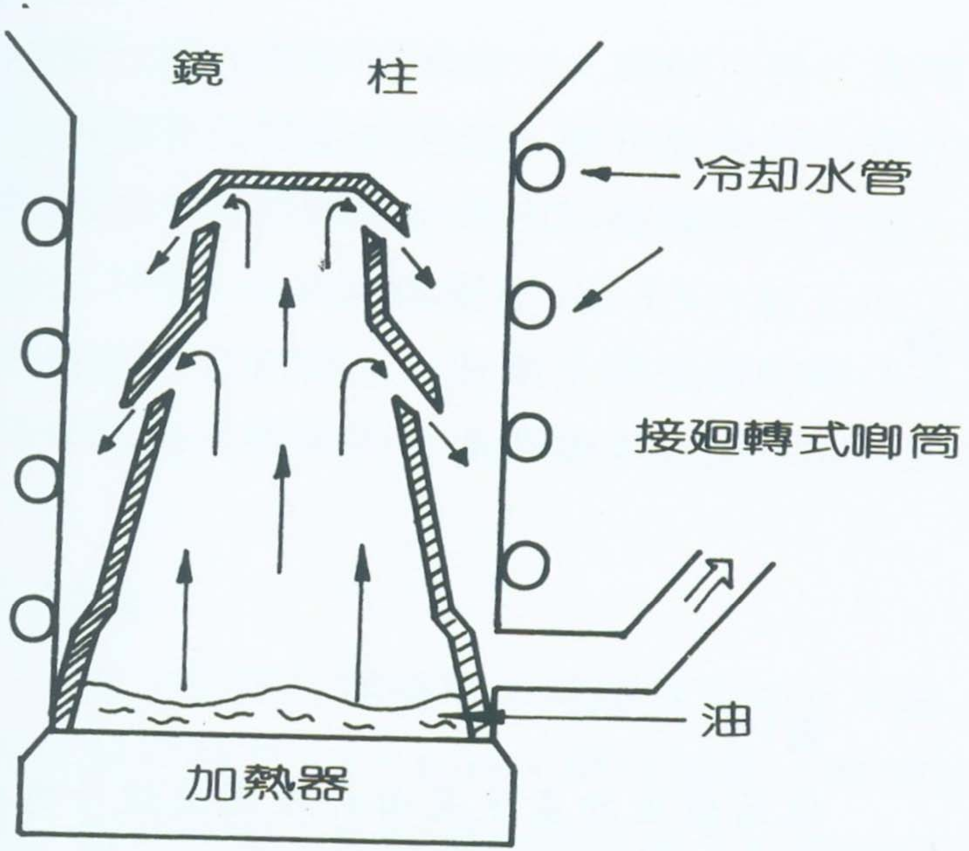



圖1.6

擴散式唧筒之構造及其作用。利用加熱汽化的油氣分子快速自傘形狹縫噴下，將空氣分子帶至底端，再由迴轉式唧筒將空氣抽出，並利用冷卻水使油氣液化循環。

擴散式唧筒(diffusion pump)：真空度可達$10^{-6}$ torr

平均自由徑(mean free distance)：電子和空氣分子從一次撞擊到下一次撞擊間所行進的距離($10^{-3}$ torr≒1公尺)

圖1-6

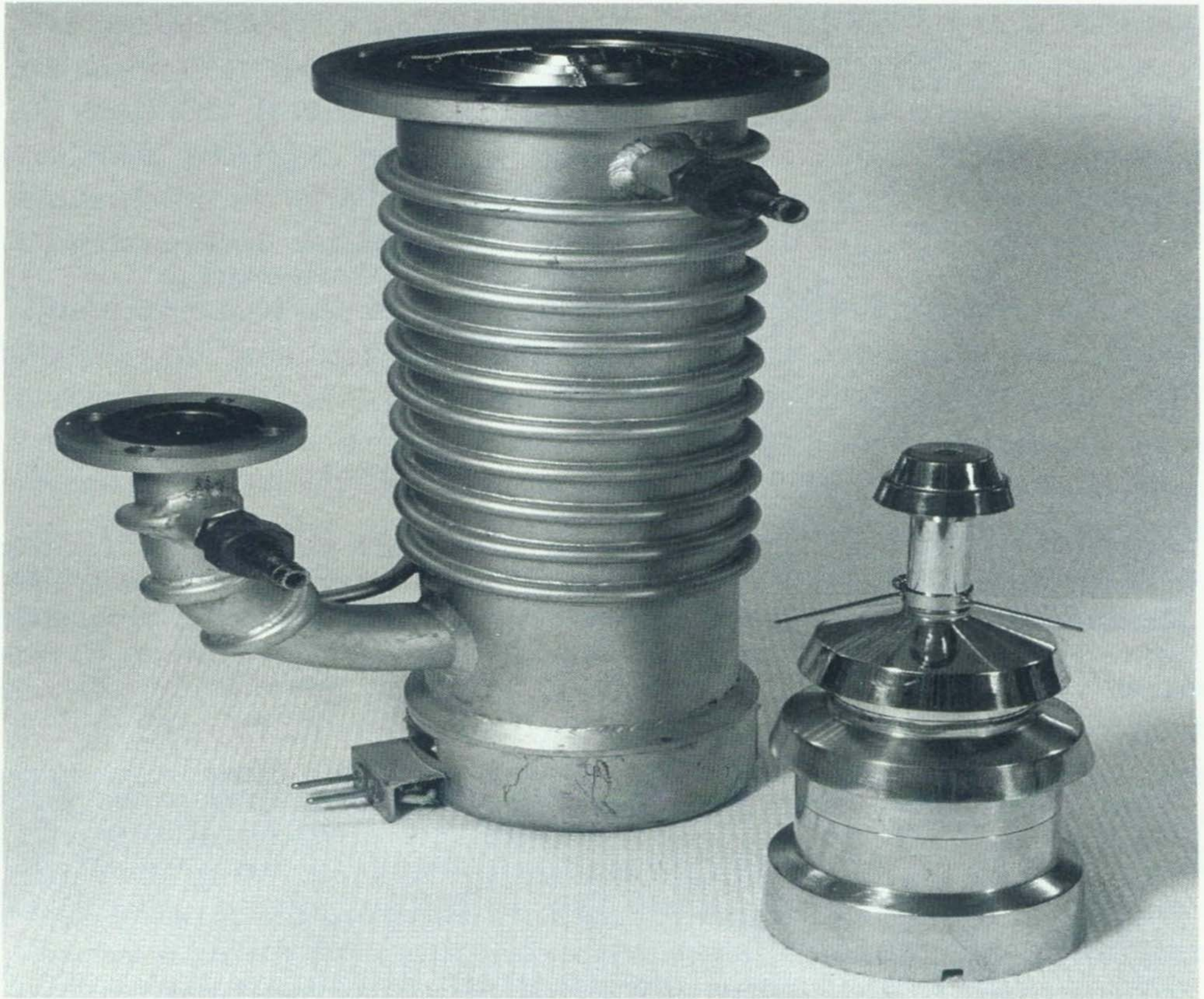
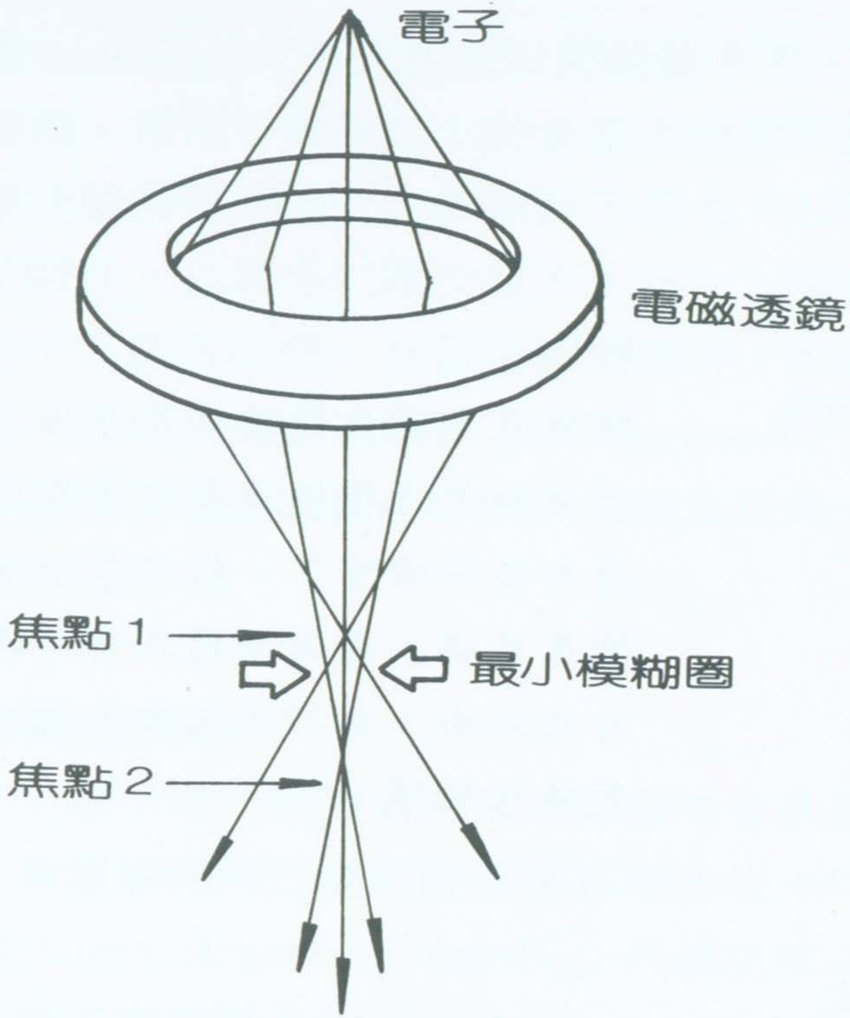


**Figure 6-38(A)** Diffusion pump. Photograph of exterior of a diffusion pump showing cooling coils surrounding body. Stacked, umbrella-like caps (lower, right) fit down into the body of the pump.




※理論上解像力可達使用光線波長的一半

※Spherical aberration

圖1.7

球面像差的產生是由於經過透鏡外緣與中心之電子偏折角度不同，因而聚集在不同的焦點上形成一最小模糊圈（空心箭頭所示）。

圖1-7

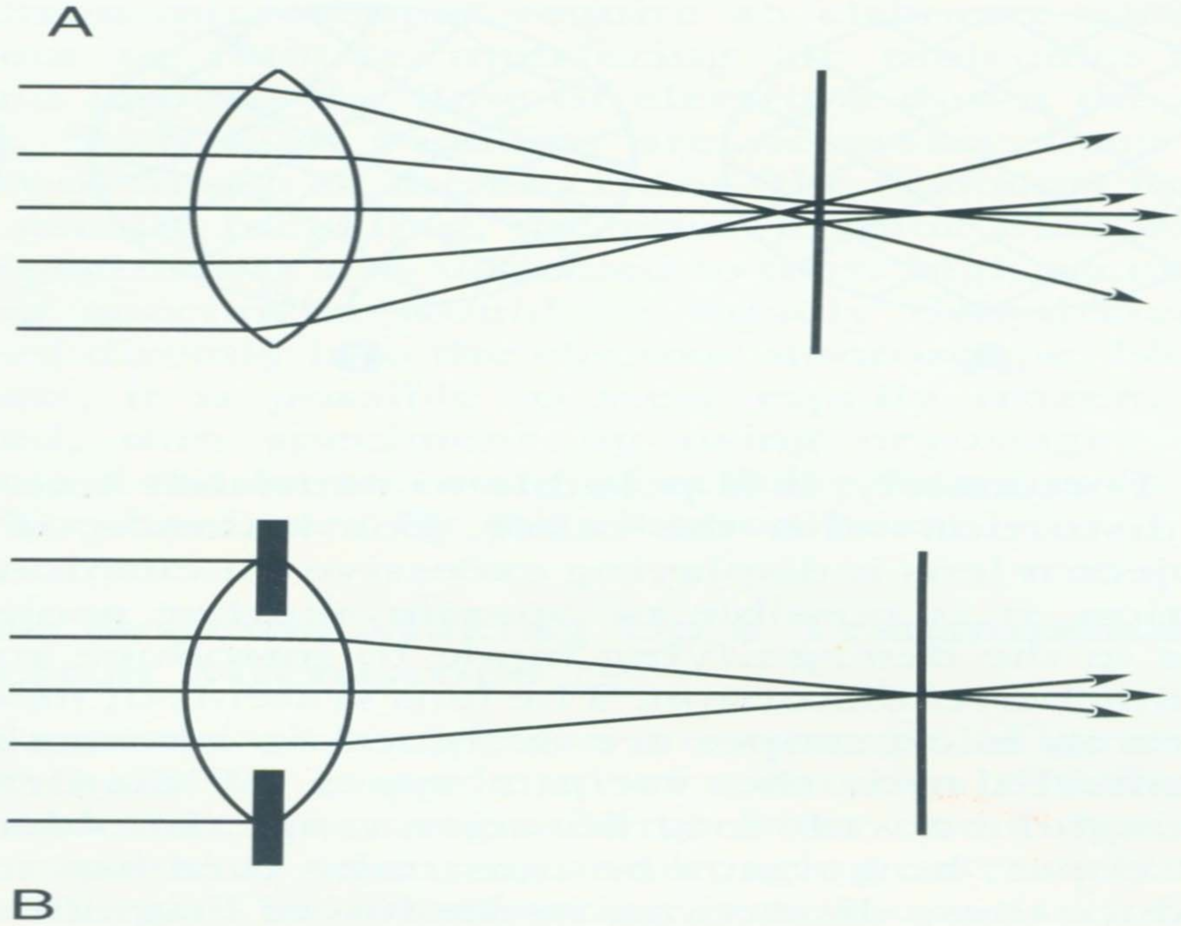



**Figure 6-17** (A) Spherical aberration in a lens. Peripheral rays are refracted more than central rays, so that all rays do not converge to a common, small focal point. Instead, an enlarged, diffuse spot like the Airy disc will be generated. The vertical line indicates the one point where the point will be smallest, i.e., having the smallest circle of confusion. (B) Correction of spherical aberration with an aperture (here shown inside the lens) to cut out peripheral rays and thereby permit remaining rays to focus at a common small imaging point. Resolution will be improved since individual image points in the specimen will be smaller.

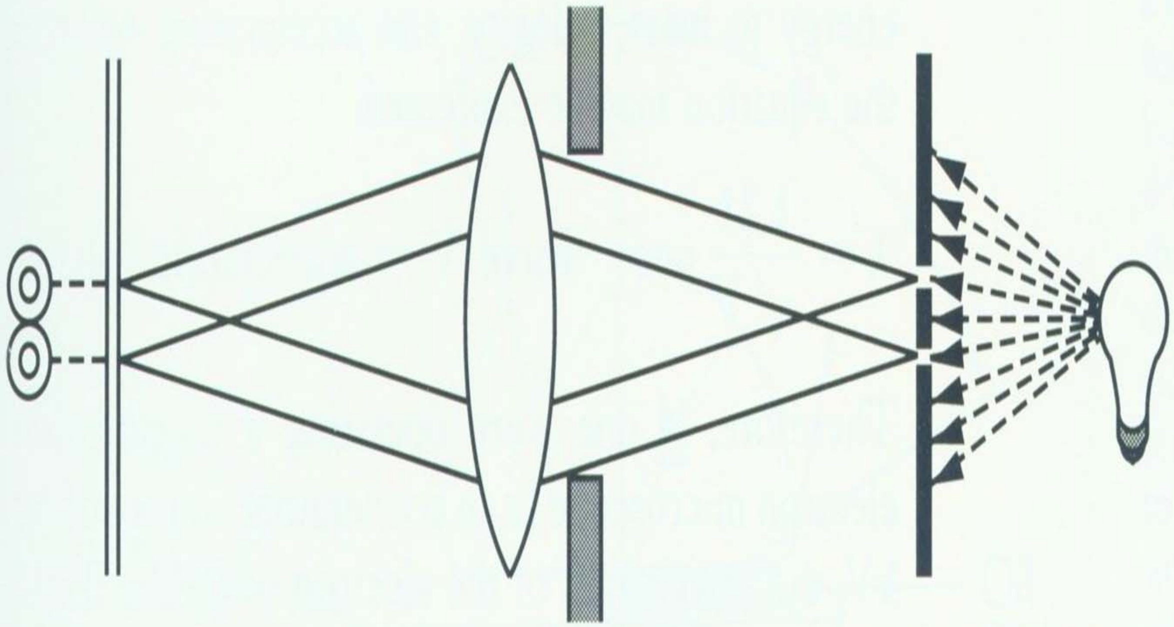


**Figure 6-5** Degradation of resolution due to diffraction. Two pinholes held in front of a light source are viewed in a lens and the image is projected onto a flat surface. Instead of two sharp, bright spots, one sees two spots surrounded by diffuse rings (left). These enlarged, indistinct spots are caused by Airy discs. The thick cross-hatched barrier in front of the lens is an aperture.

- 光是一種波動物質, 當其通過小孔徑時, 會因干涉（interference）和繞射（diffraction）現象的共同效應而產生埃氏光環(Airy’s disk), 致使解像力受影響

圖6-5

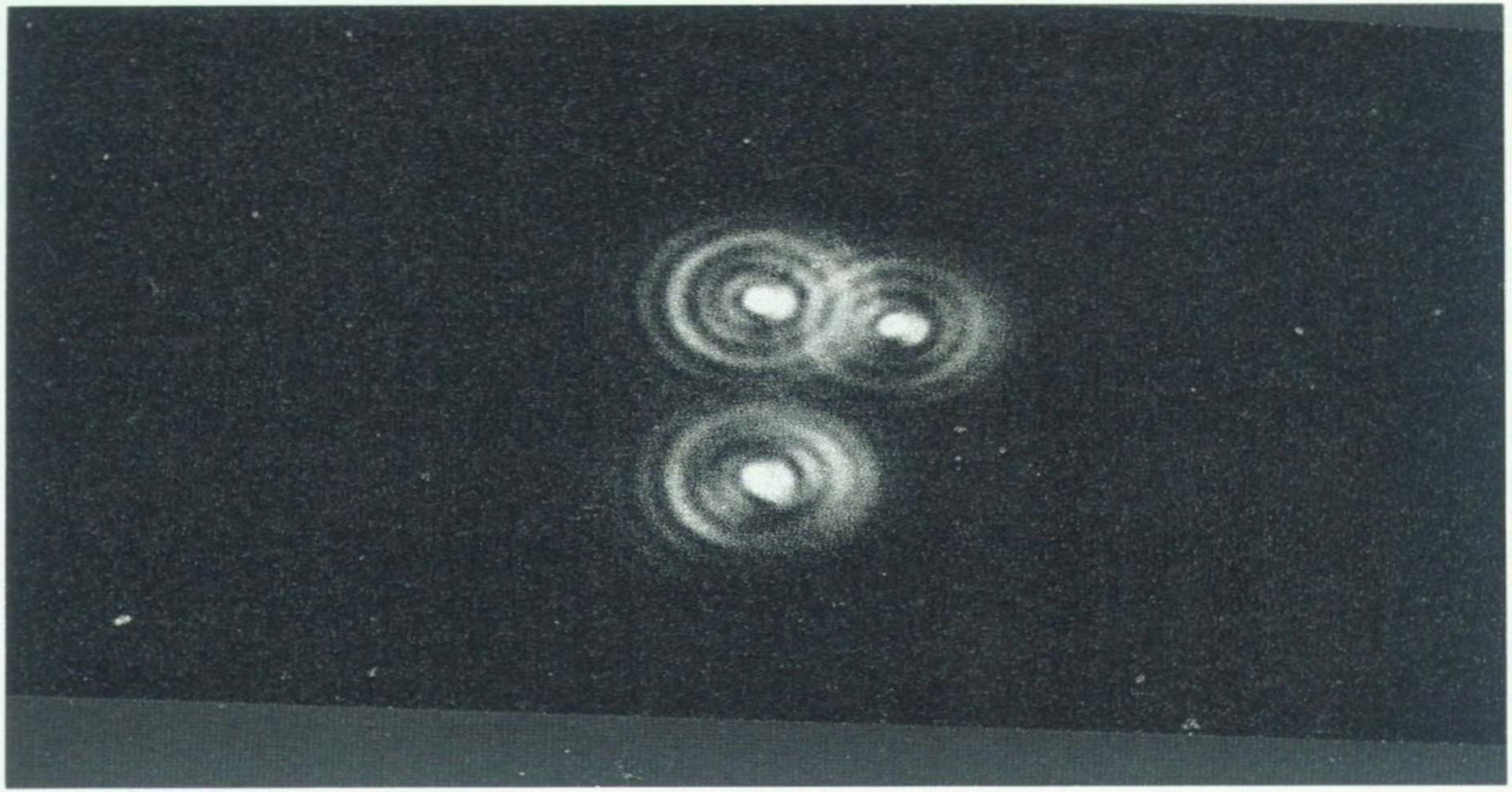


**Figure 6-6** Airy discs generated by viewing three pinholes in a light microscope. A thin film of palladium/gold was deposited onto a glass slide, and the slide was examined for naturally occurring pinholes in the film. Magnification of micrograph is 1,000×.

圖6-6

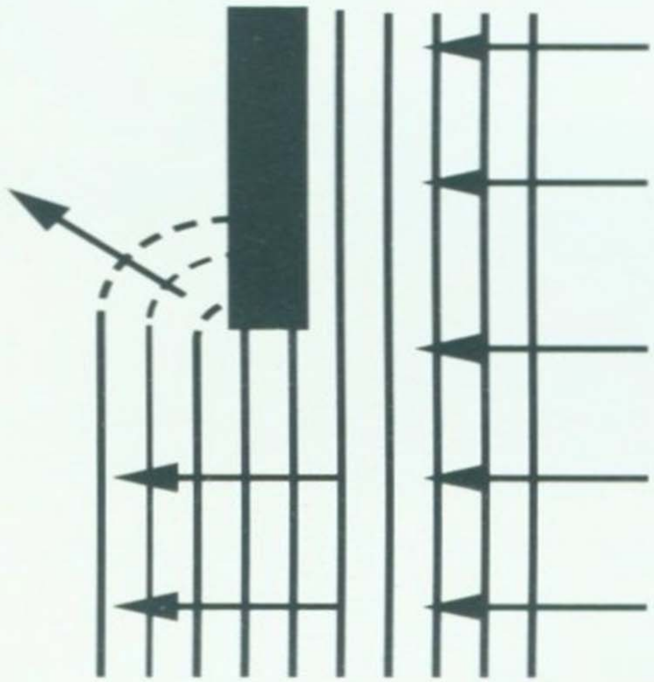
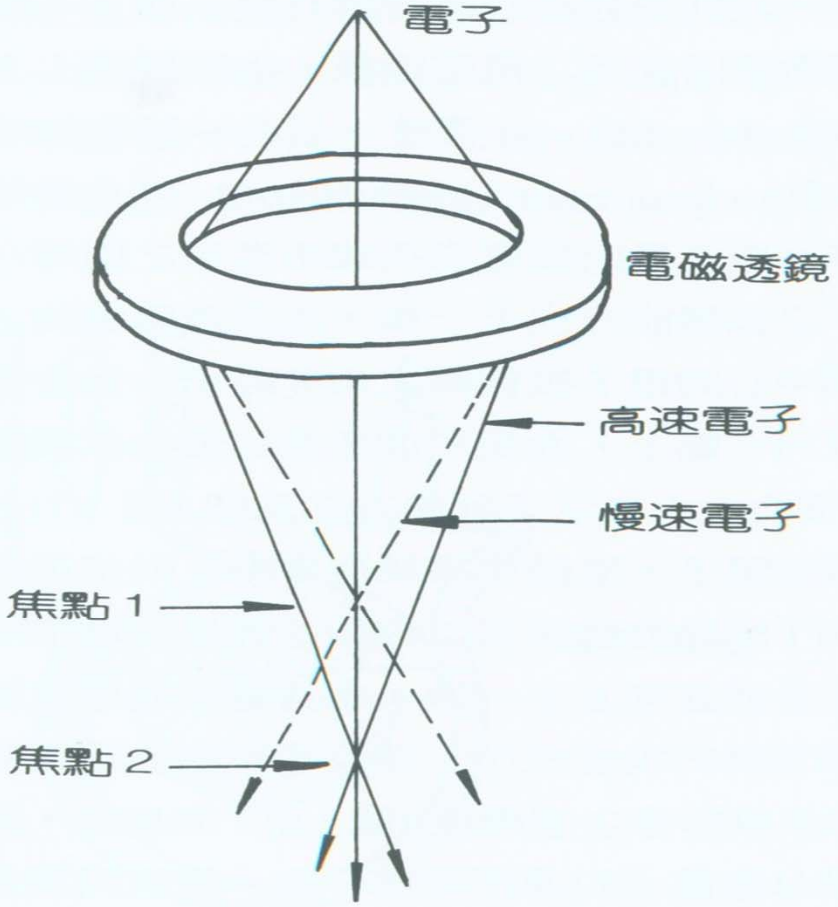


**Figure 6-2** Diffraction phenomenon demonstrated by a series of parallel waves that strike the edge of a solid object. From the edge, a new series of waves (dashed lines) are generated that merge with the original front.

圖6-2




Chromatic aberration

圖1.8

色像差的形成乃由於不同能量或速度的電子經過透鏡後聚集的焦點不一而造成影像模糊。

圖1-8

## Table 6-2 Wavelengths of Visible Light

| Color | Wavelength in nm |
|---|---|
| red | 760–630 |
| orange | 630–590 |
| yellow | 590–560 |
| green | 560–490 |
| blue | 490–450 |
| violet | 450–380 |

表6-2

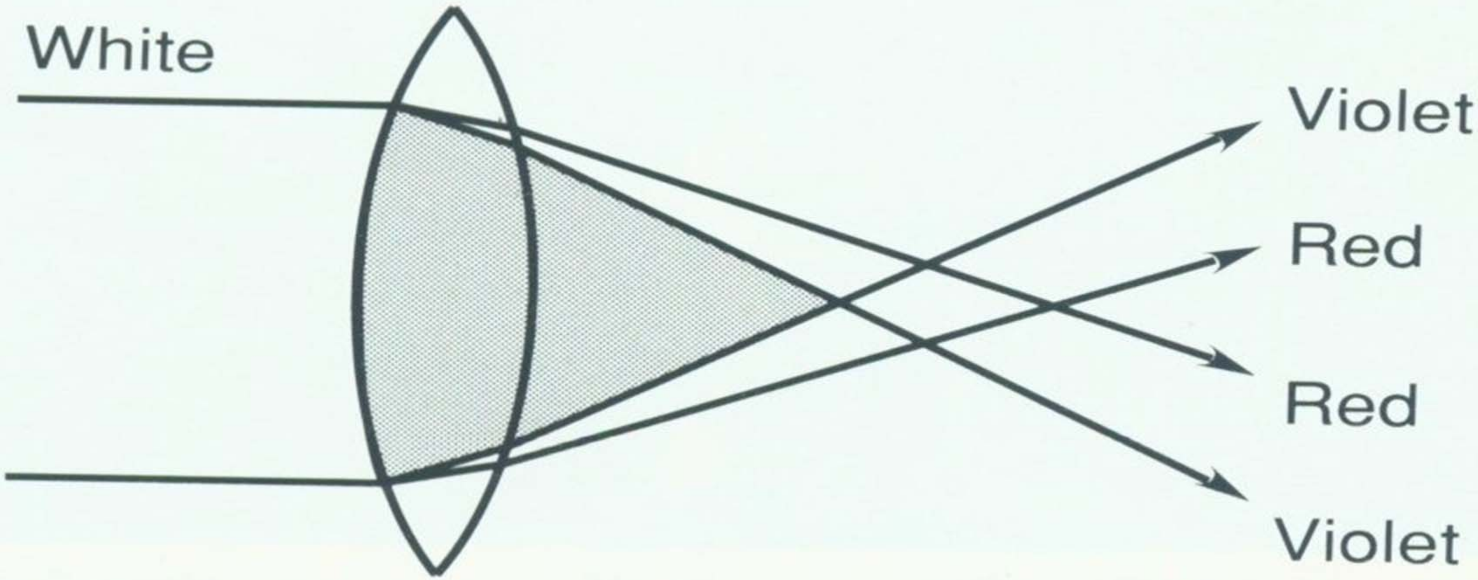
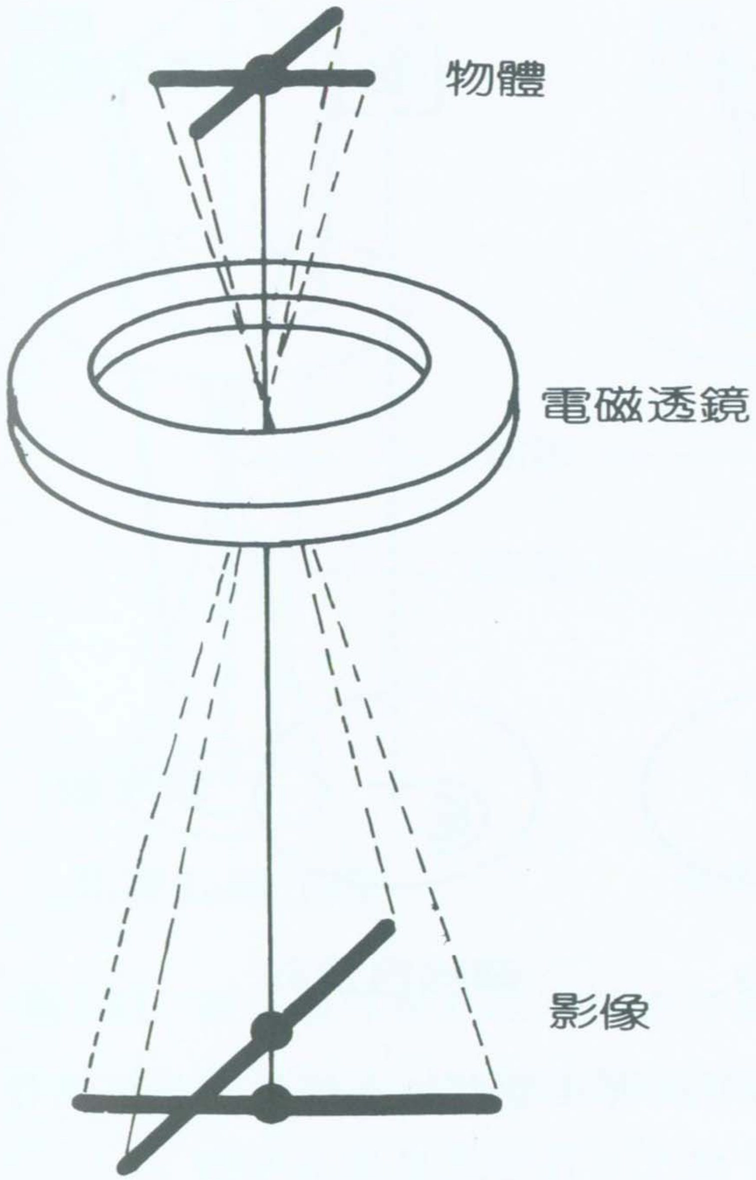



**Figure 6-15** Chromatic aberration in a glass lens. Different wavelengths do not come to focus at the same point. Note how the violet part of the spectrum (stippled) focuses at a shorter distance than does the red part of the spectrum. This results in an enlarged, unsharp point rather than a smaller, focused one. Resolution of the point will be degraded.

圖6-15



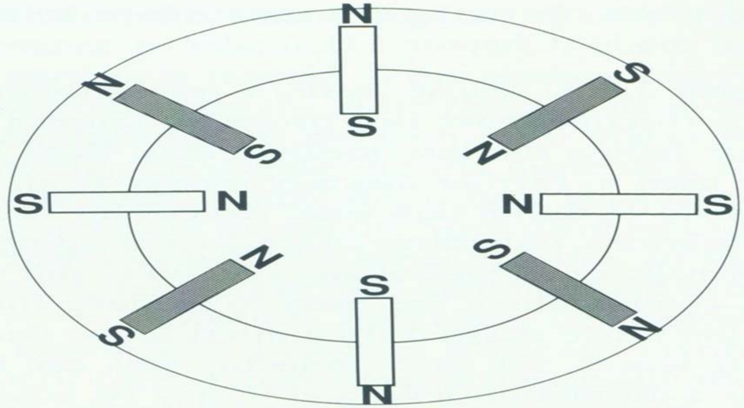

Astigmatism

圖1.9

當電磁透鏡的磁場在X軸與Y軸上不對稱時，物體經過透鏡呈像亦不在同一平面上，因而造成影像扭曲，此即像散的造成原因。

圖1-9

A




B

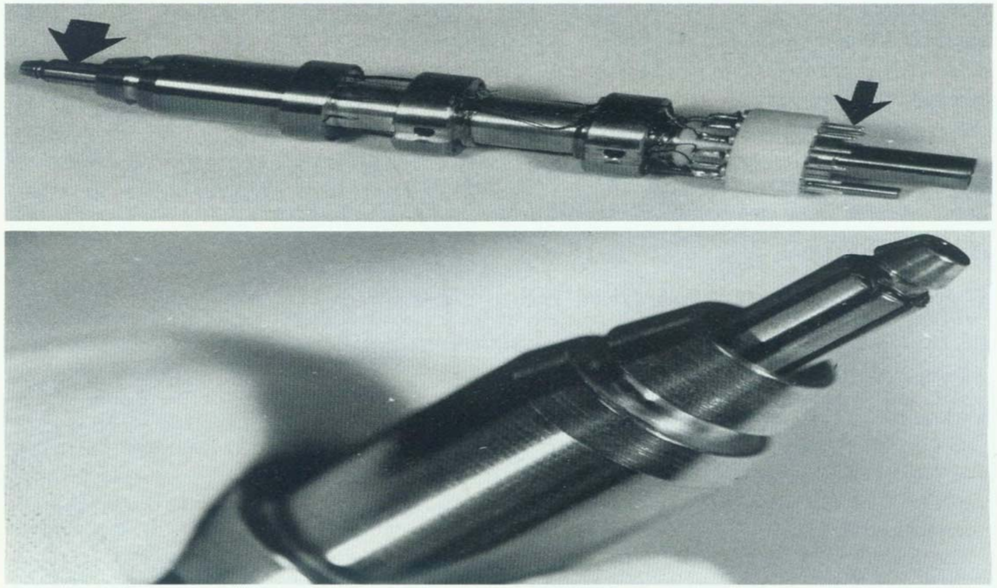


**Figure 6-35** (A) Conceptual drawing of electromagnetic stigmator showing orientation of eight electromagnets around lens axis. Strength and direction are controlled by adjusting appropriate combinations of magnets to generate a symmetrical field. The stigmator is located under the condenser and the objective lens polepieces. (B) Actual stigmator apparatus taken from an electron microscope. Large arrow indicates one of the eight electromagnetic iron slugs oriented around the central axis. The entire apparatus fits up into the bore of the objective lens so that the area indicated in the large arrow is positioned just under the specimen. The smaller arrow points out individual electrical contacts through which current flows to energize the electromagnets. The closeup photograph shows some of the electromagnets that are positioned near the specimen.

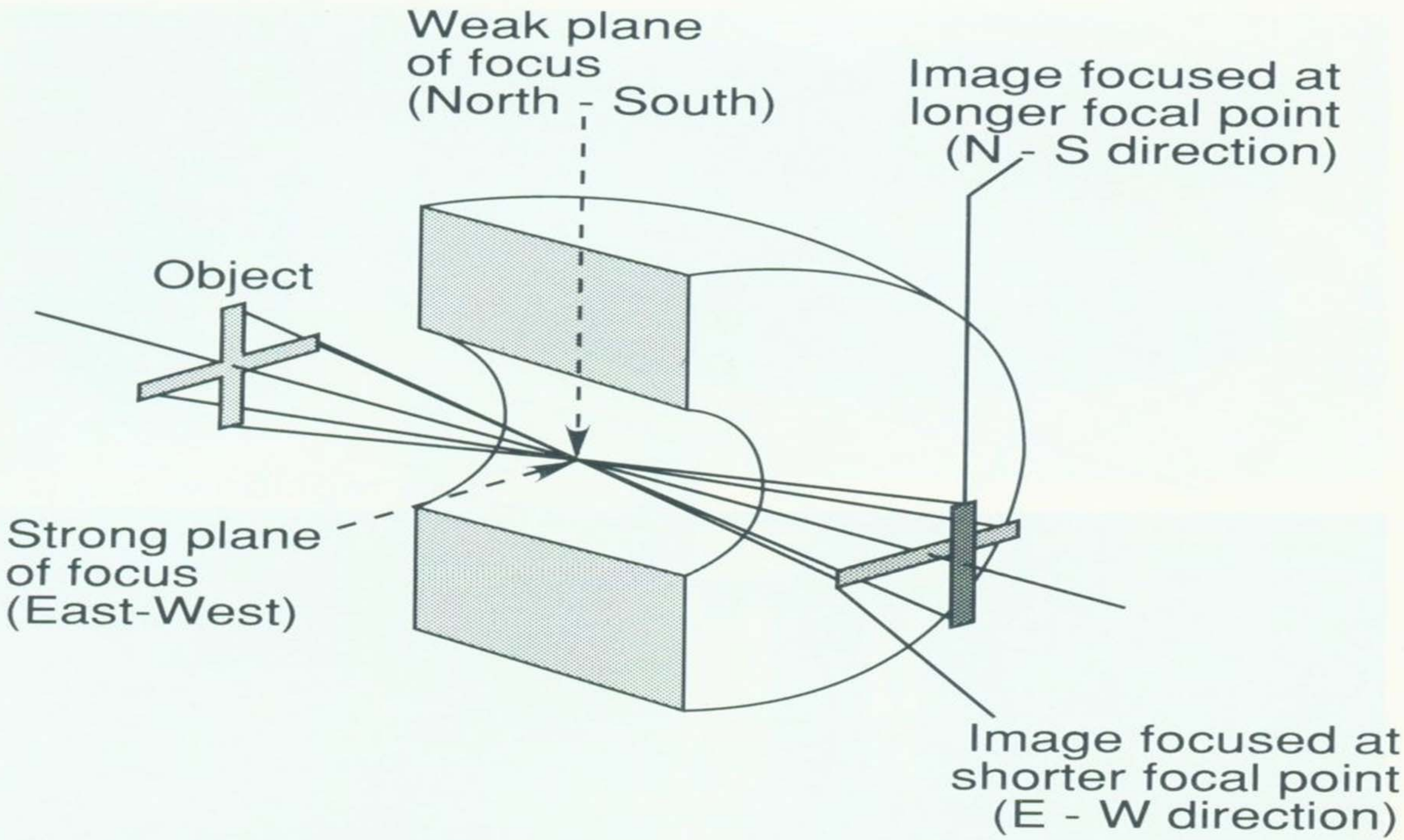



**Figure 6-14** Astigmatism in a lens. Since the lens field is asymmetrically weaker in the north/south plane, objects oriented along the north/south axis will focus at a longer distance. By contrast, due to a stronger east/west lens field, objects oriented east/west will come to focus at a shorter distance from the lens. The effect is that only some portions of the image (either north/south or east/west) will be in focus at one time. Obviously, resolution will be degraded since the image will be focused in only one plane.

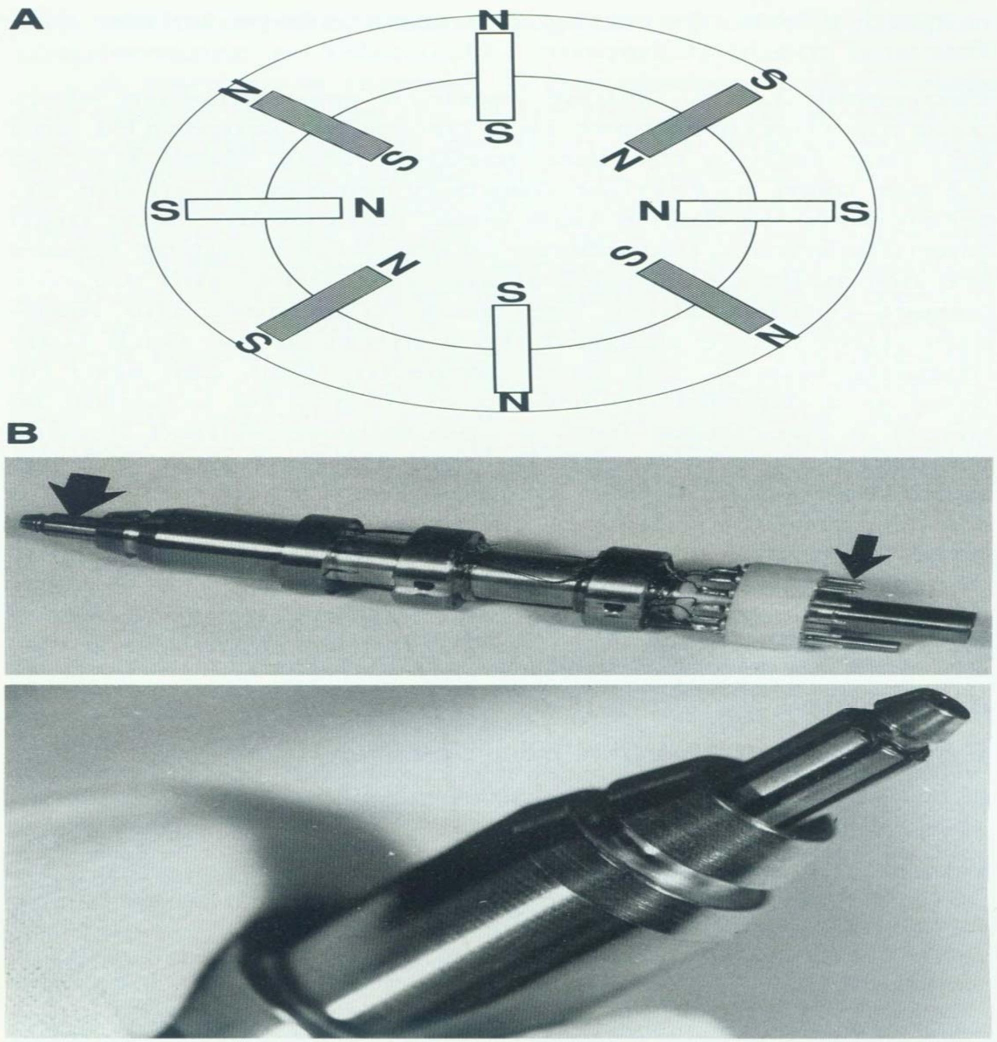
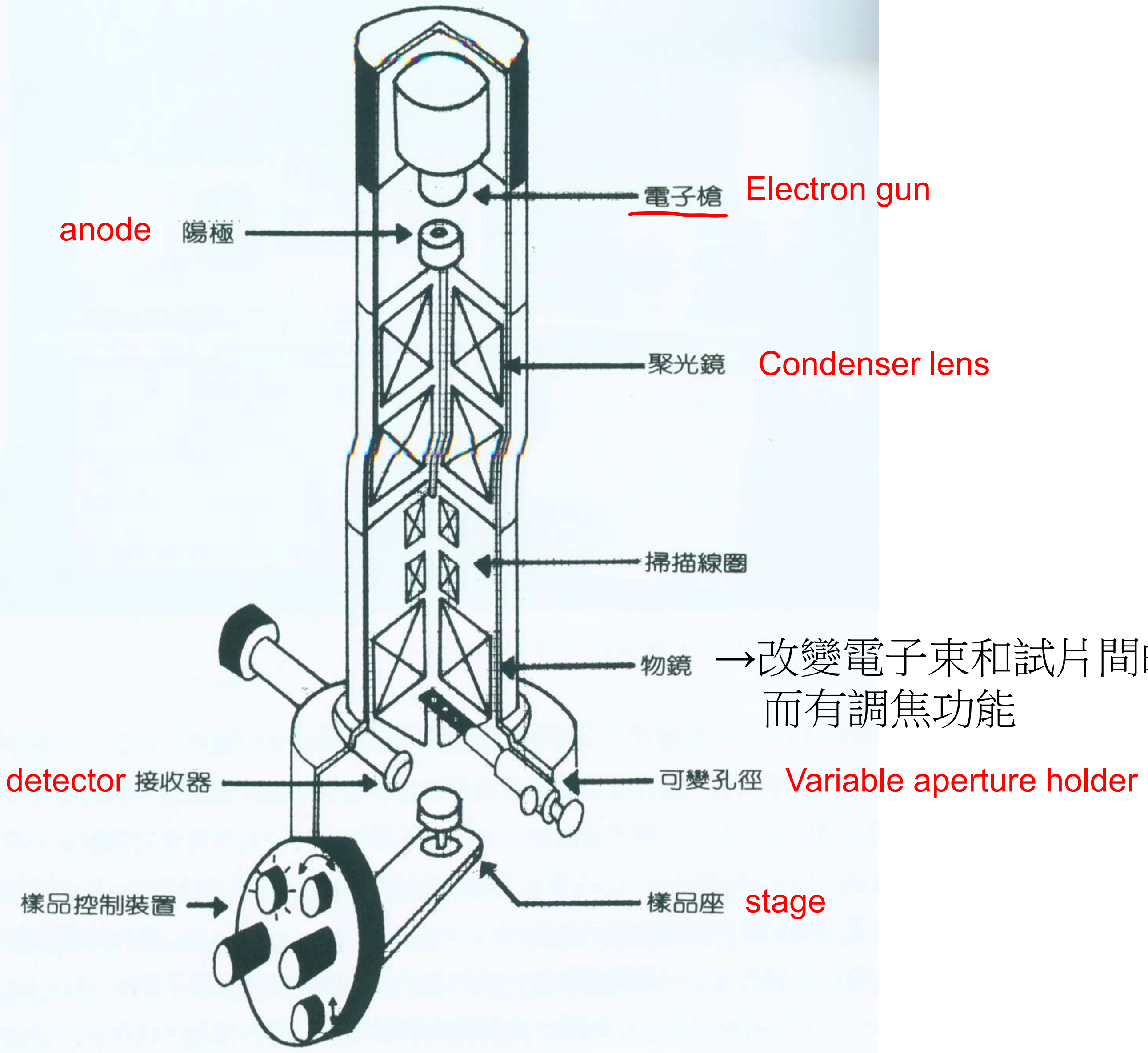
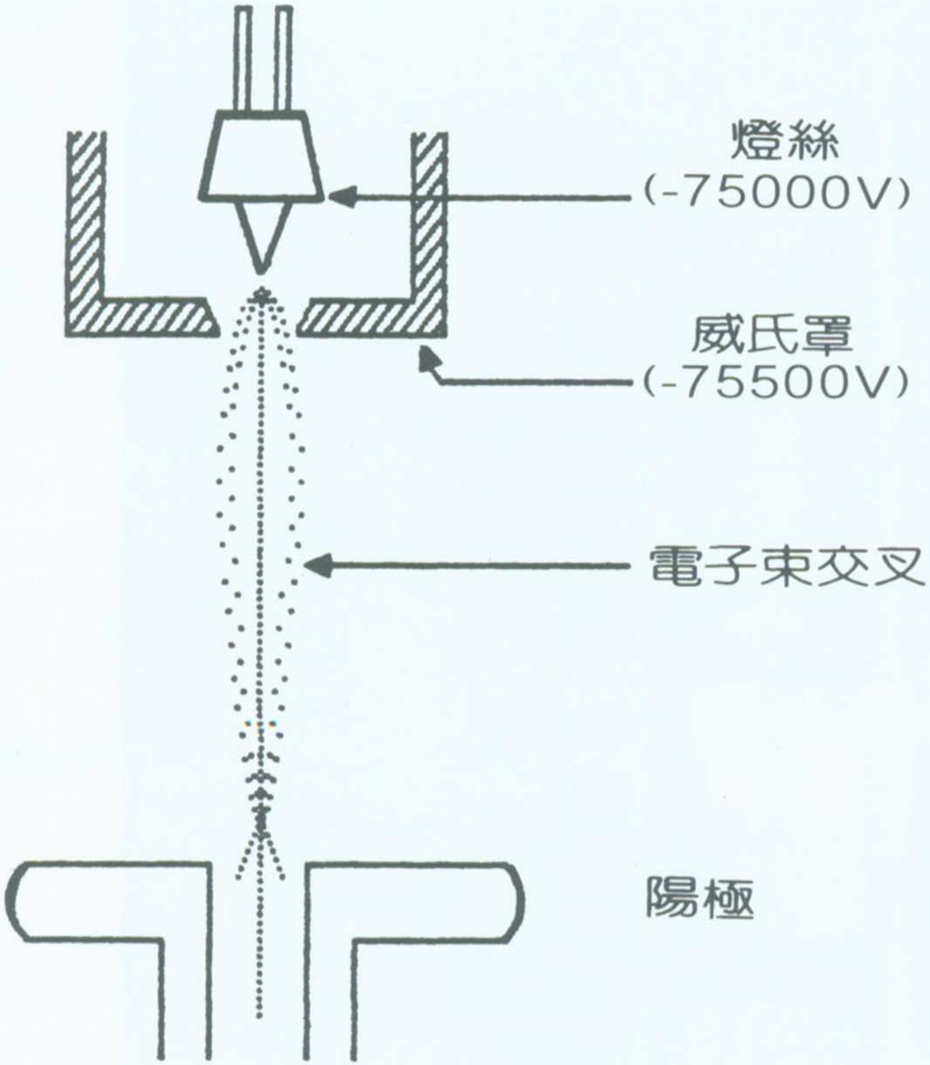



**Figure 6-35** (A) Conceptual drawing of electromagnetic stigmator showing orientation of eight electromagnets around lens axis. Strength and direction are controlled by adjusting appropriate combinations of magnets to generate a symmetrical field. The stigmator is located under the condenser and the objective lens polepieces. (B) Actual stigmator apparatus taken from an electron microscope. Large arrow indicates one of the eight electromagnetic iron slugs oriented around the central axis. The entire apparatus fits up into the bore of the objective lens so that the area indicated in the large arrow is positioned just under the specimen. The smaller arrow points out individual electrical contacts through which current flows to energize the electromagnets. The closeup photograph shows some of the electromagnets that are positioned near the specimen.




→改變電子束和試片間的距離
而有調焦功能

圖6.3　掃描式電子顯微鏡的鏡柱剖面圖。(Hitachi, LTD.)

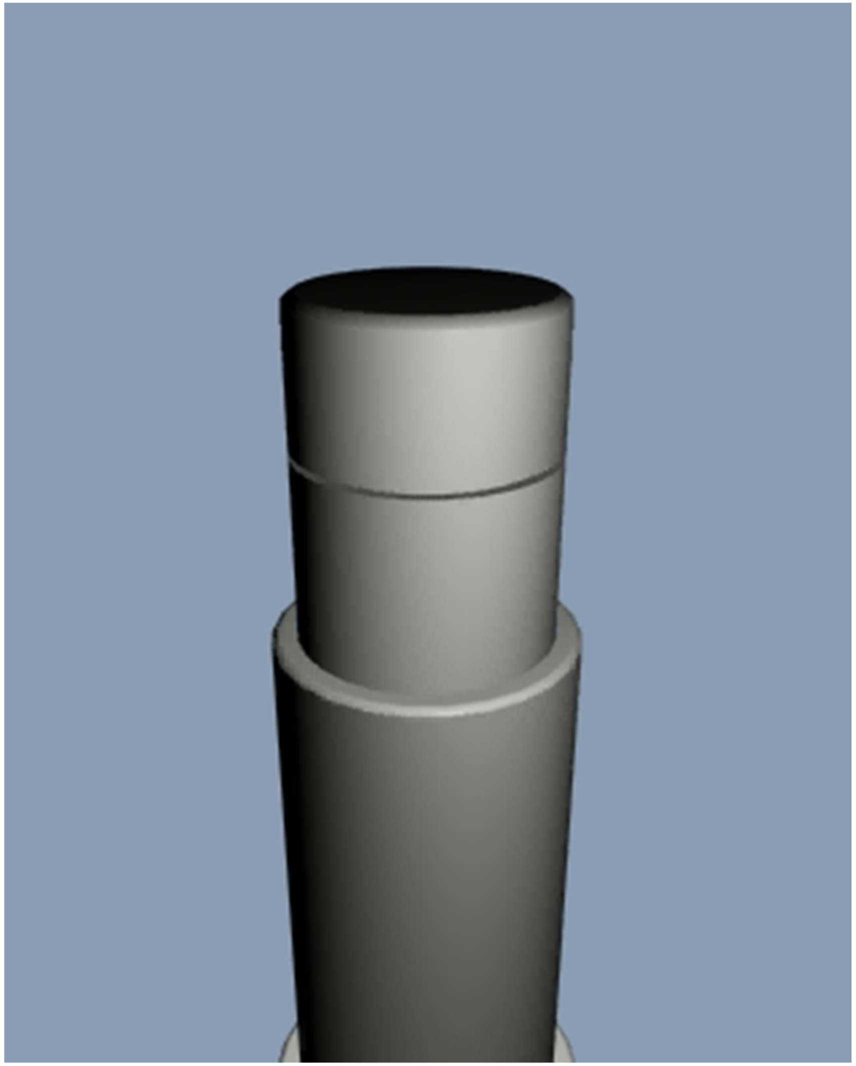



當燈絲加熱至2600℃左右時，會有大量電子自尖端釋出,通常提供燈絲負60kv至負100kv的電壓，以加速電子

圖1.3

電子槍之構造以及電子由燈絲釋出後經過威氏罩形成電子束交叉之情形。

圖1-3



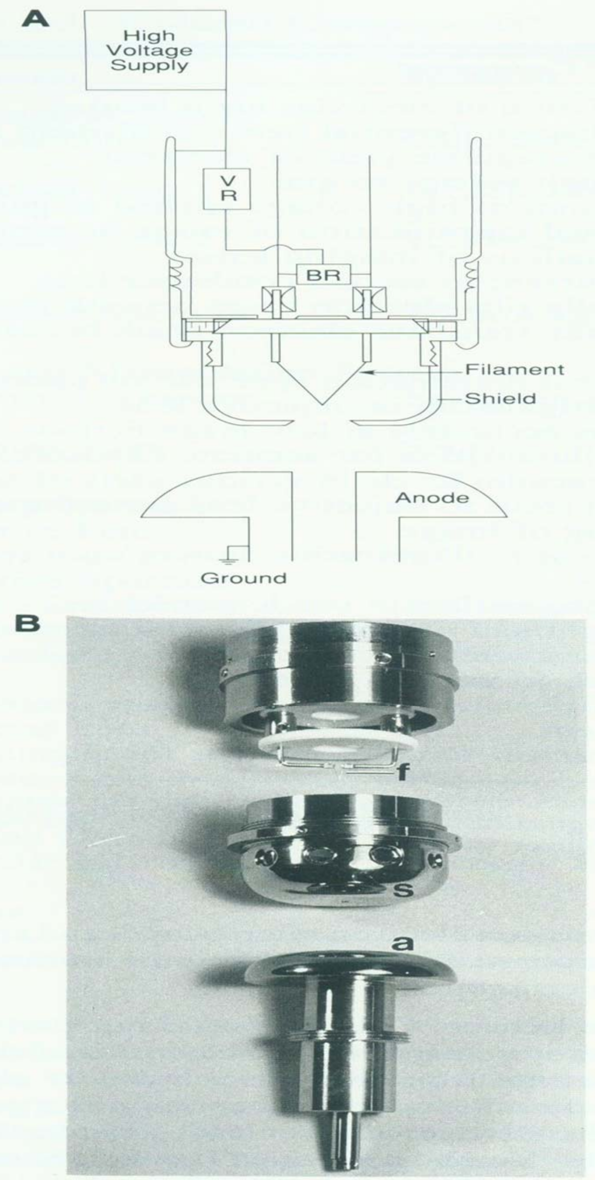



**Figure 6-21** (A) Diagram of an electron gun showing filament, shield, and anode. The shield is connected directly to the high voltage, whereas the high voltage leading to the filament has a variable resistor (VR) to vary the amount of high voltage. The output from the variable resistor is then passed through two balancing resistors (BR) which are attached to the filament. (Modified from a drawing provided by Hitachi Scientific Instruments). (B) Actual electron gun from TEM showing filament (f), shield (s), and anode (a). Compare to line drawing in 6-21(A).

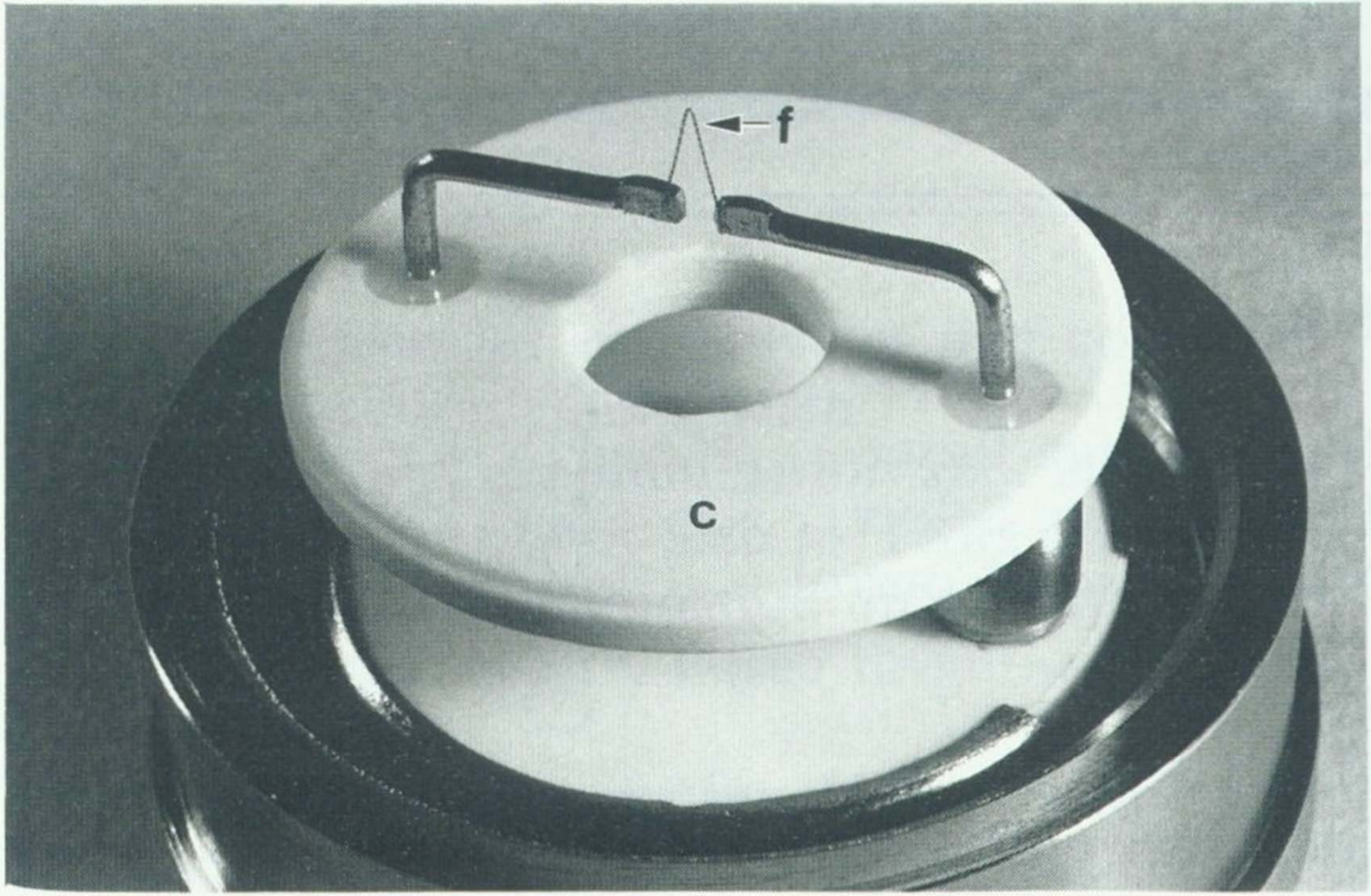



**Figure 6-22** Standard V-shaped tungsten filament (f) used in most electron microscopes. The filament is spot welded to the larger supporting arms which pass through the ceramic (c) insulator and plug into the electrical leads of the gun.

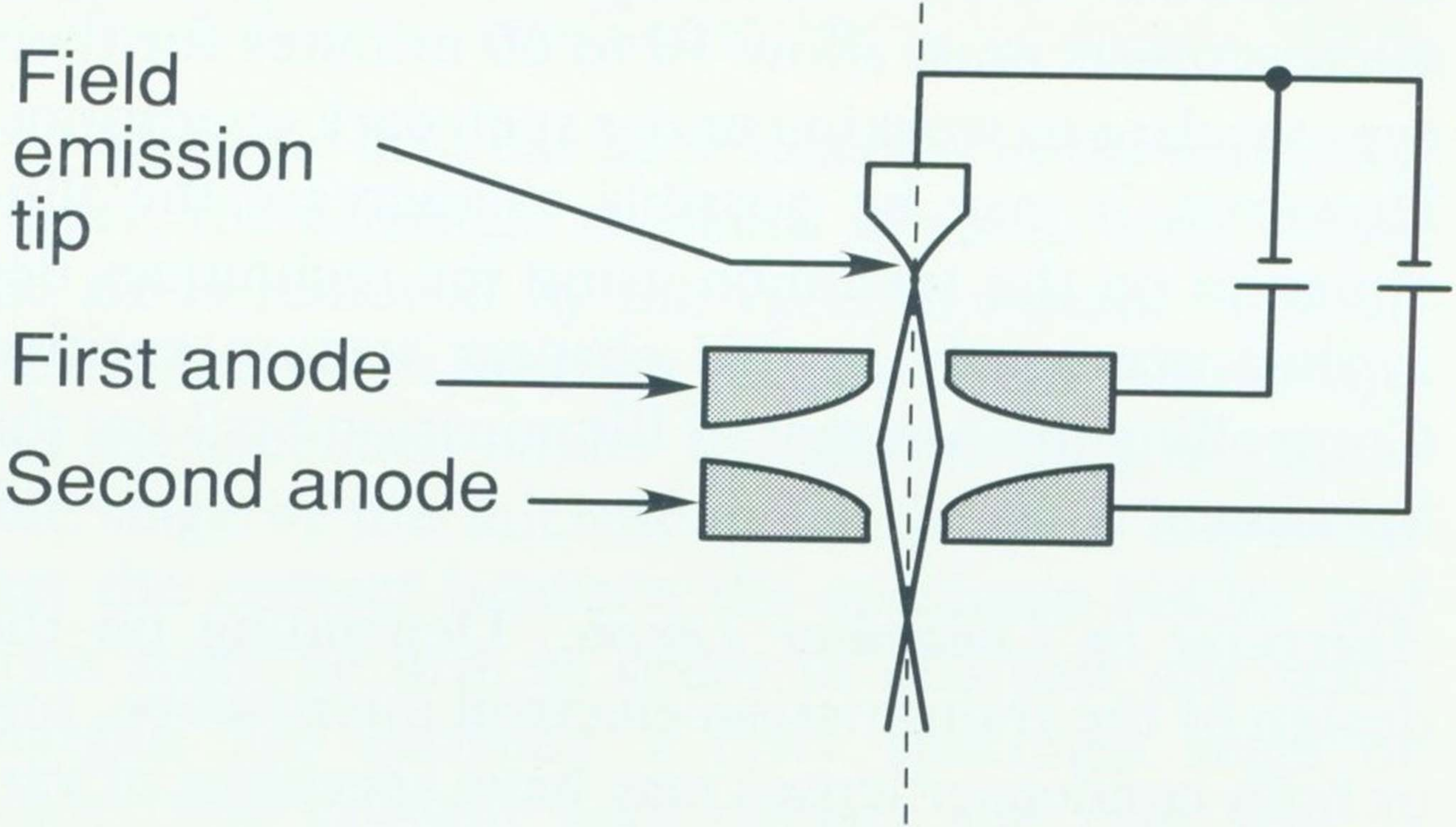



**Figure 6-27** The field emission gun. Electrons are extracted from a single crystal of tungsten by a series of anodes that are several thousands volts positive. It is not necessary to heat this type of filament.

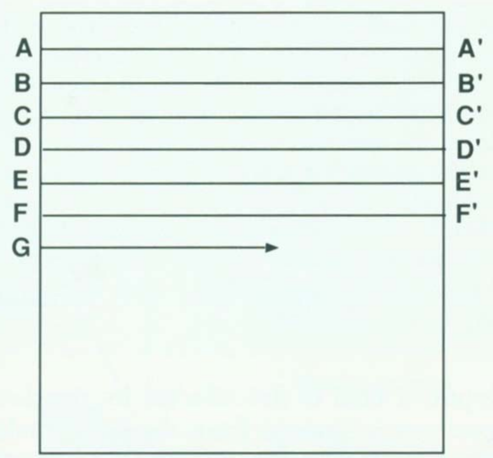



**Figure 7-4** The focused beam of electrons is scanned in a raster pattern over the specimen surface. The first scan is from A to A′, with the beam moving down and then scanning line B to B′, etc. (Redrawn from Postek, et al, 1980.)

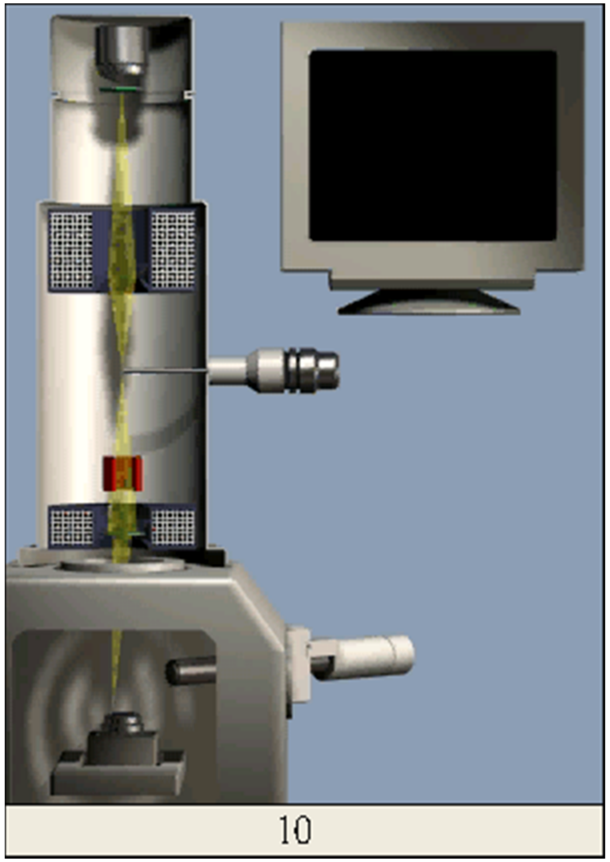
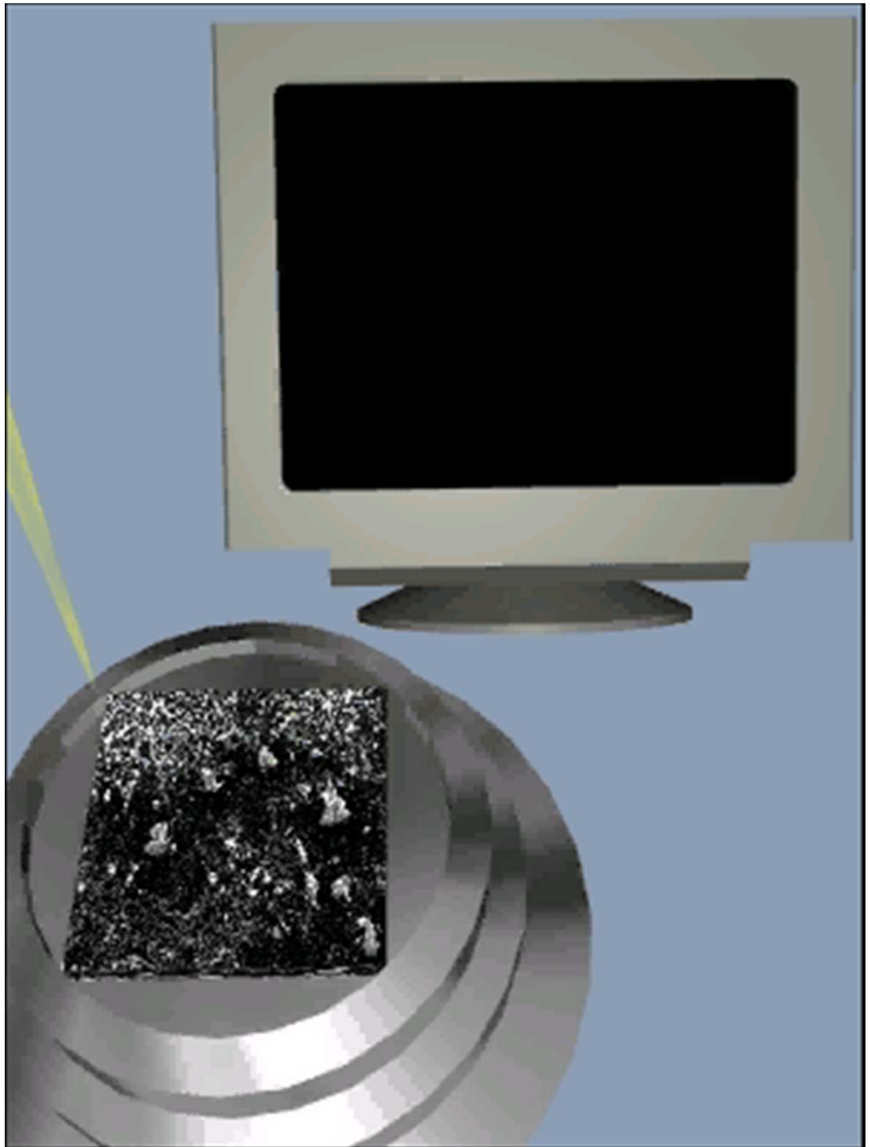
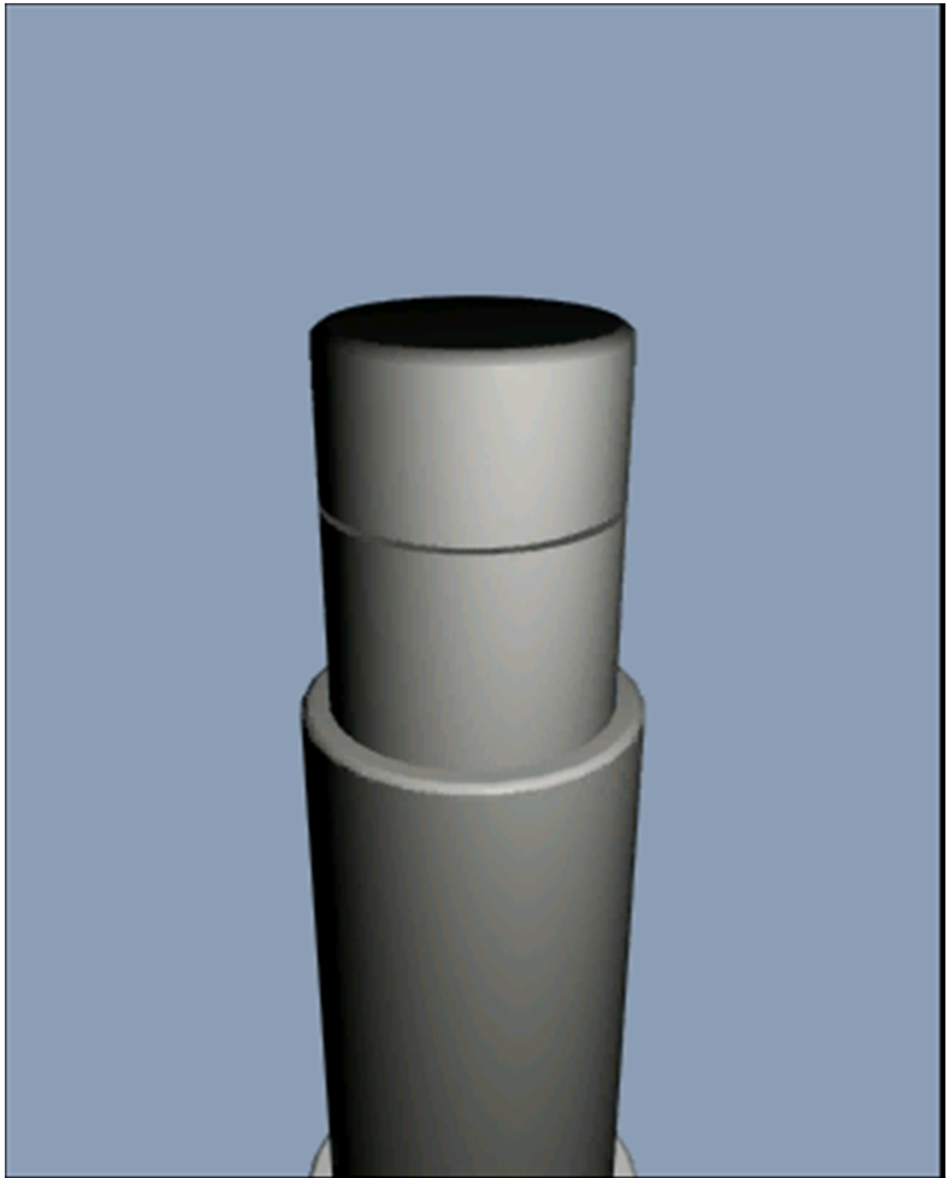



圖7-4



25



15

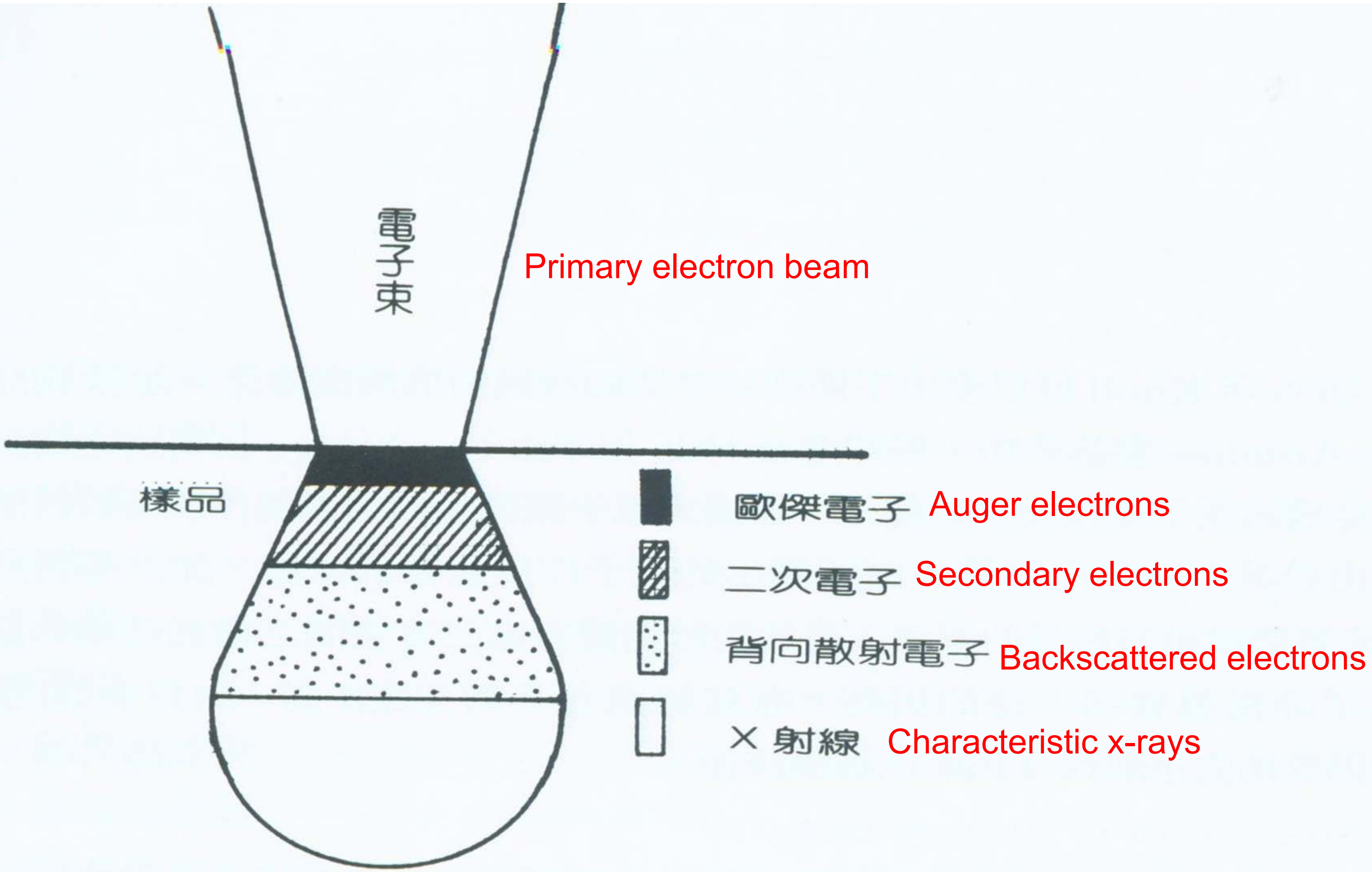
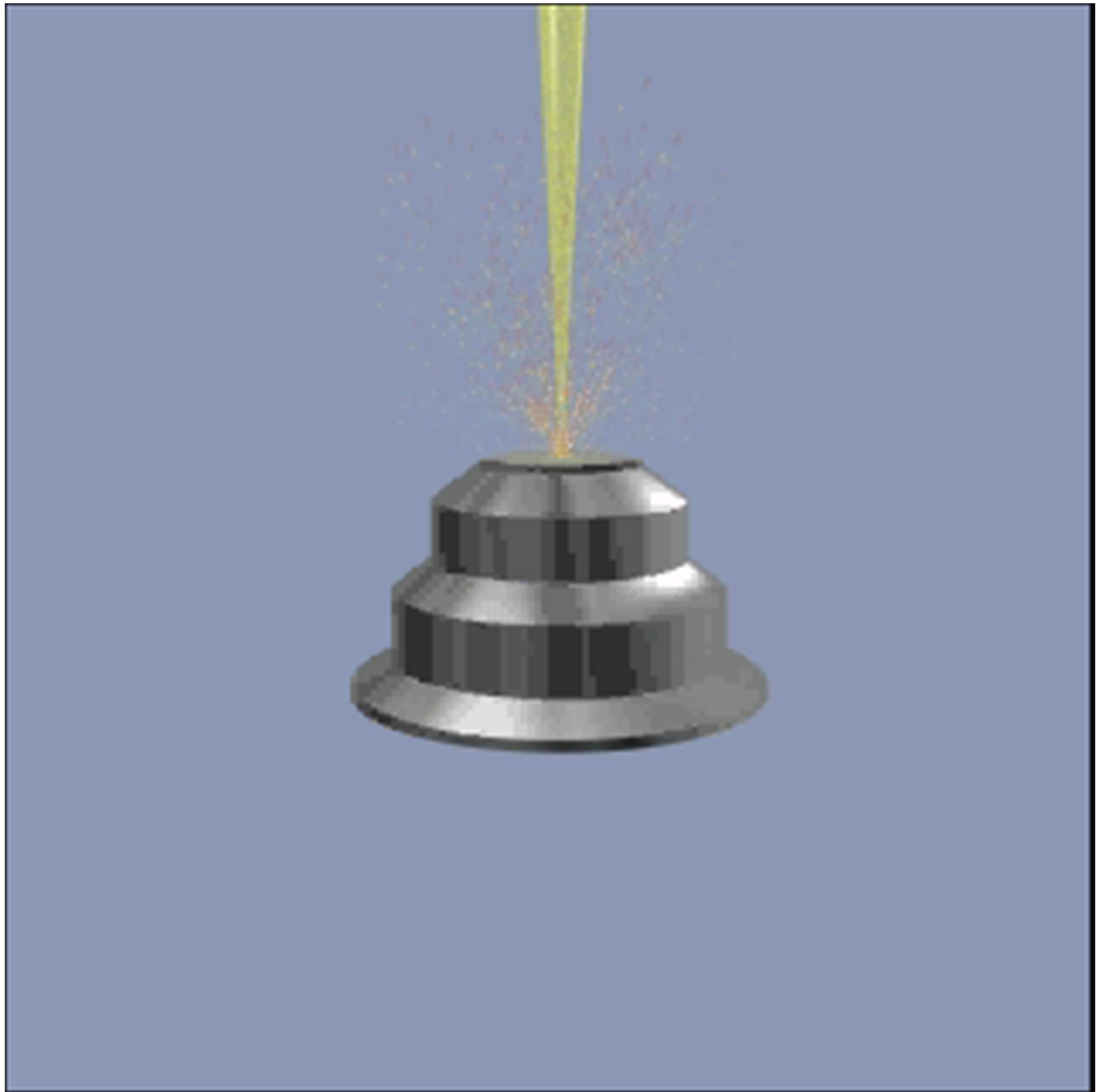


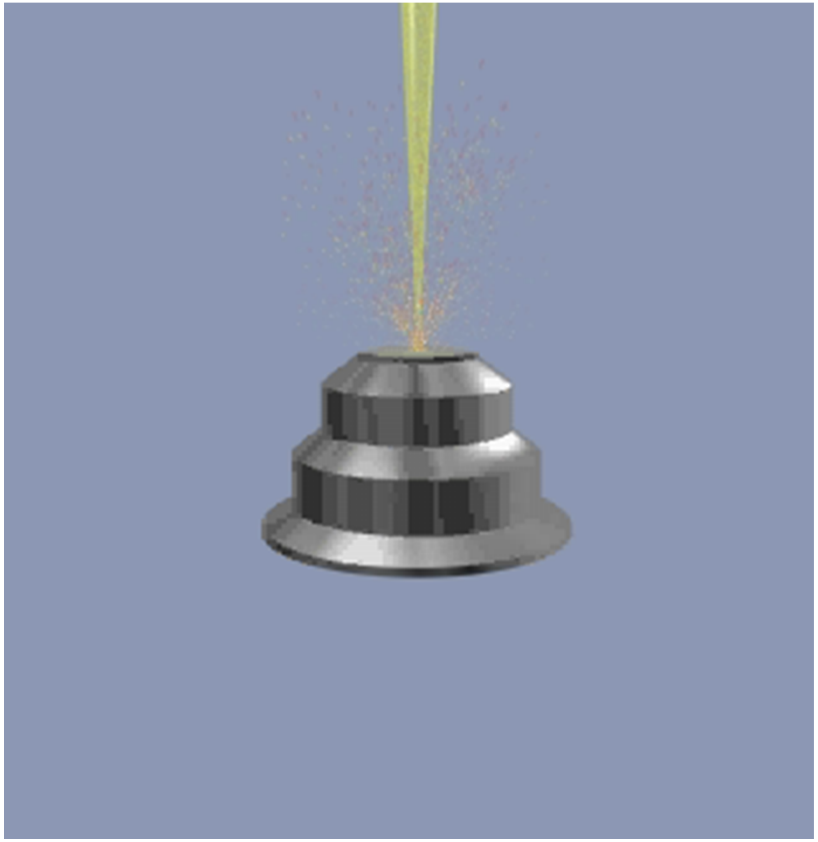

圖6.1　電子探束撞擊在樣品表面時，各種訊號電子自作用體積之不同層面釋出的情形。

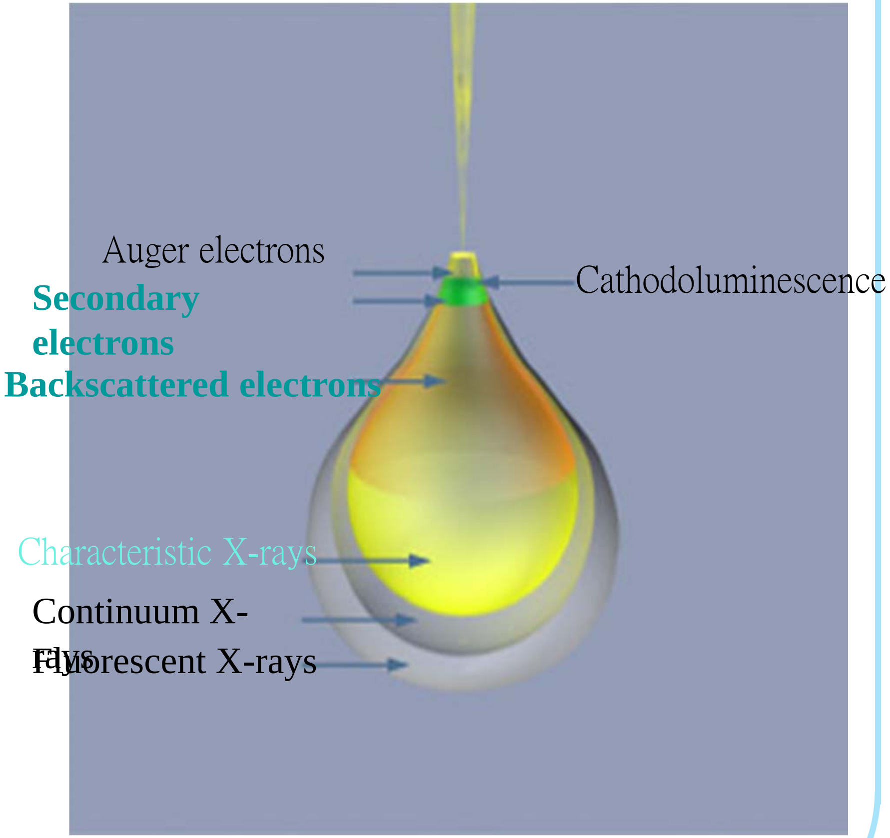
圖6-1



6

# Theory of Scanning Electron Microscope





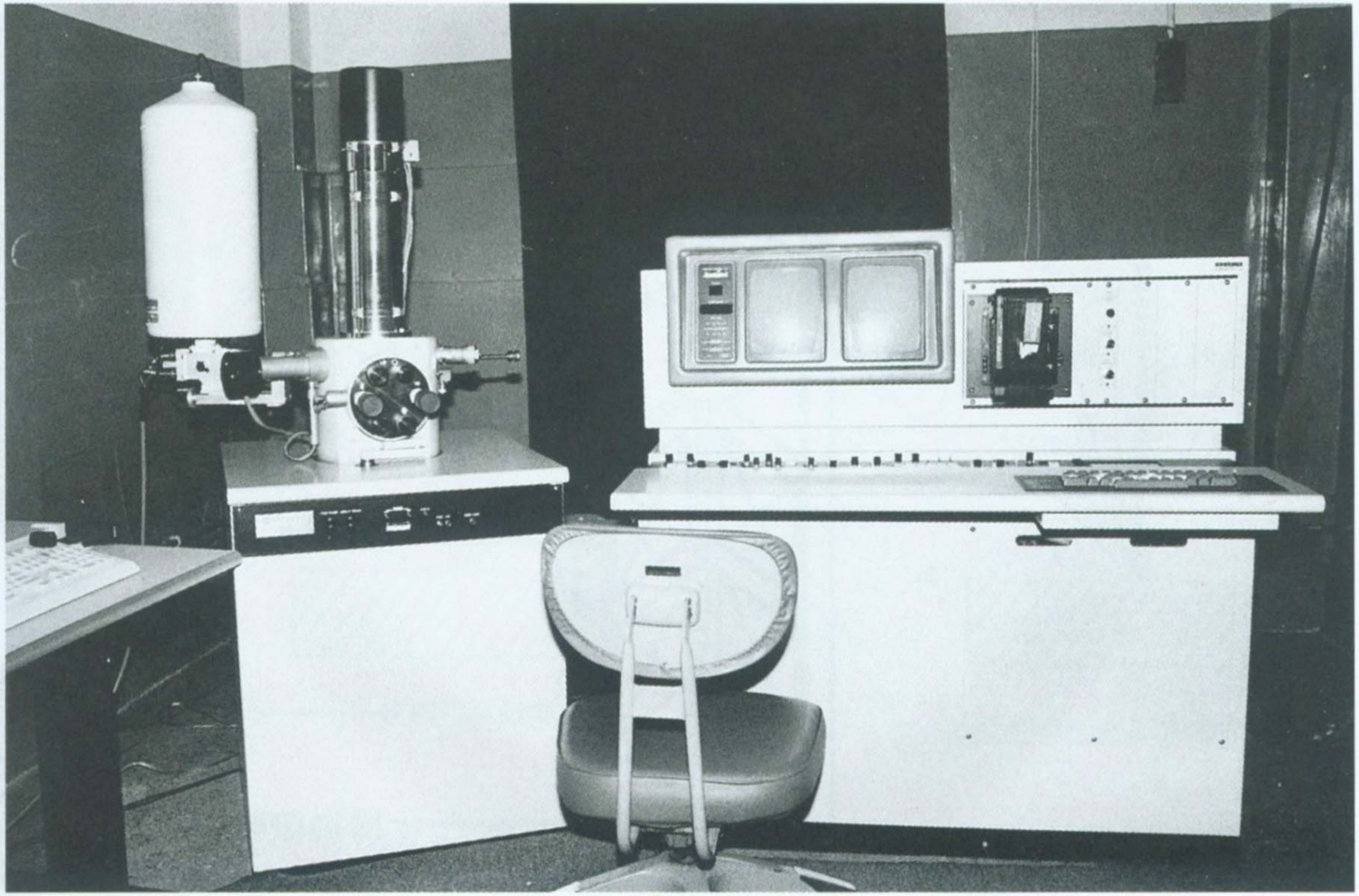


圖6.2　掃描式電子顯微鏡之外形構造圖。

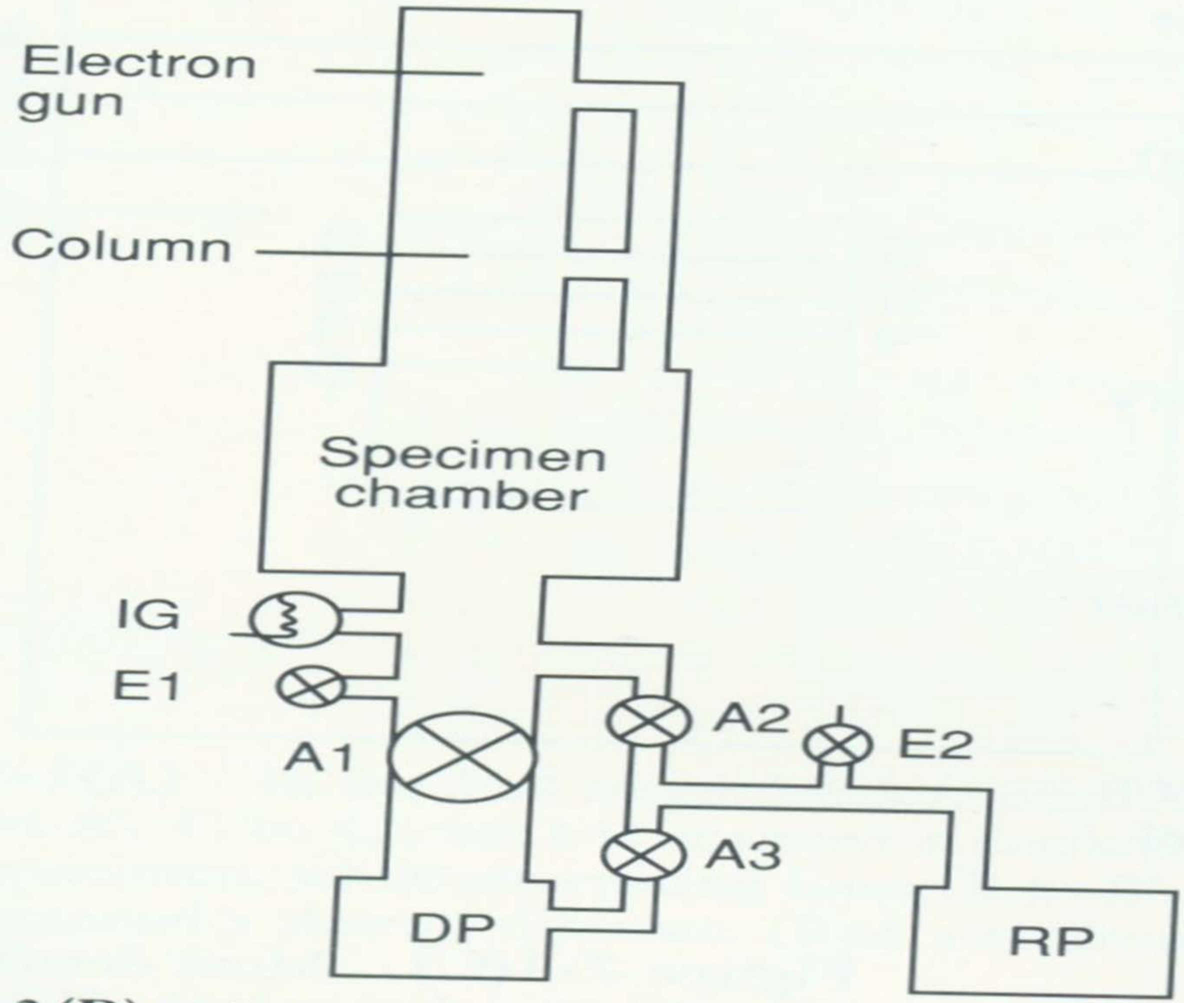



**Figure 7-3(B)** Schematic diagram of vacuum system. A1-3 = valves, DP = diffusion pump, E1, 2 = air admittance valves, RP = rotary pump, IG = ionization gauge. (Courtesy of Hitachi Scientific Instruments.)

圖7-3B

(一).電子槍的種類:

(1)FEG(field Emission Gun)

(2)Lab6(六硼化鑭)

(3)W filament(鎢陰極)

## (二).各種電子槍特性比較表:

| 電子源 | Electron source size | 20KV 之亮度（A/cm$^2$Sr） | Life Time（hr） | 操作溫度（°C） | 所需真空（Torr） | 發射電流（μA） | 特性 |
|---|---|---|---|---|---|---|---|
| 鎢絲 | 20μm | $5x10^4$ | 50~100 | 2800 | $10^{-5}$ | 10 | 穩定，大電流 |
| $LaB_6$ | 10μm | $3x10^5$ | 300~500 | 1800 | $10^{-6}$ | 50 | 高解析時具大電流 |
| FEG (cold) | 5~10nm | $10^7$ | >1 year | RT | $10^{-8}$ | 50~100 | 高解析 |

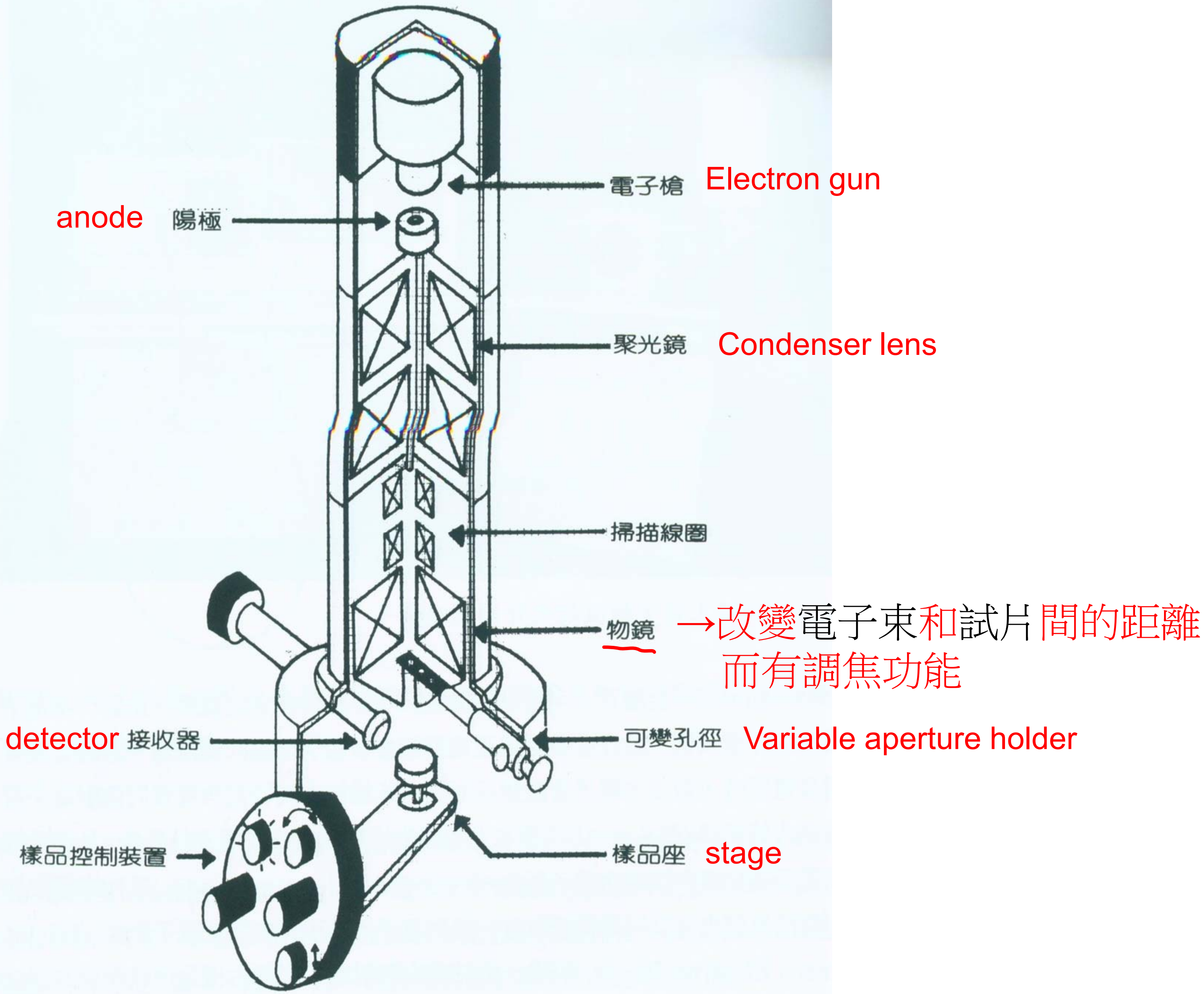
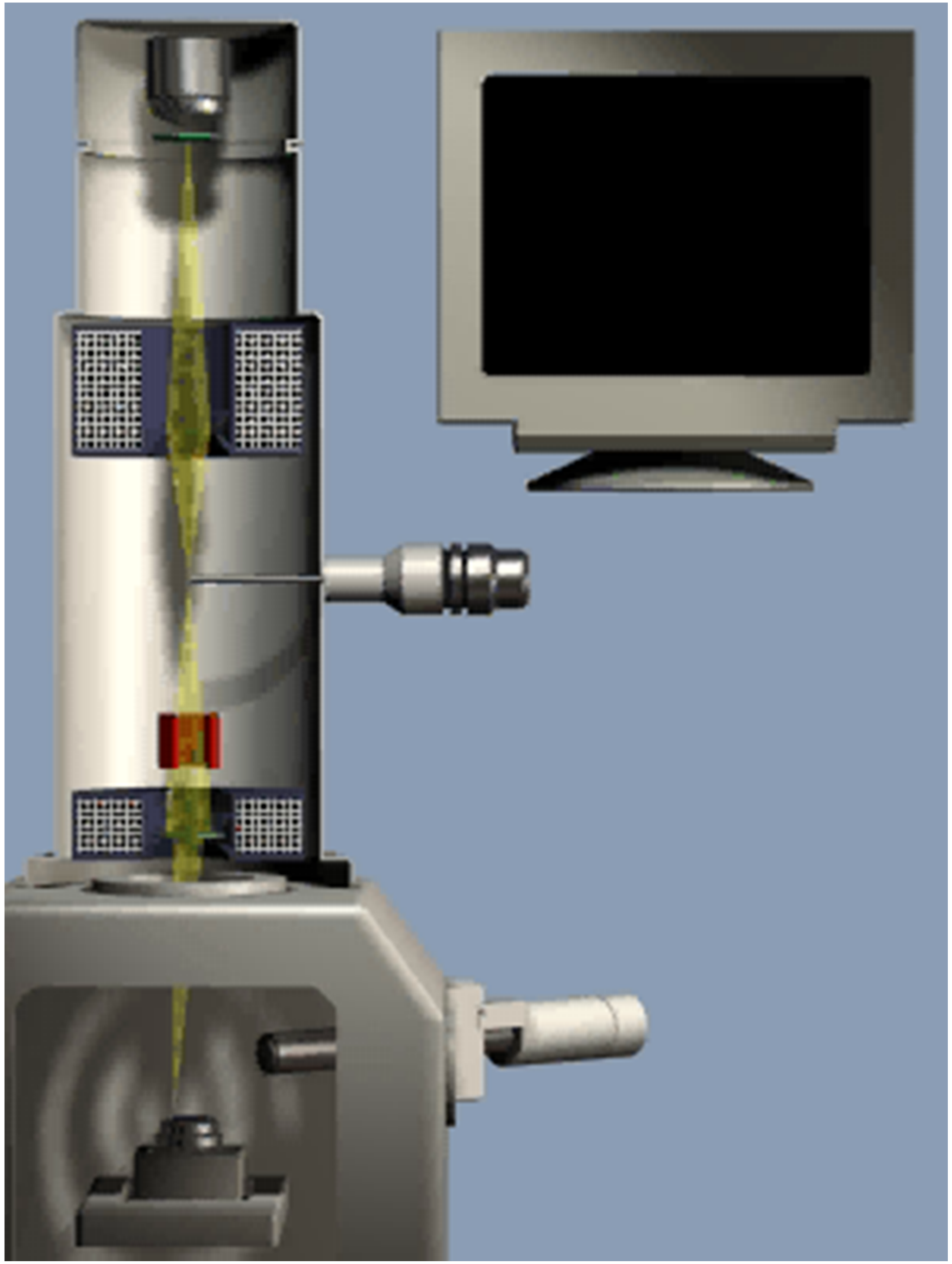



圖6.3 掃描式電子顯微鏡的鏡柱剖面圖。(Hitachi, LTD.)



18

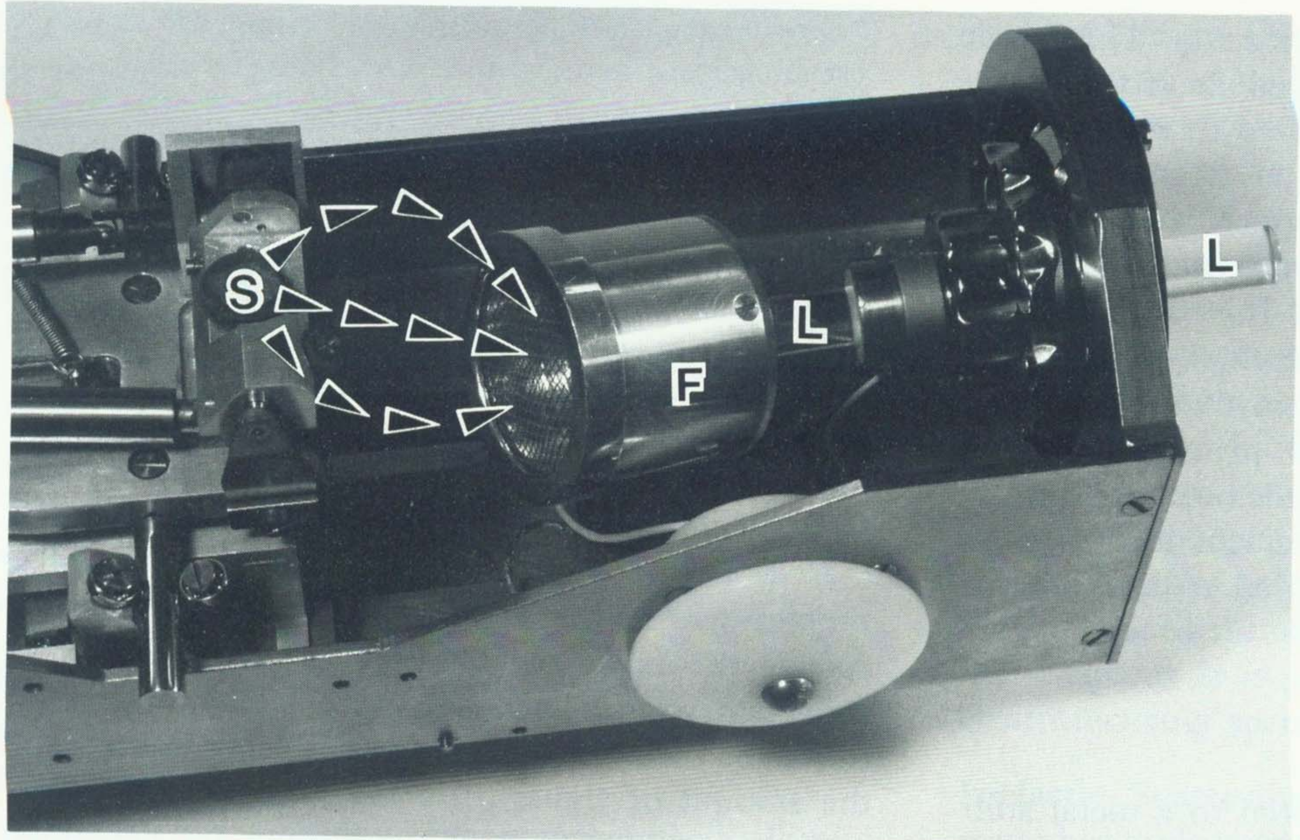



**Figure 7-10** Photograph of Everhart-Thornley secondary electron detector from a Kent-Cambridge SEM. The arrowheads show the paths secondary electrons might travel from the specimen (S) to the detector (not shown) housed inside the Faraday cage (F). After striking the scintillator of the detector, photons of light travel down the plastic light guide (L) to the photocathode of the photomultiplier (not shown).

- 電子束撞及到標本(S)上的特定位置時,所產生的二次電子or背向散射電子被位於法拉第盒(F)(Faraday cage)內的偵測器(detector)(閃爍器, scintillator)接收後,可將電子轉換成光子(photons);再經由光管(L) (light pipe)傳送至光放大器(photomultiplier) (光電倍增器)

圖7-10

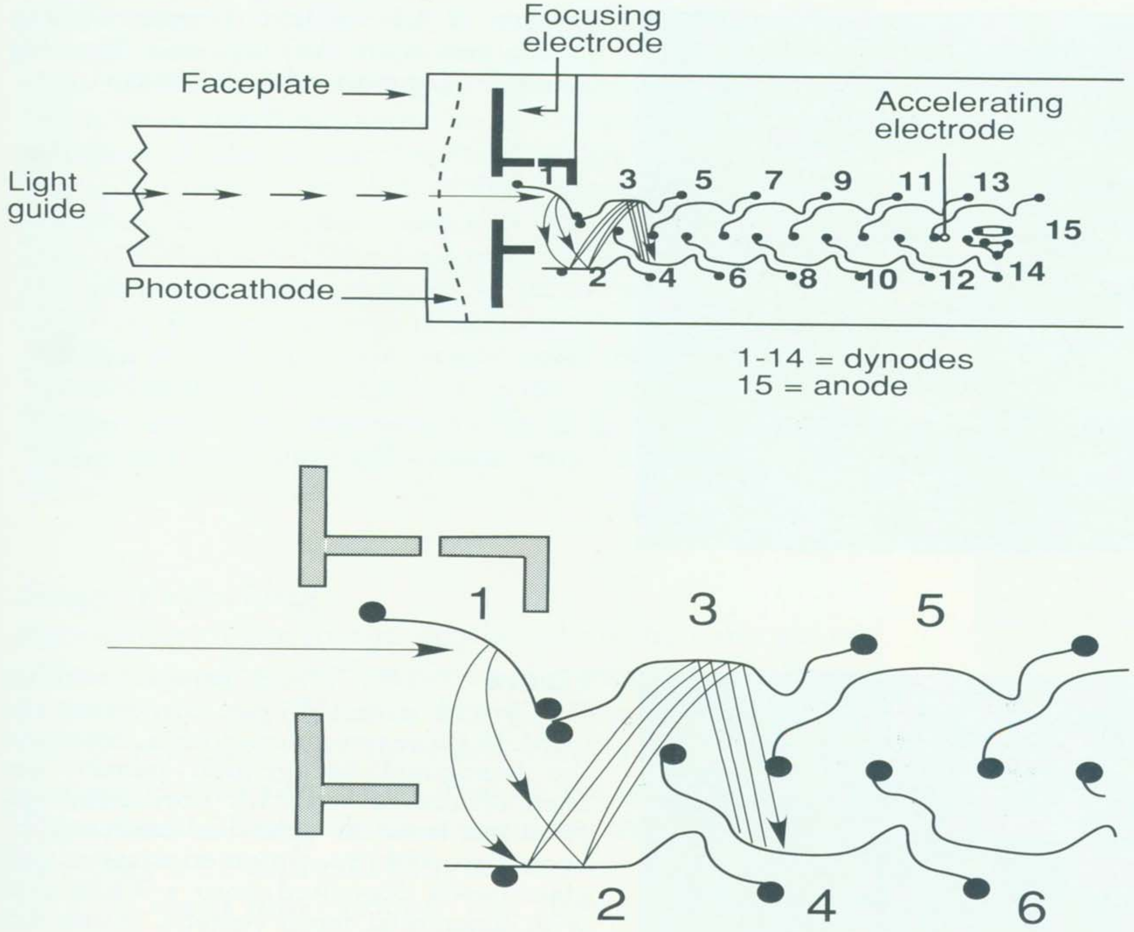
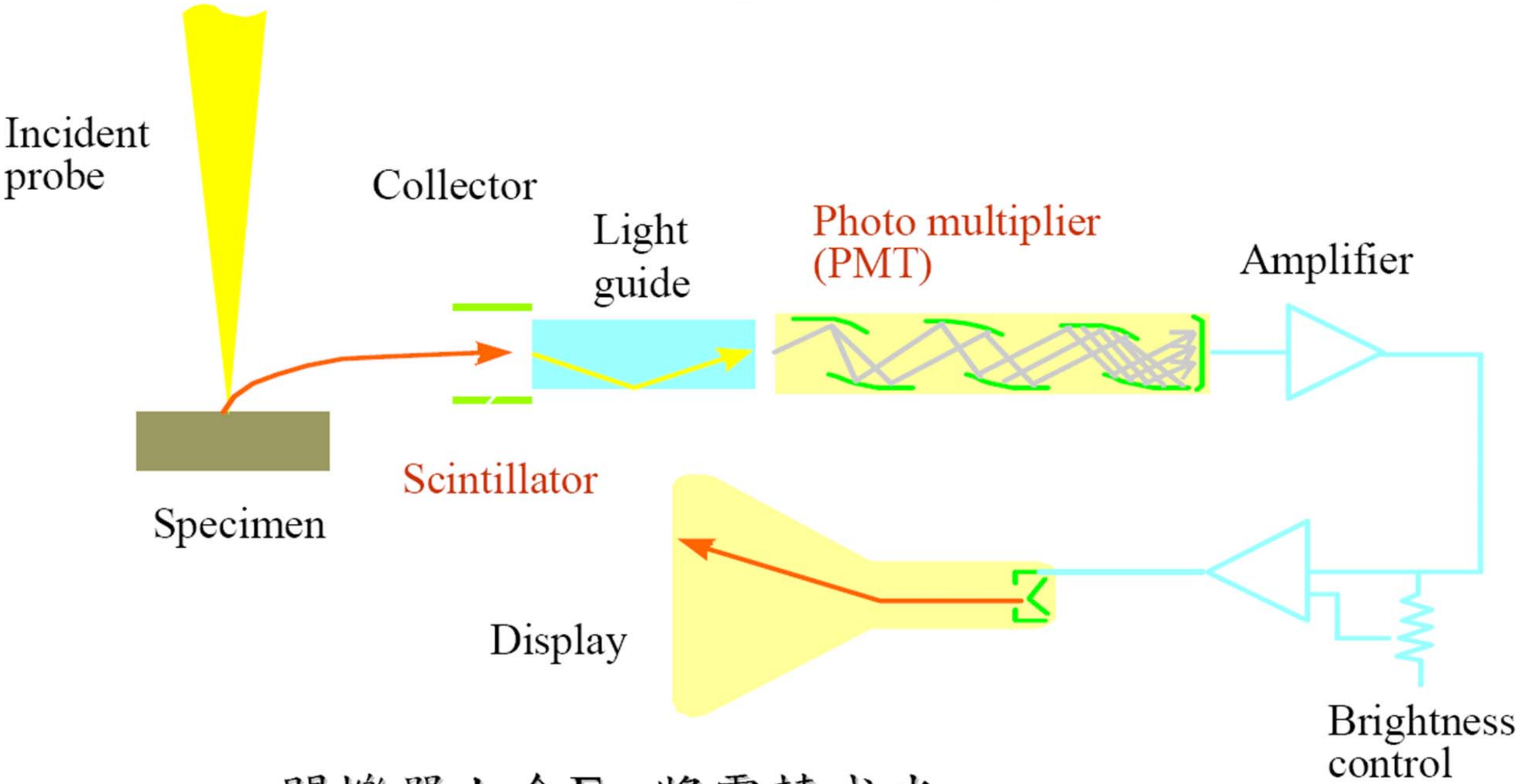


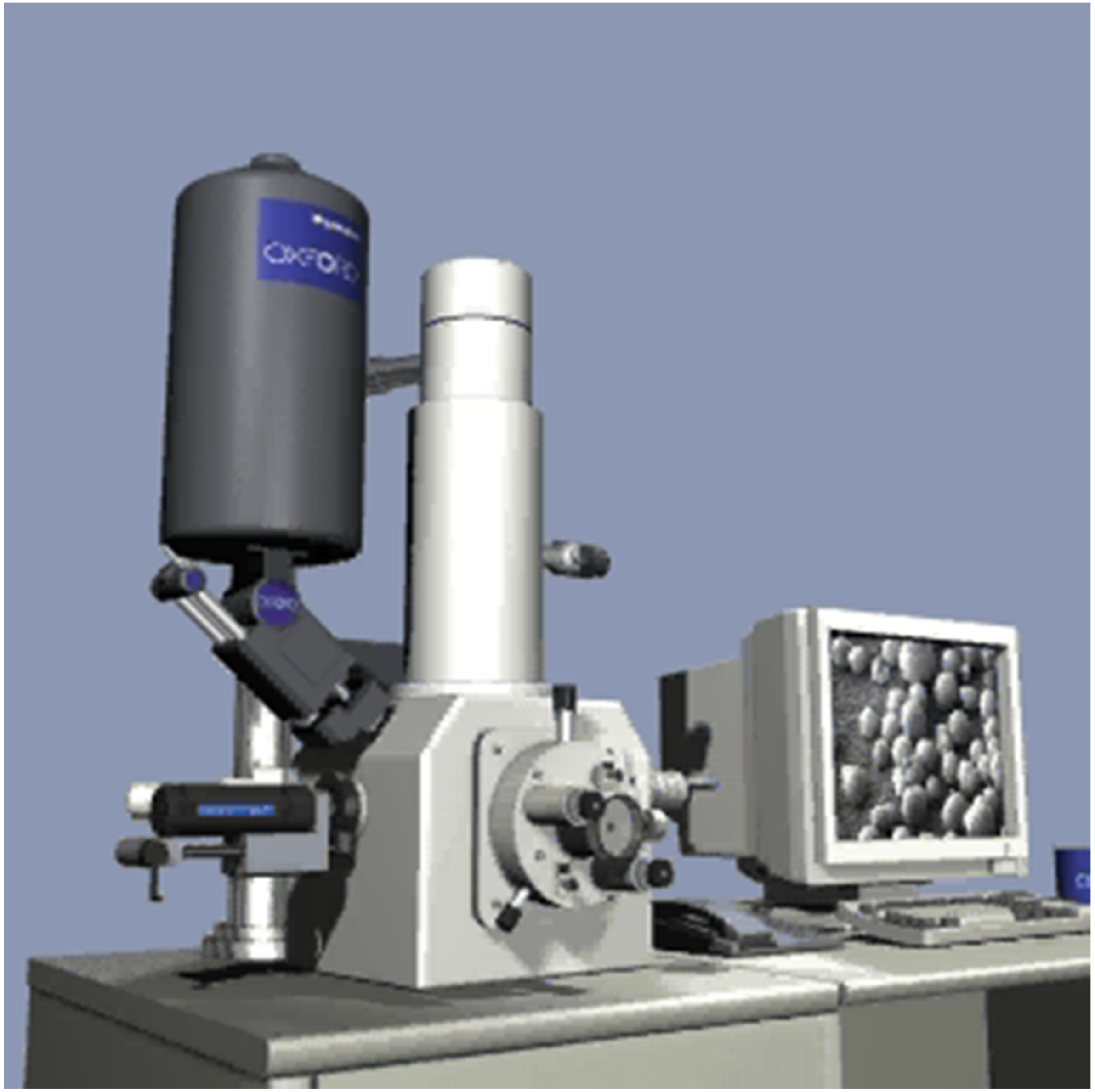

**Figure 7-11** (top) Schematic of photomultiplier where photoelectrons (generated by photons from the light guide striking the photocathode) are multiplied by striking a series of high voltage dynodes to generate more secondary electrons. (bottom) Enlarged area of photomultiplier showing entry of electron and multiplication of signal along dynodes.

圖7-11

# 二次電子偵測器




- 閃爍器上含Eu,將電轉成光
- 光電倍增管將光轉成電場(放大$10^5$~$10^6$倍)
- Eu: 銪, 原子序63, 原子量約152, 有吸收中子的作用



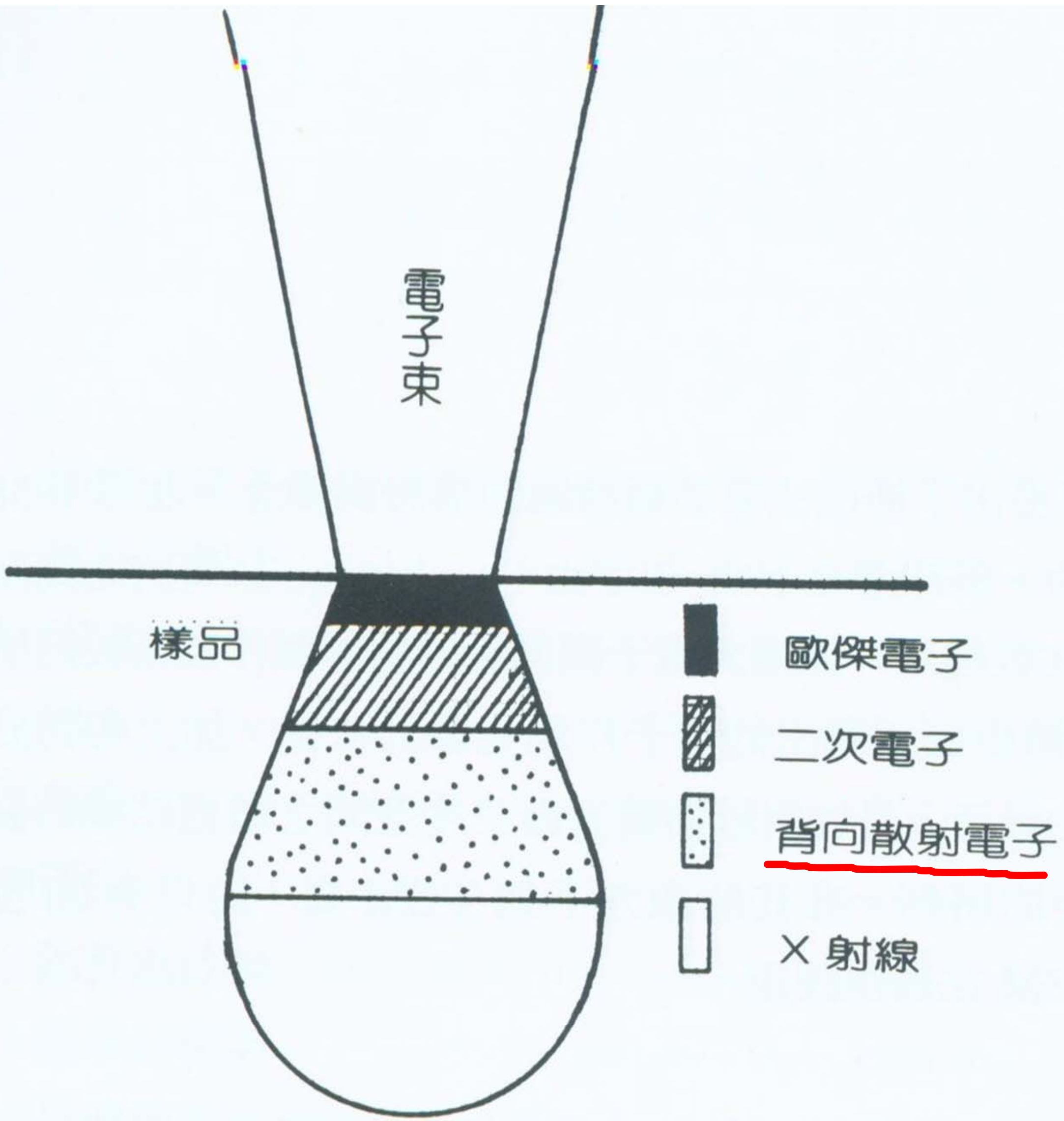



圖6.1　電子探束撞擊在樣品表面時，各種訊號電子自作用體積之不同層面釋出的情形。

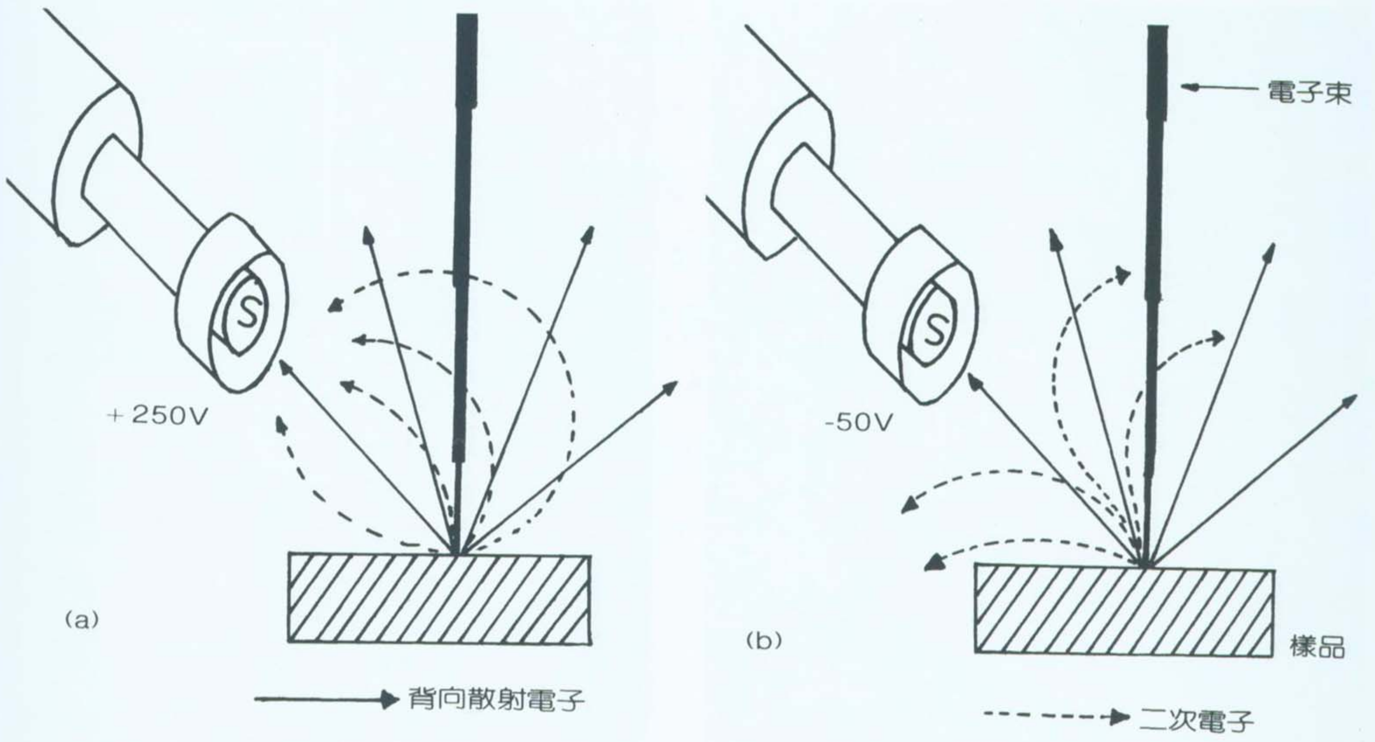


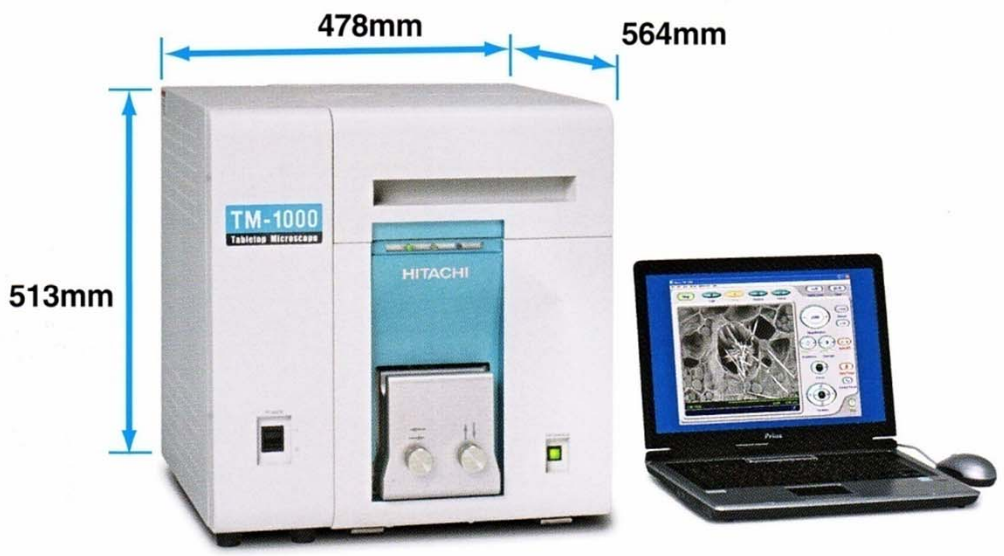

圖6.4 偵測器構造以及接收二次電子及背向散射電子之方式。(a)當閃爍器(S)外圍之金屬罩帶正電時，二次電子會被吸引而進入偵測器，(b)當金屬罩改為負電壓時，只有特定角度之背向散射電子方可被接收。

BSE可在低度真空狀態下直接觀察含有水分的樣品

## Table-top size and ready for observation anytime

The TM-1000 is ready to image at anytime using a standard power outlet. Power is only needed during use, creating an energy-saving, eco-friendly system.

The compact and portable design allows it to fit on any standard laboratory bench or desk, requiring no special room or environment.




桌上型掃瞄式電子顯微鏡

# 2 No metal coatings required for observation of non-conductive sample types

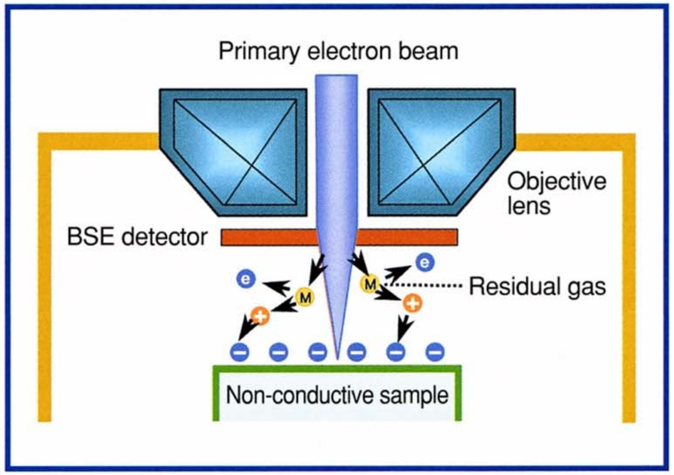
## No coating is required due to observation under variable pressure vacuum

Samples imaged by the TM-1000 require no special preparation such as metal coatings of non conductive samples, giving the ability to observe a broad variety of samples quickly and easily.

## Observation utilizing Variable Pressure vacuum

Because the Tabletop Microscope is based on Variable Pressure technology, sample throughput is high*1 and perfect for a multi-user lab. The Variable Pressure, combined with the high sensitivity backscattered electron detector, makes visual observation quick and simple.

*1 At 3 minutes after sample exchange image observation is ready.



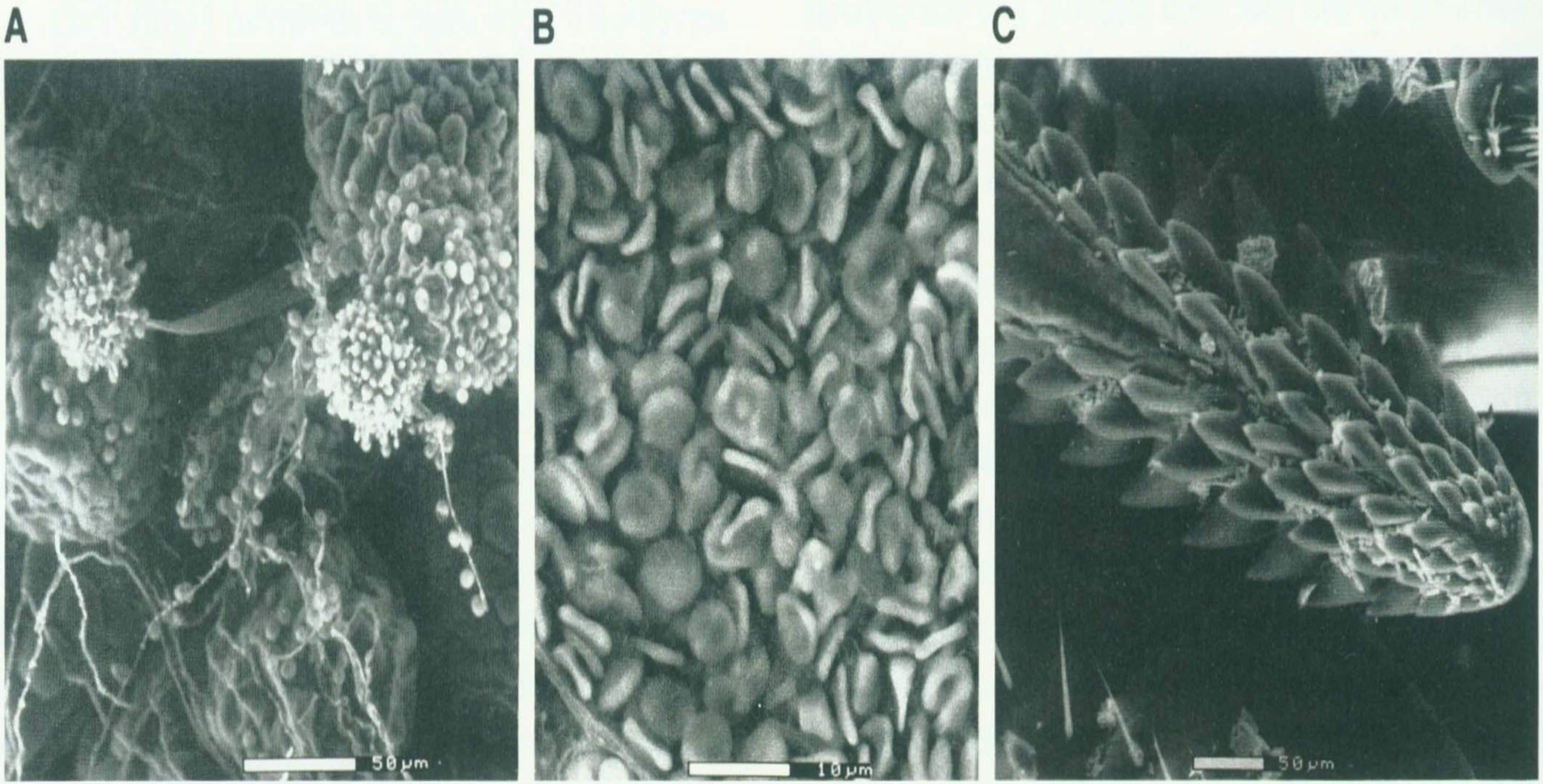



**Figure 7-33** Examples of different types of unfixed, hydrated biological specimens observed in the Environmental SEM. (A) Bread mold viewed at 315×. Accelerating voltage, 12 kV; vacuum, 3.7 Torr; temperature, −15° C. (B) Fresh frozen red blood cells in lung tissue. 1,400×; 20kV; 2.8 Torr; −15° C. (C) Mouth parts of tick. 200×; 20kV; 3.7 Torr; 24° C.

圖7-33

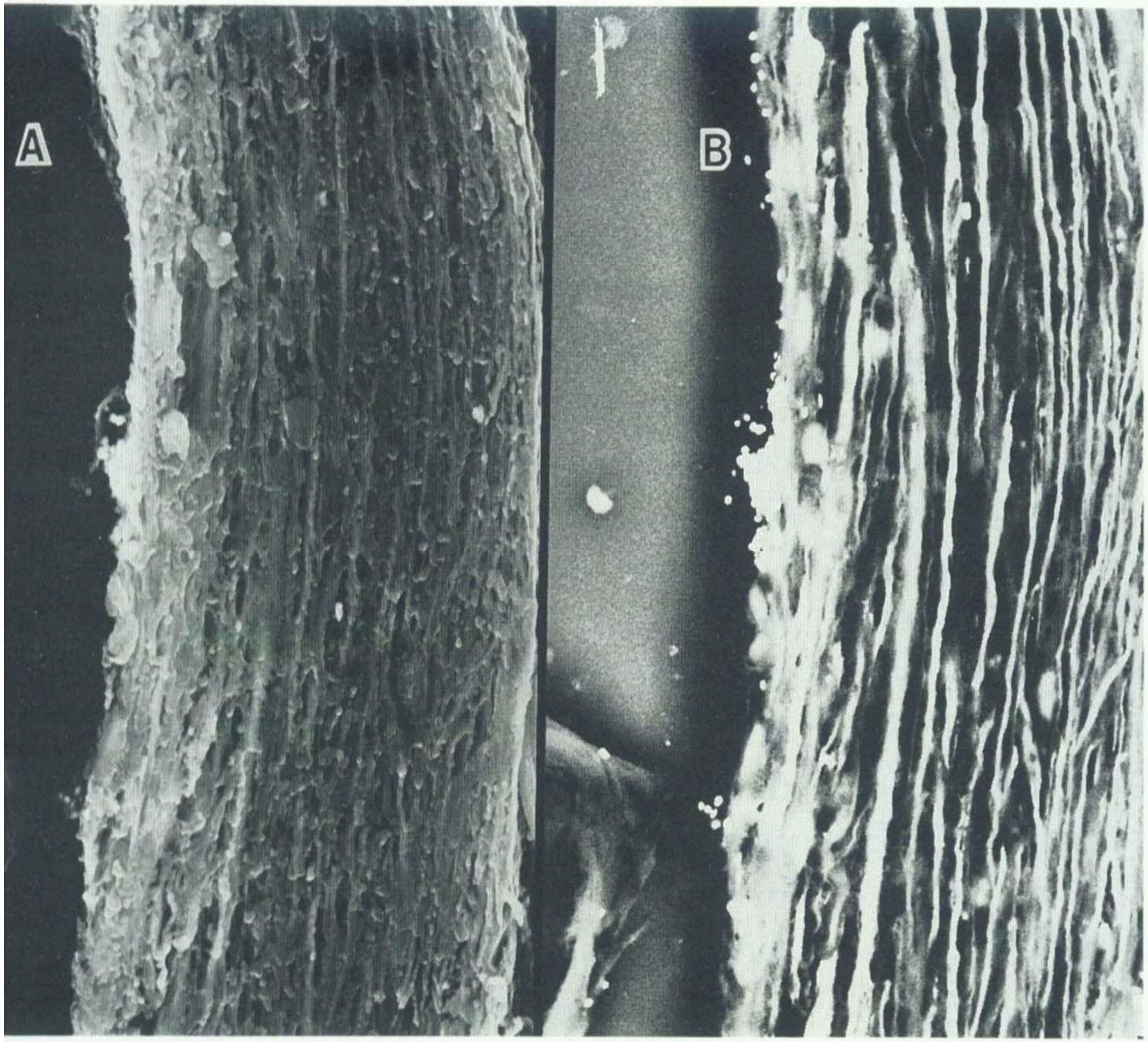
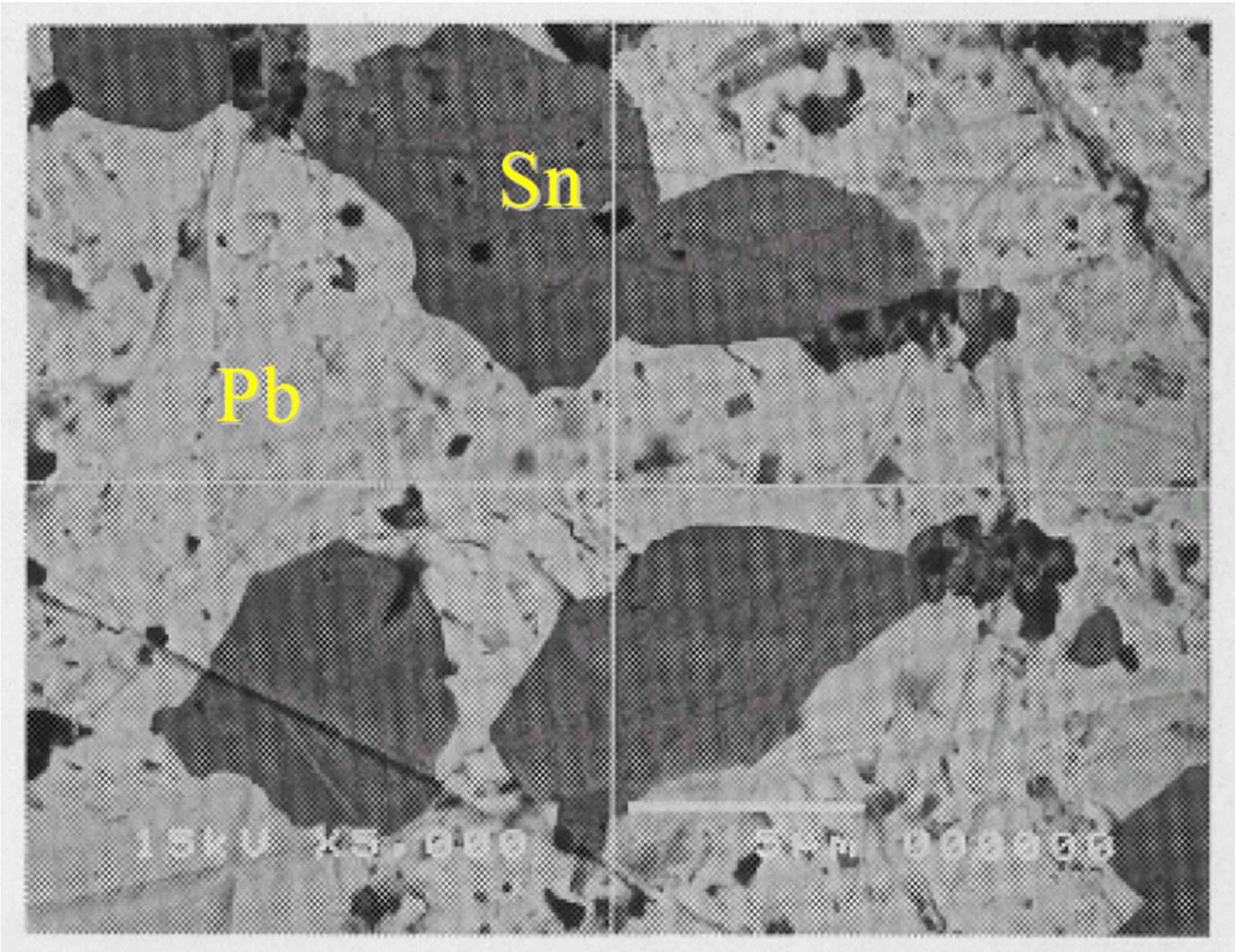



**Figure 7-24** (A) Secondary electron image of *Xenopus* (frog) optic nerve tract. (B) Same specimen as in 7-24(A), except viewed in the backscattered imaging mode. Since the nerves have been stained with silver, they appear much brighter than the background so that it is much easier to trace them throughout the tissue. (Courtesy of J. S. J. Taylor and The Williams and Wilkins Co.)

原子序較大的元素反射BSE的能力亦較強, 故呈現較亮的image

圖7-24




Sn: 錫, 原子序50; Pb: 鉛, 原子序82

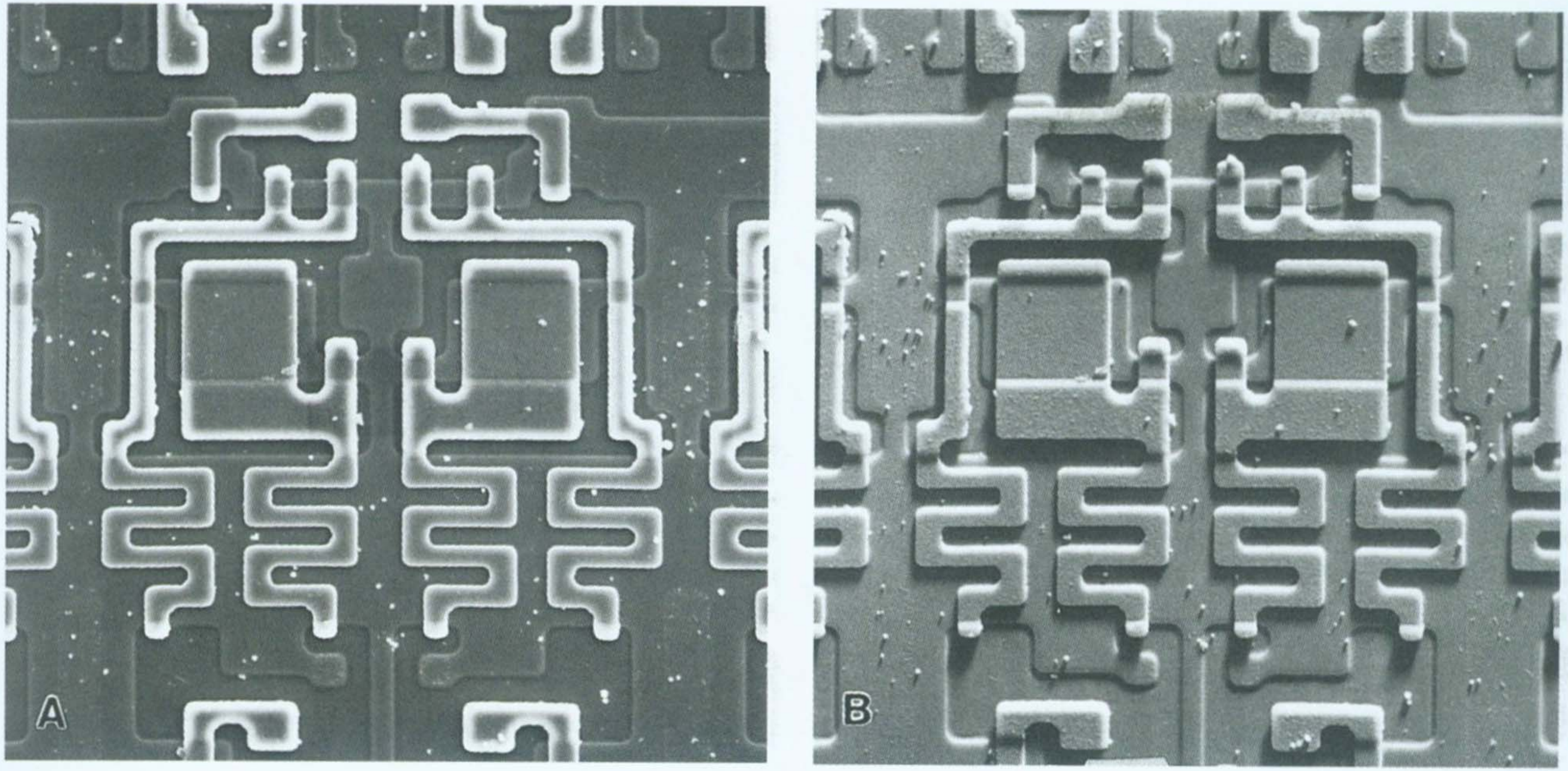



圖6.5　同一塊 IC 板以二次電子影像(A)與背向散射電子影像(B)觀察所得到影像立體構造之比較，背向散射電子影像有較明顯之立體效果。

圖6-5

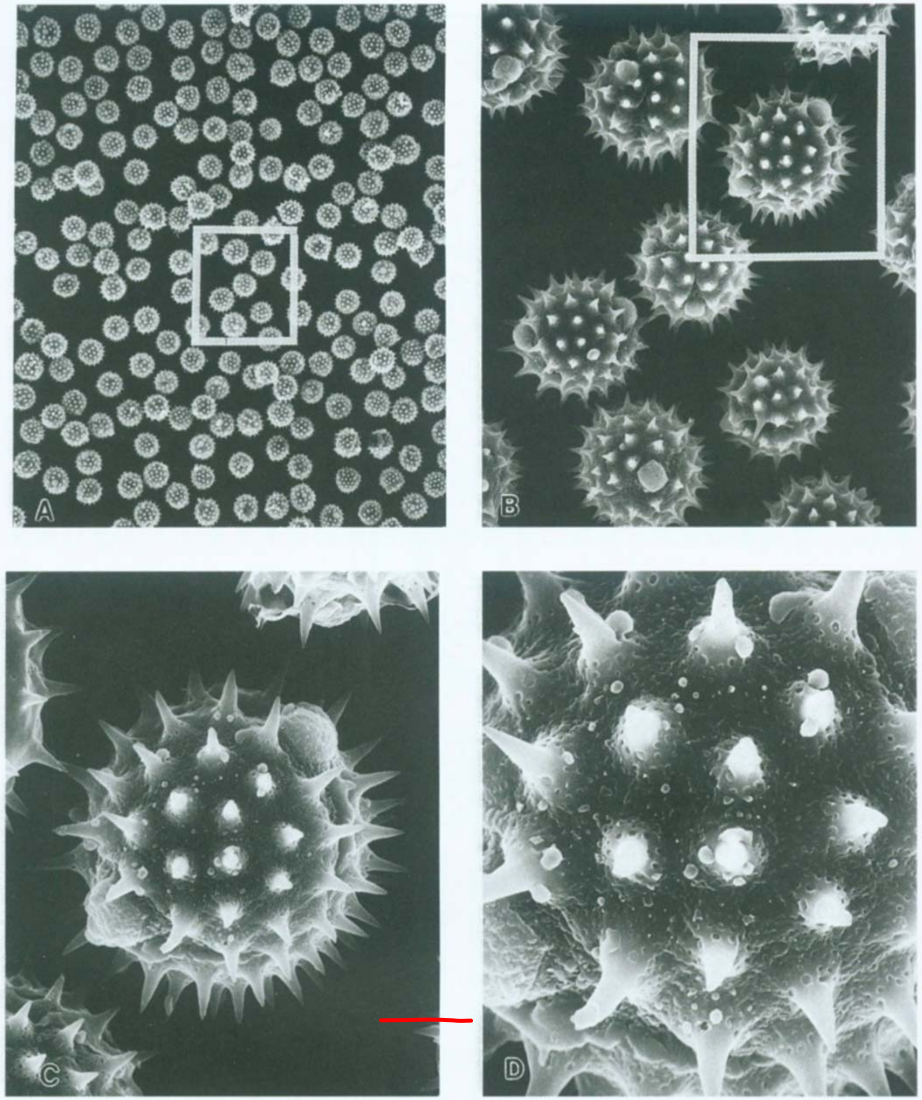
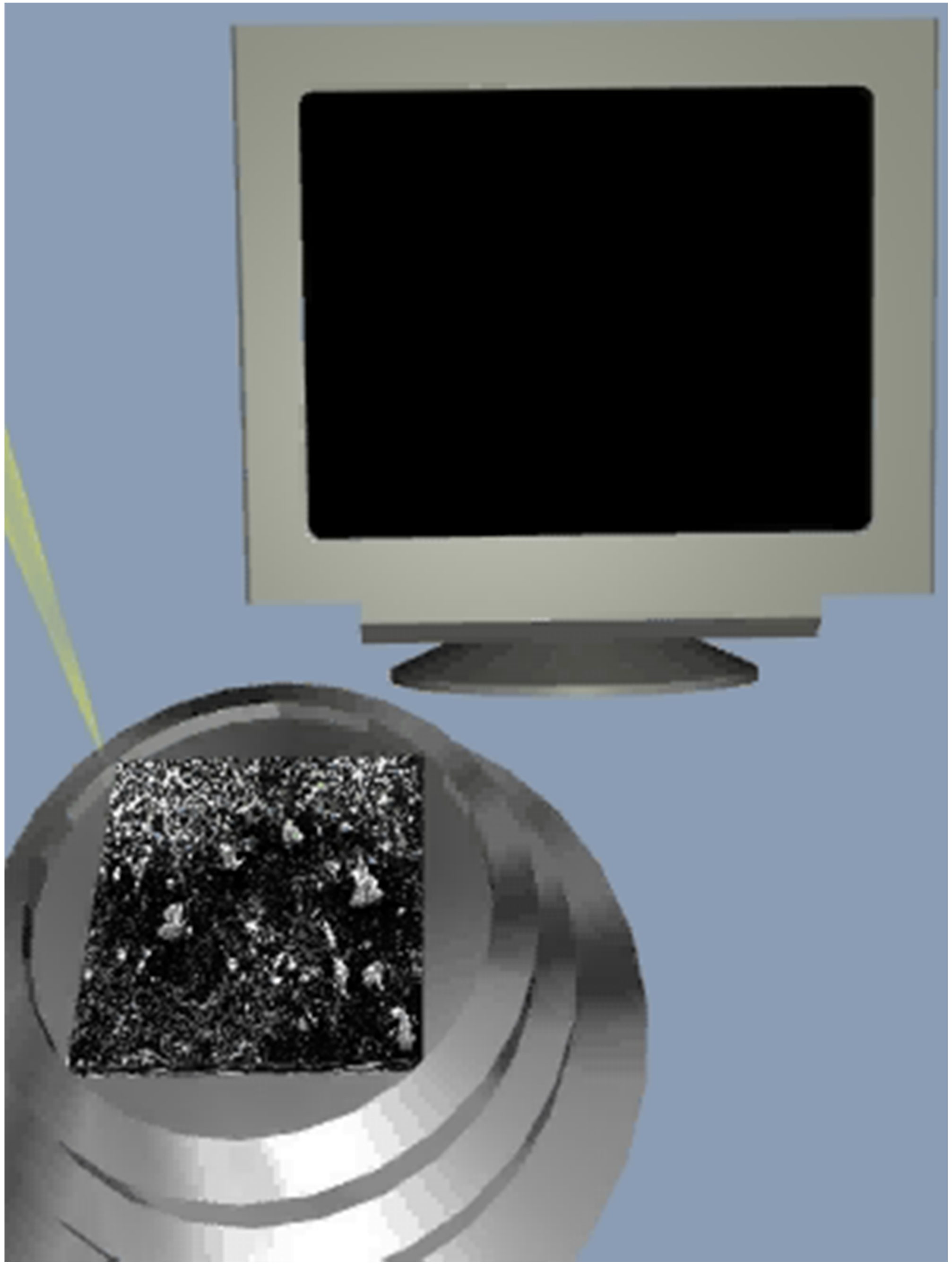



圖6.6　掃描式電子顯微鏡之放大倍率由掃描區域大小來決定，(A)圖中之方形區域即為(B)圖的掃描範圍，而(B)圖內的方形區域為(C)圖的掃描範圍，(D)圖的掃描區域最小，放大倍率最大。



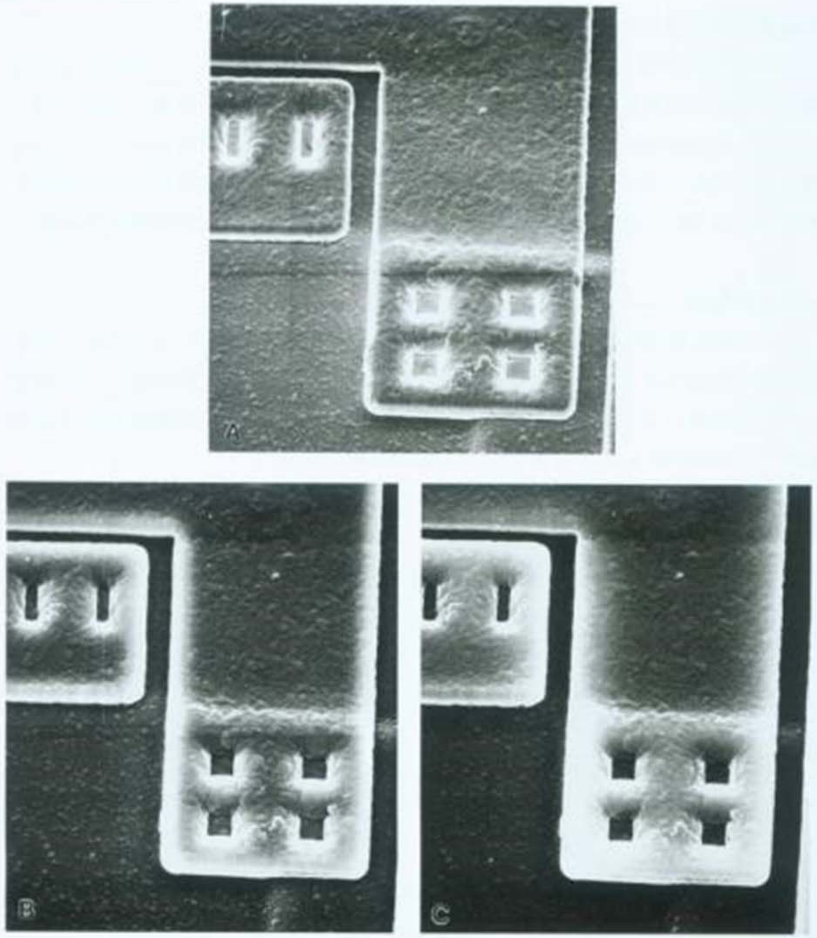



圖6.14 在不同的加速電壓下 IC 板之表面立體構造。(A)在較低的加速電壓 (5 KV)時，物體表面可見較多之細節，(B)當電壓加大時 (10 KV)，相對損失了表面訊息，(C)加速電壓繼續增加 (25 KV)可有較高之解像力，影像之邊緣輪廓清楚但表面細節喪失。

加速電壓愈大時,電子探束愈深入樣品表層,深層的二次電子過多將使亮度增強,而掩蓋了最表層的訊息

圖6-14

# 適當電壓的選擇

- 一般來說，加速電壓提高，電子束波長越短，理論上，只考慮電子束直徑的大小，加速電壓愈大，可得到愈小的聚焦電子束，因而提高解析度，然而提高加速電壓卻有一些不可忽視的缺點：

A. 無法看到試片表面的微細結構。

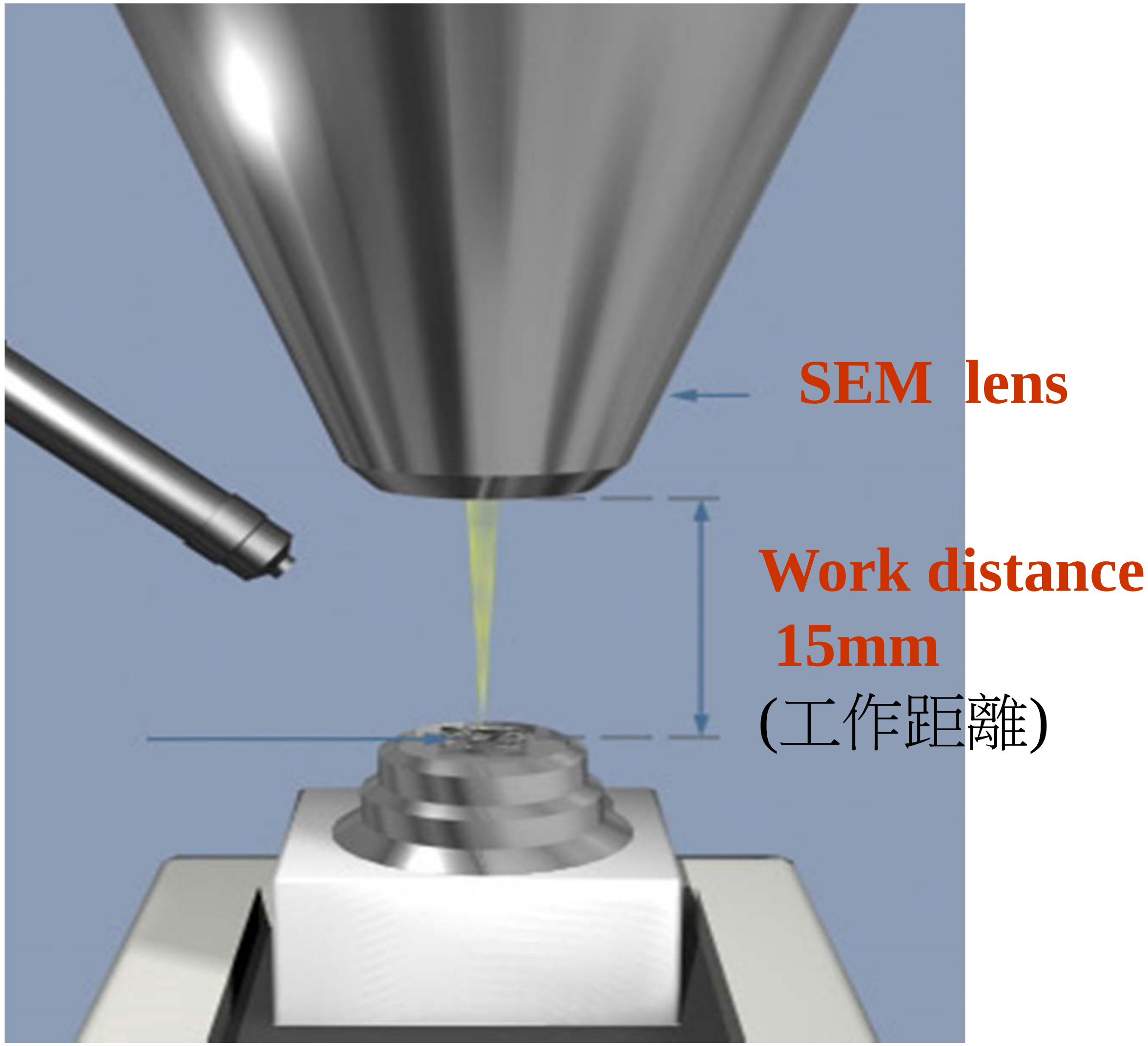
B. 會出現不尋常的邊緣效應。

C. 電荷累積的可能性增高。

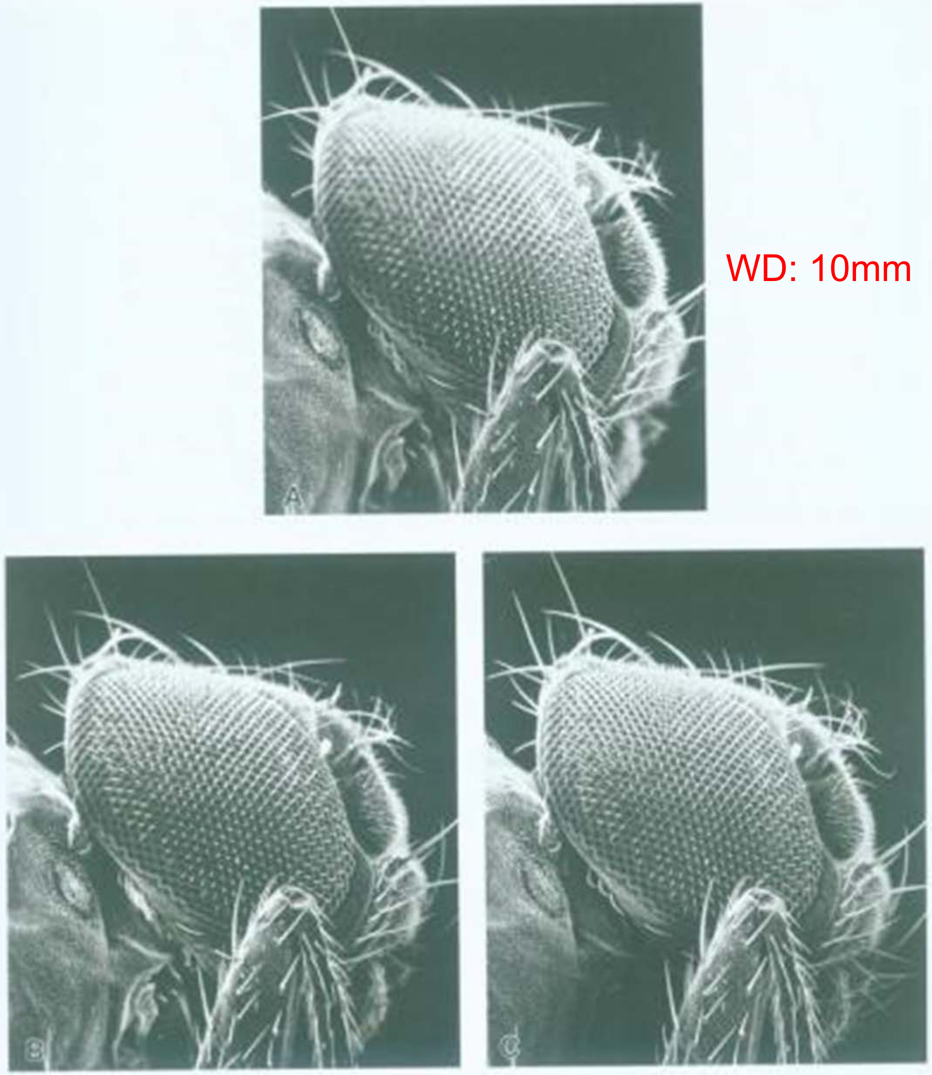
D. 試片損傷的可能性增高。

- 因此適當的加速電壓調整，才可獲得最清晰的影像。

# Theory of Energy Dispersive Spectrometer



工作距離:
標本最表面和其最
接近的透鏡間的距離



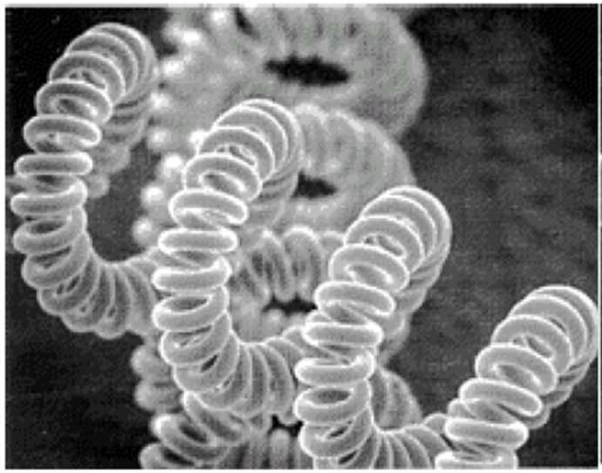

WD: 10mm

WD: 35mm
物鏡孔徑: 400um

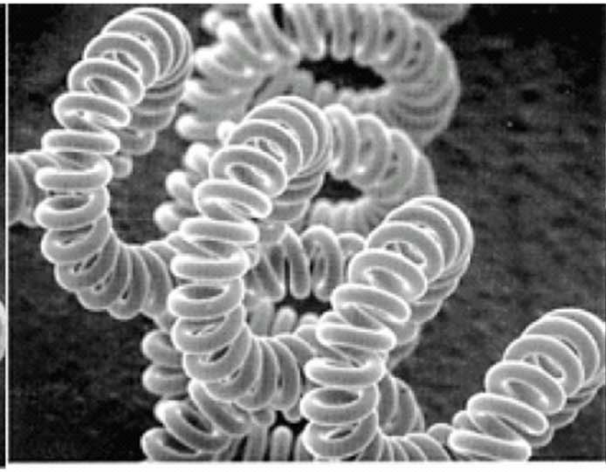
WD: 35mm
物鏡孔徑: 100um

圖6.15 工作距離的長短、物鏡孔徑的大小與景深之關係。在相同放大倍率下工作距離越短 (A, 10 mm)，標本上清楚的範圍越窄，若將工作距離變長 (B, 35 mm)，則可有較長的景深。而在相同的工作距離下 (35 mm)，選用較小的物鏡孔徑 (C, 100 $\mu$m) 又比選用較大的物鏡孔徑 (B, 400 $\mu$m) 時有更長的景深。

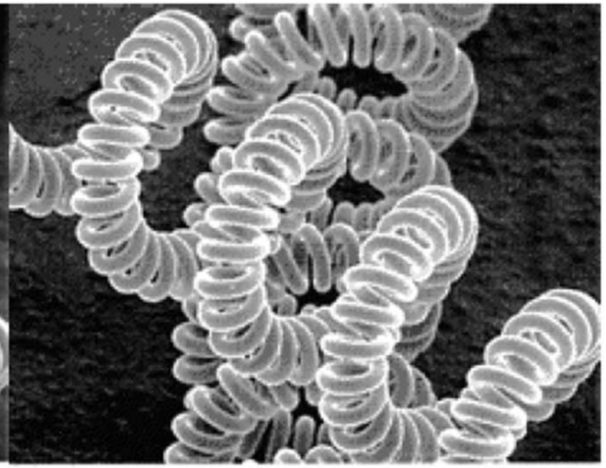
• WD,OL Aperture與景深比較



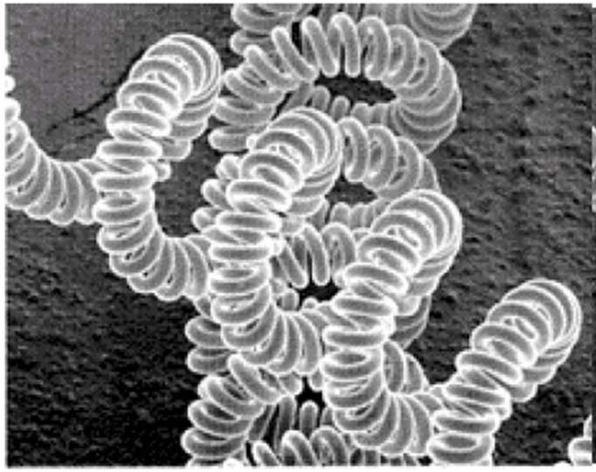
(a) OL aperature diameter: 600 μm
WD: 10mm



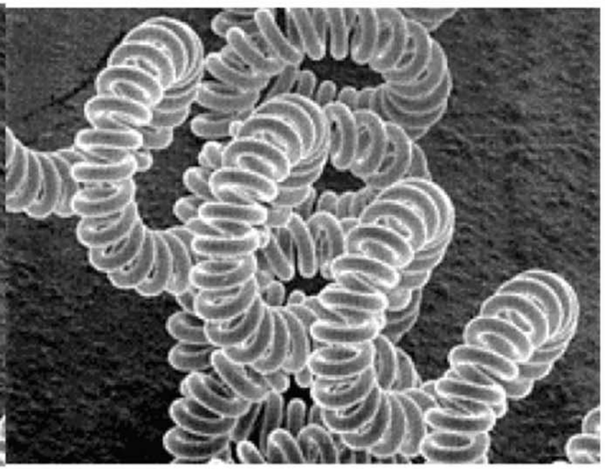
(b) OL aperature diameter: 200 μm
WD: 10mm



(c) OL aperature diameter: 200 μm
WD: 20mm



(d) OL aperature diameter: 200 μm
WD: 38mm



(e) OL aperature diameter: 100 μm
WD: 38mm

# 適當的工作距離的選擇

- 適當的工作距離的選擇，可以得到最好的影像。較短的工作距離，電子訊號接收較佳，可以得到較高的解析度，但是景深縮短。較長的工作距離，解析度較差，但是影像景深較長，表面起伏較大的試片可得到較均勻清晰的影像

## Table 7-1 Effect of Aperture Size on Depth of Field at Various Magnifications and at a 10 mm Working Distance

| Mag | Depth of Field | | |
|---|---|---|---|
| | 100 $\mu$m Aperture | 200 $\mu$m Aperture | 600 $\mu$m Aperture |
| 10× | 4 mm | 2 mm | 670 $\mu$m |
| 50× | 800 $\mu$m | 400 $\mu$m | 133 $\mu$m |
| 100× | 400 $\mu$m | 200 $\mu$m | 67 $\mu$m |
| 100,000× | 0.4 $\mu$m | 0.2 $\mu$m | 0.067 $\mu$m |

Reference: Goldstein, et al. 1981. *Scanning Electron Microscopy and X-ray Analysis: A Text for Biologists, Materials Scientists, and Geologists.* (New York: Plenum Publishing Corp.), 134.

表7-1

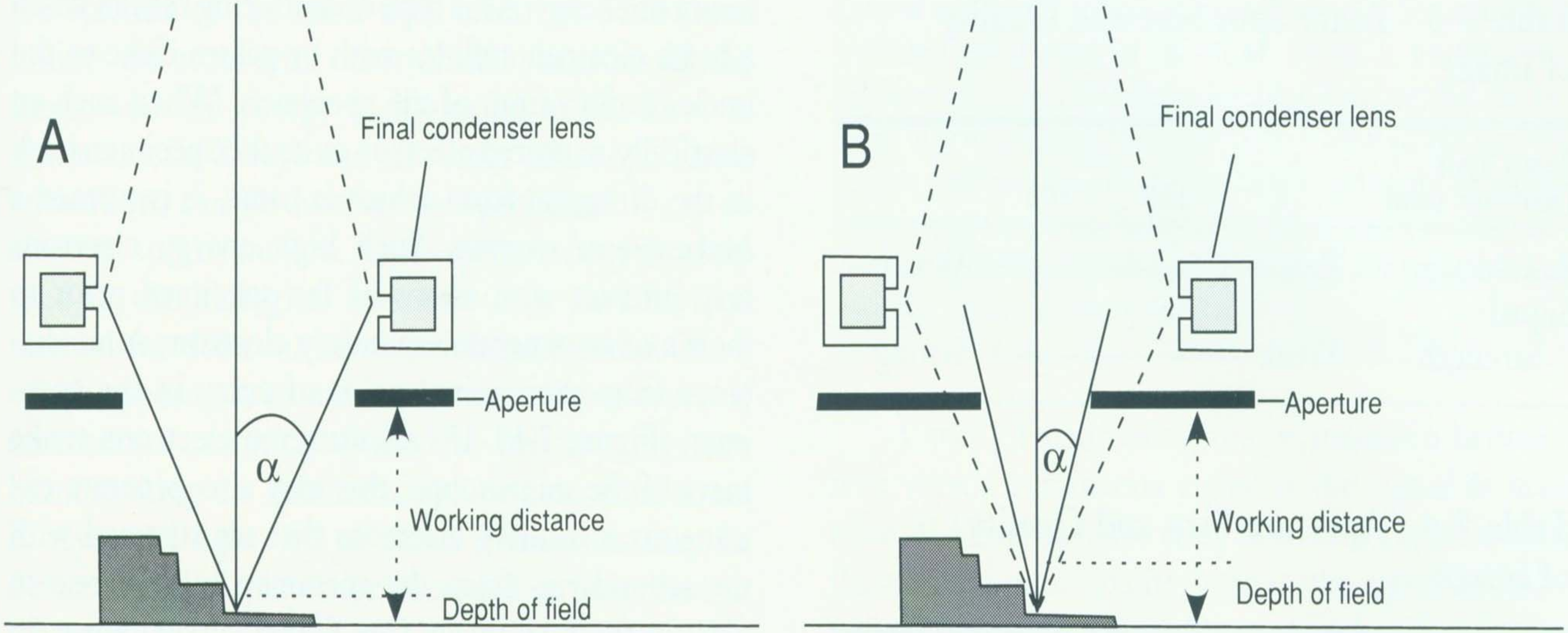



**Figure 7-6** Depth of field (the depth that is in focus in the specimen) is increased by using smaller apertures as shown on right.

圖7-6

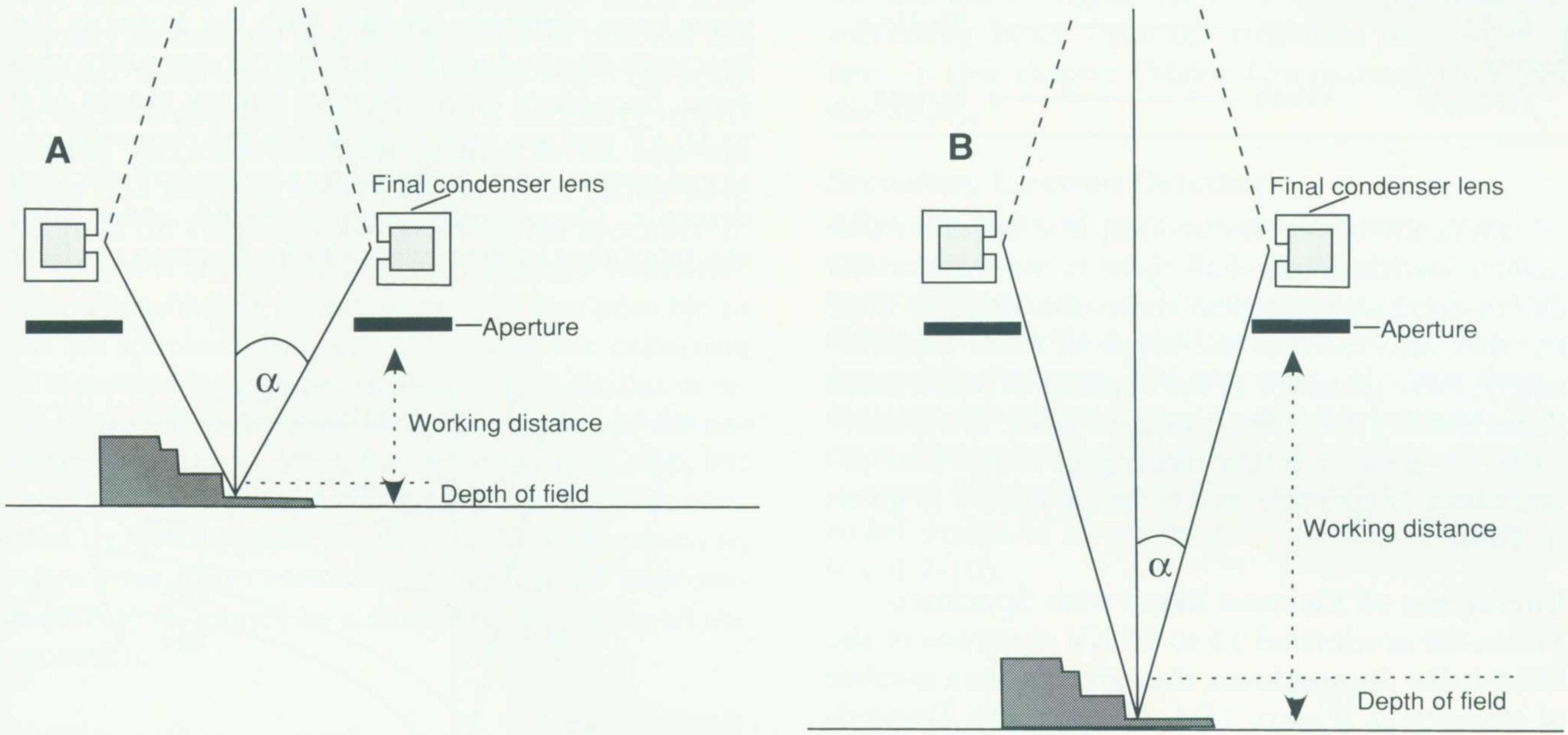



**Figure 7-7** When the working distance is increased as shown in B, this decreases the aperture angle alpha so that the depth of field is also increased.

圖7-7

## Table 7-2 Working Distance and Quality of Image

| Working Distance (mm) | 5 | 10 | 20 | 35 | |
|---|---|---|---|---|---|
| Resolution | Best ⟶ | | | | Worst |
| Depth of Field | Shallow ⟶ | | | | Deep |
| Signal Strength | Strong ⟶ | | | | Weak |

表7-2

## Table 7-3 Beam Spot Size and Quality of Image

| Beam Spot Diameter (nm) | 1 | 100 | 500 | |
|---|---|---|---|---|
| Resolution | Best | → | → | Worst |
| Signal Strength | Weak | → | → | Strong |

表7-3

## Table 7-4 Aperture Size and Quality of Image

| Aperture Diameter (μm) | 30 | 200 | 400 | 600 |
|---|---|---|---|---|
| Resolution | Best | → | → | Worst |
| Depth of Field | Deep | → | → | Shallow |
| Signal Strength | Weak | → | → | Strong |

表7-4

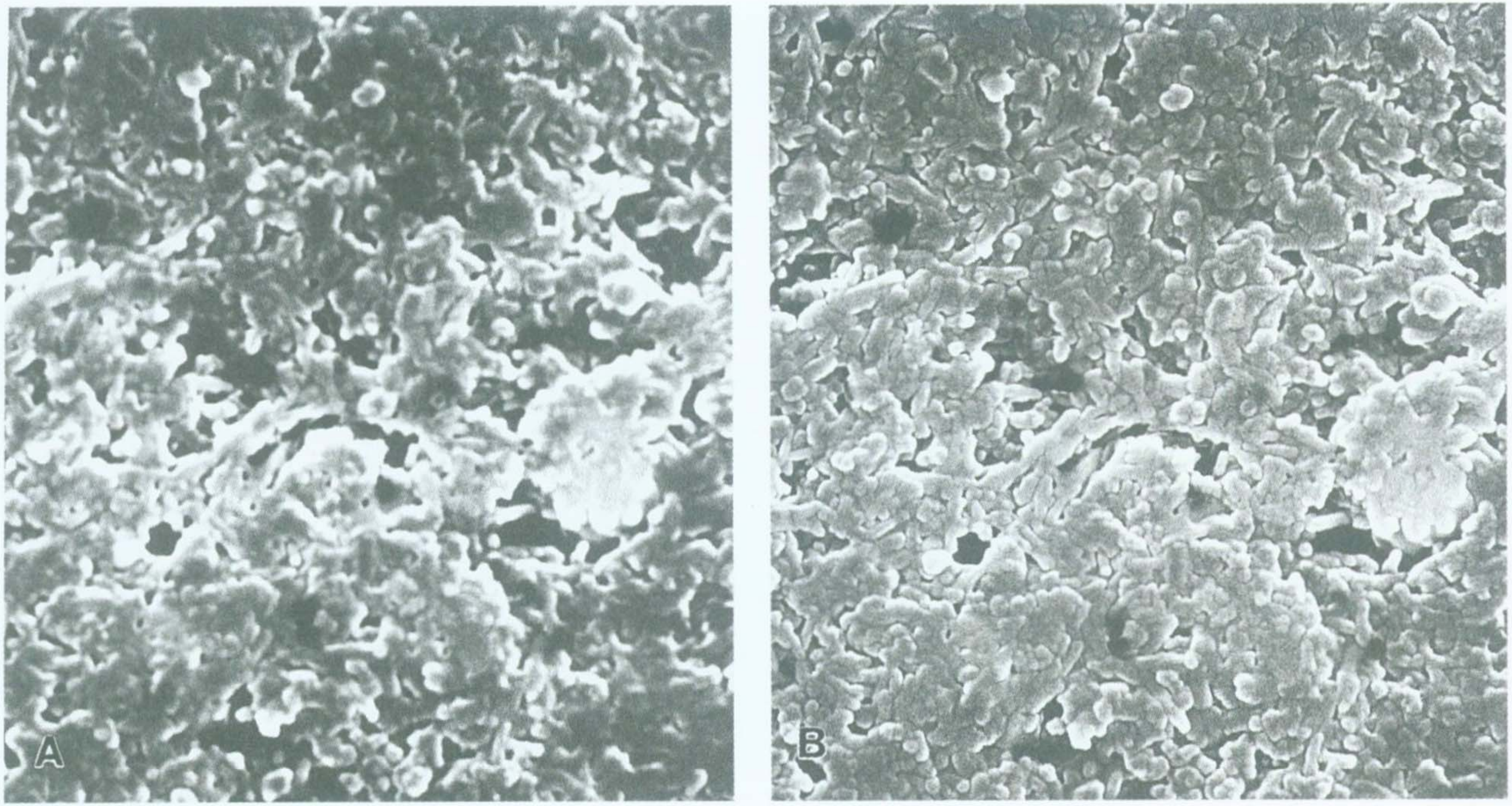


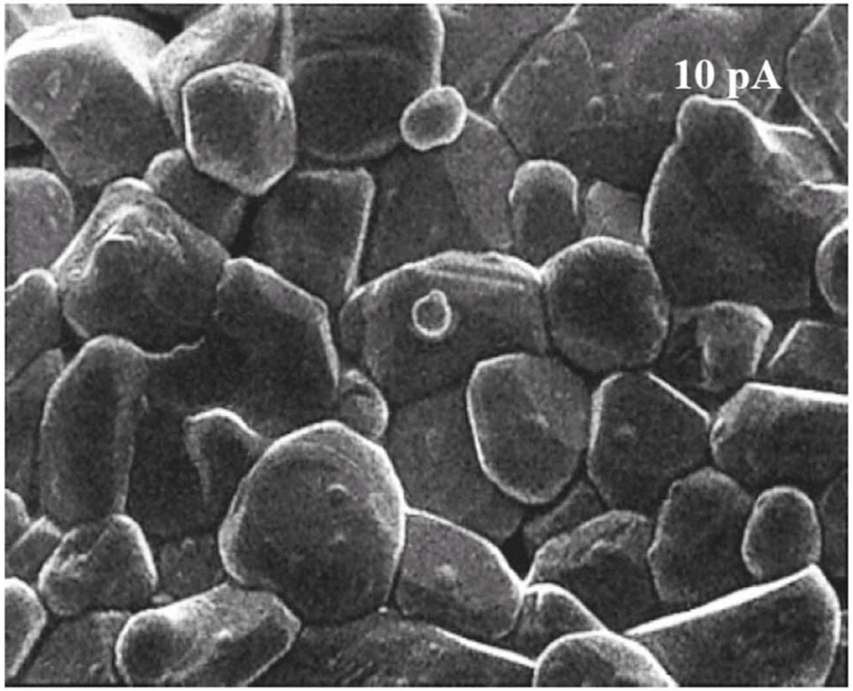
圖6.16 聚光鏡電流強度不同（即探束直徑大小）對解像力之影響。在相同的放大倍率下電流越強或探束直徑越小(A)較電流越弱或探束直徑越大(B)有較高的解像力。

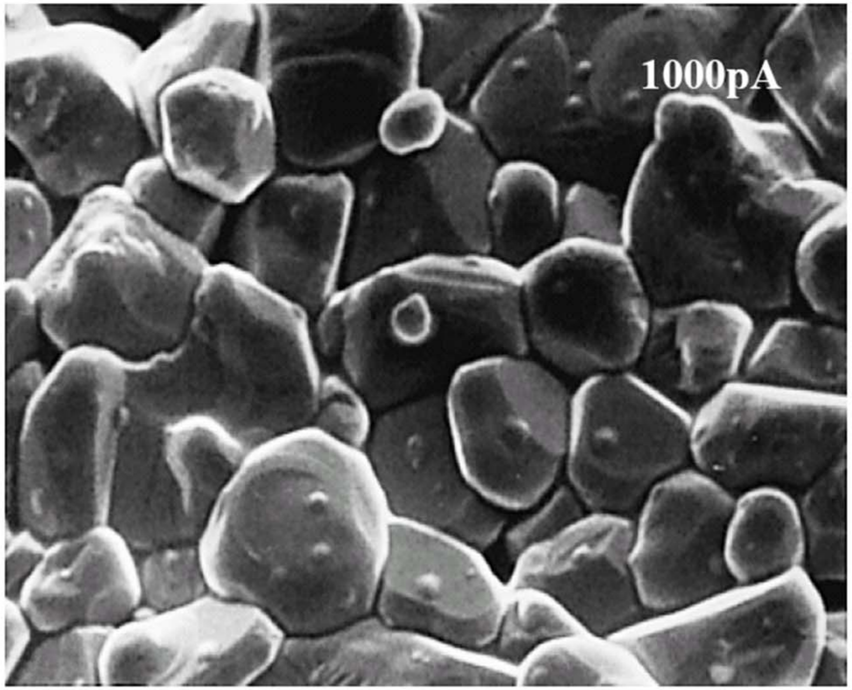
圖6-16

高電子束電流➔由於電子束密度高,所產生二次電子數目較多,影像品質較細緻

低電子束電流➔由於電子束密度低,所產生二次電子數目較少,影像品質較粗糙.

**ACCV=10kV**



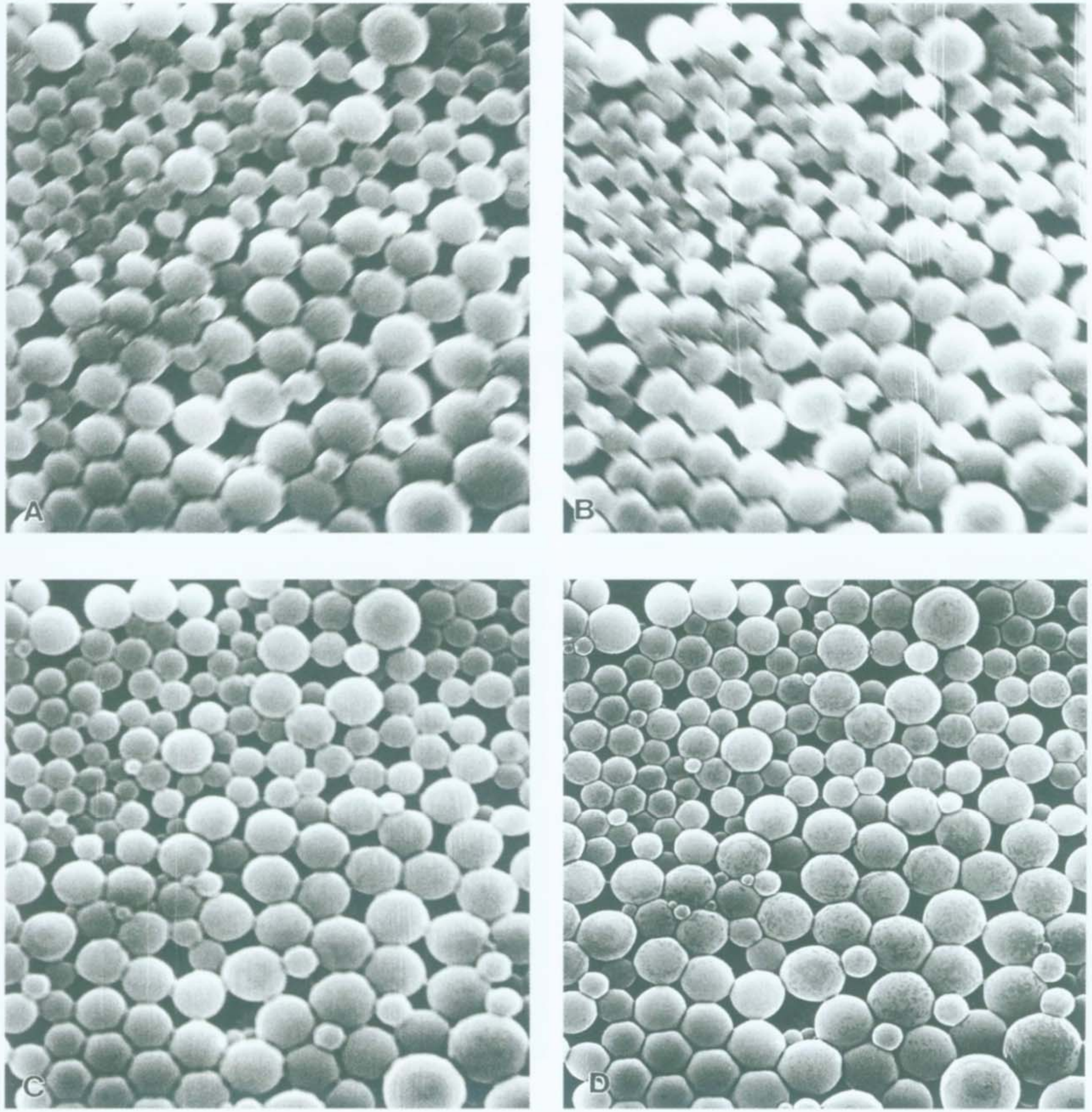
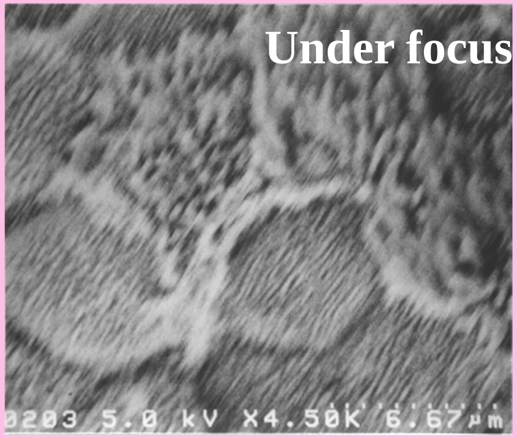
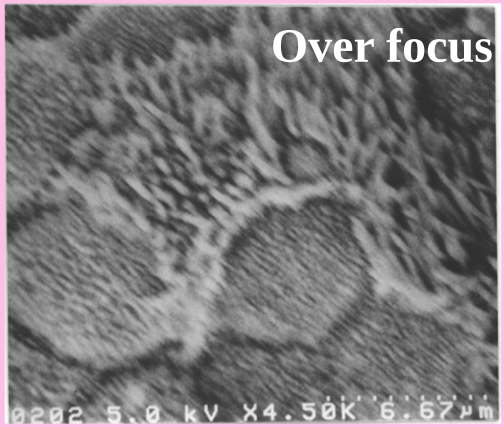



圖6.17　當透鏡垂直的兩軸面磁場強度不同時，影像常隨著焦距的改變而被拉折成線狀(A, B)，以像散校正器校正透鏡磁場可使影像不再變形(C)，再配合焦距的調整則可得到清晰的影像(D)。

# Theory of Scanning Electron Microscope








**Before correction**


**After correction**

**Specimen:Trachea of rat**

## Astigmatism correction method

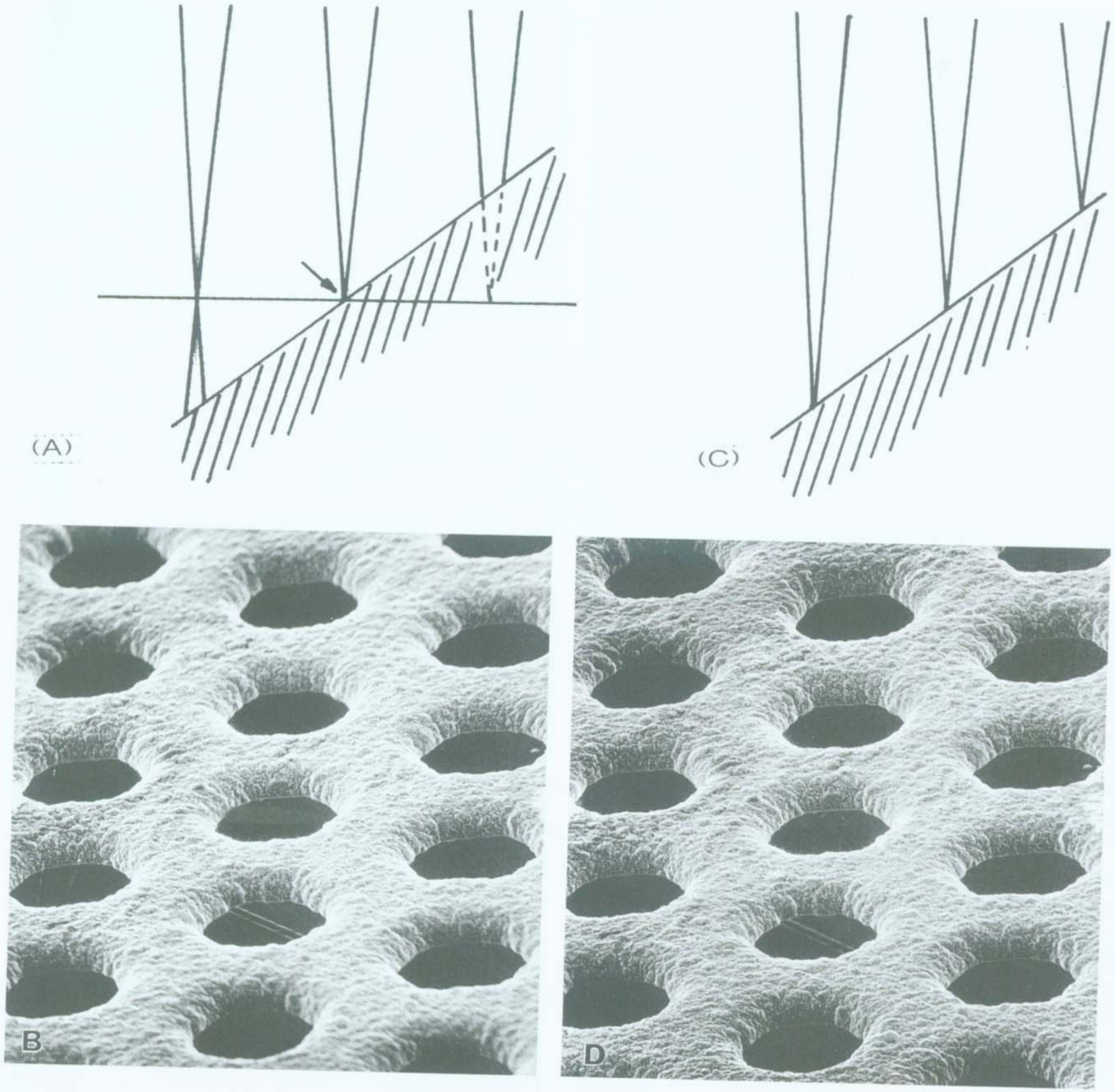
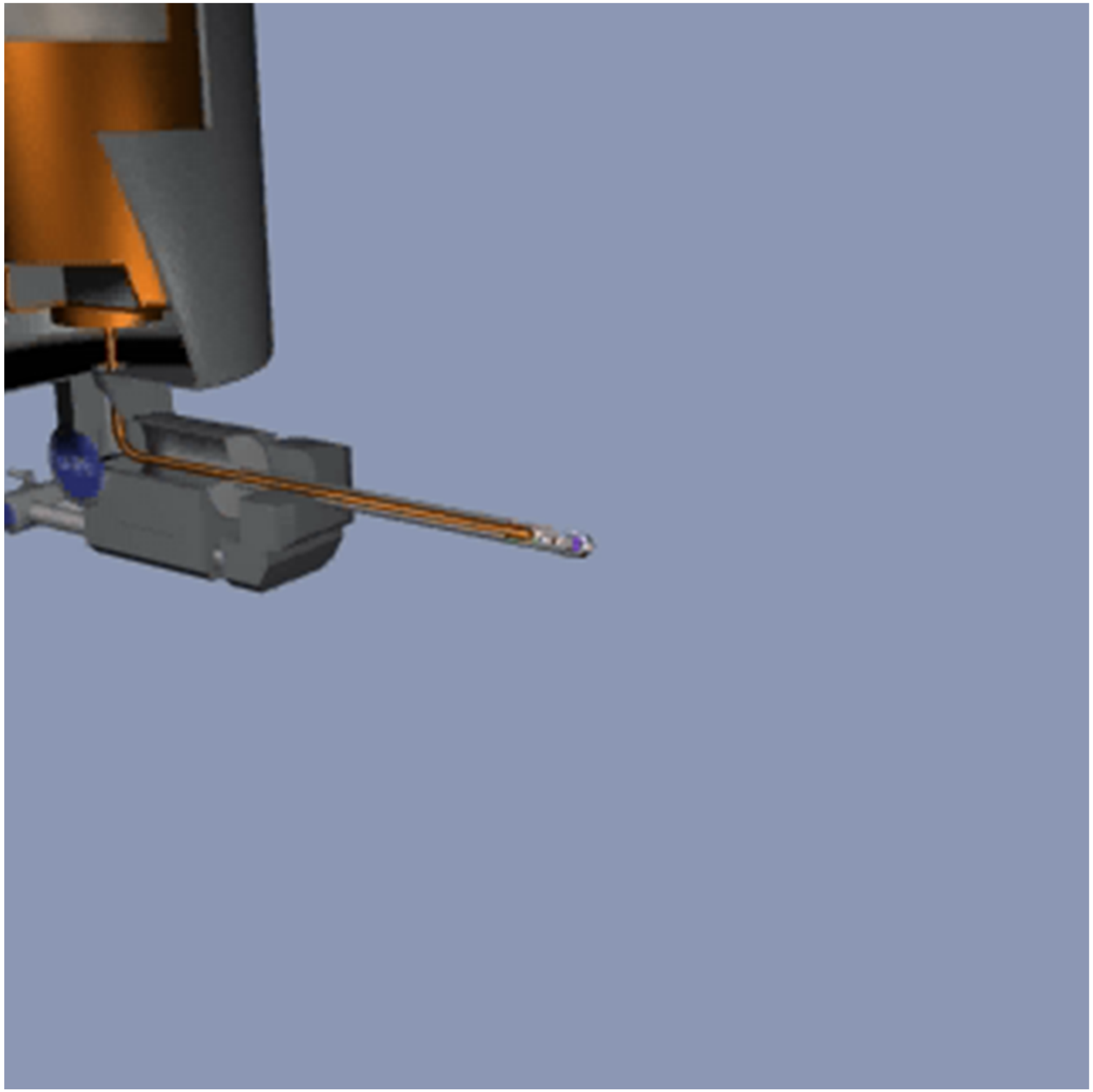


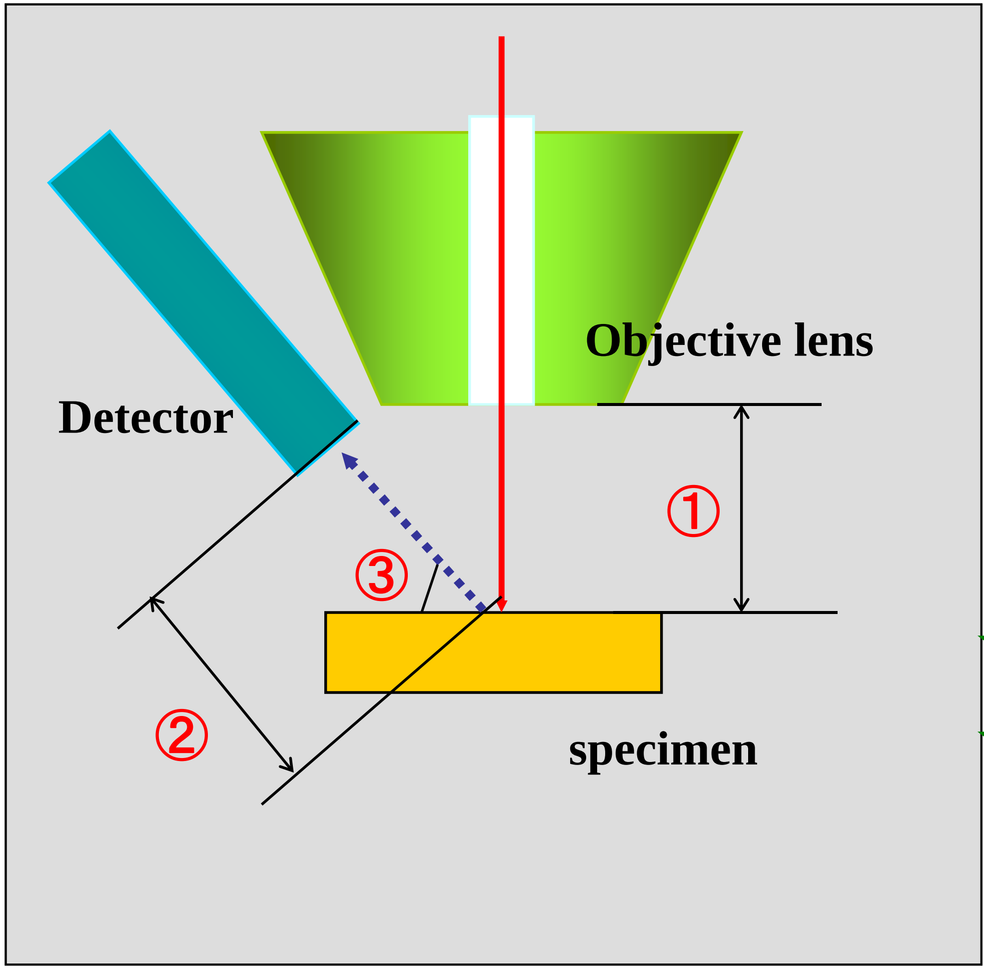

圖6.18 對於傾斜角度很大之樣品，在一般對焦方式下(A)僅有局部區域焦距正確(箭頭)，影像之景深較短，中央清楚兩端模糊(B)。若使用動態變焦，則隨著電子束掃描時焦距會跟著改變(C)，如此可使樣品在整個傾斜面上同樣清晰 (D)。

**Figure 7-1(B)** Photograph of a modern Cambridge SEM equipped to do x-ray analysis. (Courtesy of Leica.)



卻的機型推出。被電子束激發而放射出來之 X光穿過薄的鈹窗（ Beryllium Window, Be ）或超薄的高分子膜窗甚至是無窗型的偵測器中，激發矽晶接收器產生電子電洞對，再轉換成電流，經放大器（ Amplifier ）及脈衝處理器（ Pulse Processor ）的處理後，送至能量數位轉化器（ Energy-to-Digital Converter ）處理由多頻道分析儀（ Multi-channel Analyzer, MCA ）將 X光能量信號存入其對應之頻道位置。

# *Theory of Energy Dispersive Spectrometer*




**Specimen need to be placed in proper position**

① **working distance=15mm**

② **detector interdistance**

③ **X-ray take off angle=35º**

★ **Acc.voltage；15～25kV**

★ **X-ray intensity；1000~2000cps**

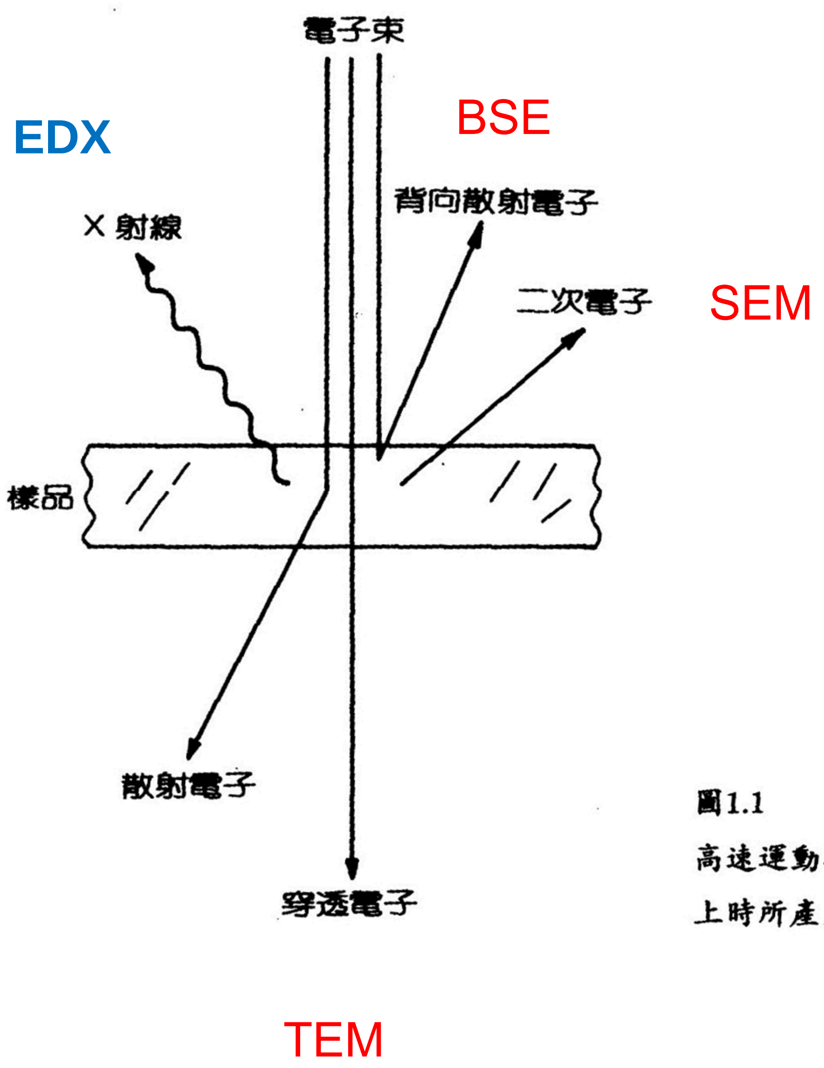
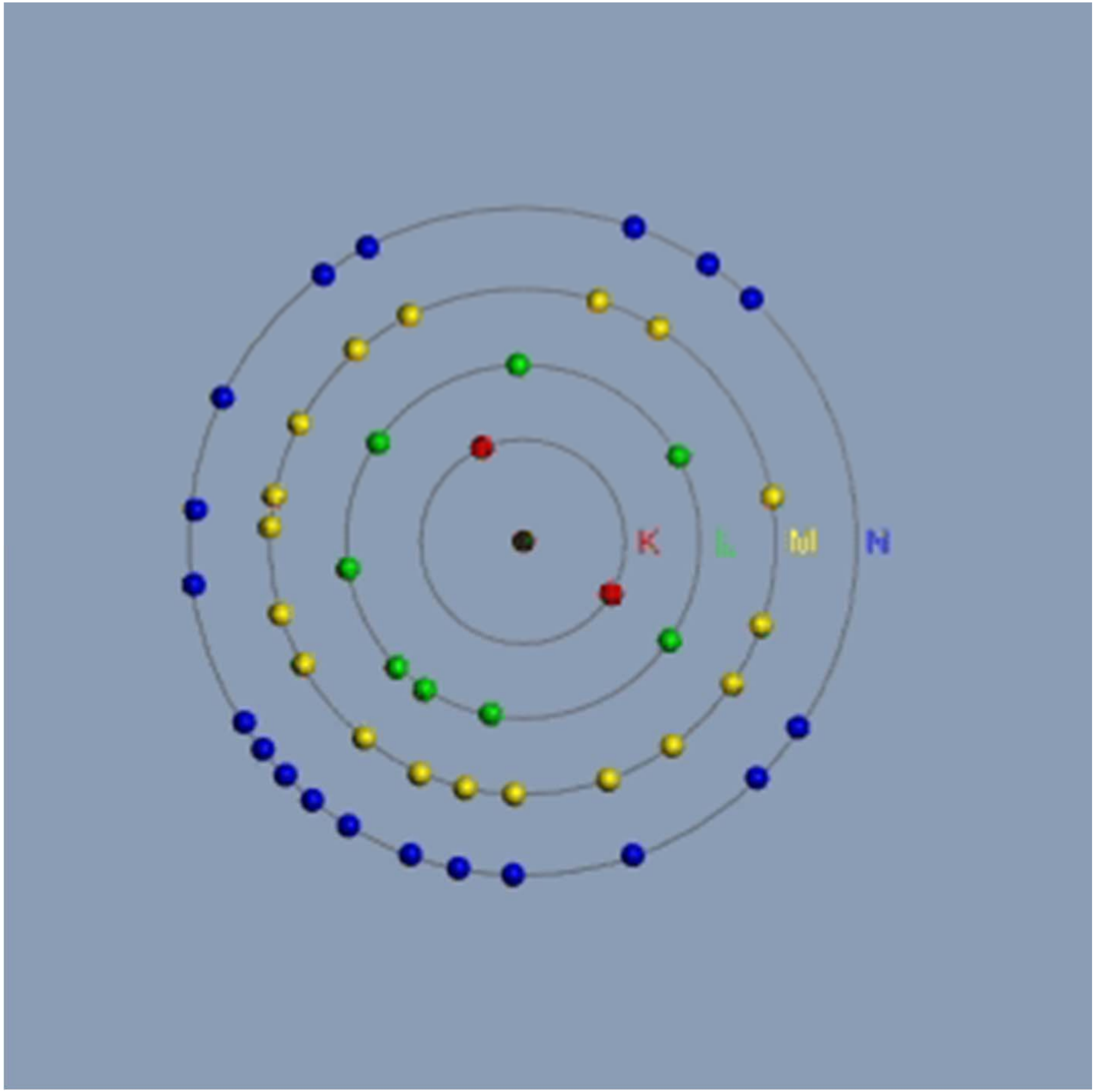
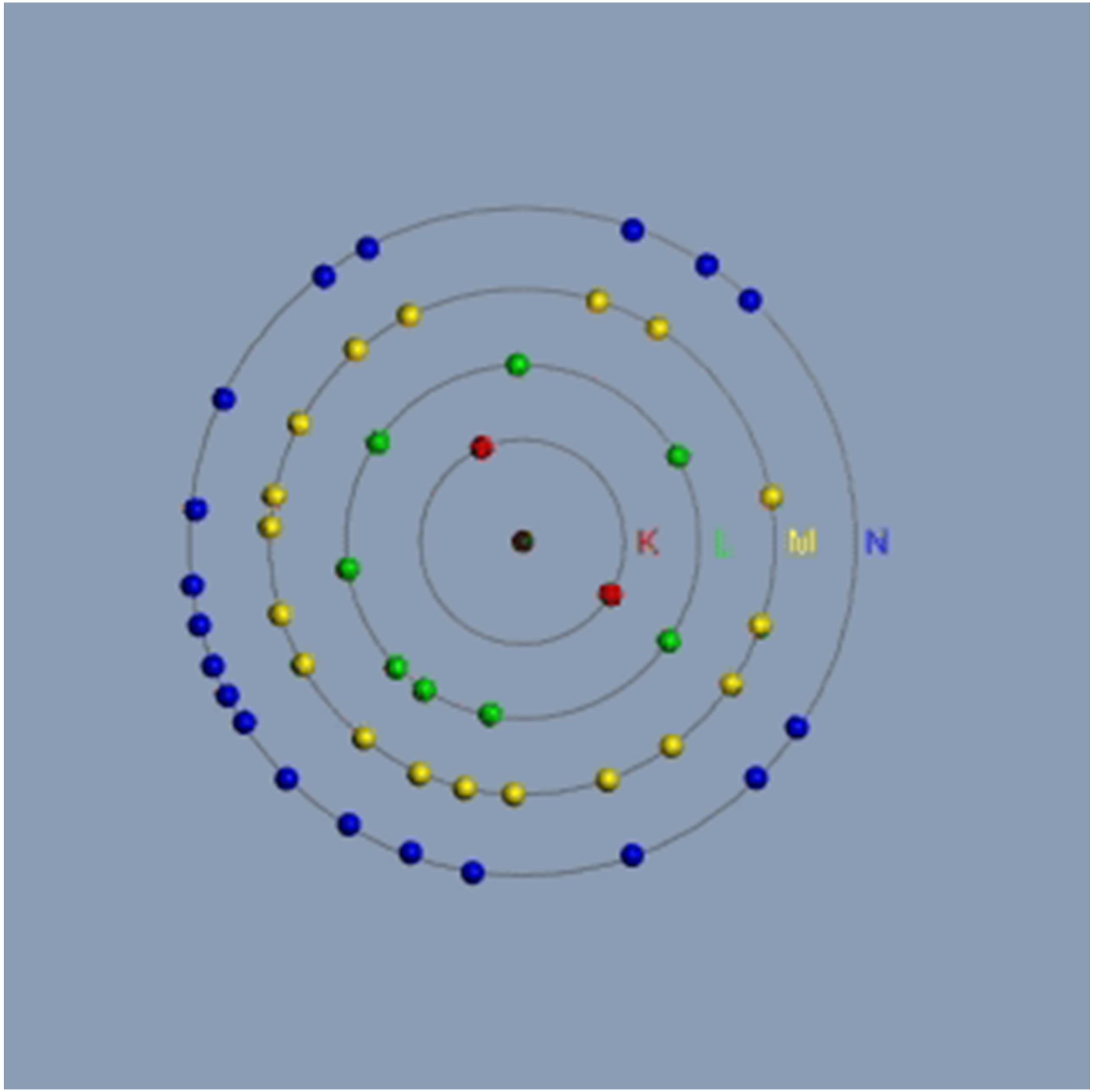


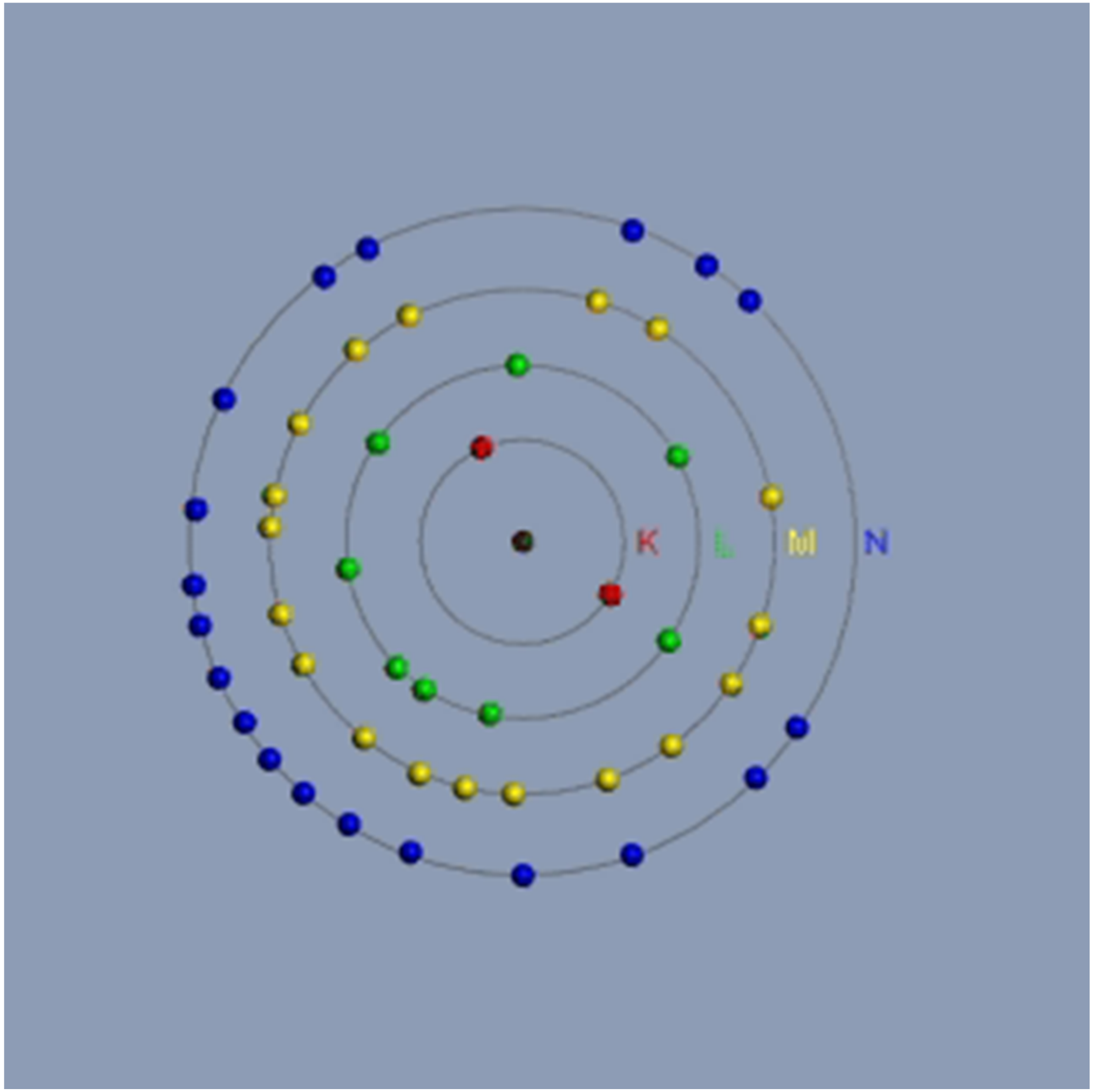

圖1.1

高速運動之電子撞擊在樣品上時所產生的各種可能狀況。

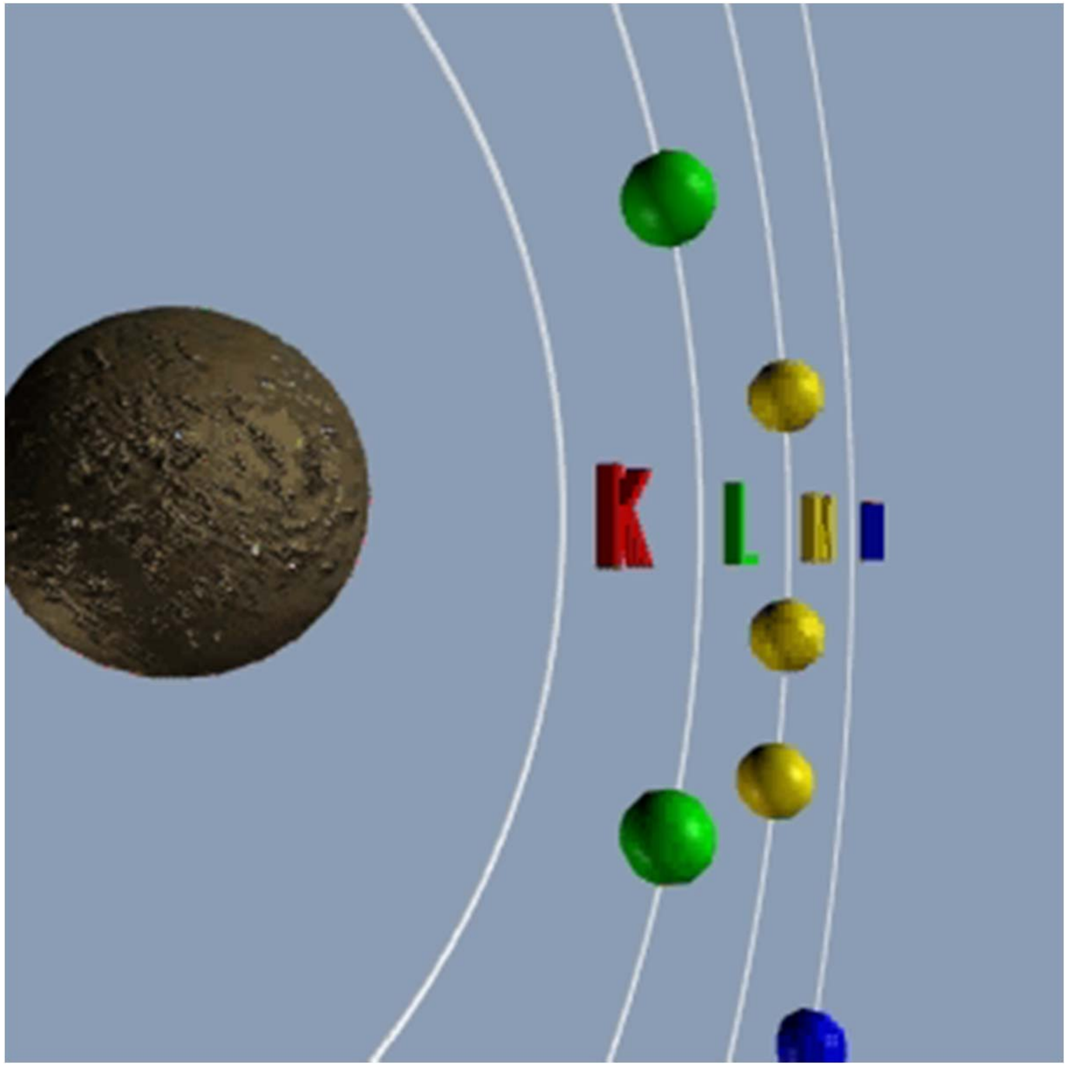

K
L
M
N




edx5




edx6

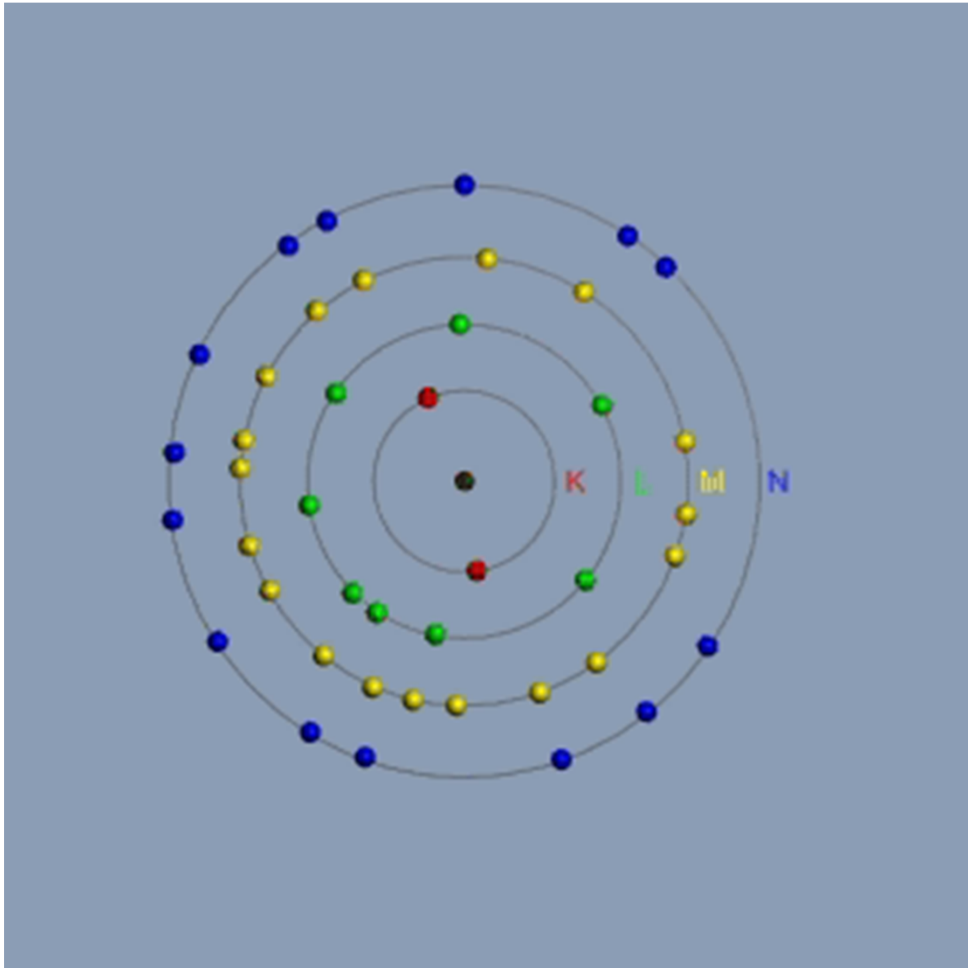



edx9

# SEM試片製備

- SEM試片製備一般原則為:
- A. 顯露出所欲分析的位置。
- B. 表面導電性良好，需能排除電荷。
- C. 不得有鬆動的粉末或碎屑(以避免洩真空時粉末飛揚污染鏡柱體)。
- D. 需耐熱，不得有熔融蒸發的現象。
- E. 不能含液狀或膠狀物質，以免揮發。
- F. 非導體表面需鍍金(影像觀察)或鍍碳(成份分析)。

金屬膜較碳膜容易鍍，適用於SEM影像觀察，通常為Au或Au-Pd合金或Pt。而碳膜較適於X光微區分析，主要是因為碳的原子序低，可以減少X光吸收




連續X光: 加速電子束撞擊到試樣元素的原子核時, 加速電子改變其方向, 於是此類電子在原子核的靜電場(electrostatic field)內逐漸緩慢下來, 並以X光形式釋放出能量, 此種X光不具有元素的特異性, 故也稱為白輻射(white radiation)或背景輻射(background radiation)

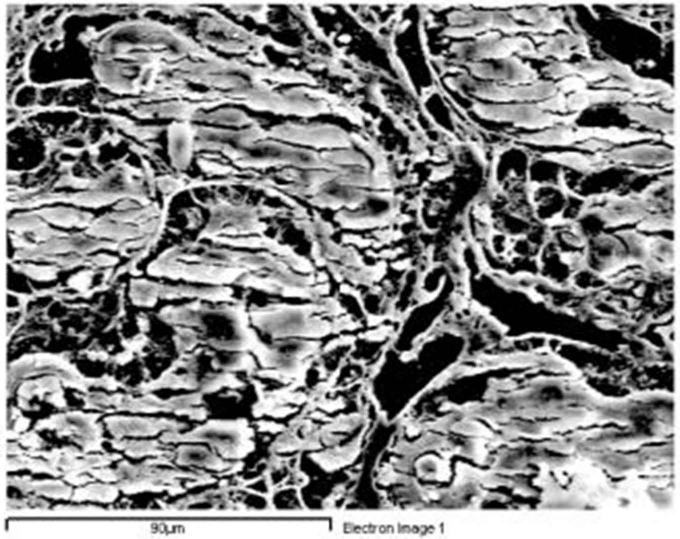
Spectrum processing :
No peaks omitted

Processing option : All elements analysed
(Normalised)

Number of iterations = 4

Standard :

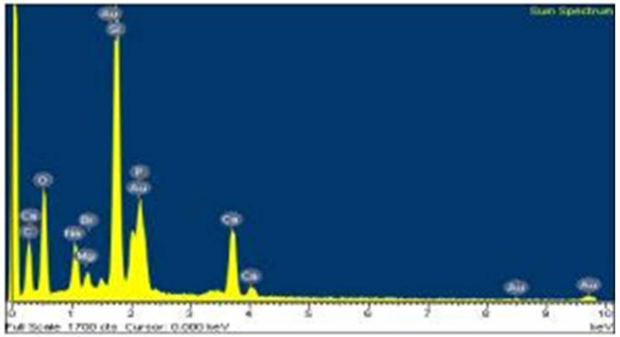
C K CaCO3 1-Jun-1999 12:00 AM
O K SiO2 1-Jun-1999 12:00 AM
Na K Albite 1-Jun-1999 12:00 AM
Mg K MgO 1-Jun-1999 12:00 AM
Si K SiO2 1-Jun-1999 12:00 AM
P K GaP 1-Jun-1999 12:00 AM
Ca K Wollastonite 1-Jun-1999 12:00 AM
Br L KBr 1-Jun-1999 12:00 AM
Au M Au 1-Jun-1999 12:00 AM




Element App Intensity Weight% Weight% Atomic%
Conc. Corrn. Sigma
C K6.920.475117.950.4833.02
O K17.300.731729.140.3840.25
Na K3.131.19023.250.093.12
Mg K0.690.96860.880.060.80
Si K14.181.072816.290.1812.82
P K4.021.30673.790.162.71
Ca K7.090.98038.910.164.91
Br L0.640.82620.950.120.26
Au M10.880.711718.840.412.11

Totals100.00

| Element | App Conc. | Intensity Corrn. | Weight% | Weight% Sigma | Atomic% |
|---|---|---|---|---|---|
| C K | 6.92 | 0.4751 | 17.95 | 0.48 | 33.02 |
| O K | 17.30 | 0.7317 | 29.14 | 0.38 | 40.25 |
| Na K | 3.13 | 1.1902 | 3.25 | 0.09 | 3.12 |
| Mg K | 0.69 | 0.9686 | 0.88 | 0.06 | 0.80 |
| Si K | 14.18 | 1.0728 | 16.29 | 0.18 | 12.82 |
| P K | 4.02 | 1.3067 | 3.79 | 0.16 | 2.71 |
| Ca K | 7.09 | 0.9803 | 8.91 | 0.16 | 4.91 |
| Br L | 0.64 | 0.8262 | 0.95 | 0.12 | 0.26 |
| Au M | 10.88 | 0.7117 | 18.84 | 0.41 | 2.11 |
| Totals | | | 100.00 | | |




Comment:尿石症(腎)
AR-01-049-b-1-700 X

# 如何得到一清晰影像

**Low Mag Focus**

方便尋找欲觀察樣品的位置，尋找到觀測樣品位置後，在附近找個明顯的標的物做影像調整

↓

**High Mag**

提高倍率至比觀察的倍率大，例如：觀察倍率為 10K，調整時倍率為 15K

↓

**Focus**

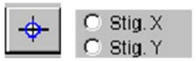
調整 Focus 時，請確認影像是否有以下的情形

影像像散 → **Stigmator X,Y**

請調整多功能旋鈕上的 stigmator X,Y 至影像清晰

調整 stigmator X,Y 影像移動 ↓

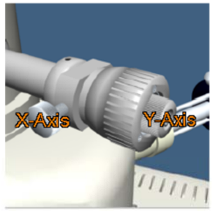
**啟動 Stigmator X,Y alignment**




請調整多功能旋鈕上的 stigmator X,Y 至影像晃動最小

影像位移 → **啟動 aperture alignment**

請調整 movable aperture X,Y 至影像晃動最小




↓

**Focus**

請確認影像是否還有位移及像散現象，若有請重複影像位移及像散調整

↓

**降低倍率**

請先將要拍照的樣品影像調整清楚，降低至拍攝倍率，執行 ABC 及 Slow Scan

↓

**Caperture Image**

敬請指正