EVO® MA and LS Series

Scanning Electron Microscopes
Operator User Guide

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ZEISS

ZEISS EVO® MA and LS Series Scanning Electron Microscopes Operator User Guide

Contents

Section 1. General Information

1. Abstract

The Operator User Guide introduces new users to the ZEISS EVO® MA and LS Series Scanning Electron Microscopes (SEM).

It provides information about important safety precautions, start up/shut down procedures, the operation of the instrument and how to get help. The basic design principles are also explained.

It is not intended to be an operator manual as all information about the operation of the SEM is contained within the comprehensive on-line help facility.

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2. Important Information

General

Software License Agreement

The software for the ZEISS EVO® MA and LS Series may be used only in compliance with the provisions of the licence agreement supplied with the software. The Carl Zeiss SMT Ltd rights for the use of the SmartSEM™ software delivered with the instrument are laid down in the agreement which is delivered with the software for the instrument and becomes an integral part of the instrument record.

Maintenance

Only correct care and maintenance of the instrument will assure optimum performance of the microscope. The maintenance work to be carried out by the user is described in *Care, Maintenance and Trouble Shooting in the Help section*. Carefully read and follow these instructions, because only then will correct operation of the microscope be guaranteed.

Only if care and maintenance are completed in compliance with these instructions, will the Carl Zeiss SMT Ltd guarantee apply. After the warranty period, ask your local ZEISS agent about a service contract. Regular servicing by ZEISS qualified engineers will guarantee that the instrument functions properly and help to obtain reliable and satisfactory results.

3. Safety Precautions (Warnings and Cautions)

Mains Voltage

When the SEM is shut down, mains AC voltage will still be present both within the instrument plinth and on some of the external cables.

ALWAYS SWITCH OFF THE SEM MAINS SWITCH BEFORE STARTING ANY MAINTENANCE WORK.

The mains CIRCUIT BREAKER is located on the rear of the SEM plinth next to the mains input cable.

 **IN AN EMERGENCY, PRESS THE RED ILLUMINATED STOP BUTTON.**

The emergency stop button is located at the top of the plinth front panel. Pressing it will interrupt the distribution of the mains supply within the plinth.

Do NOT use this switch as a means of isolating the SEM from the electricity supply, because some parts of the instrument will still be live. To isolate the SEM use the circuit breaker and the mains switch.

EHT Voltages

The EHT voltages present in this instrument can be lethal. Safety interlocks are built into the system to protect the operator and to safeguard the equipment. Do not override the interlocks.

X-Rays

The SEM is designed to ensure that any radiation of X-rays is within the permitted limits. This is achieved by the use of appropriate materials, and the fitting of radiation shields. When fitting to the chamber any accessory that has not been supplied by Carl Zeiss SMT Ltd or when installing your own equipment, make sure that X-radiation levels are checked (contact Carl Zeiss SMT Ltd for more information). If an X-ray shield has been removed for any reason, always replace it before switching on the electron beam.

Rotary Pump Exhaust

A small quantity of oil mist will be discharged from the rotary pump. This can contaminate the environment and create a health hazard in confined surroundings. Always use an exhaust system, such as an oil mist filter, or vent the pump discharge outside the building.

Venting system

The SEM can be vented using either

- Dry nitrogen gas (the preferred method)
- Atmospheric Air that has been passed through filters in a specially designed container filled with a dessicant.
- Atmospheric Air that will probably contain moisture (non-preferred).

Solvents

Careless use of solvents can constitute a health hazard. Follow the safety procedures or the Hazard Data Sheet relevant to the solvent being used. Take precautions to avoid spillage, skin and eye contact, vapour inhalation and use only in small quantities. It must be noted that some solvents may remove the paint surface from the microscope.

Corrosion

Although all the components used in the SEM are protected from corrosion by painting or plating, Carl Zeiss SMT Ltd cannot take responsibility for corrosion caused by storing or operating the SEM in adverse atmospheric conditions. To avoid corrosion of the internal components in the column, always keep the column and chamber under vacuum, even when the SEM is not being used to examine specimens.

Panels and Covers

For safety reasons and to comply with CE and EMC regulations, never operate the SEM with any panel or cover removed.

4. Notes about the Mains Supply

Input Requirements

The SEM operates only within the range of 100 – 230V @ 50-60Hz single phase with a consumption of 2.5kVA. In countries where the mains voltage is different, an autotransformer should be used. The rotary pump is powered seperately. All accessories use the same voltage as the electricity supply to the SEM.

Mains Switching

With the mains input cable connected to the electricity supply and the circuit breaker switched on, the lamp in the STOP button should be illuminated. Pressing the STANDBY button will supply power to the vacuum system components and the vacuum system will operate automatically. Power is supplied to the computer and the control electronics only when the START button is pressed.

5.Use of Liquid Nitrogen

Where the EDS accessory for the ZEISS EVO® MA and LS Series uses liquid nitrogen the following safety precautions must be strictly observed.

 **Caution:** Danger of cold burns by liquid nitrogen on the skin!

Because of the low temperature, liquid nitrogen may destroy the skin severely in a way that is similar to scalding by hot fluids or burns. Splashes or drops of liquid nitrogen generate an insulating layer of gaseous nitrogen between the skin and the drop due to the body temperature (Leidenfrost's phenomenon). However, a greater amount of liquid nitrogen which comes into contact with the skin or soaks clothes is hazardous, because it eliminates the protective Leidenfrost effect. The drastic thermosteresis of liquid nitrogen on the skin will cause the aforementioned burn symptoms.

 **Caution:** Implosion risk when pouring liquid nitrogen into glass dewar vessels!

Dewar vessels are double-walled, with a vacuum between the walls (thermos bottle principle). Always wear a face mask when handling glass vessels or, preferably, use metal Dewar vessels. These are of higher stability and withstand the temperature shock when pouring in liquid nitrogen.

 **Caution:** Explosion risk if liquid nitrogen penetrates between the walls of the Dewar vessel!

Liquid nitrogen which penetrates the insulating space through pores or fissures of a defective Dewar vessel will evaporate due to heat absorption on the inner side of the outer wall. The vessel will explode because the then gaseous nitrogen cannot escape quickly enough.

 **Caution:** Fire risk due to enrichment of liquid nitrogen by liquid oxygen!

Oxygen liquefies at -183°C, and the oxygen in the air condenses on the surface of the liquid nitrogen (-196°C) and enriches it gradually by liquid oxygen. At concentrations of more than 5% liquid oxygen, flammable material such as dust, paper, wood shavings or foam material will burn when falling into the Dewar vessel.

Therefore:

Always cover the vessel so that evaporating nitrogen can escape, but the entrance of air is inhibited and no flammable material can fall in.

Use a vessel with a narrow neck which is also more economic because of slower nitrogen evaporation.

 **Caution:** Risk of oxygen deficiency due to nitrogen enrichment of respiratory air!

The amount of evaporating nitrogen in the workroom should not noticeably reduce the relative amount of oxygen in the respiratory air. The permissible lower limit of the oxygen content is 20% vol. (normally 21% vol).

Standard values for maximum amount of liquid nitrogen in surface rooms:

Naturally aired room:	30: l /cubic meter room volume
Constantly air-conditioned room:	150:1 /cubic meter room volume

Liquid nitrogen may be used or stored in subterranean rooms only if,

- controlled mechanical ventilation is available
- evaporated nitrogen (which because of its higher density accumulates at the ground or in depressions) can drain off without damage.

6. Do's and Don'ts

Important Information

The following instructions should be followed to ensure correct operation of the instrument:

- Ventilation ports and cooling surfaces should be left uncovered and kept free from obstructions.
- Call the maintenance service in case of incidents or irregularities and if in doubt switch off the instrument to prevent extended downtime and expensive repairs.
- All safety precautions mentioned in this chapter and in connection with the routines described in this instruction manual must be strictly observed. This applies in particular to the safety precautions for the use of liquid nitrogen.

Important Prohibitions

The following prohibitions must be observed for safe operation of the instrument. Failure to comply with such prohibitions may damage your health or the instrument and also forfeit any warranty claims.

- Do not operate the instrument in case of frequent high-voltage arcs or strong instabilities.
- Do not operate the vacuum system when there are vacuum leaks.
- Do not apply force to stuck mechanical components.
- Do not unscrew sheathings and protective cover sheets.
- Do not shunt safety circuit breakers.
- Do not remove electrical safety fuses and mechanical safety devices and earth leads.
- Do not use non-approved vacuum grease and pump oil.
- Do not operate the instrument outside the installation specifications for an extended time.
- Do not mount or connect attachments that are not approved by Carl Zeiss SMT Ltd.

Section 2. Switching on the SEM

1. User Interface Description

SEM Software

Format and terminology used throughout this help text, and especially the software help are explained below in detail.

Font	Used for	Example	Explanation
BOLD CAPITAL	Command input	VENT	Push Vent key
	Acknowledgements	STORING	Data storage
	Warnings	ERROR	Error message
<BOLD CAPITAL>	Keyboard input	<ENTER>	Push Enter key
[boldface]	Optional parameters	[.TIF]	Extension which can be omitted
Boldface	Accentuation	Note:	General accentuation in text

Keyboard Entries

<Key1 + Key2>	Special codes <CTRL + Z> Push <CTRL> and <Z> jointly
<Key1, Key2>	Standard input <D,1> Push <D> first, then <1>

Other Designations

DR:	Disk drive identification	A:	Disk drive A (Floppy Disk)
\Path	Path input	C:\TEST	Subdirectory TEST on drive C
.TIF	Extension	NAME.TIF	Image named NAME

Icons

Mark for special attention.

Tip for improved operation.

Caution! Safety precautions; if not observed, danger of damage to persons or instrument.

Additional help available, click!

Reference to drop down menu.

The Mouse:
The mouse is the primary adjustment and selection control of the microscope. There are carefully defined conventions governing its use:

The left mouse button:
The normal Windows® convention of pointing and clicking is used for icon selection or menu selection, dragging is used for moving or sizing objects and for parameter adjustment.

The middle mouse button:
The middle button is used for parameter adjustment.

The right mouse button:
The right mouse button is used to display a pop-up menu while over the image. When the cursor is over an icon and the right button is pressed, the control panel associated with that icon will pop up.

The Keyboard
In addition to the mouse control of parameters, many keyboard keys have been assigned to special functions acting as “accelerators” or “short cut keys“ and giving direct access to the function. For a list of short cut keys *see Keyboard Keys in Software Help*.

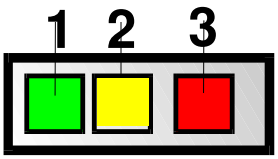
The Monitor
The monitor serves as the visible link to the user. The SEM image together with all other necessary information for the operation is shown here.
(The resolution of the monitor should be set to 1280 x 1024 pixels).

2. Start Up

Start-up from Cold

After a power failure or planned power interruption, restart the Zeiss EVO® according to these instructions:

- Turn on mains power to microscope.
- Set the Circuit Breaker on rear panel of the plinth to ON (the red switch (**3**) should illuminate).
- On the plinth front panel locate the middle Standby Button (**2**) and press it. (This applies power to the vacuum system and is considered as being the standby condition.)



- The vacuum system will start automatically.
- On the plinth front panel locate the green Start Up Button (**1**) and press it. (This applies power to the rest of the system and the computer.)
- Switch on the display monitor.

The Windows® software is loaded from the hard disk and the desktop image with the program icons and the Task Bar is displayed on the monitor.

The next and most important action is to load the user interface as described in the section below.

3. Starting the SEM Software (Log On)

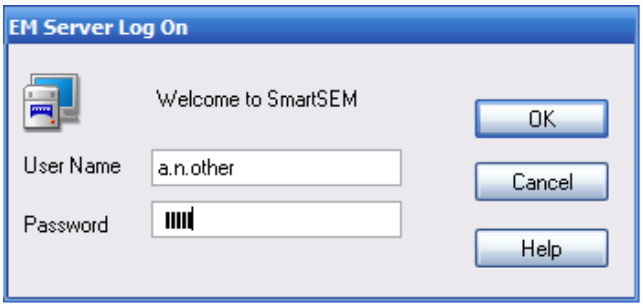
Locate the SmartSEM™ Logo Icon on the desktop if installed and double click on it

or

Select the Windows® START, All programs and navigate to SmartSEM™, and click on SmartSEM™ Interface .

The SmartSEM™ program is then started. Wait until the EM Server program (this controls the hardware) is loaded and then log on to the SEM. You must insert your name and the password that was supplied by your systems administrator or installation engineer.

Alternatively you may sign in as GUEST with no password.



- Click in the text field for User Name and enter your name.
- Change to Password box with the Tab Key  and enter your password. Since the password is hidden during input, only *** are displayed.
- Click on the OK button.

The software is now loaded, and the SEM user interface is displayed on the monitor.

When the Vac box in the taskbar displays a tick the SEM is ready to be used.

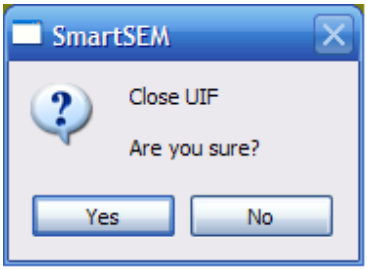
4. SEM User Interface Log Off

This function is called from the system menu, it closes the SEM user interface and puts the SEM hardware into standby mode. Any other applications using the server such as REMCON and the Administrator are also closed and you will be logged off the server.

In the Menu Bar click on File and Log Off

or

Type <ALT + F> then <G> to start the shut down sequence.



<Yes> starts the close down action.

<No> breaks it off and returns to the application.

This includes the following:

- Setting the EHT to standby conditions.
- Setting the lens currents to standby conditions.
- The EM server application is still running which can be seen on the Task Bar.

If the Zeiss icon is double clicked the log on procedure must be followed to re-establish the SEM control interface.

5. SEM User Interface Close

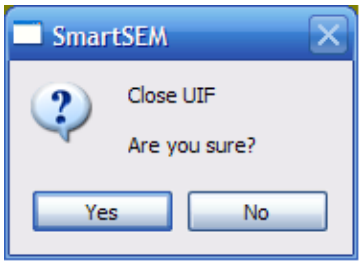
This function is called from the system menu and terminates the operation of the SmartSEM User Interface software only. Any other applications, such as REMCON and The Administrator that are using the server, remain active and logged on.

In the Menu Bar click on File and Exit

or

Type <ALT + F> then <X> to start the shut down sequence.

For safety you are requested to confirm the action and to close the User Interface.



<Yes> starts the close down action.

<No> breaks it off and returns to the application.

The action is as follows:

- Save operating conditions if required.
- Perform an orderly shutdown of each control module. This includes shutting down the high voltage set (EHT).
- Save any critical data.

- Save in files any user specific data in the User Directory, and record the current user directory.
- The server is still running and should be closed by clicking the server application on the System Tray and then clicking the application close button  .
- Windows® can then be closed in the normal way as described below, once the EM server has been closed.

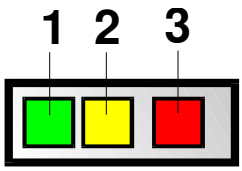
6. SEM Shutdown

This function shuts down Windows® and removes the power from the computer, the display monitor and the SEM electronics. The vacuum system stays ON.

 Before you attempt to shut down the instrument, always follow an orderly exit sequence on the SEM application by following the procedures given in section *SEM User Interface Close*. Otherwise loss of data may occur.

Click on  in the Desktop Task Bar and select <Turn Off Computer>.

On the plinth front panel locate the middle Standby Button (**2**) and press it.



The instrument electronics and the computer are powered down. You may notice a delay until the system switches OFF.

7. Emergency Shutdown 

 Use this shutdown mode only in case of an emergency!

 All current working data and conditions will be lost!

On the Plinth locate the red Emergency Button (**3**) and press it.

The software is forced to terminate immediately, the computer and the instrument electronics are switched off and the vacuum system is stopped.

The power is removed from the instrument's electronics.

 If you need a complete Power OFF shut the instrument down in an orderly fashion and then use the Circuit Breaker on the back panel of the plinth. Then turn OFF the mains power to the microscope.

8. How to use Help

There is extensive help available through the SmartSEM™ User Interface program. Use the following techniques to extract the information of interest.

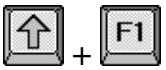
- Context Sensitive Help for the functions called from the Image Window.
- Windows® Style Help from the Menu Bar by clicking on Help.

Context Sensitive Help

Key(s):Function:

Displays help for the function that is active.

If the Help window is already open, pressing <F1> displays the "Using Windows Help" topic.



Changes the cursor to  so you can get help on a specific command, screen region, or key.

You can then choose a command, click the screen region, or press a key or key combination you want to know more about.

(This feature is not available in all Windows® applications.)

Cancels the Context Sensitive Help mode. Brings back the normal cursor.

Windows® Style Help

Select Help from the Menu Bar. A Sub-Menu opens with following topics:

- Help on Help — What you should do to get Help
- Search — How to access Help Topics
- SmartSEM™ Help (main page) — The overview of SEM specific help

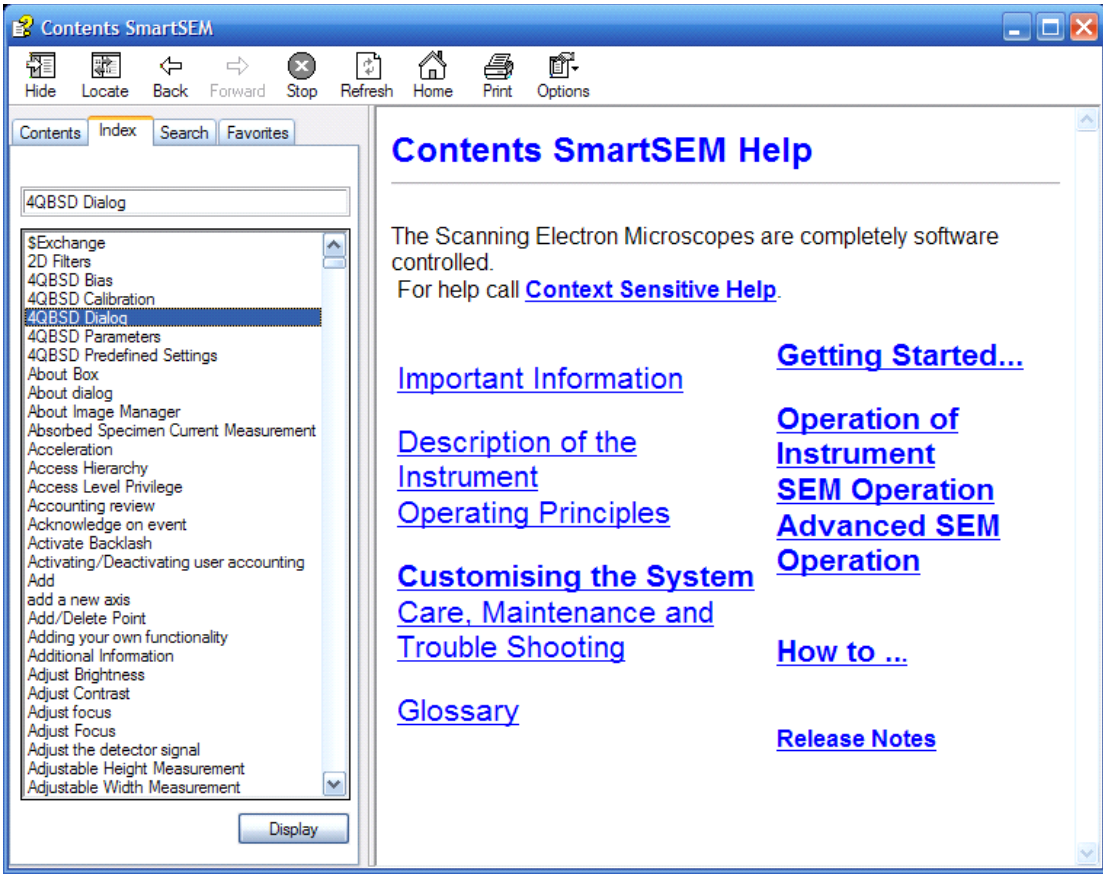
- Keys Help Explains which keys have which functions

Clicking on one of the Sub-Menus takes you into more detailed information.

Searching for Topics

The Contents tab shows the structure of the Help System as books , and pages .

- To open a book click on it.
- Pages show the paragraphs in the book.
- Click on a page to show the help text.
- The Index tab shows all topics in a list.
- Use the Scroll Bar to see the text or start typing the topic in the top window.
- Mark the topic and select Display.
- The Find tab provides full text search. Follow the directions showing up on the tabs as you go through the program.



9. Contents of Help Software

SmartSEM™ Help	cover page
Contents	contents of Help Software
Glossary	explains special terms
Important information	similar to the information given in this manual
Description of Instrument	describes the components of the instrument
SEM Operation	describes how to operate the instrument
Operating Functions	describes the principles of operation of components
Care, Maintenance and Troubleshooting	describes how to look after the instrument and achieve best results
Operating Principles	describes the function of system components

In some of the graphics in the Software Help you can point and click onto features to obtain additional information.

This is available whenever the normal cursor 

changes to the  hand cursor.

Section 3. Operation of the SEM

1. Consider the properties of the specimen

Specimens that are studied in the SEM can be divided into two main categories, namely **conductors** and **non-conductors** and these will be discussed in greater detail later. However there are several factors to consider during all specimen preparation and these are given below.

1. The size and weight might necessitate some reduction of the specimen to fit holders and to ease specimen manipulation for observation.
2. Mineral and metallurgical specimens may require polishing. Etching and electropolishing may also be required.
3. The specimen should be able to withstand the vacuum of the SEM as it might become damaged or deformed.
4. The specimen should be clean and dry if it is to be used in high vacuum mode, i.e. free of dust, moisture, oils and grease, as their presence can lead to charging, contamination and longer pump down times. Otherwise use VP or EP vacuum mode.
5. Porous samples will also take a long time to pump out in high vacuum mode.
6. Coat non-conductors to prevent charging if allowed or VP mode is not available. Observation of the specimen at low kV (start at 1 kV) is another good alternative where “charge balance” can be obtained.
7. The specimen should be firmly attached to the specimen stub or holder either mechanically or by gluing. Silver dag and carbon dag can be used as conductive glues for small samples. Carbon tabs/tape are also convenient but may not provide good mechanical stability.
8. There should be good electrical connection between the surface of the specimen and specimen stub or holder for non-conductors.
9. Health and Safety procedures regarding the handling of the specimen during its preparation should be observed.

Conductive specimens are generally observed in what might be described as a conventional SEM however **Non-conductive** specimens are being increasingly observed in a specially modified SEM called the **Variable Pressure SEM** (**VPSEM**) where gas can be introduced into the specimen chamber. The benefit is that the specimen can be viewed without the necessity to “coat” as the negative charge built up on the surface of the non-conductive specimen by the high energy primary electrons is neutralised by the positively charged ions that are produced when the gas molecules are ionised by collisions with secondary, backscattered and primary electrons. It also slows down the dehydration of moist specimens that leads to structural collapse.

However it is now possible to observe moist specimens in conjunction with cooling of the specimen and the water control option using SEMs with **EP** vacuum pumping modes.

Specimen chamber pressure range	
VP	10 to 400 Pascals
EP	10 to 3000 Pascals

2. Guide to SEM Operating Parameters

The conditions given below are suggestions and the operator may find that different values will give better information for the types of specimens that are being investigated.

Conductive specimens

1. General Microscopy

   EHT =20 kV Iprobe = 200 pA WD = 15 mm

   Filament set to the "Long Life Mode" (select the option on the Gun panel)

   Filament I set to 1st peak for magnifications < 10 kx (gives longer filament life)

   Filament I set to 2nd peak for magnifications > 10 kx (for better resolution)

   Detector = SE with collector bias > + 300 V

   MC∅ = 30 µm

   Cycle time to reduce noise = 20 sec

2. EDS

   EHT = 20 kV for metals and minerals

   EHT = 7.5 kV for semiconductors and organic materials

   Filament I set to 1st peak for Qualitative analysis

   Filament I set to 2nd peak for Quantitative analysis

   Iprobe = 1000 pA or adjust for 30 % deadtime

   WD = 8.5 mm for a 35° take off (elevation) angle

Key:

MC∅ = Mid-Column Aperture (final aperture in HV mode)

PLA = Pressure Limiting Aperture

Detector = BSD

Final aperture = 30 µm

Cycle time = 20 sec or longer for X-ray mapping

3. High resolution

EHT=30 kV Iprobe = 10 pA WD = 5 mm

Filament I set to 2nd peak and untick the "Lonflife Mode"

MC∅ = 30 µm

Detector = SE with collector bias + 400 V

Cycle time to reduce noise =1.3 mins or longer

Warning – remove the BSD to its parked position

Non-conductive specimens

1. High vacuum mode.

EHT = 1 kV Iprobe = 10 pA WD = 5 mm

Filament I set to 2nd peak

MC∅ = 30 µm

Detector = SE with collector bias + 400 V

Use scan speed 3 with frames to average = 30 to reduce noise.

2. Variable pressure (VP) mode.

EHT = 25 kV Iprobe = 250 pA WD = 8.5 mm

Filament I set to 2nd peak

MC∅ = 750 µm

PLA = 100 µm

Detector = BSD or VPSE

Cycle time = 20 sec or longer

Chamber pressure = 10 Pa for BSD detector, 40 Pa for VPSE detector or adjust to eliminate charge disturbances.

Key:

MC∅ = Mid-Column Aperture (final aperture in HV mode)

PLA = Pressure Limiting Aperture

3. Extended Pressure (EP) mode for cooled hydrated samples.

   EHT = 30 kV Iprobe = 300 pA WD = 8.5 mm

   Filament I set to 2nd peak

   MC∅ = 750 µm

   PLA = 100 µm

   Beam Sleeve® = 500 µm

   Detector = BSD or VPSE

   Cycle time = 20 sec or longer

   Chamber pressure = 650 Pa to slow dehydration

   Temperature of specimen to retain water at 650 Pa = 1°C.

4. EP mode for hydrated samples.

   EHT = 30 kV Iprobe = 300 pA WD = 5 mm

   Filament I set to 2nd peak

   MC∅ = 750 µm

   PLA = 100 µm

   Beam Sleeve® = 500 µm

   Detector = BSD or VPSE

   Cycle time = 20 sec or longer

   Chamber pressure = 2000 Pa to retain water at 20°C.

Key:

MC∅ = Mid-Column Aperture (final aperture in HV mode)

PLA = Pressure Limiting Aperture

Imaging wet samples using the Peltier coolstage

To study hydrated specimens it is recommended that a Peltier cooling stage and a water control system be fitted to the SEM. This will enable fresh specimens to be examined with little or no loss of water in the SEM environment (the sample is kept fully hydrated during the pump down).

1. Vent the chamber and remove the lens mounted BSD [if fitted] and place it in the "park" position.
2. Fit the 100µm EP upper aperture.
3. Fit the 500µm lower aperture/ BeamSleeve®.
4. Remove the round right hand blanking plate from the stage door.
5. Detach the Peltier assembly from its parking plate.
6. Feed the Peltier stage holder through the hole in the stage door and carefully rest it with its pipe work on the stage.
7. Clamp the Peltier vacuum flange to the stage door.
8. Suitably arrange the pipe work and attach the Peltier stage holder to the SEM stage with the 3mm clamp screw [Deben Manual].
9. Place the sample onto the Peltier sample stub and tighten the clamp screw but do not over tighten as this deforms the PTFE end of the screw.
10. Re-fit the BSD if required.
11. On the Aperture Control Panel click on "Select Aperture".
12. Select the EP aperture, the panel with then expand.
13. Then select the 500µm BeamSleeve®.
14. From the main menu select "Tools", "Administrator" and type your "User name" and "Password".
15. In the Administrator panel select "Configuration", "Other" and tick the "Peltier fitted" box.
16. Scroll down and select the USB connection and tick the "Humidity Option" box.
17. Remove the lens mounted BSD [if fitted] from the "park" position and place it in the "active" position.
18. Ensure that the Z is low enough before closing the stage door.
19. Pump the chamber and wait for vacuum ready.
20. Open the docking panel and from the menu select the "Extended Pressure Control".
21. On the dialog panel tick the "Peltier" option and click on the "Purge Settings" option.

The aim of purging is: i) to remove air from the water bottle (will take a while to be completed), and ii) to fill the chamber with just water vapour). Choose between step 22 and 28 depending on the condition of the water bottle.

22. If the Water Kit has recently been fitted, filled with water, or has not been used for a while:
23. Set the “Purge cycles” to 5.
24. Set Purge Max to 1000 Pa.
25. Set Purge Min to 20 Pa.
26. On the Purge Control dialog panel select “WET” if the EP Mode states “DRY”.
27. The purging then starts:
    i) The pressure in the chamber will vary between the Max and Min levels.
    ii) Bubbles can be seen in the water bottle.
    iii) All air is removed from the water bottle (will take a while to be completed).
    iv) Continue from step 29.

28. If the system has recently been used in the “WET” mode:
29. Set the “Purge cycles” to 1.
30. Set Purge Max to 950 Pa.
31. Set Purge Min to 640 Pa.
32. On the Purge Control dialog panel select “WET” if the EP Mode states “DRY”.
33. Ensure the Peltier control box is switched on.
34. On the “Extended Pressure” panel set the “Peltier Target” to 1°C and suitably adjust the “Humidity Target”.
35. Adjusting the position of the “Green Cross” in the Phase Diagram will change the environment of the sample; water vapour, water, or ice.
36. Turn the beam on and start with the imaging.

Suitable SEM operating parameters for imaging the sample

These will depend on the type of sample being examined and the nature of the detail you are expecting to see [be prepared to experiment]

EHT = 20 KV, I Probe = 200 pA or higher, WD = 8.5 mm or less, Detector = VPSE, BSD, or EPSE

“Use the Chamberscope and Stage Navigation panel for positioning the specimen”.

3. Sequence Guide to Operation of the SEM

1. Start up the SEM by pressing green Start Up button.
2. Start the SEM software by double-clicking on the SmartSEM logo icon.
3. Vent the chamber, load samples quickly and pump the chamber.
4. Wait for the vacuum "ready".
5. Switch on the beam by either selecting “File” then "Load state" or the "Gun" in the bottom right of the display.
6. Set "brightness" to 50 % and adjust "contrast" if the image is too bright/dark.
7. Check filament saturation and gun alignment.
8. Focus the beam on the sample/holder at low magnification and then position the sample as required using the joystick.
9. Progressively increase the magnification, focussing the beam until the sample is being viewed at greater than 5000x.
10. Align the final aperture if necessary utilising the Focus Wobble mode.
11. Correct for any observed astigmatism.
12. Select a suitable scanning/noise reduction mode to remove any noise from the image.
13. Suitably adjust the "brightness" and "contrast" of the image.
14. Freeze the image.
15. Annotate the image if required.
16. For printing or exporting the image, just right-click the mouse in the image area and select the appropriate option from the "send to" menu.
17. To end the SEM session the operator may LOG OFF or shut-down.

For further assistance on above select Help, then SmartSEM™ Help and SEM operation and select the appropriate topic.

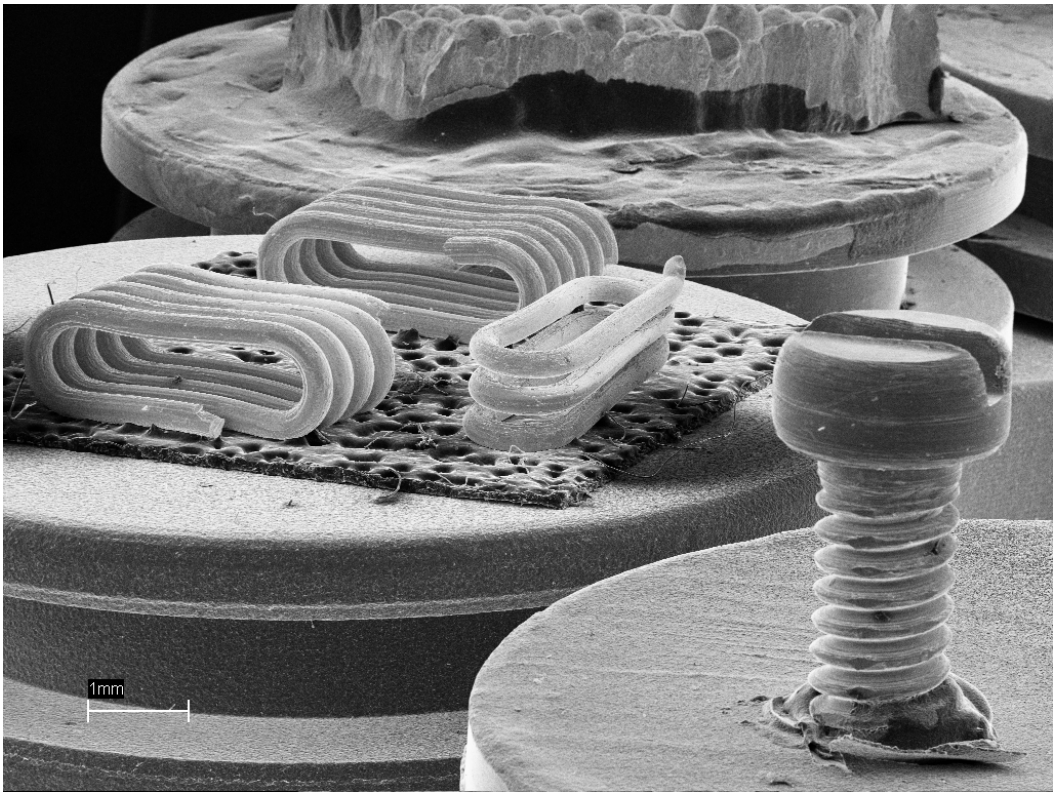
Section 4. Basic Design Principles of the SEM

1. Introduction

The scanning electron microscope (SEM) is used to examine microscopic detail of solid specimens that may have initially been viewed in a light microscope but subsequently found to require SEM examination in order to provide information not available from the light microscope.

The image is produced by scanning an extremely small focused beam of electrons (adjustable down to a few nm in diameter) across the surface of a specimen in an array of picture points (pixels) usually 1024 by 768 pixels. High-energy electron bombardment of the specimen causes signals to be emitted at each pixel. These are collected and their intensities are used to produce images of the specimen by modulating the brightness of equivalent pixels on a TV monitor.

Initial viewing of the specimen uses the secondary electron emission signal to provide an image very similar in shape to that seen in the light microscope. Unlike the light optical microscope that displays images in true colour, the SEM presents intensity images where zero signal is displayed as black, intermediate signals as shades of grey and maximum signal as white.



The SEM image shows a small screw and some broken coils of tungsten wire from a car headlamp.

In order to see why one would use an SEM it is useful to compare it with the light microscope as it has three distinct advantages - better resolution, greater depth of field and the ability to carry out X-ray microanalysis.

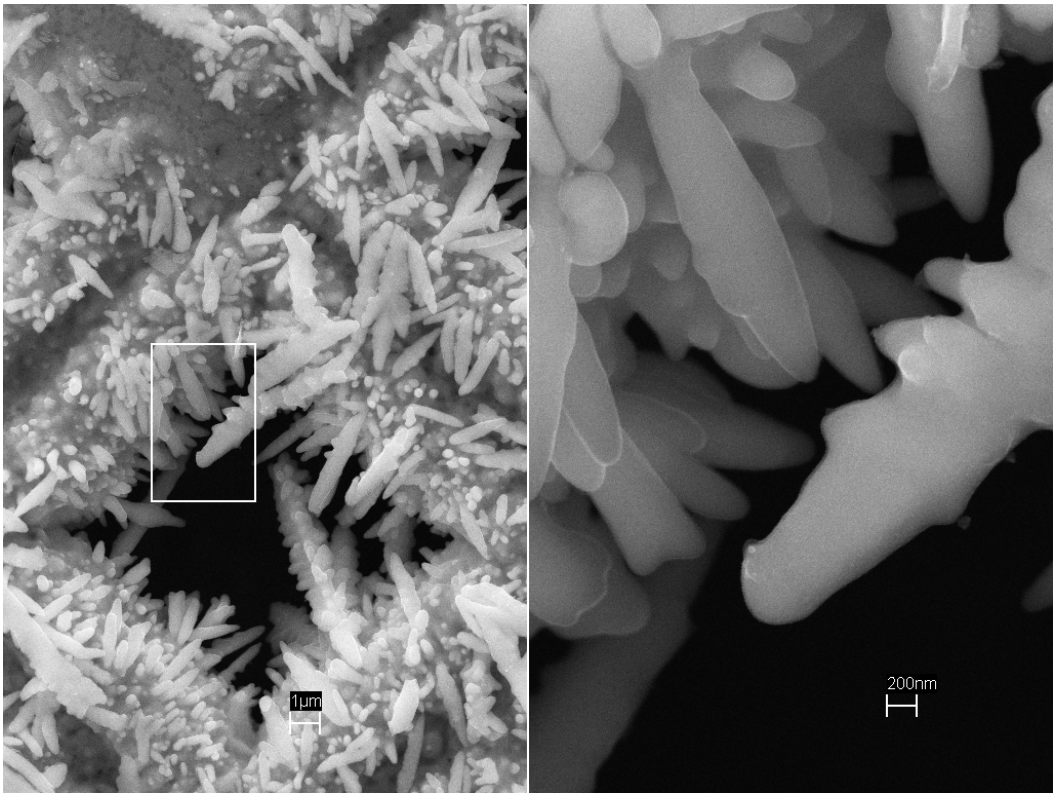
2. Resolution

The resolution limit of any microscope is defined as the minimal perceptible distance between two points and is partly dependent on the wavelength of the illumination. The wavelength of electrons is considerably smaller than that of light, hence the improvement in resolution.

The better resolution of the SEM enables surface detail not normally visible in the light microscope to be viewed at high magnifications.

Resolution of the light microscope > 200 nm (wavelength of green light = 500 nm) with magnifications up to 3,000 times.

Resolution of the SEM < 5 nm (wavelength of 30 kV electrons = 0.007 nm) with magnifications up to 1 million times.



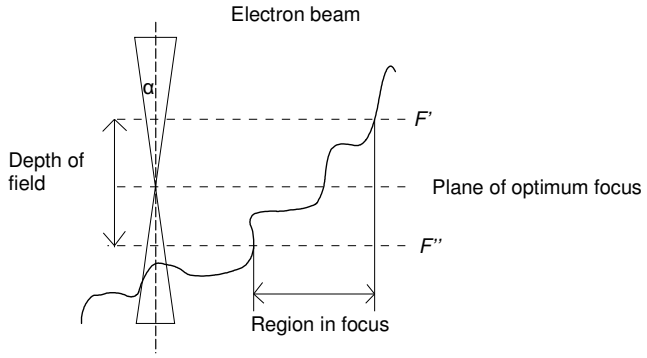
This SEM image shows silver dendrites on a copper support mesh.

The image is displayed in dual magnification mode where the area shown within the box on the left side has been further magnified on the right side. The markers on both sides of the image indicate the scale.

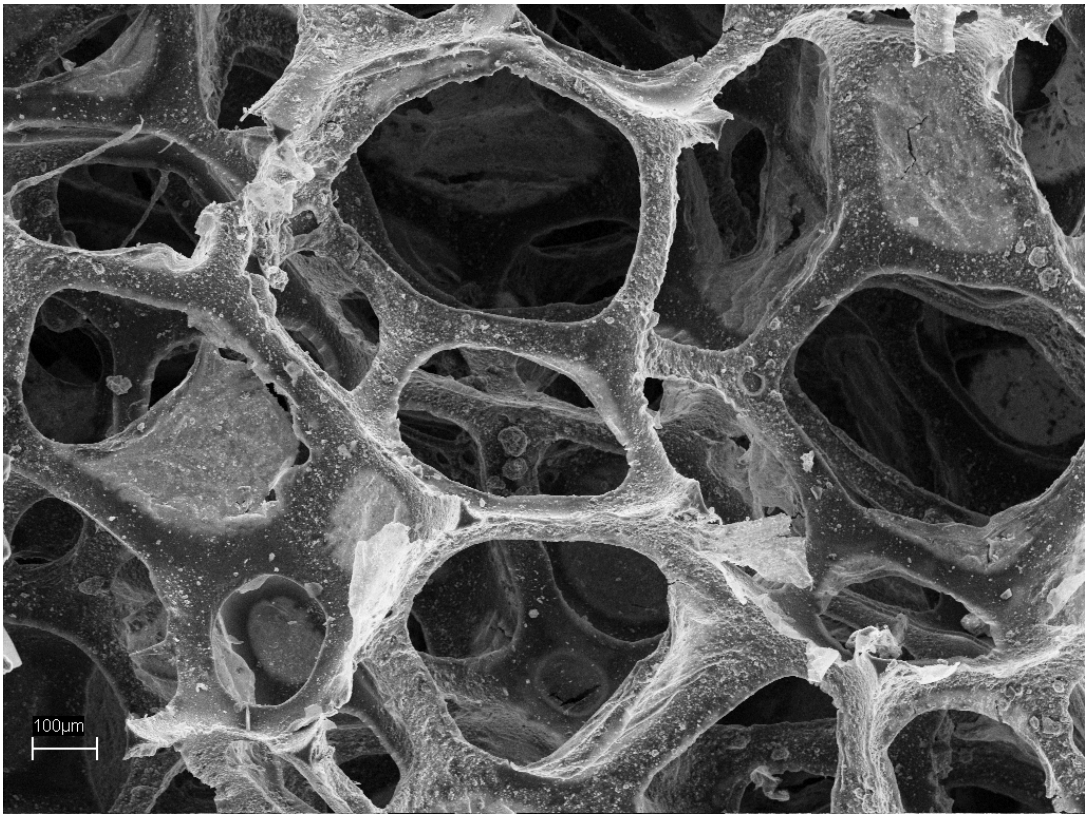
3. Depth of field

The SEM has a considerably better depth of field (DOF) than a light microscope i.e. the ability to maintain sharp focus of detail as the specimen surface height changes. This facilitates the examination of specimens that have a very irregular topography.

If the depth of field of a light microscope is said to be 1, the depth of field of the SEM is



typically 300 times better.



This image is of conductive foam where the depth between layers is approximately 0.5 mm.

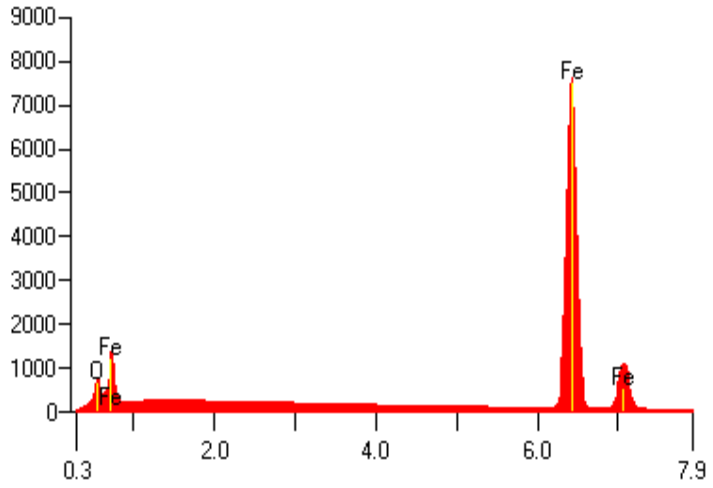
4. Microanalysis

The elemental composition of the specimen can be determined from the x-ray spectra excited by high-energy electron bombardment of the specimen. Each element has its own unique spectra that can be identified, just like a “finger print”. Particles as small as 1 micrometre can be analysed. Detection and quantification of the spectra is achieved in one of two ways.

1. EDS

An Energy Dispersive X-ray detector System (EDS or EDX) is the more popular as this detector can display all the elements present in the specimen as they are being collected and enables rapid identification to be performed. It enables the composition of the specimen to be determined to an overall accuracy of about 1% and detection sensitivity down to 0.1% by weight.

A semiconductor material is used to detect the x-rays together with processing electronics to analyse the spectrum.



The above diagram shows an EDS spectrum from iron oxide. The vertical axis displays the number of x-ray counts whilst the horizontal axis displays energy in KeV. Identification lines for the major emission energies for iron [Fe] are displayed and these correspond with peaks in the spectrum, thus giving confidence that iron has been correctly identified. Similarly there is a peak appearing at the marker for oxygen (O).

2.WDS

Where resolution and sensitivity are of the utmost importance particularly for elements of low atomic number an additional detector called a Wavelength Dispersive X-ray Spectrometer (WDX or WDS) would be required. This detector collects one discrete element at a time and hence is relatively slow in identifying elements in a specimen of unknown composition. However its detection sensitivity is in the order of 0.01 % by weight thus making trace analysis possible. Also, because its resolution is approximately 10 times better than that of an EDS, it can clearly identify elements that are severely overlapped in the EDS spectrum.

A range of diffracting crystals is used to analyse the spectrum with precision mechanics to control the positional relationship between the specimen, crystal and a gas proportional detector for counting the x-rays.

The operation of wavelength spectrometers is based upon the principle of diffraction according to Bragg's law that states:

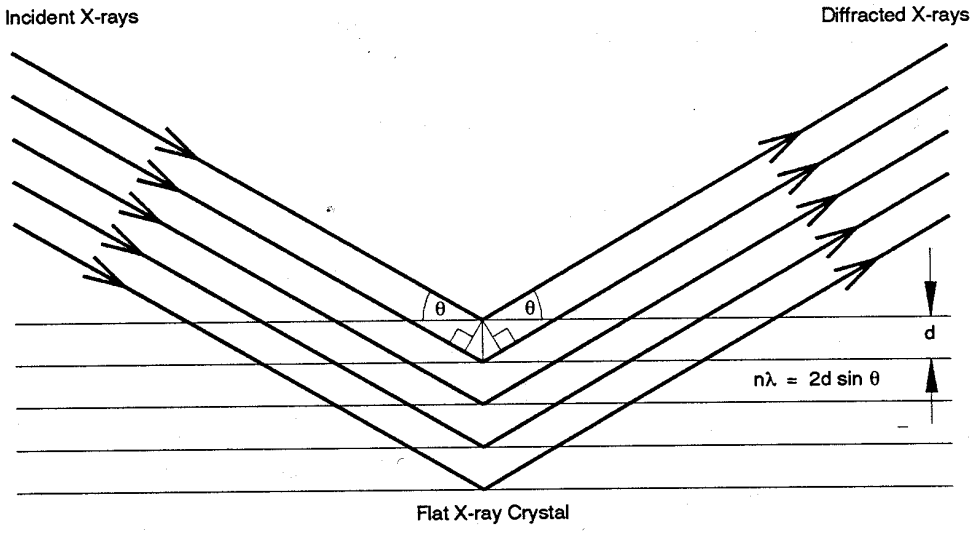
$$n\lambda = 2d \sin \theta$$

Where n = 1, 2, 3,……. The order of diffraction

λ = wavelength of the x-rays being diffracted

d = lattice spacing of the diffracting crystal

θ = Bragg angle (the angle between the crystal surface and the incident and diffracted x-rays)



The relationship between the energy of an x-ray (E) and its wavelength (λ) is given by the equation

$$E = \frac{12.396}{\lambda}$$

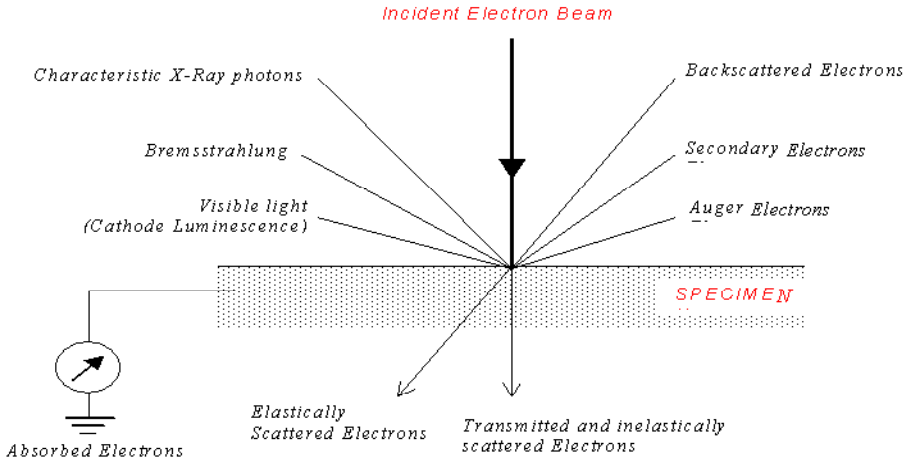
E in kev

λ in Angstroms

In both systems the number of x-rays generated by the beam for each element in the specimen is proportional to its concentration.

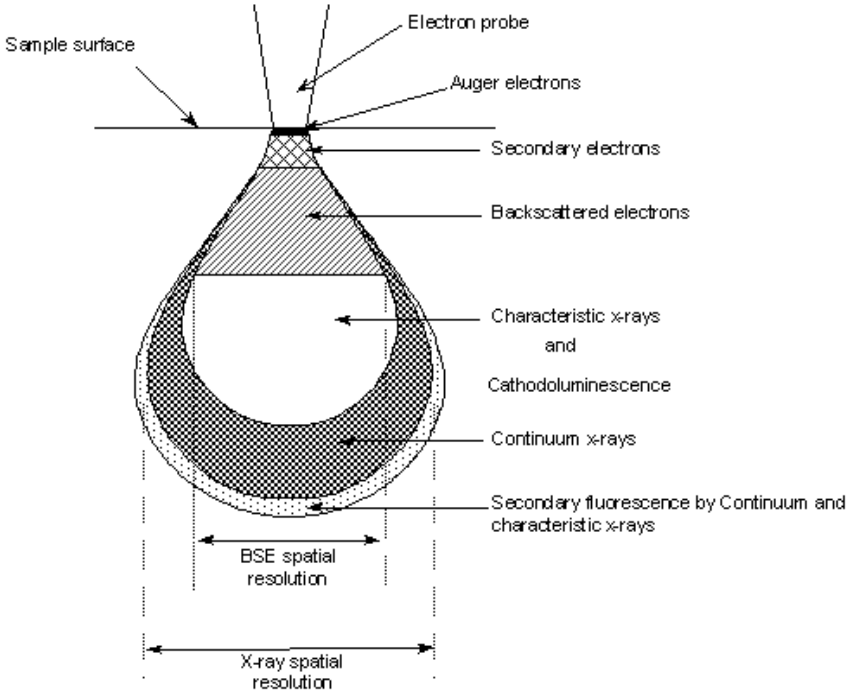
5. Beam specimen interactions

The high energy focused beam of electrons **PE** (primary electrons) excites a variety of different signals from the specimen surface. Each of these signals shown below has a detector in order that its information can be displayed as an image.



1. Secondary electrons **SE** are excited from the top surface layer of the specimen (0 to 10 nm).They are defined as having an energy range from 0 to 50 eV with the majority having an energy from 3 to 5 eV. The secondary electrons emitted at the point of impact of the beam are referred to as **SE1** type electrons and it is the amount of these electrons that are dependent on the shape of the sample.
2. Backscattered electrons **BSE** emerge from depths well below the top surface of the specimen.They are defined as those electrons having an energy greater than 50 eV with the majority having an energy approximating to ¾ of the electron beam (probe) energy. The number of **BSE**s that are emitted is highly dependent on the mean atomic number of the specimen at the point of impact of the beam. So as the atomic number increases the greater the number of electrons that are backscattered. The emergence of the backscattered electrons from the specimen surface also excite secondary electrons that are referred to as **SE2** type electrons. **BSE** give depth infomation and atomic number contrast within the image.
3. X-rays characteristic of the elements in the specimen are generated in an excitation volume in the order of a cubic micometre below the surface at the point of impact of the beam hence “microanalysis”.
4. Cathodoluminescence **CL** is light generated by some specimens that contain electro luminescent material. It is generated from a similar volume as that of X-rays.
5. Specimen current **SC** is the electron flow out of the specimen to earth and if an amplifier is placed in the return path of electrons to earth its value can be measured and also used for imaging.

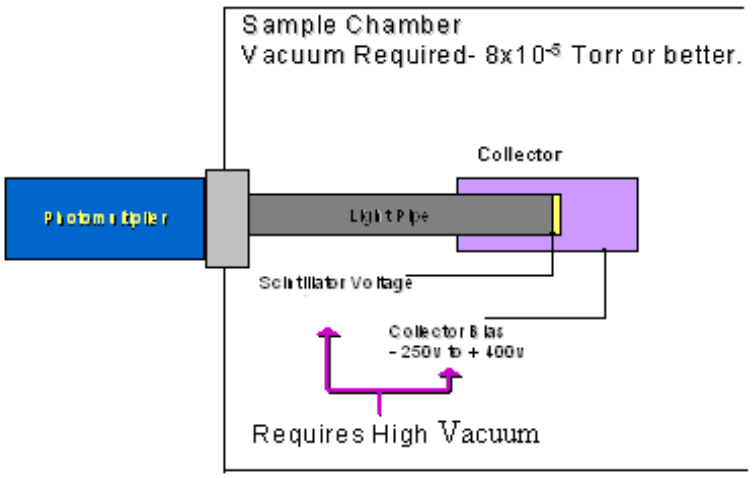
6. Transmitted electrons **TE** can be detected provided that the sample is exceptionally thin.
7. Auger electrons are low energy electrons produced in the ionisation of an atom and are characteristic of the atom.



The depth of penetration of the electron beam into the specimen will depend on the energy of the beam and the mean density of the specimen at the point of impact of the beam.

6. Detectors

Everhart – Thornley type Secondary Electron Detector



Secondary electrons are attracted by the positive voltage (up to + 400V) applied to the collector. They are then attracted to a phosphor (scintillator) biased at a high voltage (+ 8kV), the electrons then have sufficient energy to cause the phosphor to emit light. This light is totally and internally reflected through a glass light pipe into a photomultiplier.

The photomultiplier converts the light into an electrical signal and amplifies the signal so that it can be used to form an image of the surface of the specimen as if viewing it from above.

The analogy with the light microscope is that the gun is the eyepiece and the detector is the illumination.

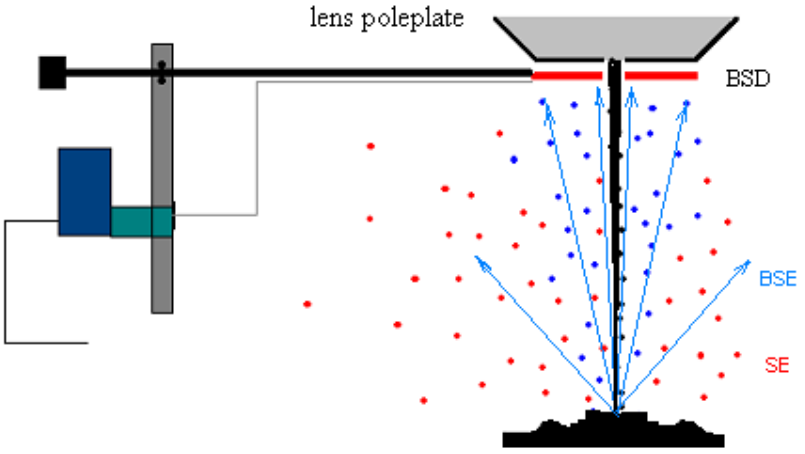
The collector can also be biased to a negative voltage (down to – 250V). This will reject all **SE** type electrons from entering the detector. However a small solid angle of **BSE** electrons will strike the phosphor and the signal produced can be used to image the specimen as if illuminating it from the side.

Backscatter Detector

There are two types of detector, either a solid state **4Q-BSD** or a **Scintillator** type. The detector can be a retractable or a fixed position type and is normally positioned beneath the pole-plate of the final lens. In this position it will collect the maximum amount of backscattered electrons provided that the specimen is flat and not tilted.

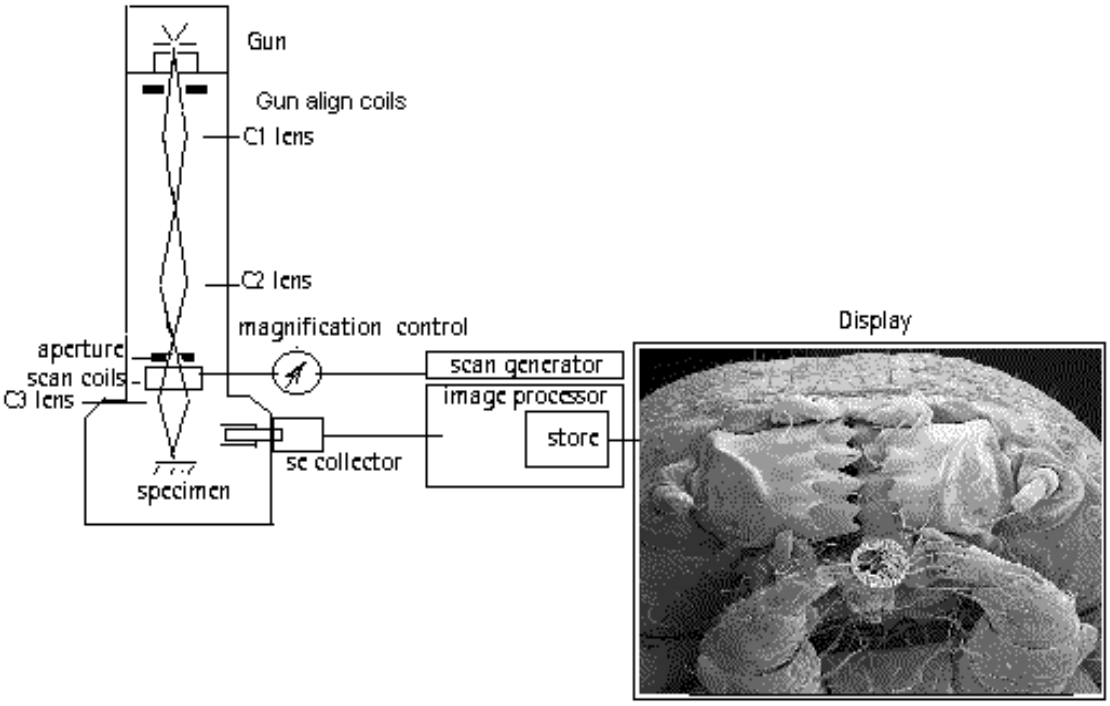
The **4Q-BSD,** when in the pole-piece position, has **4** individual elements (diodes) which are symmetrically placed around the electron beam and is usually configured so that the signals from the diodes are added together to maximise the atomic number contrast effect within the image of the specimen.Thus the image displayed will be dark for low atomic number elements and bright for high atomic number elements. It can also be easily configured, by altering the polarity of the diodes, to give a topographical image of the specimen.

The **Scintillator** type gives an image which combines the atomic number contrast with the topography of the specimen. It also has a better frequency response than the **4Q-BSD** thus enabling the beam to be scanned faster over the specimen surface.



7. How it works

The basic operating principle of an SEM is shown in the diagram below followed by a brief description.



Description

The specimen to be viewed has to be suitably adhered to a stub or clamped into a holder. A frequently used stub has a diameter of 12.5 mm which is clamped either in a single or multi-stub holder.

The operator should consult the specimen preparation guidance (next section) and operating parameters (section 3.2) if unfamiliar with an SEM, as obtaining good images is highly dependent on both.

To facilitate the fitting of the specimen or specimens, the specimen stage is retractable from the specimen chamber and before imaging can start it is necessary to pump out the air from within the chamber and column.

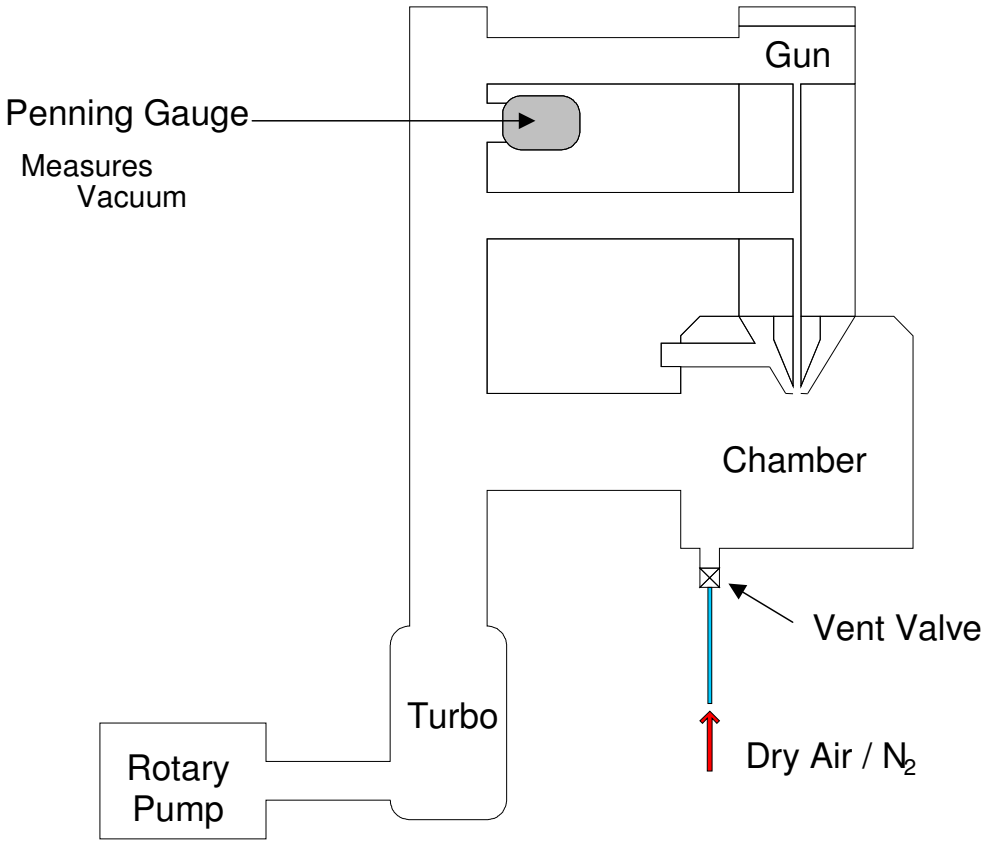
This will take a few minutes and when a sufficiently good vacuum in the column has been achieved the electron beam is switched on. The good or high vacuum in the column is required to prevent any electrical discharges occurring due to the high voltages that are used to generate the beam and to prevent the electron beam being diffused/absorbed by the gas molecules.

The stage on which specimens are mounted can be moved in a horizontal plane (X and Y), raised or lowered (Z), rotated and tilted so that each one in turn can be imaged from the most appropriate view.

Above the specimen is the electron optical column which, starting from the top, consists of:

1. An electron gun to generate a source of illumination called the electron beam.
2. A set of deflection coils to direct the electron beam straight down the column called the gun align coils.
3. Two electromagnetic lenses to focus the electron beam to a very small spot. These lenses are called condenser lens 1 and 2.
4. A final aperture to assist in defining the size and slenderness of the electron beam (similar in function to the variable aperture in a camera).
5. A set of stigmation coils to shape the beam so that it is circular in cross section (not shown in above diagram).
6. A set of scan coils to deflect the beam across the specimen surface in what is usually referred to as a raster scan, very similar to that of a TV. These coils are driven from a Scan Generator through a Magnification Control which controls the size of the area scanned on the specimen surface and hence the “magnification” of the image displayed on the TV monitor. The scan generator also controls the speed of scanning the beam, the slower the speed the less “noisy” or “cleaner” the image appears.
7. A third condenser lens, sometimes known as the objective lens, is used to focus the beam on to the specimen surface.
8. All three condenser lenses are under the control of a software program known as “**Optibeam**” that maximises the amount of electron current (probe current) in the beam as it is focused on the specimen surface. The amount of current is indicated by the parameter “Iprobe” that controls the “Spot Size”of the beam and greatly influences the resolution of the image.
9. A secondary electron collector within the specimen chamber is the detector that is normally used to produce the image of the surface of the specimen. As the beam is scanned across the specimen it attracts the low energy secondary electrons excited from its surface. The emission of secondary electrons from each point on the specimen surface is mainly dependent on its shape. Other signals are also excited from the specimen; each has its own detector and can be used to display informative images.

10. The signal from each point on the specimen surface having been collected by the appropriate detector is placed within the memory of an Image Store. This store resides within an Image Processor that reduces “noise” in the image in combination with the selection of beam scanning speed. The image displayed on the TV monitor is the output of the Store and is constantly being updated at TV frequencies. This enables changes to be seen very rapidly for example when the specimen is being moved to look for areas of interest. The image is “frozen” in the Store before it is saved/ exported to a printer or network device.
11. Beneath the specimen chamber is fitted a turbo molecular pump which removes the air to enable a high vacuum to be achieved in the column and the chamber. A rotary pump at the rear of the SEM then expels the air to atmosphere.



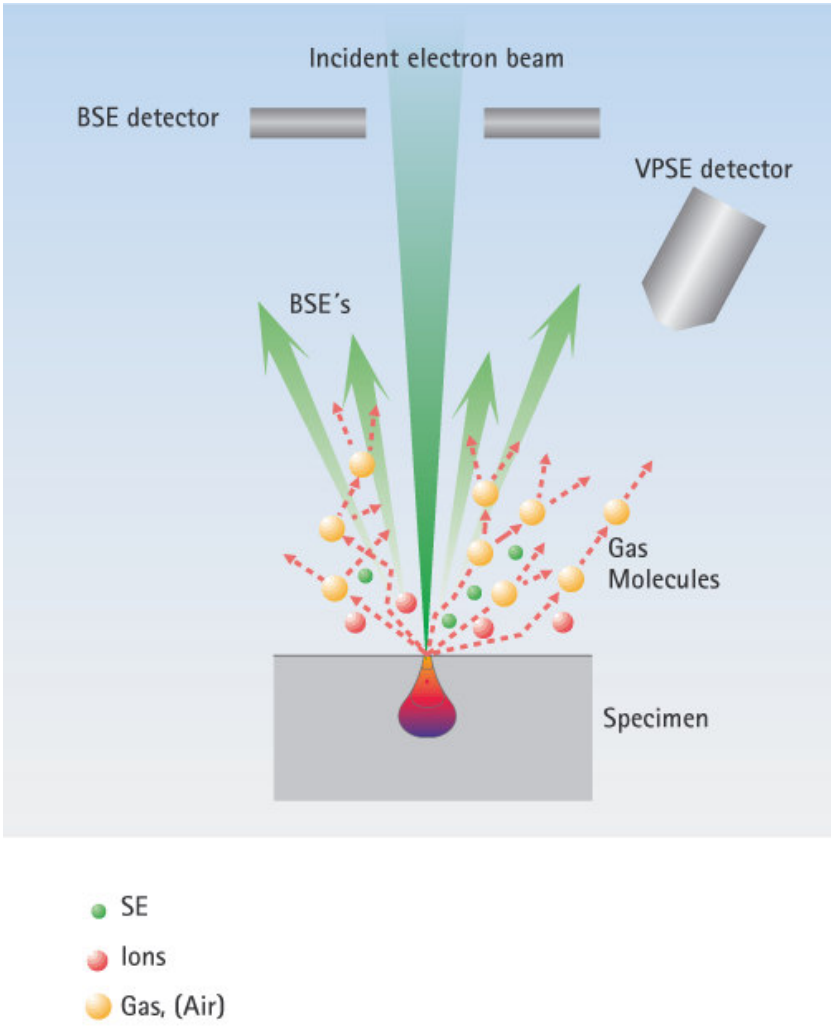
12. The specimen chamber pumping system can also be adapted to provide a controllable poor (low) vacuum within the chamber whilst maintaining the essential high vacuum within the column. The low vacuum is monitored with a Pirani gauge whilst high vacuum is monitored with a Penning gauge. This mode enables the viewing of electrically insulating and volatile specimens.

Variable Pressure Principle

When the Pressure Limiting Apertures are fitted (see Appendix 2 for sizes and vacuum range) a controllable amount of gas molecules, usually air, can be introduced into the specimen chamber whist maintaining a good vacuum in the electron optical column and the electron gun. Collisions between the high energy electrons of the beam and the gas molecules will cause the gas to ionise.The positive ions that are produced will neutralise the negative charge that has formed on the surface of an insulating material and will enable disturbance free images of the specimen to be obtained.

The secondary electrons (**SE**) passing through the gas will interact with the molecules and cause photons of light to be emitted. These photons can be collected by another detector (**VPSE** detector) to give an image of the surface of the specimen similar to that produced by the **Everhart Thornley SE** detector which cannot work in poor vacuum conditions.

The introduction of water into the chamber slows down the dehydration of specimens containing water and helps to maintain the shape of the specimen.



8. Specimen preparation guidance

Specimens which are studied in the SEM can be divided into two main categories, namely electrical **conductors** and **non-conductors**. Initial guidance has already been given in section 3.1 regarding factors for consideration before examination of specimens in the SEM.

Conductors

These fall into two groups:

1. Metallic: these are generally excellent conductors and need no preparation for surface topography examination. However for microstructural morphology studies and precise microanalysis it will be necessary to polish the specimen. Etching and electropolishing may also be a requirement.
2. Semi-conducting (semi-insulating) specimens be examined without special preparation and can be satisfactorily observed under high vacuum conditions.

Non-Conductors

This group can be further divided into non-volatiles and volatiles.

Non-Volatile

If it is not possible to obtain suitable resolution by using a low accelerating voltage and leaving the sample uncoated, then there two approaches to follow; either coat the specimen or use a Variable Pressure in the specimen chamber.

1. Coating the specimen.

For most non-conductors which contain no volatile components, eg water that would outgas in the vacuum system, it is sufficient to coat the sample with a thin layer of conducting medium such as Au, Au/Pd, etc. This layer is typically 20-30 nm in thickness and there are several reasons for coating:

a. Increased conductivity of the sample, thus minimising sample charge up, which results in deflection of the incident beam and severe degradation of the final image.
b. Increased mechanical stability of the sample due to increased heat conduction.
c. Increase in primary and secondary electron emission.
d. Decrease in beam penetration, resulting in better spatial resolution.

The two important current techniques of applying a coating are vacuum evaporation and ion sputtering.

Gold is generally used for the following reasons:

a. High secondary emission co-efficient
b. High conduction of electrons and heat
c. Does not oxidise
d. Good granularity of evaporated or sputtered particles

Carbon coating by evaporation is generally used if X-ray microanalysis is to be undertaken on the specimen unless, of course, the element under consideration happens to be carbon. Aluminium could be used in this case.

More recently Pt/Pd, Au/Pd and Cr have been used since their granularity is smaller. Aluminium can also be used, but it has low mechanical strength and can oxidise.

2. Variable Pressure

The specimen can be observed at high kV either with the VPSE, EPSE, and/or BSD detector. It will be necessary to adjust the chamber pressure to reduce beam charging effects. Under these conditions micoanalysis can be performed.

Volatile

Biological and botanical samples, by their nature, require relatively more complex preparation procedures. The specimens generally fall into two main categories: hard and soft.

1. Hard samples (eg bone, teeth, wood).

   These, if necessary, can be washed to remove extraneous fluids such as blood and mucus, dried in air and coated in the normal way or left uncoated and observed in the VP mode or at low kV in high vacuum.

2. Soft samples

   Once a living system, be it a plant or animal, is removed from its life support system it begins to degrade and deform. The aim of the microscopist is to see the specimen in its most natural state and therefore a more specialised approach is required and this will depend to a large extent on the type of equipment available. As the specimen will probably contain large amounts of water as soon as it is placed in a conventional high vacuum SEM partial collapse and distortion will occur due to the vacuum environment.

For users of a high vacuum SEM

There are several approaches to preparation of the specimen that will initially require a Chemical Pre-Treatment.
This technique involves chemical fixation of the material to strengthen the tissue. There is a large range of chemicals used in this process (eg glutaraldehyde and osmium textroxide) and there are numerous publications discussing the benefits of each. After fixation, it is necessary to replace the water in the sample by a solvent to aid drying. The method must be such that the specimen suffers no physical change. The most common drying agent used is a series of ethanol/water mixtures through to 100% ethanol. Having replaced the water present in the sample there is a choice of two methods for drying:

Critical Point Drying

The critical point has been defined as “that point at which the liquid, owing to expansion, and the gas, owing to compression, acquire the same specific gravity, and consequently mix with each other.”

The specimen is dehydrated as previously described and the solvent replaced with a liquefied gas,usually CO_2 in a small pressure vessel. The vessel is then heated to above the critical temperature of the selected gas. Under these conditions the liquid and vapour phases have the

sample physical properties so that on venting the liquid vapourises across cell boundaries and therefore minimum sample distortion occurs.

Freeze Drying

The sample is quench-frozen and maintained at low temperatures (about -130°C) until the sublimation process is complete.
The advantage of freeze drying over critical point drying is that it can be done from water. It is not necessary to treat the sample with organic solvents that might attack membranes or surface materials soluble in non-polar solvents. There is an advantage in freeze drying when X-ray analysis of ions such as calcium, potassium, sodium, etc is to be done since they will be dried in place, hopefully undisturbed by fixation and substitution of cryoprotectant.

Freeze drying involves the following steps: the sample is frozen in liquid nitrogen (N_2) or Freon 22 chilled by N_2; some specimens require the use of a cryoprotectant to avoid ice crystal damage while others (eg a leaf) do not. The frozen sample is transferred onto a cold stage in a vacuum chamber; drying occurs by sublimation with pressures less than 10^{-3} torr and at temperatures less than -70C. The drying process may take several hours (for cells in suspension) to several days (for leaf samples) depending on the size of the sample.
Most specimens must be treated with a cryoprotectant such as 16% glycerol in water or saturated chloroform water. This is necessary to avoid ice crystal damage that could poke holes in the surface of the sample. For the scanning electron microscope, chloroform water is better than glycerol water since after drying, glycerol is left on the "dry" surface of the sample, whereas chloroform water evaporates during drying.

Low Temperature Scanning Microscopy

This is the closest approach to the goal of examining fresh, untreated, biological material. It requires specialised equipment but once up and running it is a very fast technique.
A considerable amount of effort has been devoted to establishing the most suitable way to perform the freezing operation . If freezing is carried out too slowly, or if frozen material is kept at temperatures above about -80°C, large ice crystals are formed which cause structural damage to the specimen and provide artificial structures (artefacts) in the micrographs. Glycerol and other anti-freezing agents ("cryoprotectants") are often introduced into specimens to inhibit the process of crystallisation. Increasing the rate of freezing results in smaller ice crystals being formed, but the idea is to cool the water so rapidly that it remains in an amorphous vitreous (glass-like) state. For this to occur it is postulated that specimen cooling rates must be in the range 10^5-10^6 degrees per second. Since biological materials are, in general, poor conductors of heat it is obvious that the outside of a specimen will cool much faster than the interior, and a frozen specimen will have only a limited thickness for which the cooling rate is sufficiently high for vitreous ice to have formed; beyond this the specimen may be unusable because of crystalline ice formation. The vitreous region is estimated to be less than 5µm in thickness.

General Considerations

There is not one major preparative technique for biological/botanical samples. Where possible, several combinations should be tried for a particular type of sample, giving prime consideration to the information sought. Once a technique has been established, instrument parameters and specimen coating methods must be carefully considered.

The methods described above for soft tissue preparation are mainly for secondary electron imaging. The problems facing the biologist or botanist who wishes to undertake X-ray microanalysis are different in that the requirements in this case are to maintain the element(s) of interest in their original position in the sample.

For users of a Variable Pressure SEM with EP capability

To study hydrated specimens it is recommended that a Peltier cooling stage and a water control system be fitted to the SEM. This will enable fresh specimens to be examined with little or no loss of water in the SEM environment (the sample is kept fully hydrated during the pump down).

Section 5. Appendices

1. An introduction to Macros

A macro is a series of stored sequential instructions written and stored by an operator in order to assist in performing routine tasks.
It is recommended that the operator become familiar with the basic operation of the macro manager, including the different types of macro entry and how to insert and edit them. This information is available in the on-line help and will not be covered in this section.
This section will take the form of a tutorial that will develop macros to emulate normal microscope operation. Therefore there will be no straightforward progression from basics to advanced topics, but it should contain some useful real world insights into the process of macro writing that are used in the operation of the SEM.
Before starting to write a macro it is a good idea to be clear about what you are trying to achieve. In this case the objective is to create a macro to exercise various subsystems and components of the SEM, specifically to:

- Cycle the vacuum by venting and pumping
- Drive the EHT set between different voltages and perform filament ramp up and down operations
- Move the stage in X and Y axes
- Toggle detectors
- Exercise the EO board in terms of lens and coil supplies and mag range relays.
- Record images to prove the success of the above operations

Structure

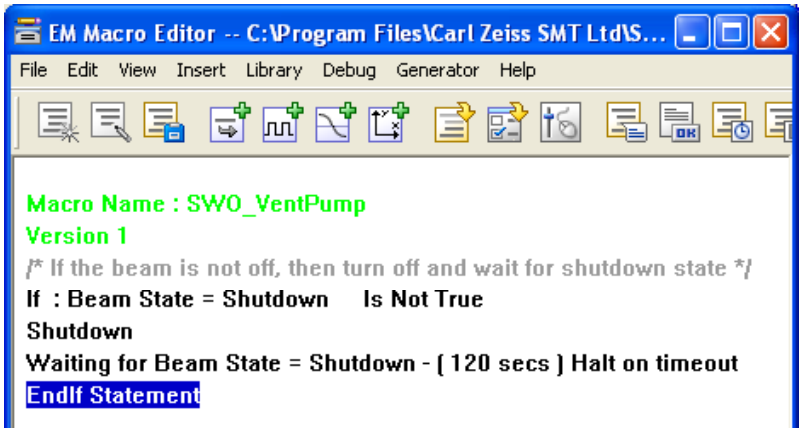
For simplicity a set of macros rather than just a single macro have been written. There will be one macro that calls a series of other macros that each perform a simple operation. Macros will be stored as files rather than in the macro library to make everything more portable. Component macros will be written first before moving on to the top level ones.

Component Macros

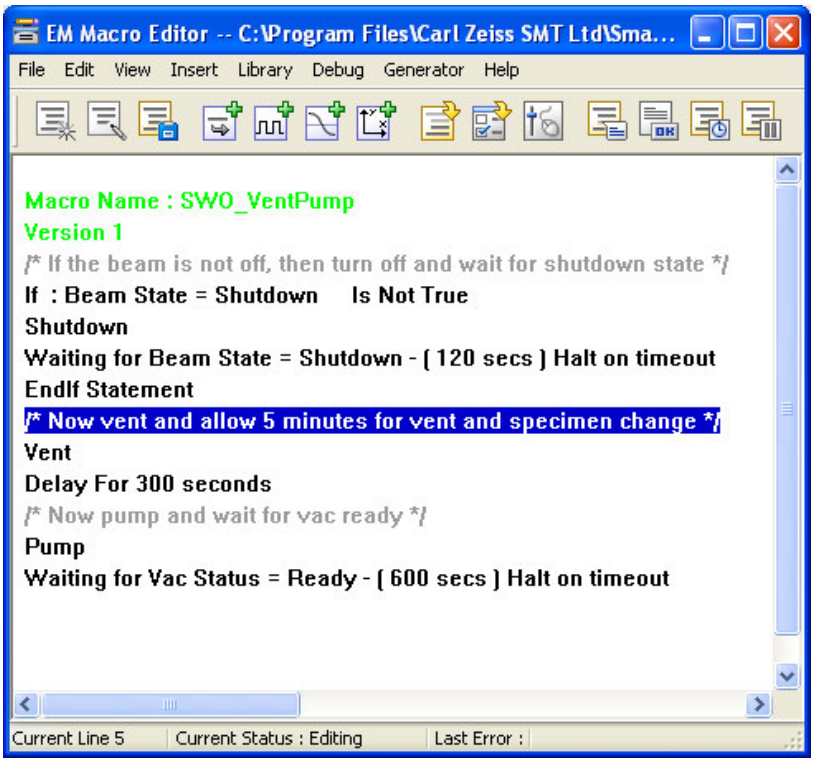
Vacuum Cycle

When starting a macro it is important to remember that the status of the instrument is unknown, therefore rather than making assumptions about the state the machine is in we need to find out.

Because the first operation we want to do is vent the chamber, we need to ensure the gun is off. So for this operation we use a decision structure with an **IF**
THEN clause followed by a **Wait For** operation on the same state.

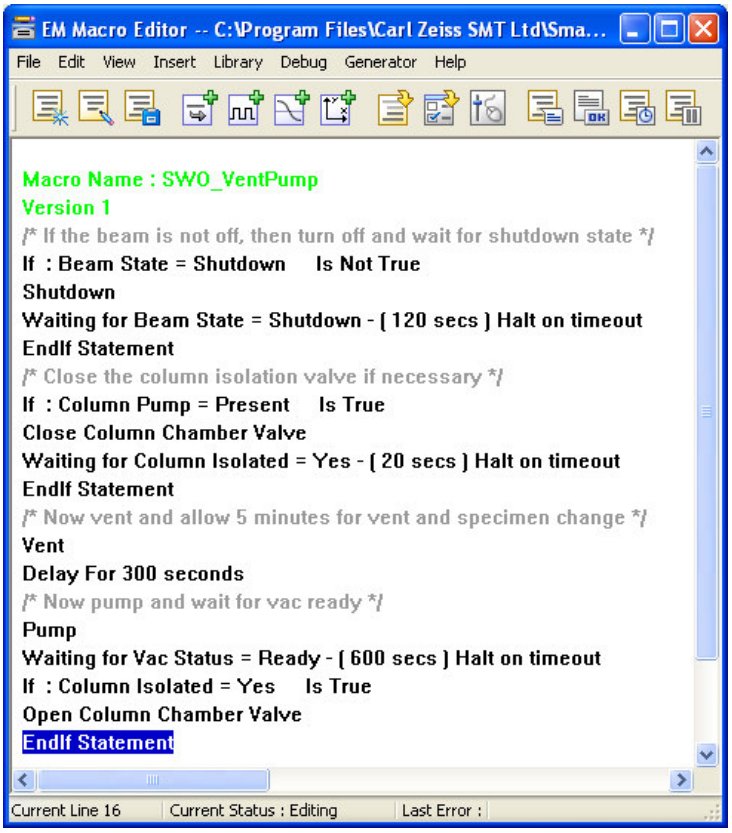


We can now proceed to vent and this time we will wait for a fixed amount of time.



This would appear to complete this simple macro, however there is one enhancement that we should consider. If the system is column pumped, then it would be sensible to close the isolation valve before pumping and open it again at the end.

Thus the full macro is as follows:



Stage Movement

The objective of the stage macro is to drive the stage in a square from its current position and leave it at the same point it started. This sounds simple enough but there are some potential hazards.

1**. Stage not initialised**
Clearly if this is the case then there is little the macro can do. Therefore the best way to handle this situation is in the top level macro when the whole test is started. If the stage isn't initialised, then the user will be prompted and asked if they wish to proceed. Therefore all that is required in this macro is a check and an exit if the stage is not initialised.

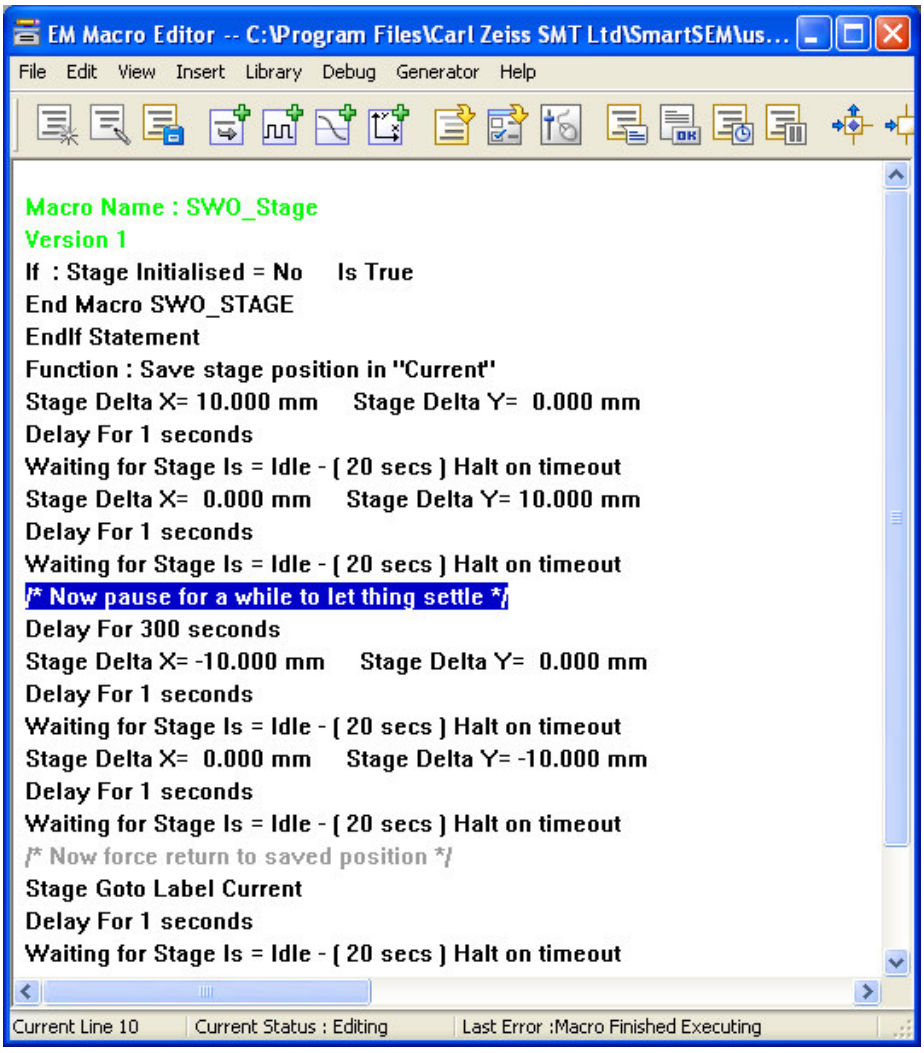
2**. Stage too near limits**
The problem here is whether the stage should be driven with absolute or relative moves. If relative moves are used, then depending on where the stage is on starting, the stage limits may come into play, which means that the stage won't travel the intended distance and might not return to the correct location.

If absolute moves are used, then returning to the original location is a problem.
Oddly enough both these problems can be solved by storing the current stage position on entry to the macro and then moving to that named position at the end.

3. **Detecting stage idle state**

This is one of the most tricky operations to do with macro. The problem is that the stage state that indicates **Stage is Busy** doesn't update immediately a command is issued to the stage. For this reason simply sending a command to move the stage and then waiting for **Stage is Idle** isn't adequate as the stage might not have gone busy yet. The situation is further complicated by the fact that for short moves there may not be a transition to a busy state at all.
The preferred solution to this problem is to wait for a fixed time (usually 1 second) before waiting for **Stage is Idle**.

Taking these points into account the following stage macro can be generated:



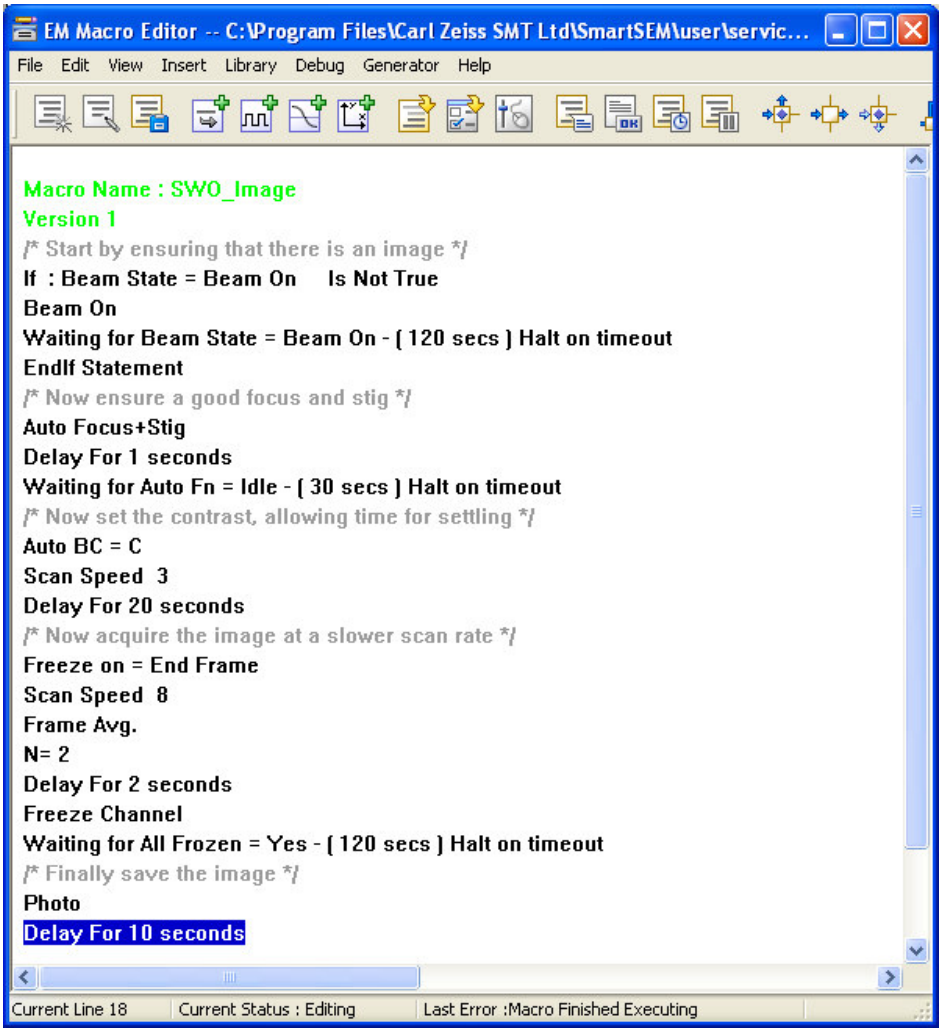
Note that the stage moves are performed using the stage delta X/Y parameter pair. If we only tried to move using X then whatever was last used in Y would also be applied. This macro would work equally well if absolute stage positions were used.

Image Save

The objective of this macro is to save an image to the hard drive in TIF format. This is easily accomplished using the **Photo** command, after setting the **Output Device** to **Display / File**. The more difficult aspect is getting an acceptable image in the first place. The sequence of operations here will be:

1. Ensure gun is run up
2. Focus the image and adjust stigmation
3. Adjust the brightness / contrast of the image
4. Scan a frame at slow scan rate and freeze the image on completion
5. Save to a file
6. Restore normal scanning conditions

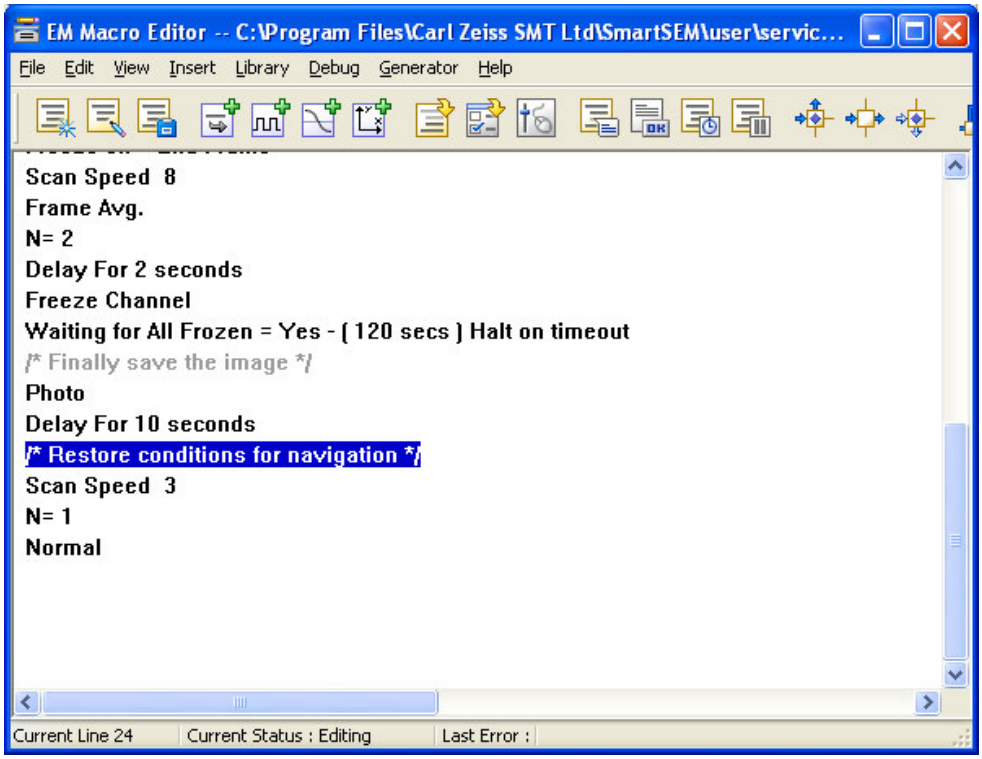
The following macro is therefore defined:



There is a similar issue to detecting the completion of the auto focus function as there is when detecting stage idle. This macro does make a number of assumptions that we should be aware of:

- A suitable detector is selected
- The probe current is at a reasonable value
- There are enough features on the sample to permit auto focus and stig to work
- The filament has already been saturated.

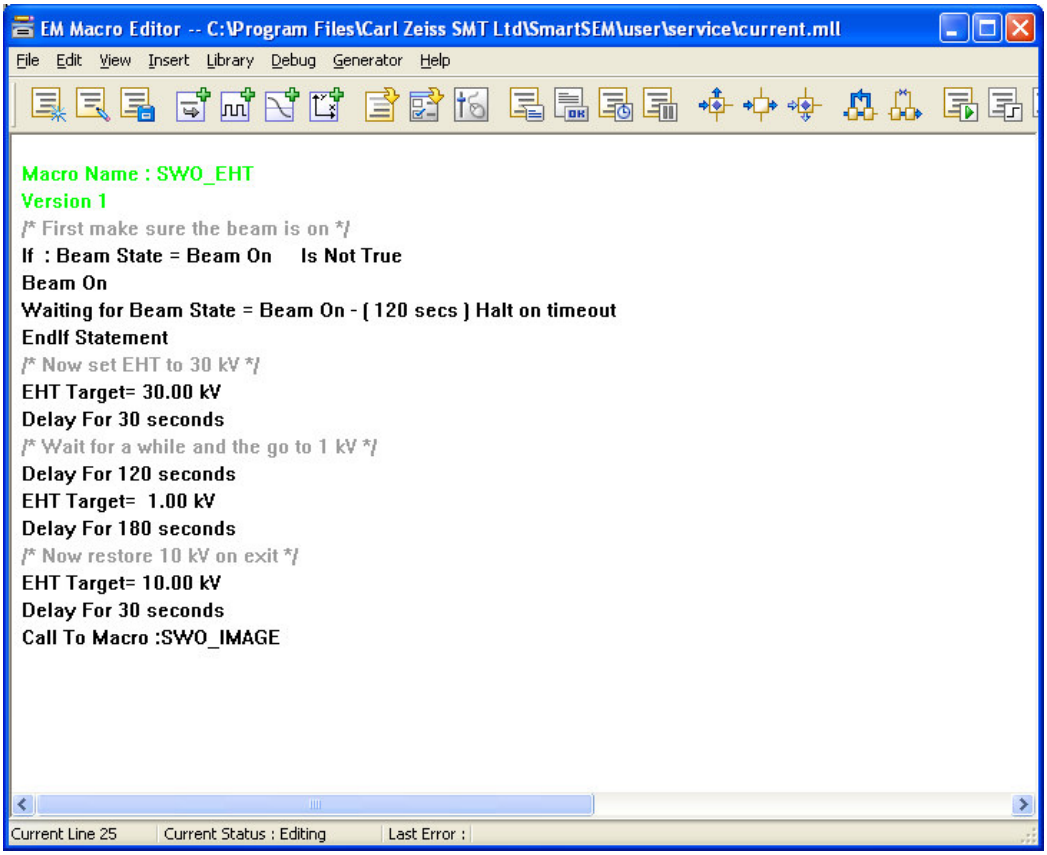
The delay following the photo command is required to ensure that the image save operation has completed before continuing.
It is good practice to unfreeze the image again before leaving the macro, so we will set up some conditions suitable for navigation.



Note that only the end part of the macro is shown here.

EHT Change

The macro to exercise the EHT set will make use of the image macro so that it is possible to change the EHT, save and image and then change the EHT again. This will effectively add a third level of nesting within the overall macro.

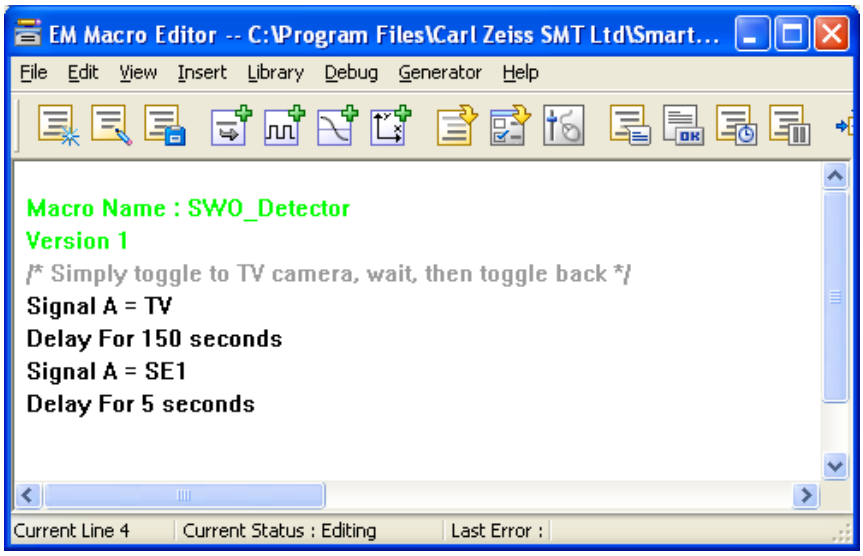


There are a couple of points to note about this macro. Firstly the delay following the setting of the EHT target is to allow time for the voltage to ramp to its new level. Unfortunately there is no mechanism to show when EHT ramping is complete, so the only option is to delay for long enough to let the ramping finish.

The other point of interest is the first example of calling a macro from within a macro. The SWO_Image macro is called at the end of this sequence. This is just like inserting the whole macro at this point. Macros can be nested to any depth, but of course recursive macros are not allowed (recursion occurs when A calls B and B calls A, or A calls A, etc.).

Detector Toggle

The objective of this macro is to toggle between different detectors to check that the relays are working properly. The difficulty here is that different machines will be configured with different detectors. Under normal circumstances this doesn't present a problem as a macro will be written for a known instrument. However for this application the solution will be to use detectors that are present on all machines, the SE1 detector and the TV camera.



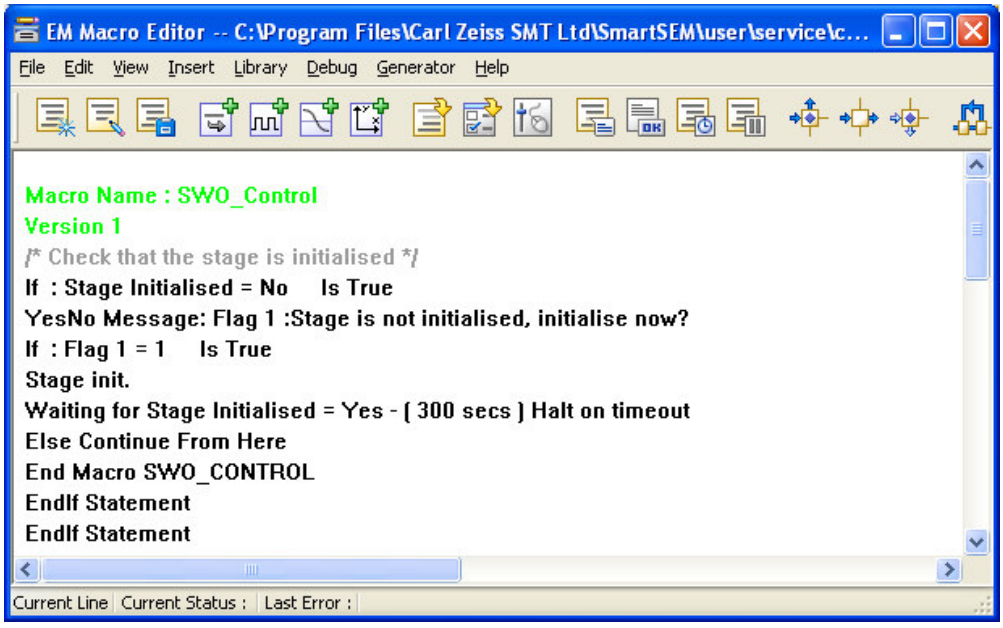
Signal A is the correct parameter to use to select the detector. The final delay is to allow time for the electronics to settle before the next operation takes place (whatever that is).

Top Level Macro

It is now possible to write the macro that calls all these others. Before the other macros can be called, the SEM must be put into a well defined state. This will be done with the user's cooperation.

Initialising the Stage

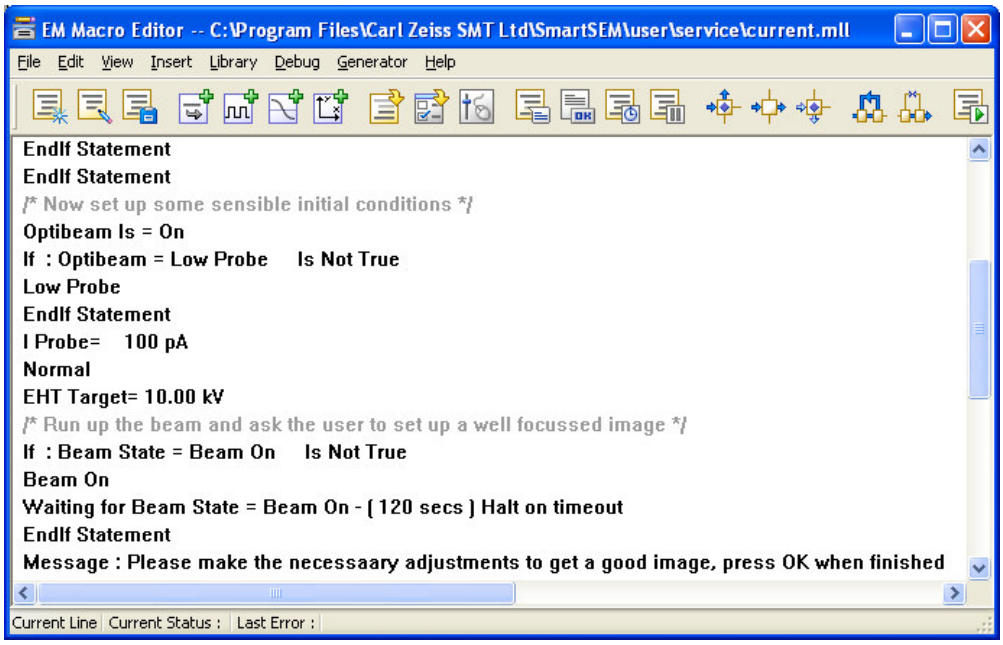
The first step is to check that the stage is initialised. Unlike the stage macro, this time we will not halt if the stage is not initialised, instead we will initialise the stage if the user agrees:



The **YesNo Message** line is a *Display Message* entry with a yes no check box selected. This allows a flag parameter to be selected for the response, 0 = No, 1 = Yes. This is also an example of nested IF statements, the ELSE clause applies to the most recent IF.

Setting Conditions

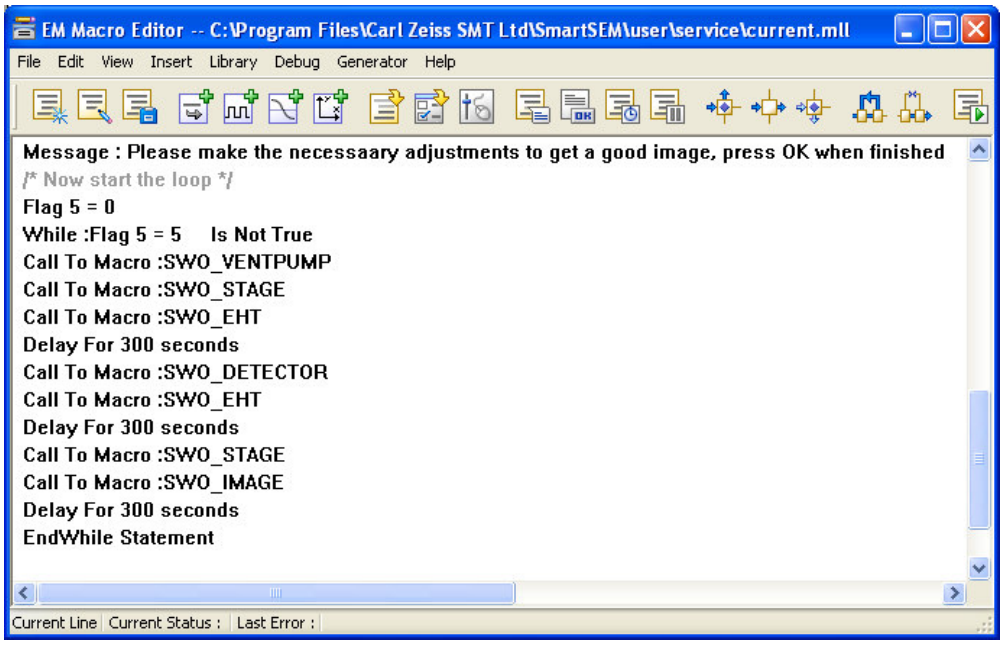
Having initialised the stage we can then set up some other parameters:



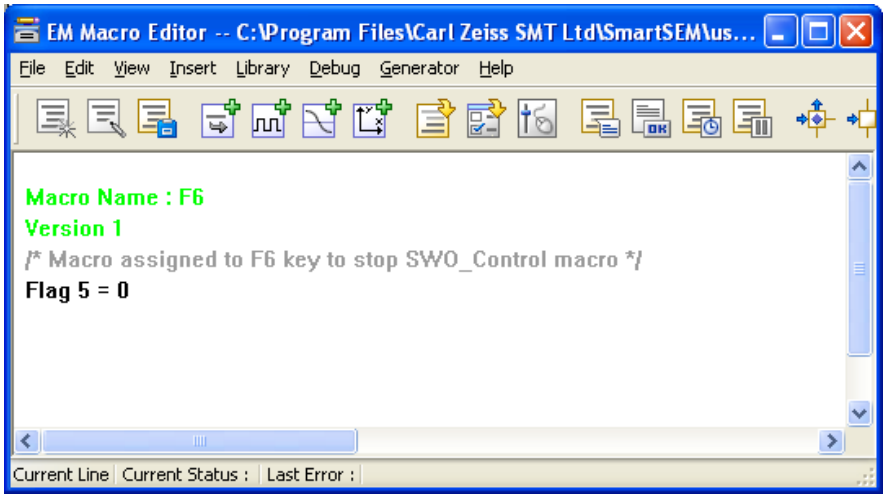
The command to put Optibeam into Low Probe mode is protected by the IF statement because if Optibeam is already in that mode, then the command will be disabled and an attempt to execute it will stop the macro.
An alternative approach to setting up the microscope would be to load a set of saved conditions. This has not been chosen as this would introduce a dependency on the detector configuration that is to be avoided in order to keep these macros general.
The message command allows the user to complete the set up of the imaging conditions by adjusting any necessary parameters. The macro will only continue when the user clicks the OK button.

The Macro Loop

Finally we can enter the main loop of the macro which will continue until stopped by the user. This is remarkably simple as all the hard work has been done by the other macros.



The body of the loop simply consists of calls to the macros we have already defined. The delays are intended to make the macro emulate normal operation to some extent.
Flag 5 is used for controlling the loop to provide an easy termination mechanism. Simply changing flag 5 to a different value will stop this macro at the end of the loop. This could be done by a macro assigned to a function key such as:



Admittedly in this particular instance the macro could take up to 50 minutes to terminate after pressing this key, but it is a useful technique for stopping a macro. In the case of the SWO_Control macro, it is best stopped using the macro editor command, as it will be run from within the editor itself.

Conclusion

The macros described in this section are a demonstration of various techniques that may be more or less obvious to control the SEM. However they are intended to encourage SmartSEM users to produce macros that will assist them in their work.

MA: Charge compensation

SEM	Application	Hardware	* Min/Max pressure	Filament
MA 10 MA 15 MA 25	Charge compensation	 VP aperture	10-400 Pa	W
		 VP aperture	10-273 Pa	LaB6
	Charge compensation + Improved image quality + High accuracy EDS analysis	 +  EP aperture 500 µm	10-400 Pa	W + LaB6

* Both “air” and “water vapour” can be introduced, as a charge compensating gas, into the chamber.

Extended Pressure MA: Dynamic processes

SEM	Application	Hardware	* Min/Max pressure	Filament
MA 10 MA 15 MA 25	Hydrated Specimen Imaging	 EP aperture +  500 µm or  500 µm	10 – 3000 Pa	W and LaB_6

* Both “air” and “water vapour” can be introduced, as a charge compensating gas, into the chamber.

LS: Charge compensation

SEM	Application	Hardware	* Min/Max pressure	Filament
LS 10 LS 15 LS 25	Charge compensation	 VP aperture	10-400 Pa	W
		 VP aperture	10-273 Pa	LaB6
	Charge compensation + Improved image quality + High accuracy EDS analysis	 +  EP aperture 500 µm	10-400 Pa	W + LaB6

* Both “air” and “water vapour” can be introduced, as a charge compensating gas, into the chamber.

LS: Hydrated specimen imaging

SEM	Application	Hardware	* Min/Max pressure	Filament
LS 10 LS 15 LS 25	Hydrated Specimen Imaging	 EP aperture +  500 µm or  500 µm	10 – 3000 Pa	W and LaB_6

* Both “air” and “water vapour” can be introduced, as a charge compensating gas, into the chamber.

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