



Instruction Manual

ZEISS MultiSEM 505/506 Series



Scanning Electron Microscope

We make it visible.

Instruction Manual ZEISS MultiSEM 505/506 Series

ZEISS MultiSEM
Original Instructions

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About this Instruction Manual

1 About this Instruction Manual

1.1 Introduction

Welcome Welcome to the ZEISS MultiSEM Instruction Manual.

ZEISS MultiSEM is the multi-beam scanning electron microscope from ZEISS.

Its multi-beam design and the intuitive workflow-oriented user interface allow you to scan large specimen areas much faster than with conventional scanning electron microscopes.

Content This Instruction Manual contains all the information you need to start the ZEISS MultiSEM system as well as to start ZEN MultiSEM.

This Instruction Manual is part of the ZEISS MultiSEM multi-beam scanning electron microscope, hereinafter referred to as "microscope". Read the instructions carefully.

This Instruction Manual is designed for operators who have been trained to operate the microscope by a ZEISS service representative. Basic operator training and safety instructions will be provided within the scope of initial start up by ZEISS. Operators of the microscope must not deviate from the instructions provided in this Instruction Manual.

The Instruction Manual contains the following chapters:

Chapter	Content
About this Instruction Manual	Introduction and overview of the documentation
Safety	Important safety information and legal information about the appropriate usage of ZEISS MultiSEM Read these instructions before working with ZEISS MultiSEM
Device Description	Overview of the ZEISS MultiSEM microscope and its main hardware components
Overview of the Software	Basic concepts including an overview of the ZEN MultiSEM user interface
Principle of Operation	Basic principles of ZEISS MultiSEM and a brief description about the main electron-optical components inside the microscope
Powering down/up the Microscope	Instructions for powering down/up ZEISS MultiSEM into/from standby

Chapter	Content
Handling of the Specimen	Information about physical constraints of a specimen and about mounting it onto a specimen holder.
Alignment	Information about aligning ZEISS MultiSEM with the help of a specially designed calibration pattern
Specimen Registration	Information about loading/unloading the specimen and the registration of the specimen
Imaging Workflow	Information about defining the scan plan and executing the experiment
Maintenance and Disposal	Instructions for ensuring optimum operation of ZEISS MultiSEM
Technical Data and Conformity	Hardware specifications of the components, conformity, and regulations
Index	List of keywords to help you find relevant information quickly

The figures in this document are for illustration purposes only. The appearance of your hardware components may vary slightly from that shown in this document.

Electronic Manual The Electronic Manual contains a complete guide to using the software as well as additional background concepts and descriptions.



You can start the Electronic Manual at any time by clicking the **Help** icon or by pressing **F1**. The Electronic Manual opens at the appropriate section that is relevant to your current workflow.

It also contains an index and full-text search to help you find the desired information quickly and easily.

Reference to Related Documents For technical data, refer to the documents Product Information and Installation Requirements. For details on the pre-vacuum components, refer to the corresponding manual delivered with the ZEISS MultiSEM.

1.2 Conventions in this Document

Text Conventions The following text conventions are used in this document:

Example	Description
Save icon	Clickable user interface elements, e.g. buttons and icons
ON button	Hardware buttons on the microscope

Example	Description
Image panel	Non-clickable user interface elements, e.g. name of a dialog box
Input text	Text to be entered by the user
Link ▶ 12]	Link to further information

Info and Tip Additional information and tips are indicated as follows:

INFO

Helpful additional information, e.g. about necessary additional actions.

TIP

Useful information, e.g. about alternative methods for achieving an aim.



Safety

2 Safety

2.1 Intended Use

ZEISS MultiSEM is to be used in a laboratory environment for scientific and/or commercial purposes.

ZEISS MultiSEM is designed for large-area microscopic examinations in the following applications:

- Neuroscience and life sciences
- Nanotoxicology
- Semiconductor research and development
- Materials Science
- Geosciences

INFO

ZEISS MultiSEM must not be used for therapeutic, treatment or medical diagnostic evidence. Not all products are available in every country. Contact your local ZEISS representative for more information.

INFO

The Instruction Manual is considered to be part of the microscope.

Users of the microscope must follow the instructions provided in this document.

Using the microscope for any purpose other than described is not allowed and could be hazardous.

2.2 General Information

Comprehensive knowledge of the hardware and software is essential for the safe operation of the microscope.

Only authorized ZEISS service representatives must unpack, install, and initially start up the product.

INFO

The user of the microscope must observe the local regulations concerning X-ray protection. According to German law, the operation of this microscope in Germany is subject to an official individual authorization process. Technical modifications may result in forfeiture of the operational approval. Outside Germany, the user of the microscope has to comply with the local regulations of the country where the microscope is installed. The microscope is equipped with several radiation protection devices, which ensure that - under regular operation conditions - the microscope operates in compliance with the German X-ray protection regulation (RöV) as well as within the EC Directive 96/29/EURATOM.

INFO

The manufacturer assumes no liability for malfunctions or damage due to either improper use of the microscope or attributed to repair and other service work performed or attempted by unauthorized service staff. Such actions shall render all warranty claims invalid, including parts which were not directly affected by this action.

INFO

The operator must observe all relevant legal accident prevention regulations.

Since the system is predominantly controlled by a computer, you should be experienced with the Windows operating system. For more information, see the Windows operating system instructions supplied with the operating system.

2.3 Safety Procedures

2.3.1 Safe Operation Conditions

INFO

Only authorized ZEISS service representatives must unpack, install, and initially start up the product.

**WARNING****Risk of injury: Unsafe operation condition**

If the product safety labels are covered or worn or if any of the safety devices are not in proper working condition, operation of the microscope can be hazardous.

- ◆ Periodically check the function of safety equipment.
- ◆ Periodically check that all protective cover panels are installed.
- ◆ Please ask your ZEISS service representative if you have questions concerning the periodical checks. The periodical checks are also performed by ZEISS service staff during each preventive maintenance.
- ◆ Always follow the instructions given on the safety labels.
- ◆ Inspect and clean the product safety labels to maintain good legibility.

2.3.2 Requirements for Personnel

**WARNING****Risk of injury: Improper operation of the microscope**

Deviating from the instructions given in this Instruction Manual and on the safety labels can be hazardous.

- ◆ Do not operate the microscope until you have completely read and understood this Instruction Manual.
- ◆ Observe all safety labels on the microscope.
- ◆ Only ZEISS service representatives, who have specialized knowledge of radiation protection, are permitted to service the microscope.

Operator Training Within the scope of initial start-up, the ZEISS service representative will perform a basic operator training. This consists of fundamental operating procedures including safety instructions. Additionally, an introduction to basic maintenance tasks will be given for an advanced operator. This person should have reasonable overall knowledge of the system. The training performed shall be documented appropriately.

Special application trainings are offered upon request.

2.3.3 Preventive Maintenance



WARNING

Risk of injury: Improper maintenance

Deviating from the maintenance tasks described in this Instruction Manual can be hazardous.

- ◆ Only perform preventive maintenance tasks described in this Instruction Manual.
- ◆ All maintenance, service, and repair tasks not described in this Instruction Manual have to be performed by an authorized ZEISS service representative.

To ensure the optimal performance of the microscope, it is essential to perform preventive maintenance on a regular basis. Moreover, it is recommended that you conclude a service contract with your local ZEISS service representative or organization.

2.3.4 Safe Handling of Spare Parts



WARNING

Risk of injury: Spare parts

Using spare parts that are not provided by ZEISS can be hazardous.

- ◆ Only genuine parts supplied by ZEISS are to be used in servicing the microscope.
- ◆ Contact your ZEISS service representative for information regarding how to order spare parts.
- ◆ Unless authorized by ZEISS, all spare parts should be installed by a ZEISS service representative.

2.4 Prevention of Hazards

This section summarizes possible hazards and recommended safety procedures.

2.4.1 Burn Hazards

CAUTION

Risk of injury: Hot surfaces during bakeout

Parts of the enclosure in the upper range of the column may become hot during bakeout, particularly after a long bakeout cycle.

- ◆ Do not touch any parts of the column.
- ◆ Do not place any combustible objects on the electron-optical column.
- ◆ After the bakeout procedure, let surfaces cool down before working near the column.
- ◆ Only advanced operators are allowed to perform the bakeout procedure.

2.4.2 Chemical Hazards

WARNING

Risk of injury: Aggressive or toxic chemicals

Aggressive or toxic chemicals can cause chemical burns.

- ◆ When handling aggressive or toxic chemicals wear suitable protective clothing, gloves, and eye/face protection.
- ◆ Do not eat, drink, or smoke while handling toxic chemicals.
- ◆ Refer to local safety regulations.
- ◆ Read and follow the instructions in the material safety data sheet of the chemical. The material safety data sheet can be obtained from the supplier of the chemicals.

WARNING

Risk of injury: Disposal of aggressive or toxic chemicals

When disposing of aggressive or toxic chemicals, there is a risk of damage to the environment.

- ◆ When disposing of waste that has been generated during a service operation (e.g. used rotary pump oil) comply with all national and local safety and environmental protection regulations.

**WARNING****Risk of injury: Reaction products**

Dangerous reaction products might be present in the specimen chamber during or after operation.

- ◆ Wear gloves when touching the inner parts of the specimen chamber or the specimen.

2.4.3 Electrical Hazards

**DANGER****Danger to life: Hazardous voltage inside the microscope**

High-voltage potentials present in the microscope can be fatal. Contact may cause burn or electrical shock.

- ◆ Ensure proper grounding. For more information refer to the Installation Requirements document.
- ◆ Only authorized ZEISS service representatives are allowed to remove the microscope enclosure.

**CAUTION****Electrocution hazard: High residual current**

High residual currents can be present in the microscope.

- ◆ Ensure proper grounding. For more information refer to the Installation Requirements document.
- ◆ Do not operate the microscope without a separate ground connection.

NOTICE

The Schottky field emitter is susceptible to startup currents.

Risk of component damage.

- ◆ Avoid switching off the gun for periods of under two days to optimize the lifetime of the Schottky field emitter.

2.4.4 Mechanical Hazards



DANGER

Risk of injury: Heavy weight of the microscope

While the microscope is being moved, it could fall over. A person or body parts of a person could be trapped and crushed under the microscope. This will lead to serious injury or death.

- ◆ Use a forklift to remove the microscope from the wooden crate.
- ◆ Maintain a safe distance from the microscope while it is being moved.



DANGER

Risk of injury: Heavy weight of the microscope

While the microscope is being lowered body parts could be trapped and crushed under the microscope. This could lead to serious injury or death.

- ◆ Do not place your hands or feet under the microscope while it is being lowered.

For more information about the weight and the dimensions of the microscope, refer to the Installation Requirements.



WARNING

Risk of injury: Moving the specimen stage

Fingers could be trapped in the moving specimen stage.

- ◆ Always close the chamber door before you move the specimen stage.



CAUTION

Risk of injury: Closing the chamber door

Fingers could be trapped between microscope and chamber door.

- ◆ Use the recessed grip to close the chamber door.
- ◆ Do not trap your fingers in the chamber door gap.

NOTICE

Risk of property damage: Transporting the microscope

- ◆ The microscope may only be transported in air-suspended vehicles.
- ◆ Moving parts must be secured during transport to prevent them from slipping or tipping over.
- ◆ Avoid rocking the crates back and forth.
- ◆ Devices for transporting the microscope must be rated to handle its full weight and dimensions.
- ◆ Note the weight information on the package and on the shipping document.

NOTICE

Risk of damage to the objective lens or the specimen

The specimen may collide with the objective lens while raising the specimen stage.

- ◆ Enter the specimen height in ZEN MultiSEM.
For more information about setting the parameter **Specimen Height**, see Stage.
- ◆ Always move the specimen stage slowly and observe its movement using the chamber CCD camera.

NOTICE

Risk of damage to ZEISS MultiSEM or the specimen: Short working distance

The specimen or the specimen stage may collide with the objective lens if they are too close to each other (i.e. the working distance is too small) when opening the chamber door.

- ◆ Ensure the working distance is sufficiently large before opening the chamber door, refer to *Working Distance* [► 147].

2.4.5 High Pressure Hazards



Risk of injury: High pressure in gas cylinders

Compressed air is used to operate several valves and the auto levelling system. Gas cylinders containing nitrogen or compressed air have a high internal pressure of approximately 200 bar. If not properly handled, the gas can escape abruptly and cause the gas cylinder to move in an uncontrollable manner.

- ◆ Observe all safety labels on the gas cylinders and all safety instruction given by the gas cylinder manufacturer.
- ◆ Always operate gas cylinders in an upright position and secure them so they will not tip over.
- ◆ Before transporting gas cylinders, place protective caps on them.

2.4.6 Radiation Hazards



Radiation hazard: X-Rays

X-rays are generated inside the microscope during operation. This is unavoidable due to electrons accelerated to an energy of up to 52 keV.

- ◆ Only authorized ZEISS service representatives are allowed to service the microscope.
- ◆ Do not remove any parts around column and chamber that are essential for radiation protection.
- ◆ Only use genuine ZEISS parts.
- ◆ Observe all safety and X-ray protection regulations.

According to German law, the operation of this microscope in Germany is subject to an official individual authorization process. Technical modifications may result in forfeiture of the operational approval.

The user of the microscope has to comply with the local regulations of the country where the microscope is operated.

The microscope is equipped with several radiation protection devices, which, under regular operating conditions, ensure that the microscope operates in accordance with the German X-ray protection regulation (RöV), the German radiation protection regulation (StrSchV) as well as with the EC Directive 96/29/ EURATOM.

Radiation Protection

For questions regarding radiation protection, contact the ZEISS Radiation Safety Officer, Carl Zeiss AG, 73447 Oberkochen, Germany

phone: +49 (0) 7364 20 0

2.4.7 Suffocation Hazards



Suffocation hazard: Lack of oxygen

Gaseous dry nitrogen is used to vent the specimen chamber during specimen exchange. Inhaling nitrogen may cause unconsciousness.

- ◆ During specimen exchange, keep the chamber door open as short as possible.
- ◆ Ensure the area around the microscope is sufficiently ventilated.
- ◆ Avoid inhaling the air from within the specimen chamber.
- ◆ If you begin to experience symptoms of asphyxia (for example: rapid breathing, loss of mental alertness and/or muscular coordination, depression of sensations, emotional instability, fatigue) leave the room immediately and inform the facility's safety officer.

Concerning the hazards of nitrogen installations and associated safety precautions refer to guideline IGC Doc 44/09/E: Hazards of inert gases, published by EIGA (European Industrial Gases Association).

To download the document:

- 1 Go to EIGA homepage www.eiga.eu.
- 2 Select **Publications > EIGA Documents**.
- 3 From the list, select **Doc. 44/09**.
- 4 Click **Download**.

2.5 Safety Equipment

2.5.1 Safety Devices

In order to prevent any risk of hazard to human health or of property damage, ZEISS MultiSEM is equipped with several safety and protective devices.

2.5.1.1 Protective Cover Panels

The following parts of the ZEISS MultiSEM cabinet are equipped with protective cover panels:

- Illumination column
- Detection column
- Specimen chamber
- Plinth below the specimen chamber

2.5.1.2 Interlock System

The interlock system provides a chamber door interlock and a vacuum interlock.

Chamber Door Interlock The chamber door interlock is located at the inner bottom front side of the specimen chamber. It ensures that the door of the specimen chamber is closed properly.

When this interlock is activated (i.e. no electrical contact), the EHT (U_{Tip}) and the specimen voltage ($U_{specimen}$) are blocked.



Fig. 2.1: Chamber door interlock

Vacuum Interlock The vacuum interlock is an internal interlock. It ensures that the gun pressure (i.e. the vacuum in the electron gun section) and the system vacuum exceed the required thresholds.

2.5.1.3 Main Switch

The main switch is essential for compliance with the SEMI standard.



Fig. 2.2: Main switch

The main switch is located at the front of the power supply rack. It cuts off all three phases of the main power supply. It has an emergency off function and can be secured against being switched on again.

2.5.1.4 EMO Button

The Emergency Off button (EMO button) is located on the right side of the microscope cabinet.

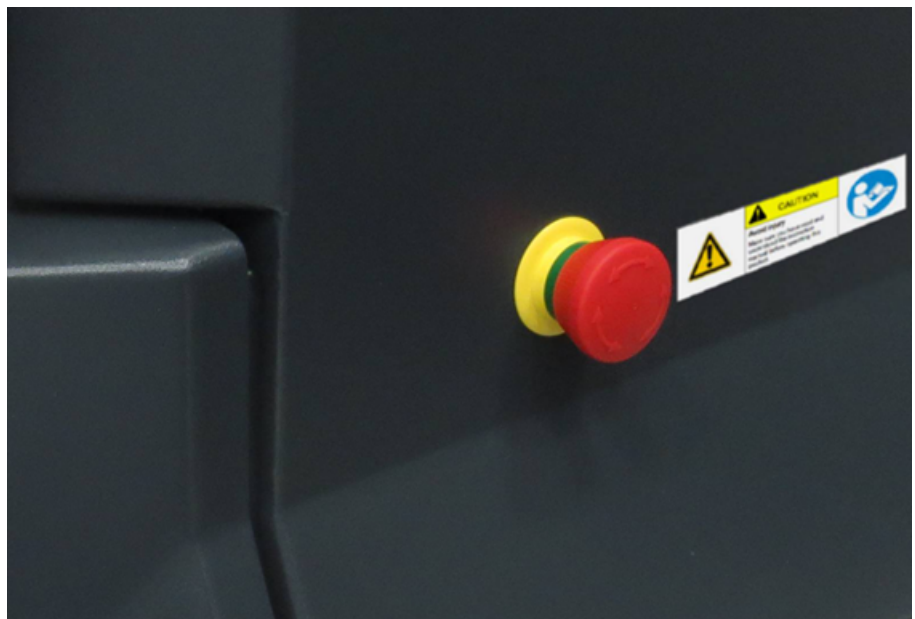


Fig. 2.3: EMO button

The EMO button is to be pressed in case of an emergency to cut off the power supply to all devices connected to the EMO box, except the image acquisition unit. It must always be freely accessible and operable.

2.5.1.5 POWER ON Button (EMO Function)

The **POWER ON** button is located at the front of the power supply rack below the main switch and to the right of the operation mode buttons. It is required to reset the EMO function.



Fig. 2.4: POWER ON button

2.5.1.6 Main Shut-off Valves

You are responsible for the installation of main shut-off valves at the site of installation.

Main shut-off valves are required for the following auxiliary supplies:

- Water supply
- Water runback
- Nitrogen supply
- Compressed air supply

The main shut-off valves have to be easily accessible. They must cut off the connections to the corresponding media when needed. The main shut-off valves have to be lockable in their OFF-position in order to prevent accidental reactivation.

As you are responsible for installing the main shut-off valves, you should also provide instructions about how to operate the main shut-off valves properly.

2.5.2 Safety Labels and Labels

Safety labels are placed on the ZEISS MultiSEM microscope cabinet to warn users of hazards. Each safety label is placed close to spots where particular hazards could occur.

Moreover, you will find several labels which provide legal information.

2.5.2.1 At the Microscope Cabinet

Chamber Door



Fig. 2.5: Safety labels on the chamber door

1	 WARNING Risk of injury Fingers could be trapped. Always close the chamber door before you move the stage.
2	 CAUTION Radiation hazard X-rays are generated inside the electron microscope during operation. Do not remove any parts. Use genuine ZEISS parts exclusively. Observe local safety and X-ray protection regulations.
3	 CAUTION Suffocation hazard The specimen chamber is ventilated with gaseous nitrogen. Ensure that the area around the electron microscope is sufficiently ventilated.
4	 CAUTION Risk of damage The FESEM or specimen could be damaged if specimen stage is at a short working distance. Move the specimen stage to a long working distance before opening the chamber door.



Right Side of the Cabinet



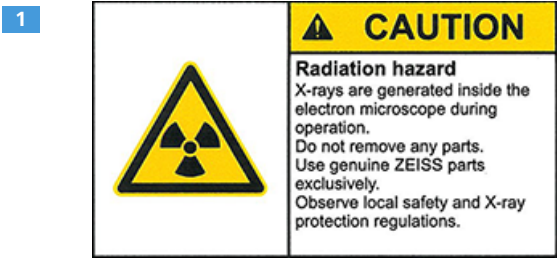
Fig. 2.6: Safety label on the right side of the microscope cabinet



Back Side of the Cabinet



Fig. 2.7: Safety label on the back side of the microscope cabinet



2.5.2.2 At the Power Supply Rack

Main Switch Area



Fig. 2.8: Safety labels at the main switch area



Bottom of the Rack



Fig. 2.9: Safety label and type plate at the bottom of the power supply rack



(Exemplary illustration of a type plate on ZEISS MultiSEM 505. The type plate on your particular instrument may slightly differ.)



Back Door



Fig. 2.10: Safety label at the back door of the power supply rack



2.5.2.3 At the Image Acquisition Rack

Front Door



Fig. 2.11: Safety label at the front door of the image acquisition rack





Fig. 2.12: Safety label at the back door of the image acquisition rack



2.5.2.4 Inside the Microscope Cabinet

Underneath the cover panels of the ZEISS MultiSEM microscope cabinet, further safety labels are placed addressing authorized ZEISS service representatives. These safety labels are described in the documents for ZEISS service representatives.

2.5.3 Material Safety Data Sheets

The document folder delivered with ZEISS MultiSEM contains material safety data sheets (MSDS) of chemicals used in combination with ZEISS MultiSEM.

2.5.4 Safety Information for Operation with the Cabinet Removed

ZEISS MultiSEM should normally be operated with the cabinet attached. For special purposes it is also possible to operate it with the cabinet removed, when the following information is considered.

- EMO Button** The Emergency Off button (EMO button) on the cabinet must be replaced by an external EMO button, connected via cable and placed visibly near the microscope.
- Safety Labels** The following safety labels placed on microscope parts are visible when ZEISS MultiSEM is operated with the cabinet removed:

- General safety label at the front of the plinth



- Burn hazard warning for the ion getter pumps (three times)



- Hazardous voltage warning at the rear of the illumination column (two times)



- Risk of injury warning on the vibrational isolation section



- Radiation hazard warning at the rear of the microscope



Health Hazard Labels The ion getter pumps create a strong magnetic field which can be dangerous for people wearing pacemakers. Therefore, the following health hazard labels are placed on the pumps.



3

Device Description

3 Device Description

3.1 Introduction

This chapter contains an overview of the main hardware components of ZEISS MultiSEM and its most important subsystems.

3.2 About ZEISS MultiSEM

ZEISS MultiSEM is a scanning electron microscope (SEM) that has been engineered for a large area to be examined with a high scan speed. To achieve this, it uses multiple electron beams to enable parallel scanning and detection and thus large area imaging in very short time.

The multi-beam array consists of several single beams in a hexagonal configuration (e.g. 61 single beams). In contrast to a conventional SEM, ZEISS MultiSEM can use this multi-beam array to scan across the specimen and thus increase the imaging speed significantly.

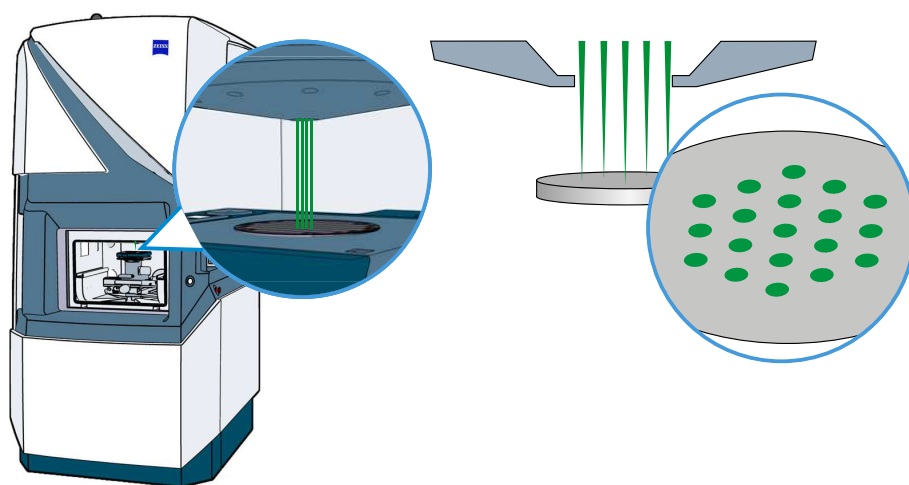


Fig. 3.1: Multi-beam array of ZEISS MultiSEM

Each individual single beam conducts its individual scan and outputs a corresponding single image. The single image is also referred to as single-beam Field of View (sFoV). ZEISS MultiSEM assembles the image data from the multiple scans in real-time. As a result, you obtain a full image: the multi-beam Field of View (mFoV) of the area that is scanned by the multi-beam array. This parallel image acquisition leads to a vastly increased throughput.

The workflow-oriented ZEN MultiSEM guides you through the necessary steps to examine a specimen as well as to stitch all the scans together to create an overall image of the entire specimen.

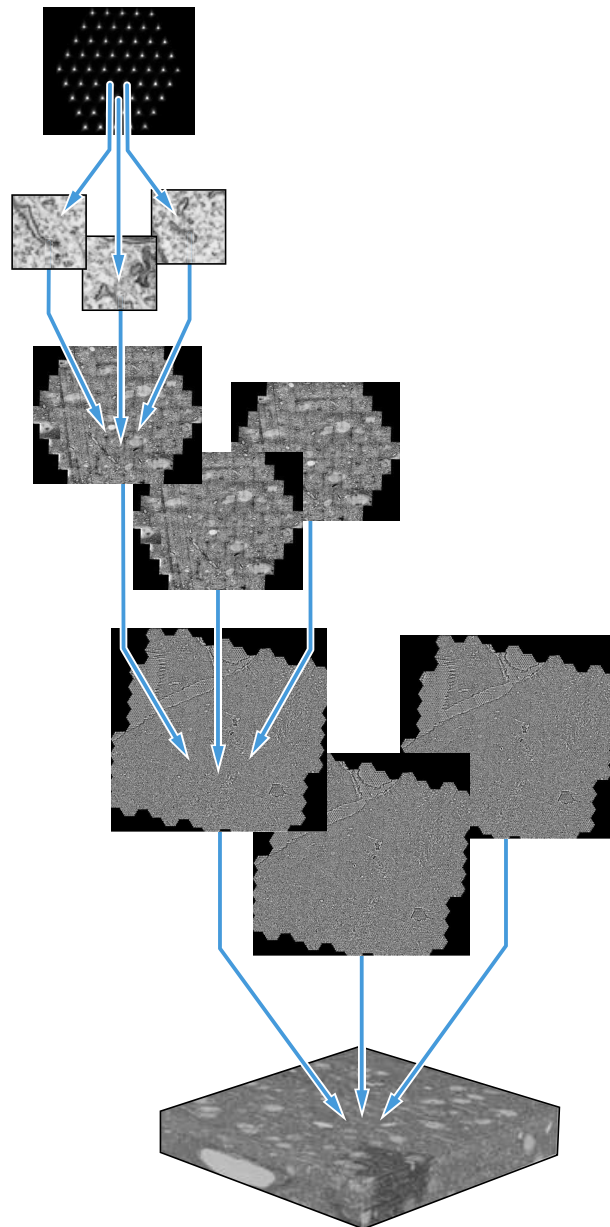


Fig. 3.2: Creation of the overall image of the specimen

3.3 Main Hardware Components

The ZEISS MultiSEM system consists of the following main hardware components:

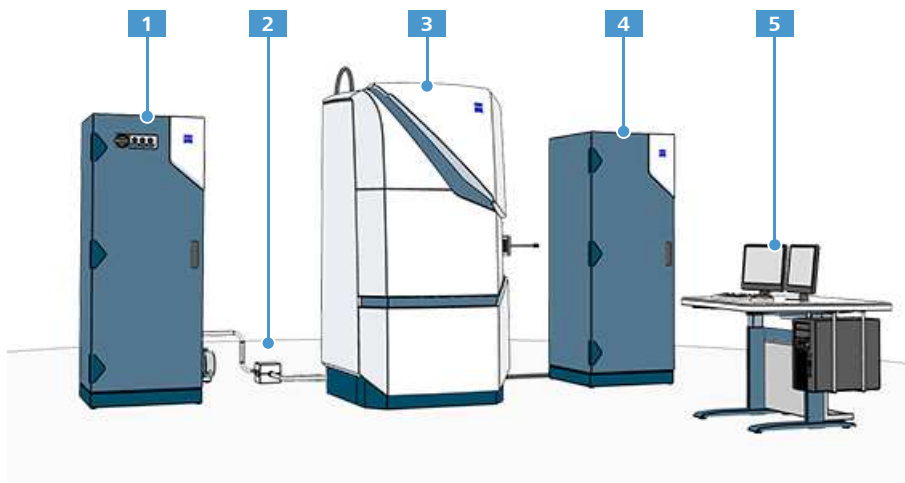


Fig. 3.3: Overview of the ZEISS MultiSEM system

- 1 Power supply rack
- 2 Pre-vacuum components
- 3 ZEISS MultiSEM microscope cabinet
- 4 Image acquisition rack
- 5 Workstation

Depending on your configuration, the ZEISS MultiSEM system may contain further optional components.

Power Supply Rack The power supply rack contains the main power supply (AC power and DC power) for the ZEISS MultiSEM microscope cabinet and the image acquisition rack.

You can set the operating status (On, STANDBY, or Off) for ZEISS MultiSEM on the front side of the power supply rack. Regardless of the set operating status, the image acquisition rack is always active.

The main switch on the front allows you to shut off the entire power supply (or to turn it on).

The power supply rack also contains the Emergency Off (EMO) unit for the system except the image acquisition rack.

Pre-vacuum Components The pre-vacuum components consist of the pre-vacuum pump for the turbo pump and a vibration damper.

A Quiet Mode unit is available (not shown in figure above) to switch off the pre-vacuum pump as needed.

ZEISS MultiSEM Microscope Cabinet The upper section of the cabinet contains the multi-beam electron optics and the light optics that transfer the converted light from the secondary electrons to the image acquisition rack.

The specimen chamber is located in the middle section. Below the specimen chamber are the microscope's controller components and the interfaces for the auxiliary supply systems, e.g. the cooling water supply.

The cabinet also contains the ion getter pumps and the turbo pump that are required to create and maintain the required vacuum.

Image Acquisition Rack The image acquisition rack detects and processes the incoming converted light from the secondary electrons, i.e. the output from the microscope.

It is connected via fiber optics to the ZEISS MultiSEM microscope cabinet. It transfers the acquired images to the ZEISS MultiSEM workstation and to the image storage.

Workstation The workstation contains the main ZEN MultiSEM software for controlling the ZEISS MultiSEM.

3.4 ZEISS MultiSEM Microscope Cabinet

The ZEISS MultiSEM microscope cabinet is the central component of the system. It is also referred to as the ZEISS MultiSEM microscope or simply as the microscope.

The ZEISS MultiSEM microscope cabinet is divided into the following sections:

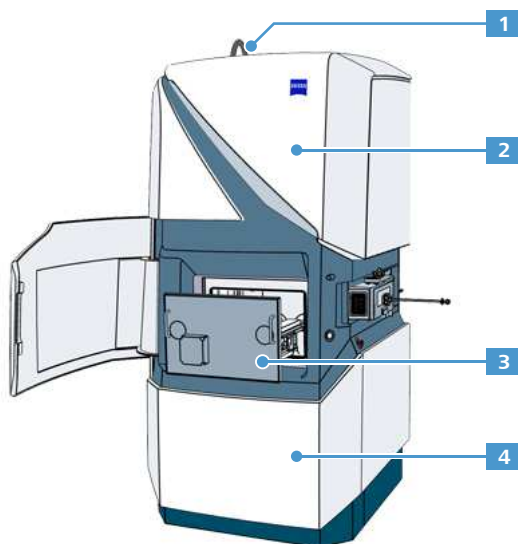


Fig. 3.4: ZEISS MultiSEM microscope cabinet

1 Fiber optics

Transfer the output signal from the microscope to the image acquisition rack

2 Upper section of the cabinet

Contains the actual multi-beam microscope together with ion getter pumps and vacuum valves for the different microscope sections

3 Middle section of the cabinet

Consists of the specimen chamber

This is the only part of the cabinet that you can access.

4 Lower section of the cabinet

The plinth below the specimen chamber contains the vacuum pump for the chamber's interior, the vibrational isolation system, as well as control electronics and the interfaces to auxiliary supply systems.

Upper Section of Cabinet The upper section of the cabinet contains the multi-beam microscope with the illumination optics for generating and guiding the primary electrons and the detection optics for collecting and detecting the secondary electrons. The multi-beam microscope consists of the following electron-optical assemblies:

- Illumination column transporting the primary electron beam
- Detection column transporting the secondary electron beam
- Objective lens with the opening to the specimen chamber

For further information on the microscope columns and their components, refer to *Illumination and Detection Optics* [► 92].

The following vacuum components are installed in the upper section of the cabinet to create and maintain the required vacuum:

- Three ion getter pumps attached to the illumination column
- One ion getter pump attached to the detection column
- Vacuum valves that separate the different vacuum sections

For further information on the system's vacuum components, refer to *Vacuum System* [► 69].

The upper section of the cabinet also contains the scan amplifier electronics.

Middle Section of Cabinet The cabinet door in the middle part provides access to the specimen chamber.

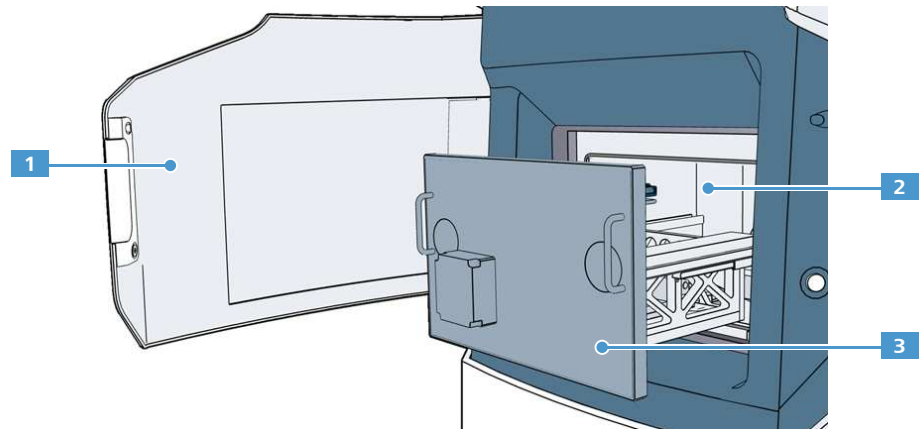


Fig. 3.5: Middle part of the Zeiss MultiSEM cabinet

- 1** Cabinet door
- 2** Specimen Chamber
- 3** Chamber door

For detailed information about the specimen chamber, refer to *Specimen Chamber* [► 47].

Depending on the configuration of your ZEISS MultiSEM, further components are available, for example:

- Airlock
- Plasma cleaner at the specimen chamber for cleaning the specimen chamber
- Plasma cleaner at the airlock for cleaning and etching the specimen inside the airlock

Lower Section of Cabinet The lower section of the cabinet contains the following hardware:

- Vibrational isolation system, including its controller

The fully automatic and self-adjusting vibrational isolation system is installed in the plinth that carries the specimen chamber and both microscope columns. It is driven pneumatically.

The vibrational isolation system prevents vibrations from inside and outside the cabinet (e.g. caused by the vacuum pump), from disturbing the scan process or the scan results.

The air pressure in the vibrational isolation system is released when you vent the specimen chamber. This causes the chamber (including the microscope columns) to slowly sink down a small amount.

- High-voltage power supplies

One power supply provides the high voltage for the electron gun (U_{Tip}).

A second power supply provides the high voltage that is applied to the specimen holder (U_{specimen}).

- Turbo pump, including controller
The turbo pump creates and maintains the required vacuum in the specimen chamber.
- Controllers for the ion getter pumps that are installed in the upper section of the cabinet
- Vacuum controller for monitoring the vacuum sections
- Electron-optics boards (EO boards)
Three EO boards drive the electron-optical components and beam alignment systems.
- Stage controller
- Interfaces and tubing for the auxiliary supply systems, e.g. cooling water

3.4.1 Specimen Chamber

The specimen chamber is located in the middle part of the ZEISS MultiSEM microscope cabinet. It is the only component within the cabinet to which you have direct access.

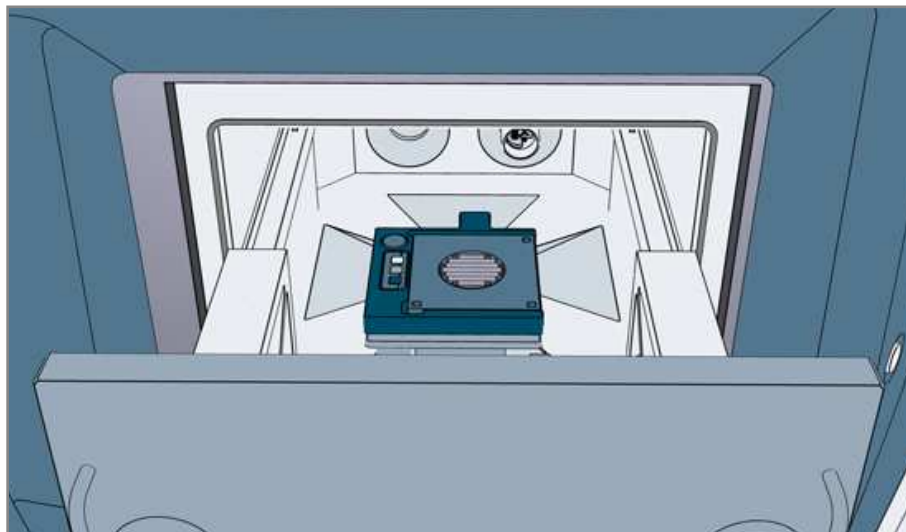


Fig. 3.6: Specimen chamber

When operating ZEISS MultiSEM, the specimen chamber is under vacuum. You have to vent the chamber before you can open the chamber door to insert or remove a specimen. Ensure that the beam is switched off before venting the chamber.

A chamber door interlock ensures that the high voltage applied to the specimen holder (U_{specimen}) is shut off when you open the door.



Suffocation hazard: Lack of oxygen

Gaseous dry nitrogen is used to vent the specimen chamber during specimen exchange. Inhaling nitrogen may cause unconsciousness.

- ◆ During specimen exchange, keep the chamber door open as short as possible.
- ◆ Ensure the area around the microscope is sufficiently ventilated.
- ◆ Avoid inhaling the air from within the specimen chamber.
- ◆ If you begin to experience symptoms of asphyxia (for example: rapid breathing, loss of mental alertness and/or muscular coordination, depression of sensations, emotional instability, fatigue) leave the room immediately and inform the facility's safety officer.

Interior of the Specimen Chamber The interior of the specimen chamber consists of the following main elements (in addition to the specimen stage and the specimen holder mount):

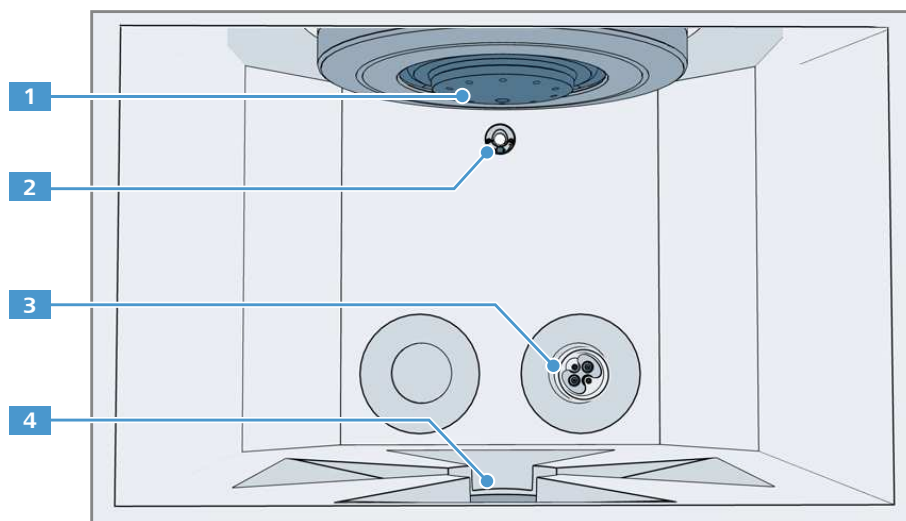


Fig. 3.7: Interior of the specimen chamber (without specimen stage)

1 Objective lens

The objective lens focuses the primary-electron beams generated by the illumination optics onto small spots on the specimen. It concurrently collects and guides the secondary electrons onto the detector at the end of the detection optics.

2 Chamber CCD camera

The chamber CCD camera monitors the stage movements and, particularly, assists you in monitoring the distance between objective lens and specimen (or specimen holder surface).

3 Connection for the high voltage applied to the specimen holder (U_{specimen}) and the lower pole piece of the objective lens

4 Turbo pump opening

The turbo pump below the chamber, backed by the upstream pre-vacuum pump, evacuates the specimen chamber.

When the front door is closed, the specimen holder mount is located beneath the objective lens.

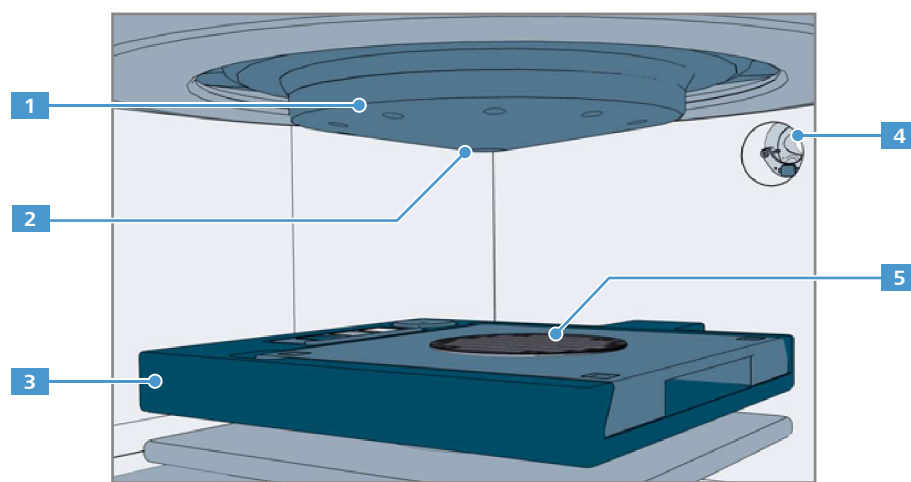
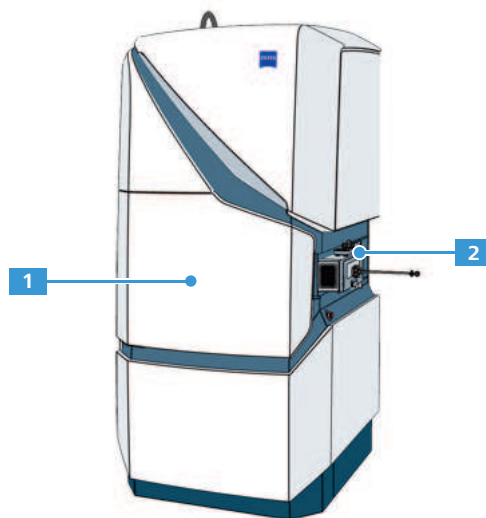


Fig. 3.8: Objective lens with opening and specimen holder mount

- 1** Objective lens
- 2** Objective lens opening
- 3** Specimen holder mount
- 4** Chamber CCD camera
- 5** Specimen holder (with specimen)

For optimal imaging, you have to set the correct working distance between the objective lens and specimen surface (see *Working Distance* [► 147]).

3.4.2 Airlock



- 1 Microscope cabinet
- 2 200 mm-Airlock with HV interlock

The 200 mm-Airlock with HV Interlock is designed to exchange standard specimens on the MultiSEM sample holder. It contains a vacuum chamber that can be operated while the specimen chamber remains evacuated. The volume of the airlock chamber is considerably smaller, which reduces the time required for ventilation or evacuation.

For more information, see the [200 mm-Airlock with HV Interlock Instruction Manual](#), Loading the Specimen via the Airlock, and Airlock Control.

CAUTION

Risk of injury or property damage due to improper operation of the 200-mm Airlock with HV Interlock.

- ◆ Read the user documentation carefully.
- ◆ Do not operate the airlock until you have completely read and understood the [200 mm-Airlock with HV Interlock Instruction Manual](#).

NOTICE

Risk of property damage due to improper maintenance, service, or repair.

The warranty might be voided.

- ◆ Follow the instructions given in the [200 mm-Airlock with HV Interlock Instruction Manual](#).

INFO

All pursuing tasks of maintenance, service, and repair not described in this help or in the [200 mm-Airlock with HV Interlock Instruction Manual](#) have to be performed by authorized Carl Zeiss Microscopy service staff only.

3.4.3 Plasma Cleaner

Two Plasma Cleaner packages are available as an option:

- Plasma cleaner at the specimen chamber for cleaning the specimen chamber
- Plasma cleaner at the airlock for cleaning and etching the specimen inside the airlock

The Plasma Cleaner units are attached to the specimen chamber or the airlock. Inside a plasma cleaner unit, a plasma of reactive gas-phase radicals is generated. From the plasma cleaner unit, the radicals migrate into the specimen chamber. They react essentially with undesired hydrocarbons, thus removing hydrocarbon contamination, either from the specimen, i.e. short cleaning cycle, or from the entire specimen chamber, i.e. long cleaning cycle.

For more information, see the [Plasma Cleaner Instruction Manual](#), *Using the Plasma Cleaners* [► 119], and Plasma Cleaner Control.

INFO

The Plasma Cleaner must only be operated by persons who have received training by an authorized ZEISS service representative. For detailed information on how to safely operate the Plasma Cleaner, refer to the Evactron 25Z Users Manual or the [Plasma Cleaner Add On Instruction Manual](#).

3.4.4 Specimen Stage

The specimen stage carries the specimen holder mount including the specimen holder. It also provides slots for one or more calibration pattern(s) and a Faraday cup.

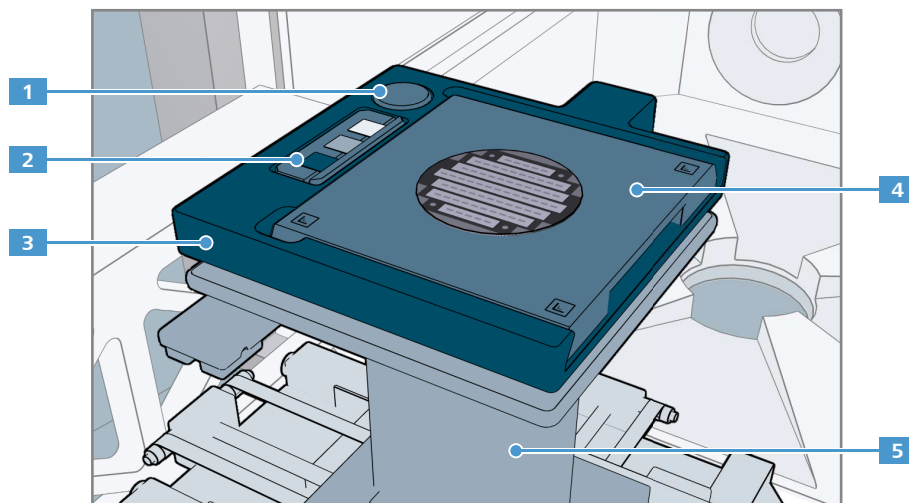


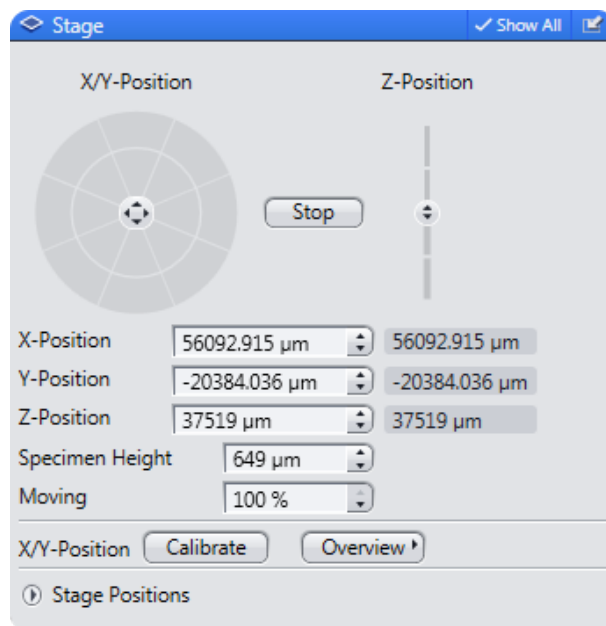
Fig. 3.9: Specimen stage

- 1 Faraday Cup
- 2 Slots for calibration patterns
- 3 Specimen holder mount
- 4 Specimen holder
- 5 Specimen stage including stage motor

Moving the Stage You can move the specimen stage vertically (along its Z axis) to focus the image or horizontally (along its X and Y axes) to move the specimen.

The three motorized axes are driven either automatically, by ZEN MultiSEM, or manually, using one of the following possibilities:

- Dual joystick panel
- **Control Panel**
- Control elements in the **Stage** tool window within ZEN MultiSEM



- Center feature within ZEN MultiSEM

Double-click a spot in a live image to center the stage on that spot.

- Arrow keys on the keyboard / control panel in certain work steps within ZEN MultiSEM.

The element that is to be controlled by the arrow keys needs to be activated.

You first have to activate the element that is to be controlled by the arrow keys by clicking the corresponding section in the tool window. For example, in the **Stage** tool window, click the **Z-Position** slider or the **Z-Position** entry field to enable vertical movements using the up/down key.

- Alternatively, you can move the stage using the Control Panel.



CAUTION

Risk of damage to stage motor due to excessive stage movement

If you move the stage too excessively, then the stage motors may heat up to critical temperatures. Due to the increased temperatures the stage motors may get damaged.

In case you perform cycles of automatic stage movement, do the following:

- ◆ Do not move the stage for a total distance of more than 6 m within a time span of 30 minutes. Typical operations of the ZEISS MultiSEM result in less than 0.5 m stage movement within 30 minutes and are not critical for the stage motors.
- ◆ In case of doubt, contact your local ZEISS service representative.

INFO

Extensive stage movements may cause image drift.
Avoid excessive stage movements to prevent image drift due to hot stage motors.

Maximum Travel Length The specimen stage has the following limits of travel, measured from the point of origin in the stage coordinate system:

Direction	Possible movements
X	± 65 mm
Y	± 65 mm
Z	0 to 36 mm ¹⁾

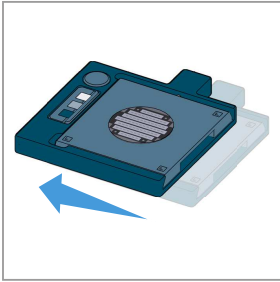
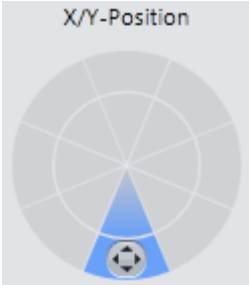
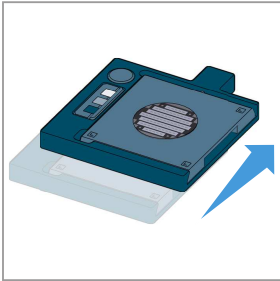
¹⁾ You can reduce the Z limit by entering the specimen height. If you enter the minimum value of 0 µm for the specimen height, the stage can move to its uppermost position. Defining the correct specimen height is particularly important to prevent collisions between the objective lens and the specimen holder.

Possible Movements You can move the specimen vertically or horizontally. Diagonal movements are possible in the horizontal plane. You can also move the specimen stage vertically and horizontally simultaneously.

Note the following when moving the specimen stage horizontally:

- The movement direction refers to the movement shown in the *live image* in ZEN MultiSEM, not to the actual stage movement.
For example, a movement into the positive X direction means, that the area shown in the live image moves/scrolls to the right. As the scanning components (e.g. the objective lens) are fixed, the specimen stage actually moves to the left.
- The chamber CCD camera shows the actual horizontal stage movements, recorded from the back of the specimen chamber (see *Chamber CCD Camera* [► 57]).

Examples:

Movement into	User Action	Actual Stage Movement
+ X		
- Y		

3.4.5 Specimen Holder

The specimen holder is the component that you remove from the specimen chamber in order to place the specimen on it. All specimen holders have dedicated fiducial marks, the ZEISS Shuttle & Find L-markers, and are suitable for correlative microscopy.

The specimen holder is inserted into the specimen holder mount.

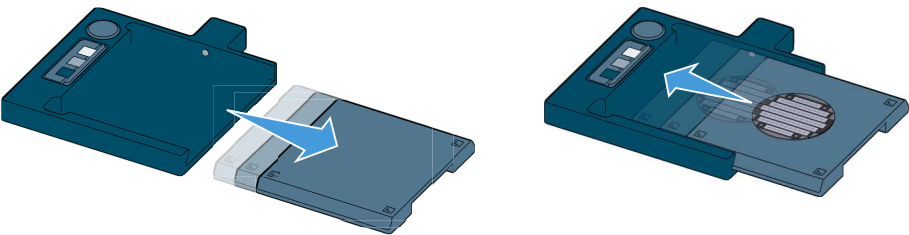


Fig. 3.10: Removing and inserting the specimen holder

The rear edge of the specimen holder has a bevel to ensure that it is inserted into the specimen holder mount with the correct orientation.

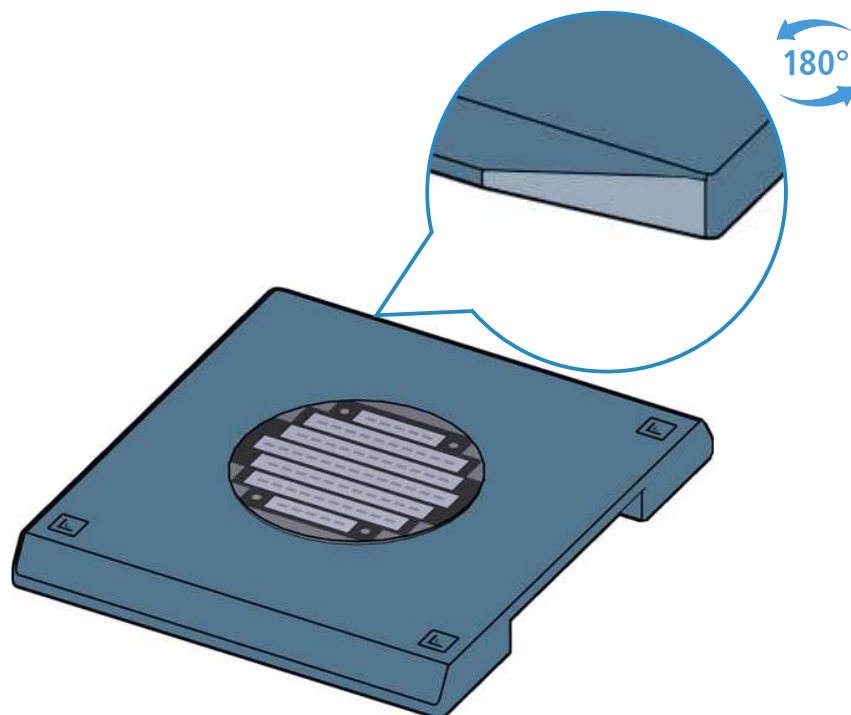


Fig. 3.11: Bevel on the specimen holder's rear edge

You typically place the specimen in the center of the holder. The specimen's diameter should not exceed 100 mm. The following figure shows the minimum distance to the edges.

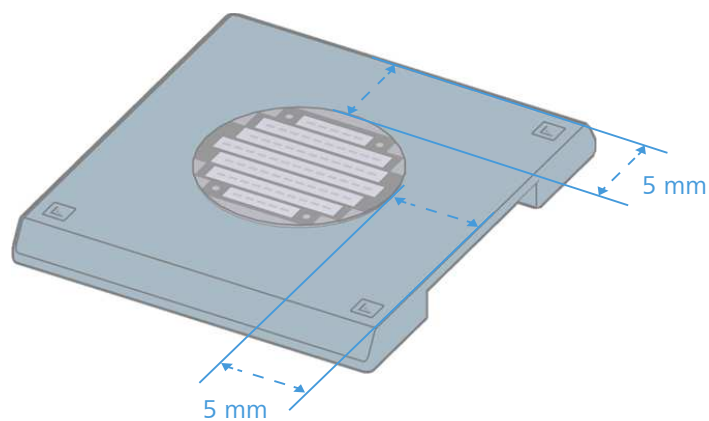


Fig. 3.12: Minimum distance to the edges of the specimen holder

Correlative Microscopy Specimen Holder The L-markers are located in the three corners of the specimen holder and are needed during the registration of the specimen. They are numbered counter-clockwise, as shown in the following figure.

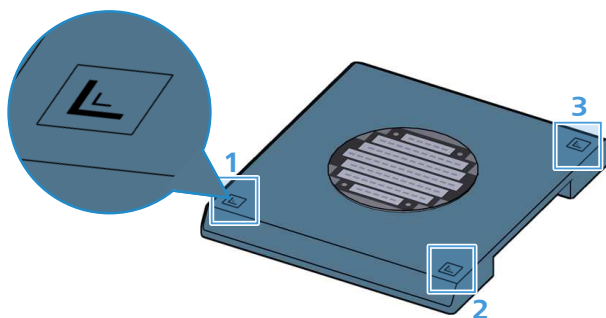


Fig. 3.13: Markers on specimen holder

Each marker consists of two right angles. The larger right angle is designed to be used as a guide to the smaller angle. The smaller angle in turn is used to register the specimen in the corresponding imaging modality (e.g. a light microscope).

For more information, see Shuttle & Find.

3.4.6 Chamber CCD Camera

ZEISS MultiSEM contains a CCD camera (charge-coupled device camera) inside the specimen chamber (see *Specimen Chamber* [▶ 47]). It is referred to as the chamber CCD camera. It allows you to monitor the position of the specimen holder mount and particularly the distance between the objective lens and the specimen holder.

The chamber CCD camera is located at the back of the specimen chamber.

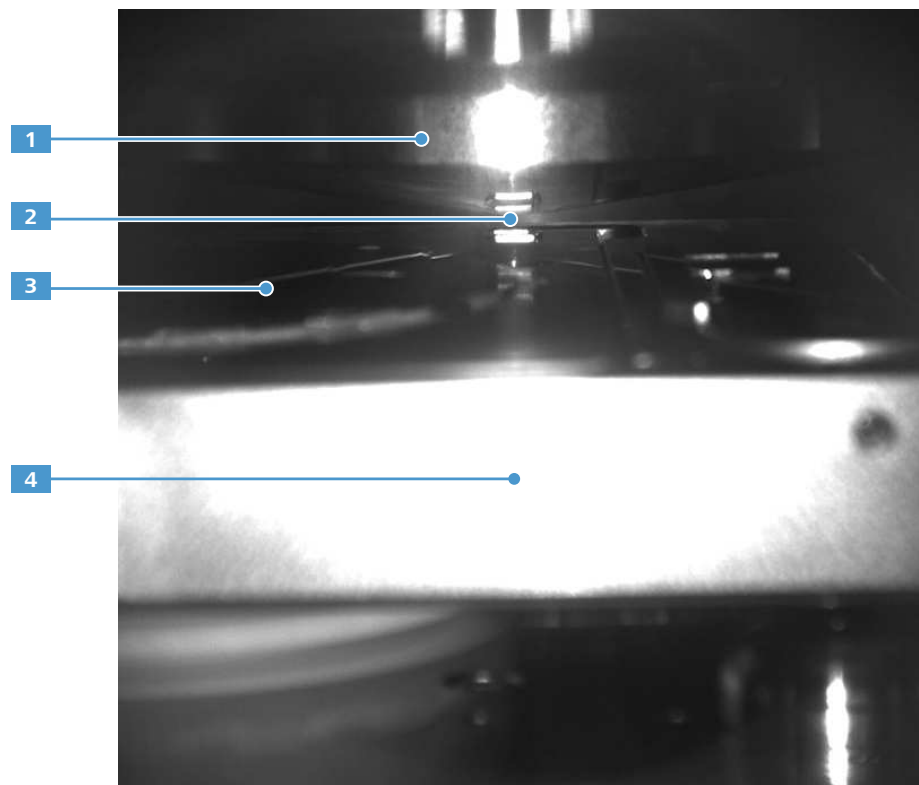


Fig. 3.14: Specimen image from chamber CCD camera

- 1 Objective lens
- 2 Objective lens opening
- 3 Specimen
- 4 Specimen holder mount (rear site)

INFO

Use the chamber CCD camera to monitor the position of the specimen during stage movements. Pay particular attention to the distance between the objective lens and the top of the specimen. This applies to vertical movements, but also to horizontal movements, because a thick specimen may collide with the objective lens from the side.

Since the camera is mounted on the rear of the specimen chamber, horizontal stage movements appear to occur in the opposite horizontal direction. For example:

- If you move the specimen stage towards the chamber door, the chamber CCD camera shows the stage moving backwards.
- If you move the specimen stage to the right, the chamber CCD camera shows the stage moving to the left.

Vertical stage movements appear as expected.

Furthermore, in correlative microscopy, the markers on the specimen holder appear as seen from the back of the specimen chamber, e.g. marker 1 is shown in the upper right corner of the specimen holder.

3.4.7 Detection CCD Camera

ZEISS MultiSEM contains a CCD camera (charge-coupled device camera) that is located behind the scintillator at the end of the detection column (see *Components in the Detection Column* [► 96]). This camera allows you to monitor the Z position of the specimen stage and helps you to set the working distance (see *Working Distance* [► 147]). It is referred to as the detection CCD camera.

The detection CCD camera acquires the light-signal output from the scintillator at the same time as the signal is coupled into the fiber optics. Each spot is the detected signal of a single beam.

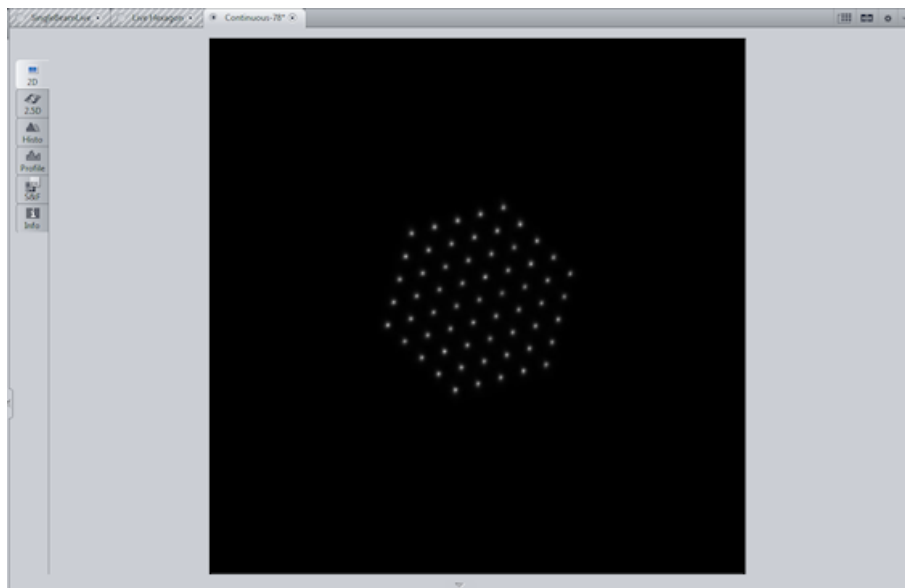


Fig. 3.15: Image from detection CCD camera

Use the detection CCD camera to monitor the coarse focus of the specimen:

- The spots are focused near the correct working distance between the objective lens and specimen surface.
- The spots become more diffuse when the deviation from the optimal working distance increases (both +Z and -Z).

You can also use the detection CCD camera to monitor the alignment of the multi-beam array, e.g. the projective alignment.

INFO

Distorted spots on the camera image

If the specimen is charged electrostatically, the spots on the camera image may appear distorted. For more information, see *Requirements for the Specimen* [▶ 117].

INFO

No spots visible

If no spots are visible on the camera image and if the beam blanker is set to auto mode (standard setting), make sure that **Single Live** or **Full Live** is active.

3.5 Workstation

The workstation is the main component of ZEISS MultiSEM you interact with. You perform all your imaging tasks at the workstation apart from loading (or unloading) the specimen chamber.

It consists of the following hardware:

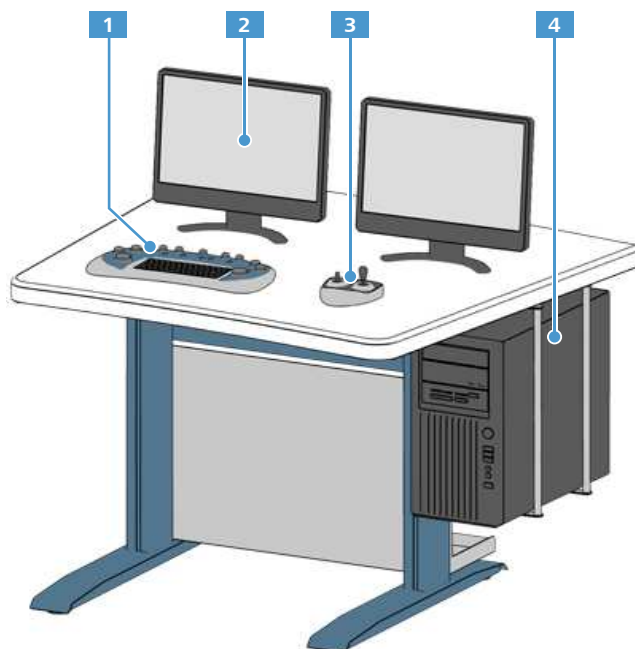


Fig. 3.16: ZEISS MultiSEM workstation

- 1 Control panel
- 2 Monitors
- 3 Dual joystick panel
- 4 Control computer

ZEN MultiSEM, and all other software required to operate ZEISS MultiSEM, is pre-installed on the control computer. It is the user interface between the microscope hardware, the controllers, the image acquisition rack, and the panels or keyboards at the workstation.

3.5.1 Control Panel

The control panel consists of a keyboard, knobs and buttons for the most important functions of the ZEISS MultiSEM.

If an experiment is started or an alignment routine is active, the knobs and buttons are disabled.

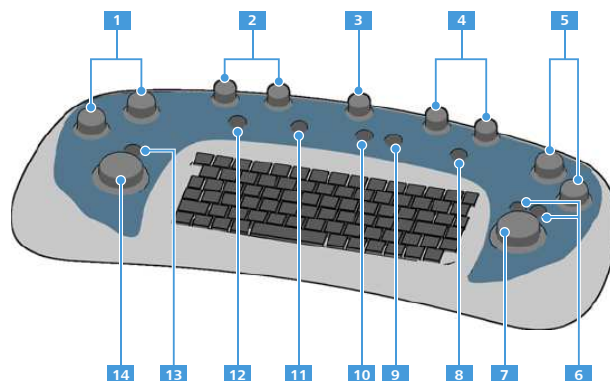


Fig. 3.17: Control Panel

1 Stigmator X / Stigmator Y

Enables you to correct astigmatism effects, such as a line focus, manually.

2 Wobble Adjust X / Wobble Adjust Y

Enables you to minimize the objective lens aberrations by adjusting the strength of the beam deflectors in an X or Y direction.

When **Focus Wobble** is activated, the field of view is constantly going in and out of focus. If the field of view is shifting, adjust with **Wobble Adjust X / Wobble Adjust Y** until the field of view goes in and out of focus but does not change in other respects.

3 Push-Button/Control Dial

This control can be used as a push-button or as a control dial.

■ Push-Button

No function.

■ Control Dial

No function.

4 Stage X / Stage Y

Enables you to navigate the specimen by moving the stage in an X or Y direction.

5 Brightness / Contrast

Changes the brightness and contrast settings of the detectors. Note that these settings directly affect the raw data to be acquired.

6 Scan Speed +/-

Changes the **Scan Speed** by one step. With each step, the integration time per pixel is doubled or halved.

Considering the required image quality, set the **Scan Speed** to a value as low as possible to optimize throughput.

The lower the **Scan Speed** number, the shorter the integration time per pixel, the faster the acquisition speed, but the lower the image quality.

7**Focus**

Enables you to set the focus by adjusting the objective lens.

This control is sensitive to its rotational speed: if you turn it fast, the focus changes in coarse steps. If you turn it slowly, the focus changes in fine steps.

8**Detector Equalization**

Equalizes the contrast or brightness between the individual single images. This routine is identical to the routine available from the **Auto Tuning** tool in the ZEN MultiSEM and is performed with the settings currently defined in the software.

9**Auto Stigmatism**

Corrects astigmatism effects, e.g. a line focus in the live image.

This routine is identical to the routine available from the **Auto Tuning** tool in the ZEN MultiSEM and is performed with the settings currently defined in the software.

10**Aufofocus**

Adjusts the focus of the specimen.

Perform this alignment routine if the live image of the specimen appears to be too blurred.

This routine is identical to the routine available via the **Auto Tuning** tool in the ZEN MultiSEM and is performed with the settings currently defined in the software.

11**Beam on Detector**

Corrects the alignment of the fiber optics.

Perform this alignment routine if ghost images are visible in the live image or if the outer regions of the multi-beam image are dark.

This routine is identical to the routine available via the **Auto Tuning** tool in the ZEN MultiSEM and is performed with the settings currently defined in the software.

For this routine to work, the beam pattern must be visible on the detection CCD camera.

12**Focus Wobble**

Enables you to minimize the objective lens aberrations.

When activated, the field of view is constantly going in and out of focus.

If the field of view is shifting, adjust with **Wobble Adjust X / Wobble Adjust Y** until the field of view goes in and out of focus but does not change in other respects.

13 Reduced

If **Single Live** mode is active, the **Reduced** button enables you to start a region of interest (ROI) scan. A reduced area in the field of view is scanned and the image update speed for this area is increased.

If you push the **Reduced** button again, the ROI scan is terminated and the entire field of view is scanned again.

The ROI can be adjusted via ZEN MultiSEM using the mouse.

14 Stage Z

Enables you to set the focus by moving the specimen in Z direction.

This control is sensitive to its rotational speed, i.e. if you turn it fast, the stage moves at a higher speed. If you turn it slowly, the stage moves at a lower speed.

3.5.2 Dual Joystick Panel**NOTICE**

Risk of damage to the objective lens or the specimen

The specimen may collide with the objective lens while raising the specimen stage.

- ◆ Enter the specimen height in ZEN MultiSEM.
For more information about setting the parameter **Specimen Height**, see Stage.
- ◆ Always move the specimen stage slowly and observe its movement using the chamber CCD camera.

The dual joystick panel controls the specimen stage and thus enables you to navigate the specimen.

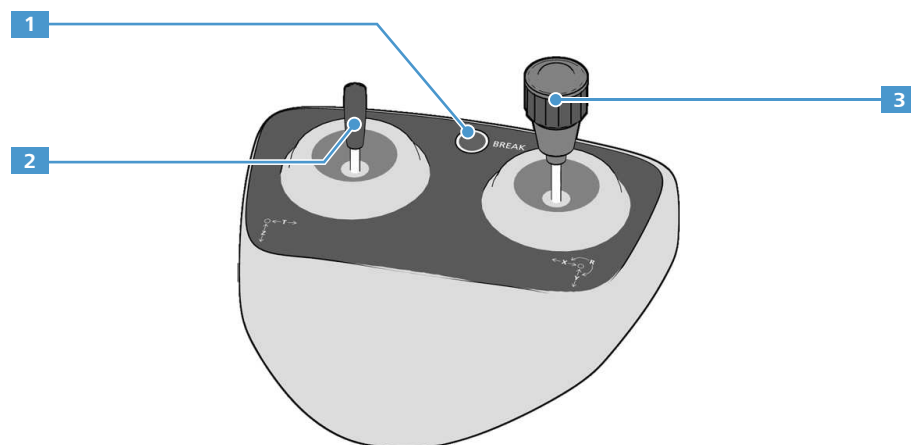


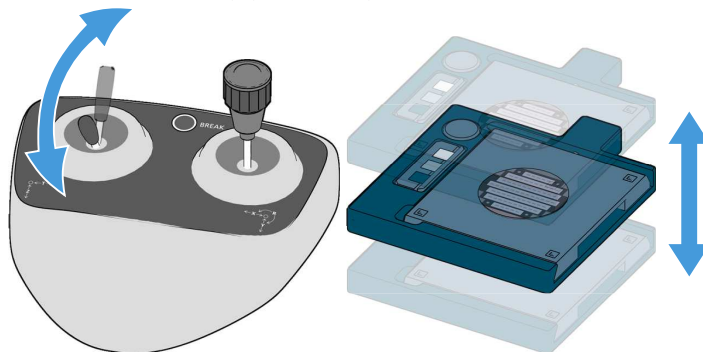
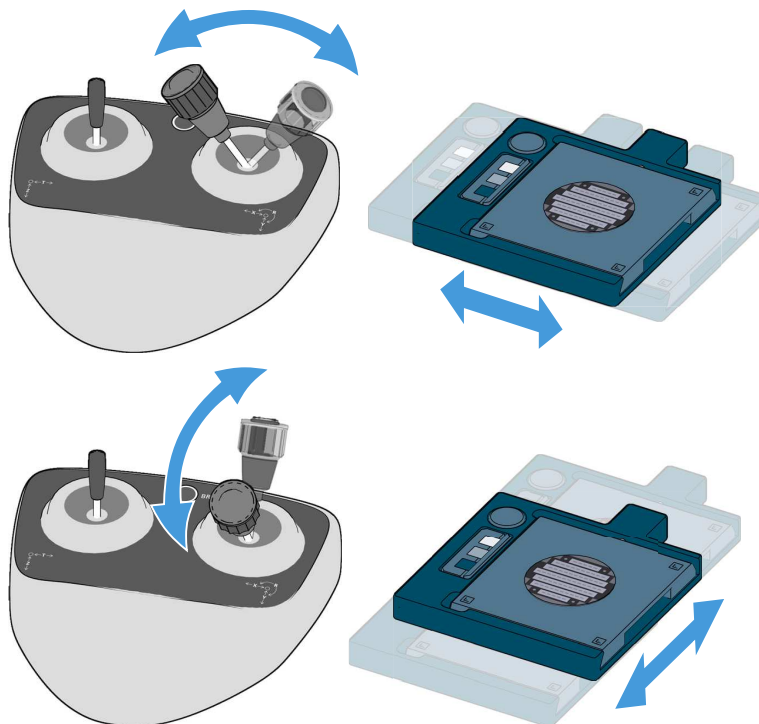
Fig. 3.18: Dual joystick panel

1 Break button

This button immediately stops the stage movement.

2 Small joystick for vertical movements

To do this, push the joystick straight upward/downward.

**3 Large joystick for horizontal movements**

You can also move the joystick diagonally to perform the corresponding diagonal movement.

General Hints for Stage Control

The following actions apply for both joysticks:

- Push the joystick gently if you want to move the specimen stage slowly in the corresponding direction.
- Push the joystick firmly if you want to move the specimen stage fast.

3.6 Power Supply Rack

The main power supply of ZEISS MultiSEM is contained within the PSU rack (power supply unit rack). Only a small number of hardware components, for example the pre-vacuum pump, are connected to additional power sockets.

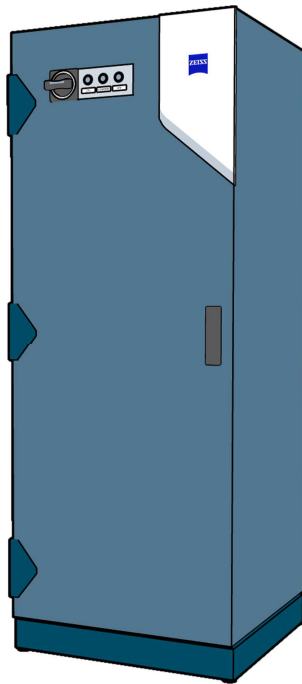


Fig. 3.19: Power Supply Rack

The PSU rack contains the following power supplies and power switches:

- Mains power supply block/unit, the so-called "PSU mains"
- Fuse box
- AC and DC power supply for the microscope's electronics and electron-optical components
- Power supply for the image acquisition rack
- Linear mains adapter for the scan amplifier
- Emergency Off (EMO) unit

Main Switch The main switch on the front of the PSU has an emergency off function: Turn it to **OFF** in an emergency to cut off all three phases of the mains power supply.

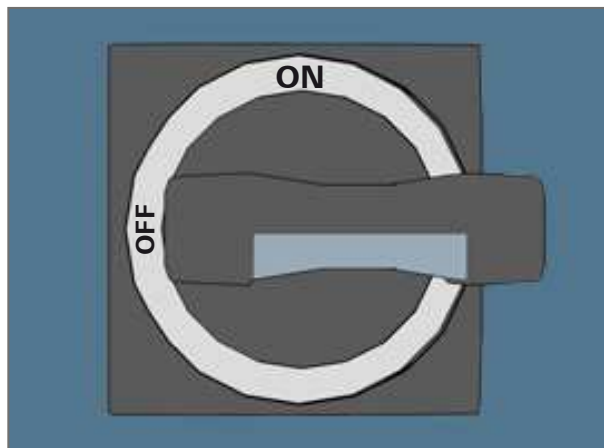


Fig. 3.20: Main switch

Operation Mode Buttons The buttons on the front of the PSU rack enable you to set one of the following operation modes:

- ON
- Standby
- OFF
- POWER ON

This button is used to switch the system on after an Emergency Off.

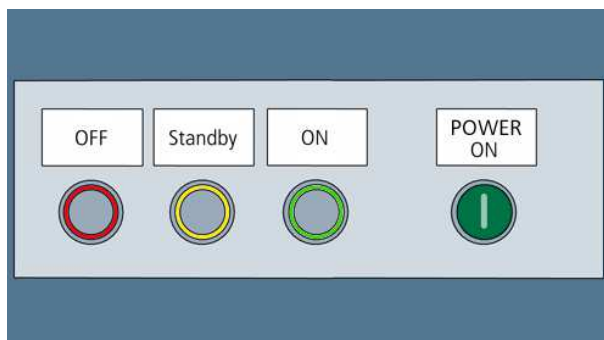


Fig. 3.21: Operation mode buttons and POWER ON button

For further information about the operating modes, refer to *STANDBY Mode and OFF Mode* [▶ 110].

For further information about the Emergency Off (EMO), refer to *Emergency Off (EMO)* [▶ 112].

3.7 Image Acquisition Rack

The image acquisition rack receives the analog scan signals from the ZEISS MultiSEM microscope and converts them into digital live images. The live images are then transferred to ZEN MultiSEM and to the image storage.

The image acquisition rack contains the following components:

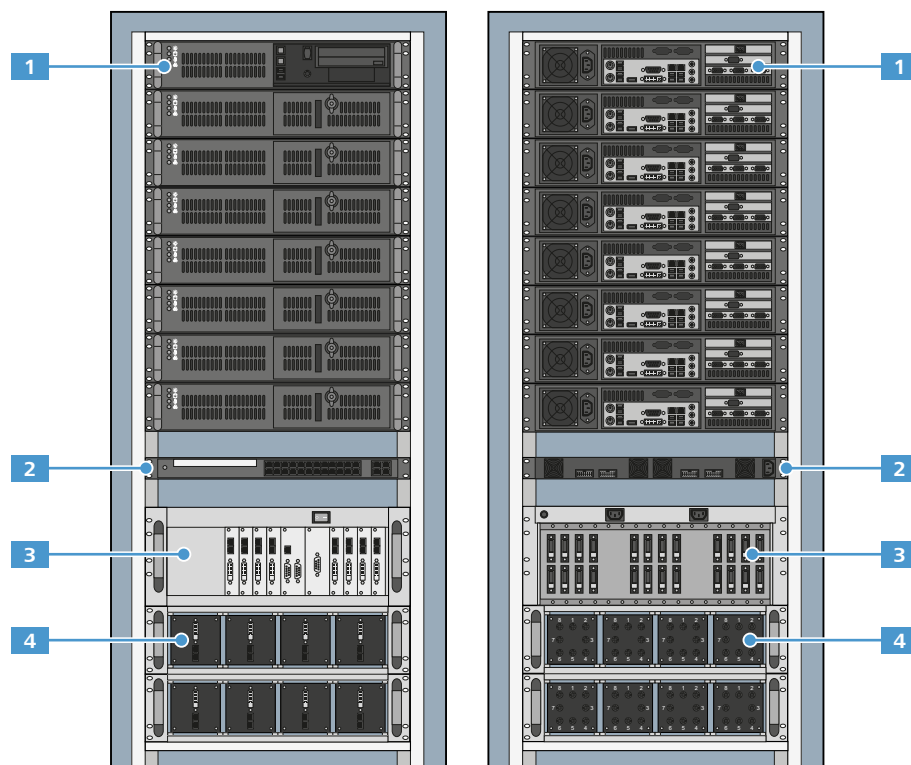


Fig. 3.22: Components in the image acquisition rack (left side: front view; right side: back view)

1 Image acquisition PCs

Process the live images and transfer them to ZEN MultiSEM and to the image storage

2 Switch

Transmits the data between the image acquisition PCs and the control computer

3 Digitalization unit

Converts the analog signals into digital signals and forwards the data to the image acquisition PCs

4 Multi-detector assembly

Detects the incoming analog signals from the microscope

The assembly consists of several detectors. Each detector can process the input from eight fiber optics, i.e. eight scan signals.

Connection to Storage System Each of the image acquisition PCs has to be connected with a 10 GBit/s connection to your image storage system. The following figure shows the connection for the ethernet cable leading to the storage.

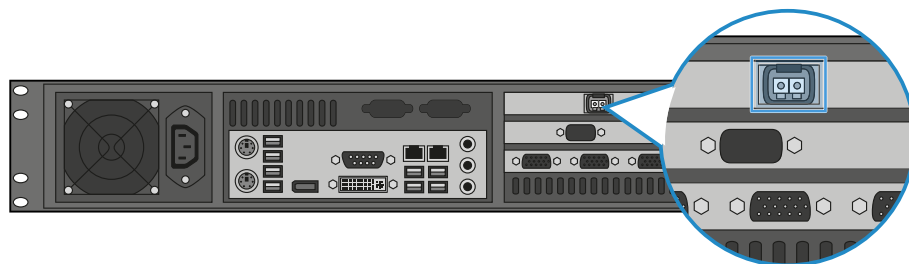


Fig. 3.23: Connection to image storage

3.8 Vacuum System

The electron gun, microscope columns (illumination column and detection column), and specimen chamber have to be evacuated. The vacuum is essential for achieving safe operation and best performance of the microscope. Both the illumination column and the detection column have several vacuum sections.

Vacuum Sections in the Microscope

To provide the required vacuum conditions in each electron-beam section, the following vacuum pumps are installed in the ZEISS MultiSEM microscope:

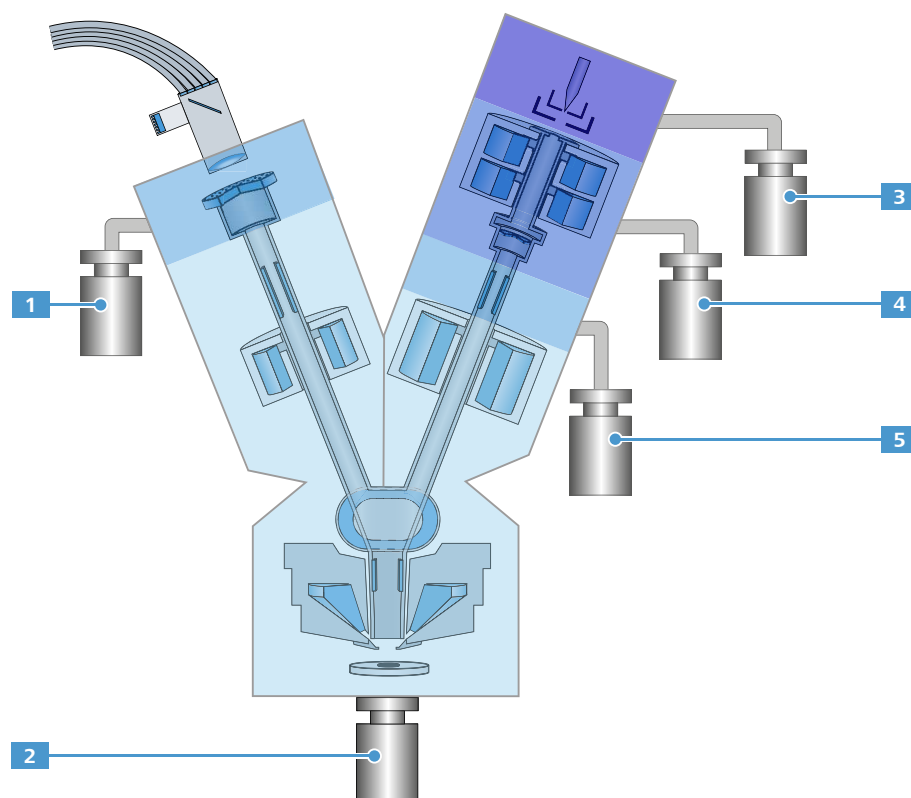


Fig. 3.24: Vacuum sections

- 1** Ion getter pump for detection column
- 2** Turbo pump for specimen chamber.
The specimen chamber is evacuated by a pre-vacuum pump and a turbo pump.

- 3 Ion getter pump for electron gun section
- 4 Ion getter pump for micro optics section
- 5 Ion getter pump for column section below micro optics

Monitoring the Vacuum A Penning gauge attached to the specimen chamber monitors the vacuum that is created by the turbo pump. The ion getter pumps are monitored by measuring their current.

You can monitor the measured vacuum in the **Vacuum Status** window in ZEN MultiSEM. Additionally, you can monitor the **Turbo Pump Speed**.

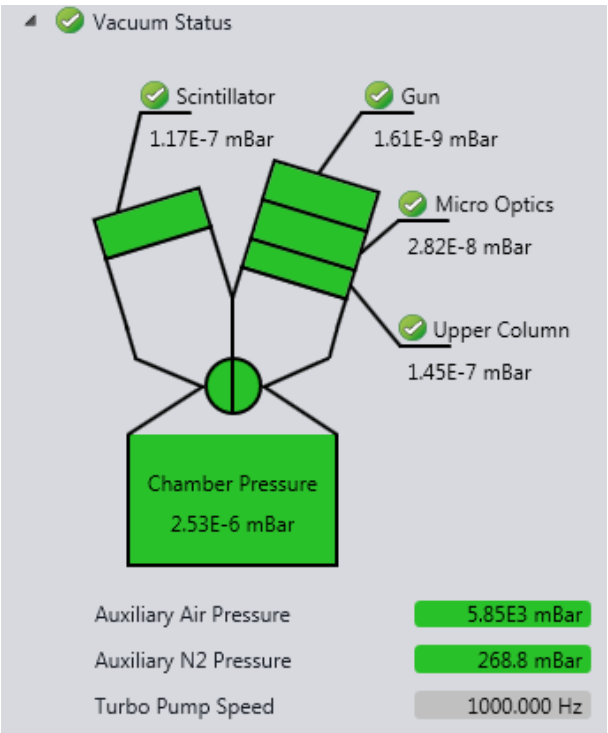


Fig. 3.25: Vacuum Status

Vacuum section	Description	Operation pressure	Pressure when beam will be switched off
Gun Pressure	Vacuum in the electron gun section	9×10^{-9} mbar	1×10^{-8} mbar
Micro Optics Pressure	Vacuum in the micro optics section	4×10^{-8} mbar	6×10^{-8} mbar

Vacuum section	Description	Operation pressure	Pressure when beam will be switched off
Upper Column Pressure	Vacuum in the upper column section above specimen chamber	7×10^{-7} mbar	1×10^{-6} mbar
Chamber Pressure	Vacuum in the specimen chamber	1×10^{-5} mbar	3×10^{-5} mbar
Scintillator Pressure	Vacuum in the detection column	4×10^{-7} mbar	1×10^{-6} mbar

If a value has a green background, then the vacuum for the corresponding section is within the operating limits.

Pre-vacuum unit The pre-vacuum unit is installed outside the ZEISS MultiSEM microscope cabinet. All other vacuum components are located inside the cabinet.

The pre-vacuum unit consists of the following components:

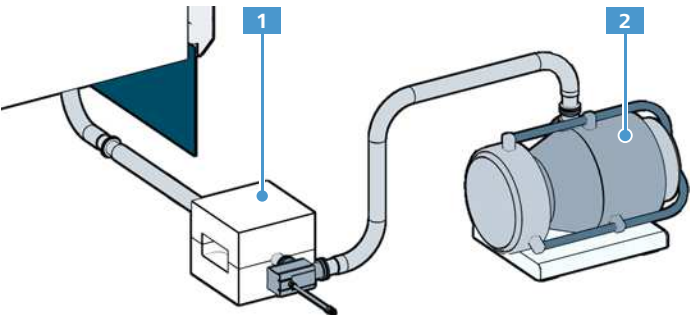


Fig. 3.26: Pre-vacuum unit

- 1 Vibration damper
- 2 Pre-vacuum pump

The pre-vacuum pump provides the pre-vacuum that is required for operating the turbo pump. A vibration damper is installed to counteract the vibrations from the pre-vacuum pump. The tube from the vibration damper is directly connected to the turbo pump inside the microscope's lower chassis.

For further information, refer to the user manual of the pre-vacuum pump.

Quiet Mode The Quiet Mode acts as a "vacuum buffer" for the pre-vacuum pump. It allows the relatively noisy pre-vacuum pump to be switched off as soon as a certain vacuum level is achieved.

Using the Quiet Mode unit allows you to run ZEISS MultiSEM for hours without using the pre-vacuum pump, which reduces the noise levels.

3.9 Overview of Auxiliary Supply Systems

ZEISS MultiSEM is connected to the following auxiliary supply systems:

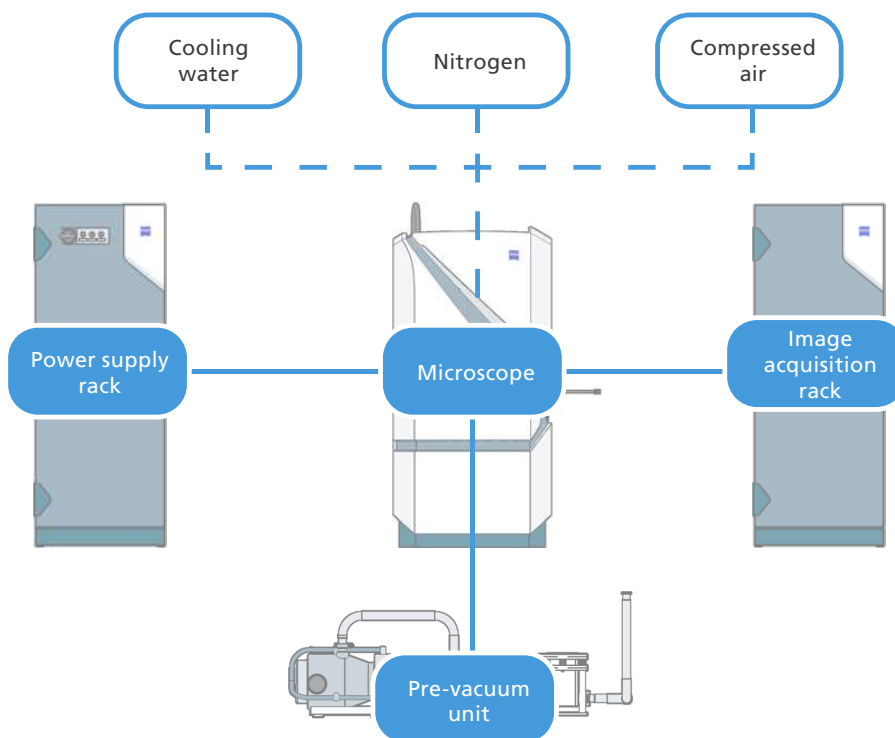


Fig. 3.27: External auxiliary supply systems (dashed lines)

■ Cooling water

Cooling water is required to prevent the following components from overheating:

- Coils of the magnetic lenses
- Microscope electronics, e.g. the specimen stage controller
- Turbo pump in the lower part of the microscope

■ Nitrogen

Nitrogen gas (N_2) is required when venting the specimen chamber.

The specimen chamber is vented with nitrogen before you can open the chamber door and access the specimen. This is necessary to avoid contamination of the chamber's inner surface, e.g. by water (vapor) molecules.

■ Air

Compressed air is required to open/close the vacuum valves and to activate/deactivate the vibrational isolation system in the lower section of the microscope.

The required gases and fluids are supplied by the customer's in-house supply systems, e.g. from the cooling water supply system. They are connected to the interface on the lower left corner on the rear of the ZEISS MultiSEM microscope cabinet.

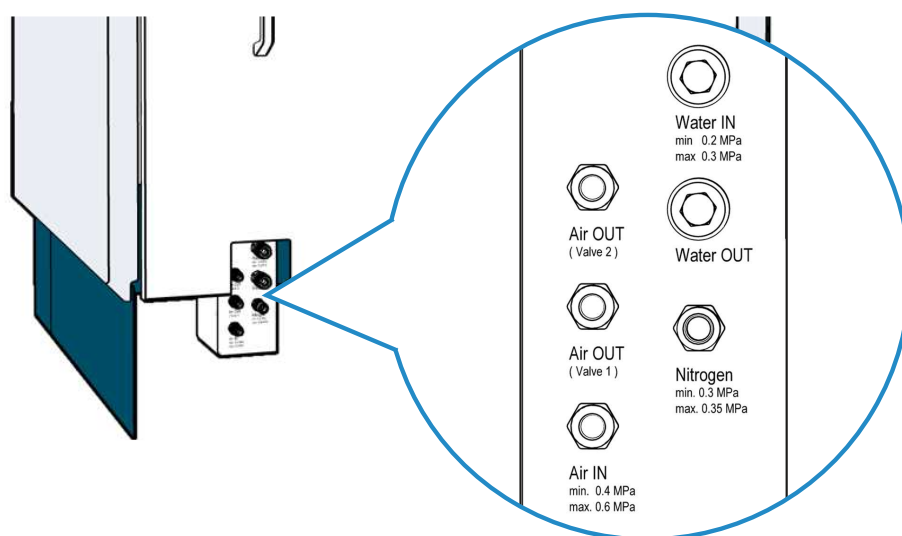


Fig. 3.28: Interface for auxiliary supply systems

3.10 Integration into Laboratory Environment

ZEN MultiSEM and the ZEISS MultiSEM microscope can be integrated into your existing lab environment.

ZEISS MultiSEM Control PC It is possible to connect the ZEISS MultiSEM control PC to your local network. If you do so, then it is your own responsibility to guarantee a sufficient network security with regard to the ZEISS MultiSEM control PC. If a sufficient network security can not be guaranteed by protection of your local network, then you are obliged to install an up-to-date antivirus software on the ZEISS MultiSEM control PC as it is described in the ZEISS MultiSEM installation requirements. The company ZEISS is not responsible and is not liable for any damages that may be caused by the presence of security vulnerabilities with regard to the MultiSEM control PC.

Network Communication The microscope can be integrated into different types of networks. For more information about supported hardware and software, such as glass fiber communication, file systems, or operating systems, contact a ZEISS service representative.

Scripting Interface You can access ZEN MultiSEM and control the microscope from your own programs or applications, for example via a scripting language such as Python. You can run your programs on the control computer or from any computer that has access to the control computer via the network. For more information, contact a ZEISS service representative.

4

Software Overview

4 Software Overview

4.1 Introduction

This chapter provides a brief introduction to using ZEN MultiSEM. It describes the general screen layout, most common global user interface elements, as well as how to start or stop the software.

ZEN MultiSEM is workflow-driven. The majority of the workflow steps have a corresponding tab and tool window.

4.2 Screen Layout

The following screenshot indicates the main elements of the ZEN MultiSEM user interface:

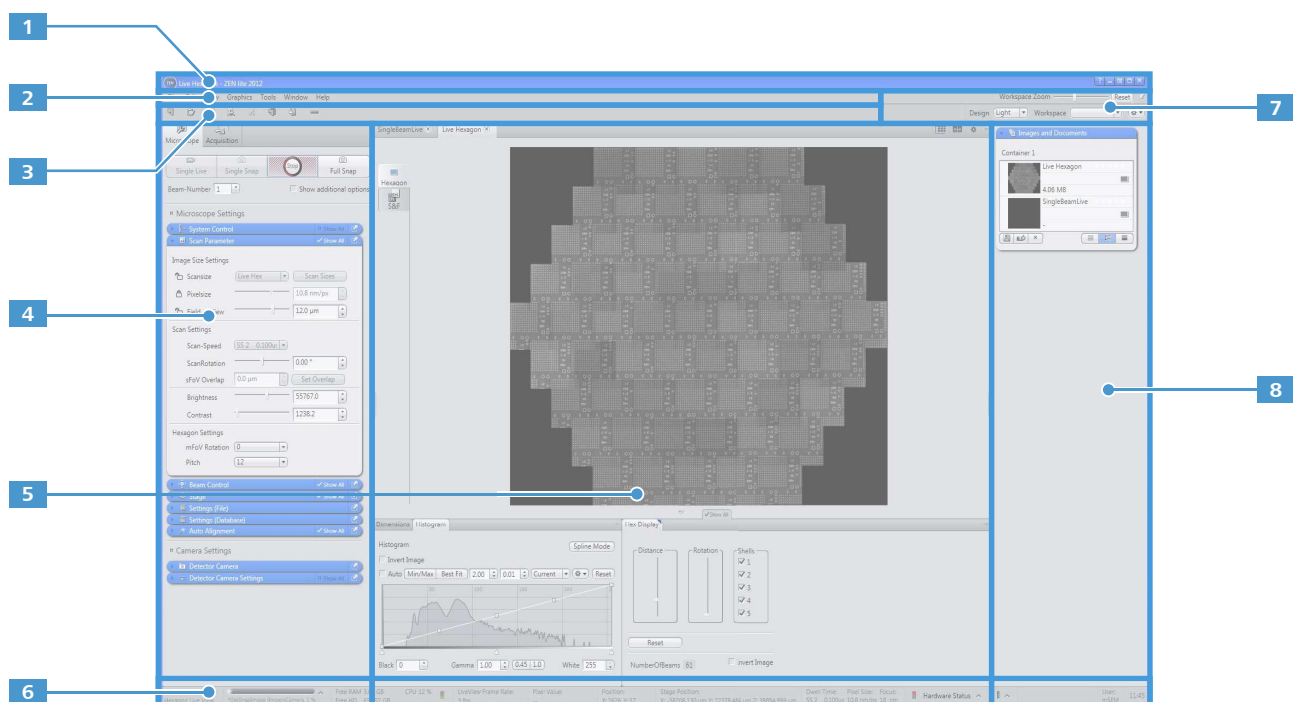


Fig. 4.1: Screen layout of the user interface

- 1 Title Bar**
Contains most basic functions such as closing or minimizing the user interface
- 2 Menu Bar**
Allows you to manage, edit, and view your work in ZEN MultiSEM

- 3 Tool Bar**
Allows you to save, open and search for files
- 4 Left Tool Area**
Contains tabs that are organized according to the current workflow.
Each tab contains tool windows that allow you to control the most important functions of the microscope.
- 5 Center Screen Area**
Displays images (live images or acquired snapshots).
This area is also used to register specimens.
- 6 Status Bar**
Shows various parameters that inform you about the system status
- 7 Workspace Configuration Area**
Allows you to configure the workspace and to save and load workspace configurations
- 8 Right Tool Area**
Contains the image library, a small image of the chamber camera, and a list of the most recent experiments

4.3 Basic Software Functions

ZEN MultiSEM allows you to adjust the user interface according to your requirements or to your favored settings.

4.3.1 Workspace Configuration Area

The **Workspace Configuration Area** allows you to configure your workspace, as well as to save and load your personal settings as a workspace configuration.

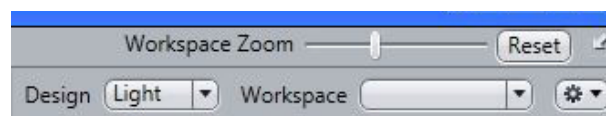


Fig. 4.2: Workspace Configuration

Workspace Zoom Use the zoom slider to vary the element size within the workspace between 70% and 150% of the original size.

Click **Reset** to reset the zoom to default (100%).

Design Select **Light** or **Dark** design to adapt the user interface to the lighting conditions in your laboratory.

Light is recommended for daylight conditions, **Dark** for low light conditions.

Workspace Save and reload all your personal settings as a workspace configuration.

Quickly load previously saved workspace configurations using the dropdown menu.

Dock all Undocked Tool Windows The **Dock all tool windows** icon in the top right corner enables you to dock all undocked tools to the **Left Tool Area** with one click.

4.3.2 Adjusting the Tool Windows

Expand/collapse Click the **expand/collapse** icon or click in the header to expand/collapse the tool window. This only works if the tool window is docked to its place in the **Left Tool Area** or **Right Tool Area**.

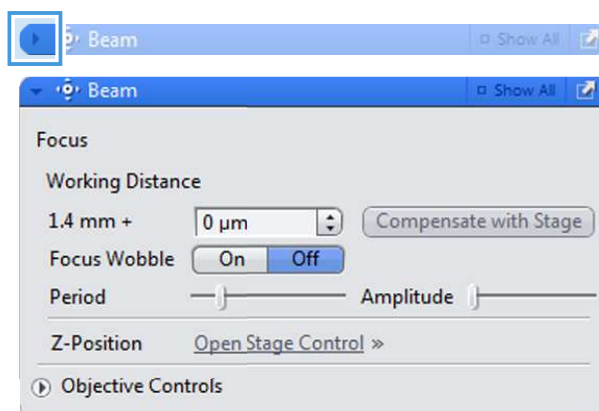


Fig. 4.3: Expanding/collapsing a tool window

You can also expand/collapse certain sections in the tool window by clicking the **expand/collapse** icon in this section.

Undock/dock Click the **undock** icon to undock a tool window.

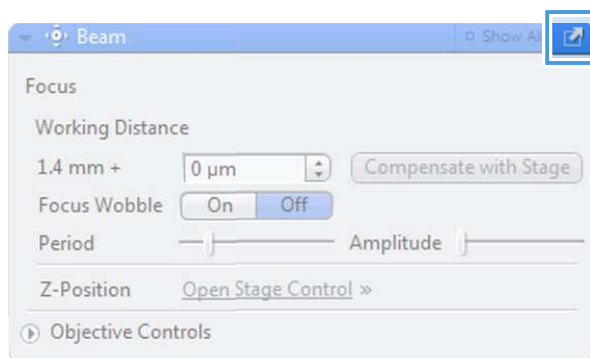


Fig. 4.4: Undocking/docking a tool window

You can also click and drag the header of the tool window.

You can position an undocked tool window anywhere on the screen, including on the second monitor.

Click the **dock** icon to dock a tool window back to its initial location.

Show All Click **Show all** in a tool window to show advanced settings or functions.

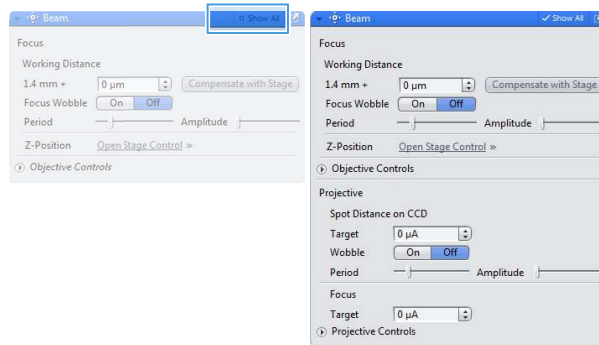


Fig. 4.5: Displaying the advanced settings of a tool window

4.3.3 Show or Hide Areas

Click the following tabs to show or hide the corresponding areas.

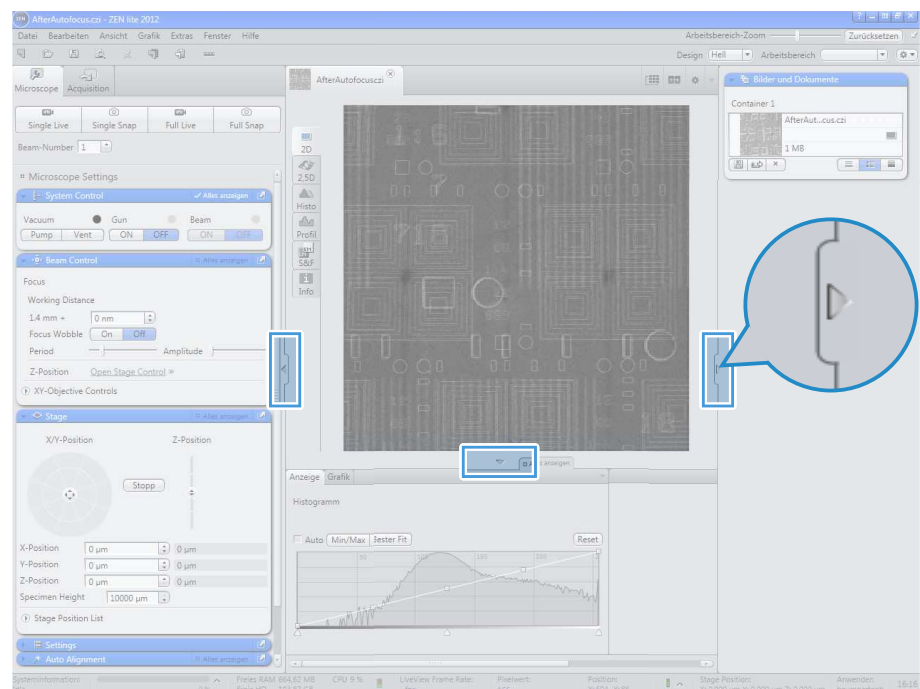


Fig. 4.6: Showing/hiding areas in the software

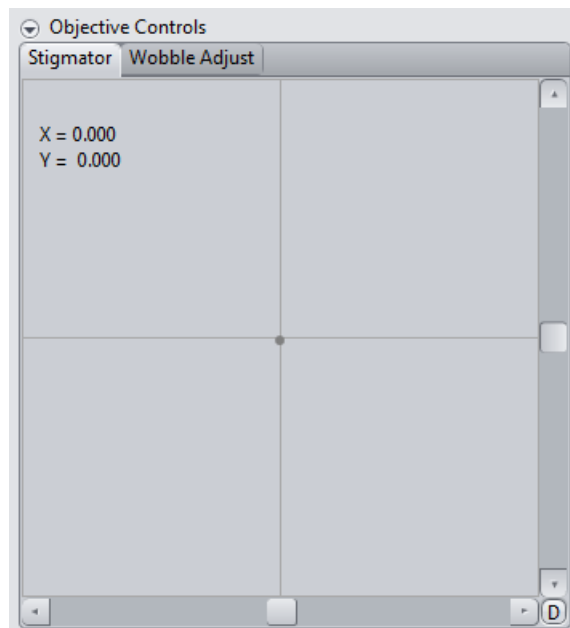
4.3.4 2D Controls

The 2D Controls enable you to use the mouse or keyboard to adjust the different beam deflectors installed in the microscope or the stage in X and Y direction. Each control displays a coordinate system containing a dot that represents the deflector, stigmator, or stage position.

Deflector Controls The deflector controls for the objective and projective are available via the **Beam** tool:

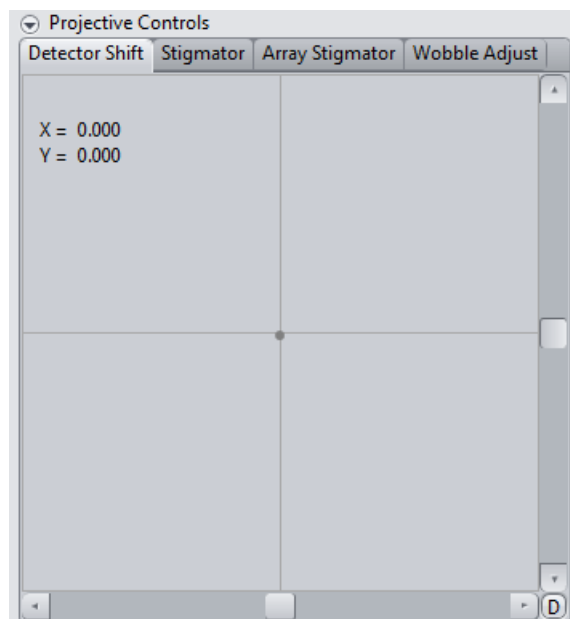
■ Objective Controls

Contains 2D Controls for each adjustable stigmator of the objective.



■ Projective Controls

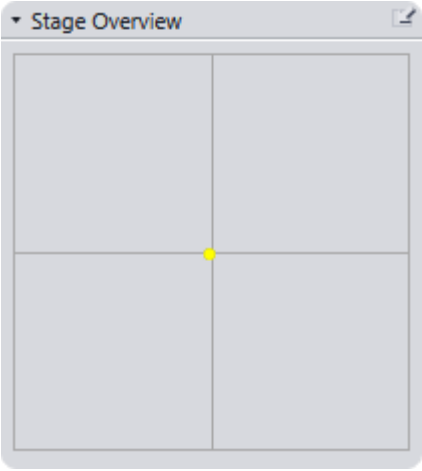
Contains 2D Controls for the adjustable projective settings.



The deflection is displayed in X and Y coordinates inside the control. The **D** button in the lower right corner resets the deflector to its default position.

For information how to operate the control using the keyboard or mouse, see **Using the 2D Controls** below.

Stage Overview Control



The **Stage Overview** is available via the **Overview** button of the **Stage** tool. You can use it as an alternative to the **Navigation Circle** control.

The resulting stage position is automatically updated in the text boxes for the X and Y coordinates.

For information how to operate the control using the keyboard or mouse, see **Using the 2D Controls** below.

Using the 2D Controls The 2D Controls can be operated using the mouse or keyboard as follows:

Input	Description
<i>CTRL</i> + Left Mouse Button	Enables you to control the deflection/stage position by dragging or clicking the dot to the desired position
Arrow Keys	Enables you to change the deflection/move the stage. To access the control via the keyboard, you need to click it once. The dot is displayed in red.
<i>SHIFT</i> + Arrow Keys	Enables you to coarsely change the deflection or move the stage. To access the control via the keyboard, you need to click it once. The dot is displayed in red. The rate of change is increased by a factor of 10.
<i>CTRL</i> + Arrow Keys	Enables you to finely change the deflection or move the stage. To access the control via the keyboard, you need to click it once. The dot is displayed in red. The rate of change is decreased by a factor of 0.1.
<i>CTRL</i> + <i>SHIFT</i> + Arrow Keys	Enables you to very finely change the deflection or move the stage.

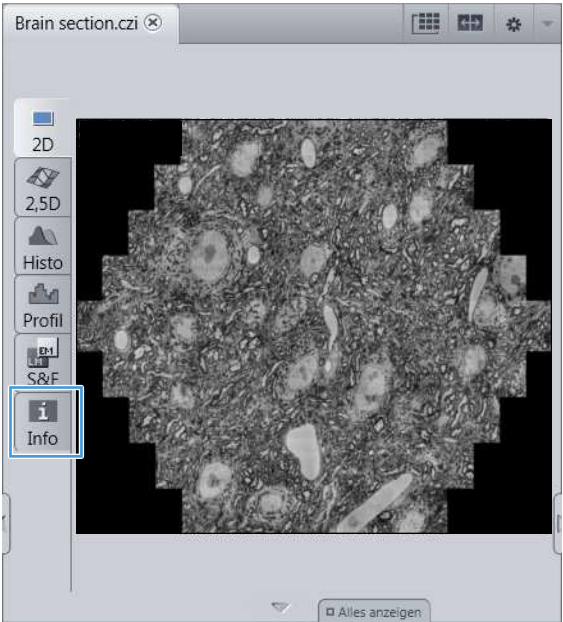
Input	Description
	To access the control via the keyboard, you need to click it once. The dot is displayed in red.
	The rate of change is decreased by a factor of 0.01.

4.3.5 Adding Meta Information to an Image

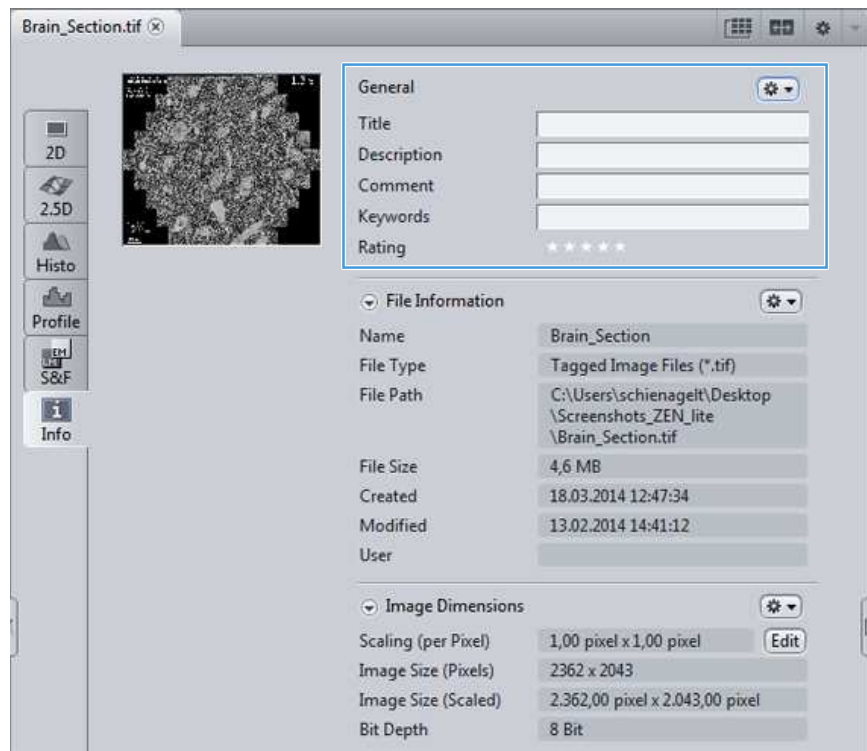
You can add meta information to an image such as a title or a description. This applies for both single images and full images.

Prerequisite ■ The image has been acquired.

- Procedure**
- 1 Open the image to which you want to add a scan note.
 - 2 At the left side of the image viewer, click the **Info** tab.



The image meta data information is displayed.



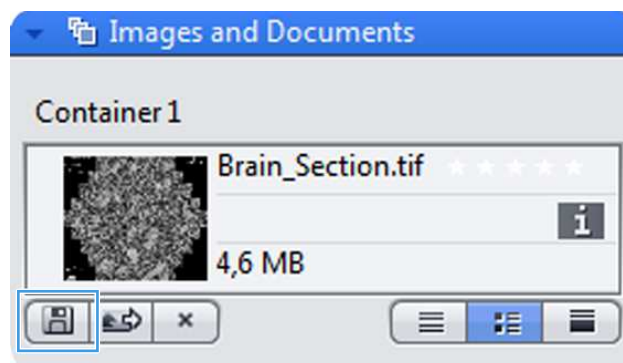
- 3 In the **General** section, enter the desired information.

You can also add graphical markers to an image (see *Basic Software Functions* [► 78]).

4.3.6 Saving an Image

Prerequisite ■ The image has been acquired.

- Procedure**
- 1 In the **Images and Documents** tool window, select the desired image.
 - 2 Click the **Save** icon.



- 3 Enter a file name and select the desired image format.

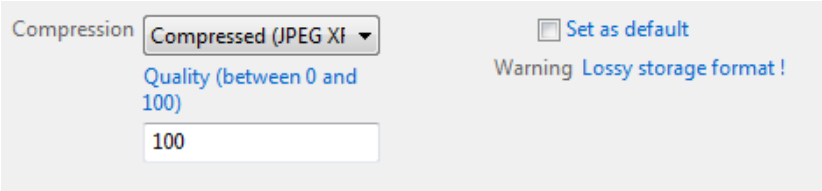
The recommended formats are:

- Carl Zeiss Image (.czi)
- Portable Network Graphics (.png)

- Windows Bitmap (.bmp)

4 If you select Carl Zeiss Image, you can compress the image:

- From the **Compression** drop-down menu, select **Compressed**.

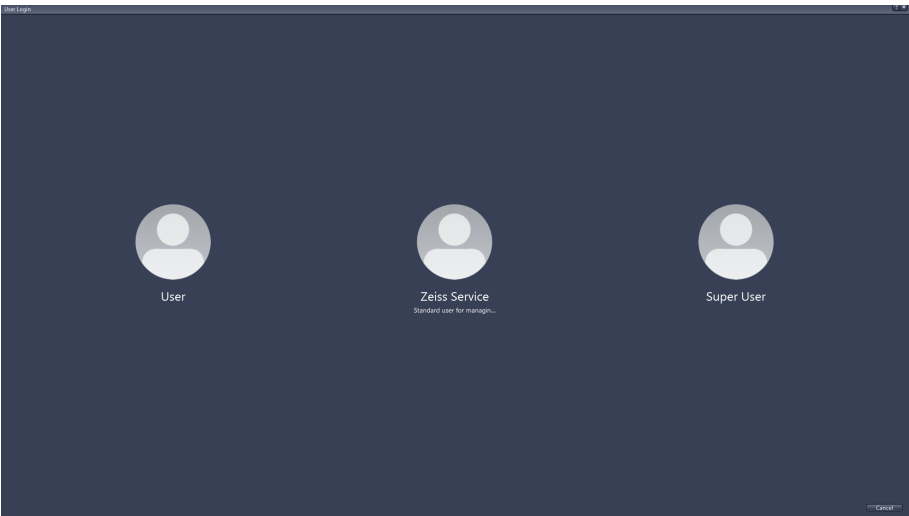


- Enter a quality level between 0 (worst) and 100 (best).

5 Click **Save**.

4.4 User Management

By default, ZEN MultiSEM is installed with user management. At the start of the software the **User Login** screen is displayed that requires you to log in with your password.



There are three different user levels available that control the actions that users may or may not perform:

User level	Description
User	Standard user class with access to standard functions required to operate the microscope
Super User	Microscope administrator with access to all user functions
Zeiss Service	Only for Carl Zeiss service representatives. Only Zeiss Service can create new user accounts.

4.5 Access Rights of Super Users

The following table gives you an overview of the software functions that are only accessible for super users:

Access	Tool/Window	Function
Microscope tab	System Control	Gun On/Off
		Slow down BeamOn
	Beam	Projective Controls
	Automated Routines	KPI Table
Acquisition tab	Tiles	Options > Tile Travel Direction
		Options > Start Position
Accessories tab	Beam Current Measurement	The Beam Current Measurement tool is only visible for Super Users.
Menu Bar > Tools	ImageAcquisition Configuration	The ImageAcquisition Configuration is only available for Super Users.

Tab. 2: Software functions limited to Super Users

4.6 Message List

The message list keeps track of the system status and displays errors and warnings:

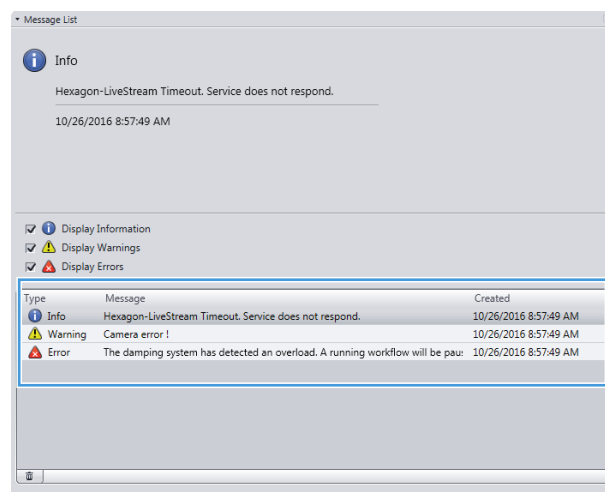


Fig. 4.7: Message list

The following notices and warnings can occur:

Icon	Type	Description
	Information	General information about the state of the system
	Warning	Warnings about failures of the system
	Error	Severe errors that need to be acknowledged by the user

Warnings and errors pop up on the screen during your work session:

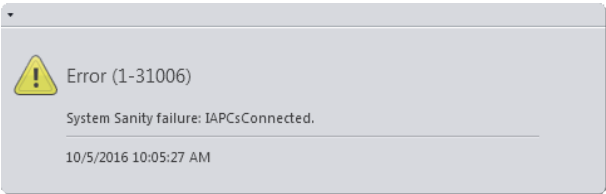


Fig. 4.8: Warning pop-up

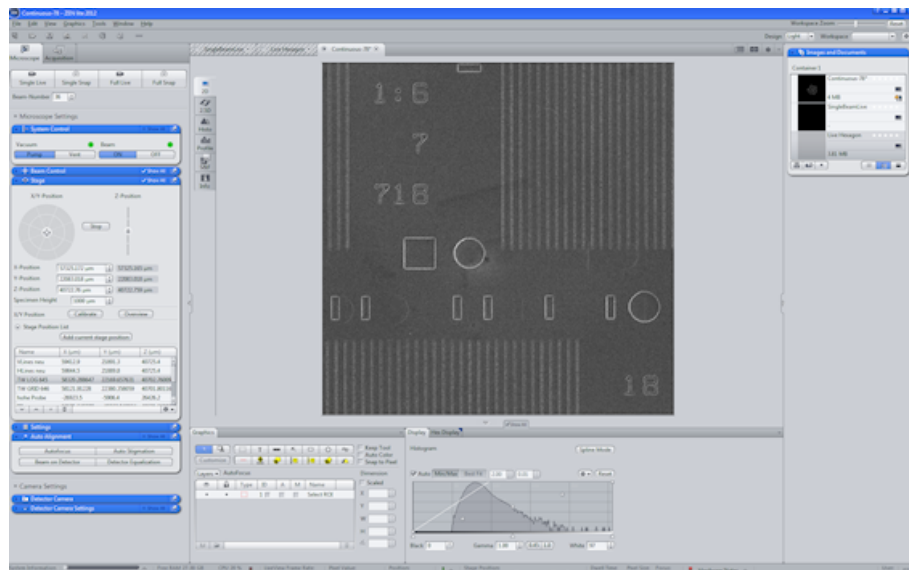
4.7 Starting the Software

The following describes how to start or restart ZEN MultiSEM. For information about how to start the complete ZEISS MultiSEM system, refer to *Powering Up the Microscope* [► 111].

- Prerequisite**
- You are logged on to the operating system on the control computer.
 - ZEISS MultiSEM is in the **ON** operating state.

- Procedure**
- 1 Double-click the ZEN MultiSEM icon on your desktop.
 - 2 Log in as a user or a super user with password.

ZEN MultiSEM including the *mEMService* software are started.



4.8 Closing the Software

Prerequisite ■ ZEISS recommends that you have finished and saved your current workflow within ZEN MultiSEM.

Procedure 1 In the upper right corner of the title bar, click the **Close** icon.

- or -

Select **File > Exit** in the menu bar.

2 Make sure *mSEMService* is shut down before you shut down or reboot the computer.

Right-click the *mSEMService* icon in the Windows **Taskbar Notification Area** and select **Shutdown Service** in the context menu.

Not shutting *mSEMService* down properly can affect some hardware modules and a reset of these modules is required.

ZEN MultiSEM and the *mSEMService* software are closed.

For information about how to power down the complete ZEISS MultiSEM system, refer to *Powering Down the Microscope* [► 111].

5

Principle of Operation

5 Principle of Operation

5.1 Introduction

This chapter introduces you to the basic principles of ZEISS MultiSEM. It provides an overview of the most important functional electron-optical components that are installed inside the microscope.

5.2 Functional Principle (Overview)

The general principle of operation of ZEISS MultiSEM is similar to that of a conventional scanning electron microscope (SEM). A conventional SEM uses a single electron beam to examine the surface of a specimen whereas ZEISS MultiSEM uses multiple electron beams in parallel. This leads to a significantly faster image acquisition.

In both types of electron microscopes, an electron source generates an electron beam, referred to as the primary-electron beam, that is guided onto the specimen. The focused primary-electron beam then scans the specimen surface.

The primary electrons interact with the atoms on, or just below, the specimen surface. Interaction products, such as secondary electrons, backscattered electrons (BSEs), or X-rays, are subsequently emitted from the specimen (see *Source of the Detection Signal* [▶ 100]). Depending on the installed detector(s), one or more of the emitted beams serve as the detection signal.

The main difference between ZEISS MultiSEM and a conventional SEM is that ZEISS MultiSEM operates with multiple electron beams. This means that multiple primary-electron beams simultaneously impinge onto the specimen. As a result, a large area of the specimen can be examined simultaneously, leading to a significantly higher throughput.

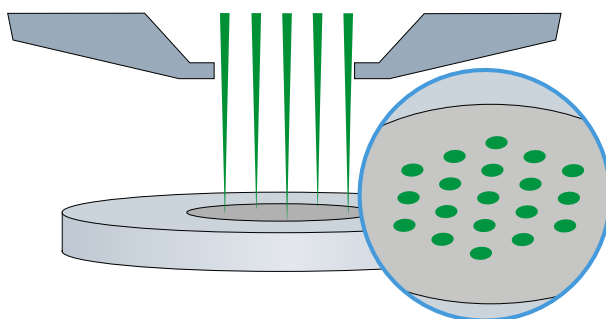


Fig. 5.1: Multi-beam principle

ZEISS MultiSEM is designed to only detect secondary electrons.

5.3 Illumination and Detection Optics

The ZEISS MultiSEM microscope is designed such that the illumination path and projection path are mostly separate.

The primary-electron beam is generated and focused in the illumination column. The secondary-electron beam is processed in the detection column.

Both beams pass through a section where both columns are joined. This section consists of the beam splitter and the objective lens. It both focuses the primary-electron beam from the illumination column onto the specimen and extracts the secondary-electron beam and directs it into the detection column.

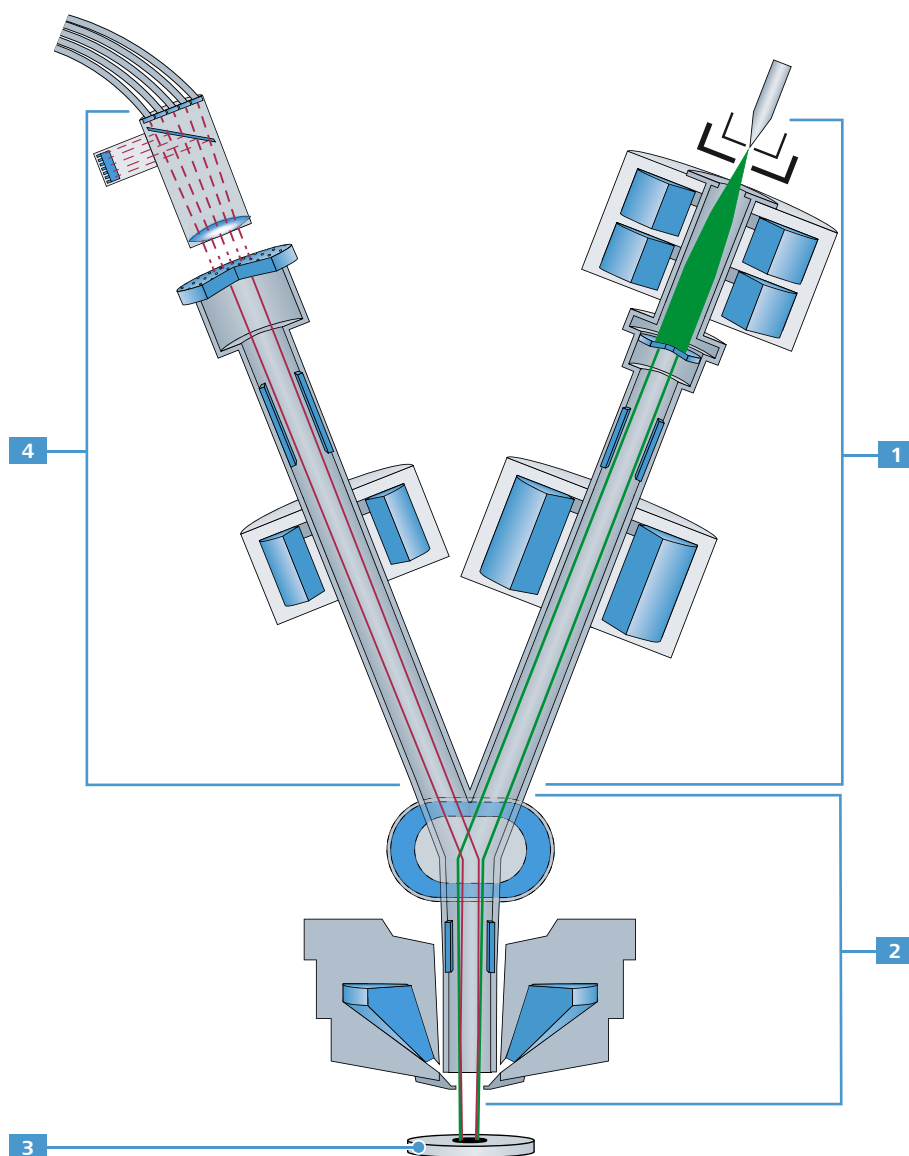


Fig. 5.2: Electron-optical layout of ZEISS MultiSEM

1 Illumination column

- 2 Section with beam splitter and objective lens
- 3 Specimen (inside specimen chamber)
- 4 Detection column

5.3.1 Components in the Illumination Column

The illumination column guides the primary-electron beam from the electron gun to the objective lens. It contains the following basic electron-optical components.

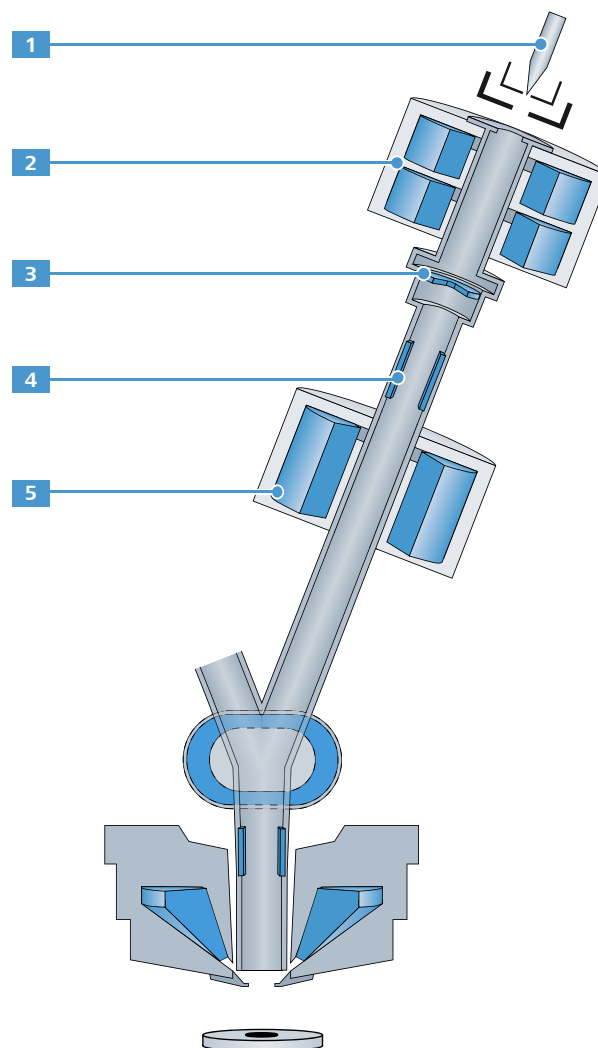


Fig. 5.3: Illumination column

- 1 Electron gun
- 2 Condenser
- 3 Micro optics
- 4 Beam blanker
- 5 Field lens system

Electron Gun The electron gun is the source of the electrons to illuminate the specimen.

A Schottky field emitter serves as the electron gun and injects the primary-electron beam into the illumination column. This comparatively small electron gun provides a beam with a low energy spread, high brightness, and long-term stability.

The actual emission source is a sharply pointed needle, the so-called tip. By applying a current to the electron gun's filament, the tip is heated to a temperature at which electrons are emitted due to thermal excitation.

In addition to the tip, the electron gun contains two further electrodes, the extractor and the anode aperture. A potential difference between tip and extractor electrode extracts and accelerates the electrons (see *Applied Voltages* [► 98]). The emitted electrons form the primary-electron beam. The primary-electron beam passes through the anode aperture before it enters the subsequent condenser. The anode aperture defines the maximum possible beam current.

The electron emission is regulated by the voltage on the extractor electrode (U_{EXT}).

The design of the Schottky field emitter requires a good vacuum of about 9×10^{-9} mbar or better in the electron section (see *Vacuum System* [► 69]).

Condenser The double condenser system controls the illumination of the subsequent micro optics.

Micro Optics The micro optics are specially designed for ZEISS MultiSEM.

The micro optics act as a beam sieve; they split the incoming beam into the configured number of single-beams, thus forming the (primary) multiple electron beam.

Beam Blanker The beam blanker deflects the primary-electron beams into a beam trap as required, such that the flow of primary electrons to the specimen can be interrupted without actually turning off the beam. This reduces the amount of radiation received by the specimen when it is not being scanned.

When a sufficiently large deflecting voltage is applied to the beam blanker (i.e. the blanker is active), the electrons are intercepted by the beam trap.

Field Lens System The field lens system in the illumination column consists of several single electromagnetic lenses.

The field lens system and the objective lens focus the primary-electron beams to the desired spot locations and spot diameter onto the specimen. Focusing in this context means the overall focusing of the multi-beam array.

5.3.2 Beam Splitter, Scan Deflector, and Objective Lens

When the primary electrons exit the field lens system in the illumination column, they pass through a section that the secondary electrons also pass through on their way to the detection column. This section contains the following electron-optical components.

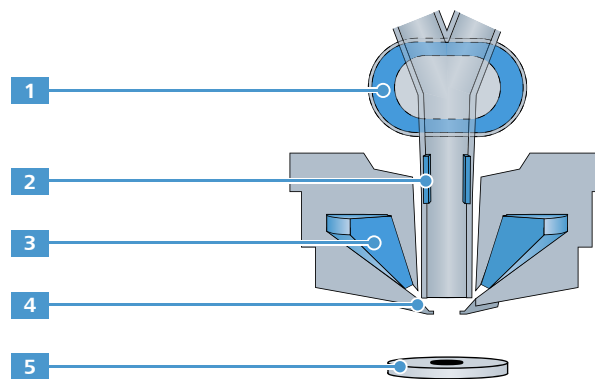


Fig. 5.4: Beam splitter, scan deflector, and objective lens

- 1 Beam splitter
- 2 Scan deflector
- 3 Objective lens
- 4 Objective lens opening
- 5 Specimen

Beam Splitter The principal function of the beam splitter is to separate the primary electron beams and the secondary electron beams.

The beam splitter is an arrangement of magnets that deflects the primary electrons from the illumination column into the objective lens. Additionally, it deflects the secondary electron beams coming from the objective lens into the detection column.

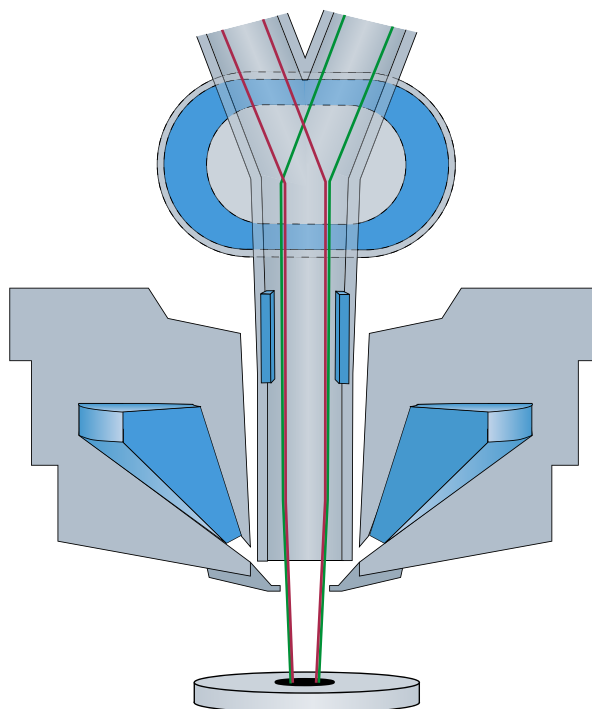


Fig. 5.5: Beam splitter (green lines: primary electrons; red lines: secondary electrons)

Scan Deflector The scan deflector is an electrostatic component consisting of capacitor plates. The electric field between the plates deflects the multi-beam array as a whole (in both the X direction and the Y direction), i.e. each single-beam is deflected by the same amount.

The strength of the electric field can be regulated in order to deflect the beam more or less. This allows the scan deflector to move the multi-beam array during imaging, i.e. to scan the specimen.

Objective Lens The objective lens is the final component that focuses the primary-electron beam onto the specimen. It controls the final size and position of the multi-beam array.

The objective lens is located such that its lower conical end extends into the specimen chamber.

The objective lens opening serves as the exit for the primary electrons and as the entrance for the secondary electrons emitted from the specimen.

Specimen The specimen is located in the specimen chamber beneath the objective lens.

When the primary-electron beam hits the specimen, secondary electrons are emitted.

5.3.3 Components in the Detection Column

The detection column guides the secondary-electron beams from the beam splitter to the light optics. It contains the following electron-optical components.

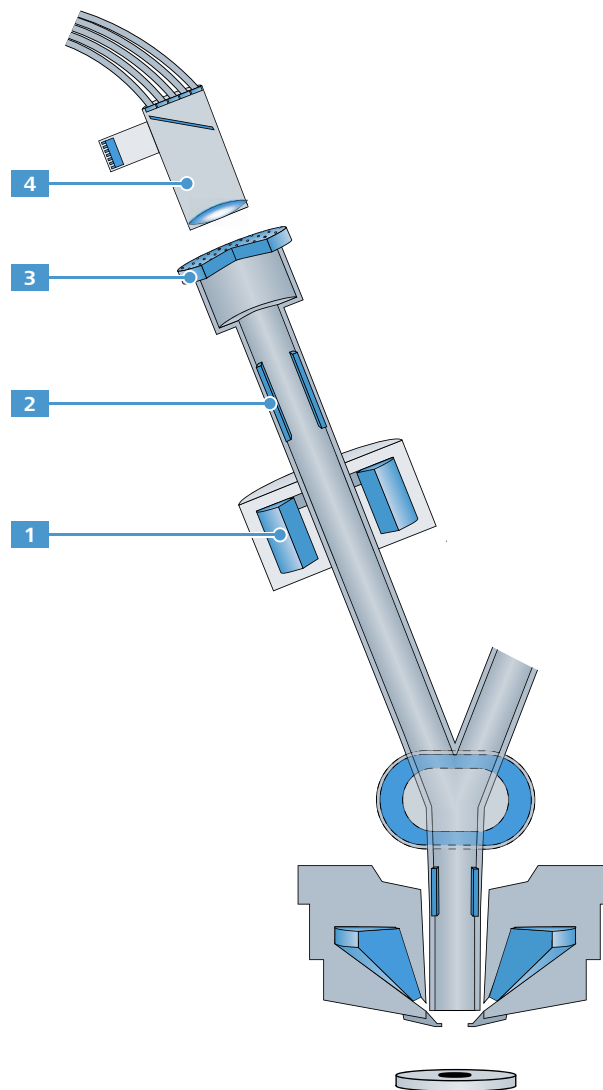


Fig. 5.6: Detection column

- 1 Projective lens system
- 2 Anti-scan
- 3 Scintillator
- 4 Light optics

Projection Path The projective lens system in the detection column focuses the secondary-electron beam onto the scintillator.

Anti Scan The anti scan is an electrostatic component consisting of capacitor plates. The adjustable electric field between the plates deflects the (secondary-electron) multi-beam array as a whole (in both the X direction and the Y direction).

The anti scan compensates the motion of the primary-electron beams on the specimen caused by the scan deflector such that the secondary electrons of each single beam always hit the same spot on the scintillator.

Scintillator The scintillator converts the secondary electrons into photons, i.e. into light signals. The result is a light-signal beam array consisting of the configured number of single-beams.

The light-signals are then further processed by the light optics.

Light Optics The light optics at the end of the detection column couple the light signals from the scintillator into the fiber-optics bundle.

A light-optical beam splitter separates parts of the light signals and thus enables the beam pattern to be monitored with the detection CCD camera (see *Detection CCD Camera* [► 59]).

The fiber optics finally transfer the light signal (i.e. the scan signal) to the detectors in the image acquisition rack.

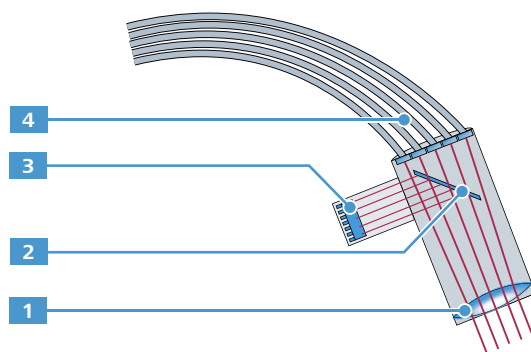


Fig. 5.7: Light optics

- 1 Optical lens
- 2 Light-optical beam splitter
- 3 Detection CCD camera
- 4 Fiber optics

5.4 Applied Voltages

The high-voltage supplies of ZEISS MultiSEM provide the high voltages that are required by some of the electron-optical components in order to extract and accelerate the electrons. These voltages are also used to adjust the landing energy of the primary electrons.

Voltages in Illumination Path The following figure shows the high voltages that are applied to accelerate the primary electrons in the illumination path.

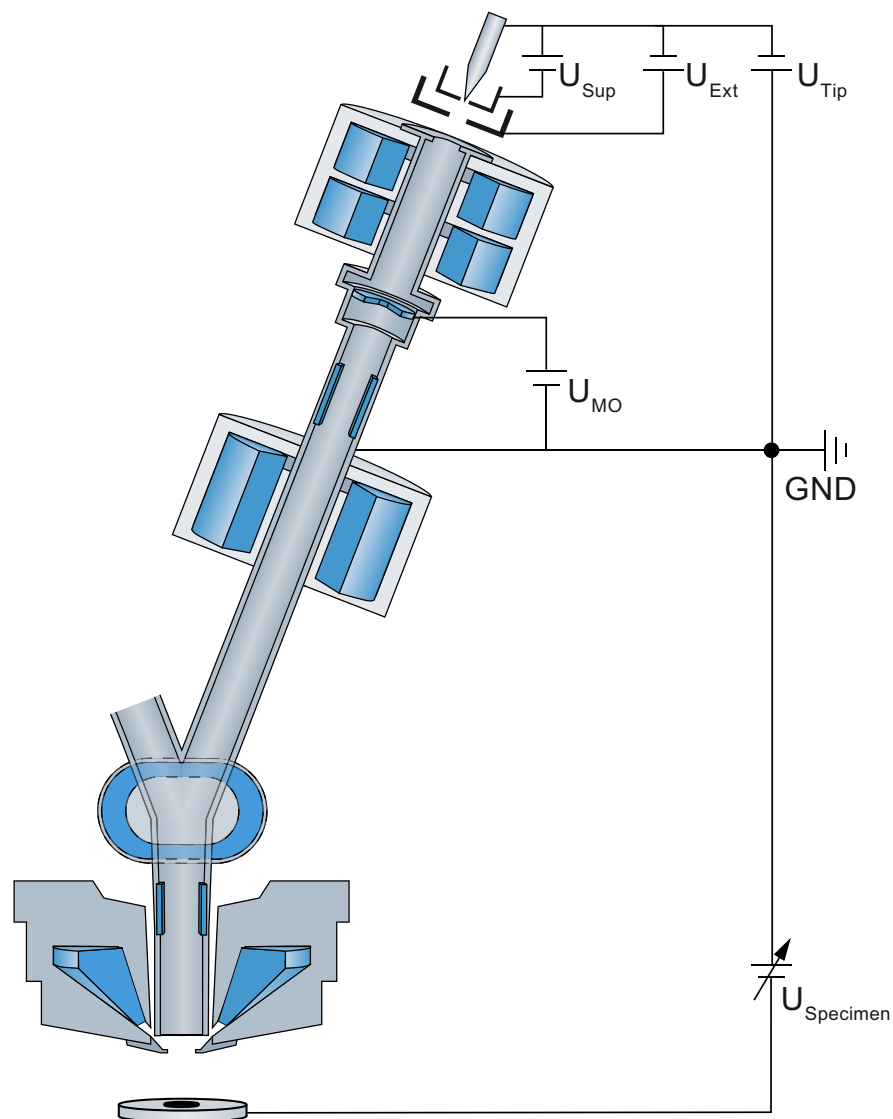


Fig. 5.8: Voltages in the illumination path

The voltage U_{Ext} is applied to the extractor of the electron gun to emit the primary electrons. Electrons from the heating filament and from the shaft of the emitter are repelled by the suppressor voltage U_{Sup} .

After the electron gun, the electrons are accelerated by the voltage $U_{Tip} = -30 \text{ kV}$.

The voltage $U_{MO} = +22 \text{ kV}$ is applied to the micro optics.

The voltage $U_{Specimen}$ is applied to the specimen holder. By setting $U_{Specimen}$ the landing energy ($U_{Tip} - U_{Specimen}$) can be adjusted between 1 keV and 3 keV.

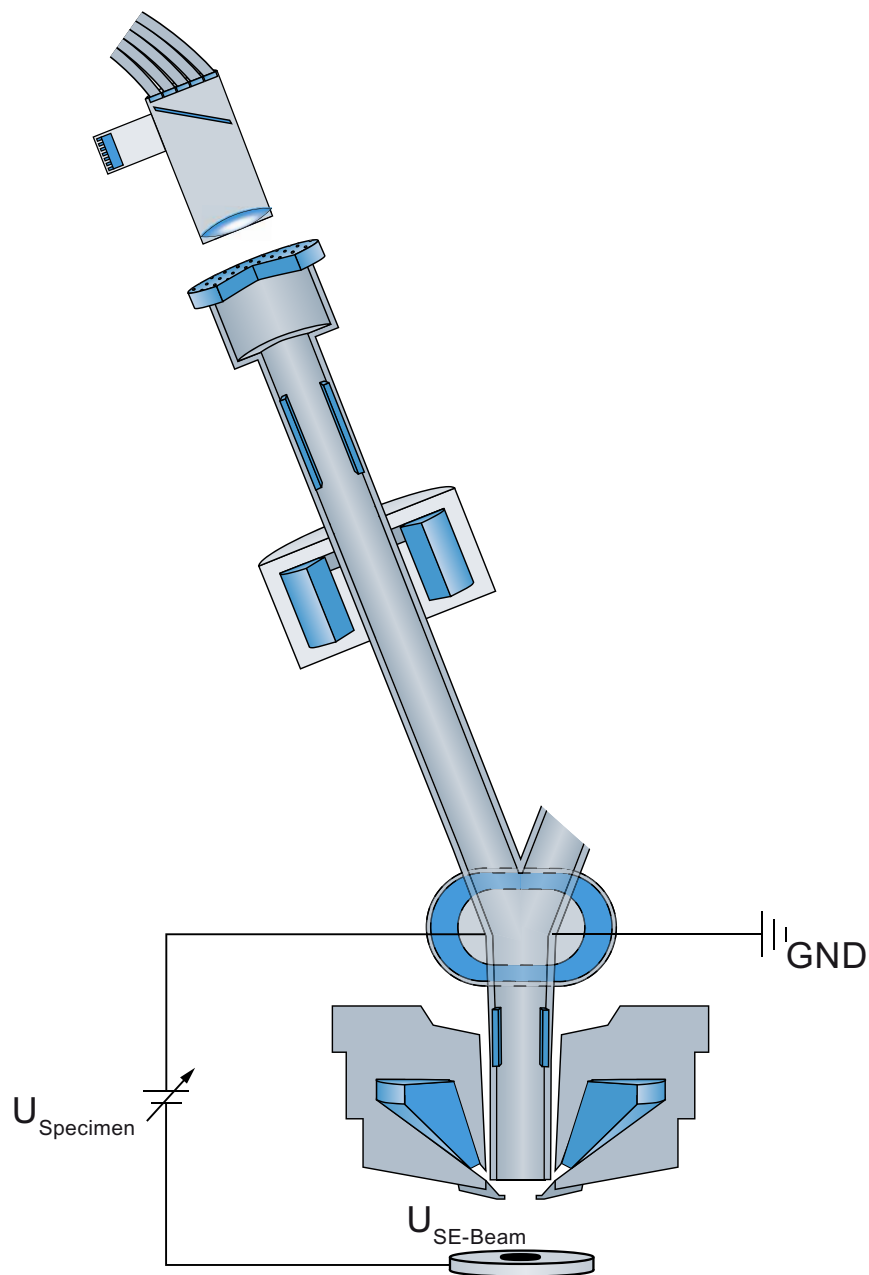


Fig. 5.9: Voltages in the projection path

5.5 Source of the Detection Signal

The incoming primary-electron beams hit the specimen with a landing energy between 1.0 and 3.0 keV (per electron). When an electron with this energy penetrates into the surface of the specimen, it is subjected to several elastic and inelastic scattering processes. The electron loses energy and slows down significantly.

The scattering and deceleration cause various types of particles and beams to be emitted from the specimen. The most important types are:

- Backscattered electrons
- Secondary electrons
- X-rays
- Bremsstrahlung

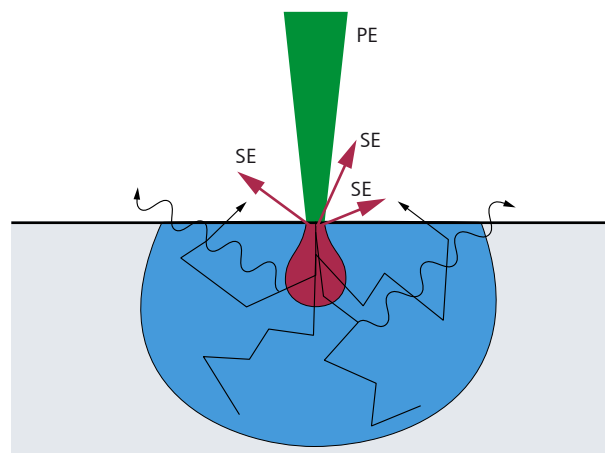


Fig. 5.10: Origin of secondary electrons (SE)

These emitted particles are typical for electron microscopy and can be used as detection signals.

In contrast to other electron microscopes, including many single-beam SEMs, ZEISS MultiSEM **exclusively detects the secondary electrons**. All other emitted particles and beams are ignored. Thus, the further explanation focuses on the secondary electrons only.

Secondary electrons Secondary electrons originate from the atoms that are located close to the specimen's surface. They are created and emitted as a result of the collision cascade between the primary electron and the specimen atoms.

Secondary electrons typically have a very low kinetic energy of between 1 eV and 50 eV (i.e. about a thousandth of the primary electron's energy).

The secondary electrons are emitted very close to the point where the incident primary electron hits the surface.

The electron optics is designed to efficiently collect these secondary electrons, which then generate the imaging signal.

Multi-beam characteristics ZEISS MultiSEM achieves its very high imaging throughput by using a multi-beam array of primary electrons. Hence, multiple single beams simultaneously hit the specimen (at different incident points) and simultaneously emit secondary electrons from the specimen (near the corresponding entry points).

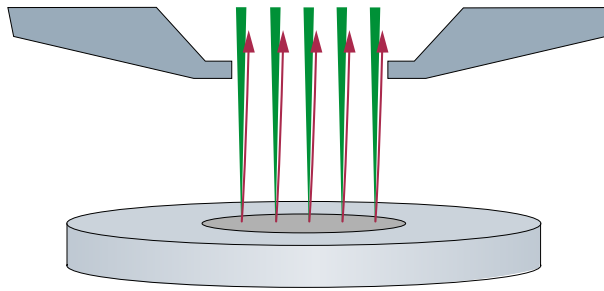


Fig. 5.11: Multi-beam principle

The secondary electrons exit the specimen surface very closely to the incident point of the corresponding primary-electron single-beam. The electron optical design ensures that they do not mix up with the secondary electrons from the other single-beams and thus provide the detection signal for the corresponding scan point.

The secondary-electron single-beams are injected into the projection path, where they are seen as separate individual scan spots.

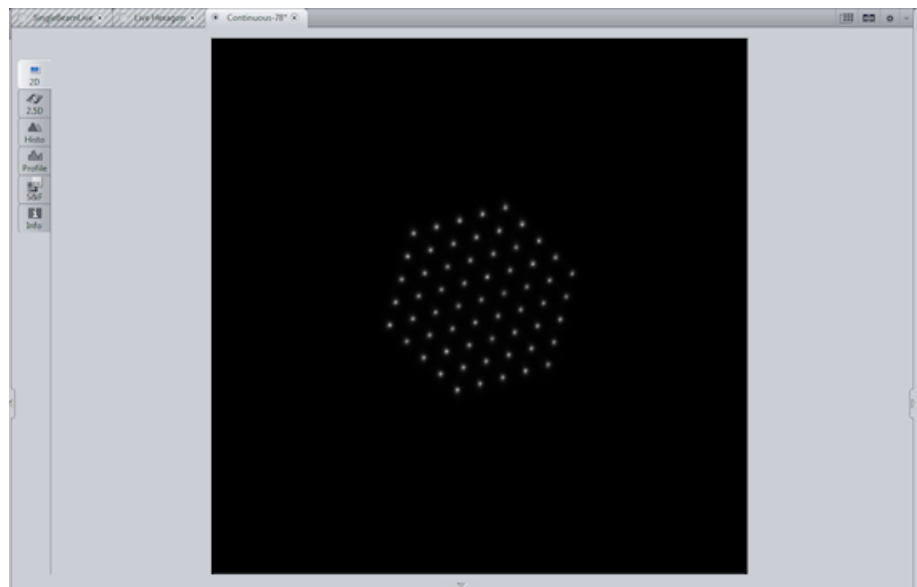


Fig. 5.12: Scan signal in detection column as seen with the detection CCD camera

5.6 Overview of Coordinate Systems

ZEISS MultiSEM contains the following Cartesian coordinate systems that are particularly relevant for beam alignment and specimen registration:

- System coordinate system
- Stage coordinate system
- Beam coordinate system
- Single image coordinate system

It is most important to understand how the coordinate systems are oriented relative to each other. Therefore, the following figures accurately reflect these orientations, whereas the location of the point of origin has sometimes been shifted for illustrative purposes.

System Coordinate System The system coordinate system is the fundamental coordinate system. It is fixed to the microscope and its axes are defined by orientation of the specimen chamber as shown in the following figure.

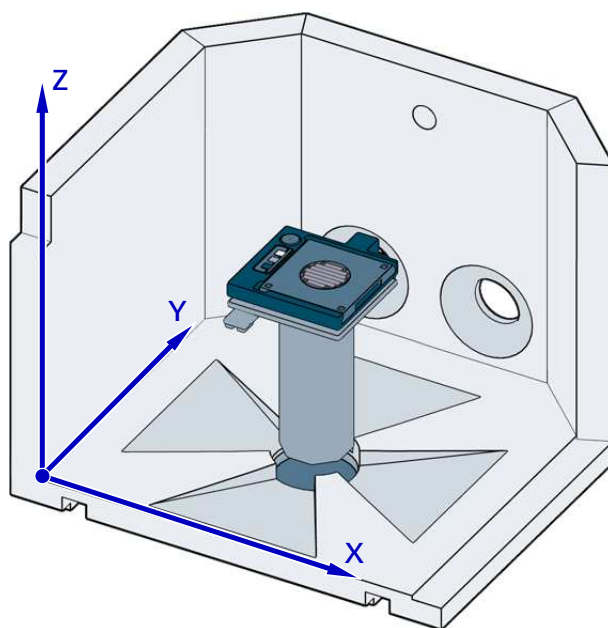


Fig. 5.13: System coordinate system

Stage Coordinate System The stage coordinate system describes the location of the specimen stage. In an ideal calibration, it has the same orientation as the system coordinate system, i.e. its X axis, for example, is parallel to the X axis of the stage coordinate system.

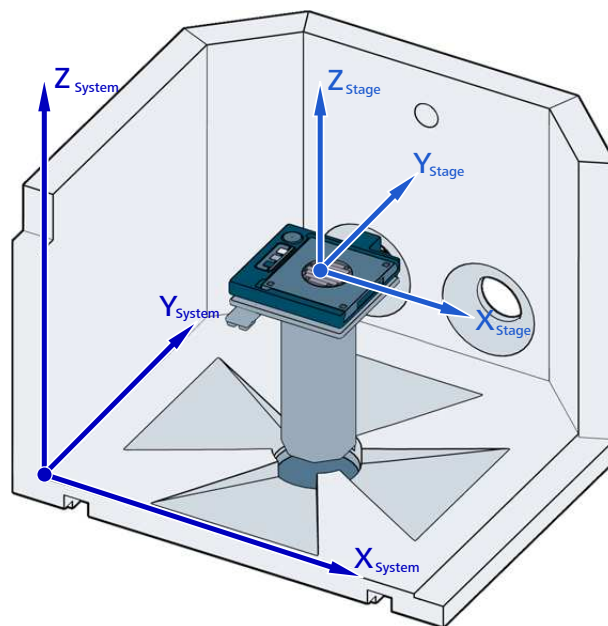


Fig. 5.14: Stage coordinate system

As the specimen stage cannot be rotated, the stage coordinate system can only be translated relative to the system coordinate system.

The lowermost possible Z coordinate is 0, i.e. you cannot set a negative Z value for the specimen stage in ZEN MultiSEM.

The following figure shows the approximate location of the point of origin with respect to the specimen holder mount.

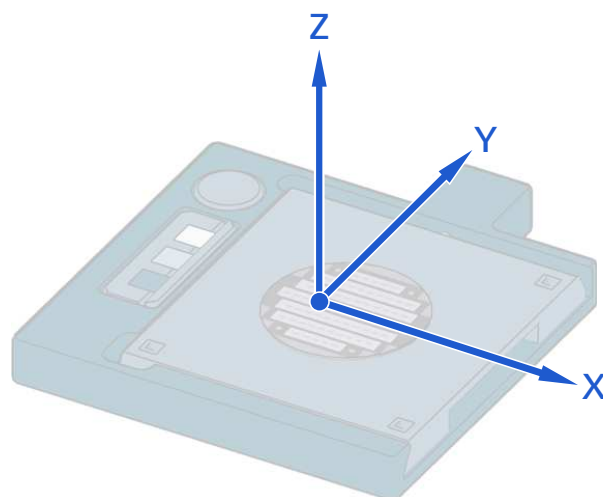


Fig. 5.15: Location and orientation of the stage coordinate system

Beam Coordinate System The beam coordinate system describes the location and orientation of the multi-beam array. It is attached to the multi-beam hexagon.

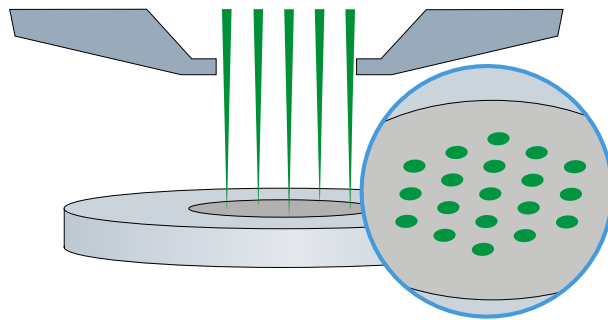


Fig. 5.16: Multi-beam hexagon

The beam coordinate system is defined as follows:

- The point of origin lies in the center of single-beam number 1.
- The X axis points from the center of single-beam number 1 to the center of single-beam number 2.
- The Y axis is perpendicular to the X axis, in the mathematically positive direction (i.e. ccw).

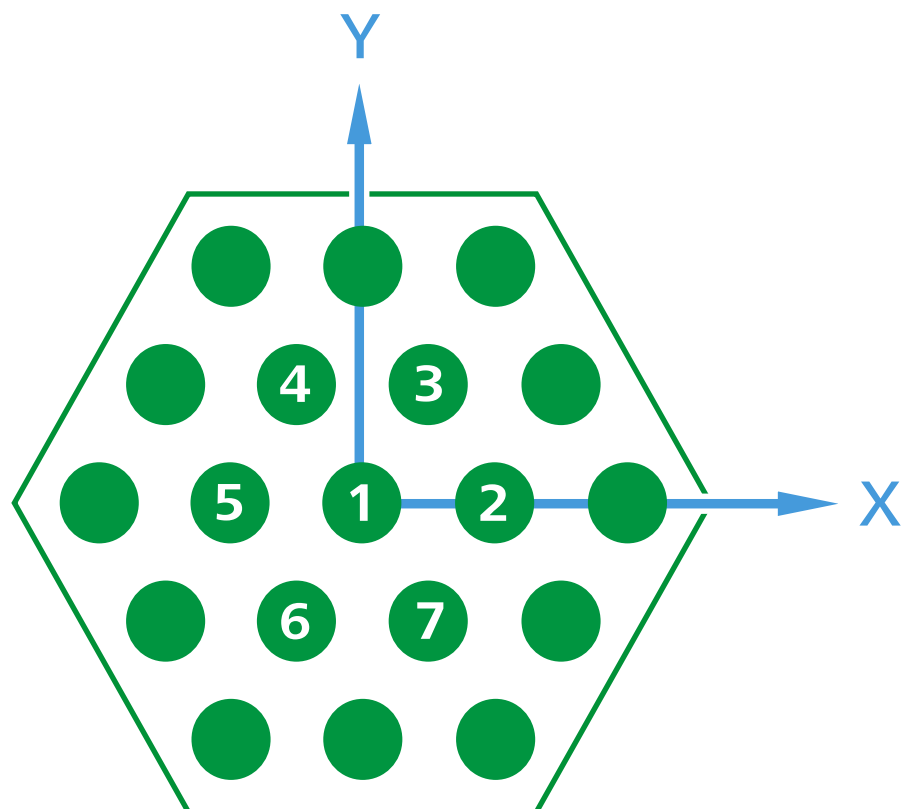


Fig. 5.17: Beam coordinate system

Single Image Coordinate System The single image coordinate system describes the coordinate system within the single images, i.e. the orientation of each of the live image of the single-beams.

The single image coordinate system is a two-dimensional coordinate system. This means, only the orientation of the X and Y axes is relevant. Note that the Y axis goes from the top left corner of the image to the lower left corner.

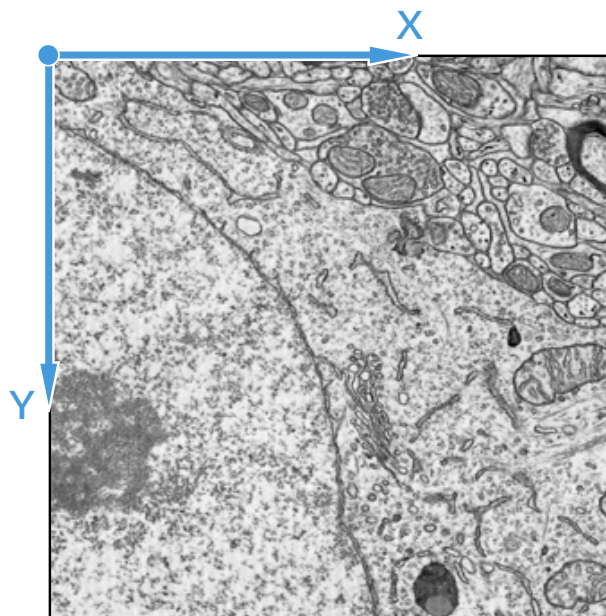


Fig. 5.18: Single image coordinate system

The single image coordinate system applies to all single images, i.e. to all single-beams. It is not possible to rotate a single image relative to another single image.

The single images are combined to form the full image. The single image is also referred to as single-beam Field of View (sFoV). The full image is also referred to as multi-beam Field of View (mFoV).

Ideal Calibration If ZEISS MultiSEM is calibrated, the coordinate systems are aligned as follows (the system coordinate system is neglected):

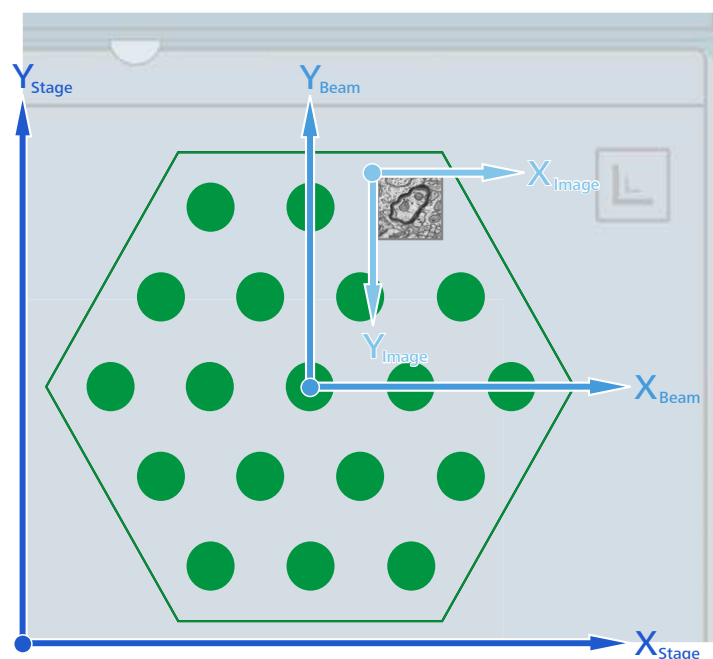


Fig. 5.19: Relationship of the coordinate systems in ideal calibration

6

Powering Down/Up the Microscope

6 Powering Down/Up the Microscope

6.1 Introduction

This chapter describes how to power down and power up ZEISS MultiSEM to and from STANDBY mode respectively.

6.2 Handling the High Voltage

The following high voltages inside the ZEISS MultiSEM can be controlled separately:

- Beam HV

This high voltage is applied in order to extract and accelerate both the primary electrons and the secondary electrons (U_{Specimen} and U_{OL}).

- Gun HV

This high voltage powers the electron gun (U_{Tip}).

For more information, see *Applied Voltages* [► 98].

You can control both high voltages in ZEN MultiSEM via the **System Control** tool window.

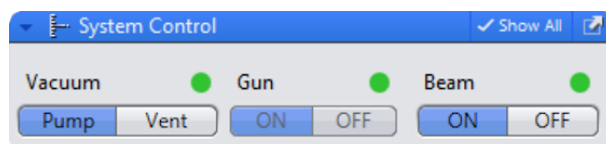


Fig. 6.1: System control tool window

High Voltages During Standby In STANDBY mode, the Beam HV is shut off, while the Gun HV remains switched on.

Safety Instructions for High Voltage ZEISS MultiSEM is secured with protective covers against hazardous voltage. Comply with the following instructions regarding the high voltage.

**DANGER****Danger to life: Hazardous voltage inside the microscope**

High-voltage potentials present in the microscope can be fatal. Contact may cause burn or electrical shock.

- ◆ Ensure proper grounding. For more information refer to the Installation Requirements document.
- ◆ Only authorized ZEISS service representatives are allowed to remove the microscope enclosure.

NOTICE

The Schottky field emitter is susceptible to startup currents.

Risk of component damage.

- ◆ Avoid switching off the gun for periods of under two days to optimize the lifetime of the Schottky field emitter.

6.3 STANDBY Mode and OFF Mode

STANDBY Mode ZEISS MultiSEM normally remains powered at all times. This means that illumination column and detection column are under vacuum, the electron gun is on, and all of the control electronics are operating.

In the STANDBY mode, the operating state of the following components changes:

- The Beam HV is off.
- The EO boards are off.
- The hardware controllers are in STANDBY mode.
- The air pressure in the vibrational isolation system is released.

The following components continue to run as in the ON mode:

- Electron gun, i.e. the Gun HV remains on and the filament will continue to be heated
- Vacuum pumps
- Image acquisition rack

OFF Mode Contact your ZEISS service representative if you want to completely shut ZEISS MultiSEM down to the operation mode OFF (see also *Basic Preventive Maintenance* [▶ 157]).

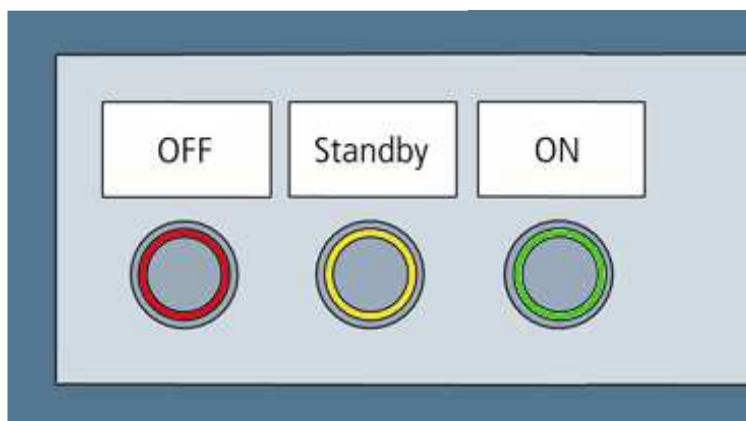
The operation mode OFF should only be used on rare occasions, e.g. during construction work in your laboratory. The OFF status means that the vacuum system is turned off completely. As a result you will have to wait for a long time until the vacuum is re-established. Additionally, all the vacuum sections will require bake-out.

6.4 Powering Down the Microscope

Powering down ZEISS MultiSEM means setting the system into the STANDBY mode. You should put the system into the STANDBY mode when ZEISS MultiSEM is not going to be used for an extended period of time, e.g. a few days.

- Prerequisite**
- ZEN MultiSEM is closed (see *Closing the Software* [► 88]).
 - The workstation computer is shut down.

- Procedure** 1 On the power supply rack, press the **STANDBY** button.



The **STANDBY** button flashes during the power-down.

- 2 Wait until the **STANDBY** button is permanently lit.

ZEISS MultiSEM is in STANDBY mode.

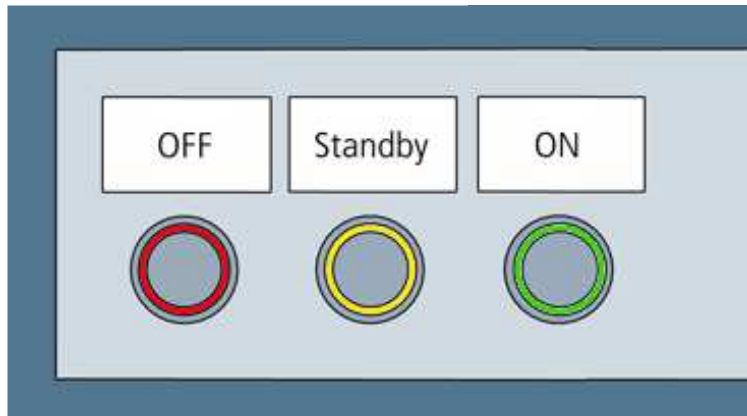
No system status information is available within ZEN MultiSEM, because there is no data transfer between the hardware and the software in the STANDBY mode.

6.5 Powering Up the Microscope

Powering up ZEISS MultiSEM will take several minutes.

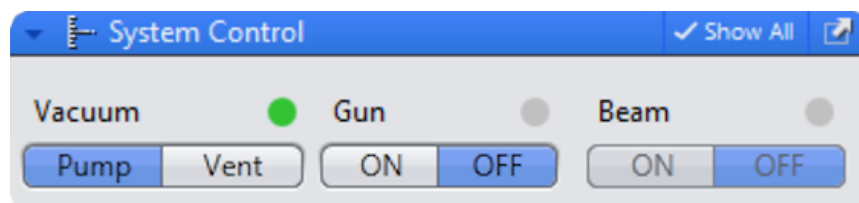
Prerequisite ■ ZEISS MultiSEM is in operation mode **STANDBY**.

Procedure 1 On the power supply rack, press the **ON** button.



The **ON** button flashes during powering on.

- 2 Wait until the **ON** button is permanently lit.
- 3 Start ZEN MultiSEM (see *Starting the Software* [▶ 87]).
- 4 In ZEN MultiSEM, check whether the electron gun is on.



If not, click **ON** in the **Gun** section.

- 5 In the **Beam** section, click **ON**.

For information about monitoring the vacuum sections, see *Vacuum System* [▶ 69].

ZEISS MultiSEM is switched on and ready for operation.

It is recommended that you perform the following checks, before you start your experiment:

- Is the beam pattern visible on the detection CCD camera, i.e. are the secondary electrons hitting the scintillator?
- Is the working distance set correctly?
- Is the specimen height set correctly?
- Is a specimen loaded into the specimen chamber?

6.6 Emergency Off (EMO)

6.6.1 Switching Off the Microscope in an Emergency

Procedure ◆ On the microscope cabinet, press the EMO button.

All power is cut off from the ZEISS MultiSEM except from the image acquisition rack.

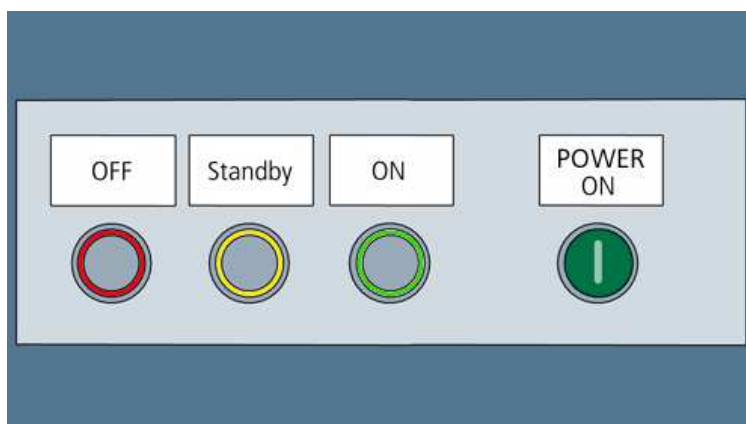
The EMO button remains in its pressed position.

6.6.2 Switching the Microscope On after an Emergency Off

INFO

Before switching the ZEISS MultiSEM on, ensure that the cause of the Emergency Off is eliminated and that it is safe to switch the ZEISS MultiSEM on.

- Procedure**
- 1 On the microscope cabinet, pull the EMO button to release it.
 - 2 Press the **POWER ON** button.



The ZEISS MultiSEM is switched on.



Handling of the Specimen

7 Handling of the Specimen

7.1 Requirements for the Specimen

In order to obtain useful images of a specimen, ensure that the specimen meets the following conditions:

Specimen parameter	Description
Area	$\leq 100 \times 100 \text{ mm}^2$ Areas up to the above value can be acquired completely. If a specimen is larger, it might fit into the microscope but cannot be imaged completely. This is due to the maximum traveling distance of the motorized stage.
Height	$\leq 30 \text{ mm}$
Flatness/Roughness	$\leq 500 \text{ nm} / 100 \text{ }\mu\text{m}$ (Peak-to-Peak)
Net weight	$\leq 0.2 \text{ kg}$
Conductivity	The specimen should be as conductive as possible. If the specimen is not conductive or not connected electrically to the specimen holder, it is charged electrostatically and the image quality is decreased.
Outgassing rate	The specimen and the related mounting equipment should have low outgassing rates. High outgassing rates may lead to a contamination of the high voltage insulators, which may cause arcing issues.

7.2 Mounting of the Specimen

To examine a specimen in the ZEISS MultiSEM, you need to mount the specimen onto a standard specimen holder before inserting it into the microscope.

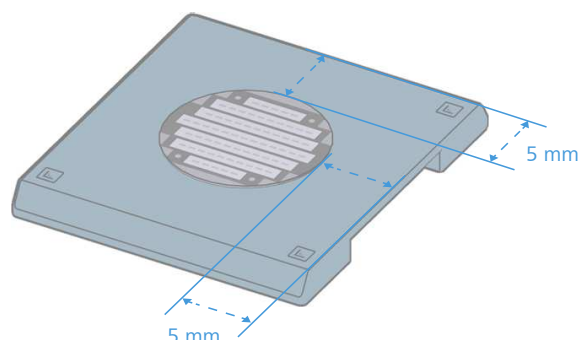


Fig. 7.1: Mounting of the specimen onto the specimen holder

Consider the following hints and guidelines when mounting the specimen onto the specimen holder:

- Make sure the specimen is connected conductively to the specimen holder.
Examples:
 - If your specimen is a piece of wafer, glue the corners to the specimen holder using an electrically conductive adhesive.
 - If your specimen consists of thin cuts of tissue, e.g. to perform serial array tomography, make sure the thin cuts are attached to a conductive substrate. Connect the substrate to the specimen holder using an electrically conductive adhesive.
- The minimum distance between the edge of the specimen and the edge of the specimen holder is 5 mm in each direction.
- Mount the specimen onto the specimen holder as flat as possible.
- Minimize the tilting of the specimen with respect to the specimen holder.
- If your specimen has a high profile and you want to acquire the entire specimen area including the edges, embed it into a frame consisting of conductive material of the same height. For example, if you want to image a wafer completely, attach pieces of the same wafer material to the edges.

High-profile specimen cannot be imaged completely due to field distortions at the edges. For example, if the specimen has a height of 1 mm, the outmost area of the specimen with a width of circa 1 mm cannot be imaged.

Embedding the specimen into a frame of the same material reduces the field distortions and improves the image quality at the edges.

INFO

You can find more detailed information about mounting specimens in the ZEISS MultiSEM Sample Preparation Guide:

https://p.widencdn.net/klyxoh/EN_wp_MultiSEM-505_Sample-Preparation-Guide

NOTICE

Risk of malfunction

Any adhesive causes a certain amount of outgassing. If you use unsuitable adhesives or if you expose too much adhesive tape to the vacuum, then the amount of outgassing may get too large. Due to the outgassing the high voltage insulators may get contaminated, which may cause high voltage arcing.

- ◆ Only use adhesives that are especially designed for vacuum applications and that have a low outgassing rate.
- ◆ Only use as much adhesive as required and avoid to expose too much adhesive tape to the vacuum.

7.3 Using the Plasma Cleaners

You can use the optional plasma cleaners to clean the specimen chamber, the airlock, or the specimen from undesired residues. Two plasma cleaners are available, one for the specimen chamber and one for the airlock.

For plasma cleaning, two different options are available:

■ Object Chamber

Activates/controls the plasma cleaner attached to the specimen chamber.

■ Multiport

Activates/controls the plasma cleaner attached to the airlock.

For both options, two cleaning modes are available:

■ Chamber cleaning

Parameter settings to clean the chamber or the airlock.

■ Specimen cleaning

Parameter settings to clean the specimen inside the chamber or the airlock.

INFO

Multiport is primarily used in combination with the **Specimen cleaning** mode to clean the specimen inside the airlock.

INFO

Object Chamber is primarily used with the **Chamber cleaning** mode to clean the specimen chamber.

INFO

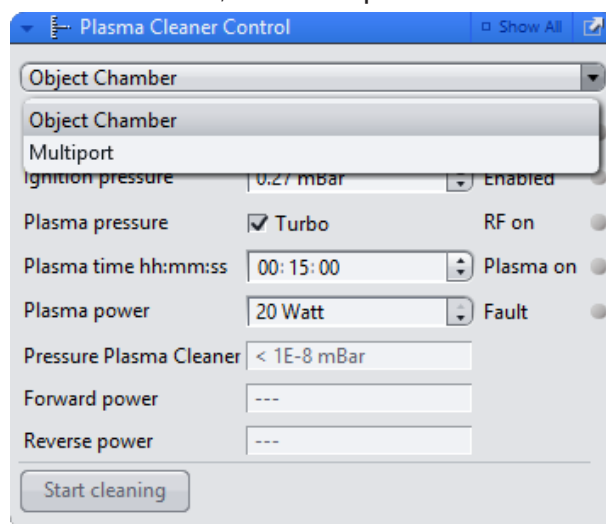
For each of the two default recipes the corresponding parameters are preset. If you select **Specimen cleaning**, the cleaning parameters are automatically set to values such that specimen damage should be avoided for most samples. To create individual recipes for plasma cleaning, you can manually adjust the parameters.

Prerequisite ■ The plasma cleaners are properly connected

Procedure 1 In the **Plasma Cleaner Control** panel, select the desired recipe:

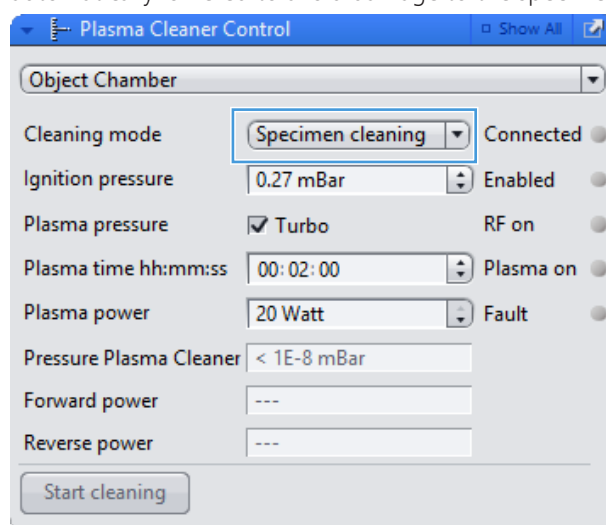
To clean the specimen chamber, select **Object Chamber**.

To clean the airlock, select **Multiport**.



2 If you want to clean the specimen itself, then select **Specimen cleaning** as the **Cleaning Mode**.

If you select **Specimen cleaning**, the **Plasma time** and the **Plasma power** are automatically lowered to avoid damage to the specimen.



- 3 If required, manually adjust the remaining parameters.
For example you can adjust a lower **Plasma time** or a lower **Plasma power** in case of very fragile specimens.
- 4 To start the plasma cleaning procedure, click **Start Cleaning**.



Alignment

8 Alignment

8.1 Introduction

This chapter describes the alignment workflow and principles of ZEISS MultiSEM. You should perform alignment when you change or insert a new specimen.

There are two different ways to perform the alignment:

- **Auto Tuning Routines**

If you insert a new specimen or navigate to a different position on the specimen, then you need to perform the **Auto Tuning Routines**.

- **Automated Routines**

If you plan to perform a sophisticated experiment, you should verify the KPIs via **Automated Routines > Check Working Point**. If the KPIs are not valid, you need to re-calibrate the working point via **Align Working Point**.

In most cases, the automated routines are sufficient to achieve an appropriate calibration of the working point and it is recommended that you mainly use the **Automated Routines** tool for alignment. Only if the automated routines are not successful, you should revert to the specialized tools, such as the calibration of coordinate systems available via the **Single Routines** tool window.

8.2 Purpose of Alignment

In order to achieve optimum image quality you need to make sure the microscope is adjusted as precisely as possible.

Auto Tuning If you are unsatisfied with the image quality, e.g. when the image is blurred, you can perform different alignment routines to improve it. The auto tuning routines are a set of one-click routines that can be applied quickly as a first measure to correct for common image errors. These routines are available via the **Auto Tuning** tool. The basic Auto Tuning functions are also accessible via the control panel.

Perform the auto tuning routines regularly, especially in the following cases:

- You have inserted a new specimen into the microscope

Whenever a new specimen is inserted, the vertical positioning is unknown and some basic settings are lost, such as the focus. Pre-adjusting using the detector CCD camera and applying the auto tuning routines can restore the basic settings.

- You have navigated to a different position of the specimen

The specimen surface differs slightly at different positions, e.g. surface angles and height can change across a specimen. The auto tuning routines re-adjust the basic settings to compensate for changes in the specimen surface.

Alignment and Calibration Certain image errors, such as ghost images in the full live image, may not be solved completely by applying the auto tuning routines. If such image errors occur or if KPIs are checked and display a red alignment status icon, align the corresponding microscope components.

If the auto tuning routines do not yield a satisfying result, use the more advanced alignment tools: Either the **Automated Routines** or the **Single Routines**. These tools give you more detailed access to microscope settings and contain the necessary alignment routines.

■ Automated Routines

The automated routines are the standard method to align the sections of the illumination and detection columns. Via the automated routines the KPIs of the aligned sections are updated. For more information about the automated routines also refer to Automated Routines.

■ Single Routines

The single routines include more specialized methods, e.g. calibration routines for the coordinate systems. For more information about the single routines also refer to *Single Routines* [► 128].

8.3 Auto Tuning Routines

Each time you insert a new specimen into the microscope, the microscope parameters need to be readjusted. The **Auto Tuning** tool contains a set of one-click routines which enable you to correct the microscope parameters automatically and to obtain a sharp image quickly.

The **Auto Tuning** tool contains the following routines which quickly optimize the scan settings:

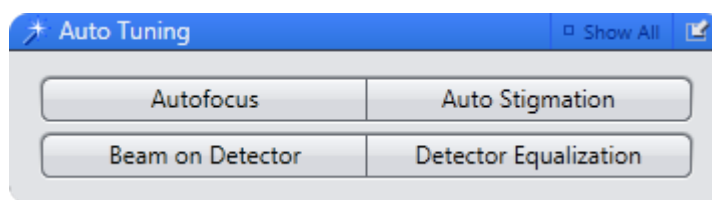
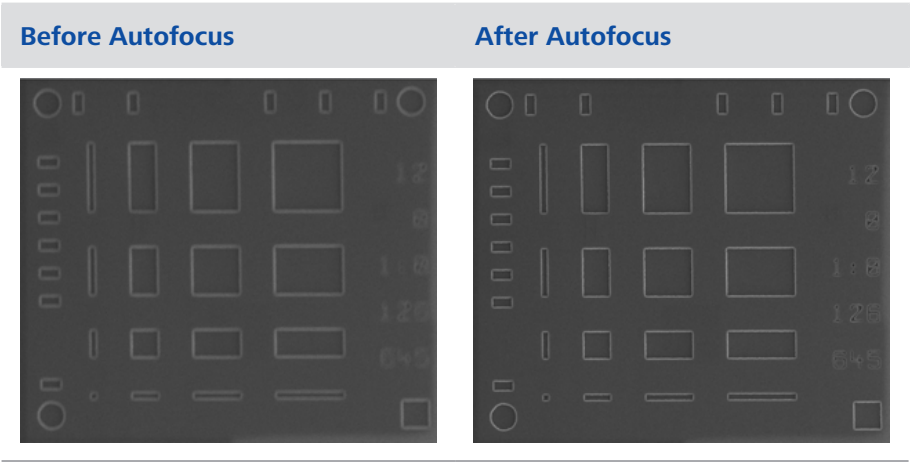


Fig. 8.1: **Auto Tuning** tool window

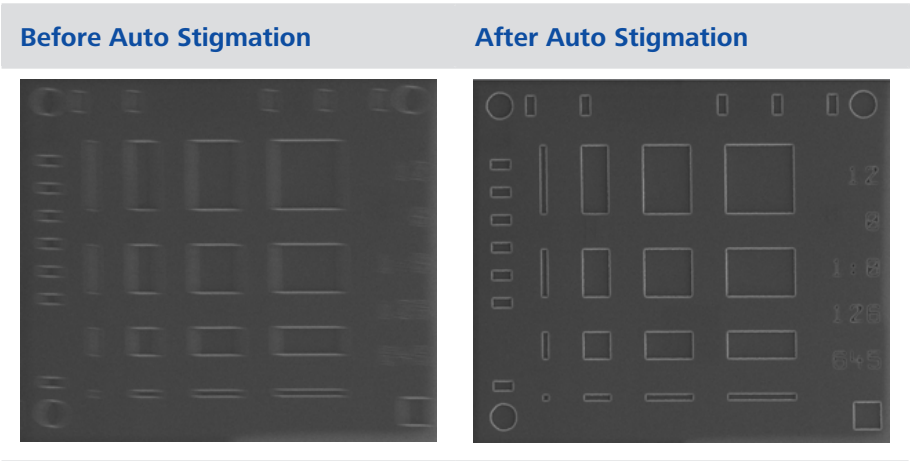
Autofocus The **Autofocus** routine adjusts the focus of the specimen.

Perform this alignment routine if the live image of the specimen appears to be too blurred. A progress bar indicates the progress of the routine.



Auto Stigmatism The **Auto Stigmatism** routine corrects astigmatism effects.

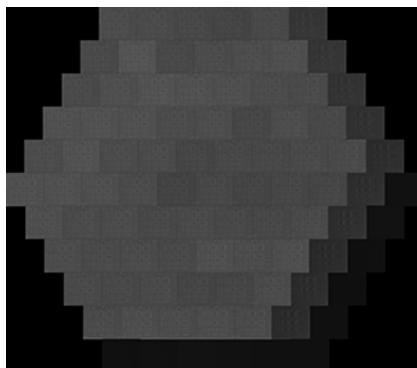
Astigmatism causes a circular electron beam to become elliptical. Perform this alignment routine if the focused live image appears to be blurred in one dimension (line focus).



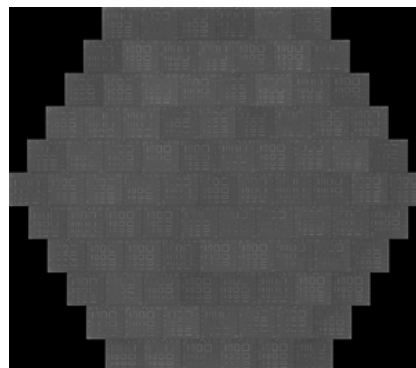
Beam on Detector The **Beam on Detector** routine corrects the alignment of the fiber optics.

Perform this alignment routine if ghost images are visible in the live image or if the outer regions of the multi-beam image are dark.

Before Beam on Detector

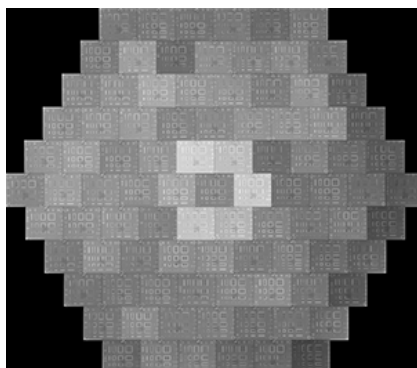


After Beam on Detector

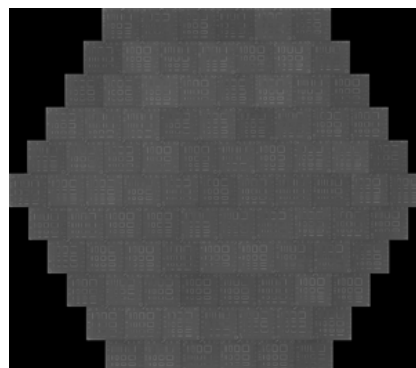


Detector Equalization The **Detector Equalization** routine equalizes the contrast or brightness between the individual single-beam images.

Before Detector Equalization



After Detector Equalization



8.4 Single Routines

The **Single Routines** tool window enables you to do the following:

■ Calibrating Coordinate Systems

During operation, the microscope uses a beam coordinate system, a stage coordinate system and a scan coordinate system. For more information about the coordinate systems, also refer to *Overview of Coordinate Systems* [► 102].

The **Single Routines** tool window enables you to optimize the calibration of these coordinate systems using the same microscope settings that you intend to use later in the experiment.

INFO When calibrating the coordinate systems, use the same settings for **Pixelsize** and **Overlap** that you intend to use later during the workflow. The calibration of the beam coordinate system and the stage coordinate system should be performed directly at the sample position of your planned experiment. This way you can

optimize the calibration on the specimen, which will not necessarily work for all specimens but is especially useful for slices of brain tissue or similar.

■ Measuring Crosstalk

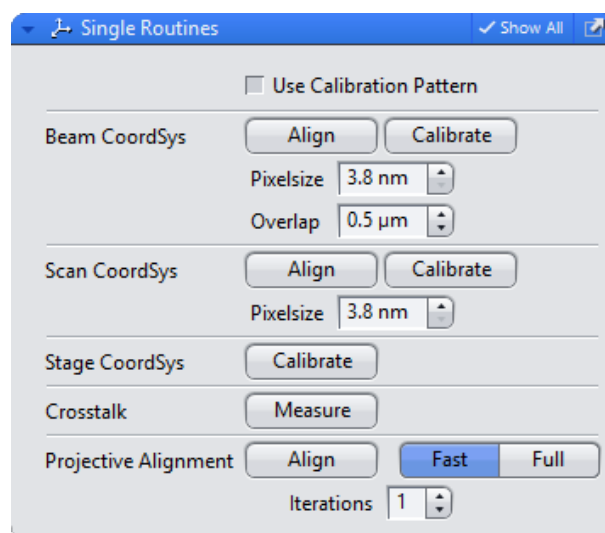
Crosstalk is an effect that may occur in the light optics (also refer to *Components in the Detection Column* [► 96]) and that may lead to ghost images.

The **Single Routines** tool window enables you to measure the amount of crosstalk that is present.

■ Aligning the Projective

The **Single Routines** tool window enables you to align the projection path in the detection column.

This may be necessary, e.g. if measuring the crosstalk does not yield satisfactory results.



INFO

For a more detailed description of the individual features of the **Single Routines** tool window, also refer to the software reference: Single Routines

8.5 Manual Operation

8.5.1 Moving the Stage

The motorized stage, which the specimen holder is placed on, enables you to move your specimen.

TIP

If you are using the Navigation Circle to navigate the specimen and find regions of interest, you can use **Single Live** or **Full Live**.

To move the stage:

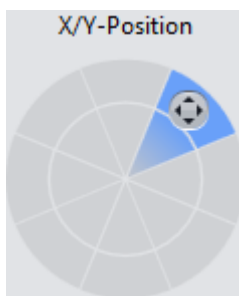
Procedure 1 Select the **Stage** tool in the **Left Tool Area**.

2 Navigate to your region of interest by dragging the **Navigation Circle (X/Y-Position)** icon in the desired direction.

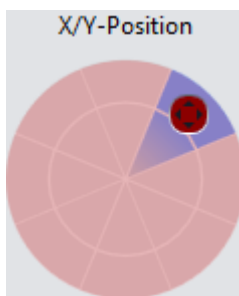
If released, the icon snaps back to the **Navigation Circle** center and the stage stops. In case the stage continues moving, click **Stop**.

The **Navigation Circle** allows for four different speeds:

- Inner segments: Slow, outer segments Medium



- Inner segments: Fast, outer segments: Very Fast



To enter high-speed mode, right-click the **Navigation Circle** icon. The **Navigation Circle** will turn red. To return to normal speed, right-click the **Navigation Circle** icon again.

3 If the live image appears blurred and out-of-focus, you can roughly readjust the focus using the **Navigation Bar (Z-Position)** and perform an **Auto Focus** afterwards.

4 If desired, store the current stage position using the **Add Current** button from the **Stage Positions** section.

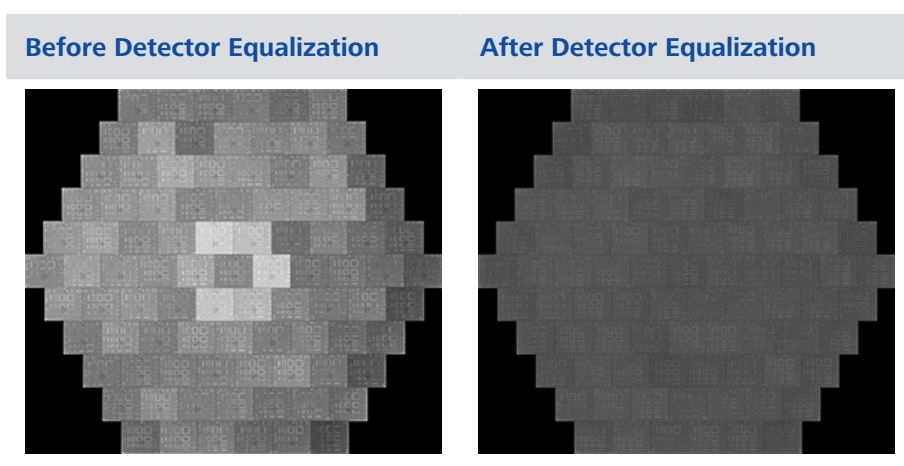
For example, you can store the marker positions of your specimen holder. If you use the **Shuttle & Find** module and change the specimen, you can easily navigate to the marker positions of the new specimen. This considerably speeds up the specimen registration.

INFO

To avoid electrical arcing, which could damage the specimen, you can set **Speed** to a value lower than 100 %. The four stage movement speeds are decreased accordingly and thus the risk of electrical sparkovers is reduced.

8.6 Detector Equalization

Detector equalization is an adjustment that is performed for each detector, i.e. for each single beam. The desired result is the same brightness and the same contrast for each single image.



8.6.1 Purpose of Detector Equalization

The purpose of detector equalization is to achieve a homogenous brightness and contrast across all images (i.e. single-beam and multi-beam images) and scan regions. If detector equalization is not performed, differences in brightness and contrast may occur between different single-beam images. Detector equalization adjusts the contrast and brightness settings of each single-beam detector such that the image of a uniform specimen area looks uniform in the multi-beam image.

The following detector equalization modes are available:

◆ **Absolute Target**

Specify a gray value to be applied to all single-beam images. The brightness is equalized.

◆ **Relative Target**

Calculate a gray level based on a single image and then apply this value to all single-beam images. The brightness is equalized.

8.7 Single Beam Contrast

8.7.1 Purpose of Single Beam Contrast

Single Beam Contrast is a feature of ZEN MultiSEM that allows you to manually fine-tune the appearance of a particular multi-beam image.

For any multi-beam image, you can individually adjust the contrast of each single-beam image. This enables you to create well matched images, for example for scientific publications.

8.8 Adjustment of Scan Rotation

8.8.1 Scan Rotation

You can specify a scan rotation if you want to scan a specimen that is slightly rotated, for example a rectangular specimen that is not exactly parallel to the edges of the stage.

Definition The scan rotation defines the degree of rotation between the beam coordinate system and the single image coordinate system.

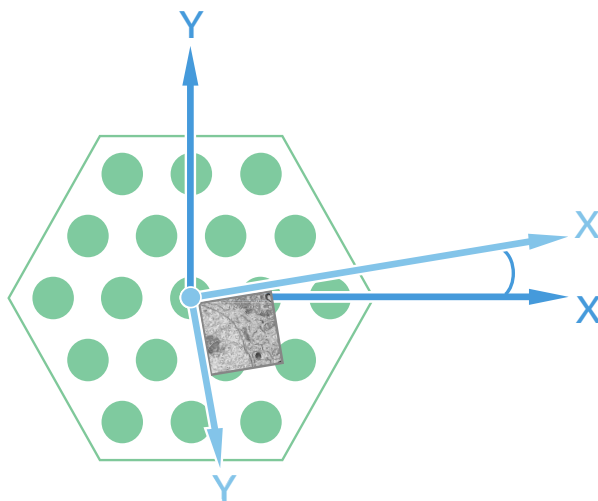


Fig. 8.2: Scan rotation

A scan rotation means that each of the single images in the full-image hexagon is rotated by the same amount, as shown in the following figure. The orientation of the hexagon itself remains the same.

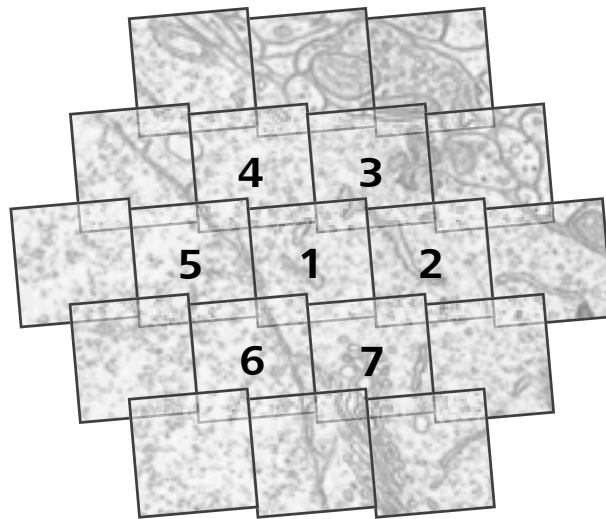


Fig. 8.3: Single images with scan rotation

Setting the Scan Rotation You can set the scan rotation in the **Scan Parameter** tool window in the **Scan Settings** section.

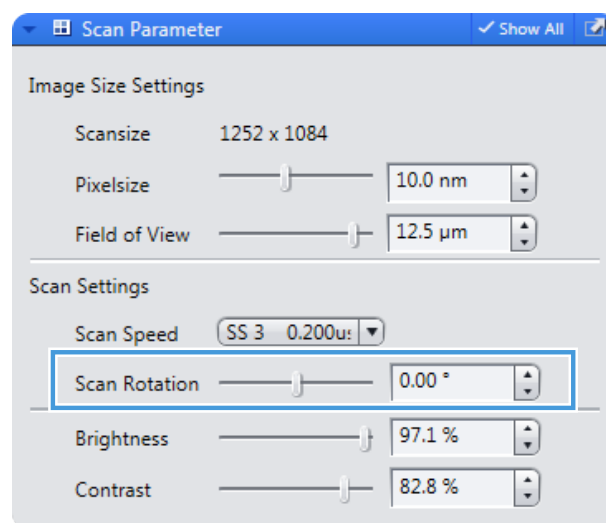


Fig. 8.4: Scan parameter tool window

For a **Scan Rotation** not equal to 0°, a certain sFoV overlap is necessary to obtain gapless multi-beam images. Note the following information about **Scan Rotation** and the sFoV overlap, which depends on the settings for **Pitch** (Beam tool) and **Field of View**:

■ **Scan Rotation = 0°:**

The Field of View must always be a bit larger than the pitch size to avoid gaps between the images and to enable stitching of the sFoVs.

■ **Scan Rotation not equal to 0°:**

If **Field of View** = **Pitch** (no overlap), gaps occur between the sFoV images and are displayed as black areas. The areas on the specimen corresponding to the gaps are not scanned.

In order to obtain a gapless scan, increase the overlap by setting **Field of View** > **Pitch**. Activate **Full Live** and increase the value of **Field of View** gradually, until no gaps are visible.

Use a scan rotation different from 0° only if necessary.

Scan Rotation in Alignment Workflow The alignment with the calibration pattern should be performed with the scan rotation set to 0°.

9

Specimen Registration

9 Specimen Registration

9.1 Introduction

This chapter describes the registration workflow, including how to load or unload the specimen chamber.

Registration of the specimen means that you locate your region of interest (ROI) on the specimen and navigate to it. Registration consists of the following tasks:

- Setting the correct focus, i.e. setting the correct vertical working distance
- Navigating to the ROI, e.g. by using the Shuttle & Find method

The purpose of the registration is to map a point or region on the specimen to the stage coordinate system. This enables you to navigate the specimen.

9.2 Loading and Unloading the Specimen Chamber

9.2.1 Unloading the Specimen Chamber

Unloading the specimen chamber is necessary if you want to remove, exchange, or insert specimens that are higher than 19 mm. Before you can open the specimen chamber, you have to vent the chamber, i.e. to break the vacuum inside the chamber.



Suffocation hazard: Lack of oxygen

Gaseous dry nitrogen is used to vent the specimen chamber during specimen exchange. Inhaling nitrogen may cause unconsciousness.

- ◆ During specimen exchange, keep the chamber door open as short as possible.
- ◆ Ensure the area around the microscope is sufficiently ventilated.
- ◆ Avoid inhaling the air from within the specimen chamber.
- ◆ If you begin to experience symptoms of asphyxia (for example: rapid breathing, loss of mental alertness and/or muscular coordination, depression of sensations, emotional instability, fatigue) leave the room immediately and inform the facility's safety officer.

NOTICE

Risk of damage to ZEISS MultiSEM: Contamination with dust particles.

Opening the chamber door exposes the chamber to dust particles from the environment. The dust particles may permanently contaminate the high voltage insulators inside the chamber, which results in an inoperable MultiSEM.

- ◆ Preferably use the airlock to unload or load the chamber.
- ◆ If you must open the chamber door, first use a wiping cloth to carefully clean the surfaces around the chamber door from dust. Remove any evident sources of dust in the vicinity of the MultiSEM. After the cleaning procedure, wait two minutes to let turbulent dust in the air fade out. Only then open the chamber door.
- ◆ If the chamber door is open, always wear a lab coat and lint-free gloves. Avoid rapid movements.
- ◆ Minimize the time span the chamber door is open.

NOTICE

Risk of damage to ZEISS MultiSEM or the specimen: Short working distance

The specimen or the specimen stage may collide with the objective lens if they are too close to each other (i.e. the working distance is too small) when opening the chamber door.

- ◆ Ensure the working distance is sufficiently large before opening the chamber door, refer to *Working Distance* [▶ 147].

INFO

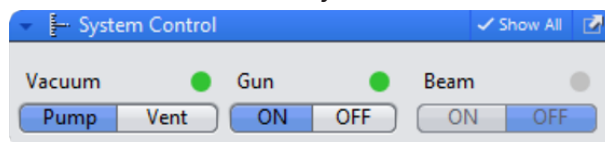
Vacuum Deterioration or Prolonged Pumping Times

Fingerprints contain hydrocarbons that deteriorate the vacuum when outgassing.

- Always wear lint-free gloves when touching the specimen, specimen holder, or stage.

Prerequisite ■ The specimen stage is lowered sufficiently far, such that it does not hit the objective lens when opening the chamber door (can be checked using the chamber CCD camera).

Procedure 1 In the **Beam** section of the **System Control** tool window, click **OFF**.



- 2 Wait until the beam is completely **OFF**.

The beam is switched off in a controlled way, which takes a few moments. The symbol next to **Beam** is flashing green.

When the symbol has stopped flashing and is displayed in gray permanently, the beam is **OFF**.

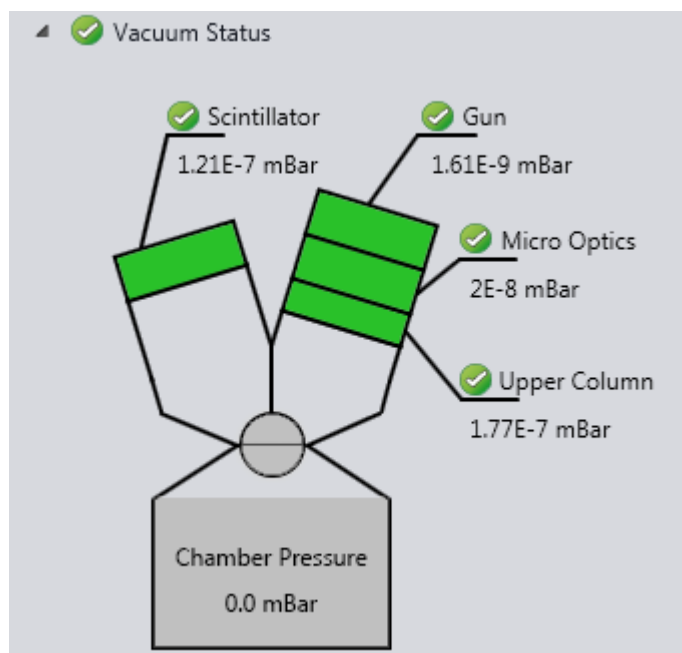
- 3 In the **Vacuum** section, click **Vent**.

- 4 In the dialog that is displayed, confirm the venting procedure.

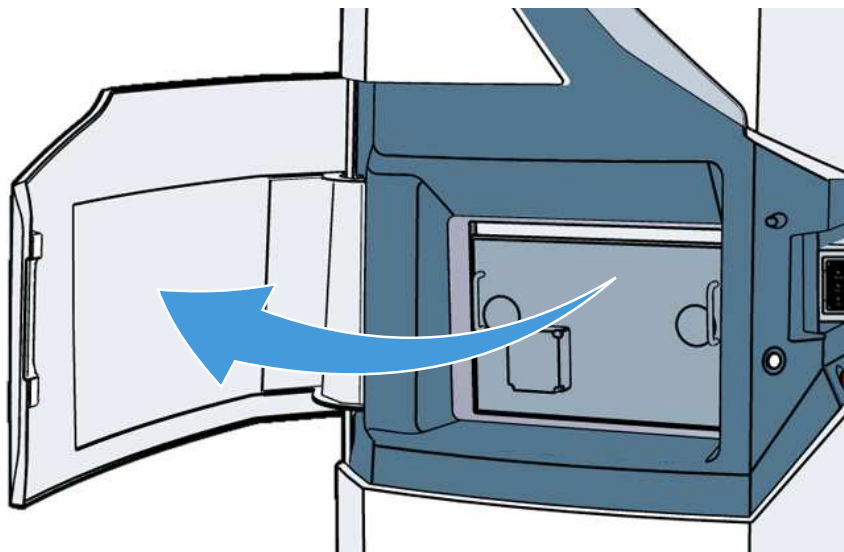
When the venting procedure is finished:

- The specimen chamber is vented.
- The turbo pump and the pre-vacuum pump shut down.
- The air pressure in the vibrational isolation system is released.
- The specimen chamber is flushed with nitrogen.
- The **Vacuum Status** displays a **Chamber Pressure** of 0 mbar.

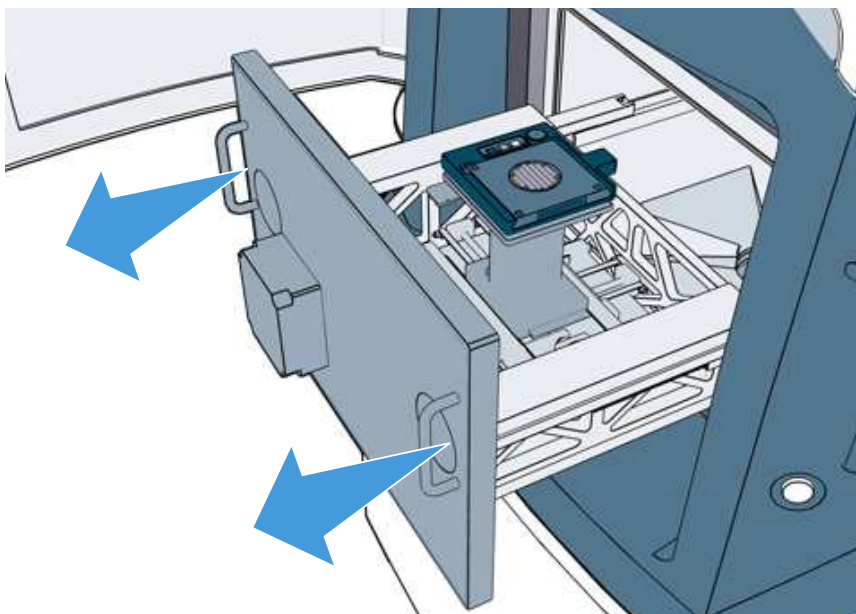
The value of 0 mbar is displayed as the now atmospheric pressure inside the specimen chamber is beyond the measurement range of the vacuum gauge head.



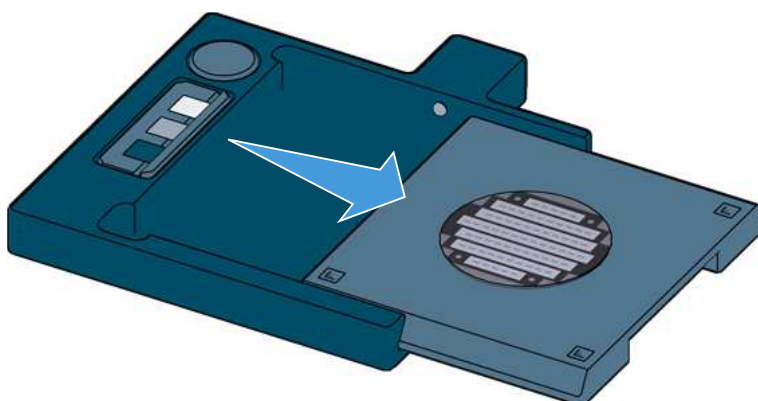
- 5** Open the cabinet door.



- 6** Open the chamber door.



- 7** Remove the specimen holder.



9.2.2 Loading the Specimen Chamber

Specimens with a height above 19 mm need to be loaded via the specimen chamber.



CAUTION

Suffocation hazard: Lack of oxygen

Gaseous dry nitrogen is used to vent the specimen chamber during specimen exchange. Inhaling nitrogen may cause unconsciousness.

- ◆ During specimen exchange, keep the chamber door open as short as possible.
- ◆ Ensure the area around the microscope is sufficiently ventilated.
- ◆ Avoid inhaling the air from within the specimen chamber.
- ◆ If you begin to experience symptoms of asphyxia (for example: rapid breathing, loss of mental alertness and/or muscular coordination, depression of sensations, emotional instability, fatigue) leave the room immediately and inform the facility's safety officer.

NOTICE

Risk of damage to ZEISS MultiSEM: Contamination with dust particles.

Opening the chamber door exposes the chamber to dust particles from the environment. The dust particles may permanently contaminate the high voltage insulators inside the chamber, which results in an inoperable MultiSEM.

- ◆ Preferably use the airlock to unload or load the chamber.
- ◆ If you must open the chamber door, first use a wiping cloth to carefully clean the surfaces around the chamber door from dust. Remove any evident sources of dust in the vicinity of the MultiSEM. After the cleaning procedure, wait two minutes to let turbulent dust in the air fade out. Only then open the chamber door.
- ◆ If the chamber door is open, always wear a lab coat and lint-free gloves. Avoid rapid movements.
- ◆ Minimize the time span the chamber door is open.

NOTICE

Risk of damage to ZEISS MultiSEM or the specimen: Short working distance

The specimen or the specimen stage may collide with the objective lens if they are too close to each other (i.e. the working distance is too small) when opening the chamber door.

- ◆ Ensure the working distance is sufficiently large before opening the chamber door, refer to *Working Distance* [▶ 147].

INFO

Vacuum Deterioration or Prolonged Pumping Times

Fingerprints contain hydrocarbons that deteriorate the vacuum when outgassing.

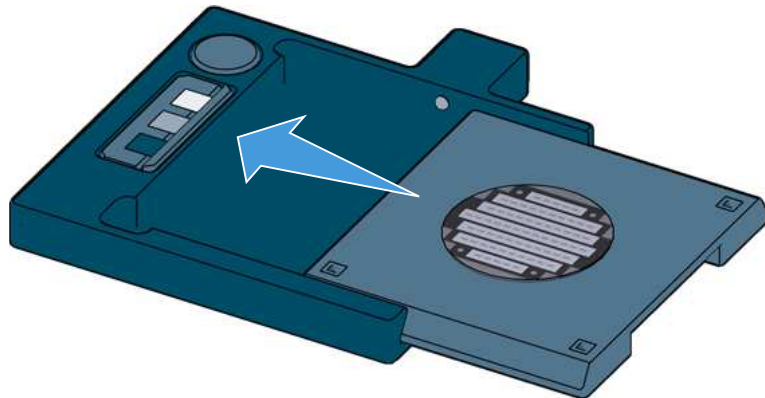
- Always wear lint-free gloves when touching the specimen, specimen holder, or stage.

INFO

If available, the specimen can be loaded into the specimen chamber via the 200 mm-Airlock with HV Interlock. For more information, see Loading the Specimen via the Airlock and Unloading the Specimen via the Airlock.

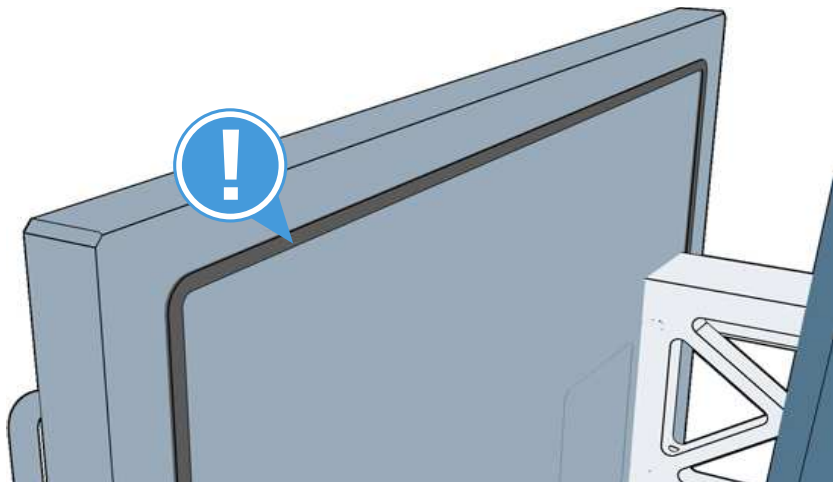
- Prerequisite**
- Specimen chamber is open and unloaded (see *Unloading the Specimen Chamber* [▶ 137])
 - Specimen is prepared
 - Specimen is properly attached to the specimen holder, e.g. with adhesive metal, conductive carbon or silver, carbon tape, or similar
 - Specimen stage is lowered sufficiently far, such that neither the specimen holder (adapter) nor the specimen hit the objective lens when closing the chamber door

- Procedure 1** Attach the specimen holder to the specimen holder mount.

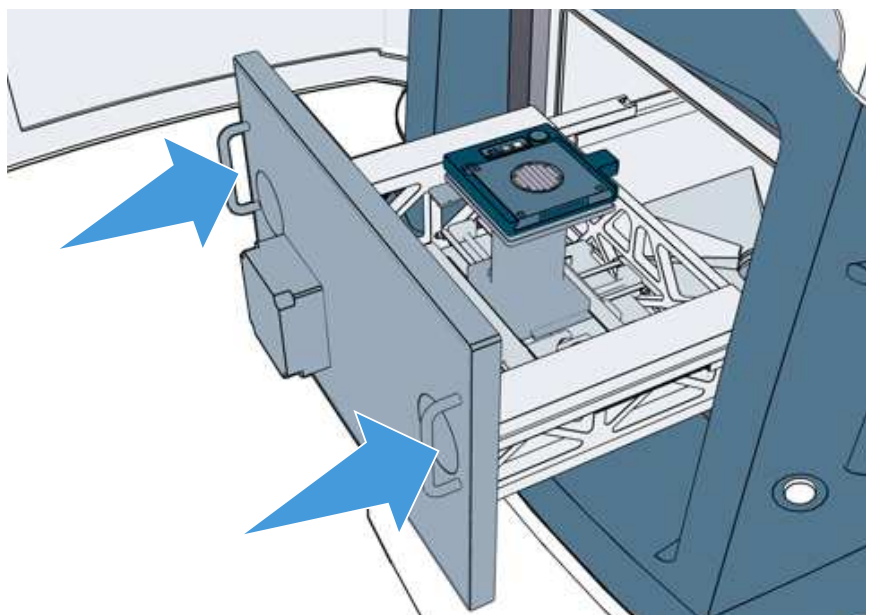


Ensure that the specimen holder is completely inserted.

- 2** Check whether the seal in the chamber door is correctly mounted.

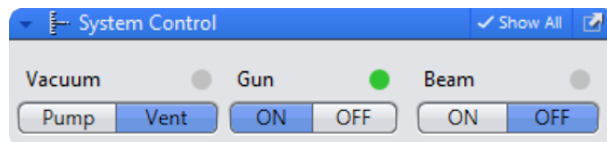


- 3** Close the chamber door.

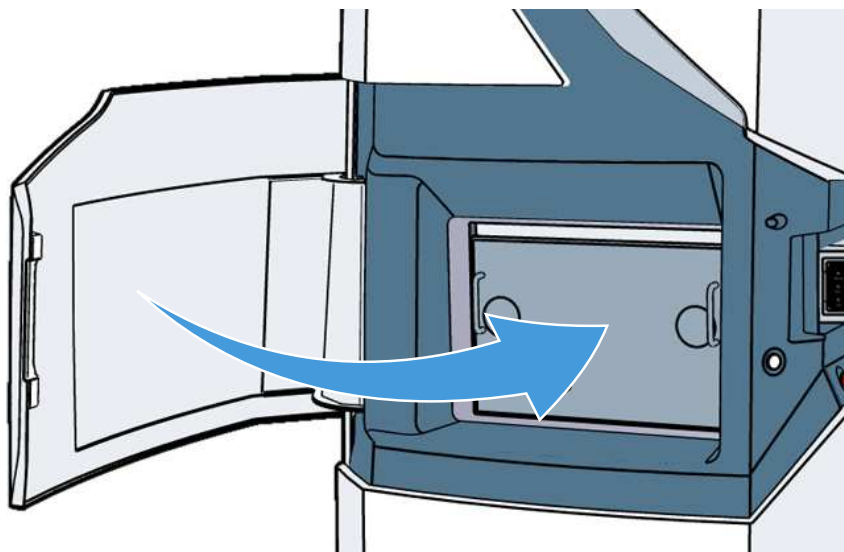


- 4 In the **Vacuum** section of the **System Control** tool window, click **Pump**.

The specimen chamber is evacuated.



- 5 Check whether the chamber door is correctly closed (if there is vacuum inside the chamber, the door cannot be opened).
- 6 Close the cabinet door.



- 7 Wait until the **Vacuum** status is displayed green.

The vacuum in the specimen chamber should be better than 1×10^{-5} mbar.

The required vacuum conditions can be reached after more than 15 to 20 minutes (depending on the specimen).

- 8 To switch the electron gun and the beam on, click **ON** both in the **Gun** section and in the **Beam** section.

The specimen chamber is loaded and the required vacuum is established. You can continue with the registration of the specimen. This typically consists of loading a valid working point.

9.3 Pre-Adjustment

9.3.1 Pre-Adjustment via Chamber CCD Camera

Before you (horizontally) move to your desired ROI, you have to set the correct Z-distance between objective lens and specimen.

It is recommended to begin with a rough visual adjustment: move the specimen stage upwards while monitoring the approach with the chamber CCD camera. You can move the specimen stage with higher speed at first.

Stop moving the specimen stage upwards, when the specimen, or the surface of the specimen holder, is near the objective lens, as shown in the following figure.

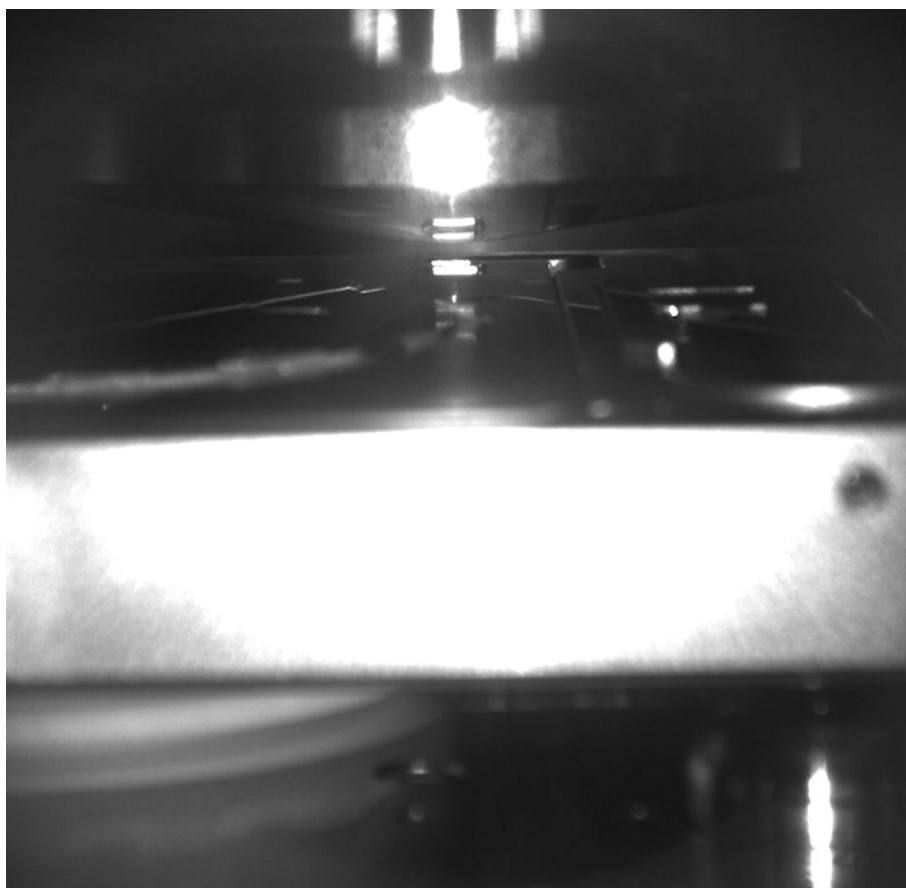


Fig. 9.1: Specimen image from chamber CCD camera

Note that the chamber CCD camera is located slightly higher than the objective lens opening. It shows the image from a slight angle.

For more information about the chamber CCD camera, see *Chamber CCD Camera* [► 57].

You should now see a signal on the detection CCD camera. Continue to adjust the specimen focus onto the specimen with the help of the detection CCD camera.

Optimizing the focus

After performing the pre-adjustment, you can optimize the specimen focus in two steps:

- 1 Perform a coarse adjustment.
You can perform the coarse adjustment in the **Stage** tool window.
- 2 Perform a fine adjustment.
You can perform the fine adjustment in the **Beam** tool window.

9.4 Vertical Adjustment of the Specimen Stage

9.4.1 Vertical Adjustment via the Detection CCD Camera

You have to set the specimen focus. To do this, adjust the Z position of the specimen stage so that the optimum working distance of ZEISS MultiSEM is set.

You can monitor this vertical adjustment on the detection CCD camera (see *Detection CCD Camera* [► 59]). The better focused the hexagon scan spots appear in the image of the detection CCD camera, the better the focus on the specimen surface.

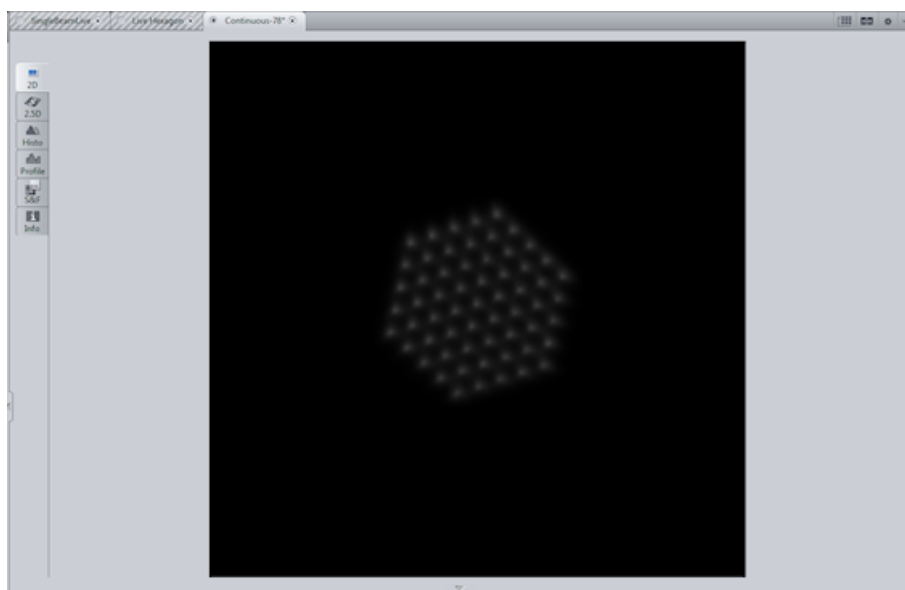


Fig. 9.2: Out-of-focus Hexagon scan spots on the detection CCD camera

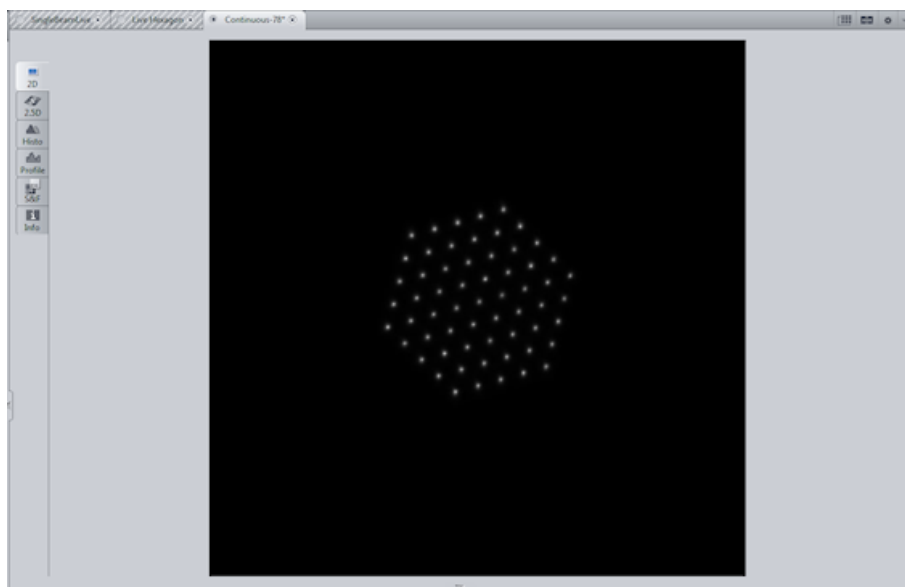


Fig. 9.3: Focused hexagon scan spots on the detection CCD camera

TIP

Use live images for the fine adjustment of the working distance.

9.4.2 Working Distance

The working distance is the distance between objective lens opening and specimen surface.

The optimum working distance of ZEISS MultiSEM in which the specimen surface is in focus is 1.4 mm ($\pm 10 \mu\text{m}$). This also means that the scintillator image and the live image are in focus.

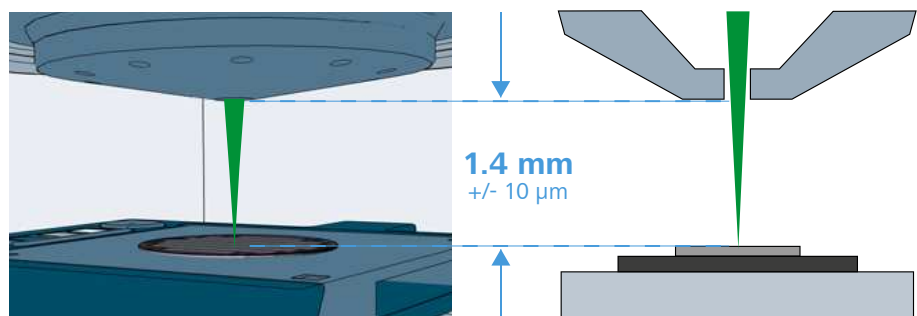


Fig. 9.4: Working distance

The live image has priority when setting the working distance: if you cannot focus both the scintillator image and the live image simultaneously, the working distance is achieved when the live image is in focus.

You can monitor the scintillator image on the detection CCD camera (see *Detection CCD Camera* [► 59]).

10

Troubleshooting

10 Troubleshooting

The following table lists possible issues and the corresponding recommended remedies. If the suggestions do not resolve the issue or if you are feeling unsure, contact your ZEISS service representative.



DANGER

Danger to life: Hazardous voltage inside the microscope

High-voltage potentials present in the microscope can be fatal. Contact may cause burn or electrical shock.

- ◆ Ensure proper grounding. For more information refer to the Installation Requirements document.
- ◆ Only authorized ZEISS service representatives are allowed to remove the microscope enclosure.

Symptom	Possible Reason	Recommended Actions
ZEISS MultiSEM does not vent	No nitrogen and/or no compressed air is supplied	Check the nitrogen supply. Check the compressed air supply.
ZEISS MultiSEM does not pump	Chamber door is not closed correctly	Close the chamber door completely
Beam or Gun cannot be switched on	Insufficient vacuum	Click Pump in the System Control tool and wait until the vacuum indicator is displayed in green
Electron gun is being switched off automatically. Gun pressure (i.e. vacuum in the electron gun section) is worse than 1×10^{-8} mbar.	Electron gun was switched off for safety reasons as the gun pressure is outside the specified range	Contact your ZEISS service representative and ask for a process description or assistance
Chamber pressure is too bad	Chamber door seal does not seal sufficiently	Replace the chamber door seal
All lights on the power supply rack are switched off after an Emergency Off	Emergency Off is not reset	Reset the Emergency-Off circuit

Symptom	Possible Reason	Recommended Actions
Arcing event	High voltage insulator contaminated	1 Plasma-clean the specimen chamber. Use default settings of the plasma cleaner (15 min).
Failure of stage		2 Via the menu bar, execute Tools > Reset Hardware Controllers > Chamber EHT .
Failure of objective lens high voltage		3 Repeat the above steps for up to three times. If not successful, contact your ZEISS service representative.
When trying to acquire live images, no images are received	Image acquisition rack is misconfigured or not running	Restart the image acquisition PCs (also refer to Image Acquisition Configuration)
Specimen stage does not move	Specimen stage is not initialized	Re-calibrate the specimen stage
	Loading or unloading the airlock is not completed	Retract the airlock rod and click Reset in the Stage tool window
Focusing via the stage is not possible	Specimen height is set too high	Set the correct specimen height via the Stage tool
Stored positions cannot be approached correctly	Specimen stage needs to realign the position	Re-calibrate the specimen stage
	Selected specimen height is too large and limits the Z position of the stage	Check if the correct specimen height is selected. If the specimen height is selected correctly, the stored position cannot be reached. Choose a different position.
The scan image (Full Live, Single Live) is displayed in black	Stage Z position is too low	Move the stage to the correct Z position
	Microscope is not adjusted correctly	Load a standard working point via the Settings tool
	Beam blanker is set to On	Set the beam blanker to Auto
	Beam or gun is switched off	Switch the beam or gun on via the System Control tool
	Contrast/brightness is insufficient.	Increase contrast and/or brightness of the detectors.

Symptom	Possible Reason	Recommended Actions
	Charging of the specimen	Make sure that the specimen is electrically conductive and is connected to the specimen holder conductively
Images are digitally distorted, or seem to consist of sub-images.	Image acquisition rack and the scan generator are out of sync.	1 Stop the image acquisition. 2 Change the scanspeed and image size to their default values. 3 Change the settings to their previous values again. 4 Restart the acquisition.
Some of the beams display a black image in an mFoV image	Beams are not correctly aligned on the detectors	Perform Beam on Detector via the Auto Tuning tool
The images acquired by running an experiment are out-of-focus	Flatness of the specimen is not sufficient for the acquisition strategy parameters	Adjust the focus parameters, depending on the acquisition strategy: ■ AfPixelSize / AfDwellTime: Get new noise constant ■ AutoAlignment: Reduce the focus interval (AfExecutionInterval) ■ FocusMap: Increase the number of focus support points per region and try a more sophisticated interpolation method
Navigation via an overview image is inaccurate	Current Shuttle & Find calibration is obsolete, e.g. a different specimen holder is used	Repeat the Shuttle & Find calibration
Brightness of the individual images in an mFoV image is different	Detector equalization is obsolete	Perform a detector equalization using the Auto Tuning tool
Autofocus routine fails repeatedly	Noise constants are obsolete	Focus manually. Via the Auto Tuning tool, click Noise Constants in the Autofocus / Auto Stigmation section.
	Structure of the specimen is too homogeneous at the current position	Ensure that the specimen is visible in the scan image and exhibits a sufficient amount of structure.

Symptom	Possible Reason	Recommended Actions
Single images of a mFoV image do not match	Unsuitable field of view	Alternatively, increase AfPixelSize . Via the Scan Parameter tool, set Field of View to the value of Pitch (Settings tool)
	Unsuitable scan rotation	Via the Scan Parameters tool, set Scan Rotation to 0°
Image displayed in the Center Screen Area is too dark or too bright	Unsuitable histogram settings	Via the Histogram tab in the Center Screen Area , click Reset



Maintenance, Shutdown, and Disposal

11 Maintenance, Shutdown, and Disposal

11.1 Introduction

This chapter describes how to maintain ZEISS MultiSEM to ensure optimal operation and how to dispose of ZEISS MultiSEM at the end of its lifecycle.

Furthermore, this chapter contains ZEISS contact addresses.

11.2 General Maintenance Information



WARNING

Risk of injury: Improper maintenance

Deviating from the maintenance tasks described in this Instruction Manual can be hazardous.

- ◆ Only perform preventive maintenance tasks described in this Instruction Manual.
- ◆ All maintenance, service, and repair tasks not described in this Instruction Manual have to be performed by an authorized ZEISS service representative.

To ensure the optimal performance of the microscope it is essential to perform preventive maintenance regularly.

It is recommended that you enter into a service contract with your ZEISS service representative. This will ensure trouble-free operation of the microscope.

Scheduled downtime is the time when the ZEISS MultiSEM is not available to perform its intended function due to planned downtime events. The scheduled downtime includes:

- Consumables and chemicals are replaced
- The operator performs basic preventive maintenance
- An authorized ZEISS service representative performs preventive maintenance

11.3 Basic Preventive Maintenance

Perform the following basic preventive maintenance tasks regularly:

- Check the existence of all protective covers.
- Inspect and clean the product safety labels to maintain good legibility.

- Replace the tip seal of the pre-vacuum pump yearly or as required.
Refer to the Instruction Manual of the pre-vacuum pump provided by the manufacturer. You can find these manuals in the "Zeiss MultiSEM" document folder.

11.4 Column Bakeout

A column bakeout is a procedure that you use to improve the vacuum inside the microscope columns.

During a column bakeout you either heat up one of the two microscope columns separately, or you heat up both microscope columns at the same time. Due to the increased temperature of the column, possible contaminations decompose and are pumped off.

You perform a column bakeout in the **Bakeout** tool window via the **Accessories** tab (also refer to Bakeout).

11.5 Shutdown

If the microscope will not be used for an extended period of time, for example several months, it should be shut down.

Contact your local ZEISS service representative if you want to put the microscope out of operation.

The ZEISS service representative will also make sure that the cooling water is completely removed from the interior of the microscope.

11.6 Disposal

11.6.1 Disposing of Consumables

The operator must ensure that consumables are disposed of and recycled in a responsible manner.

Description	Material	Disposal
Schottky field emitter	Tungsten, ceramics	Return to the emitter manufacturer.
Apertures	Platinum, iridium, gold	Very small amounts may be disposed of in accordance with local/regional regulations.

11.6.2 Disposing of the Microscope

The operator must ensure that waste products are disposed of and recycled in a responsible manner.

Refer to EC directive 2002/96/EC on waste of electrical and electronic equipment (WEEE).

The microscope consists of several modules. Be careful to separate the materials properly when disposing of the microscope.

- Materials: e.g. metals, non-metals, composite materials, process materials
- Electronic scrap material: e.g. transformers, circuit boards, cables

Comply with national and regional waste disposal ordinances.

11.7 Support

If you require support, please contact your ZEISS representative or consult one of the following websites:

- Sales:
multisem.microscopy@zeiss.com
- Support:
techsupport.multisem.microscopy@zeiss.com
- <https://www.zeiss.com/microscopy/int/service-support/microscopy-contact.html>

12

Technical Data

12 Technical Data

12.1 Declaration of Conformity

Denomination Multi Beam Field Emission Scanning Electron Microscope (MultiSEM)

Models MultiSEM 5xx Series

Manufacturer Carl Zeiss Microscopy GmbH

Carl-Zeiss-Str. 22

73447 Oberkochen

Germany

This is to declare that the machinery mentioned above fulfils all the relevant provisions of the

■ Directive 2006/42/EC.

Moreover the machinery fulfils the following directives and standards:

■ EN 61010-1

■ Directive 2004/108/EC "Electrical equipment for measurement, control and laboratory use - EMC requirements". In case of a BURST (EN 61000-4-4) or SURGE (EN 61000-4-5) event, an minor image deformation may occur. This deformation can be corrected by changing or resetting the "Field of View" value of the system.

■ Standard EN ISO 12100

■ Standard EN ISO 13849-1

■ Standard EN 60204-1

■ Standard EN 61326-1

■ Standard EN 61000-6-4

■ Standard EN 61000-6-2

Unauthorized modifications of the machinery will cancel this declaration.

CE Marking The CE conformity marking is located on the type plate of the machinery.

The production facility has a certified Quality Management system ISO 9001 and environmental management system ISO 14001.

The product was developed, tested, and manufactured in conformance with the applicable regulations and directives for environmental law of the European Union. Regarding electromagnetic immunity, the equipment shall be operated in a controlled electromagnetic environment. In terms of emission requirements, the equipment is classified in Class A.

The product and respective accessories have been rated as Class 9 instruments (laboratory equipment or comparable standard). The product and the respective accessories satisfy the requirements of EU Directives 2011/65/EC (RoHS) and 2002/96/EC (WEEE) insofar as these are applicable to the product.

ZEISS introduced a procedure for the return and recycling of the instruments within the member states of the European Union which ensures suitable recycling procedures conforming to the EU directives.

For more information on disposal and recycling please consult your ZEISS service representative.

The product may not be disposed of in the household waste or through municipal waste disposal services. If the product is resold, the seller is obliged to inform the buyer that the product must be disposed of in accordance with the regulations.

12.2 Warranty

The manufacturer guarantees that the instrument has no material or production defects when delivered. You must inform us of any defects immediately and do everything to minimize any damage. If the manufacturer is informed of such a defect, he is obligated to rectify it; it is his decision whether he does this by repairing the instrument or by delivering an instrument free of any defect.

One set of consumables is included per year. Further consumables are excluded from the scope of warranty and are invoiced separately in accordance with the incurred expenditure.

The instrument manufacturer is not liable for damage caused by faulty operation, negligence, or any other tampering with the instrument, particularly the removal or replacement of instrument components, or the use of accessories from other manufacturers. This invalidates all warranty claims.

With the exception of the work specified in this manual, no maintenance or repair of ZEISS MultiSEM may be undertaken. Repairs may only be performed by a ZEISS service representative. Should any defect occur with the instrument, please contact your ZEISS service representative.

12.3 Technical Data

Refer to the Installation Requirements document.



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