



# Service of Scanning Electron Microscopy (ESEM) USER GUIDE

*M. Marceddu, Rev. 2.0 - 03/02/2022*

## Introduction

This guide provides basic usefull informations for users with a self-service authorization to the CeSAR Service of Scanning Electron Microscopy (ESEM). Also users with full-service authorization are invited to read this guide as they will find uselfull informations.

Please also refer to CeSAR general regulation, available at:

[https://www.unica.it/unica/it/ateneo\\_s03\\_ss07.page?contentId=STR19179](https://www.unica.it/unica/it/ateneo_s03_ss07.page?contentId=STR19179)

**This guide is not intended as an extended course or training in electron microscopy.**

The ESEM Service laboratory is located at CeSAR, Building B, ground floor, room 0C3. The lab specialist is Dr. Marco Marceddu (mail: [marco.marceddu@unica.it](mailto:marco.marceddu@unica.it) /phone +390706756584/ Building A, ground floor, room CS3).

The first step to access CeSAR services is to apply for a specific authorisation. Acces levels to the lab are authorized by CeSAR Director (application forms are available at CeSAR website). Two acces levels are available: full service and self service. Self service is reserved only to UniCa staff users, with certified expertise in electron microscope systems. Expert users not familiar with our specific microscope (brand/model), will receive familiarization training on demand.

Full service is devoted to third party users and UniCA users with no specific expertise. The lab specialist will perform the measurements.

Once you get your access level you can schedule and book lab-time by e-mail to the lab specialist. In case of priority activity or extraordinary maintenance, the staff may change your booking.

Ordinary opening hours are 8 am–5.30 pm, Mon-Fri. Special needs must be authorized by the CeSAR Director. Each lab session must be recorded in the lab register. When you leave the lab and the end of your day, or have to leave the lab for some minutes, always inform the lab specialist or other CeSAR personnel. **We need to know who is in the building.**

The lab layout is intended for general purposes. **Changes of this standard configuration are not allowed.** Specifically tailored configurations must be previously authorized by the lab specialist.

Experiments involving instruments or components from other UniCa Departments must be authorized.



**Users are not allowed to change computer settings nor to plug external memories or other devices to lab computers.** Data transfer will be performed by CeSAR staff.

**Each working session must be recorded in the lab logbook, specifying the number and nature of the observed specimen.**

**Any failure and anomaly must be immediatly communicated to the lab specialist.**  
**It is raccomened not to listen to music nor use hearphone: noises often are good indicators of something going wrong.**

The lab employs UPS systems. In case of black out UPS will supply power to the microscope and beep-sound alarm will be emitted. UPS are intended to protect the instrument from voltage/current spikes and to ensure the completeness of the systems shutdown in case of emergency. Therefore UPS are not intended to continue the experiments for hours in case of back out. **In case of prolonged main power failure (say 5 min.), immediatly stop the experiment and call the lab specialist who will start the shutdown precedures if needed.**

**As a general rule of the lab: if you have doubts always refer to the lab specialist. Do not worry about disturbing or boring: this is his job.**

## 1. The lab rules

Being a service facility available to different users, the lab is organised with a general pourposes layout. For the same reason **some basic rules must be observed**. Please always remember that you are in a multi-users laboratory, not in your own lab. Please observe the lab rules and leave the lab neat and tidy as you find it. Avoid the rat-pack mentality: hurry, disorder, drab, inaccuracy are our worst enemy. We will very appratiate your collaboration to make the lab a nice and reliable workplace for as many users as possible, but alway remember that in case of problems, **the staff is authorized to deny further acces to the lab.**

**Only authorized persons are allowed in the lab.**

**Only authorized persons are allowed to use the microscope**

**The maximum number of persons allowed in the lab at once is 3, due to COVID-19 regulations.**

**Always inform the CeSAR Director if coworkers and colleagues are meant to assist the lab session.**

**Foods and drinks are not allowed in the lab:** you'll find a confortable resting area in the entrance hall of the building.



**For safety reason always wear appropriate dresses: no open shoes, no shorts, no short skirts, no rowers; long hairs must be tied and loose clothing must be secured.**

**Never handle samples, sample holders, vacuum components without gloves**

**Potentially outgassing sample cannot be inserted in the SEM chamber: always discuss with the lab specialist to find out the best suited procedures.**

**Any failure and anomaly must be immediatly communicated to the lab specialist.**

**It is raccomened not to listen to music nor use hearphones: noises often are good indicators of something going wrong.**

Mechanical tools, manuals and so on are available in the lab cabinets: feel free to use what you need, **but at the end of the day keep everything in place.**

## 2. The microscope

The lab microscope is the FEI Quanta 200, designed to work in high vacuum, low vacuum and ESEM mode. High vacuum mode is devoted to conductive, stable and not outgassing materials; low vacuum is for insulating materials and ESEM is reserved to hydrated biological materials.

The gun surce is a tungsten filament. Filament change and saturation, gun and lens allighment are strictly reserved to the lab specialist.

**Never change the gun/column settings. If needed call the lab specialist.**

**The ESEM mode is available only under the lab specialist supervision.**

The FEI Quanta 200 is equipped with the ensuing detectors:

- Everhart-Thornley detector (ETD) for secondary electron imaging in high vacuum mode;
- Solid State detector (SSD) for backscattered electron imaging in both high and low vacuum modes;
- Large Field detector (LFD) for secondary electron imaging in low vacuum mode;
- Scintillator and photomultiplier for catholuminescence imaging;
- Energy dispersive spectrometer (EDS) for x-ray microanalysis.

The microscope is equipped with two computers: the microscope PC is devoted only to microscope control, while the support PC is devoted to microanalysis and data storage. Never save data on the microscope PC hard disk.

**Working with the Quanta 200 is very easy, but damages can easely occur as well.**

Most likely and serious damages are: the x-ray detector window brake, x-ray detector damage due to excessive count rate; the SSD detector damage, contamination of the sem chamber.

#### To prevent x-ray detector window brake:

Never change the nitrogen vent pressure; open and close the chamber very gently, if the door doesn't open, never use force but vent once again; always follow the indications for powdered samples and other potentially dangerous materials (see next).

#### To prevent x-ray detector damage due to excessive count rate

Before starting the Pathfinder software always turn off the chamber camera (attention: for specific operation the camera automatically turns on – immediately turn it off); keep the detector death time below 35%

#### To prevent SSD detector damage

Never work without coupling the stage to the proper working distance; be very careful when using automatic “goto” stage function in the vertical direction; use “escape” to immediately stop the stage if it gets too close to the SSD detector. **A very dangerous bug pertains the automatic stage positioning at the working distance. Be very careful and use “escape” to immediately stop the stage, if it does not automatically stop at 10 mm. Self service users will be specifically trained on this issue.**

#### To prevent chamber contaminations

As a general rule **the sem chamber must be kept in high vacuum as long as possible**. Do not vent the chamber until your sample is ready and steadily mounted on the stub. At the end of your session switch to high vacuum mode and leave the pumps running. **Keep everything clean and vacuum compatible**. Use appropriate sem stub and clean it before usage. Use only glue authorised by the lab specialist (carbon tape or carbon paint). Specific needs (special sample holders or glues) must be authorized. **Always wear gloves when handling specimen, stubs and so on.** **Avoid samples that can likely outgass inside the vacuum chamber** (i.g. porous or volatile materials), use special precautions with powered or not stable samples. It is highly recommended to **discuss the nature of your samples with the lab specialist** in order to define the proper solution. In any case, conventional sem preparative techniques can be used. As a general rule the user must verify that the inner surfaces of the chamber are cleaned. If needed use only analytical grade acetone and clean paper (refer to the lab specialist).

**The microscope PC operating system is quite old and can easily crash.**

#### To prevent PC crashes

Always remember to start the microscope PC first, and then the support one; on the contrary the support PC must be turned off first, and then the microscope one. Do not ask for more than one operation in the same time: give one command and wait until it's done.

#### Known bugs

- Vent command not available at random (vent button looks grey and not “clickable”): to fix the bug, change the vacuum mode (switch high/low) for a while, then press vent



- A very dangerous bug pertains the automatic stage positioning at the working distance. It cannot be fixed. You must be very careful and use “escape” to immediately stop the stage, if it does not automatically stop at 10 mm. Self service users will be specifically trained on this issue.
- At shutdown the support PC doesn’t turn off (blocked in backup): force quitting
- Scan control switches to “external” at random: you cannot fix it, manually switch again to “full frame”

The following issues are strictly forbidden:

Open the gun column and cup

Change the gun filament

Change the chamber detectors

Open any SEM cabinet

Change the nitrogen pressure

## 2. Sample preparation

The FEI Quanta 200 microscope enables different vacuum working regimes, ranging from high vacuum, to low vacuum and environmental conditions. Therefore many different kind of materials can be analysed with minimal or none preparation. This definitely doesn’t mean that every kind sample can be analysed without specific care.

**When considering specimen for SEM analysis, the first issue to account with, is the sample stability inside the chamber** (in vacuum and under the electron beam): sample that can melt under the electron beam, or outgas or evaporate in vacuum, must be treated with conventional SEM preparation technique.

**Since porous material can easily outgas inside the chamber it’s recommended to keep them in a proper vacuum chamber before SEM analysis.**

**Particular attention must be devoted to fragile materials and powders:** when pumping the chamber, fragments of the specimen might be ejected hitting the EDS windows. **In this case it is mandatory to verify that the sample is hardly bound to the stub and the stage must be set in safe position before starting the pumps.**



Wet specimen must be analysed only in ESEM mode, by trained and authorised operators. Biological samples can be used in high/low vacuum mode only after proper preparation; otherwise, ESEM must be used.

Materials prepared by electrochemical etching or other similar techniques, must be previously stabilized to prevent chamber contamination.

In any case it is always recommended to discuss with the lab specialist about the properties of your sample.

**To perform a very rough first check of your sample, consider the ensuing list:**

Solid metals and metal alloys: no problems

Solid semiconductors: no problems

Nanostructures, devices, porous materials: first consider growth technique, think about stabilisation and refer to the lab specialist

Rocks and solid dry minerals: no problems

Hydrated samples: refer to the specialist

Powders: very dangerous (see previous section)

Ground/earth samples: to be treated as powders

Biological samples: must be specifically prepared or need ESEM

Plant samples: same as biological (preparation or ESEM needed)

If the sample smells: it will strongly outgas inside the chamber (preparation needed)

Potentially radioactive materials must be authorised by the lab specialist

If the sample stability is not known or you have any doubt: always refer to the lab specialist

Samples without documented "SEM" history, must be authorised by the lab specialist



### 3. Daily Operations

As a general rule the microscope is always kept in stand by mode, i.e.: cabinet powered (green light on), pumps on in high vacuum, computers off.

We define “stage safe position” the lowest position in the z direction and close to the external door, as far as possible from the EDS window (x-y direction). The stage must be placed in the safe position whenever pump are started, chamber is vented, the vacuum level is changed (switch between high/low vacuum); hold on the safe position until vacuum status gets green.

To start your session:

1. Start microscope PC and log-on with proper id and password
2. Start microscope software and wait until all traffic lights are green (otherwise call the specialist)
3. Start microscope user interface (id and password required)
4. Home the stage
5. Prepare your sample
6. Slowly open the nitrogen bottle (never change pressure settings)
7. Vent the chamber and wait the green light
8. Gently open the door (do not use force)
9. Insert the specimen
10. Gently close the door
11. Move the stage to the safe position
12. Select the suitable vacuum mode
13. Press pump and wait green light
14. Lift the stage at about 12-15 mm
15. Turn high voltage on and wait for stable filament current
16. Start scanning with desired detector and optimise the image
17. At moderate high magnification level, couple the stage to the working distance
18. Set stage to the working distance (pay attention)
19. Perform your measurements

At the end of your session:

1. Stop scanning
2. Switch high voltage off
3. Home stage/safe position (wait until it's done)
4. Vent the chamber and wait green light
5. Gently open the door (do not use force)
6. Take out your sample
7. Gently close the door
8. Select high vacuum mode, press pump and close the nitrogen bottle
9. Wait for green light
10. Switch off the camera
11. Close user interface
12. Stop the microscope software and close the window
13. Exit microscope server
14. Turn off computers and monitors



Working with the EDS microanalysis:

1. Turn on the support PC after the microscope one
2. Don't start Pathfinder until everything is fine with the microscope control
3. Switch off the chamber camera
4. Start Pathfinder
5. Set beam voltage, spot size and stage vertical position ( at about 11 mm) to optimise x-ray detector counts and death time
6. Perform measurements
7. Close Pathfinder

## 4. In case of emergency

Please refer to the official University safety and emergency rules:

General procedures:

<https://www.unica.it/static/resources/cms/documents/37862445b945116b96dcfb7d4b864e7c.pdf>

Emergency plan:

<https://www.unica.it/unica/protected/179253/0/def/ref/DOC11444/>

**As general rule, always keep in mind that in case of emergency, people's safety comes first.**

**The microscope employs high voltage components: never open the microscope cabinets.**

Due to the nitrogen usage in the lab, the room is equipped with an oxygen level monitor. This monitor has light indicators both inside and outside the lab, with green, yellow and red indicator according to the oxygen level. In case of low and very low oxygen level, the lights are yellow and red respectively and a sound alarm is emitted.

**YELLOW LIGHT:** open the room door and the windows and immediately close the nitrogen bottle; call the lab specialist. If the monitor does not get green, immediately leave the room.

**RED LIGHT: DO NOT ENTER THE ROOM OR LEAVE IT IMMEDIATELY** and then call the lab specialist.

The lab employs UPS systems. In case of black out UPS will supply power to the microscope and beep-sound alarm will be emitted. UPS are intended to protect the instrument from voltage/current spikes and to ensure the completeness of the systems shutdown in case of emergency. Therefore UPS are not intended to continue the experiments for hours in case of black out. **In case of prolonged main power failure (say 5 min.), immediately stop the experiment and call the lab specialist who will start the shutdown procedures if needed. Keep in mind that in case of black out, the oxygen level monitor does not work.**