# Leica EM VCT100

**Vacuum Cryo Transfer**

From Preparation to Analysis

Living up to Life

Leica
MICROSYSTEMS

# Versatile Specimen Transfer

Transferring specimens to the chamber of an analysis system is a very critical step for most preparation methods. It is essential to protect the samples from contamination. The Leica EM VCT100 concept was established to cross-link preparation units with various analysis systems via a transfer shuttle connected to a load-lock. Samples are transferred in a well-defined environment, e.g. protective gas or high vacuum conditions from a preparation instrument to the analysis unit. In addition, the specimen can be kept at low temperature for cryo techniques. The load-lock can mount to any vacuum operated SEM, FIB, SIMS, AFM or XPS chambers.

The sample holders used during specimen preparation and transfer are compatible with the custom-made stage of the analysis instrument. Due to the modular concept, the stage can be installed in any (ultra) high vacuum system or glove box and used for both room temperature and cryo applications. The flexible system design enables adjustments to any specific application.

EM VCT100 shuttle with specimen and holder

EM VCT100 cryo preparation workstation

**Setup Advantages and Key Features**

- Contamination-free transfer between preparation and analysis unit
- Unique shuttle system for the vacuum cryo transfer of specimens
- Special shuttle and load-lock design maintains resolution of the SEM, vibration free
- Suitable for either room or cryo transfer
- Versatile protective gas transfer such as argon to prevent oxidation
- Preparation and analysis can be performed at different locations
- Space saving design offers minimal interference with the analysis system
- Sample preparation and analysis can be performed independently without interruption of either process
- Possible to repeat preparation with the same specimen
- Adaptable to more than one SEM
- Preparation units can be linked to several Leica EM VCT100 adapted analysis units such as the EM SCD500, EM MED020 or the EM BAF060


Leica EM VCT100 control unit enables integrated safety communication for specimen transfer through the load-lock system of the preparation and analysis unit.


Freeze etch/fracture unit with the Leica EM VCT100 shuttle attached. From preparation to imaging, the EM VCT100 is a complete cryo transfer system. Its components include among others a cryo preparation workstation, transfer shuttle, docking station, a controlled cold stage (-150°C to +60°C), $LN_2$ Dewar and operating panel with touch screen. For docking to a SEM or different analysis instrument such as FIB, SIMS, XPS, etc., a cryo adaption kit with cryo stage is included.

# The Leica EM VCT100 for Cryo SEM

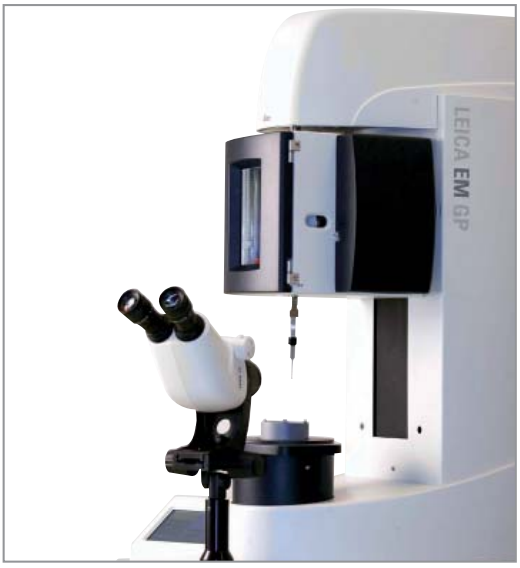
Cryo Electron Microscopy (Cryo SEM) is the process of observing non-stained and untreated vitreous samples. Cryo sample preparation is a quick sample preparation technique that has none of the artefacts associated with chemical fixation. It requires no toxic reagents and reduces beam damage due to low temperature during imaging. With the right experimental setup the entire processing of the specimen from excision to immobilization (freezing), surface preparation, coating, and imaging can be done in less than one hour.

## STEP 1: Sample Freezing

A typical preparation procedure starts with cryo fixation of the specimen using techniques like high pressure freezing with the Leica EM HPM100 or the Leica EM PACT2, jet freezing with the Leica EM JFD 030 or plunge freezing with the Leica EM CPC or Leica EM GP, depending on the nature of the specimen and application desired.

Leica EM HPM100

Leica EM PACT2




Leica EM GP


Leica EM CPC

## STEP 2: Sample Loading

The specimen is loaded under $LN_2$ onto a specimen holder using the loading chamber of the Leica EM VCT100 loading box and then retracted into the pre-cooled shuttle of the EM VCT100. Since the shuttle and loading chamber are pre-cooled with $LN_2$, the specimen is transferred to the preparation unit contamination-free without any exposure to air.

There are a variety of specimen holders to choose from, e.g. for freeze fracture, double replica, freeze drying, etc.

$LN_2$ reservoir

Loading chamber for loading specimen onto the sample holder and the Leica EM VCT100 shuttle.

Specimen and holder on the retracting arm of the Leica EM VCT100 shuttle.

# STEP 3: Sample Transfer for Fracturing, Etching and Coating

The specimen and holder are subsequently transferred under vacuum and low temperature to one of the many Leica preparation systems using the Leica EM VCT100 shuttle. The specimen can then be freeze fractured, freeze etched, freeze dried and/or coated for follow-on analysis in the cryo SEM.

Three preparation instruments are available for cryo SEM sample preparation:
the Leica EM SCD500, the EM MED020 and the EM BAF060.

**Leica EM SCD500**
The Leica EM SCD500 offers many conversion options in one single unit, all of which can be easily adapted to a variety of applications:

- high vacuum sputtering
- single and multiple carbon thread evaporation
- thermal resistance evaporation
- carbon rod evaporation
- etching and glow discharge
- cryo coating, double replica, freeze drying, freeze etching and vacuum cryo transfer with the Leica EM VCT100

**Leica EM MED020**
The modular high vacuum system can be fitted with different, optional attachments (i.e., modules) for the following sample preparations:

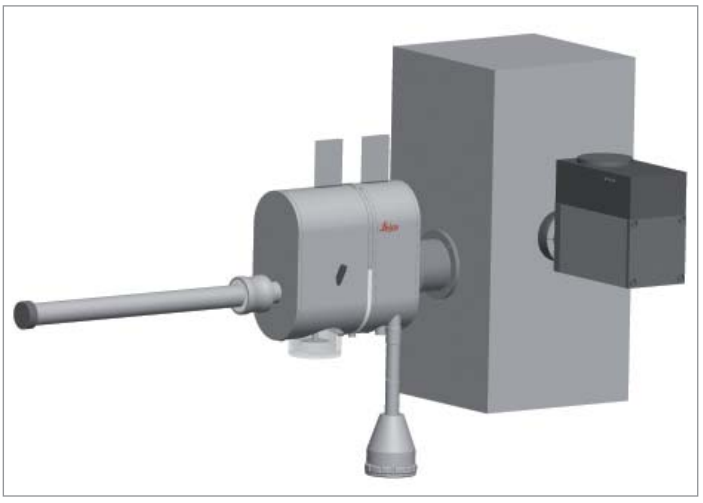
- single and triple high vacuum sputter coating
- single and multiple carbon thread evaporation
- thermal resistance evaporation
- carbon rod evaporation
- e-beam evaporation
- etching and glow discharge
- cryo preparation for freeze drying, freeze fracturing, double replica, freeze etching, cryo coating and vacuum cryo transfer with the EM VCT100

**Leica EM BAF060**
The Leica EM BAF060 is a fully automatic high-end preparation unit for:

- freeze fracturing
- freeze etching
- freeze drying
- double replica (mirror fracturing)
- high resolution carbon/metal mix coatings for TEM/SEM analysis
- specimen replication by electron beam evaporation
- double layer coated specimens for cryo SEM analysis
- cryo coating and vacuum cryo transfer with the Leica EM VCT100

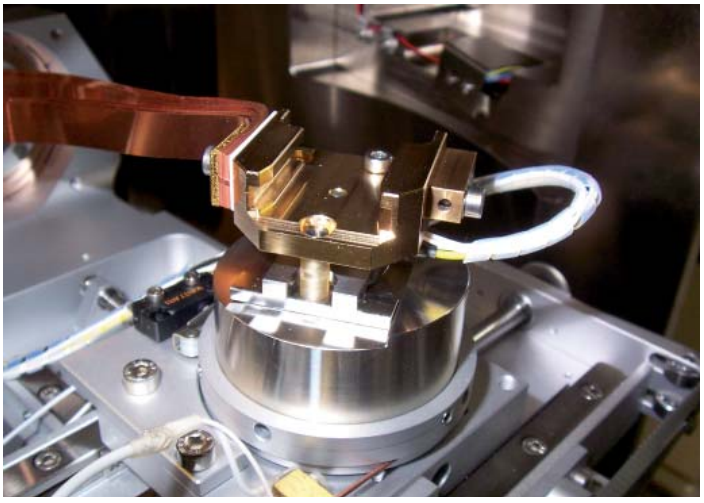
# STEP 4: Sample Transfer for Analysis

After sample preparation, the specimen is transferred still frozen and under vacuum via the Leica EM VCT100 shuttle onto the cryo stage within the SEM.



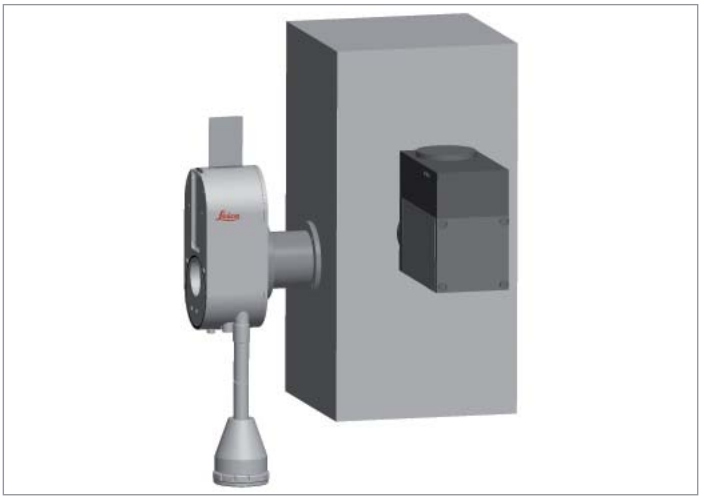
EM VCT100 docking station and shuttle with specimen and holder connected to the cryo stage.

The custom-made cryo stage slides onto the standard SEM stage and is connected via copper bands to the $LN_2$ Dewar. The specimen holder with the specimen is transferred onto the cryo stage using the EM VCT100 shuttle. The cryo stage can be used for any specimen with the advantage of improving the vacuum in the chamber and reducing beam damage. SEM resolution remains unchanged. The stage can also be used for room temperature analysis with the cryo stage in position.



Custom-made cryo stage

During SEM analysis the Leica EM VCT100 shuttle is detached from the SEM so it has no effect on the stability of the microscope.



The EM VCT100 is compatible with all current SEMs.

# Specimen Holders

A variety of specimen holders can be supplied with the system. The selection depends on the cryo fixation technique and the specimen.

① **Freeze Fracture holder**
with retaining spring for three Ø 3 x 0.8 mm gold specimen carriers.

② **Freeze Fracture holder**
with retaining spring for three Ø 3 x 4.5 x 0.8 mm copper specimen carriers.

③ **Universal Freeze Fracture holder**
with clamp for Ø 3 mm gold or aluminium specimen carriers.

④ **Double Replica holder**
with spring load for two Ø 4.6 x 0.6 mm gold specimen carrier sandwiches.

⑤ **Double Replica holder**
with spring load for four Ø 3 x 4.5 x 0.6 mm copper specimen carrier sandwiches.

⑥ **Freeze Drying holder**
with two magnetic strips for three Ø 3 mm grids.

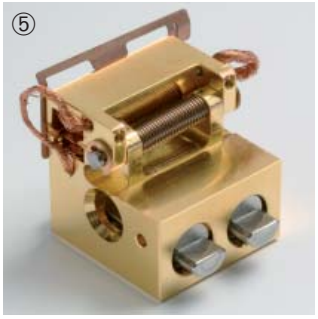
⑦ **Freeze Drying holder**
with retaining spring for four Ø 3 mm grids.

⑧ **Holder for SEM stubs**
with fastening thread (M3) for screw-on SEM stubs.

⑨ **Blank holder**
The specimen is adhered directly onto the holder.





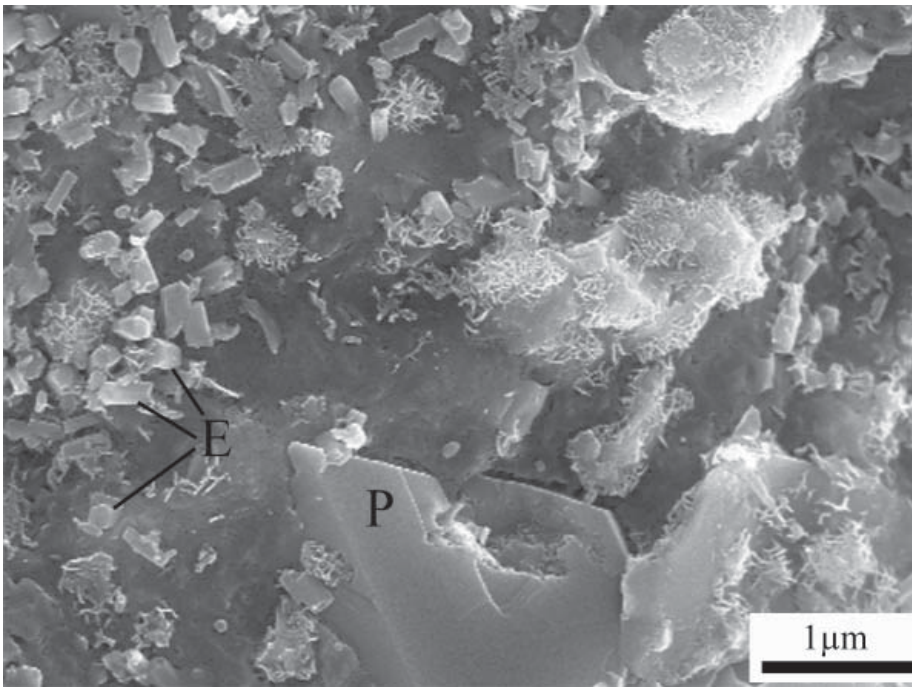






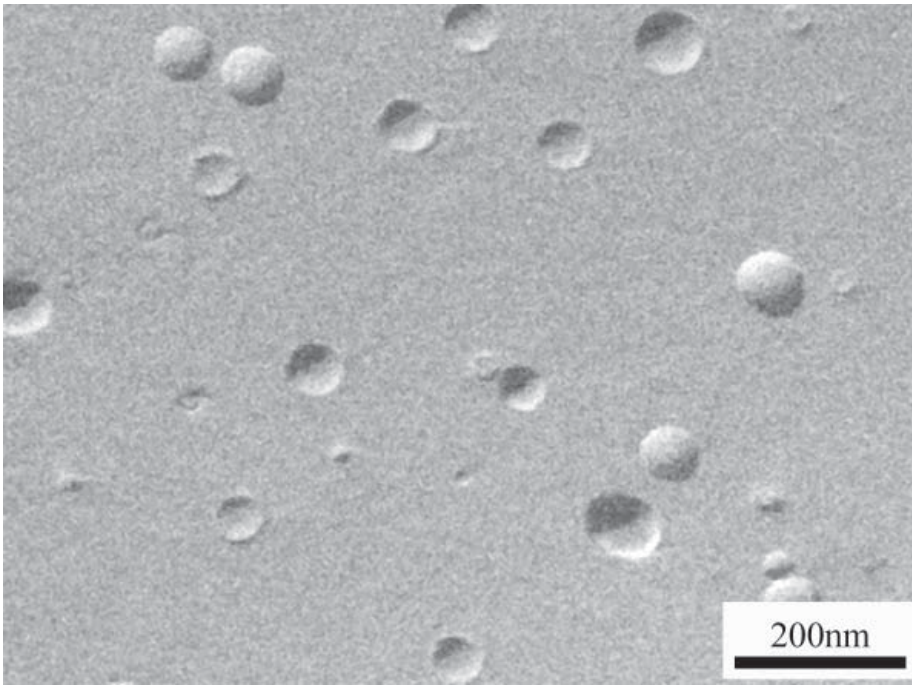
# Applications

Cryo SEM is an imaging technique for a wide range of applications in life and material sciences. Cryo SEM is not exclusively used to access the ultra-structure of an object. This technique can also be used for the analysis and localization of different components (e.g. elements).

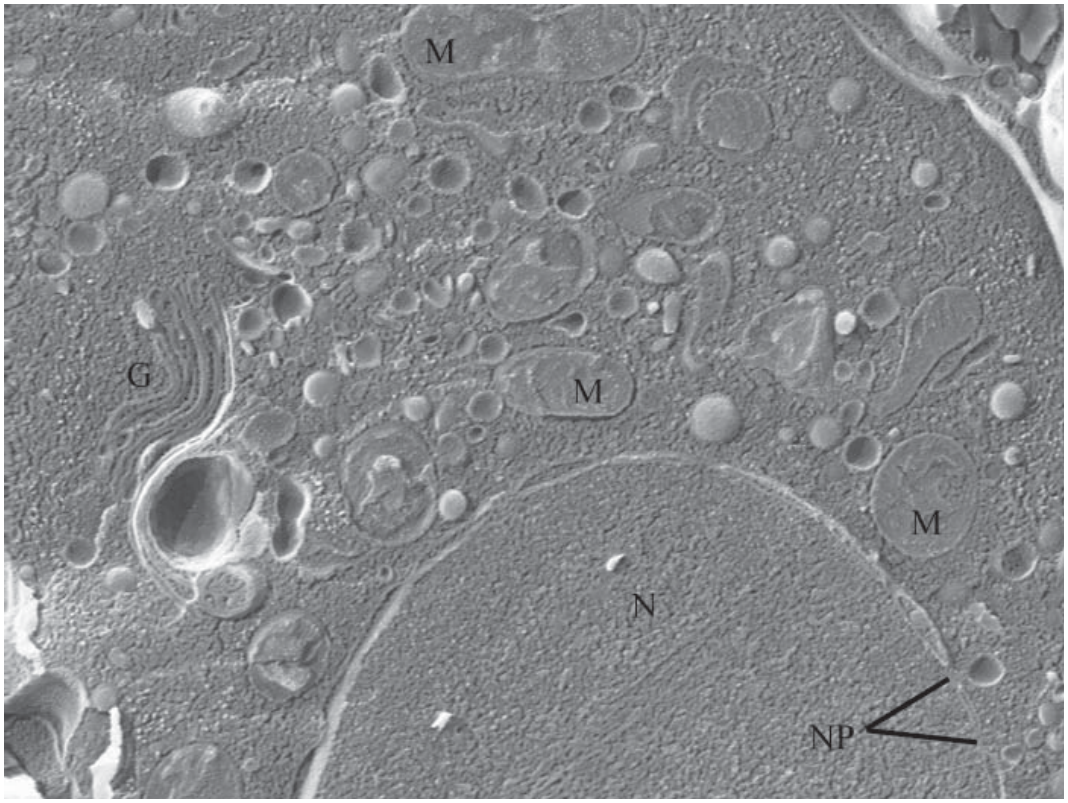



High pressure frozen, freeze fractured cement suspension. The sample was fractured at –115°C in a BAF060, etched at –105°C for 4 minutes and coated by electron beam evaporation with 3 nm of Pt/C. P…Cement particle, E…Ettringites. Electron Microscopy ETH Zurich (EMEZ).

Courtesy of L. Holzer, EMPA, Swiss Federal Laboratories for Materials Testing and Research, 3D-Mat group, Duebendorf, Switzerland.

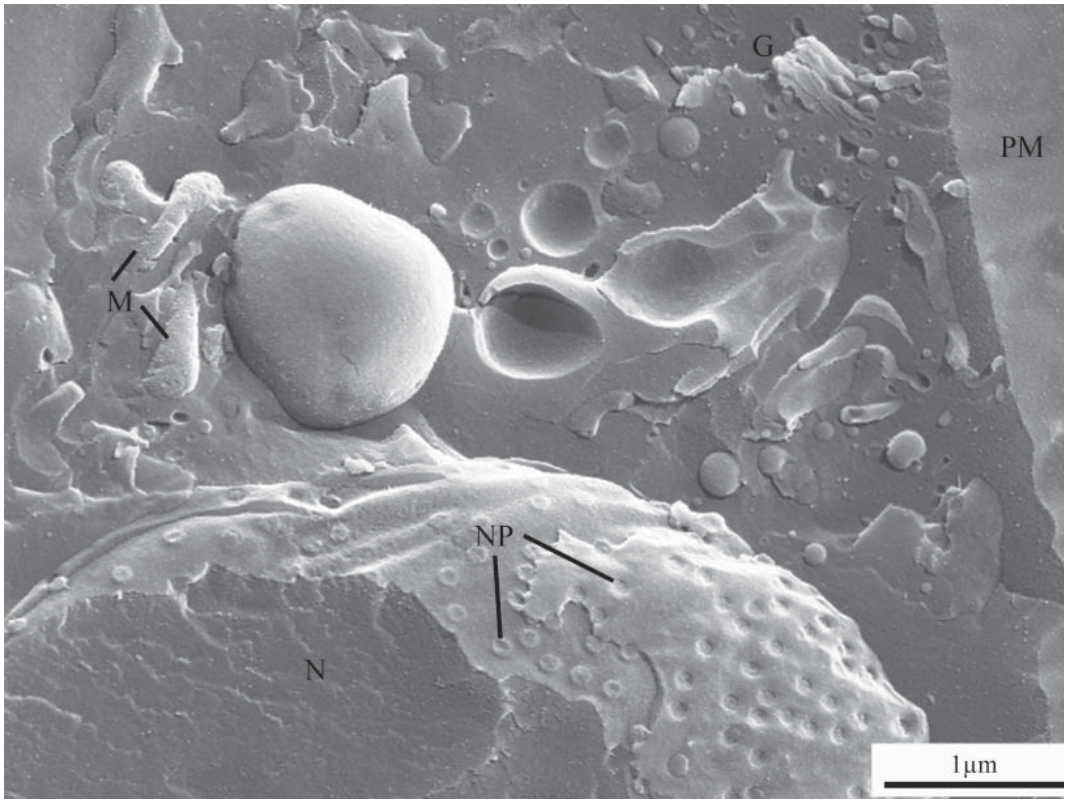



High pressure frozen, freeze fractured liposome suspension. The suspension was fractured at –150°C in a BAF060 and double layer coated by electron beam evaporation with 3 nm of Pt/C at 45° and subsequently with 6 nm of carbon. Specimen was imaged using backscattered electrons. Electron Microscopy ETH Zurich (EMEZ).



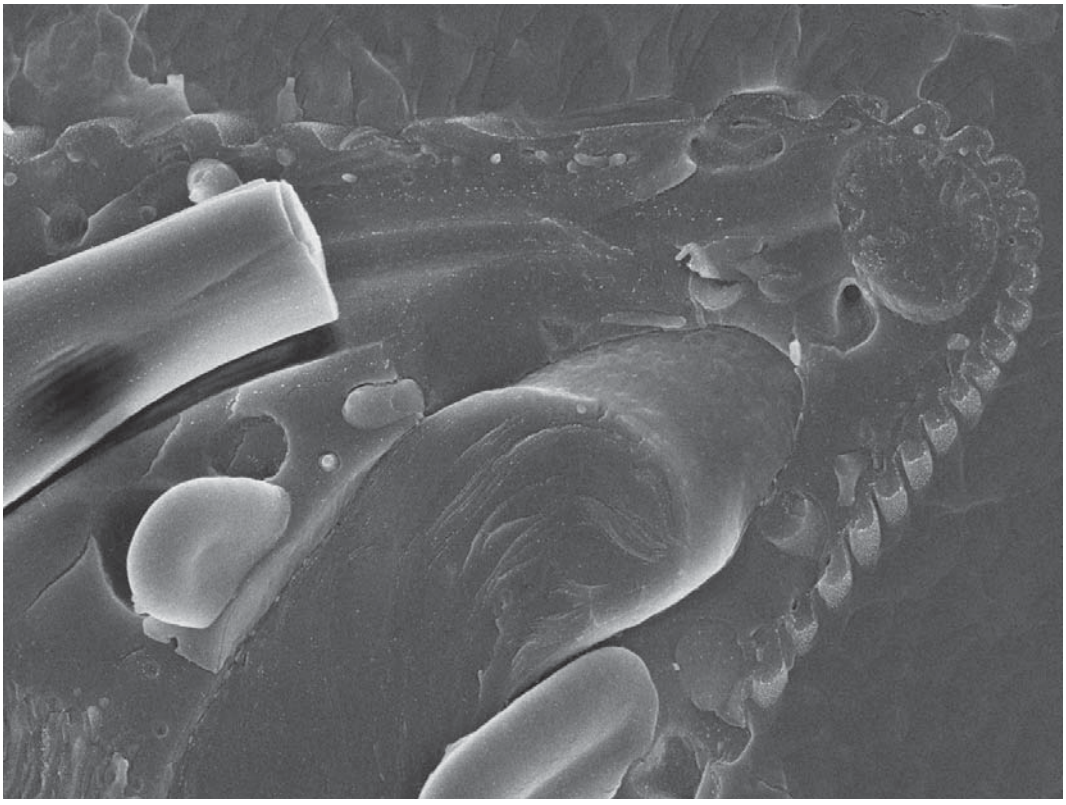

High pressure frozen, freeze fractured mouse intestine biopsy. The sample was fractured at –115°C in a BAF060, etched at –105°C for 5 minutes and coated by electron beam evaporation with 3 nm of Pt/C. M…Mitochondria, N…Nucleus, NP…Nucleopores, G… Golgi, PM…Plasma membrane. Electron Microscopy ETH Zurich (EMEZ).

Courtesy of René Fischer, Laboratory of Organic Chemistry, ETH Zurich, Switzerland.



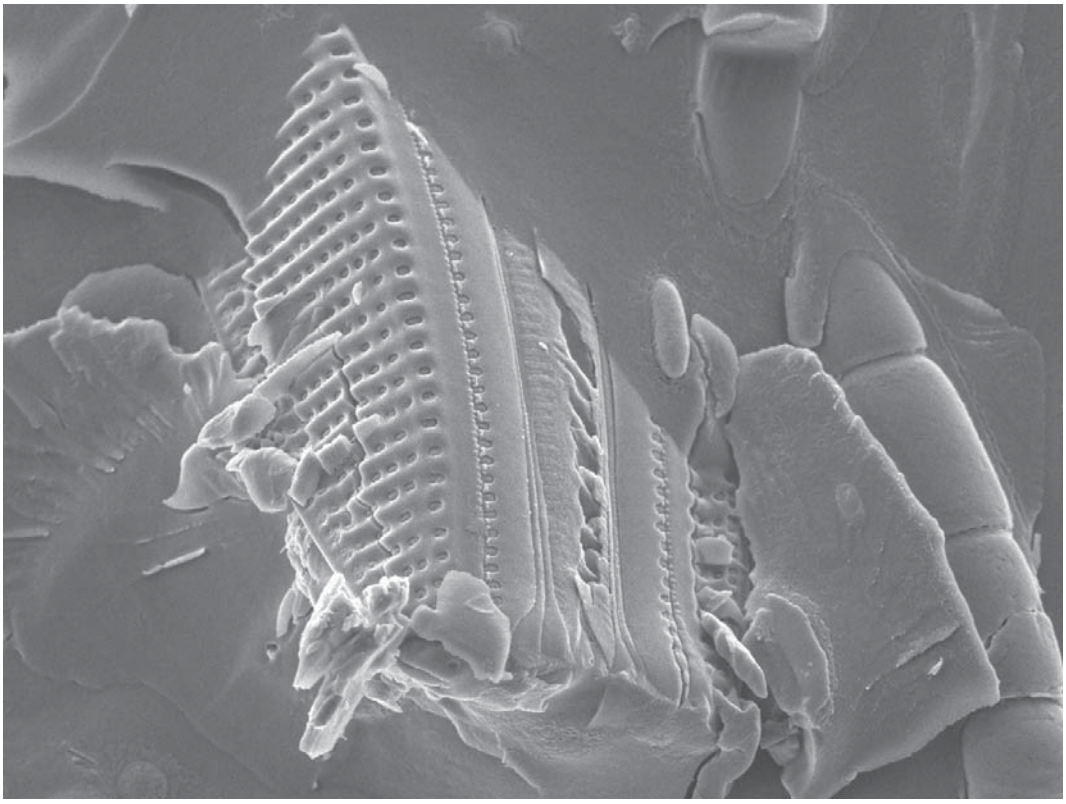

High pressure frozen, freeze fractured suspension of Vero cells. The sample was fractured at –115°C in a BAF060 and immediately coated by electron beam evaporation with 3 nm of Pt/C. M…Mitochondria, N…Nucleus, NP… Nucleopores, G…Golgi, PM… Plasma membrane. Electron Microscopy ETH Zurich (EMEZ).

Courtesy of Peter Wild, Institute of Veterinary Anatomy, University of Zurich, Switzerland.



High pressure frozen (HPM010), EM VCT100 transfer to the EM BAF060 for freeze-fracture/freeze-etching and cryo-coating (Pt/C, 3 nm) using the electron beam gun and rotating specimen holder. EM VCT100 transfer to the cryo-FEGSEM (JEOL 7401F). Euglena gracillis Klebs CCAP 1224/5Z.

Courtesy of Dr. Roland Fleck, NIBSC, Potters Bar, UK.



High pressure frozen (HPM010), EM VCT100 transfer to the EM BAF060 for freeze-fracture/freeze-etching and cryo-coating (Pt/C, 3 nm) using the electron beam gun and rotating specimen holder. EM VCT100 transfer to the cryo-FEGSEM (JEOL 7401F). Pennate diatom from a mixed culture of the protist Euplotes.

Courtesy of Dr. Roland Fleck, NIBSC, Potters Bar, UK.

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