



Leica EM BAF060

Freeze Fracture and Etching System

Living up to Life

Leica
MICROSYSTEMS

Freeze Fracture and Etching

The freeze fracture technique is a specimen preparation method in which vitreous material is physically broken apart to expose organelle surface structures for examination with an electron microscope. A metal/carbon mix coating on the fractured plane offers a replica of the specimen which in turn can also be viewed with a transmission electron microscope.

Etching, which can be carried out after fracturing, provides more information from the fracture faces by subliming superficial ice layers under vacuum to expose cellular elements that were originally hidden.

Recent technical advances in freeze fracture electron microscopy, particularly freeze replica immunolabeling (FRIL), have provided new insights into the roles of membrane proteins in dynamic cellular processes.

Features of the Leica EM BAF060

- Active LN₂ pumping system for continuous sample preparation without temperature fluctuations.
- Hydrocarbon-free operation using the turbo molecular drag pump with membrane diaphragm backing pump.
- Excellent high vacuum conditions and full range vacuum display. Preserves the sample against contamination and allows sublimation at low temperature.
- High performance microscope. Ergonomically designed for optimal viewing of all preparation processes.
- Automatic bake-out function for LN₂ cooling system. Quick release of LN₂ from the chamber Dewar.
- Programmable pre-set parameters.
- Easy and safe source adjustment.
- LED illumination available for best preparation observation.





High-End Preparation Unit

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 coating of specimens for cryo SEM analysis
- cryo coating for cryo SEM using the EM VCT100 vacuum cryo transfer system

as well as

- carbon reinforcement films on collodion or formvar coated specimen support grids
- conductive carbon films on specimens for X-ray microanalysis (EDX, WDX)
- multiple layer systems without interruption of the vacuum
- normal, portrait and rotary shadowing
- shadowing at low angles

The Leica EM BAF060 brings the freeze fracturing technique to a new level, featuring an advanced microtome, flexible shadowing options with electron beam sources and a load-lock transfer system.

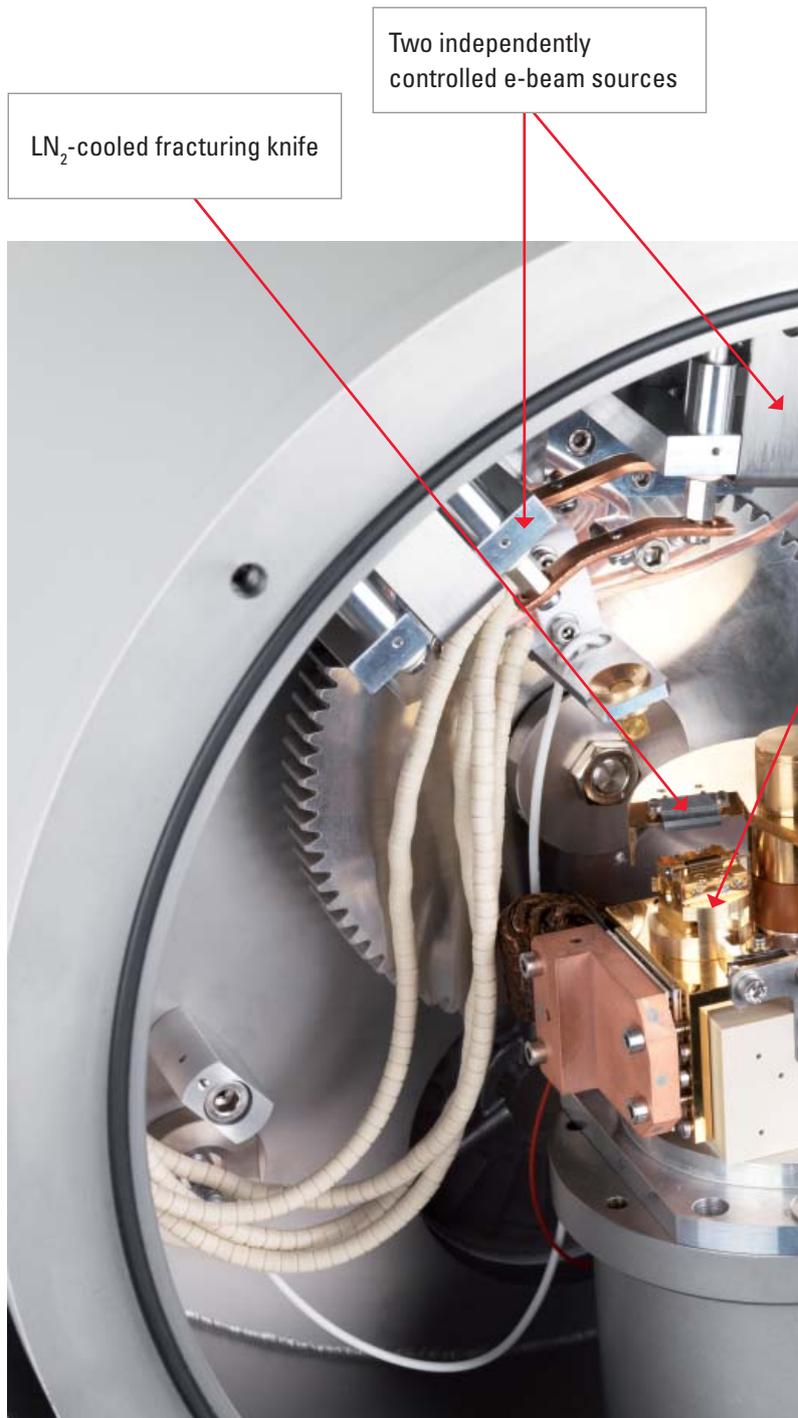
Designed to Meet Your Needs

Precise fracturing with the microtome

- Manual or automatic selectable knife advance with stepper motor between 1 μm and 40 μm . Either single step or continuous advance possible.
- Versatile knife positioning allows three samples to be cut with different parts of the knife.
- Robust rotary microtome for contamination-free freeze fracturing.

Optimal coating and flexible shadowing

- Two independently controlled e-beam sources for creating multi-layer replicas in a single run.
- The e-beam guns can be tilted during the coating process allowing for flexible change of the deposition angle.
- Electron beam source angles can be preset from 0° to 90° for shadowing and replication (carbon layer at 90°).
- Stationary or rotary shadowing is possible, offering a wide range of shadowing options, including DARS (double axis rotary shadowing) for homogeneous layers of fissured samples.
- Low angle shadowing to 0° for high resolution shadow coating of very small structures such as DNA.
- Evaporation angle adjustable during coating for more stable replicas and surfaces. Optimal for deeply fissured samples.
- Specimen stage rotation possible during coating.
- Pre-setting of individual electron beam evaporation parameters using Pt-C, Ta-W, Cr, Pt-Ir-C or C.
- Individually pre-selected high voltage, current and time values for degassing.





Precise stage, knife and shutter temperature control

- Temperature controlled cold stage for defined sublimation (+60°C to -180°C). Precise vacuum readout for optimal results.
- An LN₂-cooled shutter (cold trap) prevents contamination of the specimen.
- LN₂-cooled fracturing knife with manual or motorized motion.
- Automatic filling of the chamber Dewar with LN₂.

Exact and reproducible film thickness control

- Precise film thickness control with automatic quartz crystal thickness monitor and shutter termination for easy and safe replica production. Film thickness termination adjustable to 1 nm.
- Optimized quartz head positioning for exact thickness measurement.
- Memory functions: 5 layers with independent thickness values.
- Programmable layer sequences for multiple coating layers.
- Integrated library for multiple coating materials.

The Load-Lock System

The load-lock system is an integral part of the Leica EM BAF060. It is ideally designed for fast contamination-free preparation cycles. The load-lock transfer system allows sample and electron beam gun (evaporation source) exchange into the sample chamber without breaking vacuum.



Load-lock transfer rod for quick and easy **sample exchange**.



Load-lock transfer rod for a one button operational **exchange of the electron beam gun and coating material**. The process is extremely easy and quick.

Large Variety of Sample Holders

The Leica EM BAF060 is a highly versatile instrument which offers best results for freeze fracture, freeze etching and freeze drying techniques for TEM, SEM and cryo SEM analysis. A variety of specimen holders can be supplied with the system depending on the application.

① **Freeze Fracture holder**

with retaining spring for three $\text{\O} 3 \times 0.8$ mm gold specimen carriers.



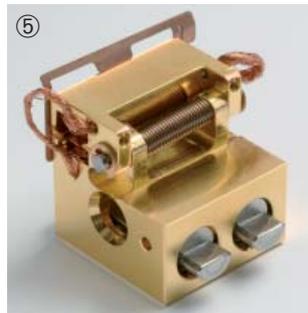
② **Freeze Fracture holder**

with retaining spring for three $\text{\O} 3 \times 4.5 \times 0.8$ mm copper specimen carriers.



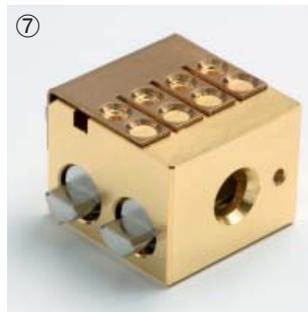
④ **Double Replica holder**

with spring load for two $\text{\O} 4.6 \times 0.6$ mm gold specimen carrier sandwiches.



⑤ **Double Replica holder**

with spring load for four $\text{\O} 3 \times 4.5 \times 0.6$ mm copper specimen carrier sandwiches.



⑥ **Freeze Drying holder**

with two magnetic strips for three $\text{\O} 3$ mm grids.

⑦ **Freeze Drying holder**

with retaining spring for four $\text{\O} 3$ 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.

STEP 3: Sample Transfer for Fracturing, Etching and Coating

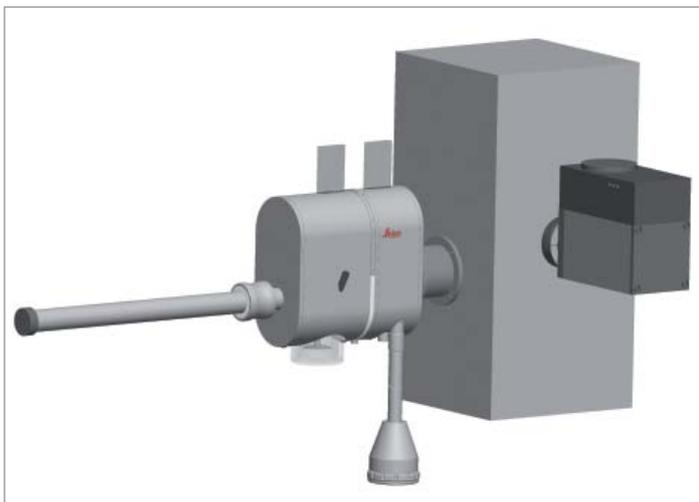
The specimen and holder are subsequently transferred under vacuum and low temperature to the Leica EM BAF060 preparation unit using the Leica EM VCT100 shuttle.



The specimen can then be fractured, etched and coated using the multiple features of the Leica EM BAF060.

STEP 4: Sample Transfer to Cryo Stage

After sample preparation, the specimen is transferred still frozen and under vacuum onto the cryo stage of the SEM.



EM VCT100 docking station and shuttle attached to an analysis instrument.

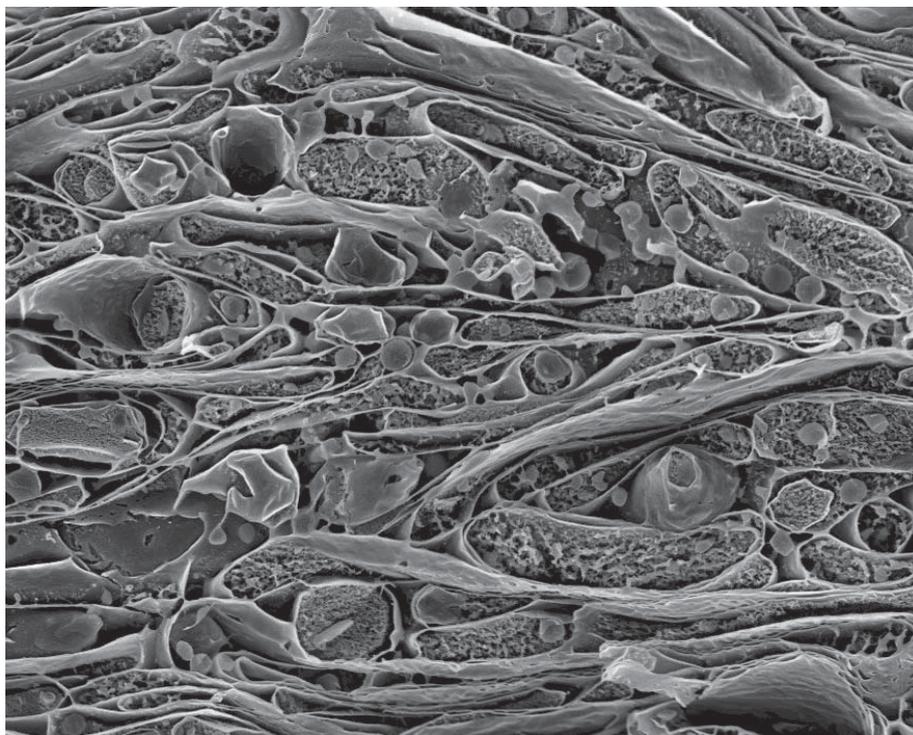
STEP 5: Sample Analysis

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



EM VCT100 docking station and cryo Dewar on an analysis instrument.

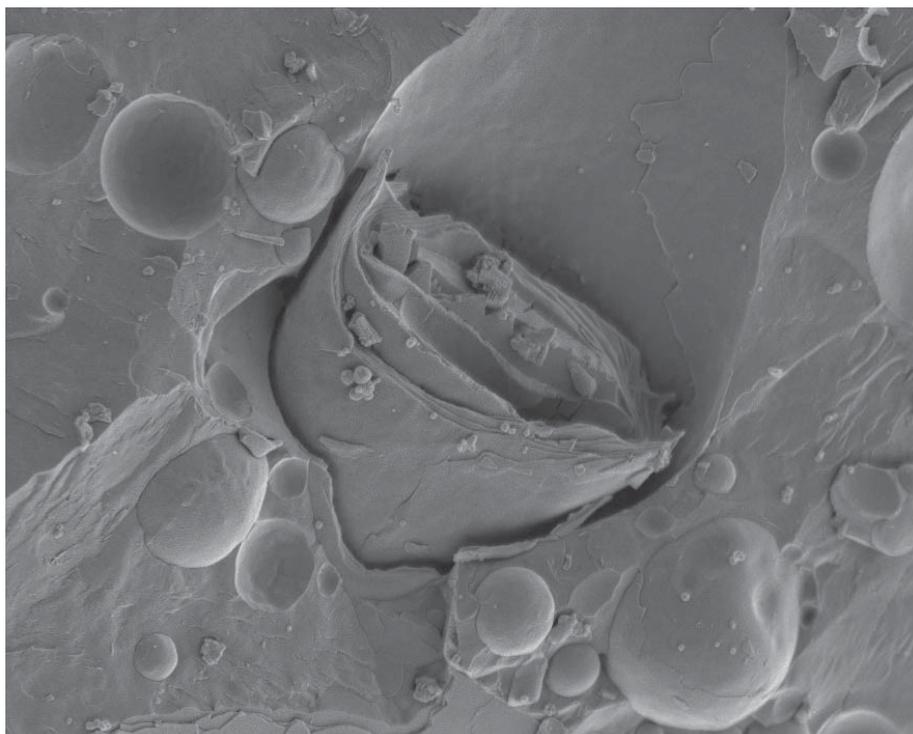
Results



HPF, EM VCT100 transfer to the EM BAF060 for freeze etching and cryo coating using the electron beam gun and a rotating specimen holder. EM VCT100 transfer to the cryo SEM.

Deo formula on an oil/water base, approx. 3 minutes by -100°C (sublimation) exposing lipid layers (rough texture wafer + emulsifier).

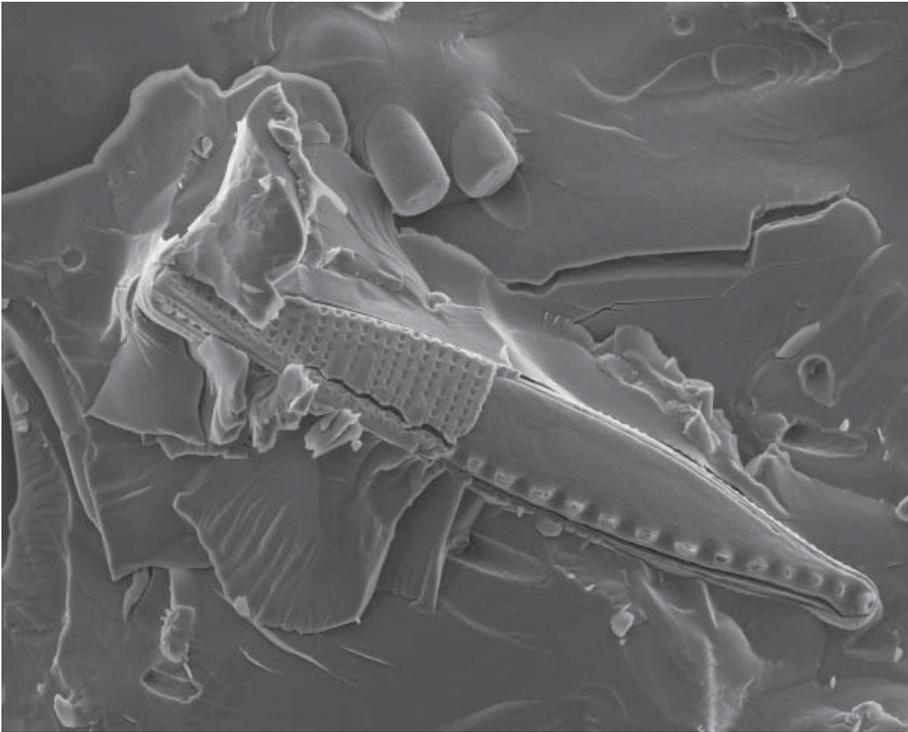
Courtesy of Dr Stefan Wiesner, Beiersdorf, Hamburg



HPF, freeze fractured, freeze etched and cryo coated with the EM BAF060/EM VCT100 transfer to the cryo SEM.

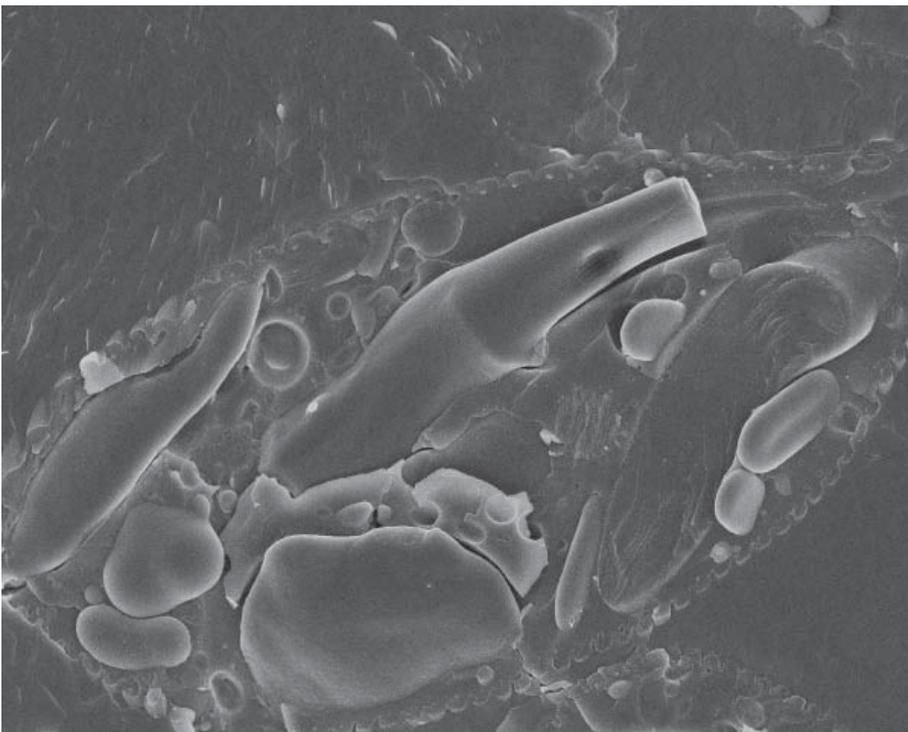
Oil/water emulsion fractured exposing onion-like composition of lamellae forming a droplet.

Courtesy of Dr Stefan Wiesner, Beiersdorf, Hamburg



HPF, EM VCT100 transfer to the EM BAF060 for freeze-fracture/freeze-etching and cryo-coating using the electron beam gun and rotating specimen holder. EM VCT100 transfer to the cryo SEM. Pennate diatom from a mixed culture of the protist Euplotes.

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



HPF, EM VCT100 transfer to the EM BAF060 for freeze-fracture/freeze-etching and cryo-coating using the electron beam gun and rotating specimen holder. EM VCT100 transfer to the cryo SEM. Euglena gracillis Klebs CCAP 1224/5Z.

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

“With the user, for the user”

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• Biosystems Division

The Leica Microsystems Biosystems Division brings histopathology labs and researchers the highest-quality, most comprehensive product range. From patient to pathologist, the range includes the ideal product for each histology step and high-productivity workflow solutions for the entire lab. With complete histology systems featuring innovative automation and Novocastra™ reagents, Leica Microsystems creates better patient care through rapid turnaround, diagnostic confidence, and close customer collaboration.

• Surgical Division

The Leica Microsystems Surgical Division's focus is to partner with and support surgeons and their care of patients with the highest-quality, most innovative surgical microscope technology today and into the future.

The statement by Ernst Leitz in 1907, “with the user, for the user,” describes the fruitful collaboration with end users and driving force of innovation at Leica Microsystems. We have developed five brand values to live up to this tradition: Pioneering, High-end Quality, Team Spirit, Dedication to Science, and Continuous Improvement. For us, living up to these values means: **Living up to Life.**

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