

From Eye to Insight

Leica
MICROSYSTEMS

AUTOMATIC PLUNGE FREEZER

Leica EM GP



Leica EM GP

Automated Plunge Freezing

For the preparation of vitrified samples for cryo-TEM.

The Leica EM GP can be used to plunge freeze biological samples in suspensions as well as industrial emulsions in aqueous or inorganic solvents.

THE BARE GRID TECHNIQUE

Many specimens for cryo-TEM can be prepared by immersion freezing, where a fluid sample is pipetted onto an EM grid (usually coated) and the excess removed until a thin film remains, before plunging into a cryogen such as liquid ethane. The grid can then be directly transferred under cryo conditions to the cryo electron microscope (cryo-TEM) for observation. This is the bare grid technique.

The bare grid technique can be used for many types of sample ranging from viruses, proteins or macromolecular complexes to industrial emulsions. Imaging macromolecular assemblies, viruses and cells in their native, hydrated environment in the cryo-TEM is the state-of-the-art technique in electron microscopy, providing maximum resolution with minimal specimen damage.



Discover the features

OUTSTANDING REPRODUCIBILITY AND SAMPLE QUALITY

Sensor controlled blotting

- › No further calibration needed
- › Blotting gives equal distributed surface on grid for perfect imaging results
- › Very soft blotting possibility to protect very sensitive samples
- › Quick and efficient blotting

Automated blotting

- › Blotting gives equal distributed surface on grid for perfect results
- › Quick and efficient blotting
- › Double side blotting for efficient removal of excessive fluid on grid for perfect cryo fixation

Multiple blotting

- › Blotting of very viscous samples for perfect cryo fixation

Environmental chamber

- › Temperature and humidity controlled protective environment variable between +4 °C and +60 °C and room humidity to 99 %
- › To provide a clear view, an anti-fogging heater keeps the glass window clear

EASE OF USE FOR MULTIUSER ENVIRONMENT

External touch screen user interface

- › Easy and accurate programming
- › For multiuser environment
- › Intuitive software with user library
- › Fast learning process

Viewing system

- › Stereomicroscope with LED to facilitate sample preparation and alignment

INCREASED SAFETY

- › Special secondary cryogen filling system for easy and accurate filling

Control Panel

PROGRAM SCREEN

All parameters can be adjusted and set for up to 10 programs. In the Setup menu the positioning of the grid relative to the blotter can be adjusted and also the transfer position after plunging.

SAFETY

The Leica EM GP operates under strict safety conditions. During any movement of the environmental chamber a large red STOP button appears on the control panel. Touching this button will immediately stop any movement. An alarm signals when either the secondary cryogen is too warm and may evaporate or the LN₂ level is too low.

BAKE-OUT

At the end of a run the bake-out cycle takes 60 minutes to dry the Dewar and environmental chamber, which allows a second run within a short time if the user does not wish to maintain the LN₂ level in the Dewar.

Main screen

Program in use → P 2 5.0/ .5/ 1.0/

Current temperature of environmental chamber → Tc 37°C

Current humidity of environmental chamber → Hr 80 %

Current temperature of ethane container → -180°C

LN₂ level → 90%

Count → 10

Load Forceps → Sets start position to accept forceps

Lower Chamber → Chamber moves downwards ready for dispensing suspension

Rotate Home Blot /A-Plunge → Blotting/plunging sequence

Plunge → Blotting/plunging sequence

Transfer → Press to raise grid in ethane and increase Dewar GN₂ production to keep out moisture

Setup Bake out Manual Rotation

Countdown for blotter exchange

Program screen

Nr.	Blotting		Time (s)		
	Sensor	A-Plunge	Delay	Blot	Hold
1	✓	✓	0.0	0.0	0.0
2	✓	✓	0.0	0.5	0.0
3	✓	✓	0.0	0.0	0.0
4		✓	0.0	0.0	0.0
5			0.0	0.0	0.0

Rotate grid after applying suspension → (row 1, column 2)

Plunge automatically after blotting → (row 1, column 3)

Blotting time → (row 1, column 5)

Program number → (row 2, column 1)

Scroll through programs 1–5 or 6–10 → (right arrow)

Rotate grid before applying suspension → (row 2, column 2)

Blotting with blot sensor activated → (row 2, column 3)

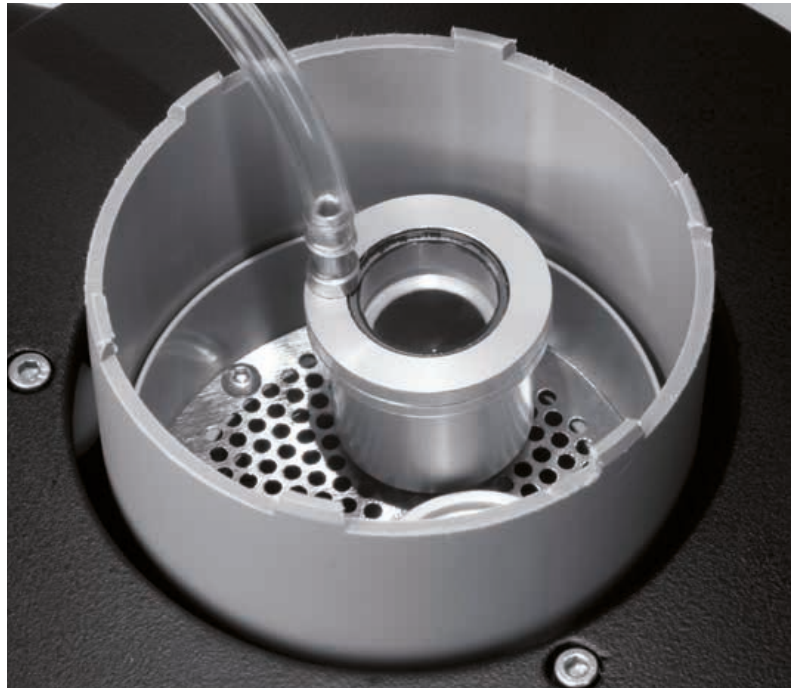
Delay time before blotting → (row 2, column 4)

Delay before freezing after blotting → (row 2, column 5)

OK

The Dewar

After switching on the Leica EM GP, the 1 liter Dewar can be filled with LN₂ before liquefying the secondary cryogen, usually ethane. A full Dewar lasts for approximately 1 hour between refilling. Liquefying the secondary cryogen is fast, easy and safe with the liquefying head. The head is connected to the secondary cryogen regulator of the gas bottle and the gas slowly fed in. It condenses within seconds, taking about one minute to fill the 2.5 ml container. A cover is provided to prevent LN₂ splashing into the ethane on subsequent refilling of the LN₂. The temperature of the secondary cryogen can be controlled precisely from the control panel. A container, filled with LN₂, sits in the Dewar to hold a grid box for transfer of prepared, vitrified samples.



LIQUEFIER IN PLACE over ethane container in Dewar



AFTER FREEZING

the grid remains in or above the ethane (depending upon user settings) ready for transfer to the grid box

EM GP Adapter for Cryo TEM Transfer

The Leica EM GP adapter for cryo TEM transfer was developed to meet the increased customer demands for an optimized cryo TEM transfer workflow for grid plunge applications.

This unique adapter can be customized to existing cryo transfer systems and ensures an easy and safe sample transport from EM GP to the cryo TEM.

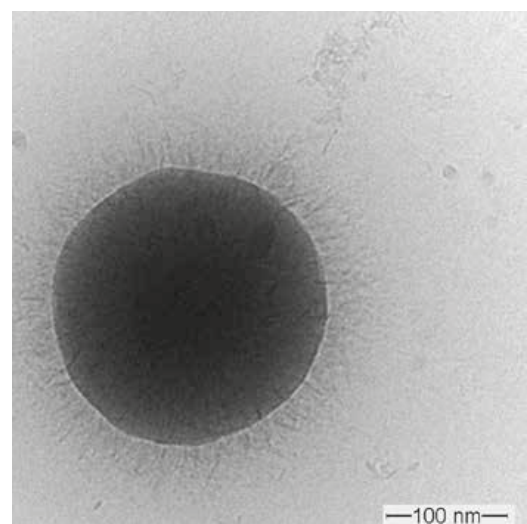
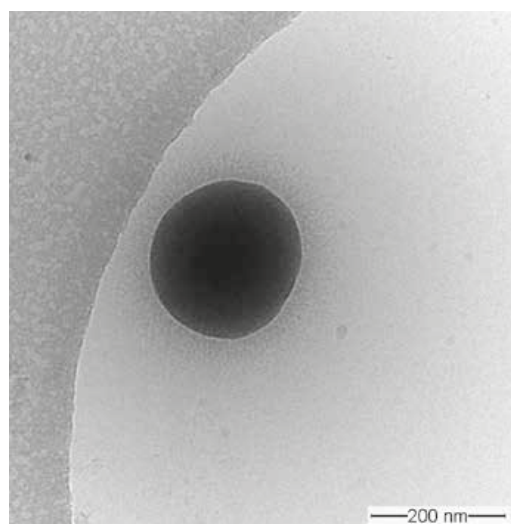
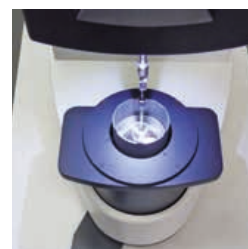
- › No “snowing” contamination
- › Fast sample transport avoids thawing and increases sample safety by preventing ice cristall formation

PH dependent self assembly of Polyacrylic Acid (PAA) brushes on Sub-micron silica spheres:

Left: Sub-micron silica spheres, covered with polyacrylic acid (PAA) brushes.

Right: The PAA of the particle is swollen and shows the typical hairy structure of polymer brushes.

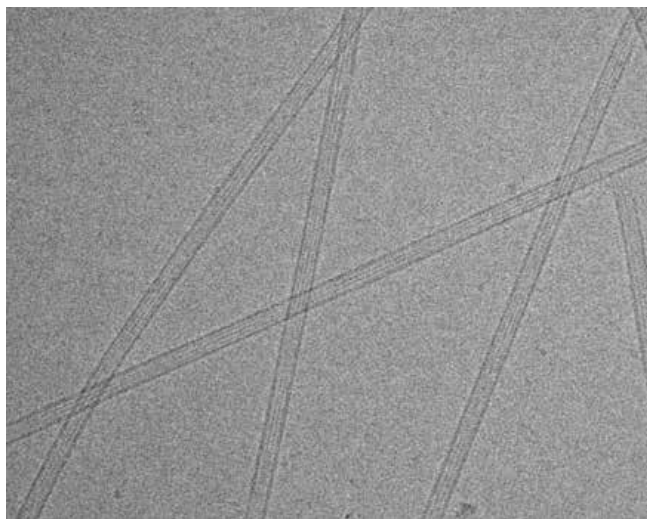
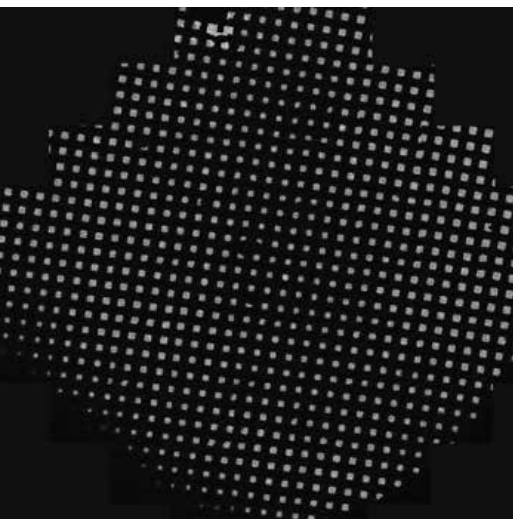
Micrographs courtesy of L. Schellkopf, Leibniz Institute of Dresden, Germany



Applications

The Leica EM GP is designed for all EM laboratories with a need to view vitrified fluid samples or extremely thin samples in the cryo-TEM, including biological research, virology, protein crystallography, pharmaceutical research, cosmetics and industrial laboratories.

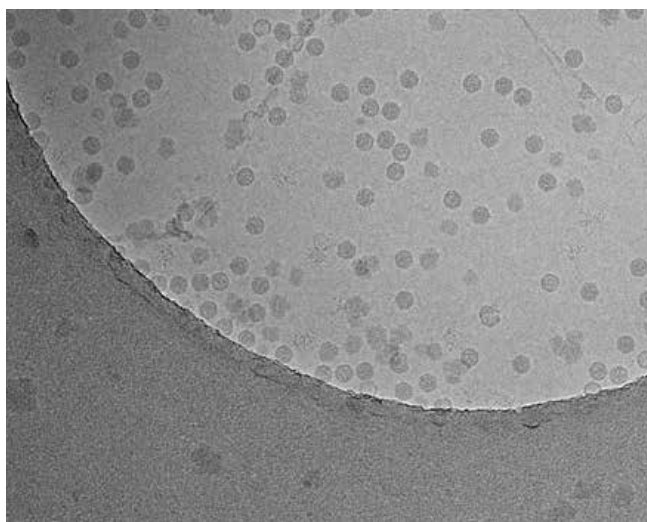
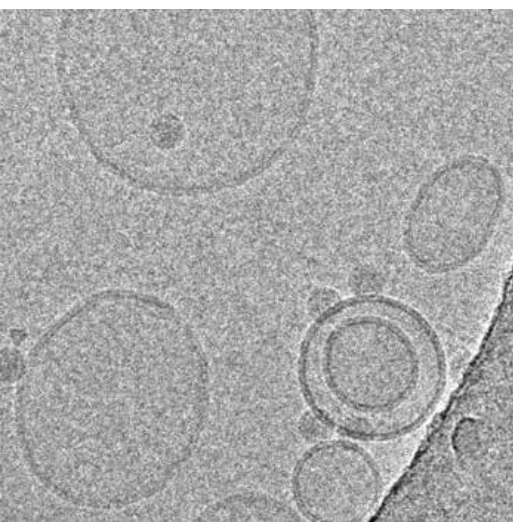
Samples that can be prepared vary for example from suspensions of viruses, liposomes, microtubules, proteins and other cellular components to paint or solutions and emulsions in both aqueous and inorganic solvents. The Leica EM GP can be used to plunge freeze samples not only on EM grids for the Bare Grid Technique, but also sapphire discs and samples in freeze fracture planchettes.



Left: Montage overview of plunge frozen grid. Note the homogeneity of the film thickness.

Right: Microtubules

Micrographs courtesy of Dr. Guenter Resch, IMP/IMBA Electron Microscopy Facility, Vienna, Austria



Left: Liposomes

Right: Rhinovirus particles on holey carbon film

Micrographs courtesy of Angela Pickl-Herk, MFPL, Vienna, Austria



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