From Eye to Insight

Leica
MICROSYSTEMS

AUTOMATIC PLUNGE FREEZER

EM GP2

EM GP2 AUTOMATIC PLUNGE FREEZER FOR EM GRIDS

REPRODUCIBLE RESULTS FOR CRYO-TEM

EM GP2 can be used to plunge freeze biological samples in suspensions as well as industrial emulsions in aqueous or inorganic solvents.

REPRODUCIBILITY AND SAMPLE QUALITY

> The Automatic Plunge Freezer EM GP2 offers flexibility by using different blotting types and allowing you to configure a range of environmental and blotting parameters individually.
> Controlled blotting conditions prevent sample and film damage
> Minimized ice contamination and devitrification thanks to GN_2 gas flow that protects sample after freezing
> Sensor controlled single-sided, flat blotting
> Consistent control of secondary cryogen temperature
> Temperature and humidity controlled environmental chamber

EASE OF USE AND SAFETY

> Window heater to keep the glass clear and ensure visibility
> Intuitive control via touch screen
> Docking station for TEM cryo transfer holders
> Intuitive software with user library
> Adjustable user interface to accommodate left and right had users
> Fast, easy and safe filling of secondary cryogen with the unique liquefying head
> Ivesta 3 Greenough Stereo Microscope for high resolution and a large depth of field
> Safety alarms for humidity and secondary cryogen temperature control

SOFTWARE CONTROL

The EM GP2 offers comprehensive control, allowing all parameters to be adjusted and set for up to 20 programs, including grid position relative to the blotter and the transfer position after plunging. The system operates under strict safety conditions, with the ability to immediately stop any movement of the environmental chamber by pressing a STOP button on the control panel. An on-screen alarm signals when the secondary cryogen becomes too warm and risks evaporation, or when the LN_2 level is too low. At the end of a working session, it takes 60 minutes to dry the Dewar and the environmental chamber through a bake-out cycle, after which the EM GP2 is ready for a new run.

THE BARE GRID TECHNIQUE

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

The bare grid technique can be used for many types of samples ranging from viruses, proteins, and 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.

SECONDARY CRYOGEN LIQUEFACTION

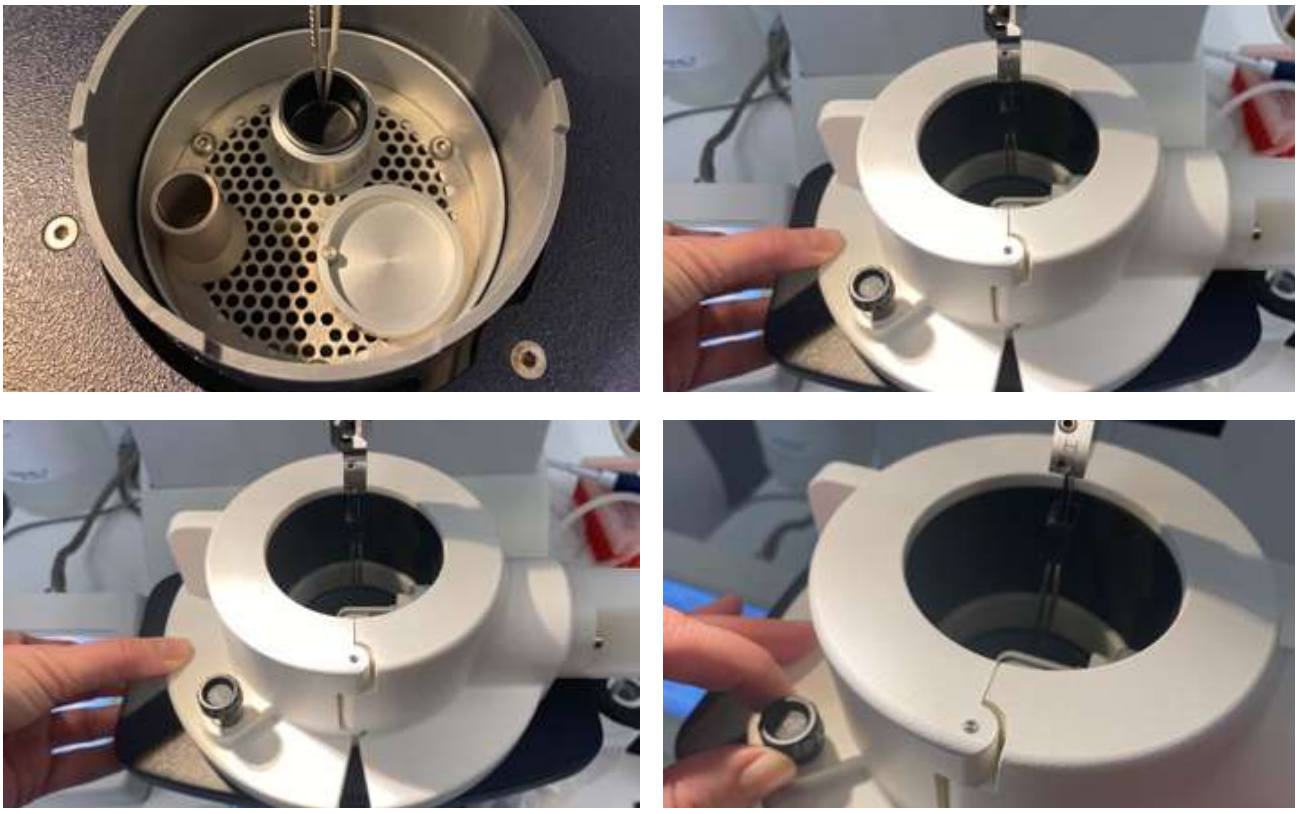
After switching on the Leica EM GP2, the 1-liter Dewar can be filled with LN_2 before liquefying the secondary cryogen, usually ethane. Liquefying the secondary cryogen is fast, easy and safe using the liquefying head. The head connects to the gas bottle's secondary cryogen regulator, and the gas is fed in slowly. It condenses within seconds, taking about one minute to fill the 2.5 ml container. A cover is provided to prevent LN_2 splashing into the ethane during subsequent refills. The temperature of the secondary cryogen can be precisely controlled from the control panel. A container filled with LN_2 is placed in the Dewar to hold a grid box for transferring prepared, vitrified samples.

Liquefier placed over the ethane container inside the Dewar.

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

EM GP2 ADAPTER FOR CRYO-TEM TRANSFER

The EM GP2 adapter for cryo-TEM transfer can be customized to fit existing cryo transfer systems, ensuring the easy and safe transport of samples from the EM GP2 to cryo-TEM. Fast sample transport increases sample safety by preventing ice crystal formation.

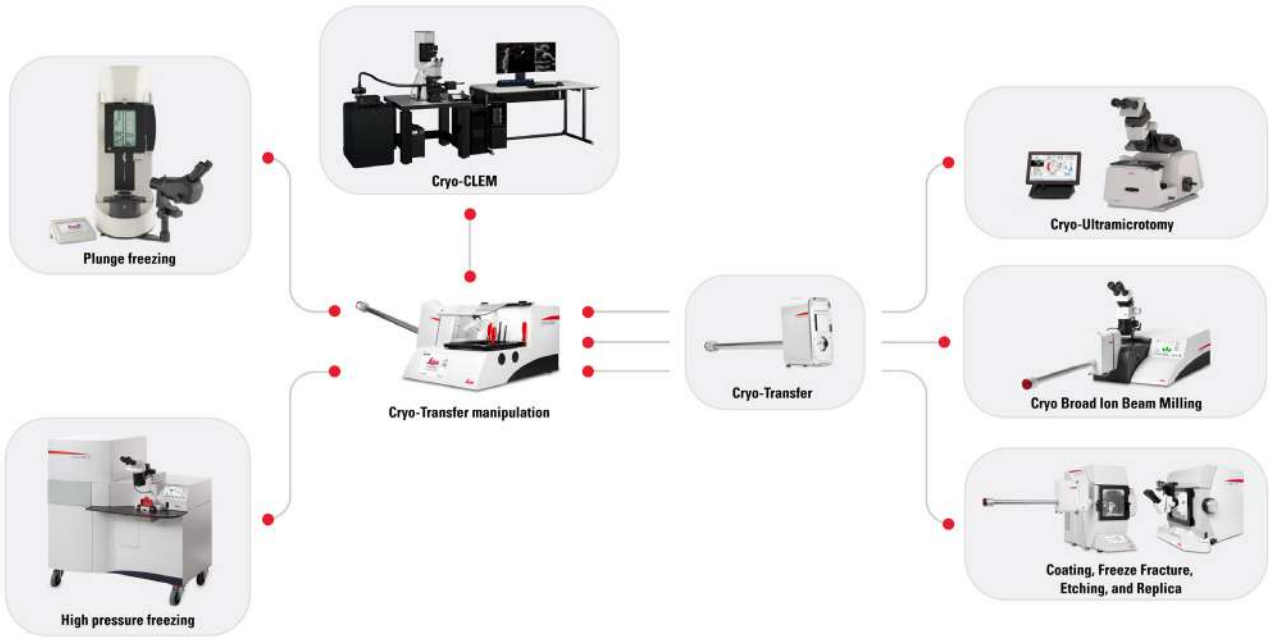


Adapter mounting process: Top left: EM GP2 Dewar without the adapter; bottom left: adapter alignment on the Dewar. Cooling platform is visible inside; top right: adapter closing using magnetic locking system; bottom right: fastening onto Dewar with screws.

EM GP2 with Cryo-TEM transfer adapter mounted. Additional Cryo-TEM support is visible below the cryo-TEM holder entry.

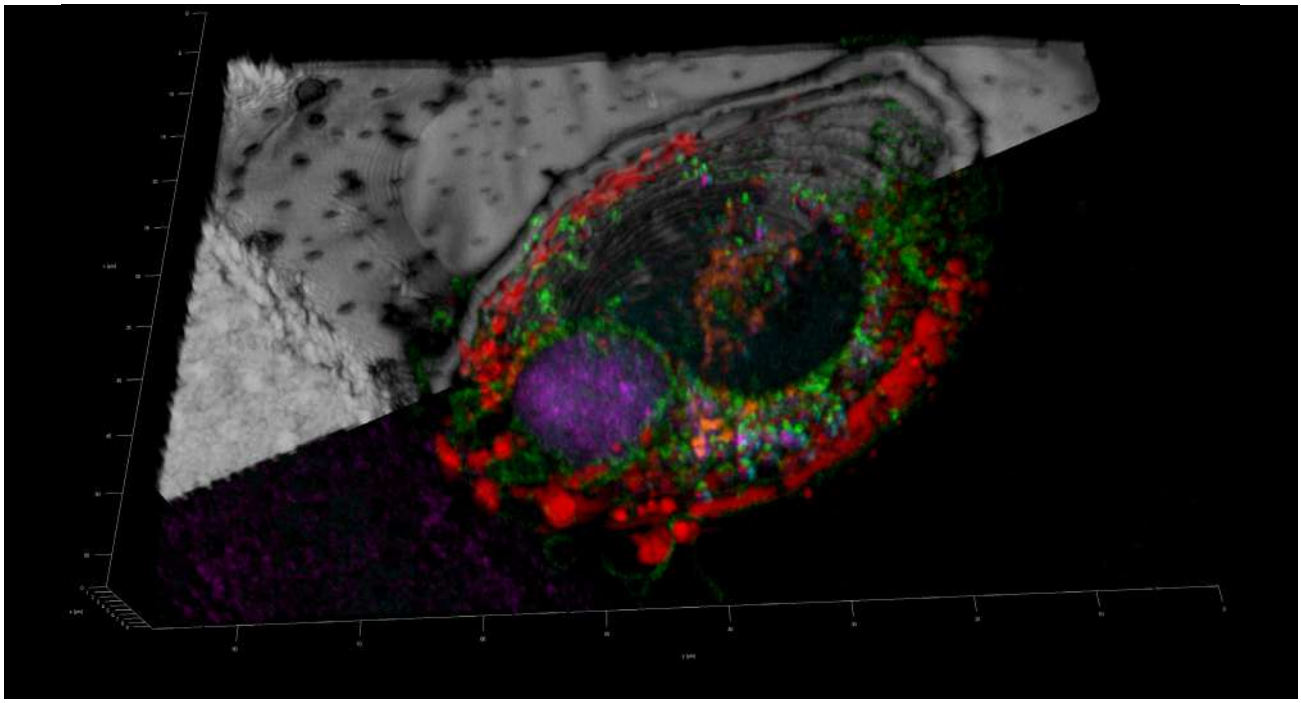
APPLICATIONS

EM GP2 automatic plunge freezer for EM grids enables vitrification of liquid or extremely thin samples for Cryo-TEM investigations. EM GP2 supports workflows used in biological research, virology, protein crystallography, pharmaceutical research, cosmetics, and industrial laboratories.

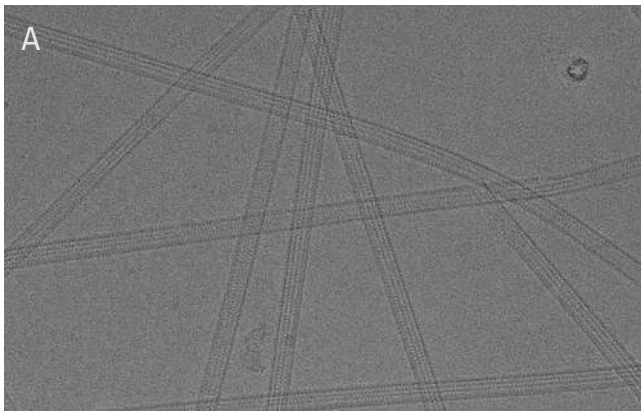


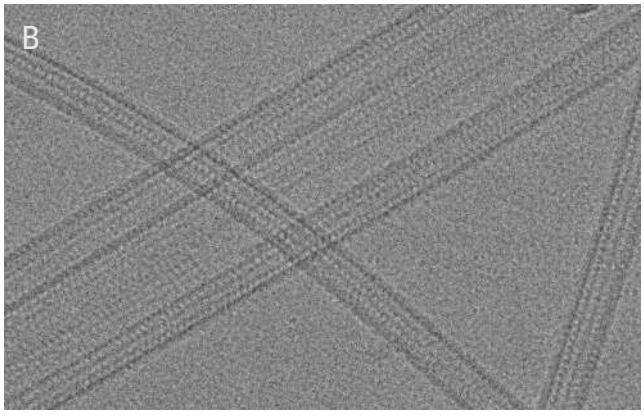
EM GP2 as a part of cryo sample preparation workflow.

Samples for grid plunging range from suspensions of viruses, liposomes, microtubules, proteins, and other cellular components to paint or solutions and emulsions in both aqueous and inorganic solvents.

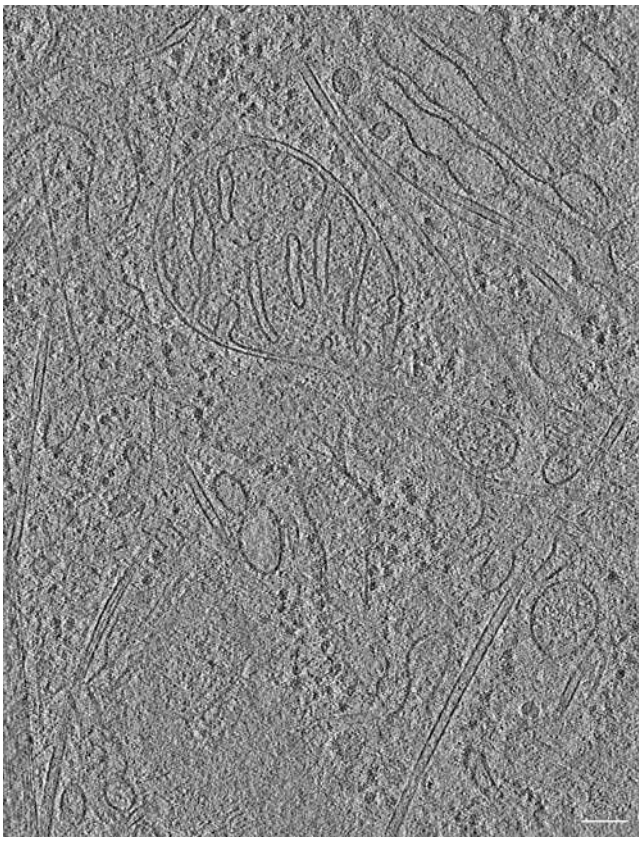


Mammalian cells plunge frozen (EM GP2) on EM grid. Cell organelles stained with: Tag BFP (nucleus, blue), mCerulean (membranes, green), hmAzami-Green (mitochondria, orange), mCitrine (Golgi, red), mCherry (ER, magenta), reflection (grid and carbon layer visualization, white). Cells courtesy of Dr. Oleg Sitsel, Max Planck Institute for Molecular Physiology, Dortmund, Germany. Confocal image acquired on STELLARIS Cryo with LIGHTNING module.
Scale bars xy: 10 µm; z: 1 µm.





Micrographs A and B show images of microtubules acquired at 20000x (A) and 39000x (B) nominal magnification in the holes of the perforated carbon film. The ice is free of exogenous contamination, the protofilaments and the individual tubulin subunits are clearly visible along the length of the microtubules. Courtesy of Dr. Guenter Resch, Nexperion e.U. - Solutions for Electron Microscopy; Dr. Thomas Heuser and Marlene Brandstetter, Vienna Biocenter Core Facilities GmbH



Full view of tomographic slices of GEM2-labeled Mito-EGFP on mitochondria. Scale bar 100nm. Image courtesy: Fung HKH, Hayashi Y, Salo VT, Babenko A, Zagoriy I, Brunner A, Ellenberg J, Müller C, Cuylen-Häring S, Mahamid J (2023). Genetically encoded multimeric tags for intracellular protein localization in cryo-EM. Nature Methods 20, pages 1900–1908.

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