Leica EM GP

Automatic Plunge Freezer for the Bare Grid Technique

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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 biological cell sub units 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.

Although a simple method, it is imperative that the suspension thickness on the grid can be reproduced and vitreous ice can be formed, otherwise much time is wasted loading useless samples into the cryo-TEM. The sample film is only tens to hundreds of nanometers thick and so can be easily influenced by temperature shifts and humidity prior to freezing. If the humidity is too low then the film breaks due to it quickly drying. If the sample is adversely affected by temperature, then the morphology may change before freezing.

Leica Microsystems has developed a plunge freezer, in conjunction with Dr. Guenter Resch of the IMP/IMBA Electron Microscopy Facility in Vienna, Austria, to standardize procedures and make the bare grid technique more reproducible.

Leica EM GP Form and Function

Leica Design by Werner Hölbl

The Leica EM GP plunge freezes samples into a secondary cryogen such as liquid ethane after removing excess fluid by automatic blotting.

After connecting the forceps holding the grid to the Leica EM GP, an environmental chamber lowers to surround the grid, providing a protective temperature and humidity controlled environment.

Access ports on both sides of the environmental chamber allow easy pipetting of solutions and suspensions for both left and right handed users.

Excess fluid is then removed by automatic blotting with filter paper from one side of the grid. Parallel, single sided blotting was developed to prevent damage to delicate support films. The blotter touches the complete grid surface in one movement. The grid can be programmed to automatically turn 180° prior to blotting to allow fluid removal from the correct side.

All parameters are displayed on the touch-screen control panel, where numerous settings can be adjusted and monitored such as blot time, grid/blotter positioning, temperature, humidity, LN_2 level, and secondary cryogen temperature.

After freezing, the grid is transferred to a pre-cooled grid box inside a transfer container filled with LN_2 located in the Dewar. This can then be taken to the sample holder of the cryo-TEM to prepare for loading into the electron microscope.

Leica EM GP Features

Environmental chamber

To provide the best conditions for pre-freezing of a suspension, the environment surrounding the grid and sample has to be precisely controlled. The environmental chamber envelopes the grid and forceps, providing a temperature and humidity controlled protective environment variable between +4° and +60°C and room humidity to 99%.

To provide a clear view, an anti-fogging heater keeps the glass window clear.

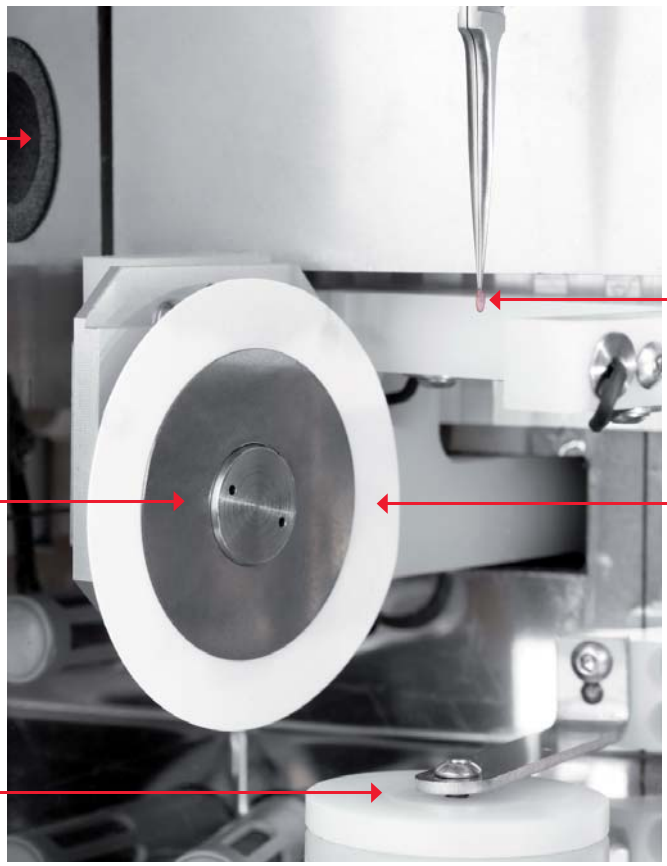
Blotting

Excess fluid is removed by automatic blotting which can be initiated in two ways:

1. **Autoblotting** – after the suspension has been pipetted onto the grid and the button pressed, the blotter automatically moves to the pre-set blot position for the user-programmed time before plunging of the grid.

2. **Sensor control** – the most automated method of plunging. After pressing the plunge button the blotter moves towards the grid. A photosensor detects the moment the droplet touches the grid and the blotting time countdown begins. The grid is then either held for the desired length of time (to allow redistribution of fluid for example) or immediately plunged into the secondary cryogen.

Blotting and plunging can be activated via the touch screen or footswitch.



Viewing system

The Leica EM GP has an optional stereomicroscope to aid alignment and sample preparation. The LED illumination, fixed both inside the environmental chamber and beaming down onto the Dewar, provides excellent high light levels for observation of the complete preparation and plunging process.

The Dewar

After switching on the Leica EM GP, the 1 liter Dewar can be filled with LN_2 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 LN_2 splashing into the ethane on subsequent refilling of the LN_2. The temperature of the secondary cryogen can be controlled precisely from the control panel. A container, filled with LN_2, 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

Control Panel

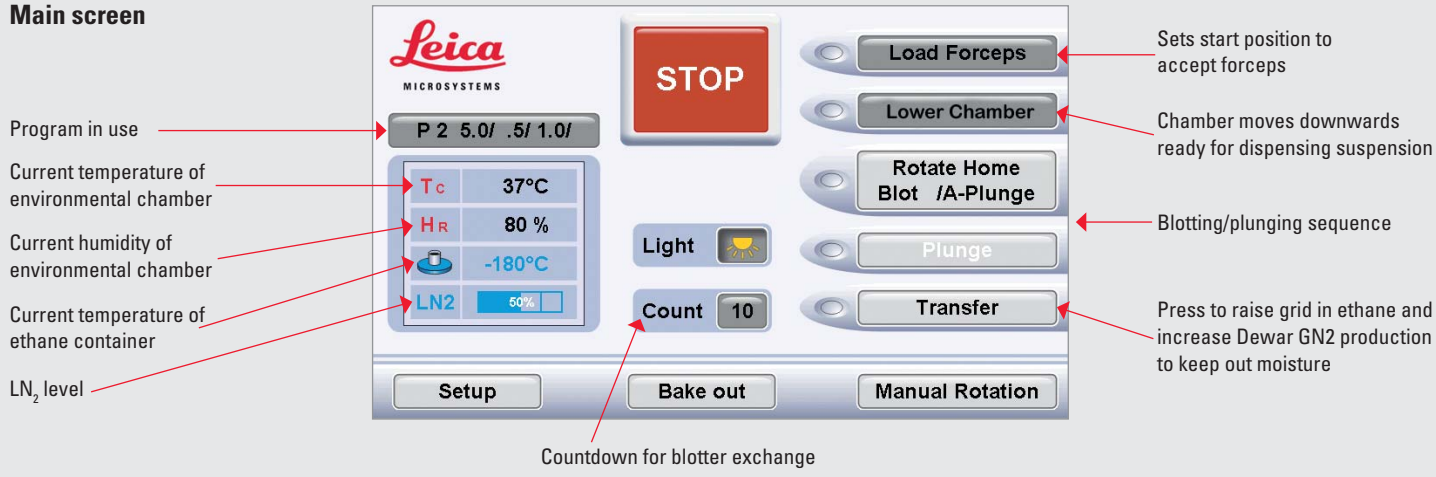
Operation is via touch screen control with all adjustable parameters visible.

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.

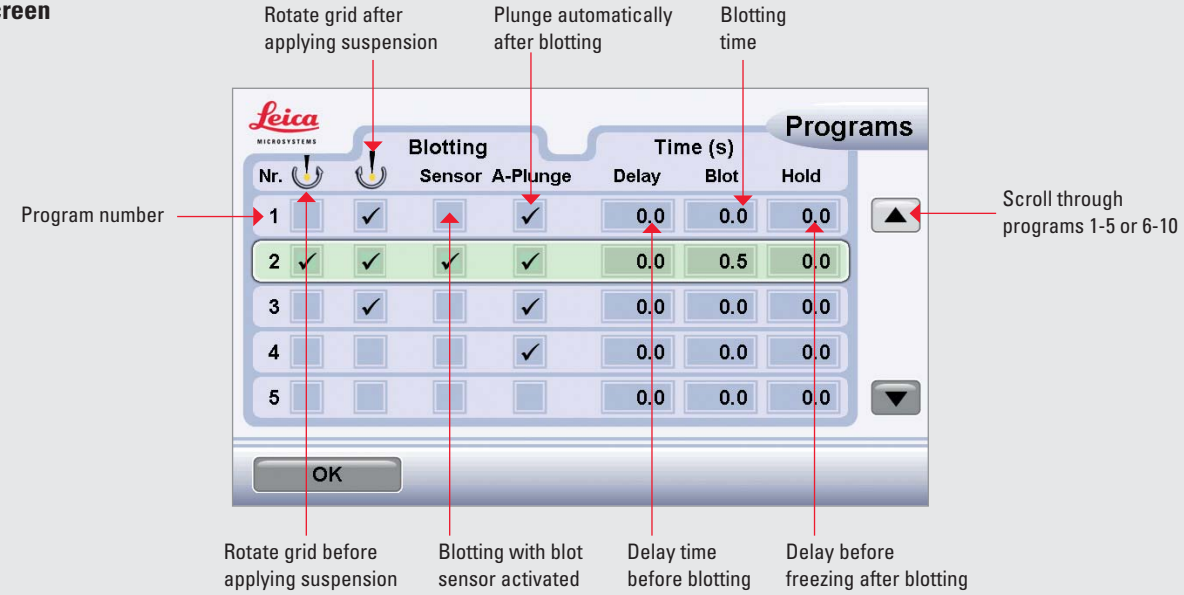
Safety
The Leica EM GP operates under strict safety conditions. During any movement of the environmental chamber a large red STOP button appears on the control panel. Touching this button will immediately stop any movement. An alarm signals when either the secondary cryogen is too warm and may evaporate or the LN_2 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 LN_2 level in the Dewar.

Main screen



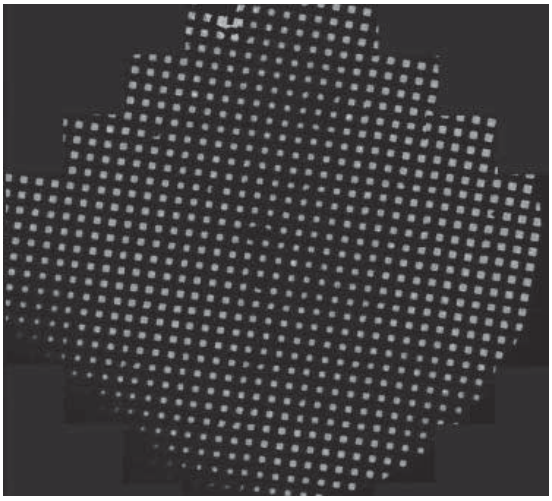
Program screen



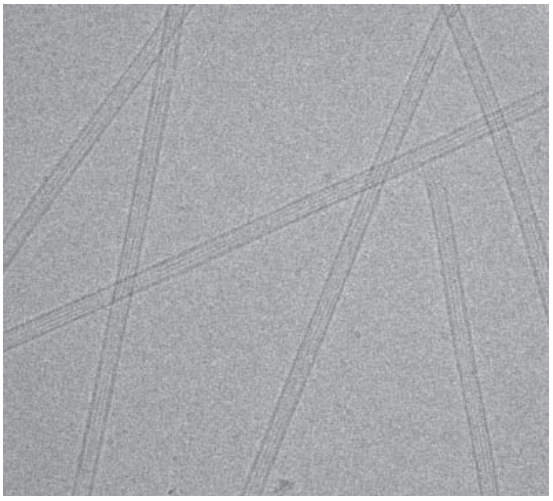
Applications

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.

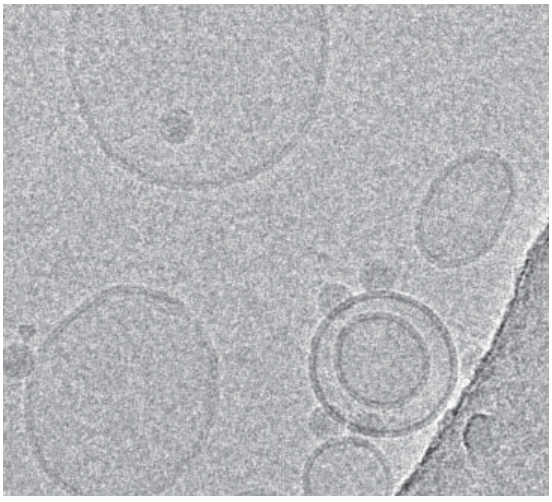


Montage overview of plunge frozen grid.
Note the homogeneity of the film thickness.

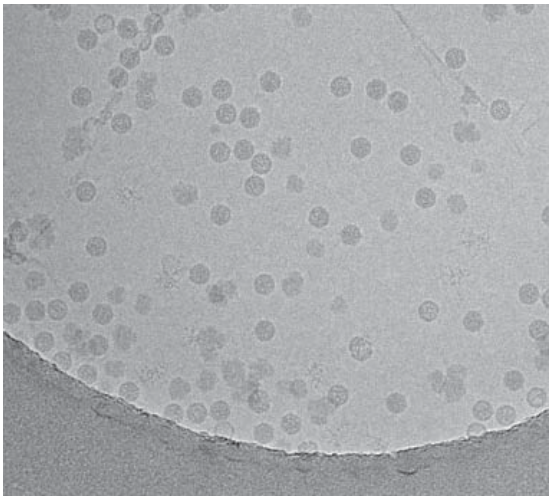


Microtubules

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



Liposomes



Rhinovirus particles on holey carbon film

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

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Netherlands:	Rijswijk	Tel. +31 70 4132 100	Fax +31 70 4132 109
People’s Rep. of China:	Hong Kong	Tel. +852 2564 6699	Fax +852 2564 4163
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Singapore		Tel. +65 6779 7823	Fax +65 6773 0628
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