



Leica EM CPC

Universal Cryofixation and Cryopreparation System

according to Neumann

Leica
MICROSYSTEMS



The CPC is a "State of the Art" multitask system for the preparation of specimens for:

- Plunge Freezing**
- Bare Grid Technique**
- Metal Mirror Freezing**
- Freeze fracture**
- Sample Preparation for e.g. cryoSEM**
- Freeze Substitution**
- Progressive Lowering of Temperature**
- UV-Polymerisation**

The CPC is a totally new design with improved ergonomics and increased tasking ability.

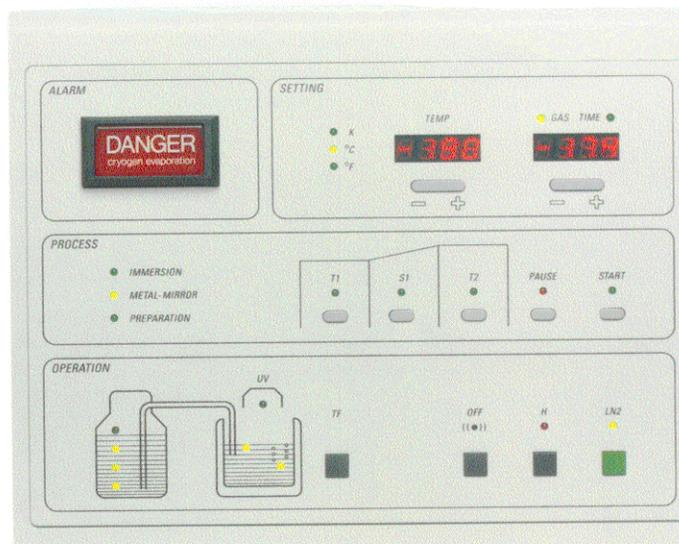
The working area with preparation plate as standard allows any low temperature manipulations to be carried out.

The well, conveniently offset towards the user, contains liquid nitrogen for the pre-cooling of forceps, scalpels etc.

The CPC is filled with LN2 via the dewar pump which automatically maintains the level of LN2. Accurate control of this helps reduce LN2 consumption and allows precise temperature control.

The modular design of the CPC-Cryoworkstation facilitates quick and easy change of the modules for Cryofixation, Cryopreparation and Observation.

The separate control unit of the CPC allows it to be placed out of the way of other experimental equipment or reagents.



The panel of the control unit is divided into four areas:

OPERATION

A schematic display shows the level of LN2 in the CPC and in the Dewar of the filling system. Pressing the **TF** button causes extra GN2 to be evaporated from the LN2 reservoir to avoid condensation during preparation.

Depressing the **H** key causes the system to close down – heaters are activated and the automatic “bake-out” heating cycle is started.

This avoids ice condensation inside the instrument and prepares it for its next operation.

The **OFF** control button allows to switch-off the acoustic warning when LN2 is low.

Pressing the LN2 button starts the LN2 pump and switches the CPC from its stand-by to its operating mode and back.

PROCESS

Operation of the CPC is simplified by the automatic detection by the CPC of the module attached.

The technique IMMERSION
METAL MIRROR
PREPARATION

used is indicated by the illuminated LED.

The logic circuit built into the CPC simplifies and automates operation by closing down non-required areas of the control panel.

Three buttons allow to set a ramp for freeze substitution or the PLT-Technique or simply a temperature higher than the steady-state GN2 temperature for sample preparation.

T1, **S1** and **T2** are used to set processing temperature and duration. **START** activates the programme and **PAUSE** interrupts or cancels the programme depending on how long the button is pressed.

Leica EM CPC

The Cryoworkstation

designed for

Performance,

Convenience and

Versatility

SETTING

The display **TEMP** normally indicates the present actual temperature in °C, °F or K. When using propane or other secondary cryogen the container has to maintain a temperature warmer than that of LN2 to hold the secondary cryogen just above its freezing point.

This temperature is set with the bar below the **TEMP** display and is automatically maintained by the system.

The display **GAS/TIME** shows the actual gas temperature at working height in the chamber or the time in **PREPARATION** mode. The bar below the display allows setting of the desired time.

ALARM

This is a safety feature for use with secondary cryogen. An alarm gives an acoustic and flashing signal when the temperature rises above -70°C or when the “bake-out” cycle is activated.

Immersion Cryofixation



The injector system for the plunge freezing of specimens on FC-series specimen pins has been designed to have minimum mass and hence warming up times after use is rapid.

The system comes with two injector heads to allow a fast turnaround operation between one specimen and the next and a deep propane container, raisable to a click-stop position, to allow a long path length of the sample through cryogen. For easy access to the propane container, the whole injector system can be moved sideways.

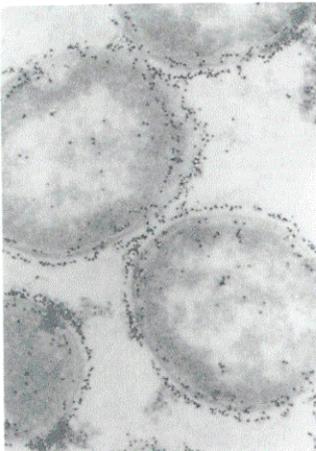
The viewing system is designed around the Leica GZ6 stereozoom microscope which has a long working distance of 115 mm and a magnification range of 10x to 60x.

Its specially designed shell reduces static effects which makes it eminently suitable for cryowork.

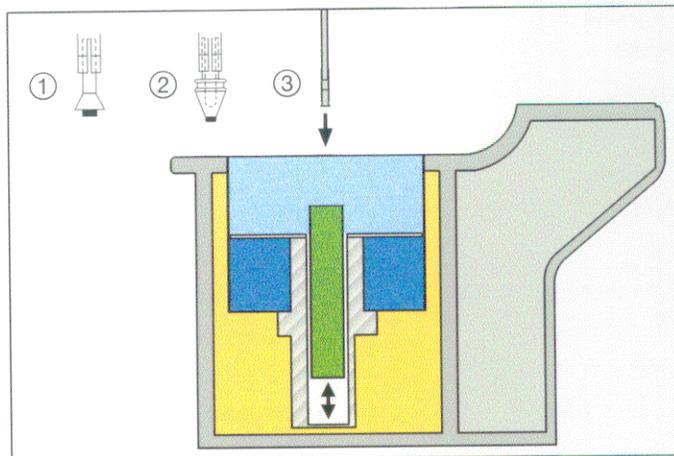
The microscope can be positioned either over the chamber for sample manipulation or, if an injector head is in use, on the rotatable carrier immediately in front of the user.

This latter position is especially useful for the Bare Grid and Metal Mirror Techniques.

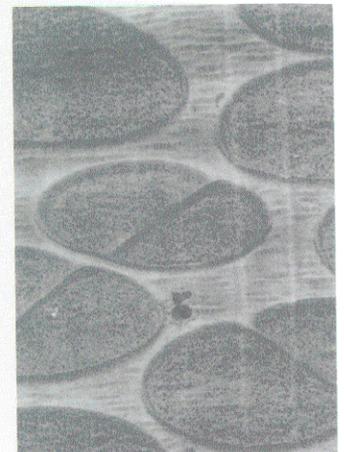
Aeromonas salmonicida;
immunogold labelled cryosection



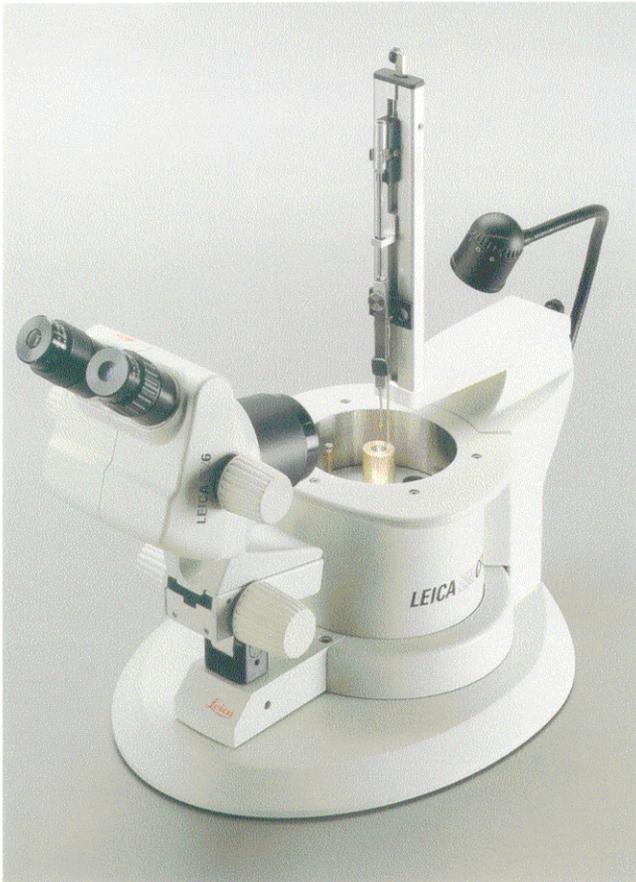
- | | | |
|---------------------|-----------|-----------------------|
| ① Tissue holder | ● GN2 | ● Aluminium container |
| ② Suspension holder | ● LN2 | ● Insulation |
| ③ Injector | ● Cryogen | ● CPC |



Staphylococcus aureus;
immersion cryofixation-cryo-TEM



Ice Embedding / Bare Grid Technique



The BG injector system comes complete with two pairs of fine forceps and a smaller cryogen container suitable for plunge freezing grids into propane or ethane.

The container is of minimal volume to facilitate the use of cryogens such as ethane.

The vessel is connected to the injector system by a rod and can simply be raised in order to bring it to the correct height for plunging grids. Thus detrimental pre-cooling of the sample by travelling through cold GN2 is avoided.

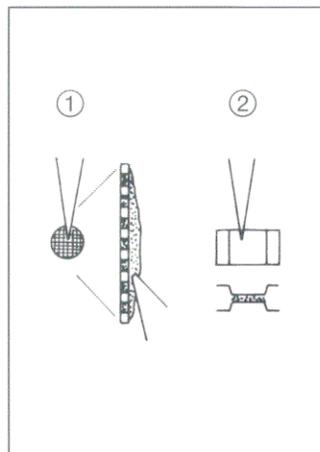
The propane container is removed from the CPC with a special tool and placed into an optional separate disposal unit for safe burning.

For easy access to the cryogen container the whole injector system can be moved sideways. This can also facilitate removal of the grid.

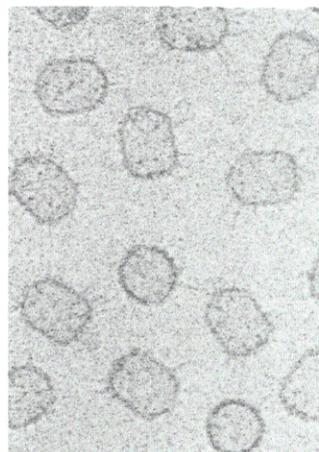
Cardiac muscle; immersion cryofixation, freeze fracture
J. A. Post + A. J. Verkleij,
Univ. Utrecht, NL



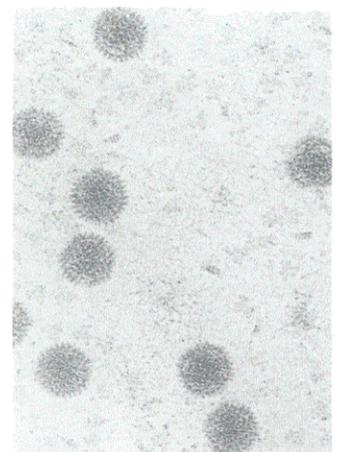
- ① Bare grid method
- ② Sandwich preparation



Preheads of bacteriophage \varnothing 29; immersion cryofixation
M. Adrian et al.,
EMBL, Heidelberg, D



Adeno viruses; vitrified by immersion cryofixation
M. Adrian et al.,
EMBL, Heidelberg, D



Metal Mirror – Impact Cryofixation



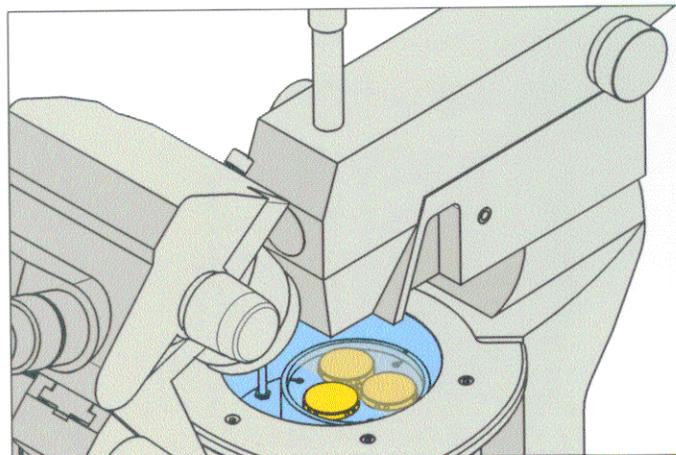
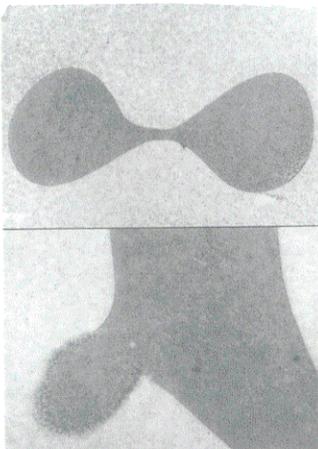
The Leica EM CPC was designed to take the proven MM80 Metal Mirror Freezing System. Improvements in the impact freezing system are to be found in the working chamber where the support for the Metal Mirror is not simply a flat plate but a rotating turret which houses and cools the three metal mirrors simultaneously.

A cover prevents them from frosting.

The copper blocks used for cooling are **gold-plated**. Without polishing they can be cleaned with alcohol. Thus turnaround times are shortened and hence efficiency is improved.

Red blood cell; damaged by high slamming speed (bottom)
E. Morgenstern,
Univ. d. Saarlandes, D

-  GN2
-  Gold coated metal mirrors
-  CPC/MM80



Gerbil parotid gland acinal cells
A. & M. Ichikawa,
Yokohama University



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