

Rat, kidney, Epon. D. Studer et al., Bern, Switzerland

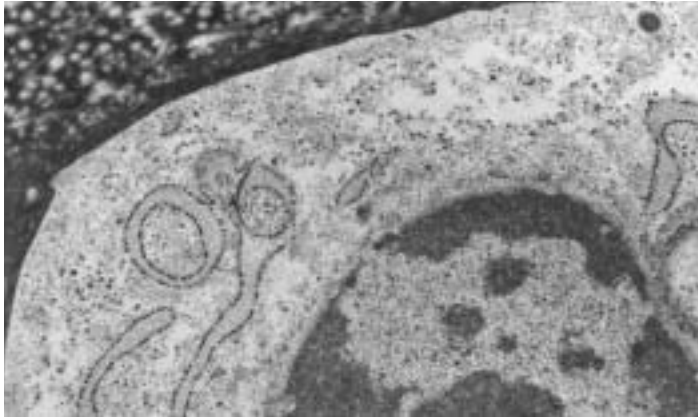
Leica EM PACT

High Pressure Freezer

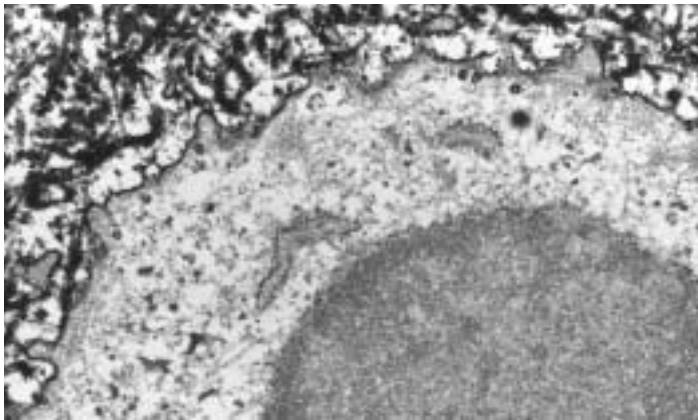
according to Studer



Why High Pressure Freezing?



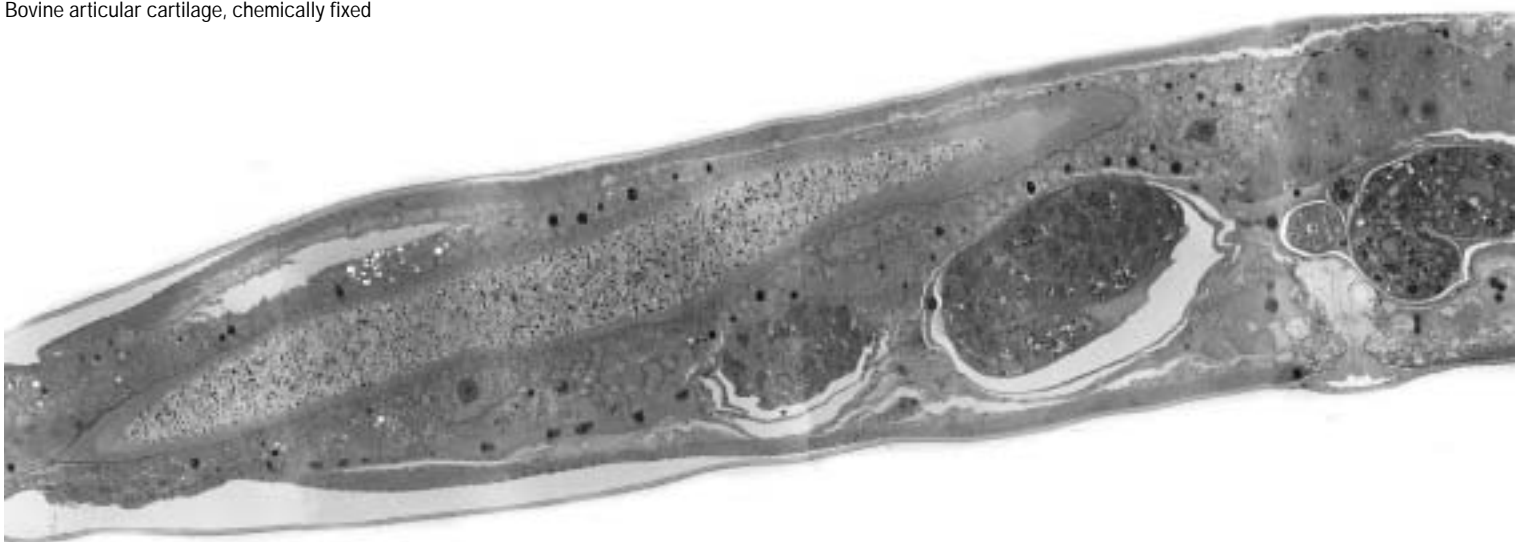
Bovine articular cartilage high pressure frozen



Bovine articular cartilage, chemically fixed

High Pressure Freezing (HPF) is THE way to localise or characterise organelles, subcellular components and gene products in EM without the disadvantages of classical fixation methods:

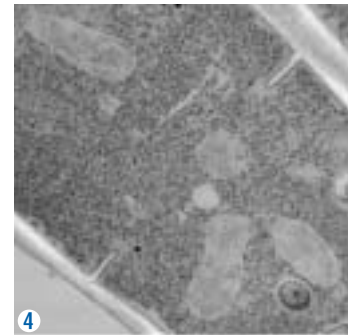
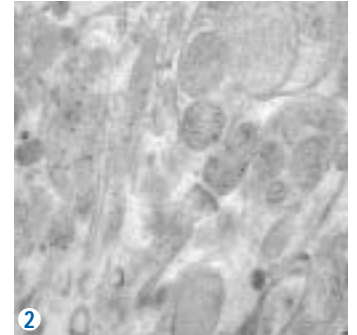
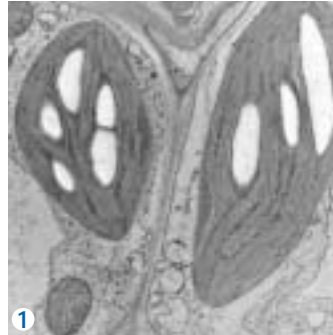
- HPF gives better structural preservation and retains antigenicity far better than conventional chemical fixation.
- HPF gives around 200 μm depth of good freezing – with all room pressure cryofixation techniques, (plunge, slam and jet freezing) samples can only be vitrified to depths of 5–20 μm . This is a ten-fold increase in the depth of amorphous ice!



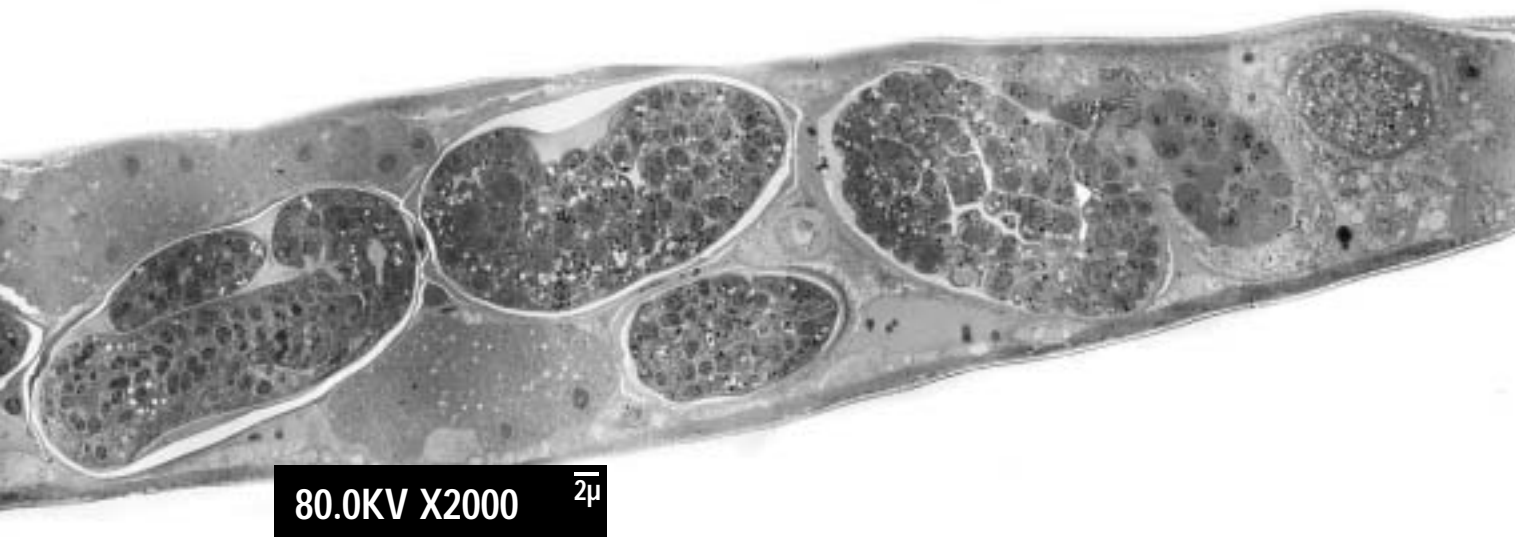
260 μm

It's fast, it's deep, it's perfect!

The Leica EM PACT high pressure freezer serves the needs of molecular and cell biologists and all researchers who want an "in vivo" impression of their cellular structures and functions in question – without the usual artefacts of chemical fixation. The Leica EM PACT gives you the possibility to get the high resolution information of EM immunocytochemistry, frozen hydrated sections and freeze fracturing in perfectly preserved tissue.



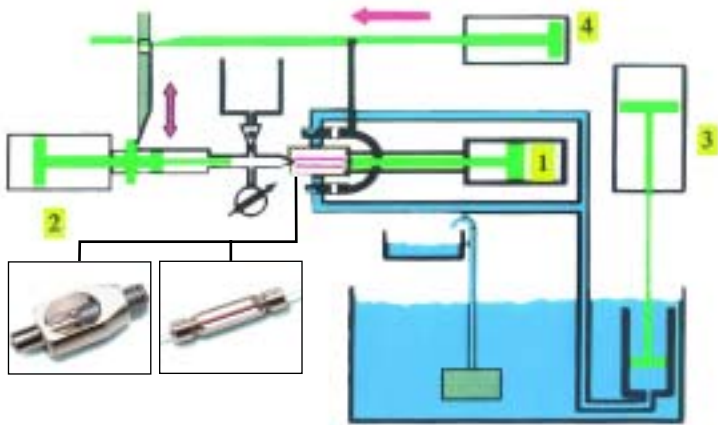
1. *Arabidopsis thaliana*, Epon. D. Studer et al., Bern, Switzerland
2. Rat, brain, HM20. Labelling against Synapsin. K. Richter et al., Magdeburg, Germany
3. *S. cerevisiae*, FF replica, P. Messner et al., Vienna, Austria
4. *S. pombe*, Epon, K. McDonald, Berkeley, USA



C. elegans
Prof. Dr. A. Coomans, Prof. G. Borgonie, M. Claeys
(Ghent, Belgium)

Leica EM PACT – Instrument Design and Function

- **Easy to install**
 - compact
 - mobile
 - standard electrical supply
 - quick release connection to compressed air
- **Easy to use**
 - touch-sensitive screen
 - menu prompts
 - automatic specimen ejection into LN₂ bath
 - temperature/pressure curve displayed for each run
- **Safe and convenient**
 - Low LN₂ consumption
 - No ethanol used
 - Low noise



Function: All You Need is LN₂...

The specimen is locked (1) and set under pressure (2) just before freezing (3) via the synchronisation mechanism (4). Only the tissue in the specimen holder is under pressure and not the complete specimen chamber. The intensity of the pressure is adjustable from 1 to 2100 bar. As methylcyclohexane (MCH) is used as hydraulic fluid, ethanol vapour is avoided.



Operation: Ready, Steady, Go!

A menu on the touch screen guides the user through the operation. Just follow the prompt in the lower black line. Keys are only illuminated when they can be pressed (the LOCK button must be pressed to illuminate the START key).

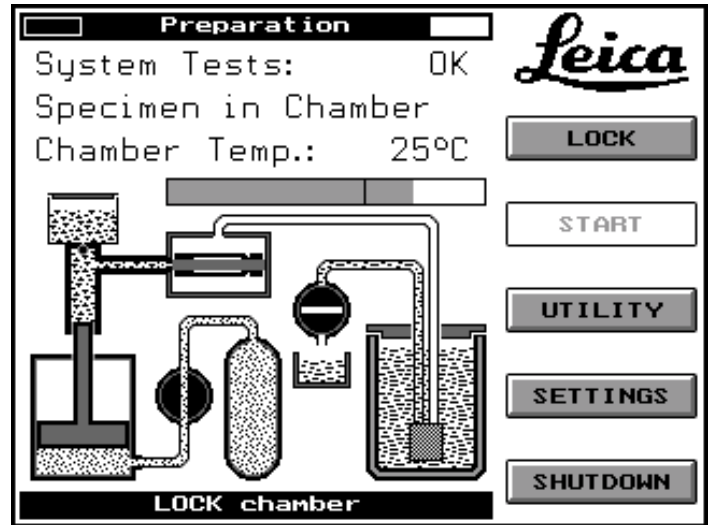
Once your specimen is inserted, you only need to press 'Lock' and 'Start'. The specimen is frozen before being ejected automatically into a bath of liquid nitrogen. Within less than 60 seconds the EM PACT will accept the next specimen (and it will tell you!).

Freezing... Is Simplicity Itself

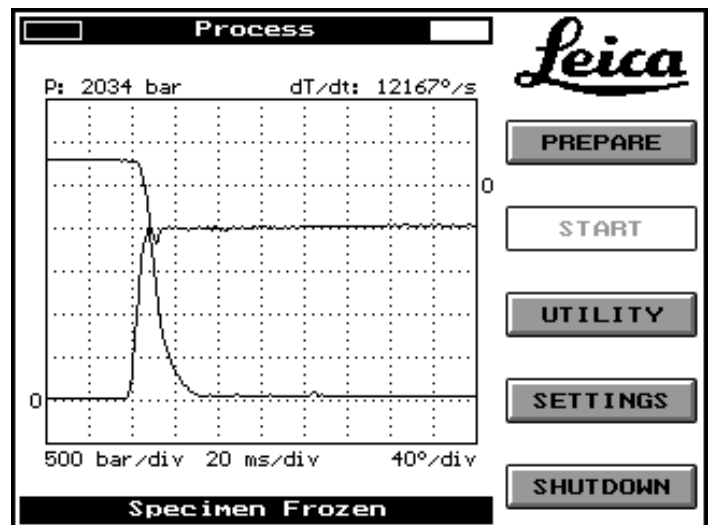
After the ejection of the cryofixed specimen into a bath of LN₂ the result of the shot is shown as a temperature/pressure curve. While the specimen chamber automatically heats up for the next run this curve can be stored, printed and transferred to your PC. The parameters in this curve show the exact physical values your specimen was exposed to. Once you have prepared the number of samples you require, simply transfer them to the "follow on procedures" of your choice via the unloading device and the transfer box. Preparation of your specimens is thus maintained from the beginning to the end with the Leica Total System Solution. Finally „SHUTDOWN" leads the EM PACT to a bakeout-cycle that heats and dries automatically all parts in contact with LN₂.

There are numerous techniques to follow excellent high pressure cryo fixation from minimal processing to:

- Freeze Substitution
- Freeze Drying
- Frozen Hydrated Sectioning
- Freeze Fracturing for TEM and SEM



A schematic drawing shows the status of all parameters



A diagram shows the freezing data after ejection of the sample

Leica EM PACT

Application Solutions

Leica EM PACT Flat Specimen Holders. Preparation of bulk specimens made simple: brain, tissue slices, cell cultures, leaves, paint, polymers ...

Prepare your sample (eg. leaf) by punching out the area of interest ...



... to fit into the specimen carrier (ø 1.2 mm, depth 200 µm).



With the help of loading station ...



... the carrier is placed into the pod and tightened securely with the supplied torque wrench.



With the loading device the specimen can be inserted into the instrument.



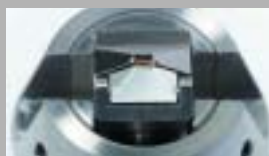
After cryofixation the carriers are collected in the LN₂ bath of the EM PACT before Freeze Substitution in the Leica EM AFS.



Alternatively, the carriers can be trimmed with a cutter...



... for Frozen Hydrated Sectioning in the cryoultramicrotome.





Leica Design by W. Hölbl

The Tube Holder for every fluid you can think of ... blood, milk, cell suspensions ... nematodes – and more!

Directly suck the suspension into copper tubes (inner \varnothing 350 μm) already mounted in their holder.

Alternatively, take up sample into cellulose microcapillary.

With the loading device the specimen can be inserted into the EM PACT.

After cryofixation the samples are stored under LN_2 in the transfer box.

Sample Punch: punching out of the central part of the specimen tube ready for Frozen Hydrated Sectioning or ...

... peeling away of the top of the copper tube for Freeze Substitution.

Storage of the copper tubes is carried out under LN_2 .

The specimen tube holders are recycled by reloading them with copper tubes.



Leica EM PACT

Application Solutions

Taking microbiopsies for EM has never been so fast!

Prepare the Microbiopsy Transfer Station under the Optical Workstation ...

... before taking a biopsy with the biopsy gun.

Insert the biopsy gun into the transfer station ...

... and transfer the tissue into the biopsy carrier.

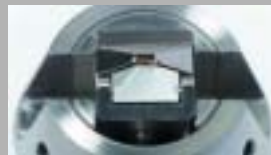
The carrier is placed into the pod and tightened securely with the supplied torque wrench.

With the loading device the specimen can be inserted into the instrument.

After cryofixation the carriers are collected in the LN₂ bath of the EM PACT before Freeze Substitution in the Leica EM AFS.

Alternatively, the carriers can be trimmed with a cutter ...

... for Frozen Hydrated Sectioning in the cryoultramicrotome.





The Freeze Fracture Holder for everything that can be fractured ...

Prepare the Freeze Fracture Station under the Optical Workstation ...

... and preload the freeze fracture carrier. A copper ring is put on top of the loaded carrier.

Carrier and copper ring are sandwiched securely in the pod with the supplied torque wrench.

With the loading device the specimen can be inserted into the instrument.

After cryofixation the carriers are collected in the LN₂ bath of the EM PACT before Freeze Fracturing.

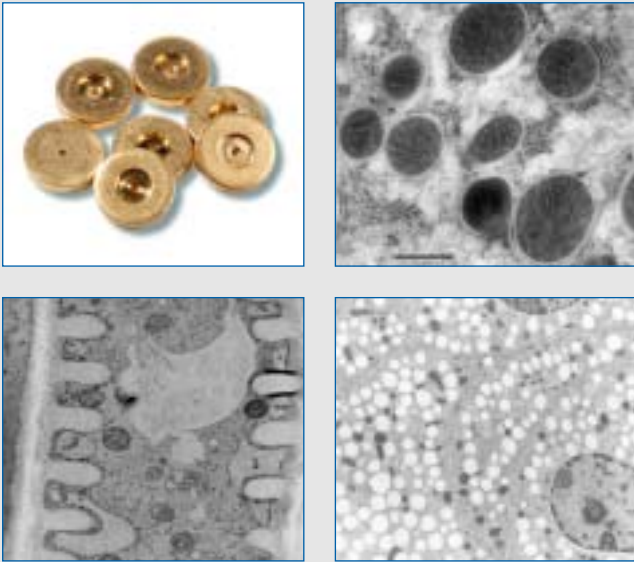


Application Solutions

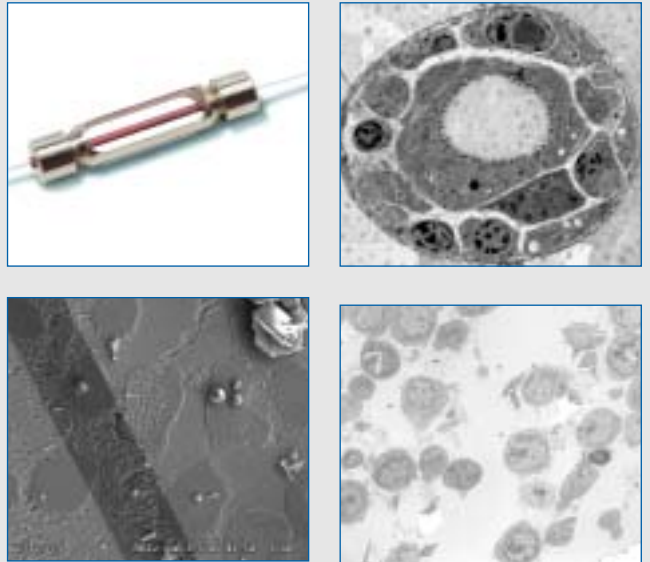
- 1 Flat Specimen System
- 2 Specimen Tube System
- 3 Microbiopsy Transfer System
- 4 Freeze Fracture System

The four Preparation Systems allow a diversity of samples to be high pressure frozen. Tissues, cell mono layers and suspensions can be frozen in the Flat Specimen System. In the Specimen Tube System suspensions and longitudinal objects, e.g. nematodes or cellulose microcapillaries can be vitrified. The Microbiopsy Transfer System allows rapid transfer of tissue biopsies from donor to the Leica EM PACT. A Freeze Fracture System is available for suspensions or tissues to be prepared in the cryoSEM or as replicas.

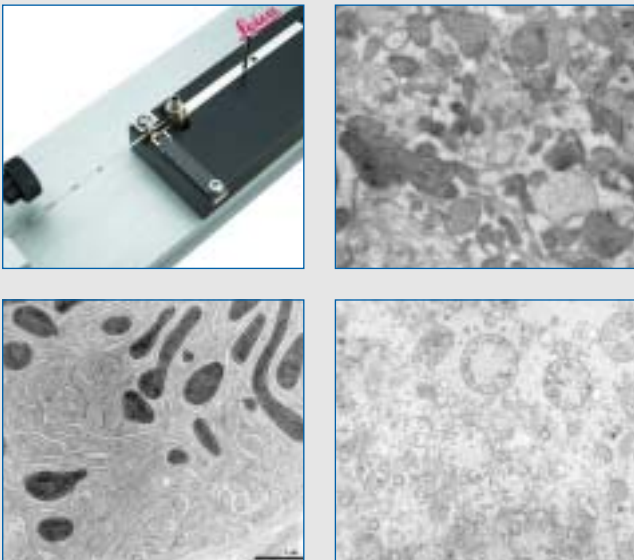
1



2



3



4



Leica Microsystems – the brand for outstanding products

Leica Microsystems' mission is to be the world's first-choice provider of innovative solutions to our customers' needs for vision, measurement, lithography and analysis of microstructures.

Leica, the leading brand for microscopes and scientific instruments, developed from five brand names, all with a long tradition: Wild, Leitz, Reichert, Jung and Cambridge Instruments. Yet Leica symbolizes innovation as well as tradition.

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● Specimen Preparation


We provide comprehensive systems and services for clinical histo- and cytopathology applications, biomedical research and industrial quality assurance. Our product range includes instruments, systems and consumables for tissue infiltration and embedding, microtomes and cryostats as well as automated stainers and coverslippers.

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