HPM 010 High Pressure Freezing Machine

Features

- Highest quality freezing of tissue samples in thickness ranges of up to 0.2 mm without requiring freeze protective additives.
- Array of specimen carriers available, i.e. for suspensions (capillary tubes), tissue extracted by fine needle biopsy and carriers for monolayer cell cultures.
- In-situ real time measurement of temperature and pressure.
- Fast 90 second process cycle allows expeditious application.
- Automatic, microprocessor controlled operation for routine work.
- Compact, sturdy unit with soundproof and vibration-free housing.
- Simple and safe operation due to quick-locking action of specimen holder and clearly arranged operational controls.
- Processing datas are recorded on digital display, such as the actual temperature, time and pressure, thus allowing the user to exactly evaluate current operational status (sample quality control).
- Officially approved materials and high pressure components, as well as twice controlled sample holders with quick-lock action ensure highest level of safety for user.
- Simple maintenance with removable cover plates and rack system for control units.
- Extensive accessory program.
- Large number of publications documenting high performance of HPM 010.

Applications

- Freezing of large tissue specimens without requiring structure-altering freeze protective additives.
- Ideally suited for subsequent replication by freeze fracturing in a freeze etching system for TEM applications or subsequent cryo sectioning for cryo SEM and TEM investigations.
- Best suited in conjunction with subsequent freeze substitution, followed by low temperature embedding and polymerisation for sectioning in a conventional ultramicrotom.
- Suitable for samples destined for subsequent thin sectioning in their frozen state by cryo-ultramicrotom for cryo - TEM analyses.
- For samples intended for subsequent freeze drying and SEM or TEM investigations.

The High Pressure Freezing Method

Excellent freezing of the specimens intended for examination in the electron microscope is one of the most important prerequisites for achieving reproducible results from the various subsequent cryopreparation methods.

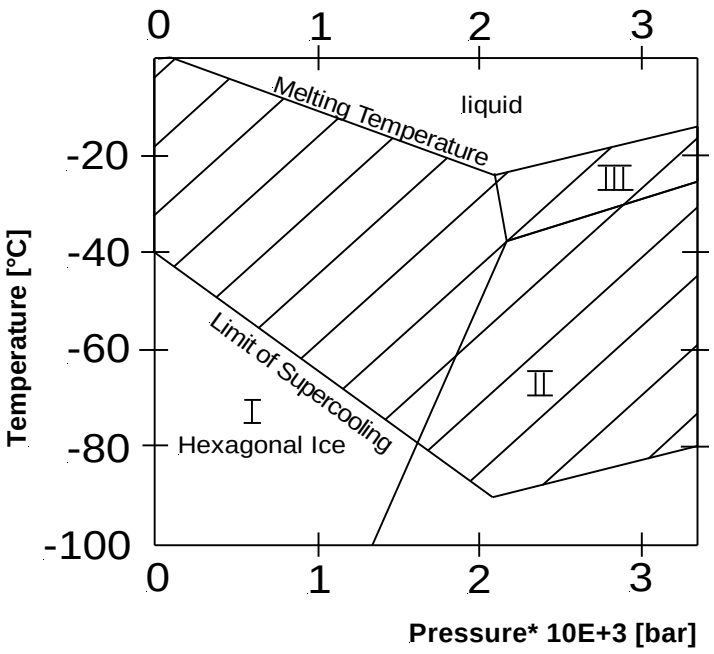
The freezing method should produce microcrystalline or amorphous ice from the specimen water. To achieve this, the specimens must be frozen as quickly as possible at a freezing rate no lower than 10'000°C/s.

The conventional freezing methods in use are plunge freezing, jet spray and cold block (slamming) cryo fixation. However, due to the poor heat conductance of water, these methods can only satisfactorily freeze specimens measuring up to between 10 and 20µm.

Thicker specimens (such as tissue samples) could only be frozen in the past, if a cryoprotectant was added to lower the freezing point of the water in the specimen. The disadvantage of chemical cryoprotectants is that they often affect certain cell structures, causing different types of undesirable artefacts.

By using the high pressure freezing method developed by Prof. Moor (ETH Zurich) in conjunction with BAL-TEC AG these artefacts can be eliminated. The high pressure method is based on an entirely physical phenomenon that lowers the freezing point of water. This method functions as follows:

At 2'100 bar the melting point of water drops from 0°C to -22°C. Under normal atmospheric conditions homogeneous nucleation (supercooling) begins at -40°C. Under high pressure this nucleation doesn't begin until the water has reached -90°C (see H_20 Phase Diagram). At 2'100 bar water is 1'500 times more viscous than at atmospheric pressure, which drastically reduces the nucleation and thus the crystal growth rate. This means that the extremely high freezing rate (min. 10'000°C/s required for satisfactory freezing by the methods previously mentioned is not necessary with the high pressure method. The high pressure method allows specimens up to 0.2mm thickness with a total volume of approx. $1mm^3$ to be vitrified or up to 0.5mm thickness to be adequate frozen at a low freezing rate of 200°C/s without requiring the addition of cryoprotectants.



H_20 Phase Diagram

1 Supercooling capability curve
2 Melting point curve

Principle of Operation

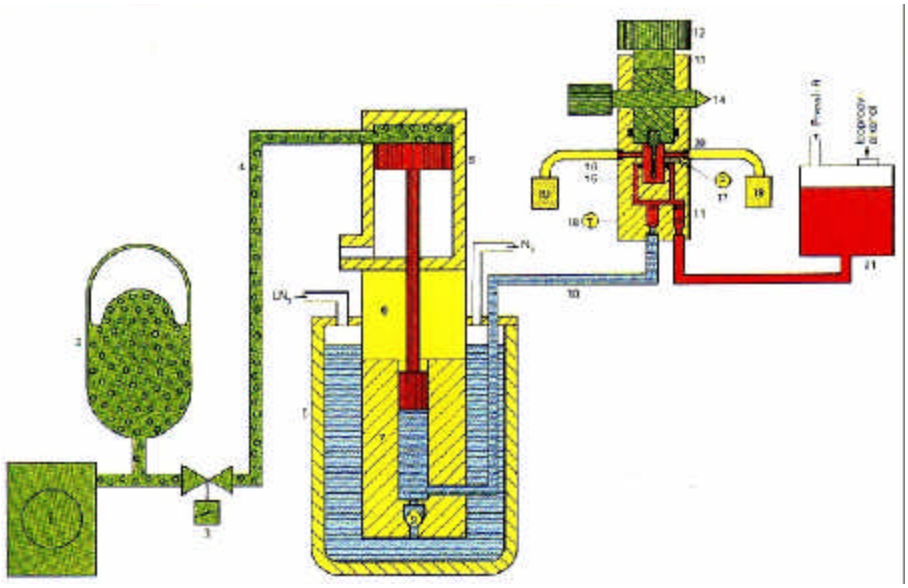
The specimens are sandwiched between two specimen carriers which are mounted in the recess of a hinged specimen holder. The holder is closed and placed in the high pressure chamber so that the specimen carriers in the holder are located exactly between the two LN_2 entry apertures. A quick fastening bolt locks the holder in place, and this mechanical fastening releases an electrical interlock - the system is now ready for operation.

When the start key is pushed, the entire freezing process is run through automatically:

A pressure valve opens, allowing hydraulic oil under a pressure of 300 bar to flow from a bladder-type accumulator into the low pressure cylinder. The pressure piston transfers this pressure to the liquid nitrogen in the high pressure chamber. The special design of this piston increases the pressure at this transfer to a value considerably higher than the oil pressure. The liquid nitrogen is introduced into the specimen pressure chamber at this high pressure. The isopropyl alcohol in the chamber is first compressed and then forced out, causing a delay, after which the liquid nitrogen (at a pressure of 2'100 bar) reaches the "specimen sandwich".

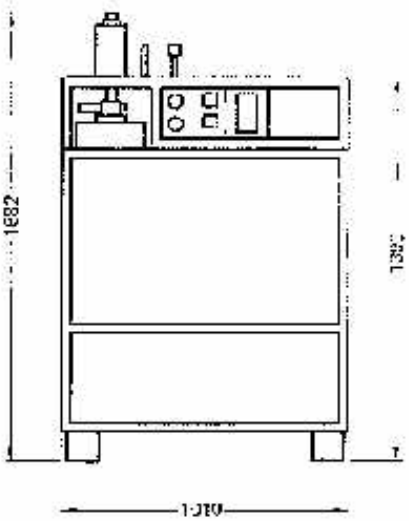
The liquid nitrogen exits the specimen pressure chamber through the outlet aperture after which it expands and escapes into the ambient air after first passing through a silencer.

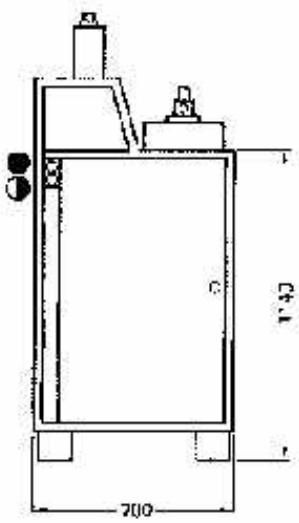
Design



1 Oil pressure pump
2 Bladder-type pressure accumulator
3 Pressure valve (electromagnetic)
4 Low pressure line
5 Low pressure cylinder
6 Pressure piston
7 High pressure cylinder
8 LN2 Dewar
9 Non-return valves
10 High pressure line
11 Non-return valves
12 Specimen holder
13 Specimen pressure chamber
14 Quick fastening bolt
15 Specimen
16 LN_2 entry apertures
17 Pressure sensor
18 Temperature sensor
19 N2 exhaust with silencer
20 Outlet apertures
21 Isopropyl alcohol reservoir

Scale drawing





Specifications

1. Housing

Consisting of:

- 1 Tubular steel frame with removable sound absorbing plate panels
- 1 Locking side door with storage shelf for sample holder
- 1 Alcohol reservoir with filling port
- 1 Collector receptacle for excess alcohol
- 1 Power distribution box
- 3 Connection ports for compressed air, LN_2 and heating water
- 4 Cushioned vibration-free machine legs.

2. Pressure Generation System

Consisting of:

- 1 Low pressure system with:
  - Radial plunger pump
  - Oil reservoir
  - Valve unit with non-return valve
  - Pressure accumulator with safety valve
  - Low pressure cylinder
  - Hydraulic circuit
- 1 High pressure system:
  - LN_2 Dewar with automatic filling control
  - High pressure cylinder (immersed in LN_2 Dewar)
  - Non-return valve
  - LN_2 cooled high pressure circuit

3. Specimen Pressure Chamber

Consisting of:

- 1 High pressure chamber with non-return valves
- 1 Quick-action locking bolt for sample holder
- 1 Pressure gauging probe
- 1 Temperature sensor
- 2 LN_2 inlet jets
- 2 Interchangeable LN_2 outlet jets
- 2 Exhaust lines with silencers
- 2 High pressure line ports for LN_2 and alcohol
- 1 Combined measuring and specimen holder for 0.5mm specimen carriers
- 1 Specimen holder for 0.8mm specimen carriers

4. Temperature / Pressure Control System

Consisting of:

- 1 HPC 010 Measuring and recording unit
- 1 Pressure gauging probe
- 1 Set of connecting cables

Technical Data

Dimensions and Weight	
Dimensions	See "Scale Diagram
Weight	approx. 450 kg
Working Data	
Working pressure	2300 - 2600 bar
Maximum pressure	2800 bar
Duration of working pressure, at least	500 ms
Cooling time from 0°C to -50°C (measured between 3 mm copper disc)	10 ms
Specimen Dimensions	
Sample thickness, up to max. of	0.6 mm
Sample volume, approx.	1 mm^3
Connection Data	
Voltages, frequencies	3 x 380/220 V, 50 Hz 3 x 208 V, 60 Hz
Power input, approx.	3kVA
Compressed air (5 bar)	G 1/4" outer thread
LN_2 (1 bar excess pressure)	G 1/4" outer thread
Heating water (rubber hose)	diam. 7/14 mm
Operational Data	
Hydraulic oil reservoir	40 liters
Hydraulic oil bias pressure	140-250 bar
LN_2 Dewar (in system)	7 liters
LN_2 consumption	10 - 20 liters/hour
Initial system cooling, approx.	15 min.
Max. processing sequence, approx.	40 shots/hour
Isopropyl alcohol reservoir, approx.	0.5 liters

5. Operation and Display Panel

Consisting of:

- 1 Adjustment and display panel for:
  - Analogue display of pressure in alcohol reservoir
  - Digital display of alcohol fill status
  - Digital display of specimen pressure chamber temperature
  - Analog display of low pressure system oil pressure
  - Control unit with digital adjustment and display
- 1 Operation panel with:
  - LED display for various process-ready parameters
  - JET-key to activate freezing process
  - Push-buttons for LN supply, plunger forward and reverse.
  - Preset key for manual or automatic operation
  - System START and STOP keys
  - Operation keys for manual operation

6. Hot Water Circuit

Consisting of:

- 1 Circulating bath thermostat with NOMINAL value control
- 1 Temperature control with sensor on specimen pressure chamber
- 1 Thermometer
- 1 Circulating pump
- 1 Set of connecting lines

The circulating bath thermostat is set up outside of the HPM 010.

7. Safety system

Consisting of:

- 1 Proximity switch for specimen holder
- 1 Proximity switch for quick-action lock bolt

8. Set of spare parts and tools

Consisting of:

- 1 Set of spares (BU 012 120-T) with:
  - 2 Plunger gaskets
  - 2 Plunger springs
  - 6 Guide belts
  - 2 Injector slider
  - 2 Injector springs
  - 20 O-rings 6.75 x 1.78
  - 2 O-rings 12.42 x 1.78
  - 2 O-rings 60.05 x 1,78
  - 2 Support rings
  - 5 Spheres with diam. of 2 mm
  - 5 Spheres with diam. of 2.5 mm
  - 1 Can of vacuum grease
- 1 Set of Tools (BU 012 118 -T) with:
  - 1 Adjustable nut wrench 43 x 375
  - 1 Adjustable nut wrench 52 x 450
  - 1 Hexagon socket screw wrench 12
  - 1 Hook spanner B
  - 1 Screw driver
  - 1 Expanding mandrel
  - 1 Reservoir filler
  - 1 Torque wrench 30 - 300 NM
  - 1 Torque wrench 10 - 100 NM
  - 1 Hexagon socket screw insert 17
  - 1 Hexagon socket screw insert 14
  - 1 Hexagon socket screw insert 6
  - 1 Hexagon socket screw insert 5
  - 1 Hexagon socket screw insert, cap head 6
  - 1 Extension

Ordering Information

HPM 010 Basic Unit according to specifications 1 through 8.

Order No.

3 x 380/220 V, 50 Hz	BU P02 260
3 x 208W60 Hz	BU P02 261

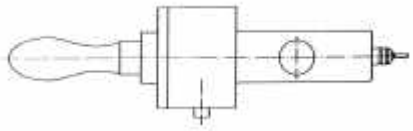
Accessories

Diaphragm for media outlet

Serves to control the outlet flow speed of the freezing medium.
The standard diaphragm is a 0.4 mm. (included in the basic unit)
The smaller diaphragm is 0.35 mm and causes a longer duration of working pressure.

2 per pack

Perforation	Order No.
0,4 mm (already build in).	B 8010 120 72
0,35 mm	B 8010 120 73



Combined measuring and specimen holder

For real time recording in high pressure freezing

Consisting of:

- Temperature sensor
- Specimen carrier clamp
- Connection plug
- High pressure seal
- Handle

The specimen can be fixed in an optimal way. Handling is simple and safe.

Included in the basic unit.

Order No.	BU 012 309 -T

Memory Recorder

For registration of process parameters in high pressure freezing

- Consisting of:
- Memory recorder
- Printer
- 10 Coils recording paper
- Accumulator
- Battery charger for installation in HPM 010 unit
- Connection cable
- Measuring cable kit
- Short manual

Order No.	BU 012 311 -T

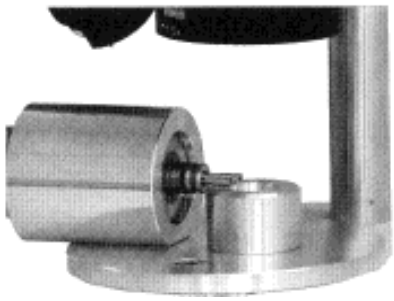
LN_2 Dewar

Aluminum dewar specially designed in conjunction with the manufacturer in order to fulfill BAL-TEC cryotechnique requirements (No compressed air required).

Consisting of:

- 1 LN2 Dewar
- 1 Roller frame base
- 1 Optical filling level indicator
- 1 Internal pressure system
- 1 Pressure control valve
- 1 Pressure gauge
- 1 Filling and extraction valve
- 1 Mounting ring and gasket
- 1 Hand valve connection port

	Order No.
60 liters	B 8010 120 01
100 liters	B 8010 120 02



Loading Device

Used for damage-free holding of the sample holder during the manipulation at the specimen carrier-mounting fixture. It helps position the specimen carriers and the precisely set up of the specimen i.e. with the help of a binocular.

Order No.	BU 012 121 –T

Unloading Device

Is used to open the specimen carrier-mounting fixture under liquid nitrogen. The specimen carrier sandwich with the frozen specimen can now easily be detached from the mounting fixture with a pair of tweezers.

Consisting of:

- 1 LN_2 Dewar with insulation
- 1 Unloading unit

The specimens may now proceed to a CRYO - TRAC system sample container for intermediate storage before further treatment. (See CRYO - TRAC system).

Order No.	BU 012 122 -T

Cryo-Box Set

7 Styrofoam boxes in various sizes with covers to keep your specimens in LN_2.

Order No.	B 8010 051 17

Tweezers

To open the specimen carrier mounting fixture and to remove the specimen carrier sandwich.

Order No.	B 8010 030 26

Tissue Puncher

Is used to punch out tissue discs without causing damage.

Designed to last a lifetime provided they are carefully cleaned and maintained.

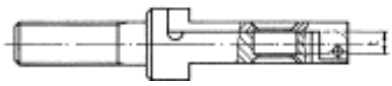
Ø2 mm Order No.	LH 01849 KN
Ø3 mm Order No.	LH 01850 KN

Disposable Tissue Puncher

It punches out tissue discs without causing damages.

10 per pack.

Ø2 mm Order No.	LH 01847 KN
Ø3 mm Order No.	LH 01848 KN



Specimen Carrier Mounting Fixture

To insert in the conventional sample holder.

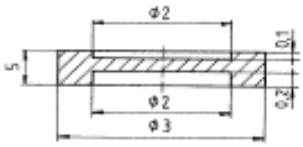
Is used to receive and position the specimen carriers.

Size		Order No.
Ø 3 x 0.8 mm (included in the basic unit)	1.6	BU 012 110 -T
Ø 3 x 0.5 mm	1.0	BU 012 117 -T

Consumables

Specimen Carriers, Aluminum

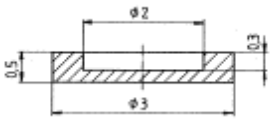
These specimen carriers can be arranged with various tissue sample combinations ranging from 0.1 up to 0.6 mm (e.g. intended for subsequent freeze substitution in the FSU 010). For fitting in the combined measuring- and specimen holder (BU 012 009 -T, included in the basic unit HPM 010) or in specimen carrier mounting fixture BU 012 117 -T.



Specimen Carriers, Type A

Made of aluminium, Ø3 x 0,5 mm.
10 per pack.

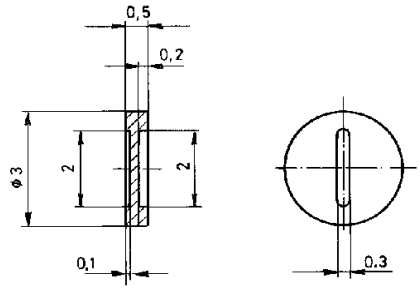
Order No.	BU 012 125 -T



Specimen Carriers, Type B

Made of aluminium, Ø3 x 0,5 mm.
10 per pack.

Order No.	BU 012 126 -T



Specimen Carriers, Type C

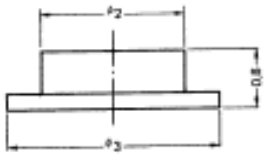
Made of aluminium, Ø x 0,5 mm.
10 per pack.

Order No.	BU 012 138 -T

Specimen Carriers, Gold

The specimen carriers for freeze fracture may be used in conjunction with the BAL-TEC object table BU 012 099 -T (for freeze etching / freeze fracturing.

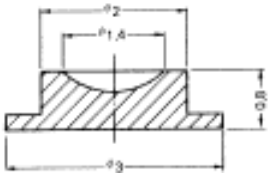
For fitting in the specimen carrier mounting fixture BU 012 11O -T.



Specimen Carriers

Made of gold with flat surface,
Ø3 x 0,8 mm.
10 per pack.

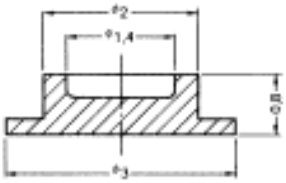
Order No.	BU 012 128 -T



Specimen Carriers

Made of gold with dome - shaped indentation, Ø3 x 0,8 mm.
10 per pack.

Order No.	BU 012 129 -T



Specimen Carriers

Made of gold with cylinder-shaped indentation, Ø3 x 0,8 mm.
10 per pack.

Order No.	BU 012 130 -T

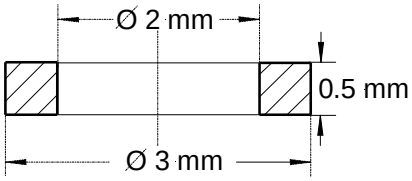
Gold Tubes

Cylindrical samples surrounded by thin walled gold tubes provide a better freezing of aqueous biological samples. The tube is held tightly between two copper rings in the specimen holder (see below).

Outside Diameter	0.3 mm
Wall Thickness	0.05 mm
Inside Diameter	0.2 mm

Made of gold (99,95), Ø x 40 mm.
1 per pack.

Order No.	LH 01846 VN



Clamp Ring

The gold tube is jammed between two clamp rings.
Made of steel, non magnetic,
Ø 3 mm x 0,5 mm.
10 per pack.

Order No.	LH 01842 VN

Cellulose Capillary Tubes

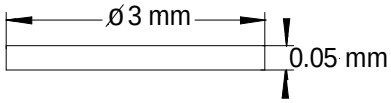
Preparing with cellulose capillary tubes is an easy to handle method for a real *in situ* fixation.
Biological suspension can be sucked into the transparent and porous micro capillary by capillary force. Micro culture of cells inside the capillaries as well as minimizing of intercellular water without increasing the ion concentration or chemical fixation is possible.
After freezing the capillaries are easy to handle for freeze-substitution.

Polymer	Cellulose
Fiber length	500 mm
Wall thickness (dry)	8 µm
Inner Diameter (dry)	200 µm
Permeability (cut off)	> 5.000 Dalton
pH range during operation	3 - 11

For High pressure freezing it may be used in conjunction with the specimen carrier, Type A (BU 012 125 –T) or Type C (BU 012 138 –T) in combination with the carrier Type B (BU 012 126 –T)

About 100 fibers per pack.

Order No.	LH 01843 VN



Synthetic Sapphire Platelets

Sapphire has an excellent thermal conductivity and is used for particular applications. For example for cell cultivations. Cut out the grown over platelet, put some Hexadecene on it and cover it with a specimen-carrier (e.g. BU 012 125 –T). To get the holder thickness, complete the sandwich with the copper Ring (B 01842 VN).
Platelets made of sapphire.
Ø 3 mm x 0.05 – 0.06 mm
Packed in Isopropanol
10 per pack.

Order No.	LH 01845 VN

Transparent Specimen Carriers

These specimen carriers from Sapphire combine excellent thermal conductivity and the transparent property of the polished material. They are used for flashlight experiments with subsequent freezing-technique.
Size of sandwich Ø3 mm x 1 mm
Size of cavity Ø1,5 mm x 0,2 mm
10 carriers and 10 lids per pack.

Order No.	LH 01916 VN

Dipalmitoyl-Lecitin

When plunged in this solution after having been frozen, specimen carriers are easy to separate from the specimen. 250 mg.

Order No.	B 8010 130 12

1-Hexadecene

To fill possible gaps during mounting of specimen carrier sandwiches. May also be used to fill extracellular gaps in the specimens. 500 m[

Order. No.	B 8010 130 40

Publications

[1] Kirschning, E., G. Rutter and H. Hoenberg
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Heinrich-Pette-Institut für Experimentelle Virologie und Immunologie
D - 20251 Hamburg
High-Pressure Freezing and Freeze-Substitution of Native Rat Brain: Suitability for Preservation and Immuno-Electron Microscopic Localization of myelin Glycolipids.
J. Neuroscience Res. 53, 465-474 (1998)

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High-pressure freezing for immunocytochemistry
J. Microsc. 192, 248-258 (1998)

[3] Hohenberg, H., M. Tobler and M. Müller
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High-pressure freezing of tissue obtained by fine-needle biopsy.
J. Microsc. 183, 133-139 (1996)

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High-Pressure Freezing of Cell Suspensions in Cellulose Capillary Tubes.
J. Microsc. 175, 34-43 (1994)

[5] Shimoni, E. and Müller, M.
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Acta Microsc. 2, 37-46 (1992)

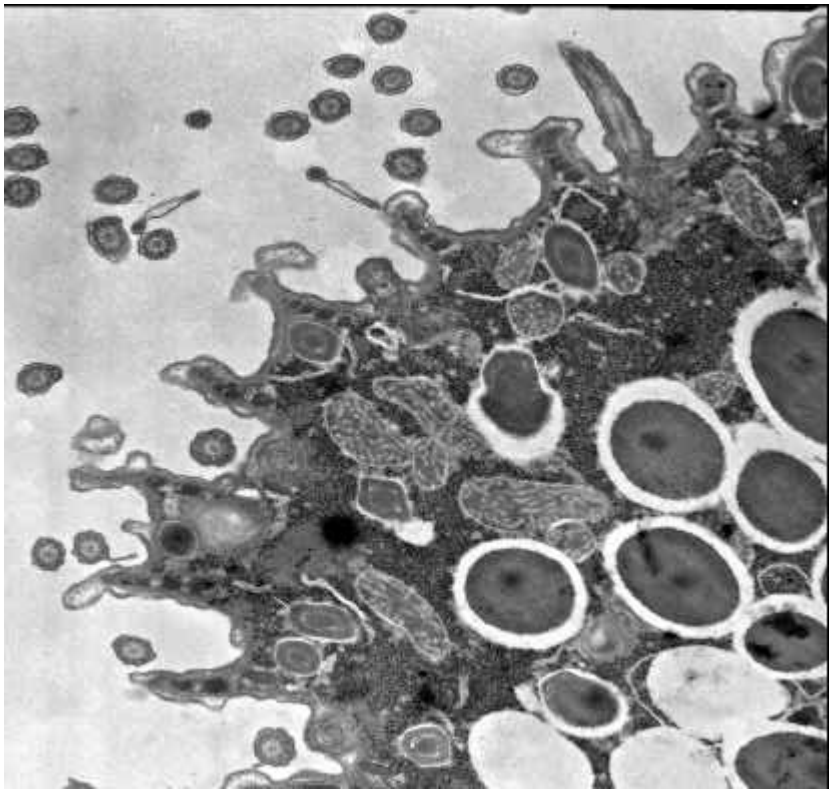
For further publications concerning High-Pressure-Freezing please see our brochure BU 800 287 D

Electron micrographs of specimens prepared in the HPM 010 high pressure freezing machine

Comparison of chemically fixed and High Pressure frozen specimen

Chemical fixed and at room temperature dehydrated Paramecium cells

Regulary distribution of Ribosomes in the Cytoplasm, granular Glykogen is scarcely visible. Trichocystenbodies (TK) and Trichocystentips (TS) exhibit shrinkage. Surface membranes (M) are crinkled.

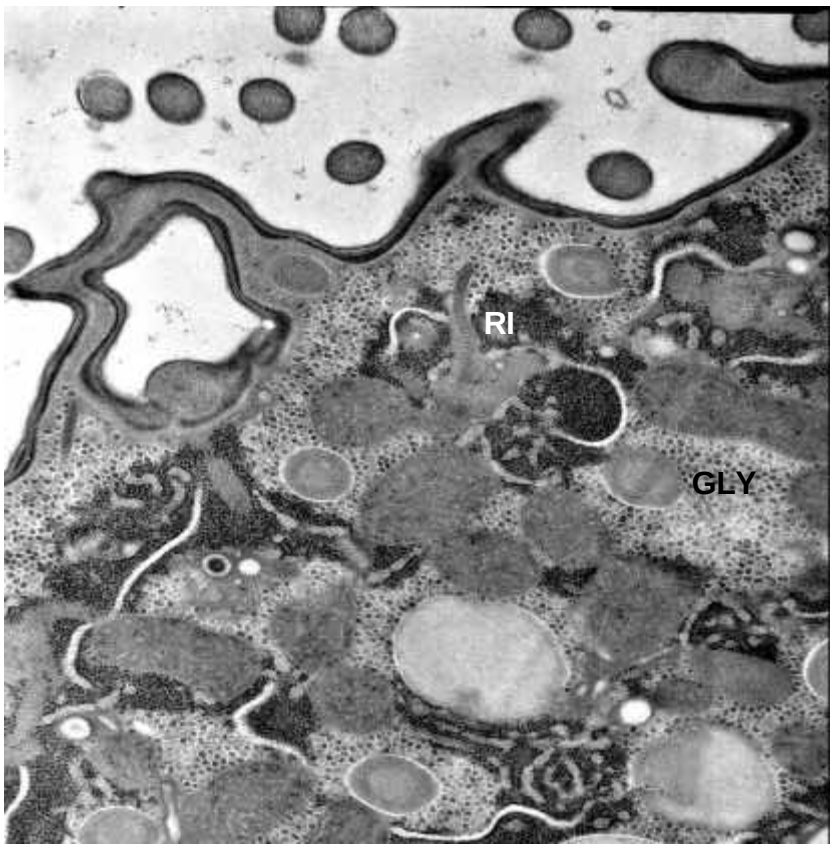


High Pressure frozen and freeze substituted Paramecium cells Preparation in Cellulose Capillary tubes

Entire Paramecium cell is adequately cryo-preserved.

Glykogranula (GLY) has high density and occurs in delimited areas, as well as the Ribosomes (RI).

Surface membranes are smooth and tight arranged.

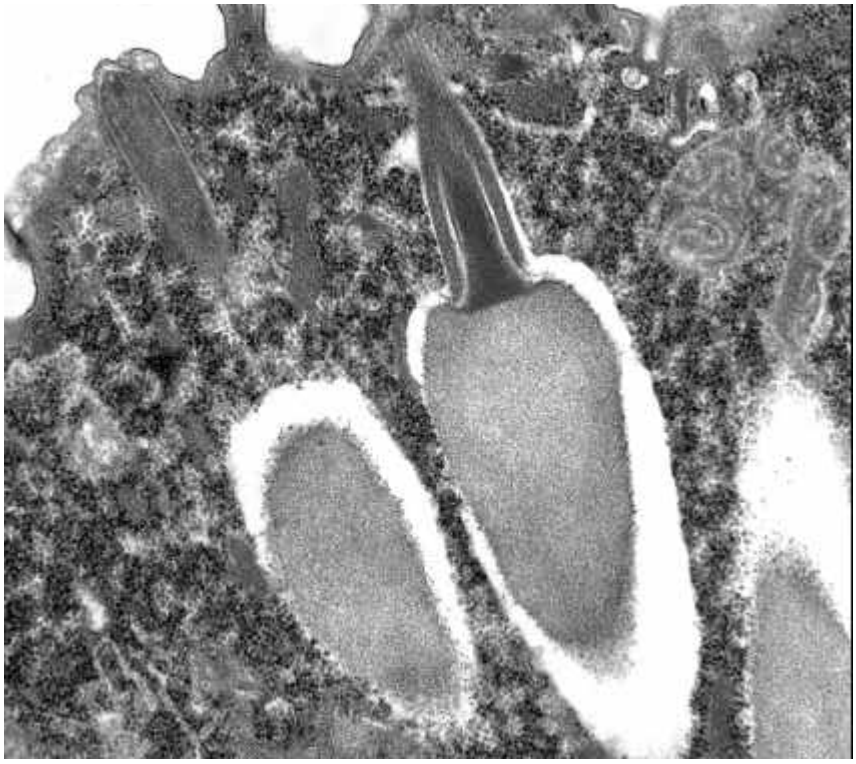


Photos:
Dr. Heinrich Hohenberg
ETHZ Zurich
Lab. For EM1
CH-8902 Zurich
Switzerland

Cross section of Paramecium cells after chemical fixation

Trichocyst bodies (TB) is detatched all round from the matrix

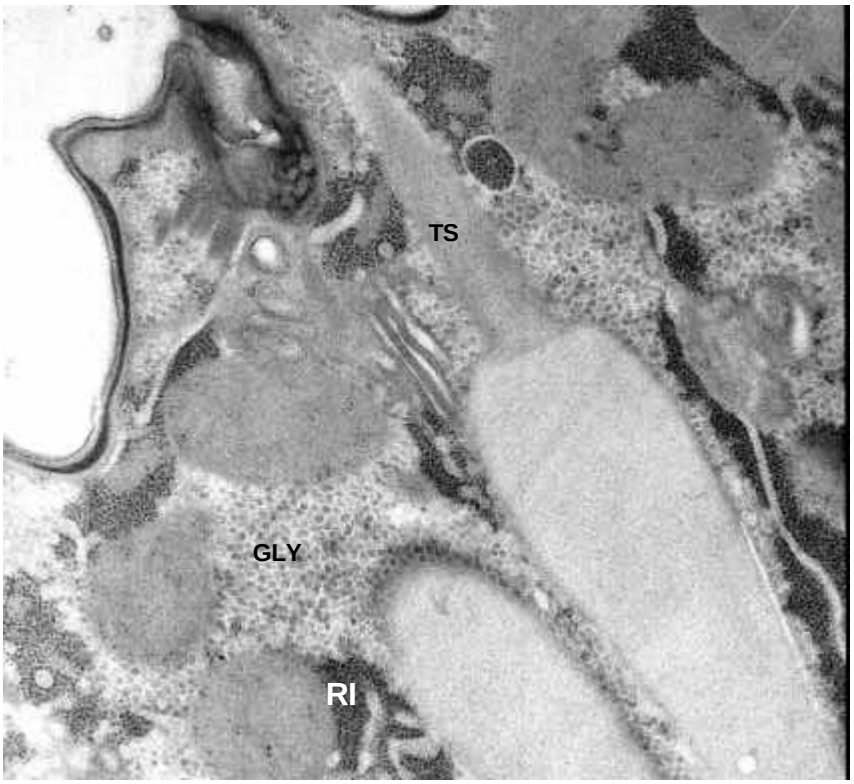
Glykogengranula is mixed with the Ribosomes.



High pressure frozen and freeze substituted Paramecium cells
Preparation inCellulose Capillary tubes

No certainable shrinkage of the Trichocysts. Bodies and tips are firmly connected.

Glykogengranula (GLY) is delimited from the Ribosome-areas(RI).



Photos:
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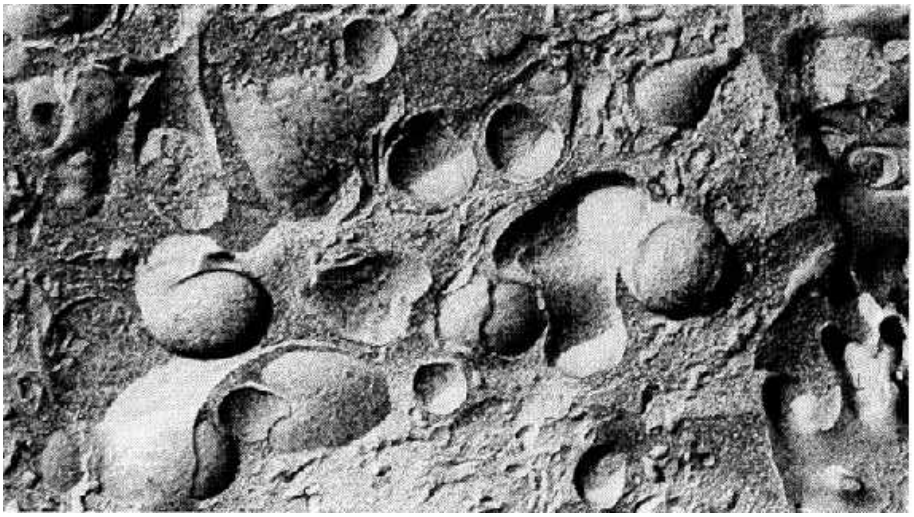
Switzerland



Liver tissue (rat)
Freeze fracture at -110°C
Evaporation coated with 2 nm Pt/C at 45°
Magnification: 33'200 x
Author: Th. Hillmann,
ETHZ Zurich
Lab. For EM1
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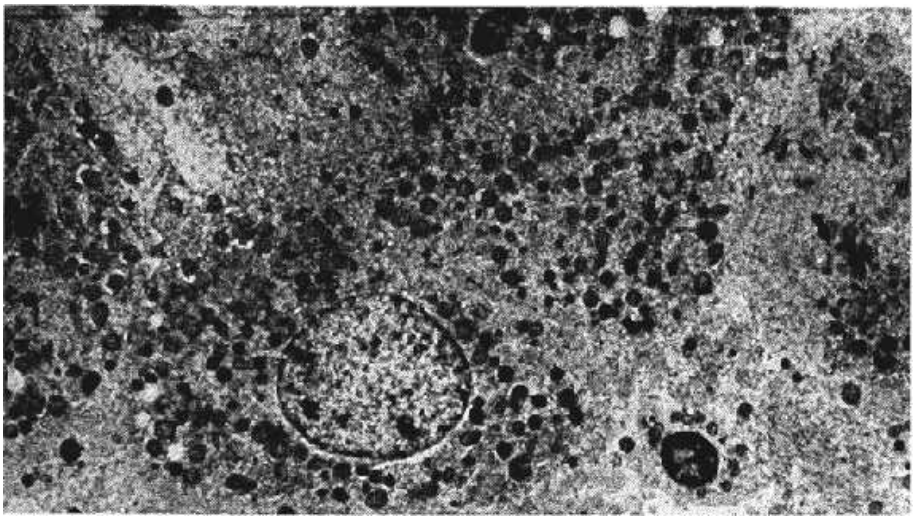
Switzerland



Liver tissue (rat)
Freeze substitution with methanol / OsO_4 / uranyl acetate/glutaraidehyde
Embedded in Araldit-Epon
Polymerization at +60°C
Post contrasted with lead according to Reynolds and uranyl acetate
Magnification: 2'800 x
Author: Th. Hillmann
ETHZ Zurich
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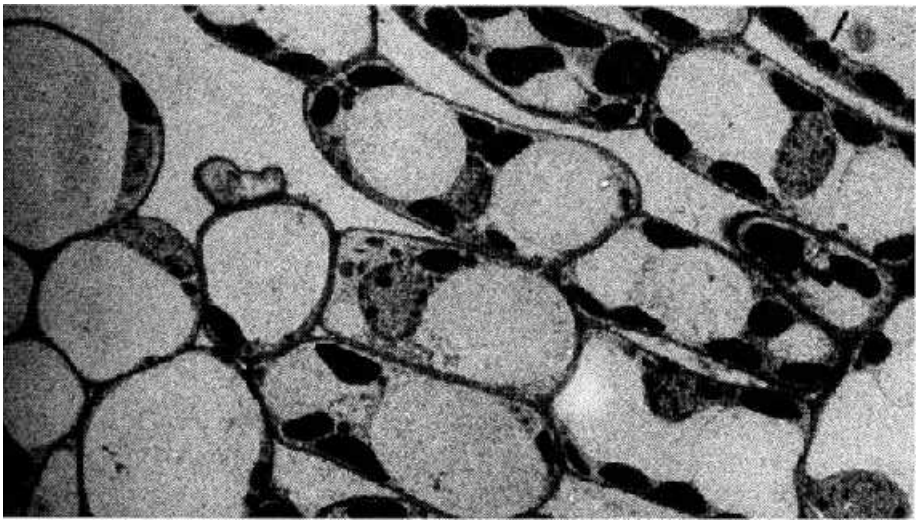
CH-8902 Zurich
Switzerland



Apple leaf (Golden Delicious)
Freeze substitution with acetone / OSO_4 (2%)
Embedded in Araldit-Epon
Polymerization at +60°C
Post contrasting with lead according to Reynolds and uranyl acetate
Magnification: 2'150 x
Author: M. Michel
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