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# Leica EM SPF

Self Pressure Freezer


Leica Design by W. Hölbl

# Leica EM SPF: A New Dimension in High Pressure Freezing

The Leica EM SPF Self Pressure Freezer is a system suited for:

› Cryo-immobilization of biological samples

› Follow-on procedures for cryo- and room temperature TEM

## GENERAL

The Leica EM SPF is based on the principle of Self Pressurized Rapid Freezing introduced by Leunissen and Yi (J. Microsc. 235: 25–35 [2009]). It uses the tendency for water inside a sealed specimen carrier to expand upon cooling, thereby generating pressure intrinsically instead of using an external hydraulic system. This pressure is likely to be the result of crystalline and low density ice formation within the sealed specimen carrier. To achieve pressure (2010 bar) where the melting point of ice is lowered to 251 K (Kanno et al. 1975, Science 189: 880–881 [1975]) 60 % of the water inside the specimen carrier needs to be converted to low density ice.

## PRINCIPLE

The Leica EM SPF works with U-tubes as a specimen carrier. The aim is to keep the low density ice located predominantly in the leg areas, whereas the arc area of the U-tube freezes last and while pressurized. This approach for cryo-fixation allows freezing of biological specimens in their native environment without any specific preparation or addition of cryo-protectants, which can alter the initial physiological balance of the sample. Almost any type of cells, free-living bacteria, yeast cells, unicellular organisms etc., can be cryo-immobilized directly after being isolated from their natural habitat.

The Leica EM SPF is a unique entry-level product offering an alternative cryo-fixation method.

## YOUR ADVANTAGES

Enhanced safety for your sample

› No cryo-protectant required: Specimens can be cryofixed in their native environment

› No Leidenfrost phenomenon: Using propane or ethane as a cryogen prevents the Leidenfrost phenomenon

Trusted reliability

› Control over freezing parameters

› Unique U-tube sample carrier

› Suitable for samples in suspensions

› Defined area of well preserved sample

› Reproducible results

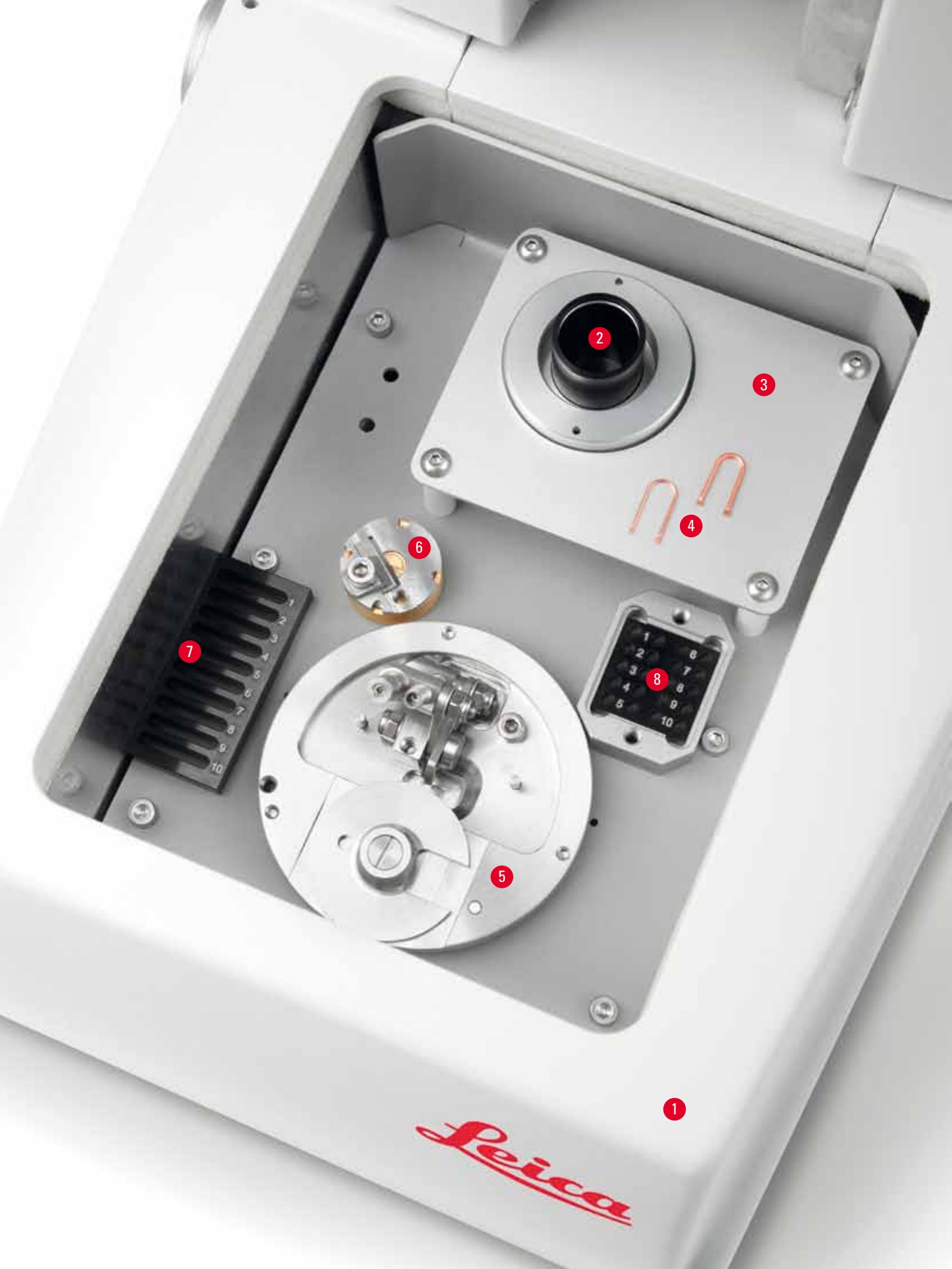
Ease of use

› Intuitive operation via touch screen panel

› Quick and economical

› Compact, mobile and very quiet during freezing

The instrument was developed in close cooperation with Dr. J. Leunissen, co-inventor of the U-tube specimen carrier.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Leica

# Special Features

1 Extra large cryo-chamber
providing a comfortable environment for manipulating the U-tubes after cryo-fixation.

2 Cryogen container
for ethane or propane

3 Platform
for blotting excess propane

4 U-tube
(sample carrier)

5 Segmenting tool
Designed to make the perfect cut (for precision in segmentation and to reduce mechanical damage during the cutting process)

6 Peeling tool
Smooth cutting through the frozen sample and copper with a tungsten carbide knife.

7 U-tube separation and temporary storage

8 Cryo-transfer container
for CEMOVIS or freeze substitution






# Freezing – Operation and Principles

The Leica EM SPF operation is via touch-screen display where every important parameter is adjustable.

## Operation

### BEND, FILL AND SEAL

U-tubes are made from copper tubes (42 mm long, 0.8 mm OD × 0.4 mm ID) using the bending tool. Filling the U-tube with sample is a standard micropipette procedure after which the open ends are sealed by clamping the leg ends shut using the sealing tool provided.

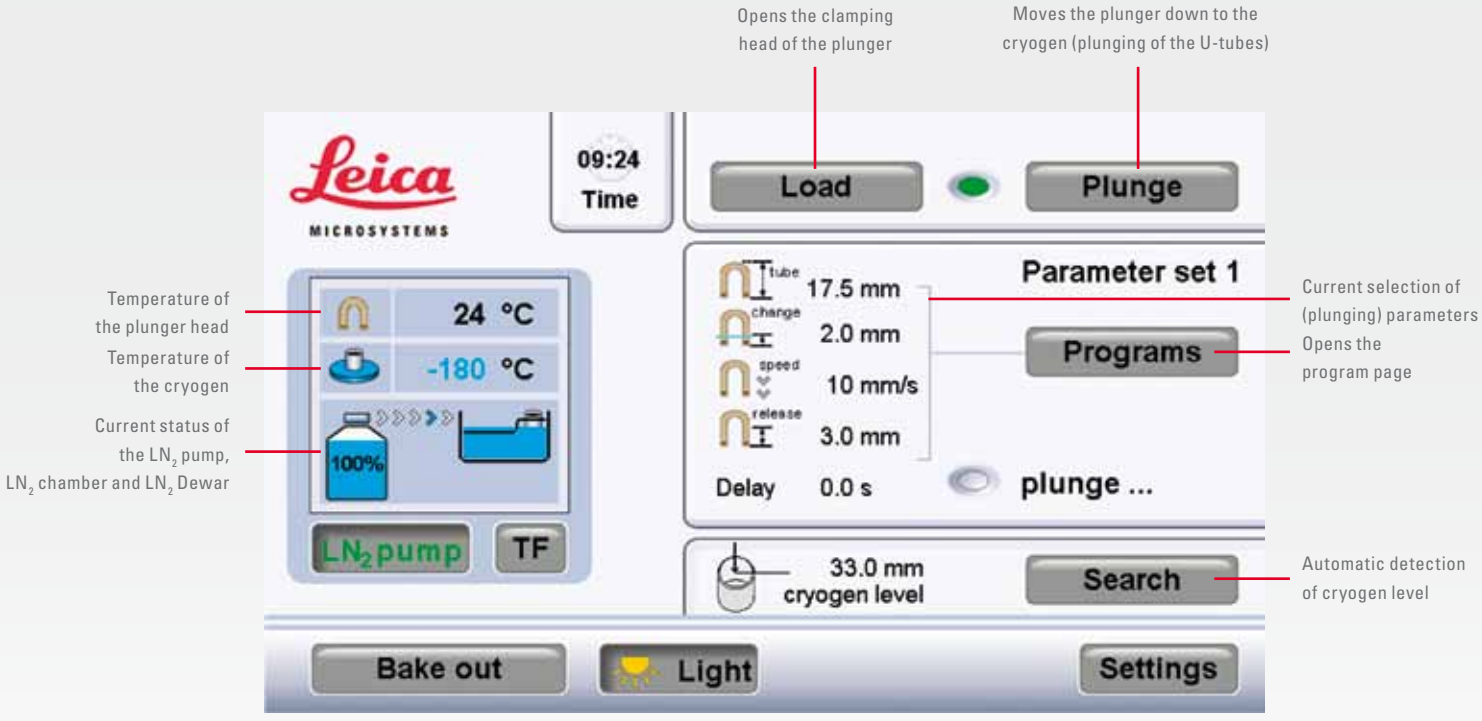
## Principles

### UNIQUE U-TUBE CARRIERS

The use of U-tubes in the Leica EM SPF facilitates spatial separation between the area of low density ice formation (predominately in the legs) and the area being pressurized (the arc of the U-tube). The arc of the U-tube is, therefore, the area where the best-preserved specimens are located.

### THROUGH THE LOOKING GLASS OF PHYSICS

Low density ice formation causes a volume expansion relative to liquid water. Numerical simulations show that freezing along the tube walls proceeds freezing in the center, producing a strongly curved ice front. This effect is prominent when the immersion speed is the highest. The separation between regions containing low density ice and well-frozen or vitrified parts is considered to be best when the ice front is as flat as possible. This can be achieved by alteration of the freezing parameters for each specific case within the Leica EM SPF program interface. During the immersion movement, the ice formation front inside the tube is always above the level of the cryogen surface. The distance of this ice front depends on the immersion speed.


Opens the clamping head of the plunger
Moves the plunger down to the cryogen (plunging of the U-tubes)
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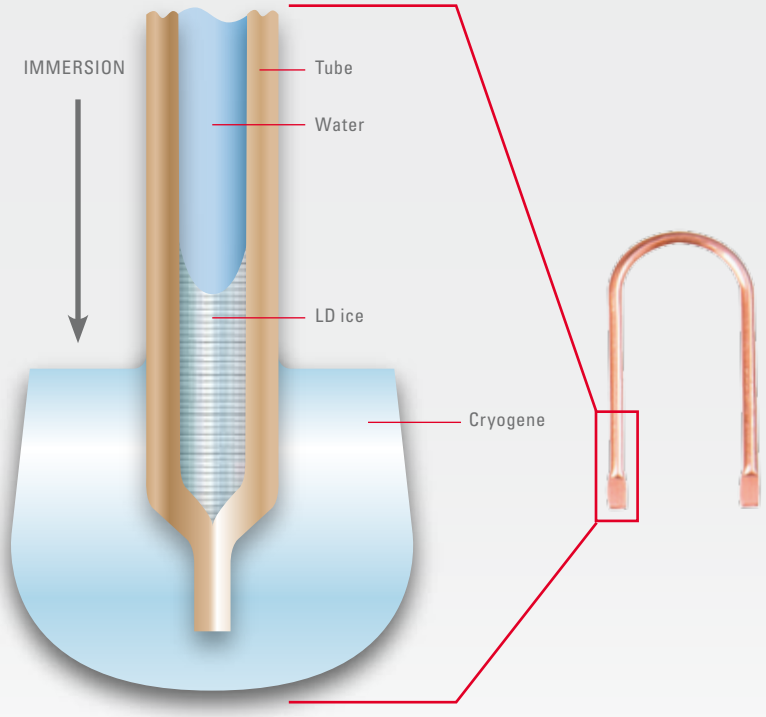
09:24
Time
Load
Plunge
Temperature of the plunger head
Temperature of the cryogen
Current status of the LN2 pump, LN2 chamber and LN2 Dewar
24 °C
-180 °C
100%
LN2 pump
TF
Tube
17.5 mm
change
2.0 mm
speed
10 mm/s
release
3.0 mm
Parameter set 1
Programs
Current selection of (plunging) parameters
Opens the program page
Delay
0.0 s
plunge ...
33.0 mm
cryogen level
Search
Automatic detection of cryogen level
Bake out
Light
Settings


IMMERSION
Tube
Water
LD ice
Cryogene

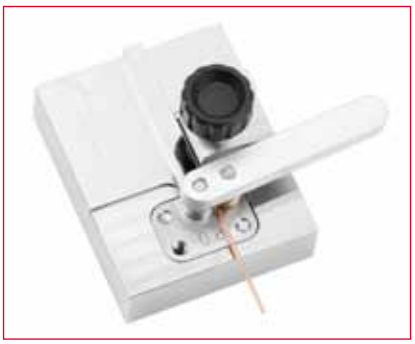
# Details

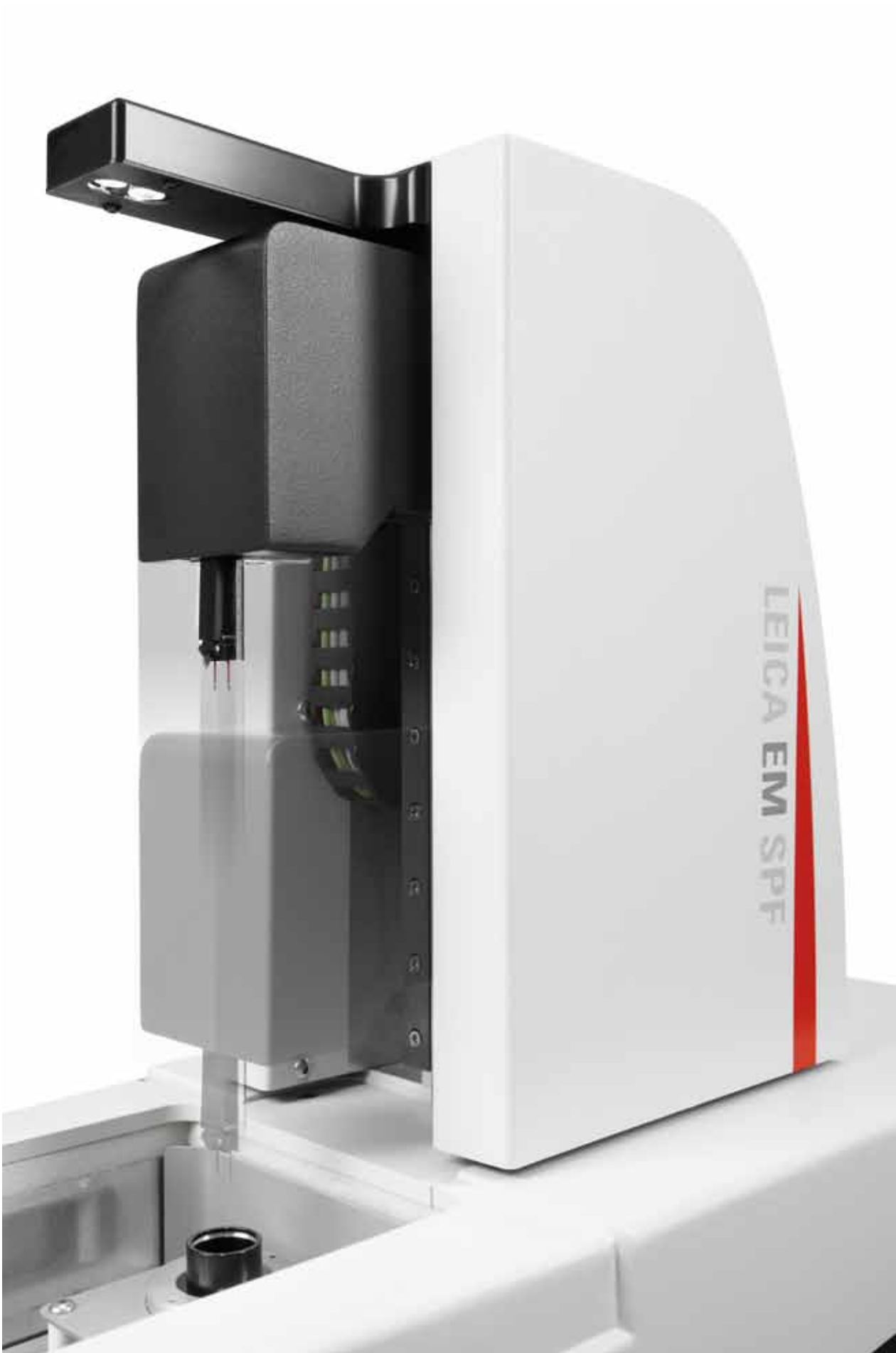
## BENDING THE COPPER TUBE

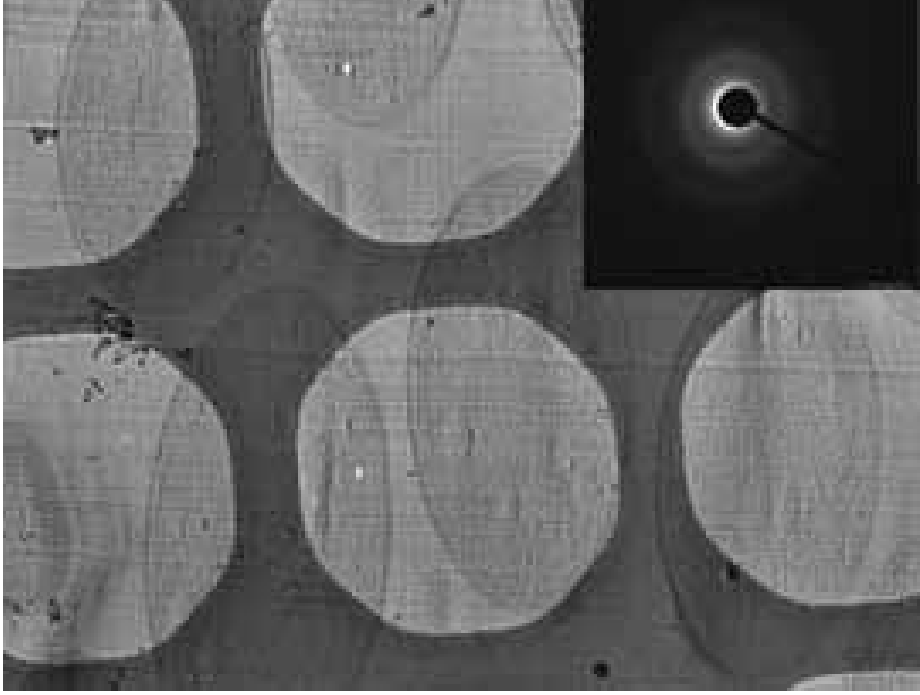
Bending the copper tube in U-shaped carrier is simply one turn without inducing any stress and deformations in the arc.

## OTHER ADVANTAGES

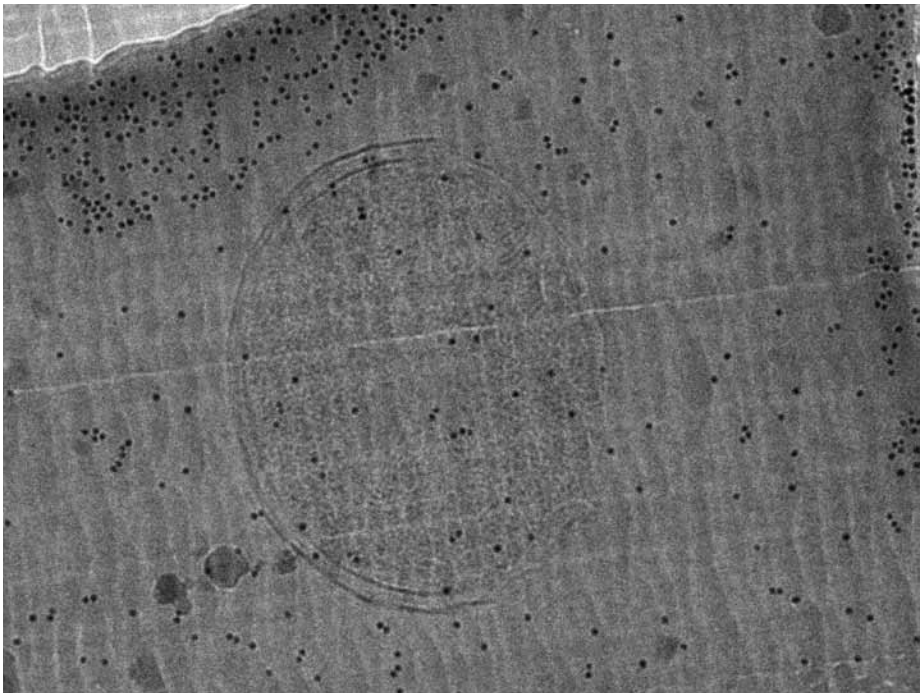
- LED illumination over the entire work area
- Thermally controlled injector head
  – ensures conditions for the specimen
  – prevents the arc from pre-cooling
- Two-step plunging speed



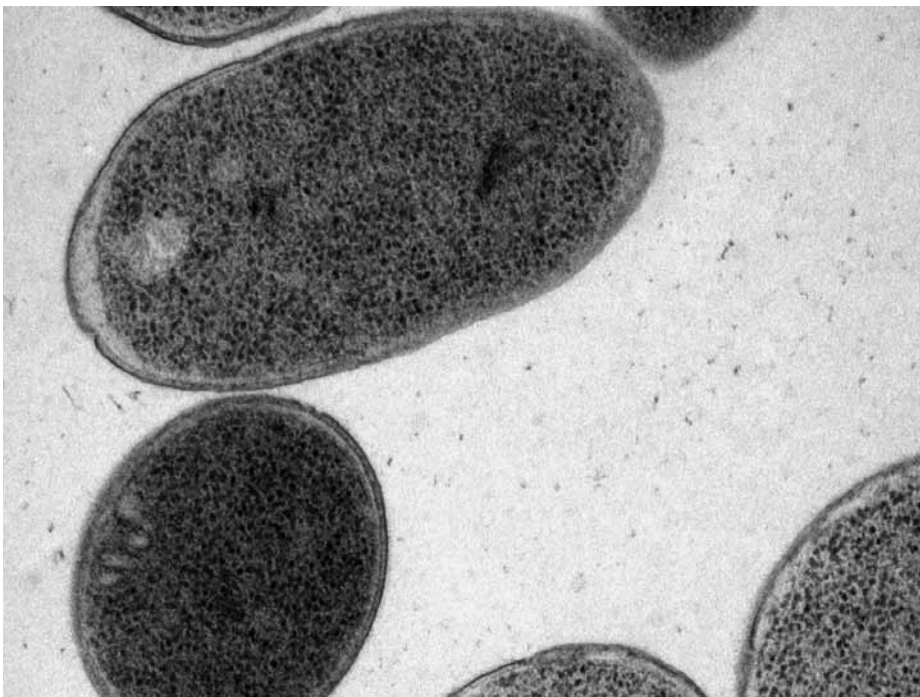

LEICA EM SPF



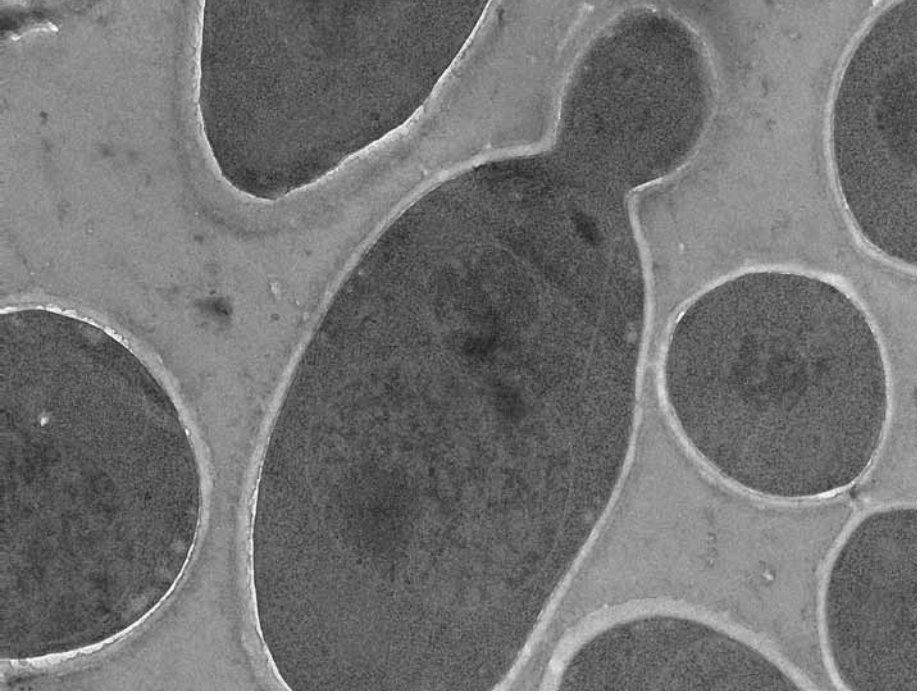
CEMOVIS of *Saccharomyces cerevisiae*
(Courtesy A. Al-Amaudi, DZNE, Bonn)



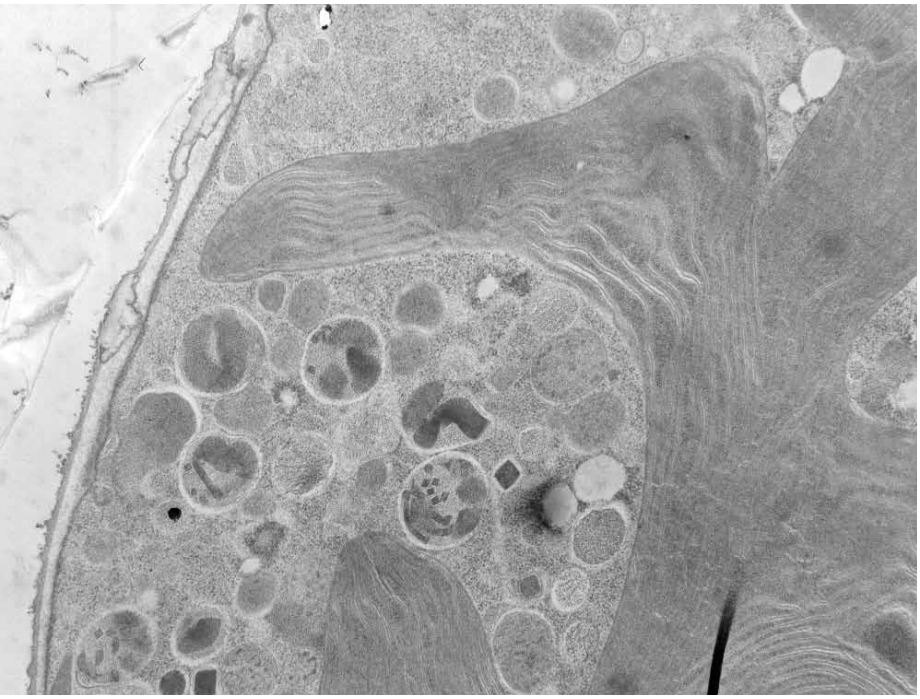
CEMOVIS of *Pseudomonas deceptionensis,* 30 % dextran in PBS (Courtesy Carmen López-Iglesias lab, Scientific & Technological Centres (CCiT), University of Barcelona)



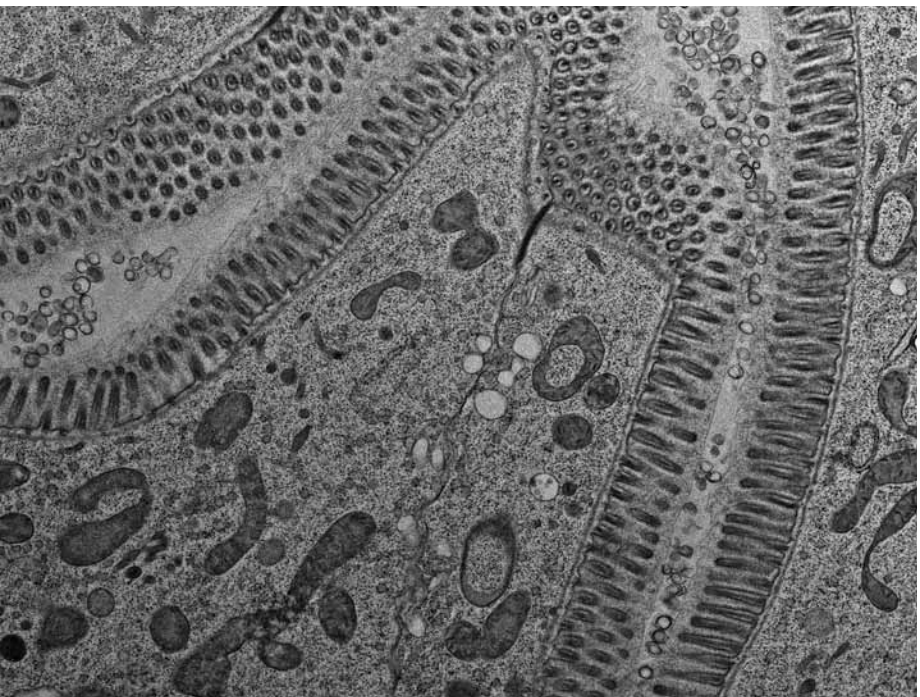
Freeze Substitution of *Pseudomonas deceptionensis*
(Courtesy Carmen López-Iglesias lab, Scientific & Technological Centres (CCiT), University of Barcelona)



Freeze Substitution of *Lingulodinium polyedra (extrusomes)* (Courtesy Elena Lindemann, Fraunhofer IGB [Functional Genomics], Stuttgart)



Freeze Substitution of *Lingulodinium polyedra* (Courtesy PD Dr. Mike Schweikert, University of Stuttgart)



Freeze Substitution of *Caenorhabditis elegans* (Courtesy of the Delaware Biotechnology Institute Bio-Imaging Center (Shannon Modla, Scott Jacobs, Jeff Caplan and Kirk Czymmek) and University of Pennsylvania (Jessica Tanis)

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