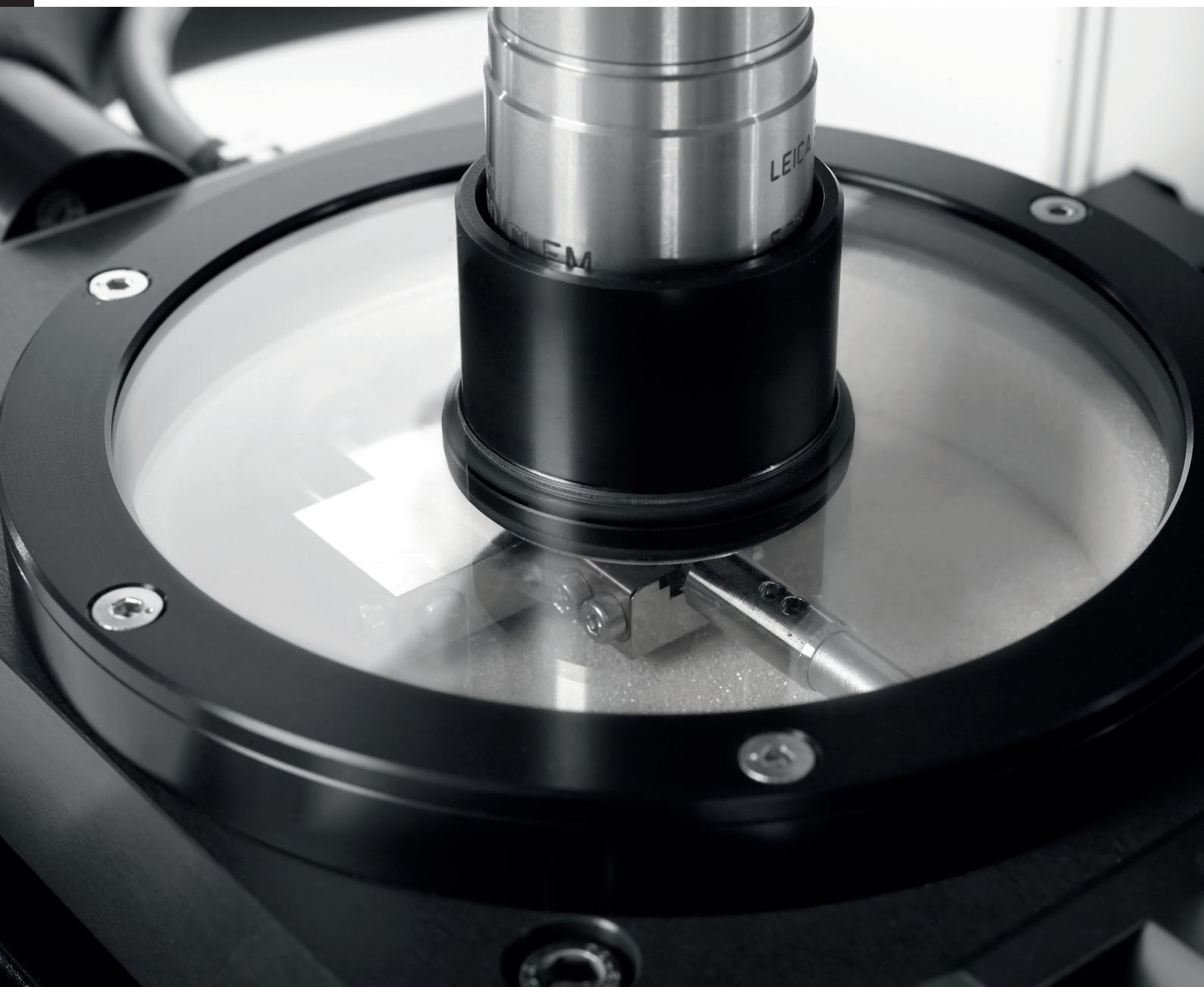
Living up to Life

Leica
MICROSYSTEMS

Leica EM Cryo CLEM

One System to Serve Your Workflow Needs in Correlative Light and Electron Microscopy



Correlative Light and Electron Microscopy

Researchers can only optimise results when their samples are well-preserved during the entire process from sample preparation to imaging. To enable you to focus on what really matters, Leica Microsystems has developed a solution for the complete workflow from cryo fixation to cryo fluorescence light microscopy. This unique solution offers premium instrumentation from electron microscopy sample preparation to fluorescence light microscopy, including analysis capabilities with the Leica Application Suite microscopy software platform.

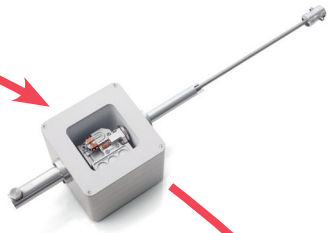
- **Correlative Light and Electron Microscopy (CLEM)** combines fluorescence light microscopy and electron microscopy (EM) imaging of the same sample.
- **Electron Microscopy (EM)** delivers structural information and also the context in which the target structures are embedded at very high resolution. But EM provides very limited information in terms of living biological processes and functions.
- **Fluorescence Light Microscopy (FLM)** on the other hand is a very sensitive method to observe and analyze biological processses and functions inside fixed and living biological samples. In addition, FLM allows rapid screening of large areas and fast determination of regions of interest (ROI) which can be quickly recognized in the electron microscope.
- **Cryo Fixation** is the sample preparation method to maintain samples in the most life like state as possible.
- **Cryo-CLEM** connects the benefits of all these techniques. It combines the individual advantages from cryo fixation, FLM and EM by time-effective imaging of identical, artefact-free samples and overlaying the complementary information to win greater understanding.

High Pressure Freezing

Leica EM ICE

Cryo-Ultramicrotomy

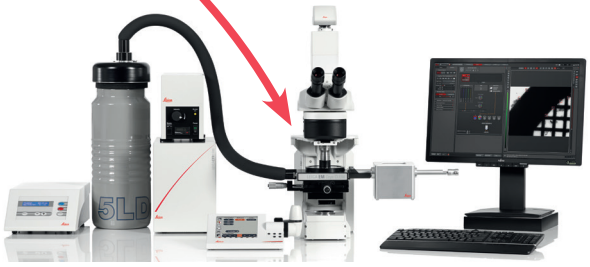
Leica EM UC7
Leica EM FC7



Cryo-Transfer-System

Grid Plunging

Leica EM GP



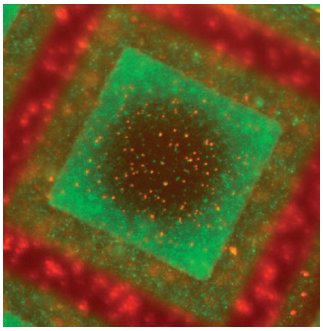
Cryo Light Microscopy

Leica EM Cryo CLEM
on Leica DM6000 FS

Leica LAS Widefield Software
for manual correlation of LM marked structures in EM

Cryo-Electron Microscopy

Image analysis



Leica EM Cryo CLEM

The Leica EM Cryo CLEM System ensures the fast, safe, contamination-free sample transfer and loading from cryo sample preparation instruments to the Leica fix stage light microscope DM6000 FS. The system consists of a cryo stage, which is developed to seamlessly integrate to the fixed stage microscope (not included), the cryo transfer shuttle, and cryo objective. With the perfect Leica camera and software portfolio, Leica can provide a complete and integrated cryo CLEM workflow from cryo sample preparation to cryo fluorescence light microscopy imaging for CLEM.

Leica EM CRYO CLEM – INNOVATIVE FEATURES

Trusted reliability

- Reproducible process through contamination-free and controlled cryo sample transfer and loading method to the cryo stage
- High image quality and specific localization of target structure in LM with the world's first cryo objective with low working distance for high resolution

Time saving

- Reduced sample loading and unloading time through new sample transfer and loading system
- Reduced cryo EM operation and user interaction time during EM analysis due to well preserved sample quality and accurately located target structures

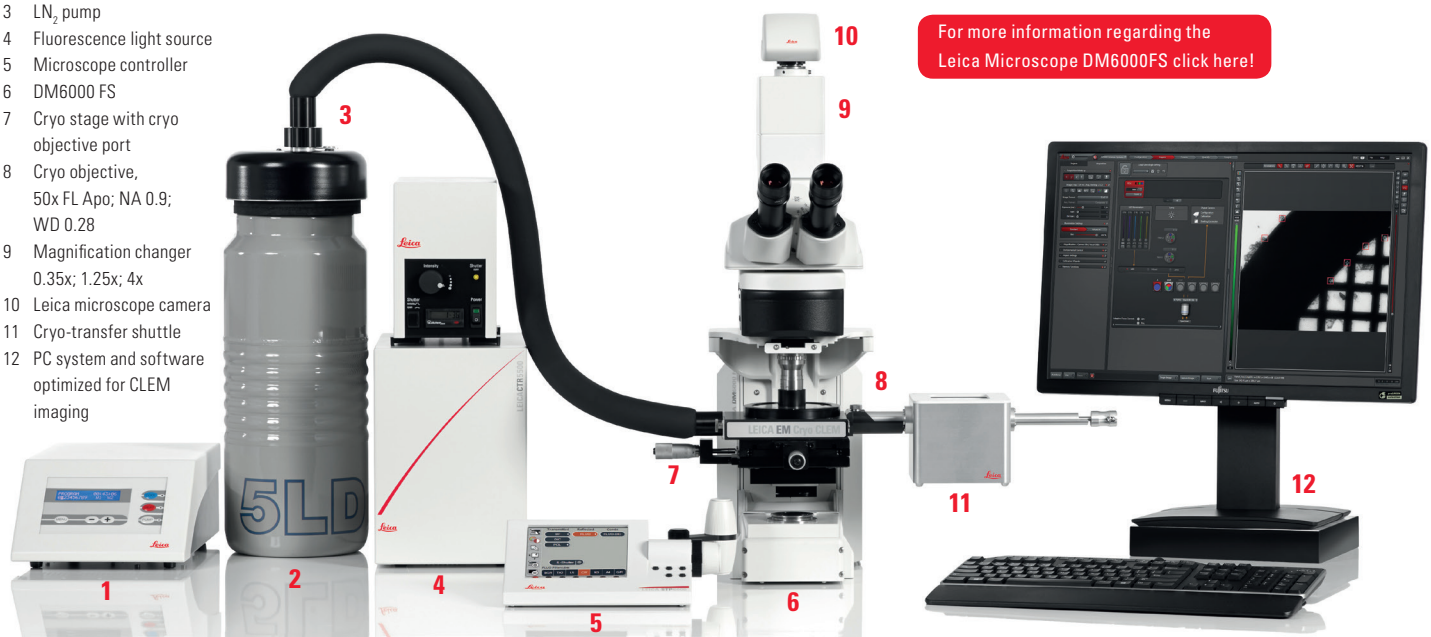
Ease of Use

- Sensor controlled stage temperature function
- Ease of use with the new intuitive sample transfer and loading system

Cost Savings

- Significantly reduced cryo EM user operation costs due to reduced target structure search time

1 LN_2 pump controller
2 5L LN_2 dewar
3 LN_2 pump
4 Fluorescence light source
5 Microscope controller
6 DM6000 FS
7 Cryo stage with cryo objective port
8 Cryo objective, 50x FL Apo; NA 0.9; WD 0.28
9 Magnification changer 0.35x; 1.25x; 4x
10 Leica microscope camera
11 Cryo-transfer shuttle
12 PC system and software optimized for CLEM imaging

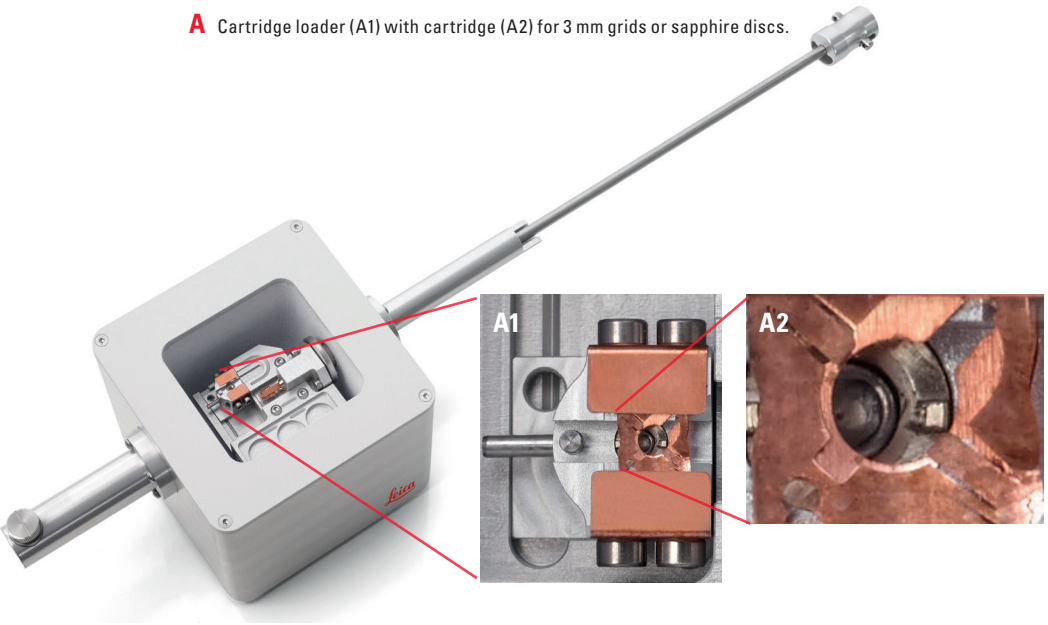


For more information regarding the Leica Microscope DM6000FS click here!

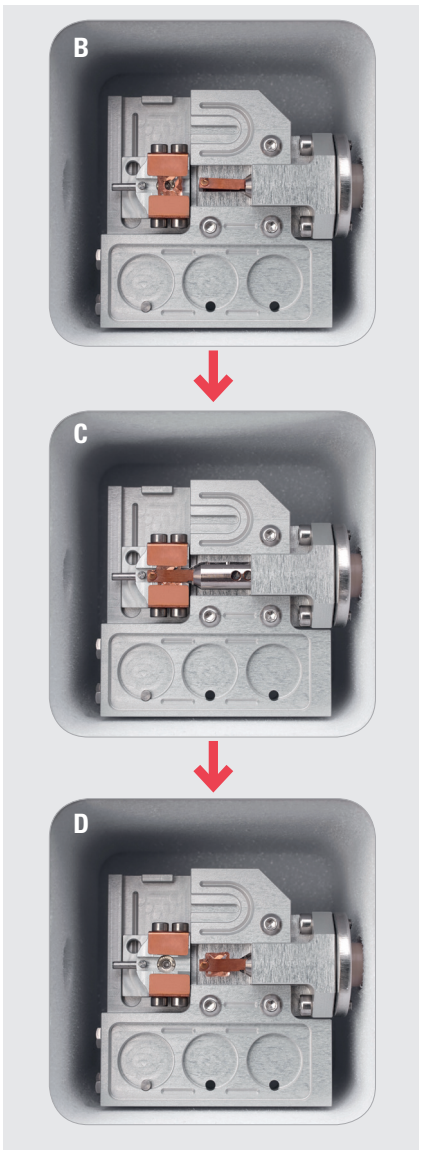
Leica Cryo Transfer Shuttle

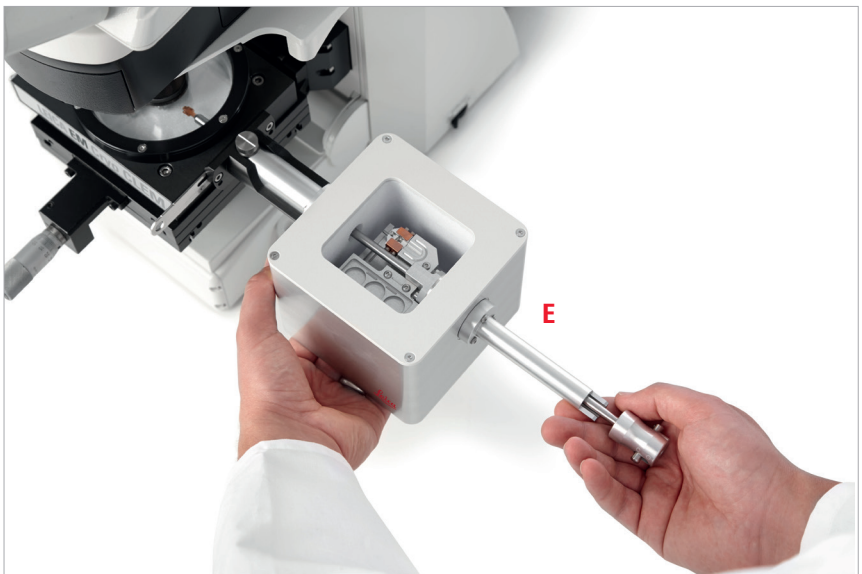
The transfer shuttle for Leica Cryo CLEM ensures fast, easy, and contamination-free sample loading and transfer (A). It consists of three functional parts: a cartridge loader, a storage area for three standard grid transfer boxes, both located in the dewar part of the cryo transfer shuttle, and third a rod with gripper for sample transfer.

A Cartridge loader (A1) with cartridge (A2) for 3 mm grids or sapphire discs.



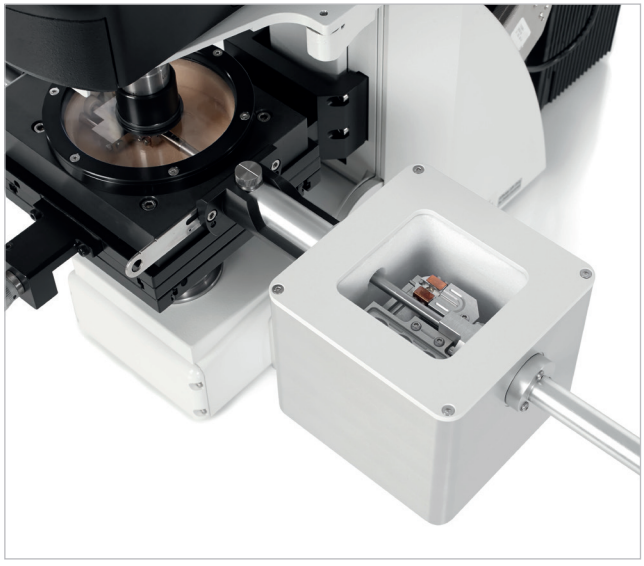
The sample loading is divided in four steps. At first the grid or sapphire disc is mounted on the cartridge (B). Second, the gripper of the transfer rod clamps the cartridge (C + D), thrid the transfer shuttle is docked on the loading port of the cryo stage and the rod with the cartrige is transferred through the loading port of the cryo stage into the cartridge intake of the cryo stage (E).





Components

Leica cryo stage and cover with cryo objective port. The stage allows cryo objective approach to the sample even with a low working distance, necessary for high resolution cryo imaging. Mechanically and thermally it is exceptionally stable which leads to long-term focus stability. Integrated sensors monitor stage temperature. Cooling range from –195 °C to +60 °C.



Leica EM Cryo CLEM on fixed stage microscope DM6000 FS. The cryo stage with inserted cryo objective is developed to seamlessly integrate to the Leica DM6000 FS fixed stage microscope; cryo transfer shuttle is docked to cryo stage.

Leica HCX PL APO 50x / 0,90 CLEM objective, apochromatically corrected with numerical aperture of 0.9, and a low working distance of 0.28 mm for high resolution cryo imaging. Maximum resolution of 364 nm.

All following pictures were taken with the CLEM objective!

Results

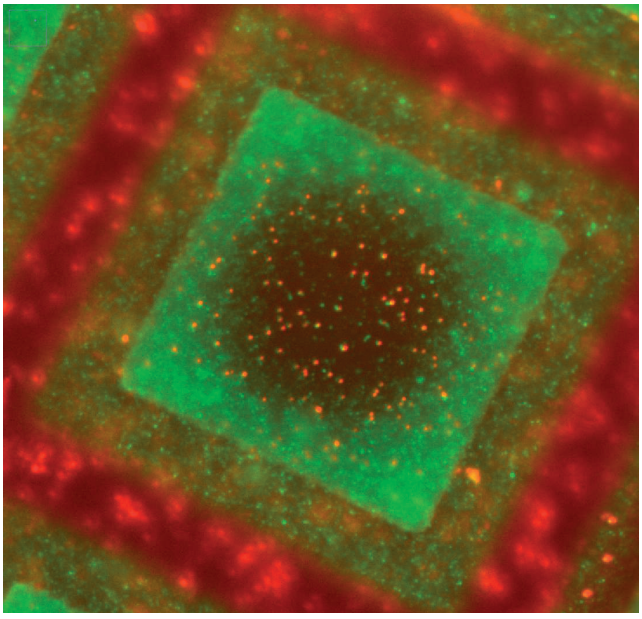


Fig 1: Multi Fluorescence cryo LM overview image of grid square. Transport vesicle with fiducial markers.

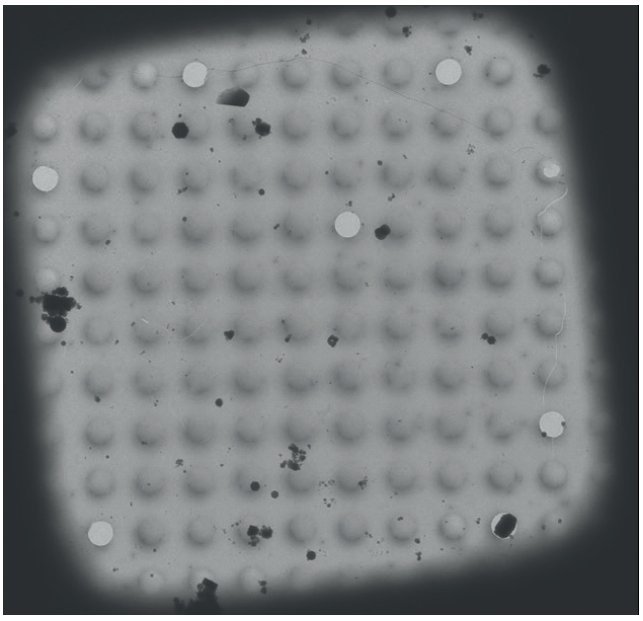


Fig. 2: Cryo TEM overview image of grid square. Perfect preservation of the vitrified sample through the transfer process from grid plunging to cryo LM and cryo EM. Granular texture of the image is vitrified ice.

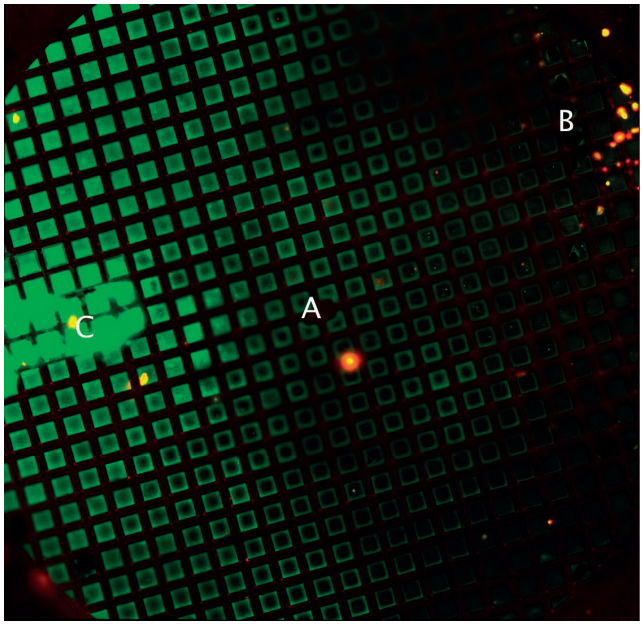


Fig. 3: Complete mosaic scan of a 300 mesh pluge-frozen TEM copper grid. The sample is fluorescein-labeled liposomes on a holey carbon film pre-treated with multifluorescent polystyrene beads. One can nicely see the distribution of ice thickness along the diagonal. "A" labels the grid center mark that is used for initial coordinate alignment of the mosaic. "B" indicates grid mesh with ruptured support film that can be used as orientation reference markers. The bright fluorescent feature underlying "C" is thick crystalline ice. Due to its distinct shape one can assume that it was caused by touching this area with the tweezers while handling the grid.

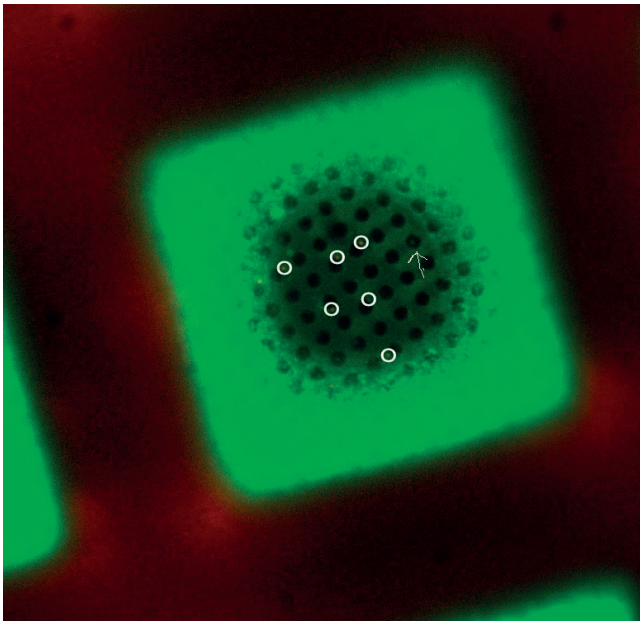


Fig. 4: A 100 % zoom in image section of the mosaic shown before. The holey carbon support film is visible due to its autofluorescent properties.
The strong fluorescence signal along the rim of the square is due to the increased ice thickness and thus the locally higher presence of particles compared to the thin ice film in the center of the grid square. White circles indicate the fluorescence signal of the beads used for coordinate transformation. The arrow points at a signal of interest that only exists in the green channel.

Images: Courtesy of Dr. J. Briggs and Dr. M. Schorb, EMBL Heidelberg, Germany.

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The statement by Ernst Leitz in 1907, "With the User, For the User," describes the fruitful collaboration with end users and driving force of innovation at Leica Microsystems. We have developed five brand values to live up to this tradition: Pioneering, High-end Quality, Team Spirit, Dedication to Science, and Continuous Improvement. For us, living up to these values means: Living up to Life.

LIFE SCIENCE DIVISION – NANO TECHNOLOGY LNT

The Leica Microsystems Nano Technology Division's focus is to provide the most compehensive product portfolio for the preparation of biological, medical and industrial samples for investigation in the Electron and Light Microscope. Excellent Sample Preparation is a prerequisite for perfect microscopy. Your image starts here!

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