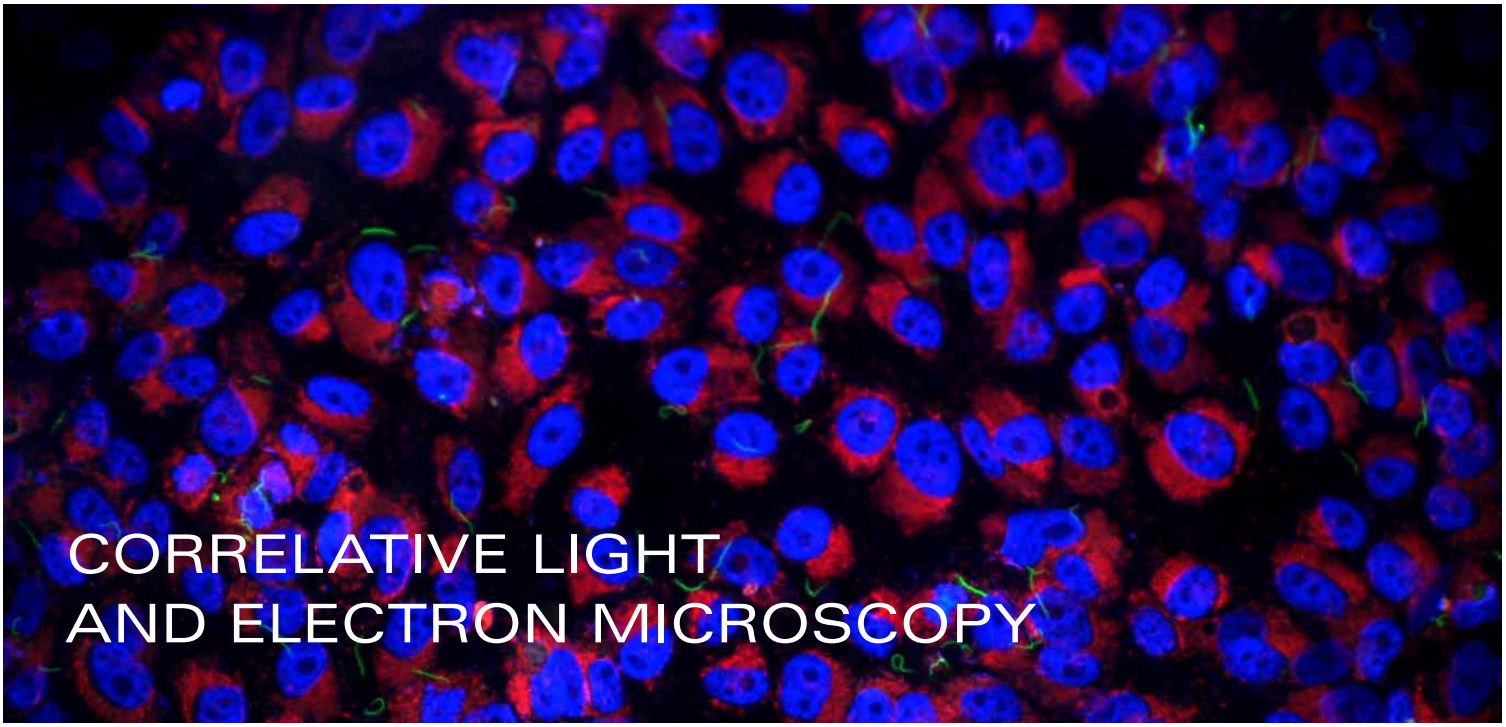
Leica
MICROSYSTEMS

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.

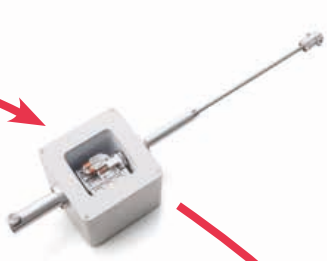
> Fluorescence Light Microscopy (FLM)
on the other hand is a very sensitive method to observe and analyze biological processes and functions inside fixed and living biological samples. In addition, FLM allows rapid screening of large areas and fast determination of regions of interest (ROI) which can 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.

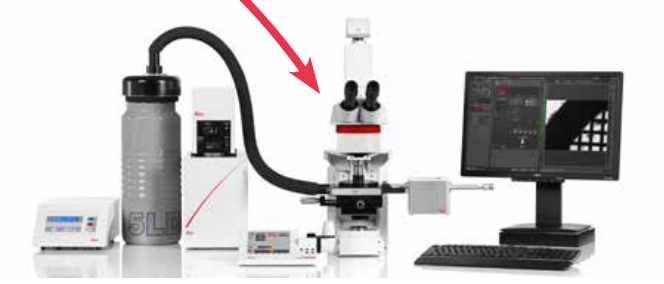
High Pressure Freezing
Leica EM ICE

Cryo-Ultramicrotomy
Leica EM UC7
Leica EM FC7



Cryo-Transfer-System

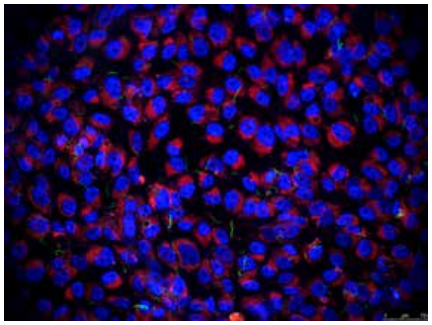
Grid Plunging
Leica EM GP



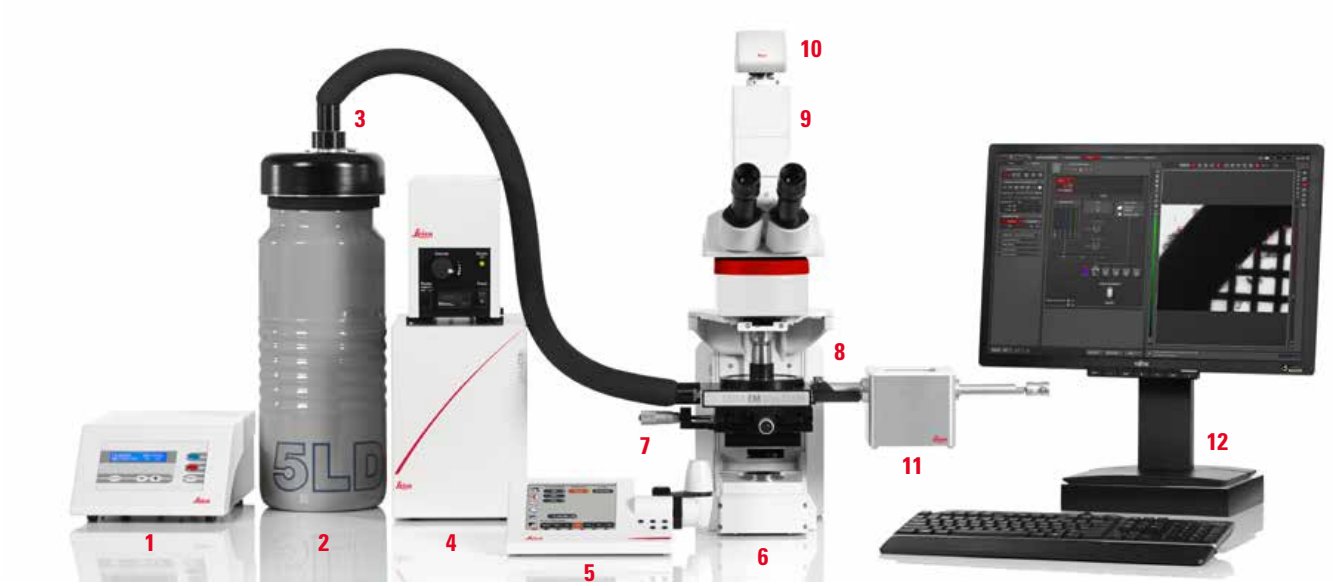
Cryo Light Microscopy
Leica EM Cryo CLEM
on Leica DM6 FS

Leica LAS X CLEM Software
for manual and automated correlation
of LM marked structures in EM

Image analysis



CRYO-ELECTRON MICROSCOPY



1 LN_2 pump controller; 2 5L LN_2 dewar; 3 LN_2 pump; 4 Fluorescence light source; 5 Microscope controller; 6 DM6 FS; 7 Cryo stage with cryo objective port; 8 Cryo objective, 50x PI 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

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 DM6 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

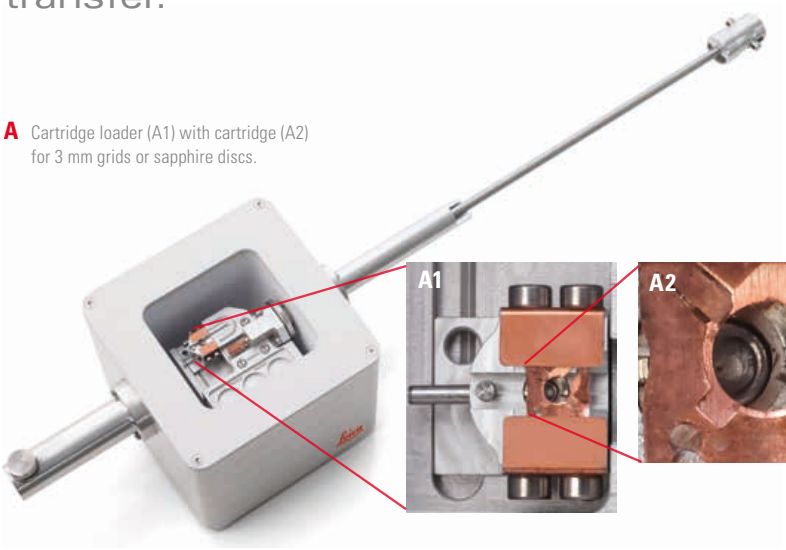
> Sensor controlled stage temperature function
> Ease of use with the new intuitive sample transfer and loading system

Cost Savings

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

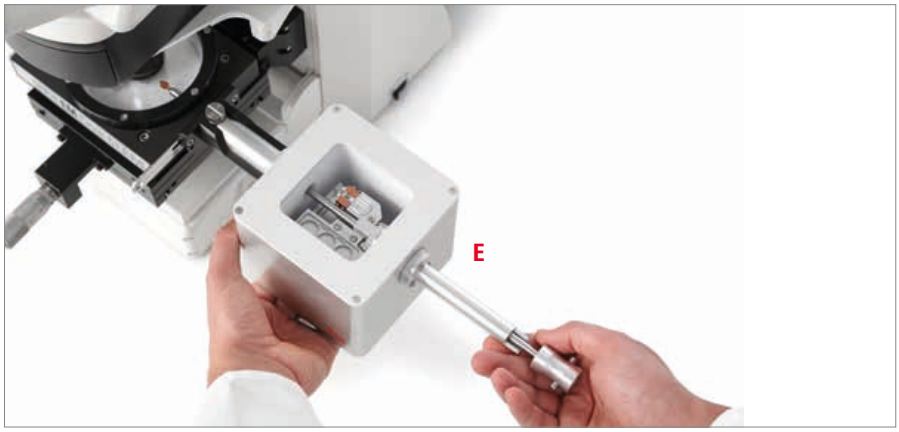
LEICA CRYO TRANSFER SHUTTLE

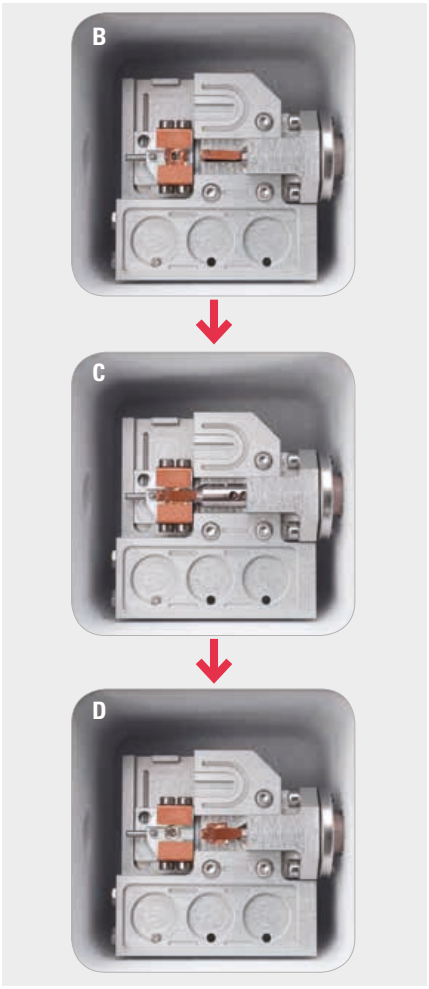
The transfer shuttle for Leica Cryo CLEM ensures fast, easy, and contamination-free sample loading and transfer (A). It consists of three functional parts: a cartridge loader, a storage area for three standard grid transfer boxes, both located in the dewar part of the cryo transfer shuttle, and third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



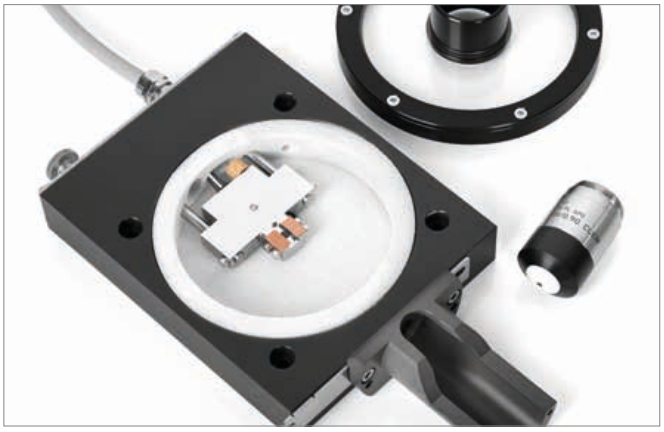
A Cartridge loader (A1) with cartridge (A2) for 3 mm grids or sapphire discs.

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), third the transfer shuttle is docked on the loading port of the cryo stage and the rod with the cartridge is transferred through the loading port of the cryo stage into the cartridge intake of the cryo stage (E).

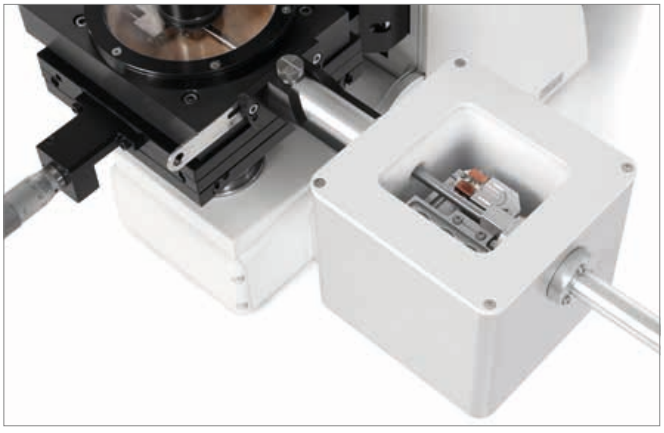




COMPONENTS



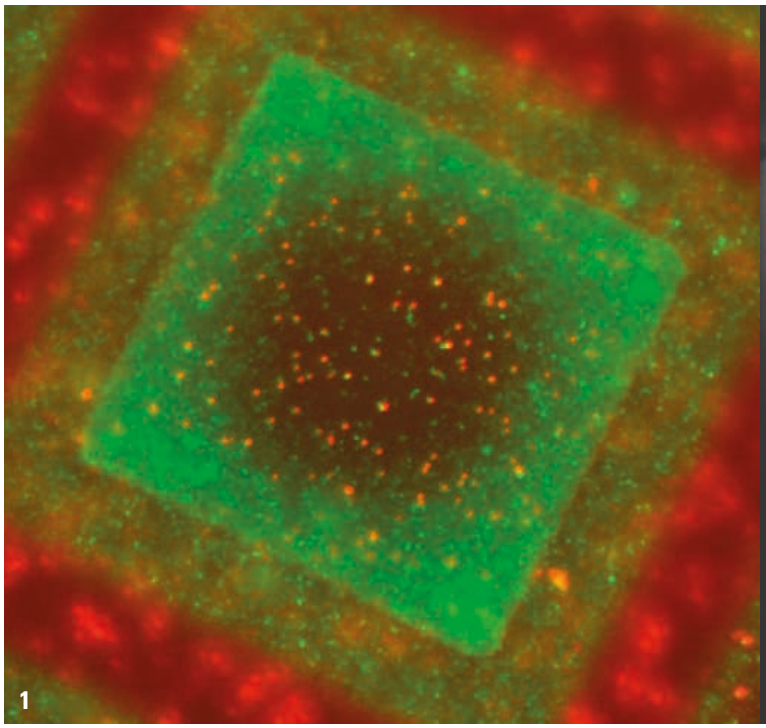
Leica cryo stage and cover with cryo objective port
The stage allows cryo objective approach to the sample even with a low working distance, necessary for high resolution cryo imaging. Mechanically and thermally it is exceptionally stable which leads to long-term focus stability. Integrated sensors monitor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

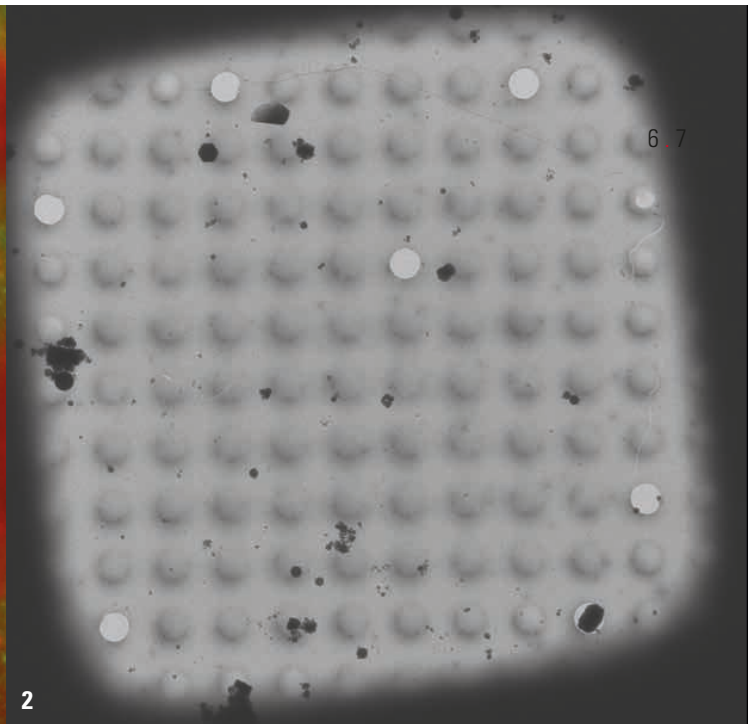


Leica EM Cryo CLEM
on fixed stage microscope DM6 FS. The cryo stage with inserted cryo objective is developed to seamlessly integrate to the Leica DM6 FS fixed stage microscope; cryo transfer shuttle is docked to cryo stage.

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 512 nm.

All following pictures were taken with the CLEM objective!





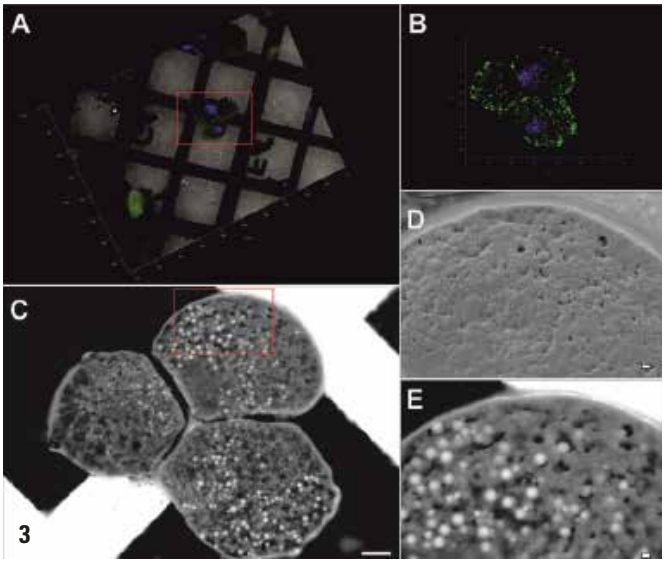
RESULTS

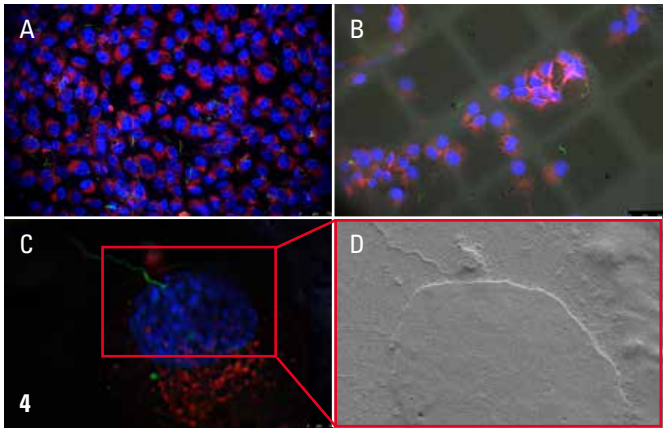
Fig 1: Multi Fluorescence cryo LM overview image of grid square. Transport vesicle with fiducial markers. Courtesy of Dr. J. Briggs and Dr. M. Schorb, EMBL Heidelberg, Germany.

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

Fig. 3: **A, B:** Cryo FLM of digestive cells stained with Hemoglobin-pHrodo® (green) and Hoechst 33342 (blue). **C:** SEM image of same cells in BSE mode at 15.0 kV, scale 10 µm. **D, E:** Close-up look at the same cells at different kV (D: 15 kV, E: 4 kV). Courtesy of Jana Schrenková, Biology Centre of CAS, Ceske Budejovice, Czech Republic.

Fig. 4: Correlative cryo-fluorescence and cryo-scanning electron microscopy of Borrelia burgdorferi-GFP (green) on the surface of **A:** human glioblastoma and **B, C, D:** mouse neuroblastoma cells grown on carbon-coated sapphire discs. Cells were counterstained with Hoechst 33342 (blue) and LysoTracker (red). Courtesy of Martin Strnad, Biology Centre of CAS, Ceske Budejovice, Czech Republic.





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