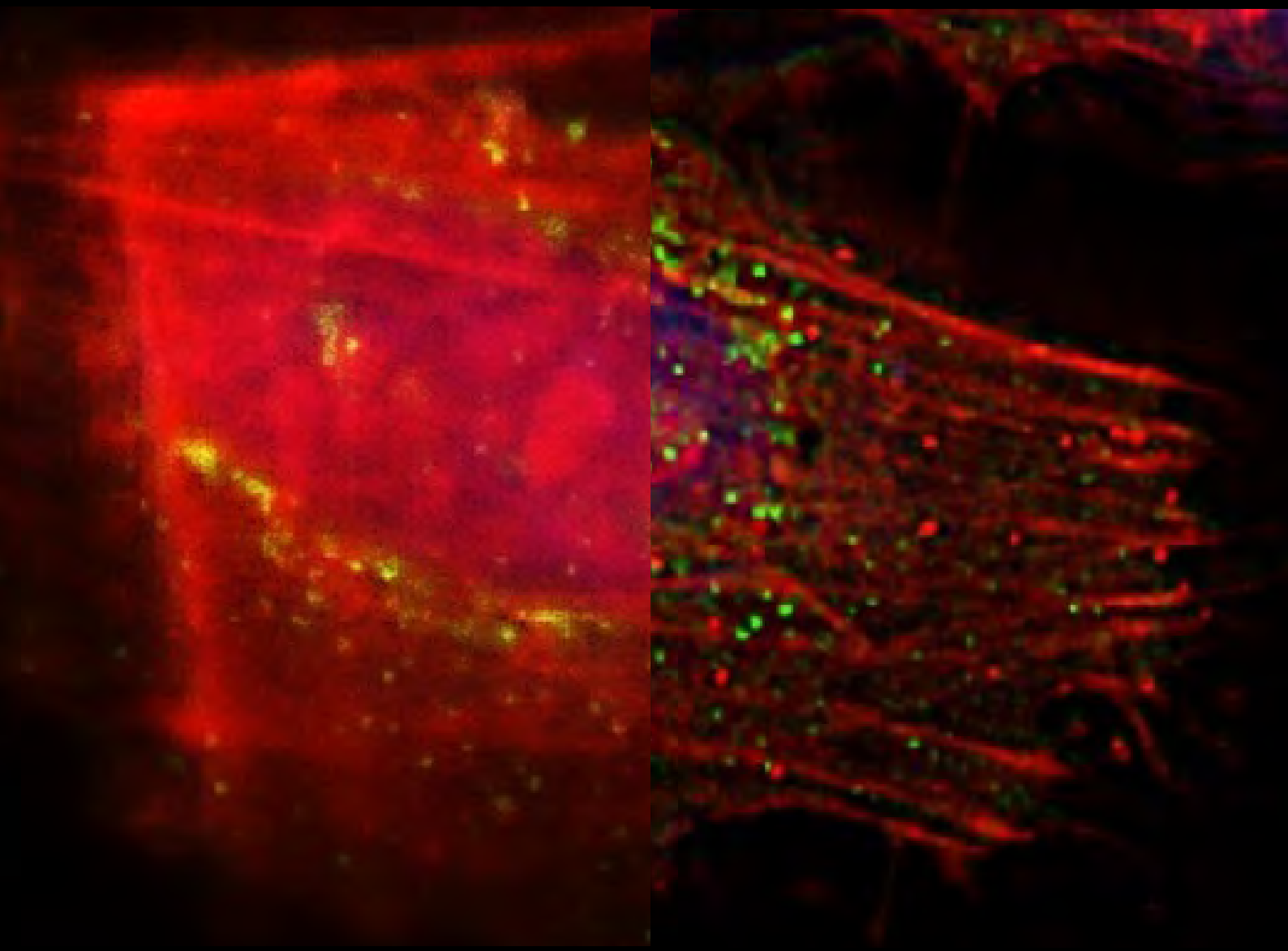
Leica
MICROSYSTEMS

CRYO LIGHT MICROSCOPE
THUNDER IMAGER EM CRYO CLEM

Obtain an in-depth understanding of cellular structural biology



THUNDER IMAGER EM CRYO CLEM

MISSION

QUALITY

PRECISION

SAFETY

RELIABILITY

“ The EM Cryo CLEM is a key element in our cryo workflows. I do not want to miss it.”

Dr. Yannick Schwab, Team Leader and Head of Electron Microscopy Core Facility, EMBL Heidelberg, Germany.

MISSION

Data courtesy of Dr. B. Engel, Department of Molecular Structural Biology, Max Planck Institute for Biochemistry, Martinsried, Germany. Original Publication: “Albert S, Schaffer M, Beck F, Mosalaganti S, Asano S, Thomas HF, Plitzko JM, Beck M, Baumeister W, Engel BD. Proteasomes tether to two distinct sites at the nuclear pore complex. PNAS, Dezember 2017.”

UNDERSTANDING CELLULAR STRUCTURAL BIOLOGY

Mankind knows many diseases. For finding effective treatments, the underlying cellular mechanisms have to be investigated. Recent developments in cryo electron-microscopy workflows enable 3D data of the protein sociology of cells to be obtained with an unprecedented resolution below 1 nm.

To increase the reliability of such workflows in terms of generating the desired data, cryo light-microscopy is an essential tool. In general, it is used to check the sample quality and identify target sites for cryo electron-microscopy, but in particular for cryo electron tomography.

The THUNDER Imager EM Cryo CLEM is a well developed solution which fulfills these tasks.

QUALITY

The THUNDER Imager EM Cryo CLEM ensures that your sample is suitable for the next steps of the cryo EM workflow. Highly resolved images with different imaging modalities help you check the quality of the support film, judge the ice thickness, and finally identify structures of interest and their accessibility for subsequent EM steps, e.g., FIB milling. The early quality check saves you time and reduces costs.

> Assess the quality of the support film.

> Judge the ice thickness.

> Check the distribution and position of targets.

CRYO-LIGHT-MICROSCOPY MODES

Reveal the sample quality

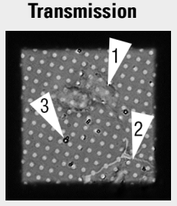
The quality of a sample can be affected by several factors.

The integrity of the support film (e.g., carbon film with holes), the thickness and distribution of the vitrified ice, and, of course, the integrity of the specimen. Also, the specimen's position in relation to the grid bars, the intensity of the fluorophore (i.e., the expression of the target protein) and the frequency of occurrence are important.

ALL of these parameters can be determined and assessed by the THUNDER Imager EM Cryo CLEM.

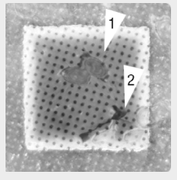
- Save time and effort thanks to identification of irrelevant samples
- Find and mark structures of interest

Transmission



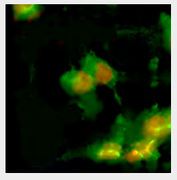
Transmitted Light Mode is used to visualize the specimen according to its thickness and transparency. Cells and their edges are revealed (1). Cracks in the film (2) and contamination by cristalline ice (3) is visible.

Reflection



Reflection Mode shows the surface of the grid or film by reflecting the light shone onto the grid. Defects in the support film are visualized (1) and the vitrified ice on top of the cells appears in layers (2).

Fluorescence



Fluorescence Mode exhibits selectively the different fluorophores applied to the sample. From the fluorescence intensity the expression level can be estimated and the distribution and position of the structure of interest can be determined.

QUALITY

Software Workflow

To ensure a constant experiment quality when using imaging systems, new users have to be intensively trained. To avoid long trainings and provide an easy transfer of experiment parameters, the THUNDER Imager EM Cryo CLEM comes with a supportive software workflow allowing users to reload complete experiment setups by one click and helping unexperienced users during the workflow.

- Full quality ensurance by reload of all experiment settings
- Reduced effort and time for training
- Support for inexperienced users

PRECISION

The THUNDER Imager EM Cryo CLEM utilizes Computational Clearing, a Leica exclusive opto-digital method and part of the THUNDER technology. It eliminates the haze in traditional widefield images by removing the out-of-focus signals without compromising the raw images. In addition, adaptive deconvolution enhances the resolution.

> Razor sharp images by haze removal through THUNDER technology.

> Precise identification of cellular targets.

> Super resolution images for correlation and publication.

PRECISION

Out-of-focus light

Although widefield microscopy is a very sensitive technique and, therefore, very well suited for cryo imaging, an image background can be observed. This is mainly originating from out-of-focus regions, significantly reducing the contrast and the potential signal-to-noise ratio (SNR) of the system. The recorded images show a typical haze and may not provide the level of detail needed for proper targeting of structures of interest.

Computational Clearing removes the blur

To address this, Leica Microsystems developed a family of imaging systems, the THUNDER Imagers, which utilize Computational Clearing as the core technology. Each time an image is acquired, the out-of-focus background is detected and removed making the signal of interest directly accessible. At the same time in the in-focus area, the edges and intensity of the specimen features remain.

THUNDER Imagers offer three modes to choose from:

Instant Computational Clearing (ICC)
Small Volume Computational Clearing (SVCC)
Large Volume Computational Clearing (LVCC)

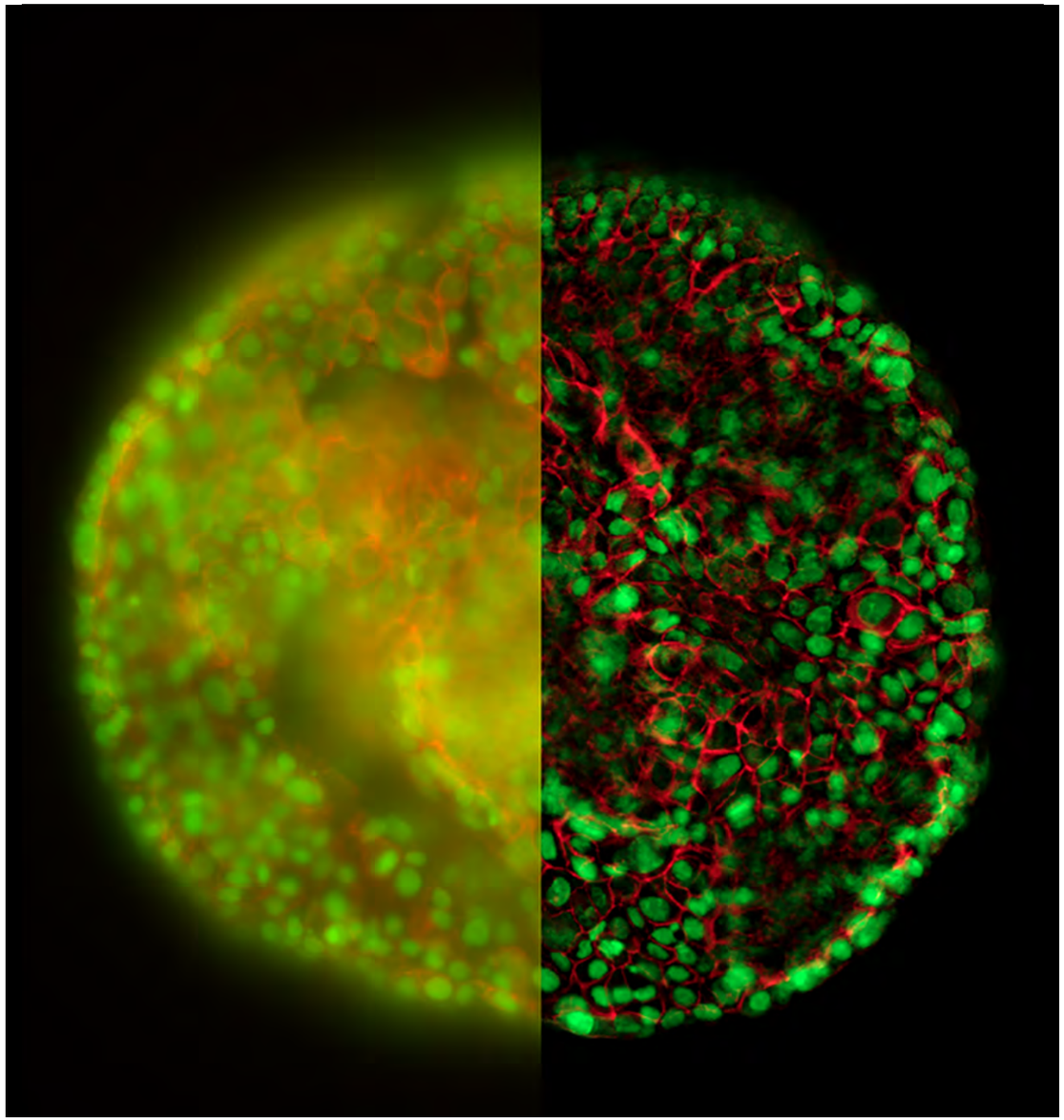
ICC corresponds to computational clearing as described above. SVCC and LVCC are combinations of computational clearing and decision-mask-based 3D deconvolution dedicated to either thin samples (SVCC) or thick samples (LVCC).

Adaptive Deconvolution improves the resolution

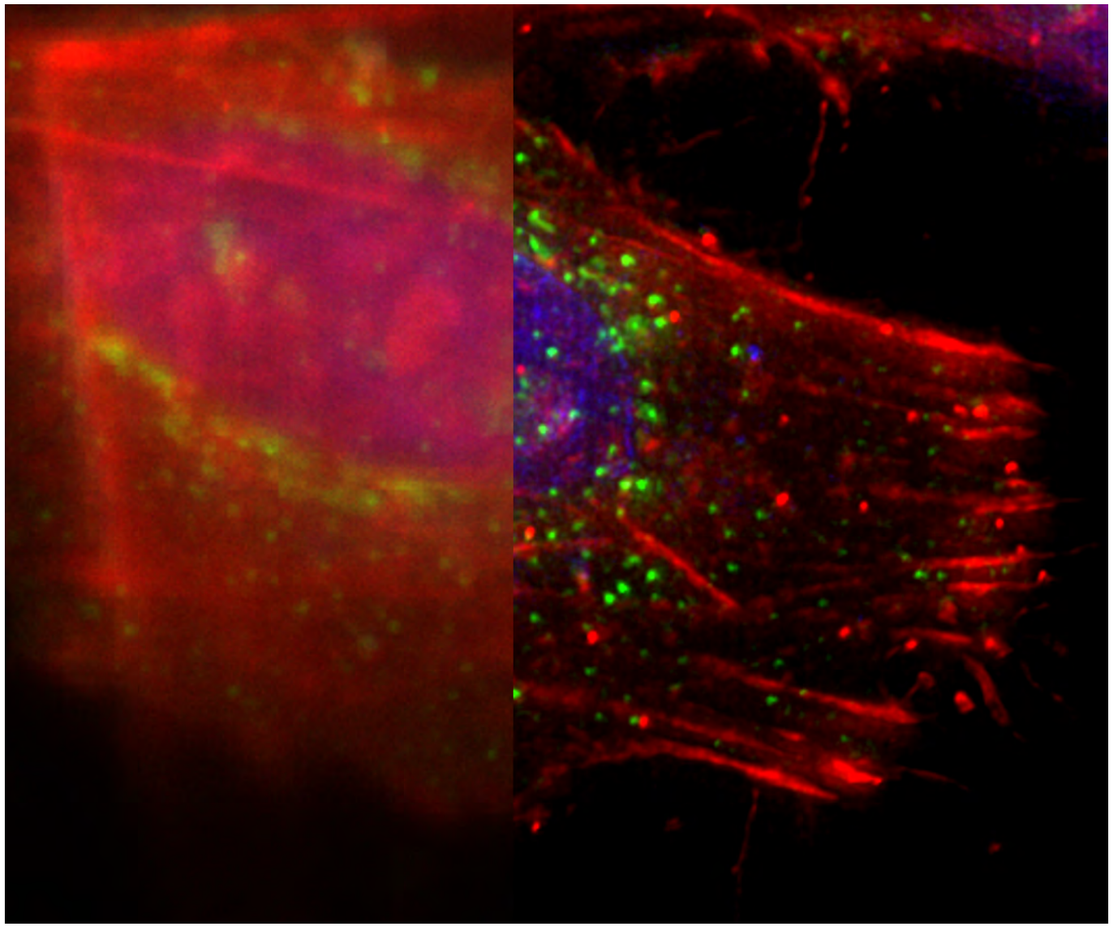
The adaptive image information extraction of the deconvolution methods in THUNDER follows a concept which evolved from LIGHTNING, Leica Microsystems´ adaptive deconvolution method, originally developed for confocal microscopy.

LIGHTNING uses a decision mask as a base reference to calculate an appropriate set of parameters for each voxel of an image series.

Hence, the resolution can be enhanced 2 times laterally and 2.5 times axially improving the potential to identify and target structures of interest. More information can be found here: https://downloads.leica-microsystems.com/Leica%20TCS%20SP8/Publications/LIGHTNING_WhitePaper.pdf).



THUNDER image of a HeLa cell spheroid stained with Alexa Fluor 568 Phalloidin (Actin) and YOYO 1 iodide (Nucleus).



THUNDER under cryo conditions for precise identification
For a precise identification of structures of interest, the widefield-inherent blur has to be removed. THUNDER technology is removing the haze, enabling a precise identification of relevant structures, which otherwise would have been inacessible. THUNDER Cryo CLEM offers:

- Increased resolution and contrast in comparison to standard images.
- Precise identification of target structures for retrieval in the EM.
- Perfect image quality for overlays with EM images.

PRECISION

CRYO CLEM objective for precise targeting

As a basis for proper targeting and subsequent correlation of cryo light and electron images, a very high image quality is needed. To develop an objective for such a requirement, many parameters have to be considered.An objective of the highest apochromatic class performing well under low temperatures needs to be used.

Use of a dry lens must be favored, as immersion liquids are prone to contaminating the sample, so better to avoid them.

The numerical aperture (NA) should be as high as possible for a dry lens.

A flexible working distance for safe sample handling should bae achieved.

To fulfill all these needs, Leica Microsystems has developed the widely used CRYO CLEM objective HC PL APO 50x/0.90 CRYO CLEM.

- Apochromatic objective with excellent color correction for precise imaging.
- Highest possible NA of 0.90 to achieve super-resolution without liquid immersion.
- Working Distance of 280 µm for flexible sample handling.
- The only commercially available cryo objective.
- Tuned for usage together with the Cryo Stage.

Throughout all cryo workflows the samples need to be maintained under cryogenic and dry conditions as soon as the sample is vitrified.

The EM Cryo CLEM set ensures a fast, intuitive sample loading and transfer while maintaining optimal cryo conditions for the specimens at all times.

A separation of loading and imaging ensures maximum reliability, i.e., sample preparation and bake out of the Transfer Shuttle do not affect the imaging.

> Safe sample conditions.

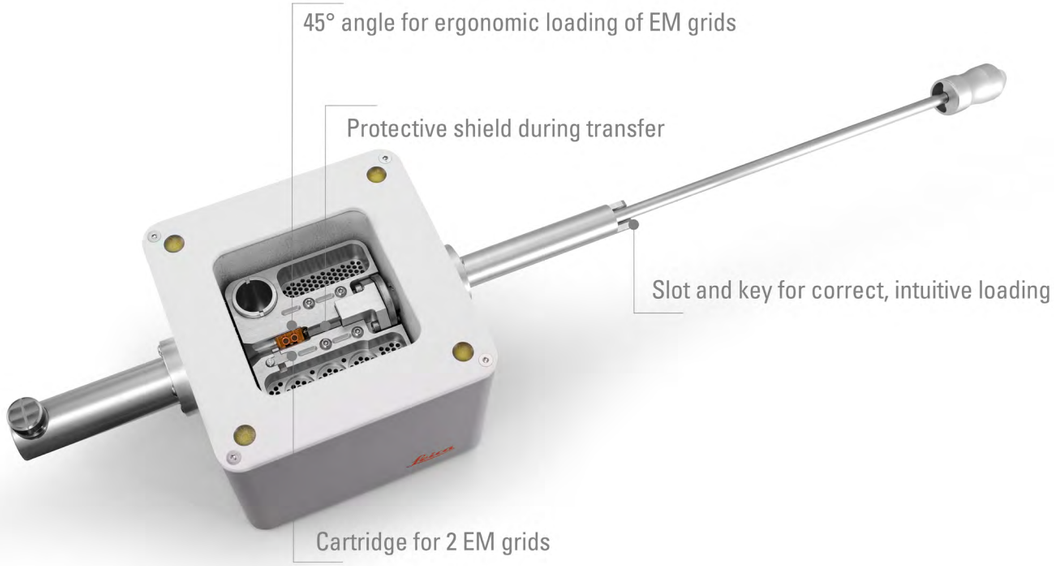
> Intuitive loading under full visual control.

> Straightforward transfer.

> Maximum sample safety with separation of loading and imaging.

PRECISION

THE TRANSFER SHUTTLE

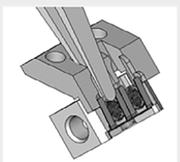


Cartridge

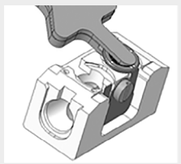
For bare grids

For clipped grids

Intuitive Loading



Loading bare grids with a Loading Aid



Loading of clipped grids

SAFETY

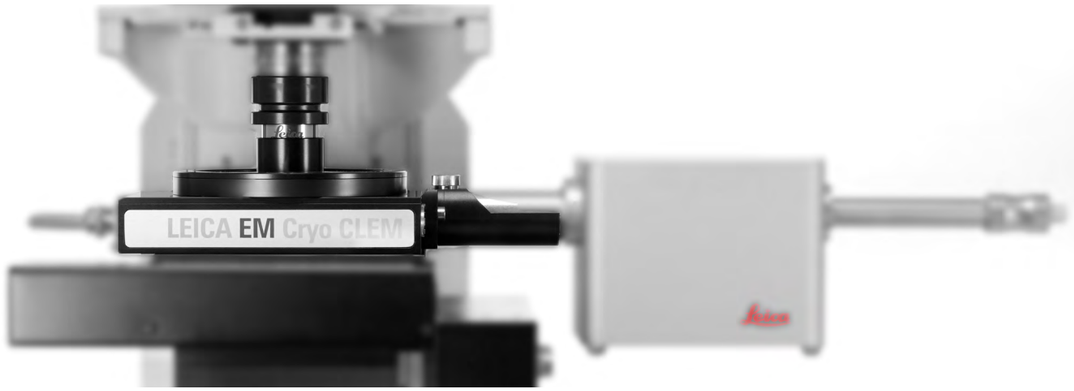
Full visual control while working under gaseous nitrogen

Full visual control

To keep the sample vitrified at all times, it is crucial to work under cryogenic conditions. One strategy is to work under liquid nitrogen. Unfortunately, the visibility of the sample within the nitrogen is poor and contamination by ice flakes taken up on the surface can occur.

Therefore, Leica Microsystems is providing cryo components since more than 30 years which enable researchers to work with samples in gaseous nitrogen. This ability ensures full visual control and reliable handling of the samples reducing the risk of sample contamination or loss.

- Full visual control
- Safe sample handling
- Increased reliability of the workflow

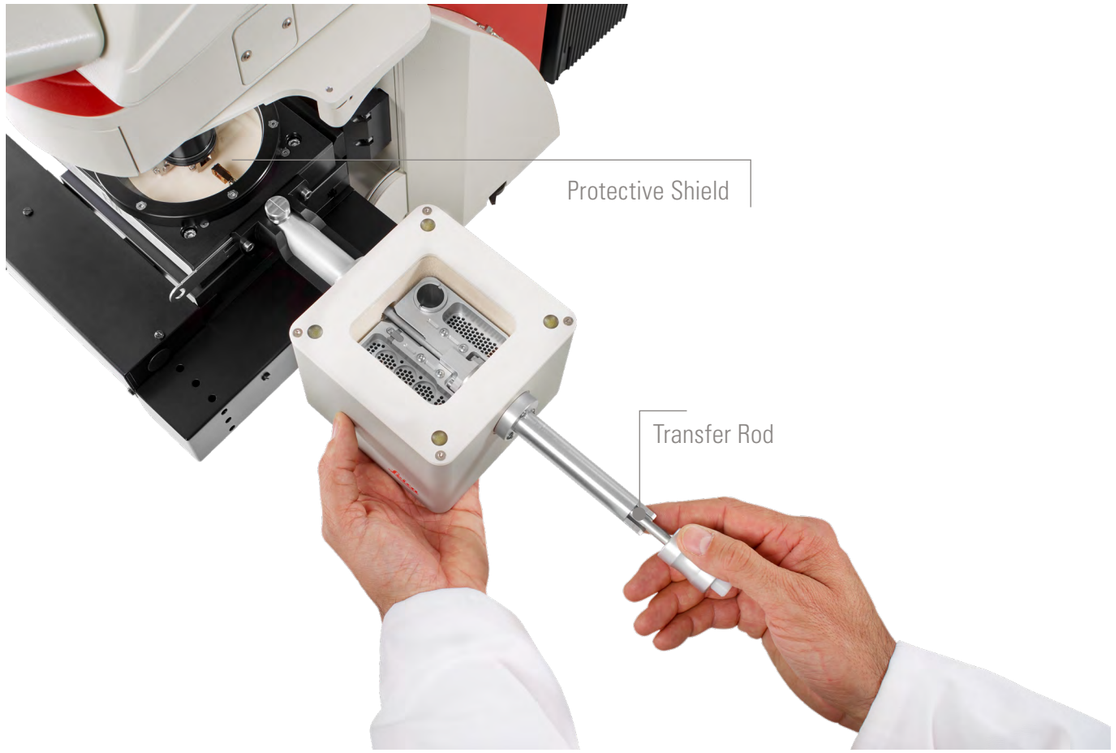


Cryo Stage

The well designed Cryo Stage enables microscopy under cryogenic conditions while allowing xyz movements for 3D imaging.

- Keeps the sample safe during imaging
- There is a constant light positive pressure of gaseous nitrogen against the surrounding atmosphere
- Includes bake-out function

SAFETY



Docking and transfer

The Shuttle-Stage ensemble allows an intuitive docking and transfer of the sample.The cartridges are covered by a shield to protect them during the transfer process. The Transfer Rod with the fixed cartridge is gently pushed into the Cryo Stage.

After release, the cartridge is automatically fixed within the Cryo Stage.

- Intuitive, easy transfer to increase safety and reliability
- Sample holder is covered by protective lid to ensure safe sample conditions
- Cryo Stage and Shuttle are separated to avoid contamination of the imaging chamber in case of sample issues during loading.

RELIABILITY

Cryo Electron Microscopy Workflows consist of many steps. A little mistake at any stage can cause the complete loss of the precious sample, which wastes both time and money. Therefore, the reliability of each step needs to be as high as possible to ensure the success of the complete process. Each step made easier and safer increases the overall success rate of the workflow. Leica Microsystems provides solutions bridging gaps between workflow steps and different systems to increase the reliability of Cryo Electron Tomography workflows in particular as well as Cryo CLEM Workflows in general.

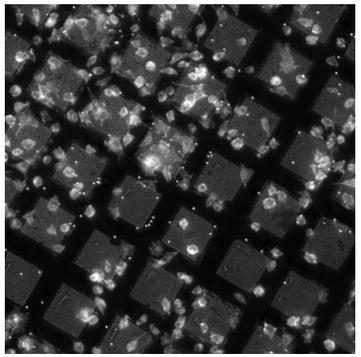
> Improve the reliability of the workflow by improving each step.

> Flexible On-Grid Lamella Workflow for flexible choice of EM workflows.

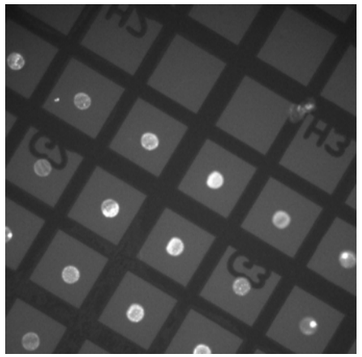
> Integrated On-Grid Lamella Workflow for reliable results.

Cryo FIB lamella - Overlay of SEM and fluorescence image. Target structure in yeast cells (nuclear pore proteine Nup159-Atg8-split Venus, red) marked by an arrow. Scale bar is 5 µm.
Allegretti et al., Nature 586, 796-800 (2020).

ADHESION CONTROL



Irregularly distributed cells (mouse A9 fibroblast cells labeled with Alexa Fluor 488 Phalloidin, visualising F-Actin).



Precisely positioned cells (fibroblasts adhering on fibrinogen micropatterns; alvéole in collaboration with Prof. Dr. Kay Grünewald, CSSB, Hamburg, Germany).

PRIMO Micropatterning for cell preparation

For access to cellular samples for successful FIB Milling and subsequent analysis in the cryo-TEM, it must be ensured, that the cells are located in or close to the center of the grid squares. Unfortunately, cells tend to grow on grid bars and are, therefore , not suitable for analysis with electron transmission. To overcome this challenge, the PRIMO micropatterning system by Alvéole allows users to control the location and spreading of cells on the carbon film, increasing the yield and reliability of the related workflows.

> Precise cell positioning by automatic alignment with the grid mesh

> Optimized cell spreading for reduced sample thickness

> Contactless micropatterning without damage to the carbon layer

alvéole

REPRODUCIBLE PLUNGE FREEZING

Automatic Plunge Freezer EM GP2

One of the first steps in complex cryo workflows is plunge freezing. To ensure reproducible results from the cryo workflow, take advantage of the EM GP2, the next generation Automatic Plunge Freezer from Leica Microsystems. Among the advantages it offers you:

> Automatic sensor-controlled blotting for equal sample distribution

> Temperature and humidity control of the sample until freezing

> Temperature stability of the secondary cryogen

RELIABILITY

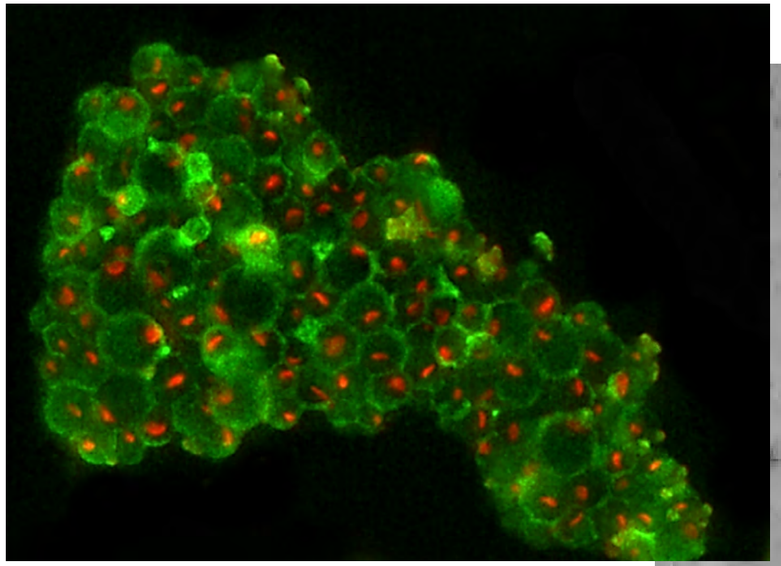
RELIABILITY

Marker Transfer for easy retrieval of target coordinates

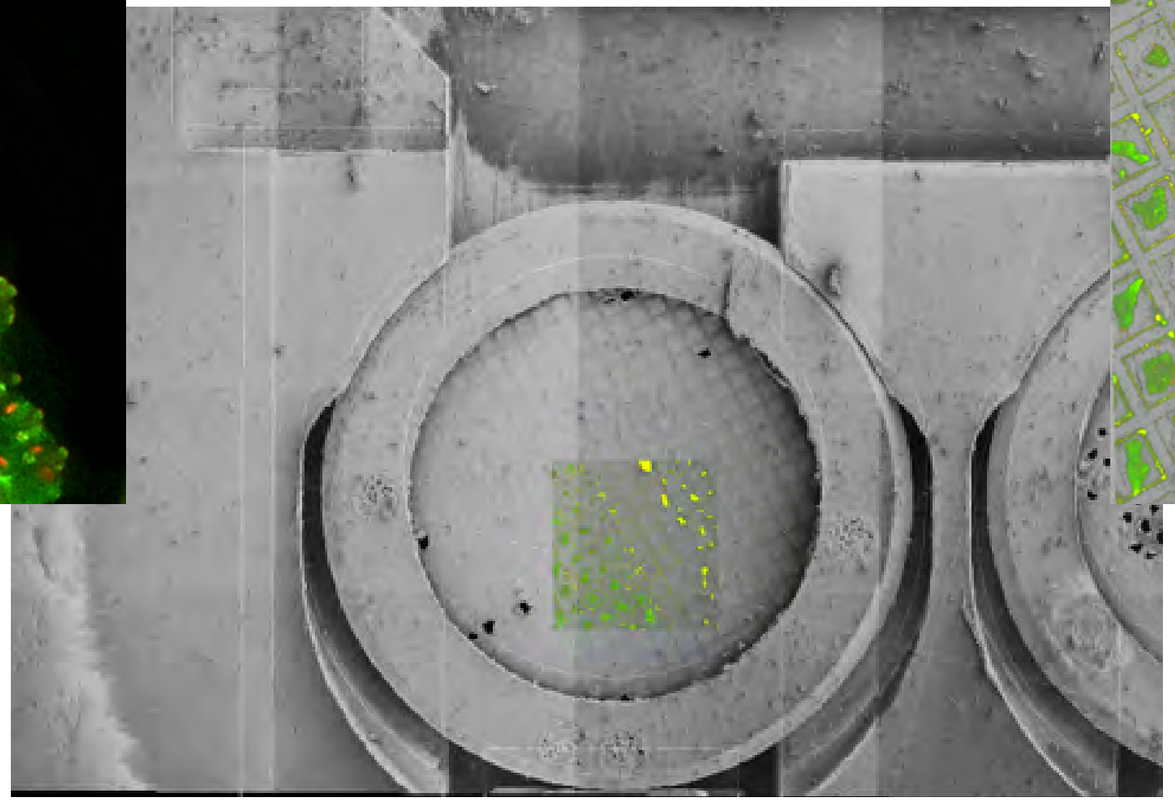
The THUNDER Imager EM Cryo CLEM allows a precise identification of fluorescently labeled target structures. Unfortunately, the fluorescence is not visible under EM conditions. Therefore, it is very helpful to mark the target coordinates on the cryo LM and transfer the coordinates together with the images to the SEM or TEM, respectively.

The software workflow allows users to mark and export the marker files on unprocessed images (outside of the THUNDER workflow). Images and coordinates can be exported automatically towards SerialEM and Thermo Scientific MAPS. An open format for embedding the coordinate retrieval is provided as well.

- Marking of target structures on non-processed images
- Fast and easy retrieval of marker coordinates for EM

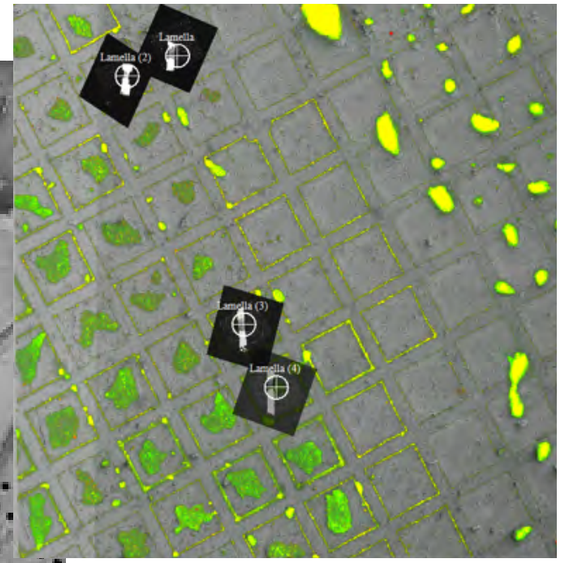


THUNDER Imager EM Cryo CLEM



Cryo-FIB SEM - Overview

Cryo-FIB SEM - Targeted lamella position



RELIABILITY

Cell Adhesion Control

Alvéole PRIMO

Automatic Plunge Freezing

Leica Microsystems
EM GP2

Cryo-LM

Leica Microsystems
THUNDER Imager
EM Cryo CLEM

Cryo-Transfer

Leica Microsystems
EM VCT500
EM VCM

Cryo-Coating

Leica Microsystems
EM ACE600
EM ACE900

Cryo-FIB SEM

TESCAN
JEOL
Zeiss
Thermo Fisher

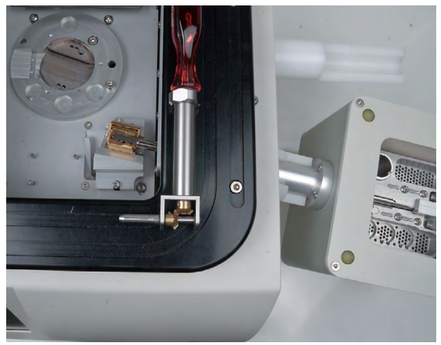
Cryo-TEM

JEOL
HITACHI
Thermo Fisher

Direct docking of Transfer Shuttle

To allow a reliable transfer to holders of different EM systems in a flexible way, the Transfer Shuttle can be directly docked to the EM VCM Vacuum Manipulation Station.

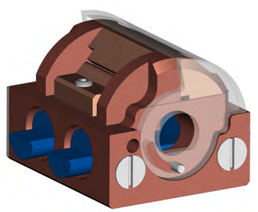
> Cartridges can be directly transfered into the EM VCM - no transfer into the grid box is necessary

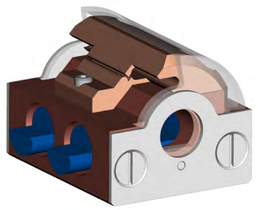


Flexible loading into pre-tilted sample holders for all EM manufacturers

> THUNDER Imager EM Cryo CLEM can be embedded into many different workflows

> EM VCM serves as gateway to many EMs and their respective holders





RELIABILITY

INTEGRATED CRYO TOMOGRAPHY WORKFLOW

Cell Adhesion Control → **Automatic Plunge Freezing** → **Identification** → **Cryo-FIB Milling** → **Cryo-Tomography**

Alvéole PRIMO

Leica Microsystems
EM GP2

Leica Microsystems
THUNDER Imager
EM Cryo CLEM

Thermo Scientific
Aquilos

Thermo Scientific
Krios

Direct sample transfer

As cryo workflows consist of many different steps, unnecessary manipulation and transfer steps must be strictly avoided. Leica Microsystems and Thermo Fisher Scientific have developed a specific cryo-FIB shuttle for the Thermo Scientific Aquilos which can host the Leica autogrid cartridges.

> No manipulation of autogrids between LM and cryo-FIB SEM

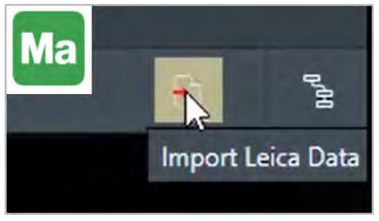
> Reduced risk of sample loss - Increased reliability

Direct marker transfer by one click

To retrieve target coordinates in the EM, the cryo light microscope images have to be imported together with the marker coordinates. Without any communication between both modalities, importing images can be cumbersome. Using the THUNDER Imager EM Cryo CLEM, images and target coordinates are imported into Thermo Scientific MAPS with just one click.

> Import images reliably with the correct coordinates

> Easy-to-use enabling error-free targeting



Screenshot of Thermo Scientific MAPS.
Import Images and Coordinates with one click.

Leica
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