From Eye to Insight

Leica
MICROSYSTEMS

CORAL CRYO
3D TARGETING SOLUTION FOR CRYO ELECTRON TOMOGRAPHY

Access what matters

PRECISION

You can count on

PERFORMANCE

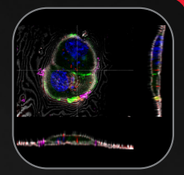
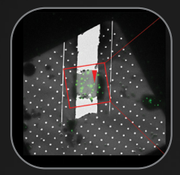
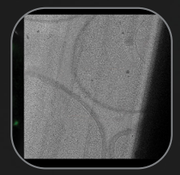
For your imaging

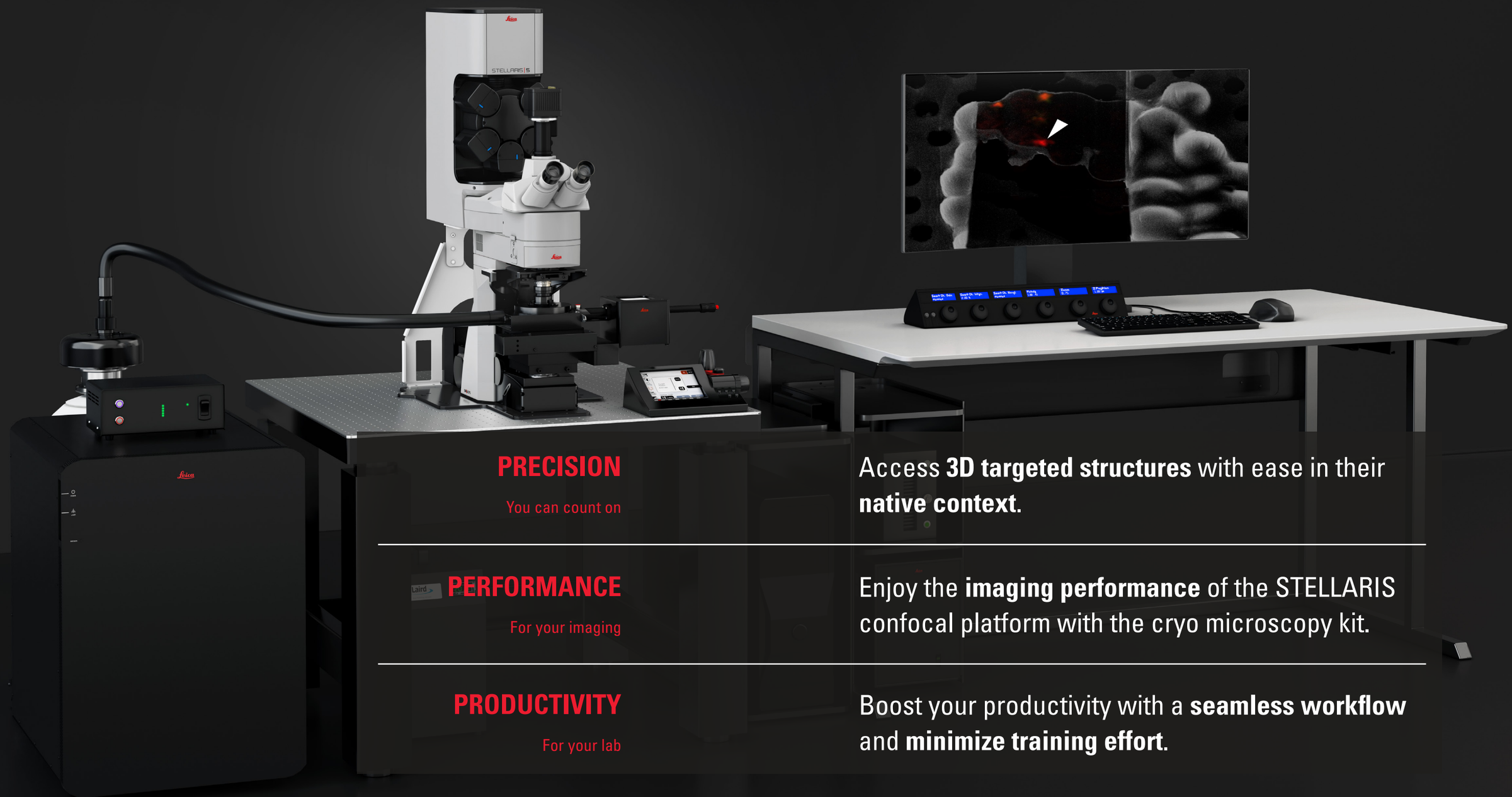
PRODUCTIVITY

For your lab

CORAL CRYO

Flexible solution

Cell Positioning	Plunge Freezing	Cryo Light Microscopy	Targeting	Cryo Coating	Cryo Transfer	Cryo FIB/SEM	Cryo TEM
							
Alveole PRIMO	EM GP2	STELLARIS Cryo	Coral Cryo Software	EM ACE 600	EM VCT500 and EM VCM	Major EM vendors	Major EM vendors



PRECISION

You can count on

Access **3D targeted structures** with ease in their **native context.**

PERFORMANCE

For your imaging

Enjoy the **imaging performance** of the STELLARIS confocal platform with the cryo microscopy kit.

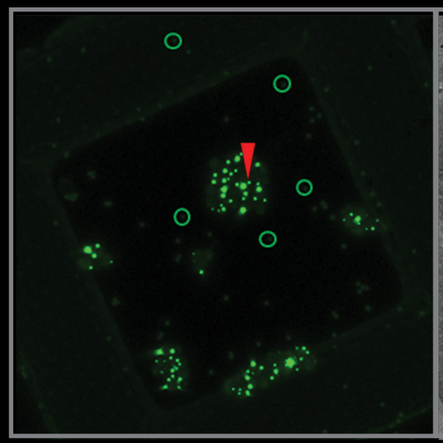
PRODUCTIVITY

For your lab

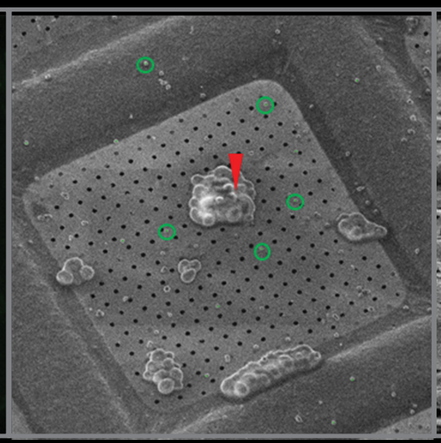
Boost your productivity with a **seamless workflow** and **minimize training effort.**

ACCESS 3D TARGETED STRUCTURES WITH EASE IN THEIR NATIVE CONTEXT

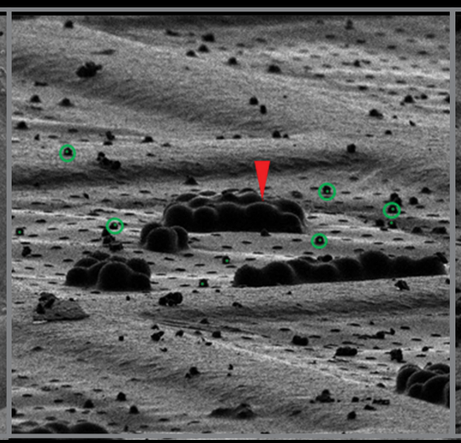
1. 3D Cryo fluorescent imaging



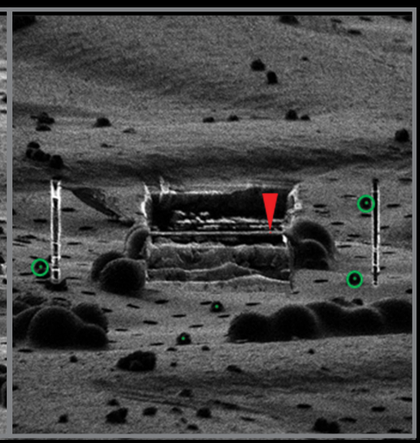
2. Cryo SEM imaging

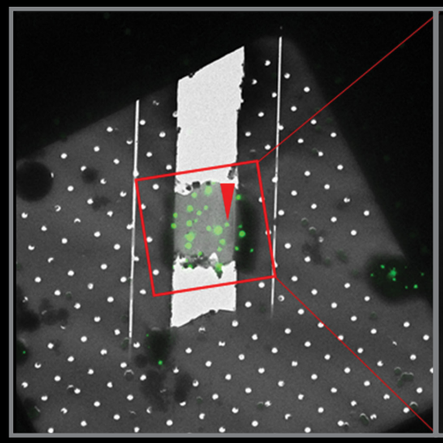


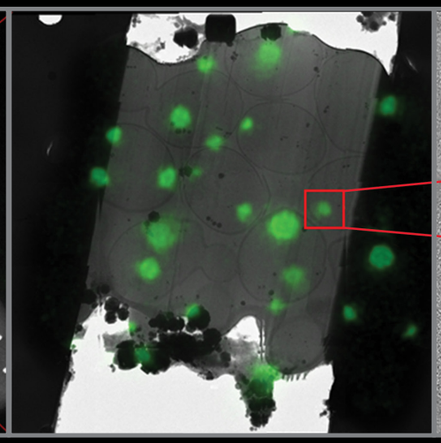
3. Cryo FIB view

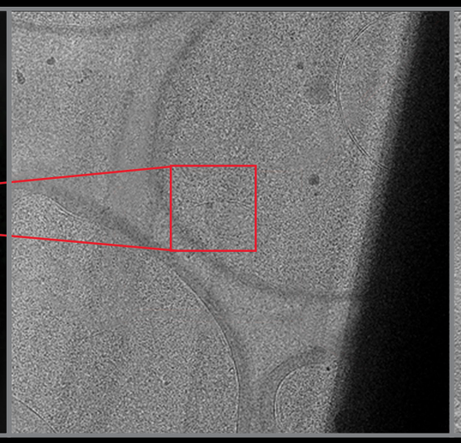


4. Cryo FIB milling









5. Lamella overview

6. FM and EM overlay

7. High resolution cryo TEM

8. Cryo ET

1. 3D Cryo fluorescent imaging:
Using the STELLARIS Cryo, super-resolved 3D fluorescence data of the target structure is recorded. Beads (green circles) serve as coordinated supporting points for retrieving the target structure in 3D for FIB milling. The width of the grid square is 90 µm.

2. Cryo-SEM imaging:
Coordination retrieval for cryo-FIB/SEM is done using a suitable correlation software (e.g., 3DCT or SerialFIB). The beads can be correlated between the two modalities (light microscopy and scanning electron microscopy). Holes: 1 µm, distance between holes 4 µm (this applies also to the other images).

3. Cryo FIB view:
After the targeted coordinates (red arrow) and beads retrieval in the SEM view, they are projected and marked in FIB view before milling.

4. Cryo FIB milling:
The milling windows can be placed above and below the target coordinate to mill a lamella containing the target structure (red arrow points to correlated target).

5. Lamella overview:
The grid with the thin lamella is then inserted into the cryo-TEM

6. FM and EM overlay:
An overlay of the TEM overview image and the LM is produced to better target the region of interest in high resolution cryo-TEM,

7. High resolution cryo TEM:
The highly resolved TEM image reveals the cell boundaries. Length of the insert: 2 µm.

8. Cryo ET:
One image of the tilt series is shown. Highly resolved structures become visible by their different contrasts and can be segmented for the rendering. The tomogram depicting the endocytic protein deposit (green in images 1, 5, and 6), showing that the protein is encapsulated by a membrane (red line).

Sample:
Saccharomyces cervisiae, yeast cells, eGFP-Ede1.
Kindly provided by Anne Bieber and Cristina Capitanio, Max-Planck-Institute for Biochemistry, Martinsried, Germany; STEM by Florian Wilfling

BOOST YOUR PRODUCTIVITY WITH A SEAMLESS WORKFLOW AND MINIMIZE TRAINING EFFORT

Cell Positioning

Alvéole PRIMO

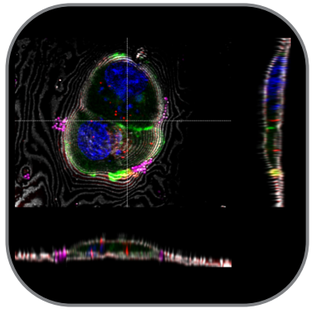
Plunge Freezing

EM GP2

Cryo Light Microscopy

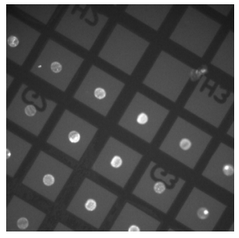
STELLARIS Cryo

Targeting



Coral Cryo Software

ADHESION CONTROL



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

PRIMO Micropatterning for Cell Preparation

The PRIMO micropatterning system by Alvéole allows users to control the location and spreading of cells on the carbon film in contrast to a random cell seeding on a grid. The cells can be located in or close to the center of the grid squares, allowing access for successful FIB Milling and subsequent analysis in the cryo TEM.

- 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
- Increased yield of accessible cells for EM

REPRODUCIBLE PLUNGE FREEZING

Automatic Plunge Freezer EM GP2

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

- Automatic **sensor-controlled blotting** for equal sample distribution
- **Back side blotting** for better cell vitrification
- **Temperature** and **humidity** control of the sample until freezing
- Temperature **stability** of the secondary cryogen
- Controlled ethane temperature to achieve the desired melting point

GUIDED WORKFLOW & 3D TARGETING

3D Targeting with LAS X Coral Cryo Software for Targeted Guided Workflow

Registering the exact **target coordinates** identified in the cryo light microscope for subsequent electron microscope steps without further means is done in the STELLARIS Cryo microscope with **open access coordinates** for greater system interoperability.

The user-friendly **LAS X Coral Cryo software** module featuring innovative interpolation-based 3D targeting allows **precise positioning** of open format coordinate markers in the **nanometer** scale. Different marker types serving different functions are also available for landmark, lamellae and bead marking.

The LAS X Coral Cryo software module offers an easy transfer of experiment parameters.

- Reload complete experiments with one click
- Guided workflow from overview imaging right through to 3D targeting
- Offline mode for image processing and targeting
- Export open access coordinates for greater system interoperability

COUNT ON THE SMOOTH OPERATION OF THE CRYO MICROSCOPY KIT

Cryo Microscopy Kit

STELLARIS Cryo

CRYOGENIC MICROSCOPY

Cryo Stage

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

> Stable gas flow for low stage vibrations

> Constant over-pressure of gaseous nitrogen to avoid any contamination by ambient humidity

> Laser-safe

SUPER RESOLUTION IMAGING

Cryo Objective

> The **only** commercially available cryo objective.

> Apochromatic objective with excellent **color correction** for precise imaging under cryo conditions.

> The HC PLAPO 50x/0.90 CRYO CLEM in combination with LIGHTNING software, provides **super-resolution imaging** without liquid immersion

SAFE TRANSFER

Transfer Shuttle

> Intuitive, easy transfer to increase safety and reliability

> Sample holder is covered by protective lid to ensure safe sample conditions, avoiding ice contamination.

SAFE SAMPLE HANDLING

Cover Box

A cover box hosting the transfer shuttle creates low humidity conditions during sample loading. Thanks to the dry atmosphere, the sample is kept free from ice contamination and therefore lowers the risk of sample loss. Within the cover box, samples are loaded inside the established cryo transfer shuttle under gaseous nitrogen to provide you with full visual control and reliable handling of the samples.

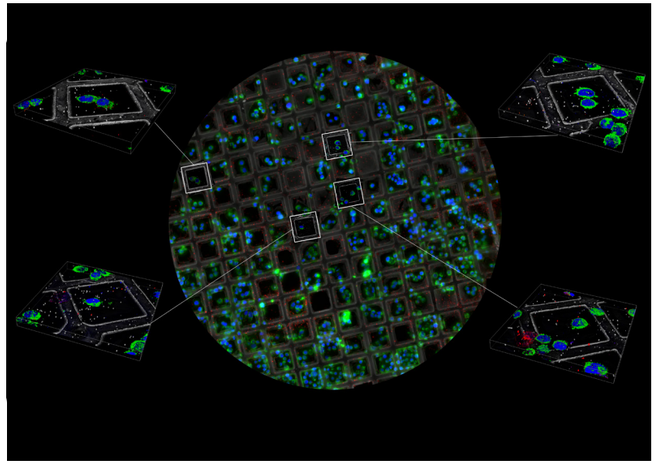
> Full visual control

> Safe sample handling, even in high humidity environments

> Decreased risk of ice contamination

ENJOY THE IMAGING PERFORMANCE OF THE STELLARIS CONFOCAL PLATFORM.

QUALITY & IDENTIFICATION



STELLARIS Cryo is equipped with a fast sCMOS camera to create overview images of the whole grid in less than one minute with full resolution. This helps to check the quality of the sample and identify cells of interest very early in the experimental pipeline. The dedicated cells will then be marked for confocal z-stack acquisition.

- Rapid overview scans for quality checks
- Fast identification of cells of interest for further preparation using cryo FIB
- Confocal z-stacks of cells of interest

Complete camera overview of EM grid recorded with 3 channels. Inserts displaying the positions, where superresolved 3D confocal images were recorded. 3D renderings of these positions are shown in the zoomed inserts. Fluorescence channels (nuclei by Hoechst, blue; mitochondria by MitoTracker Green, green; lipid Droplets by Bodipy and Crimson Beads, red). Width of a grid square is 90 µm, width of a grid bar is 35 µm. Samples kindly provided by Ievgeniia Zagoriy, Mahamid-Group, EMBL Heidelberg, Germany.

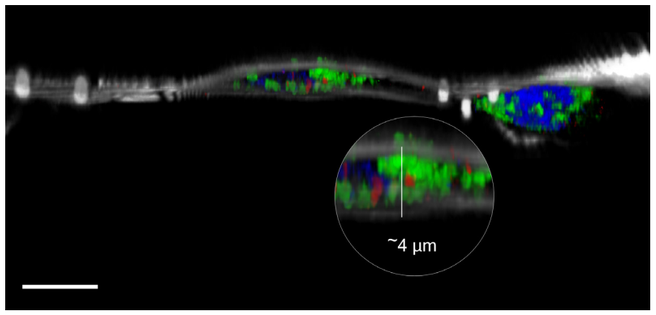
ICE THICKNESS

The STELLARIS Cryo offers two modes for ice checking: a fast camera overview (see above) and a confocal reflection mode to determine the ice thickness in 3D.

The confocal reflection mode allows to analyze the ice thickness in the XZ plane and to calculate its thickness

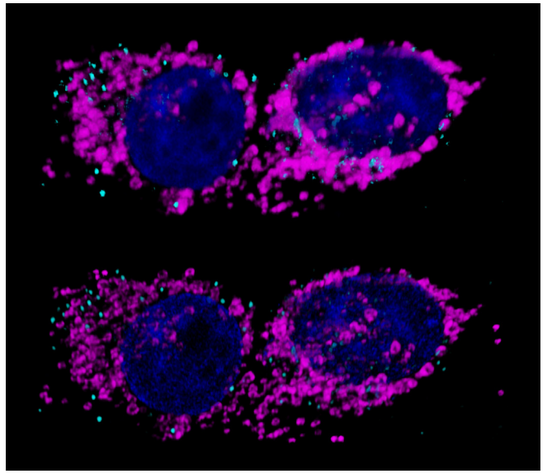
Importance for Coral Cryo workflow:

A thick ice layer can cause issues during milling. If the ice is too thin, this may affect sample stability.



HeLa-cells displaying Hoechst (blue, Nucleus), Mitotracker Green (green, Mitochondria), Bodipy (red, lipid droplets), beads (magenta, 1 µm), Reflection (white). Scale bar 10 µm: Cells kindly provided by Ievgeniia Zagoriy, Mahamid Group, EMBL Heidelberg, Germany

SUPER RESOLUTION

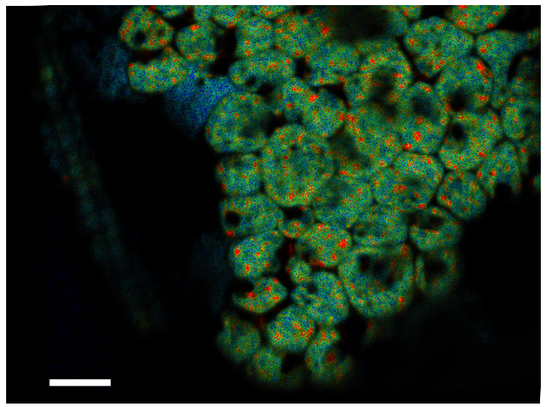


Maximize the information you extract from your vitrified specimen and get in-depth answers to scientific questions with the unique LIGHTNING detection concept, an adaptive extraction process that reveals fine structures and details in the image information that are otherwise simply not visible. This allows you to target structures of interest more precisely for the next steps in your EM microscopy workflow.

HeLa cells labeled with dark blue – Hoechst, Nuclei; magenta – MitoTracker Green, Mitochondria; turquoise - Bodipy, lipid droplets. Cells kindly provided by Ievgeniia Zagoriy, Mahamid Group, EMBL Heidelberg, Germany.

LIFETIME INFORMATION

Within the STELLARIS Cryo, our unique TauSense technology allows you to extract an extra layer of information from every sample and increase the scientific impact of your research. In addition to the spectral color information, the average arrival time of the photons can be visualized. This can be used to reveal hidden components just by the different fluorescent lifetimes TauContrast. Thanks to their different average photon arrival times, TauSense allows you to separate your target fluorescent marker from overlapping fluorophores or autofluorescence. In addition, unwanted reflections can be excluded, caused for example by the reflection of the grid and the carbon layer.



Cells on EM grids, display under TauContrast. Average Arrival times of the photons are displayed in false color. Early arrival times (–0.2 ns) in turquoise, late arrival times (2.4 ns) in red. Red signal: VIPP1-mCherry. VIPP1 is avesicle inducing protein in plastids possibly involved in the assembly of thylakoid membrane core complexes.
Two different components of autofluorescence of the chloroplast can be distinguished (turquoise and yellow in the image). UVMV4 strain provided byKlumpe et al.
(Cell, 2021, 184 (14): 3634-3659). Scale bar: 10 µm.

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