

# A cryo workflow combining light, electron and soft x-ray microscopy provides targeting of unlabeled features

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## Background incl. aims

While Electron Microscopy (EM) reveals extensive subcellular information in exquisite detail the technique has some limitations; lack of 3D cellular context, limited field of view and demanding sample preparation protocols compared to other techniques such as light or x-ray microscopy. To overcome these limitations correlative workflows have been developed that combine light with volume EM techniques such as focused ion beam scanning electron microscopy (FIB-SEM), serial block face scanning electron microscopy or array tomography, thus facilitating the localization of specific regions of interest within an extended sample volume. Nonetheless, these workflows remain largely confined to chemically fixed samples requiring labor intensive workflows, as the contrast from frozen-hydrated samples can be limited, making direct SEM imaging of native samples challenging. Soft x-ray tomography (SXT), on the other hand, is a unique x-ray imaging modality which enables imaging of frozen-hydrated specimens like entire mammalian cells or thick tissue sections with a few tens of nanometers spatial resolution and minimal sample preparation. The recent development of a laboratory scale SXT microscope opens the possibility of integrating this novel technique into light and electron imaging workflows. The SXT microscope features an integrated light microscope for overview imaging and fluorescence targeting, allows for swift acquisition of 2D and 3D images covering extensive areas on the specimen, and enables efficient and rapid identification of cells of interest. The (x,y,z) feature location can be recorded and the specimen passed to a cryo FIB-SEM for lamella extraction and subsequent cryo-ET imaging at ultra-high resolution.

## Methods

Cryo SXT was used to identify target regions of interest within whole, frozen hydrated cells. Soft x-rays from 284 to 543 eV (2.34 to 4.4 nm) allow SXT to retrieve quantitative x-ray absorption information of protein content in biological cells with high native contrast. The resulting 3D datasets were imported to the correlative module in the TESCAN AMBER cryo FIB-SEM and used as a reference for targeted extraction of cryo lamella from the specimen.

## Results

Resulting cryo-ET tomograms provide proof of concept for presented workflow.

## Conclusion.

This workflow of correlative light, electron and soft x-ray microscopy (CLEXM) combines the strengths of both SXT and EM while also avoids any adverse effect of chemicals used for fixation in traditional EM methods, and therefore is particularly suited for studying rare events or features which cannot be labelled with fluorescent tags. We will discuss recent progress in this novel workflow development.

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CLEM, Cryo-FIB, Cryo-ET, SXT, CLEXM