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Integrative Cryo-Correlative Microscopy Approaches

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In its broadest sense, correlative microscopy is a term used to describe the visualization of microscopic features using distinct, complementary imaging modalities. Direct correlation uses an instrument equipped with two or more imaging devices and/or detectors. Examples of this approach include most microspectroscopy techniques that allow one to superimpose maps of elemental composition over topographical images. Indirect correlation, in contrast, uses one instrument to record the coordinates of a feature, and other instruments with distinct imaging principles to modify and record complementary information from the same coordinates. The coordinates of a fluorescently-labelled target structure recorded by correlative light microscopy provide a convenient and efficient means for subsequent recording of nanometre-scale images using the electron microscope [1, 2]. Thus cryo-fluorescence microscopy can be exploited to navigate the cellular landscapes for features of interest before zooming in on these areas by cryo-electron tomography (cryo-ET).

In combination with cryogenic focused ion beam (FIB) milling [3], cryo-correlative microscopy is instrumental in expediting searching and targeting appropriate milling sites [4]. While the FIB technique allows site-selective removal of material with very high precision, targeting is typically done by cryo-scanning electron microscopy (SEM), which is essentially limited to the study of surface structures. Hence it is difficult to accurately localize underlying cellular features, which are hidden under a thick layer of amorphous ice. Here the fluorescent signal, ideally based on clonable labels such as green fluorescent protein (GFP), offers an independent and unambiguous confirmation of the identity of a feature of interest and at the same time facilitates the search for low-copy-number features. A conversion of the spatial coordinates across the different imaging modalities ensures relocalization for thinning by cryo-FIB and subsequent analysis by cryo-ET.

In the aforementioned cryo-workflow still a number of issues remain to be resolved which are mainly associated with handling and manipulation of frozen-hydrated specimens. Some of these issues include the prevention of mechanical damage and avoidance of ice (frost) contamination during transfers. In addition radiation damage exhibits an ultimate limit to the amount of structural information that can be extracted from a frozen hydrated specimen. Therefore it is critical to minimize the cumulative electron dose in SEM imaging as well as in cryo-ET. Correlative light/electron microscopy approaches at cryogenic temperatures provide the advantages described thus far as well as compatibility with the low-dose imaging requirements of cryo-EM.

However, to identify suitable structures for ‘thinning’ vitreously frozen biological samples, to distinguish different cell types or to even target subcellular structures, cryo-fluorescence microscopy is the only available method for a fast and supervised preselection. Here we will

report on our recent findings regarding the integration of correlative approaches for cellular cryo-ET.

References
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[5] This research was supported by the European Commission's 7th Framework Programme (grant agreement HEALTH-F4-2008-201648/PROSPECTS) and from the Federal Ministry of Education and Research (BMBF).

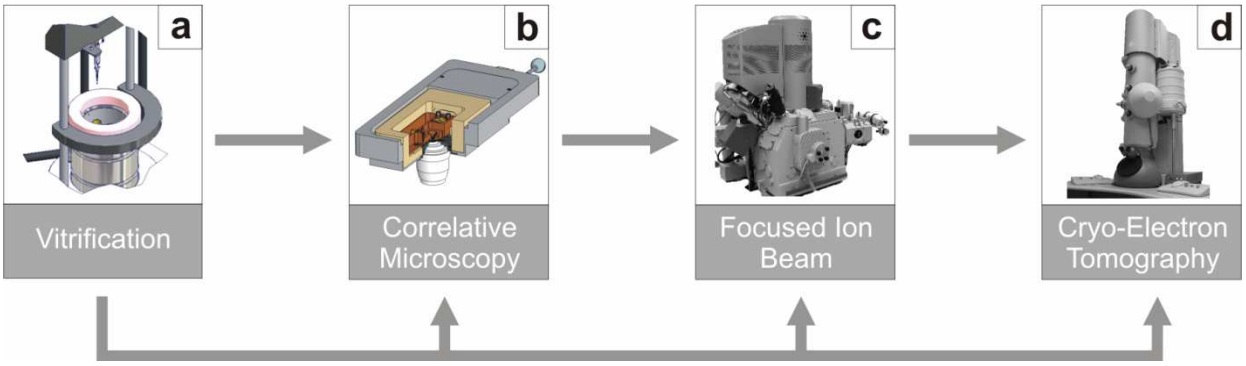

FIG. 1: Workflow for imaging cryogenic specimens by cryo-ET.

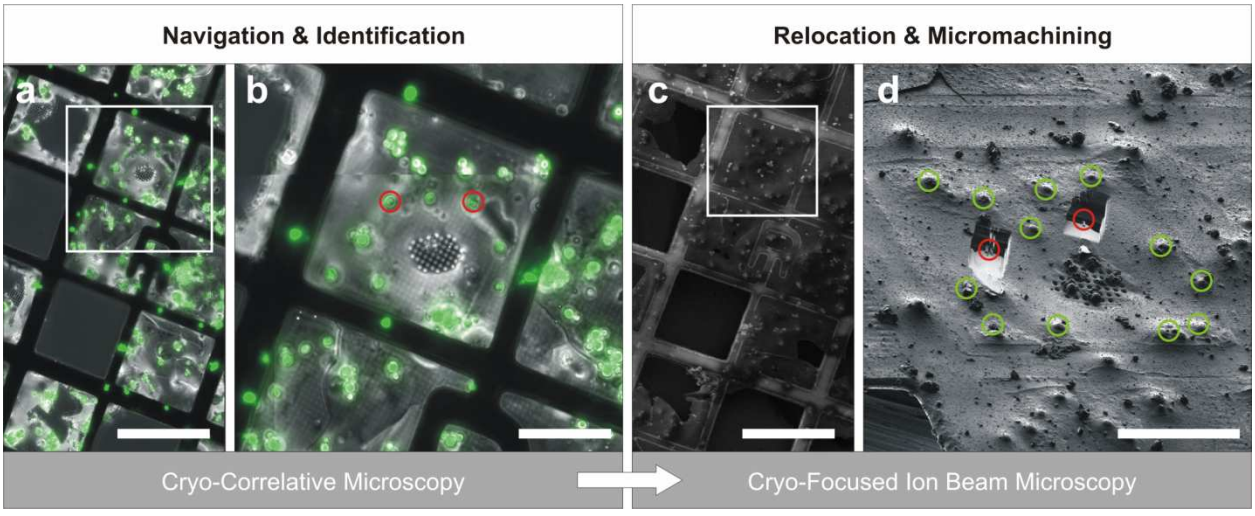

FIG. 2: Targeting areas of interest for focused ion beam milling. (**a** and **b**) Merged cryo-phase contrast and cryo-fluorescence image of prion-infected yeast cells (GFP). Red circles indicate the sites selected for FIB milling. (**b**) Cryo-scanning electron micrograph of the same area where the milled areas (corresponding to the red circles) can be recognized easily. The green circles correspond to the yeast cells found within the mesh in **b**. Scale bars: (**a**, **c**) 100 µm, (**b, d**) 40 µm.

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