Sample preparation for correlative light, soft X-ray tomography, and cryo FIB-SEM imaging of biological cells

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In this collaborative endeavor between SiriusXT and King's College London (KCL), we present our efforts to refine high-pressure freezing (HPF) protocols tailored to facilitate correlative imaging workflows integrating cryo-fluorescence microscopy, lab-based soft X-ray cryo-tomography (SXT), and cryoFIB-SEM for high-resolution three-dimensional (3D) imaging of biological cells. This refined protocol is followed by correlative light fluorescence, SXT, and FIB-SEM studies of biological cells, aiming to compare sample quality vitrified by plunge freezing and high-pressure freezing. Our motivation stems from the persistent uncertainty surrounding the efficacy of plunge freezing in adequately vitrifying thicker cellular components for high-resolution imaging purposes.

The primary objective of our study is to develop strategies to regulate ice thickness in high-pressure frozen samples, making them suitable for correlative imaging by light, SXT, and cryo-FIB-SEM techniques. Subsequently, we employ correlative imaging to investigate regions of interest, initially utilizing light fluorescence and soft X-ray tomography, followed by identification of regions of interest and further imaging of these regions using cryo FIB-SEM. We also compare the quality of frozen samples between high-pressure and plunge-frozen specimens. For our study we use two distinct biological organisms: the nannochloropsis microalgae and single-cell flagellate eukaryotes of the Euglena genus.

Optimal ice thickness for direct imaging by SXT without the need to thin the sample typically falls below fifteen micrometers, a threshold often exceeded by the conventional HPF "waffle" method, resulting in ice thicknesses around 20-25 micrometers. However, through modifications to the freezing procedure - such as liquid wicking and removal of planchettes spacer - we demonstrate the achievement of HPF ice thickness as low as 5-15 micrometers across significant fractions of the grid area.

This improvement eliminates the need to thin high-pressure frozen samples for SXT imaging. Using the lab-based SXT-100 for rapid 3D imaging of large areas with resolutions of 50-60 nm full-pitch, this approach enhances the throughput of cryo FIB-SEM imaging of high-pressure frozen samples by streamlining the process of identifying regions of interest for higher resolution imaging, albeit at a significantly slower imaging pace by cryo FIB-SEM.

Keywords:

SXT, cryoFIB-SEM, correlative microscopy, tomography

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