New and Notable

Further Closing the Resolution Gap: Integrating Cryo-Soft X-Ray and Light Microscopies

Dorit Hanein[1,*]

[1]Bioinformatics and Structural Biology Program, Sanford-Burnham Medical Research Institute, La Jolla, California

Structural biologists are increasingly focused on the integration of micro-, meso-, and macroscale information to gain a comprehensive mechanistic understanding of dynamic biological processes. The last few years have witnessed a veritable quantum leap in our ability to perform imaging experiments on all resolution scales, including super-resolution light microscopies, a new generation of electron transmission microscopes, novel direct electron detectors, powerful scanning electron microscopes, and newly emerging soft x-ray microscopes. These developments, together with the evolution of correlative methods for integrating information across all relevant resolution scales, provide new opportunities for linking dynamic biological processes to their underlying structural framework in situ.

Having these image acquisition toolboxes in place, one of the key challenges is how to quantitatively link the macroscopic cellular outputs investigated to the high-resolution structural information derived in the same window of time and space. The reliability of such correlations rely on three-dimensional (3D) imaging and their respective image analysis methods, such as electron tomography (ET) for cellular imaging via electron microscopy, 3D scanning electron microscopy techniques such as array tomography (3D-SEM) and 3D soft x-ray tomography (SXT) (1). These high-resolution imaging modalities need to be complemented by cryo-imaging capabilities (i.e., cryo-ET, cryo-3D-SEM, and cryo-SXT) to enable the examination of fully hydrated samples.

Of the 3D cryo-imaging techniques providing correlative information of assemblies fully hydrated in cellular contexts, correlative light and ET (cryo-CLEM) is the most mature (2–4). In this approach, proteins of interest are fused to fluorescent protein to allow recording of functional history and spatial positioning by fluorescence light microscopy. Subsequently, the samples are imaged by cryo-ET and the fluorescence image is superimposed with the cryo-ET reconstruction to spatially localize, albeit coarsely, the macromolecular assemblies of interest. A particularly exciting example is the recently reported correlated cryo-ET with cryo, super-resolution light microscopy (cryo-Palm), which provides the means to effectively expand the spatial registration to a range of a few hundred nanometers (5). The derived cryo-ET reconstructions, though not isotropic due to tilting restrictions to $\pm 70°$, provide maps of the correlated assemblies within the 2–4 nm resolution range. In silico segmentation and merging of molecular structures obtained by crystallography and NMR with the cryo-ET maps (6) represent an enormously insightful integration of information from the atomic (i.e., Angstroms) to molecular (i.e., tens of nanometers) ranges, and is coarsely tied to the dynamic biological process via light microscopy.

The next imaging technique to employ correlative imaging is cryo-SXT, which uses diffractive optics to image a specimen in the x-ray water window (7,8). The samples remain hydrated, albeit due to the sample thickness (10–15 μm) the aqueous milieu surrounding the sample contains not amorphous rather crystalline water. At the resolution range of the SXT technique (~60 nm), which is currently an order of magnitude lower than EM-based imaging of cells, the possible effect of the cryogenic environment damage cannot be visualized or determined. Nevertheless, cryo-SXT offers clear advantages, as samples as thick as 15 μm can be imaged, and identification of molecular/cellular components can be performed quantitatively through segmentation of particles/organelles according to their unique linear absorption coefficients (9). Although cryo-SXT is relatively new, it has been applied successfully to a range of biological samples ranging from individual vaccinia virus particles, yeast, and mammalian cells (10).

The use of cryo-SXT was significantly enhanced by employing, similar to CLEM, fluorescence microscopy (cryo-CLXM) techniques (11), through localization of organelle-resident fluorescent proteins before freezing and imaging with cryo-SXT. In this issue of the *Biophysical Journal*, Smith, Le Gros, and Larabell (12) advanced this technology one step further through the implementation of a cryogenic light microscope. Cryogenic optical-microscope systems are available; with the most advanced using a color-aberration corrected air-dried objective lens with a numerical aperture of 0.9 (13). Here, the authors implement a cryogenic immersion lens instead, which represents a significant enhancement over cryogenic light microscopy using air lenses. The use of high-numerical aperture objective lenses minimizes mismatch in refractive indices, an effect that commonly degrades both the maximum spatial resolution and fidelity of the projection image. Furthermore, Smith et al. report a custom-built cryo-rotation stage allowing isotropic resolution for cryo-confocal light microscopy, as well as cryo-SXT. Finally, the use of fiducial markers, visible in both the linear model and soft x-ray microscope data sets, enable accurate registration of the two complementary imaging modalities. Employing this correlative imaging technology, Smith et al. suggest

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*Correspondence: dorit@bumham.org

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that X-chromosome inactivation is not accompanied by distinct or global spatial organization of the nucleus, or chromosome patterns, rather by unique cellular changes.

A complete molecular understanding of complex biological processes can only be realized with models that incorporate structural information on all biologically relevant length scales. New hardware and technological developments, such as those described by Smith et al., are bringing this aim closer to reality by further bridging the resolution abyss. Many more exciting correlates lie ahead that would allow tying seamlessly the atomic, molecular, and cellular resolution ranges.

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