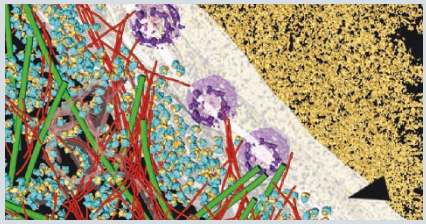
Cryo-electron tomography

Cryo-electron tomography may facilitate *in situ* structural biology on a proteomic scale.

Single-particle cryo-electron microscopy (cryo-EM) has gotten a lot of attention for recent technical advances that enable it to help solve macromolecular structures at near-atomic resolution, an achievement we celebrated as the Method of the Year 2015. These methodological developments are also benefitting cellular ultrastructure imaging using cryo-electron tomography (cryo-ET).

In a cryo-ET study, a biological sample—a cell, tissue, or organism—is flash frozen, thinned to an appropriate thickness, and then imaged using an electron microscope. The freezing process preserves the sample in a hydrated, close-to-native state. Multiple images are captured as the sample is tilted along an axis. The images are then aligned and merged using computational techniques to reconstruct a three-dimensional picture, or tomogram.



A cryo-ET view of the nuclear periphery of a HeLa cell. From Mahamid, J. *et al. Science* **351**, 969–972 (2016). Reprinted with permission from AAAS.

With its capability to obtain nanometer-scale information about macromolecular complexes in their native cellular environment, cryo-ET provides a bridge between light microscopy and *in vitro* structure determination methods. This is important because many complexes cannot be purified, and knowing both the structure and location of macromolecular complexes is crucial for understanding cellular function.

Biological material is very sensitive to radiation damage by an electron beam, however, so researchers have had to devise many ways to improve resolution without destroying samples. Recent hardware developments vital for enhancing the resolution of single-particle cryo-EM, including direct electron detectors and novel phase plates, are also improving cryo-ET imaging. Computational tricks originally developed for cryo-EM, such as beam-induced motion correction algorithms and subtomogram averaging, also help sharpen structural features visualized by cryo-ET. These developments synergize with advances in cryo-ET sample preparation, most notably the application of focused ion beam (FIB) milling to thin samples to ideal thicknesses for imaging.

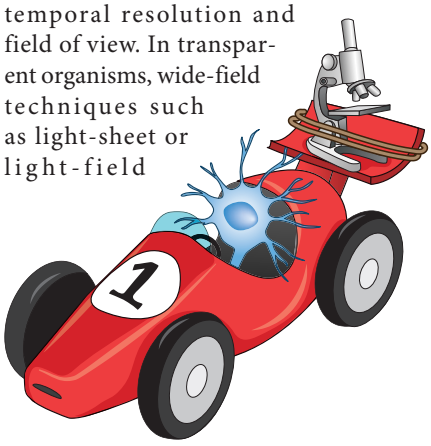
In principle, cryo-ET can image the entire proteome of a cell, but new data analysis methods are needed to parse such dense pictures. In one promising approach, called template matching, a template structure is used to locate matching structures in the tomogram. While this method has been successful for mapping the locations of relatively large structures such as ribosomes, sensitivity and accuracy improvements are needed for its broader applicability. We look forward to the development of new approaches that will enable *in situ* structural biology on a proteomic scale.

Allison Doerr

Faster brain imaging

Higher volumetric imaging rates shed light on the dynamics in neuronal networks.

Calcium- or voltage-sensitive probes have made it possible to watch neurons in action. But observing larger neural networks together at the same time is challenging, as many relevant imaging techniques are afflicted by a trade-off between temporal resolution and field of view. In transparent organisms, wide-field techniques such as light-sheet or light-field microscopy can resolve this conundrum. But the situation is more challenging in the light-scattering rodent brain, where imaging neuronal activity with conventional approaches such as two-photon scanning is slow.



Marina Corral Spence/Springer Nature

Speeding up the imaging of neural activity.

Several strategies are available to increase the number of imaged neurons or the temporal resolution, with each strategy having its own advantages and drawbacks. The most intuitive option is to simply increase the scan speed, but this comes at the cost of shorter pixel dwell times, thereby requiring brighter probes. Alternatively, if the neurons of interest are sparsely distributed, they may be selectively imaged by random-access microscopy, which avoids scanning 'empty' areas. Tissue movement can compromise the signals, but this problem can be overcome with a random-access scanning approach (*Nat. Methods* **13**, 1001–1004, 2016; *Neuron* **92**, 1–16, 2016).

Another possible means for increasing the imaging speed is multiplexing: i.e., using multiple imaging beams, which may be temporally interleaved or spatially separated. In sparsely labeled brain regions, multiple layers can be scanned together (by splitting the beam with a spatial light modulator) while the emitted light from the different layers is collected together, and later the signals can be separated computationally (*Neuron* **89**, 269–284, 2016; *Neuron* **89**, 285–299, 2016).

Finally, reducing the number of voxels per volume speeds the imaging process up as well. Instead of scanning a diffraction-limited spot across the sample, the voxel size can be adapted to approach the size of a neuronal cell body, which substantially increases the imaging speed (*Nat. Methods* **13**, 1021–1028, 2016). Alternatively, the axial extent of a voxel may be increased, as in extended-depth-of-field imaging.

While the described general strategies have been around for a while, the recent developments in microscope hardware and the combination of these strategies with advanced computational approaches have certainly moved the field forward. It will be interesting to watch how the boundaries in volumetric imaging speeds are further stretched when the different strategies are combined.

Nina Vogt