New technologies in imaging

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Visualization of cellular and molecular processes is an indispensable tool for cell biologists, and innovations in microscopy methods unfailingly lead to new biological discoveries. Today, light microscopy (LM) provides ever-higher spatial and temporal resolution and visualization of biological process over enormous ranges. Electron microscopy (EM) is moving into the atomic resolution regime and allowing cellular analyses that are more physiological and sophisticated in scope. Importantly, much is being gained by combining multiple approaches, (e.g., LM and EM) to take advantage of their complementary strengths. The advent of high-throughput microscopies has led to a common need for sophisticated computational methods to quantitatively analyze huge amounts of data and translate images into new biological insights.

In vivo imaging requires carefully balancing conflicting parameters to achieve high imaging speed, low photobleaching and phototoxicity, good three-dimensional resolution, high signal-to-noise ratio, and excellent physical coverage. Light-sheet microscopy provides outstanding performance in all of these categories and has become a key imaging method for the life sciences. **Philipp Keller** (Janelia Farm) showed how entire *Drosophila* embryos can be imaged throughout embryogenesis at a spatiotemporal resolution that enables comprehensive cell tracking. The use of automated approaches for computational image analysis enables systematical reconstruction of cell lineage information from such recordings in real time.

Progress in the light-sheet microscopy field is faster than ever, and further improvements in temporal and spatial resolution are expected in the near future. These capabilities will directly synergize with the rapid progress in related fields, such as the development of advanced fluorescent reporter strategies, powerful approaches to high-throughput data analysis, and computational tools for biophysical modeling, opening up exciting new opportunities for microscopy-based research in the life sciences.

Superresolution imaging is enabling the visualization of intracellular relationships unobtainable using traditional fluorescent microscopy. However, different superresolution techniques are based on distinct physical principles to break conventional light microscopy limitations, and these principles determine the apparent size of the biological structure being imaged. The 25-nm-microtubule diameter appears to be between 30 and 120 nm, depending on the technique used. These specific principles also define the acquisition speed of each method, resulting in a family of techniques, with each technique optimized for both size (e.g., organelles vs. transmembrane receptors) and dynamics (e.g., slow transport vs. diffusion). Recent advances that combine multiple approaches to address a biological question show great promise for overcoming these limitations. **Catherine Galbraith** (National Institutes of Health) showed how live-cell superresolution imaging of single molecules can be combined with computer-vision tracking and conventional microscopy. This integration provides a dense functional dynamics map that can be used to correlate the behavior of multiple proteins to that of the entire cell, while visualizing biochemistry at the single-molecule level. Because biology is the complex integration of multiple systems, the combination of multiple imaging approaches presents the greatest opportunity for making meaningful discoveries.

Cellular transmission EM has traditionally suffered from the need to section the sample. Sectioning of resin-embedded material is relatively easy but can suffer from poor sample preservation, while cryosectioning of frozen-hydrated samples is inefficient and hampered by cutting and compression artifacts. A recent breakthrough is the use of focus ion beam (FIB) methodology to carve out cellular slices, thus opening a window into deep regions of the eukaryotic cell without displaying the shortcomings of previous methods.

Although EM methodologies cannot provide live imaging, they can produce snapshots that allow inference of biological transitions. The resolution and number of snapshots have been increasing with the high-throughput automated data collection and analysis. **Eva Nogales** (University of California–Berkeley, Howard Hughes Medical Institute, and Lawrence Berkeley National Laboratory) reported the structure of GMPCPP and GDP microtubules at 5-Å resolution, showing how hydrolysis of GTP results in an accordion-like deformation of protofilaments that is overcome by the presence of Taxol.

New developments (silicon-based cameras for direct electron detection, several phase-plate implementations for in-focus EM imaging, and innovative image analysis algorithms) will extend the scope of molecular and cellular EM studies, opening the door for higher-quality structures and quantitative conformational descriptions.

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