New and Notable

Cellular Diffraction: Scanning X-Ray Nanodiffraction from Living Cells

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Cellular diffraction, or scanning x-ray nanodiffraction applied to cells, is a new technique that promises insight into cellular organization at length scales of 1–200 nm, covering the features of molecules and organelles. Filling a niche between the length scales probed by optical and electron microscopy, cellular diffraction rasters a nanofocused x-ray beam across living cells in an aqueous medium and records a two-dimensional image of, for instance, the small-angle scattering signal at each position. This results in a real-space image of the cell where the contrast is based on the average intensity of the small-angle x-ray scattering (SAXS) signal (dark-field image), or phase contrast, or other modalities such as the x-ray fluorescence intensity, with spatial resolution determined by the step-size of the raster scan ranging from submicron to a few microns. But the individual diffraction patterns can also be analyzed, providing higher-resolution limited by the signal/noise of the scattering pattern, currently ~6 nm. With the cells bound to a solid support, the SAXS patterns do not result from isotropic averaging of the scatterers, and no staining or labeling is required for the technique.

The development of cellular diffraction is spearheaded by two groups from the Institute for X-Ray Physics at the University of Göttingen, led by Sarah Köster and Tim Salditt, who exploit recent advances in x-ray detectors, x-ray optics, and microfluidics in order to apply scanning SAXS imaging (1) to hydrated, living cells and address subcellular dimensions. In a collaborative first effort at beamline ID13 of the European Synchrotron Radiation Facility, they imaged freeze-dried epithelial cells and saw distinctive scattering from the network of keratin fibers in the cell (2). More recent work using a specially developed instrument and beamline P10 of PETRA III imaged both fixed and live cells. The Köster team, together with collaborators from the European Synchrotron Radiation Facility, HASYLAB, and Ghent University, observed that the azimuthally averaged SAXS signal from the cells follows a power-law decay, with different exponents for living and fixed cells (3). Moreover, the scattering decays more rapidly after repeated exposure, so the value of the exponent serves as a diagnostic for beam damage in the sample. This issue of the *Biophysical Journal* contains a report from the Salditt team and collaborators from the Max Planck Institute for Dynamics and Self-Organization about their studies on living and fixed amoeboid cells (4), one which provides the best realization yet of the full potential of this exciting new technique.

Chemotaxis in amoeboids is accomplished by extending pseudopods with controlled polymerization of actin fibers and the retraction of receding edges by depolymerization of actin and contraction of myosin motor proteins, but the details of the actin networks remain unknown. The molecular signature of this mechanism is revealed by cellular diffraction, which shows highly anisotropic scattering streaks resulting from oriented fiber bundles in the periphery of the cell. Using an automated algorithm to convert this anisotropy to a contrast neatly outlines the advancing edges of the cell. Further modeling of the scattering streaks is necessary to characterize quantitatively the nature of the fiber bundles that give rise to the SAXS signal, but the richness of the data should allow the extraction of meaningful parameters such as the number of fibers in the bundle, their spacing, and the density of cross-links.

The authors note that their data and instrument are both complementary to and compatible with x-ray coherent diffraction imaging, so that with suitable modifications, users could first apply coherent diffraction imaging to image the whole cell and then scanning nanodiffraction to examine particular regions in greater detail. Indeed, the team has already demonstrated this approach by examining freeze-dried bacterial cells with both ptychography and cellular diffraction at the coherent SAXS beamline of the Swiss Light Source (5). With the synchrotron community preparing for fourth-generation facilities that approach the diffraction limit, which will greatly improve the coherence of the x-ray beam and the ability to focus the beam (6), cellular diffraction will surely grow in application and impact.

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