the block face is approximately 5 nm, the resolution along the z-axis in SBF-SEM is limited by the minimum slice thickness of around 25 nm. We have explored the feasibility of improving the z-resolution in SBF-SEM by recording images at more than one primary beam energy, thus sampling different depths below the block surface. We used Monte Carlo simulations of SEM images from an epoxy block containing 5-20 nm diameter carbon spheres stained with 14% osmium positioned at different depths, as a model for small biological structures. A linear relationship was found between the depth of the spheres and the ratio of backscattered signals at primary beam energies of 1.4 keV and 6.8 keV, which allowed us to generate 3D tomograms with a depth resolution of around 5 nm. Experiments are in progress to test this technique using a Zeiss Sigma-VP SEM equipped with a Gatan 3View SBF system. Sub-surface SBF-SEM could potentially match focused ion beam (FIB) SEM in terms of z-resolution, but with the added advantage of providing higher throughput and larger tissue volumes. The research was supported by the intramural program of NIH/BIB.

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Fixed Path Length Sample Holders Enable Robust Cryosaxs Measurements from Sub-Microliter Sample Volumes
Andrea M. Katz1, Jesse B. Hopkins2, Steve P. Meisburger3, Matthew A. Warkentin2, Robert E. Thorne2, Lois Pollack2
Cornell University, Ithaca, NY, USA.

Small angle x-ray scattering (SAXS) gives structural information about biological molecules in solution. However, large (~30 microliter) sample volumes are needed to mitigate radiation damage, limiting the use of SAXS in studying rare molecules. By cryocooling SAXS samples, radiation damage and required sample volumes are reduced by orders of magnitude [1], but challenges in creating identical-sized frozen samples complicate background subtraction. Here we present microfabricated silicon sample holders for cryoSAXS. These rigid solid holders have a fixed x-ray path length, simplifying background subtraction. Less than 800 nL of sample are required, facilitating measurements on expensive or hard-to-express molecules. These fixed path length, low volume sample holders make cryoSAXS a more accessible technique capable of probing a wide range of biological molecules.


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3D Dynamical Observations of Single Molecule Motions by X-Rays, Electron and Neutron
Yuji C. Sasaki1, Keigo Ikezaki2, Kouhei Ichiyanagi3, Hiroshi Sekiguchi3, Naoto Yagi4
1The University of Tokyo, Tokyo, Japan; 2KEK, Tsukuba, Japan; 3Research & Utilization Div., JASRI/SPring-8, Sayon-gun, Japan.

We have proposed that single molecule techniques using short wavelengths, for example, X-rays, electrons, and neutron [1]. Especially, Diffracted X-Ray Tracking (DXT) using normal synchrotron orbital radiation (SR) source (not XFEL) has been developed for obtaining the information of the 3D internal motions of single proteins with both high time-resolution (micro-seconds) and high precision (nm/1000) [2, 3]. DXT can be monitored through trajectories of the Laue diffraction spots from the nanocrystal which was labeled on the individual proteins. This concept can apply to utilize by using both electrons and neutron. Instead of the Laue diffraction using white X-ray, the Electron Back-Scattered Diffraction Pattern was adopted to monitor the 3D orientations of the nanocrystals linked to the single protein molecules[4]. We called Diffracted Electron Tracking (DET). Additionally, we call Diffracted Neutron Tracking (DNT) for new single molecule measuring method in which the long time observation from the non-destructivity of a neutron is possible. DXT, DET and DNT are assigned to labeling techniques through the nanocrystals. The size effect between intramolecular motions of individual proteins and the labeled nanocrystals becomes very important. We succeeded in the analysis of the quantitative size effects. As a result, we pointed out that determinations of the intramolecular motions without labeled nanocrystals are carried out quantitatively. Additionally, by progressing of the automatic DXT analysis corresponding to huge diffraction information, we obtained the time-resolved dynamical information that statistical reliability is sufficiently high.