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X-Ray Tomography Generates 3-D Reconstructions of the[1]

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Soft X-ray microscopy is an emerging new technique that can image whole, hydrated, biological specimens with a spatial resolution 5-10 times better than that obtained with light microscopy. X-ray imaging at photon energies below the K-absorption edge of oxygen exploits the strong natural contrast for organic material embedded in a mostly water matrix. With a transmission X-ray microscope using Fresnel zone plate optics, specimens up to 10 microns thick can be examined. The highest X-ray transmission in hydrated samples is obtained at a wavelength of 2.34 nm but, due to the low numerical aperture of zone plate lenses operated in first order diffraction mode (NA$\sim$0.1), the structures resolved are much larger than the X-ray wavelength. To date, soft X-ray microscopy has been used to resolve 30 nm structures in frozen hydrated specimens. Because of the low NA of X-ray lenses, combined with the effect of polychromatic illumination and a wavelength dependant focal length, the effective depth of field is large (6-10 microns). In this paper, we show tomographic reconstructions of rapidly frozen live cells in a 10 um diameter glass capillary (200 nm wall thickness). The image sequences span 180 degrees and consist of either 45 or 90 images spaced by 4 or 2 degrees, respectively. Computed tomographic reconstructions generate 3-D images of whole cells at better than 50 nm isotropic resolution. Using the x-ray linear absorption coefficient, quantitative information is obtained from the reconstructed data. Data sets containing 180 images, made possible by our new fully automated cryo-rotation stage, will generate images at resolution approaching 30 nm. This stage also enables collection of data in less than 3 minutes, making soft x-ray tomography the first high-throughput, high-resolution imaging technique for biological specimens.

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