Soft X-ray microscopy can be used to 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 µm 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~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 µm). This permits tomographic reconstruction of X-ray microscope images analogous to electron tomography. We show here tomographic reconstructions of rapidly frozen yeast in a 10 µm diameter glass capillary (200 nm wall thickness). The image sequence spanned 180 degrees and consisted of 45 images spaced by 4 degrees. The Spider software package was used to align the images to a common axis (with the aid of 60 nm diameter gold beads) and computed tomographic reconstruction was used to obtain the 3-D X-ray linear absorption coefficient. Volume rendering and animation of reconstructed data was performed using the 3-D program, amira (www.tgs.com). Using this technique, we show cryo X-ray tomography of whole yeast (Saccharomyces cerevisiae) at ~50 nm resolution.

References