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X-Ray Cryo-Tomography of Whole Yeast at 60 nm Resolution

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X-ray microscopy can be used to image whole, hydrated, biological specimens with a spatial resolution 5-10 times better than that obtained using visible light microscopy(1). X-ray imaging at photon energies below the K- absorption edge of oxygen, referred to as the water window, 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. Thus, the technique is ideal for imaging biological specimens such as cells. As with electron microscopy, the specimen must be maintained at low temperatures to avoid radiation damage. 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(2). 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(3).

The experiments presented here were performed at the Advanced Light Source soft X-ray synchrotron using the full field transmission X-ray microscope, XM-1(4). This microscope employs a bend magnet X-ray source and zone plate condenser and objective lenses. The condenser zone plate acts as a monochromator and the X-ray images are recorded directly on a cooled, back-thinned 1024x1024 pixel CCD camera. It is also equipped with a cryo stage previously used to examine rapidly frozen, whole cells(5). The sample holder was a rotationally symmetric glass tube, prepared using a standard pipette puller and mechanically stabilized by coating all but the final 200 μm with epoxy. The region containing the sample was 10 μm in diameter with a wall thickness of 200 nm. Live yeast were loaded into the micropipette along with 60 nm-diameter gold beads (high-contrast markers typically used to align tomographic tilt series). They were then placed in the X-ray microscope cryo-tilt stage, rapidly frozen by a blast of liquid nitrogen-cooled helium gas, and maintained at -140°C by a steady flow of cold helium. The image sequence spanned 180 degrees and consisted of 45 images spaced by 4 degrees. After each rotation the sample was manually repositioned and refocused using a 4x4 binned mode of the CCD to reduce radiation damage. The Spider(6) software package was used to align the images to a common axis and computed tomographic reconstruction was used to obtain the 3-dimensional X-ray linear absorption coefficient. Volume rendering and animation of reconstructed data was performed using the 3-D program, amira (www.tgs.com). Acquisition time with manual repositioning was 2hrs, but an automated system is being developed. With the relatively large mechanical tolerances involved in cryo X-ray tomography (~1 μm), refocusing and repositioning after each rotation should be unnecessary, enabling collection of a 45-image sequence in < 20 min. The low radiation damage of cryogenic samples will also enable collection of more projection images at smaller angular spacing, improving the resolution of the reconstructed 3-D volumes. Using this technique, we performed cryo X-ray tomography of whole, hydrated yeast (*Saccharomyces cerevisiae*) at various stages of yielding images at ~60 nm resolution.

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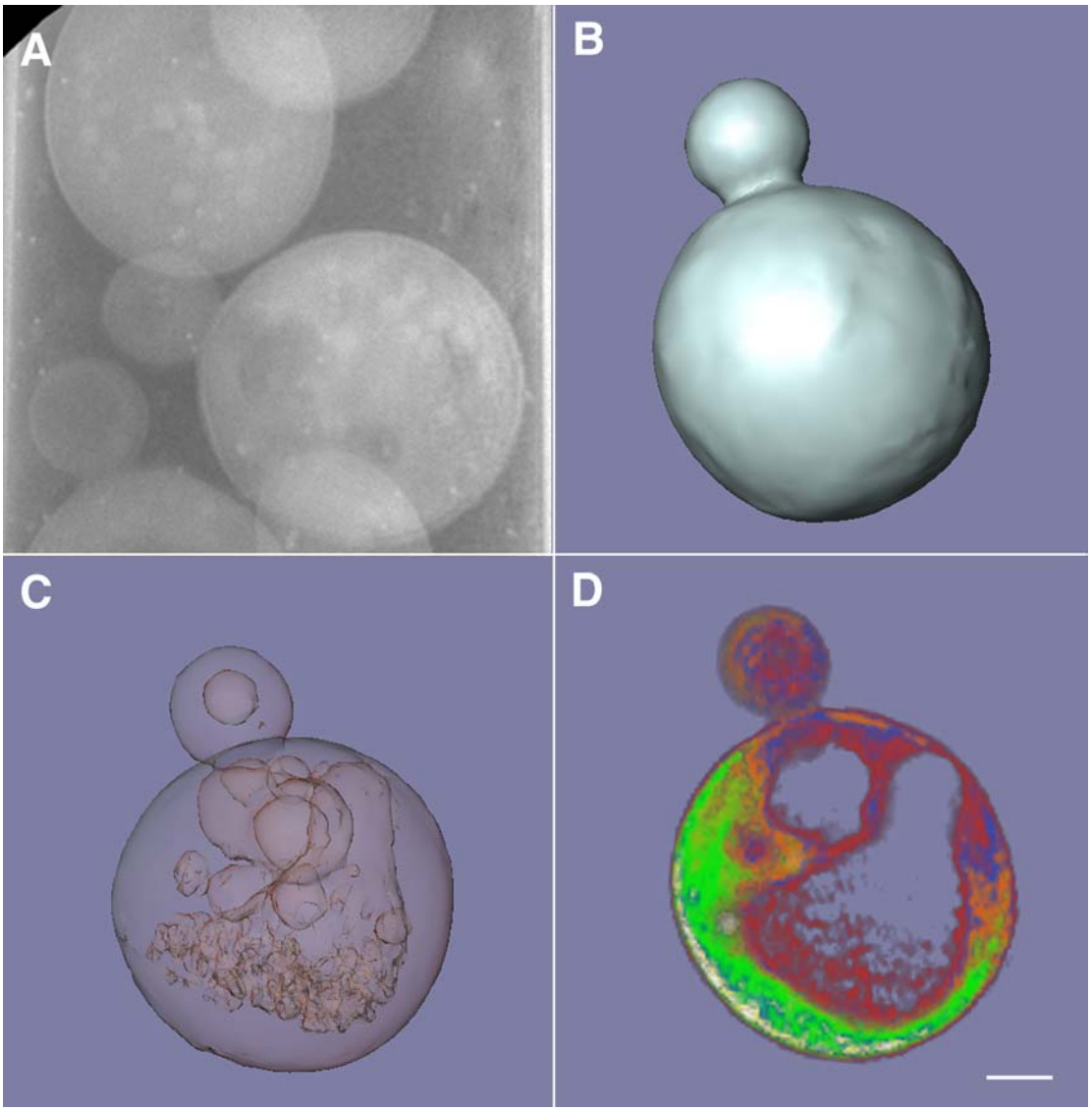

FIG. 1 (A) Single projection image of rapidly frozen yeast in capillary tube. (B) Same yeast after full reconstruction of 3-D data showing cell surface. (C) Transparent surface view of yeast showing internal organelles and vesicles. (D) Volume-rendered 10-slice section of cell structures of yeast assigned color based on X-ray absorption coefficient. Bar = 1 μm.

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