X-ray Tomography of Whole Cells at 50 nm Resolution
C. A. Larabell and M. A. Le Gros

*Department of Anatomy, University of California at San Francisco, larabel@itsa.ucsf.edu

#Physical Biosciences Division, Lawrence Berkeley National Laboratory, MALeGros@lbl.gov

Soft X-ray tomography is an imaging technique that can examine whole, hydrated, biological specimens up to 10 microns thick at better than 50 nm resolution. In the energy range of X-rays used to examine cells, photons with energies between the K shell absorption edges of carbon (284 eV, \( \lambda = 4.4 \) nm) and oxygen (543 eV, \( \lambda = 2.3 \) nm), organic material absorbs approximately an order of magnitude more strongly than water. These photons readily penetrate the aqueous environment while encountering significant absorption from carbon- and nitrogen-containing organic material. This generates a quantifiable natural contrast in fully hydrated cells and eliminates the need for chemical fixatives or contrast enhancement reagents to visualize cellular structures. We have used this imaging technique to reveal remarkable details of the nuclear and cytoplasmic architecture of fully hydrated whole cells[1]. We have also localized molecules in the nucleus and cytoplasm of these cells using immunogold labeling protocols[1]. We show here that cryo X-ray tomography of cells held in micro capillaries generates three-dimensional reconstructions of cells in their native state at better than 50 nm isotropic resolution. With X-ray imaging, the internal structures are not masked by ice and the resulting images are inherently of greater contrast. We present three-dimensional tomographic reconstructions of yeast cells, *Saccharomyces cerevisiae*, and bacteria, *E. coli*, revealing details of their internal structural organization at approximately 50 nm resolution. Data collection is extremely fast, with a complete data set for tomographic reconstruction requiring less than 3 minutes. In conclusion, X-ray tomography is an exciting new high-throughput approach for obtaining 3-D, quantifiable information from whole, hydrated cells.

Figure 1. Computer generated sections through X-ray tomographic reconstructions cells that were rapidly frozen just prior to imaging. X-ray absorption coefficients are colored light to dark, where dark is most transparent, light most X-ray absorbing (and hence most dense). (A) budding yeast cell (B) *E. coli*. The data for the complete 3-dimensional reconstructions comprised 45 images, collected through a total of 180 degrees of rotation.