# Biological Nano-Tomography

Carolyn A. Larabell[1,2] and Mark A. Le Gros[2]

[1]Department of Anatomy, University of California at San Francisco, 513 Parnassus, Box 0452, San Francisco CA 94143
[2]Physical Biosciences Division, Lawrence Berkeley National Laboratory, 1 Cyclotron Rd., MS 6-2100, Berkeley CA 94720

Soft X-ray microscopy is an emerging new imaging technique that can examine whole, hydrated, biological specimens up to 10 microns thick with a spatial resolution up to 15 times higher than that obtained with light microscopy. In the energy range of X-rays used to examine cells, organic material absorbs approximately an order of magnitude more strongly than water. This produces 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 approach to reveal remarkable details of the nuclear and cytoplasmic architecture of fully hydrated whole cells. We have also localized molecules in the nucleus and cytoplasm of whole, hydrated cells using immunogold labeling protocols. Using cryo X-ray tomography of cells held in micro capillaries, we have obtained 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. In addition the proteins, lipids and nucleic acids are detected by the amount of carbon and nitrogen they contain, generating quantifiable data based on their absorption coefficient. Three-dimensional tomographic reconstructions of the yeast, *Saccharomyces cerevisiae*, reveal high fidelity views of the internal architecture of these eukaryotic cells. Tomographic reconstructions of several bacteria reveal unprecedented details of their internal structural organization. All data obtained using this unique imaging approach are quantifiable by calculating the x-ray linear absorption coefficient. Using immunolabeling combined with tomography, we will be able to obtain 3-D information about the spatial distribution of proteins throughout the entire cell. Data collection is extremely fast, with a complete data set for tomographic reconstruction requiring less than 3 minutes. Consequently, X-ray tomography is an exciting new high-throughput approach for obtaining 3-D, quantifiable information from whole, hydrated cells.