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Compact Soft-X-Ray Imaging of Hydrated Biological Materials

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Soft x-rays have been shown to be useful for imaging whole cells or microns-thick sections at better than 50-nm resolution with useful native contrast and without the need for staining.[1] Soft x-ray radiation within the so-called "water-window" energy regime (between the absorption edge of C (285 eV) and O (540 eV)) produces good absorption contrast from intercellular components but ~10x less contrast from surrounding water.[2] The brightest and most intense sources for soft-x-ray radiation are by far synchrotrons which can produce sub-second images at wavelengths tunable through the soft-x-ray regime. In comparison, the few laboratory sources available currently are limited to one or a few wavelengths and exposure times are source-brightness limited to many seconds or minutes.[3] However laboratory-based (aka "compact") soft-x-ray microscopes have distinct advantages in cost, size, and ability to be located close to the biological laboratory. This paper demonstrates results from the compact soft-x-ray microscope (Gatan Model 550 SXM) we have built for both 2D and 3D imaging of hydrated biological material with 50-60 nm full-period resolution.

The SXM has a laser-pulsed plasma (LPP) source[4] based on an ultrafast laser that is focused on a target stream of methanol. The superheated target produces an intense source of soft-x-ray radiation emanating from a region tens of microns across and dominated by the C-VI line (368 eV) with an estimated brightness of $>10^{10}$ photons/sr/μm^2/s. Other transitions are present but are filtered by the condenser system which produces hollow cone illumination at the sample and is subsequently imaged onto a 2Kx2K CCD using an objective zone-plate. The SXM can host multiple objective zone plates (to trade off resolution against depth-of-field) – the current system has a 30-nm zone plate (resolution image shown in Fig. 1a) for high-resolution 2D, and a 48-nm zone plate for tomography.

Specimens for the SXM can either be placed on whole TEM grids, 600 μm-wide TEM grids for full-tilt tomography, or inside pipettes. Specimens are then plunge-frozen in liquid ethane to cryo-protect the cells in vitreous ice. Once transferred into the SXM, samples are typically viewed with a built-in fluorescence light-microscope which is extremely useful for locating areas of interest. Switching between light objectives and x-ray objectives takes seconds and positions are precisely correlated. Fast soft x-ray imaging with quality sufficient for navigation can be several images per second and high-quality images take 10-100 seconds.

In the experiment shown in Figures 1-3, we sought to measure the thylakoid membrane volume in cyanobacteria – these membranes are essentially invisible in 2D whole cell imaging except where membranes break spherical symmetry. In Figures 1bc, as-acquired projections show the bacteria embedded in ice supported by a lacey carbon film. These films are useful in supporting "droplets" of water containing bacteria and the lacey filaments also serve as a built-in resolution standard throughout the tomogram. The projection series acquired covered +/-71 deg (tilt-limit of 200 mesh grids) and the reconstructed tomogram is shown in Figure 2. Internal structures of the cyanobacteria are shown more clearly in Figure 3.

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[5] Cyanobacteria samples courtesy of R. Roberson, ASU.

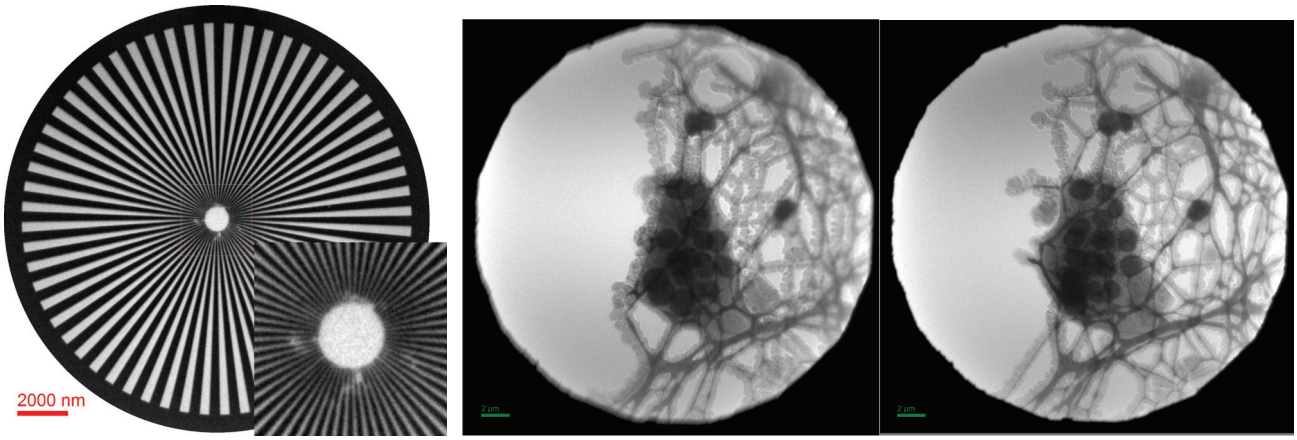

FIG. 1 (a) Siemens star resolution image demonstrating 25-nm half-period resolution achieved. (b) -28 deg projection image (29 μm FOV) of bacteria embedded in vitreous ice (c) +28 deg projection.

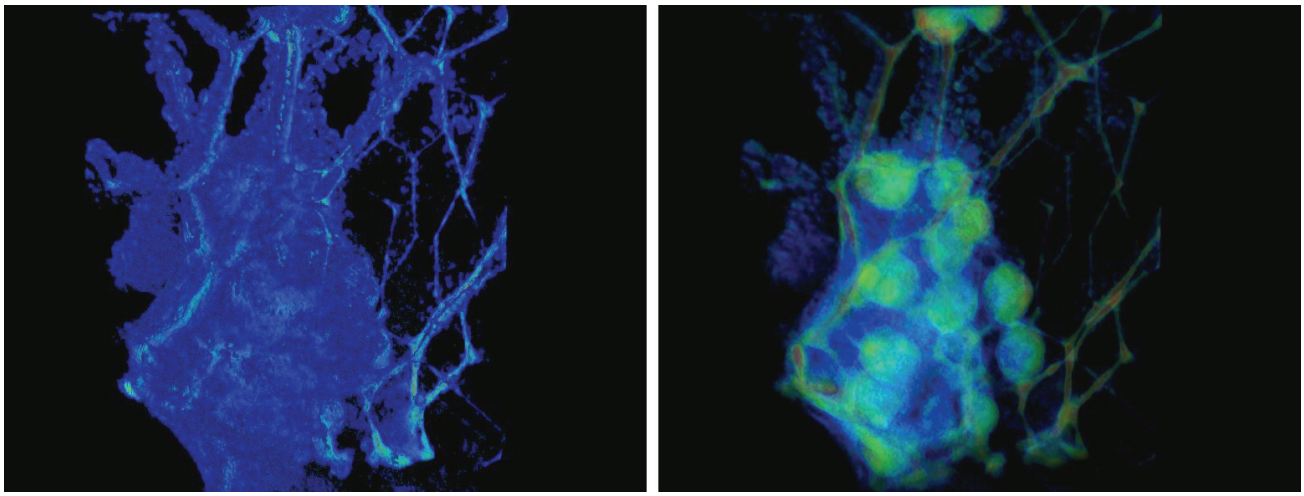

Fig 2. (a) Volume rendering of tomogram reconstruction thresholded to show ice volume (max thickness ~8 microns). (b) Thresholding and transparency set to expose cells embedded in ice.

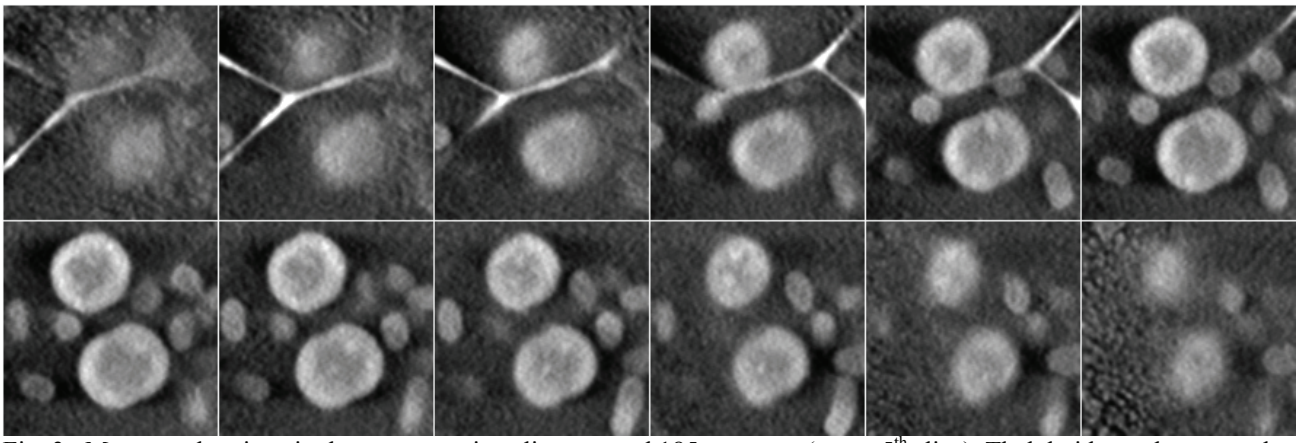

Fig. 3. Montage showing single reconstruction slices spaced 185 nm apart (every 5th slice). Thalykoid membranes and central structures in the cyanobacteria are clearly resolved.

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