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High Throughput Tender 3D X-ray Imaging of Cells for Correlative Microscopy

Katarzyna Matusik, Sylvia Lewis, David Vine, Jeff Gelb and S.H. Lau

Sigray, Inc., Concord, California, United States

X-ray microscopy has emerged in the past few years, driven by intrinsic advantages of nondestructive high resolution imaging and enabled by major advances in high-resolution x-ray optics and sources. The technique has become the **leading 3D nano-imaging approach for intact cells in their native or near-native hydrated state** [1] and has facilitated bridging the critical resolution gap between electron and light-based approaches (Fig. 1) [2].

One of the major advantages of the x-ray microscopy is its potential for correlative imaging [3-5]. X-rays provide 3D information about whole, hydrated cells without requiring staining or serial sectioning, which makes it an **ideal correlative technique**. This includes as a structural context for interpreting cryo super resolution light microscopy results [5-6] that lack structural context and as a complement to electron microscopy for aligning the hundreds of slices required for a large cell or to prescreen cells or regions of interest [7].

Laboratory and synchrotron-based cellular x-ray microscopes have primarily operated in the "water window" (285-543 eV) [8]. In the "water window," water is relatively transparent because the x-ray energies are less than the oxygen K-shell absorption edge energy of 543eV, but carbon-containing organic matter is highly absorbing because the x-ray energies are above the carbon K-shell absorption edge energy of 284eV. Such water window systems have provided powerful capabilities for imaging prokaryotic (e.g. yeast, bacteria, algae) cells and a sub-set of small mammalian cells. However, the system's **utility is limited for many medical applications** because of fundamental limitations of water window x-rays: it is easily attenuated and has a shallow depth-of-field (DOF) for high resolution imaging, which impedes practical imaging of adherent cells and cells above 10µm in diameter; most mammalian cells are at least 10-20µm in diameter.

Here we present a recent laboratory development using the phase contrast of tender x-rays (e.g. 2-6 keV) for rapid ultrastructural imaging of large mammalian cells at 30 nm spatial resolution. This system is based on a key recognition that phase contrast between organic matter and water at **~2.5 keV x-rays is comparable to or even higher** than the respective absorption contrast at x-ray energies in the water-window. This realization has motivated developments at synchrotrons (e.g. Diamond Light Source and BESSY) to develop tender x-rays as an option for their water window x-ray microscopes.

The system enables several key advantages for mammalian cell imaging, including:

- Fast **30-minute** tomography imaging with 30nm resolution
- High natural phase image contrast between water and organic matter
- Ability to image large (up to 80µm) unstained cells and fixed stained cells

We will review recent data acquired from Sigray's 2.7 keV BioLambda x-ray microscope system and discuss developments toward facilitating workflows using 3D x-ray tomographies of cells.

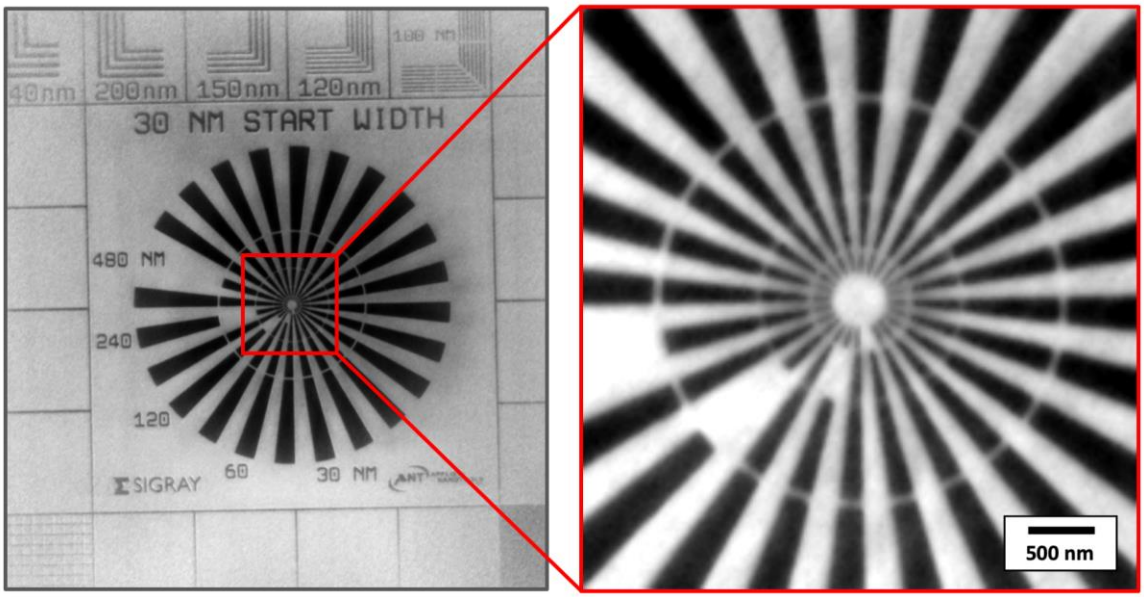

Figure 1. Resolution capabilities of the 2.7 keV x-ray microscope with 30nm spatial resolution

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