Laboratory Cryo Soft X-ray Tomography:

Progress in the development of a commercial microscope

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Introduction

Cryo-soft X-ray tomography (cryo-SXT) is the only technique that allows the imaging of an entire cell in its fully hydrated state. Whole cells up to 10-15 microns thick can be imaged at a 3D resolution approaching 30 nm. Cryo-SXT preserves volatile structures, and since the cell is fully hydrated, avoids artefacts associated with sample shrinkage during dehydration. Cryo-SXT can also image the thickest parts of the cell, including the perinuclear region that contains many of the cell's organelles, which cannot be imaged in 3D by other techniques. Great progress has been made over the last decade in developing cryo-SXT as an imaging technique on synchrotron hosted microscopes [1-4]. Workflows have improved which allow non-synchrotron researchers to access the technique, and significant expertise has been developed in correlating SXT and cryo fluorescence data [5-7]. This amalgamation of techniques integrates 3D molecular localisation data with a high-resolution, 3D reconstruction of the cell. Here we report on the development of a compact lab based microscope that aims to deliver synchrotron performance in a system that will turn cryo-SXT into an affordable, efficient laboratory tool, thus increasing the scope and throughput of possible research projects. The key to this is the development of a sufficiently bright and compact source of soft X-rays. We show data on light source performance and first images from our microscope.

Why Soft X-rays?

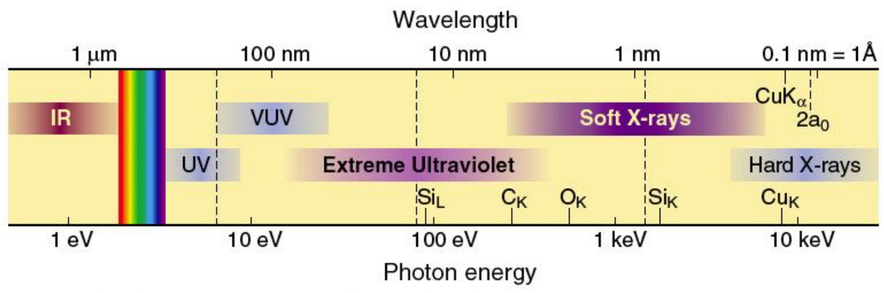

Figure 1: The Electromagnetic Spectrum, depicting the soft X-ray region.

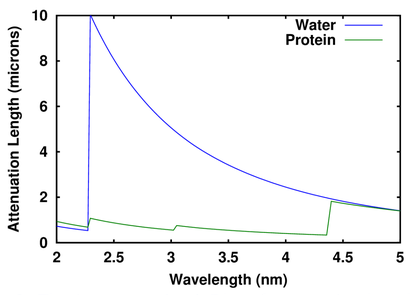

Figure 2: The 'water window'. Plot of attenuation length against photon wavelength for soft X-rays in a biological sample.

- Whole, hydrated cells 10-15 μm thick (Cryo-immobilized)
- Natural contrast – no staining, dyes etc.
- Quantitative
- Fast - five minutes per cell
- Resolution <40 nm (isotropic)
- Localization of molecules using correlated fluorescence and X-ray tomography

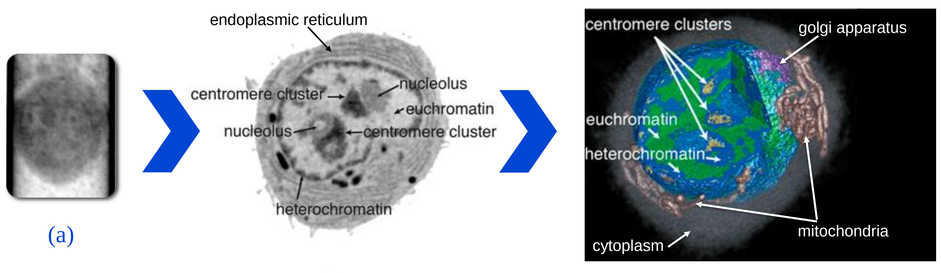

Figure 3: The sample to be imaged is rotated in front of the beam of soft X-rays. A series of 2D projection images (a) is generated and computer algorithms are used to reconstruct the 3D cell image. (b) shows a 2D virtual slice through the reconstructed cell, shown volume rendered and segmented in (c). Courtesy of Prof. Carolyn Larabell, and published in Proc. Natl. Acad. Sci. USA 2016; 113(12): E1663-72.

Cryo-SXT Applications

With a field of view of ~10–20 × ~10–20 μm, a penetration depth of ~10 μm and a resolution of ~30 nm^3, the soft X-ray microscope neatly fits between the imaging capabilities of light and electron microscopes. The Cryo-SXT niche can be summarized as follows [8]:

- Complex 3D structures in whole cells (mitochondrial networks, nuclear morphology…)
- Volatile structures that are difficult to capture with chemical fixatives (autophagosomes, tubulated endosomes…)
- Samples where accurate volumetric measurements are important (organelle volumes, cell volumes, gross morphological changes due to drug treatment or disease…)
- Samples in which compositional information is relevant (iron, nanoparticles, metal labels…)

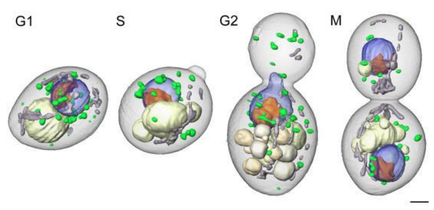

Figure 4: Yeast cells at the four main stages of the cell cycle. Courtesy of Prof. Carolyn Larabell of NCXT, LBL, and published in Yeast 2011; 28: 227–236.

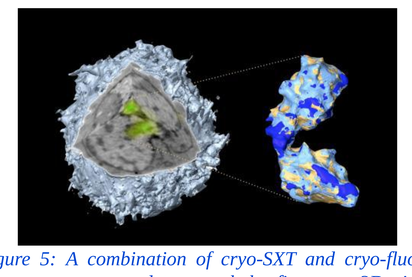

Figure 5: A combination of cryo-SXT and cryo-fluorescence microscopy was used to reveal the first ever 3D view of the inactive X chromosome. Courtesy of Prof. Carolyn Larabell and published in Biophysical J., Oct 2014, Vol 107, pgs. 1988-1996.

Our Technology

The key technology at the core of the SiriusXT instrument is a high-performance soft X-ray light source based on laser-produced plasma emission with the appropriate size, wavelength and brightness, combined with smart optics whose optical quality is not degraded by the debris generated by the plasma. This unique combination enables the deployment of a lab-scale stable and robust light source suitable for cryo-SXT. The technology works by focusing a high power pulsed laser onto a solid target made from appropriate metals, producing a tiny million degree plasma. This laser plasma is hot enough to emit soft X-rays sufficient for efficient cell imaging, but it also produces a lot of metal debris. Delicate optics are required to collect the soft X-rays for use in the microscope and for focusing the laser, and these would be very quickly destroyed by the debris from the plasma. The company has developed self–healing soft X-ray and laser focusing optics, which means that the optics can last indefinitely even in these extreme conditions.

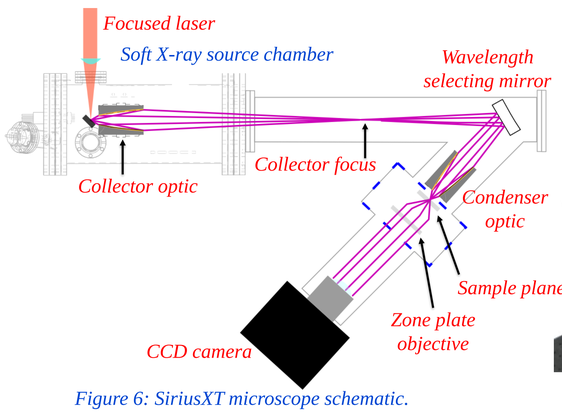

Figure 6: SiriusXT microscope schematic.

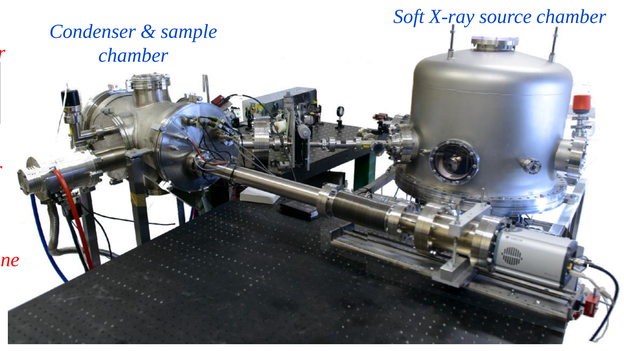

Figure 7: SiriusXT prototype microscope.

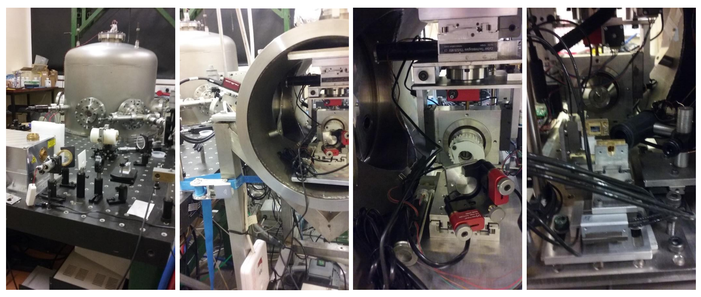

Figure 8: Components of the prototype microscope from l-r; Laser optics & source chamber (far left); condenser chamber showing bandwidth selecting optic and condenser mirror (middle images); 'dry' sample chamber showing sample and zone plate objective (far right).

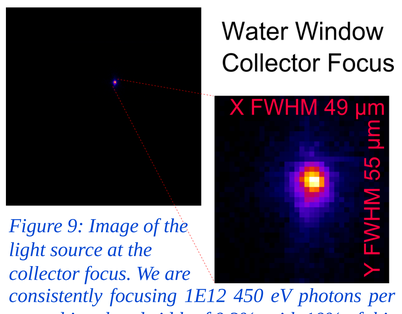

Figure 9: Image of the light source at the collector focus. We are consistently focusing 1E12 450 eV photons per second in a bandwidth of 0.3%, with 10% of this appearing on average in a 60 micron by 60 micron area which can be used for imaging. This corresponds to greater than > 1E7 photons/square micron/per second at the sample. This is enough to produce high resolution 3D images of good contrast on 10 micron thick samples in < 1 hour.

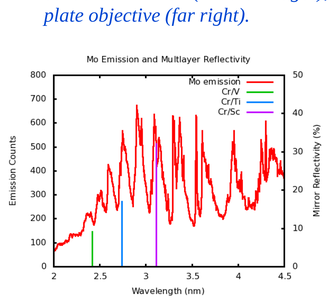

Figure 10: Measured source spectrum, showing positions of available multilayer optics.

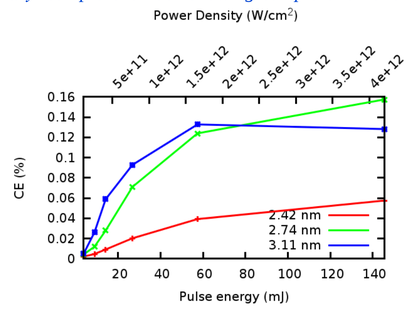

Figure 11: Measured Mo conversion efficiency of input laser into useful photons (0.2% BW), as a function of laser pulse energy and source wavelength.

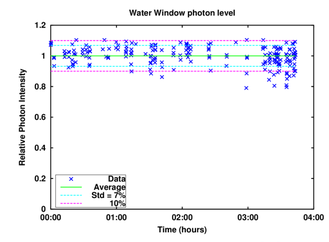

Figure 12: Source output stability over a 4 hour continuous run.

Summary & Future Plans

- SiriusXT has developed a bright and compact soft X-ray light source that will propel soft X-ray tomography into labs worldwide for 24/7 access.
- Current source output consistent with <1 hour tomograms.
- Clear roadmap to 10 min tomograms with commercially available improved optics.
- Cryo sample stage integration and first 'wet' full cell tomograms scheduled for Q4 2016.
- SiriusXT will provide an imaging service to academic and industrial research groups from Q1 2017.
- SiriusXT will provide full microscope products from Q2 2017.

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Acknowledgements

The authors would like to thank the staff of the UCD School of Physics and Mechanical Workshop, for their continued contribution to this effort. In addition we would like to thank Prof. Carolyn Larabell (NCXT, Berkeley), Dr. Lucy Collinson (Francis Crick Institute), Dr. Eva Pereiro (ALBA, Barcelona) and Dr. Gerd Schneider (BESSY, Berlin) for their support and advice, on a range of aspects including microscope design, implementation, use of images, and advice on biological applications of soft X-ray tomography.