

## Development of a Commercial Laboratory Scale Soft X-ray Microscope

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Cryo-soft X-ray tomography (cryo-SXT) is an extremely powerful technique that allows the imaging of an entire cell in its fully hydrated state with natural contrast. 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.

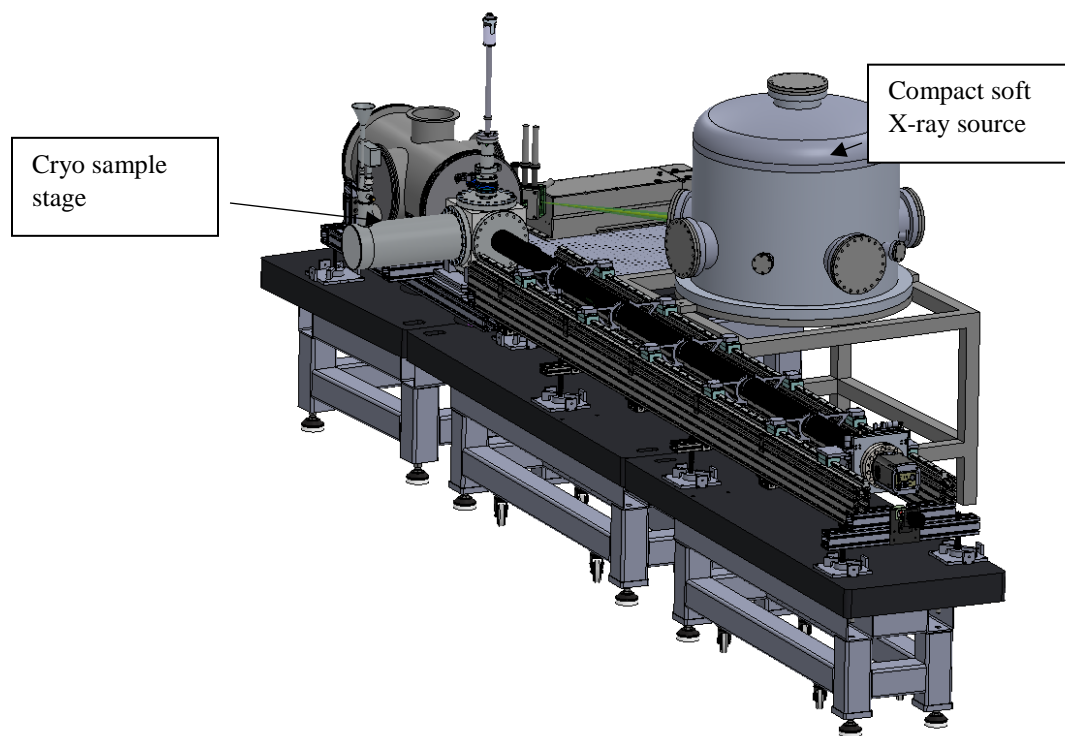
The key technology at the core of the SiriusXT instrument is a high-performance soft X-ray light source based on laser-produced plasma emission with the appropriate size, wavelength and brightness, combined with smart optics whose optical quality is not degraded by the debris generated by the plasma. 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 a self-healing liquid coating for both the soft X-ray and laser focusing optics, which is continuously replenished and means that the optics can last indefinitely even in these extreme conditions.

With a field of view of ~10–20 μm x ~10–20 μm, a penetration depth of ~10 μm and a resolution of ~30 nm<sup>3</sup>, the soft X-ray microscope neatly fits between the imaging capabilities of light and electron microscopes. The cryo-SXT niche can be broadly divided into the following four categories [8]; complex 3D structures in whole cells (mitochondrial networks, nuclear morphology etc.), volatile structures that are difficult to capture with chemical fixatives (autophagosomes, tabulated endosomes etc.), samples where accurate volumetric measurements are important (organelle volumes, cell volumes, gross morphological changes due to drug treatment or disease etc.), and samples in which compositional information is relevant (iron, nanoparticles, metal labels etc.).

We present data on light source performance and first images from our microscope [10].

## References:

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**Figure 1.** Conceptual design of the compact soft X-ray source and microscope.