# Laboratory-scale cryo soft X-ray tomography

elmi2021
Abstract ID: 185

Kenneth Fahy[1], Dunja Skoko[1], William Fyans[1], Tony McEnroe[1] , Fergal O'Reilly[1,2] and Paul Sheridan[1]

[1]SiriusXT Ltd., 9A Holly Avenue, Stillorgan Industrial Park, Blackrock, Co. Dublin, Ireland
[2]School of Physics, UCD, Belfield, Dublin 4, Ireland
Email: *info@SiriusXT.com* Web *www.SiriusXT.com*

## Introduction

SiriusXT has developed a commercial bench-top cryo soft X-ray microscope for 3D cryo-soft X-ray tomography (cryo-SXT). Cryo-SXT uses X-rays in the 'water window' that extends from the K-absorption edge of carbon to the K-edge of oxygen, that is from about 282 eV (λ = 4.4 nm) to 533 eV (λ = 2.3 nm). Water is transparent to these X-rays, but organic molecules are absorbing. Therefore, these X-rays can be used as the basis for microscopy of whole cells in their near-native (frozen) state, without need for any contrast enhancing agents. A 3D tomogram with resolution between 25 nm to 60 nm (full pitch) is produced by rotating the cell over a range of angles, with an image acquired at each tilt angle [1, 2, 3]. The concept is equivalent to a medical CT scan applied at the nanoscale.

## Our Technology

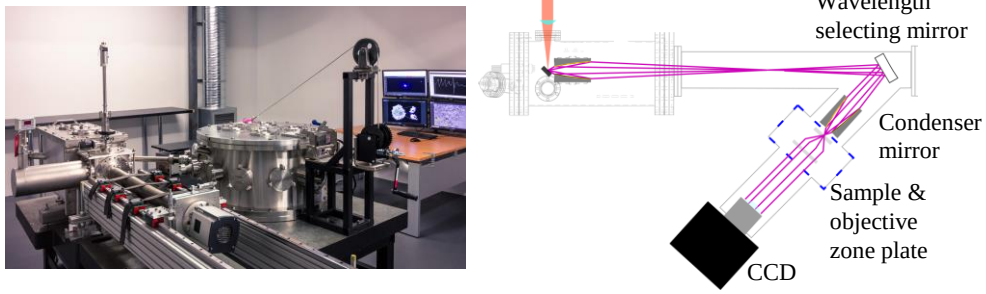


*Figure 1. SiriusXT microscope (left) and schematic of main microscope components (right).*

- High-performance soft X-ray light source based on laser-produced plasma emission
- Small source size ~ 10 µm
- Power scalable with laser input power
- Multilayer optic selects wavelength for imaging (λ=2.73 nm , λ/ Δλ ~ 350)
- Cryo stage with long term thermal & spatial stability
- Spatial resolution 60 nm (full pitch) with a 35 nm zone plate
- System footprint 3 m x 2 m

## CryoLM workflow

- Cell culture on 3mm TEM finder grids.
- Cryo-preservation by plunge freezing.
- Grids transferred to cryoLM stage and inspected using fluorescent microscopy.

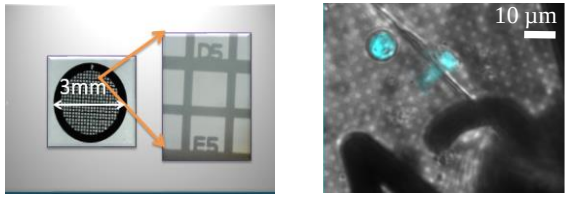


*Figure 2. TEM finder grid (left) and CryoLM image of Chlamydomonas reinhardtii alga (right) prepared in the SiriusXT lab, imaged using a 100X, 0.9 NA objective in our homebuilt cryostage.*

## CryoSXT

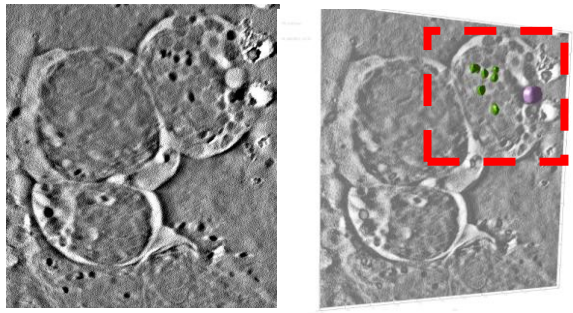

*Figure 3. CryoSXT of chlamydomonas reinhardtii algae cluster; 2D orthoslice (left), and partial volume segmentation (right). Data was taken over a ±40° tilt range, at 2.5° increments with a 35 nm zone plate objective.*

## Summary

- Initial cryoSXT demonstrated on algae samples with optimisation ongoing.
- CryoLM workflow developed for screening of biological samples.
- CryoSXT to be extended to mammalian cells in the near future.

## References

[1] Schneider G, Guttmann P, Heim S *et al* 2010 *Nature Methods* **7** 985-987
[2] Müller WG, Heymann JB, Nagashima K *et al* 2012 *J Struct Biol* **177** 179
[3] McDermott G, Fox DM, Epperly L *et al* 2012 *BioEssays* **34** 320

## Acknowledgements

The authors would like to thank the SiriusXT staff and the staff of the UCD School of Physics, UCD Conway Institute and Mechanical Workshop at the School of Physics, for their continued contribution to our work. In addition we would like to thank Prof. Carolyn Larabell (NCXT, Berkeley), Dr. Eva Pereiro (ALBA, Barcelona), Dr. Liz Duke (Diamond Light Source, UK), Dr. Gerd Schneider (BESSY, Berlin), Dr. Lucy Collinson (Francis Crick Institute, UK) and Dr. Dimitri Scholz (Conway Institute, UCD) 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.
**This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No. 959776 and 101017116.**