The ARC Centre of Excellence for Coherent X-ray Science (CXS) Annual Workshop 2011:
Physicists and Biologists Working Together – Facilitating Imaging and Biophotonics

Report by Leann Tilley, Harry Quiney and Robert Scholten

The ARC Centre of Excellence for Coherent X-ray Science (CXS) Annual Workshop 2011 was held at the Bio21 Institute, in Melbourne, Australia, 10-12 October, 2011. This cross-disciplinary workshop brought together physical and biological scientists at a very exciting time, when there are major developments emerging in structure determination and imaging techniques based on X-rays, visible photons and electrons. The workshop participants included most of the members of the CXS "family" as well as many other members of the physics, biology and chemistry communities. There were a total of 157 registrants, including 15 international speakers from the USA, UK, Switzerland, the Netherlands, Germany, Japan and New Zealand, as well as prominent local speakers from the CXS and premier institutions in Australia. It was a fantastic opportunity to meet within and across disciplines and countries. A summary of some of the talks is given below, concentrating on the presentations from the overseas invited speakers

Professor Keith Nugent, CXS Scientific Director opened the meeting welcoming the travellers from near and far and explaining the goals of the workshop and its fit with the activities of the CXS.

Day 1

New X-ray Free-electron Laser sources (XFELs) have recently become available. XFELs produce X-ray pulses of femtosecond duration with a peak energy level 10 billion times that of current synchrotron sources. Physicists and biologists are working together to apply XFELs to biological problems. It is anticipated that they will have profound impact in biological imaging, structure determination and femtosecond biology. Ever wondered what happens when photons strike a light harvesting complex in a photosynthetic organism? Or puzzled about how cellular machinery synthesises proteins, or generates ATP, or reproduces itself? Our current understanding of molecular machinery is limited by our ability to induce proteins of interests to form large crystals suitable for X-ray crystallography. Recent work provides proof-of-concept that XFELs can be used to obtain a diffraction signal from much smaller assemblies of macromolecules than is currently possible. The potential also exists eventually to image single molecules. CXS members are contributing to efforts to apply XFELs to obtain atomic scale structural information for proteins that cannot be crystallized. A highlight of the first day of the CXS Workshop was presentations about some of the important technical barriers that are being overcome so that XFEL imaging of macromolecular machines can become a reality.

Dr Tatiana Latychevskaia, ETH-Zurich, provided a stimulating start to the workshop, graciously touching on all the workshop themes: biological, diffractive, electron and X-ray based imaging. Her research group is working on imaging of individual molecules at atomic resolution, using low-energy electrons to avoid sample damage. Lensless imaging is achieved with both holographic and CDI (coherent diffractive imaging) approaches. The talk summarised the methods and thus provided a foundation for many of the later talks in the conference which are based on CDI and related approaches, and also recent promising results.

Professor Abbas Ourmazd is Distinguished Professor of Physics and Electrical Engineering at the University of Wisconsin-Milwaukee. The problem of structure determination from XFEL diffraction data using the "diffract and destroy" approach is complicated by the random orientation with which each molecule presents itself to the femtosecond X-ray pulse. While many approaches have been proposed to categorize the individual diffraction images by molecular orientation, Ourmazd and collaborators have adapted concepts borrowed from general relativity and Riemannian geometry to a manifold analysis of the complete set of data. Ultra-low-signal snapshots are placed on a Riemann surface that may include internal conformational information as well as orientation, yielding information about both structure and dynamics of evolving systems.

Associate Professor Yukio Takahashi, Osaka University has been developing coherent diffractive X-ray microscopy for applications to biology and materials science. He reported a three-dimensional reconstruction of silver nanoparticles by X-ray tomography with a resolution of 4.2 nm. The need to account for positional errors in X-ray ptychography has led to the development of the technology necessary to reconstruct the complex transmission function of extended, two dimensional objects. An element-specific variant of this approach was reported that has been used to map silver-enriched regions of composite silver-gold nanoparticles using anomalous X-ray scattering.

Professor Bonnie Wallace is Professor of Molecular Biophysics in the Department of Crystallography at Birkbeck College, University of London, UK, and a CXS International Advisory Board member. She spoke about AcrB, a multidrug efflux pump found in pathogenic *E. coli*. This membrane protein is a problem contaminant in efforts to generate 2-D crystallization of exogenous membrane proteins expressed in *E. coli*. Bonnie's lab has identified at least eight unique crystal forms of AcrB and showed examples of how it has been studied by single particle imaging electron microscopy, electron diffraction from 2D crystals, and high resolution X-ray crystallography and can be used to compare the advantages, disadvantages and limitation of the different currently available techniques for structure determination.

Professor John Spence is Regent's Professor of Physics at Arizona State University. In recent years his group has pioneered the development of coherent diffractive imaging including the delivery of samples to the beam. In a wide-ranging talk, Professor Spence covered recent results from LCLS on the structures of fully hydrated samples of submicron protein nanocrystals. The first new biology from LCLS has yielded new insights into the enzyme cathepsin, while clear indications were given in the presentation that pump-probe experiments would soon yield the first stroboscopic molecular movie of chemical dynamics. Careful analysis of Bragg spots from nanocrystal samples and of intensity variations between Bragg spots offers a new solution to the phase problem that does not depend on *a priori* structural information.

Dr Garth Williams is a former CXS researcher and is now an instrument scientist at the Linac Coherent Light Source, SLAC National Accelerator Laboratory, working on the Coherent X-ray Imaging instrument, which serves as the primary structure-determination instrument at the world's first hard X-ray free electron laser user facility. For slightly more than one year, the Linac Coherent Light Source has been providing highly brilliant hard X-rays in the range of 4-10 keV. The current and potential capabilities of the facility were presented by Dr Williams, together with the general philosophy of its science-driven programme and some recent research highlights. There was also a discussion of the unique opportunities for imaging at sub-nanometre resolution that that are available at the CXI beamline.

Professor Rick Millane is a Professor and Head of the Department of Electrical and Computer Engineering at the University of Canterbury in Christchurch, New Zealand. Professor Millane presented a discussion of the relationship between the inversion problem for coherent diffractive imaging and for crystallography. For both problems uniqueness and effective algorithms need to be considered. Professor Millane provided a method for quantising uniqueness and presented an overview of reconstruction algorithms; an application of solving for molecular envelopes was presented.

Day 2

An important aim of CXS is the development of new forms of very high resolution X-ray and optical microscopy. Our aim is to obtain high resolution 3D images of molecular machinery in a whole cell context. The resolution of conventional light microscopy is limited by the degree to which light can be focussed - effectively about half of the wavelength of the light used (i.e. about 250 nm). Recently new techniques that use ingenious methods such as interfering light beams or photo-activation of fluorescent chromophores have pushed the achievable resolution towards the nanometre scale. These so-called "super-resolution" methods are providing details of cellular architecture that were beyond imagining only a few years ago. In the second day of the workshop the talks explored a range of new developments in X-ray and optical microscopy, with particular highlights in the areas of soft cryo X-ray microscopy and super-resolution microscopy.

Professor Carolyn Larabell holds a joint position as Professor in the Department of Anatomy at the University of California, San Francisco School of Medicine, and Faculty Scientist in the Physical Biosciences Division at the Lawrence Berkeley National Laboratory. She is also the Director of the UCSF/LBNL National Center for X-ray Tomography. She has worked with colleagues (including Dr Mark LeGros, who also attended the meeting) to commission an X-ray tomographic microscope at the Advanced Light Source (ALS). The microscope operates in the water window (which gives natural contrast between carbon and water) and the samples are cryo-stabilised, making it well-suited for biological applications. It is capable of imaging whole, hydrated cells at a resolution of about 35 nm. Caroline showed us some fantastic 3D reconstructions of cells ranging from pathogenic fungi to mammalian cells. Areas of heterochromatin and euchromatin in mammalian nuclei are readily distinguished based on their different X-ray absorption profiles. The rendered tomograms revealed an intricate network of interconnected regions of active and silenced nuclear material.

Dr Peter Kner is a Physics and Electrical Engineering graduate from MIT and UC Berkeley. He undertook post-doctoral work at UCSF (working with Mats Gustafsson, John Sedat and David Agard) where he helped pioneer the development of Structured Illumination Microscopy before taking up an independent position at University of Georgia. He explained the theory and showed experimental examples of how 3D-SIM can achieve a 2-fold increase in resolution in all 3 dimensions, as well as removing out-of-focus light. He described recent work using a spatial light modulator (SLM) for pattern creation which permits live TIRF SIM at 11 Hz for an 8 μm × 8 μm field of view. His images of GFP-kinesin marching along tubulin filaments are quite mesmerising and herald the possibility of video rate imaging of cellular events at an unprecedented level of resolution.

Professor Markus Sauer holds a Chair in the Biotechnology and Biophysics at the Julius-Maximilians-University Würzburg, Germany. He explained that PALM, STORM and dSTORM rely on photoactivation, photoconversion, photobleaching or blinking processes to turn fluorescent molecules on and off. Because photoactivation is a stochastic process, only a few well separated molecules are turned on at any one time. Each emitter is thereby individually captured in an image without overlap. These molecules are turned off and a new set turned on until the entire image is captured. The “resolution" for this technique (where resolution is defined as the distance between objects that can be reliably measured rather than the true optical resolution) reaches ~10 nm. Markus is the inventor of direct-STORM, which uses small molecular weight photoswitchable fluorophores (including Cy5, Alexa Fluor 647, and ATTO fluorophores) that undergo switching in the presence of reducing agent. He stressed the importance of high labelling densities for obtaining reliable super-resolution information. For example one fluorophore per 10 nm permits a "resolution" of ~20 nm, and approaches the level resolution that is achieved by electron microscopy.

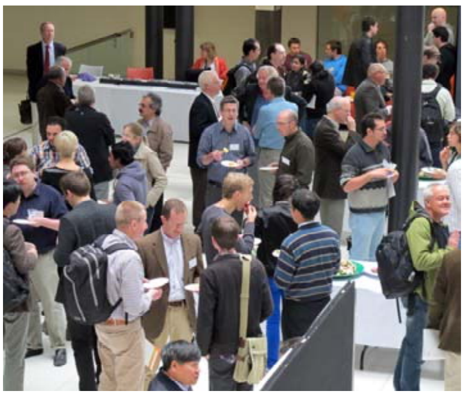
Associate Professor Christian Soeller is at the Department of Physiology, University of Auckland, New Zealand. Originally a physicist from the Max-Planck Institute for Biophysical Chemistry, Göttingen, he now uses fluorescence super-resolution methods, PALM and STORM, to look at calcium signalling in heart muscle cells. He described methods for imaging optically thick mammalian tissue samples in 3D, using multiple marker molecules, with a lateral resolution of ∼15 nm. His imaging revealed micro-clusters of ryanodine receptors in muscle cells and has led to a new concept of calcium sparks caused by the concerted triggering of clusters of receptors. Christian assembles his own instruments and is keenly involved in developing mathematical models to analyse the super-resolution information.

Associate Professor Peter Carlton was Postdoctoral Fellow at the University of California, San Francisco, where he worked with John Sedat and other colleagues to undertake the first applications of 3D-SIM to biological samples. In 2010 he became an iCeMS Kyoto Fellow as a Research Group Leader at the iCeMS (Institute for Integrated Cell-Material Sciences) of Kyoto University. He is applying super-resolution optical microscopy methods to studies of meiotic chromosome pairing, chromosome structure and dynamic behaviour. He is trying to probe the differences between pluripotent and differentiated cells, to understand how chromosomal regions destined for pairing come together, and work out how *C. elegans* avoids meiotic mayhem.

Day 3

Coulomb repulsion limits conventional electron imaging to low frame rates, but Professor Jom Luiten of the Eindhoven University of Technology (Netherlands) and colleagues have shown that the problem is not absolute. For example, they have recently demonstrated that the expansion of a high-density charge bunch can be reversed to allow single-shot diffractive imaging with sub-picosecond temporal resolution. Cold atom electron sources promise the spatial coherence and brightness to extend the imaging to macromolecular crystals, and thus provide molecular imaging capability effectively equivalent to a “mini-XFEL”.

The same technology used to create highly coherent electron bunches from cold atoms can easily be applied to producing highly coherent ion beams. Focused ion beams (FIBs) are an important tool for nanometre scale imaging and fabrication but have been restricted to gallium. Dr Jabez McClelland from the National Institute of Standards and Technology (USA) showed competitive imaging results obtained with a new ion source based on laser cooled atoms. He also put a new twist on imaging with electrons, using beams with high orbital angular momentum in a transmission electron microscope, with possible applications in phase-contrast and magnetic domain imaging.



Other events
The attendees of the workshop enjoyed lively discussions during the lunchtime and evening poster sessions - fed and watered by the excellent catering of "Mary and Steve". Many members also attended the conference dinner which was held at University House, with some very good wines and wide-ranging discussions about the best time and length scales for important biology questions. An interactive meeting was also held between members of the Scientific Advisory Board and the post-doctoral fellows and students of the CXS. This session proved to be a valuable source of feedback from the perspective of the early career researchers and generated some excellent initiatives for enhancing the inter-disciplinary nature of the CXS. In addition, groups of attendees were taken on tours of the electron microscopy and other platform facilities of the Bio21 Institute, as well as a much-appreciated tour of the synchrotron. Many members of the Workshop attended a Round Table Discussion the day after the Workshop and provided suggestions and feedback on potential scientific directions for a "CXS of the future". It was agreed that any new Centre should build on the scientific and collaborative successes of CXS and that it should retain its inter-disciplinary character: “Physicists and Biologists working together”.

The Organizing Committee for the workshop (Leann Tilley, Rob Scholten (Program Chair), Harry Quiney, Trevor Smith, Keith Nugent, Grant van Riessen, Mike Ryan, Tania Smith and Fabienne Perani) thank all of the participants for contributing to such an excellent program. We also thank our AV team, Paul, Megan and Mauro and give particular credit to Fabienne Perani, who made it all happen from a practical point of view.

We hope you enjoyed the event and found it to be a worthwhile experience and are now more excited than ever about the prospects for close interactions between the biological and physical sciences. From the feedback we have received so far, everyone from students to Principal Investigators agreed that it was a terrific workshop due to the extremely high standard of presentations and the friendly, interactive spirit of all involved. We thank everyone who made oral and poster presentations, initiated discussions in the sessions, and continued those discussions over a beer.

We value your feedback on how the Workshop met your expectations and are interested to hear of suggestions for our next conference. Please let us know if there are speakers, sessions or themes you would like us to pursue.

Photos and the report from the workshop are available at http://www.coecxs.org/