# 2010 ANNUAL REPORT

## ARC CENTRE OF EXCELLENCE FOR COHERENT X-RAY SCIENCE

CXS

ARC Centre of Excellence for
COHERENT X-RAY SCIENCE

www.coecxs.org

CXS would like to acknowledge the support of the Australian Research Council. We would also like to acknowledge the financial and in-kind support provided by our collaborators –University of Melbourne, La Trobe University, Monash University, Swinburne University of Technology, Griffith University and the Australian Commonwealth Scientific and Industrial Research Organisation (CSIRO). We are grateful for the financial support received from the Science, Technology and Innovation (STI) Initiative coordinated by the Office of Science and Technology within the State Government of Victoria.

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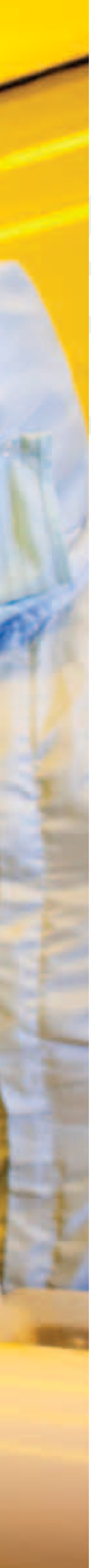
# MISSION STATEMENT

## TO BE THE WORLD LEADER IN THE DEVELOPMENT OF COHERENT X-RAY DIFFRACTION FOR IMAGING BIOLOGICAL STRUCTURES



# ABOUT CXS

The Australian Research Council (ARC) Centre of Excellence for Coherent X-ray Science (CXS) brings together leading Australian researchers in the fields of X-ray physics; the design and use of synchrotron radiation sources; and the preparation, manipulation and characterisation of biological samples.

Its aim is to open a new frontier in biotechnology – the non-crystallographic structural determination of membrane proteins. These proteins mediate the activity of pharmaceuticals in human medical therapies. Their structures, however, are still mostly unknown because they do not form crystals suitable for analysis using the conventional crystallographic techniques that have driven almost all the progress in structural biology. A breakthrough in this area would revolutionise rational drug design through the insight gained into the function of membrane proteins. This would have far-reaching consequences for the pharmaceutical industry. CXS's research is driven by its access to existing third-generation synchrotron light sources and to the Australian Synchrotron. CXS is also exploring the application to imaging problems of short wavelength high-harmonic generation sources and free-electron X-ray lasers that are under development worldwide.

When combined with non-crystallographic diffractive imaging techniques, the brightness and intensity of these sources gives us the opportunity to take snapshots of biomolecules. CXS is exploring the fundamental issues in the use of these light sources, including the nature of the interaction between intense coherent X-rays and electronic matter. The efficiency of diffraction processes in these highly coupled light-matter systems, the detection of the scattered light, the preparation and handling of suitable biological samples, the management of radiation damage throughout the interaction, and the design of algorithms to extract structural information from diffraction data is also under exploration.

It is an ambitious interdisciplinary program of research.

# DIRECTOR'S REPORT

CXS can reflect on 2010 as another year of outstanding achievement. The centre continues to adhere to its commitment to achieve an inter-disciplinary research collaboration of the highest international standing, while engaging with the broader Australian community through its outreach program.

Without question, one of the highlights of 2010 was the privilege of hosting a lunch with Nobel Laureate, Professor Ada Yonath, at the Lorne Protein Structure and Function Meeting in February. Professor Yonath spoke inspirationally to a group of women in protein science of her passion for research in biomolecular chemistry.

A similar passion for curiosity-driven research was uncovered in a group of secondary school students through the Centre's *Growing Tall Poppies (GTP)* initiative, a partnership with Santa Maria College, Northcote, CXS and Akorn Education Services. The GTP program provides an authentic research experience for students to demystify the transition between secondary school and a university environment. It is clear from participants' comments that the program is of immense value to both the students and their CXS mentors. We were particularly fortunate to have had the active participation of two distinguished physicists, Professor Henry Kapteyn and Professor Margaret Murnane of the University of Colorado, in our end of year *GTP* Conference event. Margaret gave the students a personal and uplifting insight into her research as a physicist, encouraging all of the students at the event to persevere if their goal is to achieve a career in science.

The video *"Röntgen: A bright spark"*, produced as part of our outreach program, won the prestigious 'Sleek Geek' Eureka Prize for 2010. Developed by our node at La Trobe University and ten students from St Helena's School, the film making project was funded by CXS and the Victorian Department of Education and Early Childhood Development. Winners were thrilled to attend a glamorous award ceremony, where they met the original Sleek Geek, Dr Karl Kruszelnicki and a number of eminent Australians. We are extremely proud of the success of our outreach program and the positive impact that it is having on the lives of young Australians.

The outstanding achievements of CXS members were recognised by a number of prestigious awards during 2010. The Deputy Director, Professor Leann Tilley, was awarded the Bancroft-Mackerras Medal of the Australian Society of Parasitology for her contributions to malaria Research. Associate Professor Rob Scholten was awarded the Alan Walsh Medal for his services to industry by the Australian Institute of Physics and made a presentation to the AIP Congress about MOGlabs, the company formed to commercialise this research. Dr Connie Darmanin received the 2010 Payne-Scott Award which commemorates a champion of the rights of working women at CSIRO. We also heartily congratulate Associate Professor Andrew Peele, the leader of the CXS Experimental Methods Program, on his appointment in December 2010 to a national leadership role as the Science Director of the Australian Synchrotron.

The Centre is fortunate to have access to the wise counsel of a Scientific Advisory Board whose members are deeply committed to the continuing success of CXS. I would like to take this opportunity to thank Professor John Helliwell, Dr Stephen Lane and Professor Bonnie Wallace for their continued involvement with our activities, over what is now a long period of time and over distances that have always been very long indeed.

A number of initiatives were undertaken in 2010 in the field of super-resolution fluorescence microscopy, in which we are providing leadership to the Australian biological imaging community. CXS organised a Super-Resolution Optical Microscopy Workshop at the Melbourne Convention Centre on 1 October as part of the international OzBio2010 Conference. The workshop was attended by leading physicists, chemists and biologists in this field and offered a glimpse of the potential of these emerging imaging technologies. The centre is actively participating in the development of these technologies through its laser physics programs and its management of the Cellular Nano-Imaging Consortium (CNIC). We are committed to ensuring that all Victorian

scientists have access to the super-resolution microscopy tools they require to be internationally competitive as research leaders.

The application of X-ray free electron laser technologies in biological imaging promises to revolutionise the determination of biomolecular structures for rational drug design in pharmacology. I am, therefore, delighted that CXS signed a memorandum of understanding with our colleague, Professor Tetsuya Ishikawa of the RIKEN Harima Institute, Spring-8 Centre, Japan. Our regional engagement included the inclusion of CXS members as invited speakers at the 3rd Asia-Pacific Workshop on FEL Science in Hokkaido during October. We will reciprocate this honour by organising the 4th workshop in the series in Cairns in August 2011.

This was the first year in which the Attosecond Physics Program at Griffith University has been an official node of CXS and we warmly welcome group leaders Associate Professor David Kielpinski and Associate Professor Robert Sang, with their staff and students to the centre. This project is already producing impressive results, including fundamental studies of the strong-field interaction physics of atomic hydrogen and a scheme to measure time intervals measured in zeptoseconds. The group were gracious hosts of an enjoyable workshop in Brisbane and we are looking forward to a fruitful collaboration involving our newest CXS node.

The research across CXS has been outstanding, as evidenced by the body of published work featured in highly ranked journals. There have been numerous invitations to participate in international conferences and we have developed a stronger presence within the international X-ray and optical imaging communities. It is particularly pleasing to note that CXS can now point to strong collaborations and a number of inter-disciplinary publications across the physical, chemical and biological sciences. It is my view that this success is a direct consequence of the openness of our collaboration and the importance that we place on frequent communication between the nodes. It hasn't always been an easy process, but there is no doubt that our claim to be world-leaders in this field is supported by our achievements.

I wish to thank all of the participants of CXS for creating and maintaining such an exciting and vibrant research environment. Having reviewed the content of the Annual Report for 2010, my excitement and enthusiasm for our work is renewed by seeing what can be achieved when physicists and biologists set their minds to working together. It is a strong indication that the collaborations within CXS are growing stronger and is a positive indicator for the success of future interdisciplinary collaborations within Australia.

PROFESSOR KEITH NUGENT
DIRECTOR

## CXS WELCOMES GRIFFITH UNIVERSITY

"THE INCLUSION OF THE ATOMIC AND LASER PHYSICS PROGRAM AT GRIFFITH WITHIN CXS WILL ENABLE US TO ENGAGE IN FUNDAMENTAL STUDIES OF STRONG-FIELD INTERACTION PHYSICS AND ATTOSECOND PHYSICS IN SIMPLE ATOMIC AND MOLECULAR SYSTEMS."

ASSOCIATE PROFESSOR HARRY QUINEY

INCREASING

# RESEARCH PROGRAMS

## ATTOSECOND SCIENCES PROGRAM

The Attosecond Science program (ASP) at Griffith University, Brisbane, Australia, began collaborating with CXS in June 2009. The program offers new opportunities for coherent X-ray science that are unique within Australia. The innovative and rapidly expanding field of attosecond science is based on recent revolutionary developments in ultrafast optics that gave rise to the award of the Nobel Prize in 2005.

It is now possible to generate high-energy infrared light pulses consisting of only a few cycles of the electric field and to control the optical electric field waveform within the light pulses. Such optical pulses have been used to generate isolated soft X-ray bursts with durations below 100 attosecond (1 as = $10^{-18}$ s). The pulses can also provide information on atomic and molecular dynamics on the attosecond timescale and have been used to map the electronic structure of molecules. The Australian Attosecond Science Facility (AASF) is the unique vehicle in Australia for attosecond science investigations. The facility is directed by Associate Professor Kielpinski, who has lead the ASP since January 2010. Fundamental to the research undertaken by the facility is a laser source providing 6 fs, 300 µJ, phase-stabilised laser pulses. This core equipment was commissioned in 2007 through an ARC LIEF grant.

In 2009, the AASF experimental group began a close collaboration with the CXS Theory and Modelling Program (TMP) group, to investigate the response of atomic hydrogen to strong few-cycle laser pulses. Atomic and molecular dynamics in strong optical fields plays a crucial role in many CXS activities, from the Biological Sciences Program's goal of molecular structure retrieval from single-molecule X-ray diffraction, to the high-harmonic generation work of the Short Wavelength Laser Source program (SWLP). However, theory and experiment in this area rarely give quantitative agreement. As the only attosecond science group with access to atomic hydrogen, the AASF group has a unique opportunity to benchmark strong-field theories with the help of the TMP.

As part of CXS, the AASF group will also pursue the generation of isolated attosecond X-ray pulses, which have already proved useful as tools for probing electronic structure of atoms, molecules, and surfaces. Currently only four research groups in the world have this capability. Isolated attosecond pulses can help unravel the problem of nonlinear X-ray back-action on molecular diffraction imaging, a key step in realising CXS goals in bio-molecular structure determination. Modelling of back-action during the long X-ray pulses from synchrotrons and free-electron lasers (FELs) requires simultaneous incorporation of several mutually interacting, many-body effects – a highly challenging task. In contrast, attosecond pulses provide a window into short-time dynamics, effectively decoupling the many-body effects. Attosecond interactions can also selectively incorporate or exclude particular processes. Although the total energy delivered in an attosecond pulse is much lower than that expected at a FEL, the peak X-ray intensity can be nearly as high because of the short pulse duration.

### GOALS

The goals of the Attosecond Science program are twofold:

1) To generate isolated attosecond pulses of XUV light for time-resolved X-ray science. Such pulses are presently the unique means of access to attosecond dynamics and are currently available at only four laser facilities worldwide. CXS anticipates that XUV pulses of duration <500 as and peak intensities of 100 $GW/cm^2$, with wavelength in the

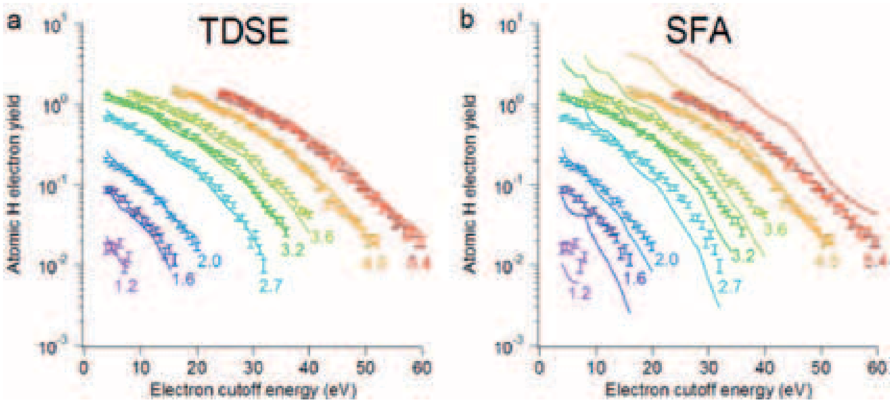



*Figure 1.*

10-20 nm range, will be achievable in the next two years. Isolated attosecond pulses have already proved useful as tools for probing electronic structure of atoms, molecules, and surfaces. CXS will extend these studies to specific chemical and biological applications of interest to Centre members. Due to attosecond science being so new, basic experimental methods are still under development. Close collaboration between our group and end-users in CXS will prove essential in realising the promise of attosecond science, with new methods rapidly taken up worldwide. The centre has already generated XUV radiation at wavelengths as short as 30 nm by focusing the AASF laser through an argon gas jet and are preparing to investigate the temporal properties of the XUV light. Nonlinear X-ray processes in helium gas have been observed in other laboratories with isolated attosecond pulses and will be readily observed for the much larger dipole moments of chemical and biological samples. Generalising commonly used ultrafast pump-probe techniques to the attosecond domain will eventually enable us to evaluate the full dynamic structure factor of chemical and biological samples under X-ray irradiation for detailed comparisons with back-action models.

2) To investigate the effects of atomic structure on strong-field interactions through quantum control of the ultra-cold metastable neon atoms currently generated in our laboratory. High-order harmonic generation (HHG) data on the exotic electronic structure of metastable neon will be critically sensitive to poorly understood atomic physics effects in HHG. Quantum state control of the atoms involved in HHG isolates specific atomic processes for detailed tests of theoretical HHG models. The novel computational techniques of the TMP group will transform data into optimised designs for HHG-based X-ray sources of specific spectral and temporal characteristics. The SWLP can take advantage of these designs for their XUV imaging source, tailoring the XUV source to their particular goals.

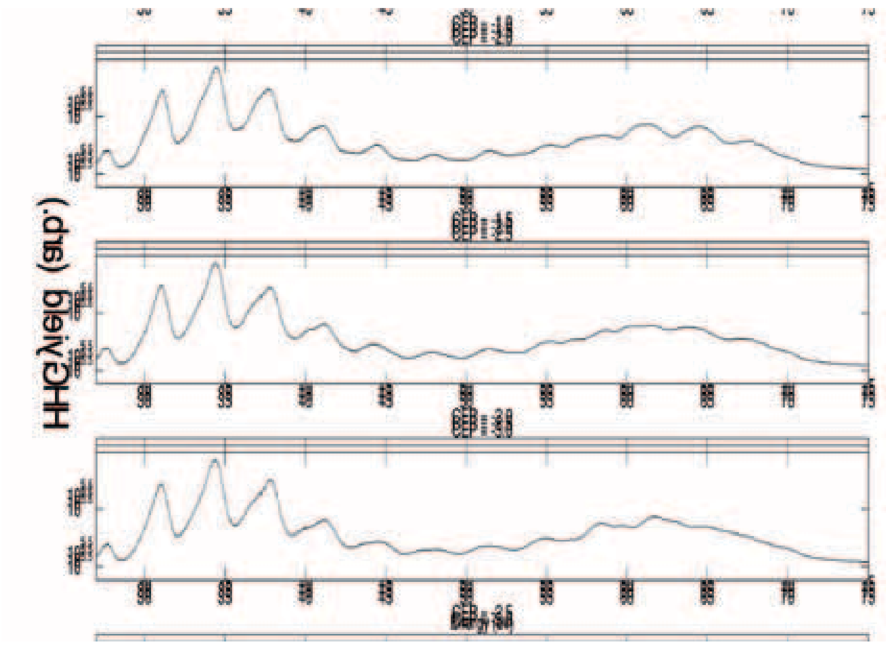
The single-atom dipole response to a strong IR field is a crucial input for optimisation of HHG sources. A recent experiment has shown that interference between the dipole amplitudes of different atomic species in a mixture of gases can boost HHG by over three orders of magnitude. However, the standard theory of HHG is inadequate for describing atomic structure effects and numerical predictions of the HHG spectrum vary by a factor of two (2) according to the exact methods used. The CXS TMP group has developed a new theory of atomic HHG based on free-field atomic states. Current experimentation examines ionisation of atomic hydrogen with few-cycle laser pulses, and the TMP is currently working to interpret these results. Because the hydrogen atom is so simple, subsequent HHG studies in hydrogen will be an excellent benchmark for HHG modelling.

## FEW-CYCLE PHOTOIONISATION OF ATOMIC HYDROGEN

The interaction of intense few-cycle laser pulses and matter gives access to electronic, molecular and condensed matter dynamics that happen on timescales below $10^{-15}$ seconds. Commonly used data, for example, the energy-momentum distribution of the freed electrons, encode structural and dynamic information through the non-perturbative laser-atom interaction, and retrieving the physical quantities of interest requires detailed and accurate simulations of the electronic dynamics. The strong-field regime is particularly challenging for simulations as a photoelectron driven by intense long-wavelength radiation can travel a distance hundreds of times further than the size of the parent atom. Complete simulations can be carried out only for atomic H due to its simple electronic structure.

CXS has performed the first successful comparison between experiment and theory on ionisation with few-cycle pulses by using an atomic hydrogen target. The comparison incorporates a detailed model of detector response and laser intensity effects. Figure 1 demonstrates the excellent agreement between experimental data and *ab initio* simulations performed at the Australian National University and at Drake University (USA). Remarkably, only two global fit parameters are needed to obtain agreement at the 10% level over >250 data points. Comparison to a calculation of the widely-used strong-field approximation shows poor agreement. The few-cycle

*Figure 2: XUV generation with phase-stabilised IR pulses. The absence of fringe contrast indicates generation of isolated attosecond pulses in the 50-70 eV range.*



experiment is able to discriminate between competing theories of this fundamental situation in strong-field atomic physics. Other phenomena of strong-field few-cycle interactions, such as high-harmonic generation and molecular orbital tomography, may now be approached accurately and quantitatively using CXS methods. The precise calibration of CXS apparatus using atomic hydrogen, provides confidence in the quantitative accuracy from the same apparatus when using other atomic or molecular targets.

## TOWARD ISOLATED ATTOSECOND LIGHT PULSES

When an atom is exposed to an electric field in which the field strength approaches the Coulomb binding energy of the outer electrons, the atom can undergo tunnel ionisation. The free electron will then be accelerated away from the atom by the electric field and, depending on the time of ionisation, will undergo one of several processes. High-order harmonic generation (HHG) occurs when the free electron recombines with its parent ion, resulting in the emission of an extreme ultraviolet (XUV) photon with energy determined by the trajectory of the electron in the accelerating electric field. When the laser pulse is only a few optical cycles long and the laser carrier-envelope phase is stabilised, it is possible to create an isolated XUV pulse of attosecond duration by gating the HHG emission. Such isolated attosecond pulses are now widely used to study physical, chemical, and biological phenomena.

The laser system at the Australian Attosecond Science Facility is the only one in Australia with the requisite degree of control to achieve attosecond XUV pulses. CXS has observed the characteristic signatures of the gating process: the oscillation of high-harmonic fringe contrast with carrier-envelope phase and the appearance of a smooth spectrum at high energies for particular phases (see Fig. 2). Direct temporal characterisation of the pulses has not yet been achieved, but is planned for 2011. While in principle, incoherent XUV emission from plasma could also generate smooth spectra, this mechanism was ruled out by observing that the XUV intensity dropped to zero when the laser polarisation was rotated slightly.

## ATTOSECOND SCIENCES PROGRAM CASE STUDY

### ZEPTOSECOND TIMING RESOLUTION FROM A GOUY PHASE INTERFEROMETER

The ASP has demonstrated a new interferometry technique that uses the Gouy phase shift of a focused laser beam to generate two HHG pulses with a tightly controlled variable delay. The Gouy effect is a rather esoteric consequence of the theory of focused laser beams, with few known practical applications. Simply put, the velocity of light near a focus is slowed down very slightly as compared to unfocused light. This velocity shift affects the carrier-envelope phase of the laser and therefore the HHG generation.

In the experiment, a few-cycle laser pulse is focused through two gas jets with a variable separation. An HHG pulse is created when the laser passes through the first jet. The laser and HHG propagate together to the second jet. The laser pulse is still focusing and slows down due to the Gouy effect, but the HHG pulse is unfocused and does not. When the laser pulse strikes the second jet, another HHG pulse is created slightly before the first pulse. The time delay is set by the distance between the two jets and can vary up to one (1) femtosecond – much longer than the duration of isolated attosecond pulses. The time delay can be measured by observing the oscillations of the HHG spectrum with varying jet distance.

# BIOLOGICAL SCIENCES PROGRAM

Methods for imaging cellular architecture and, ultimately, macromolecular complexes and individual proteins within a cellular environment are important goals for cell and molecular biology. The Biological Sciences Program (BSP) involves the participation of biochemists, structural biologists and cell biologists who are undertaking specific research in the biomedical area. As part of work undertaken within CXS, BSP members collaborate closely with members of the Experimental Physics Program in the development and implementation of novel imaging techniques to provide new insights into the structures of cells and cellular compartments. Members of this program also interact with members of the Structure Determination Methods Program and the Theory and Modelling Program (TMP) to optimise techniques for determining the structures of membrane proteins and other components of biological interest.

The groups within this program conduct world-class research in the following areas:

## MALARIA AND REMODELLING OF THE RED BLOOD CELL

The most deadly of the human malaria parasites, *Plasmodium falciparum*, invades red blood cells (RBCs) and initiates a remarkable series of morphological rearrangements. The mature red blood cell is effectively a floating sack comprising a membrane that encloses the oxygen-transporting protein, haemoglobin. Unlike other cells, RBCs have no nucleus and cannot make or traffic proteins. In order to colonise and remodel the red blood cell, the parasite generates a series of novel structures that are involved in the export of virulence proteins to the surface of the host cell. These include extensions of the parasite's vacuolar membrane, known as the tubulo-vesicular network, and structures referred to as Maurer's clefts. These membrane structures play an important role in the trafficking of virulence proteins to the host cell surface, however their ultrastructure is only partly defined and there is on-going debate regarding their origin, organisation and connectivity. Parasite endocytic processes are also poorly understood. The parasite consumes host haemoglobin in order to support its own growth. Packets of hemoglobin are transferred from the host cell cytoplasm to a parasite digestive vacuole for hemoglobin digestion and heme detoxification, however the precise mechanism for uptake is debated. One of the aims of CXS is to image these compartments and to develop an understanding of their function and the way in which they are formed. Such research could lead to new avenues for drug and vaccine design to combat the serious problem of malaria.

## MITOCHONDRIA: UNDERSTANDING THE POWERHOUSE AND THE POISON CUPBOARD

Mitochondria are the generators within our cells, synthesising chemical energy in the form of the molecule ATP. They also act as 'poison cupboards', releasing certain proteins that kill cells as part of programmed cell death if the mitochondrial outer membrane is opened. Defects in mitochondria cause energy-generation disorders and are also implicated in other conditions, including Parkinson's and Alzheimer's disease. In addition, efforts to activate the machinery involved in mitochondrial permeation can act as anti-cancer agents Work within CXS seeks to understand some of the events involved in remodelling mitochondrial membranes during disease and to provide potential new insights into the formation of pores that lead to cell death. In addition, work is being undertaken to provide insight into the structure of mitochondrial membrane proteins and their complexes.

## GOALS:

- Prepare and optimise cellular samples for use as test-beds for X-ray coherent diffraction imaging and for other pioneering imaging techniques.

- Use X-ray imaging and other imaging modalities to gain novel insights into cellular architecture and function.
- Prepare samples of soluble and membrane proteins and determine their structural characteristics using both conventional and novel X-ray-based approaches.

## ACHIEVEMENTS

The malaria parasite *Plasmodium falciparum* has been imaged using cryo transmission X-ray microscopy, and the data have been correlated with other imaging modalities. In particular, the group has performed pioneering efforts in the development of whole cell electron tomographic imaging, X-ray tomographic imaging and super-resolution fluorescence imaging.

## IMAGING INSIDE CELLS

Three-dimensional structured illumination microscopy provides resolution beyond the optical diffraction limit and permits analysis of fluorescently labelled whole cells. Cryo transmission x-ray microscopy in the "water window" of photon energies has recently been introduced as a method that exploits the natural contrast of biological samples. Immunoelectron tomography offers the possibility of high resolution imaging of individual ultrastructural features in a cellular context. Combined with serial sectioning and immunogold labelling, this technique permits precise mapping of whole cell architecture.

*P. falciparum* develops within human RBCs. As it grows, the parasite establishes a membrane network outside its own limiting membrane in the cytoplasm of its host cell. These membrane structures play an important role in the trafficking of virulence proteins to the host cell surface. Using serial section electron tomography and super-resolution optical microscopy Dr Eric Hanssen, Dr Bohumil Maco and Dr Nick Klonis showed that the exported secretory system of *P. falciparum* comprises a series of modular units, comprising Golgi-like structures, known as Maurer's clefts, tubular connecting elements, two different vesicle populations and electron-dense structures that have fused with the erythrocyte membrane. The membrane network is not continuous, pointing to an important role for vesicle-mediated transport in the delivery of the virulence determinant, PfEMP1, to the RBC surface. Super-resolution optical microscopy and electron tomography showed that the membrane complex is assembled at the apical end of developing daughter cells and plays a role in merozoite invasion of RBCs.

Electron tomography studies provided novel insights into the process of haemoglobin digestion by the malaria parasite. The work shows that haemoglobin digestion is initiated in the early ring stage of development in pre-digestive vacuoles, which then fuse to form a mature digestive vacuole. The timing of the onset of haemoglobin digestion has implications for the action of anti-malarial treatments. The BSP solved the structure of the malaria parasite pigment, hemozoin, revealing novel hematin-hematin self-association states that initiate and stabilise the crystal.

The BSP has also undertaken techniques to image mitochondria and their re-modelling within cells. This has been performed using electron microscopy and novel confocal techniques that utilise photo-conversion of specific fluorescent proteins to track intracellular movements. PhD students Laura Osellame and Catherine Palmer have also investigated the function of two novel membrane proteins that are located on patches on the mitochondrial surface and are involved in causing mitochondrial division. They have identified patches on these proteins on the mitochondrial surface, which recruit the Dynamin related protein 1. Structural studies on these proteins are underway (see below). We are also investigating the localisation of other proteins – including cell death proteins – on the mitochondrial surface.

## STRUCTURAL AND BIOCHEMICAL STUDIES OF MEMBRANE PROTEINS

Using biochemical methods, we have investigated the association of the cell death protein, Bak, at the mitochondrial surface. We have found that that Bak is prevented from undergoing accidental activation by binding to a membrane protein termed VDAC2 (voltage-dependent anion channel 2). We are now purifying and reconstituting the Bak-VDAC2 complex to understand how they interact together. This is being conducted by Dr Diana Stojanovski and Mr Boris Reljic. The structure of membrane proteins Tom40 and its relationship to a mouse model of disease is also being investigated. This is being performed by Ms Viviane Richter who joined Mike Ryan's group in 2011 as a new PhD student.

G-protein coupled receptors (GPCRs) belong to a large group of membrane proteins that are involved in various physiological functions. They have a common structure that is composed of 7-transmembrane alpha-helices. This family of membrane proteins represents targets for 50% or more of pharmaceutical drugs. Despite the importance of GPCRs in drug development, only a few structures have been solved. GPR41 and GPR43 are activated by short chain fatty acids. Lynn Liang, a PhD student supervised by Connie Darmanin (CSIRO) and Leann Tilley, has been optimising conditions to express and purify GPR41 and GPR43 in baculovirus/insect expression systems. She has purified the proteins and examined lipidic cubic phases to facilitate meso crystallisation of these GPCRs. The bicontinuous cubic phase consists of curved lipid bilayers extending in three dimensional with interpenetrating but unconnected water channels. The group has also optimised the expression and purification of new proteins involved in the assembly of the first mitochondrial generator, Complex I. These proteins are now being used in crystallisation trials for conventional protein structure determination and can be used as future test beds with complex I assemblies in novel X-ray approaches.

## BIOLOGICAL SCIENCES PROGRAM CASE STUDY

### WATER WINDOW CRYO TRANSMISSION X-RAY MICROSCOPY

Cryo transmission X-ray microscopy in the "water window" of photon energies has recently been introduced as a method that exploits the natural contrast of biological samples. With Professor Carolyn Larabell from the, National Center for X-ray Tomography, Lawrence Berkeley Laboratory, USA, The BSP has undertaken tomographic X-ray imaging of whole hydrated cells at different stages of growth of *P. falciparum*. The group examined whole hydrated cells and defined some of the structures with different X-ray density, including the parasite nucleus, cytoplasm, digestive vacuole and the hemoglobin degradation product, hemozoin.

It was shown that as the parasite develops from an early cup-shaped morphology to a more rounded shape, puncta of hemozoin are formed and subsequently coalesce in the mature trophozoite into a central compartment. In some trophozoite stage parasites, invaginations of the parasite surface were observed and, using a selective permeabilization process, it was shown that these remain connected to the RBC cytoplasm. Some of these invaginations have large openings consistent with phagocytic structures and we observed independent endocytic vesicles in the parasite cytoplasm which appear to play a role in hemoglobin uptake. In schizont stage parasites, staggered mitosis was observed and X-ray-dense, lipid-rich structures were evident at the apical ends of the developing daughter cells. Treatment of parasites with the antimalarial drug artemisinin appears to affect parasite development and their ability to produce the hemoglobin breakdown product, hemozoin.

*Figure A: The design of new branch on the soft X-ray beamline at the Australian Synchrotron is underway. This will be the new home for our FRIEND endstation.*

# EXPERIMENTAL METHODS PROGRAM

The Experimental Methods Program (EMP) group has members based at La Trobe University, the University of Melbourne and Monash University. Through its broad spectrum of members and activities, the EMP is ideally suited for interaction across other CXS programs. Through regular interaction with the Theory and Modelling Program (TMP), the EMP has established standing research activities with most of the other programs in CXS:

**Biological Sciences Program (BSP)** – this is a fundamental CXS interaction. The work of the EMP focuses on three-dimensional imaging of biological samples. This foundation work is undertaken by both the EMP and BSP groups and is assisted by the TMP in analysing imaging data obtained from biological samples. With the bulk of their membership based at La Trobe University, EMP and BSP staff meet regularly to develop imaging methods. To further facilitate the mutual translation of physics and biology, BSP staff attend EMP experiments at overseas facilities, which has led to the exploration of access to other imaging techniques and facilities around the world. The EMP and BSP also hold seminars and meetings designed to encourage interaction and collaboration between students and staff from the two groups. In 2010, ARC Super Science Fellow Michael Jones was jointly appointed between the EMP and BSP to work on nano-imaging the cellular architecture of the malaria parasite.

**Theory and Modelling Program (TMP)** – The TMP and EMP regularly collaborate. The EMP provides experimental data to which the TMP can apply new methods of analysis, and the TMP provides new directions for the experimental work. Members of the TMP are co-located at the University of Melbourne with several of the EMP group, enabling easy and frequent interaction.

**Short Wavelength Laser Source Program (SWLP)** – the SWLP provides a novel source of coherent photons at wavelengths approaching X-ray. With strong interaction from the TMP and the EMP, the SWLP has a standing experimental activity based around pursuing the limits of imaging with these sources.

The design of new branch on the soft X-ray beamline at the Australian Synchrotron is underway. This will be the new home for our FRIEND endstation (Fig. A).

**Ultra-Cold Plasma Source Program (UPSP)** – the UPSP was formed within CXS to exploit techniques developed by the EMP and TMP that demonstrate imaging using a bright coherent source of high-energy electrons. With membership based at the University of Melbourne, the UPSP and EMP have a high degree of interaction.

## ACHIEVEMENTS

In 2010 the EMP conducted a wide range of experiments at several facilities, (synchrotron sources in four different countries, the X-ray free electron laser at Stanford and the Swinburne High Harmonic Generation source), as well as consolidating its activities in commissioning the Fresnel Imaging ENDstation (FRIEND) at the Advanced Photon Source in Chicago. During this time, the EMP has published several results demonstrating new optical techniques. The group has also made major progress toward making Fresnel coherent X-ray diffractive imaging an efficient technique of utility in materials and biological sciences.

In addition to its stated program goals, the EMP continued its overall mandate to explore new methods in imaging and coherence. Some of the results achieved in 2010 include:

- Extending methods based on the understanding of the role of partial

*Figure C: The first Fresnel coherent X-ray tomographic reconstruction of a test object in the soft X-ray regime (Isaac Peterson, unpublished). The diameter of the object is 4 μm at its base. Data were collected with FRIEND at the Advanced Photon Source.*

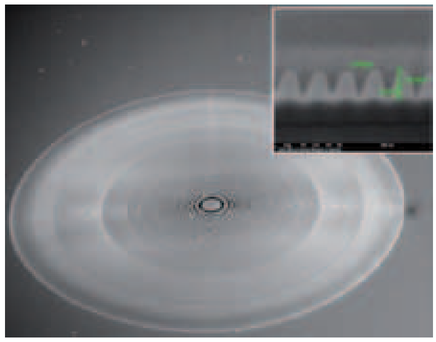


*Figure Y: High resolution 92 um diameter Fresnel zone plate (15 nm outer zone width) fabricated with focussed ion beam milling. The inset shows that a sinusoidal high-resolution profile is achievable.*

coherence in image formation to demonstrate coherent diffractive imaging with partial temporal coherence (B. Abbey).

- Performing the first tomographic FCDI reconstructions in the X-ray regime (I. Peterson).
- Presenting the first demonstration of biological tomographic FCDI, with a reconstruction of malaria infected red blood cells at spatial resolution better than 30 nm (G. Cadenazzi). This demonstrates achievement of one of the key goals of the EMP.
- Creating measurements to characterise the coherence properties of the SXR beamline at the first X-ray Free Electron Laser, LCLS. This was done as part of an international collaboration.
- Demonstrating novel momentum-transfer X-ray diffraction imaging techniques by imaging embedded nano-objects with resolution below 10 nm (A. Nikulin, D. Pelliccia).

## NEW FRIENDS, NEW PLACES

The FResnel Imaging ENDstation (FRIEND) is a custom-designed experimental that was commissioned at the Advanced Photon Source in Chicago under a partnership arrangement. It comprises twenty-one axes of motion, all housed within a vacuum chamber, for positioning optics, specimens and detectors in various geometries to accommodate three common implementations of diffractive imaging: plane wave illumination; scanning focused (ptychographic); and defocused-probe (Fresnel diffractive imaging). The strength of FRIEND lies in its stability and in-vacuum operation which allow for long exposure times, high signal-to-noise and large dynamic range, two-dimensional intensity measurements to be acquired.

In 2010 CXS used FRIEND to demonstrate for the first time, several extremely challenging measurements that combine phase-diverse and tomographic methods with Fresnel coherent diffractive imaging. To allow experimentation to keep pace with the developments in the theoretical description of coherent X-ray imaging by CXS, major upgrades to the facility commenced during 2010 that will continue through the next year. Importantly, these developments will draw upon the collective experience in CXS to deliver to the Australian Synchrotron a facility routinely capable of extremely high resolution diffractive imaging to meet the ever-growing demand from biological sciences.

In parallel, we have begun work towards FRIEND2, a new endstation that will be more stable and flexible than its predecessor, and will allow specimens to be cryogenically cooled.

Having achieved the aims of our partnership with the Advanced Photon Source, our period of Partner User access to beamtime will soon draw to a close. However, as one door closes, another opens. The EMP has been working with Australian Synchrotron scientists and engineers to build a new permanent home for FRIEND at the end of a new branch on the Soft X-ray beamline and FRIEND will be relocated to the Australian Synchrotron in 2012.

## ANOTHER DIMENSION TO FRESNEL COHERENT DIFFRACTIVE IMAGING

The use of coherent diffraction microscopy employing brilliant synchrotron sources is now well established for 2D imaging of materials science and biological samples at resolutions below 100nm. The penetrating power of X-rays also opens the possibility of 3D tomography techniques. Important progress has been made with iterative reconstruction algorithms that provide significant computational advantages in Fresnel coherent diffraction imaging (FCDI) tomography (Corey Putkunz). We have experimentally demonstrated these advantages with both laser light and synchrotron soft X-ray radiation (Fig. C).

In conventional 3D CDI, an object must be isolated so as to be contained within the illumination. Only then can the corresponding, highly detailed, coherent diffraction pattern be adequately sampled by the detector. This prevents the approach familiar to microscopists of obtaining low resolution images over a large area and then zooming into regions of interest. Adaptation of CDI to image extended objects was earlier made possible by CXS researcher Brian Abbey, who recognised that an illumination which is finite in extent also satisfies the conditions necessary to reconstruct an image of the object. Another approach to imaging objects that extend beyond the illumination is ptychography,

*Figure Z: 3D phase retrieval images after segmentation step for 200µm aerosil grain (upper) and 300µm cylindrical structure of secondary osteon in human cortical bone (lower).*

*Dr Mark Junker.*

whereby a multitude of diffraction patterns from overlapping regions of the sample are recorded (work by David Vine). This has been generalised in FCDI though the concept of phase diversity that includes not only lateral translation of the illumination on the sample, but also longitudinal translation which affects the curvature of the illuminating wavefield (Corey Putkunz). Our work with these methods has culminated in 2010 with FCDI tomography at FRIEND incorporating phase diversity methods at each of the many projections comprising a tomographic measurement of malaria infected red blood cell. A spatial resolution of 30nm was achieved.

## EXPERIMENTAL METHODS PROGRAM FACILITIES

In addition to FRIEND, the EMP maintains laboratory facilities for microfabrication (Eugeniu Balaur) where novel X-ray optics and bespoke samples for experiments are created; where student projects can be tested prior to a synchrotron visit and for tomography (Benedicta Arhatari); and where conventional X-ray imaging and 3D methods can be trialled. Very productive work in these laboratories was performed in 2010, producing papers, data and samples for CXS research. In one example of microfabrication, high resolution Fresnel zone plates were fabricated by simply milling a layer of gold sputtered onto a membrane using a focused ion beams (Fig. Y).

## PHASE TOMOGRAPHY

During 2010, the EMP studies using lab based X-ray sources focused on the development and testing of the theory of optimising phase imaging performance. Test work was performed using simulation, experimental and analytic work to analyse models of coherence and polychromaticity as applied to general imaging. The studies of the imaging system were undertaken in the presence of real world systematics and demonstrate 3D phase imaging of real samples (Fig. Z).

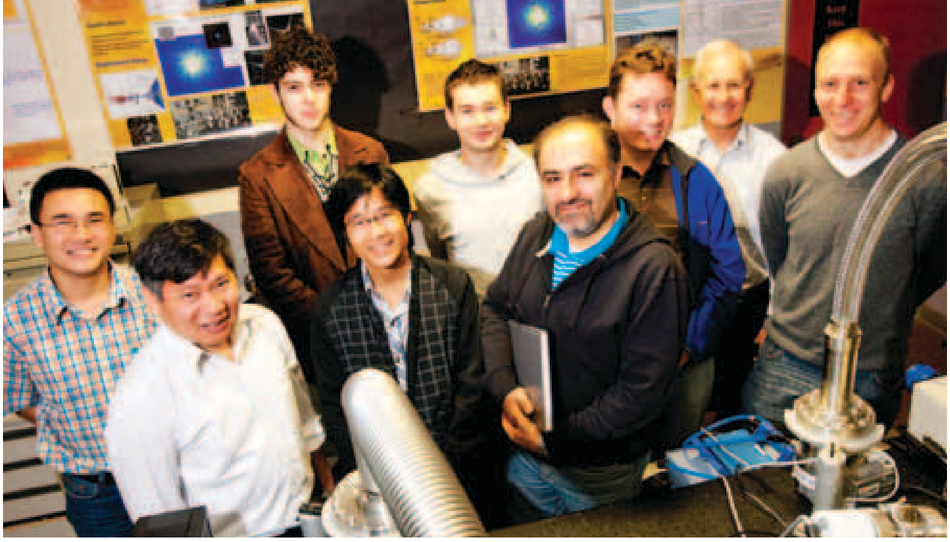
## HIGH RESOLUTION DIFFRACTOMETRY AND IMAGING

The experimental activity at Monash University during 2010 focused on the combination of high resolution diffractometry techniques and x-ray imaging. Of particular note is a novel momentum-transfer X-ray diffraction imaging technique, which has proven to be extremely powerful through its imaging of embedded nano-objects with resolution below 10 nm (Andrei Nikulin). The method is based on the collection of the diffracted signal from large number of nanoparticles in a matrix. The diffraction pattern is recorded directly in the momentum transfer space using a crystal analyser and a detector as in reciprocal space mapping. The corresponding real space image is then reconstructed applying phase retrieval methods as in CDI. Most recently, the technique has been used to measure the statistically average form, orientation and dimensions of nanoscale precipitate structures embedded in a continuous matrix (Daniele Pelliccia).

The maximum length scale of the statistical information that can be gained by this new approach depends on the available coherence length of the X-ray beam. Limitations are expected, therefore, for highly poly-disperse samples. We again find that the problem can be alleviated by the knowledge of the coherence properties of the beam. Methods have been developed to quantify the coherence properties of both laboratory and synchrotron X-ray sources to enable partial coherent x-ray diffraction imaging experiments (Daniele Pelliccia).

## FROM SYNCHROTRONS TO FREE-ELECTRON LASERS

In 2010 EMP researchers (B. Abbey and G. Cadenazzi) participated in an experiment at the first soft X-ray free electron laser, the Linac Coherent Light Source (LCLS) facility, at Stanford Linear Accelerator Centre in the United States. The aim of the experiment, led by Prof. Ivan Vartanyants (DESY, Hamburg), was to characterise the coherence properties of the soft X-ray femtosecond pulses at the LCLS. This experiment successfully demonstrated that the pulses measured at the SXR beamline had a high degree of coherence (75%) essential for phasing the scattered intensity distribution from a sample.



*The Short Wavelength Laser Source Program Team.*

# SHORT WAVELENGTH LASER SOURCE PROGRAM

The SWLP has investigated the generation of extreme ultraviolet (XUV) and soft X-ray pulses by high harmonic generation (HHG) and applied these sources in atomic and molecular spectroscopy, condensed matter physics, and imaging on the micron- and submicron-scale. These compact, (table-top), femtosecond pulsed sources will complement imaging studies using X-ray free-electron laser (XFEL) sources currently under development at large international facilities.

By their nature, HHG sources produce a laser-like beam that consists of a number of harmonic orders. Therefore, a harmonic source with just a few intense orders (ideally a single harmonic order) may be advantageous for many applications as they can be used directly without additional spectral selection optics.

The high harmonic generation process can be explained in terms of a semi-classical three-step model. In this model, under interaction of a strong laser field, the active electrons first tunnel through the potential barrier, are then accelerated in the first half of the optical cycle of the laser field, and then are pulled back and finally recombine with parent ions to emit high-energy photons in the second half of the cycle. The electronic acceleration processes and the variation of the molecular or atomic ground state throughout the interaction with the driving laser field play important roles in quantum systems and need to be studied in more detail.

Unlike atoms, molecules are not spatially isotropic systems. For randomly aligned molecules, their HHG spectrum has been shown to have characteristics similar to that produced by atoms, but for aligned molecules, which can be realised by using another laser field, the HHG, which is influenced by the angle between the molecular frame and the polarisation vector of the femtosecond laser field. An investigation to clarify the roles of intramolecular quantum processes in field-free aligned molecules is highly desirable, in order to obtain an improved understanding of the underlying physics which is the basis of future applications.

Due to the low efficiency of the HHG process, phase-matched propagation of the fundamental and harmonic radiation throughout a macroscopic sample is required to obtain a measurable signal. The degree of phase-matching depends on the harmonic order and several experimental parameters, including the focusing characteristics of the laser beam, the absorption coefficient of the target gas at the harmonic frequencies, the ionisation fraction of the gas, and the difference in the refractive index at the fundamental and harmonic wavelengths. We have been investigating ways of optimising the phase-matching.

The high harmonic spectrum and intensity contains information about the electronic structure of the atom or molecule and other quantum processes involving the free and bound electrons. Studies of the process of high harmonic generation provide a better understanding of the microscopic and macroscopic process and may lead to additional information about the electronic structure of the atom or molecule.

## ACHIEVEMENTS

### HIGH HARMONIC GENERATION AND COHERENT DIFFRACTIVE IMAGING

The phase-matched propagation of the fundamental and harmonic radiation, (i.e., the coherent construction of the harmonic field in the macroscopic medium), is reflected in the observed harmonic intensity. For high intensity output of the harmonics, an increased interaction length is necessary but it is not easy to obtain the phase-matching condition in a long interaction length. The degree of phase-

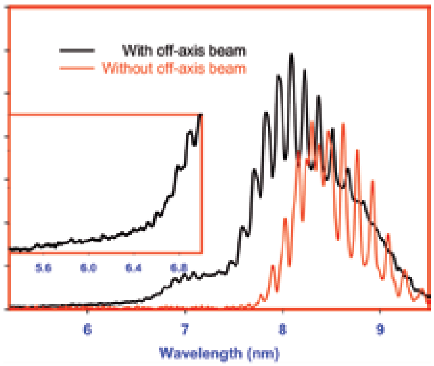



*Figure 1: Off –axis beam for quasi-phase matched HHG in a helium gas cell.*

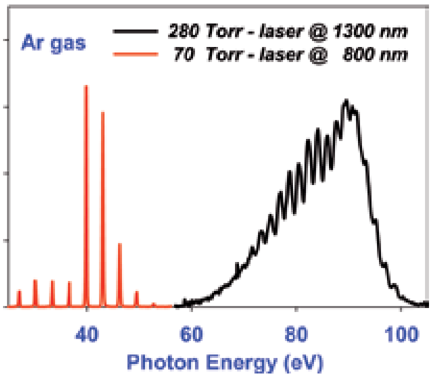



*Figure 2: HHG with fundamental laser at wavelength of 800 nm and 1300 nm.*

matching depends on the harmonic order, the wavelength of the driving pulse and a number of other experimental parameters including the atomic and molecular dispersion, the absorption coefficient of the target gas at the harmonic frequencies, the ionisation fraction, and the gradient of the atomic or molecular dipole phase.

The theoretical cut-off rule for the highest photon energy produced in the high harmonic generation (HHG) process is given by: $h\nu_{max} = U_i + 3.17\ U_p$, where $U_i$ is the ionisation potential of the gas and $U_p$ the laser ponderomotive potential. For a monochromatic wave the ponderomotive potential depends on the laser intensity, I, and the wavelength, $\lambda$, scaling as $\sim I\lambda^2$. Since the phase mismatch between the harmonic and the driving laser fields increases in the high photon energy region the efficiency of the HHG process decreases rapidly at high photon energies. The highest photon energy with reasonable flux is much lower than that predicted by existing theoretical estimates. Efficient phase-matched HHG has been demonstrated only at photon energies of less than 50 eV and less than 100 eV for argon and helium gas, respectively, when a fundamental laser pulse at 800 nm is applied.

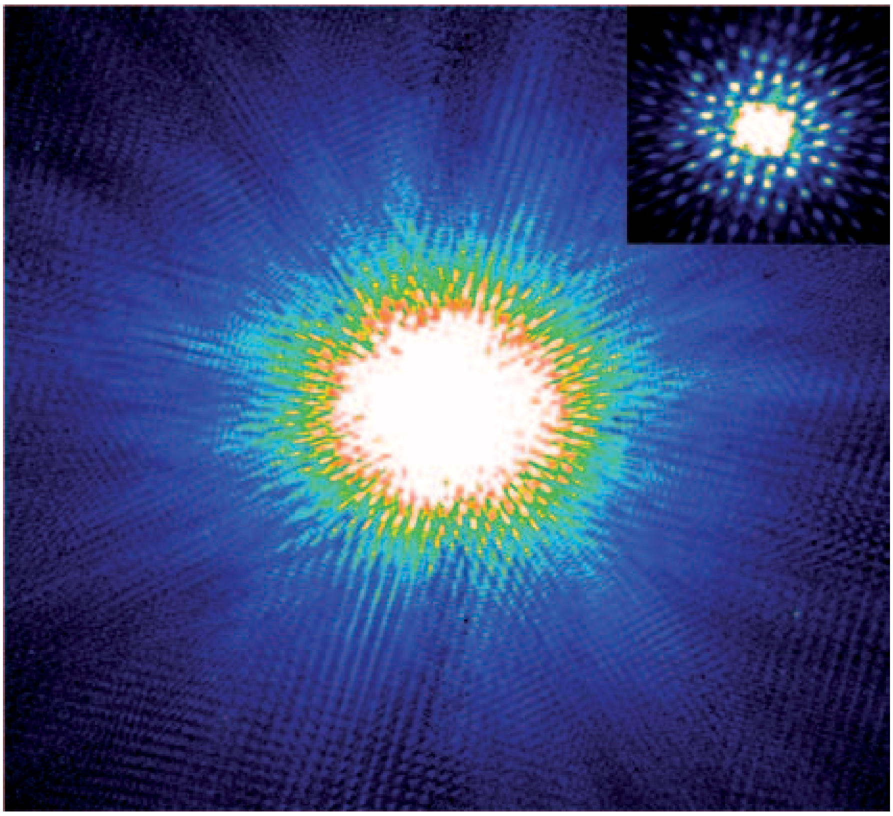


*Figure 3: Diffraction imaging of a 2D array of pinholes test sample illuminated with a 13 ± 1 nm source. The inset shows the centre part of the image.*

Quasi-phase matching techniques have been employed for generating high photon energy harmonics at ionisation levels where true phase matching is not possible. In the quasi-phase matching configuration of HHG, the nonlinear process in the region of the medium where the polarisation and the harmonic field are initially out-of-phase is cancelled. An off-axis beam is applied to vary the phase-matching condition of the HHG process in a semi-infinite gas cell which leads to enhancement of the cut-off energy and, therefore, higher photon energies are generated, as shown in Figure 1.

As a consequence of the cut-off frequency for the generation of XUV/soft X-ray radiation scaling approximately as $\lambda^2$, longer wavelength driving fields can be used to provide a route to increasing the HHG photon energy. When an infrared pulse (1200 – 1600 nm) is used to generate the high harmonics, true phase-matching is possible in order to obtain wavelengths down to the water window region (~ 4 nm). A high power optical parametric generation system has been built to extend the capacity of our laser system to the infrared region (> 1 mJ, 1300 nm, 40 fs). Figure 2 shows the enhancement, by a factor of two, of the cut-off photon energy generated in an argon gas cell when laser pulses at 1300 nm are applied, compared with an 800 nm beam.

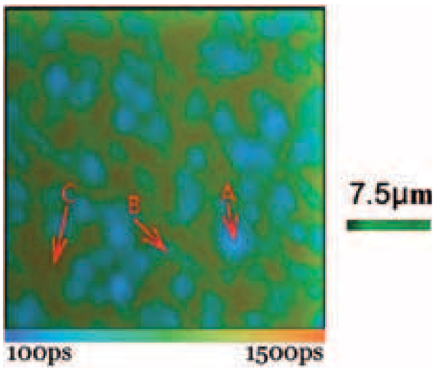



*Figure 4: Fluorescence "lifetime" map for MEH-PPV film cast from toluene. Colour represents fluorescence decay time and the three labelled regions exhibit different emission decay characteristics.*

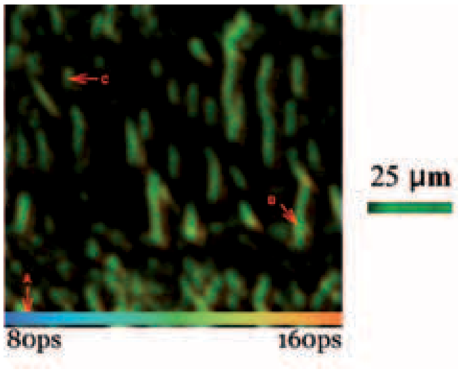



*Figure 5: Confocal fluorescence images of (a) drop-cast and (b) friction transferred films of DPS-PPV, and (c) fluorescence "lifetime" map of the friction transferred film. Colour represents fluorescence decay time and the three labelled regions exhibit different emission decay characteristics.*

The SWLP has used an extreme ultraviolet source at 13 nm for coherent diffractive imaging with the aim of enhancing the spatial resolution achievable. The XUV radiation is focused onto the sample by a small bandwidth (1 nm) mirror which selects ~ 3 harmonic orders centred at 13 nm. The diffraction pattern from test sample consisting of a 2D array of pinholes (1 µm diameter and 3 µm separation) is shown in Figure 3. The diffraction pattern has a strong qualitative resemblance to the pattern that would be produced by an extended periodic sample. Because the coherence length of the source is short, some distortion can be seen in the diffraction pattern. The coherence and the stabilisation of the source need to be improved and work to achieve this is progressing.

## SUPER-RESOLUTION OPTICAL MICROSCOPY

In conjunction with high resolution X-ray/XUV imaging, we are also interested in the rapidly developing field of "super-resolution" *optical* microscopy. These methods are potentially capable of providing resolution comparable to that achievable by the X-ray imaging methods of interest to CXS and provide complementary information using optical methods on samples prepared using standard protocols. Within the past few years, methods including structured illumination microscopy (SIM), scanning near field optical microscopy (SNOM), stimulated emission depletion (STED) microscopy, photoactivated localisation microscopy (PALM) and stochastic optical reconstruction microscopy (STORM), have been developed by various groups around the world.

The team has been active in establishing several of these techniques in association with the BSP. To date, concentration has largely been focussed on structured illumination microscopy and the construction of a SIM system. Current work involves investigating methods that might improve the resolution achievable by SIM by operating in the non-linear regime. This can be done by either illuminating the sample with very high laser intensities or by saturating the response of the system to the illumination pattern by judicial selection of the fluorophore. Other super-resolution techniques are also currently under development within our group, including STED (and variants) and SNOM.

A major thrust of the SWLP work is in adapting the various available imaging modes, and combining these methods to study a variety of samples of importance in both the biological and materials sciences. One area of research that is benefiting from this approach is the study of small molecule and conjugated polymer films of particular relevance to organic photovoltaic applications. Inhomogeneities in the film can occur on a wide range of spatial scales from tens of nanometres to many micrometres and adversely affect the efficiency of electron-hole separation and injection. Scanning near-field excitation has been coupled with fluorescence detection, to investigate thin films of these materials. Additionally, time-resolved confocal fluorescence imaging methods have been used to measure the morphological changes in these films formed under different conditions. For example, the "lifetime" map for the polymer MEH-PPV cast from toluene (Fig. 4) shows how the emission decay varies on the micron scale.

Studies have also included the morphology of films of another conjugated polymer, DPS-PPV, prepared using drop-casting compared with a friction transfer method, using confocal fluorescence microscopy (Fig. 5). There is clearly a strong dependence of the morphology on the casting method, which is also reflected in the time-resolved image. The polymer becomes highly aggregated in fibres that align (surprisingly) perpendicular to the direction in which the polymer is drawn. These aggregated regions show rapid fluorescence decay behaviour.

CXS is also attempting to correlate super-resolution optical imaging with CDI measurements on these polymer films. This work proceeds despite the challenge to achieving high spatial resolution that the complex morphology of these films represents.

A picosecond X-ray source that is activated by a high repetition rate femtosecond laser amplifier is also currently under construction. This source may find subsequent applications in pump-probe X-ray experiments and will provide a low power, lab-based source of short X-ray pulses more energetic than we can achieve with HHG.

CXS has been instrumental in initiating the Cellular Nano-Imaging Consortium (CNIC). The consortium seeks to bring together as many interested parties as possible to collaborate on the various super-resolution optical microscopy approaches and their applications. As part of this initiative we have hosted a super-resolution microscopy workshop at the Melbourne Convention Centre and CNIC is generating a high level of interest through many institutions.

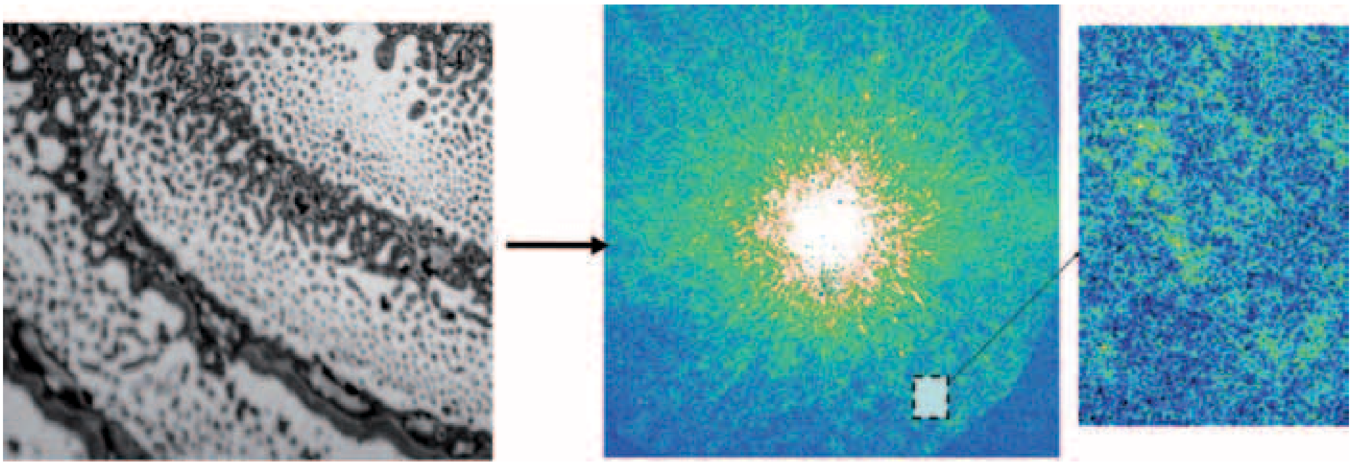


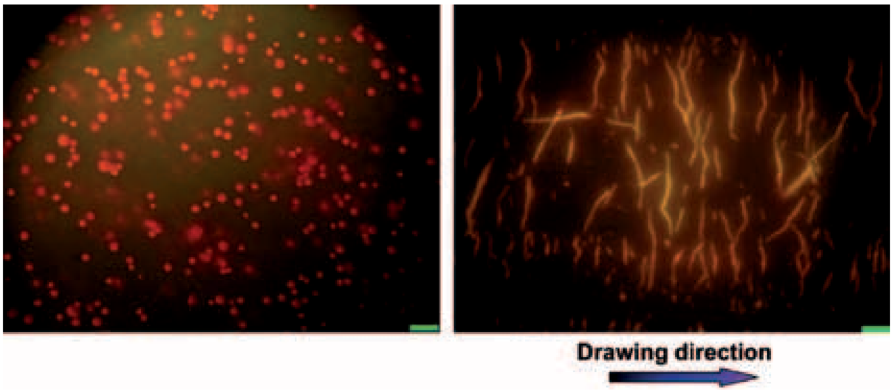
*Figure 6 (above and below): Scanned laser transmission image and diffraction pattern from a conjugated polymer film.*

## SHORT WAVELENGTH LASER SOURCE PROGRAM CASE STUDY

Structured Illumination Microscopy (SIM) is one of the new "super-resolution" fluorescence microscopy techniques proposed in the past few years [1]. In SIM, a grating pattern is projected onto the sample, and Moire fringes are used for gathering small details of the sample [2]. Although the resolution is limited to ~100 nm, data acquisition is quick enough to image living cells [3] and there are no restrictions on fluorophores, unlike with some other "super-resolution" methods [1].

Although commercial SIM microscopes are available, construction of our own experimental system allows easy implementation of extensions of this technique. These include Saturated Structured Illumination Microscopy (SSIM) [4], which can offer resolution on the scale of few tens (10s) of nms by breaking the linear relationship between excitation and emitted intensity, and picoSIM [5] that can speed up the image acquisition process by a factor of three (3).

The samples used for imaging are provided by our collaborators in the BSP. This close association allows direct comparison of images obtained by high-resolution optical microscopy methods with those obtained via coherent diffractive imaging (CDI). We are also collaborating with researchers from other Institutions and a variety of disciplines.





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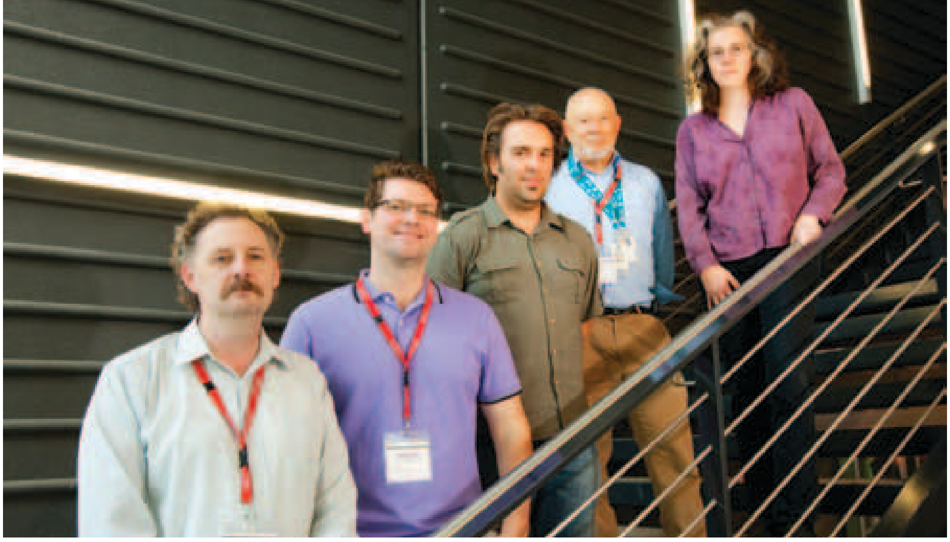
[2] Heintzmann, R., Cremer, C. Laterally modulated excitation microscopy: Improvement of resolution by using a diffraction grating (1999) Proceedings of SPIE – The International Society for Optical Engineering, 3568, pp. 185-196.

[3] Hirvonen, L.M., Wicker, K., Mandula, O., Heintzmann, R. Structured illumination microscopy of a living cell (2009) European Biophysics Journal, 38 (6), pp. 807-812.

[4] Heintzmann, R., Jovin, T.M., Cremer, C. Saturated patterned excitation microscopy – A concept for optical resolution improvement (2002) Journal of the Optical Society of America A: Optics and Image Science, and Vision, 19 (8), pp. 1599-1609.

[5] Wicker, K., Heintzmann, R. Single-shot optical sectioning using polarisation-coded structured illumination (2010) Journal of Optics, 12 (8), art. no. 084010.



*The Structure Determination Methods Program Team.*

# STRUCTURE DETERMINATION METHODS PROGRAM

The Structure Determination Methods Program (SDMP) consists of CSIRO researchers working broadly within the fields of X-ray and electron crystallography in collaboration with other CXS Centre members. Its main aim is to develop novel experimental techniques and data analysis methods for extracting structural information from 2-D crystals and 3-D nanocrystals, especially relating to the determination of the structure of the pharmaceutically very important class of proteins known as integral membrane proteins. This program brings with it internationally recognised expertise in the preparation, purification, crystallisation and handling of these samples.

The ongoing study of *purple membrane*, a naturally occurring 2-D crystal of the membrane protein *bacteriorhodopsin*, serves as a useful test case because there is high-resolution structural information available from 3-D X-ray crystallography and 2-D cryo-electron microscopy that can be used for comparison. Collaboration within CXS has helped link into expertise in developing and applying computer programs for de-convoluting data for diffraction from 2-D crystal powders, and has led to alternative ways to explore the use of 2-D crystal samples in the context of different X-ray diffraction techniques.

Development of novel experimental and related theoretical methods for the preparation and analysis of powder samples for integral membrane proteins has commenced. These techniques include preparation and collection of data from various 2-D crystal powders – a little-explored approach. These methods offer the exciting possibility of providing alternative and easier paths to the X-ray structure determination of this very important class of proteins that have mostly resisted efforts based on conventional 3-D single-crystal methods.

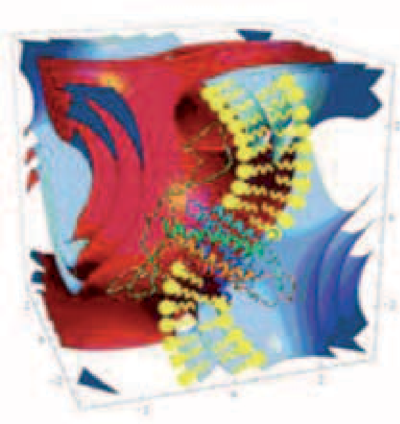
On the CSIRO Molecular & Health Technologies (CMHT) side, work has progressed on the preparation of a number of different types of powder samples of integral membrane proteins consisting of preferentially and randomly oriented 2-D crystal layers.

Work at CSIRO Materials Science and Engineering (CMSE) has been continued with the development of analytical methods for structure determination using X-ray diffraction with two-dimensional (2-D) protein crystals in powder samples. The research broadly sits in three areas:

- The first is concerned with fitting 2-D powder diffraction data using a non-empirical approach based on a physical model of the scattering process.
- The second and third areas are closely linked: phase retrieval and refinement, and structure determination. While these are separate problems, they are generally best treated together. Structure determination in the 2-D crystal powder diffraction context amounts to reconstruction of a 2-D projection map of the electron density in the crystal. This can be viewed as a technique spanning coherent diffractive imaging and 3-D crystallography and is aimed at high-resolution 3-D structure determination. The advantage of the technique being developed here is that it does not require 3-D crystals, nor does it require 2-D crystals of the size needed for structure determination by electron diffraction.

## ACHIEVEMENTS

- High throughput *in cubo* crystal screening has been established within CSIRO where it is possible to rapidly produce crystallisation and small angle X-ray scattering plates using various lipids and membrane proteins. Two proteins have been characterised, dopamine 2L receptor and bacteriorhodopsin, and the outcomes have been published.



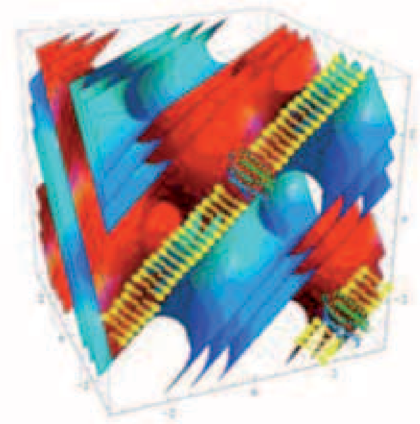


*Figure 1: A schematic model of (A) A schematic of the dopamine D2L receptor embedded within a $QII_D$ bicontinuous cubic phase. (B) A schematic of bR embedded within a $QII_G$ bicontinuous cubic phase. Models are not scaled relative to each other.*

- Lynn Liang has expressed and purified two butyrate GPCRs (GPR41 & GPR43) as part of her PhD project. She has entered both proteins into crystallisation trials and small angle X-ray scattering trials.
- In partnership with Ruben Dilanian and collaborators at The University of Melbourne The SDMPis obtaining powder diffraction data and analysis from bacteriorhodopsin. The groups are also working together to obtain higher resolution powder diffraction data for lysozyme, bacteriorhodopsin and other GPCRs.
- Connie Darmanin has hosted a crystallisation workshop for a group of six Tall Poppy program participants from Santa Maria. The students attended the workshop CSIRO in Parkville and used beamlines at the Australian Synchrotron to take pictures of the protein as part of the first secondary school student lead experiment at the facility.
- Connie Darmanin has received the CSIRO Payne-Scott Award, which assists researchers who have taken career breaks to care for family to re-enter their field.
- Powder diffraction data for hemazoin and beta-hematin has been collected by Victor Streltsov at Australian synchrotron and analysed by Ruben Dilanyan in collaboration with the CSIRO and La Trobe University groups. This has resulted in the hemazoin structure at 2Å resolution, new interpretation of hemazoin crystal formation and a joint publication [1].
- Victor Streltsov has collected X-ray absorption (XAFS) data for beta-hematin using Australian National Beamline Facility (ANBF) at Photon Factory, Japan. Combined X-ray powder diffraction and X-ray absorption results have been presented at the Asian crystallographic conference.

## STRUCTURE DETERMINATION PROGRAM CASE STUDY

The dopamine D2L receptor and bacteriorhodopsin (bR), which are integral membrane proteins, have been incorporated within bi-continuous cubic meso phases formed by the lipid monoolein, which is the standard lipid used for in meso crystallisation experiments. In both cases, the incorporated membrane protein was found to promote a structural transition within the underlying cubic phase. The structural effects observed were found to depend strongly on the size and shape of the membrane protein. For incorporation of bR the structural transition observed, from a $QII_G$ to a $QII_D$ cubic architecture, is consistent with the effect of the hydrophobic domain of bR on the curvature of the polar–apolar interface and results in a thicker bilayer, alleviating hydrophobic mismatch between the protein and the lipid bilayer.

The significantly increased hydrophilic domain size associated with the D2L receptor results in the reverse transition, from a $QII_D$ to a $QII_G$ phase, with a concomitant increase in water channel diameter. The observed structural effects have ramifications in designing in meso crystallization trials where the underlying cubic structure must be retained.

# THEORY AND MODELLING PROGRAM

The Theory and Modelling Program (TMP) is responsible for developing the theoretical and computational physics required to support the experimental programs in CXS. The group's focus incorporates:

(i) the solution of inverse problems;

(ii) the characterisation of partial spatial and temporal coherence in short wavelength light sources;

(iii) the relativistic formulation of molecular electronic structure and quantum electrodynamics;

(iv) the dynamical description of non-linear interactions between molecules and strong coherent fields;

(v) coherent energy transfer processes in biomolecules; and

(vi) the design of efficient computational algorithms.

## GOALS

The Theory and Modelling Program (TMP) collaborates closely with all of the other programs in the centre, especially in identifying fruitful directions for the experimental programs to pursue and by supporting these activities with theoretical and computational tools. The key aims of TMP involve the development of:

- Image reconstruction algorithms for diffraction data obtained using sources exhibiting partial spatial or temporal coherence;
- Quantum electrodynamical models of high-harmonic generation in atomic systems using visible and infra-red light sources and of the interaction of molecules with strong-field high-frequency X-ray free-electron laser (XFEL) sources; and
- Non-interferometric phase recovery techniques in photon echo spectroscopy.

## ACTIVITIES AND OUTCOMES

The TMP collaborates closely with the physics-based experimental activities in CXS, i.e. the EMP, the SWLP, the UPSP and the ASP. In 2010, the TMP also contributed to publications on biochemistry and structural biology, in collaboration with the BSP and the Structure Determination Program. This is regarded as a particularly significant outcome, since it demonstrates the group's commitment to comprehensive theoretical support of the entire CXS experimental research program. It has also contributed to the cohesiveness of the entire research portfolio, since the involvement of the TMP has led to increased interdisciplinary collaboration across CXS.

The partially coherent imaging algorithms developed by the TMP found widespread use in the analysis of diffractive imaging experiments performed by the EMP. The introduction of a modal analysis of the optical properties of the wave fields involved in these experiments has made the use of partially coherent light sources a routine and computationally efficient tool in the imaging repertoire of CXS. It was shown that even a small deviation from full spatial coherence in the illumination of a diffractive imaging experiment could lead to the formation of significant artefacts in the reconstruction. A modal correction for this partial coherence was shown to eliminate these artefacts at minimal computational cost. It was also demonstrated that a similar approach could be used to successfully employ spectrally broad sources in diffractive imaging, emphasising the comprehensive nature of the algorithms within the framework of optical coherence theory.

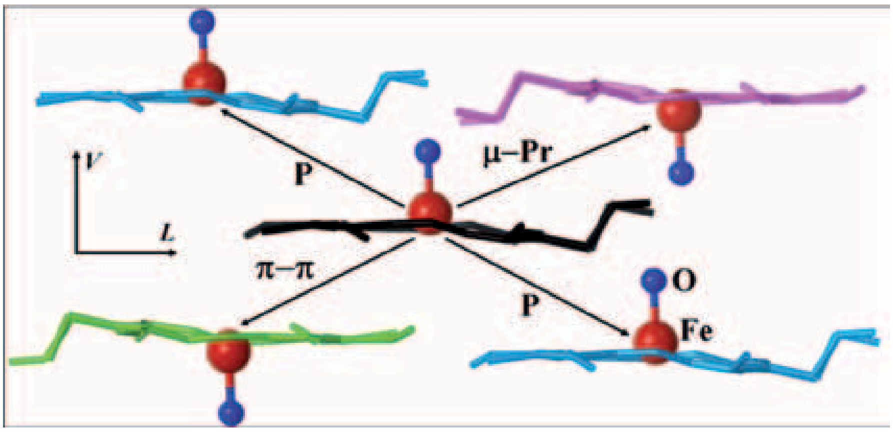



*Figure 1: Individual FP Fe(III) units (black) are involved in a range of metalloporphyrin interactions with the surrounding FP Fe(III) units. These involve μ-Pr (black to pink), π- π (black to green) and P-type (black to blue) interactions.*

The TMP's contribution to the research of ASP and SWLP has centred on the development of numerical solutions of the time-dependent Schrödinger equation, and the modelling of non-linear interactions between atoms and strong-field laser pulses of femtosecond duration. The particular challenge here concerns the need to represent the evolving wave function over spatial domain extending about 1000 bohr from the target atom, and the representation of the electric dipole interaction in the so-called 'velocity gauge'. The implicit form of the short-time propagation equation

$$\psi\left(\boldsymbol{r},t+\delta t\right)=\exp[-i\hat{H}(t+\delta t/2)\delta t]\psi\left(\boldsymbol{r},t\right)$$

for the time evolution of the wave function, $\psi(\boldsymbol{r},t)$, over the time interval $\delta t$ may be written in general, as the solution of a large system of linear equations. Special algorithms exist for the solution of these equations in the case of the length-gauge form of the interaction, but in the velocity gauge one must adopt some numerical approximation scheme that exploits the sparseness of the matrix representation of the time-dependent Hamiltonian operator, $\hat{H}(t)$. The Theory and Modelling Program group has adopted the conjugate gradient method to solve these equations, since one may always construct a reasonable approximation to $\psi\left(\boldsymbol{r},t+\delta t\right)$ by explicit propagation, which is then refined iteratively by its implicit solution. This approach is rapidly convergent because the matrix representation of $\exp[-i\hat{H}(t+\delta t/2)\delta t]$ is 'preconditioned' in the sense that its inverse differs from the identity matrix by terms proportional to $\delta t$. This approach exhibits the additional feature that it is rather general, in that a single procedure may be used propagate the solutions of either the Dirac equation or the Schrödinger equation, differing in cost only because of the increased complexity of relativistic spinor amplitudes. These methods have been applied to modelling the laser-atom interaction physics in high-harmonic generation processes in SWLP and the few-cycle interactions that characterise the interests of ASP.

The development of methods in protein polycrystallography have enabled TMP to engage with BSP and SDMP on a number of projects involving the determination of molecular structures of biomolecules, including membrane proteins. The approach involves a careful re-examination of the methods used to subtract the background in powder diffraction studies and the functional forms used to fit powder diffraction peaks. This is followed by a novel method, devised by Ruben Dilanian, for resolving overlapping reflections in protein powder diffraction patterns, enabling molecular structures to be determined without the use of high-quality single crystals. This approach was applied to the determination of the structure of β-hematin and the identification of the likely self-association states of the monomer as it forms crystals.

Significant progress towards the realisation of one of the central research goals of CXS was made by the TMP. The group has developed a model of electronic damage processes in biomolecules interacting with XFEL sources as well as an algorithm to determine molecular structure directly from a three-dimensional diffraction pattern in such cases, without resorting to the explicit construction of the molecular one-electron density function. The approach was based on the realisation that the time-evolution of the electron density of a molecule undergoing such interactions leads to a scattered X-ray signal that possesses the characteristic signature of a partially coherent source, even if the illumination incident on the target molecule is fully coherent. The partially coherent character of this scattered wave is incorporated within our reconstruction algorithm, motivated by our earlier experience in partially coherent diffractive imaging.

The TMP collaboration continued with the Ultra-Cold Plasma Source Program, who use an algorithm developed by the group to image and characterise their ultra-cold atom clouds. This is a critical aspect of the subsequent steps in which ultra-cold electron pulses are produced for eventual use in coherent electron imaging experiments. The TMP has also collaborated in the development of models to simulate the diffraction of coherent electron pulses from molecules and nanocrystals, as a part of the preliminary work necessary to use the ultra-cold electron source in imaging experiments.

As a service to the wider diffractive imaging community, the TMP engaged the services of Ms Nadia Davidson, who is an experienced computer programmer as well as accomplished particle physicist. Nadia has been involved in the development of CXS

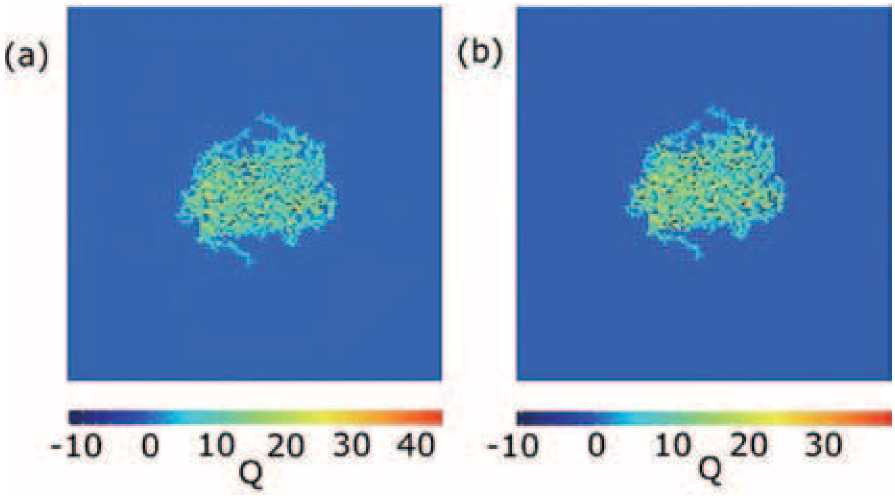



*Figure 2: Comparison of the exact solution of the structure determination problem (a) with the reconstruction obtained using our new imaging scheme, (b), for bacteriorhodopsin in the presence of the electronic damage encountered in the interaction process under realistic interaction conditions. The quantity displayed is the two-dimensional projection of the Fourier transform of the three-dimensional nuclear structure factor,* $T(\mathbf{q})$*. This factor is sufficient to locate and identify the atomic positions and, hence, the biomolecular structure.*

coherent diffractive imaging software, with the purpose of producing packages that are sufficiently robust to enable distribution under an open source licence. This new facility is likely to accelerate the progress of students beginning their research careers in imaging as well as standardising the software and data handling protocols that we employ.

## ACHIEVEMENTS

- Development of electronic damage models in XFEL-biomolecule interactions and the detailed characterisation of the partially coherent character of the diffraction signal.
- Development of electrodynamical model of diffractive imaging of biomolecules that enables molecular structures to be determined in the presence of electronic damage without explicit construction of the ground-state electron density.
- Calibration of biomolecular imaging algorithms in XFEL experiments to accommodate the effects of electronic damage.
- Establishment of bootstrap method for molecular structure determination from powder diffraction data, resolving overlapping reflections.
- Generation of teoretical insights into the formation hemazoin crystals from the self-assembly of β-hematin molecules.
- Initiating an error regularisation scheme for handling measurement errors in reconstructing images from diffraction data.
- Foundation of partially coherent diffractive imaging using modal representations of optical coherence functions.
- Established a generalised propagation scheme for time-dependent strong-field electron dynamics using implicit representation, solved using sparse matrix methods for linear equations.
- Development and standardisation of software for eventual distribution to the diffractive imaging communities.

## THEORY AND MODELLING PROGRAM CASE STUDY

The determination of membrane protein structures using diffraction data obtained from XFEL sources is the central goal of CXS. The LCLS source at Stanford became operational in 2009, making the analysis of biomolecular diffractive imaging a priority in the TMP group

Earlier proposals to use XFEL sources in diffractive imaging experiments had identified that the interaction between the biomolecule and an intense femtosecond pulse causes several electro-dynamic processes to occur. These include inner-shell photoionisation, Auger emission, and electron recapture, which in turn, cause secondary valence shell electron impact ionisation. As a consequence, it is not possible to regard molecular imaging as falling within the usual formulation of coherent diffractive imaging subsequent to this kind of interaction, as the scattering of photons is determined by the local electron density, which becomes strongly time dependent in this circumstance. The restriction applies even if the interaction time is so short that the nuclear positions do not have time to adjust to the changing electronic environment. This time interval is estimated to be of the order of a few femtoseconds.

In preliminary work in CXS, a form of diffractive imaging was developed in which the partially coherent character of the illumination was incorporated in the reconstruction algorithm in the form of a modal expansion. In the case of bio-molecular imaging using XFEL sources, it was shown that the scattered wave has the characteristics of a partially coherent secondary source, even if the incident illumination is fully coherent. This formulation is a synthesis of CXS expertise in the development of detailed quantum chemical models of the electrodynamics of the XFEL-molecule interaction and of the detailed characterisation of the partially coherent optical properties of imaging sources.

In the course of this research, it was realised that one may make a distinct choice of strategy in extracting molecular structure – which is classically regarded as being the knowledge of the equilibrium positions of the nuclei – from a diffraction pattern that includes the effects of the electro-dynamic processes that have been identified. The conventional approach seeks to restore the effects of damage in the diffraction data, so that one obtains an approximation to the ground state electron density. From this knowledge, one must

then fit the molecular structure by building an electronic model of the molecule within the reconstructed electron density.

The approach developed by the team, however, is based on the assumption that the duration of the pulse is short enough for the nuclear positions to remain unchanged throughout the interaction. In this case, the coherent information is contained within the nuclear positions, which are modulated by the partially coherent electronic information. Rather than restore the ground-state electron density the nuclear positional information was isolated from the electronic information and the molecular structure problem recast in terms of the nuclear positions alone. This procedure was possible only because the structure problem involved a sample of finite extent, rather than the periodic samples that are assumed to exist in crystallography. The iterative recovery procedure returns a three-dimensional map of the molecular structure, with $\delta$-function distributions appearing at the positions of the nuclei, scaled by the corresponding nuclear charge. This approach circumvents the need to build an atom-based model to fit the structure to the molecular electron density.

This research was recently published in *Nature Physics,* and makes a significant contribution to ongoing international efforts to realise the use of XFEL sources as the basis of a "molecular microscope" with atomic resolution.

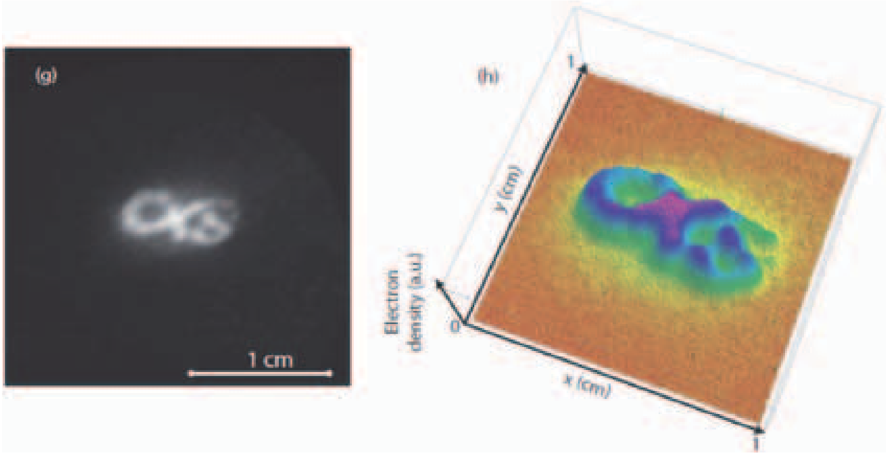



*Figure 1: Cold electrons extracted from an ultra-cold plasma. The plasma was formed from cold atoms, at a temperature of 70μK. Atoms were selectively excited in the spatial distribution of the CXS logo using a narrowly tuned laser beam shaped by a spatial light modulator. The cold atom cloud was then illuminated uniformly with an intense pulse of blue light, which photo ionised only those atoms which had previously been excited. The electrons, with temperature blow 10 K, were then extracted from the plasma with a uniform electric field, and propagated to a charge multiplier and phosphor detector. The grey scale image at left was acquired with a cooled CCD camera, and shown as a false-colour rendered surface in the image on the right.*

# ULTRA-COLD PLASMA SOURCE PROGRAM

The Ultra-Cold Plasma Source Program (UPSP), formed within CXS in 2007, is developing an ultra-bright, coherent source of electrons for imaging of biologically relevant targets. By applying technical developments taken from the ultra-cold atom community, and the theoretical algorithms developed in the TMP, the UPSP will enable a new approach to electron imaging. The enhanced probe-molecule interaction strength that a coherent electron source offers, combined with an improvement of four orders of magnitude in brightness over existing electron sources, will enable high-resolution imaging of biological targets with atomic scale resolution.

The most significant aspect of the ultra-cold plasma source and the basis of the dramatic enhancement in brightness that it promises is the origin of the electrons: they will be extracted from ultra-cold atoms, just a few millionths of a degree above absolute zero. The brightest conventional electron sources start with hot material, by blasting a target with a high-energy laser pulse. The hot electrons then expand like steam from a kettle, and are equally difficult to tame and control. Electrons extracted from ultra-cold atoms can be accelerated and focused with unprecedented resolution. The comparison is like that of a conventional light bulb and a laser: we need laser-like coherence and brightness to image molecular structure with atomic resolution.

The UPSP team has strong expertise with ultra-cold atom technology, with conventional optical imaging, and with electron optics. The team is collaborating with the world-leading research group in this area, at the University of Eindhoven in The Netherlands. The project is strongly connected with the TMP, and the groups have jointly published work based on the centre's imaging approaches for applications in characterising the cold atom cloud. The UPSP is now collaborating with the TMP group to employ their expertise on partially coherent X-ray sources for modelling our now-operational electron source. The theoretical formalism of partial coherence has not previously been applied to electron imaging, but recent development of new sources has made partial coherence highly relevant. TCP modelling will be used to design the imaging component of the ultra-cold plasma source system, firstly to enable verification that the electron source is indeed coherent and bright, and secondly to enable imaging applications. In the longer term, collaboration with the TMP will be essential to unravel electron-molecule interactions so that target structural information can be separated from the complexity of the diffraction data. The ultimate goal, the high-impact demonstration of electron diffraction from molecules, will require close liaison with the BSP, to determine the optimum biological targets and the appropriate sample preparation strategies. The initial collaboration with the BSP has established two-dimensional crystals of bacteriorhodopsin as a promising target for the first experiments. Such inter-program collaborations, the envy of our colleagues at Eindhoven, are simply not available to other groups around the world and will allow the UPSP team to rapidly achieve high-impact results across disciplines.

## ACHIEVEMENTS

The CXS UPSP is developing a new type of electron source for imaging at the nanometre scale. An ultra-cold plasma is generated by photo ionisation of atoms which have been cooled to a temperature of a few millionths of a degree above absolute zero. An electric field applied to the ultra-cold plasma then extracts free electrons. The electrons are cold, and thus have small transverse momentum, corresponding to high spatial coherence. This high coherence will enable enhanced diffractive imaging, in particular using the partially coherence diffractive imaging approaches developed by CXS.

Electron (and X-ray) sources are normally assumed to be incoherent at the source; coherence is obtained by reducing the beam

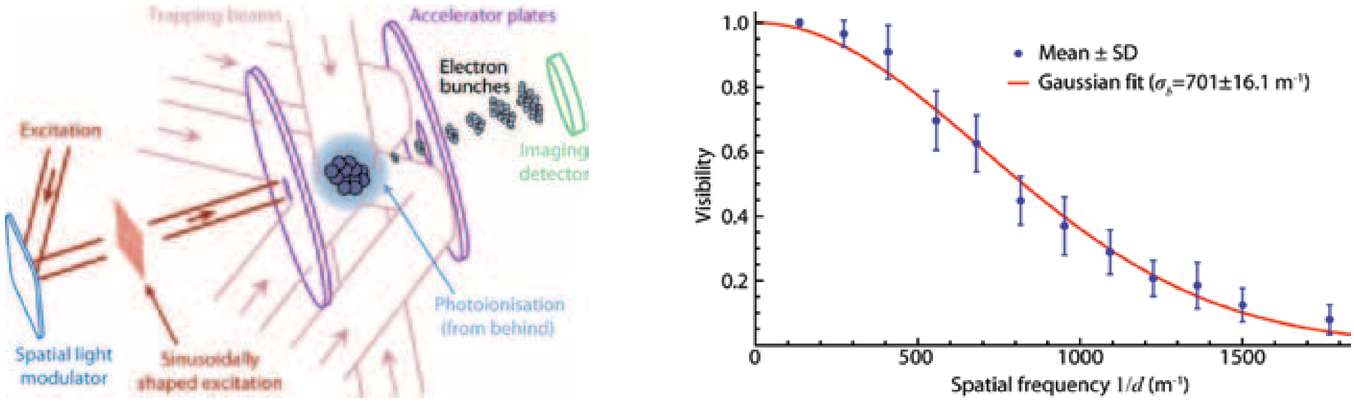



*Figure 2: Measurement of the coherence of a cold electron source. Left: a narrow line width laser is tuned to the first dipole excitation of rubidium atoms. A spatial light modulator creates sinusoidal patterns of that light on a cloud of cold rubidium atoms suspended between accelerator electrodes in a magneto-optic trap. The atoms are uniformly illuminated by an intense pulse of blue laser light, which photo ionises the excited atoms. The electrons are electrostatically accelerated and propagate to a charge multiplier and phosphor screen, where the pattern is recorded with a CCD camera. Right: the visibility of the pattern depends on the transverse momentum spread of the electrons: that is, on the temperature and spatial coherence length of the electrons. A fit of the visibility with respect to the spatial frequency of the sinusoidal pattern then gives us the transverse coherence length of the electrons,* $l_c = \sqrt{2}\hbar t / m_e\sigma_b$.

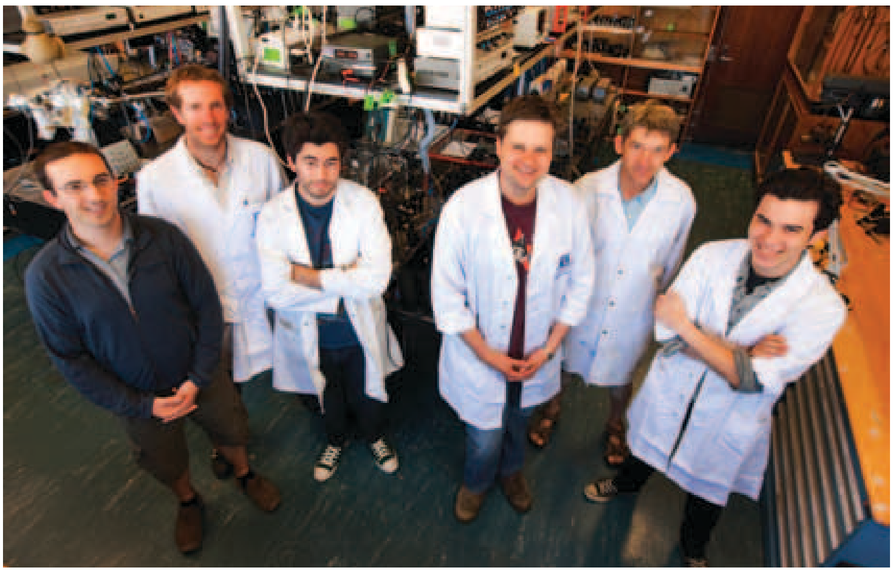
with apertures. The ultra-cold plasma electron source is inherently coherent at the source. Extraction of bunches of cold electrons has been demonstrated, with an upper limit on their temperature of 10 K with an uncertainty of ±5 K determined. At that temperature, the transverse coherence length is greater than (10 ± 3) nm – large enough for imaging crystalline structure and even small viruses.

Aside from the low temperatures, an ultra-cold electron source offers the unique ability to arbitrarily change the spatial distribution of electron emission in real-time, by optically controlling the distribution of cold atoms. The UPSP has demonstrated such shaping using a diffractive spatial light modulator to excite and photo ionise the cold atoms, for example, into the CXS logo as shown in Figure 1. The arbitrary control of electron bunch shape is a major step towards the alleviation of electron source brightness limitations due to Coulomb explosion. The Coulomb-driven expansion of normal electron bunches cannot be reversed, but if the bunch is shaped into a uniform density ellipsoid, the expansion can be reversed using standard electron optics, thus promising exceptionally bright electron bunches. The ability to dynamically shape the electron source itself and to observe that pattern in the propagated electron bunch is also a remarkable experimental demonstration of the intrinsically high spatial coherence of a cold electron source

The results shown in Figure 1 demonstrate, for the first time since electrons were first discovered by JJ Thomson in 1897, that it is possible to make a pattern with electrons and see that pattern after the electrons have propagated across free space. We take such imaging for granted with light, for example, with a film or digital video projector, but electrons have until now been so hot that any pattern is immediately lost. The UPSP has now used that ability to efficiently characterise a new source, and now looks forward to novel forms of diffractive imaging which have hitherto not been feasible.

## ULTRA-COLD PLASMA SOURCE PROGRAM CASE STUDY

The coherence properties of an ultra-cold plasma electron source are unusual, in that the electrons are spatially coherent at the source, but the source is extended, in the sense that it has a finite size. Laser beams are coherent but are effectively point sources, in that they are fully coherent across the beam. We have shown that the coherence of the cold but thermal electrons is described by a quasi-homogeneous partial coherence function. Using the unique ability to shape the ultra-cold plasma and hence electron bunches (see Fig. 1), the visibility of sinusoidal patterns of electrons after propagation has been measured. The visibility is directly linked to the partial coherence function and, hence, the transverse coherence length of the new electron source has been determined. A lower limit to the coherence length of 9.7 ± 1.8 nm is measured, consistent with an electron temperature of below 10K. The coherence length compares well to the typical lattice spacing of crystalline materials (a few nm), and is even large enough for diffraction from small viruses without the losses inherent in obtaining coherence through beam expansion.



*The Ultra-Cold Plasma Source Program Team*

*Below: CXS students Nor Azah Abdul Aziz and Ba Khuong Dinh*

STUDENTS AT CXS GAIN VALUABLE EXPERIENCE AND WORK ON PROJECTS WITH CUTTING EDGE TECHNOLOGIES.

# EXPANDING

# STUDENT LIFE @ CXS

## EXPANDING HORIZONS

### KHUONG BA DINH, SHORT WAVELENGTH LASER SOURCE PROGRAM

SWINBURNE UNIVERSITY OF TECHNOLOGY

I joined CXS in July, 2008 as a PhD student and it truly turns out to be a milestone in my research career. As I was not involved with laser physics field as my background was in Telecommunications (Bachelor) and Nanotechnology (Master), I found it really challenging at the beginning. However, the further I progress with the High Harmonic Generation project, the more this field catches my true interest. As a member of the CXS, I have had opportunities to take part in various conferences, both national and international. These activities have offered me valuable opportunities to obtain and broaden a great deal of advanced knowledge, which contributes significantly not only to my PhD thesis but also to my future career. In addition, continuous and great support from CXS members, (my supervisors, co-workers, and other friends), always inspires my work. I feel grateful to CXS for helping me fulfil my dreams.

### LYNN LIANG, STRUCTURE DETERMINATION PROGRAM

CSIRO, PARKVILLE

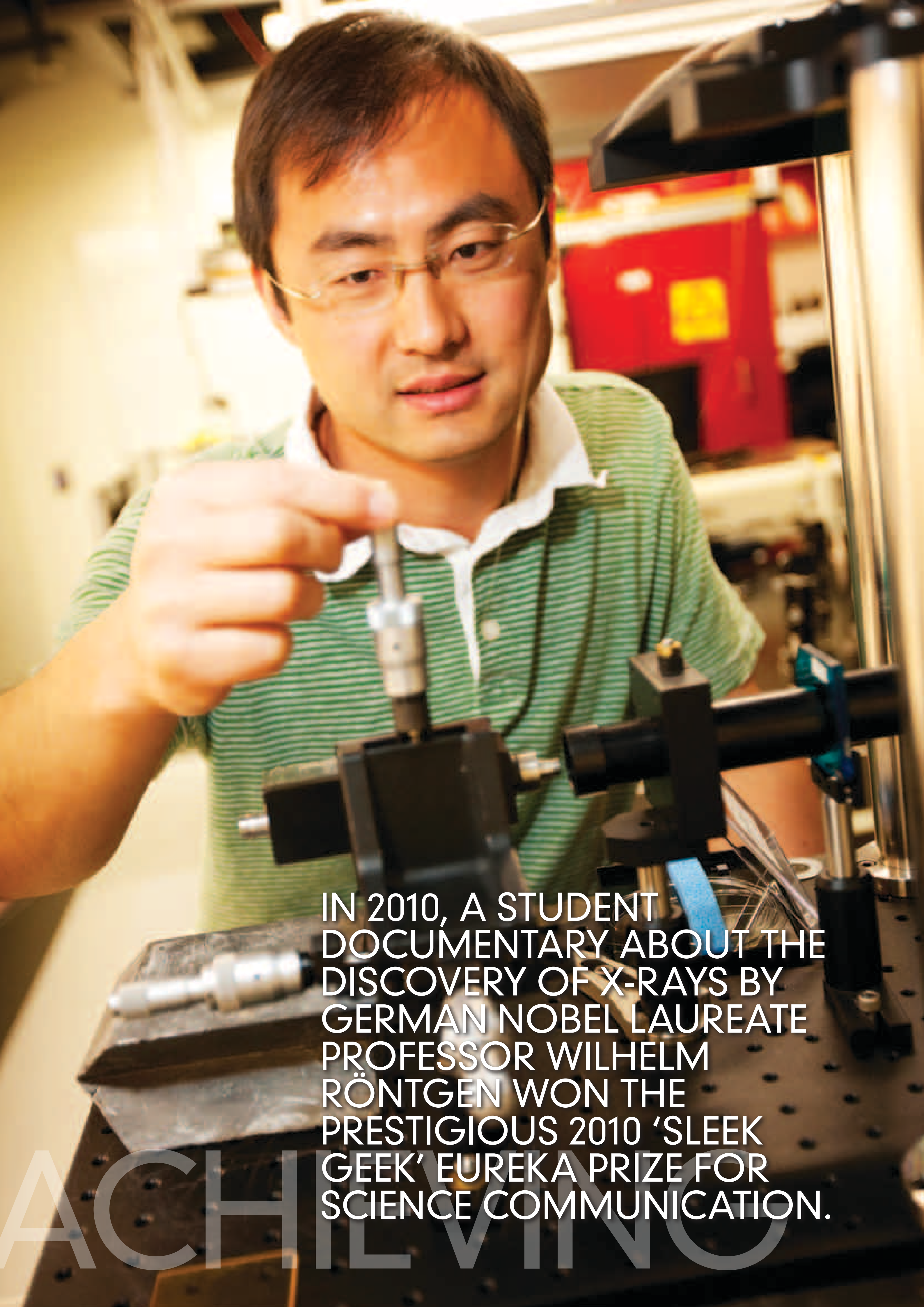
I have been a CXS member for the last three years and I have genuinely enjoyed the experiences I have gained through the workshops. These workshops had speakers from all around the world, which expanded my knowledge. I have met intellectual members in the field and have kept in contact for discussion regarding my project. As a member of CXS, I was given the opportunity to attend a crystallisation course that helped a lot in the progress of my PhD project and work that was done at the Australian synchrotron. Besides the financial support that I have received from CXS, it has also broadened my network to allow me the opportunity to grow and advance as a research scientist.

### MAURO MAIORCA, BIOLOGICAL SCIENCES PROGRAM

LA TROBE UNIVERSITY

My first year as a PhD student in Prof. Leann Tilley's Lab was focused on a multidisciplinary project, which aims to contribute to the fight against malaria through improving specific image processing/analysis techniques and applications. In particular, my investigations include improving electron tomographic reconstructions using multiple scattering estimation and correction; supervised 3D segmentation of specific structures of interest; automatic classifications of infected erythrocytes; and high resolution structural analysis of specific targets (i.e. nuclear membrane structure and apical compartments of merozoites). Highlights have included generating a model of a complete schizont-infected red blood cell with 32 daughter merozoites) and generating a filtering algorithm that takes into account the different scattering contributions at different tilt angles in electron tomography tilt projections.

My multidisciplinary background in both computer sciences (BSc) and biomedical image science (MSc), together with my previous working experience in the medical imaging area makes my life easier. However, in order to achieve my goals I constantly need feedback from experts in both biochemistry and physics and from a supervisor with the "big picture" in mind. Being part of CXS and having Leann as the main supervisor is certainly a necessary condition for allowing my project to move ahead.



IN 2010, A STUDENT DOCUMENTARY ABOUT THE DISCOVERY OF X-RAYS BY GERMAN NOBEL LAUREATE PROFESSOR WILHELM RÖNTGEN WON THE PRESTIGIOUS 2010 ‘SLEEK GEEK’ EUREKA PRIZE FOR SCIENCE COMMUNICATION.

# ACHIEVING

# CXS MANAGEMENT & GOVERNANCE

CXS is a collaborative research program between the University of Melbourne, La Trobe University, Monash University, Swinburne University of Technology and CSIRO, funded under the Australian Research Council (ARC) Centre of Excellence program and the Victorian Government's Science, Technology and Innovation (STI) Initiative.

As Lead Administering node, the University of Melbourne manages the grants and distributes funds in accordance with the signed agreements. These agreements cover CXS management, collaboration and intellectual property arrangements.

All collaborating organisations are represented within CXS boards. Commercial expertise is represented on the CXS Intellectual Property Committee and Sub Committee. A Scientific Advisory Board and a General Advisory Board have been established and meet annually.

## CENTRE MANAGEMENT

The CXS Management team and its Executive Committee are responsible for administration as it pertains to centre policy, performance, financial matters, research output, research training and professional education of members, partnerships, national and international liaison, commercialisation and outreach.

The management team is:

PROFESSOR KEITH NUGENT
Director of Research

PROFESSOR LEANN TILLEY
Deputy Director of Research

MS TANIA SMITH
Chief Operating Officer

## EXECUTIVE COMMITTEE

During 2010, the administration of CXS was overseen by the Executive Committee, which comprises:

MS ROSSLYN BALL
Executive Officer to Committee

ASSOCIATE PROFESSOR DAVID KIELPINSKI
Attosecond Science Group Leader

PROFESSOR KEITH NUGENT
Research Director

ASSOCIATE PROFESSOR ANDREW PEELE
Experimental Methods Group Leader

ASSOCIATE PROFESSOR HARRY QUINEY
Theory and Modelling Group Leader

ASSOCIATE PROFESSOR MIKE RYAN
Biological Sciences Group Leader

ASSOCIATE PROFESSOR ROBERT SCHOLTEN
Ultra Cold Plasma Source Group Leader

MS TANIA SMITH
CXS Chief Operating Officer

ASSOCIATE PROFESSOR TREVOR SMITH
Short Wavelength Laser Source Group Member

PROFESSOR LEANN TILLEY
Deputy Research Director

DR VICTOR STRELTSOV
Structure Determination Methods Group Leader

PROFESSOR LAP VAN DAO
Short Wavelength Laser Source Group Leader

## ADVISORY BOARD

The CXS Advisory Board met in December 2010 at the University of Melbourne CXS Office. The meeting focussed on the recommendations of the CXS Scientific Advisory Board and discussed matters relating to the long-term future of CXS, industry and community outreach, and the leadership of the Centre leading into 2011.

CXS would like to thanks Ken Ghiggino, Gareth Moorhead, Peter Hannaford and Ned Pankjurst for their contribution to this years meeting.

MR DAVID KRENUS (CHAIR)
Chief Executive Officer Cyclotek

PROFESSOR PETER RATHJEN
Deputy Vice Chancellor (Research)
The University of Melbourne, or nominee

DR CAL DRUMMOND
Chief of CSIRO Materials Science
and Engineering

PROFESSOR ANDREW FLITMAN
Pro Vice-Chancellor (Research) Swinburne University of Technology, or nominee

PROFESSOR JOHN HELLIWELL
Professor of Structural Chemistry
University of Manchester

PROFESSOR TIM BROWN
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La Trobe University, or nominee

DR STEPHEN LANE
Chief Science Officer
NSF Centre for Biophotonic, Science & Technology, UC Davis

PROFESSOR EDWINA CORNISH
Deputy Vice Chancellor (Research)
Monash University, or nominee

PROFESSOR BONNIE WALLACE
Professor of Crystallography
Birkbeck College

BRUCE WHAN
Chairman of INNOVIC
(Victorian Innovation Centre Ltd) & Director
Swinburne Knowledge

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Professor of Crystallography
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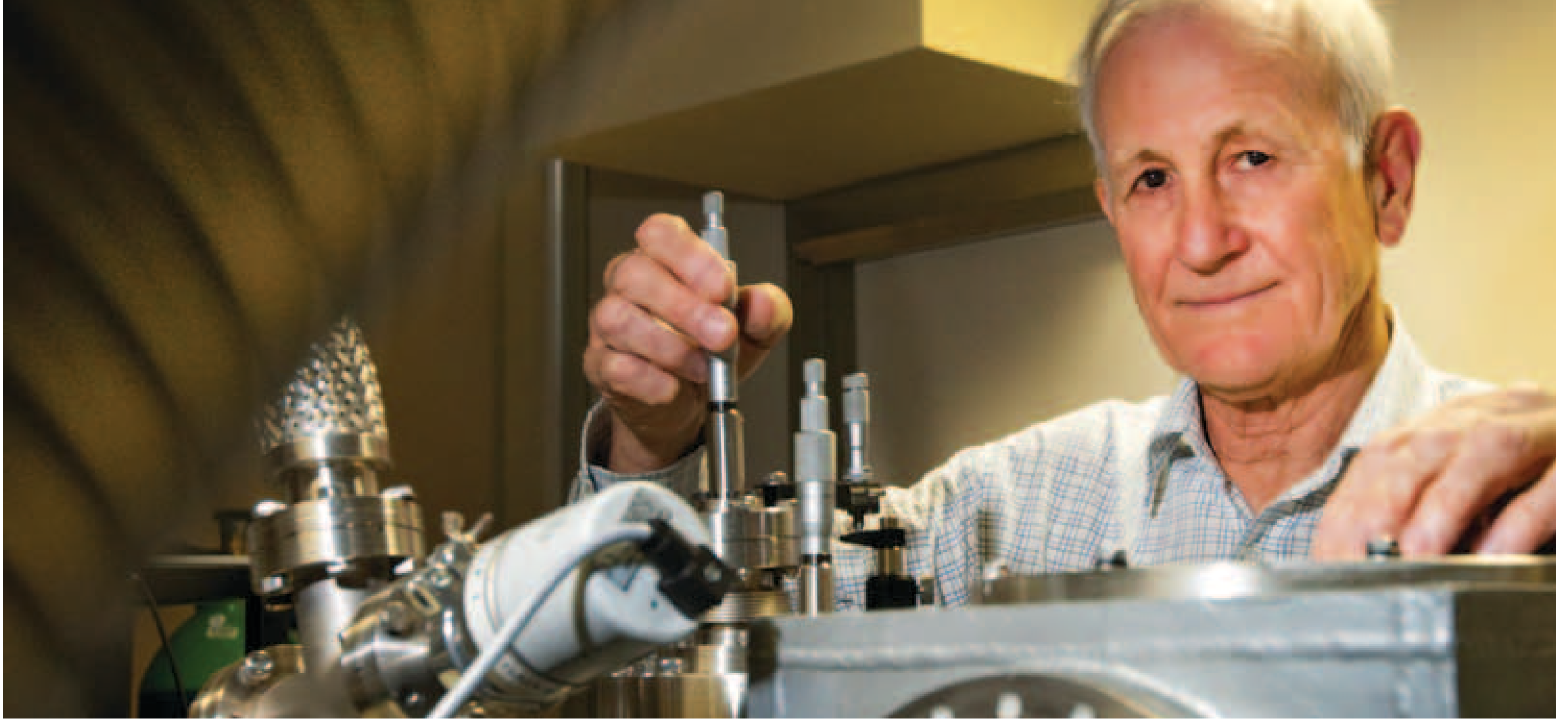
Honours Student, University of Melbourne



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DR DAMIAN E. MYERS
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CSIRO, Clayton

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Group Leader – CSIRO, Clayton

DR JOSE VARGHESE
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DR JANELLE WILLIAMS
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ASSOCIATE PROFESSOR HARRY QUINEY
Program Leader, University of Melbourne

DANIEL WELLS
MSc Student, University of Melbourne

# ORGANISATIONAL CHART AS OF JUNE 2010

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PhD Student, University of Melbourne

GABRIELLE FEJES
MSc Student, University of Melbourne

DR MARK JUNKER
Research Fellow, University of Melbourne

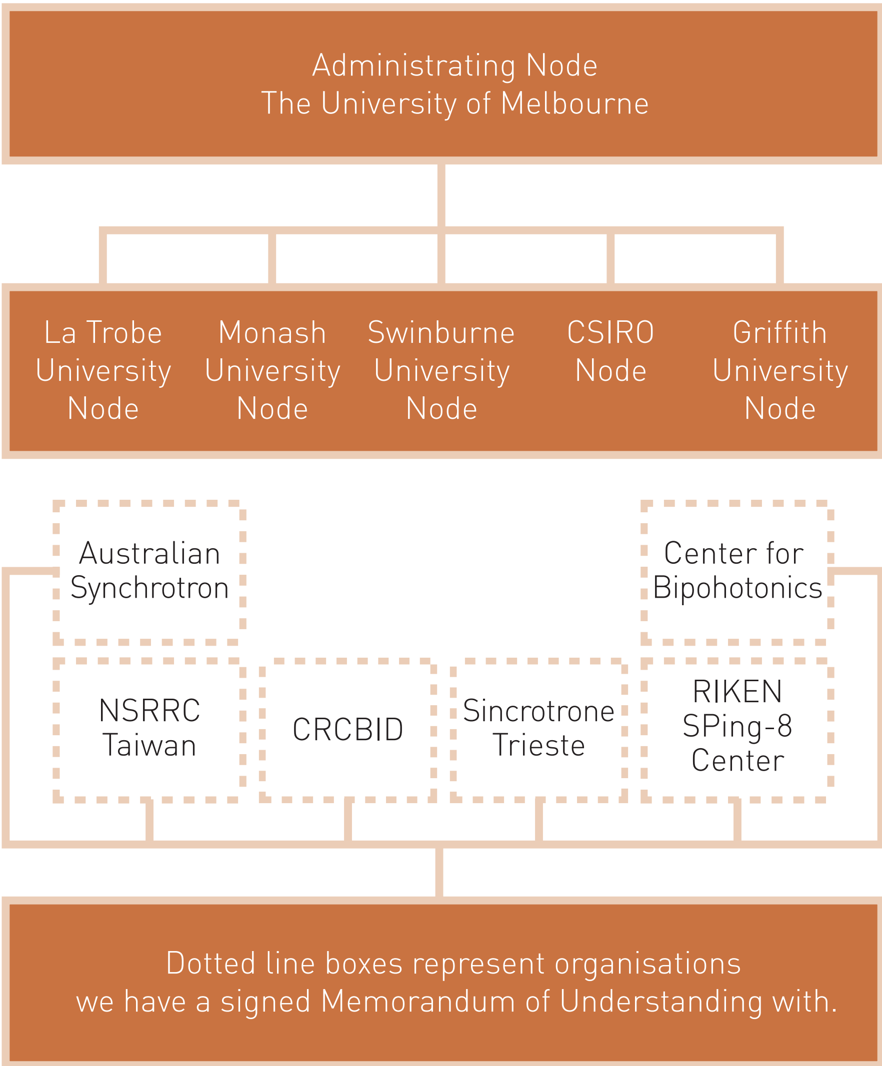
ANDREW MCCULLOCH
PhD Student, University of Melbourne

DR COREY PUTKUNZ
Research Fellow, University of Melbourne

SEBASTIAN SALIBA
PhD Student, University of Melbourne

ASSOCIATE PROFESSOR ROB SCHOLTEN
Program Leader, University of Melbourne

DR DAVID SHELUDKO
Research Fellow, University of Melbourne



185 PEOPLE ATTENDED THE 2010 CXS SUPER-RESOLUTION OPTICAL MICROSCOPY WORKSHOP; AN OPPORTUNITY TO MEET WITHIN AND ACROSS DISCIPLINES AND COUNTRIES.

# EXTENDING

# PRESENTATIONS, CONFERENCES & LABORATORY VISITS

DR BRIAN ABBEY

- Speaker – *Synchrotron X-ray tomographic investigation of internal structure of individual Flax fibres,* 6th World Congress of Biomechanics, Singapore, August 2010
- Speaker – High Harmonic Generation Workshop, Melbourne, Australia, October 2010
- Poster – *Diffractive Imaging using wide-bandwidth X-ray sources,* Australian Synchrotron Users Meeting, Melbourne, Australia, November 2010

DR BENEADICTA ARHATARI

- Attended – *Midbrain responses to Microstimulation of the cochlea using multi-channel thin film electrodes,* Australian Neuroscience Society Conference, Sydney, Australia, February 2010
- Attended – *Midbrain responses to Microstimulation of the cochlea using multi-channel thin film electrodes,* Frontiers 2010, Melbourne, Australia, July 2010
- Poster – Simulation of the effects of an indirect imaging detector used for imaging of corrosion in Aluminium Alloys, AIP Congress, December 2010

SIMON BELL

- Attended – Ultrafast Laser Users' Workshop, University of Melbourne, Australia, February 2010

GUIDO CADENAZZI

- Attended – Ultrafast Laser Users' Workshop, University of Melbourne, Australia, February 2010
- Attended – *Higher order Fresnel zone plate characterisation,* Australian Synchrotron Users Meeting, Melbourne Australia, February 2010

EVAN CURWOOD

- Attended – Ultrafast Laser Users' Workshop, University of Melbourne, Australia, February 2010
- Attended – KOALA Conference, University of Otago, New Zealand, November 2010

DR JEFFERY DAVIS

- Presentation – *Two-colour multi-dimensional spectroscopy of semiconductor nanostructures and light harvesting molecules and complexes,* Ultrafast Laser Users Workshop, Melbourne Australia, February 2010
- Attended – 17th International Conference on Ultrafast Phenomena, Colorado, USA, July 2010

KHUONG BA DINH

- Attended – CXS PostDoc and Student workshop, Melbourne, Australia, June 2010
- Attended – 22nd International Conference on Atomic Physics, Cairns Australia, July 2010

MATTHEW DIXON

- Invited Speaker – International Congress of Parasitology, Melbourne, Australia, August 2010
- Invited Speaker – OzBio 2010 Conference, Melbourne, Australia, September 2010

DR WILFRED FULLAGAR

- Oral presentation – *Lab-based ultrafast molecular structure,* Ultrafast Laser Users' Workshop, Melbourne, Australia, February 2010
- Poster – *Transient optical grating for ionising radiation – revisiting Bragg's X-ray microscope,* Ultrafast Laser Users' Workshop, University of Melbourne, Australia, February 2010

PROFESSOR PETER HANNAFORD

- Speaker – *Four-wave mixing as a sub-kHz probe for ground-state atomic coherence,* 22nd International Conference on Atomic Physics, Cairns Australia, July 2010
- Speaker – *Coherent blue light generation in Rb vapour,* 22nd International Conference on Atomic Physics, Cairns Australia, July 2010

DR ERIC HANSSEN

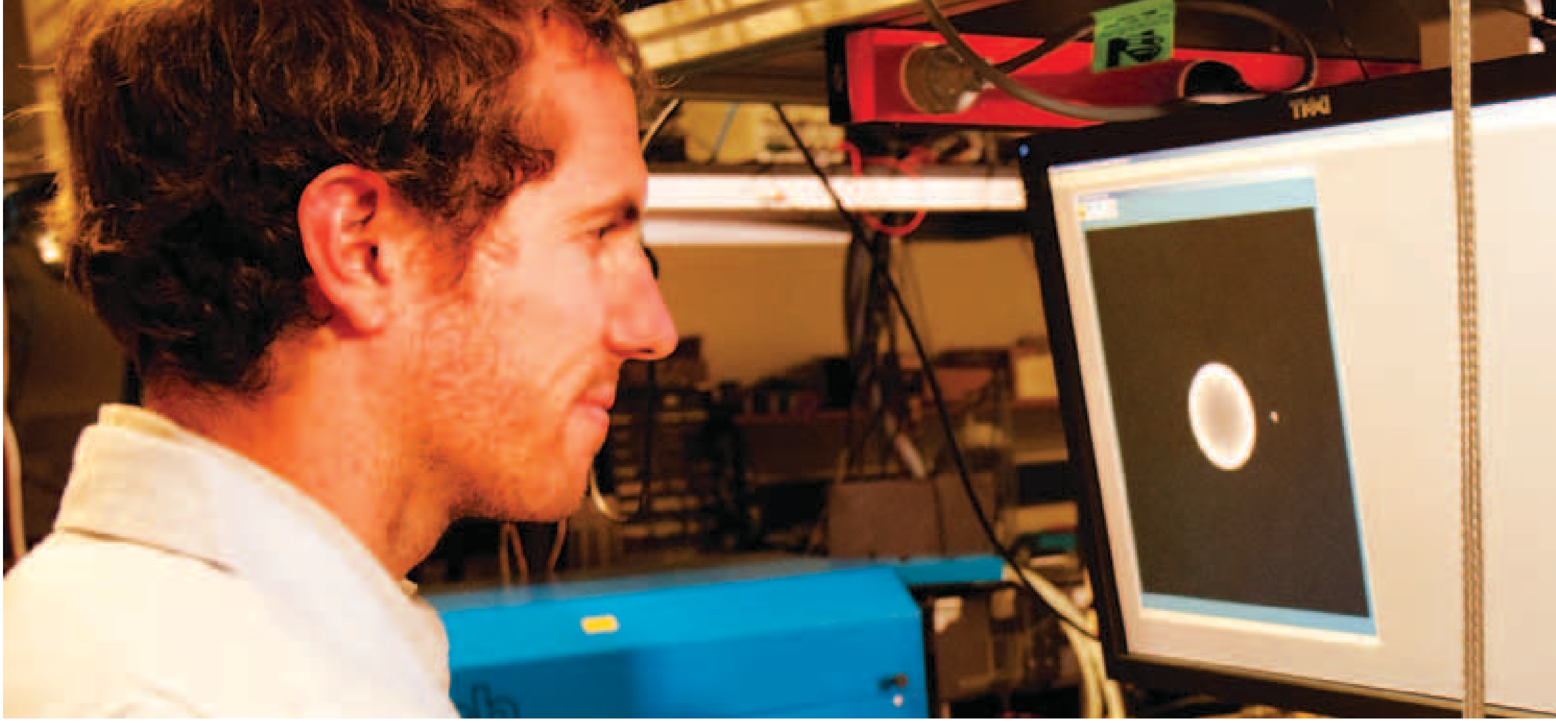
- Speaker – CXS X-ray Microscopy Workshop, La Trobe University, Melbourne, Australia, February 2010

CLARE HENDERSON

- Attended – Ultrafast Laser Users' Workshop, University of Melbourne, Australia, February 2010

DR LIISA HIRVONEN

- Speaker – *Light microscopy of living cells at nanometre scale resolution,* Biophysical Chemistry Workshop, Adelaide, Australia, April 2010
- Attended – Microscience 2010, London, England June 2010



- Attended – IUPAC Symposium on Photochemistry, Ferrara, Italy, July 2010

DR MARK JUNKER
- Attended – CXS Postdoc/Student Workshop, Melbourne, Australia, June 2010

ASSOCIATE PROFESSOR DAVID KIELPINSKI
- Invited Speaker – Annual Meeting of the Division of Atomic, optical and Molecular Physics of the American Physical Society, Texas, USA, May 2010
- Attended – International Conference on Atomic Physics, Queensland, Australia, July 2010
- Invited Speaker – *Strong-field ionisation experiments with atomic hydrogen,* Max Planck Institute, Munich, Germany, October 2010

DANE LABAN
- Attended – Ultrafast Laser Users' Workshop, University of Melbourne, Australia, February 2010
- Attended – International Conference on Atomic Physics, Queensland, Australia, July 2010

ANDREW MCCULLOCH
- Attended – Ultrafast Laser Users' Workshop, University of Melbourne, Australia, February 2010
- Speaker – *Partially coherent electron diffraction of biomolecules using an ultra cold plasma electron source,* International Conference of Atomic Physics, Queensland, Australia, July 2010
- Attended – KOALA Conference, University of Otago, New Zealand, November 2010

LACHLAN MCKIMMIE
- Speaker – *Three pulse photon echo studies of core-shell semiconductor quantum dots,* Ultrafast Laser Users' Workshop, University of Melbourne, Australia, February 2010
- Poster – *Examining the vibrational modes of CdSe nanocrystals,* 6th Asian Photochemistry Conference, New Zealand, November 2010
- Poster – *A picosecond X-ray source based on an amplified femtosecond titanium laser,* 6th Asian Photochemistry Conference, New Zealand, November 2010
- Poster – *Time-resolved fluorescence measurements of scorpions,* 6th Asian Photochemistry Conference, New Zealand, November 2010

DR PAUL MCMILLAN
- Invited Speaker – International Congress of Parasitology, Melbourne, Australia, August 2010

BEN MORRISON
- Speaker – *Picosecond X-ray source based on femtosecond Ti:Sapphire Laser,* Ultrafast laser Users Workshop, University of Melbourne, February 2010
- Attended – CXS Postdoc/Student Workshop, Melbourne, Australia, June 2010
- Poster – *A picosecond X-ray source based on an amplified femtosecond titanium laser,* 6th Asian Photochemistry Conference, New Zealand, November 2010

ASSOCIATE PROFESSOR ANDREI NIKULIN
- Invited Speaker – *X-ray diffraction studies of layered nanostructures and 3D diffraction imaging of nano-scaled materials,* ALBA Synchrotron, Barcelona, Spain, June 2010
- Visited – London Centre of Nanoscience, London, England, June 2010
- Invited Speaker – X-ray Optics Workshop, Chernogolovka, Russia, October 2010

PROFESSOR KEITH NUGENT
- Attended – BSR/MASR, Melbourne, Australia, February 2010
- Seminar Presentation – Illinois Institute of Technology, Chicago, USA, March 2010
- Seminar Presentation – University of Wisconsin, Milwaukee, USA, March 2010
- Presentation – American Physics Society meeting, Portland, Oregon, USA, March 2010
- Committee Member – Laureate Fellowship Selection Advisory Committee, Canberra, Australia, April 2010
- Lecture – Australian Institute of Physics, Victoria, Australia, April 2010
- Invited Presentation – National Synchrotron Light Source 2, New York, USA, May 2010
- Attended – Australian Synchrotron SAC Meeting, Melbourne, Australia, May 2010
- Attended – National Committee of Crystallographers Meeting, Sydney, Australia, May 2010

- Attended – Bragg Institute Advisory Committee Meeting, Sydney, Australia, May 2010
- PhD Opponent – Uppsala University, Sweden, May 2010
- Panel Member – 2010 Victoria Prize and Fellowship Awards, Victoria, Australia, May 2010
- Invited Speaker – Coherence 2010 Conference, Rostock, Germany, June 2010
- Attended – XRM 2010 Conference, Chicago, USA, August 2010
- Paper Presentation – International Microscopy Congress, Rio de Janeiro, Brazil, September 2010
- Symposium Chair – International Microscopy Congress, Rio de Janeiro, Brazil, September 2010
- Master of Ceremonies – Super Resolution Microscopy Workshop, Melbourne, Australia, October 2010
- Invited Speaker – 3rd Workshop on XFEL Science, Hokkaido, Japan, October 2010
- Attended – NAB Impact Award Ceremony, Melbourne, Australia, October 2010
- Colloquium – University of Sydney, New South Wales, Australia, October 2010
- Attended – Australian Institute of Physics Congress, Melbourne, Australia, December 2010

ASSOCIATE PROFESSOR ANDREW PEELE

- Invited Speaker – 4th Italian Australian Workshop, *Advances in Coherent Diffractive Imaging,* Victoria, Australia, February 2010
- Visited – Advanced Light Source, Lawrence Berkeley National Laboratory, USA, June 2010
- Attended – Coherence 2010 Conference, *Particle Coherence, Phase Diversity and Coherent X-ray Imaging,* Rostock, Germany, June 2010
- Attended – Workshop on X-ray diffraction imaging of embedded nanoparticles, Bari, Italy, June 2010
- Invited Speaker – Melbourne Centre for Nanofabrication Seminar Series, *High resolution optics for X-ray imaging,* Melbourne, Australia, July 2010
- Invited Speaker – SPIE, *High resolution X-ray phase tomography,* San Diego, USA, August 2010
- Invited Speaker – XRM, *Fresnel coherent Diffractive Imaging,* Chicago, USA, August 2010
- Invited Speaker – LCLD Users Meeting, Frontiers in Biology with XFELS, Stanford, USA, October 2010
- Master of Ceremonies – Super Resolution Microscopy Workshop, Melbourne, Australia, October 2010

DR DANIELE PELLICCIA

- Speaker – Microscience 2010, *3D X-ray diffraction imaging with 5nm resolution of embedded nanoparticles,* London, UK, June 2010
- Visited – Institute for Photonics and nanotechnologies, Rome, Italy, June 2010

MICHAEL PULLEN

- Attended – *Experimental tunnelling ionisation of H using few-cycle pulses,* International Conference on Atomic Physics, Queensland, Australia, July 2010

COREY PUTKUNZ

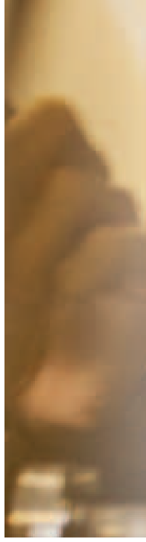
- Speaker – Biology and Synchrotron Radiation, *2D and 3D Fresnel Coherent Diffractive Imaging,* Melbourne, Australia, February 2010

ASSOCIATE PROFESSOR HARRY QUINEY

- Invited Speaker – 4th European XFEL Users' Meeting, *Diffraction imaging using partially coherent X-rays,* Germany, January 2010
- Invited Speaker – Coherence 2010 Conference, *Biomolecular imaging and electronic damage using XFEL sources,* Germany, June 2010
- Visitor – University of Oxford, United Kingdom, June 2010
- Visitor – Imperial College London, United Kingdom, June 2010
- Invited Speaker – 3rd XFEL Asia-Pacific Users' Meeting, Japan, October 2010

PROFESSOR MIKE RYAN

- Session Chair – Annual Lorne Conference on Protein Structure and Function, Lorne, Australia, February 2010
- Invited Speaker – Sansom Institute, University of South Australia, Australia, February 2010
- Invited Speaker – Peter MacCallum Cancer Institute Seminar Series, Melbourne, Australia, March 2010
- Invited Speaker – SFB Seminar, Freiburg, Germany, June 2010

- Guest Speaker – Department of Genetics, University of Melbourne, Melbourne, Australia, July 2010
- Program Chair – OzBio 2010 Conference, Melbourne, Australia, September 2010
- Invited Speaker – John Curtin School of Medical Research, Australian National University, Canberra, November 2010

REBECCA RYAN
- Attended – CXS Postdoc/Student Workshop, Melbourne, Australia, June 2010

SEBASTIAN SALIBA
- Attended – CXS Postdoc/Student Workshop, Melbourne, Australia, June 2010
- Speaker – International Conference of Atomic Physics, *Extending Coherence of Nitrogen-Vacancy in Diamond,* Queensland, Australia, July 2010

PROFESSOR ROBERT SANG
- Attended – Ultrafast Laser Users Workshop, University of Melbourne, Australia, February 2010
- Attended – International Conference on Atomic Physics, Queensland, Australia, July 2010

ASSOCIATE PROFESSOR ROB SCHOLTEN
- Attended – Meeting on Structural Dynamics: Ultrafast Dynamics with X-rays and Electrons, Banff, Canada, February 2010
- Visit – American Physical Society Division of Atomic Molecular and Optical Physics, Houston Texas, USA, May 2010
- Speaker – 52nd International Field Emission Symposium, *Diffraction imaging with electrons from an ultra cold plasma source,* Sydney Australia, July 2010
- Poster – *Analytic model for the effects of absorption on relative intensity squeezing,* International Conference of Atomic Physics, Queensland, Australia, July 2010
- Attended – Haemozoin Workshop, Victoria, Australia, August 2010
- Presentation – Techische Universiteit Eindhoven, *Ultra Cold Ion Source,* Netherlands, August 2010

DAVID SHELUDKO
- Attended – Ultrafast Laser Users' Workshop, University of Melbourne, Australia, February 2010

TANIA SMITH
- Invited Speaker – Melbourne Research Office Seminar, COEs' – An insiders Perspective, University of Melbourne, Australia, February 2010

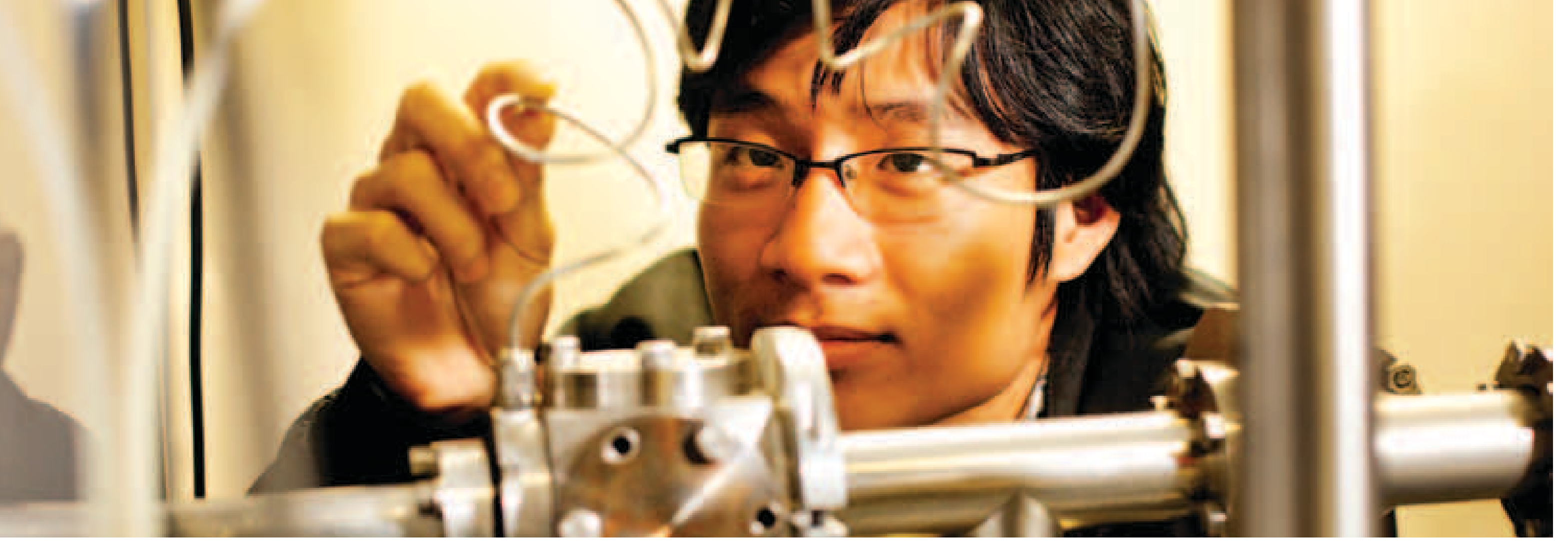
ASSOCIATE PROFESSOR TREVOR SMITH
- Speaker – Biophysical Chemistry Workshop, *Time-resolved fluorescence measurements of bimolecular adsorption,* Adelaide, Australia, April 2010
- Attended – Nikon Super-resolution Workshop, New South Wales, Australia, October 2010
- Speaker – Super Resolution Microscopy Workshop, Melbourne, Australia, October 2010

DR DIANA STOJANOVSKI
- Oral Presentation – Gordon Research Conference on Mitochondria & Chloroplasts, Barga, Italy, July 2010

DR VICTOR STRELTSOV
- Visited – Photon Factory Synchrotron, Tsukuba, Japan, June 2010
- Attended – Asian Crystallographic Association meeting, Korea, October 2010

DANIEL THOMPSON
- Attended – KOALA Conference, University of Otago, New Zealand, November 2010

PROFESSOR LEANN TILLEY
- Speaker – MASR2010/BSR2010, Melbourne, Australia, February 2010
- Session Chair – Annual Lorne Conference on Protein Structure and Function, Lorne, Australia, February 2010
- Speaker – 10th hunter Cell Biology Conference, Imaging Workshop, New South Wales, Australia, March 2010
- Speaker – Issues in Nanotechnology Seminar Series, Physics Department, La Trobe University, Melbourne, Australia, April 2010
- Chair – OzBio 2010 Conference, Melbourne, Australia, September 2010
- Speaker – Super Resolution Microscopy Workshop, Melbourne, Australia, October 2010
- Invited Speaker – Biochemistry and Molecular biology Department, Monash University, Melbourne, Australia, October 2010



ANGELA TORRANCE

- Attended – Ultrafast Laser Users' Workshop, University of Melbourne, Australia, February 2010

PROFESSOR LAP VAN DAO

- Attended – Ultrafast Laser Users Workshop, Melbourne Australia, February 2010
- Invited Speaker – 12th International Conference on X-ray Lasers, *Generation of small bandwidth coherent extreme ultraviolet radiation and its applications,* Gwangju, Korea, May 2010
- Attended – 22nd International Conference on Atomic Physics, Cairns Australia, July 2010
- Attended – *High harmonic generation for study of rotational raman,* 17th International Conference on Ultrafast Phenomena, Colorado, USA, July 2010
- Attended – 6th Asian Symposium on Intense Laser Science, Beijing, China, October 2010
- Course Lecture – International Conference on Photonics, China, November 2010
- Attended – International Conference on Photonics and Applications, Vietnam, November 2010

DR DAVID VINE

- Speaker – Biology and Synchrotron Radiation, *Fresnel Coherent Diffractive Imaging: A new technique for bio-imaging,* Australia, February 2010

WILLIAM WALLACE

- Poster – *Ionisation of atomic hydrogen with few-cycle pulses,* Ultrafast Laser Users' Workshop, University of Melbourne, Australia, February 2010
- Attended – International Conference on Atomic Physics, Queensland, Australia, July 2010

LACHLAN WHITEHEAD

- Attended – Ultrafast Laser Users' Workshop, University of Melbourne, Australia, February 2010

JEFF YOEMAN

- Attended – Ultrafast Laser Users' Workshop, University of Melbourne, Australia, February 2010

# AWARDS & HONOURS

CXS recognised a number of its members for their work in 2010. We extend our congratulations to each of them for their efforts and awards in the following honours:

- Catherine Palmer and Jeff Yeoman were awarded the ABAS Student Poster Prizes at OzBio2010 Conference, Melbourne, Australia, September 2010
- Jesse Clark and Corey Putkunz received the student bursaries award to attend the Coherence 2010 Conference.
- Professor Leann Tilley received the Bancroft-Mackerras Medal 2010 from the Australian Society for Parasitology for outstanding contributions of its members to the science of parasitology.
- Dr Michael Baker received the Alexander von Humboldt Research fellowship for his postdoctoral work in Cologne, Germany.
- Dr Alex Maier was a finalist for the 2010 University of New South Wales Eureka Prize for Scientific Research for his work on adaptive strategies of the malaria parasite.
- Dr Marc Kvansakaul received a Tall Poppy award at Bio21.
- Dr Connie Darmanin was awarded the CSIRO Payne Scott Award 2010
- Associate Professor Robert Scholten received the Alan Walsh Medal for service to industry 2010 by the Australian Institute of Physics.

*Professor Leann Tilley receives the Bancroft-Mackerras Medal from Professor Terry Spithill, President of the Australian Society for Parasitology.*

# EUREKA!
# CXS VIDEO PROJECTS


## 2010 saw the completion of the centre's two video projects, one of which achieving national recognition!

The three minute documentary student film, *Röntgen: A Bright Spark* about the discovery of the first X-ray by German Nobel Laureate Professor Wilhelm Röntgen won the prestigious 2010 'Sleek Geek' Eureka Prize for science communication.

As part of the outreach program of CXS, administered at the University of Melbourne, CXS embarked upon a film making project in which six students from St Helena Secondary College in Eltham created the three minute documentary.

Professor Wilhelm Conrad Röntgen was a German physicist, who, on 8 November 1895, produced and detected electromagnetic radiation in a wavelength range today known as X-rays, or Röntgen rays – an achievement that earned him the first Nobel Prize in Physics in 1901. *Röntgen: A Bright Spark* was funded by CXS and the Victorian Department of Education and Early Childhood Development (DEECD) and was developed and delivered by the CXS node at La Trobe University.

The winning St Helena year ten students; Betty Cheregi, Matt Dalla Rosa, Paul Dalla Rosa, Steven Megaloudis, Byron Mihailides and Evan Raif worked collaboratively to plan the film's content, create a storyboard, write a script and perform before the cameras. While the project required a large and dedicated group of stakeholders, the extent of the school's interaction and the students' commitment and the quality of the final product ensured its success.

To better understand the science, the students visited the University of Melbourne, the Australian Synchrotron and delved into the scientific research world by interviewing a number of scientists who work with X-rays.

The students were treated to a glamorous night at the Eureka Prize Awards Dinner in Canberra, where they met the original Sleek Geek Dr Karl Kruszelnicki, and mingled with well-known Australian such as Cate Blanchett, Peter Garrett, MP, Jennifer Byrne, Amanda Keller and Sandra Sully.

A second video, *X-rays and the Magic of Coherence,* was developed to overview the history and basics of X-rays, and explain their current applications in arts, medicine and other areas, as well as their use by members of CXS to determine biological structures.

As part of producing the supplementary piece, the students visited several universities to interview researchers as well as the Royal Children Hospital and the National Gallery of Victoria.

The 13 minute video will be a useful marketing tool for CXS activities, directed to year 12 and first year physics students and is available for viewing on the CXS website.

CXS Director and Professor of Physics at the University of Melbourne, Professor Keith Nugent said CXS is proud of its extensive outreach program, and in particular its well established relationship with secondary school students:

*"We are dedicated to the communication of the importance of our way of doing science and showing that the physical sciences have an impact in all areas, particularly in the biosciences.*

*"We believe that modern science, if it is to address the problems we face in the 21st century, must harness the expertise of interdisciplinary teams prepared to work together to solve the big problems.*

*"We have an excellent outreach program that has achieved considerable recognition, including the inaugural Victorian State Impact Award from the Schools First Program run by the National Australia Bank and now this Eureka Prize.*

*"The movie is delightful and captures the CXS emphasis on the excitement of the science that can be performed with X-rays. We congratulate the St Helena College students involved."*

To view *Röntgen: A Bright Spark*, follow the link to the University of Sydney website: www.science.usyd.edu.au/outreach/eureka/

To view *X-rays and the Magic of Coherence* please visit the CXS website: www.coecxs.org

# SCHOLARSHIPS & STUDENTSHIPS

We would like to congratulate the following students for their successful applications in 2010:

- Alex Lowdin, CXS Vacation Studentship, *Shape formation within the sexual stages of the human malaria parasite, Plasmodium faciparum,* Biological Sciences Program, La Trobe University
- Darren Smith, Universitas21 joint PhD student from University of Edinburgh, Short Wavelength Laser Source Program, Swinburne University of Technology
- Ben Morrison received a University of Melbourne PhD scholarship to undertake X-ray generation and super-resolution microscopy with the Short Wavelength Laser Program and the University of Melbourne
- Sebastian Saliba had his scholarship with the Ultracold Plasma Source Program at the University of Melbourne extended for six months
- David Sheludko had his scholarship with the Ultracold Plasma Source Program at the University of Melbourne extended for three months

# RESEARCH TRAINING & PROFESSIONAL EDUCATION

The Centre met all of its recruitment and professional education targets for 2010, and has exceeded expectations in the areas of *Postgraduate Recruitment* and *Presentations to Schools and/or Teaching Communities.*

## CXS SPONSORED EVENTS

CXS sponsored the following events in 2010:

- Lorne Conference on Protein Structure and Function, Lorne Australian, February 2010
- The 3rd annual Conference on Optics, Atoms and Laser Applications – ION – KOALA 2010, Dunedin New Zealand, November 2010
- Eureka Prize Sleek Geeks Winning Documentary Launch, Melbourne Australia, December 2010
- The 19th Australian Institute of Physics Congress incorporating the 35th Australian Conference on Optical Fibre Technology(AIP/ACROFT), Melbourne Australia, December 2010

## WORKSHOPS

CXS conducted the following interdisciplinary workshops in 2010:

- Ultrafast Laser Physics Workshop, University of Melbourne, Australia, 11–12th February 2010
- X-ray Microscopy Workshop, La Trobe University, Australia, 19th February 2010
- Super Resolution Microscopy Workshop, Melbourne, Australia, 1st October 2010
- Growing Tall Poppies Conference, Melbourne, Australia, 3rd December 2010
- CXS End of Year Overview, Melbourne, Australia, 3rd December 2010

# CXS SUPER-RESOLUTION OPTICAL MICROSCOPY WORKSHOP

"It is very easy to answer many fundamental biological questions; you just look at the thing". This is quote from Richard Feynman (Nobel Prize in Physics) in a talk to the American Physical Society in 1959. He went on to suggest that what physicists should do to help biologists is to make better microscopes. Indeed, in the last few years optical microscopy methods have undergone breathtaking developments. In particular, cell biologists are very excited about new light microscopy techniques that use ingenious approaches to overcome the "diffraction limit" of light. These new techniques enable imaging of cellular processes at a level of resolution up to an order of magnitude beyond the current limits.

The impact of the new 'super-resolution' microscopy methods is being recognised around the world. To inform Australian scientists about the new methods, CXS organised a 'Super-Resolution Optical Microscopy Workshop' at the Convention Centre, in Melbourne, on 1st October 2010. The workshop formed part of the highly successful international OzBio2010 Conference, which was held in the same venue over the course of the week. The CXS workshop aimed to bring physicists and biologists together at a very exciting time for optical microscopy. There were 185 registrants and it was a fantastic opportunity to meet within and across disciplines and countries.

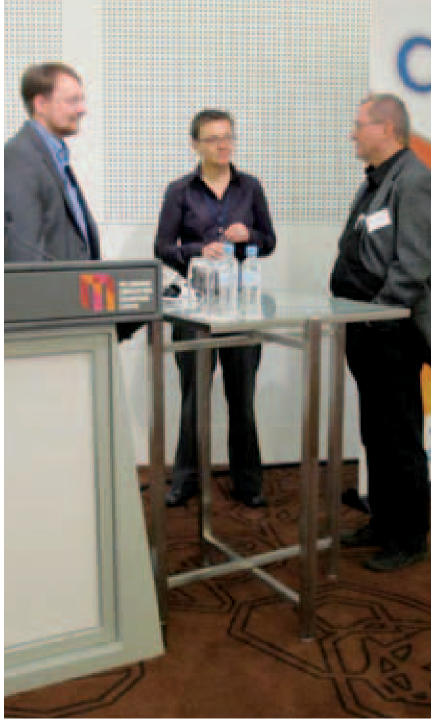
Professor Keith Nugent opened the meeting explaining the goals of the workshop. He gave an overview of some of the activities within CXS and explained how physicists, chemists and biologists work together to achieve the aims of the Centre. He explained that CXS, established to develop X-ray imaging, has seen the exciting opportunities offered by the new Super-Resolution visible light methods and is keen to provide leadership and scientific contributions in this area.

The workshop featured a talk by Associate Professor Sam Hess, from the University of Maine, USA. Associate Professor Hess is an inventor of one of the new super-resolution microscopy techniques as well as excellent presentations by leading Australian proponents. The aim of the workshop was to provide an overview of the new techniques as well as providing high-level insights into the development and application of particular techniques. Here are some highlights from the excellent program of speakers:

**Professor Guy Cox**, Sydney University, served as Deputy Director of the Electron Microscope Unit, University of Sydney (now the Australian Centre for Microscopy and Microanalysis) from 2001 to 2005. He explained the laws described by Raleigh and Abbé, dictating that the resolution of conventional light microscopes is restricted to about half the wavelength of the exciting light. Thus conventional microscopes can resolve 200 – 250 nm in the lateral plane and ~600 nm in the vertical direction. This is about the size of a mitochondrion or a bacterium and is a major limitation on the ability of cell biologists to study sub-cellular events.

Prof. Cox provided an overview of the new imaging techniques that use different strategies to overcome the diffraction limit. He offered a more in-depth analysis of stimulated emission depletion (STED) microscopy, explaining the use of non-linear de-excitation of fluorescent dyes to increase resolution. Professor Cox went on to explain how the technique employs a conventional excitation laser in combination with a STED laser to produce a torroidal or doughnut-shaped illumination pattern The STED laser deactivates the fluorescence outside a small central zone, reducing the effective excitation area from which fluorescence emission can occur. A resolution of ~80 nm in xy can be readily achieved, with ~20 nm the best reported (2, 8).

**Associate Professor Sam Hess**, University of Maine, USA, invented a technique known as fluorescence photoactivation localisation microscopy (FPALM). FPALM and the related technique stochastic optical reconstruction microscopy (STORM) separate fluorophores in time that are

*Leann Tilley and Keith Nugent.*

inseparable in space and are sometimes called "pointillist" techniques (1, 4). They rely on photo activation processes to turn fluorescent molecules on and off (1, 3). With only a few well separated molecules "turned on" each emitter is individually captured in a given image without overlap. 2-D Gaussian functions are fit to accurately determine the location of each emitter in that image. These molecules are then turned off and a new set turned on which are reimaged and located. This process is repeated thousands of times until the entire image is reconstructed. The technique enables imaging of subcellular structures in living cells with a "resolution" of 20–30 nm, which is well below the diffraction limit (where "resolution" is defined as the distance between objects that can be reliably measured rather than true optical resolution).

Associate Professor Hess described the application of FPALM to the question of how the influenza virus exploits cell membrane organisation for infection and assembly. His work is providing unprecedented views of the clustering and dynamics of hemagglutinin, the fusion protein from influenza.

**Associate Professor Katharina Gaus** is an NHMRC Senior Research Fellow and leads the Cell Membrane Biology group at the Centre for Vascular Research, University of New South Wales. Associate Professor Gaus uses PALM and STORM to study the dynamic mechanisms that underlie membrane signalling. These single molecule imaging techniques can be used to quantify the number of proteins participating in signalling clusters, the number of clusters and the ratio of proteins within clusters. She described the threshold parameters used to distinguish fluorescent signals from background and noted that labelling density is an important determinant of the performance of these pointillist methods. Associate Professor Gaus' work has provided important insights into the principles that control the organisation of signalling proteins in the T cell membrane.

**Associate Professor Trevor Smith**, University of Melbourne, described the more established techniques of scanning near-field optical microscopy (SNOM) and total internal reflection fluorescence microscopy (TIRFM), both of which can be classified as "near-field" techniques. SNOM was the first optical method capable of overcoming the diffraction limit and is based on squeezing the excitation light through the very small aperture, some tens of nanometres in diameter, of a drawn optical fibre, restricting the region of the sample that generates emission to these dimensions. TIRFM can provide imaging with axial resolution on the tens of nanometres, and some new approaches based on this method such as Harmonic Excitation Laser Microscopy (HELM) or Standing Wave Illumination, are reported to achieve ~90 nm lateral resolution (5) or better. Associate Professor Smith discussed the combination of some Super-Resolution techniques in addition to the extension of super-resolution microscopy through the use of time-resolved emission detection, providing picosecond temporal resolution in addition to the sub 100 nm spatial resolution. He applies these techniques to biological as well as non-biological samples, including luminescent polymer films.

**Associate Professor Cynthia Whitchurch** is a NHMRC Senior Research Fellow in the ithree Institute (previously the UTS Institute for Biotechnology of Infectious Diseases, (IBID)) and is Director of the UTS Microbial Imaging Facility at the University of Technology, Sydney. Associate Professor Whitchurch outlined her work that uses 3-D structured illumination microscopy (3D-SIM) to study bacterial cell biology. In 3D-SIM applications the sample is illuminated with a periodic pattern that interacts with the fluorescence from small features in the sample to generate Moiré images with resolvable spatial features (5–7). An algorithm is used to reconstruct an image with a 2-fold increase in resolution over conventional optical microscopy (xy ~100 nm, z ~250 nm).

Bacteria are very small with rod-shaped species having a typical width of 0.5 – 1.5 microns and a length of 1 – 10 microns. As a result, studying microbial cell biology using conventional light microscopy is a major challenge. Using the Delta Vision OMX microscope, Associate Professor Whitchurch has been able to ask questions about cell division, cellular differentiation and protein trafficking in bacteria.

**Professor Leann Tilley** is Director of Research at the La Trobe Institute for Molecular Science. Her laboratory is using a range of high resolution microscopy techniques to image malaria parasite-infected erythrocytes. Professor Tilley was an early adopter of 3D-SIM. She travelled to UCSF to use the developmental instrument of Prof. John Sedat to study sub-structure within the exomembrane system of malaria

*Delegates at the Super Resolution Optical Microscopy Workshop.*


paraiste-infected erythrocytes. More recently she has worked with Associate. Professor Cynthia Whitchurch to apply 3D-SIM to study 90 nm knob structures that are important *P. falciparum* virulence determinants. In other examples she showed that 3D-SIM is able to resolve apical pores (~150 nm diameter) in GFP-labelled merozoites and ~100 nm striations in the sub-pellicular membrane complex of *P. falciparum* gametocytes. Professor Tilley discussed with workshop attendees some of the challenges associated with obtaining good 3D-SIM images and stressed the potential for artefacts with any imaging technology. She commended the use of multi-modal or correlative imaging methods, which greatly increase confidence in the data.

The attendees of the workshop enjoyed lively discussion during question time, as well as breaks. The advantages and disadvantages of the various super-resolution techniques and conventional imaging techniques were discussed at length. Issues explored by attendees, and that continue to be evaluated, include the ideal optical resolution; whether existing fluorophores and labelling protocols are compatible with particular imaging methods; the time required to acquire data and reconstruct an image; and the need for live cell imaging.

It became apparent from these discussions that access to a range of instruments and techniques is imperative for scientists, in order to trial various alternate techniques to determine the best application within a specific circumstance. The instruments and techniques currently available are not universally applicable to all research environments, with each modality possessing its own unique advantages and disadvantages, which cannot be explored without access to a range of available options.

CXS has sponsored the development of the Cellular Nano-Imaging Consortium (CNIC) as an affiliation of scientists with interests in Super-Resolution Optical Microscopy. CNIC brings together institutions and research leaders with cross-disciplinary expertise and an interest in using and/or developing nano-imaging optical methods. It provides on-line access to information about conventional and super-resolution optical imaging techniques and details the resources that are currently (and potentially) available to interested parties. Through CNIC additional workshops and conference sessions will be organised to inform Australian scientists about new high-resolution imaging modalities.

The Organising Committee for the workshop (Leann Tilley, Trevor Smith, Fabienne Perani, Keith Nugent and Tania Smith) thank the session chairs, Sarah Russell (Swinburne University / Peter MacCallum Cancer Centre) and Mark Prescott (Monash University) and all of the participants for contributing to such an excellent program. The Committee would also like to thank a number of commercial companies whose generous sponsorship made the workshop possible. These are: Coherent Scientific (Nikon), Zeiss, Leica Microsystems, Berthold Australia Applied Precision), FABLS and La Trobe Institute for Molecular Science (LIMS). Photos are available at www.coecxs.org.

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# VISITORS TO CXS

1. ANNE AGGARWAL, Santa Maria College, Australia
2. KLAUS BARTSCHAT, Drake University, USA
3. XAVIER BUTCHER, St Bernard's College, Australia
4. XAVIER DE BRUYN, St Bernard's College, Australia
5. GWENNETH CHEALE, St. Catherine's School, Australia
6. JAMES DEAN, Euroa School College, Australia
7. NICOLE DO, Santa Maria College, Australia
8. ASH DONNISON, Euroa School College, Australia
9. PROFESSOR TIM EGAN, University of Cape Town, South Africa
10. WILL FLEMING, St Bernard's College, Australia
11. IVAN GALIC, St Bernard's College, Australia
12. PROFESSOR TIM GILBERGER, Bernard nocht Institute, Hamburg, Germany
13. KEELY HAUSLER, Euroa School College, Australia
14. ASSOCIATE PROFESSOR ANITA JONES, University of Edinburgh, Scotland
15. PROFESSOR HENRY KAPTEYN, University of Colorado, USA
16. TESS KIRKINIS, Santa Maria College, Australia
17. VAN LE, Santa Maria College, Australia
18. YVONNE LIU, Santa Maria College, Australia
19. JORDAN LOPRESTI, St Bernard's College, Australia
20. DR BOHUMIL MACO, University of Queensland, Australia
21. ANDRIAN MARCATO, St Bernard's College, Australia
22. PROFESSOR MARGARET MURNANE, University of Colorado, USA
23. ISSIE MCBETH JEPHCOTT, Star of the Sea College, Australia
24. LUCY MCNEILL, Star of the Sea College, Australia
25. LISA MILLER, Brookhaven National Laboratory, New York, USA
26. GEORGIA O'CONNOR, Star of the Sea College, Australia
27. MATTHEW PAIGE, University of Saskatoon, Saskatewan, Canada
28. MEGAN POOL, Star of the Sea College, Australia
29. NATALIE RELF, St. Catherine's School, Australia
30. MS VIVIANE RICHTER, University of New South Wales, Australia
31. GARRY RUMBLES, National Renewable Energy Laboratories, Colorado, USA
32. PROFESSOR ROBERT SAINT, University of Melbourne, Australia
33. RAMONA SARKIS, Santa Maria College, Australia
34. PROFESSOR JOHN SEDAT, University of California, USA
35. ANGELA SELLECK, St. Catherine's School, Australia
36. EVGENY SHAPIRO, University of British Columbia, Canada
37. ALESSIA TAMBORRIELLO, Star of the Sea College, Australia
38. WENDY TO, Santa Maria College, Australia
39. ALISOUN TOWNSEND, Euroa School College, Australia
40. MY CHAU TRAN, Santa Maria College, Australia
41. NHUNG TRAN, Santa Maria College, Australia
42. MS. NORA VOGTELE, University of Freiburg, Germany
43. TONY WARWICK, Advanced Light Source, California, USA
44. GRACE WHITBREAD-PHEE, Star of the Sea College, Australia
45. LAURA WHITNEY, Euroa School College, Australia
46. TYSON WICKS, Euroa School College, Australia
47. MARIA WU, Santa Maria College, Australia
48. KAI YANG, Santa Maria College, Australia
49. PROFESSOR ADA YONATH, Weizmann Institute, Israel
50. KATIE ZHANG, Katie Zhang, Gwenneth Cheale

# COLLABORATIONS

*Professor Keith Nugent and Dr Tetsuya Ishikawa signing the Memorandum of Understanding.*

A number of ongoing collaborations continue to develop with the following groups:

- **RIKEN Harima Institute, Spring-8 Centre, Japan**
  CXS signed a Memorandum of Understanding with RIKEN Harima Institute, RIKEN Spring-8 Centre, Japan.
- **Physical and Theoretical Chemistry, University of Oxford**
  Associate Professor Harry Quiney discussed the teaching of mathematics and quantum mechanics to undergraduate chemists and the preparation of a manuscript on The Dirac-Coulson-Fischer Wavefunction in Quantum Chemistry.
- **Imperial College London**
  Discussions with the Laser Physics group on high harmonic generation, theoretical modelling of non-linear optical processes and the development of laser facilities at Imperial College.
- **Carola Hunte Laboratory, Frieburg, Germany**
  Investigating membrane protein structure and potential collaborations.
- **University of Oxford**
  Collaboration with Brian Abbey, Felix Hiofmann and Nikos Baimpos on Laue Orientation tomography and high-energy differential aperture microscopy.
- **Consiglio Nazionale delle Ricerche (CNR), Italy**
  CXS Director Professor Keith Nugent, attended collaborative meetings with CNR to implement a Human Frontiers Project.
- **ASML, The Netherlands**
  The Ultra Cold Plasma Source Program held discussions with Kenian Domen on new developments in ultra cold plasma.
- **Center for Nanoscale Science and Technology, Maryland, USA**
  Associate Professor Robert Scholten visited this facility to discuss ultra cold ion beam systems and participate in the ongoing collaboration on quantum optics and lasers.
- **Juniata College, Pennsylvania, USA**
  Associate Professor Robert Scholten participated in the ongoing collaboration with J White on laser-atom interactions.
- **University of Maryland, USA**
  Associate Professor Robert Scholten attended the Joint Quantum Institute to discuss ultra cold plasma.
- **ANSTO, Australia**
  Associate Professor David Kielpinski met with Adi Patterson, Richard Garrett and John Boldeman to discuss collaborations on the proposed Queensland laser Accelerator.

# CELLULAR NANO-IMAGING CONSORTIUM

The Cellular Nano-Imaging Consortium (CNIC) is an affiliation of scientists with interests in Super-Resolution Optical Microscopy managed under the auspice of CXS. Its inception is the direct result of a joint initiative undertaken by CXS Director Professor Keith Nugent, Deputy Director Leann Tilley and CXS member Associate Professor Trevor Smith, School of Chemistry, University of Melbourne to bring together institutions and research leaders with cross-disciplinary expertise and an interest in using and/or developing nano-imaging optical methods.

CNIC provides online access to information about conventional and super-resolution optical imaging techniques and what resources are currently (and potentially) available to interested parties. Through CNIC, workshops and conference sessions are organised to inform Australian scientists about new high-resolution imaging modalities. CNIC aims to co-ordinate efforts to generate a super-resolution imaging capability in Victoria, providing information and access to the new techniques.

CNIC is working to ensure that all Victorian scientists have access to the Super-Resolution Microscopy format they need to be competitive as international research leaders.

The CNIC website can be visited at www.coecxs.org/cnic

# SCIENTIFIC LINKAGES

CXS is pleased to announce the signing of a Memorandum of Understanding with:

*Australian Synchrotron*

*National Synchrotron Radiation Research Center of Taiwan*

*ELETTRA*

*RIKEN*

*CRC for Biomedical Imaging Development*

*The Centre for Biophotonics Science and Technology*

# COMMERCIALISATION

Professor Keith Nugent continued to head the development team of Iatia Ltd. Using their globally patented QPI technology, Iatia has continued to expand into life sciences, nanotechnology, ophthalmology and defence markets, with customers including GE Healthcare, Columbia University, Oxford University, the Federal Bureau of Investigations (FBI) and the Australian Defence Force.

The UPSP developed the MOGlab's range of external cavity diode laser (ECDL) controllers. Each MOG unit provides everything needed to run an ECDL and lock it to an atomic transition. Marketing material has been produced and a targeting marketing strategy was developed in 2008. All revenue derived from this activity will be the property of The University of Melbourne and one student inventor. There were 22 sales made in 2010, down from previous years, and a license agreements has been in place with the University of Melbourne since June 2008.

Dr Chanh Tran obtained an Australian Provisional Patent, "Imaging Method and apparatus", No. 2008901157.

CXS and Melbourne Ventures have produced a DVD on the commercialisation of IP.

*Professor Henry Kapteyn and Professor Margaret Murnane.*

# OUTREACH

As part of the CXS Outreach Program a number of key initiatives took place in 2010; expanding on existing activities:

- The Theory and Modelling Program hosted the Growing Tall Poppies Program "X-ray Sudoku" with students from St Bernard's Catholic Boys' School in Essendon.
- Professor Leann Tilley gave a talk at the Post-PhD career pathways in Molecular Sciences workshop, La Trobe University on *Tips for the Early Career Researcher.*
- Matt Dixon was involved in the Out-of-Classroom Program with Ivanhoe Grammar School.
- Samantha Deed gave a talk to a Year 9 Secondary College Program on *How malaria parasites are grown in the lab for research studies.*
- Dr Brian Abbey produced an article for Hilda's Chronicle on *Science with Synchrotrons.*
- Associate Professor Andrew Peele recorded a podcast on the work of CXS for Broadcast in the La Trobe University podcast series.
- CXS Sponsored Professor Margaret Murnane from the University of Colorado, USA at the Australian Institute of Physics Congress.
- A 4th year graduate student X-ray course was offered by the Experimental Methods Program at La Trobe University.
- Summer student Camille Mucha completed a project making web pages for the Experimental Methods tomography facility.
- Professor Robert Sang gave an overview of the Attosecond Science Facility at Griffith University for a promotional video.
- Dr Eugeniu Balaur delivered the practical component of the La Trobe University Masters subject – Advanced Nanomaterials and Fabrication, which consisted of four two week laboratory projects.

*Dinner with Professor Yonath.*

# WOMEN IN PROTEIN SCIENCE WITH THE 2009 NOBEL PRIZE WINNER, PROFESSOR ADA YONATH

Professor Ada Yonath is an Israeli crystallographer best known for her pioneering work on the structure of the ribosome. She is the current director of the Helen and Milton A. Kimmelman Center for Biomolecular Structure and Assembly of the Weizmann Institute of Science. In 2009, she received the Nobel Prize in Chemistry along with Venki Ramakrishnan and Tom Steitz for her studies on the structure and function of the ribosome, becoming the first Israeli woman to win the Nobel Prize, the first woman from the Middle East to win a Nobel prize in the sciences, and the first woman in 45 years to win the Nobel Prize for Chemistry.

Professor Yonath was invited to Australia to present plenary talks at the Lorne Protein Structure and Function Meeting (Feb 7–11) and the Biology of Synchrotron Radiation Meeting (Feb 14–18). Her talk at the Lorne Proteins meeting was a tour de force.

The presentation provided an explaination of ribosomes as a complex assembly of macromolecules that perform protein biosynthesis and that are made of several RNA molecules, assisted by a number of distinct protein molecules. Professor Yonath described her work on the determination of the complete high-resolution structures of the two ribosomal subunits. The audience heard how she discovered, within the otherwise asymmetric ribosome, a universal symmetrical region that provides the framework for and navigates the process of polypeptide formation. She described ribosomes as factories and explained that the ribosome has enzyme activity (i.e. it is a ribozyme). placing the tRNA-bound amino acid substrates in the correct stereochemistry to facilitate peptide bond formation. Professor Yonath illustrated the path taken by the nascent polypeptides through the ribosomal tunnel, and the dynamic elements that control the rate of polypeptide elongation and direct the nascent chain into a space where it can begin to fold.

Professor Yonath enthusiastically supports the RNA-world hypothesis; that is, that RNA predated both proteins and DNA during the origin of life. She points to evidence for an ancient RNA machinery (a proto-ribosome) residing within the core of the modern ribosome. She believes that this proto-ribosome was the pre-biotic RNA synthesising machine and that the ribosome was initially an RNA replicase that was hi-jacked by amino acids to produce polypeptides. Indeed the RNA core of the ribosome is highly conserved in all divisions of life, which has led her to the view that there are 1000s of pre-historic machines lying hidden within every cell.

Professor Yonath commenced her pioneering work on the structure of the ribosome over twenty years ago despite considerable scepticism from the international scientific community, maintaining her enthusiasm in the face of endless setbacks. At the initiative of Professor Leann Tilley (Deputy Director, CXS), Professor Yonath was invited to meet with some of the women protein scientists attending the conference who had been inspired by her work. Over an enjoyable meal, Prof. Yonath reflected to the group that the main requirements for winning a Nobel Prize are curiosity, determination and passion. In talking to her it also became evident that an ability to work super-human hours, a deep interest in people and in human nature, a wide ranging interest in science and an optimistic personality have also contributed to her success.


# GROWING

THE 2010 GROWING TALL POPPIES (GTP) ALUMNI CONFERENCE DAY WAS A RESOUNDING SUCCESS AND WILL GO DOWN IN THE CXS AND GTP HISTORY BOOKS.

# THE GROWING TALL POPPIES IN SCIENCE PROGRAM

## PARTNERSHIPS BETWEEN SCHOOLS AND COMMUNITIES PROVIDE A CONNECTED LEARNING EXPERIENCE FOR STUDENTS.

### PARTNERS WITH A PURPOSE

*'Growing Tall Poppies: an authentic science experience for secondary school students'.* Growing Tall Poppies (GTP) is a collaboration between Santa Maria College (SMC) Northcote, CXS and Akorn Educational Services. The initial implementation of the program was supported by the Catholic Education Office Melbourne (CEOM). The program has grown from a shared goal of providing a positive and engaging experience for students to enhance their learning of science, with the greater aim of increasing the number of students studying science. Working in partnership with the wider community in this way has been essential for realising this goal.

The program title, 'Growing Tall Poppies'; has evolved from the euphemism for those whose achievements conspicuously exceed – or grow above – those of others around them. The program emphasises the potential that students possess to achieve above and beyond average, given the opportunity to do so. The purpose of the GTP program is to help young Australians, who have the potential, to flourish and become 'Tall Poppies' in science. It is genuinely about growing new Tall Poppies for Australia.

SMC is a Catholic girl's school in the inner north of Melbourne and its vision is to ensure that students are fully able to realise their potential in all areas of academic endeavour, so as to enrich their lives and the lives of the members of the community of which they are a part. CXS as an Australian Research Council Centre of Excellence has an imperative to share its interdisciplinary research and to encourage students to pursue careers in the sciences. An important feature of the partnership formed between SMC and CXS is that students from many schools have also been able to participate in the program through an agent (Akorn) that connects educational programs and school communities.

In developing the GTP program, CXS has focused on providing secondary school students, especially girls, an authentic experience of the physical sciences. Through this enquiry-based experience, students are given the opportunity to explore the limits of their curiosity by being placed in a real scientific research environment. The experience offers a genuine feel for how interesting and relevant the physical sciences are to their work and their future. It allows students to not only act upon their inquisitiveness, but also to create knowledge and to practice team-building and leadership skills.

When students have the opportunity to experience science with real scientists, grappling with real problems, they are more likely to view their classroom activities in context, and so feel more connected in their classroom learning experience. This enables teachers to engage students with the relevance of science to society and so make what would otherwise be an esoteric pursuit into a tangible one. Through the partnership developed between CXS and SMC we have been able to engage Year 10 and 11 students with science in a unique way, and through this increased level of engagement, to improve their classroom wellbeing.

Students crave excitement in their learning environment and they search for life or career choices where they can make a contribution to the world. Young adults in our care deserve the opportunity to see how science is constructed in real time and how the advances being made right now can effect change that can cure disease, solve climate change and benefit society, the community and individuals in many other ways.

### THE GROWING TALL POPPIES PROGRAM

The GTP program is a series of authentic science projects that connect students with the science community to explore scientific processes and knowledge along with its formation and relevance. Students are mentored by professional scientists and do real experiments to obtain first-hand data that is, in some cases, the first

*Anne Aggarwai, Santa Maria College student.*

of its kind collected in the world. Students are immersed in labs that are working at the forefront of science. They formulate questions and search for the solutions together with the scientist. GTP focuses student learning on the scientific process as a way of building knowledge that improves the quality of life and solves problems that confront society today. Students experience meaningful learning by linking the science content to the relevance of science to everyday human life. They are also able to expand their understanding of the technologies used in research in Australia, such as laser facilities and the Australian Synchrotron.

The GTP curriculum allows students to focus on the interconnectedness of science with other facets of life and society. This builds an understanding of the interdisciplinary nature of science, as well as society's dependence on scientific knowledge and understanding. Through experimental investigation and reflective discourse with experts, students learn the issues associated with science and have the chance to synthesise individual opinions and critical thinking. The confidence students gain from working in this environment arms them with skills for future learning as well as developing them as global citizens for the challenges of the 21st century.

## PROGRAM OUTCOMES

In partnership with the broader science community CXS is improving students' sense of self and connectedness with the world they live in while they are learning science. Although students have the opportunity to undertake practical experiments in the conventional science classroom, these are historically re-enactments, sometimes with an inquiry dimension, and therefore often perceived as lacking real-world relevance. The partnership with scientists gained through the GTP program overcomes this classroom constraint and broadens students' experiences. Students have developed new, positive ways of viewing science and they have been engaging more actively in the classroom. Students have displayed an increased inclination to ask questions about the class topics and are self-confident in seeking answers. They become proactive in posing new inquiry questions to investigate, and have published their work both on the web and in the Science Teachers' Association of Victoria *Labtalk* magazine. Students are eager to participate in multiple GTP programs and to mentor not only other students but also teachers to participate with them.

The student outcomes of the project are aligned with the Victorian Essential Learning Standards (VELS), the national Curriculum and the International Baccalaureate middle Year program curriculum. The GTP curriculum outline and student projects can be viewed on the GTP website www.coecxs.org/growingtallpoppies/.

The GTP program is also providing a unique opportunity for scientists to reflect on their role as advocates of science in the community. Furthermore, teachers have been inspired to experience this authentic science experience to stimulate and invigorate their classroom teaching practice. This GTP component of teaching professional learning, which places groups of teachers with scientists to investigate cutting-edge technology and the relevance of the scientific research, contributes to positive student outcomes.

Through a further partnership, an online learning environment has been developed to capitalise upon the contemporary and preferred mode for student communication. The GTP online forum is the outcome of another partnership with the Victorian e-Research Strategic Initiative (VeRSI) and can be accessed at http://gtp.versi.edu.au/forum. The web-based environment enables students and scientists to work as partners on projects via the web and to remotely link into scientific facilities, for example the Australian Synchrotron, to perform experiments. This is consistent with current scientific methodology.

The partnership established between CXS and SMC is a new and exciting mode of delivering a living science curriculum in secondary schools. Connections with the professional science fraternity, and ultimately with the wider community, creates positive outcomes for students and school communities alike.

## BREAKING NEW GROUND

A recent development in the GTP program has been engaging a group of year 11 secondary students to run the first secondary student experiment at the Australian Synchrotron. The students first prepared samples of protein crystals at the CSIRO and then used beamlines at the synchrotron to take pictures of the protein. This new collaboration will lead to further authentic projects for students at the Australian Synchrotron. The development offers the opportunity to truly change the way students engage with the physical sciences and promises to change their views of the relevance and importance of science in all of our lives.

# GTP STUDENT REFLECTIONS

*"My experience helped me see the importance of science and relevant applications of the things you study at school. You can also see the links with the different areas of study. It also made me learn new things about proteins: for example I learned that you can make protein crystals using different methods. I also learned the different techniques that are involved in examining the crystals by using X-rays. What was good too was that we were able to use and see some of the advanced technologies that are not usually found at school."*
*(KRISTINE PICARDAL, 17 YEARS)*

*"The excitement of participating in Growing Tall Poppies is always intoxicating. The experience made me realise that it is actually fun to learn. Not only do you realise science is awesome but also that the different branches of science such as biology, physics and chemistry are all joined together. Each project has so many things to look forward to, including getting to know how scientists do their investigations and how they create new knowledge. I really feel I can do this type of study as a career."*
*(MY CHAU TRAN, 17 YEARS)*

*"Before the Growing Tall Poppies program, I thought that physics, biology and chemistry were separate from each other. I was surprised to find that science is more interdisciplinary than that. The program allowed me to have an overview of science and to see the connections between my subjects much more vividly because I can see some of the connections with the real world. It has helped me throughout my learning including my non-scientific subjects!"*
*(RAMONA SARKIS, 17 YEARS)*

*"The program has helped me see science-in-action and what scientists really do. It has helped me stay interested in the science we learn at school because it has given it a real context and application. Each project shows how all the science subjects we learn at school, such as biology, chemistry and physics, all link to each other and [how] we need to use all of them in order to solve a real-life problem. I have been able to learn different scientific techniques, which has been very useful in the classroom to make what I learn more real."*
*(NHUNG TRAN, 17 YEARS)*

*"Growing Tall Poppies gives students the opportunity to experiment with what happens in the real world of science. It has given me a better understanding of scientists. Before the program my definition of a scientist was a man in a white lab coat; now I see that scientists are all sorts of people with a wide range of interests and really creative minds. If I had the choice to do GTP all year, I would choose it. I loved working in a group to find out about real projects that people don't have an answer for yet. At school, we are just learning basic knowledge (like every other student) with no application and no creativity."*
*(VAN LE, 17 YEARS)*

*"Growing Tall Poppies was an important program. Not only did it help me explore my own capabilities, explore new interests in the field of physics, and explore science outside the classroom, it also demonstrated the importance of science in today's society and has inspired me to continue to study science. It was fantastic to be able to publish the work in Labtalk (Kirkinis & Barone-Nugent 2010)."*
*(TESS KIRKINIS, 16 YEARS)*

# GTP MENTOR SCIENTISTS' REFLECTIONS

*"The Growing Tall Poppies program provides a great mechanism through which students get to see behind the scenes of a working science laboratory. It helped us to identify ways of communicating and highlighting what we do to student who are (currently) non-specialists. Participation in this program reinforced the importance of science education and of invigorating interest in science. It also helped to illustrate how our (sometimes apparently esoteric) scientific investigations are directly applied to real-world problems."*
*(ASSOCIATE PROFESSOR TREVOR SMITH)*

*"The students' enthusiasm and engagement with the work I think was a direct result of the choice they had in formulating it. Although experiments were designed by us for the students to work on, they developed their own questions and methods for bringing the experiment into the context of a broader research project.*

*'I was surprised by the students' ability to negotiate and listen to each other. Together they developed goals, timelines, workloads, responsibilities, colour schemes, and deadlines – and all done with great care and fairness. They seemed overjoyed with the opportunity to structure their time in their own way.*

*"By the end of the week spent with us I had started to think of them as university undergraduates, not Year 10 secondary students."*
*(CLARE HENDERSON, PHD STUDENT)*

*ST CATHERINE'S SCHOOL STUDENTS*

*Hosted by Short Wavelength Laser Source Program.*

Ultra-Violet (UV) Semiconductors and Extreme Ultra-Violet (XUV) Ultrafast Lasers

***Student Participants:*** *Natalie Relf, Angela Selleck, Katie Zhang, Gwenneth Cheale, Sarah Lockwood*

***CXS Mentors:*** *Christopher Hall and Khuong Dinh*

*ST BERNARD'S COLLEGE STUDENTS*

*Hosted by the Theory and Modelling Program.*

Mathematics and Physics Meeting Popular Culture

***Student Participants:*** *Will Fleming, Andrian Marcato, Xavier Butcher, Xavier de Bruyn, Jordan LoPresti and Ivan Galic*

***CXS Mentors:*** *Evan Curwood and Rebecca Ryan*

*EUROA SECONDARY COLLEGE STUDENTS*

*Hosted by the Ultracold Plasma Source Program.*

I can see a rainbow

***Student Participants:*** *Alisoun Townsend, Ash Donnison, Keely Hausler, James Dean Laura Whitney and Tyson Wicks*

***CXS Mentors:*** *Andrew McCullock, Simon Bell and Sebastian Saliba*

*STAR OF THE SEA COLLEGE STUDENTS*

*Hosted by the Experimental Methods*

Figures in the Dark

***Student Participants:*** *Georgia O'Connor, Grace Whitbread-Phee, Megan Pool, Issie McBeth Jephcott, Lucy McNeill and Alessia Tamborriello*

***CXS Mentors:*** *Clare Henderson and Angela Torrance*

*SANTA MARIA COLLEGE STUDENTS*

*Hosted by the Short Wavelength Laser Source Program*

***Project group I:*** What is all the Wining About/ Drunken Projector

***Project group II:*** What is all the Wining About Part 2

***Student Participants:*** *My Chau Tran, Nhung Tran, Maria Wu, Nicole Do, Van Le Wendy To, Tess Kirkinis, Anne Aggarwal, Ramona Sarkis, Yvonne Liu and Kai Yang*

***CXS Mentors:*** *Daniel Dias, Liisa Hirvonen and Trevor Smith*

# MEDIA COMMENTARIES

## NEWSPAPER AND MAGAZINE ARTICLES

- The Australian, *New scientific experts to guide synchrotron*, 22 February 2010
- The Age, *More strife at synchrotron*, 28 February 2010
- The Australian, *Researchers probe the depths of deadly parasite*, 28 April 2010
- The Age Higher Education Supplement, *Researchers probe the depths of deadly parasite*, 28 April 2010
- The Australian, *Federal light may shine on synchrotron*, 21 April 2010
- The Diamond Valley Leader, *National prize thrills St Helena*, 24 August 2010
- ARC/NHMRC Research Network for Parasitology Newsletter, Vol 21, Issue 4, October 2010 http://parasite.org.au/wp-content/uploads/2010/10/ASPnewsletterV21i4.pdf
- Learning Matters, Vol 15, Issue 2, 2010, *The Growing Tall Poppies in Science Program*, December 2010
- Dr Brian Abbey produced an article for Hilda's Chronicle on *Science with Synchrotrons*, 2010

## ELECTRONIC MEDIA

- Australian Life Scientist, *Super resolution microscopy breaks the light barrier*, 13 December 2010, www.lifescientist.com.au/article/371098/feature_super_resolution_microscopy_breaks_light_barrier/
- MediLexicon, *Despite Damage, Membrane Protein Structure Can Be Seen Using New X-ray Technology, Study Reveals*, 21 December 2010, www.medilexicon.com/medicalnews.php?newsid=212075
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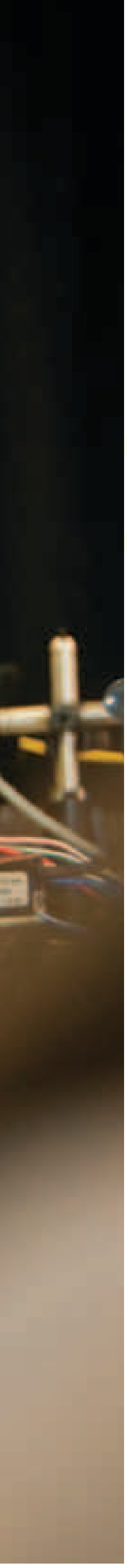
## BROADCASTS

- Professor Leann Tilley was interviewed by Linda Burns on ABC 774, September 2010
- Associate Professor Andrew Peele recorded a podcast on the work of CXS for Broadcast in the La Trobe University podcast series.

THE RESEARCH ACROSS CXS HAS BEEN OUTSTANDING, AS EVIDENCED BY THE BODY OF PUBLISHED WORK FEATURED IN HIGHLY RANKED JOURNALS, WITH STRONG COLLABORATIONS AND A NUMBER OF INTER-DISCIPLINARY PUBLICATIONS ACROSS THE PHYSICAL, CHEMICAL AND BIOLOGICAL SCIENCES.

# BROADENING



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## CONFERENCE PROCEEDINGS

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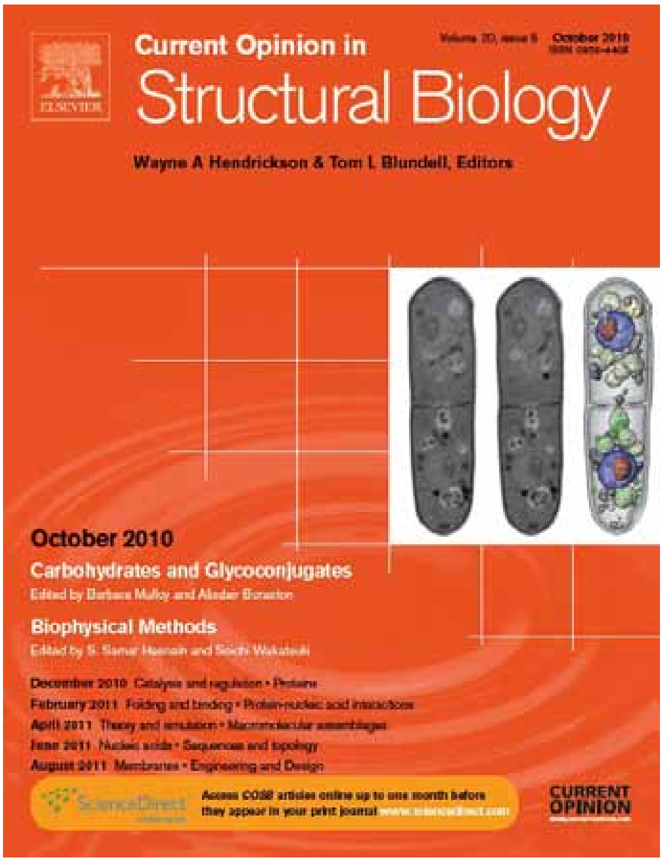
Science Precinct
Dean of Science
Faculty of Science Office
School of Physics
Materials Engineering

# 2010 COVERS


Current Opinion in
Structural Biology
Wayne A Hendrickson & Tom L Blundell, Editors
October 2010
Carbohydrates and Glycoconjugates
Biophysical Methods
December 2010 Catalysis and regulation • Proteins
February 2011 Folding and binding • Protein-nucleic acid interactions
April 2011 Theory and simulation • Macromolecular assemblages
June 2011 Nucleic acids • Sequences and topology
August 2011 Membranes • Engineering and Design
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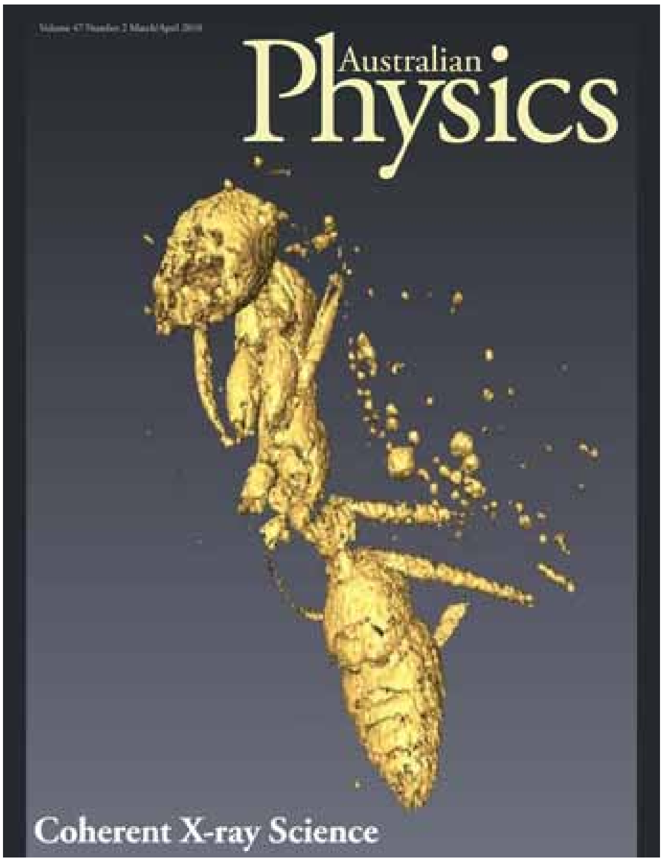
CURRENT OPINION


Australian
Physics
Coherent X-ray Science

# GRANTS

CXS members attracted $3,310,706 in additional support in 2010:

| | |
|---|---|
| **ARC** | |
| Transporting proteins to and within mitochondria | $328,348 |
| High-resolution electron diffraction imaging for the nanosciences | $750,000 |
| **Australian Synchrotron** | |
| International Synchrotron Access Program | $11,300 |
| **Superscience Fellowship** | |
| Nanoimaging the cellular architecture of the malaria parasite, Plasmodium falciparum | $278,400 |
| **Clive & Vera Ramaciotti Foundation** | |
| Shape, form and function of the human malaria parasites sexual stages | $61,888 |
| **ANZ Trustees** | |
| Matthew Dixon | $19,000 |
| **AMMRF** | |
| Correlative fluorescence and EM Imaging of the Malaria Parasite Plasmodium falciparum | $3,900 |
| **NHMRC** | |
| The role of assembly factors in mitochondrial complex I biogenesis and their defects in disease | $586,875 |
| Defining the Genomic basis of mitochondrial complex I deficiency | $616,995 |
| The Na+/H+ exchanger and V-type pyrophosphatases of the malaria parasite | $640,000 |
| **ISAP** | |
| Travel grant | $14,000 |

## PARKVILLE CAMPUS

Corner Swanston Street and Tin Alley, Parkville

### PHYSICS BUILDING

CXS Head Office

The Experimental Methods Program (also at La Trobe University)

The Theory and Modelling Program

The Ultra-Cold Plasma Source Program

### PARKING

'Scratch & Display' car parking permits are available for the use of official visitors to the campus and nearby University parking areas. Upon notification, CXS staff can arrange permits in advance.

## BUNDOORA CAMPUS

Kingsbury Drive, Bundoora

### PHYSICAL SCIENCES BUILDINGS 1 AND 4

The Biological Sciences Program

The Experimental Methods Program (also at University of Melbourne)

### PARKING

Parking for visitors at there is on a fee-paying basis. Tickets can be purchased at car parks from the ticket machines. Upon notification, CXS staff and visitors can arrange daily temporary permits in advance.

## CLAYTON CAMPUS

Wellington Road, Clayton

### PHYSICS BUILDING

The Detector and Beamline Development Program

### PARKING

Parking permits are required during weekdays and short-term parking zones are also available.

Parking without a permit is available in the blue, red and yellow zones after 5pm on weekdays and all weekend.

# CXS LOCATIONS

## HAWTHORN CAMPUS

John Street, Hawthorn

CENTRE FOR ATOMIC OPTICS AND ULTRAFAST SPECTROSCOPY

The Short Wavelength Source Program

PARKING

Parking in university car parks is on a fee-paying basis only. Tickets can be purchased in car parks from the ticket machines or from multi deck car park.

This campus is also situated a couple of minutes walk from the Glenferrie train station & tram stops.

## NATHAN CAMPUS

170 Kessels Road, Nathan QLD 4111

SCHOOL OF BIOMOLECULAR AND PHYSICAL SCIENCES

PARKING

Griffith University offers a variety of parking options on the Nathan campus. Casual visitors can choose from $5/day parking permits, metered parking or pay and display parking.

## CLAYTON

Gate 5, Normanby Road, Clayton

MANUFACTURING AND INFRASTRUCTURE TECHNOLOGIES

The Structure Determination Methods Program

## PARKVILLE

343 Royal Parade, Parkville

MOLECULAR AND HEALTH TECHNOLOGIES

The Structure Determination Methods Program

# FINANCIAL STATEMENT

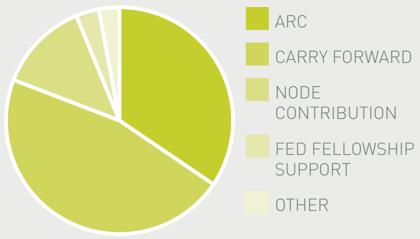
## CXS FINANCIAL REPORT JANUARY – DECEMBER 2010

| | 2010 REPORTING PERIOD ($) | | 2011 REPORTING PERIOD (ESTIMATED) ($) | |
|---|---|---|---|---|
| Carry Forward | $ 2,711,770 | | $2,603,014 | |
| Other Funds | $2,000,000 | ARC Income | $2,200,000 | ARC Income |
| | $160,350 | ARC Indexation | $127,212 | ARC Indexation |
| | $750,000 | Node Contribution | $825,000 | Node Contribution |
| | $200,000 | ARC Federation Fellow Support | $200,000 | ARC Federation Fellow Support |
| **Total Income** | **$5,822,120** | | **$5,955,226** | |
| Expenditure | $1,999,840 | Salaries | $2,000,000 | Salaries |
| | $332,919 | Equipment | $1,500,000 | Equipment |
| | $347,152 | Travel, Accommodation and Conference | $450,000 | Travel, Accommodation and Conference |
| | $257,613 | Materials, Provisions and Services | $300,000 | Materials, Provisions and Services |
| | $120,093 | Scholarships | $160,000 | Scholarships |
| | $126,553 | Marketing, Outreach and Sponsorship | $150,000 | Marketing, Outreach and Sponsorship |
| | $34,936 | General | $50,000 | General |
| | **$3,219,106** | | **$4,610,000** | |
| **Balance** | **$2,603,014** | | **$1,345,226** | |

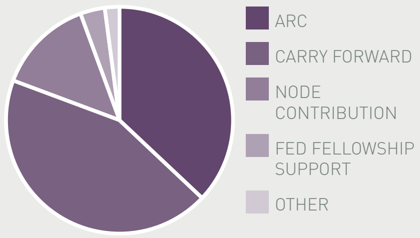
## IN-KIND REPORT JANUARY – DECEMBER 2010

| | |
|---|---|
| University of Melbourne | $2,116,570 |
| La Trobe University | $1,722,244 |
| Monash University | $294,806 |
| Swinburne University of Technology | $530,977 |
| Griffith University | $449,186 |
| CSIRO | $335,643 |
| **Total** | **$5,449,426** |

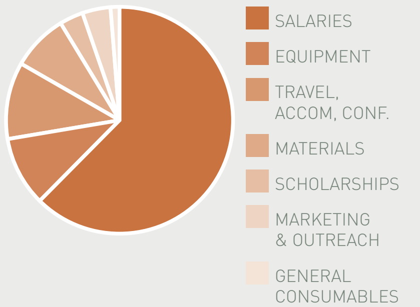
## 2010 INCOME BY SOURCE



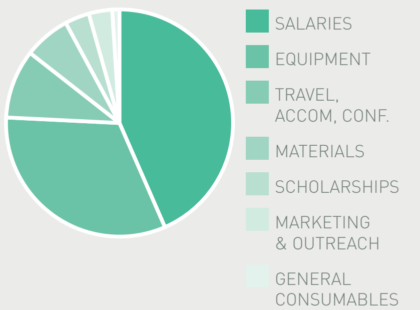

## 2011 INCOME FORECAST



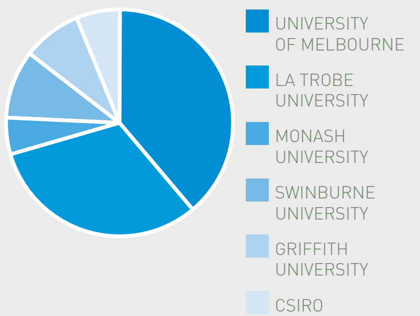

## 2010 EXPENDITURE




## 2011 EXPENDITURE FORECAST




## 2010 IN-KIND CONTRIBUTION



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