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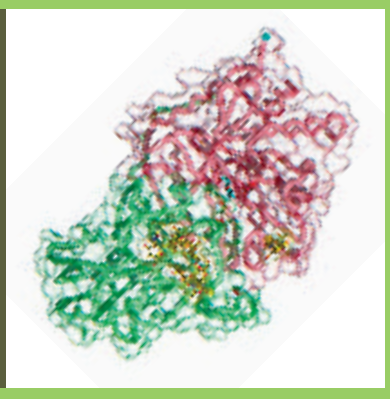

New science enabled by the synchrotron

IMAGE: Two domain exoglucanase enzyme that removes glucose from plant cell walls and is used by plants for softening the plant skin so that new shoots can grow.

Image by Jose Varghese, CSIRO Structural Biology Program

Chapter 03

New science enabled by the synchrotron

Australian Nobel Prize winners William Lawrence Bragg and William Henry Bragg. Courtesy Edgar Fahs Smith. Memorial Collection, Special Collections, University Pennsylvania Library

There are many fields of new science where access to a synchrotron is essential. It is noteworthy, for example, that the Nobel Prize for Chemistry this year for 'discoveries concerning channels in cell membranes' was supported with research done at synchrotrons in the USA and France for solving the structure of the potassium ion channel.

This chapter provides an insight into the opportunities in just a few of the fields that will be possible for researchers who have access to the proposed beamlines on the Australian Synchrotron. The examples are illustrative, not comprehensive, and have been sourced from Australian research where possible.

Structural Biology

Tremendous strides have been taken over the past twenty years in the understanding of biology and the processes that make life possible. Central to this has been the understanding of the role of genes, and the sequencing of the DNA code. Recent unravelling of the human genome had led to the perception that this knowledge will help cure many intractable diseases, and that the control and manipulation of biota is possible.

Understanding the genome is important, but of greater interest in the post-genomic era is understanding the structure and function of the many proteins that are expressed by the gene. This is a very large undertaking – it is estimated that there are over one million different protein products expressed by the human genome alone.

Nobel Prizes for research with x-rays

The Australian Synchrotron will provide x-rays over a wide energy range. Since their discovery in 1895, x-rays have had an extraordinary effect on society. There have been no fewer than fifteen Nobel prizes awarded for x-ray research, listed below.

X-rays are already vital to research ranging from molecular biology through to high-energy astrophysics and there is continuing potential for exciting new science.

1901	W. C. Roentgen in Physics for the discovery of x-rays.
1914	M. von Laue in Physics for x-ray diffraction from crystals.
1915	W. H. Bragg and W. L. Bragg in Physics for crystal structure derived from x-ray diffraction.
1917	C. G. Barkla in Physics for characteristic radiation of elements.
1924	K. M. G. Siegbahn in Physics for x-ray spectroscopy.
1927	A. H. Compton in Physics for scattering of x-rays by electrons.
1936	P. Debye in Chemistry for diffraction of x-rays and electrons in gases.
1962	M. Perutz and J. Kendrew in Chemistry for the structure of haemoglobin.
1962	J. Watson, M. Wilkins, and F. Crick in Medicine for the structure of DNA.
1964	D.C. Hodgkin in Chemistry for the structure of vitamin B_{12} and penicillin
1979	A. McLeod Cormack and G. Newbold Hounsfield in Medicine for computed axial tomography.
1981	K. M. Siegbahn in Physics for high resolution electron spectroscopy.
1985	H. Hauptman and J. Karle in Chemistry for direct methods to determine x-ray structures.
1988	J. Deisenhofer, R. Huber, and H. Michel in Chemistry for the structures of proteins that are crucial to photosynthesis.
2003	R. MacKinnon in Chemistry for structural and mechanistic studies of ion channels.

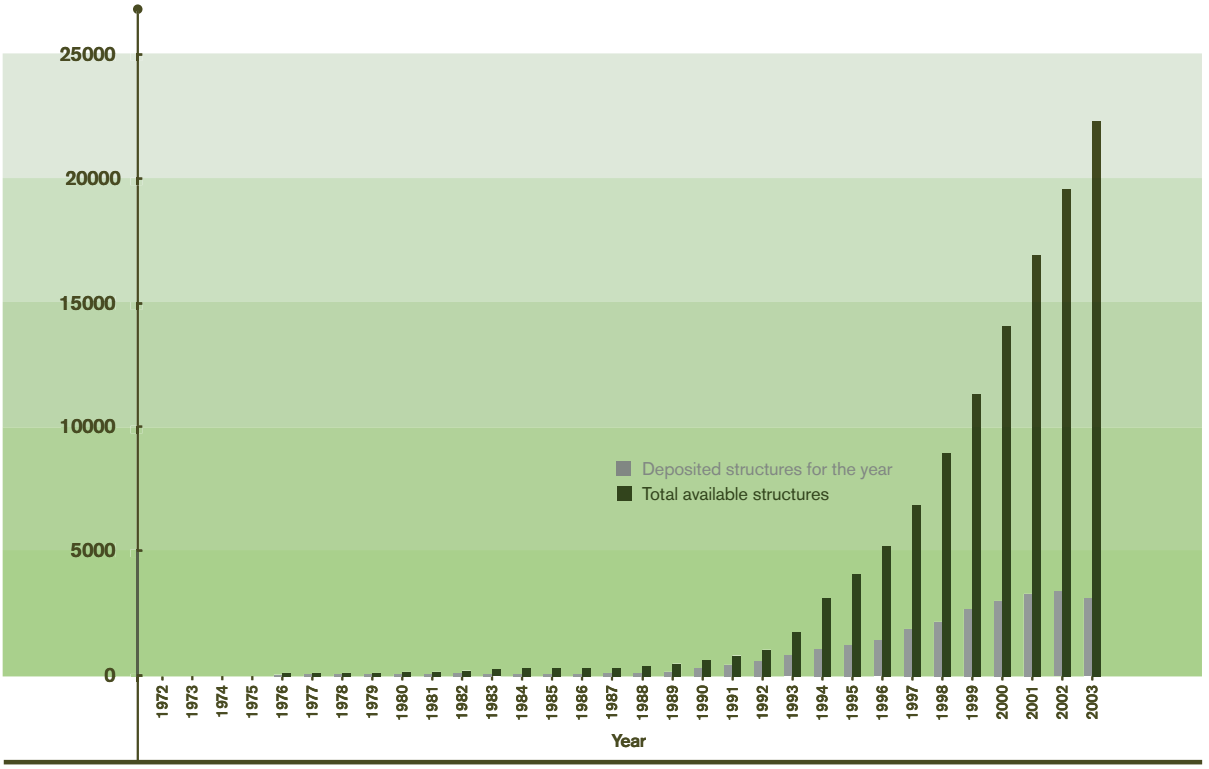

Figure 3.1. *Number of protein structures lodged with the Protein Data Bank over the period 1972 to December 20031*

Thus a new field of 'proteomics' has emerged that is the systematic characterisation of the gene products of entire organisms.

Beyond proteomics, there are many more proteins produced by the immune system, and in addition to proteins, there are other complex macromolecules of key importance in biological processes, such as viruses and nucleic acids.

The determination of the three-dimensional structures of these complex macromolecules is known as 'structural biology'. Perhaps the best-known technique employed by structural biologists is protein crystallography using single-crystal x-ray diffraction, which provides the 'primary' structural information – that is, the structure of the molecule. While knowledge of the molecular structure of the crystal alone is useful, of additional and sometimes greater importance is to elucidate the shape of the molecule and how the molecule is folded – the so-called secondary or 'conformation' structure.

Although nuclear magnetic resonance (NMR), mass spectrometry and cryo-electron microscopy do provide valuable information on the primary and secondary structure of complex macromolecules, x-ray diffraction, small angle scattering, circular dichroism and microspectroscopy are the key techniques for structural biology.

Early attempts to analyse macromolecular structures used conventional x-ray and laboratory light sources; the most famous of these is probably the determination by Watson and Crick of the structure of DNA. However it was not until developments over the past decade in cloning and over-expression of proteins, more effective methods of protein crystallisation, better data collection and manipulation, cryogenic cooling of the crystals (to minimise degradation by the radiating beam), advances in computer and detector technology and, in particular, access to synchrotron light that structural biology on a large scale has been possible.

The latest developments in synchrotron techniques, using the finely focussed, high intensity beam coupled with multiple wavelength anomalous dispersion (MAD) techniques, are enabling the analysis of smaller and weakly diffracting crystals, which has previously been impossible to do.

The growth in activity has been spectacular. Figure 3.1 shows the number of protein structures registered in the international Protein Data Bank over the period 1972 to June 2002. At 7 December 2003 the number of proteins in the data bank was 23,552 of which 20,018 were determined by x-ray diffraction. The remarkable rise after 1992 has coincided with the commissioning of a number of synchrotron-based protein crystallography beamlines (see figure 3.2, which shows the proportion of protein structures determined by synchrotron techniques to 1999. It is now believed to be close to 100%.)

1 Annual Report of the Protein Data Bank, 2002, http://www.rcsb.org/pdb/annual_report02.pdf

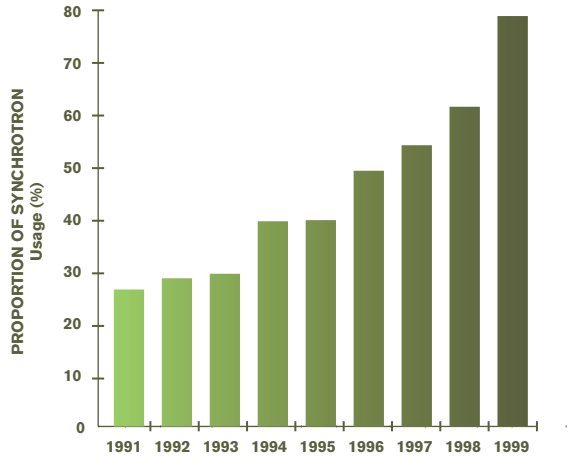

Figure 3.2. *Proportion of protein structures determined via synchrotron-based techniques*

Australia has been a significant player in proteomics – indeed the term 'proteomics' was first coined by researchers at Macquarie University – and access to the protein crystallography, SAXS/WAXS and circular dichroism beamlines on the Australian Synchrotron will be vital to remaining at the forefront of the field.

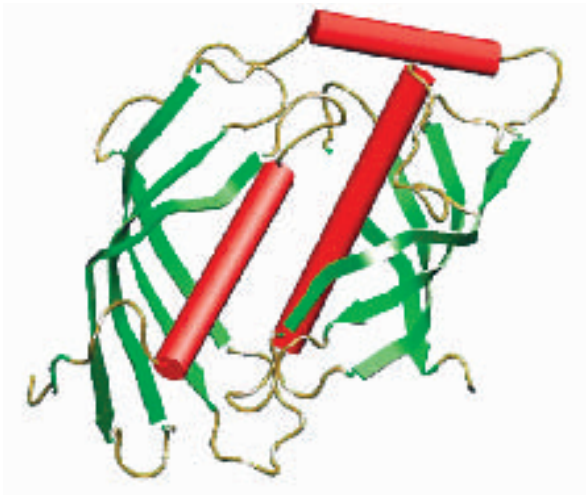

Figure 3.3. *Structure of mouse latexin, solved as a part of the structural genomics of macrophage proteins initiative at The University of Queensland. Latexin's known function is as carboxypeptidase inhibitor, and the protein is expressed at high levels in mouse macrophages and the expression increased in response to lipopolysaccharide*[2].

Cellular Biology

In concert with the advances in structural biology there has been spectacular progress in understanding the molecular basis of a number of processes in the fields of cellular and developmental biology. The structure of the major histocompatibility antigen (MHC) and its complexes with other immuno-modulating factors has revolutionised molecular immunology, and similar new insights are being made into other biological processes. The structures of many hormone–receptor, protein–protein and protein–DNA complexes have now been determined, and these have laid down the foundations of the mechanisms of cellular processes.

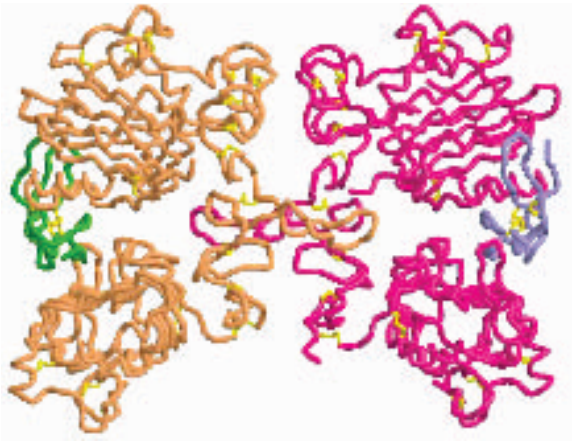

Figure 3.4. *Crystal structure of a dimer of a truncated epidermal growth factor receptor extracellular domain bound to transforming growth factor alpha*[3]*. This receptor–ligand complex is involved in the growth of many cancers. It was determined by a collaboration between the Walter and Eliza Hall Institute, Ludwig Institute for Cancer Research and CSIRO.*

An important new area, which until the advent of the synchrotron has been impossible to research, is the study of how biological signals are transmitted across cellular membranes. Areas of study include pore-forming toxins, receptors and channels.

Fundamental to further progress in this area will be the ability, using the synchrotron, to image cellular and molecular events in both normal and pathological processes. Significant advances in this field will be made by coupling knowledge of the physical sources of contrast, technological capabilities of the instrumentation and cellular and/or molecular processes.

Two major approaches are possible using synchrotron techniques – infrared microspectroscopy and x-ray microspectroscopy – and Australian researchers are at the forefront of both.

Projects at the Royal Women's Hospital in Melbourne and Monash University School of Chemistry have demonstrated that synchrotron-based infrared microspectroscopy can add a new capability to the diagnosis of cervical cancer.

Researchers, primarily at The University of Sydney, are making major advances using x-ray absorption microspectroscopy at overseas synchrotrons to image the uptake and metabolism of metal-containing pharmaceuticals in cells and tissue.

In the next few years, progress in x-ray microspectroscopy will be limited by the sensitivity of the detectors, as radiation damage issues become more important; by the precision with which the scanning can be performed; and by the ability to produce a very small x-ray focus in the scanning system.

Beamline 9 on the Australian Synchrotron will be designed with the latest technology for x-ray focussing to obtain a spot size of 0.2 microns on the sample, and with new detector systems to enable this important program to advance rapidly.

2 A. Aagard, P. Listwan, N. Cowieson, T. Huber, C. Wells, T. Ravasi, D. Hume, B. Kobe & J. Martin. University of Queensland.

3 T.P. Garrett, N.M. McKern, M. Lou, T.C. Elleman, T.E. Adams, G.O. Lovrecz, H.J. Zhu, F. Walker, M.J. Frenkel, P.A. Hoyne, R.N. Jorissen, E.C. Nice, A.W. Burgess, C.W. Ward. Cell, 110(6) (2002) 763-73.

Rational Drug Design

Many medicines have been developed by traditional 'drug discovery' methods in which the myriad of naturally occurring compounds have been surveyed for their ability to control disease. However the explosion in the fundamental knowledge of biological protein interactions has enabled a rational approach to drug design based on theory and structural biology. The impact of structural biology on the design of medically important drugs has been exemplified by the development of the anti-influenza drug Relenza. This work was carried out within CSIRO and was the first structure-based anti-viral drug to be developed, and also a very early example of the rationally based drug design methodologies. Subsequently the new generation of drugs active against HIV such as the HIV-protease inhibitors were developed by similar methodology. Other examples have been the development of the anti-inflammatory inhibitors that were selective inhibitors of the COX-2 enzyme. There are many drugs undergoing late stage clinical trials at present for a number of human diseases ranging from cardiovascular disease to cancers that are based on the information discovered by structural biology. It is expected that this approach to finding solutions to human health problems will accelerate in the future, as it is becoming increasingly important in the fight against newly emerging and re-emerging viral and microbial diseases.

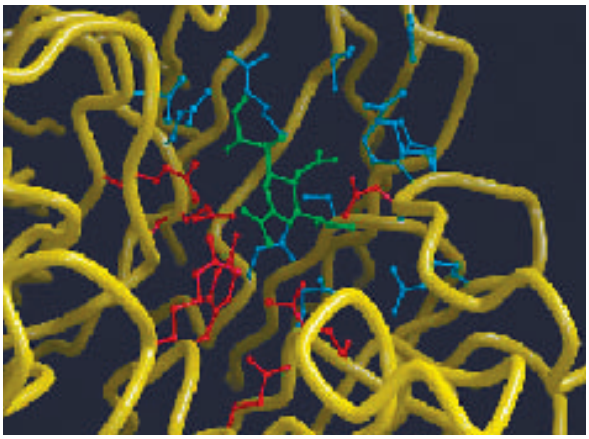

Figure 3.5. *The anti-influenza drug Relenza™ in the conserved active site of influenza neuraminidase*[4].

Rational drug design is currently being applied to many areas of drug development. Anti-viral drug developments include efforts to abate the HIV pandemic; the serious human health threat posed by hepatitis C virus, which is mutating at a rate that makes vaccine treatment ineffective; and measles, which continues to kill over a million children per year in Africa alone. Currently no drugs are available for many Third World protozoan pathogens such as sleeping sickness, and malaria is becoming increasingly resistant to current drug therapy, as are several microbial diseases such as tuberculosis and *staphylococcus aureus* infections.

Almost all drugs used in the treatment of cancer cause serious side effects because they lack selectivity for tumours over normal tissues. Selective activation relies on successful exploitation of the differences between the environment in tumours and that in healthy tissues. Tumour hypoxia, the lower than normal oxygen levels present in solid tumours, is the result of the rapid growth and poor vascularisation of tumours. For a drug to be activated in a hypoxic environment it must have an inactivated higher oxidation state and an activated lower oxidation state. To date, the development of hypoxia-selective agents has been carried out in the absence of information on the oxidation status of the agents in tumours and, in particular, how this status is affected by the degree of hypoxia. Extensive investigations of Co and Pt anti-cancer drugs using x-ray absorption spectroscopy are in progress to determine the oxidation state in-situ in different regions of tumours and in models of hypoxic tumours. This will enable the rational tuning of the reduction potentials to achieve activation in the desired regions of the tumours. Simultaneously, the project will provide information on the relationship between reduction potential and the extent of activation in hypoxic environments.

Infrared spectroscopy and circular dichroism are complementary techniques that are able to monitor the up-take of anti-cancer drugs and their effect on the conformational changes that these cause in critical proteins. Synchrotron light will add a new dimension to these studies because it will be possible to follow these processes in real time.

Co, Cu, Ni and Zn anti-inflammatory drugs are potent veterinary drugs and are likely to enter human clinical trials in the near future. X-ray absorption spectroscopy and powder diffraction have been used extensively in the characterisation of new drugs in the solid state, solution, pharmaceutical formulations and biological fluids. This research has been essential in determining the stability of the drugs in pharmaceutical preparations[5], and is providing a better understanding of the pharmacology of these drugs for the development of better and safer systems.

Toxicology

The synchrotron is a unique and powerful tool for determining the speciation of chemicals, with applications in the fields of environmental science, forensics and medicine.

Of all carcinogens, Cr has the widest occupational exposure to workers and is of growing environmental concern. Although Cr(VI) is the carcinogenic form of Cr, it does not interact with DNA in the absence of cellular reductants. X-ray absorption spectroscopy is being used to characterise for the first time the structures of a range of reactive Cr(VI), Cr(V) and Cr(IV) complexes with biological reductants. Many Cr(III) complexes, which are the ultimate products of the reductions, have also been characterised[6].

4 J. Varghese, V. Epa & P. Colman, Protein Science, 4 (1995) 1081–1087.

5 J.E. Weder, T.W. Hambley, B.J. Kennedy; P.A. Lay, G.J. Foran & A.M. Rich, 'Determination of the structures of anti-inflammatory copper(II) dimers of indomethacin by multiple-scattering analyses of XAFS', Inorg. Chem., 40 (2001), 1295–1302.

6 C.T. Dillon, T.W. Hambley, B.J. Kennedy, P.A. Lay; J.E. Weder, Q. Zhou, 'Copper and zinc complexes as anti-inflammatory drugs' in A. Sigel & H. Sigel, (Eds) Metal Ions and Their Complexes in Medication, Vol. 41 of Metal Ions in Biological Systems, Marcel Dekker, Inc., New York, in press.

Using microfocus synchrotron radiation induced x-ray emission (Micro-SRIXE), researchers at Sydney University and ANSTO have been able to follow the uptake of Cr(III) and Cr(VI) chemical species into individual cells with sub-micron resolution[6].

In a collaboration between the Schools of Biological Sciences and Chemistry at Monash University, synchrotron based infrared spectroscopy techniques are being developed to monitor the chemistry and toxicity of microalgae and to use infrared signatures for classification of species. Apart from toxological effects, the study of microalgae is relevant to environmental research; microalgae, as marine phytoplankton, perform more photosynthesis in the world's oceans each year than do the tropical rainforests on land, and thus their life cycle has a primary effect on greenhouse gas control.

Biosystems

The electronic properties and interactions of matter at an atomic level in biological environments is very much unknown. Yet the detailed understanding of these systems is crucial to the successful development of many new technologies that have direct impact on biosystems. These include medical implants, delivery systems (for example of radiopharmaceuticals), bio-sensors and chips for diagnostics, biomimetic materials (such as the construction of artificial skin or organs) and novel artificial photosynthetic devices. Vacuum ultraviolet (VUV) light is able to probe the valence and low-lying core states of many elements in the periodic table. The interaction of such states ultimately controls the complex interactions and properties observed in biological systems.

The high flux and small spot size produced by the VUV beamline will allow for many ground-breaking experiments and studies to be performed on biosystems. Studies will initially focus on more traditional (but still not understood) systems such as the electronic properties and structure of amino acids (an essential building block) on various surfaces. One significant new direction would be the in-situ study of liquid–solid interfaces and multi-layered systems. As most biosystems are made of several functioning parts, small spot microscopy of objects as tiny as only a few nanometres to as large as a few microns in size would be of tremendous importance in order to determine the electronic state of each part accurately.

Surface science, which is supported by soft x-ray, VUV and infrared spectroscopy techniques, has recently been increasing in prominence in the biomedical area, based on the fact that many biological reactions occur at surfaces. Thus any fundamental understanding of the biocompatibility of a medical device must take into account the properties of proteins and cells at interfaces, and the characteristics of local biological reactions. Principles worked out in surface science laboratories are likely to become the basis for ways of improving the function and durability of materials featured in a wide range of medical products.

As an example, the hemocompatibility of synthetic surfaces can be improved by various biologically active substances, of which heparin is perhaps the most promising. To immobilise heparin onto biomaterial surfaces, its physiochemical properties are modified by incorporation of a specific binding agent onto the heparin molecules. The resulting modified-heparin coating material has a high affinity for a variety of synthetic surfaces, and retains all biological properties of the unmodified heparin. This offers the prospect of heparin-coated bypass circuits for use in open heart surgery, for example.

Biotechnology

Access to synchrotron light is becoming increasingly important for researchers developing industrial applications of biotechnology, in such areas as bio-remediation and biological sensors. Enzymes to degrade industrial and environmental pollutants are being designed to alter their substrate specificity by modification of the active sites of natural enzymes that degrade similar chemical moieties, by protein engineering based on the three-dimensional structure of the native enzyme. These modified enzymes themselves, or the genes that code for them, can be inserted into biological organisms that can then be used in the remediation of contaminated environments. These techniques can also be applied to the removal of toxic metals and the concentration of metals from low-grade ores. The engineering of the thermostability of enzymes used for industrial purposes at various temperature regimes can be carried out based on structural information.

The design of insecticides with increased efficacy and species specificity is also being investigated through structural biology. The new classes of insecticides target insect hormone receptors, enabling the disruption of normal insect growth, by the design of agonists or antagonists to modulate the function of these receptors. Such biotechnological applications can have an enormous impact on the environment and the rural sector, for example by the control of specific insect pests in the agriculture and livestock industries.

Nanotechnology

Nanotechnology, the science and engineering of the small or, equivalently, the study and manufacture of structures and devices of nanometre-scale dimensions, has the potential to impact our way of life profoundly. Nanoparticles (with distinctly different properties from bulk material) can be exploited in a multitude of potential technological applications (for example, non-linear optical switches). Accordingly, funding agencies worldwide are allocating considerable resources towards nanoscience and, in Australia, the Australian Research Council has designated nanoscience as a research priority area.

Synchrotron-based powder diffraction combined with x-ray absorption spectroscopy (XAS) is the ideal technique, for determination of the local arrangement of atoms within the nanoparticles.

6 A. Levina, R. Codd, C.T. Dillon & P.A. Lay, 'Chromium in Biology: Nutritional Aspects and Toxicology', Prog. Inorg. Chem., (2003), 51145–250.

Many nanomaterials have a disordered structure and conventional powder diffraction (e.g. Rietveld refinement) is unsatisfactory, so it is necessary to use pair distribution function (PDF) analysis. Such analysis requires the data to be collected to a much higher q-range than is possible with a conventional powder diffractometer and high energy synchrotrons are emerging as the only x-ray source capable of this.

XAS also provides information on short- and medium-range order and disorder, and studies using this technique have been initiated by Australian scientists to study both semiconducting and metallic nanoparticles in a variety of matrices for application to photonic devices and chemical catalysis, respectively. An example of semiconducting nanoparticles is shown in figure 3.6. Given that both the optical and catalytic properties are governed by the structural properties, XAS structural determinations have the potential to yield fundamental insights into the unique nature of science at the nanoscale[7].

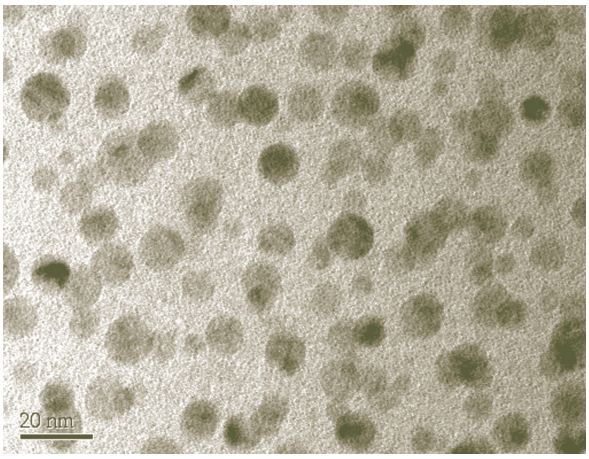

Figure 3.6. *Semiconducting Ge nanoparticles in a SiO_2 matrix for application in advanced photonic devices in telecommunications and the electronics industry[7].*

In addition the ability to focus the x-ray spot size down to the order of 0.2 microns in the microspectroscopy beamline means that for the first time it will be possible to analyse the chemical composition of individual nanoparticles.

There is clear overlap in biotechnology and nanotechnology when biological molecules are used for non-biological purposes. Knowledge of the three-dimensional structure of antibody molecules obtained through the use of synchrotron-based techniques is leading to the development of biosensors that are able to detect both biological and other chemical moieties. Rapid developments in the field of diagnostics are enabling the design and construction of biosensor chips using these antibody molecules as the detection front-end to facilitate the diagnosis of cancer and other disease states. This is proving useful in particular for future medical diagnostics 'point of care' technology. There is also an increasing interest in using biological molecules as sensors for the detection of chemicals and pathogens. This has particular relevance in the area of detection of chemical and biological weapons. Currently there are programs within CSIRO and several CRCs that are directed towards finding solutions to these goals.

Devices exploiting nanotechnology developments, such as biosensors, usually require micro-machined substrates to support the nano layers, and provide intelligence or other functions such as microfluidics. Synchrotron-based lithography provides unequalled capability to produce such substrates with truly deep three-dimensional structures and optically flat surfaces, and will become an essential tool as nanotechnology develops.

The chemical compatibility and reactivity of surfaces is also an important factor in producing nanolayers, and the synchrotron-based soft x-ray techniques will be important for characterising these surfaces as well as the layers.

Advanced Materials

Solid metal oxides – magnetic, superconducting and battery materials

The majority of advanced inorganic materials used in magnetic, conductivity, superconductivity, ferroelectric, catalytic and battery applications are solid metal oxides. Numerous groups across Australia are actively studying the properties and structures of metal oxides. In general such inorganic materials are prepared and used as polycrystalline ceramics or powders. Powder x-ray diffraction is a key characterisation method and synchrotron radiation is often required.

Metal oxide chemistry is dominated by classes of materials having crystal structures derived from a simpler parent structure, such as perovskite or rutile. Small lattice distortions, which are critical to the key electronic and physical properties of these oxides, usually lead to lower symmetries and superstructures. These distortions are characterised by subtle peak splittings and the appearance of weak superlattice reflections in the diffraction data. The detection and understanding of such distortions using powder diffraction methods usually requires high resolution that is only afforded by synchrotron radiation. Typical examples include the polar distortions in bismuth oxide ferroelectrics, Jahn-Teller distortions in manganese oxide battery materials and valence ordering in CMR (colossal magnetoresistance) materials. Phase transitions between the distorted structural variants influence the stability and processing of the materials. Such distortions often lead to twinning making it impossible to synthesise diffraction-quality single crystals easily. This is critical in emerging areas. Powder diffraction does not suffer from this problem and historically has been the tool of choice to study novel oxides.

An underlying feature of many of the most interesting materials is the strongly correlated behaviour of the electrons and coupling of the electronic charge and spin degrees of freedom with those of the electron orbitals and the lattice. The greatest potential for functionality is

7 M.C. Ridgway, G. de M. Azevedo, C.J. Glover, D.J. Llewellyn, R.G. Elliman, B. Johannessen, D.A. Brett & G.J. Foran, 'EXAFS characterisation of Ge nanocrystals in silica', Nucl. Instrum. Meth., submitted (2003) (invited contribution).

in materials at the edge of a structural and/or electronic instability, where small changes in chemical or physical conditions lead to a major change in properties. Establishing the role of these perturbations requires careful variation. The success of such parametric studies is reliant on rapid data collection (about 5 minutes per data set) without compromise of data quality. The importance of such studies is illustrated in the identification of an intermediate thermal phase in the unusual 4d ferromagnet $SrRuO_3$. This phase was only correctly identified after collecting high resolution data in 5°C temperature intervals over a wide temperature range[8].

Australian researchers have an enviable reputation in the study of incommensurate structures. Previously such studies have been limited to samples where high quality single crystals are available. However, the presence of structural modulations can by themselves preclude the formation of suitable single crystals. The superb signal-to-noise at synchrotron powder diffractometers can reveal the extremely weak superlattice reflections associated with such modulations, and considerable effort is being directed towards the analysis of such structures from powder diffraction data. A number of key materials exhibit modulated structures, including Cu superconductors and layered Bi oxide ferroelectrics[9].

Electronic and opto-electronic materials

A niche area in which Australian research has made a significant contribution is the development of thin film materials for electronic and optoelectronic devices. Thin films of materials with particular chemical and/or physical properties such as piezoelectricity are typically deposited onto an appropriate substrate by one form of chemical vapour deposition, and during the development phase for both precursor and deposition conditions the physical and chemical properties of the film must be determined. As in most surface characterisation, conventional x-ray photoelectron spectroscopy (XPS) is used for initial chemical analysis, but XPS is rarely able to reveal the orientation of film crystallites. Synchrotron-based variable-angle XAS is the most effective technique for determining this important characteristic.

A major thrust for new knowledge and understanding of spin-dependent phenomena in atoms, clusters, ferromagnetic films and surfaces is developing from the 'two-particle coincidence reflection spectroscopy' technique which leads to surface information unobtainable by any other method. The technique allows a description of spin-dependent interaction potentials and electron correlations which determine the enhanced magnetic moments of atoms, surfaces and magnetic coupling between, for example, a magnetic and a non-magnetic material or magnetic layers separated by a spacer layer. This information is the basis of spin-electronics (or magneto-electronics) in which the spin as well as the charge of the electron is a determining factor. For example, a system of alternating ferromagnetic and non-magnetic metal layers can change its electrical resistance from small (with parallel magnetisation) to large (with anti-parallel magnetisation) to form a 'spin-valve' in the 'read head' of hard disks. It allows the 'write/read head' to be made smaller and the storage density on hard disks to increase to above 10^{10} bits/cm^2. Research using a synchrotron is expected to provide a basis for further reduction in size and greater density.

Trace metallic impurities at ultra-dilute levels have become a significant concern for silicon microchip manufacturers seeking ever improving performance. Recent research has shown that it is possible to trap these impurities on the inner surface of nanocavities in the silicon substrate. Figure 3.7 shows an example of nanocavities that effectively getter or trap the metal impurity atoms at depths beyond the device's active region. The very high intensity of a beam derived from a wiggler, planned for the XAS beamline (beamline 5), enables measurement of concentrations of these impurities at parts per billion level using XAS and can be used to study the trapping mechanism.

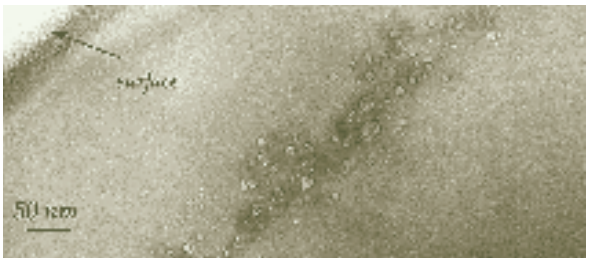

Fig 3.7 Nanocavities for entrapping and immobilising impurities at the parts per billion level in silicon substrates for ultra-high performance micro-circuits (Source: M. Ridgway, ANU)

Catalysts

Catalysts are vital for many industrial, biotechnological and food manufacturing processes, but how they work is often not well understood. Understanding their reaction mechanisms on an atomic scale is a key part of developing new and better catalysts. Combining microspectroscopy and imaging will provide new information on the chemistry and physical structure of a material's surface, which will give important clues to how catalysts function.

The redox state dependence of the reactions and reactivity of transition-metal complexes is a key distinguishing characteristic of the d-block elements. This characteristic is pivotal to their remarkable ability to act as catalysts for an extraordinary range of reactions and explains the vital role of transition metals in a broad range of enzymes. Both in biological and abiological contexts transition-metal catalysis is essential for life as we know it. Metalloenzymes are examples of exquisitely designed low-temperature, low-pressure, low-volume catalysts. In contrast, the catalysts typical of the chemical industry are capable of high-volume chemical transformation but at the cost of high-temperature and/or pressure. Elucidation of the molecular details of the chemistry associated with enzyme catalysis is thus important because of the potential this offers for the

8 B.J. Kennedy, B.A. Hunter & J.R. Hester, 'Synchrotron X-ray diffraction reexamination of the sequence of high temperature phases in $SrRuO_3$', Phys. Rev. B., (65), 224103 (2003).

9 C.D. Ling, J.G. Thompson, R.L. Withers & S. Schmid, Acta Crystallographica., B55 (1999) 306–312.

discovery of energy-efficient large-volume catalysts and the light that these investigations cast on our general understanding of chemistry. Accordingly, XAS studies are in progress to improve understanding of the influence of redox or charge state on the electronic and molecular structure of metal complexes or clusters so as to better anticipate (and ultimately control) the reactions and reactivity of transition-metal catalysts. The metal clusters that lie at the active site of the nitrogenase and hydrogenase enzymes provide challenging, but important, target molecules for this work. Techniques, including the use of infrared, ultraviolet and electron paramagnetic resonance spectroscopies, have been developed to permit in situ spectroscopic examination of reactive electro-generated species to complement direct structural characterisation with XAS.

XAS studies will be used to identify efficient abiological catalysts to provide a low-cost, energy-efficient production of ammonia and catalysts for H_2 fuel cells based on cheap and abundant chemicals. Progress depends upon the determination of the molecular structure of reactive intermediates that, with the aid of the increasingly powerful suite of theoretical techniques, can then be used to drive catalyst design. XAS studies enable time-resolved XAS measurements of dynamic systems. These advances will be essential toward the implementation of the catalyst into a process control environment.

Microporous materials such as zeolites have great potential as catalysts, sorbants and micro-reactors. These materials typically have large unit cells and often contain large voids that are invariably responsible for their key properties. Analysis of their structures is vital to understand the way they function, but is very difficult. It is not uncommon to observe weak and complex x-ray diffraction patterns due to low symmetry or subtle distortions. The small molecule diffraction end station on beamline 2 will be an important new tool for this task.

Metals and Alloys

The development and production of metals and alloys are of fundamental importance for any advanced society that is dependent on sophisticated elaborately transformed manufactures.

Many different types of metals and alloys are now available, each tuned with the required combination of physical, mechanical and chemical properties to suit a specific application. These combinations of properties are achieved by the development of highly complex microstructures through the addition of alloying elements, together with thermal and mechanical treatments.

The understanding of the role of microstructures and their influence on the alloy properties has been made possible by access to a wide range of measurement and imaging techniques, especially optical and electron microscopes and microprobes, x-ray imaging and x-ray diffraction. As a result, remarkable progress has been made, but there are still many aspects of alloy design not fully understood and plenty of opportunity for further improvement. The advanced techniques possible with the synchrotron are bringing new tools to this task.

Particular techniques that will make valuable new contributions are:

- x-ray absorption spectroscopy to obtain knowledge on the short and medium range order, coordination numbers and local coordination geometry of the elements at an atomic level in complex alloys
- higher resolution x-ray diffraction on a micro scale to be able to understand subtle changes to crystal structure that occur with the addition of alloying elements and to be able to measure internal thermal and residual strain distributions in microstructures
- phase contrast enhanced hard x-ray imaging to be able to see microstructures and defects in structures inside the material on a three-dimensional basis
- in-situ imaging of the liquid to solid transformation that occurs during the casting of an alloy, to observe the nucleation and growth of grains and dendrites and the segregation of alloying elements
- in-situ imaging of the microstructural transformations that occur on thermal treatment or mechanical deformation of alloy systems
- in-situ observation of the corrosion mechanisms that occur at the surfaces of alloys under a wide range of environmental conditions.

Australia is a major producer and exporter of metals and alloys, particularly for manufactured goods such as motor vehicles. While the major material used in a modern automobile is steel, the move to improve fuel economy in order to reduce greenhouse gas emissions has led to a search for high strength, low density alloys. Australia is responding to this with the Light Metal Action Agenda and initiatives such as CSIRO's Light Metals Flagship. Major programs are under way to develop new low-cost magnesium, aluminium and titanium alloy systems. Synchrotron techniques will be extensively used by the researchers in these programs because of the ability of high energy, high brightness x-rays to penetrate deeply into these metals.

In some of the most advanced light metals the incorporation of ultra-fine refractory fibres has been considered to strengthen the material. The performance of this type of strengthening mechanism depends critically on the residual strains and the efficiency of stress transfer at the fibre/matrix interface. Synchrotron x-ray diffraction techniques are an excellent method for measuring internal stresses, particularly for monitoring in real time the changes that can occur when straining or thermally treating the material.

Engineered Components

The measurement of residual strain fields in the surface and subsurface (0.01 to 1 mm) region is important in understanding the long-term performance of mechanical engineering components. This depth range is where most

of the degradation of mechanical components during service originates. It also covers the thickness range of many protective coatings (for example, thermal barrier coatings) and surface engineering treatments (for example, laser shot peening).

The large flux of high energy x-rays coupled with the ability to scan components in beamline 10 will enable the two-dimensional mapping of strain and grain texture in practical times. It will be a major advance over the alternative techniques that are currently used – neutron methods which have insufficient spatial resolution (1 mm) and laboratory sourced moderate energy x-rays which are limited to investigating the top 0.01 to 0.05 mm.

Earth and Environmental Sciences

It is clear that earth and environmental sciences are of crucial importance to Australia. Environmentally sustainable ore extraction, mineral processing, coal combustion and soil use are merely a few of the areas that will continue to require significant research support. Synchrotron techniques are already opening up new ways to address the complex problems arising from earth resource utilisation, and this contribution is expected to increase.

Evaluation of an ore body

The economic viability of a potential new ore body depends on many factors. One of the most important factors is the ease of mineral processing. Mineral bodies contain a number of crystalline and amorphous phases that contain complex distributions of metal cations. The optimising of mineral processing conditions is often dependent on the precise composition of the ore and this generally requires very high resolution data. Although the presence of major phases is often easily established using conventional x-ray methods, the quantification of all the species present, including establishing the distribution of the metal cations, requires extremely high resolution coupled with high signal-to-noise data. The use of lower grade ores with increasing complexity in mineralogy is accelerating this requirement.

Often, an understanding of mineralogical changes that occur during processing is derived from equilibrium studies that only provide information about the final product in an idealised 'steady state' operation. However, few mineral processing operations operate in this mode. In order to understand chemical and physical properties of minerals, it is important to obtain information under conditions that emulate the 'real' processing conditions. This information can be derived from so called in-situ experiments where the sample is subjected to elevated temperature, pressure (typically hydrothermal pressure), sample pH and so on during powder x-ray diffraction data collection.

Two recent Australian examples of the use of in situ XRD (x-ray diffraction) experimentation in the mineral processing area are:

- the pressure acid leaching of Ni-laterites at real processing conditions of 250°C and 600 psi in H_2SO_4. The experiments showed the formation of an intermediate phase kieserite $MgSO_4.H_2O$ that cannot be observed in ex situ experiments due to its negative temperature coefficient of solubility[10].
- the formation of silico-ferrite of calcium and aluminium (SFCA). SFCA is the major bonding phase for the iron-ore sinters used in the production of iron and steel. The experiments have allowed study of the mechanism of SFCA formation, observation of intermediate phases directly with respect to time and temperature, and derivation of the order and comparative rates of phase formation throughout the experiments.[11]

Mineral beneficiation – flotation

The surface chemistry of metal sulfides is of major importance in the separation of the valuable and unwanted components in base metal ores, in the hydrometallurgical processing of a concentrate to produce the corresponding metal from the sulfide, and in the leaching of rejected material in waste heaps. Over the past thirty years, conventional x-ray photoelectron spectroscopy (XPS) has provided a wealth of information in these areas, however, because of the several nanometre analysis depth, establishing the chemical nature of the true surface layer has been difficult. Since the application of synchrotron XPS to mineral fracture surfaces, the importance of surface chemical states arising from relaxation of the outermost layer following fracture has become evident. Enhanced surface sensitivity is achieved by determining the S 2p spectrum from a sulfide mineral fracture surface with ~200 eV synchrotron x-rays compared with 1,487 eV x-rays in conventional XPS. For example[12], in the case of pyrite, the additional states present at the surfaces are believed to be S^{2-}, arising from broken S–S bonds, and S_2^{2-}. The relevance of surface states to industrial-scale processes lies in their influence on surface reactivity, and this reactivity can be monitored by synchrotron XPS when mineral fresh fracture surfaces are subsequently exposed to different environments.

The enhanced surface sensitivity provided by synchrotron XPS, as well as the ability of angle-dependent NEXAFS (near edge x-ray absorption fine structure) to reveal orientation, have also assisted elucidation of the mechanism by which flotation reagents interact with the surface of minerals. By contrast, investigation of passivation layers that slow the dissolution kinetics in the hydrometallurgical processing of mineral concentrates is usually hindered not by a lack of surface sensitivity but because a near-surface, yet buried, interfacial layer must be characterised. In that situation, it is the non-destructive chemical depth profiling ability of NEXAFS spectroscopy that is exploited, and attempts are currently being made to augment the XAS data with threshold XAES (x-ray Auger-electron spectroscopy) measurements. The principle underpinning the threshold XAES approach is that, by incrementing the photon energy through the

10 Unpublished data from CSIRO Division of Minerals, supplied by I. Madsen et al.

11 I. Madsen, et al., paper submitted to J. Applied Cryst.

12 A.G. Schaufuss, H.W. Nesbitt, I. Kartio, K. Laajalehto, G.M. Bancroft, & R. Szargan, 'Reactivity of surface states on pyrite', Surf. Sci., 411, (1998) 321-328; A.G. Schaufuss, H.W. Nesbitt, I. Kartio, K. Laajalehto, G.M. Bancroft, & R. Szargan, 'Incipient oxidation of fractured pyrite surfaces in air', J. Electron Spectrosc. Relat. Phenom., 96 (1998) 69–82.

absorption edges for the different species present, it should be possible to identify an interfacial species under resonance conditions, even if that species might be present in only a thin interfacial layer.

Environmental issues

Mineral weathering resulting in the release of acid mine drainage (AMD) is now of considerable environmental concern. It is estimated that in Australia remediation costs will be in the order of $900m over the next 15 years[13]. The dual XRF–XRD (x-ray fluorescence–x-ray diffraction) mapping facility of the Microdiffraction and Fluorescence Probe will enable the identification of reaction and re-precipitation layering on mineral surfaces as a functioning of weathering. Understanding the evolution of these layers in terms of both their elemental composition and crystalline phase is important to the prediction and control of AMD[14]. This combination of analyses will enable significant contributions to be made to the understanding of the release of toxic elements, which often accompanies AMD, and their bioavailability[15].

Another example is mine tailings containing arsenic. Arsenic (As) can exist structurally bound in compounds such as $FeAsO_4$ or adsorbed onto the surface of minerals such as goethite (FeOOH). The manner in which the As is incorporated has a strong impact on its 'availability' to the environment and hence on the steps that must be taken in the remediation of contaminated sites. The synergistic use of the XRF-XRD mapping facility together with x-ray absorption spectroscopy (EXAFS) is important in understanding the nature of contaminant metals at the molecular level so that appropriate action can be taken at the macro level.

Imaging

Perhaps better than any other method, images provide us with an intuitive understanding of the subject. It is therefore perhaps surprising that the exploitation of synchrotrons for imaging arrived rather later than their use for diffraction and spectroscopy. Nevertheless, the three largest synchrotrons now have substantial numbers of people and several beamlines dedicated to advanced x-ray imaging techniques, and imaging is one of the most rapidly expanding areas of synchrotron science. The use of synchrotrons for imaging has led to the development of new approaches that provide unprecedented resolution and contrast of nature's smallest details. Australian scientists, based largely in Melbourne, have helped pioneer the development of many of these techniques and are regarded as among the world leaders in the field. One aspect of this work relates to the development of phase-contrast based imaging techniques that transcend the conventional reliance on absorption to produce contrast. A second relates to the development of theory to make these imaging techniques quantitative. These developments provide the basis for major advances in x-ray imaging science that are not only relevant for synchrotron-based imaging but also to radiography with conventional sources.

Biomedical imaging

Despite being by far the most popular medical imaging modality, lack of soft tissue contrast is a significant problem in both medical and biomedical x-ray imaging. The relatively small variations in density and composition of soft tissues mean that their x-ray attenuation characteristics are very similar. Conventional radiography produces images through the differential absorption of x-rays, and so provides very little soft tissue contrast unless high doses are employed as in computed tomography. Synchrotron-based imaging techniques produce high resolution images using differences in the refraction and scatter of x-rays as they pass through tissue. Genuine soft tissue contrast with micrometre-scale resolution is possible.

Furthermore the collimation and monochromaticity of an imaging beamline allows high resolution images to be recorded at far lower doses than required by conventional equipment. This capability permits longitudinal studies (serial imaging) to be performed for investigations where the dose required by conventional imaging would confound the experiment.

The power of these imaging techniques is particularly suited to the study of living processes. It will be possible to exploit the proximity between the Australian Synchrotron, Monash University, CSIRO, Melbourne University and the Monash Medical Centre to bring together the expertise and facilities that will make the imaging beamline (beamline 10) one of only two beamlines in the world capable of work on live animals. The studies of live animals for medical research is an area that is impractical under overseas access programs, and so relates to an essentially new and numerous Australian synchrotron user class that has not been served in the past.

One of the problems at present is that animals are often sacrificed in order to obtain anatomical information at high resolution. The proposed imaging beamline will allow in vivo imaging of small animals and so provide the major advantage of allowing longitudinal studies to be carried out. This has the significant advantage of following the same animal through the process and also dramatically reducing the number of animals sacrificed in a study.

Mammography

Two major areas where soft tissue contrast is vital are breast and lung imaging. Both breast and lung cancer are major killers and better methods of imaging these diseases would have a major impact on health care.

Screening for breast cancer, which is the biggest killer of women in the 35 to 55 age group, is based entirely upon soft-tissue x-ray absorption contrast. As a result,

13 G. Parker, 'A critical review of acid generation resulting from sulfide oxidation: Processes, treatment and control', in Acid Drainage. Australian Minerals and Energy Environment Foundation, Melbourne, 1–182 (1999).

14 J.L. Jambor, J.E. Dutrizac & T.T. Chen, 'Contribution of specific minerals to the neutralisation potential in static testes', Proc. Fifth Int. Conf. Abatement of Acid Mine Drainage (Denver, May 2000) Soc. Min. Metall. Explor. Inc. (SME), U.S.A. 1, (2000) 551–565.

15 S. Kurunczi, S. Torok & P. Chavallier, 'A micro-XRF study of the elemental distribution on the growth front of mussel shells', Mikrochimica Acta 137, (2001) 41–48; M. Labrenz et al., 'Formation of sphalerite (ZnS) deposits in natural biofilms of sulfate-reducing bacteria', Science, 290, (2000) 1744-1747 'Reactivity of surface states on pyrite', Surf. Sci., 411, (1998) 321-328; A.G. Schaufuss, H.W. Nesbitt, I. Kartio, K. Laajalehto, G.M. Bancroft, & R. Szargan, 'Incipient oxidation of fractured pyrite surfaces in air', J. Electron Spectrosc. Relat. Phenom., 96, (1998) 69–82.

mammography, while having been proven to reduce mortality, suffers from some major deficiencies. In particular it is non-specific, resulting in a very large number of unnecessary biopsies, and it does not work well in women below the age of 50. The potential benefits of phase contrast imaging to improving the success of mammography in detecting cancer are enormous. Work by others has shown that the contrast increases by as much as 25 times by employing phase contrast.[16] The beamline to be constructed would be used as a 'gold standard' facility to develop improved techniques for breast imaging.

Lung Imaging

Anyone who has seen a chest x-ray knows that the lungs are largely invisible to all but the highly trained eye of a radiologist. However, the air–tissue interfaces in the lung appear with startling clarity using phase contrast imaging (illustrated in figure 3.8 showing the differences between conventional and two synchrotron phase contrast images of mouse lungs). The resolution of the synchrotron images is around 10 microns, which enables individual alveoli to be visualised. This is particularly applicable to human babies, and an Australian project at a SPring-8 beamline is planned to investigate the potential for developing technology to image lung clearance at birth.

Phase contrast techniques offer enormous opportunities for the study of lung function and disease in both humans and animals. Examples include:

- The detailed study of the development of respiratory function in marsupials that are born in an embryonic state and yet can still breathe
- Longitudinal studies of the effect of anti-cancer therapies on mice and other animal models.

In addition to the dramatic improvements offered by phase contrast, workers at the ESRF have demonstrated xenon contrast respiration-gated synchrotron radiation computed tomography (SRCT) with a spatial resolution at the level of the respiratory lobule (terminal bronchiole and alveoli). This technique allows direct quantification of xenon as an inhaled contrast agent based on K-edge subtraction imaging and hence the dynamics of xenon wash-in can be used to calculate regionally specific quantitative maps of lung ventilation. Examples of the use of this technique are:

- Identifying local variations in lung function caused by diseases such as asthma and chronic obstructive pulmonary disease
- Testing the efficacy of pharmaceuticals on respiratory dysfunction.

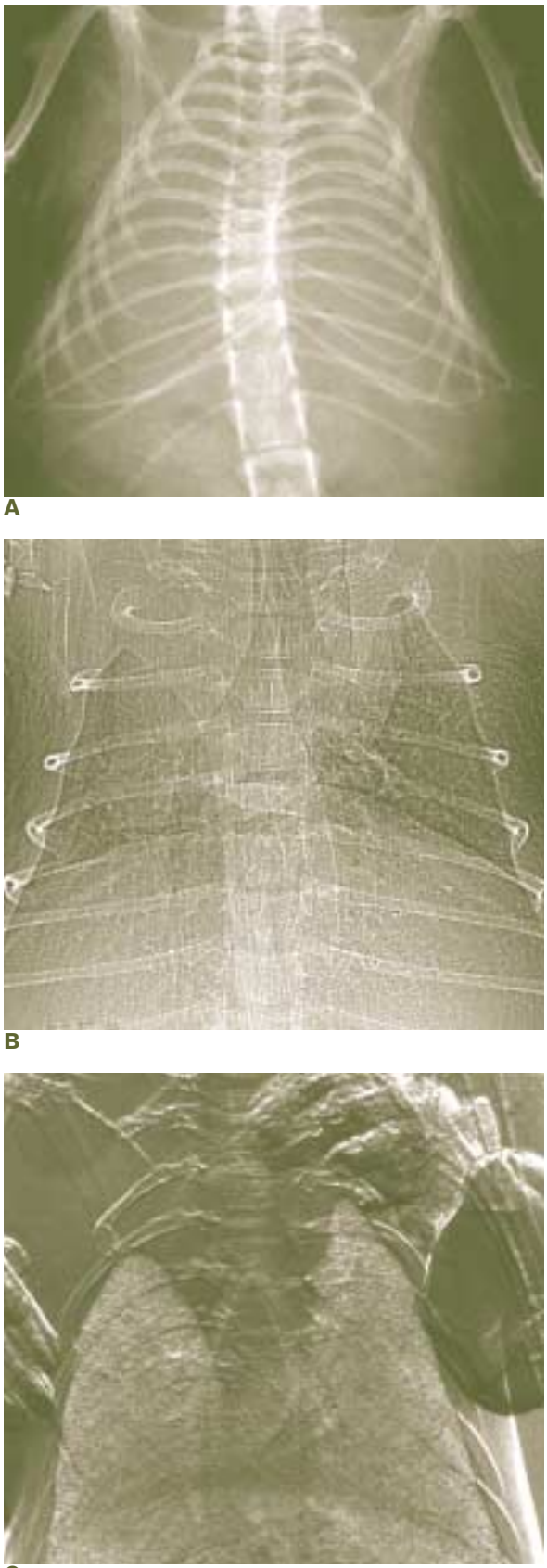

Figure 3.8. *X-ray images of mouse lungs, showing the differences between the conventional x-ray absorption image (A) and two synchrotron phase contrast images (B, C). Source: Image A was obtained by R. Lewis with a Facitron. Images B and C taken by R. Lewis et al, Monash University, at SPring-8, Japan.*

16 M.Z. Kiss, D.E. Sayers, Z. Zhong 'Measurement of image contrast using diffraction enhanced imaging', Physics in Medicine & Biology, 48 (3), 325-40, 2003. F. Arfelli et al "Phase detection techniques for possible developments in mammography with synchrotron radiation", Radiology, 215, (2000), 286–293.

Imaging of advanced materials and manufactured products

The high contrast and microtomography capabilities can be exploited to great effect in the areas of materials science, non-destructive testing and mineralogy.

Examples include:

- studies of precipitation and voids in industrially important light metal alloys
- the study of membranes for use in advanced fuels cells
- studies of fracture in ceramics
- investigation of micro/nano structured devices by micro-CT, e.g. for use in automotive applications
- the use of high resolution computed tomography for the study of porosity in oil-bearing rocks. By tuning to different energies it will also be possible to image the amount of residual oil left in the rock following extraction
- the study of advanced materials following and during various stresses, both mechanical and environmental. Many advanced materials, for example those in aerospace applications, are composed of materials that cannot be imaged with conventional x-ray techniques due to lack of contrast (figure 3.9 image of graphite fibres in aluminium). These features would be essentially invisible in conventional absorption contrast radiography.

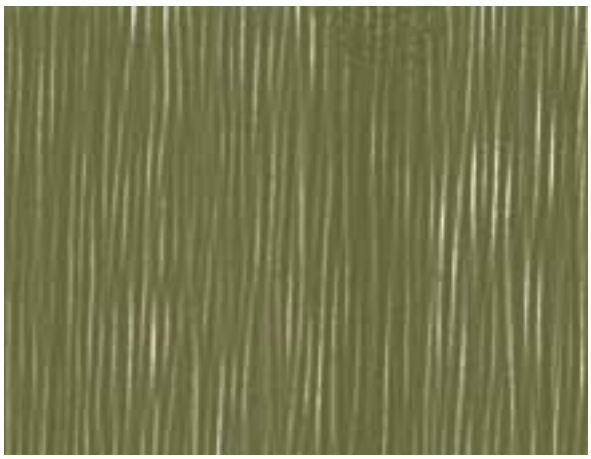

Figure 3.9. *Synchrotron phase contrast image of graphite fibres in aluminium*[17]

Imaging of plants

The contrast mechanisms employed to visualise soft tissues in animals can also render visible many of the structures inside plants. An enormous range of studies is envisaged, but of particular interest is the study of drought- and salt-tolerant species, with a view to developing more efficient crops for Australia. Phase contrast computed tomography techniques will be employed to study the development of root structures without removing the plant from the soil, while K-edge imaging will be used to study protein hormone flow dynamics.

Further developments of imaging

Imaging may be regarded as any process by which spatial structural information about an object is acquired. A paradigm shift occurred with the Nobel Prize winning work of Gabor where he demonstrated that the information in a coherent wave could be captured on film and then decoded afterwards.

Australian researchers have already made major contributions to the fundamental understanding of this, leading to the algorithms for decoding the information and thus the ability to extract readily interpretable information about a sample from the images that have been described above. However there is still much to be done, and access to the Australian Synchrotron will be important for advancing the field.

Some fundamental topics that will be pursued are the non-crystallographic phase problem and complete wavefield recovery, leading to the detailed structural analysis of a sample.

This work will be of great value in obtaining structural information from proteins that are impossible to crystallise, such as membrane proteins. It will also enhance the ability to obtain very high resolution three-dimensional images of objects with poor contrast such as biological cells and tissue.

Progress in this field is currently limited by lack of detailed theoretical understanding of the problems of coherence and phase recovery; by the need for custom optics, such as cylindrical lenses; by the sophisticated software required to recover the multi-dimensional image information; and by access to a suitable imaging detector. Beamline 10 will be designed for flexibility to support the development of the optics systems and detectors that are needed.

Radiotherapy

In cancer biology, imaging and therapy are inextricably linked. In the case of beamline 10 (the imaging and medical therapy beamline) also, the capabilities designed for excellent imaging are ideally suited for the study and development of novel radiotherapy techniques.

The major problems with radiotherapy lie in determining the extent of the spread of the disease and delivering sufficient radiation to the tumour without damaging surrounding healthy tissues. These problems are particularly acute in tumours where the surrounding tissue is extremely sensitive. Synchrotron radiation is able to deliver high dose only to the targeted area significantly better than current clinical techniques. Three methods are currently under investigation at overseas synchrotrons: photon activation therapy (PAT), computed tomography (CT) therapy, and microbeam radiation therapy (MRT).

Photon activation and CT therapy both use specific x-ray energies that are preferentially absorbed by an element that has been delivered into the tumour. In PAT a chemical agent (e.g. cis-platinum, which is also used for chemotherapy) is introduced and concentrates in the

17 A.W. Stevenson et al. "Phase-contrast x-ray imaging with synchrotron radiation for materials science applications", Nucl. Instr. and Methods in Phys. Res. B 199 (2003), 427–435.

tumour. By choosing the correct energy the x-ray beam interacts preferentially in the tumour and delivers a high localised dose. CT therapy also uses a contrast agent (e.g. iodine) that concentrates in the tumour but takes advantage of beam spreading effects and stereotactic methods to spare normal tissues.

Perhaps the most exciting possibility is MRT. Here extremely large radiation doses are applied to tissues in an array of micrometres-thick highly collimated x-ray beams (figure 3.10).

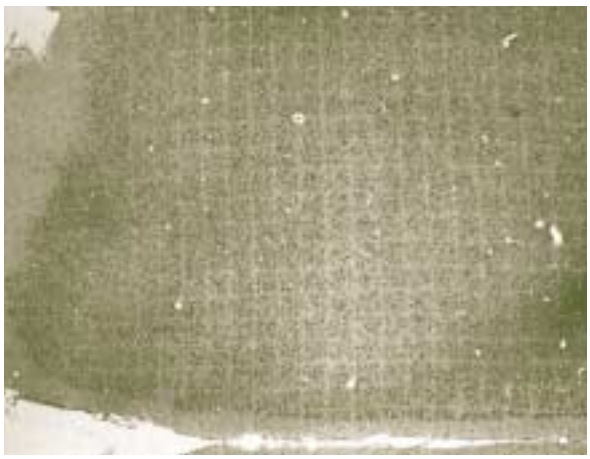

Figure 3.10. *The right cerebral hemisphere of a young adult rat after cross irradiation with x-ray beams from a multi-slit collimator. Two arrays of vertical, 0.5 cm-high and approx. 25 mm-wide microbeams with centre-to-centre distances of 210 mm were used. Subsequent examination showed that normal tissue was able to regenerate the blood vessels after one month, even in the irradiated slices. Tumours, however, do not recover from this treatment*[18].

The extraordinary aspect of microbeam radiation is that it spares healthy tissue far better than large-area beams of the same dose and yet the tumour is still damaged. The method has been used with great effectiveness to deliver doses in excess of 1000 Gy to live animals. (Note that 10 Gy delivered in a conventional method is lethal.) The reason for this effect is unknown, and is a fertile area for study.

It is possible that therapies utilising this effect may revolutionise the treatment of some kinds of cancers, which are currently untreatable. A strong programme of research into the nature of this effect, together with determining the most effective way of delivering the dose, is planned to be a significant activity on the Australian Synchrotron. However it should be noted that much research will be required before MRT could be considered for clinical application.

Future Beamline Possibilities

The flexibility of the Australian Synchrotron as a light source means that beyond the initial suite of beamlines there are other possibilities for new techniques that could be added to the facility in the future.

One of the new techniques is imaging in the terahertz (THz) region of the spectrum. So-called 'T-ray imaging' uses pulsed, far-infrared light. It has great potential as a medical imaging tool because there is no ionisation hazard for biological tissue and Rayleigh scattering is many orders of magnitude less for THz wavelengths than for the neighbouring infrared and optical regions of the spectrum[19].

The THz frequencies correspond to energy levels of molecular rotations and vibrations of DNA[20] and proteins[21], and these may provide characteristic fingerprints to differentiate biological tissues in a region of the spectrum not previously explored for medical use. In addition THz wavelengths are particularly sensitive to water[22], which can indicate tissue condition.

Another possibility is to build a micro-focussed soft x-ray beamline, which utilises the unique capabilities of the toroidal geometry electron spectrometer that has been developed by researchers at La Trobe University[23]. This would bring a new approach to the imaging of self-assembling structures on the nanometre scale and enable researchers to follow the self-assembly process in real time. Not only could imaging be achieved but it should also be possible to investigate the electronic and magnetic properties of self-assembling systems using the techniques that have been developed in recent years for 'bulk angle resolved spectroscopy'.

A second approach to imaging self-assembling systems that could be incorporated on this beamline involves illuminating a macroscopic area of sample and relying on the spatial resolution obtainable using a low energy electron microscope column. This is the PEEM or XPEEM (x-ray/photoelectron emission microscopy) system pioneered by Ernst Bauer and others[24].

18 J.A. Laissue, N. Lyubimova, H-P. Wagner, D.W. Archer, D.N. Slatkin, M. Di Michiel, C. Nemoz, M. Renier, E. Brauer, P.O. Spanne, J-O. Gebbers, K. Dixon, H. Blattmann, 'Microbeam radiation therapy', In H. Bradford Barber, H. Roehrig (Eds), Medical Applications of Penetrating Radiation, Proceedings of SPIE, 3770 (1999) 38–45. See also http://www.pathology.unibe.ch/Forschung/microbeam/microbeam.htm

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21 M. Walther, B. Fischer, M. Schall, H. Helm & P. Uhd Jepsen. Chemical Physics Letters, 332 (2000) 389–395.

22 D.M. Mittleman, R.H. Jacobsen & M.C. Nuss. IEEE Journal of Selected Topics in Quantum Electronics, 2(3) (2000) 679–691.

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