02

Introduction to synchrotron science

IMAGE: Australian Synchrotron floorplan

Layout of the Australian Synchrotron showing the positions of the LINAC injector, booster synchrotron, storage ring, x-ray shielding and beamlines. It will be possible to accommodate more than 30 beamlines in the longer term.

Chapter 02

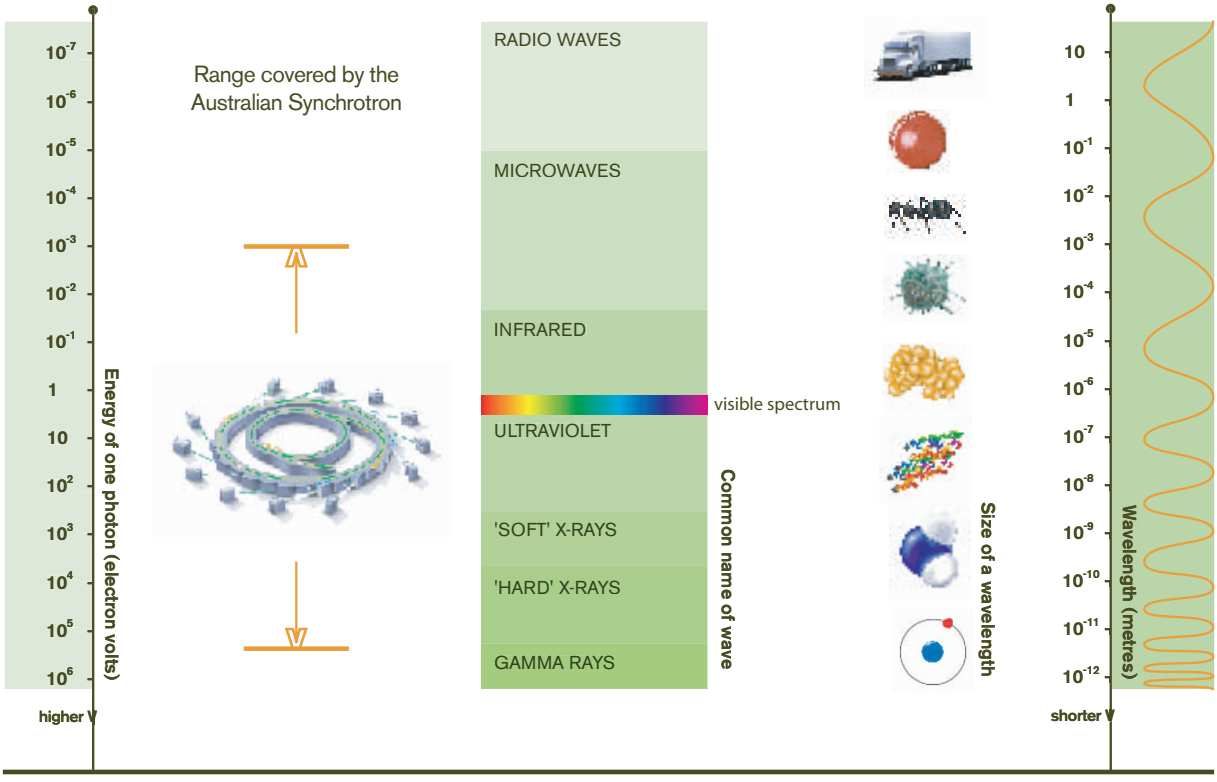
Introduction to synchrotron science

Synchrotron light (electromagnetic radiation) is emitted when charged particles, usually electrons or positrons, moving at velocities close to the speed of light, are forced to change direction under the action of a magnetic field. The electromagnetic radiation is emitted in a narrow cone in the forward direction, at a tangent to the orbit.

Characteristics of Synchrotron Light

Synchrotron light has a number of unique properties. These include:

- **High brightness:** synchrotron light is extremely intense (hundreds of thousands of times more intense than that from conventional x-ray tubes) and highly collimated.
- **Wide energy spectrum:** synchrotron light is emitted with energies ranging from infrared light to hard, energetic (short wavelength) x-rays.
- **Tunable:** through sophisticated monochromators and insertion devices (see below) it is possible to obtain an intense beam of any selected wavelength.
- **Highly polarised:** the synchrotron emits highly polarised radiation, which can be linear, circular or elliptical.
- **Emitted in very short pulses:** pulses emitted are typically less than a nano-second (a billionth of a second).



EMR The Electromagnetic Spectrum

Figure 2.1. *The electromagnetic spectrum, showing the energy range produced by the Australian Synchrotron*

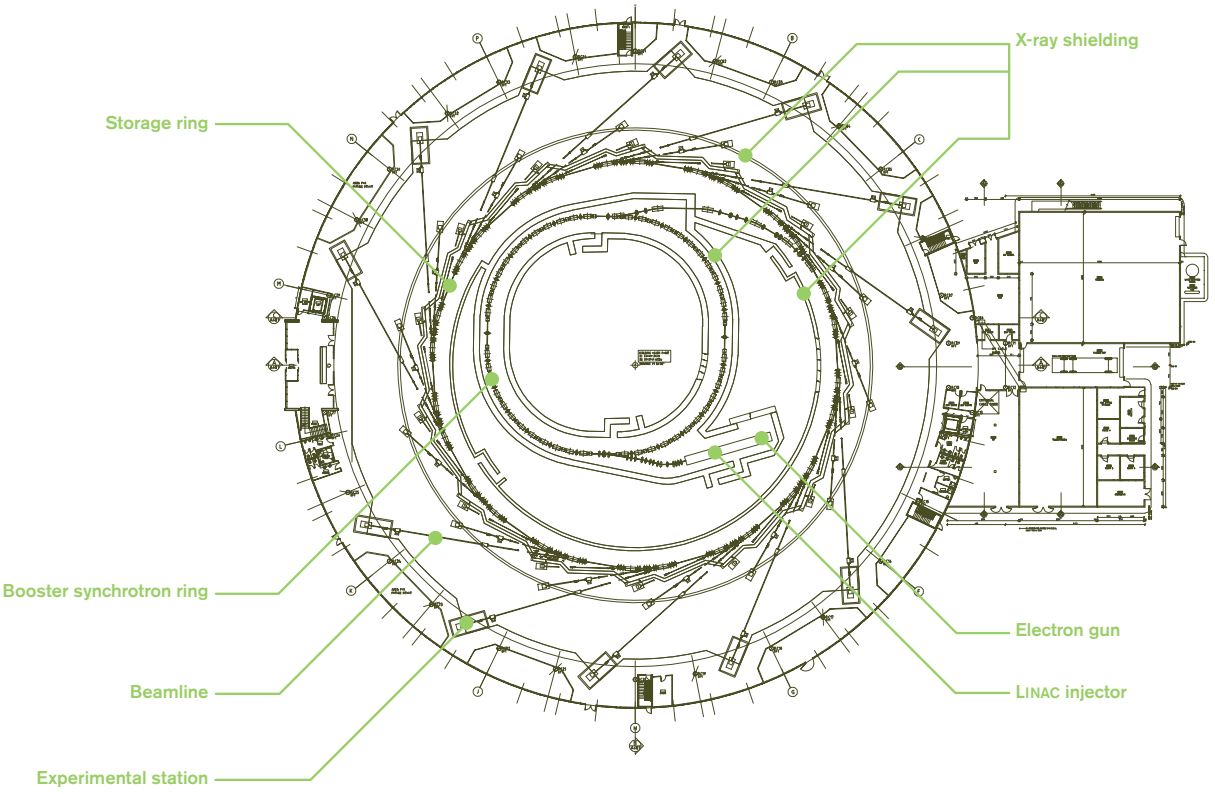
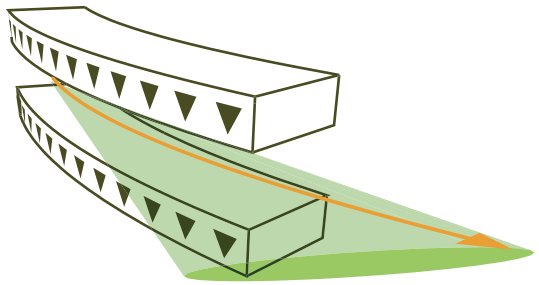


Figure 2.2. *Layout of the Australian Synchrotron showing the proposed positions of the LINAC injector, booster synchrotron, storage ring, x-ray shielding and beamlines. It will be possible to accommodate more than 30 beamlines in the longer term.*

Many techniques that provide information from materials on a microscopic and molecular scale require that the wavelength of the illuminating radiation must be of the same order as the structure. As shown in figure 2.1, synchrotron radiation is able to span the entire field of scientific interest – from lifesize imaging, down to nano-, molecular and atomic scale.



BENDING MAGNET Sweeping Searchlight

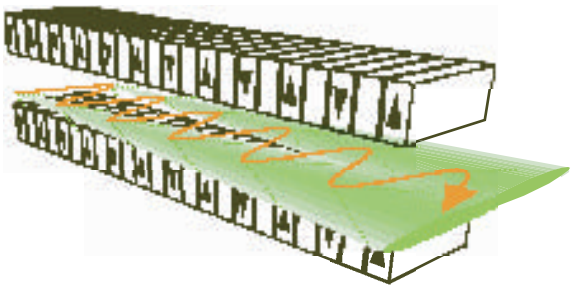
Figure 2.3. *A typical bending magnet – at each deflection of the electron path a beam of 'light' is produced. The effect is similar to the sweeping of a search light.*

Creation of Synchrotron Light

Figure 2.2 shows a plan view of the Australian Synchrotron. The electrons are generated in the centre (electron gun) and accelerated almost to the speed of light by the linear accelerator (abbreviated 'LINAC') and the booster ring, and then transferred to the outer storage ring. The electrons are confined to the circular orbit by a series of bending magnets separated by straight sections. When electrons moving at close to the speed of light are deflected they give off electromagnetic radiation, so that at each bending magnet a beam of synchrotron light is produced (see figure 2.3). These beams can be captured and used to perform a wide range of experiments – from x-ray diffraction to x-ray and light spectroscopy to x-ray imaging – by selecting parts of the beam's spectrum.

Synchrotron design has evolved rapidly over the past 20 years. In early designs the principal sources of synchrotron light were the bending magnets.

Although the intensity of synchrotron light from bending magnets is very high it was found that this can be increased by many orders of magnitude by the use of 'insertion devices' in the straight sections of the ring. These consist of two rows of small magnets with alternating polarity perpendicular to the electron beam, which move the beam from side to side. This sinusoidal trajectory causes more radiation to be emitted over and above that resulting from moving through the circular arc imposed by the storage ring's bending magnets.



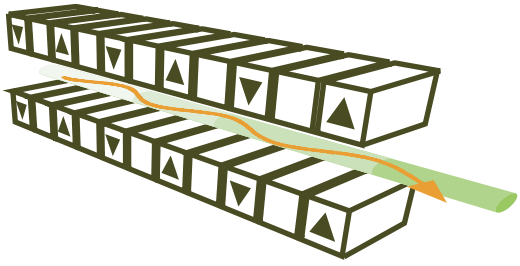
WIGGLER Incoherent Superposition

Figure 2.4. *A multipole wiggler – at the peak of each wave a beam of light is emitted. These beams reinforce each other and appear as a broad beam of incoherent radiation when viewed in the horizontal plane at a distance ahead of the wiggler.*

There are two classes of insertion devices. One is a multipole wiggler (MPW) in which a cone of light is emitted at each bend in the 'wiggle' so that the cones of light superimpose on each other, the intensity increasing with the number of bends (see figure 2.4).

The second type of device is an undulator that uses less powerful magnets to produce gentler undulations of the beam. In this case, the light cones just overlap and interfere with each other, so that certain wavelengths of light are enhanced perhaps by 10,000 times. These wavelengths can be changed by altering the gap between the component magnets so that the light is tunable to specific wavelengths (see figure 2.5).

Current ('third generation') designs of synchrotrons aim to optimise the intensity that can be obtained from insertion devices. In particular, attention is given to the size and positioning of the straight sections that accommodate the insertion devices.



UNDULATOR Coherent Interference

Figure 2.5. *An undulator – the poles produce less deflection of the electron beam. This results in a narrow beam of coherent synchrotron light, with certain frequencies amplified by up to 10,000 times.*

In the Australian Synchrotron it is likely that the undulator magnets will be placed inside the vacuum of the storage ring; this will allow very close spacing of the magnets and extremely high light intensities. Conventional magnets can be used for the wigglers, but they may be wound with super-conducting wire, again enabling extremely high light intensities and the ability to easily tune the frequency of the light.

Thus the Australian Synchrotron, which is an advanced 'third generation' design, will be able to accommodate all the different types of light sources (bending magnets, multipole wigglers and undulators) to enable a wide range of advanced experiments or measurements to be carried out.

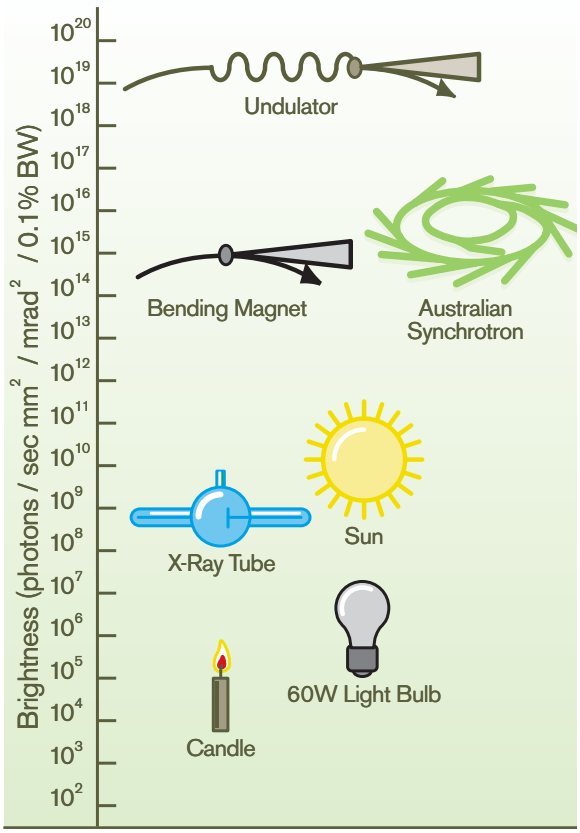


Figure 2.6. *Intensity of light that can be obtained from the synchrotron compared to other sources.*

Experimental Techniques

To carry out experiments a beam originating from one of the sources travels out of the ring and down a tube that is under high vacuum, called a 'beamline'. The appropriate type of radiation is selected from the spectrum and then directed to the experimental 'end station'. The radiation can be light from deep infrared through to extreme ultraviolet light, or x-rays from soft to very hard (0.2 to 120 keV).

The experiments or measurements that can be carried out fall into four main categories:

- Diffraction/scattering for crystallography, including protein crystallography
- Spectroscopy for analysis of chemical composition and speciation in the bulk material and at surfaces, down to nanometre dimensions
- Polarimetry for measuring the shape of complex molecules, especially proteins, and the properties of magnetic materials
- Imaging from highly detailed imaging of small animals, and ultimately humans, down to the substructure of biological and physical material, using light from infrared through to hard x-rays.

It will be possible to combine imaging with analytical techniques to provide additional information in four of the beamlines – the Microspectroscopy, the Microdiffraction and Fluorescence Probe, the Infrared Spectroscopy, and the Imaging and Medical Therapy beamlines.

Apart from these measurement and imaging techniques it is possible to use the highly collimated hard x-rays for medical therapy and for micro-machining materials to micron sized dimensions, with exceptionally high depth to width aspect ratios.

Diffraction

X-ray diffraction is the most widely used approach for 'imaging' substances at atomic resolution and elucidating their structures. These substances include metals and alloys, chemical compounds, minerals, and molecular crystals ranging from several atoms to macromolecular assemblies of hundreds of thousands of atoms.

In a crystal the constituent atoms are arranged in regular arrays. When x-rays pass through the crystal they may be elastically scattered by the electron structure of the atoms. Because of the regular arrangement of the atoms the scattered waves periodically reinforce each other to form a diffracted beam (see figure 2.7). By rotating the crystal through all angles with respect to the x-ray beam, a complete diffraction pattern can be built up that is uniquely characteristic of the crystal. From this pattern it is possible to determine accurately the crystal structure by theoretical analysis and reference to molecular and crystallographic databases.

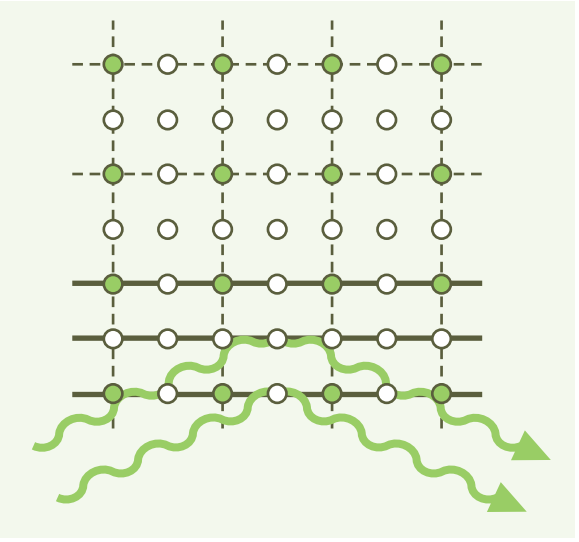


Figure 2.7. *The x-ray beam scattered by the electrons associated with the atoms in the crystal structure. Because of the regular array the scattered x-rays reinforce each other to form a diffracted ray, whose angle from the incident ray provides a measure of the distance between the planes of atoms.*

When a single crystal is examined, as will be the case for the protein crystallography and small molecule beamlines, a pattern of spots is produced on the detector – see figure 2.8.

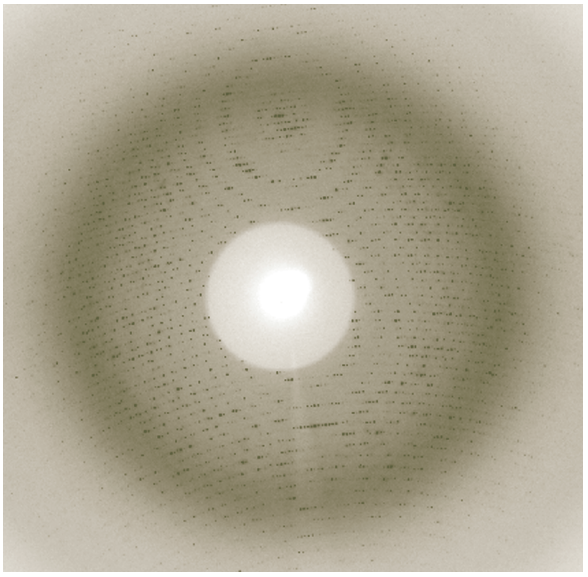


Figure 2.8. *A high resolution x-ray diffraction pattern obtained from crystals of a paramyxoviral haemagglutinin-neuraminidase. Courtesy of M.C. Lawrence, CSIRO Health Sciences and Nutrition, Parkville, Victoria*

Because the x-rays are scattered by the electrons in the crystal structure it is possible to compute not only the atomic structure of crystal but also its electron density map.

Single-crystal diffraction is the preferred method for determining the structure and electron density map. However, the preparation of diffraction-quality crystals often lags months or even years behind pioneering scientific breakthroughs, such as high temperature

superconductivity. This is particularly true of many materials of major industrial or commercial importance, including zeolites, catalysts, pigments and pharmaceuticals, as well as many minerals. The ability of the synchrotron to produce intense, highly collimated x-rays and to focus them with x-ray optics to a very small spot size enables diffraction to be performed on small single crystals of many of these materials where previously this has been impossible.

Powder diffraction

When single crystals cannot be obtained, the material can be ground into a powder of tiny micro-crystals and analysed by powder diffraction. Powder diffraction is frequently chosen when the behaviour of the crystal structure under external conditions such as high pressure or high or low temperature is to be observed. In this technique a three-dimensional diffraction pattern is collapsed onto two dimensions by spherical averaging, and as a result, reflections, which would otherwise be separately measured, overlap. The resulting pattern is a collection of concentric rings, often with fine structure from the individual crystallites in each ring (see figure 2.9).

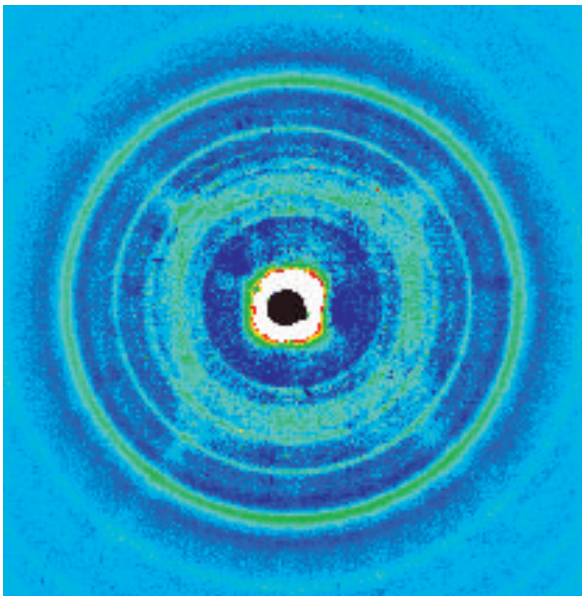


Figure 2.9. *Powder diffraction pattern of a human heart valve, showing some collagen structure and also the calcification that led to the failure of valve. (courtesy of Prof. R. Lewis (Monash University) and Dr K Rogers (Cranfield University), taken at the Synchrotron Radiation Source, Daresbury (UK).).*

The resolution that can be obtained from powder diffraction is limited by the degree of monochromaticity, the signal to background noise level ('noise' can result from scattering by the cell or air) and by the range of observations (d-range). Synchrotron radiation with its high intensity and highly collimated incident beam can be of great assistance in resolving overlaps of the diffraction rings experimentally; it also improves the signal to noise level and can increase the range of observations. Thus diffraction data obtained from synchrotron-based instruments provide much greater accuracy and resolution compared with conventional instruments.

Anomalous dispersion

Another important aspect of synchrotron light is its tunability, enabling anomalous dispersion effects to be exploited. This means that by the use of a monochromator the wavelength of the beam can be selected to maximise the visibility of certain elements in the sample. Many advanced materials contain elements that may be disordered over a number of sites within the crystalline phases, either as a result of the processing conditions or due to deliberate doping. Such disorder is often inherent in mineral samples. Anomalous dispersion effects produce element-specific information, enabling such disorder to be identified and quantified.

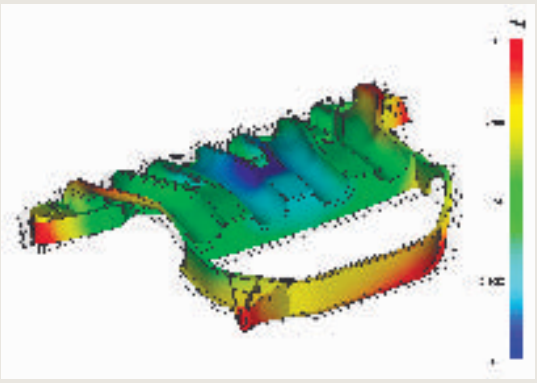
Anomalous dispersion is particularly useful for protein crystallography, especially of proteins containing seleno-methionine, where selenium replaces sulfur in the amino-acid methionine. In this technique, called 'multiple wavelength anomalous dispersion' (MAD), the diffraction patterns are measured at a number of wavelengths close to the x-ray absorption edge. The technique has formed the basis of many structural genomics initiatives, where the gene products of entire genomes are produced with seleno-methionine.

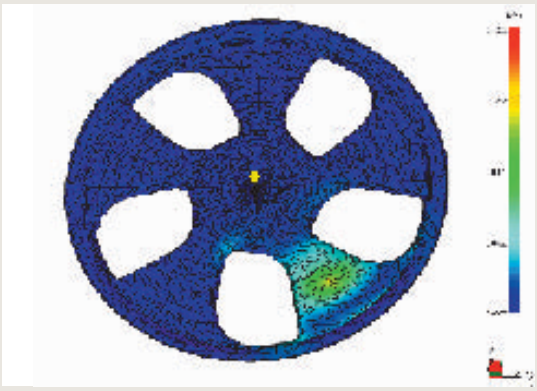
Small and wide angle scattering

Small angle scattering (SAXS) is the process of elastic scattering of x-rays from a sample, where those scattered at a small angle with respect to the original direction of the beam are detected. The information gained is primarily structural, especially for materials whose features are in the range 500 nm to 0.1 nm. Because of the inverse relationship between scattering and size, x-rays scattered at small angles give information about the size and shape of relatively large structures such as polymers and proteins.

For many experiments (such as nucleation and crystallisation) it is necessary to observe both the SAXS and the wide angle scattering (WAXS) from growing crystallite phases, e.g. from hydrothermal processes such as zeolite formation or Bayer liquor crystallisation. The wide angle diffraction gives information about structure at far smaller scales, similar to that obtained from the more standard x-ray diffraction technique. At a synchrotron beamline it is possible to collect SAXS and WAXS data simultaneously with specially designed detectors.

One of the first instruments to be built at the Replacement Research Reactor at Lucas Heights, NSW, will be a small angle neutron scattering instrument. The x-ray and neutron scattering methods complement one another, and the simultaneous refinement of data produced using a combination of both methods on the one system is very powerful. The major benefit of such analysis is the removal of potential ambiguity in the interpretation of data.





Structural analysis of a plastic part showing warp, and a plastic hubcap showing maximum stresses.

Improving plastic products

Australian researchers are using synchrotron SAXS and WAXS techniques on the Australian beamline in Japan to improve injection-moulded polymers.

- In Australia, injection-moulded plastics are an $8b industry. They are used in cars, medical devices, mobile phones, computers, TVs, toys and many other consumer goods.
- The industry needs accurate information about how polymers set after injection into the moulds.
- Monash University researcher Dr Graham Edward works with the CRC for Polymers. He says "using a synchrotron means we take days to complete work that would need months in the lab and the synchrotron results are more accurate".

Sources: G Edward, Monash University; CRC for Polymers; Moldflow Corporation

Spectroscopy

When photons pass through any material a proportion of their energy will be absorbed by the atoms. Depending on the amount of energy absorbed an electron in an inner orbital may be ejected completely from the atom, figure 2.10a, or may be raised to a higher energy, unoccupied orbital state, figure 2.10b.

This gives rise to a number of spectrographic techniques, including:

- X-ray photoelectron spectroscopy (XPS) where the emission of photoelectrons is measured.
- X-ray absorption spectroscopy (XAS), where the degree of x-ray absorption is measured as the energy level of the radiation is increased. A typical plot that is obtained is shown in figure 2.12; it can be seen that the absorption suddenly changes at a specific energy level, which is related to the threshold energy at which an electron is ejected from its base orbital state. The position of the absorption edge is characteristic of a particular element and can be used to discriminate between elements with a high degree of precision.

When an electron is ejected from its base orbital state, a 'hole' or vacancy is created which is subsequently filled by an electron moving from a higher energy state to the lower state. During that process energy is given off by the electron, which can be in the form of a photon, see figure 2.10c, and the energy of the photon is characteristic of the particular atomic element. This gives rise to a third spectrographic technique termed x-ray emission spectroscopy (XES). (Sometimes XES is known as x-ray fluorescence (XRF)).

Alternatively the excess energy may be transferred to another electron in an outer orbital so that this electron now has sufficient energy to escape from the atom, see figure 2.10d. These escaped electrons are known as Auger electrons, and measurement of the emission of Auger electrons is known as Auger electron spectroscopy (AES).

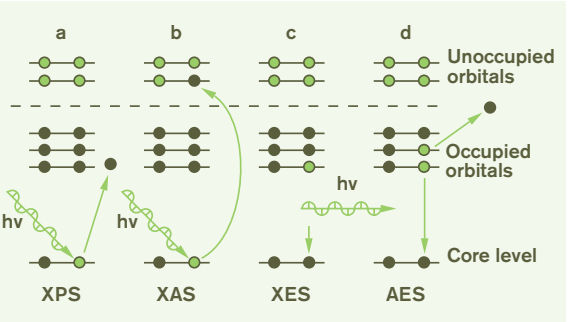


Figure 2.10. *Illustration of the changes in electron state when a photon is absorbed by an atom. These mechanisms enable (a) x-ray photon spectroscopy, (b) x-ray absorption spectroscopy, (c) x-ray emission spectroscopy and (d) Auger electron spectroscopy.*

X-ray absorption spectroscopy (XAS)

X-ray absorption spectroscopy (XAS) is a well-established, quantitative analytical technique used by both academia and industry to garner atomic-scale structural information for a wide range of systems in both liquid and solid form. XAS probes both the short and medium range order of a sample and measures disordered samples, and as such is complementary to x-ray diffraction.

As a crystal is not needed for XAS, it is not only possible to study liquids but also to analyse the elemental structure of most biological (cells, plant roots, etc.) and environmental (soils etc.) samples.

XAS measurements are made over the energy range covered by soft, intermediate and hard x-rays. Intermediate and hard x-radiation is used for studying elements from atomic number Z=20 (calcium) upwards. Soft x-radiation is used for studying the lighter elements, and also to obtain additional chemical information about elements above Z=20. For example, in the case of chromium, K-edge (~6 keV) XAS is carried out on a hard x-ray beamline, but L-edge (~0.6 keV) XAS, which provides valuable additional information, is carried out on a soft x-ray beamline.

The physical processes governing x-ray absorption are shown schematically in figure 2.11 – a photon of energy *hv* is absorbed by an atom and a photo-electron is ejected. The latter is considered quantum mechanically as an out-going spherical wave that may be scattered by neighbouring atoms. At the absorbing atom, out-going and scattered waves interfere, modulating the absorption coefficient. Accordingly, an absorption spectrum such as that shown in figure 2.12 exhibits oscillations or fine structure extending beyond the absorption edge. These gradually die away as the x-ray energy increases. The oscillations, which occur relatively close to the edge (within about 40 eV), are known as NEXAFS (near edge x-ray absorption fine structure) or XANES (x-ray absorption near edge structure), and those further out are termed EXAFS (extended x-ray absorption fine structure).

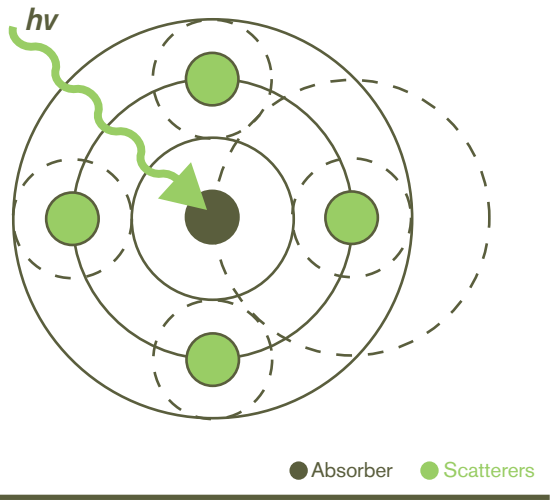


Figure 2.11. *Schematic representation of the x-ray absorption process. A quantum of light is absorbed by the central atom and scattered by adjacent atoms.*

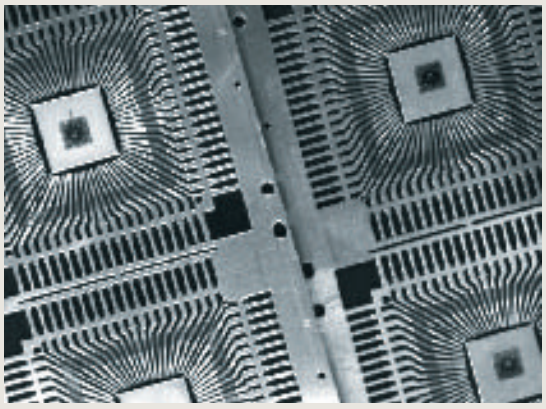
Analysis of the extended x-ray absorption fine structure (EXAFS) yields structural information such as bond lengths and coordination numbers. In closer proximity to the absorption edge, analysis of the electronic transitions and multiple-scattering processes that dominate XANES yields chemical information such as the local coordination geometry and oxidation state of the absorbing atom.

Though the fine structure of an absorption spectrum was first observed experimentally approximately 100 years ago, it was not until the 1970s and the availability of the intense, tunable source of photons from synchrotron light that the underlying physics was correctly established and XAS forever changed from a qualitative observation to a quantitative analytical technique. Despite a 30-year history and current widespread usage, the XAS technique continues to evolve both experimentally (for example, time-resolved measurements) and theoretically (for example, improved ab-initio calculations).

X-ray emission spectroscopy (XES)

XES provides similar and complementary information to XAS and XPS. A wide variety of materials including solids, liquids and gases can be analysed.

The characteristic photons measured in XES are those that arise when an electron vacancy in an inner shell is filled by an electron from one of the outer shells. The energy, E, of the characteristic photon is equal to the difference in the binding energies between the two electron levels involved in the transition. Lines are called K series lines if the initial ionisation is in the K shell, L series lines if it is in the L shell and so on.



Semi-conductor image captured with a Redlake MegaPlus Camera. Used with permission from Redlake http://www.redlake.com

Semiconductor Science

Australian materials scientists are using XAS to determine the nature of ion-implantation-induced disorder in semiconductor substrates.

- This research impacts the fabrication of both electronic and photonic devices.
- The ion implantation process is one of several basic processes used in the production of all such modern devices. The form and extent of ion-implantation-induced disorder ultimately governs device performance.
- Synchrotron XAS is the only analytical technique capable of unambiguously identifying and quantifying the homopolar bonding observed before the crystalline-to-amorphous phase transformation. (See chapter 3 for further details.)

Source: Dr Mark Ridgway, Australian National University

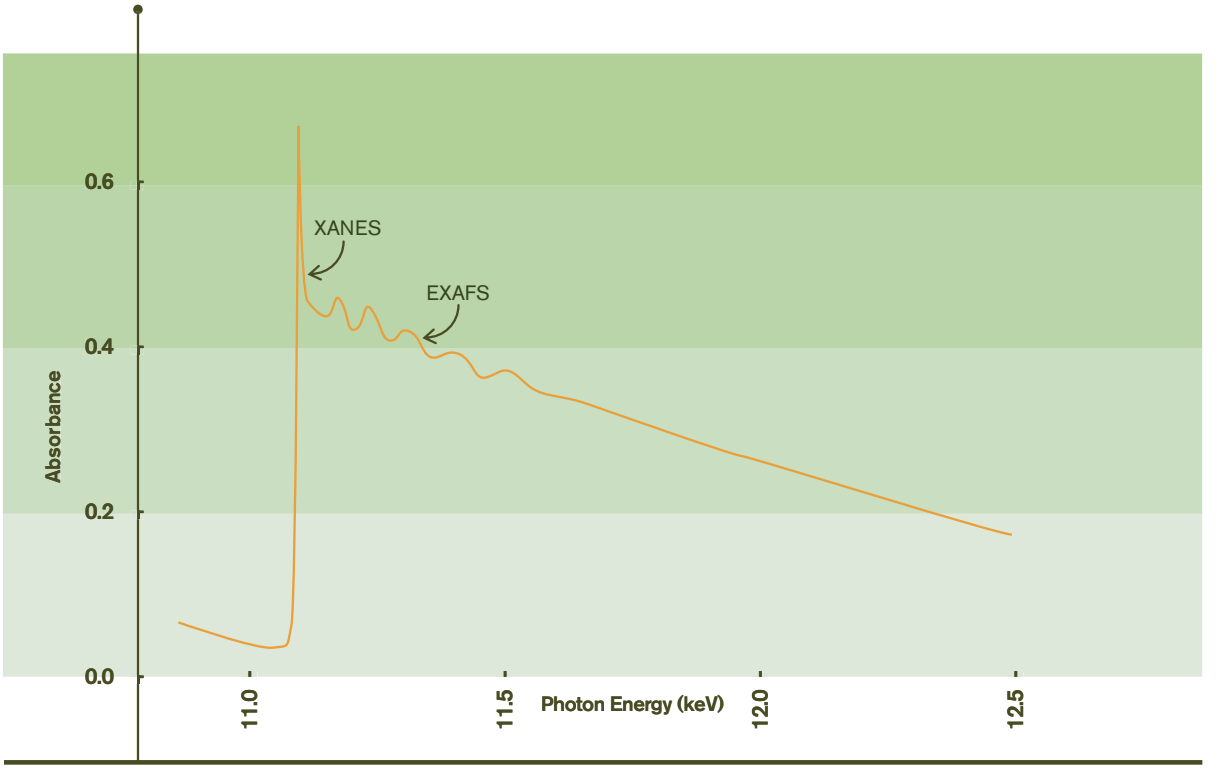


Figure 2.12. *A typical x-ray absorption spectrum*

Because XES measures photons that arise from transitions between inner electron energy levels in atoms, the technique can provide very detailed, element-specific information about the valence electron states in a compound or alloy.

The interpretation of XES spectra obtained from conventional laboratory x-ray sources, is sometimes made difficult by overlaying lines due to satellite transitions or close-lying core holes. Also, irrelevant inner core transitions may accidentally fall in the wavelength region under study. These problems can be removed by using monochromatised synchrotron radiation.

Thus the use of XES has developed rapidly in the past decade as access to tunable soft x-ray synchrotron radiation has become more readily available. Most work has been on solids, particularly electronic materials, but recently the technique has been applied to investigating metalloproteins where light elements contained in peptide chains or water molecules can be detected.

X-ray photoelectron spectroscopy (XPS)

X-ray photoelectron spectroscopy (XPS) is the most important and versatile technique for the chemical characterisation of the surface of a material. In conventional XPS, soft x-rays of fixed energy are obtained from an aluminium anode, and because this photon energy is near 1.5 keV, depending on the binding energies of the core electrons of interest, the photoelectron kinetic energies are such that the typical analysis depth is 2–5 nm. Clearly only a small proportion of this analysis depth can be considered to be the true surface layer.

In synchrotron XPS, the photon energy can be tuned to vary the kinetic energy of the ejected photoelectrons, thereby varying the analysis depth. In particular, a photon energy can be selected to result in the photoelectrons of interest having a kinetic energy near 45 eV, the energy for which the inelastic mean free path is a minimum. In this way, the surface sensitivity of XPS can be maximised and an analysis depth of two atomic layers can be achieved. By increasing the photon energy, the analysis depth is increased and information for a non-destructive chemical depth profile can be obtained.

In synchrotron XPS, the photon energy can also be tuned to alter the photo-ionisation cross-section for the electrons of particular interest. In practice, it is often highly desirable to optimise the cross-section to enhance the sensitivity for a particular element, or to change the relative cross-section for a sub-shell in two elements, but of course in both cases, a concomitant change in the depth analysed would occur. Thus, in synchrotron XPS, it is the tunability of the photon energy that is of greatest importance, but of almost equal importance is the high photon flux, because this allows high energy resolution to be selected in the trade-off between monochromator resolution and transmission. The high flux also allows photoelectron diffraction measurements to be made.

Angular Resolved Ultraviolet Photoelectron Spectroscopy (ARUPS)

Angular resolved ultraviolet photoelectron spectroscopy (ARUPS) is the pre-eminent technique for the elucidation of the electronic structure of atoms, molecules and solids. The method enables the determination of absolute binding energies of electrons in solids.

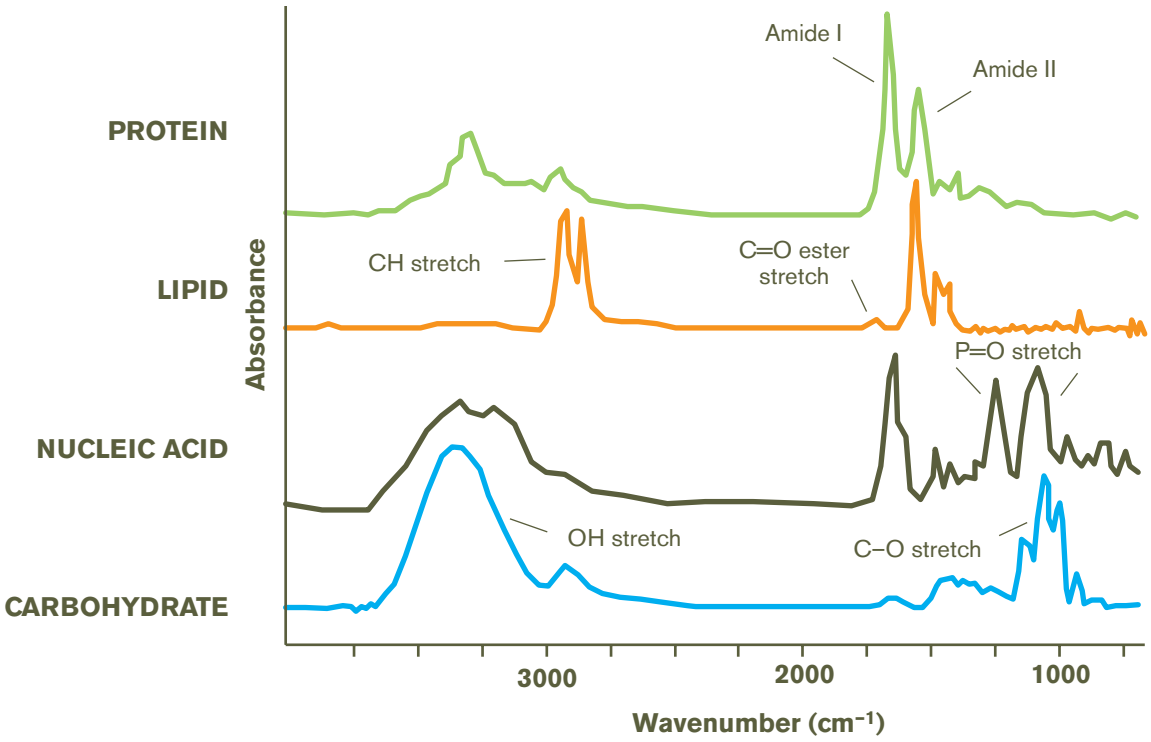


Figure 2.13. *Typical infrared spectra of a protein, lipid, nucleic acid and carbohydrate. Each has a separate, characteristic profile because of the different types of chemical bonding in the structure.*

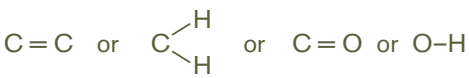
For ARUPS the sample is irradiated with VUV light (energy range from 5 to 100 eV) to excite photoelectrons into the vacuum above the surface of the sample.

In general, the emission pattern of photoelectrons is not isotropic in space, but produces a characteristic angular distribution of energies. From measurement and analysis of the kinetic energy and angular distribution of the photoelectrons, information on the electronic structure of the material in the sample, particularly Fermi surfaces, can be determined.

Due to the limited mean free path of the photoelectrons, ARUPS is a surface sensitive technique, and is especially suited to the analysis of thin films.

Infrared Spectroscopy

Infrared illumination causes atoms in a molecule to vibrate. The frequency of vibration is specific to the type of interatomic bond. For example, in a biological or polymer molecule the bonds are mostly between carbon, hydrogen and oxygen.



Each of these bonds vibrates at a different, characteristic frequency. Typical infrared absorption spectra for a protein, lipid, nucleic acid, and carbohydrate obtained by Fourier Transform infrared spectroscopy (FTIR) are shown in figure 2.13. The types of bonds present and their intensity provides a unique 'signature' for each molecule.

By analysing the spectra it is possible to identify the structures and types of molecule with a high degree of discrimination. If a characteristic peak is selected and then imaged it is possible to build a picture of the distribution of specific molecules in the sample.

Infrared spectroscopy is very widely used in Australia. Every reputable research or analytical laboratory (chemistry, physics, materials, biochemistry and microbiology) would possess an infrared system and many production facilities use the technique for quality control.

Laboratory-based microspectroscopy instruments are usually driven by globar light sources, which limit the achievable spatial resolution to 20–30 microns. Synchrotron light, which is highly collimated, polarised and much more intense (at least 100 times more intense) and stable (see figure 2.14), vastly increases the potential of the techniques:

- Using a synchrotron, spatial resolution down to the diffraction limit of 5–10 microns can be obtained. As a result it is possible to locate and analyse individual components in a sample with dimensions typical of biological cells.
- Recent advances in focal plane array detectors, when coupled with high intensity illumination from a synchrotron and scanning confocal microscopy, will enable high resolution infrared imaging in three dimensions in a realistic time frame, leading to wide application in anatomy and forensic science.
- The high brightness enables high resolution infrared spectroscopy, which is important in atmospheric monitoring, gas analysis and molecular structure determination.

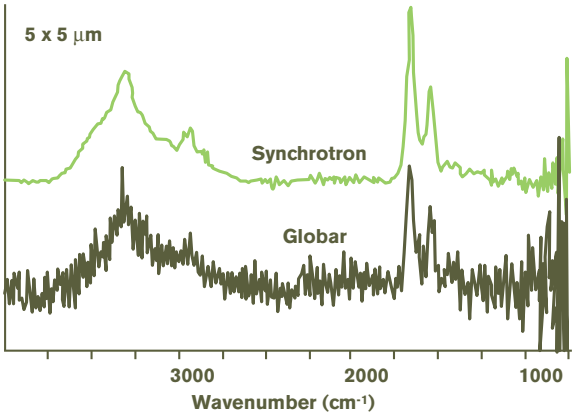


Figure 2.14. *Comparison of infrared spectra obtained from the same sample using a conventional globar-based laboratory instrument and a synchrotron IR beamline. The signal to noise ratio is substantially improved due to the greater stability of the synchrotron source.*

In isolation or when combined with other techniques, particularly diffraction and fluorescence, infrared spectroscopy is highly effective for characterising the structure, composition and state of samples – from minerals through to biological tissue.

Polarimetry

X-ray circular dichroism (MXCD)

It is possible to obtain circularly polarised x-radiation from a synchrotron which, with the appropriate design of insertion device, can be rapidly switched between both helicities.

This can be used to obtain information on a wide range of magnetic properties of materials. Examples include measurement of the spin and orbital components of element-specific and site-specific magnetic moments, measurement of element specific hysteresis loops, determination of Lz/Sz ratios, and determination of absolute local moments.

Magnetic X-ray Circular Dichroism (MXCD) arises because a magnetic material may have a different photon absorption cross-section at a particular photon energy for left- and right-circularly polarised light, see figure 2.15. This is known as the Faraday effect. By comparing the absorption spectra measured with the two opposite signs of polarisation, the MXCD spectra is obtained, which can then be interpreted to determine the various magnetic properties. Thin films, bulk materials and the interfaces between thin films can all be analysed.

The technique is essential for development of the next generation of hard disk storage devices.

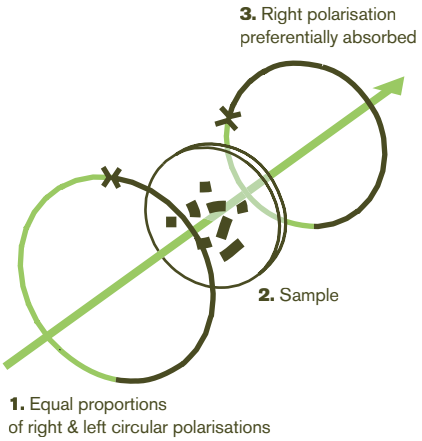


Figure 2.15. *Illustration of the principle of 'circular dichroism'. The sample may absorb circularly polarised light asymmetrically because of its electronic spin-orbit structure (in the case of magnetic materials), or its molecular bond structure (in the case of complex molecules such as proteins).*

VUV circular dichroism (VUV CD)

In the ultraviolet region of the spectrum circular dichroism can be used to analyse the conformational structure (i.e. shape) of complex molecules – for example the folding of proteins. It can also be used for examining the binding of ligands and drugs to proteins, as well as for deciphering the nature of interactions between proteins and other macromolecules. It is particularly valuable for examining both soluble and membrane proteins, which are difficult to analyse by other means because of the difficulty in crystallising them. Membrane proteins are important for drug development, because most drug delivery systems involve transfer across membranes using them.

Circular dichroism (CD) spectroscopy is possible in these materials because their polypeptide backbones are optically active and differ in their absorption of left- and right- circularly polarised UV light.

Conventional laboratory based CD instruments have been available since the 1960s and typically cover from about 190 to 250nm wavelengths. While these provide very valuable information, further information exists in the vacuum ultraviolet (VUV) wavelength region (below 190 nm), but its measurement is generally limited in a conventional instrument by the high absorption of the sample, buffer and solvent, and the low intensity of the light source.

Over the past few years synchrotron radiation has been used to extend CD measurement into the VUV region. Apart from the additional information provided it has been found that synchrotron radiation is a much more stable source so that the spectra are much clearer and provide more detail (see figure 2.16).

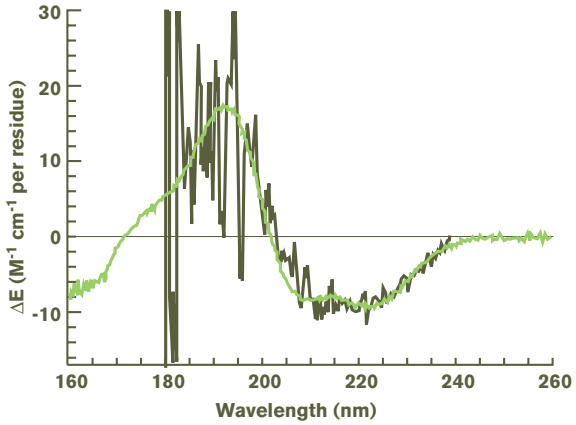


Figure 2.16. *Comparison of CD spectra for the same sample from a conventional laboratory instrument (black line) and from a synchrotron VUV CD beamline (green line). Note the improved signal to noise ratio, and the additional information at wavelengths shorter than 180 nm provided by the synchrotron. Courtesy G.R. Jones and D.T. Clarke, SRS, Daresbury, UK.*

In addition, it has recently been demonstrated that VUV irradiation in an SRCD instrument is non-damaging to protein integrity.

Parallel developments in bioinformatics are providing better definition and classification of a wide range of protein structural types, and the rapid growth in crystal structure analyses has provided a large number of protein structures from which more comprehensive reference databases can be constructed.

Taken together, these developments mean that SRCD is becoming a vital part of the suite of techniques required for modern structural biology.

Imaging

X-ray imaging

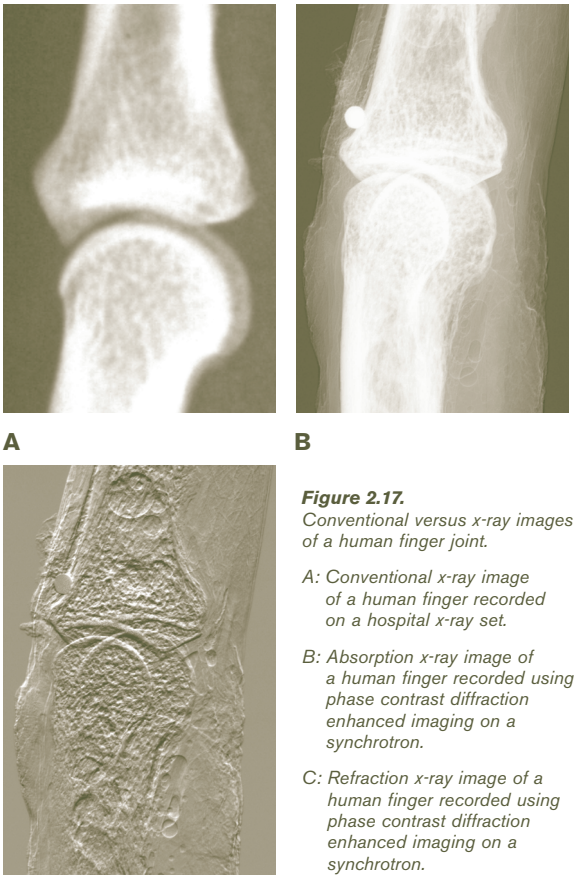
Conventional imaging with x-rays based on the absorption of the radiation has been in use for over 100 years. The contrast produced in a conventional x-ray image results from differing absorption by components in the object caused by varying composition, thickness or density. Effectively, a shadowgraph is obtained, which works well when there are very large differences in absorption between constituents – such as bone and soft tissue. However, conventional x-ray imaging of soft tissues, such as skin, cartilage, ligaments, tendons, lungs, breast tissue and tumours, produces information of poor quality.

Phase contrast

Pioneering work at The University of Melbourne, CSIRO,[1] Monash University and several synchrotron laboratories has shown that a suite of techniques, collectively known as phase contrast x-ray imaging, can exploit the valuable information that arises from the refraction of the x-ray beam by the soft tissue[2]. The different refractive indices of the various types of soft tissue cause changes in the direction and phase of the illumination and this can be detected when the highly collimated, tuned radiation of a synchrotron is used. The magnitude of the phase shift effect is approximately 1,000 times larger than the absorption, allowing much greater contrast from weakly absorbing objects such as soft tissue.

Diffraction enhanced x-ray imaging

Diffraction enhanced imaging[3] is one type of phase contrast imaging and relies upon the very small range of angles over which a perfect crystal reflects x-rays. If the crystal is placed just after the sample as an analyser and rocked through this small angular range (approximately 3 microradians) it can separate out the refraction or phase information from the absorption information in the image. The resulting images have greatly improved contrast.



A **B**

C

Figure 2.17.
Conventional versus x-ray images of a human finger joint.

A: Conventional x-ray image of a human finger recorded on a hospital x-ray set.

B: Absorption x-ray image of a human finger recorded using phase contrast diffraction enhanced imaging on a synchrotron.

C: Refraction x-ray image of a human finger recorded using phase contrast diffraction enhanced imaging on a synchrotron.

Images: R. Lewis, Monash University, obtained using Elettra synchrotron, Italy

Time Dependent Studies

A major advantage of a synchrotron source is that, because of the extremely high beam intensities, it is possible to study processes that are changing with time. These dynamic processes are of enormous interest in both the life sciences and physical sciences, and they represent an area where synchrotron sources are contributing valuable knowledge, unobtainable by other experimental techniques.

1 S.W. Wilkins, T.E. Gureyev, D. Gao, A. Pogany & A.W. Stevenson, 'Phase-contrast imaging using polychromatic hard X-rays', Nature, 384, 1996 335–338.

2 R.A. Lewis, C.J. Hall, A.P. Hufton, S. Evans, R.H. Menk, F. Arfelli, L. Rigon, G. Tomba, D.R. Dance, O. Ellis, A. Evans, E. Jacobs, S.E. Pinder & K.D. Rodgers, 'X-ray refraction effects: application to the imaging of biological tissues', British Journal of Radiology, 76 (2003), 301–308.

3 D. Chapman, W. Thomlinson, R.E. Johnston, D. Washburn, E. Pisano, N. Gmür, Z. Zhong, R. Menk, F. Arfelli & D. Sayers, 'Diffraction enhanced x-ray imaging',. Phys. Med. Biol., 42 (1997) 2015–2025.

Time dependent studies can be done using all of the techniques that have been described. They usually require sample containment chambers that will change an external factor to the sample, for example increase or decrease temperature or pressure, vary the atmosphere surrounding the sample, introduce a chemical or biological reactant or irradiate the sample with a laser.

Some of the beamlines (for example the powder diffraction beamline) will have an environmental chamber as a standard part of their set-up, and others (for example the infrared and the imaging beamlines) will be sufficiently flexible to accommodate special purpose experimental chambers.

Micro-machining by X-ray Lithography

Lithography is the process of making mechanical parts and structures by photographically exposing a light-sensitive material (usually a photo-resist) to create patterns that may be either directly used, or act as shields to allow selective etching of lower layers. Alternatively it can produce moulds to fill with metals, ceramics, polymers, glasses or even bio- and nano-engineered materials. Lithography is the cornerstone of the semiconductor industry where the world's top ten manufacturers have combined sales in excess of US$4b annually.

The semiconductor microchip is a planar device, and the lithography techniques used are essentially two-dimensional. The emerging field of microtechnology, or MEMS, where additional functions such as actuation, sensing or microfluidics are integrated with intelligent microchips, has brought the need to manufacture devices with three-dimensional structure. Techniques used for this to date include excimer laser micromachining, UV lithography, electrodischarge machining and electrochemical machining. However none of these are capable of manufacturing structures with high depth to width aspect ratios.

In 1986 researchers at the German atomic research centre (Forschungzentrum Karlsruhre) announced the development of a deep x-ray lithography technique using synchrotron light.

They called the technique LIGA, a German acronym that stands for lithography, electroplating and replicating by injection moulding or embossing.

LIGA is a major advance; it produces structures with:

- micron sized features
- aspect ratios (depth : width) of more than 100 – this is essential for power transfer in micro-devices
- optically smooth side walls – vital for telecommunication applications
- almost perpendicular walls – made possible by the highly collimated synchrotron x-ray beam.

By way of comparison excimer laser lithography achieves aspect ratios in the region of 4–16, has side wall roughness of around 75 nm R.A. or more, and produces a 7°–12° wall angle.

LIGA was developed using polymethylmethacrylate (PMMA) as the x-ray sensitive material. Pre-cast sheets of PMMA are glued to a previously plated holder and exposed to x-rays. PMMA requires exposure times of about 2–3 hours on the synchrotron. More recently an epoxy-based resist has been used that requires exposure times of the order of 60 seconds. This completely transforms the cost analysis of LIGA and has brought the process into prominence as a competitive mass production process.

Capabilities of the Proposed Beamlines

The proposed initial suite of beamlines has been selected to perform all of the techniques described in this chapter. The capabilities of each beamline are described in table 2.1.

Further information on the beamlines is given in chapters 4 and 10.

Table 2.1. Techniques available using the proposed initial suite of beamlines

Beamline Name	Techniques	Capabilities
Beamline 1 High Throughput Protein Crystallography	Medium energy, multiple wavelength anomalous dispersion XRD	Dedicated facility for crystallography of large protein crystals, set up with robotic loading and centring, and for remote operation
Beamline 2 Protein microcrystal and small molecule diffraction	Medium energy, multiple wavelength anomalous dispersion XRD	Facility with finely focussed x-ray beam for determining the crystal structure and electron density maps of weakly diffracting, hard–to-crystallise proteins, nucleic acids, and for small molecules
Beamline 3 Powder diffraction	Medium to high energy powder XRD	Versatile high resolution powder diffraction facility equipped with sample chambers for a wide range of in-situ experiments
Beamline 4 Small and wide angle x-ray scattering	Medium energy SAXS/WAXS	Measurement of long range order in complex molecules and materials
Beamline 5 X-ray absorption spectroscopy	Medium and high energy XAS, XANES, XAFS and XES	Measurement of short and medium range order, bond lengths, coordination numbers and local coordination geometry, and the oxidation state of atoms from atomic number Z=20 upwards
Beamline 6 Soft x-ray spectroscopy	Low energy XAS, XES, XPS and AES	As above, for the light elements , $Z < 20$. Also for the analysis of surfaces and thin films
Beamline 7 VUV spectroscopy	ARUPS, MXCD	Determination of the electronic structure and surface characteristics of solid, soft matter and gas phase substances
Beamline 8 Infrared spectroscopy	FTIR spectroscopy and IR microspectroscopy	Analysis of bond structures in complex molecules, biological materials, minerals and band structures in certain semiconductors
Beamline 9 Microspectroscopy	XAS, XANES, XAFS and XES at a submicron scale	For producing high resolution maps of elemental distribution in a sample. Also for determination of the oxidation state and coordination geometry of atoms in particles down to sub-micron size.
Beamline 10 Imaging and medical therapy	Phase contrast enhanced high energy x-ray imaging	Very flexible beamline for research into high contrast imaging of objects from small animals through to engineering components. For research into the physics and biophysics of cancer therapy techniques
Beamline 11 Microdiffraction and fluorescence probe	Simultaneous medium energy micro-XRD and fluorescence	Fast mapping of micro-XRD and fluorescence information. Especially intended for the minerals industry, environmental sciences, and manufacturing investigations
Beamline 12 Circular dichroism	VUV CD	Determination of the 'secondary' structure of biological molecules, e.g. protein folding
Beamline 13 Lithography	LIGA	Production of micro-components with very high depth to width ratio and excellent surface finish