X-ray scattering is well established for probing the structure of matter at the atomic level in the materials sciences. In particular, coherent X-ray scattering (X-ray diffraction) has developed to become an indispensable tool for crystal structure determination. The level of technological advancement is such that, coupled with the high fluxes offered by synchrotron sources, a high degree of automation in the data collection process is possible. In the biomedical sciences, X-ray diffraction techniques are now essential to the characterisation of macromolecules in growth areas such as structural genomics. The demand for such structural characterisation techniques across all areas of contemporary science is amply demonstrated by the fact that of the 13 beamlines envisaged for the first stage of the Australian Synchrotron, 5 will be dedicated to various diffraction techniques.

In contrast, X-ray scattering is usually regarded as a problem for radiographic imaging, where it is considered to be a major contributor to reduced image quality. The familiar X-radiograph relies on the differential absorption of X-rays by materials of varying densities, and considerable effort is directed towards minimising X-ray scatter during image acquisition and the subsequent correction of its effects. However, it is readily apparent to diffraction specialists that this scattered radiation must contain considerable information about the sample. It is only relatively recently that the increased availability of synchrotron sources for imaging applications has permitted these apparently disparate approaches to X-ray scattering to be united. These techniques are able to utilise scattered radiation to provide novel imaging methods that are able to substantially increase image contrast or provide material specific analysis.

**X-ray Diffraction**

Macromolecular crystallography – the elucidation of the atomic structure of a single crystal – relies on the constructive interference between X-rays scattered from the regular arrangement of atoms in the crystal. Rotation of the crystal in the X-ray beam allows the formation of a complete and characteristic diffraction pattern, and hence solution of the atomic structure and mapping of the electron density. The difficulty of crystallising proteins and viruses has especially driven demand for synchrotron based X-ray diffraction. These crystals are generally not only small, but also tend to be intrinsically weakly diffracting. The high brightness, collimation, tunability and pulsed time structure of synchrotron sources permits not only better quality data acquisition, and hence increased precision and accuracy, but also allows studies that are either not possible with laboratory sources.

Time-resolved crystallography exploits these unique characteristics to study dynamic or transient species. Rather than attempting to chemically or physically stabilise intermediates over increased lifetimes, the pulsed time structure of the synchrotron storage ring and extremely brilliant source is used to provide very short exposure times with synchronised laser stimulation to initiate reactions (the 'pump-probe' technique) (1). This method has been used to examine the photodissociation and rebinding of the carbon monoxy myoglobin with picosecond X-ray pulses (2).

**Non-Crystalline X-ray Diffraction**

Many materials of biological interest are unable to be crystallised sufficiently for crystallographic study. However at larger length scales, where features of interest are in the 0.1 to 500 nanometer range, X-rays scattered at small angles to the incident beam direction can yield useful structural information. This technique is known as Small Angle X-ray Scattering (SAXS). At length scales intermediate between these and the atomic resolution probed by crystallography is the regime of Wide Angle X-ray Scattering (WAXS). SAXS and WAXS are important techniques in the study of polymers, colloids and emulsions with widespread synchrotron applications. They are particularly useful for the study of biological or even living organisms since samples can be maintained in wet environments, or in dilute solution for the study of dynamic conformational processes.

These characteristics make SAXS ideal for the study of fibrous tissues of the body. Both collagens and muscle fibres, for example, have long been studied using SAXS methods (see (3) and (4) for reviews). However, the long exposures required on conventional sources limited functional studies in areas of physiological interest such as muscle contraction or tendon deformation, or were impractical when tissues contained smaller amounts of less ordered fibres. With the increased flux available at synchrotron sources, time-resolved studies have become possible, yielding detailed information on the structural changes underlying macroscopic processes. Recent work has demonstrated sub-microsecond time resolution in studies of skeletal muscle contraction (5). The SAXS patterns indicate complex early changes in the relative configurations of the actin and myosin filaments as tension is developed in the muscle following an electrical stimulus (Fig. 1).
An illustrative and impressive example of the potential of synchrotron based SAXS is given by Pearson et al.'s in situ measurements of cross-bridge dynamics in cardiac muscle (6). The complex arrangement of muscle fibres in the heart generally result in poorer quality scattering patterns from cardiac muscles compared to those from skeletal muscles, since the sample is effectively less well orientated when the scattering is averaged over the increased sample thickness. By exposing the left ventricular wall of spontaneously beating rat hearts to the incident X-ray beam, it was observed that filament dynamics (the mass transfer of myosin heads to actin) preceded changes in the lattice spacing of myosin filaments during systole in non ischemic regions of the heart. These changes were not observed in ischemic regions. This in situ, real time analysis was made possible by the use of a beam line optimised for the highest possible flux at the SPring-8 third-generation synchrotron radiation source in Japan.

Our group at the Monash Centre for Synchrotron Science and others are working towards a novel application of SAXS that explores the potential of SAXS data as a marker for breast cancer and other malignancies (7,8). Preliminary results suggest that breast malignancies can consistently be distinguished from benign lesions and normal tissues by examining alterations in the collagen fibril structure and organisation. This is believed to arise from the disruption of the tissue structures with the invasion of cancerous cells. Significantly, changes were observed in the scattering patterns of tissues, considered healthy by the pathologist, even when the tissues were taken at significant distances (several centimeters) from the malignancy site (7). This finding has interesting implications for both diagnostic methods and therapeutic interventions in the treatment of cancers.

Coherent Scatter Imaging

In conventional X-ray imaging, X-ray scattering is considered to be an artifact of the imaging process that should be minimised or removed if at all possible to maximise the image quality. 'Conventional' in this context is taken to mean those images that are formed principally by the differing attenuation of X-rays by materials of different densities. This is the basis of diagnostic radiography as well as computed tomography (CT), in which multiple images from many different directions are used to reconstruct three dimensional maps of X-ray attenuation. The obvious clinical usefulness of these methods is dependent upon the highly penetrating and non-destructive nature of X-rays. The number of scattered X-rays that reach the detector is commonly minimised by mechanical means in which slits or grids simply block any X-rays that do not follow a direct path from source to detector.

Yet as suggested by the discussion of diffraction materials characterisation methods above, the fraction of incident radiation that is coherently scattered is both significant and contains valuable information. This is particularly true for the relatively low energies used to image the soft tissues such as the breast. This scattered radiation can be used to provide chemical or crystallinity mapping throughout the sample in a method known as coherent scatter imaging or diffraction tomography (9). This method is more discriminating than conventional CT which is ambiguous when materials of similar density have differing chemical compositions, and hence is particularly useful when examining biological materials (10).

To measure the coherent scatter signal from the sample, the detector is moved to a characteristic scattering angle at some angle away from the conventional placement in the path of the transmitted X-ray beam. The scattering...
intensity is measured for many orientations of the object as for conventional CT, and this data can then be used to create tomographic reconstructions which map the distribution of the scattering material. Although conventional polychromatic sources have been used for diffraction tomography (11), the technique is considerably improved in both quality and acquisition times by the use of monochromatic high-flux synchrotron sources. The chemical sensitivity of the method has found application in fields as diverse as bone mineral content to explosives detection (12).

A more sensitive method of diffraction tomography uses a narrow beam and an analyser crystal to select the scattering angle (13). The analyser crystal ‘reflects’, or more correctly diffracts, only those X-rays which fall within a narrow band of incident angles. Although the use of a narrow beam and analyser crystal demands higher incident flux, it has the advantages of higher chemical specificity and improved mapping accuracy, since only coherent scattering arising directly from the object is detected, whilst other events such as incoherent or multiple scattering are rejected. An example of the excellent discrimination of the technique is given in Fig. 2, which shows reconstructed diffraction images of a polymer phantom.

A slightly different approach to diffraction imaging is being undertaken at the Daresbury Synchrotron Radiation Source in the UK. This approach combines the experimental arrangement for the SAXS study of breast cancer (7) with an imaging detector at the centre of the SAXS detector where the beam stop would otherwise be located (14). A transmission image is recorded simultaneously with the scattering image as the sample is stepped through the beam. In this way a complete transmission image is built up and deconvolution techniques can be used to create diffraction images as a function of the scattering length. These diffraction images are able to clearly distinguish tissue types, for example between skin, fat and duct tissues, based upon their respective collagen content.

**Scatter Enhanced Imaging: Phase and Refraction Contrast**

Whilst diffraction tomography directly measures X-ray scattering in order to reconstruct a chemically specific scattering map, several other imaging methods use X-ray scatter to actively enhance image contrast. These are known collectively as phase contrast imaging, since it is changes in the phase of the X-ray wave as it passes through composite materials that give rise to the scattering or refraction of X-rays from their incident direction. In a process analogous to hologram formation, in-line phase contrast imaging exploits the radiation scattered in the forward direction (15,16). This scattered radiation interferes with the transmitted X-rays, and if sufficient propagation distance is allowed between the sample and the detector, a fringe pattern results at the interfaces between different materials. For edge-enhancing interference to occur, the technique requires that the X-ray source size be small, a demand which is easily achievable at synchrotron sources.

Another means of exploiting X-ray refraction effects is known as Analyser Based Imaging (ABI) (17-19). The reflectivity of the analyser crystal not only has a narrow angular acceptance range of the order of only a few microradians, but generally has an approximately Gaussian shape as a function of incident angle of the X-rays to the crystal surface. This reflectivity profile means that ABI is extremely sensitive to phase changes. X-rays scattered by the sample that fall outside this range are not reflected, providing a means of scatter rejection. However, scattered X-rays that fall within this range will be reflected with a probability dependent on the

![Fig. 2. An example of coherent scatter tomography reconstructions of a phantom composed of a nylon cylinder, with smaller cylindrical inserts of lexan (top left), perspex (top right) and air (bottom).](image)

A conventional transmission computed tomograph image mapping the differing attenuation of the composite materials of the phantom is shown in (a). The relatively low contrast between the three materials is compared with the diffraction images reconstructed using the scattered radiation characteristic of nylon (b) and lexan (c). Images kindly supplied by Dr Cameron Kewish, School of Physics and Materials Engineering, Monash University.
reflectivity curve. By tuning the analyser crystal to an appropriate angle, the degree of refraction contrast that contributes to the final image (consisting of both absorption and refraction effects) can be controlled. In the case of a Bragg analyser crystal, a simple algorithm can then be used to separate these two effects for quantitative analysis (19). In common with in-line imaging, ABI has the effect of enhancing edges in the acquired images.

The potential for imaging biological samples is illustrated in Fig. 3, which shows a mouse thorax imaged using a conventional set-up, the in-line phase contrast technique and the DEI method. The in-line and DEI images show the lungs, nearly invisible in the conventional image, with startling clarity since the air-tissue interface provides considerable scattering effects.

Summary

Synchrotron based X-ray scattering techniques are finding wide application in the biomedical sciences. The extreme brightness of these sources means that well established coherent scattering techniques such as X-ray crystalline diffraction and Small Angle X-ray Scattering are now able to yield real-time analysis of dynamic biological processes. Additionally, the monochromaticity and highly collimated nature of these sources has permitted the implementation of exciting imaging techniques particularly suited to the study of tissues such as coherent scatter imaging and phase contrast imaging.

References


Fig. 3. Conventional (left), in-line phase contrast (centre), and analyser-based (right) images of a mouse thorax.

The conventional image was acquired on a Faxitron unit, whilst the phase contrast images were acquired at SPring-8, Japan. Images kindly supplied by Prof. Rob Lewis and Mr Marcus Kitchen, School of Physics and Materials Engineering, Monash University.