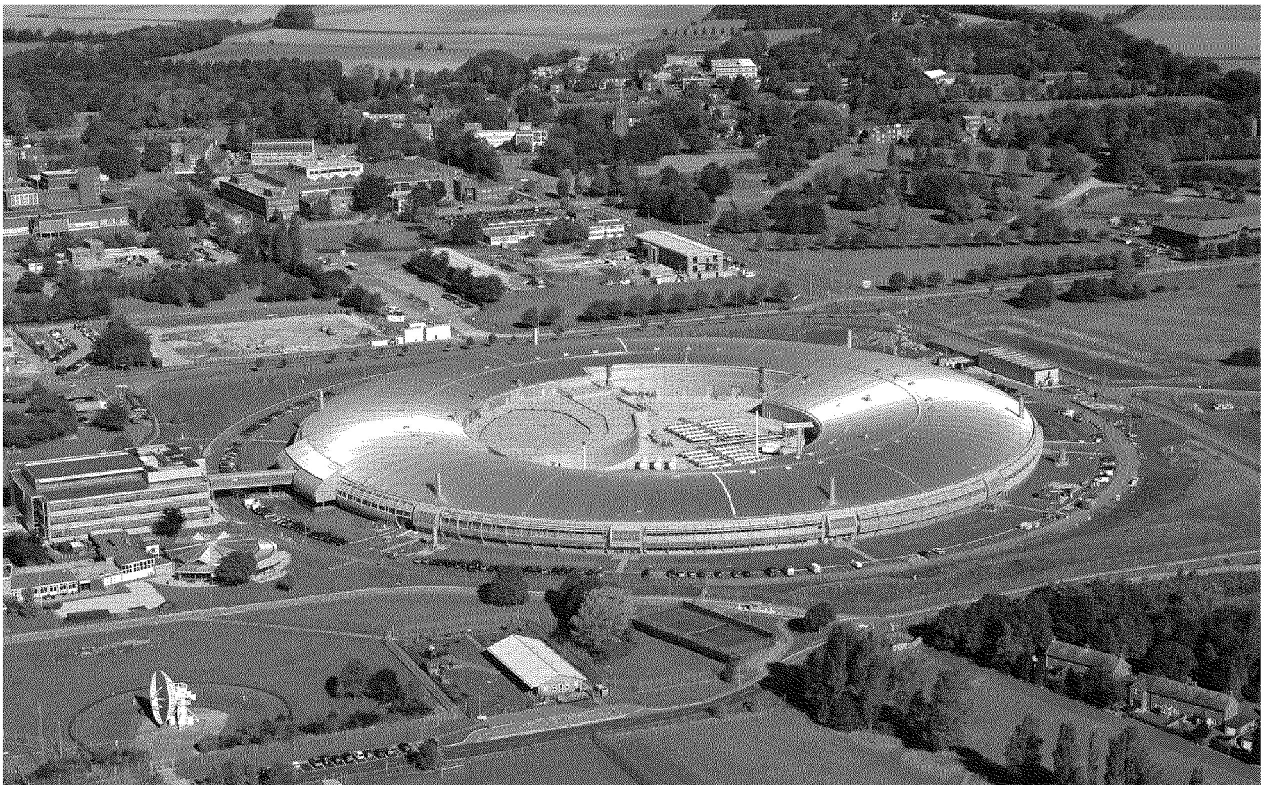
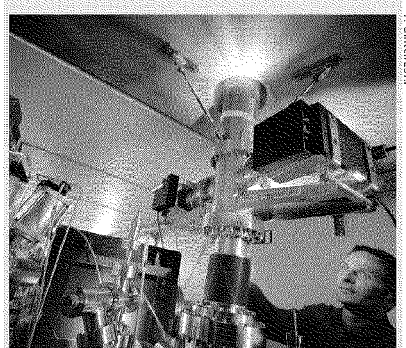
Synchrotron science



Diamond light source: the largest UK funded scientific facility built for 40 years, based in Oxfordshire, UK

In Brief

- Synchrotron light provides high spatial and temporal resolution
- There are roughly 70 synchrotron facilities worldwide
- Structural characterisation using X-rays accounts for much of the research
- Crystals with dimensions of a few cubic microns are routinely measured



P. Ginter/ESRF

IR light extraction: from the ESRF storage ring

Shining examples

Brilliant synchrotron radiation is shedding new light on some thorny chemical problems, writes Elizabeth Shotton

In 1997, Sir John Walker was awarded the Nobel prize for chemistry for his work to elucidate the synthesis of the body's energy storage molecule adenosine triphosphate (ATP). Fundamental to this work was the determination of the structure of the F_1ATP-ase enzyme, carried out at the Synchrotron Radiation Source at Daresbury Laboratory in the UK. This was followed in 2006 with a second Nobel prize in chemistry awarded to Roger D. Kornberg, for determining how DNA's genetic blueprint is read and used to direct the process of protein manufacture. Kornberg carried out a significant part of this research at the Stanford Synchrotron Radiation Laboratory in California, US, again using macromolecular crystallography.

As these examples illustrate, synchrotron light is ideal for analytical problems that require high spatial or temporal resolution, or problems that are simply intractable using conventional instruments.

Globally, there are around 70 synchrotron science facilities, all offering research opportunities across the disciplines. While large science facilities such as Diamond Light Source have traditionally been used by universities and higher education institutions for pure R&D, access for industrial users is increasing. Clarity on IP ownership and the introduction of service-mode access where synchrotron-based scientists do the experiments on behalf of industry have been the major factors increasing industrial usage. While the pharmaceutical sector has been the early adopter of synchrotron technology, the potential for the chemical industry is only starting to be realised. Already, roughly 25% of UK synchrotron research is chemistry related.

A synchrotron accelerator produces infrared, ultraviolet and X-ray beams of exceptional quality and brightness to scientific end-stations, referred to as beamlines. These beams are of high intensity, extremely well collimated and tuneable. This allows researchers to collect data substantially faster, on smaller samples and using different wavelengths than they can in their home laboratories.

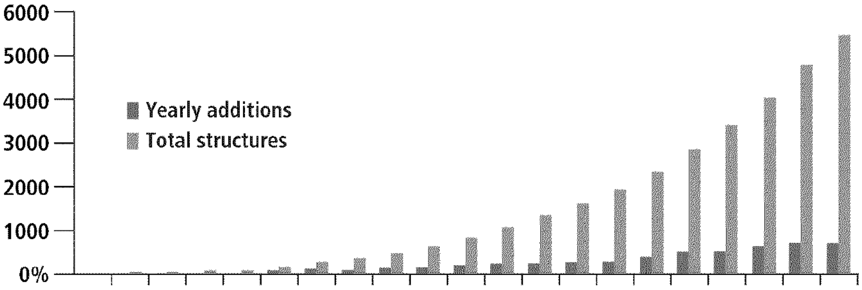


Figure 1: The rise in the number of structures in the PDB archive

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Structural characterisation using X-rays forms a major proportion of the research done at synchrotrons, with applications ranging from protein crystallography through small molecule crystallography to high resolution powder diffraction. Figure 1 shows the increase in submissions of structures to the Protein Data Bank (PDB) over the last 20 years, primarily due to advances in synchrotron facilities. Small molecule crystallography is the gold standard technique for determining crystal structure but unfortunately, not all samples co-operate during crystallisation and chemists are left with crystals that are too small to be studied using standard X-ray diffractometers. In addition, with samples that do diffract well, the benefits of the synchrotron are significantly higher resolution and very fast data collection.

Because of the higher intensity of the X-rays, smaller crystals can be used for structural studies, with samples of a few cubic microns routinely measured. Moreover, time-resolved crystallography – involving laser excitation – can be used for the determination of excited-state structures with very short lifetimes. Additional facilities allow samples to be studied at high or low temperatures, high pressure and under controlled humidity conditions.

The solution of more complex structures from powder diffraction data is a newer technique. While it is possible to solve structures using standard laboratory based diffractometers, the low symmetry of many organic materials such as pigments and drug candidates can make analysis of the patterns troublesome due to severe peak overlap. The high resolution data obtainable with synchrotron light can usually overcome this problem. With the incorporation of robotic sample changers on beamlines, along with state-of-the-art detector systems, high quality diffraction patterns can be collected in a fraction of the time that it would take using a standard laboratory diffractometer.

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Imaging technique for breast cancer

Scientists using Elettra, the Italian synchrotron, have developed a mammography method that supplies high quality diagnosis images, while at the same time reducing the delivered radiation doses. The clinical trial involved over 50 women for whom conventional techniques gave uncertain diagnosis. For these women, the only alternatives would be a biopsy, or a follow up examination after six months.

So what sets this novel mammographic approach apart from the traditional method performed in hospitals? Both use X-rays but those generated by the synchrotron are tuneable and their high spatial coherence permits new techniques such as the 'phase contrast' radiography, resulting in improved image quality and radiation doses up to 50% lower.

Breast cancer is the most common form of cancer in women, and it is the fifth most common cause of cancer death. Mammography is the main diagnosis tool for breast cancer and screening programmes are carried out routinely on large numbers of women.

Conventional X-ray radiology is based on the absorption properties of the sample and the image contrast is generated by variations in the density, composition or thickness of the sample. One of the main drawbacks of this technique applied to mammography is the poor contrast in the breast tissues. 'Phase-contrast radiography is, however, based on the observation of interference phenomena between diffracted and undiffracted radiation waves passing through the tissue, explains Elisa Quai, medical physicist at the Elettra Laboratory. 'These variations are usually more relevant at the edges of structures within the sample itself resulting in an "edge enhancement" effect in the images. Therefore the technique is very appropriate to detect and characterise nodules and lesions with low contrast.'

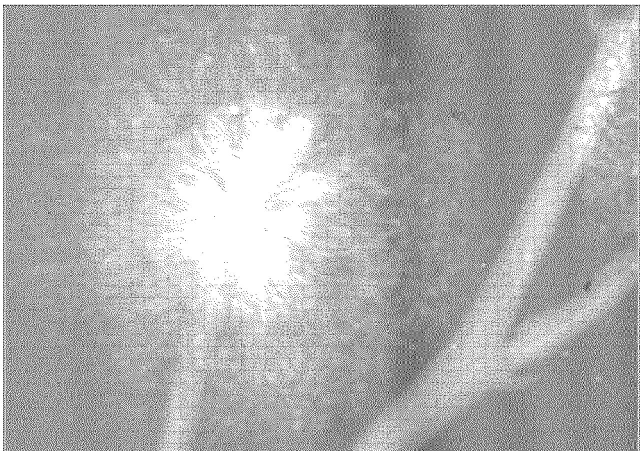
UV light studies at Diamond

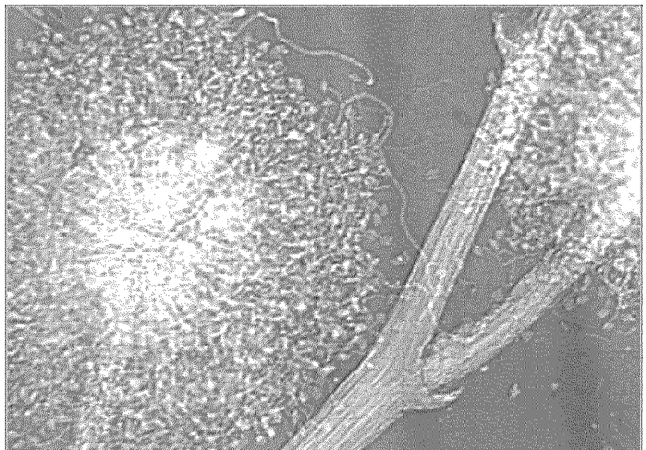
The UK's Diamond Light Source introduced its 13th beamline (B23) last year, and it comes with a difference: UV light, perfect for such studies as determining the structure of proteins in solution. Designed for experiments requiring circular dichroism (CD), a technique that allows the study of biological structures that do not readily form crystals, B23's UV light will permit the investigation of structural and dynamic interactions of molecules such as proteins, nucleic acids and small chiral molecules.

B23's first users are a team of scientists from the University of Reading carrying out research into the cause of neurodegenerative diseases such as Alzheimer's and CJD. One of the main contributing causes of these diseases is thought to be the formation of hard, insoluble plaques between brain neurons. In a healthy brain, protein fragments including the so-called amyloid beta protein are broken down and eliminated but in Alzheimer's disease and CJD, these structural protein fragments (fibrils) accumulate to form troublesome amyloid plaques.

Led by Ian Hamley, a joint appointment professor at Diamond, the Reading team has synthesised samples of short peptides based on the human sequence, modifying their design with the aim of enabling them to bind to the fibrils in the brain and disrupt the formation of plaques. The team used the beamline to investigate the structure of the short peptides – an essential step in their research; without knowing the structure of the synthesised protein fragments, the group cannot determine how they function.

'It was excellent to be the first team to use Diamond's circular dichroism beamline. We were able to do everything we needed to do and collected some good results,' Hamley says.





Elisa Quai/Elettra

Breast cancer imaging: by conventional radiology (left) and phase contrast (right)

Whilst powder diffraction is important for investigating long range order in systems such as pigments, other chemists are more interested in studying local order during product development. Understanding the near neighbour environment of active sites in catalytic systems is important and X-ray absorption spectroscopy is routinely used to study systems such as zeolites and electrode surfaces. In 2007 Diamond Light Source, the UK synchrotron facility in Oxfordshire, opened a microfocus X-ray absorption spectroscopy beamline that extends this technique to allow chemical mapping of samples followed by detailed investigation of areas of interest to get oxidation state and local environment information.

Using this beamline allows researchers to map every element in the periodic table above phosphorous. Samples studied in the last 12 months, for example, include artificial hips, pigments, pieces of the *Mary Rose* and model corrosion systems.

Synchrotron infrared microspectroscopy is emerging as a powerful analytical tool, with the high spatial resolution and significantly improved signal-to-noise ratios achievable over conventional systems being exploited. Applications have included chemical mapping of hair cross-sections to evaluate the effects of hair care products, forensic science and oil distribution in fried foods.

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Elizabeth Shotton is industrial liaison manager at Diamond Light Source in Didcot, Oxfordshire, UK.

From synchrotron light to the pharmacy

Research by drug companies accounts for roughly a quarter of user activity at the European Synchrotron Radiation Facility's macromolecular crystallography beamlines. Sanofi-Aventis, for example, is currently carrying out clinical trials for a new medicine for type 2 diabetes, designed with the help of X-ray crystallographic structures using data collected at the ESRF.

The new drug is an 'agonist' of the nuclear receptor protein PPAR delta, which increases its activity and is expected to have a beneficial effect in the treatment of type 2 diabetes.

To optimise the design of the drug, researchers crystallised the ligand-binding domain of PPAR delta with a series of agonist molecules. X-ray diffraction data collected at the ESRF allowed them to understand the structural basis of interactions between PPAR delta and the agonist molecules, and showed that the agonist-binding pocket of the protein is large and exhibits plasticity. This information provided the key for the team to develop novel agonists with improved properties.

Catalytic converter research

Researchers from University College London (UCL) interested in analysing heat-treated nano-ceramics for better automotive catalyst support materials have been harnessing the powder diffraction capabilities of Diamond.

The group has produced a large number of nano-powder samples, each with a slightly different composition, using a novel high throughput synthesis method followed by heat-treatment. Analysis of these samples allowed them to establish stable compositions that may be of use as supports in catalytic converters – by effectively simulating the conditions that a car would experience after 100,000 miles on the road.

Due to the large number of samples the group can produce in a day, the faster they can gain high quality X-ray powder diffraction data, the better.

The group successfully ran 66 nano-powder samples on the robot, which would have taken two to three months to do on a laboratory instrument. After commissioning and further fine tuning, the robot on the powder diffraction beamline will be able to run up to 200 samples in one to two days.

Commenting on the efficiency of Diamond's powder diffraction beamline, principal investigator, Jeremy Karl Cockcroft, said: 'We were delighted with the performance of the high throughput system on I11. Our experiments went very well and were successful.'