HEXITEC

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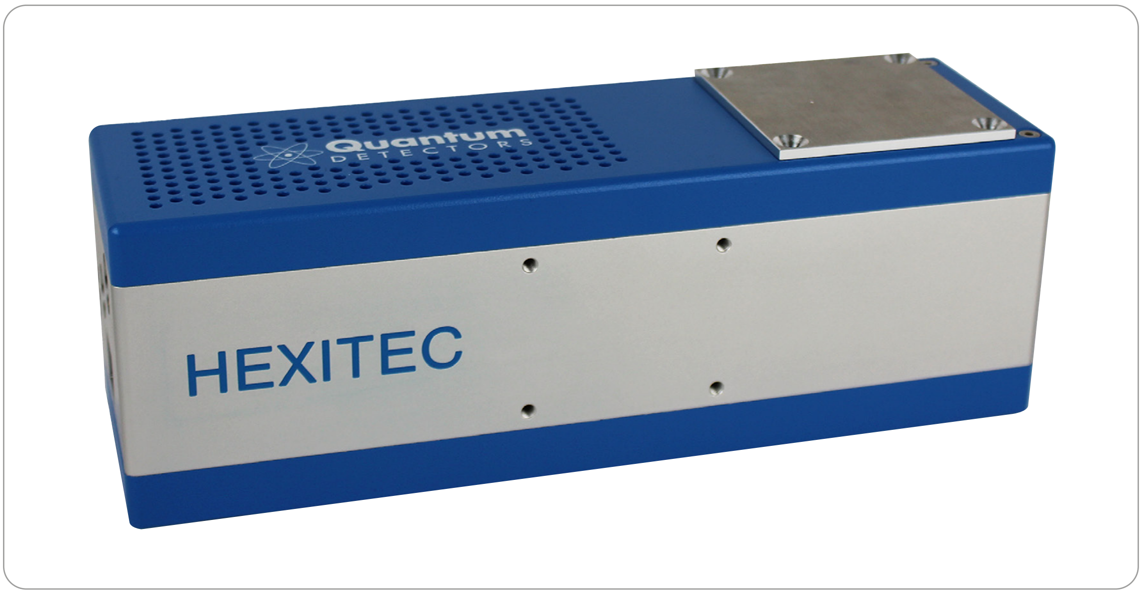
Spectral Small Angle X-ray Scattering (sSAXS) Application Note



High-energy hyperspectral pixelated area detector

HEXITEC is a hyper-spectral high-energy x-ray area detector. Its 4 cm^2 CdTe sensor measures the energy and position of every incident photon in the 4-200 keV range. Each one of its 6400 (80x80) pixels provides a full energy spectrum with an average FWHM resolution of 800 eV per pixel (~1 keV aggregate) at 60 keV.

Here, we present the application of HEXITEC to the Small Angle X-ray Scattering (SAXS) technique, in a lab setting. The hyper-spectral capability of HEXITEC is utilised to make use of the wide range of the X-ray energies produced by a lab-based source. The increase in flux, compared to a monochromatic source, allows the use of the SAXS technique in applications where previously it would have been impractical. In the research highlighted here, HEXITEC is used to successfully detect the presence of β-amyloid (βA) plaques in mice models, potentially facilitating the diagnosis and early treatment of neurodegenerative conditions such as Alzheimer's disease.

Small Angle X-ray Scattering (SAXS)

The scattering of light by the electrons of a material can be used to derive atomic, molecular and nanometer-scale structural information [1]. Photons incident upon a sample elastically interact with the sample and are deflected, or scattered, from their initial path in all directions. If a detector is placed to collect the scattered photons from a single atom, a circularly symmetric pattern of photon intensity is formed (Fig 1.a). The scattering from two closely spaced atoms results in a pattern of light and dark areas (Fig 1.b). The path difference, P, between the scattered light from each of the atoms produces wholly constructive interference where the difference is an integer multiple of the wavelength, λ, (Fig 2) such that,

$$P = n\lambda \qquad (1)$$

In the case where the distance to the screen is large compared to the separation of the atoms, the path difference can be written as $P = d \sin\theta$, and we arrive at an expression for the spacing of the atoms,

$$\delta = n\lambda/\sin\theta \qquad (2)$$

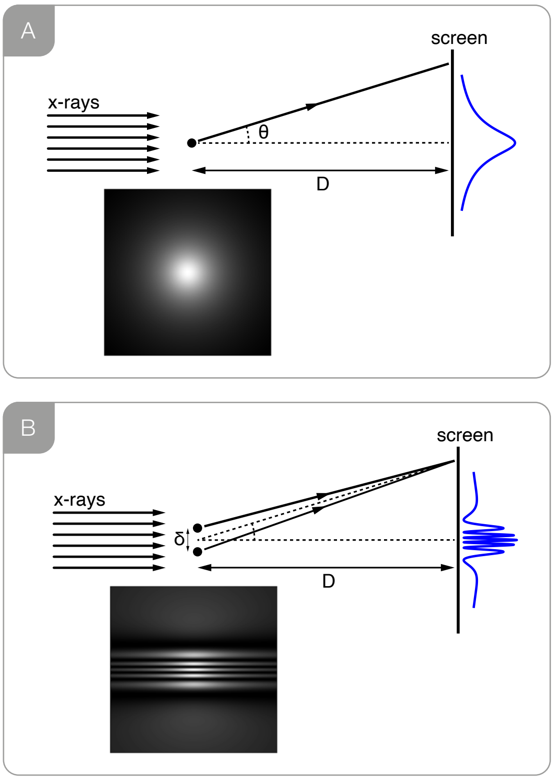


Fig 1. The principles behind SAXS. (a) A single atom creates a circularly symmetric scattering pattern. The X-ray intensity seen on the screen, in the plane of the page, is shown in blue. Below this is a representation of what would be observed on an 2D area detector. (b) The patterns from two atoms interfere due to a path (and thus phase) difference in the X-rays from each atom. In a biological sample the atoms are randomly oriented and so create a circularly symmetric pattern, rather than the asymmetric pattern seen here.

Thus, armed with a monochromatic X-ray source and a suitably large detector, one can accurately derive the spacing of constituent atoms in a sample by measuring the angle at which the bright bands occur.

Small-angle X-ray scattering relies on this principle but concerns itself with the information contained in the patterns seen at smaller angles. At small angles $\sin\theta \approx \theta$, which gives us,

$$\delta \approx n\lambda/\theta \qquad [3]$$

By inspecting eqn. 3 one can see that θ is inversely proportional to δ. Eqn. 3 broadly indicates that information on larger scale features are present at smaller angles. Consequently, small angle scattering is used to derive, or detect, molecular and nanometer-scale structural information.

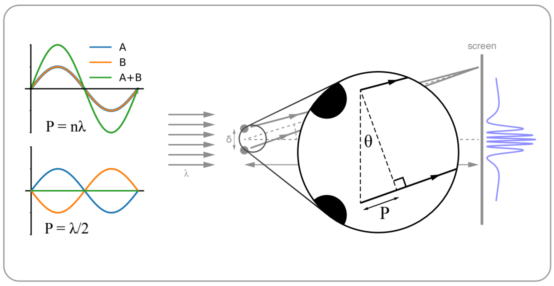


Fig 2. The path difference, P, dictates the angles at which maxima occur in the pattern of interference created by two closely spaced atoms. When the path difference is a whole multiple, n, of the wavelength, constructive interference occurs, leading to bright fringes on a screen. The position of which can be used to calculate the spacing of the atoms.

The SAXS technique does not rely on highly ordered systems (crystals) to be effective which has made it useful in the study of biological systems [2,3]. In a typical experiment, a highly collimated beam of monochromatic X-rays is transmitted through a sample, typically of millimetre thickness. The scattered X-rays are collected on a 2-dimensional area detector and a variety of computational techniques are then applied to the patterns of intensity recorded on the detector to derive information on the structure of the sample, or confirm that a particular species is present in the sample.

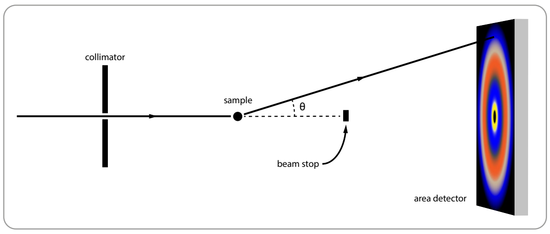


Fig 3. A typical SAXS experimental setup. A narrowly collimated x-ray beam is aimed at the sample, creating a circularly symmetric intensity pattern on the detector, placed behind.

Spectral Small Angle X-ray Scattering (sSAXS)

Spectral Small Angle X-ray Scattering (sSAXS) is an extension to the standard SAXS technique that dispenses with the requirement for a monochromatic source of X-rays, employing a wide range of X-ray energies from a polychromatic source. The sSAXS technique is particularly useful for lab-based X-ray sources, which are limited in the flux of X-rays they can produce. Using a wider band of energies results in higher fluxes and enables techniques that would otherwise have not been practical.

If we look back to equation 3, we can see that δ, the atom spacing, is proportional to λ, the wavelength of the X-ray, which itself is inversely proportional to the X-ray energy. When using a monochromatic source this wavelength is known, but if one wishes to use a polychromatic source, with a wide band of energies, then one must measure the energy of each event. It is here where the unique utility of HEXITEC becomes clear. Unlike other area detectors, HEXITEC measures the exact energy of each photon and thus allows one to use a wide band of x-ray energies. This would not be possible with standard area detectors which typically measure the position of events above a specified energy threshold.

Breedlove et al. in the group of Aldo Badano developed this technique [4,5], applying it to the detection of amyloid plaques, which are suspected to be causative agents in the development of Alzheimer's disease. Fig 4, below, taken from [4] shows the intensity pattern from (a) a PMMA phantom containing β-amyloid protein aggregate model and (b) a PMMA phantom with no model. Subtracting these patterns from each other results in (c) the recovered signal from the amyloid model alone. The results from this study clearly showed that it is possible to detect the presence of amyloid plaques in relatively thick samples.

In a related study [5], the group experimentally demonstrated the capability for identifying the presence of amyloids in excised mouse heads with an energy range from 30 to 45 keV. The hope is to extend this technique to enable the detection of these proteins in humans to aid the early prediction of Alzheimer's, permitting prompt treatment and improving patients' prognosis.

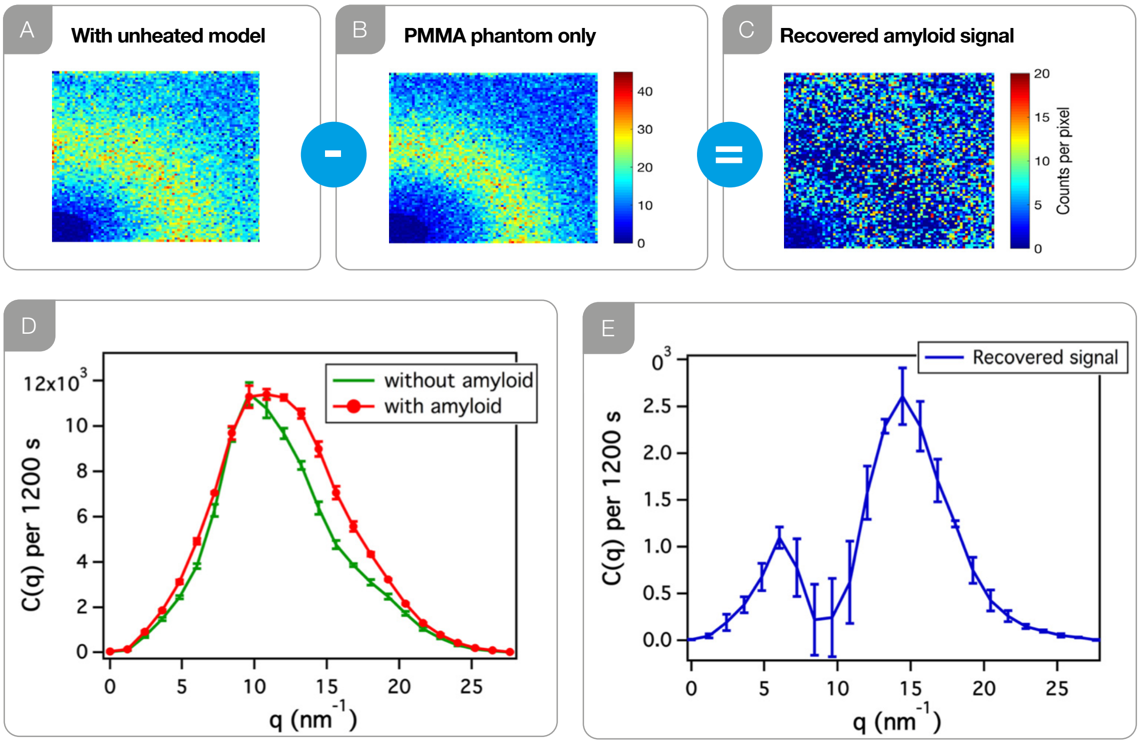


Fig 4. Characterization of the unheated ßA model in a cylindrical PMMA phantom. **(a)-(c)** 2D detector data before and after background subtraction. **(d)** Scattering signal from the PMMA phantom with and without the unheated ßA model. **(e)** Recovered scattering peaks associated with amyloids after background subtraction. Error bar represents standard deviations from n = 3 measurements [4].

Conclusion

HEXITEC is a hyper-spectral area detector which measures the energy and position of every incident photon in the 4-200 keV range. The sensitivity and hyper-spectral imaging features have been exploited to develop an extension to the SAXS technique, sSAXS, which makes better use of a polychromatic lab source. The subsequent increase in effective flux available has allowed the use of the SAXS technique where otherwise it would not have been practical.

Acknowledgements

Fig 3. was reproduced under the Creative Commons 4.0 licence from [4] Breedlove et al., Small-angle X-ray scattering characterization of a β-amyloid model in phantoms. Minor changes were made to match the style of this document.

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