Conversion of the oldest XAFS station at the first dedicated SR source to a state-of-the-art XAFS facility

Kan-Cheung Cheung,[a,b] Richard Strange,[b] Ian Harvey,[a,b] Barry Dobson,[b] Gareth Derbyshire,[c] Jim Kay,[b] Norman Binsted,[b] Roger Linford[a] and Samar Hasnain[a,b]

[a]*Faculty of Applied Science, De Montfort University, Leicester, LE1 9BH, UK.*
[b]*CCLRC Daresbury Laboratory, Warrington, Cheshire, WA4 4AD, UK.*
[c]*CCLRC Rutherford Appleton Laboratory, Didcot, Oxfordshire, OX11 0QX, UK.*

Station 7.1 was the first XAFS station to operate at the Synchrotron Radiation Source (SRS) starting in 1981. A major upgrade of 7.1 has been taking place aimed towards a multi-dimensional XAFS and single crystal facility for 3d elements (Ca to Zn). The optical upgrade included a collimating mirror and QuEXAFS monochromator, also a highly compact and rapid count-rate multi-element solid state detector has been designed, built and recently commissioned. A stopped-flow system is commissioned and a diffractometer stage is planned. The necessary analytical tools for 3D XAFS refinement using crystallographic data as the starting 3D model have been developed for solution XAFS.

Keywords: XAFS; EXAFS; QuEXAFS; monochromator; optics; stopped-flow; instrumentation.

1. Introduction/Historical Notes

The first experimental station to begin user operation at the Daresbury Synchrotron Radiation Source (SRS) was the XAFS station 7.1; the first scientific publication from this dedicated SR source came from work carried out on 7.1 which was reported in Nature in 1981 (Greaves *et al.*, 1981). The first fluorescence detection system at the SRS using scintillation counters was established on 7.1 in 1983 (Hasnain *et al.*, 1984) and the first biological XAFS studies using them was reported in 1983 for the enzyme superoxide dismutase (Blackburn *et al.*, 1984), producing results that have stood the test of time (Murphy *et al.*, 1997).

The station became "unsupported" in June 1990, resulting in nominal resources not even sufficient for maintaining the aging equipment let alone any development. A five yearly review of the SR science and funding in the UK recommended that wherever possible the Laboratory should form partnership with Higher Education Institutions (HEI), namely universities, in order to achieve better integration of SR technology in higher education (Munro, 1997). De Montfort University, Leicester, UK, entered into an agreement with the Daresbury Laboratory in 1993 with the aim of extending and improving the XAFS capabilities on Station 7.1. This paper reports on the upgrade achieved so far.

2. Optics: The optical elements (Fig. 1) have been designed to provide both high intensity and high energy resolution over a small sample area. The design is unique to the SRS, incorporating the focusing optics and energy range of the XAFS Station 8.1 (Van der Hoek et al., 1986) on the dipole and the QuEXAFS monochromator of XAFS Stations on the wigglers: 9.2 (Murphy *et al.*, 1995), 9.3 and 16.5.
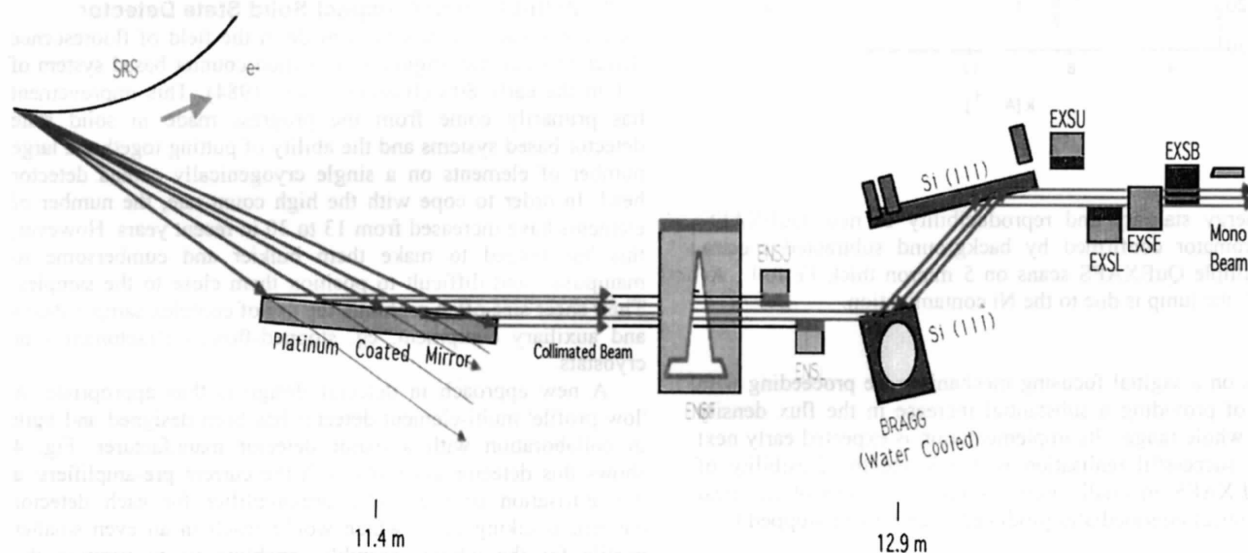

Figure 1
Optical layout showing the flat platinum coated pre-monochromator mirror operated in collimating mode on SRS Station 7.1. The double crystal QuEXAFS monochromator is water cooled. The sample is located at 16.8 m. ENS=Entrance Slit. EXS=Exit Slit. B=Back, F=Front, L=Lower and U=Upper.

2.1 QuEXAFS Monochromator

A rapid scanning double-crystal QuEXAFS monochromator has been implemented establishing station 7.1 as the only rapid scanning XAFS station on a dipole at the SRS. The monochromator, using Si (111) crystals, accepts 3.3 mrad of X-ray beam and has water cooling on the first crystal to minimize temperature effects. A water flow of 270 litres/hr is sufficient for maintaining a stable temperature across the diffracting surface of the crystal. The water channel is directly cut in the crystal as a single cylindrical bore. The second crystal is 100 mm long and is kinemetically mounted on three piezoelectric "Inchworm" drives (Burleigh Instruments) which control the alignment of the second crystal with respect to the first crystal: parallel for maximum flux and slightly offset for obtaining harmonic rejection. The smallest step increment of 2 nm on the Inchworm drives gives an angular accuracy of 0.02 µrad. The stability of the Inchworm drives along with the rest of the monochromator assembly mean that it can be operated without active servoing of the second crystal position. Due to the mechanical stability of the system, high quality data can be collected over an extended range (k=14 Å^{-1}) very quickly (30 s for a metal foil) making QuEXAFS mode highly practical. The stability of the monochromator is shown by recording one hundred successive QuEXAFS scans of an iron foil (Fig. 2).
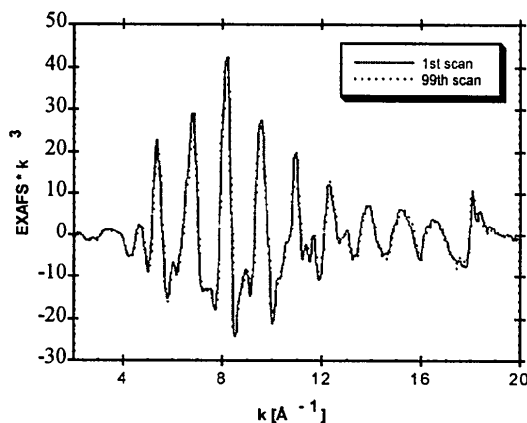

Figure 2

High energy stability and reproducibility of new QuEXAFS monochromator confirmed by background subtracted spectra from multiple QuEXAFS scans on 5 micron thick Fe foil. At k~18 Å^{-1}, the jump is due to the Ni contamination.

Tests on a sagittal focusing mechanism are proceeding with the aim of providing a substantial increase in the flux density over the whole range. Its implementation is expected early next year, its successful realisation will enhance the feasibility of polarised XAFS on small single crystals and XAFS of chemical and biological intermediates produced in an on-line stopped flow system.

2.2. Platinum Coated Collimating/focussing Mirror

A 580 mm long and 82 mm wide, platinum coated, Zerodur flat mirror was installed in January 1997. The mirror (at 11.4 m) can provide 2.1:1 vertical focusing for higher flux on small

samples of sub-millimeter size (at 16.8 m). It can be alternatively used in the collimating mode for higher flux on larger samples so that energy resolution is independent of the beam height used. The insertion of the mirror has not affected the energy range on the station. The high photon energy cut-off, governed by the mirror coating material (Pt) and the angle of incidence (5 mrad), is higher than that available from the source. The measured flux profile (Fig. 3) shows that the flux at 12 keV is still as high as that at 4 keV the low energy cut-off, which is primarily determined by the thickness of the Be windows of the beam line. The x-ray energy range provided by the station is thus essentially the same as before, 4 to 12 keV.
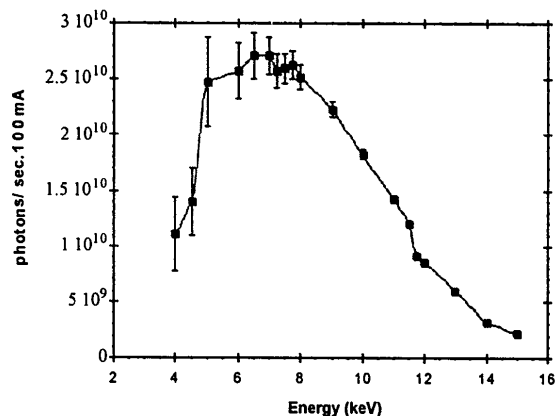

Figure 3

Intensity profile on Station 7.1 (full beam height, 10 mm width at exit slits). No harmonic rejection was applied and appropriate gas mixture (20% absorbance) was used for each energy.

3. A Multi-Element Compact Solid State Detector

Substantial progress has been made in the field of fluorescence detectors since the original scintillation counter based system of 7.1 in the early 80's (Hasnain *et al.*, 1984). This improvement has primarily come from the progress made in solid state detector based systems and the ability of putting together a large number of elements on a single cryogenically cooled detector head. In order to cope with the high count-rate, the number of elements have increased from 13 to 30 in recent years. However, this has tended to make them bulkier and cumbersome to manipulate and difficult to position them close to the samples. Their sheer size often prohibits the use of complex sample stages and auxiliary equipment, eg. stopped-flow, diffractometers or cryostats.

A new approach in detector design is thus appropriate. A 'low profile' multi-element detector has been designed and built in collaboration with a major detector manufacturer. Fig. 4 shows this detector assembly with the current pre-amplifiers: a miniaturisation of the bulky pre-amplifier for each detector element is taking place which would result in an even smaller profile for the whole assembly, enabling us to position the detector close to a stopped flow system as well as integrate it in a diffractometer and faster pulse counting electronics. A typical energy resolution of 170 eV has been achieved using X-rays of 5.9 keV with Gaussian shaping time of 0.5 msec, this should be compared with 260 eV of the 30-element SSD on Station 16.5. This improvement is extremely valuable for obtaining high

quality XAFS spectra at lower energy. Together with the XSPRESS (X-ray Signal PRocessing Electronics for Solid State detectors) digital electronics developed at Daresbury Laboratory, the detector is expected to give a high photon count rate capability in the region of several MHz. A fuller description of the system will be published separately.
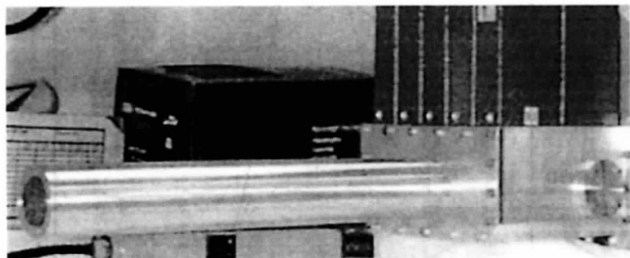

Figure 4
A 'low profile' multi-element solid state detector designed for Station 7.1 and built in collaboration with a major detector manufacturer. (A miniaturisation of the bulky pre-amplifier for each detector element is taking place.)

4. A Stopped-Flow System For Fluorescence XAFS

For studying structural changes in chemical and biochemical reactions with millisecond time-resolution, the time-slicing method combining fluorescence XAFS and stopped-flow is identified to be the most suitable choice to cater for all dilute systems (<10 mM). This combination satisfies the criteria on data acquisition mode (fluorescence), time-resolution (few msec) and sample economy in order to study the intermediates in reaction kinetics. A standard UV/visible stopped-flow from Hi-Tech Scientific Ltd has been adapted by fitting of an x-ray transparent window to the cell to let the incident x-ray and the emitted x-ray fluorescence pass through. The x-ray transparency of a number of materials have been investigated. Exotic but very strong materials like PBN (pyrolytic boron nitride), diamond wafer and CVDD (chemical vapour deposited diamond) are composed of light atoms and thus have very good x-ray transmission. The most suitable material is found to be PBN (Inada *et al.*, 1994). The material is chemically stable and has high tensile strength. Coupled with its relatively high x-ray transparency (90% at 8 keV for 300 μm thickness), it is able to withstand the pressures generated during mixing as well as is suitable for the chemicals to be used. Accordingly, the stopped-flow unit is modified by the substitution of a PBN window (250 μm thick). Tests show that the window is mechanically strong and stable against chemical attack except very strong bases (high pH values). A test Zn K-edge XAFS data have been collected using this system demonstrating that this stopped flow system is capable of obtaining fluorescence XAFS as well as the optical spectrum simultaneously on the same sample.

5. Development And Application Of 3D-EXAFS Analysis Approach

X-ray diffraction techniques can yield three dimensional (3D) structural information on materials in the crystalline state. The accuracy and precision in determining atomic distances depend upon the resolution of the structure. Atomic resolution is routinely achieved in small molecule crystallography but is only seldomly possible for protein molecules. EXAFS is capable of providing such information around an atomic centre, generally a metal atom. The resolution of EXAFS is the same and is

irrespective of the size of the molecule or the state of the sample, whether it is crystalline or solution. However, no geometrical information is provided by standard data analysis other than cases where multiple scattering exists.

A new approach to EXAFS analysis of metal centres (Cheung, 1998) exploits the vast structural resources available in the Chemical Database Service (CDS) and the Protein Structure Database (PDB). The method recognises that 3D structural information are contained in the EXAFS data in the form of inter-ligand as well as intra-ligand multiple scattering (MS) (Binsted & Hasnain, 1996). The atomic coordinates from CDS or PDB data files are used as the initial parameters for a 3D-EXAFS refinement of the metal coordination. The models revealed by protein crystallography and 3D-EXAFS can be examined using molecular graphics.

This 3D-EXAFS approach has been tested on an iron haem model compound and then on the active iron sites of myoglobin in various states (Cheung, 1998). Constrained and restrained EXAFS refinements were applied. The 3D-EXAFS refinement method was then applied to a type-1 copper protein, azurin II, from *Alcaligenes xylosoxidans*. XAFS of oxidised and reduced protein shows a substantial difference but the crystal structure of the oxidised and reduced protein at 1.75 Å resolution shows no significant difference. The 3D structures which emerge from the EXAFS analysis shows that the increased charge at the reduced site results in a 0.1 Å movement of copper out of the N(His) plane and towards the O(Gly) along the O(Gly)-S(Met) vector. We note that such movement is unlikely to be quantifiable by crystallography at current resolutions of 1.75 Å.

Conclusions

The oldest XAFS station at the first purpose built SR source has undergone a complete refurbishment including a vertically collimating/focussing mirror, a directly water cooled QuEXAFS monochromator and a low profile high energy resolution multi-element SSD. These instrument capabilities together with a 3D-refinement approach of EXAFS data open the possibility of undertaking multi-dimensional structural studies of chemical and biological systems at atomic resolutions.

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