

Changing and challenging times for service crystallography

Simon J Coles,*^a and Philip A Gale*^a

Electronic Supplementary Information

Instrument descriptions and experimental protocols

The following firstly describes the instrumentation available to the NCS in the home laboratory in Southampton and provides a generalised strategy for conducting experiments both there and at beamline I19 of the Diamond Light Source synchrotron. Secondly, a comparison experiment is presented that highlights the difference in data collections on the same crystal between DLS beamline I19, the Southampton rotating anodes and a sealed tube source.

A. Instrumentation Descriptions

i) NCS Home Laboratory

Data can be collected using one of the following four instruments currently installed in the laboratory in Southampton:

- 1) The Rigaku FR-E+ Very High Flux Diffractometer: the left-hand port on the new FR-E+ SuperBright molybdenum X-Ray generator, has a highly focused beam (70 μ m) achieved with the new VariMax VHF (Very High Flux) optics. The setup is completed with an AFC12 goniometer and an enhanced sensitivity (HG) Saturn 724+ 18bit CCD detector.
- 2) The Rigaku FR-E+ High Flux Diffractometer: the right-hand port on the new FR-E+ SuperBright molybdenum X-Ray generator is equipped with VariMax HF (High Flux) optics and the beam is slightly de-focused (100 μ m). This diffractometer also features the AFC12 goniometer and an enhanced sensitivity (HG) Saturn 724+ 18bit CCD detector.
- 3) The Rigaku 007 HF (High Flux) diffractometer: a copper rotating anode, equipped with a Saturn 944+ enhanced sensitivity (HG) detector and an AFC11 quarter chi goniometer.
- 4) The Rigaku R-AXIS SPIDER diffractometer: molybdenum sealed tube X-ray generator combined with an RAPID image plate detector system and a quarter chi goniometer.

ii) Diamond Light Source

Beamline I19 is situated on an undulator insertion device and the beam is optically conditioned using a combination of a double crystal monochromator, vertical and horizontal focussing mirrors and a series of beam slits (primary white beam and either side of the focussing mirrors). The experimental hutch used for service

crystallography (EH1) is equipped with a Crystal Logic 4-circle kappa geometry goniometer with a Rigaku Saturn 724+ CCD detector and an Oxford Cryosystems Cryostream plus cryostat (80-500K). For conventional service crystallography the beamline operates at a typical energy of 18 keV (Zr K absorption edge) and a Rigaku ACTOR robotic sample changing system is available.

B. Generalised Experimental Strategy

Sample preparation

A single crystal is mounted in paraffin or paratone oil on a Mitegen micromount which is then cooled under a cold stream of nitrogen to 100K using an Oxford Cryosystems Cobra (Mo rotating anodes) or 700 Series CryoStream (Cu rotating anode). Data using the sealed tube (Mo radiation) image plate system is routinely collected at 120K using an Oxford Cryosystems 600 Series CryoStream.

Data collection strategy at Southampton

i) Rotating Anodes

A routine unit cell determination is achieved by means of a single omega scan. For this 15 images corresponding to 1 degree slices are collected in 5 degree steps at a default exposure time of 20 seconds. The exposure time may be adjusted according to crystal size, composition and diffraction quality after assessment of the initial diffraction images. Images containing overloading reflections are re-measured at a fraction of the original exposure time and a scaling factor applied.

These 15 images are harvested for the strongest reflections (observation criterion of $I > 5$ to $10 \sigma(I)$) which are then used to determine the crystal symmetry, orientation matrix and unit cell dimensions, forming the basis for the calculation of a full data collection strategy which is bespoke for each individual crystal.

The strategy targets are to cover reciprocal space for the asymmetric unit to a minimum of 99% completeness at a resolution of 0.77\AA with a minimum of 2-fold redundancy using molybdenum radiation. Images are collected as 1 degree slices, with a standard detector to sample distance of 45mm. The time per frame is chosen according to the observed diffraction strength of the unit cell images.

The strategy calculation using copper radiation is the same, except for the detector being placed at a minimum distance of 41mm from the sample and the maximum resolution being limited to 0.85\AA due to the geometry of the diffraction equipment.

ii) Sealed Tube

The unit cell determination relies on a total of 4 images taken as 1 degree slices at 5 degree steps. The default exposure time of 2 minutes may be adjusted to a more suitable time per frame after inspection of the initial diffraction pattern.

The strategy calculation is aimed at a minimum of 99% completeness at a resolution of 0.77\AA with a minimum of 2-fold redundancy. Images are collected as 4 to 5 degree slices, with a fixed detector to sample distance of 127.40mm. The time per frame is

increased in accordance with the wider slicing compared with the unit cell collection strategy. Further adjustments of the exposure time are based on the observed diffraction intensity of the unit cell images.

NCS Data collection strategy at Diamond Light Source

The unit cell is determined from twenty 1° omega scan images, each separated by 5° spacing (wavelength of 0.6889Å). All measurements are performed at 100K. The data collection strategy is composed of four omega scans covering a hemisphere of data and is employed for all measurements (see table below). These scans cover 535° in omega* and are collected as 1 degree images with a detector offset of 30° and distance of 60mm at one or two second exposure. For images containing overloading reflections a time-based re-measurement, as per the laboratory systems, is used, but also a physical attenuation may be applied using any combination of 0.25, 0.5, 1.0 or 2.0mm of aluminium sheets. Data are processed and corrected for absorption effects using the REQAB algorithm in the d*trek package from within the CrystalClear software suite.

Scan	Phi	Omega	Kappa	Length
1	0	-115	0	175
2	0	-60	-50	120
3	120	-60	-50	120
4	240	-60	-50	120

Software & Data Processing

The CrystalClear suite of programs (at the time of writing v2.0, but with an early adoption of v3.1 planned for the start of 2012) is used throughout the whole diffraction experiment, from diffractometer control, through data collection strategy calculation to integration of the data collected in Southampton. Data is routinely integrated and corrected for absorption by the multi-scan approach using FS_PROCESS available within CrystalClear. Other options available are d*trek and TwinSolve.

C. Comparison Data Collection

Data Collection & Refinement Parameters

A light yellow fragment (0.07 x 0.05 x 0.02 mm) of 1,3-bis(3,5-dinitrophenyl)urea tetramethylammonium acetate (**1**) was glued to a Mitegen micromount loop (30µm) using epoxy resin. Data were collected at room temperature throughout to avoid repeated heating and cooling and thereby thermal stressing of the crystal and to ensure the same sample could be used for all experiments.

In order to compare the capabilities of the rotating anode-based equipment with standard laboratory and synchrotron sources, data for **1** were collected on the sealed tube system and the FR-E+ rotating anode diffractometer based at Southampton as well as at beamline I19 at the DLS.

Since the wavelength available at the DLS beam line I19 is similar to Mo-radiation the I19 data collection strategy was also adopted for the collection on the FR-E+ with some minor adaptation due to instrument geometry.

The following experiments were performed:

1) I19: The crystal was exposed to the X-ray beam for 1s and the beam was further attenuated using a 1mm Al foil yielding a transmission of 29.2% at $\lambda=0.6889\text{\AA}$.

2) FR-E+: Two exposure levels (10s and 30s) were used to test performance of the instrument.

3) Completing the study, some reflection data were collected using the R-AXIS SPIDER image plate system. In light of the much-reduced intensity of the X-ray beam of the sealed tube instrument, only the determination of the unit cell was attempted. This was done at three different exposure times per frame (4h, 6h and 12h), with an exposure of 12 hours per frame needed to produce a sufficient number of intense reflections for the reproduction of the unit cell dimensions with $d \cdot \text{trek}^1$. However, even then the number of observations was too small to provide meaningful standard deviations for the unit cell parameters to be estimated. The strategy calculation estimated that a full data collection at this level would require 5 months!

Data were collected according to the following scan strategies:

DLS I19

Detector 2theta offset = 30° , unit cell collection time = 1s/frame, data collected at 1s/frame, attenuator = 1mm Al foil

Scan	Phi	Omega	Kappa	Detector Distance	Length
uc	0	-60	0	60	20
1	0	-60	0	60	175
2	0	-60	-50	60	120
3	120	-60	-50	60	120
4	240	-60	-50	60	120

Rotating anode VHF system

Detector 2theta offset = -30° , unit cell and data collection time = 10s & 30s/frame

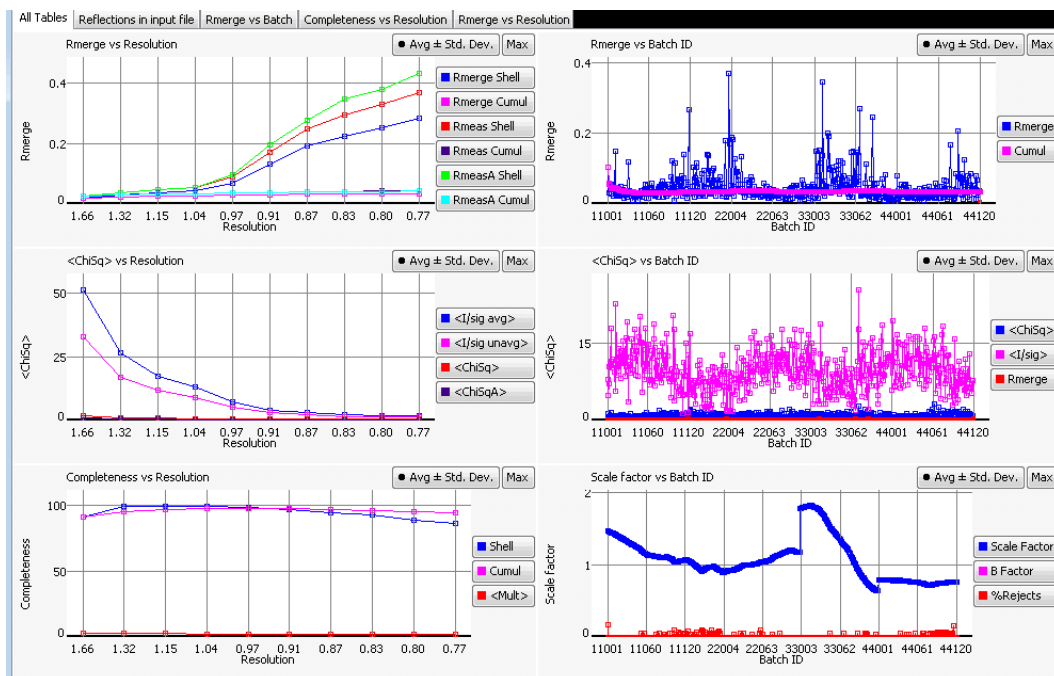
SPIDER sealed tube image plate

N.B. Data were only collected for a unit cell determination

Scan	Phi	omega	Kappa	Detector Distance	Length (no of images)
uc	0	0	0	127.40	4

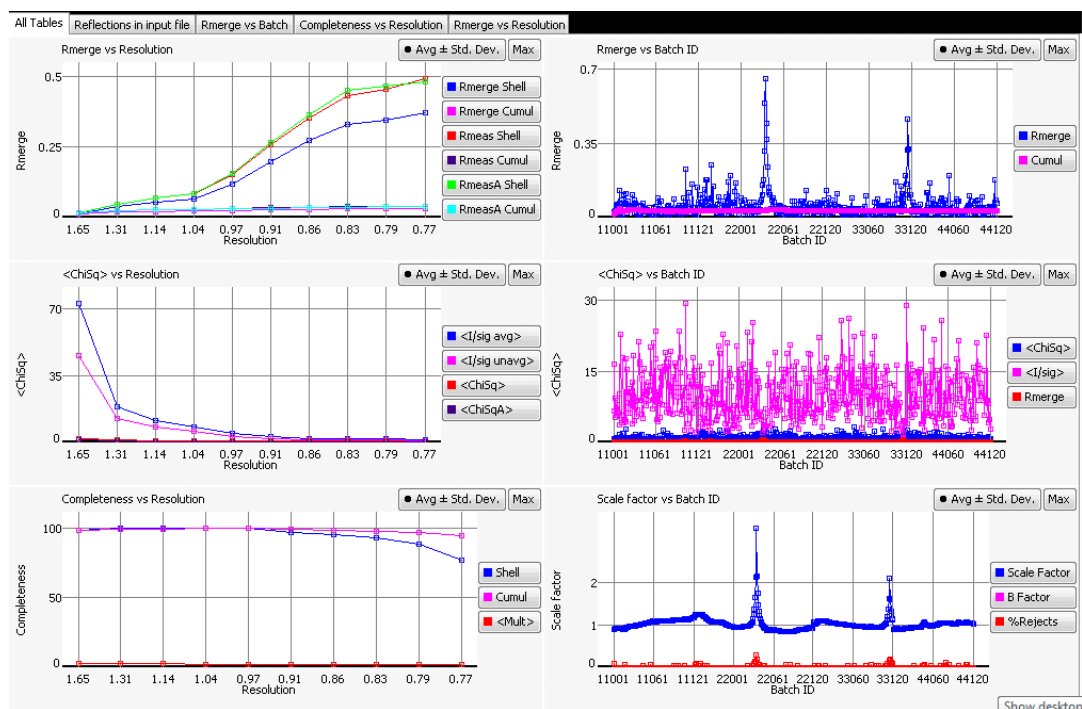
All data were processed using the d*trek software package and a comparison of the statistical outputs from the integration and scaling processes is shown below.

DLS I19

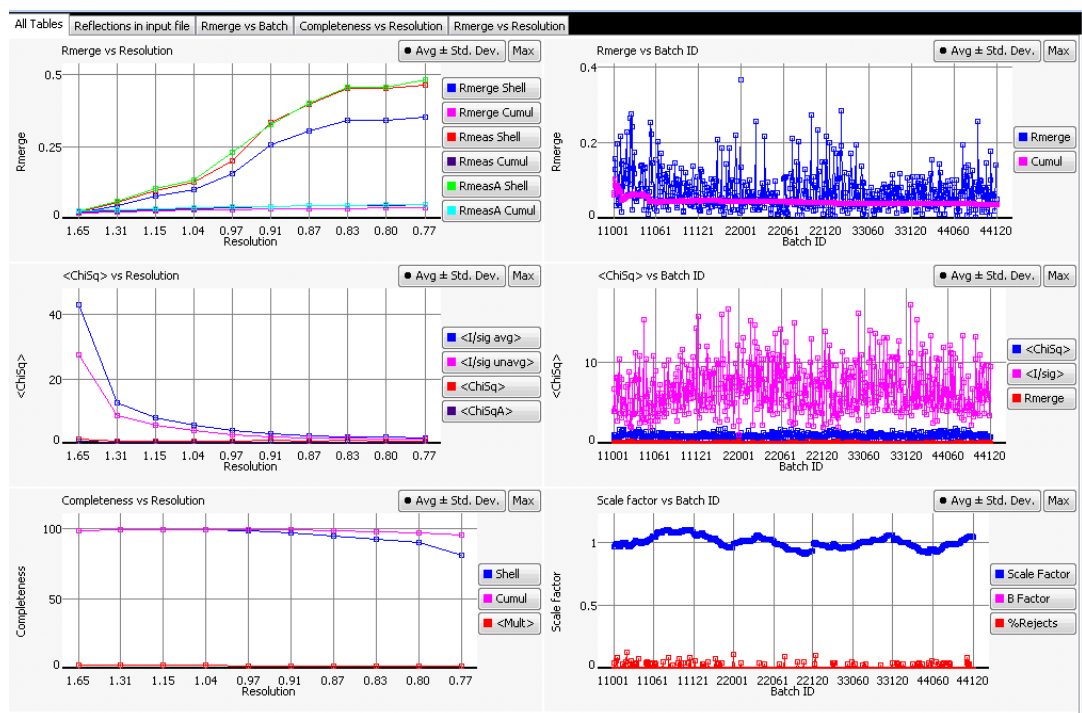


Rotating anode VHF system (10 second data collection)

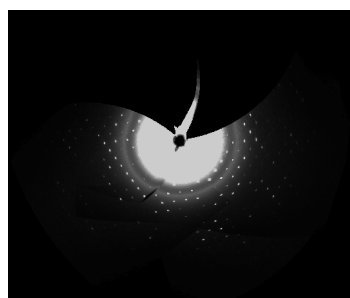
Scan	Phi	Omega	Kappa	Detector Distance	Length (no of images)
uc	0	-100	0	45	15
1	0	-120	0	60	180
2	0	-60	50	60	120
3	120	-60	50	60	120
4	240	-60	50	60	120



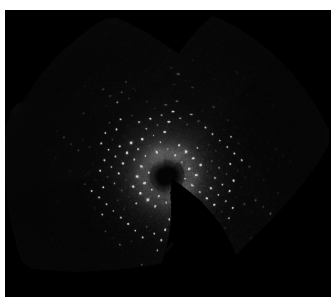
Rotating anode VHF system (30 second data collection)



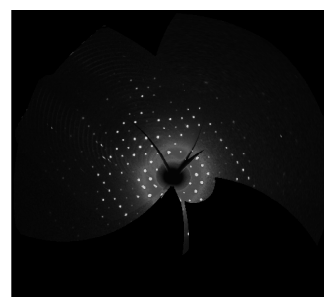
From these plots it can be seen that the completeness and resolution of the data arising from the two rotating anode collections is comparable, but the scaling and merging statistics are somewhat better for the longer data collection. The statistics also compare well with the 1 second data collection on the synchrotron, with slightly better merging statistics. The precession photographs of the OKL layer of the dataset (taken from the data collection images themselves) shown below, give a visual and qualitative comparison of the three data collections.



I19 (1 second)



FR-E+ (10 seconds)



FR-E+ (30 seconds)

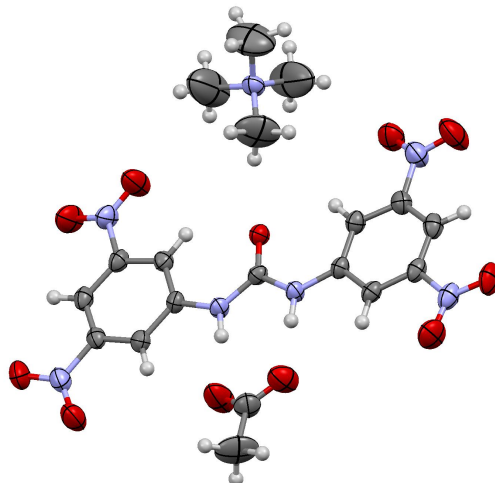
The structures were solved by direct methods with SHELXS-97ⁱⁱ and refined on F^2 using SHELXL-97ⁱⁱ as implemented in the WinGXⁱⁱⁱ suite of programs. Anisotropic displacement parameters were assigned to all non-hydrogen atoms. Hydrogen atoms bonded to carbon atoms were included at calculated positions and the thermal parameters were constrained to ride on the atom to which they are bonded. The urea hydrogen atoms were located on the difference map and the bond distances were restrained to idealised values. Thermal parameters for those hydrogen atoms were refined isotropically. Details regarding the crystal and data collection parameters are summarised and compared below.

<i>Instrument</i>	<i>I19</i>	<i>FR-E+</i>	<i>FR-E+</i>
<i>Exposure</i>	1s	10s	30s
λ [Å]	0.6889	0.71073	0.71073
<i>Temperature [K]</i>	293(2)		
<i>Sum formula</i>	C ₁₉ H ₂₃ N ₇ O ₁₁		
<i>Formula weight</i>	525.44		
<i>Crystal Size [mm]</i>	0.07 x 0.05 x 0.02		
<i>Crystal System</i>	Triclinic		
<i>Space Group</i>	P $\bar{1}$		
<i>Z</i>	2		
<i>a</i> [Å]	9.537(7)	9.510(17)	9.525(12)
<i>b</i> [Å]	11.248(9)	11.31(2)	11.380(15)
<i>c</i> [Å]	12.439(10)	12.46(2)	12.488(16)
α [°]	107.701(9)	107.583(19)	107.619(15)
β [°]	97.463(6)	97.533(14)	97.507(10)
γ [°]	104(937)(10)	104.719(19)	104.894(16)
<i>V</i> [Å ³]	1196.4(16)	1203(4)	1214(3)
<i>Reflections used in unit cell refinement</i>	2318	2974	2547
<i>θ range for unit cell</i>	1.9-26.6	3.0-27.5	3.0-27.4
<i>All data</i>	11746	11790	11747
<i>θ range for all data</i>	2.43-26.60	2.96-27.48	2.95-27.46
<i>\int</i>			
<i>Ave I/σ(I) 1.31-1.15Å</i>	17.5	8.1	11.3
<i>Ave I/σ(I) 1.31-1.15Å</i>	4.0	3.0	3.0
<i>Unique data</i>	5223	5276	5265
<i>R_{int}</i>	0.0345	0.0389	0.0325

Observed data	3330	3619	3188
<i>R1/wR2 [I>2σ(I)]</i>	0.0810/0.2357	0.1249/0.2658	0.0855/0.2300
<i>R1/wR2 [all data]</i>	0.1108/0.2662	0.1709/0.2959	0.1329/0.2674
<i>GoF</i>	1.072	1.237	1.109
<i>Residuals [eÅ⁻³]</i>	0.389/-0.318	0.407/-0.212	0.434/-0.254

From this table it can be seen some aspects are better for synchrotron data in general (e.g. the error on unit cell measurements) but when considering all factors (i.e. comparing merging and final refinement parameters) the synchrotron and 30 second rotating anode measurements are very comparable. However, the final refinement arising from the 10 second rotating anode measurements still provides a publishable structure and gives both a useful benchmark for data quality and an indication of the difference between synchrotron and high flux laboratory sources.

A thermal ellipsoid plot of the resulting structure is shown below (ellipsoids drawn at 30% probability level) as obtained from the 10s data set collected on the FR-E+ diffractometer. There is some minor disorder of the ammonium cation, but this was not included in the model. CIF files for each of the 3 compared data collections are also provided as supporting information.



ⁱ The unit cell dimensions obtained were $a = 9.273 \text{ \AA}$, $b = 11.436 \text{ \AA}$, $c = 112.405 \text{ \AA}$, $\alpha = 107.18^\circ$, $\beta = 97.56^\circ$, $\gamma = 104.69^\circ$ based on 60 reflections.

ⁱⁱ G. M. Sheldrick "A short history of SHELX", (2008), *Acta Cryst.*, A64, 112-122.

ⁱⁱⁱ L. J. Farrugia, *Journal of Applied Crystallography*, **32**, 837-838 (1999).