The XIPHOS system in Durham is now operational, allowing crystal structures to be determined routinely at temperatures down to 2K. [1] In order to reach these temperatures, the sample is isolated from the environment using vacuum shrouds made from beryllium. These shrouds contain unevenly distributed crystalline parts, resulting in powder rings of non-uniform intensity. When collecting data with a weakly diffracting crystal where the reflections from the beryllium are strong in comparison to those reflections from the crystal, the uneven intensities from the rings can distort the intensities calculated by integration programs.

This can be avoided, and improved data quality obtained by masking out the regions of the diffraction image which are affected by beryllium scattering. Any reflections which overlap with the masked region are discarded by the integration program. As the scattering from the beryllium shrouds and the crystal originates from different positions, overlapping reflections can be separated by changing the detector distance. By using masks and recovering the lost data with these different detector distances, data quality can be improved compared with data obtained at one distance and without masking.

Masquerade has been written to generate the mask files required for this technique quickly and accurately. The positions of the beryllium rings are calculated using an *ab-initio* model of the position of the shrouds in 3D space, and include goniometer rotations as well as centring offsets for the shrouds themselves. The program is written in C++ and uses multithreading to make full use of modern multi-core processors. It runs on a variety of platforms and can generate thousands of mask images for a full data collection in minutes.

The use of this program along with the data collection at multiple detector distances has been found to improve the data quality obtained from weakly diffracting crystals, showing clear improvements in R_1, wR_2 and Rsigma values over data integrated without the masks.

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X-ray-induced cooperative atomic movements in protein crystals

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A specially designed diffraction experiment with controlled radiation damage to proteins makes it possible to investigate X-ray-induced changes in protein molecules and their crystals at the atomic level. We present the results of several experiments in which multiple data sets corresponding to increasing absorbed doses were collected from the same crystals at atomic resolution ([1], [2]). A comparison of the refined models of a protein molecule that corresponded to different levels of damage allowed us to study progressively increasing local and overall radiation damage effects. It was shown that irradiation causes displacements of big parts of the protein molecule. These movements have a cooperative character: big parts of protein molecules are displaced approximately as rigid bodies. The collective movement of the atoms of the protein leads to the expansion of the protein globule, which occurs synchronously with, and in the same direction as, the expansion of the unit cell.

It was also shown that water molecules in the vicinity of protein surface move in the same direction and in concert with the nearest protein atoms. It appears as if the atoms of the expanding protein molecule pull the hydrogen-bonded network.

Displacements of protein domains occur simultaneously with the X-ray-induced damage to protein residues that participate in the contacts between different domains (decarboxylation of Asp and Glu residues, disruption of S-S bridges). An analysis of atomic ADPs revealed that, for the atoms with initially isotropic ADPs, an increase in anisotropy of ADPs occurs in the same directions as atomic displacements. This indicates that radiation-induced atomic displacements occur with different speed in different cells of the crystal and lead to an additional increase in crystal static disorder.

We studied the radiation-induced local and global damage not only at 100K, which is a usual temperature of data collection at modern synchrotrons, but also at as low as 15K. In the last case, crystals were cooled by a cold helium stream. Both kinds of radiation damage, local and overall, evolve on approximately the same time scale and similarly diminish as temperature decreases from 100K to 15K. Lowering the temperature from to 100 to 15K decreases the disulfide-bond deterioration and atomic displacements, the decrease being somewhat greater than twofold.

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Characterization of radiation damage in macromolecular crystals at cryo and room temperature

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Radiation damage caused by ionising radiation is a constant source of concern when collecting X-ray data from biological samples. Its effects are sample dependent and are proportional to the absorbed dose. Accurate dose estimation is essential to determining the crystal dose tolerance and data collection strategy. A reliable and reproducible method to automatically characterise the radiation sensitivity of macromolecular crystals based on a preliminary experiment sacrificing a whole or part of a sample has been recently developed [1]. The method was implemented at the ESRF beamlines through an automated procedure using the EDNA online data analysis framework [2] and the MxCuBE data collection control interface [3]. The information extracted from this procedure is directly used for optimal planning of data collection by strategy software program BEST [4]. Using test crystals with well known radiation sensitivity, the procedure can also be used at the beamlines to verify and calibrate X-ray flux and beam size.

The method has been recently applied to a systematic study of radiation damage at room temperature. Reproducibility of the measurements, dose rate effects, radiation damage correlation with crystal properties and the diffraction intensity degradation model have been investigated, analysed and compared with the results obtained at cryo-temperature.

Experimental measurements and theoretical modelling has been also carried out to understand the effect of the X-ray beam shape on the absorption dose estimation and the radiation damage model.

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Potential of UV in phasing and its implementation for crystal centering at PF

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The continuously increasing demand for synchrotron beam-time, both from academic and industrial users, is a direct outcome of the exponential growth of macromolecular crystallography. Fully automated procedures at every level of the experiments are being implemented at all synchrotron facilities, allowing the screening of a profusion of sample crystals for more and more projects. However, the sample recognition and centering in the X-ray beam represents one of the major obstacles to achieving such automation.

Several independent algorithms have been developed to achieve crystal recognition and centering. The most popular method relies on pattern recognition of the loop encircling the crystal [1]. Ideal for high-throughput data collections, this frequently used routine has the advantage to allow the screening of plenty of samples in a timely and efficient manner. Nevertheless, when dealing with crystals of small sizes or shifted from the loop center, it suffers from a lack of precision. A non-exhaustive list of other techniques includes diffraction-based analysis crystal centering [2], increase of crystal-to-surrounding contrast by differential lights [3], X-ray fluorescence [4] and UV-fluorescence recognition [5].

UV-based crystal centering takes advantage of the properties of UV-light that specifically reacts with aromatic residues present in proteins or with DNA base pairs. Although very efficient for visualizing protein crystals, a well-known side effect of illuminating biological samples with strong UV-sources resides in the damages induced on the exposed crystals [6]. While these damages can affect the inner structure of the irradiated samples, the structural alterations generated can be extracted and provide new phasing information for solving macromolecular structures, also known as UV radiation induced phasing (UV-RIP).

In the present study, the effectiveness of a softer UV-light for crystal centering, by taking advantage of low power light-emitting diode (LED) sources was investigated. The impact of such UV-LED on the biological crystals was carefully analyzed, notably in regards to the resulting radiation damages occurring after irradiation. The optimum set-up for crystal centering as implemented at the Photon Factory showed no distinguishable damages on any of the tested crystals.

Additionally, to allow an efficient use of UV for macromolecular structure determination, the minimum dose necessary for obtaining enough damages leading to significant phase information needs to be determined with care. Based on the resulting investigation, a consensus methodology for practical use of UV-RIP at the Photon Factory is proposed.

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MX Radiation Damage and Swept Volumes: improvements in dose estimation

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In order maximize the quality and quantity of data obtainable from a single macromolecular crystal, understanding radiation damage progression is paramount. To effectively manage the problems associated with it, routine on-line software must be available to predict the likely rate of damage for a given optimised data collection strategy (eg. [1]).

Dose (gray=J/kg) is the appropriate unit for the amount of energy absorbed per unit mass. It is thus a powerful metric against which to plot radiation damage indicators for quantifying the extent of radiation damage in a crystal [2].

The widely used dose estimation program RADDOSE [3-6] provides an accurate method for calculating the absorption and attenuation cross sections of macromolecular crystals. However, in order to calculate the dose absorbed by a crystal, we need both the total deposited energy and the mass of the exposed region. This is currently well-modeled for crystals smaller than the beam since the whole crystal is exposed for all the images. For crystals larger than the beam, current models can lead to a significant over-estimation of the dose.

When we have beams smaller than the crystal (e.g. [7]), as is often the case in micro-beam work, we must take into account the total swept volume during the experiment: not just the size of the static beam-crystal intersection. As the crystal is rotated, we are both bringing new regions of the crystal into the beam (increasing the mass in the denominator of the dose equation) and continually exposing the centre of rotation of the crystal, leading to a highly inhomogeneous exposure profile.

Two levels of implementation in RADDOSE will be offered: firstly, a routine on-line version which will require no new inputs other than total data collection angles and, secondly, a fully parameterized version which will use actual images of the crystal and experimentally determined beam profiles to generate a finite element model of the crystal-beam exposure tomography.

We will report on our progress in updating the RADDOSE software to provide users with both an exposure map of their crystal and improved dose estimation for each of these implementations. This new information will enable crystallographers to plan data collection strategies that will optimize their crystal real estate while minimizing radiation damage. For radiation damage research, this will also enable a better quantification of dose. We will also discuss the effect that taking a dose density approach to the differential damage throughout a crystal can have on our approach to data collection strategies for mitigating radiation damage.

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