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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