

A Guide to Dose-Aware Data Collection for Crystallography Users at Diamond Light Source Beamline I04 (March 2025)

Introduction

X-ray crystallography is a powerful technique used to determine the 3D structures of biological macromolecules, which plays a crucial role in understanding their functions and interactions. The quality of the data collected from X-ray diffraction experiments is critical for the success of structure determination and subsequent analyses. With advancements in technologies, such as brighter synchrotron sources and more sensitive detectors, the limitation in data collection has shifted from the capabilities of the instruments to the radiation damage of the sample.

The Impact of Dose

Radiation damage, caused by the absorption of X-ray photons by the crystal, is a major concern in macromolecular crystallography. The absorbed energy can lead to various deleterious effects, such as global damage to the crystal lattice and specific damage to individual amino acids in the protein molecules that might be involved on the biological question being researched. These effects can manifest themselves in the diffraction data as reduced resolution, decreased intensity, and increased noise.

The amount of absorbed dose depends on several factors, including the composition and size of the sample, as well as the properties of the incident X-ray beam, such as energy, flux, size, and shape. Samples containing high-Z elements, such as selenium, are more susceptible to radiation damage due to their higher absorption cross-sections. Lower energy (longer wavelength) beams also deposit more energy into the crystal, leading to increased

damage. The beam size and shape also influence the distribution of dose within the crystal, with a top-hat profile being more favourable for uniform dose spreading.

Strategies for Dose Mitigation

To mitigate the effects of radiation damage and maximize data quality, several strategies have been developed.

- **Dose spreading:** Distributing the dose across multiple crystals, by increasing the angular range of data collection, or by using multiple sweeps with different crystal orientations, can help to reduce the damage to any individual crystal.
- **Sample diffracting power:** The dose should be adjusted based on the diffracting power of the sample, where e.g. a higher dose could be applied with poorly ordered samples since the high-resolution information is already unavailable whereas for well-ordered, highly diffracting samples a more cautious approach with lower dose should be applied (Figure 1)

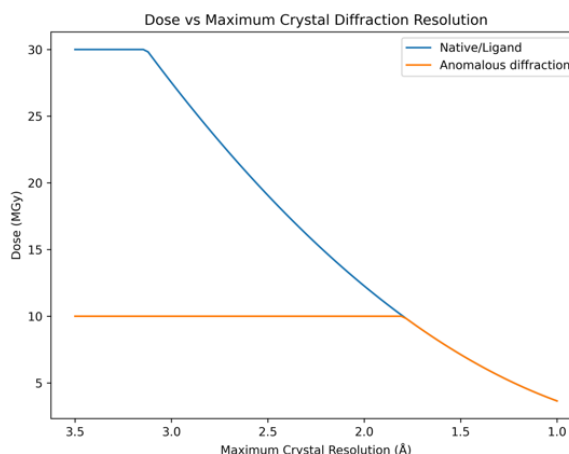


Figure 1: Relationship between Dose & Diffraction Resolution

Figure 1: This graph provides a guide on the maximum dose to use for data collection as a function of the maximum diffraction resolution of the crystal. The maximum dose is lower for higher-resolution crystals and higher for lower-resolution crystals, as higher-resolution crystals are more susceptible to radiation damage. For native experiments (blue line), two sweeps of 360 degrees at different crystal orientations are recommended, with a maximum dose of 15 MGy per sweep. For ligands, a single 360-degree sweep with a maximum dose of 30 MGy is recommended. For anomalous diffraction experiments (orange line), three sweeps are recommended, with a maximum dose of 3.33 MGy per sweep. It is important to note that this graph provides a guide only, and the values do not need to be followed exactly. The actual dose used may vary depending on the specific experiment and the properties of the crystal.

- **Experimental aim:** The dose should also be tailored to the specific experimental aim, as different goals may have different priorities and requirements in terms of data quality and resolution.

Optimizing X-ray Exposure

Determining the optimal X-ray exposure for a given crystal has always been a challenge, as it requires balancing the need for sufficient signal to achieve the desired resolution with the need to minimize radiation damage. The optimal exposure depends on various factors, including the intrinsic diffraction limit of the crystal, which can vary significantly between samples and even within the same sample under different growth conditions.

Modern approaches to dose optimization involve using readily available beamline data, user-friendly tools, and knowledge of sample absorption and tolerance limits to calculate the optimal exposure and transmission parameters. This personalized approach allows for tailoring the dose to the specific needs of each crystal, protecting highly diffracting crystals from excessive damage while ensuring that sufficient data are collected to achieve the experimental goals.

Dose Awareness at Diamond Light Source beamline I04

The I04 beamline at Diamond Light Source provides advanced dose awareness tools to assist users in optimizing their data collection strategies. The beamline control software offers two main modes of operation (Figure 2):

- **Set target exposure:** In this mode, the user defines the desired

exposure time and transmission, and the software estimates the corresponding dose that will be deposited on the sample. This allows users to be aware of whether they are using too little or too much dose.

- **Set target dose:** In this mode, the user specifies the maximum dose they want to deposit on their sample, and the software automatically calculates the appropriate exposure time and transmission to achieve that dose. This mode utilizes the RADDOS-3D program to perform dose calculations in real-time, using live beamline data and making approximations when necessary.

Start	0.00
Oscillation	0.100
Total oscillation	360.0
Delta	0.00
Chi	0.000
Phi	0.000
Dose and Exposure	
Number of Images	3600
<input checked="" type="radio"/> Set Target Dose <input type="radio"/> Set Target Exposure	
Exposure Time	0.0020
Total Exposure Time	7.2
Dose / Dataset	7
First Image Number	1

Figure 2. Dose and Exposure Control Panel at Beamline I04

The "set target dose" mode is particularly useful when changing beam size or energy,

Figure 2: This figure shows the graphical user interface (GUI) panel, in the GDA software, for controlling dose and exposure settings at Diamond Light Source beamline I04. The red box highlights the radio buttons for selecting between two modes of operation: "Set Target Dose" and "Set Target Exposure." In "Set Target Dose" mode, the user specifies the desired dose, and the software automatically calculates the appropriate exposure time and transmission. In "Set Target Exposure" mode, the user defines the desired exposure time and transmission, and the software estimates the corresponding dose. Dose units are in MGy, exposure in seconds and angles in degrees.

as it ensures that the beamline operates at a fixed dose, regardless of the changes in beam parameters. This is important because the flux profile across the energy range is not linear or constant (Figure 3). Even for a particular energy, the flux can vary on different days due to variations in beamline commissioning and machine ring current (Figure 4). Working at a fixed dose and using the live flux at the time of the experiment allows for more rigorous comparisons between experiments on the same sample conducted months apart. This ensures that the diffraction power of the crystals remains consistent, which is not the case when working at fixed exposure.

leading to more accurate and reliable structural insights. The advanced dose awareness tools available at beamlines like I04 at Diamond Light Source further empower users to optimize their data collection strategies and achieve the best possible results.

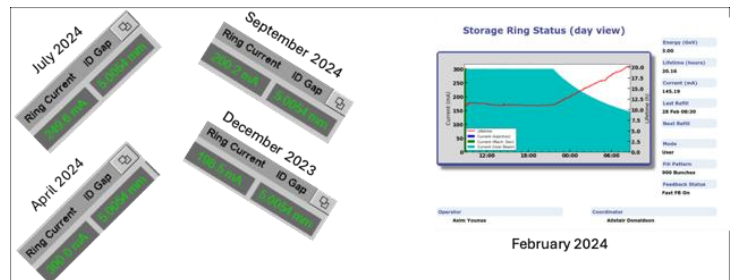


Figure 4. Variation of Diamond Light Source Ring Current

For more information please talk to I04 beamline staff <https://www.diamond.ac.uk/Instruments/Mx/I04/>

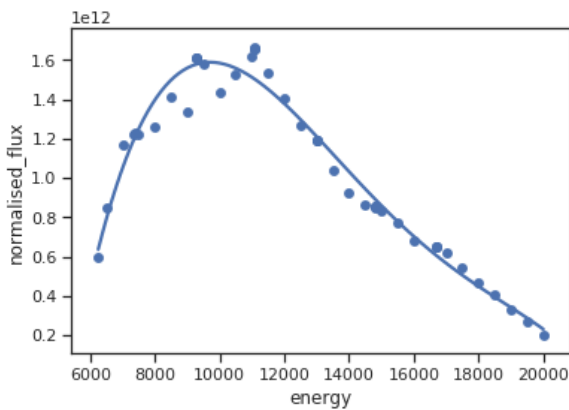


Figure 3. Flux as a Function of Energy at Beamline i04

Conclusion

Dose is a critical parameter in macromolecular crystallography that significantly impacts the quality of the diffraction data and the success of structure determination. By understanding the factors that contribute to dose and employing appropriate dose mitigation strategies, researchers can maximize data quality while minimizing radiation damage,

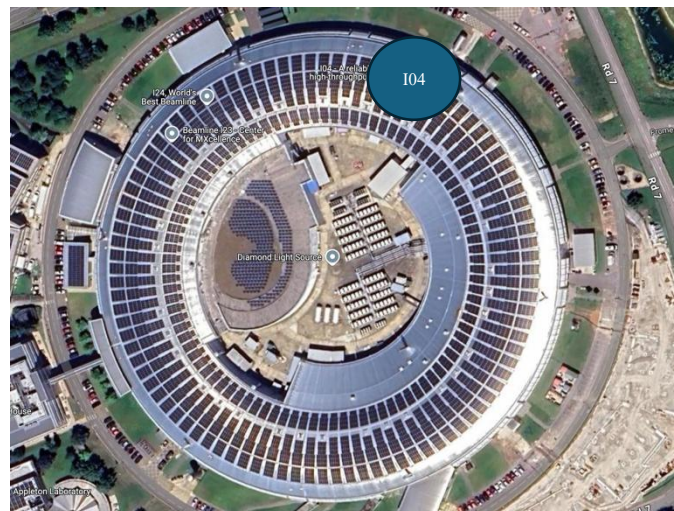


Figure 3: This figure shows the variation of the flux (number of photons per second) as a function of energy (eV) at Diamond Light Source beamline I04. The data were collected for a fixed beam size of 20 microns vertical and normalised to a 300 mA synchrotron ring current. The dots represent the real measured flux, while the smooth line is to illustrate the difficulty of fitting the data with a simple algorithm. The non-linear and non-constant nature of the flux profile is evident, highlighting the importance of using dose-aware data collection strategies.

Figure 4: This figure shows the variation in the Diamond Light Source ring current over time. The left panels illustrate examples from December (2023), April, July, and September 2024, where the ring current varied significantly between and within synchrotron runs, often unnoticed by users. The right panel shows an example of a "decay mode" event, where the ring current steadily decreased over an 11-hour period due to the lack of electron re-injection. In such situations, the "Set Target Dose" mode is crucial for maintaining the consistency of experiments, as the exposure time will be automatically adjusted to compensate for the drop in flux.