

Instrumentation and Software Support for X-Ray

Radiation Sensitivity Studies of DNA Crystals

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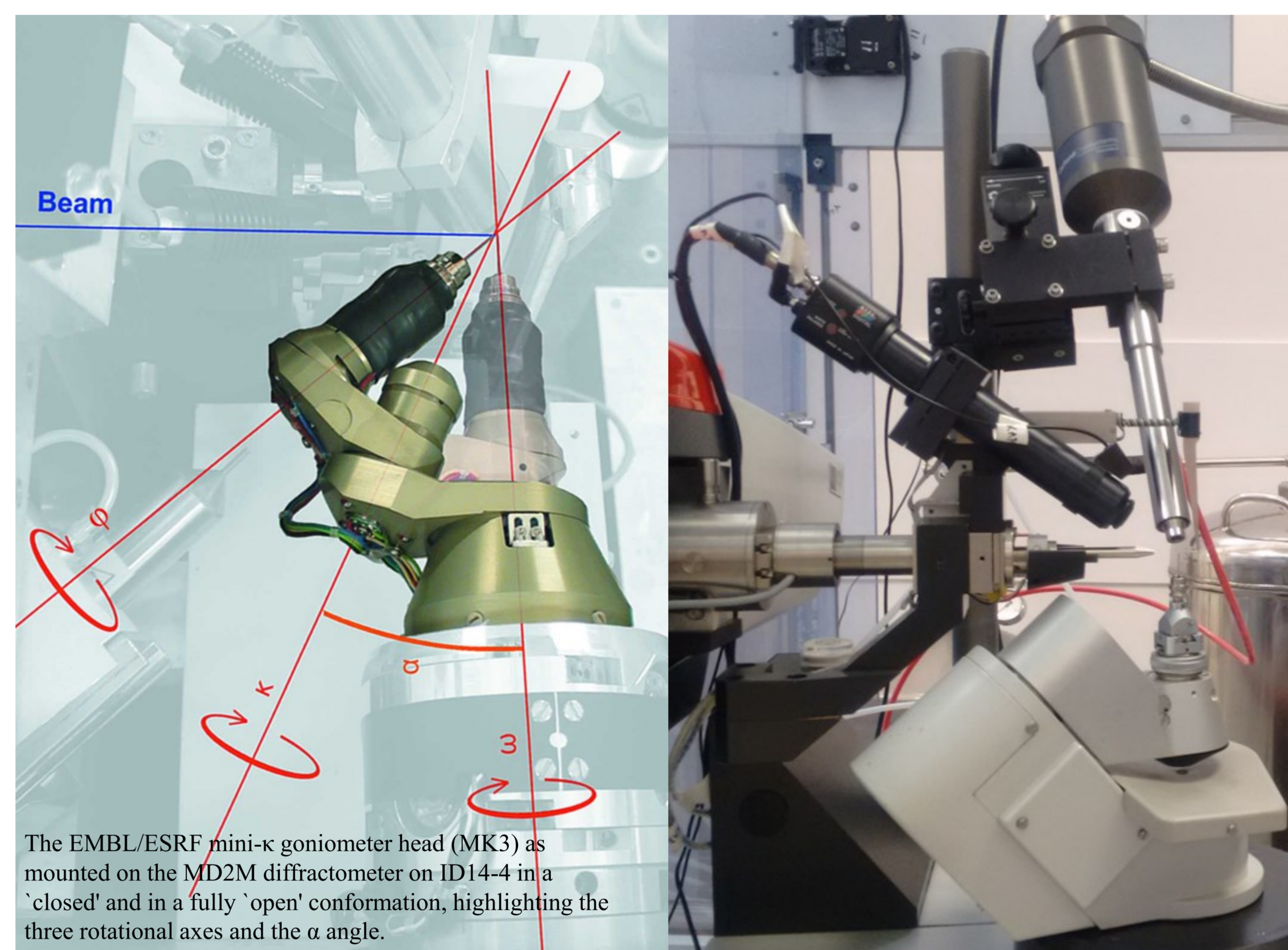


Introduction

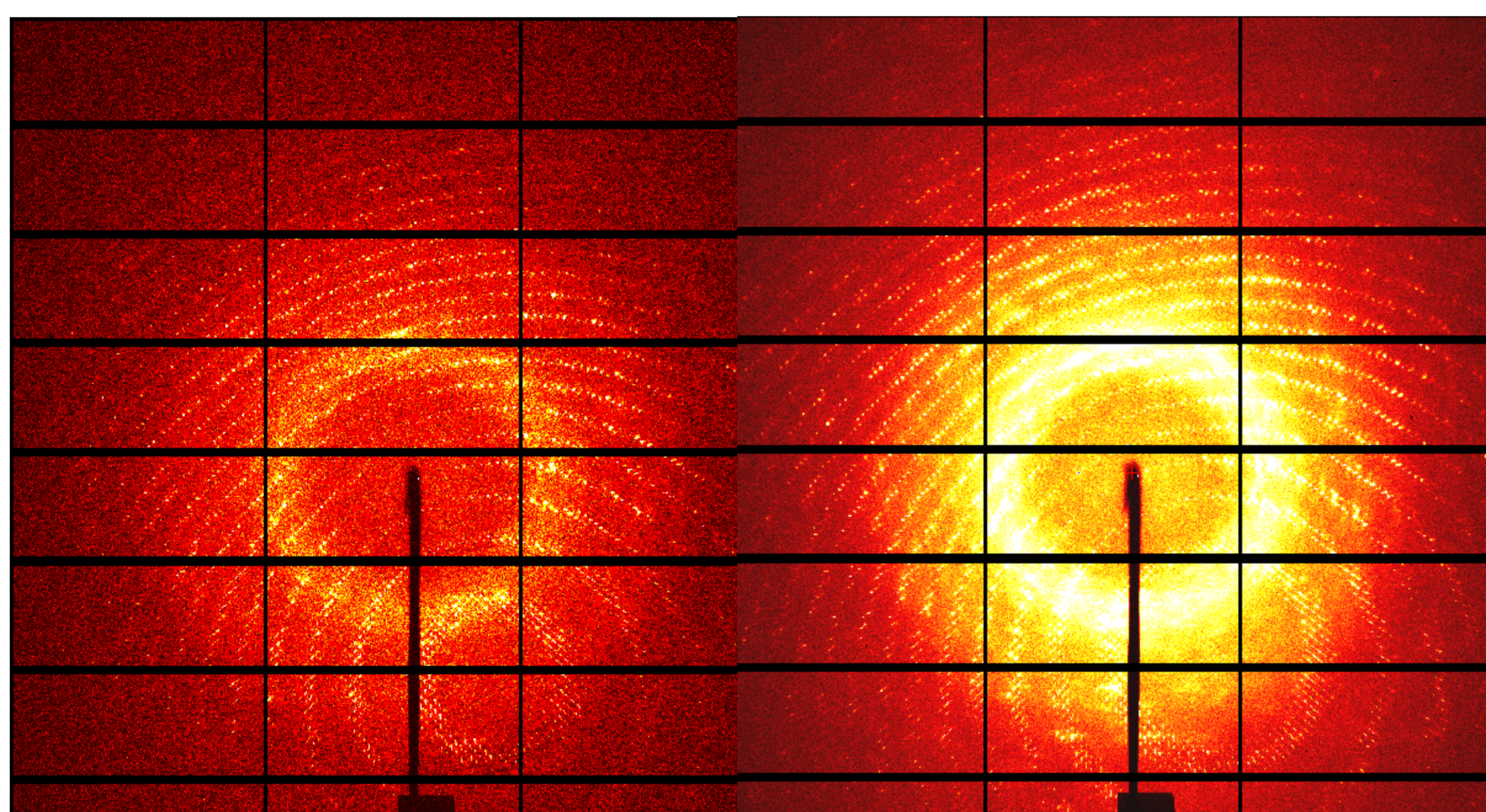
As a revival of κ goniometry, Brockhauser *et al.*'s [1] mini- κ goniometer head enables the reorientation of a macromolecular crystal for optimal X-ray data collection e.g. measuring Bijvoet pairs without the systematic error of radiation dose differences. Current third generation synchrotrons are producing more than enough flux density for MX experiments. Hence, the limitation for solving big complex structures is radiation damage. While κ goniometry can be used for minimising this problem by careful reorientation of the samples, it also supports systematic studies of radiation damage effects. The purpose of this work is to study the sensitivity of DNA crystals and optimise data collection methodologies. As well as sample reorientation, we also focus on the positive effects of off-axis data collections [2].

K-goniometer calibration proc.

The installation of multi-axis goniometers such as the ESRF/EMBL mini- κ goniometer system has allowed the increased use of sample reorientation in macromolecular crystallography (MX) [1]. Old and newly appearing data collection methods all require precision and accuracy in crystal reorientation. The proper use of such multi-axis systems has necessitated the development of new methods for rapid and easy to perform methods for establishing and evaluating device calibration. A diffraction-based method meeting these criteria has thus been developed for the calibration of the motors responsible for rotational motion [3]. This method takes advantage of crystal symmetry by comparing the orientations of a sample rotated about a given axis, and checking the magnitude of the real rotation to check it is the same as the calculated angle between the two orientations. Hence, the accuracy and precision of rotational motion can be assessed. This rotation calibration (RC) procedure has been performed on several beamlines at the ESRF and other synchrotrons.



The EMBL/ESRF mini- κ goniometer head (MK3) as mounted on the MD2M diffractometer on ID14-4 in a 'closed' and in a fully 'open' conformation, highlighting the three rotational axes and the α angle.

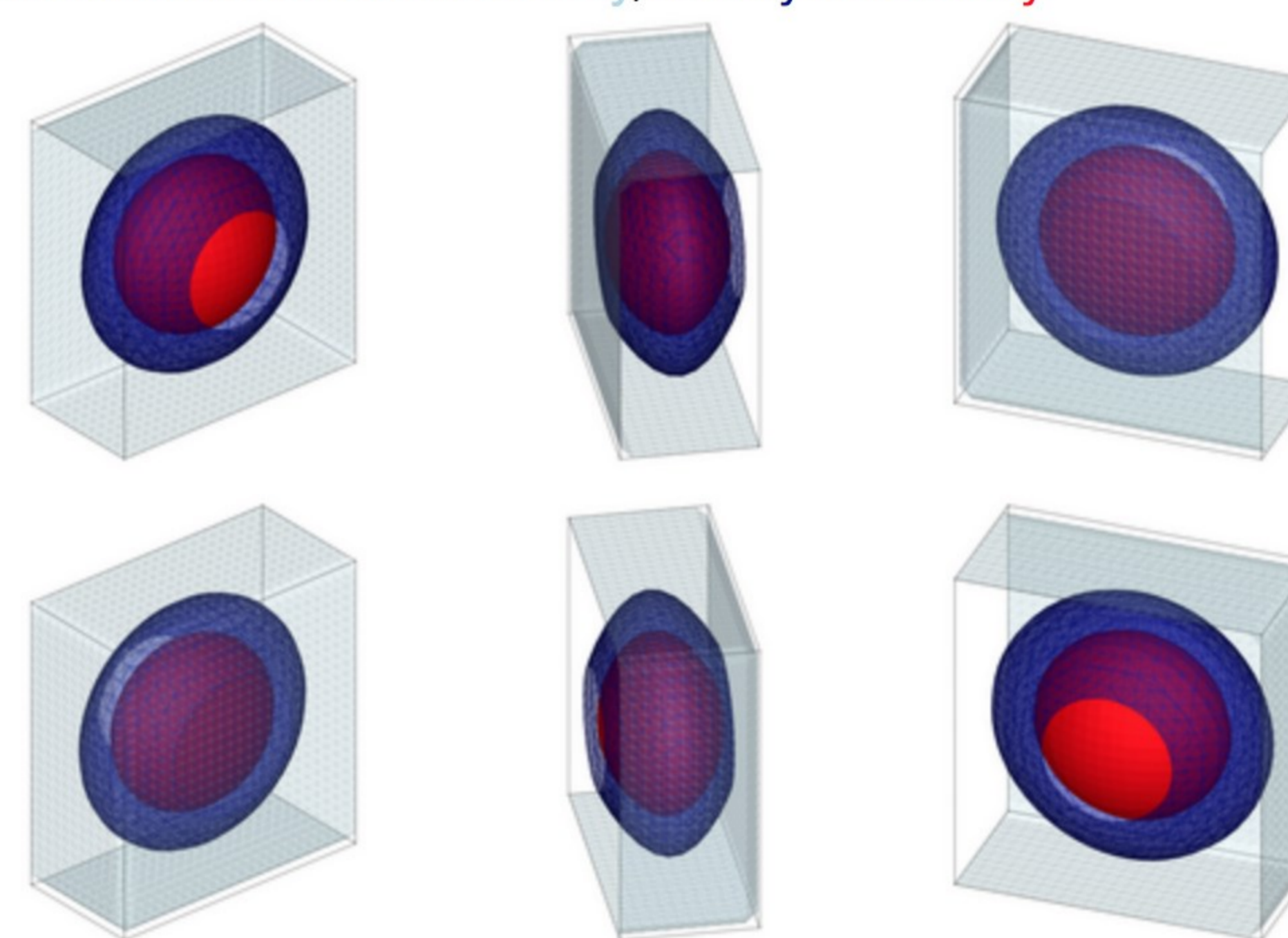


Alignment of rotation calibration at XDR2 beamline in ELETTRA synchrotron

Off-axis data collection

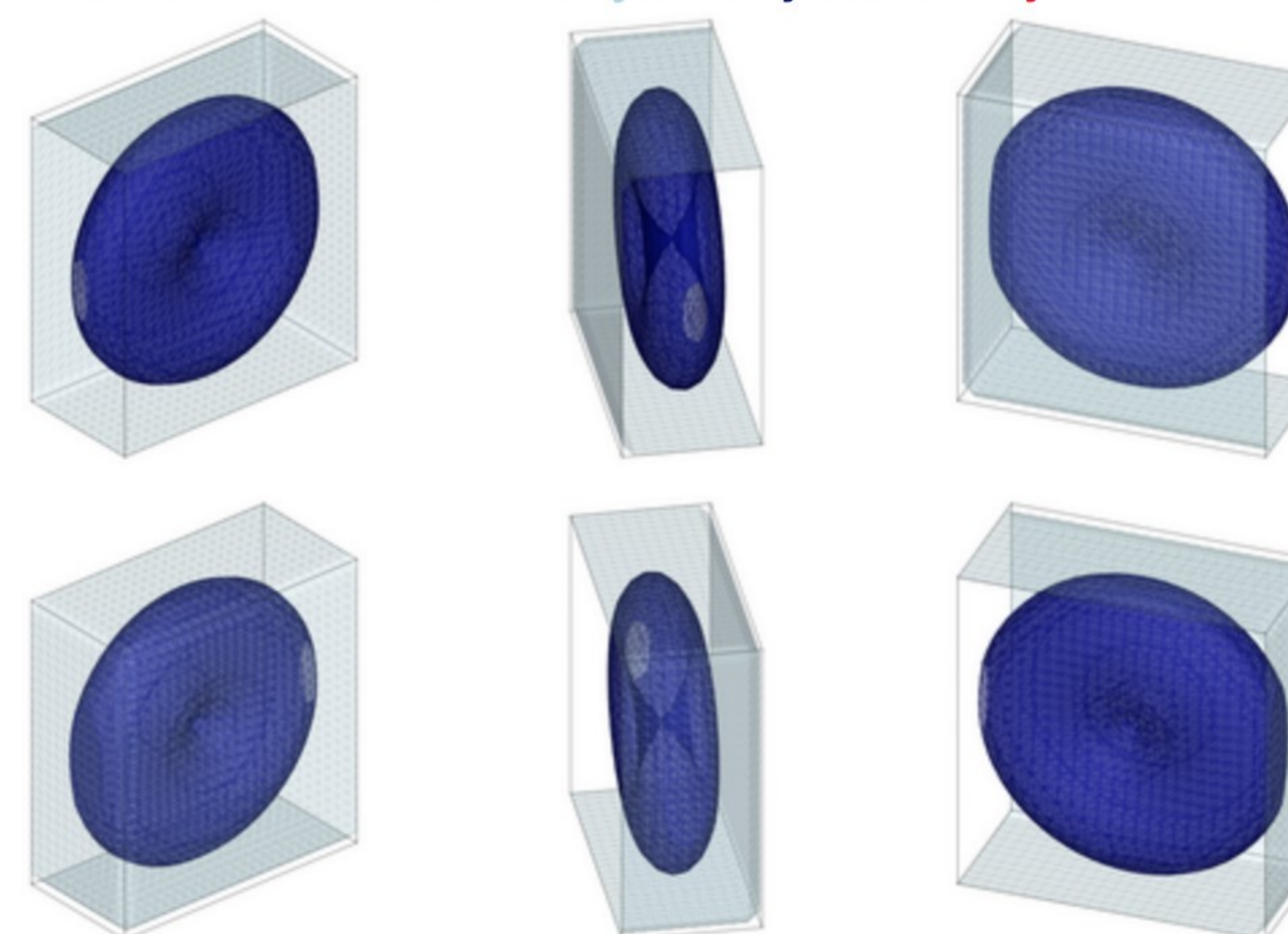
The use of a microfocus beam results in a hot central region which suffers high levels of radiation damage while other regions of the sample not continuously exposed to the X-ray beam show much less damage. Next to helical scans, off-axis data collection also allows a smearing of the damage effects of radiation dose and an optimal use of the available crystal volume [2]. Diffraction Weighted Dose (DWD), calculated by RADDOSE-3D [4], describes the diffraction capabilities of the sample with inhomogeneous dose history and its volumetric distribution. We are working on systematic studies to verify the usability of this model and extend it for the optimisation of data collection strategies for crystals of DNA and DNA binding complexes. During these studies, we use the mini- κ goniometer to align the samples with their front face perpendicular to the beam direction and compare the data quality as well as the specific damage while collecting with normal centring and with an off-axis setup.

Dose isosurfaces drawn at 0.1 MGy, 20 MGy and 30 MGy.



Crystal	
Type	Cuboid
Dimensions	34 15 37
PixelsPerMicron	0.7
AbsCoeffic	RD3D
UnitCell	
	37.4 37.4 163.0
	90 90 120
NumMonomers	18
NumResidues	16
ProteinHeavyAtoms	P 16
SolventHeavyConc	Ca 600 Cl 1200
SolventFraction	0.45
Beam Type	Gaussian
Flux	3.7e12
FWHM	15 15
Energy	12.81
Collimation	Rectangular 100 100
Wedge	0 360
ExposureTime	4
ROTAXBEAMOFFSET	9

Dose isosurfaces drawn at 0.1 MGy, 20 MGy and 30 MGy.



References:

- [1] The use of a mini- κ goniometer head in macromolecular crystallography diffraction experiments S Brockhauser, RBG Ravelli, AA McCarthy Acta Cryst. D (2013) 69 (7), 1241-125
- [2] Predicting the X-ray lifetime of protein crystals OB Zeldin, S Brockhauser, J Bremridge, JM Holton, EF Garman, PNAS (2013) 110 (51), 20551-20556
- [3] Rotation Axis Calibration of Multi-Axis Goniometers in Macromolecular Crystallography KI White, V Bugris, A McCarthy, RBG Ravelli, A Cassetta, S Brockhauser J. Appl. Cryst. (2018) 51 (5), 1421-1427.
- [4] RADDOSE-3D: time- and space-resolved modeling of dose in macromolecular crystallography OB Zeldin, M Gerstel, EF Garman. J. Appl. Cryst. (2013) 46, 1225-1230