

## Low background serial crystallography experiments

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Latest generation (upgraded) synchrotron sources and XFELs allow generating extremely small, high-intensity X-ray beams. In the field of serial crystallography this allows performing experiments with even smaller crystals and achieving higher resolution in the diffraction experiments. For time resolved laser pump-probe experiments this significantly relaxes the requirements on the pump laser power, as only a much smaller sample volume needs to be optically excited.

Another important parameter with strong influence on the quality and achievable resolution in a diffraction experiment, and which has not received too much attention over the last years, is background scattering. Background scattering arises from the interaction of X-rays with non-sample material, including air, sample supports, and liquids around the sample. Consequently an ideal experiment requires a naked sample to be measured in a high-vacuum environment, similar to the experimental conditions in electron microscopy or in the early days of serial crystallography with liquid jets at FEL sources running in high-vacuum.

We have developed several methods with the corresponding instrumentation to significantly reduce background scattering levels in serial crystallography experiments. For the reduction air scattering, we have developed the concept of a capillary beamstop, where the direct X-ray beam is enclosed by thin-walled tantalum capillaries shortly before and behind the sample. This reduces the free-path of the beam in air to a few millimeters. Flushing this remaining free path with helium further reduces background scattering. For sample delivery we have developed micro-perforated sample holders from single crystalline silicon allowing for efficient removal of mother liquor by blotting and which do not contribute to any background scattering. More recently and following this blotting approach we have developed a low-background tape drive system for sample delivery. Here a micro-crystal suspension is applied to micro-perforated polycarbonate tape, which transports the micro-crystal suspension to the X-ray interaction point. By attaching filter paper to the backside of the perforated tape all liquid from the suspension is efficiently removed and background scattering levels are significantly reduced.

Diffraction experiments with polychromatic X-rays performed at the BioCARS instrument at the APS and ID09 at the ESRF, France, have demonstrated the applicability of these low-scattering background diffraction methods for time-resolved serial crystallography experiments and have yielded high-quality datasets.