Macromolecular crystallography application

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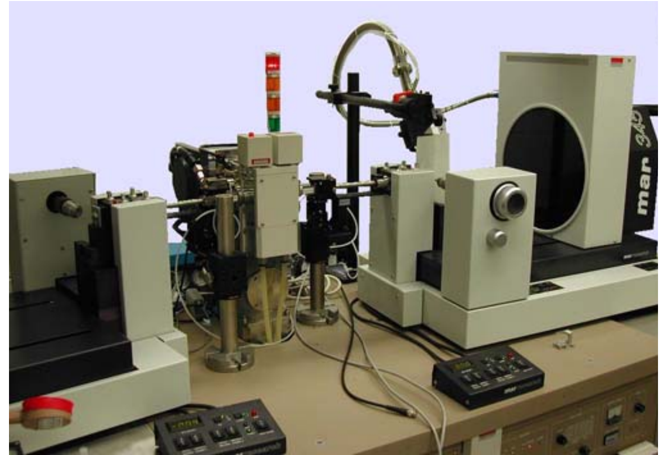
Courtesy of Mark A. Rould, Ph.D .



In June of 2004 we replaced the mirror systems on each of the two ports of a Rigaku RUH3R generator (each with a MAR-345 image plate detector) with the Xenocs *FOX2D CU 12_38P* optics. The previous optics were the MAR-Harvard side-by-side single-layer mirrors designed by John Genova (Harvard Medical School), and provided a tight, hot beam with little divergence and good spectral purity for a single-layer mirror. Before Xenocs replaced the mirrors, we collected complete cryo-diffraction datasets on two crystals: the benchmark standard tetragonal lysozyme, and a weakly-diffracting protein crystal with a relatively large cell edge (270A primitive) and relatively high mosaicity (1.2 degrees.) We collected data on the identical crystals under identical collection conditions (e.g., crystal-to-detector distance, crystal orientation, rotation angle width) and even collected the identical sweeps of data before and after installation of the Xenocs mirrors.

There were two main questions we aimed to address with these experiments:

- For well-diffracting crystals, what increase in throughput can we attain with the Xenocs mirrors (i.e., by what factor can we reduce data collection time without any sacrifice in data quality)?
- For more typical weaker-diffracting crystals with large unit cells, how much of an improvement in diffraction limit (resolution) can we attain for the same amount of exposure time as before ?

From others' experience with the Xenocs optics, we anticipated a three-fold increase in data collection throughput, and thus to address the first question we collected 3 minute exposures per frame with the previous optics and recollected the same data with 1 minute exposure times after installation of the Xenocs optics. A complete 360 degrees of data, 0.5 degree per frame, was collected in both cases. The table below provides the relevant data reduction statistics:

	Before	with Xenocs optics
Exposure time per frame	3 min	1 min
R_{merge} (20.-1.73A)	5.8%	5.1%
R_{merge} (1.79-1.73A)	19.0%	16.9%
<I>/<sigI> (20.-1.73A)	62	76
<I>/<sigI> (1.79-1.73A)	8.4	11.7
R_{anom} on Intensities	3.2%	2.9%
R_{anom} on Amplitudes	2.6%	2.4%

The identical lysozyme crystal was used for both datasets ($P4_32_12$: a=b=78.9A, c=37A). 720 frames of 0.5deg oscillation angle were collected per dataset, 3 minutes per frame using the MAR/Harvard monolayer side-by-side focusing mirrors, and 1 minute per frame using the Xenocs multilayer optics.

Clearly, we were able to increase our data collection throughput three-fold, and achieved a significant improvement in data quality at the same time.

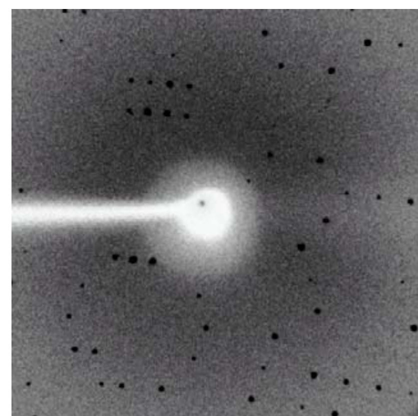


Figure 1 - Detail of Lysozyme Diffraction at Low Resolution
The excellent spectral filtering properties made possible by the multi-layer optics of the Xenocs mirror system are evident in this close-up image of the low resolution region of diffraction from a lysozyme crystal. Streaking is absent, as are reflections due to Cu-Kbeta wavelengths. (The lowest order reflection visible here corresponds to a Miller plane spacing of approximately 16 Angstroms.)

DMC-040818 - FOX2D Macro Xstall Application Note - 02

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Xenocs - A spin off company from Institut Laue Langevin

Application Note

FOX2D CU 12_38P focusing optics

In the second part of our tests, we used a weakly-diffracting crystal with a large cell edge (P422: a=b=77A, c=270A) to see how much the Xenocs optics improve data quality for a more typical crystal, all other conditions held constant. As can be seen from the statistics below, the Xenocs optics extended the resolution limit from 2.9A to 2.7A (determined as the highest resolution shell which gives <I>/<sigI> greater than 2), and significantly improved the quality of the entire dataset. Although the spot size at the detector is larger for the Xenocs optics than for the MAR-Harvard mirrors, we are still able to well resolve the closely spaced reflexions along the c*-axis.

	Before	with Xenocs optics
R_{merge} (15-2.7A)	24.3%	11.7%
R_{merge} (2.8-2.7A)	46.4%	21.4%
<I>/<sigI> (15-2.7A)	8.2	16.5
<I>/<sigI> (2.8-2.7A)	1.2	3.4
R_{anom} on Intensities	12.4%	5.6%
R_{anom} on Amplitudes	13.1%	4.9%

Statistics are given for all observations (no cutoffs, no rejections) in the range 15-2.7A. The identical crystal was used for both datasets, and the identical frames were collected with the same exposure time per frame: 360 frames of 0.5deg oscillation angle were collected per dataset, 10 minutes exposure per frame. With the previous optics, the resolution limit of usable data (ie, highest resolution with I/sigma > 2) was barely 2.9A.

The Xenocs optics extended the resolution limit beyond 2.7A, while providing substantially better data throughout the entire resolution range.

The crystal was mounted with its large (270A) unit cell axis aligned with the spindle (rotation) axis in order to keep the close reciprocal lattice spacing in the plane of the detector.

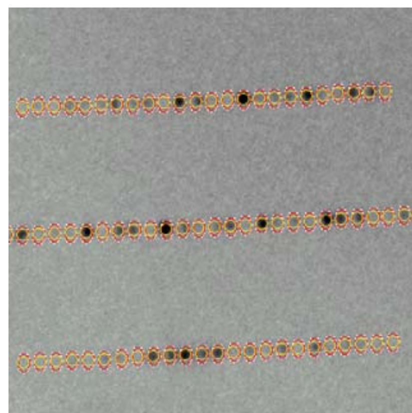

Figure 2 - Spatial Resolution of Diffraction Along a 270 Angstrom Cell Axis
Even at a crystal-to-detector distance of 250mm, reflections along the closely-spaced reciprocal cell axis are resolved on a MAR 345 image plate detector using the Xenocs *FOX2D CU 12_38P* optics. The space group of this crystal is P422, with cell parameters a=b=77A, c=270A. The spacing between reflections is about 10 pixels (1.5mm).

Four other aspects of the Xenocs design further increase its value. Re-alignment of the mirrors after a filament change or other generator maintenance is reduced to a straightforward two knob adjustment that takes no more than a few minutes. By keeping the mirror assemblies under a vacuum, damage to the mirror surfaces due to x-ray induced ionization of air is drastically reduced, much more so than is possible with helium-filled assemblies. By virtue of the excellent monochromatization possible with multi-layer optics, the beam from the Xenocs mirrors is essentially pure K-alpha, with no visible K-beta, allowing accurate measurement of the very low resolution reflexions free of spectral streaking. And lastly, the compact design of the Xenocs optics assembly greatly reduces the room space required to accommodate the entire x-ray diffraction equipment.

Conclusion

The Xenocs optics allow us to collect data three times faster than with single-layer optics, or to collect data with approximately two-fold improvement in signal-to-noise ratio given the same exposure times compared with one of the best single-layer optics available. Viewed from a financial perspective, with the two Xenocs optics, we got the equivalent of two more generators and four more detectors for a small fraction of the cost, and without tripling the physical space required. From all angles, we see the Xenocs optics as one of the most important and cost-effective purchase a macromolecular x-ray diffraction lab can make.

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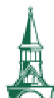

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