

Complete Data Sets Acquired with Different Polycapillary Optic - Source Configurations

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Abstract

The work described in this paper concerns a systematic study of the applications of both collimating and slightly focusing polycapillary optics to x-ray crystallographic structure determination of egg-white lysozyme using a standard rotating anode source and a low power tabletop microfocusing source. The results summarize a series of measurements comparing duplicate data sets obtained from an individual crystal. Intensity and data quality are discussed for measurements with pinhole collimator, collimating polycapillary optic, and focusing polycapillary optic. The collected data were analyzed using conventional analysis software. Opportunities and limitations on the use of conventional analysis software for data from focused beams will be discussed.

Introduction

Polycapillary x-ray optics can be utilized to optimize the characteristics of x-ray beams for special applications. They collect x rays over a wide solid angle from a divergent source and collimate them to a parallel beam (collimating optics) or focus them to a convergent beam (focusing optics) thus increasing the flux at the sample while maintaining the necessary angular divergence and cross section. Different polycapillary optics configurations have been used successfully in the past for a variety of single crystal diffraction applications yielding gains of one to three orders of magnitude in direct beam flux compared to conventional data collection methods using highly collimated beams created with pinhole collimators.¹

Setup

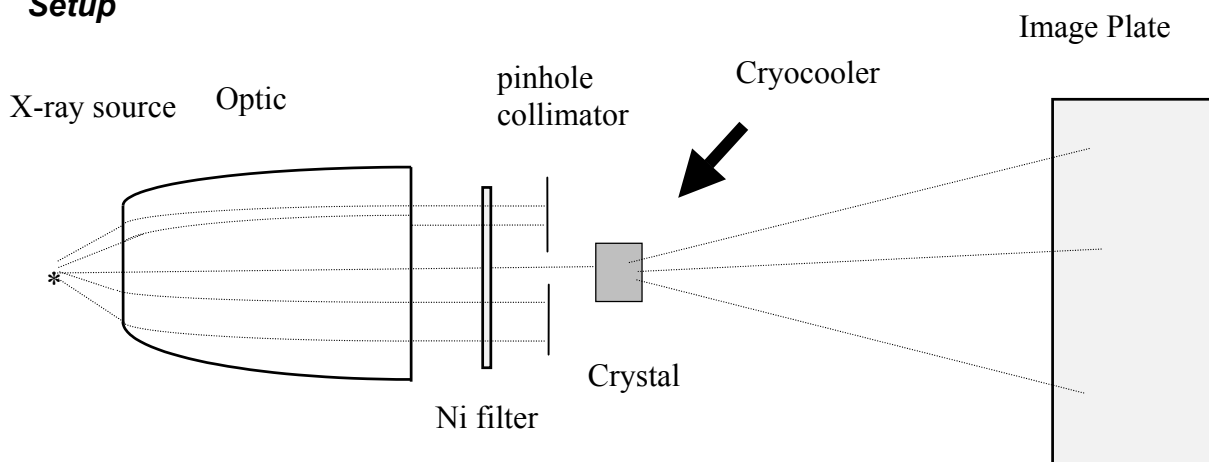


Figure 1. Schematic of setup including collimating optic.

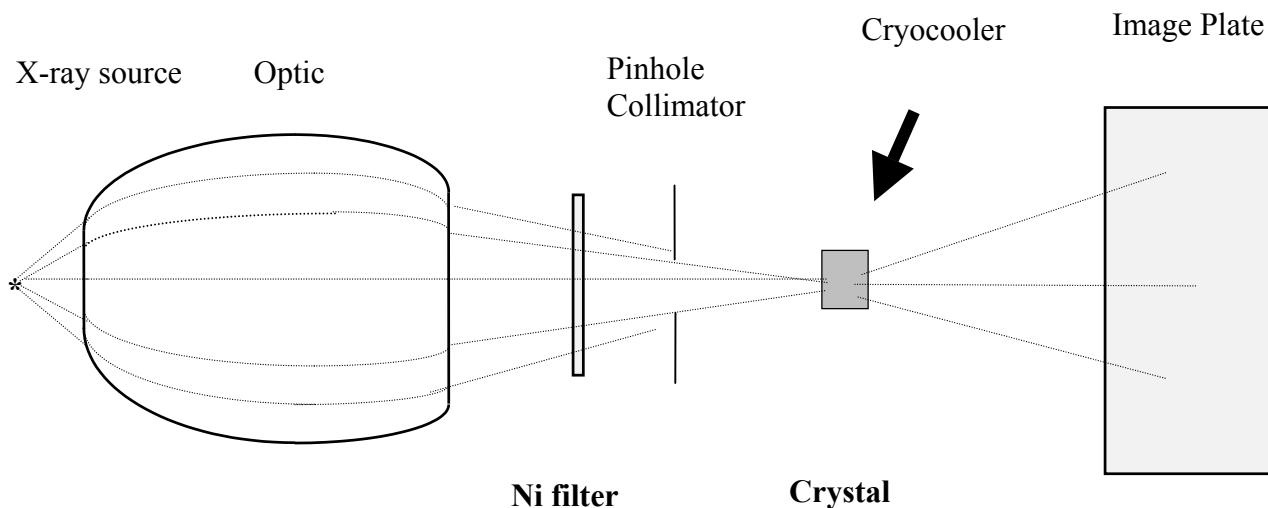


Figure 2. Schematic of setup including slightly focusing optic.

Source and Beam Requirements

Before designing a polycapillary optic for macromolecular crystallography one has to keep the source and beam requirements in mind. General requirements are a small, monochromatic and parallel beam. Most macromolecular crystals are between 0.3 mm and 0.5 mm in size. Pinhole collimators of the same size are used to minimize background. Usually the beam is then monochromated by diffracting the x rays from, e.g., the (002) planes of a graphite crystal. Also, a filter of the appropriate material is placed in the beam (e.g. Ni foil for Cu anode sources subtracts Cu K_{β} x rays). The divergence of the beam at the crystal is usually determined by the size of the pinhole collimator and the distance of the source, and is usually below 5 mrad. The beam can be manipulated by other x-ray optics, which may generate a focused beam with high convergence (10 mrad or more). Furthermore, there are source specific requirements. Most macromolecular crystallographers use rotating anode x-ray sources with anode to window distances of 30 to 40 cm and spot sizes ranging from 0.1 mm x 1.0 mm to 0.5 mm x 10 mm. We first take a look at polycapillary optics fabricated for such a rotating anode source.

Optic Characterization

Two optics were manufactured taking the above requirements into account. The properties of these optics are described in Table 1 and a schematic of the collimating optic setup and the slightly focusing optic setup are shown in Figure 1 and Figure 2, respectively. The rotating anode source used in this experiment has a modified shortened source to window distance of about 25 mm.

Property	Collimating optic	Slightly focusing optic
Output D (mm)	4.02	3.56

Input d (mm)	3.22	3.89
Length L (mm)	25	22
Input focus length (mm)	35	50
Output focus length (mm)	N/A	150
Input capture angle ω (rad)	0.09	0.08
Transmission	~ 28 %	~ 20 %

Table 1. Properties of polycapillary optics fabricated for rotating anode source.

Gain

If one talks about the gain of an optic one has to specify the exact definition; there is direct beam intensity gain, diffracted beam intensity gain, signal-to-noise ratio, etc. This paper discusses diffracted beam intensity gain for complete data sets, taken from a chicken egg-white lysozyme protein crystal. Special emphasis of the discussion of the data quality is made, since a gain in diffracted beam intensity with a corresponding loss in data quality is pointless. On the other hand a gain in direct beam intensity is impaired if part of the beam is not used.

To measure diffracted beam intensity gain, the optic was implemented in an existing protein diffraction setup, consisting of a Rigaku RU-300 rotating anode x-ray source with a 0.3 mm x 3.0mm source spot. The optic was mounted on micrometer controlled stages, which allowed alignment in the x-ray beam and positioned with the optic axis at a approximately 6 to 8 degree take-off angle. Direct beam intensities were monitored with a Molecular Structure Corporation (MSC) PIN diode, which converts beam intensities into milliamps of current. It has

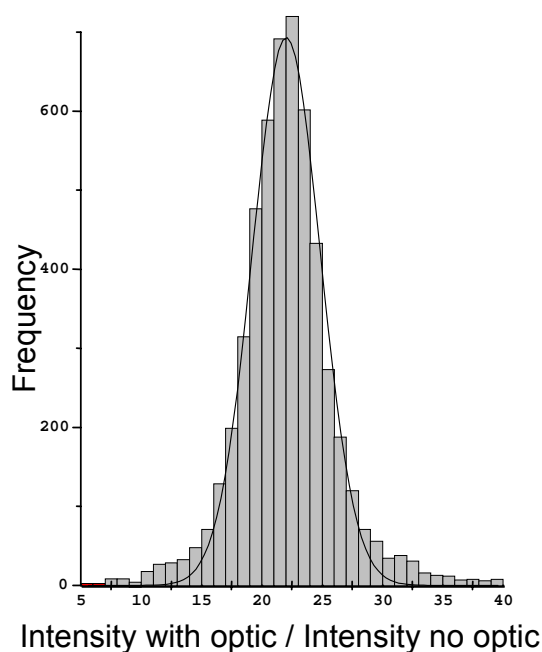


Figure 3. Histogram of ratios of intensities for common reflections (slightly focusing optic).

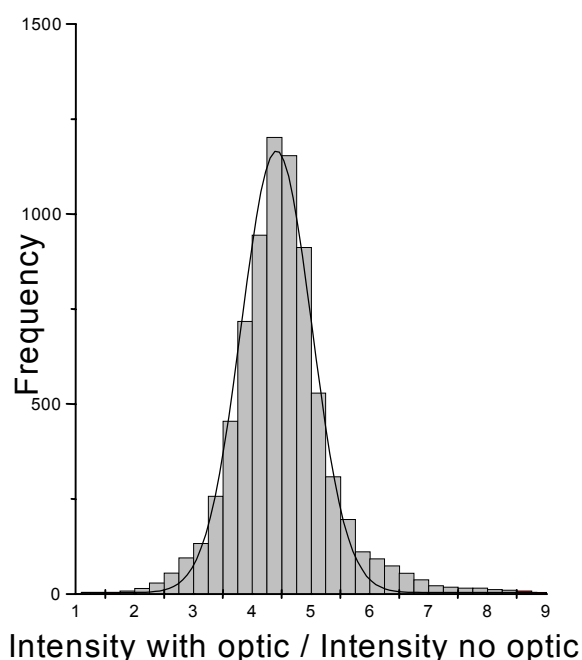


Figure 4. Histogram of ratios of intensities for common reflections (collimating optic).

a range of six orders of magnitude, with the “1 scale“ the least sensitive scale and the “6 scale” the most sensitive scale. The direct beam intensity from a Rigaku RU-300 rotating anode source operating at 40 kV and 60 mA through a 0.3mm pinhole collimator yielded intensities from 0.9 to 1.0 on the 4 scale. Note that usually one uses a monochromated beam for protein crystallography. Such a monochromator would decrease the direct beam intensity. Measurements of such a beam yielded 5 on the 6 scale¹. Here, for the purpose of comparison, 1.0 on the 4 scale is used as a standard for calculations through the remainder of the paper.

For the collimating optic, the direct beam intensity through a 0.3mm pinhole collimator is 4.2 on the 4 scale, equivalent to an intensity gain of 4.2. For the slightly focusing optic, the direct beam intensity through a 0.3mm pinhole collimator is 2.0 on the 3 scale, equivalent to an intensity gain of 20.

The diffracted beam intensity was determined using the same protein crystal at the same orientation for both measurements, one with optic and one without optic. Setup related restraints made it necessary to acquire the data set with the optic before the data set without optic. Since protein crystals undergo a continuous deterioration process after being exposed to ionizing radiation, the protein crystal often loses its diffraction ability before the end of the experiment. Besides keeping the crystal in a stream of cold nitrogen to slow down the deterioration process, it was necessary to monitor the degree of deterioration throughout the data acquisition process and reject bad data sets. Rejection of data sets usually occurred when the scale factor dropped below 90 % of the original scale factor. Choosing only those diffraction spots that appear in both data sets to be compared, the average intensity for the spots for each data set was determined. Figure 3 and Figure 4 show histograms of ratios of intensities for common reflections including the exposure time factor. These histograms were obtained by dividing the intensity of each of the reflections acquired with the optic, through the intensity of the corresponding reflection acquired without optic and then this ratio was multiplied by the ratio of the exposure time per frame. Fitting these histograms with a Gaussian, a diffracted beam intensity gain of **4.4** was recorded for the collimating optic and a diffracted beam intensity gain of **21.9** was recorded for the slightly focusing optic. Table 2 and Table 3 show summaries of the results for both of those optics.

	with collimating optic	without optic
Optic	collimating optic + 12.5 μm Ni	0.3 mm pinhole collimator + 12.5 μm Ni
sample	chicken egg-white lysozyme – frozen	chicken egg-white lysozyme - frozen
source setting	40 kV 60 mA	40 kV 60 mA
oscillation angle	1.25 deg (30 frames)	1.25 deg (30 frames)
time / frame	15 min	30 min
average intensity of common reflections	11676 \pm 884	4141 \pm 240
PIN diode intensity of direct beam	4.2 on 4 scale	1.0 on 4 scale
linear R-factor	0.093	0.089
resolution	1.6 \AA	1.7 \AA

Table 2. Comparison of collimating optic data.

	with slightly focusing optic	without optic
Optic	slightly focusing optic + 0.3 mm pinhole collimator + 12.5 μm Ni	0.3 mm pinhole collimator + 12.5 μm Ni
sample	chicken egg-white lysozyme - frozen	chicken egg-white lysozyme - frozen
source setting	40 kV 60 mA	40 kV 60 mA
oszillation angle	1.25 deg (31 frames)	1.25 deg (31 frames)
time / frame	4 min	30 min
average intensity of common reflection	10082 \pm 491	3911 \pm 255
PIN diode intensity of direct beam	2.0 on 3 scale	1.0 on 4 scale
linear R-factor	0.069	0.064
resolution	1.6 \AA	1.6 \AA

Table 3. Comparison of slightly focusing optic data.

All the data was analyzed with conventional software. No decline in data quality was observed. One indication for the quality of the data is the crystallographic R-factor for indicating the correctness of a model structure (Equation 1). The R-factor is calculated from a set of observed structure factors F_{obs} and a set of calculated structure factors F_{calc} ; k is a scale factor.

$$R = \frac{\sum_{hkl} \left| |F_{\text{obs}}| - k|F_{\text{calc}}| \right|}{\sum_{hkl} |F_{\text{obs}}|} \quad \text{Equation 1}$$

R-factors for data sets with and without optic are comparable and indicate good quality of the measurements (6.9% and 6.4% for the protein crystal used for the slightly focusing optic comparison, 9.3 % and 8.9% for the protein crystal used for the collimating optic comparison). Figure 5 and Figure 6 show internal R-factor versus resolution for the two different optics. Here internal R-factors compare the data set with the optic and the data set without optic among each other and the results are displayed versus different resolution ranges. The \blacklozenge series represents all the common diffraction spots while the \blacksquare series neglects some diffraction spots with high ΔF values. The internal overall R-factor for the collimating optic is 9.4 % and the internal overall R-factor for the slightly focusing optic is 8.4 %.

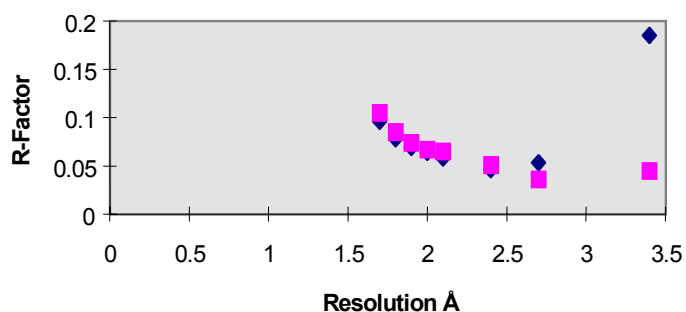


Figure 5. R-Factor versus resolution for collimating optic.

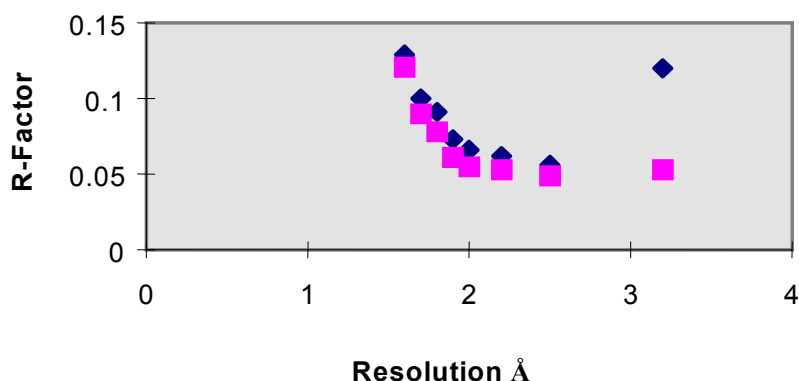


Figure 6. R-Factor vs. Resolution for slightly focusing optic.

Low power polycapillary based system for protein crystallography

While the rotating anode – polycapillary optic combination has been proven a powerful tool for macromolecular crystallography, there is ongoing work to develop a system with the highest possible x-ray flux that is small, reliable, convenient and extremely affordable to use for laboratory based rapid evaluation of crystal growth techniques and for determination of the purity and structural properties of protein crystals prior to eventual synchrotron based high resolution measurements. A systematic study of the applications of polycapillary optics to x-ray crystallographic structure determination of standard system chicken egg-white lysozyme is under way. Using a low power tabletop microfocus x-ray source (Oxford X-ray Instruments) designed for optimum coupling with polycapillary collimating optics, one has obtained an x-ray power with 8 keV (Cu K_{α}) x-rays of 4.5×10^{-5} Watts/mm², comparable to that obtained with a 4.5 kW rotating anode source. Table 4 shows the results for the two complete data sets that have been acquired with this source - optic combination. The direct beam intensity of these data sets, measured with a MSC PIN diode at 1.0 on 4 scale, compares to the direct beam intensity obtained with the rotating anode source from the measurements described above (see Table 2 and Table 3). Figure 7 shows a diffraction image taken with this source – optic combination. The exposure time per frame was 10 minutes and one can detect diffraction spots up to 1.9 Å resolution.

	data set 1	data set 2
Optic	collimating optic + 600 μ m pinhole collimator + 12.5 μ m Ni	collimating optic + 600 μ m pinhole collimator + 12.5 μ m Ni
sample	chicken egg-white lysozyme at room temperature	chicken egg-white lysozyme at room temperature
source setting	38 kV 20 W	38 kV 20 W
oscillation angle	2.0 deg (45 frames)	2.0 deg (45 frames)
time / frame	10 min	10 min
PIN diode intensity	1.0 on 4 scale	1.0 on 4 scale
resolution	1.9 Å	1.9 Å

Table 4. Low power source – collimating optic diffraction data.

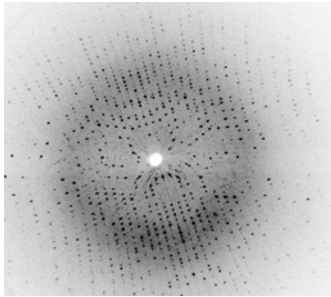


Figure 7. Lysozyme diffraction image taken with microfocus source – polycapillary optic combination.

Conclusions

Performance and testing of polycapillary x-ray optics for macromolecular crystallography advances rapidly. The diffracted beam intensity gain was determined for a collimating optic and a slightly focusing optic and the results compare to the direct beam intensity gain previously obtained. The collimating optic yields a gain of 4.4 and the slightly focusing optic yields a gain of 21.9 in diffracted beam intensity over the same system with no optic.

Further work is being done to test the performance of a low power polycapillary based tabletop system for macromolecular crystallography.

Acknowledgments

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References:

¹ S.M. Owens, J.B. Ullrich, I. Yu. Ponomarev, D.C. Carter, R.C. Sisk, J.X. Ho, and W.M. Gibson, "Polycapillary x-ray optics for macromolecular crystallography", *SPIE Proceedings*, vol. **2859**, 200-209, (1996).