Micro-Crystallography Developments at GM/CA-CAT at the APS*

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Abstract. Recently, several important structures have been solved using micro-crystallographic techniques that previously could not have been solved with conventional crystallography. At GM/CA-CAT we continue to develop micro-crystallographic capabilities for difficult problems such as small crystals of large macromolecular complexes or membrane proteins grown in the lipidic cubic phase. This paper will describe three major upgrades to our arsenal of tools, "mini-beam" collimators, active beamstop, and an improved goniostat. Our "mini-beam" collimators have evolved to a new triple-collimator fabricated from molybdenum as a uni-body. This has significantly improved the robustness, ease of initial alignment, and reduction of background. More recently, two prototypes of a quad-collimator have been developed and fabricated to provide a selection of mini-beams of 5, 10, 20 µm and a 300 µm scatter-guard on a single body. The smaller beams and samples have increased the demand on the tolerances of our goniostat. To meet these challenges we have designed and implemented a goniostat with a 1-micron peak-to-peak sphere of confusion. This is a significant improvement over the previous 6 micron sphere of confusion of the commercially available air-bearing and XY stages. Finally, an "active beamstop" has been constructed. This will provide non-invasive, real time feedback at the sample during data collection.

Keywords: Mini beam, Mini Collimator, Goniostat, Active beam stop
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INTRODUCTION

A trend in macromolecular crystallographic projects is to focus on difficult biological problems such as multi-protein complexes, large RNA molecules, or membrane proteins. Many of these studies start with micron sized crystals. Investigators are left pondering whether to grow larger crystals or pursue the tiny crystals that they have in hand. At GM/CA-CAT we continue to develop micro-crystallographic capabilities for these difficult problems. Three of these tools, mini-beam collimators [1, 2, 3, 4], an improved goniometer, and an active beamstop [5], have enabled these investigators to advance their research on small crystals that may not have been possible in the past.

MINI BEAM COLLIMATOR

In 2007, we offered single collimators for mini-beams of sizes 5, 10, and 20 µm as well as a 300 µm scatter guard to accommodate the fully focused beam [2]. The experimenter was able to optimize the signal/background ratio by matching the beam size to the crystal size. For larger crystals (>30 µm), the user defines the size of the focused beam with slits, then uses the 300 µm collimator to serve as a scatter guard. The advantages to the experiment were obvious: better signal/background and the capability to scan large "bad crystals" to locate smaller "good regions" that can be exploited with a mini-beam [3]. The mini-beam proved to be a technical and popular success, however the switching of the single collimators often times involved beamline staff intervention [1, 4].

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In February of 2008, a triple-collimator apparatus was developed [2]. This implementation incorporated two mini-beam collimators and a 300 µm scatter-guard on one mount. Improvements in the mechanical reproducibility/stability of the collimator stage and the software integration of the selection of the appropriate beam size have resulted in a one-button "User" feature [4]. This triple-collimator assembly has been in routine use on both undulator beamlines. The device has proven to be stable during routine operation. The convenience of the switching between beam sizes has further increased the number of experimenters that use this feature. This triple collimator was successful but too fragile for our high throughput user program.

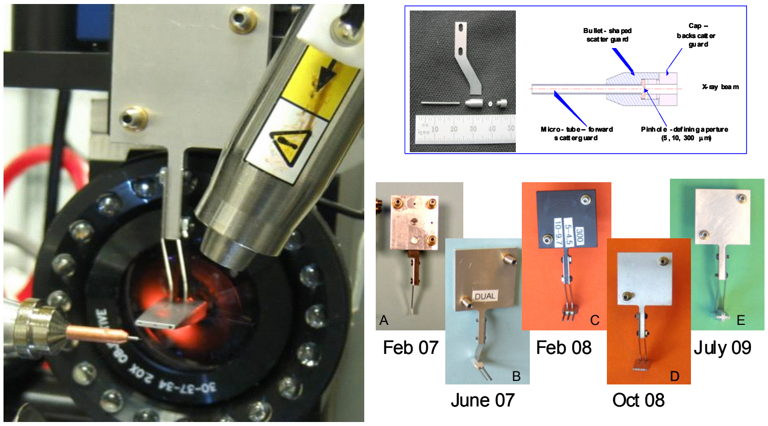


FIGURE 1. . Mini-beam collimators. A triple, uni-body mini-beam collimator on the ID-lines shown on left. The schematic diagram of assembly of each mini-beam collimator shown on right top. (A) Single, (B) Dual, (C) Triple, (D) Uni-body triple and (E) Uni-body quad collimator

Although the support and positioners are reliable and reproducible, the apparatus was vulnerable to mechanical impact during manual sample mounting or sample washing with liquid nitrogen. In order to address this problem, a new type of triple-collimator was developed and fabricated from a single piece of molybdenum in October of 2008. It significantly improved the robustness, ease of initial alignment, and reduction of background. Figure 2 shows a triple-collimator with the new "uni-body" design that had fewer parts, was easier to assemble, provided a higher degree of concentricity of the beam-defining pinhole and the exit aperture, and had better co-linearity of the three collimators. In addition to these advances, it had an insignificant impact on the sample visualization when compared to its predecessor collimator. In February 2009 uni-body collimators were fabricated and implemented on both ID-lines (Fig. 1). Experimenters were still left with a choice of either a (5, 10, 300 µm) - or a (10, 20, 300 µm)-triple collimator. In order to avoid any loss of beamtime, the experimenters felt compelled to choose one set over the other at the start of their experiment.

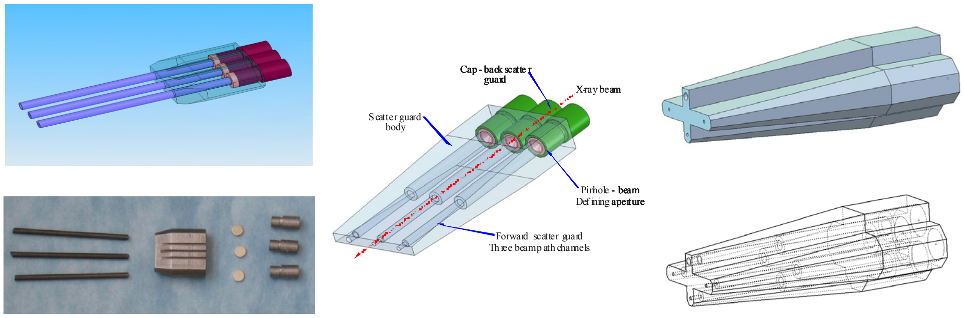


FIGURE 2. Upgrade design of collimators. Left shown a triple collimator with tubes, Center is Uni-body tripe collimator and the right shown a new uni-body quad collimator.

In July of 2009, a prototype of the quad-collimator [Fig.2 right] were designed and fabricated. The quad collimators allow experimenters to quickly select 5, 10, or 20 micron mini-beams or the 300 µm scatter guard (70 x 20 μm^2 FWHM (H x V, 23-ID-D) or 120 x 25 μm^2 (H x V, 23-ID-B)).

The mini-beam has been successfully implemented at GM/CA CAT. The triple collimators provide a variety of sizes and intensities ranging from the 5 micron beam (FWHM) with an intensity of ~7x10^{10} photons/sec, to a 20 micron beam (FWHM) with an intensity of ~1x10^{12} photons/sec, as seen in the table in Figure 3. Experimenters require the capability to switch back and forth between "full"- and "mini"-beam depending on the size and nature of their samples. A significant contribution to the popularity of its use is the convenient, single button control of the collimator motion. We have designed and included this feature in our user-friendly Blu-Ice GUI software. Eighty percent of our users take advantage of the mini-beam for data collection. The design can be exported to standard beamlines to enable micro-crystallographic capabilities [Fig. 3].

	23-ID-B			23-ID-D		
	Beam Size	**Intensity (f/s)**	**Flux Dens. (f/s/μm^2)**	**Beam Size**	**Intensity (f/s)**	**Flux Dens. (f/s/μm^2)**
Natural Focus	25 μ x120 μ	5 x 10^{12}	2 x 10^9	20 μ x70 μ	1 x 10^{13}	4 x 10^9
Mini Beam collimator	Ø20μ	5 x 10^{11}	2 x 10^9	Ø20μ	1 x 10^{12}	3 x 10^9
	Ø10μ	1 x 10^{11}	2 x 10^9	Ø10μ	2 x 10^{11}	2 x 10^9
	Ø5μ	4x 10^{10}	2 x 10^9	Ø5μ	7x 10^{10}	4x 10^9

FIGURE 3. Measured beam sizes, intensities and flux densities are listed above. The pinhole selects the central part of the focused beam.

GONIOMETRY IMPROVEMENTS: MEMBRANE-BASED SAMPLE XY POSITIONER

Micron sized (5-20 μm) beams and similarly sized crystals require accurate positioning and reduced goniometer wobble or sphere-of-confusion (SOC). The general rule of thumb is that the SOC should not exceed 10% of the size of the X-ray beam or sample. Thus, for the 10-μm X-ray beam the SOC should be less than a 1μm. Such a requirement is hard to achieve, especially in the case of a goniostat with a horizontal rotation axis. The major problem is that when the goniometer rotates, the force of gravity acting on the sample positioning stages change direction, contributing to an increase in the SOC. Figure 4 shows the SOC achieved with commercial off the shelf stages (~6 μm peak-to-peak) versus the GM/CA designed stages (~1 μm peak-to-peak). We have achieved this by implementing several improvements in the goniometer sphere-of-confusion as well as in the sample positioning stages.

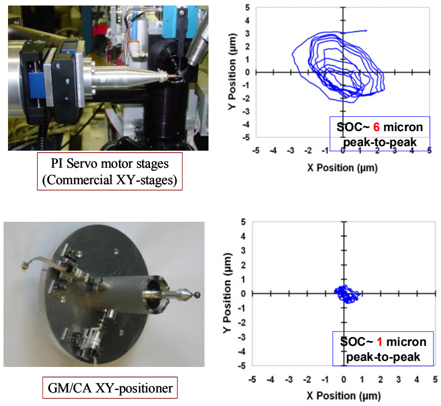


FIGURE 4. On the left: sample-XY positioners built with PI M-III XY-stages (top) and GM/CA-designed and built XY-positioner (bottom) with a tooling ball at the sample position for SOC measurements. On the right: plots for the measured XY trajectory of a tooling ball as the goniometer with sample positioner was rotated by a few turns. The SOC of the positioner with commercial XY-stages exhibits a peak-to-peak excursion of about 6 μm (top) with 4-turn rotation, and the SOC of the GMCA-built positioner exhibits a peak-to-peak excursion of about 1 μm with 2-turn rotation (bottom).

Since September 2008, we had a working prototype of the GM/CA sample XY positioner with a peak-to-peak SOC of about 2 microns. Since then, we have disassembled and rebuilt the air bearings (goniometer) and improved the rigidity of the sample XY stages. The improved sample XY positioners were installed on the 23ID beamlines last fall and the SOCs (measured several times over the year) were 1.3 μm and 1.0 μm, for the 23ID-B and 23ID-D beamlines, respectively. The effects of prolonged use on the SOC remain to be determined. Following the generally accepted rule that the SOC should be 0.1 times the size of the beam or crystal, whichever is bigger, we have achieved our goal for the 10-μm X-ray beam. However, for the 5-μm X-ray beam we need a SOC to be less than 0.5 μm. A design of a new device to meet this specification is nearly complete. It will be fabricated and tested in the next few months.

ACTIVE BEAMSTOP-BASED ON PHOTOCURRENT

The I_0 monitor, which is located downstream of the beam defining slits and the timing shutter, provides a measure of the incident flux for "large" beam experiments. The mini-beam collimator is downstream of the I_0 monitor so a new monitor was needed for mini-beam experiments. Initial tests showed that a PIN diode positioned to record X-rays scattered from the air between the mini-beam collimator and the sample was unreliable as an intensity monitor because it also picked up scattered X-rays from the sample and beamstop. Thus, we chose to develop an active beamstop to measure the intensity of the beam transmitted through the sample (Fig. 5). The sample only absorbs a few percent of the incident beam so the transmitted intensity is a useful monitor of the incident intensity. A plot of intensity vs. time, and therefore image frame, would allow the user to monitor beam intensity during data collection and serve as a warning when the beam alignment drifts (Fig. 6).

In order to maintain a stable microbeam, it is essential that an accurate reading of the beam intensity be available at the sample position. We have developed an active beamstop which provides a signal of a few nanoamps that is proportional to x-ray flux. The signal, based on the photoelectric effect, provides a non-invasive, real-time intensity monitor at the sample during data collection for mini-beam and automated realignment. The 1.0 mm diameter active beamstop consists of a 1.0 mm diameter beam stop and a 1.0 mm diameter concentric collector, with an applied voltage that drives electrons from stop to collector (Fig.5).

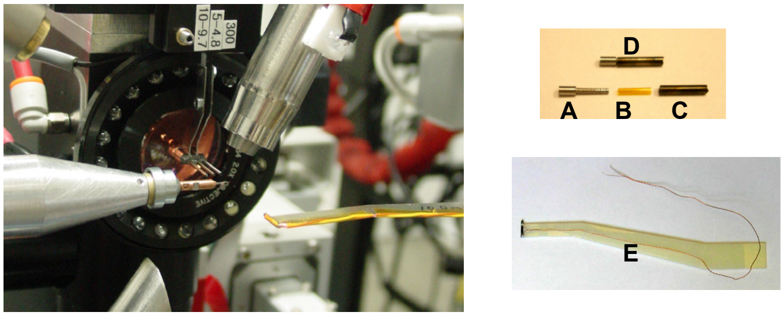
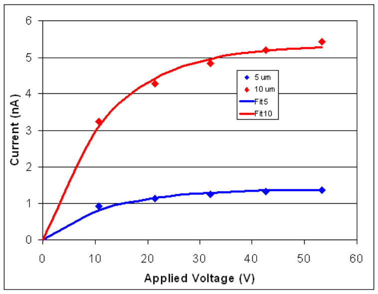


FIGURE 5. . A 1.0 mm diameter active beamstop installed on 23ID beamlines. Right top: the beamstop is shown both before and after assemble (A) Beam stop (B) Insulator, (C) Collector and (D) Assembled active beamstop, Right bottom: the fully assembled beamstop is shown mounted on a electrical insulating support arm (E) with attached wires to measure the photocurrent.



Pinhole size (μ)	Intensity (photons/sec)	Measured current with 53 V bias (A)
300	5.50E+12	1.46E-07
10	2.18E+10	5.43E-09
5	4.58E+09	1.38E-09

FIGURE 6. Our design incorporates an electron collector (Fig. 5), that provides a signal that is linearly proportional with the incident intensity

Generally a 1.0-mm diameter beamstop is used, but plans to implement a 0.5 mm diameter beamstop at half the distance from the sample are underway. Reducing the path length of the direct beam in air will reduce the background intensity of X-rays scattered by air thereby improving the signal-to-noise ratio in the diffraction pattern.

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