

# Development of a real-time timing-shutter performance monitor for protein crystallography

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In order to accurately monitor shutter timing events and long-term shutter performance, a timing-shutter monitor has been developed. This monitor uses a photodiode to capture X-ray-induced fluorescence from the shutter blade in synchrony with goniometer rotation to measure shutter opening and closing delay times, as well as the total time that X-rays are exposed to the sample during crystallographic data frames.

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**Keywords:** X-ray shutter; timing; protein crystallography.

## 1. Introduction

One of the many instrument challenges facing protein crystallography synchrotron beamlines is the synchronization of fast sample rotation with shutter timing. Because data sets are collected as the sum of sequential images, errors in timing effect all reflections that are not fully recorded in a single image (partials). When exposure times are 1 s per image or less, over- or under-exposed partial reflections due to timing errors begin to significantly impact the integrity of the data set, even when these errors are only a few milliseconds. The magnitude of the effect is small for samples with moderate (0.4°) or larger mosaic spreads. However, as crystal perfection improves, the angular range covered in a few milliseconds becomes an increasingly larger percentage of the total reflection, leading to larger integration errors.

Table 1 demonstrates the effect of timing errors on data sets taken at the SBC beamline 19BM at the Advanced Photon Source from frozen lysozyme. Data were collected using 1° frames exposed for 1 s each. Sample mosaicity (full width) after refinement using *HKL2000* (Otwinowski & Minor, 1997) was determined to be 0.166°. Data were collected over identical 100° ranges on the same sample. Shutter timing was set correctly (zero offset) for the reference data set. The closing delay time was then re-programmed so that the shutter closed 10 and 20 ms before the frame ended, and data were collected using each offset closing delay time. As can be seen from the table, a modest 10 ms timing error can degrade low-resolution data. At 20 ms, the impact is noticeable throughout the entire data set. Since low-resolution reflections are of prime importance for structure solution, minimizing errors due to timing is essential.

Typical pneumatic timing shutters contain O-ring seals around the cylinder shafts to maintain vacuum integrity. O-rings create a sticking force around the cylinder shaft that varies depending upon the frequency of shutter use and the air pressure applied. We have measured a time difference of as much as 3–5 ms between the first exposure and subsequent exposures of a shutter due to this sticking force, even if the shutter has only been idle for a few minutes. As a further complication, when exposure times lengthen, e.g. 10 s or more, the sticking force partially returns, causing variability in the

delay time. Multiple blade shutters use one blade to open and a separate blade to close, thus doubling the potential for error. With age, O-rings begin to degrade and/or lose lubrication, resulting in a gradual reduction in shutter performance that may go undetected without routine monitoring.

To minimize these problems, we have started using an electrically activated Uniblitz X-ray shutter (Vincent Associates, Rochester, NY, USA). The Uniblitz shutter blade is activated by an electronic pulse and is held against a rubber stop until closed *via* a spring return. Rather than use the built-in signalling device to indicate when the shutter is 80% open or closed, we have designed a shutter-monitoring device that measures fluorescence from the shutter blade. This allows us a real-time monitor of shutter performance and provides an accurate measure of shutter opening and closing delays measured against the actual focused X-ray beam.

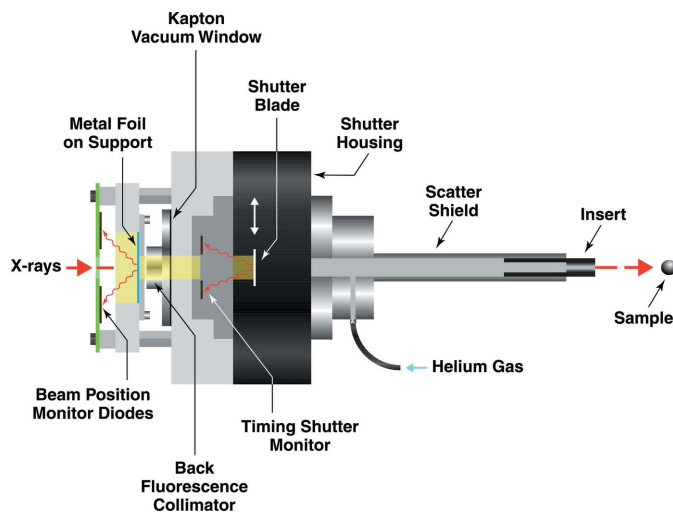
## 2. Timing-shutter monitor design

The Uniblitz X-ray shutter, model XRS6, contains a single 0.25 mm PtIr blade with a 6 mm clear aperture and a specified opening time of 4.4 ms. Fig. 1 shows the detailed environment around the timing shutter. The beam-defining slits (not shown) are located 15 mm upstream of the beam-position monitor (bpm) (Alkire *et al.*, 2000). A

**Table 1**

*R*-factor squared in different resolution shells for frozen lysozyme data sets collected with 0, 10 ms and 20 ms timing offsets.

Resolution (Å)		<i>R</i> -factor squared		
Lower limit	Upper limit	0 ms	10 ms	20 ms
50.0	3.23	0.0270	0.0350	0.0490
3.23	2.56	0.0320	0.0380	0.0510
2.56	2.24	0.0430	0.0450	0.0560
2.24	2.04	0.0530	0.0540	0.0630
2.04	1.89	0.0760	0.0740	0.0830
1.89	1.78	0.113	0.112	0.125
1.78	1.69	0.150	0.152	0.165
1.69	1.62	0.190	0.194	0.212
1.62	1.55	0.235	0.236	0.262
1.55	1.50	0.287	0.289	0.317



**Figure 1**  
Timing-shutter monitor environment.

Kapton end-window terminates the vacuum at a distance of 100 mm from the sample. The Uniblitz housing is oriented so that the shutter blade opens vertically, parallel to the smallest beam dimension. Prefocus beam dimensions (full width at half-maximum) at the shutter are approximately 0.120 mm (vertical)  $\times$  0.700 mm (horizontal). Beam size at focus is about 0.070 mm  $\times$  0.070 mm.

Built into the end-cap of the vacuum enclosure on the atmospheric side is an array of four 5 mm<sup>2</sup> photodiodes (UDT Sensors, Hawthorne, CA, USA; model S-25CL) mounted onto a printed-circuit board. Diode capacitance is low (95 pF at  $-5$  V bias) in order to maintain high speed. Currently only one of the four diodes is used in the timing-shutter monitor. The others are available for beamline diagnostics. The distance from the diode to the shutter blade is approximately 10 mm. To isolate bpm signals from shutter-blade fluorescence, a back-fluorescence collimator has been installed between the two devices.

A scatter shield with removable insert is attached to the downstream side of the shutter housing. At 0.5 mm diameter, the insert opening is small enough to keep all forward-scattered radiation from the slits, bpm metal foil and Kapton end-window within the 1 mm vertical beamstop profile (Alkire *et al.*, 2004) without interfering with the focused X-ray beam. To further reduce absorption and scatter from air, a slow flow of helium gas is fed through the scatter shield and shutter housing. The sample-to-insert distance is  $\sim$ 14 mm.

If the main beam energy is above the  $L_3$  edge of either Ir (11.215 keV) or Pt (11.564 keV), fluorescence from the shutter blade is captured by the photodiode. The photodiode is used in photoconductive mode with a voltage bias provided by a variable-gain low-noise current amplifier (Femto, model DLPCA-200, Electro Optical Components, CA, USA). At 12.66 keV, an amplifier gain of  $10^8$  and an incident photon flux of  $1.4 \times 10^{11}$  X-ray photons s<sup>-1</sup>, the output voltage is 1.6 V. Output from the amplifier is fed into a custom-designed discriminator with comparator. The comparator output goes high when input from the diode is above a preset level, typically 1 V. Comparator and timing-shutter signals are input to a National Instruments PCI-6602 80 MHz counter/timer card in a Windows-based computer that performs the timing measurements. Timing output is displayed on the computer *via* a Visual Basic graphical interface.

Perfect shutter-goniometer synchronization happens when the shutter opens at precisely the starting rotation angle of a particular

frame and closes at precisely the ending angle of the frame. Achieving this situation requires an accurate measure of the time difference between the shutter activation signal and the time when the shutter actually opens or closes, *i.e.* the shutter delay time. Knowing the desired angular width and exposure time for a frame and appropriate shutter delay times, the exact goniometer encoder values needed to synchronize the shutter and goniometer can be calculated for the frame.

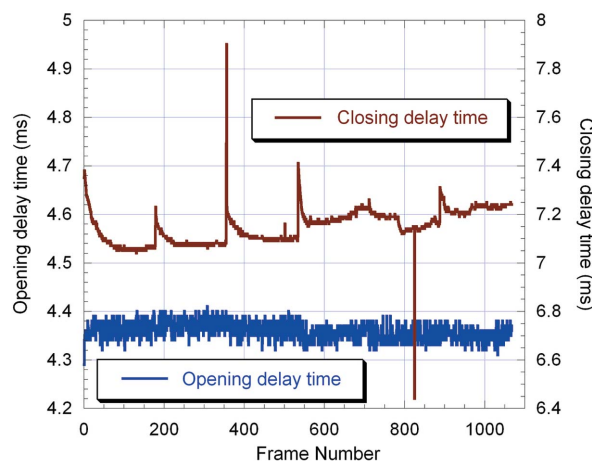
At SBC beamlines, the goniometer motor (Heidenhain encoder, resolution 180000 counts deg<sup>-1</sup>) is controlled by a PMAC interface (Delta Tau Data Systems). When the calculated encoder target for the opening angle is reached, custom hardware is used to generate a 1 ms-wide pulse that initiates a logic level shutter open signal. The shutter signal stays high until the closing target value is reached, whereby a second pulse is issued to de-activate the shutter signal.

As the shutter opens, the timing-shutter monitor output signal drops below 1 V, causing the comparator signal to fall. The time difference between the shutter activation signal and comparator transition is measured by the PCI-6602 card, producing the shutter opening delay time. The duration of the comparator low-level state is recorded by a second counter, measuring the total time that the sample is exposed to X-rays during the frame. A third counter measures the total length in time of the logic level shutter pulse. The closing delay time is calculated from measurements made by the three counters. 19BM software uses separate parameters for the opening and closing delay times to account for different opening and closing shutter speeds.

### 3. Results and discussion

Opening and closing delay times recorded by the timing-shutter monitor are shown in Fig. 2. Data from six 180-frame data sets, 1° frame<sup>-1</sup>, are presented. The first three sets were collected using 1 s frames, followed by 10, 1 and 5 s frames. Opening delay times for all data average  $4.36 \pm 0.02$  ms. No obvious breaks occur between data sets, indicating shutter opening to be quite uniform, regardless of exposure time.

Closing delay times, however, show considerably more structure, averaging  $7.15 \pm 0.07$  ms over the six data sets. Each data set starts with a noticeably longer closing time for the first image, followed by



**Figure 2**  
Opening and closing delay times measured using the timing-shutter monitor for six data sets with 1, 1, 1, 10, 1 and 5 s exposure times, respectively. Opening delay times average  $4.36 \pm 0.02$  ms for all frames, while closing delay times average  $7.15 \pm 0.07$  ms.

measurably shorter times as the data set progresses. Only between the fourth and fifth data sets is the transition relatively smooth. Changes in closing delay times between the first and last frame of each data set are modest, averaging 0.26 ms for all six data sets. Variations in closing times are most likely attributable to the spring return mechanism or the rubber stop that, according to the manufacturer, may cause some sticking problems under the right conditions. Future shutters of this design are being modified with Teflon-coated stops to eliminate the potential sticking problem.

Data monitored during every shutter cycle are displayed in real time on the computer and written to a log file. Output includes opening and closing delay times, X-ray exposure time of each frame, a time stamp for each exposure and an error flag if one of the delay times exceeds a pre-determined limit. The average X-ray exposure times per frame measured by the shutter monitor for the 1, 5 and 10 s frames were 0.9995 (15), 5.0007 (6) and 10.00112 (6), respectively.

While the monitor works well as described, attenuating the incident beam can cause the output voltage to fall below the comparator threshold. Software modifications to adjust amplifier gain automatically to match attenuator selection are pending. It is important to avoid saturating the amplifier because amplifier recovery time

lengthens the measured opening delay time by as much as 1–2 ms. We are in the process of designing a custom amplifier with much finer gain control to more closely match attenuator selection. We are also working with the manufacturer to coat the Uniblitz PtIr shutter blade with chromium to generate continuous fluorescence at energies above 6 keV. The goal for this device is to track every exposure in real time with instant feedback to data collection.

This work was carried out at the Argonne National Laboratory Structural Biology Center beamline 19BM at the Advanced Photon Source and was supported by the US Department of Energy, Office of Biological and Environmental Research and Office of Basic Energy Sciences, under Contract W-31-109-ENG-38.

### References

- Alkire, R. W., Rosenbaum, G. & Evans, G. (2000). *J. Synchrotron Rad.* **7**, 61–68.
- Alkire, R. W., Schuessler, R., Rotella, F. J., Gonczy, J. D. & Rosenbaum, G. (2004). *J. Appl. Cryst.* **37**, 836–840.
- Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.