How to collect best MX data

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Outline

- Characteristics of X-ray detectors
- Accurate data – best practice
  - Fine $\varphi$-slicing
  - Low intensity
- Think about your experiment
The meaning of HPC

Hybrid Photon Counting = Hybrid pixels + photon counting
**Hybrid pixel = sensor + readout**

- **Sensor**: CdTe or Silicon
- **Readout chip**: (CMOS ASIC)
  - Indium bump 18 µm
- **X-rays**
  - Hybrid after bump bonding
Modular HPC detectors

Module active area: $8 \times 4 \, \text{cm}^2$
100k pixels on PILATUS (172 $\mu$m)
500k pixels on EIGER (75 $\mu$m)
Photon detection in hybrid pixels

Sensor pixel
- Direct detection of X-ray photons
  -> one e-/hole pair per 3.6 eV
- Charge is captured by electric field

Readout electronics
- Counting of charge pulses
Superiority of direct detection

Direct detection

- X-ray
- Electrical signal

Indirect detection

- X-ray
- Light (Scintillator)
- Electron (Photodiode)
- Electrical signal
Superiority of direct detection

**Direct detection**
- Charge captured in electric field
  - All photons captured
  - Signal remains in pixel

**Indirect detection**
- Radiation scattered in scintillator
  - Signal spread across pixels
  - Light partially lost
Superiority of direct detection

Charge captured in electric field

- No photon loss
- Sharpest reflections
70S ribosome on EIGER X 16M

\[ a = 210 \text{ Å}, \quad b = 450 \text{ Å}, \quad c = 620 \text{ Å} \]

Diffraction to 2.3 Å, Y. Polikanov, UIC

How to collect best MX data

2019-12-01
Photon counting – one by one

- Photons absorbed in sensor pixel
Photon counting – one by one

- Photons absorbed in sensor pixel
- Charge pulse proportional to energy
Photon counting – one by one

- Photons absorbed in sensor pixel
- Charge pulse proportional to energy
- Threshold to discard noise

- Signals above threshold are counted
  - Suppression of dark signal
  - Suppression of electronic noise
Photon counting – one by one

- Photons absorbed in sensor pixel
- Charge pulse proportional to energy
- Threshold to discard noise

- Signals above threshold are counted
  - **Suppression of dark signal**
  - **Suppression of electronic noise**

- On-the-fly digitization in digital counter
  - **No readout noise**
  - Fast readout
  - High dynamic range
**Photon counting vs. integration**

**Photon counting**
- Electrical signal vs. photon energy over time
-_counts vs. time

**Charge integration**
- Electrical signal vs. photon energy over time
- Charge vs. time
Counting with 50% threshold

All signal counted within one pixel

1 photon

100% charge
1 count

1 photon

60% charge
1 count

40% charge
-
Negligible background

Protein crystallography in vacuum

PETase @ I23.

Austin et al. (2018)
Accuracy of intensity estimates

Less background

=> Better data

<table>
<thead>
<tr>
<th>Integrated intensity [photons]</th>
<th>Background [photons]</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>500</td>
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<tr>
<td>1000</td>
<td>0</td>
</tr>
<tr>
<td>10000</td>
<td>0</td>
</tr>
</tbody>
</table>

\[
Q \quad : \quad \text{Number of photons}
\]
\[
\text{var}(Q) = Q
\]
\[
\sigma(Q) = [\text{var}(Q)]^{1/2} = Q^{1/2}
\]
\[
I = \Sigma(\text{Peak}_i - \text{Bkg}_i)
\]
\[
\text{var}(I) = \Sigma[\text{var}(\text{Peak}_i) + \text{var}(\text{Bkg}_i)]
\]
\[
R_{\text{err}} = \frac{\sigma(I)}{I}
\]
Pros and cons of fine $\varphi$-slicing

The finer things in X-ray diffraction data collection

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X-ray diffraction images from two-dimensional position-sensitive detectors can be characterized as thick or thin, depending on whether the rotation-angle increment per image is greater than or less than the crystal mosaicity, respectively. The expectations and consequences of the processing of thick and thin images in terms of spatial overlap, saturated pixels, X-ray background and $l/\sigma(l)$ are discussed. The $d^*TREK$ software suite for processing diffraction images is briefly introduced, and results from $d^*TREK$ are compared with those from another popular package.

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doi.org/10.1107/S090744499900935X
**Fine \( \phi \)-slicing minimizes background**

**Wide \( \phi \)-slicing**
- \( \Delta \phi > \xi \)
- Lots of background
- Few images

**Fine \( \phi \)-slicing**
- \( \Delta \phi \ll \xi \)
- Minimal background
- Many images
Advantages of fine $\phi$-slicing

Optimal fine $\phi$-slicing for single-photon-counting pixel detectors

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Accepted 21 November 2011

The data-collection parameters used in a macromolecular diffraction experiment have a strong impact on data quality. A careful choice of parameters leads to better data and can make the difference between success and failure in phasing attempts, and will also result in a more accurate atomic model. The selection of parameters has to account for the application of the data in various phasing methods or high-resolution refinement. Furthermore, experimental factors such as crystal characteristics, available experiment time and the properties of the X-ray source and detector have to be considered. For many years, CCD detectors have been the prevalent type of

doi.org/10.1107/S0907444911049833
Fine $\varphi$-slicing improves data quality

$\Delta \varphi < \text{mosaicty improves:}$
- overall statistics
- anomalous signal
- highest-shell statistics
- number of overlaps

on PILATUS.
Fine $\phi$-slicing with EIGER

EIGER detector: application in macromolecular crystallography

Arnau Casanas, Rangana Warshamanage, Aaron D. Finke, Ezequiel Panepucci, Vincent Olieric, Anne Nöll, Robert Tampe, Stefan Brandstetter, Andreas Förster, Marcus Mueller, Clemens Schulze-Briese, Oliver Bunk and Meitian Wang

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Accepted 29 July 2016

Keywords: X-ray detectors; EIGER detector; macromolecular crystallography; data-collection strategy.

The development of single-photon-counting detectors, such as the PILATUS, has been a major recent breakthrough in macromolecular crystallography, enabling noise-free detection and novel data-acquisition modes. The new EIGER detector features a pixel size of 75 $\times$ 75 $\mu$m, frame rates of up to 3000 Hz and a dead time as low as 3.8 $\mu$s. An EIGER 1M and EIGER 16M were

doi.org/10.1107/S2059798316012304
Smaller pixels improve data quality

Reflection on EIGER

Reflection on PILATUS
Smaller pixels improve data quality

Reflection on EIGER

Better high-resolution data
Smaller pixels allow for finer ϕ-slicing

$\Delta \phi \approx 1/10$ mosaicty improves:

- overall statistics
- highest-shell statistics
- anomalous signal
- number of overlaps

on EIGER.
Get best data by

- Using EIGER and PILATUS detectors
- Using fine φ-slicing
- Minimizing other sources of error
Decrease absolute background

bad!

good!

bad!
Decrease absolute background
Get best data by

- Using EIGER and PILATUS detectors
- Using fine $\varphi$-slicing
- Minimizing absolute background
- Collecting 360° of data
Low-intensity data collection

How best to use photons


Received 12 October 2018
Accepted 13 March 2019

Keywords: radiation damage; data collection; data processing; data analysis.

Supporting information: this article has supporting information at journals.iucr.org/d

Strategies for collecting X-ray diffraction data have evolved alongside beamline hardware and detector developments. The traditional approaches for diffraction data collection have emphasised collecting data from noisy integrating detectors (i.e. film, image plates and CCD detectors). With fast pixel array detectors on stable beamlines, the limiting factor becomes the sample lifetime, and the question becomes one of how to expend the photons that your sample can diffract, i.e. as a smaller number of stronger measurements or a larger number of weaker data. This parameter space is explored via experiment and synthetic data treatment and advice is derived on how best to use the equipment on a modern beamline. Suggestions are also made on how to acquire data in a conservative manner if very little is known about the sample lifetime.

doi.org/10.1107/S2059798319003528
The American method

Collect 360° of data
- To get more precise intensity estimates
- To avoid space group frustration
- To avoid getting burned

90° @ 10 img/s (0.1°/img) 90 s
360° @ 40 img/s (0.1°/img) 90 s
Attenuate beam 4-fold + 360° @ 40 img/s (0.1°/img) 90 s
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Are these spots?

Fine $\varphi$-slicing + low-intensity data collection make for very weak spots
There is always anomalous signal

**Current paradigm**
- Molecular replacement
- In case of failure
  - Se-Met substitution
  - Heavy atom soaking

**Always measure anomalous differences**
- Experimental phasing
- Improved MR phases
- Identify metal ions
- Without extra work

**Always measure 360° of data.**
Get best data by

- **Using PILATUS and EIGER detectors**
- **Using fine \( \varphi \)-slicing**
- **Minimizing absolute background**
- **Collecting 360° of low-intensity data**
Sophisticated strategies

**Collect 360° of data**

- From starting angle suggested by strategy software
- With detector at correct distance
- With optimized exposure time/attenuation
- At non-standard energy
- With inverse beam method
- From aligned crystal
- Multiple times (dose fractionation)

**Never measure less than 360° of data.**
Simple native SAD strategy

Making routine native SAD a reality: lessons from beamline X06DA at the Swiss Light Source

Shibom Basu, a Aaron Finke, b Laura Vera, a Meitian Wang a and Vincent Olieric**

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Native single-wavelength anomalous dispersion (SAD) is the most attractive de novo phasing method in macromolecular crystallography, as it directly utilizes intrinsic anomalous scattering from native crystals. However, the success of such an experiment depends on accurate measurements of the reflection intensities and therefore on careful data-collection protocols. Here, the low-dose, multiple-orientation data-collection protocol for native SAD phasing developed at beamline X06DA (PXIII) at the Swiss Light Source is reviewed, and its usage over the last four years on conventional crystals (>50 μm) is reported. Being experimentally very simple and fast, this method has gained popularity and has delivered 45 de novo structures to date (13 of which have been published). Native SAD is currently the primary choice for experimental phasing among

doi.org/10.1107/S2059798319003103
Maximize anomalous signal

- **Multiplicity amplifies anomalous signal**
  - Change crystal orientation for true multiplicity

- **Radiation damage must be avoided**
  - Low-intensity data collection
  - Collect more images at lower dose

- **Combine data from multiple crystals**

*Detector must be free of readout noise.*
Get best data by

- Using PILATUS and EIGER detectors
- Using fine $\phi$-slicing
- Minimizing absolute background
- Collecting at least 360° of low-intensity data
- Thinking about your experiment
Use beam time effectively

- Mount crystal – 1 min
- Center crystal – 1 min
- Collect a dataset – 1 min
- Eight-hour shift – 480 min

- **Person A:**
  - Collects 82 datasets
  - Takes 1 TB of data home

- **Person B:**
  - Thinks about experiment
  - Talks to beamline scientist
  - Has a tea to focus
  - Collects a dataset
  - Has a chat with her mate
  - Collects two more datasets
  - Solves structure
Conclusions

- Optimize your sample!

- With PILATUS or EIGER, collect \( n \times 360^\circ \) of data with weak beam

- With PILATUS or EIGER, \( \Delta \phi \leq \frac{1}{2} \) mosaicity (XDS) for best data

- There is no native data

- Ask your beamline scientist / think!
Read more


- Förster and Schulze-Briese. *A shared vision for macromolecular crystallography over the next five years.* *Struct Dynam.* 2019; 6:xxxx.
Thank you for your attention!

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