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detecting the future

How to collect best MX data

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CCP4 Workshop, DLS, 2019-12-01

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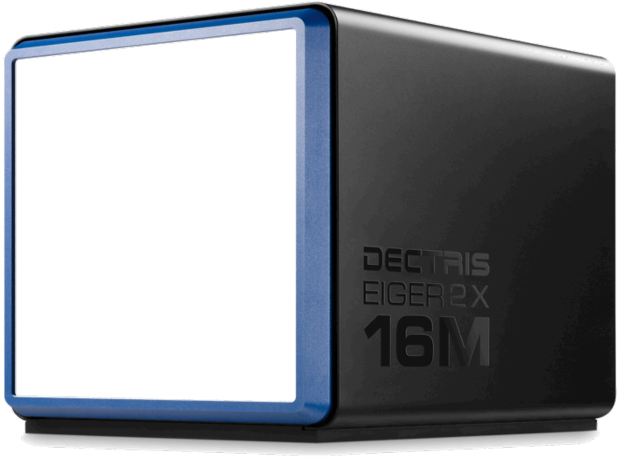
Outline

- *Characteristics of X-ray detectors*
- *Accurate data – best practice*
  - *Fine φ-slicing*
  - *Low intensity*
- *Think about your experiment*



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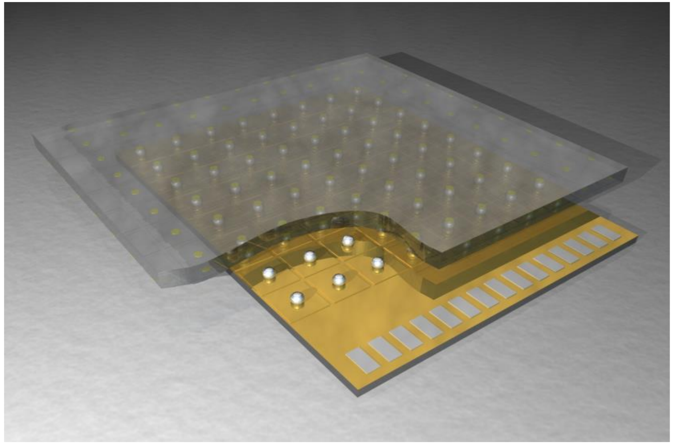
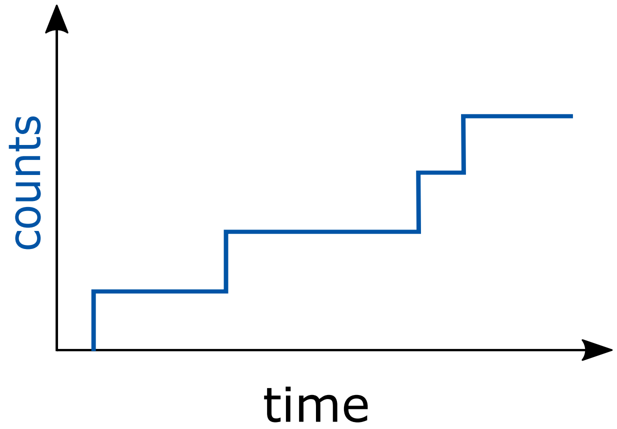
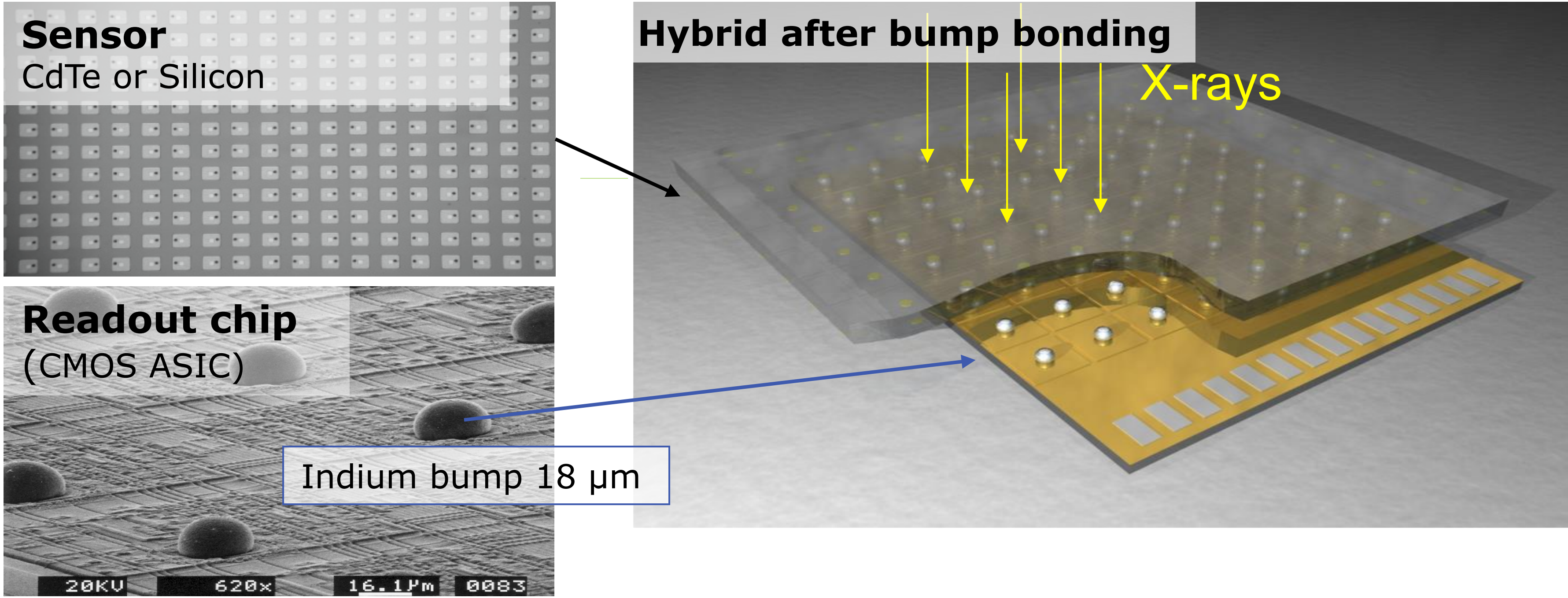
The meaning of HPC

Hybrid Photon Counting = Hybrid pixels + photon counting

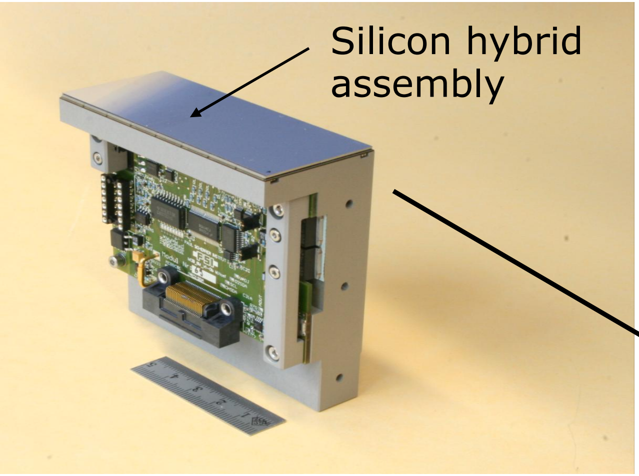


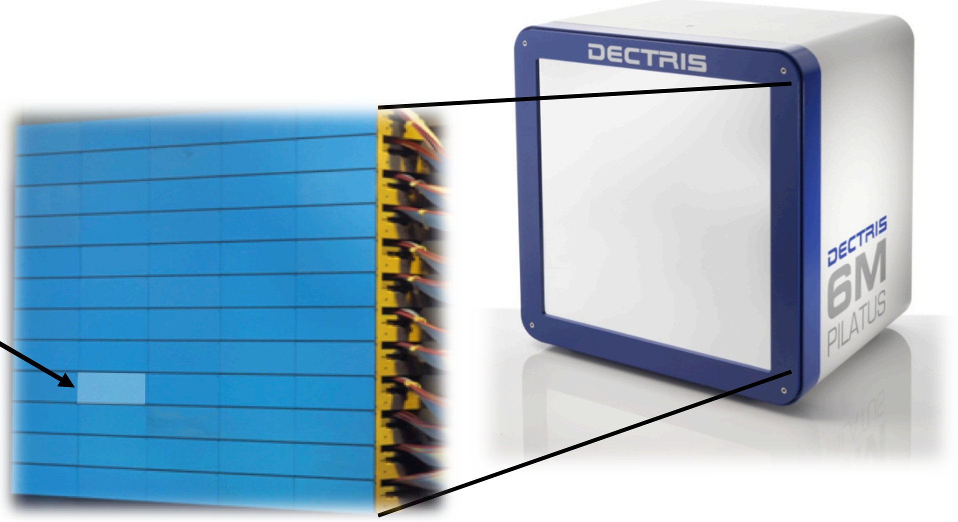


Hybrid pixel = sensor + readout



Modular HPC detectors



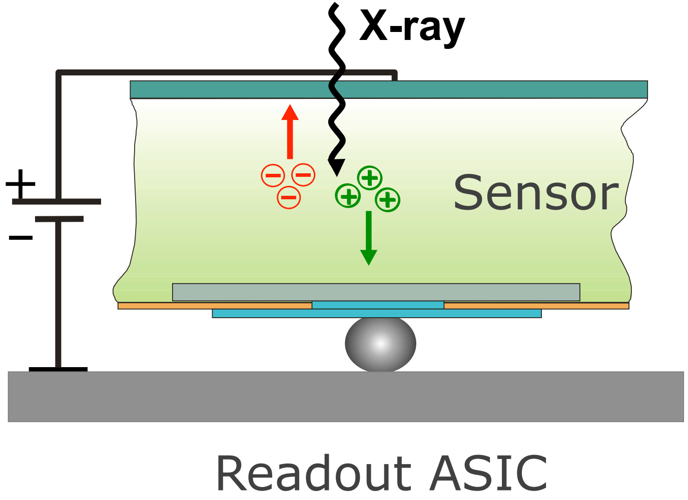


Module active area: 8 × 4 cm²

100k pixels on PILATUS (172 µm)

500k pixels on EIGER (75 µm)

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*

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Superiority of direct detection

Direct detection

X-ray

↓

Electrical signal

Indirect detection

X-ray

↓

Light (Scintillator)

↓

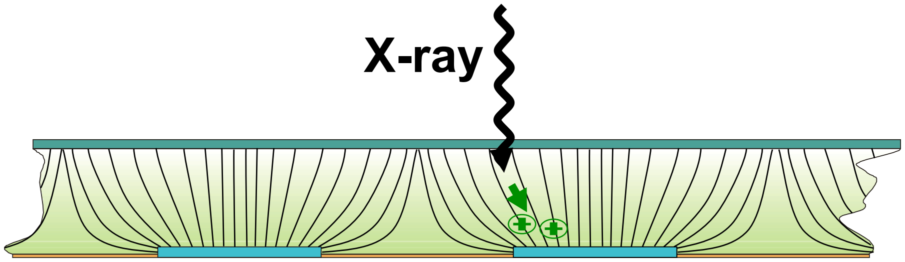
Electron (Photodiode)

↓

Electrical signal

Superiority of direct detection

Direct detection

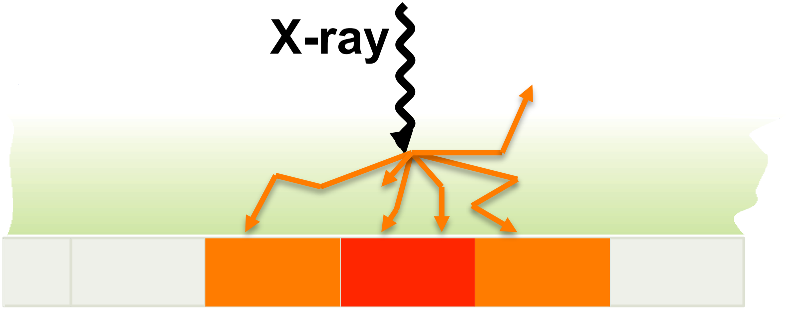


– *Charge captured in electric field*

=> ***All photons captured***

=> ***Signal remains in pixel***

Indirect detection



– *Radiation scattered in scintillator*

=> ***Signal spread across pixels***

=> ***Light partially lost***

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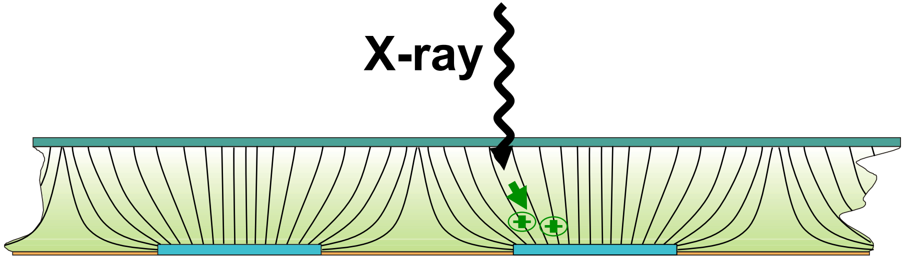
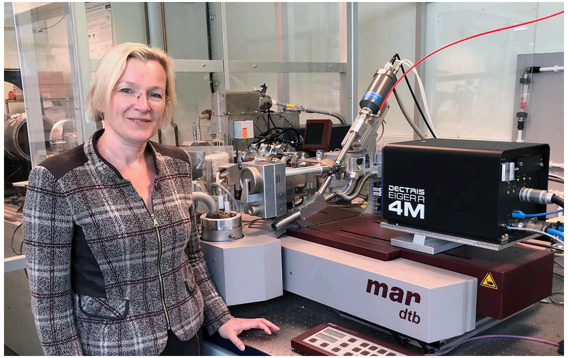
Superiority of direct detection

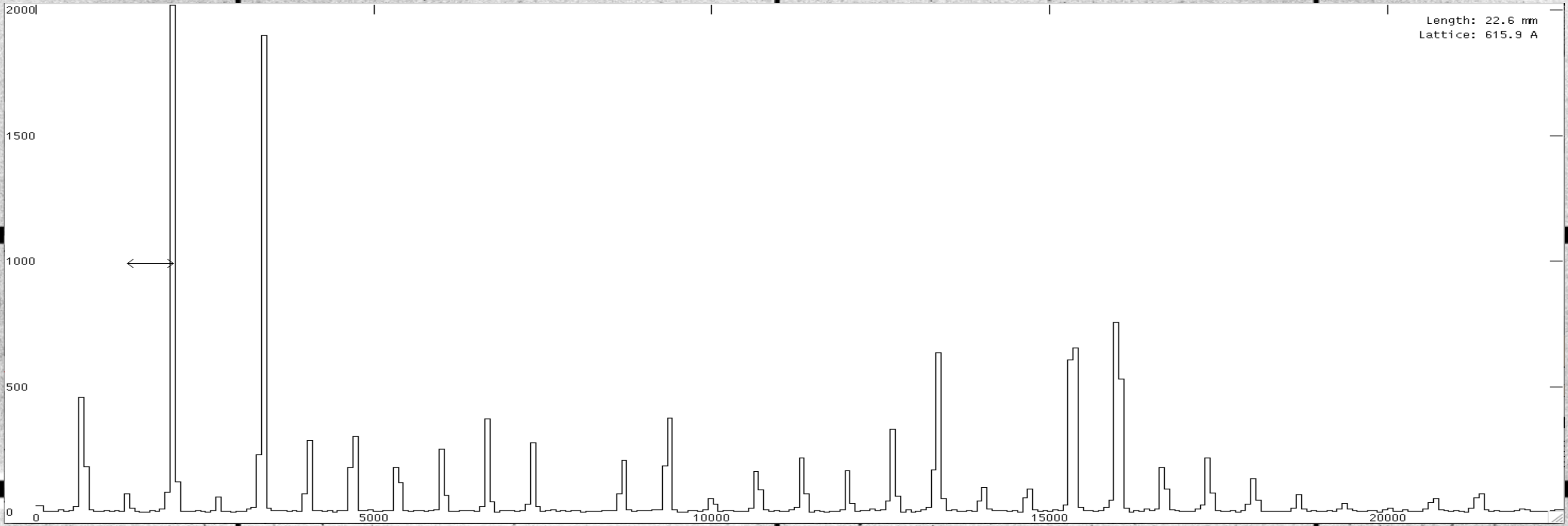
Charge captured in electric field





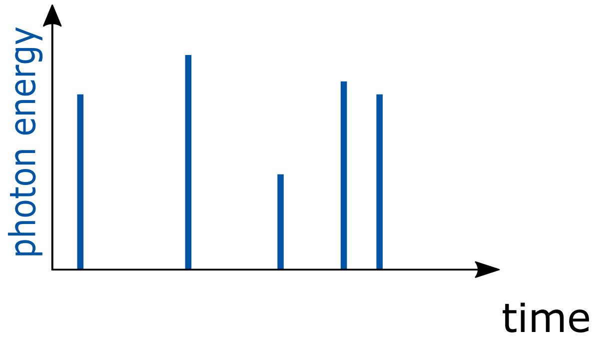
- *No photon loss*
- *Sharpest reflections*

70S ribosome on EIGER X 16M



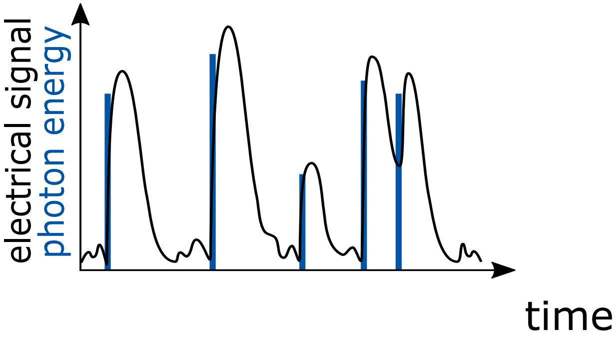
a = 210 Å, b = 450 Å, c = 620 Å
Diffraction to 2.3 Å, Y. Polikanov, UIC

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*

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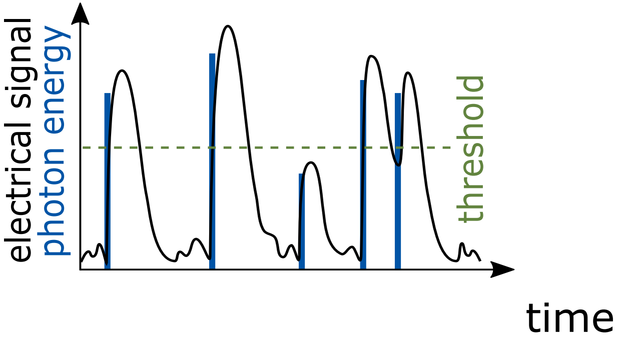
Photon counting – one by one



- *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*

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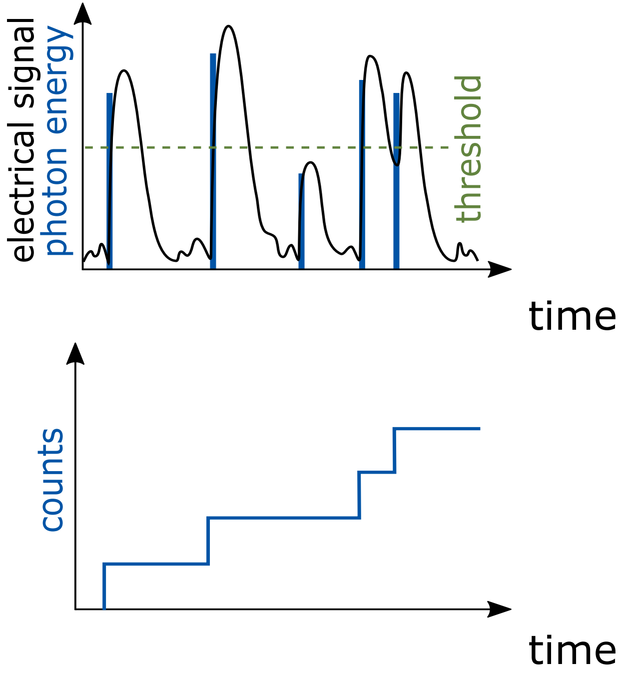
Photon counting – one by one



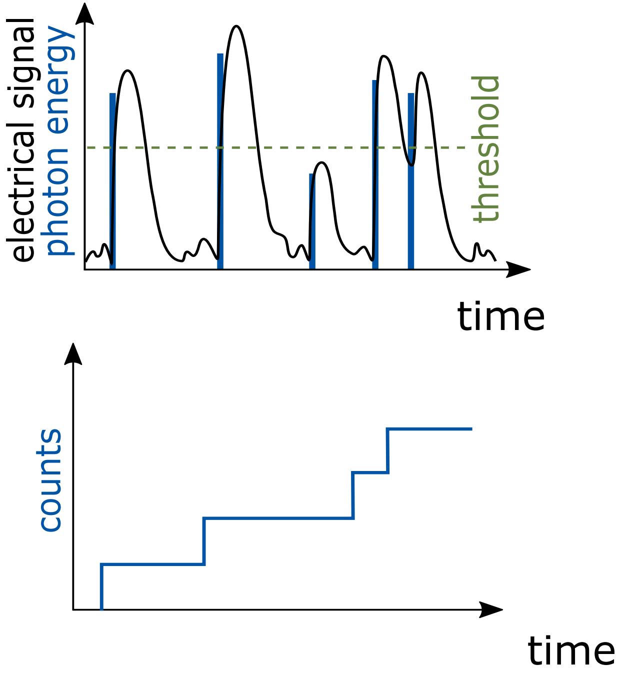
- *Photons absorbed in sensor pixel*
- *Charge pulse proportional to energy*
- *Threshold to discard noise*

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

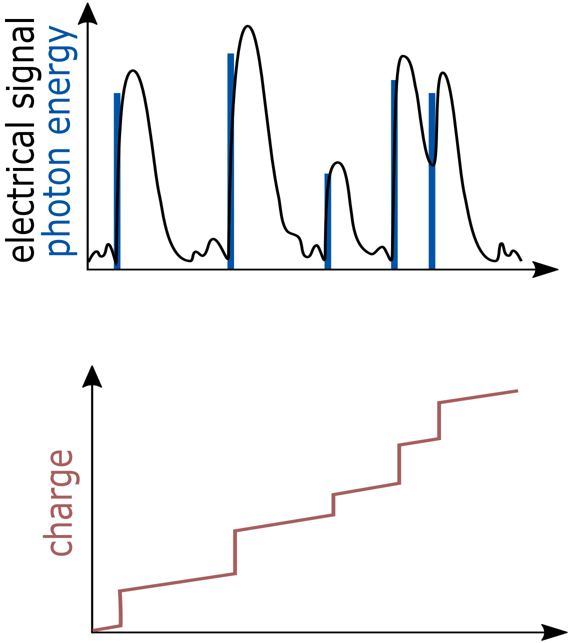
- *On-the-fly digitization in digital counter*
  - ***No readout noise***
  - *Fast readout*
  - *High dynamic range*

Photon counting vs. integration

Photon counting



Charge integration



Counting with 50% threshold

All signal counted within one pixel

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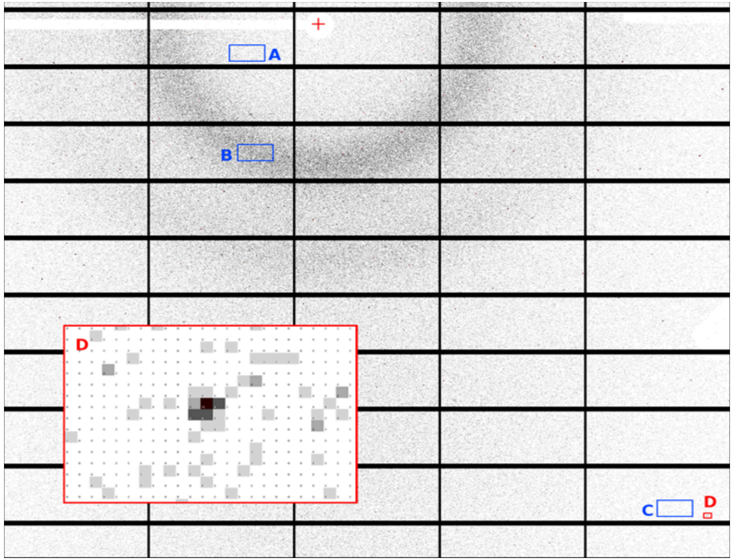
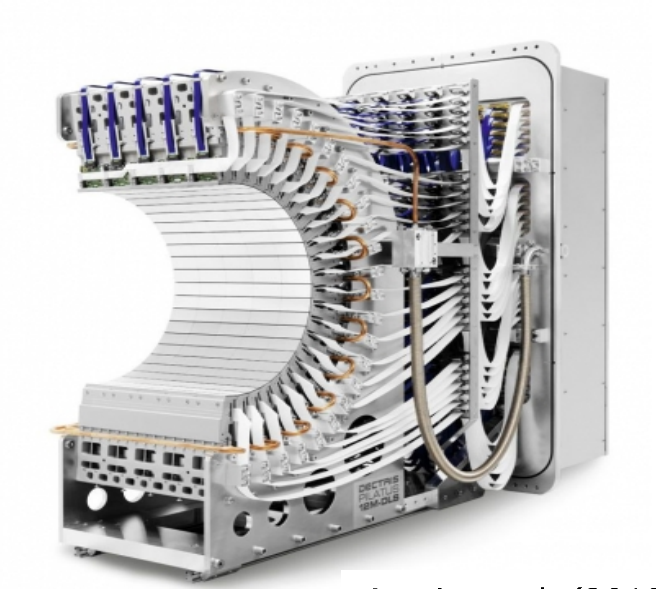
Negligible background

Protein crystallography in vacuum

PETase @ I23.





Austin et al. (2018)

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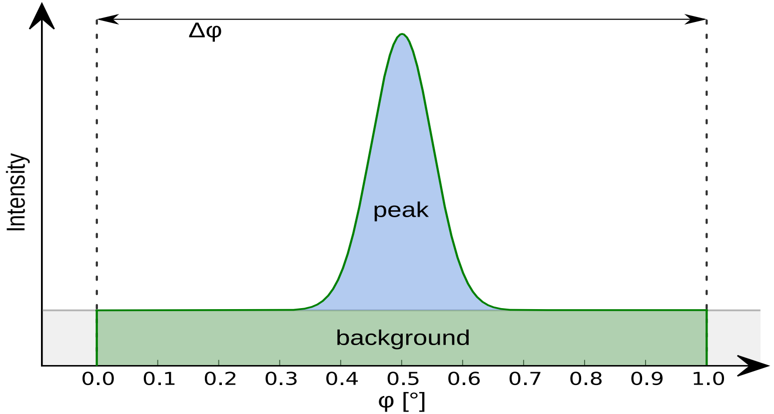
Accuracy of intensity estimates



Q : Number of photons

$var(Q) = Q$

$\sigma(Q) = [var(Q)]^{1/2} = Q^{1/2}$

$I = \Sigma(Peak_i - Bkg_i)$

$var(I) = \Sigma[var(Peak_i) + var(Bkg_i)]$

$R_{err} = \sigma(I)/I$

Relative error [%]	Background [photons]		
Integrated intensity [photons]	0	500	1000
100	10.0	33.2	45.8
500	4.5	7.7	10.0
1000	3.2	4.5	5.5
10000	1.0	1.0	1.1

Less background

=> Better data

Pros and cons of fine φ-slicing

Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

The finer things in X-ray diffraction data collection

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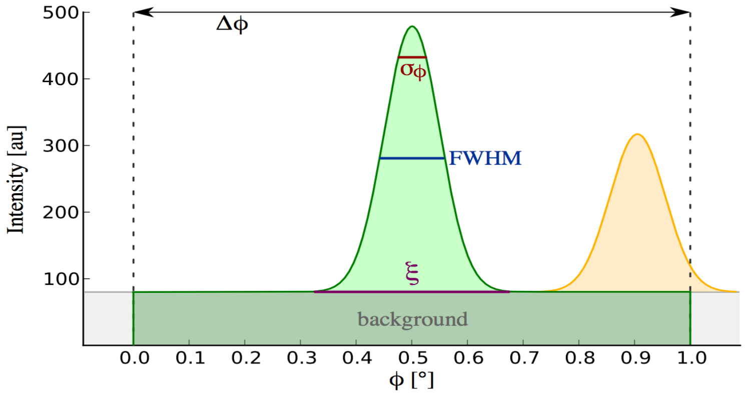
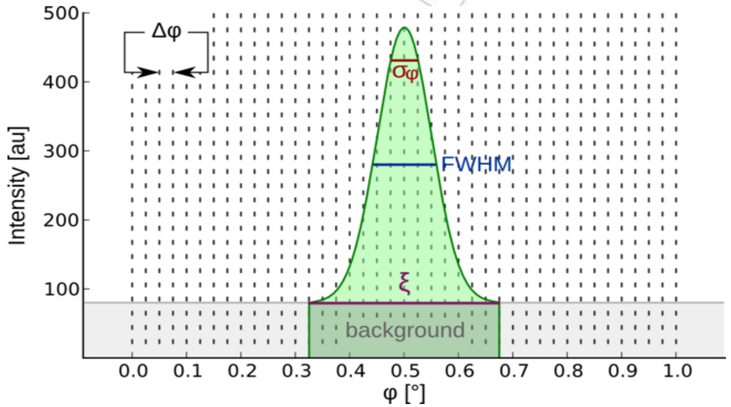
X-ray diffraction images from two-dimensional position-sensitive detectors can be characterized as thick or thin, depending on whether the rotation-angle increment per image is greater than or less than the crystal mosaicity, respectively. The expectations and consequences of the processing of thick and thin images in terms of spatial overlap, saturated pixels, X-ray background and $I/\sigma(I)$ 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.

Received 6 May 1999
Accepted 5 July 1999

doi.org/10.1107/S090744499900935X

Fine φ-slicing minimizes background





Wide φ-slicing

- $\Delta\varphi > \xi$
- Lots of background
- Few images

Fine φ-slicing

- $\Delta\varphi \ll \xi$
- Minimal background
- Many images

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Advantages of fine φ-slicing

research papers

Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Optimal fine φ-slicing for single-photon-counting pixel detectors

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Received 17 June 2011
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

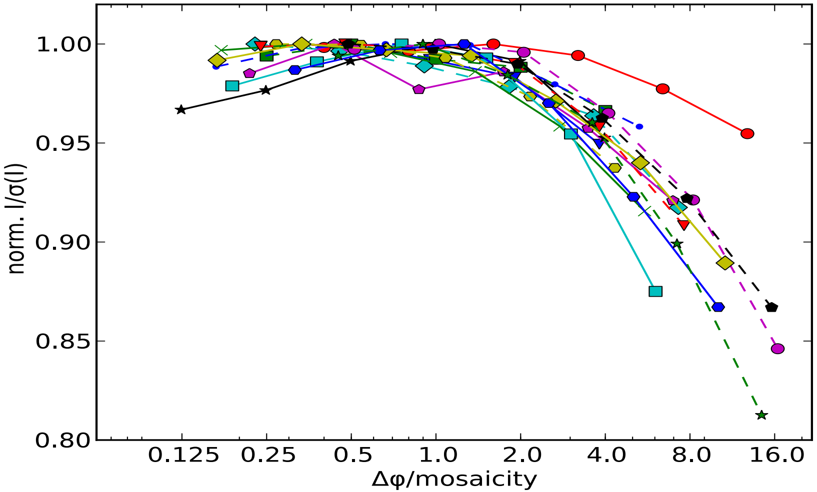
Fine φ-slicing improves data quality

Δφ < mosaicity improves:

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

on PILATUS.

higher I/sig(I)



Fine φ-slicing with EIGER

research papers

CrossMark

Acta Cryst D
STRUCTURAL BIOLOGY

ISSN 2059-7983

EIGER detector: application in macromolecular crystallography

Arnau Casanas,[a] Rangana Warshamanage,[a] Aaron D. Finke,[a] Ezequiel Panepucci,[a] Vincent Olieric,[a] Anne Nöll,[b] Robert Tampé,[b] Stefan Brandstetter,[c] Andreas Förster,[c] Marcus Mueller,[c] Clemens Schulze-Briese,[c] Oliver Bunk[a] and Meitian Wang[a]*

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Received 9 June 2016
Accepted 29 July 2016

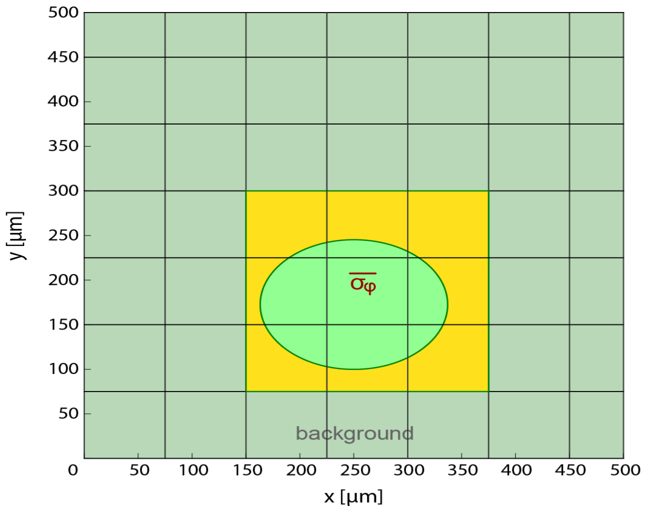
Edited by K. Diederichs, University of Konstanz, Germany

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

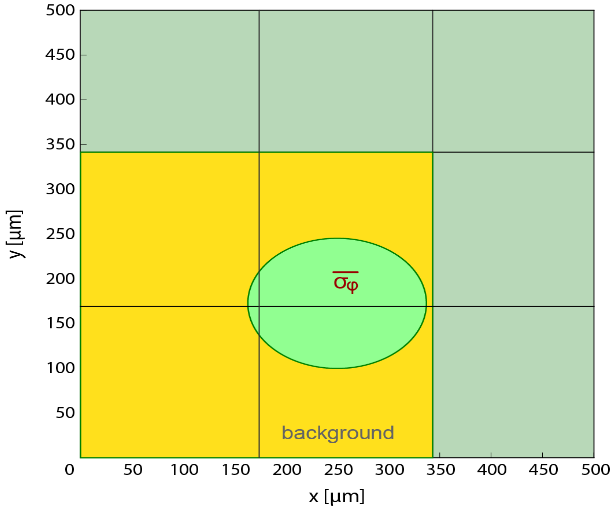
The development of single-photon-counting detectors, such as the PILATUS, has been a major recent breakthrough in macromolecular crystallography, enabling noise-free detection and novel data-acquisition modes. The new EIGER detector features a pixel size of 75 × 75 µm, frame rates of up to 3000 Hz and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

doi.org/10.1107/S2059798316012304

Smaller pixels improve data quality



Reflection on EIGER



Reflection on PILATUS

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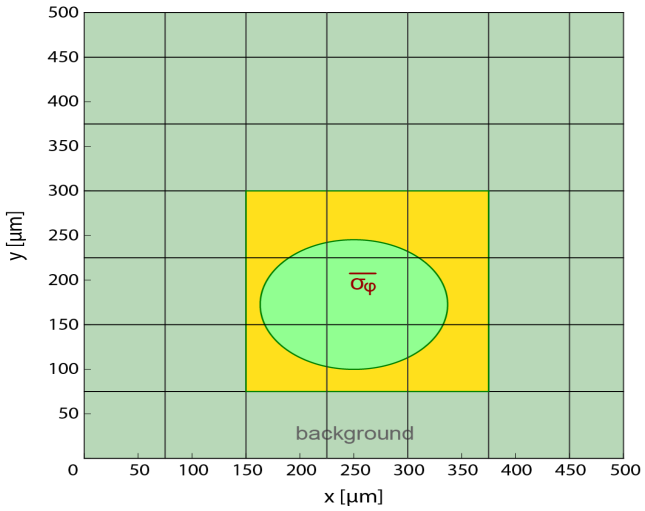
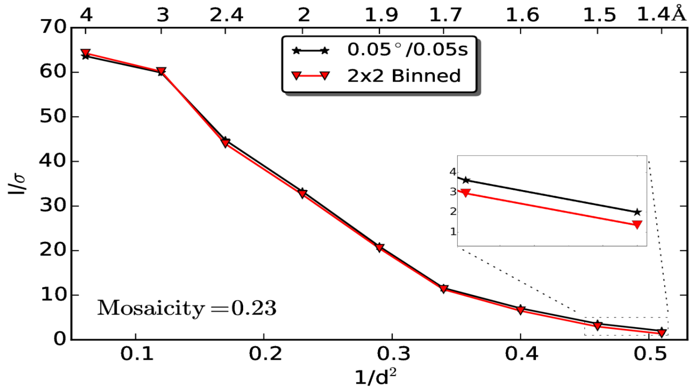
Smaller pixels improve data quality



Reflection on EIGER



Better high-resolution data

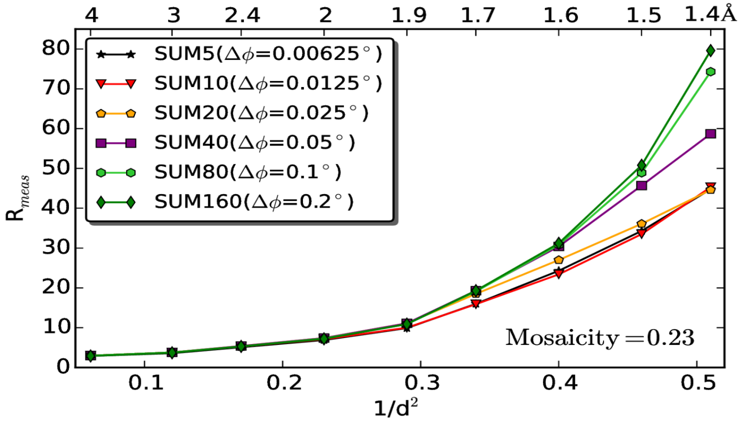
Smaller pixels allow for finer φ-slicing

Δφ ≈ 1/10 mosaicity improves:

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

on EIGER.

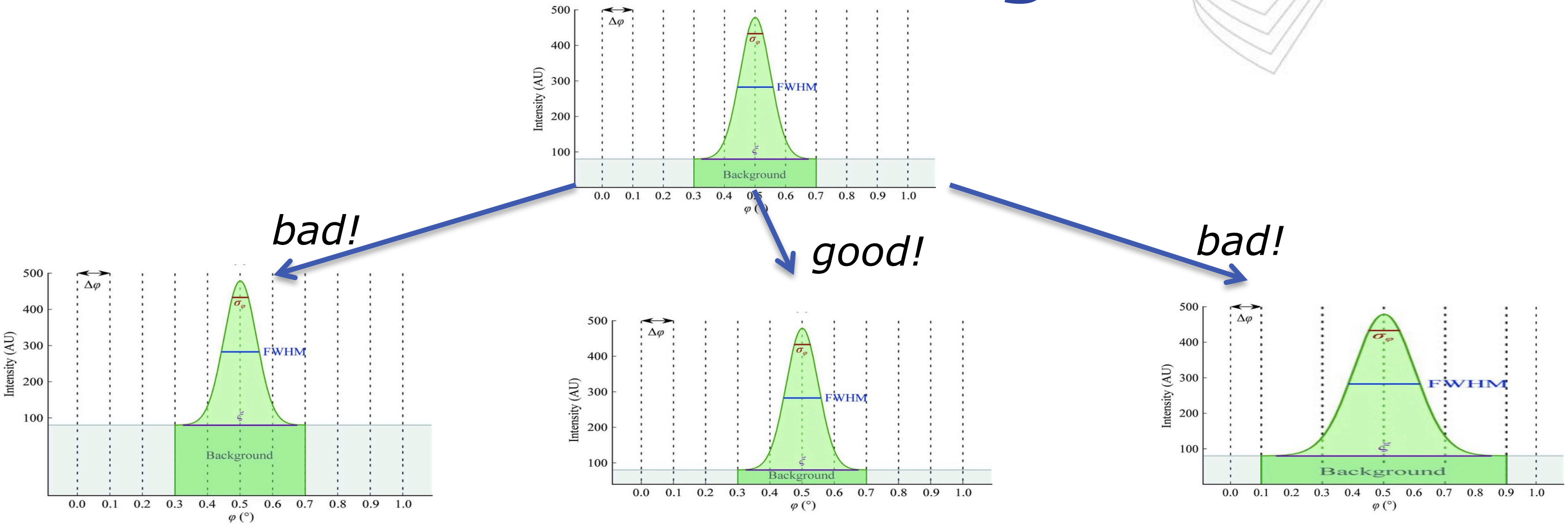
lower R_{meas}



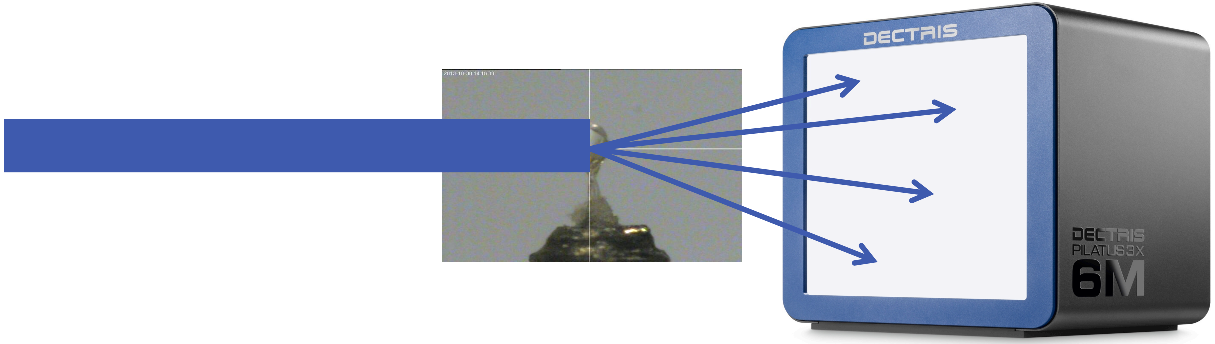
Get best data by

- *Using EIGER and PILATUS detectors*
- *Using fine φ-slicing*
- *Minimizing other sources of error*

Decrease absolute background



Decrease absolute background



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Get best data by

- *Using EIGER and PILATUS detectors*
- *Using fine φ-slicing*
- *Minimizing absolute background*
- *Collecting 360° of data*

Low-intensity data collection

research papers

Acta Cryst D STRUCTURAL BIOLOGY

ISSN 2059-7983

How best to use photons

Graeme Winter,* Richard J. Gildea, Neil G. Paterson, John Beale, Markus Gerstel, Danny Axford, Melanie Vollmar, Katherine E. McAuley, Robin L. Owen, Ralf Flaig, Alun W. Ashton and David R. Hall

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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

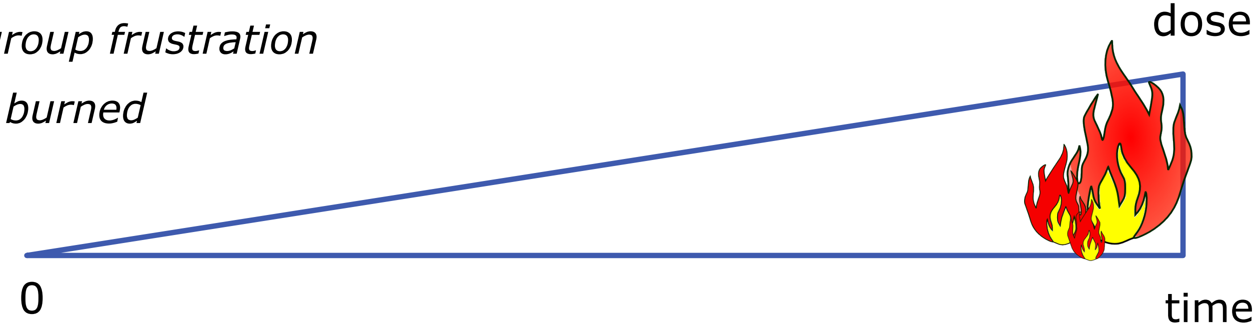
Strategies for collecting X-ray diffraction data have evolved alongside beamline hardware and detector developments. The traditional approaches for diffraction data collection have emphasised collecting data from noisy integrating detectors (*i.e.* film, image plates and CCD detectors). With fast pixel 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*

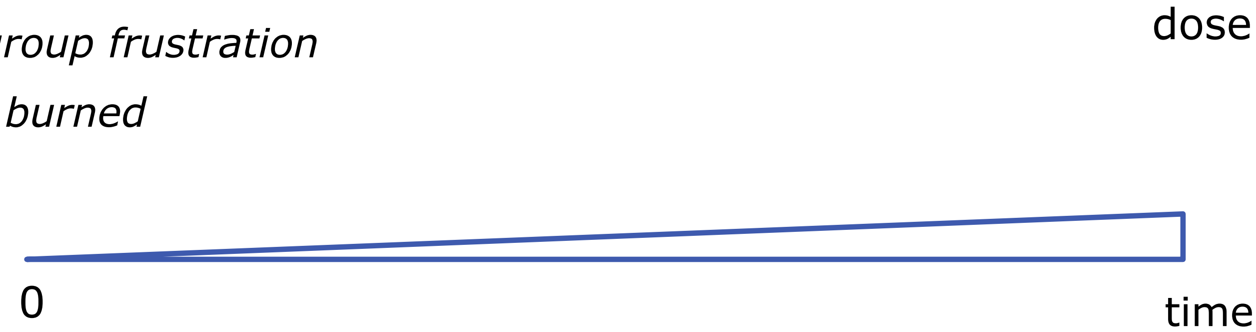


	90° @ 10 img/s (0.1°/img)	90 s
	360° @ 40 img/s (0.1°/img)	90 s
Attenuate beam 4-fold +	360° @ 40 img/s (0.1°/img)	90 s

The American method

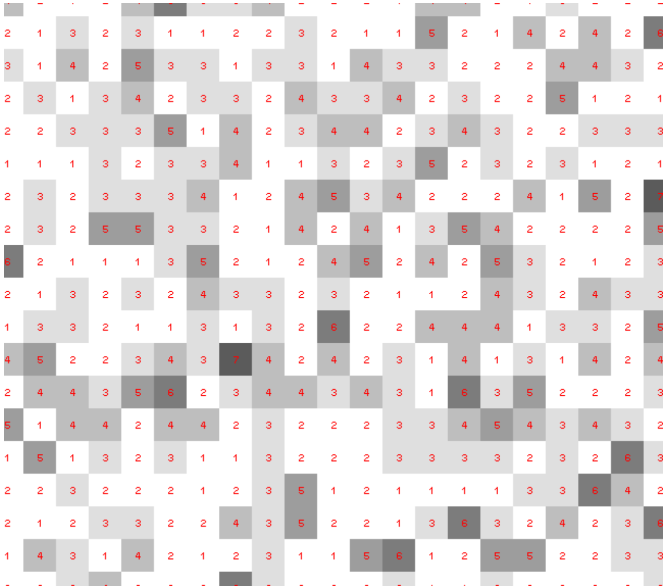
Collect 360° of data

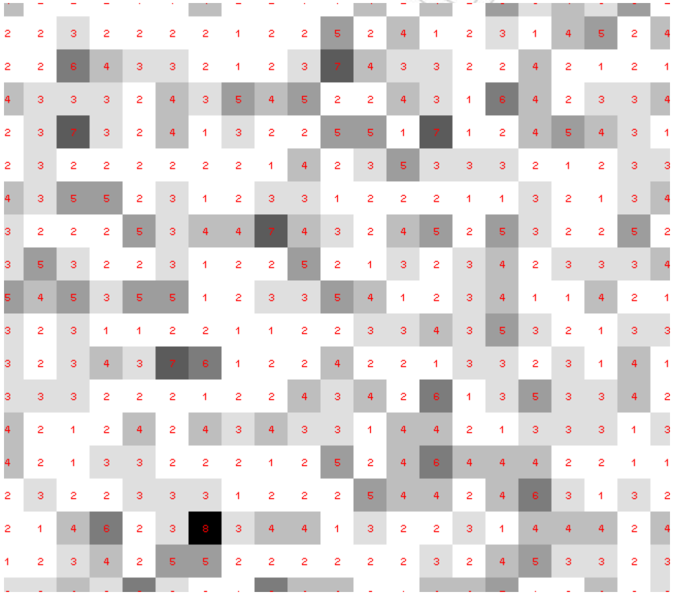
- *To get more precise intensity estimates*
- *To avoid space group frustration*
- *To avoid getting burned*



	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

Are these spots?





Fine φ-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

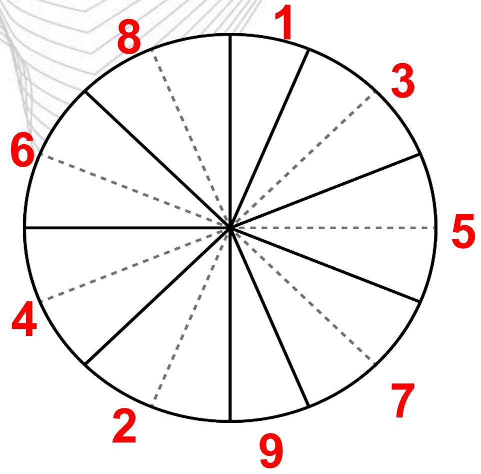
- *Experimental phasing*
- *Improved MR phases*
- *Identify metal ions*
- *Without extra work*

Always measure 360° of data.

Get best data by

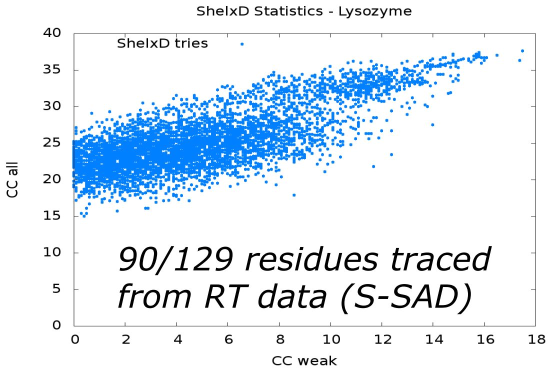
- *Using PILATUS and EIGER detectors*
- *Using fine φ-slicing*
- *Minimizing absolute background*
- *Collecting 360° of low-intensity data*

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)*



30 kGy of X-rays

Never measure less than 360° of data.

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Simple native SAD strategy

research papers

Acta Cryst D
STRUCTURAL BIOLOGY

ISSN 2059-7983

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

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Received 24 August 2018
Accepted 1 March 2019

Keywords: native SAD phasing; anomalous signal; data-collection strategy; multi-axis goniometer; data multiplicity.

PDB reference: streptavidin–biotin, 6m9b

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

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 φ-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

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Conclusions

- *Optimize your sample!*
- *With PILATUS or EIGER, collect $n \times 360°$ of data with weak beam*
- *With PILATUS or EIGER, $\Delta\phi \leq$ ½ mosaicity (XDS) for best data*
- *There is no native data*
- *Ask your beamline scientist / think !*

Read more

- *Förster et al.* ***Transforming X-ray detection with hybrid photon counting detectors****.* *Philos Trans A. 2019;377:20180241.*
- *Pflugrath.* ***The finer things in X-ray diffraction data collection.*** *Acta D. 1999;55:1718.*
- *Mueller et al.* ***Optimal fine φ-slicing for single-photon-counting pixel detectors****.* *Acta D. 2012;68:42.*
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- *Winter et al.* ***How best to use photons****.* *Acta D. 2019;75:242.*
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