X-ray and electron detection - technology & how to get best data

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ReNaFoBis workshop, IGBMC, 2021-01-13

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Outline

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

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X-ray detection

Mode of detection

- *Direct detection*
  → No intermediates
- *Indirect detection*
  → Conversion into species that can be detected

Mode of quantification

- *Counting*
  → Each photon as it is detected
- *Integration*
  → Accumulation of signal that is proportional to number of photons

More information

- *Watch https://www.youtube.com/watch?v=sxyjqGN8R18 for a historic overview over X-ray detection.*
- *Slides at https://slideplayer.com/slide/12089838/.*

Photographic film

Indirect detection

– *Blackening of silver halide*

Integrating

– *Estimation of spot intensity*
  → *Comparison by eye*
  → *Film scanners*

More information

– *Limited dynamic range, linearity, uniformity.*

– *Slow because of film development.*

– *Inaccurate when estimating intensities.*

Multiwire proportional counters

Direct detection

- *Absorption of photons in gas (Xe) generates detectable electrons*

Counting

- *Charge pulses induced in wires at site of incidence.*
- *Delay lines make it possible to deduce wire/position from arrival time of pulse.*

More information

- *Developed in 1970s (ADSC, Xentronics).*
- *Low spatial resolution (1 x 2 mm pixels).*
- *Excellent temporal resolution (1 µs).*
- *Up to 50 spots per image.*

Image plates

Indirect detection

- *Absorption of photons in storage phosphor ($BaF(Br,I):Eu^{2+}$).*

Integrating

- *Excitation of visible light by laser scan.*
- *Emitted light proportional to absorbed X-ray intensity.*

More information

- *From early 1990s (marresearch, Rigaku).*
- *Large area, linear, simple.*
- *Large dynamic range.*
- *Slow, large point-spread function.*

CCD detectors

Indirect detection

- *Absorption of photons in phosphor (Gd-based) generates visible light.*
- *Light usually travels down taper to small detector.*

Integrating

- *CCD collects light throughout exposure.*

More information

- *From the mid-1990s (ADSC, Rayonix, Rigaku).*
- *Small but can be tiled, fast.*
- *Small dynamic range, needs to be cooled.*
- *Large point-spread function, noisy.*

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Pixel array detectors

Direct detection

- Absorption of photons in semiconductor (Si, CdTe) generates electron/hole pairs.
- Electric field drives electrons or holes into readout circuit.

Counting or integrating

- Depends on readout circuitry.

More information

- From early 2000s (PSI, Medipix, DECTRIS).
- Small but can be assembled larger, with gaps.
- Extremely fast, simple.
- Single-pixel point-spread function, no background noise.
- Huge dynamic range.
- Large count-rate capacity.

The optimal detector

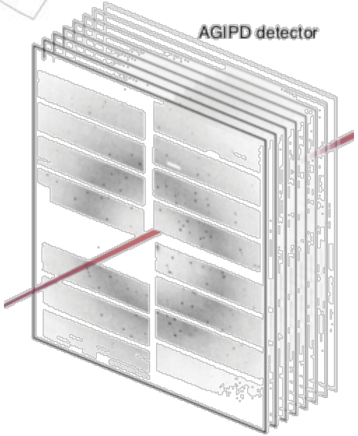
Direct or indirect detection

- *Indirect detection always limited.*
- *Direct detection can achieve*
  → *Single-pixel point spread.*
  → *Near theoretical MTF.*
  → *Highest quantum efficiency.*

Counting or integrating

- *Depends on application.*
- *Counting: best when photons can be counted.*
- *Integration: best when photons arrive simultaneously.*

Integrating detectors



@EuXFEL

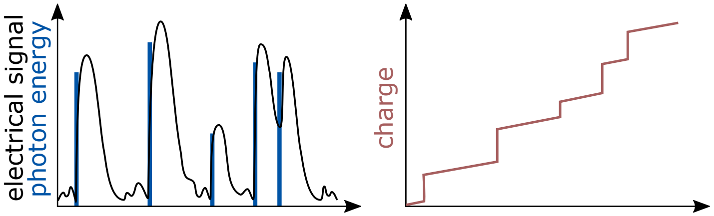
Direct detection – CS-PAD, JUNGFRAU, AGIPD, ePIX

- *Development projects.*
- *Noise suppression difficult (low operating temperature).*
- *Conversion from numbers on image to photons expensive.*
- *Extremely high uncompressed data rates.*

2021 - JUNGFRAU 10M **2.2 kHz**

46 GB/s

@PSI



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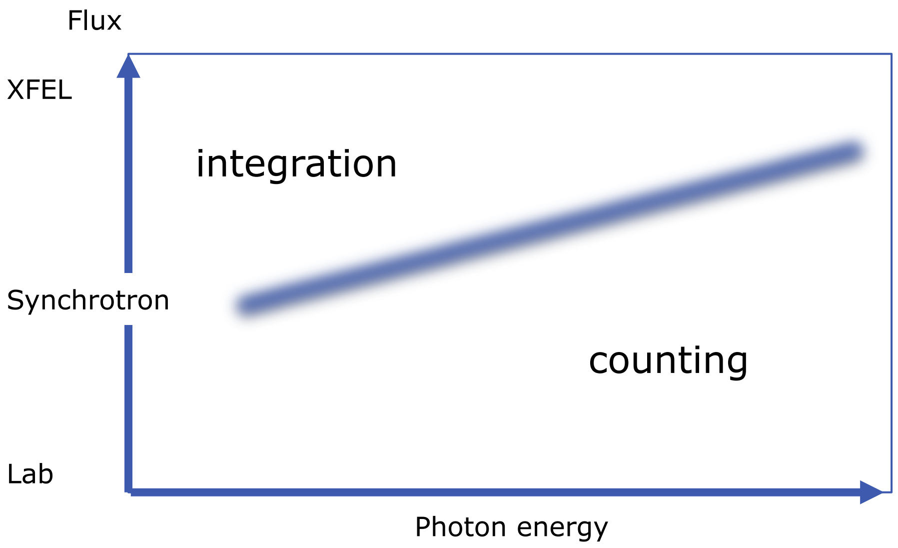
Counting vs. integration?

Detector depends on the source

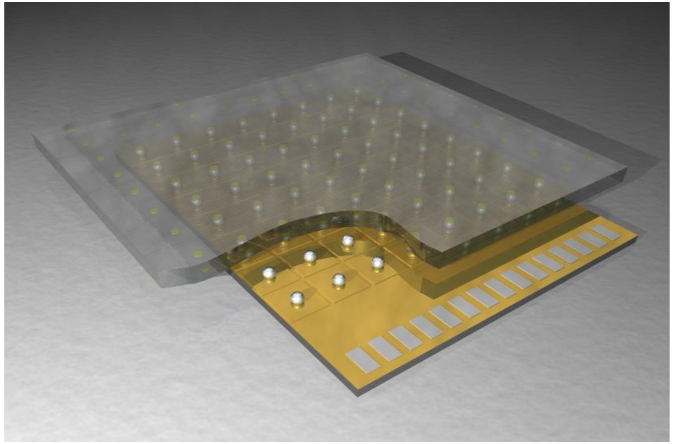
- *Flux vs. energy*
- *Computational effort*
- *Pulses vs. continuous beam*

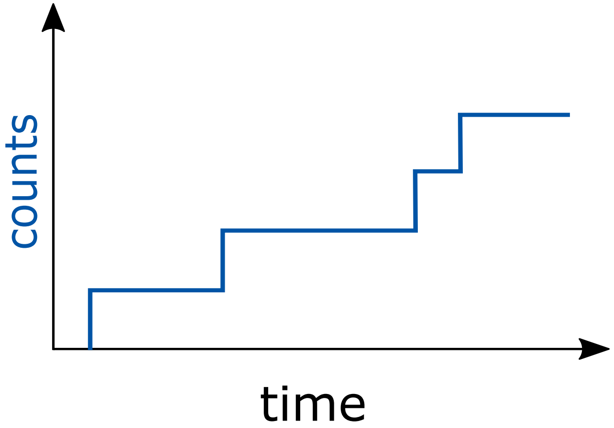


Philip Leonarski, PSI

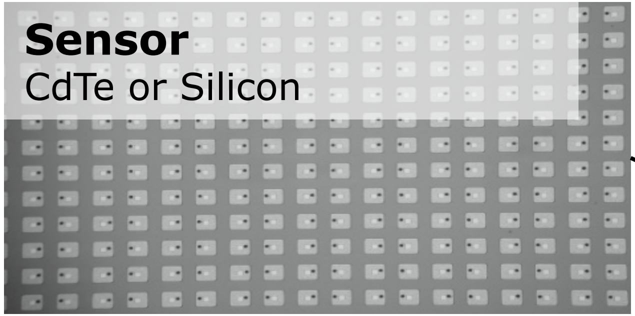
The meaning of HPC

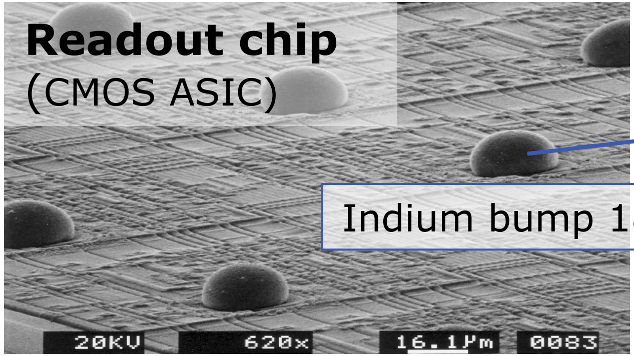
Hybrid Photon Counting = Hybrid pixels + photon counting

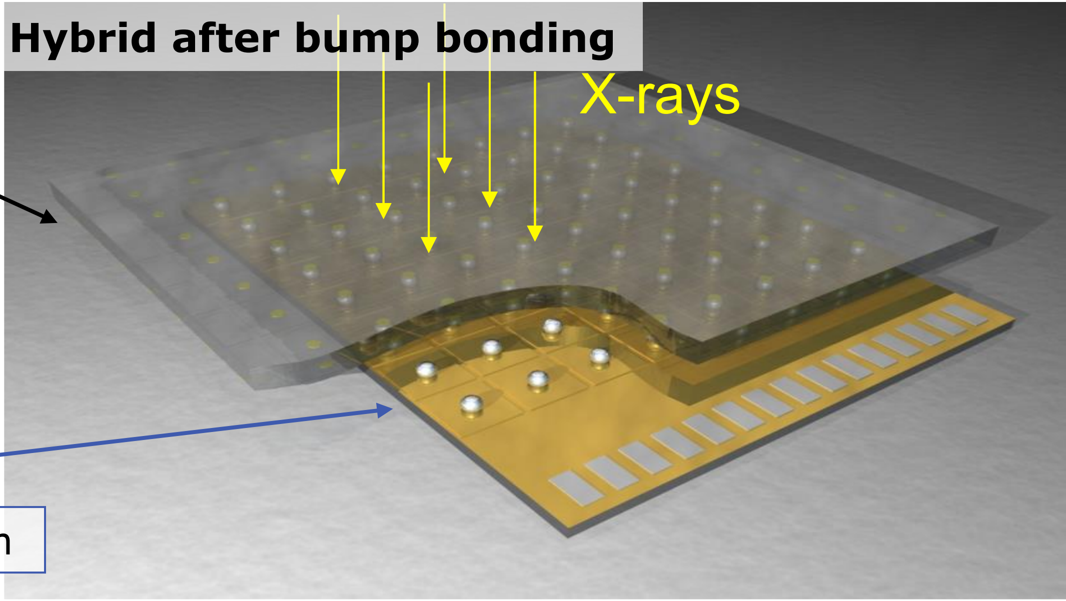




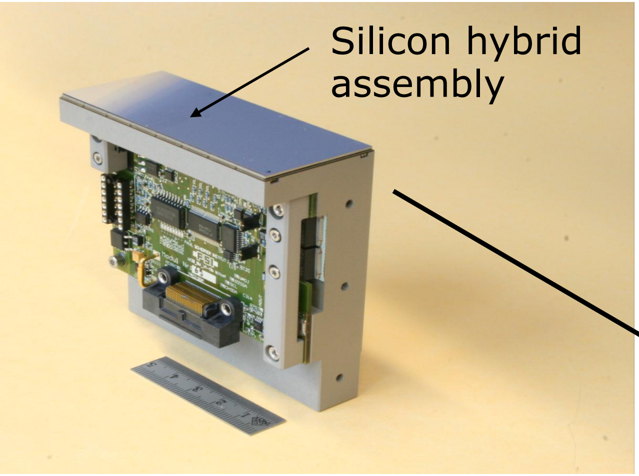
Hybrid pixel = sensor + readout

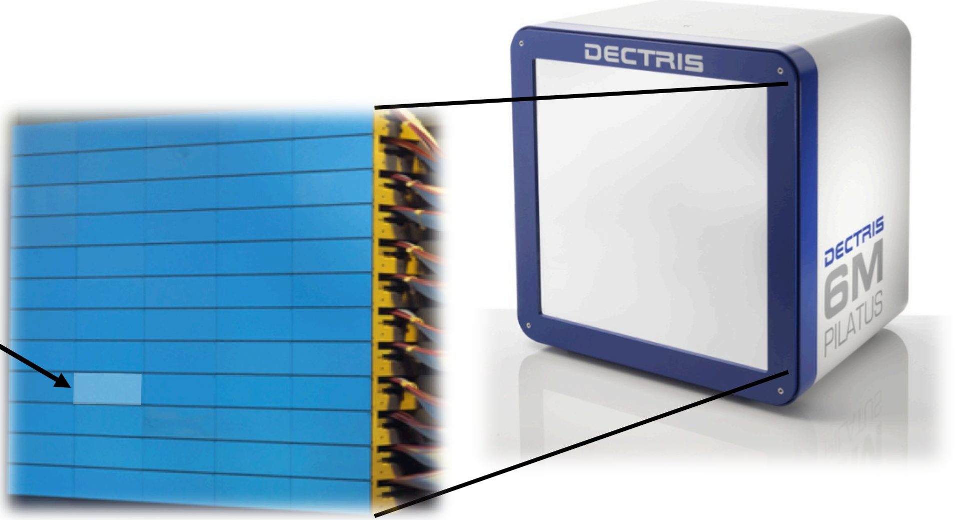






Modular HPC detectors



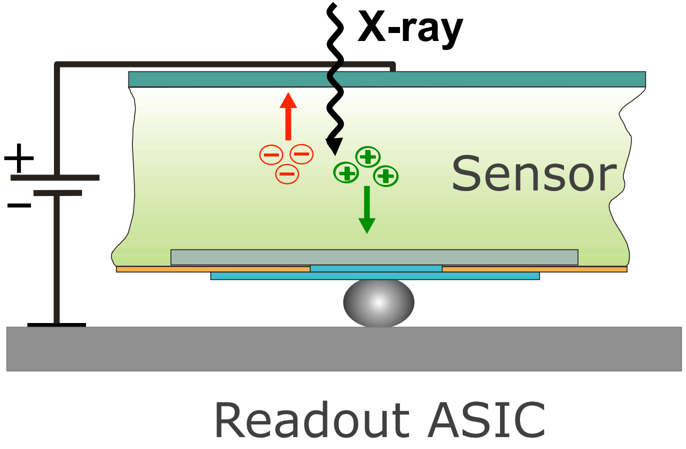


Module active area: 8 × 4 cm²

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

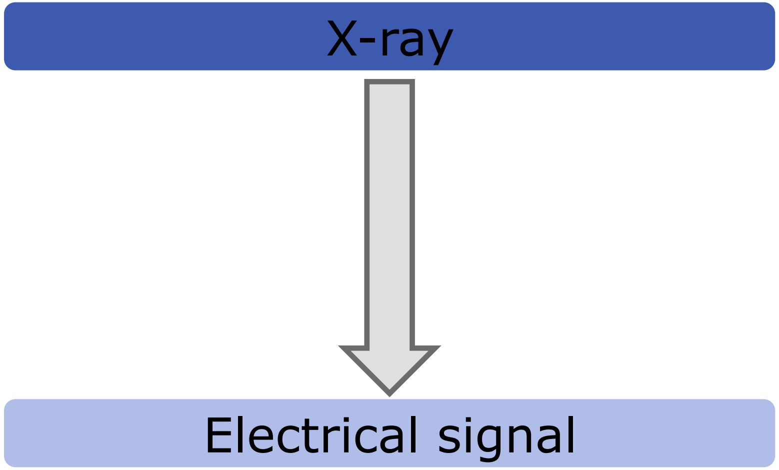
– *Charge is captured by electric field*

Readout electronics

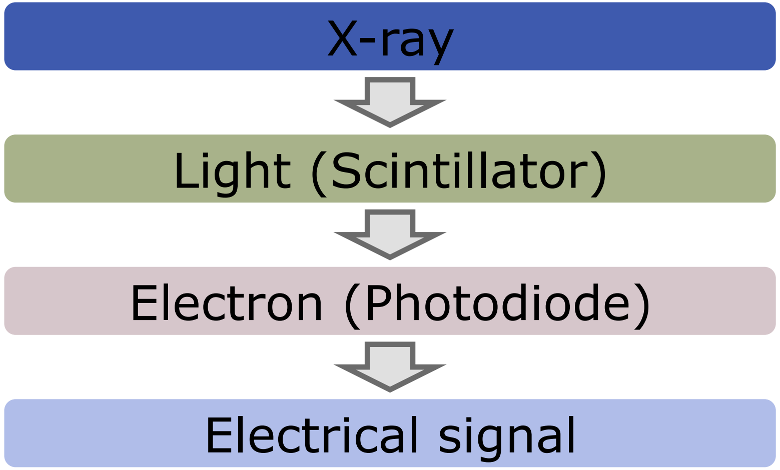
– *Counting of charge pulses*

Superiority of direct detection

Direct detection

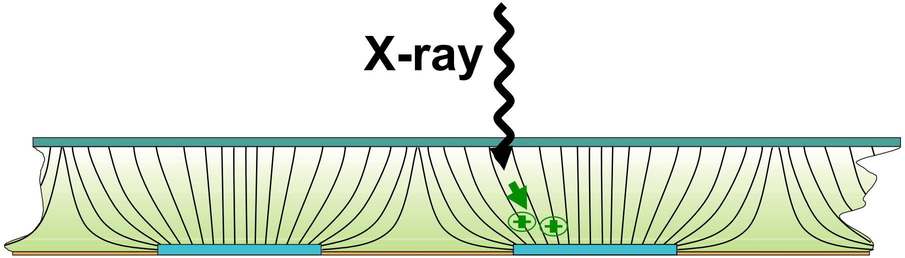


Indirect detection



Superiority of direct detection

Direct detection

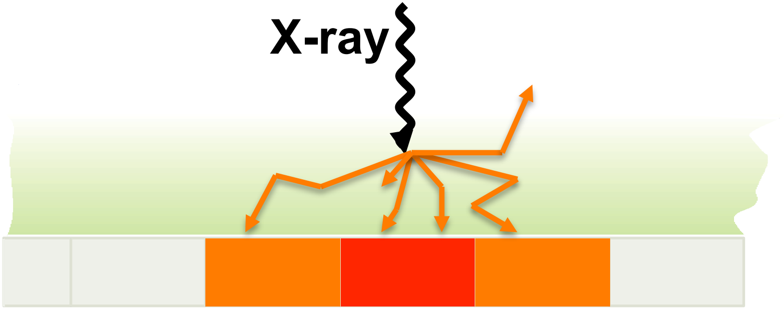


– *Charge captured in electric field*

=> ***All photons captured***

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

Indirect detection



– *Radiation scattered in scintillator*

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

=> ***Light partially lost***

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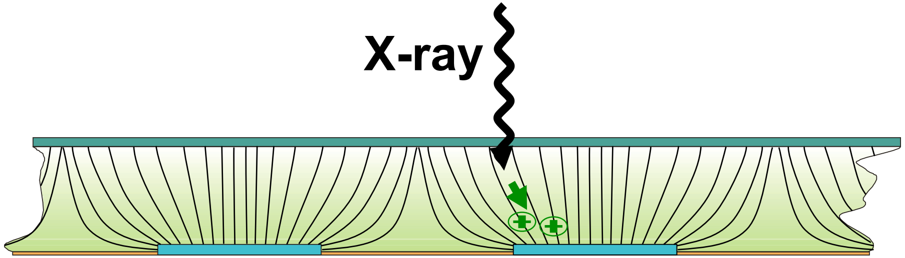
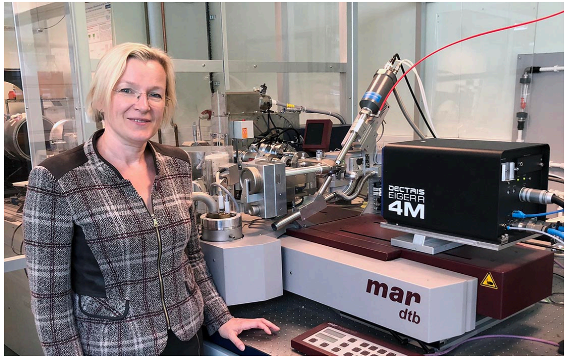
Superiority of direct detection

Charge captured in electric field





- *No photon loss*
- *Sharpest reflections*

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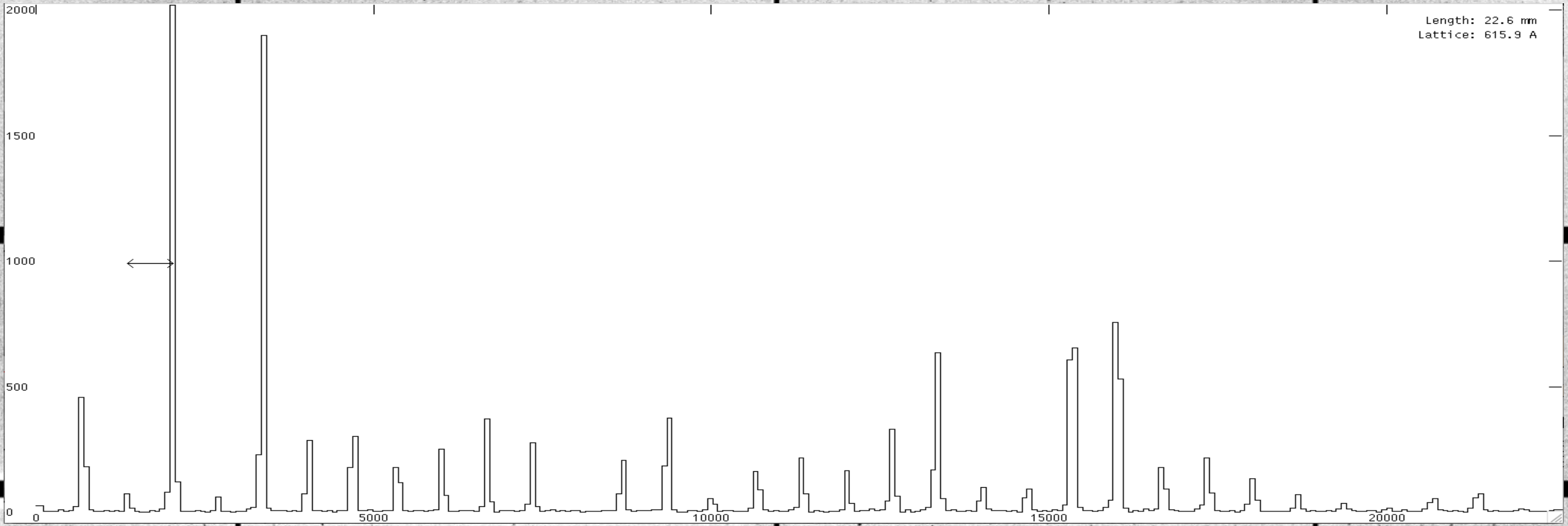
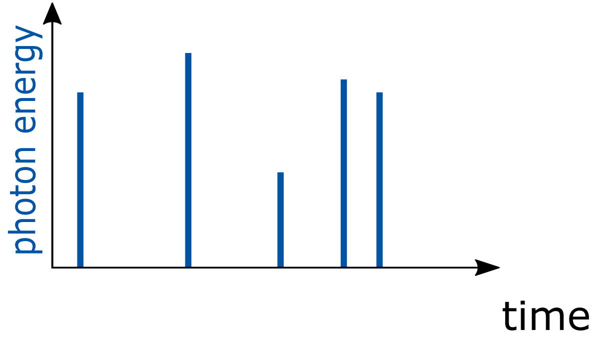
70S ribosome on EIGER X 16M



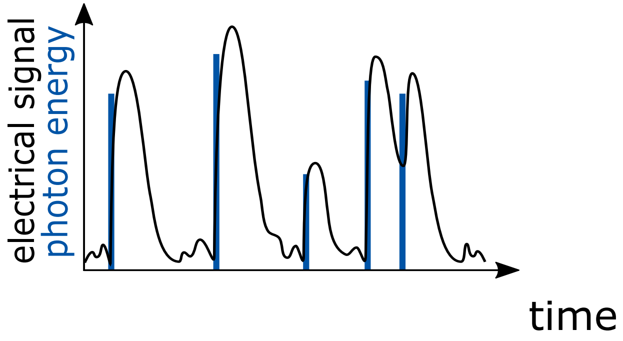
a = 210 Å, b = 450 Å, c = 620 Å
Diffraction to 2.3 Å, Y. Polikanov, UIC

Photon counting – one by one



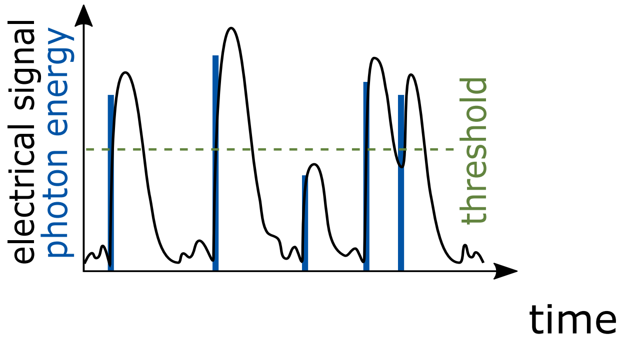
– *Photons absorbed in sensor pixel*

Photon counting – one by one



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

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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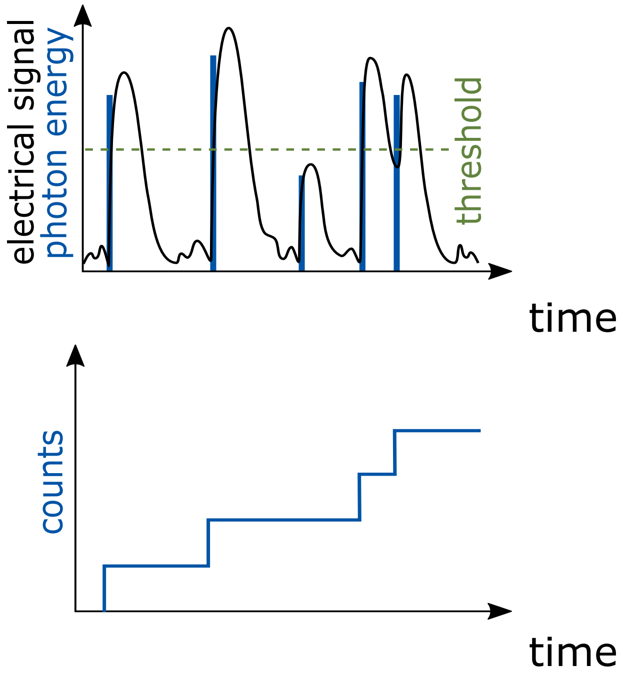
Photon counting – one by one



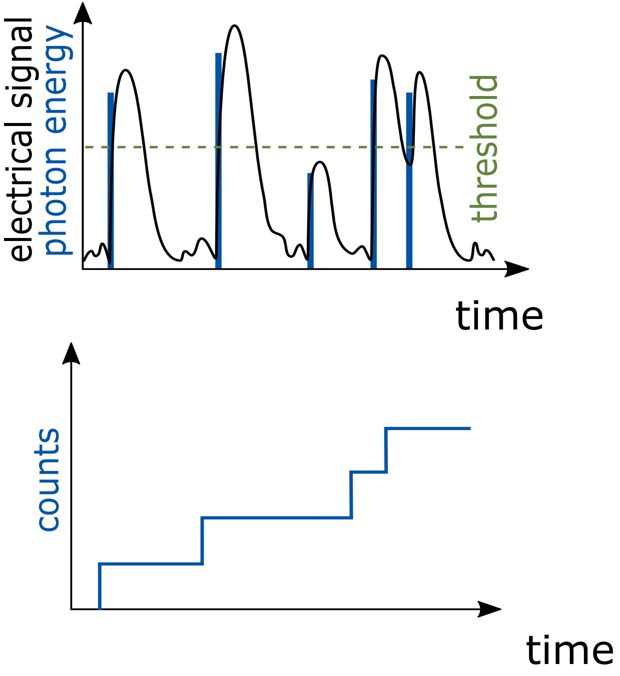
- *Photons absorbed in sensor pixel*
- *Charge pulse proportional to energy*
- *Threshold to discard noise*

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

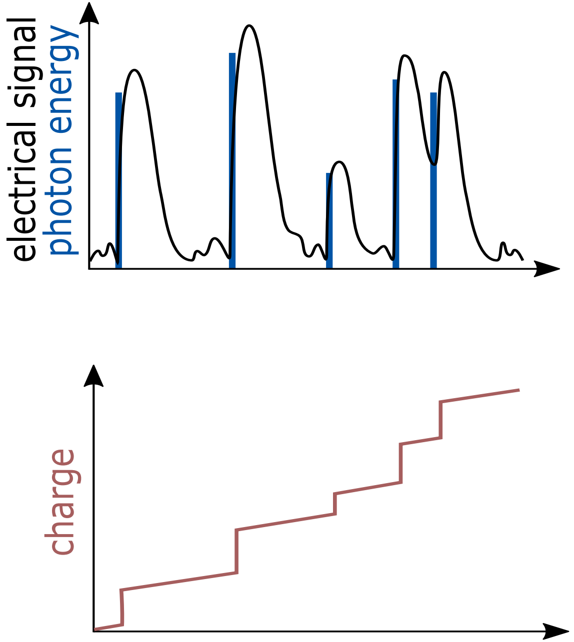
- *On-the-fly digitization in digital counter*
  - ***No readout noise***
  - *Fast readout*
  - *High dynamic range*

Photon counting vs. integration

Photon counting



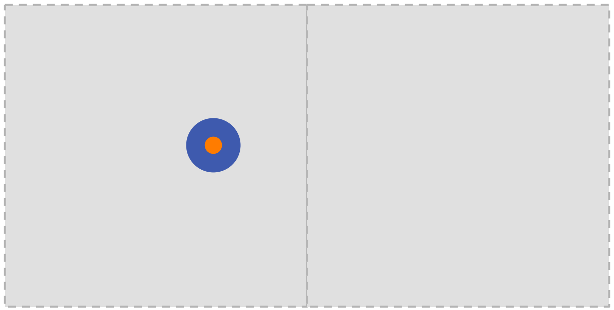
Charge integration



Counting with 50% threshold

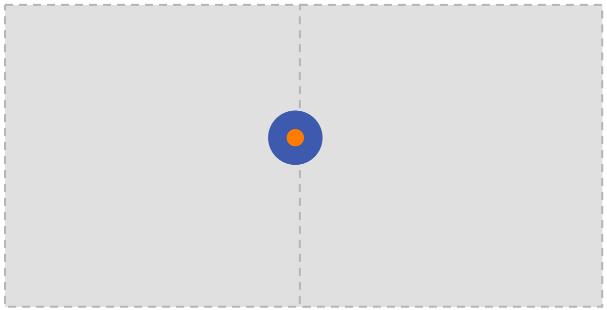
All signal counted within one pixel

1 photon



100% charge
1 count

1 photon

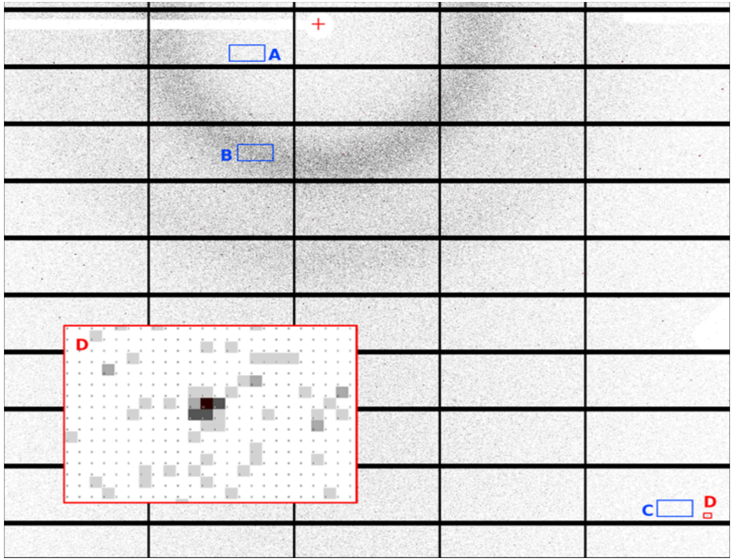


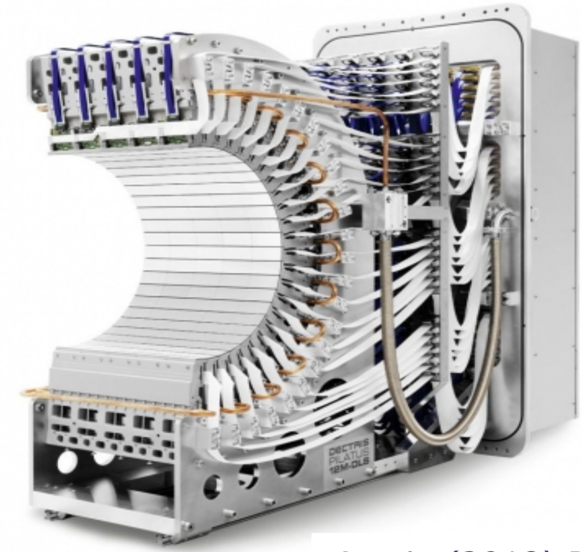
60% charge | 40% charge
1 count | -

Negligible background

Protein crystallography in vacuum

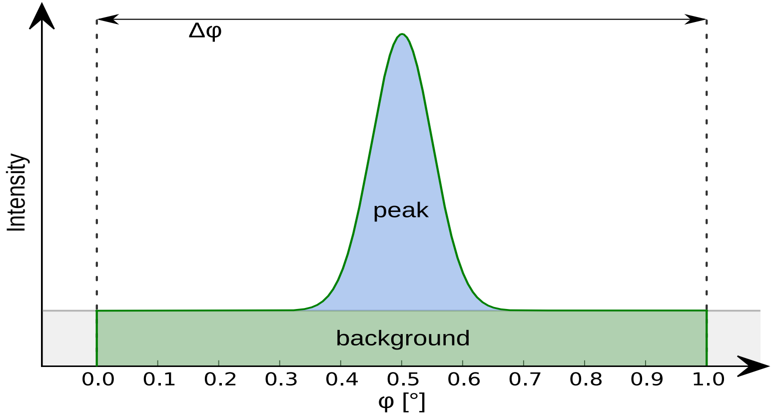
PETase @ I23.





[Austin (2018) PNAS](#)

Accuracy of intensity estimates



Q : Quantity of photons

$var(Q) = Q$

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

I : Observed intensity

$I = \Sigma(Peak_i - Bkg_i)$

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

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

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

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Pros and cons of fine φ-slicing

research papers

Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

The finer things in X-ray diffraction data collection

J. W. Pflugrath

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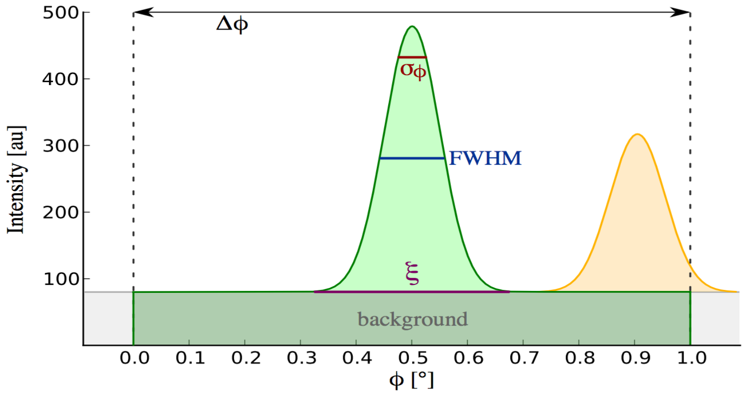
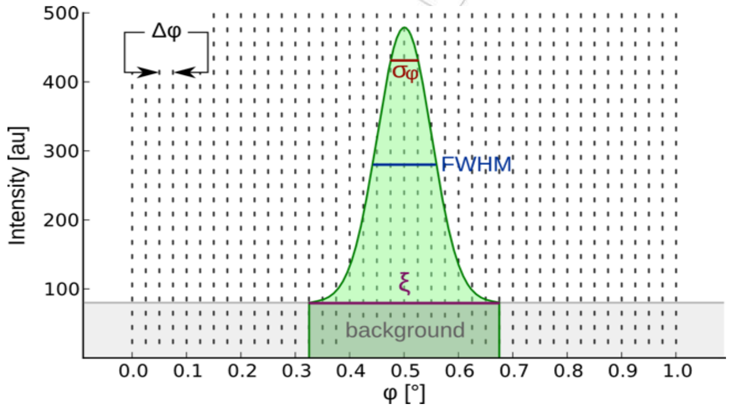
X-ray diffraction images from two-dimensional position-sensitive detectors can be characterized as thick or thin, depending on whether the rotation-angle increment per image is greater than or less than the crystal mosaicity, respectively. The expectations and consequences of the processing of thick 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

Pflugrath (1999) Acta D

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
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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

Müller (2011) Acta D

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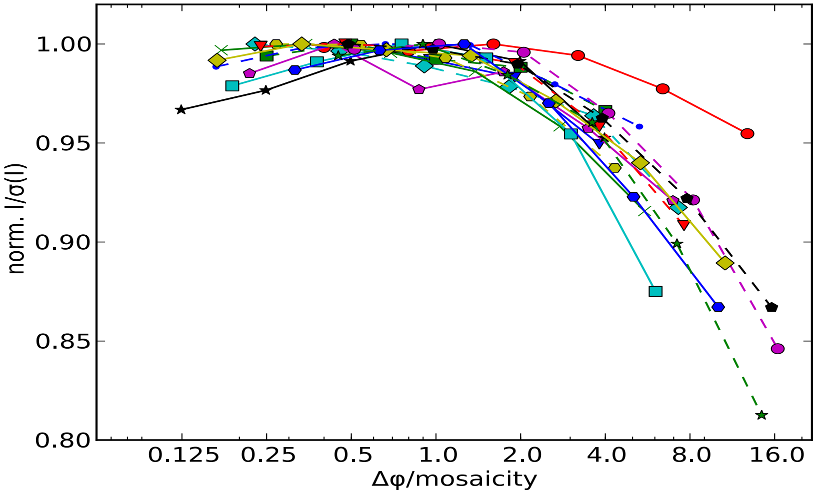
Fine φ-slicing improves data quality

Δφ < mosaicity improves:

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

on PILATUS.

higher I/sig(I)



Start with 0.1°/img

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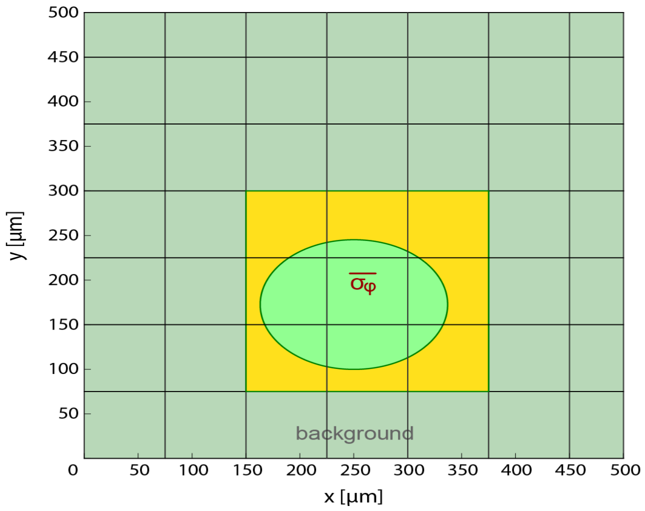
Keywords: X-ray detectors; EIGER detector; macromolecular crystallography; data-collection strategy.

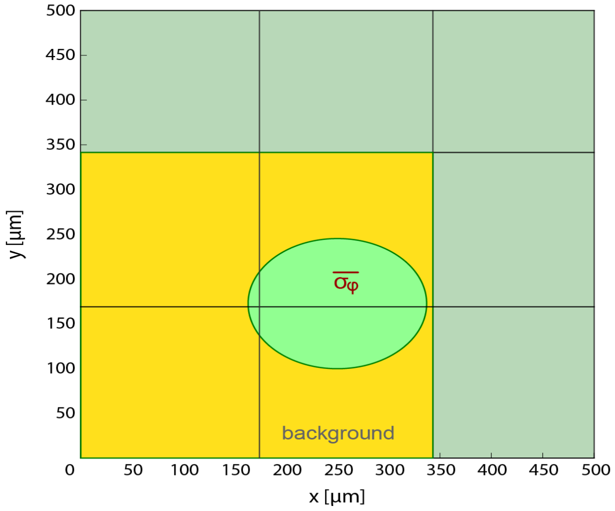
The development of single-photon-counting detectors, such as the PILATUS, has been a major recent breakthrough in macromolecular crystallography, enabling noise-free detection and novel data-acquisition modes. The new EIGER detector features a pixel size of 75 × 75 µm, frame rates of up to 3000 Hz and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

Casanas (2016) Acta D

Smaller pixels improve data quality

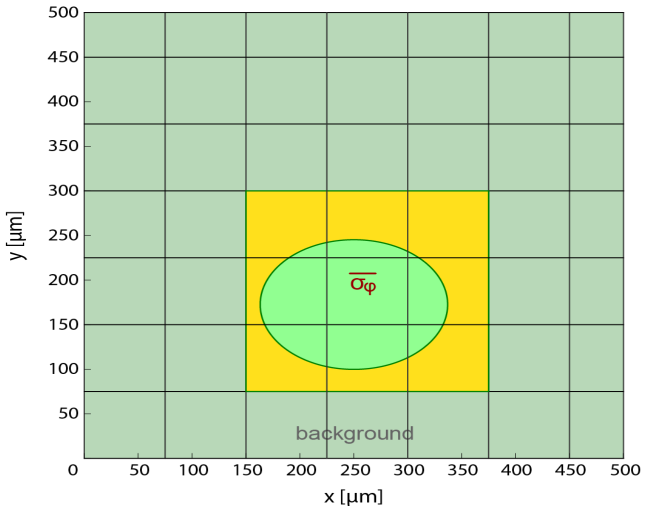


Reflection on EIGER

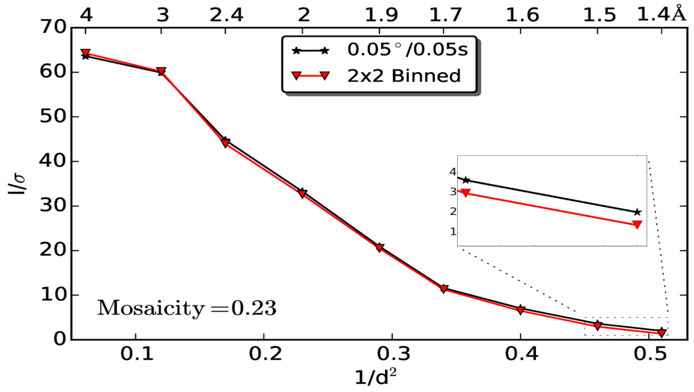


Reflection on PILATUS

Smaller pixels improve data quality



Reflection on EIGER



Better high-resolution data

Get best data by

- *Using EIGER and PILATUS detectors*
- *Using fine φ-slicing (0.1°/img)*
- *Minimizing other sources of error*

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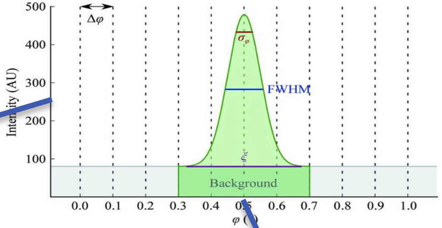
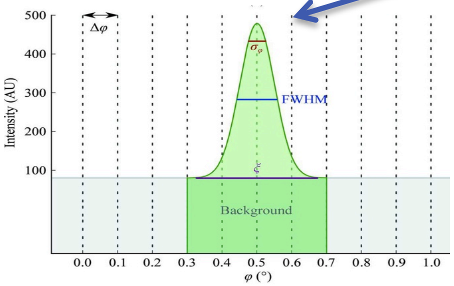
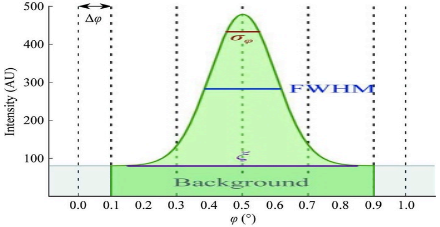
Decrease absolute background

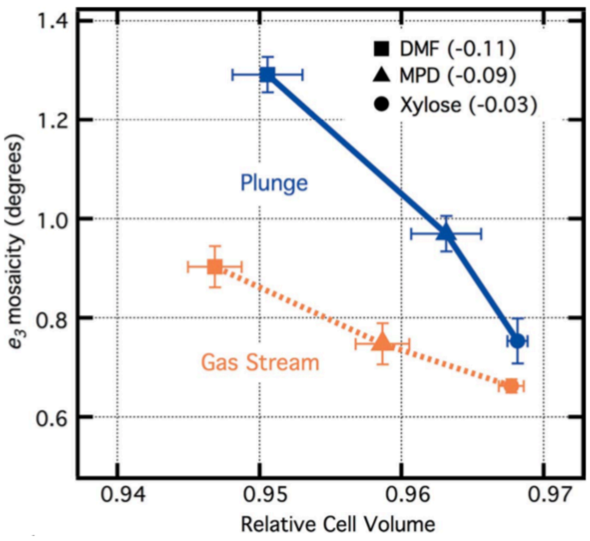


bad!



bad!

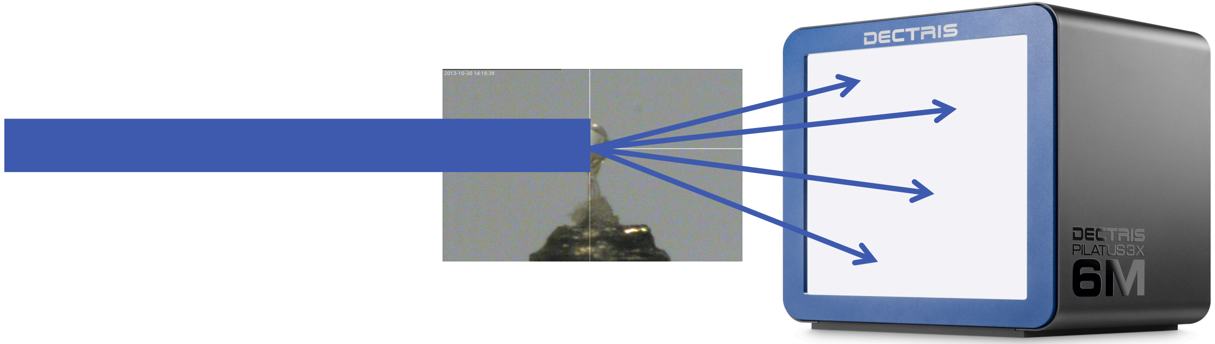




Reduce mosaicity!!

[Harrison (2019) J Appl Cryst]

Decrease absolute background



Get best data by

- *Using EIGER and PILATUS detectors*
- *Using fine φ-slicing (0.1°/img)*
- *Minimizing absolute background*
- *Collecting 360° of data*

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Low-intensity data collection

research papers

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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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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.

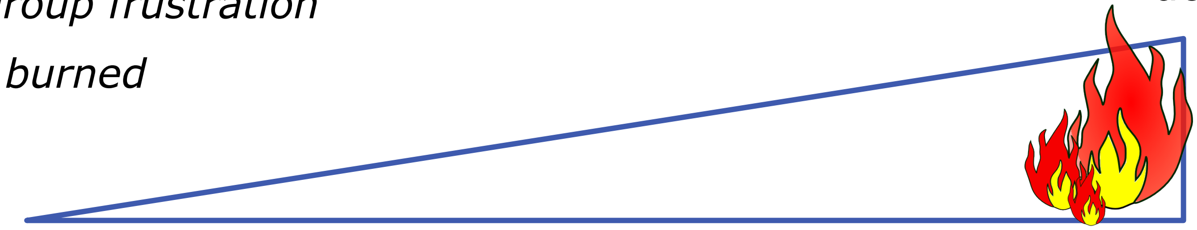
Winter (2019) Acta D

DECTRIS detecting the future

The American method

Collect 360° of data

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



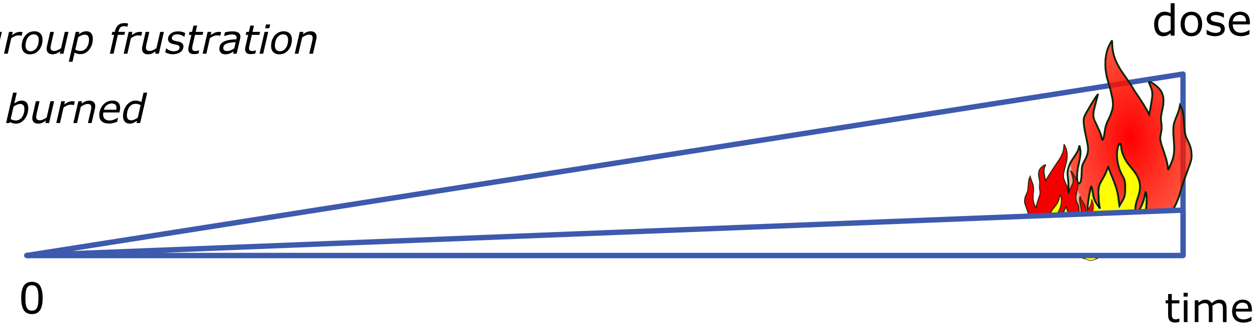
90° @ 10 img/s (0.1°/img) 90 s

360° @ 40 img/s (0.1°/img) 90 s

The American method

Collect 360° of data

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

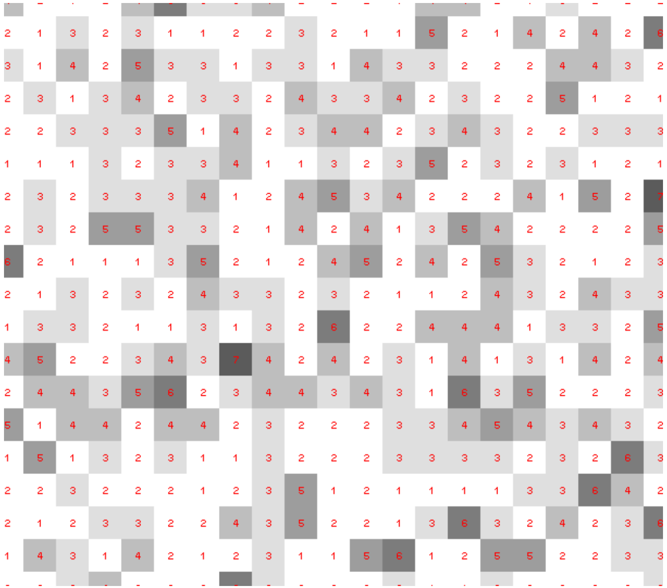


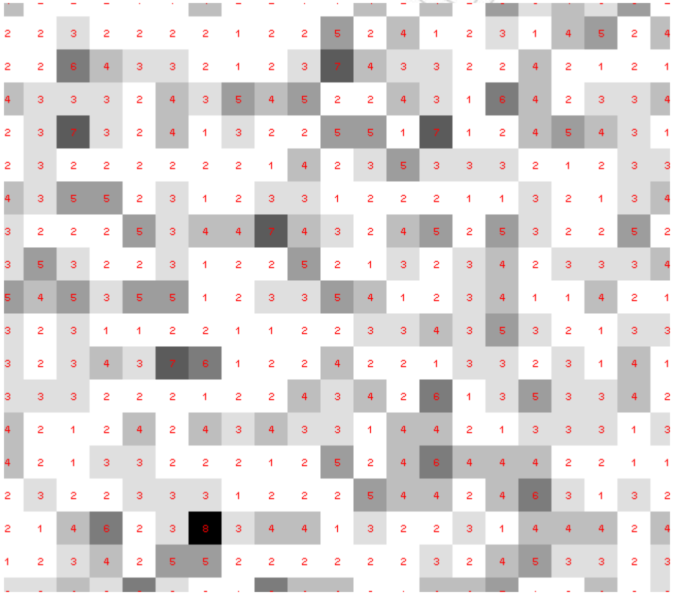
90° @ 10 img/s (0.1°/img) 90 s

360° @ 40 img/s (0.1°/img) 90 s

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

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 (0.1°/img)*
- *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

research papers

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

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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

Basu (2019) Acta D

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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 (0.1°/img)*
- *Minimizing absolute background*
- *Collecting at least 360° of low-intensity data*
- *Thinking about your experiment*

Use beam time ~~efficiently~~ 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 about hardware/best strategy
  - 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, collect 360° of data with weak beam*
- *With PILATUS, $\Delta\phi \approx$ ½ XDS mosaicity for best data*
- *There is no native data*
- *Ask your beamline scientist / think !*

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Ask if things are unclear

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Read more

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