DECTRIS®

detecting the future

Getting best data using Hybrid Photon Counting detectors

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Diamond-CCP4 Data Collection and Analysis workshop, 2014-12-11

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Outline

PILATUS features and how to exploit them:

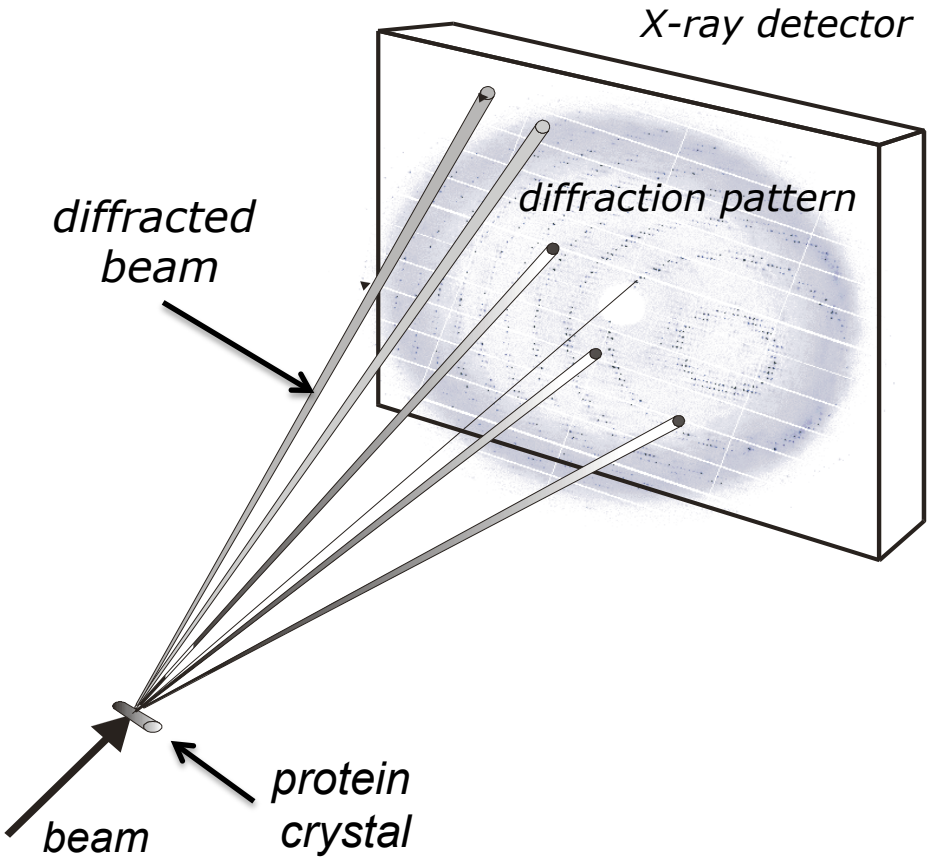
- ***Characteristics of Hybrid Photon Counting detectors***
- ***What is fine φ-slicing?***
- ***Why should I use it?***

Fine φ-slicing: Experimental results

- ***How much do I gain?***
- ***What are the limits?***
- ***What about my real life crystals?***

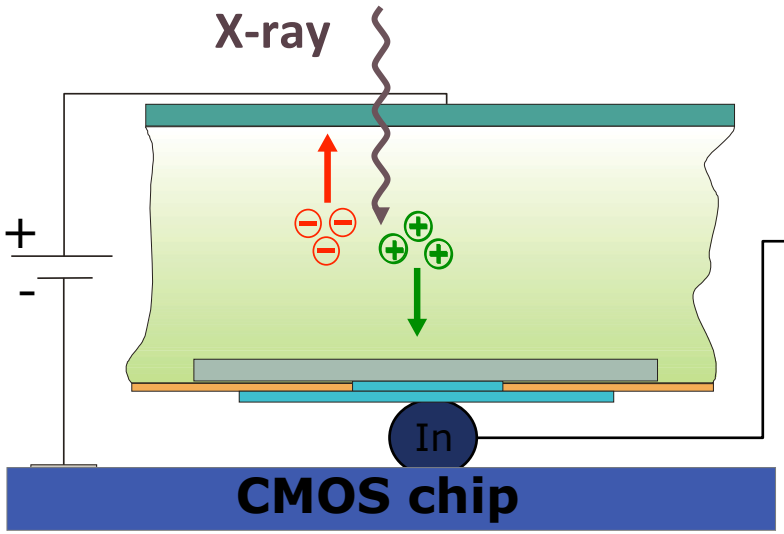
Detector requirements

- Large difference between weak and strong reflections
  -> **high dynamic range**
- Measure weak reflections accurately
  -> **low detector background**
- Collect all photons which are scattered from protein
  -> **high quantum efficiency**
- Minimize overlap with background
  -> **good spatial resolution, low point-spread function**
- continuous rotation to eliminate shutter jitter and fine φ-slicing to minimize overlap with background
  -> **short readout time, high frame rate, no readout noise**

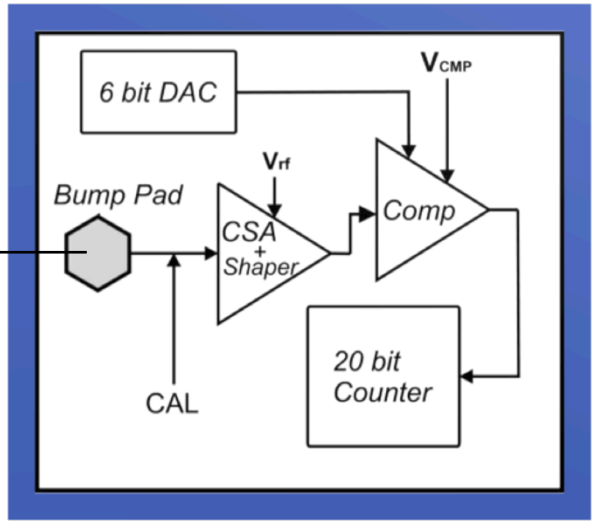


Hybrid Photon Counting Technology

Sensor pixel



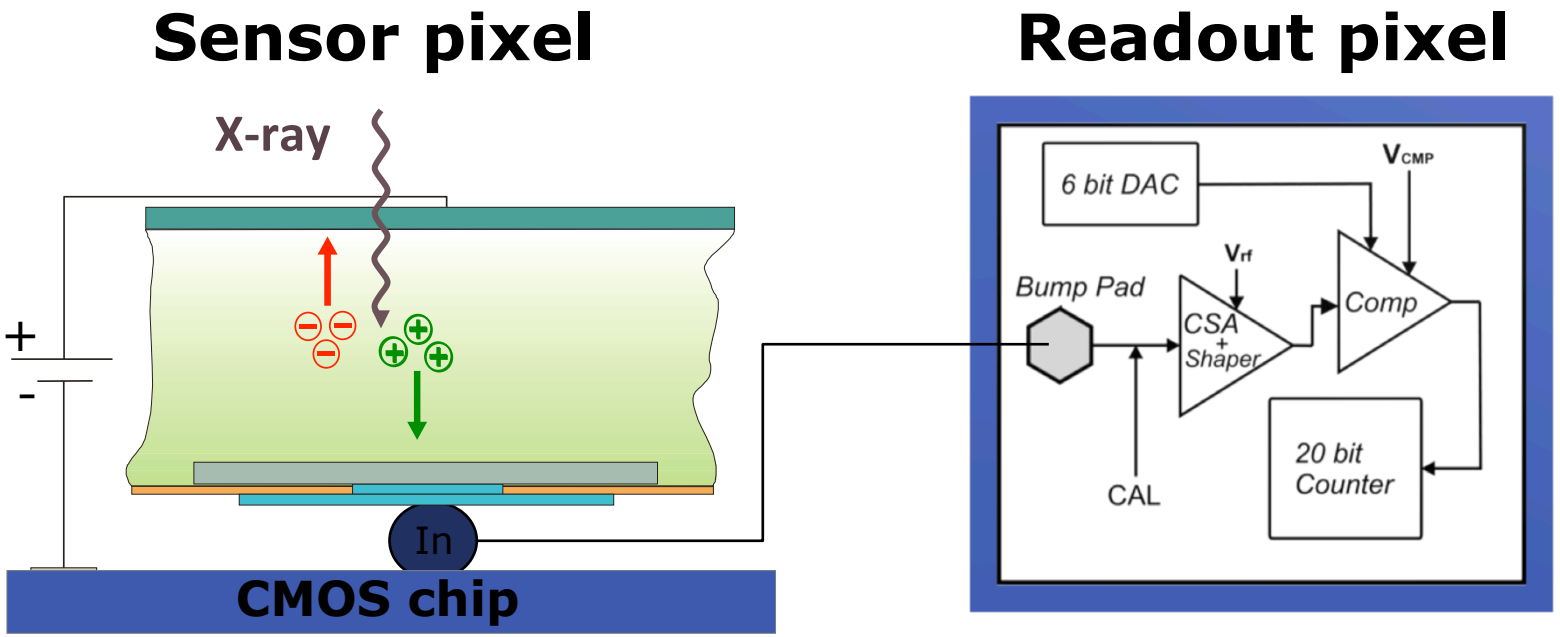
Readout pixel



Direct Detection of X-rays in silicon sensor
→ Point Spread Function of 1 pixel

Single Photon-counting in CMOS
→ no readout noise & dark current
→ high dynamic range (20 bit)
→ fast readout

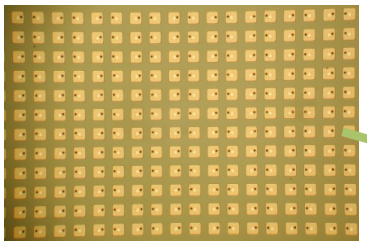
Hybrid Photon Counting Technology



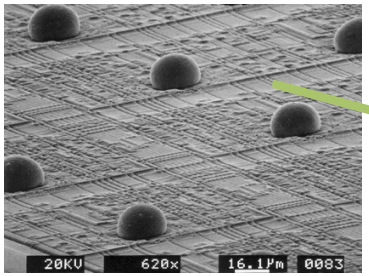
Hybrid pixel + single-photon counting
= Hybrid Photon Counting

Hybrid Photon Counting Technology

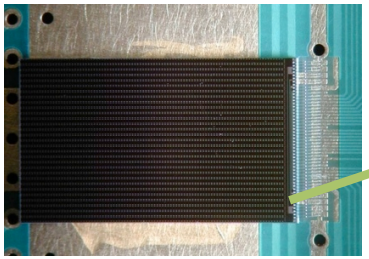
Silicon sensor

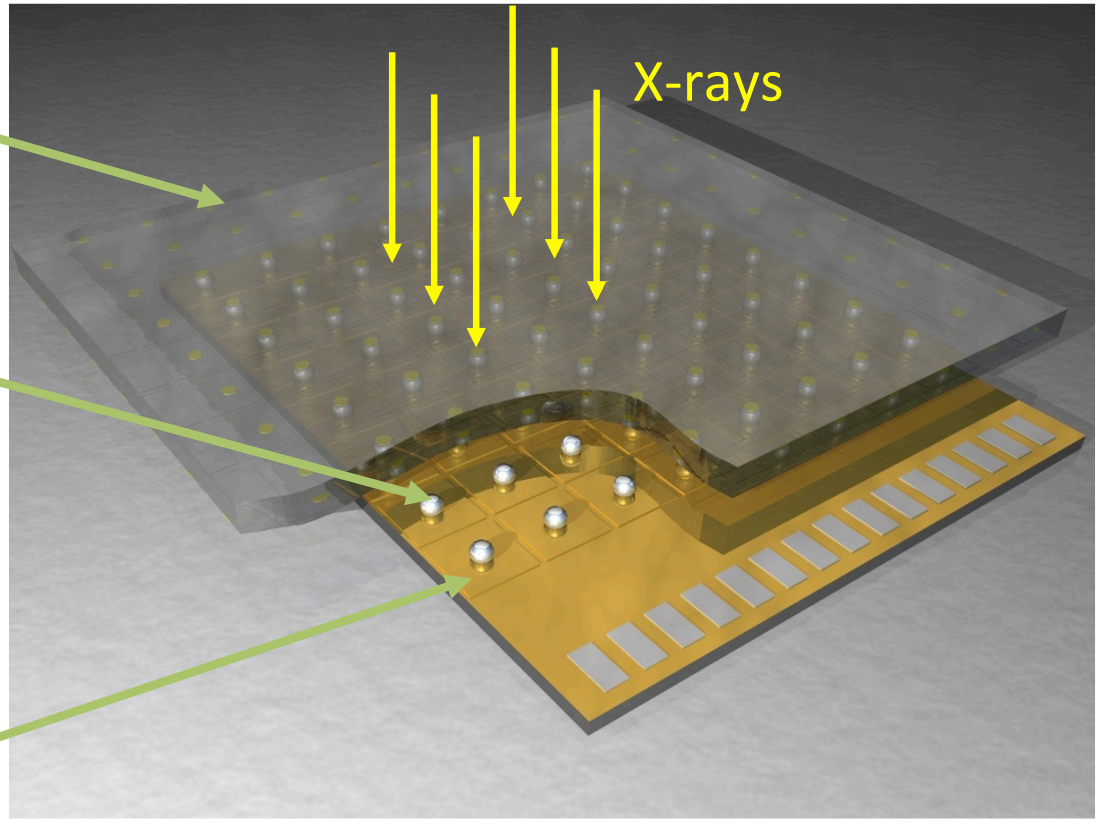


Indium bump bonds (18 µm)



CMOS chip

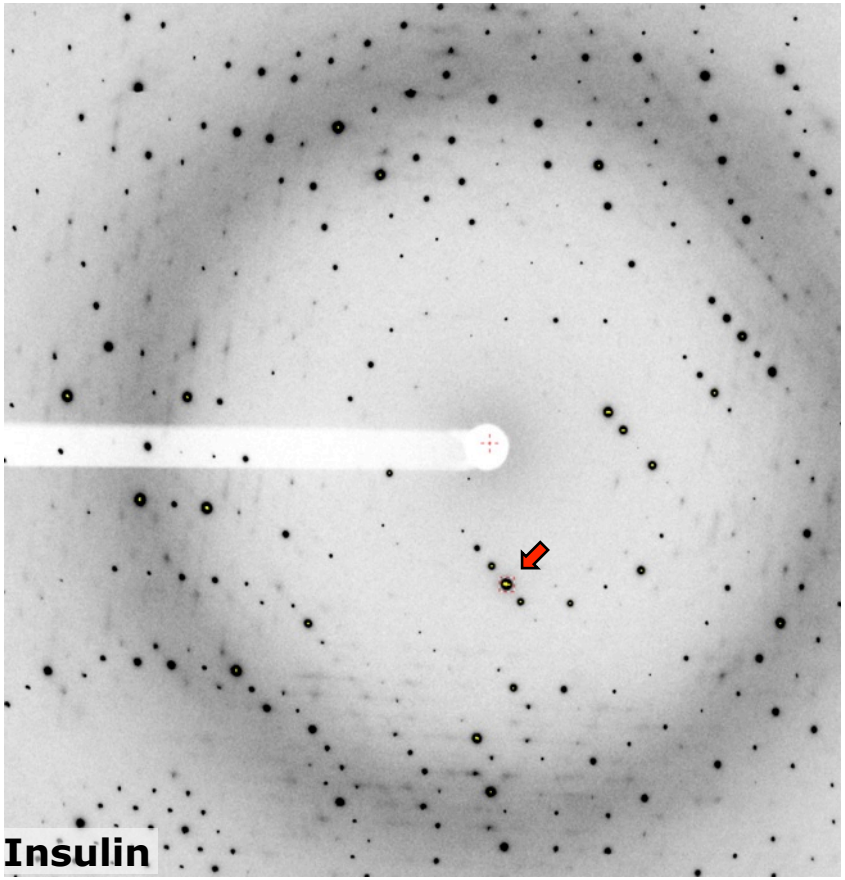




Dynamic range and point spread

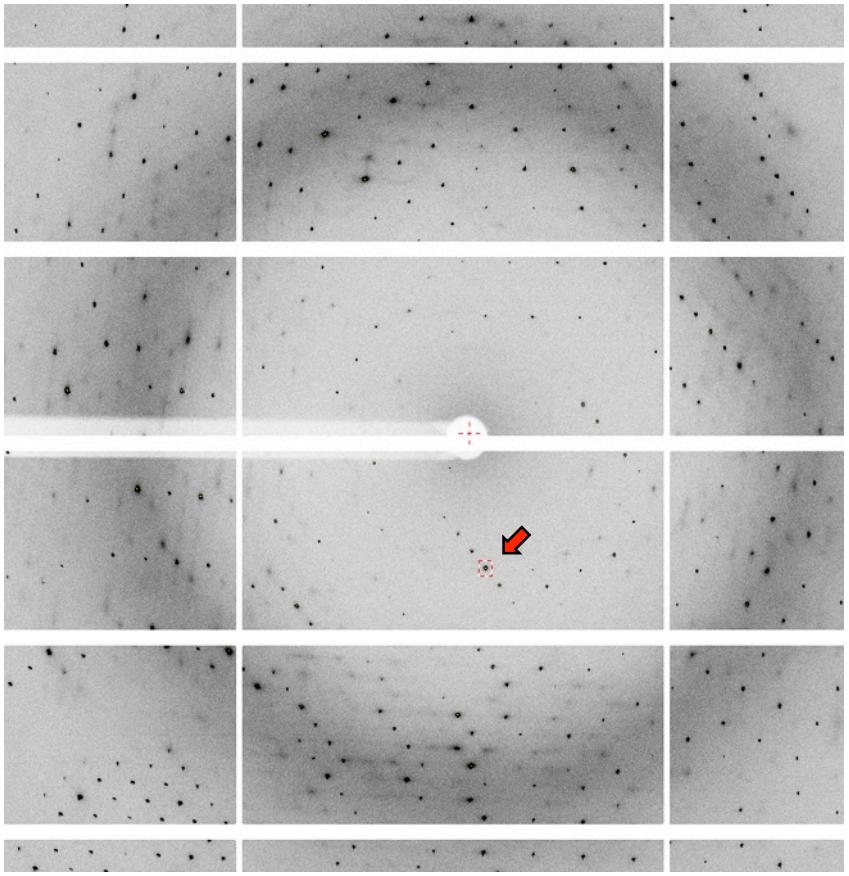
CCD

16 bit (65 535 ADU)



PILATUS

20 bit (1 048 575 counts)



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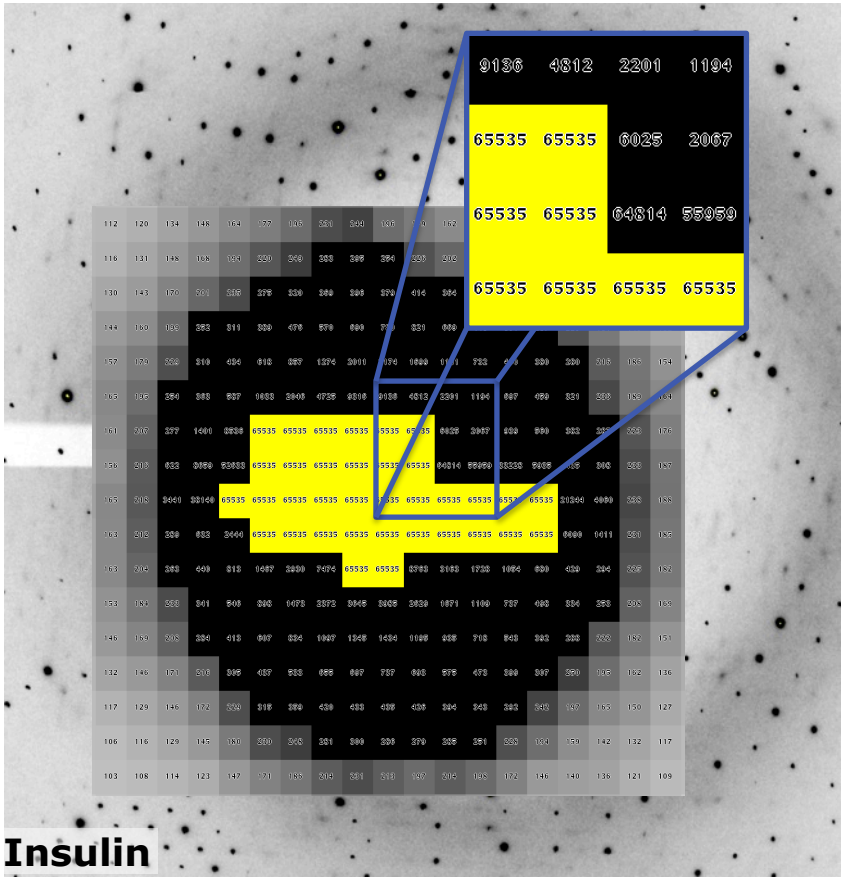

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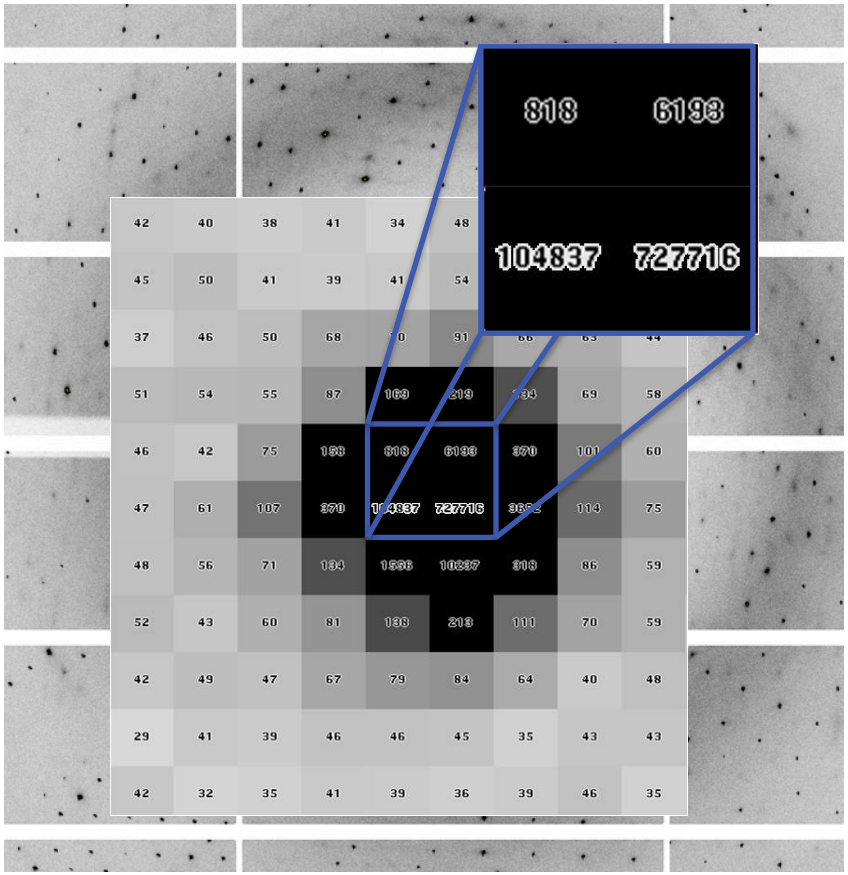


Fast readout and high frame rates

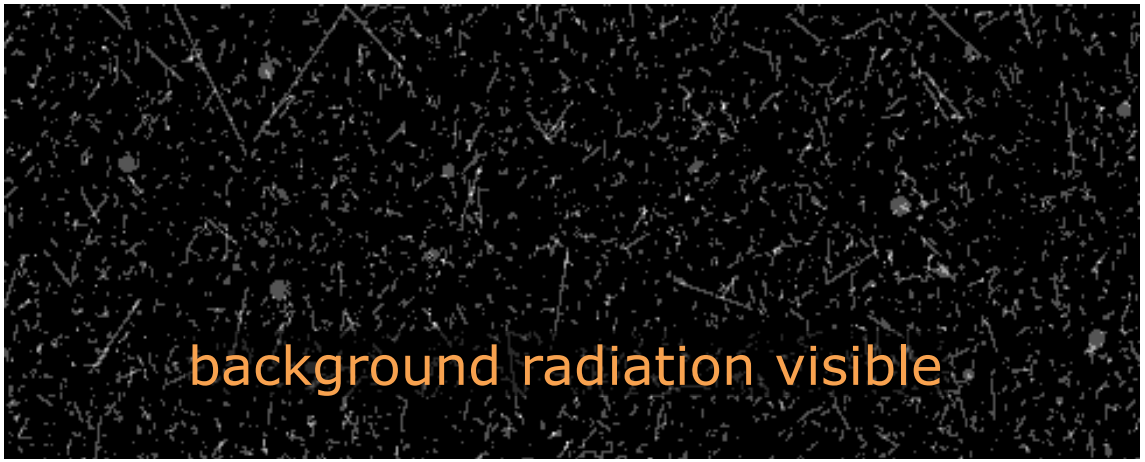
- Fast readout (2.3 ms, PILATUS3: 0.95 ms)
  - ➔ shutter-free, **continuous rotation data collection**
  - ➔ **no systematic errors** from shutter-spindle synchronization

- High frame rates (6M: 12.5 Hz, PILATUS3 6M: 100 Hz)
  - ➔ complete data sets in less than 10 s
  - ➔ highly redundant, fine-sliced data sets in a few minutes
  - ➔ **High-throughput** data collection
  - ➔ Outrun radiation damage at room temperature:
    Acta Cryst. (2012). D68, 810-818

Noise-free detection

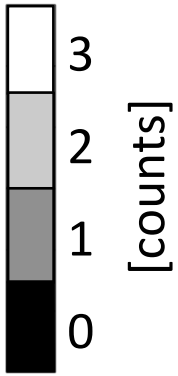
100 ms

1 hour



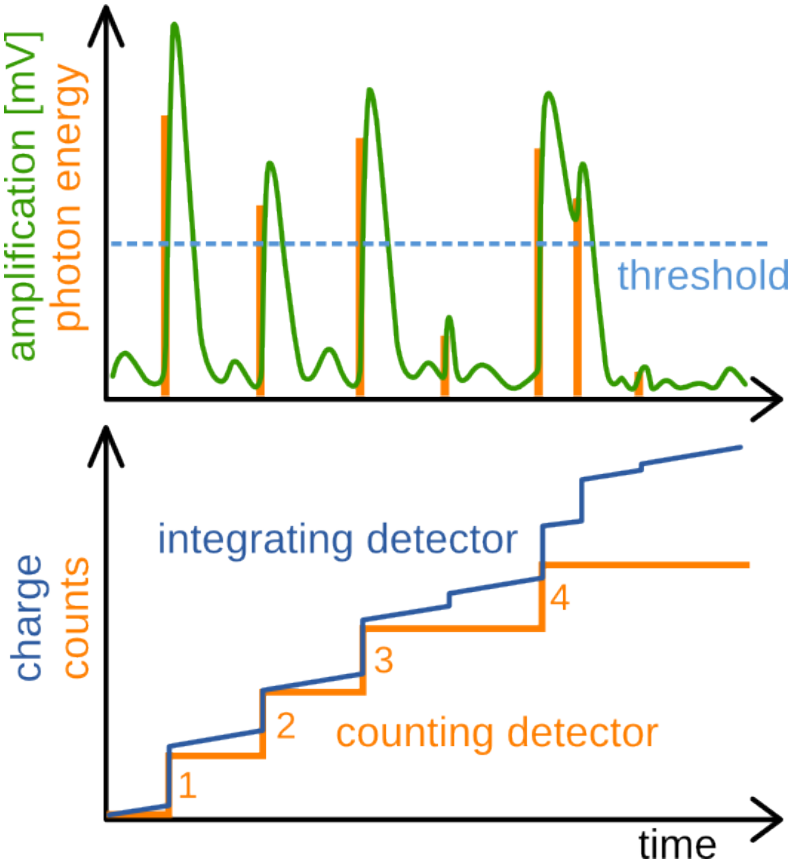
No readout noise

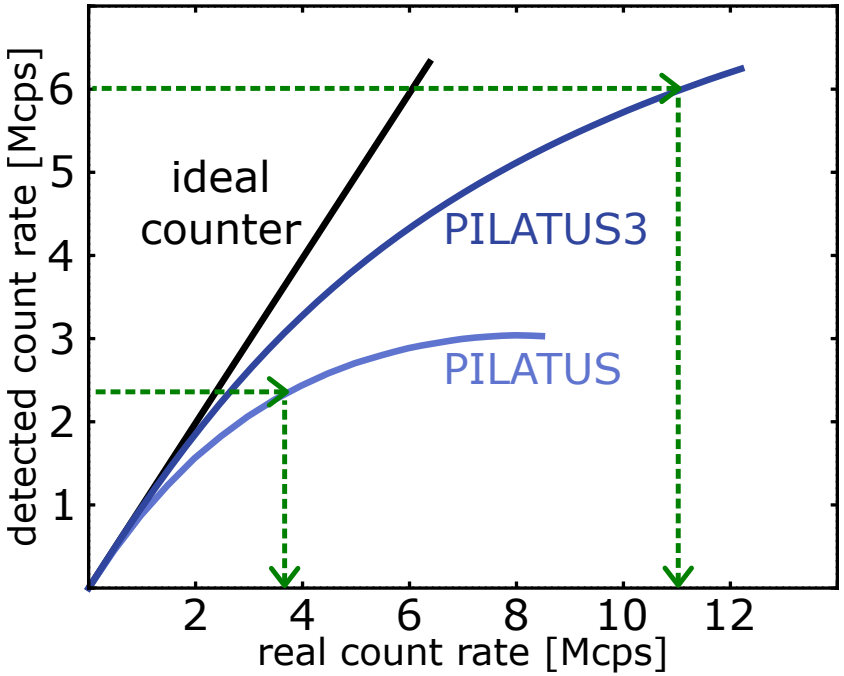
No dark current



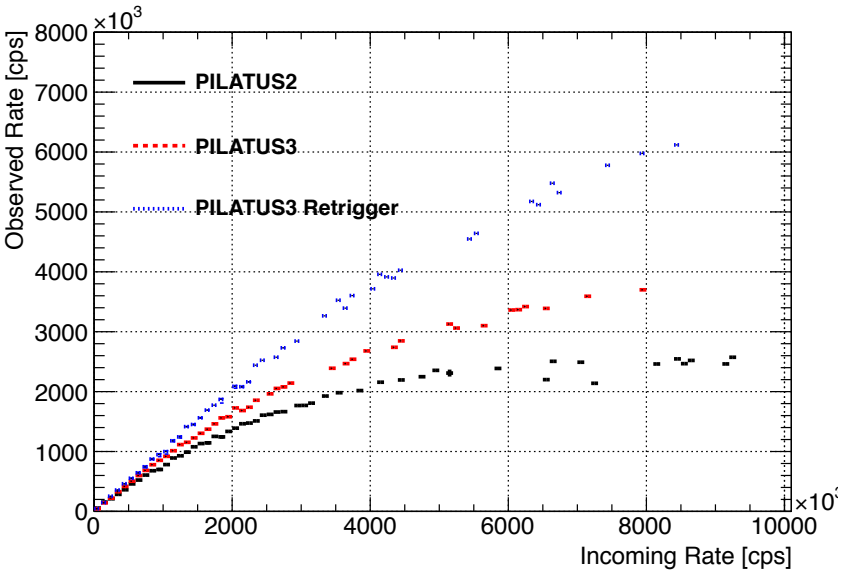
100K detector

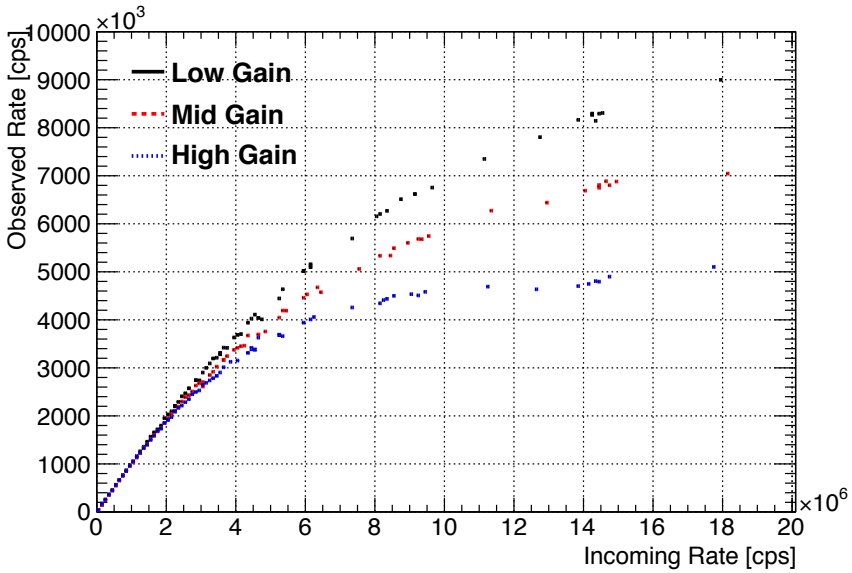
Photon counting detector





DECTRIS Instant Retrigger Technology: PILATUS3





Accuracy of intensity estimates

Q : Quantity of photons

$\mathrm{var}(Q) = Q$

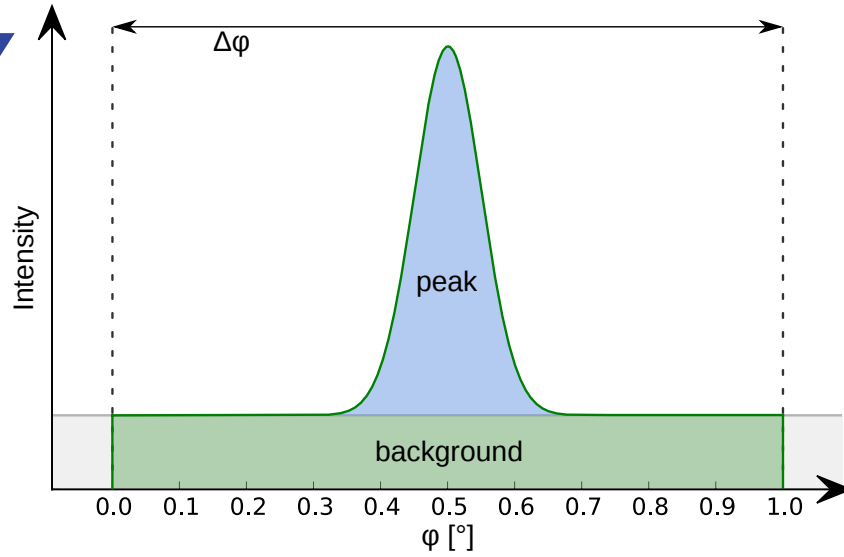
$\sigma(Q) = [\mathrm{var}(Q)]^{1/2} = Q^{1/2}$

I : Observed intensity

$I = \Sigma(\mathrm{Peak}_i - \mathrm{Background}_i)$

$\mathrm{var}(I) = \Sigma[\mathrm{var}(\mathrm{Peak}_i) + \mathrm{var}(\mathrm{Background}_i)]$

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

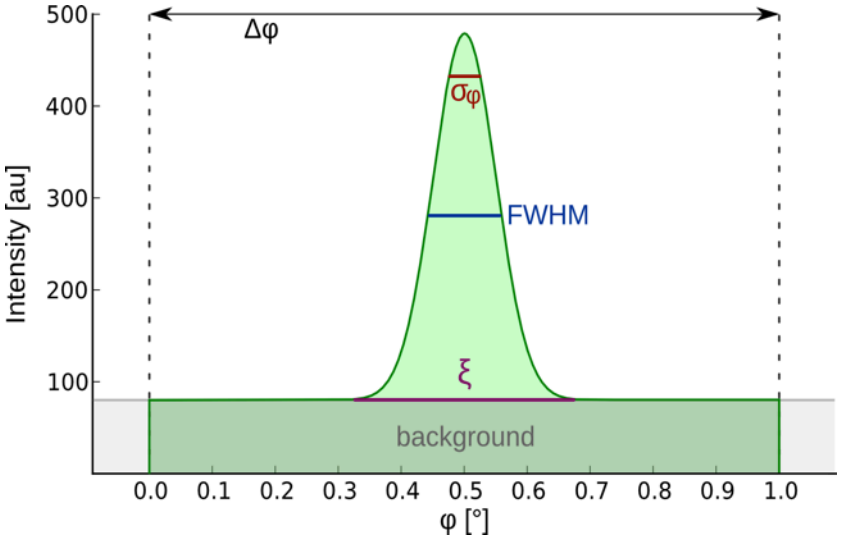


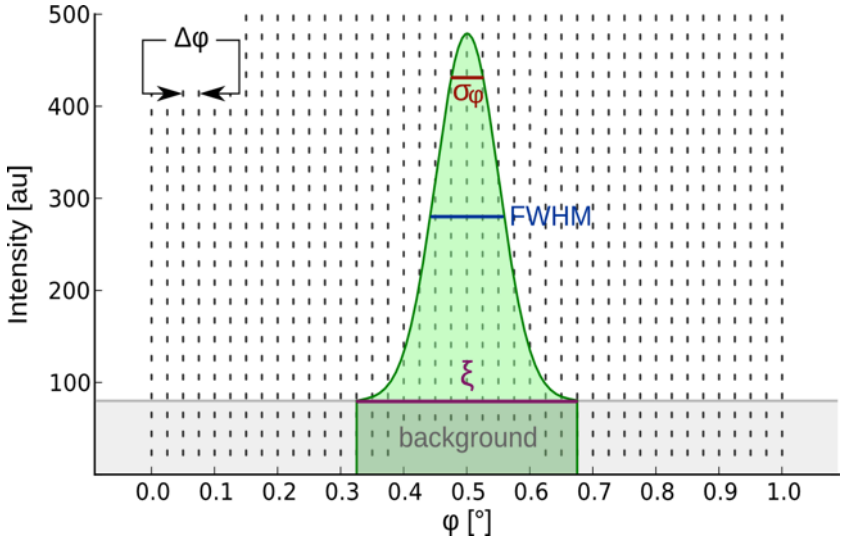
Relative error [%]	Background [photons]		
Integrated intensity [photons]	0	500	1000
100	10.0	33.2	45.8
500	4.5	7.7	10.0
1000	3.2	4.5	5.5
10000	1.0	1.0	1.1

Pflugrath, J.W. (1999). Acta D 55, 1718–1725.

Less background => better data

Fine φ-slicing: minimizing background overlap





Wide φ-slicing

- Large Δφ (Δφ > ξ)
- Large overlap of reflections and background along φ
- Few images

Fine φ-slicing

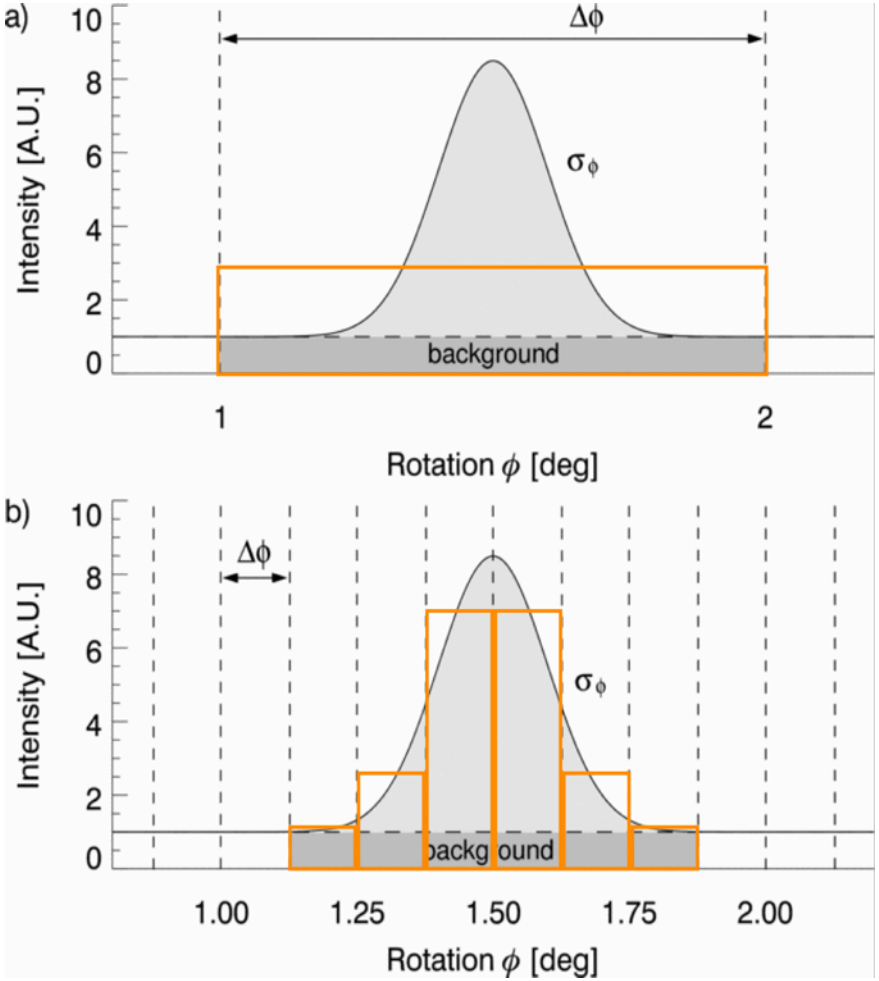
- Small Δφ (Δφ << ξ)
- Minimal overlap of reflections and background along φ
- Many images

Fine φ-slicing: exploit absence of readout noise

Detector with readout noise (CCD)

Hybrid pixel detector
without readout noise

Fine φ-slicing: improving count rate correction



$$\text{Count rate} = \frac{\text{number of counts}}{\text{exposure time}}$$

Data collection and processing

- Test crystals: insulin, thaumatin, lysozyme, thermolysin
- Beamline X06SA at SLS
- Wavelength: 1.000Å, thermolysin: 1.282Å (zinc edge)

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- Beam size: 100 µm * 100 µm, crystals > beam
- Flux: 6e08 – 1e11 ph/s
- 0.3 – 8 MGy per series of 5 to 7 data sets
- Interleaved data acquistion scheme to distribute dose evenly over all data sets of a series of data sets

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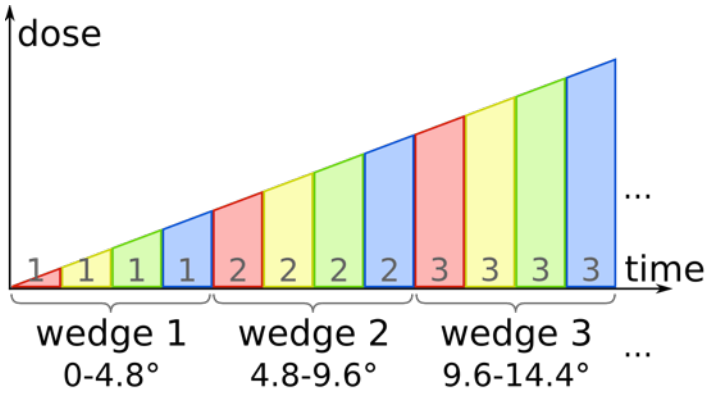
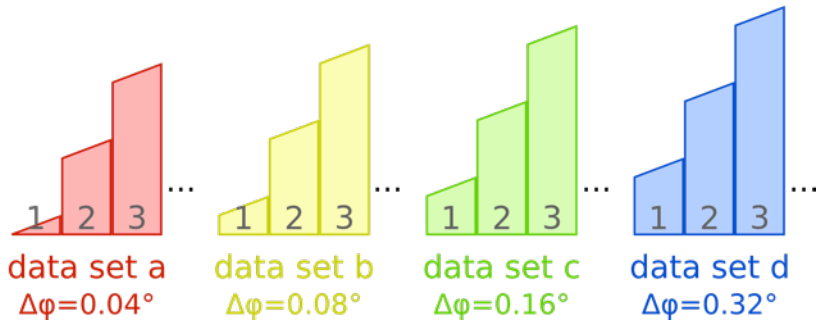
- Processed with XDS

Fine φ-slicing: data sets





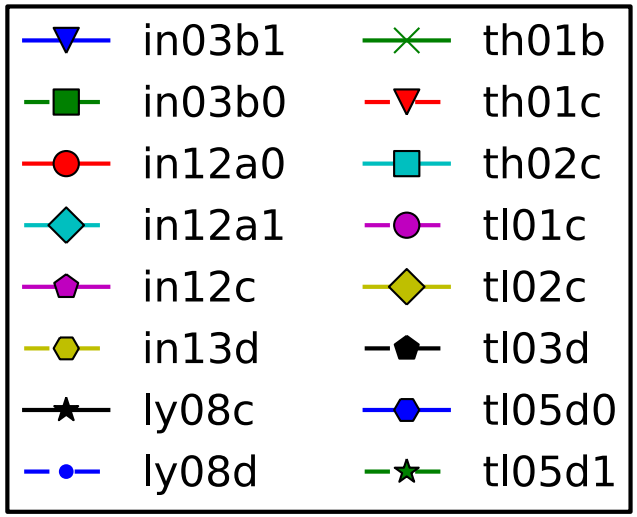
data set	a	b	c	d
ΔΦ [°]	0.04	0.08	0.16	0.32
t [s]	0.1	0.2	0.4	0.8
images	1200	600	300	150
Φ [°]	48	48	48	48

Each series:

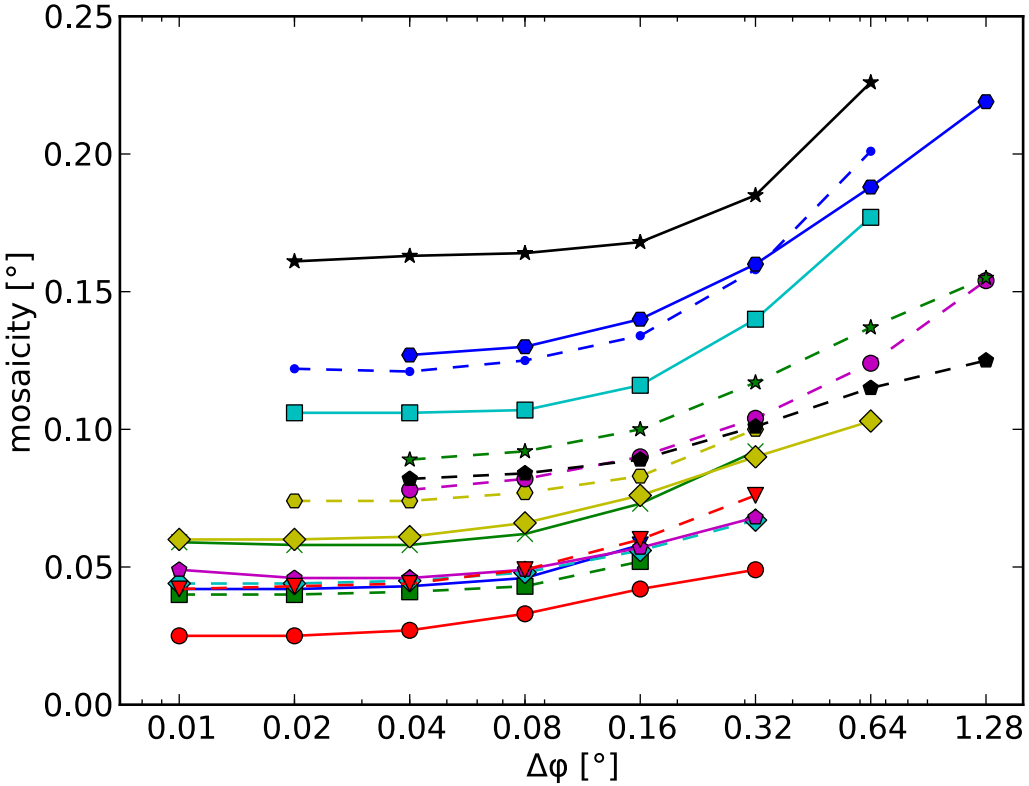
- *Same position of single crystal*
- *Same angular speed* and dose rate*
- *Interleaved data collection:*
  Dose is distributed evenly over the compared data sets.

* Diederichs, K. (2010). Acta Cryst. D 66, 733–740.

Refined mosaicity and Δφ



16 series of data sets with increasing Δφ, each with 5 to 7 data sets



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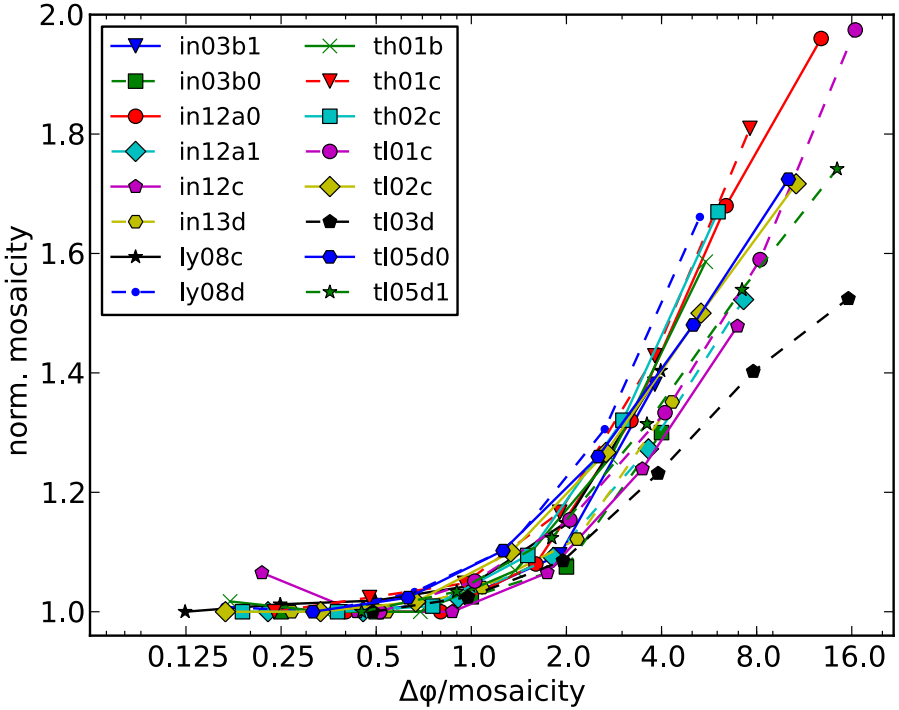
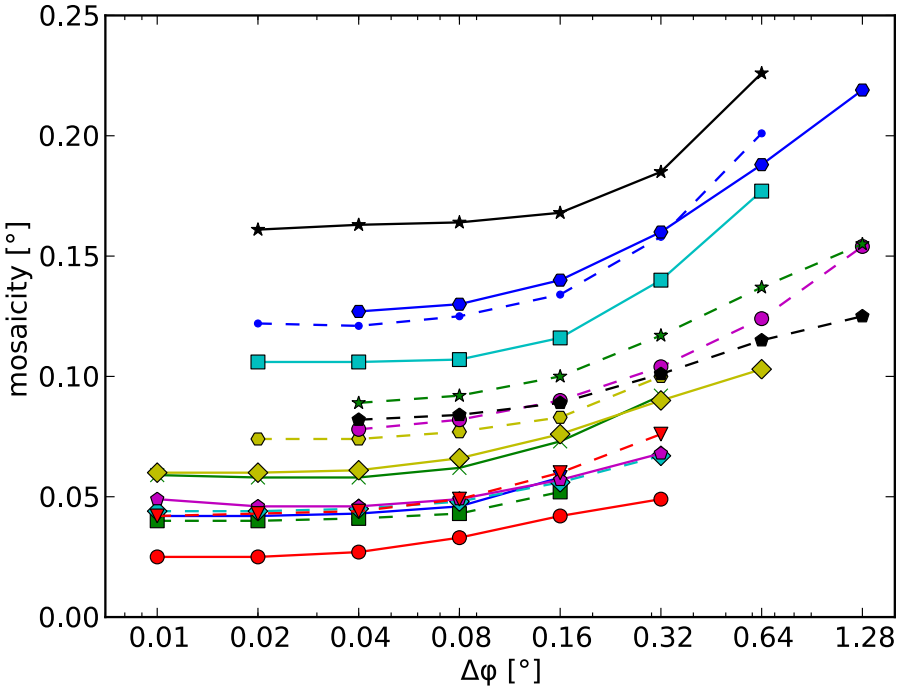
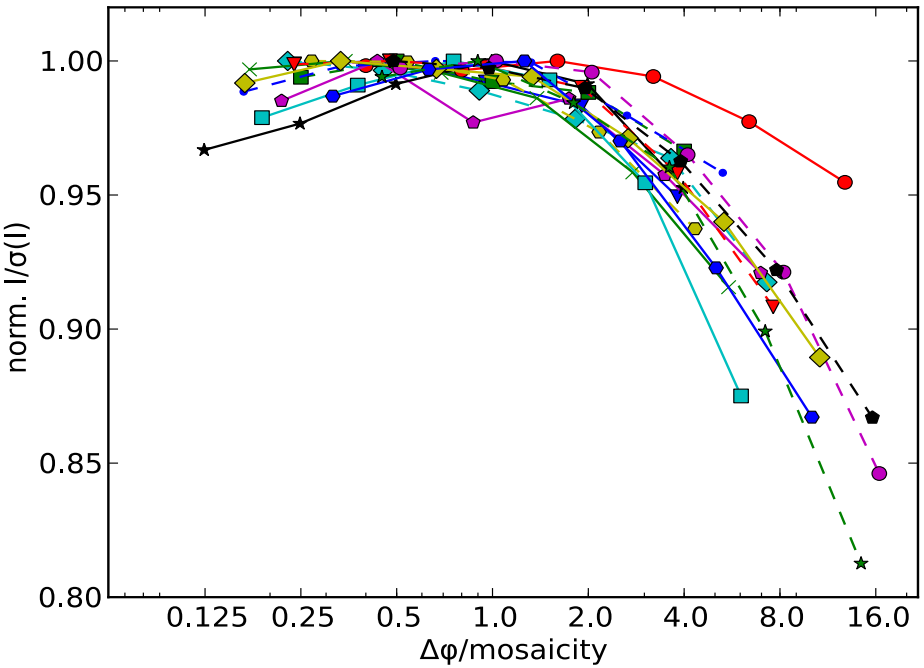
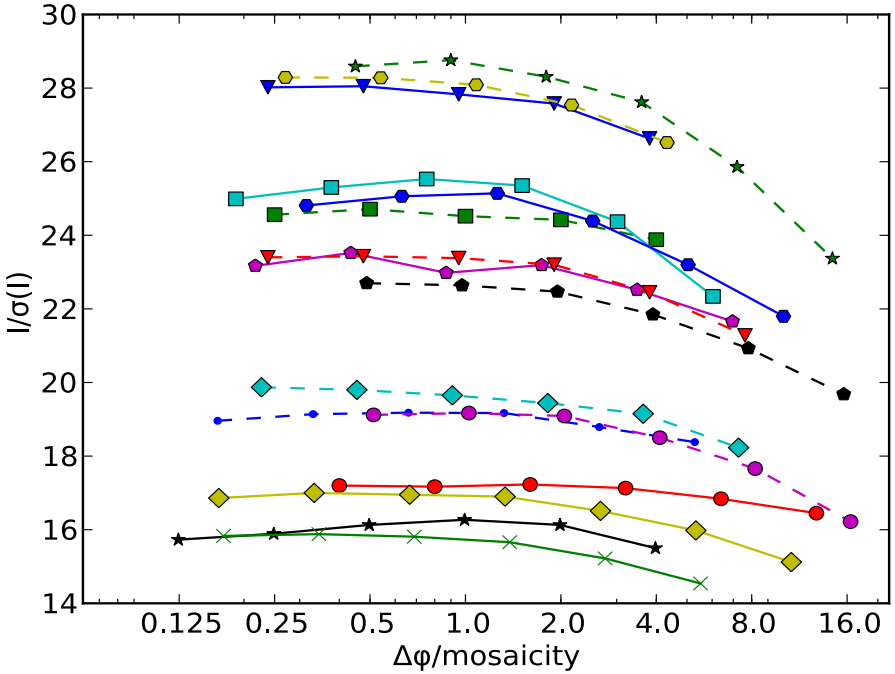




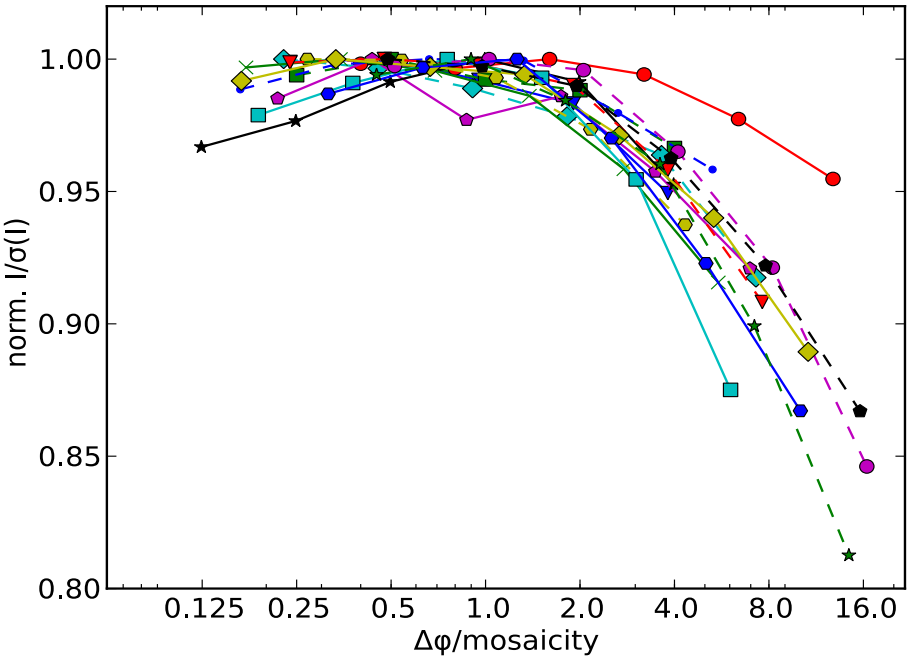
- *Smaller mosaicity for finer Δφ.*
- *Asymptotic minimum for small Δφ.*
- *0.03° < σ_φ < 0.16°*
- *0.06° < σ_φ < 0.23° from wide-sliced data*

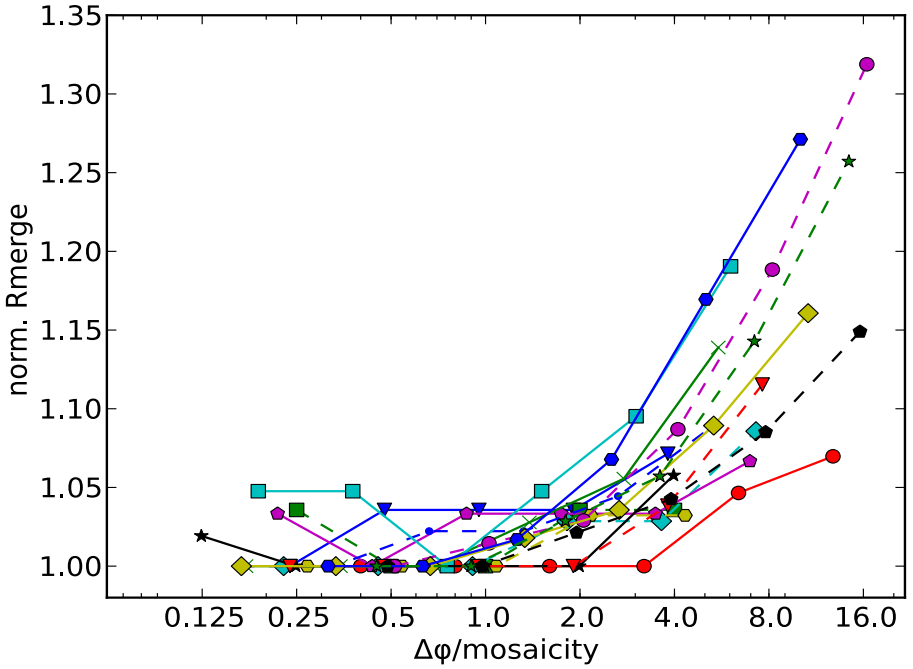
Fine φ-slicing: Overall I/σ(I)



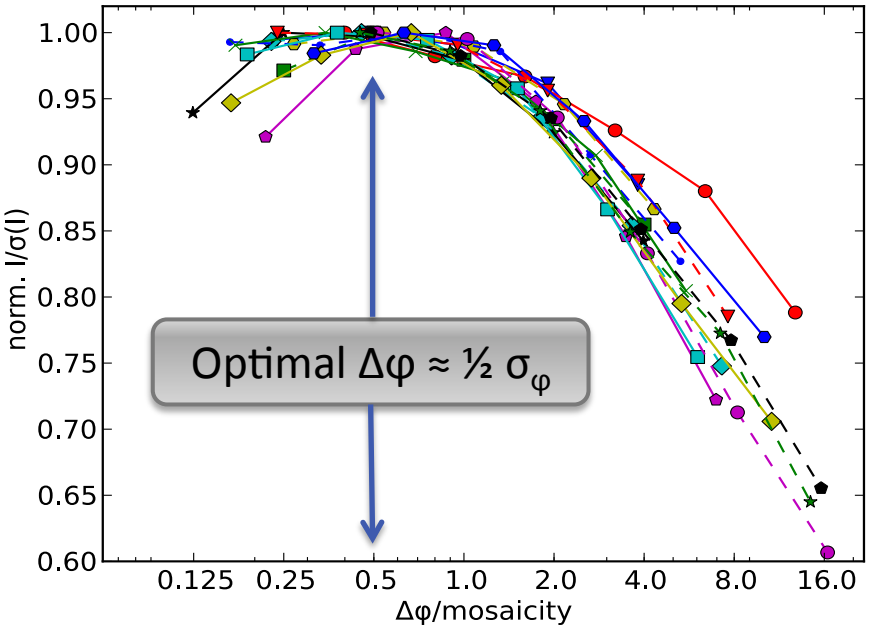


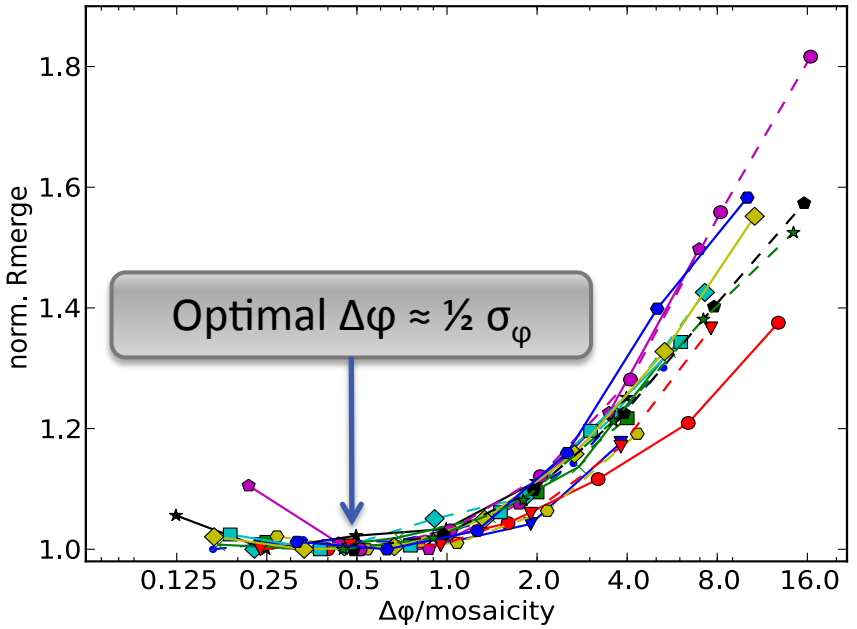
Fine φ-slicing: normalized overall statistics





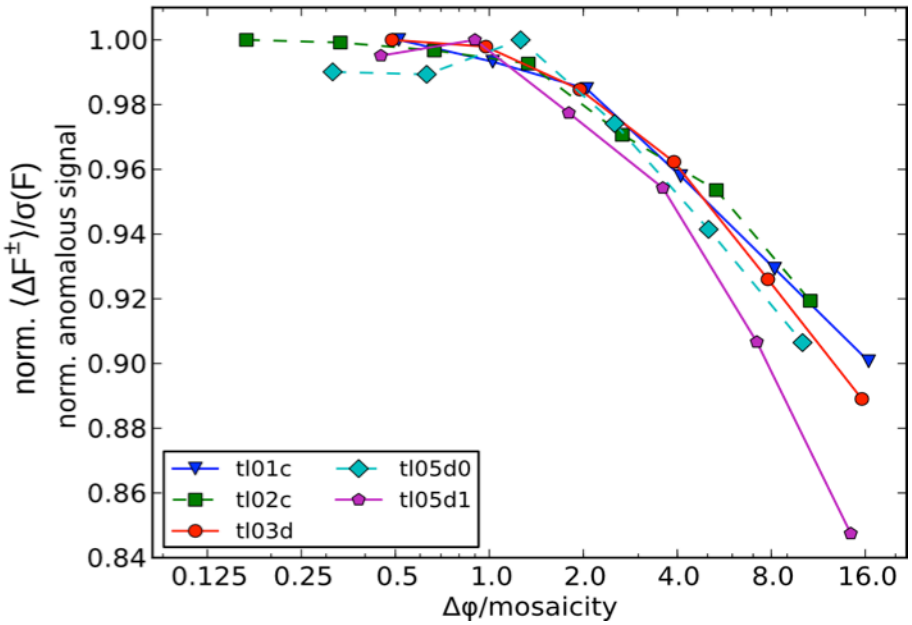
Fine φ-slicing: highest shell statistics

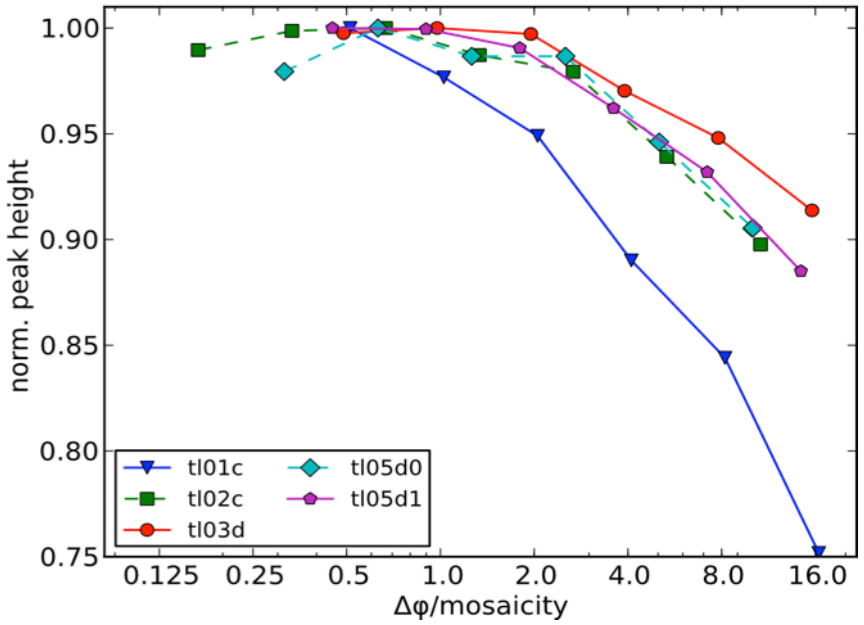




- Best data at Δφ ≈ ½ mosaicity (σ_φ)
- Highest shell statistics improve substantially => fine-slicing extents maximum resolution of data set

Fine φ-slicing: anomalous signal





Fine-slicing improves quality of anomalous data

Fine φ-slicing: Summary

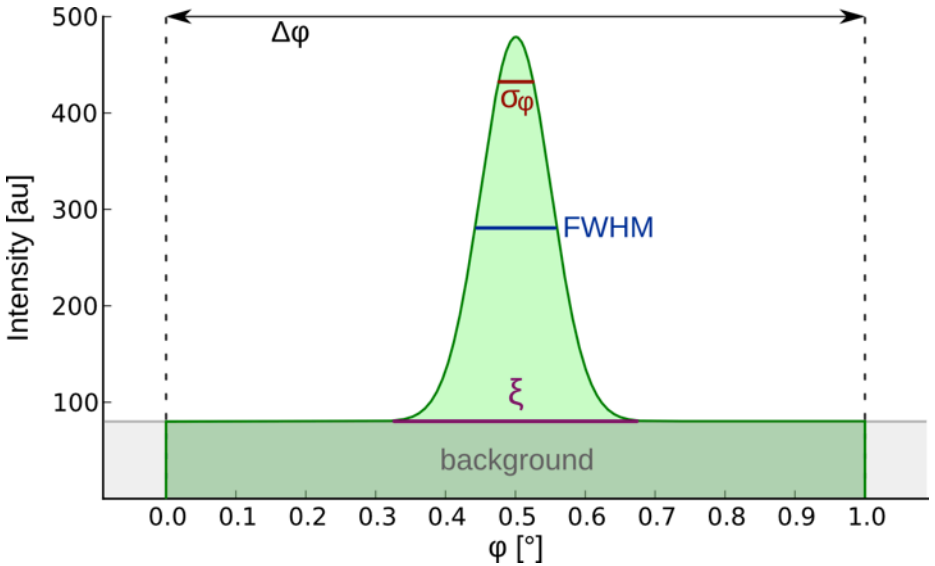
Fine φ-slicing with Δφ ≈ ½ mosaicity (σφ) improves:

- ***overall statistics***
- ***highest-shell statistics***
- ***anomalous signal***

Use a detector without readout noise!

Be aware of your integration software's definition of mosaicity!

Less overlaps.



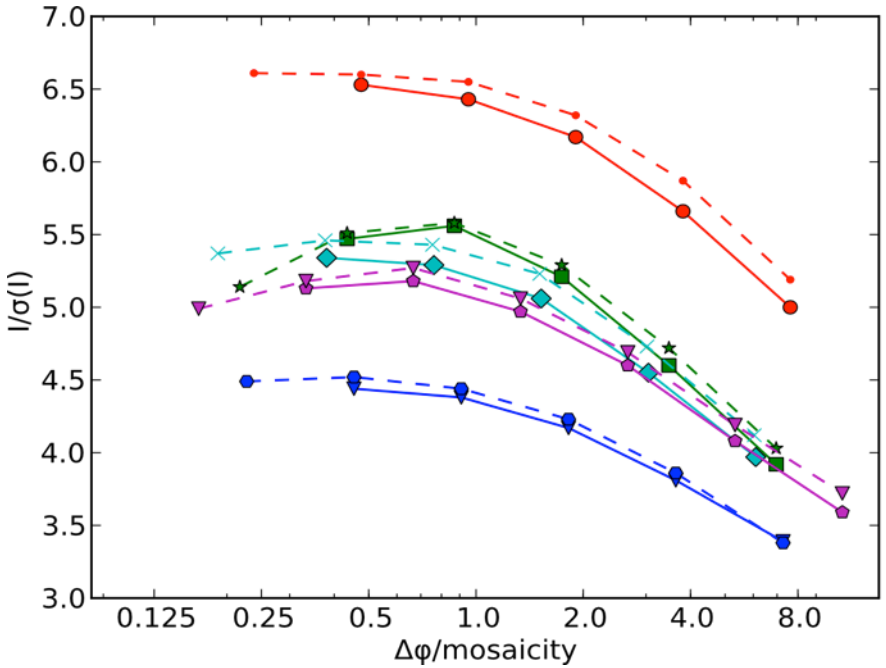
Mosaicity in XDS and MOSFLM

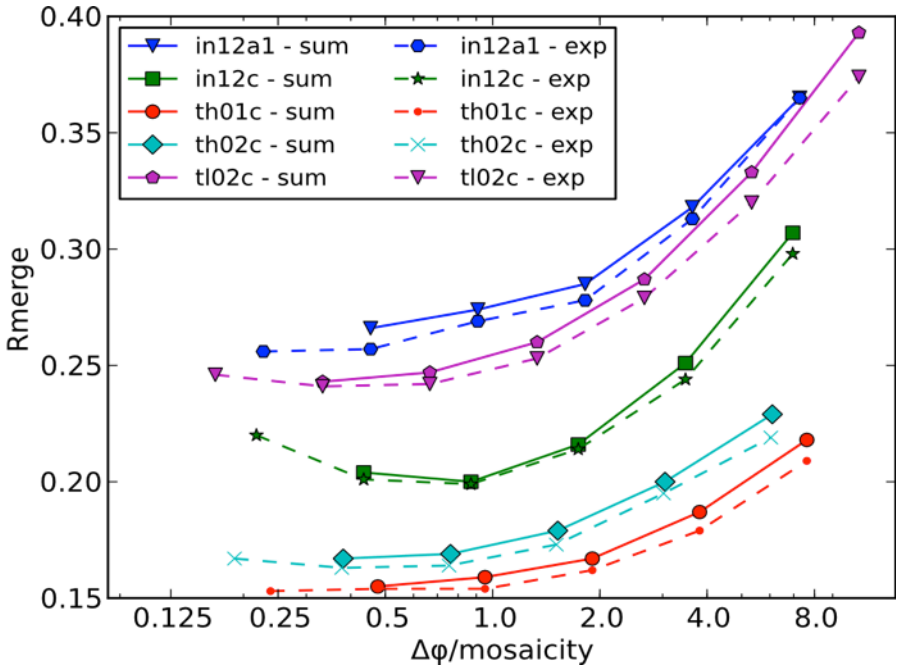
Crystal	in12a1	in12c	ly08c	th01c	th02c	tl02c
XDS σ_φ [°]	0.07	0.07	0.23	0.08	0.18	0.10
MOSFLM mosaic spread [°]	0.18	0.14	0.68	0.20	0.48	0.31
Ratio MOSFLM/XDS	2.6	2.0	3.0	2.5	2.7	3.1

Open questions

1. Why is there an optimum at $\Delta\varphi \approx$ ½ mosaicity ($\sigma\varphi$) ?
2. What about my "real life" crystals?

Summation of fine-sliced data



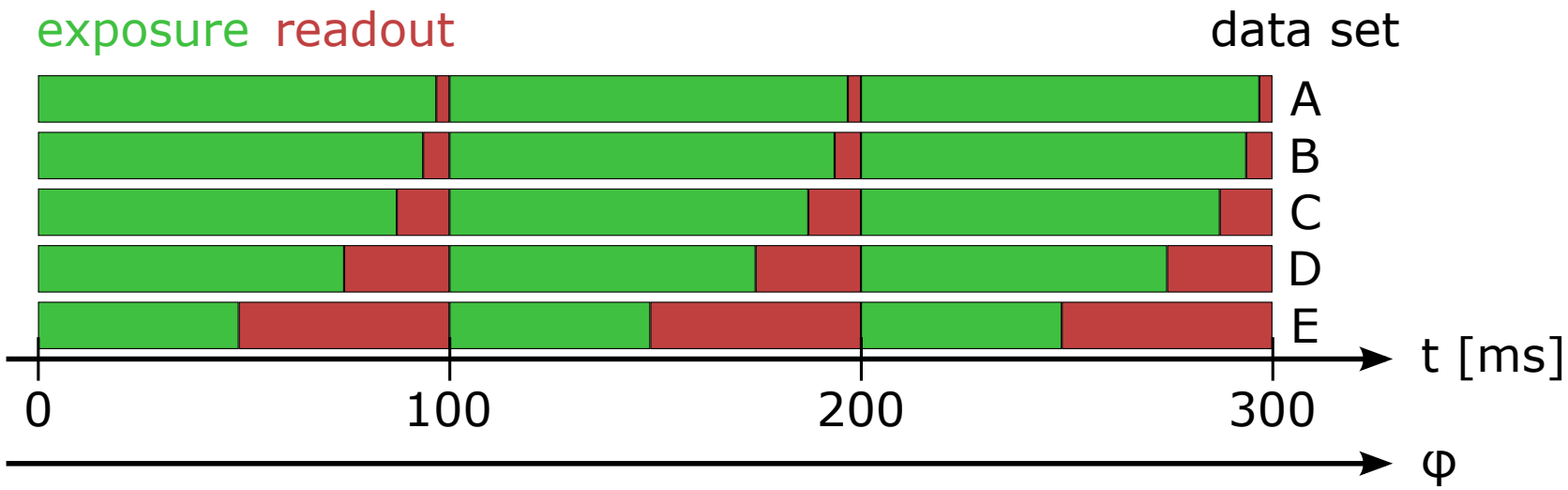


Optimum at ½ σ_φ is a result from

- ***intensity estimation by integration software***
- ***experimental and systematic errors in the highly fine-sliced data.***

Readout time and data quality: procedure

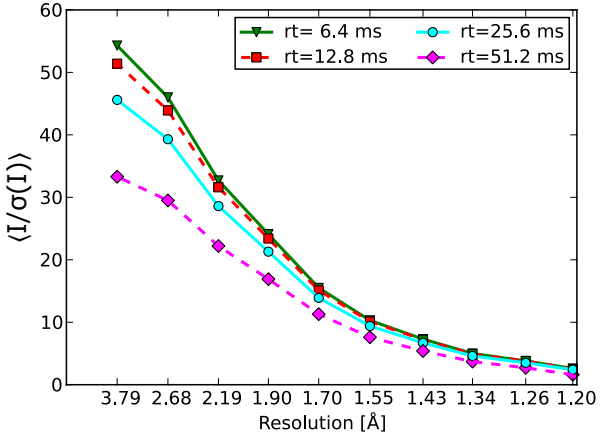
- ***Interleaved acquisition of data set series with …***
  - constant period of 100 ms between images
  - constant oscillation width of 0.05° or 0.25° during the 100 ms period
  - increasing readout time (rt) per image for each data set, ranging from ~3 to ~50 ms

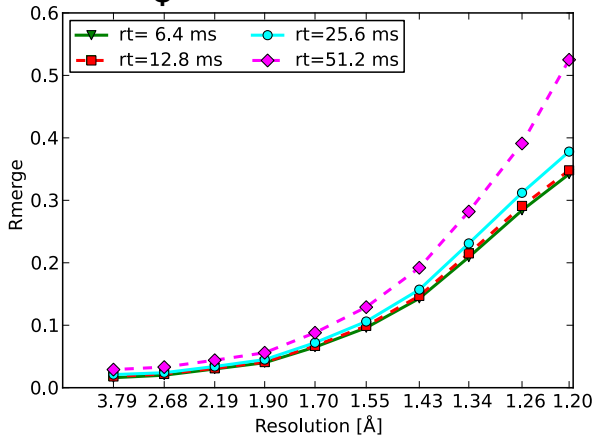


Readout time and data quality: thaumatin

fine sliced

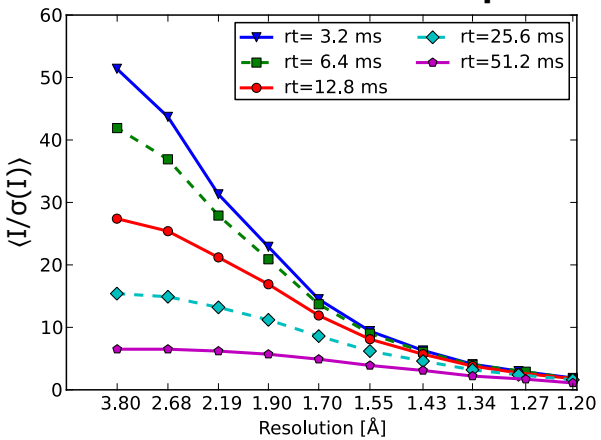
Δφ = 0.05° ≈ 0.6 σ_φ

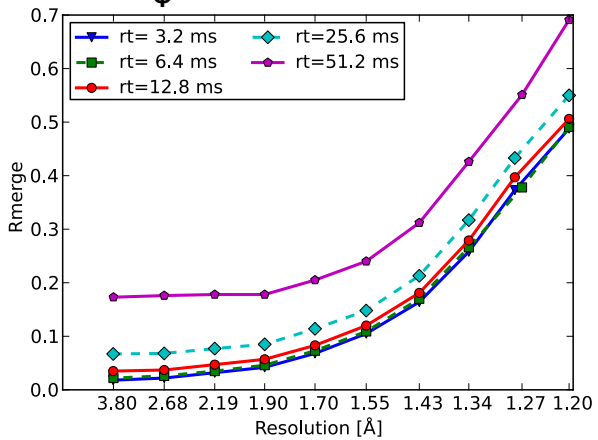




wide sliced

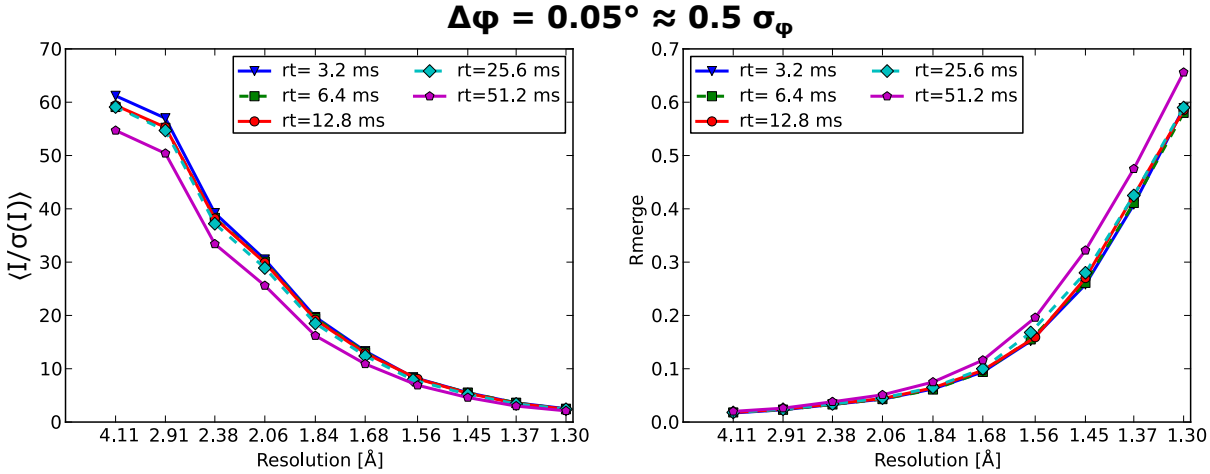
Δφ = 0.25° ≈ 3.2 σ_φ



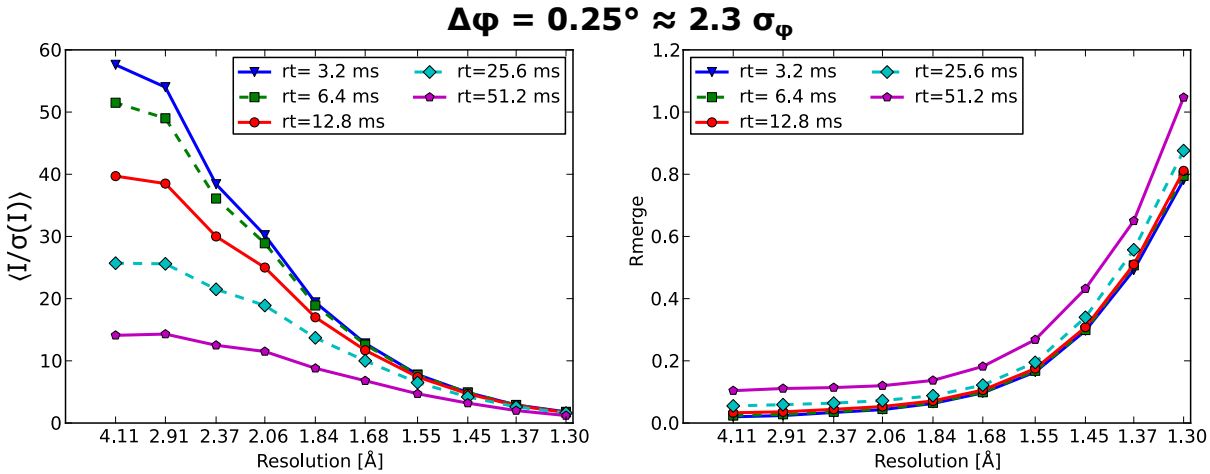


Readout time and data quality: insulin

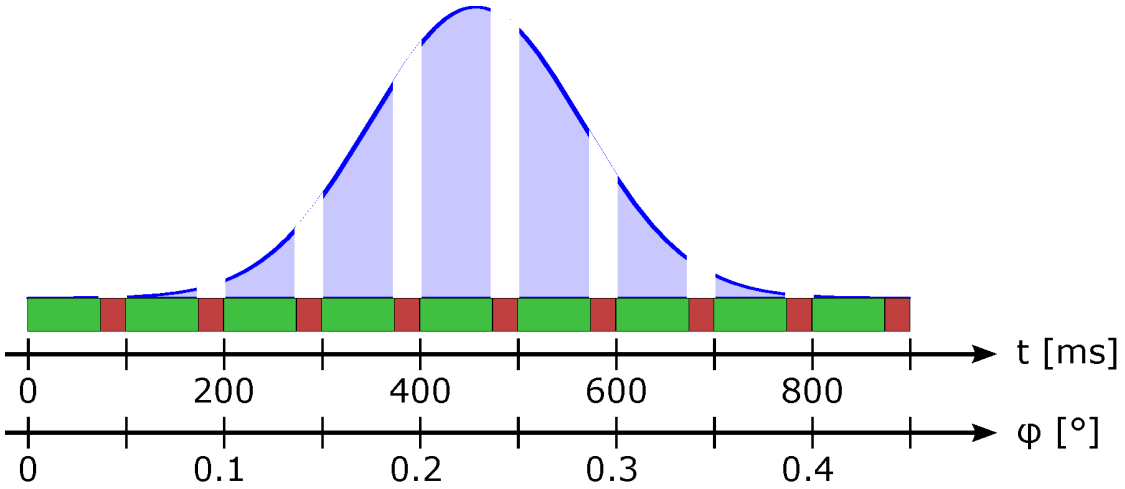
fine sliced



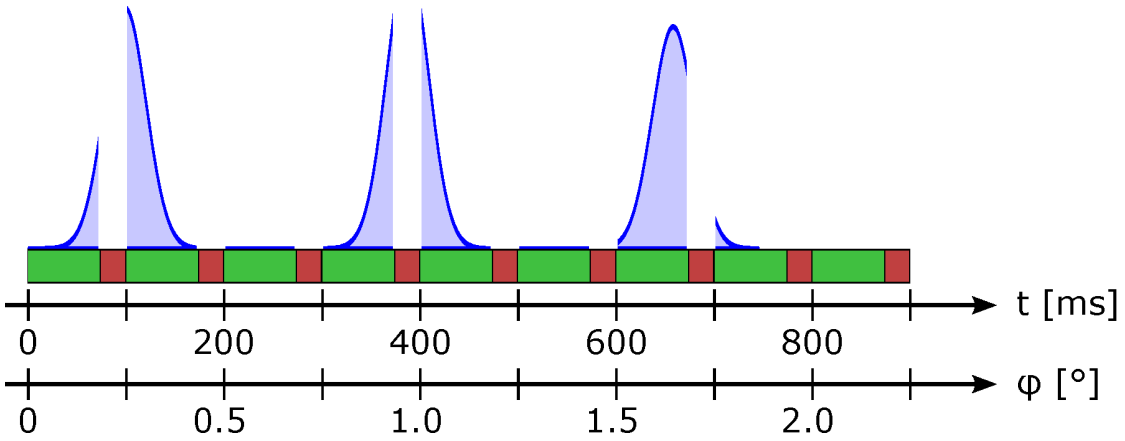
wide sliced



Readout time and data quality: conclusion



fine slicing and slow rotation => **small effect** of readout time

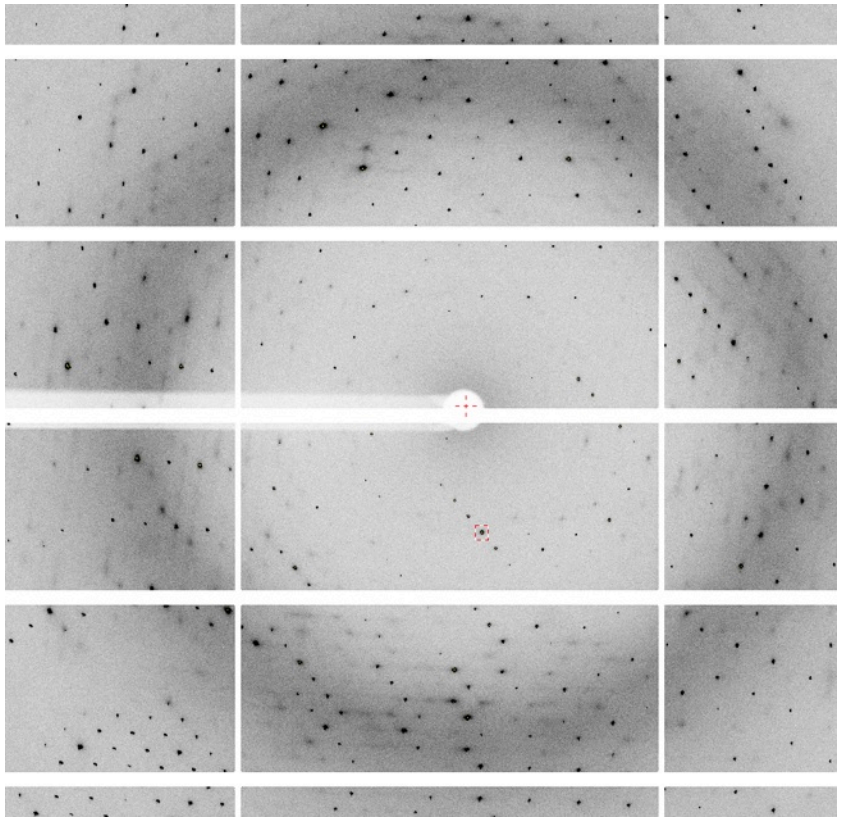


wide slicing and fast rotation => **large effect** of readout time

What about my "real life" crystals?

Benefits of fine-slicing should be more pronounced when crystals show poorer diffraction and stronger scattering:

- High Wilson-B factor
- Large solvent content
- Small crystal in large drop of solvent
- High resolution limit is in the region of solvent ring



Conclusions

- ***Δφ ≈ ½ mosaicity (σ_φ) for best***
  - overall statistics
  - highest shell statistics
  - anomalous signal
- ***be aware of your mosaicity definition***
- ***when slicing was too fine, summing images can yield better scaling statistics***
  XDS: merge2cbf
- ***maximum relative readout time at highest frame rate of 6% (6M-F) to 12% (2M-F) has minimal impact on quality of fine sliced data***

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

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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 detectors used in macromolecular crystallography. Recently, hybrid pixel X-ray detectors that operate in single-photon-counting mode have become available. These detectors have fundamentally different characteristics compared with CCD

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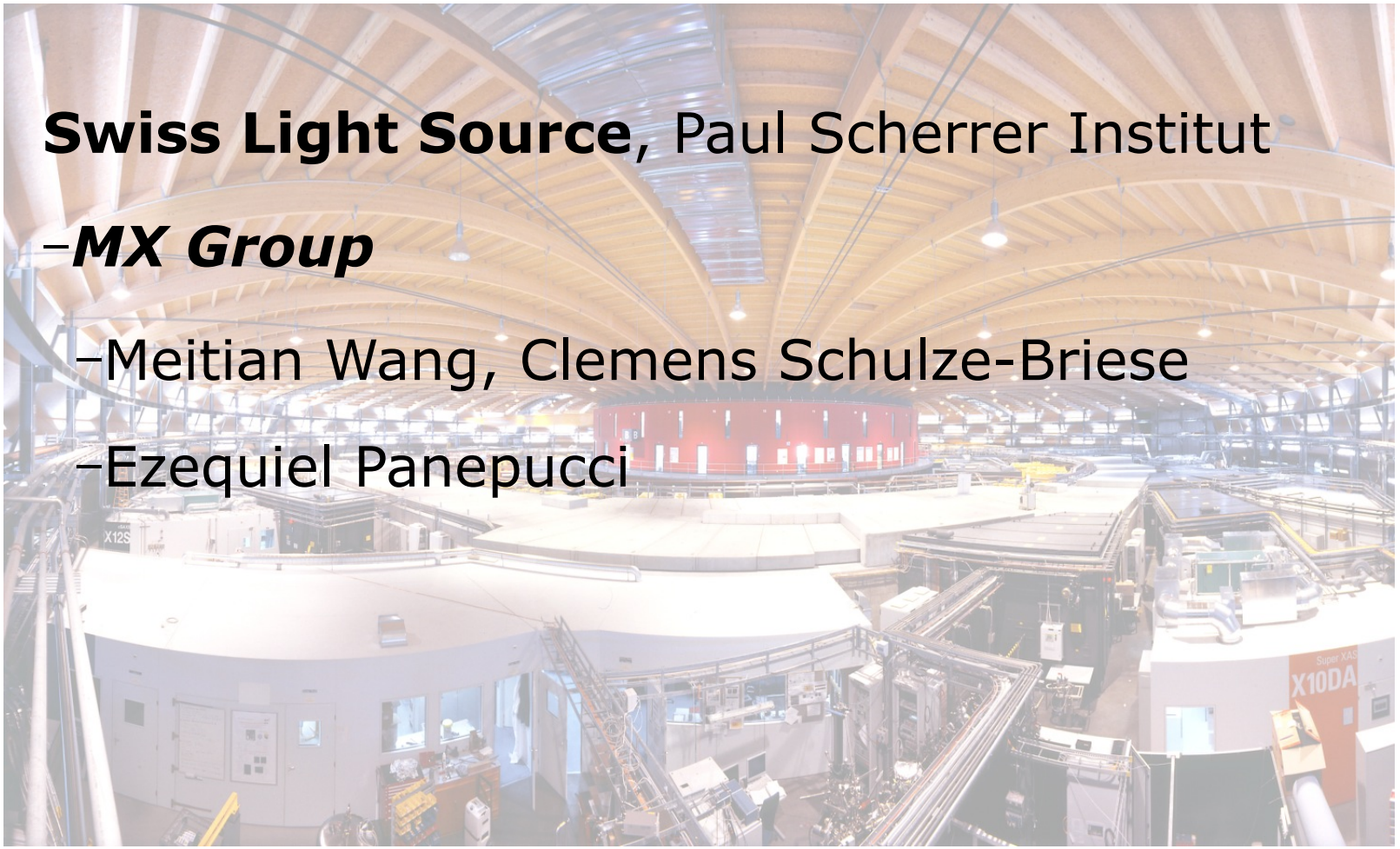

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