DECTRIS®

detecting the future

Optimizing data quality with Hybrid Photon Counting detectors

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CCP4 workshop, 2015-12-02

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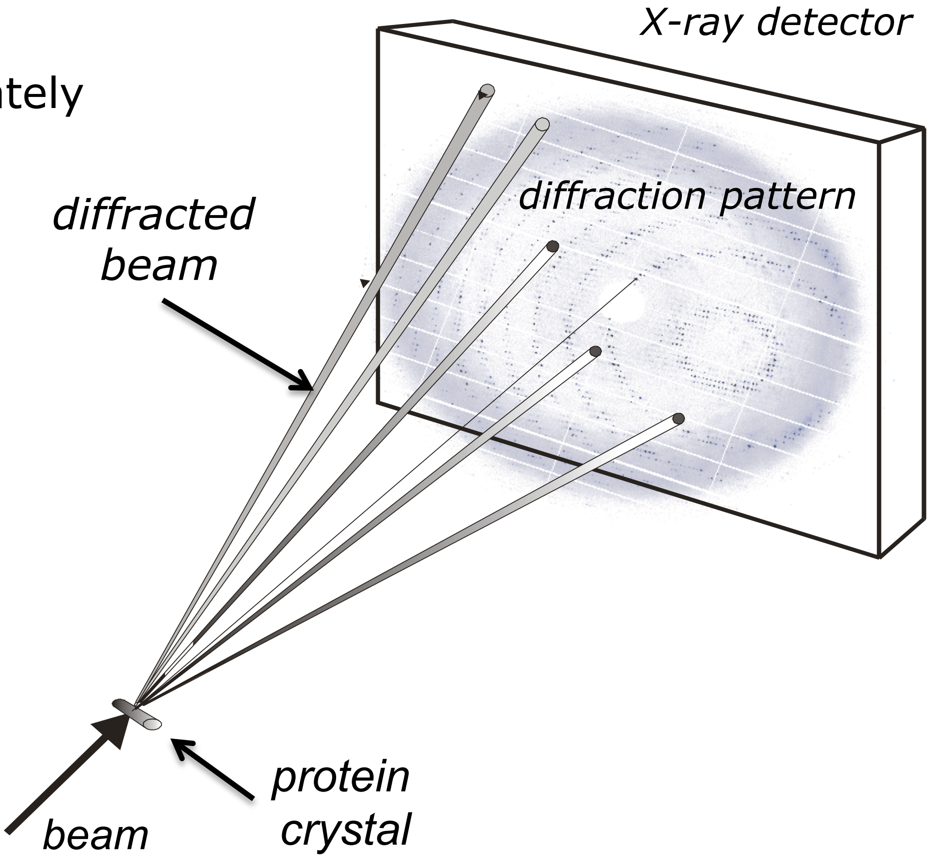
Outline

- ***Characteristics of HPC detectors***
- ***Data collection – best practice***
  - *Accurate data*
  - *Fine φ-slicing*
  - *Anomalous data*
  - *Low intensity/high multiplicity*

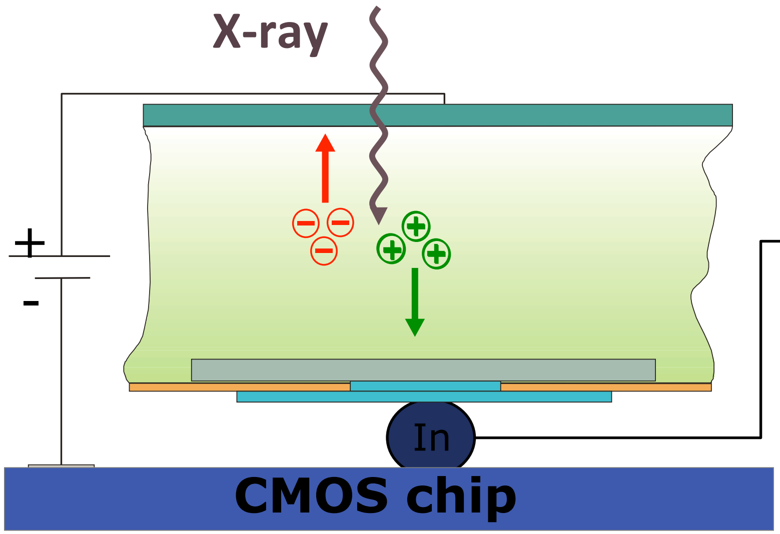
The perfect detector should have

- **High dynamic range**
  Measure weak and strong reflections simultaneously
- **No readout noise**
  Measure weak reflections accurately
- **Uniformity**
  Measure differences accurately
- **High quantum efficiency**
  Minimize dose
- **High spatial resolution**
  Resolve large unit cells
- **High frame rate**
  Continuous rotation collection

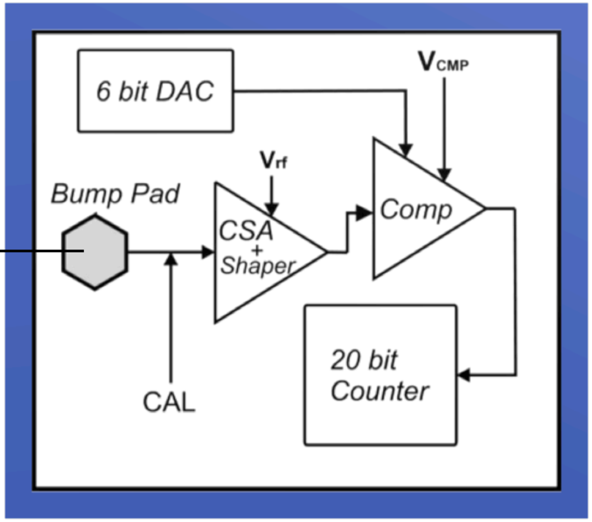


Hybrid Photon Counting technology

Sensor pixel



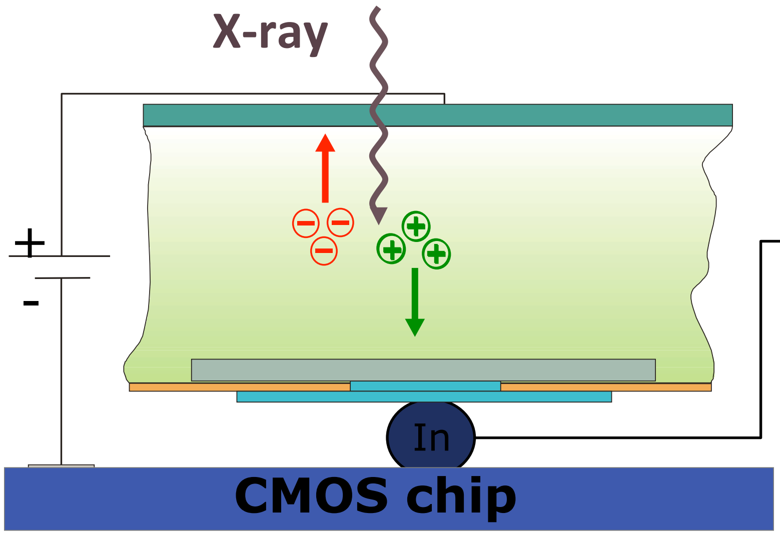
Readout pixel



Hybrid pixel + single-photon counting
= Hybrid Photon Counting (HPC)

Hybrid Photon Counting technology

Sensor pixel

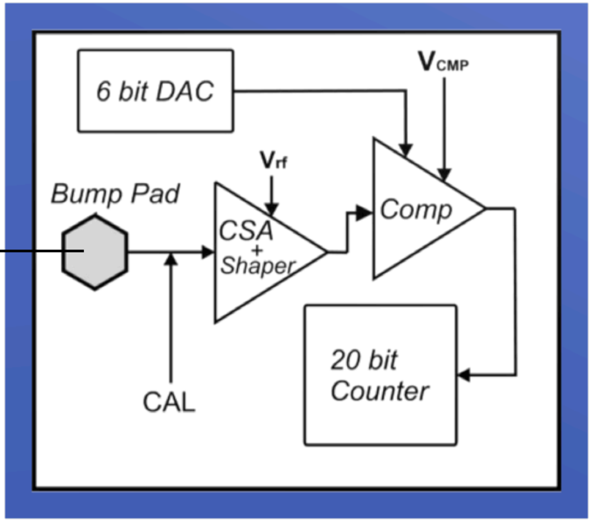


Direct detection of X-rays in silicon sensor

→ Quantum yield nearly 100%

→ Point spread function of 1 pixel

Readout pixel



Single photon-counting in CMOS

→ No readout noise & dark current

→ High dynamic range (20 bit)

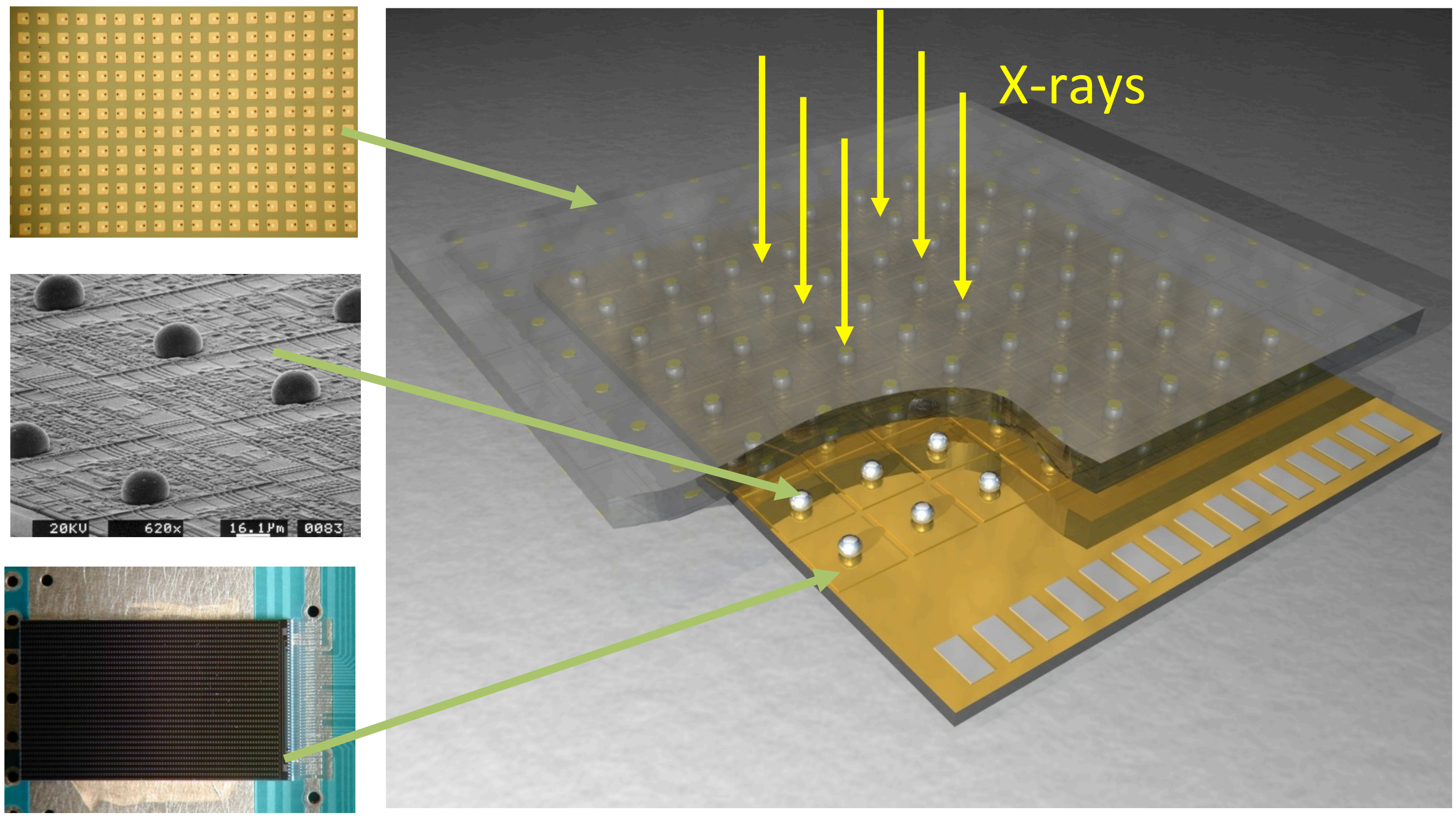
→ Fast readout

Hybrid Photon Counting technology

Silicon sensor

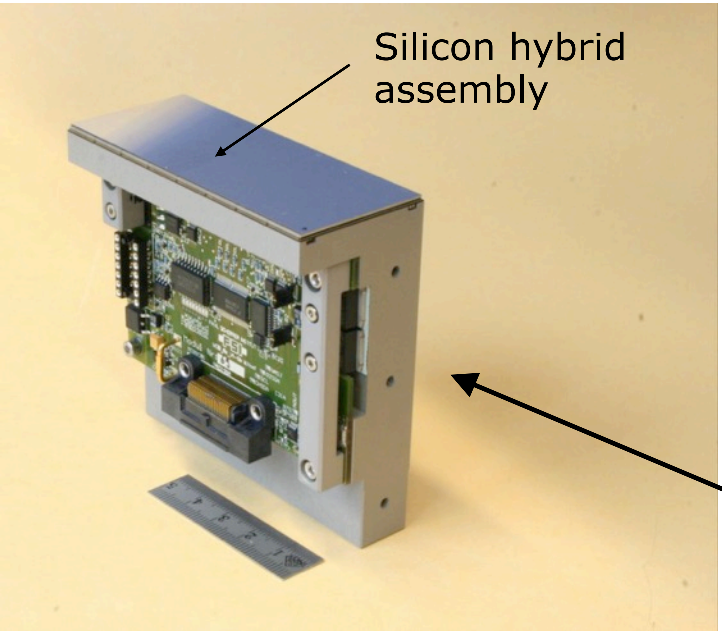
Indium bump bonds (18 µm)

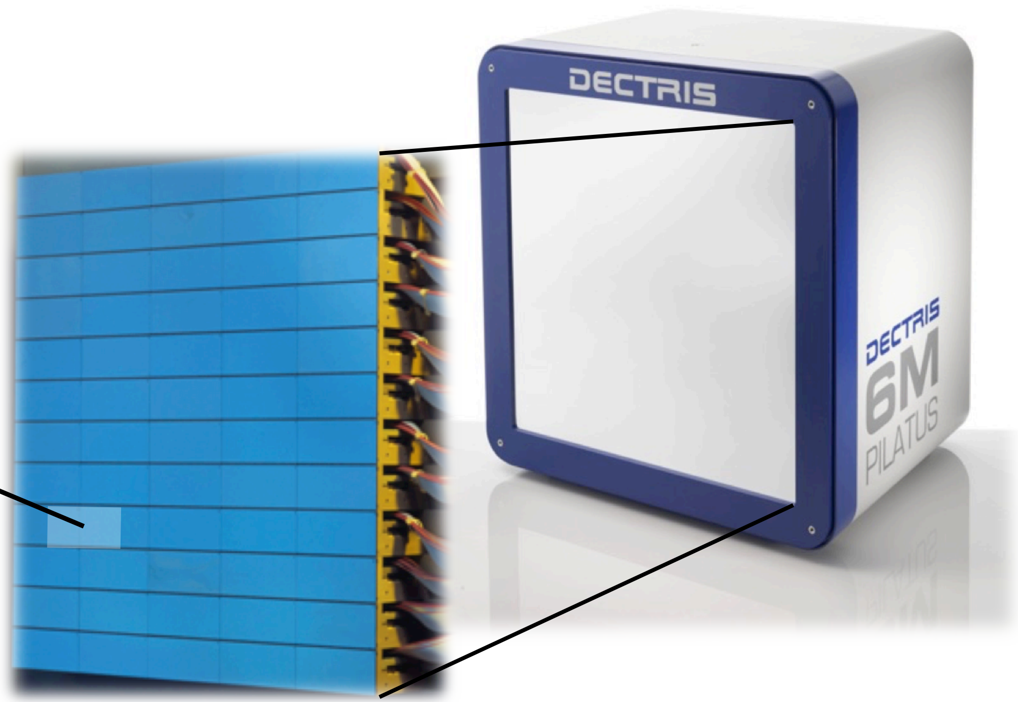
CMOS chip



Modular assembly of HPC detectors

PILATUS detectors are built as arrays of **PILATUS modules**.





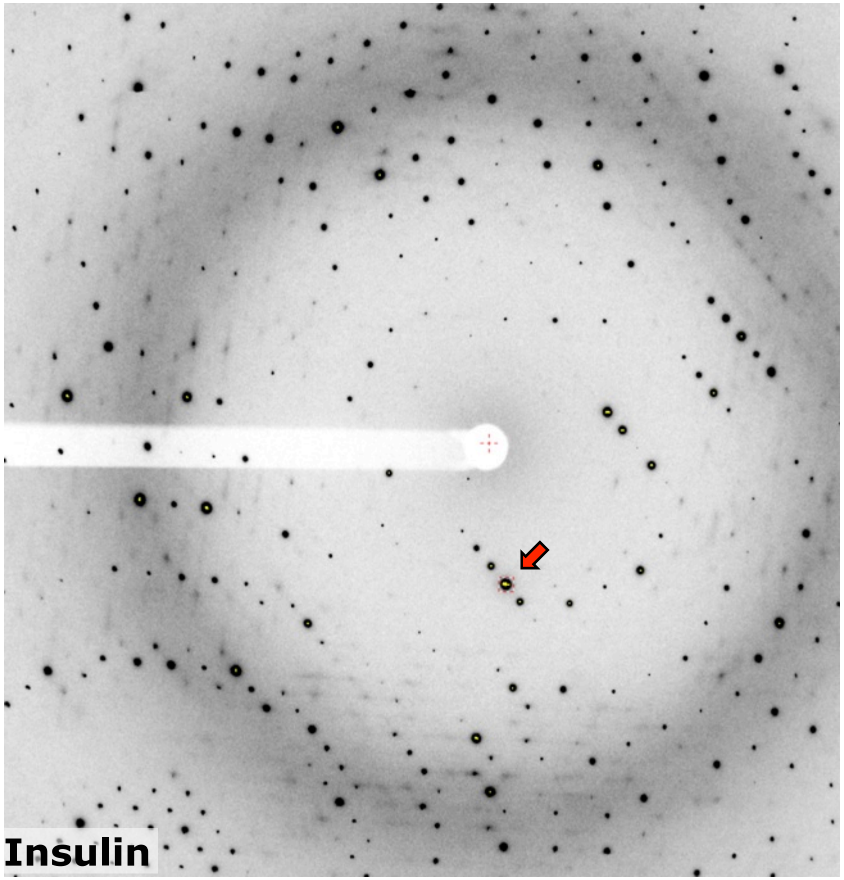
Module active area: 83.8×33.6 mm^2

487×195 pixels = ~100k pixels

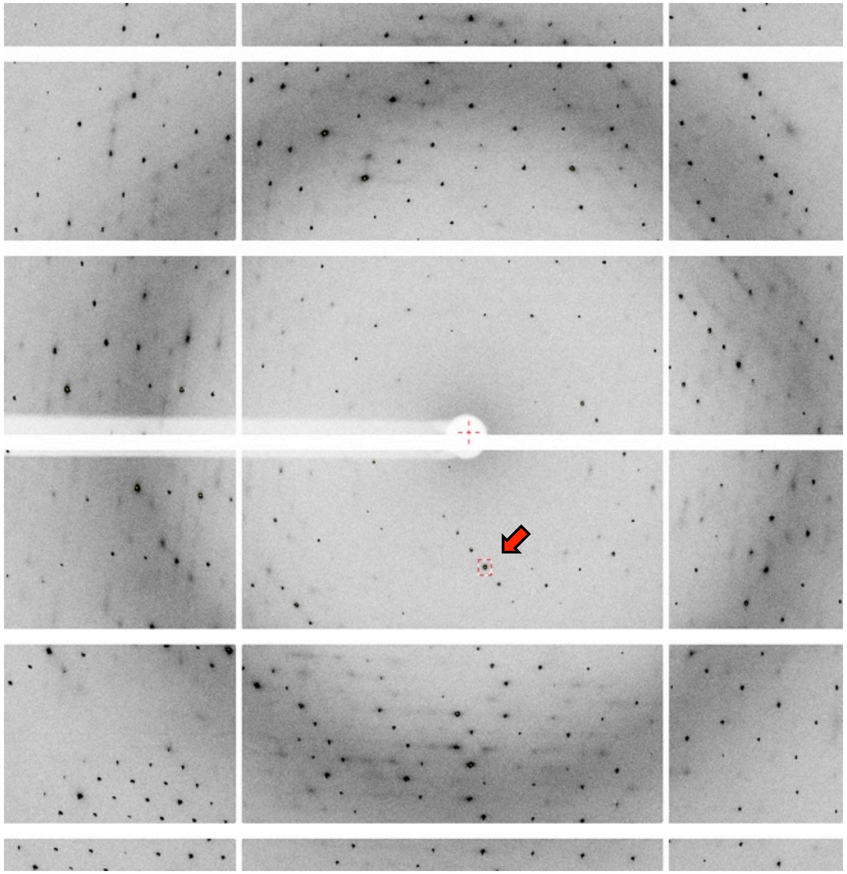
Pixel pitch 172 µm

High dynamic range, no point spread

CCD
16 bit (65 535 ADU)



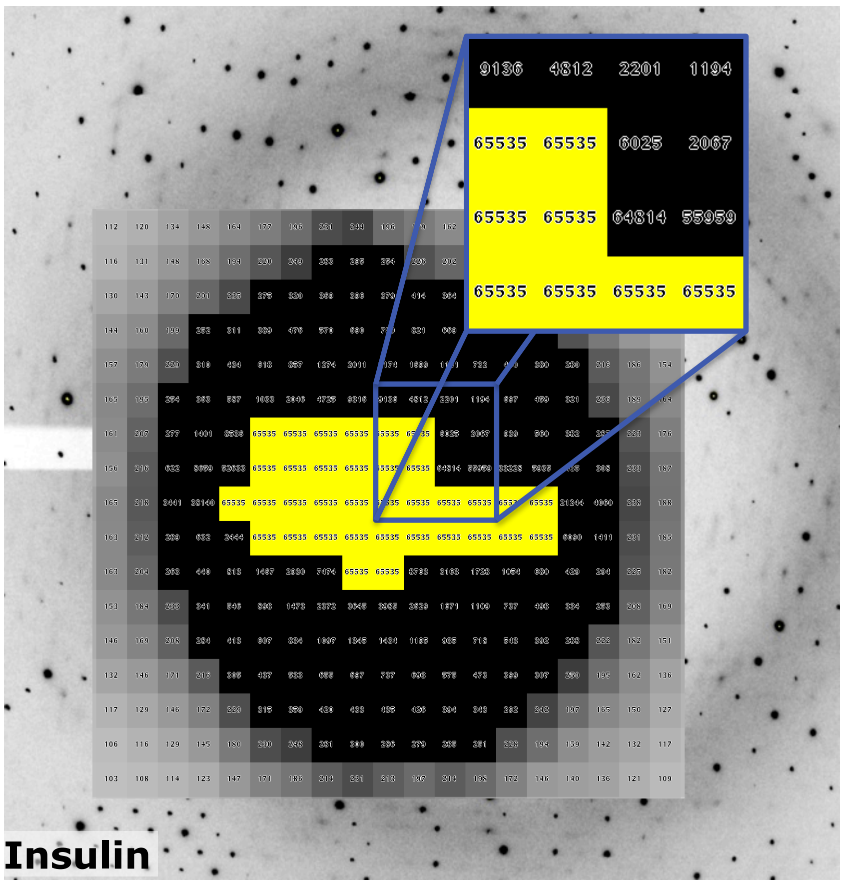
PILATUS
20 bit (1 048 575 counts)



High dynamic range, no point spread

CCD

16 bit (65 535 ADU)



PILATUS

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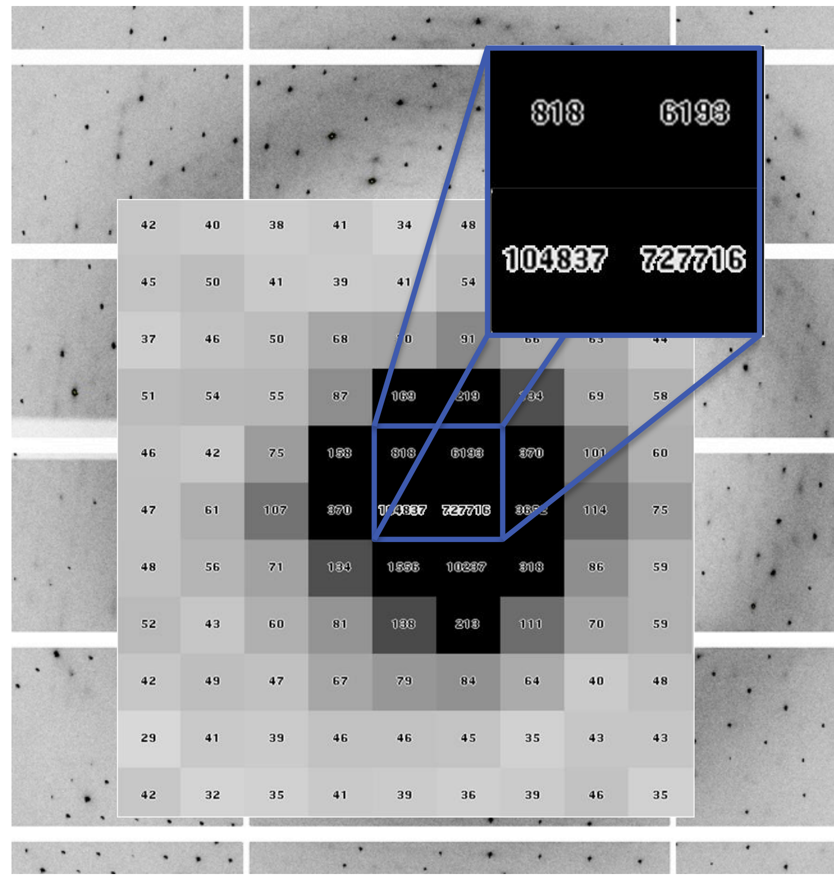
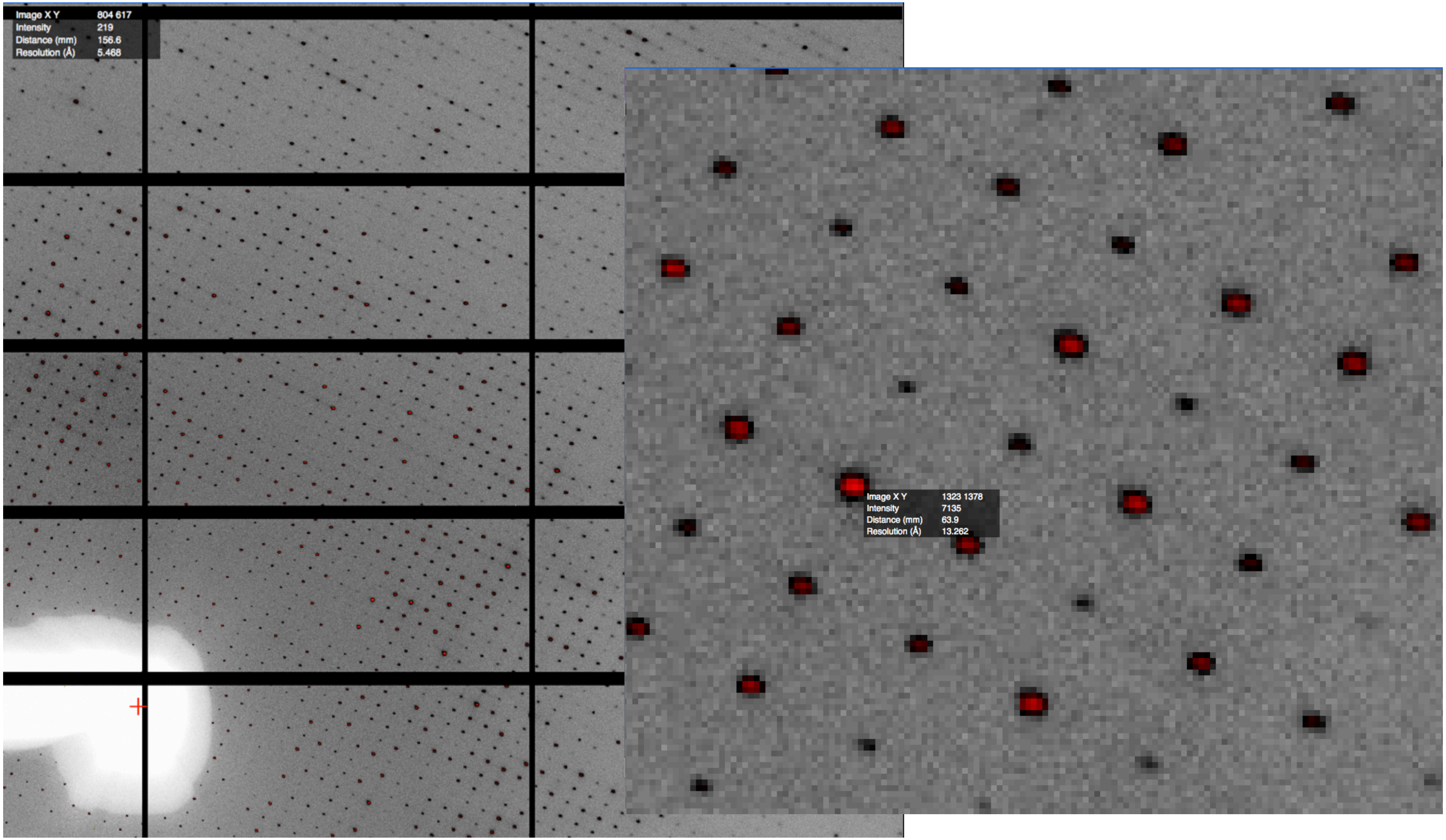
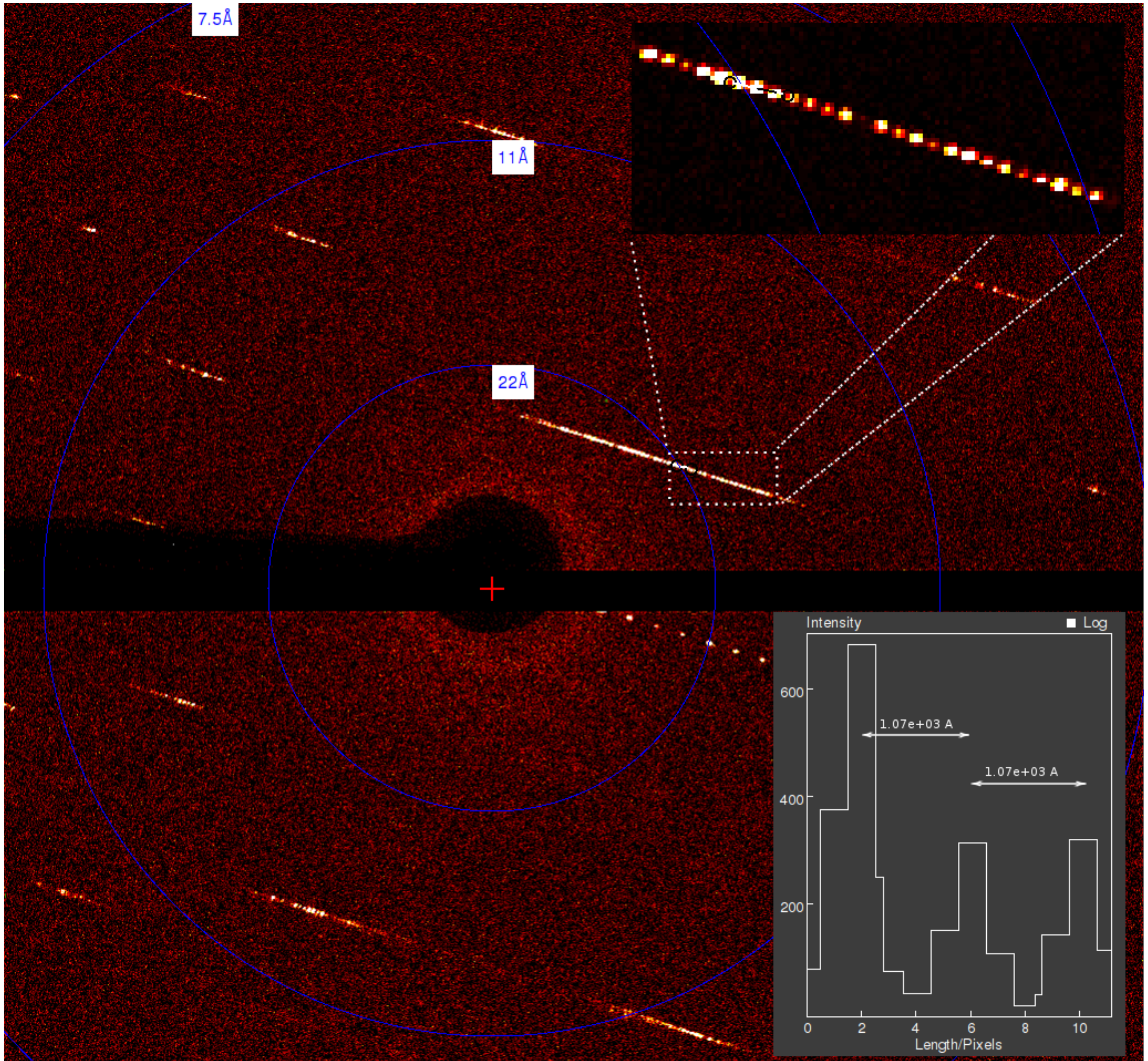


Resolution of large unit cells





EIGER

Resolution of spots from a 1000 Å unit cell with diffraction to 2.3 Å

Images courtesy of Arnau Casanas @ PSI

Fast readout and high frame rates

- Fast readout (PILATUS3 X: 0.95 ms; EIGER X: 4 µs dead time)
  ➔ shutter-free, continuous rotation data collection

- High frame rates (>100 Hz with EIGER and PILATUS3)
  ➔ complete datasets in less than 10 s

Position 5

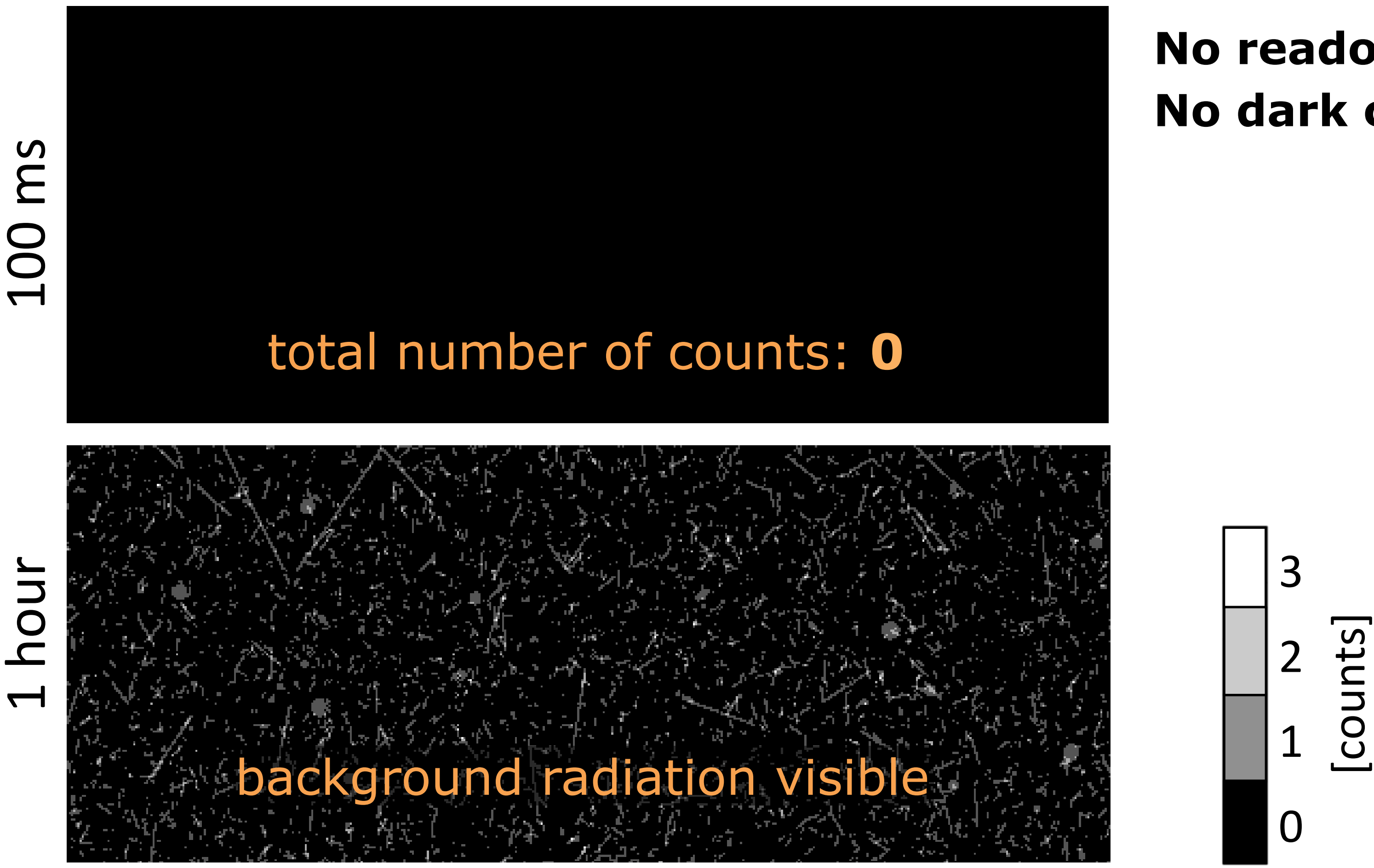
4.48	92.9%	3.1%	36.98	**Nb images: 600**
3.18	93.3%	3.8%	31.59	Exp. time: 0.004 s, **250Hz**
2.60	97.0%	4.8%	24.49	Phi range: **0.20 º**, --> 50º/s
2.25	92.1%	7.1%	17.71	**Flux: 5.78E12 ph/sec**
2.01	92.0%	10.9%	12.35	Transmission: 48.96
1.84	87.1%	19.9%	7.15	**Total expo time: 2.40 s**
1.70	93.1%	27.9%	4.25	
1.59	93.2%	44.0%	2.41	
1.50	87.7%	73.3%	1.50	PILATUS3 2M
total	**91.6%**	**6.4%**	**11.49**	

P. Carpentier

The European Synchrotron | ESRF

Noise-free detection

No readout noise
No dark current

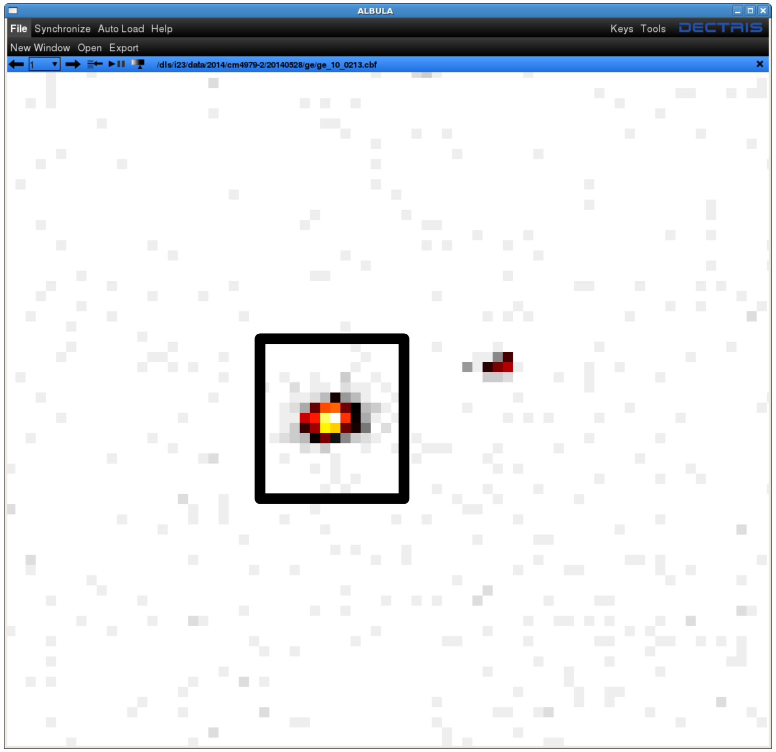


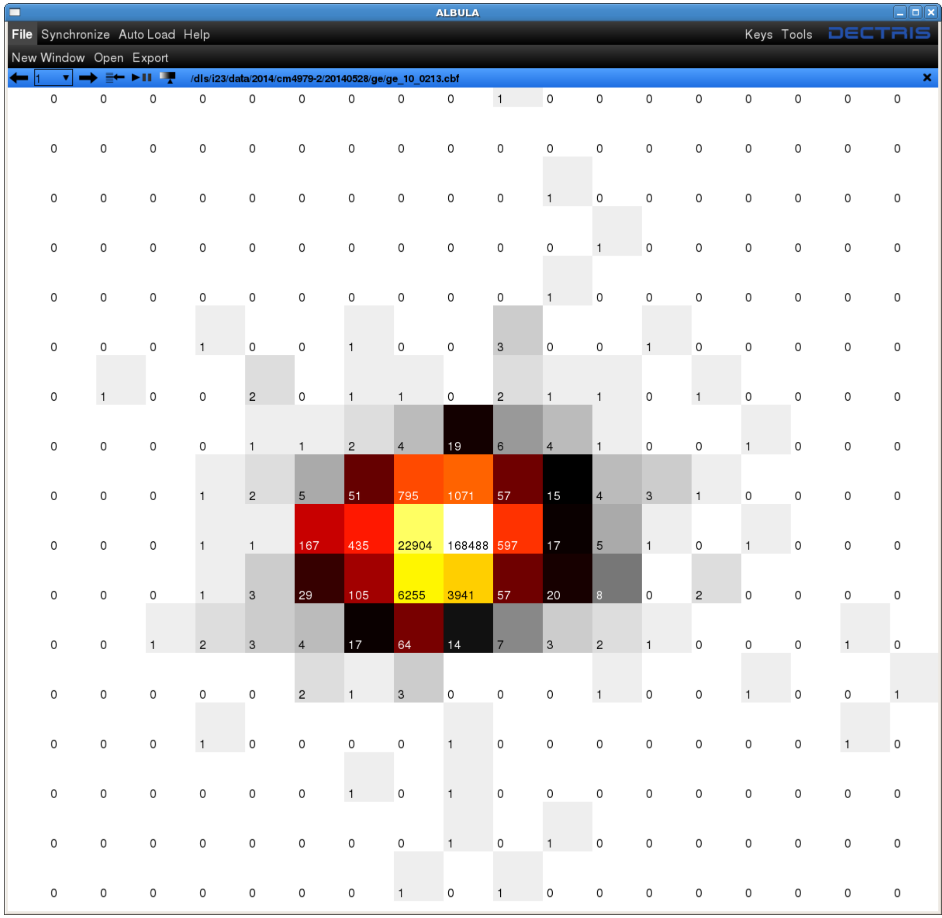
100K detector

Negligible X-ray background

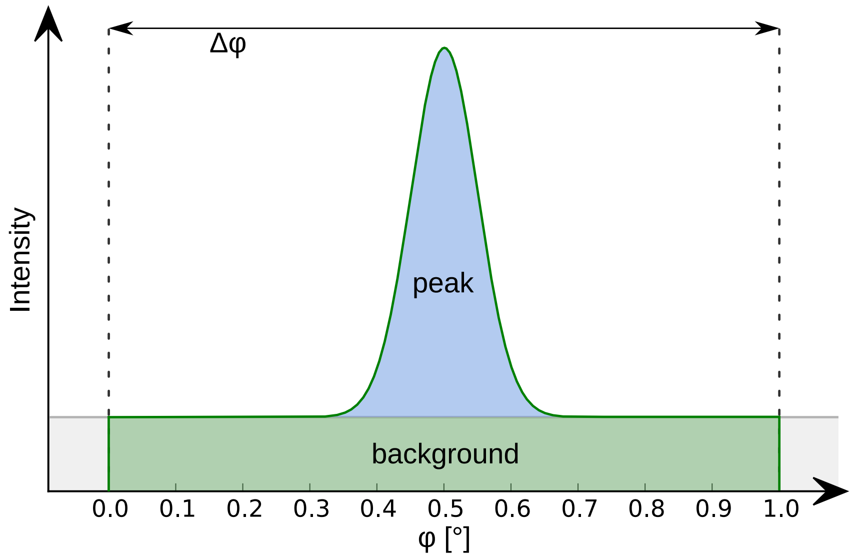
PILATUS 12M in vacuum (I23 @ DLS)
No air absorption, no scattering (no solvent)
Near perfect background of zero counts

Bragg scattering from germanate crystal.





Accuracy of intensity estimates



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

I : Observed intensity
$I = \Sigma(Peak_i - Bkg_i)$
$var(I) = \Sigma[var(Peak_i) + var(Bkg_i)]$
$R_{err} = \sigma(I)/I$

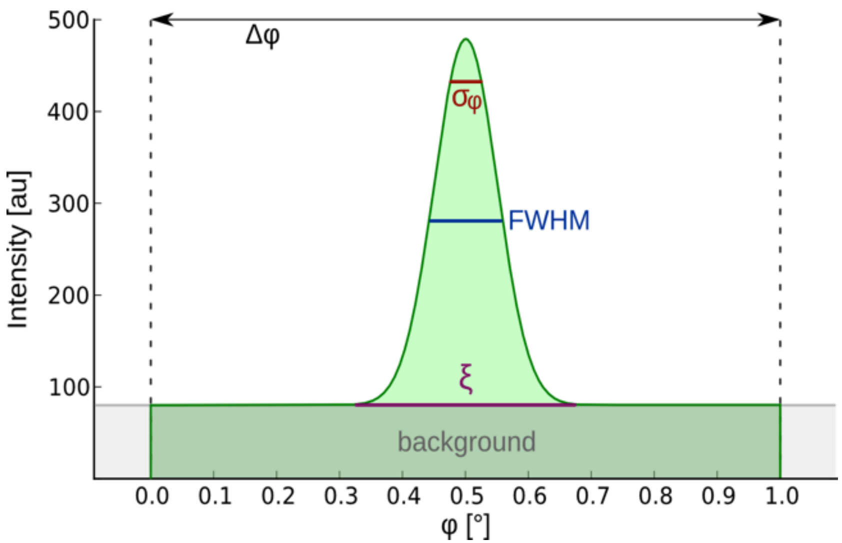
Less background

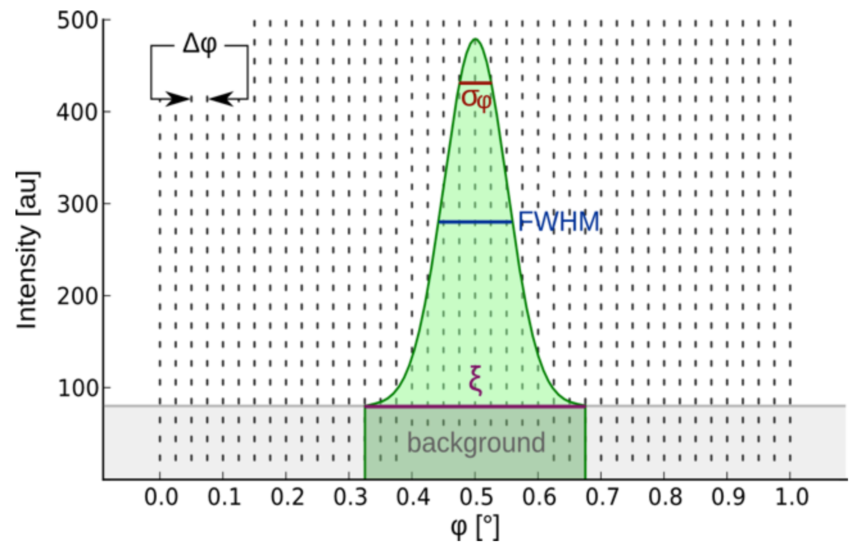
=> better data

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.

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

Fine φ-slicing without readout noise

Detector with readout noise (CCD)

minimize readout noise ← compromise → minimize background

→ more images and smaller Δφ

Hybrid pixel detector without readout noise

take full advantage of fine φ-slicing

minimize background

→ more images and smaller Δφ

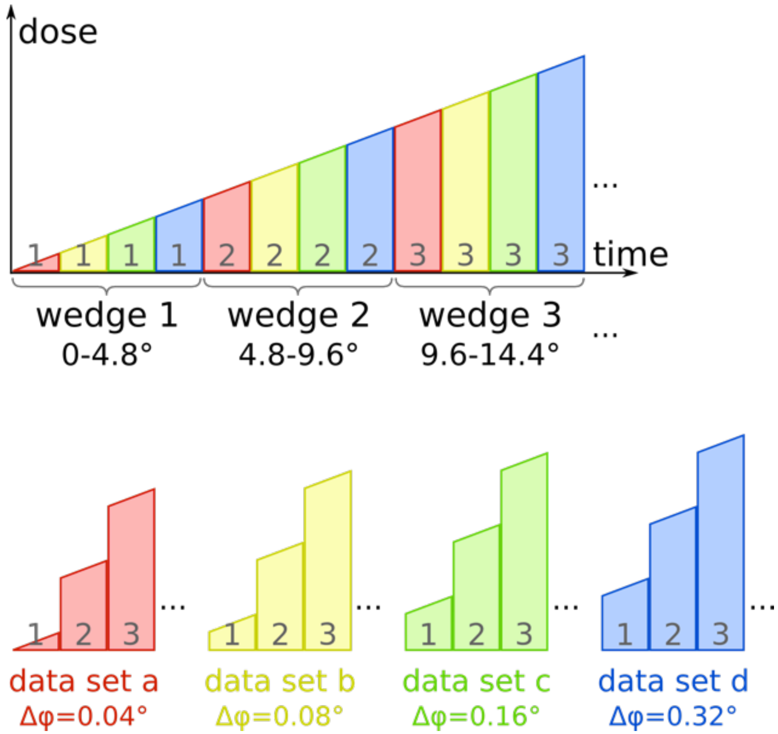
Data collection and processing

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

Swiss Light Source, Paul Scherrer Institut

- ***MX Group, Meitian Wang***
- *Marcus Müller, Clemens Schulze-Briese*
- Ezequiel Panepucci

Interleaved data collection



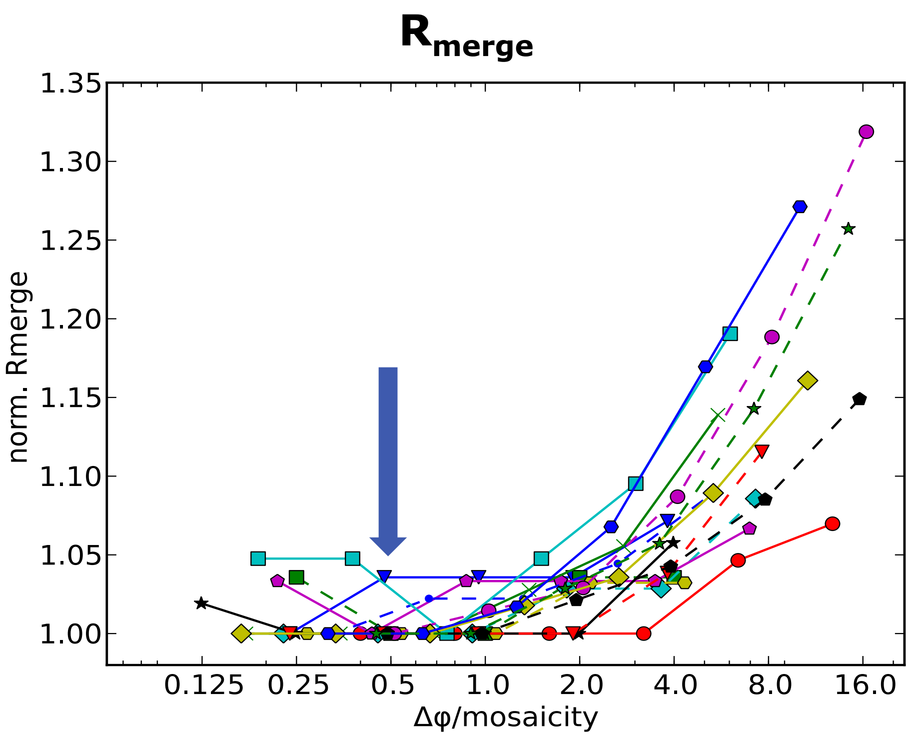
data set	a	b	c	d
Δφ [°]	0.04	0.08	0.16	0.32
t [s]	0.1	0.2	0.4	0.8
images	1200	600	300	150
φ [°]	48	48	48	48

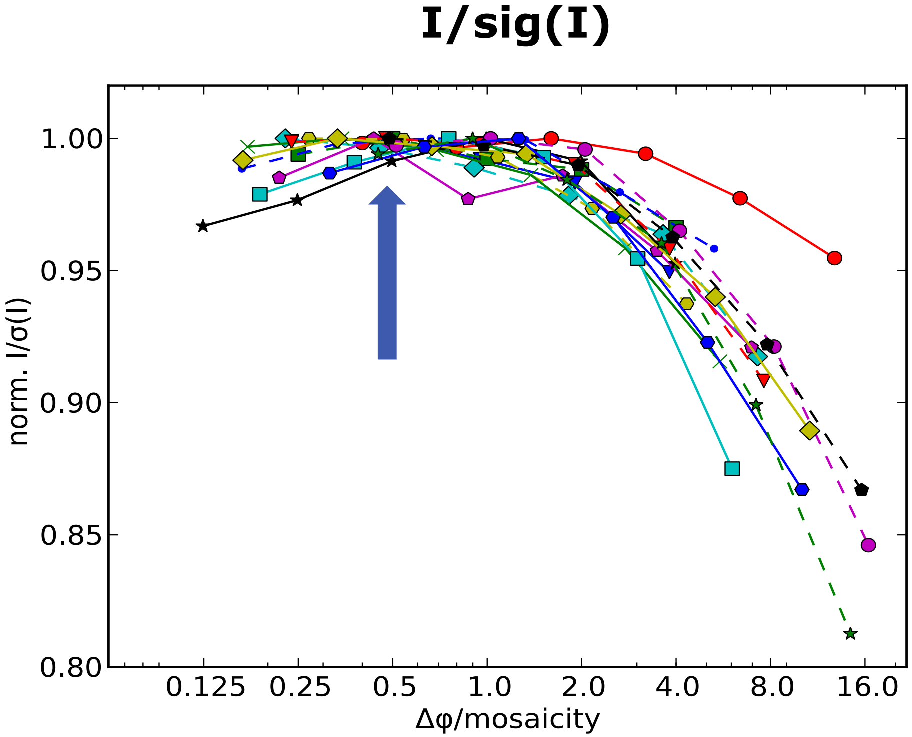
16 series of 5 to 7 datasets with increasing Δφ

Each series:

- Same position of single crystal
- Same angular speed and dose rate
- Interleaved data collection: Even dose distribution over compared data sets.

Fine φ-slicing increases overall I/σ(I) and decreases R_{merge}

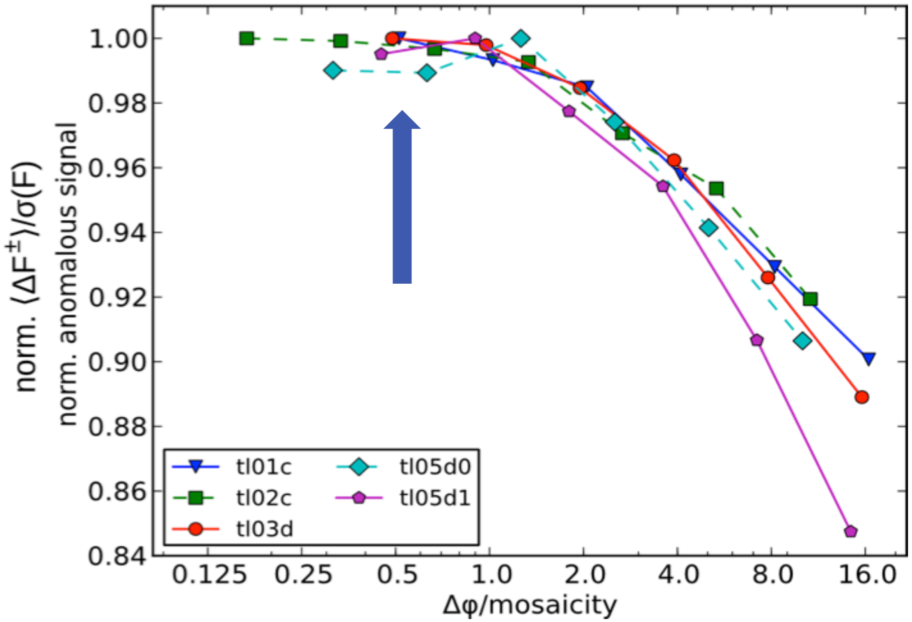




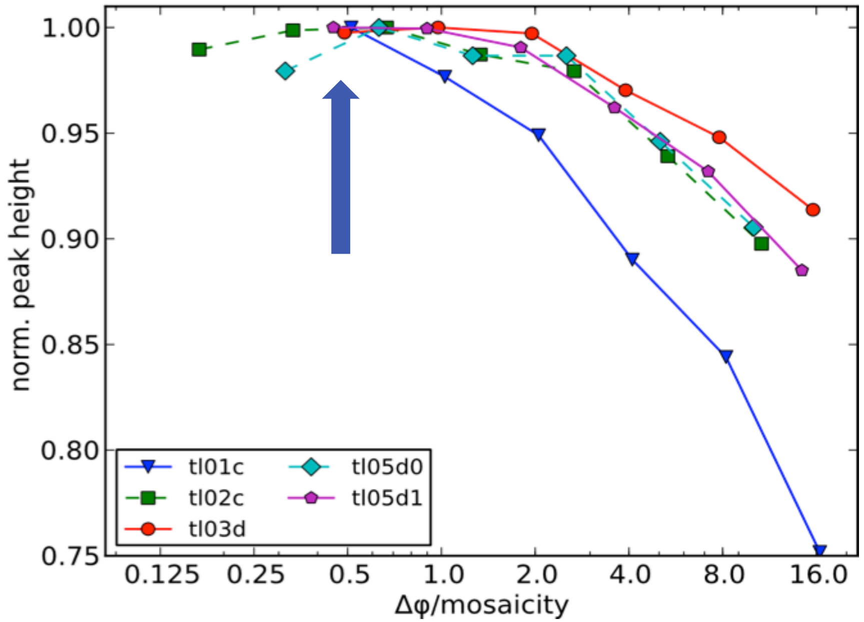
normalized values

Fine φ-slicing improves anom. signal

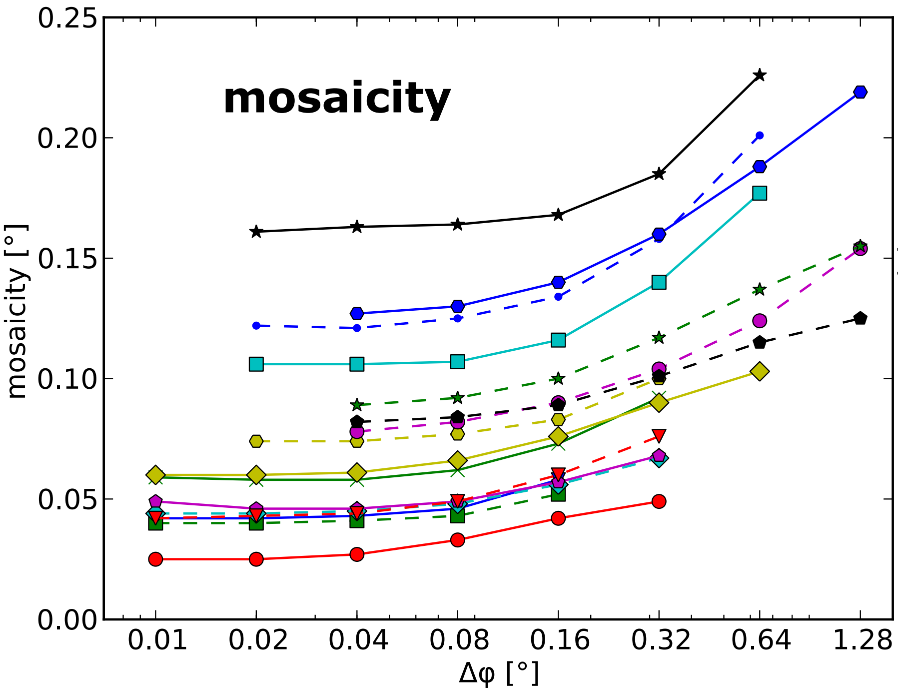
anomalous signal/noise

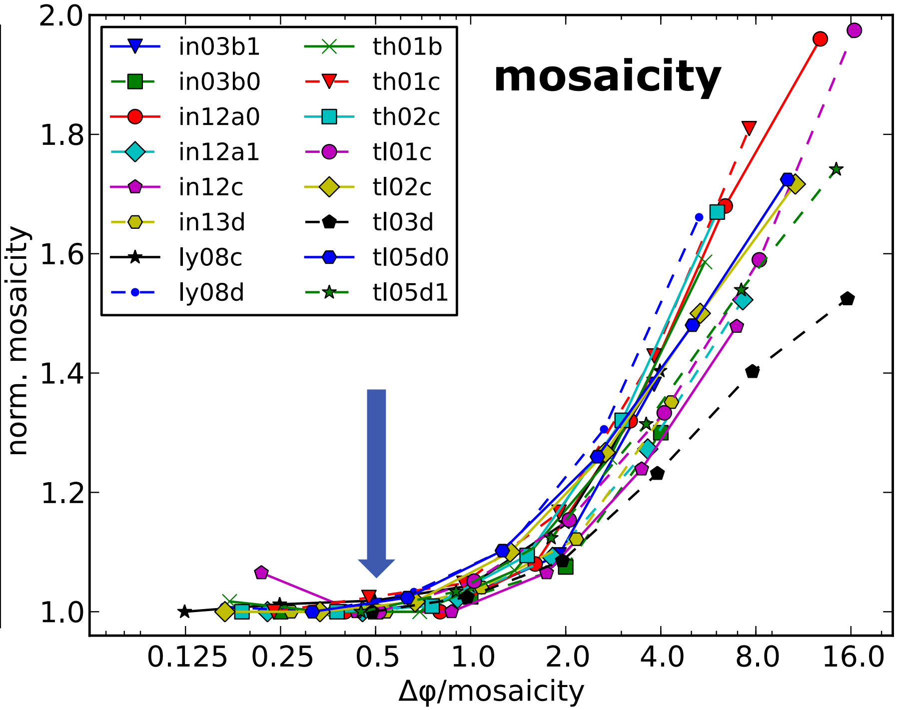


anomalous peak height



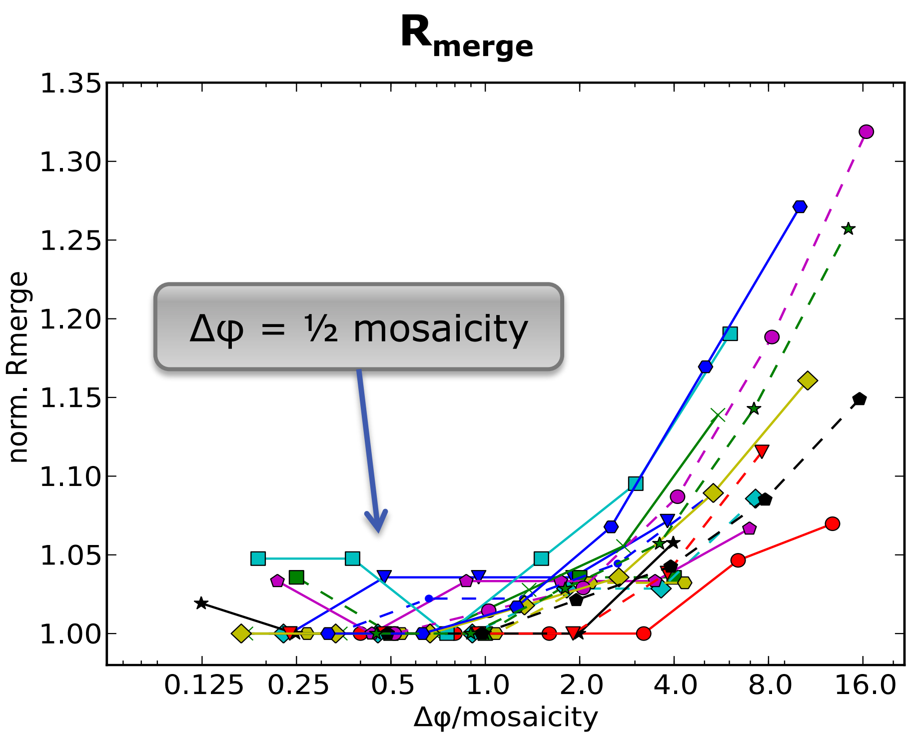
Fine φ-slicing lower mosaicity values

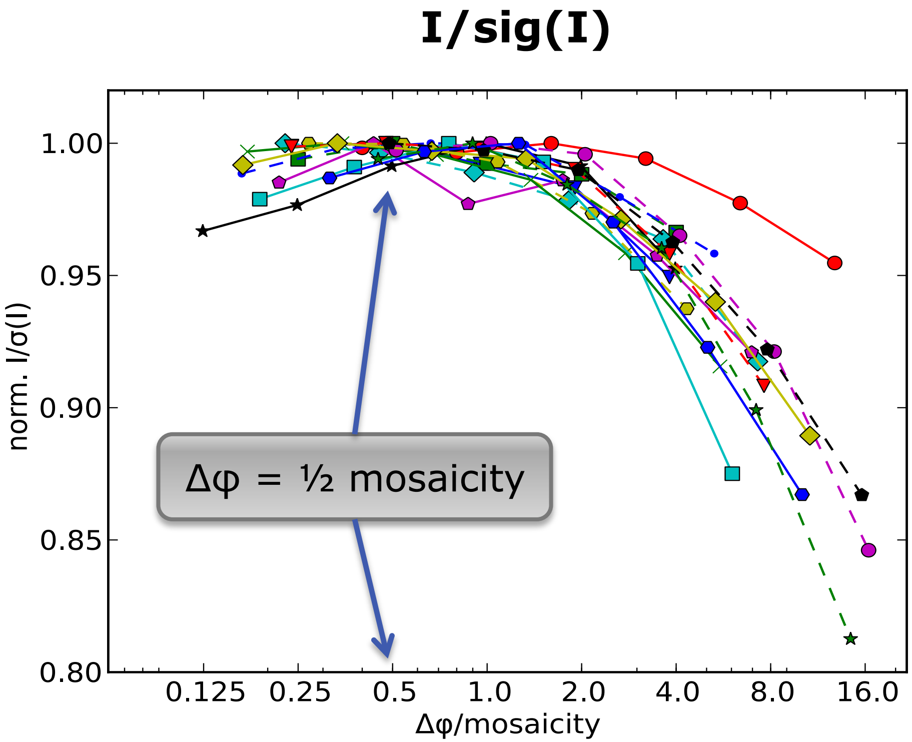




- More accurate spot centroids, better sampling of intensities
- Better estimate of crystal mosaicity for finer Δφ.
- Asymptotic minimum for small Δφ.

Best data at Δφ ≈ ½ mosaicity



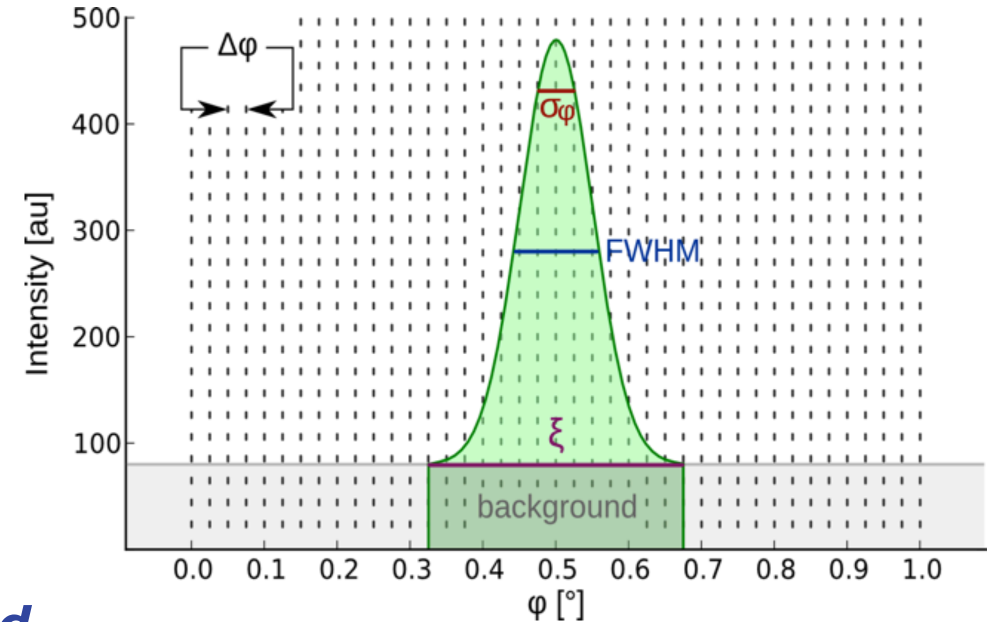


Highest shell statistics improve substantially
=> fine-slicing extends maximum resolution of data set

Fine φ-slicing improves data quality

Δφ ≈ ½ mosaicity improves:

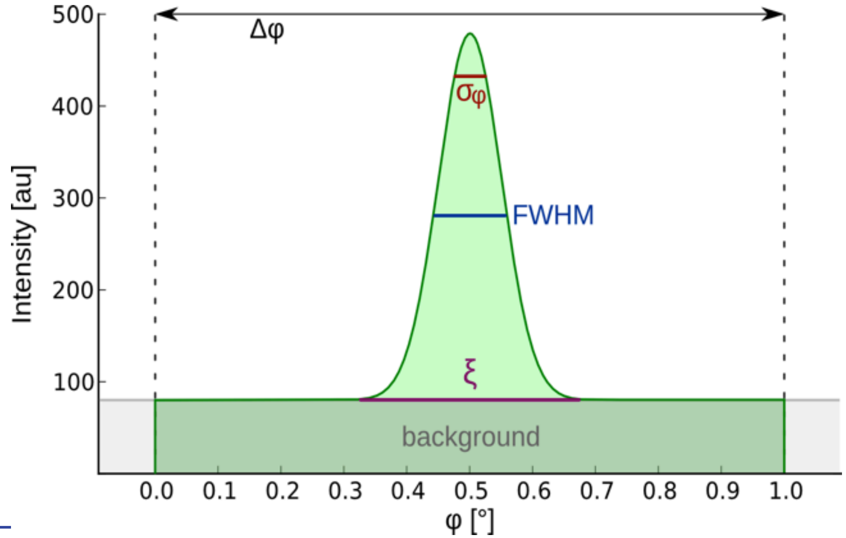
- ***overall statistics***
- ***highest-shell statistics***
- ***anomalous signal***
- ***number of overlaps***



Detector must be free of readout noise.

Computer resources required.

Mosaicity in XDS and Mosflm



XDS and Mosflm differ
in their definition of mosaicity.

Crystal	in12a1	in12c	ly08c	th01c	th02c	tl02c
XDS [°]	0.07	0.07	0.23	0.08	0.18	0.10
Mosflm [°]	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

Processing is difficult – use XDS

- Fewer photons per spot
  → ***XDS gets better all the time***

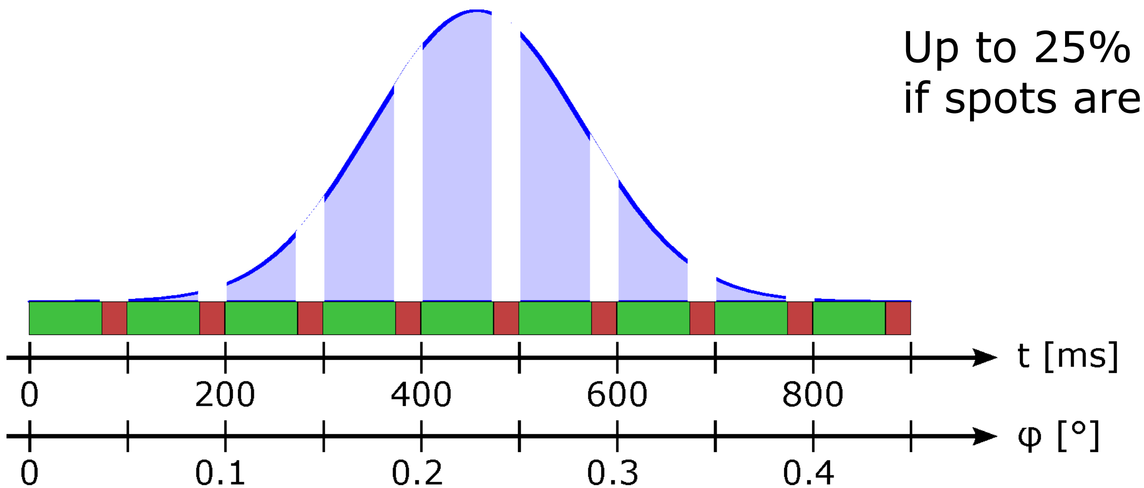
- More images to assemble spot profiles

  → ***use finer grid for profile fitting***

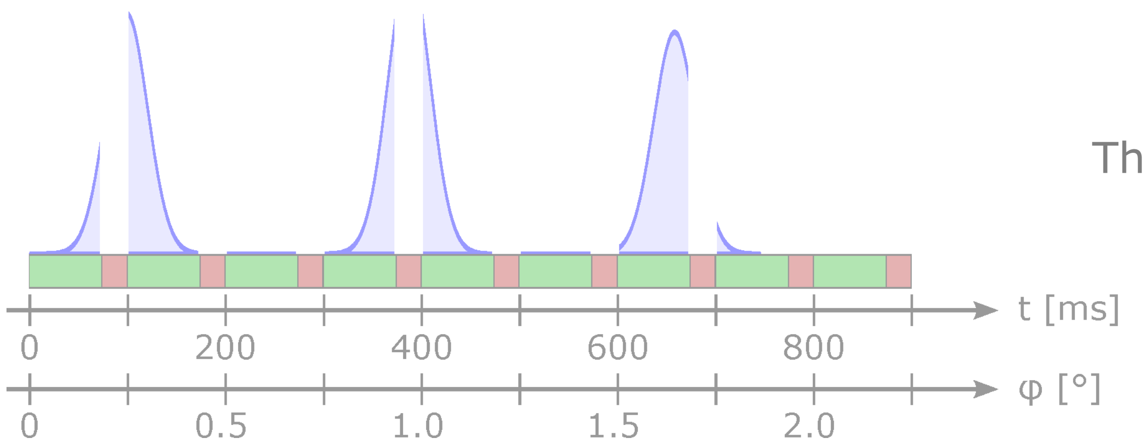
```
NUMBER_OF_PROFILE_GRID_POINTS_ALONG_ALPHA/BETA= 21
NUMBER_OF_PROFILE_GRID_POINTS_ALONG_GAMMA= 21
```

or use DIALS
Diffraction Integration for Advanced Light Sources

Readout time affects data quality



Up to 25% readout time is ok
if spots are sampled finely enough



This is not good!

EIGER measures without dead time

4 µs between successive images

- Negligible dead time
- Higher frame rates
- Smaller pixels
- More pixels

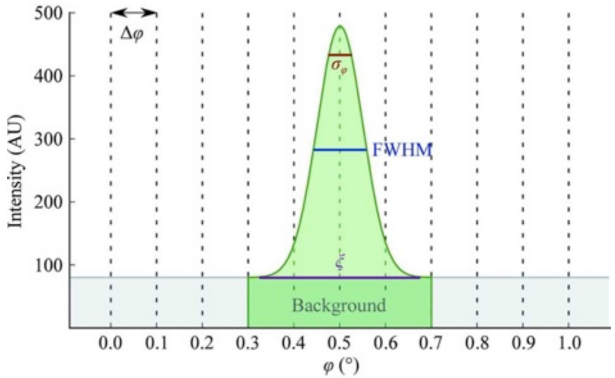
EIGER X 16M @
PSI PXI and **NSLS** II

EIGER X 9M @
Soleil Proxima 2

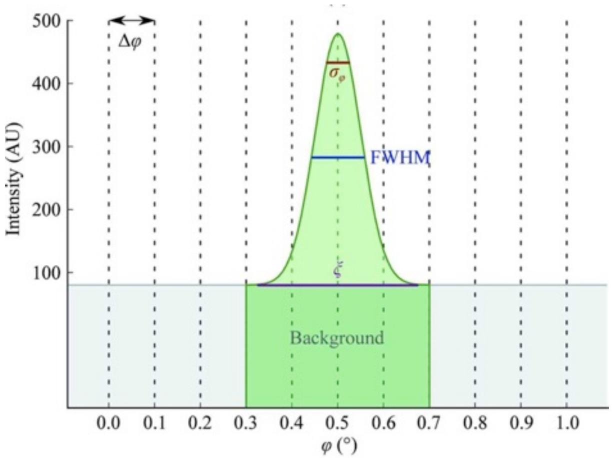
Improve your data by

- ***Using Hybrid Photon Counting detectors***

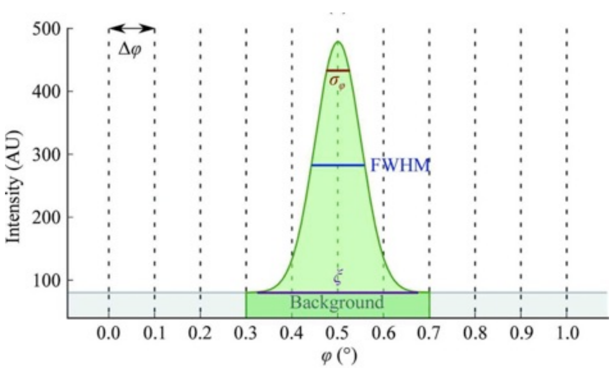
Decrease absolute background



rubbish!



good!



Improve your data by

- ***Using Hybrid Photon Counting detectors***
- ***Moving the detector as far back as possible***
- ***Minimizing solvent around crystal***
- ***Matching beam size to crystal***

With accurate data

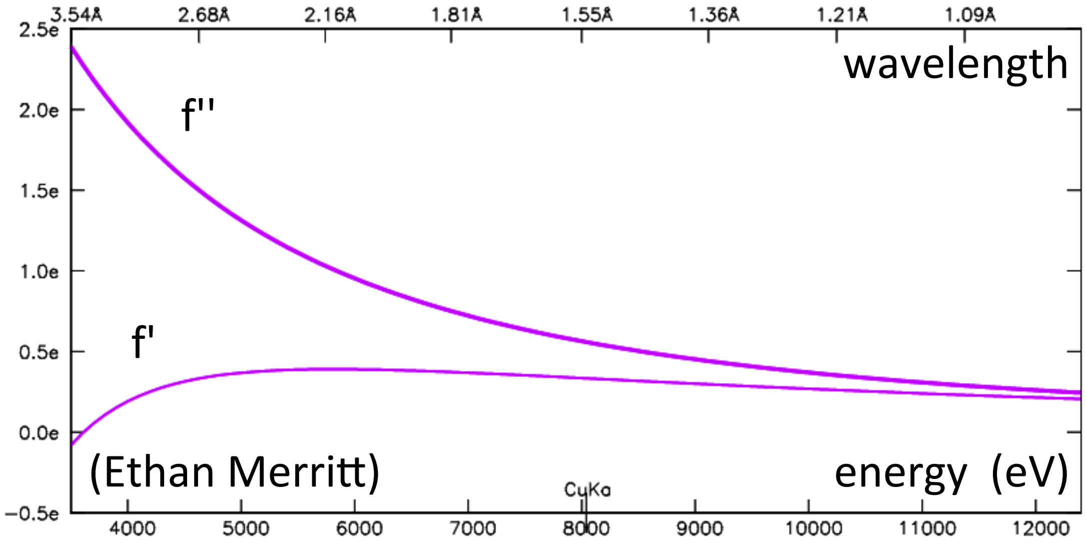
Go solve a structure

- ***default: 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*

Native SAD phasing



Some strategy considerations

- ***Inverse beam?***
- ***Which wavelength?***
- ***How to distribute dose?***
- ***How to achieve multiplicity?***
  => kappa, chi, 2-theta

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.

Improve your data by

- *Using Hybrid Photon Counting detectors*
- *Moving the detector as far back as possible*
- *Minimizing solvent around crystal*
- *Matching beam size to crystal*
- *Lowering intensity and collecting more images*
- *Thinking about your experiment*

Use beam time efficiently

- ***mount crystal – 1 min***
- ***center crystal – 1 min***
- ***collect a dataset – 1 min***
- ***five-hour shift – 300 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
  - collects two more datasets
  *- **solves structure***

Conclusions

– ***Collect accurate data***

– ***Δφ ≈ ½ XDS mosaicity for best***
  - *overall statistics*
  - *highest-shell statistics*
  - *anomalous signal*

– ***There is no native data***
  - *especially at high multiplicity*

– ***Ask your beamline scientist / think !***

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 detectors used in macromolecular crystallography. Recently, hybrid pixel X-ray detectors that operate in single-photon-counting mode have become available. These detectors have

Acta Cryst. (2012). D68, 42-56

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