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How to collect best MX data

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Outline

- *Characteristics of HPC detectors*
- *Data collection – best practice*
  - *Accurate data*
  - *Fine φ-slicing*
  - *Anomalous data*
  - *Think about your experiment*

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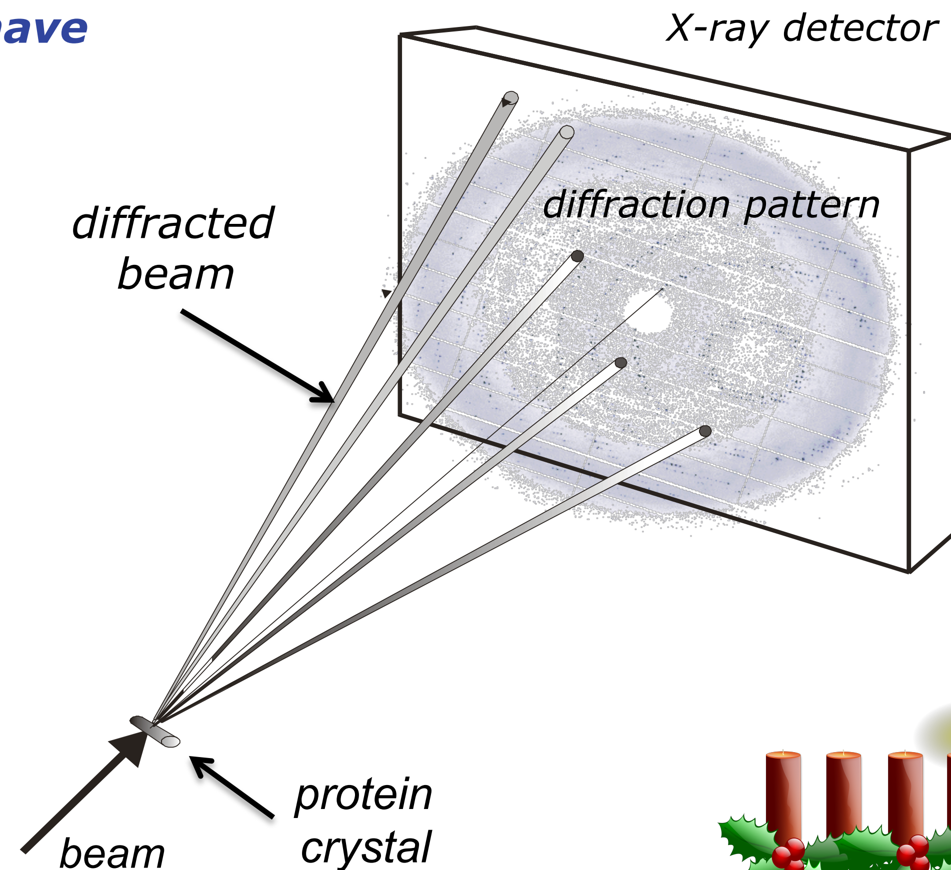
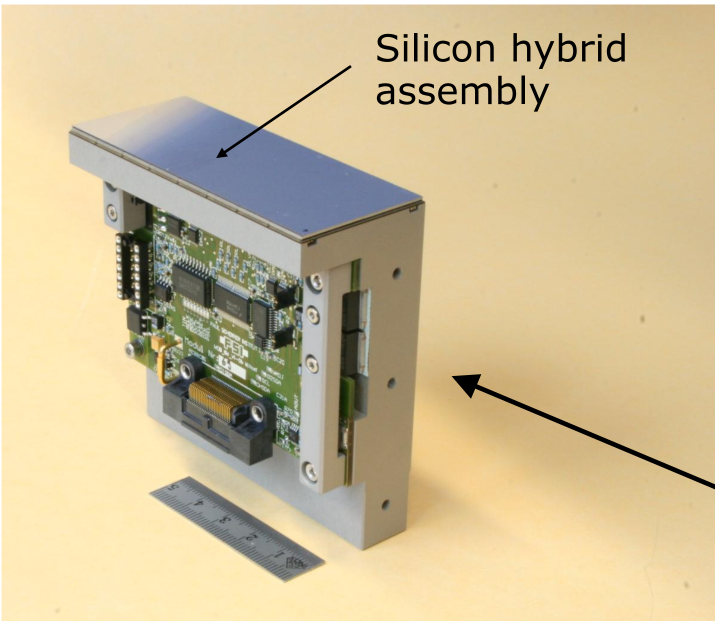
The perfect detector

The perfect detector should have

- *High dynamic range*
- *No readout noise*
- *Uniformity*
- *High quantum efficiency*
- *High spatial resolution*
- *High frame rate*
- *High count rate*
- *Large active area*



Elements of a detector



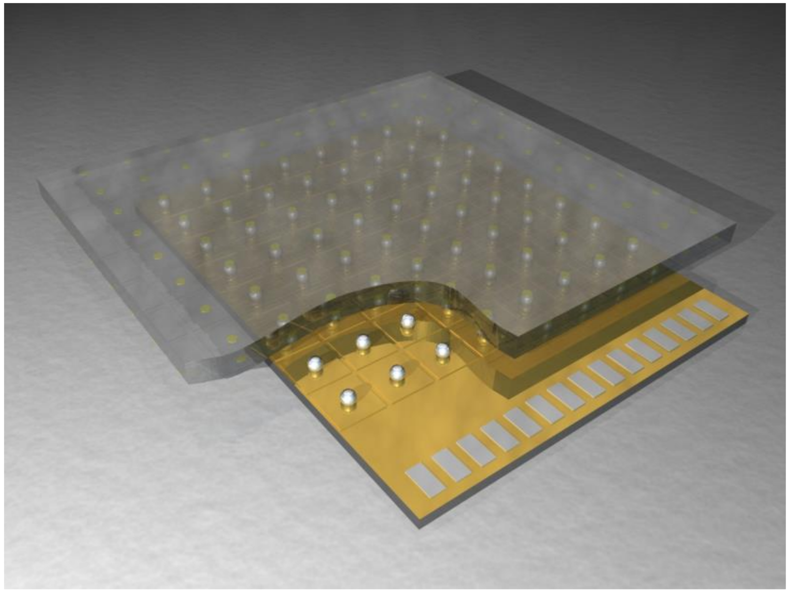
Module active area: 8 × 4 cm²

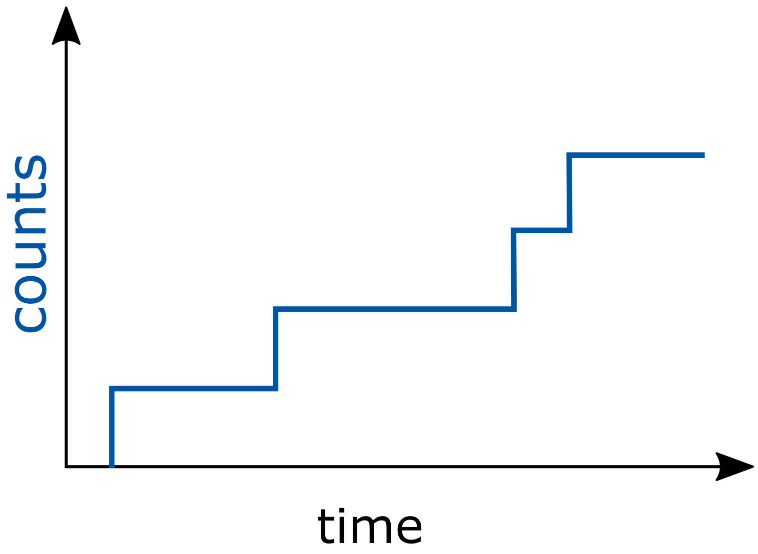
100k pixels on PILATUS (172 µm)

500k pixels on EIGER (75 µm)

The meaning of HPC

Hybrid Photon Counting = Hybrid pixels + photon counting





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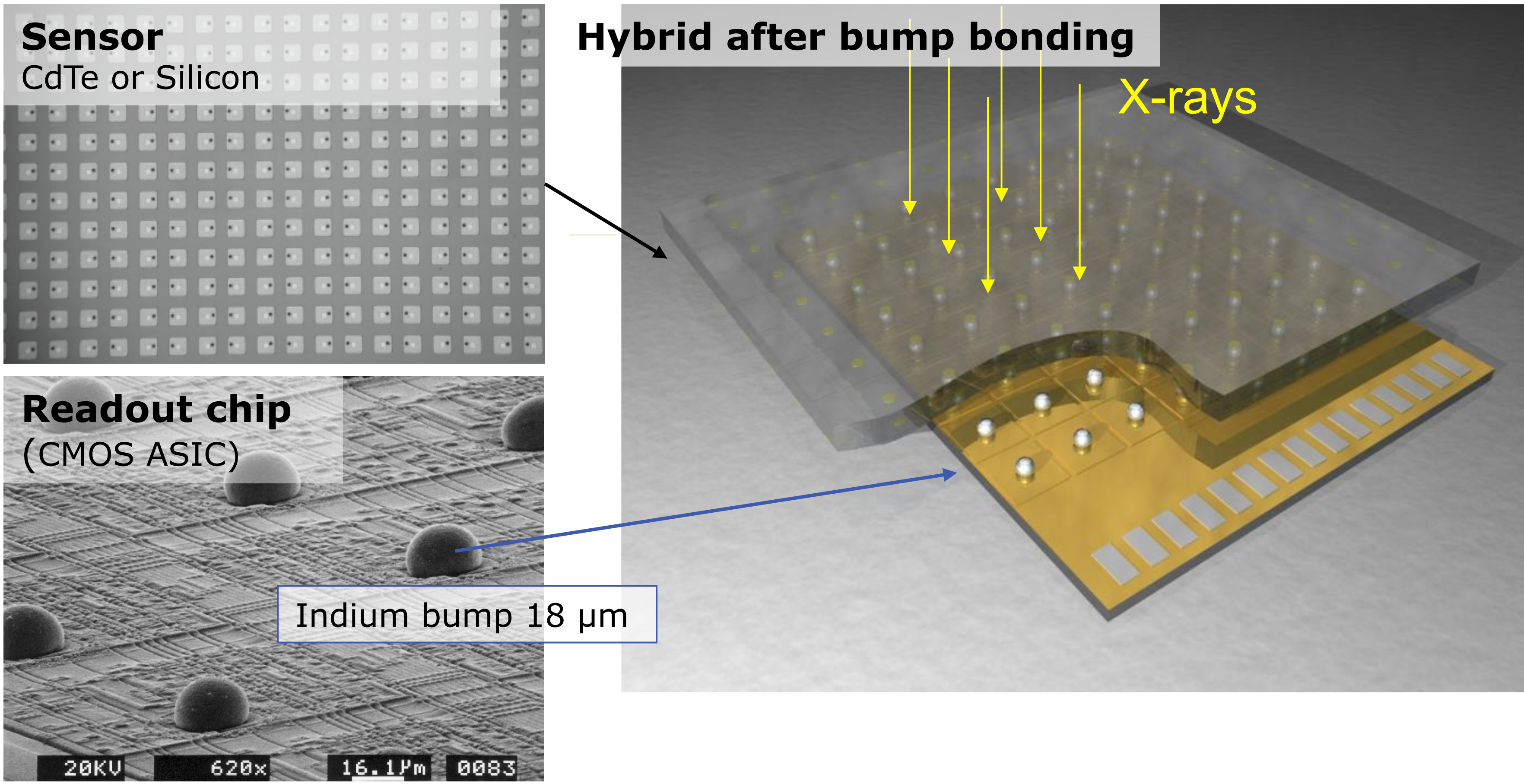
Detector module = sensor + readout



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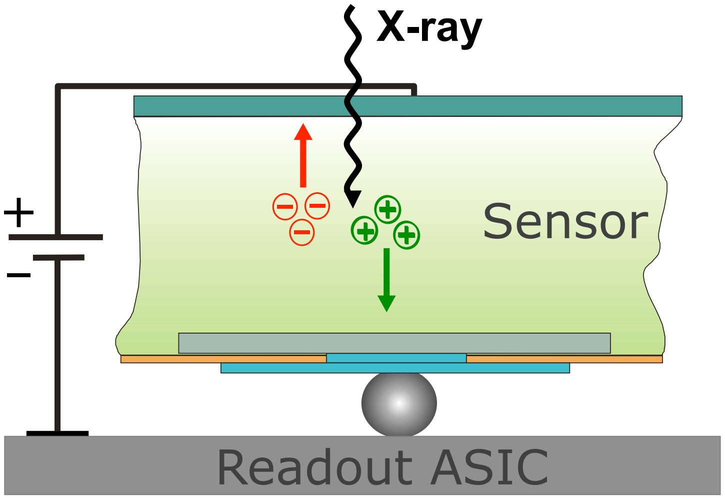
Photon detection in hybrid pixels



Sensor pixel

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

Pixel electronics

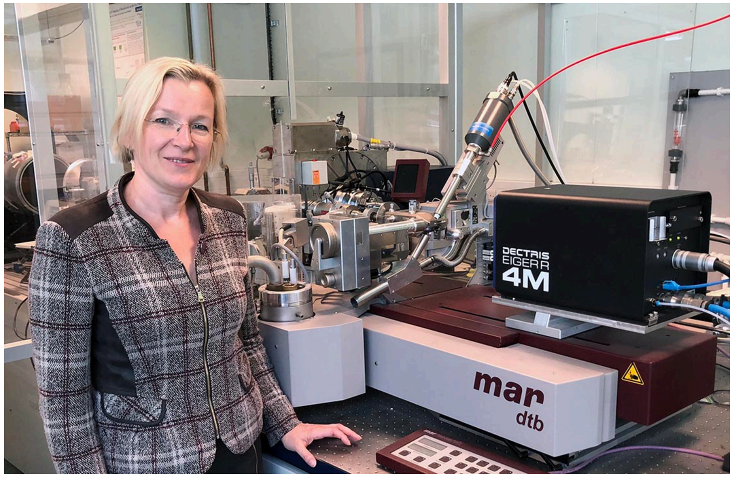
- *Counting of charge pulses*

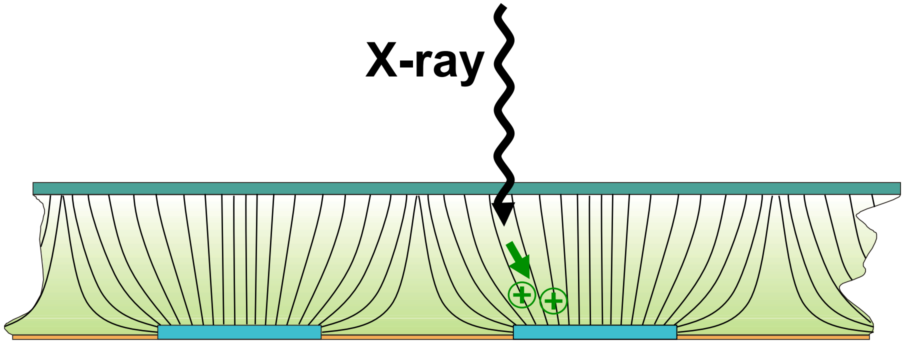
Direct detection of photons

Charge captured in electric field

- *All photons captured*
- *Signal remains in pixel*

- *No photon loss*
- *Sharpest reflections*





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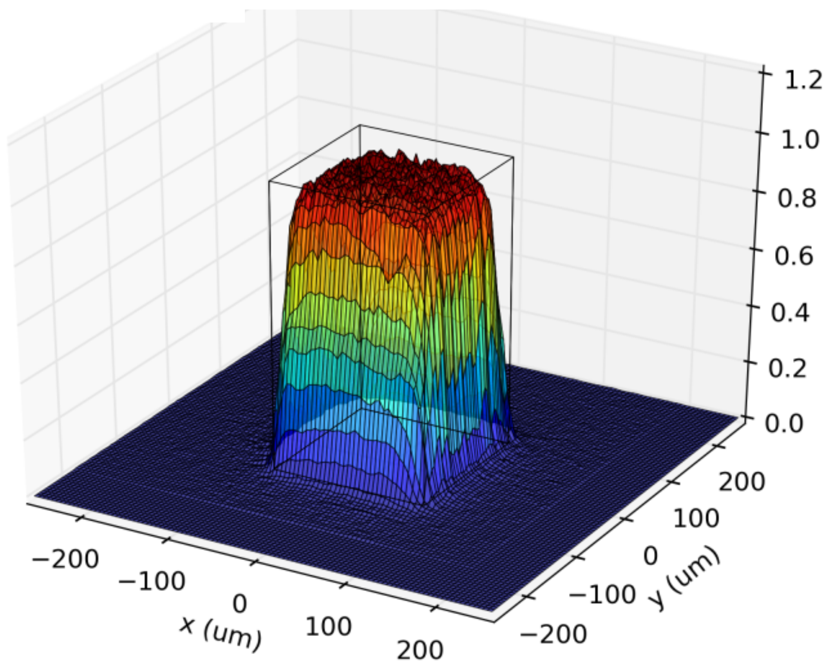
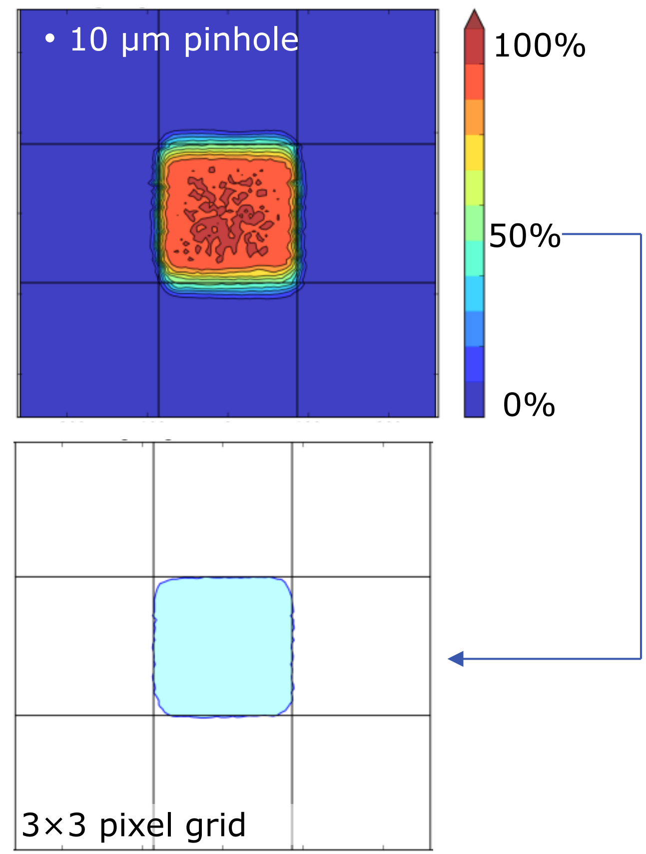
Spatial resolution PILATUS3

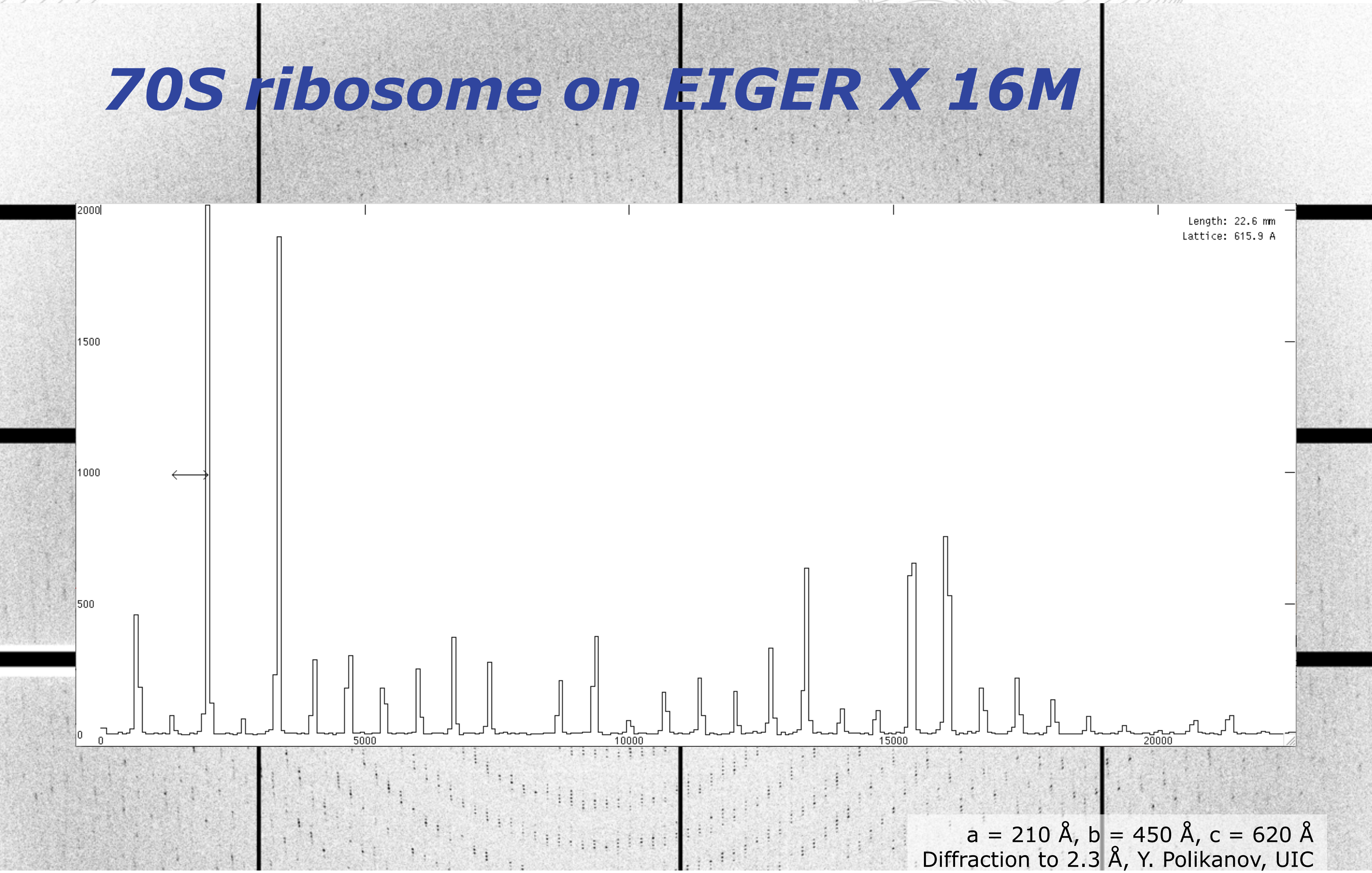
CdTe sensor (same for silicon)



10 µm pinhole at Ag Kα wavelength

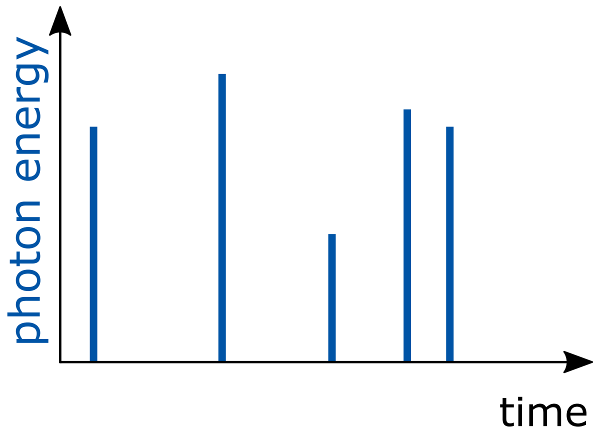


70S ribosome on EIGER X 16M



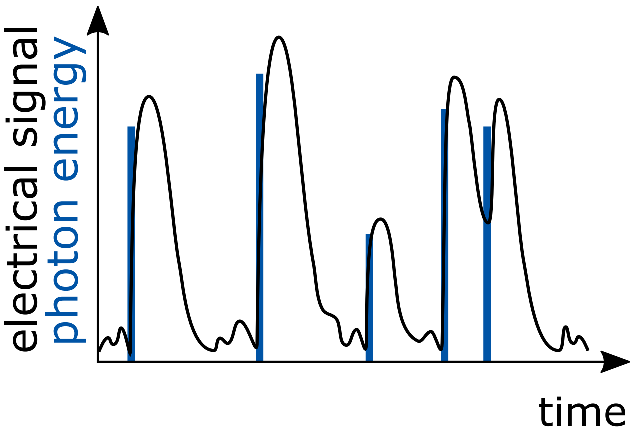
a = 210 Å, b = 450 Å, c = 620 Å
Diffraction to 2.3 Å, Y. Polikanov, UIC

Photon counting – one by one



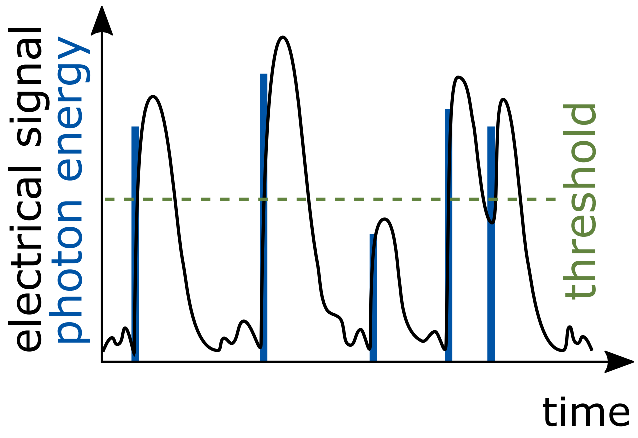
– *Photons absorbed in sensor pixel*

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*

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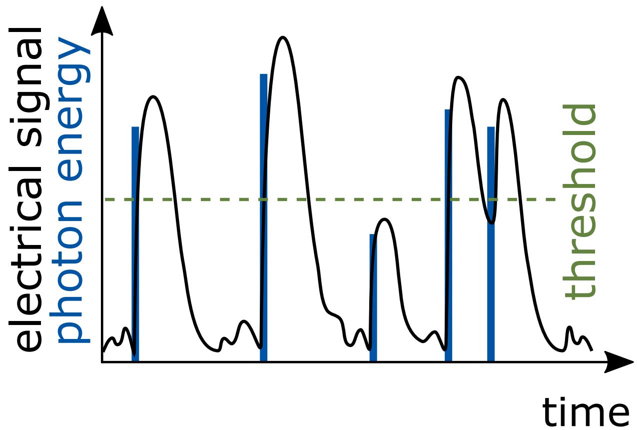
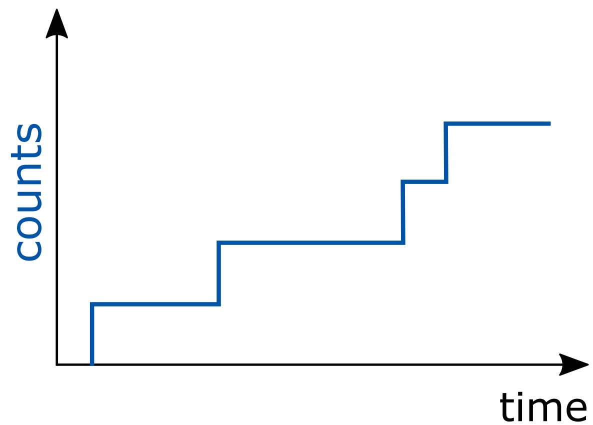
Photon counting – one by one





- *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 fluorescence*
  - *Suppression of electronic noise*

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

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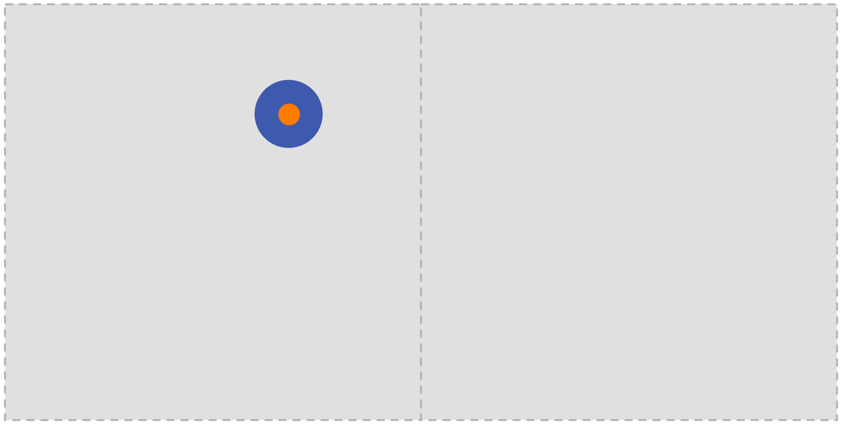
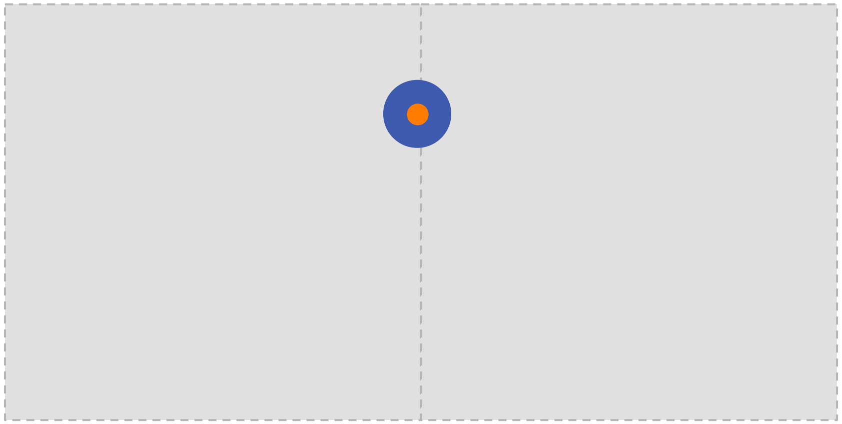
Threshold at half photon energy

All signal counted within one pixel



1 photon
100% charge
1 count



1 photon
60% charge — 40% charge
1 count — -

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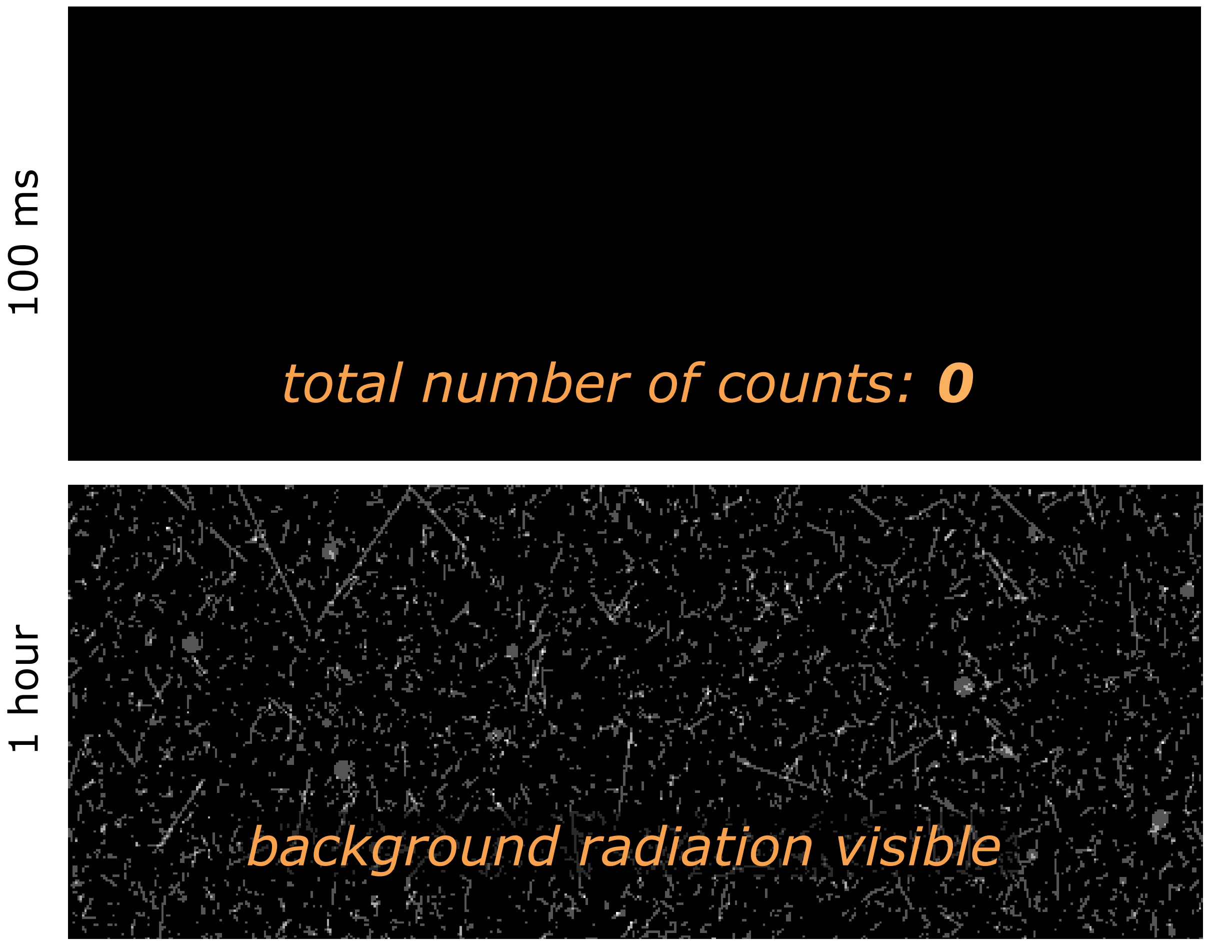
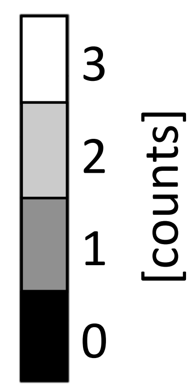
Noise-free detection

No readout noise
No dark signal





100K detector

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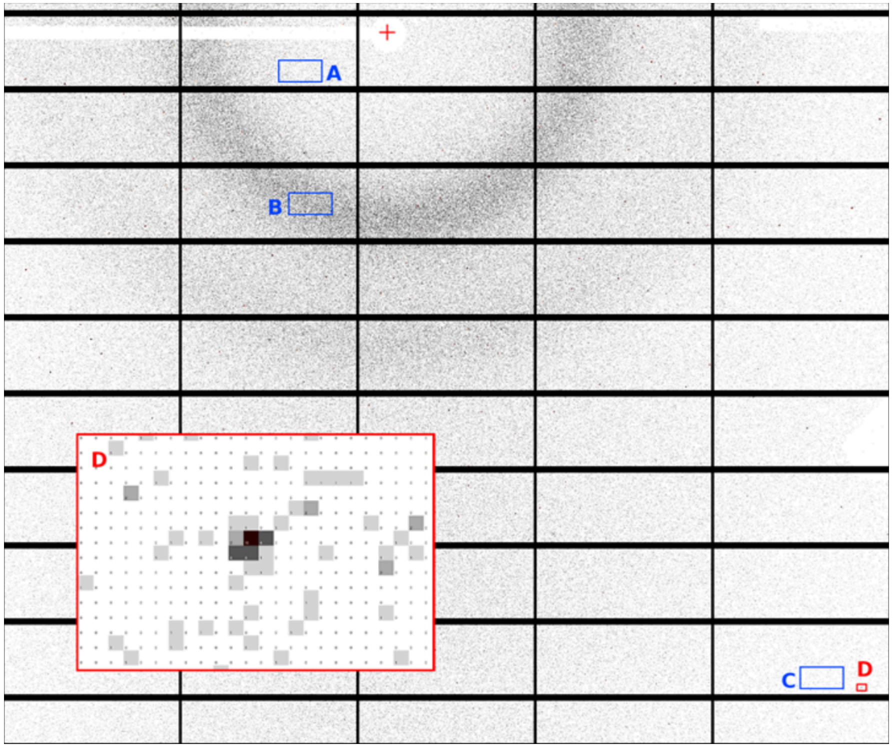
Negligible background

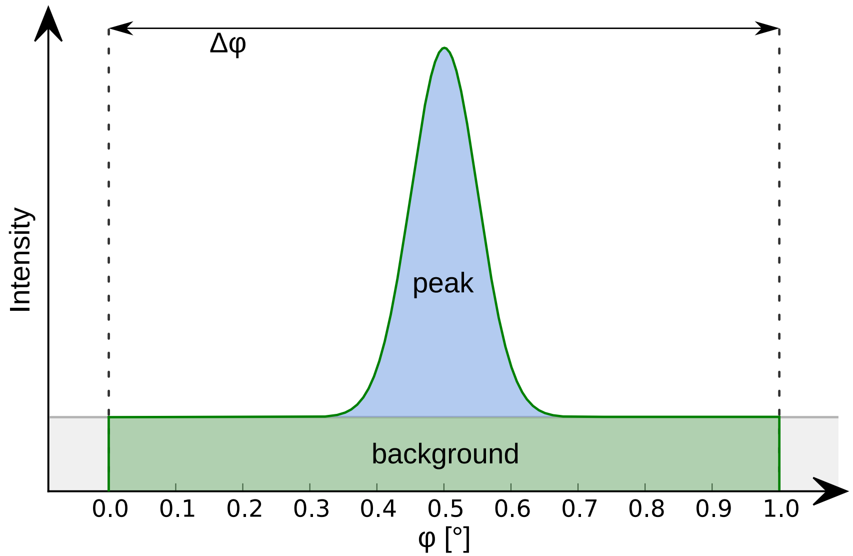
Protein crystallography in vacuum

PETase @ I23.



Austin et al. (2018)

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

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

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

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

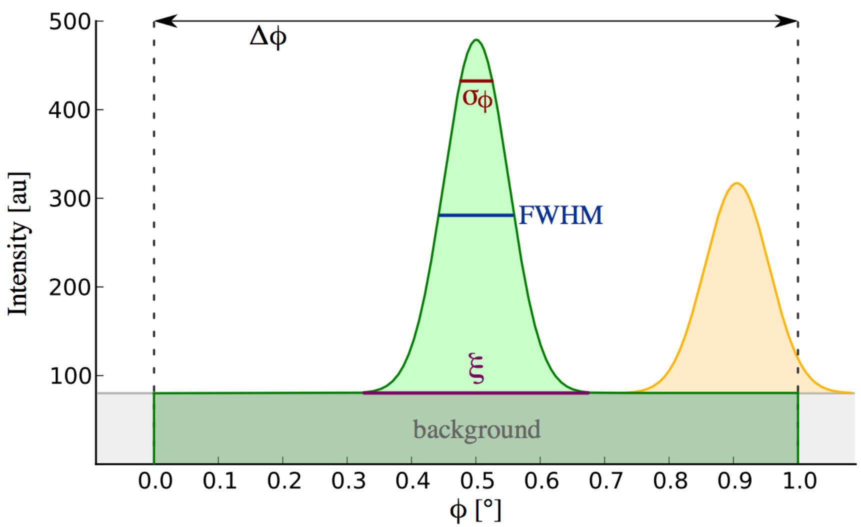
Relative error [%]	Background [photons]		
Integrated intensity [photons]	0	500	1000
100	10.0	33.2	45.8
500	4.5	7.7	10.0
1000	3.2	4.5	5.5
10000	1.0	1.0	1.1

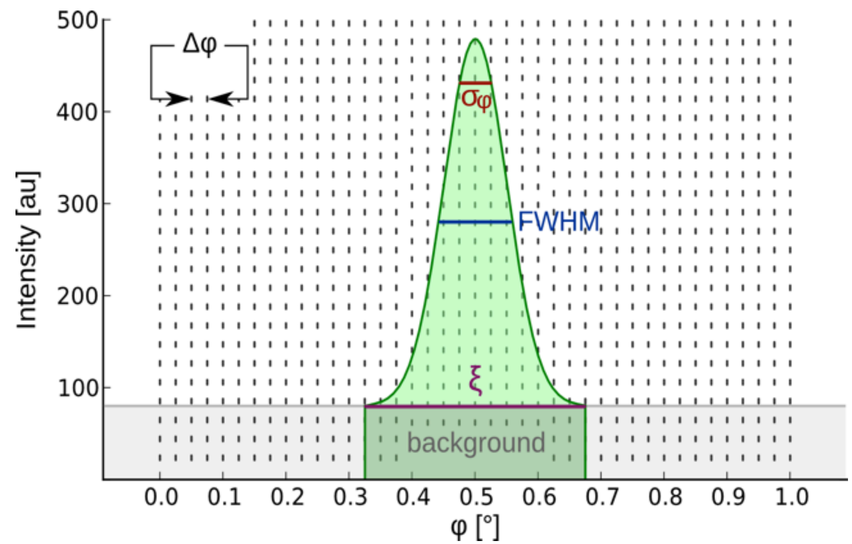
Less background

=> Better data

Pflugrath (1999)

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

Mueller et al. (2012)

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

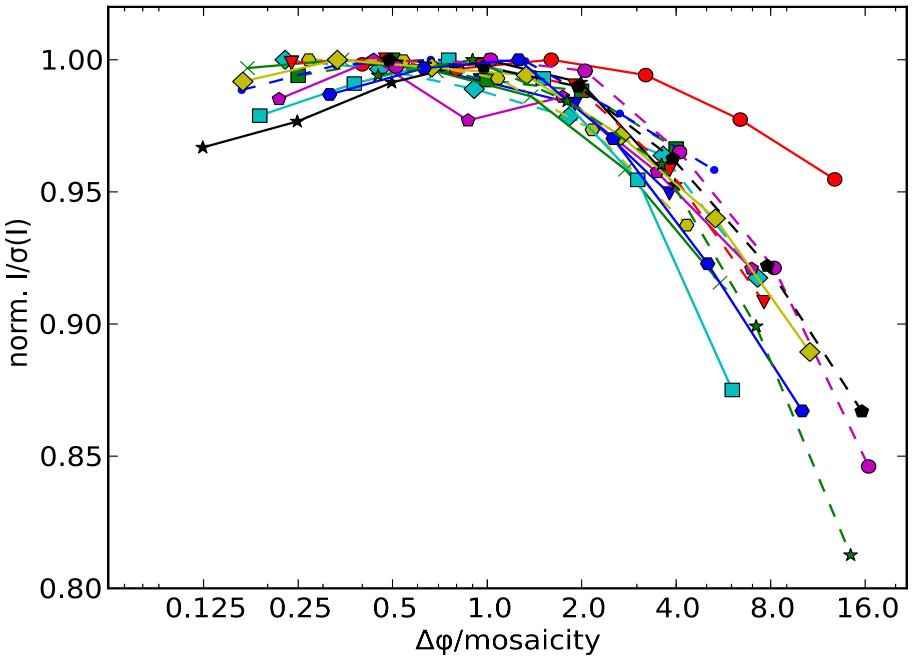
Fine φ-slicing improves data quality

Δφ < mosaicity improves:

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

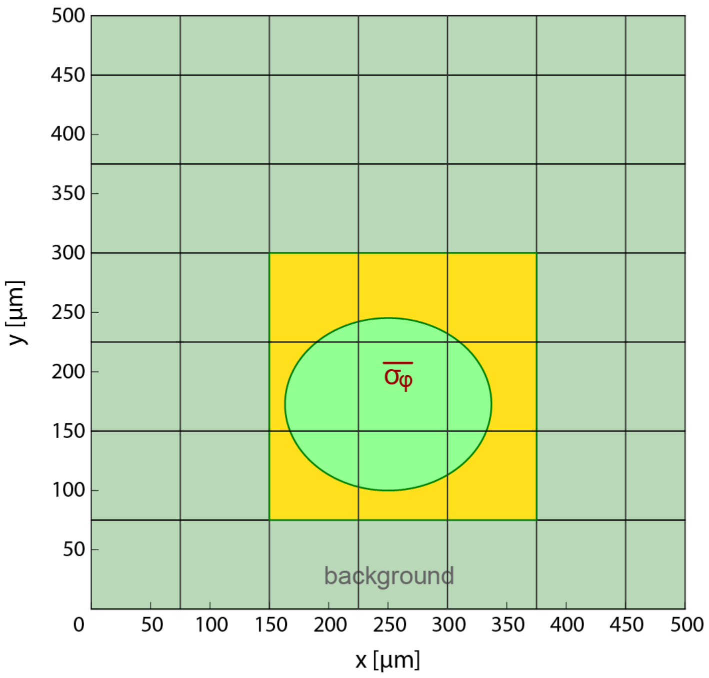
on PILATUS.

higher I/sig(I)

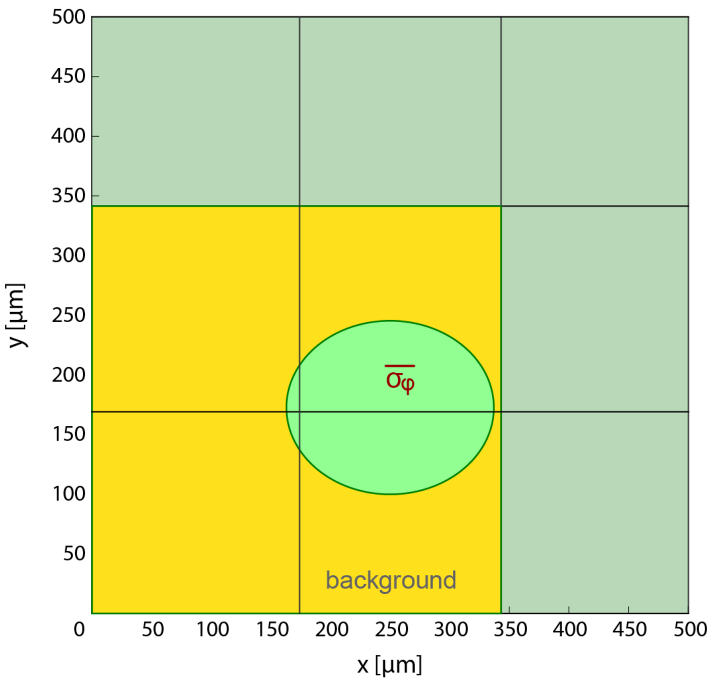


Mueller et al. (2012)

Smaller pixels improve data quality



Reflection on EIGER



Reflection on PILATUS

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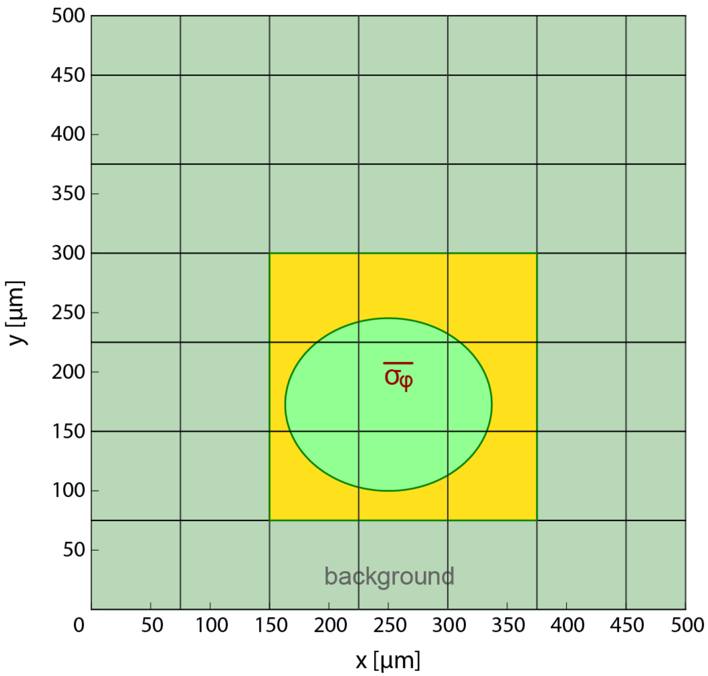
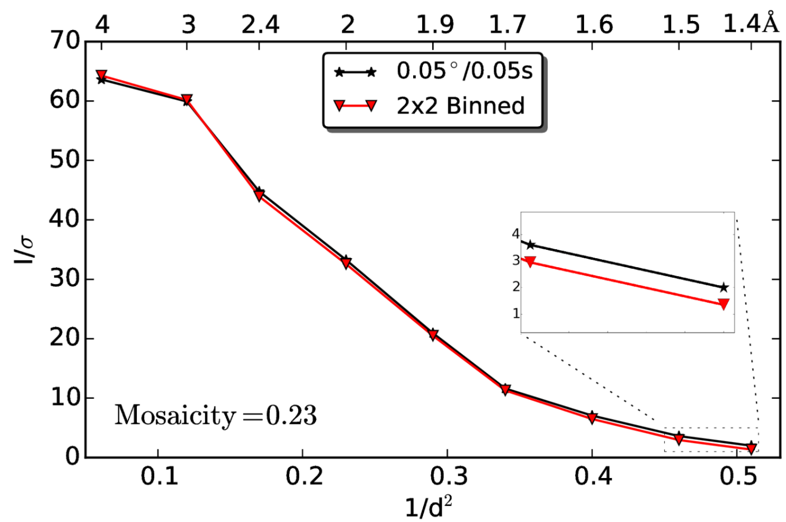
Smaller pixels improve data quality



Reflection on EIGER



Better high-resolution data

Casañas et al. (2016)

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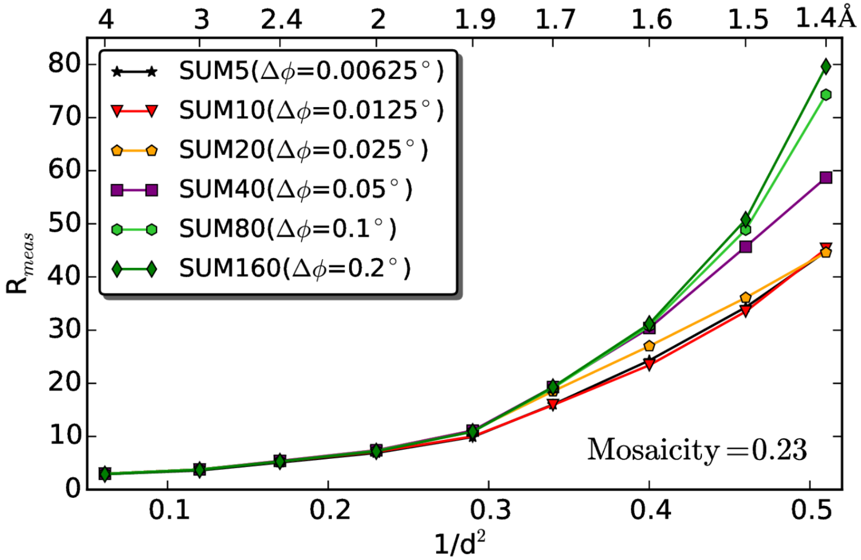
Smaller pixels allow for finer φ-slicing

Δφ ≈ 1/10 mosaicity improves:

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

on EIGER.

lower R_{meas}



Casañas et al. (2016)

Get best data by

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

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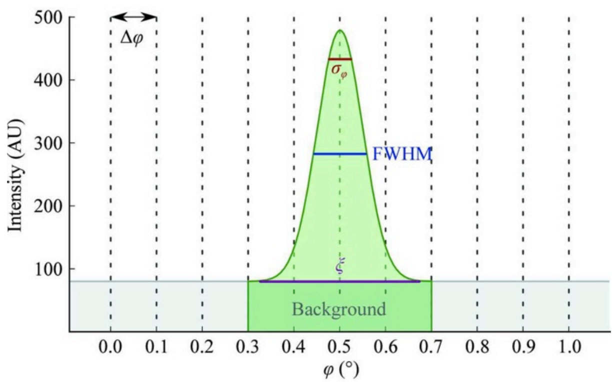
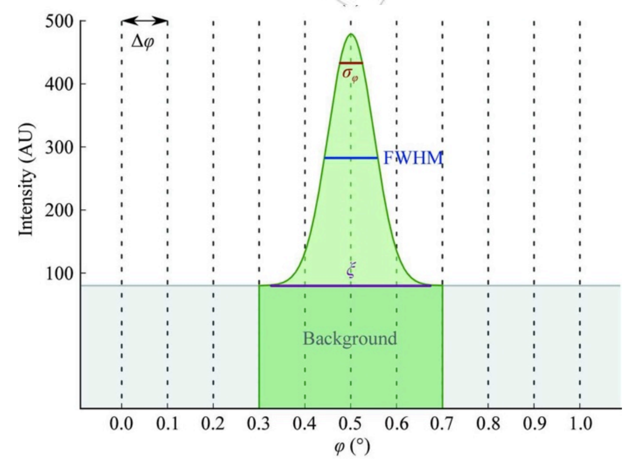
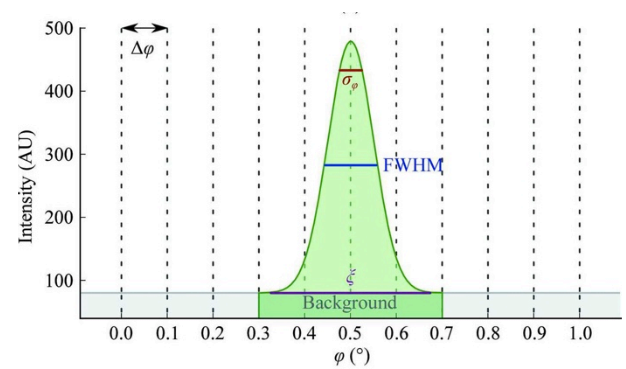
Decrease absolute background

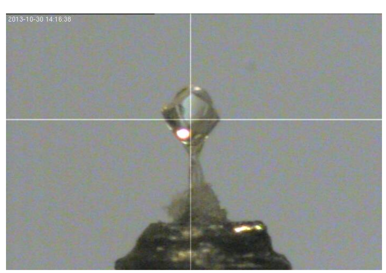


bad!



good!





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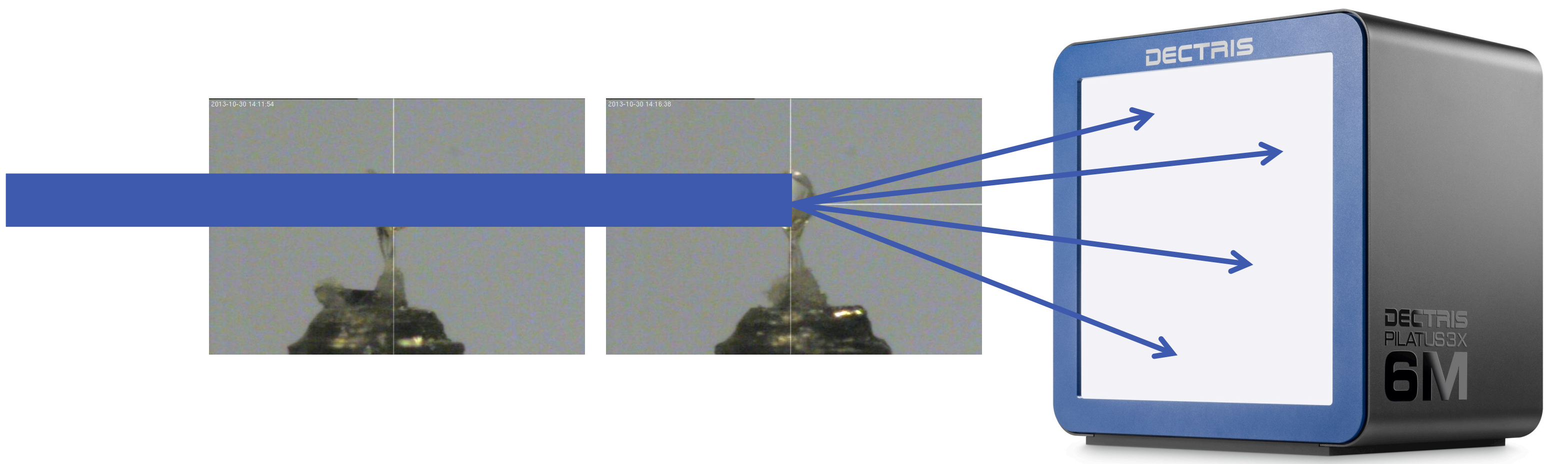
Decrease absolute background



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Get best data by

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

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Obsolete strategy

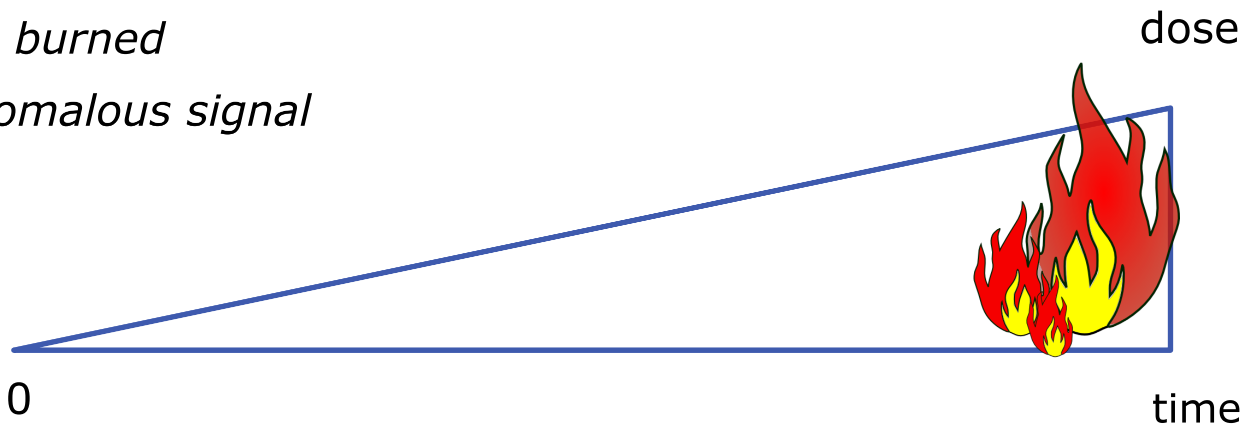
Once upon a time

- Strategy software for minimal sweep
  - 47 images at 1°/img from φ_0 = 115.4°
- Few images to minimize noise
- Long exposures to lift weak reflections above noise
- Only if using CCDs

Wild West revival

Collect 360° of data

- To get more precise intensity estimates
- To avoid space group frustration
- To avoid getting burned
- To maximize anomalous signal



90° @ 10 img/s (0.1°/img)	90 s
360° @ 40 img/s (0.1°/img)	90 s

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There is always anomalous signal

Current paradigm

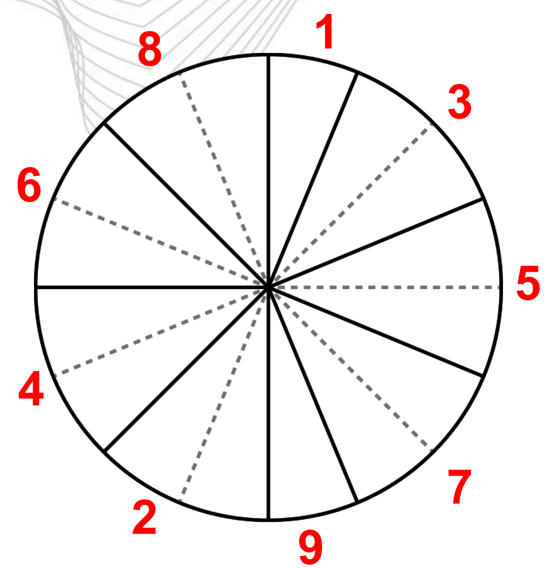
- *Molecular replacement*
- *In case of failure*
  - *Se-Met substitution*
  - *Heavy atom soaking*

Always measure anomalous differences

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

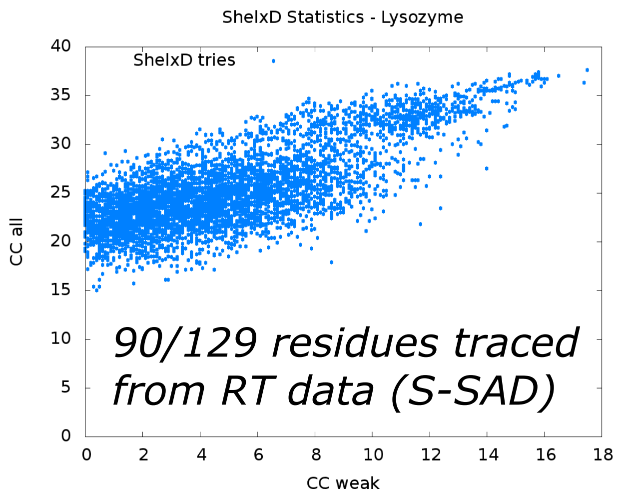
Always measure 360° of data.

Sophisticated strategies



Collect 360° of data

- ***From starting angle suggested by strategy software***
- ***With detector at correct distance***
- ***With optimized exposure time***
- ***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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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.

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Get best data by

- *Using PILATUS and EIGER detectors*
- *Using fine φ-slicing*
- *Minimizing absolute background*
- *Collecting at least 360° of data*
- *Thinking about your experiment*

Use beam time *effectively*

- *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 360° of 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 !*

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Thank you for your attention!

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