Practical MX data collection & Strategies

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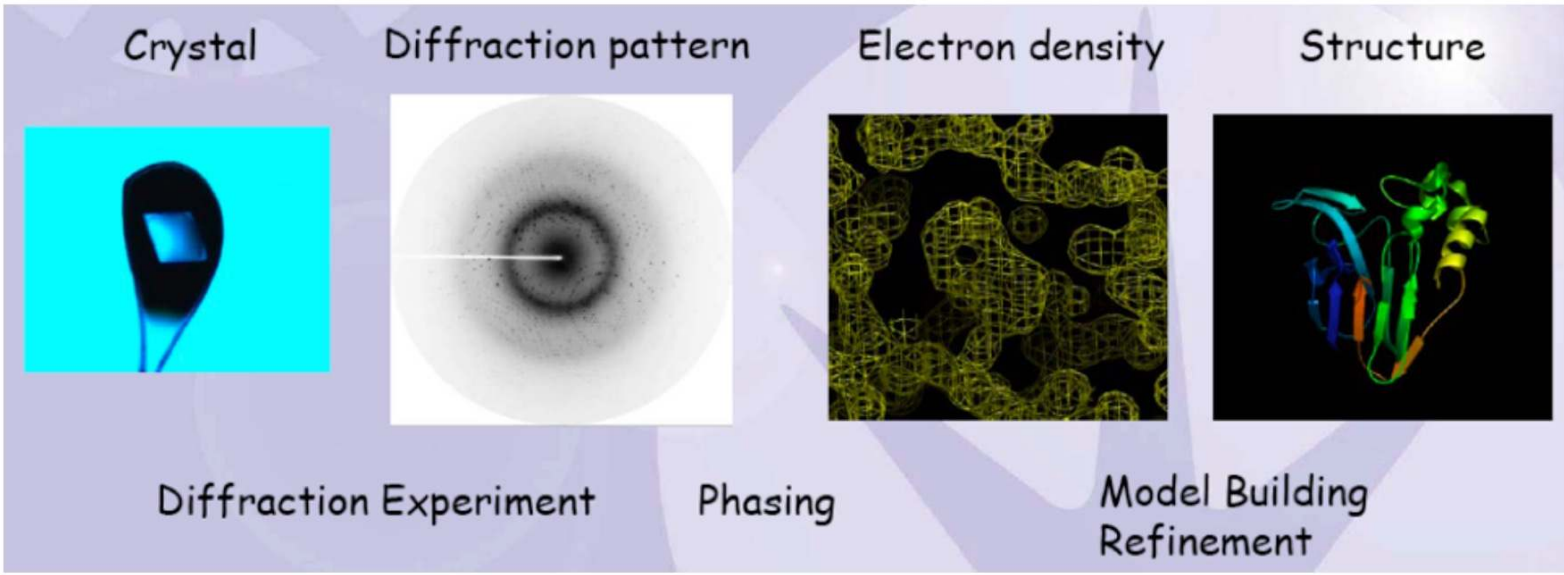
for slides & ideas

$$\rho(xyz) = \frac{1}{V}\sum_{hkl}|F_{hkl}|\exp(i\alpha_{hkl})\exp[2\pi i(hx+ky+lz)]$$



$$I(hkl) = I_0 \cdot \frac{\lambda^3}{\omega V_{cell}^2} \cdot V_{cr} \cdot L \cdot P \cdot T_r \cdot r_e^2 \cdot |F_{hkl}|^2$$

Data collection is important!

<u>This is the last experimental step</u>

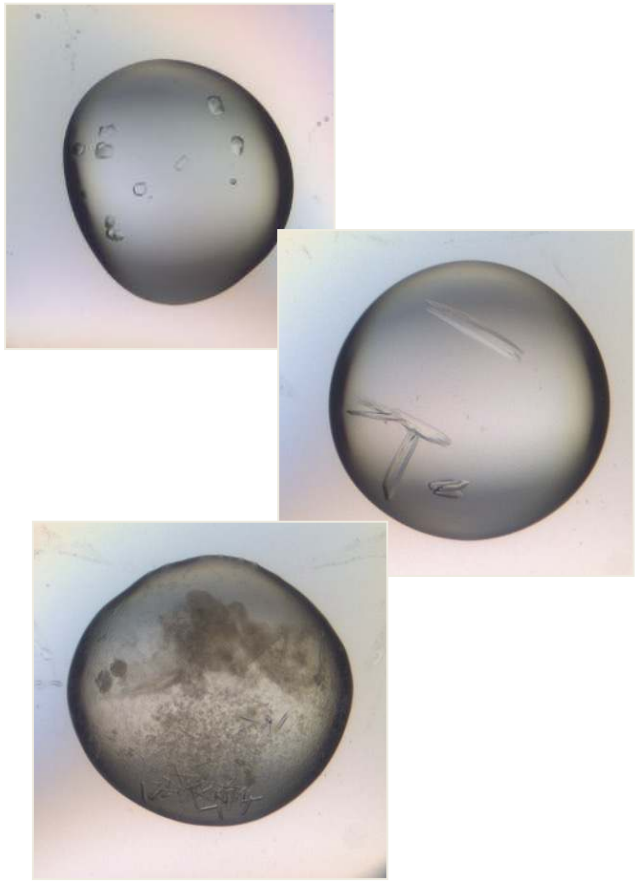
Time taken now to collect the best possible data can prevent pain and possible failure later on.

"One good friend is better than a thousand poor ones." Indian Proverb

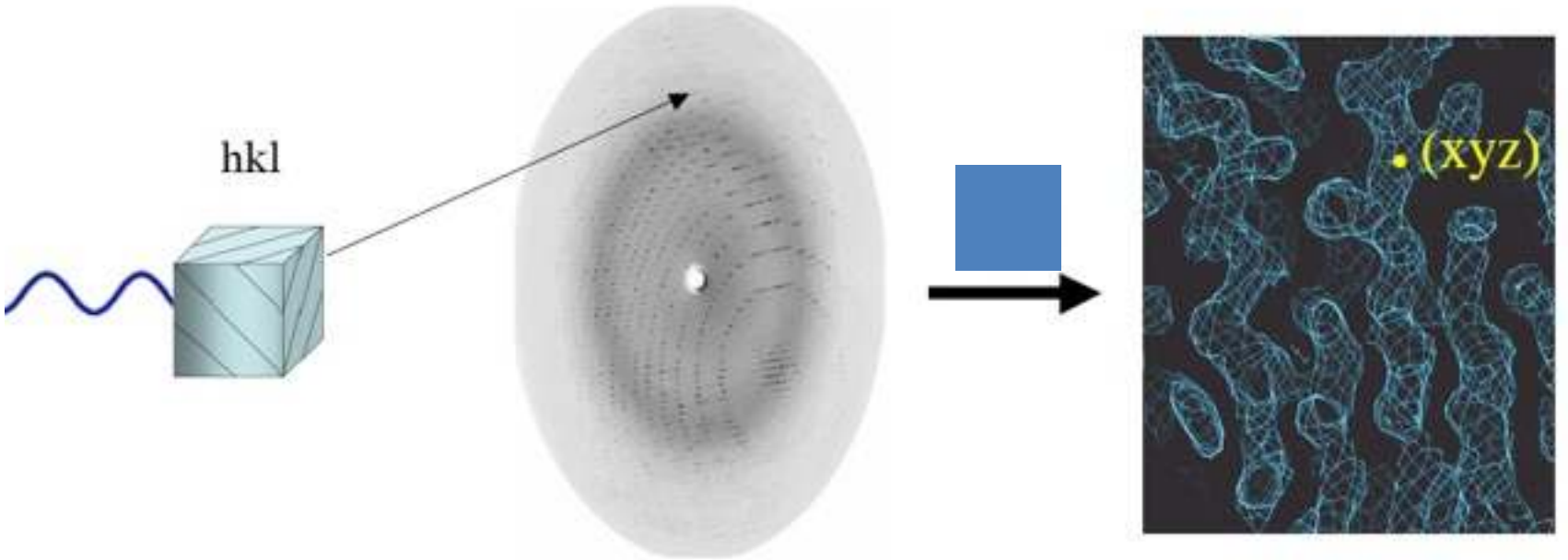
If the original data are poor then not much can be done about it later.

GIGO/RIRO paradigm

With fast sample changers, goniometers and detectors a lot of data can be collected very fast.
Quantity ≠ Quality

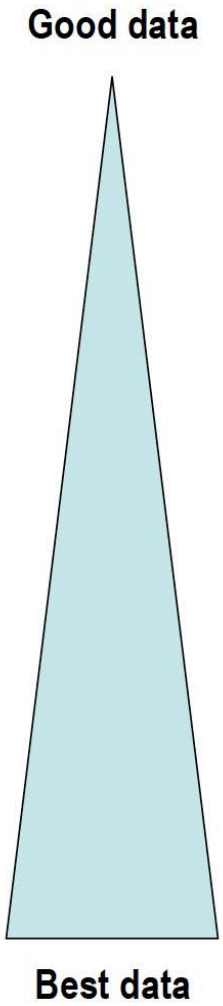


Data collection: the goal



- record complete set of X-ray diffraction intensities from a crystal
- the better the data (high resolution, high completeness, low noise), the easier the following steps and the better refinement works
- like all previous steps this might need some optimisation and a lot of patience

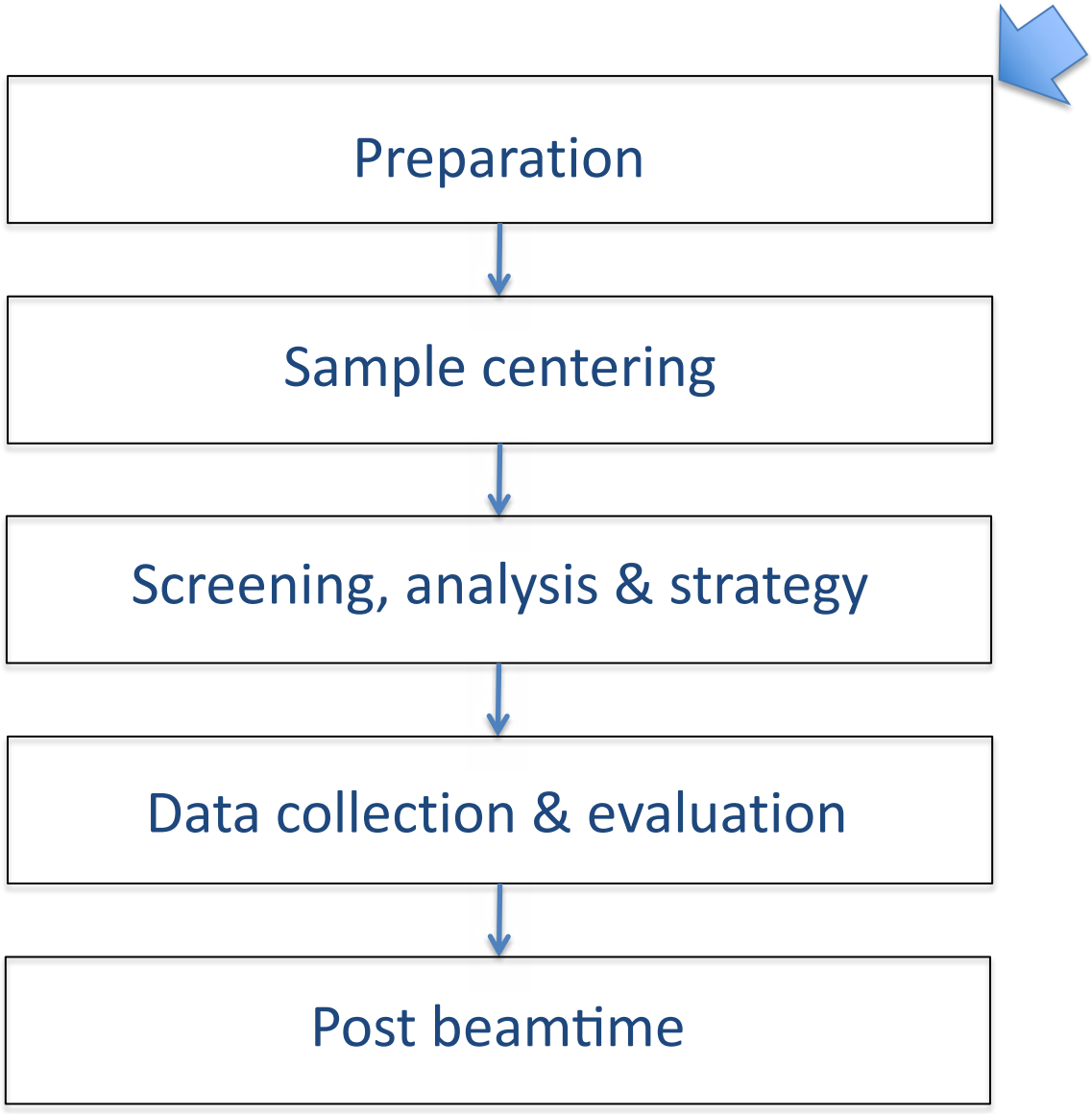
- Molecular substitution
- Molecular replacement
- Isomorphous replacement (Hg, Pt)
- MAD/SAD phasing
- Native-SAD phasing (Sulfur-SAD)



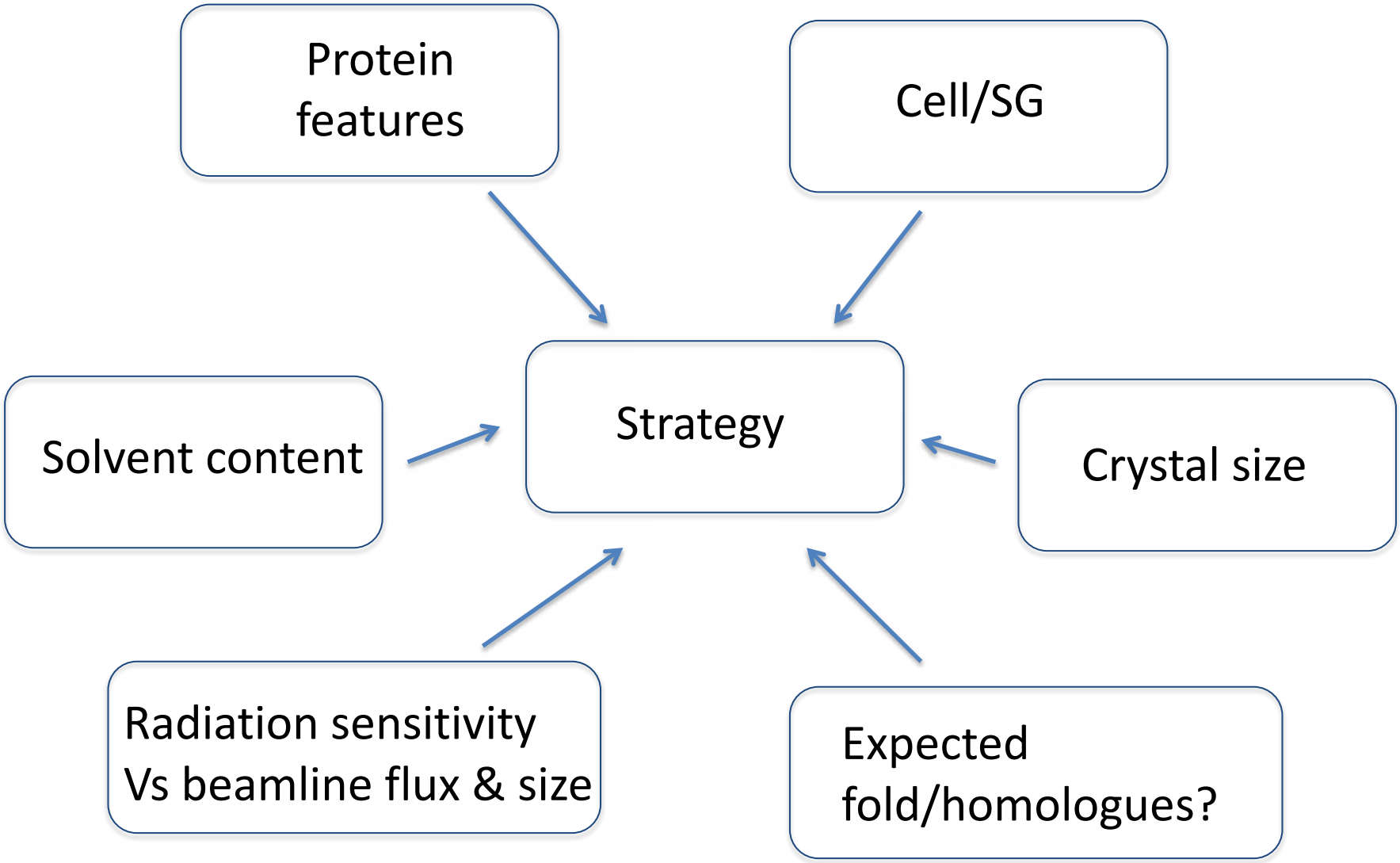
What's the goal?

Aim	High priorities	Lower priority	**Max Dose per dataset** (e.g. per 120, 180 or 360 degrees) For a ~2A resolution dataset
Native data collection	High resolution (e.g < 1.8 A) Complete data	High redundancy	20 MGy
Multi-wavelength anomalous dispersion (MAD) Single Anomalous dispersion (SAD)	Accurate, complete and highly redundant Very little radiation damage Choice of wavelength	High resolution data	1 - 5 MGy (heavy atom will increase dose for same exposure)
Sulphur SAD	As above + extra high redundancy (40-200fold) if not on I23 beamline		0.3-0.5 MGy
Molecular replacement	Completeness at low resolution (< 3Å) Good quality low resolution	High resolution data High redundancy	~20 MGy
Ligand/mutation	Medium resolution (e.g. 2 A), complete data. High throughput (FAST)	High redundancy	10 - 20 MGy

Trying to collect everything at once will usually result in failure

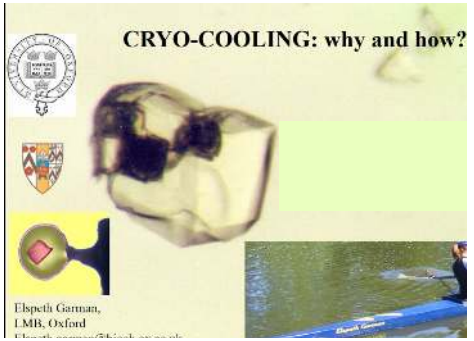

Preparation
Sample centering
Screening, analysis & strategy
Data collection & evaluation
Post beamtime

Preparation - Prior knowledge



Data collection – cryo protectant

Talk later today

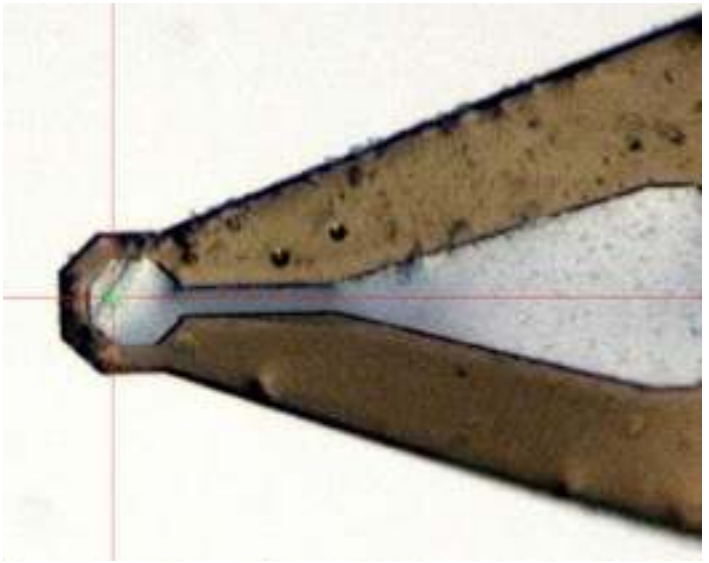


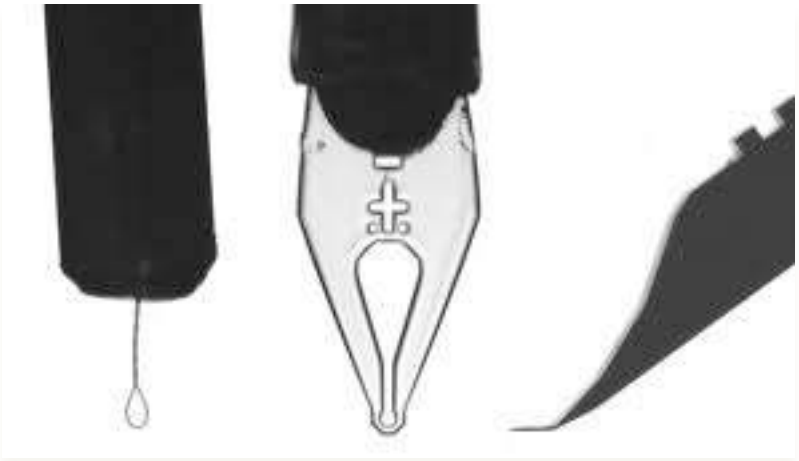
• see A. Evdokimov's protocol for complete practical approach (but better is to attend Elspeth Garman's talk)
https://mcl1.ncifcrf.gov/nihxray/Tips-and-Tricks_CryotectionOfDelicateCrystals-ArtemEvdokimov.pdf

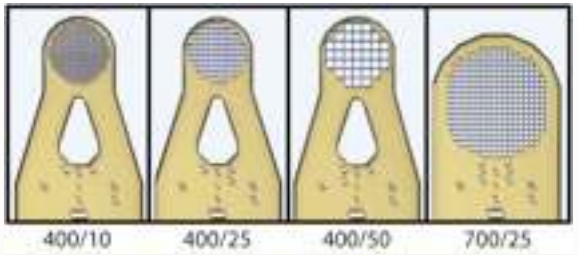
• Pre-synchrotron: test new cryoprotectant in X-ray beam / cryo-stream before mounting crystal

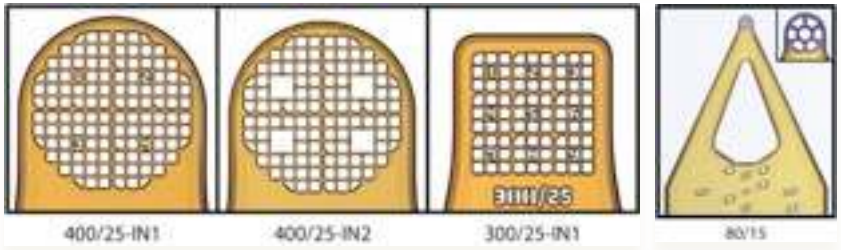
• consider mounting + shooting crystals at room temperature (particularly on a home source) to know how well they diffract without potentially damaging cryoprotectants (MiTeGen MicroRT sleeves)

Data collection - crystal mounting



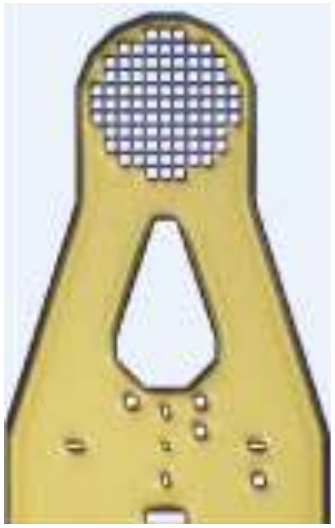


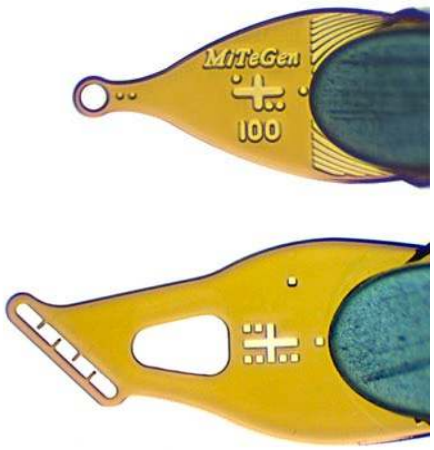


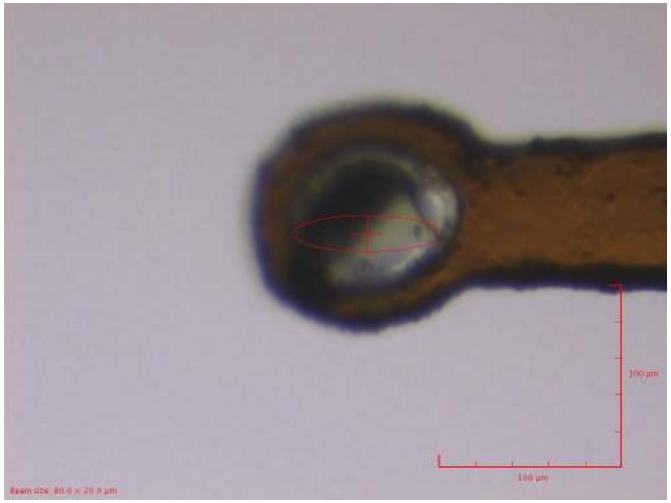


http://www.mitegen.com/

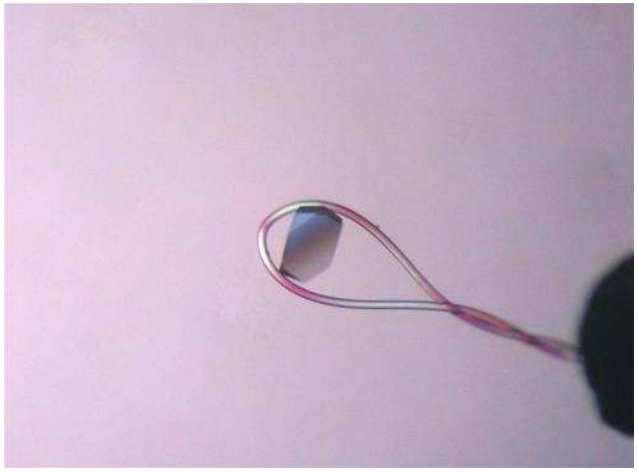
Preparation - Sample mounting

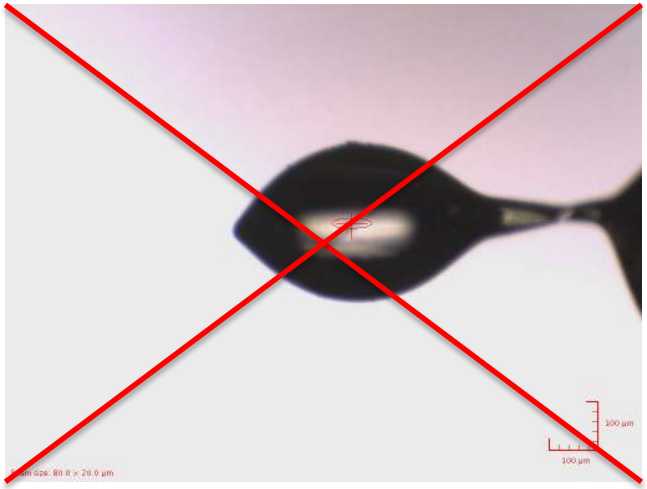






Do not just use a tool because a lab mate says that's is! (or is the only available in the lab) – I bet you will hear similar wording by Elspeth Garman on cryoprotection talk because I learned this from her ☺





Preparation - Choice of beamline

- Energy range – does it cover what you need?
- Multi-axis goniometer? P1, experimental phasing
- Biological containment?
- Cryo or room temperature collection?
- Can the beam size match your crystals?
- Flux (seconds exposure for max dose)
- – important for radiation damage

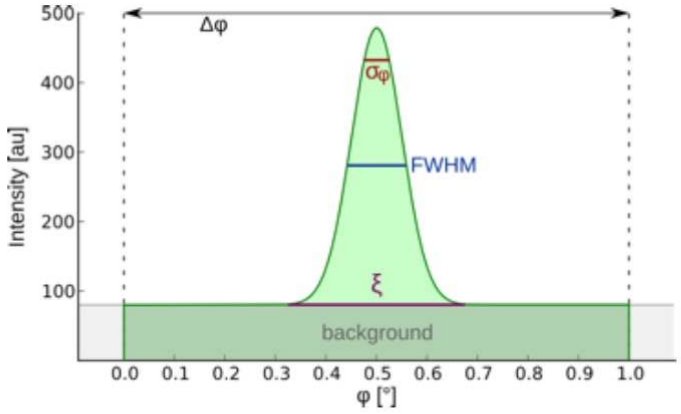
Random errors, counting statistics

$\sigma_{count} = N^{1/2}$

$I = N_p - N_b$ **Signal is the difference**

$\sigma_I = (\sigma_p{}^2 + \sigma_b{}^2)^{1/2}$

$\sigma_I = (N_p + N_b)^{1/2}$ **Uncertainty is the sum**



Relative errors (%) for hypothetical integrated intensity and background values.

Example: Intensity = 100, Background = 500, therefore Peak = 600 and $R_{err} = \sigma(I)/I = (600 + 500)^{1/2}/100 = 33.2\%$.

Integrated intensity (photons)	Background (photons)		
	0	500	1000
100	10.0	33.2	45.8
500	4.5	7.7	10.0
1000	3.3	4.5	5.6
10000	1.0	1.0	1.1

Pflugrath, *Acta Cryst*. **D55**, 1718 (1999)

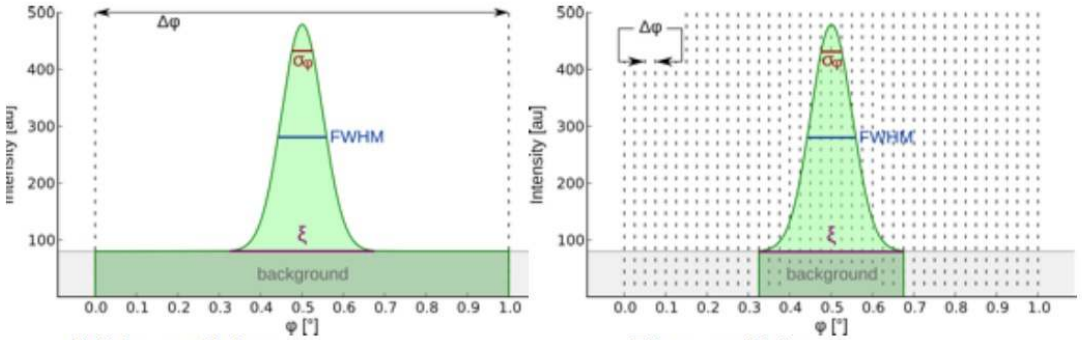
Random errors, counting statistics

$$\sigma_{count} = N^{1/2}$$

$$I = N_p - N_b \qquad \textbf{Signal is the difference}$$

$$\sigma_I = (\sigma_p{}^2 + \sigma_b{}^2)^{1/2}$$

$$\sigma_I = (N_p + N_b)^{1/2} \qquad \textbf{Uncertainty is the sum}$$

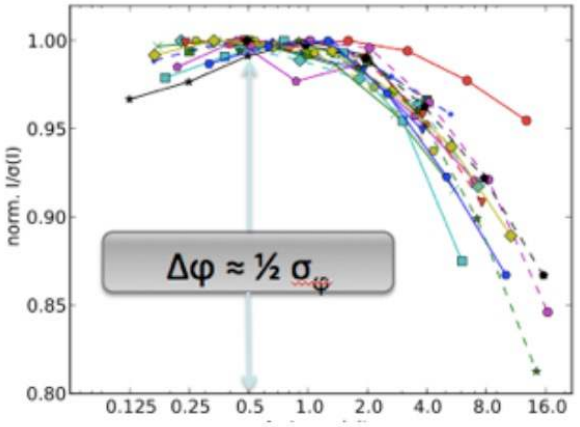


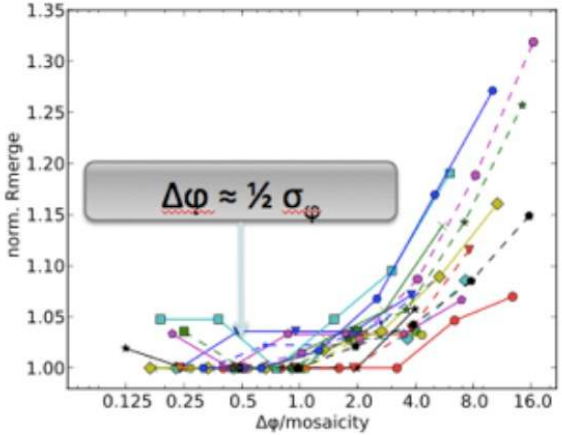
Wide φ-slicing

- Large $\Delta\varphi$ ($\Delta\varphi > \xi$)
- Large overlap of reflections and background along φ
- Few images

Fine φ-slicing

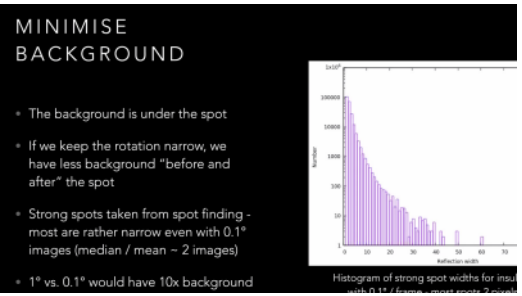
- Small $\Delta\varphi$ ($\Delta\varphi << \xi$)
- Minimal overlap of reflections and background along φ
- Many images



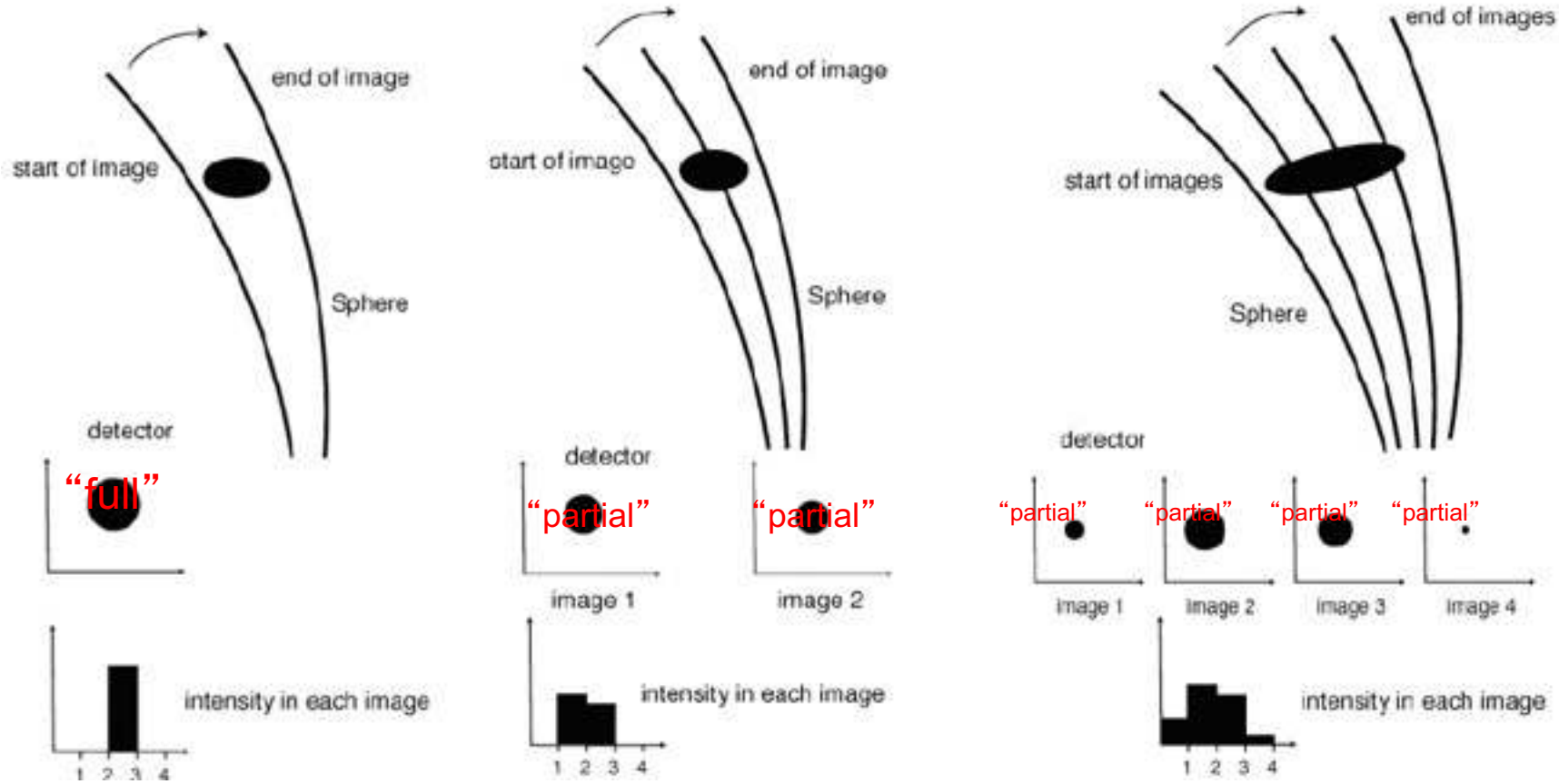


Fine-phi slicing data collection is enabled by the pixel array detector (PILATUS, EIGER), which has single-photon sensitivity and no readlout noise

Mueller, *et al*, *Acta Cryst*. **D68**, 42 (2012)

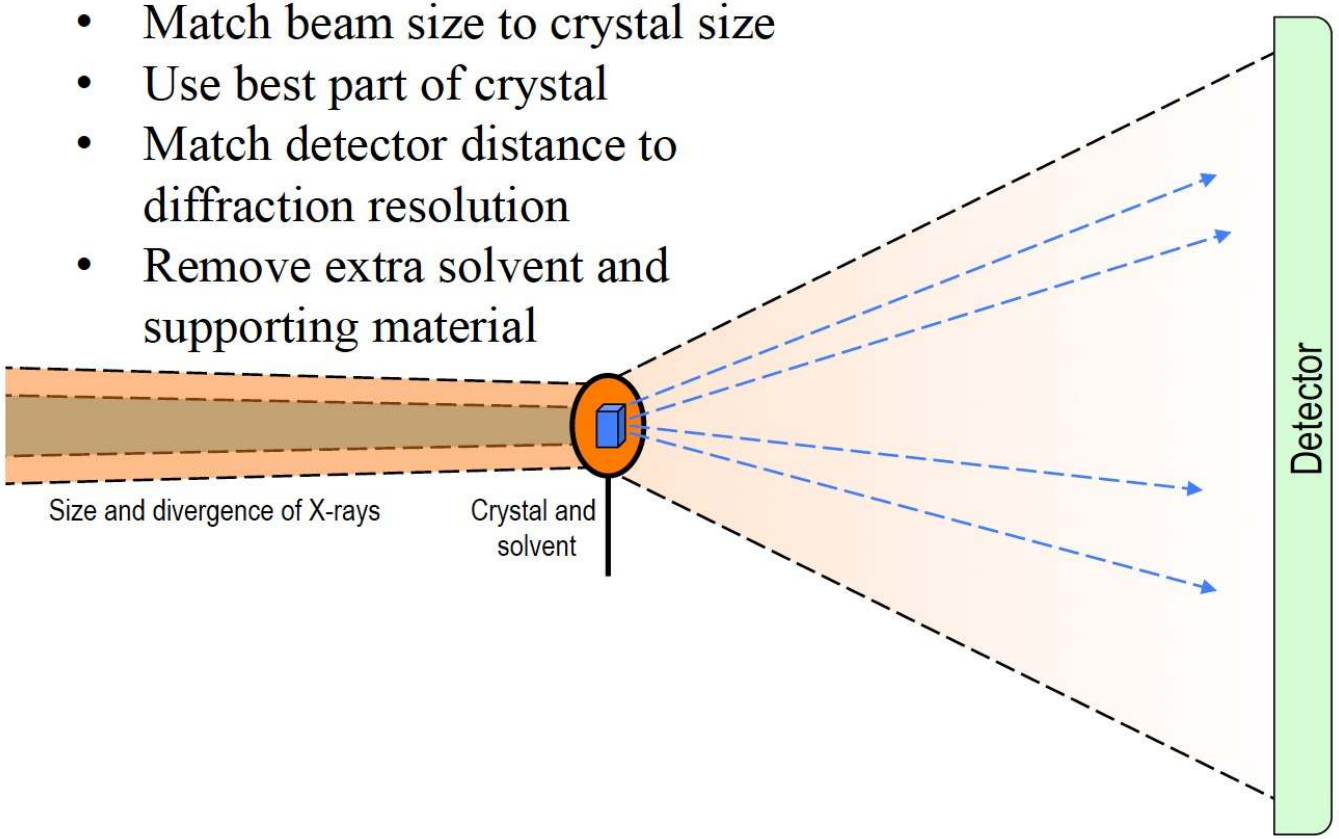


Data collection: the rotation/oscillation method

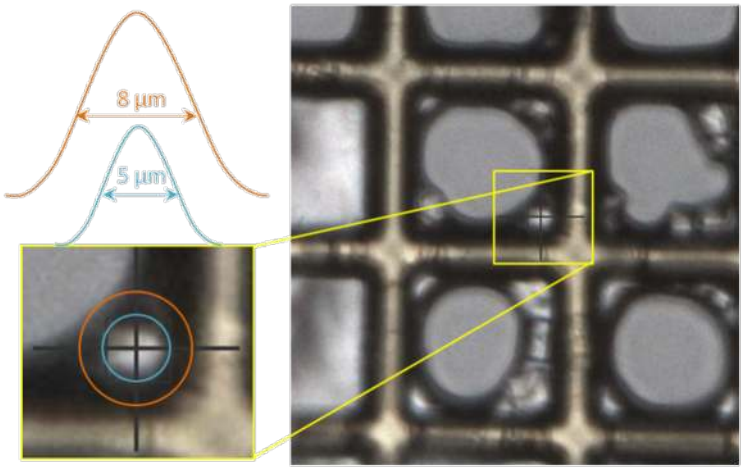


- spot size is equally affected by mosaicity, beam size and beam divergence
- programs that work with 3-d profile fitting (e.g. XDS, DIALS, …) are better for dealing with "partials" spread over many images

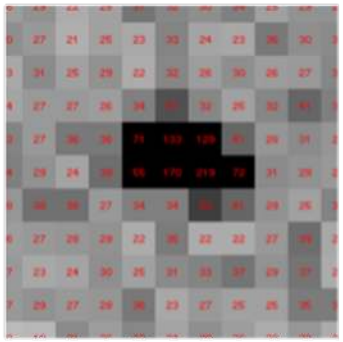
- Match beam size to crystal size
- Use best part of crystal
- Match detector distance to diffraction resolution
- Remove extra solvent and supporting material

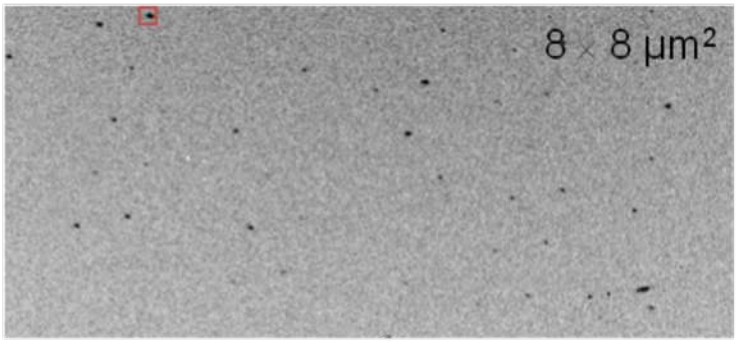


Match beam to sample

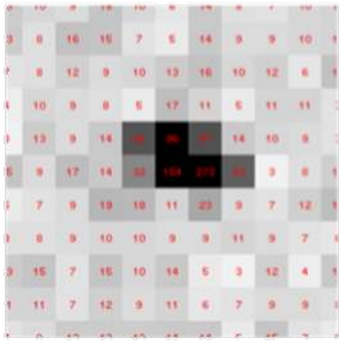


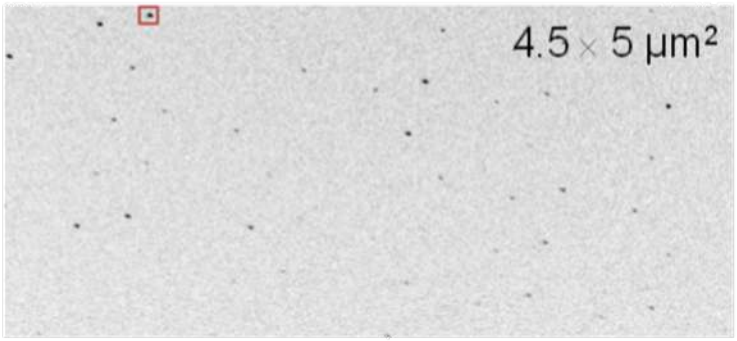
AcMNPV polyhedra crystals approx 5×5×5 μm^3 in size were mounted on a micromesh grid. Data were collected with two beamsizes: 8×8 μm^2 and 4.5×5 μm^2





I/ σ(I) = 1.5 at 2.9Å





Average background reduced by 3

I/ σ(I) = 1.5 at 2.5Å

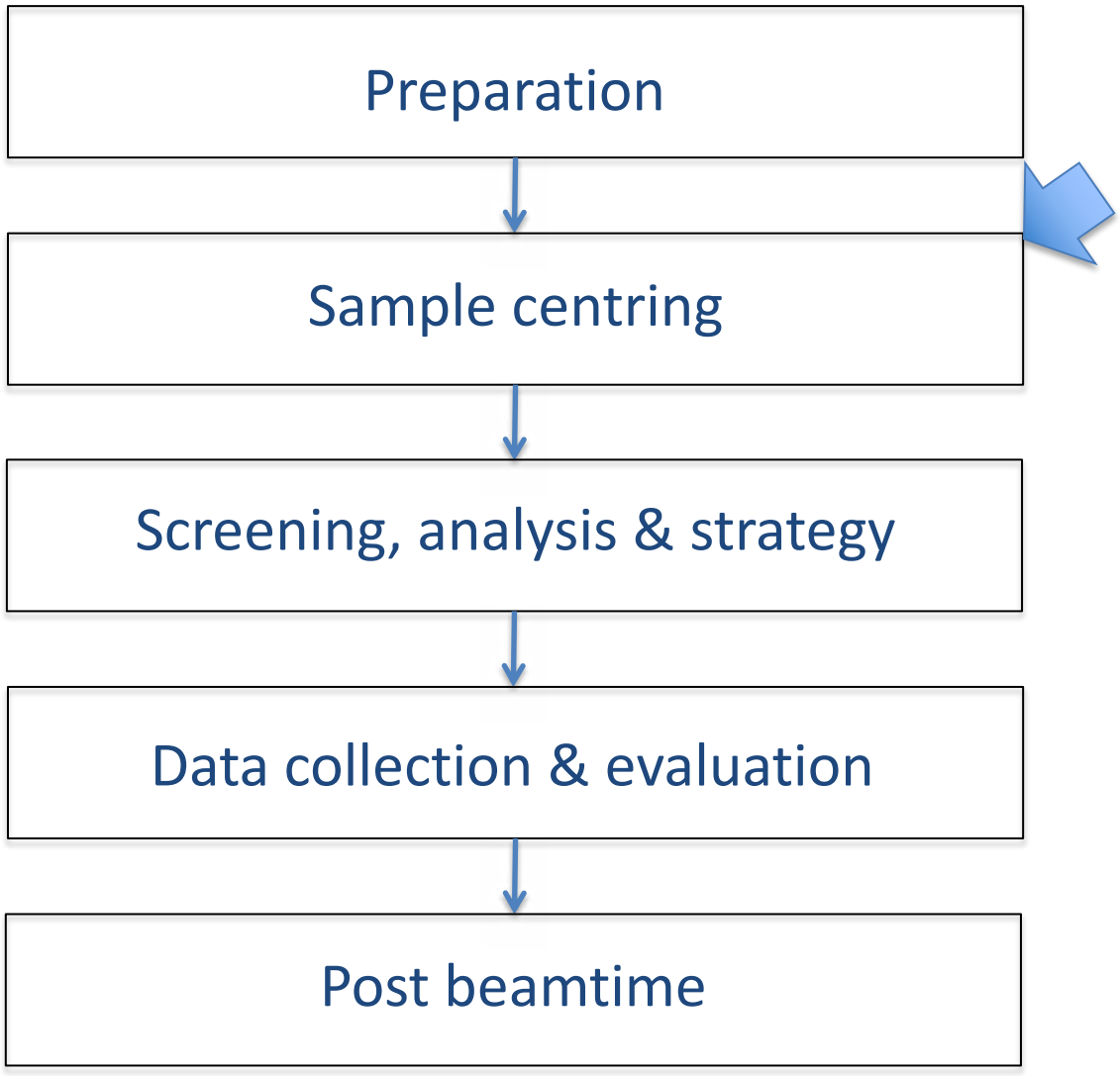
Beam size and flux density

Be mindful of how the beamline generates a small beam size. Focusing (I04 +I24) can increase the flux density massively compared to apertures (I03)

Radiation damage is related to the flux density so small beam sizes on I04/I24 can kill a crystal very quickly.

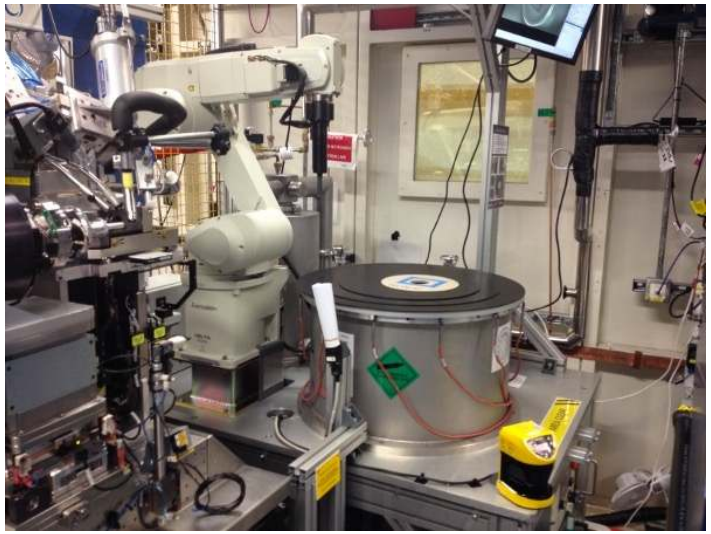
E.g. 50x50 μm^2 beam will deliver around 45x less dose than 9x6 μm^2 beam on I24

So match beam to crystal, but attenuate when required

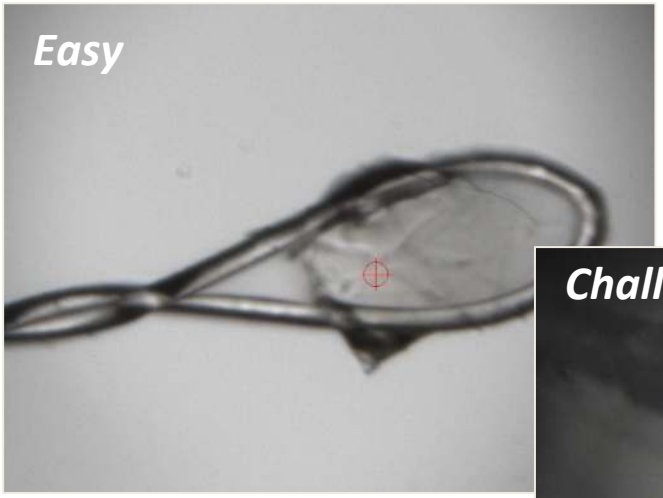

Preparation
Sample centring
Screening, analysis & strategy
Data collection & evaluation
Post beamtime

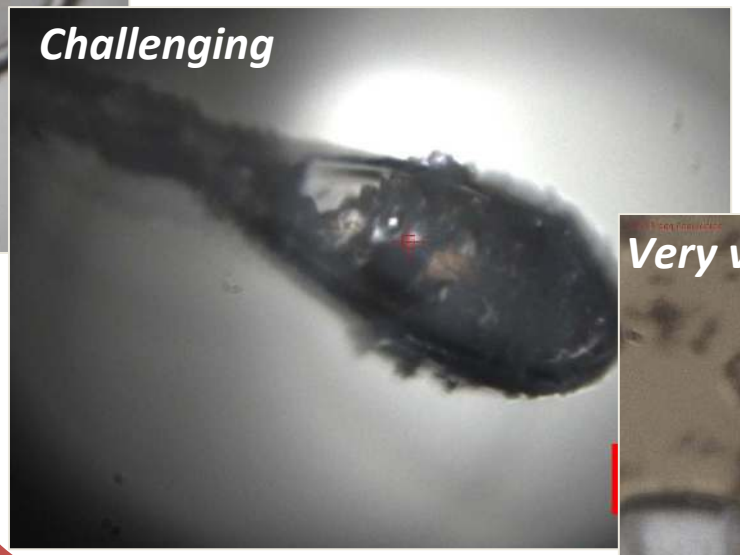
Mount and centre crystal

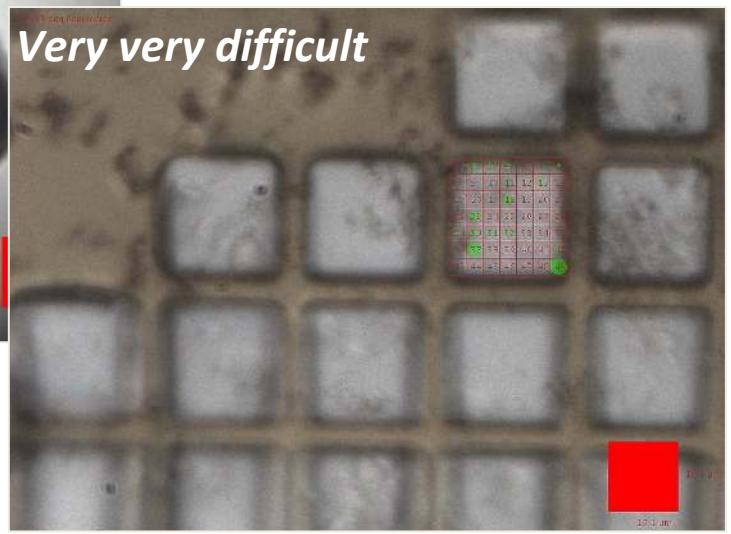
Mounting now automated at most beamlines.



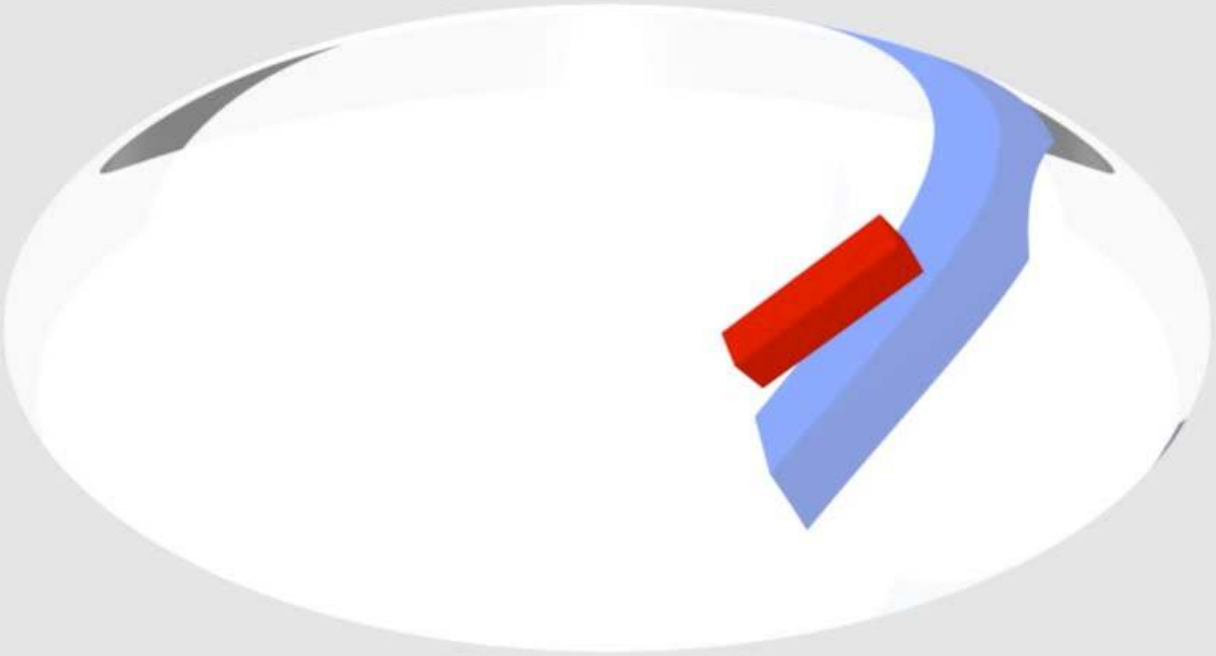
Centring can be:





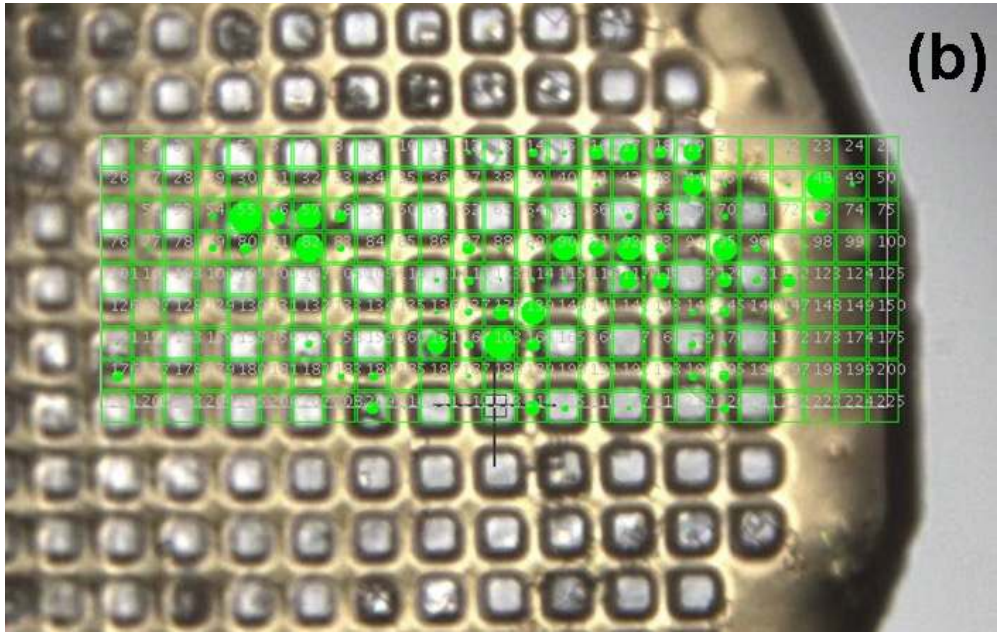


Make the most of tools developed to make these cases easier



Centring in difficult cases

Making this easier: grid scanning



Allows large areas to be quickly rastered over and small or hard to see crystals found.

Also eliminates optical effects: crystal may not be where it seems..

X-ray centring option in GDA

Try to crudely centre first and collect a test shot. Is it worth spending a lot of time on this sample?

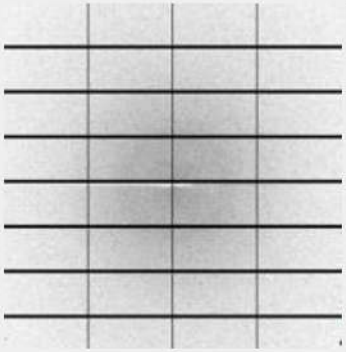
If yes ⇨ Spend more time

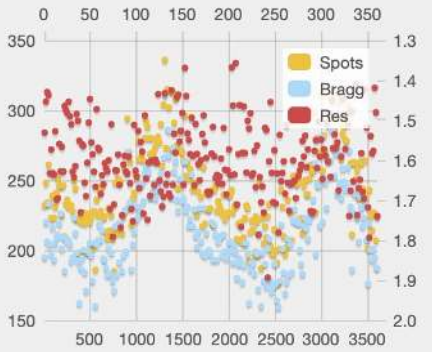
If no ⇨ Move on

If maybe ⇨ Take advantage of the robot: dismount and see what your other samples are like

0 0 28-11-2019 21:05:04 - 20191128/Sethaumatin/Sethau_14/stepped/Sethau_14_1_master.h5

Sample: Sethau_14	Flux: 3.04e+11
Ω Start: 0.0°	Ω Osc: 0.10°
Ω Overlap: 0°	No. Images: 3600
Resolution: 1.33Å	Wavelength: 0.9763Å
Exposure: 0.002s	Transmission: 100.00%
Beamsize: 32x20µm	Type: SAD
Group: 4 Data Collections	
Comment: (34,-126,78) Aperture: Large	

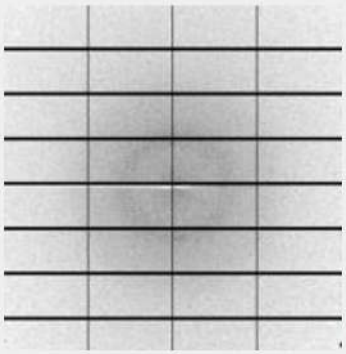


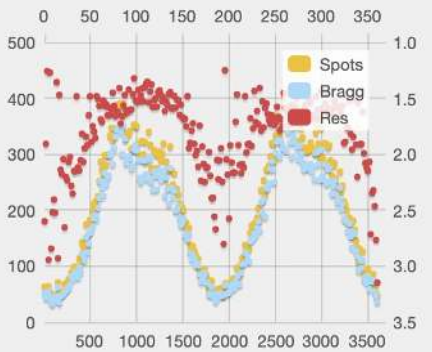


Strategy2 Wedge1 Standard Anomalous Dataset Multiplicity=3 I/sig=2 Maxlifespan=200 s 105 0.05 1.16 1.04 24.9 24.9 0.010 3000

0 0 28-11-2019 20:58:37 - 20191128/Sethaumatin/Sethau_13/stepped/Sethau_13_1_master.h5

Sample: Sethau_13	Flux: 3.06e+11
Ω Start: 0.0°	Ω Osc: 0.10°
Ω Overlap: 0°	No. Images: 3600
Resolution: 1.33Å	Wavelength: 0.9763Å
Exposure: 0.002s	Transmission: 100.00%
Beamsize: 32x20µm	Type: SAD
Group: 4 Data Collections	
Comment: (-1111,-315,146) Aperture: Large	

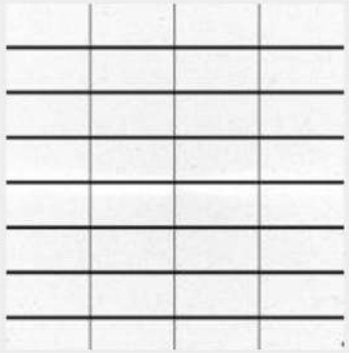


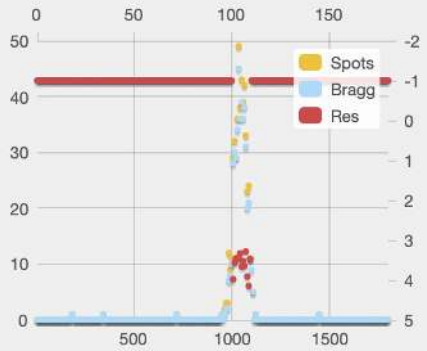


Res 2.0

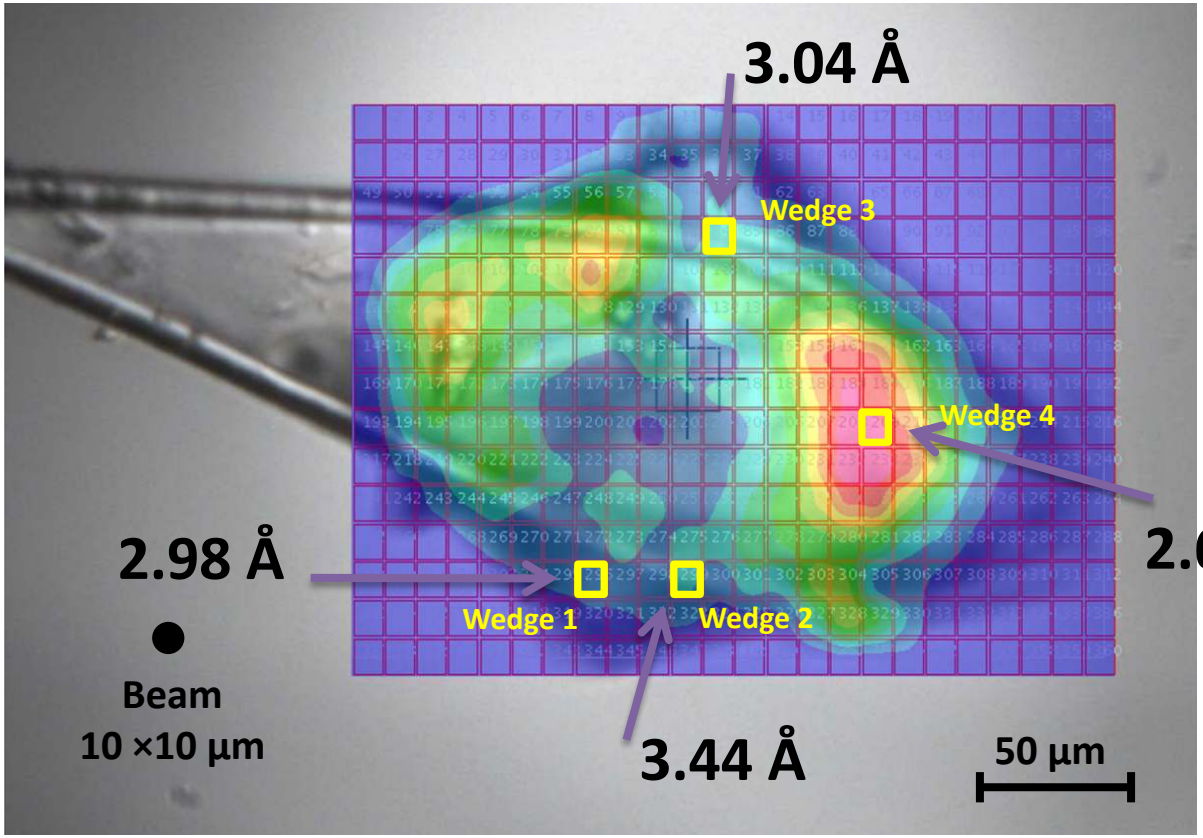
0 0 29-11-2019 09:14:05 - 20191129/Sethaumatin/Sethau_6/Sethau_6_700_1_master.h5

Sample: Sethau_6	Flux: 3.14e+11
Ω Start: 0.0°	Ω Osc: 0.10°
Ω Overlap: 0°	No. Images: 1800
Resolution: 4.32Å	Wavelength: 0.9795Å
Exposure: 0.010s	Transmission: 100.00%
Beamsize: 32x20µm	Type: SAD
Comment: (0,0,0) Aperture: Large	





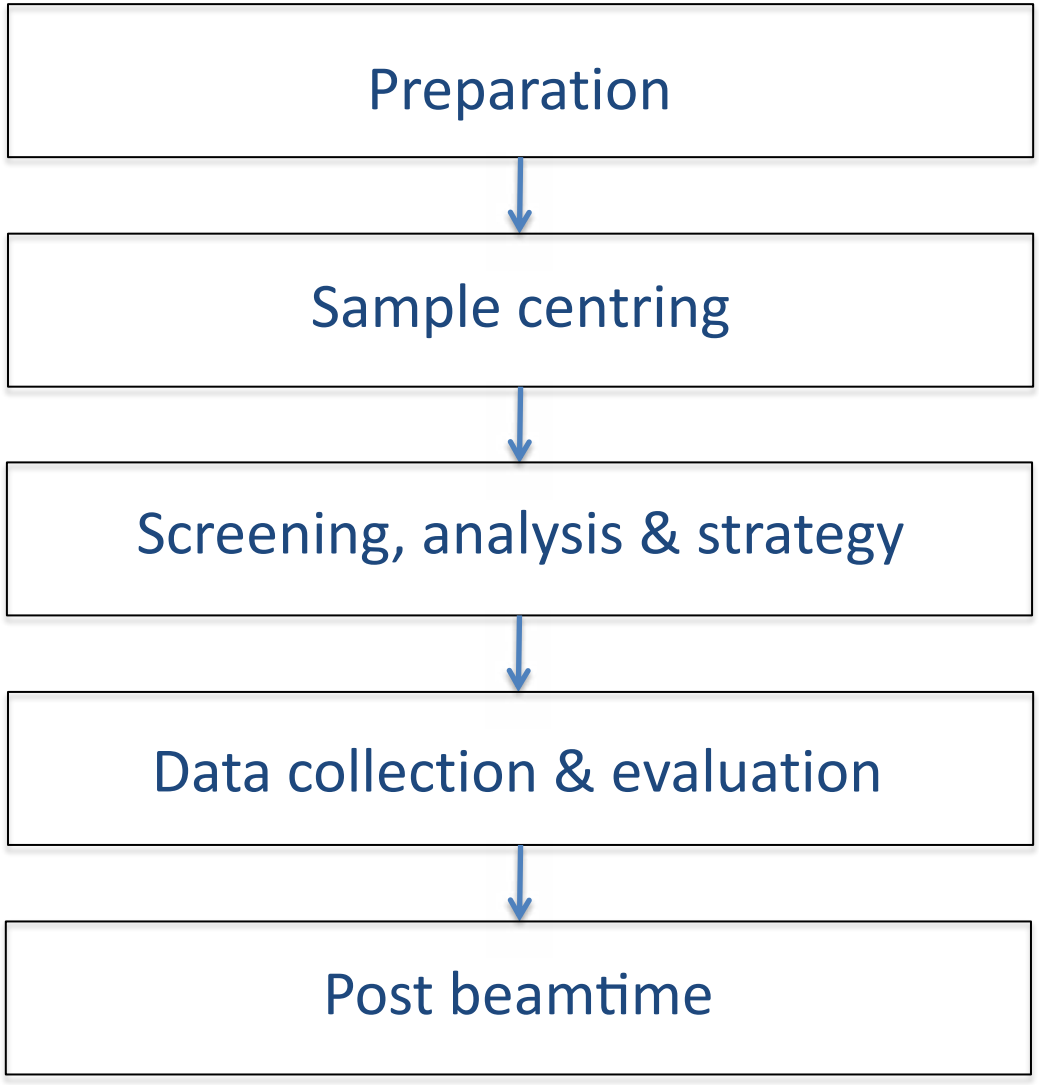
Even the same crystal can show a lot of variation…



Sometimes worthwhile, sometimes not. Learn when to move on.

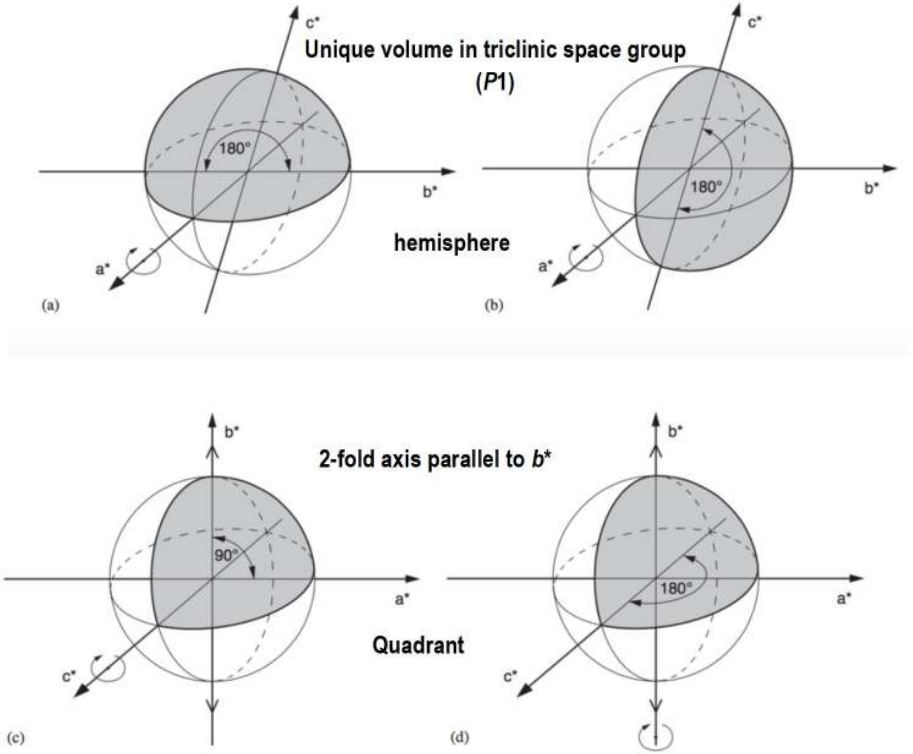
Data collection - think!

- before starting data collection make sure xtal is properly centered and does not "walk" out of beam
- consider exposure time, attenuation, wedge angle, detector distance, wavelength ... before it is too late!


Preparation
Sample centring
Screening, analysis & strategy
Data collection & evaluation
Post beamtime

Completeness - crystal symmetry

- reciprocal space symmetry = crystal symmetry plus
- Friedel's law : I (h k l) = I (-h -k -l)



thus even for P1 only 180º are needed to obtain complete data set of unique reflections - unless there is anomalous scattering

for N-fold symmetry along rotation axis, need 180/N degrees of data to obtain complete data set (assuming Friedel's law holds)

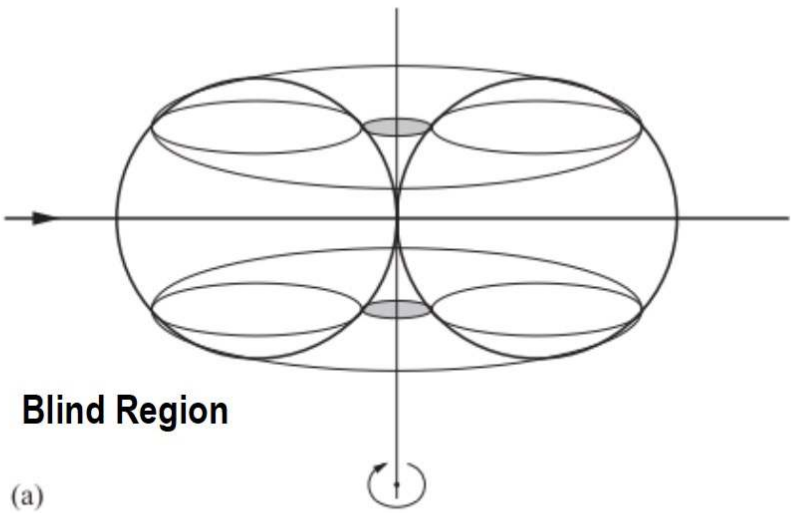
Table 1

Rotation range (°) required to collect a complete data set in different crystal classes.

The direction of the spindle axis is given in parentheses; *ac* means any vector in the *ac* plane.

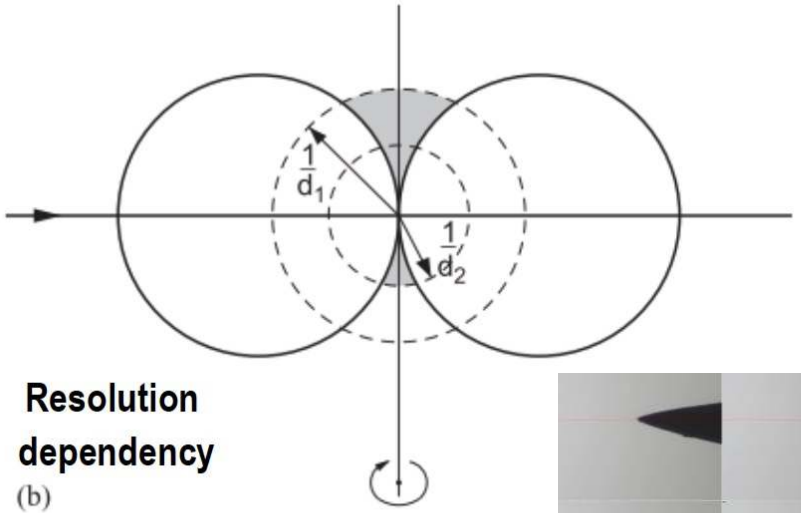
Point group	Native data	Anomalous data
1	180 (any)	$180 + 2\theta_{max}$ (any)
2	180 (*b*); 90 (*ac*)	180 (*b*); $180 + 2\theta_{max}$ (*ac*)
222	90 (*ab* or *ac* or *bc*)	90 (*ab* or *ac* or *bc*)
4	90 (*c* or *ab*)	90 (*c*); $90 + \theta_{max}$ (*ab*)
422	45 (*c*); 90 (*ab*)	45 (*c*); 90 (*ab*)
3	60 (*c*); 90 (*ab*)	$60 + 2\theta_{max}$ (*c*); $90 + \theta_{max}$ (*ab*)
32	30 (*c*); 90 (*ab*)	$30 + \theta_{max}$ (*c*); 90 (*ab*)
6	60 (*c*); 90 (*ab*)	60 (*c*); $90 + \theta_{max}$ (*ab*)
622	30 (*c*); 90 (*ab*)	30 (*c*); 90 (*ab*)
23	~60	~70
432	~35	~45

Dauter, *Acta Cryst.* *D***55,** 1703 (1999)



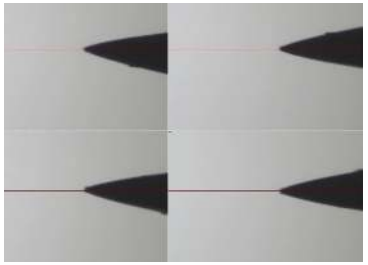
Blind Region

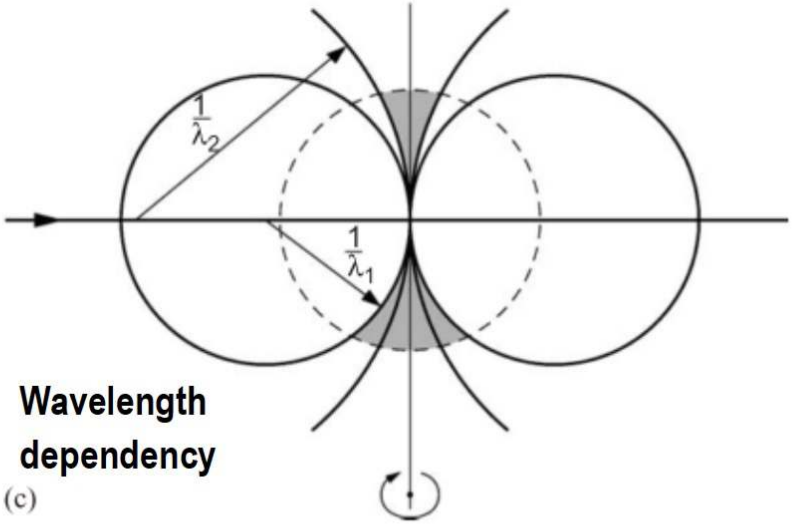
(a)



Resolution dependency

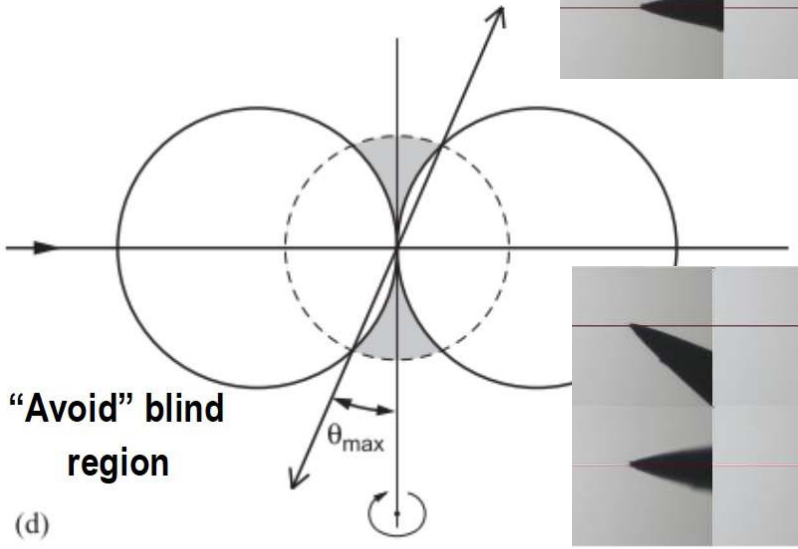
(b)





Wavelength dependency

(c)



"Avoid" blind region

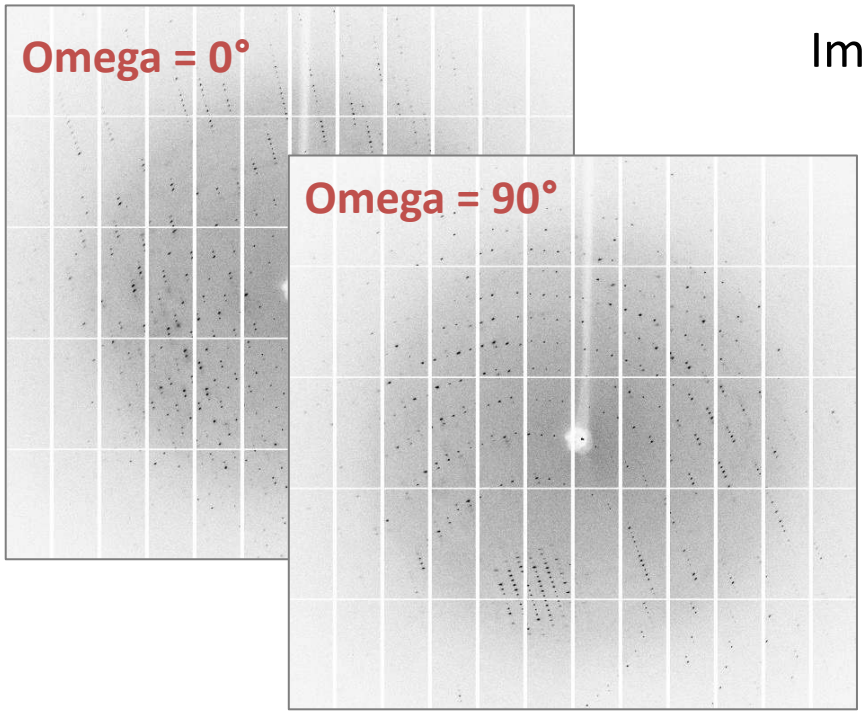
(d)

Dauter, *Prog. Biophys Mol. Bio.* **89,** 153 (2005)

Screening and analysis

Determine crystal diffraction properties, orientation matrix and appropriate settings for data collection

Usually by collecting 3 or 4 images, each separated by 45 degrees but can also efficiently be done by collecting an attenuated small wedge of 20 degrees for example.



Important to check the images:

Spot quality – circular, well spaced
Similar at 0° and 90°?
Resolution appropriate?
Autoindexing successful?

Screening ?

- strategy / crystal assessment prior to dataset?

Depends if you are eager for speed, data quality or unattended data collection.

  - 3 images at 0, 45 and 90 degrees are slow to take (3 arming of the detector) and slow to get results from but are useful particularly for new projects – implemented in GDA
  - A small wedge of data (e.g. 20 degrees) that does not need to be “thrown away” but later merged with the dataset is also a good way – Gives more accurate resolution

- Stepped transmission? (*)

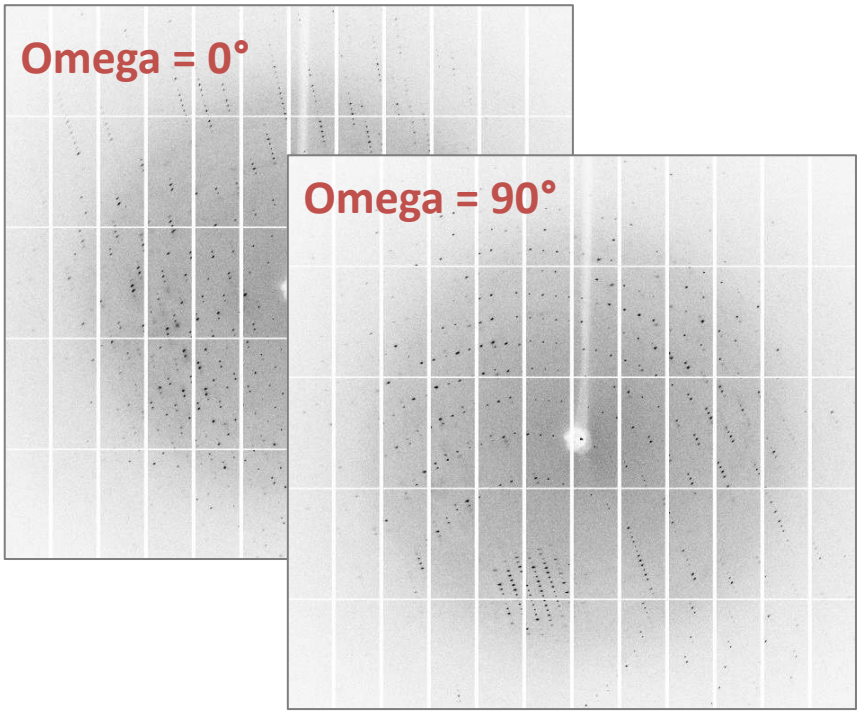
Is a good idea and prune to automation.

  - Does not require any calculations on dose and knowing a priori beam flux, size or crystal composition as it collects several datasets from very conservative doses to higher doses.
  - Does require analysis of which dataset is best, sometimes merging of datasets.
  - Takes a little longer

(*) ”How best to use photons” (2019) Acta Crystallogr D Struct Biol. 1;75:242-261. doi: 10.1107/S2059798319003528

Strategy

Programs such as BEST & Raddose 3D go further than just providing a start angle and oscillation range..



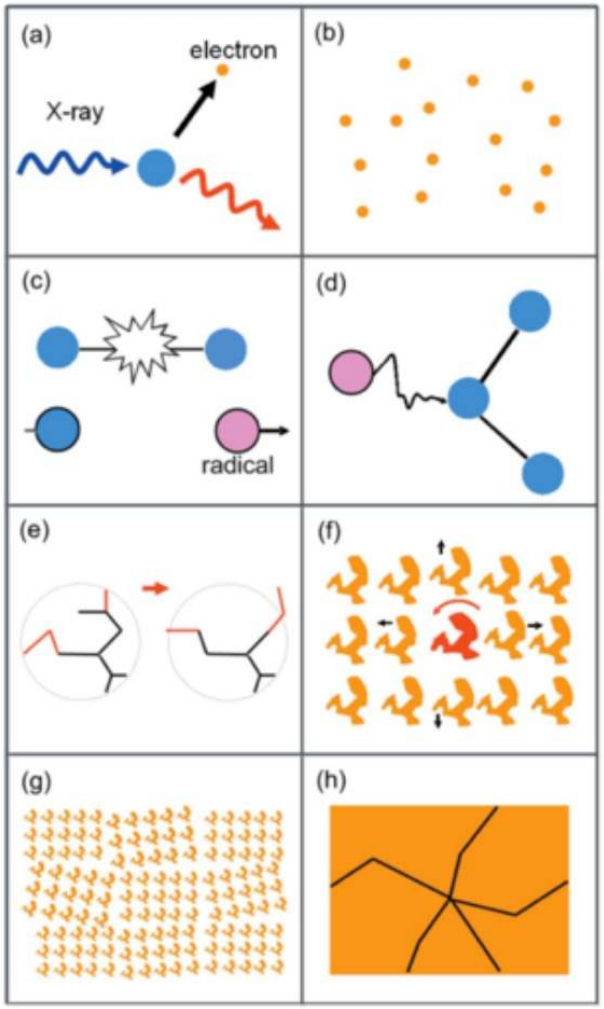
plus

- Estimate of crystal lifetime
- Aim of experiment (*eg* native or anomalous data collection)
- Desired multiplicity

gives

Comprehensive strategy including omega start and end, but also suggested oscillation angle, exposure time and filter transmission and possible a step transmission strategy

BEST: Bourenkov and Popov *Acta Cryst*. (2010). **D66**, 409-419



M. Warkentin *et al. J. Synchrotron Rad.* **20,** 7 (2013)

Room temperature 298 K

(0.1 – 0.5 MGy)

- Owen, *et al. Acta Cryst.* **D68**, 810 (2012)
- Warkentin, *et al. J. Synchrotron Rad*, **20**, 7 (2013)

Cryo-temperature 100 K

Native data collection (20 MGy)

- Henderson, *Proc. R. Soc. B.* **241**, 6 (1990)
- Owen, *et al. Proc. Natl. Acad. Sci. USA*, **103**, 4912 (2006)

Experimental phasing (< 5 MGy)

- Holton, J. M. *J. Synchrotron Rad.* **14**, 51 (2007)
- Olieric, *et al. Acta Cryst.* **D63**, 759 (2007)

Rule of thumb

- Resolution dependency of 10 MGy / Å, Howells *et al. J. El. Spect. & Rel. Phen.* **170**, 4 (2009)
- Does estimation, Holton, *J. Synchrotron Rad.* **16**, 133 (2009)

$$Dose = (t_{expo} \times flux) / (k_{dose} \times I_{H\text{-}beam} \times I_{B\text{-}beam})$$

$$k_{dose} = 2000\lambda^{-2}$$

Radiation damage estimation at 100 K

$Dose = (t_{expo} \times flux) / (k_{dose} \times I_{H\text{-}beam} \times I_{B\text{-}beam})$; $k_{dose} = 2000\lambda^{-2}$ (Gy, sec, photon, μm)

e.g. 12.4 keV (1.0Å), 4×10^{11} photon/sec,

dose-rate =

4×10^{11} p/s / (2000 × **100 μm × 100 μm**) = 0.02 MGy/s

- Another > flux beamline
- Phasing experiment

Single crystal crystallography

4×10^{11}p/s / (2000 × **10 μm × 10 μm**) = 2 MGy/s

10 seconds dataset
360 Hz (3600 images at 0.1 oscillation, 100 transmission)

Micro-crystallography

4×10^{11}p/s / (2000 × **5 μm × 5 μm**) = 8 MGy/s

7.2 seconds dataset
500 Hz (3600 images at 0.1 oscillation, 35% transmission)

Serial crystallography

4×10^{11}p/s / (2000 × **1 μm × 5 μm**) = 40 MGy/s

7.2 seconds dataset
500 Hz (3600 images at 0.1 oscillation, 7% transmission)

Serial crystallography

4×10^{11}p/s / (2000 × **1 μm × 1 μm**) = 200 MGy/s

7.2 seconds dataset
500 Hz (3600 images at 0.1 oscillation, 1.4% trans)

Serial femtosecond crystallography (xFEL)

2020

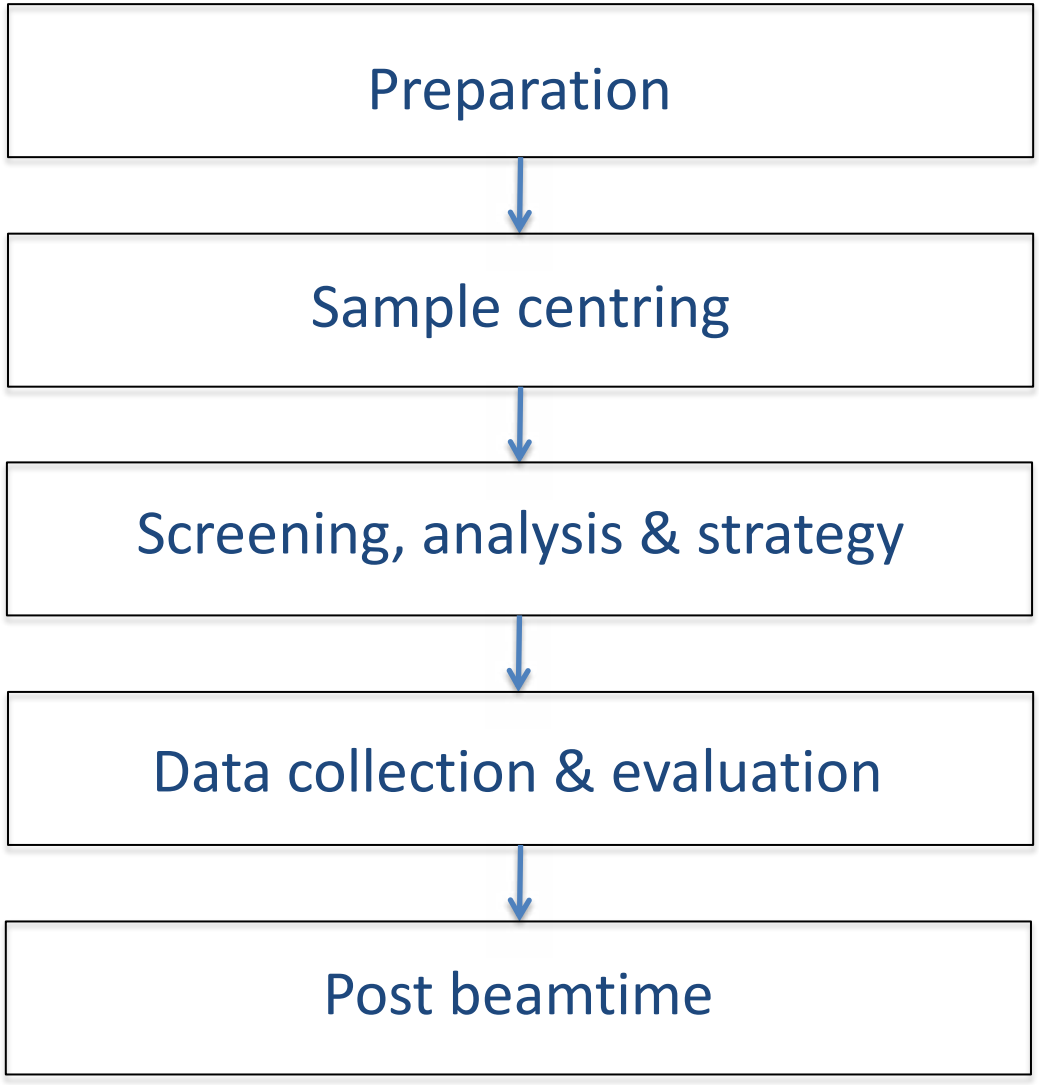
Does estimation, Holton, *J. Synchrotron Rad.* **16**, 133 (2009)

Online calculator: https://bl831.als.lbl.gov/xtallife.html (allows resolution as a factor)

expected crystal lifetime calculator
https://bl831.als.lbl.gov/xtallife.html

Beamline settings	Flux (ph/s)	Beam size (um)	Exposure (s)	Dose (MGy)
A	4.5x10^11	100 x 100	1316	20
B	3.1x10^11	30x 20	80	20
C	3.0x10^12	80x15	19	20
D	3.0x10^12	8x6	1	20
E	3.0x10^12	20x20	6	20

Assumptions:
Beamline energy at 0.97 A
Crystal of the size of the beam
100% transmission
“standard” sample = no heavy metals (e.g. no Se, no As, average amount of S)


Preparation
Sample centring
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Data collection & evaluation
Post beamtime

Decision time

Remember the aim of your experiment – will this sample give you what you need, or get you closer to your goal? If not, move on.

Experimental phasing is the most critical at this point, a trivial case can be made borderline by bad choices. Most common error is overexposing the crystal, next is not collecting enough data.

Remember that low energy = stronger diffraction and faster radiation damage. Transmission of <5% not uncommon. If the edge is very different to the screening energy then consider repeating the screening shots.

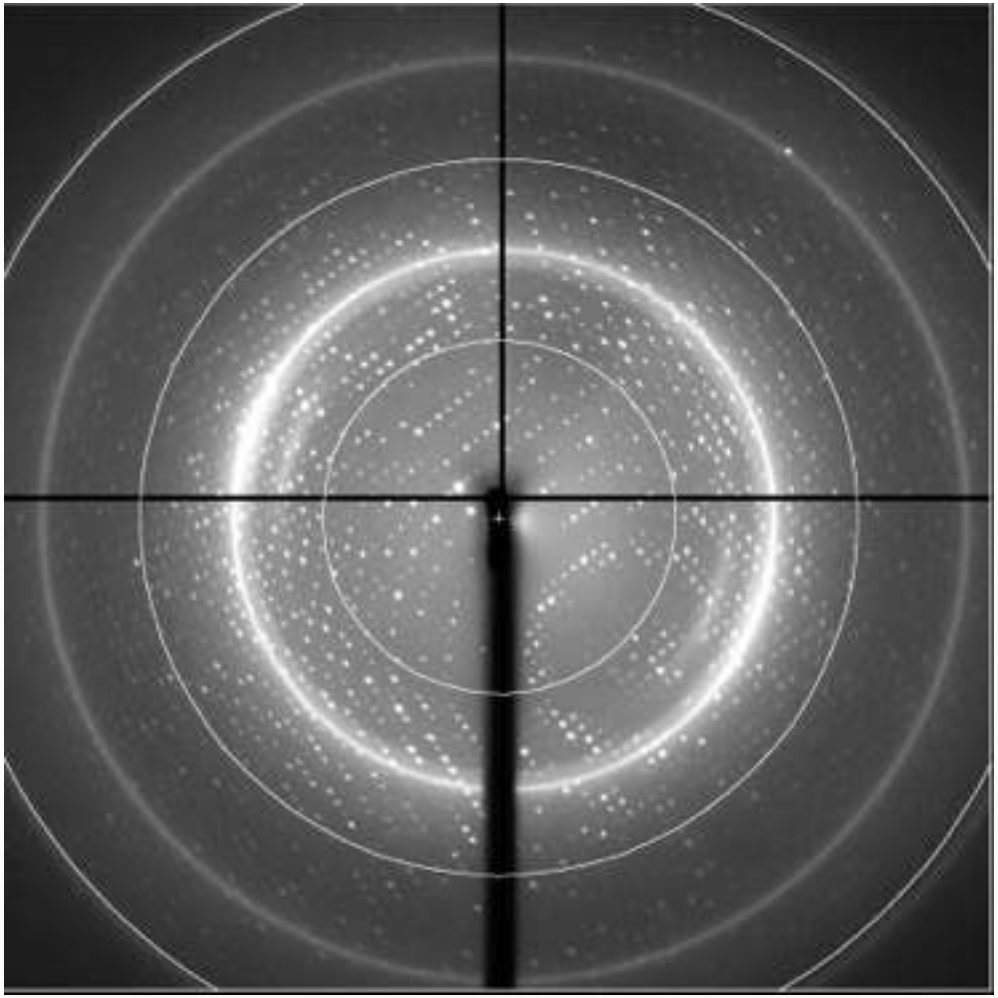
Data collection - the “native” data set

if you have solved your structure and need a “native” data set of the highest possible resolution:

- need complete (> 90%) but not highly redundant data set
- optimise resolution - “fry” as much as possible
- may have to collect multiple “wedges” - STRATEGY to calculate start and end angles to minimise number of images
- or collect datasets from multiple xtals and merge the most isomorphous ones until complete (extreme: XFEL - 1 crystal per image)

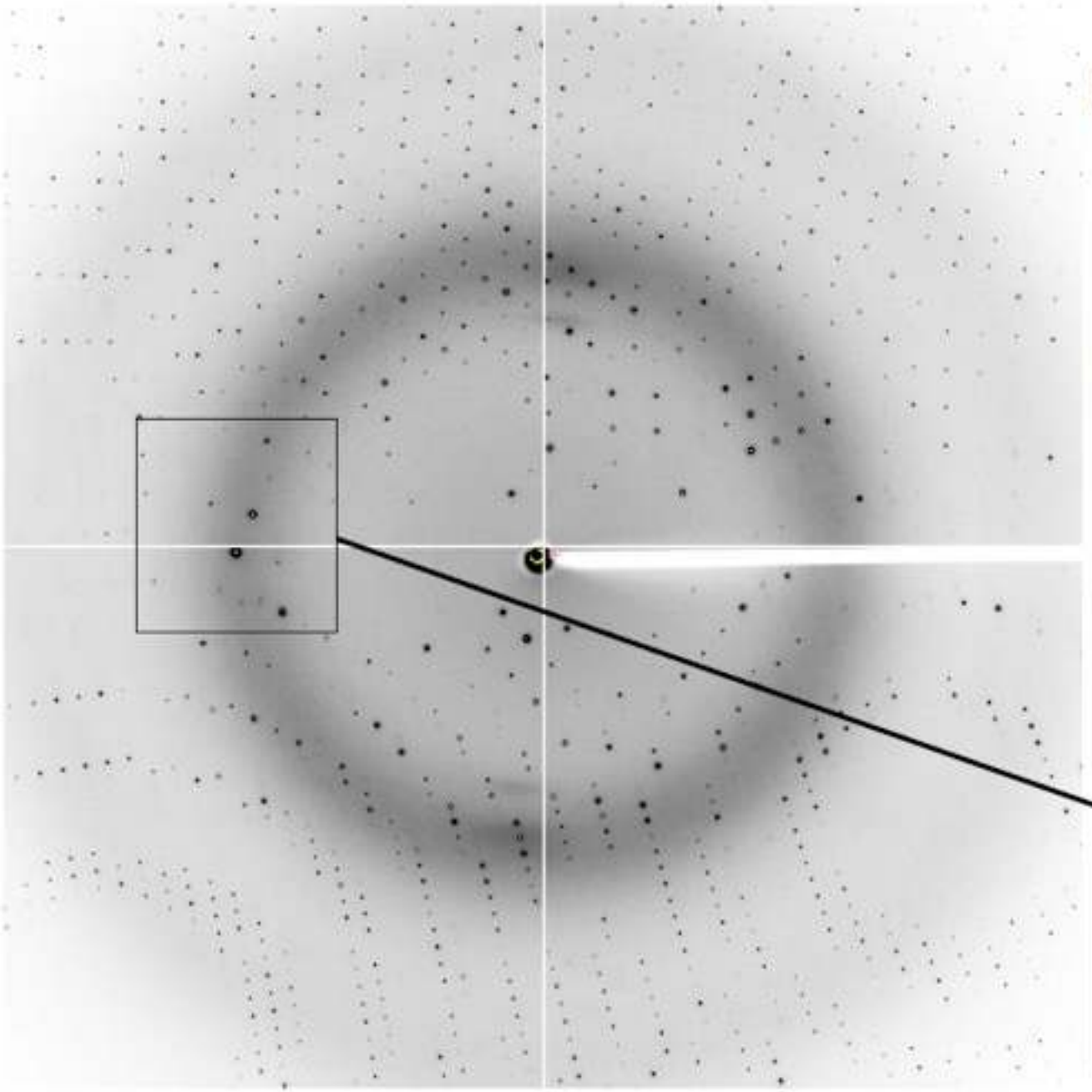
Data collection - molecular replacement

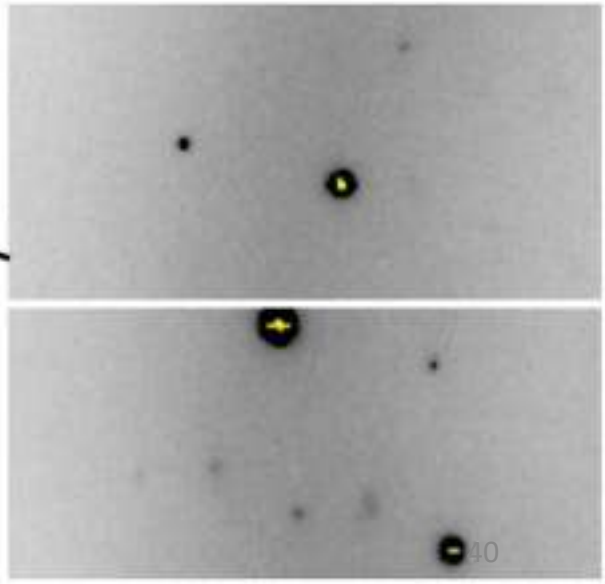
- needs good + complete low resolution data!
- take some time to adjust beam stop - move as far away from xtal as possible without exposing detector to primary beam
- be careful with overloads/count rate correction + spot overlaps
- overall redundancy of data not so important, but as always should be > 90% complete



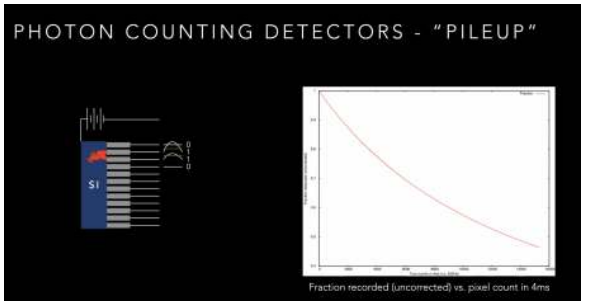
Overloads

Many reflections in central portion of image are overloaded, need to ***adjust exposure time or attenuate beam.*** If we don't then data set will be incomplete at low resolution





Saturated detectors



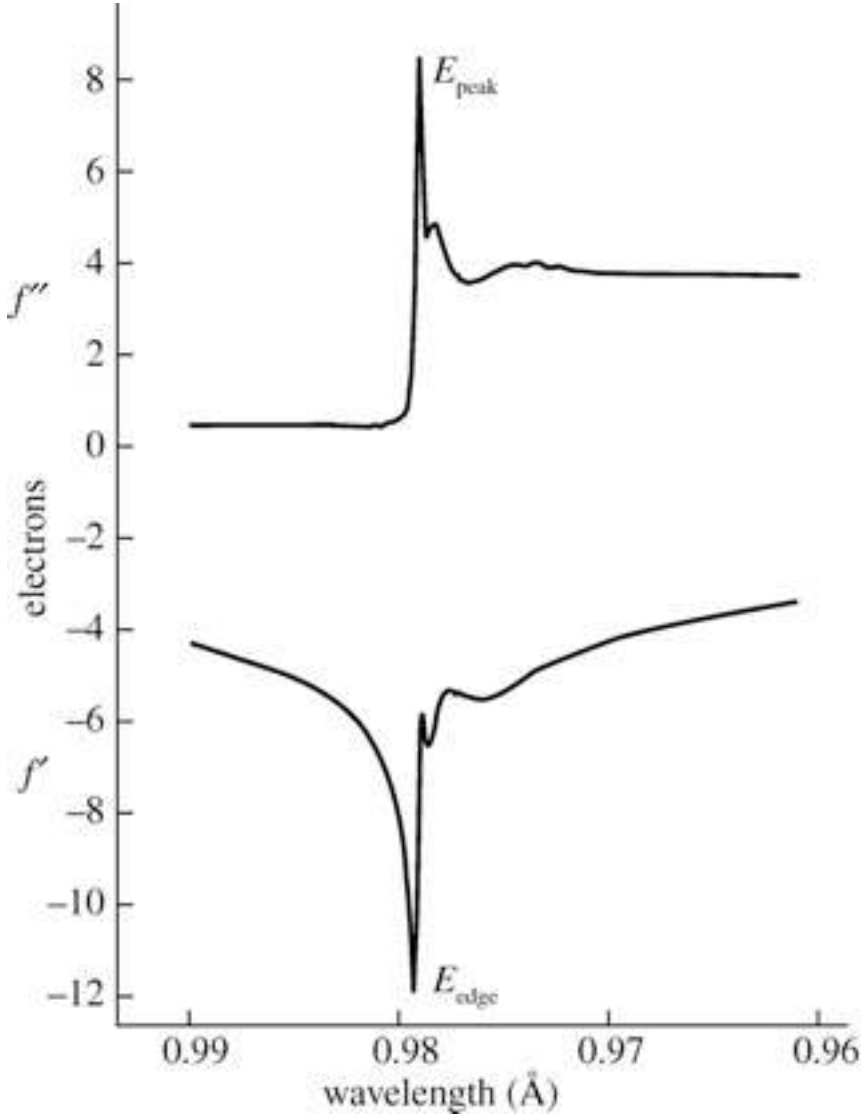
The Pilatus 6M and Eiger 16M have a large dynamic range.

When the dynamic range is exceeded, the counter starts from zero again. The only indication for this is that when extreeme holes may be observed in a few peaks.

I worse issue is also that some highly count rate corrected peaks won't show any visible pathology.

Conclusion: Even if there is no radiation damage one might have had "too much photons on our detector" resulting in damaged data – ATTENTION: Flux dependent not exposure dependent, fine slicing helps but not totally fixes it

Data collection - anomalous diffraction



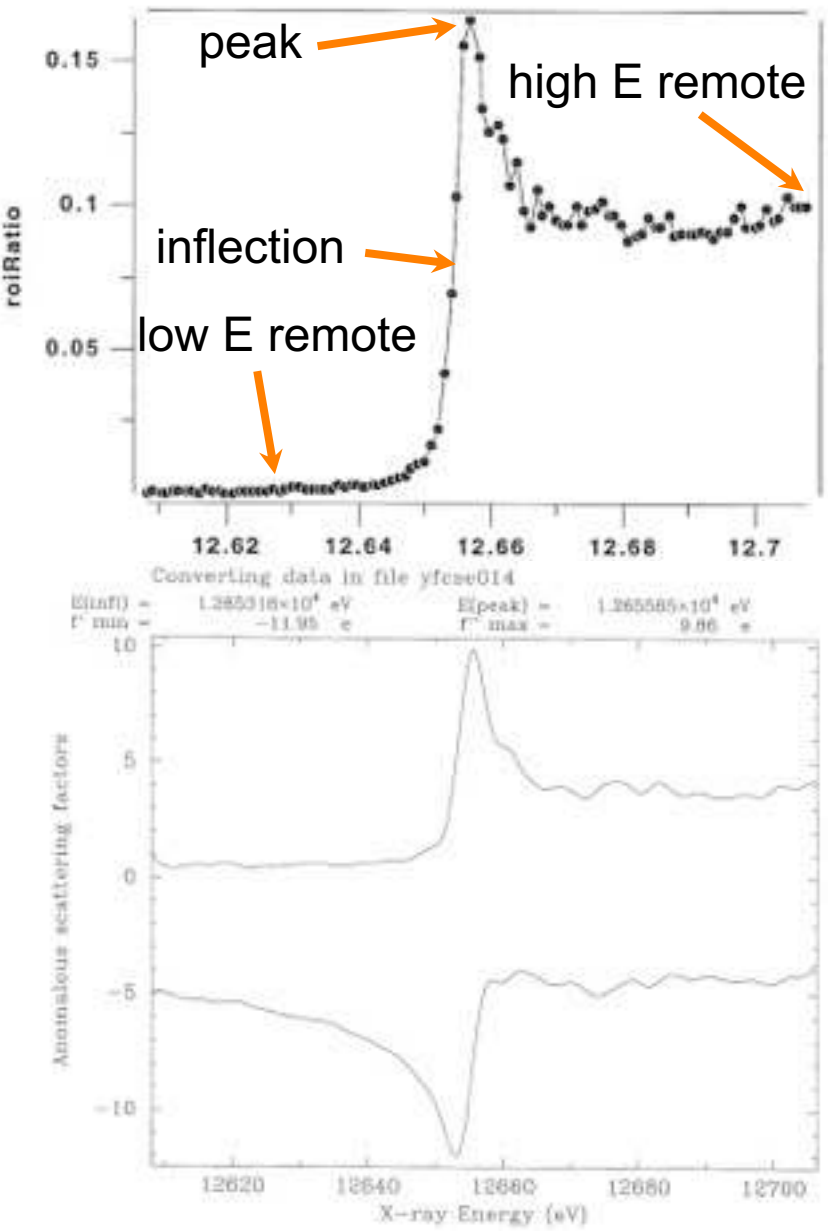
MAD/SAD:

- breaking Friedel's symmetry: I(hkl)≠I(-h-k-l)
  - Friedel pairs become Bijvoet pairs
- detecting anomalous signal is tricky
  - very weak ~ 2 - 4 % of total signal!

Practical considerations:

- especially for SAD: the more **redundancy** (without radiation damage), the better!!!

Data collection - anomalous diffraction



MAD: 3-4 data sets of one crystal at different wavelengths around absorption edge of heavy atom

SAD: 1 data set of 1 crystal at absorption peak of heavy atom (usually Se)

- MX beamlines: automated scans of metal edges
- otherwise see e.g. http://skuld.bmsc.washington.edu/scatter/AS_index.html

Consider effects of changing wavelengths:

f'' – less absorption of X-rays by air at short wavelength / high energy

– radiation damage

– also check max. resolution and spot overlaps after changing wavelength (Bragg's Law: nl=2dsinth)!

f'

- Please calculate Dose (e.g. Raddose3D)

Should I use the same recipe for any energy?

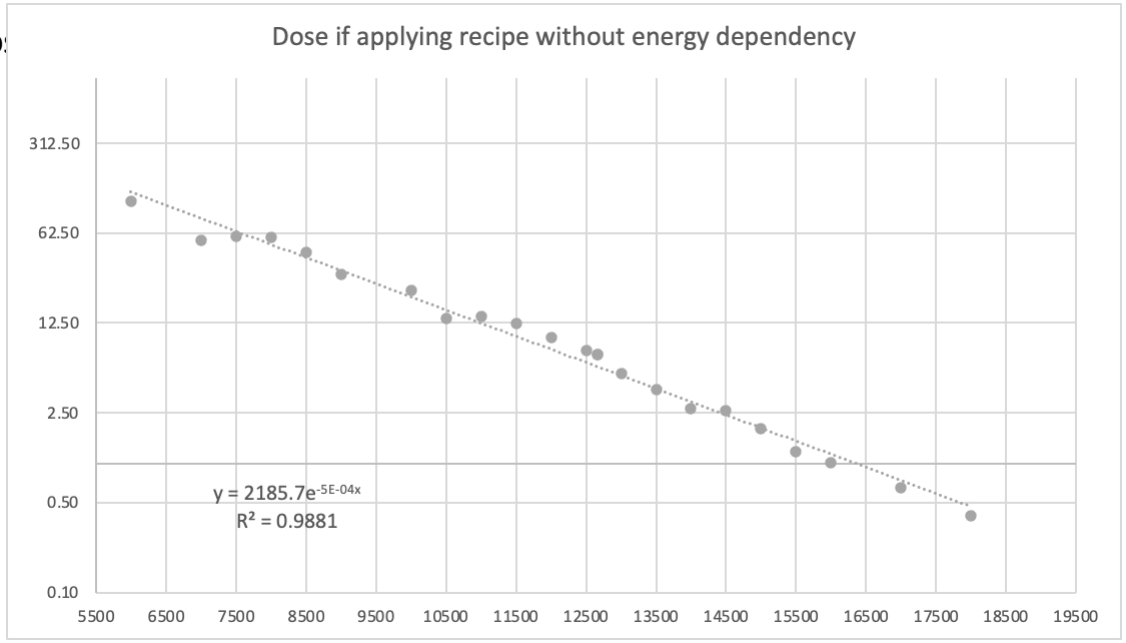
- 36 total exposure (two datasets of 18 seconds)
- 0.005 s/frame
- 7200 frames (2x 3600)
- Beam ~30x20 microns
- Energy: varying
- Same sample (for calculation assuming lysozyme but could be standard protein without any metals or special scatterer).

I04 Dose (*) vs Energy profile

(fixed exposure)

Set point 20 µm vertical

Log Do
(Mgy)



energy	Wavelegth	beamsize.F	n_lenses		Dose if applying recipe without energy dependenc
6000	2.070313	[28.6, 17.4]	9		109.76
7000.1	1.774528	[34.0, 21.9]	12		54.74
7500	1.65625	[29.7, 18.4]	14		59.44
8000.1	1.552715	[28.6, 17.4]	16		57.66
8500.1	1.46138	[29.5, 18.2]	18		44.46
9000	1.380208	[31.8, 20.1]	20		29.90
10000.1	1.242175	[28.6, 17.4]	25		22.23
10500	1.183036	[34.0, 21.9]	27		13.57
11000	1.129261	[30.8, 19.2]	30		14.04
11500.1	1.080154	[29.1, 17.8]	33		12.33
12000.1	1.035148	[28.6, 17.4]	36		9.71
12500	0.99375	[29.0, 17.7]	39		7.57
12658	0.981346	[28.9, 17.7]	40		7.14
13000	0.955529	[30.1, 18.7]	42		5.07
13500.1	0.920132	[31.8, 20.1]	45		3.79
14000	0.887277	[34.0, 21.9]	48		2.70
14500	0.856681	[31.4, 19.7]	52		2.58
15000	0.828125	[29.7, 18.4]	56		1.90
15500.1	0.801406	[33.3, 21.3]	59		1.25
16000.1	0.776362	[32.7, 20.8]	63		1.02
16999.9	0.730703	[33.1, 21.2]	71		0.65
18000.1	0.6901	[31.8, 20.1]	80		0.40

(Outliers removed)

110 MGy

Energy
(eV)

0.4 MGy

110X

(*) as calculated with raddose using current i04 recipe (2x 0.005s*3600frames = 2x 18 seconds = 36 seconds exposure) and a few assumptions (E.g. xtal size = beam size, standard protein composition etc)

Trickier cases

Low solvent content, low resolution, low anomalous signal cases may require more than 1 dataset or even crystal to solve.

Low solvent content usually requires MAD phasing, 2 or 3 energies

From a data collection point of view you have options here:

- Collect all on same part of crystal, interleaved. Chief concern is radiation damage.
- Collect each energy on a separate part of the same crystal, small beam and large crystal.
- Collect data from several crystals and combine (isomorphous?).

Practical rules

These seem to work well on a majority of cases

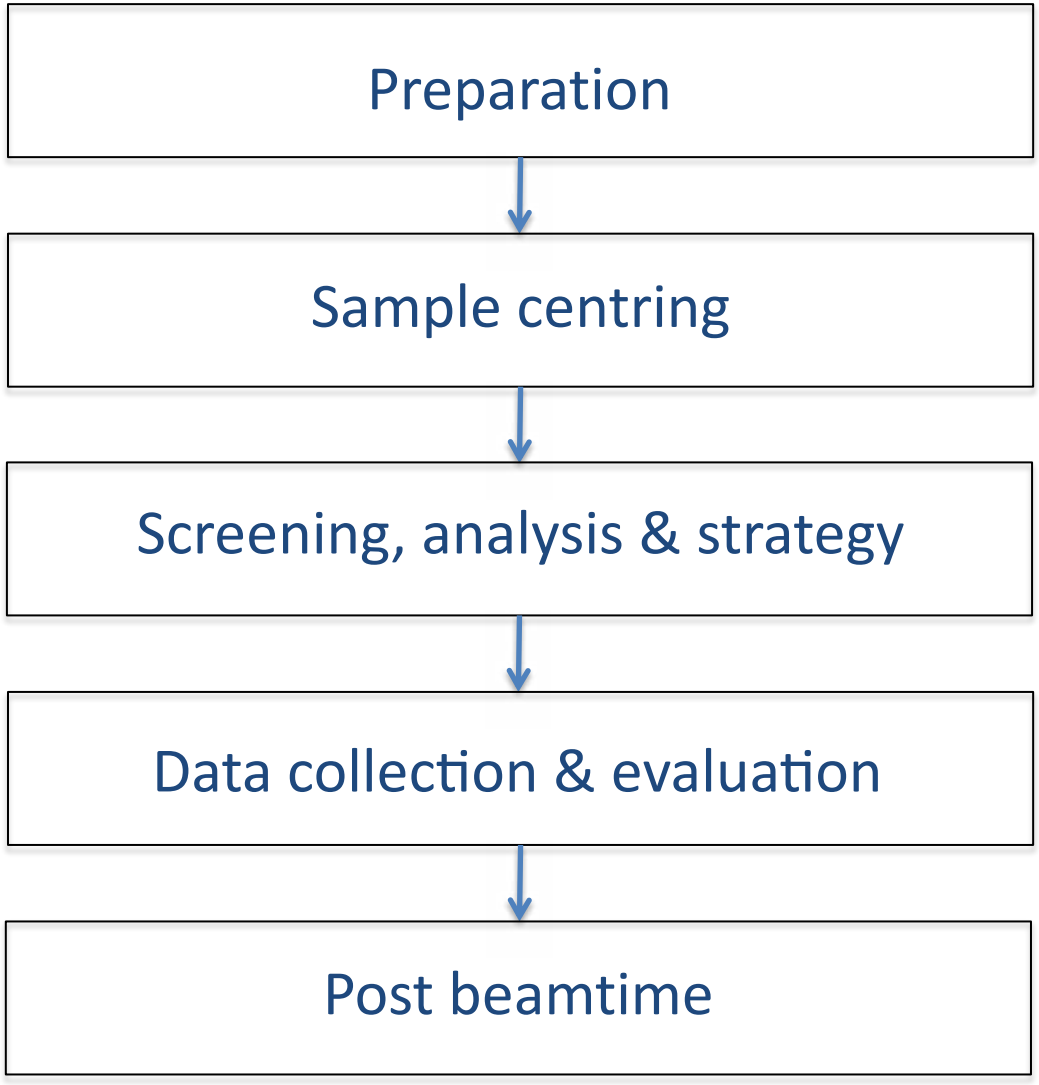
- Default total angle: 360 ° per dataset (*)
- Default oscillation: 0.1° for all datasets
- Use total exposure for dataset. For example for a beamline with flux and beamsize of I04 (***)
  - MR → 20 MGy (greedier on Max resolution), 10 MGy or 5MGy (greedier on time per dataset)
  - SAD, MAD → 5 MGy (***) in reasonable time
  - Low anomalous signal SAD, MAD (e.g. S, P etc) → Spread the dose through more images

Issues:

- Needs to index and as you drop flux this can be harder but trust that indexing happens in 3D so if you need to rationalise then merge images to 1° using ADXV, Albula, Dials and do not look at fine sliced images.

(*) even for high symmetry space groups but if possible get best starting angle from strategy. Also consider collecting datasets with different Chi/Phi if goniometer allows

(***) Don't think in terms of exposure per frame and think in total dose you want to load the crystal with. That directly translates to a number of seconds full beam for each particular beamline / crystal composition, size and maximum resolution you need to obtain . Estimate this number using the crude online calculator (https://bl831.als.lbl.gov/xtallife.html) or even better the online Raddose 3D interface (http://www.raddo.se/rd3d/prepare.php)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Post beamtime

Ideally you'd want to confirm on the beamline that your structure is solved and gives you what you need. Doesn't always happen.

Check structure solution pipelines, FastEP, Longer phasing pipelines (AutoSharp, Crank2, etc), Dimple, MrBump (*)

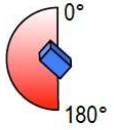
Post beamtime is your chance to take your time and do things carefully. Reprocessing, exclusion of radiation damage, data combination etc. Don't assume that autoprocessing failure means your data is useless.

How to do this? That's what you'll learn through the rest of this course ☺

(*) Heads up that Diamond does not run EP pipelines if you don't define a heavy atom on your pucks/pins & won't run MR pipelines if you don't give a PDB (code or coordinates)

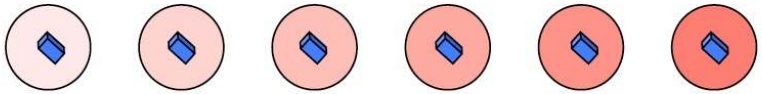
Traditional high-dose coarse-phi slicing strategy

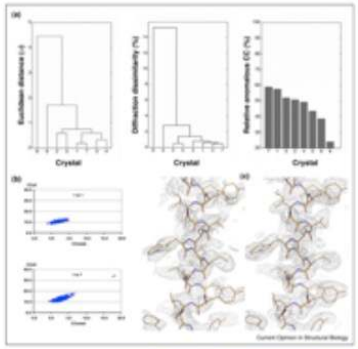
- 0.5-1° per image,180°, 4-fold redundancy, and 10 MGy
- CCD detector

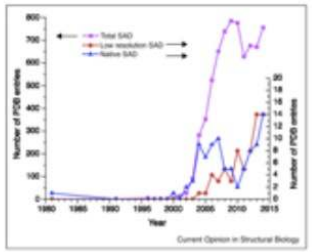


low dose, fine-phi slicing, high redundancy strategy

- ½ mosaicity slicing, *n* × 360° Mueller, *et al*, *Acta Cryst*. **D68**, 42 (2012)
- Distribute x-ray dose to multiple data sets. Liu *et al. Acta Cryst.* **A67**, 544, (2011)
- PILATUS detector





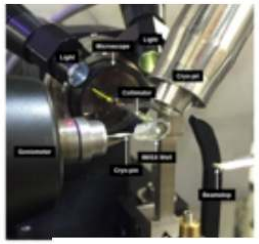


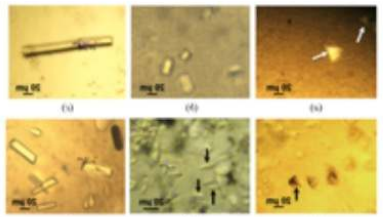
Multi-orientation and multi-crystal strategy

- Distribute x-ray dose to multiple data sets and multiple crystals
- Liu & Hendrickson *Curr. Opin. Struc. Bio.* **34**, 99 (2015)
- Weinert *et al. Nature Methods* **12**, 131, (2015)

Micro-crystallography

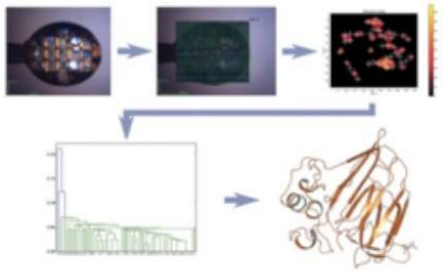
- 10-20° data per crystal × *n* crystals
- Smith *et al. Curr. Opin. Struc. Bio.* **22**, 602 (2012)





Serial crystallography

- Injector method. Botha *et al. Acta Cryst.* **D71**, 397 (2015), Nogly et al IUCrJ, **2**, 168 (2015)
- *In-situ* method. Huang *et al. Acta Cryst.* **D71**, 1238 (2015), **D72**, 93 (2016)
- Scanning method. Gati *et al. IUCrJ*, **1**, 87 (2014), Zander *et al.*, *Acta Cryst.* **D71**, 2328 (2015)



References on dose choice

10 MGy/A resolution:

Howells MR, Beetz T, Chapman HN, Cui C, Holton JM, Jacobsen CJ, Kirz J, Lima E, Marchesini S, Miao H, Sayre D, Shapiro DA, Spence JHC & Starodub D (2009*J. Electron Spectrosc. Relat. Phenom.* **170**, 4-12.

1% non-isomorphism per MGy:

Banumathi S, Zwart PH, Ramagopal UA, Dauter M & Dauter Z (2004) *Acta Cryst. D* **60**, 1085-1093.

200 kGy for Room Temperature:

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Barker AI, Southworth-Davies RJ, Paithankar KS, Carmichael I & Garman EF (2009*J. Sync. Rad.* **16**, 205-216.

Blake CCF & Phillips DC (1962)., pp. 183-191. Vienna: IAEA.

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Holton JM (2007*J. Synch. Rad.* **14**, 51-72.

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Ramagopal UA, Dauter Z, Thirumuruhan R, Fedorov E & Almo SC (2005).", *Acta Cryst. D* **61**, 1289-1298.

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Murray JW & Garman EF (2002*J. Sync. Rad.* **9**, 347-354.

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rough rotisserie factor:

Holton JM (2009)."A beginner's guide to radiation damage", *J. Synch. Rad.* **16**, 133-142.

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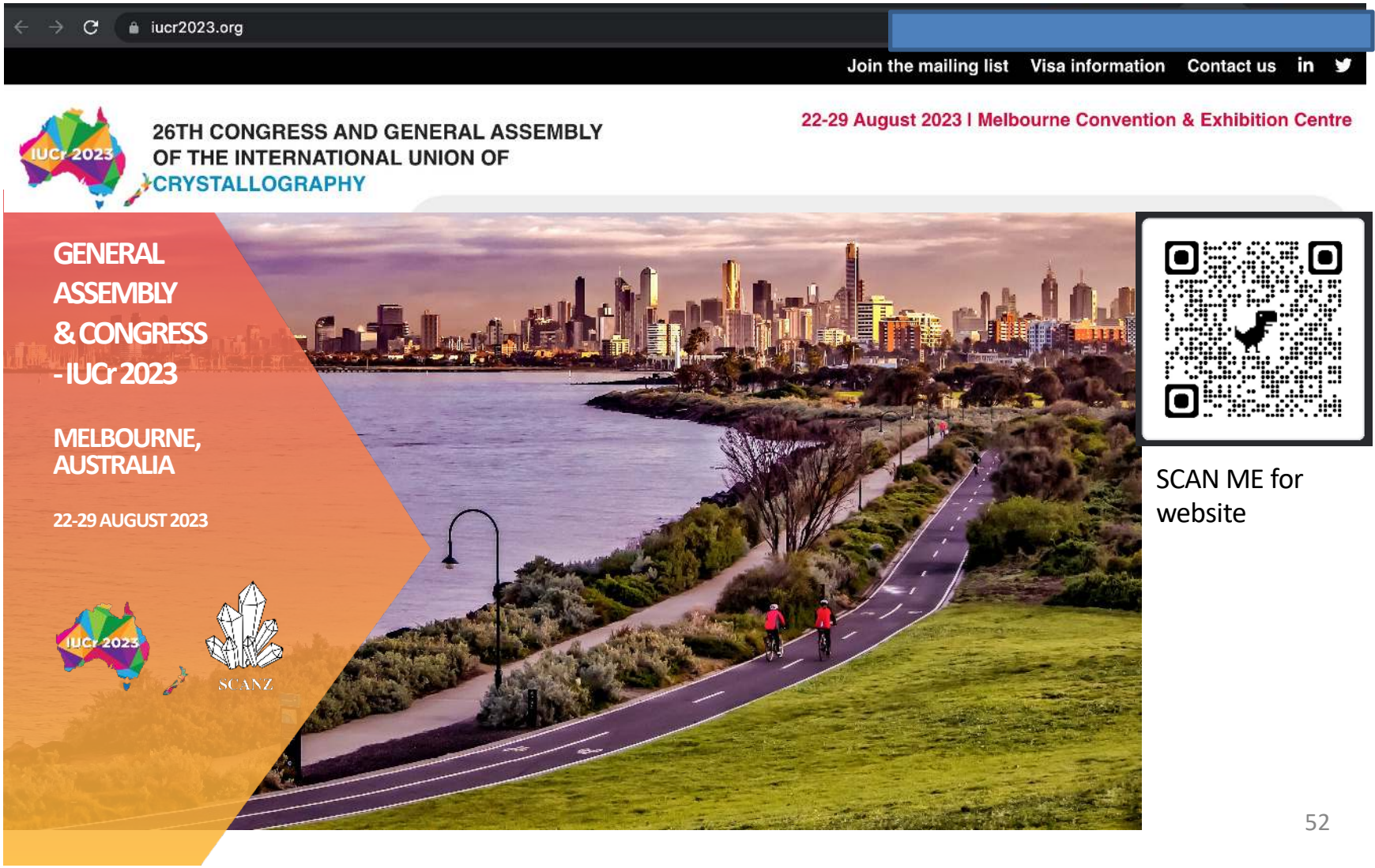

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