

Designing an X-ray experiment

Gwyndaf Evans

Diamond Light Source
Harwell Science and Innovation Campus
Didcot OX11 0DE

Gwyndaf.Evans@diamond.ac.uk



Contents

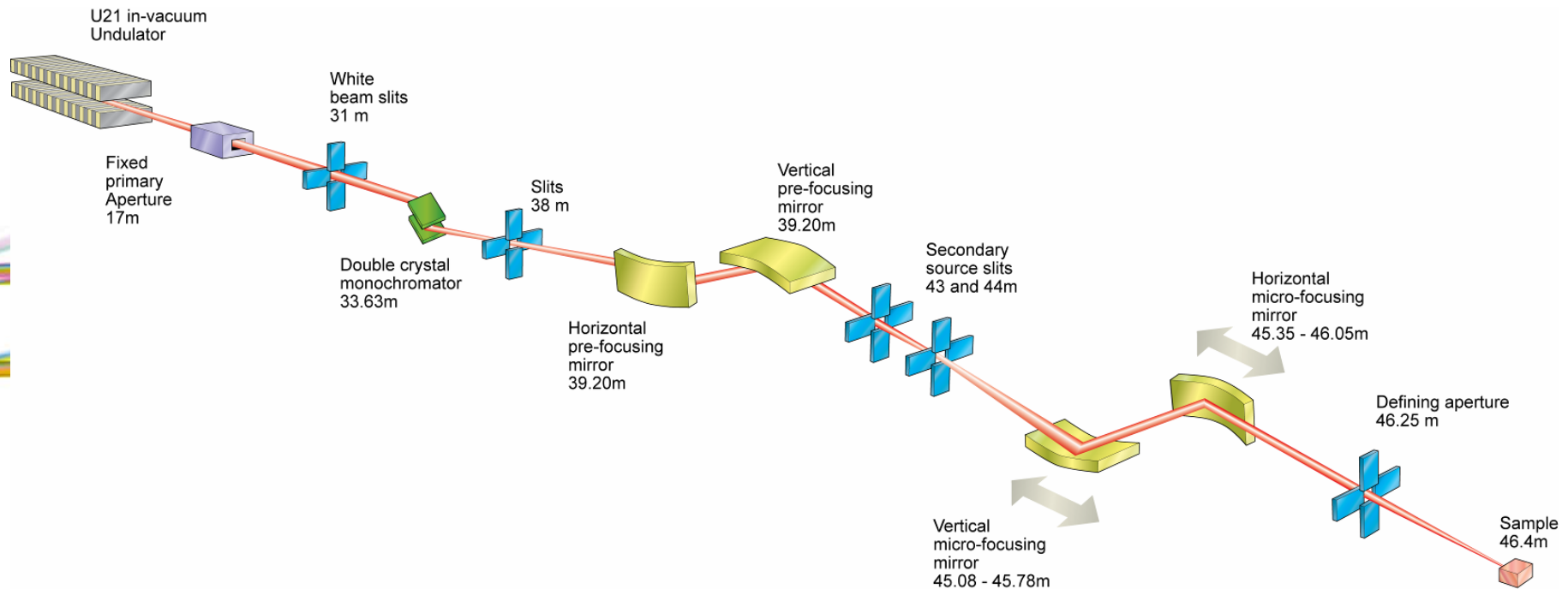
- choose you weapon
 - beamline variety
- good data
- how the beamline and sample interact to affect data quality
- tools that are available at beamlines to assist in making your measurements
- small beam vs. large beam
- brief word about MAD and SAD strategies

Diamond MX beamline suite

Beamline	I02/3/4	I04-1 (Apr 2010)	I24	I23 (2012/13)
	MAD	fixed- λ	MAD μ -focus	long- λ
λ -range (Å)	0.5 – 2.5	0.916	0.5 – 1.9	~2.0 – 4.0
Beamsize (μm)	30 – 200 (v×h)	50 × 50	< 5 – 100 (v×h)	~50 – 200 (v×h)
Flux (ph/s)	10 ¹²	10 ¹¹	10 ¹²	TBD
Automation	Rigaku ACTOR & OSCAR (I03)	Irelec CATS	Irelec CATS	TBD
Target area	Multipurpose / S(M)AD phasing	Ligand binding studies/MR/SAD	Membrane proteins / Multiprotein complexes	native protein phasing, MAD, SAD
Notes	I03 is CL3 compatible	CATS sample changers currently being commissioned		

Beamline anatomy

- Monochromators to select X-ray energy
- Mirrors focus source to sample in horizontal and/or vertical plane
- Beam conditioning to attenuate, shape and size the beam while cleaning up scatter around main beam region



Define the experiment

- Single crystal diffraction experiment
 - characterize samples
 - structure solution
 - extend resolution
 - identify a bound ligand
 - test new apparatus or new methods
- Objectives are
 - answer the questions you are asking
 - to measure the best data to enable you to answer these question

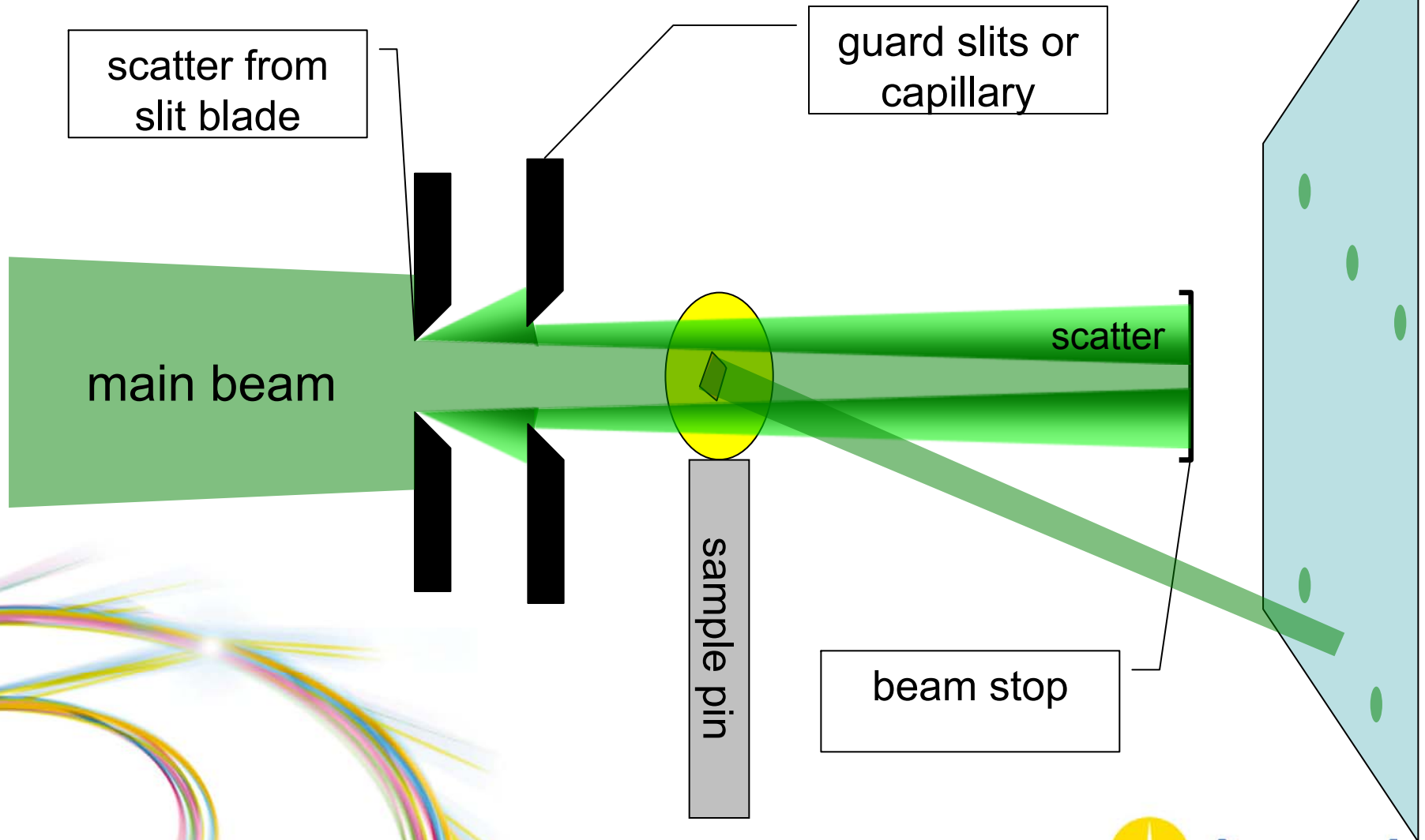
What is good data?

- the best signal to noise possible
 - no single number can be suggested because this is very case dependent
 - usually dictated by phasing signal requirements and resolution needs
- extends down to low resolution ($> 30\text{\AA}$)
 - always good practice and useful for effective solvent flattening and molecular replacement
- as complete as possible
 - you might compromise on this in some cases e.g. ligand detection
- free of radiation damage
 - you want the structure to be the same at the start and end of data collection if possible
- answers your scientific question
 - data for structure solution might be of very different quality than that required to identify a bound substrate

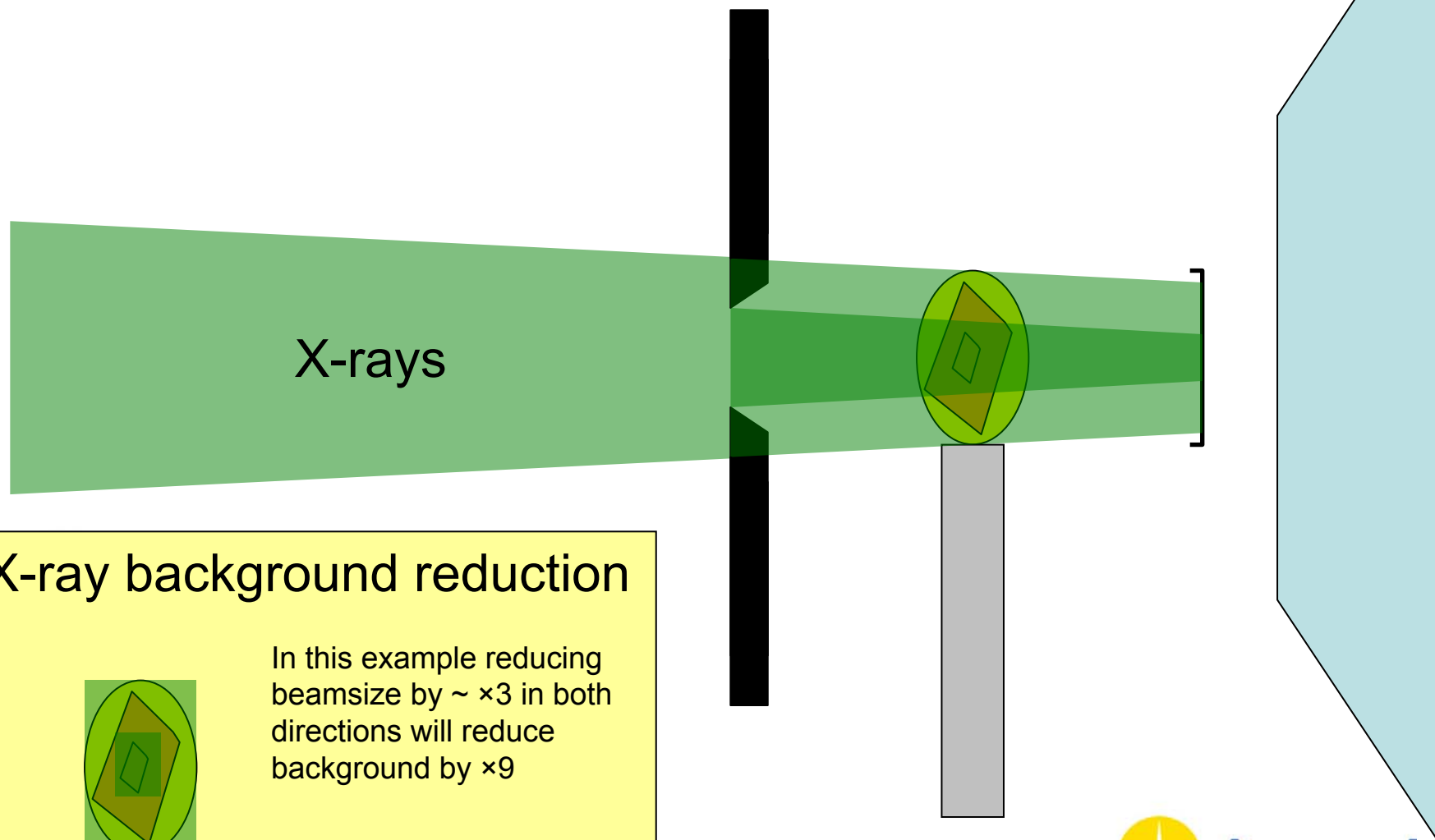
Signal to noise

- we want to measure signal
 - signal comes from our crystal diffraction
- we do not want noise or errors
 - random noise arises from (amongst other things)
 - detector errors
 - beamline problems: high frequency flux variation
 - sample loop vibration
 - X-ray background from non-crystalline material
 - X-ray background from air or N₂ cryostream
 - systematic errors arises from
 - sample and air absorption
 - radiation damage
 - beamline problems: low frequency flux variation

sample environment geometry



Beam size and shape at sample

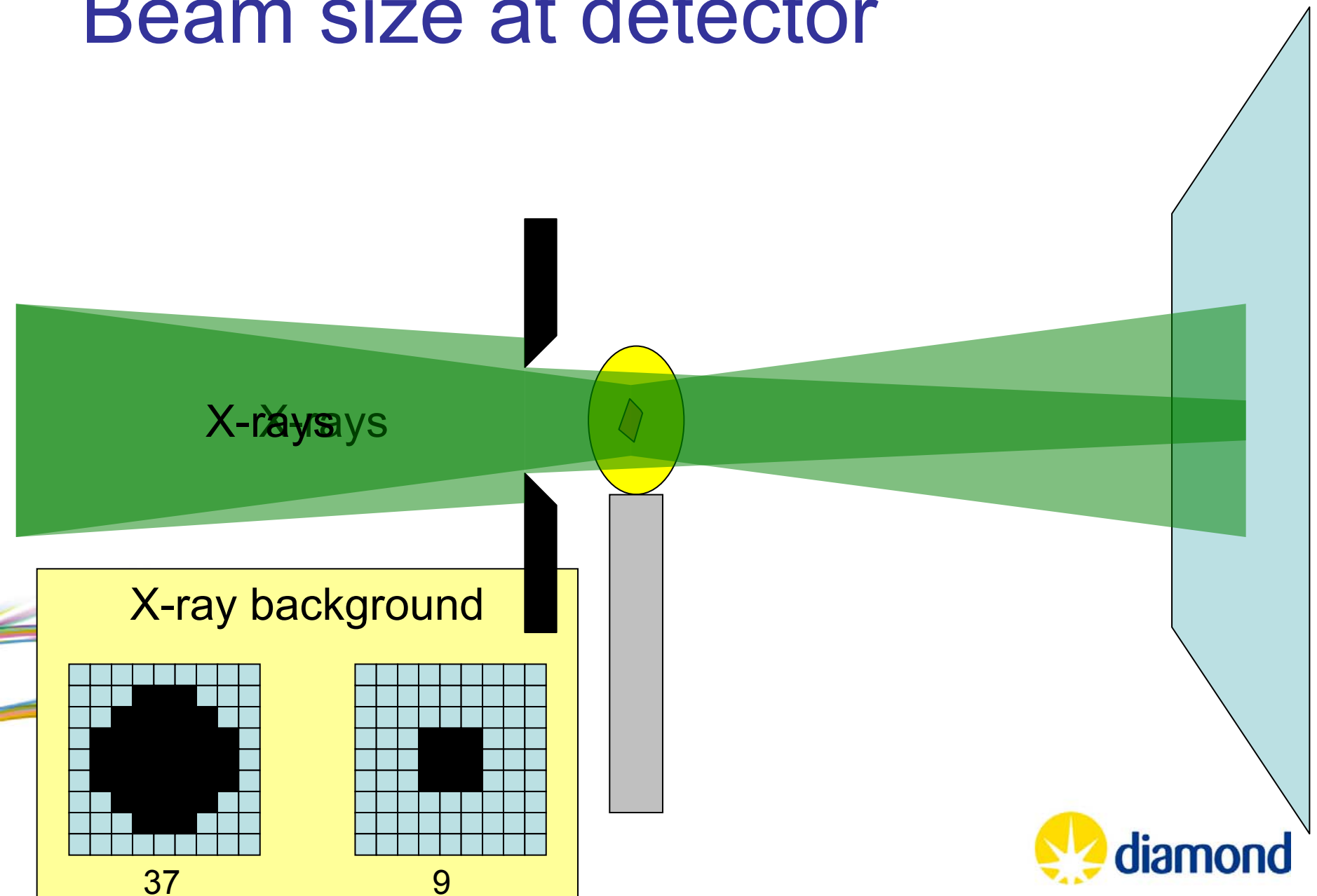


X-ray background reduction



In this example reducing beamsize by $\sim \times 3$ in both directions will reduce background by $\times 9$

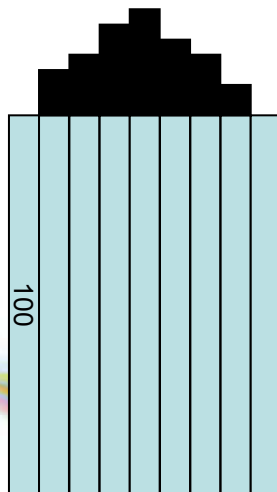
Beam size at detector



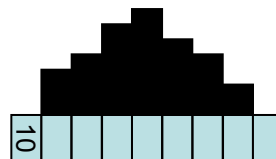
Effect of background and spot size on signal to noise ratio

e.g. $I_s = 150$; 2D spot with 9×9 pixels

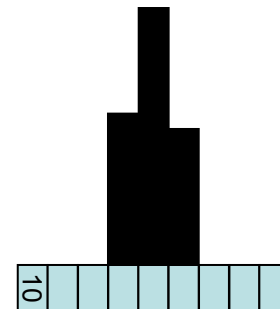
$I/\sigma = 1.3$



$I/\sigma = 4.0$



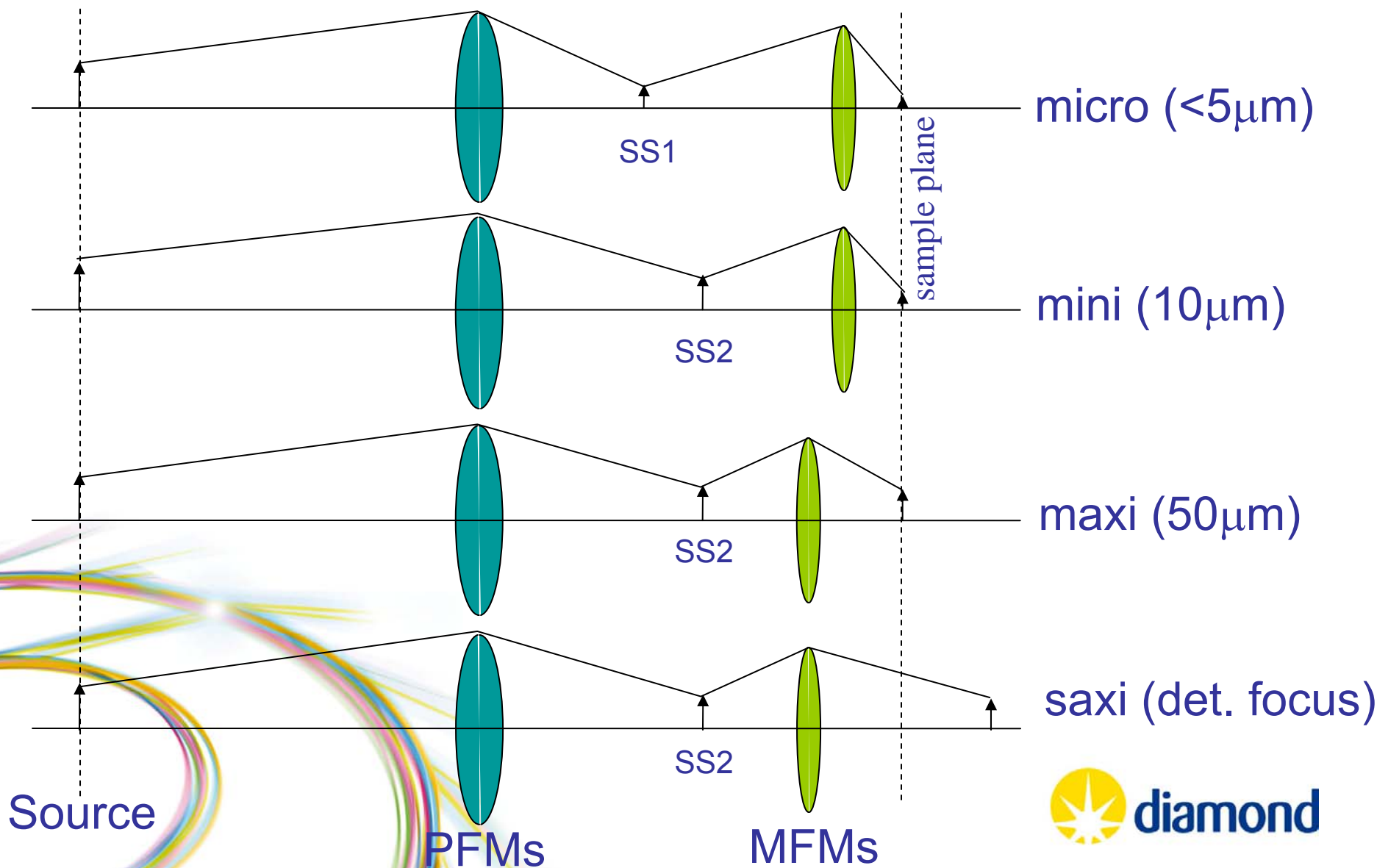
$I/\sigma = 9.5$



What does this mean in practice

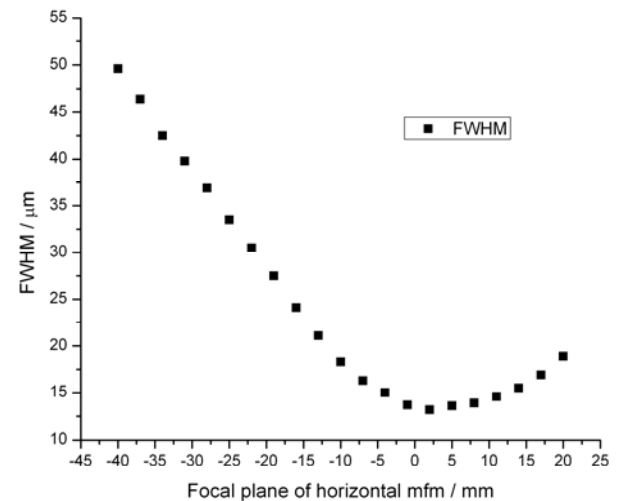
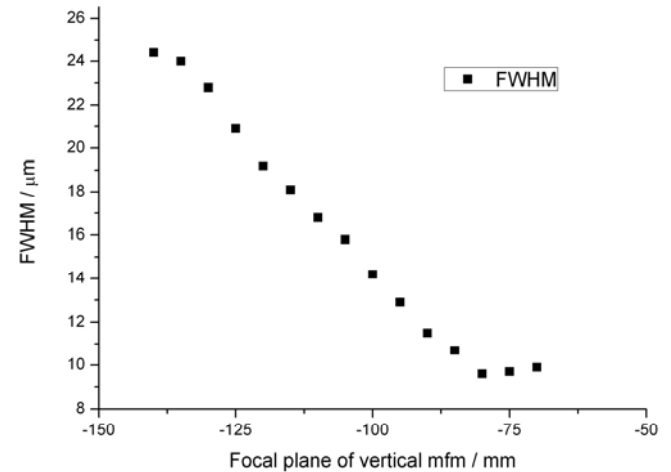
- On standard MAD beamlines
 - I02, 3 and 4
 - Slits settings should be adjusted
 - low divergence means that beam size is essentially constant between sample and detector
- On microfocus beamlines
 - I24
 - relatively high divergence means defocusing X-rays can play a role in signal to noise

I24 optical configuration pre-sets



I24 variable beam size

- Adjustable within 5 mins
 - vertically 10 – 25 μm
 - horizontally 10 – 50 μm
 - 1×10^{12} ph/s
- Microbeam
 - $6 \times 6 \mu\text{m}$
 - 5×10^{12} ph/s
- More configurations will be added over coming year



Sample loop vibration

- This has been observed by groups at SLS, ESRF, APS and elsewhere

Journal of
Applied
Crystallography
ISSN 0021-8898

Received 21 April 2008
Accepted 8 October 2008

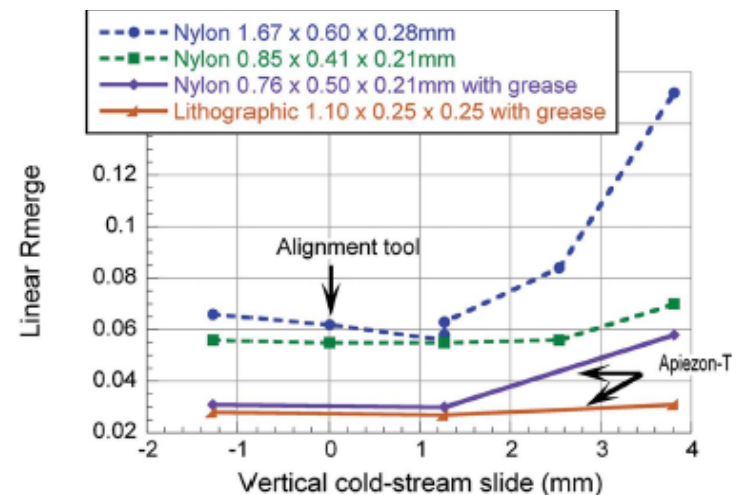
J. Appl. Cryst. (2008). **41**, 1122–1133

Assuming that the beamline optics and the X-ray source are performing within specifications and the sample is of good quality, processing statistics can be used to look for evidence of loop motion. Loop stems should always be kept as short as possible to reduce motion and, when practical, reinforced with epoxy or grease to increase rigidity.

Is your cold-stream working for you or against you? An in-depth look at temperature and sample motion

R. W. Alkire,* N. E. C. Duke and F. J. Rotella

Structural Biology Center, Argonne National Laboratory, USA. Correspondence e-mail: alkire@anl.gov



Optimising signal to noise

- Sample preparation
 - use a short and stiff loop or other mount
 - minimize the amount of solvent/cryoprotectant around your crystal
 - careful choice of loop can help
- All beamlines have defining slits before the sample
 - use the slits to size the beam to your crystal and avoid generating background from your sample mount and solvent
 - if you can change the beamstop distance then consider putting it as close to the sample as tolerable (think about your low resolution data though)
- If the option exists then consider X-ray focusing at the detector
 - e.g. Diamond I24

Measuring complete data

- Strategies

Acta Cryst. (1999). D55, 1703–1717

research papers

Acta Crystallographica Section D

**Biological
Crystallography**

ISSN 0907-4449

Data-collection strategies

Zbigniew Dauter

National Cancer Institute, Frederick and Brookhaven National Laboratory, Building 725A-X9, Upton, NY 11973, USA

Correspondence e-mail: dauter@bnl.gov

The optimal strategy for collecting X-ray diffraction data from macromolecular crystals is discussed. Two kinds of factors influencing the completeness of data are considered. The first are geometric, arising from the symmetry of the reciprocal lattice and from the experimental setup; they affect quantitatively the completeness of the measured set of reflections. The second concern the quality, or information content, of the recorded intensities of these measured reflections.

Received 28 January 1999

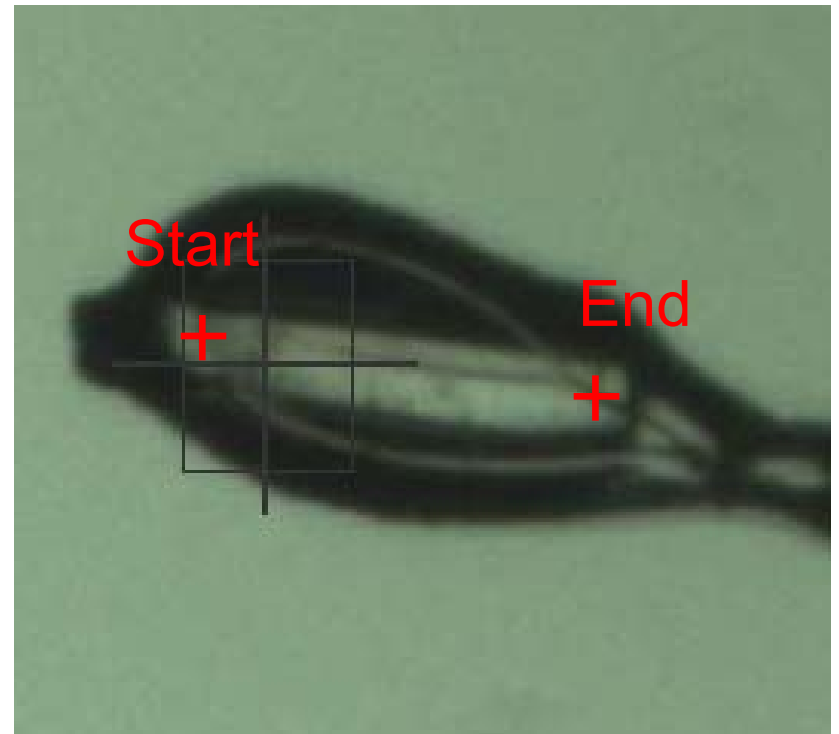
Accepted 22 June 1999

Radiation damage

- A real problem for small crystals and very little we can do about it
 - accept that we will suffer
 - measure data from many crystals and merge
- Using RADDOSE or a sacrificial crystal can provide with estimates of lifetime
- For larger blocky homogenous crystals use a larger beam
- For needles or plates can use trajectory data collections or helical scans

Trajectory (helical or line) scanning

- To alleviate radiation damage where dose can be distributed across a crystal
- Define start and end points of data collection
- Straight line trajectory
- More general trajectories will be added for general crystal shapes



Line scanning

The screenshot displays a software interface for line scanning. The main window shows a diffraction pattern with a central spot and a series of spots along a curved path. Two red squares mark specific positions, labeled "k position" and "k position". The interface includes a menu bar (File, View, Look and Feel, Layout, Help) and a toolbar with buttons for "Start line scan" (green) and "Stop line scan" (red). The "Other controls" panel includes a "Zoom" dropdown set to 4.1 and a "Backlight" button labeled "Out". The "Experiment parameters" panel includes fields for "Run Number" (2), "Number of Images" (10), "Exposure" (1.000 s), "Start angle" (320.000 deg), "Angle range" (0.500 deg), and "Angle overlap" (0.000 deg). The "Alignment" panel includes a "Rotation" section with a "Phi" field set to 325.14 deg and buttons for +15, +90, -15, and -90 degrees. The "Visit Directory" field shows "/dls/i24/data/2009/0-0" and the "Folder" field shows "test2009shutdown4". The "File Prefix" field shows "linediffraction20090617".

Should I illuminate the whole crystal?

- This depends on
 - crystal size relative to maximum beam size available
 - on I02, 3 and 4 this is $\sim 100\mu\text{m} \times 100\mu\text{m}$
 - on I24 currently $\sim 30\mu\text{m} \times 50\mu\text{m}$ (v \times h)
 - diffraction quality of the crystal throughout its volume i.e. diffraction homogeneity
- In general it is best to have a constant diffracting volume as this will improve scale factors
- some evidence that for a large homogeneous crystal use of a large beam results in better data quality
 - Sanishvilli *et al.* *Acta Cryst.* D**64**, 425-435 (2008).

Large beam vs. mini-beam

Acta Crystallographica Section D
Biological
Crystallography
ISSN 0907-4449

A 7 μm mini-beam improves diffraction data from small or imperfect crystals of macromolecules

Ruslan Sanishvili,^{a*} Venugopalan Nagarajan,^a Derek Yoder,^a Michael Becker,^a Shenglan Xu,^a Stephen Corcoran,^a David L. Akey,^b Janet L. Smith^b and Robert F. Fischetti^a

A simple apparatus for achieving beam sizes in the range 5–10 μm on a synchrotron beamline was implemented in combination with a small 125 \times 25 μm focus. The resulting beam had sufficient flux for crystallographic data collection from samples smaller than 10 \times 10 \times 10 μm . Sample data were collected representing three different scenarios: (i) a complete

Received 12 December 2007
Accepted 16 January 2008

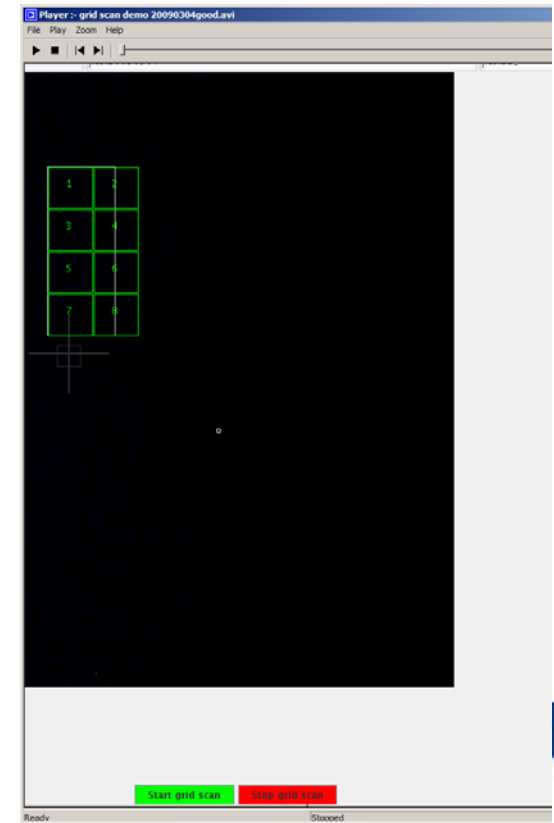
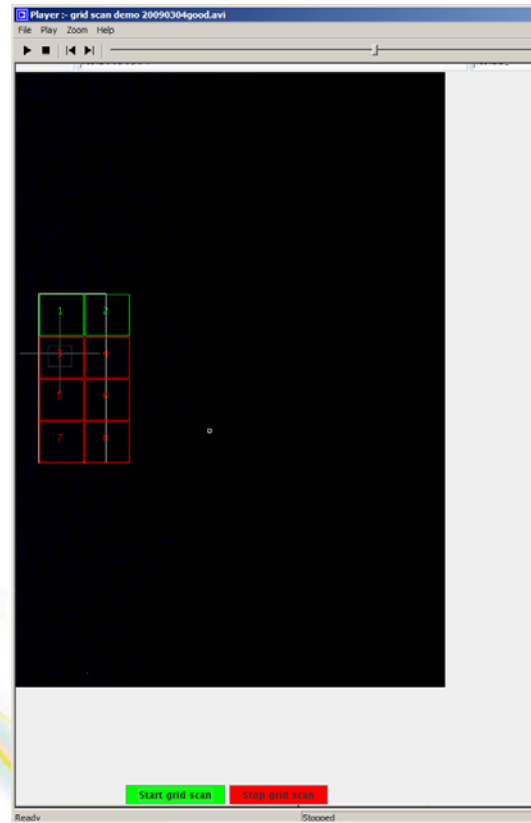
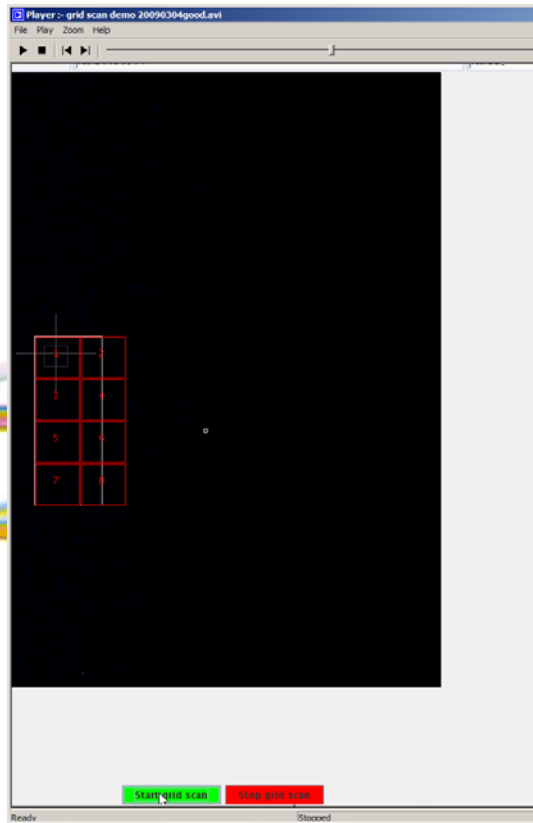
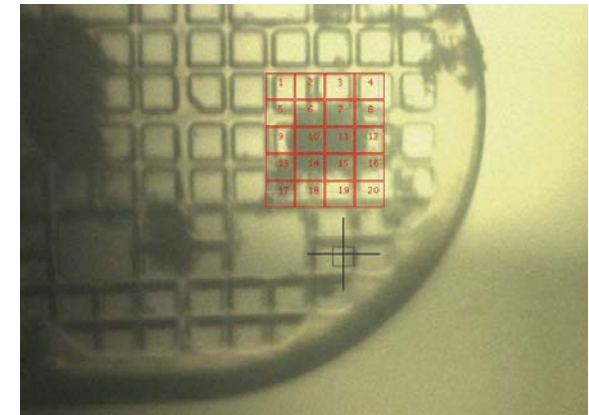
Acta Cryst. (2008). D64, 425–435

Both data sets measured with the larger beam were of superior quality to the mini-beam data set (Table 4, Fig. 7), demonstrating that the larger beam produces better data from large homogeneous sample crystals. We expected the data

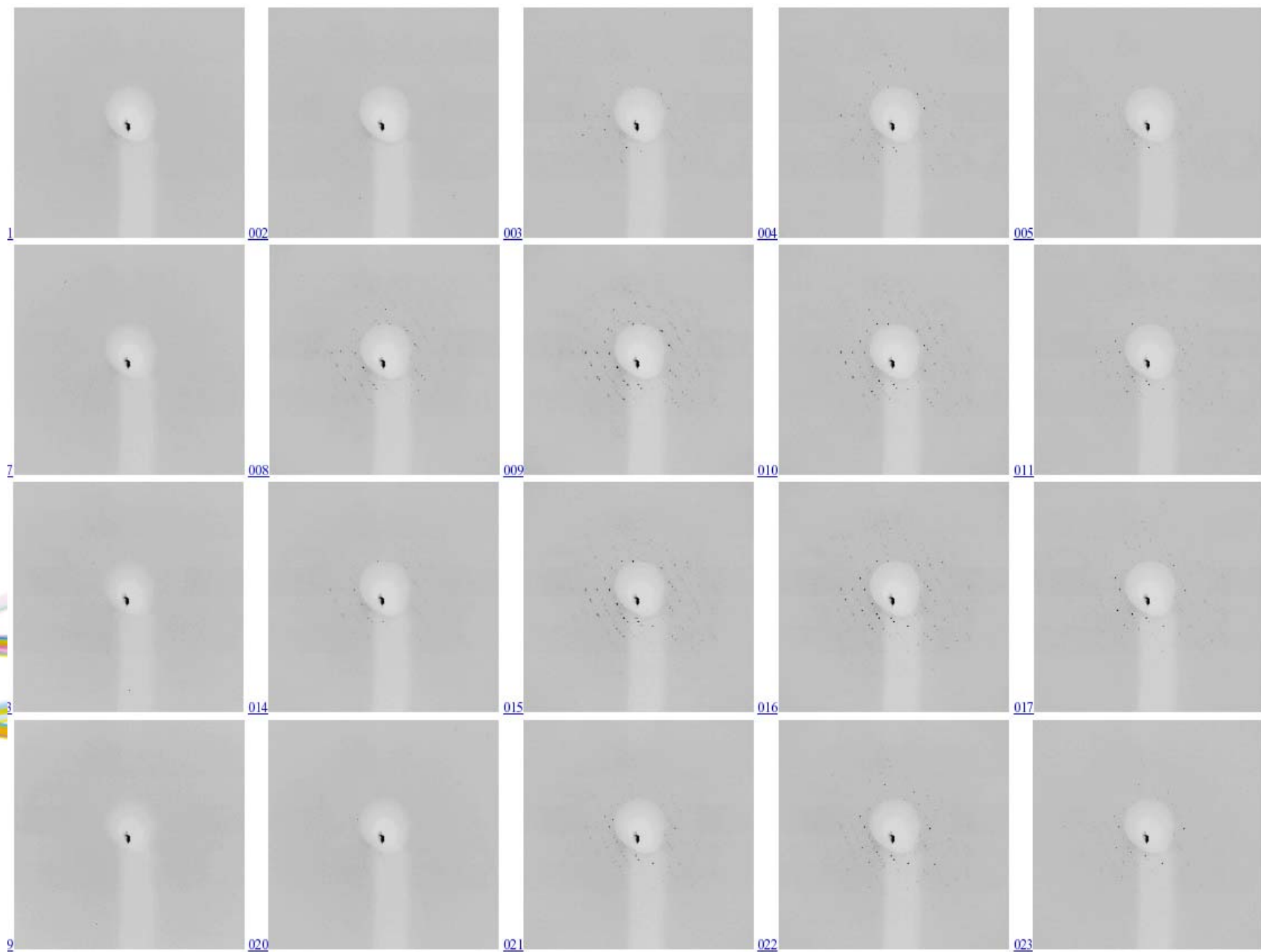
unchanged. This illustrates how the larger beam and larger diffracting volume allowed the use of lower flux density, thus better preserving the sample.

Inhomogeneous crystals

- Sequence of diffraction images at same crystal orientation but different position of crystal or loop use to
 - find the crystal(s)
 - find the best part of the crystal
- Use still images – usually enough spots from mosaic protein samples to index/characterize diffraction

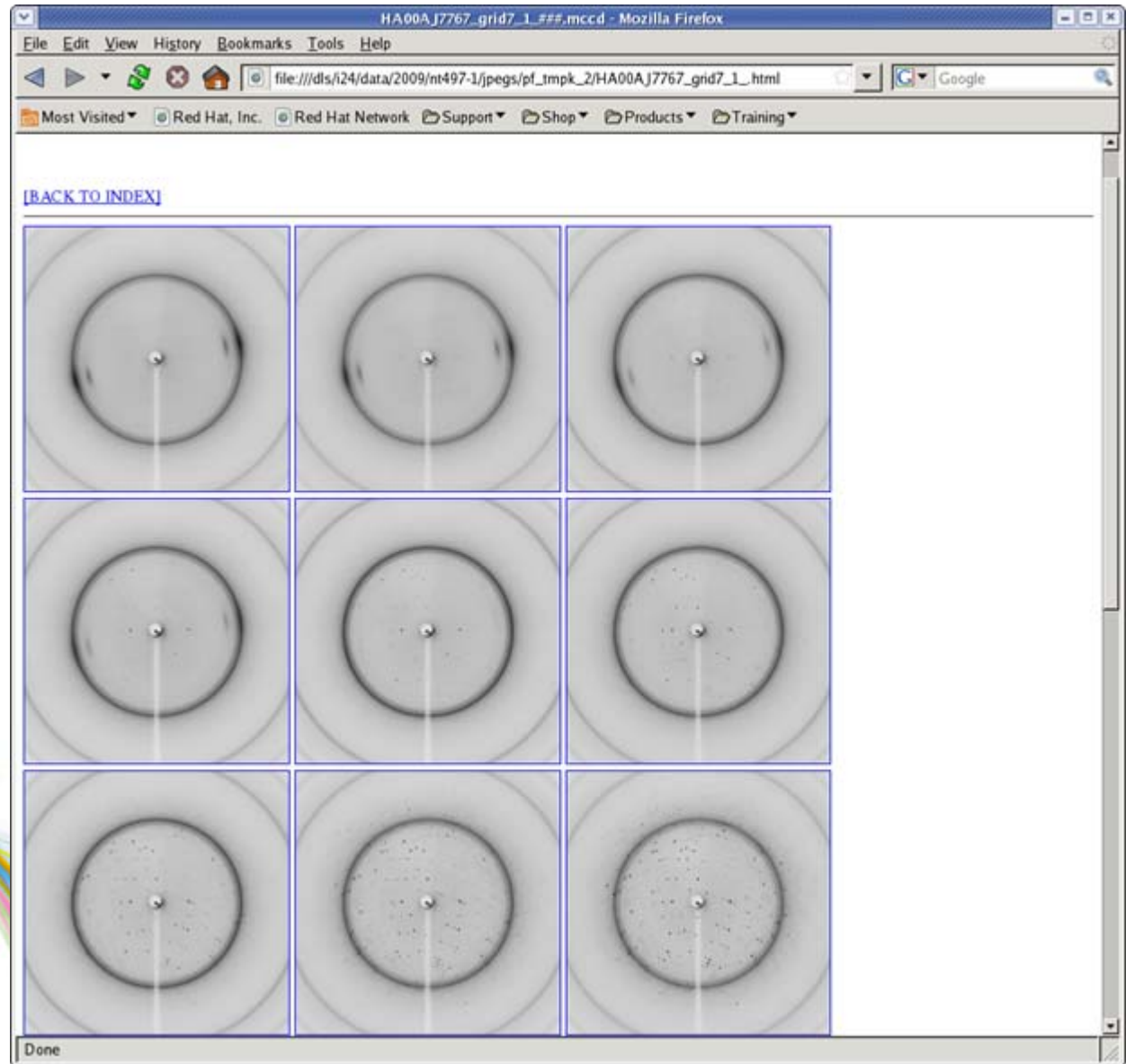


Grid scan: results



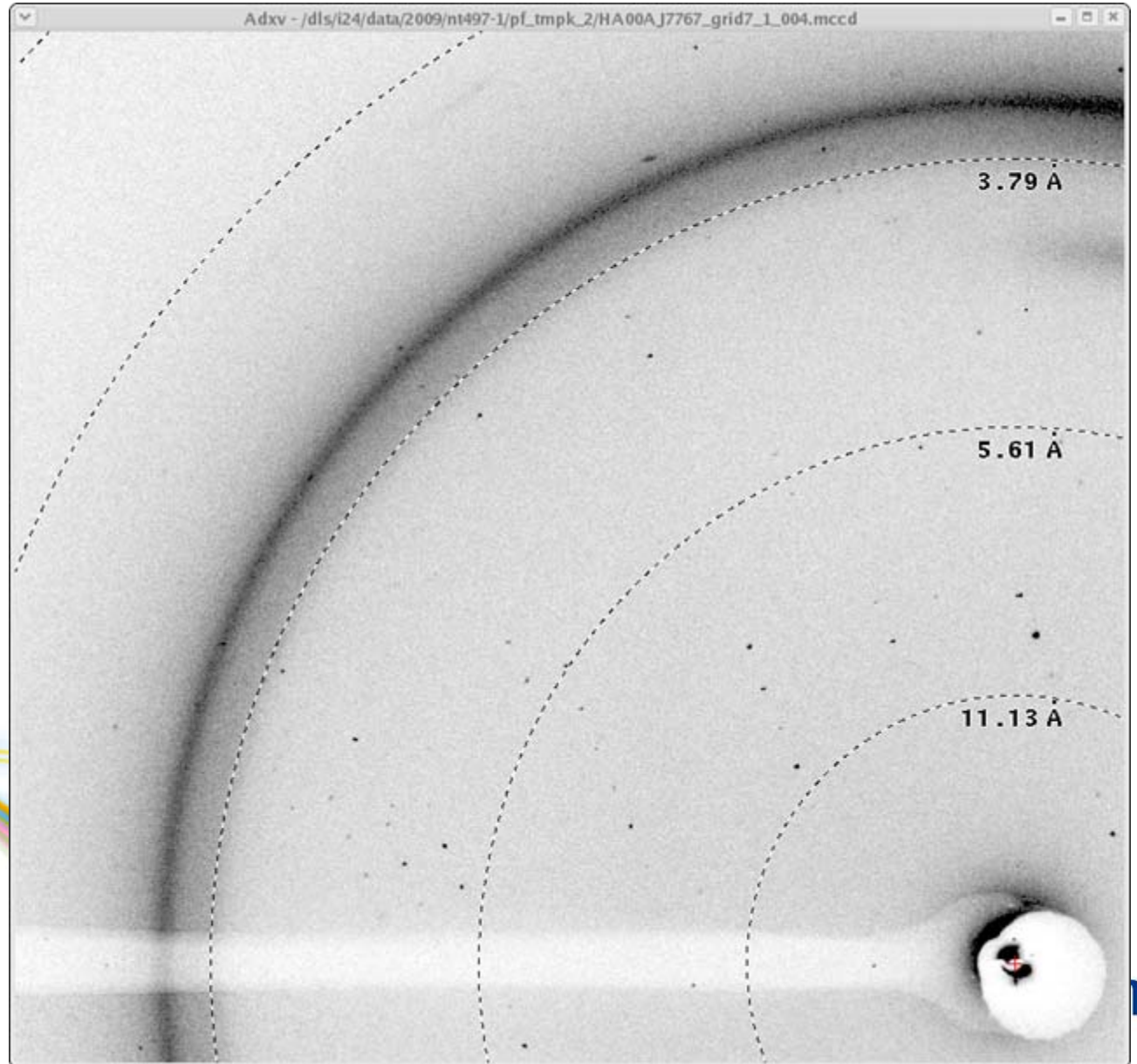
Grid scan: scanning over a large crystal

1	2	3
4	5	6
7	8	9
10	11	12
13	14	15
16	17	18



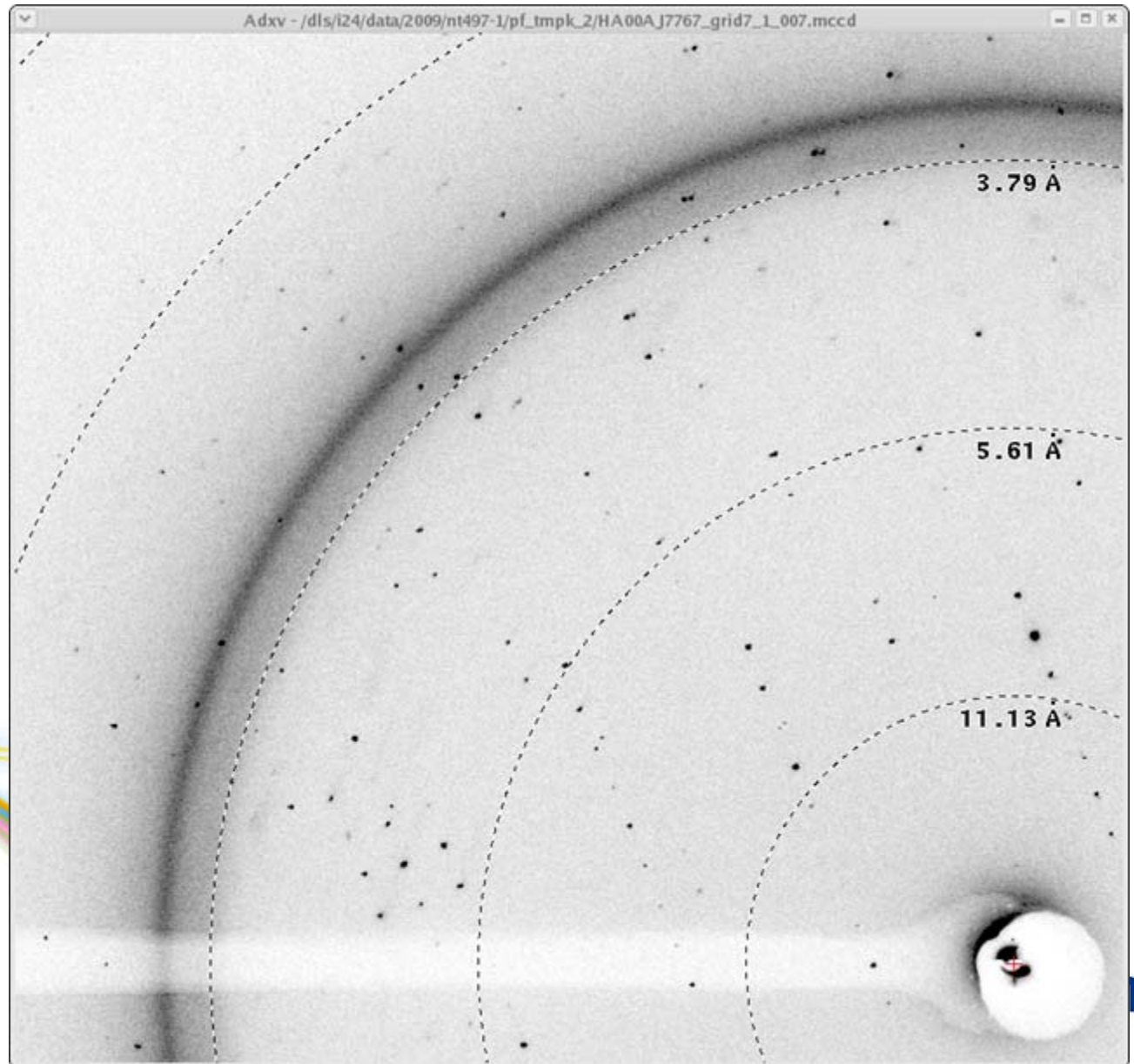
Grid scan: scanning over a large crystal

1	2	3
4	5	6
7	8	9
10	11	12
13	14	15
16	17	18



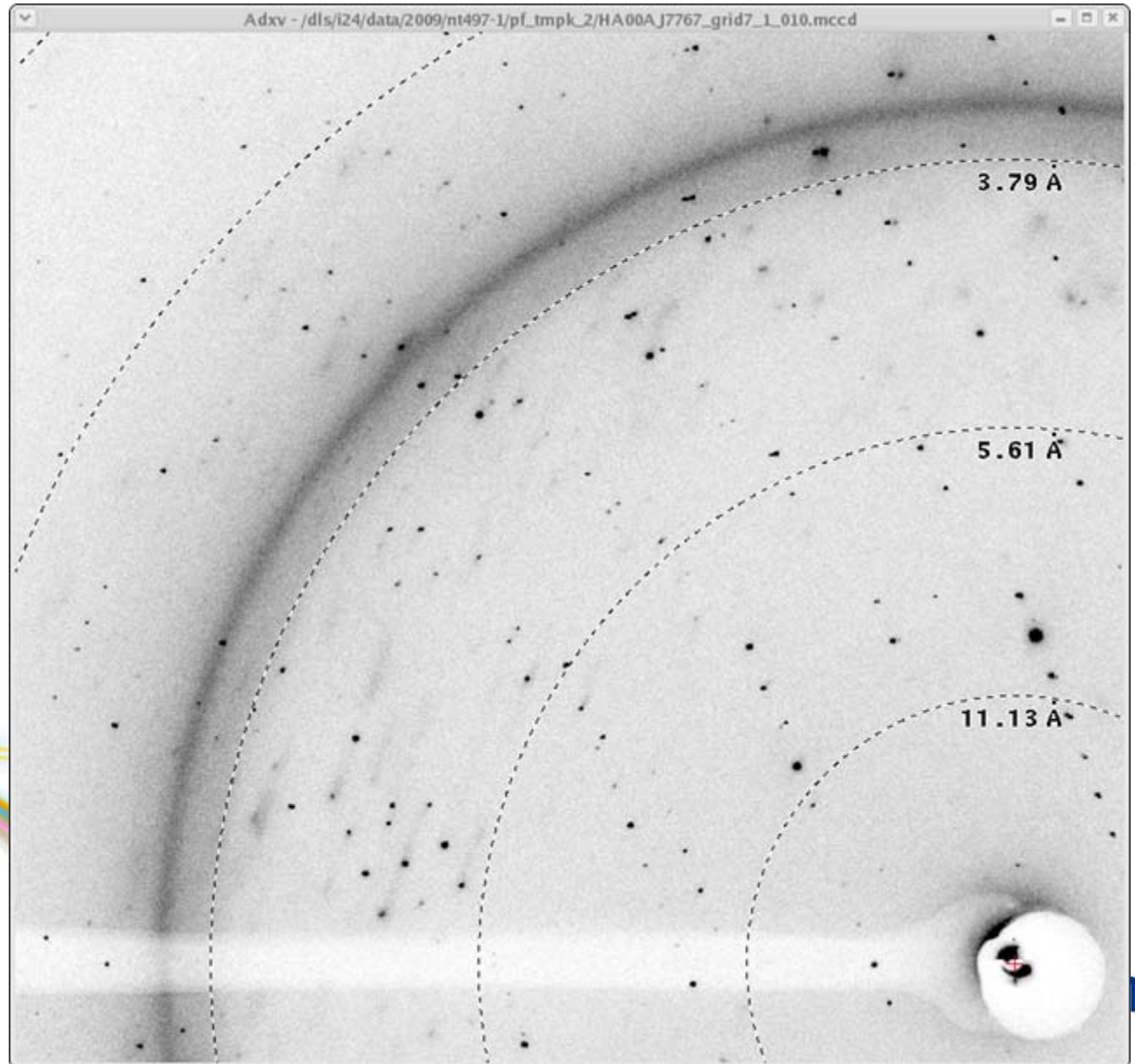
Grid scan: scanning over a large crystal

1	2	3
4	5	6
7	8	9
10	11	12
13	14	15
16	17	18



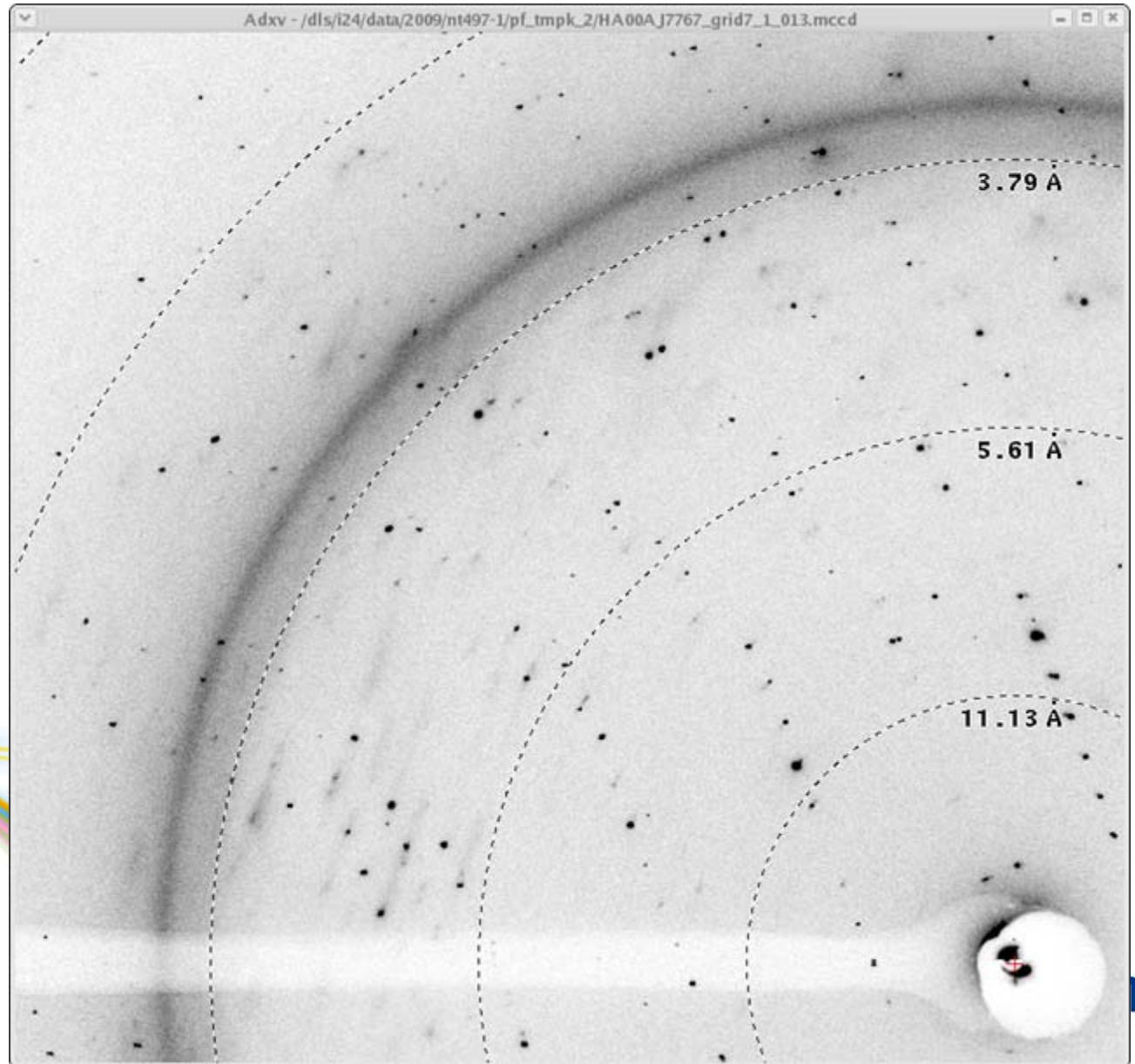
Grid scan: scanning over a large crystal

1	2	3
4	5	6
7	8	9
10	11	12
13	14	15
16	17	18



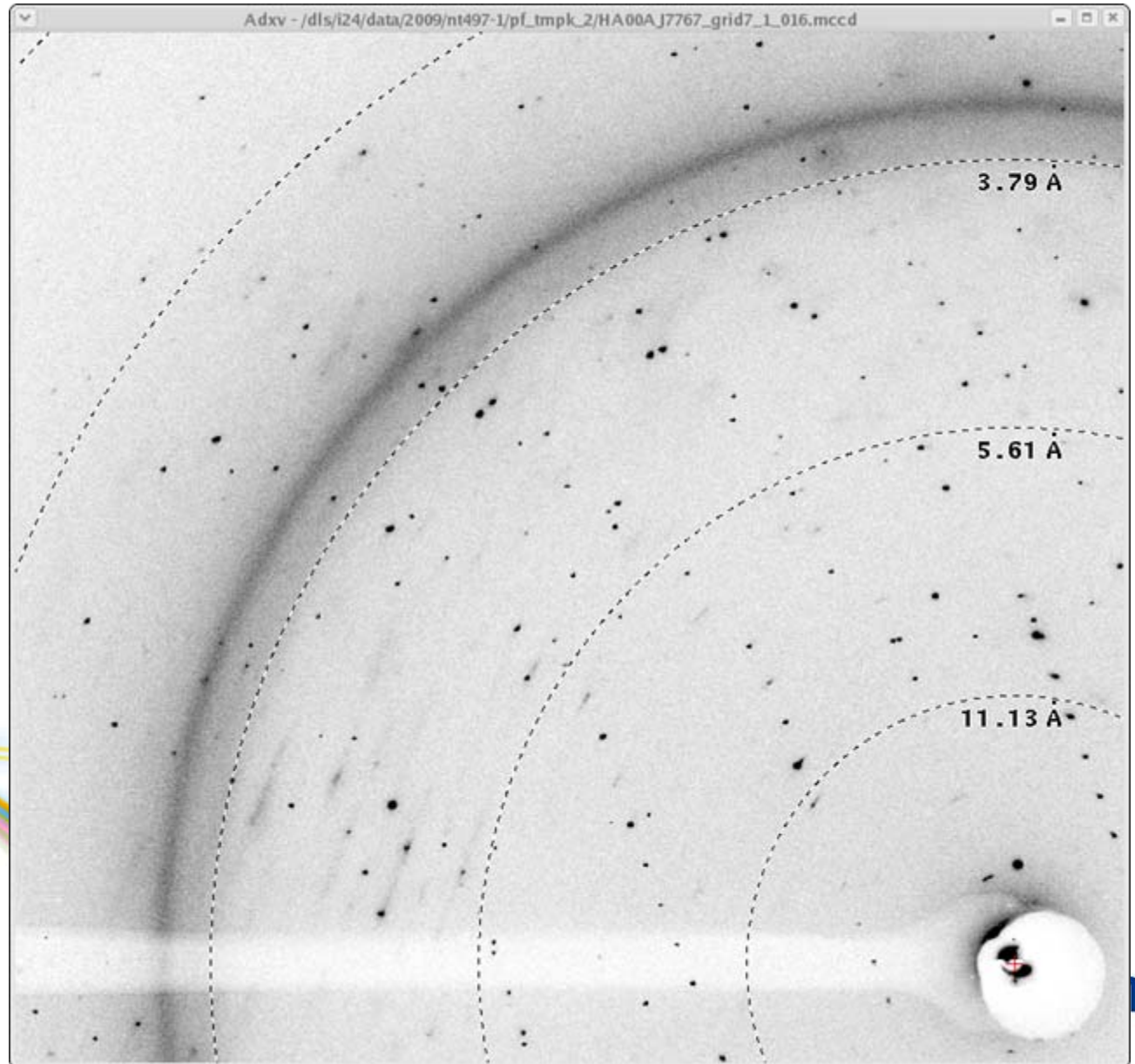
Grid scan: scanning over a large crystal

1	2	3
4	5	6
7	8	9
10	11	12
13	14	15
16	17	18



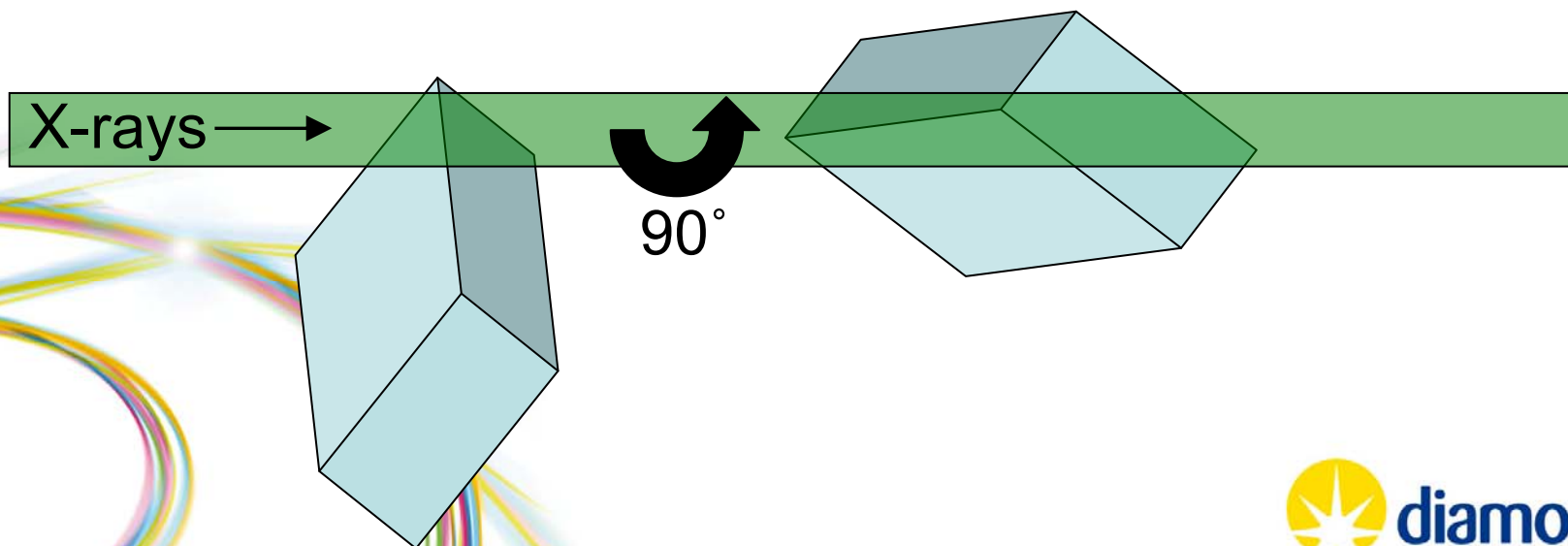
Grid scan: scanning over a large crystal

1	2	3
4	5	6
7	8	9
10	11	12
13	14	15
16	17	18



Decisions based on grid scanning

- If crystal has sweet spots
 - select a small beam and record from best parts
 - needs careful though for a blocky crystal since on rotation additional material enters the beam path
 - you may only be able to measure a small sweep of data before
- Carefully plan your measurements at this stage
 - a grid scan at two orthogonal orientation may be need to completely assess crystal



So what should you do?

- A strategy might be
 - take two test exposures using large beam and test diffraction quality
 - if not good then perform grid scan using smaller beam to isolate good regions
 - record data set from smaller regions using small beam
 - if good then record data set
- In practice if you test your crystal using I02, 3 or 4 and find the quality is poor then
 - either cut beam size with slits and perform grid scan
 - significantly lower flux but may be high enough
 - save crystal for I24 microfocus beamline visit
 - higher flux in smaller beam

MAD and SAD

- Great care needs to be taken to avoid radiation damage
 - heavy atoms are sensitive (highly absorbing) and your signal is related to their occupancies and B-factors
- Remember therefore that good phases need only be recorded to lower resolution $\sim 2.5 - 3.5 \text{ \AA}$
 - use modest exposure levels and ensure your crystal survives the distance
- Take extra care in determining your strategy
- They can then be extended against a purpose measured higher resolution (possibly native) data set

Aligned crystal or inverse beam

- Aligned crystal
 - Use of Kappa geometry can allow your crystal to be aligned so that Bijvoet mates are measured simultaneously
 - This can improve Bijvoet difference measurements by removing some sources of systematic error
 - radiation damage
 - absorption (sometimes)
 - You may need to realign to measure blind region
 - Can make scaling less precise
- Inverse beam
 - Record Friedel pairs close together in time
 - Again reduces sources of systematic error
 - radiation damage
 - absorption (sometimes)
 - Does not required Kappa geometry and therefore a random orientation may be ok.
- These methods may be more appropriate where radiation damage is extreme and unavoidable
- Alternatives might be merging MAD/SAD data from multiple crystals
 - last resort since isomorphism becomes critical
 - essential if you have microcrystals

Important!

- Data collection is the last experimental step
 - “no amount of data massaging is going to turn bad data into a structure”
- So take your time
- Think about what your doing
- Use the available facilities (and staff) to your advantage
- Know what is in your sample
 - request a fluorescence measurement for elemental analysis
- Index and integrate your first images
- Determine how radiation sensitive your sample is
- Do not over expose your crystal
- Calculate a strategy
- Use assisted data collection programs like DNA/EDNA and/or BEST
- Scale your data as you go along if possible
 - inspect autoprocessing output

Finally

- Thanks to
 - Randy Alkire (SBC)
 - Nukri Sanishvilli (GM/CA-CAT)
 - Zbyszek Dauter (ANL)

 - Keith Wilson (U. of York)
 - Ana Silva (U. of York)