Radiation Damage - Minimising the Dose and Maximising the Signal

Colin Nave, Diamond Light Source

Crystallography at 1 micron scale

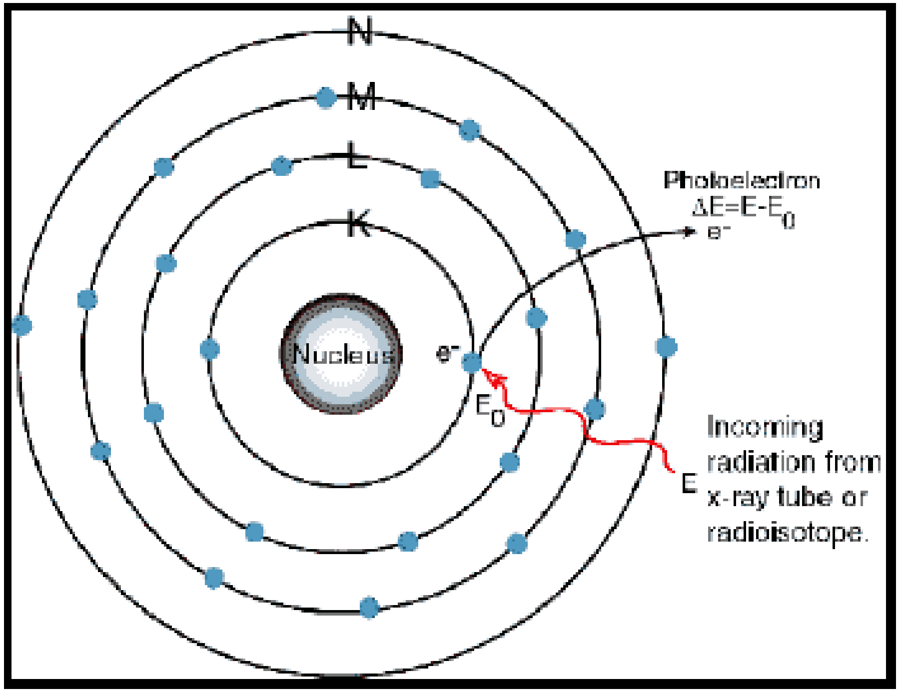
- Use micron size beam and micron size sample
- Use micron size beam and larger sample
- Use parallel (perhaps coherent) beam to reveal crystal structure at (sub) micron scale.

Possibilities to exploit some of these for reducing radiation damage and maximising signal to background

First a short introduction to radiation damage

Primary Damage

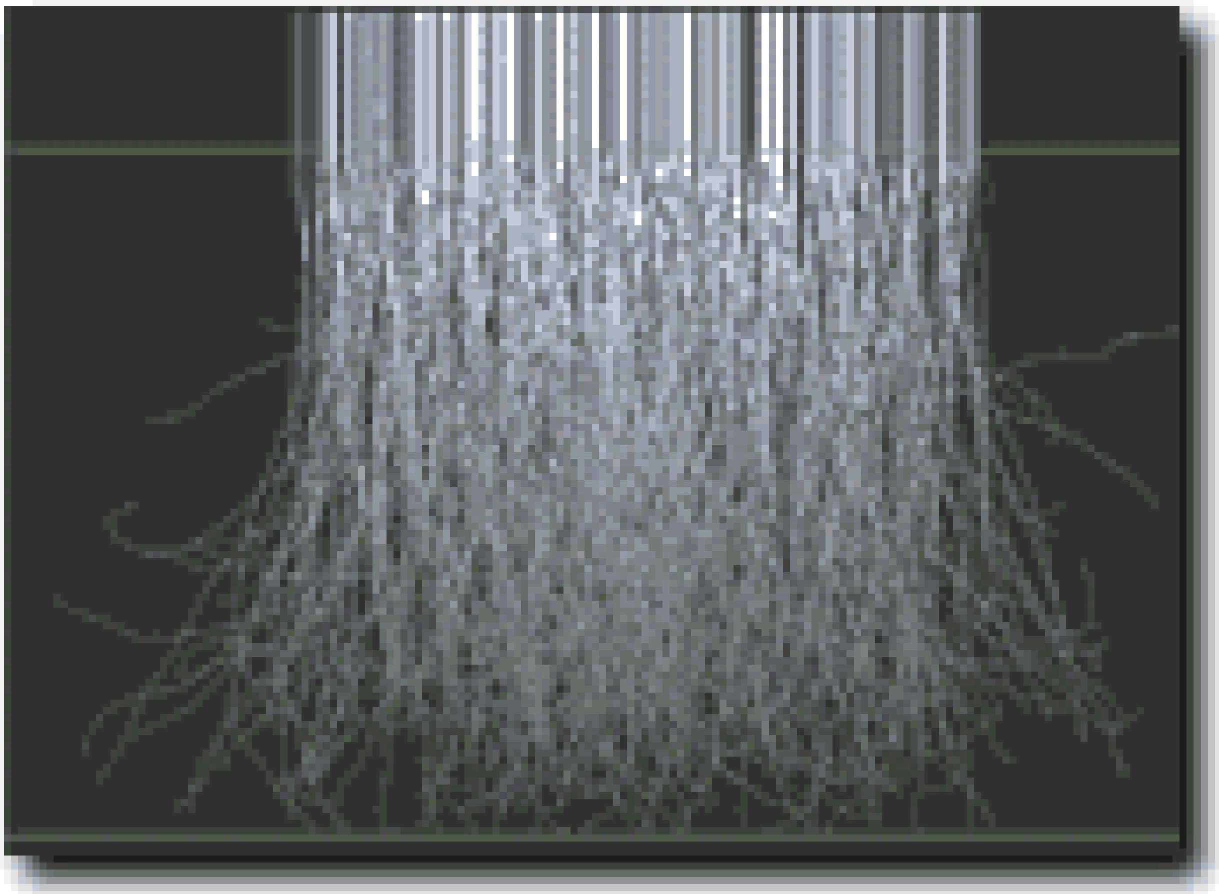
- X-ray absorbed by light atom – produces photo-electron



http://www.lightsource.ca/education/images/science/xas.gif

Not prevented at cryo-temperatures

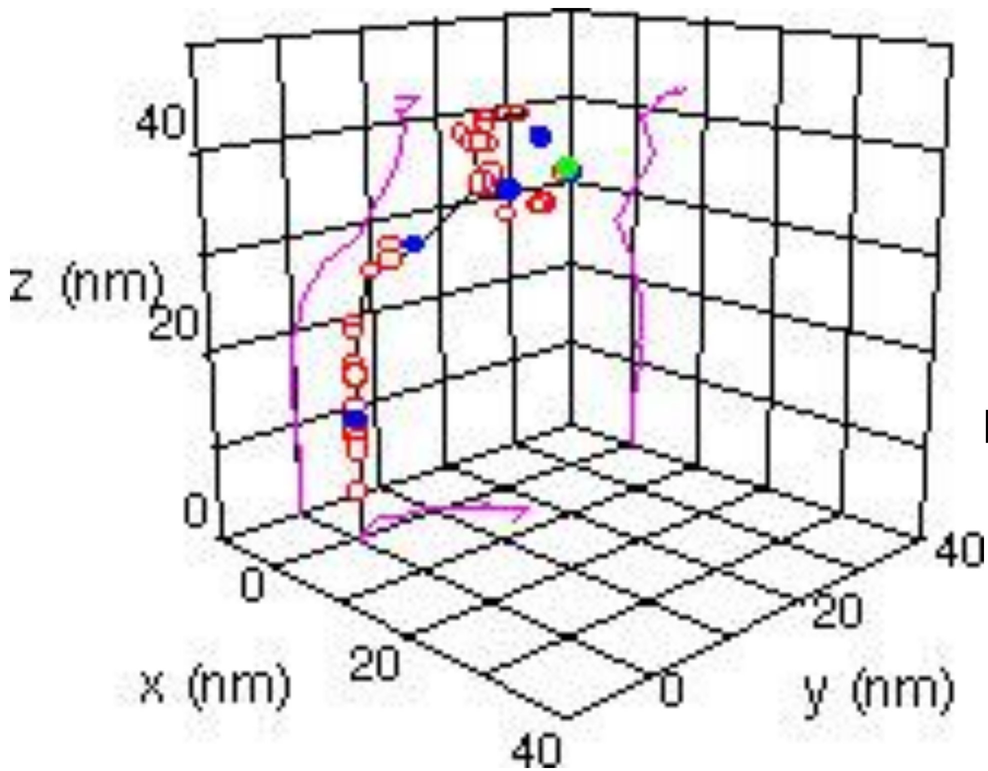
Electrons undergoing inelastic scattering



Several hundred inelastic scattering events for each 10KeV photo-electron

1 KeV electron slowing down

The oscillator strength of molecules peaks at approximately 20 to 30 eV and decreases to very low values at 100 eV.



From S Pimblott

Damage more concentrated at end of photo-electron path

What happens next?

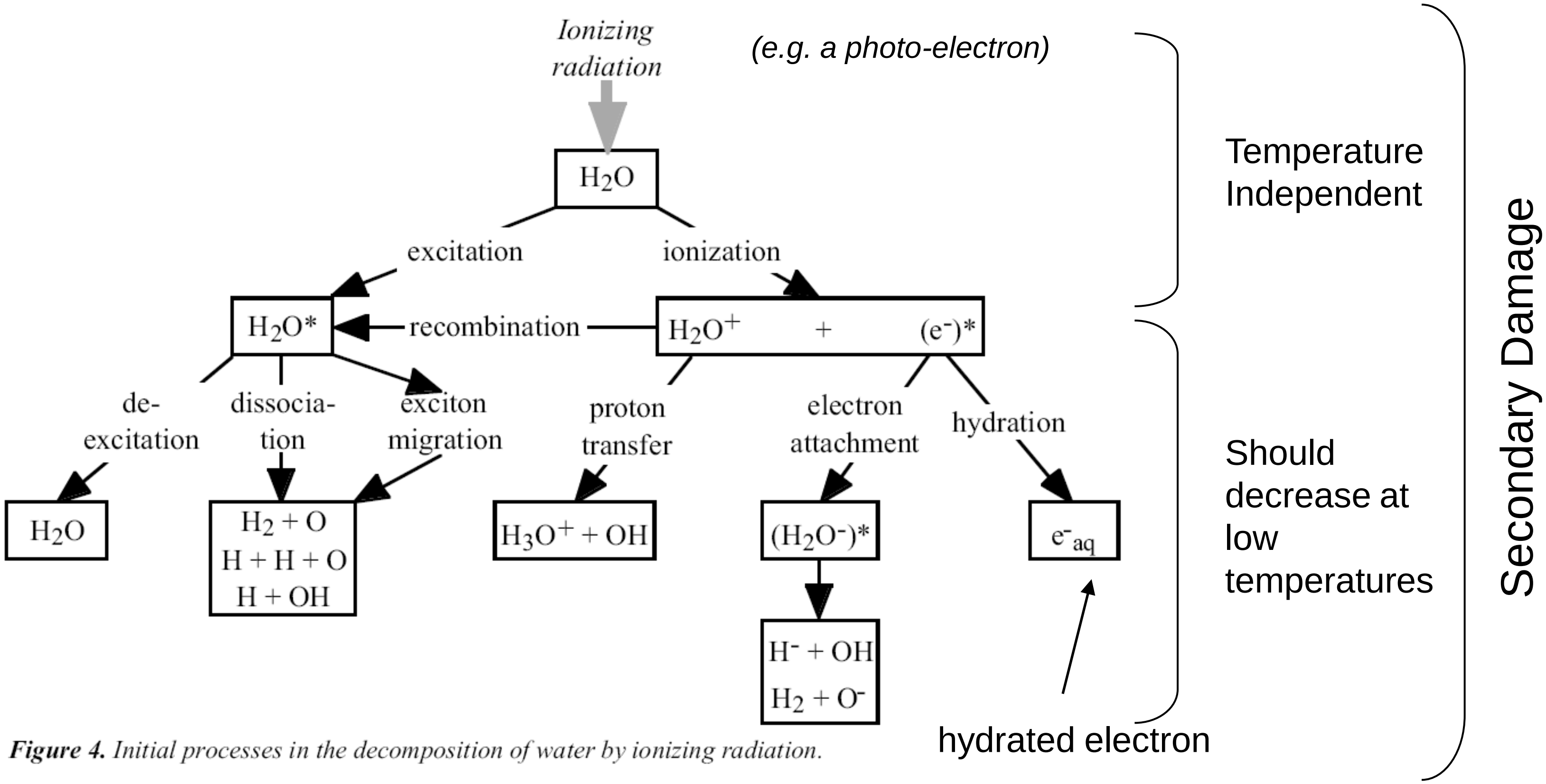
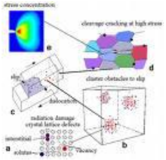


Figure 4. Initial processes in the decomposition of water by ionizing radiation.

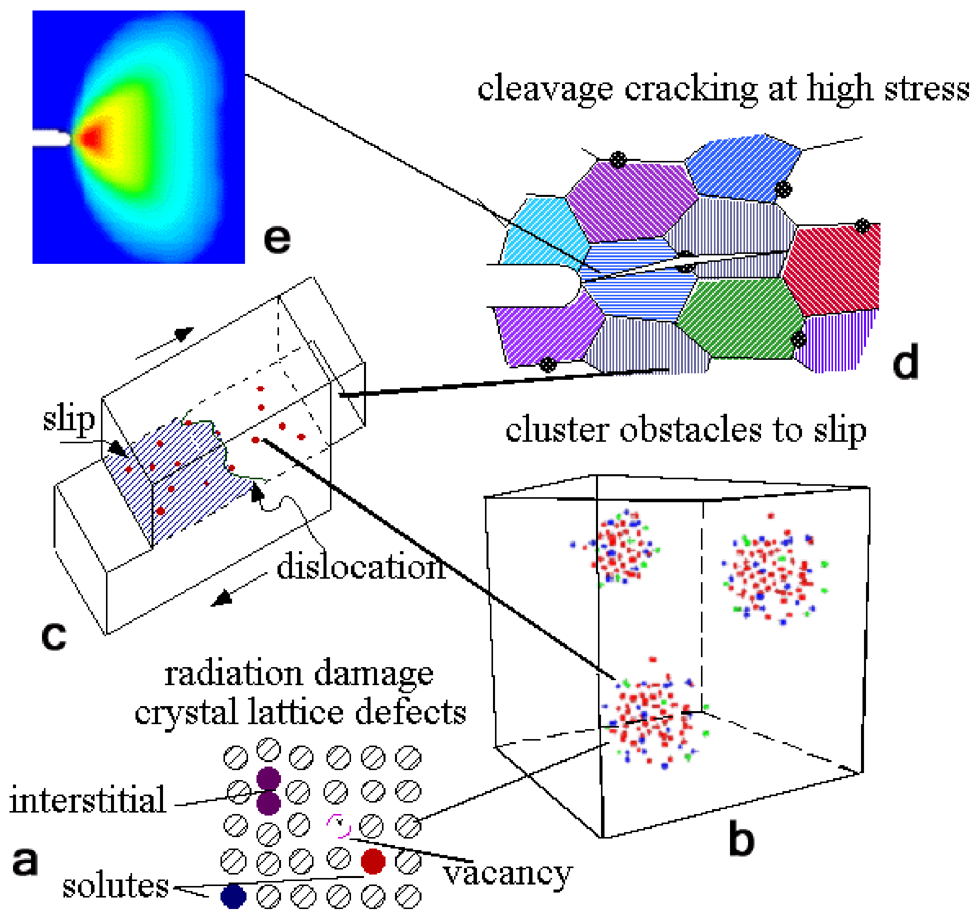
From Workshop Report Understanding the Role of Water on Electron-Initiated Processes and Radical Chemistry
Pacific Northwest National Laboratory, September 26-28, 2002. see also Chem. Rev. 2005, 105, 355-389

In a protein crystal, presence of organic molecules* and the dimensions of confined water alter the reactions significantly

* e.g. Alke Meents, Birger Dittrich and Sascha Gutmann J. Synchrotron Rad. (2009). 16, 183–190.



Tertiary Radiation Damage



Embrittlement of Nuclear Reactor Pressure Vessels. G.R. Odette and G.E. Lucas *JOM,* 53 (7) (2001), pp. 18-22

Some Timescales in Water

Radiation traversing a typical protein molecule	10^{-17} s
Energy transfer (ionization and excitation events)	$10^{-17} – 10^{-16}$ s
Lifetimes of above	10^{-15}- 10^{-14} s
Thermalisation of electrons	10^{-13} s
Subsequent reactions (room temperature)	10^{-12} - 10^{-7} s
Pulse from x-ray free electron laser	10^{-14}- 10^{-13} s

Global and specific damage

Global – Loss of resolution

Specific – damage identified at specific sites

- disulphides, carboxyl groups

Methods to Examine Radiation Damage

Arwen R. Pearson and Robin L. Owen Biochemical Society Transactions (2009) Volume 37, part 2

- X-ray diffraction
- X-ray scattering
- XANES
- EXAFS
- Fluorescence
- Raman
- Resonance Raman
- FTIR

These probe different length and time scales -
e.g. only some sensitive to changes in redox state.

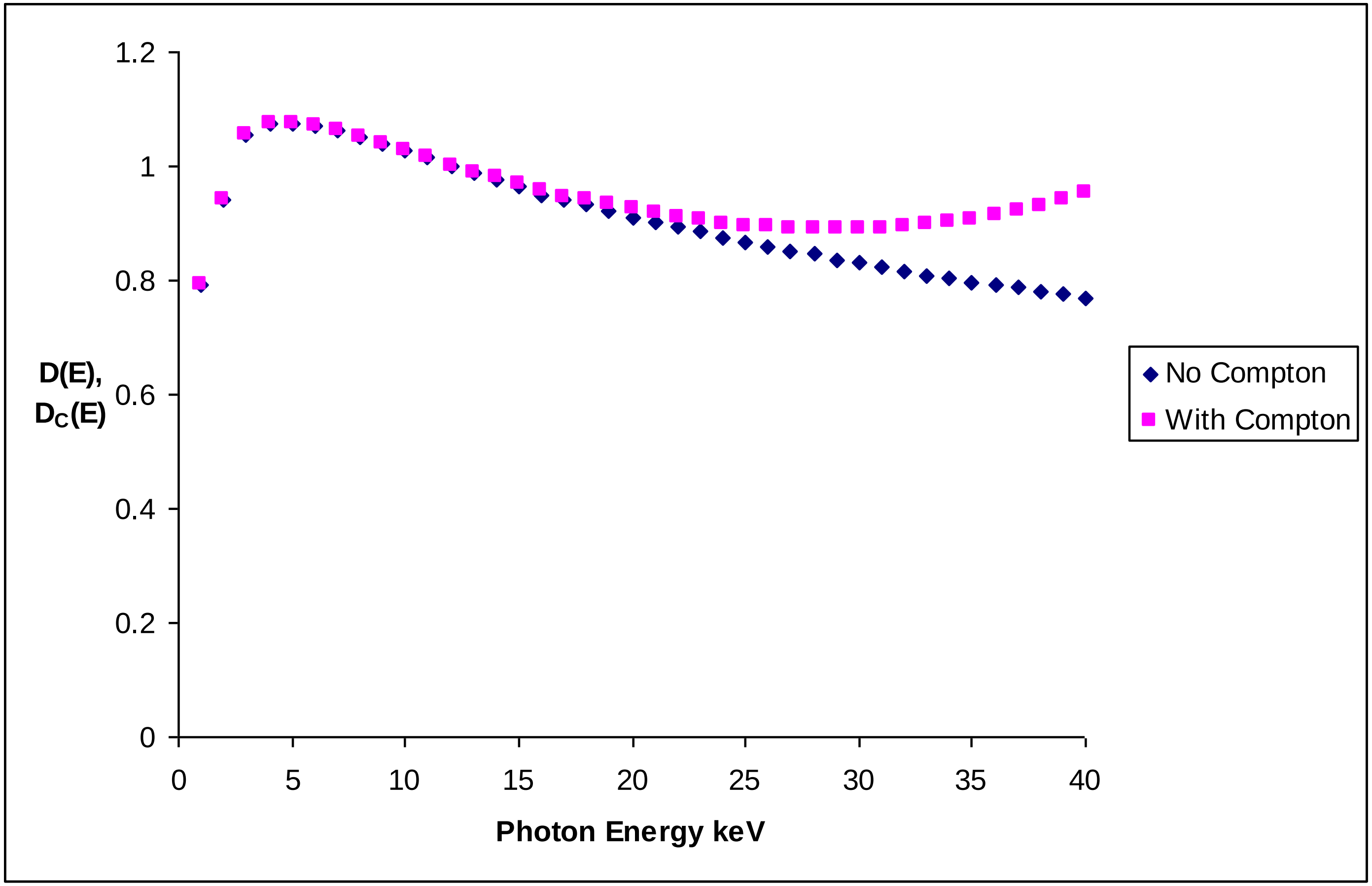
Modelling Photo-electron Escape

- Escape of Photo-electrons – C Nave and M Hill *Journal of Synchrotron Radiation, 2005, 12, 299-303***.**
- Inclusion of Compton Scattering
- Energy Retained in the K shell

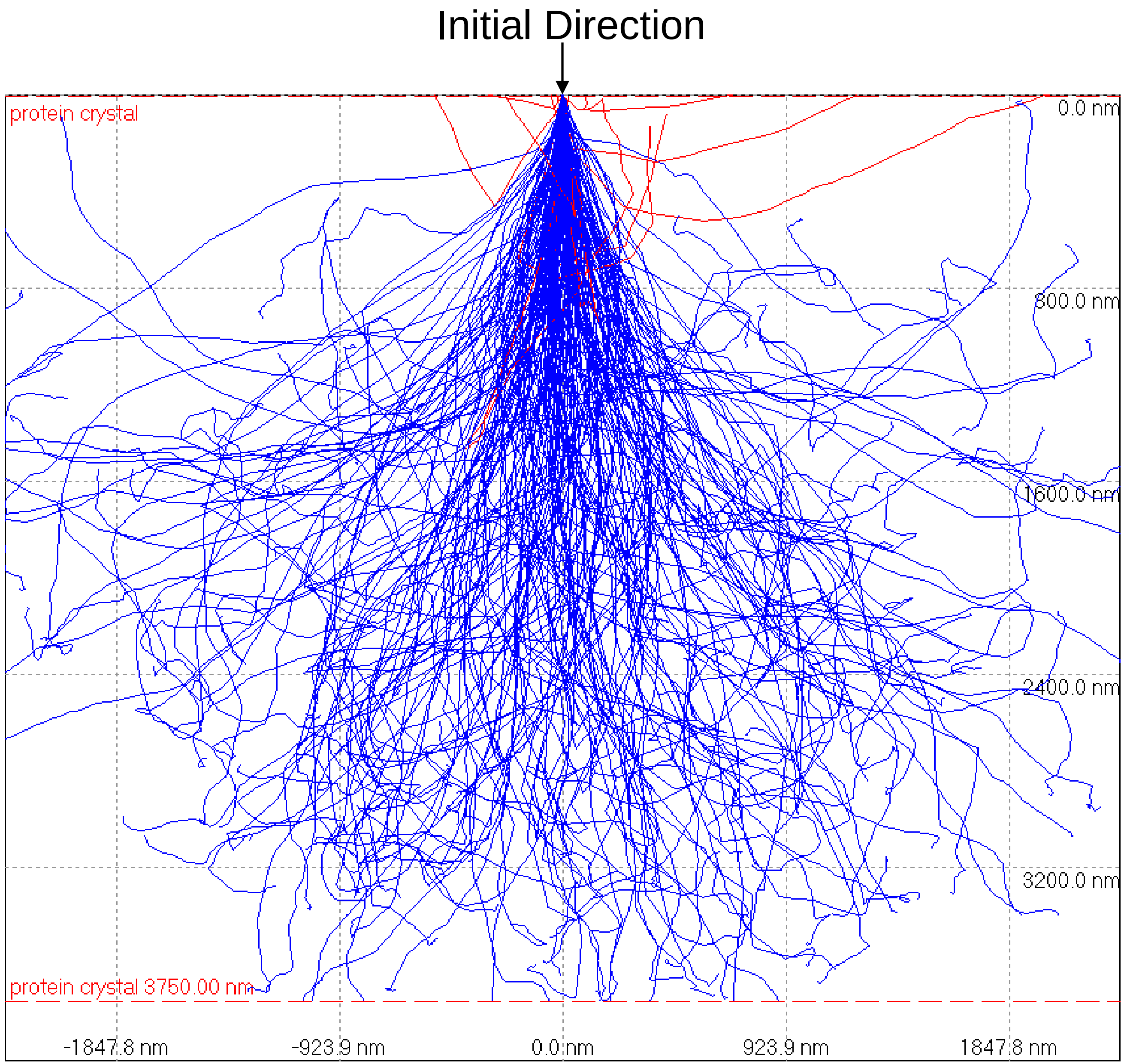
Work done with John Cowan, Daresbury

Cowan, J. A. & Nave, C. (2008). J. Synchrotron Rad. 15, 458–462.

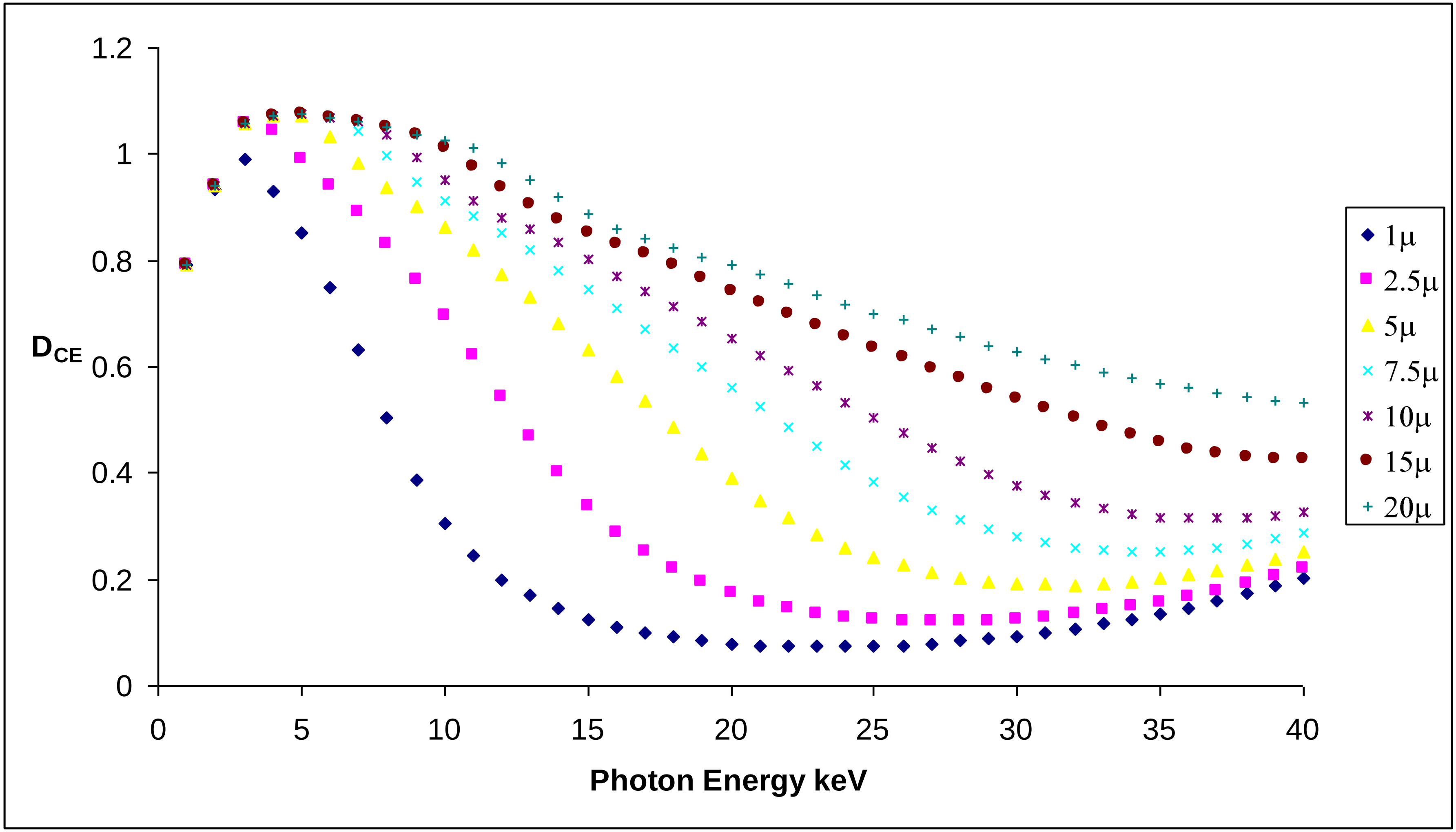
Dose absorbed Scaled to Intensity of a Diffraction Spot



Track Distributions for 15KeV Photo-electrons

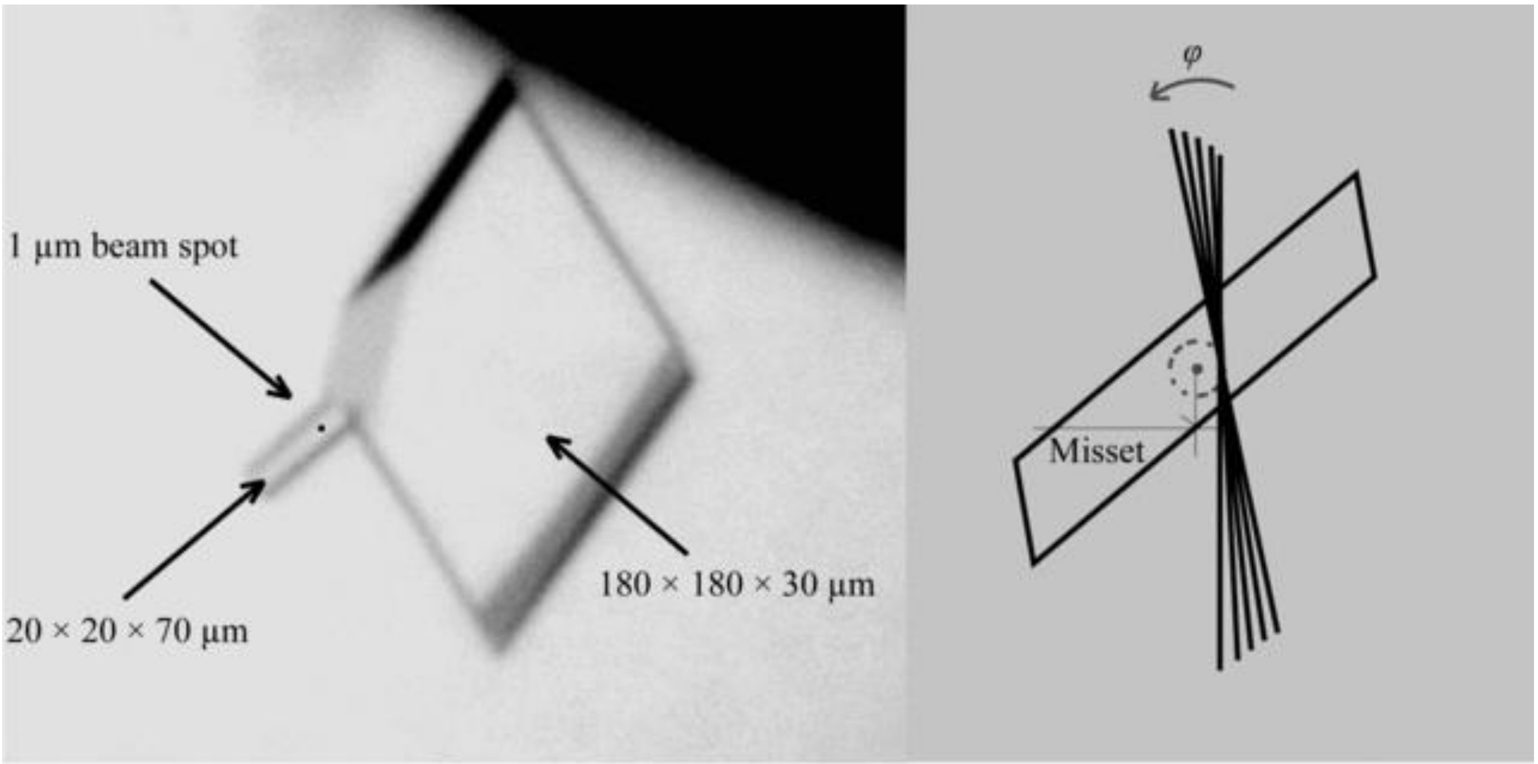


Include Photo-electron escape



Limit - Approx 0.45 keV energy retained in K shell

People are Trying It!

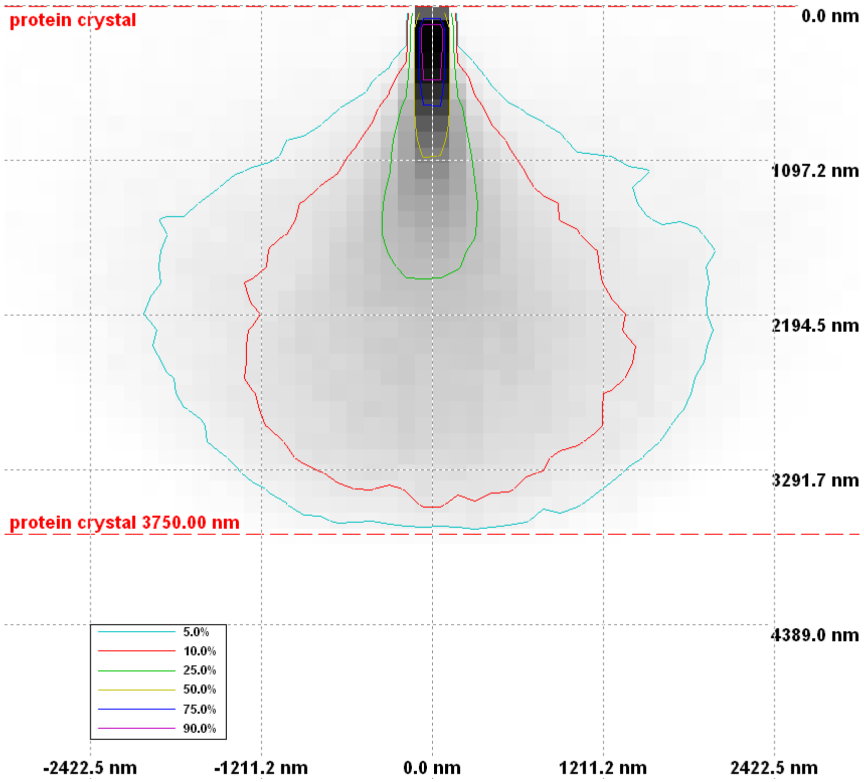


(a) (b)

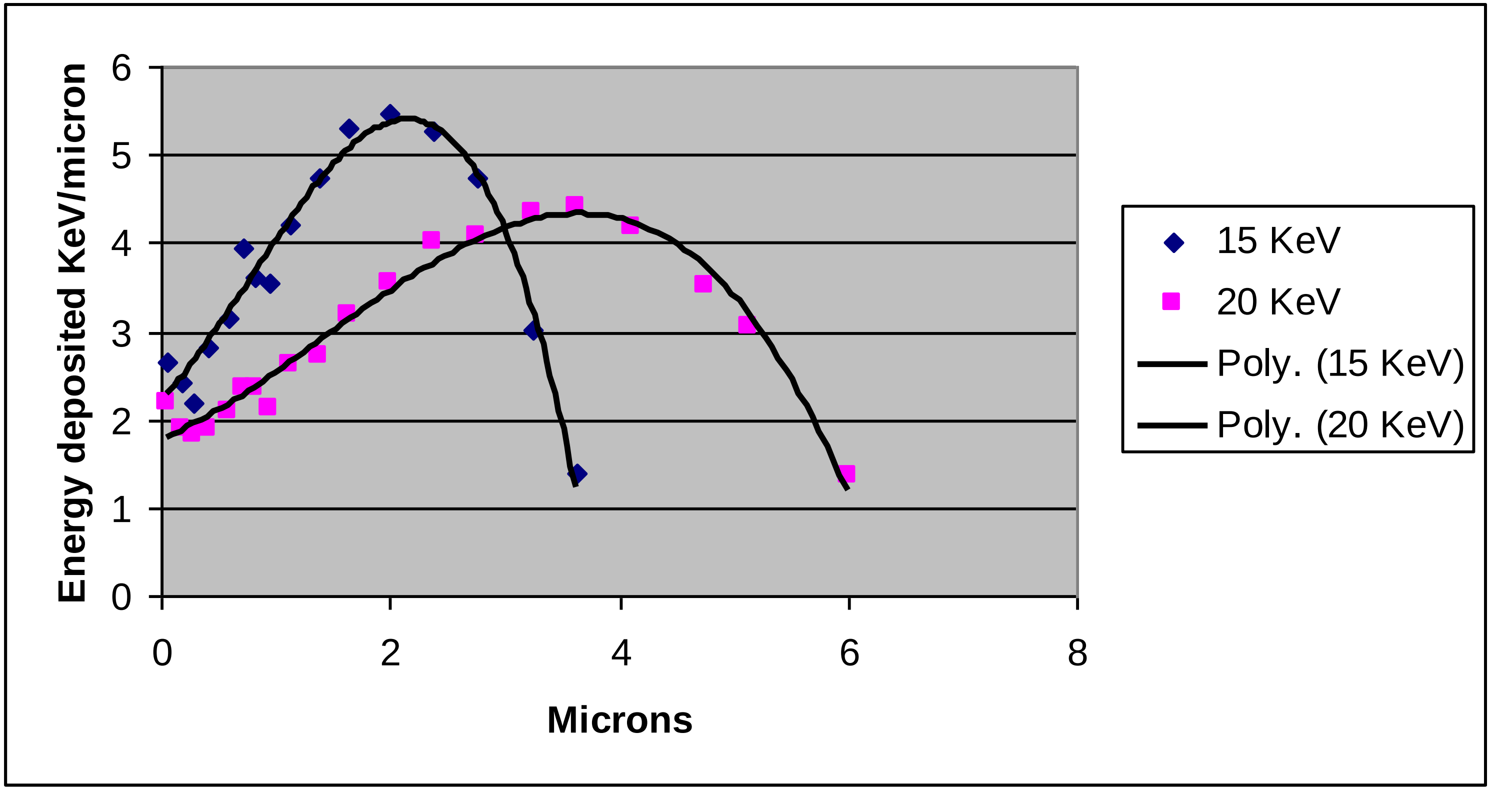
Moukhametzianov, R., Burghammer, M., Edwards, P.C., Petitdemange,S., Popov, D., Fransen, M., McMullan, G., Schertler, G. F. X., & Riekel, C. (2008). Acta Cryst. *D64, 158-166.*

See also presentation by Bob Fischetti

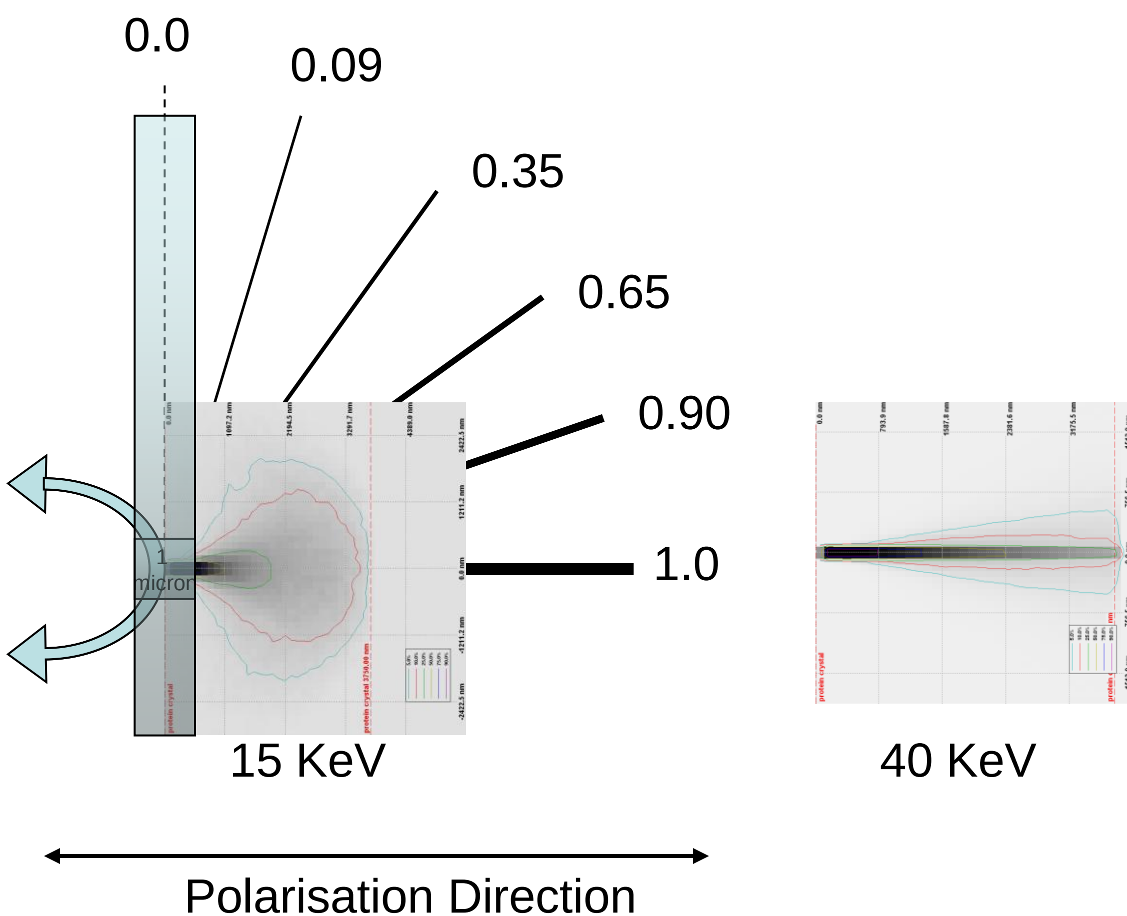
Energy Distribution from 15 KeV Photo-electron



Energy Deposition with Distance



Cos^2 theta weighting



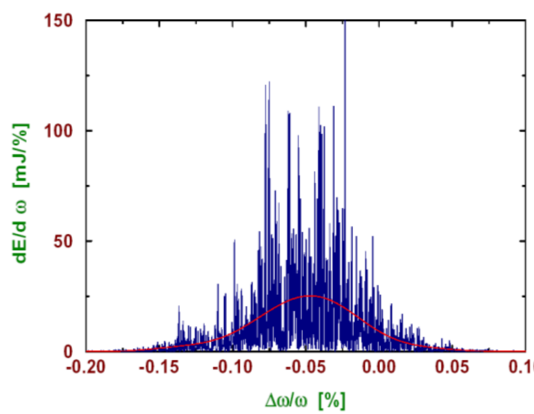
See also Stern, E. A.,. Yacoby, Y , Seidler, G. T. , Nagle, K. P., Prange, M. P., Sorini, A. P. , Rehr, J. J. and Joachimiak, A. (2009) *Acta Cryst* . D**65**, 366-374.

Pulsed or Continuous Source?

Assume Ideal Conditions

$3x10^{11}$ photons in 100nm diameter spot gives 3.9Å resolution from lysozyme crystal 5X5X5 unit cells (Neutze, R., et al., Nature, 2000. **406**(6797): p. 752-7)

Cross over point between continuous source and pulsed approximately 100nm crystal of lysozyme.



James Holton Talk for realistic estimate? Also talk by John Spence?

Small crystals with continuous source

- Beam size matched to size of crystal
- Intrinsic Divergence for a perfect crystal given by crystal size - implies coherent incident beam
- Need detectors optimised for photon energies up to 30keV?
- Multi-circle goniometer to optimise photo-electron escape

Suppressing Radiation Damage

Physical processes reasonably well understood

To mitigate

- Exploit photo-electron escape
- Use very short pulsed (sub ps) intense sources

Both require further experimental verification

Chemical Processes Complex

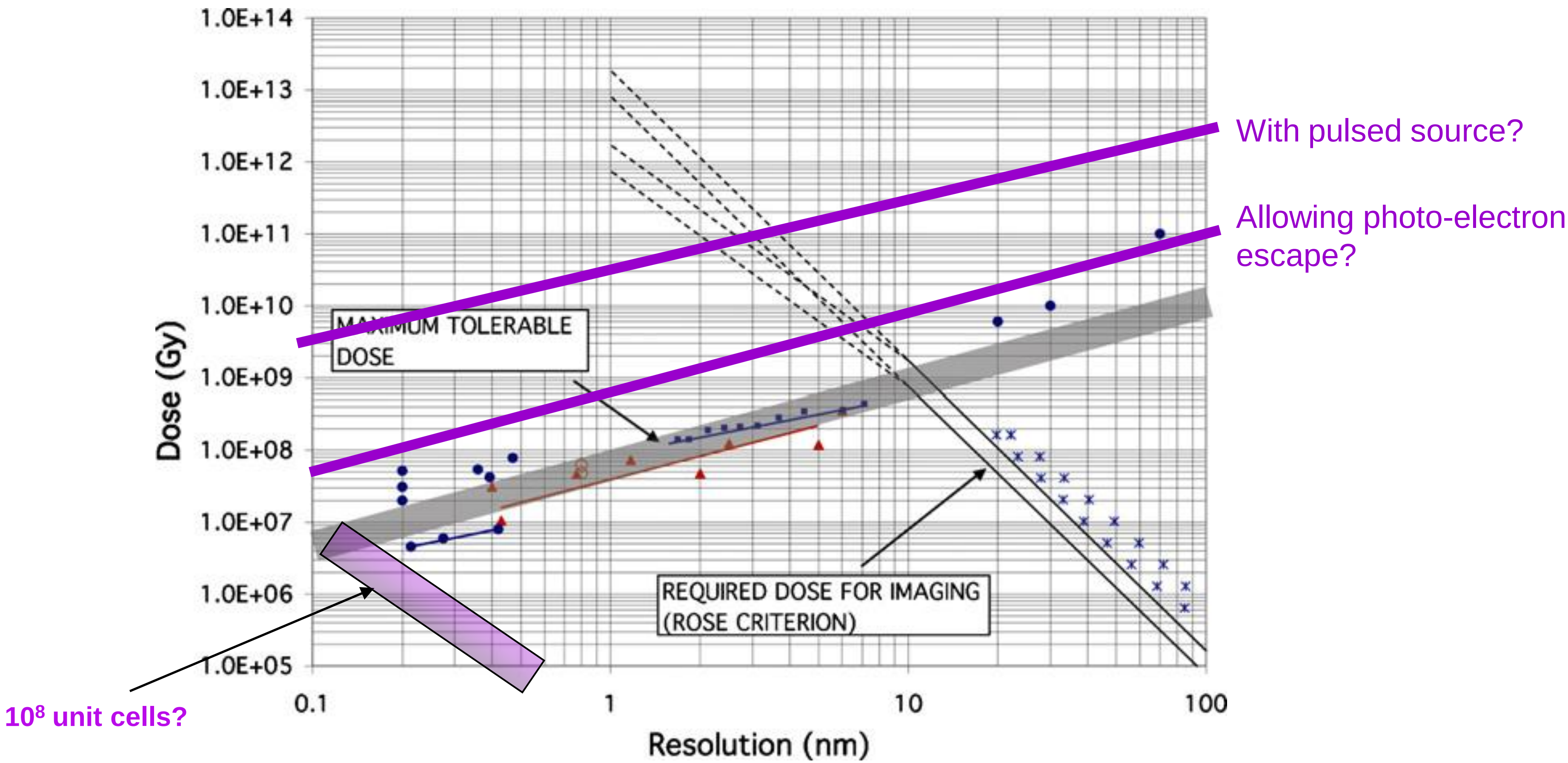
To mitigate

- Try scavengers (bigger effect at room temperature, pH dependent?)
- Try helium temperatures (e.g. 20K) for specific damage, metal reduction

These likely to be very sample dependent

More details in talk by Elspeth Garman?

Dose, damage and resolution



Howells, M.R., Beetz, T., Chapman, H.N., Cui, C., Holton, J.M., Jacobsen, C.J., Kirz, J., Lima, E., Marchesini, S., Miao, H., Sayre, D., Shapiro, D.A., Spence, J.C.H. & Starodub, D. Journal of Electron Spectroscopy and Related Phenomena 170, 4-12 (2009).

See also Q. Shen, I. Bazarov and P. Thibault, *J. Synchrotron Radiat.* **11** (2004), pp. 432–438

Towards Ideal Conditions

Maximise Spot to Background

What does a spot look like?

Domain Structure in Protein Crystal at Cryo-temperatures

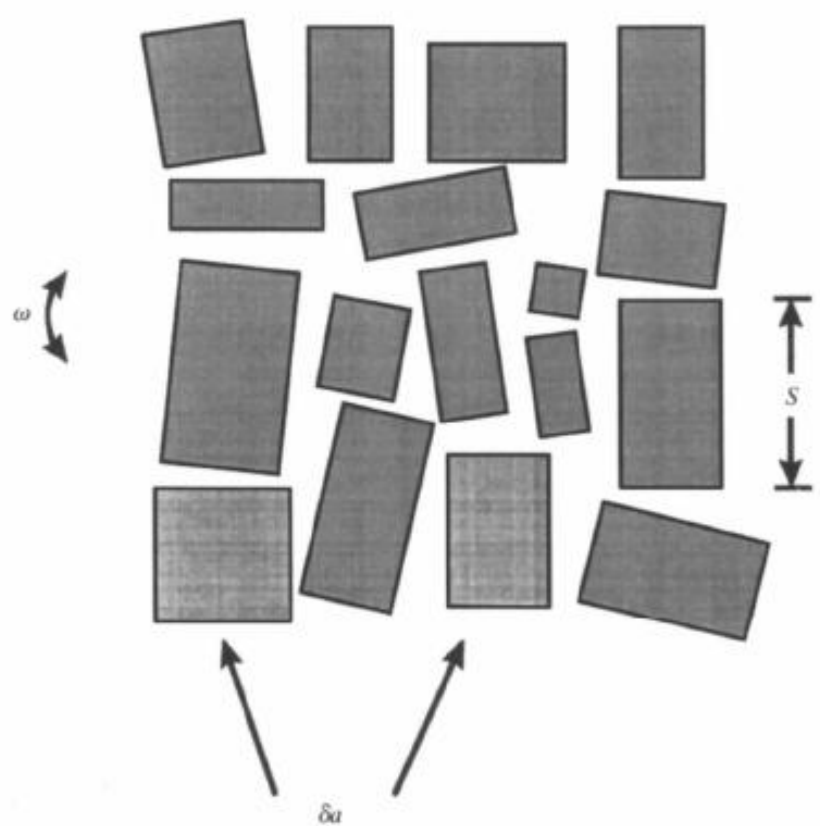


Fig. 1. A mosaic-block model of a crystal showing a spread in the orientation ω of the mosaic blocks, a spread in the size s of the blocks and a variation δa in the cell dimensions between different blocks.

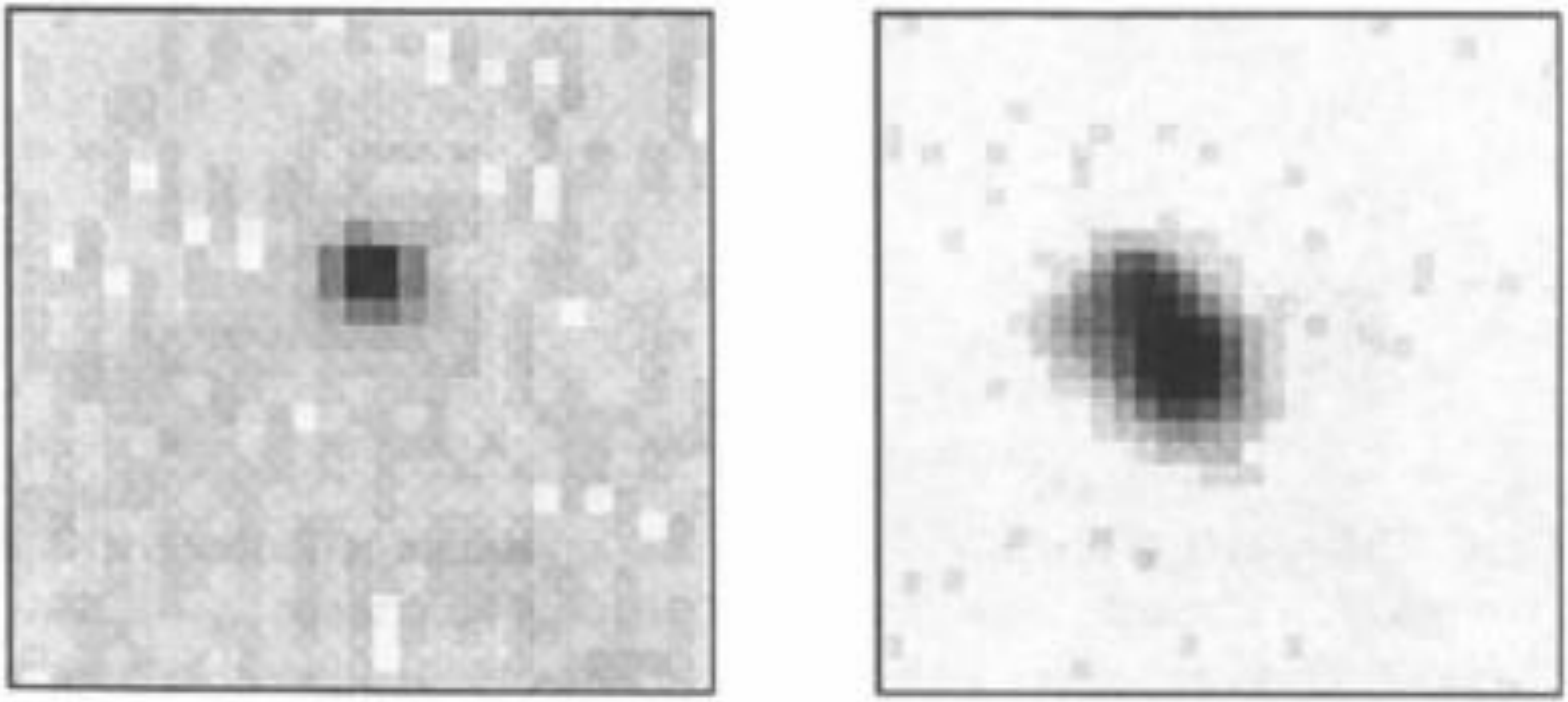
C. Nave (1998) Acta Cryst., **Vol. D54**, pp. 848 - 853.

Should high pressure freezing be more widely adopted?

Kim CU, Chen YF, Tate MW, Gruner SM. 2008. Pressure-induced high-density ice in protein crystals. *J. Appl. Crystallogr.* 41:1–7

Kim, C. U.; Barstow, B.; Tate, M. W.; Gruner, S. M. Evidence for liquid water during the high-density to low-density amorphous ice transition
PNAS, vol. 106, issue 12, pp. 4596-4600

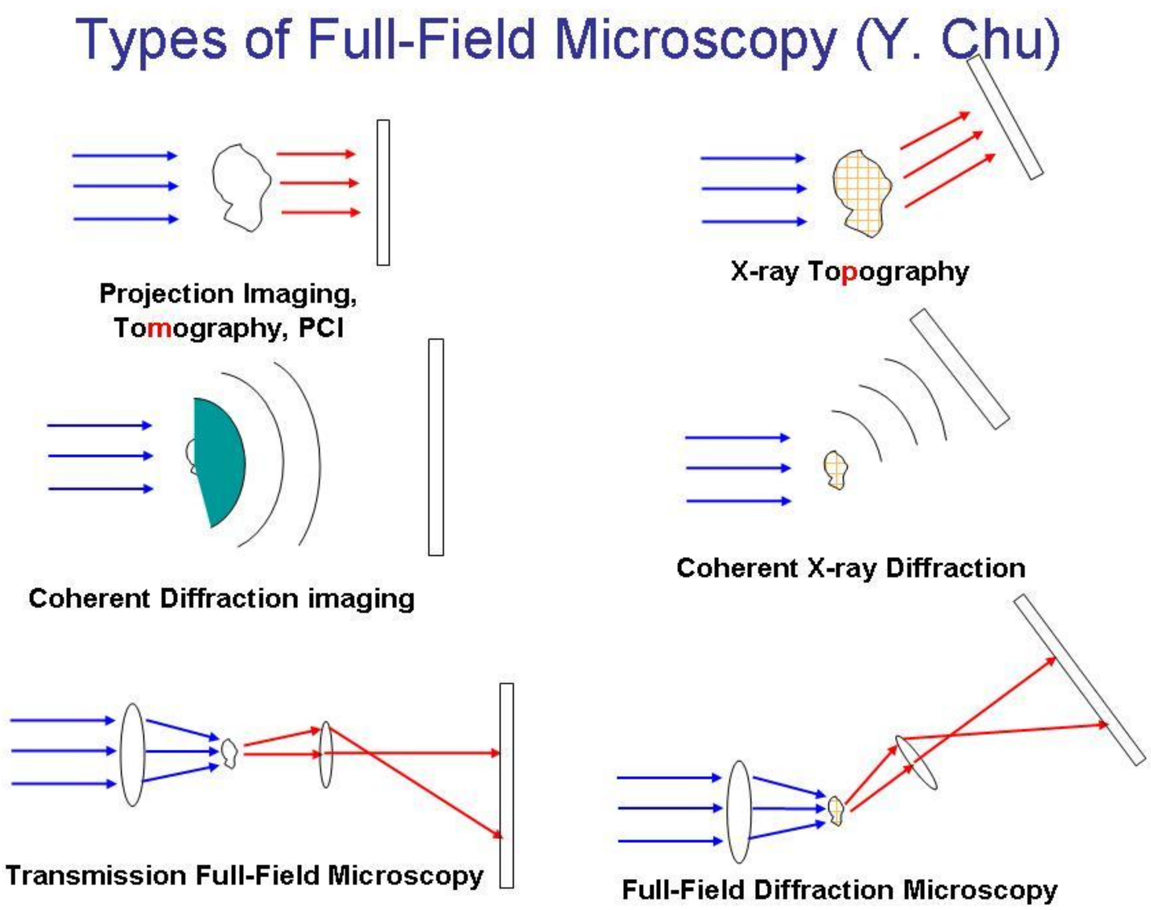
See more detail if increase detector distance with parallel beam



(d) 250mm (e) 1000mm

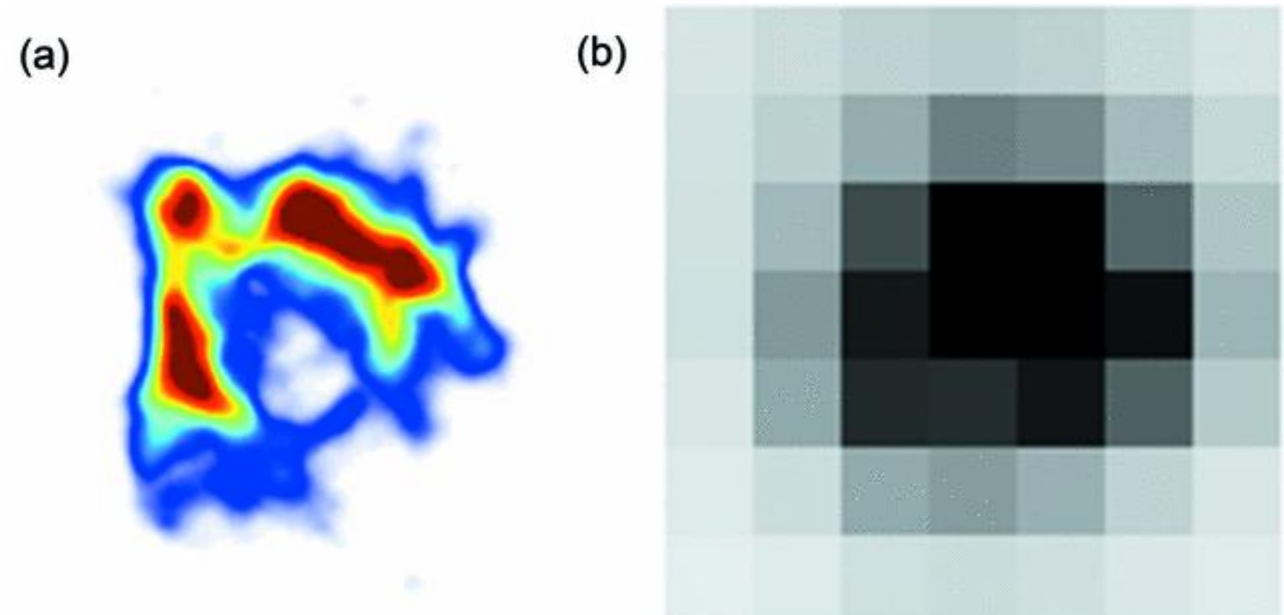
Improved signal to background, especially if can model spot shape properly

Obtaining Detailed Images of the Crystal



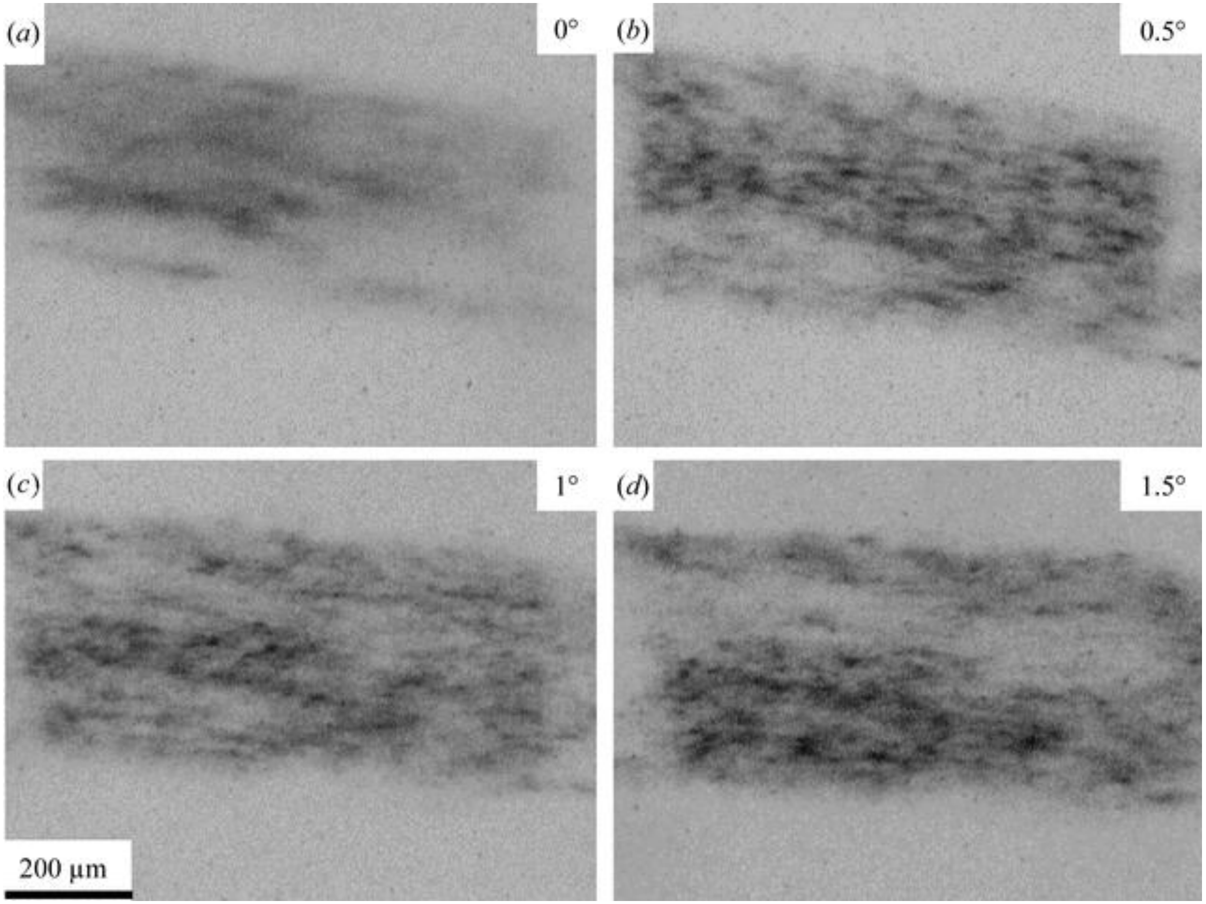
Obtain model from strong spots or straight on beam, then apply it to get better estimates for weak spots

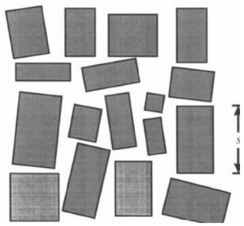
Tracking reflections through cryogenic cooling with topography



J. J. Lovelace, C. R. Murphy, R. Pahl, K. Brister and G. E. O. Borgstahl
J. Appl. Cryst. (2006). 39, 425-432

Sequences of topographic images from lysozyme Crystal 100K

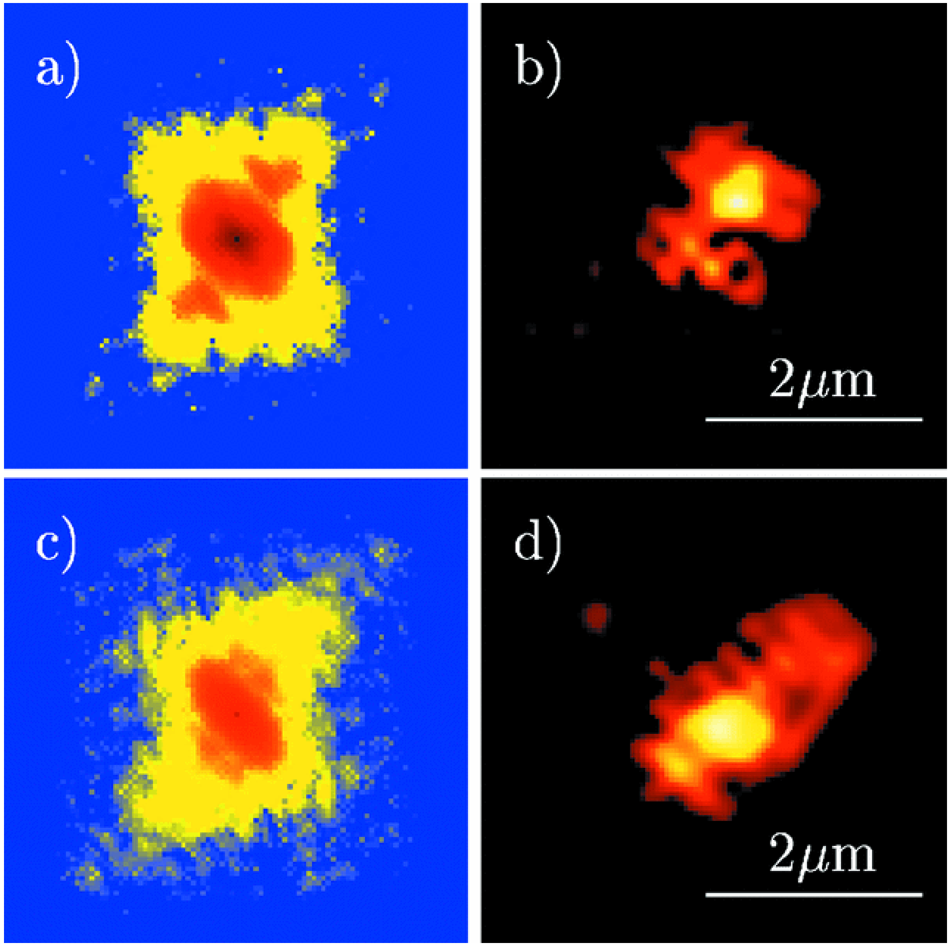




Compare with Model!

Kriminski, S., Caylor, C. L., Nonato, M. C., Finkelstein, K. D. & Thorne, R. E. (2002). *Acta Cryst.* D**58**, 459-471

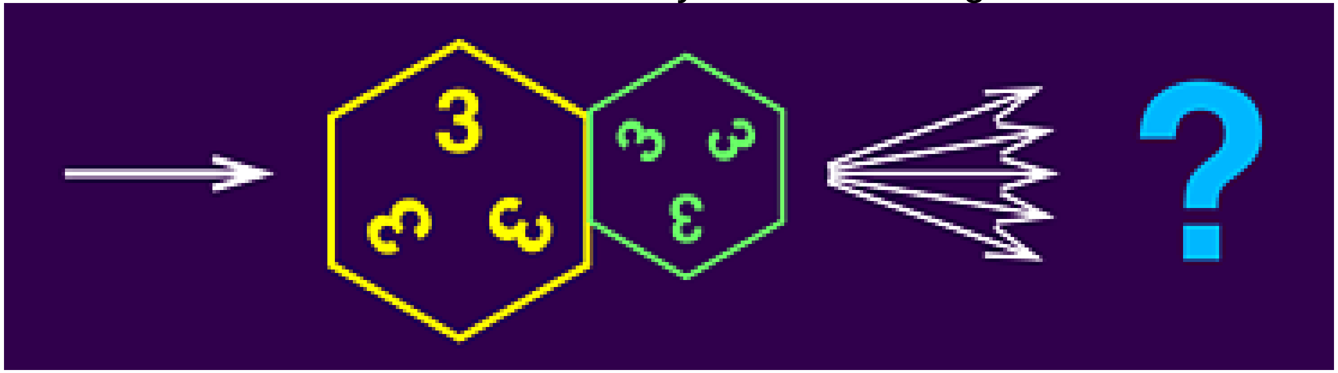
Coherent X-ray Diffraction - Reconstructed diffraction patterns and real-space images.



Boutet, S. & Robinson, I. K. (2008) *J. Synchrotron Rad.* **15**, 576-583

Image Components of Merohedral Twins?

Picture from Todd Yeates, Barry Fam Twinning Server



hkl and khl superimposed giving 6 fold symmetry if twin fraction 50%.

Imaging could resolve individual contributions

Summary

Microfocus beams could allow reduced radiation damage for small crystals

Parallel beams could allow better signal to background to be obtained

Both types of beam will be available at NSLS2

Will the detectors?

Prior Probabilities of Methods Being Widely Adopted

- Photo-electron escape – 40%
- Tomographic Imaging – 30%
- Topographic Imaging – 20%
- Coherent Diffraction Imaging – 10%
- Separating Merohedral Twins – 5%

Priors to be updated as people attempt to apply the above.

However, they already sum to over 100%!