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... for a brighter future

U.S. Department of Energy

UChicago Argonne LLC

Office of Science
U.S. DEPARTMENT OF ENERGY

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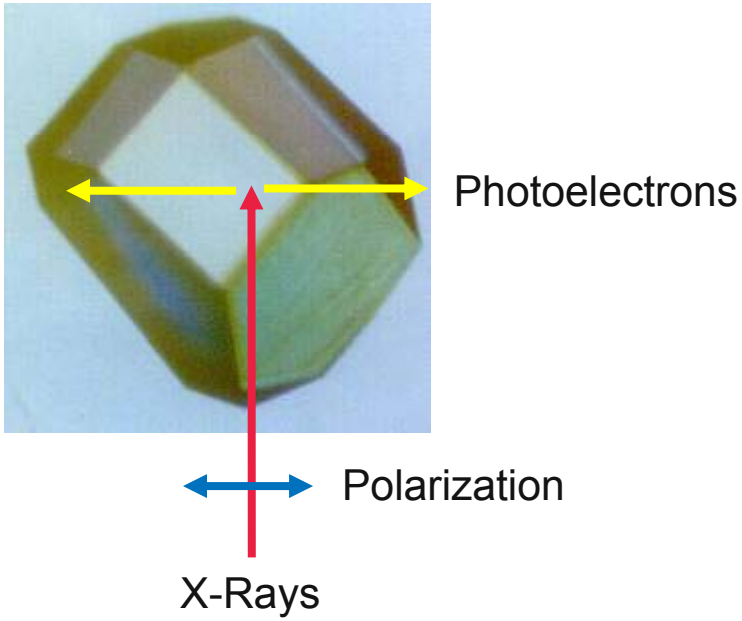
Microcrystallography and Mapping Radiation Damage with a 1-micron Beam at GM/CA-CAT



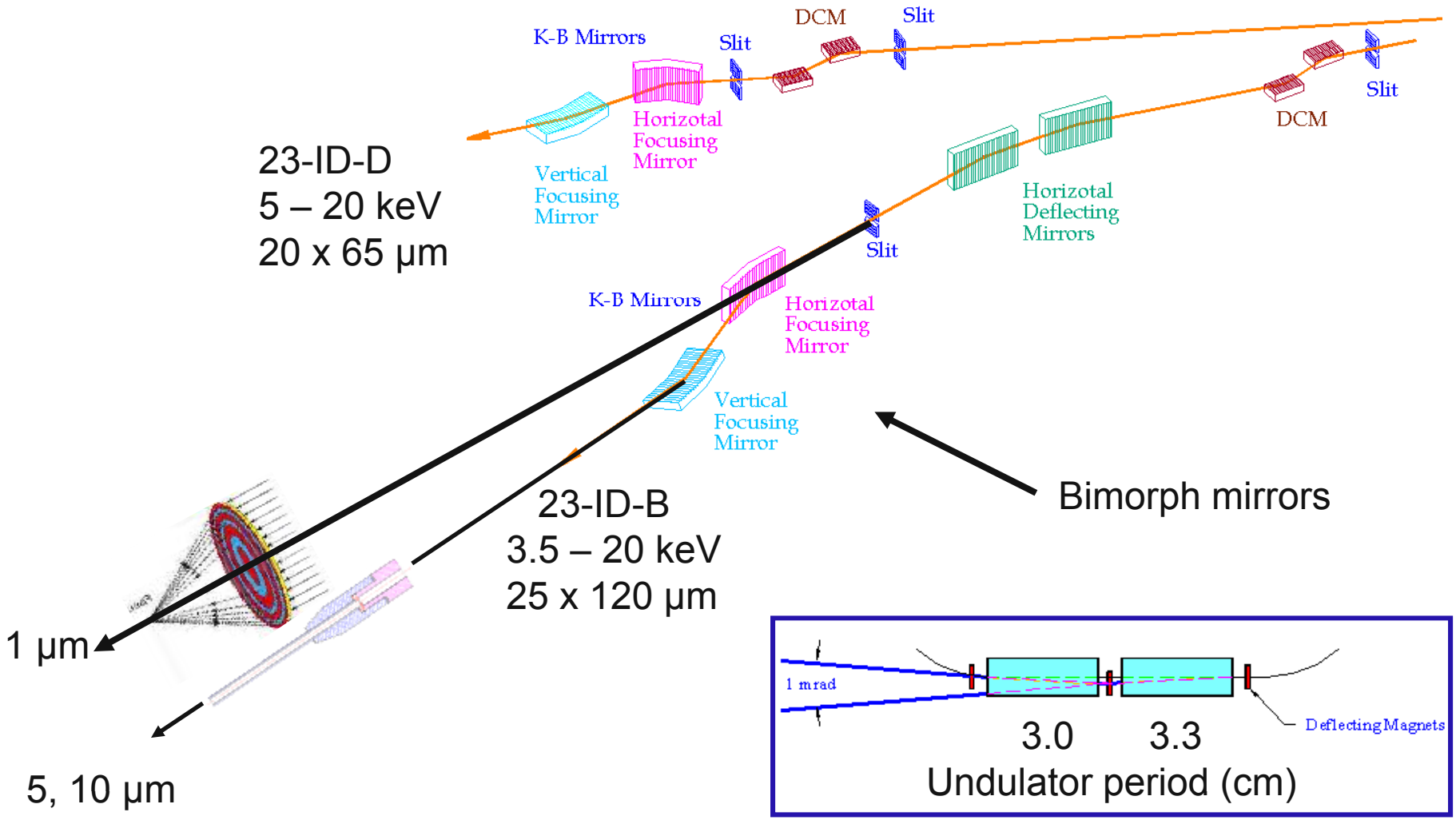
Bob Fischetti
Associate Director
GM/CA-CAT
Argonne National Laboratory

MX Frontiers at the 1-micron Scale
NSLS, Upton, NY
July 22, 2009

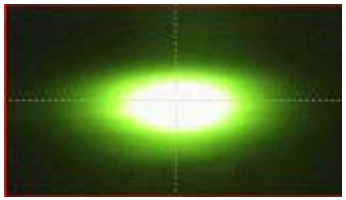
Micro-crystallography capabilities around the world (partial list)

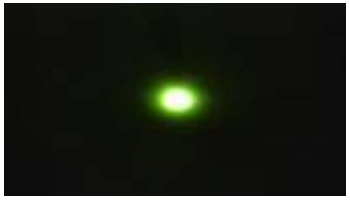
- ESRF
  - ID-13 “pioneering”
  - ID23-2: 7 microns
- APS
  - GM/CA-CAT: 1, 5, 10, 20 microns
  - Several sectors with MD2s: 10 , 20 microns
- Diamond
  - ID24: 5 microns
- Swiss Light Source
  - X06SA: 5 x 25 microns
- SSRL
  - 5 x 70 microns
- Petra-III “1st Light July 2009”
  - Planned micro-diffraction capabilities – 2010
- NSLS-II
  - Planned micro-diffraction capabilities

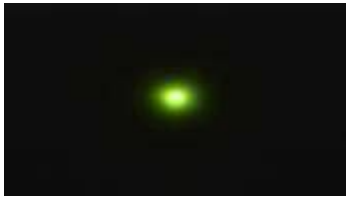
GM/CA-CAT dual canted undulator beamlines at the Advanced Photon Source



Beam properties







Beam	Size at sample, FWHM µm	Intensity Photons/sec	Flux density* Photons/sec/µm²	Convergence µ-radians
Full	25 x 120 20 x 65	1.0×10^{13} 2.0×10^{13}	3.3×10^{9} 1.5×10^{10}	176 x 95 305 x 172
10- **µm**	10.6 x 11.6 10.5 x 10.8	1.3×10^{11} 5.2×10^{11}	1.1×10^{9} 4.6×10^{9}	103
5- **µm**	4.8 x 6.2 5.0 x 5.1	2.7×10^{10} 5.4×10^{10}	9.1×10^{8} 2.1×10^{9}	
1- **µm**	1.1 x 1.2	3.0×10^{9}	2.2×10^{9}	310

* Flux density = total intensity / beam FWHM

How long can you collect on one spot?

Henderson limit[1] ~ 2×10^7 Gray

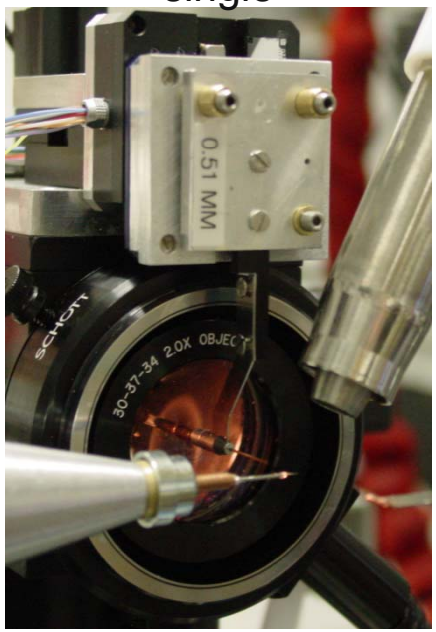
Deposited energy in sample – not incident!

Beam	Size at sample, FWHM (µm)	Intensity (Photons/sec)	Dose Rate [2] (Grays/sec)	Time to Henderson Limit (sec)
Full	25 x 120 20 x 65	1.0×10^{13} 2.0×10^{13}	0.13×10^7 0.61×10^7	15.4 3.3
10- **µm**	10.6 x 11.6	1.3×10^{11}	0.42×10^6	47.6
5- **µm**	4.8 x 6.2	2.7×10^{10}	0.36×10^6	55.6
1- **µm**	1.1 x 1.2	3.0×10^9	0.5×10^6	40.0

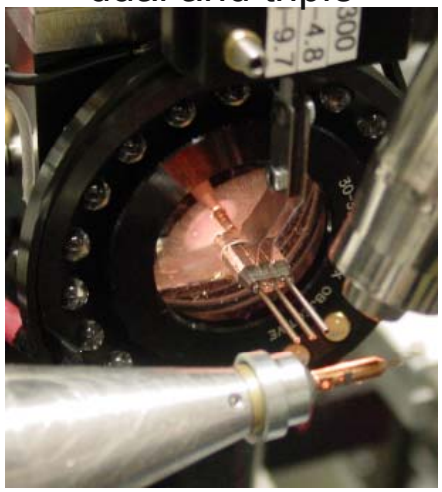
[1] Henderson (1990) Proc. R. Soc. Lond. B 241, 6-8
[2] RADDOSE http://biop.ox.ac.uk/www/garman/lab_tools.mtml

Evolution of the mini-beam collimator

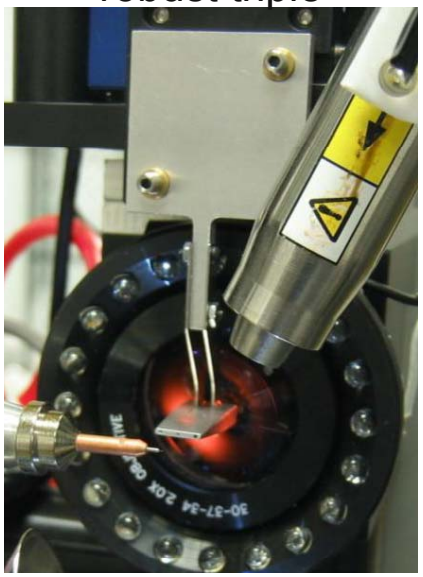
Feb 2007
single



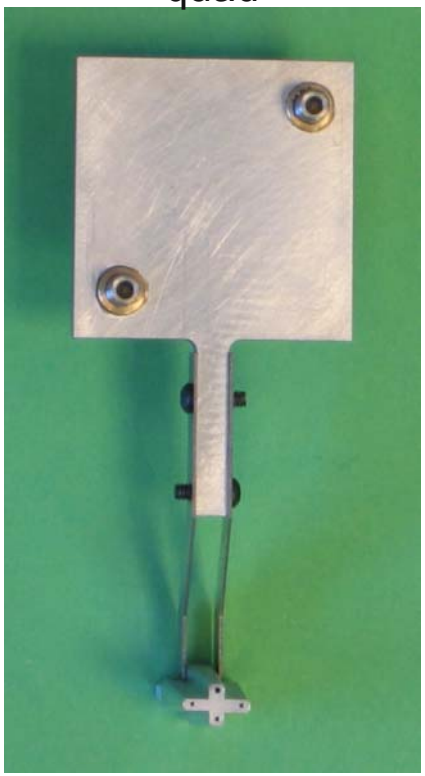
Feb 2008
dual and triple

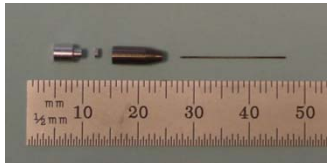


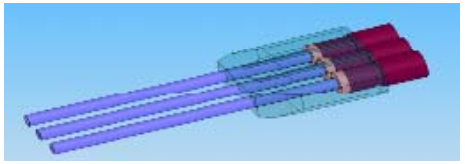
Feb 2009
robust triple

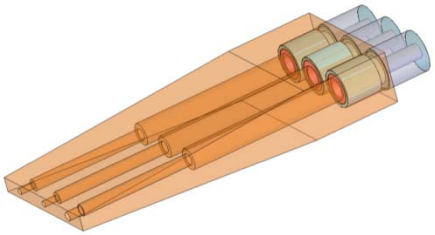


Jul 2009
quad







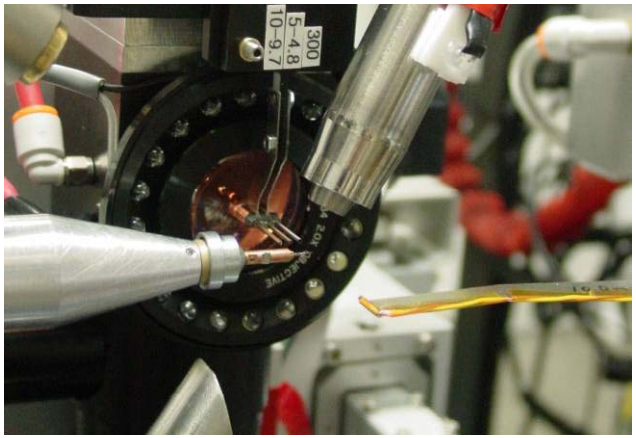


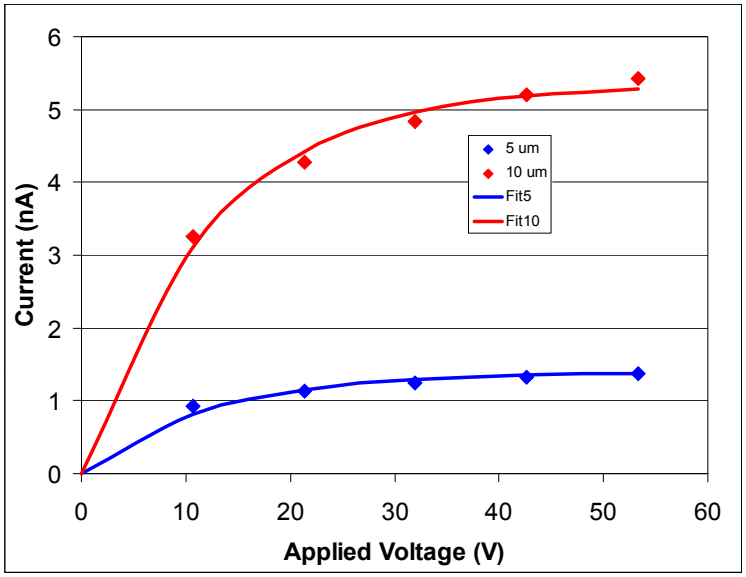
5, 10, 20 micron and scatter guard pin holes

Fischetti, R.F., Xu, S., Yoder, D.W., Becker, M., Nagarajan V., Sanishvili, R., Hilgart, M.C., Stepanov, S., Makarov, O. and Smith, J.L. (2009) Mini-beam collimator enables micro-crystallography experiments on standard beamlines, JSR 16, 217-225.

Active beamstop – based on photocurrent

Provides real time intensity monitor for mini-beam & automated realignment





Construction

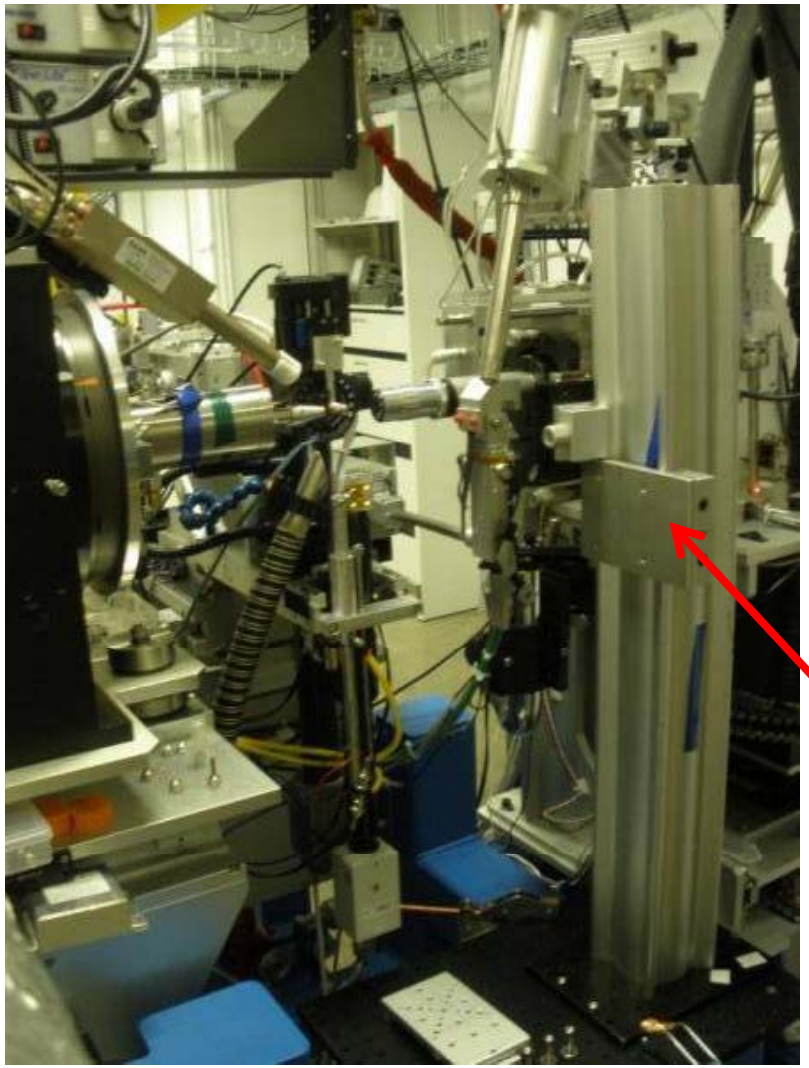
- 0.5 mm diameter beam stop
- 1.0 mm diameter concentric collector
- Applied voltage to drive e^- from stop to collector

Developing 0.5 mm diameter beamstop
"1 mm air = 1 μm of crystal" Colin Nave
-> He box maybe He jet at 100 K

Pin Hole	Intensity (photons/sec)	Measured current with 53 V bias (A)
300 micron	5.50E+12	1.46E-07
10 micron	2.18E+10	5.43E-09
5 micron	4.58E+09	1.38E-09

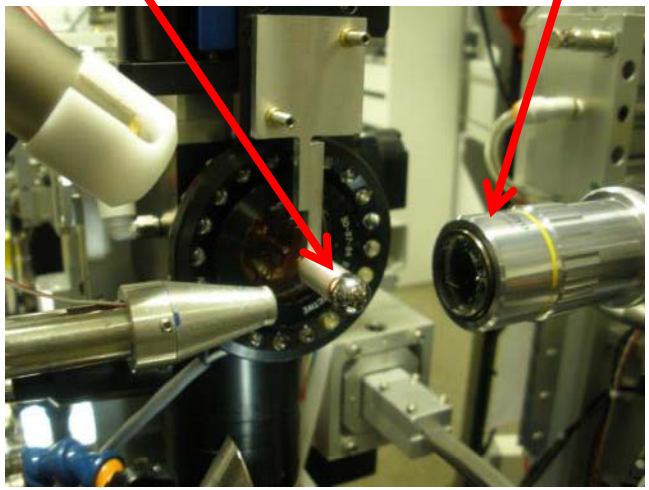
Lin Yang, Macromolecular Research, **13**, 1-4 (2005).

"Auto-collimator" device for non-contact measurement



Precision tooling ball

Objective lens added to Newport Optical Autocollimator

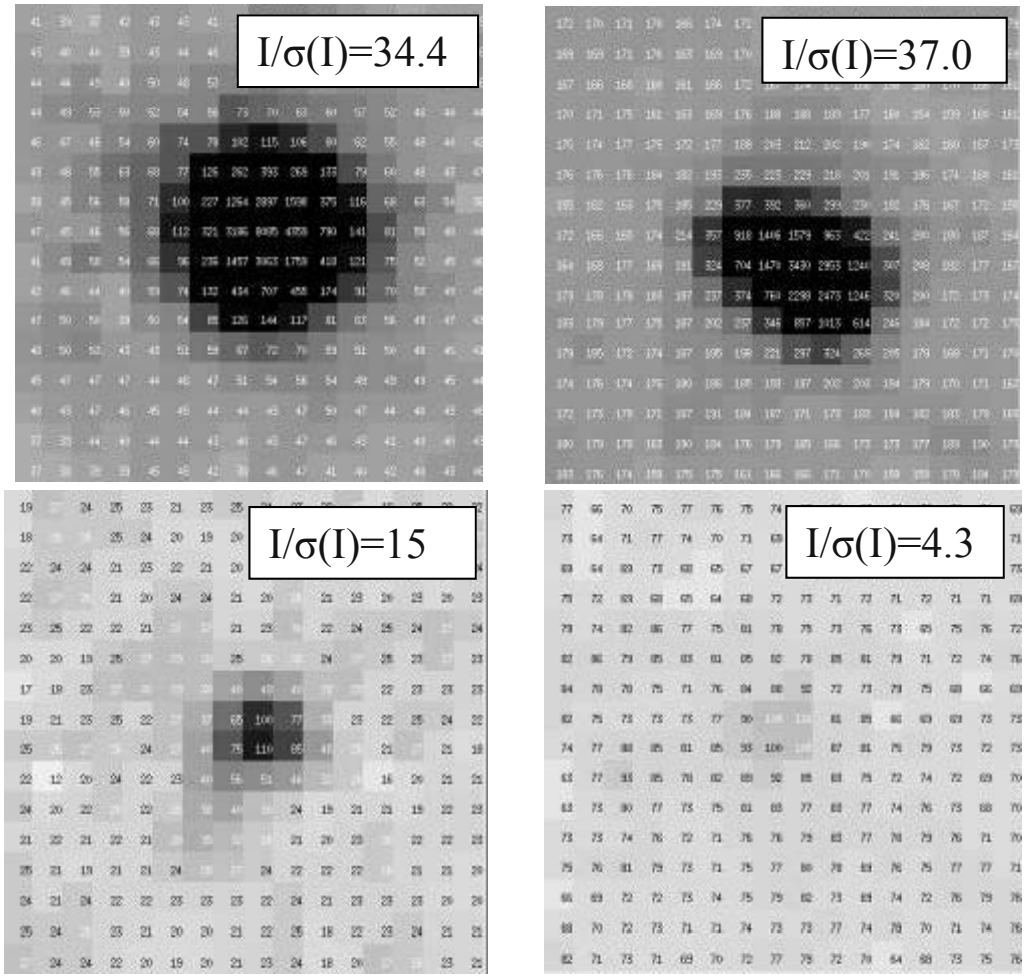


Temporary support for autocollimator

Benefits of the mini-beam (I/σ)

Same reflection with

mini-beam standard beam



At ~10 Å resolution

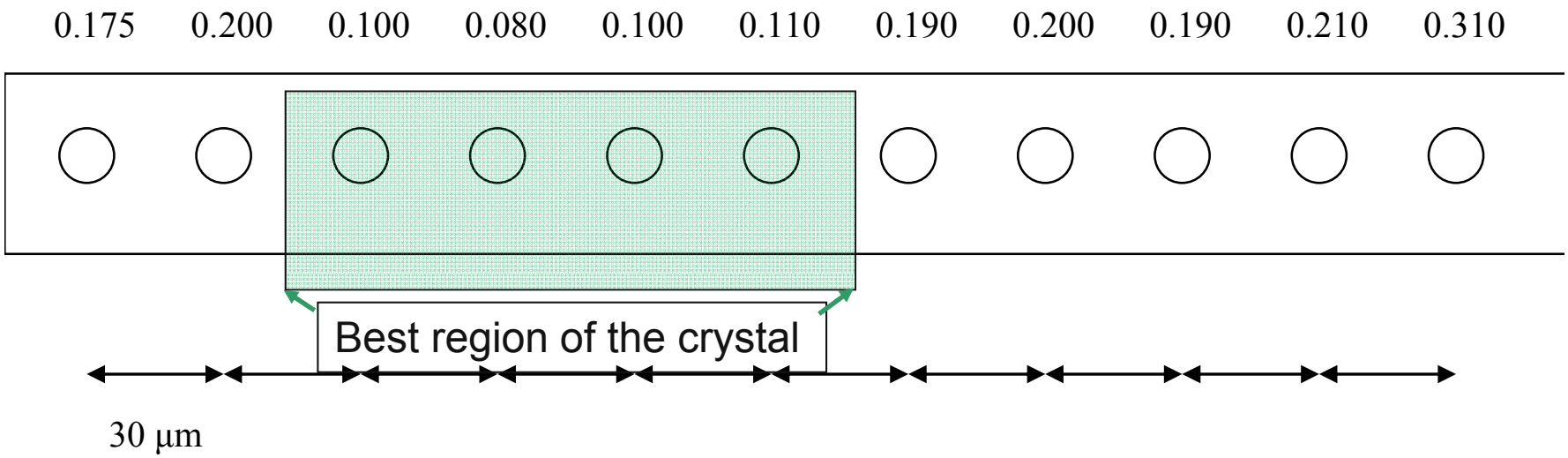
At ~2.7 Å resolution

Optimized volume ratio:
Crystal / Support

Nukri Sanishvili

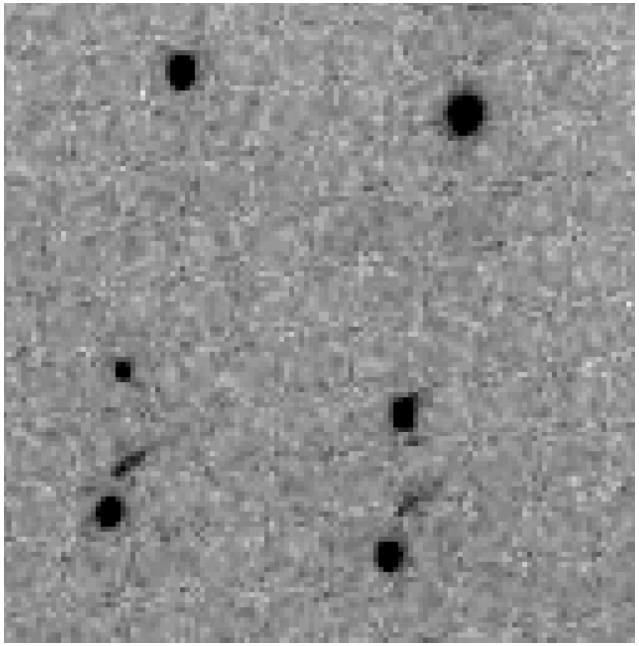
Additional benefits of the mini-beam
Finding best spots of the crystal for data collection

10 frames of 0.2° data were collected from each of the 11 spots along the 250 micron long crystal.
Mosaicity values are shown as refined by HKL2000

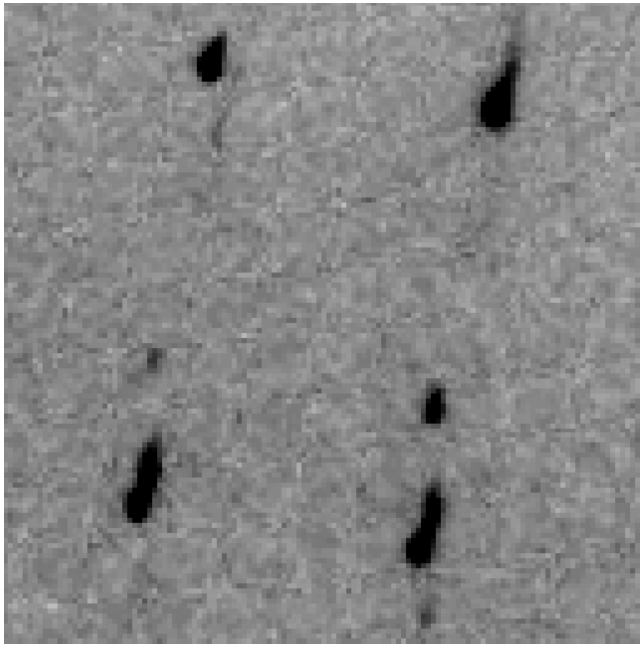


Nukri Sanishvili

Additional benefits of the mini-beam
Better spot resolution and improving spot shapes



A



B

Same slice of Ewald sphere generated by

A) mini-beam (8 x 6 μm)

B) “standard” beam (140 x 30 μm)

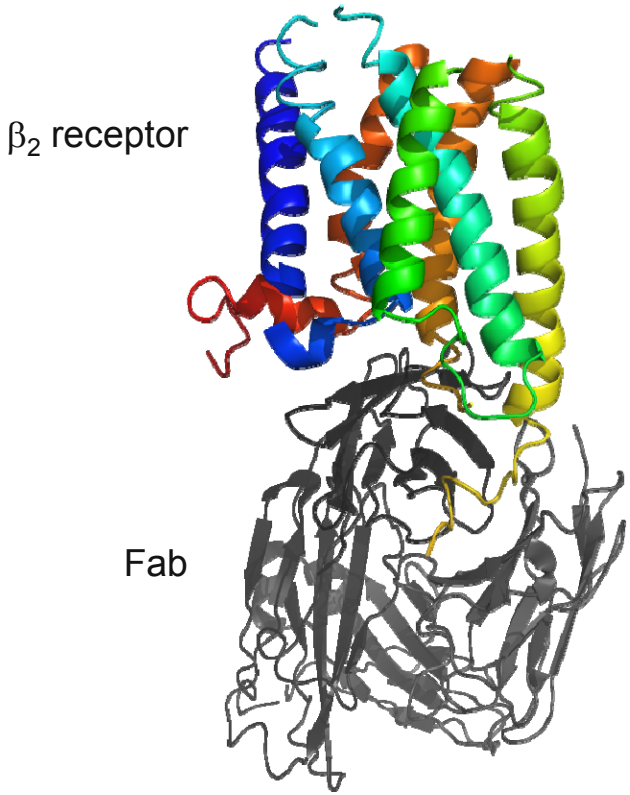
Imperfect sample crystal was either cracked or had a satellite.

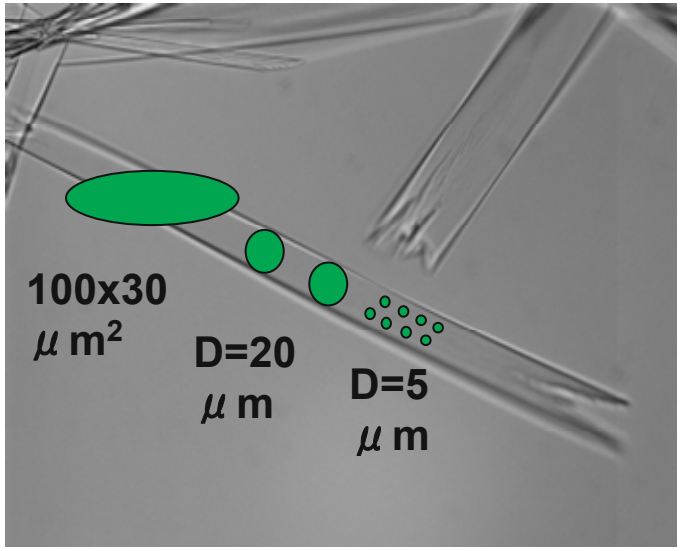
A single-crystal region is illuminated with the mini-beam.

Nukri Sanishvili

User Needs Drove Development of the Mini-beam - Radiation Sensitivity

S. G. F. Rasmussen, H.-J. Choi, D. M. Rosenbaum, T. S. Kobilka, F. S. Thian, P. C. Edwards, M. Burghammer, V. R. P. Ratnala, R. Sanishvili, R. F. Fischetti, G. F. X. Schertler, W. I. Weis, **B. K. Kobilka**, "Crystal structure of the human β_2 adrenergic G-protein-coupled receptor", *Nature* **450**, 383-387 (2007).





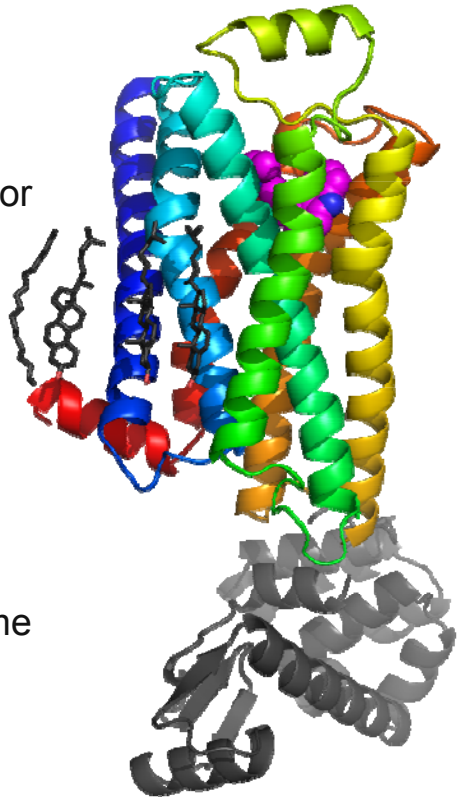
User Needs Drove Development of the Mini-beam - Microcrystals

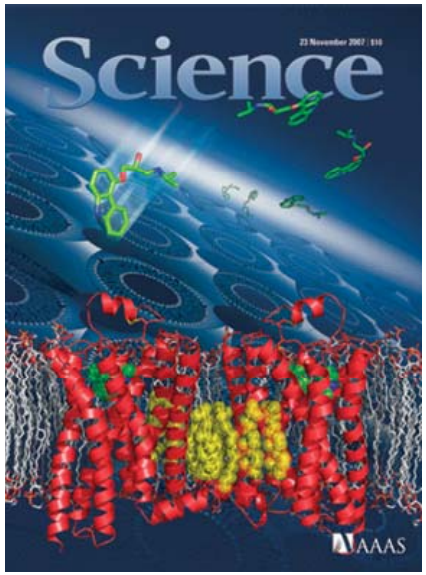
D. M. Rosenbaum, V. Cherezov, M. A. Hanson, S. G. F. Rasmussen, F. S. Thian, T. S. Kobilka, H.-J. Choi, X.-J. Yao, W. I. Weis, R. C. Stevens, **B. K. Kobilka**, "GPCR engineering yields high-resolution structural insights into β_2 adrenergic receptor function" *Science* **318**, 1266-1273 (2007).

V. Cherezov, D. M. Rosenbaum, M. A. Hanson, S. G.F. Rasmussen, F. S. Thian, T. S. Kobilka, H.-J. Choi, P. Kuhn, W. I. Weis, B. K. Kobilka, **R. C. Stevens**, "High-resolution crystal structure of an engineered human β_2 adrenergic G-protein-coupled receptor" *Science* **318**, 1258-1265 (2007).

β_2 receptor

T4 lysozyme





Lipidic cubic phase - microcrystals

Micro-crystals mean lots of screening

SSRL's BluIce modified to communicate directly with EPICS Berkeley/ALS sample automounter – Thomas Earnest and Carl Cork

TYPICAL user group: screens 120 crystals and collects 20 data set

BLU-ICE: Beamline ID-D Version 702

File Network Options Help

Hutch | Sample | Collect | Screening | Scan | Users | Setup

Spreadsheet: /mnt/staffhome/spothineni/ALS_ten Import Distance: 908.172 mm B-warmup: 0 sec

Automounter status

Current: cool_down, 9.971V > 2.500V

-1: unloading current sample Command: load A 2 Ready Paused

-2: ready

None	Load	Port	ID	Protein	Comment	Directory	Freezing
0		A	1	Protein1		Dir1	
1		A	2	Protein2		Dir2	
2		A	3	Protein3		Dir3	
3		A	4	Protein4		Dir4	
4		A	5	Protein5		Dir5	
5		A	6	Protein6		Dir6	
6		A	7	Protein7		Dir7	
7		A	8	Protein8		Dir8	
8		A	9	Protein9		Dir9	
9		A	10	Protein10		Dir10	
10		A	11	Protein11		Dir11	
11		A	12	Protein12		Dir12	
12		A	13	Protein13		Dir13	
13		A	14	Protein14		Dir14	
14		A	15	Protein15		Dir15	
15		A	16	Protein16		Dir16	
16		B	1	Protein17		Dir17	

Directory: /mnt/evagfs1/user0 Browse

Task list

- Mount Next Crystal
- AutoCenter Crystal
- Pause4Center
- Collect Image 0
- Collect Image 45
- Collect Image 90
- Pause4Inspection
- Analyse with Web-Ice

Start Resume Pause

A2 MountNextXtal
A2 Paused4Center
A3 MountNextXtal
A3 Paused4Center
A4 MountNextXtal
A4 Paused4Center
A5 MountNextXtal
A5 Paused4Center
A6 MountNextXtal
A6 Paused4Center

Sample status

Mounted: A 1

Unmount sample

Mount history:

-0: A 1
-1: F 4
-2: F 3
-3: F 2
-4: F 1
-5: C 1
-6: B 10
-7: B 9
-8: B 8
-9: C 16

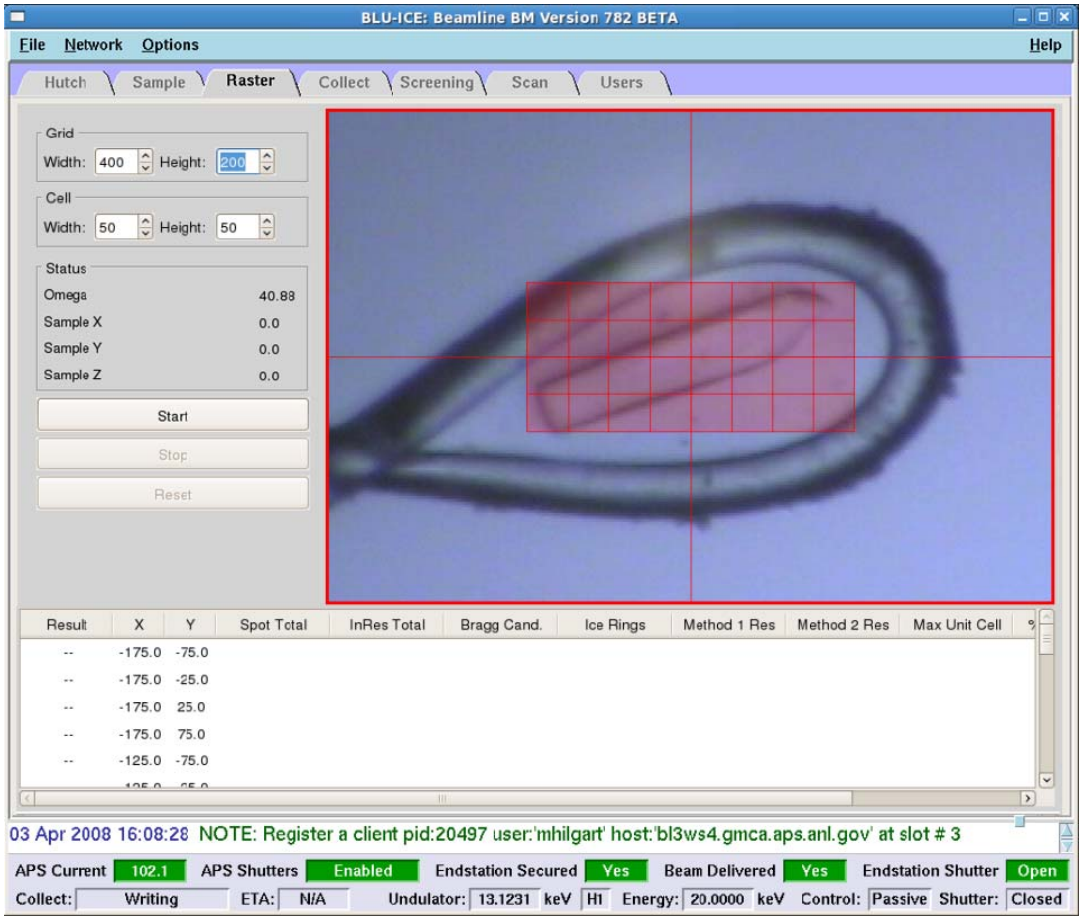
Puck type: ALS

12 Feb 2008 10:34:31 NOTE: Crystal list updated

Collect: Writing ETA: N/A Undulator: 15.4264 keV H1 Energy: -22.3509 keV Control: Active Shutter: Closed

Low dose - diffraction based centering

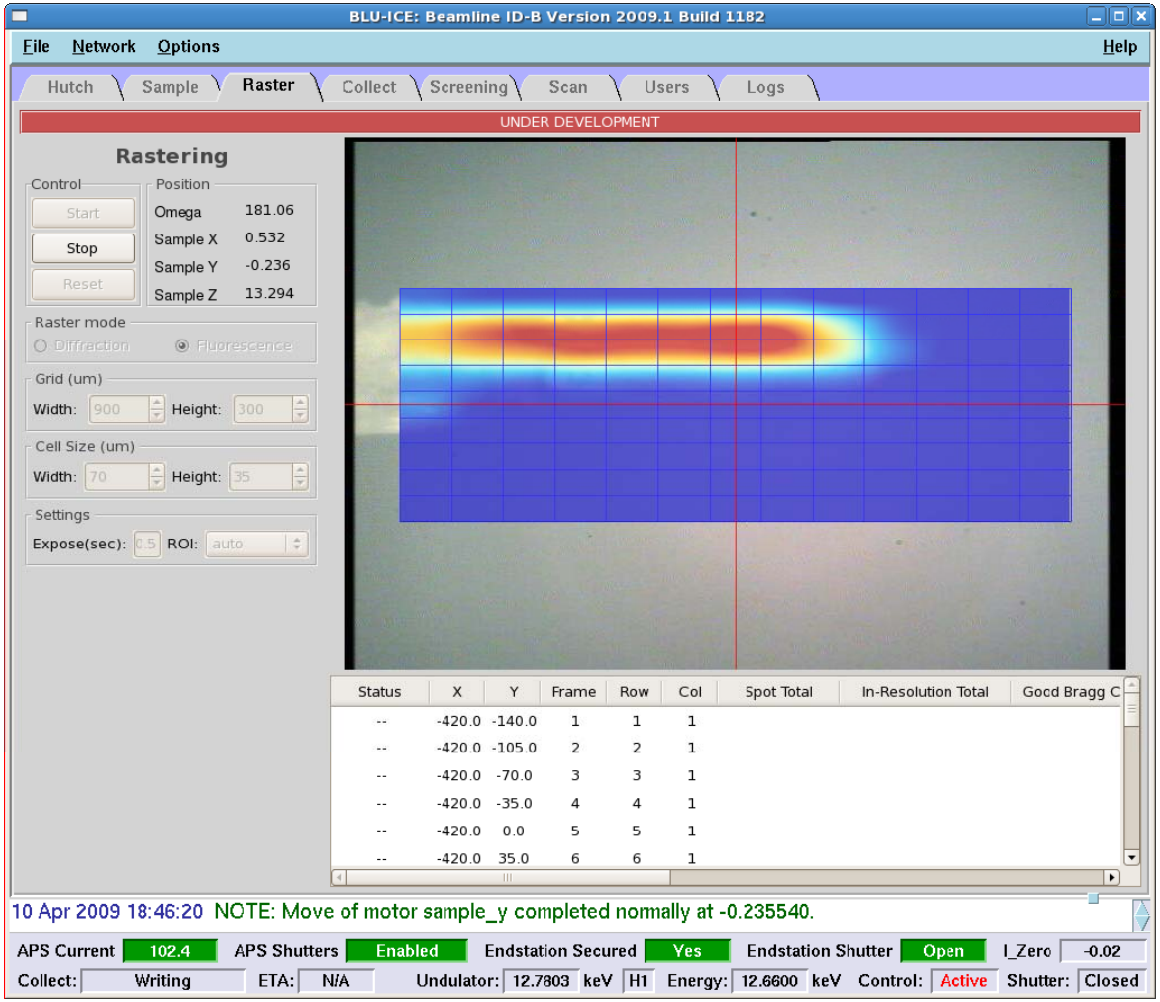
Probe with highly attenuated beam

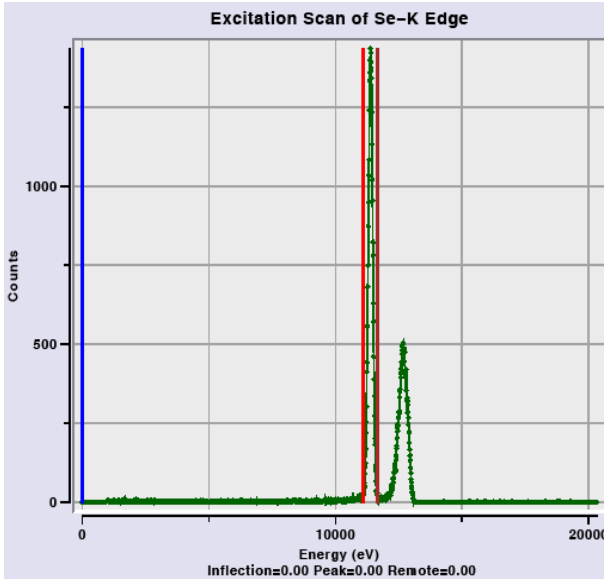


- Micro-crystals can be difficult to center optically
  - refraction effects
  - lipidic cubic phase
- Configurable grid and cell size
- Diffraction is scored by distl
- Iterate full beam and mini-beam

X-ray fluorescence rastering implemented

Fluorescent rastering is fast → lower radiation dose load on the sample





Candidate proteins

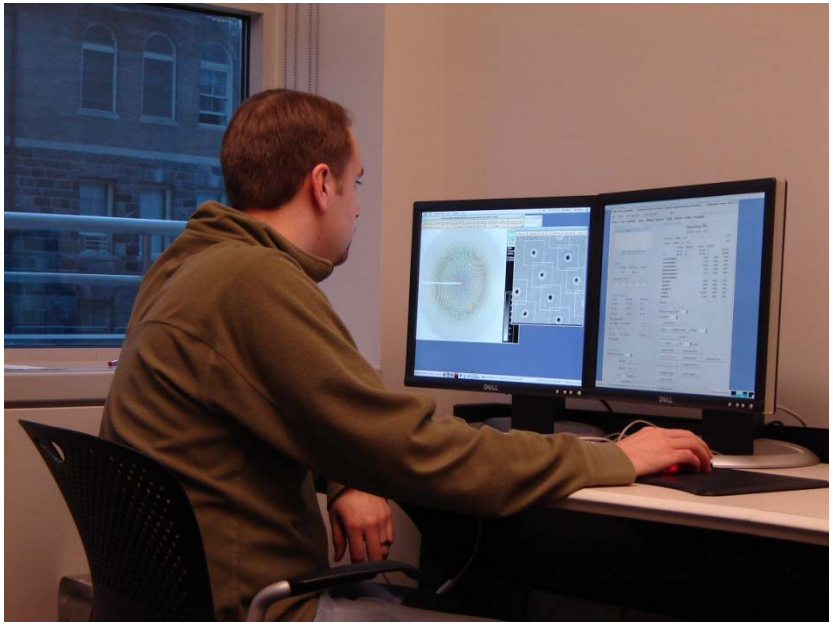
- Almost 1/3 of all proteins contain metals
- Se-derivatives
- Crystals back soaked with a heavy-atom

Process

- Fluorescence window is chosen depending on the element of interest
- 2D grid is defined by user and automatically scanned

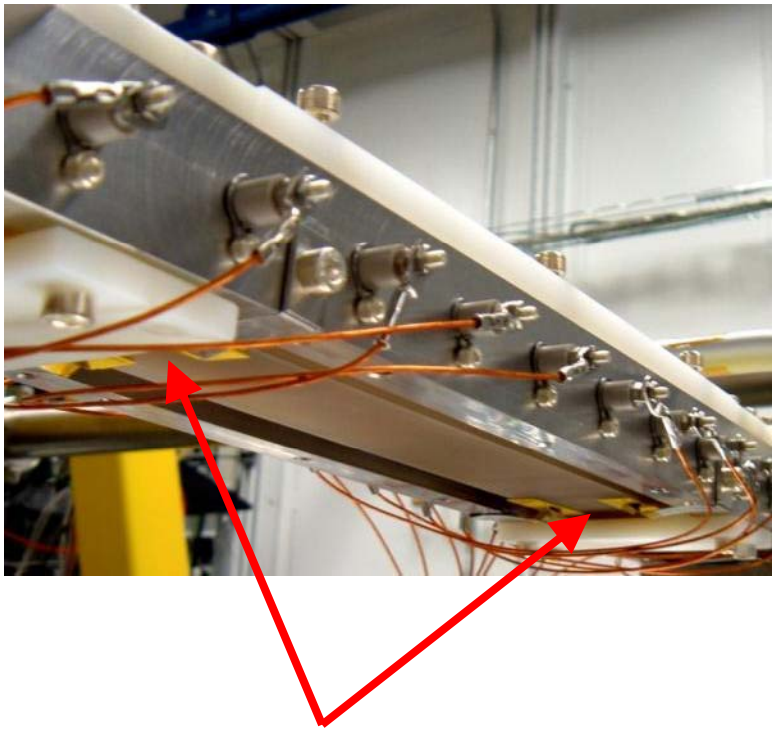
Remote Access: Technology

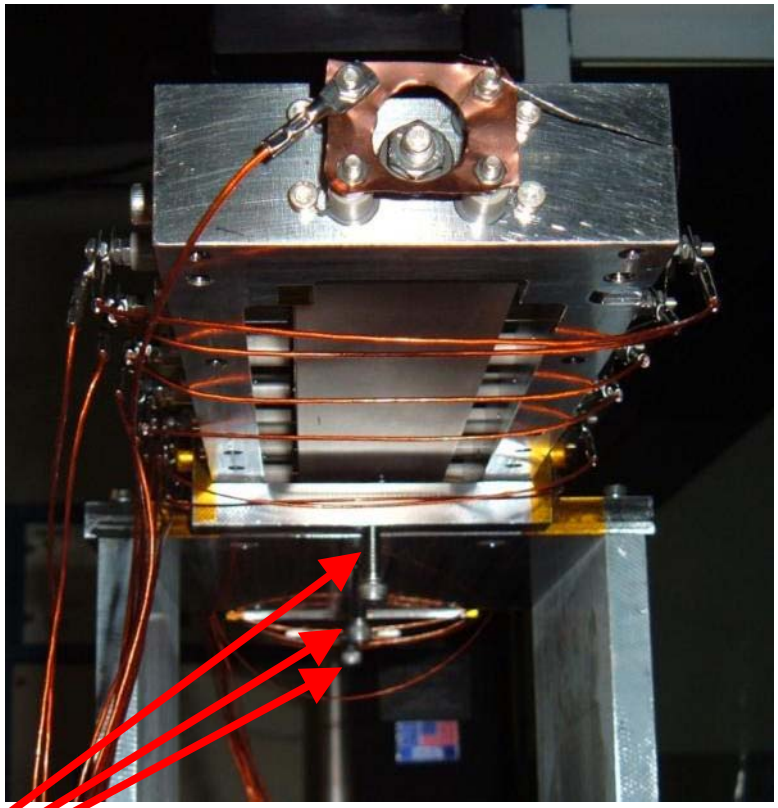
- **Relies on automation already in place:**
  - 100% automated beam optimization
    - Latest additions include:
      - Beam recentering
      - Fluorescence detector
  - Automated screening
  - Reliable robot operation
- **NOMACHINE (NX) servers:**
  - Same as at SSRL, SER-CAT, ALS, others
  - Session is preserved if connection is lost
  - Two computers open per beamline (one for data collection & one for processing)
  - Security through IP address filtering
- **WebIce:**
  - Porting is complete, testing has started
  - Blu-Ice integration will begin soon



Remote processing with HKL2000

Re-polished mirrors and redesigned gravity compensation



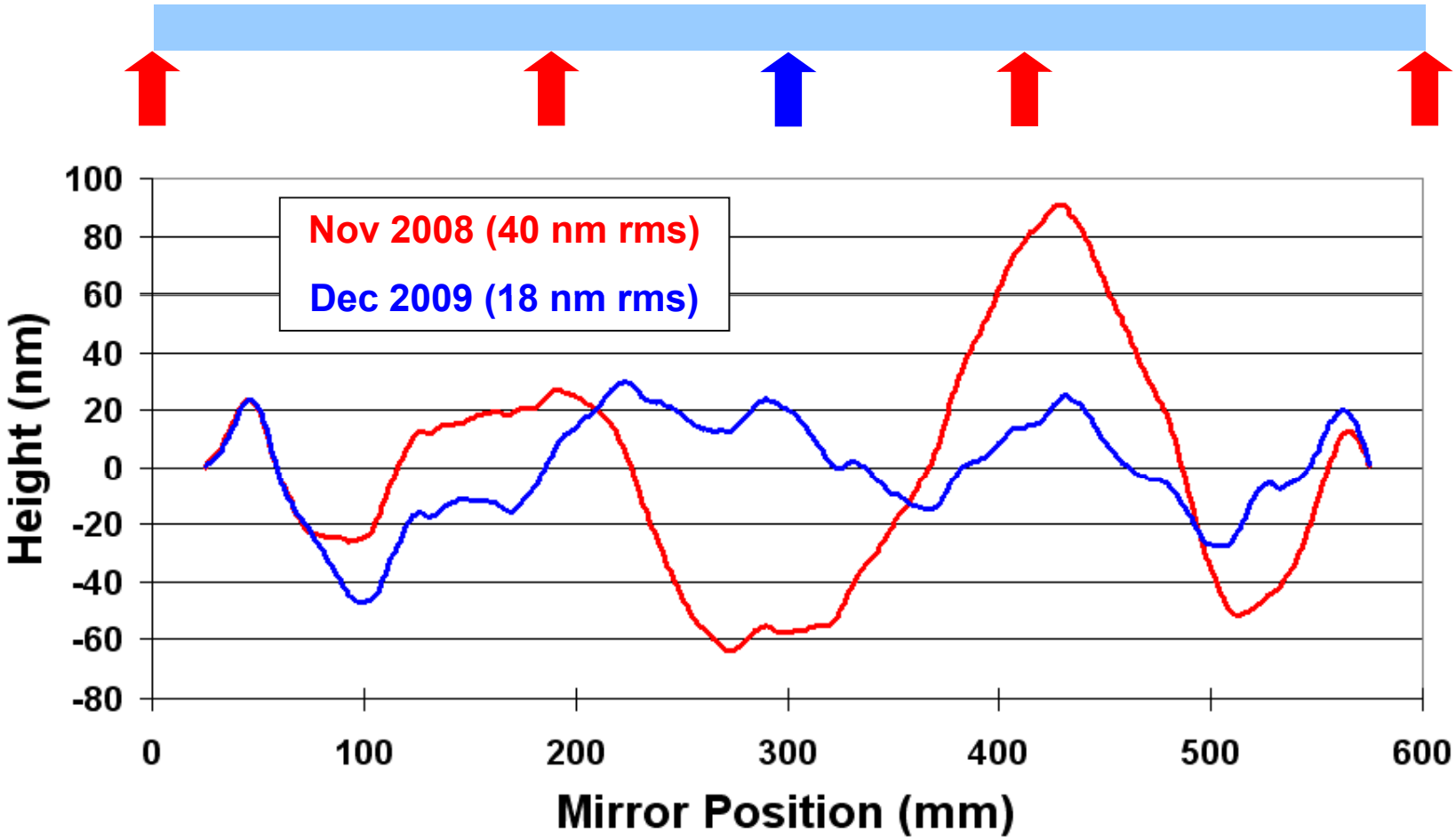


Nov 2008 – Two locations adjusted with shims

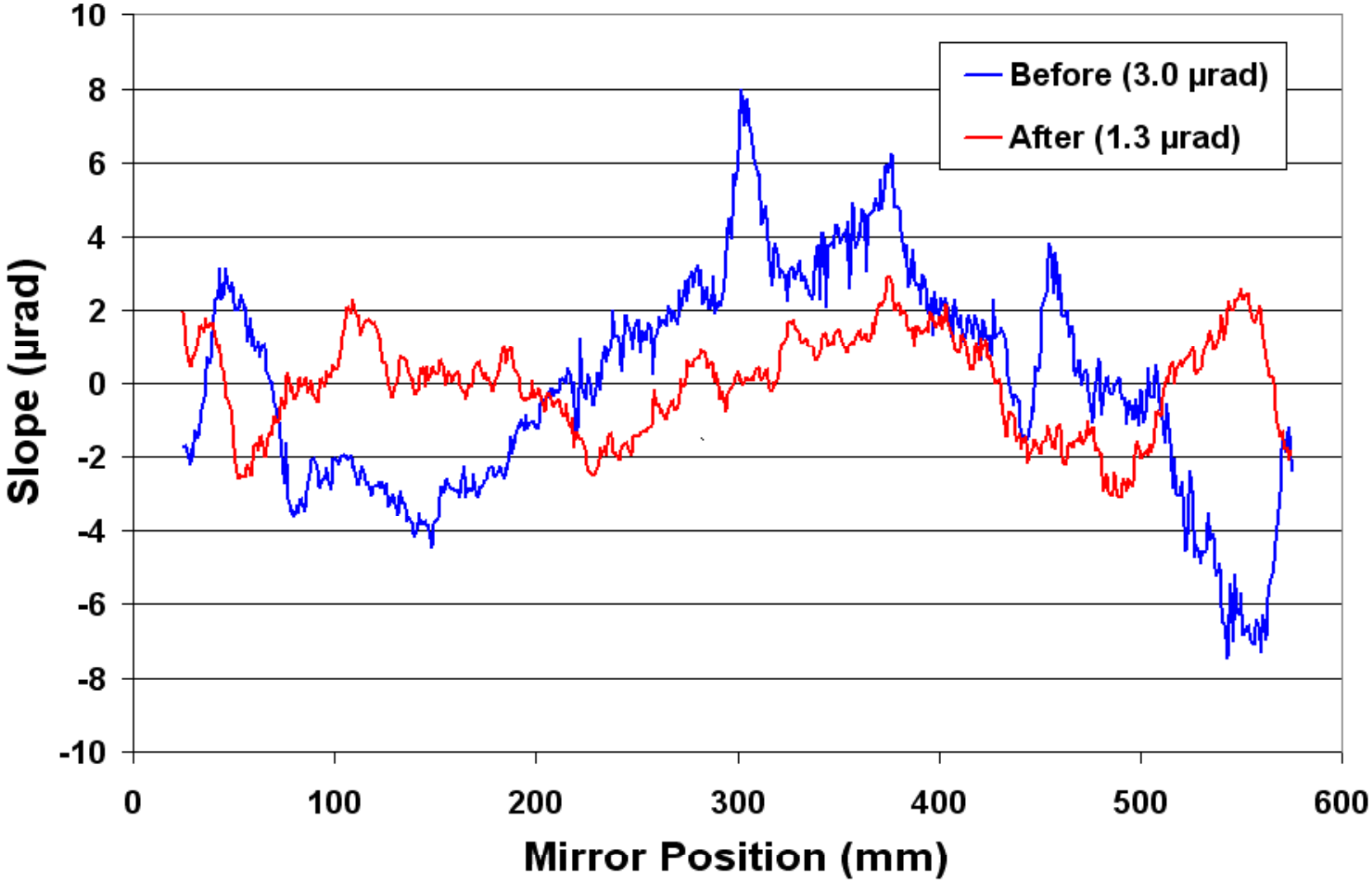
Jan 2009 – Three locations with screw adjustment

Improved Height – Jan 2009

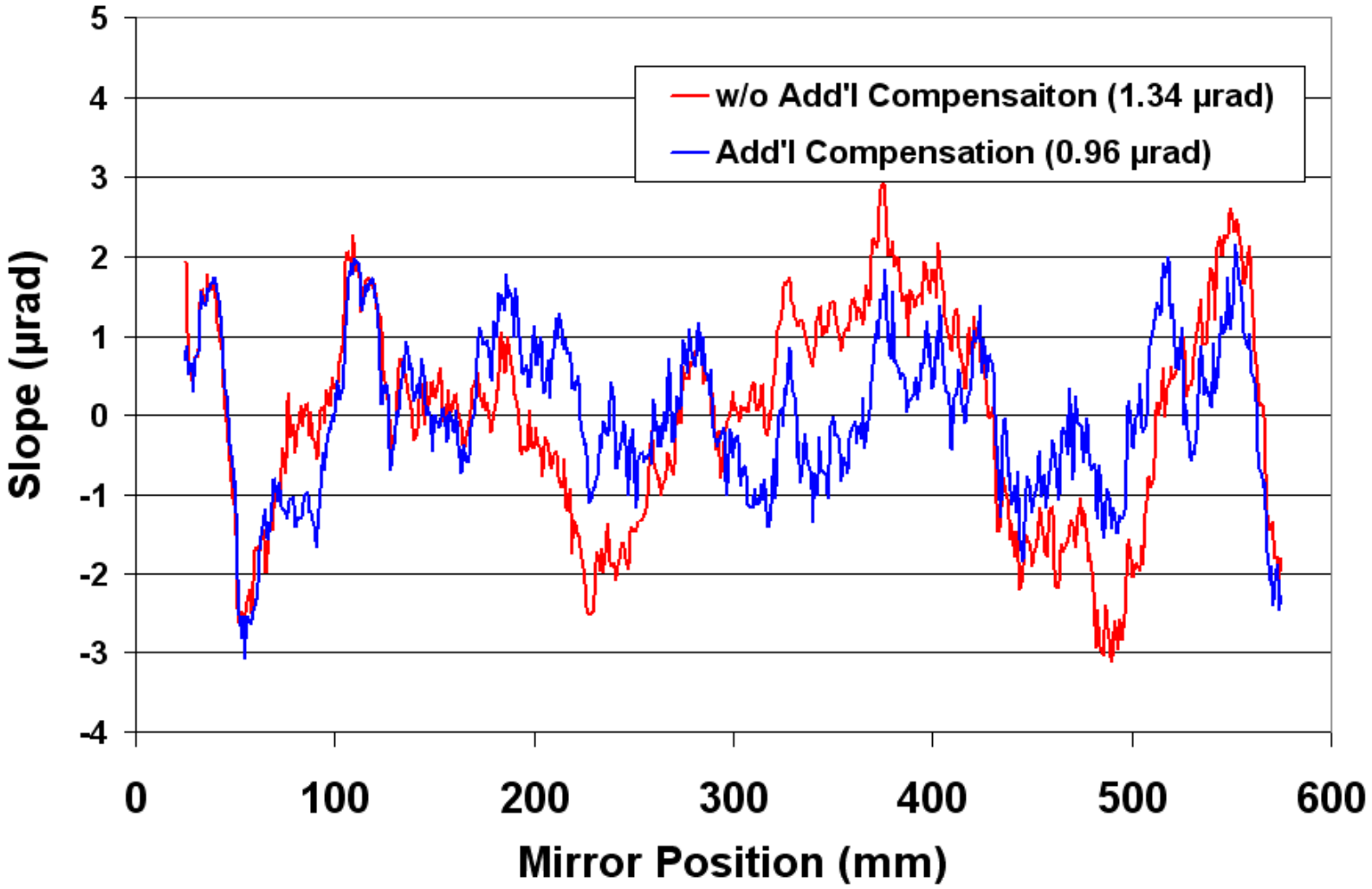
Locations of gravity compensation:



Slope Error of $VFM_{ID\text{-}D}$ – Before and After Repolishing

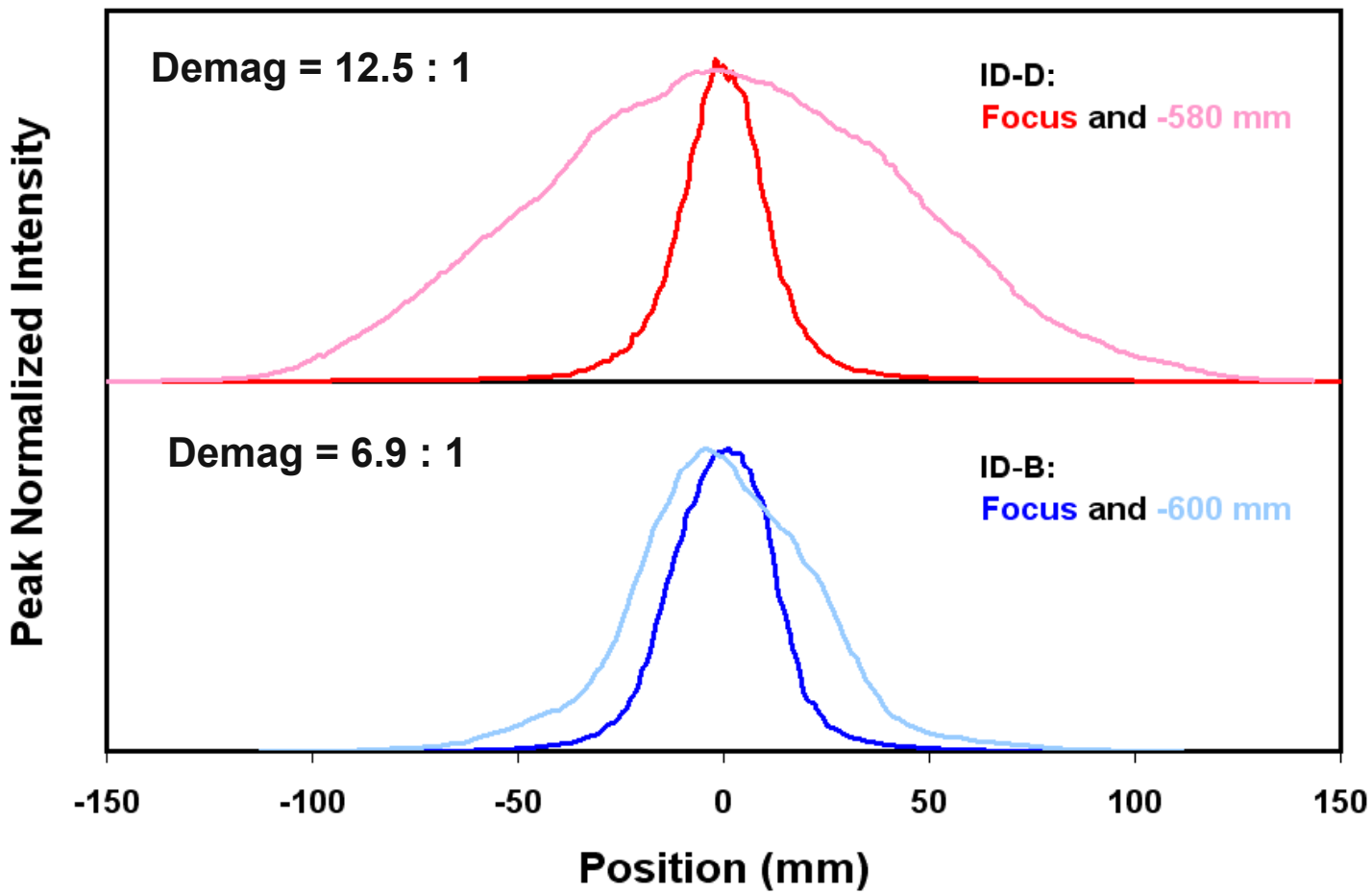


Improved Slope Error – Jan 2009



Vertical Focus, Beamline Comparison – February 2009

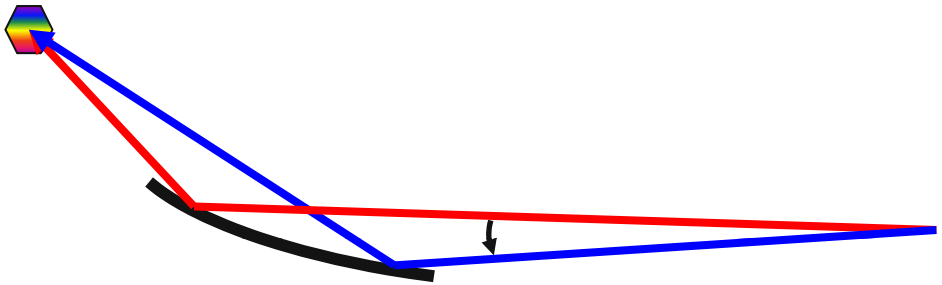
Result: excellent beam profile both on-focus and off-focus



Slope error effects beam size AND positional stability

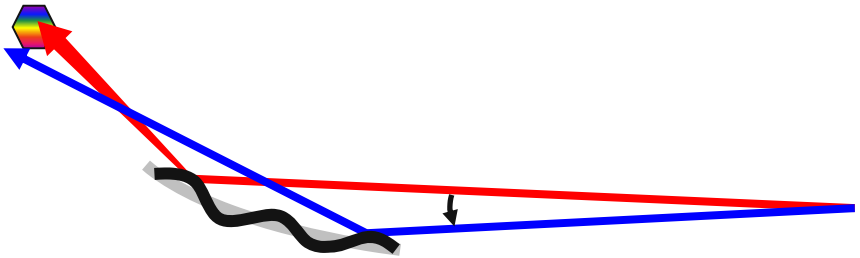
Ideal mirror (with zero slope error):

- **All beamlets focus to same point**
- **Direction of beamlet insensitive to change in angle of source**
- **Source transverse motion is demagnified at the focus**

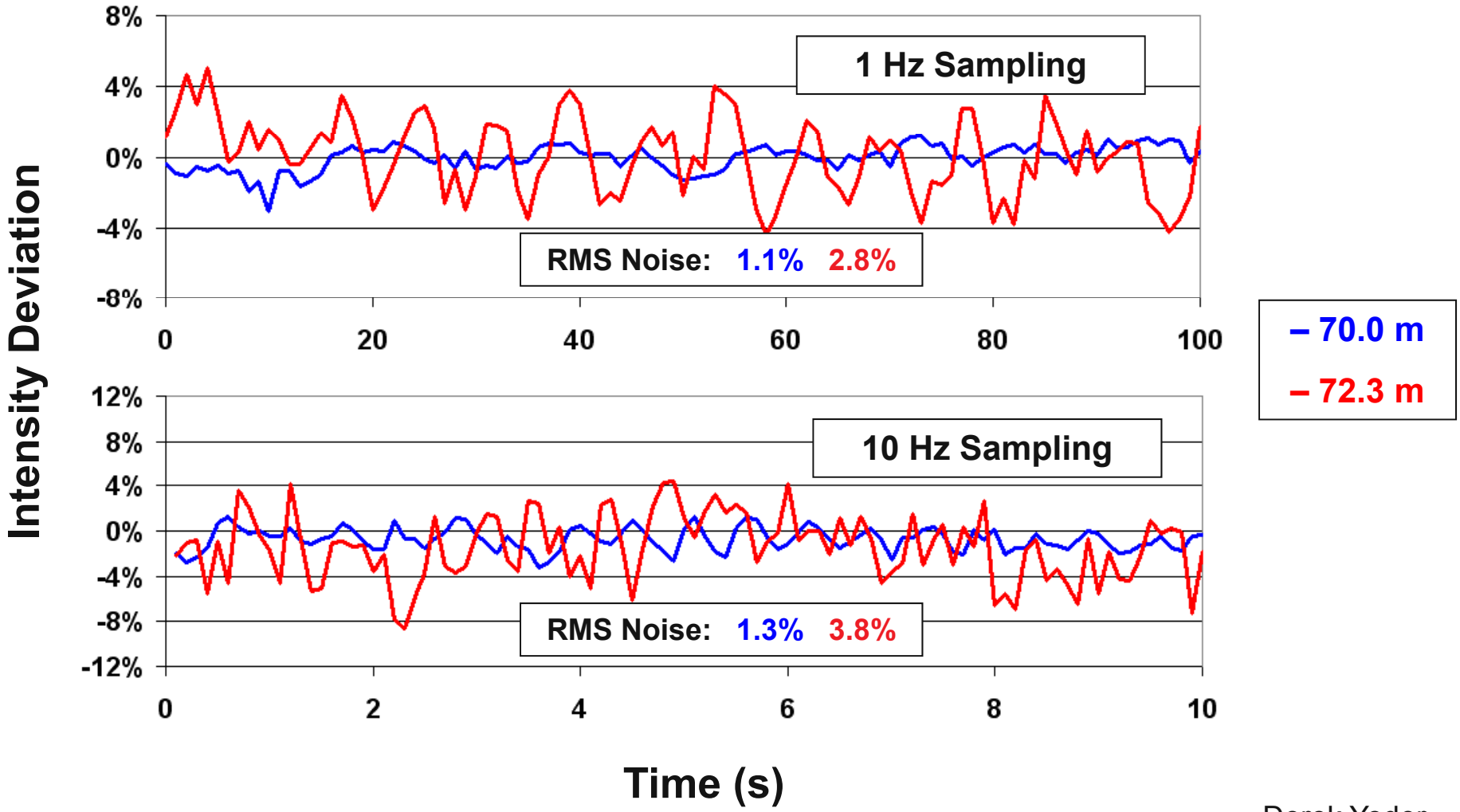


Real mirror (with slope error exaggerated for this image):

- **Beamlets are missteered**
- **Changes in source angle or position cause the beam to strike a different part of the mirror. The local angle (slope error) re-directs the beam angle.**
- **The effect is magnified at greater distances from the mirror.**



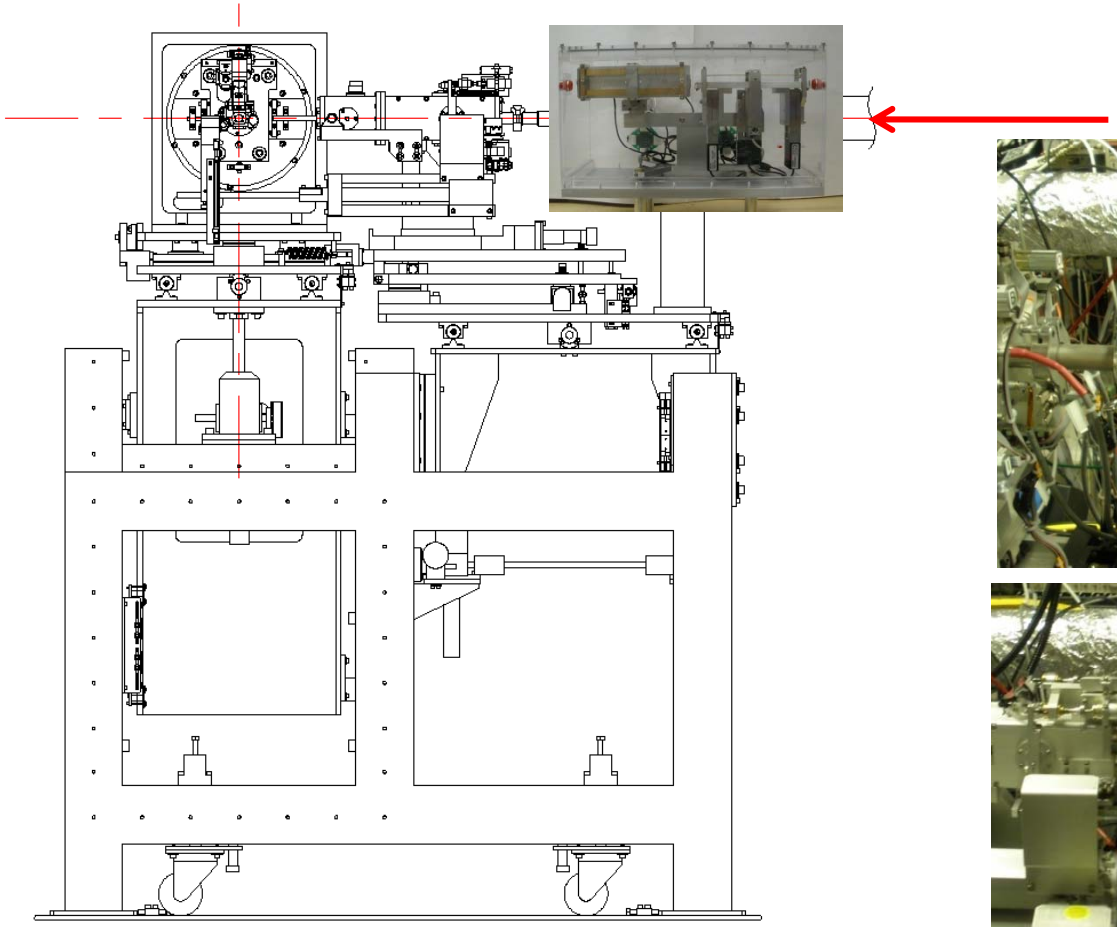
Compare intensity fluctuations at two different distances from the mirror (Vertically-sensitive 10-micron slit, 1 Hz sampling)



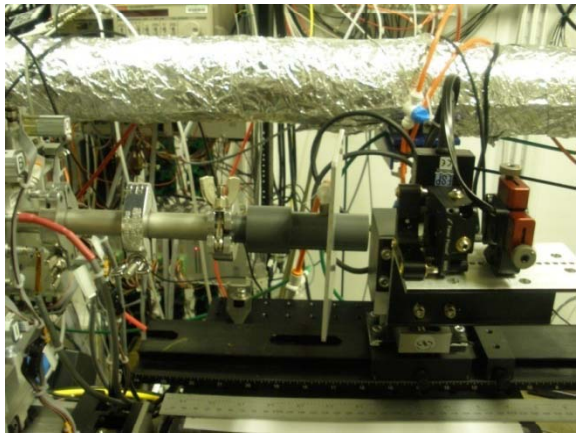
Recorded on 23-ID-D. Similar measurements with a horizontal slit show significantly less noise

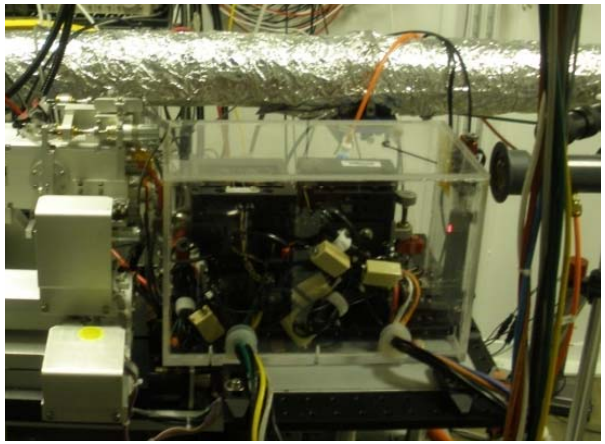
Derek Yoder

Micro-focusing with secondary optics

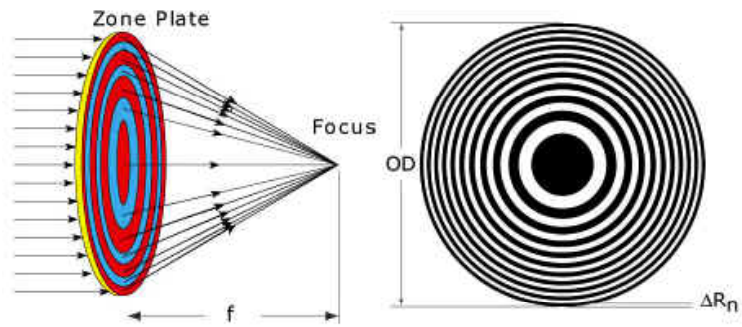


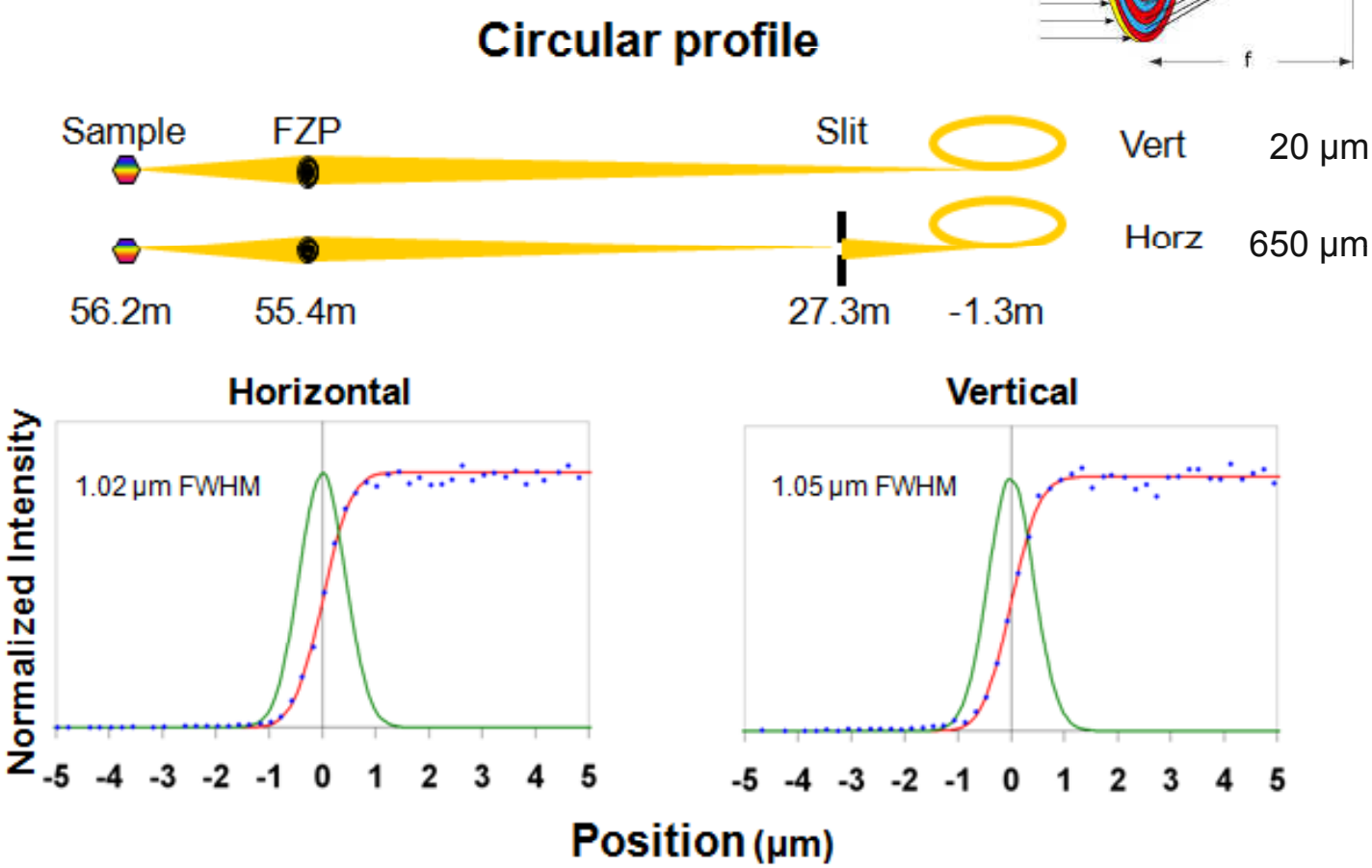
Beam direction





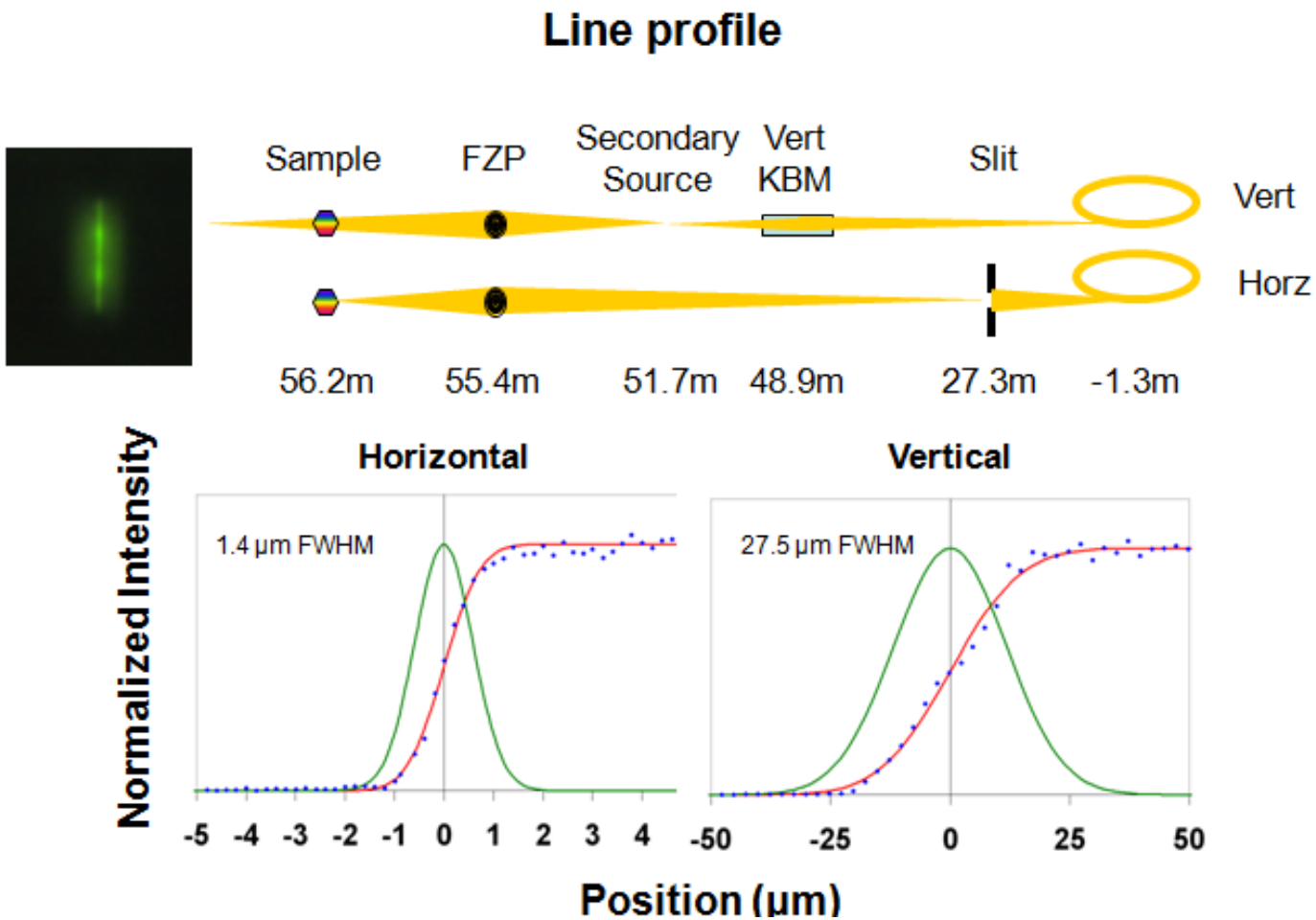
Making a 1-micron beam for MX





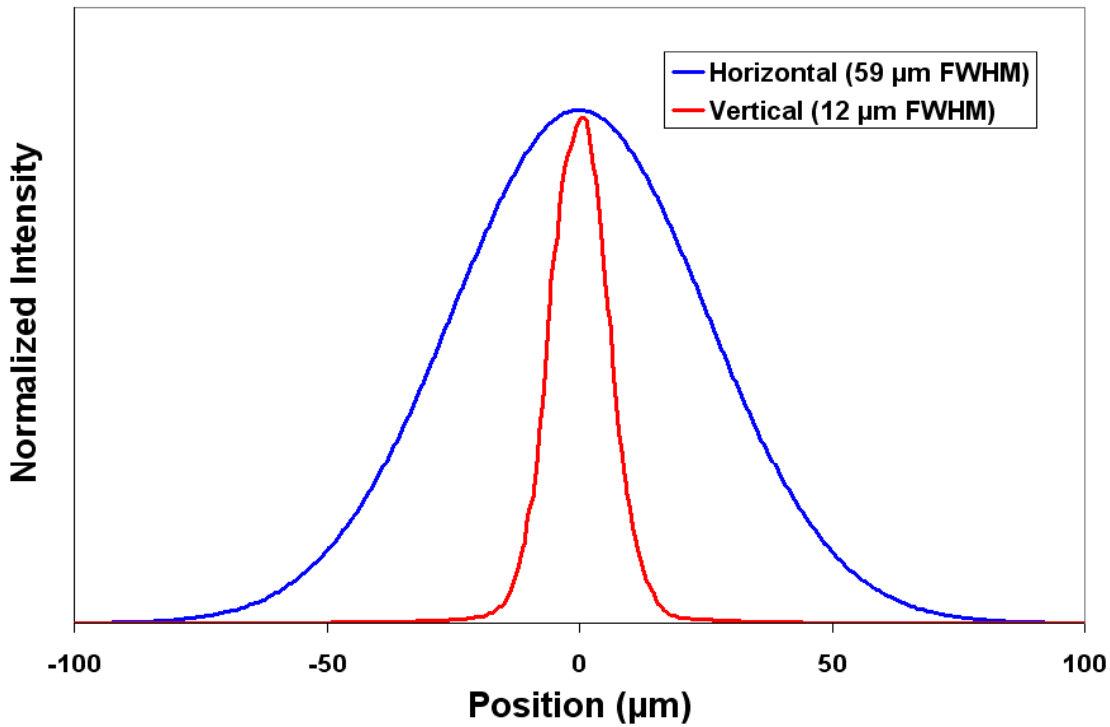
Zone Plate courtesy of Stefan Vogt, Advanced Photon Source

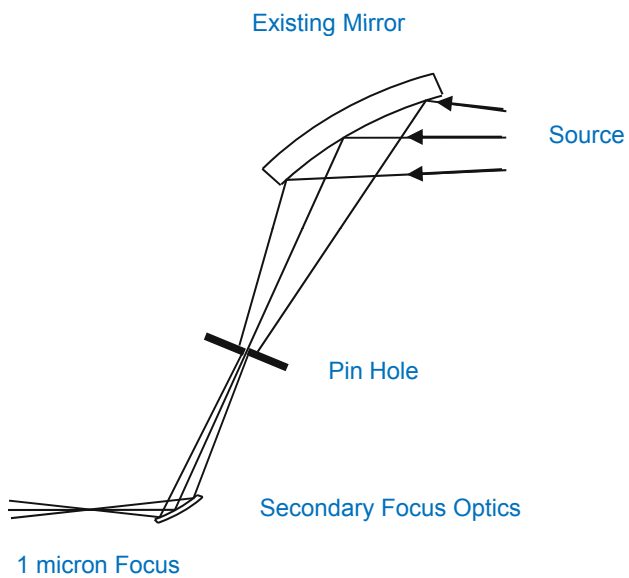
Compound optics



Re-imaging a 2nd source

- Beam focused 4.0 m upstream of normal (sample) position
- Place a pin hole in the focused beam
- Focus the image of the pin hole with another set of optics



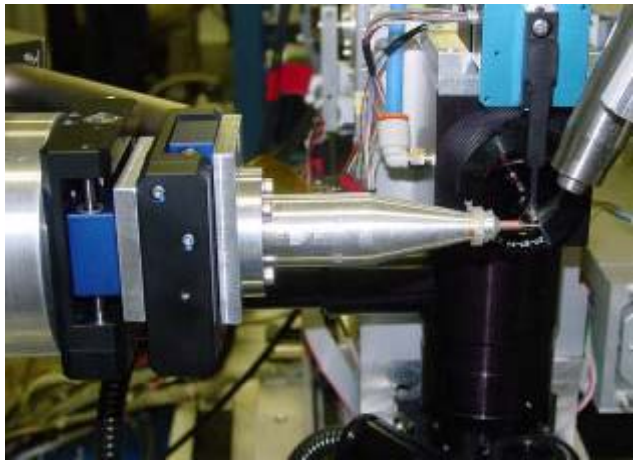


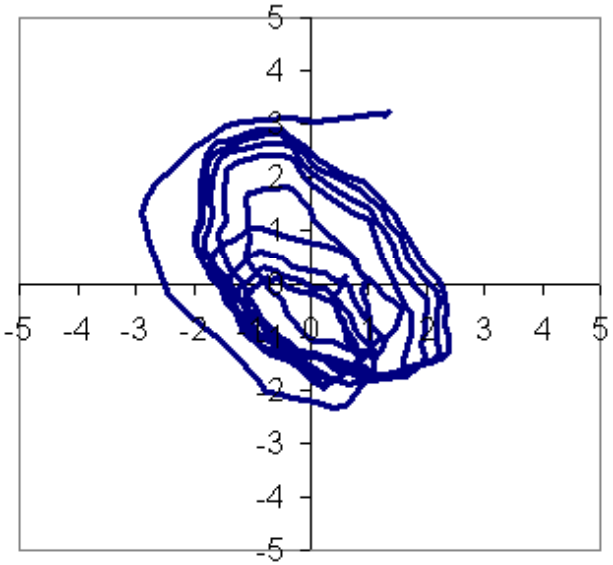
Intensity stability significantly improved by running feedback on intensity thru the pin hole!

Auto-focused in 3 hours

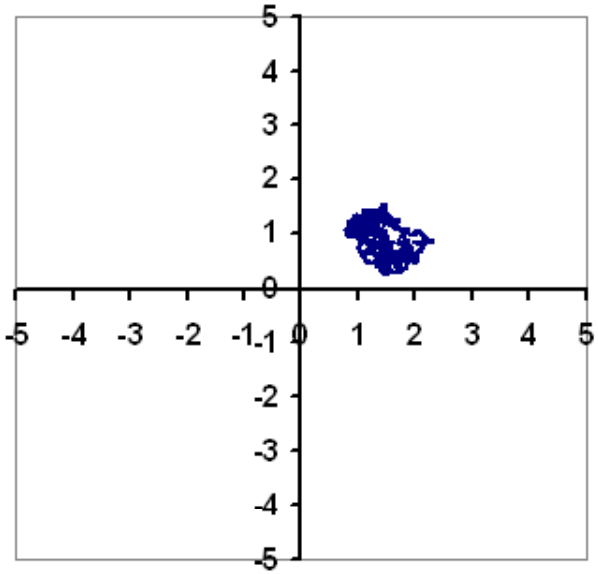
Nominal focus on 23-ID-B at sample position: 20 x 120 microns (FWHM)

Reducing the sphere-of-confusion





6 microns
peak-to-peak



1 micron
peak-to-peak

Nano-positioning goniometer head

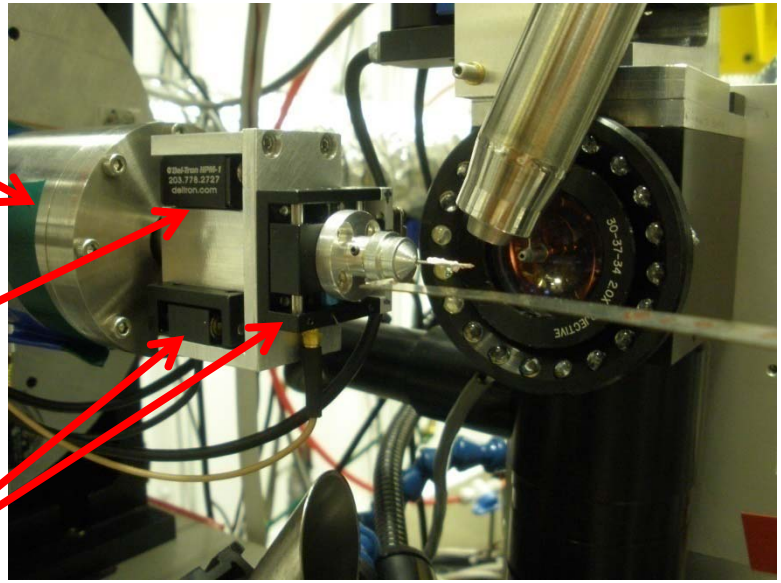
Features

- Horizontal and vertical motions
- 7 nm encoding
- 100 nm repeatability
- Rapid settling time <1 sec

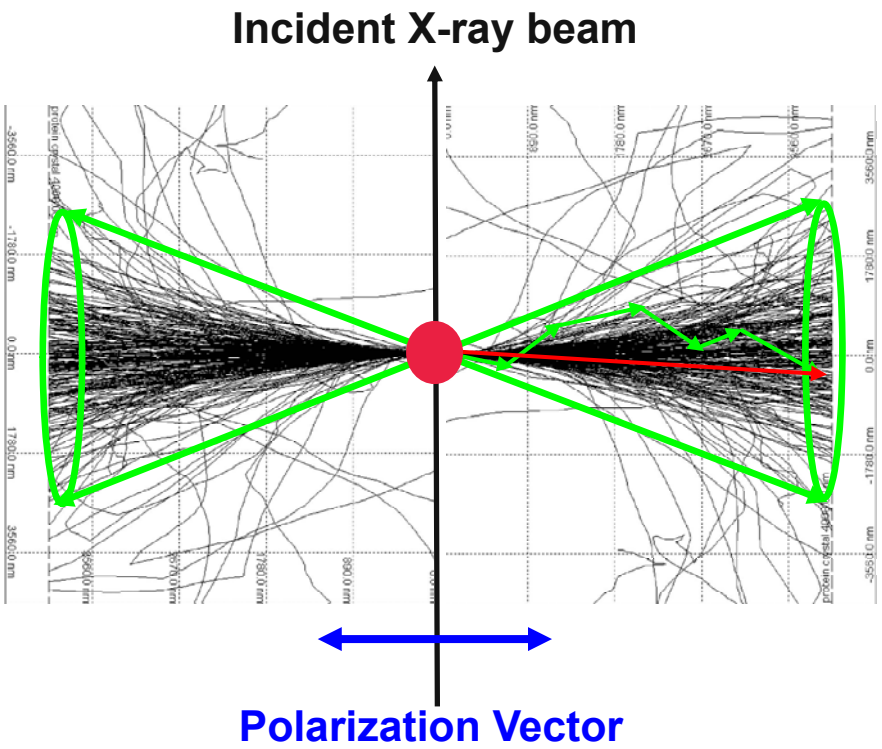
Membrane XY asssembly

DELTRAN linear stage

MICOS piezo translator stages with MicroE optical encoders



Radiation damage and small beams: Three postulates of photoelectron escape theory



1. Photoelectrons are preferentially ejected parallel to the polarization vector and propagate within a cone.

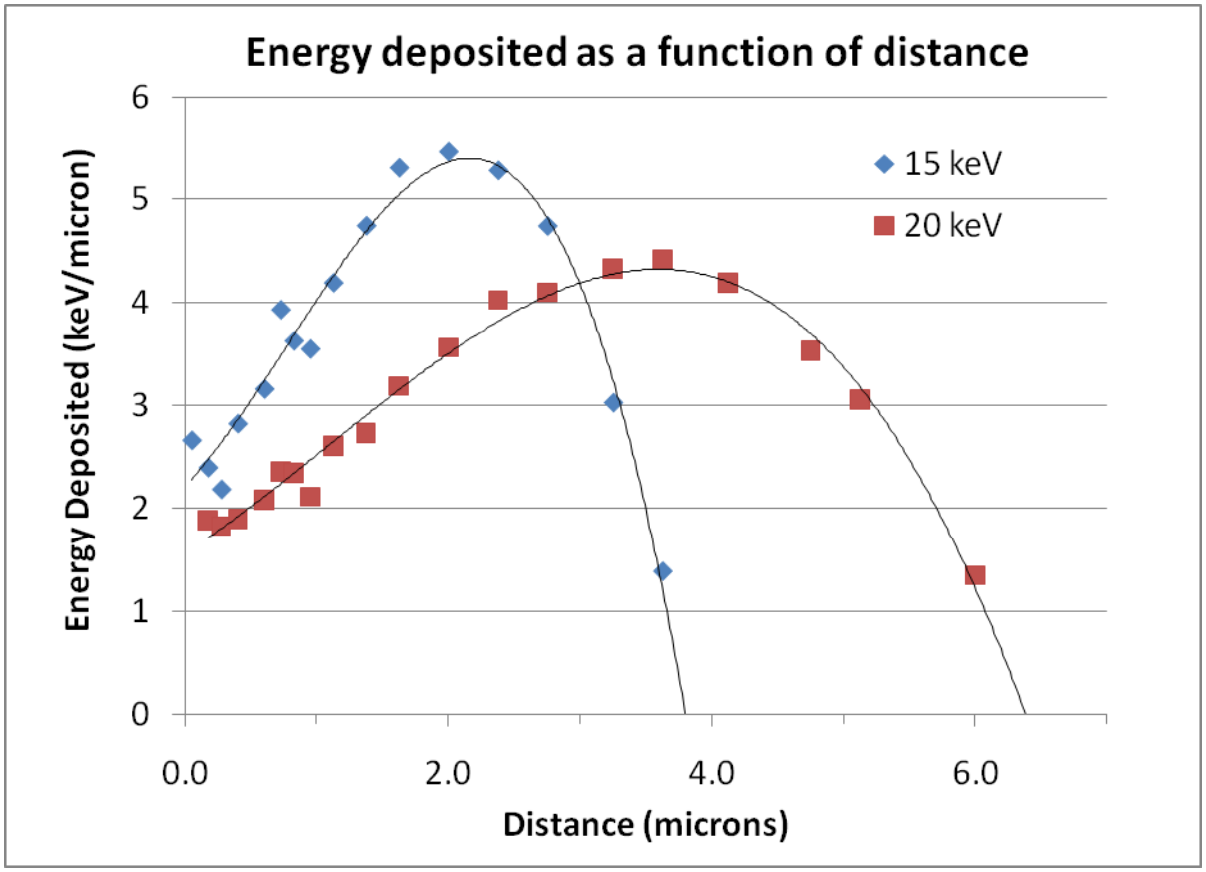
2. Photoelectron may escape from the illuminated volume before substantial damage occurs.

3. Radiation damage abruptly ends a few microns from the point of photoelectron ejection, and most of the damage is concentrated in the last few tenths of a micron.

Caution: electrons undergo a random walk as they interact with the sample. Thus one must consider the difference between the path and the vectorial distance).

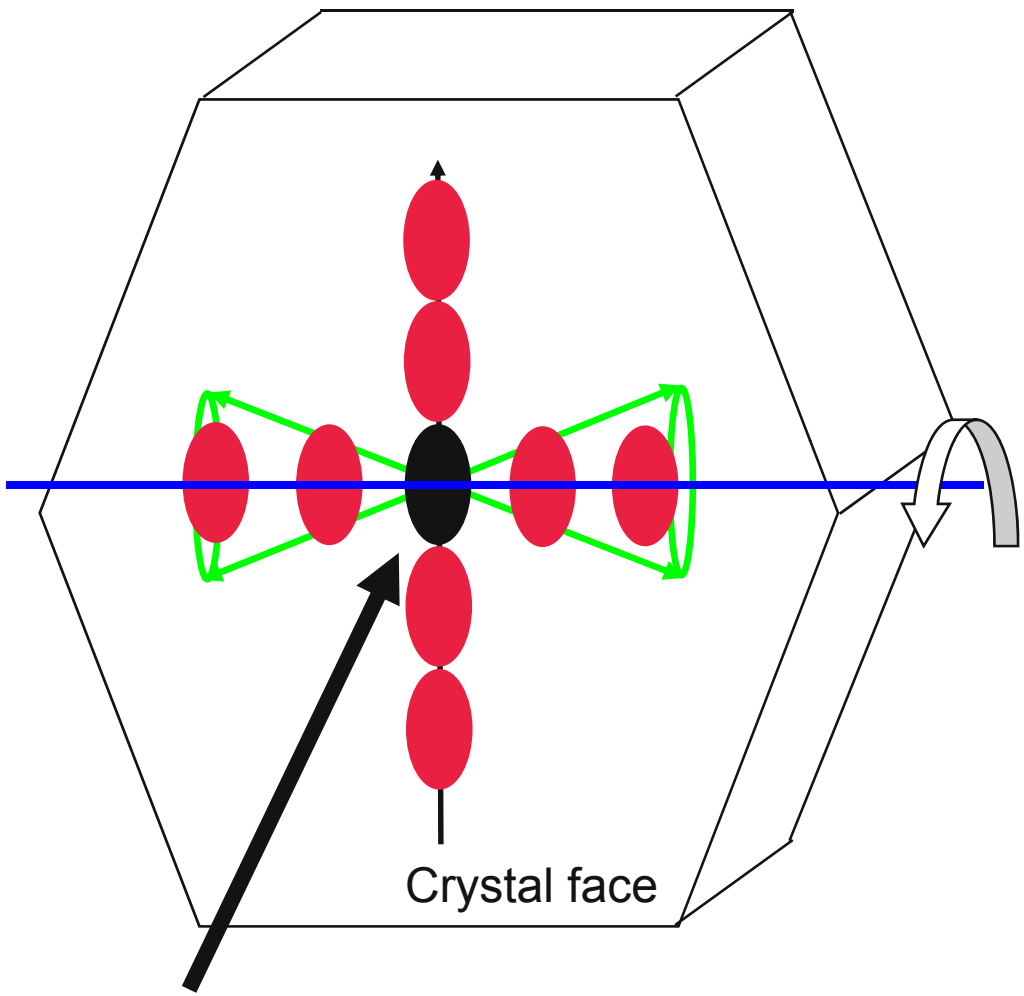
Monte Carlo simulations of photoelectron trajectory in a protein crystal after being ejected from an atom.
C. Nave and J. Hill, *J. Synchrotron Rad.* (2005). 12, 299–303

Photoelectron energy loss



Data courtesy of Colin Nave

Experimental protocol – 1: contiguous probe



Focal spot	3×2 (V, H)
Photon energy	15.4 keV

Data collection

- Record low dose patterns at 3 μm spacing
- Each pattern is over the SAME 1º range
- "Kill" the center with 10 frames of 1º
- Record low does patterns again
- Iterate until "significant" damage observed

Data analysis

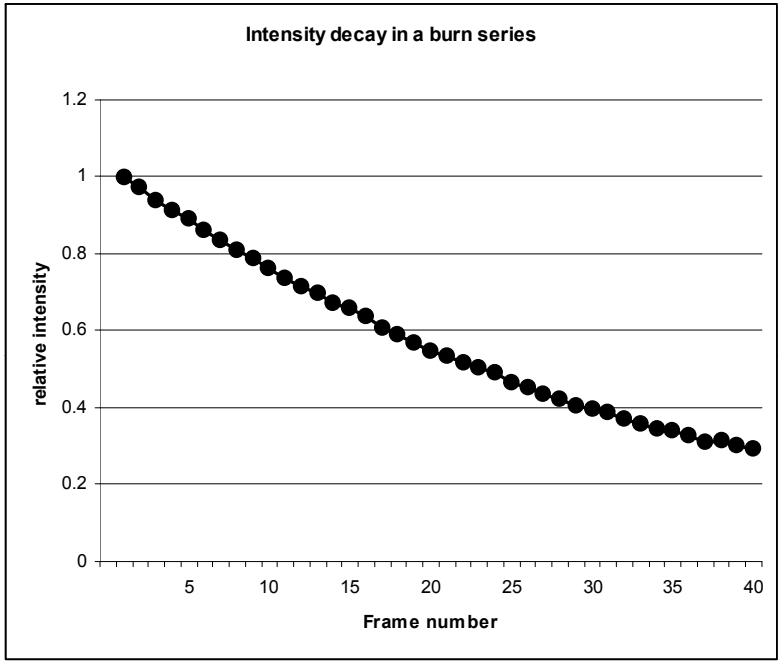
Initial

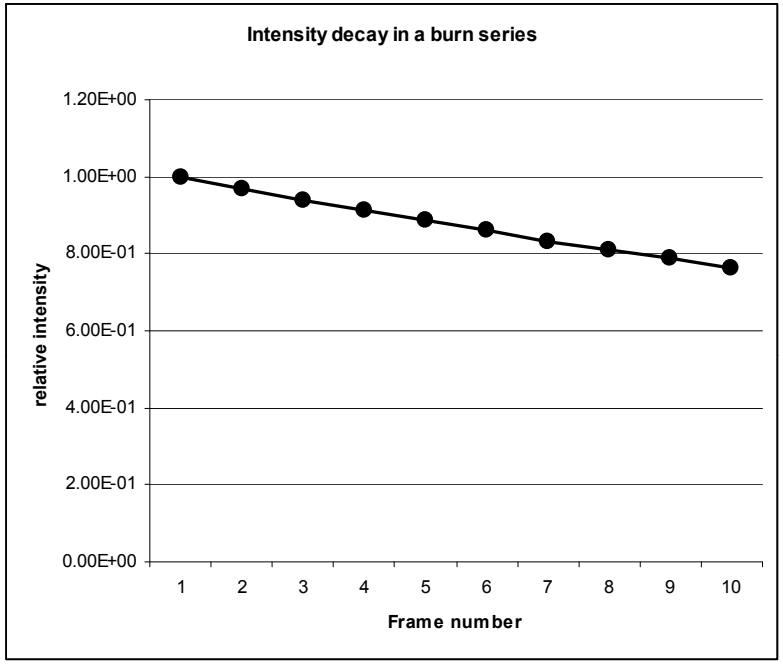
- Integrate the intensity from all reflections (fulls and partials)
- Bin the reflections into equal volume shells
- Normalize the total intensity per shell by the intensity in the First low dose measurements
- Plot normalized intensity as a function of resolution shell and dose
- Poor statistics: too few reflections per shell

Final

- Integrate all reflections (full and partial) w/ I/σ >2 (2D profile fitting HKL2000)
- **Plot fractional loss of normalized intensity vs distance and dose**

Intensity decay as a function of dose

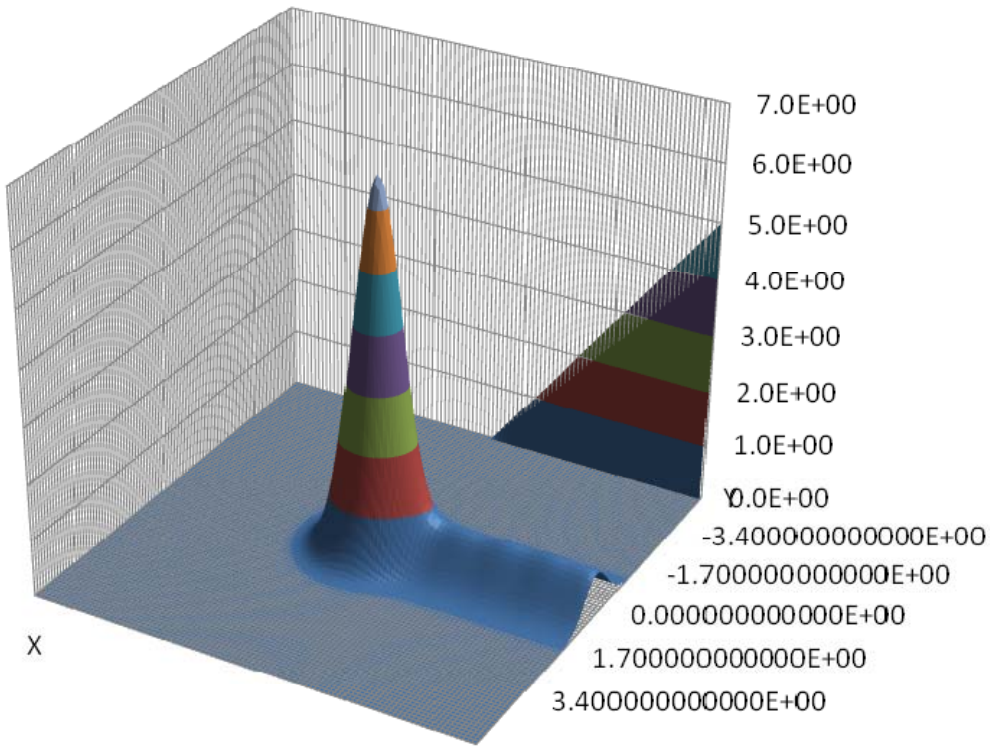


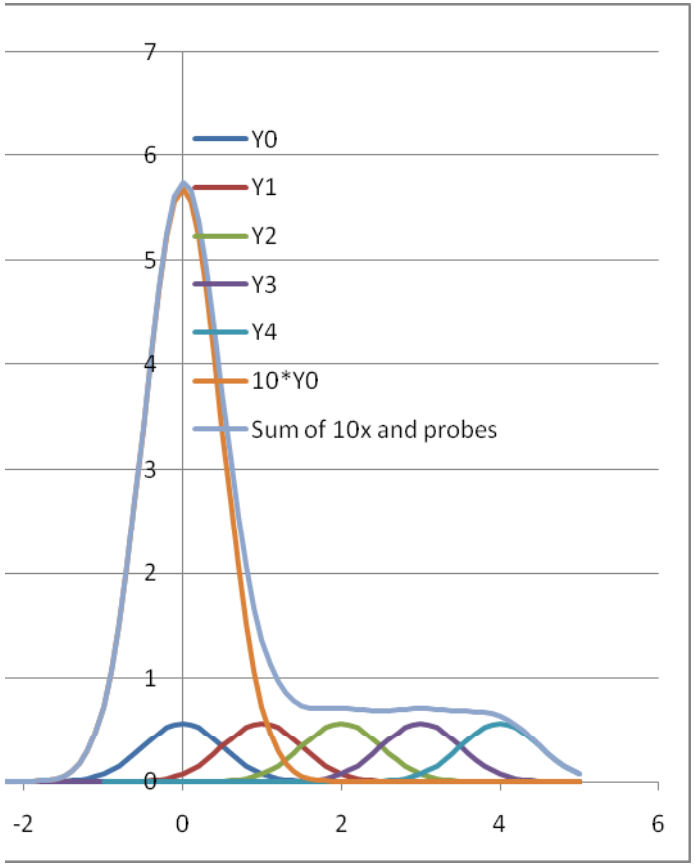


Decay of total diffraction intensity as a function of frame number.
Total of 40 frames were accumulated in 4 burn series of 10 frames each.

Within each series of 10, the decay is linear while for the whole experiment it has more asymptotic profile.

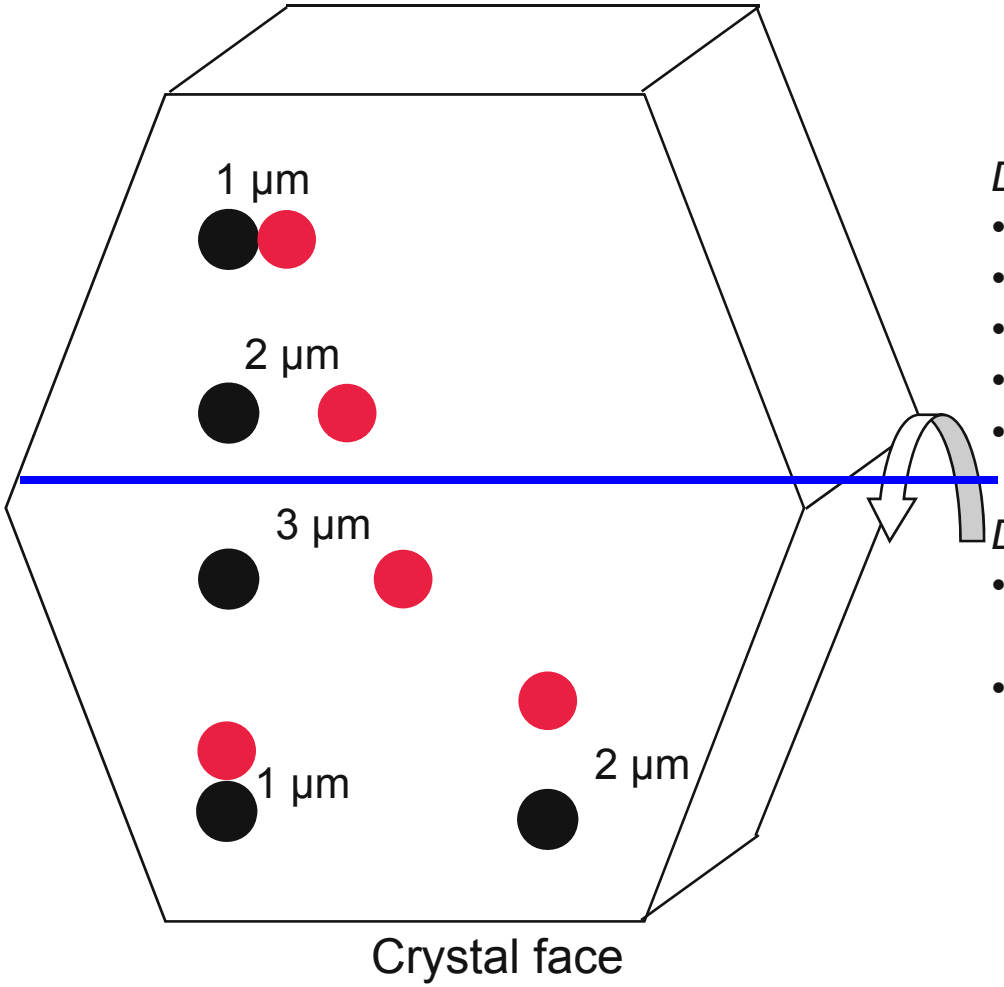
Overlapping incident "burn" and "contiguous probes" beams





Beam size 1.16 x 1.18 microns (H x V, FWHM)

Experimental protocol – 2: isolated probe



Data collection modified

- Record low dose patterns at a position
- Each pattern is over the SAME 1º range
- "kill" a spot 1, 2, 3… µm away: 10 x dose
- Record low dose patterns again
- Iterate until "significant" damage observed

Data analysis – same as before

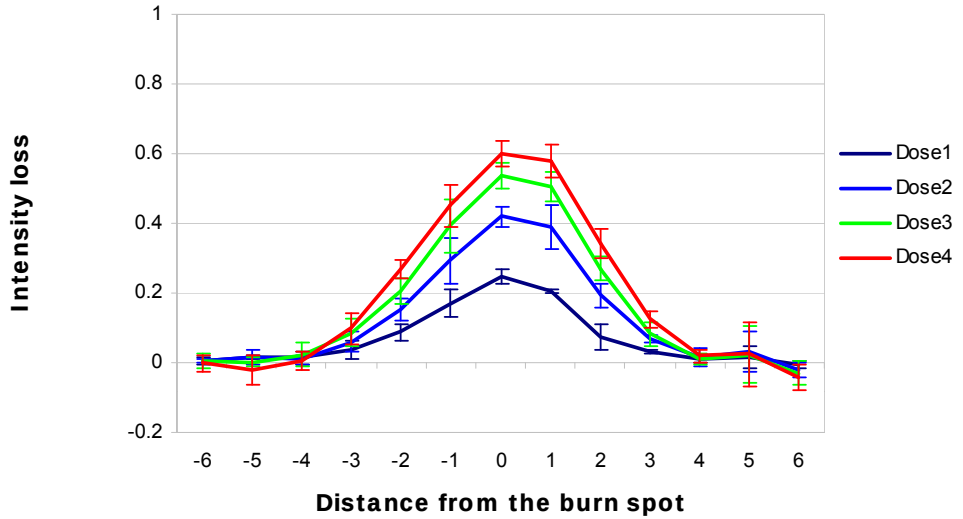
- Integrate all reflections (full and partial) w/ I/σ >2
- Plot normalized intensity vs distance and dose

Focal spot	1×1 (V, H)
Photon energy	15.4 keV

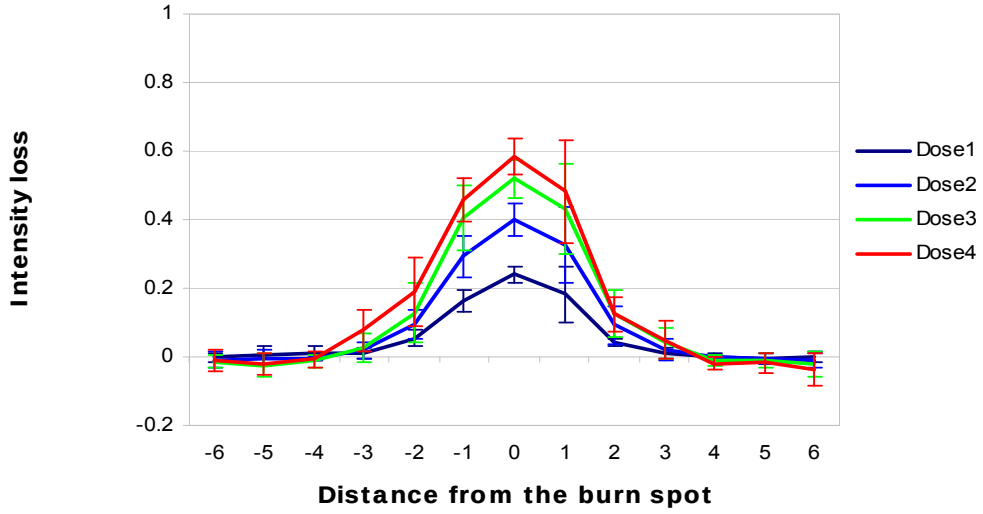
Comparison of horizontal and vertical decay

Error bars: sigma from average of 10 crystals

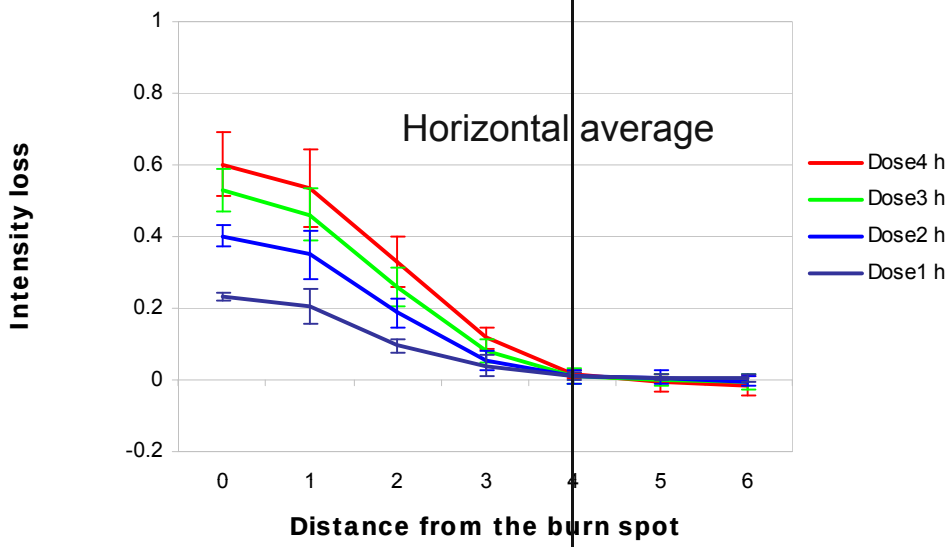
Horizontal

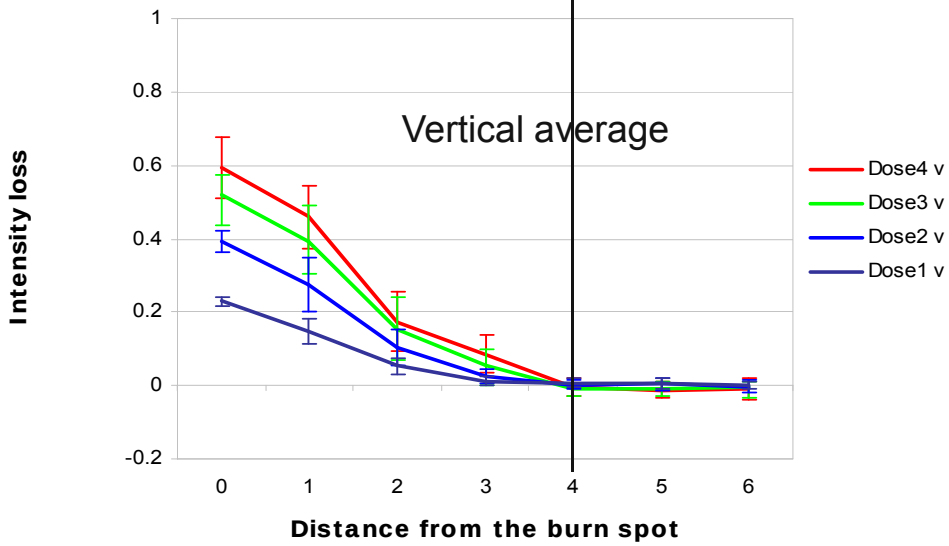


Vertical



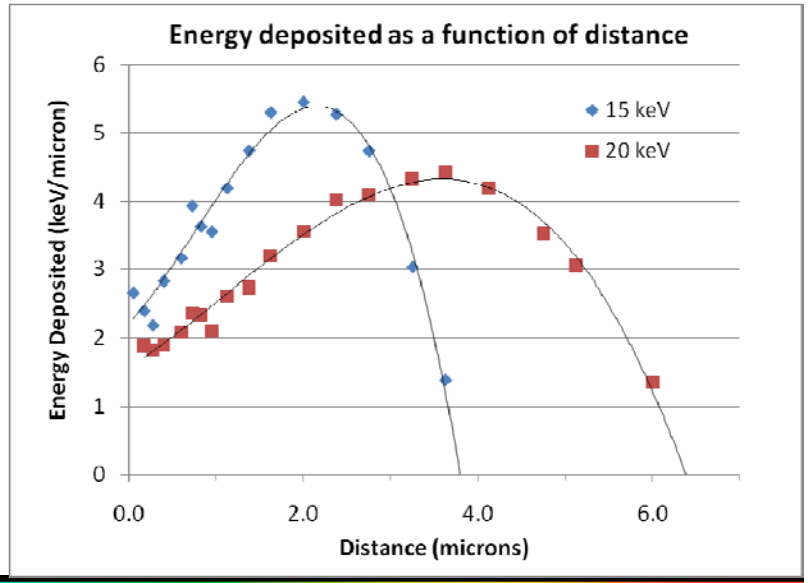
Average of horizontal (left, right) and vertical (up, down)





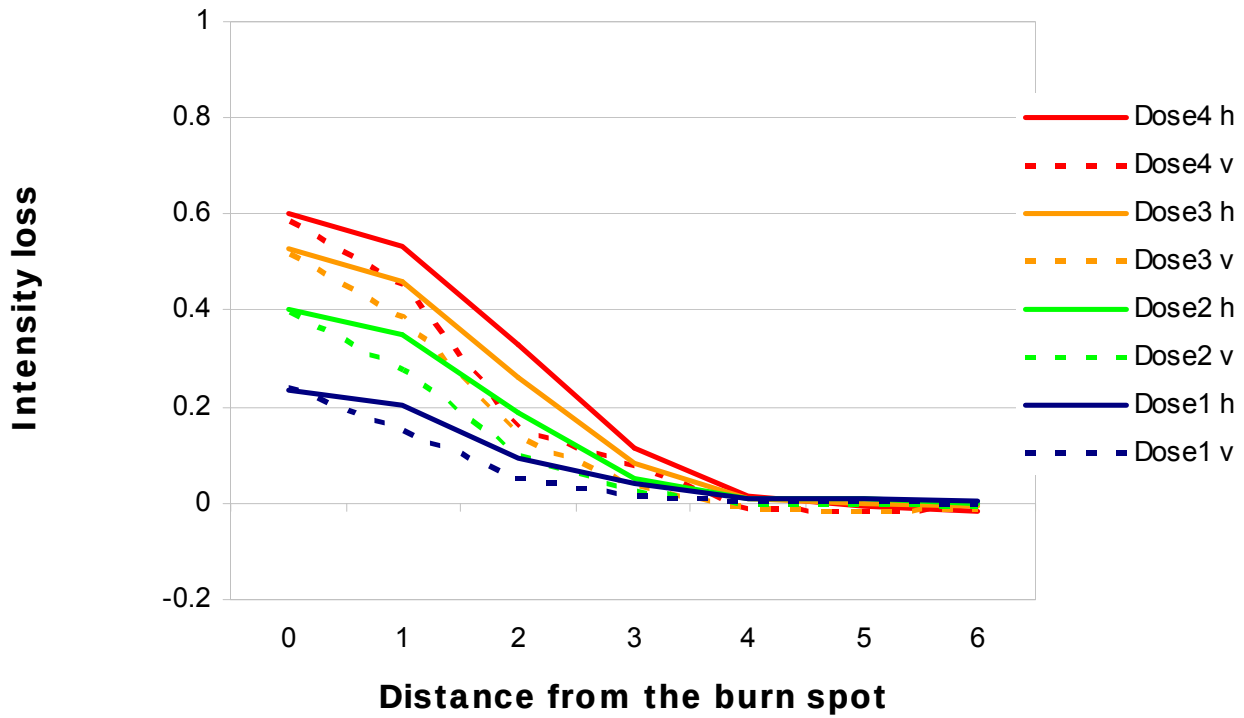
Conclusions

- Damage does not extend beyond 4 microns
- Do not observe reduced damage at center
- Do not see "capture" peak
- Need smaller beam or higher energy

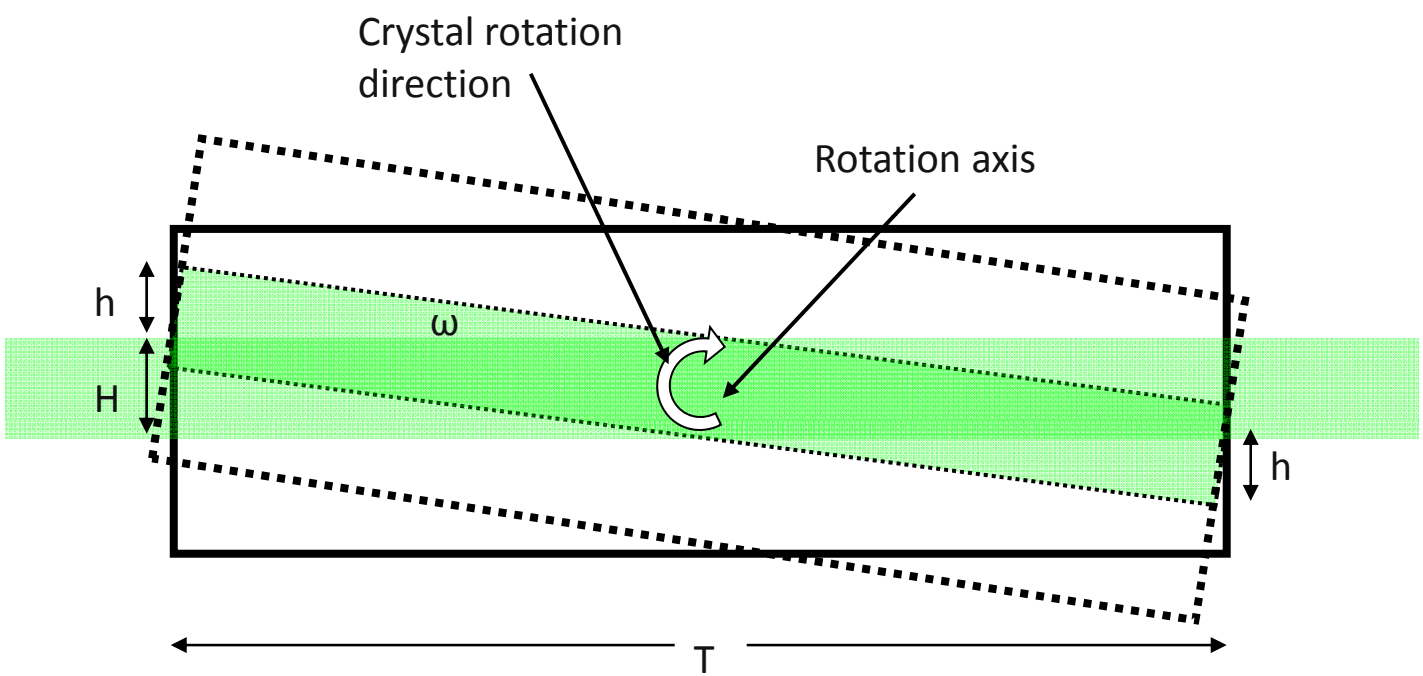


Anisotropy of radiation damage

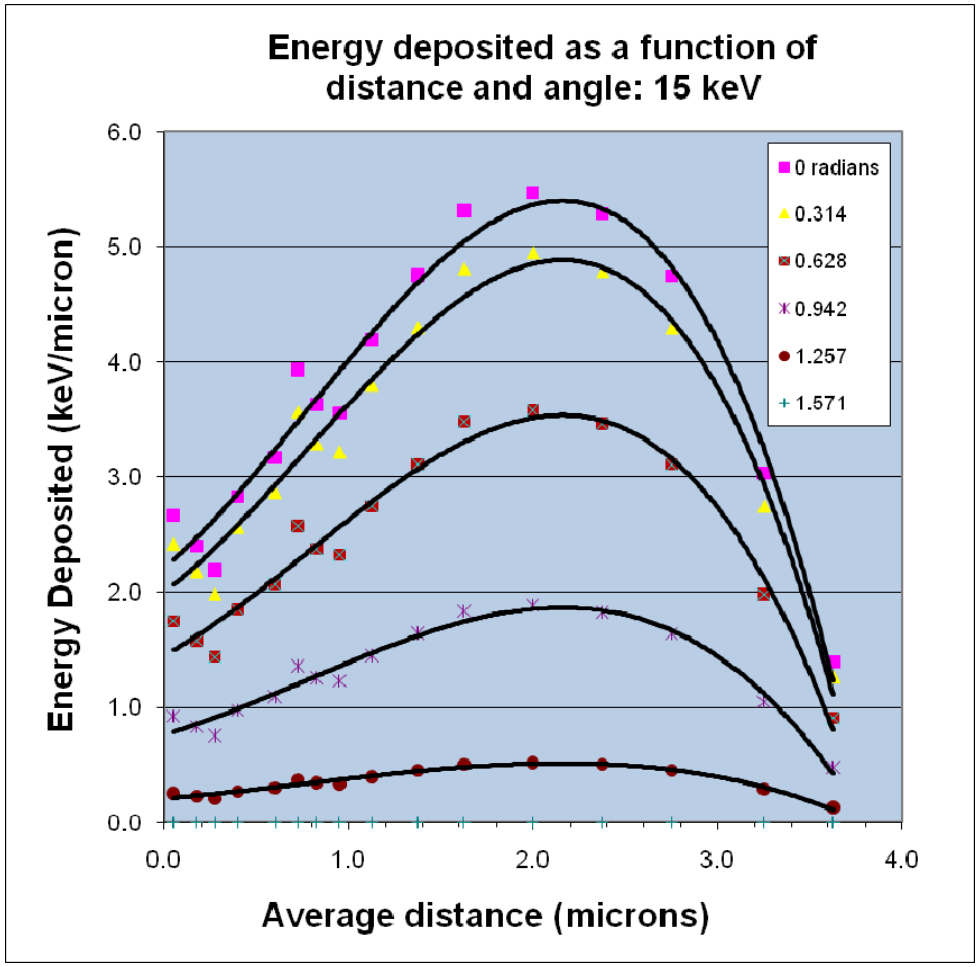
Solid lines - horizontal: left and right averaged
Dashed lines - vertical: up and down averaged

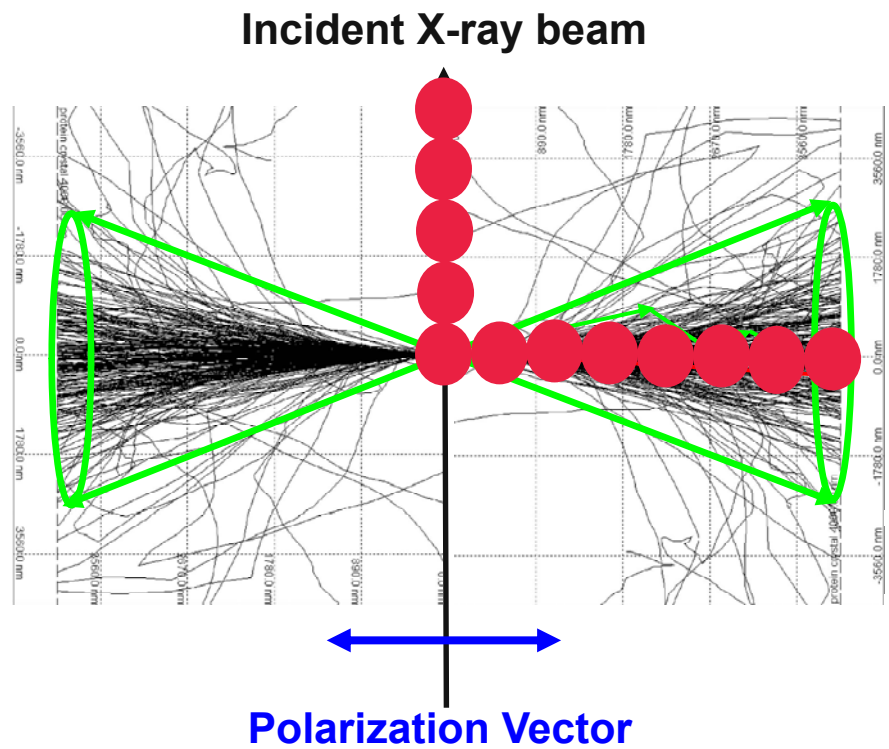


Crystal thickness "blurs" vertical measurements



Photoelectron energy loss: angular component

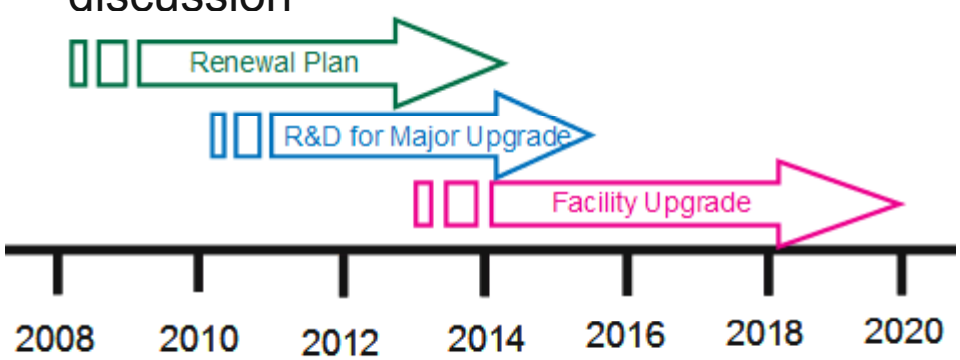




Data courtesy of Colin Nave

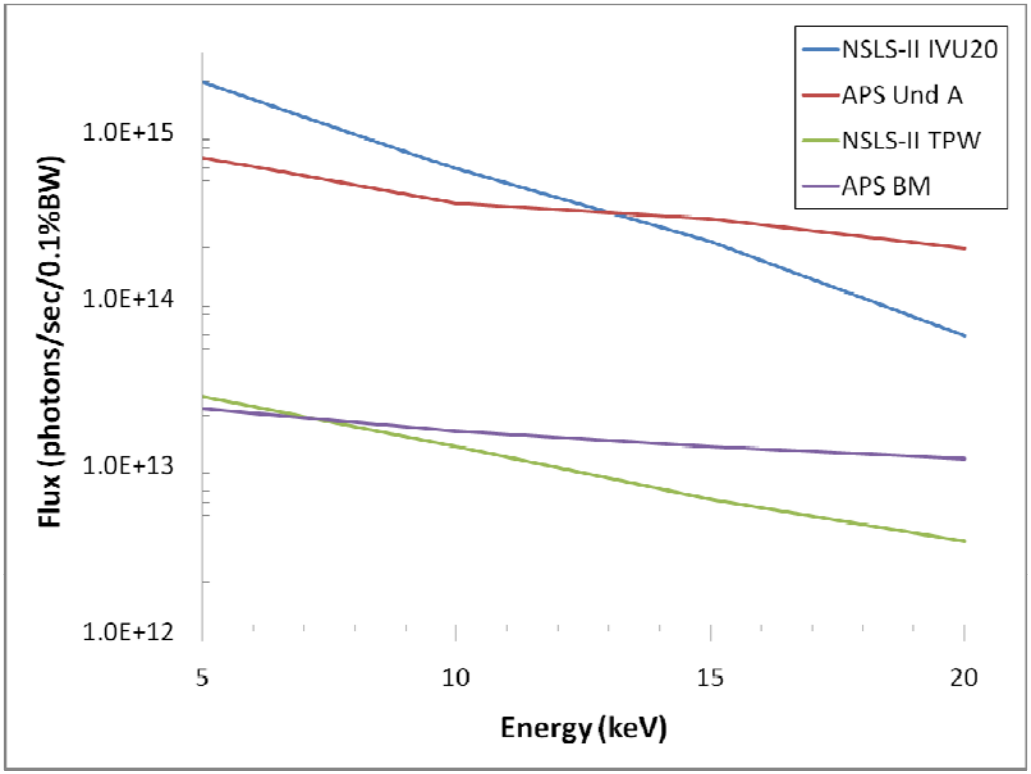
Future plans - APS

- Construct permanent 1-micron capability (funds in hand)
- Reduced horizontal beam size: σ_x = 280 μm to 120 μm
  - Only available on a few sectors
  - Cuts horizontal focal size in half
- Renewal plan
  - 5 year plan to invest in beamlines, storage ring (200 mA), and infrastructure
  - science case based
  - CD-0 submitted May 30, 2009
- Upgrade plan: 5 – 10 year plan super storage ring, ERL, FEL open for discussion



http://www.aps.anl.gov/Renewal/

NSLS-II



K-B mirrors
0.25 μ-radian slope error

16:1 demag
5 x 0.5 microns

81:1 demag
1 micron horizontal
-> slits or staged focus

Beam	Size at sample, FWHM (microns)	Intensity (photons/sec)	Time to Henderson Limit - GMCA (sec)	Time to Henderson Limit NSLS-II (sec)
Full	20 x 65	2.0×10^{13}	3.3	
10- μm	10.6 x 11.6	1.3×10^{11}	48	
5- μm	4.8 x 6.2	2.7×10^{10}	56	0.08
1- μm	1.1 x 1.2	3.0×10^{9}	40	0.005

My NLSL-II wish list

- Brilliance, brilliance, brilliance.
- Build world leading beamlines for MX that fully exploit the unique capabilities of NSLS-II.
- Low-β straight section provides the highest brilliance, but should consider the possibility of a “focused” long straight section if it provides higher brilliance
- A pair of canted-undulator beamlines for MX is strongly preferred over investing in TPW beamlines
- Largest cant angle possible: 2 – 5 mrad desirable
- Smallest horizontal beam size possible
- Convergence/divergence 500-1000 micro-radians
- Incorporate longer period undulator (21 or 22 mm) to avoid tuning curve gaps and minimize power load jumps when switching harmonics
- Energy range should 3.5 – 20 keV with the possibility of energies up to 30 keV.

Funding Acknowledgment

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Administration

Janet Smith
Director

Robert Fischetti
Assoc. Director

Sheila Trznadel
Admin Specialist

Crystallographic Support

Craig Ogata
Crystallographer

Nukri Sanishvili
Crystallographer

Michael Becker
Crystallographer

Naga Venugopalan
Beamline Specialist

Computing Support

Sergey Stepanov
Beamline Controls Scientist

Oleg Makarov
Beamline Instrumentation Scientist

Mark Hilgart
Software Developer

Sudhir Pothineni
Software Developer

Engineering & Technical Support

Shenglan Xu
Engineer

Derek Yoder
Beamline Scientist

Rich Benn
Engineering Specialist

Steve Corcoran
Engineering Asst.