Micro-crystallography at the GM/CA-CAT Canted Undulator Beamlines

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... for a brighter future

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NSLS-II
Macromolecular Crystallography Workshop July 18, 2007

U.S. Department of Energy

UChicago Argonne LLC

Office of Science
U.S. DEPARTMENT OF ENERGY

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Outline

- GM/CA-CAT construction approach
- Scientific Mission
- Beamline capabilities
- User program and results
- Micro-diffraction
- Relevance to NSLS-II

Funding and Community Served

GM/CA CAT is funded by the US National Institutes of Health's partnering institutes the National Institute of General Medical Sciences (GM), and the National Caner Institute (CA) to build and operate a facility for macromolecular crystallography.

Beamtime is allocated in the following categories

- GM special grantees – Protein Structure Initiative (20.5%)
- CA special grantees – Structure based drug design (12.5%)
- General user facility (including DOE 25%) (42.0%)
- CAT Director, SAB members, R&D and staff research (25.0%)

Scientific & Technical Vision

Wide energy tunability for MAD

3.5 - 35 keV (wavelength 3.5 - 0.35 Å)

Variable beam size to match to sample size

Easily varied in the range 5 - 200 µm

Small beam capability

Down to 5 µm routinely

High stability of beam position and wavelength

Small beam stable throughout a user's experiment

Wavelength tunable without beam position changing

User-friendly beamline operation

Easily understood & operated computer interface

Automation to speed sample screening

For both difficult and high-throughput projects, and for beamline setup

Not just high throughput

Larger macromolecules & complexes, small crystals, weakly diffracting crystals such as membrane proteins

GM/CA-CAT oversight and planning

Technical Advisory Committee (advises GM/CA-CAT)

Peter Kuhn, Scripps (formerly Stanford, SSRL), current chair

Lonny Berman, NSLS

Thomas Earnest, ALS and Berkeley

Jim Viccaro, ChemMat-CARS, University of Chicago

Synchrotron Advisory Board (advises NIGMS, NCI)

Jack Johnson – Scripps, chair

Funding/administrative oversight

Charles Edmonds – NIGMS, NIH

John Sogn (retired) – NCI, NIH

Randy Knowlton – NCI, NIH

Dennis Dougherty – SAIC

Jeff Derge - SAIC

ACCEL Collaborators

Wolfgang Diete

Markus Schwoerer-Boehning

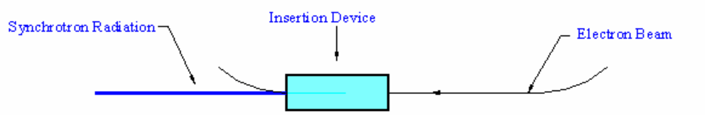
Leif Schroeder

Riccardo Signorato

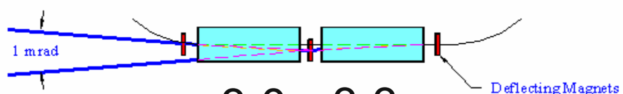
Chitra Venkataraman
Timm Waterstradt
Oliver Clauss
Artur Greschik
Ulf Schwabe
Adrian Olejnik
Werner Koenighaus
Juergen Bach
Guido Schneider
Udo Neugebauer
Harald Buechel
Wolfgang Opterweidt
Michael Poier
Gerd Gruetzmacher
Juergen Schultheiss
Nissel
Anja Kraemer
Helmut Schulten
Ronald Frahm

1st APS Sector Based on Hard X-ray Dual Canted Undulators

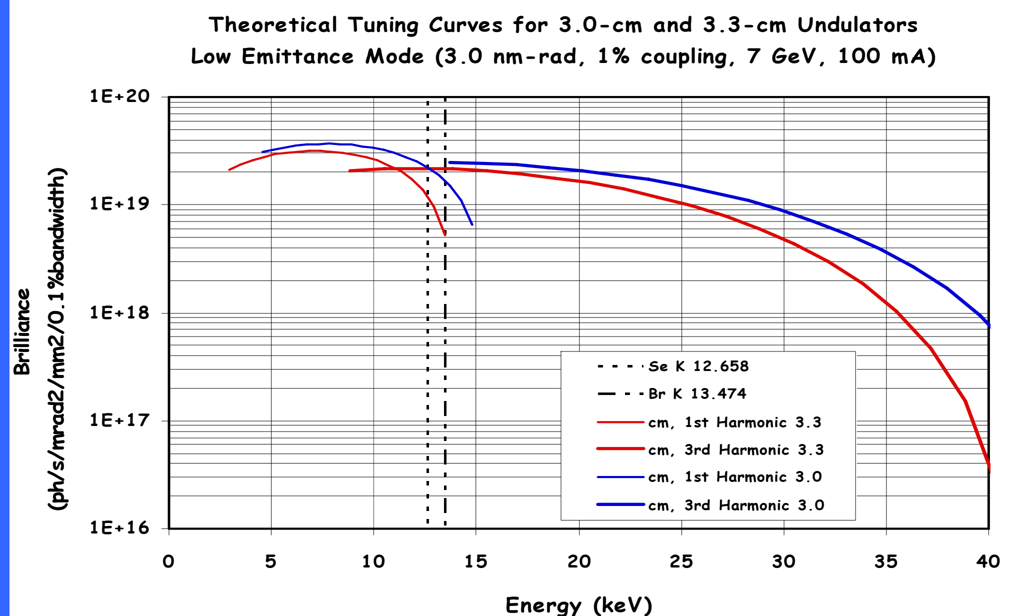
Typical Single Undulator



Dual Canted Undulator



3.0 3.3
Undulator period (cm)



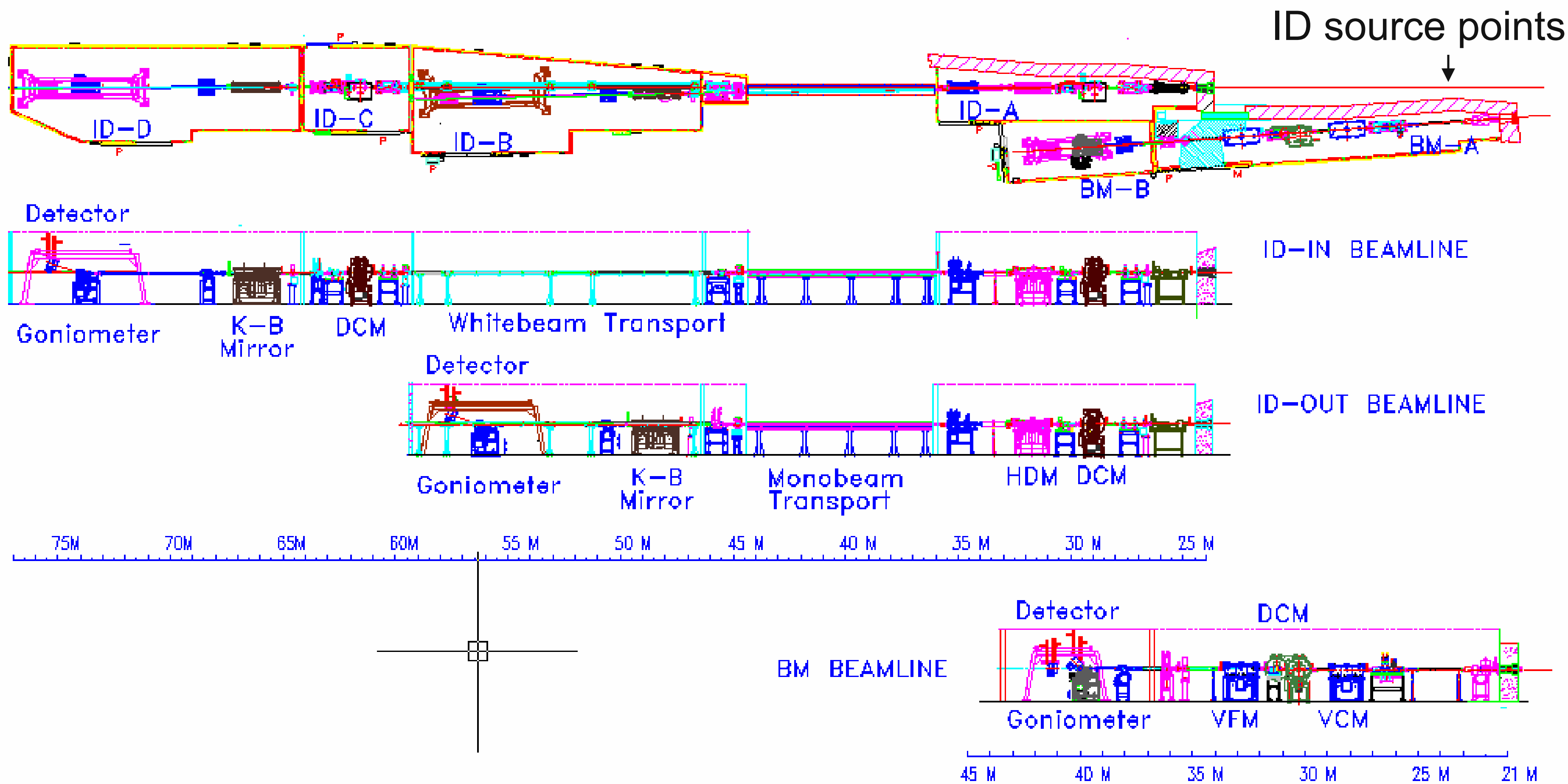
Independent steering: 3 RF-BPMs
Stable: 1 X-ray BPM at 22 m
Increased flexibility:

- 3.3 cm – standard UA
- 3.0 cm – optimized for Se and Br

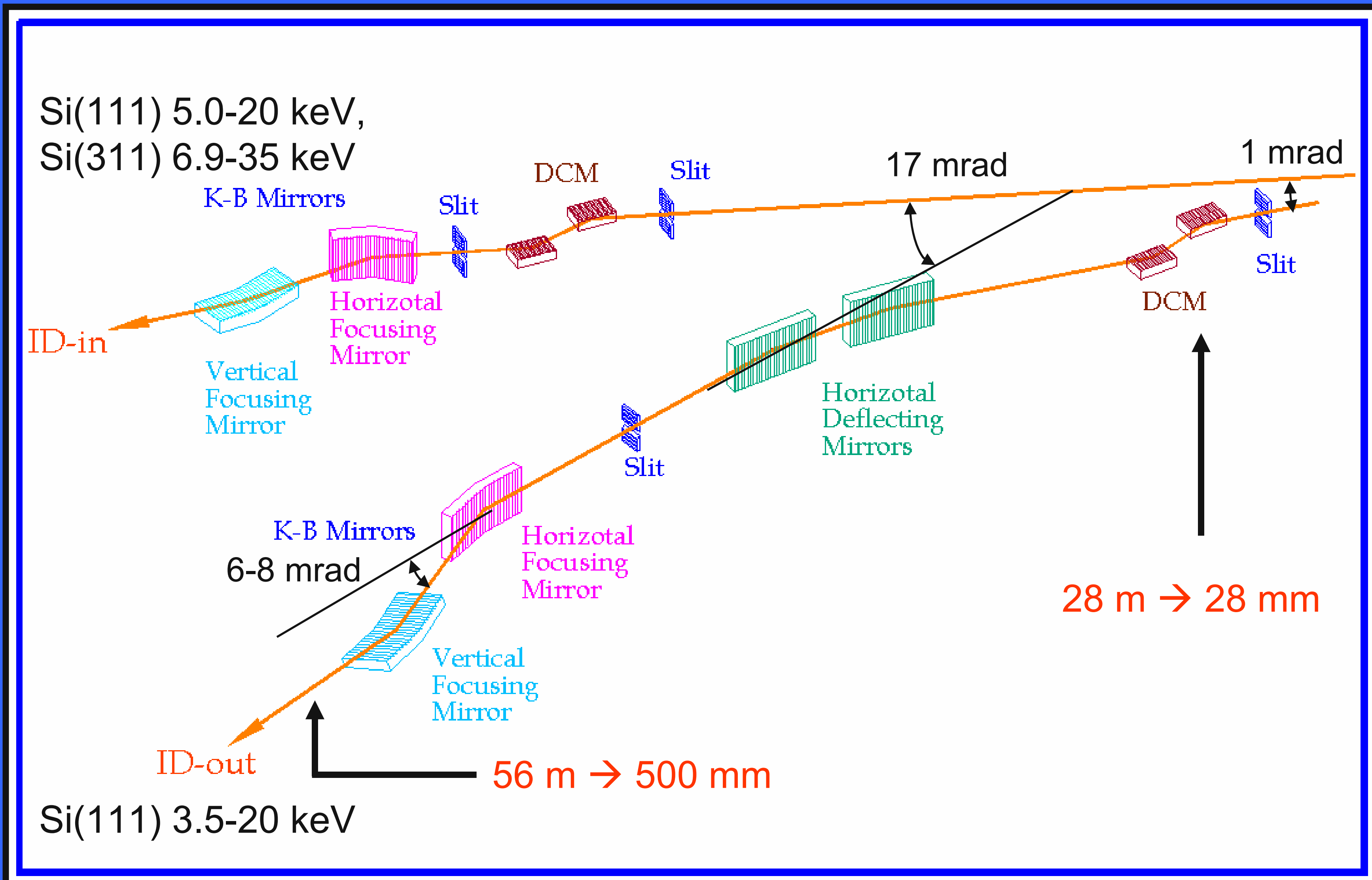
GM/CA Sector Layout

Two ID-lines based on canted-undulators

One BM – accepting 1 mrad of 6 mrad horizontal fan

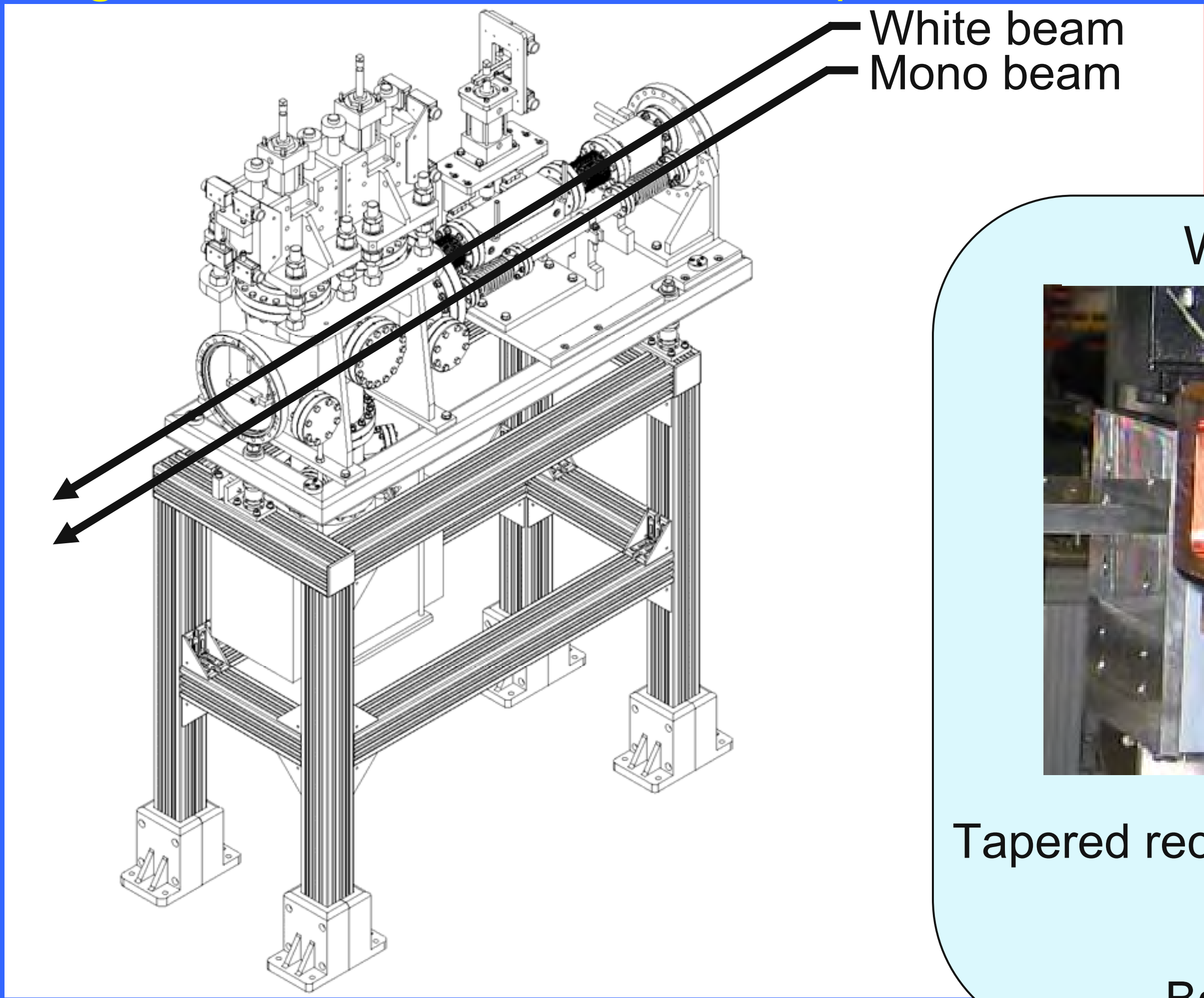


Schematic of Canted ID Beamlines



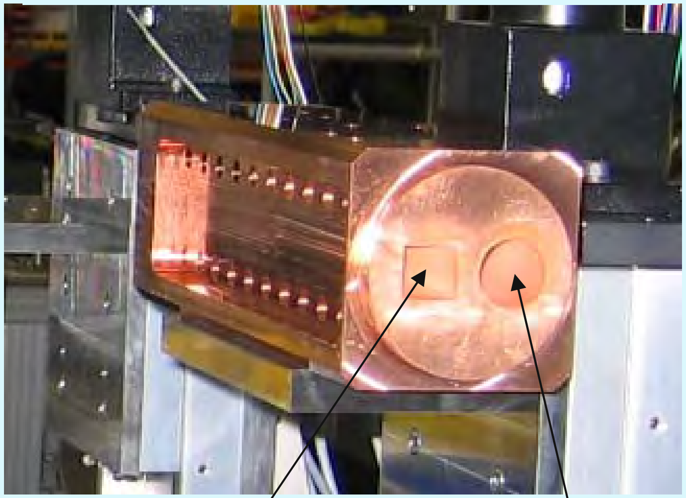
Unique components for canted undulator beamlines

Integral Beam Shutter - Provides independence of two ID-lines



Based on APS P4-30 single beam shutter

White Beam slits

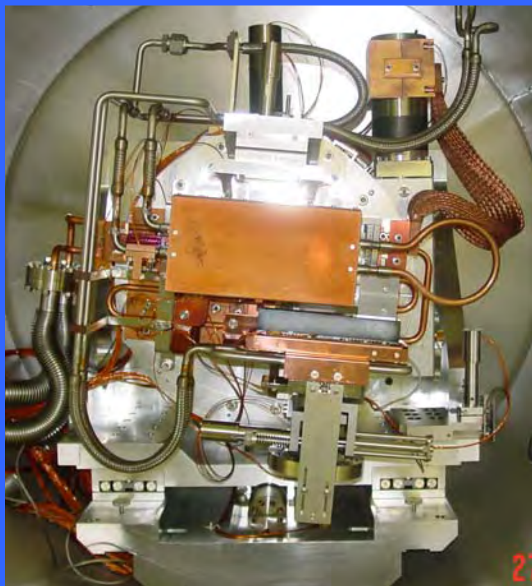


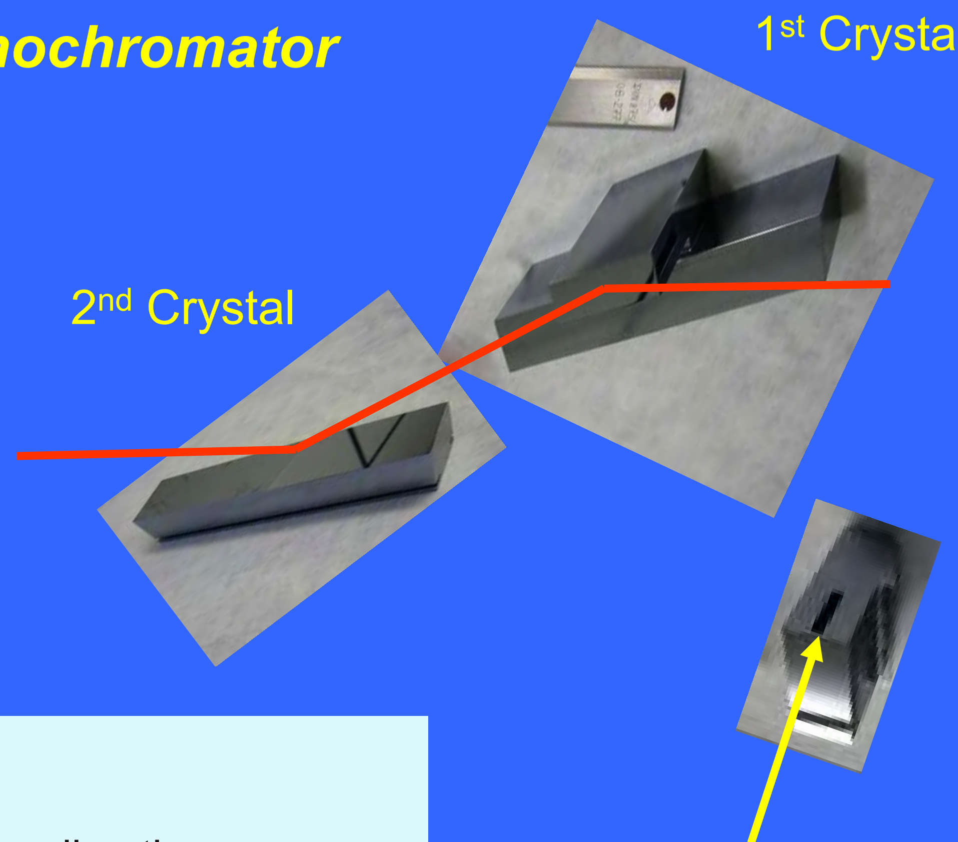
Tapered rectangular aperture

Beam pass-thru

Based on APS L5-92

ACCEL - Double Crystal Monochromator





- Constant exit height
- Long 2nd crystal to minimize tune error
- 2nd crystal translates in Bragg perpendicular direction
- 1st and 2nd crystals are both indirectly, cryogenically cooled
- Compton scatter shield around 1st crystal and 2nd crystal mount for improved thermal stability
- In-vacuum Huber 430 rotation axis – avoids rotary feedthrough
- Si (111) or Si(311) for higher energy and/or resolution

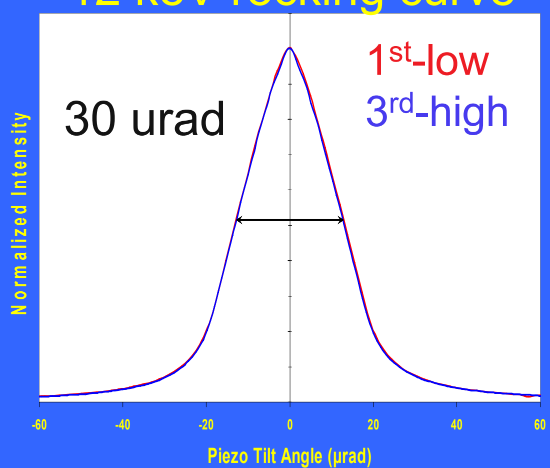
Thin web under cut

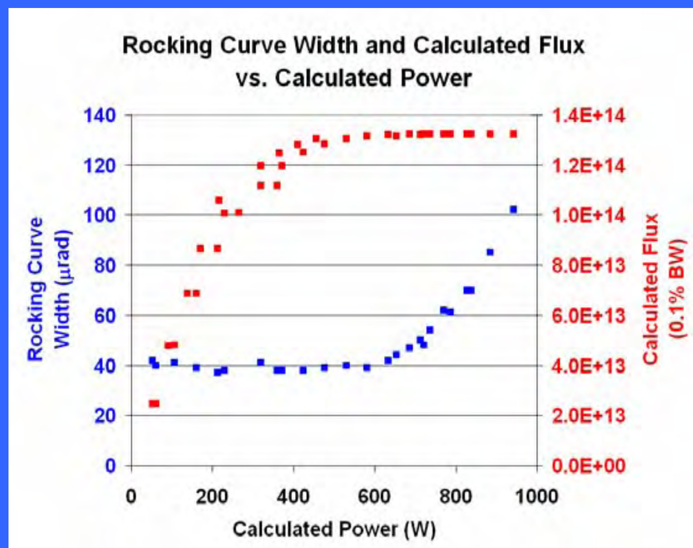
Reduces absorbed power and amount of Compton scattering produced

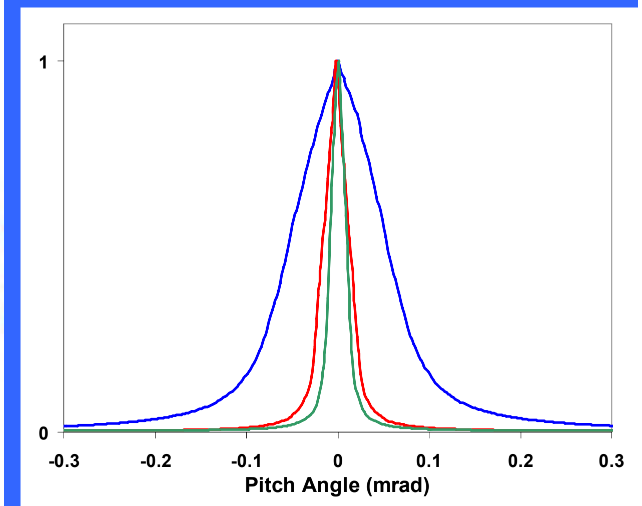
Monochromator Performance

- **Energy resolution in good agreement with Si (111)**
- **High heat load tolerance**
  - **No broadening of rocking curve with 600 W on 1st crystal at 100 mA**
  - **Can accept 90% of central cone at 200 mA (FEA calculations)**
- **Minimal rocking curve dispersion or “detuning” of 1st and 2nd crystals *vs*. energy**
- **Beam position stable to less than +/- 5 µm over the range 4 – 20 keV**

12 keV rocking curve

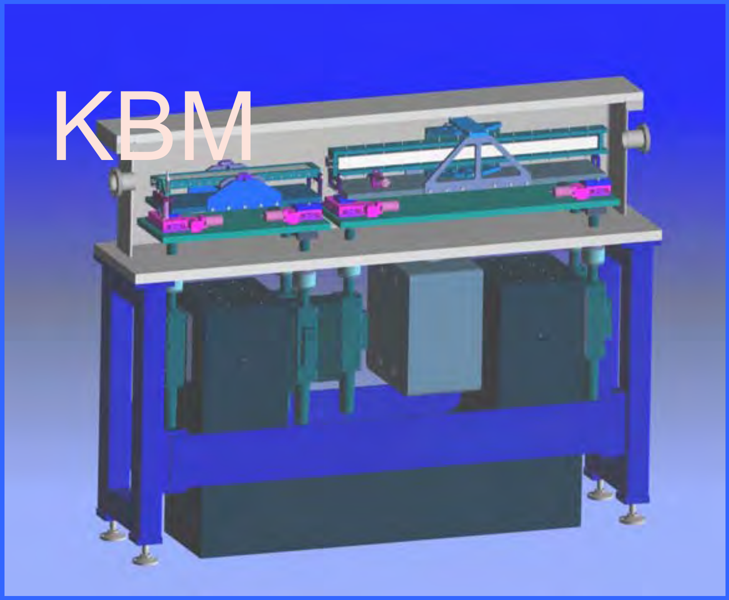






Fischetti, R.F. , et. al. (2007) "Optical Performance of the GM/CA-CAT Canted Undulator Beamlines for Protein Crystallography," Synchrotron Radiation Instrumentation, J.-Y. Choi, S. Rah, eds., American Inst. of Phys. 754-757.

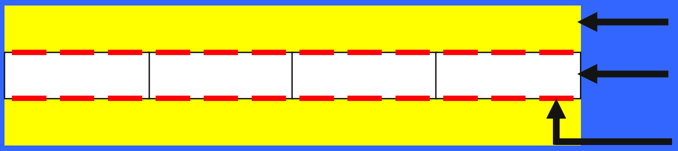
Compact K-B “bimorph” mirrors



Why use bimorphs mirrors

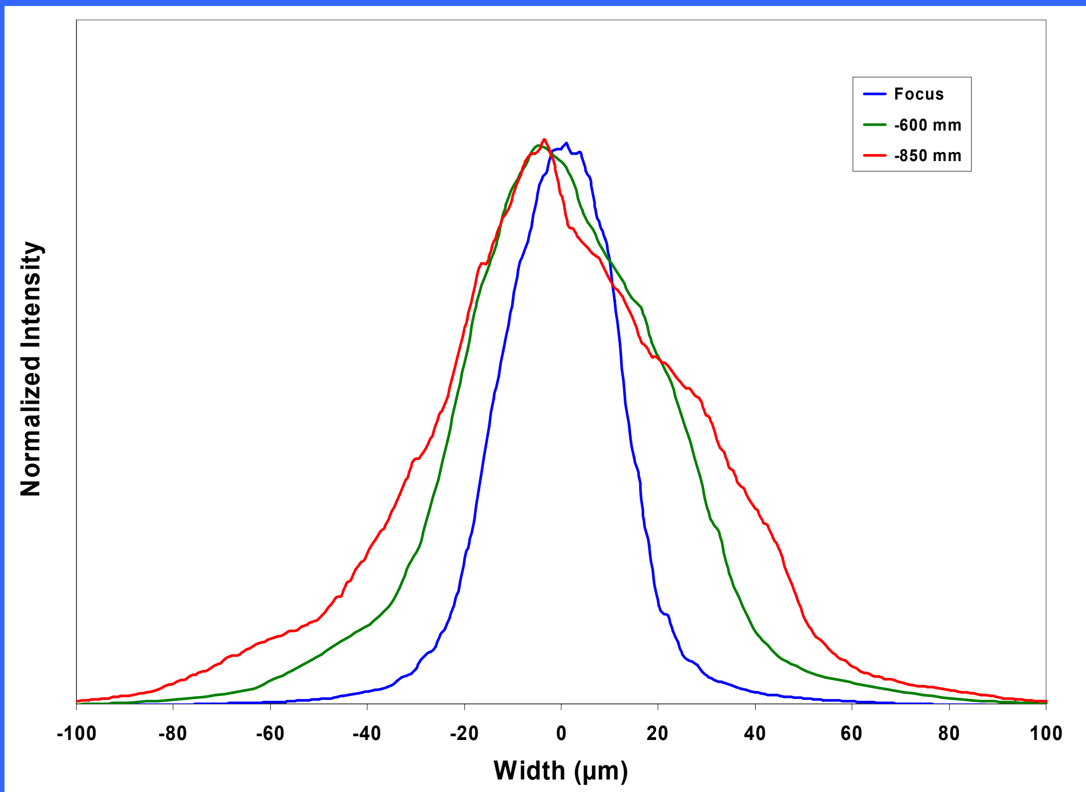
- •Insitu adjustment of slope error
- •Not just small beam
- •Uniform profile “off-focus”

Recently developed automated deterministic focusing procedures



	Length (mm)	# of segments	Electrodes / segment	Total Electrodes	Demag
HFM	1050	7	2	14	6:1 – 10:1
VFM	600	4	4	16	7:1 – 12.5:1

Vertical beam profiles over 850 mm range



Position	Distance (mm)	Beam Profile FWHM (µm)
Focus	600	30
Sample	0	48
Slits	-250	53

To shift the beam focal position:

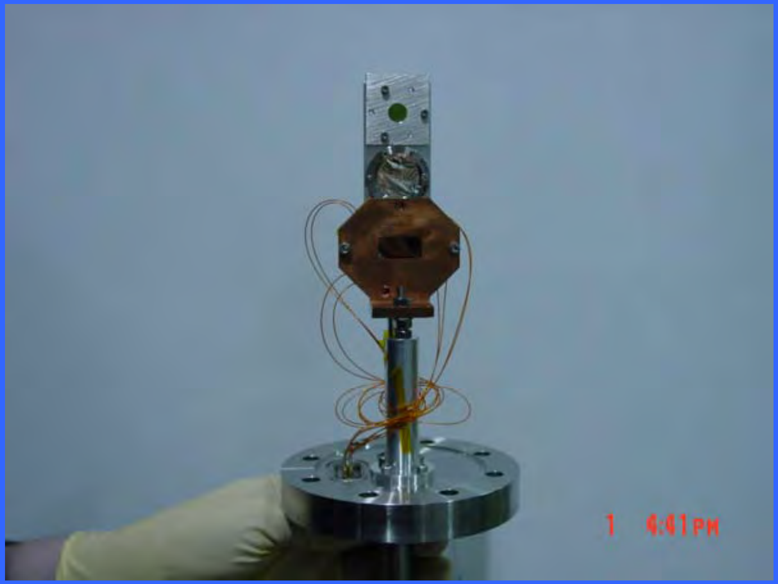
•Using automated focusing tools for a new position → 3 – 4 hours

•Using a lookup table for a previously determined position → minutes

Beam Position Monitors – after each optical component

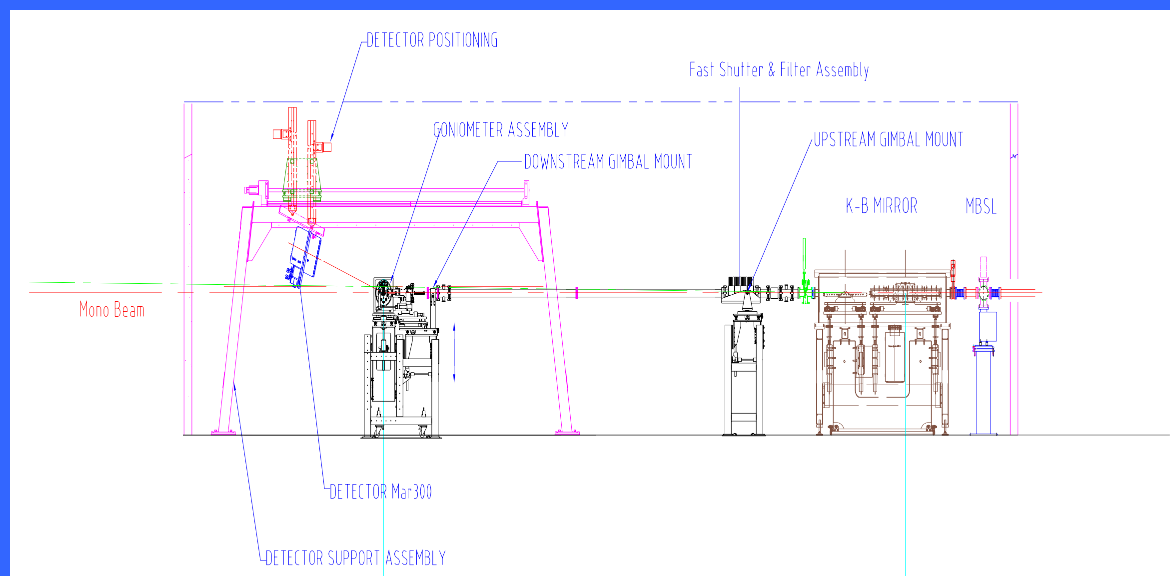
Intensity and positional information
Metallic foil source of fluorescence or scatter, quadrant diode array
(R.W. Alkire, G. Rosenbaum, G. Evans *J. Synchrotron Rad*. (2000) **7**, 61-68)

Real time image of beam
YAG crystal, right angle prism or mirror, vacuum window, and external video camera
(Xu, S., Fischetti, R.F**.,** Benn, R., Corcoran, S., (2007) Synchrotron Radiation Instrumentation, J.-Y. Choi, S. Rah, eds., American Inst. of Phys. 1403-1406.)

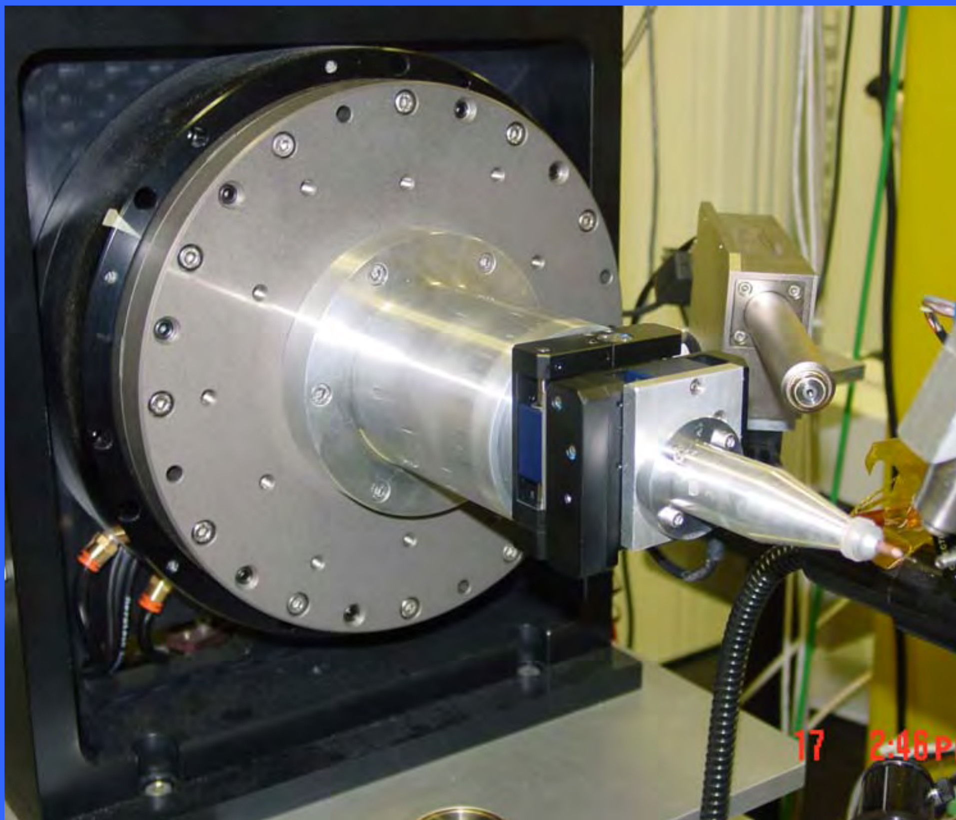


- Intensity feedback stabilizes I(0) – DCM 2nd crystal piezo
- Positional feedback locks position – VFM and HFM piezos

Detector support, beam delivery and goniometer support



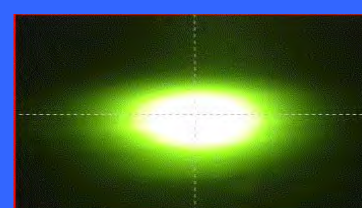
Goniometry and Visualization



On-axis-camera:
- beam passes thru optical axis of high resolution microscope
- non-parallax view

- AeroTech ABR1000 air bearing: <1 um wobble
- PI XY sample positioner: 2 – 3 micron “tumble”

Image of 20x70 µm beam on YAG as seen by high resolution microscope. (Image of unattenuated beam is saturated.)

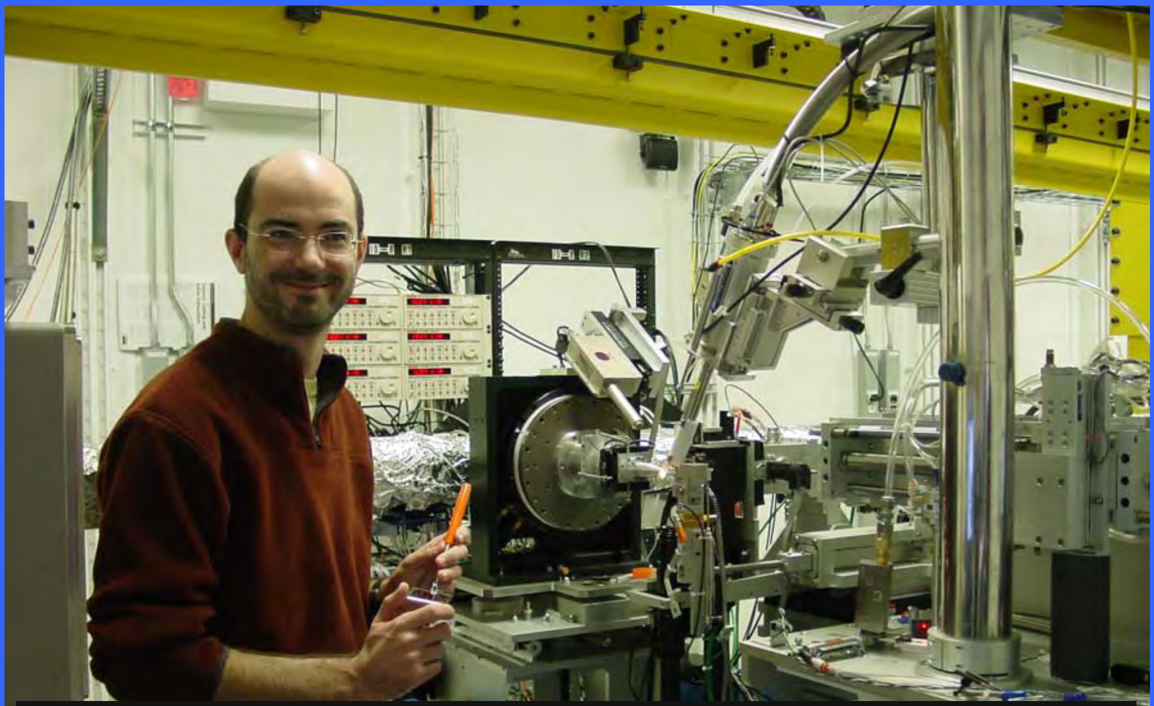


150 µm

← 200 µm →

Automated sample handling

Modified ALS robot design, collaborated with LRDesign to incorporate improvements suggest by Thomas Earnest, Carl Cork and others, and to customize for our goniometry.

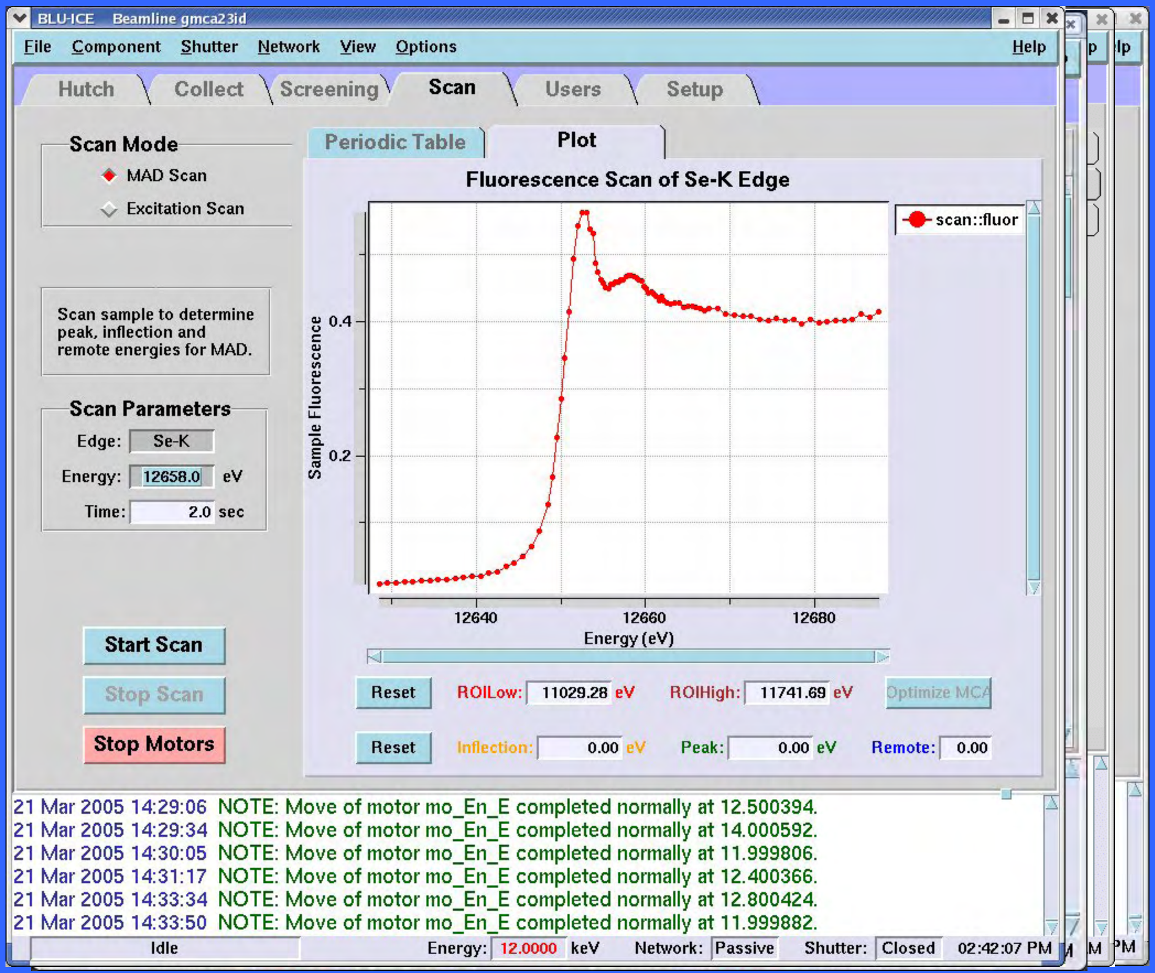


Beta tester Gyorgy Snell (Takeda San Diego)

Installed on all three beamlines
Available to users on ID-lines
Compatible with either ALS/SSRL (96 pins), or Rigaku (72 pins) pucks
Screening takes < 3 min/crystal with “point & click” centering
Implementing automated sample centering (hired Sadhir Babu Pothineni from Victor Lamzin’s group - XREC

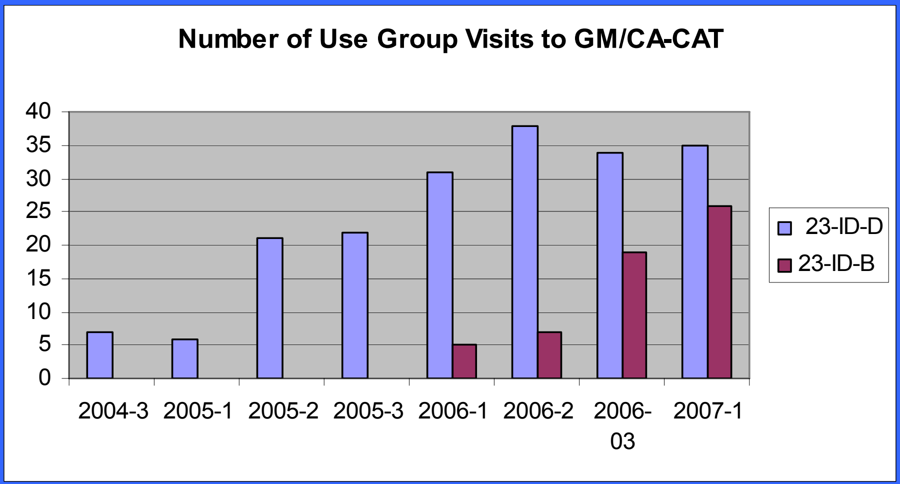
1st user screened >400 crystals in 3 days
2nd user screened >100 crystals in 1 day

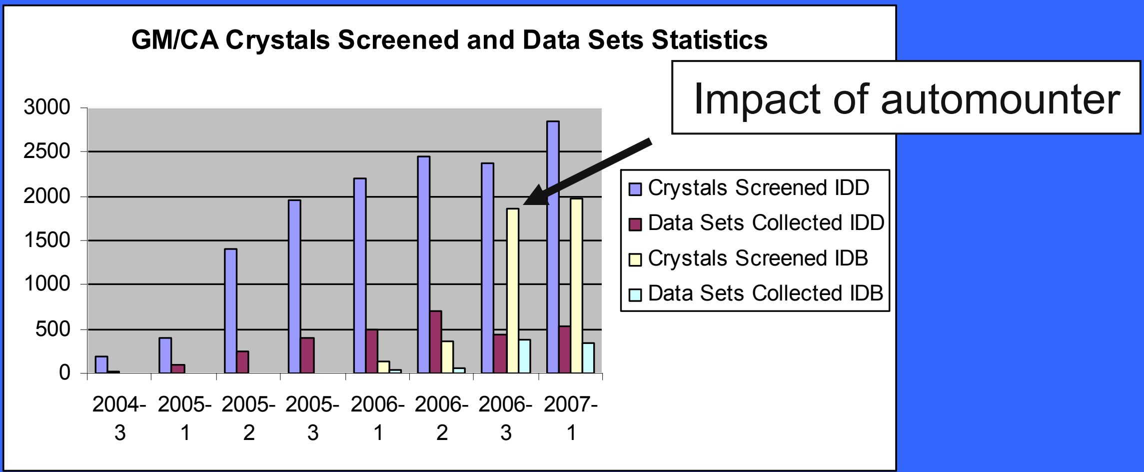
Blu-Ice/EPICS user interface



BluIce - Stanford Synchrotron Radiation Laboratory (SSRL)
Structural Biology Macromolecular Crystallography Program (SMC)
EPICS interface - GMCA

Ramp Up of the User Program





Total Number for all Runs

	Crystal Screened	Sets Collected	PDBs
ID-D	13826	2919	
ID-B	4328	806	
Total	18154	3725	102

Highlights of user activity

Viruses

Vijay Reddy and coworkers from Scripps conducted preliminary experiments with crystals of human adenovirus - Unit cell (892, 892, 2122.0 Å)
Resolving spots along the 2000-A axis was not a problem
After improvements in the crystallization and crystal-handling techniques, this group is returning to collect better data.

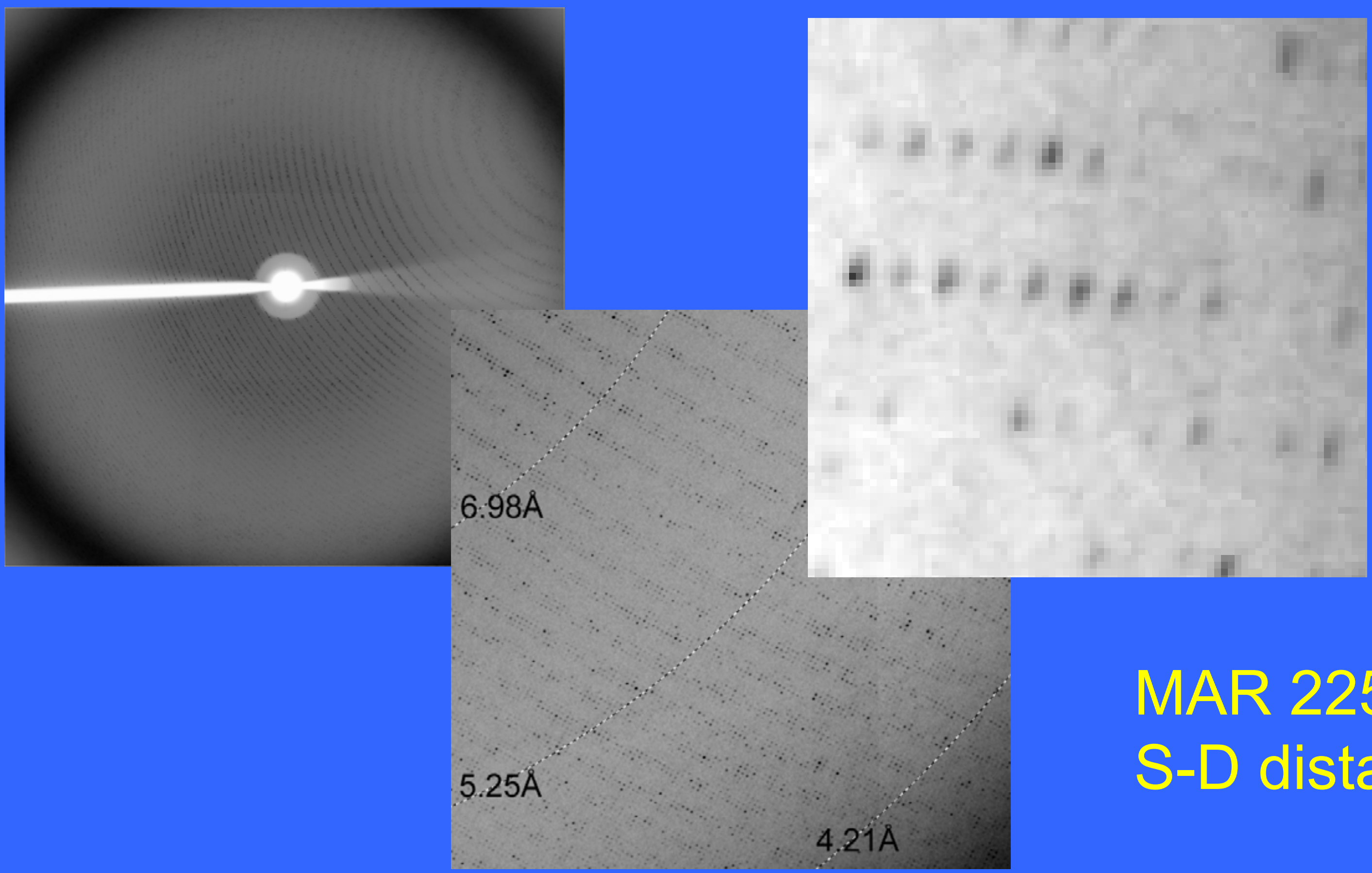
Stunning diffraction from the HK97 virus capsids, obtained by Jack Johnson's group from Scripps.

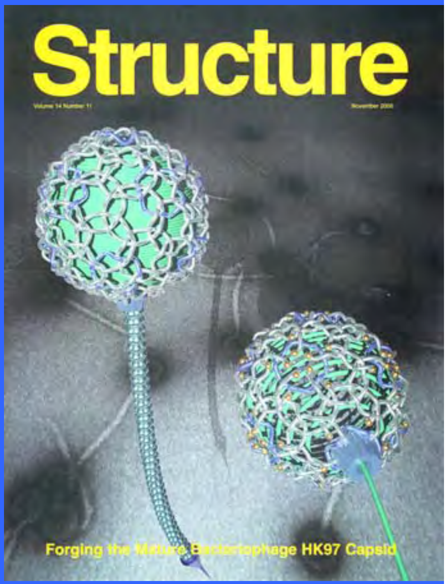
Ribosomes

Harry Noller's group from UC-Santa Cruz collected data from the crystals of whole ribosome soaked in various ligands. High quality data are being analyzed. Sergei Trakhanov has visited 4 times to collect data on a new crystal form of the 70S ribosome.

Large Unit Cells

Diffraction pattern from HK97 virus capsid.
Unit cell dimensions: 1010 x 1010 x 732 Å





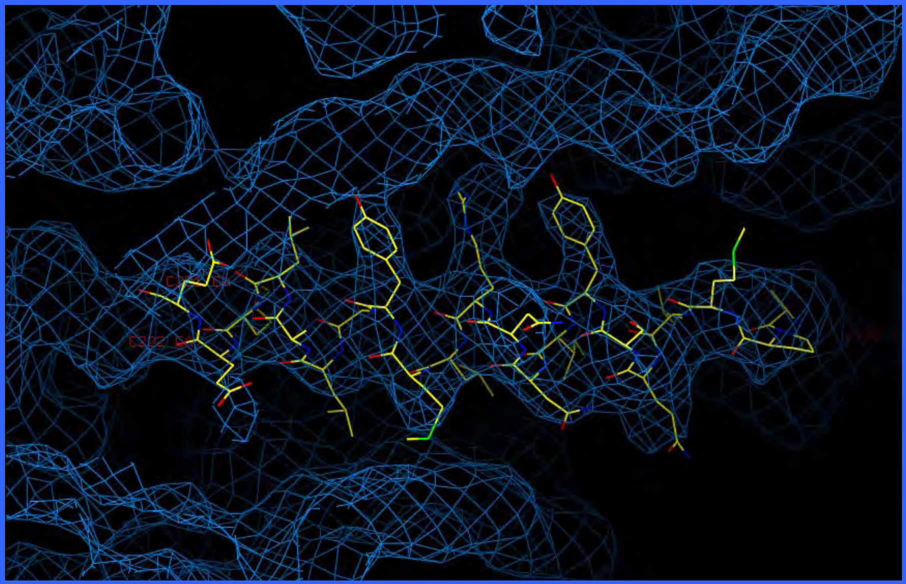
MAR 225
S-D distance 680 mm

L. Gan, *et al*. & J. E. Johnson *Structure* 14, 1655-65 (2006)

Large Unit Cells, continued

Model fitting into electron density map of HK97 capsid (Jack Johnson/Lu Gan)

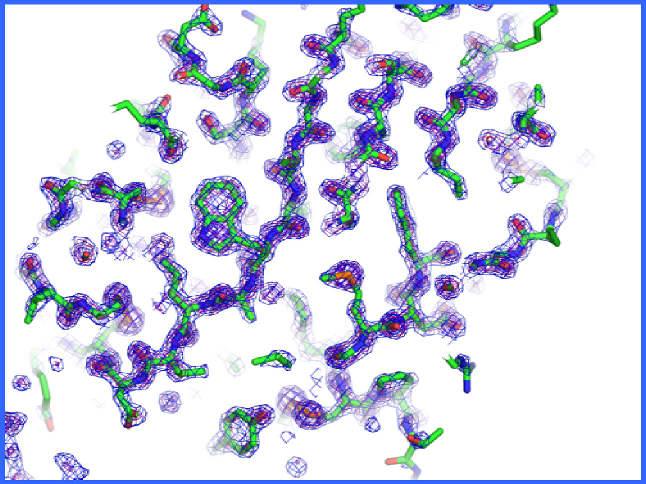
"…Figure below shows a section of electron density from the 4.2Å map of Head II. The quality is superb and bulky side chains can be visualized even at this resolution…."



Highlights of the user program, continued

New technology

Crystal never handled!



In-situ x-ray data collected using capillary microfluidics approaches.
1.61-Å map
Yadav & Kuhn

Mini-beam Capability

Requirements

- Beam size: 5 – 10 μm FWHM, Gaussian-like profile
- Beam positional stable ~1 μm
- Goniometer center of rotation stable to ~1 μm
- Accurate alignment of beam and center of rotation
- Effective visualization and lighting for ~5 μm samples for easy alignment
- Sample position stable on goniometer to ~1 μm
- All above attributes stable through sample changes

Advantages of mini-beam

- Raster the beam on crystals with a long dimension to overcome radiation damage
- Select best part of crystal – mosaicity or macro-twining
- Reduce background by better matching beam and crystal size
- Collect useful data on projects that produce only small crystals
- Reduced beam cross fire – especially in the horizontal direction

Disadvantages of mini-beam

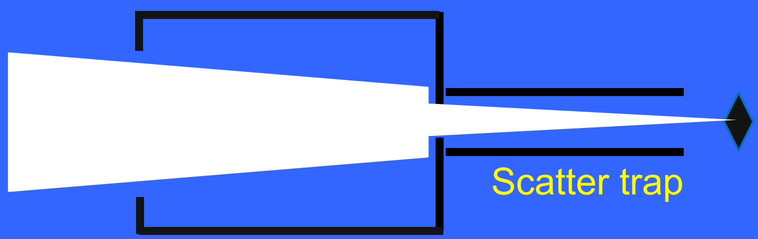
- Diffraction from small crystals is weaker than from big crystals
- The flux density is similar to that of the “big beam” so the trade-off between obtaining stronger diffraction vs. greater radiation damage is trickier
- May have to merge partial data sets from multiple crystals
- Achieving stability requirements routinely

Approaches to achieving small beams

Approach #1: overfill an aperture

Focus to 20x70 µm at sample

5-30 µm pin hole reduces beam size



"Small beam"

Advantages

- Tolerates beam positional instabilities
- Provides clean beam shape w/ low background
- Reduces beam divergence to detector

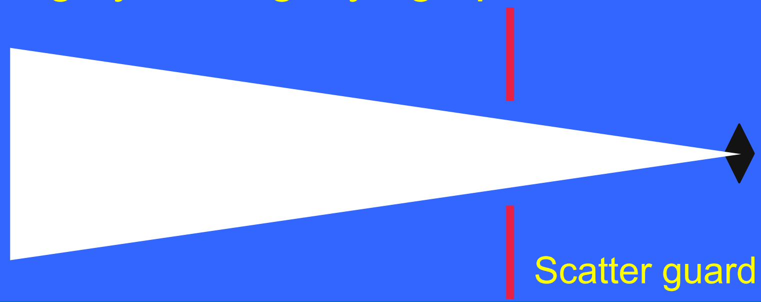
Disadvantage

- Limited to ~5 µm beam size

Approach #2: focus to desired size

Focus to 1 – 10 µm at sample

Highly demagnifying optics



"Small focus"

Advantage

- Suitable for 1 µm beam size

Disadvantages

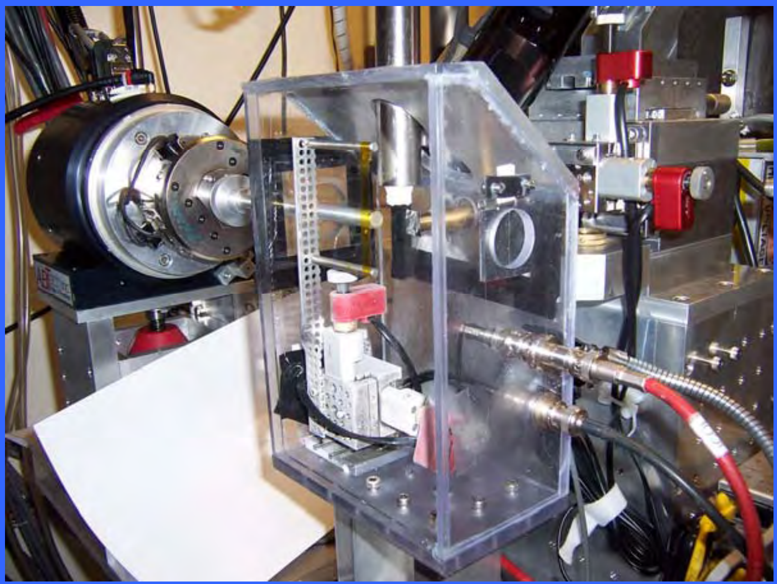
- Sensitive to beam positional instabilities
- Large beam divergence to detector

Micro-diffraction around the world (not all listed)

Current capabilities

- ESRF ID-13: recently rebuilt as nano-focus beamline (David Eisenberg, amyloid)
- ESRF ID23-2: micro-focus, ~7 microns, 1x10^11 photons/sec
- SLS: MD2
- APS GMCA-CAT (23ID-B, -D): ~5 micron, ~7x10^10 photons/sec
- MacCHESS (F1): ~20 micron, 1x10^10 photons/sec

MacCHESS F1

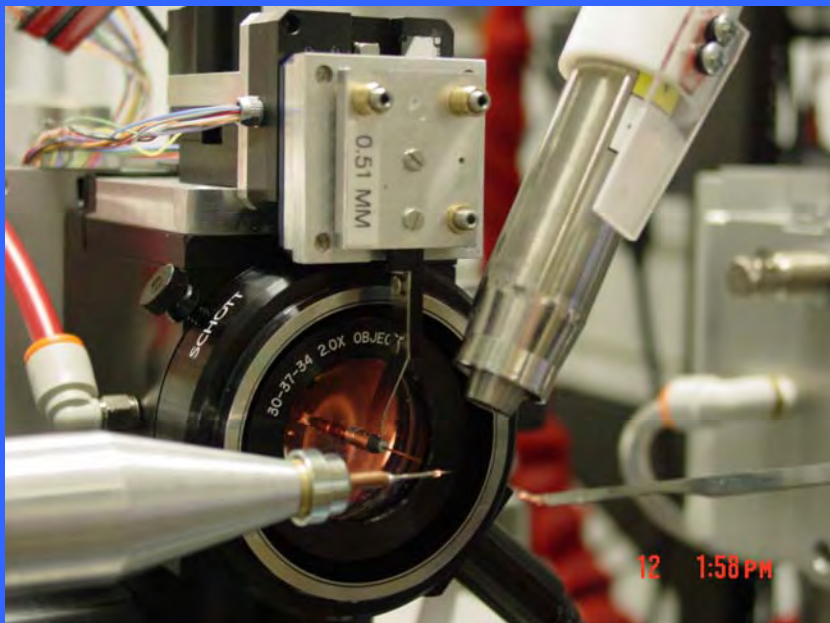


Courtesy Richard Gillian

Planned capabilities

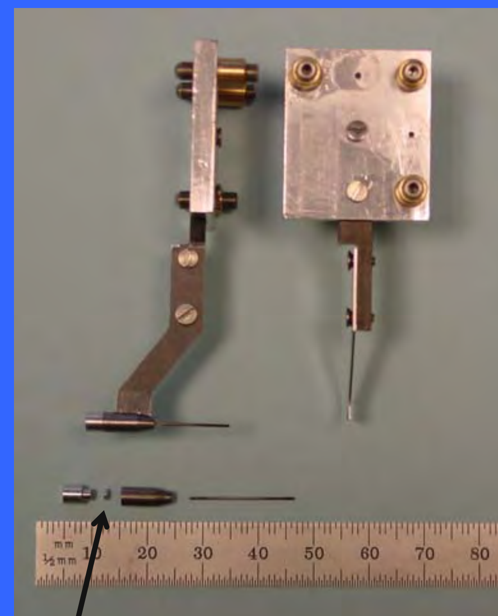
- MacCHESS (F2): ~ 5 micron
- Diamond – micro-focus beamline
- APS NE-CAT (24ID) – MD2
- APS LS-CAT (21ID) – MD2
- APS SBC-CAT (19ID) – new beamline with 1 micron focus

Mini-beam apparatus: small beam-defining pinholes



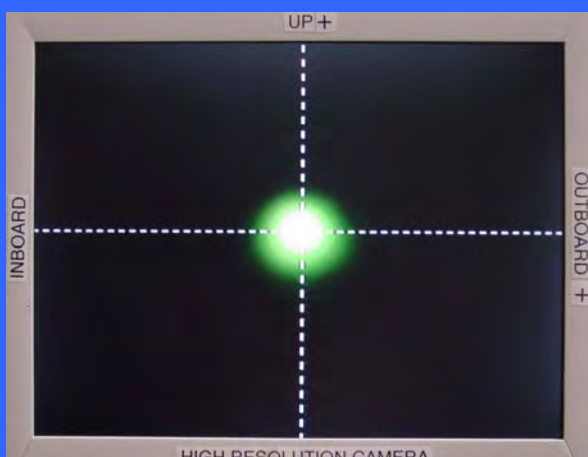
Kinematic mount
- rapid interchangeability
- 15 minute re-alignment

New dual collimator
- one click exchange
- auto-align routines



5, 10, 30 μm apertures

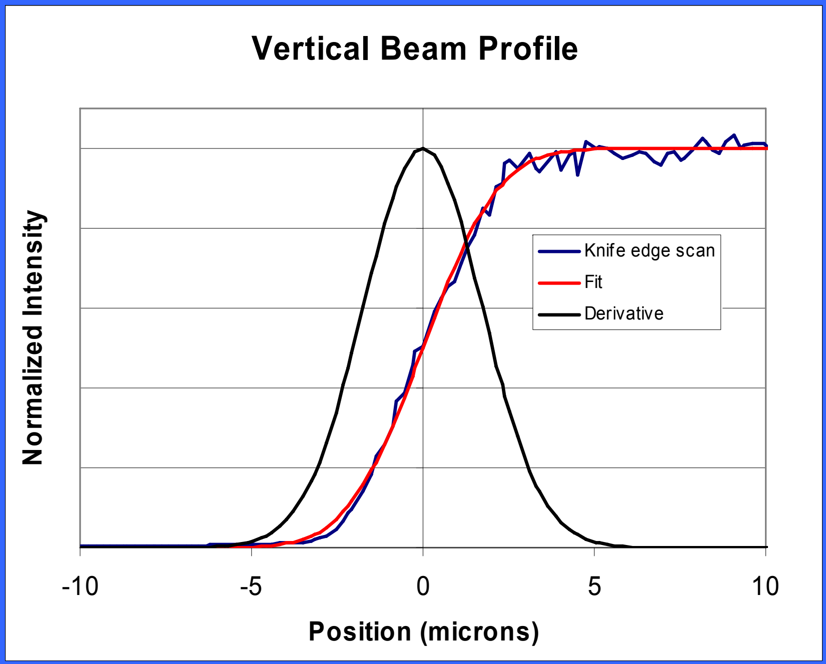
5 & 300 μm



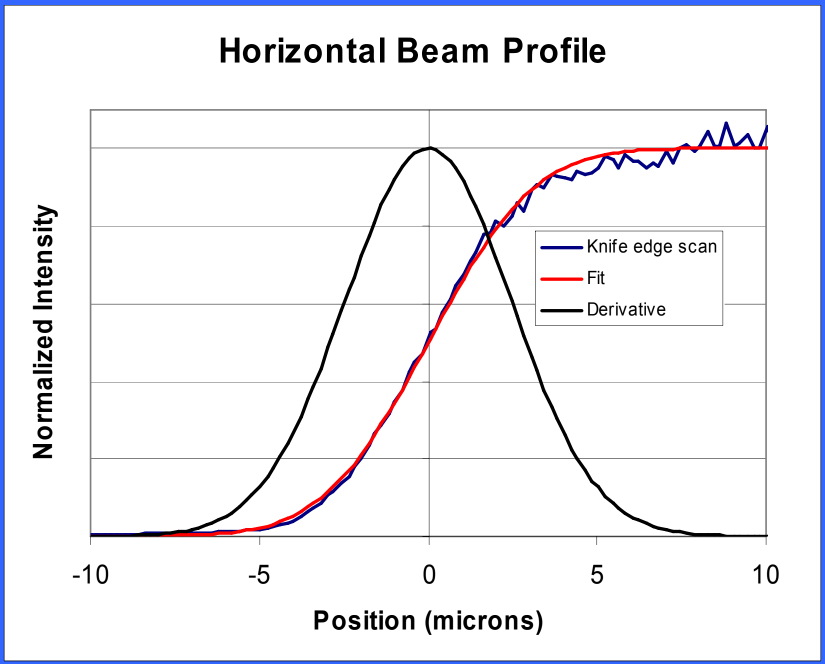
~7 μm beam (FWHM)
Viewed on YAG crystal at sample position
Beam focused at sample position
10 μm aperture

Knife Edge Scans of "Mini-beam" through 5 µm pinhole

FWHM = 4.1 µm



FWHM = 5.8 µm



- Flux : ~ 6.7×10^{10} photons/sec/100 mA – often needs to attenuate!
- Intensity stability – <1% RMS

Visualization of small crystals

7 x 7 x 15 µm^3 lysozyme crystal in 30 µm MiTeGen loop

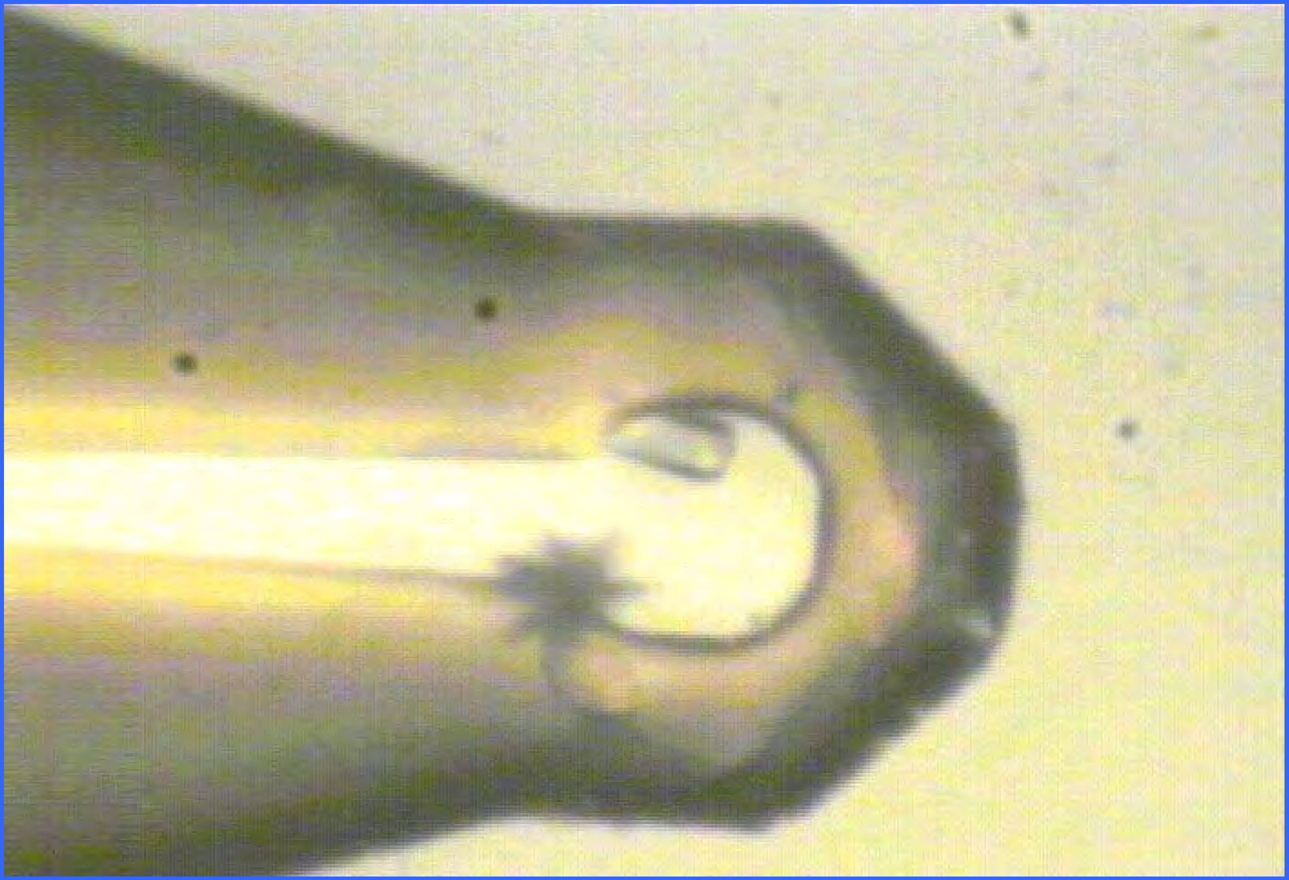
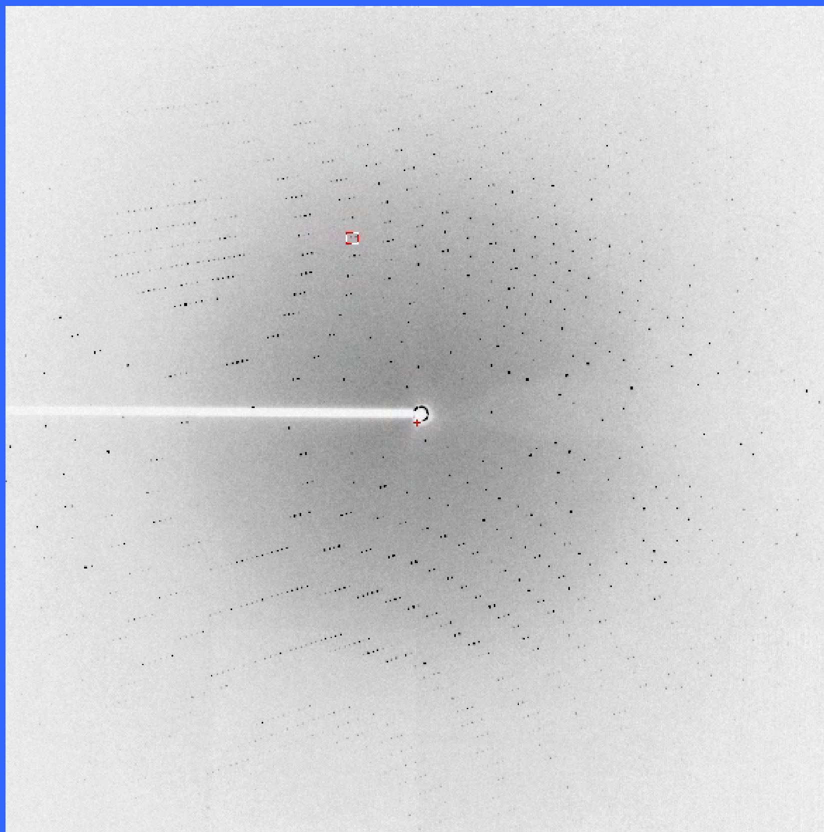


Image recorded with mini-beam apparatus in position

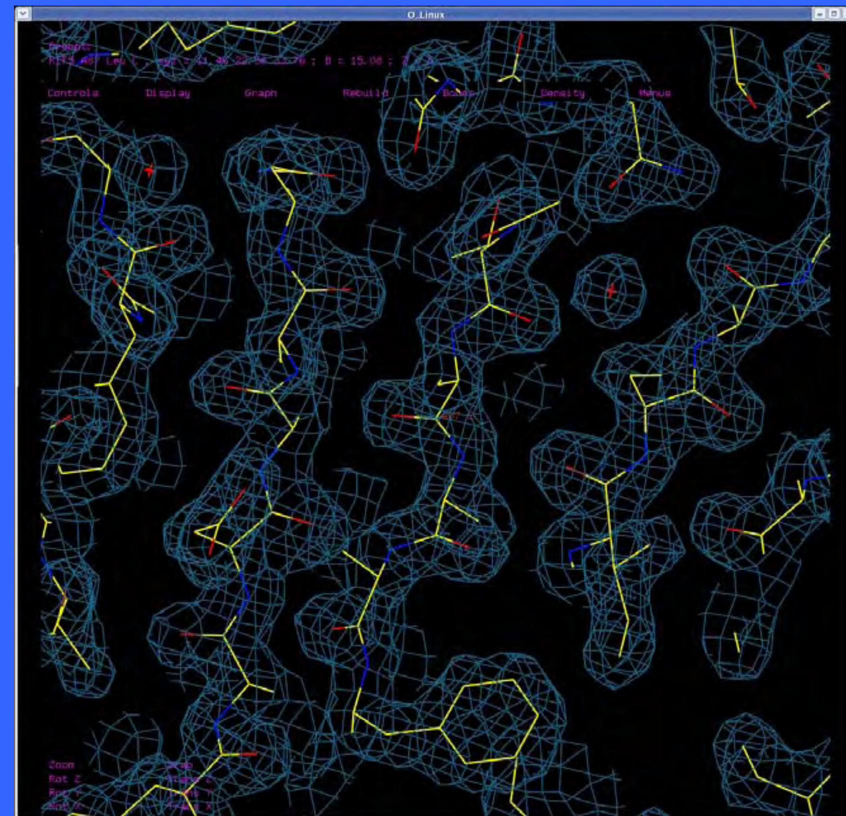
Diffraction patterns from small thaumatin crystals

10x4x<4 μm^3 thaumatin crystal



Diffraction from small crystals is weak, but measurable. Background is low → S/N is high!

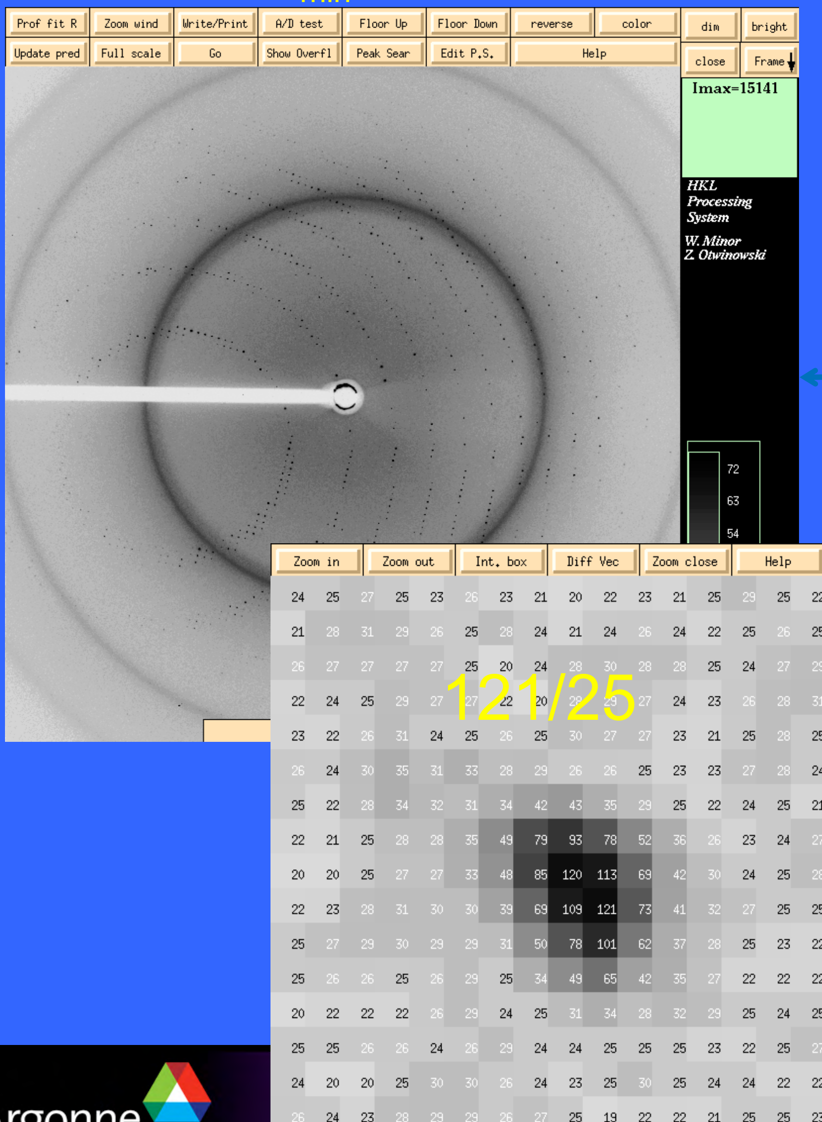
Electron density model refined against 1.94 Å data from 6 small crystals.



2F$_o$-F$_c$ electron densities contoured at 1 rmsd.

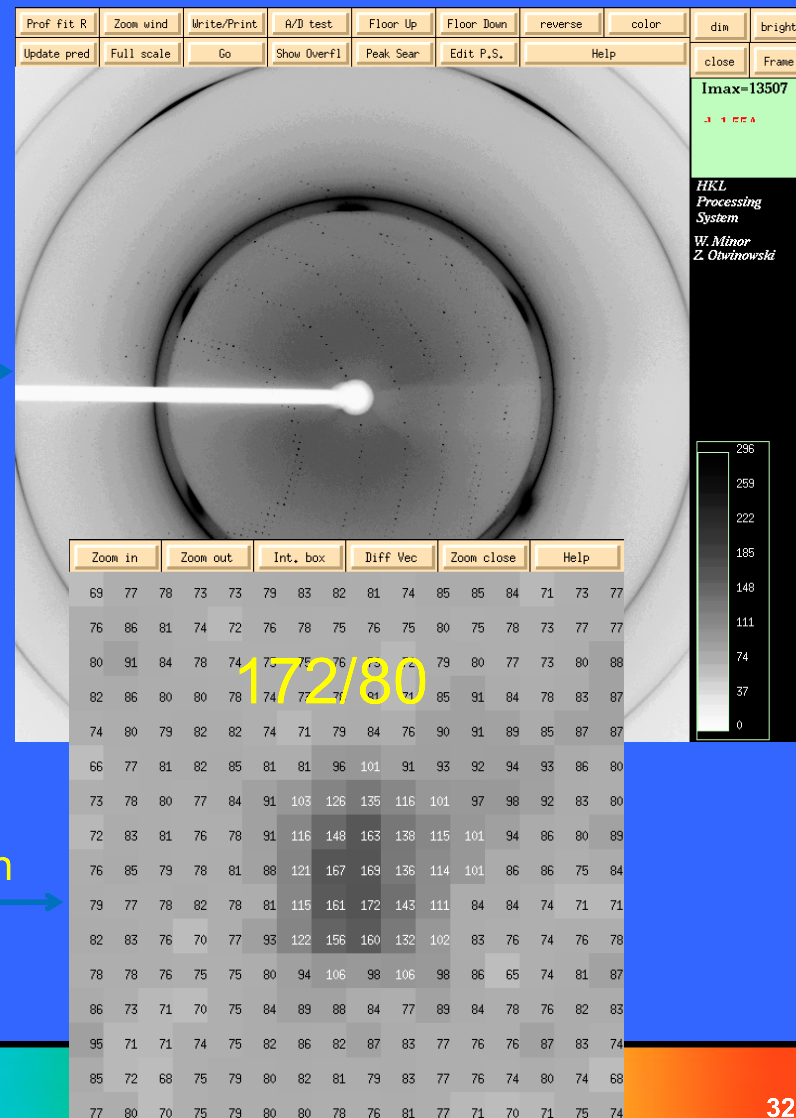
Improved Signal/Noise from Thioesterase Sample - ~8x8x150 µm

7-µm beam
d_{min} = 2.4 Å



Same crystal & setting

75-µm beam
d_{min} = 2.6 Å



Same reflection

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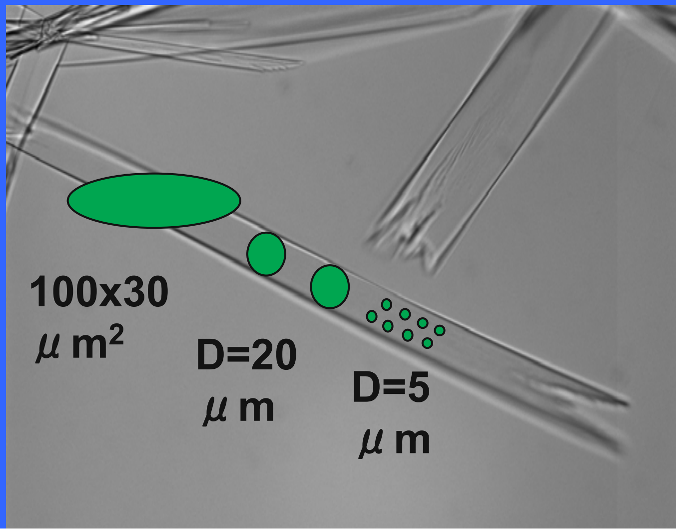
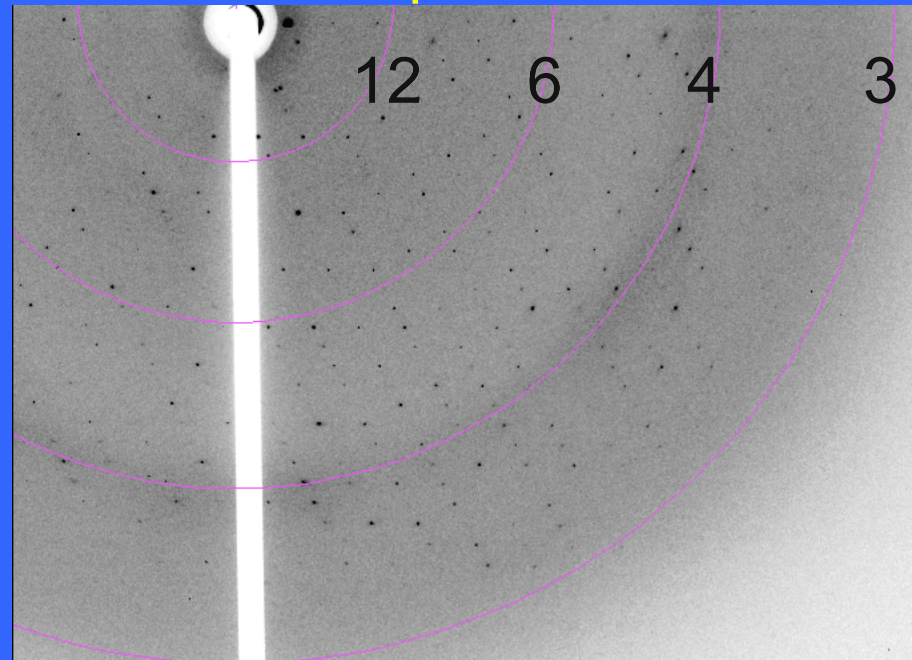
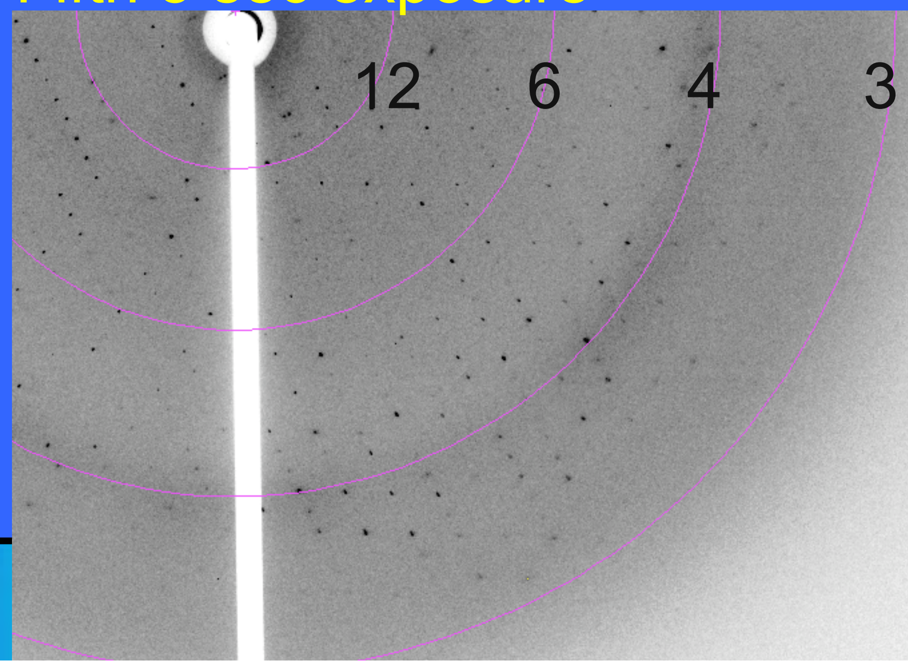
Beware of radiation damage



First 5 sec exposure



Fifth 5 sec exposure



The two diffraction patterns show some radiation induced broadening of reflections after 5 frames. Exposure time was 5 sec/frame with 6.7x10^10 photons/sec.

A full data set was merged using the 5 μm beam and rastering in a zig-zag motion. Only 6 degrees of data could be collected at each location.

Images courtesy Brian Kobilka and Bill Weis, Stanford University.

NSLS-II

Suppose we put GM/CA-CAT on a flat bed truck and drove it to NSLS-II. What performance can we expect?

	APS (microns) *	NSLS-II Low β (microns) *	NSLS-II High β (microns) *	NSLS-II Low β - Optimized (microns) **
Beam size at mirror	1044 x 431	1290 x 348	466 x 297	1450 x 384
Theoretical focus	49 x 2.4	6.4 x 0.63	22 x 1.1	0.92 x 0.14
Focus w/ slope error	62 x 20	38 x 20	44 x 20	1.49 x 1.65

* Reduced the length of the mirrors, positioned mirrors closer to sample and reduced mirror slope error to 0.25 micro-radian.

Conclusions

- Maintaining source brilliance will be a major challenge
- Slope error of focusing mirrors will dominate the minimum focal size
- One can achieve beam sizes on the order of 1 micron with approximately a 10-fold increase in intensity over GM/CA

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