

Microcrystallography Developments at the APS and Around the World

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Outline

- Statistical highlights
- Aspects of micro-crystallography
- Scientific highlights
- Automation
- SONICC
- Micro-beams and radiation damage
- APS-U (150 mA)
- Putting it all in perspective



Macromolecular Crystallography at the APS

Operator	BM Line	ID Line(s)			Technique
BIOCARS	14-BMC	14-ID			MX, Laue, TR scattering, BSL2/3
IMCA-CAT	17-BM	17-ID			MX, 17-BM: powder diffraction
SBC-CAT	19-BM	19-ID			MX
LS-CAT		21-IDD	21-IDF	21-IDG	MX, Bionano-probe
SER-CAT	22-BM	22-ID			MX
GM/CA	23-BMB	23-IDB	23-IDD		MX, 23-BMB: WAXS
NE-CAT		24-IDC	24-IDE		MX
LRL-CAT		31-ID			MX

Beamlines previously used for MX

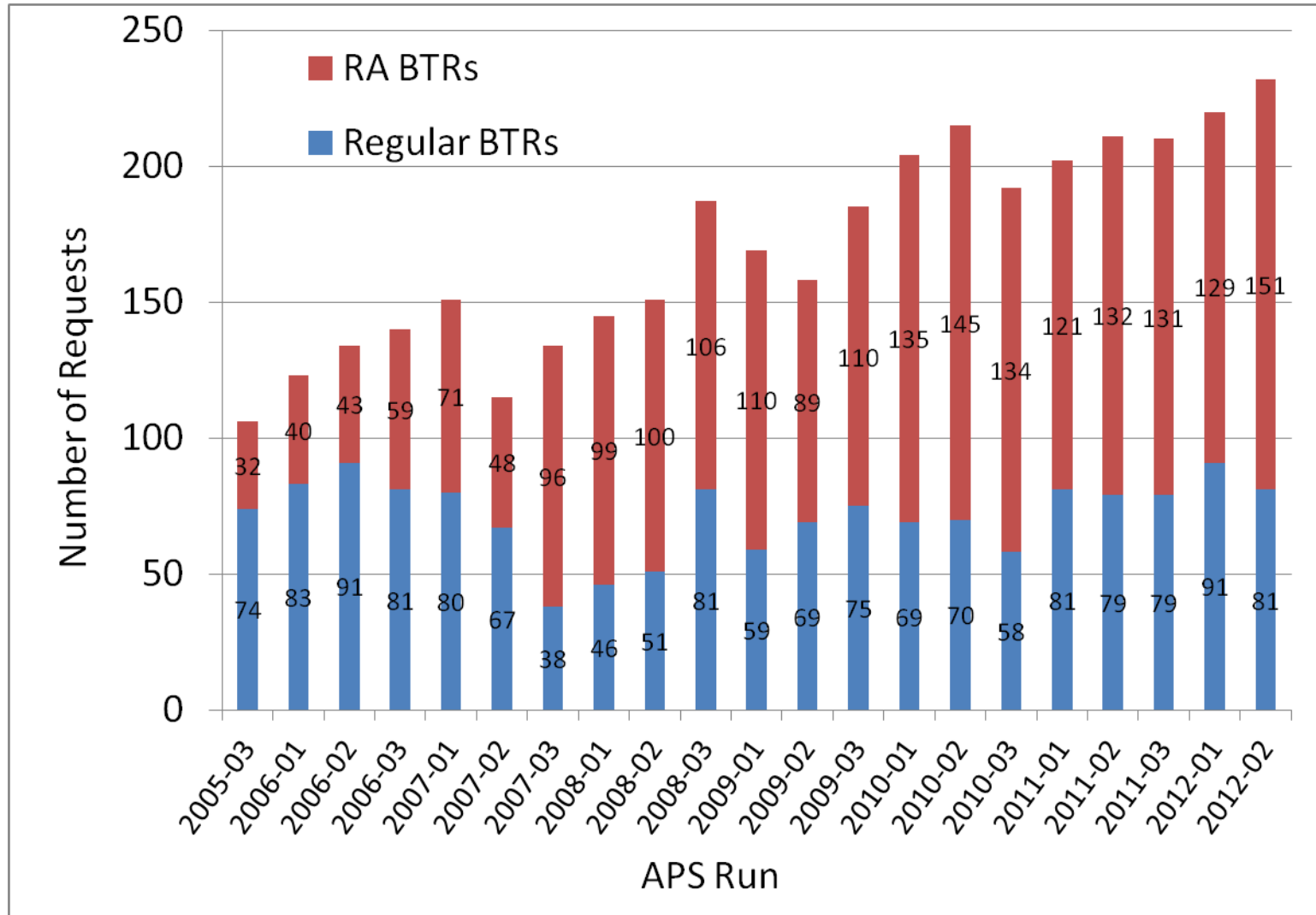
5ID

8BM

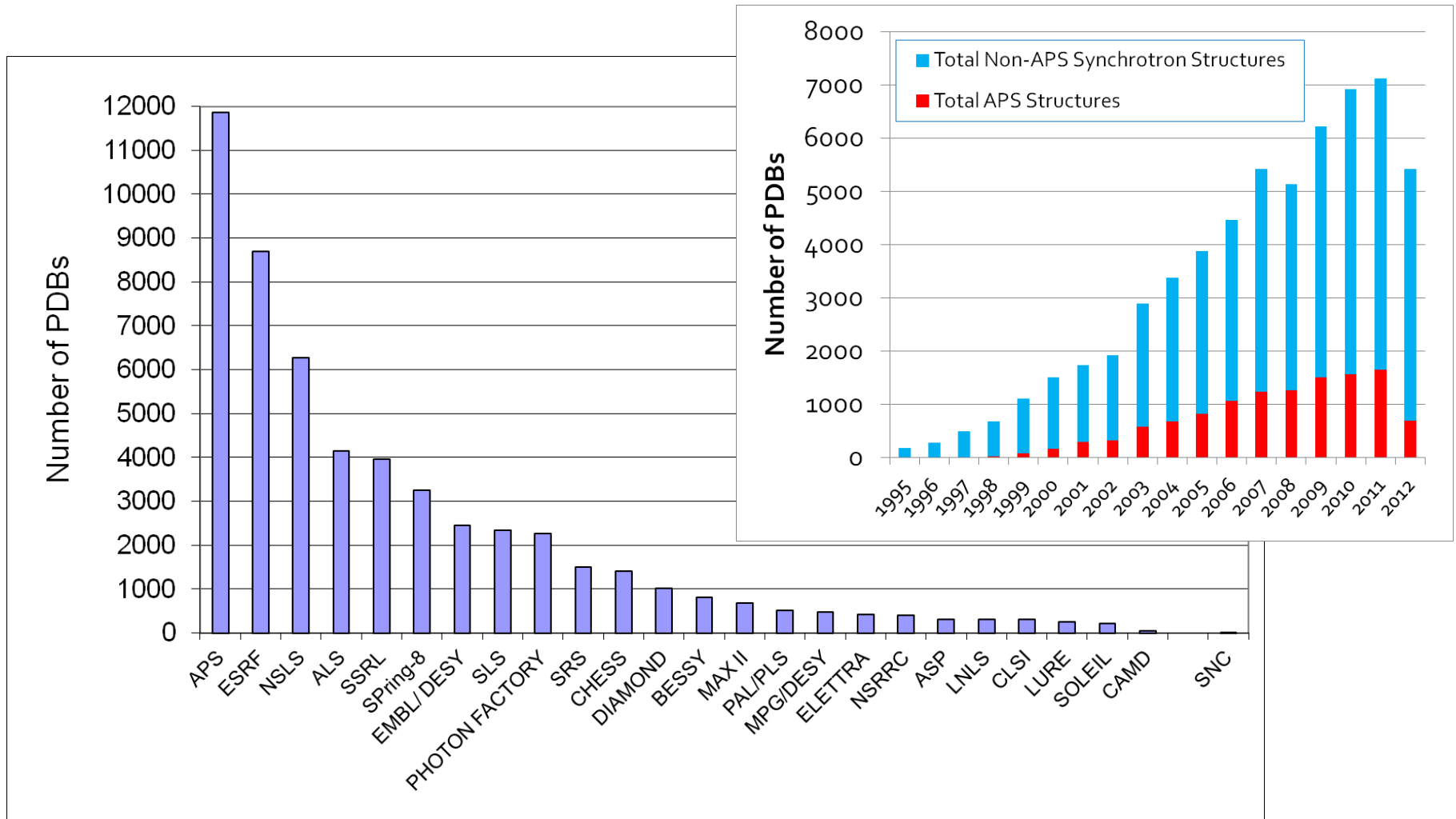
14-BMD



Beamtime request continue to grow



APS world leader in Protein Data Bank depositions



Over 25% of all structures from synchrotron source are from APS



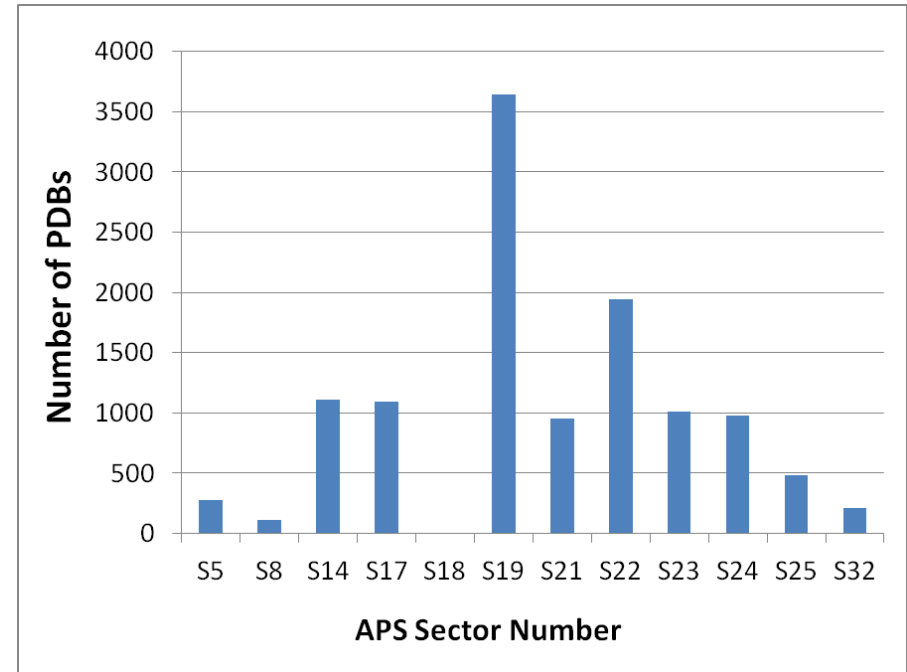
1000 PDB club

APS 1000 PDB club membership

- SBC-CAT has 3629 deposits since 1997
- SER-CAT has 1943 deposits since 2002
- BioCARS has 1108 deposits since 1998
- IMCA-CAT has 1943 deposits since 1998
- GM/CA has 1005 deposits since 2005
- NE-CAT has 982 deposits since 2004
- LS-CAT has 905 deposits since 2008

National 1000 PDB club membership

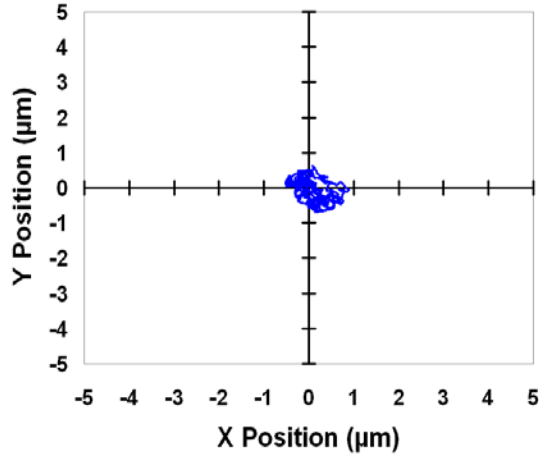
- 5 [+ 2] sectors at the APS
- 2 sectors at ALS
- 3 sectors at NSLS
- 2 sectors at SSRL



Micro-crystallography developments

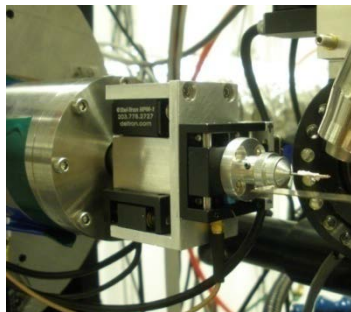
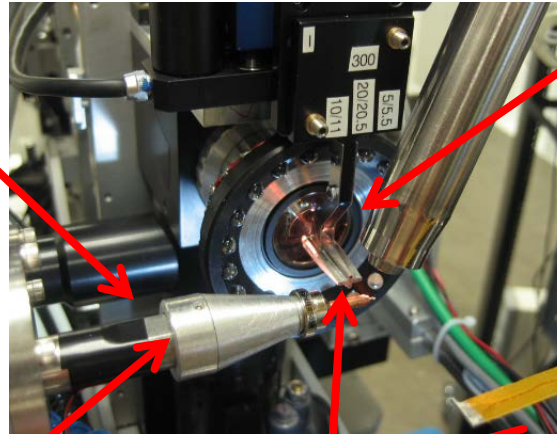
On-axis sample visualization

Goniometer:
1 μm SOC peak-to-peak



SONICC

Sample environment



Goniometer head:
nano-positioning



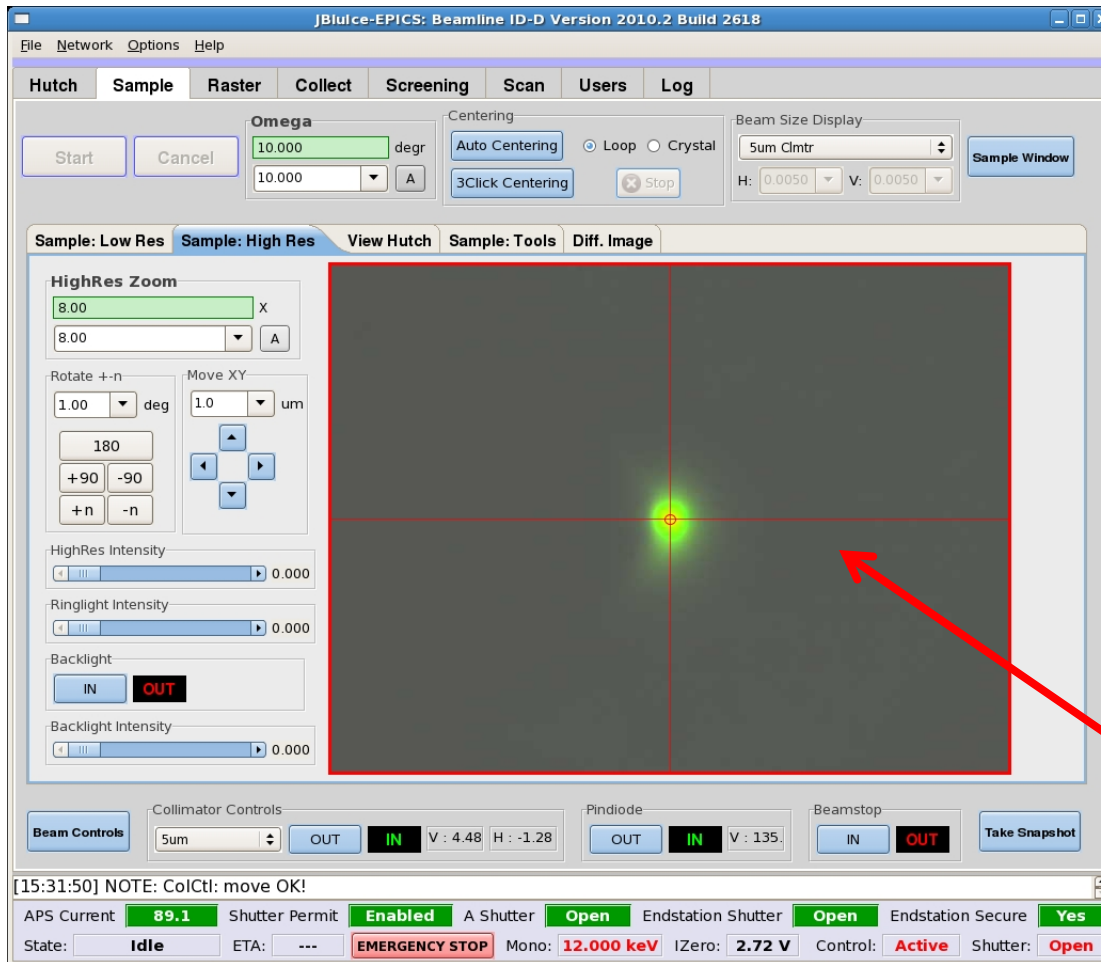
Quad mini-beam collimator:
5, 10, 20- μm beams and
300- μm scatter guard



Active beamstop:
Photoelectric effect

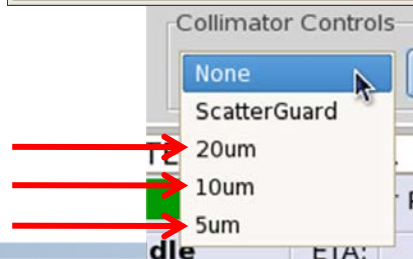
Rapid beam size selection

JBlulce-EPICS GUI



Beam size FWHM (μm)	Intensity (Ph./sec)
20 x 65	2.0×10^{13}
20 \emptyset	1.0×10^{12}
10 \emptyset	5.2×10^{11}
5 \emptyset	5.4×10^{10}
1 \emptyset	3.0×10^9

Image of beam at sample position on YAG crystal



Quad mini-beam collimator

- match beam and crystal size
- use small beam to probe large crystal

Finding/centering invisible crystals or mapping quality

The screenshot shows the JBluice-EPICS Beamline ID-B software interface. The main window is titled "JBluice-EPICS: Beamline ID-B Version 2010.3 Build 2850". It features a menu bar (File, Network, Options, Help) and a toolbar (Hutch, Sample, Raster, Collect, Screening, Scan, Users, Log). The interface is divided into several sections:

- Control:** Start, Pause buttons.
- Display:** Grid #s (unchecked), Smoothing (checked), Position sync (checked).
- Position:** Omega = 125.802, Sample X = 0.479, Sample Y = -0.443, Sample Z = -0.947, Detector = 300.001, Atten. = 19.989, Beamstop = 34.999.
- Run 1 (rastering):** Default, Update, Delete, Reset buttons. Raster mode: Diffraction (selected), Fluorescence. Prefix: csd1_raster_1. Dir: /mnt/share1/naga/csd1. Grid Width: 100 μm, Grid Height: 60 μm, Cell Width: 10 μm, Cell Height: 10 μm, Time: 1.0 sec, Delta: 1.000 deg, Distance: 300.000 mm, Attenuation: 20.00 factor, Processing: checked.
- Diffraction Image:** A central image showing a diffraction pattern with a blue grid overlaid. A red crosshair is centered on the image.
- Data Table:** A table with columns: Status, X, Y, Frame, Row, Col, Spot Total, In-Resolution Total. The table contains 7 rows of data, with the 4th row highlighted in blue.
- Status Bar:** [16:45:40] NOTE: DevFPE: start=0 opStat=0 abort=0, sending start=1. APS Current: 102.0, Shutter Permit: Enabled, A Shutter: Open, Endstation Shutter: Open, Endstation Secure: Yes, State: Rastering, ETA: ---, EMERGENCY STOP, Mono: 12.659 keV, IZero: 0.02 V, Control: Active, Shutter: Closed.

Status	X	Y	Frame	Row	Col	Spot Total	In-Resolution Total
DONE	-45.0	-5.0	30	3	1	394	378
DONE	45.0	5.0	31	4	10	24	24
DONE	35.0	5.0	32	4	9	216	183
DONE	25.0	5.0	33	4	8	472	451
PROCESSING	15.0	5.0	34	4	7		
WRITING	5.0	5.0	35	4	6		
COLLECTING	-5.0	5.0	36	4	5		

Grid search developed at ID13

Big beam – beam sample implemented at SSRL (*J. Syn. Rad.* (2007) **14**, 1891-195)

GM/CA large-beam (coarse grid) and mini-beam (fine grid) implementation (*J. R. Soc. (2009) Interface* , **6**, S587-S597)

Diamond and now many others have implemented rastering *Acta Cryst. D*, **66**, 1032-1035 (2010)

Ranking by “distl”
Nick Sauter

M.Hilgart, R.Sanishvili, C.Ogata, M.Becker, N.Venugopalan, S.Stepanov, O.Makarov, J.L.Smith, and R.F.Fischetti, Automated sample scanning methods for radiation damage mitigation and diffraction-based centering of macromolecular crystals, *JSR* (2011) **18**, 717-722



Polygon rastering

The screenshot shows the JBIUCE-EPICS Beamline ID-B software interface. The main window displays a grayscale image of a sample with a blue polygon overlaid. The polygon is divided into numbered cells (1-81). A table at the bottom right lists the status, coordinates (X, Y, Z), Omega, Frame, Row, Col, and Spot Total for each cell. The status for all cells is 'DONE'.

Status	X	Y	Z	Omega	Frame	Row	Col	Spot Total
DONE	0.920	-0.112	-5.606	-0.000	152	16	4	538
DONE	0.920	-0.112	-5.586	-0.000	153	16	3	156
DONE	0.920	-0.112	-5.566	-0.000	154	16	2	54
DONE	0.920	-0.092	-5.626	-0.000	155	17	5	34
DONE	0.920	-0.092	-5.606	-0.000	156	17	4	83
DONE	0.920	-0.092	-5.586	-0.000	157	17	3	453
DONE	0.920	-0.092	-5.566	-0.000	158	17	2	357

Below the table, the status bar shows: APS Current: 102.4, Shutter Permit: Enabled, A Shutter: Open, Endstation Shutter: Open, Endstation Secure: Yes, State: Idle, ETA: ---, EMERGENCY STOP, Mono: 12.000 keV, IZero: 0.04 V, Control: Active, Shutter: Closed.

Coordinates can be transferred automatically for data collection

M.C. Hilgart, R. Sanishvili, C.M. Ogata, M. Becker, N. Venugopalan, S. Stepanov, O. Makarov, J.L. Smith and R.F. Fischetti J. Synchrotron Rad. (2011). 18, 717-722

Fluorescence rastering - fast slew scan mode

Slew mode ~30 sec

The screenshot shows the JBIUC-EPICS Beamline ID-D software interface. The main display window shows a grayscale image of a cell with a blue and yellow heatmap overlay representing the fluorescence raster scan. The heatmap is shaped like a large 'L' or a similar pattern. The interface includes a menu bar (File, Network, Options, Help) and several tabs (Hutch, Sample, Raster, Collect, Screening, Scan, Users, Log). The Raster tab is active, showing control buttons (Start, Pause) and display options (Grid #s, Smoothing, Position sync, Show beamsize, Color map parameter). The Interactive tab is also active, showing a Run 0 (inactive) section with buttons (Default, Update, Delete, Reset) and various parameters (Prefix, Dir, Polygons, Cell size, Beam size, Time, Distance, Attenuation, Energy, ROI). A data table is visible at the bottom of the interface, showing the status of the scan.

Status	X	Y	Z	Omega	Frame	Row	Col	Fluorescence
DONE	-0.000	0.093	36.394	180.000	1	1	4	218
DONE	-0.000	0.093	36.414	180.000	2	1	3	602
DONE	-0.000	0.093	36.434	180.000	3	1	2	668
DONE	-0.000	0.073	36.374	180.000	4	2	5	151
DONE	-0.000	0.073	36.394	180.000	5	2	4	525

[14:46:20] NOTE: [collimatorCtrl.pl]

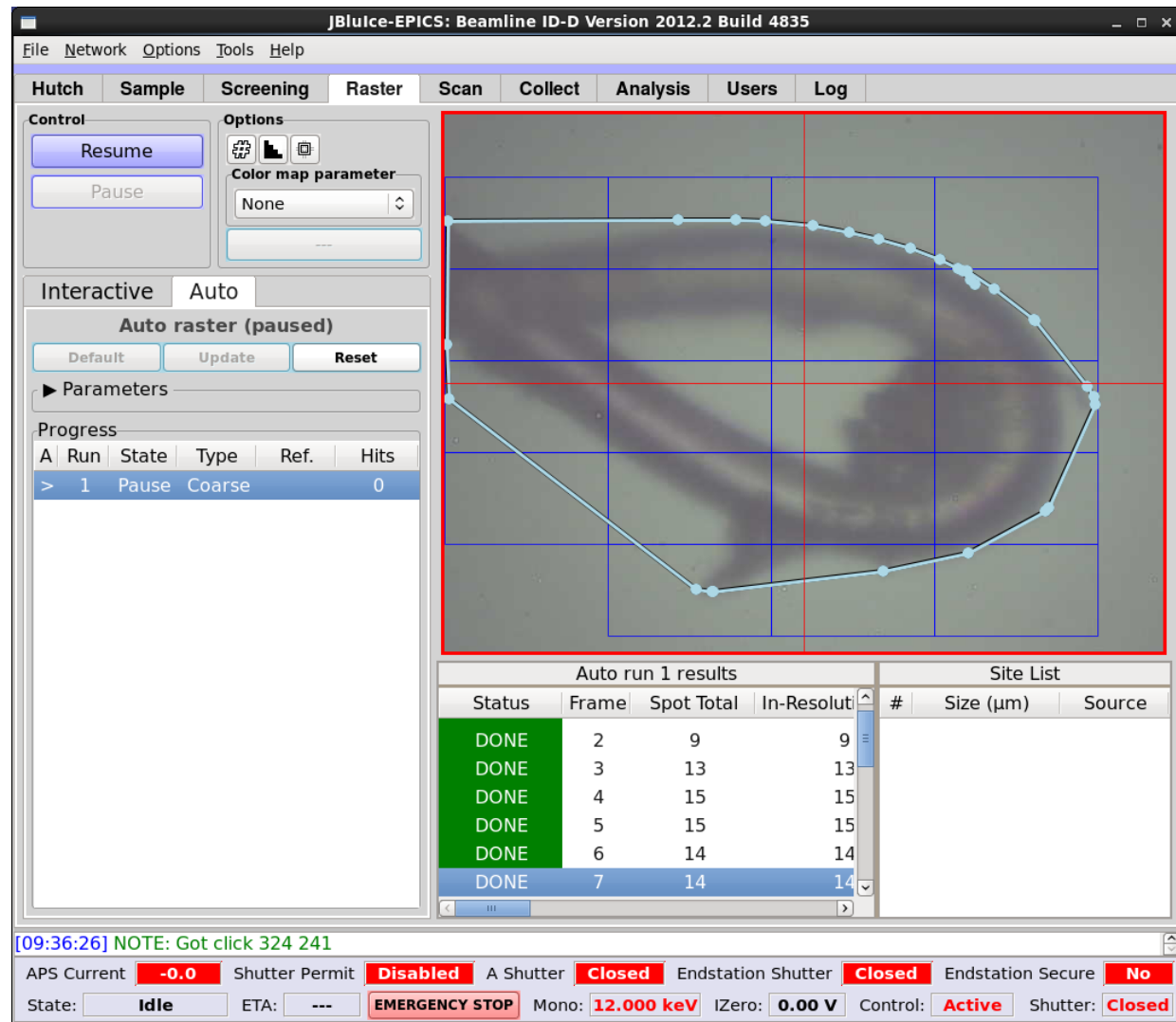
APS Current: 102.3 Shutter Permit: Enabled A Shutter: Open Endstation Shutter: Open Endstation Secure: Yes
State: Idle ETA: --- EMERGENCY STOP Mono: 10.000 keV IZero: 0.05 V Control: Active Shutter: Closed

The cell and the beam size are 20 μ m.

Fast fluorescence techniques for crystallography beamlines Stepanov, S., Hilgart, M., Yoder, D., Makarov, O. Becker, M., Sanishvili, R., Ogata, C., Venugopalan, N., Aragão, D., Caffrey, M., Smith, J.L. and Fischetti, R.F. Acta. Cryst. D., **44**, 772-778, (2011).

AutoFind

- Produces a search area (polygon) definition
 - First performs optical centering if needed
 - Takes four images at angles 0, 30, 60, 90
 - Uses XREC to generate loop outline
 - Sets the sample to face-on orientation
 - Total time is about 40 seconds
- Adds a critical link from screening to analysis
- The next step in automation is to link this to the screening tab



JBlutce-EPICS: Beamline ID-D Version 2012.2 Build 4835

File Network Options Tools Help

Hutch Sample Screening Raster Scan Collect Analysis Users Log

Control

Resume

Pause

Options

Color map parameter: None

Interactive Auto

Auto raster (paused)

Default Update Reset

Parameters

Progress

A	Run	State	Type	Ref.	Hits
>	1	Pause	Coarse		0

Auto run 1 results

Status	Frame	Spot Total	In-Resoluti
DONE	2	9	9
DONE	3	13	13
DONE	4	15	15
DONE	5	15	15
DONE	6	14	14
DONE	7	14	14

Site List

#	Size (μm)	Source
---	-----------	--------

[09:36:26] NOTE: Got click 324 241

APS Current: -0.0 Shutter Permit: Disabled A Shutter: Closed Endstation Shutter: Closed Endstation Secure: No

State: Idle ETA: --- EMERGENCY STOP Mono: 12.000 keV IZero: 0.00 V Control: Active Shutter: Closed

AutoFind automatically generates a search polygon



Dealing with radiation damage - automated collection along a user defined vector

Efficient use of large homogeneous crystals

The screenshot displays the JBIUCE-EPICS Beamline ID-D software interface, Version 2010.2 Build 2618. The main window is titled "Vector Diffraction" and shows a diffraction pattern with a green vector line indicating the collection direction. The vector length is 124 μm. The interface includes several control panels:

- Current position:** Gonio = 180.802, Detector = 300.011, Attenuation = 1.000, Beamstop = 39.998.
- Resolution Predictor:** 1.64, 2.13.
- Run Sequence:** A list of frames with filenames and angles, ranging from V_1306_1_1.0 180.00 to V_1306_1_1.0 190.00.
- Run 1 (inactive):** Prefix: RVFV_1306_1, Dir: /mnt/share2/jsmith/data1/d, Distance: 300.011 mm, Beamstop: 39.998 mm, Attenuation: 1.00 factor, Axis: gonio_phi, Delta: 1.00 deg, Time: 1.00 sec.
- Status Bar:** APS Current: 98.9, Shutter Permit: Enabled, A Shutter: Open, Endstation Shutter: Open, Endstation Secure: Yes, State: Idle, ETA: ---, EMERGENCY STOP, Mono: 12.660 keV, IZero: 0.02 V, Control: Passive, Shutter: Close.

Mark Hilgart and Craig Ogata

Strategy Extended

- Multiple potential space groups are displayed with their associated strategy calculations
 - Solutions for each space group are computed in parallel
- Anomalous and inverse beam modes are supported
- MOSFLM or BEST can be chosen as the strategy program

JBluice-EPICS: Beamline BM Version SIM-MODE Build 1 *Simulation Level 2* (on mark.gmca.aps.anl.gov)

File Network Options Tools Help

Hutch Sample Screening Raster Scan Collect Analysis Users Log

Diffraction Strategy

Collect Pause

Run 0 (inactive)

Copy Update Delete Reset

Collect Param. ---

Prefix: test

Dir: /home/mhilgart

Distance: 800.000 mm

Atten.: 1.00 factor

Site: None

Beam size: 43 x 43 μm

Delta: 1.00 deg

Time: 1.00 sec

Frame Gonio

Start: 001 0.00

Strategy

Energy: 13.6240 keV

Current position

Gonio = 0.000
Detector = 800.000
Attenuation = 1.000
Beamstop = 30.500

Resolution Predictor

4.65
6.52

Run sequence >>

Filename	Angle	En
test_0.0001	0.00	13.624

Strategy All Results

Done

Dir: /mnt/share2/test1

warning:

Images: test_1.0001 test_1.0091

Space group: (2) P121 P1211-->57.62,89.26,62.83,90.00,109.65

Native Anomalous Inverse

Osc. start: 104.00 85.00 104.00

Min. Coverage: 190.10 210.45 190.10

Osc. end: 190.10 335.90 190.10

Completeness: 98.53% 96.96% 98.53%

Osc. delta: 0.70

Det. dist.(pred): 477.9

Res. (pred): 3.41 A

Mosaicity: N/A

Unit cell: 57.62 89.26 62.83 90.00 109.65 90.00

Create run 1 Export to run 0 Solution

[09:02:41] NOTE: Unknown command: setGraphXLimit 12628.50 12687.50

APS Current: 101.3 Shutter Permit: Enabled A Shutter: N/A Endstation Shutter: Open Endstation Secure: Yes

State: Idle ETA: --- EMERGENCY STOP Mono: 13.624 keV IZero: 0.00 V Control: Active Shutter: Closed



Analysis Tab

- XDS, POINTLESS, SCALA and TRUNCATE are run automatically in the background as each collect run completes
- Results populate the analysis tab as they finish
- Previous results can be flipped through using back/forward arrows
- An overview is shown along with graphs on the right
- Full text logs are available by clicking buttons at the bottom

JBluice-EPICS: Beamline ID-D Version 2012.2 Build 4803

File Network Options Tools Help

Hutch Sample Screening Raster Scan Collect Analysis Users Log

1 of 1 Export All

Data Quality All Results

Done

Sample Name: beth1_2

Dir: /mnt/staffhome/spothineni/testxsderrors//beth1_2_xds

Images: /mnt/staffhome/spothineni/testxsderrors//beth1_2.####

warning: DONE

Unit Cell: 49.61 55.26 84.24 90.00 90.00 90.00

Space Group: P 2 21 21

	Overall	InnerShell	OuterShell
Low Res.:	46.21	46.21	1.46
High Res.:	1.39	4.39	1.39
Rmerge:	0.085	0.043	0.685
Completeness:	97.4	94.7	98.1
Anom.Completeness:	93.2	91.7	94.4
Multiplicity:	4.1	3.9	4.1
Anom.Multiplicity:	2.2	2.3	2.2
I/Sigma:	9.7	24.8	1.8

Rmerge vs Resolution

I/Sigma vs Resolution

For more plots click on Scala Log button

XDS Log Scala Log Truncate Log

APS Current 102.4 Shutter Permit Disabled A Shutter Closed Endstation Shutter Closed Endstation Secure No

State: Idle ETA: --- EMERGENCY STOP Mono: 12.809 keV IZero: 0.00 V Control: Active Shutter: Closed



JBlulce-EPICS publications

Cherezov, V., Hanson, M.A., Griffith, M.T., Hilgart, M.C., Sanishvili, R., Nagarajan, V., Stepanov, S., Fischetti, R.F., Kuhn, P. and Stevens, R.C. (2009) Rastering strategy for screening and centering of microcrystal samples of human membrane proteins with a sub 10 micron size X-ray synchrotron beam,
J. R. Soc. Interface, **6 Suppl 5:S587-97** PMID 2843980

Stepanov, S., Makarov, O., Hilgart, M., Pothineni, S., Urakhchin, A., Devarapalli, S., Yoder, D., Becker, M., Ogata, C., Sanishvili, R., Nagarajan, V., Smith, J.L. and Fischetti, R.F. (2011) JBlulce-EPICS control system for macromolecular crystallography,
Acta. Cryst. D67, **176–188** PMID 3046456

Stepanov, S., Hilgart, M., Yoder, D., Makarov, O., Becker, M., Sanishvili, R., Ogata, C., Venugopalan, N., Aragão, D., Caffrey, M., Smith, J.L. and Fischetti, R.F. (2011) Fast fluorescence techniques for crystallography beamlines,
J. Appl. Cryst., **44**, **772-778**

Hilgart, M., Sanishvili, R., Ogata, C., Becker, M., Venugopalan, N., Stepanov, S., Makarov, O., Smith, J.L. and Fischetti, R.F. (2011) Automated sample scanning methods for radiation damage mitigation and diffraction-based centering of macromolecular crystals,
J. Synchrotron Rad. **18**, **717-722** doi:10.1107/S0909049511029918

Video tutorials on-line and code is available for download

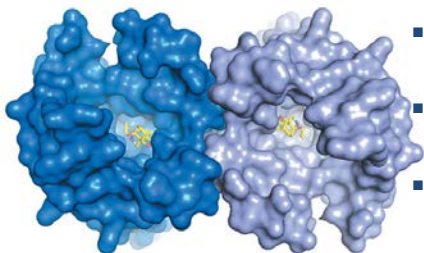
GM/CA co-sponsors with CCP4 a “hands on” school

www.gmca.aps.anl.gov

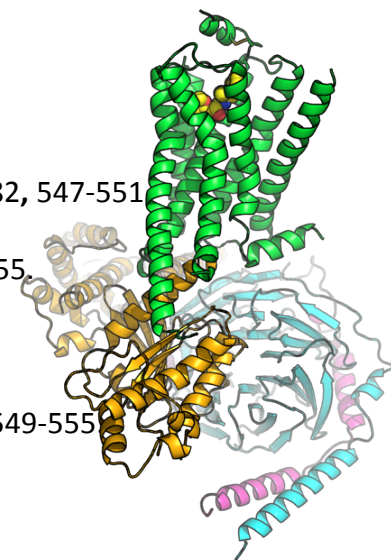


GPCR Highlights from 2012 GM/CA

A pair of μ -opioid receptors



- Liu, W., ..., Cherezov, V., and Stevens R.C. (2012), *Science* 337, 232-236.
Human A_{2a} adenosine receptor
- Chun, E., ..., Cherezov, V., Hanson, M.A., and Stevens, R.C. (2012), *Structure* 20, 967-976.
Methods
- Manglik, A., ..., Weis, W.I., Kobilka, B.K., and Granier, S. (2012), *Nature* 485, 321-326.
 μ -opioid receptor bound to a morphinan antagonist
- Wu, H., ..., Cherezov, V., and Stevens, R.C. (2012), *Nature* 485, 327-332.
Human κ -opioid receptor in complex with JD1c
- Thompson, A.A., ..., Cherezov, V., and Stevens, R.C. (2012), *Nature* 485, 395-399.
Nociceptin/orphanin FQ receptor in complex with a peptide mimetic
- Granier, S., ..., Weis, J. and Kobilka, B.K. (2012), *Nature* 485, 400-404.
Delta-opioid receptor bound to naltrindole
- Kruse, A.C., ..., Weis, W.I., Wess, J. and Kobilka, B.K., (2012), *Nature* 482, 552-556.
M3 muscarinic acetylcholine receptor
- Haga, K., ..., Weis, W.I., ..., Kobilka, B.K., Haga, T., & Kobayashi, T., (2012), *Nature* 482, 547-551
Human M2 muscarinic acetylcholine receptor bound to an antagonist
- Hanson, M.A., ..., Kuhn, P., Rosen, H. and Stevens, R.C. (2012), *Science* 335, 851 – 855.
Lipid G Protein-Coupled Receptor
- Li, D., Lee, J. and Caffrey, M. (2011), *Cryst Growth Des* 11, 530 – 537
Methods
- Rasmussen, S.G., ..., Weis, W.I., Sunahara, R., and Kobilka, B.K.(2011), *Nature* 477, 549-555
 β_2 adrenergic receptor-Gs protein complex

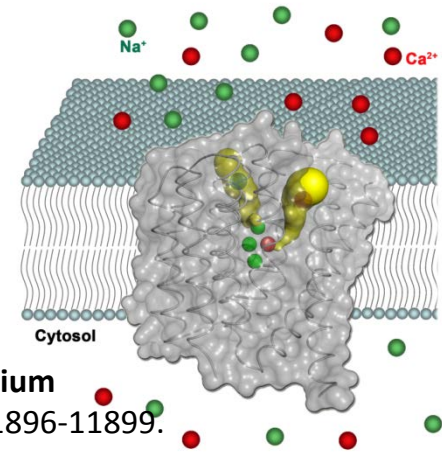


β_2 adrenergic receptor-Gs protein complex

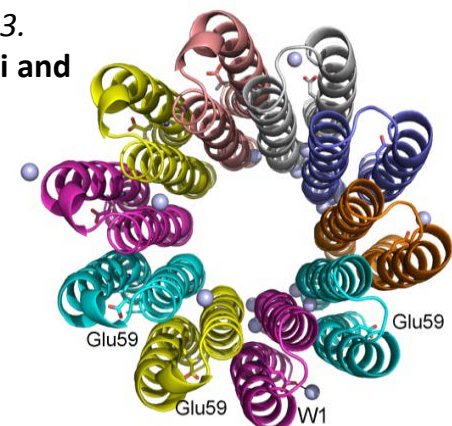


Other Membrane Protein Publications

- Liao, J., ..., and Jiang, Y. (2012), *Science* 335, 686-690.
Sodium/calcium exchanger
- Brohawn, S. G., ..., and MacKinnon, R. (2012), *Science* 335, 436-441.
Human K2P TRAAK, a lipid- and mechano-sensitive K⁺ ion channel
- Whorton, M. R., and MacKinnon, R. (2011), *Cell* 147, 199-208.
Mammalian GIRK2 K⁺ channel and gating regulation by G proteins, PIP2, and sodium
- Uysal, S., ..., Kossiakoff, A. A., and Perozo, E. (2011), *Proc Natl Acad Sci U S A* 108, 11896-11899.
Activation gating in the full-length KcsA K⁺ channel
- Shi, N., ..., and Jiang, Y. (2011), *J Mol Biol* 411, 27-35.
Determinants of K channel conductance and gating.
- Sauer, ..., and Jiang, Y. (2011), *Proc Natl Acad Sci U S A* 108, 16634-16639.
Protein interactions central to stabilizing the K⁺ channel selectivity filter.
- Derebe, M. G., ..., and Jiang, Y. (2011), *Proc Natl Acad Sci U S A* 108, 598-602.
Tuning ion selectivity of tetrameric cation channels by changing the number of ion binding sites
- Noinaj, N., ..., and Buchanan, S. K. (2012), *Nature* 483, 53-58.
Structural basis for iron piracy by pathogenic Neisseria
- Fairman, J. W., ..., Cherezov, V., and Buchanan, S. K. (2012), *Structure* 20, 1233-1243.
Outer Membrane Domain of Intimin and Invasin from Enterohemorrhagic E. coli and Enteropathogenic Y. pseudotuberculosis
- Oldham, M. L., and Chen, J. (2011), *P Natl Acad Sci USA* 108, 15152-15156.
Maltose transporter during ATP hydrolysis
- Symersky, J., ..., and Mueller, D. M. (2012), *Nat Struct Mol Biol* 19, 485-491
c(10) ring of the yeast mitochondrial ATP synthase in the open conformation
- Tiefenbrunn, T., ..., and Cherezov, V. (2011), *PLoS One* 6, e22348.
ba3 cytochrome c oxidase from Thermus thermophilus in a lipidic environment



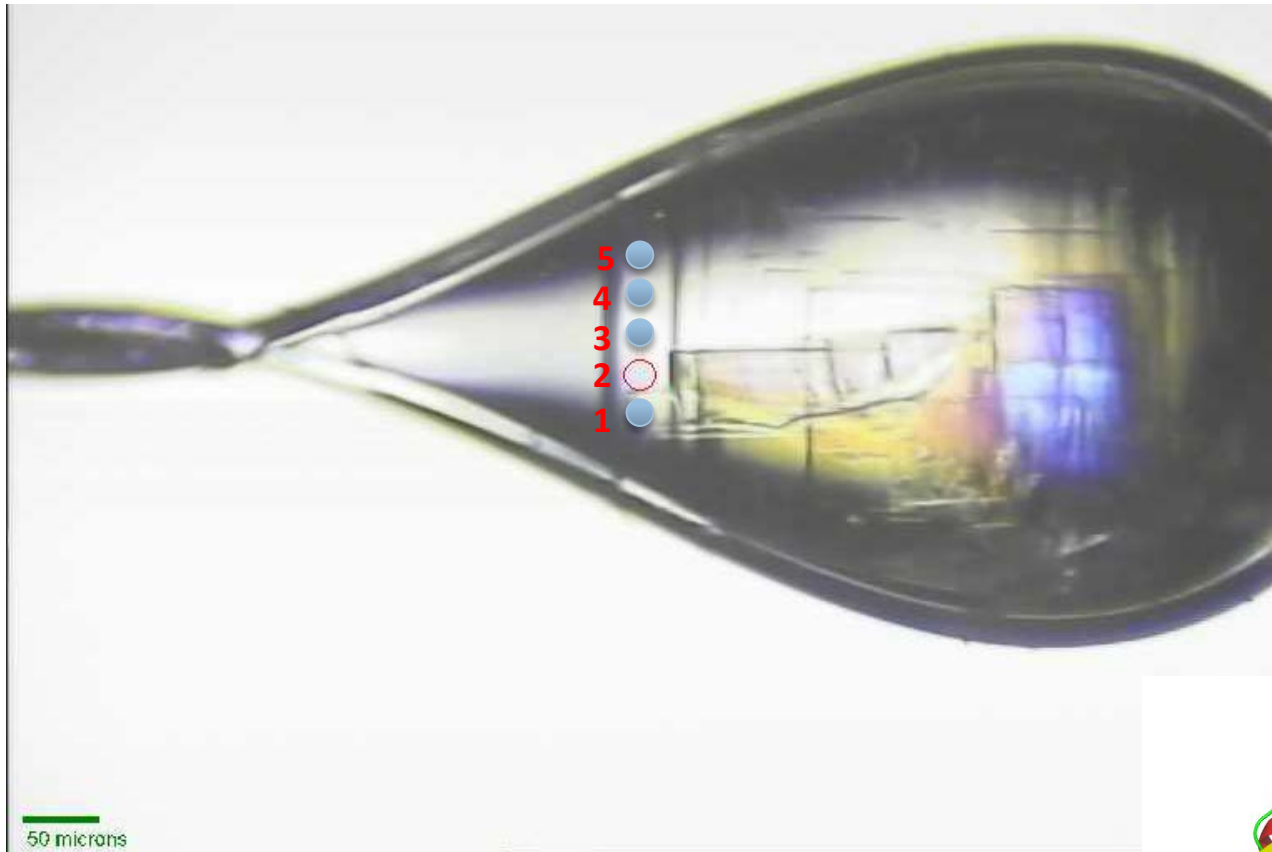
Crystal structure of the Na⁺/Ca²⁺ exchanger embedded in a membrane bilayer



Yeast mitochondrial ATP synthase c10-ring at pH 8.3

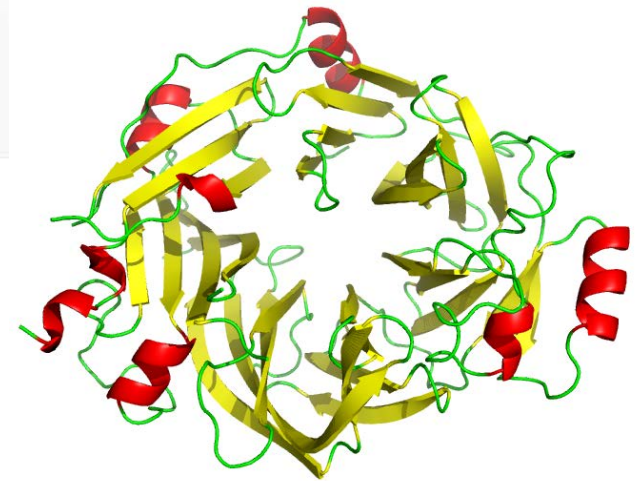


Thomas Schwartz (MIT): Structure of nucleoporin complex components



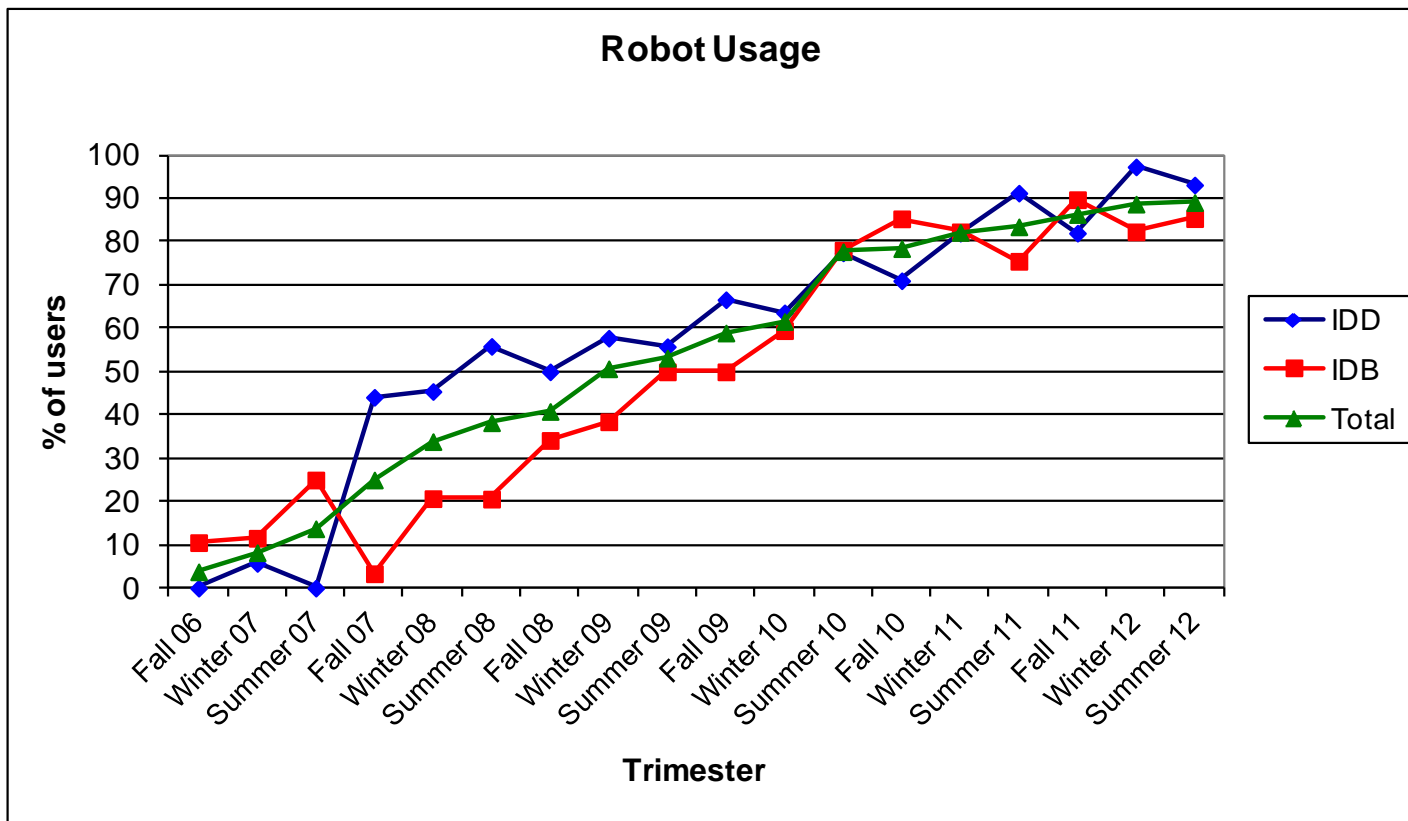
Space group $P2_1$, $a=52$, $b=78$, $c=59\text{\AA}$, $\beta=106^\circ$

NE-CAT



Automounter usage at GM/CA (% of user visits vs. APS trimester)

Over 90% of groups use the automounter
Over 40% collect data remotely
→ 5000 mounts/APS run cycle/beamline



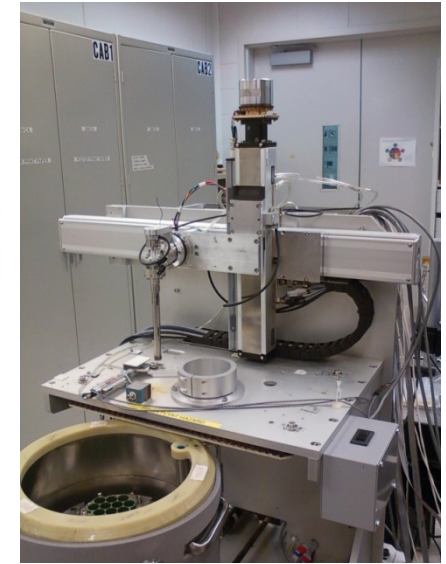
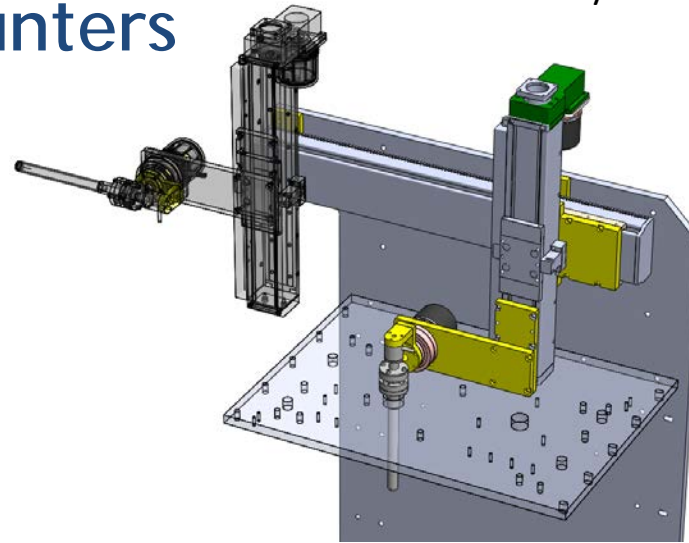
Berkeley Automounters

(T. Earnest and C. Cork)

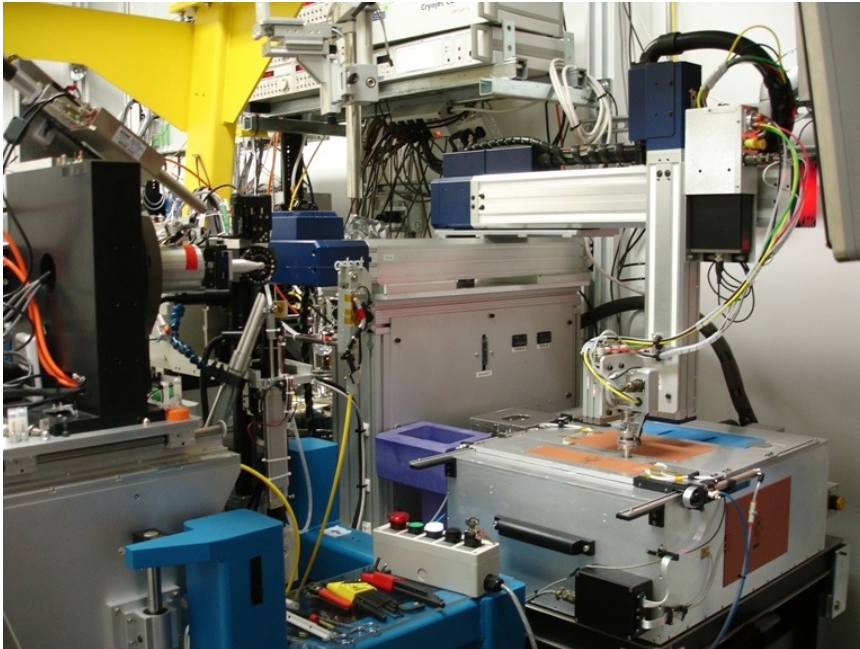
Benefits:

- Larger Dewars
- Increased throughput
- Reduced vibrations
- Automated alignment

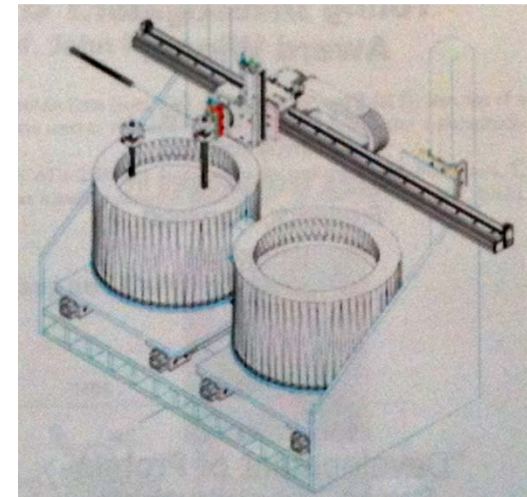
BAM-1 GM/CA modified Cartesian



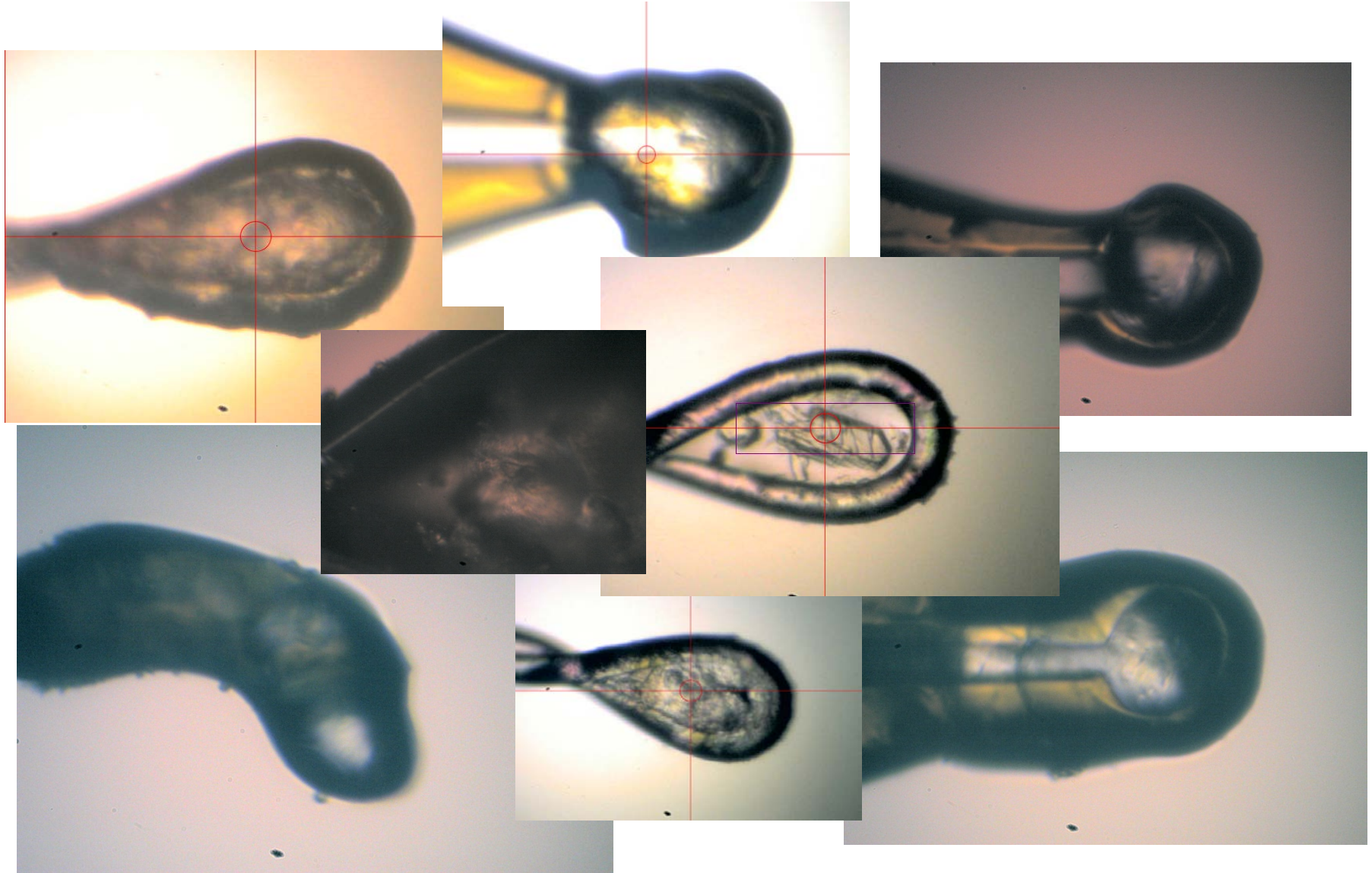
BAM-2 GM/CA Cartesian



SER-CAT Cartesian w/ dual-Dewars



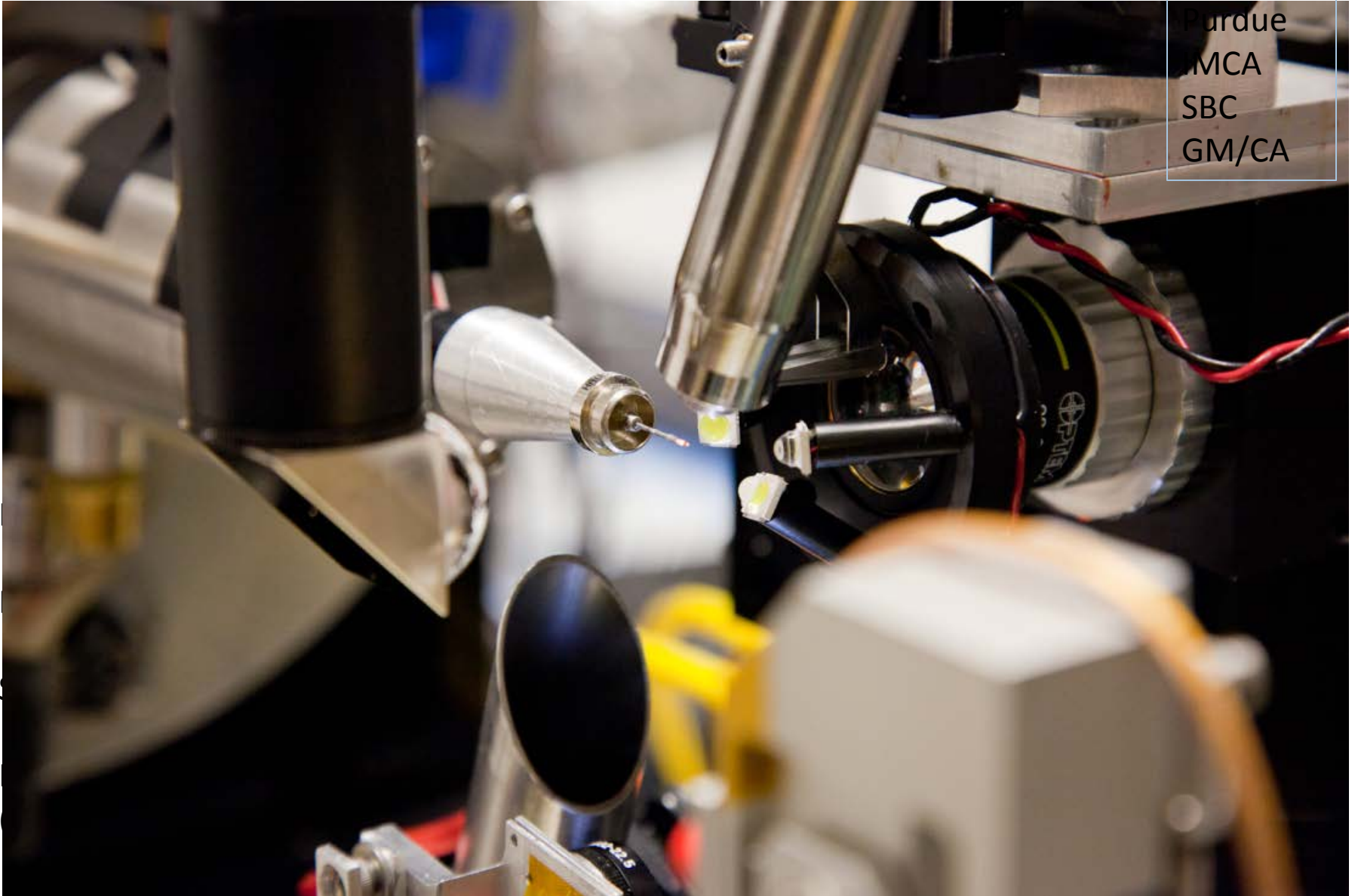
Do I have a crystal? Where is it?



Chris Dettmar



SONICC on the beamline



Purdue
IMCA
SBC
GM/CA

Mike Becker & Chris Dettmar



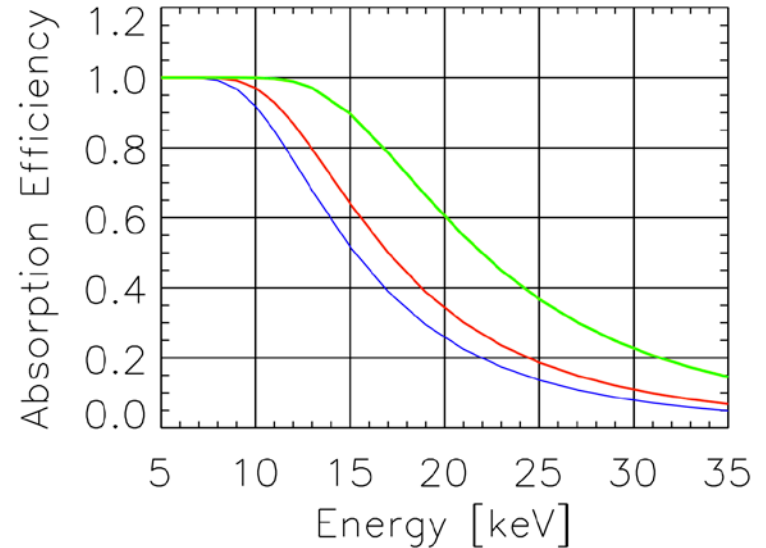
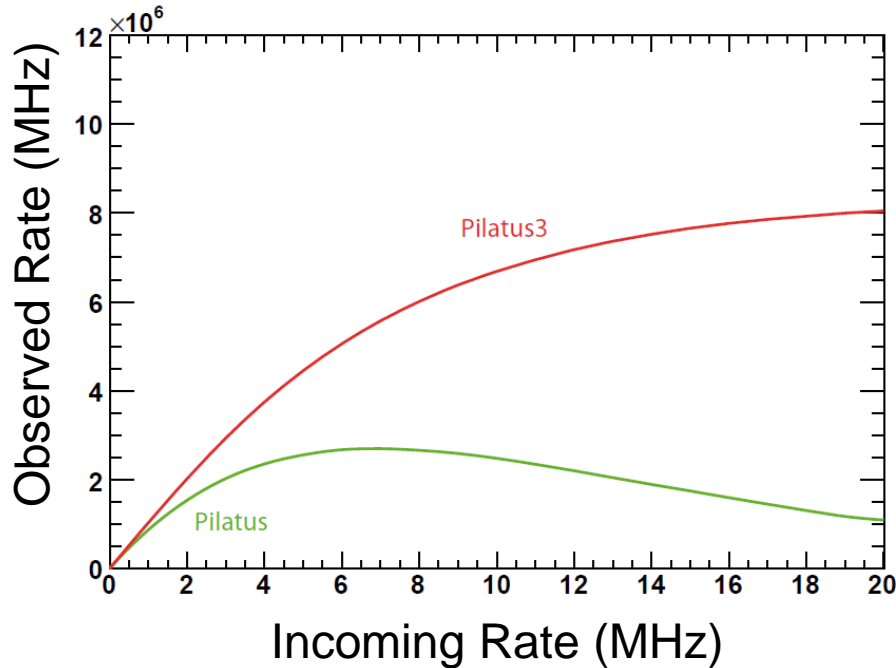
Faster, higher sensitivity detectors

CAT upgrades

BioCARS	Fast-CCD (on order)
IMCA	Pilatus 6M
SER-CAT	FAST-CCD (delivery soon)
GM/CA	Pilatus3 6M (on order)
NE-CAT	Pilatus-F 6M

Pilatus3 6M

- Improved dead time correction
- High count rate (10 MHz)
- High frame rate (100Hz)
- Improved efficiency with thicker sensor



More efficient than 40 μm “thick” phosphor



Microfocus Upgrade Motivation

Provide more intensity for challenging projects

Membrane proteins in meso-phase

Small (5-10 μm) and weakly scattering crystals

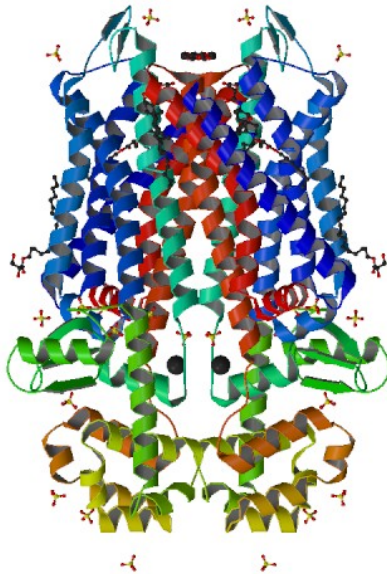
Provide routine access to microfocus beam - $\sim 1 \mu\text{m}$

Exploit APS high energy source properties

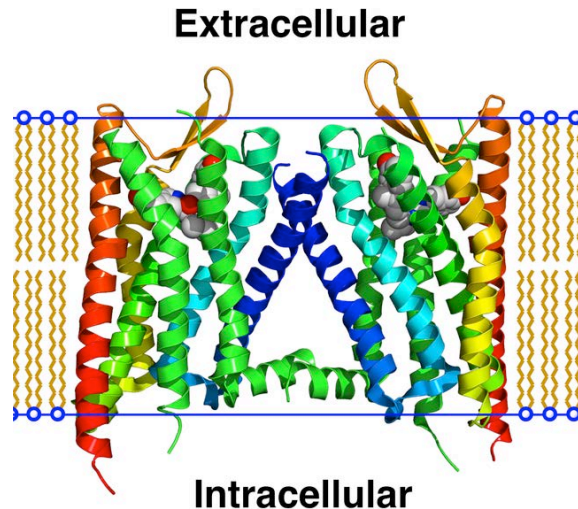
Provide high energy and/or small beams

Study radiation damage at higher energies

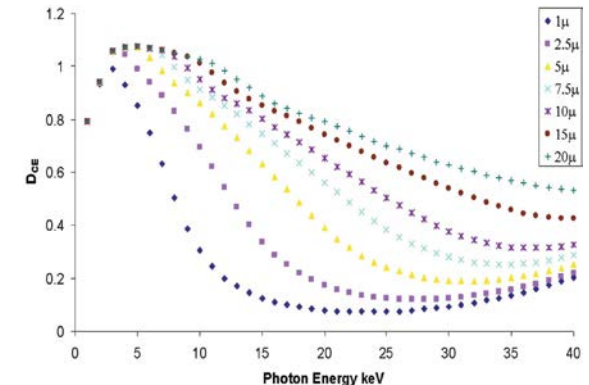
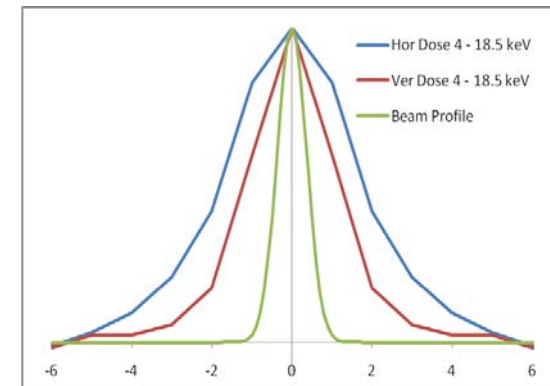
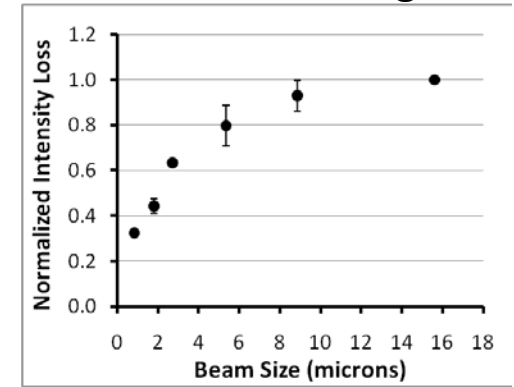
μ -opioid GPCR
Brian Kobilka's lab



k-opioid GPCR
Ray Steven's lab



Radiation damage



Microfocus Upgrade Optical Specifications

Optical Specifications

Beam size (FWHM):

micro-beam mode - beam size can be varied from 1 – 5 μm in a few seconds

mini-beam mode - beam size can be varied from 3 – 20 μm in a few seconds

mode switch <10 minutes

Energy range: 6 – 35 keV

using Si(111) and Si(333)

or Si(311)

Harmonic rejection

>10²

existing KBM system will provide sufficient harmonic rejection

Intensity in 1 μm beam at sample position:

2×10^{10} photons/s, < 500 μrad^2 @ 12.0 and 18.5 keV

5×10^{10} photons/s, < 1000 μrad^2 @ 12.0 and 18.5 keV

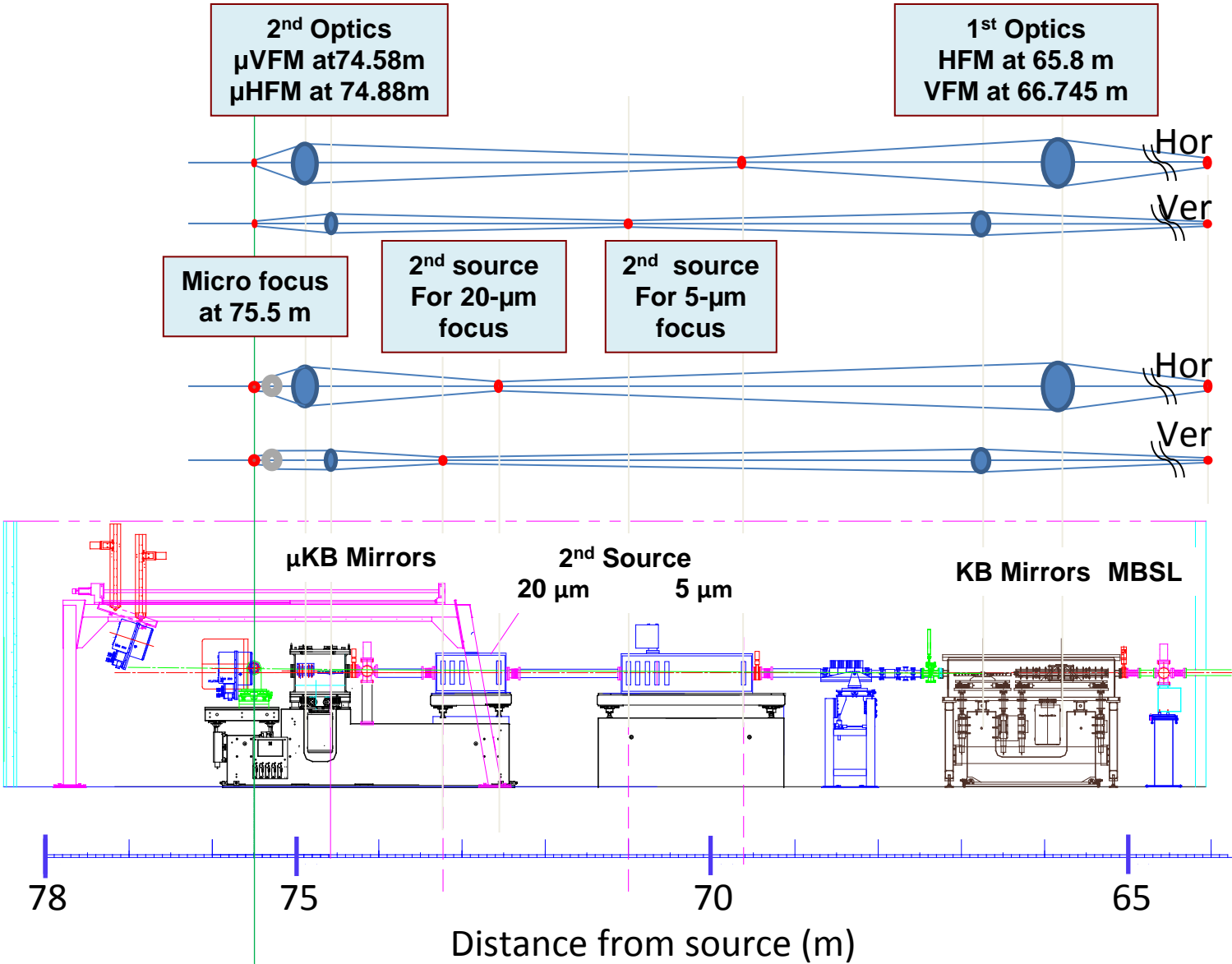
Increase mini-beam intensity 5-fold over current 23-ID-D

Positional stability: 10% RMS of focal size, 1 – 100 Hz

Intensity stability: 1% RMS noise, 1 – 100 Hz

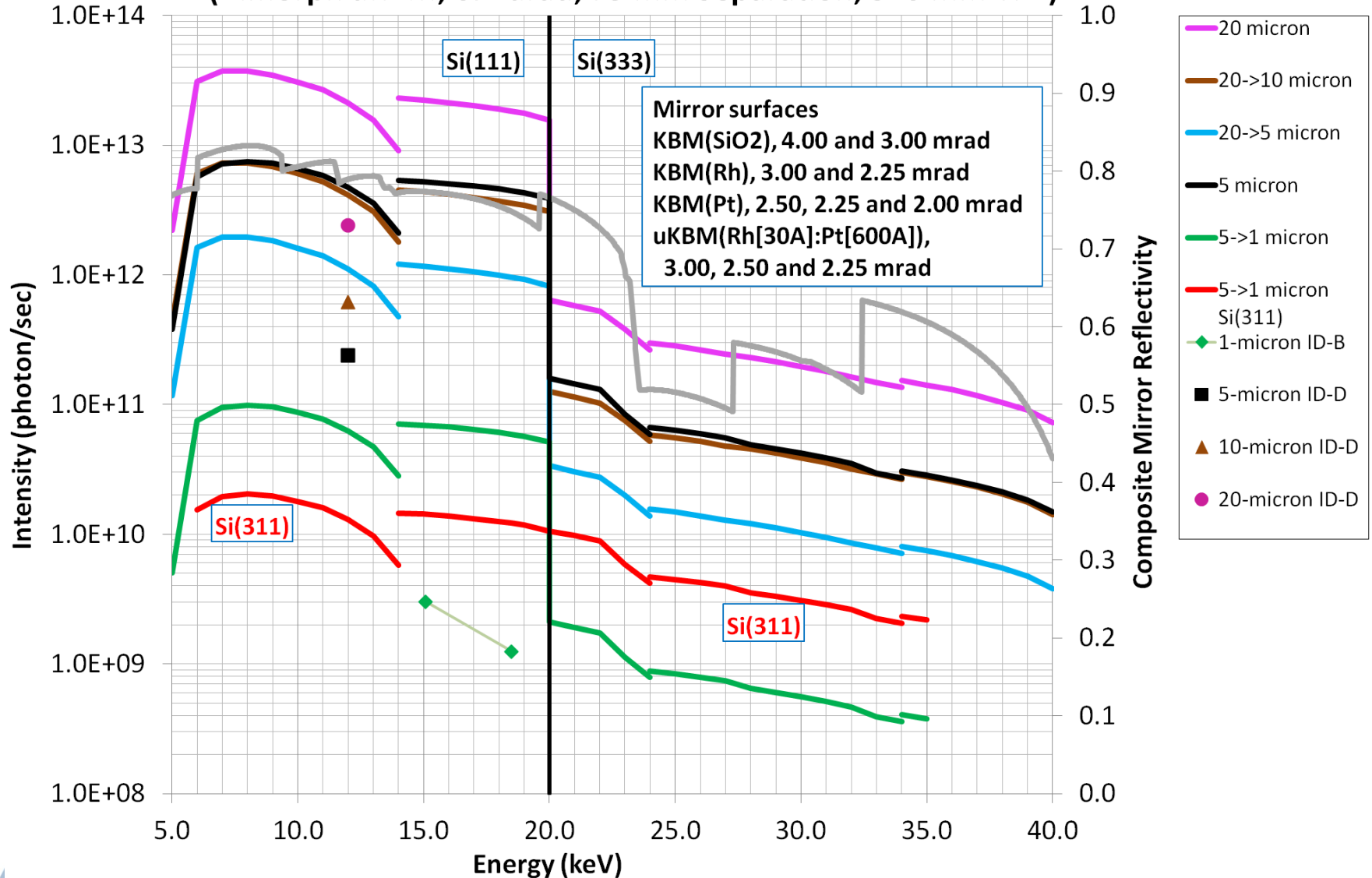


Microfocus Upgrade Layout



Microfocus Upgrade: Intensity vs. Energy

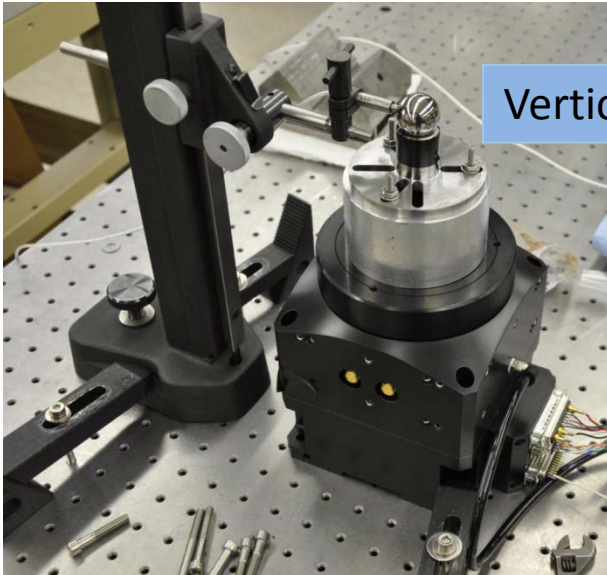
Intensity Spectrum for mini- and micro-beams at 23-ID-D
(Bimorph uKBM; 0.2 urad; 75 mm Separation; 520 mm WD)



Air bearing performance

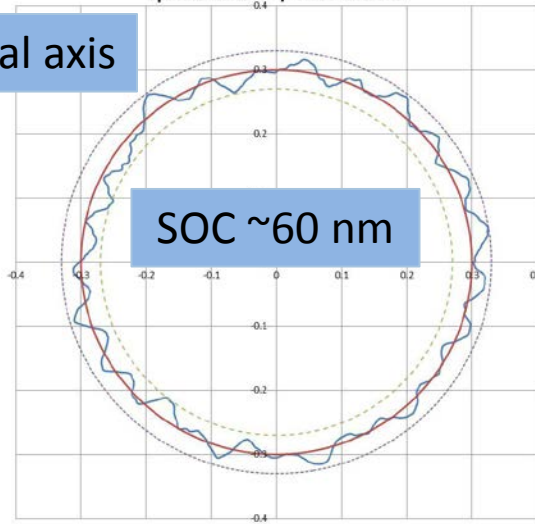


precision air bearing positioning



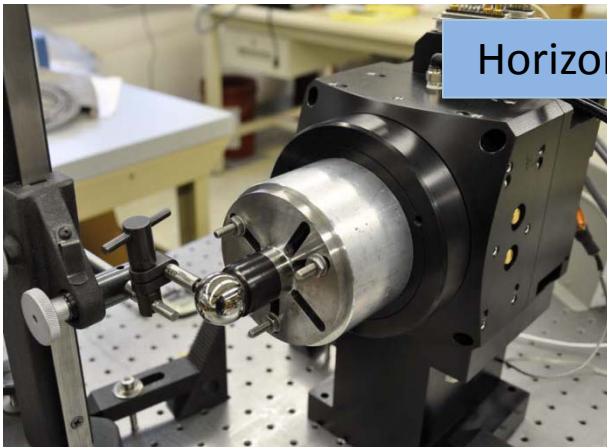
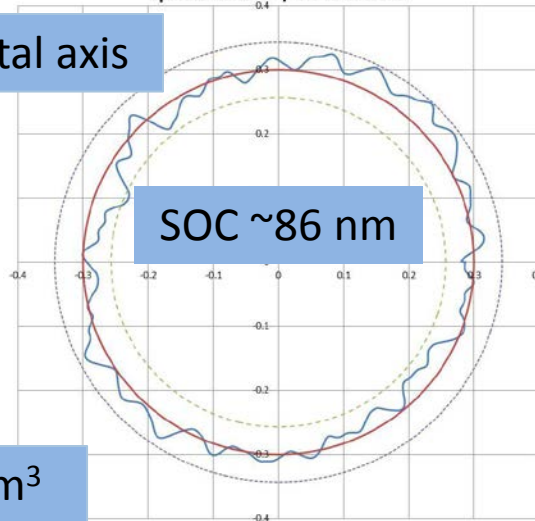
Vertical axis

Final Thingap RT125-M-013, Vertical, 4" from Face
Spindle Runout +/-0.030 microns



SOC ~100 nm

Final Thingap RT125-M-013, Horizontal, 4" from Face
Spindle Runout +/-0.043 microns



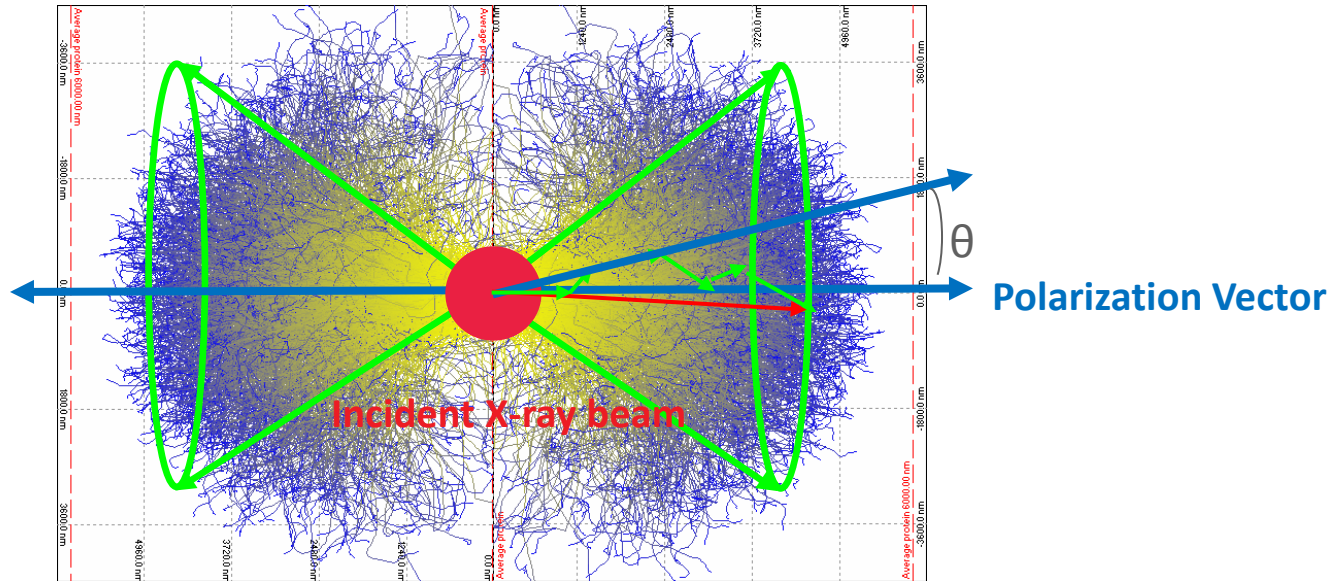
Horizontal axis

Dimensions: 140 x 140 x 165 mm³

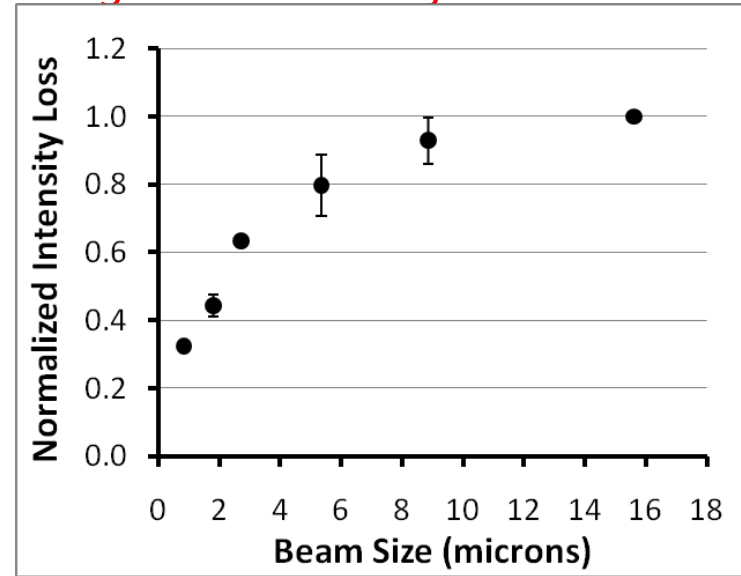
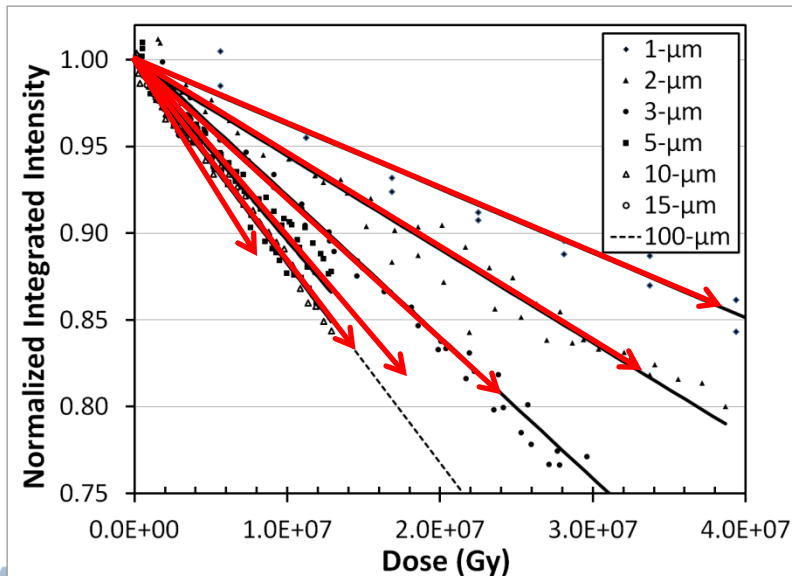
- SOC measurements
- 100 mm off face
 - peak-to-peak
 - mostly synchronous error



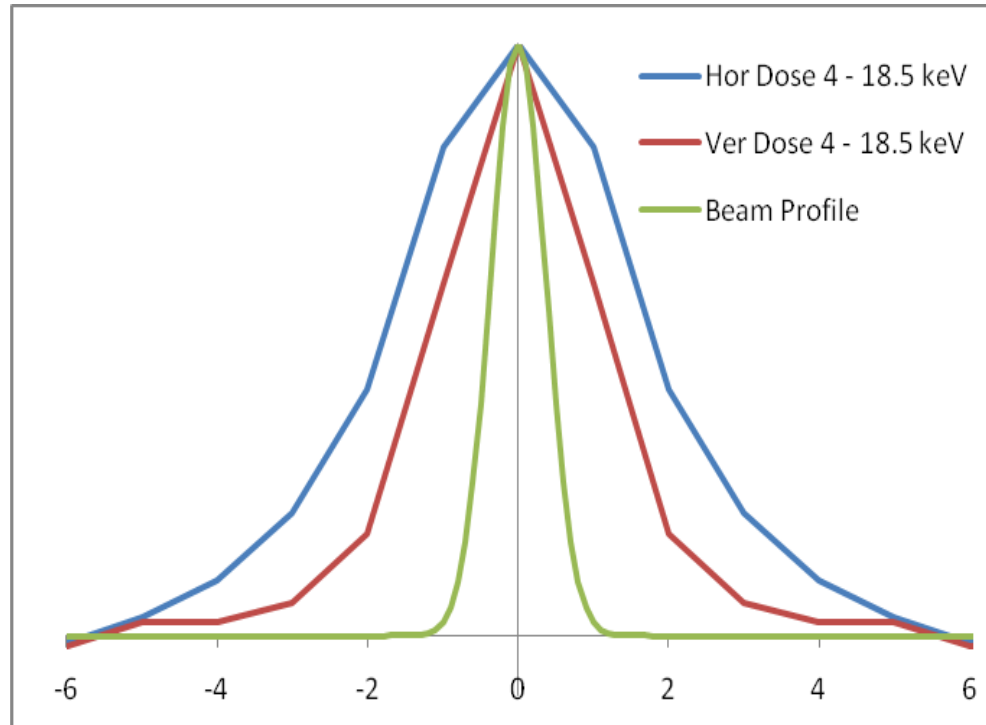
Intensity loss as a function of beam size and dose



Damage decreases 3-fold with beam size



Distribution of damage is wider than beam



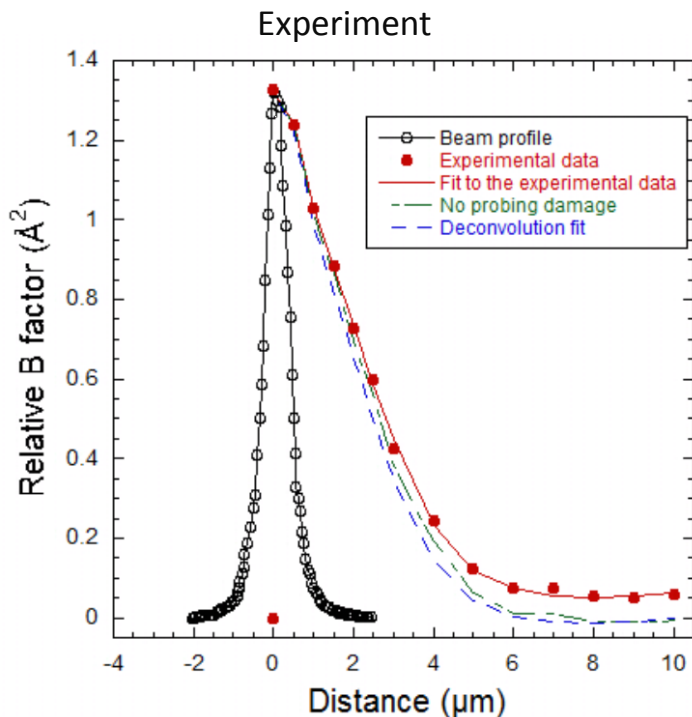
Photoelectron
CSDA $\sim 8 \mu\text{m}$
Monte Carlo $\sim 4 \mu\text{m}$
Auger $\sim 0.1 \mu\text{m}$
Compton

	HWHM (μm)	Ratio
Beam profile	0.42	1.0
Horizontal distribution	2.02	4.8
Vertical distribution	1.19	2.8



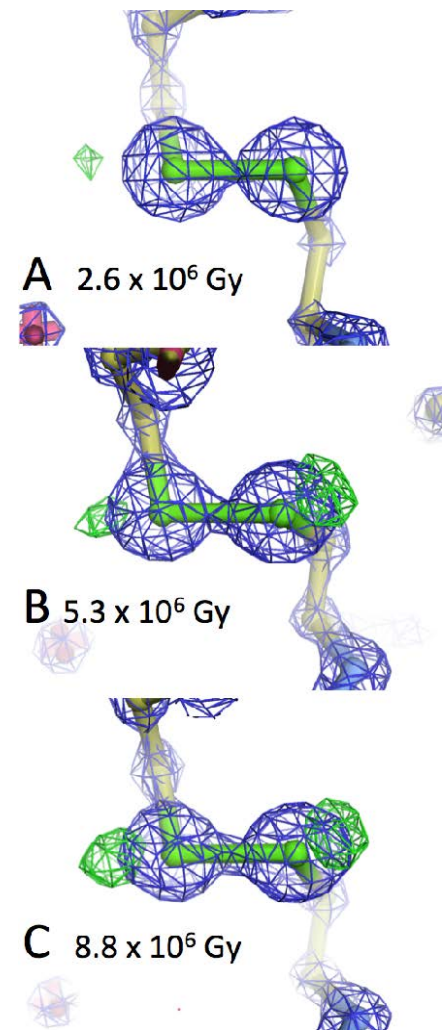
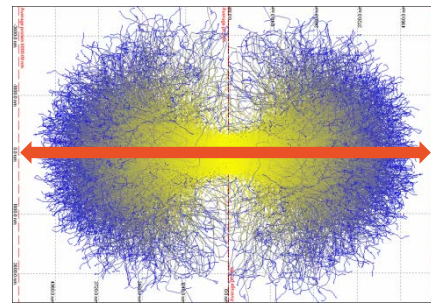
Mitigation of Radiation Damage Using Line Focus Beam

- A new strategy to reduce primary X-ray damage in macromolecular crystallography uses the basic principle of separating, as much as possible, the X-ray irradiated region, where the diffracted signal originates, from the region where damage accumulates.
- Photoelectrons causing radiation damage accumulate predominantly outside the irradiated region of the crystal exposed with a line focused beam leading to a 4.5 factor decrease in radiation damage.



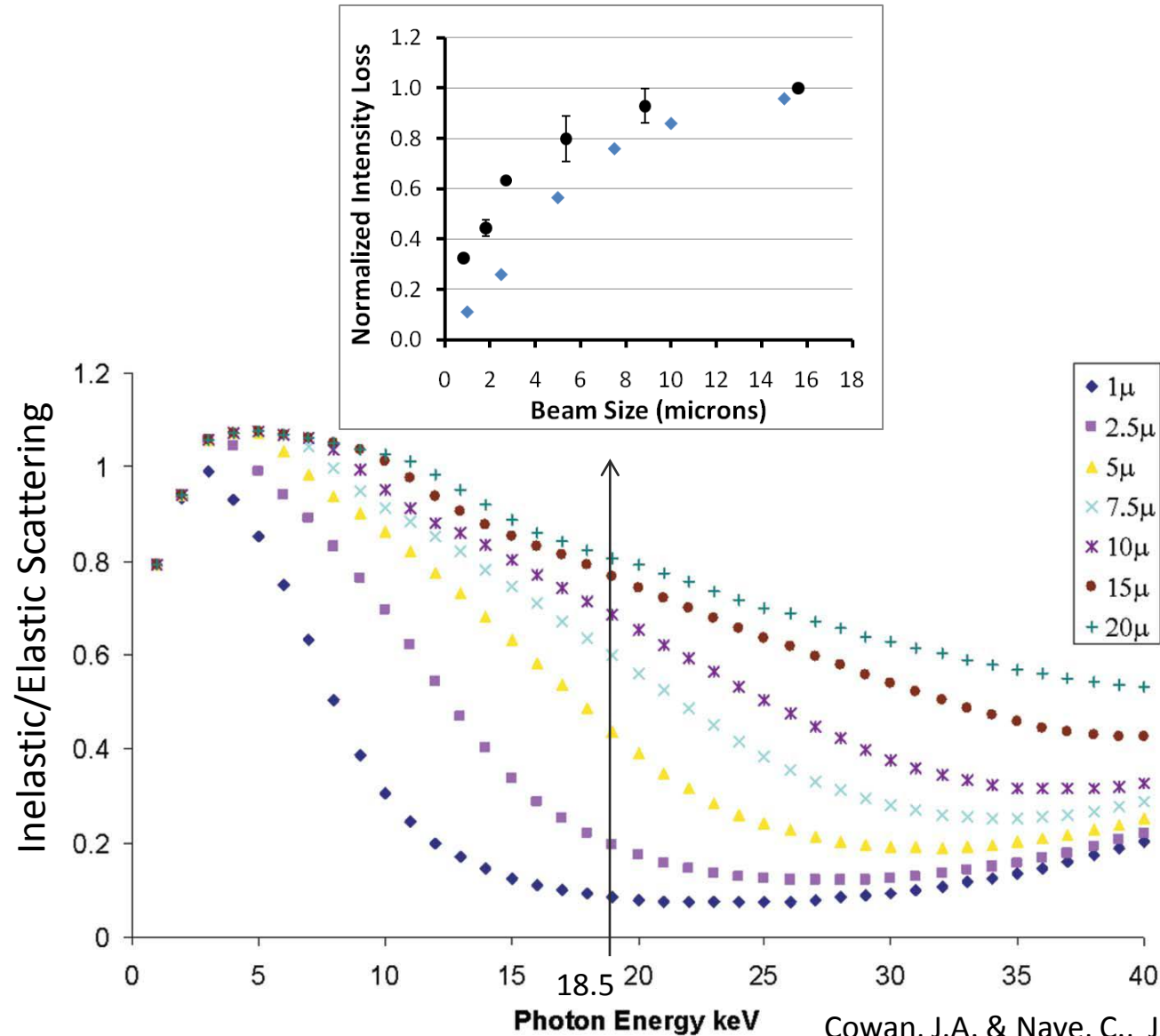
Plots of the measured lens focus profile, spatial dependent damage, the deconvoluted spatial dependent damage, and the spatial dependent data with the probing damage removed.

Simulation



Electron density maps contoured at 3σ for the region near C64-C80 disulfide bridge of three lysozyme structures determined from the data obtained with 19ID line focus beam at three different doses.

Comparison of Monte Carlo simulations and our data



Cowan, J.A. & Nave, C., JSR **15**, 458-62 (2008).



Benefits of the APS Upgrade

Increased beam current

Pro - Increase intensity in to focus

Con - Additional heat load on DCM

Improved beam stability

Pro - Better spatial and/or temporal stability

Con - N/A

New revolver undulator

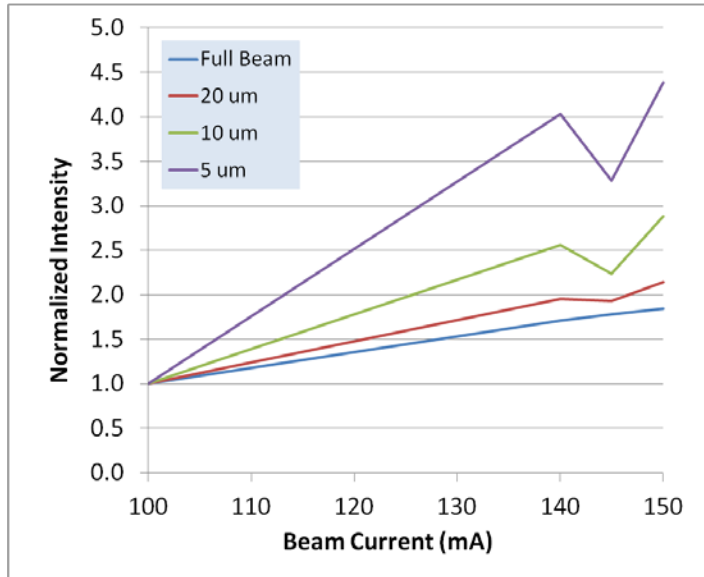
Pro – better match spectrum to experiment

Con - \$\$

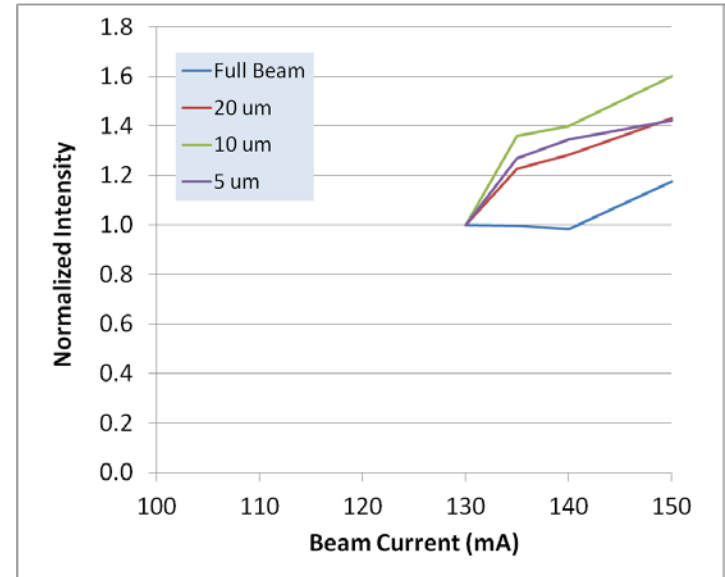


APS-Upgrade - higher current

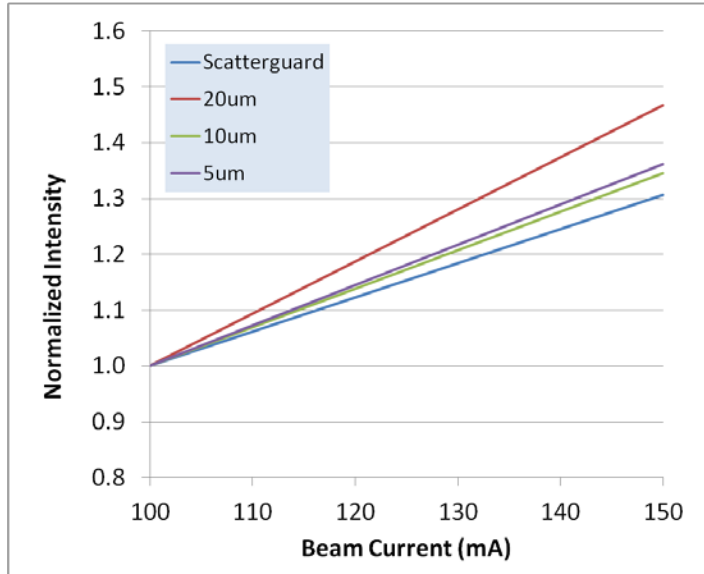
12.0 keV 1st



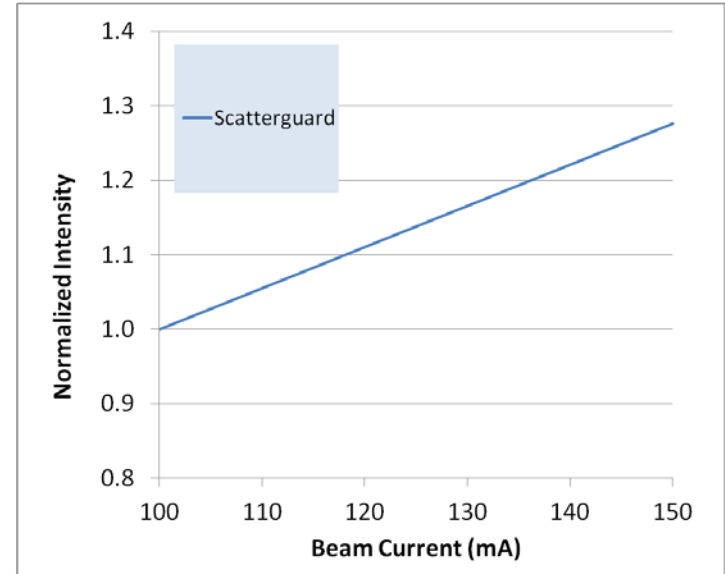
15.3 keV 3rd



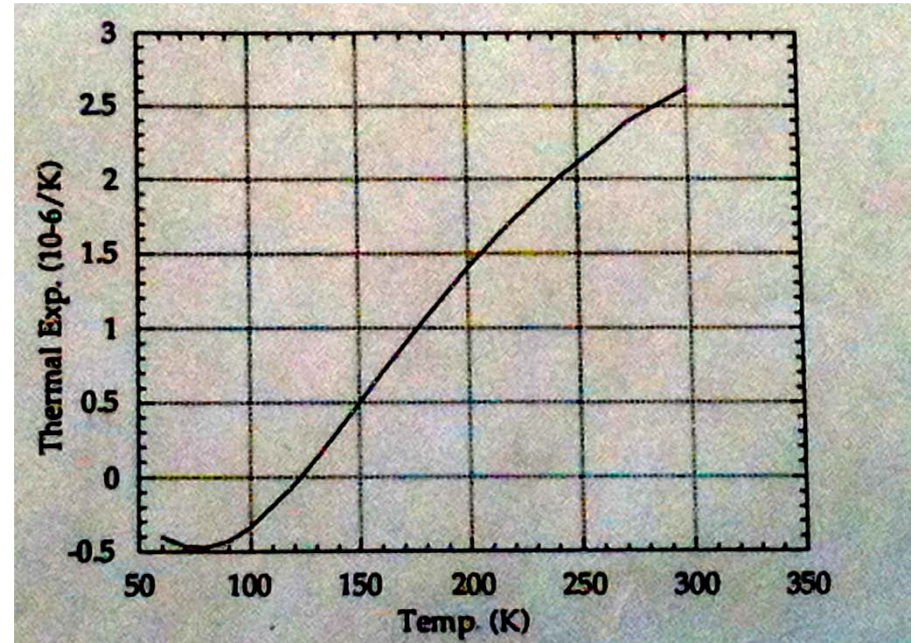
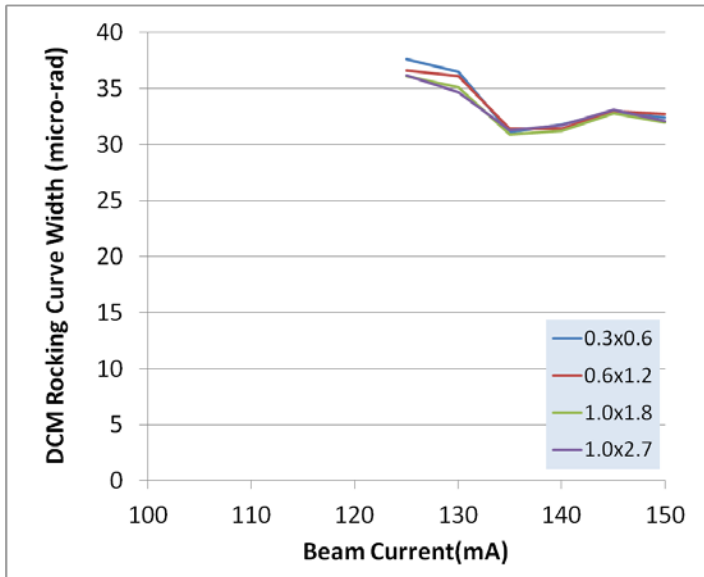
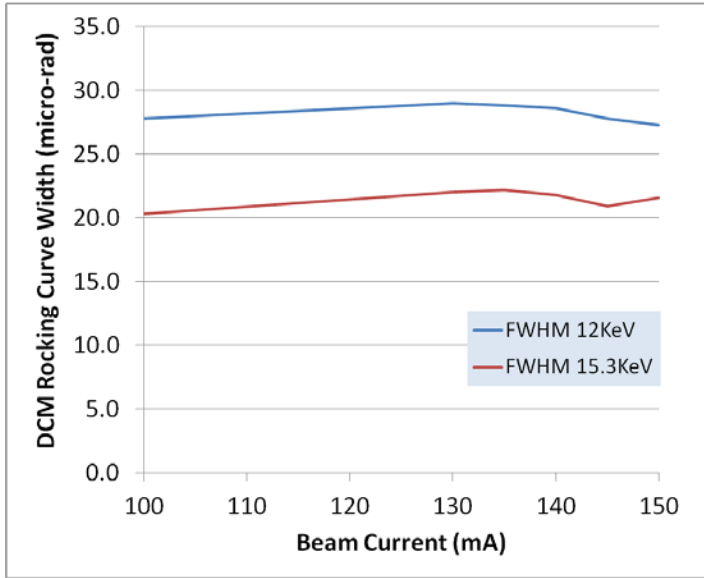
12.0 keV 1st



10.5 keV 3rd



APS-Upgrade - higher current (continued)



Operating micro-crystallography beamlines

Facility & beamline	Target beam size	Energy range	Approach
APS 23ID-B	5, 10, 20 μm	3.5-20 keV	Aperture
APS 23ID-D	5, 10, 20 μm	5-20 keV	Aperture
APS 17ID-B	10, 20 μm	6-20 keV	Aperture
APS 19ID	5, 10, 20 μm	6-17 keV	Aperture
APS 24ID-E	5-20 μm	12.66 keV	Aperture
APS 31ID	20 μm	9-13.8 keV	Aperture
Australia MX2	10 μm	5.5-28 keV	Aperture
CHES A1	<20 μm	12.68 keV	Direct focus
CHES F1	<20 μm	13.50 keV	Direct focus
CHES F2	<20 μm	7-16 keV	Direct focus
Diamond I02	20 μm	5-25 keV	Aperture
Diamond I03	20 μm	5-25 keV	Aperture
Diamond I04	2x8 μm^2	13.1, 7.15 keV	Aperture
Diamond I24	7-10 μm	6.5-18 keV	Secondary source
ESRF ID13 EHII	1 μm	5-17 keV	Direct focus
ESRF ID23-2	10 μm	14.2 keV	Direct focus
ESRF ID29	10, 20 μm	6-20 keV	Aperture
Photon Factory BL-17A	20 μm	5.9-13.8 keV	Aperture
Photon Factory BL-1A	10 μm	2.7-3.0 keV	Aperture
SPring-8 BL32XU	1-10 μm	8.5-20 keV	Divergence-limited source
SPring-8 BL41XU	10 μm	6.5-35 keV	Aperture
SLS X06SA	(15)x5 μm^2	5.7-17.5 keV	(Aperture) direct focus
SSRL 12-2	7, 10, 20 μm	6.7-17.2 keV	Aperture

All dimensions are FWHM. (HxV) Selectable beam sizes are designated by comma-separated discrete sizes or by a size range. Beamlines with beams of dimension 20 μm or smaller; some also produce larger beams.



Micro-crystallography beamlines - under development

Beamlines in process			Status
ALBA BL13	300x7 μm^2	5-21 keV	Commissioning
Diamond I02	20, 10 μm	7-17 keV	Commissioning
Diamond I03	20, 10 μm	7-17 keV	Commissioning
Diamond I04	20, 10 μm	7-17 keV	Commissioning
PETRA III MX1	5, 10 μm ; 28x13 μm^2	5-17 keV	Commissioning
PETRA III MX2	4x1 μm^2	7-35 keV	Commissioning
APS 23ID-D	1-20 μm	6-35 keV	Construction
NSRRC PX	1-50 μm	5.7-20 keV	Construction
SOLEIL PX2	20 μm^2	5-15 keV	Construction
SSRF NFPS	10x5 μm^2	5-18 keV	Construction
MAX IV BioMAX	20 μm	5-25 keV	Design
NLSL II FMX	1-100 μm	5-20 keV	Design
NLSL II AMX	5-300 μm	5-25 keV	Design
NLSL II NYX	5-50 μm	3.5-17.5 keV	Design



GM/CA@APS Staff

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From left to right:
Mark Hilgart
Craig Ogata
Robert Fischetti
Sergey Stepanov
Dale Ferguson
Janet Smith
Oleg Makarov
Shenglan Xu
Michael Becker and
Sudhir Babu Pothineni

Insets left to right:
Sheila Trznadel
Ruslan (Nukri) Sanishvili
Naga Venugopalan and
Stephen Corcoran

Thank you for your attention



Thank you for your attention

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