DLSR
Opportunities and Challenges
for
Macromolecular Crystallography

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Diffraction Limited Storage Ring Workshop
SLAC National Accelerator Laboratory
Menlo Park, Ca
December 9, 2013

Scientific Opportunities

- ☐ Membrane proteins (~30% of all proteins) – many grown in lipidic cubic phase (LCP)
  - Receptors (GPCR) – signaling across cellular membrane (>800 proteins in family)
  - Kinases – regulatory control via ATP driven phosphorylation (>500 proteins in family)
  - Transporters – move ions, small molecules or macromolecules across membrane (active and diffusion)
  - Neurotransmitter transporters – transport neurotransmitters across neuron membranes
  - Ion channels - Na, K, Ca, and proton pumps

- ☐ Understanding protein synthesis and better antibiotics
  - Ribosomes – large complex that synthesis proteins and are the target of most antibiotics
  - Polysomes - complexes of ribosomes acting in concert

- ☐ Large macromolecular complexes and molecular machines
  - Nuclear pore complexes (>120 nm in diameter) responsible for molecular trafficking
  - Kinetochores: large complex assemblies (50-100 nm) that attach chromosomes to microtubules during mitosis
  - Transcription initiation machines – large, multicomponent assembly that aligns DNA for transcription

Improving human health

Scientific opportunity

Membrane proteins and proteins complexes mediate cellular responses, act as cellular gateways, have been implicated in many diseases, and are the target of many drugs.

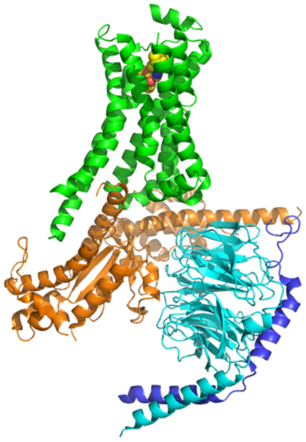
Breakthrough techniques

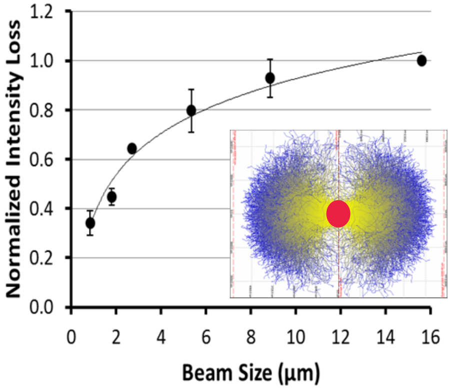
Microcrystallography has recently enabled the determination of high impact, 3D structures of these complexes. Crystals tend to be small (<10 µm), inhomogeneous and weakly scattering. Recording full data sets requires merging partial data sets from many crystals.

APS MBA enabler

Will allow one to obtain critical data from nano-crystals (>500 nm), and to mitigate the effects of radiation damage by exploiting both the high brightness and high X-Ray energy of the new MBA-lattice.

β_2 adrenergic receptor-Gs protein complex – the first structure of a human GPCR





Submicron beam size reduces radiation damage

Brian Kobilka shared the 2012 Nobel Prize in Chemistry for studies of G-Protein-Coupled Receptors

Understanding protein synthesis and better antibiotics

Scientific Opportunity

Ribosomes are large, complex molecular machines that translate the genetic code of mRNA to synthesize proteins. Antibiotics block protein synthesis killing bacterial infections. Polysomes (ribosome clusters) simultaneously read mRNA to synthesize proteins.

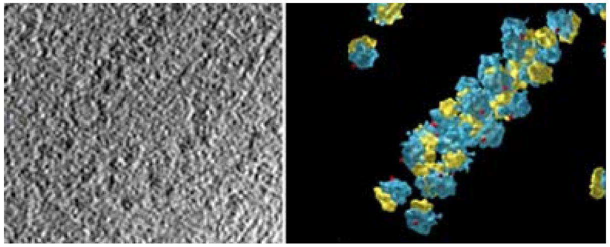
Breakthrough Techniques

Highly collimated beams were required to solve the ribosome structure. Weak scattering and extreme unit cell dimension (2700 Å) from polysome crystals require enhance microcrystallography techniques.

APS MBA Enabler

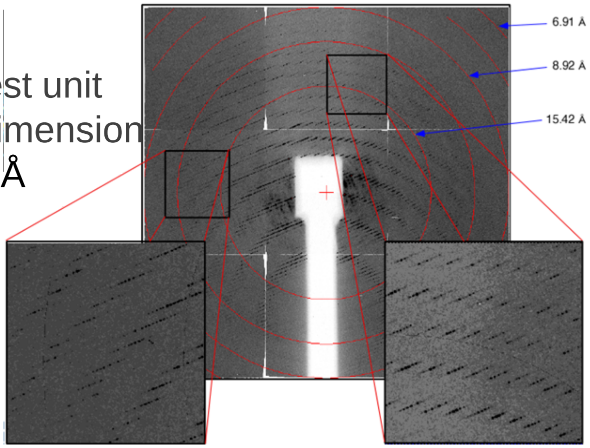
The large unit cell and weak scattering can be combated by the intense, extremely collimated beam to probe micro-crystals or well-ordered regions of larger crystals.

EM reconstruction of polysome



Diffraction pattern from a polysome (5 ribosomes)

(Courtesy of Dr. Jaime Cate, U. of California Berkeley)



Largest unit cell dimension 2700 Å

Ada Yonath shared the 2009 Nobel Prize in Chemistry for studies of the structure and function of the ribosome.

Molecular trafficking through the nuclear membrane

Scientific Opportunity

The nuclear pore complex (NPC) mediates the exchange of macromolecules between the nucleus and the cytoplasm. It is one of the largest supramolecular assemblies in the cell (120 nm in diameter, 120 Mega-Daltons, 30x larger than the ribosome). It is composed of many copies of 30 unique proteins.

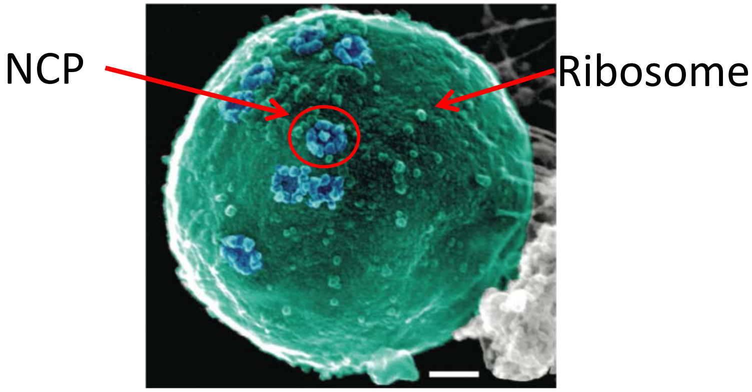
Breakthrough Techniques

Enhanced microcrystallography techniques and low noise detectors are required to solve the structure of subunits and the intact NPC.

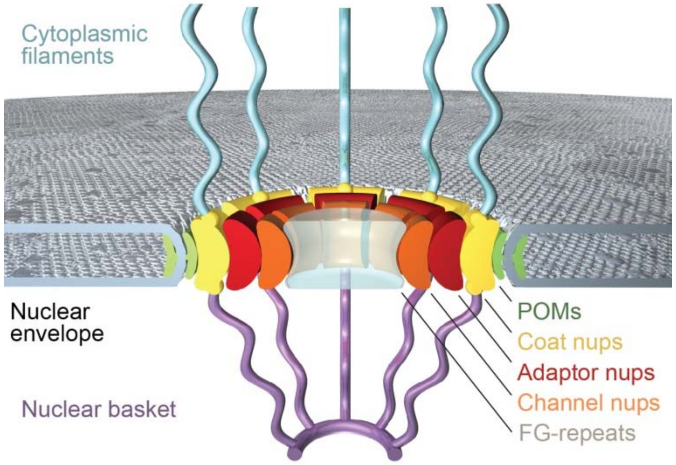
MBA-lattice Enabler

The high brightness will provide an intense, extremely collimated beam necessitated by the 120 nm dimension and to probe micro-crystals or well-ordered regions of larger crystals.

Cross section of the NPC



Elena Kiseleva provided the image to Tom Schwartz



André Hoelz, California Institute of Technology

MBA challenges

Higher flux density → radiation damage occurs faster
- Reach Garman limit in 0.3-100 msec (cryo-cooled)
- Can one outrun 2nd order radiation damage at RT?
- RT *in-situ* screening
- Multi-crystal data sets
- High multiplicity to overcome "noise" of multi-crystal

How to deal with partials
- Increased bandwidth (pink beam)
- Increased convergence

Need new sample handling/delivery/alignment/data collection methods
- Acoustic drop ejection – on grids, tape, or capture with laser tweezers
- Slow LCLS type ejector
- SONICC

Improved stability
- Beam stability
- Sample and goniometry

High speed (frame rate and "count rate"), high sensitivity detectors
- Photon counters vs. charge integrators?

Complementarity of Synchrotron vs. FELs MX in the future

In situ Data Collection

Scientific Opportunity

In Situ screening will provide important diffraction feedback on limited quantities of biological material at an early stage in crystallization trials.

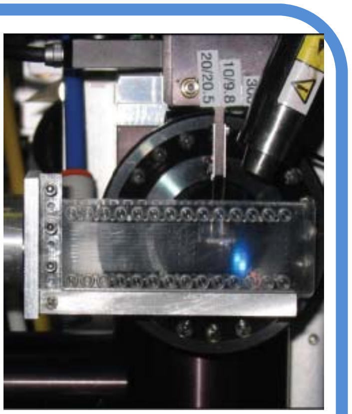
Breakthrough Techniques

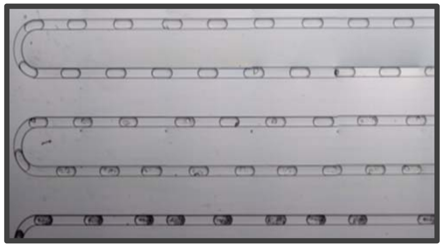
- Samples introduced by novel delivery systems (e.g. acoustic drop or microfluidics)
- Data collection on large number of micro-crystals complexed to a variety of compounds.
- Data collection on high symmetry space groups (e.g. viruses)

MBA-lattice Enabler

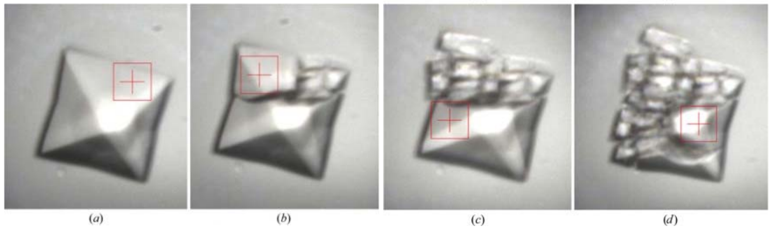
Higher brightness and faster detectors employed in the search for every shrinking crystals of increasing complexity and biological importance.

Microfluidic crystallization card on a goniometer with a mini-beam collimator





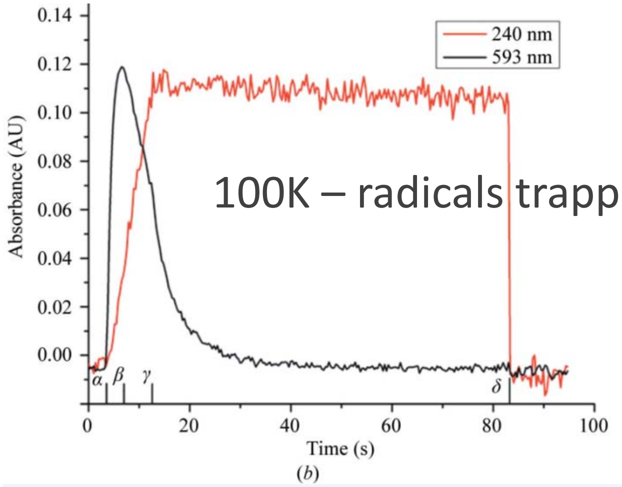
Many crystals in micro-channels for rapid data collection



Crystallization tray - containment for virus crystals. Radiation damage occurs quickly (<100 ms) requiring many crystals

In situ data collection from a virus crystal at 3 different Positions. D. Axford et. al., Acta Cryst. (2012) D68, 592-600

Outrunning 2nd Rad Dam at Room Temp – Beware!



100K – radicals trapped

Crystal lifetime

Start-stop

40 msec exp; 4 sec dead time → 0.186 MGy

Shutterless

40 msec exp → 0.373 MGy

Compare cryo-cooled → 30 MGy

Black – generation of aqueous or solvated e-, followed by 1st order decay

Red – postulated to be hydroxyl radical

Cooling to -20 C

- Just above protein crystal phase transition
- Significantly reduces spread of radiation induced damage
- Increases sample lifetime

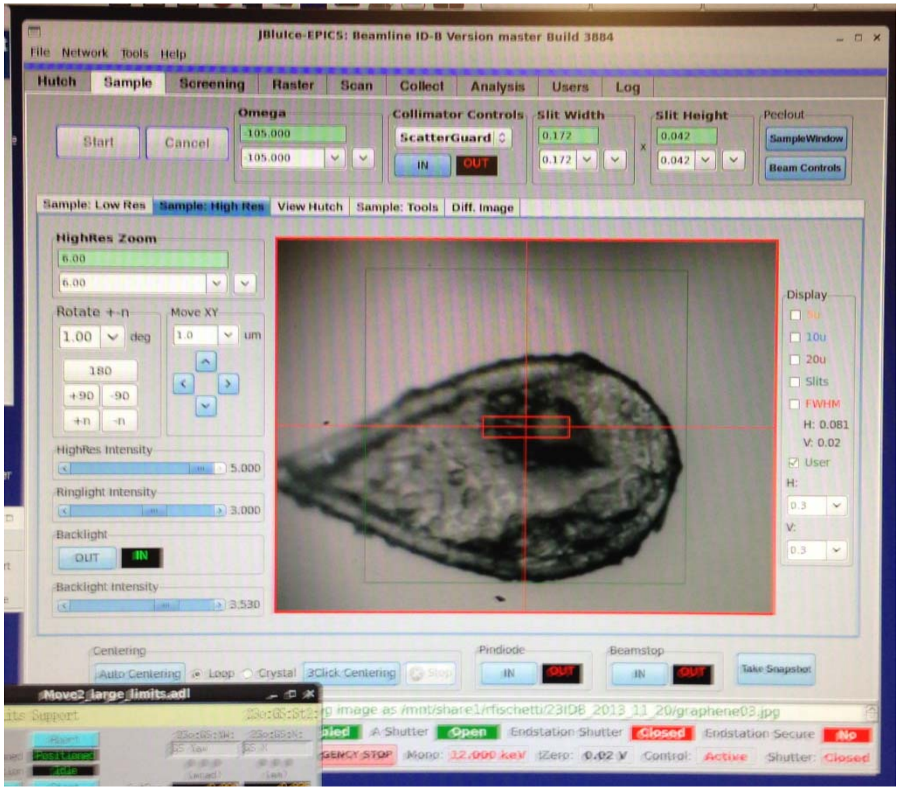
Beware of sample heating!

Owen *et. al.* Acta Cryst. (2012) D**68**, 810-818

Reduce experimental background

Graphene wrapped crystals

- 3-5 layers to ensure hermitic
- Minimizes mother liquid surrounding the sample
- Prevents dehydration



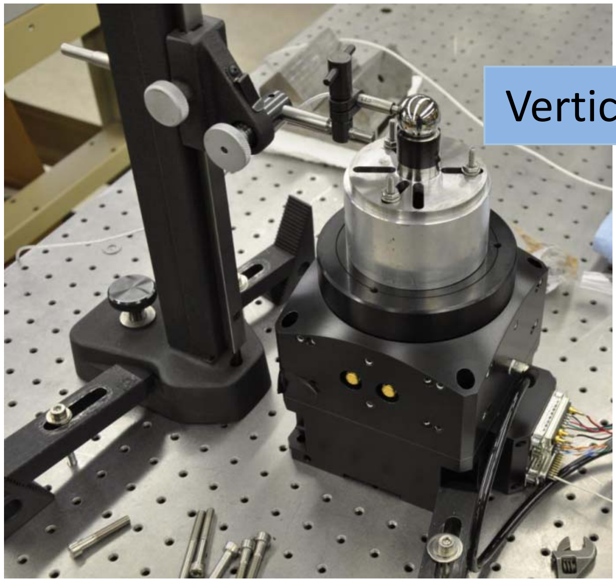
Omega: 105.000
Slit Width: 0.172
Slit Height: 0.042
HighRes Zoom: 6.00
Rotate +-n: 1.00 deg
Move XY: 1.0 um
HighRes Intensity: 5.000
Ringlight Intensity: 3.000
Backlight Intensity: 3.530
FWHM H: 0.081 V: 0.02
User H: 0.3 V: 0.3
g image as /mnt/share1/rfischetti/23IDB_2013_11_20/graphene03.jpg
A Shutter: Open
Endstation Shutter: Closed
Endstation Secure: No
Mono: 12.000 keV
IZero: 0.02 V
Control: Active
Shutter: Closed

J. Wierman, et. al. and So, Gruner J. Appl. Cryst (2013) 46, 1501-1507

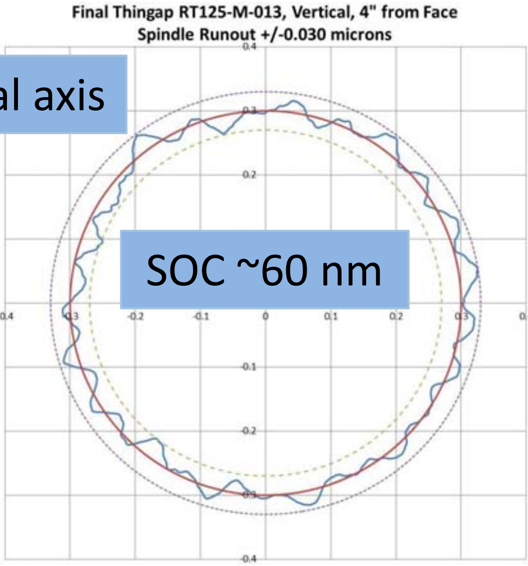
Goniometer performance

NELSON AIR CORP.

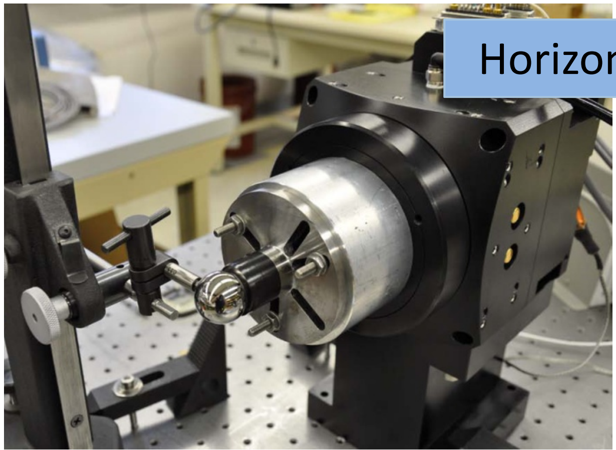
precision air bearing positioning



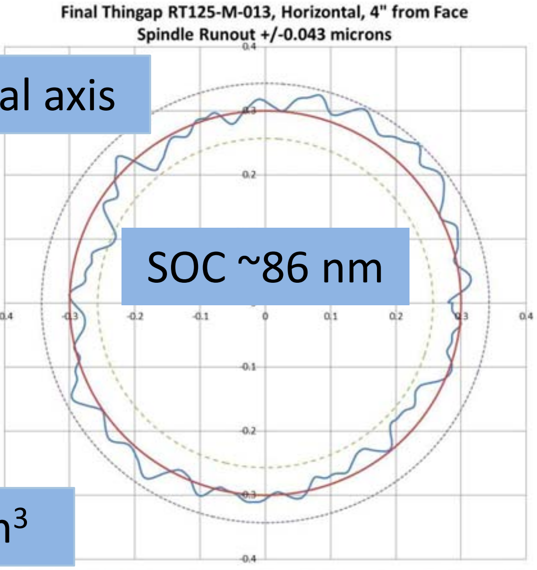
Vertical axis


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

SOC ~60 nm



Horizontal axis


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

SOC ~86 nm

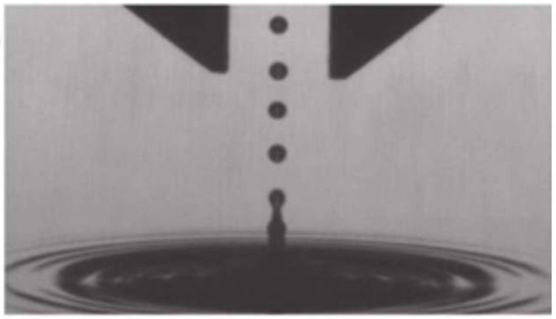
SOC ~100 nm

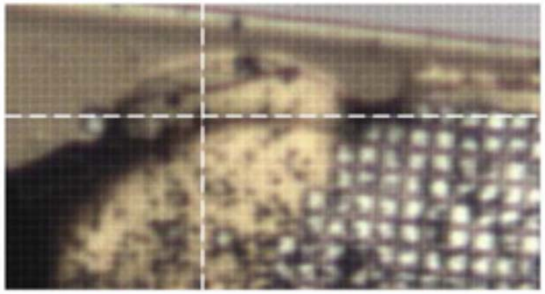
SOC measurements
- 100 mm off face
- peak-to-peak
- mostly synchronous error

Dimensions: 140 x 140 x 165 mm^3

Acoustic droplet ejection

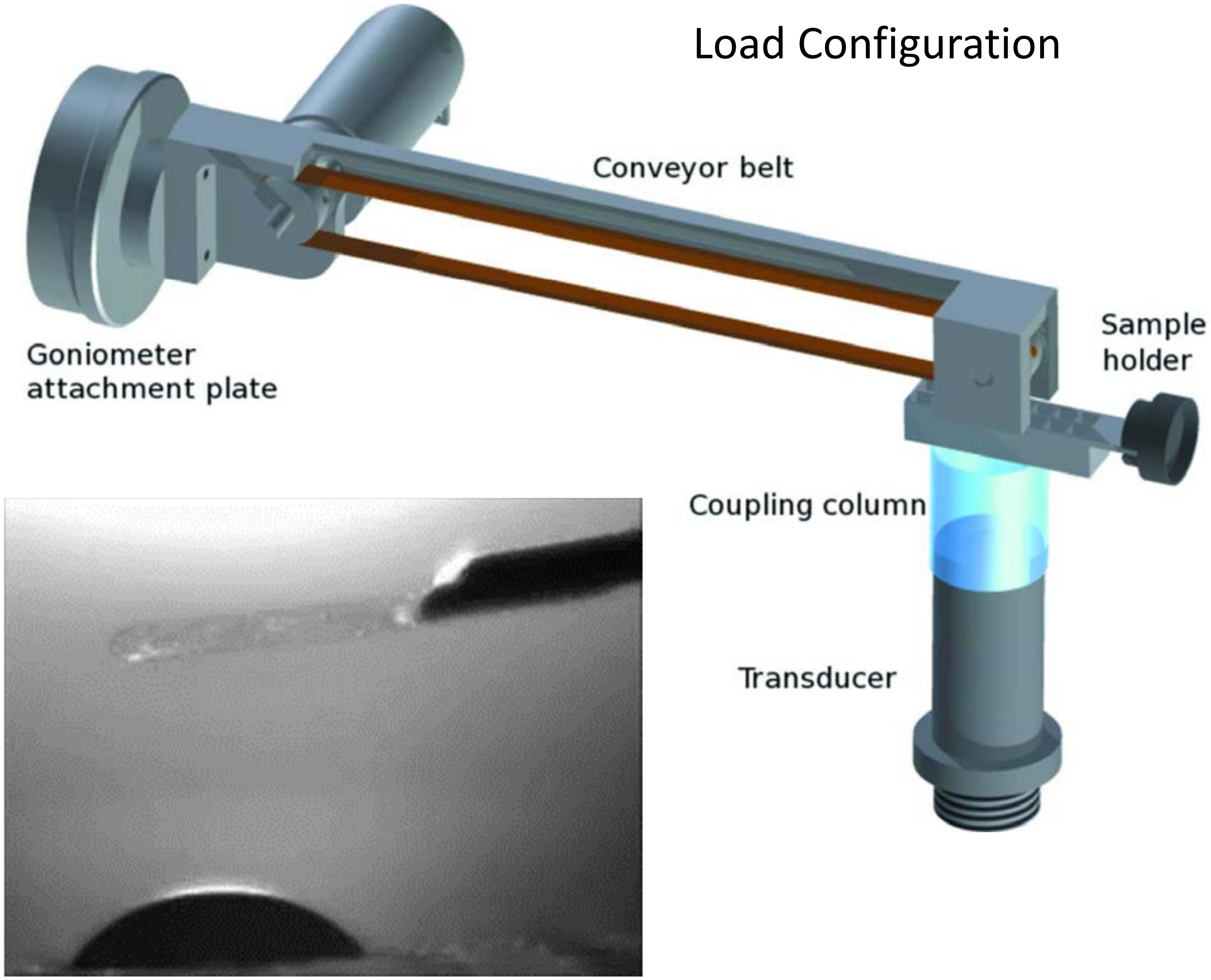
2.5 nl ejected droplet
175 µ diameter





Slurry of insulin microcrystals
Supported by 25 x 25µm grid

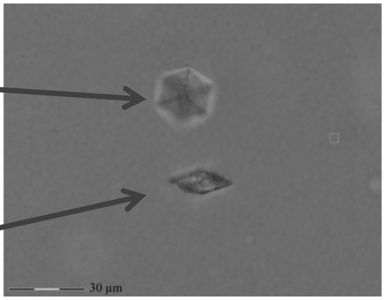
Load Configuration



Alexei Soares, BNL/NSLS

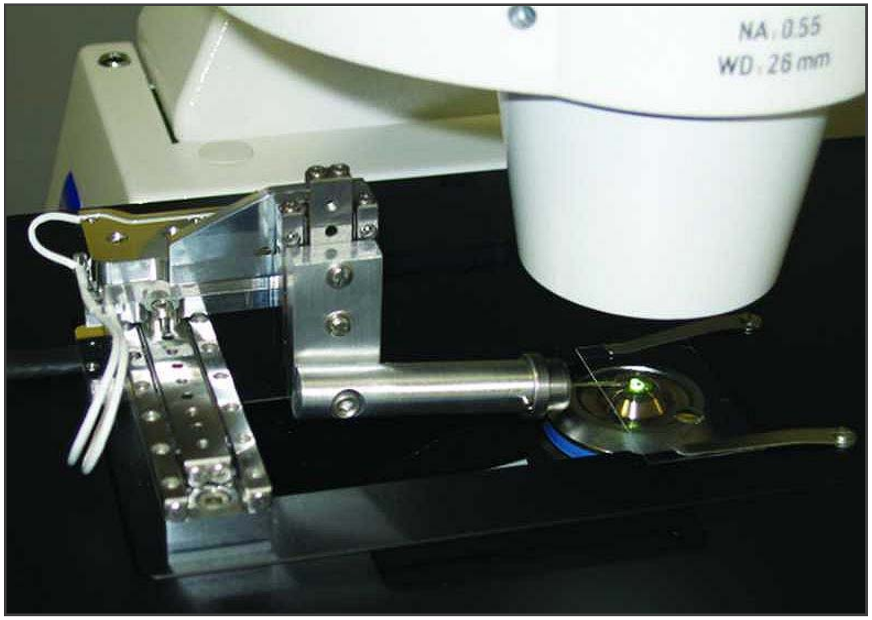
Optical laser "tweezers"

Strong E field gradient at waist of focused laser beam -> repulsive or attractive forces on dielectric particles with refractive index mismatch to solvent. Potential minimum at focus of beam.

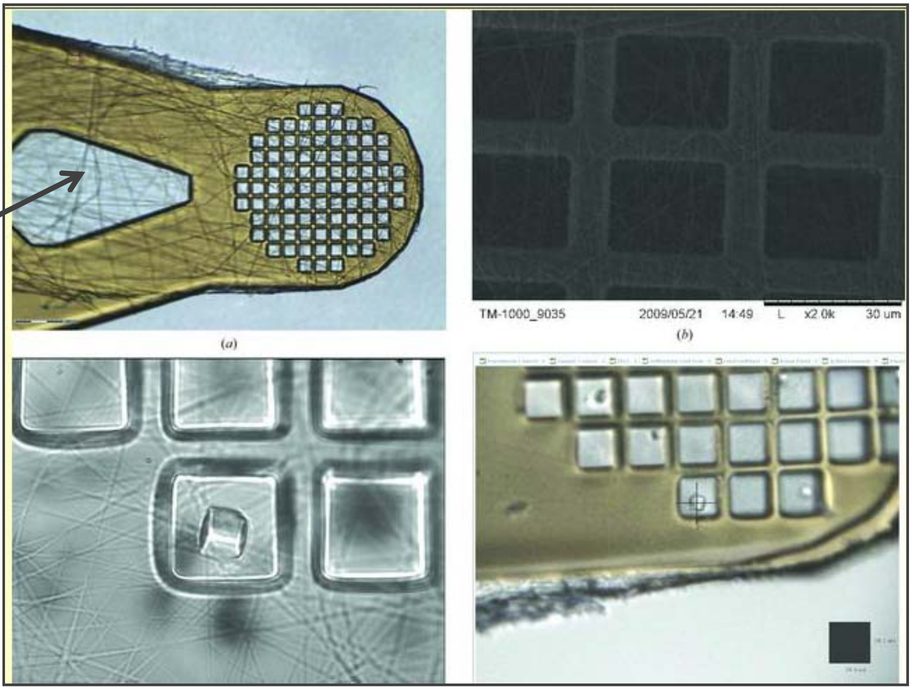


Crystal on Cover Slip

Trapped Crystal



Electro-spun PMMA fibers provide
Floor to micromesh wells



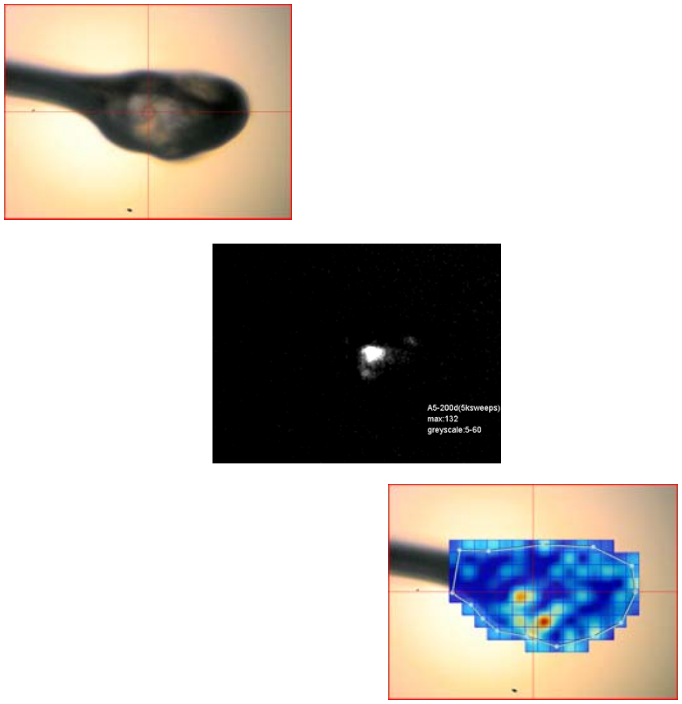
DLS

A. Wagner, et al Acta. Cryst. D
Vol 69, pp 1297-1302, Jul 2013

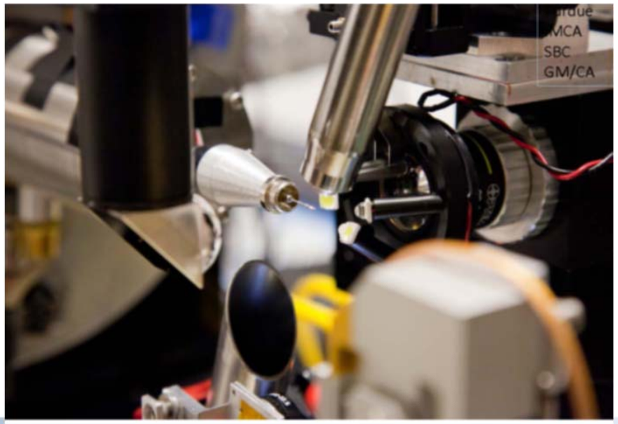
Second Order Non-linear Imaging of Chiral Crystals (SONICC) / Two-Photon Excitation UV Fluorescence

- High-sensitivity technique to detect sub-micron sized crystals, even in turbid media such as lipidic cubic phase (LCP).
- Measures Second Harmonic Generation (SHG) signal that arises from interaction of high-field laser with anharmonic polarizability tensor of chemical bonds.
- Multi-CAT collaboration

  GM/CA, IMCA, SBC
- Pioneered by Garth Simpson's group at Purdue University.

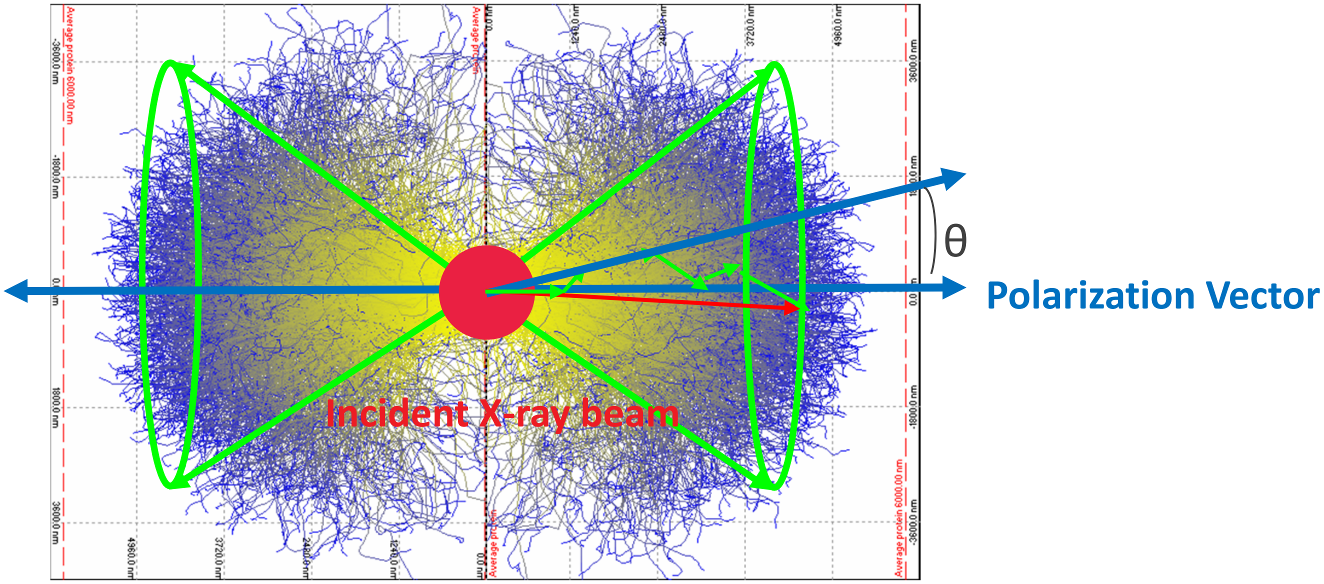


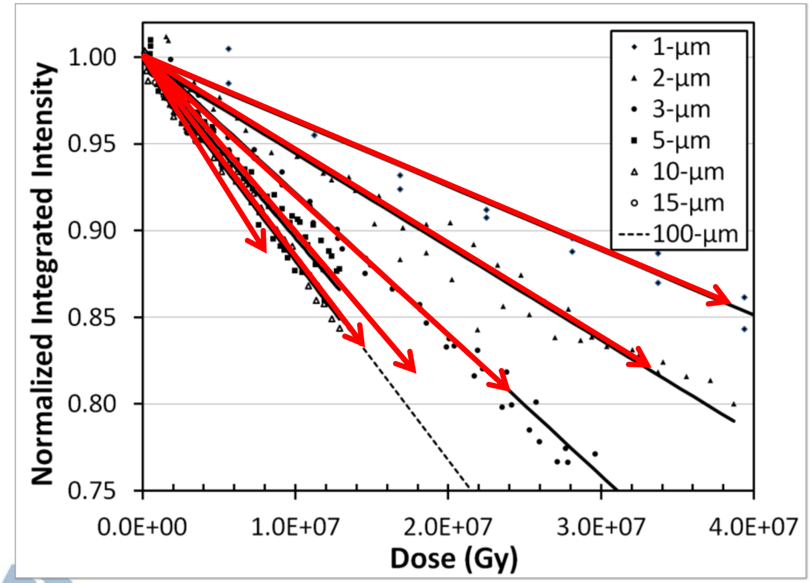
Sample containing human opioid receptor in lipidic cubic phase from Vadim Cherezov (TSRI).
Top: sample in bright field
Middle: SONICC image in laser focal plane
Bottom: Diffraction raster in JBluice .



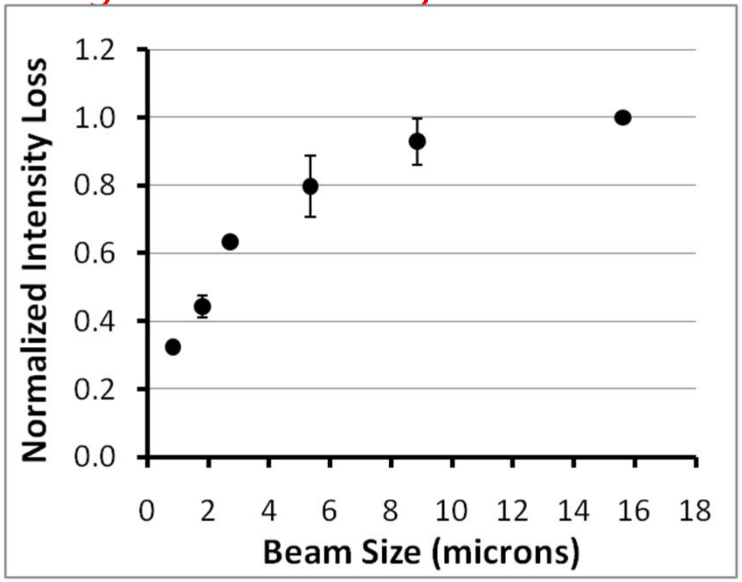
MICHAEL BECKER, ANL

Crystal decay (intensity loss) *vs.* beam size and dose



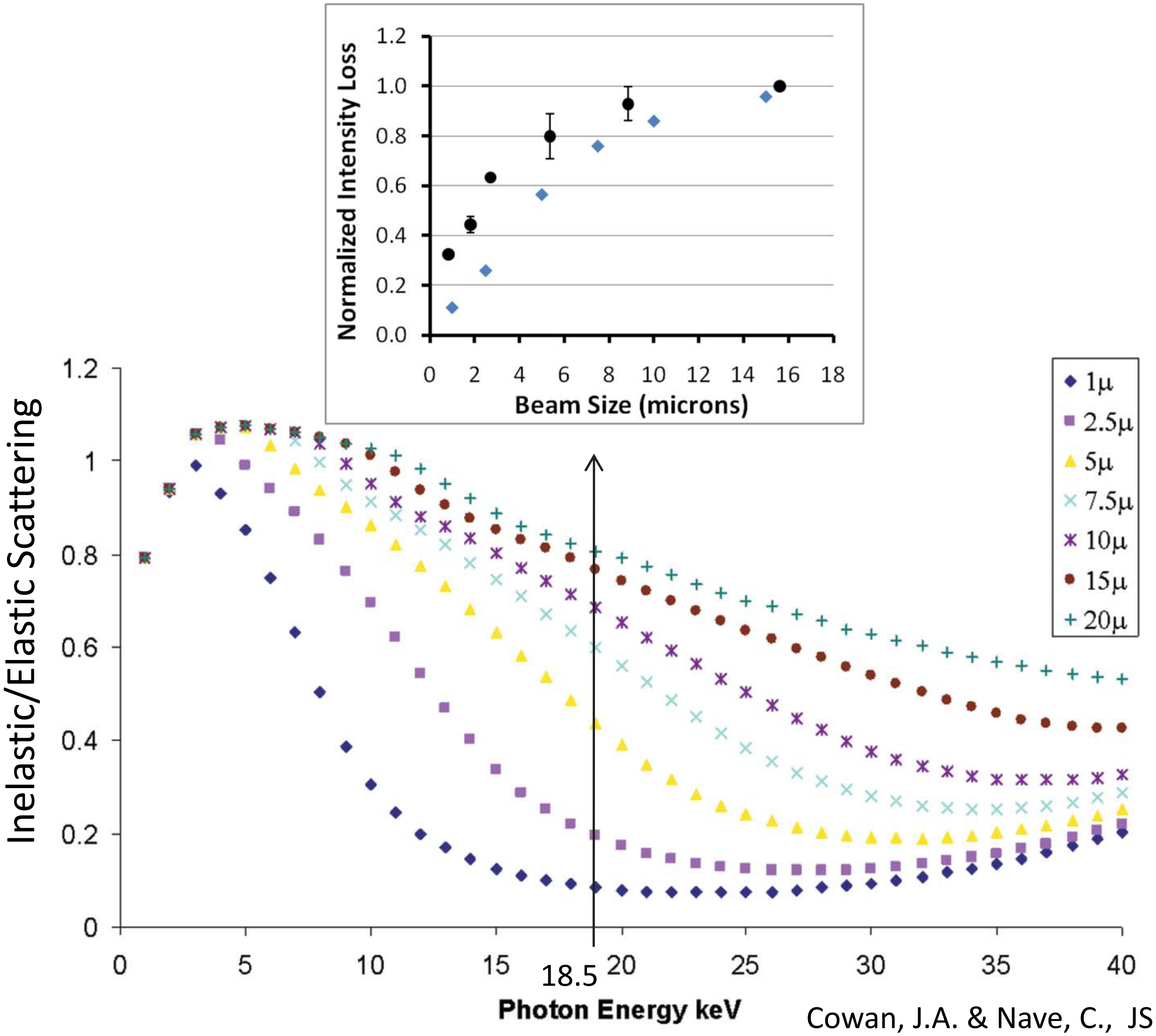


Damage decreases 3-fold with beam size



Nukri Sanishvili and Derek Yoder

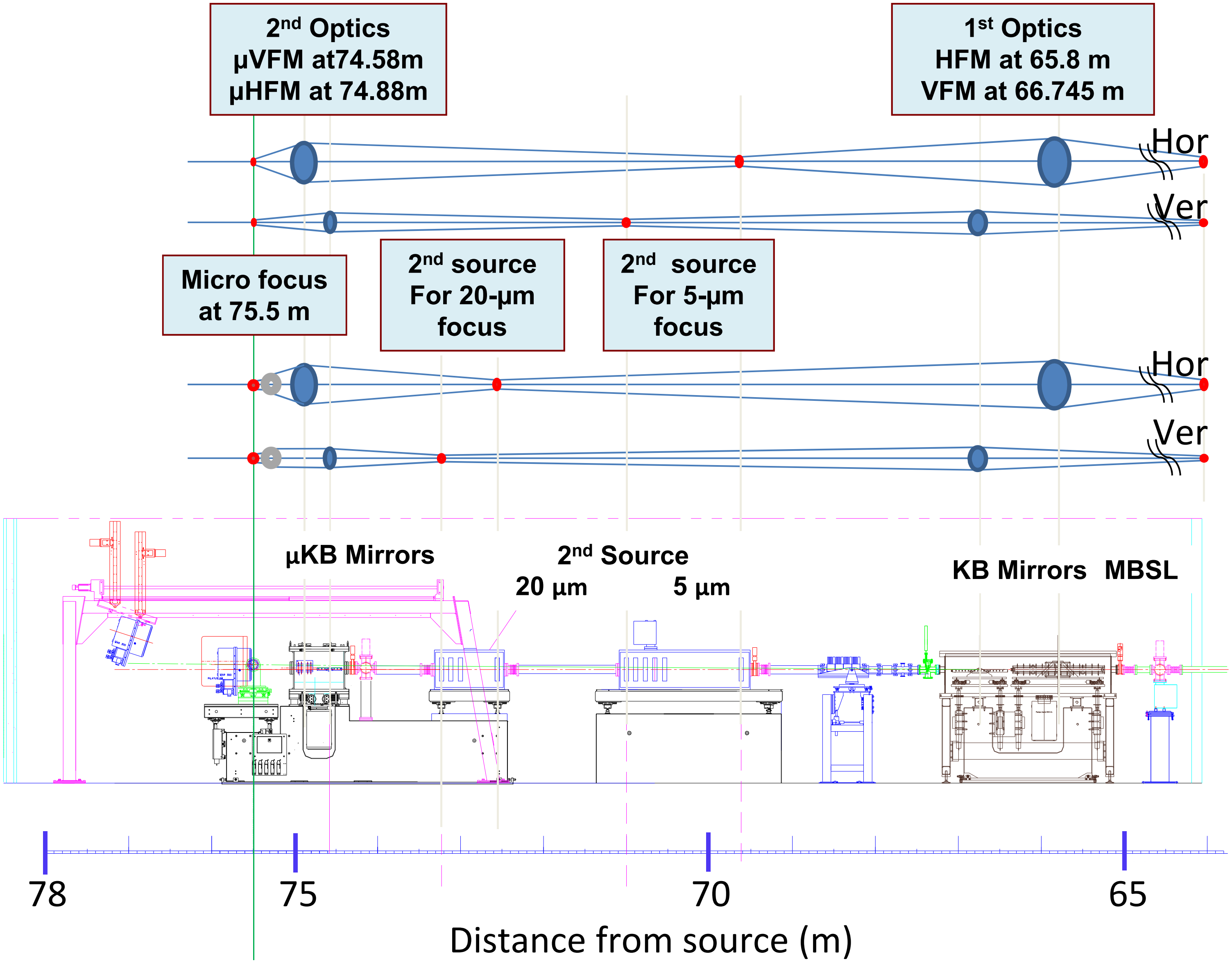
Comparison of Monte Carlo simulations and our data



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

Thank you for your attention

Microfocus Upgrade Layout



2nd Optics
µVFM at74.58m
µHFM at 74.88m

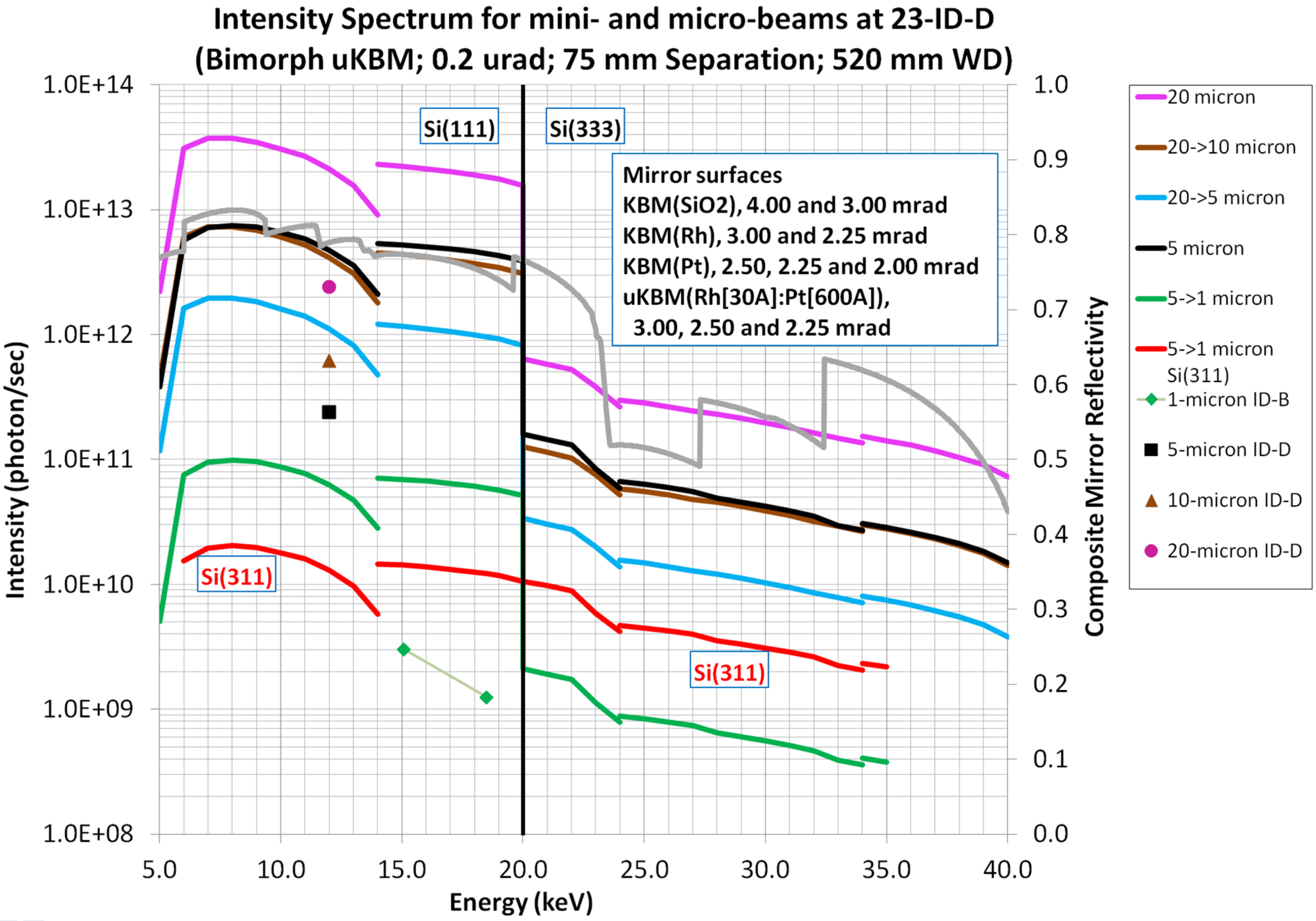
1st Optics
HFM at 65.8 m
VFM at 66.745 m

Micro focus
at 75.5 m

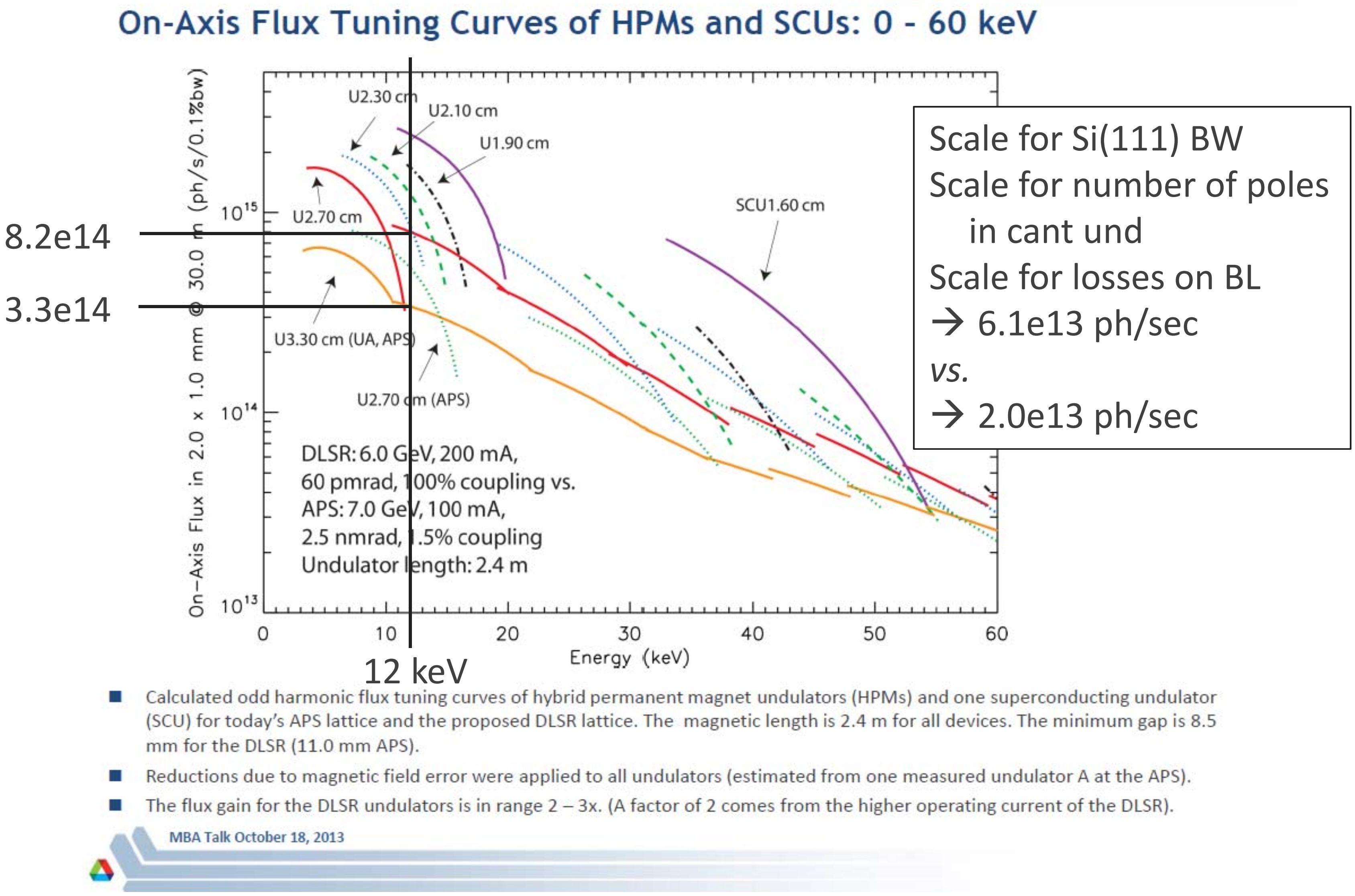
2nd source
For 20-µm
focus

2nd source
For 5-µm
focus

Microfocus Upgrade: Intensity vs. Energy



Beamline Performance



On-Axis Flux Tuning Curves of HPMs and SCUs: 0 - 60 keV

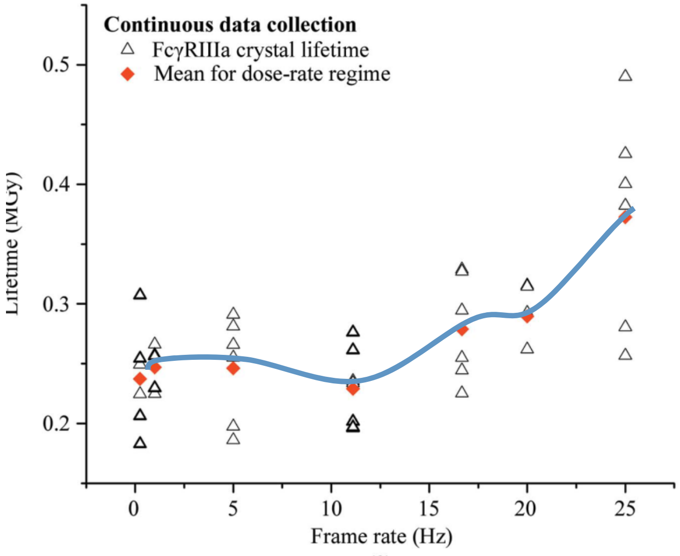
- Calculated odd harmonic flux tuning curves of hybrid permanent magnet undulators (HPMs) and one superconducting undulator (SCU) for today's APS lattice and the proposed DLSR lattice. The magnetic length is 2.4 m for all devices. The minimum gap is 8.5 mm for the DLSR (11.0 mm APS).
- Reductions due to magnetic field error were applied to all undulators (estimated from one measured undulator A at the APS).
- The flux gain for the DLSR undulators is in range 2 – 3x. (A factor of 2 comes from the higher operating current of the DLSR).

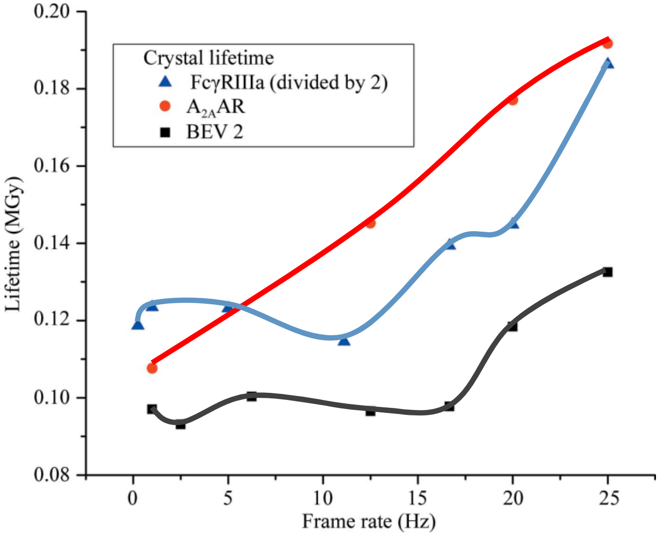
MBA Talk October 18, 2013

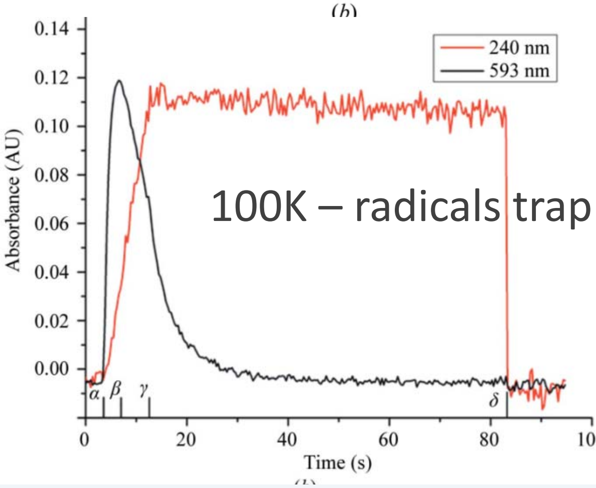
Scale for Si(111) BW
Scale for number of poles
in cant und
Scale for losses on BL
→ 6.1e13 ph/sec
vs.
→ 2.0e13 ph/sec

Roger Dejus

Outrunning 2nd Rad Dam at Room Temp – Beware!







100K – radicals trapped

Crystal lifetime

Start-stop

40 msec exp; 4 sec dead time → 0.186 MGy

Shutterless

40 msec exp → 0.373 MGy

Compare cryo-cooled → 30 MGy

Black – generation of aqueous or solvated e-, followed by 1st order decay

Red – postulated to be hydroxyl radical

Owen *et. al.* Acta Cryst. (2012) D**68**, 810-818

Time to the Garman Limit (cryo-cooled)

Garman limit[1] ~ 3.0 x 10^7 Gray (35% intensity loss)
Deposited energy in sample – not incident energy!

E ~ 12.68 keV

Beamline	Divergence (µrad, FWHM)	Smallest beam width (µm)	Smallest beam height (µm)	Flux (ph/sec)	Flux density (ph/s/µm2)	Dose rate (Gy/s)	Time to Garman limit (msec)
APS-U MBA 23-ID-D	3200 x 1200	0.40	0.50	5.3E+13	3.4E+14	1.0E+11	0.29
NSLS-II FMX‡	1700 x 700	1.00	0.50	5.0E+12	1.3E+13	3.9E+09	7.60
DLS VMX¥		0.50	0.50	1.0E+12	5.1E+12	1.0E+09	29.90
APS-U MBA 23-ID-D	270 x 180	6.10	5.20	6.1E+13	2.4E+12	7.6E+08	39.52
Petra3 MX2	500 x 300	4.00	1.00	5.0E+12	1.6E+12	4.9E+08	60.81
SPring8 BL32XU§	1520 x 980	0.90	0.90	6.2E+10	9.7E+10	3.0E+07	992.99
Petra3 MX1	200 x 150	28.00	13.00	1.0E+13	3.5E+10	1.1E+07	2766.63
APS 23-ID-D*	400 x 100	5.00	5.00	5.4E+11	2.8E+10	8.5E+06	3518.81
DLS I24	2000 x 50	8.00	8.00	1.0E+12	2.0E+10	6.2E+06	4864.40
ESRF ID23-2†	550 x 360	7.50	7.50	4.0E+11	9.1E+09	2.8E+06	10688.38
APS 23-ID-D*	400 x 100	70.00	25.00	2.00E+13	1.46E+10	4.50E+06	6650.55

*APS 23-ID intensities are for 12.0 keV except where noted
§SPring8 BL32XU intensities area at 12.398 keV
†ESRF Upgrade may have changed these numbers
‡NSLS-II AMX/FMX intensities are at 12.7 keV

[1] Owen, R.L., Rudino-Pinera, E. & Garman, E.F. *Proc Natl Acad Sci U S A* **103**, 4912-7 (2006)
[2] RADDOSE http://biop.ox.ac.uk/www/garman/lab_tools.html