Harvesting, Handling & Alignment

M. Capel

MBA Lattice Upgrade Workshop

MX Breakout Session

October 21, 2013

Microbeam Crystallography

Remit:

- Structure determination using micro crystals (1-20 micrometers) – probably using serial, multi-crystal data collection with flux density generated by MBA Lattice optimized beam lines.
  - Efficient / automated sample preparation (sampling / handling).
  - Efficient location and alignment of microcrystals in opaque cryomedia.
- Scanning larger crystals with micro beams to generate spatial assays of diffraction quality…use of high quality regions for data acquisition.
- Spatial scanning large crystals with small beams during data collection to mitigate radiation damage effects on diffraction data quality.

Requirements:

- High beam flux density.
- High positional stability for x-ray beam and sample (time domain: msec - minutes).
- Ability to precisely & reproducibly manipulate sample position relative to beam.
- High sensitivity, low noise detection.
- Minimization of diffraction background.
- Facile manipulation and alignment of microcrystals, including optically opaque preparations.

Agenda

- Survey of emerging techniques for manipulation of microcrystals.

  Harvesting Survey Data Collection

- Review of new methods for microcrystal visualization and alignment.

- Scanned Data Collection Methods

  Sample location & alignment.

  mitigation of radiation damage.

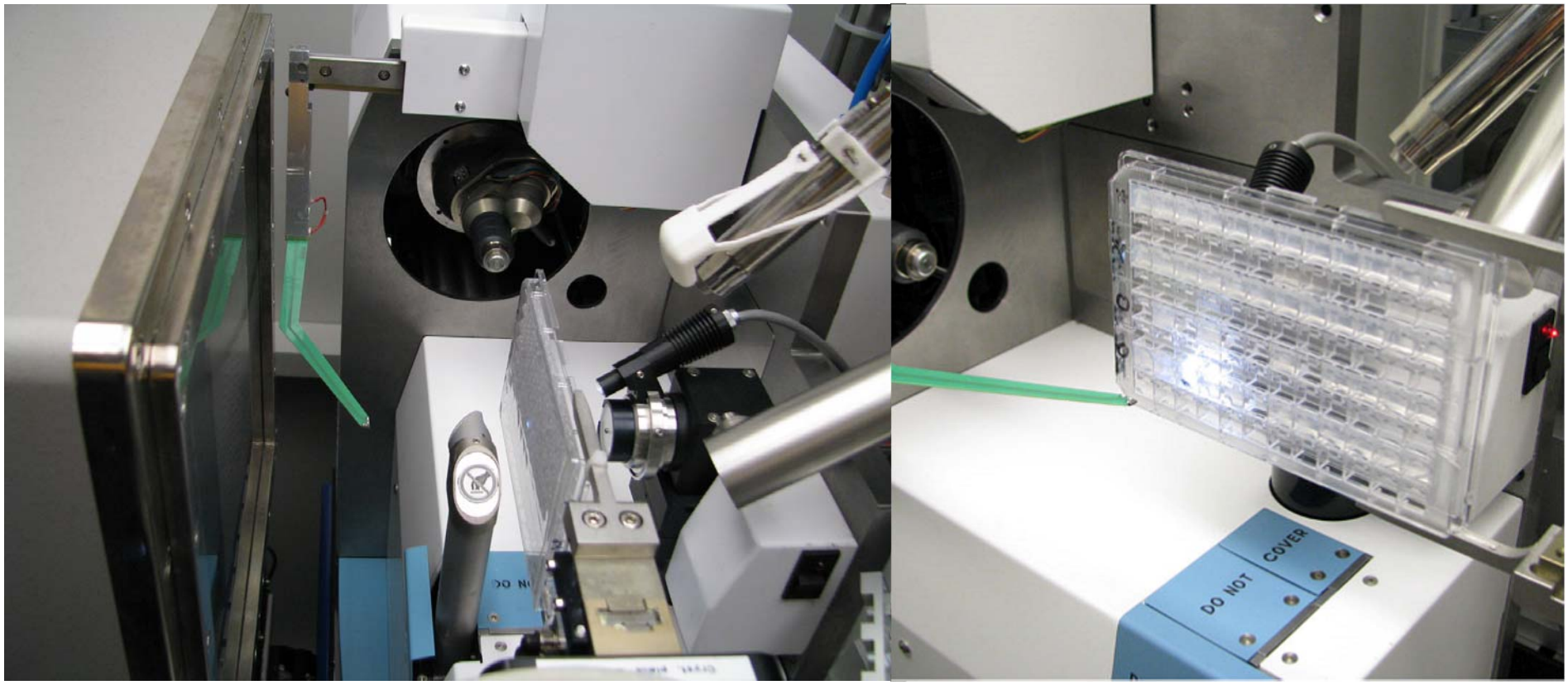
- Improvements in sample and beam positional stability

  Mandatory for extremely small samples.

Mounting/Handling Modes

- In-situ survey, data collection
  - crystallization plates
  - Micro fluidic plates
  - Crystal Direct (Photo ablation of support films)
- Acoustic ejection
  - grid mounts
  - belt handler
- Optical Traps (Laser tweezers)
- Enabling technologies for time-efficient serial microcrystallography

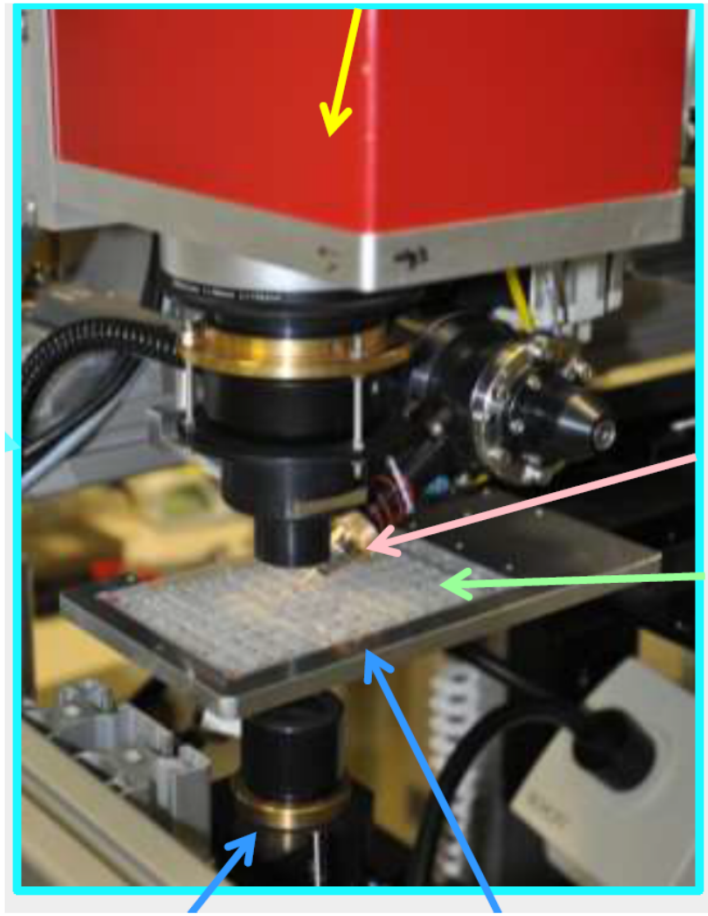
In-situ Screening/Collection



HZB Helmholtz Zentrum Berlin

CrystalDirect

fs Laser Scanner



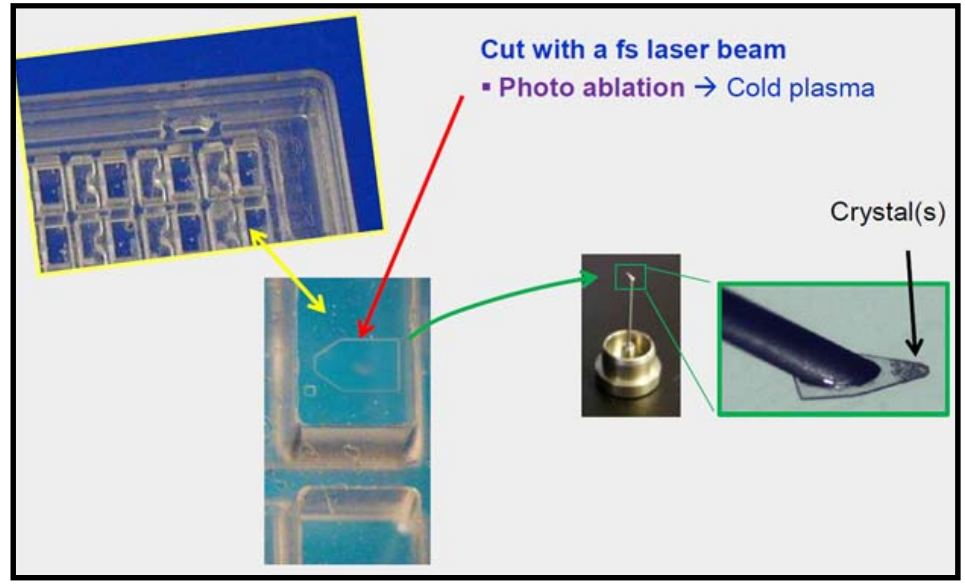
Base Holder

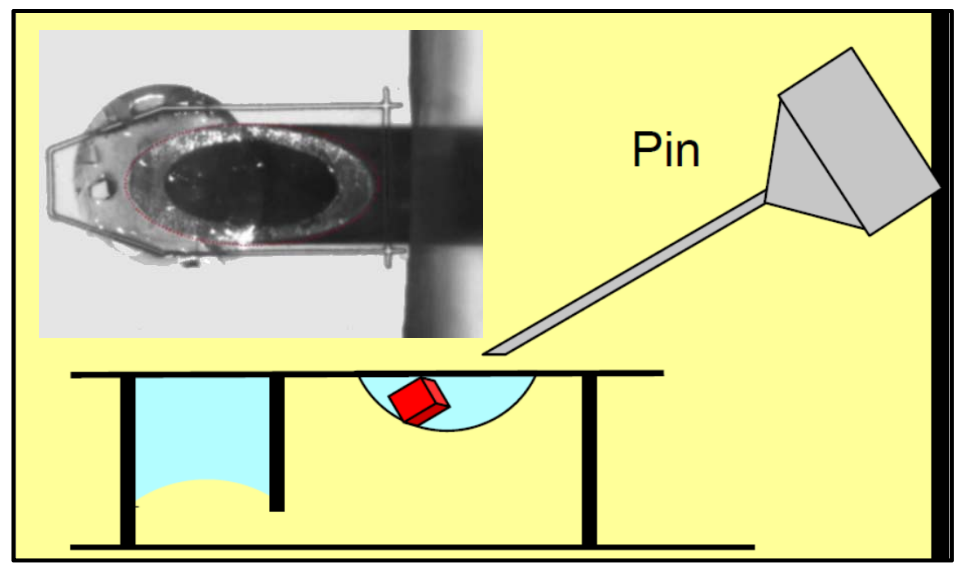
CD Plate

Video Microscop

XYZ Table

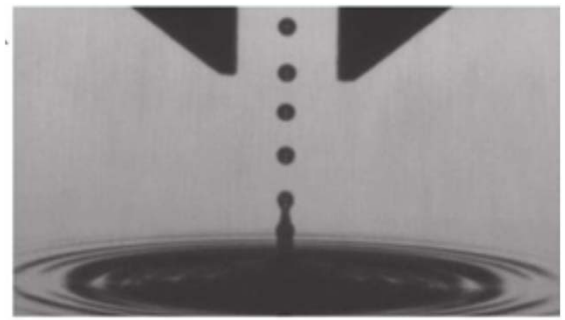
Cipriani Group, EMBL/ESRF

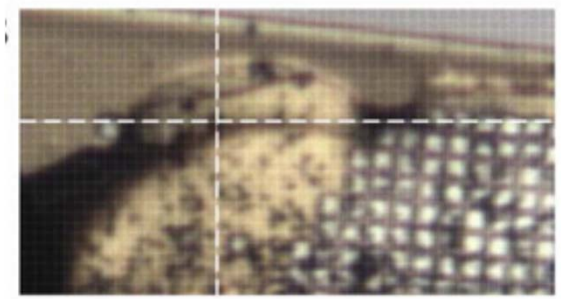




Acoustic Droplet Ejection

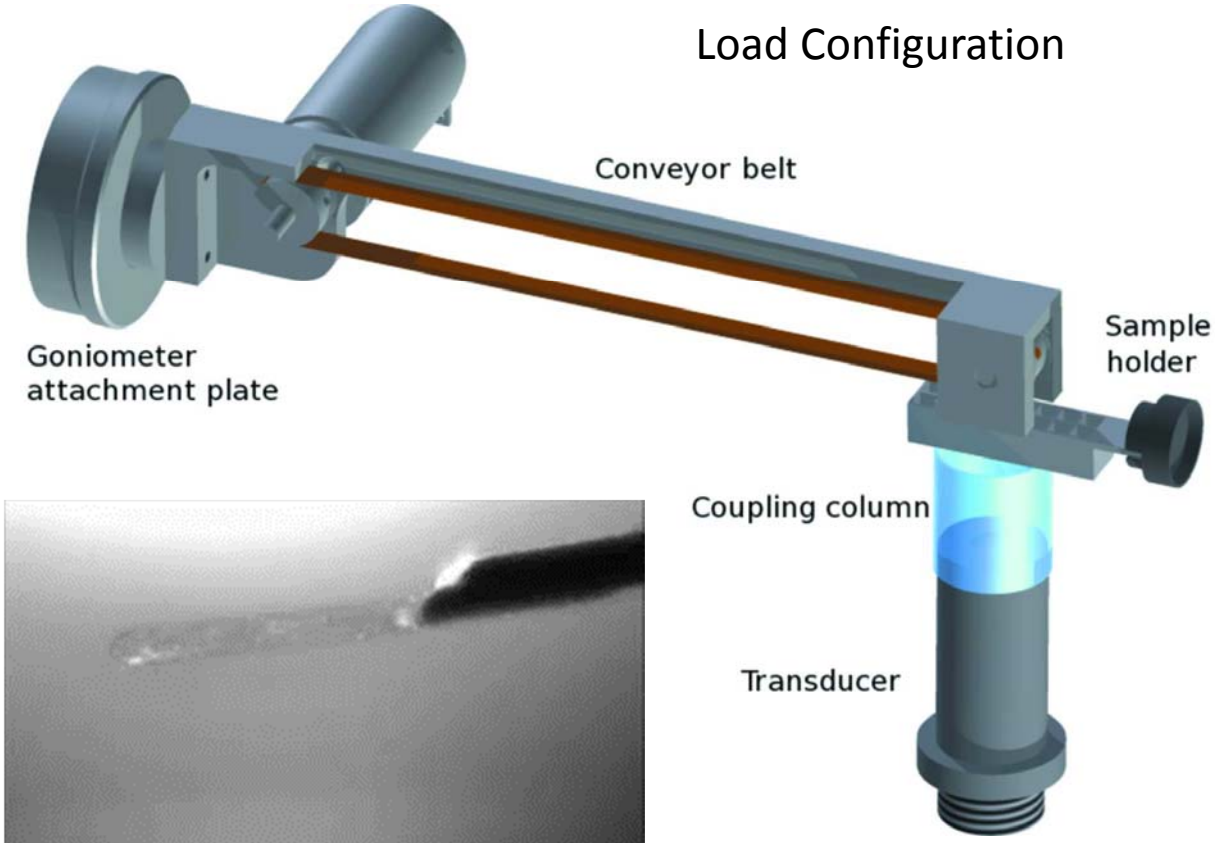
2.5 nl ejected droplet
175 µ diameter

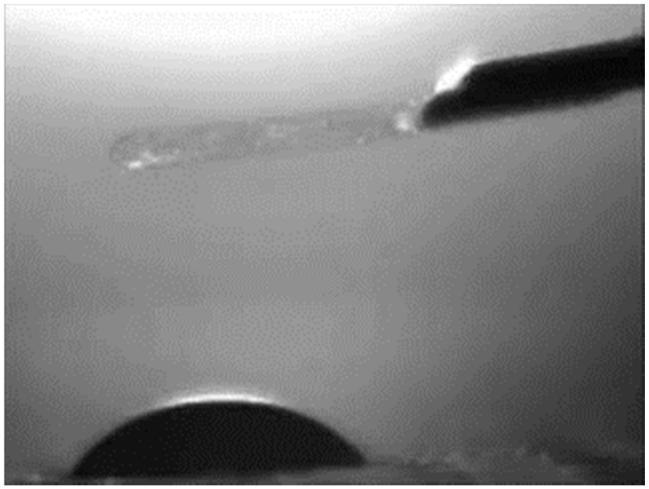




Slurry of insulin microcrystals
Supported by 25x25µ grid

Load Configuration

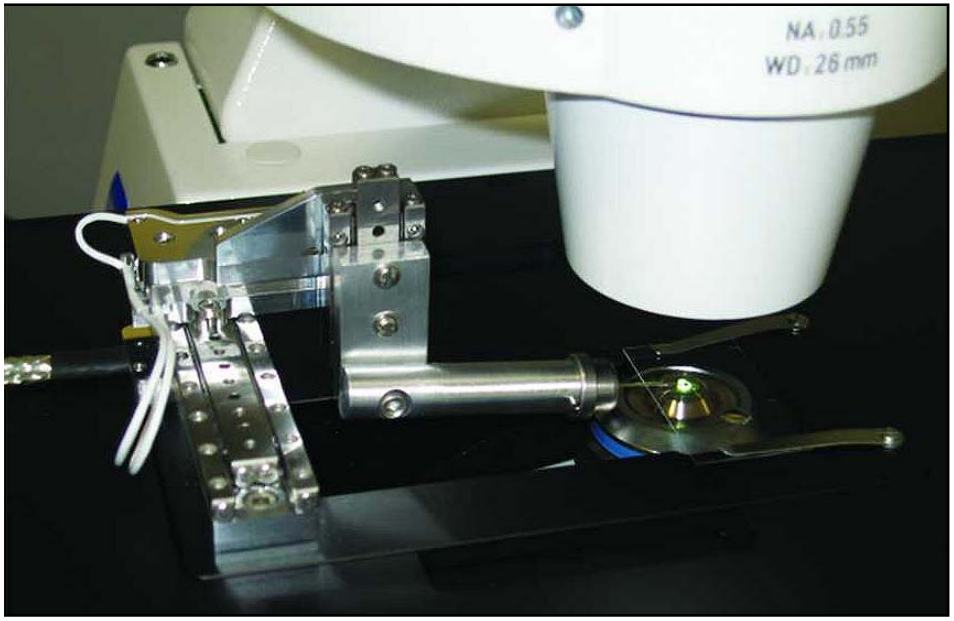


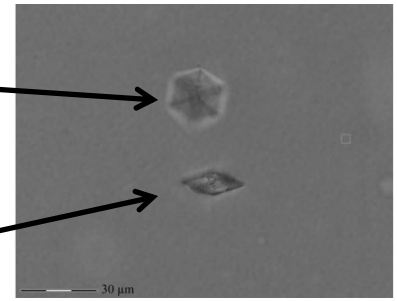


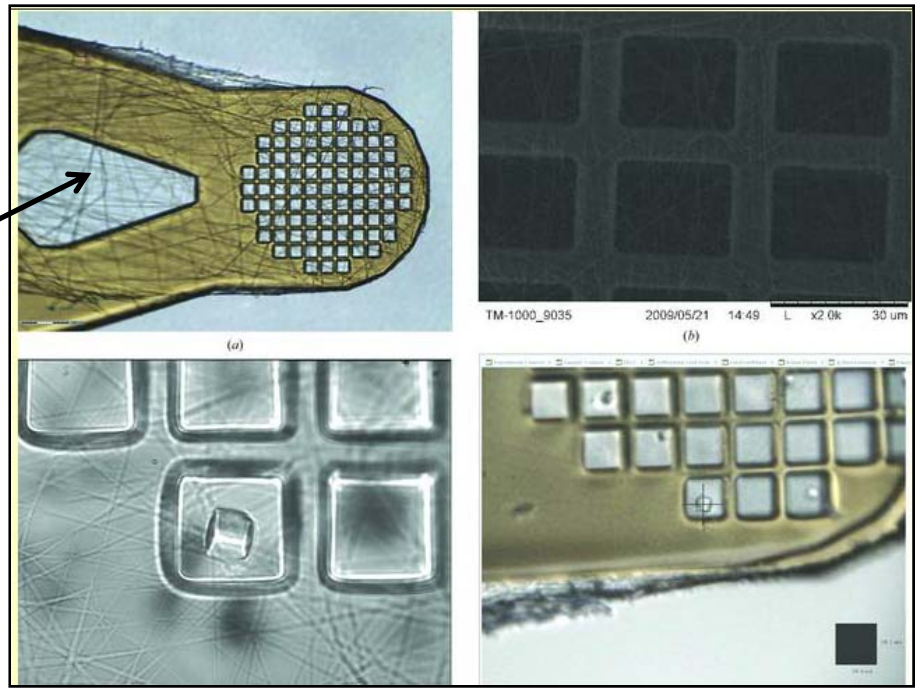
Alexei Soares, BNL/NSLS

Optical Traps

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.







Electro-spun PMMA fibers provide Floor to micromesh wells

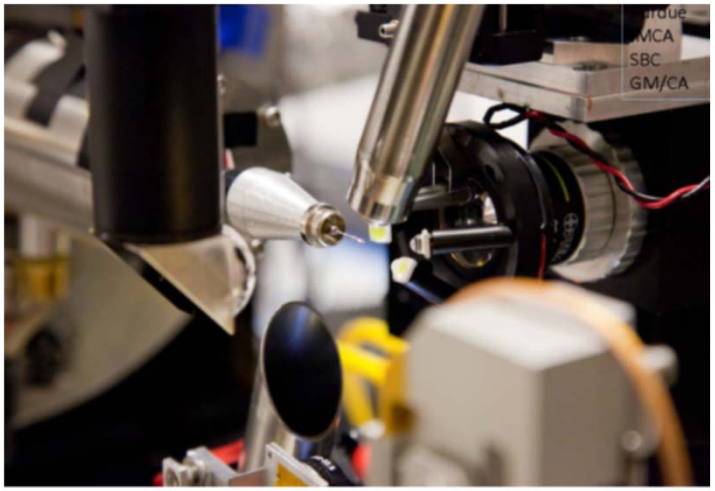
A. Wagner, et al Acta. Cryst. D Vol 69, pp 1297-1302, Jul 2013

Location/Visualization Modes

- TPE-UVF / SONICC
- Low-does Radiography / Tomography
- Diffractive alignment/survey
- Automation – Image analysis
- Problem cases:
  - Frost / mother liquor precipitation
  - LCP
  - Miniscule crystals

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

Crystal system	Point group/ Crystal Class		Space groups (international short symbol)	Generates SHG	% of known structures (A)	% of structures in the space group that do not have any tryptophan residues (B)
	Intl	Schönflies				
Triclinic	1	C_1	P1	yes	4.04	18.34%
Monoclinic	2	C_2	P2, $P2_1$, C2	yes	24.78	16.88%
Orthorhombic	222	D_2	P222, $P222_1$, $P2_12_12$, $P2_12_12_1$, $C222_1$, C222, F222, I222, $I2_12_12_1$	yes	36.36	16.83%
Trigonal	3	C_3	P3, $P3_1$, $P3_2$, R3	yes	4.04	20.24%
	32	D_3	P312, P321, $P3_112$, $P3_121$, $P3_212$, $P3_221$, R32	yes	7.3	18.18%
Tetragonal	4	C_4	P4, $P4_1$, $P4_2$, $P4_3$, I4, $I4_1$	questionable	2.36	19.26%
	422	D_4	P422, $P42_12$, $P4_122$, $P4_12_12$, $P4_222$, $P4_22_12$, $P4_322$, $P4_32_12$, I422, $I4_122$	questionable	10.02	17.33%
Hexagonal	6	C_6	P6, $P6_1$, $P6_5$, $P6_2$, $P6_4$, $P6_3$	questionable	3.6	19.46%
	622	D_6	P622, $P6_122$, $P6_522$, $P6_222$, $P6_422$, $P6_322$	questionable	4.26	20.07%
Cubic	23	T	P23, F23, I23, $P2_13$, $I2_13$	questionable	1.22	19.88%
	432	O	P432, $P4_232$, F432, $F4_132$, I432, $P4_332$, $P4_132$, $I4_132$	questionable	.59	25.85%



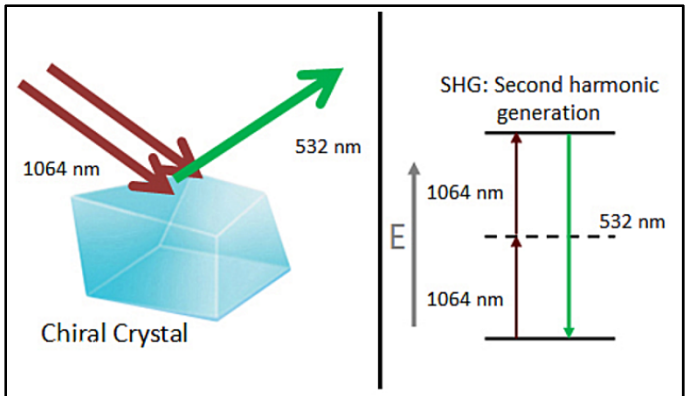


Figure 1. Two photons of IR (1064 nm) interact with a chiral crystal to generate SHG (532 nm).

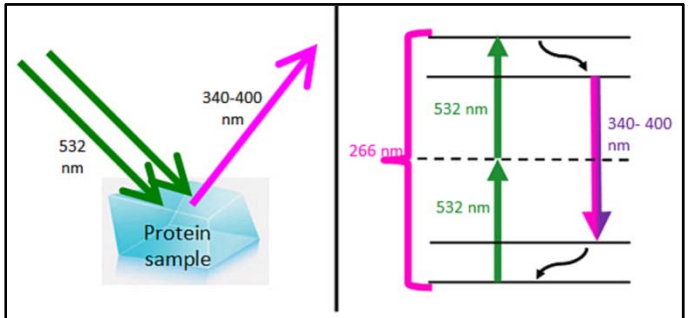
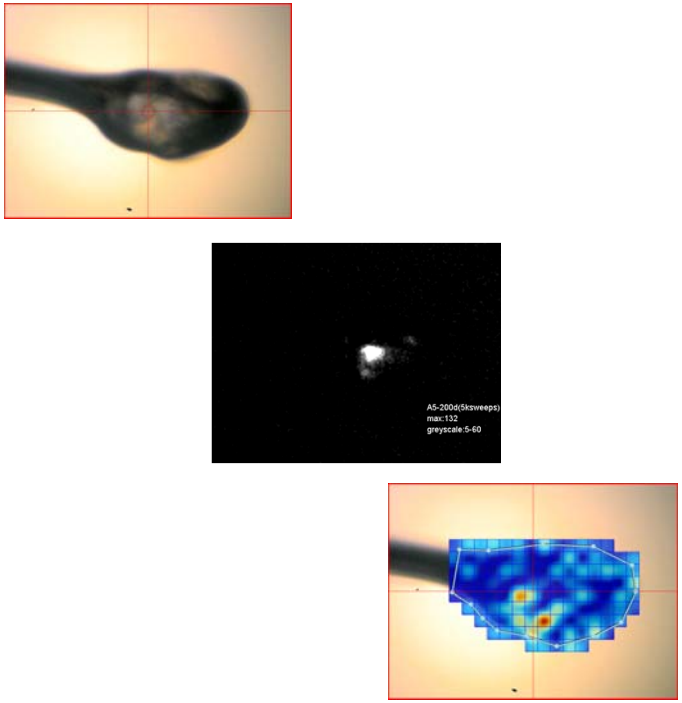


Figure 2. Depiction of UV-TPEF where two photons of green interact with a protein sample to generate UV excited fluorescence

Haupert, L. M.; Simpson, G. J., Chirality in nonlinear optics. *Annual Review of Physical Chemistry* **2009**, 60, 345-365

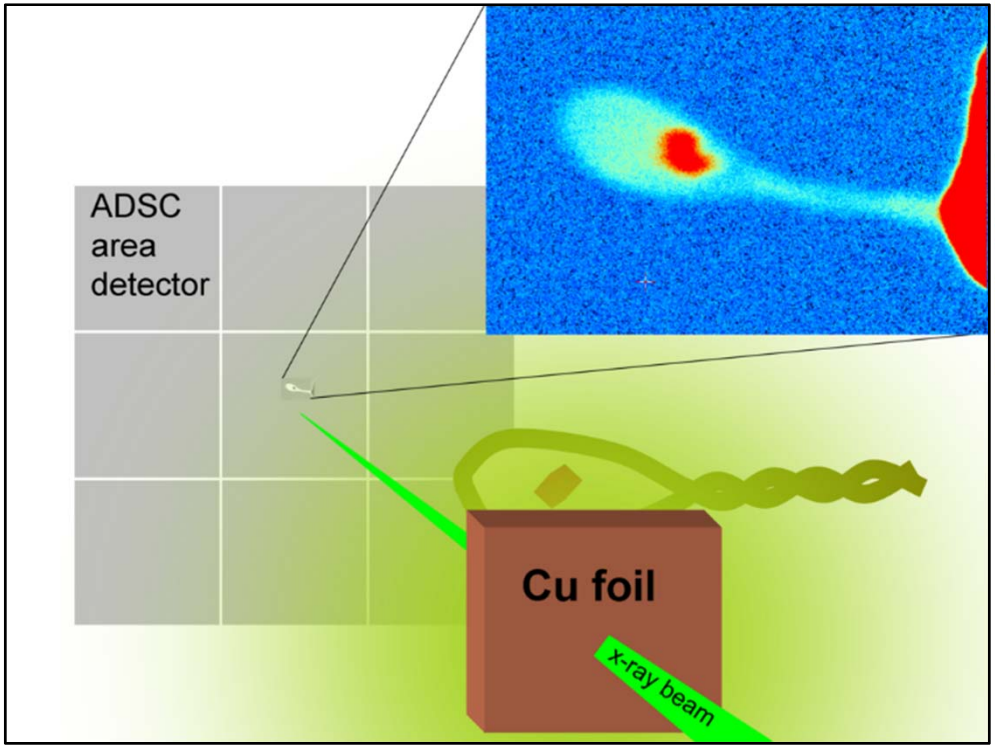
- High-sensitivity technique to detect sub-micron sized crystals, even in turbid media such as lipidic cubic phase (LCP).
- Measures Second Harmonic Generation (SHG) signal that arises from interaction of high-field laser with anharmonic polarizability tensor of chemical bonds.
- Laser is scanned across the sample at high frequency to minimize damage.
- Pioneered by Garth Simpson's group at Purdue University.

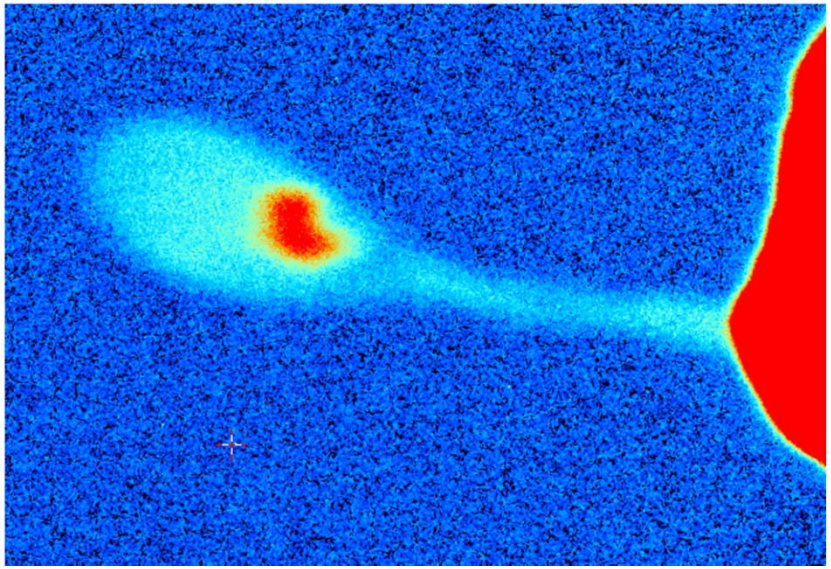


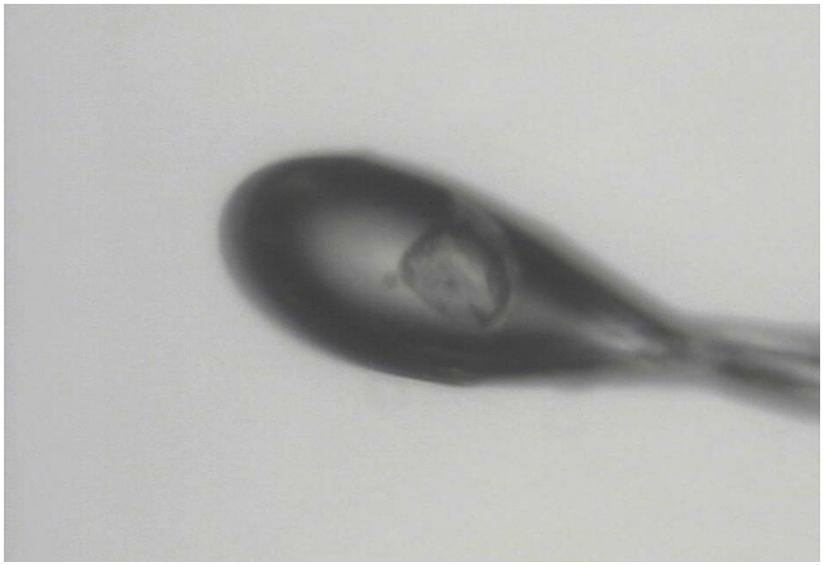
Sample containing human opioid receptor in lipidic cubic phase from Vadim Cherezov (TSRI). Left: sample in bright field; middle: SONICC image in laser focal plane; right: smoothed, corrected Bragg candidates from diffraction raster in JBluice .

MICHAEL BECKER, ANL

Radiography / Tomography

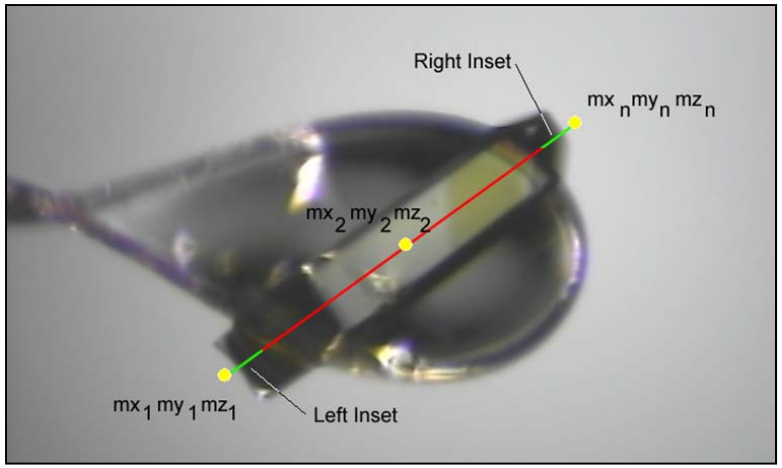




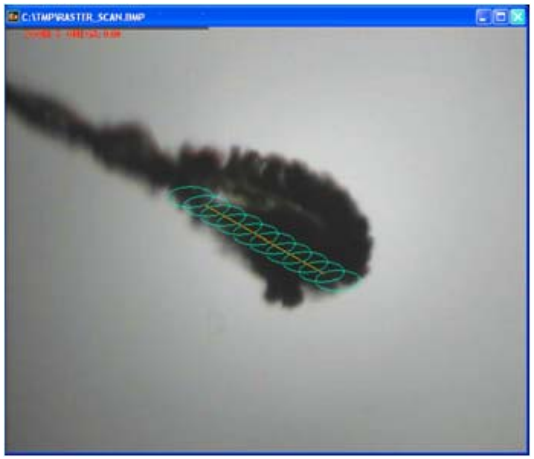


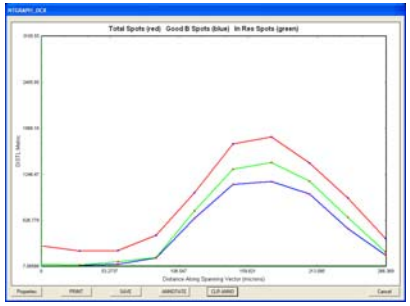
James Holton, LBL ALS

Scanned Data Collection



Definition of Scanning Vector

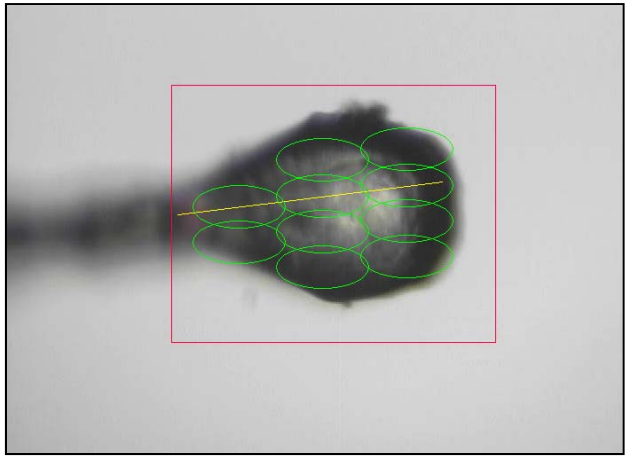




RasterSnap Linear Location / Quality Assay

Continuous Vector Scan

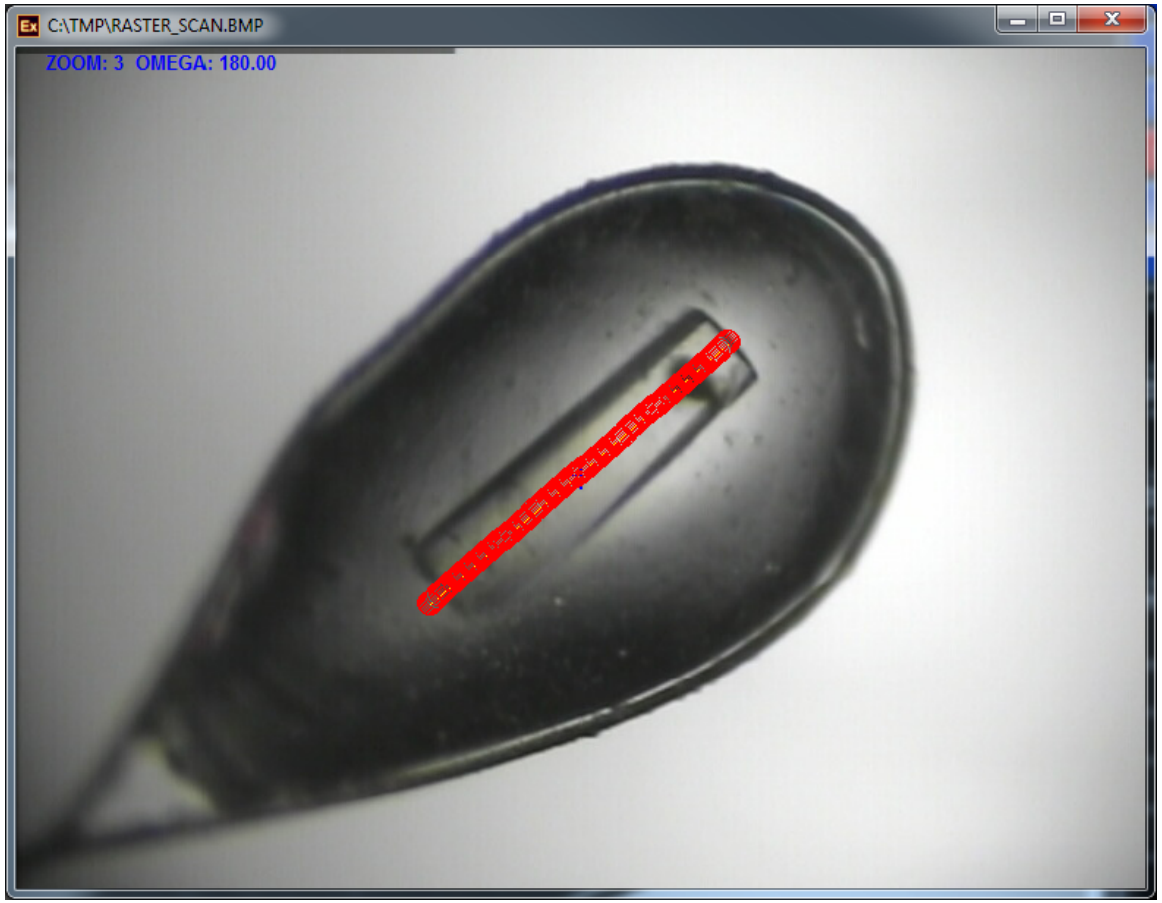
Discrete vector Scan

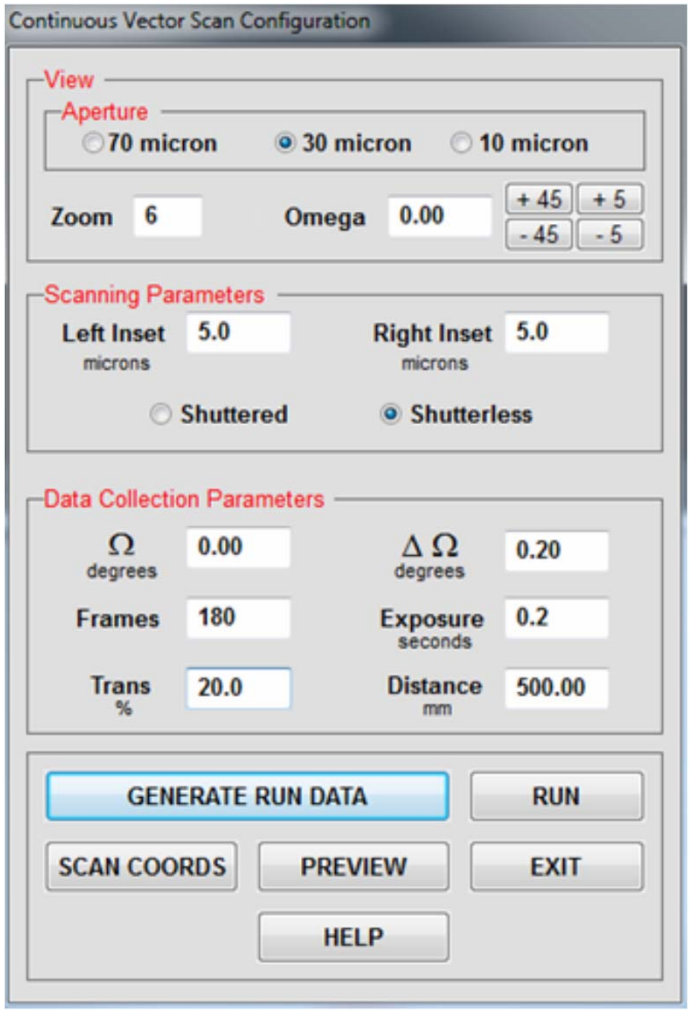


2D Scan for Location / Quality

Shutterless Continuous Vector Scan 70S Ribosome Crystal

SETUP


C:\TMP\RASTER_SCAN.BMP
ZOOM: 3 OMEGA: 180.00


Continuous Vector Scan Configuration
View
Aperture
70 micron
30 micron
10 micron
Zoom
6
Omega
0.00
+ 45
+ 5
- 45
- 5
Scanning Parameters
Left Inset
microns
5.0
Right Inset
microns
5.0
Shuttered
Shutterless
Data Collection Parameters
Ω
degrees
0.00
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Frames
180
Exposure
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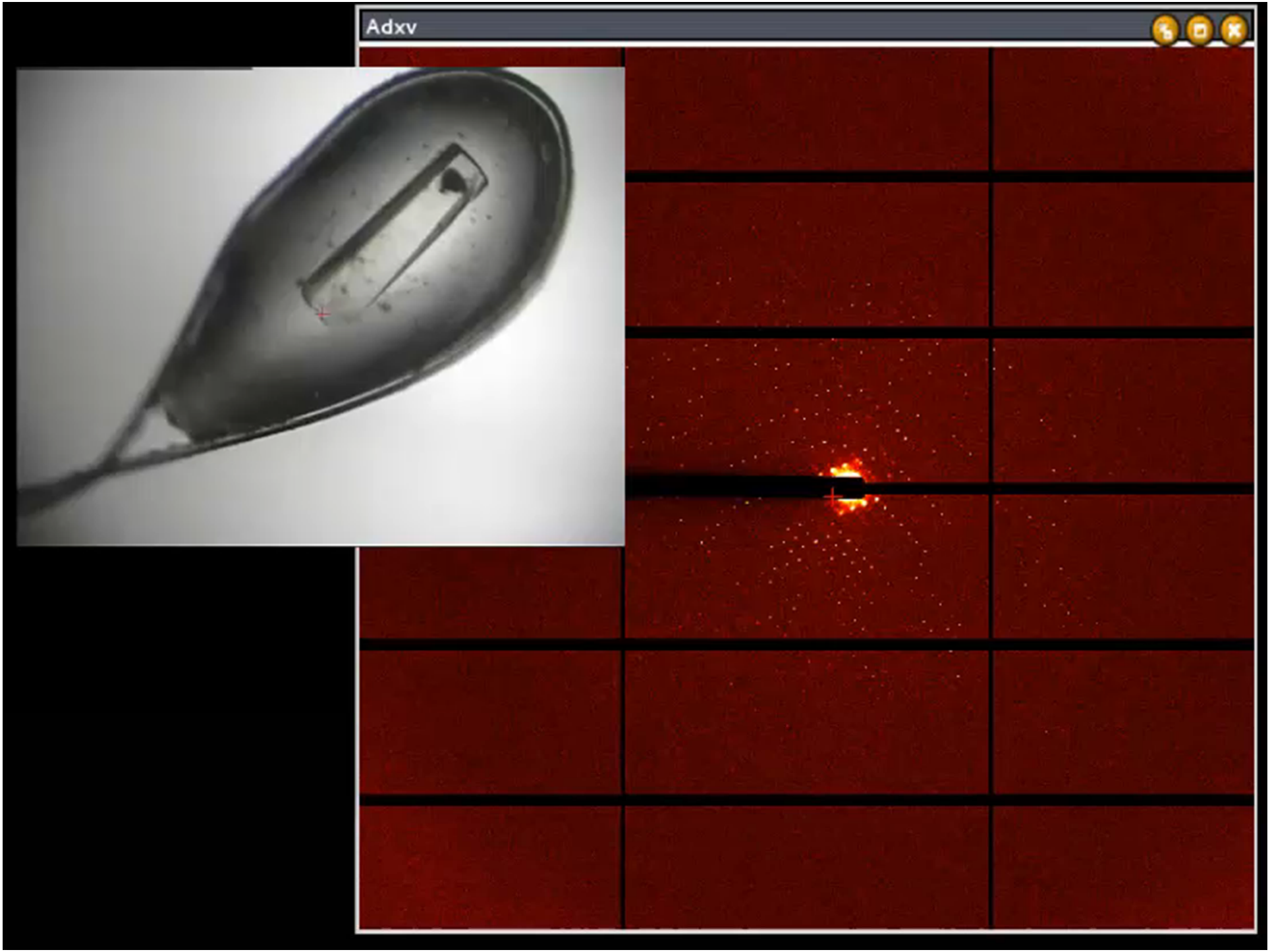
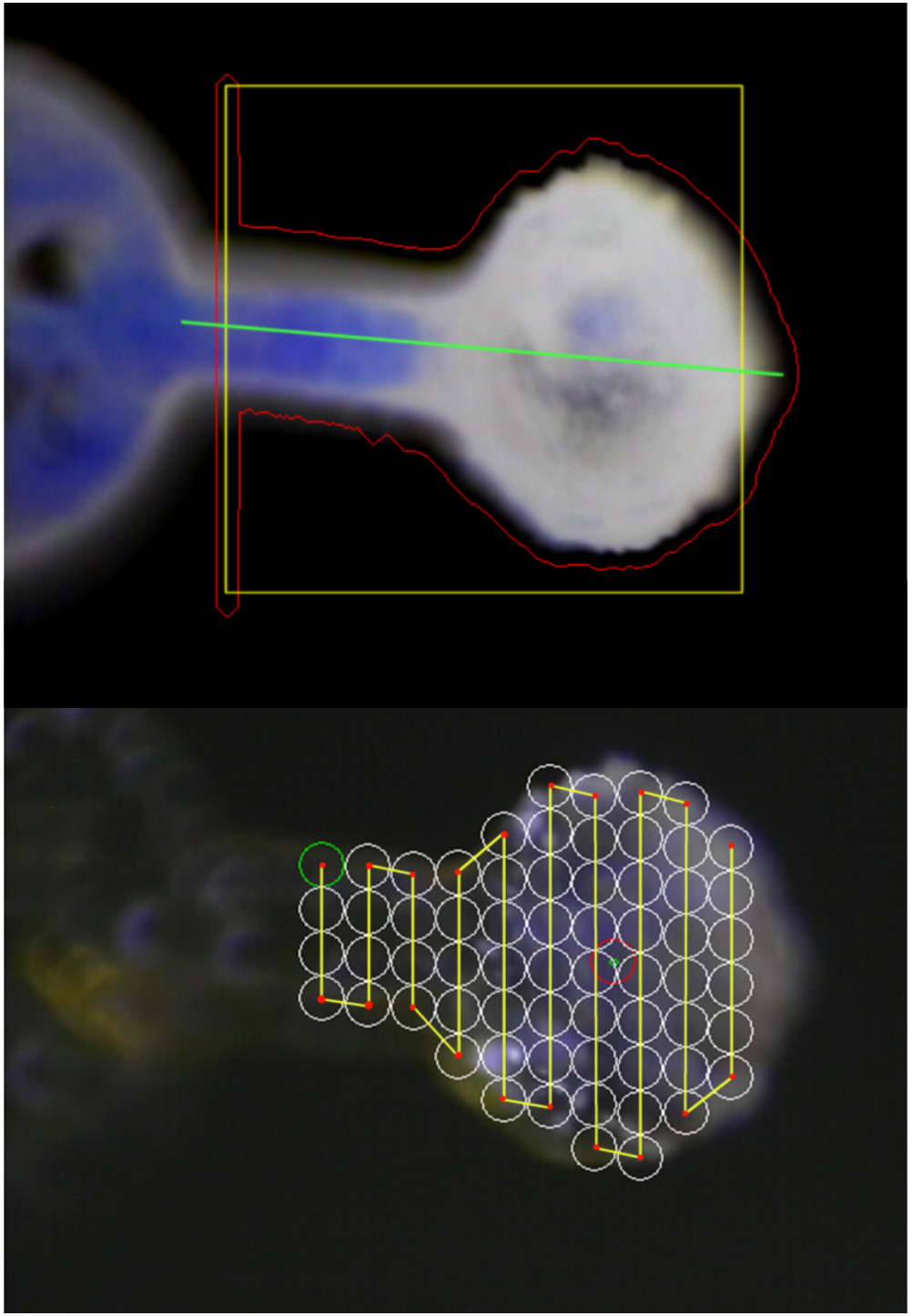
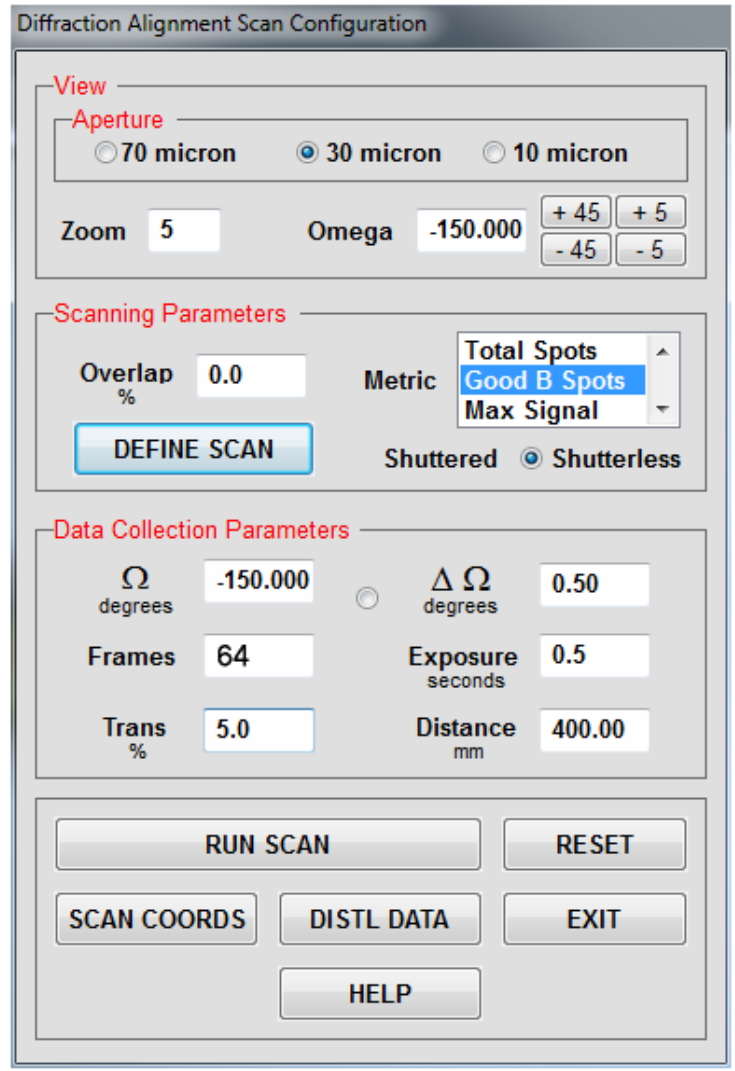
GENERATE RUN DATA
RUN
SCAN COORDS
PREVIEW
EXIT
HELP


Adxv

Shutterless Diffractive Crystal Alignment

Setup

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30 micron
10 micron
Zoom
5
Omega
-150.000
+ 45
+ 5
- 45
- 5
Scanning Parameters
Overlap
%
0.0
Metric
Total Spots
Good B Spots
Max Signal
DEFINE SCAN
Shuttered
Shutterless
Data Collection Parameters
Ω
degrees
-150.000
Δ Ω
degrees
0.50
Frames
64
Exposure
seconds
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Trans
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Distance
mm
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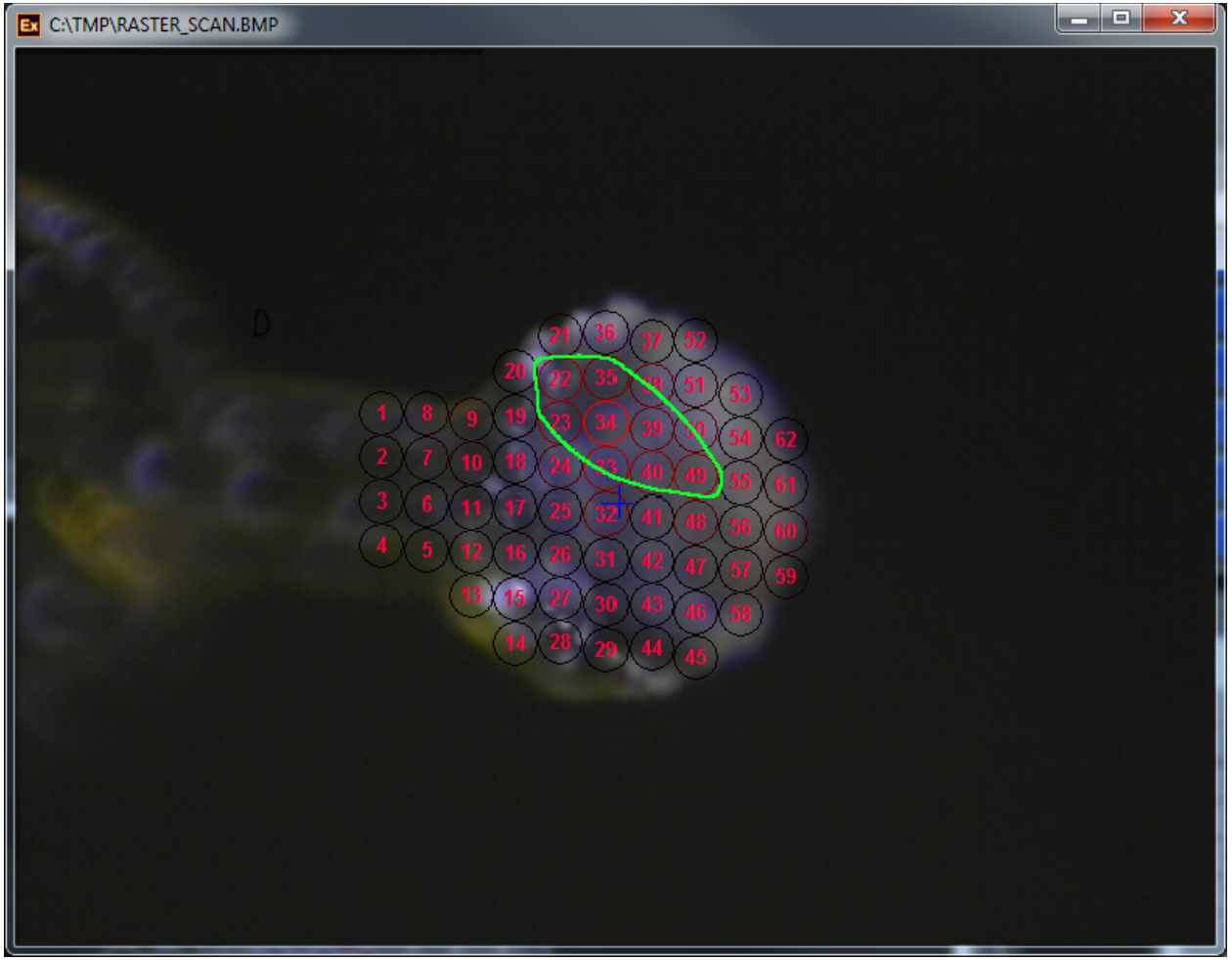
RUN SCAN
RESET
SCAN COORDS
DISTL DATA
EXIT
HELP



Distl Results

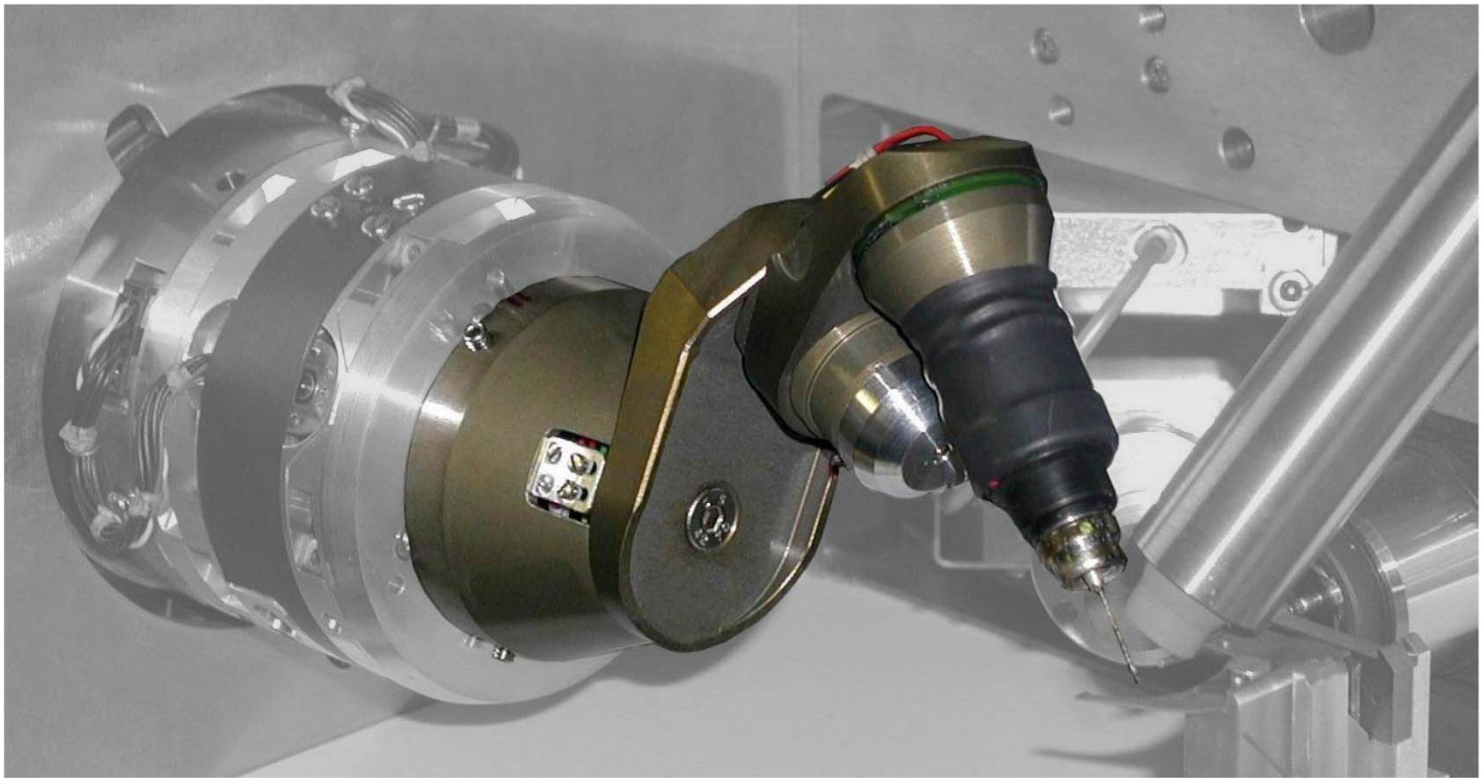
Diffractive Alignment DISTL Results

	DISTL Res	LABLEIT Res	Overloads	Total Spots	Good B Spots	In Res Spots	Max Signal	Mean Signal	Min Signal
31	10.0000	0.0000	0	4	0	2	0.0000	0.0000	0.0000
32	4.5500	0.0000	0	19	17	17	28991.	7718.1	704.50
33	5.9600	4.9000	0	39	26	26	0.23215E+06	35700.	511.00
34	4.2200	4.9000	0	69	42	43	0.15329E+06	16748.	147.30
35	4.0800	4.9000	0	43	19	19	25276.	7086.0	378.00
36	10.0000	0.0000	0	5	3	3	847.30	548.40	208.70

EXIT PLOT MOVE TO SAVE FRAME DATA

Improved Sample Stability & Goniometry

Opportunities Arising from Vertically Polarized Beam



Current Horizontally Oriented MD2 Kappa Stage: 10-20 micron SOC

Vertically Oriented Kappa Goniometry to Minimize Gravitational Sag

Arinax MD3

SOC < 0.6 micron

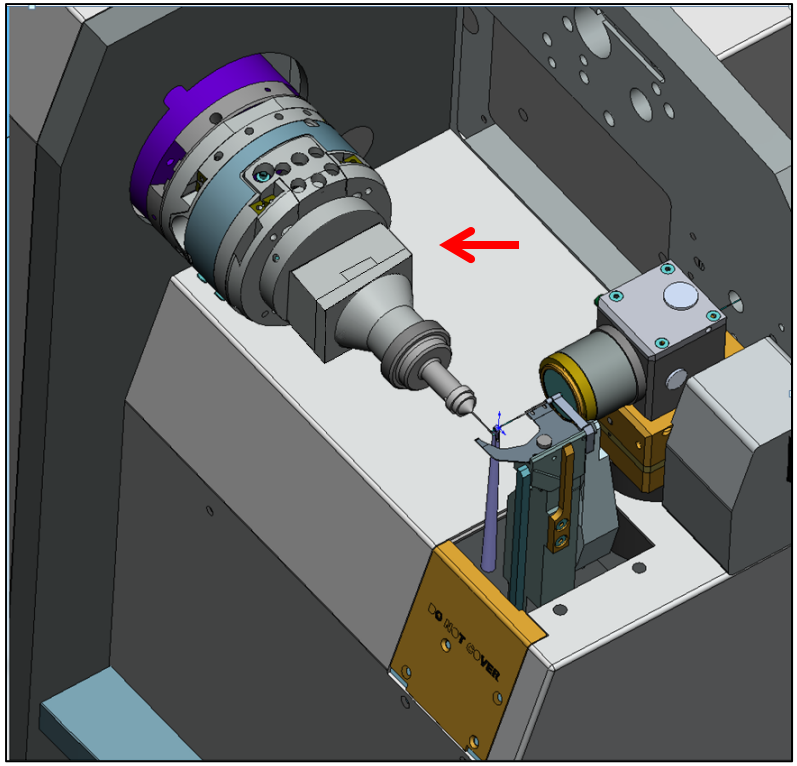
“Lorentz Axis” still aligned
With omeg axis.

Kinder, Gentler Robots!

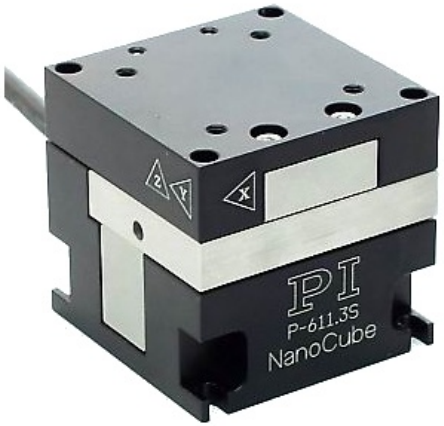
Improved stability and accuracy of the MD2 spindle alignment system (E-line)

MD2 Centering Stage Mechanical Performance Metrics:

	X	Y	Z	
Precision:	1.5,	1.5,	1.0	micron
Reproducibility:	2.6,	2.6,	1.5	micron



PI Nanocube Nanopositioning Stage



Dimensions: 44 x 44 xx 44 mm
Travel: 100 x 100 x 100 micron
Resolution: 1 x 1 x 1 nm
Repeatability: < 10 nm
Mass: 320 g
Input conductor count: 9

Active-SOC Compensation?

- Use SOC measurements to correlate omega axis position and SOC excursions (amplitude).

- If SOC amplitude is a consistent and reproducible function of omega use PI nanocube to actively correct for SOC excursions in real-time (nanocube has a response time of a few milliseconds).

- Potential show stopper: sample vibration induced by nanocube corrections.

Improved positional sample stability

Objective measurements of real-time aerodynamic stability of sample mounts and beam position via high speed, high resolution videography (dark field, laser fluorescence).

Real-time aerodynamic stability measurements will provide engineering data for design and testing of more Stable loops.

Cryosystem optimizations using results of stability measurements

Operating parameters:

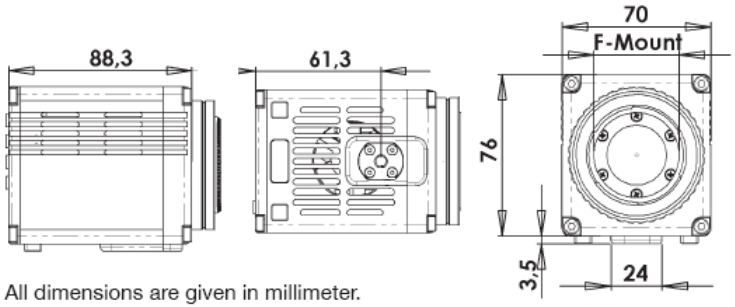
Flow Orientation, flow rates, external factors

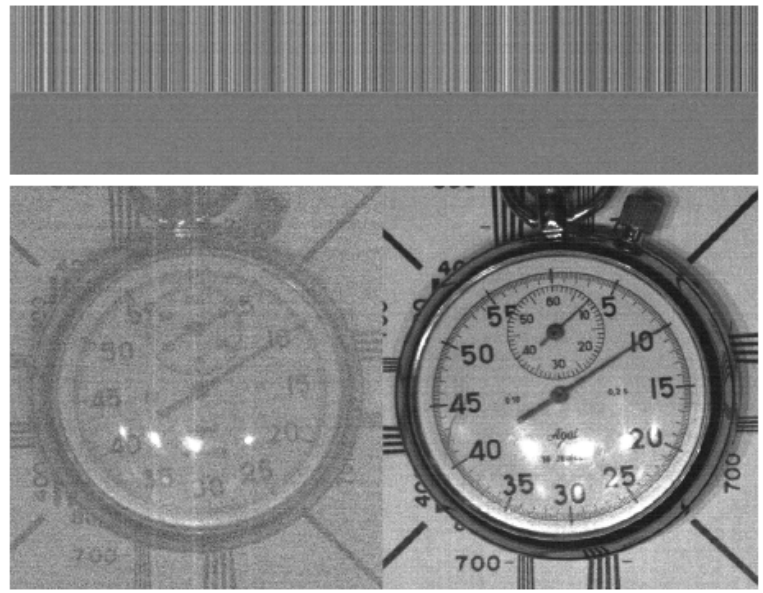
Measurement of beam position with millisecond time resolution and sub-micron spatial precision.

Potential problem: Discrimination against Schlieren effects induced by cryosystem.

Real –Time Sample and Beam Position Measurements

Improved Sample Visualization – Qualify Loop Aerodynamic Stability
Replace MD2's OVA CCD camera with High performance CMOS Sensor





Sensor Characteristics:

Max resolution (pixels)	2560 x 2160
Dynamic range	22000:1 (86.9 dB)
Dark current	2 – 6 e^- /sec @5°C

Available Frame Rates

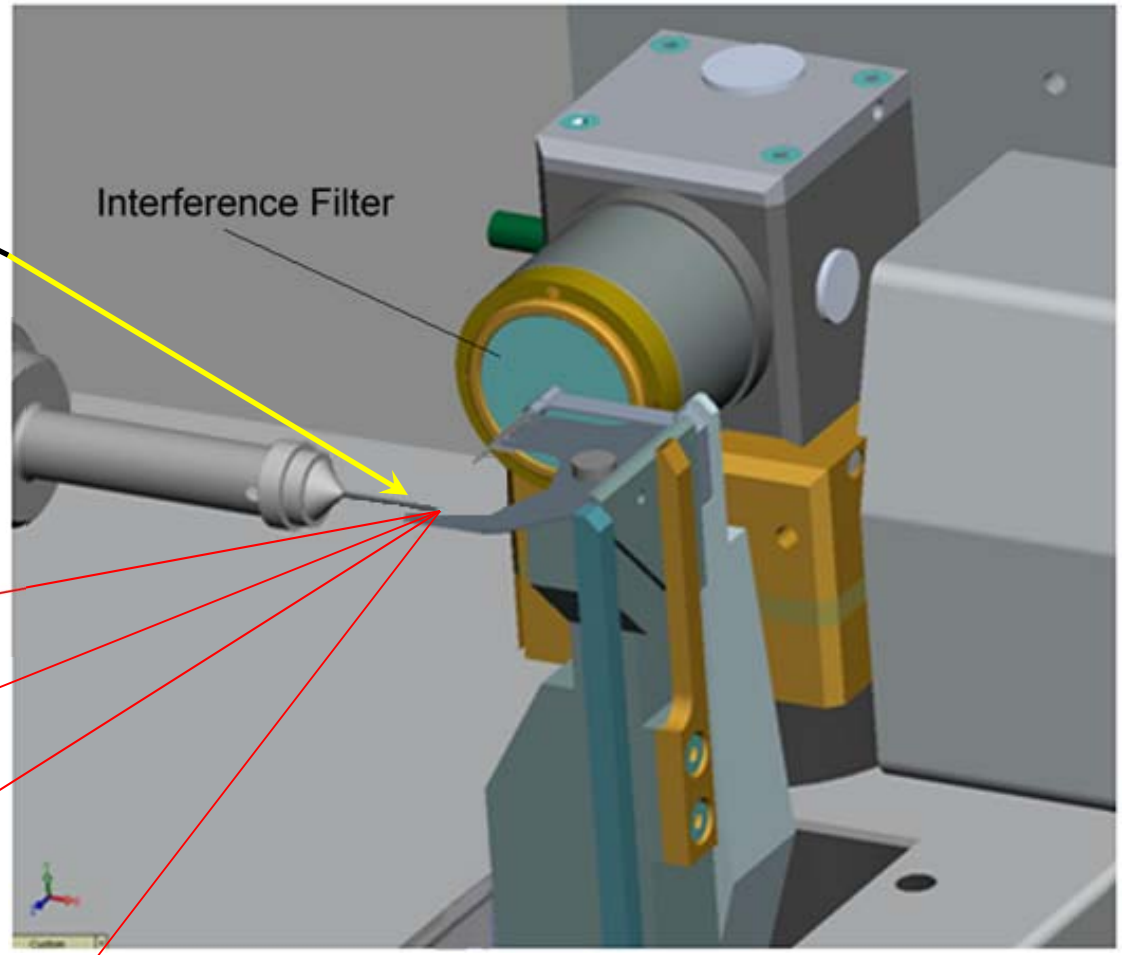
2560 x 2100	100 fps	(full frame)
1920 x 1080	200 fps	(ROI)
1280 x 1024	210 fps	(ROI)
640 x 480	450 fps	(ROI)
320 x 240	900 fps	(ROI)

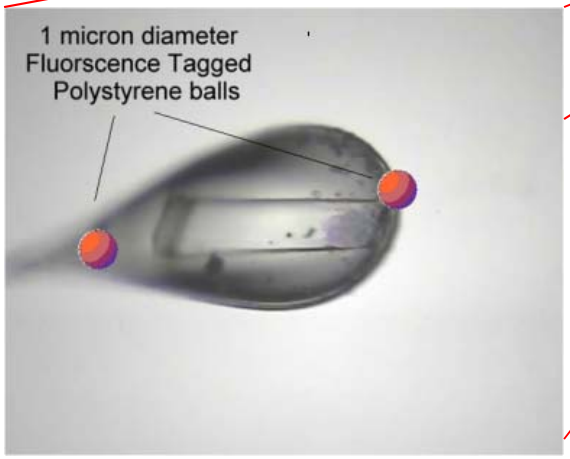
Real-time Sample Loop Position Fluctuation & Spindle Axis SOC Measurements

Fiber optic Excitation Source

Inplane motion ∝ Δ centroid

Outplane motion ∝ Δ Spatial Freq power spectrum (tag image focus)





SOC measurements use (rigid) pin-mounted fluorescent spheres (no cold stream).