Next Generation Opportunities:

From single particles to simultaneous spectroscopy/diffraction

Mike Bogan, PULSE Institute SLAC National Laboratory

U.S. DEPARTMENT OF ENERGY
Office of Science

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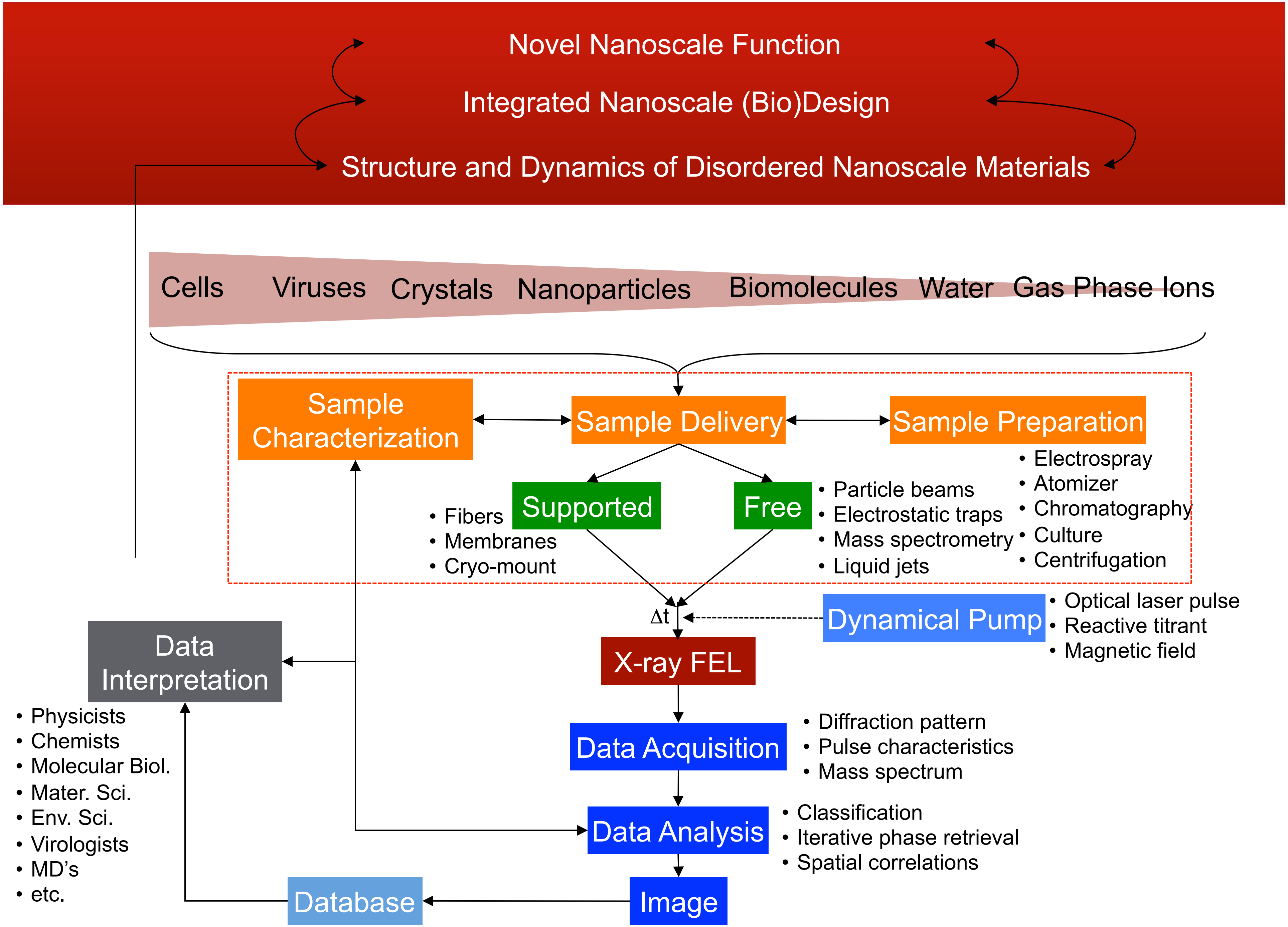
SLAC NATIONAL ACCELERATOR LABORATORY



Novel Nanoscale Function

Integrated Nanoscale (Bio)Design

Structure and Dynamics of Disordered Nanoscale Materials

Cells Viruses Crystals Nanoparticles Biomolecules Water Gas Phase Ions

Sample Characterization

Sample Delivery

Sample Preparation

- Electrospray
- Atomizer
- Chromatography
- Culture
- Centrifugation

Supported

- Fibers
- Membranes
- Cryo-mount

Free

- Particle beams
- Electrostatic traps
- Mass spectrometry
- Liquid jets

Δt

Dynamical Pump

- Optical laser pulse
- Reactive titrant
- Magnetic field

X-ray FEL

Data Acquisition

- Diffraction pattern
- Pulse characteristics
- Mass spectrum

Data Analysis

- Classification
- Iterative phase retrieval
- Spatial correlations

Image

Database

Data Interpretation

- Physicists
- Chemists
- Molecular Biol.
- Mater. Sci.
- Env. Sci.
- Virologists
- MD’s
- etc.

Overview: serial femtosecond diffraction experiments

SLAC

Sample Heterogeneity:

Variety of targets:

Individual molecules

Biomolecular complexes

- Covalent & noncovalent
- DNA duplexes, protein-protein, protein-drug, etc.
- Biomolecule-nanoparticle

Viruses, carboxysomes

Bioengineered nanoparticles

- Biomolecular clusters

Intact cells (living)

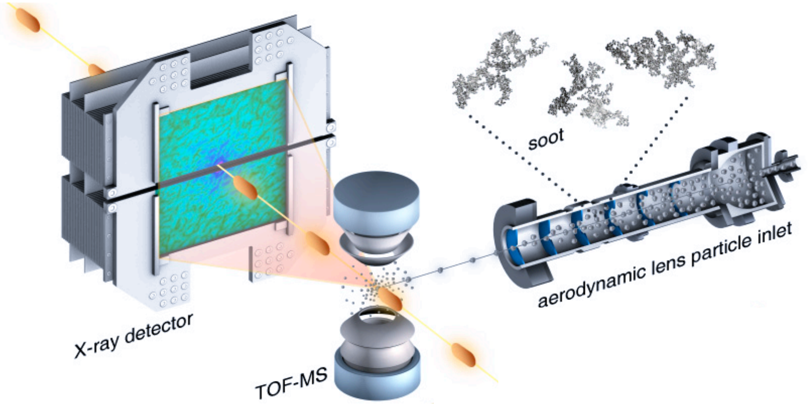
Class of Imaging Experiment:

- 2D single image of unique biotargets (i.e., cells):
  - Any conformation/orientation
- 3D biomolecule/virus reconstruction:
  - Identical molecules/viruses
  - Identical known conformation
  - Different orientations
- 4D pump-probe/reaction dynamics experiments

One "injector" cannot solve all problems

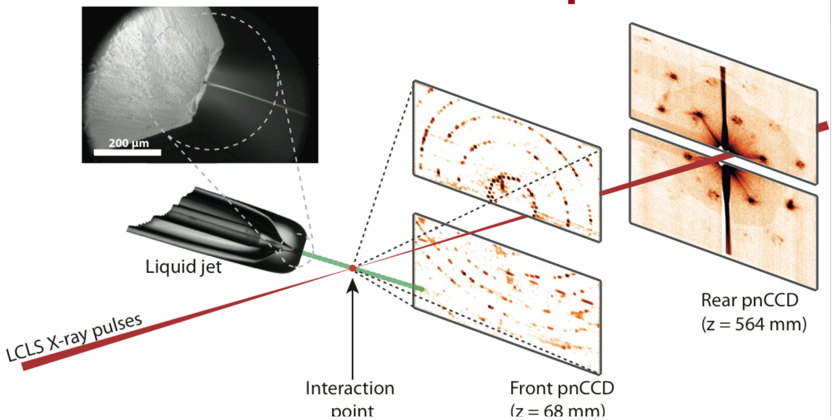
Proven Technology for Serial Femtosecond Diffraction

Gas-Focused Jet: Aerosols



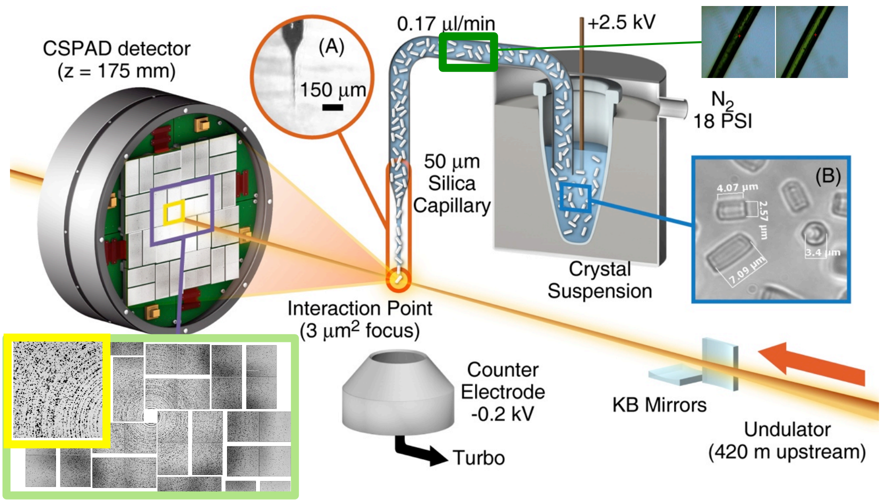
Loh, NTD *et al. Nature* **486**, 513-517 (2012).

Gas-Focused Jet: Liquid Beam



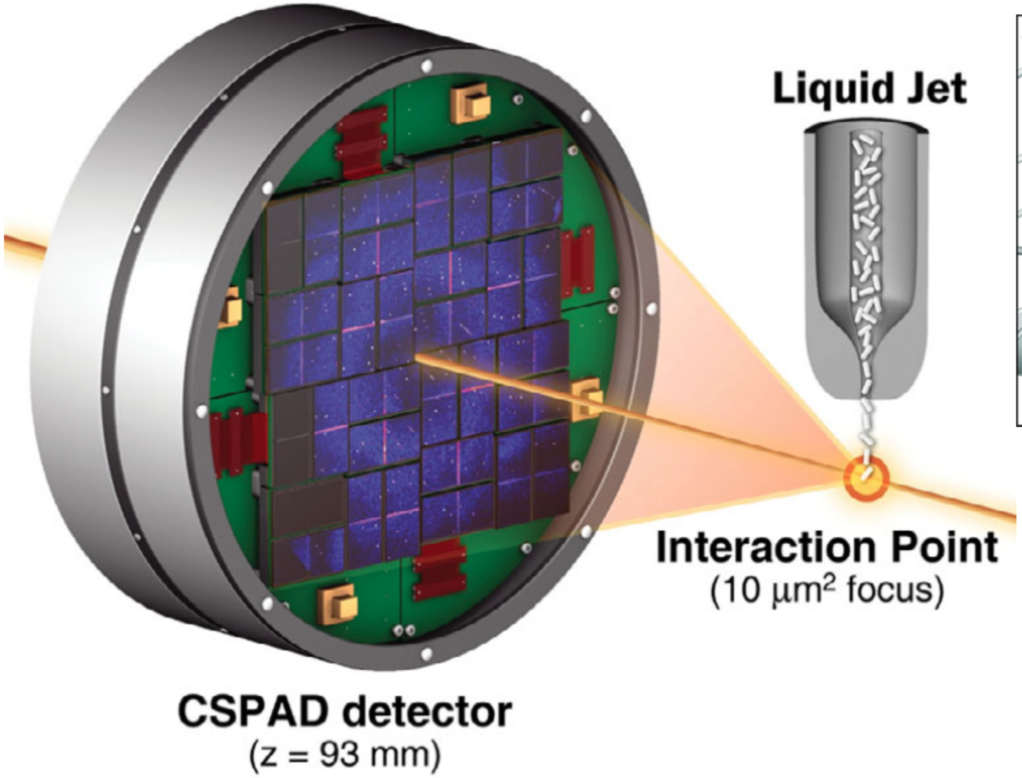
Chapman, *et al. Nature* **470**, 73-77 (2011).

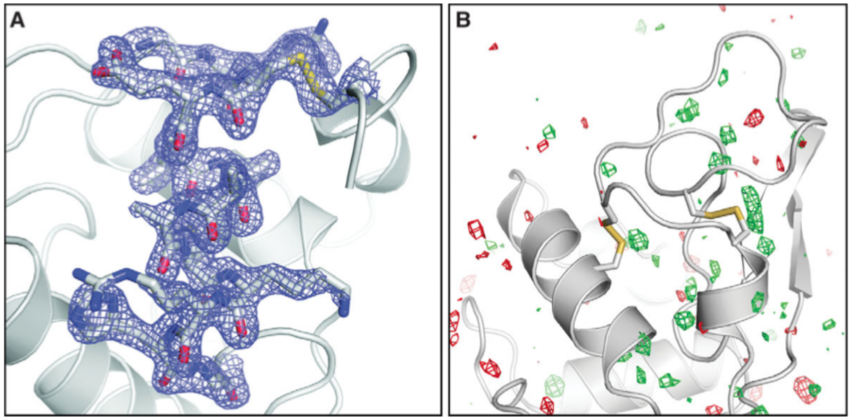
Electric-Field Focused Jet: Liquid Beam



Sierra, *et al.* (2012). *Acta Cryst D*, D68, 1584-1587.

Serial femtosecond crystallography at LCLS: SUCCESS!!





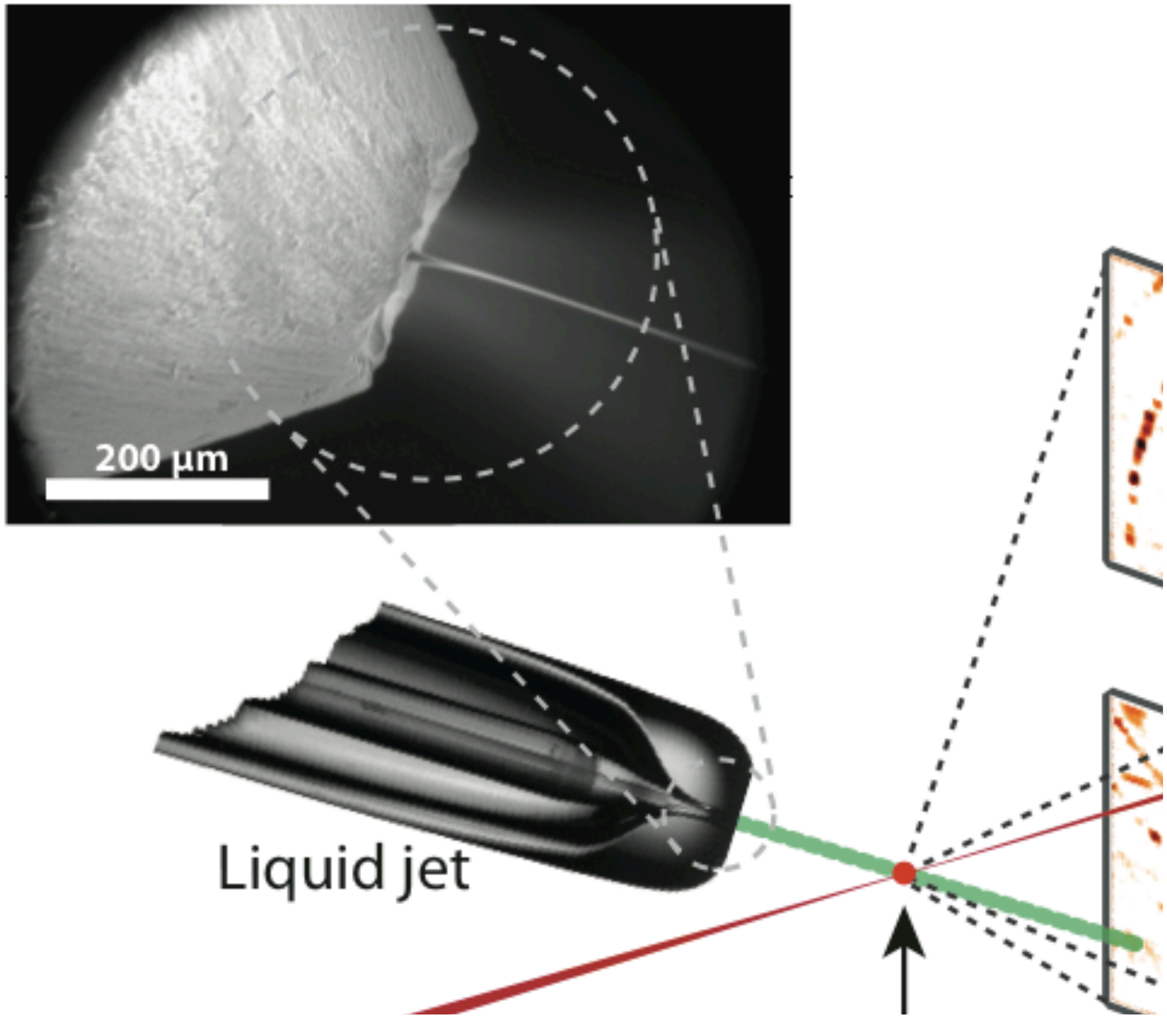
- 1.9 Angstrom structure
- no interpretable differences with synchrotron data
- SFX collected at room temperature
- HUGE opportunity for dynamic structural biology

Koopmann, *Nat Methods* 2012;Johansson *Nat Methods* 2012; Boutet, *Science* 2012; Aquila *Opt Exp* 2012; Lomb *Phys Rev B* 2011; Chapman *Nature* 2011; Barty *Nature Phot* 2011

Gas Focused: Liquid Beam

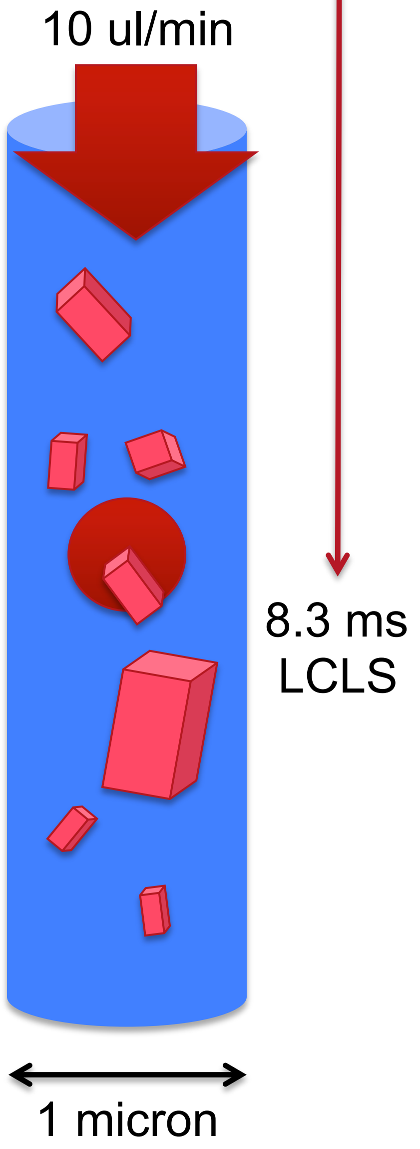
Developed by Arizona State University, based on Ganan-Calvo flow focusing
DePonte, D. P. *et al*. *J. Phys. D: Appl. Phys.* **41**, 195505 (2008)
Weierstall, Spence, and Doak, Review of Scientific Instruments 83 (3), 035108 (2012)

The Pioneering Liquid Jet for SFX: Gas Dynamic Virtual Nozzle (GDVN)



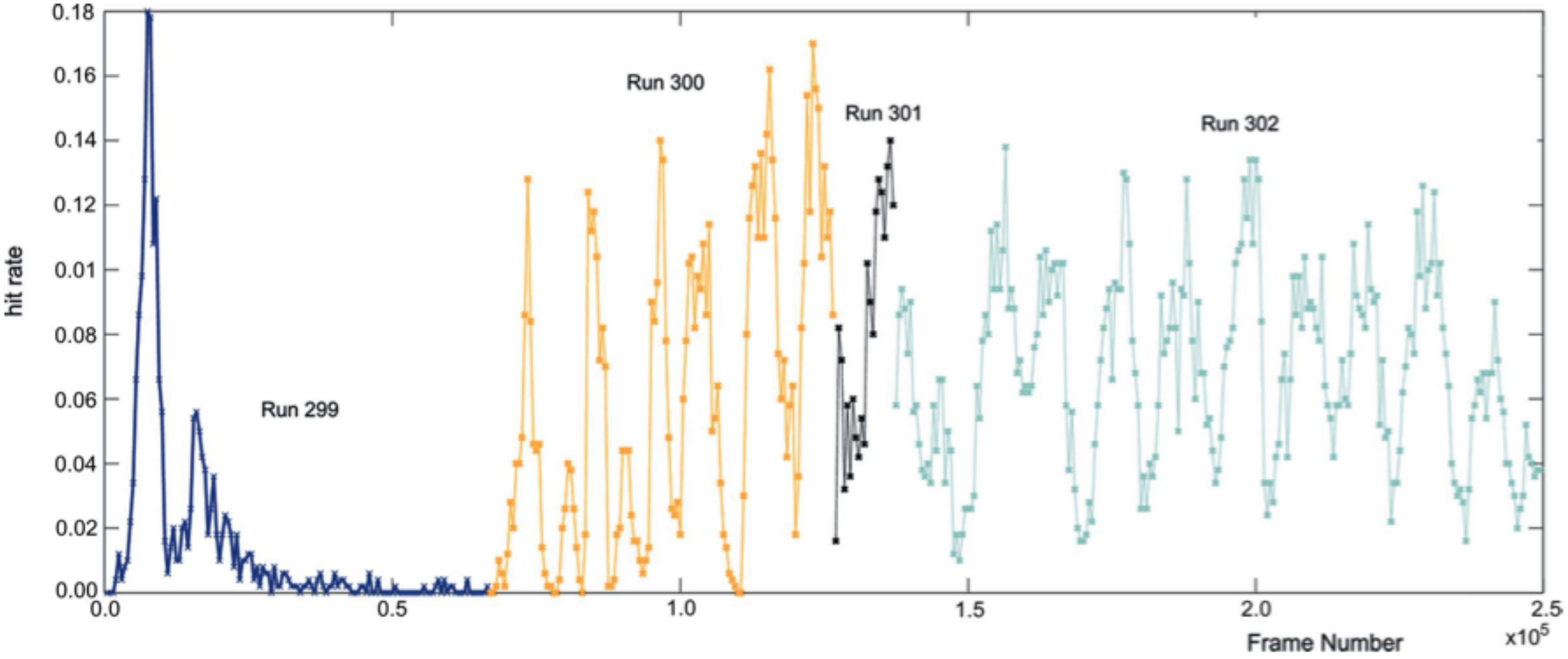
Chapman, H. *et al.* Femtosecond x-ray protein nanocrystallography. *Nature* **470**, 73-77 (2011)

What do the x-rays see?



Hit Rate = (number of particles per cm^{-3}) × (Interaction volume) × (XFEL repetition rate)

Must mitigate settling of sample

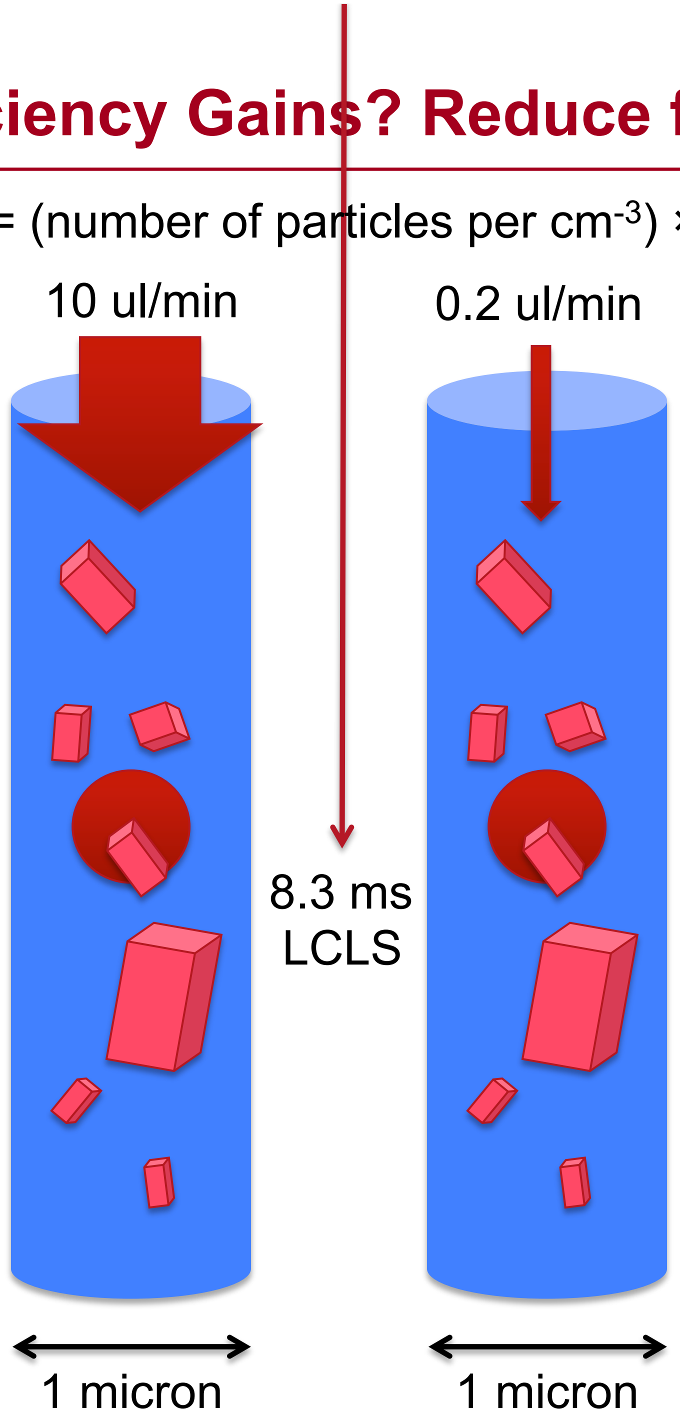


Use rotating syringe pump

Lomb, L., Steinbrener, J., Bari, S., Beisel, D., Berndt, D., Kieser, C., Lukat, M., Neef, N. & Shoeman, R. L. (2012). *J. Appl. Cryst.* **45**, 674-678.

Efficiency Gains? Reduce flow rate for same size jet

Hit Rate = (number of particles per cm^{-3}) × (Interaction volume) × (XFEL repetition rate)



<u>Reduce Sample Consumption?</u>

Slow down the flow rate feeding the same size jet

Cone-jet physics: Gas Flow Focusing vs. Electrospray

Gas-focused: Liquid Beam

PHYSICAL REVIEW E **79**, 066305 (2009)

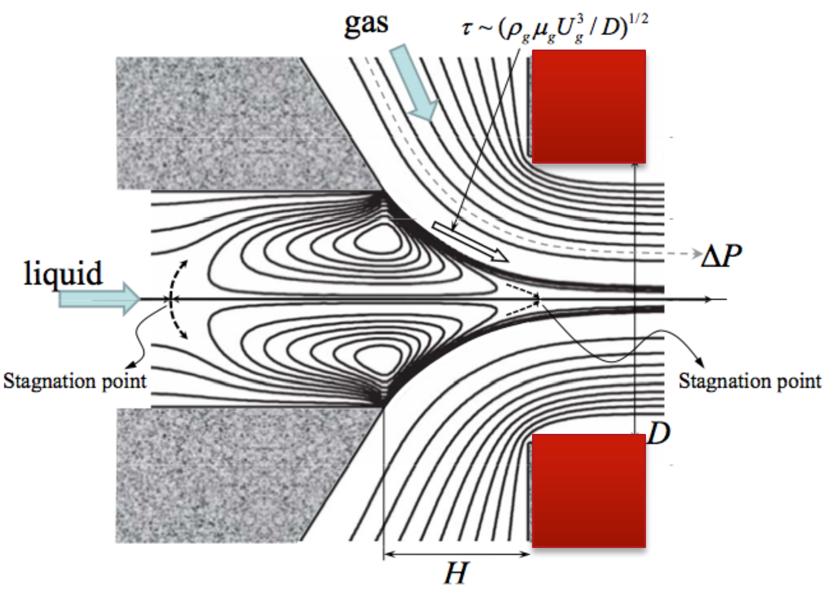


FIG. 1. (Color online) Typical flow pattern in FF. Streamlines of the liquid and gas phases [43].

Electric Field-Focused: Liquid Beam

ALFONSO M. GAÑÁN-CALVO AND JOSÉ M. MONTANERO

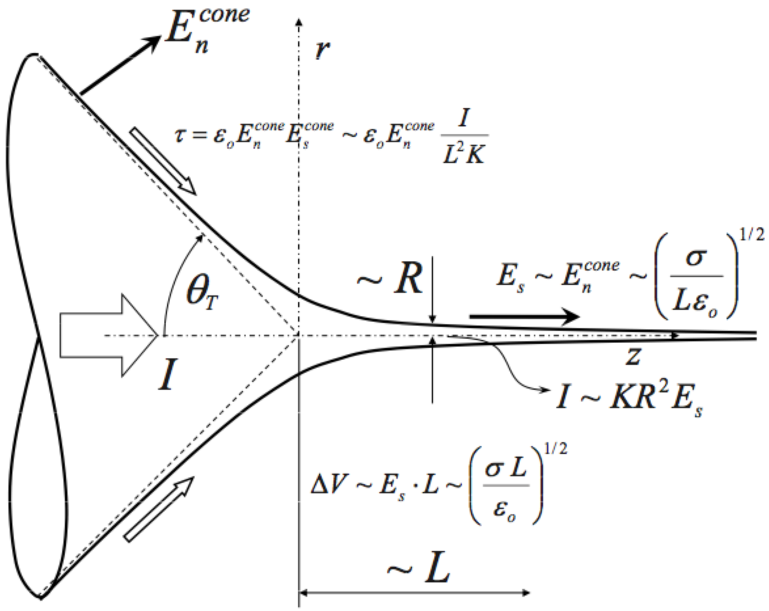
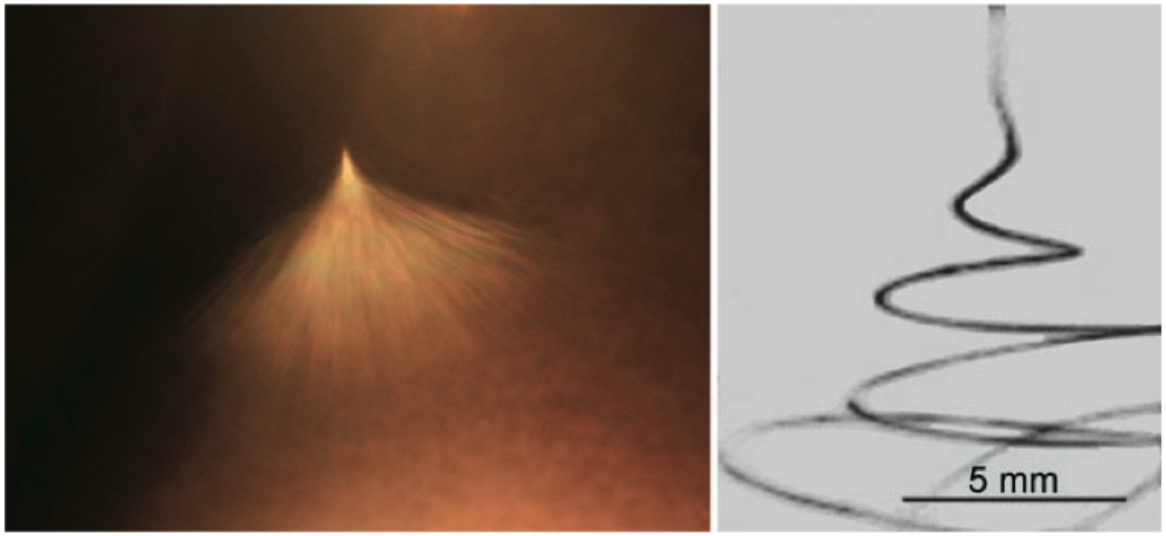
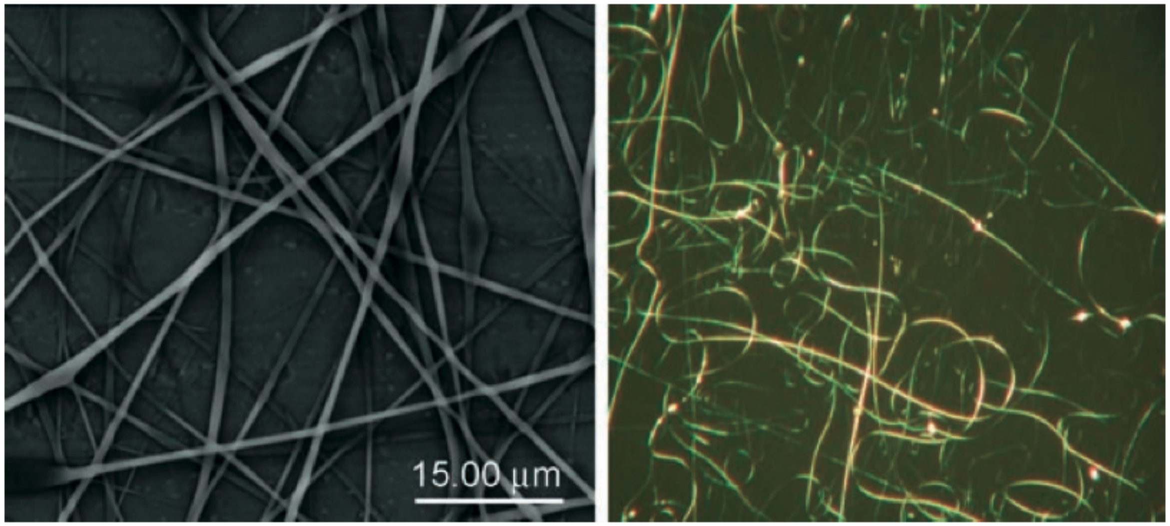


FIG. 2. Sketch of the Taylor cone-jet region. The solid angle θ_T represents the underlying Taylor's conical solution. The rest of the quantities are defined in the main text.

Use principle of electrospinning to elongate jet



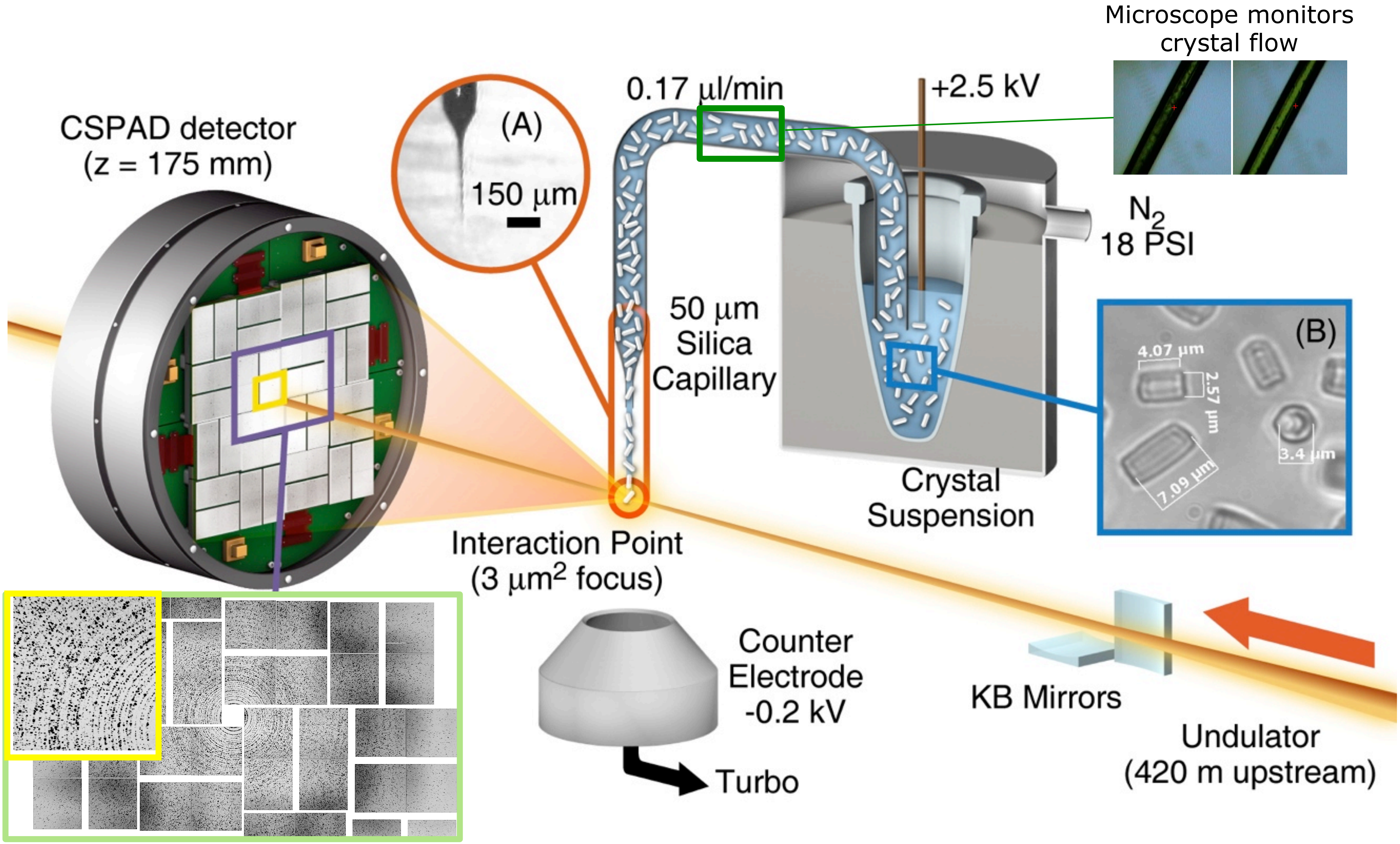
Normal (left) and high speed (right) photographs of a jet of PEO solution during electrospinning



Electrospun fibers of collagen and chitosan

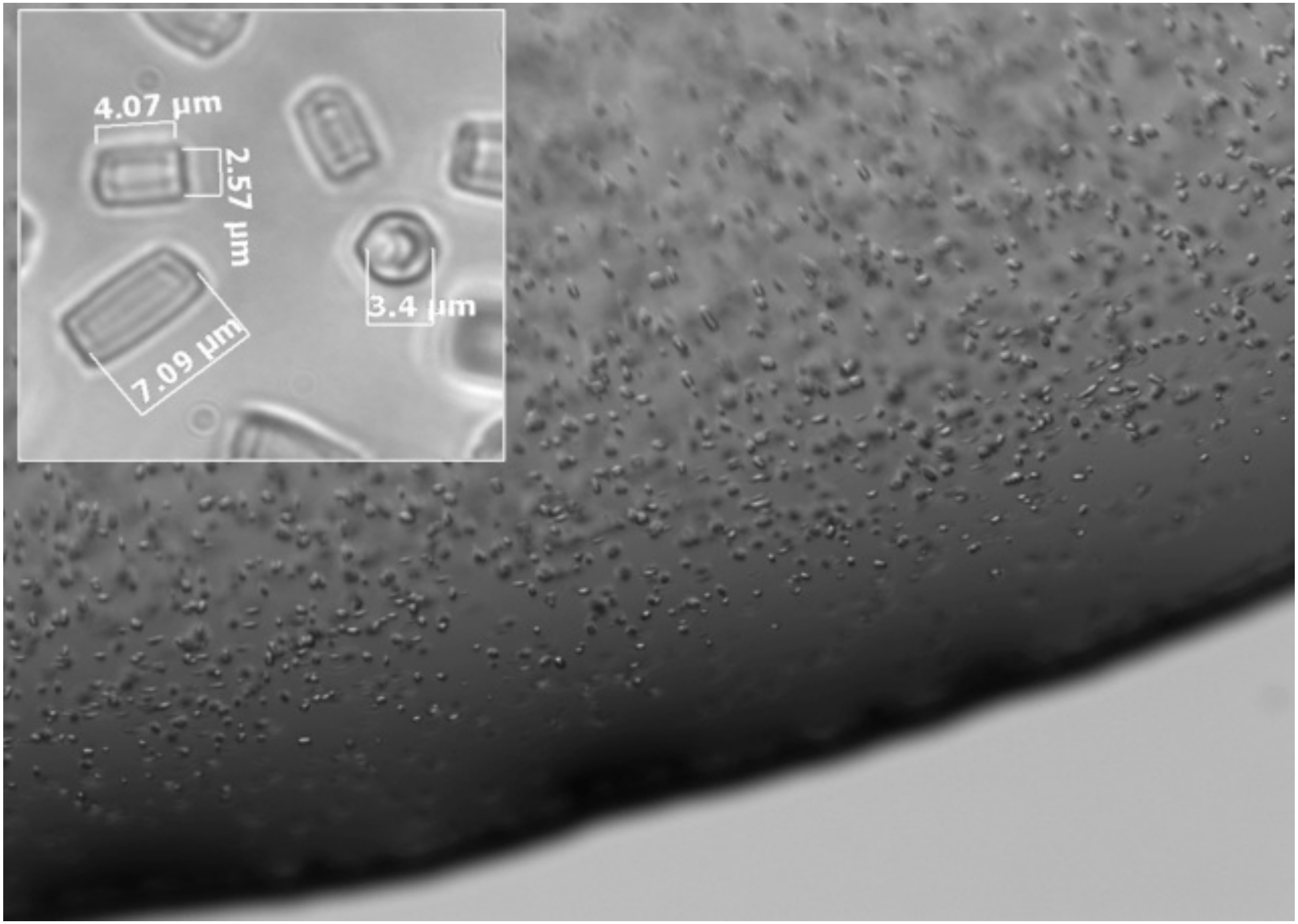
Andreas, G. & Wendorff, J. H. *Angewandte Chemie* **46**, 5670-5703 (2007).

Nanoflow electrospinning serial fs crystallography

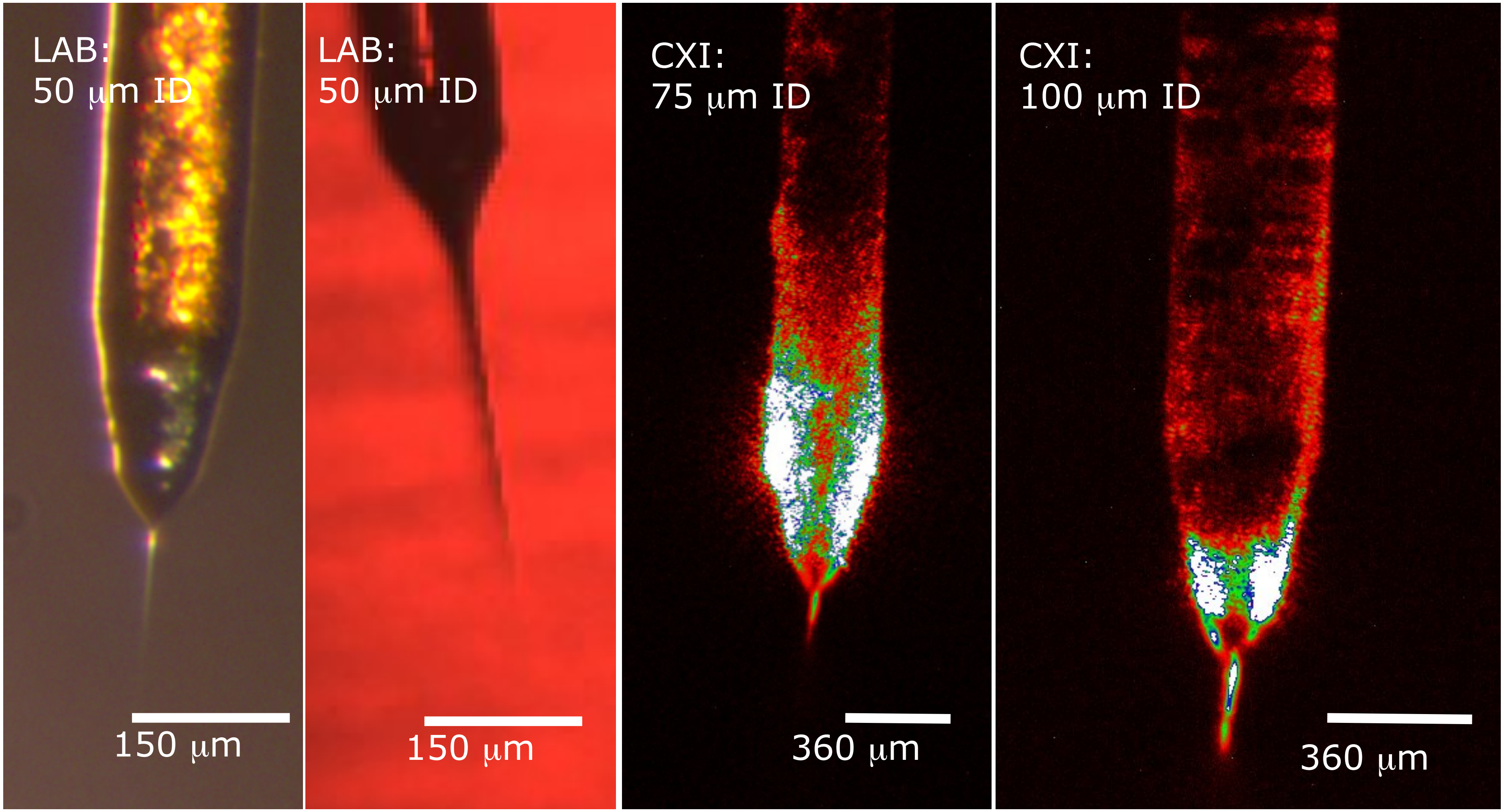


Sierra, RG; Laksmono, H; et al 2012, Acta Crystallographica D, D68, 1584-1587

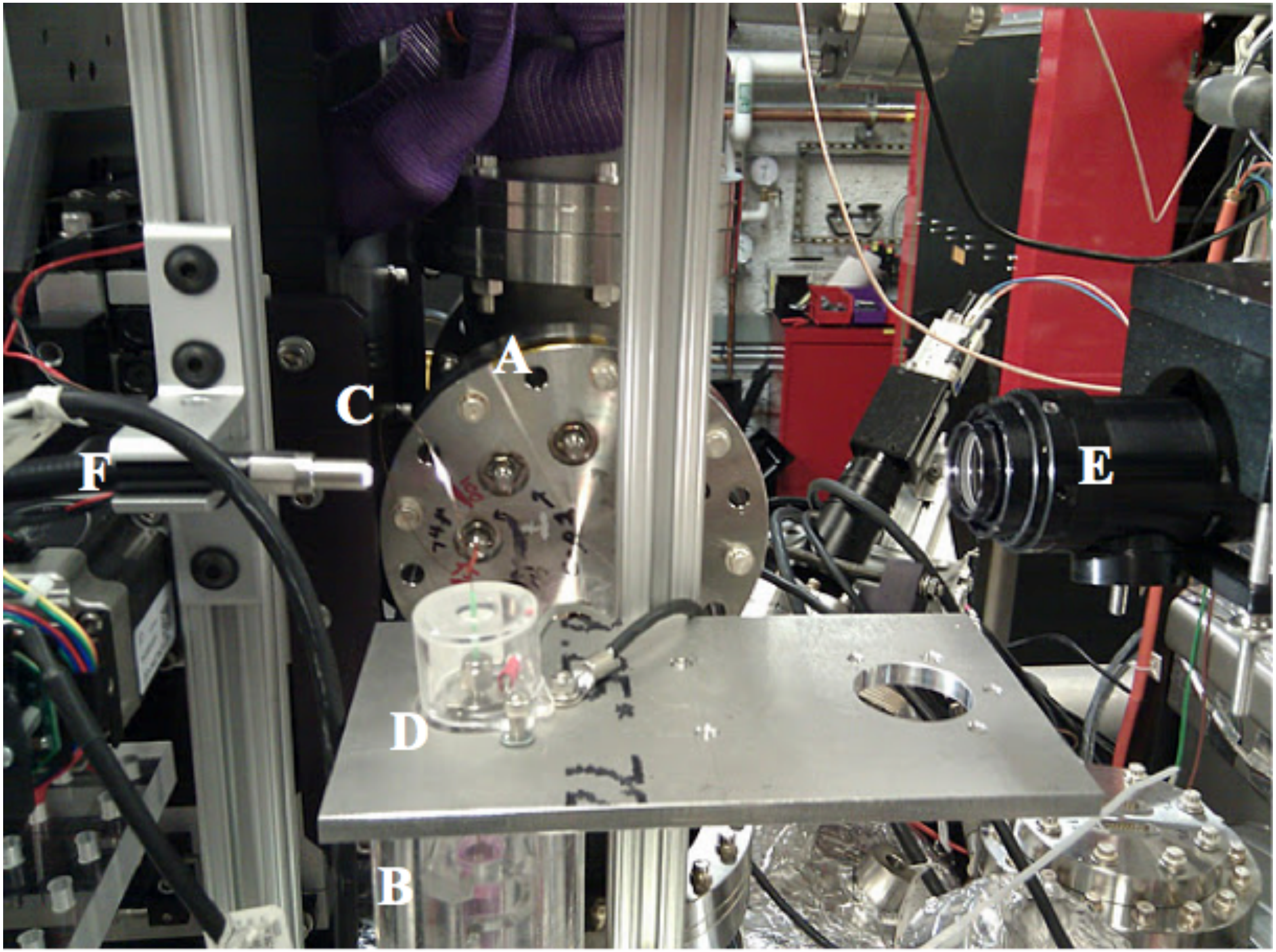
Thermolysin crystal slurry



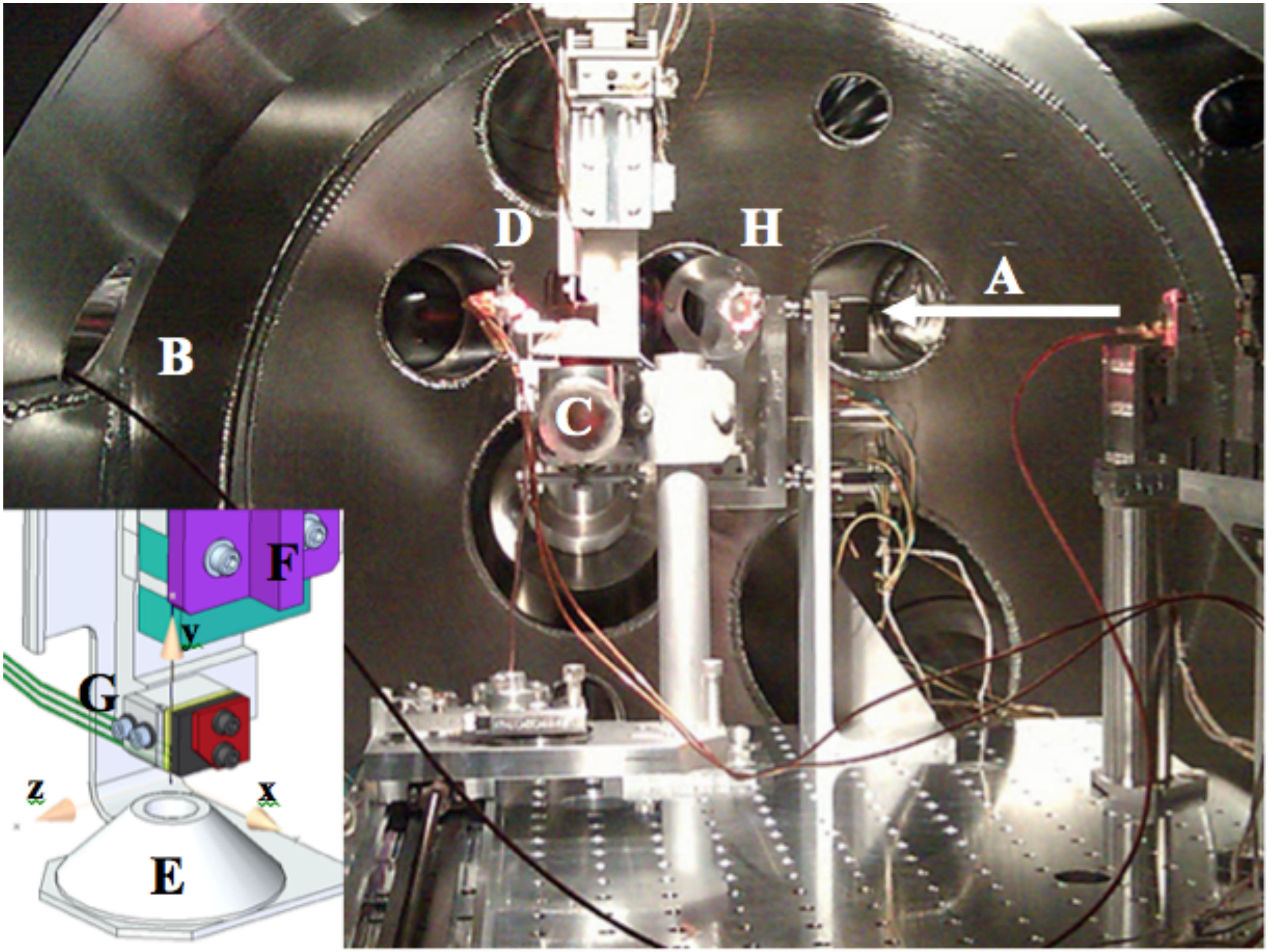
Images of electrospun jets of protein crystals



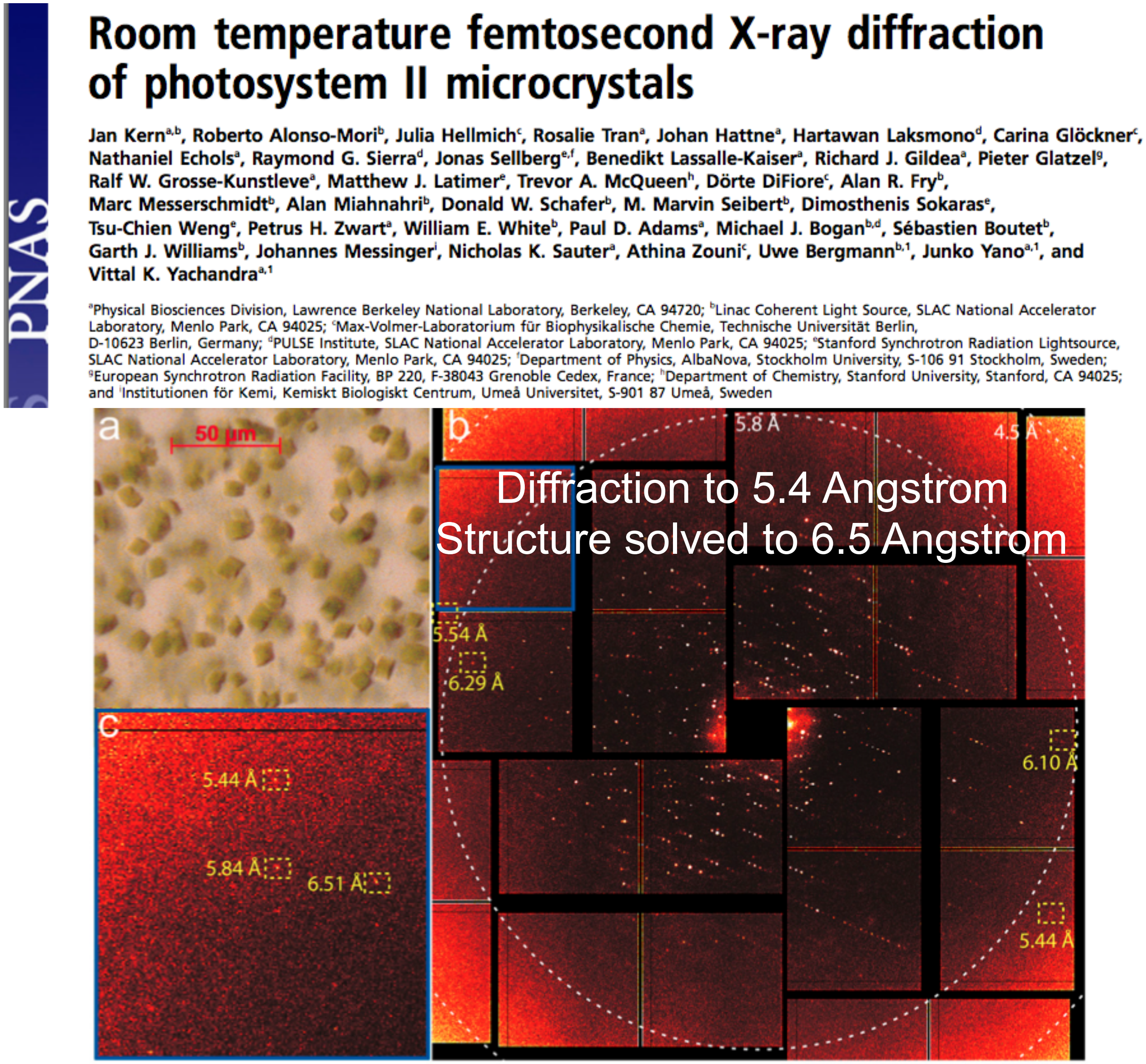
Experimental setup at LCLS CXI Endstation



Experimental setup at LCLS CXI Endstation



Microflow electrospinning SFX of Photosystem II



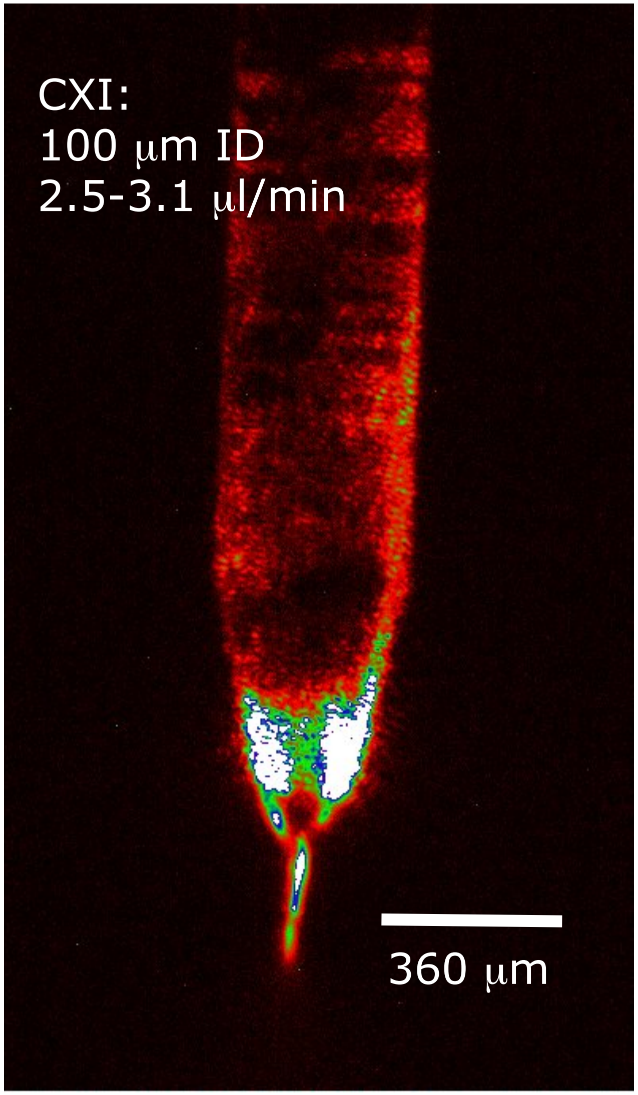
PNAS

Room temperature femtosecond X-ray diffraction of photosystem II microcrystals

Jan Kern[a,b], Roberto Alonso-Mori[b], Julia Hellmich[c], Rosalie Tran[a], Johan Hattne[a], Hartawan Laksmono[d], Carina Glöckner[c], Nathaniel Echols[a], Raymond G. Sierra[d], Jonas Sellberg[e,f], Benedikt Lassalle-Kaiser[a], Richard J. Gildea[a], Pieter Glatzel[g], Ralf W. Grosse-Kunstleve[a], Matthew J. Latimer[e], Trevor A. McQueen[h], Dörte DiFiore[c], Alan R. Fry[b], Marc Messerschmidt[b], Alan Miahnahri[b], Donald W. Schafer[b], M. Marvin Seibert[b], Dimosthenis Sokaras[e], Tsu-Chien Weng[e], Petrus H. Zwart[a], William E. White[b], Paul D. Adams[a], Michael J. Bogan[b,d], Sébastien Boutet[b], Garth J. Williams[b], Johannes Messinger[i], Nicholas K. Sauter[a], Athina Zouni[c], Uwe Bergmann[b,1], Junko Yano[a,1], and Vittal K. Yachandra[a,1]

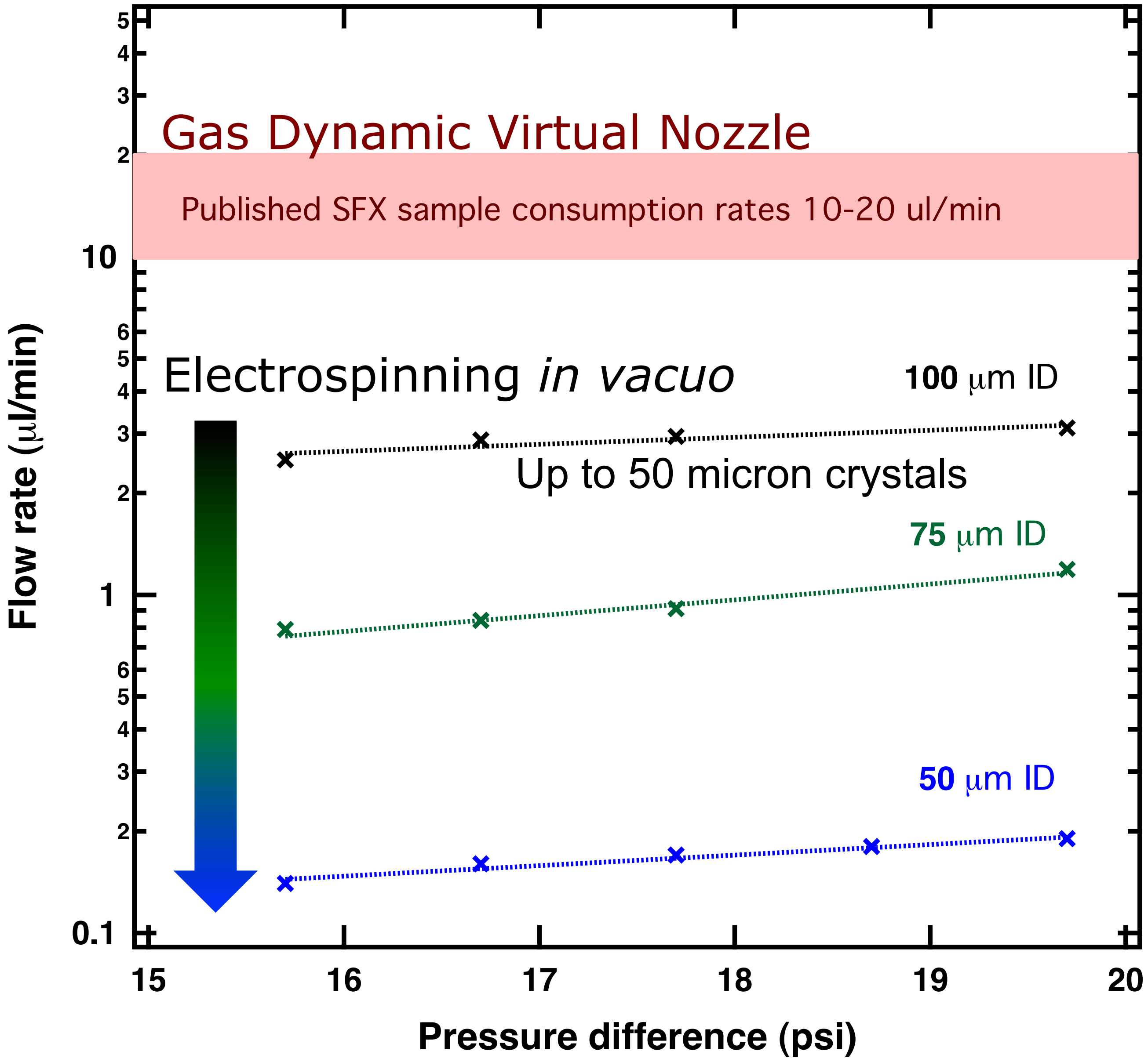
[a]Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720; [b]Linac Coherent Light Source, SLAC National Accelerator Laboratory, Menlo Park, CA 94025; [c]Max-Volmer-Laboratorium für Biophysikalische Chemie, Technische Universität Berlin, D-10623 Berlin, Germany; [d]PULSE Institute, SLAC National Accelerator Laboratory, Menlo Park, CA 94025; [e]Stanford Synchrotron Radiation Lightsource, SLAC National Accelerator Laboratory, Menlo Park, CA 94025; [f]Department of Physics, AlbaNova, Stockholm University, S-106 91 Stockholm, Sweden; [g]European Synchrotron Radiation Facility, BP 220, F-38043 Grenoble Cedex, France; [h]Department of Chemistry, Stanford University, Stanford, CA 94025; and [i]Institutionen för Kemi, Kemiskt Biologiskt Centrum, Umeå Universitet, S-901 87 Umeå, Sweden

Diffraction to 5.4 Angstrom
Structure solved to 6.5 Angstrom



CXI:
100 μm ID
2.5-3.1 μl/min

Sample consumption rates for SFX at LCLS

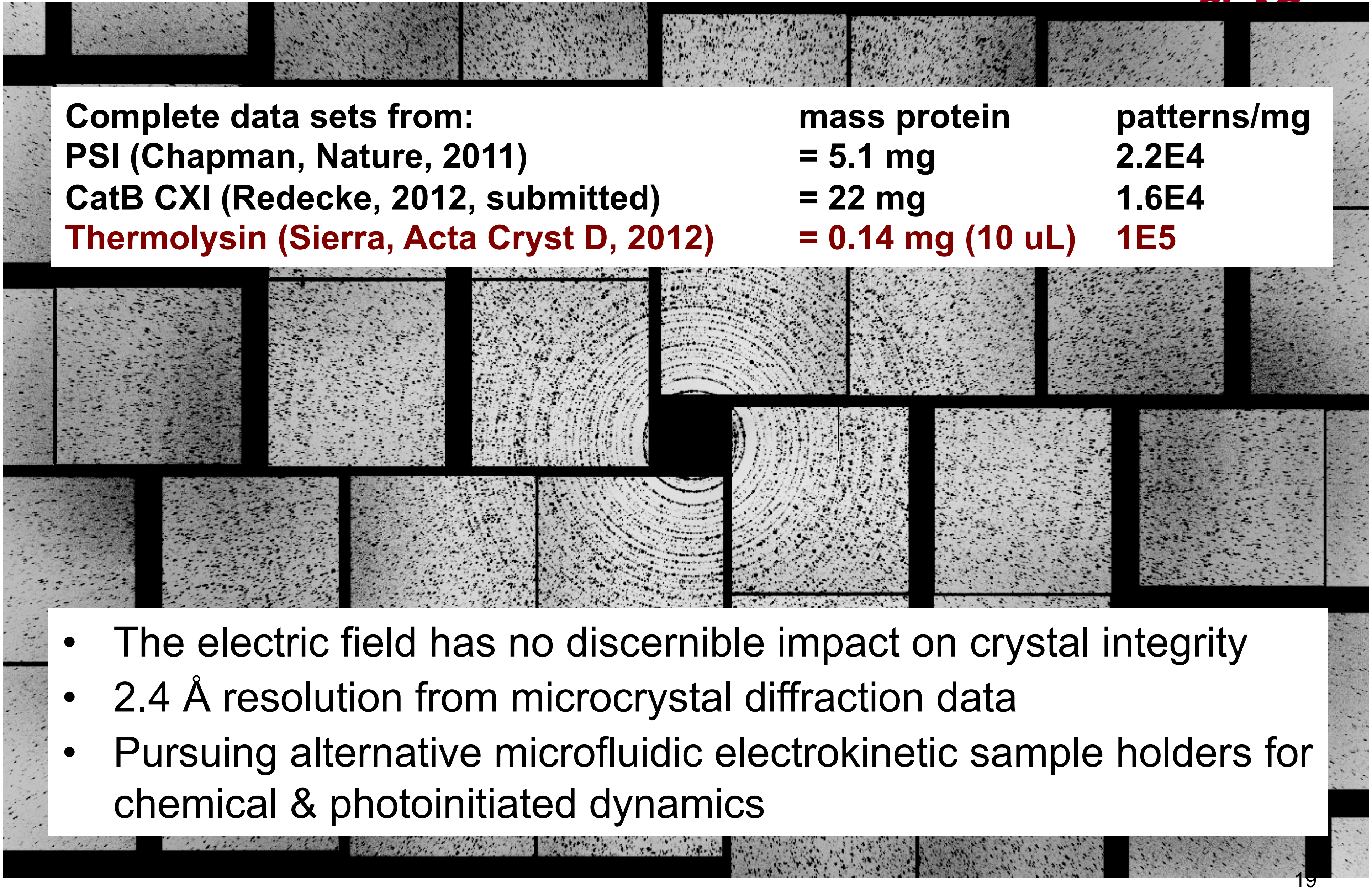


Microflow Regime:
Koopmann *Nat Methods* 2012
Johansson *Nat Methods* 2012
Boutet, *Science* 2012
Aquila *Opt Exp* 2012
Lomb *Phys Rev B* 2011
Chapman *Nature* 2011
Barty *Nature Phot* 2011

Low Microflow Regime:
Kern, J. *et al. PNAS* **109**, 9721-9726, (2012).

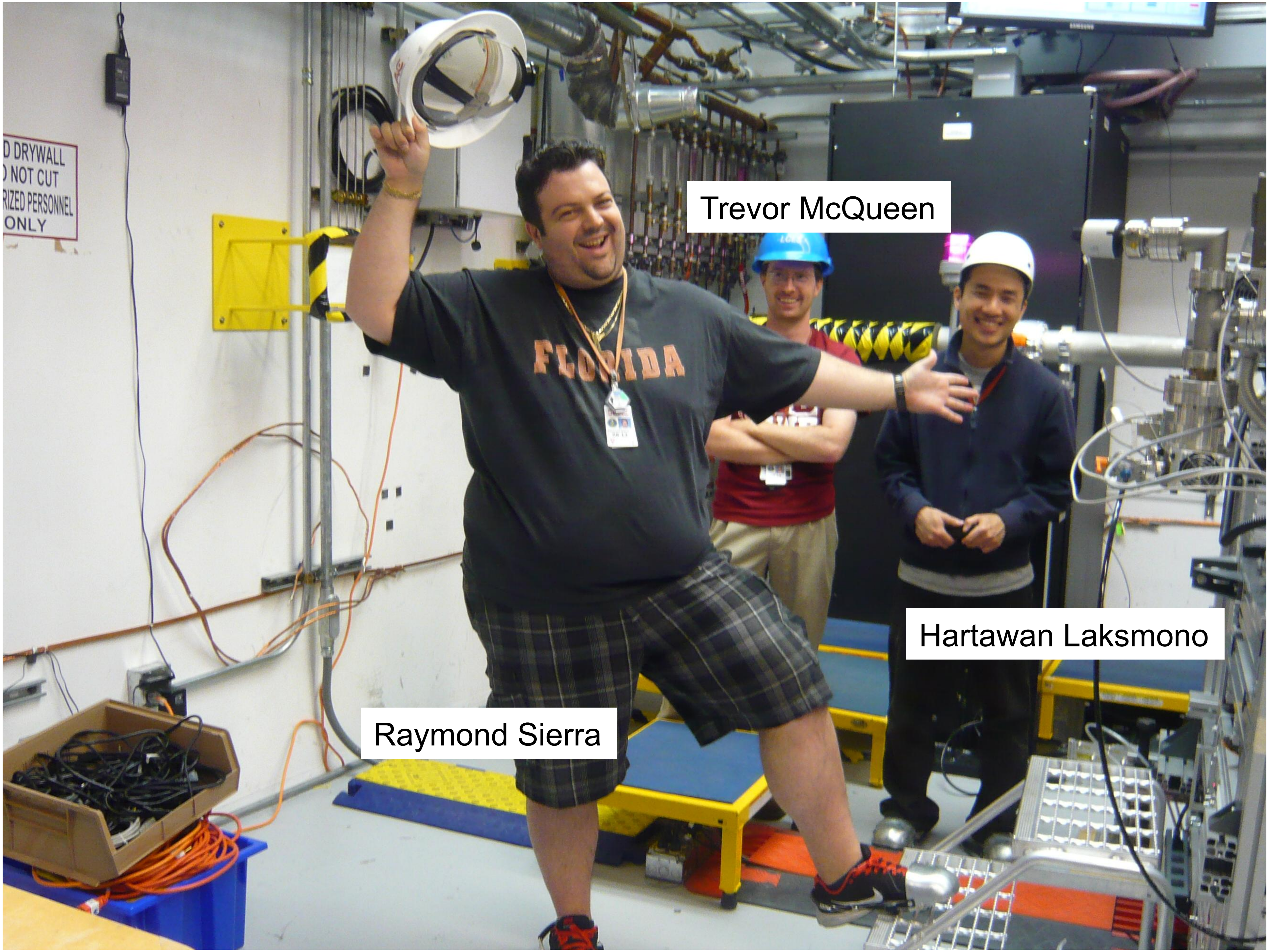
Nanoflow Regime:
THIS WORK
Sierra, R. G. et al. Acta Crystallographica D, 2012 D68, 1584-1587

Virtual powder pattern: 1024 single shots, single crystals

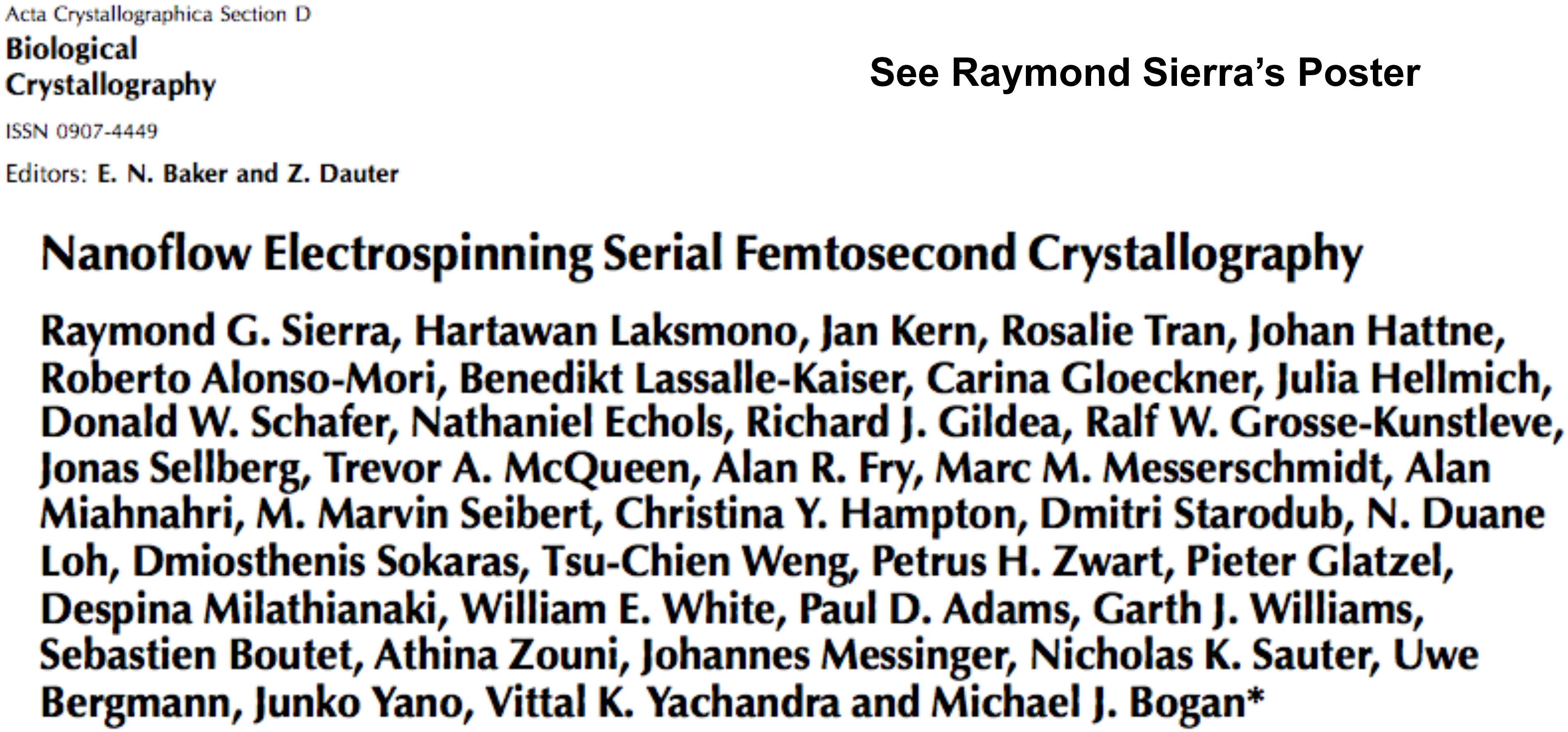


Complete data sets from:	mass protein	patterns/mg
PSI (Chapman, Nature, 2011)	= 5.1 mg	2.2E4
CatB CXI (Redecke, 2012, submitted)	= 22 mg	1.6E4
Thermolysin (Sierra, Acta Cryst D, 2012)	= 0.14 mg (10 uL)	1E5

- The electric field has no discernible impact on crystal integrity
- 2.4 Å resolution from microcrystal diffraction data
- Pursuing alternative microfluidic electrokinetic sample holders for chemical & photoinitiated dynamics


D DRYWALL
D NOT CUT
RIZED PERSONNEL
ONLY
Trevor McQueen
FLORIDA
Hartawan Laksmono
Raymond Sierra

See Raymond Sierra’s Poster



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Biological Crystallography
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Nanoflow Electrospinning Serial Femtosecond Crystallography

Raymond G. Sierra, Hartawan Laksmono, Jan Kern, Rosalie Tran, Johan Hattne, Roberto Alonso-Mori, Benedikt Lassalle-Kaiser, Carina Gloeckner, Julia Hellmich, Donald W. Schafer, Nathaniel Echols, Richard J. Gildea, Ralf W. Grosse-Kunstleve, Jonas Sellberg, Trevor A. McQueen, Alan R. Fry, Marc M. Messerschmidt, Alan Miahnahri, M. Marvin Seibert, Christina Y. Hampton, Dmitri Starodub, N. Duane Loh, Dmiosthenis Sokaras, Tsu-Chien Weng, Petrus H. Zwart, Pieter Glatzel, Despina Milathianaki, William E. White, Paul D. Adams, Garth J. Williams, Sebastien Boutet, Athina Zouni, Johannes Messinger, Nicholas K. Sauter, Uwe Bergmann, Junko Yano, Vittal K. Yachandra and Michael J. Bogan*

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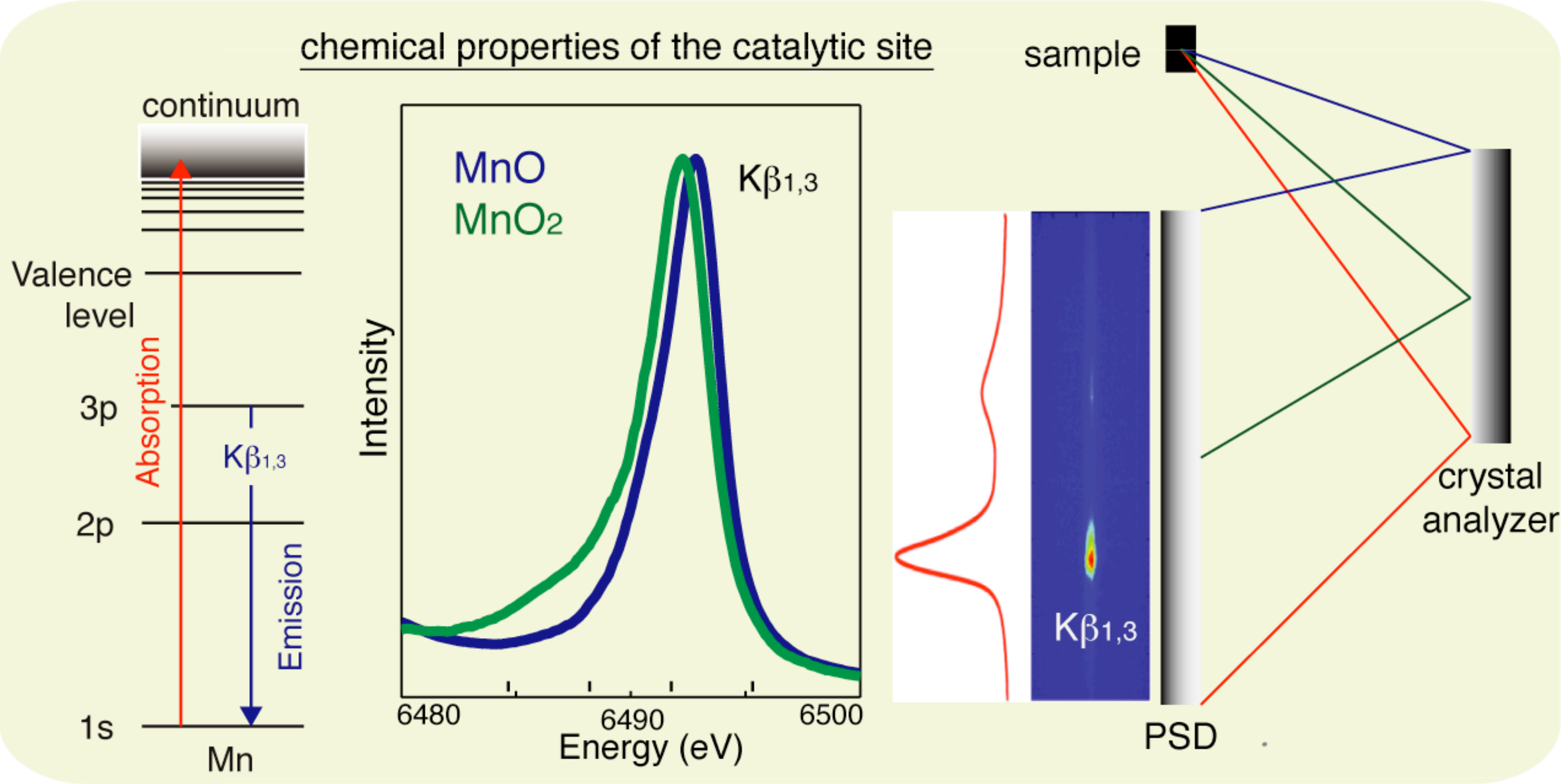
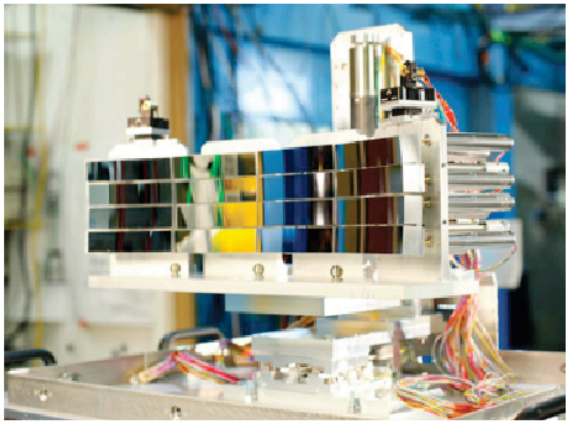
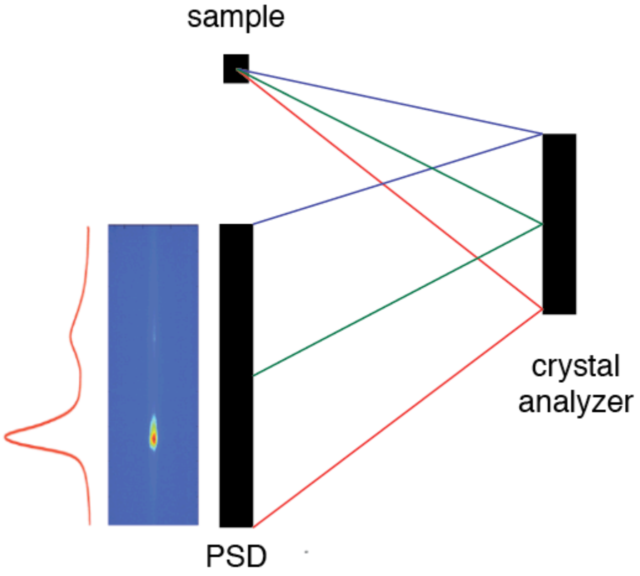
X-ray Emission Spectroscopy – Probe Metal Centers

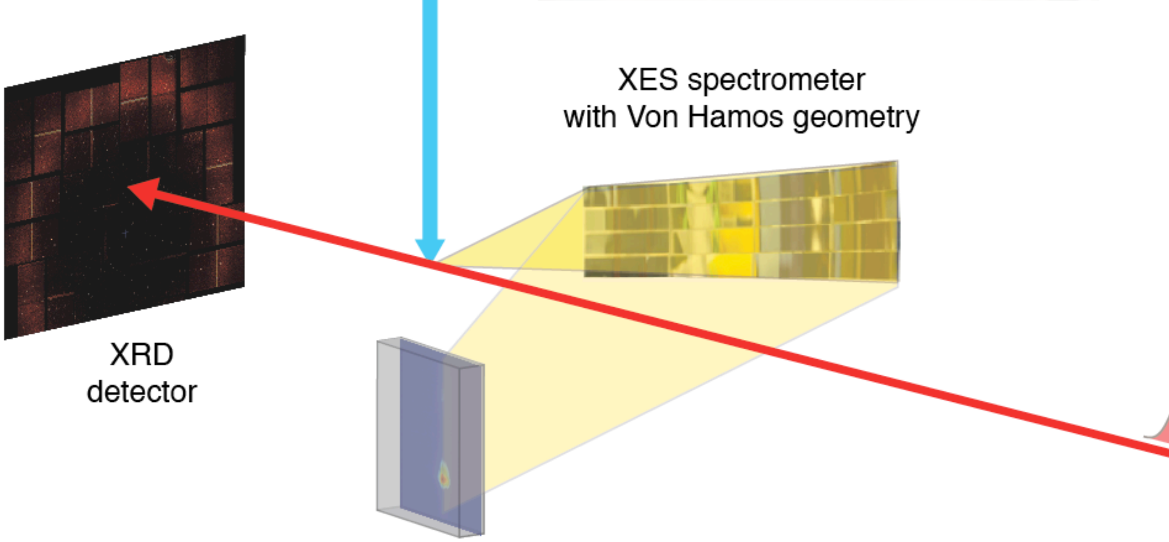


X-ray Emission Spectroscopy – Probe Metal Centers

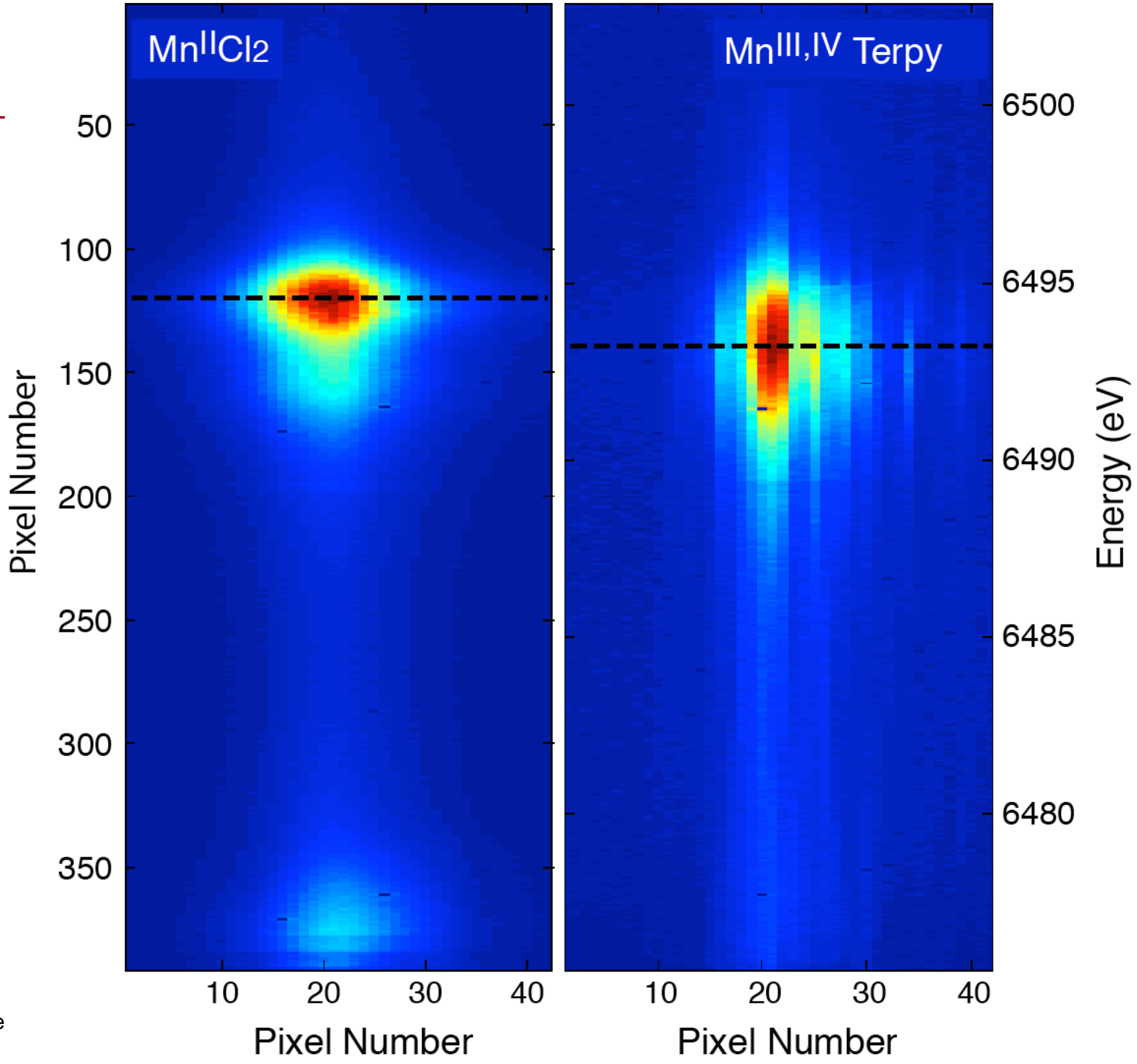
Energy-dispersive X-ray emission spectroscopy using an X-ray free-electron laser in a shot-by-shot mode







Alonso-Mori et al, PNAS, 2012, accepted


Mn^II Cl2
Mn^III,IV Terpy
Pixel Number
50
100
150
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Pixel Number
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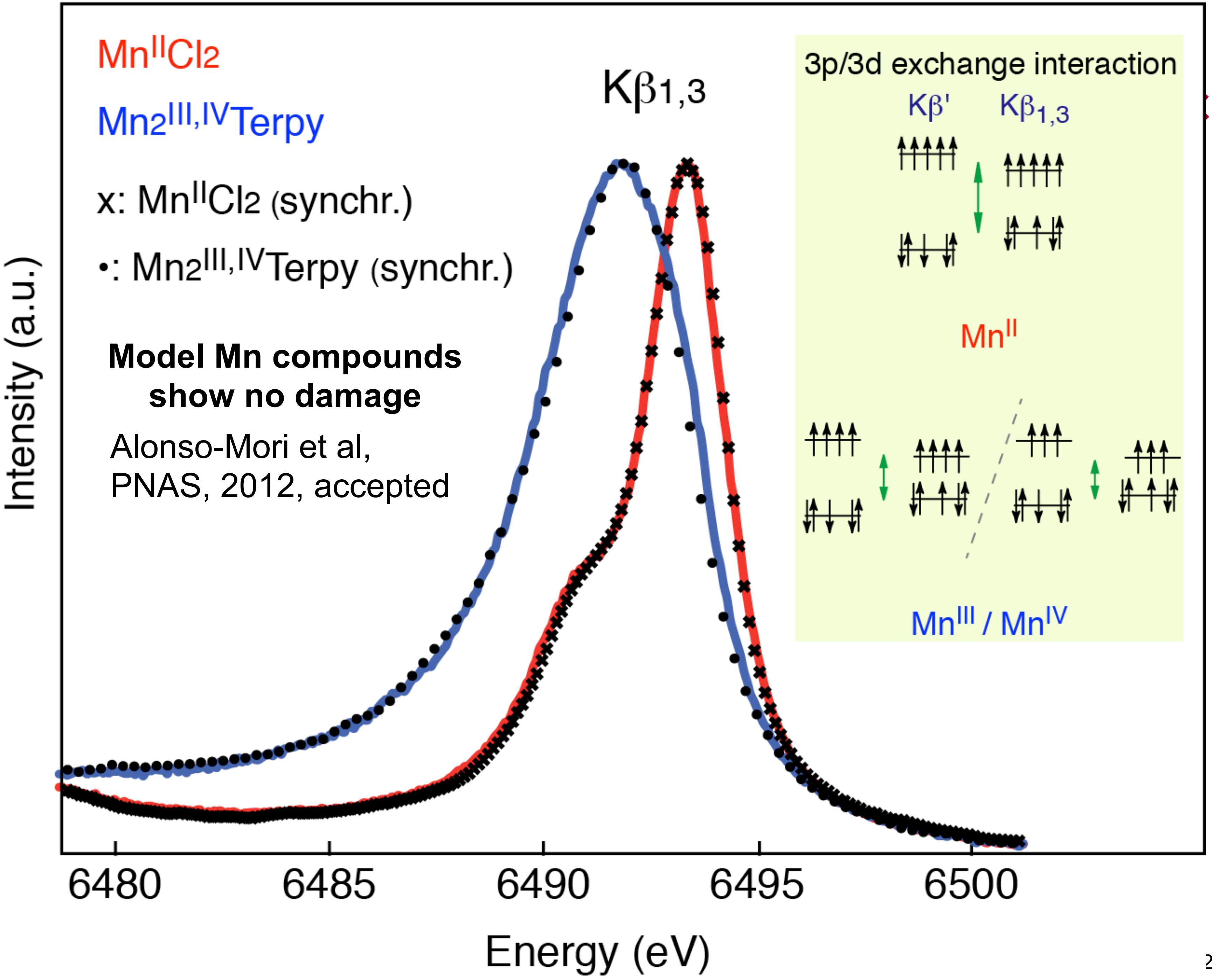
6495
6490
6485
6480
Energy (eV)

Insert Pre



Model Mn compounds show no damage

Alonso-Mori et al, PNAS, 2012, accepted

Comparison of ES jet, GDVN, Acoustic Droplets

Nanoflow Electrospinning: 14000 patterns from 10 ul in 58 minutes

- 170 nl/min flow rate for 58 minutes, 8um filtered crystals
- 140 ug protein required

Acoustic droplet dispensing: 14000 patterns from 35 ul in 2 minutes (UNTESTED)

- Droplet size = 2.5 nl = 17*5 um diameter* (Soares Biochemistry, 2011, 50, 4399)
- 2.5 nl per drop at 120 Hz (LCLS rep rate) = 18 ul/min
- 14000 diffraction patterns = 14000 drops = 35000 nl, 35 ul
  - Assuming every drop has a crystal that is hit
- 490 ug protein required
- Collection time = 2 min
- Huge solvent scattering background issues may preclude Bragg detection

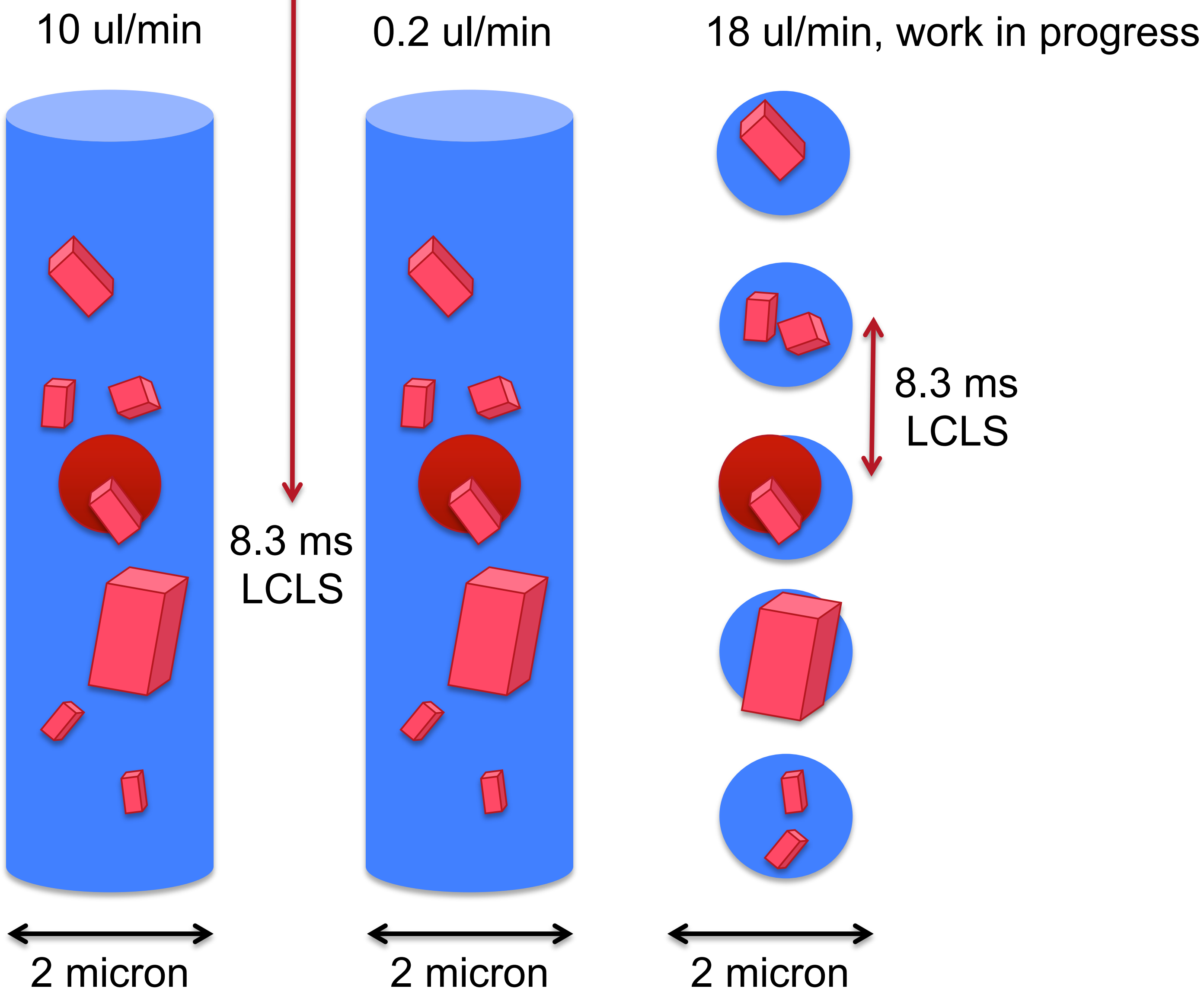
What if you wanted to deliver 10um crystals?

- 10 micron diameter droplet = 0.5pl,
- Need to reduce droplet size by 3-4 orders of magnitude, research in progress

Gas Dynamic Virtual Nozzle: 14000 patterns from 580 ul in 58 minutes

- 10 ul/min flow rate for 58 minutes
- Assume same jet size and solution used for ES jet above
- 8100 ug protein required

Need to increase efficiency



10 ul/min

0.2 ul/min

18 ul/min, work in progress

8.3 ms
LCLS

8.3 ms
LCLS

2 micron

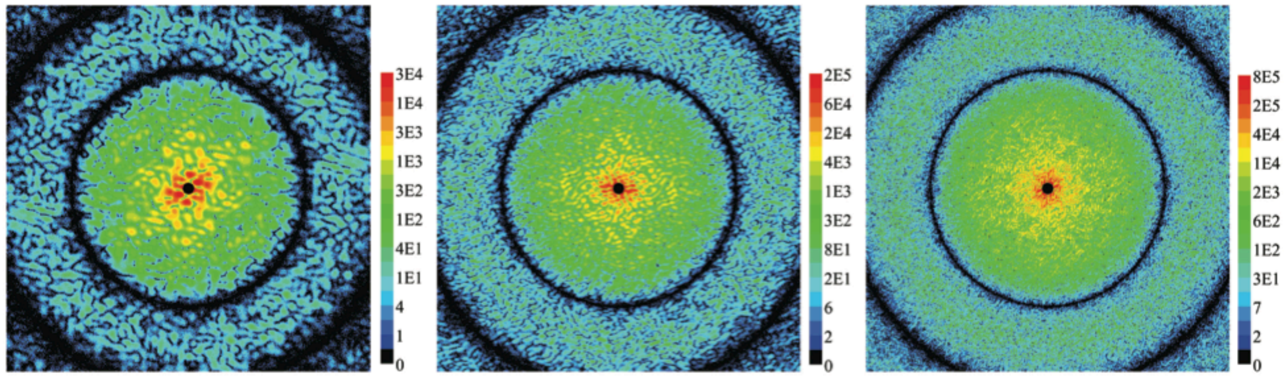
2 micron

2 micron

LCLS: We envisioned a new type of aerosol dynamics study

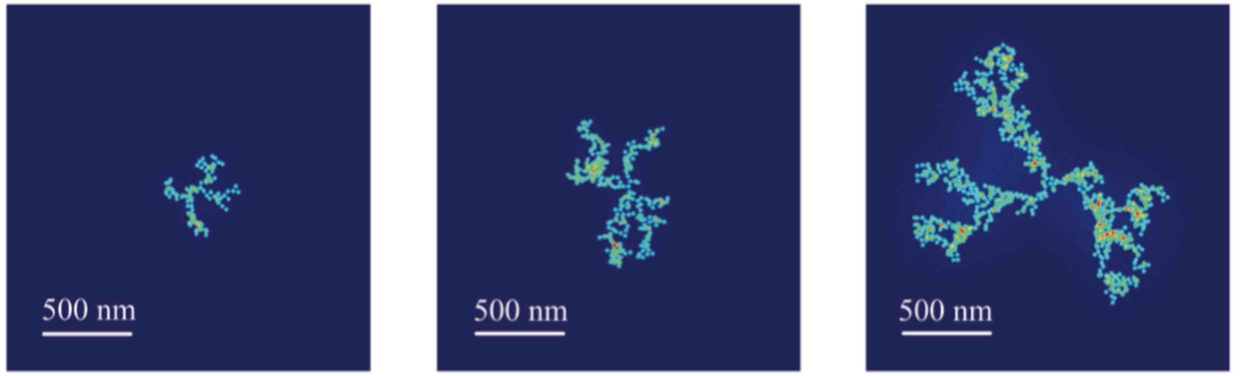
- EACH PARTICLE IS UNIQUE – SINGLE SHOT EXPERIMENT
- DIFFRACT BEFORE DESTROY
- ATOMIC RESOLUTION NOT REQUIRED TO SET NEW STANDARD FOR IMAGING PM2.5

Calculated diffraction patterns



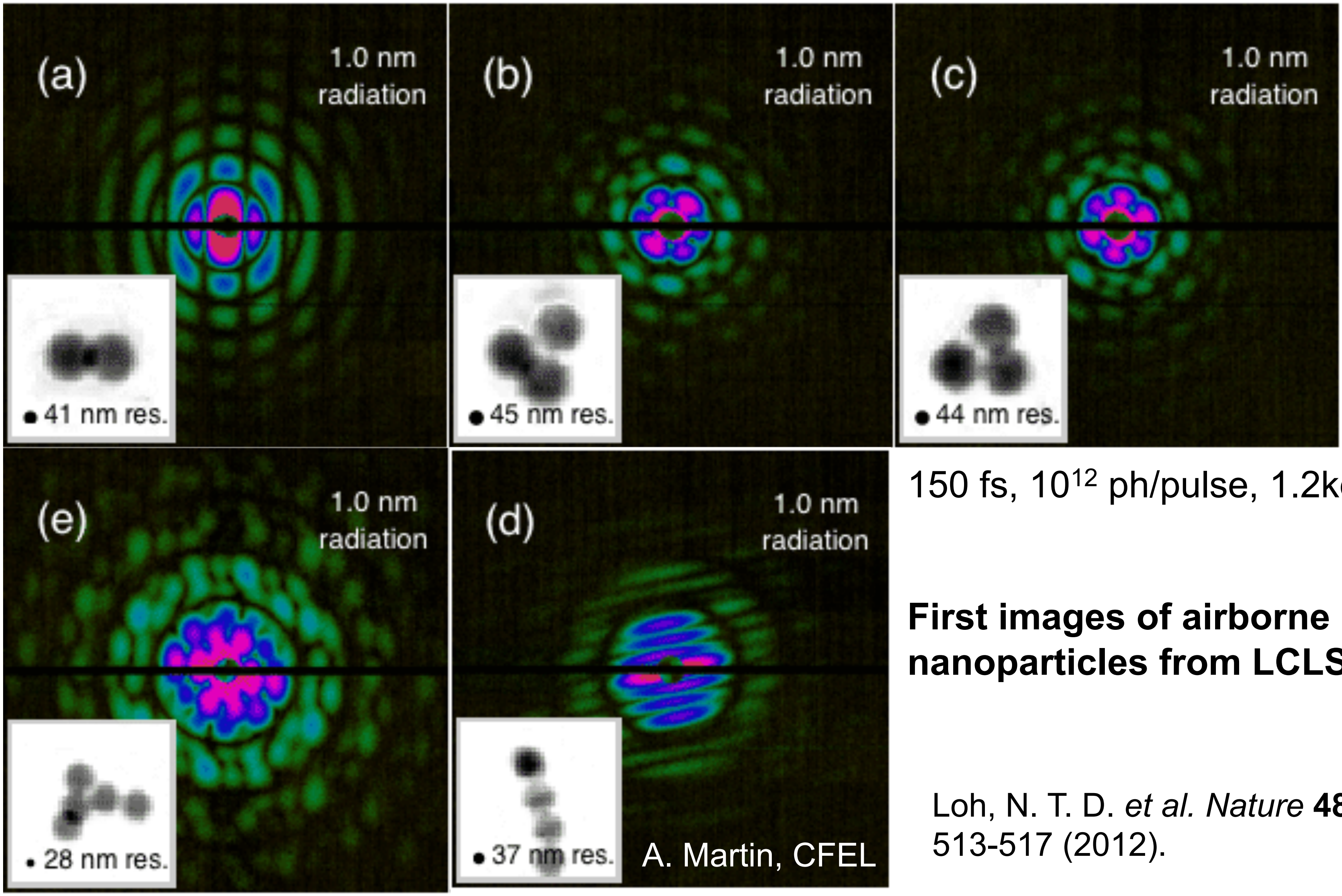
Simulated FLASH data: 7nm, 10^{12} ph/pulse, 10 μm

Reconstruction of the electron density of individual soot particles



Bogan, Starodub, Hampton, Sierra, J. Phys. B. 2010, 43, 194013, 2010

Our test model: Unknown structures of known components

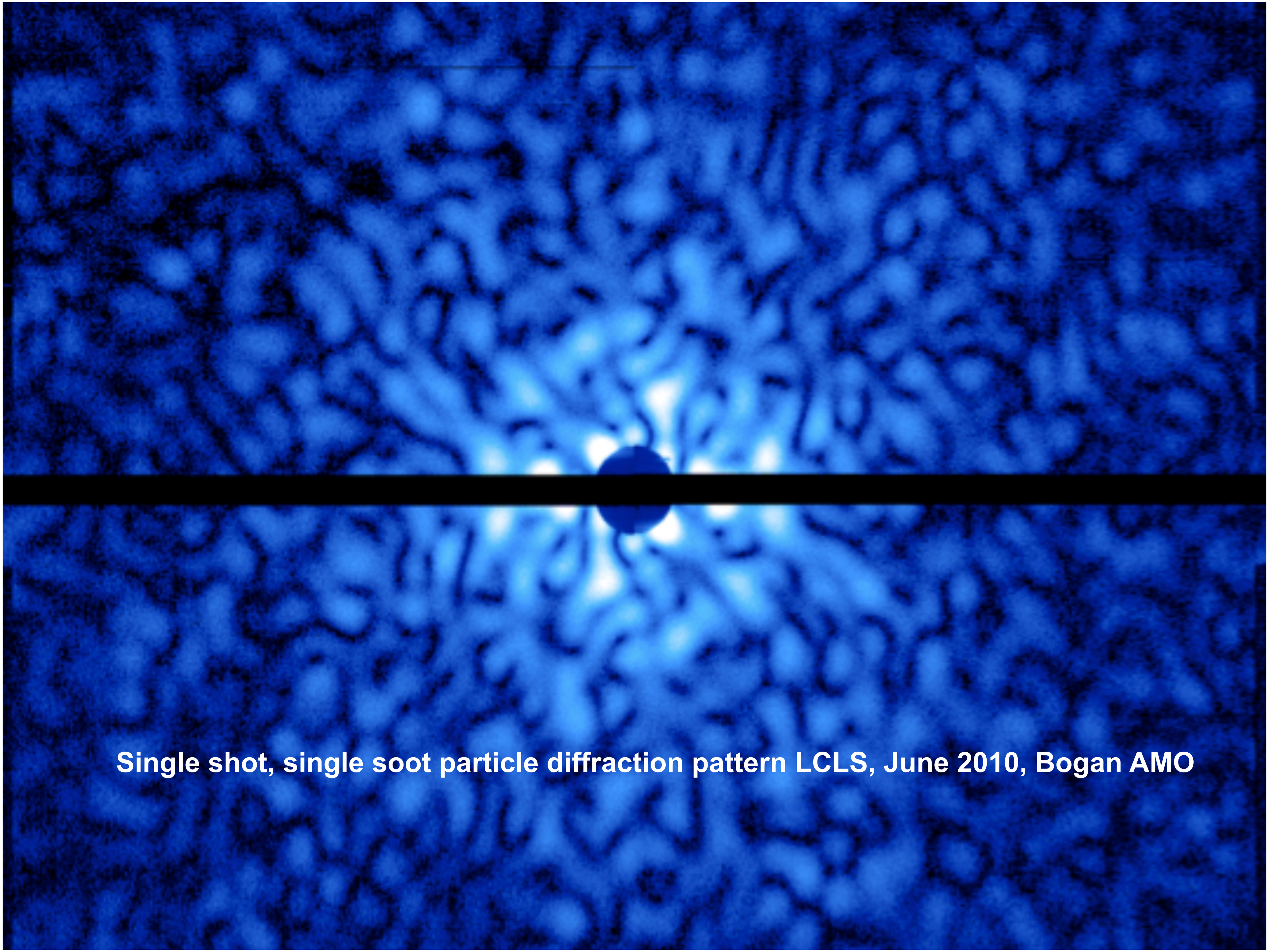


A. Martin, CFEL

150 fs, 10^{12} ph/pulse, 1.2keV

First images of airborne nanoparticles from LCLS

Loh, N. T. D. *et al. Nature* **486**, 513-517 (2012).

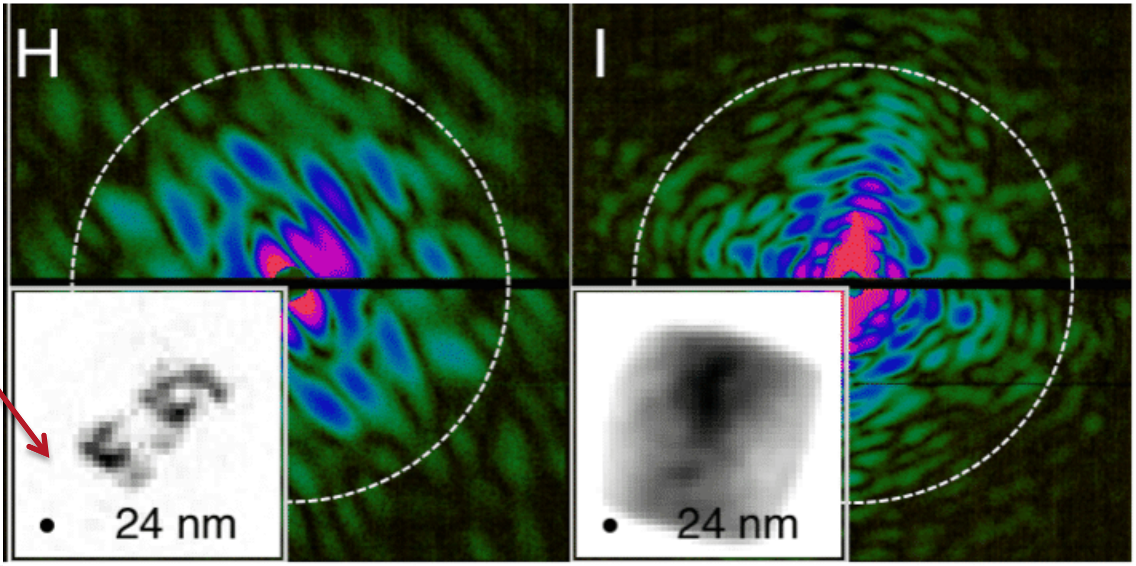


Single shot, single soot particle diffraction pattern LCLS, June 2010, Bogan AMO

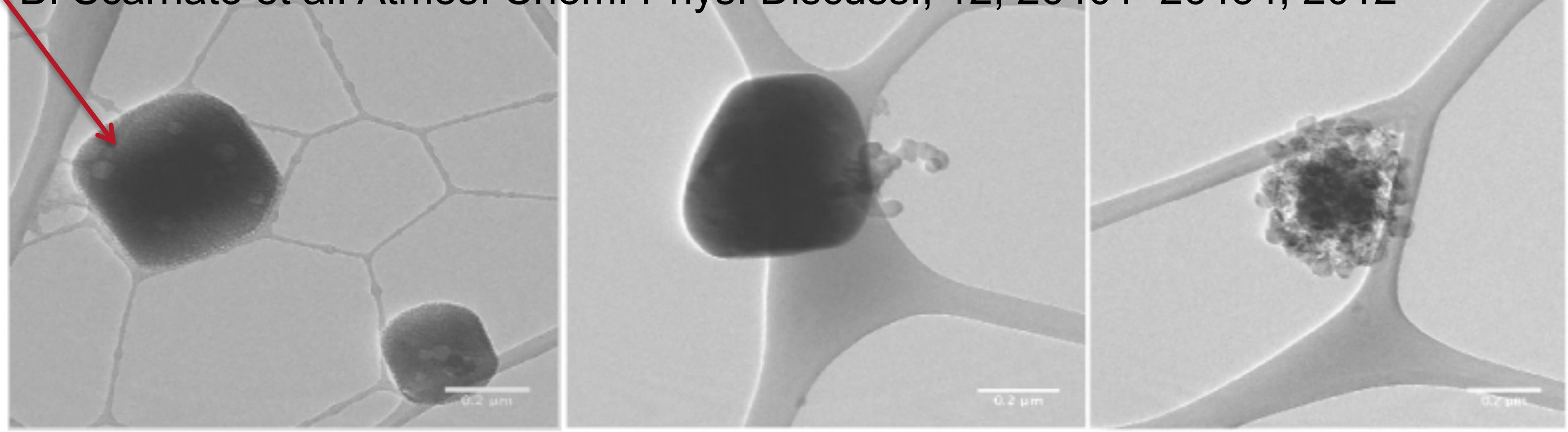
LCLS Imaging vs. Transmission Electron Microscopy

Airborne, in vacuum for microseconds, moving 150 m/s

In vacuum minutes-hours, captured stationary on target



B. Scarnato et al. Atmos. Chem. Phys. Discuss., 12, 26401–26434, 2012



(a) *BC immersion in NaCl*

(b) *BC immersion in and surface contact with NaCl*

(c) *NaCl immersion in BC*

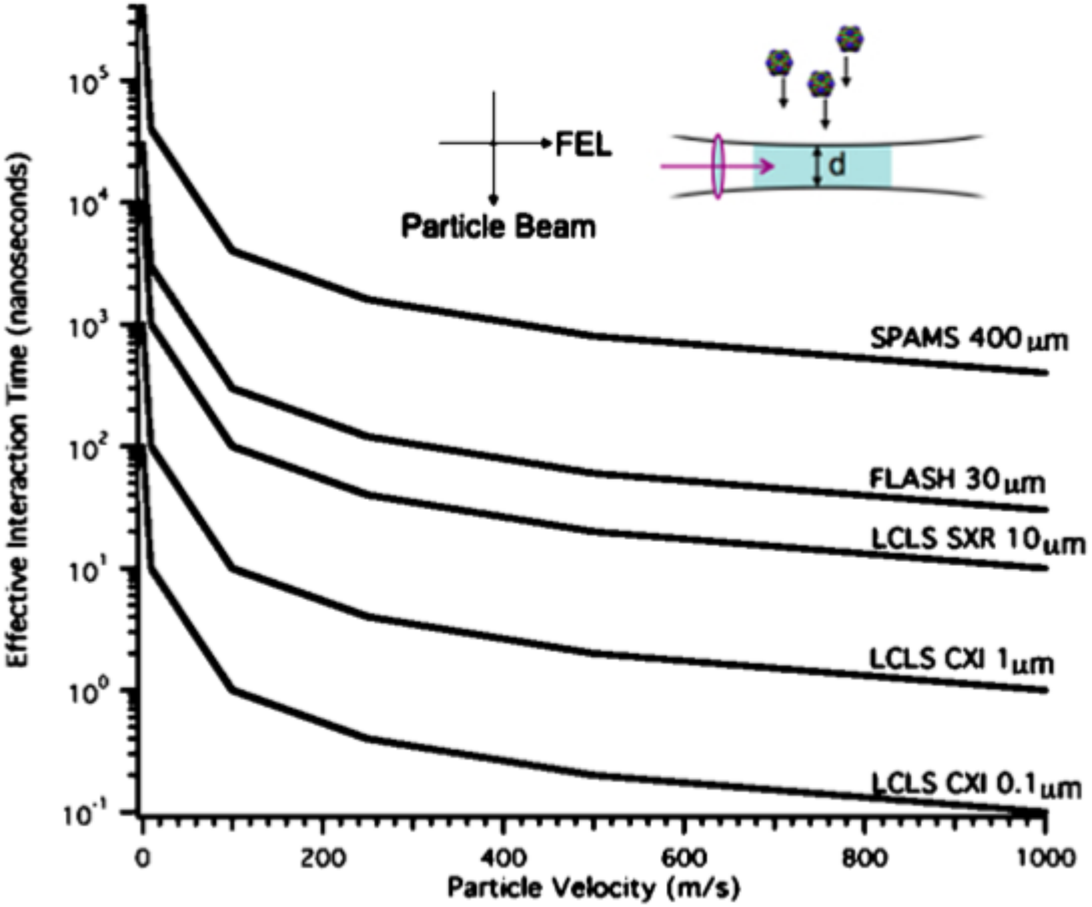
Time-Vernier Mode

Proposed for aerosols based on aerosol mass spectrometry:

SPAMS achieves 100% hit rate for 1 micron particles. Typically 10^8/ml sample.

Bogan, M. J., *et al.* (2010). *Physical Review Special Topics - Accelerators and Beams* **13**, 094791.

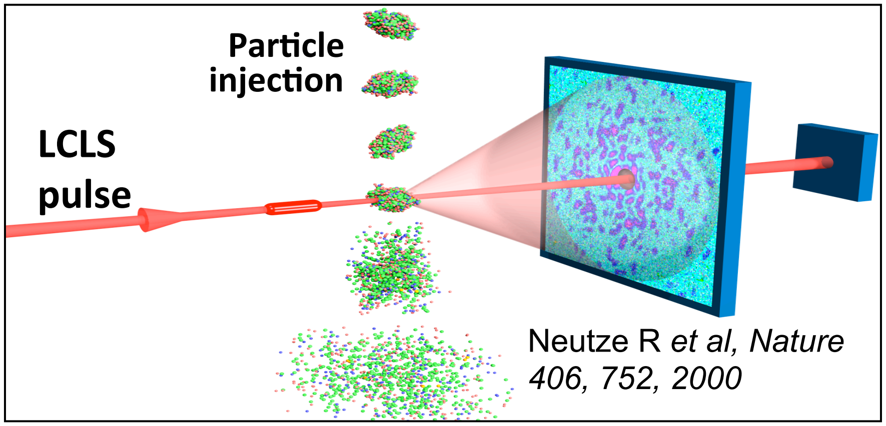
Bogan, M. J., Starodub, D., Hampton, C. Y. & Sierra, R. G. (2010). *Journal of Physics B: Atomic, Molecular and Optical Physics* **43**, 194013.



Worth it at SwissFEL? – yes for strong scattering single particles

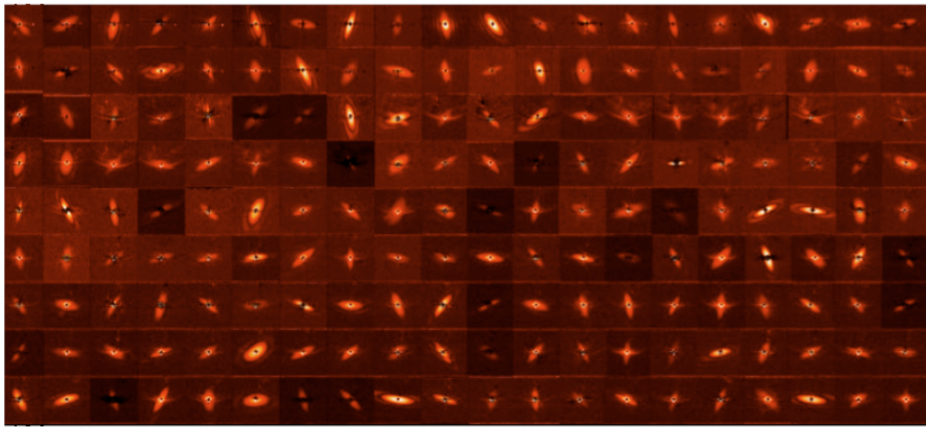
What about 3D structures? First Experimental Demonstration

Proposal



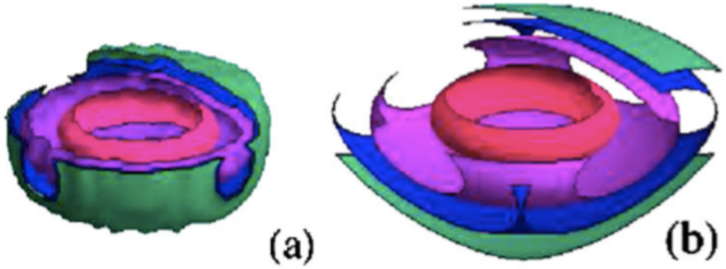
Neutze R *et al, Nature 406, 752, 2000*

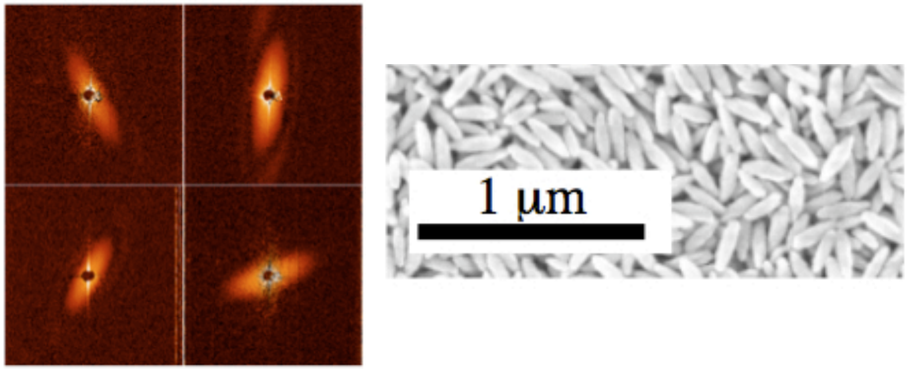
First experimental validation of data



Bogan; Boutet, et al. Physical Review Special Topics: Accelerators and Beams, 13, 094701, 2010

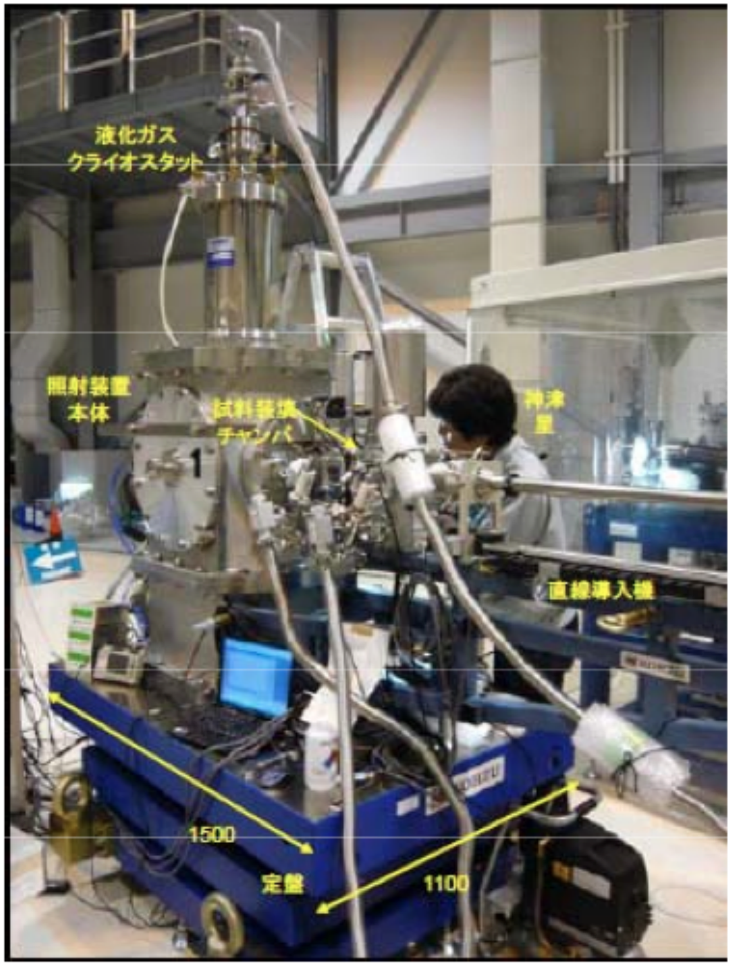
First reduction of single-shot data into 3D structure: Cryptotomography



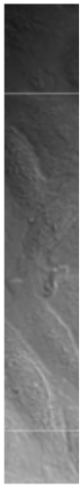


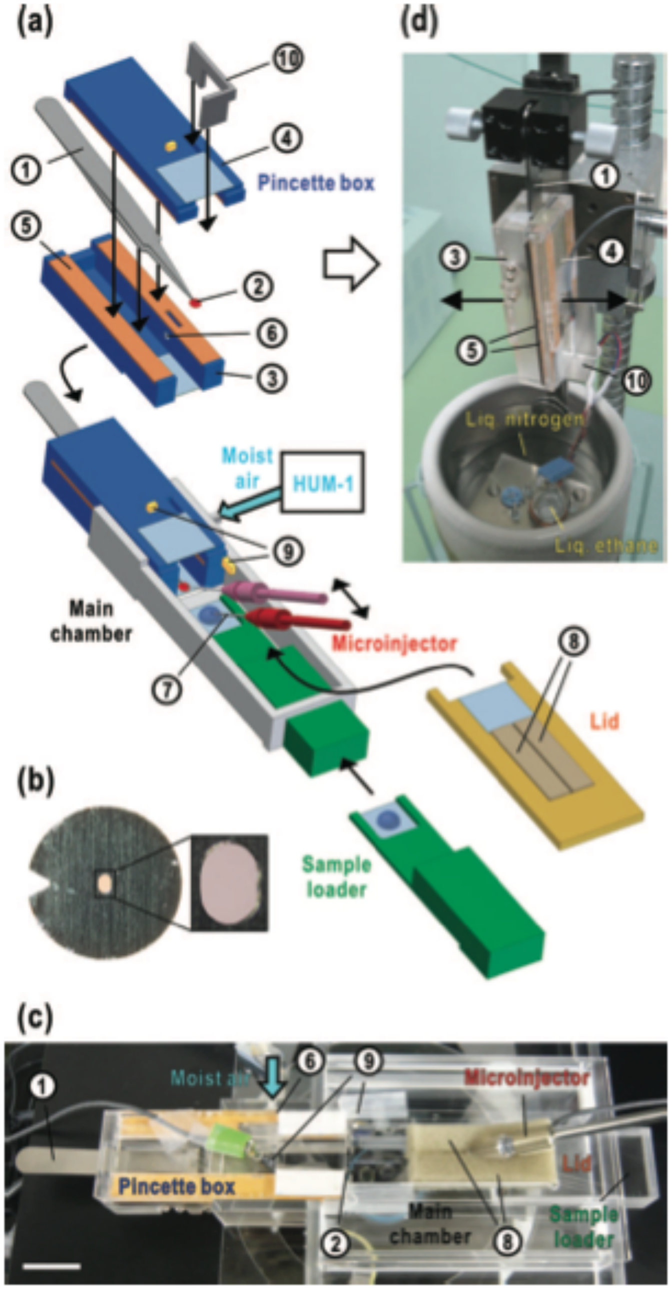
Loh; Bogan et al. Physical Review Letters, 104, 225501, 2010

Scanning stage for cell imaging



EM-derived XFEL instrument by M. Nakasako et al. (Keio Univ) to be installed in SACLA, Harima, Japan





Takayama, Y. & Nakasako, M. (2012). *Rev. Sci. Instr.* **83**, 054301.

The CAMP chamber was designed and commissioned by the Max Planck CFEL Advanced Study Group

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