Diamond UK Sept 3 60 mins 2014

Molecular imaging and dynamics at the LCLS

John C H Spence*

ASU Physics/LBNL/NSF BioXFEL STC Director of Science

*and many others, especially

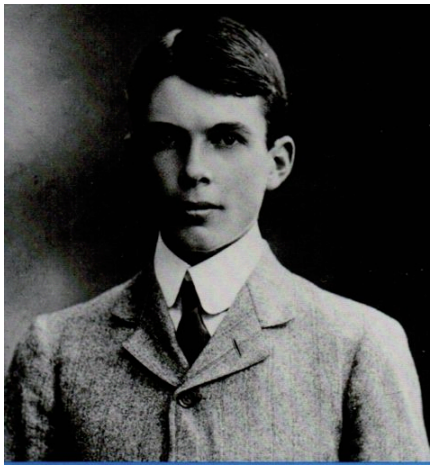
H. Chapman, U. Weierstall, B. Doak, A. Barty, I.Schlichting, J. Hajdu, P. Fromme. R. Kirian, T. White

OUTLINE

***Summary of milestones since 2009.**

*** Current research**

100 Years of Crystallography



William Lawrence Bragg. 1890-1971

Wilhelm Roentgen 1845 - 1923

Max von Laue 1879 - 1960

See Rep Prog Phys, Spence, Weierstall, Chapman. 2012 for review

Milestones in bioXFEL

A summary of achievements since 12/2009

The LCLS near Stanford was the world's first hard X-ray laser. It produces 9 kV X-rays in 70 fs pulses, about 1E12 photons/pulse.

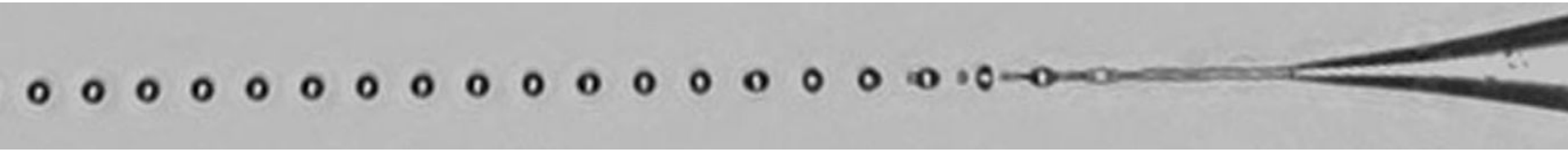
XFEL led to a new method of protein crystallography : SFX*

Discovery : Brief pulses out-run radiation damage.

*Result : Since data quality and resolution in MX have always been limited by damage, this *breaks the nexus between resolution, damage and sample size which has limited all MX in the past*. Consider δ(t).

The use of femtosecond pulses instead of freezing to avoid damage opens the way to the study of protein dynamics at room temperature, at atomic resolution, without damage.

A continuously refreshed supply of hydrated protein nanocrystals must then be supplied by spraying them across the pulsed X-ray beam. This "diffract-then-destroy" mode then also allows the study of protein nanoxtals from proteins which fail to grow xtals large enough for MX.



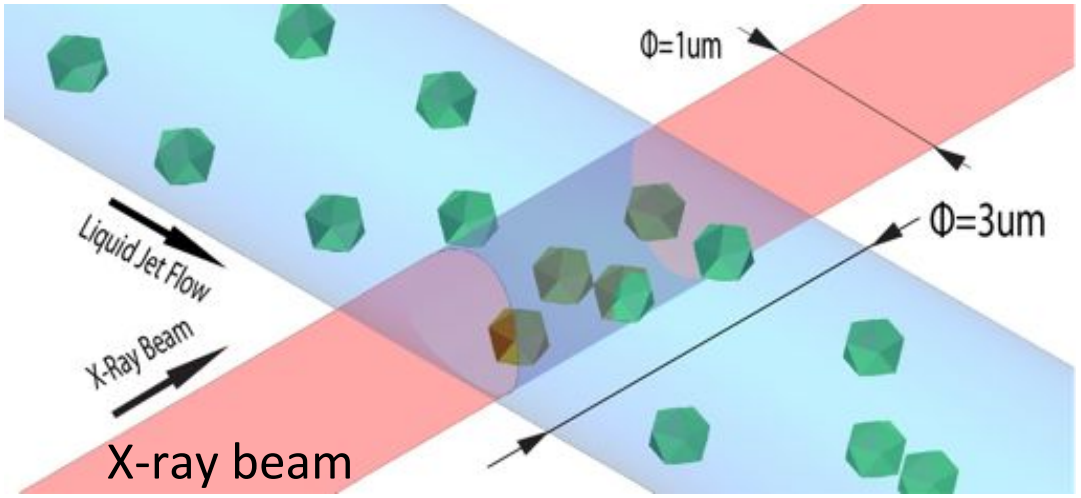
*Solem 1986, Neutze…Hadju 2000, Chapman et al 2006 – first experiment, low resolution.

*Chapman, Fromme……Spence Nature 2011; Spence , Weierstall, Chapman Rep Prog Phys 2012 (Review).

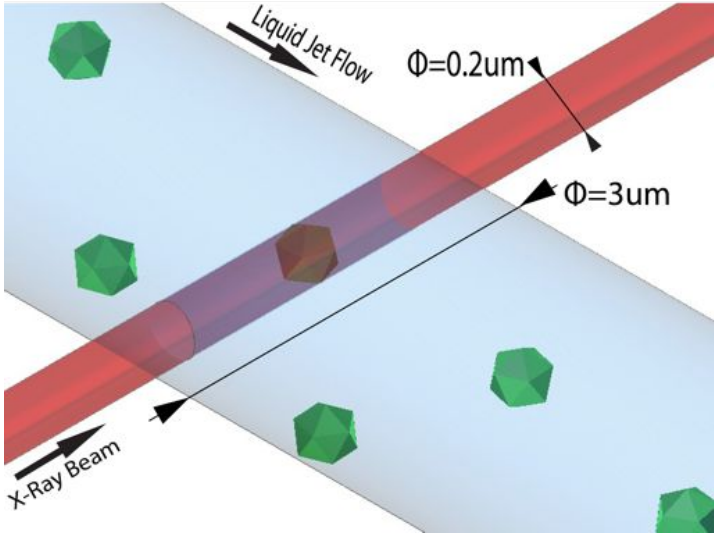
The STC does four kinds of snap-shot diffraction experiments .

Note dimensions in **microns**.

SFX, SP, FSS, 2D Xtals and TR variants.



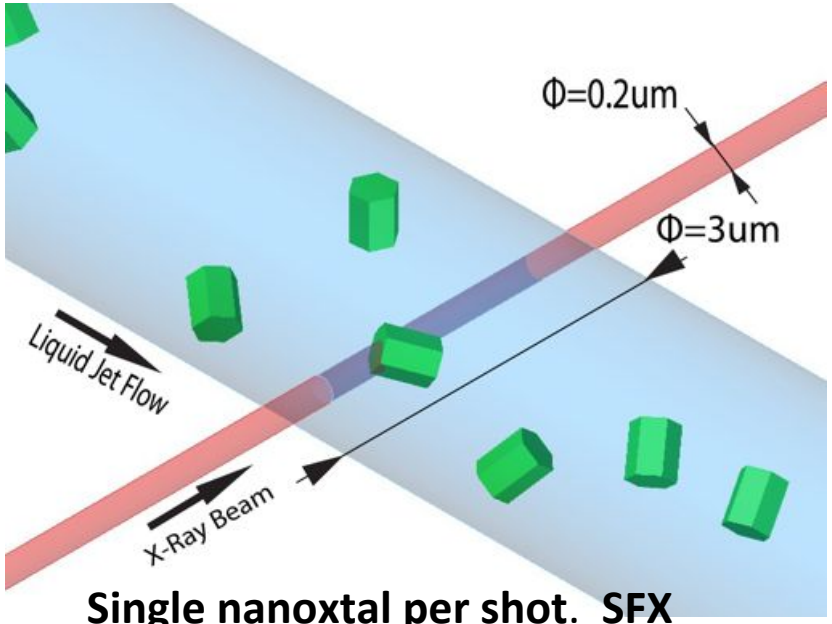
Fast Solution Scattering (WAXS) FSS



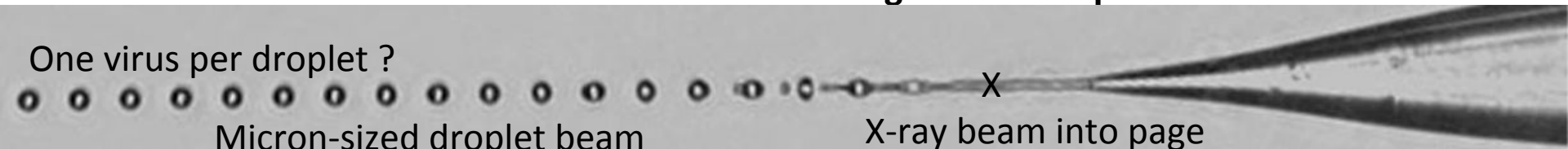
Single particle (eg virus) per shot . SP

Many X-ray shots do not hit a crystal

Plus 2D xtals on fixed targets (M. Frank)

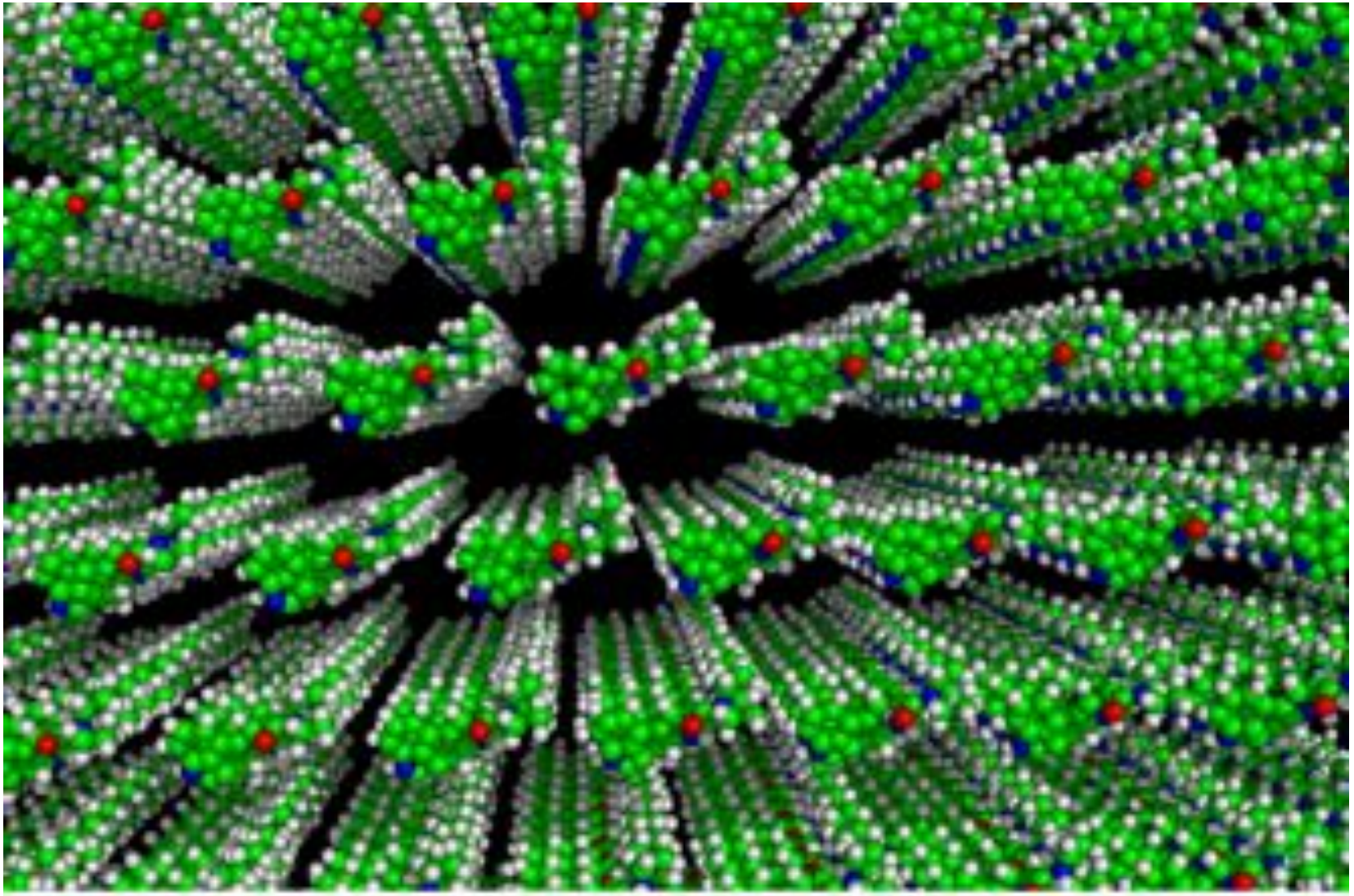


Single nanoxtal per shot. SFX



The time-evolution of damage during SFX may be simulated.

Lysergic acid 2 kV, 70 fs flat top, 1E17 W/cm2, Plasma simulation. Barty, Caleman et al 2012.



Fine detail is destroyed first

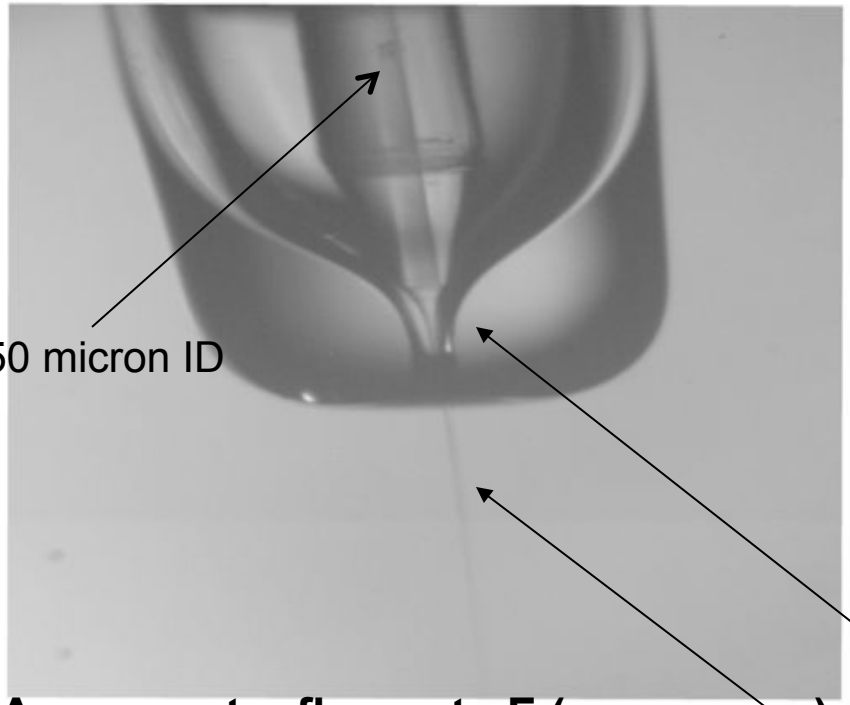
High order Bragg spots fade first

Resolution is 1.2* 2 pi = 7.6 Angstroms at 70 fs. Better at 10 kV. Dose 100 times safe dose

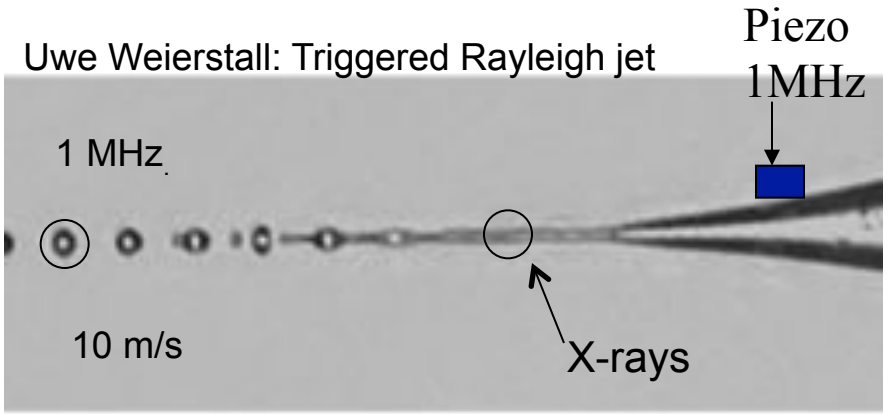
Hydrated particle delivery is needed for proteins

Our Liquid jet uses gas focusing to make a micron jet from bigger nozzle.

Gas focusing prevents clogging - get *submicron droplets* from a 15 micron nozzle
Absence of fields (electrospray) prevents charge artifacts on proteins.
Liquid feed ! No goniometer !



Uwe Weierstall: Triggered Rayleigh jet



LCLS Rep Rate ~100 Hz
Droplet frequency 1 MHz. v = 10 m/s = 10 mic/micsec
Droplet diam 1 micron
LCLS beam diam 0.1 micron or 4 microns.
Flow rate F= 10 microL per minute. v = F/A
Need 1 MHz rep rate to hit every particle.

Liquid cone enhances flow alignment
(from HPLC syringe pump)

Av = const = flow rate F (mass cons)
Gas accelerates liquid
Making cone as shown.
Higher pressure for smaller droplets

~1 MHz single-file micron-sized droplet beam. Big nozzle makes Small droplets

Allows study of *irreversible* processes in *fully hydrated* biomolecules

M. Frank, Mike Bogan, have gas-phase jet

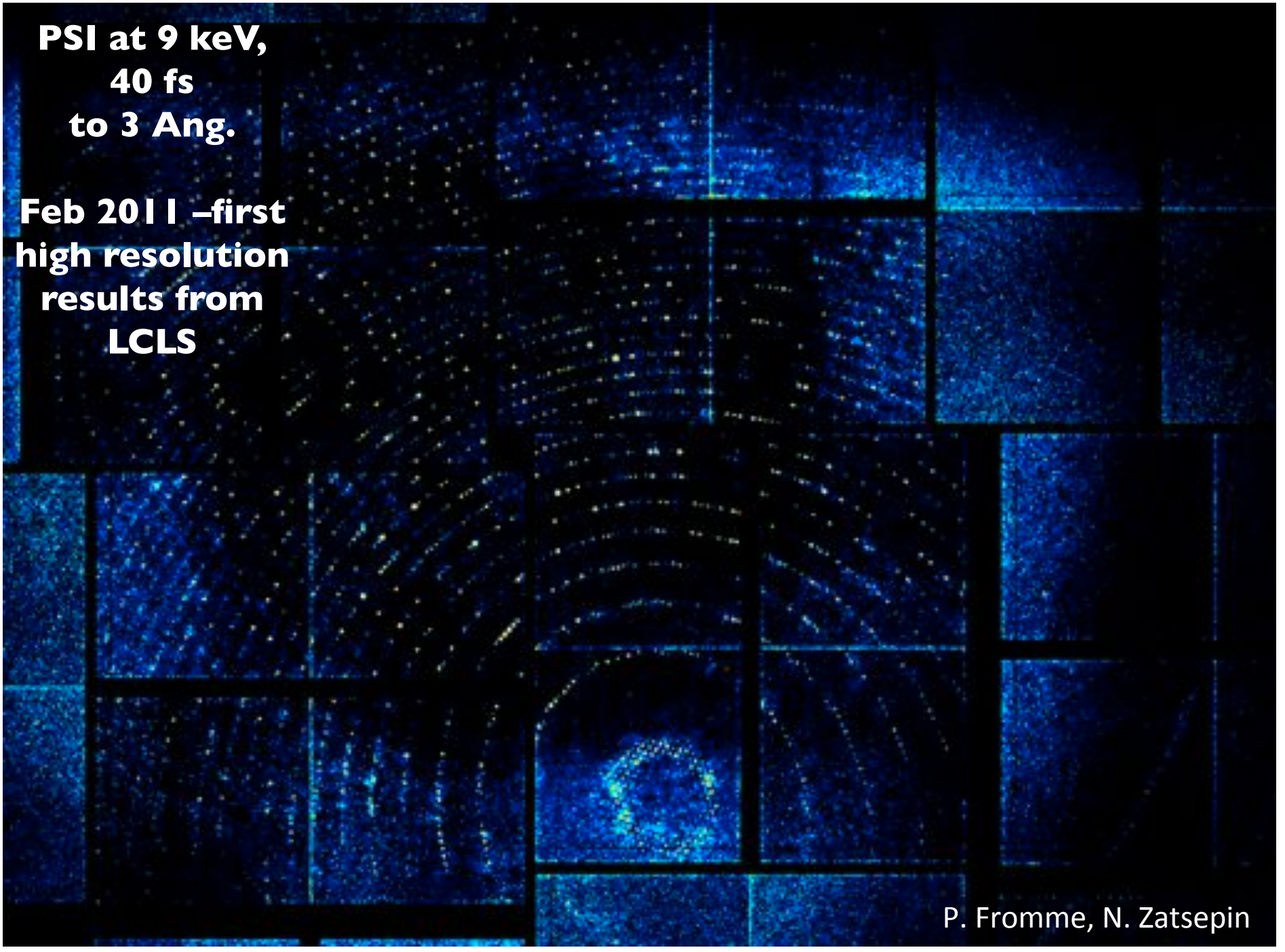
Agilent, Medical Rayleigh 1890

Spence, Doak, Phys Rev Letts. 92, 198102 (2004). Weierstall, Doak, Spence Rev Sci Instr. (2012)

Milestones

1. Can "diffract-and-destroy" (sFX) give atomic resolution ?

LCLS has about 6 Petabytes of storage (about $200K/Pbyte)
1.8 Terabytes per hour are generated by the 120 Hz detector readout.
Use www.globus.org to move large data sets.


PSI at 9 keV,
40 fs
to 3 Ang.
Feb 2011 –first
high resolution
results from
LCLS
P. Fromme, N. Zatsepin

Can diffract-and-destroy give atomic resolution ?

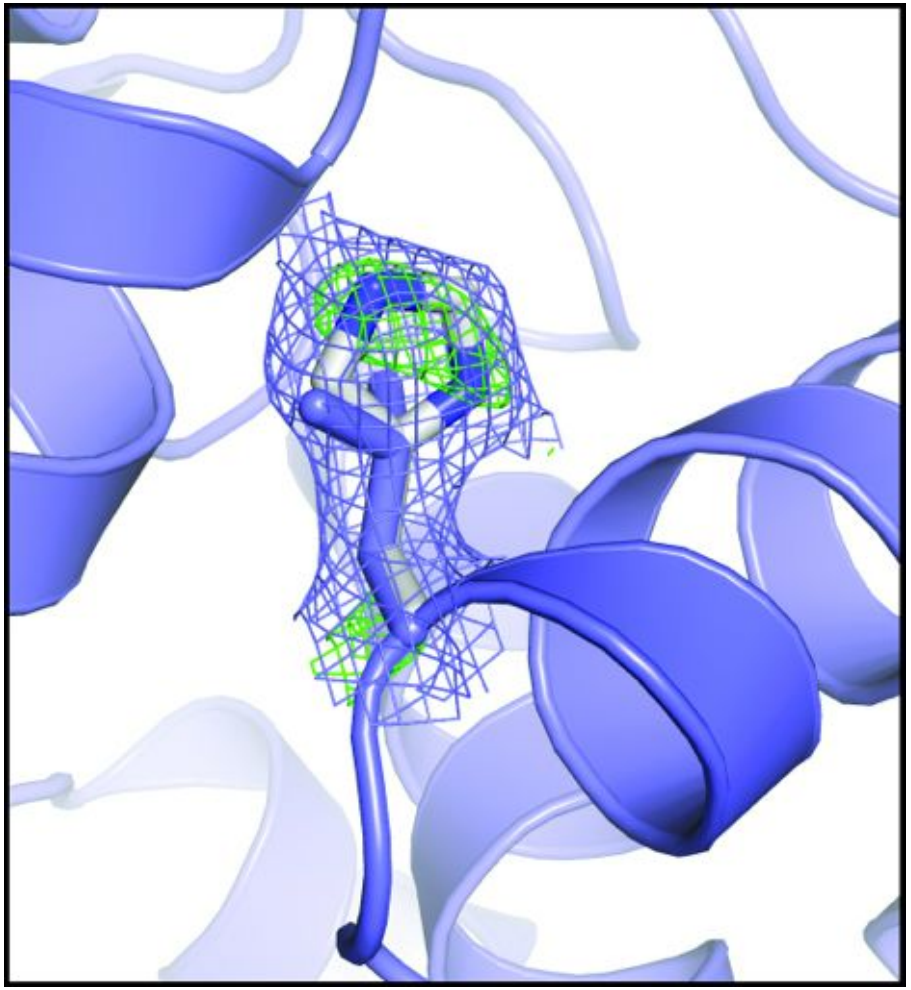


Fig. S3

Quality of the molecular replacement map. The region around Leu15 in the turkey egg white lysozyme model (blue carbon atoms) is shown. In the actual hen egg white lysozyme structure obtained of the presented SFX data, this is a histidine (white carbon atoms). Both the $2mF_{obs}$-DF_{calc} (blue, 1.5 σ) and Fobs-DFcalc (green, +/- 3 σ) maps *(10)* clearly show the difference between the search model and the actual structure obtained from the SFX data.

XFEL data can distinguish Turkey from Hen.

Hen Lysozyme, 1 x 1 x 3 microns xtallites at 9.4 kV with 40 fs pulses, Dose 33 Mgy. CrystFEL . **RESOLUTION 1.9 ANGSTROMS**.

Serial Femtosecond Crystallography SFX

Weighted R-factor between LCLS and SLS data is about 10%

Turkey Blue
Hen White

D.C.Phillips, L.N. Johnson, 1965 first enzyme solved.

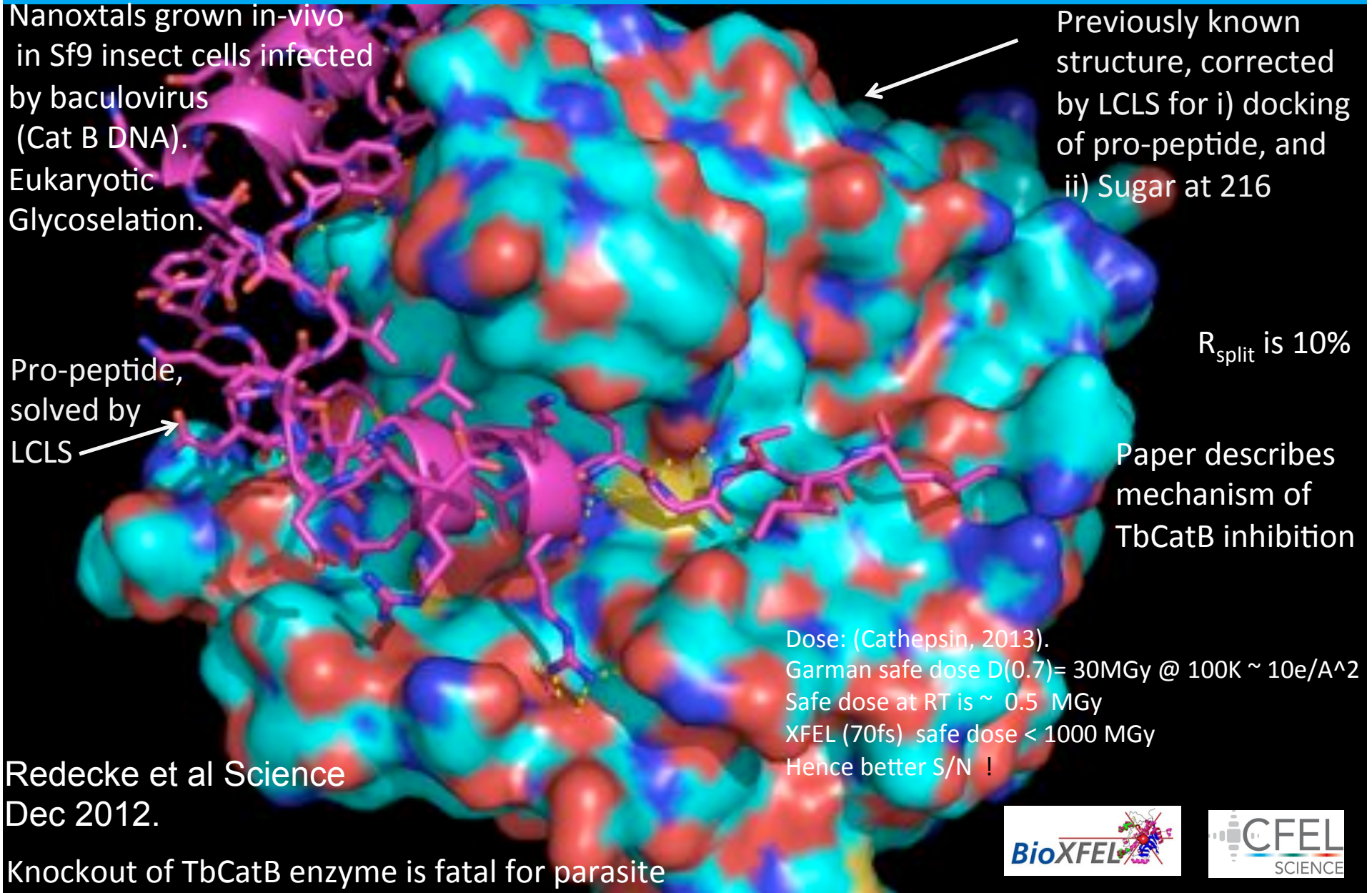
S. Boutet,.....I. Schlichting. Science 2012

Milestones

2. Can "diffract-and-destroy" (sFX) give New Biology ?

The previously-unknown structure of Cathepsin B determined to 2.1 Å resolution

A drug target for sleeping sickness



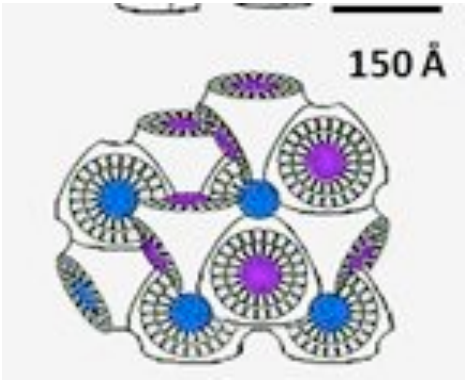
Redecke et al Science Dec 2012.

Knockout of TbCatB enzyme is fatal for parasite

A G-protein Coupled Receptor studied by SFX in LCP jet

Human serotonin.
Regulates mood, appetite, sleep, memory, learning…..

Lipidic Cubic Phase



- GPCR are mem prot. and **60% of drug targets**
- Do signal transduction, cellular response
- Very difficult to crystallize, except micron-sized in **LCP.**
- LCP offers native environment, low solvent content, higher crystalline order
- Only 19 receptor structures solved so far; most crystallized in LCP. 2012 Kobilka Nobel prize.

- **We can do SFX with nanocrystals of GPCRs in LCP**

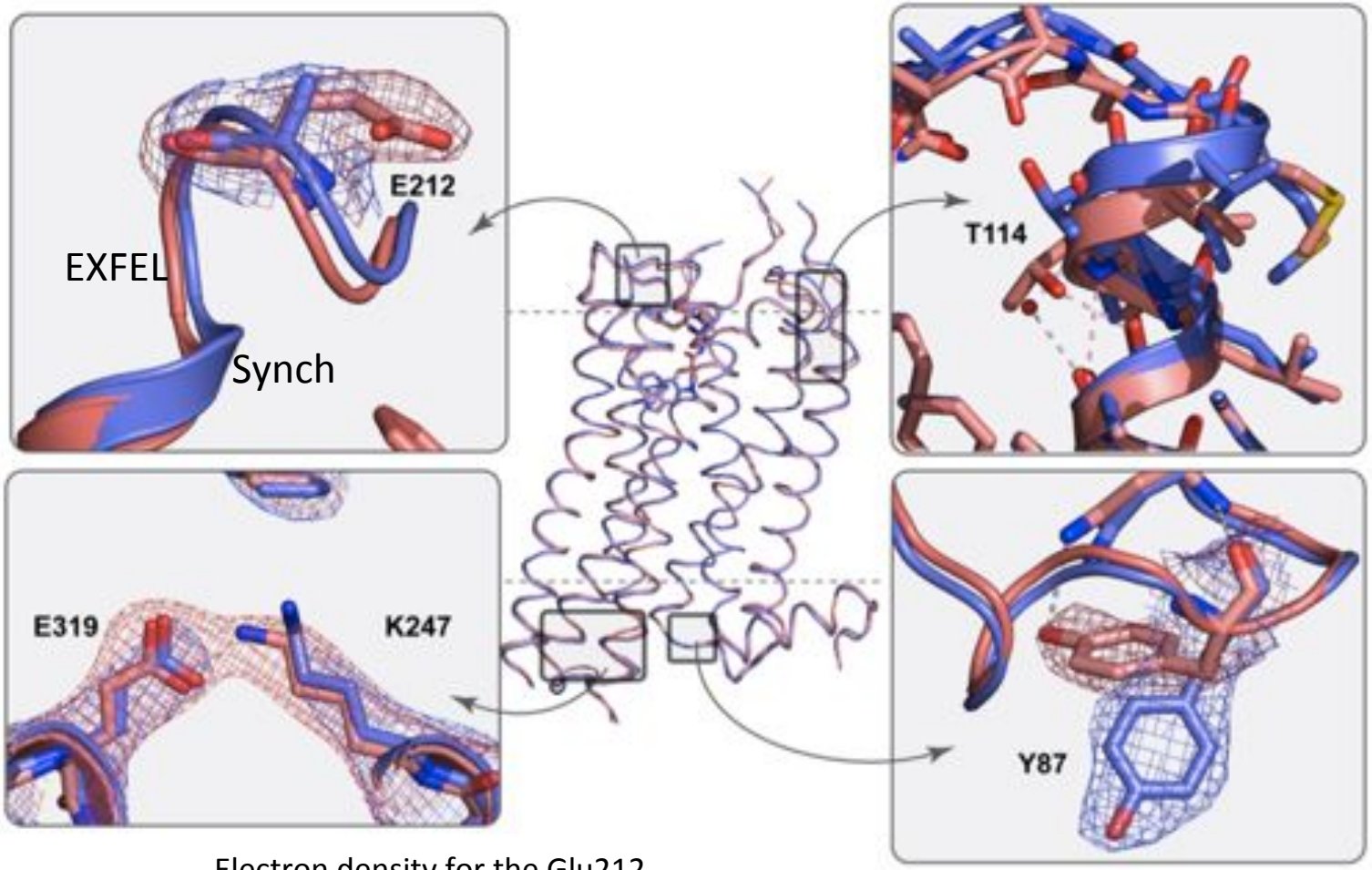
Liu,Cherezov et al
Science Dec 2013

Synchrotron (frozen) and XFEL (RT) structures found to differ.

Human Serotonin receptor
5-HT_{2B} + Ligand

RT structure more accurately represents the conformational ensemble for this receptor under native conditions

Ligand binding site also imaged



A salt bridge links intracellular parts of helices in XFEL structure, not present in syn. structure

Differences most likely originate from cryo-cooling

Electron density for the Glu212 **side-chain is missing** in syn. structure; fully resolved by XFEL.

2.8 Ang Resolution

Sidechains have different conformations

hydrogen bond is broken and Tyr87 adopts a different rotamer conformation in synch. structure

The largest backbone deviations are observed in the loop regions

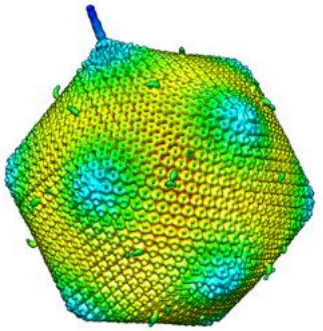
Liu, Cherezov et al Science Dec 2013

Milestones

3. Single-particle (virus) diffraction.

Progress has been made with Single Particle (SP) imaging

One particle per shot.



The latest single-particle results (with Schlichting) extend to 120 Angstrom resolution. (vs 300 A in 2009).

Hogue group (Liu, Lawrence..) for Virus Nanoxtals

Cannot be worse than SAXS !

Image reconstruction and phasing from inorganic nanoparticles

See also I. Robinson group, ICL

Preliminary reconstruction
Pd-Cu_2O nanoparticle

Use 3 simultaneous orthogonal beams ?

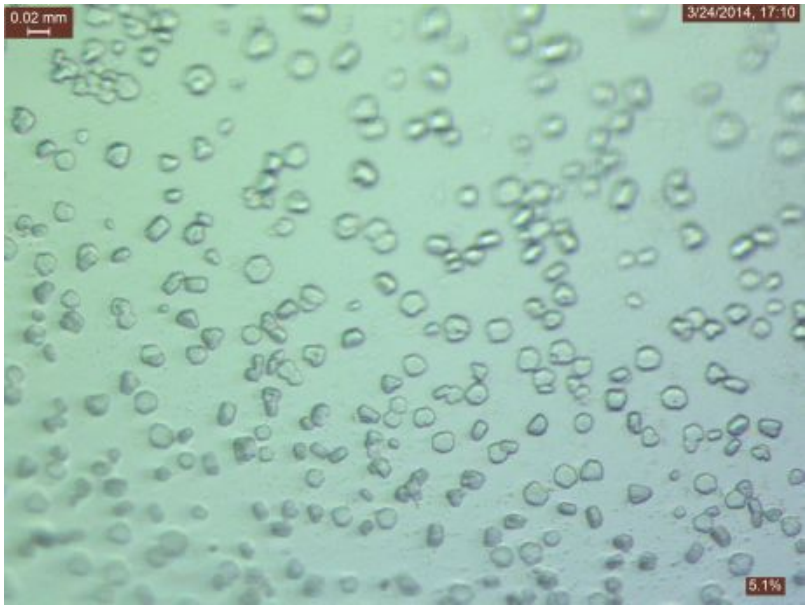
Hogue, Liu, Lawrence,ASU team, et. al.

Milestones

4. Virus nanocrystals.

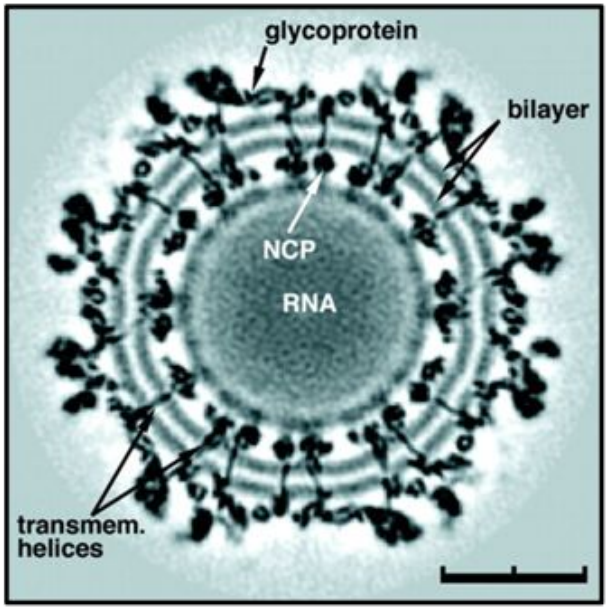
Virus crystallography

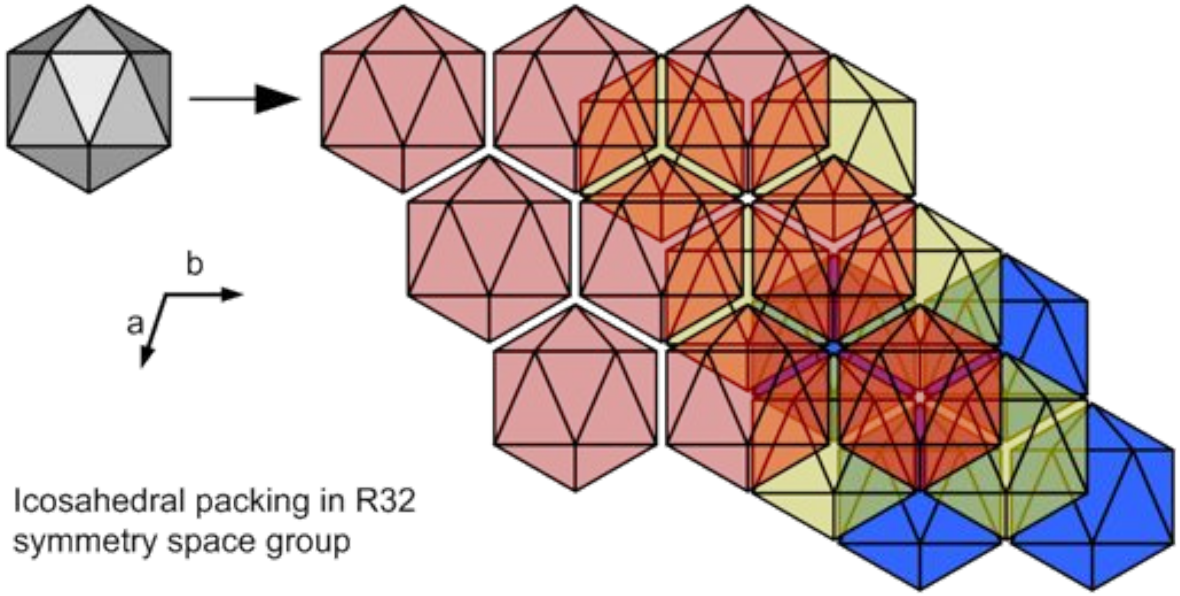
- Enveloped and non-enveloped
- Fixed icosahedral geometry
- Macro-crystals used for synchrotron studies



Sindbis virus crystals

Sindbis virus EM





Icosahedral packing in R32 symmetry space group

Zhang, W. et al. J. Virol. 2002; 76:11645-11658

Sindbis Virus: Indexed pattern w/ rings

B. Hogue, R. Lawrence, ASU

	LCLS XFEL Measurements
Resolution	~4 Å
Unit Cell	386 Å, 90° 386 Å, 90° 497 Å, 120°
Space Group	R32?

One intact enveloping virus per unit cell

	Synchrotron Measurements
Resolution	30 Å
Unit Cell	640 Å, 90° 640 Å, 90° 640 Å, 90°
Space Group	R32

Cowpea Mosaic Virus (CPMV): Indexed pattern

Vijay Reddy, Scripps

	LCLS XFEL Measurements
Resolution	2.5 Å
Unit Cell	384 Å, 90° 396 Å, 90° 386 Å, 90°
Space Group	I41, cubic

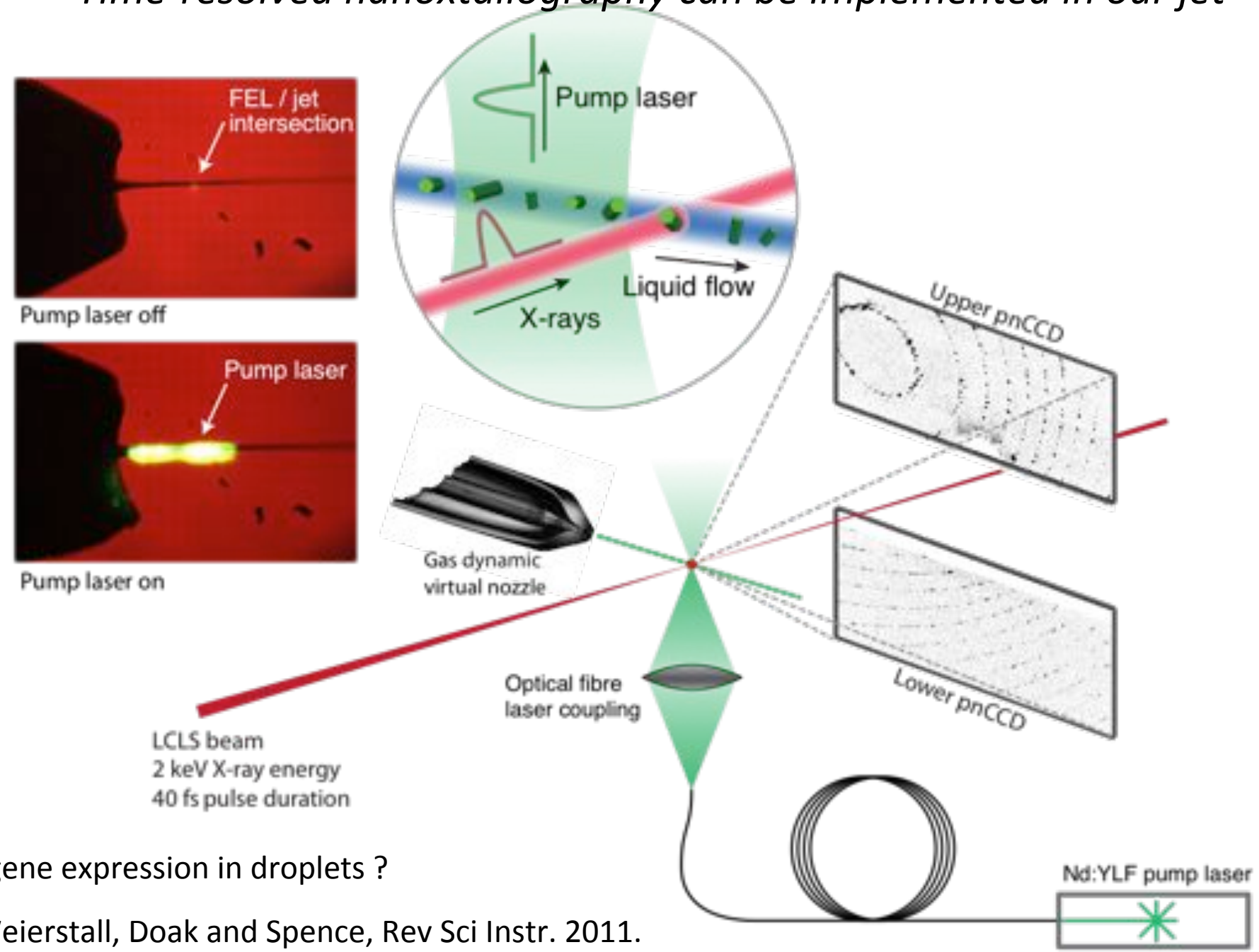
One intact non-enveloping virus capsid per unit cell

	Synchrotron Measurements
Resolution	3.0 Å
Unit Cell	656 Å, 90° 656 Å, 90° 572 Å, 90°
Space Group	I41

Milestones

5. Time-resolved protein crystallography.

Time-resolved nanoxtallography can be implemented in our jet



gene expression in droplets ?

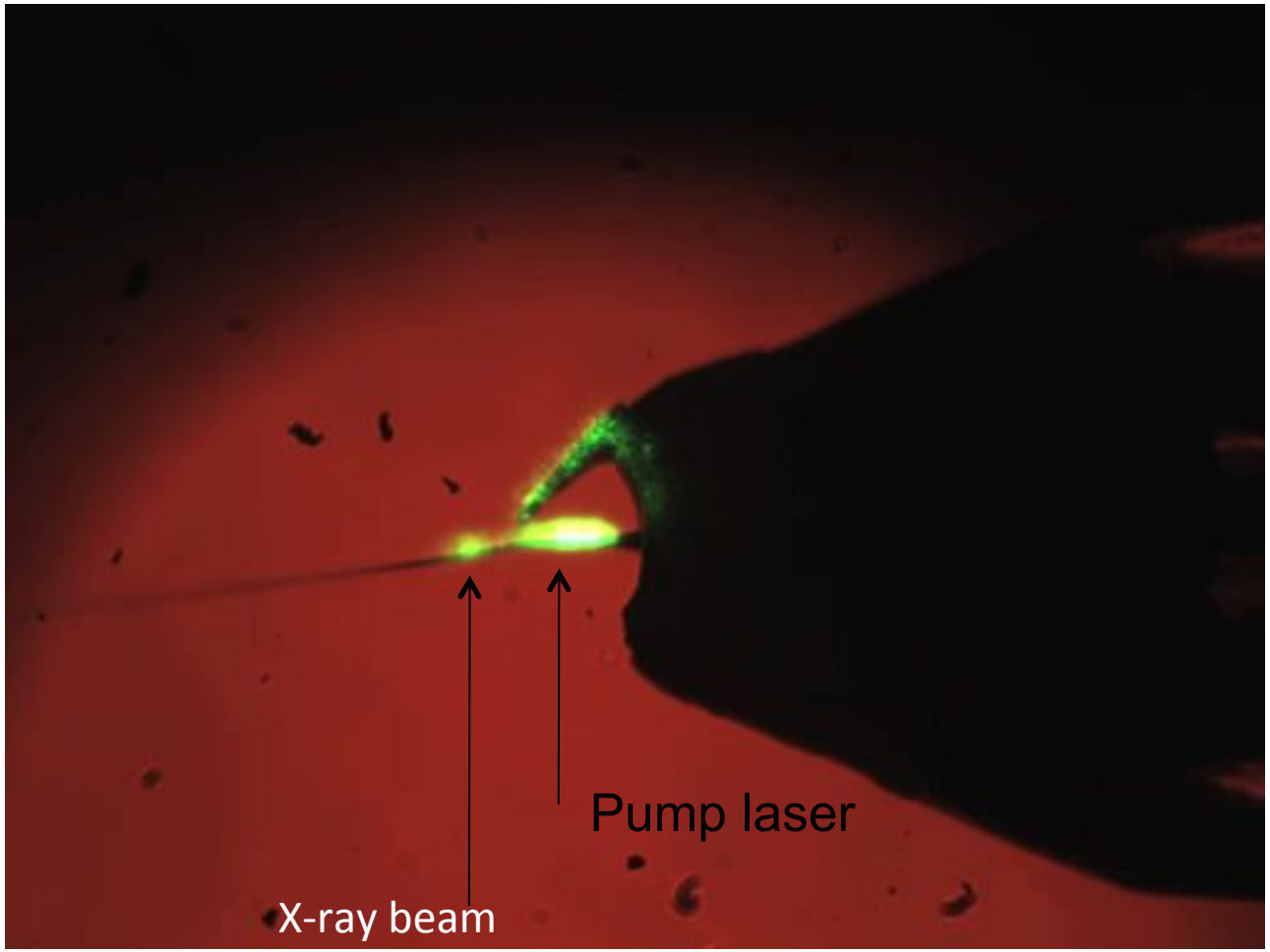
Weierstall, Doak and Spence, Rev Sci Instr. 2011.

Pump-probe experiments are possible with the liquid jet. PSI-ferre.

Pump laser and XFEL on jet - exploding nanoxtals

Like sunlight on a leaf….snapshots of the excited state density

Movie



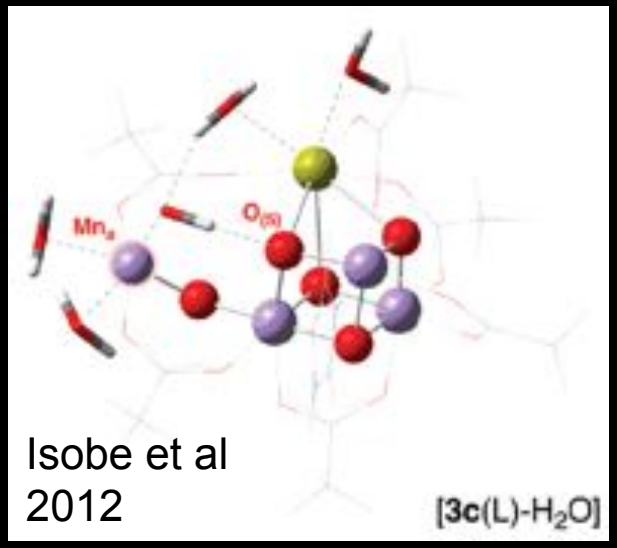
pump laser:
532nm,
10ns pulse, 8
microjoules,
focused to 380
micron spot,
fiber coupled,

Time delay
between 70 fs
X-ray pulse and
laser 0 - 10μs

7 micron beam
0.5-2 mic. xtals
4 micron jet

To observe undocking of ferredoxin from PSI, excite nanoxtal 10 microseconds before XRD snapshot
Travelling at 10 m/s, nanoxtals go 100 microns, less than width of 400nm doubled Jedai fs beam
Flow rate 10 microliters/min.

Conformational changes at PSII water-splitting site in S3



Isobe et al 2012

P. Fromme: results of time resolved SFX of the oxygen evolving complex OEC in Photosystem II

Red: Oxygen
Purple Mn
Turquoise Ca
Oxygen release in S4
527 nm pump,
210 microsec delay

Kok cycle

Distance from dangler Mn to cubane increases which would allow a water molecule to bind between the dangler Mn and the O5 as predicted by DFT calculation by Isobe 2012

Position of dangler Mn changes

Cluster shrinks in S3, expected as all Mn are Mn IV, so no Jahn-Teller distortion

Damage-free snapshots should show Mn^{+++}, not reduced (e- gain) SFX images true chemical state ?

Diff maps in two projections

S1 (dark)

S3 (double pumped)

Dark S1 and double excited S3 state at 5 Å resolution Kupitz

Dangler Mn

Kupitz et al Nature , July 2014.

Time-Resolved Serial Femtosecond Crystallography with Near-atomic Resolution

Difference Maps of Photoactive Yellow Protein at XFEL and Synchrotron Agree

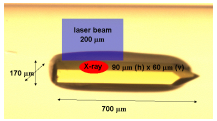
PYP is Fundamental to Light Perception/ Light Absorption and Photosynthesis

Marius Schmidt (STC PI) et al. June 2014

1.6 Ang resolution !

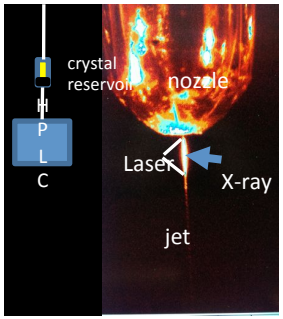
ASU, CFEL, Imperial College, LCLS, LLNL, UB, U of C, UWM

Advanced Photon Source, BioCARS
100 ps X-ray pulses
Crystal size: 170 μm
Pump laser: 4 ns pulses
Photo-initiation: 4.5 mJ/mm^2 at 485 nm



APS: 16 ns

Linac Coherent Light Source, CXI
40 fs X-ray pulses
Crystal sizes: 1 – 3 μm
Pump laser: 4 ns pulses
Photo-initiation: 800 μJ/mm^2 @ 45



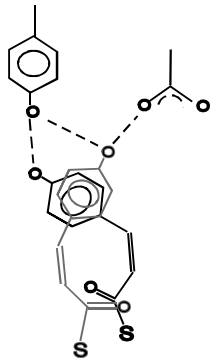
LCLS: 10 ns

LCLS beamtime LD62
June 5th to 9th, 2014
- preliminary results -

Red: negative
Green: positive
Contour: +/- 3 σ
Yellow: dark state model

APS: 1 μs

1 μs structural interpretation



LCLS: 1 μs

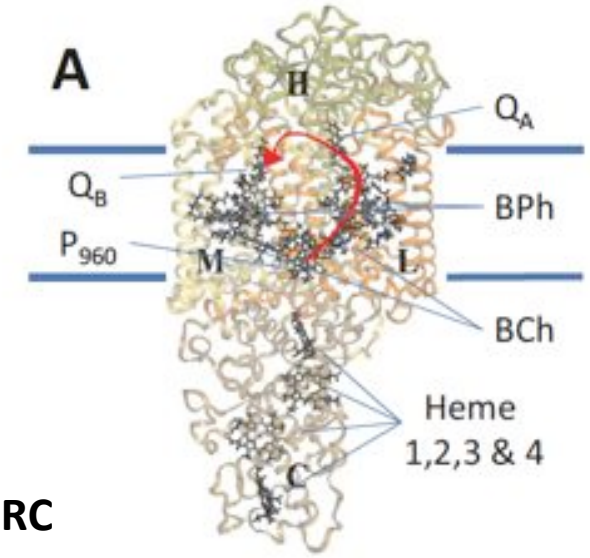
Milestones

6. Fast solution scattering (FSS)

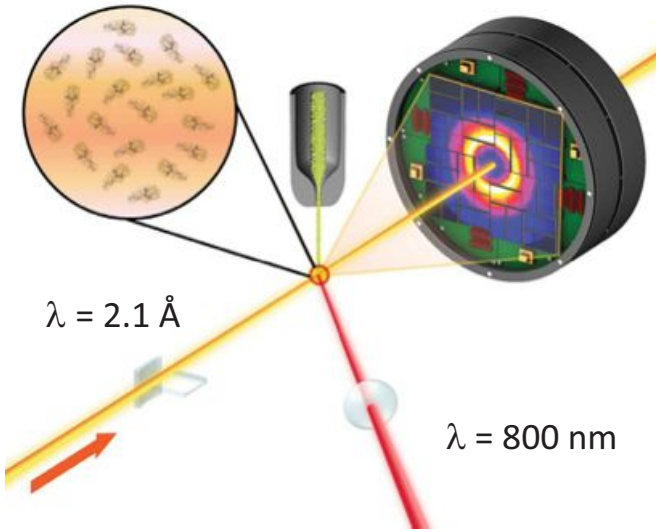
Time-resolved fast solution scattering (FSS) : exciting first results from Neutze group: Fast (ps) motions matter in Photosynthesis.

Aims: To understand how PS proteins avoid unfolding when solar photons arrive.
To determine if fast (ps) nuclear motions are involved in photosynthesis.

- Single molecules of Reaction Center (RC) in solution study conformational change triggered by visible laser
- Many molecules per snapshot – "snapshot SAXS"
- Low q is early "quake" motion, high q is heat



***Blastochloris viridis* RC**



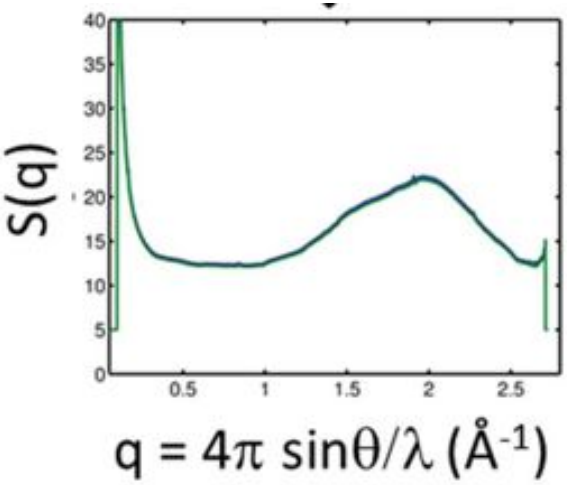
Arnlund….Neutze et al. Nature Methods '14
STC Collaboration. Published. Aug 2014.

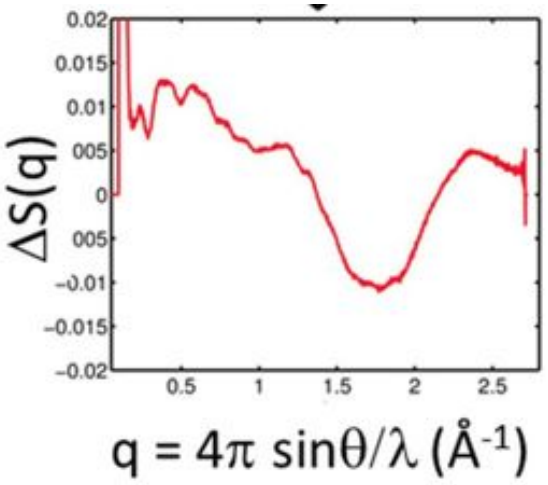
Pump-probe fast solution scattering in our water jet.

Blastochloris Viridis RC.

FSS – Fast Solution Scattering

Neutze group + STC 2014. Nature submitted.





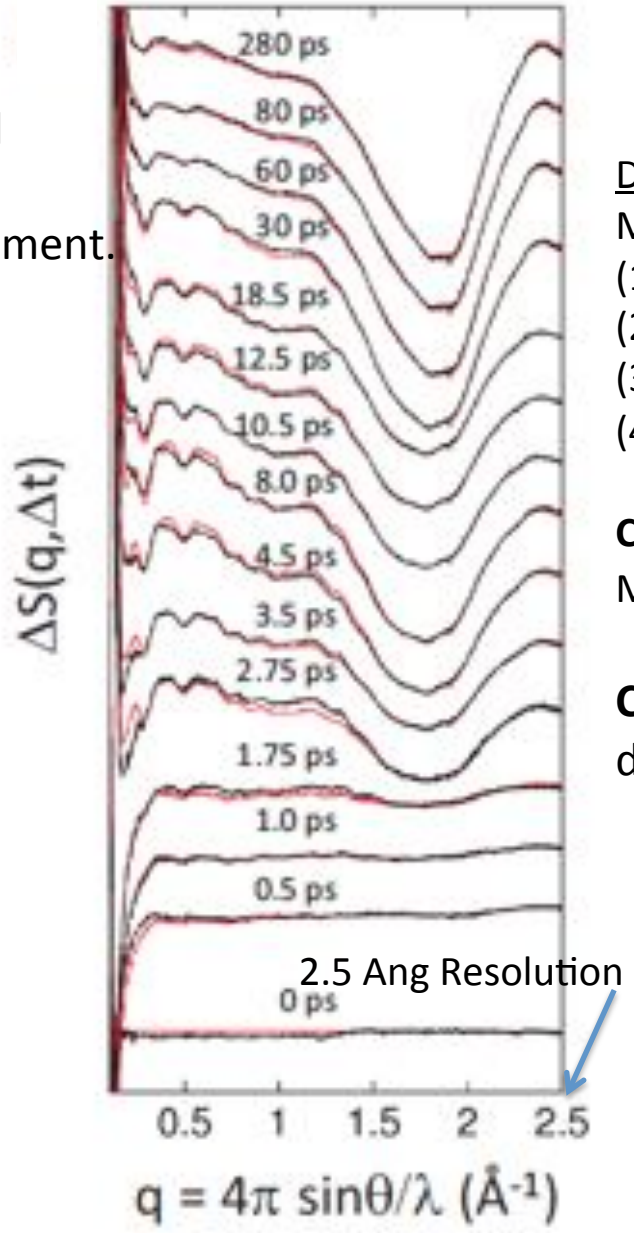
Scattering Pattern

WAXS profile

Diff = WAXS – $WAXS_{ref}$

FSS at EXFEL finds evidence for fast nuclear motions in photosynthesis

Black: model
Red: experiment.



Compare change in FSS due to pump with model

Data analysis:
Modified SVD to find dominant components:
(1) Describe conformation populations using rate equations
(2) Extract components
(3) Check whether the model can explain the observed changes
(4) Conformation sampling using MD simulations

Conclude: Global conformational change damped within 80 ps. Movie from MD.

Conclude: ps nuclear motions occur (low q) as quake to dissipate solar photons. Pump laser power titration done.

High spatial resolution obtained through modeling
Higher time resolution than synchrotron (100ps)

Conclude: 3 Ang model from TR-FSS with 500 fs time resolution supports a "quake" (low q) model of energy dissipation, followed by heat (high q) pulse.

Neutze group. Nature Meth 2014 Arnlund et al .Aug 12

The time for SFX data collection and analysis has been reduced greatly between 2011 and 2014.

Protein	2011 Cathepsin	2014 Phycocyanin
Sample injection	GVDN	LCP
Protein size	37 kDa protease	209 kDa hexameric antenna complex
Year of experiment	2011	2014
Data collection took	**5 days**	**2.5 hours**
Crystal hits	293,195	18,794
Indexed hits	**178,875**	**6,629**
Time data took to analyze	**1 year**	**3 months**
Resolution	2.1 Å	1.95 Å

The improvement is due to many incremental advances and high quality nanocrystals.

Phycocyanin: SFX results of crystals delivered in the liquid jet and embedded in LCP were in good agreement, but showed significant difference from results on similar samples recorded from cryo-cooled samples at a synchrotron.

Phycocyanin in LCP:
Only 6 µL of crystal suspension of globular protein transferred into LCP

Ginn, Stuart, Garman et al . **used 6000 patterns in 30 mins to get 1.74 Ang.** map of Polyhederin CPV17 (Aug 2014). Adjust λ for max # of spots, optimize orient matrix

This greatly advances our goal of increased capacity for SFX work and XFEL availability for biology

Current Research

Making Nanoxtals

BioXFEL is developing new methods to make and identify nanoxtals

The ability to obtain diffraction patterns without damage allows us to study protein nanoxtals, which would be destroyed before providing a useful pattern at synchrotron

Contact: Ed Snell at Hautpman-Woodward Institute , Buffalo NY or Prof Alex Ros, ASU

Kupitz, Fromme et al Phil Trans (2014) Review.
Spence, Weierstall, Chapman (2012) Rep Prog Phys review.
Snell/Luft H-W. Poster, this conference (2014)
Ros , Abdalllah et al ACS Nano (2013).
Caffrey, Cherezov, Weierstall Growth in LCP.

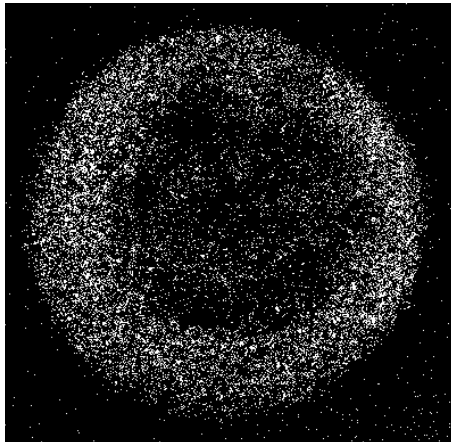
A new imaging technology can identify invisible submicron crystals: Sonicc.

Sonicc: Garth Simpson, Purdue (second order non-linear imaging of chiral crystals)

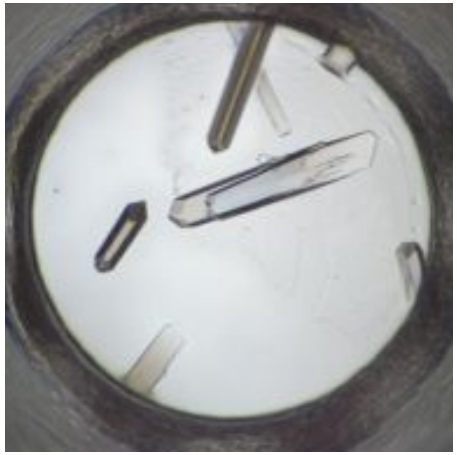
Second-harmonic Generation (SHG) doubles light freq if acentric.

protein from the pyruvate dehydrogenase complex

Brightfield



SONICC



Optimized Crystals

Aim is automated identification of nanoxtals in ***1536-condition hi-throughput*** screening lab at HW by SONICC and Trp fluor.

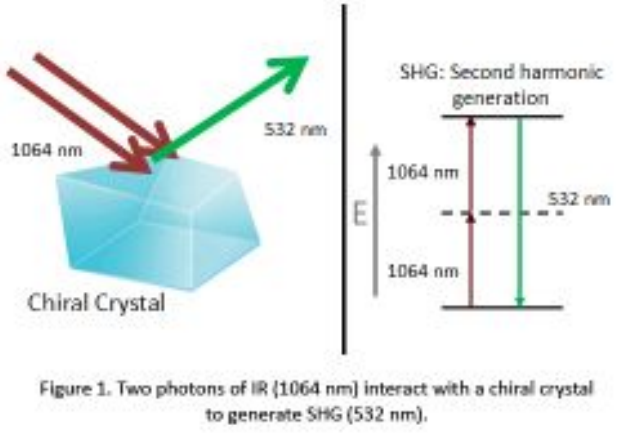


Figure 1. Two photons of IR (1064 nm) interact with a chiral crystal to generate SHG (532 nm).

Snell and Luft, Buffalo. 2014

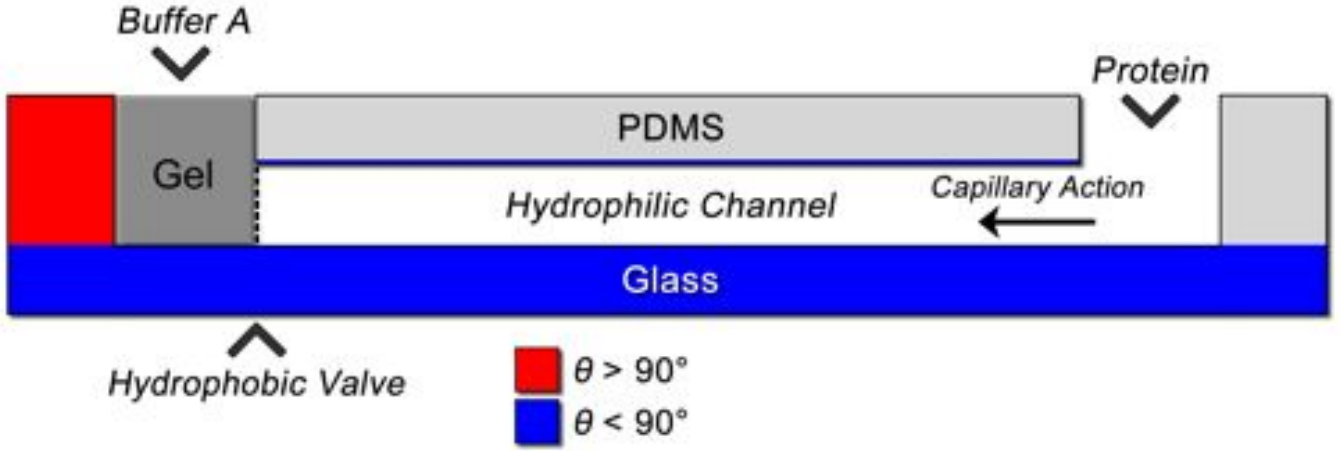
*SHG Finds all acentric xtals, some weak
*low background
*detects submicron xtals
*Doubled green incident light also excites tryptophan in UV if protein.

Annu Rev Anal Chem. 2011 ; 4: 419–437.

The Ros group at ASU has developed microfluidics for growing nanoxtals under SONICC observation.

» Controlled parameter search for conditions to **grow nanocrystals** of membrane proteins

Device Schematic



Crystal growth trends and conditions

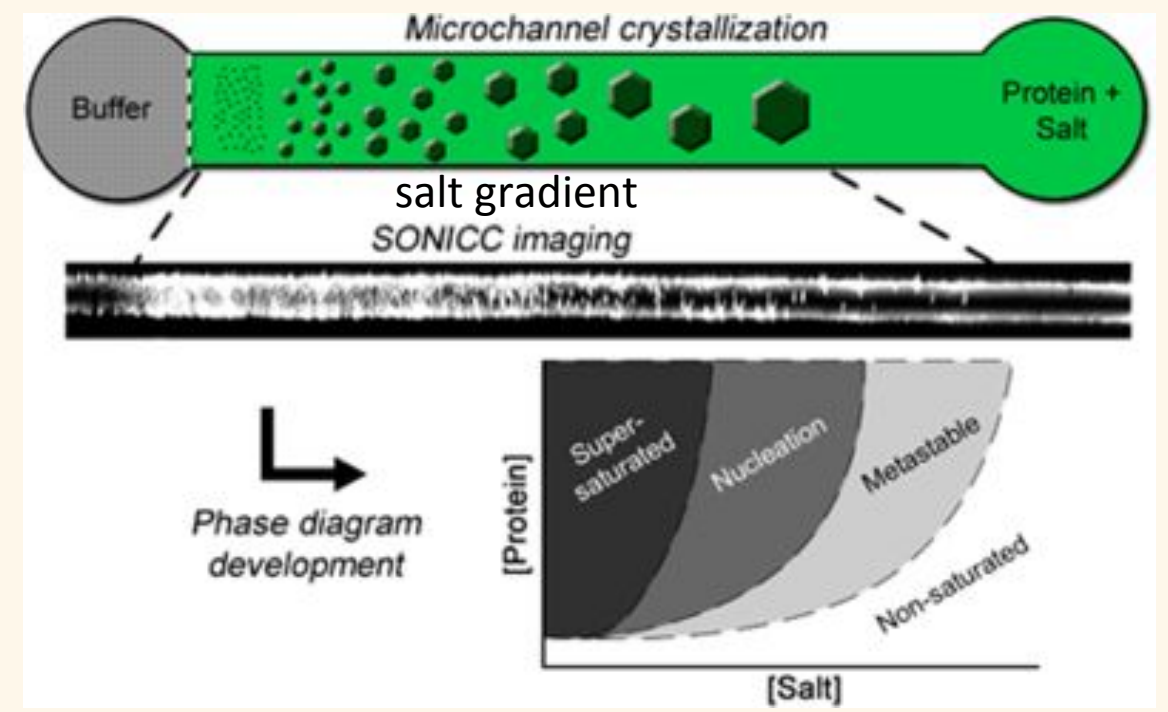


Image salt gradient from above by SONICC. Get phase diagram.

Prof A. Ros, ASU chemistry.

B. G. Abdallah, C.Kupitz, P. Fromme, A. Ros, ACS Nano (2013) 7, 10534-10543

This movie shows microcrystals sorted by size.

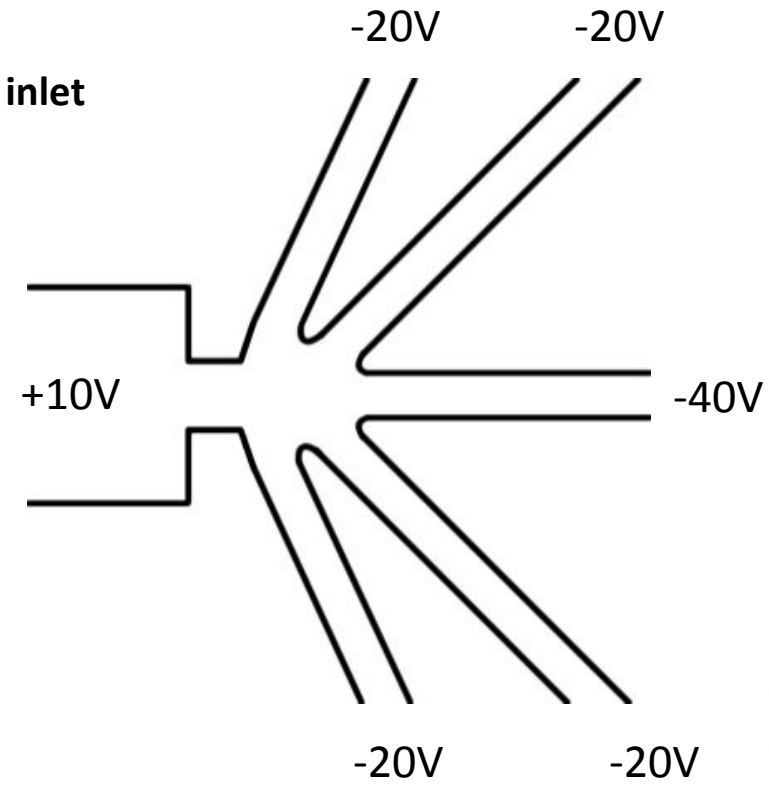
Alex Ros group ASU

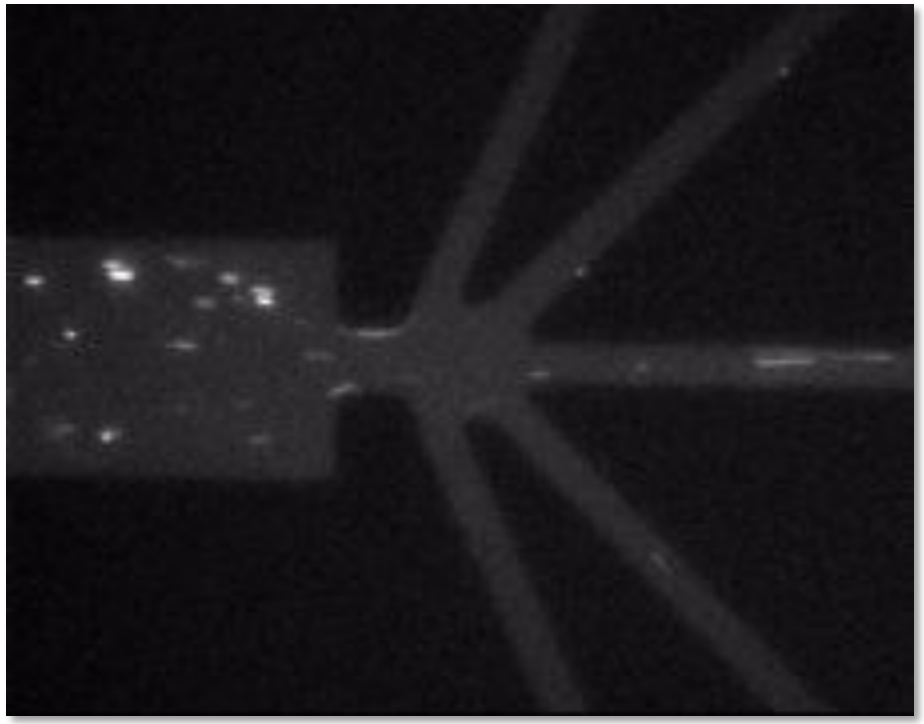
Dielectrophoretic (DEP) Sorting

This sorter will feed sample delivery at LCLS.

Photosystem I Crystal Sorting Experiment

(use smallest xtals for phasing ?)





➜ **Bright channels**: fluorescent PSI nanocrystals
➜ **Bright particles**: fluorescent PSI microcrystals

B. G. Abdallah, T.-C. Chao, C.Kupitz, P. Fromme, A. Ros, ACS Nano (2013) 7, 9129-9137

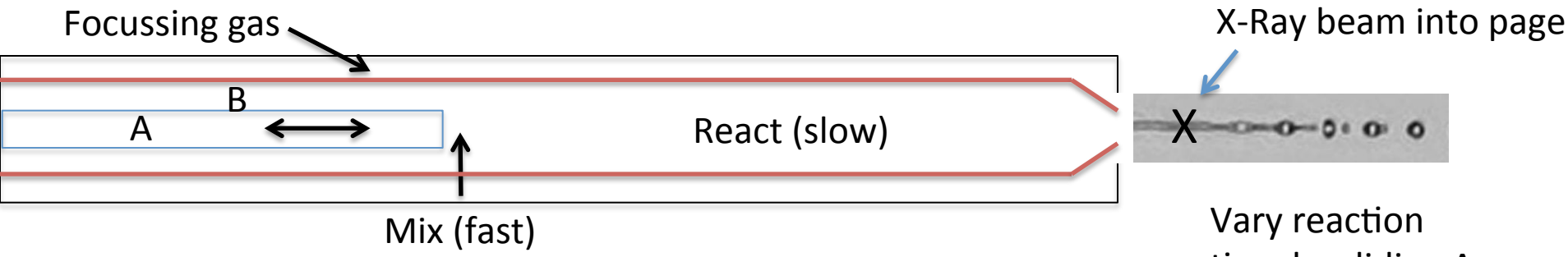
Improved sample delivery devices

2. A double-focusing mixing jet has been built for the XFEL.

(eg. study of enzyme/substrate reactions, folding of DNA, tRNA)

If no
sharp trigger..

D.Wang, J. Spence, U.Weierstall, L.Pollack.

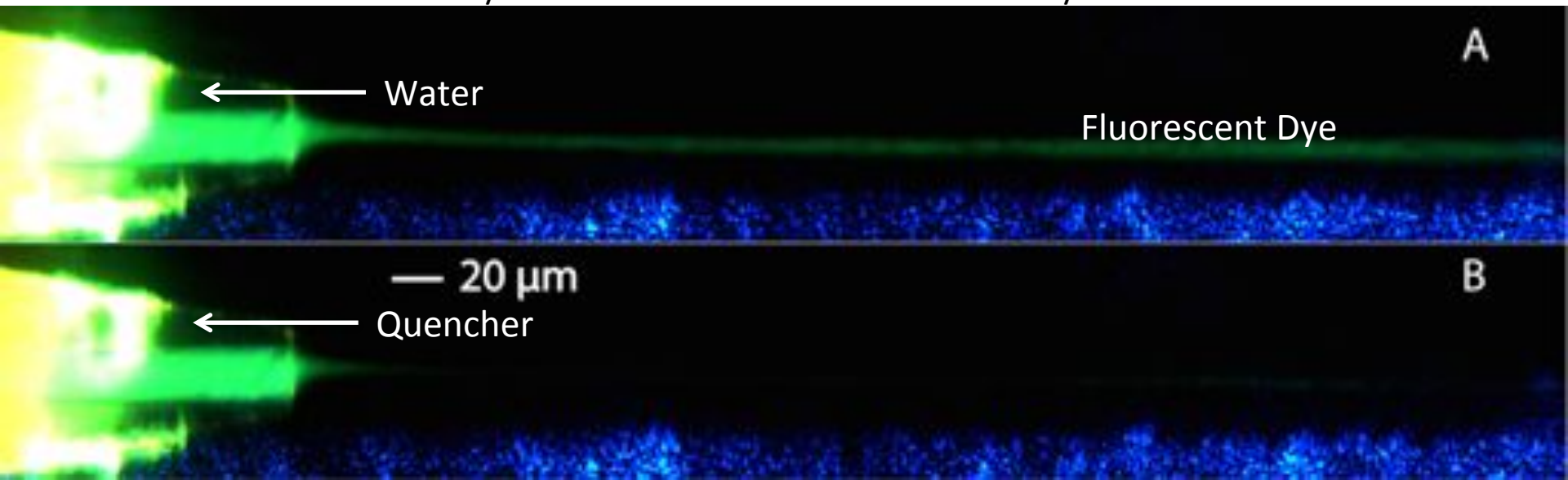


Vary reaction
time by sliding A

Diffusion into nanxtals is fast

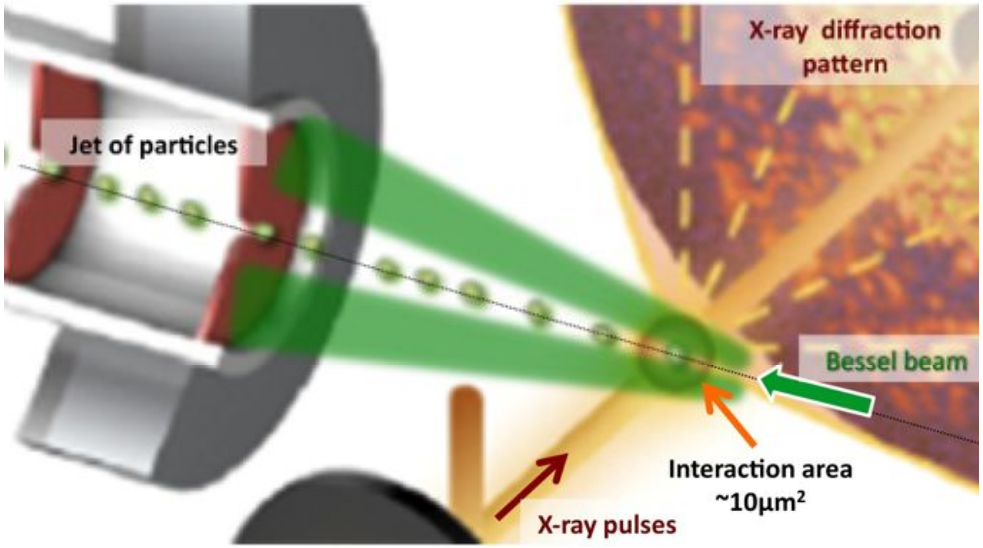
Mixing time (time resolution) is 200 microseconds
Reaction time adjustable from 200 microsec to 1 sec.
Justification for XFEL: 2D patterns ? Reduce damage

Outer flow rate 50 microliters/min. Inner flow rate 0.1 microliters/min.

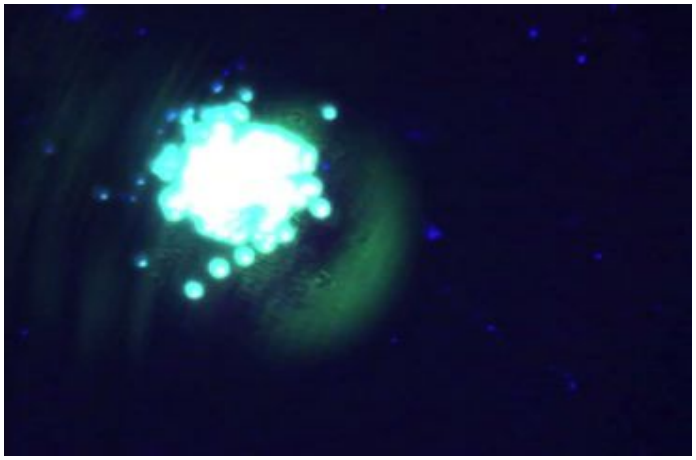


Single-particles can be run along a hollow light pipe (Bessel beam).

This is Kirian's STC project at ASU Physics (and DESY).

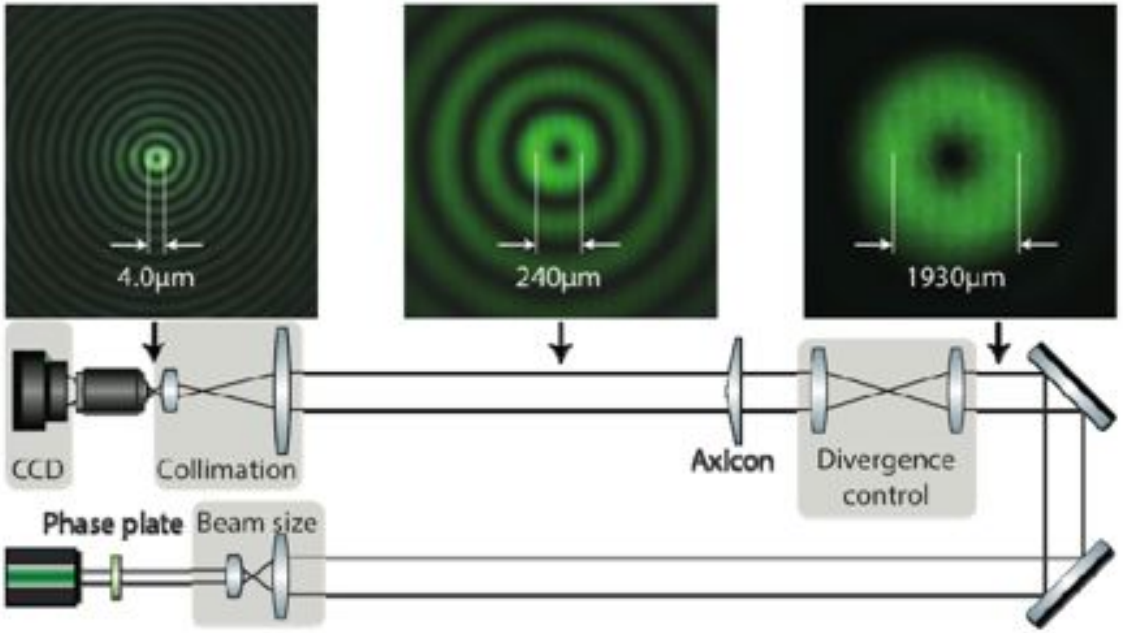


Movie – view along beam



2 micron polystyrene spheres steered

Ekerskorn, Kirian et al Optics Express 21, (2013)



This advances our aim of SP imaging by increasing the hit rate.

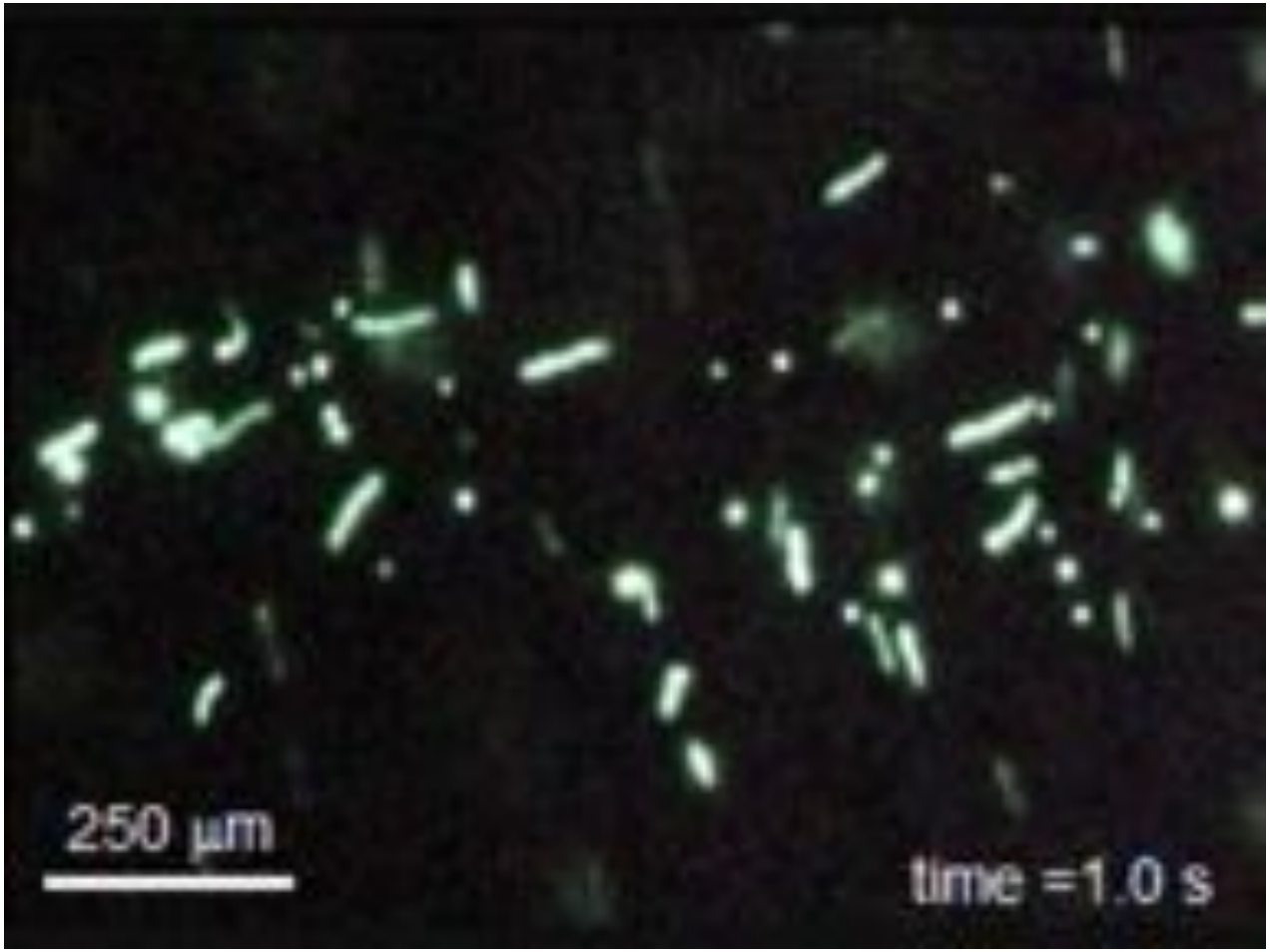
A new kind of optical trap may be useful for single-particle imaging

It is based on the Photophoretic force

Side of ball heated by optical absorption recoils more than cold side.

Atoms traps don't work for large hydrated ptcles

Laser tweezers (gradient force) normally reply on viscous damping. Hard to fill in vacuum.

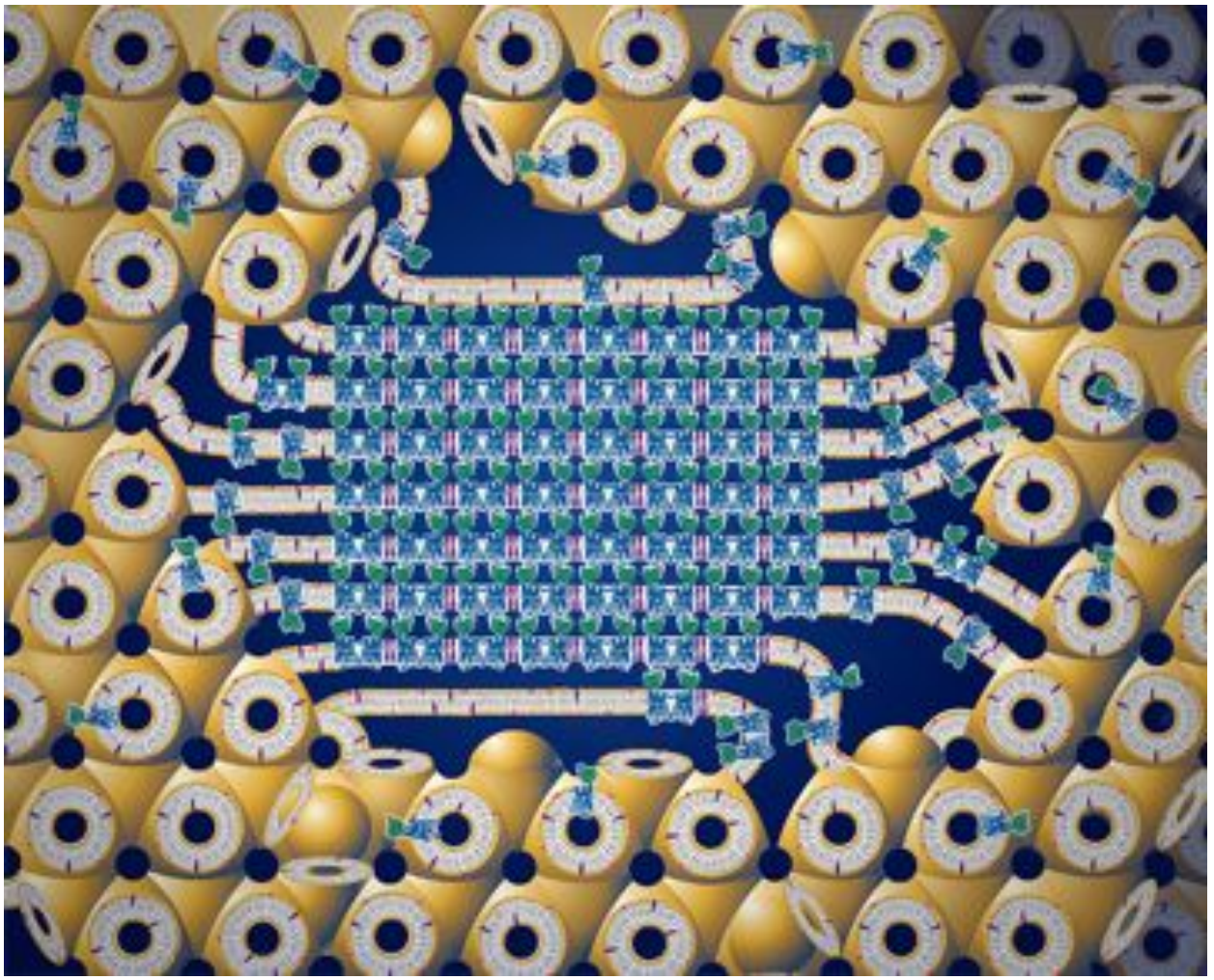


2 micron carbon ptcles trapped in speckled "bottle-beam". Movie.

Schvedov et al al Optics Express 2012.

LCP injector: wastes less protein, high hit rate, nanoxtal growth medium.

Membrane protein embedded within lipid cubic phase (viscosity of car grease !)

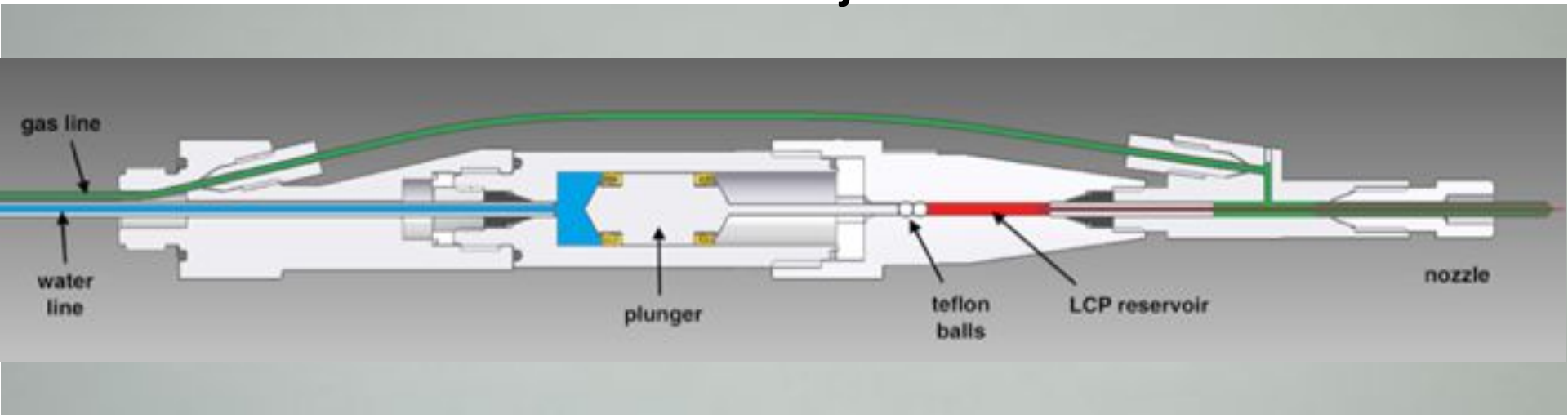


Martin Caffrey

Water jet flows at 10/s (10 microns/microsec). Can higher viscosity match nanoxtal flow rate to arrival of X-ray pulses at 100 Hz ? TOOTHPASTE JET !!!!

3. SFX in LCP works at high res – Time-resolved SFX in LCP.

The LCP injector



*LCP provides a **growth** medium for many proteins, including membrane proteins.

*LCP jet delivers ptcls at about the **rate** of X-ray pulses – high hit rate.

*Low flow rate avoids **wasted** protein (1-300 nl/min vs 10 microL/min in water jet).

*Use less protein; microliters not milliliters, at a few mg/ml of precious human protein

The ASU shop is building six for STC/other users.

Merck, Agilent

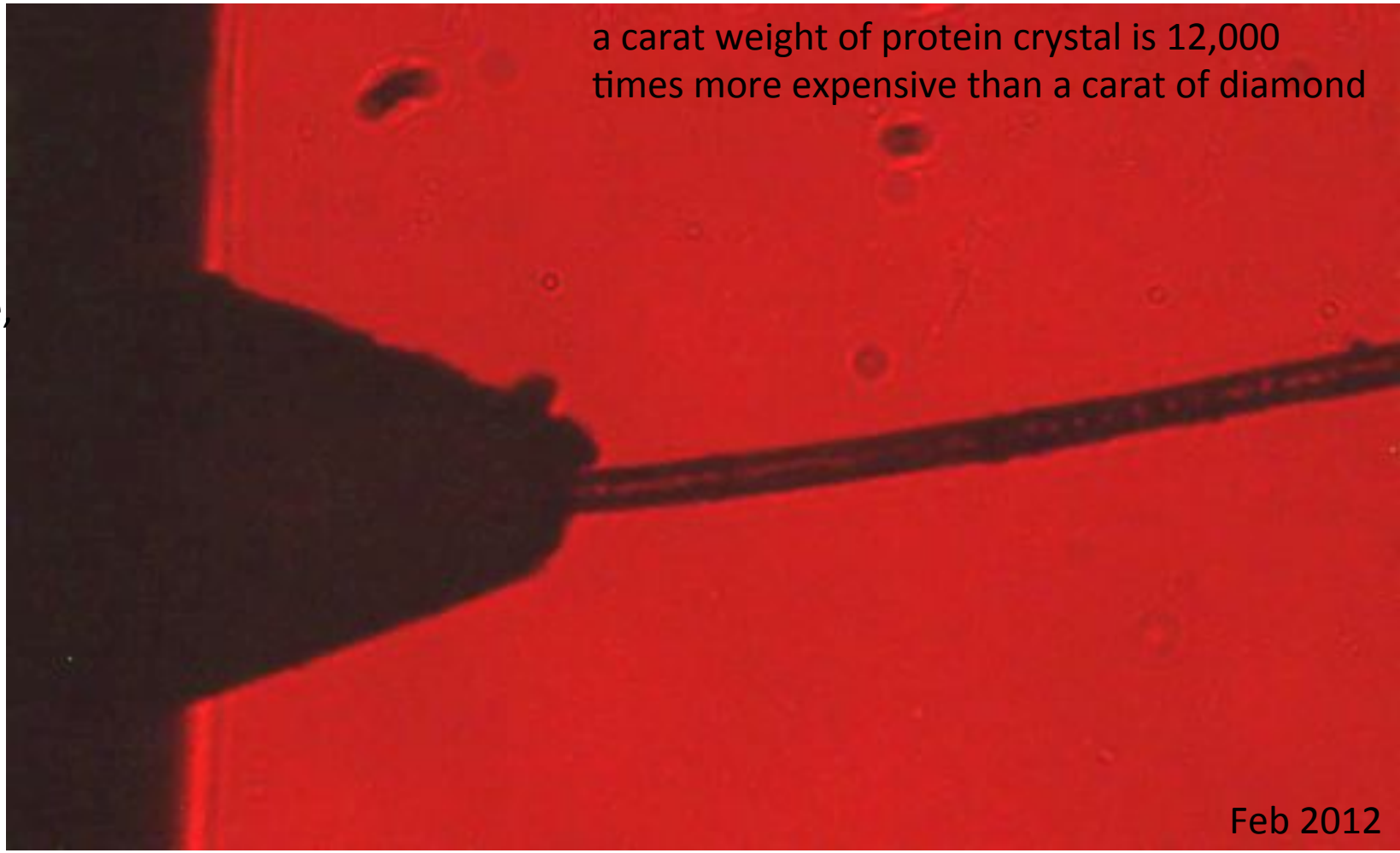
Constant pressure, or constant flow rate, modes (with HPLC pump). Pressure amplifier.

Used to solve Cyclopamine GPCR binding to smoothen receptor (3 A).

Uwe Weierstall, Dan James (ASU PhD), Martin Caffrey, et al Nature Coms Feb. 2014

Slow LCP "toothpaste" jet wastes less protein, good for GPCRs Grows nanoxtals, high hit rate.

GPCR in viscous LCP at 300 picoliters per minute. LCLS at 1 Hz. 9.4 kV 7%
50 microliters total used Later 5 microliters/min Adenosine A2A



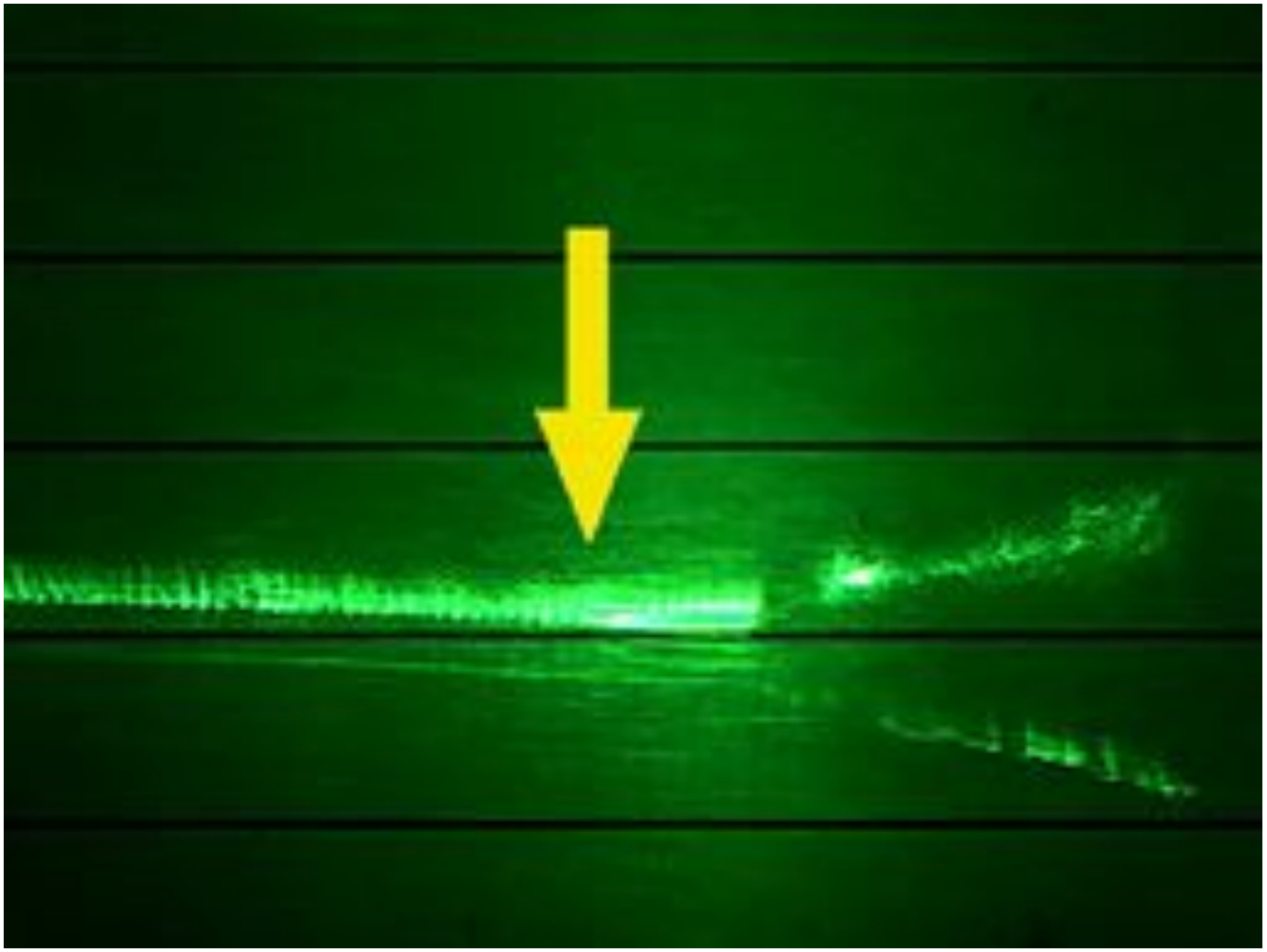
Optimize
conc, rep rate,
viscosity,
particle size,
jet size,
for each
sample.

V = F/A
AV = const.

Vadim Cherezov. High rep rate of NGLS helps

Uwe Weierstall

We can image the LCP stream hit by the XFEL beam



green laser illumination synchronized with the LCLS X-ray pulses at 120 Hz

movie

1.6 mm/sec (190 nL/ min)

LCLS at 120Hz

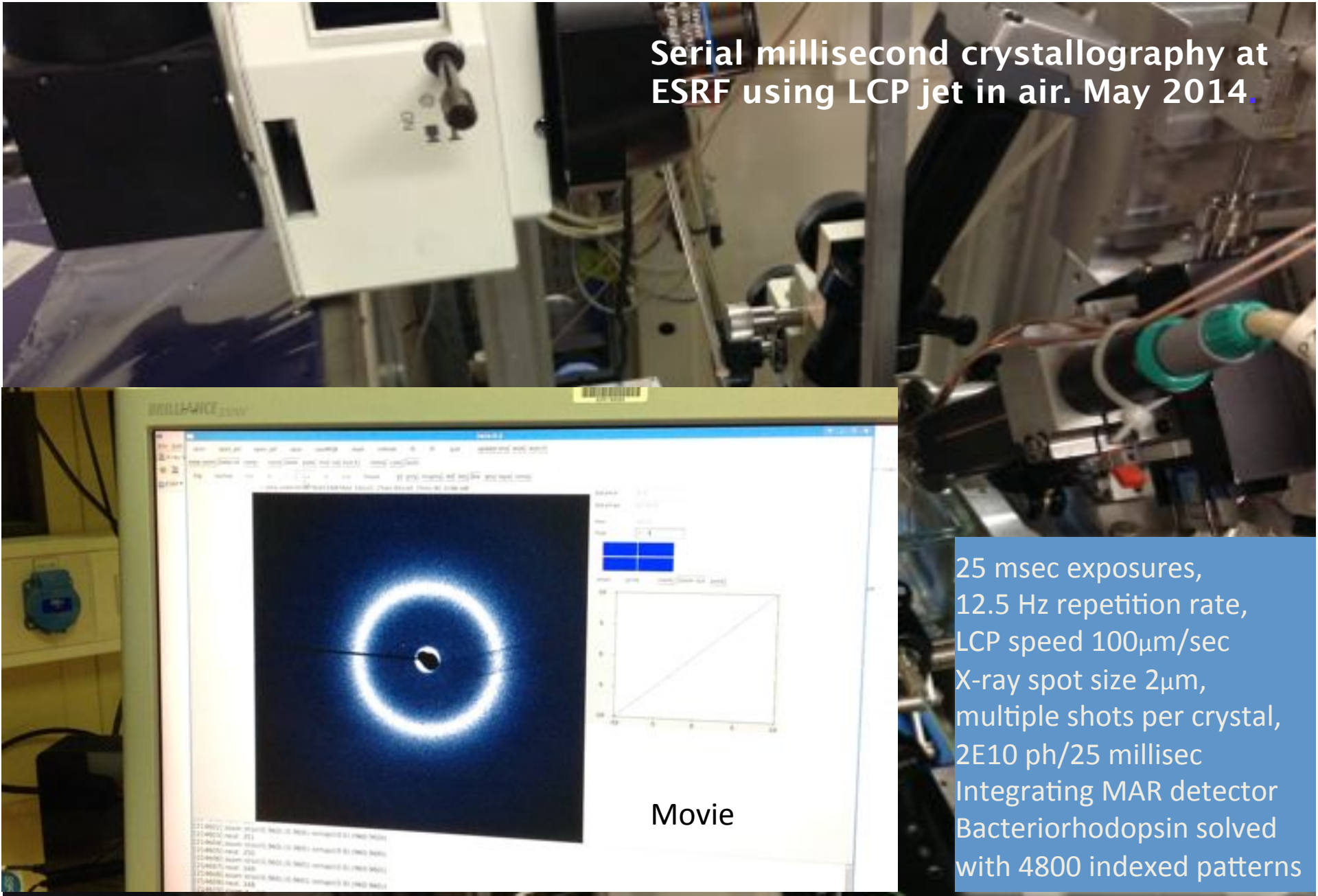
Works at synchrotrons…

must clear debris before next shot.

Optical props of LCP for pump light.

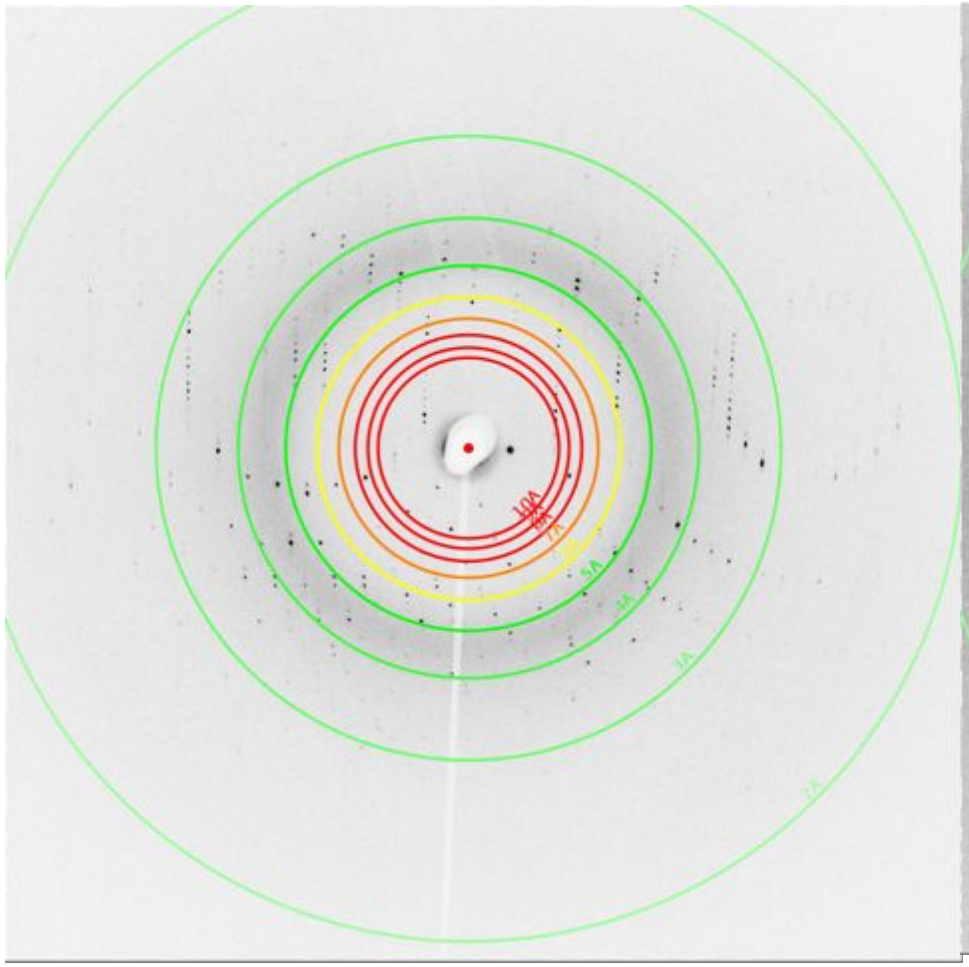
REF: Weierstall et al

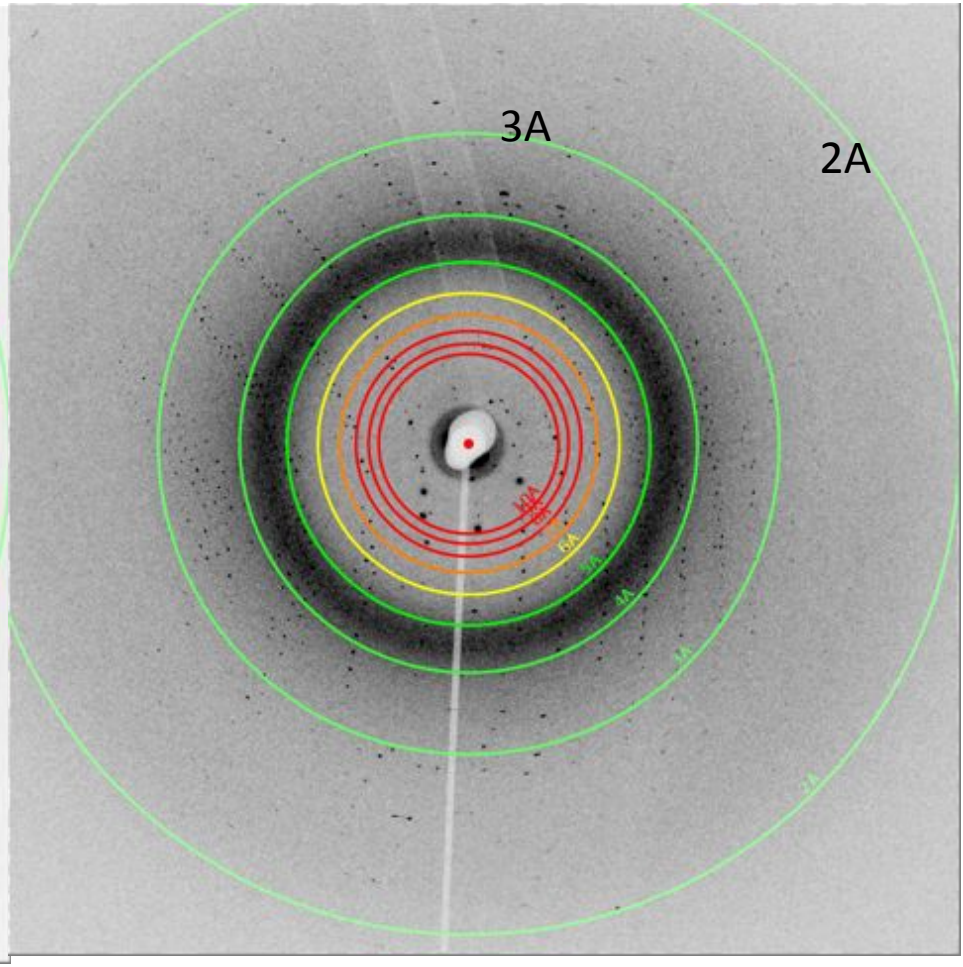
Our LCP jet works on a synchrotron at atmos. pressure with fast shutter



25 msec exposures,
12.5 Hz repetition rate,
LCP speed 100μm/sec
X-ray spot size 2μm,
multiple shots per crystal,
2E10 ph/25 millisec
Integrating MAR detector
Bacteriorhodopsin solved
with 4800 indexed patterns

The resolution of LCP-in-air data extended to 2 Ang at ESRF





Lysozyme and bR in LCP in air at ESRF, May 2014. Joerg Standfuss, Uwe Weierstall, Schertler et al

“Snapshot SAXS”

* Extracting an image of one particle
  using the scattering from many copies*

  (ab-initio, without SAXS modelling)

* If particles are frozen in space or time, the normally isotropic WAXS pattern becomes 2D

- Hit rate 100% ! No orientation determination !

*identical and randomly oriented. By summing the angular correlation functions we unscramble the orientational disorder.

Fluctuation cross correlations

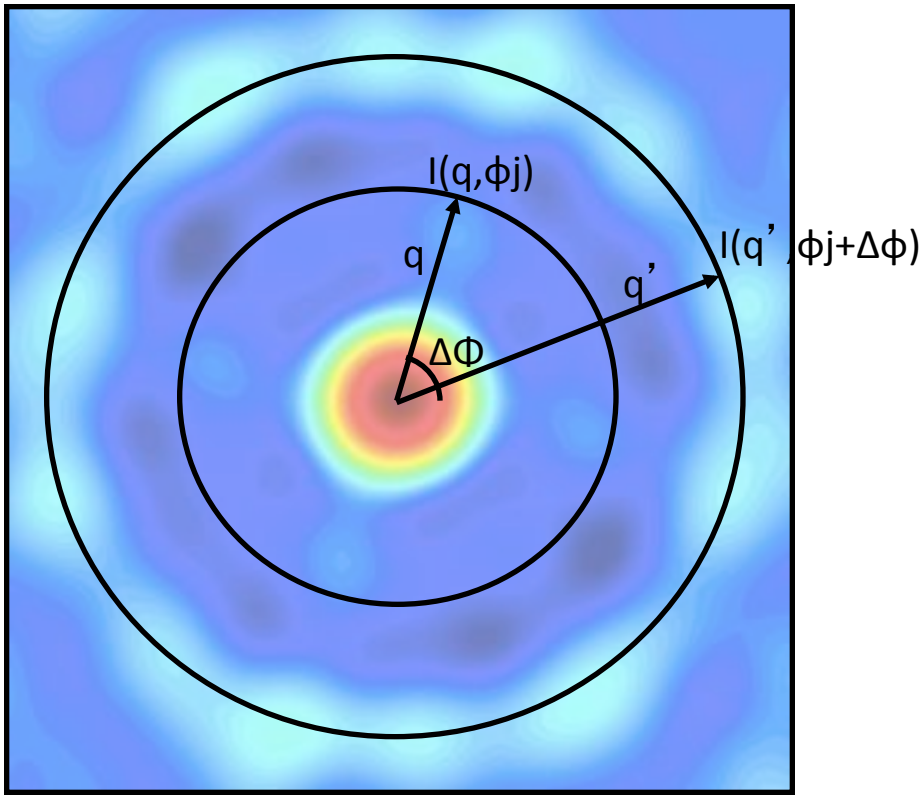
The experiment consists of detecting scattered intensity at TWO points on a ring, and multiplying these together. Then summing around the ring.

Averaged pair correlations are azimuthally symmetric, they depend only on Δϕ. We measure the angular cross correlation functions in the intensity *fluctuations*:

$$C_2(q,q',\Delta\phi) = \left\langle I'(q,\phi) I'(q',\phi+\Delta\phi) \right\rangle_\phi$$

$$I'(q,\phi) = I(q,\phi) - \left\langle I(q,\phi) \right\rangle_\phi$$

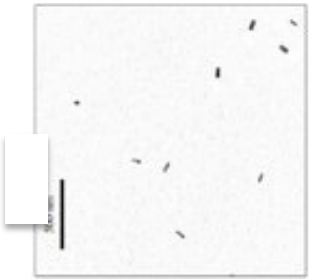
intensity fluctuation (mean subtracted ring intensity)



D. Saldin 2010

For identical, randomly oriented particles frozen in space or time we can reconstruct an image of one particle, using the scattering from many…

Kam's method unscrambles orientational disorder, ab-initio, without modelling.



Many identical gold rods

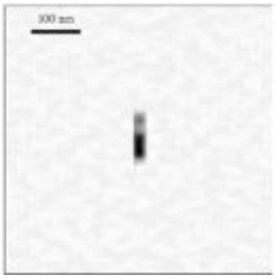
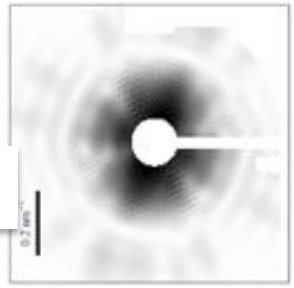


Image of ONE gold rod !!

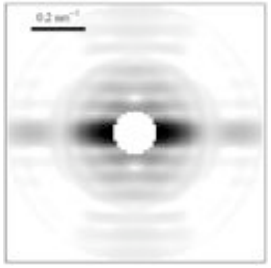
FT

FT

FT, phasing



No "SAXS" modelling !



FT, phasing

Angular Autocorrelation Function

+

Angular Autocorrelation Function

+ ……..

=

Angular Autocorrelation Function of ONE partilce

Kam, 1978 ; Saldin, Spence, Kirian PRL 2011, Phys Rev B81, 174175 (2010) Starodub et al 2011.

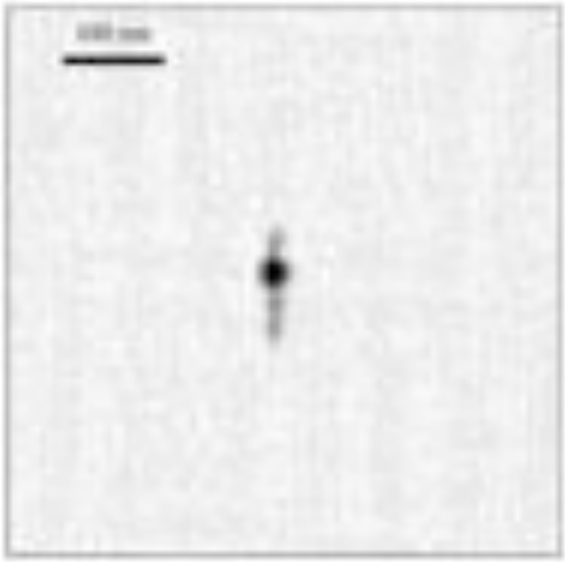
Experimental demonstration – in two dimensions.

Diffraction patterns were collected from 90nm gold rods
at ALS using coherent 2nm X-rays. Single-axis alignment

TEM Image

Reconstruction from XRD

Now apply this to an enzyme in our mixing jet. Doniach.



Conformations separated !

FIG. 1: Electron microscope image of a typical sample from which soft x-ray diffraction patterns are measured. Each sample consists of a set of about 10 gold nanorods of approximately 90 nm × 25 nm projection in random orientations about the normal to a transparent SiN substrate. There is a small admixture of ~ 25 nm diameter gold nanospheres.

FIG. 7: Projection of the electron density of a single ~ 90 nm × 25 nm rod reconstructed from the diffraction pattern of Fig. 6 after 500 iterations of the reciprocal-to-real-space phasing algorithm. The bulge near the center is probably a superposition of an image of a nanosphere, also found in the experimental sample (see Fig. 1).

Both beam noise and Kam fluctuations are propn to sqrt number of particles/shot, so ratio S/N isn't. Hence S/N is independent of number of particles/shot Kirian et al (2012). Elser. Wochner.

Saldin, Kirian, Marchesini, Spence…. Phys Rev Letts. 106, 115501 (2011).

The KAM angular correlation method for FSS was demonstrated without modeling.

The diff pattern for one oriented dumbbell was reconstructed from the angular correlation fn of many patterns from dumbells in random orientations, then phased iteratively.

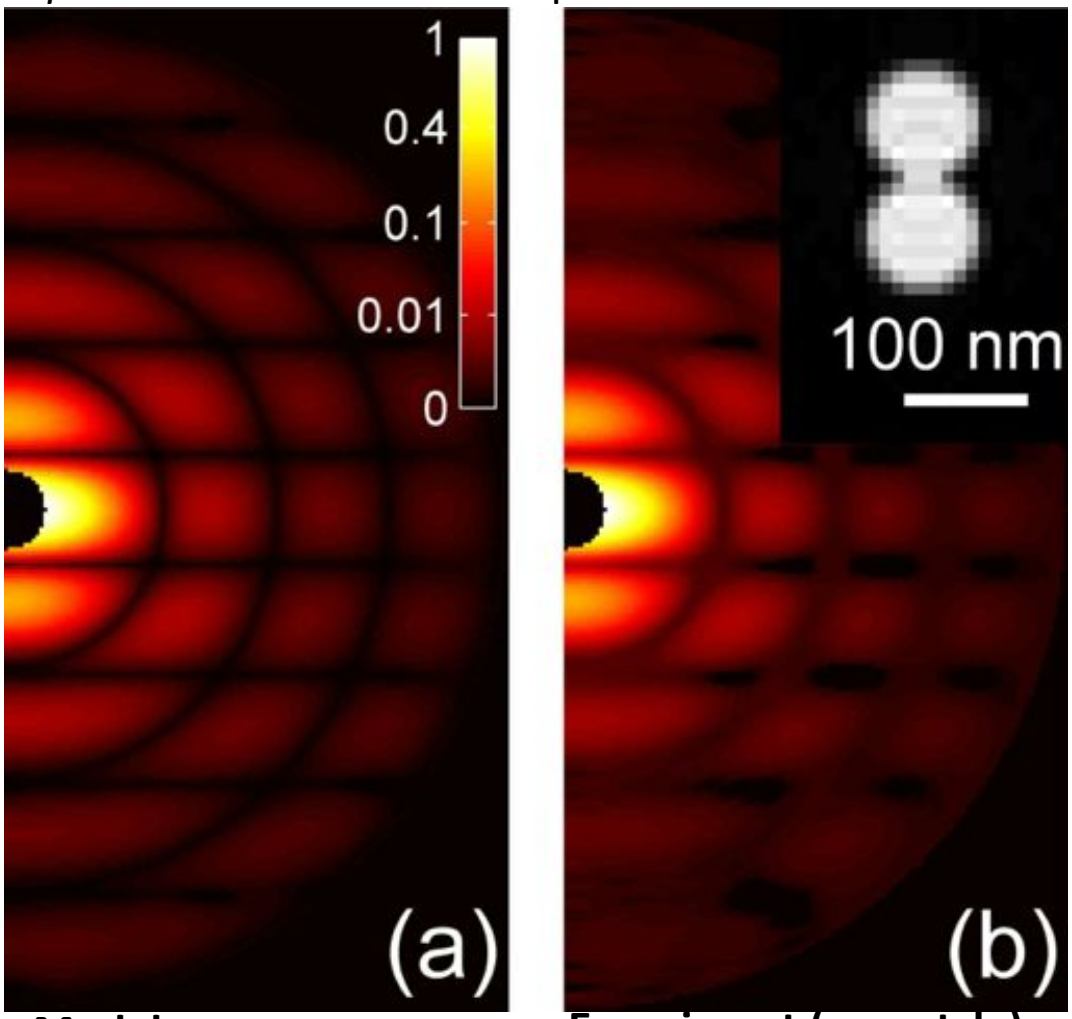
Each diff pattern comes from *one particle per shot* in solution. 635 patterns.

Two angles required
to define orientation.

Ewald sphere curvature included
Cylindrical harmonic basis
Triple correlations from squared intensities used to get signs (phases)

Polystyrene Dumbbells
Each sphere has diam 91 nm.
Resolution 10 nm.

Hit rate if many particles/shot is 100% !!!!



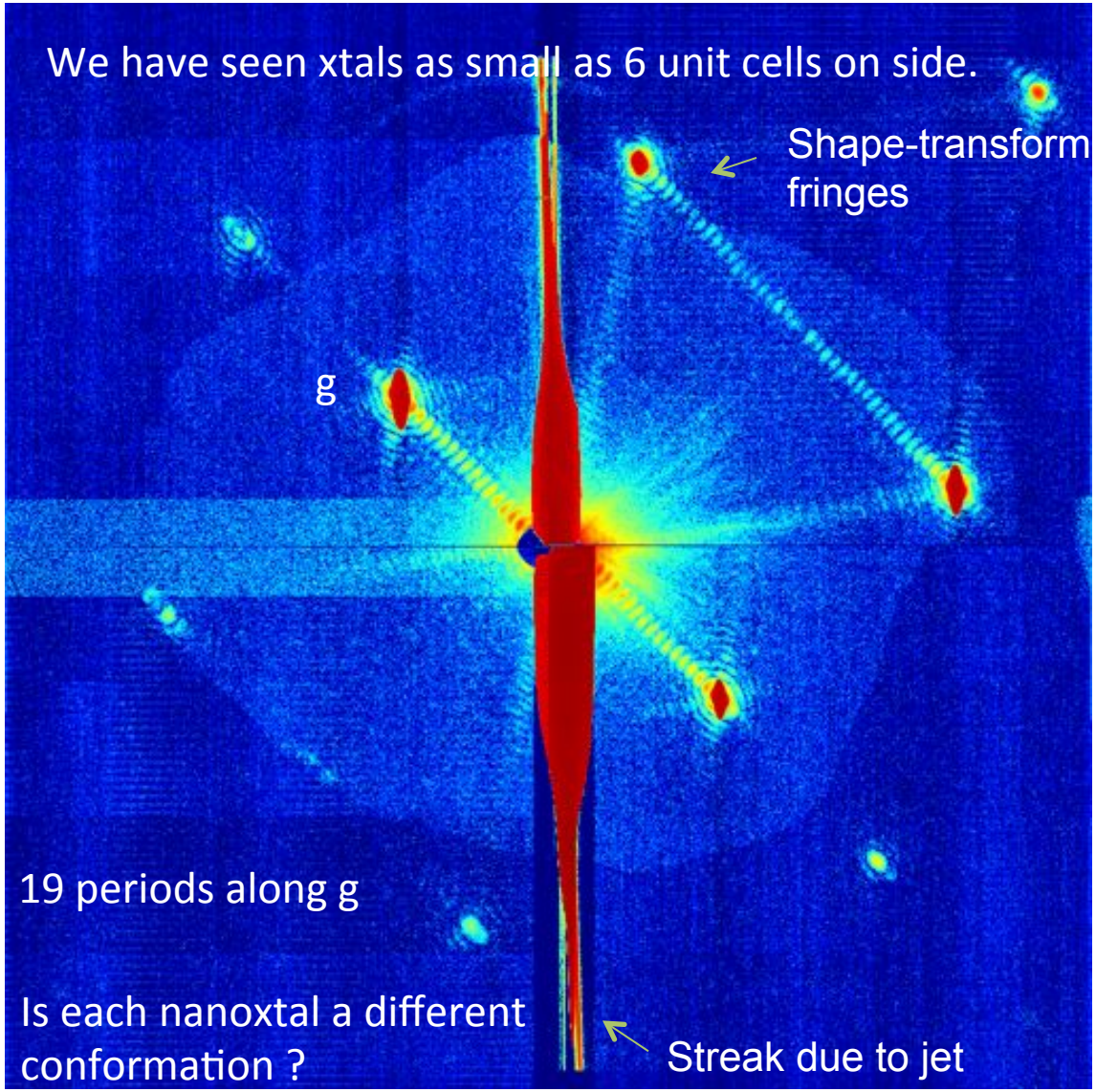
Model **Experiment (one ptcle)**

Starodub, Bogan, Spence… Nature Comms 2012

A new solution to phase problem – ab initio Image nucleation and xtal growth ?.

Spence

BioXFEL

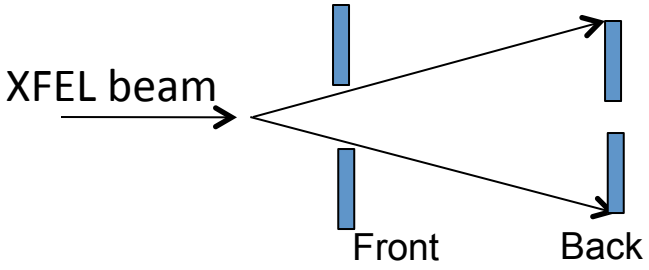


Phasing by shape transforms
- Does not need atomic res.
- No chemical mods to sample
- Ab initio, PDB not used,
"a new measurement"

Fast SAD also possible ?

To phase, "divide out" particle size distribution.

Every nanoxtal is in a different (random) orientation.

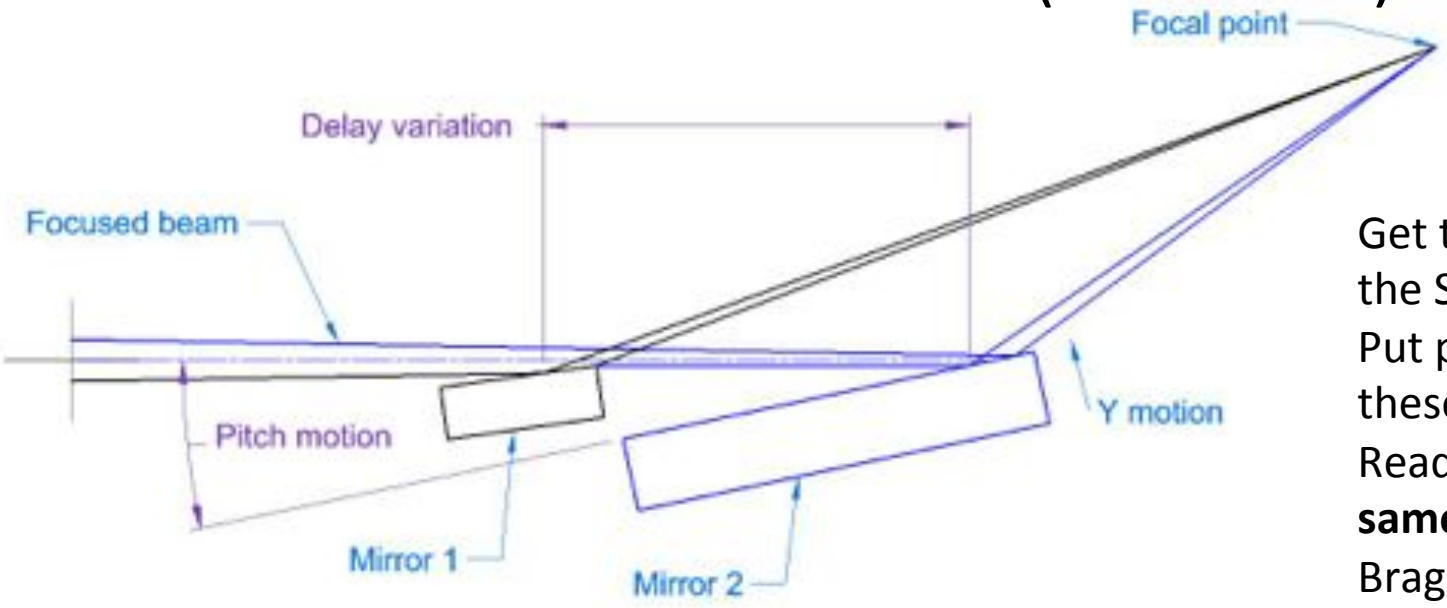


Photosystem I nanocrystals at 2 kV (6.9 Ang wavelength).
Single Shot (10^{12} photons incident).

See Spence et al Optics Express, 2011

Split and delay, two color methods

Split-and-delay, 2 Color. More accurate Time-resolved SFX ?. 2 Color for SAD ? (Wakatsuki)



Get two hits from the SAME particle.
Put pump laser between these X-ray hits.
Readout both hits on **same** detector readout.
Bragg spots slightly shifted

Currently works up to 2 kV. Delays up to 20 ps. Longer are better done at synchrotrons.
Use either "2 color" or change of beam direction to get two shots on same detector readout.
Pump laser can occur between X-ray shots (needs very long optical path at 3mm in 10 ps)
Errors due to variations in xtal orientation, size and shot-to-shot are mostly elliminated.
If pumped between X-ray pulses, first pulse must not damage xtal.

Notes:

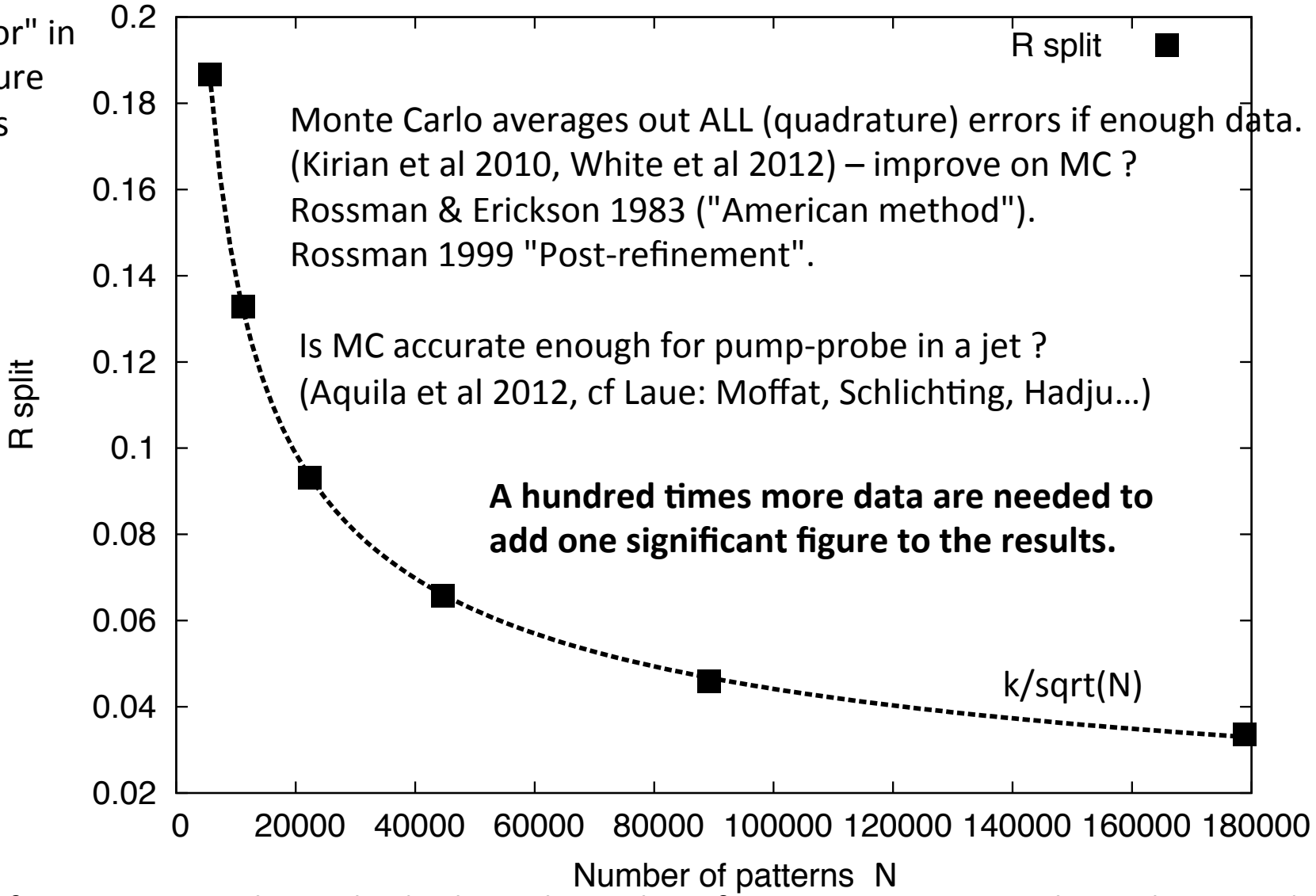
1. Other split-and-delay, **2 color schemes** under development (split undulator, slotted foil)
2. Diamond crystal self-seeded beam. Same direction, focus. Hard Xrays < 150 fs delay (Soichi)
4. Use LCP jet for sample delivery. Bragg spots from shot pairs displaced on detector.

Can we improve on the Monte Carlo method for TR-SFX ?

Evidence that S/N improves as 1/sqrt(N) for N shots from Cathepsin data. (K.Nass Feb 2013)

XFELS cause big errors in SFX (Shot-to-shot 15%, xtal orientation, size)

~ "Error" in structure factors



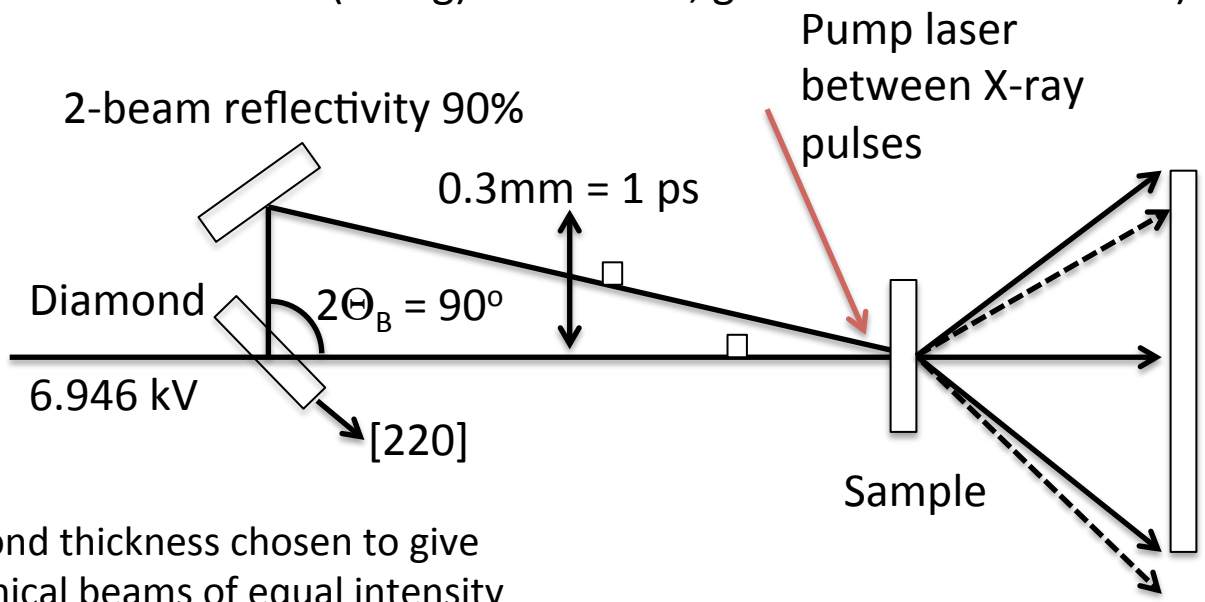
Validity of our Monte Carlo method is best shown by R factor comparison with synchrotron data

4. Split-delay improves accuracy for time-resolved diffraction

* Pump between XFEL pulse and delayed pulse
* These pulses have equal intensity no shot-to-shot fluctuations.
* Measure (almost) same ***partials*** on ***same*** detector recording
* First pulse does not destroy sample, so scattering weaker.
* Use LCP jet. Splitters produce extreme collimation and monochromation.

(So use Mirror at 4 kV (3 Ang) or 2-colors, generated in undulator)

Or: Two color.
(max delay 150 fs)



This measures change in partial reflections due to pump laser with little error. Given a model, this is all you need !

Diamond thickness chosen to give dynamical beams of equal intensity via Bormann effect.

10 ps ~ 3 mm
2ns ~ 0.6 m path diff
2 microsec ~ 600 m path diff.
Rot Diff Time ~ 10 ps for mol (Arg, RT, Water)
Rot Diff Time ~ 100 ms (E Coli)
Use LCP ?

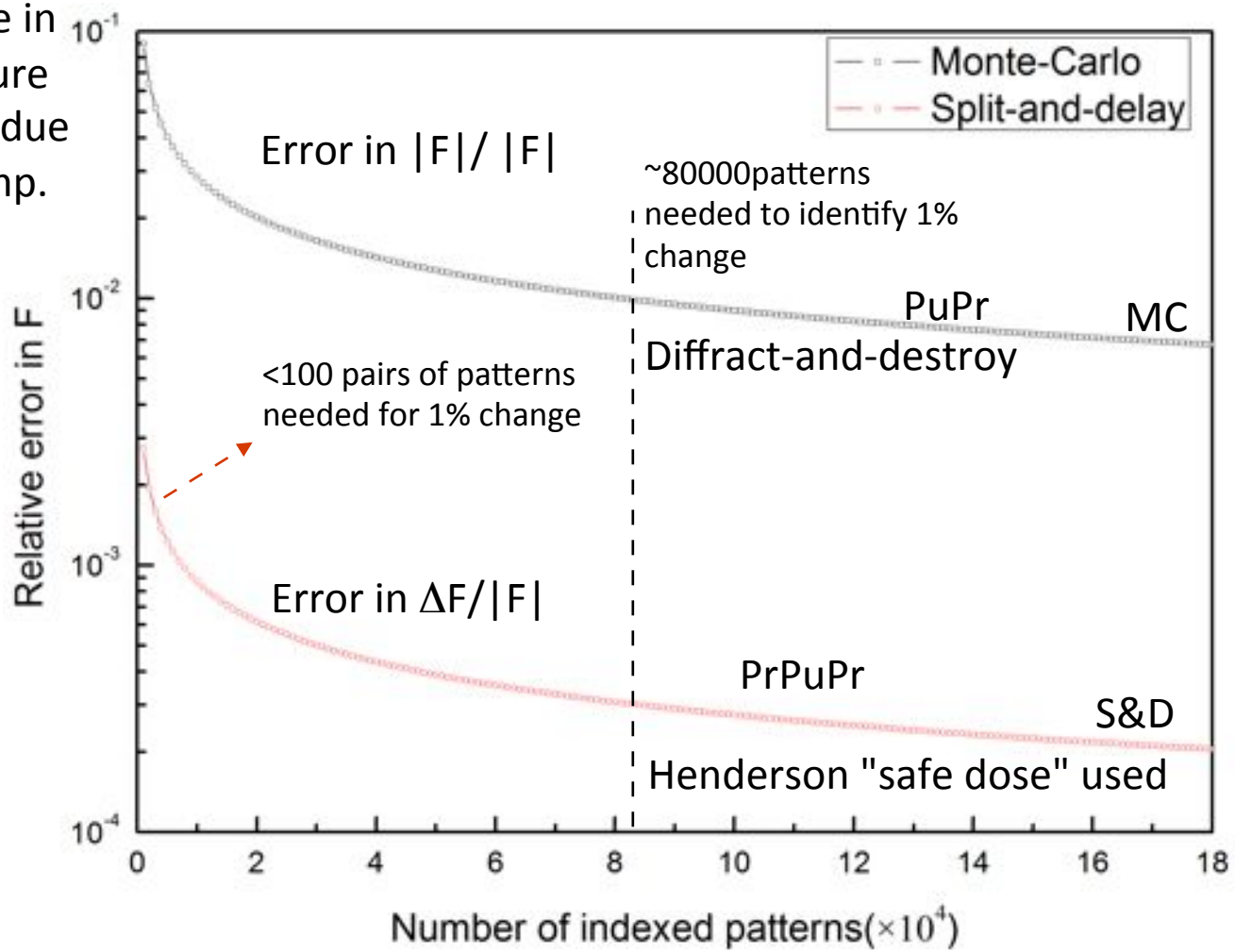
Notes:
*Here we measure only differences between "same" partials. Shot-to-shot variation of 15% eliminated. Str factor change due to pump Is < 15%. Orientation, xtal size errors elliminated.
*Laue, CB wont work through beamsplitters. (Mirrors work below 2 kV now, more later ?).
*Jitter between pump and LCLS now > 50 fs, but <10 fs with "measure and sort"

Error in two-color, delay, falls more rapidly with number of shots than Monte Carlo

Relative Error comparison : MC v.s. S&D

BioXFEL

Error in change in structure factor due to pump.



$$\frac{\sigma_{MC}^{(N)}(|F|)}{\langle |F| \rangle} \approx 2.85 \frac{1}{\sqrt{N}}$$

$$\sigma(R') \approx 0.087 \frac{1}{\sqrt{N}}$$

X-P-X

$$R_{(hkl)}^{(i)} = \frac{\Delta(|F_{(hkl)}|)}{|F_{1,(hkl)}|} = \frac{|F_{2,(hkl)}| - |F_{1,(hkl)}|}{|F_{1,(hkl)}|}$$

Chufeng Li and JCHS

A movie of the rad damage processes in SFX can be made using 2-color.

Without any pump pulse,

1. First pulse initiates damage, but gives undamaged pattern.
2. Second pulse (different λ) gives image of sample after delay. (does add damage).
3. Both patterns impressed on same detector readout. Wavelength change moves spots

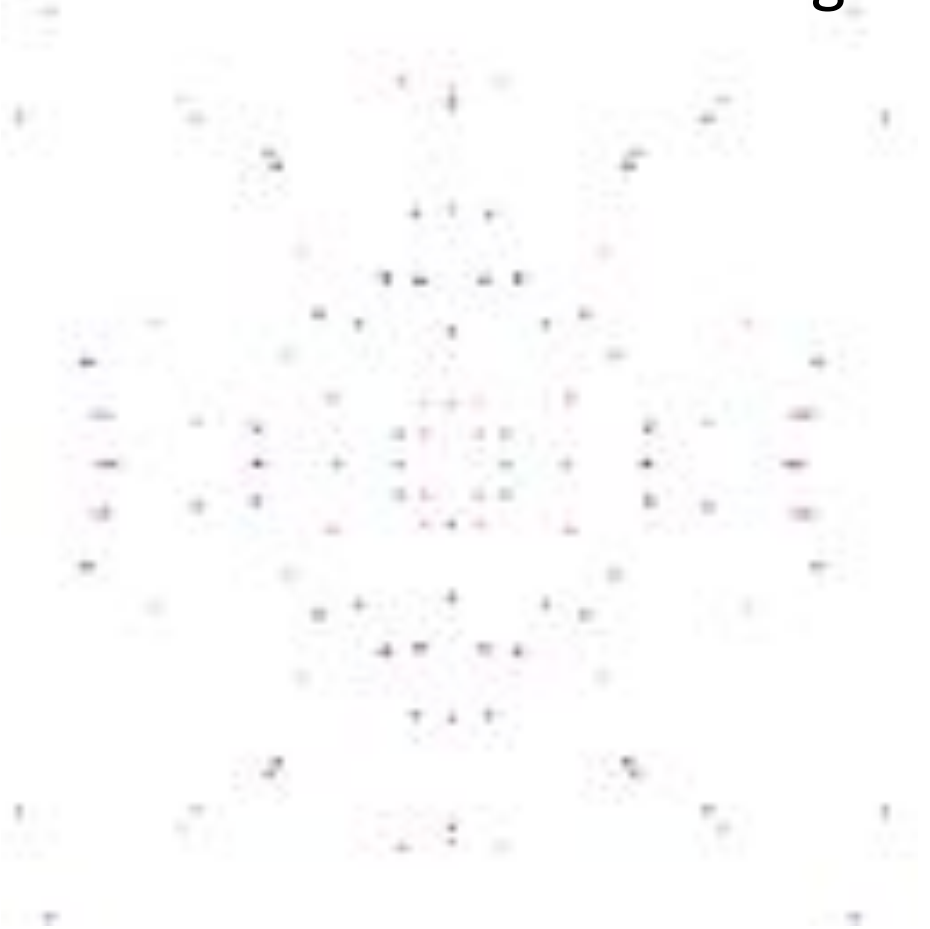
Collect many patterns at one delay for 3D image.
Collect many delays, one for each frame of movie.

This proposal (Spence, Chapman…) has been submitted for LCLS beamtime.
Use pairs of pulses generated at the photocathode.

SAD phasing may be possible using 2 –color split and delay. (Soichi Wakatsuki).

Two-color splitting in crystals of I_3C at 6.6 kV will make a dramatic movie of radiation damage occuring

Simulation



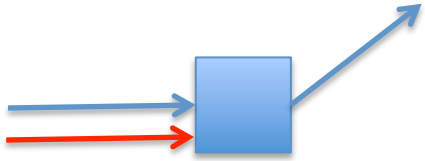
"Magic triangle", I_3C molecule (5-Amino-2,4,6-triiodoisophthalic acid monohydrate) has 3 iodine atoms in triangle, producing a strong shower of photoelectrons above Iodine L1 edge 5.5 kV. (0.9, 1.57, 1.88 nm, orthorhombic). Max delay 200 fs (overcompression), 90 ev pulse separation.

F

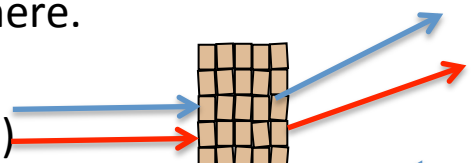
Data Analysis

The four cases of SFX diffraction.

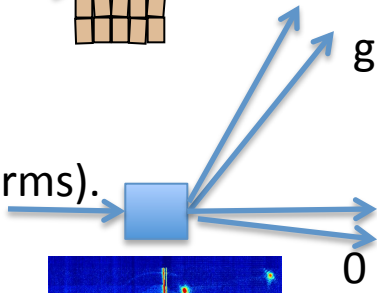
1. Big perfect crystal, polychromatic beam. Sample monochromates.
   Plane-wave to plane-wave. Picks out one wavelength)
   *J.Holton & Frankel Acta D66, 393 (2010)

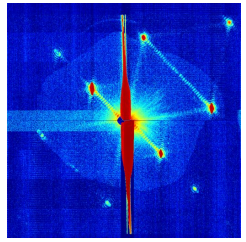


2. **Big crystals*** (>1 micron) , mosaicity. Every wavelength goes somewhere.
   *J.Holton & Frankel Acta D66, 393 (2010);
   *J.Hattne….Sauter Nature Meth. (2014), W. Kabsch Acta D. (2014)
   *T.White Phil Trans Roy Soc. B 369 (2014)
   *E. Snell Meth Enzymol (2003). *What is mosaicity* ?

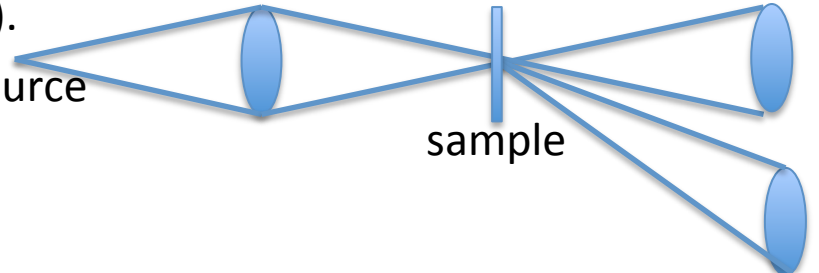


3. Nanocrystal (< micron). Monochromatic beam give big spots (shape transforms).
   *Kirian et al Optics Express 18, 5713 (2010); Acta A 67, 131 (2011).
   *Spence et al Optics Express 19, 2866 (2011); Kirian et al (2014) in press
   (for phasing from shape transforms).



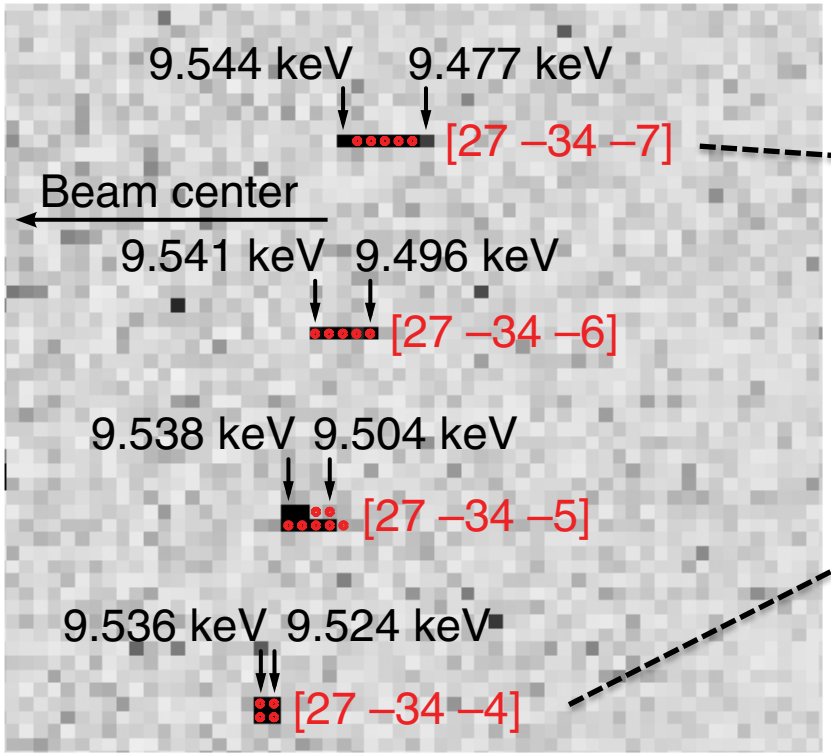


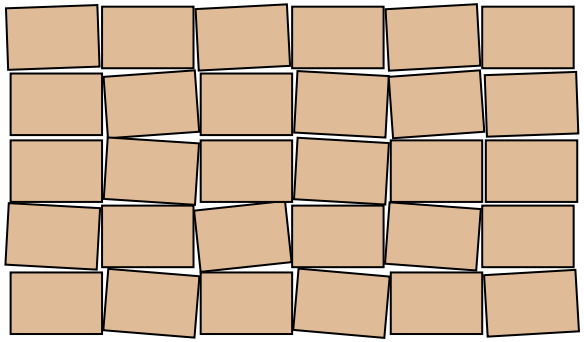
4. Beam smaller (eg 0.1 micron) than one mosaic block. Same as 1, but beam divergence (CCBD).
   *J.Spence et al Phil Trans *B* **369**, 20130325 (2014).
   Small coherent focussed beam.

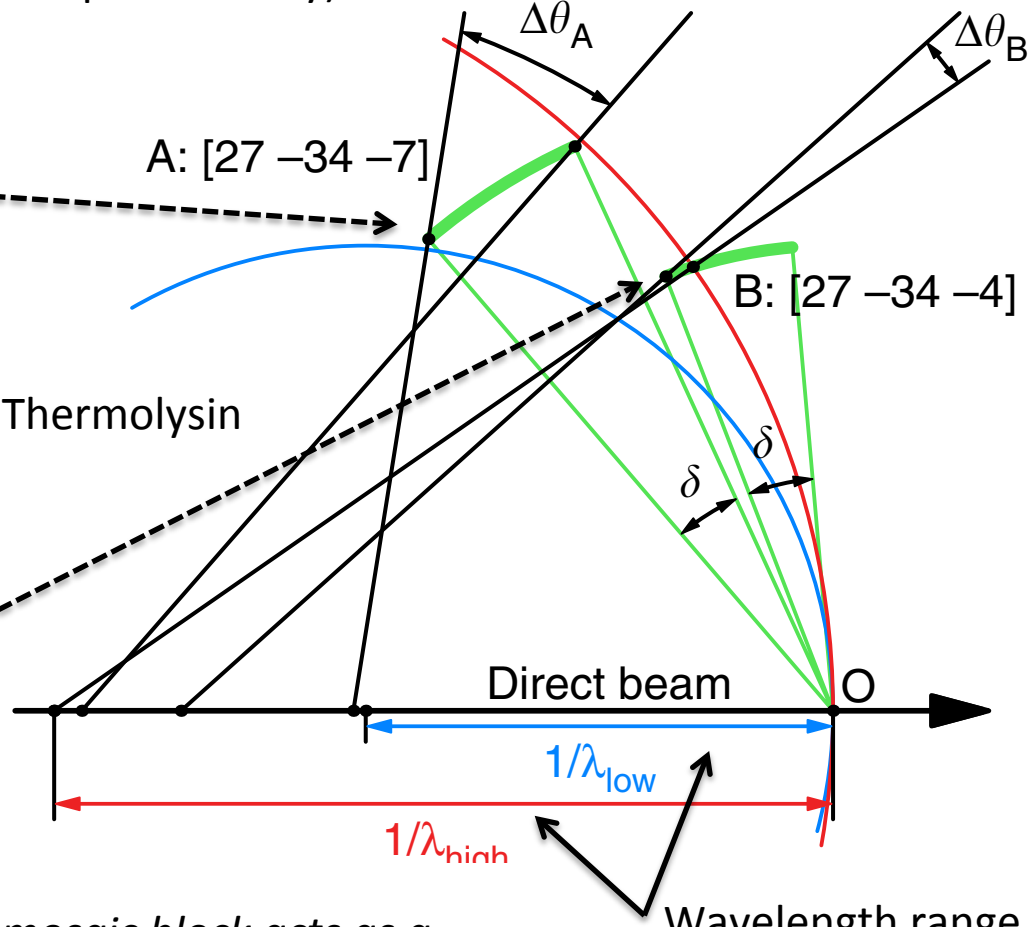


The wavelength spread in XFEL beam spans wider spots at higher angle.

so different fractions of different reflections are uncovered by the range of wavelengths.
(some reflection blobs are just clipped, others spanned fully)







Each tilted mosaic block acts as a monochromator for a different component wavelength in the beam..

From Hattne/Sauter et al Nature Methods 2014

A CXIDB Data Base for XFEL data has been established at http://cxidb.org/index.html

Used for STC SFX BioXFEL data analysis Workshop LBNL , Aug every year

Including these SFX data sets…
CXIDB #15 (Lysozyme Boutet)
CXIDB #21 (Serotonin/ergotamine)
CXIDB #22 (Gd-lys SAD from Heidelberg)
CXIDB #23 (thermolysin from Sauter et al)

Indexing ambiguity (symmetry of lattice higher than structure):
See Brehm and Diederichs Acta D70, p101 (2014)

Oct 2013: There are 15 data sets, both nanoxtal and single-particle..

F. Maia Nature Methods 9, 854 (2012)

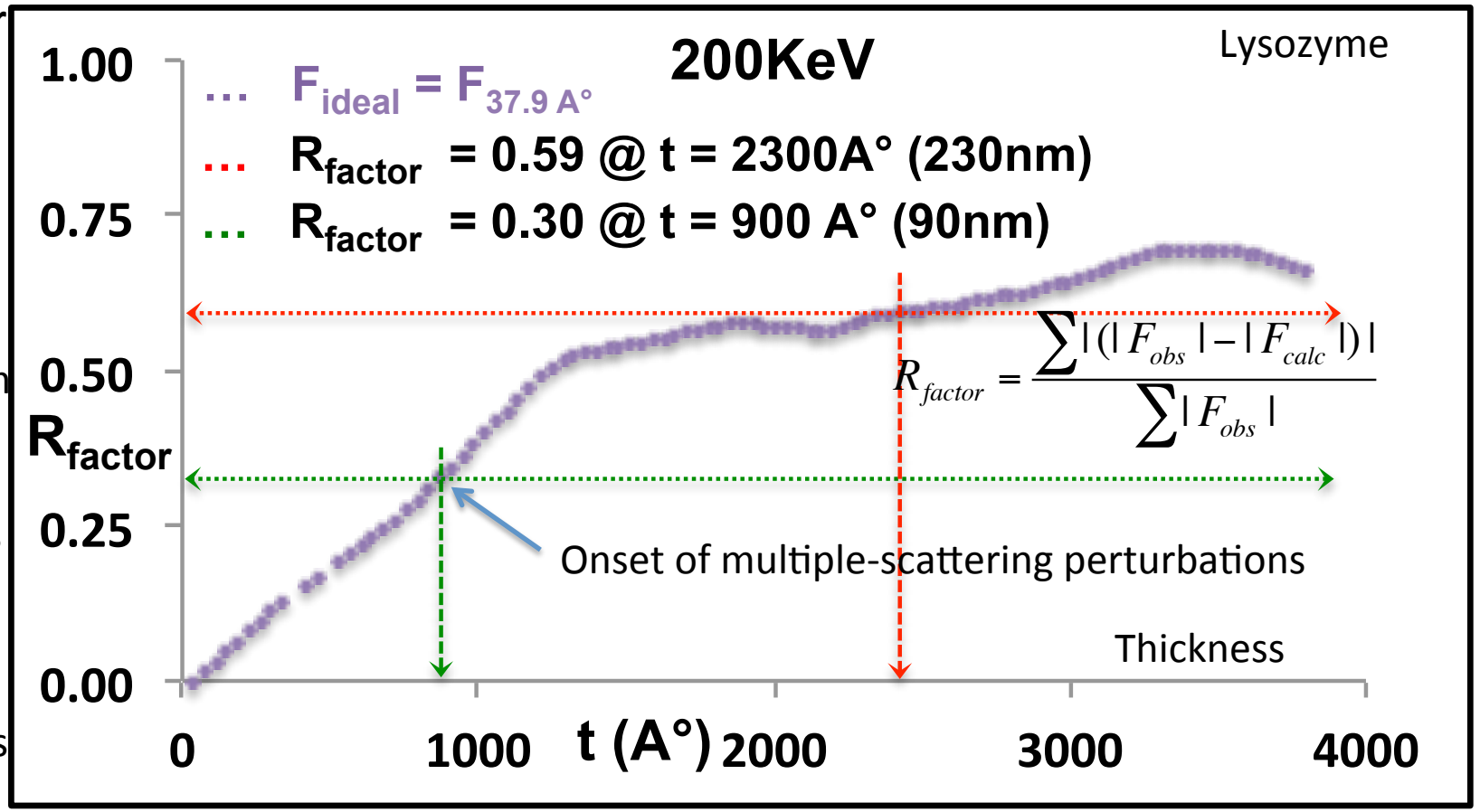
Protein nanoxtals are solved by electron diffraction (TEM) but size is limited by multiple scattering.

R-factor vs thickness for electron diffraction from Lysozyme

R factor (R>0.59 is junk)

Diamond new TEM facility ! (cf Superstem Daresbury)

Can electrons outrun damage ? See Spence Faraday Trans (2014)



Conclude: TED for protein nanoxtals works up to 100nm thickness at 200 kV. More (eg 200 nm) if zone axes avoided, and most information from phases, by modeling (MR) from PDB. Subramanian, Liu, Spence Ultramic (2014); **Shi, Gonen**, eLife (2014)

The US NSF BioXFEL Science and Technology Center

http://www.bioxfel.org

Established late 2013 among 7 US universities, typically runs for a decade

SUNY Buffalo. Director Ed Lattman

ASU. J.Spence (Director of Science),Fromme,Weierstall,Hogue

Stanford. R. Kornberg

U. Milwaukee. A. Ourmazd, D. Saldin, M. Schmidt

Cornell. L. Pollack

UCSF. R. Stroud

Rice. G. Phillips.

Plus Education, Outreach, Diversity, Technology Transfer, Patents.

POSTDOC POSITIONS !

"Application and development of XFELs to biology".

Hosts annual international conference , annual data analysis workshop.

Conference on BioXFEL in Puerto Rico Jan 14 2015.

Web pages: Roy Soc Conference: http://royalsociety.org/events/2013/x-ray-lasers/

Phil Trans "BioXFEL" issue : http://bit.ly/PTB1647

THIS NSF STC EXISTS TO HELP YOU WITH YOUR PROJECTS AT LCLS !!!

Second International BioXFEL Conference

A National Science Foundation Science and Technology Center

January 14 - 16, 2015

Ponce Hilton, Puerto Rico

Join us for the second annual international conference of the BioXFEL NSF Science and Technology Center. This series is aimed at spotlighting the advances in applying the X-ray free-electron laser to the study of biological systems. Following the previous meeting held at the Royal Society, London in October 2013, this second edition will focus on the recent advances in the field, and the potential possibilities moving forward. Faculty members, research scientists, post-docs, students and industry partners who pursue research in this field are invited to participate.
Registration Fee $300 ($200 for students) NO FEE FOR BioXFEL CENTER MEMBERS.

Topics:

- INSTRUMENTATION
- NANOCRYSTALLOGRAPHY
- SINGLE PARTICLE IMAGING
- SOLUTION SCATTERING
- TIME-RESOLVED SCATTERING
- SPECTROSCOPY

Conference Co-chairs:
Ed Lattman (UB) and Abbas Ourmazd (UWM)

Session Chairs:
Henry Chapman (CFEL)
Majed Chergui (EPFL)
Petra Fromme (ASU)
Elspeth Garman (Oxford)
Keith Moffat (University of Chicago)
Michael Rossmann (Purdue)
Ilme Schlichting (MPI Heidelberg)
John Spence (ASU)
David Stuart (Diamond)

Invited Speakers:
Brian Abbey (University of Melbourne)
David Arnlund (Gothenburg)
Axel Brunger (Stanford)
Marco Cammarata (University de Rennes)
Leo Chavas (CFEL)
Fasseli Coulibaly (Monash)
Ali Dashti (UWM)
Lorenzo Galli (University of Hamburg)
Max Hantke (Uppsala)
Stefan Hau-Reige (LBNL)
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Stefan Kassemeyer (MPI Heidelberg)
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Ian Robinson (UCL)
Lars Rudecke (DESY)
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Nick Sauter (LBNL)
Gebhard Schertler (ETH)
Marius Schmidt (UWM)
Sang-kil Son (CFEL)
Yun-Xing Wang (NIH)
Bill Weis (Stanford)
Uwe Weierstall (ASU)
Junko Yano (LBNL)

For more information: www.bioxfel.org/events/details/6

The three most common questions regarding XFELs

1. Why use an XFEL instead of a synchrotron ?
   (*XFEL proposals for work which could be done on a synchrotron are rejected* !)

* Avoid damage (not electronic, assuming MR, 2/3 info comes from phases, 2Ang)
* RT, native environment. Use short pulses instead of freezing to avoid damage.
* Time resolution (ps), study dynamics because not frozen.
* Try new ideas. (2 color SAD, pump-probe, FSS, SP, SFX, Shape transform phasing)
* Xtals large enough for MX not available
* Rapid diffusion into nanoxtals, irreversible processes

2. How to make nanoxtals ? See bibliography at end

3. How does data analysis differ for SFX ?

Summary

* Serial crystallography (SFX) uses short pulses to avoid damage, instead of freezing. This allows RT sample, controlled envir, hence dynamics, with x1000 RT dose (wrt RT safe dose at synchrotron). Quinney ?

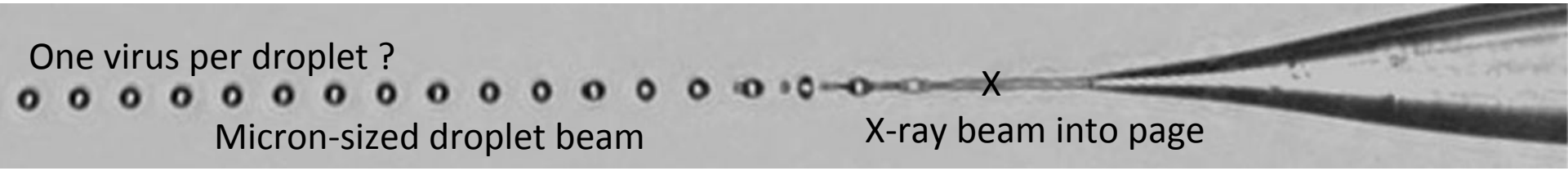
* "Toothpaste" (LCP, GPCR) injector improves hit rate, less protein, grows nanoxtals ! Works in air at synchrotrons.

* Pump-probe time-resolved nanoxtallog at 5 Ang (PS II) and 3 Ang (PYP).

* "Snapshot" solution scattering (FSS) at ps time and 3 Ang spatial resolution (Neutze)

* Mixing jet, Two-color, Angular correlations, Virus xtals, .

The NSF BioXFEL Science and Technology Center is a US consortium of 7 universities with faculty devoted to the use of XFELs for structural biology



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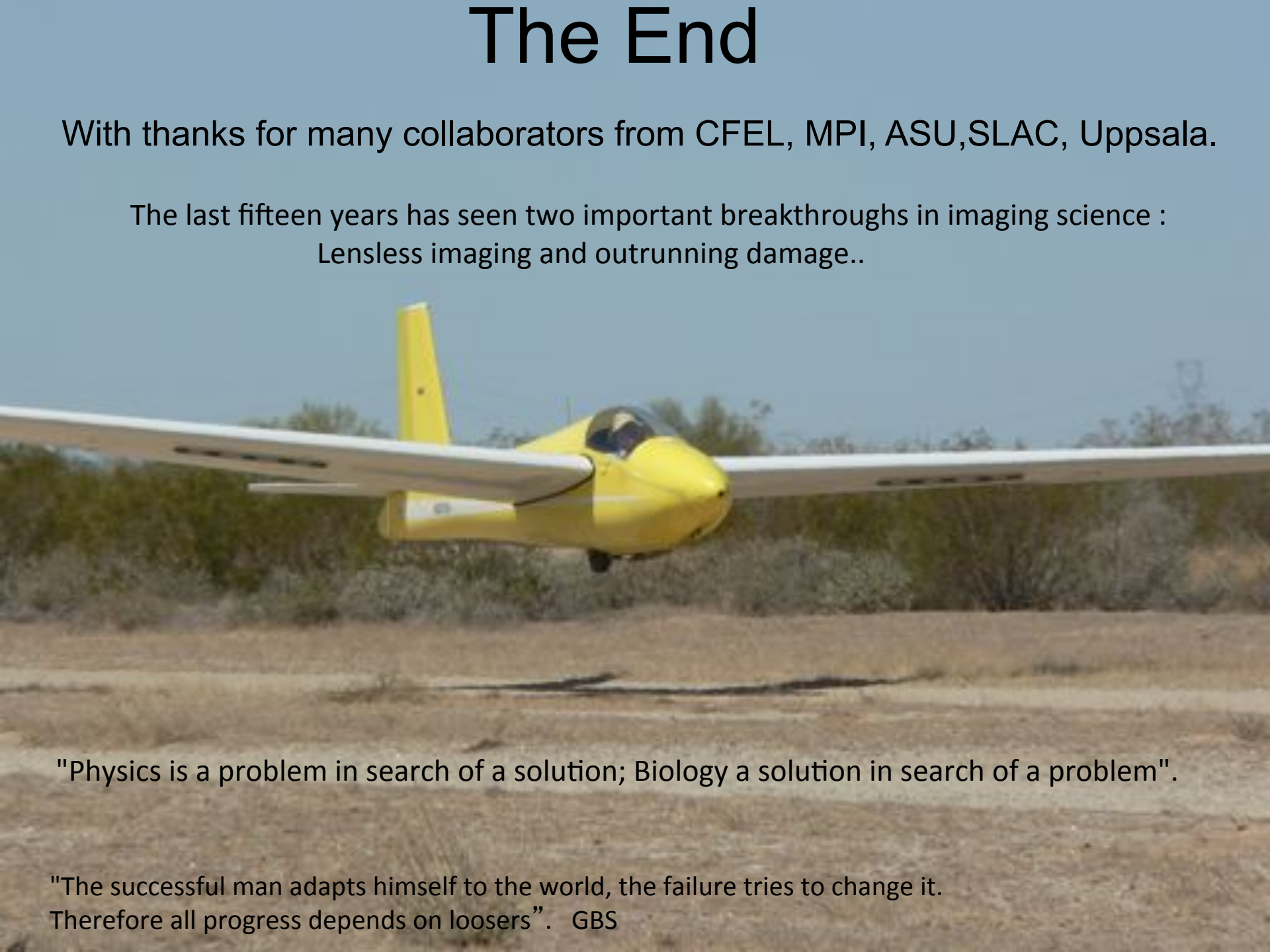
The End

With thanks for many collaborators from CFEL, MPI, ASU,SLAC, Uppsala.

The last fifteen years has seen two important breakthroughs in imaging science :
Lensless imaging and outrunning damage..



"Physics is a problem in search of a solution; Biology a solution in search of a problem".

"The successful man adapts himself to the world, the failure tries to change it.
Therefore all progress depends on loosers". GBS