New approaches to biology using hard X-ray lasers

BioXFEL
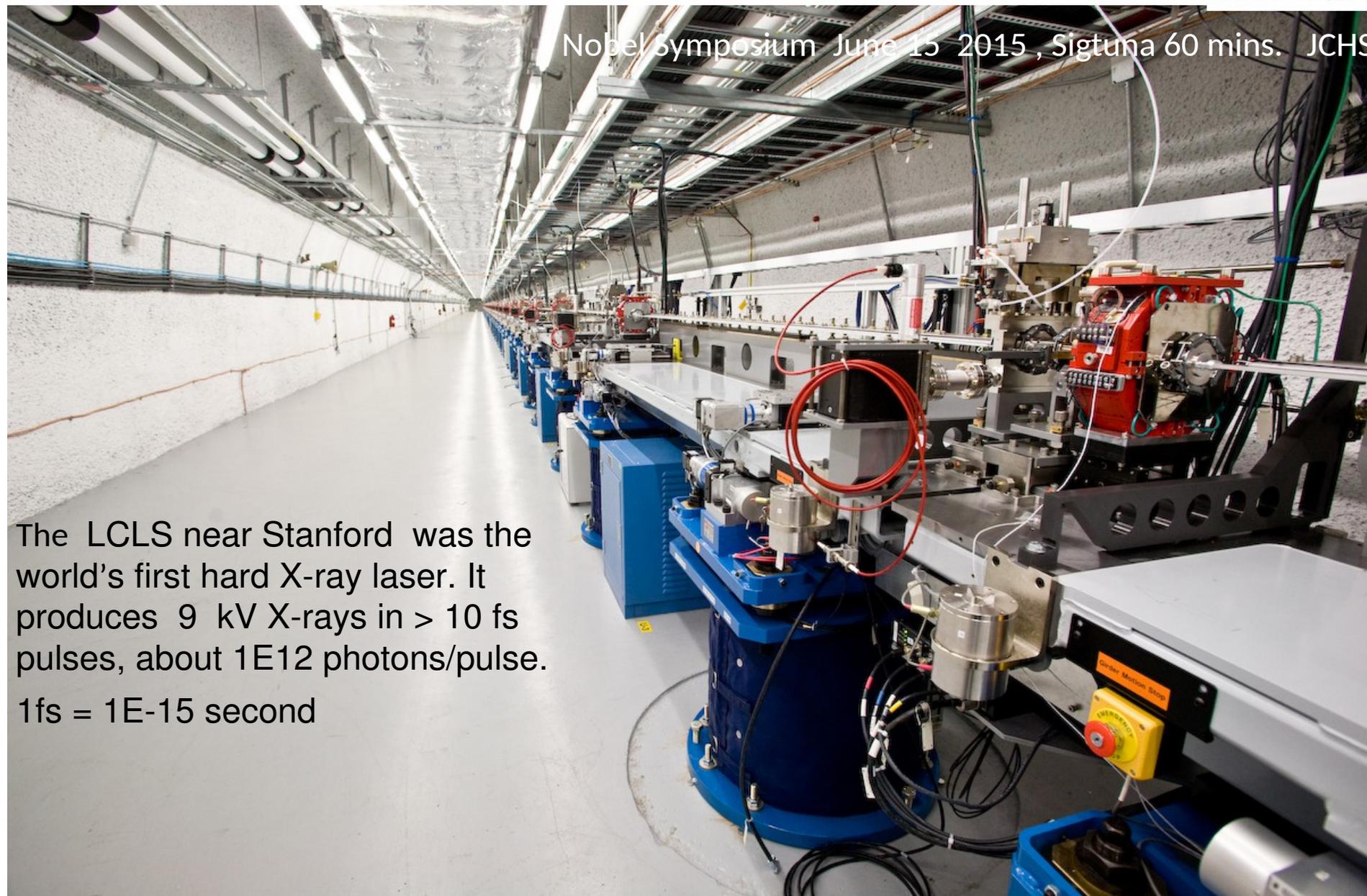
The LCLS near Stanford was the world's first hard X-ray laser. It produces 9 kV X-rays in > 10 fs pulses, about 1E12 photons/pulse.
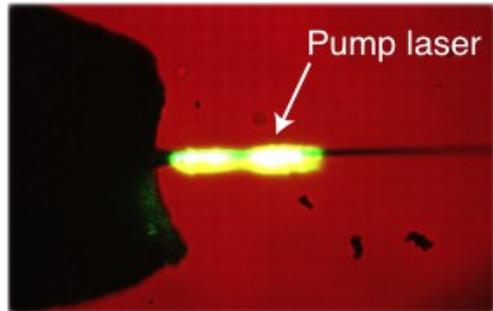
1fs = 1E-15 second

Madey 1972 Stanford; Saldin,, Bonefachio, Sessler 1985 Microwaves; Pellegrini optical 1991; Leutl 1997 Argonne; Flash Matterlink 1997 ; Murphy Brookhaven 2001; (SASE mode)

Nanoxtals are sprayed across the pulsed X-ray beam.
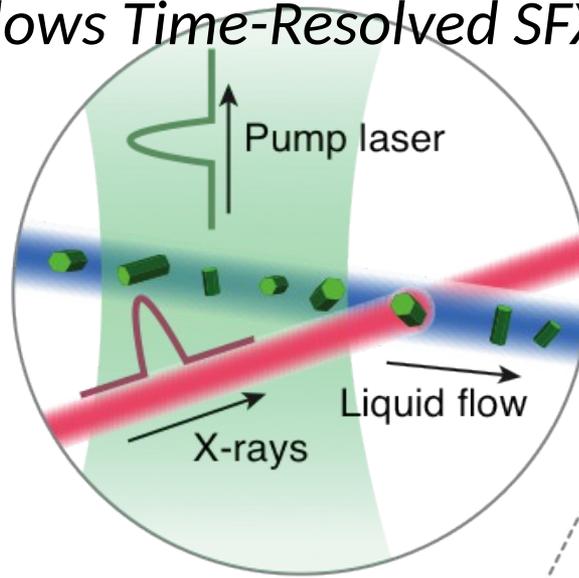*This also allows Time-Resolved SFX (serial fs xtallog).*

FEL / jet intersection
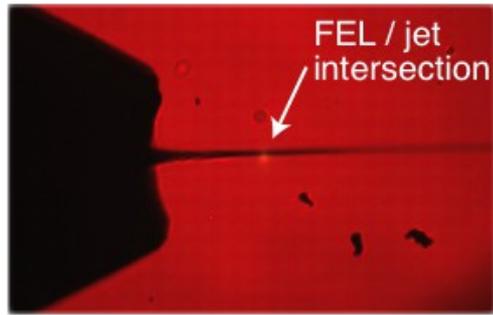
Pump laser off

Pump laser

Pump laser on

Pump laser

Liquid flow

X-rays

The 10 fs XFEL pulses should allow us to improve on the ~ 100 ps time resolution of SR (at RT , without damage).

Upper pnCCD

Lower pnCCD

"Diffraction before destruction"
(next talks, Hadju, Chapman, Neutze)

Gas dynamic virtual nozzle

Optical fibre laser coupling

LCLS beam
2 keV X-ray energy
40 fs pulse duration
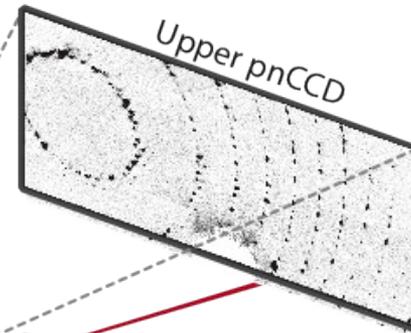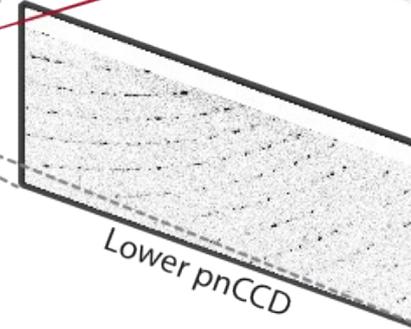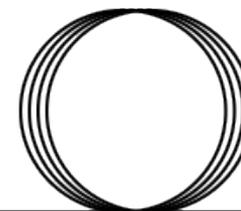
Nd:YLF pump laser

Linear Coherent Light Source at SLAC > 2009

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

- Avoid radiation damage. The smallest xtals fry before they'd give Bragg spots on synchrotron, reverse at XFEL.

- Room temp structures, avoid "freezing".

- Better time resolution (ps).

- Irreversible reactions can be studied (eg PSI-ferredoxin).

- Xtals large enough for MX not available.

- Optical pump laser absorption length comparable to nanoxtal size.

- Diffusion times short for nanoxtal in mixing jet. Diffusive mixing possible.

- Higher resolution for some proteins. (Higher dose, 100X Safe Dose)
  (Are smaller xtals or beam better ? ***Dose, defects, diam of beam, DW***.
  Depends on type of defect (SRO, LRO))

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

Note dimensions in **microns**.

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

molecules

Liquid Jet Flow

X-Ray Beam

Φ=1um

Φ=3um

X-ray beam

Fast Solution Scattering (WAXS) FSS

Liquid Jet Flow

Φ=0.2um

Φ=3um
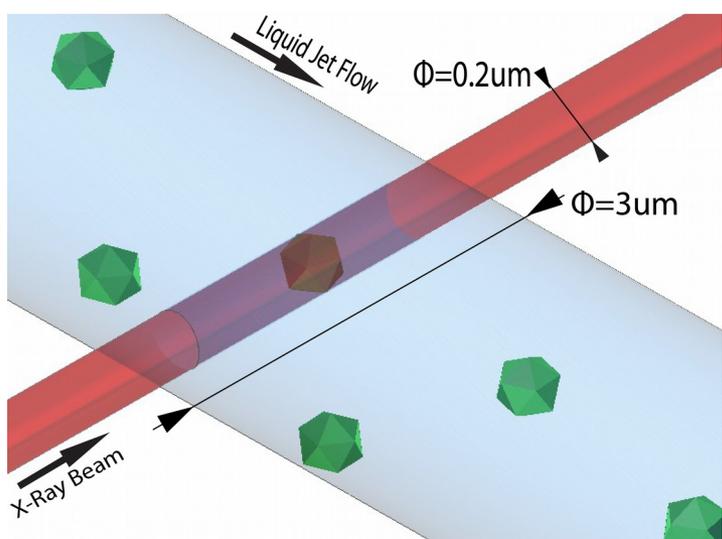
X-Ray Beam

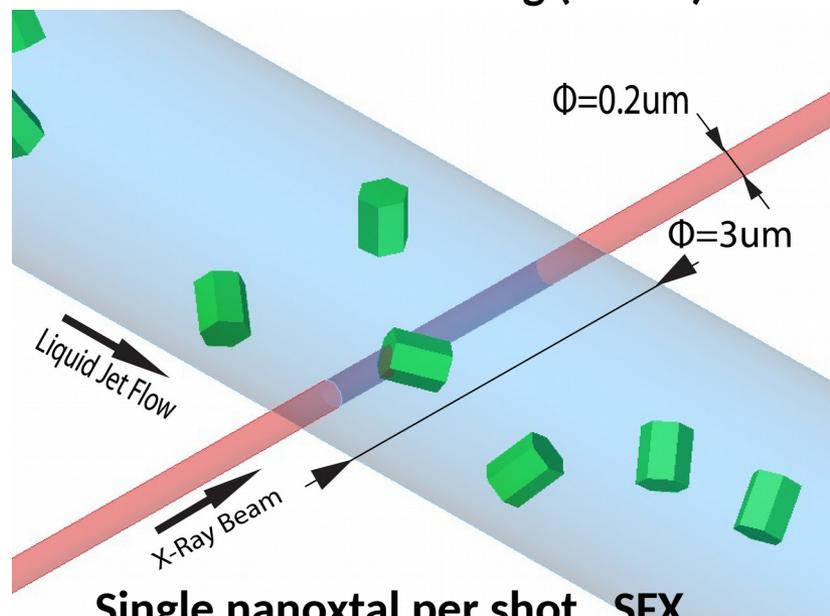**Single particle (eg virus) per shot . SP**

Many X-ray shots do not hit a crystal

Plus 2D xtals on fixed targets (M. Frank)
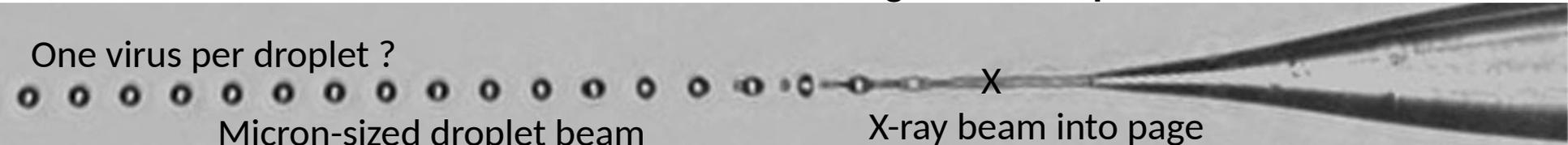
Φ=0.2um

Φ=3um

Liquid Jet Flow

X-Ray Beam

Single nanoxtal per shot. SFX

One virus per droplet ?

X

Micron-sized droplet beam

X-ray beam into page

Why is crystallography hard to beat ?

Current XFELs are not powerful enough to image single mols at high resolution

Bragg boost needed for atomic resolution, to see building blocks, how molecular machines work. Coherent amplification, so I ~ n^2 at one detector point, like 1D grating, **SASE Boost**: XFEL also gives N^2 (for N electrons/bunch)
  - *both N^2 needed to see atoms.*

Not possible using XFEL with SP (5 -12nm best in 2D, 3D worse) or FSS. (I ~ Θ^{-4}). Modelling !

Crystallography is a filter for conformation.

Xtallog spreads the dose to avoid damage – less than damage dose per mol. XFEL solves this.

BUT the XFEL "stills" give wrong ratio of structure factors because of angular width of spots

And TR-SFX can only study limited protein motions which don't destroy the crystal.

Building blocks of matter are larger for biology than in condensed matter – secondary structure.

self-amplified *spontaneous* emission

"Diffract-before-destruction" agrees with SR results.

The 14 high-resolution XFEL crystal structures published to March 2015.

Date	Citation	Structure	Total Number of shots	Number of Indexed pattern used	Amount of protein	Comments
Jul-2012	Boutet	1.9 Å Lysozyme	66,442	12,247		
Jan-2013	Redecke	2.1 Å Cathepsin B	293,195	178,875		
Dec-2013	Liu	2.8 Å Serotonin receptor	152,651	32,819		
Jan-2014	Barends	2.1 Å Gadolinium lysozyme	191,060	59,667		
Feb-2014	Weierstall	3.2 Å Smoothens GPCR	152,651	32,819	**0.5 mg**	
May-2014	Hattne	2.1 Å Thermolysin	14,932	11,455		
Sep-2014	Sawaya	2.8 Å Cry3A Toxin	380,650	78,642		
Dec-2014	Tenboer	1.6 Å Photoactive Yellow Protein	36,632	22,678	**300 mg**	CASPAD hi/low gain
Dec-2014	Cohen	1.6 Å Hydrogenase	162	158		Postrefinement
Dec-2014	Cohen	1.36 Å Myoglobin	932	739		Goniometer, Mar
Mar-2015	Ginn	1.75 Å CPV17 Polyhedrin	144,803	5,787		Postrefinement LCP jet
Forthcoming	Xu	3 Å GPCR-arrestin complex	8934	2047		

Vadim Cherezov

- These span the cell membrane. About human 800 GPCRs respond to many extracellular signaling molecules and transmit signals into the cell

- 40% of drugs target GPCRs. 70% of recent drug approvals were GPCRs

- Challenges: low expression yields, low receptor stability after extraction from native membranes with detergent, high conformational heterogeneity

- 19 receptor structures solved so far
- most were crystallized in LCP
- crystals often limited in size, sub 10 micron

- microfocus beamlines have been used, radiation damage severe, merge data from multiple crystals.

Brian Kobilka Nobel Prize
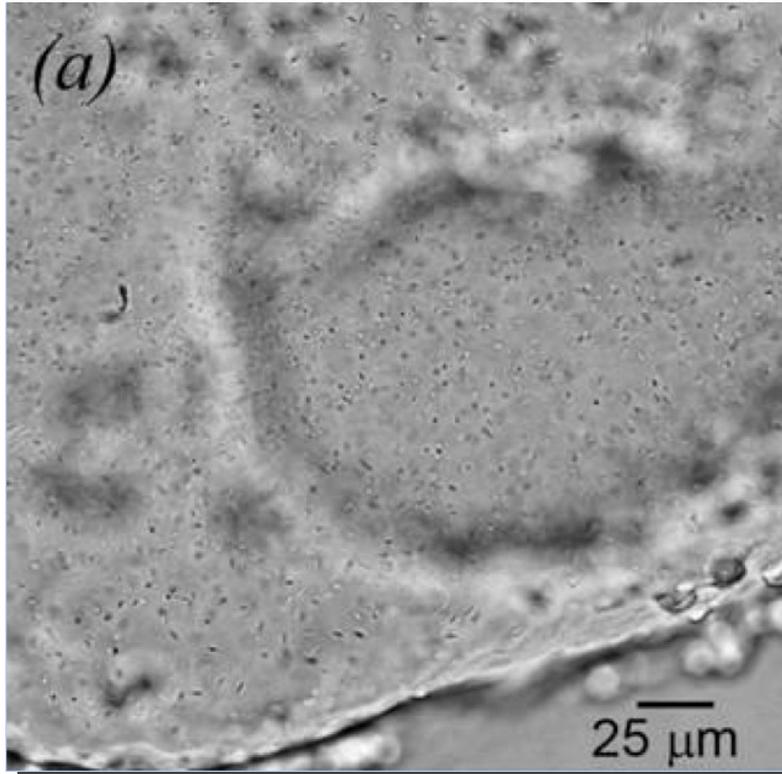
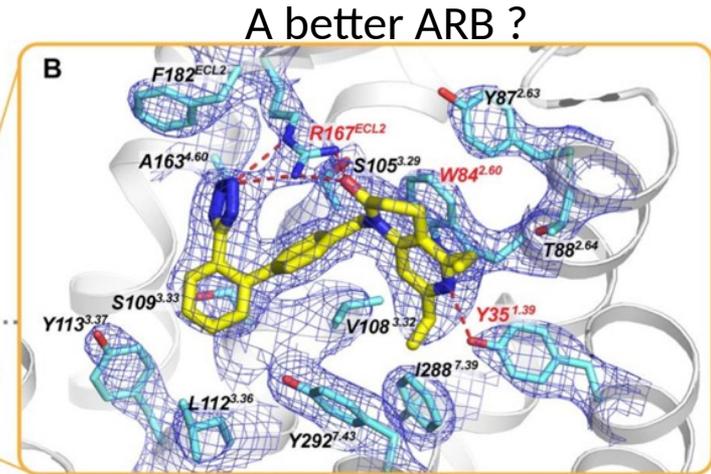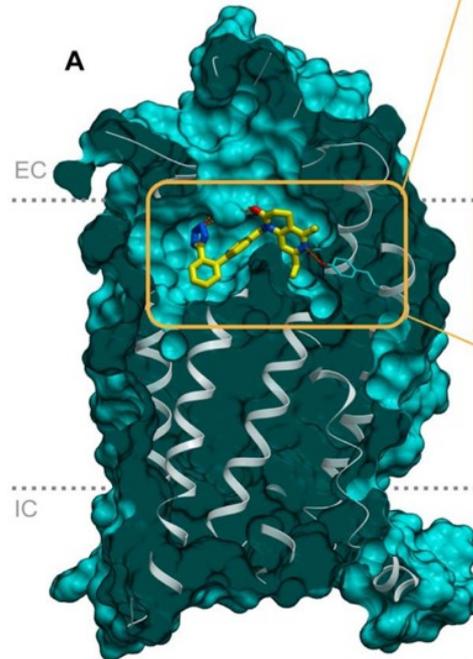A typical initial hit contains high-density of 3x1x1 μm^3 crystals suitable for XFEL.
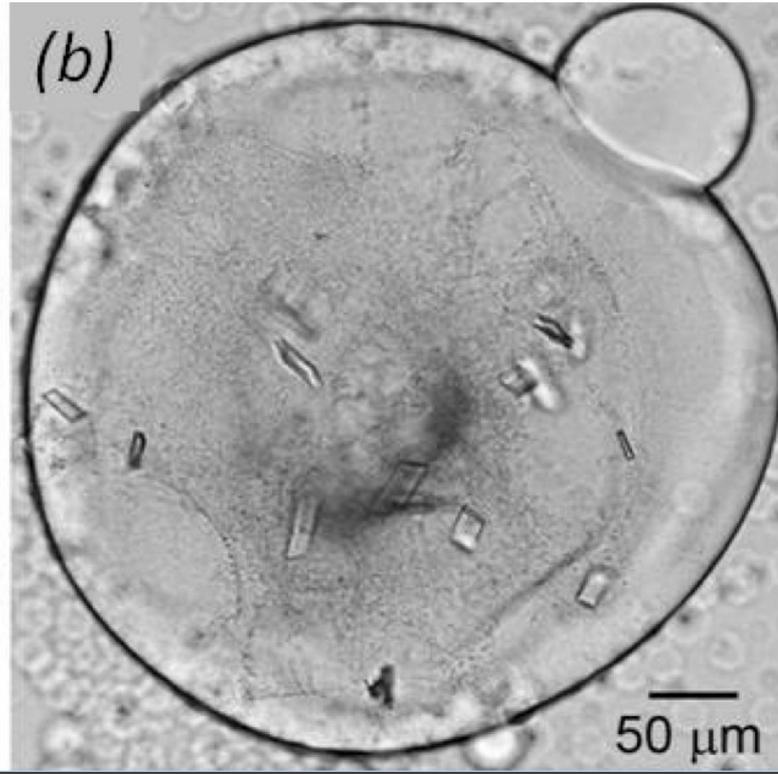
Substantially larger crystals (40x20x7 μm^3), required for microfocus synchrotron data collection, were produced after one year of intensive optimization studies.

Serotonin.

Vadim Cherezov

GOAL 1. Develop SFX.

A better ARB ?

- Serves as a primary regulator for blood pressure maintenance

- In complex with a selective antagonist ZD7155

- We got 2.9 Å resolution (vs 4 Ang with SR, twinned)

- **Docking simulations of the clinically used AT$_1$R blockers into the AT$_1$R structure show distinct binding modes for anti-hypertensive drugs**

- **Results provide fundamental insights into AT$_1$R structure-function relationship and structure-based drug design**

AT1R - ZD7155 (yellow) complex

Despite its medical importance, structure is unknown, due to limited crystal size.
*First novel GPCR structure solved with SFX
Better res than SR.

THE SCRIPPS RESEARCH INSTITUTE

Fusion proteins added assist crystallization.
Serotonin, δ-opiod, plus 4 other GPCRs....

Zhang, Cherezov et al. **2015**, Cell.

Additional SFX results from microcrystals in LCP
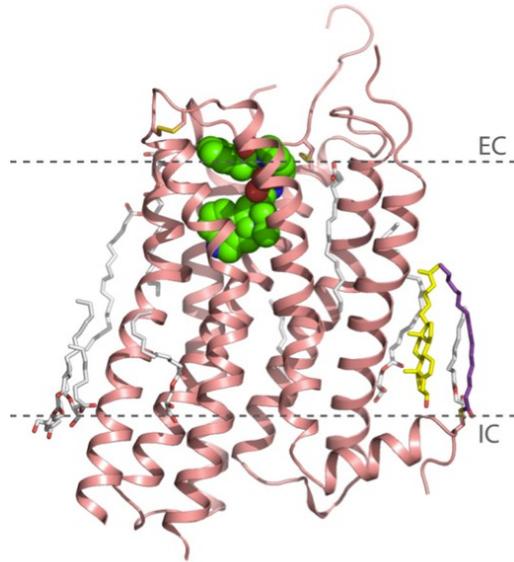
Serotonin receptor with
ligand ergotamine
(2.8 Å)
indexed patterns: 32,819

Wei Liu….Science 20, 2013:
342 1521-1524
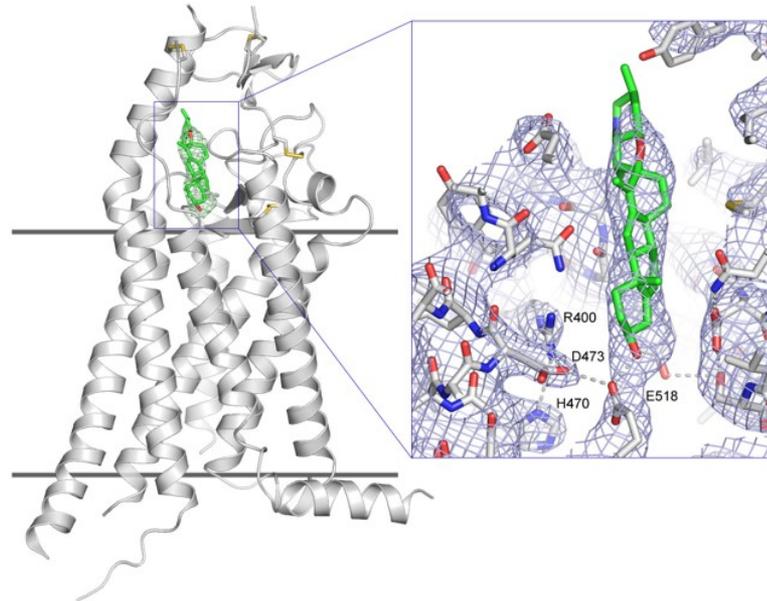
Smoothened receptor with
ligand cyclopamine
(3.8 Å)
indexed patterns: 40,036

Weierstall….Nature Communications
5: 3309, 2013
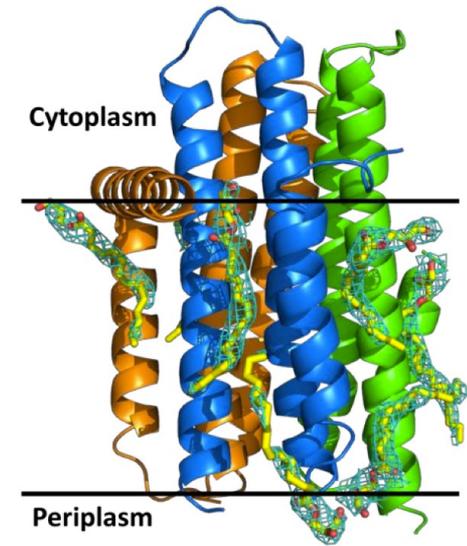
Membrane Kinase DgKA
(2.8 Å, March 2013)
indexed patterns: 66,165

unpublished

Also: δ-Opiate receptor* (+ DIPP-NH2), and human rhodosin bound to arrestin (Xu et al)

0.3 mg protein for full data set, compare to sample consumption with
GDVN injector: 10 - 15 mg of protein.   *reduce pain-killer addiction

Vadim Cherezov,
Martin Caffrey

Data Analysis

What the nanoxtal Bragg XFEL data looks like

SFX Each point is the sum of all pixels contributing to one partial reflection on one shot.
This sum is seen to vary by orders of magnitude for the same reflection on different shots.
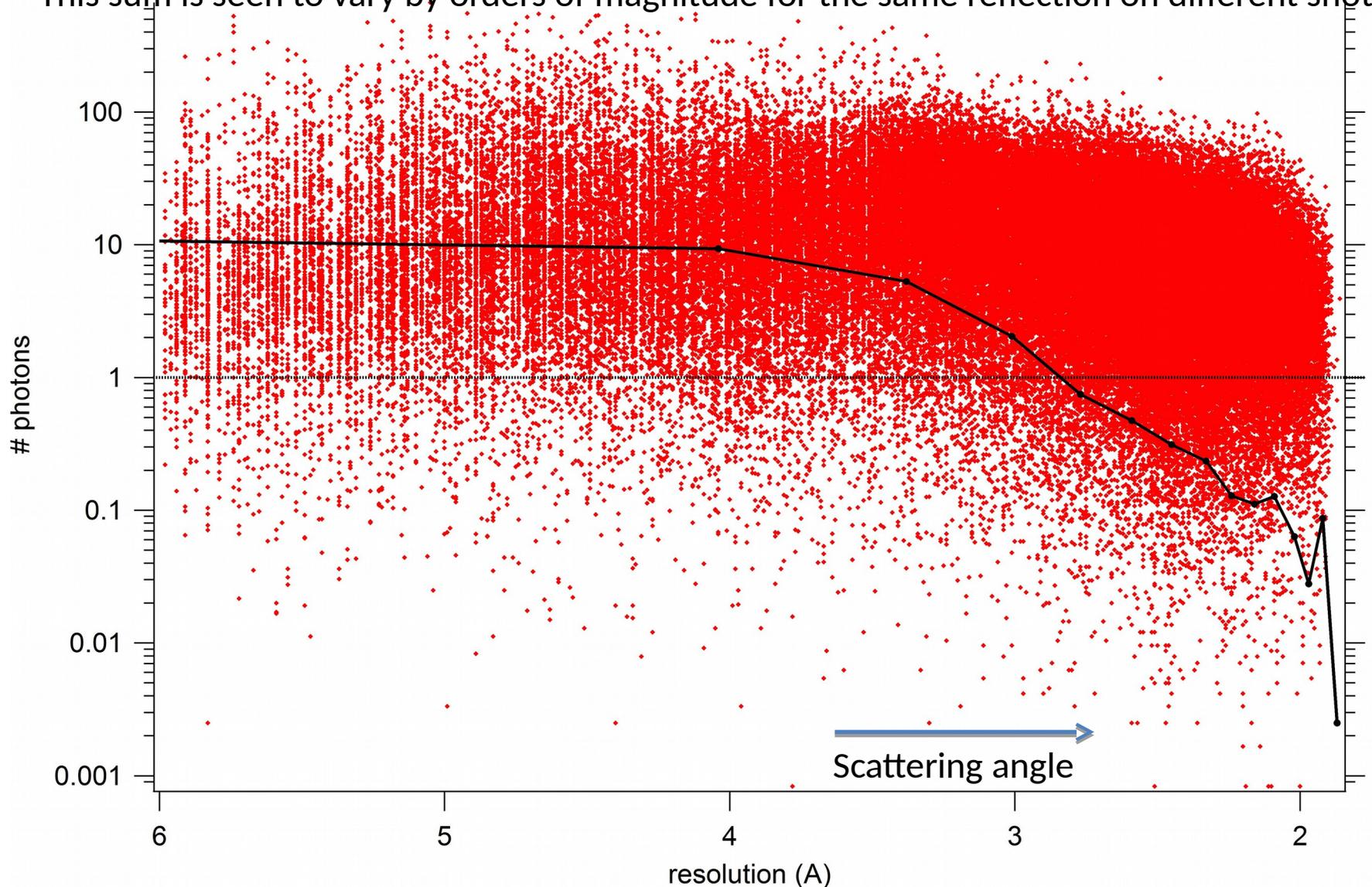
Granulo Virus nanoxtal data. Tom White. Negative intensities shift mean. Nov 2014.

New data merging algorithms take account of partial reflections.

Snapshot diffraction has no goniometer !   Rossman's "American Method".

In XRD the Ewald sphere takes a SLICE through the 3D FT of the nanoxtal shape

But the structure factors are proportional to the VOLUME of the shape-transform.

Ewald sphere

K_i

K_s
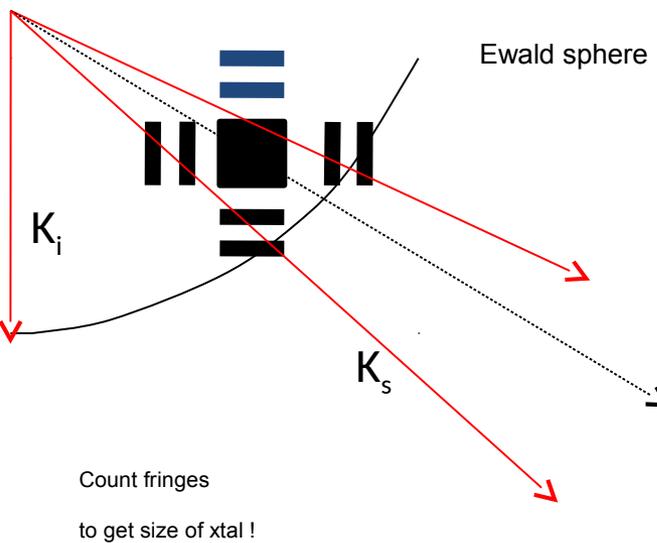
No scattering

in this Bragg direction

Count fringes

to get size of xtal !

OR

Wavelength ~6.88 Angstroms

2δ

Important

quantity for

data analysis !

This is a pattern simulated for

one PS1 needle in random orientation

showing sinc function shape

transforms at each lattice point. J. Holton
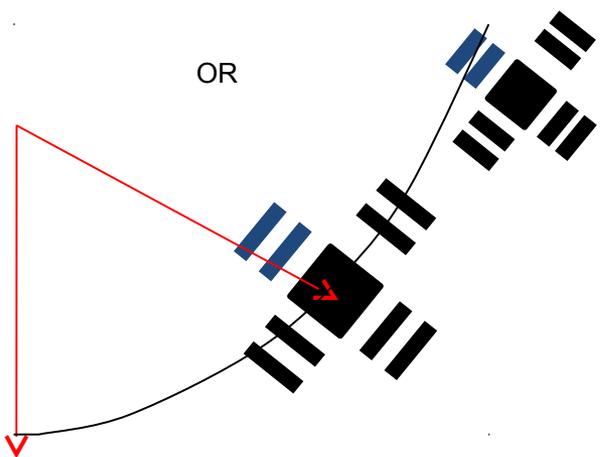
Ratio of Bragg intensities completely wrong !
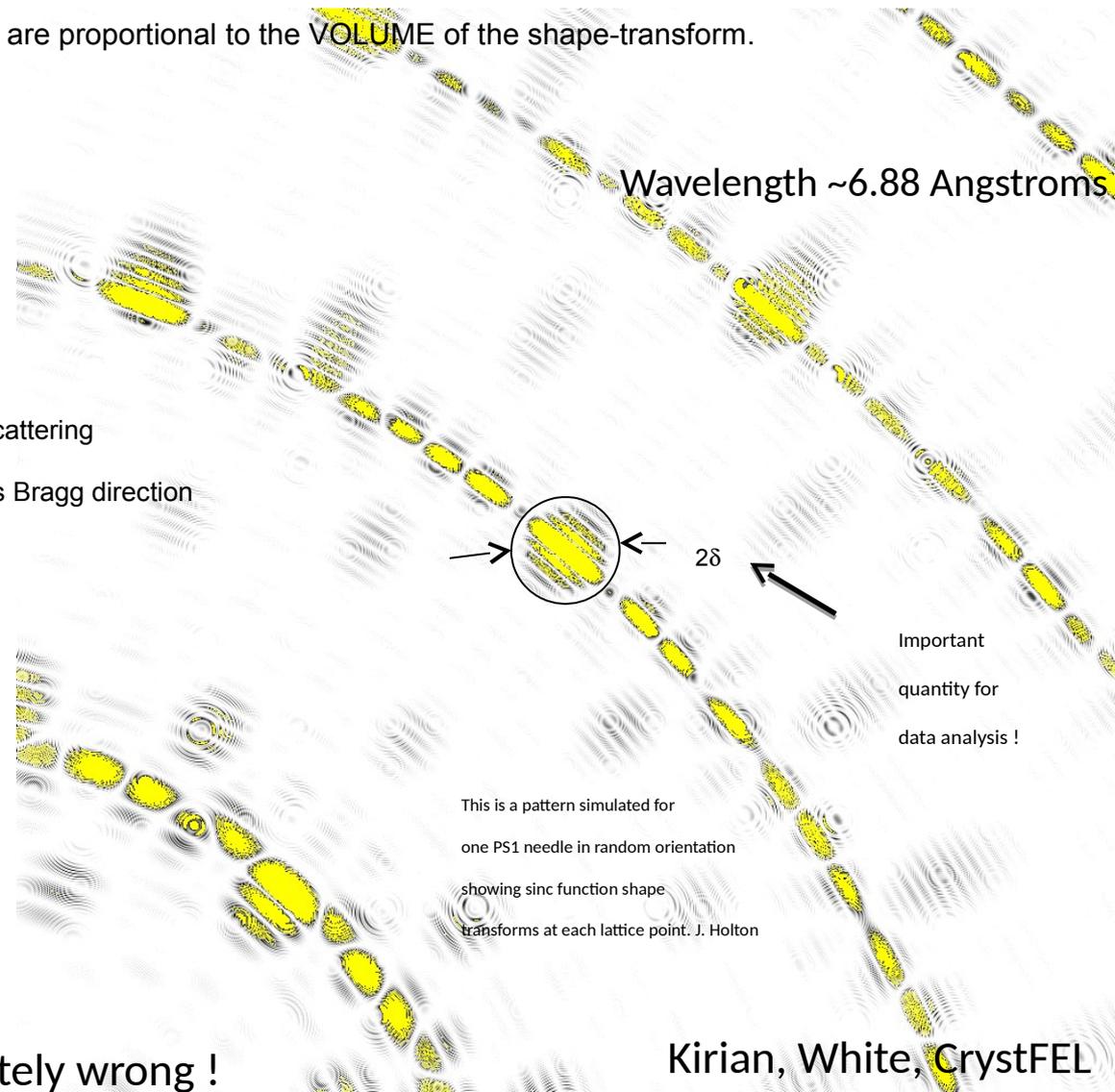
Kirian, White, CrystFEL

Data for Cathepsin, LCLS

~ "Error" in structure factors

R split

Monte Carlo averages out (quadrature) errors if enough data.
(Kirian et al 2010, White et al 2012)
Rossman & Erickson 1983 ("American method").
Rossman 1999 "Post-refinement".

Errors in SFX are shot-to-shot 15%, xtal orientation, size

A hundred times more data are needed to add one significant figure to the results.

Reducing k allows more people to use LCLS/time
(Only two XFEL expts at a time in the world !)

Karol Nass.

k/sqrt(N)

R split

Number of patterns N
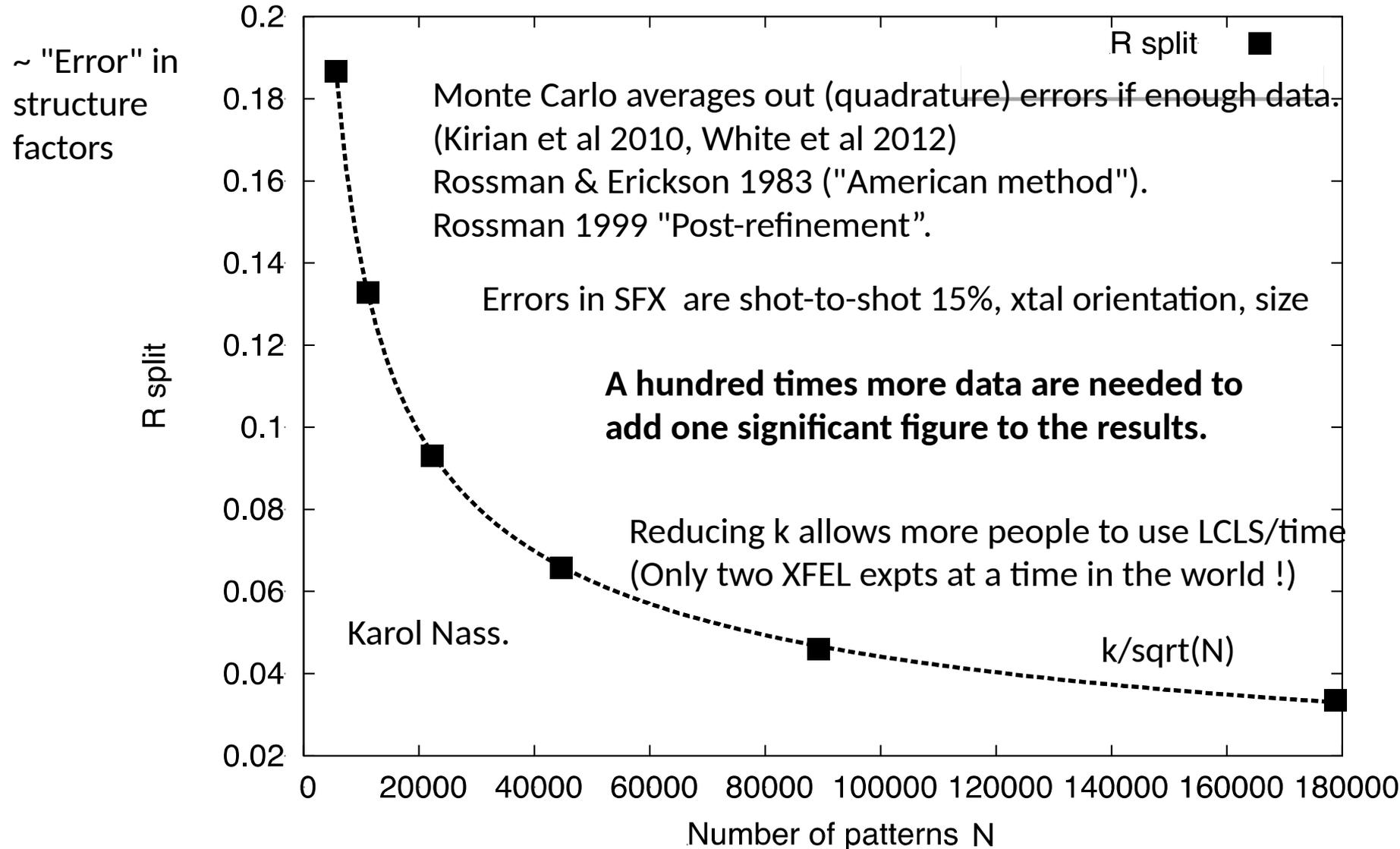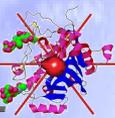
New algorithms in Year 2 take account of partiality and mosaic spread

SFX data sets now small enough to allow iterative optimization of experimental params (λ, S_g) on *every* shot – beyond Monte Carlo (MC) averaging. (White, Ginn, Sauter, Brunger, Kapsch…)

Partiality = Red/Blue
(volumes, in full theory)

Spherical
cap traced out
by mosaic spread

K_f

Each block acts as a monochromator
for a different wavelength in the beam

g (reciprocal lattice vector)

K_I

Origin of reciprocal lattice

's 1922 mosaic block model

Spread of wavelengths
and directions in XFEL beam

MC is first guess. Then refine each pattern against
this, and update MC. More users at LCLS !

Rossman et al. (1999).

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)

$\Delta\theta_A$

$\Delta\theta_B$

9.544 keV   9.477 keV

[27 −34 −7]

A: [27 −34 −7]

Beam center

B: [27 −34 −4]

9.541 keV 9.496 keV

[27 −34 −6]

Thermolysin

9.538 keV 9.504 keV

Detector

[27 −34 −5]

δ

δ

9.536 keV 9.524 keV

[27 −34 −4]

Direct beam   O

$1/\lambda_{low}$

$1/\lambda_{high}$
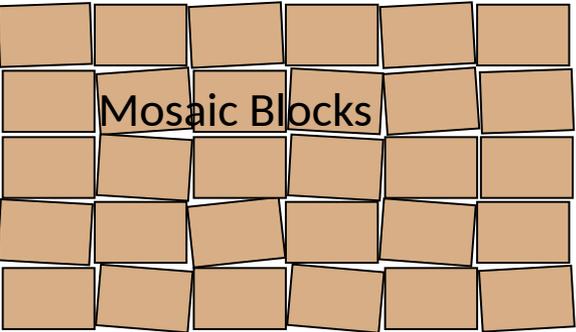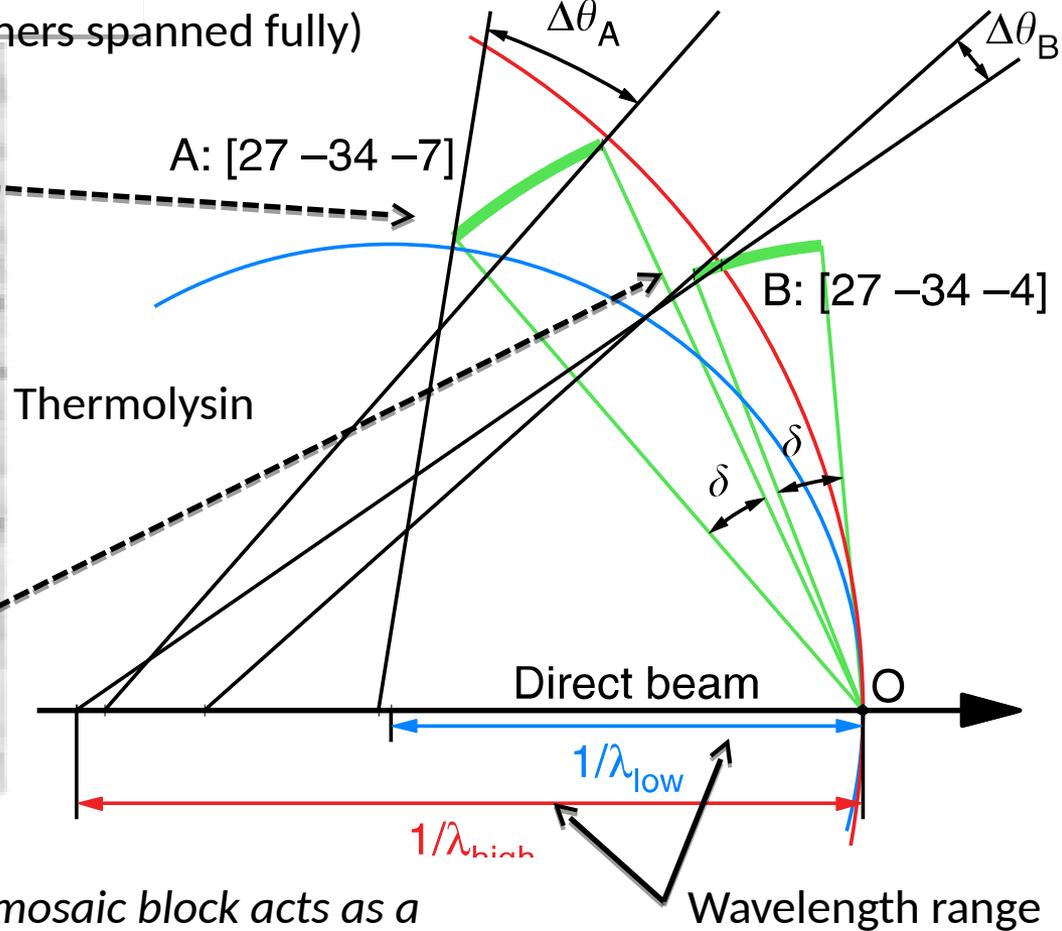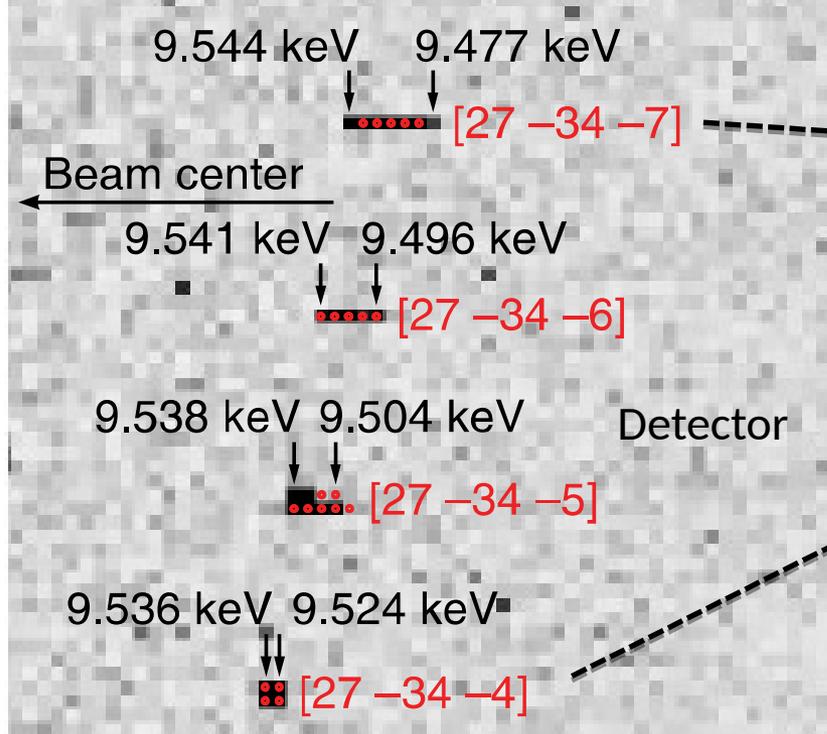
Mosaic Blocks

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

Wavelength range

From Hattne/Sauter et al Nature Methods 2014

Developments in sample delivery

How to deliver bioparticles to a pulsed X-ray laser ?

6   (Jack Strutt)   Lord Rayleigh *on the*   [Nov. 14,

The coefficient A is to be determined from the consideration that the outwards normal velocity at the surface of the cylinder is equal to $\dot{a}\cos\kappa z$. Hence

$$i\kappa A J_0'(i\kappa a) = \dot{a} \quad\ldots\ldots(10).$$

Denoting the density by ρ, we have for the kinetic energy the expression

$$T = \tfrac{1}{2}\rho \int 2\pi a \cdot \phi \frac{d\phi}{dr_{(-a)}} dz;$$

or, if we reckon it in the same way as V per unit of length,

$$T = \tfrac{1}{2}\rho\pi a^2 \frac{J_0(i\kappa a)\,\dot{a}^2}{i\kappa a\, J_0'(i\kappa a)} \quad\ldots\ldots(11).$$

Thus, by Lagrange's method, if $a \propto e^{qt}$,

$$q^2 = \frac{T_1}{\rho a^3}\frac{(1-\kappa^2 a^2)\cdot i\kappa a \cdot J_0'(i\kappa a)}{J_0(i\kappa a)} \quad\ldots\ldots(12),$$

which determines the law of falling away from equilibrium for a disturbance of wave-length λ. The solutions for the various values of λ and the corresponding energies are independent of one another; and thus, by Fourier's theorem, it is possible to express the condition of the system at time t, after the communication of any infinitely small disturbances symmetrical about the axis. But what we are most concerned with at present is the value of q^2 as a function of κa, and especially the determination of that value of κa for which q^2 is a maximum. That such a maximum must exist is evident *a priori*. Writing x for κa, we have to examine the values of

$$\frac{(1-x^2)\cdot ix \cdot J_0'(ix)}{J_0(ix)} \quad\ldots\ldots(13).$$

Expanding in powers of x, we may write, for (13),

$$\tfrac{1}{2}x^2(1-x^2)\left\{1-\frac{x^2}{2^2}+\frac{x^4}{2^4.3}-\frac{11x^6}{2^6.3}+\frac{19x^8}{2^8.3.5}+\ldots\right\}\ldots(14).$$

or

$$\tfrac{1}{2}\left\{x^2-\tfrac{3}{2}x^4+\frac{7}{2^2.3}x^6-\frac{25}{2^5}x^8+\frac{91}{2^6.3.5}x^{10}+\ldots\right\}\ldots(15).$$

Hence, to find the maximum, we obtain by differentiation

$$1-\tfrac{3}{2}x^2+\frac{7}{2^4}x^4-\frac{100}{2^6}x^6+\frac{91}{2^6.3}x^8+\ldots = 0 \ldots\ldots(16).$$

If the last two terms be neglected, the quadratic gives $x^2 = \cdot4914$. If this value be substituted in the small terms, the equation becomes

$$\cdot98928 - \tfrac{3}{2}x^2+\tfrac{7}{16}x^4 = 0,$$

whence

$$x^2 = \cdot4858\ldots\ldots(17).$$

1878.]   *Instability of Jets.*   7

The corresponding value of λ is given by

$$\lambda = 4\cdot508\times 2s\ldots\ldots(18),$$

which gives accordingly the ratio of wave-length to diameter for the kind of disturbance which leads most rapidly to the disintegration of the cylindrical mass. The corresponding number obtained by Plateau from some experiments by Savart is 4·38, but this estimate involves a knowledge of the coefficient of contraction of a jet escaping through a small hole in a thin plate, and is probably liable to a greater error than its deviation from 4·51.

The following table exhibits the relationship between x^2 or $\kappa^2 a^2$ and the square root of expression (13) to which q is proportional:—
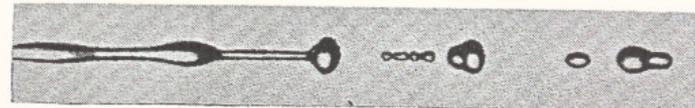

Fig. 5.15

Rayleigh's experiments with tuning fork

and spark-gap flash photography 1878

Necking instability has period = 4.5 D.

(consider KE and Surf En of liquid column)

Rate of droplet formation given by buzzer.

* Phil. Mag., Vol. xxxvi. 1868.   † Phil. Mag., Nov. 1871.

column unstable wrt wavelengths longer than circumference of column

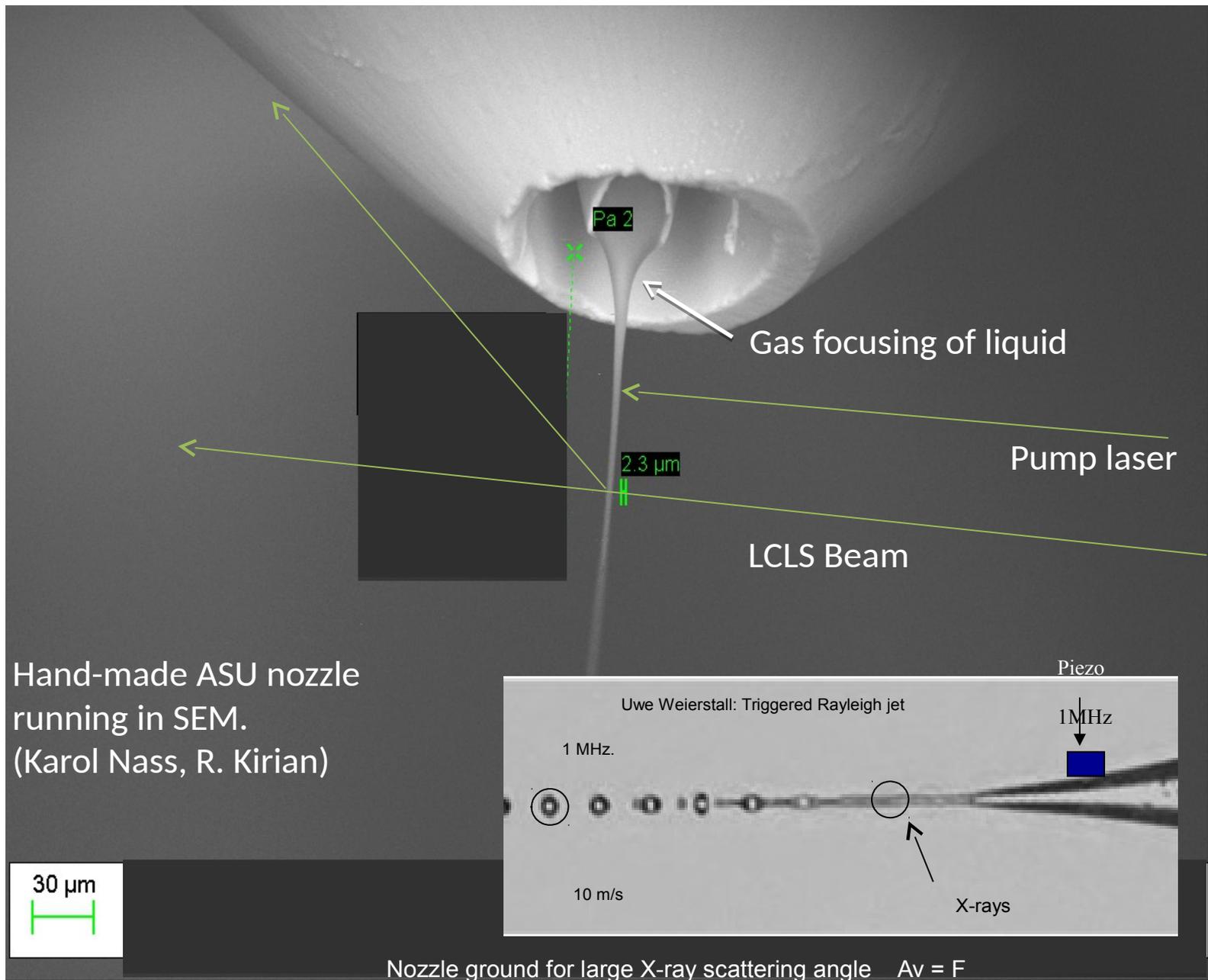# TR Protein nanoxtal sample delivery uses a liquid jet

Droplets freeze at 10^6 o/sec.

in vacuum

to vitreous

ice if cryo

protectant used

Flow velocity

about 10 m/sec

Area * velocity

= flow rate

= constant.

Pa 2

2.3 µm

Gas focusing of liquid

Pump laser

LCLS Beam

Hand-made ASU nozzle running in SEM.
(Karol Nass, R. Kirian)

30 µm

Uwe Weierstall: Triggered Rayleigh jet

Piezo

1 MHz.

1 MHz

10 m/s

X-rays

Nozzle ground for large X-ray scattering angle   Av = F
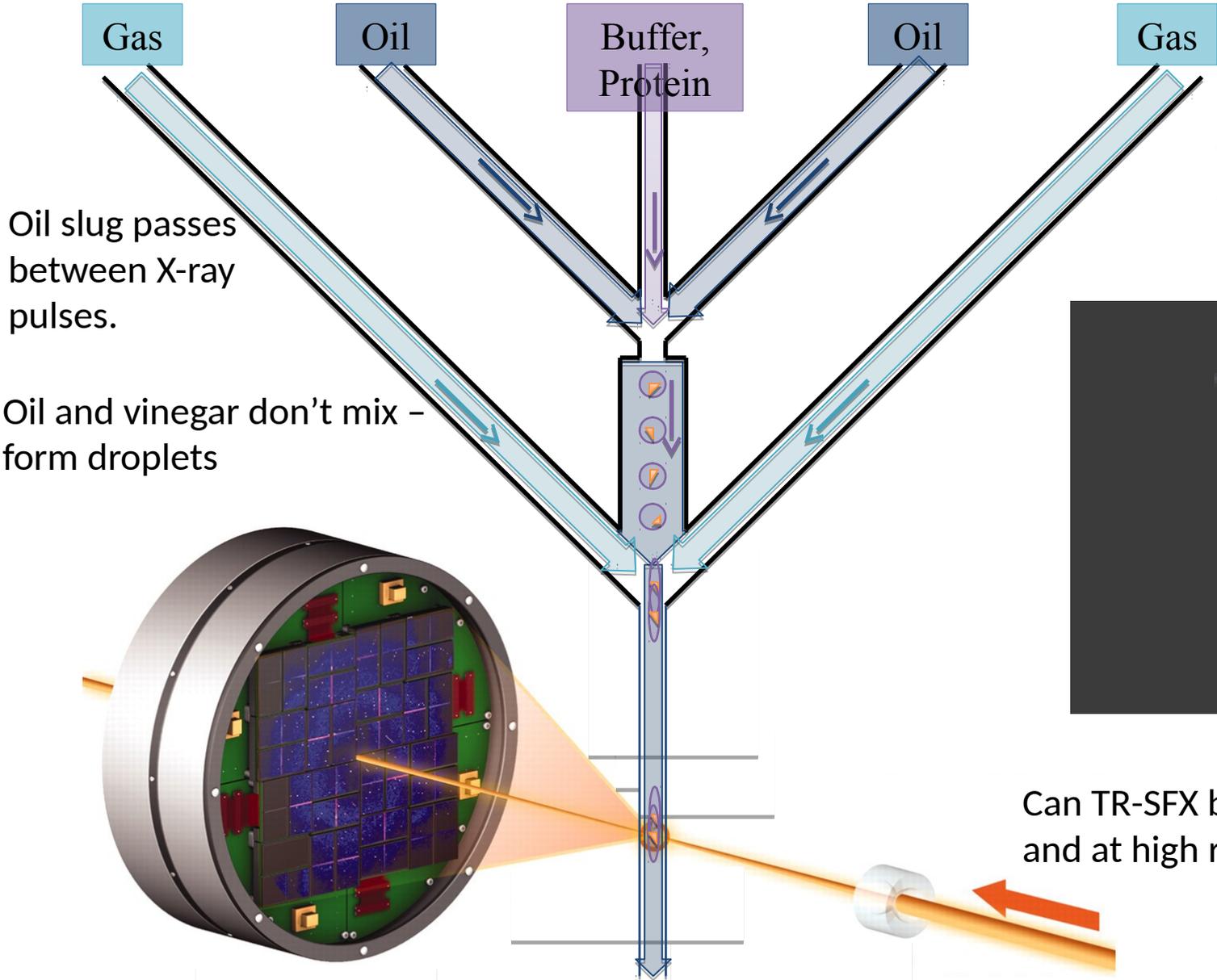
movie

This waste can be greatly reduced by switching off the jet between X-ray pulses.

We are developing a switching jet to reduce sample volume for liquid jet **(TR-SFX)**

Gas   Oil   Buffer, Protein   Oil   Gas

BioXFEL

The ASU Switching GDVN jet alternates oil and protein slugs across X-ray beam

Oil slug passes between X-ray pulses.

Oil and vinegar don't mix – form droplets
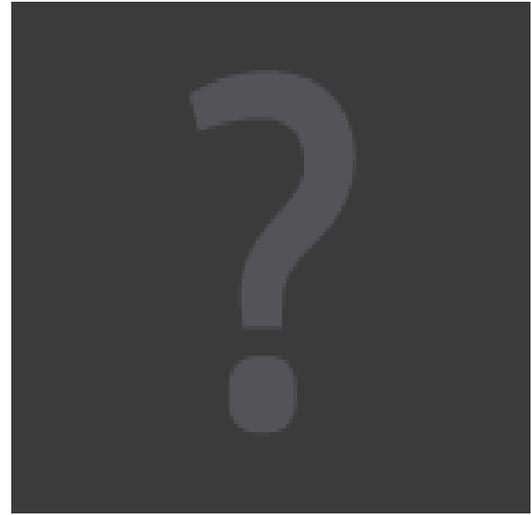
Can TR-SFX be done with this and at high rep-rate for LCLS 2 ?

Switching jet. Jan 2015 Alex. Ros, Austin, Nelson, JCHS, Weierstall.

Some membrane protein nanoxtals (& others) grow in Lipid Cubic Phase.

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

Works for soluble proteins too..

0.1 mg of Lys. used rather than 15 mg in Boutet (2012) with GDVN OR 6,000 xtas of phycocyanin to 1.95 A.
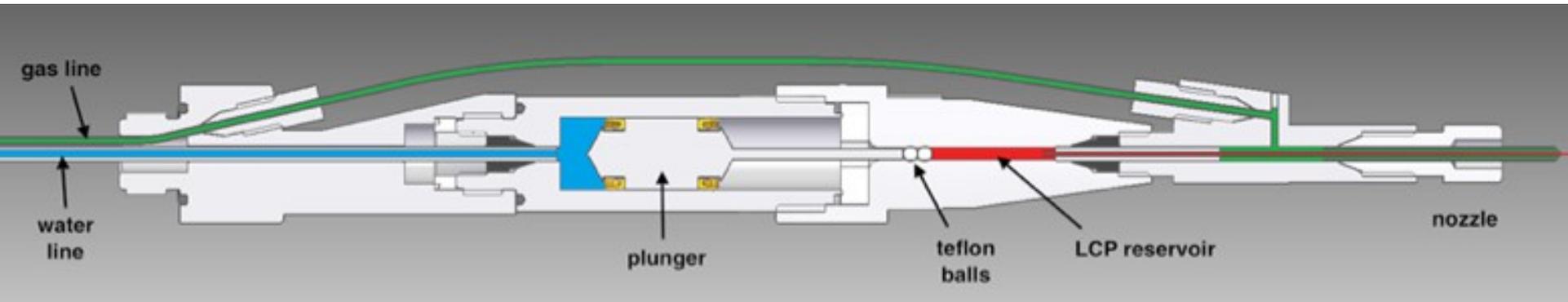
Cherezov Talk !!
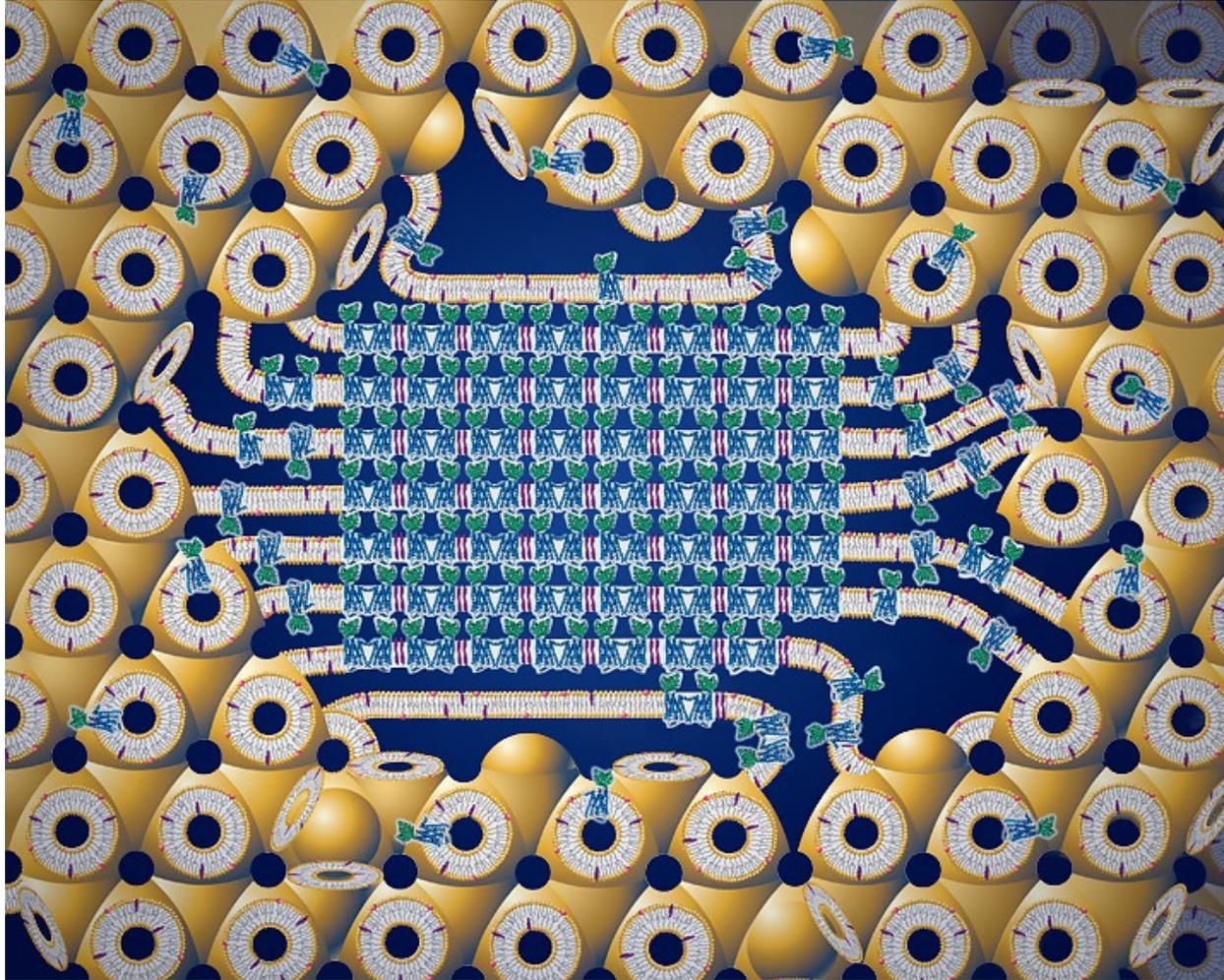
Martin Caffrey

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 !!!!
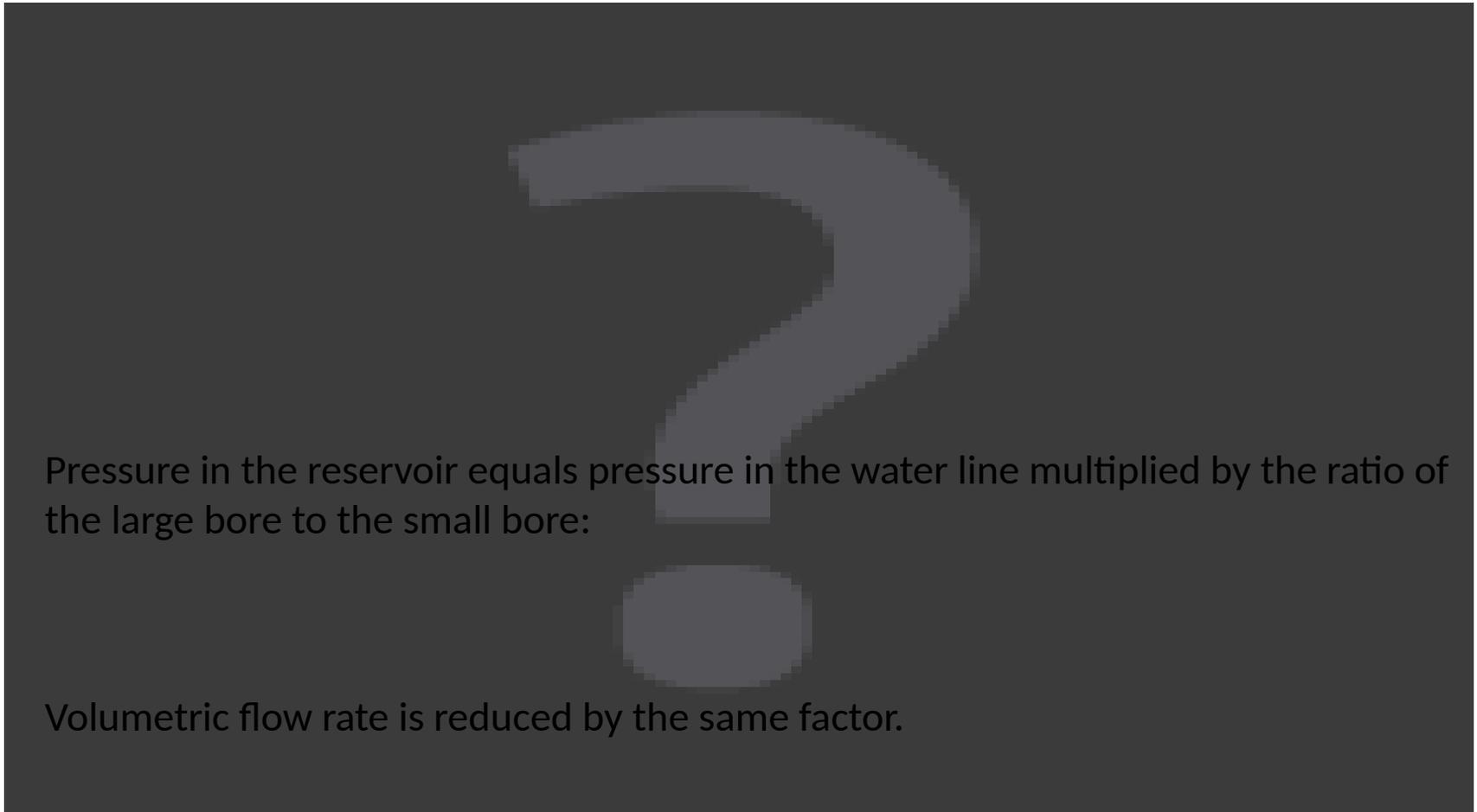
A grease gun to deliver nanocrystals to the XFEL in LCP.

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

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

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

*Use less protein of precious human protein, eg 0.3 milligrams

Used to solve Cyclopamine GPCR binding to smoothen receptor (3 A) Angiotensin, δ-OR, Serotonin.
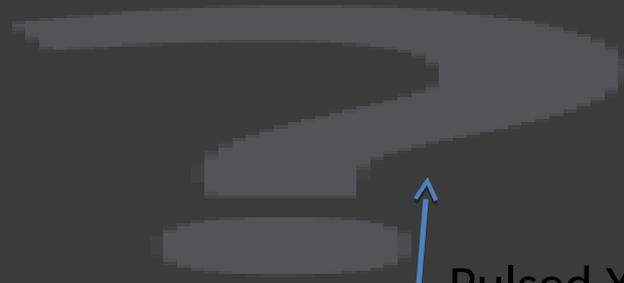**New media support PSI, PSII, Phycocyanine, Cytochrome C oxidase, Rhodopsin, Sindbis nanoxtals.**

Uwe Weierstall, Chelsie Conrad, Dan James , Martin Caffrey, et al Nature Coms Feb. 2014

The asymmetric piston provides an amplification in pressure.

Pressure in the reservoir equals pressure in the water line multiplied by the ratio of the large bore to the small bore:

Volumetric flow rate is reduced by the same factor.

"Toothpaste" jet

LCP jet operating at LCLS

Pulsed X-ray beam

$50\ \mu m$

Optimize conc, rep rate,
viscosity, chemistry, particle
size, jet size, for each sample.
V = F/A   AV = const.

XFEL beam drills 120 holes/sec across LCP tube.
Adjust flow speed to avoid shrapnel from last hole
Gaps due to time-structure of LCLS pulses.

Agarose also works for soluble proteins.
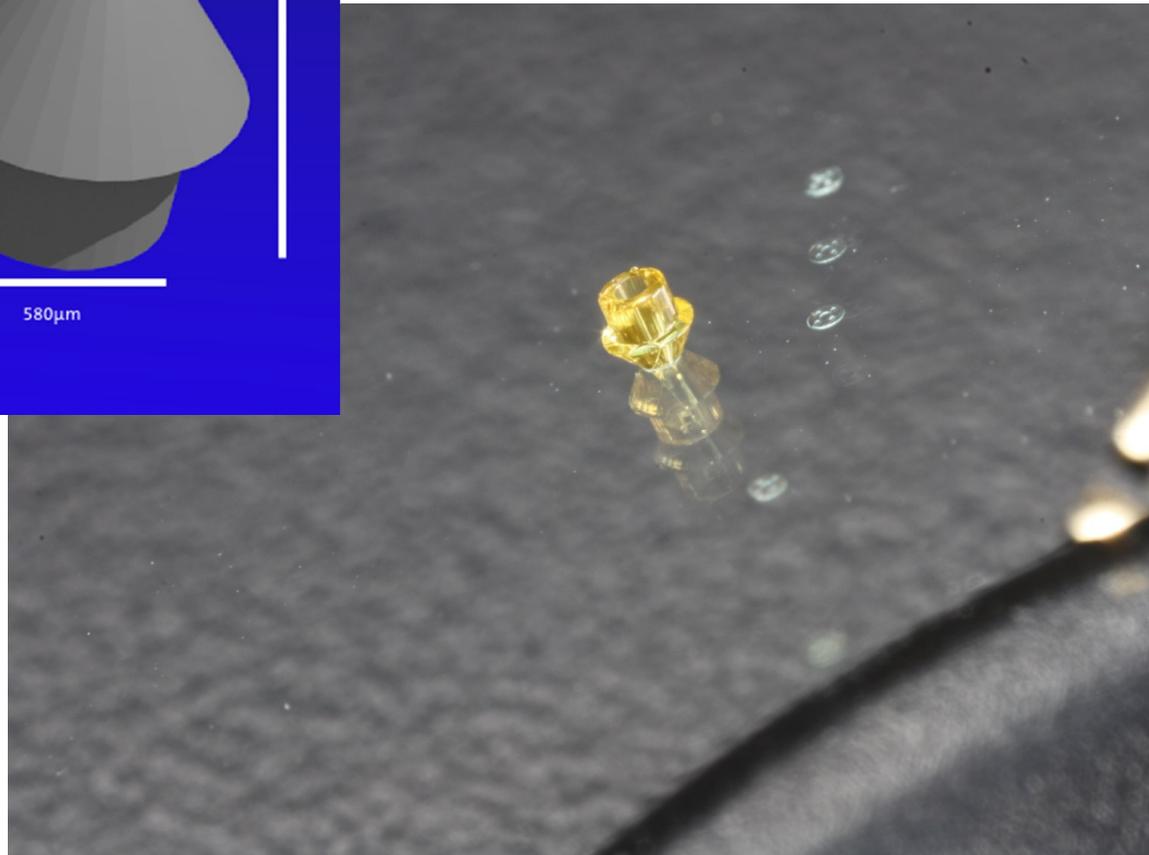Conrad et all IUCrJ in press. Phycocyanine to 2.6 Ang.

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

Also works at synchrotrons ! MSX 2 Ang Resolution in bR @ ESRF – Standfuss, Schertler.

CAD design and result after printing
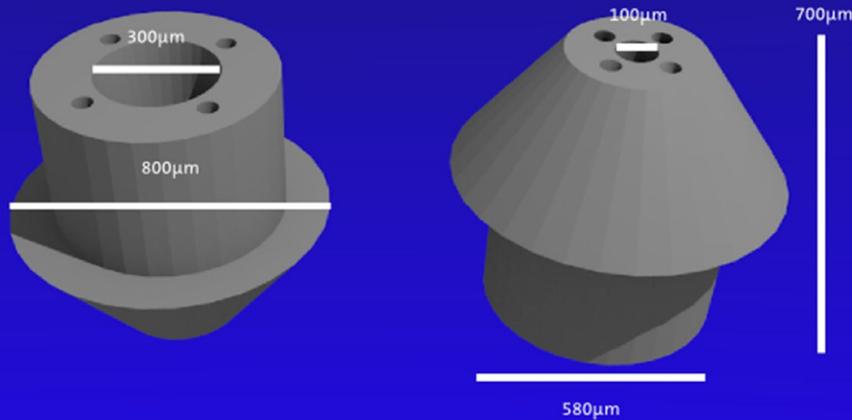
STC Collaboration with Nanoscribe, Germany.

Top: Dimension of the nozzle

Two-photon polymerization is a direct-write process with 100nm resolution.

Right: 3D printed nozzle on substrate

Garret Nelson, ASU graduate student, PhD in Physics

First result of 3D printed GDVN nozzle, made to ASU design at Nanoscribe, Germany

Enables flexible prototyping, optimization from CAD of mixing, switching, sheet jets etc.

Proposals for 3D printer sent to : NIH High-end Instrumentation; NIH Shared Instrumentation ; NSF MRI ; Continue till funded.
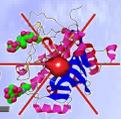Cost: $500K
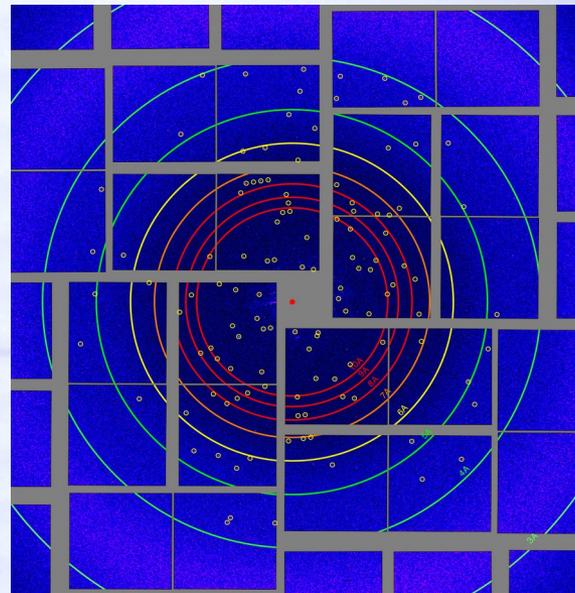
Cytochrome C Oxidase D. Rousseau
Extends to 4 Ang SFX LCLS

FIRST 3D (2PP) PRINTED NOZZLE RUNNING FEB 2015

Garret Nelson

Fabrication time 4 hours. Cost ~ $1000. 2PP Printer cost $500K (Handmade take 4 hours, "free")
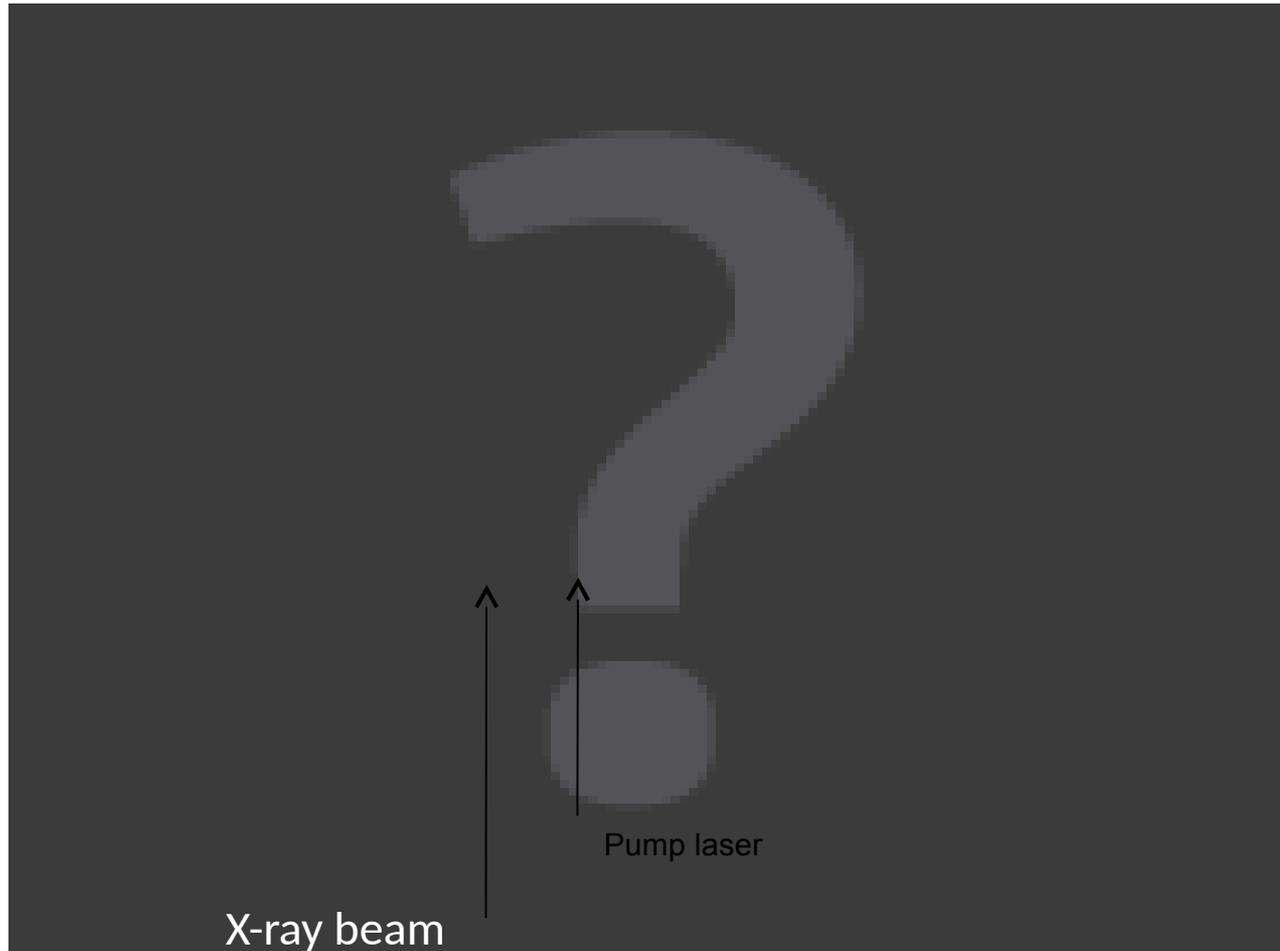- Integrated with microfluidics (Ros Lab) – nanoxtal size sorter using electrophoretics..

Development of Time-Resolved SFX

For *irreversible processes* – eg catalysis, enzymes

Pump laser and XFEL on jet – exploding PS I nanoxtals

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

Movie

Aquila
Optics
Express
2012

Kupitz,
Fromme
et al
Nature
2014

X-ray beam

Pump laser

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 xtal 10 microseconds before XRD snapshot

Travelling at 10 m/s, nanoxtals go 100 microns, less than width of 400nm doubled Jedai fs beam

TR-SFX of Photoactive Yellow Protein (now 1.4 Ang resolution).
Blue light photoreceptor mechanism at 1 µsec & 10 ns time delays.

Pump light saturates nanoxtal

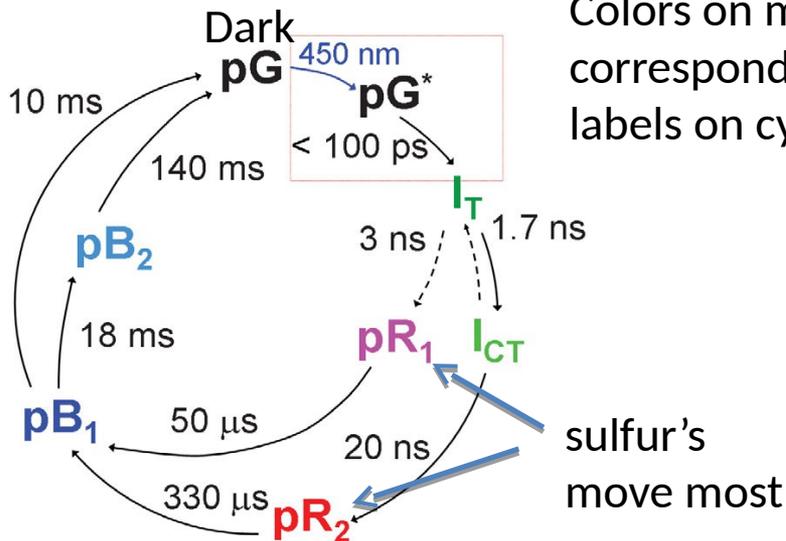A light-sensor in purple **photosynthetic** bacteria

Science Dec 2014
Tenboer…. Marius Schmidt et al
group (Milwaukee) +STC

Delays 10ns and
1 microsec.
pR1 and pR2 are maximally
occupied at 1 microsec.

Red is PR1
then later
PR2. Not a
diff. map.

Colors on map
correspond to
labels on cycle

Dark
pG — 450 nm → **pG***

10 ms

140 ms   < 100 ps

pB₂

18 ms

I_T

3 ns   1.7 ns

pR₁   **I_CT**

pB₁   50 µs   20 ns

330 µs **pR₂**

sulfur's
move most

reaction rate lifetimes, exp decay. SVD
Six stable intermediates

Photoactive Yellow Protein, pR₁, pR₂, imaged at LCLS
Shows total density of intermediates that accumulate
and decay during the photocycle. Chromophores.

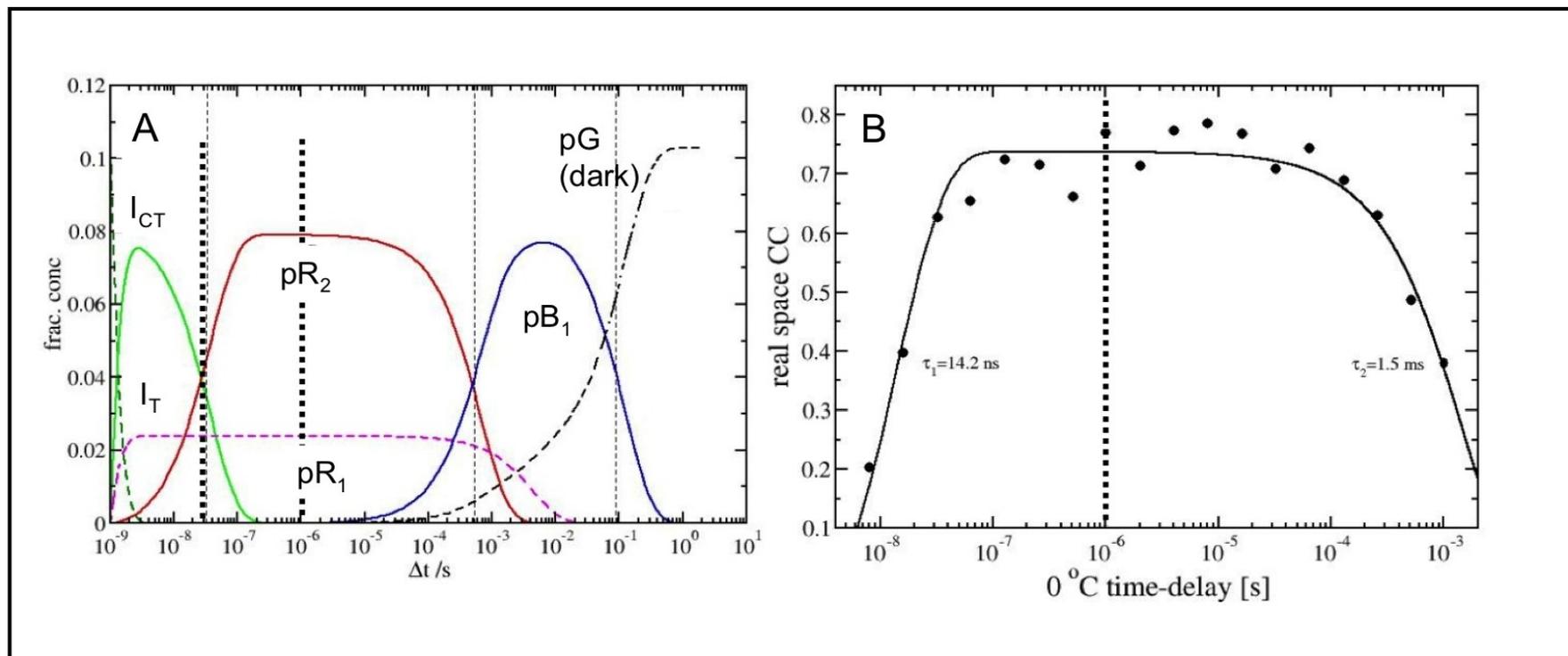# Time dependence of concentration of intermediate states

Fig. S5. (A) Approximate time course of concentrations of the intermediates in the PYP photocyle calculated from the mechanism show in the main text. Thick vertical lines: time-points in this study. (B) Real space correlation coefficients of DED maps from a time-series at 0 °C (solid spheres) collected at BioCARS (Laue) versus the 1 μs DED map from CXI (TR-SFX); positive DED features selected. Exponential rising and decaying phases are shown (solid line). Relaxation times are marked. Dotted vertical line: time-delay of the TR-SFX experiment at CXI.

Tenboer, Schmidt et al Science Dec 2014
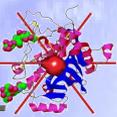
Preliminary Time Resolved SFX-LCP data from LCLS

~ 40000 indexable patterns were collected in 12h of beamtime with 8000-10000 per time point

1 millisec time delay probes M1 state
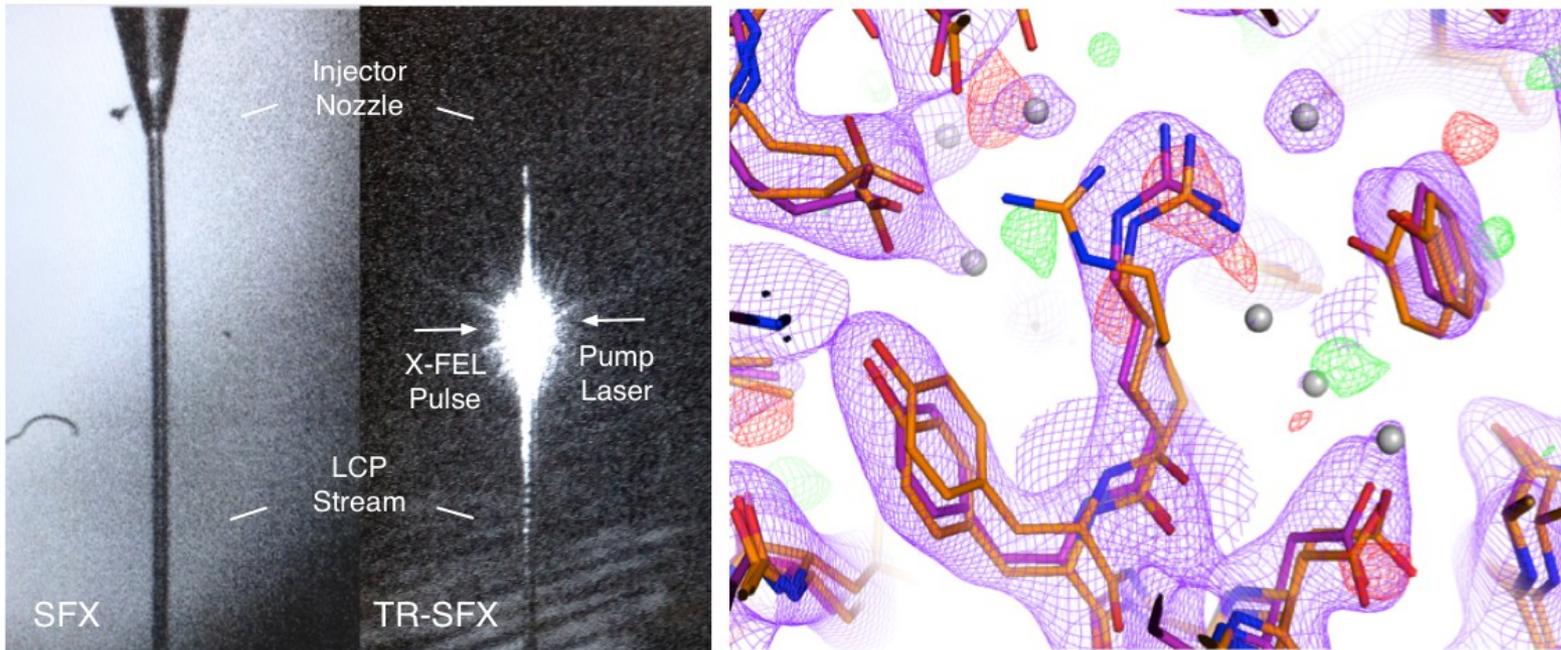
Fo-Fo difference maps at 3 sigma. Compatible with known conformational changes on bR activation for example a rotamer change of Arg82, a key residue in the proton transfer chain.

This is time-resolved (pump-probe) SFX at LCLS in LCP

Standfuss, Weierstall, Schertler et al 2015

For the future, BioXFEL will develop 2-color methods for SFX.

BioXFEL

- Two successive hits from same xtal (first below damage threshold) with different wavelengths on the same camera readout. (Damage movie).

* Pump pulse occurs between these.

* No errors due to xtal size, orientation in difference

* 2 Color also useful for SAD phasing (Wakatsuki)
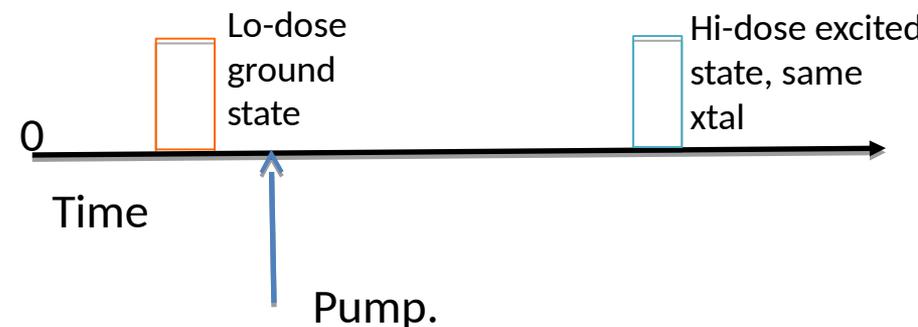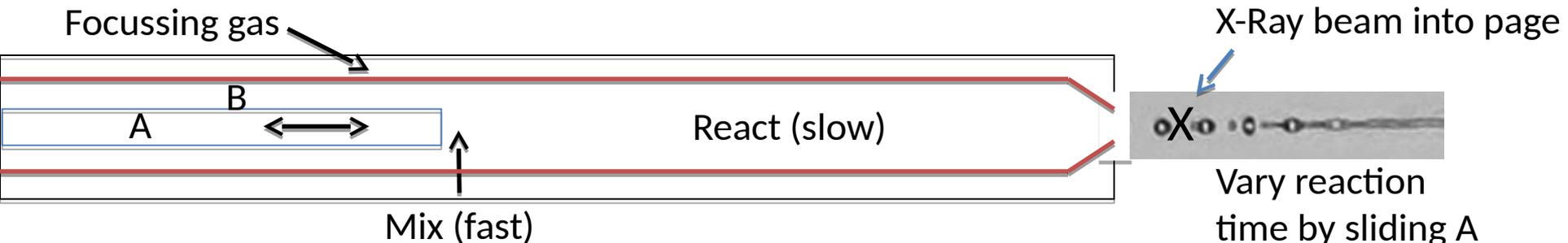

Two-color patterns will improve TR-SFX accuracy.

Two-wavelengths from two successive pulses on same readout simulated for I$_3$C xtal ("Magic triangle").

Chufeng Li PhD, Struct Dyn. Submitted
Submitted papers by Wakatsuki, Schlichting

BioXFEL is developing a mixing jet for dynamics

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

D.Wang, J. Spence, U.Weierstall, L.Pollack J. Synch Rad. 2014.

BioXFEL

Focussing gas

X-Ray beam into page

B

A

React (slow)

X

Mix (fast)

Vary reaction time by sliding A

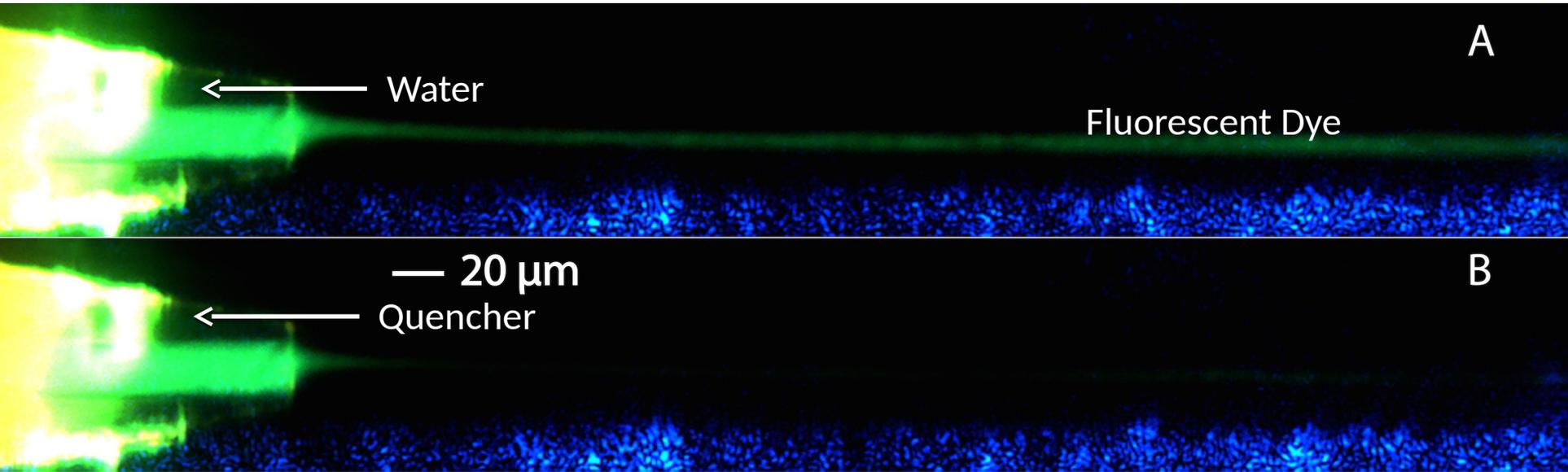Mixing time (time resolution) is 200 - 300 microseconds
Reaction time adjustable from 200 microsec to 1 sec.
Diffusive mixing is possible using protein nanoxtals !

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

Diffusion into nanxtals is fast : 17 microsec for glucose into 0.5 micron xtal

A

← Water

Fluorescent Dye

— 20 μm

← Quencher

B

Fast Solution Scattering (FSS)

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)
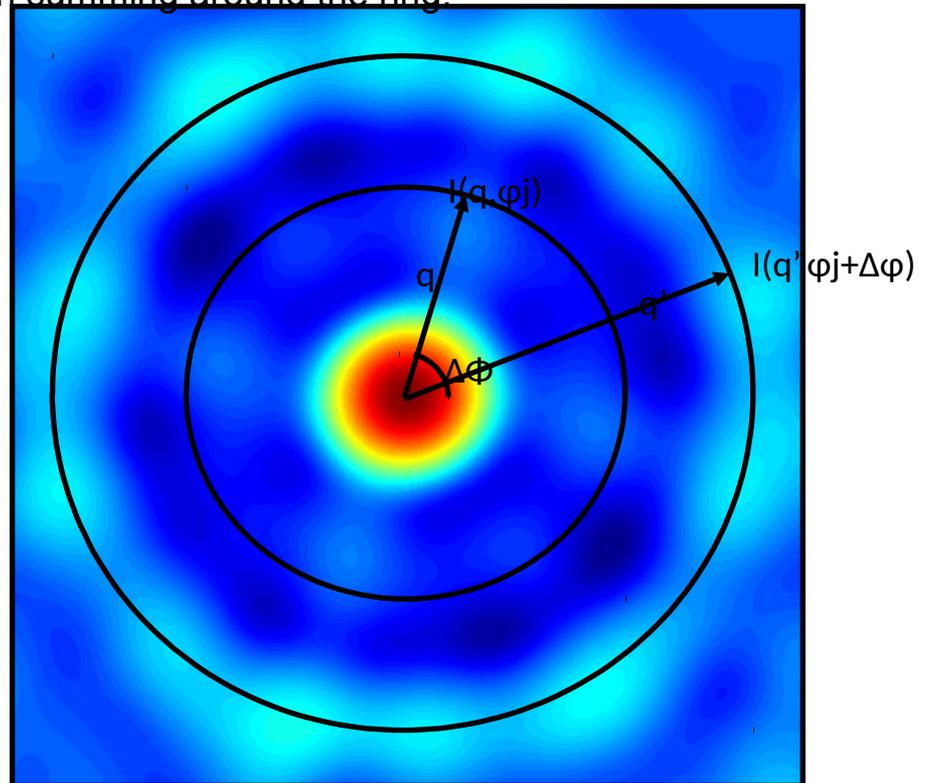
Donateli, Sethjan, Zwart PNAS 2015 have new "one-step" phasing for Kam AC patterns. FSS is 2D for particles frozen in time time or space. Better inversion to 3D.

KAM's AC method for FSS has been demonstrated experimentally.

The image of one dumbell has been reconstructed using XFEL snapshot scattering from randomly oriented dumbells in solution.

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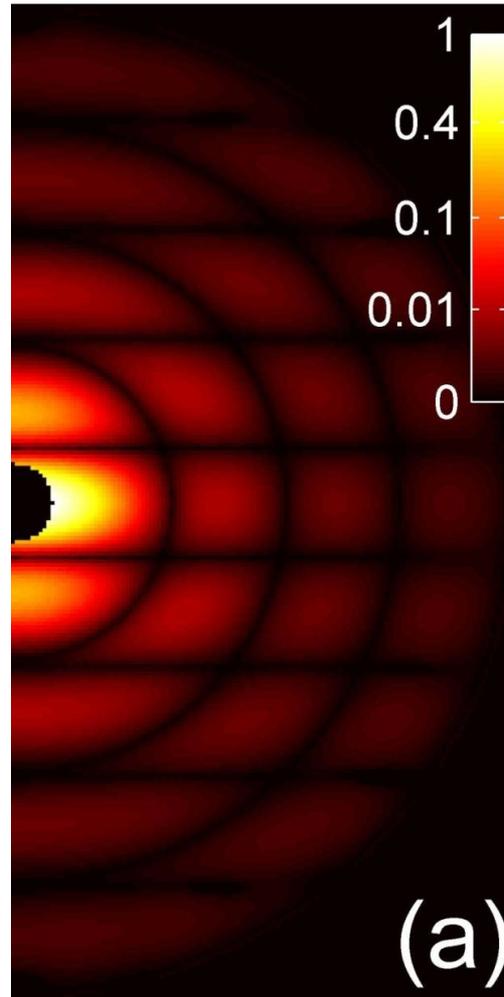*one particle per shot* in solution.
635 patterns.

Two angles required
to define orientation.

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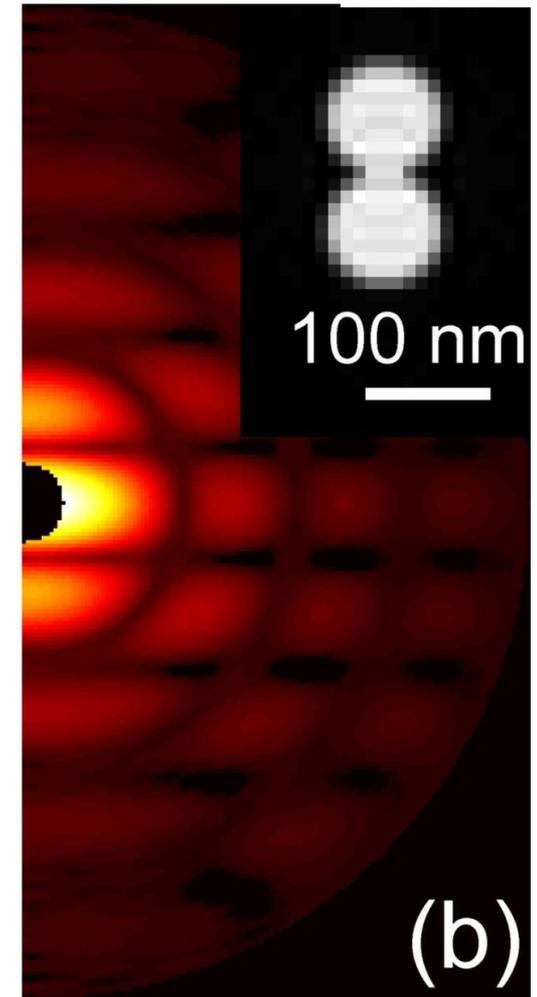intensities used to get signs (phases)

More recent (Pedrini) work had many
particles per shot.

Polystyrene Dumbbells
Sphere diam 91 nm.
Resolution 10 nm.

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

100 nm

(a) Model

(b) Experiment (one ptcle)

Starodub, Bogan, Spence... Nature Comms 2012

*BioXFEL*

Fast Solution Scattering (FSS)

BioXFEL is developing the Angular Correlation (AC) method.

(This extracts image of one particle from scattering from many, randomly oriented)

Doubling FSS beam size gives same signal.
FSS has 100% hit rate, avoids chamber vibration,
beam position fluctuations. No aiming needed !

"Random hits with beam same size as ptcle
give same signal/time as the same beam
broadened over many particles".

But targeted head-on SP hits are better,
- less background more signal/time. Then we need to aim.

Molecules

Beam
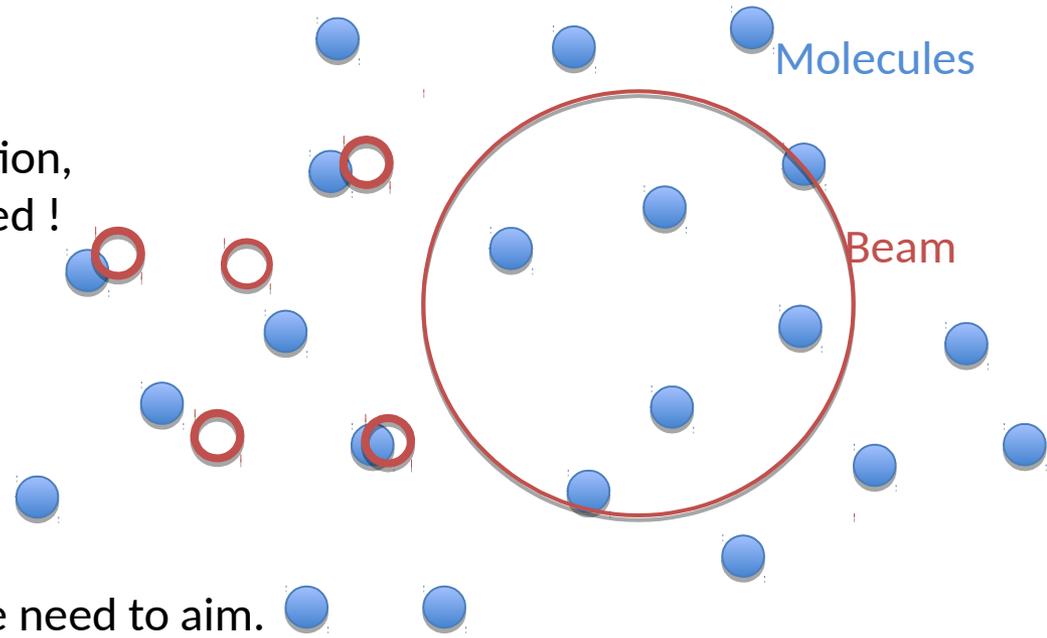
Pump-probe AC FSS difference method is most powerful
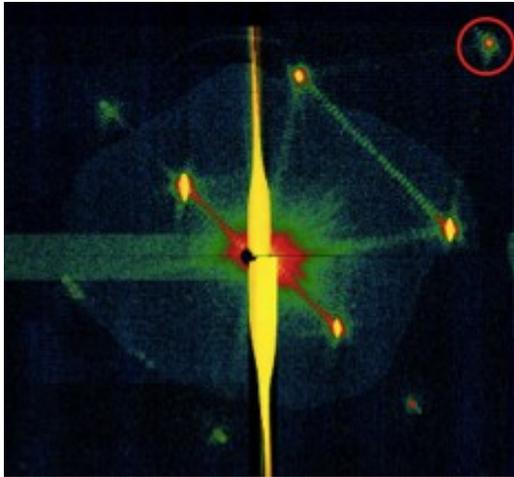for structures known to high resolution by MX

We seek anisotropy in the Neutze FSS LCLS data set   (Zwart , H. Liu, Spence).

Modelling can be combined with AC Kam method.

*Fast solution scattering

Molecules

Unwanted streak

LCLS beam into page
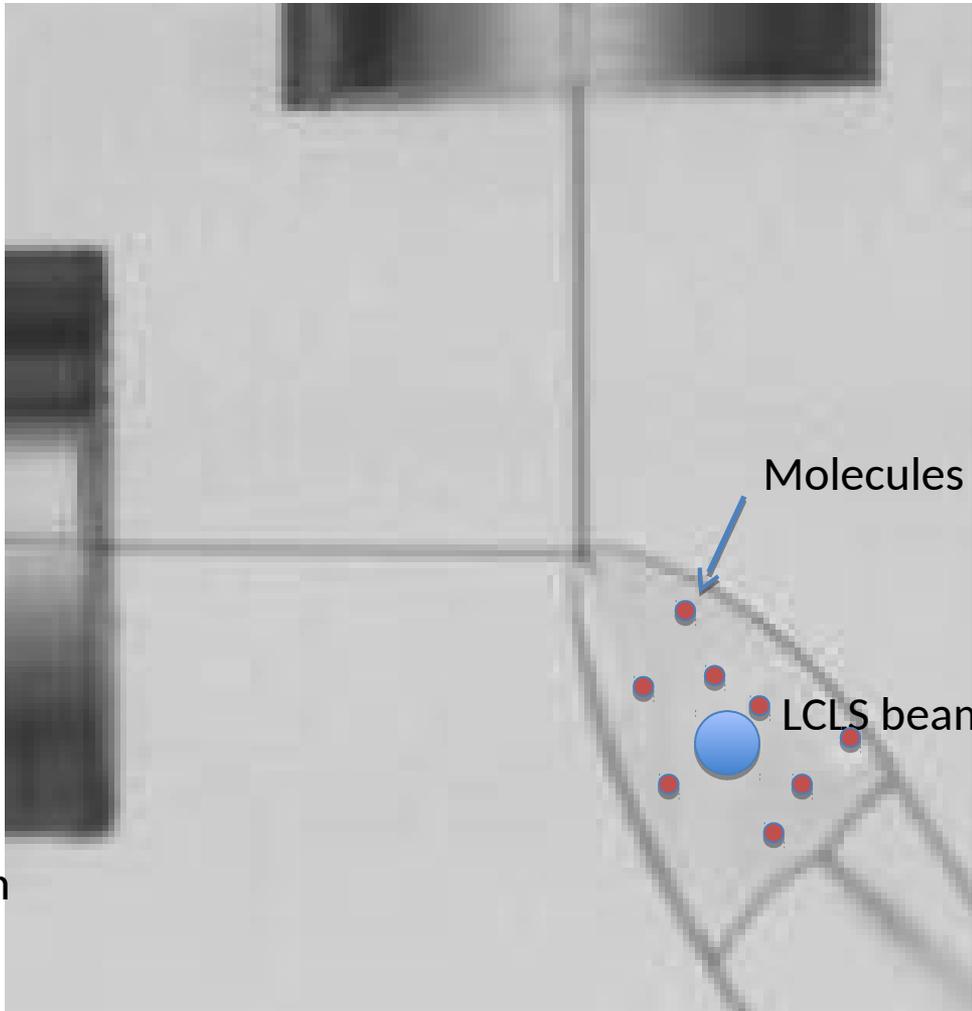
Fabricate using 2PP 3D printer with sub-micron resolution

Microliquids 2011

Sheet jets would eliminate the diffraction streak from the jet for FSS

Conc of mols
is low.
Thickness of
sheet 0.1 micron.

3D printed nozzles !

Nozzle

r

16 mm

Rim

5 mm
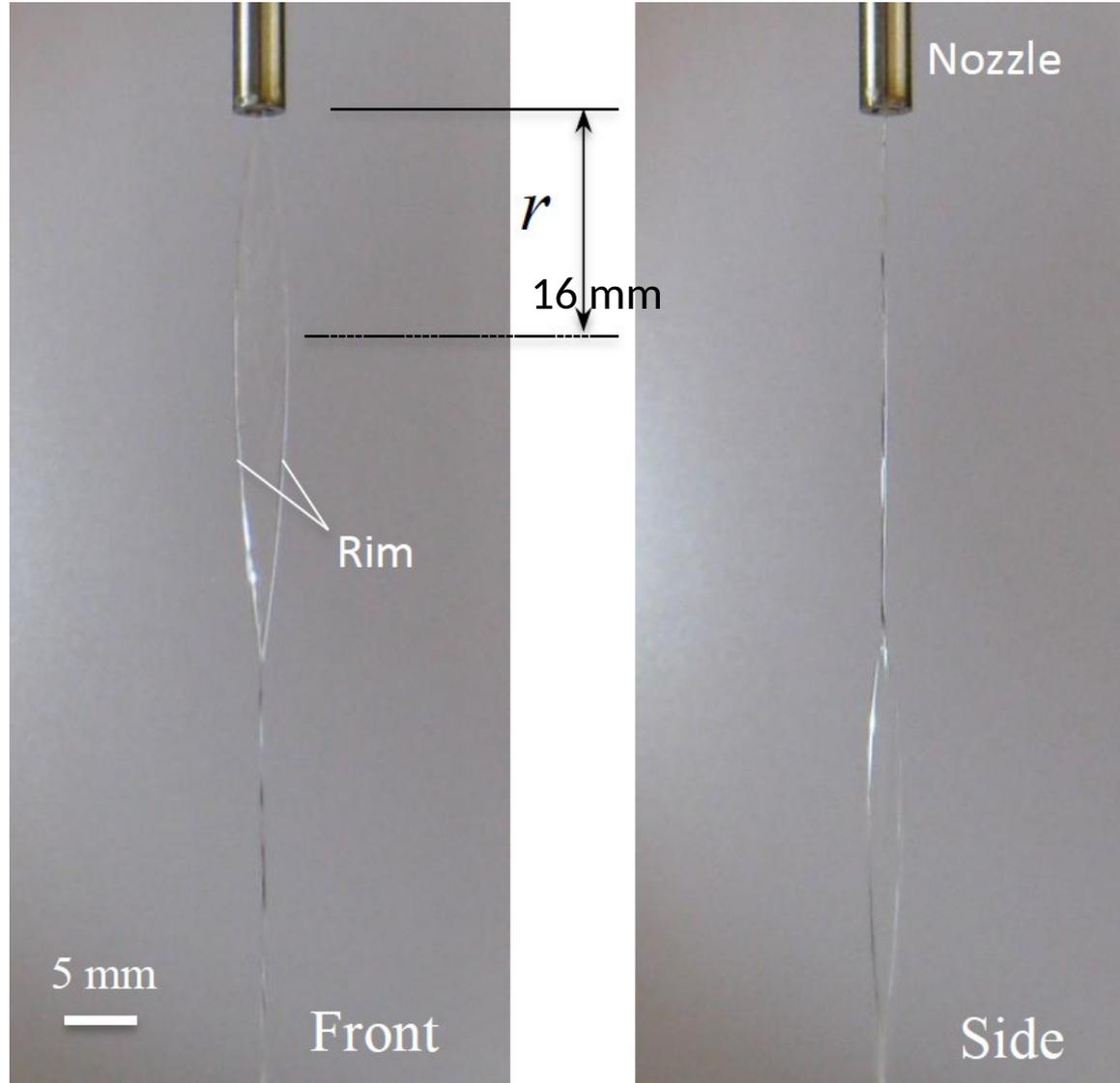
Front

Side

Ohba et al 2014
G.I. Taylor 1959
Savart 1833.

Fig. 3. Front- and side-view photographs of liquid sheet of water with flow rate of 130 mL/min. r: the distance from the nozzle exit.

Compact Light Source started at ASU (MIT design)

Graves.....Kaertner, Moncton. Phys Rev (2014)

Incoherent, phase 1
Linac + Inverse compton.
Linac cavities fed separately
X-band. 18 MeV electrons
< 12 kV X-rays
Laser, phase 2
$\lambda_x = \lambda_L/\gamma^2$
Inverse Compton.
THz Linac.
Emittance exchange from
spatially patterned beam
for lasing.

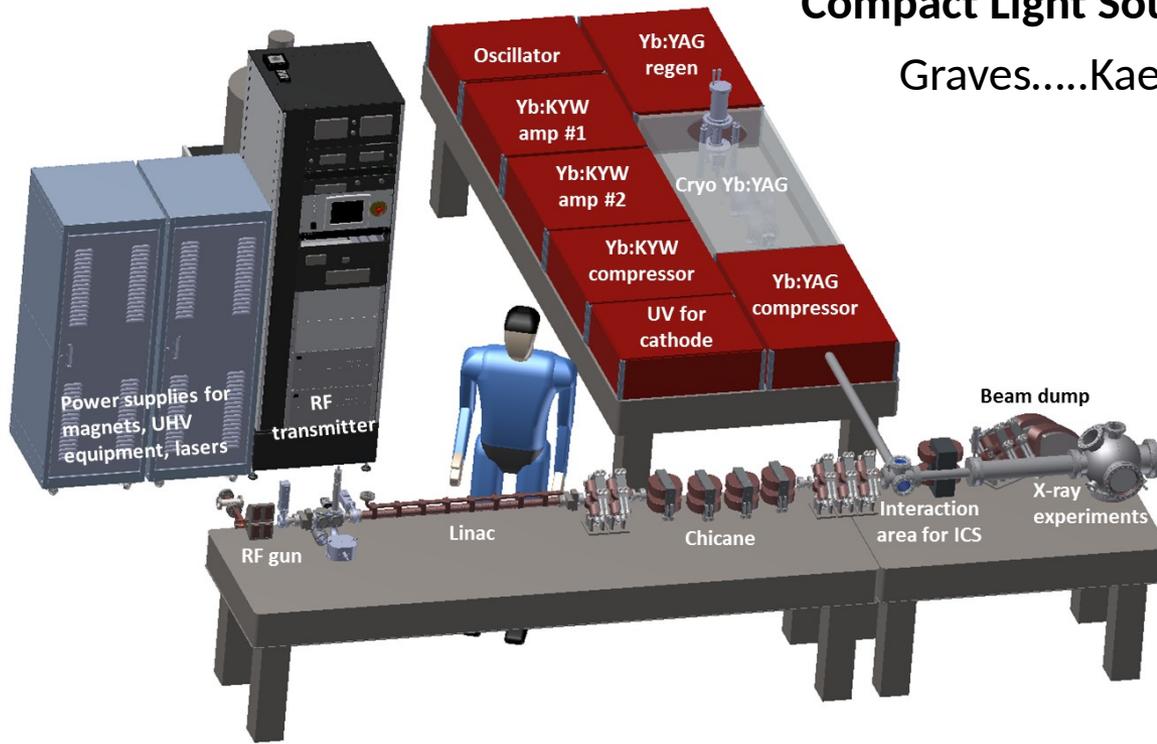FIG. 2: CAD layout of the components for the compact x-ray source showing the lasers including Yb:KYW that produces electrons via photoemission and the cryo Yb:YAG amplifier used for ICS. Accelerator components shown include the RF gun, short linac and transport magnets. The cabinets house the RF transmitter and power supplies for magnets, vacuum equipment, and lasers.

$$\lambda_x = \frac{\lambda_L}{4\gamma^2}(1 + a_0^2 + \gamma^2\Delta\theta^2),$$

$$\left(\frac{d^2U}{d\omega d\Omega}\right)_e \simeq \alpha h a_0^2 N_L^2 \gamma^2, \quad \text{for N}_L \text{ laser periods}$$

For SASE:

$$\frac{\Delta\lambda}{\lambda} = \frac{1}{N_u},$$

$$I = I_0 \exp\left(\frac{ut}{L_G}\right) = I_0 \exp\left(\frac{x}{L_G}\right).$$

If coherent, $I_x \sim N^2$
If incoherent $I_x \sim N$

$$L_G = \text{constant} \times \left(\frac{i}{\Sigma}\right)^{-1/3} B_0^{-2/3} L^{-1/3}\gamma,$$

$N_x \sim q$ V/bunch

$$\lambda = \frac{L}{2\gamma^2}\left(1 + \frac{K^2}{2}\right),$$

Σ – bunch cross section
N e- per bunch.

Summary of 12 keV parameters

(incoherent ICS, undulator-like radiation)

| Parameter	0.1% Bandwidth	5% Bandwidth	Units
Average flux	2×10^{10}	5×10^{11}	photons/s
Average brilliance	7×10^{12}	2×10^{12}	photons/(s .1% mm²mrad²)
Peak brilliance	3×10^{19}	9×10^{18}	photons/(s .1% mm²mrad²)
RMS horizontal size	2.4	2.5	microns
RMS vertical size	1.8	1.9	microns
RMS horizontal angle	3.3	4.3	mrad
RMS vertical angle	3.3	4.3	mrad
Photons per pulse	2×10^5	5×10^6	
RMS pulse length	490	490	fs
Repetition rate	100	100	kHz

Compact incoherent source for ASU, not lasing. Graves et al Phys Rev 2014

Lasing achieved later by patterning the beam, emittance exchange, Short THz linac (Graves et al Conf. 2014).

Attosecond XFEL avoids damage, gives full Braggs, not partials.

$$\Delta t \text{ (fs)} = 4.14/\Delta E \text{ (eV)}$$

"Compact attosecond X-ray sources and their applications"
Workshop CFEL June 2015
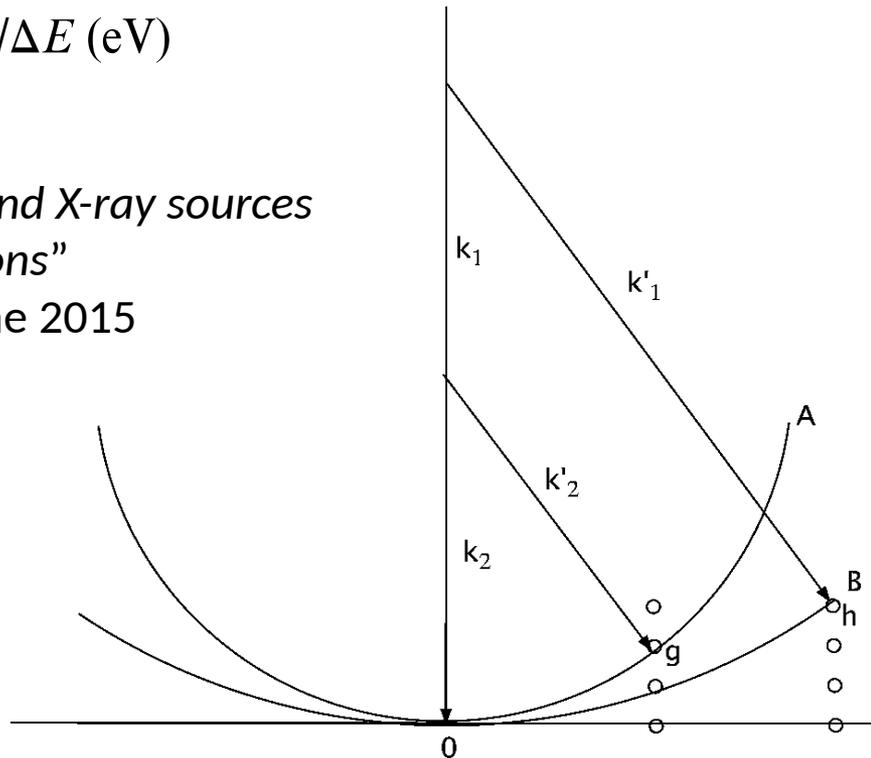
Different wavelengths cause different Bragg reflections to interfere on same detector pixel , if pulse duration less than beat period.
This gives structure factor phase information .

Attosecond XFEL gives 300 eV = 3% bandwidth at 10 kV with 14 as pulses, hence full reflections, without damage.
Coherence length L = λ E/ΔE ~ 3nm, less than sample thickness !
3-phase sums (as above , plus Friedel conjugate of sum) are origin independent.

Spence, Trans Faraday Soc 2015

Can electron beams outrun radiation damage ?

When focusing a 500 micron diameter beam on an approximately 0.1 micron "virus" we find less than one electron scattered per 100 fs shot. (3MeV, Murooka's system).

Or 49,043 elastically scattered electrons if beam could be focused down to 0.1 microns.

Cryo-em "dose" is about 10 e$^-$/Ang^2
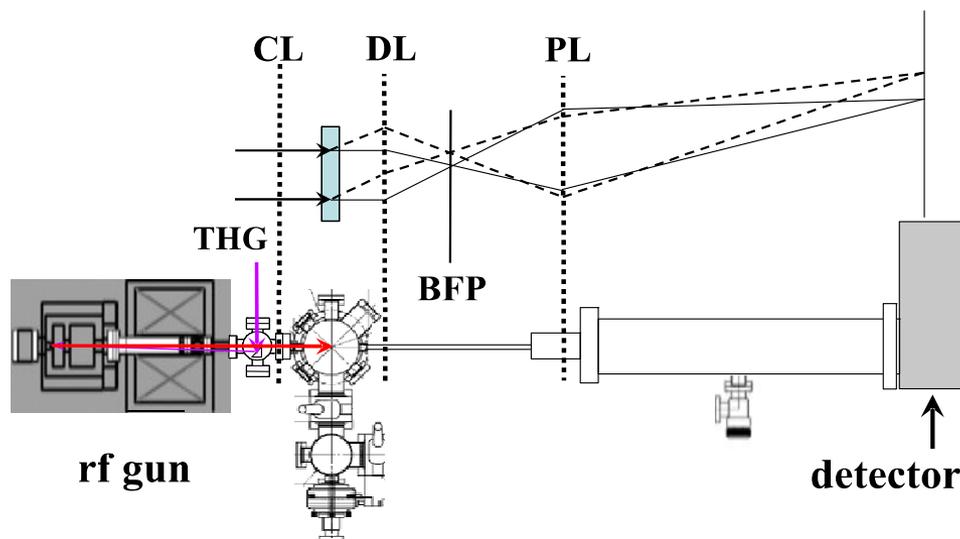
Electron Diffraction Camera. 3 MeV, 1.06E6 e$^-$/pulse, 100 fs, 500 μ beam
Murooka et al 2011.

Electrons which loose energy continue to detector to make background, unlike X-rays !

Spence, Musumeci, Subramanian, "Applied Physics" 2015. submitted.

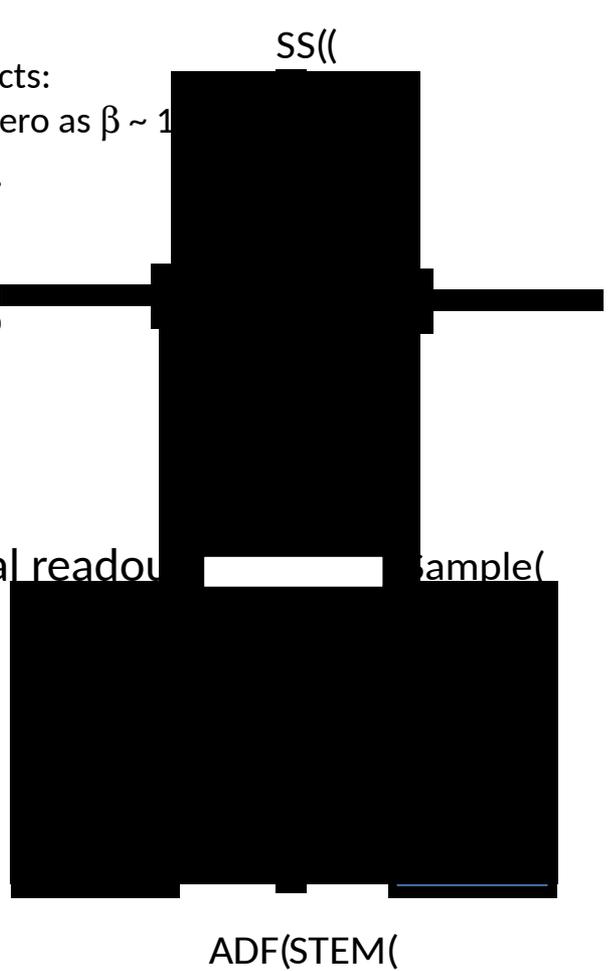# "Hollow cone" allows *large incoherent* source for fast e⁻ imaging at hi-res.

Atomic-resolution imaging does ***not*** require coherent illumination, which wastes electrons.

Space-charge effects:
Lorentz force to zero as β ~ 1

1. One e-/shot **.**
2. Dark field.
3. High energy
4. Spherical cap

SS((

TS(

Spence et al, Applied
Physics 2015. submtd.

Scan for serial readou... Sample(
of image

OA(

Full-field image
simultaneous in
every pixel (camera)

TD(

ADF(STEM(

Hollow(cone(TEM(

1. Under reciprocal apertureing conditions (identical objective lenses) the STEM scanned image is identical to that produced immediately at every pixel in the TEM.
2. *The number of electrons arriving per unit time at **every** pixel of the HC-TEM image is the same as that arriving at the whole STEM detector, for **one** STEM probe position, if the brightness of the extended HC source is equal to the STEM point source. (may be reduced by*

Crystal undulators for lazing using Coherent bremsstrahlung

Counts

Reciprocal space

80 kV

3

Si

4

Cu

n=3

4

5

6

7

2

120 kV

5

Cr

Fe Co

FOLZ

n=2

n=1

n=0

(ooo)

111

Cu

Cu

kV

Lines absent due to zero structure factors !

2   4   6   8

kV

Tunable, monochromatic X-ray emission from 0.1 MeV beam
passing thro' thin xtal of diamond

−n

γ EDS

g

d

θ

L

Bragg
angle
90°

d = λ/2
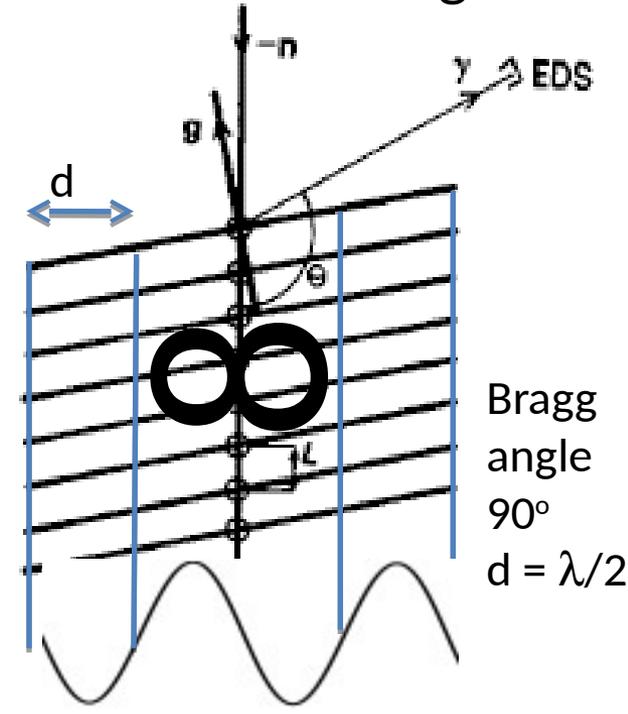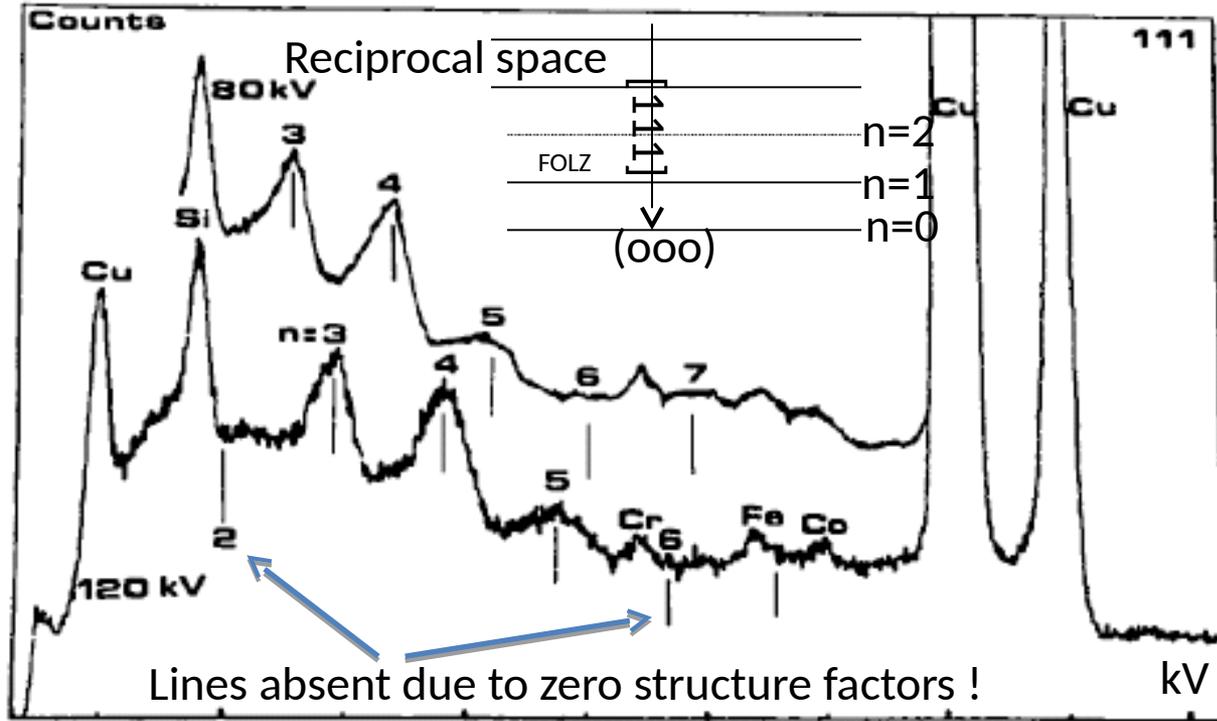
Frequency at which beam e⁻
encounters atoms in its path is

$\omega = 2\pi v / L = 2\pi c \beta / L$

$$E = 12.4\ \beta\ /\ L(1-\cos\theta)$$

Continuous Brem from glassy target concentrates into lines for xtal, one for each extinction dist.

Harmonics may be labelled with Miller indices for layer of reciprocal lattice points responsible

Spence and Reese, Acta A42, p.577 (1984)

*New data analysis team: Zatsepin, Grant, Messerschmidt

BioXFEL

http://www.bioxfel.org

The NSF BioXFEL STC Research Consortium

NSF

Biologists

University@Buffalo HWI
E. Lattman, E. Snell, T. Grant*
Outreach, nanocrystals, data

ASU
J. Spence. Zatsepin*
Science, Data analysis

Techniques (Methods Group)

established Oct 2013

U. Wisc. Milwaukee
M. Schmidt
Energetics of Bioreactions

Center for Applied Structural Discovery (CASD) ASU

Rice
G. Phillips:
conformational variability

UCSF
R. Stroud
membrane proteins

BioXFEL STC

ASU
P. Fromme:
membrane proteins
B. Hogue: viruses

Stanford
R. Kornberg
D. Bushnell: Pol II, S.P. transcription complex

Puerto Rico International conference,
Data Analysis Workshop,
LCP injector sales (17), Beamtimes, LCLS 2.
Crystallization workshops at HWI.

Cornell
L. Pollack:
mixing reactions

LBNL, LLNL
Unfunded collaboration
J. Holton, M. Frank, S. Hau-Riege

U. Wisc. Milwaukee
A. Ourmazd, D. Saldin:
SP data analysis

ASU
injectors
Weierstall,
Kirian

DESY
Unfunded collaboration
Chapman, Messerschmidt*

Current BioXFEL work includes…

1. GPCRs for drug design. Cherezov, Weierstall, Stevens collab.
2. Beyond Monte Carlo – optimization and partiality. White, Li, Zatspin.
3. Time-resolved SFX for photosynthesis. Schmidt, Fromme.
4. Fast Solution Scattering (FSS).   Mixing jet, Kam method, Angular correlations.
5. Viscous jets. LCP with XFEL or SR, in helium, Agarose for solubles.

Building blocks have explanatory power for understanding mechanisms in matter.
eg The α-helix in bio - need only 6 Ang resolution to see it.
**   - Atoms at 2 Ang for mat sci., cond matter. Eg kink landscape.**

Current XFELs need Bragg Boost (& lasing) to see them (or modelling, Bayesian)
So either learn to make 10x10x10 xtals or build 1E6 times more powerful XFEL.

TR-SFX in Bio can image molecular machines inside one molecule in one hydrated unit cell (hence get Bragg boost), not possible in a continuously bonded silicon wafer
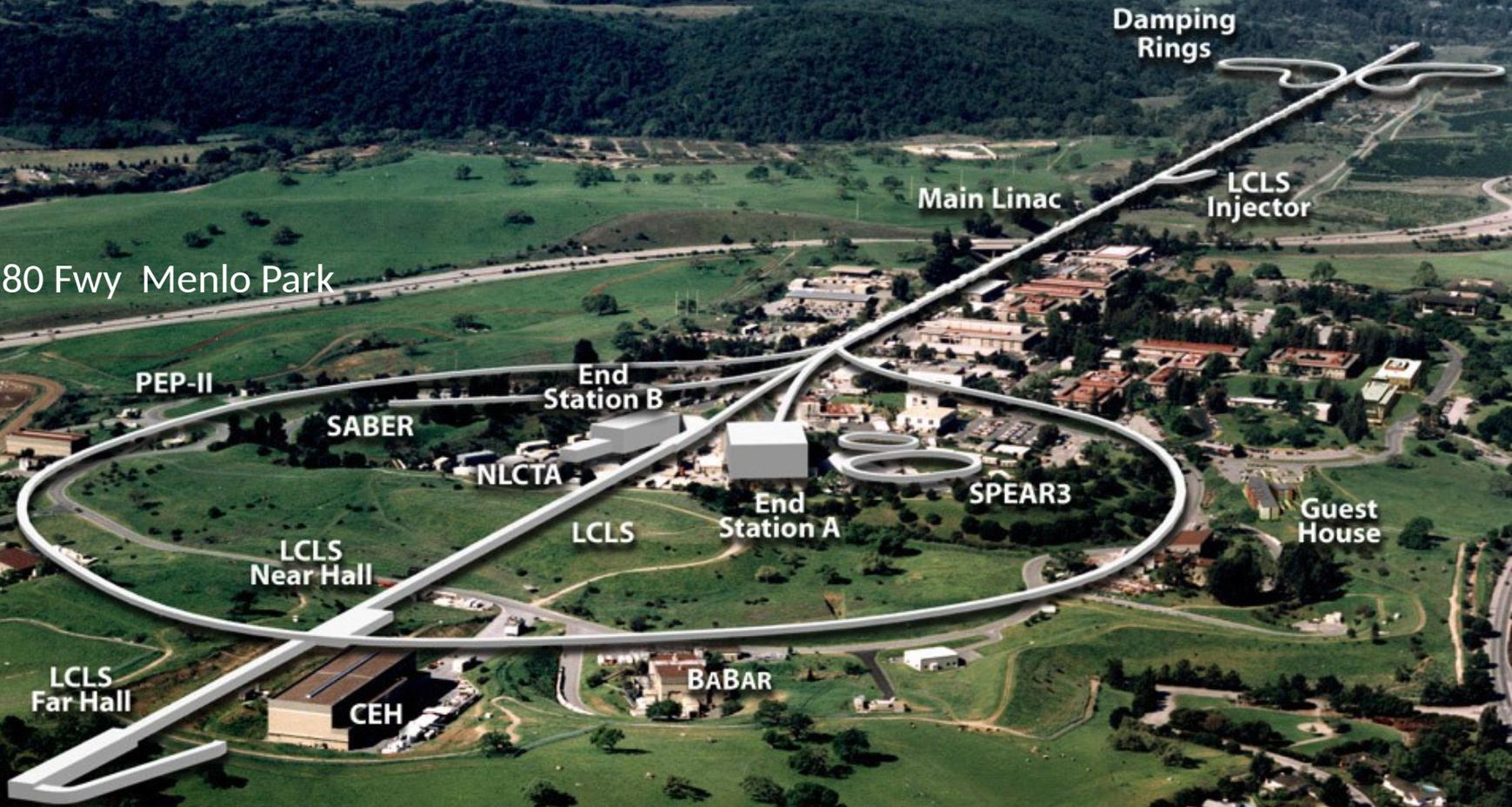
The transition from SP (one ptcle per shot, needs aiming) to a few per shot (no aiming) is continuous.

**   Exciting times ahead !**

The End

The birth of a new field

280 Fwy Menlo Park

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