Cornell XDL 2011. Nanoxtals. 30 mins June 13, 2011

John Spence ASU Physics/LBNL.

Why nanoxtals ?

1. Solve more proteins. Bottleneck. Took 13 years to make big xtals of PSI.

2. Nanoxtals may be more perfect (Von Dreele). Resolution limited by xtals and damage.

3. Iterative phasing – a new direct method not requiring atomic resolution.

4. Faster diffusion of reactants ? In-jet solution chemistry at variable temps ?

5. Time-resolved nanocrystallography – will it be better or worse ?

Nanoxtal challenges

1. Damage. "A 1 micron xtal gives 4 photons (S/N=2) at 2 Ang on gonio before Henderson 'Safe Dose limit' " (Holton 2009).

2. So, to get more info, use Laue. Cornaby et al. recently solved a nanoxtal using 5 single-shot Laue patterns from 5, 20-mic. xtals. (Acta D66, 2 (2010)).
Or, use short pulses, increasing Henderson "safe dose" by 100, hence smaller xtals.

3. Handling , mounting, Goniometer. So use continuous flow and snapshots. Then one must merge partial reflections. (coherent, incoherent). Hence Serial Crystallography.

5. If using XFEL, flow must be windowless – hence free liquid jet in vacuum, freezing.

4. Hit rate problem. Use submicron beam focused onto submicron xtal. All protein flowing between Xray pulses is wasted. Most shots miss xtals. Need better injectors, higher rep. rate to minimize wasted protein. On demand ? Synchronous, Asynchronous ? Snap-shot SAX ? Feedback on jet height (micron-sized beam hits micron particle ?)

- **Questions:**

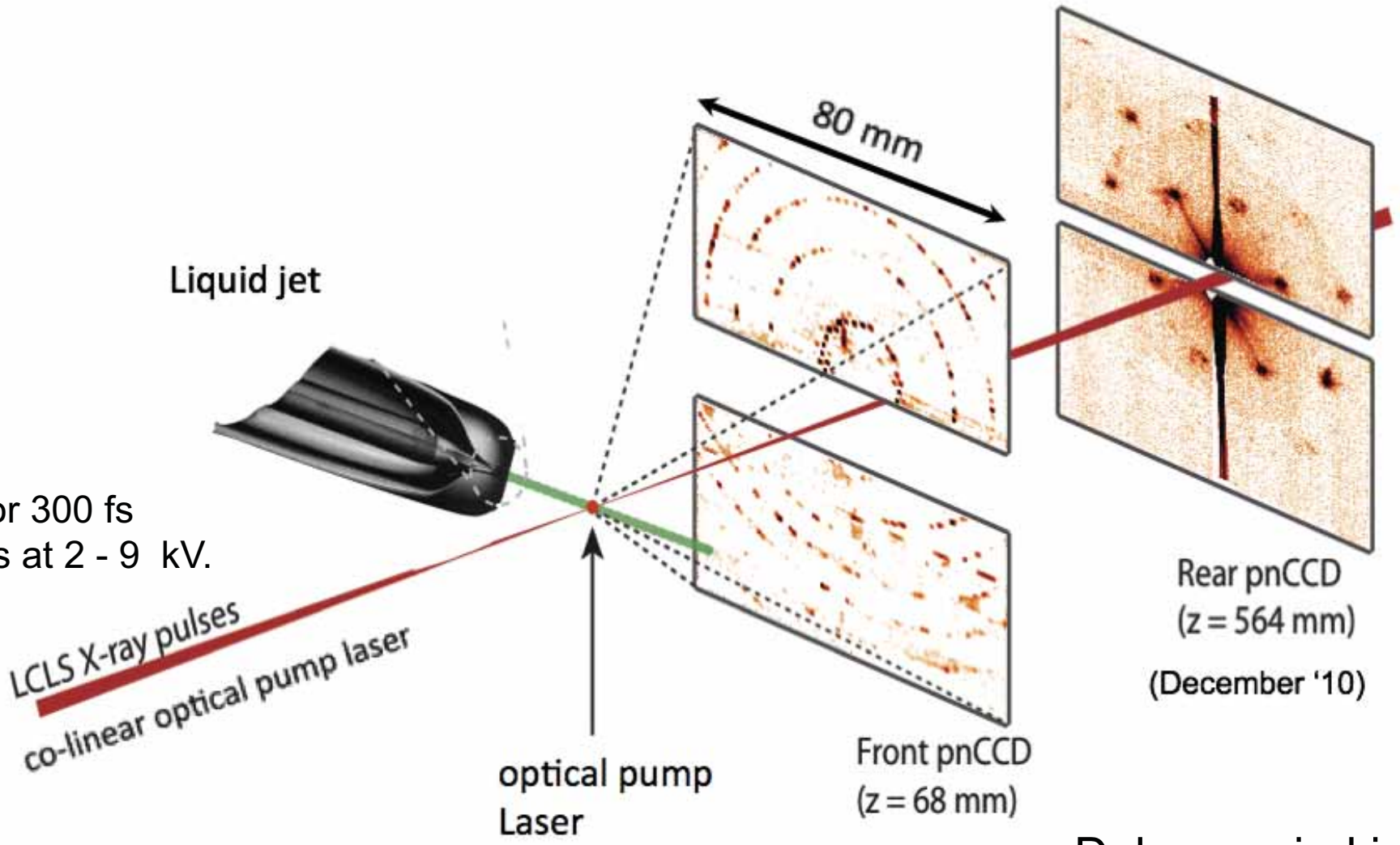
- **1. Is there an arrangement for ERL or XFEL which wastes no protein or photons ?**

- **2. Can snapshot data give highly accurate structure factors ? How to analyse data obtained without a goniometer in the form of partial reflections, especially for time-resolved work with nanoxtals.**

Current arrangement for an XFEL

AIMS:

- **OUTRUN DAMAGE**
- **SOLVE SUBMICRON XTALS**
- **REAL-TIME SNAPSHOT CHEMISTRY AT ROOM TEMPERATURE ?**
- **PUMP-PROBE WITH VERY HIGH TIME RESOLUTION.**



Debye period is ~100fs

Useful words

1. **Asynchronous**. From the days of the teleprinter (1930) when clocks were inaccurate, "Asynchronous" means that a clock is not used. A special start pulse is sent before each word , to allow the timing between words (injected proteins, viruses) to be variable. (eg our "On Demand" mode).

2. **Synchronous**. Everything is slaved to the same clock at transmitter and reciever. (requires equally spaced proteins in the jet).

3. **Gated X-ray detectors.** CCDs, Cornel detectors integrate charge; Pilatus counts photons – up to a million per pixels per sec. , 20 bits. (no good for XFEL). Denes's femtoPix has electronic shutter – a gated, integrating area detector.

4. **Gated sources**. XFEL photocathode could be triggered by TTL pulse (?). ERL runs continuously at 1 GHz rep rate (or 100 kHz), can make periodic bunches.

4. **Efficiency of nanocrystallography**. At LCLS we currently use a few percent of data , and need "buckets" of protein. The data acquisition rate at an XFEL depends on **THREE** things – source, scatterer and detector. Can these be "synchronized" to reduce waste of photons, waste of protein ?

Nanoxtals at ERL - below damage threshold.

ERL gives in 10 microseconds of GHz pulses the same fluence as LCLS gives in 10 fs.

Don't need high coherence for nanocrystallography ! ($X_c > d_{001}$).

Require: 1. Pulse duration less than rotational diffusion time of nanoxtal in flowing buffer
(by choice of buffer viscosity. Microseconds). Use rotation as goniometer ?
2. Repetition rate matched to flow rate of nanoxtals (don't waste protein)
(buffer viscosity fixes flow rate (300 microns/s – 10 m/s), and background)

Examples.

*Flowing: 1 micron xtals 10 micron apart at V=1 m/s, 1 micron beam, Need Rep Rate 100 kHz
Hit rate problem. Conc is possible (how to measure particle conc ?).

So…..

3. Detector readout (>200 ns) matched to flow rate, rep rate.
4. Operate below damage threshold – "1 micron xtal gives 4 photons at 2 Ang"
(but we have snapshots, not gonio. This is angle-integrated). In high charge
mode, ERL gives 6E6 photons per pulse for 2ps pulse, 0.1% bandwidth.

Conclude: Buffer chemistry is critical – controls speed for given pressure, background, thickness (viscous thin capillary tube may require unattainable driving pressure), clogging, detector readout rate. Thicker give more background. Control viscosity by addition of glucose ?

A. Synchronous, on-demand droplets, "Big" xtals for time resolved*.

(Time-resolved MX requires very accurate structure factor measurement).

ERL demands droplets periodically

*Use High Coherence mode of ERL. Rep Rate 1000 MHz (1 ns). Gate photocathode to give bunches of 1000, 2 ps pulses (1E8 photons/pulse) every millisecond (readout time). Use 30 micron X-ray beam.

*Bunch duration of 1 microsecond is less than rotational diffusion time. (depend on viscosity of buffer). Droplet travels VT= 1 mic/micsec * 1 mic = 1 micron during expsr

*Dose is 0.25 MGy, << "Safe Dose" of 30 Gray J/Kgm (30 mic. beam, 9 kV)

*TTL from photocathode generates one, 30 micron droplet, on-demand, every millisec., often containing one 20 micron microxtal.

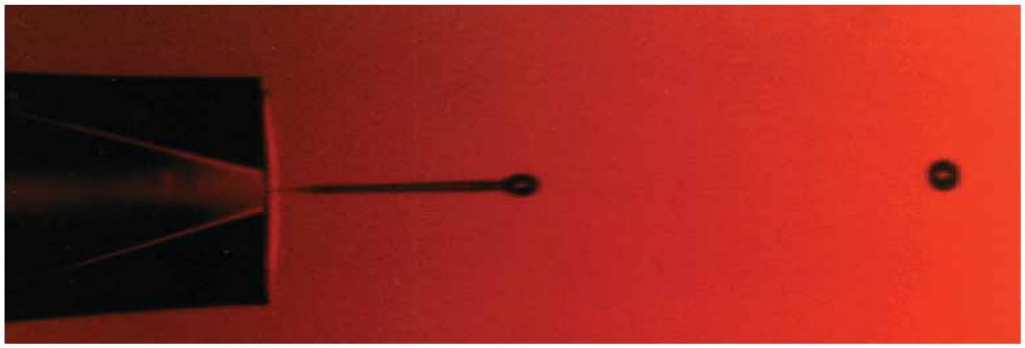


Figure 4: Drop-on-demand type ink-jet device generating 50• m diameter drops at 2kHz.

Problems:

1. Lots of background ?.
2. Empty droplets waste photons but every nanoxtal is hit.

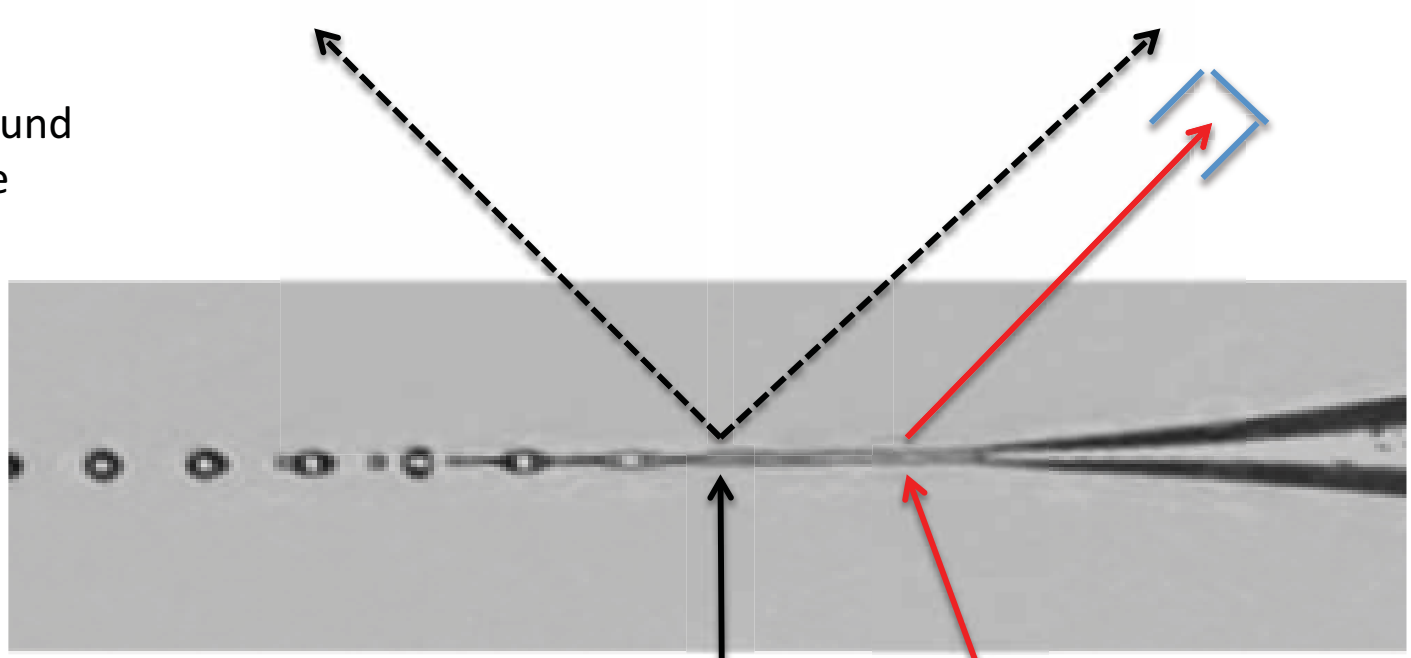
*Below damage threshold.

B. Asynchronous, laser detection of nanoxtals. (For submicron xtals, phasing)

Detector opens when particle is detected emerging from jet, ERL runs continuously at GHz

Vacuum environment reduces X-ray background and gives temperature variation along jet.

*Can femtoPix count fast enough ? Pilatus cannot.



Wastes photons, not protein.

ERL beam, 9 kV.

Laser beam detects nanoxtals (Anfinrud). SONICC ?

*Use 1 micron X-ray beam, submicron xtals. Feedback on jet height
*ERL runs continuously. High Coherence mode (allow phasing)
*Viscous jet (2.5 mm/sec) has 1 millisec rot diffusion time. Toothpaste.
Electronic shutter opens for 1 millisec when xtal detected. Readout every 4 millisec.

Conclusions

- Synchronous **ERL**/On-demand Injector is fine for 10 micron xtals. Periodic. This wastes no protein, but wastes photons (some droplets are empty). (Not destructive, so could use capillary tube. Clogging ?)

- Asynchronous gated detector, **ERL** running continuously, wastes no protein, wastes photons (when detector shutter is closed).

- Synchronous **XFEL**/On demand injector OK as above. (XFEL demands droplet periodically).

- Asynchronous **XFEL** responding to particle-detecting laser at jet.

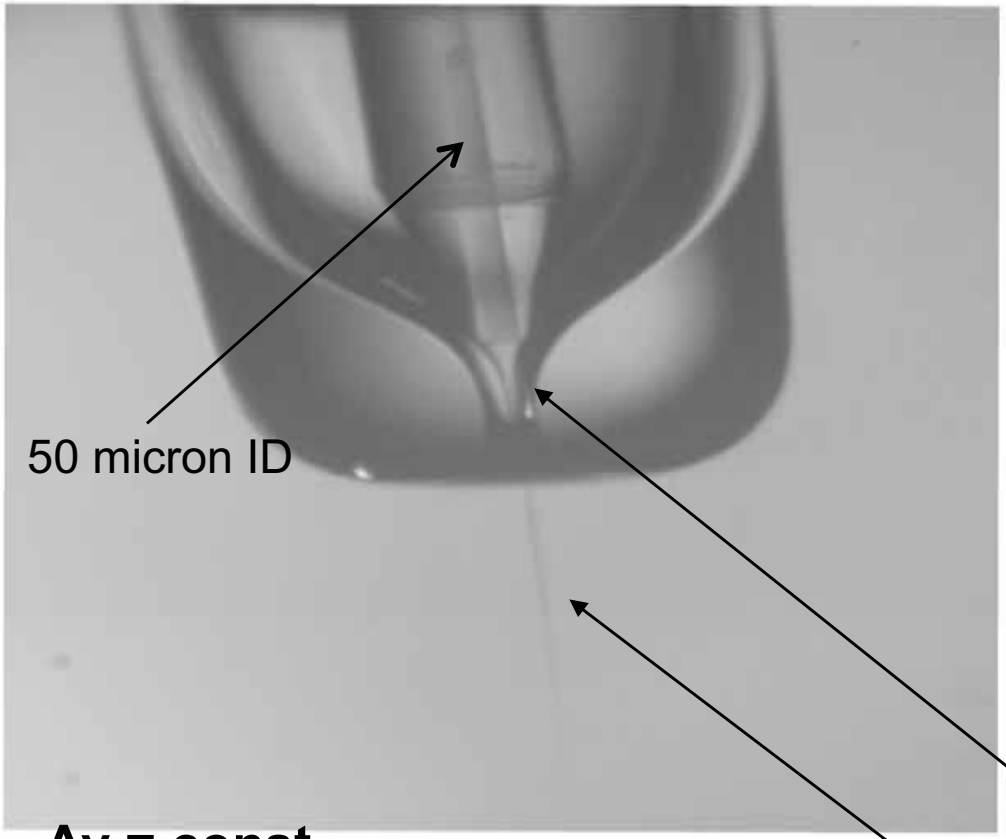
  Wastes nothing. **Perfect solution !**. (other users ?.

Asynchronous triggered detector at ERL or asynchronous triggered photocathode at XFEL are the designs which waste no protein.

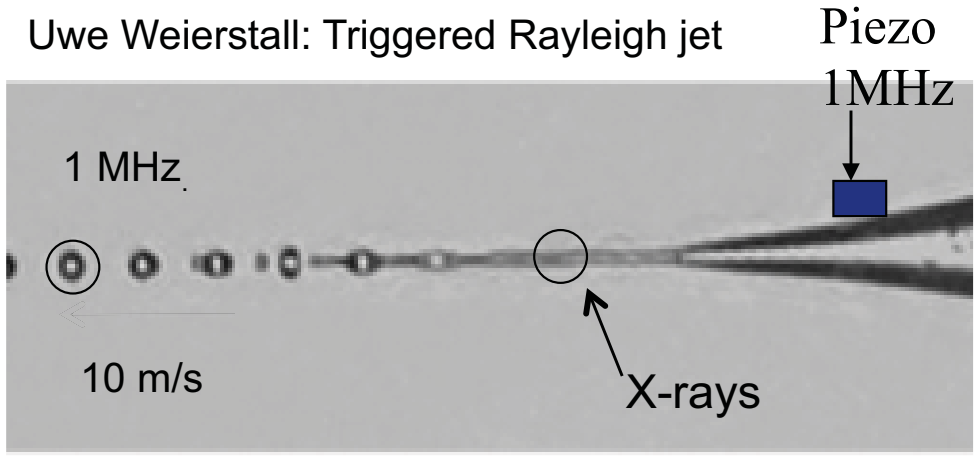
Existing injectors for fully hydrated bioparticles

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.



Uwe Weierstall: Triggered Rayleigh jet



LCLS Rep Rate 30 Hz
Droplet frequency 1 MHz. v = 10 m/s
Droplet diam 1 micron
LCLS beam diam 3 microns, v = c
Flow rate F= 12 microL per minute. v = F/A

Av = const
Gas accelerates liquid
Making cone as shown.
Higher pressure for smaller droplets

Liquid cone enhances flow alignment
(from HPLC syringe pump)

~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

DePonte, Doak, Weierstall Spence

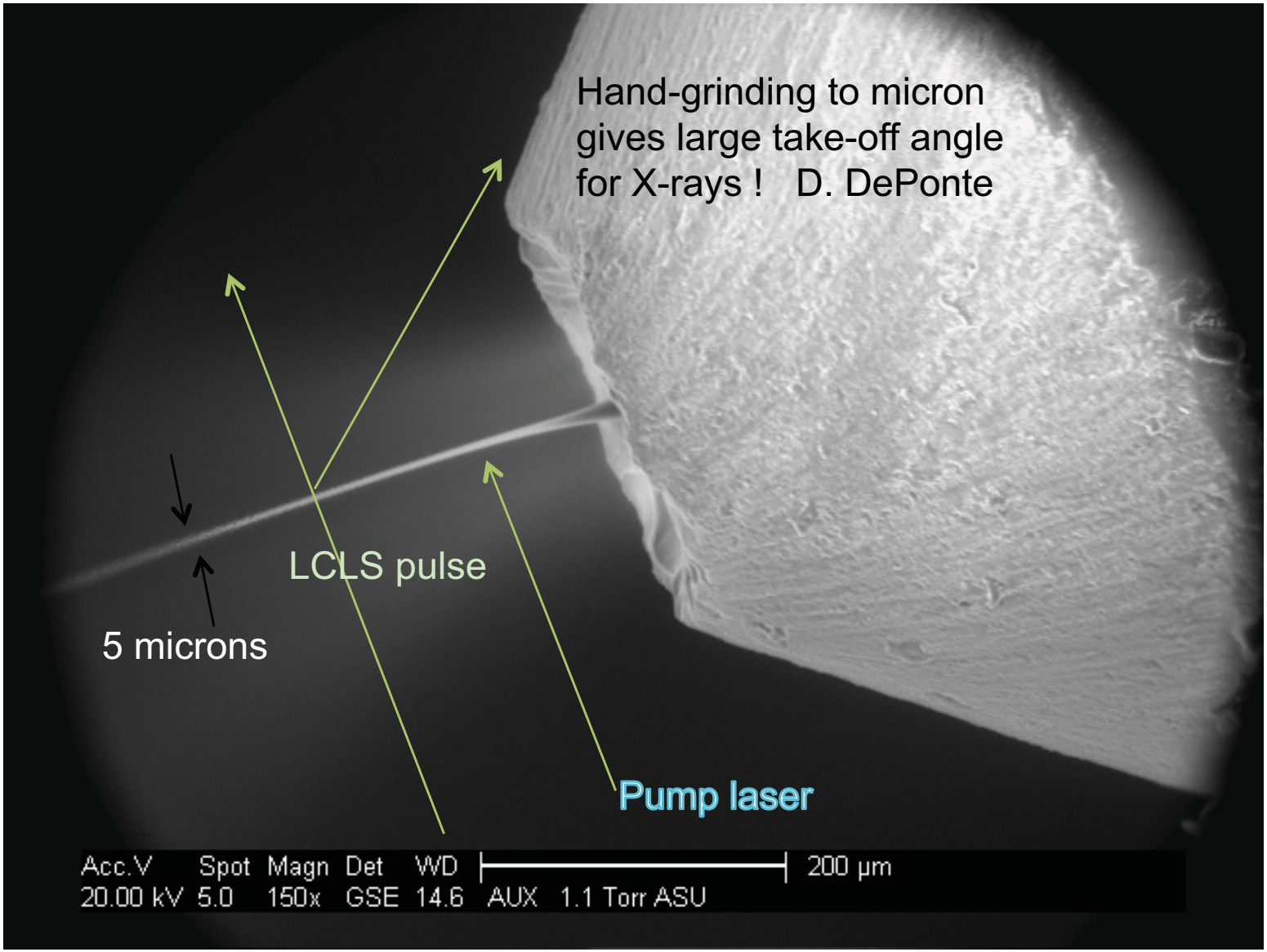
Spence and Doak, Phys Rev Letts. 92, 198102 (2004).

An environmental SEM image of our operating protein-beam injector.

Nozzle ground to provide large X-ray diffraction angle

The use of short pulses to prevent damage instead of freezing allows us to do real-time snap-shot chemistry at room temperature.

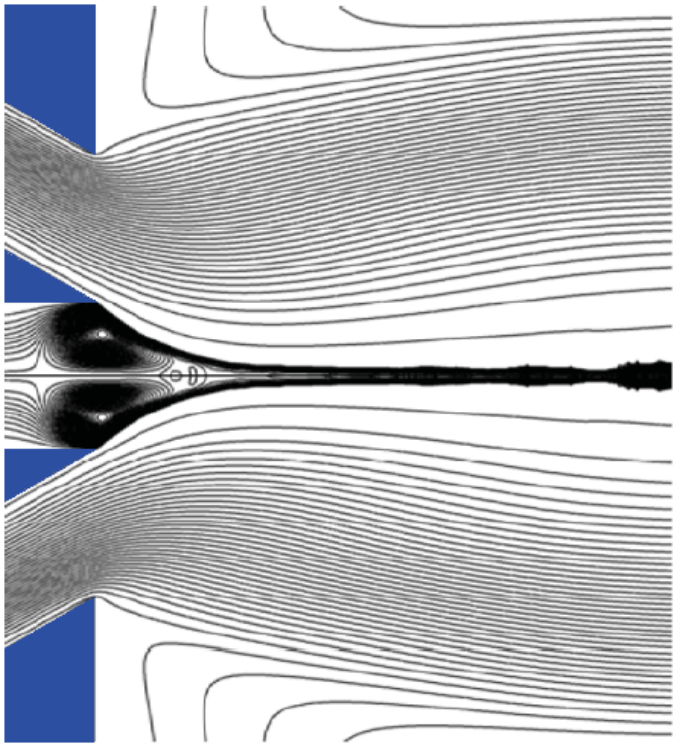
Droplets freeze at 10^6 °/sec. in vacuum to vitreous ice if cryoprotectant added.



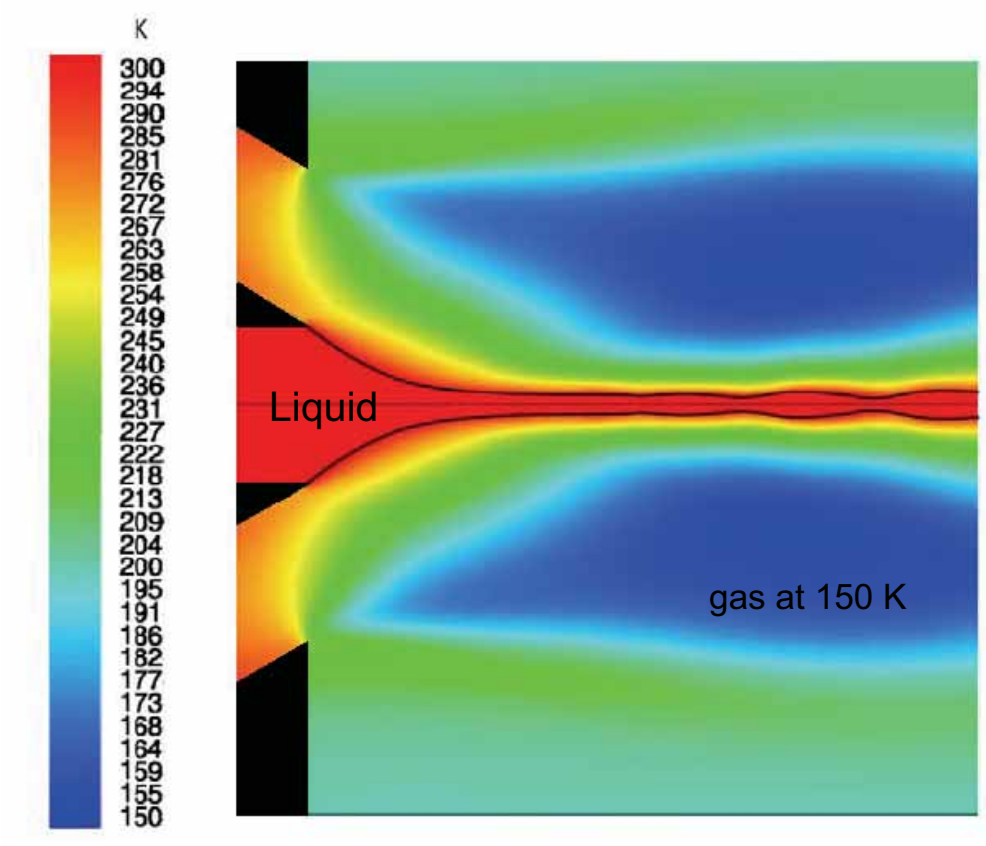
Flow rate 12 microliters per minute. Applications to analytical chemistry, electronic printing

GDVN fluid calculations (Injection into free-jet)

Flow Streamlines



Temperature

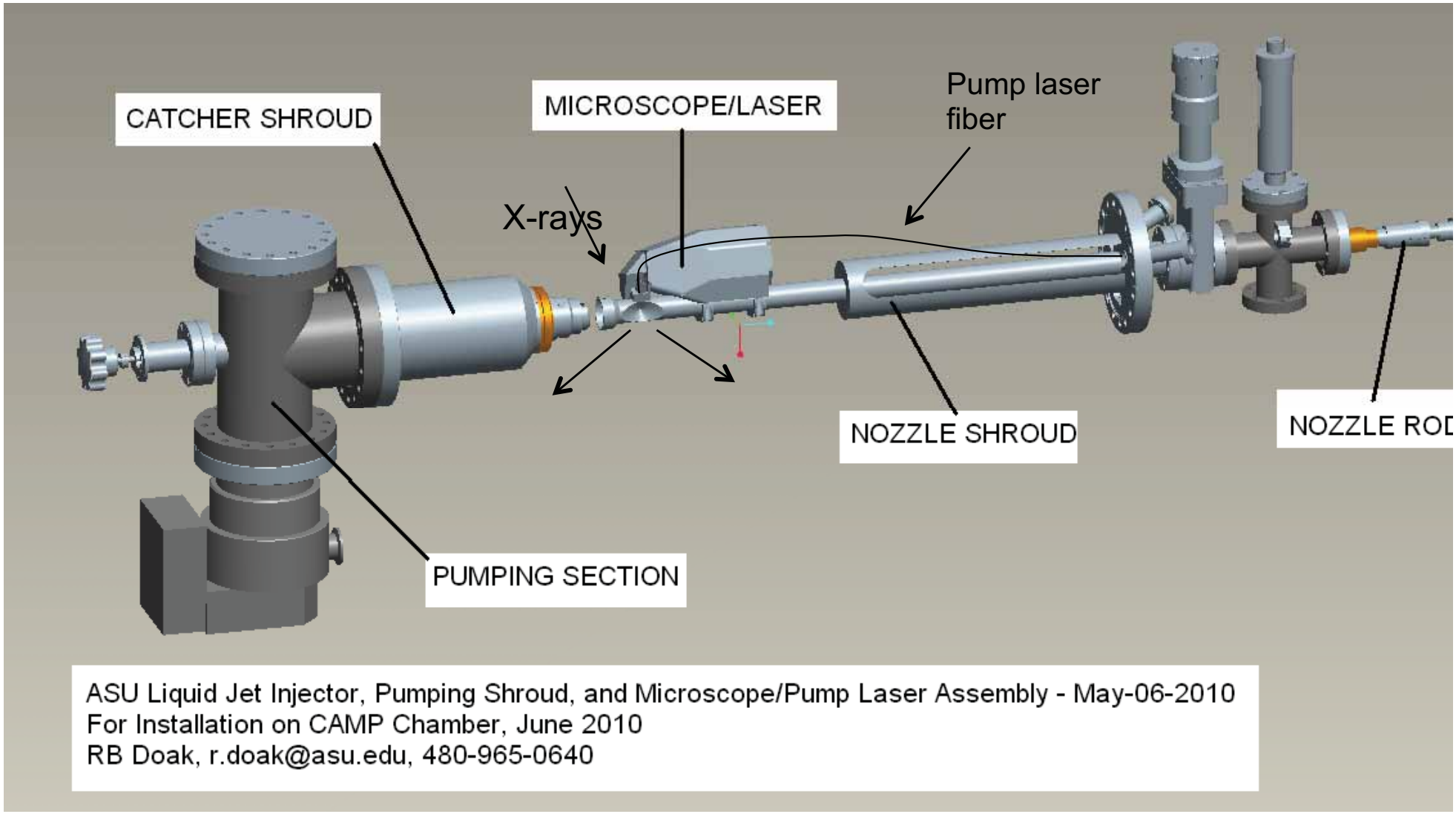


*A.M. Gañán-Calvo, et al., Small, **6**, 822-824 (2010).*

Solve Navier-Stokes equation

ASU particle injector with pump laser.

New ASU Doak RR jet for June 2010 had microscope, retract nozzle, pump laser

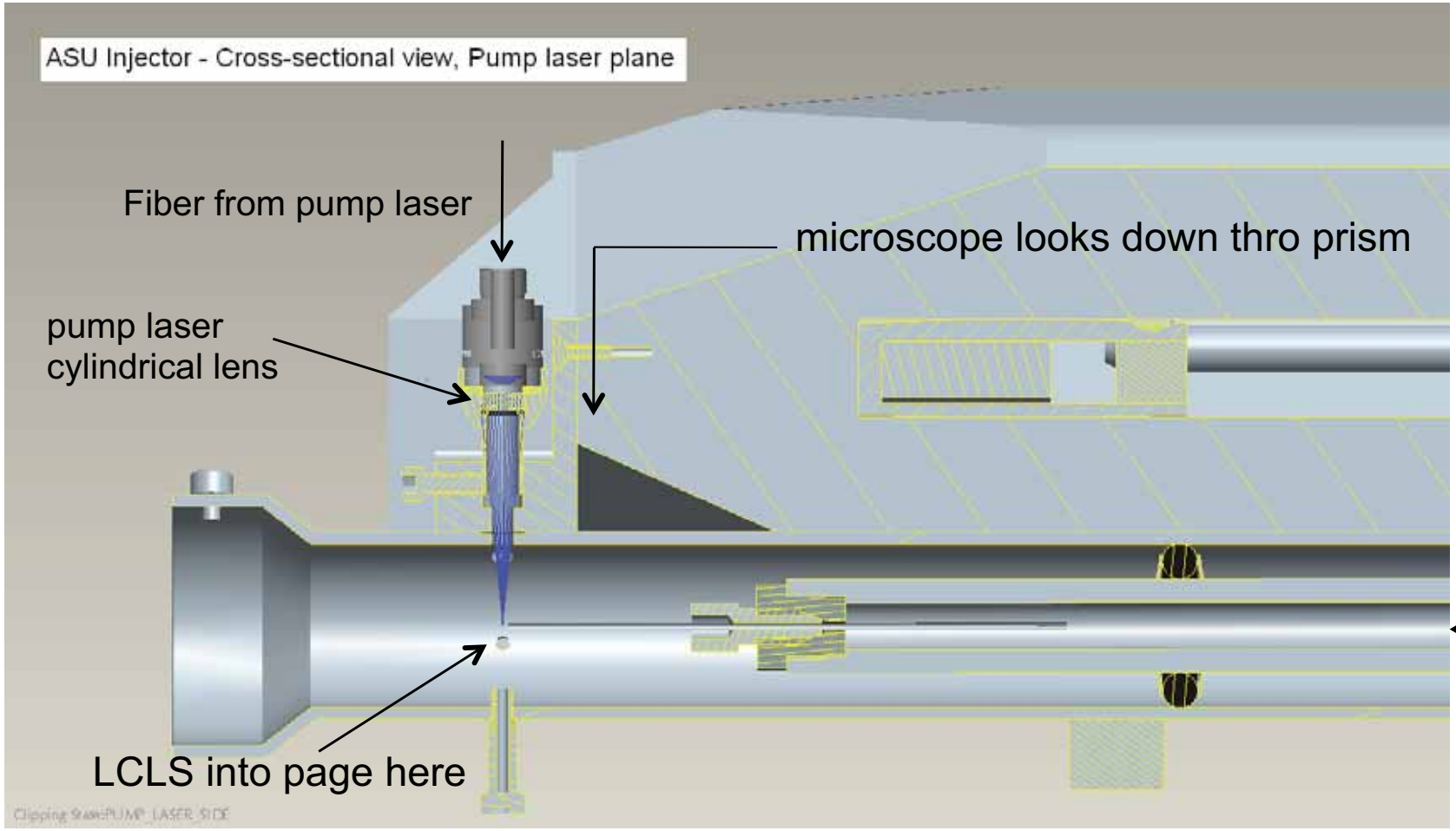


To observe undocking of ferredoxin from PSI, excite nanoxal 10 microseconds before XRD snapshot at 400nm. Travelling at 10 m/s, nanoxtals go 100 microns, less than width of doubled Jedai fs beam

Droplets freeze in vacuum at 10^6 $^oK/s$

B. Doak, U. Weierstall

ASU injector showing pump laser section



Lipid Xtal Phase jet takes 10 millisecs for protein to traverse a 3micron beam diam.
Hence plenty of time to read out two diffraction patterns from same nanoxtal
if dose below damage threshold. Use larger xtals, eg 2 micron.

Drop-on-Demand (DoD) – Typical Specifications

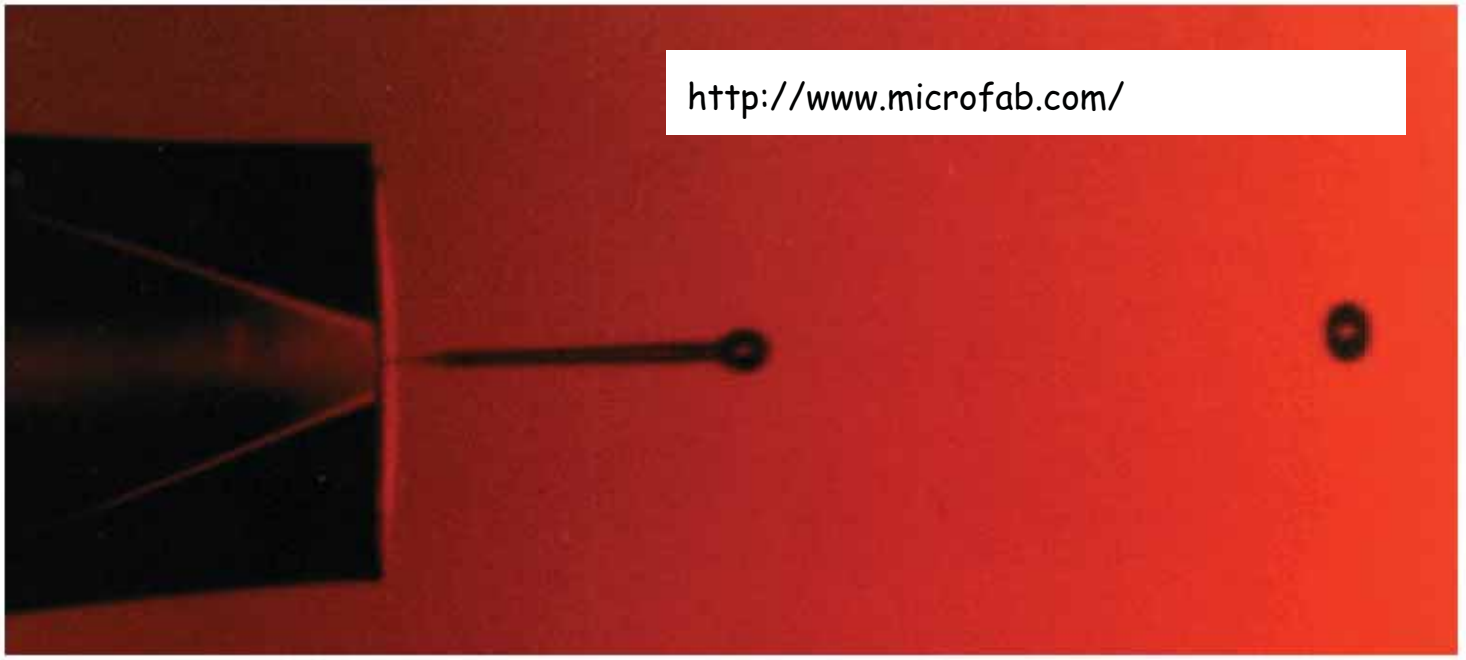
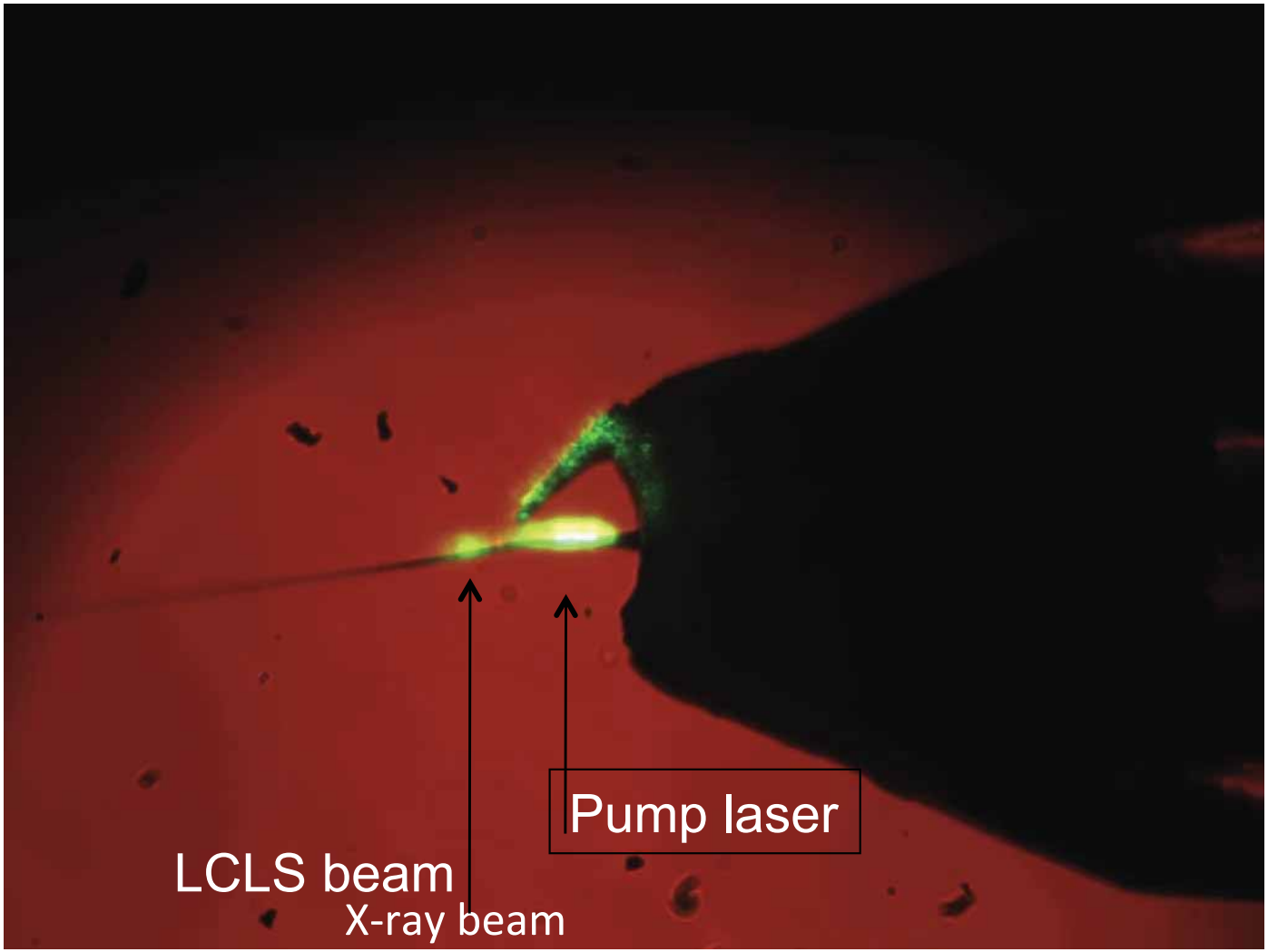


***Figure 4**: Drop-on-demand type ink-jet device generating 50• m diameter drops at 2kHz.*

Droplet velocity: 0.1 to 5 m/s
Fluid flow rate: 240 nl/min, e.g. (40 micron droplets at 120 Hz)
Droplet diameter: 40 to 60 micron (smaller possible)
Temperature: RT (near nozzle) to 200 K (droplets in vacuum)
Triggerable: Yes (can be run as triggered Raleigh Droplet Beam)
Environment: Gas only, no vacuum (freezes up)
Clogging: Usually not

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

Pump laser on jet



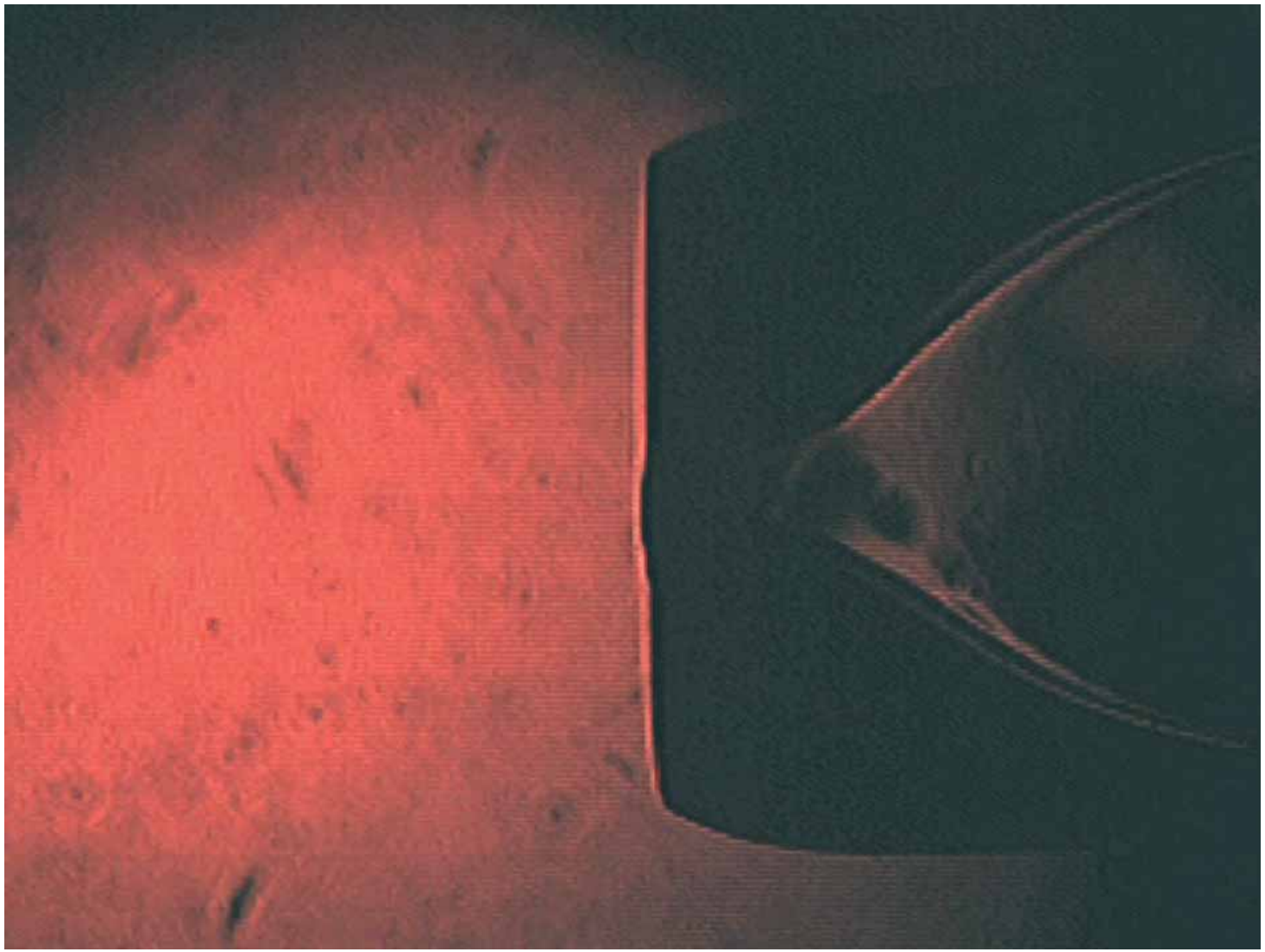
pump laser:
532nm,
7ns pulse, 10 microjoules,
focused to 100 micron spot,
fiber coupled,

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

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

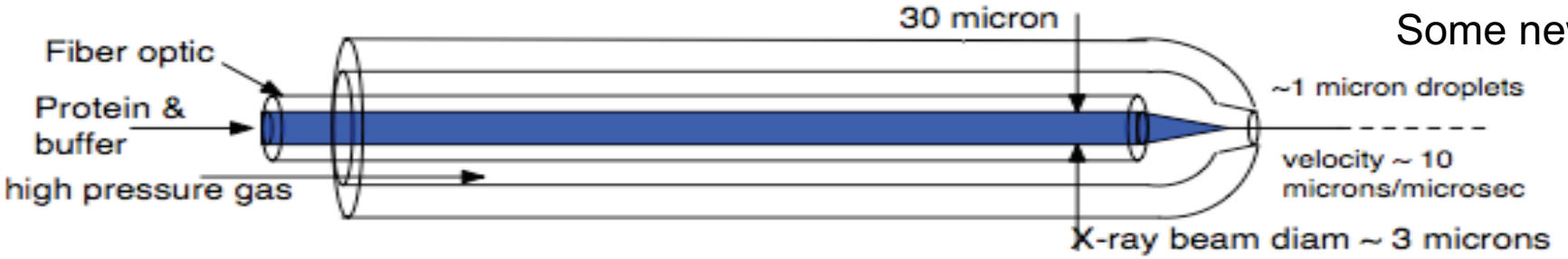
Strobe movie of “Droplet-on-demand”

DoD - tiny reservoir, no gas bottles, no hand-made nozzles
No fiber-optic, freezes in vacuum, use push-pull pulse.
We find xtals (PSI, PSII) survive. Use Neutral Bouancy against settling (sucrose).
BUT, cant make droplets smaller than 30 microns.



New injector possibilities for hydrated bioparticles under development at ASU.

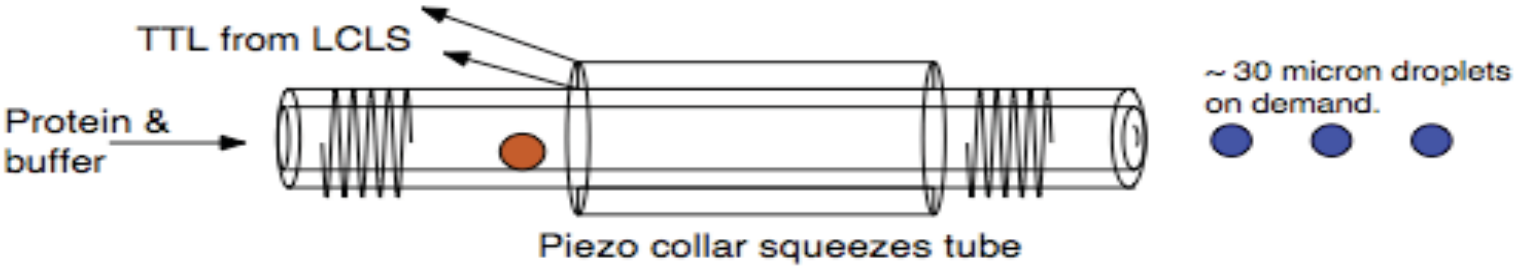
Some new ASU injectors



10 micL/min

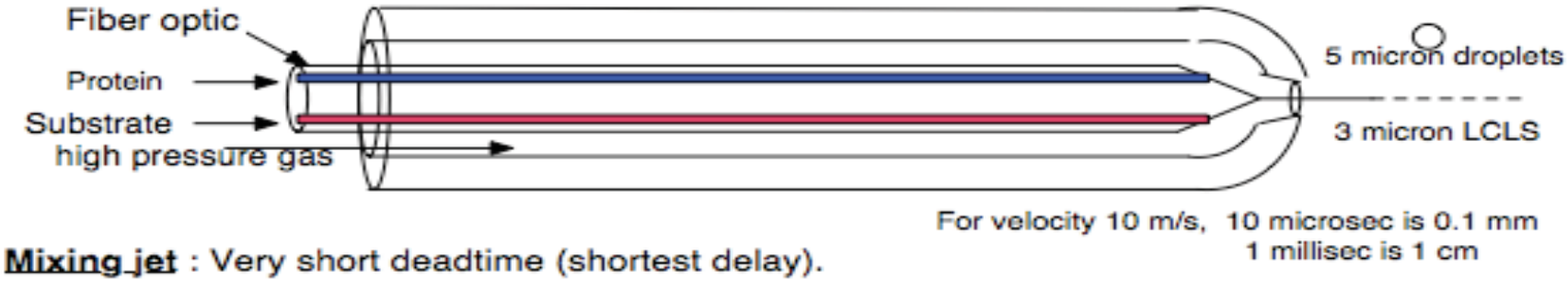
GDVN : Avoids clogging by gas focussing; smallest droplets for soft X-rays. Freeze in vacuum. Could be synchronized. Wasteful. Settling ? Flow rate 10 microliters/min

Now add pump-probe to any of these. (June 2010)

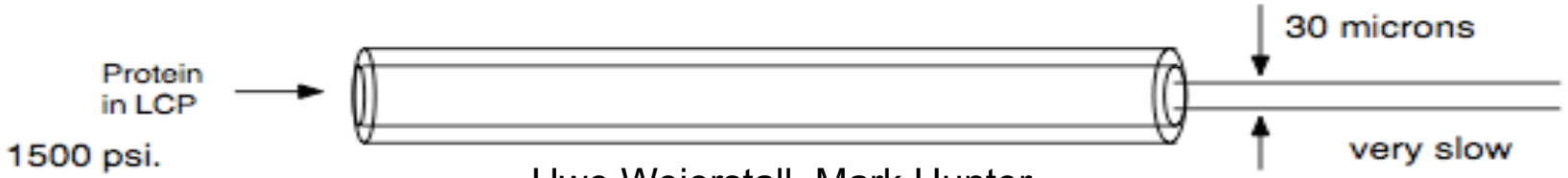


DoD : Waste no liquid. Large droplets for hard X-rays in air, no freezing. No gas bottles, reservoir, 1 ml lasts a week. Neutral bouancy avoids settling. Or use solenoid-driven red ball agitator. Flow rate 10 nL/min.

Strobe microscope from above sees flash, to synchronize



Mixing jet : Very short deadtime (shortest delay).



35 nL/min

300 microns/sec

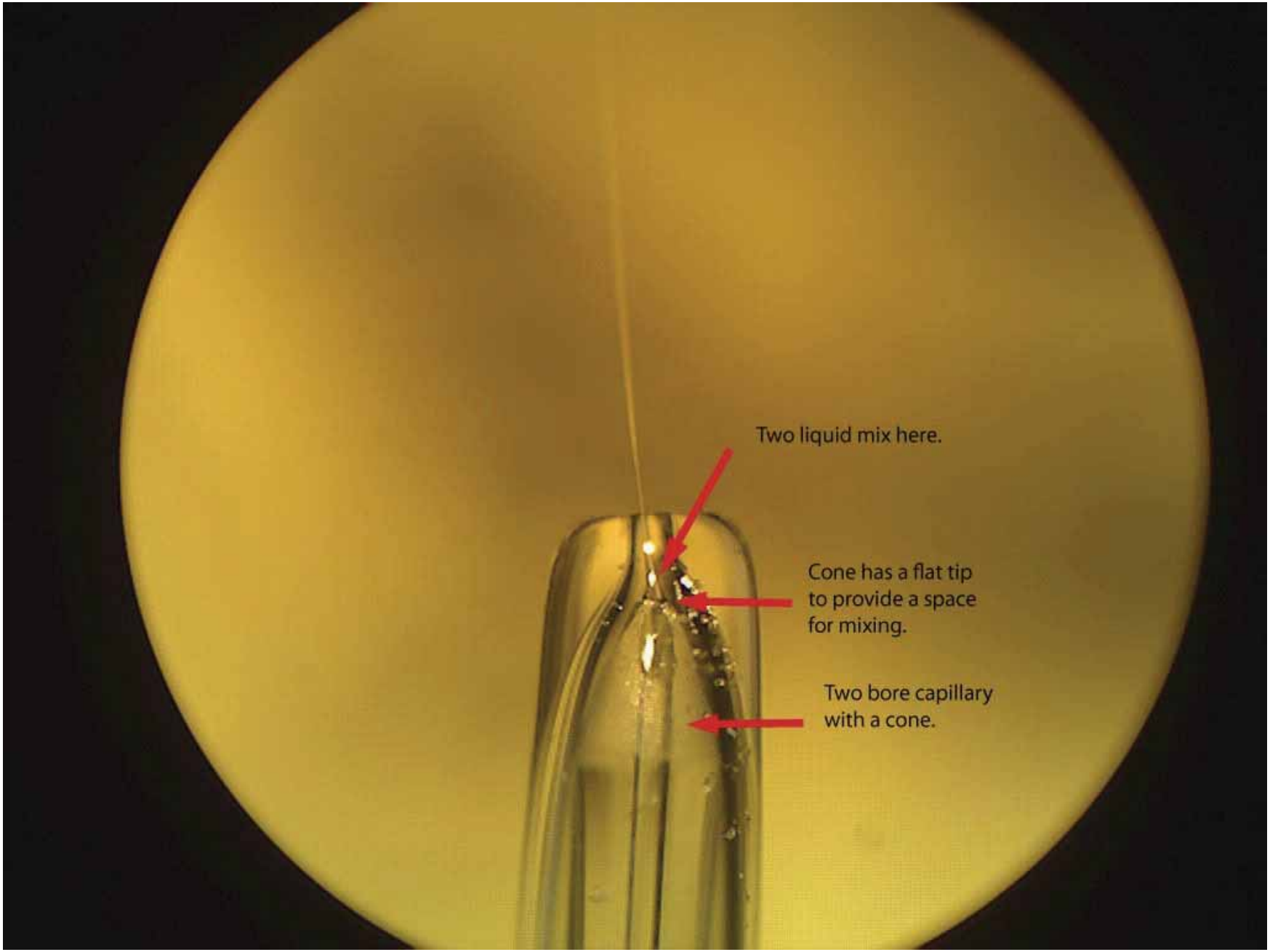
Uwe Weierstall, Mark Hunter

Sponge phase

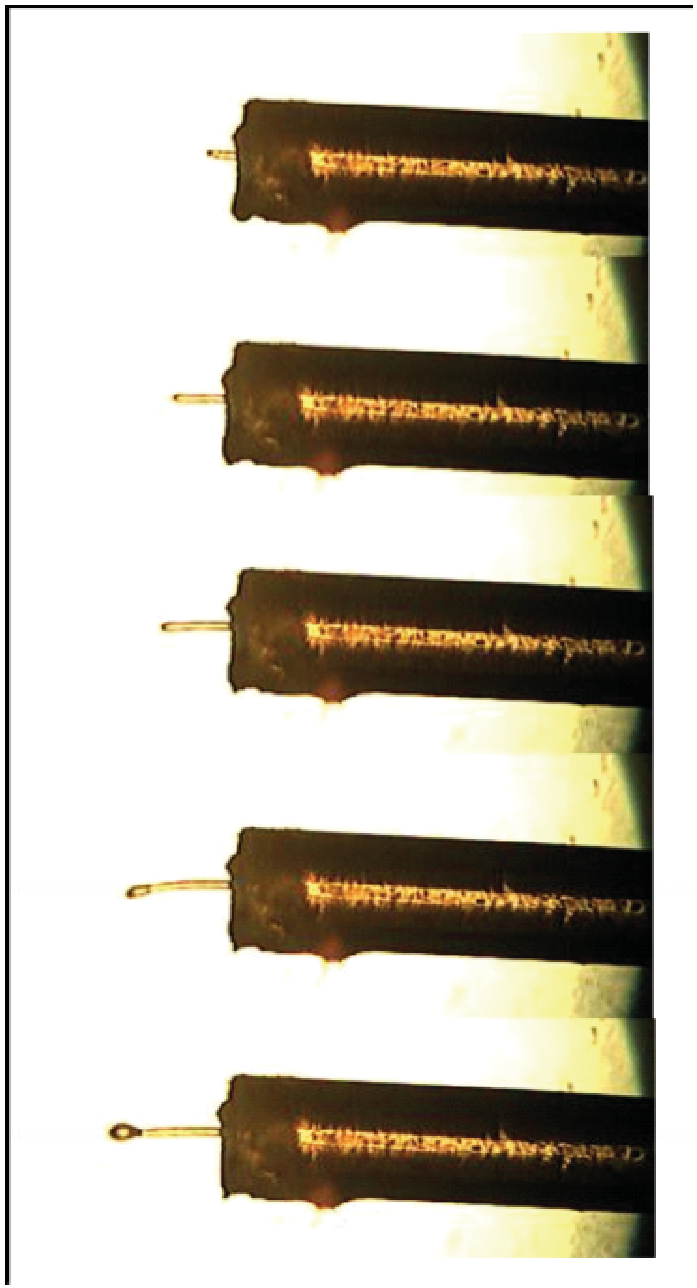
Lipid cubic phase (toothpaste) : no settling, flow rate 35 nL/min, no sychronization needed. At 100 Hz with 5 micron X-ray beam, all solution is hit at this flow rate.

Bruce Doak, Uwe Weierstall, JS ASU

The dual-bore mixing jet, with gas focusing



Dingjie Wang. June 2011



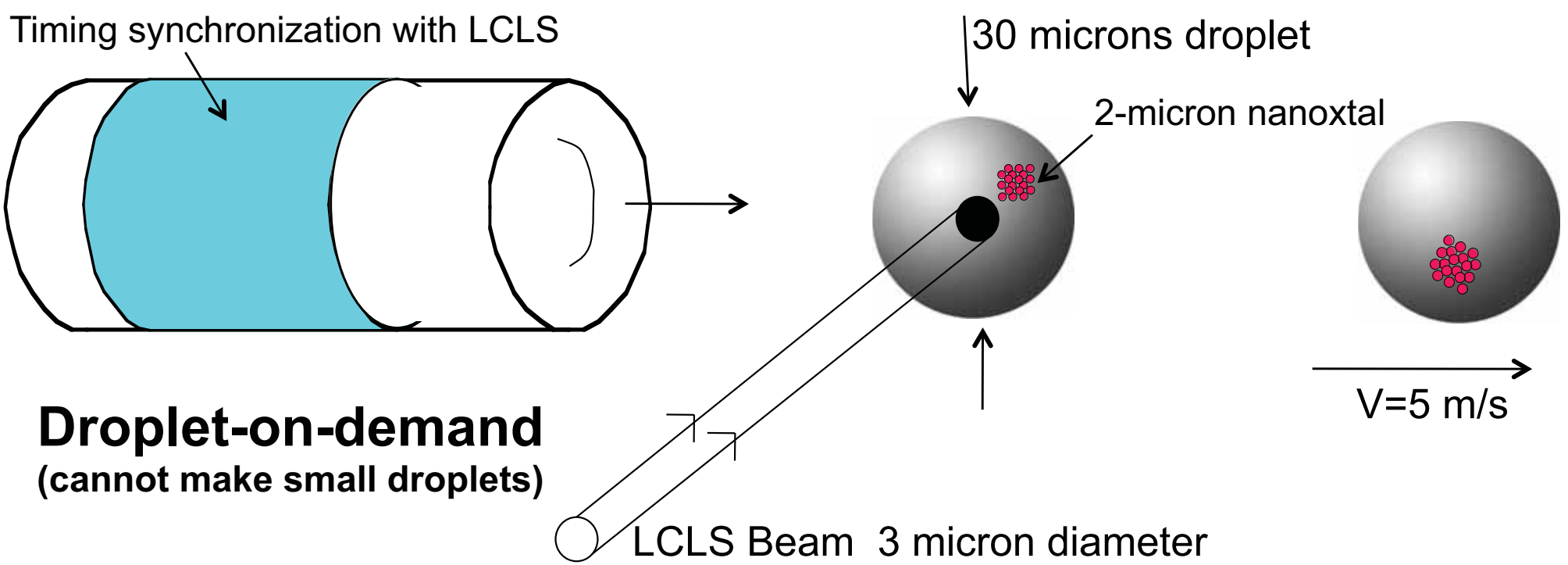
“Toothpaste” LCP injector, showing lipid cubic phase emerging in 30 micron stream at 300 microns/sec. (1500 psi)

This is 3 microns (LCLS beam diam) every 1/100 sec.

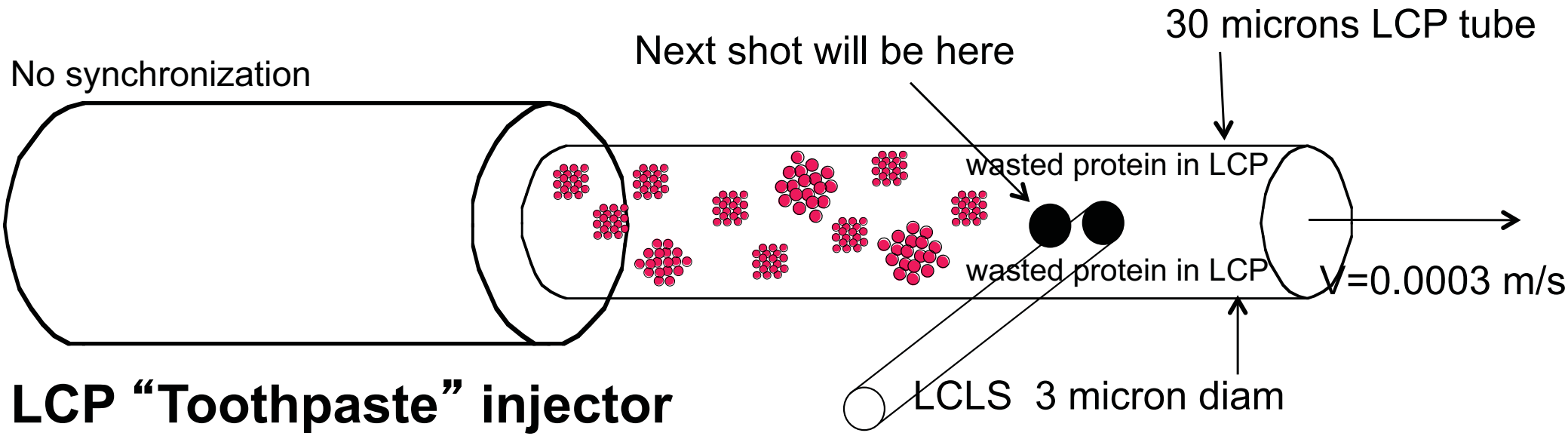
LCLS pulses can arrive at 100 Hz.

U. Weierstall, M. Hunter, ASU.

Dimensions



Droplet-on-demand
(cannot make small droplets)

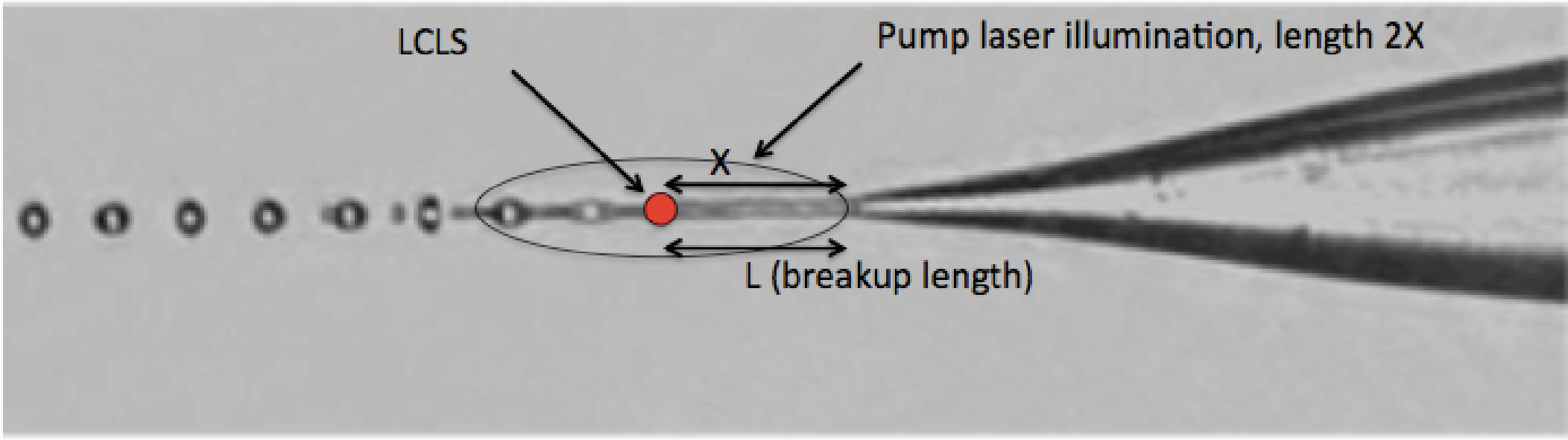


LCP "Toothpaste" injector

Note: To avoid wasted protein need slower, hence more viscous jet. This needs larger diam. hence more sucrose background. Higher Rep Rate allows faster thinner jet without waste.

Pump-probe in a jet

Bio times are 10 microsec to 1 millisec. At V= 10 m/sec this is 100 microns to 1 cm.
DoD droplets go 1 cm in air at 1-3 m/s, dispersed after a few mm.



L= Breakup length = 7.3 mm for 25 micron stream at 13 m/s (560 microsecs)
= 1.8 mm for 10 micron stream
= 650 microns (~0.5 mm) for 5 micron stream (50 microsec)

Notes: *Particle hit by LCLS must have been within pump laser illumination circle at earlier time $\Delta T<X/V$ before hit. Longest delay (0.5 millisec) needs big stream to keep within breakup length. Use cylindrical lens for more pump intensity.

*<3 micron LCLS beam may be smaller than stream diameter. Long pathlength.
*Alternative method using flow times, off-center laser, has worse timing error.
*First experiments done in June 2009 (PSI-ferre).
*Pump laser can be aligned by jet lateral motions while viewing microscope.
*Jet height is aligned with LCLS by viewing vaporization flashes on microscope.
*Use X-ray, Pump, X-ray on same particle in toothpaste mode, 200 ns readout

Data Analysis for partial reflections, time-resolved nanocrystallography.

An international collaboration

CFEL-DESY

H. Chapman, *J. Schulz, A. Barty, M. Liang, A. Aquila, T. White, D. Deponte, S. Stern, N. Coppola, S. Bajt, M. Barthelmess, A. Martin, C. Caleman, K. Nass, F. Stellato, H. Fleckenstein, L. Gumprecht*

ASU

J. Spence, *P. Fromme, U. Weierstall, B. Doak, M. Hunter, R. Kirian, K. Schmidt*

SLAC

M. Bogan, *S. Boutet, G. Williams, D. Starodub, R. Sierra, C. Hampton, J. Kryzwinski, C. Bostedt, M. Messerschmidt, J. Bozek*

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LLNL

S. Hau-Riege, M. Frank

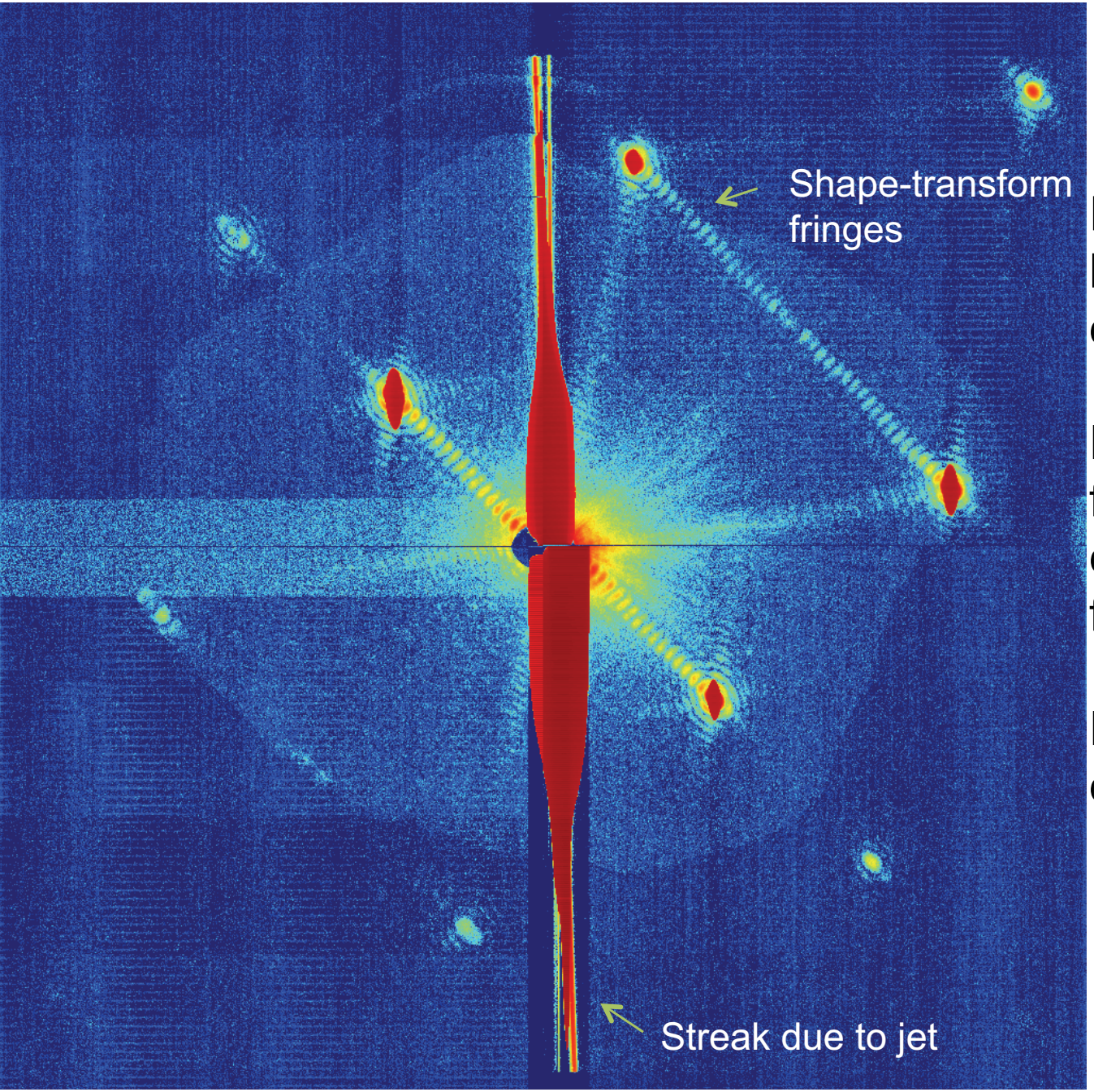
LBNL

S. Marchesini, ***J. Holton***

Max Planck CFEL Advanced Study Group

I. Schlichting, *R. Shoeman, L. Lomb, M. Bott, D. Rolles, S. Epp, A. Rudenko, L. Strüder, R. Hartmann, L. Foucar, N. Kimmel,* ***J. Ullrich***

What the back (high angular resolution) detector data looks like.

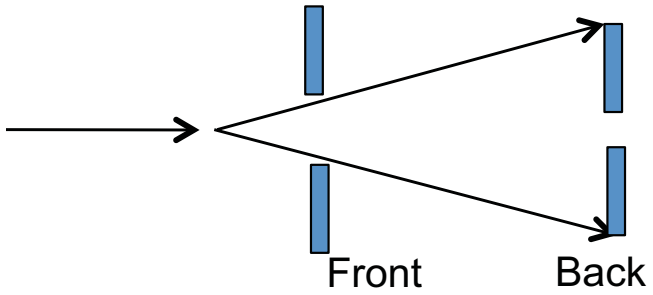


Coherence width spans entire nanoxtal, not cell.

How can structure-factors be extracted from this data ?

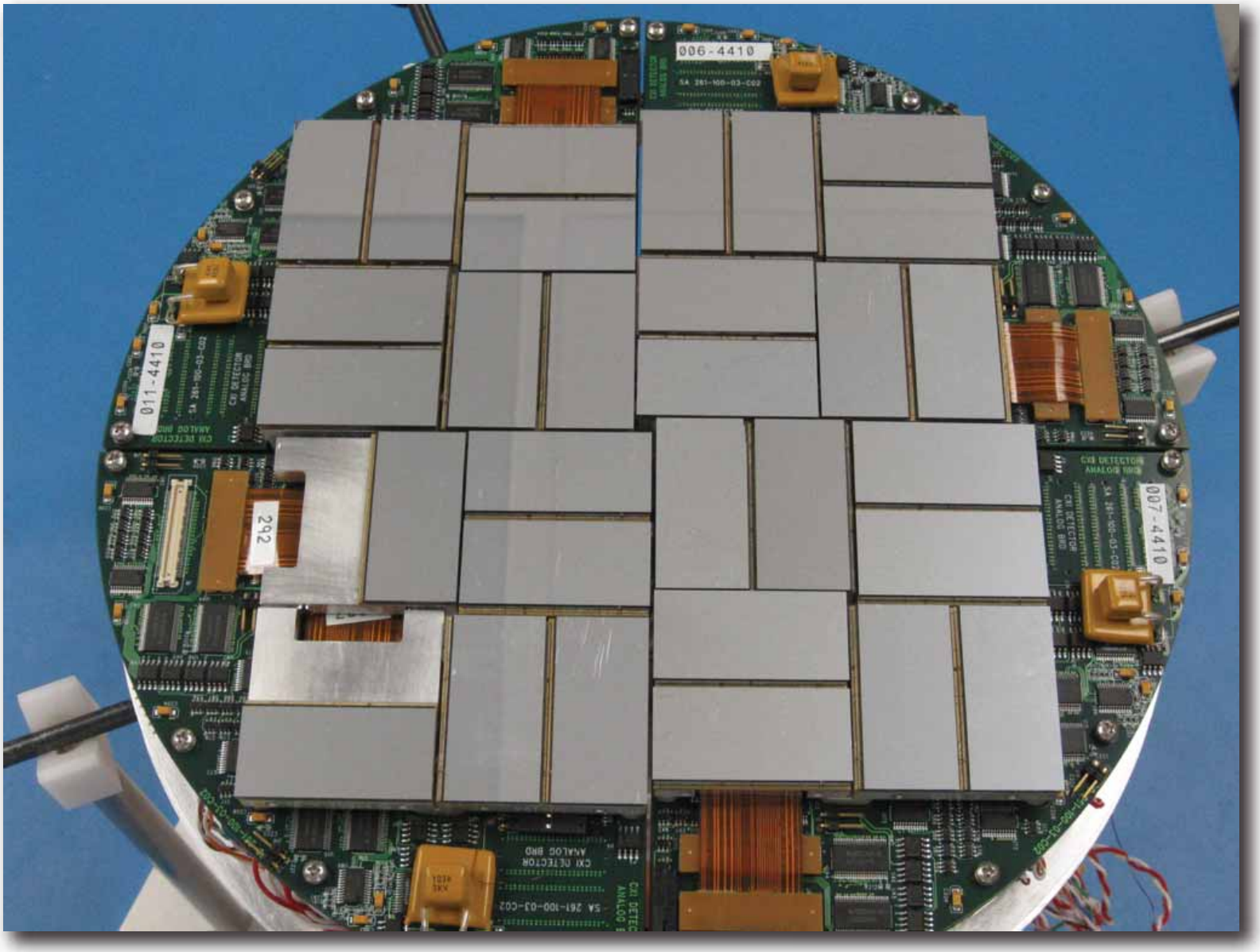
Every snap-shot comes from a nanoxtal of different size, with different fine structure on each spot

Every nanoxtal is in a different (random) orientation.



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

Pixel array detector (Cornell)



silicon diodes

1516 x 1516 pixels

110x110 micron pixels

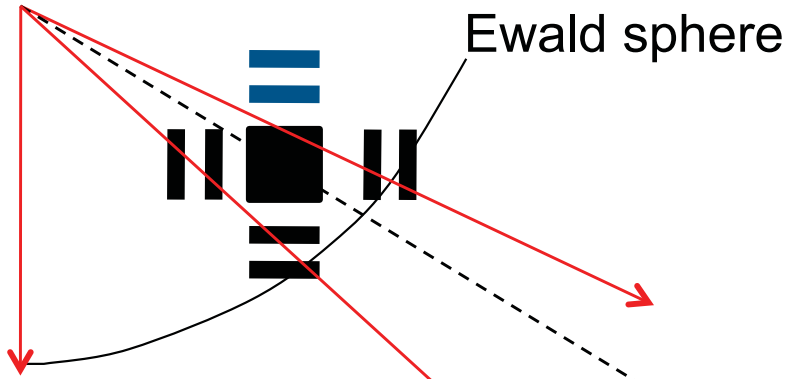
frame rate >120Hz

Sol Gruner et. al, “Pixel array detector for X-ray free electron laser experiments”, Nuc. Inst. Meth. A .

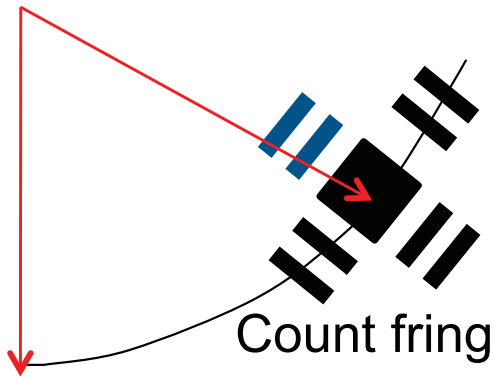
Shape-transform effects. PSI nanoxtals at 2 kV, random orientation.

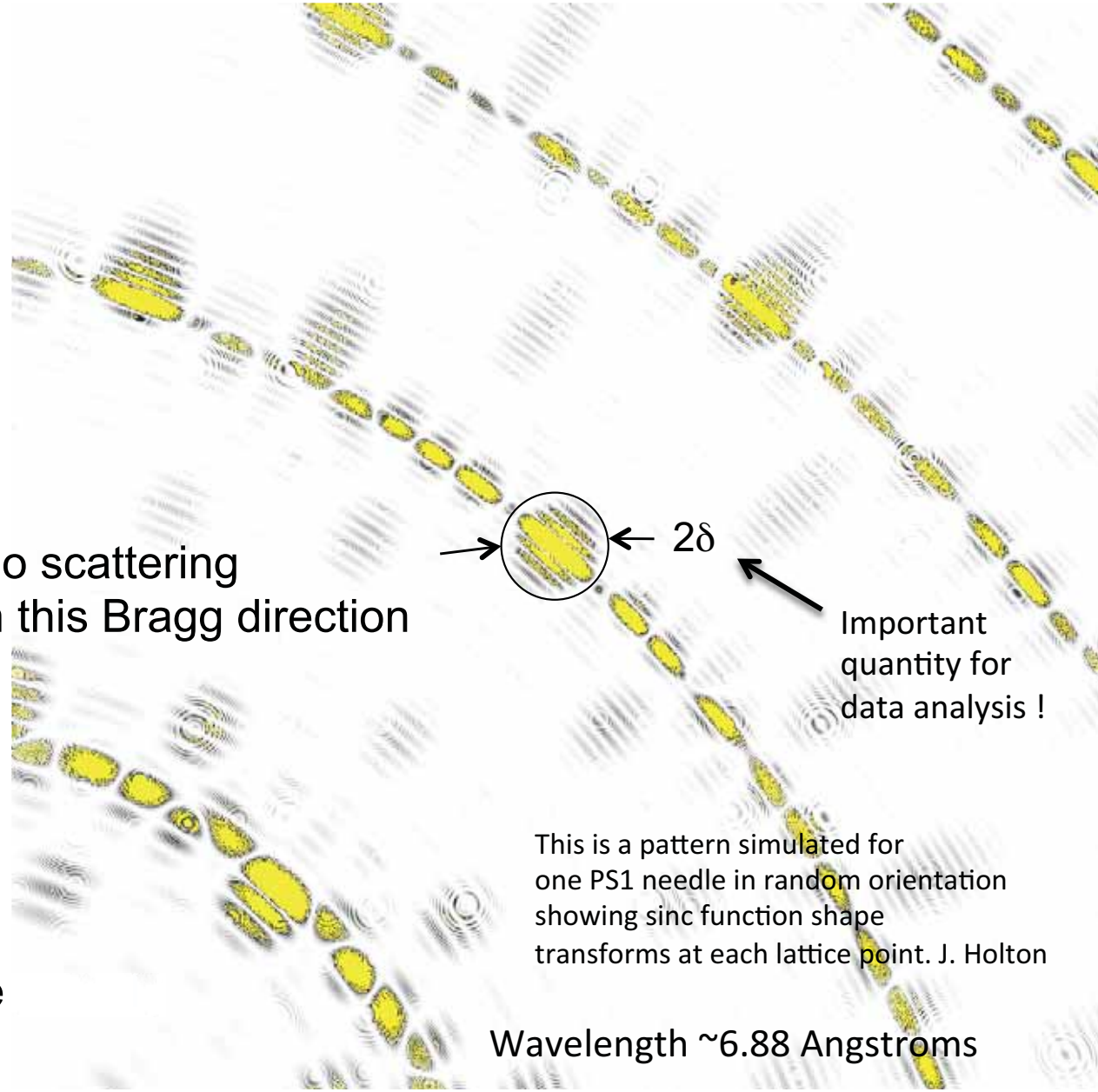
In XRD the Ewald sphere takes a SLICE through the 3D FT of the nanoxtal shape
The structure factors are proportional to the VOLUME of the shape-transform.

Confuse autoindexing programs !



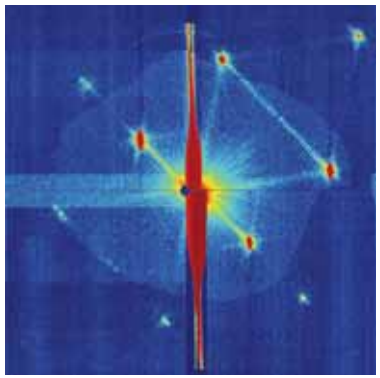
OR





How can we extract structure factors from this new kind of data ?

To extract structure-factors from partials we must sum over shape-transform volume – over xtal orientation *and size. Add Braggs from different xtals with same index*



Does this sum converge ? Test using Monte-Carlo integration.
(It must converge, because Powder diffraction exists).

Diffraction from one crystallite is

$$I_n(\Delta\mathbf{k},\mathbf{k}_o,\alpha,\beta,\gamma,N_i) = J_o \mid F(\Delta\mathbf{k}) \mid^2 r_e^2 P(\mathbf{k}_o) \frac{\sin^2(N_1\Psi_1)}{\sin^2(\Psi_1)} \frac{\sin^2(N_2\Psi_2)}{\sin^2(\Psi_2)} \frac{\sin^2(N_3\Psi_3)}{\sin^2(\Psi_3)} \Delta\Omega$$

One crystallite, smaller than a mosaic block.
Xtals too small for extinction. Assume no twins
α, β, γ are euler angles between xtal and Δk.

Sum over size and orientation from many is

$$I_{hkl}^{\exp}(m,\delta_t) = \sum_{n=1}^{m} \sum_{\{j\}_{m,\,hkl,\,\delta_t}} I'_n(\Delta\mathbf{k}_j)$$

Subtract water background

Sum over xtals
various size, orientn.

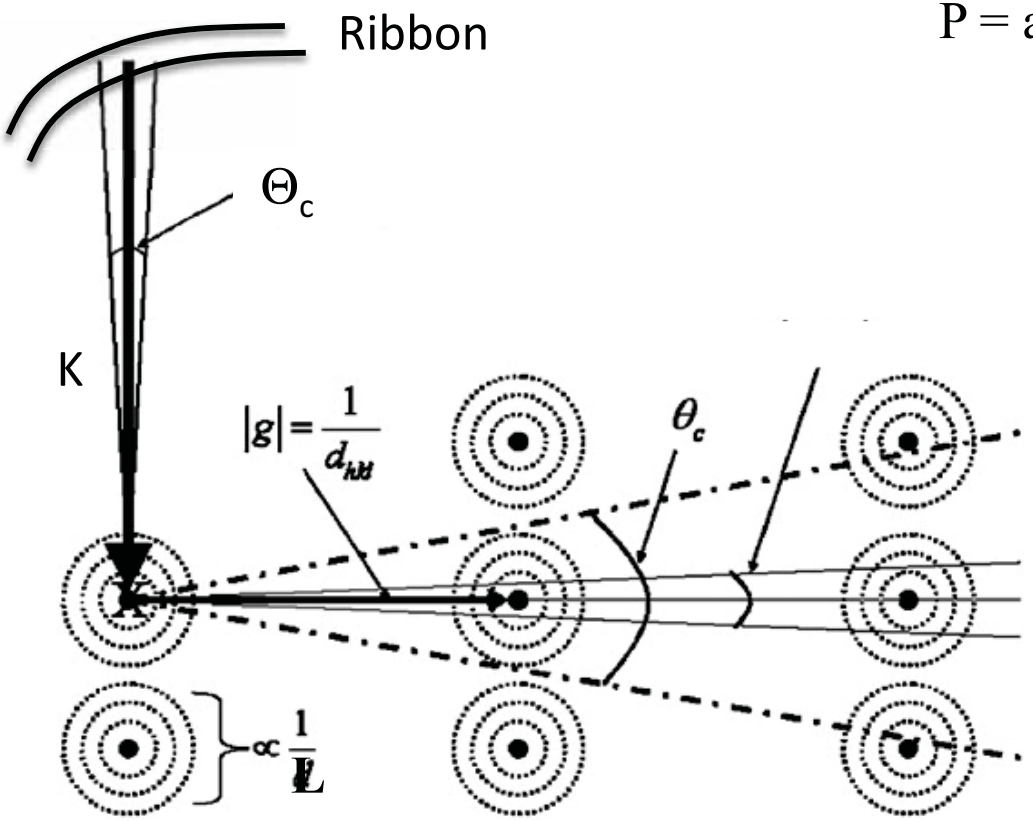
Sum over pixels around Bragg

$$\Psi_1 = 2\pi a \sin(\theta)\cos(\alpha)/\lambda$$
$$\Psi_2 = 2\pi b \sin(\theta)\cos(\beta)/\lambda$$
$$\Psi_3 = 2\pi c \sin(\theta)\cos(\gamma)/\lambda$$

This is similar to Powder diffraction, so must converge
Use average, not sum, to avoid flow alignment,

Increase beam divergence ?. Lorentz factor. Kirian et al , Optics Express (2010)

Chance P of hitting Bragg condition for nanoxtal in random orientation



P = area of ribbon around sphere / area of sphere.

Width of strip

$S = |K|\,\Theta_c = |K|\,\Delta K/g = |K|\,(d_{hkl}/L)$

Circumference of ribbon ~ $2\pi\,|K|$

Area strip/Area sphere = $2\pi\,|K|^2(d_{hkl}/L) / 4\pi|K|^2$

= Proby of hitting Bragg

$P = M\,\Theta_c / 2 = M\,(d_{hkl}/L)/2$

M= spot degeneracy
L= size of nanoxtal
d_{hkl} = d – spacing

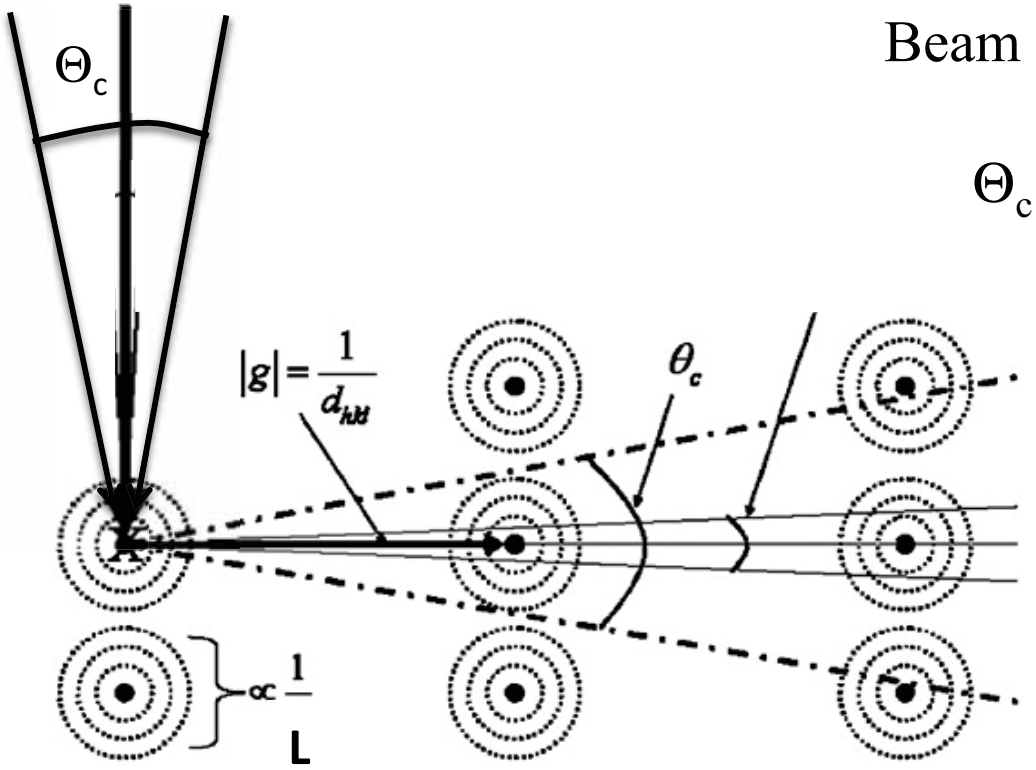
Low orders more frequent (big angle Θ_c)
Small xtals (small L) give more spots.

Remember:
For an nanoxtal, an incident plane-wave gives scattering into *many* directions.
For an ideal slab xtal, an incident plane-wave gives a scattered plane-wave

Will convergent-beam mode integrate partial reflections ?

*XFEL Beam energy spread 0.1% - too small for Laue

*Can ERL do Laue ? Wiggler ?



Beam divergence needed is

$\Theta_c = (1/L) / g = d_{hkl}/L = 30\text{nm} / 1000\text{ nm}$

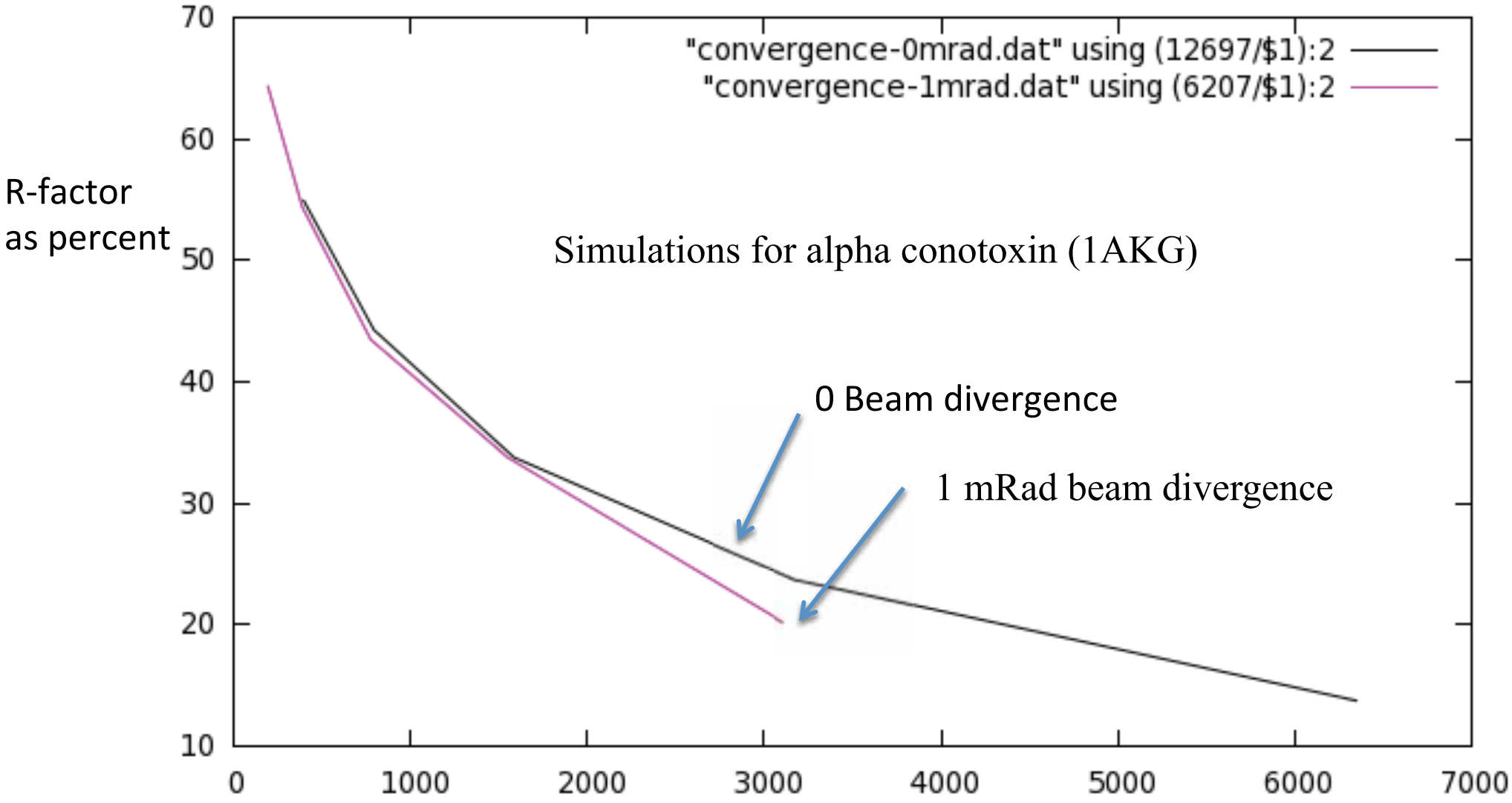
= 30 milliradians

for PSI xtal of one micron, first order

= 3 millirad for tenth order.

Conclude: With 1 mRad divergence at LCLS our high-order reflections are already CB-XRD

Does Monte-Carlo converge better using CB-XRD ?.



Now – 1. Compare coherent and incoherent partials – LCLS is coherent
2. Replace Laue for Time-resolved PX at XFEL with Post-refinement of CB-XRD

Rossman's post-refinement. Scaled partials. Better than CBXRD ?

A. XDS – Profile fitting.

1. Selecting larger xtals without big shape transforms
2. Finding by chance a reflection which is at bragg condition and get its profile.
3. Using this profile to model all the other reflections on that shot.
4. Repeat for every shot. In 3D !

B. Rossmann method : Scaled partials with post refinement" (Tom White).

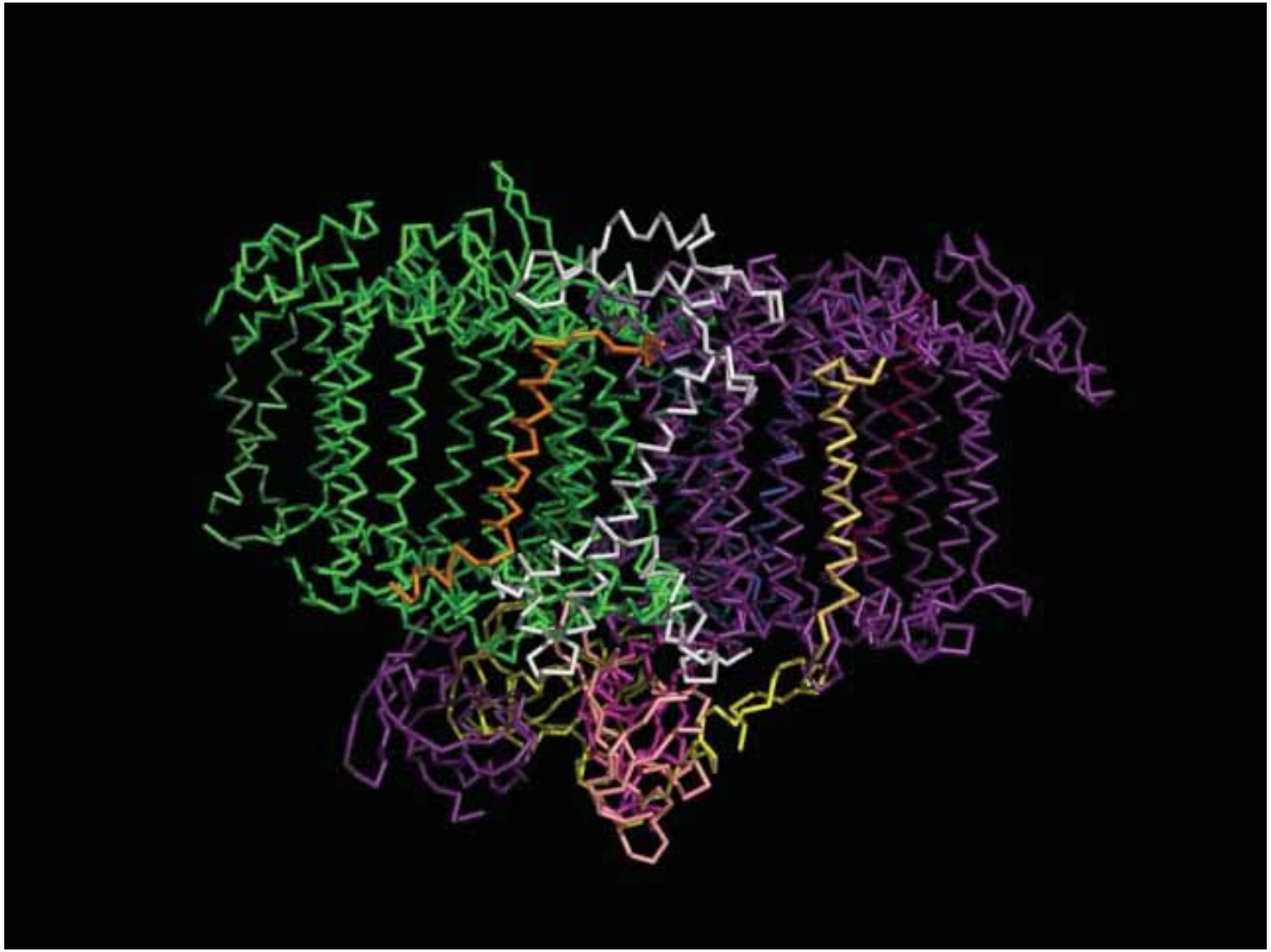
1. Index patterns
2. Estimate "full" intensities using the profile model and the estimated reciprocal lattice from the indexing.
3. Calculate the mean of all full intensity estimates for each reflection in the asymmetric unit (the "apparent" asymmetric unit if there's an indexing ambiguity).
4. Optimize the reciprocal unit cell and other parameters such as bandwidth, mosaicity, divergence to get the best agreement between each pattern and the mean.
5. If indexing is ambiguous – optimise "twin assignments" to improve fit, using to the current fit as a lower bound.
6. Repeat from step 2 until converged.

Step 3 also includes a determination of overall scaling factors for each pattern in the same way as conventional XRD. Steps 4, 5 and 6 are classic expectation maximisation algorithm. The profile width itself (i.e. the crystal size, if that's what's dominating the size of the profile) is also optimised in step 4.

Now do this with Convergent-beam data for fuller reflections…(instead of Laue).

Pump-probe in a jet
First attempted June 2010

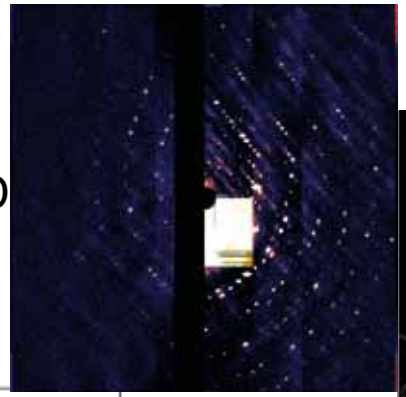
Pump-probe on PSI-ferre

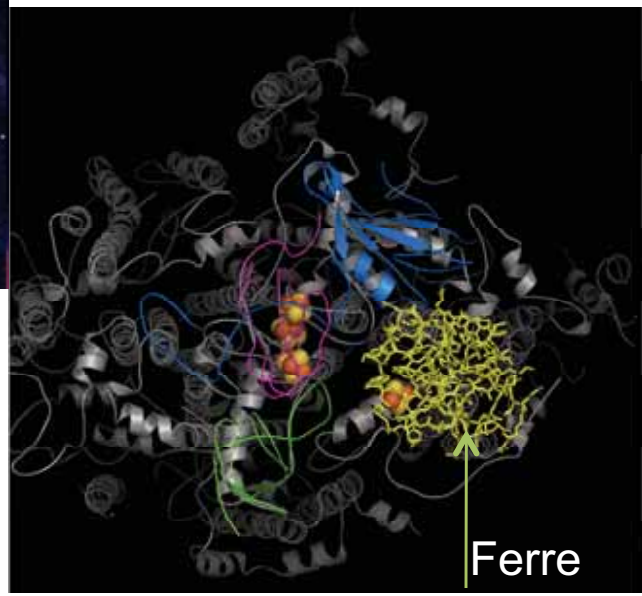


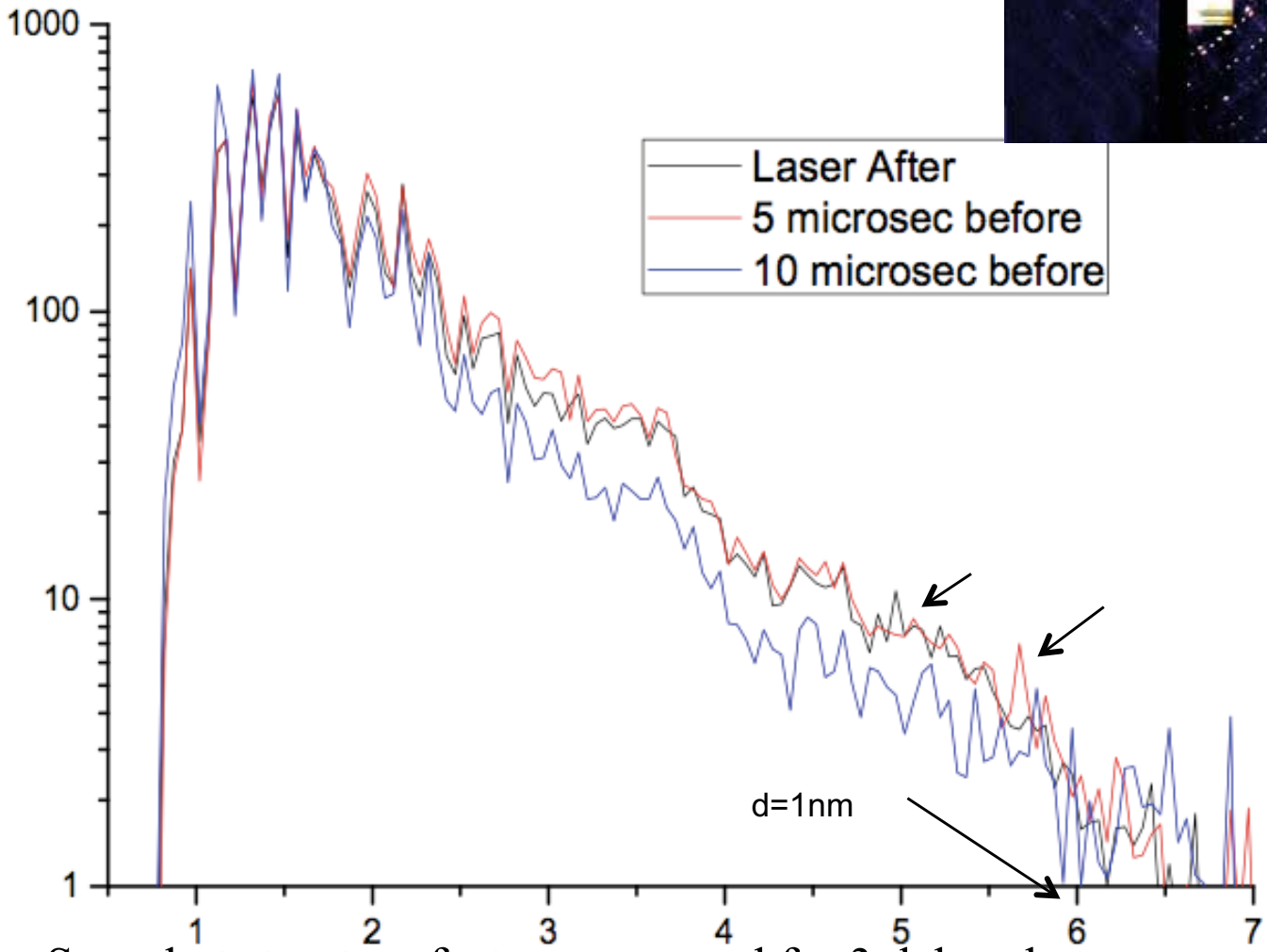
Movement of ferredoxin molecule on Photosystem 1 during electron transfer.

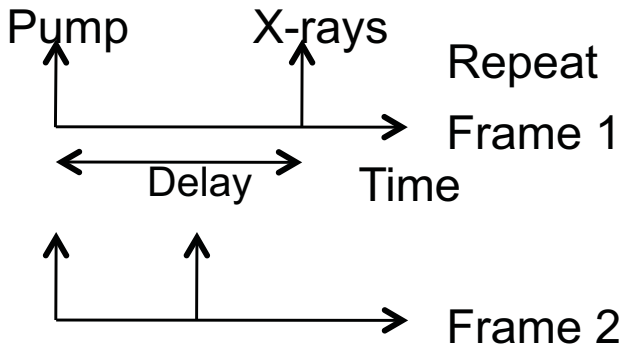
We aim to make a movie of the ferredoxin molecule undocking from PSI

Electron transfer causes undocking. Ferre is responsible for entire redox regulation in plants.
PSI-ferre solved recently by Fromme at 3.8 A -3KCD









Snapshot structure factors measured for 3 delays between pump laser and 70 fs X-ray pulse. Visible-light (photosynthesis) pump laser causes electron transfer, which causes undocking. Then entire xtal falls apart (but not the molecule). Indexed patterns show small change in lattice constants consistent with 3deg temp change, up to 10 microsecs, indicating no serious damage.

Phasing nanoxtals

Phasing Nanoxtals 1.

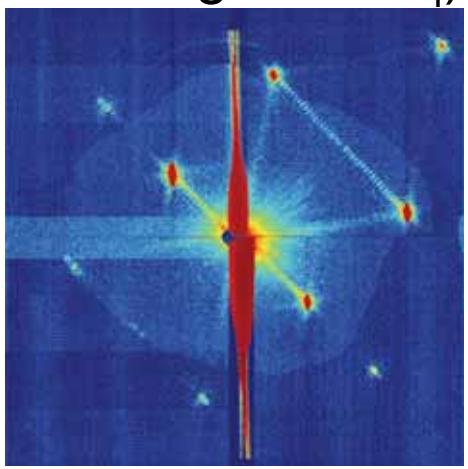
$$\Psi_1 = 2\pi a \sin(\theta)\cos(\alpha)/\lambda$$
$$\Psi_2 = 2\pi b \sin(\theta)\cos(\beta)/\lambda$$
$$\Psi_3 = 2\pi c \sin(\theta)\cos(\gamma)/\lambda$$

XRD from an acentric nanoxtal

$$I_n(\Delta\mathbf{k},\mathbf{k}_o,\alpha,\beta,\gamma,N_i) = J_o \,|F(\Delta\mathbf{k})|^2 \; r_e^2 P(\mathbf{k}_o) \frac{\sin^2(N_1\Psi_1)}{\sin^2(\Psi_1)} \frac{\sin^2(N_2\Psi_2)}{\sin^2(\Psi_2)} \frac{\sin^2(N_3\Psi_3)}{\sin^2(\Psi_3)} \Delta\Omega$$

$$= c\,|F(\Delta\mathbf{k})|^2 \; S_n(\Delta\mathbf{k})$$

Since I(Δk) is known, and interference fn. S(Δk) is known (count fringes for N_i) we can find the continuous molecular transform F(ΔK)…..



$$|F(\Delta k)|^2 = \frac{I_n}{S_n} = \sum_n \frac{I_n}{S_n}$$

where sum avoids problems due to division by zero.

Then phase F iteratively. Reconstruct density for one molecule.

The shape transform is the same around every lattice point for one xtal.
Every xtal has the same molec transform, independent of size.
This allows them to be disentangled.

-Don't need atomic resolution. Molecular (unit cell) density is real. Support is unit cell.
-Normal to 2,4,6-fold axis, phases are signs for origin on axis.

Phasing Nanoxtals . 2.

**Index & assemble data in 3D diffraction volume, including inter-bragg intensities (use fractional indexing)*

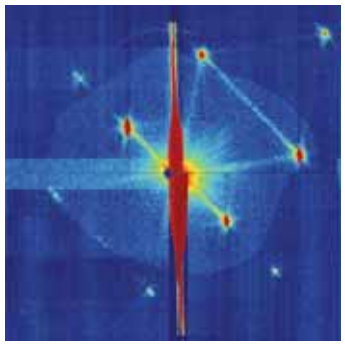
For one xtal..

$$I_n(\Delta\mathbf{k},N^{(n)}) = c\,|F(\Delta\mathbf{k})|^2\; S_n(\Delta\mathbf{k},N^{(n)})\Delta\Omega$$

**Now sum over particle size at each voxel….but Mol Transform is indep. of size.*

$$< I_n(\Delta\mathbf{k},N^{(n)}) >= c\,|F(\Delta\mathbf{k})|^2 < S_n(\Delta\mathbf{k},N^{(n)})\Delta\Omega >$$

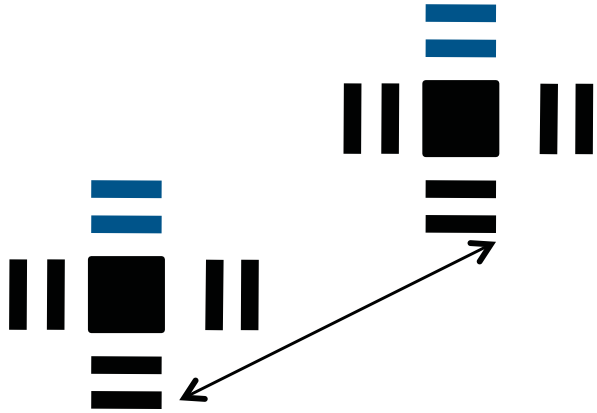
$$c\,|F(\Delta\mathbf{k})|^2 = F^2 = \frac{< I_n(\Delta\mathbf{k},N^{(n)}) >}{< S_n(\Delta\mathbf{k},N^{(n)})\Delta\Omega >} = \frac{Ex}{Av}$$



To get denom, sum corresponding voxels around each recip lattice point. (molecular transform then washes out**)*

** Particle size distribution was extracted from shape transforms in patterns. Self-normalizing !!!!!*

Now use iterative phasing on molec transform.



Spence et al Optics Express 19, 2866 (2011)

Now phase molecular transform in doubled cell by
iterative methods (Gerchberg, Saxton, Fienup, Marchesini's shrinkwrap)
cf Solvent flattening, Density modification , with feedback !

This method will fail if ….

1. xtal is too big (no inter-Bragg scattering)
2. xtal too small (can't index and merge data)
3. dynamic range insufficient (related to 1)
4. pixel density too low (sampling for Monte-Carlo is sufficient)

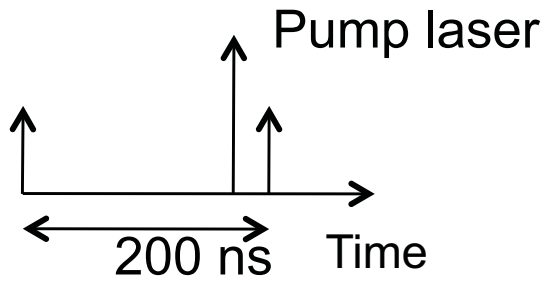
(maximum would be 4L/d on a side for largest xtal width L, res d).

Structure factors require the converging sum of the *volumes* of the shape transforms
Phasing requires 3D mapping of the continuous scattering from one molecule

WILD IDEAS…

Can we hit each nanoxtal twice (below destruction threshold) ?

Anfinrud suggests this for pump-probe. Holton will suggest anti-parallel beams.



Nanoxtal travels 0.2 microns in 200ns at 1 m/s

So readout two frames, 200ns apart. Pump laser between.

Repeat at 60 Hz.

This is possible using Pixel Array Detector.

Only useful for pump-probe delays less than 200 ns.

How to get 100% hit-rate – use “Fluctuation WAXS”

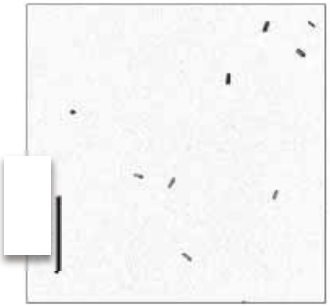
* Extracting an image of one particle
  from the scattering from many copies*

  (ab-initio, without SAXS modelling)

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

*identical and randomly oriented.

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



Many identical gold rods

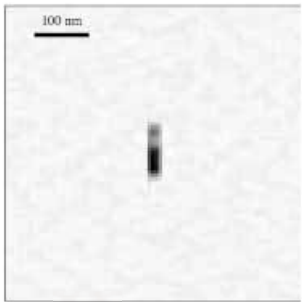
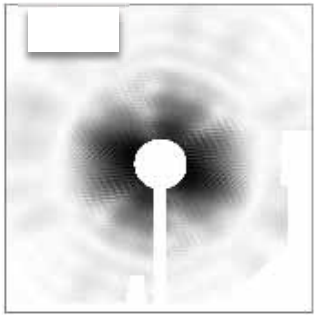


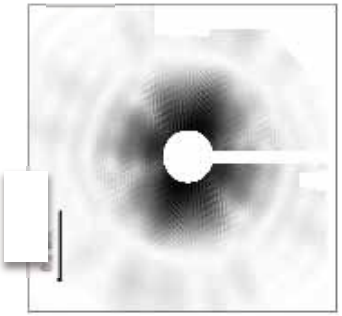
Image of ONE gold rod !!

FT

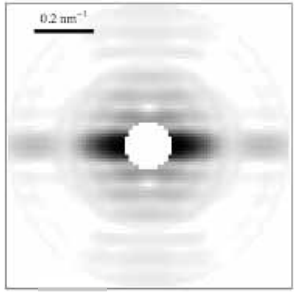
FT

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. Our movie shows Ang Corr fn on ring converging !

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

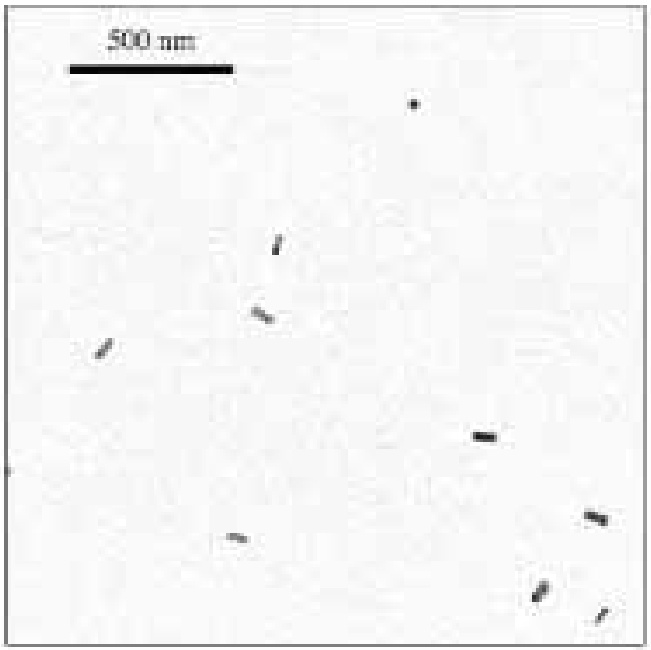


FIG. 1: Electron microscope image of a typical sample from which soft x-ray diffraction patterns are measured. Each sample consists of a set of about 10 gold nanorods of approximately 90 nm × 25 nm projection in random orientations about the normal to a transparent SiN substrate. There is a small admixture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

Reconstruction from XRD

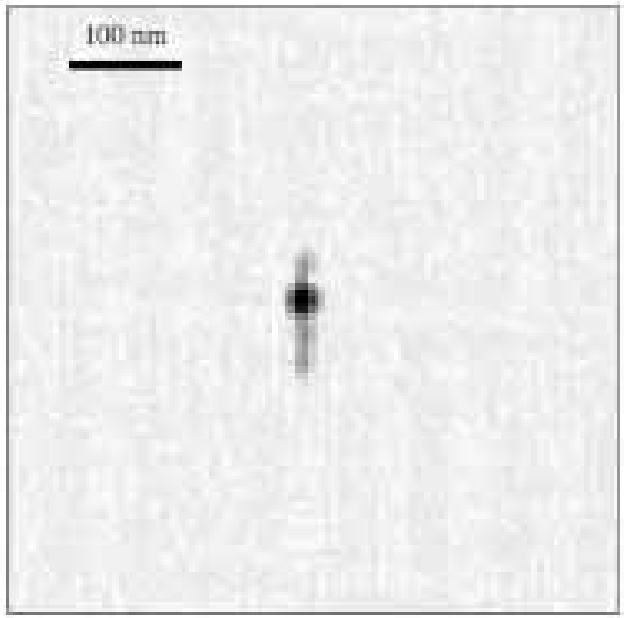


FIG. 7: Projection of the electron density of a single ∼ 90 nm × 25 nm rod reconstructed from the diffraction pattern of Fig. 6 after 100 iterations of the reciprocal-to-real-space phasing algorithm. The bulge near the center is probably a superposition of an image of a nanosphere, also found in the experimental samples (see Fig. 1).

In 3D, both beam noise and Kam fluctuations per shot are propn to no. of particles, so ratio isn't.
Hence if one particle doesn't work, many won't. Kirian et al Phys Rev E. Elser. Wochner.

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

Summary

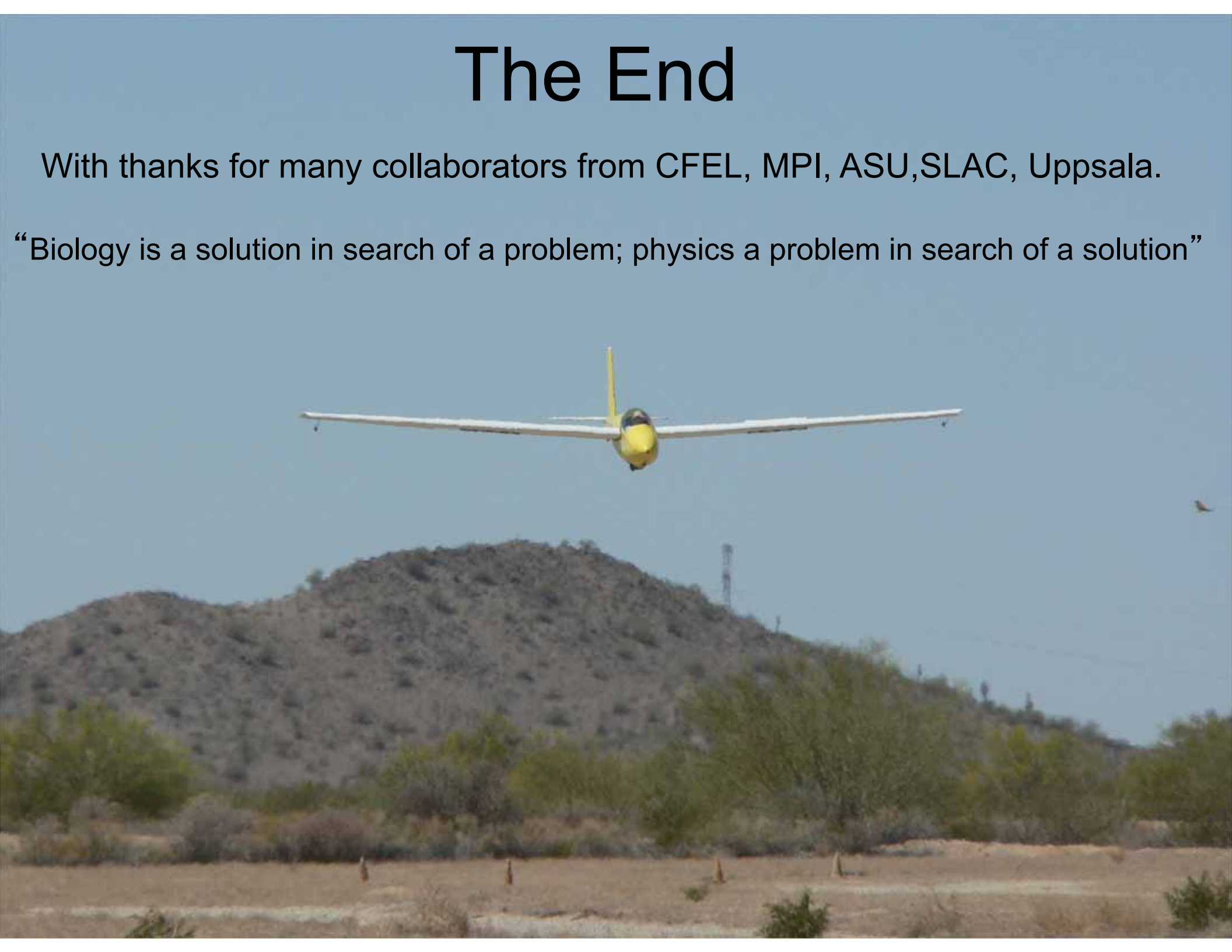
- "Diffract-and-destroy" (FSX) works at high resolution (0.3 nm) for delicate membrane proteins, fully hydrated. (Catepsin B, PSI, reaction center, Lyz)
- Solve invisible xtals in mother liquor ? Reduce crystallization bottleneck in protein crystallography. (SONICC). More perfect xtals for better resolution ?
- Use **short pulses instead of freezing** to reduce damage, **work at RT.**
- Hence control chemical conditions in jet at room temp, integrated with microfluidics, HPLC. Snap-shot chemistry.
- Dynamic studies - pump-probe for irreversible processes.
- Do Laue at ERL for time-resolved nanoxtals ?
- **Snapshot WAXS gives 100% hit-rate – solves hit-rate problem of FSX**.
- **To avoid wasted protein: XFEL photocathode triggered by asynchronous injector particle-detector. For ERL, trigger electronic shutter from injector**.
- A **new solution to the phase problem** ? Direct method at low resolution.

Nature - 2 papers – single particles, nanoxtals. Phasing PRL.

The End

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

“Biology is a solution in search of a problem; physics a problem in search of a solution”



. . . so intense, they drill through stainless steel