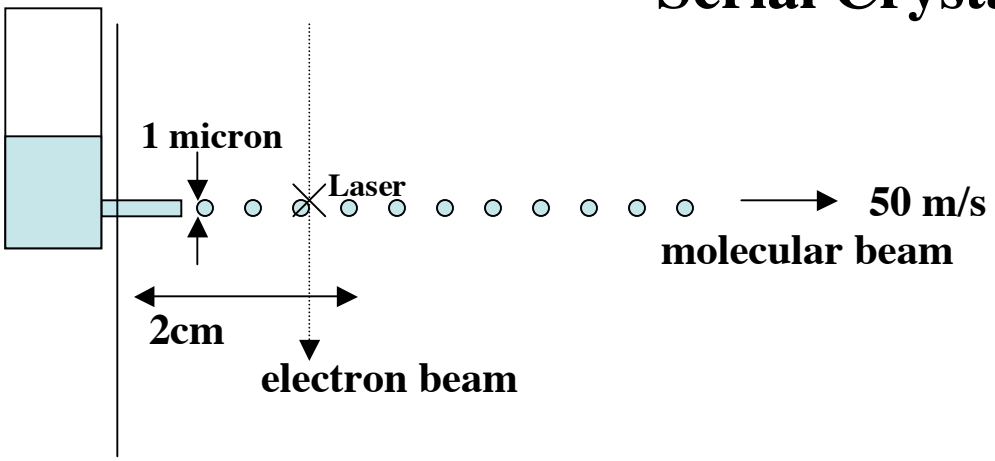
ANL Workshop, May 9 2007. 30 mins

Serial Crystallography.



We* have built apparatus to obtain X-ray (and electron) diffraction patterns from a beam of ice-jacketted proteins.
Molecules will be aligned by a polarized laser beam. All 3 beams run *continuously*.

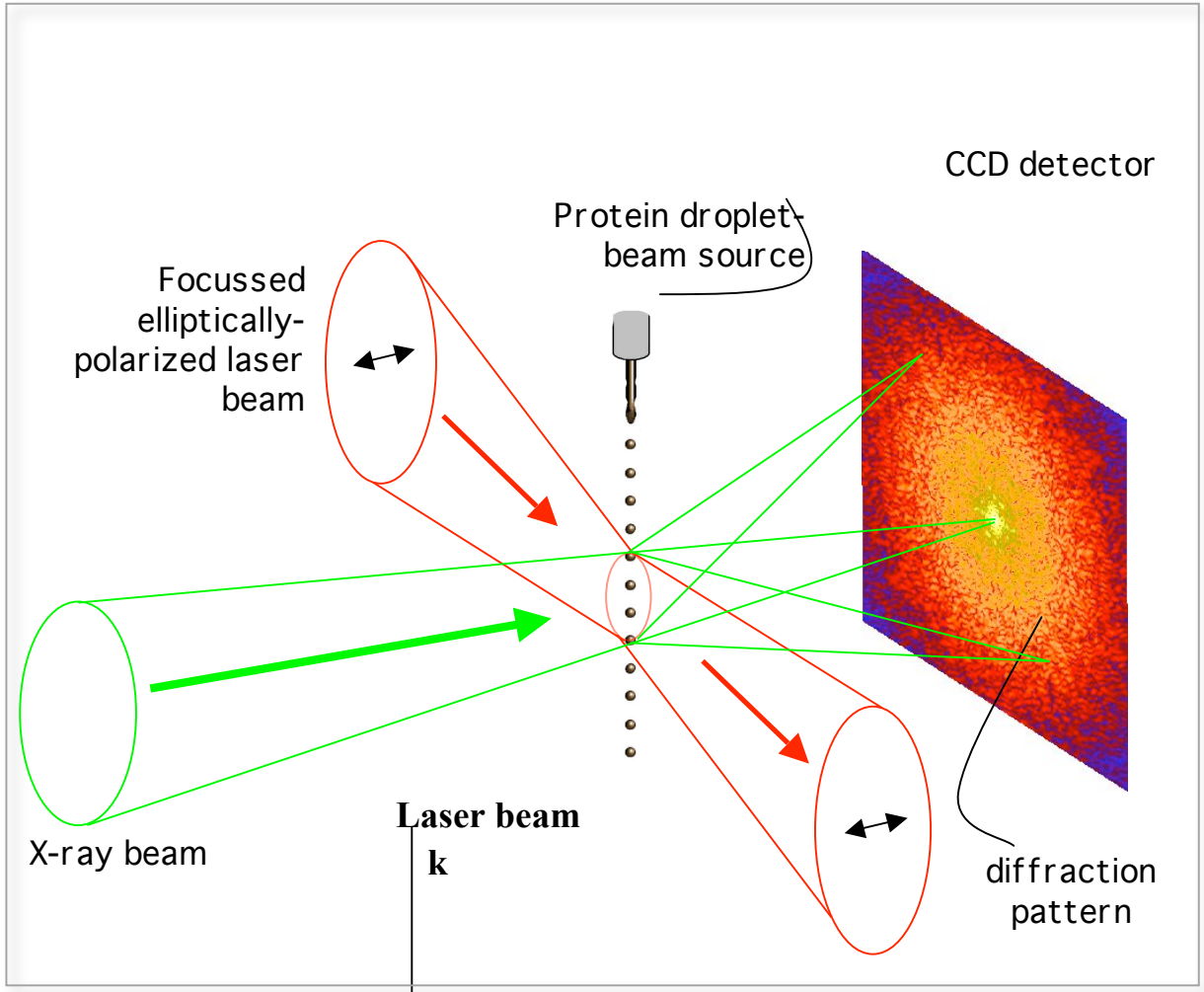
AIM: To solve protein structures which cannot be crystallized.

"The most important problem in structural biology"

1. *Droplet physics.*
2. *Laser-alignment*
3. *Phase problem -*
4. *Aligned SAX, Birefringence*

Spence and Doak, P.R.L. 92, 198102 '04. Acta A61, 237. '05. J.Chem Phys 123, 244304 '05

***Spence, Doak, Weierstall, Starodub, DePonte, Schmidt, Fromme,Hembree. ASU NSF IDBR $.**
***Chapman, Spence, Howells, Shapiro, Weierstall, Doak. ALS, ASU XRD CBST $**

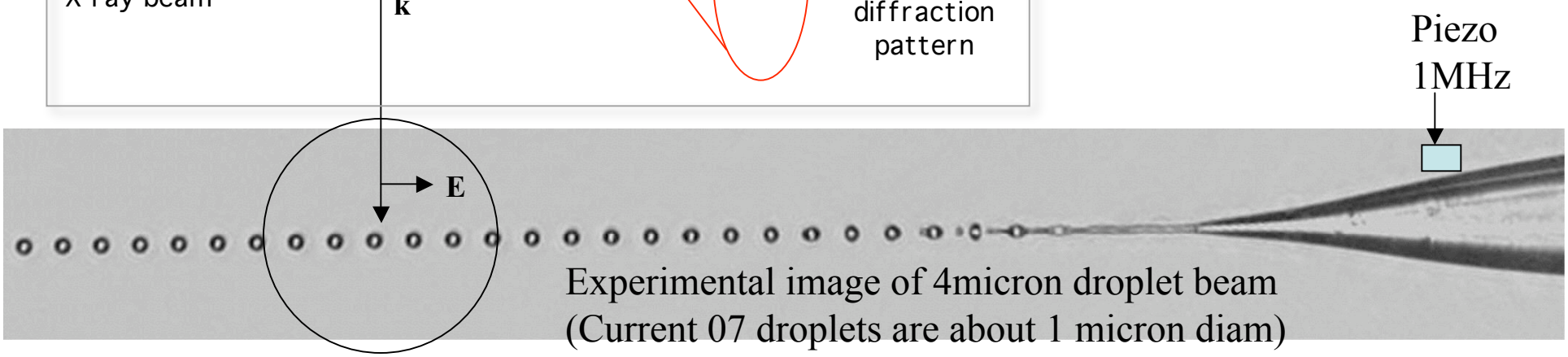


Three droplet beam methods

1. Rayleigh
2 Electrospray.
3 Aerojet.

Four alignment methods

1. **Laser**
2. Static field
3. Magnetic field
4. Flow alignment.



Experimental image of 4micron droplet beam
(Current 07 droplets are about 1 micron diam)

Electron beam, into page.

Main idea of serial xtallog

Consider a single-file stream of identical , aligned molecules traversing an X-ray beam. Assume their spacing exceeds the width of the Xray beam, so there is only one mol in the beam at any instant.

Because the *motion* of one molecule across the collimated X-ray beam has no effect on the diffraction pattern, the pattern is equivalent to that from one stationary immortal molecule, which never damages*.

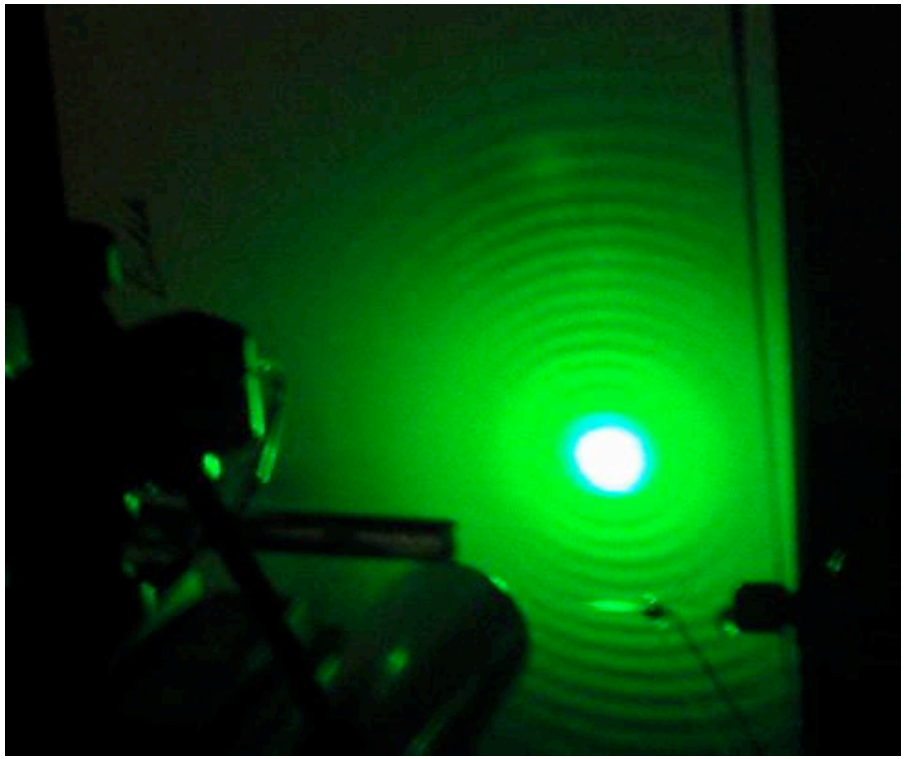
Plan of talk

1. Laser alignment
2. Exposure times, Phasing.
3. Droplet sources, results

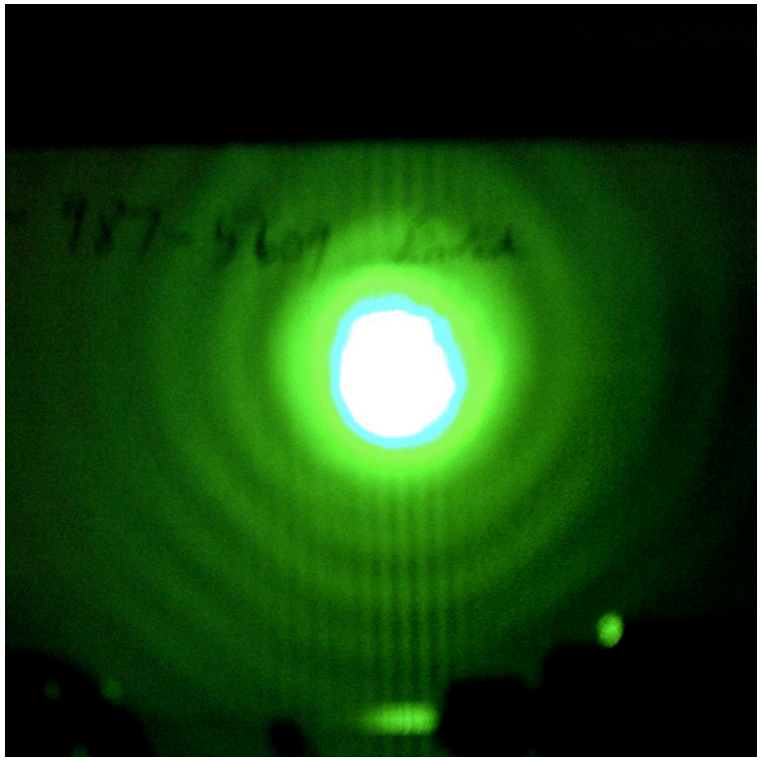
*The transit time for one mol travelling at 50m/sec across a ten micron beam is 200ns.

We have obtained laser diffraction patterns from a droplet beam.

Translational motion of droplets does not affect diffraction pattern



Laser diffraction pattern obtained from a single-file monodispered 8 micron Rayleigh droplet beam of water.



Young's fringes seen when Laser beam spans several droplets

Mie scattering possible for smaller droplets. Laser alignment of 1.5 micron e-coli in progress.

Weierstall, Shapiro, Spence, July 05. LBL.

Experimental demonstration of laser-alignment of a molecular beam

***The non-resonant laser induces a dipole moment p for polarizability α.**

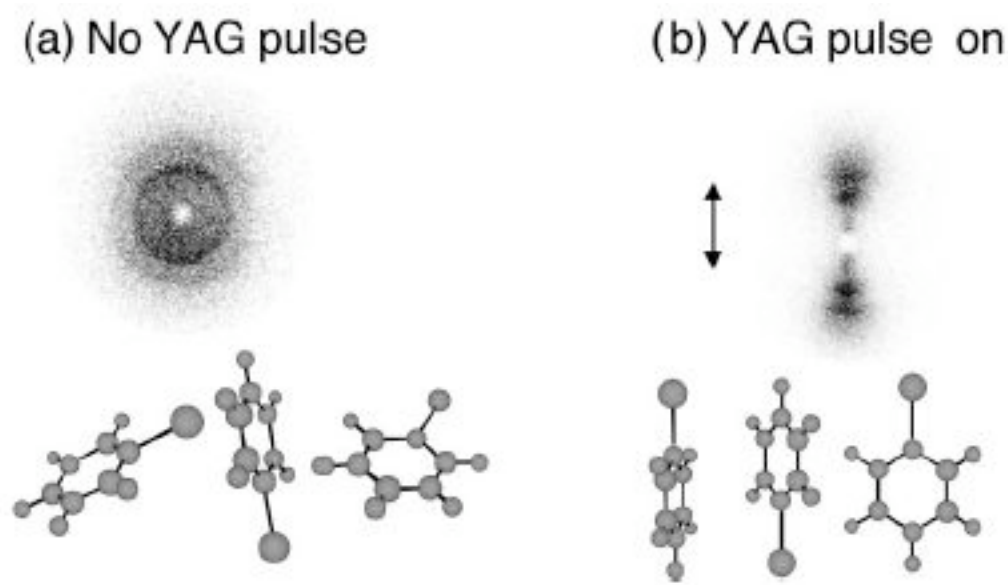
$$\mathbf{p_i(t)} = \alpha_{ij}(\theta)\, E_j(t)$$

***Because the induced moment is not parallel to the field which caused it, there is a torque τ**

Torque is $\boldsymbol{\tau} = \mathbf{p} \times \mathbf{E}$

When elliptically polarized light is used, all 3 molecular axes are aligned in direction, but not sense (Elser 06).

There are no absorption lines in protein at one micron. At 10^8 watts/cm^2 no damage is expected.



Molecules of Iodobenzene in a gas jet aligned by linearly polarized light. After alignment, mols are ionized and exploded by circularly polarized laser pulse, producing the pattern shown.

Rotational temp 20 K

Larsen, J. Chem Phys 111, 7774 (1999); Stapelfeldt, Seideman, '03 etc.

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The laser wrench :A laser-driven nanomotor.

By rotating the direction of polarization (lying in the plane of the page) this glass nanorod can be rotated in water at will. (An external quarter-wave plate is rotated)

This is a movie.

Glass rod in water.
Length 2.5 microns.

E

X k

Keith Bonin et al . Optics Express, 2002. Glass rod is 2.5 microns long, 260nm wide. Polarised laser 20mW CW, lambda = 514nm, focussed to 0.5 micron.

The laser power and temp needed for given resolution can be found from equipartition.

- Brownian motion causes angular blurring of diff pattern, limiting resolution in reconstruction. Like Kelvin's mirror galvanometer.
- Mol. in beam is harmonic oscillator, oscillating about C of M. Need 0.5 kT per degree of freedom. "Spring constant" is propn. to laser power
- Use **$0.5\ kT = 0.5\ K \langle\Delta\theta\rangle^2$** for torsion pendulum with spring const K

$$\Delta\theta = \frac{Temp}{LaserIntens * Volume}$$

$K = 4\pi\ (I_o/c)\ (\gamma * V)$

polarizability

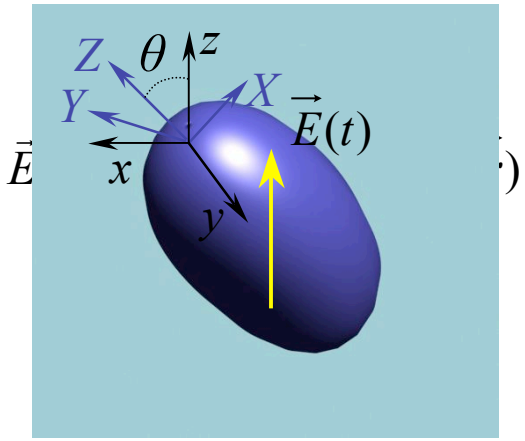
$\Delta\theta$ = 15 degrees for ribosome at 150K with 100 W laser focussed to 5 micron spot.

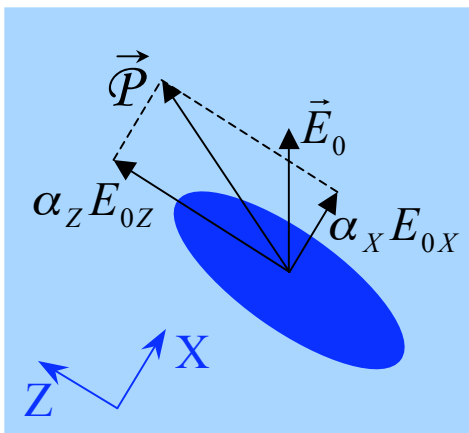
Period $2\pi(I/K)^{1/2} \sim 1$ ns. Transit time ~ 200 ns

D. Starodub et al J. Chem Phys 123,244304 (05)

Classical calculation of polarizability for laser vs DC (SAX).

D. Starodub. Also in progress based on lattice dynamics





$$E_i = \frac{\varepsilon_m}{\varepsilon_m + L_i(\varepsilon - \varepsilon_m)} E_{0i} \qquad E_i = E_{0i} - 4\pi L_i P_i$$

Ellipsoid dipole moment:

$$\mathcal{P}_i = \varepsilon_m P_i V = \underbrace{\frac{V}{4\pi} \frac{\varepsilon - \varepsilon_m}{\varepsilon_m + L_i(\varepsilon - \varepsilon_m)}}_{\text{polarizability } \alpha_i} \varepsilon_m E_{0i}$$

Torque is

$$\vec{\mathcal{K}} = \vec{\mathcal{P}} \times \vec{E}_0 \qquad \mathcal{K}_Y = \mathcal{P}_Z \cdot E_{0X} - \mathcal{P}_X \cdot E_{0Y}$$

$$\mathcal{K} = \frac{V}{8\pi} \frac{(\varepsilon - \varepsilon_m)^2 (1 - 3L_Z) \sin 2\theta}{[\varepsilon_m + L_Z(\varepsilon - \varepsilon_m)][\varepsilon + \varepsilon_m + L_Z(\varepsilon_m - \varepsilon)]} \varepsilon_m E_0^2$$

$$L_Z = \frac{1 - e^2}{e^2}\left(-1 + \frac{1}{2e} \ln \frac{1+e}{1-e}\right) \qquad e^2 = 1 - \frac{b^2}{a^2}$$

ε_w(static)=80, ε_p(static) = 12 (?) ε_{ice}(static) = 3.2

ε_w(NIR) =1.74 ε_p(NIR) = 2.34 ε_{vacuum} = 1.

Conclude: for a cigar shaped protein in water, $\Delta\alpha \sim \varepsilon_w (V/4\pi) [\varepsilon_w - \varepsilon_p]$

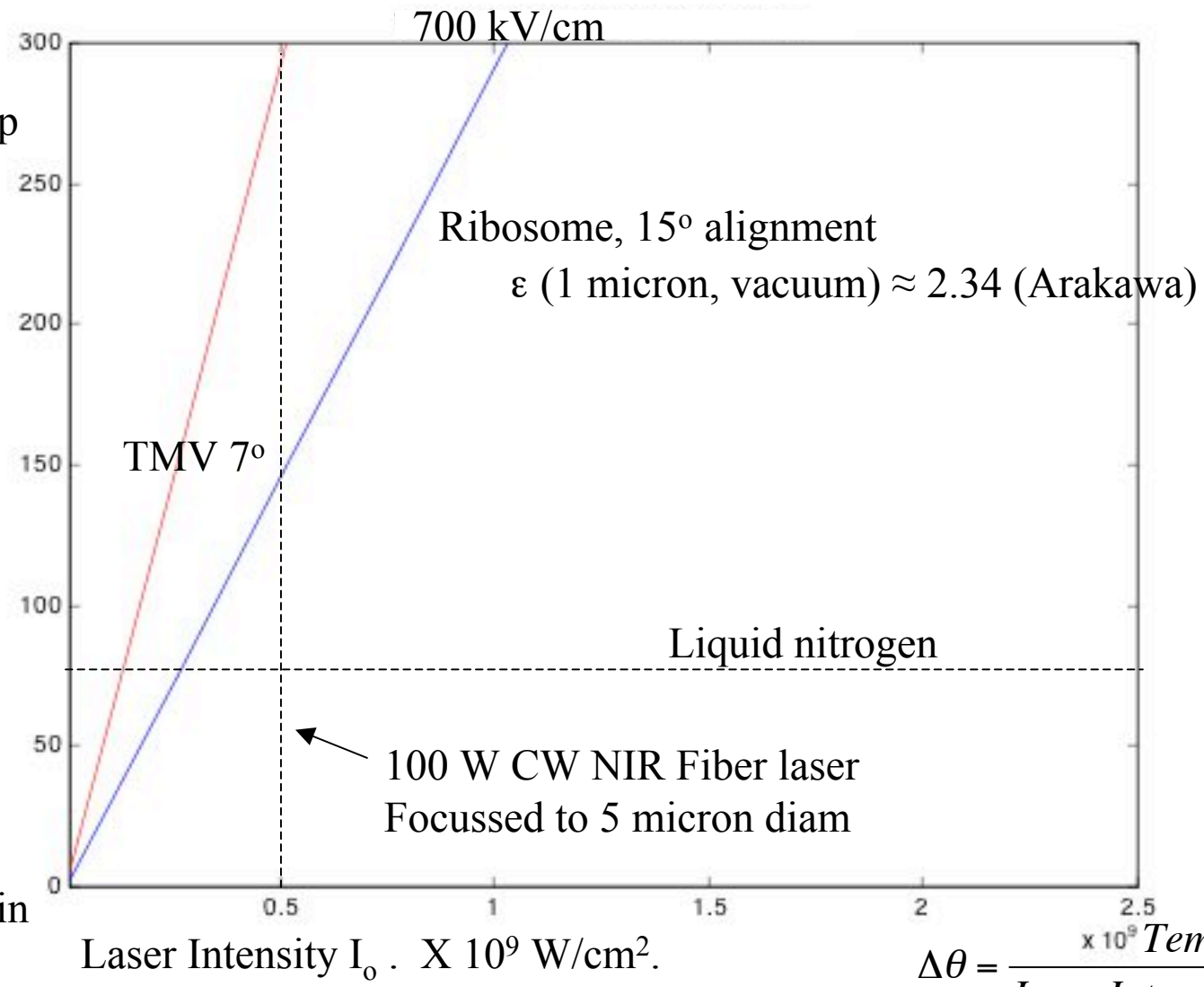
Hence a large torque enhancement in water at DC ! 80(80-12) vs 1(1-2.34)

BUT: These static Δα are 100 X less than <u>O'Konski</u> measures. Dielectric theory ignores dipole layers ? Laser fields in vacuum may be orders of magnitude higher than static in water. Induced as E^2.

Temp vs Laser Intensity for TMV and Ribosome.

$kT = 0.5\ K \langle\theta^2\rangle = -2\pi (I_o/c)(\gamma * V) \langle\Delta\theta^2\rangle$ by equipartion.

Hence T propn to laser intensity I_o.

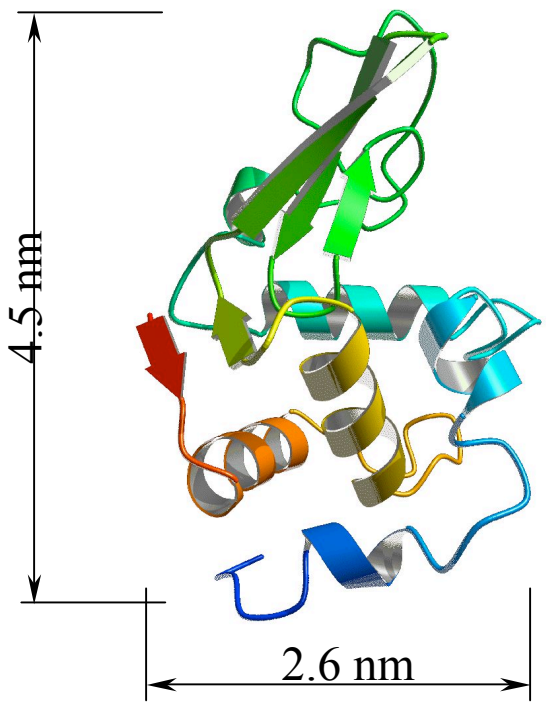


Koch aligned TMV to 20° half-angle by electric SAX (5 kV/cm) with high background in one dim.

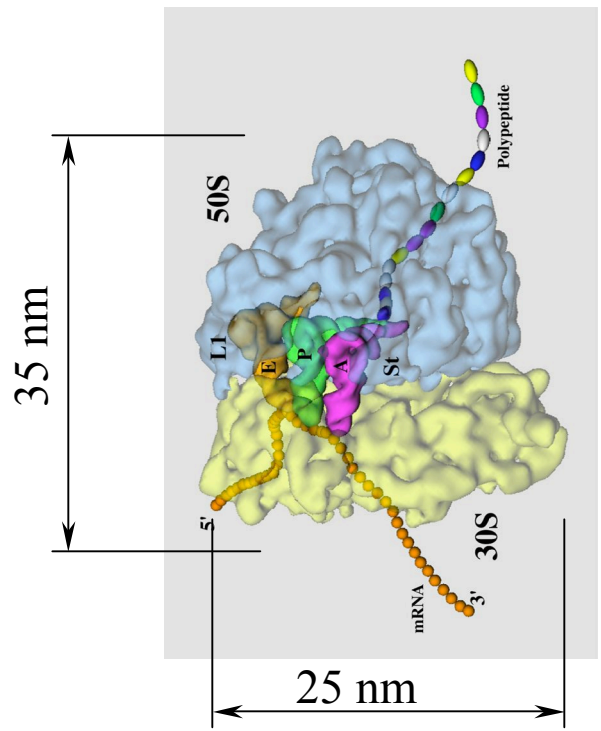
$$\Delta\theta = \frac{Temp}{LaserIntens * Volume}$$

Resolution requirements

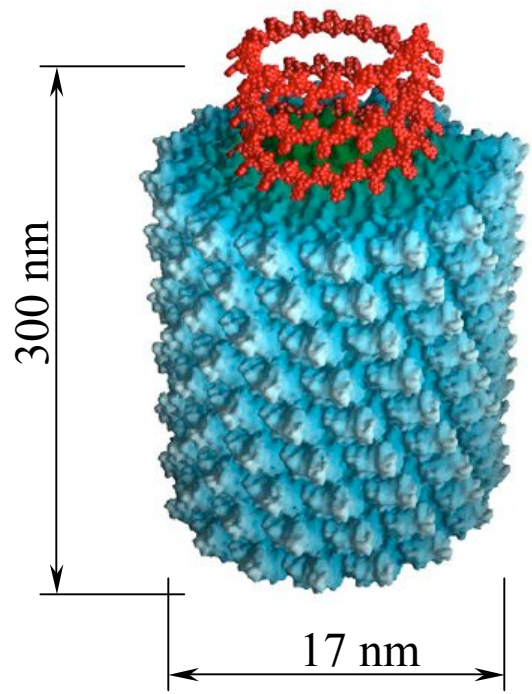
Lysozyme



Ribosome



Tobacco Mosaic Virus



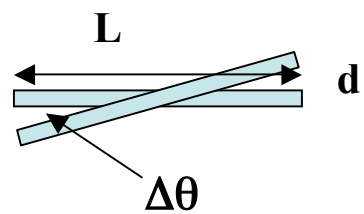
Thermal fluctuations set the limit to resolution *d*:

$$d = \sqrt{\langle \theta^2 \rangle} \cdot \frac{a}{2}$$

d = 2 nm: overall shape is resolved

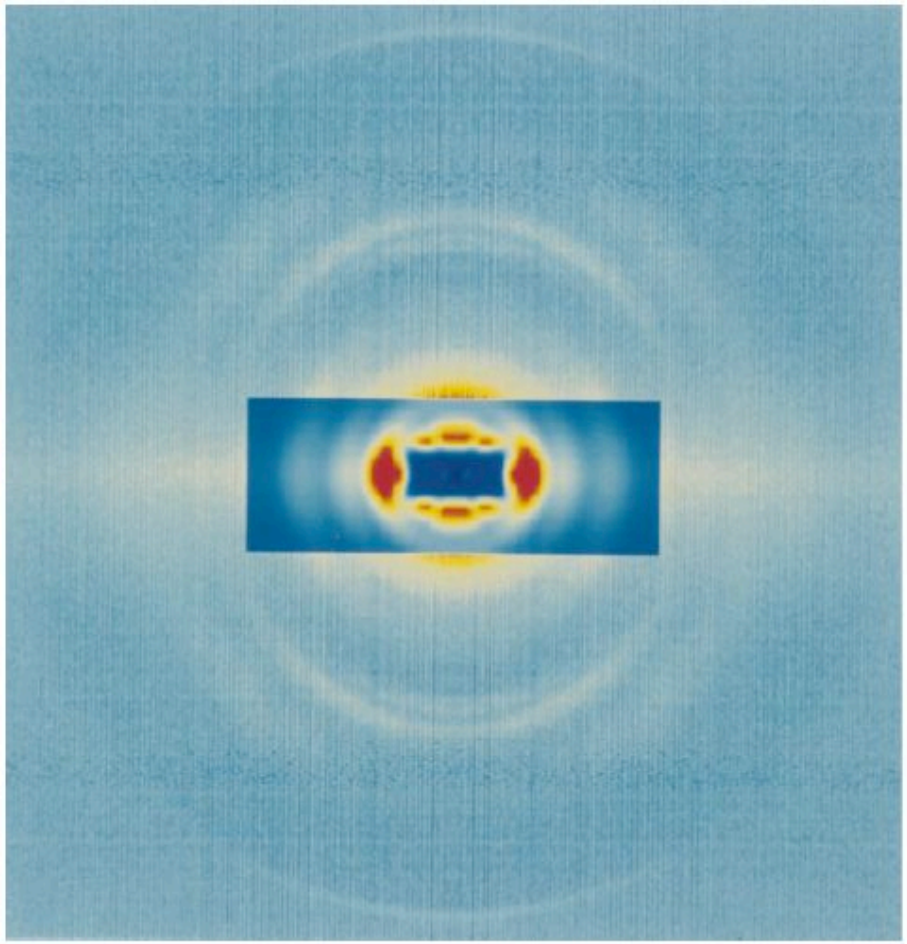
d = 0.7 nm: secondary structure (α-helices, β-sheets)

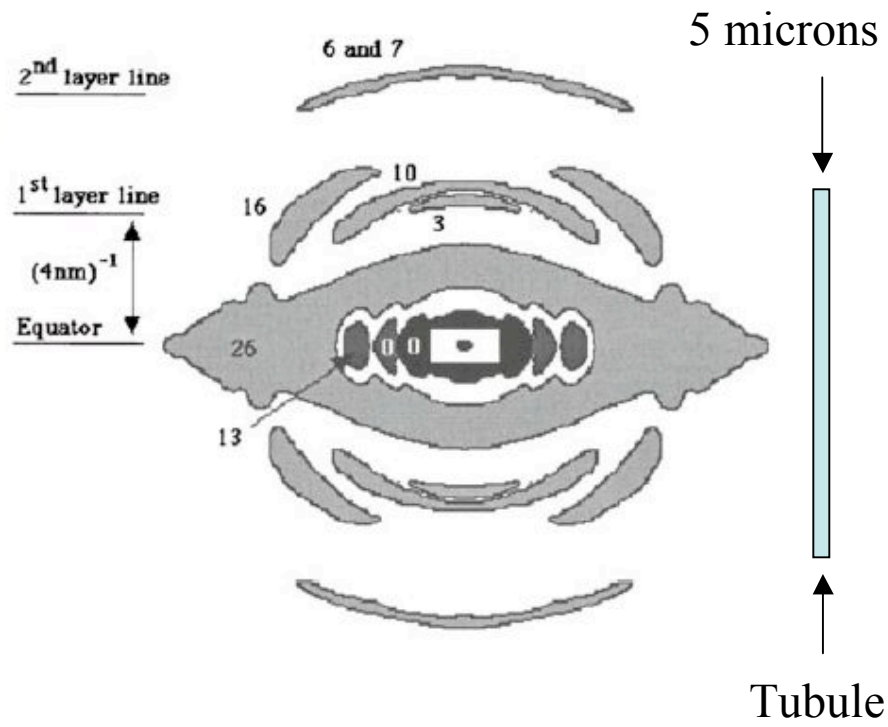
Resolution = 0.5 L Δθ



SAX from tubules after alignment by 11 Tesla field.

Align with Magnetic field





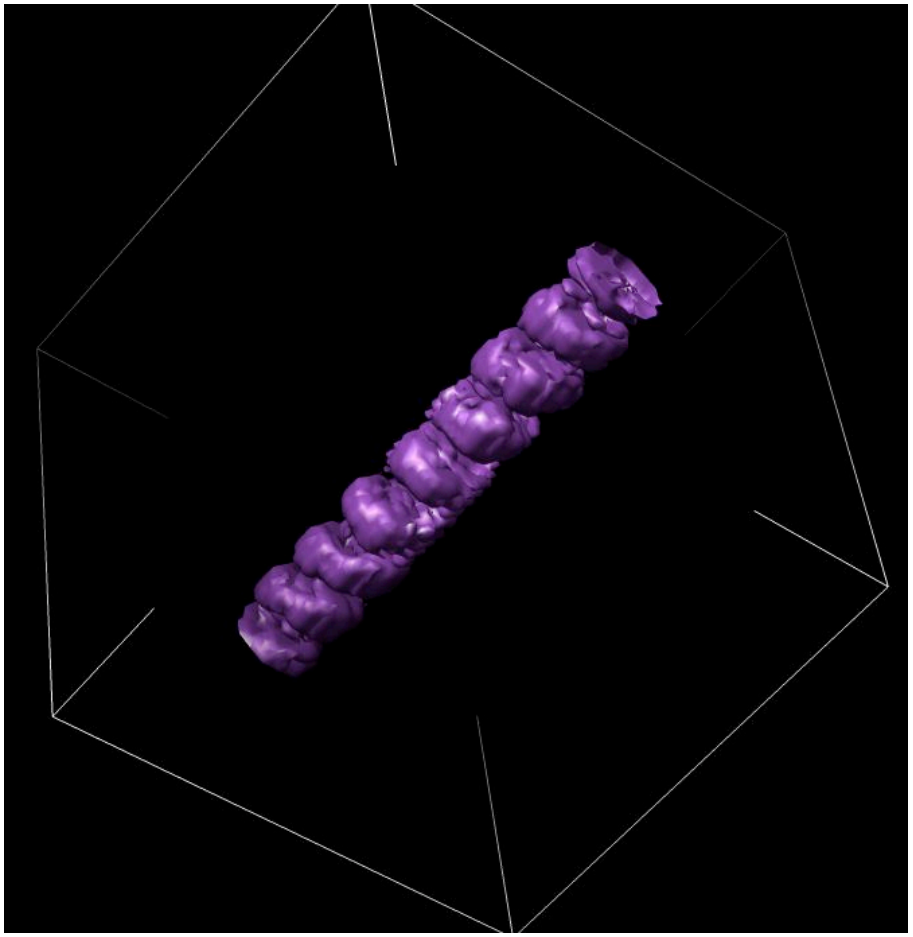
Fiber diffraction

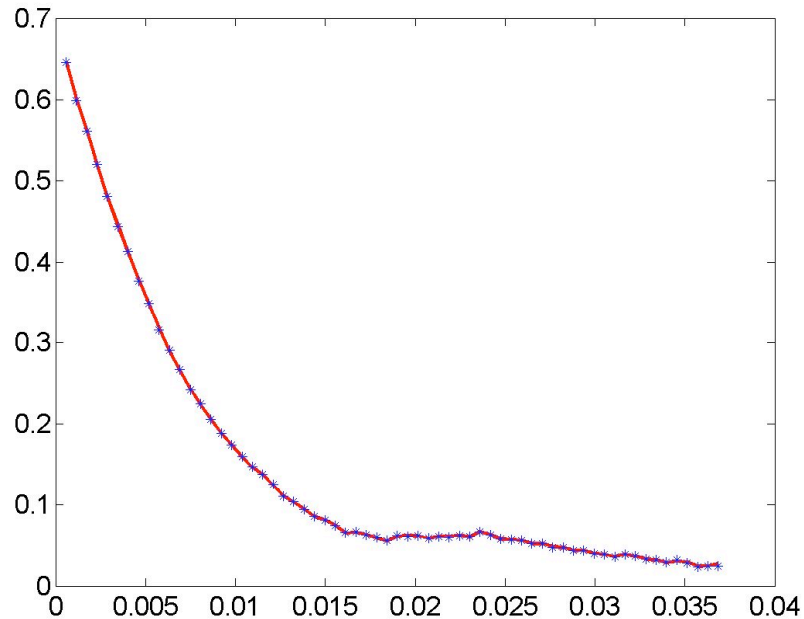
FIGURE 8 Low-resolution x-ray fiber diffraction pattern of an oriented solution of hydrated microtubules. Data shown are the average of data collected from six individual samples that were each exposed to the x-ray beam at a number of spots to avoid radiation damage. Total data collection time was 4 h. The contribution of the buffer solution to the diffraction pattern has been subtracted. The right-hand panel is a stylized trace indicating the main diffraction features. The rectangular box in the center represents an area with a higher threshold value (100×).

W. Bras et al. (1998). Misalignment is estimated as 10° RMS. X-ray spot scanned (damage).

Resolution ~ L $\Delta\Theta/2$ L=5 micron, $\Delta\Theta=10^o$ Res = 300nm. **Actually 5nm.** Sax res limited by Ewald sphere curvature, misalignment, typically 4nm. Water subtracted. **Birefringence !**

Use both aligned SAX and un-aligned data in Fienup HIO algorithm to find density.
Set density outside envelope to zero in iterations. Assume known rod diam.





Original SAX data from microtubules.
Random orientations give 1D data.
X-axis in Ang^{-1}.

Cf Eva Nogales

Now refine this with HiO, using support found by flipping. Sign constraint also.
Real object. Friedel symmetry. Diam 15nm

Koch et al (1989) did E-aligned SAX of TMV.

Conclusion: New ab-initio iterative algorithms make better useof a-priori info., and can extract more information from partially aligned data. Try fishing line and spiders web ?

Some Times.

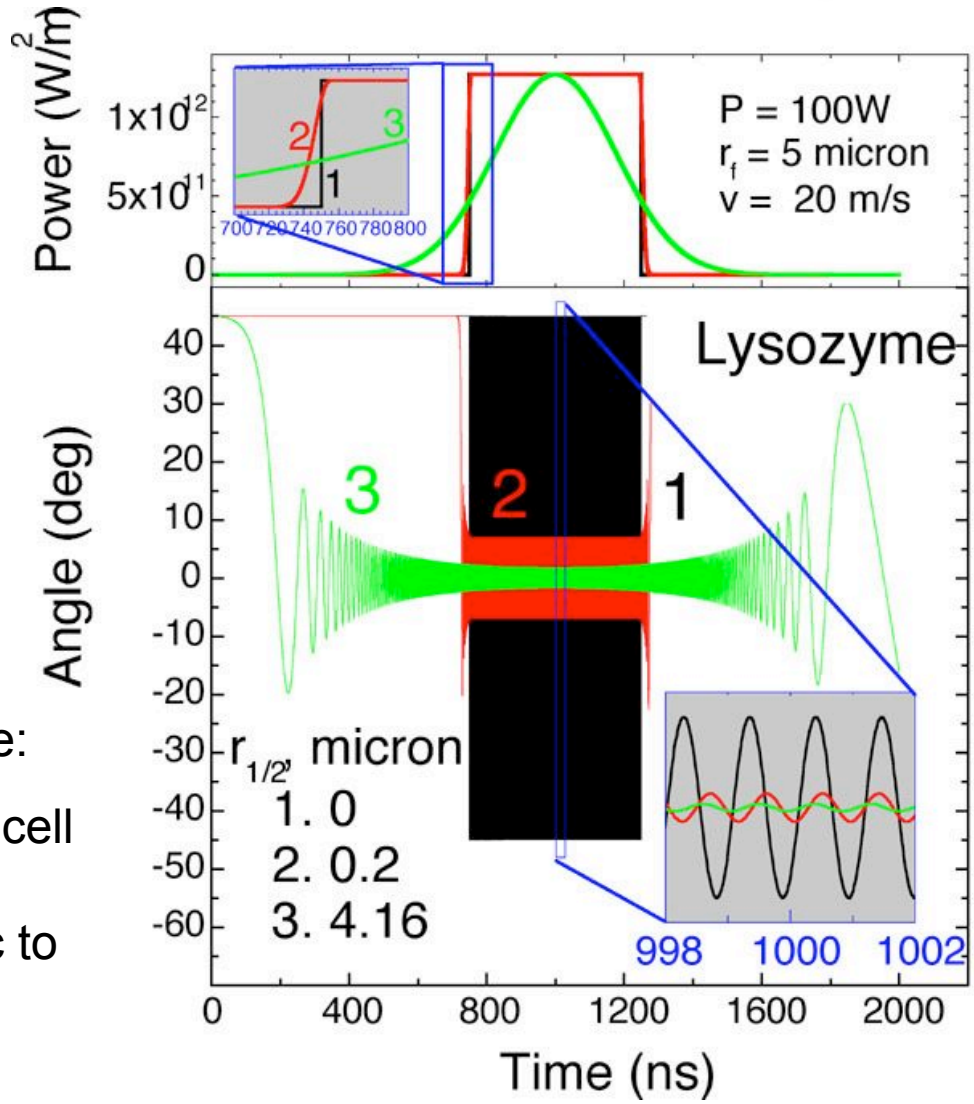
Molecule	Oscillation Period (50W)	Damping time (gas,STP)	Transit time (50 m/sec)
TMV (l=300nm, γ =10)	16ns	5.5 underdamped	200 ns
Ribosome (l=35nm, γ=0.93)	6.8ns	3.5 ns underdamped	
Lysozyme (r=1.9nm, γ = 1.6)	0.63ns	0.46ns overdamped	

Conditions:
50 Watt laser, 10 micron spot, viscosity $\eta = 1.8 \times 10^{-5}$ Pa sec (Nitrogen at STP), mfp = 100nm

Conclude:
XRD in air OK.
TED needs environmental cell, filled with helium at near one atmosphere. Align before diffraction ?

Adiabatic ? Risetime of laser field is less than oscillation period, hence no damping needed ?

Adiabatic damping in vacuum



Numerical solution of Langevan eqn. D. Starodub

Violent oscilations if laser switch-on time too short !

Adjust with laser profile and mol. beam speed

Conclude:

Use gas cell and/or adiabatic to damp.

Adiabatic if rise-time of laser field long compared with oscillation period. Then gas damping not needed - molecule does not overshoot.

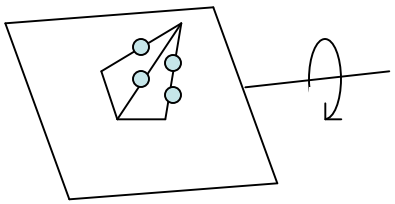
Exposure time and phasing algorithms

Note: we assume no interference between XRD from different , mols,
so loose the "coherent amplification" of Bragg diffraction ~ N^2.
But XRD from one protein at low angles is propn to $(Z=60,000)^2$!

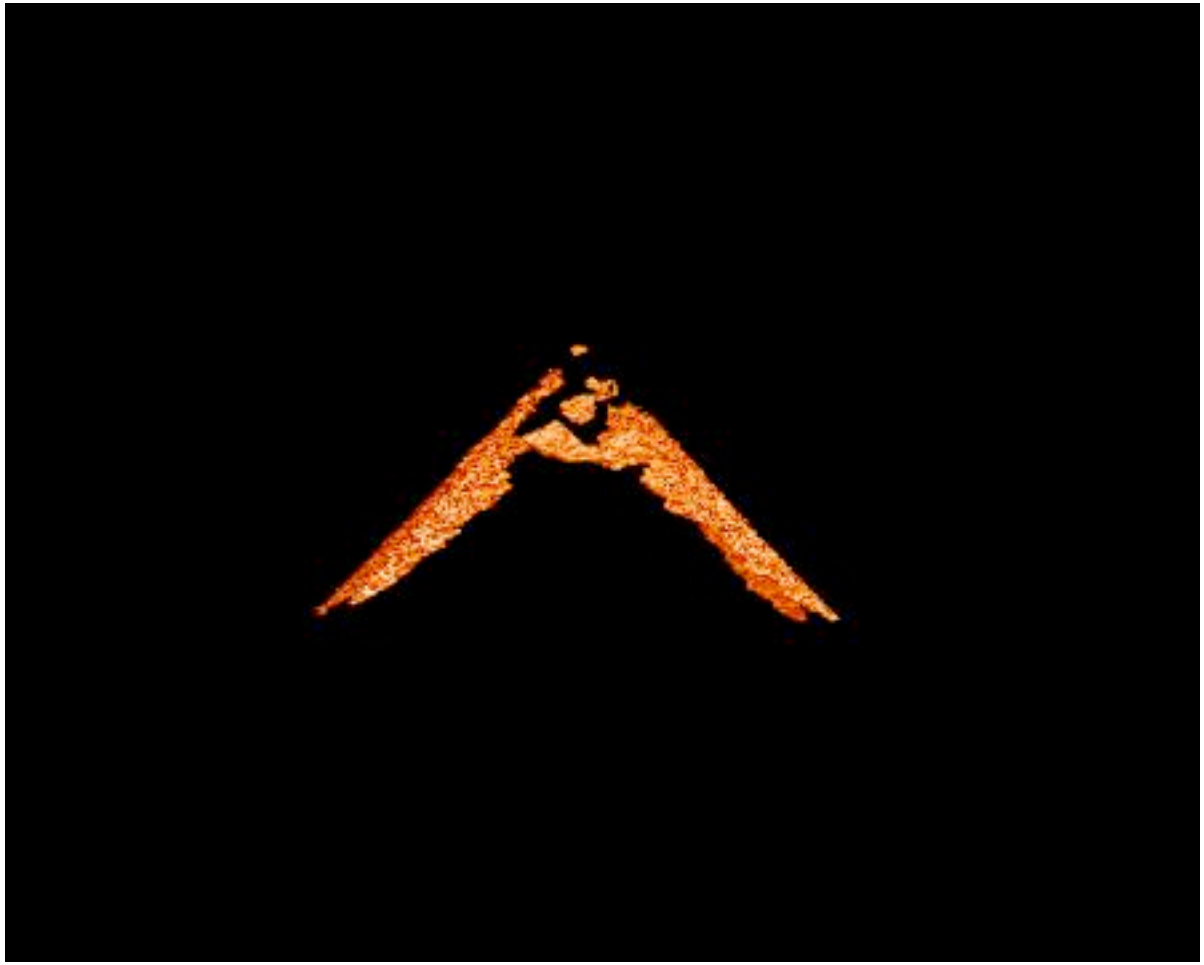
Previous work:Gerchberg, Saxton, Fienup, Elser, Bauschke, Millane, Barakat,Nugent, Miaow etc.

Lensless (diffractive) soft-X-ray tomography at 25nm resolution.

Object is 50nm gold balls decorating the sides of a SiN pyramid



SiN



The X-ray beam energy is 650 eV. (Oxygen edge is 580 eV)

Wavelength about 1.8nm

Undulator on synchrotron (ALS, LBL, Berkeley)

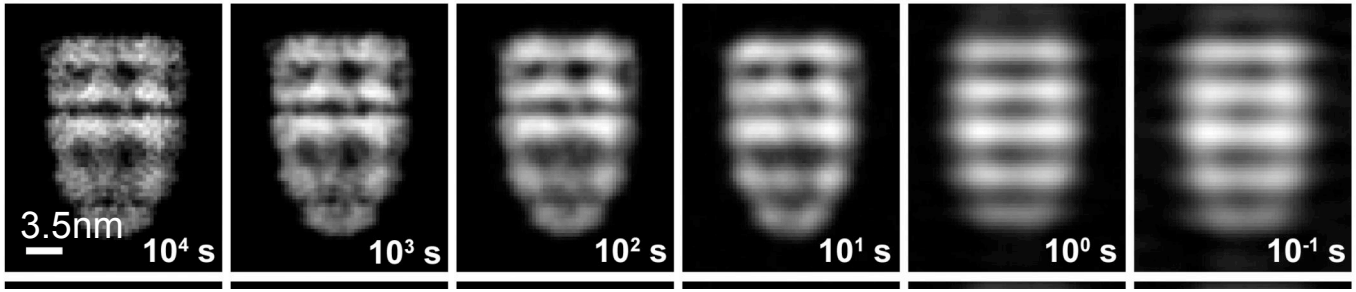
3D reconstruction of real-space density from phased 3D diffraction data.

Interations consist of several hundred 1024^3=8GB complex FFT. 5 arrays.

Takes 20 hrs for 3000 iterations on 16 node Mac G5 cluster at LLNL (Barty)

Chapman, Barty, Marchesini, Cui, Howells, Spence, Weierstall et al J. Opt Soc Am. 23, p 1179 (2006)

How does resolution d depend on exposure time for given incident flux and number of mols N in beam at any instant ?



Average of 200 2D HIO reconstructions of GroEL (60,000 atoms) for exposure times shown.

Incident flux 3×10^8 photons/(nm^2 s) (Cornell ERL) and X-ray energy 8 keV. **One GroEL protein (20 x 14.4 nm) in the X-ray beam at any instant**. Exposure time reduced N-fold for N mols in beam. Resolution depends on exposure (Rose criteria). $GroEL_{14}$–$GroES_7$–$(ADP{\cdot}AlF_x)_7$ complex. Doyle-Turner. Poisson noise in beam. 200 HIO random starts.

APS 10^6 ph/sec/nm^2 at 4.5kV (0.275nm) (0.1% bandwidth), L_c = 30nm.
ALS 2.5 x 10^3 ph/sec/nm^2 at 1.5 kV. (0.826nm).

Note: Our analytic result for spherical protein gives exposure scaling as d^{-4} and λ^{-2} consistent with simulations above (based on atom coords and X-ray scat factors).Bad news ! (Henke).

D. Starodub et al. J. Synch Res. In press. 07 compares this with earlier work by Shen, Howells et al.

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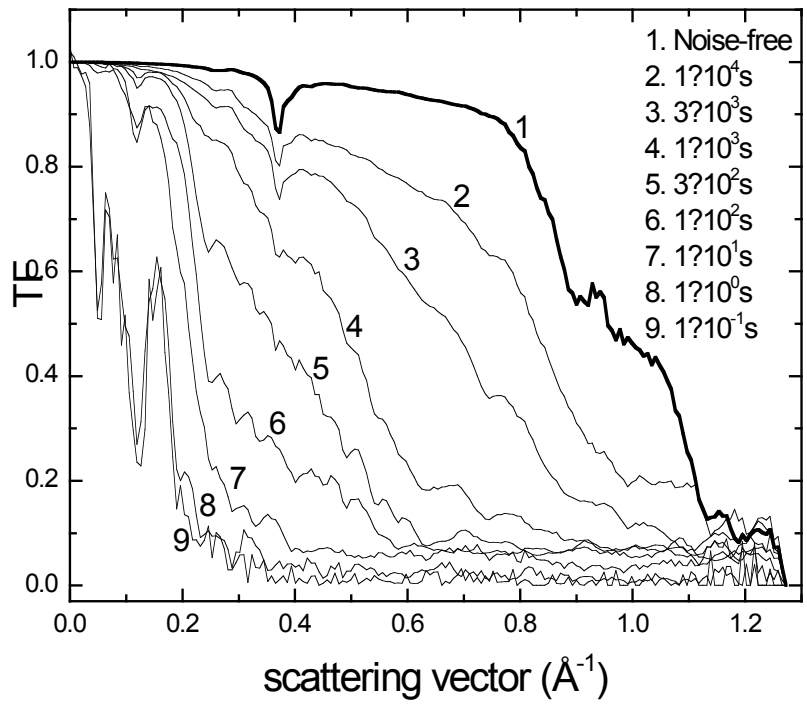
HIO Transfer function for different exposure times (Elser)

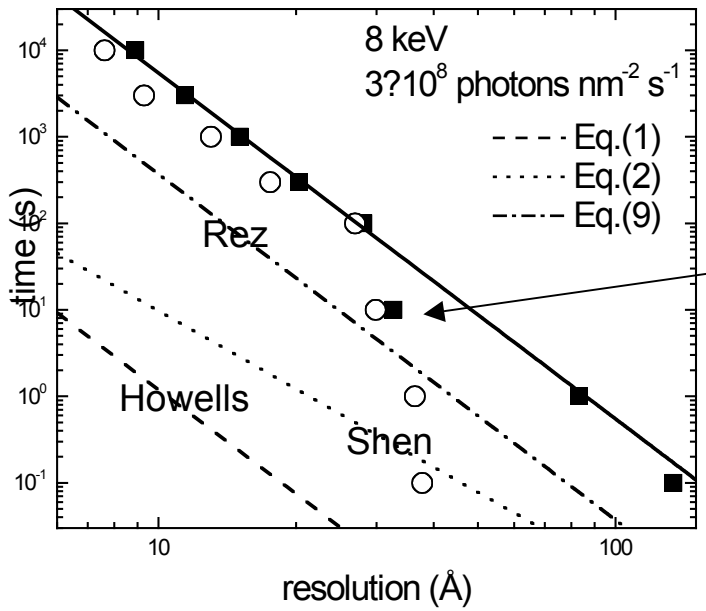


Fourier transforms of average of many GroEL density map reconstructions for different exposure times at the Cornell ERL.

Define resolution as spatial freq (scattering vector) for which TF = 0.5.

Scaling of exposure time T with resolution d and λ

T



Our full simulations with HiO inversion, noise using 50,000 GroEL atomic SF give k = 9.8E6

$$T = \frac{s^2 k}{N I_0 d^4 \lambda^2}$$ nm. sec

From full simulations an inversion of GroEL, define d = Transfer Fn = 0.5

s = oversampling ratio > sqrt(2)
N = Number of mols in beam at any instant
I_o = Incident X-ray flux
d = resolution
λ= Xray wavelength
k = 9.8E6 (nm, sec)

Starodub et al 07 J. Synch Res (Acta) 07

Exposure times* (Full HIO sim with noise. Scat Fac. for GroEL)

0. ERL (Cornell, planned) 8kV, Flux 3E8

Resolution	N	Exp time
2nm	1	30 seconds
0.7 (α-helix)	10	204 sec.

1. Current APS capability, 4.5 kV Undulator, Flux 7.7E5 ph/sec/nm^2.(0.1% BW)

Resolution	Number of mols. N	Exp Time.
0.7 nm	1	2.5E5 sec
2nm	1	3.8E3
1nm	10	6.1E3 =1.6 hrs

2. Summer 07 ALS upgrade. 1.5 kV, Flux 1E4 ph/sec/nm^2.

Resolution	Number of mols.	Exp time.
2 nm	100	324 sec
1.5 nm	5	2E4

Divide this exposure time by 1000 if ALS 9.0.1 optimized for our experiment.

*Scaling: $T = s^2 k / (N^* I_o \; d^4 \lambda^2)$ with k = 9.8E6 (nm, sec) . Oversampling ratio s -sqrt (2) above. For constant T, d goes as $N^{-1/4}$. Analytic result (Rez, Howells) for S/N=5 gives 100 times shorter

Factors affecting exposure time T

- Synchrotron ID. ALS 2.5E3-2.5E6 ph/nm^2/s, APS 10^6(now), Cornell ERL 10^8 (future).
- N, number of mols in beam at any instant.
- S, oversampling factor. $T \sim S^2$
- More constraints in algorithm allow smaller S>sqrt(2) (Histogram, Support..)
- More efficient phasing algorithms (Elser, Shrinkwrap..)
- Optimizing spatial Lc and chromatic ΔE coherence (now too much !).

Need about the same conditions as for PX, where Lc ~ unit cell size D.

$Lc \sim \lambda/\Theta_c \sim 2D$, $E/\Delta E \sim n$ for n linear number of pixels.

**Hegerl-Hoppe Dose fractionation - 3D exposure time is same as a 2D section*

Fundamental problem

$$T = s^2 \ k \ / \ (N \ I_o \ d^4 \lambda^2)$$

Exposure time depends on steep power of poorly defined quantity d (resolution).

*Resolution cannot be defined for phase-contrast because it is a property of the sample, not the instrument.

*Eg, scattering at angle theta depends on size of molecule. Ability to distinguish adjacent points in image depends on the phase shift they introduce. (Use CTF, Elser, or cross-correlate with known)

*s=oversampling ratio, k constant, I_o = flux, N mols.

Solution : Do experiments !

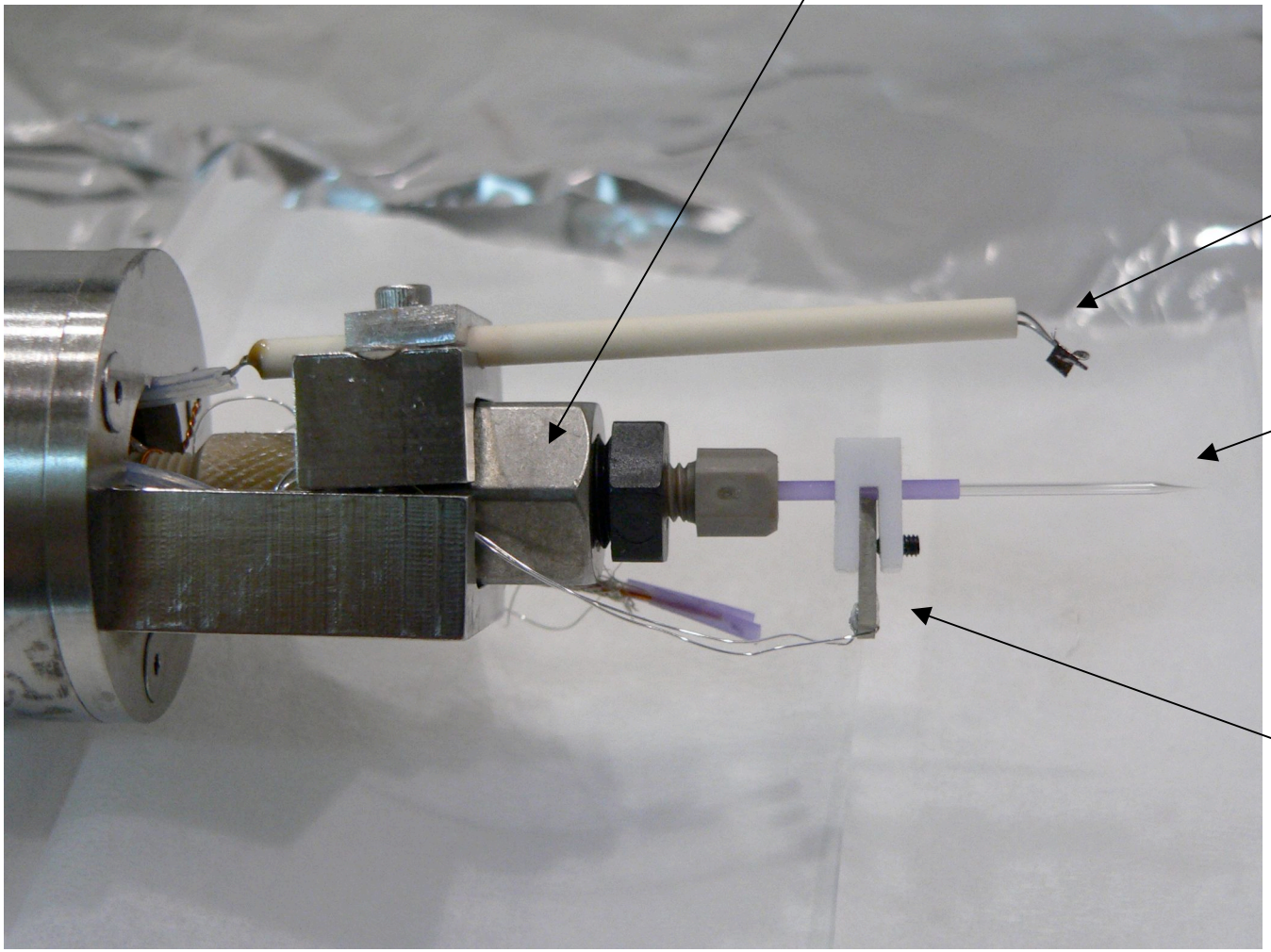
Droplet sources and results

Over the past 2 years we have evaluated 3 main types..

Rayleigh, piezo triggered (as used for liquid helium and some ink-jets.

Electro-spray, as used for hydrated protein-beam TOF spectroscopy.

Aerojet. This we have built for the ALS for trials in summer 2007.



Rayleigh (or Electrospray) droplet source for XRD operation in vacuum

All HPLC (Chromatography) peek tubing components.

Starodub/Weierstall et al

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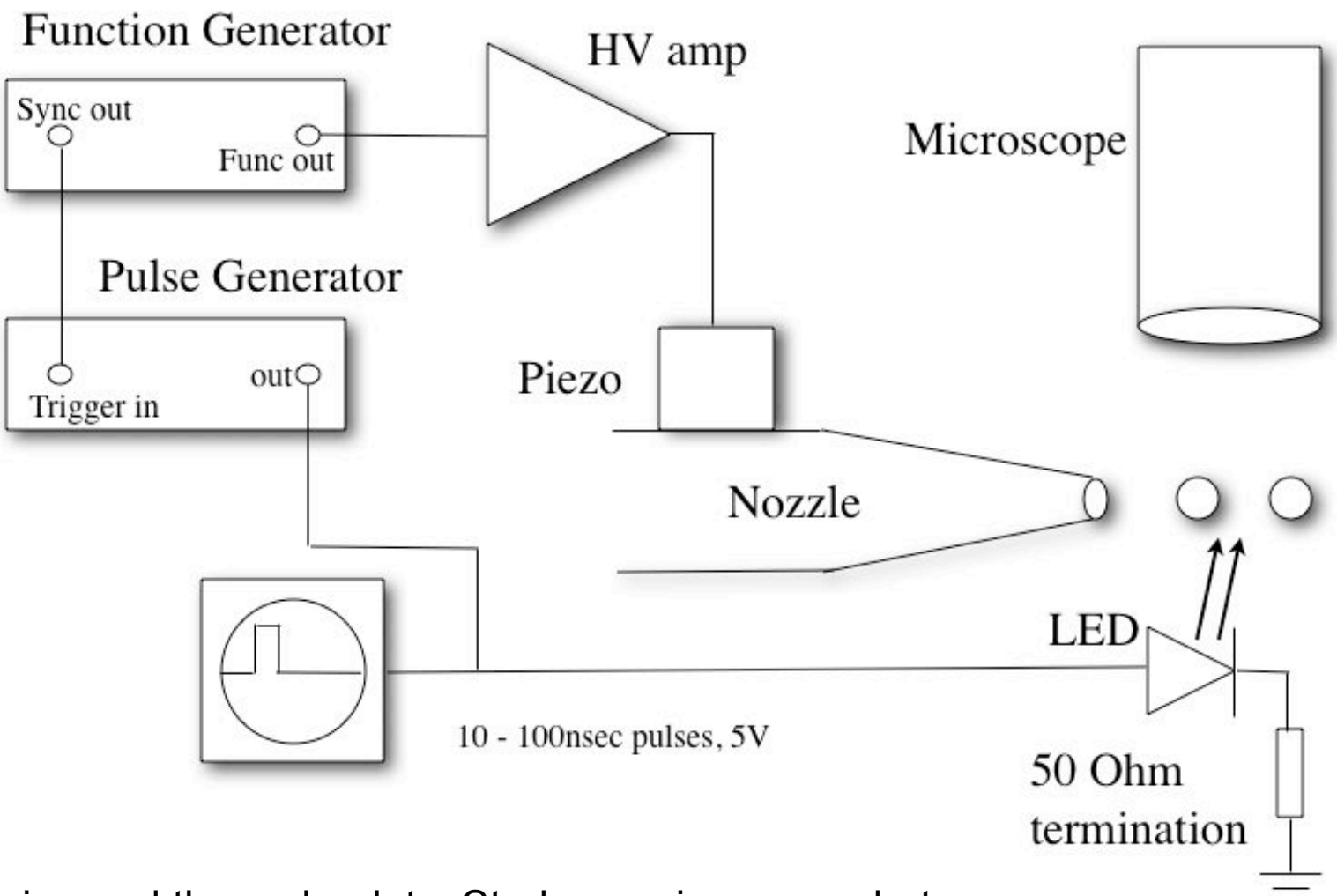
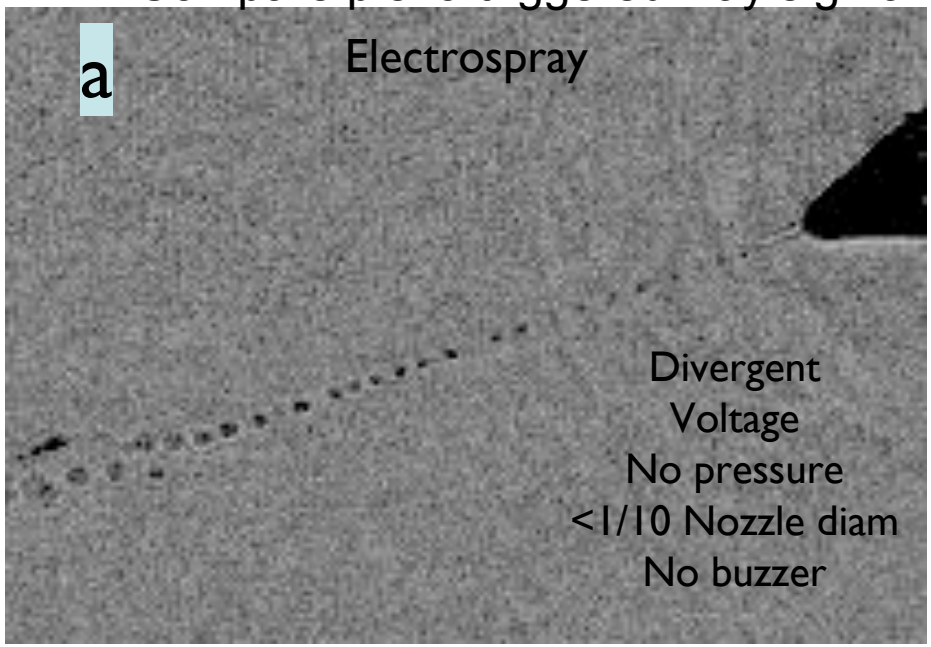
Imaging Droplets

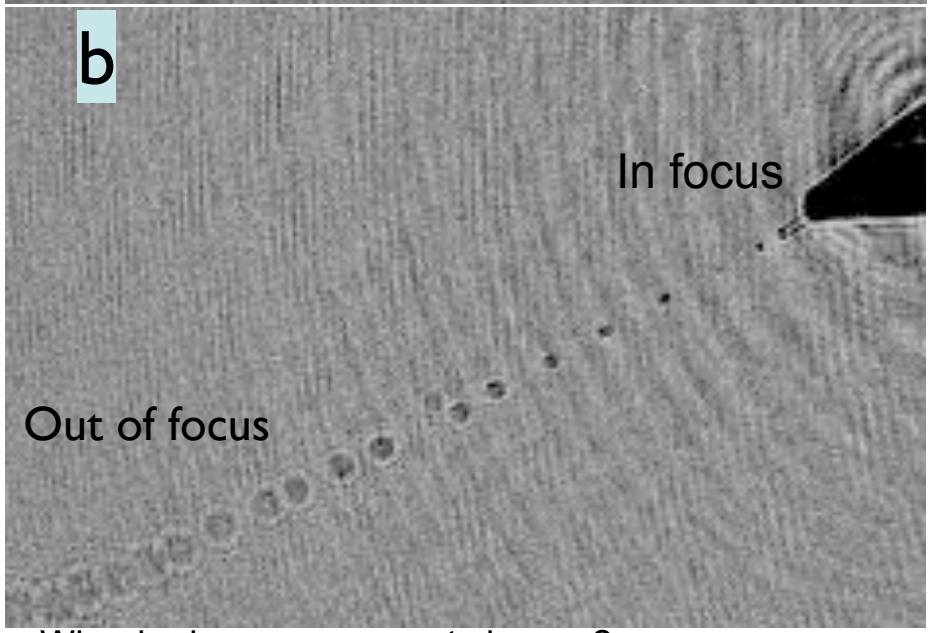


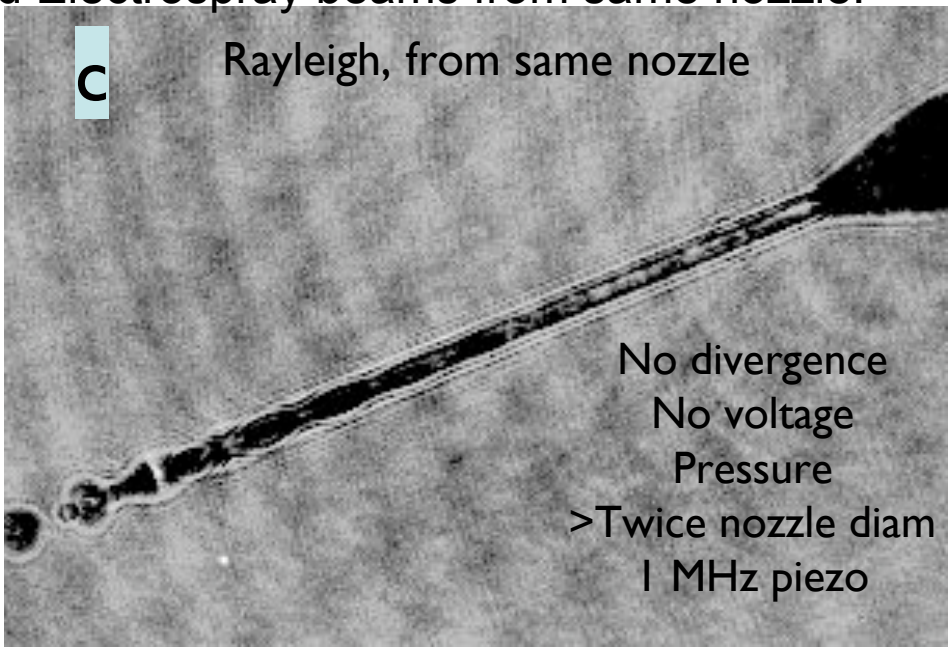
How we imaged these droplets. Stroboscopic or one-shot.

U. Weierstall.

Compare piezo-triggered Rayleigh and Electrospray beams from same nozzle.







(a) and (b): Electrospray of water from 8 micron nozzle at 2.6kV (Cone jet mode). Droplets are in focus at the nozzle and out of focus further downstream.(Gilbert 1600, Zeleny 1914, Taylor 1964)

(c) Triggered Rayleigh Droplet Beam from 8 micron nozzle at 100psi and 600kHz trigger frequency. (no voltage applied). Diameter of jet: 8 micron, Droplet diameter ~16micron.

(Single shot imaging with 100nsec exposure time)

Electrospray possible if nozzle clogged for Rayleigh.

U.Weierstall et al 07 in press

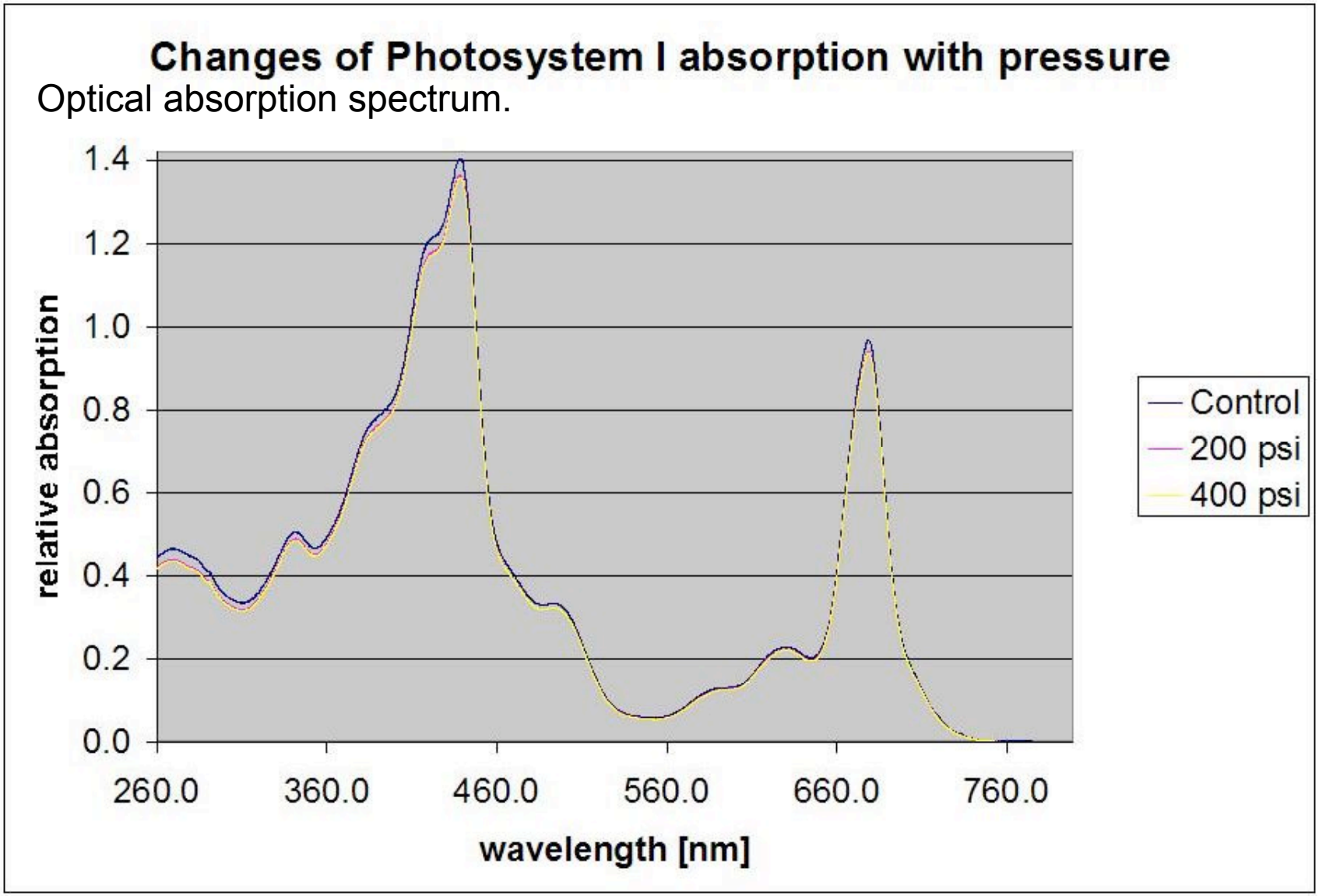
Why do drops carry a net charge ?

Why does electrospray diverge ? Rayleigh radius. Freeze before explode ?

Q

Does droplet beam damage proteins ?

Photosystem 1 before and after…..

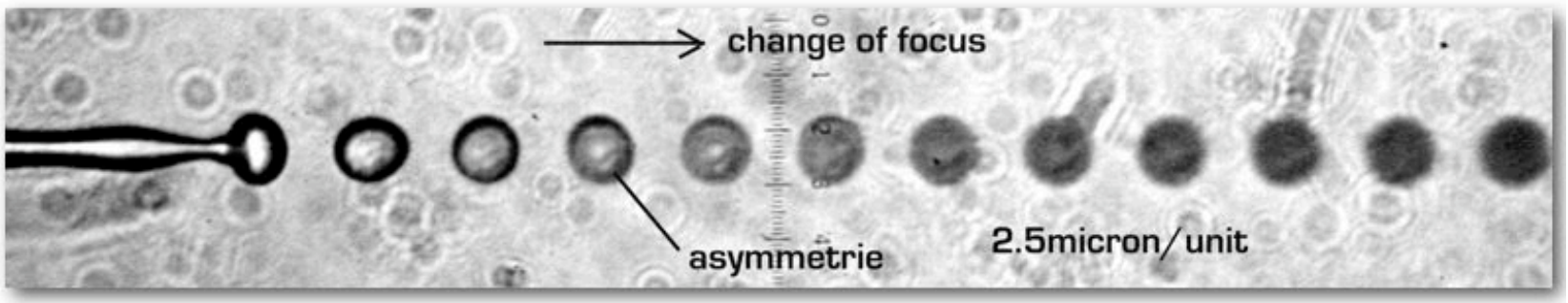


Ex Thermosynechococcus Elongatus (cf cyanobacteria). P. Fromme, D, Starodub et al 05

Q

Water droplets doped with e-coli bacteria

E-coli can serve as a test object for measuring alignment in a strong laser field. Its dimensions (~2 x 0.5micron) make alignment possible at room temperature (large shape factor).



Stroboscopic image of doped droplets shows an asymetrie not observed for empty droplets. Maybe an average over many e-coli?

The presence of e-coli in the droplets was confirmed by examining the water collected on a slide. Similar test have also been done with 250nm Au balls.

E-coli pass a 5micron filter without clogging!

Alignment measurements with a linear polarized 1 Watt IR laser are under way at LBL. Look for asymetrie in laser optical diffraction pattern from visible laser.

Note: Lindinger '99 did spectroscopy on amino acids in helium droplet beam.

U. Weierstall , D. Shapiro et al

Electrospray in air and vacuum

Air

Vacuum

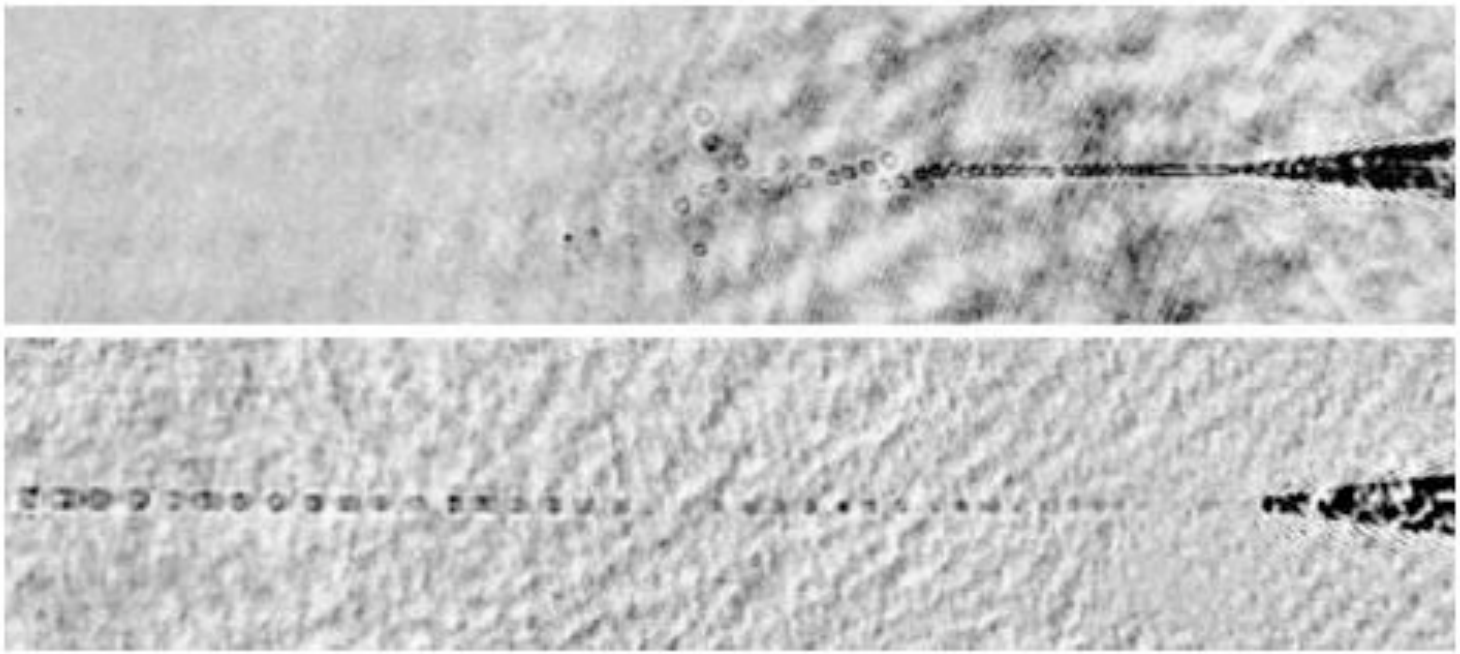
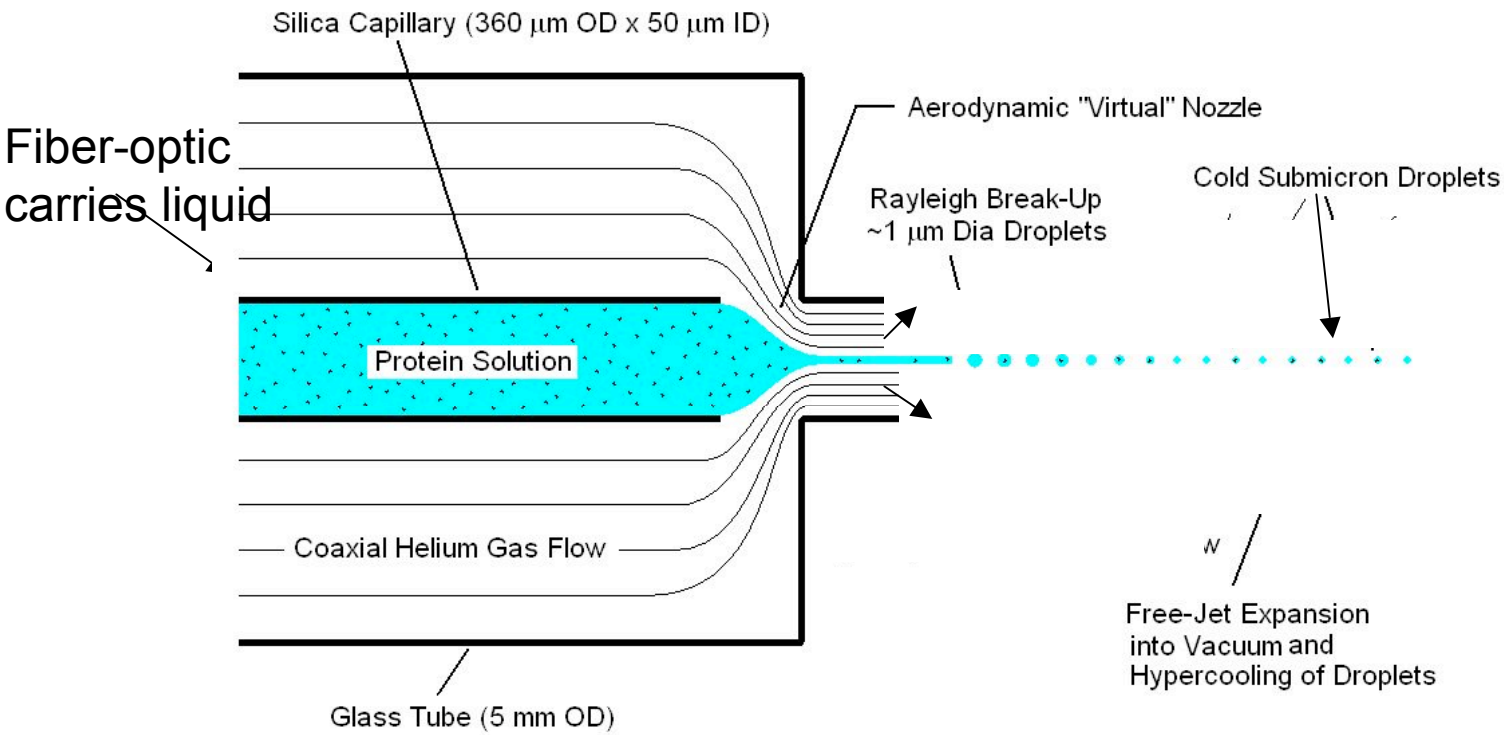


Figure 16:
Top: electrospray of water into stagnant air. Dispersion of the jet is due to slowdown caused by air drag and coulomb forces between drops. Drops in the dispersion region are much slower than at the nozzle. This can be visualized by using a long time exposure time (~700 ns). Then the droplets close to the nozzle were washed out to an apparent jet, but the droplets in the breakup region could still be resolved. Nozzle diameter 1 μm, 2 kV, 70 psi. Single shot exposure 100 ns with laser diode.
Bottom: ES in vacuum, $p = 10^{-5}$ Torr, same nozzle, 2 kV, 70 psi. To start the electrospray, the nozzle was heated in vacuum with a tungsten filament close to the nozzle (to thaw the ice generated by evaporative cooling). The HV has to be turned off during pumpdown to prevent discharges in the low vacuum corona discharge region. The droplets stay unidirectional at least up to the extraction electrode (about 2 mm). The laser diode light (50 mW) was focused into the vacuum chamber just below the droplet stream by a lens.

A line of like charges is unstable with respect to lateral instability. Coulomb explosion ?.

Virtual Gas Dynamic Nozzle (Aerojet)



Rotn temp of Iodobenzene 20 K

Laminar flow forms effective "Taylor cone".

Large nozzle (doesn't clog) but small droplets (submicron).

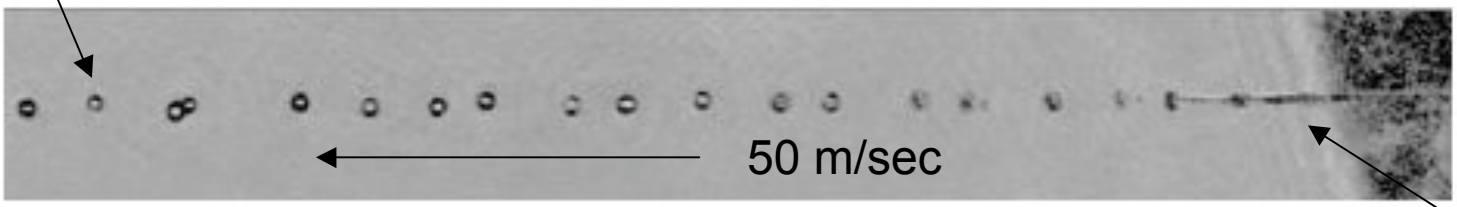
Size measurement - 1. XRD. 2. Concentration of gold balls, SEM imaging. 3 AFM

Previous work on larger droplets: Gannan-Calvo et al.

B. Doak, D. DePonte et al.

Triggered Aerojet Source - on demand ?

Ten-micron droplets



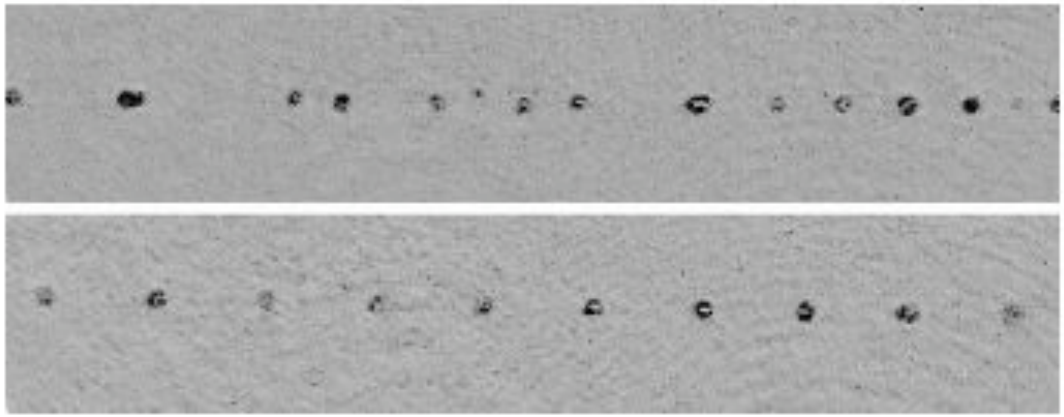
Rayleigh jet liquid column is half diam of droplets.

Figure 21: Fast exposure (100 ns) of water droplet jet ejected from aerojet focused source. The source is on the right. The dimensions of the apertures are smaller than in Figure 20: The capillary supplying the water has an ID of 50 μm. The diameter of the jet before breakup is about 5 μm, i.e. a reduction by a factor of 10. The gasflow aperture has a diameter of 100 μm. The droplet diameter is about 10 μm. Water pressure 20 psi, gas pressure 6 psi (measured at the gas supply bottles, the water pressure at the nozzle is reduced due to the use of a 0.5μm filter in the water line). By reducing the water pressure, i.e. the flow rate, the droplet diameter could be reduced to about 5 μm.

Now trigger Aerojet with piezo….

Untriggered

Triggered

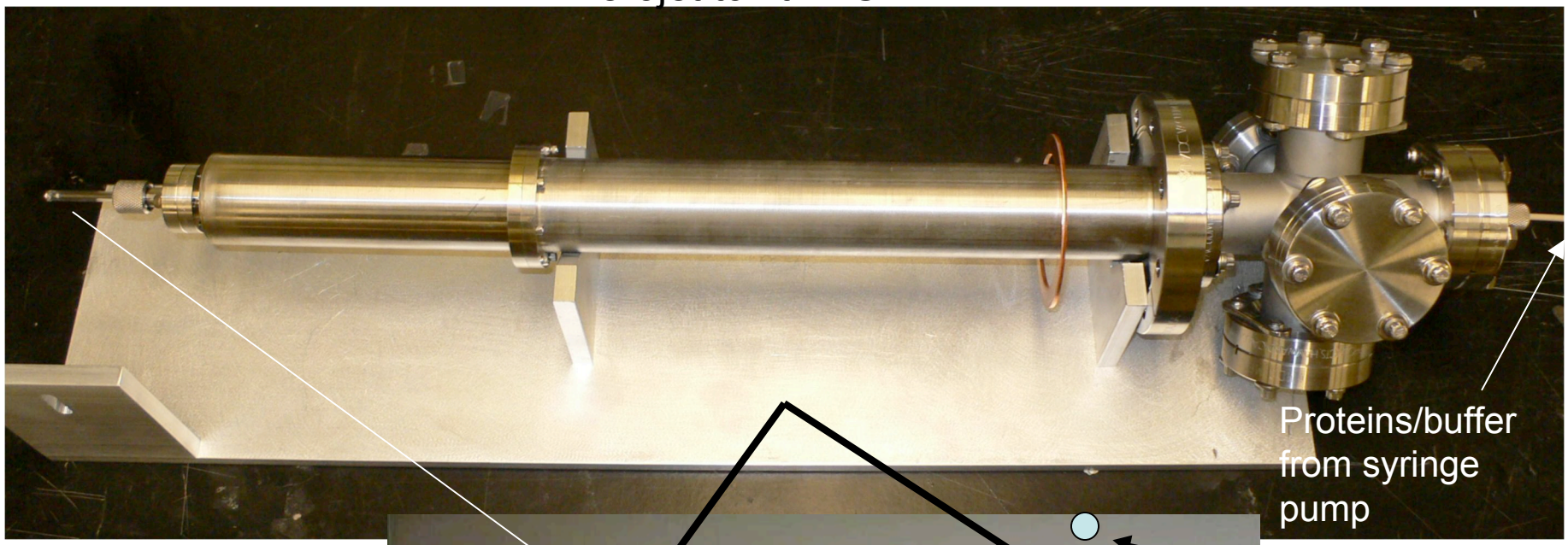


Our latest aerojet gives sub-micron droplets, not visible optically (2007).

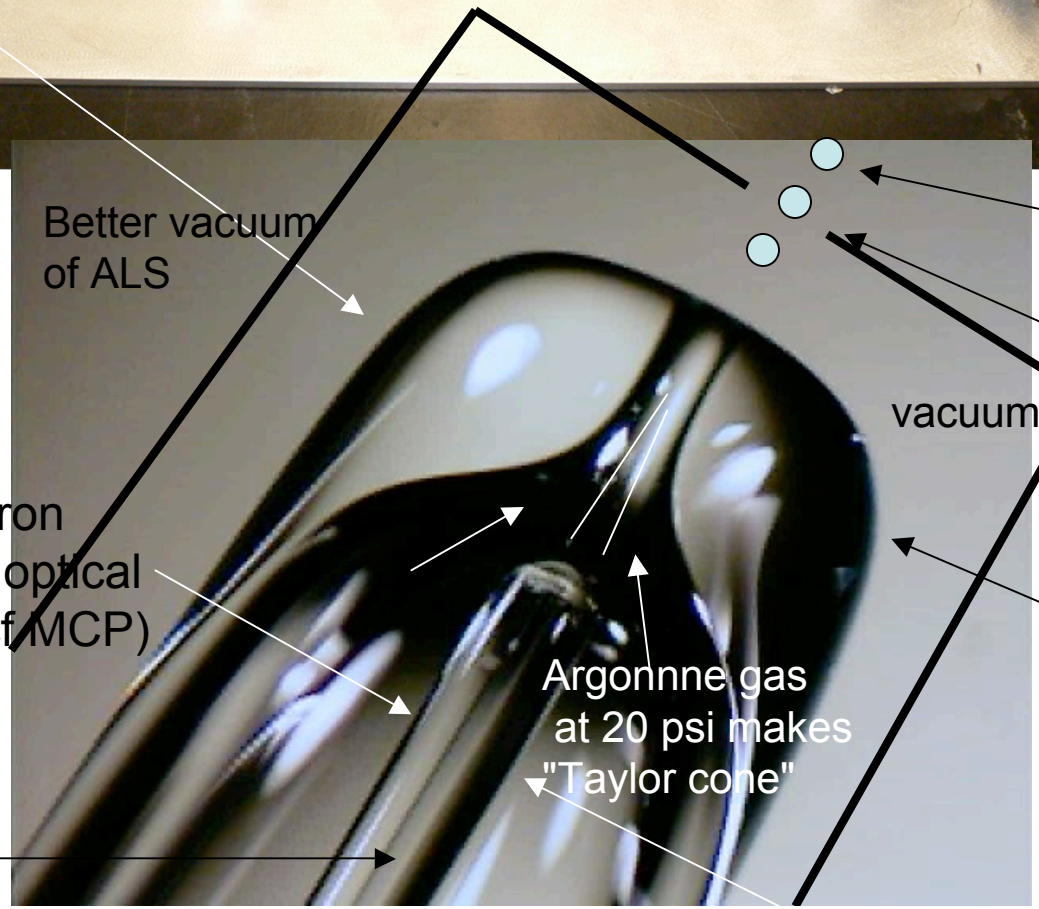
Figure 22: Untriggered (top) versus triggered (bottom) droplet breakup from an aerojet focused source. The source is on the right. Exposure time: 200 ns, water pressure: 25 psi, gas pressure: 5 psi, piezo trigger frequency: 405 kHz, droplet diameter 10 μm. Source dimensions are the same as in Figure 21.

Weierstall et al 2007 in press

Aerojet to fit ALS.



Liquid forms Rayleigh jet which is tapered by gas (Taylor cone), then breaks up by necking instability.



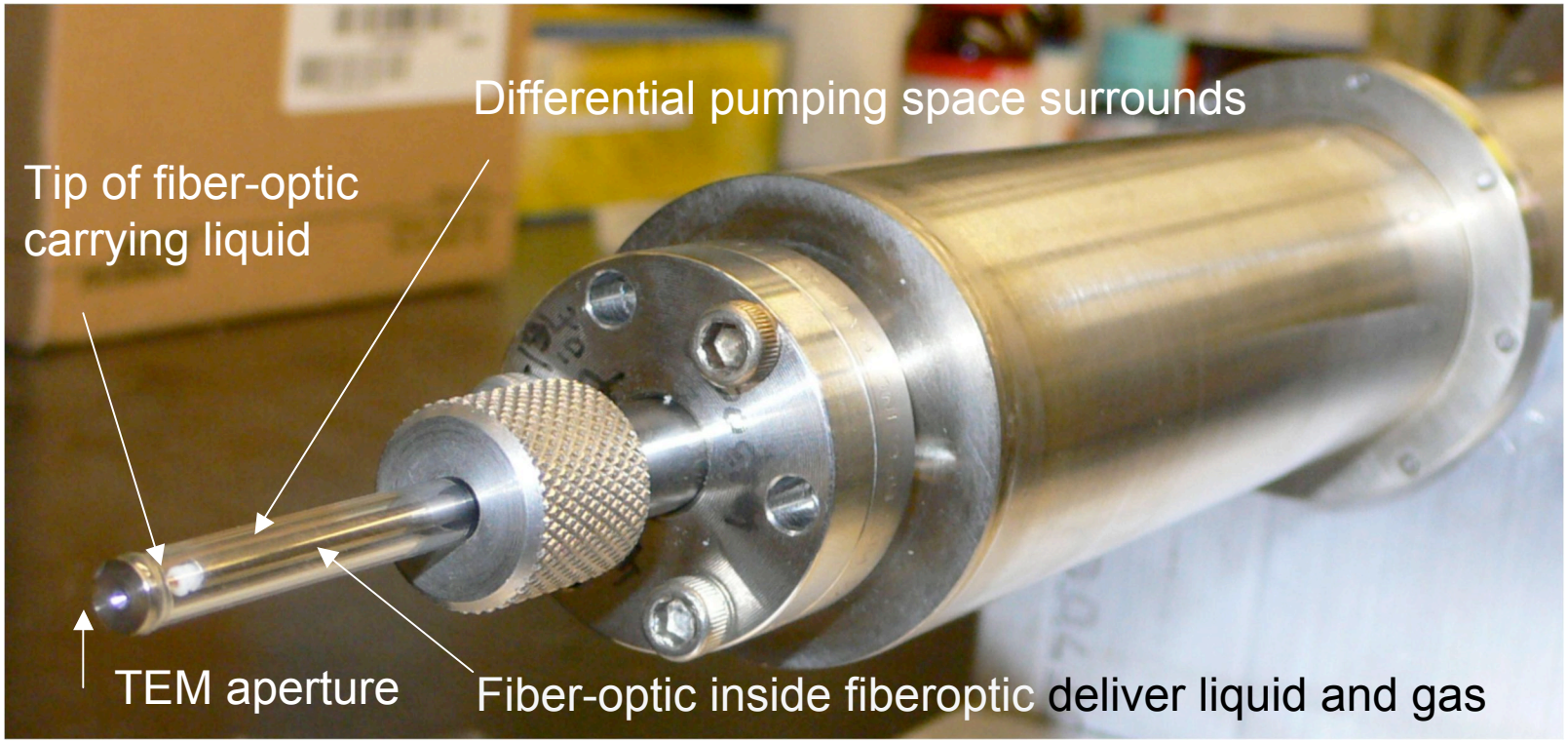
One micron droplets

Diff pumping Aperture,200mic

Capillary or f/o closed down as for NSOM, single-cell, gives laminar flow.

Dan Deponte

Aerojet to fit ALS - first trial summer 2007



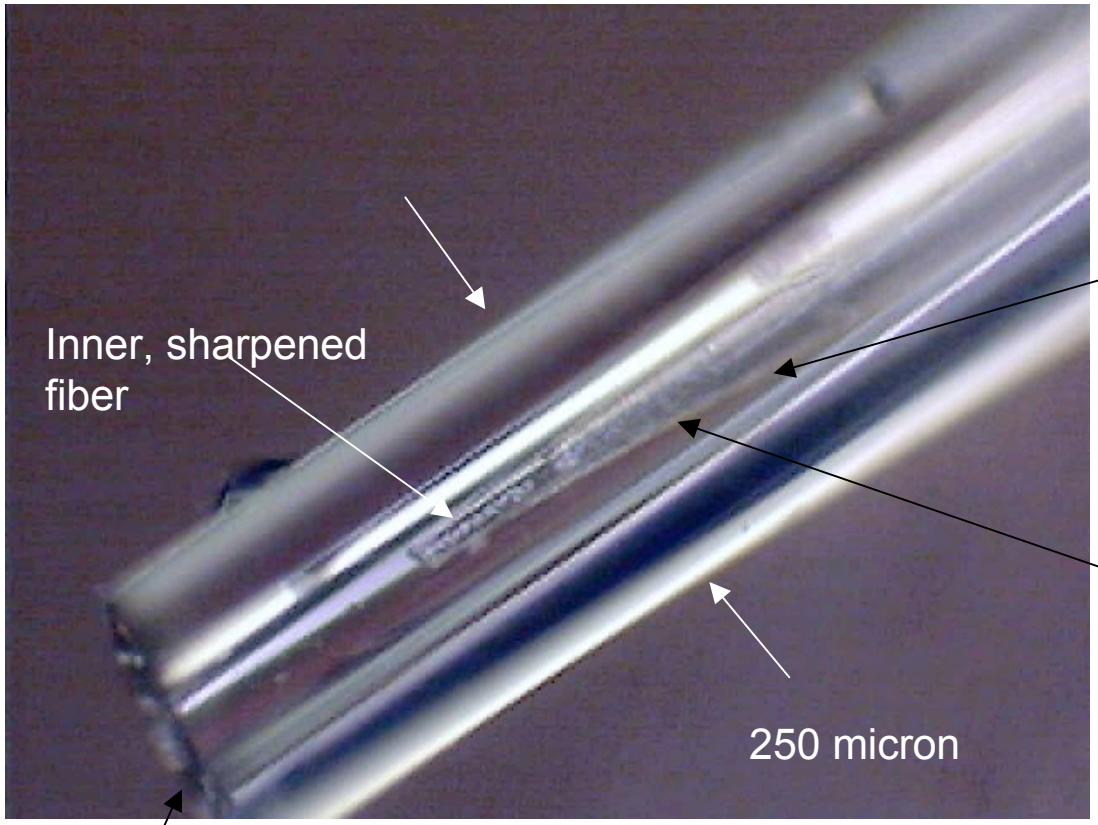
Summary of work on droplet sources.

1. Rayleigh does least damage to proteins (uncharged, pH OK), but needs much water shedding, clogs if < 8 micron with 0.5 micron filter. Onsager.

2. Electrospray may unfold some proteins (charge), no clogging, smallest droplets (35nm,TSI), a large Taylor cone would freeze in vacuum, coulomb explosion destroys collimation unless Po source used (TSI - density too low). Explosions usefully shed water.

3. Best is Aerojet. Lowest temp., no clogging, no Coulomb explosion. Now submicron ('07).

Fiber-in-fiber Aerojet



5 micron gap provides adequate alignment (because gas flow self-aligns liquid) and space to gas to get past.

Inner 90 micron OD hollow fiber brings liquid.

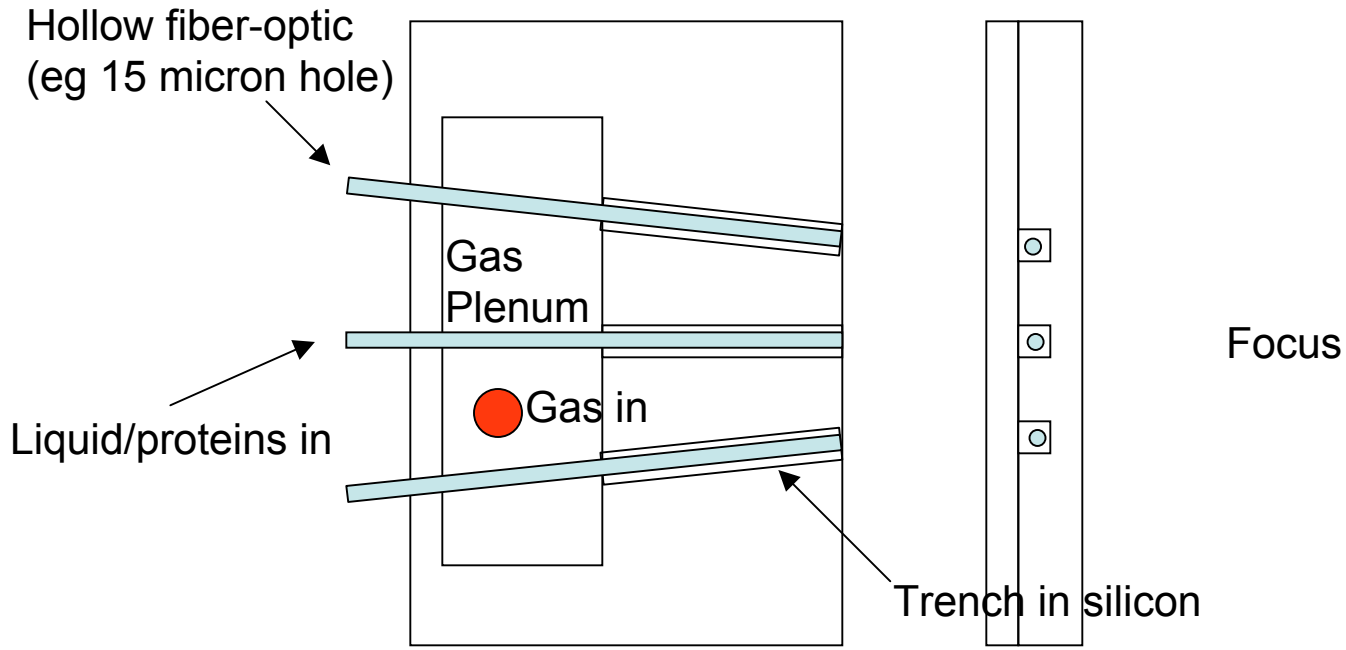
Sharpened with HF etch.

Heat glass to close off, and form smooth gas nozzle.

These can be bundled together to form a shower-head array.

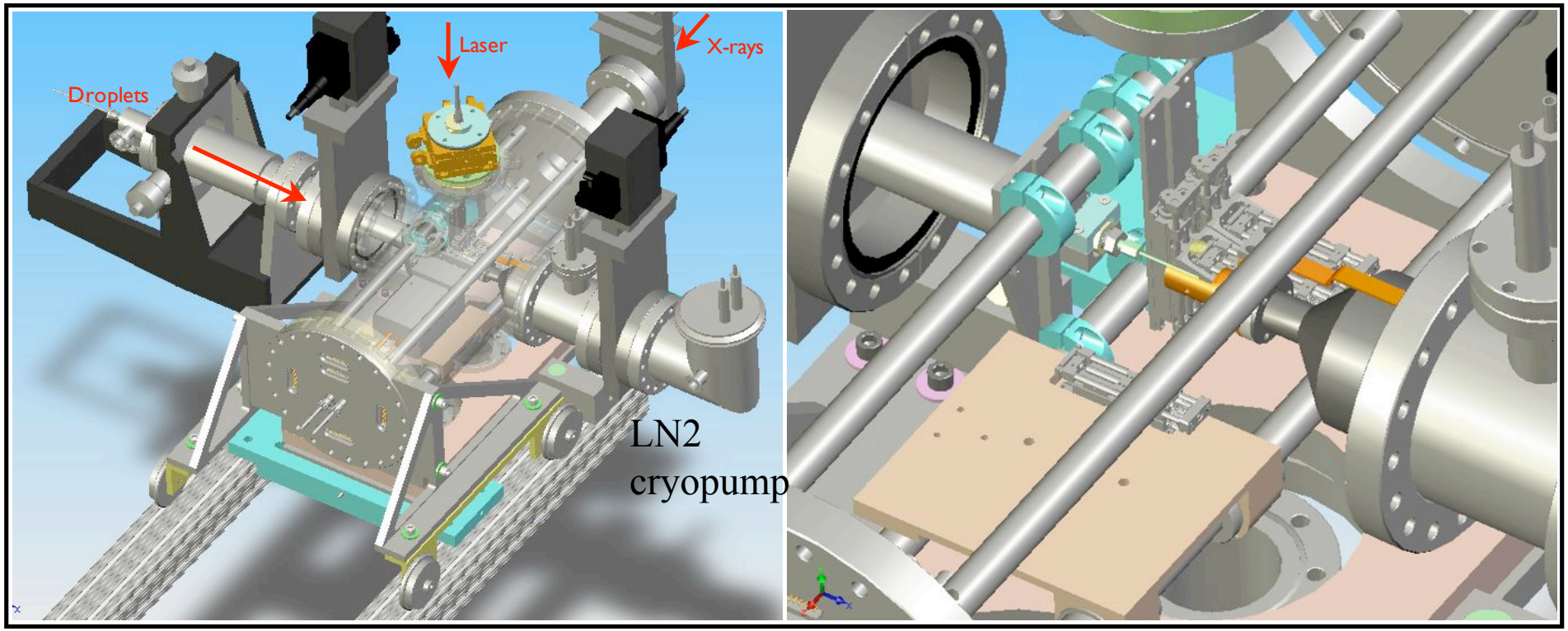
Dan Deponte, B Doak

Nozzle arrays
(Need depends on synchrotron)



Droplet X-ray Diffraction Camera. ALS June 2006

- Retractable nozzle and cold trap
- Adjustable sample/detector distance (3.5-5.5 inches)
- Computer controlled optics/laser positioning
- Maximum spatial frequency corresponds to 2 nm pixel size (after upgrade to 1.5 keV)
- Fully enclosed laser system

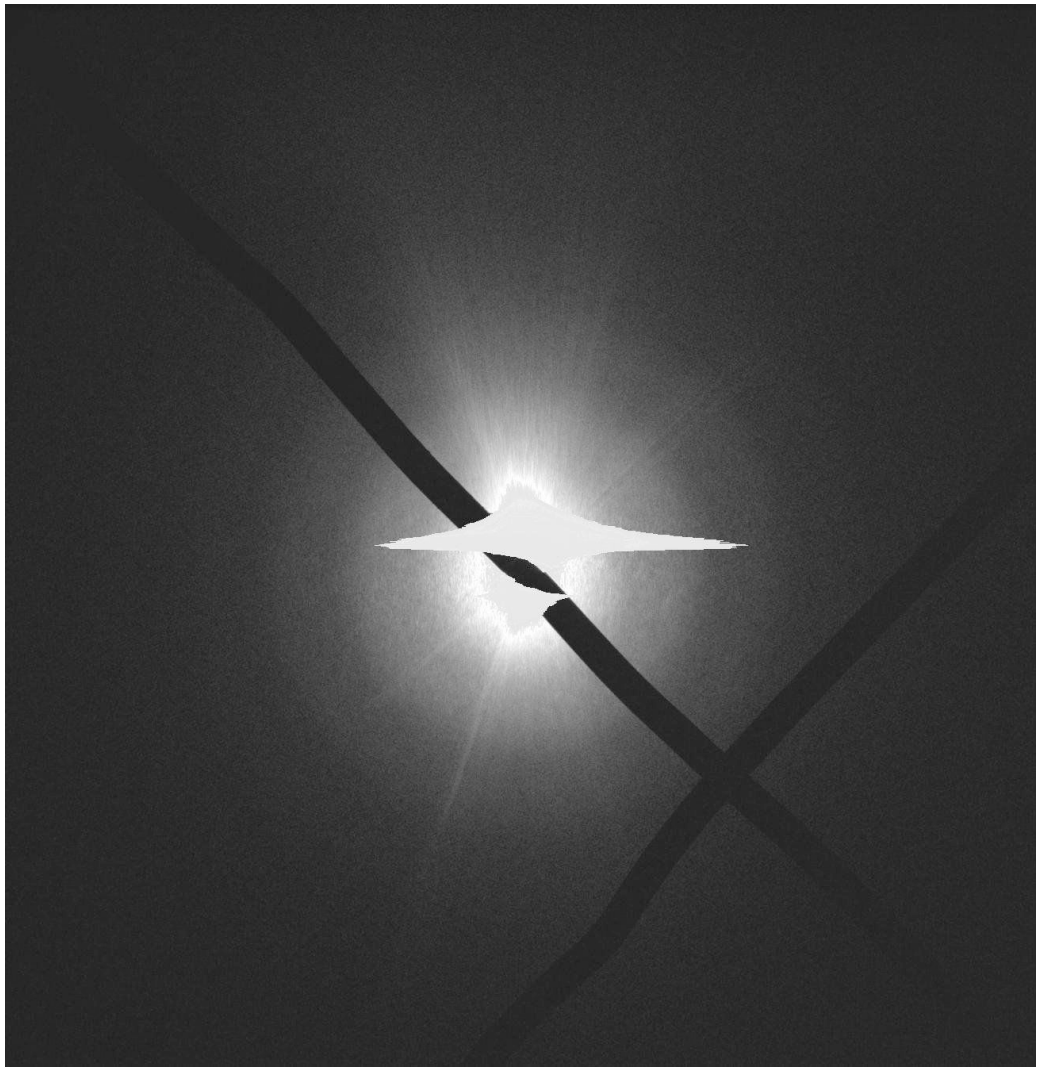


First experiments with soft X-rays at 2 nm wavelength at LBL Berkeley

D. Shapiro et al

First results - 50nm gold balls in 8 micron HPLC water droplets

Airey's disc
soft X-ray
pattern from
100nm
gold balls in water
droplet beam,
(possibly within
Ice, producing
radial streaks.
Some detector
saturation. Beam stops.
15 sec exposure.
2nm wavelength.



Summary

- 1. Rayleigh/Aerojet droplet beams don't damage proteins.
- 2. 3D diffractive imaging achieved (pyramid of gold balls).
- 3. Laser-alignment achieved with small organic mols
- 4. Proteins, virus aligned for SAX by E, M fields.
- 5. Electron diffraction achieved from ice-ball beam. (Bartell, 91)
- 6. Rayleigh, ES ,Aerojet imaged in vacuum for first time.(Single shot and strobe.)
- 7. X-ray, e^- chambers built, tested, (Photosystem 1, **Au balls**, No laser).
- 8. HiO, Shrinkwrap tested for aligned SAX, flipping for powder XRD (Wu).
- 9. New Aerojet sources designed, tested which don't clog, makes smallest ever uncharged, submicron droplets
- Water-shedding before freezing without lose of collimation (droplet beam brightness) appears to be happening with fiber-in-fiber design, diff pump.
- Exposure times are reasonable based on FULL simulations with noise

Do molecular replacement from **nanoxtals** in droplets ?. $I_g = (3X3X3)^2 = 729$ if angle resolvd !!

The End

Publications

1. JCHS and B. Doak, PRL. 92, 198102 (2004)
2. JCHS et al Acta Cryst A 61 237 (2005)
3. J. Wu et al. Acta. Nature Materials 06. Charge-flipping for Powder XRD.
3. Starodub et al J. Chem Phys. 123, 244304 (2005). Laser alignment
4. Starodub et al J. Synch. Res. (2007) Exposure times.
5. Weierstall et al. J. Fluids. (2007) Submitted. Droplet sources.

People.

B.Doak, U.Weierstall. P Fromme, K.Schmidt, D.Starodub, D.DePonte,G.Hembree,
J. Spence (ASU),
M.Howells (LBL), D. Shapiro (UCD), A, Barty, H. Chapman (LLNL)

Money.

NSF SGER, **NSF IDBR > Nov 06.** NSF **CBST.**

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