Serial crystallography – recent results, challenges and prospects

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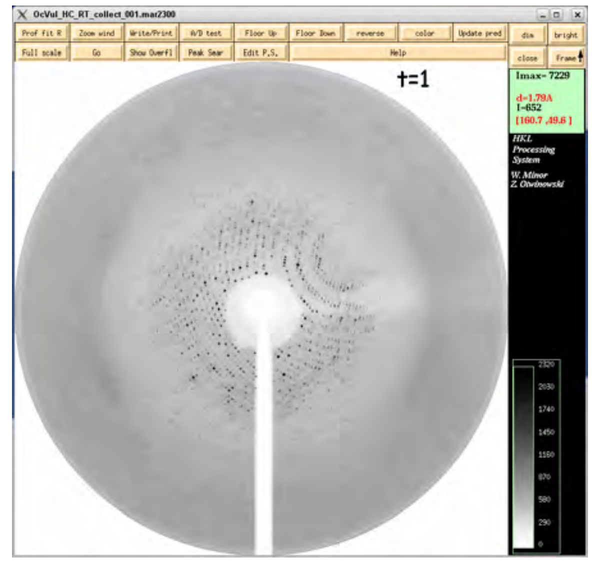
Serial crystallography

Serial collection of partial datasets of different crystal(position)s

- mitigate the effects of **radiation damage**

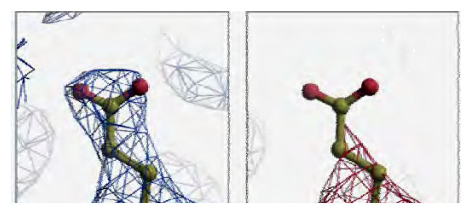


Global damage

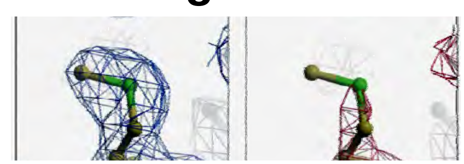
- Loss of resolution
- Increase of mosaicity
- Change in unit cell constants

Local damage

- Decarboxylations, S-S bond breakage
- Photo-reduction of redox systems, e.g. metal centers, flavins,…



Local damage to structure



From: Garman and Owen (2005), *Acta Cryst.* D**62**, 32-47

Serial crystallography

Serial collection of partial datasets of different crystal(position)s

- mitigate the effects of **radiation damage**
- **time-resolved** pump probe measurements

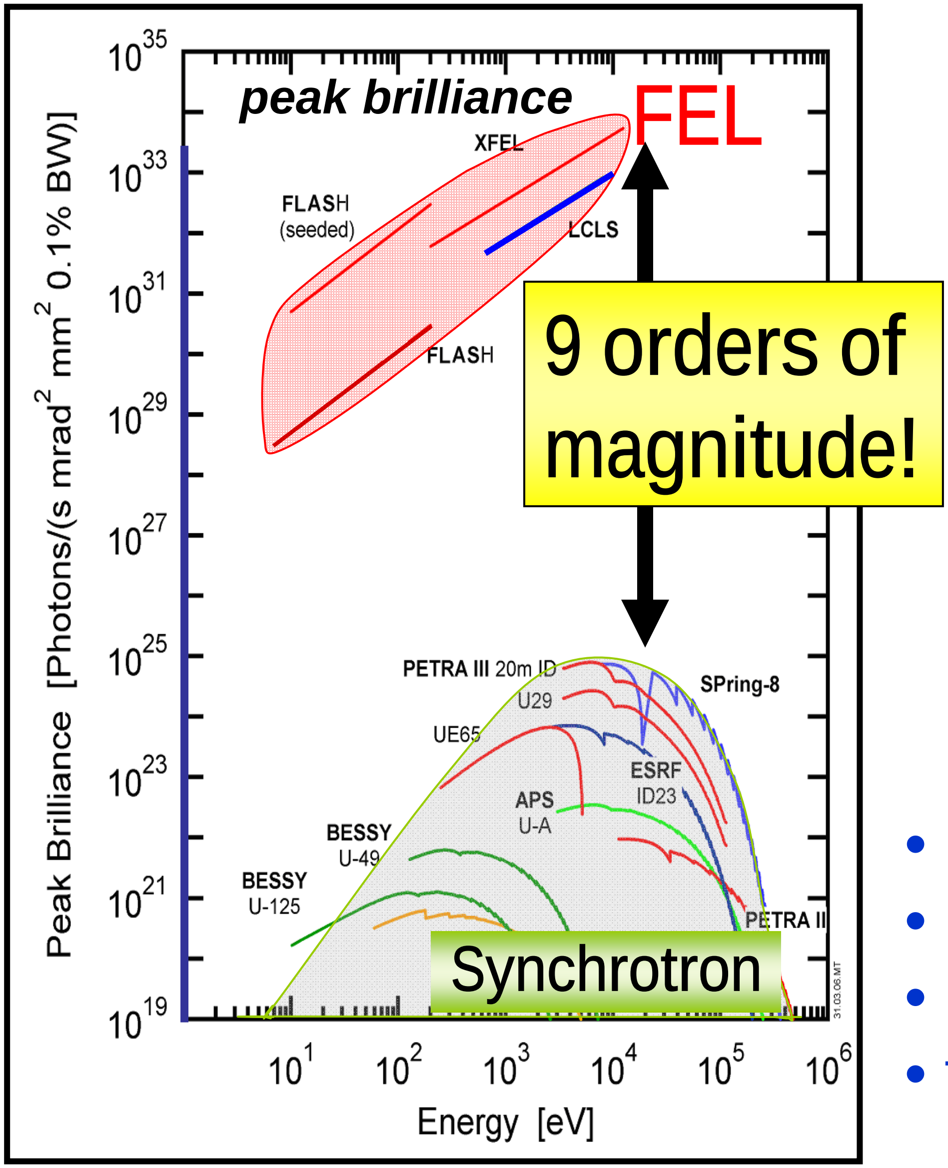
has been performed for a very long time …

- New light sources require much faster sample change due to short lifetime of sample

XFELs **10^{12} photons/pulse, few fs exposures**

- Time-resolved measurements on irreversible reactions

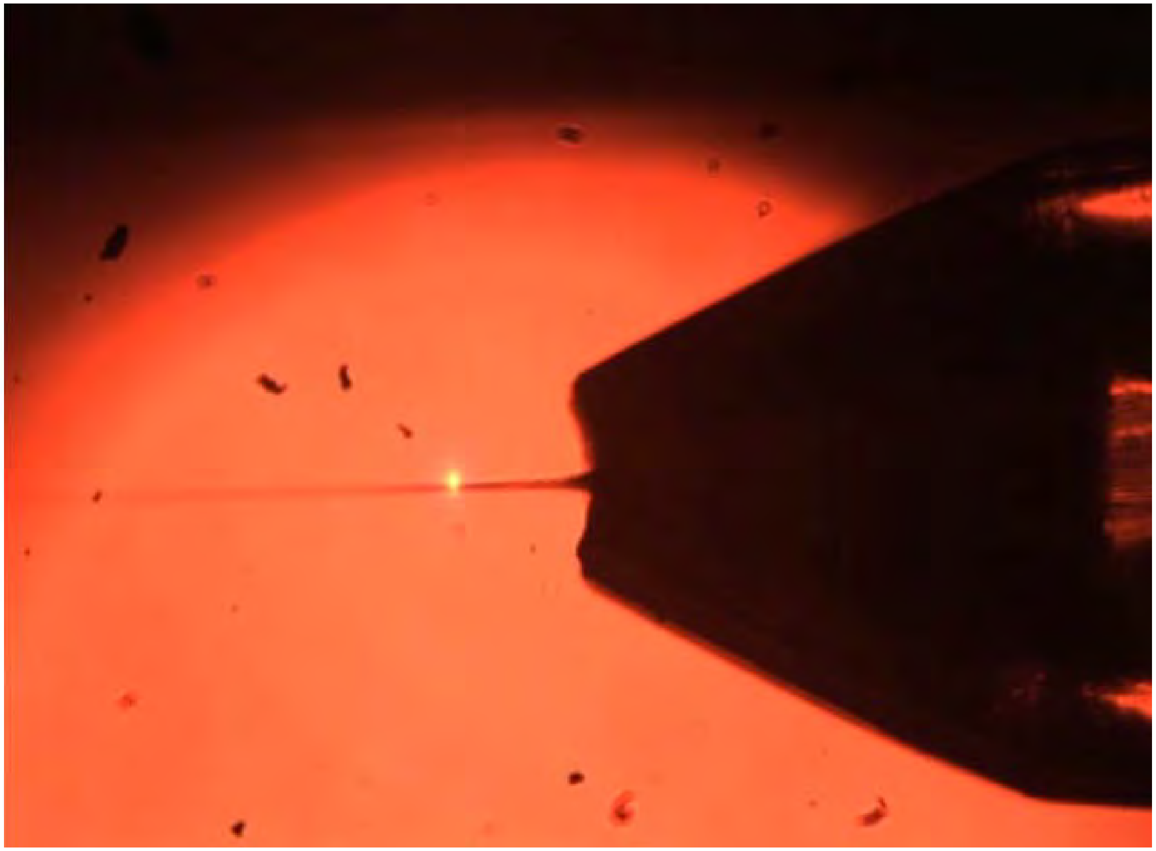
X-ray free-electron lasers



- FLASH: 2005
- Fermi: 2009
- LCLS: 2009
- SACLA: 2011
- Fermi 2011
- XFEL: 2017
- PSI, LCLSII, KVI, Shanghai,…

- $10^{12\text{-}13}$ photons ~ 3-500 fs pulses
- repetition rate: 120 Hz
- photon energy: 300 eV-10 keV
- transversally fully coherent

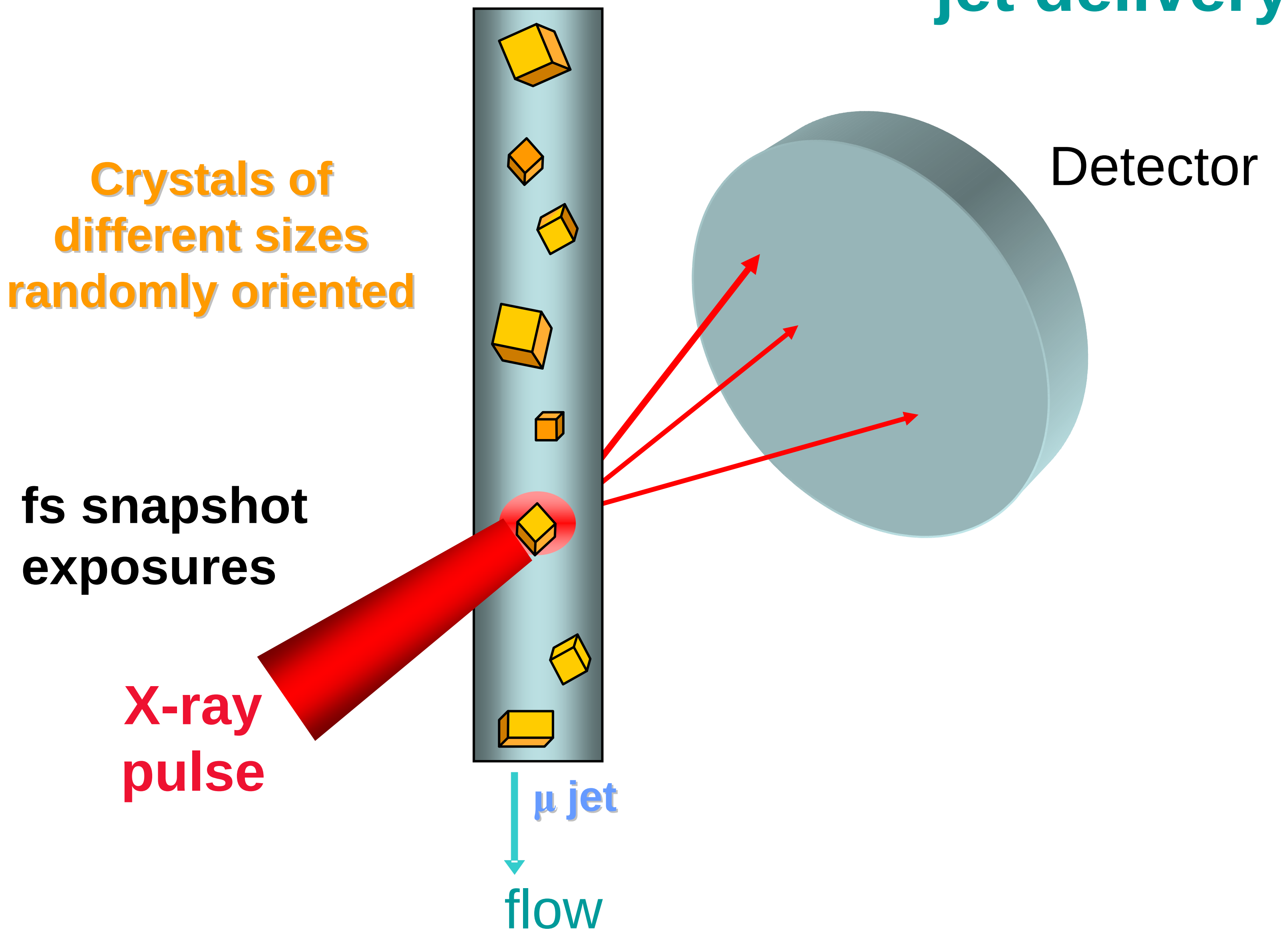
Samples are destroyed upon interaction with the full FEL beam



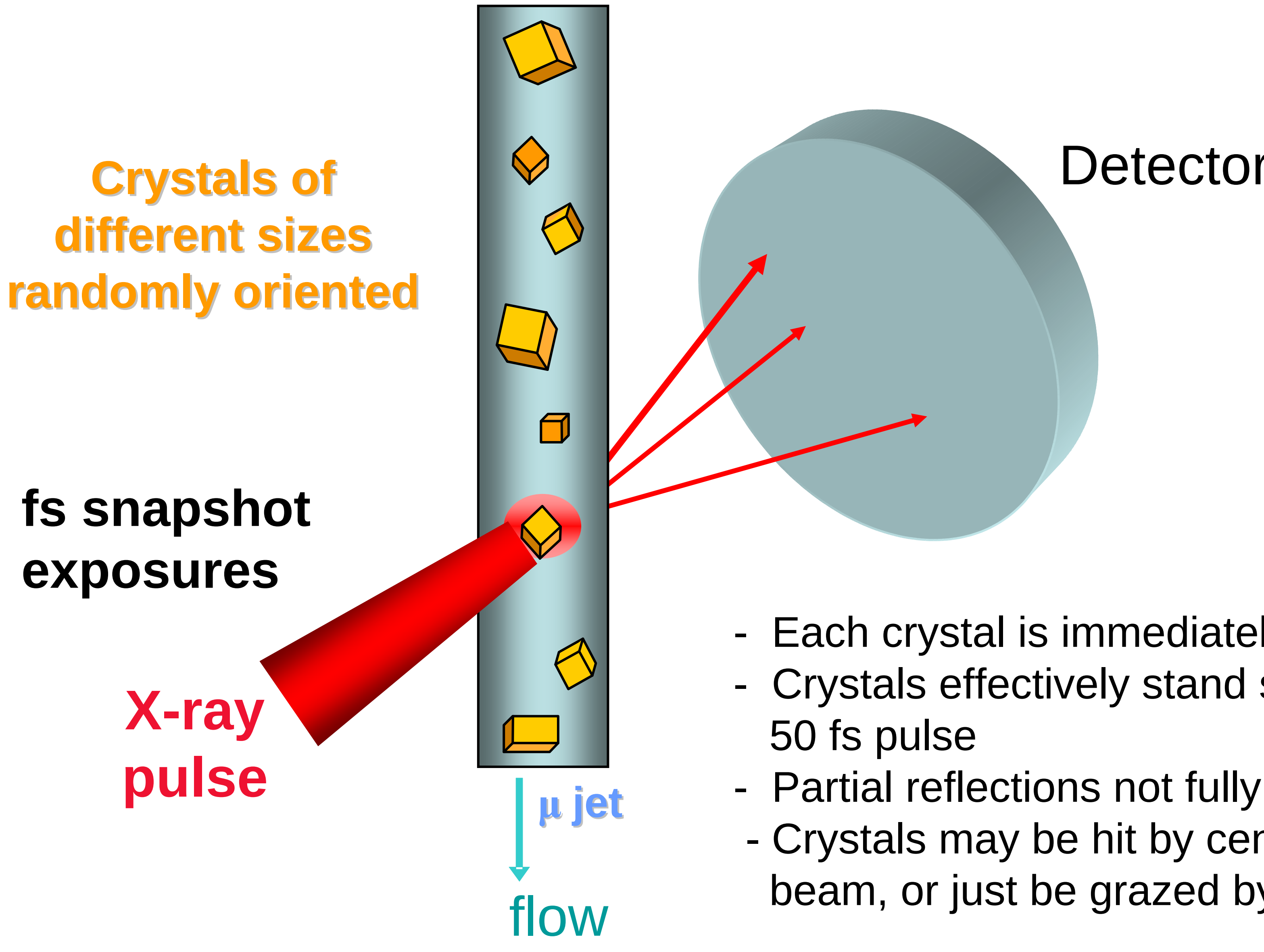
Plasma glow at FEL liquid jet interaction zone

Aquila *et al* Optics Express **20**:2706 (2012)

Serial femtosecond crystallography
jet delivery

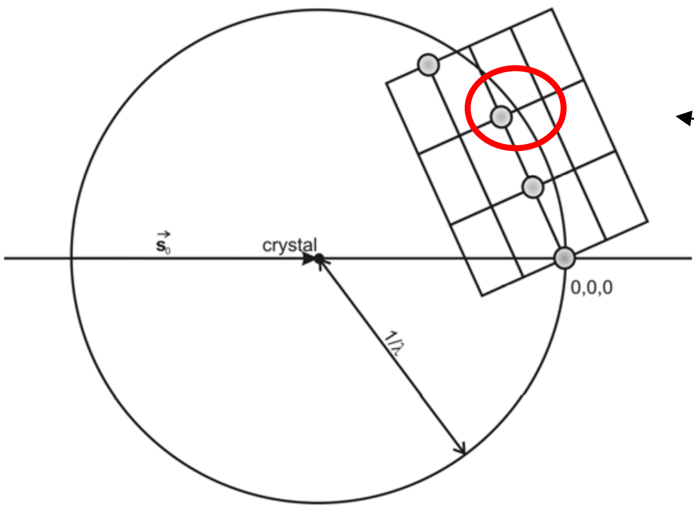


Serial femtosecond crystallography



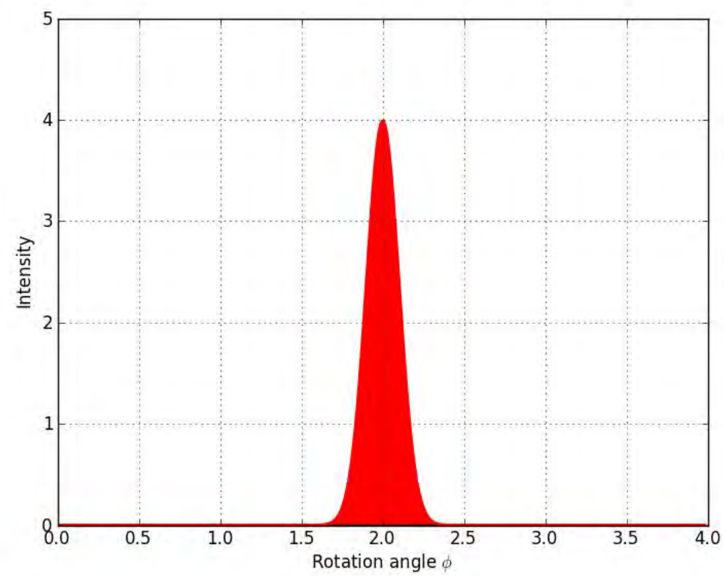
- Each crystal is immediately destroyed
- Crystals effectively stand still during a 50 fs pulse
- Partial reflections not fully integrated
- Crystals may be hit by center of the beam, or just be grazed by it

Indexing and integrating reflections: rotation/oscillation method

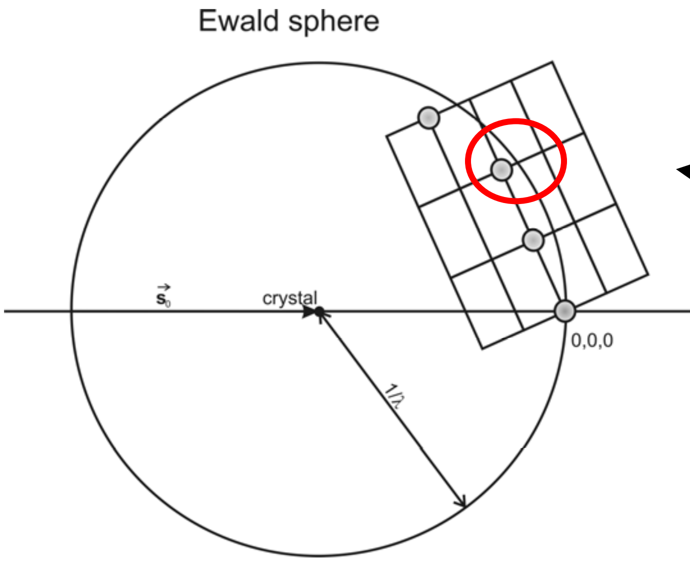


Accurate determination of profile → accurate I and σ(I)

Rotation method
-rotate xtal over finite range
-calculate orientation matrix from observed spot positions
Can fully integrate whole reflections!

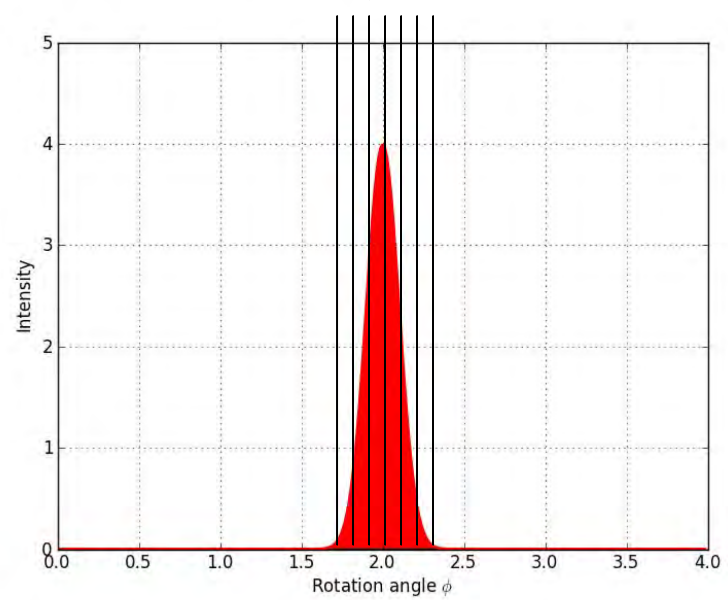


Indexing and integrating reflections: rotation/oscillation method



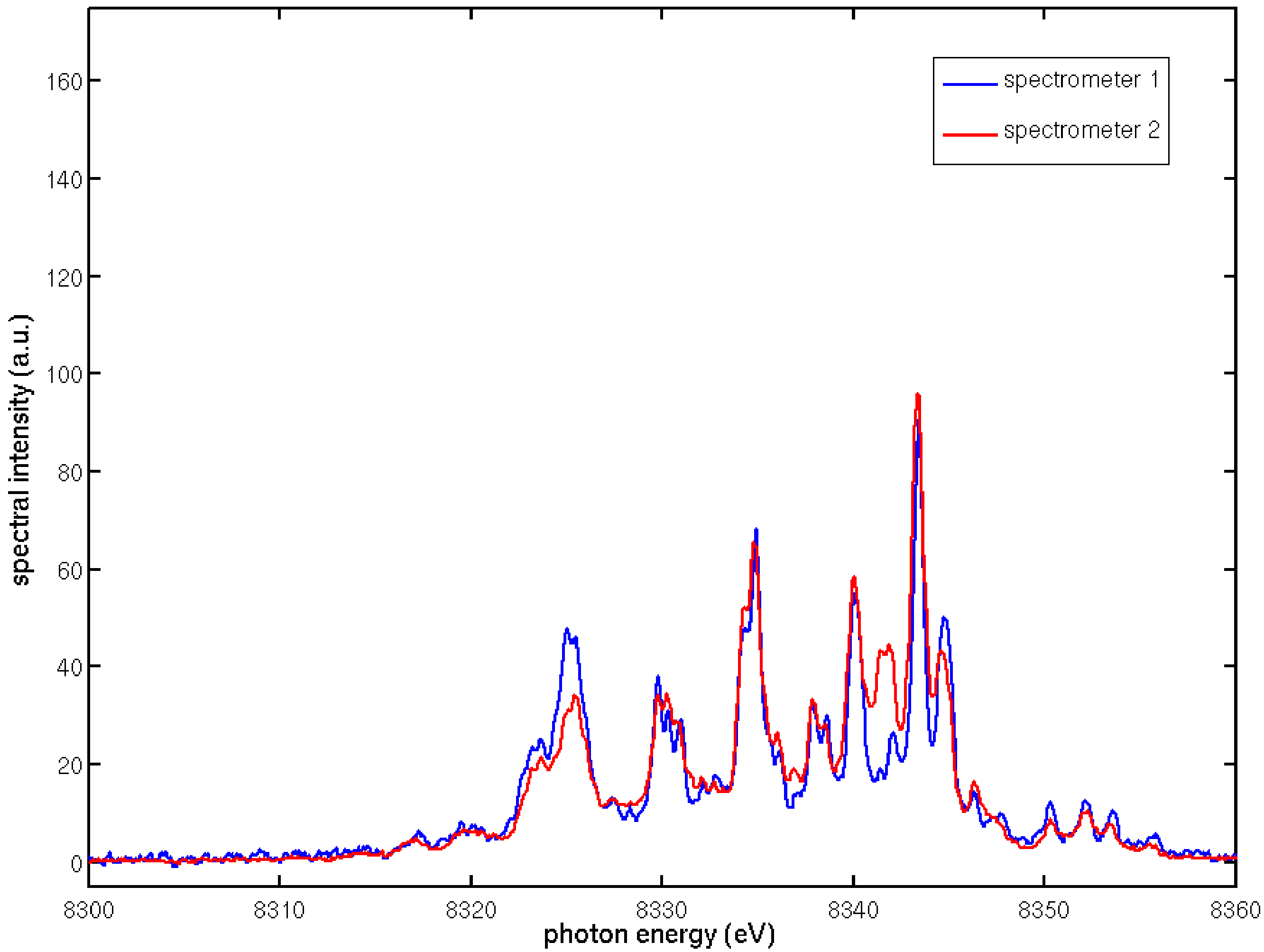
Accurate determination of profile → accurate I and σ(I)

(fine) slicing



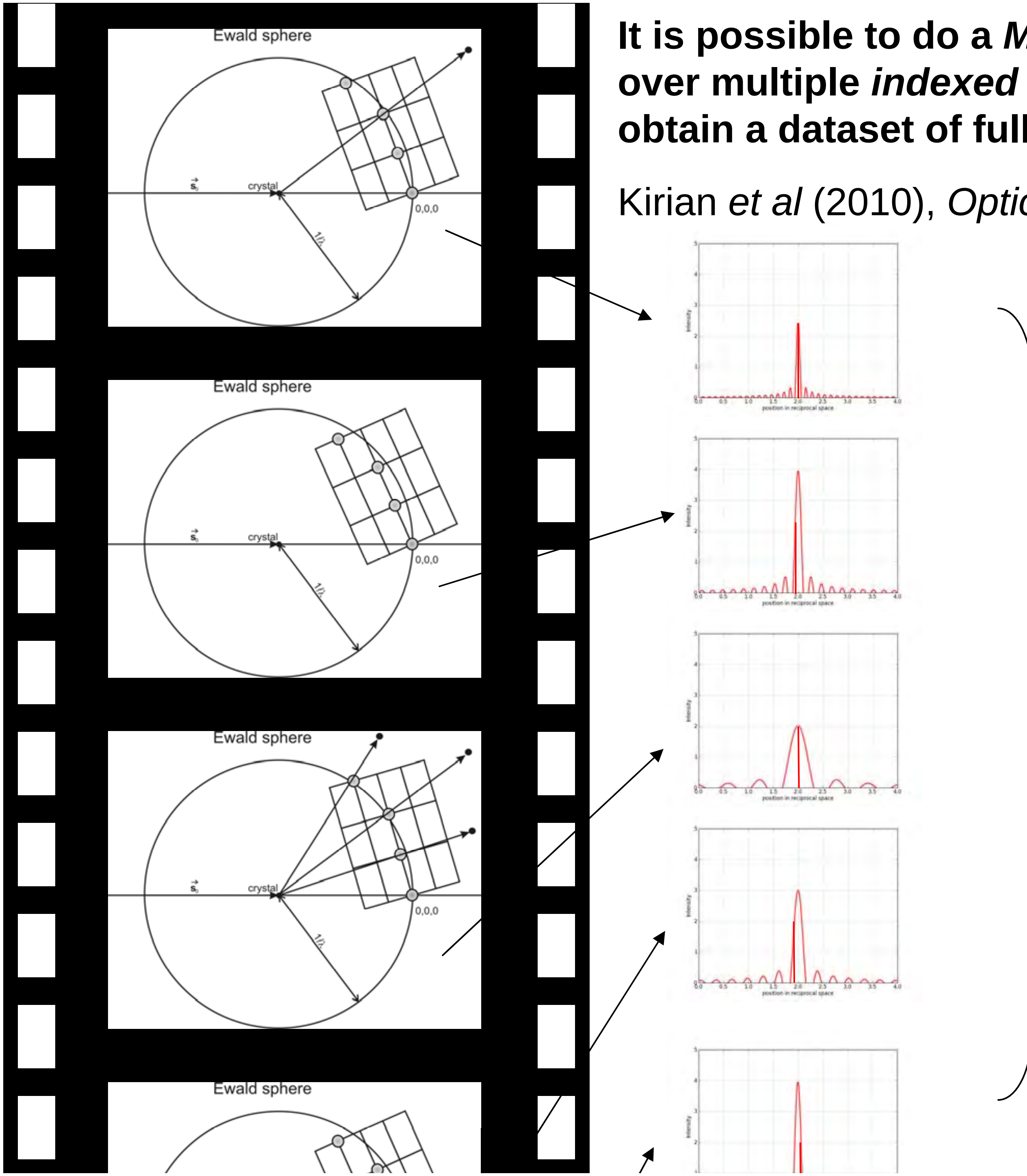
Rotation method
-rotate xtal over finite range
-calculate orientation matrix from observed spot positions
Can fully integrate whole reflections!

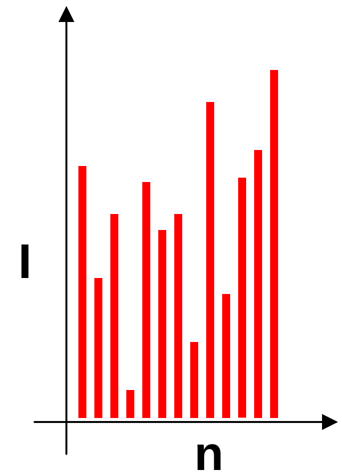
More fluctuations: SASE FEL spectrum varies from shot to shot, too



It is possible to do a *Monte Carlo* integration over multiple *indexed* femtosecond images and obtain a dataset of fully integrated reflections

Kirian *et al* (2010), *Optics Express*,**18**, 5713-5723:

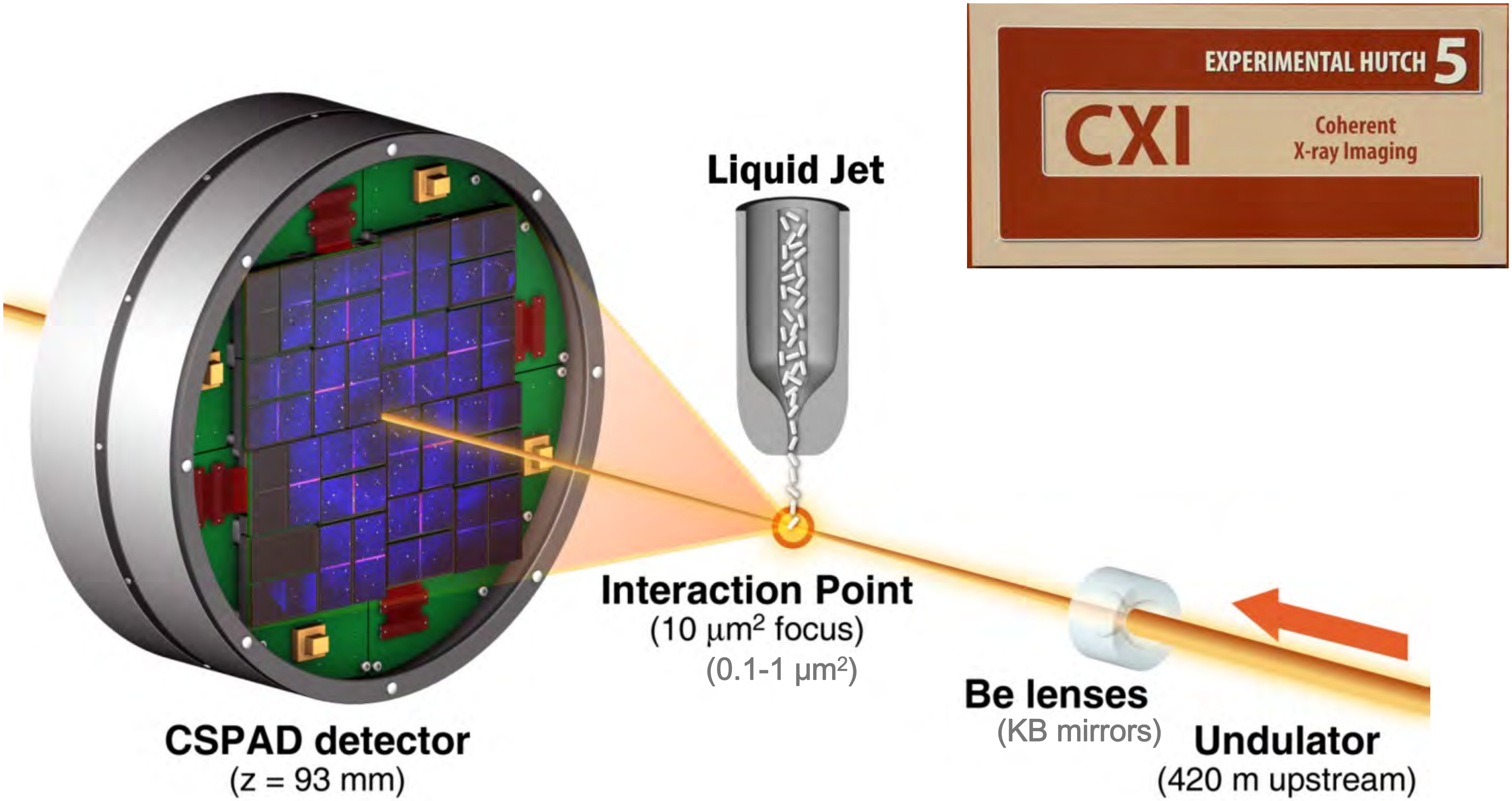




$\langle I[hkl] \rangle = \Sigma I_n / n$

…how good are these I? What are their sigma's?

High resolution serial femtosecond crystallography

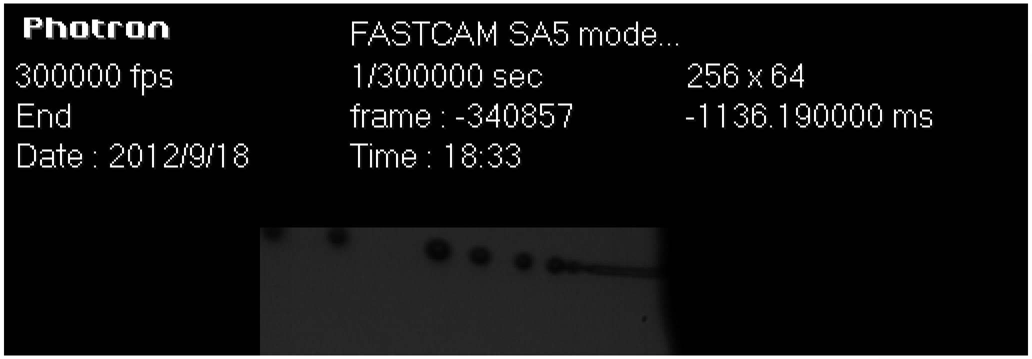


Cornell-SLAC Pixel Array Detector

Boutet et al Science **337**:362 (2012)

Gas-focused liquid microjets

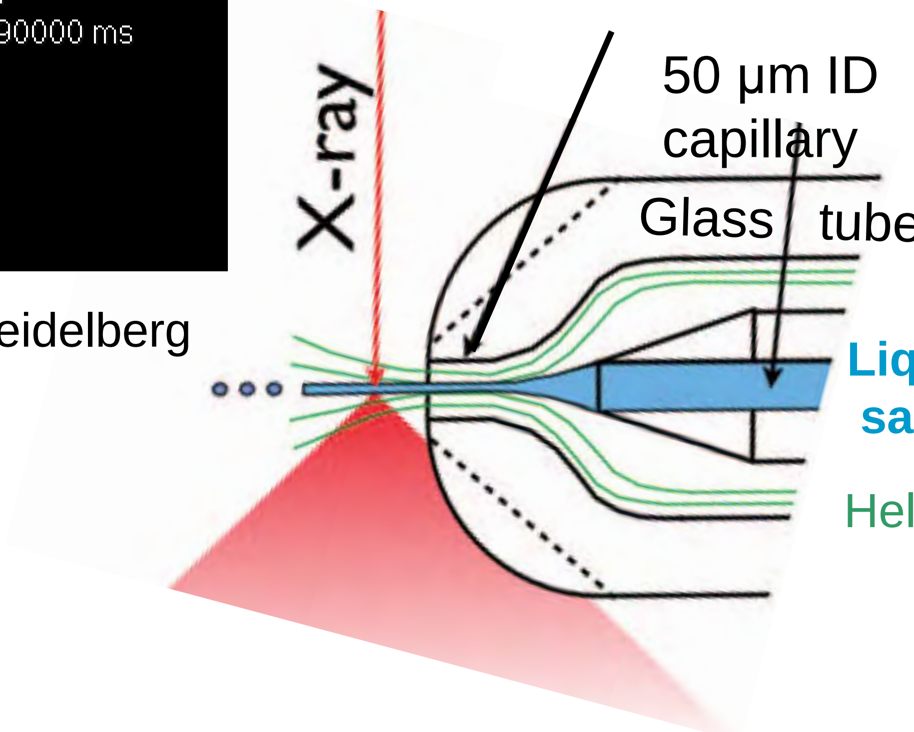
Daniel DePonte, Uwe Weierstall, John Spence, Bruce Doak



S. Botha, R. Shoeman, R.B. Doak Heidelberg

Q=30 μL/min
PHelium=30 psi

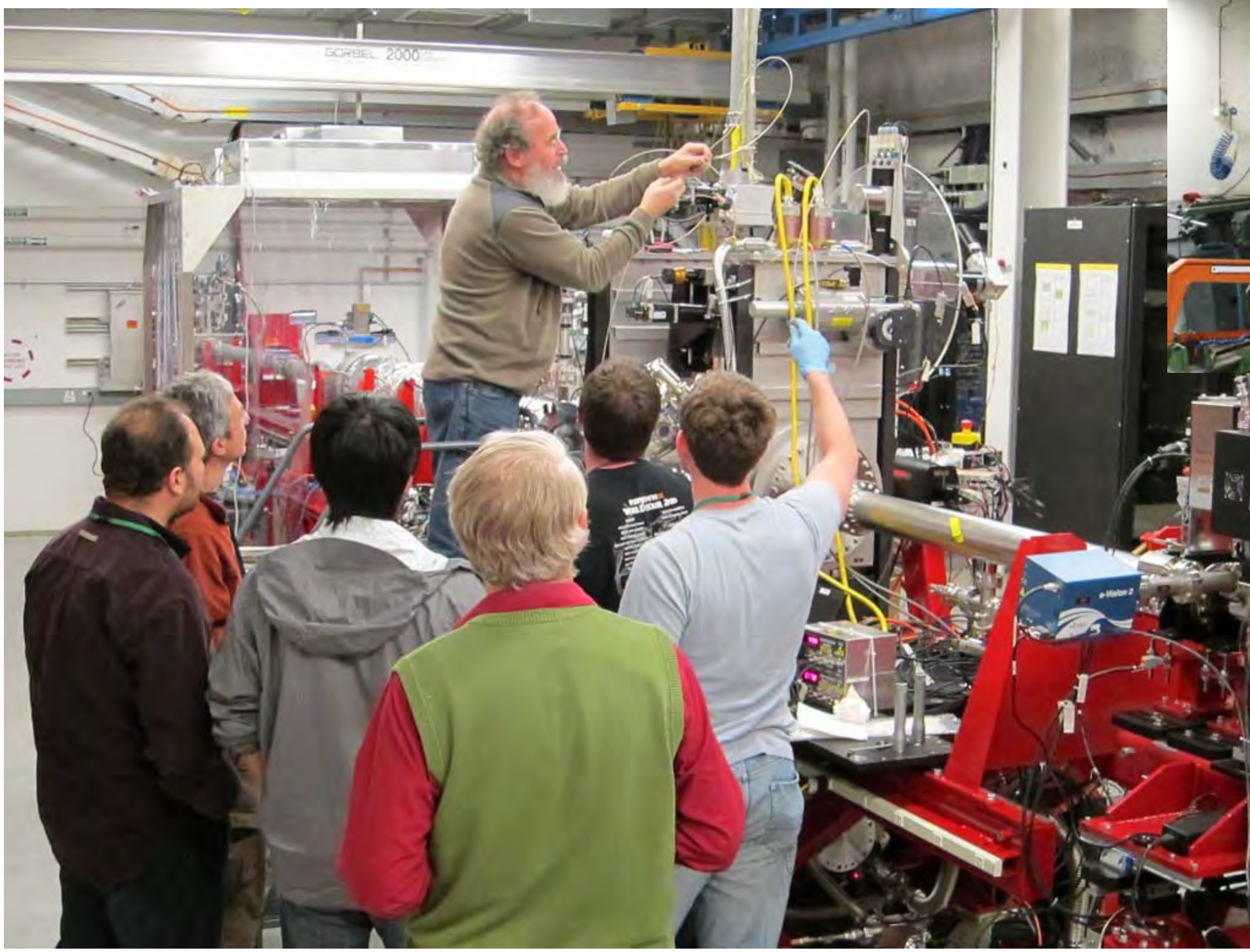
Frame Rate= 300 000 fps
Play Back= 30 fps

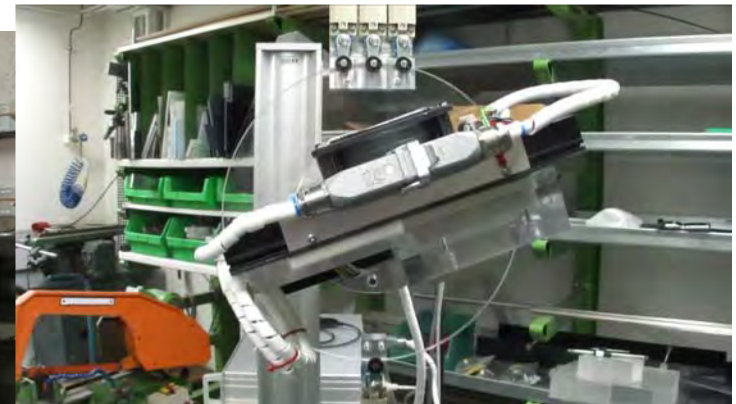


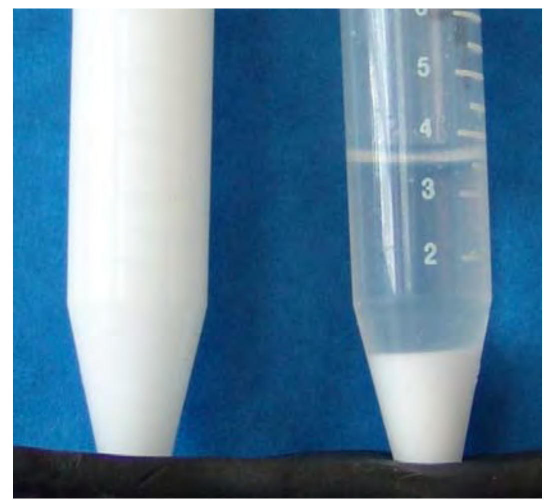
Gas-dynamic virual nozzle
Sample at room temerature, in solution

Weierstall, Spence, Doak, Rev. Sci. Inst **83**, 035108 (2012)

Samples are injected vertically, crystal settling prevented by rotating temperature-controlled syringe pump



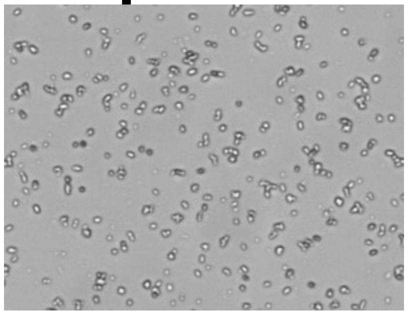




Lomb *et al.* J. Appl. Cryst. **45,** 674 (2012).

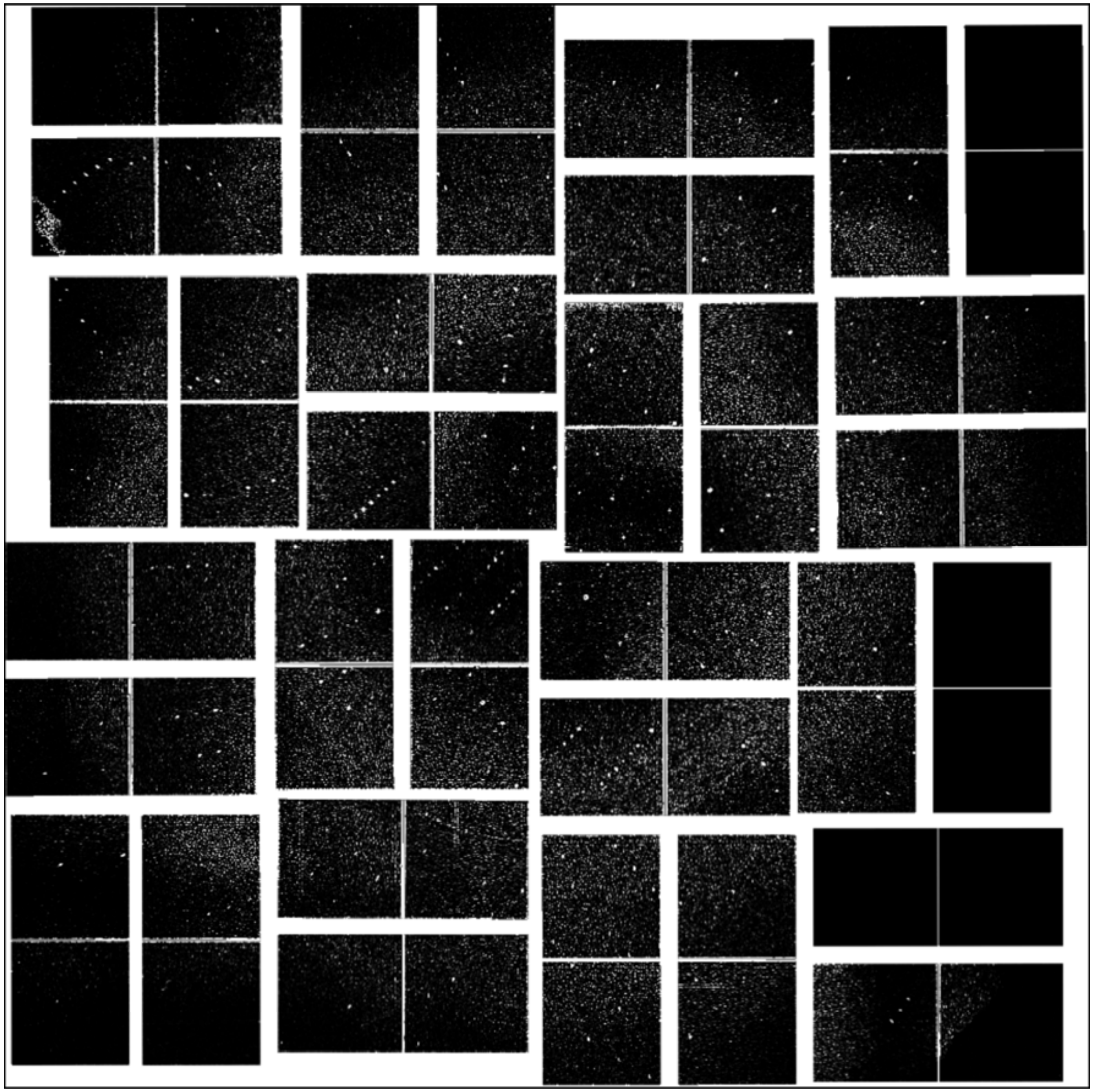
High resolution femtosecond diffraction of micron-sized lysozyme crystals

Lysozyme crystals
1-2 μm Ø



40 fs pulse*, 3 mJ/pulse
10 μm^2 focus
Transmission 15%
0.6 mJ/sample
33 MGy/pulse
9.4 keV , λ =1.32 A
Resolution 1.9 Å

*electron bunch length



Comparison of FEL and synchrotron data

	40 fs	5 fs	Synchrot.
Dose / crystal	33 MGy	3 MGy	0.02 MGy
Dose rate [Gy / s]	8.3 x 10^{20}	5.8 x 10^{20}	9.6 x 10^2
Number of DP	~1.5 x 10^6	~2 x 10^6	100
Hits	66442	40115	100
Indexed DP	12247	10575	100
B-factor [Å^2]	28.3	28.5	19.4
R/R_{free} [%]	19.2 / 22.09	18.5 / 22.7	16.8 / 20.0

Resolution limit: 1.9 Å

R-factor *vs* resolution

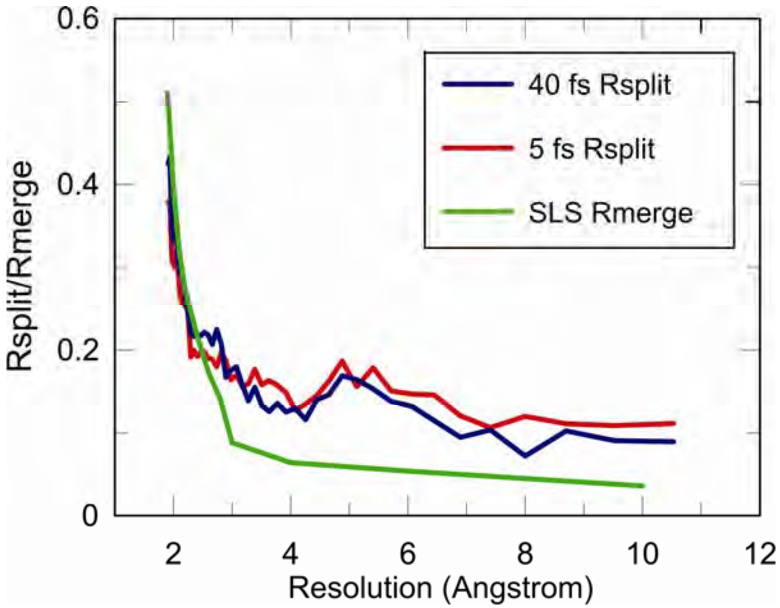
40 fs LCLS data

(1 µm lysozyme crystals)

and

SLS synchrotron data

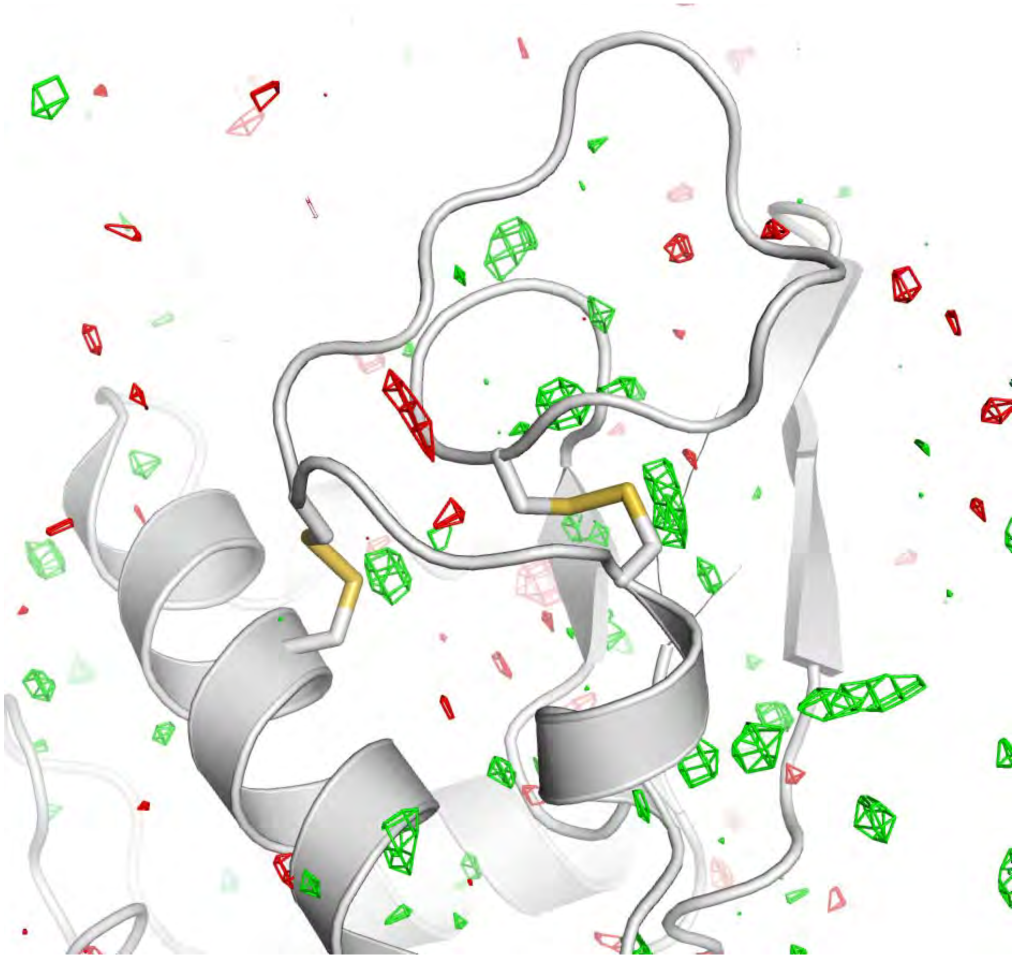
(200 µm lysozyme crystal, room temperature)



Boutet et al Science **337**:362 (2012)

Serial femtosecond crystallography yields undamaged high resolution structures

No difference density Fobs (synchrotron (SLS) – Fobs (LCLS))



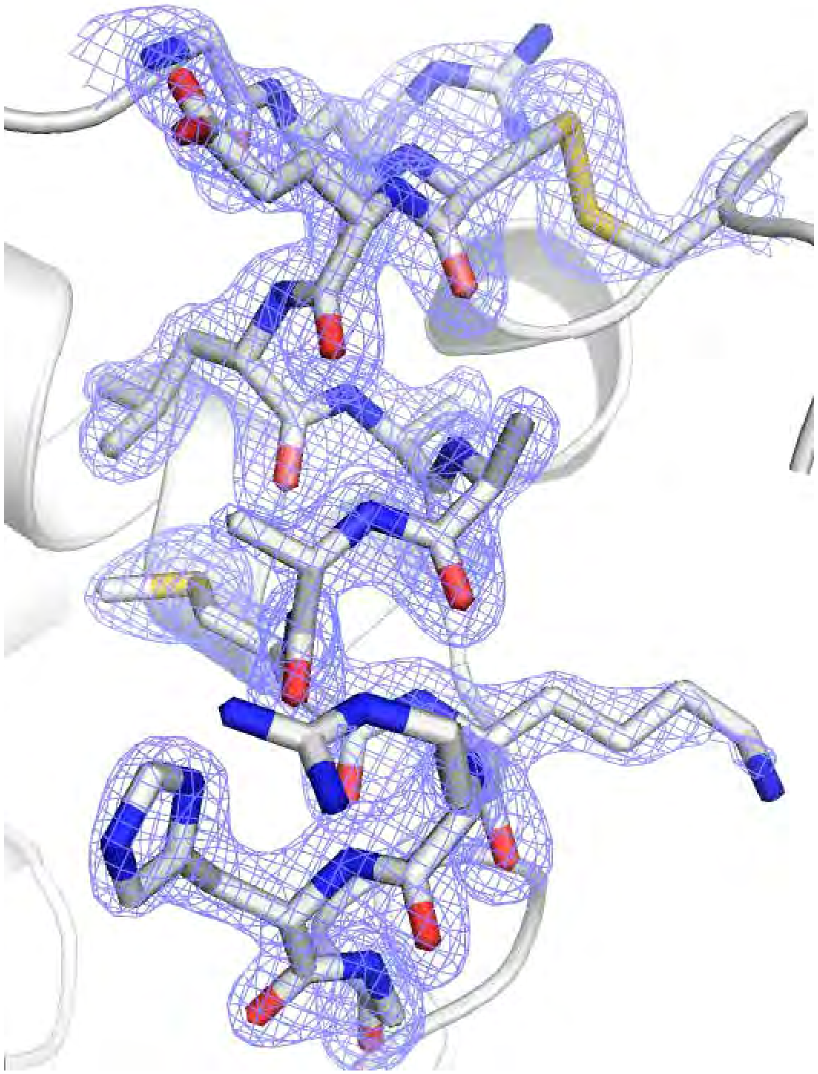
Boutet et al Science **337**:362 (2012)

FEL derived intensities provide high resolution structures

Molecular replacement-phased density, 1.9 Å resolution

-Resolution better than 2 Å because S-atoms in disulfides can be resolved separately, S-S distance is 2 Å

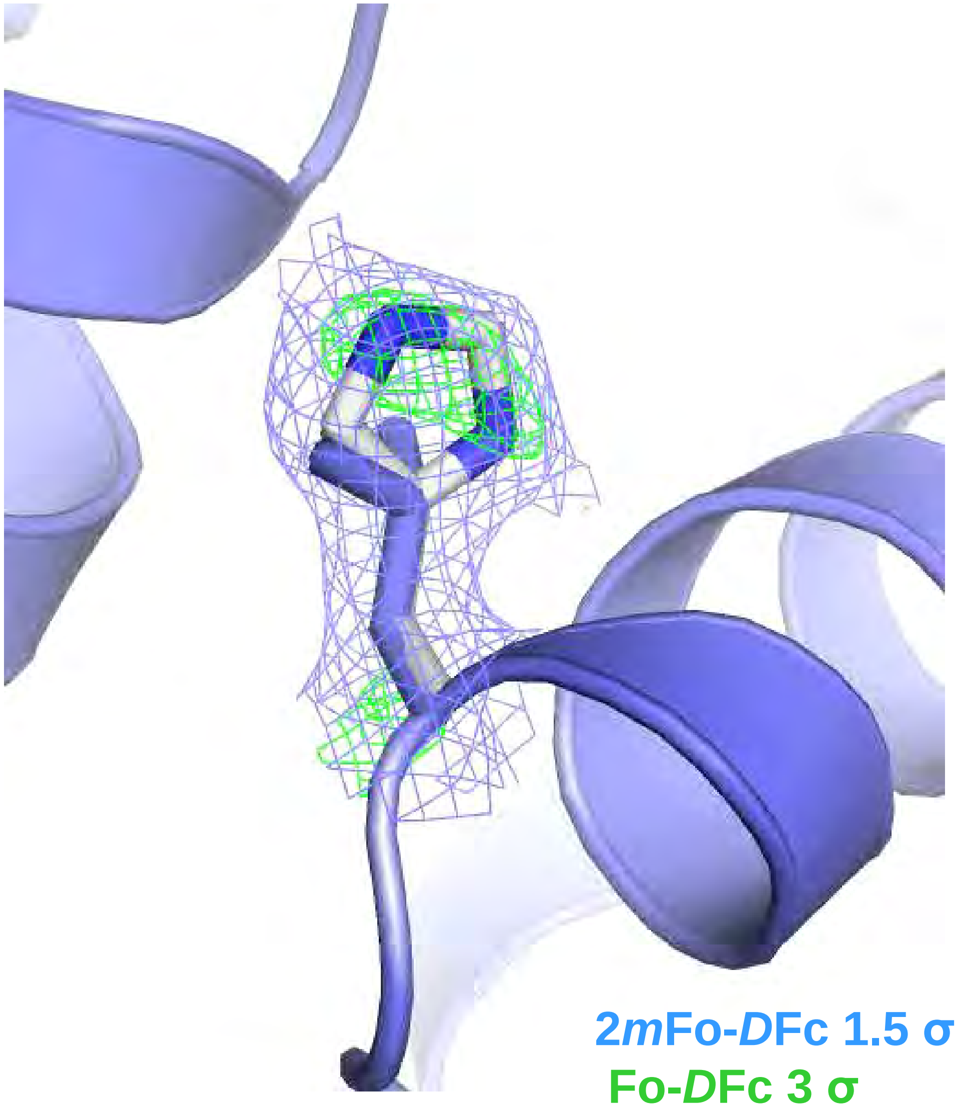
-Good definition of side chains



1.5 σ 2*m*Fo-*D*Fc

FEL derived intensities are good enough to see small differences

Molecular replacement with turkey lysozyme (Valine where there should be histidine)

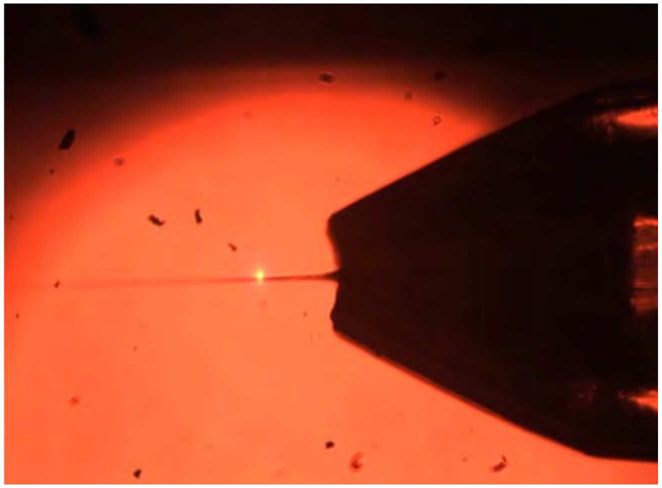


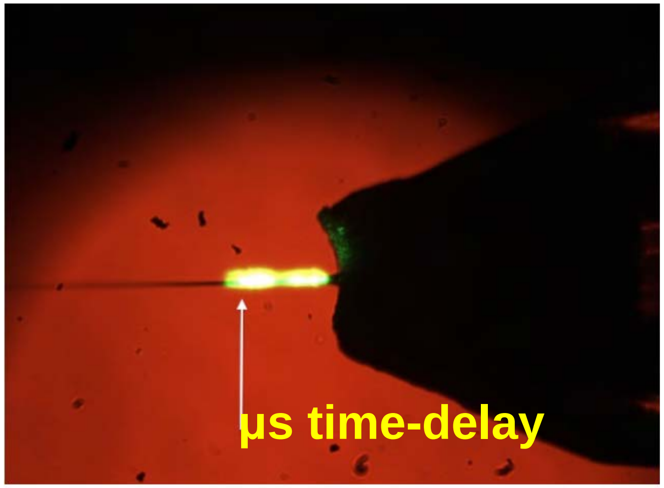
2*m*Fo-*D*Fc 1.5 σ

Fo-*D*Fc 3 σ

Applications of serial femtosecond crystallography

- Analysis of (sub)micron crystals, including membrane proteins in sponge (Nature Meth. **9**: 263 (2012)) or lipidic cubic phase (Science **342**: 1521 (2013))
- SAXS and WAXS measurements
- Time-resolved pump-probe studies on light-sensitive systems





Placement of pump laser beam determines time delay

Aquila *et al* Optics Express **20**:2706 (2012)

FEL beam hitting LCP-jet at 1 Hz

PIs: Raymond Stevens, **Vadim Cherezov** *The Scripps Research Institute*

LCP Jet: **Uwe Weierstall,** Bruce Doak, John Spence *Arizona State University*

Serial ~~femtosecond~~ crystallography at synchrotrons

Longer exposure times

- Crystals do not necessarily stand still during exposure

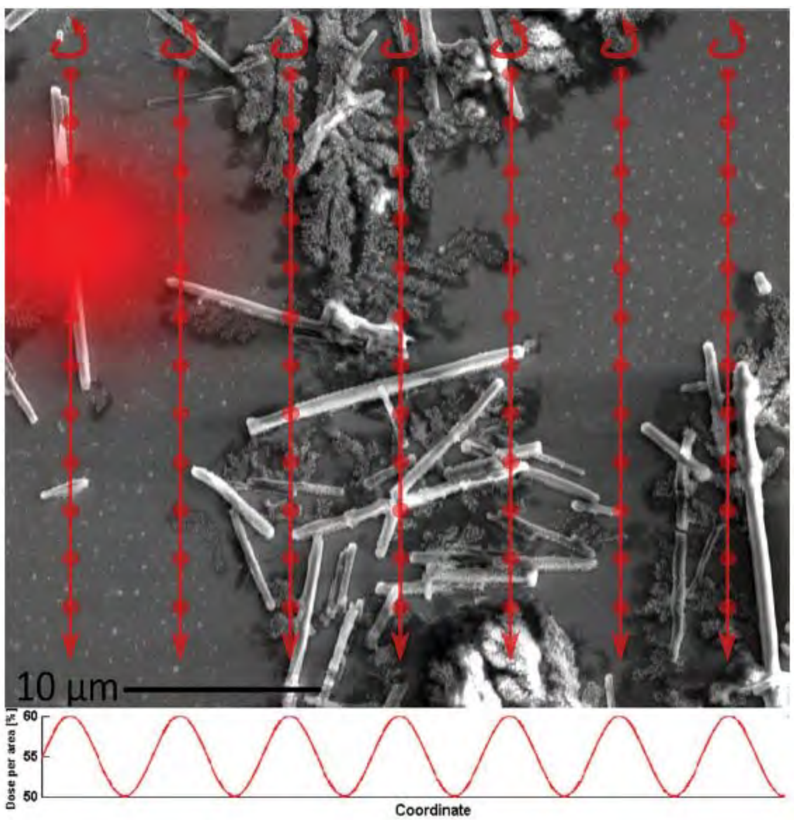
- Tumbling potential problem
- Rotation for data collection possible

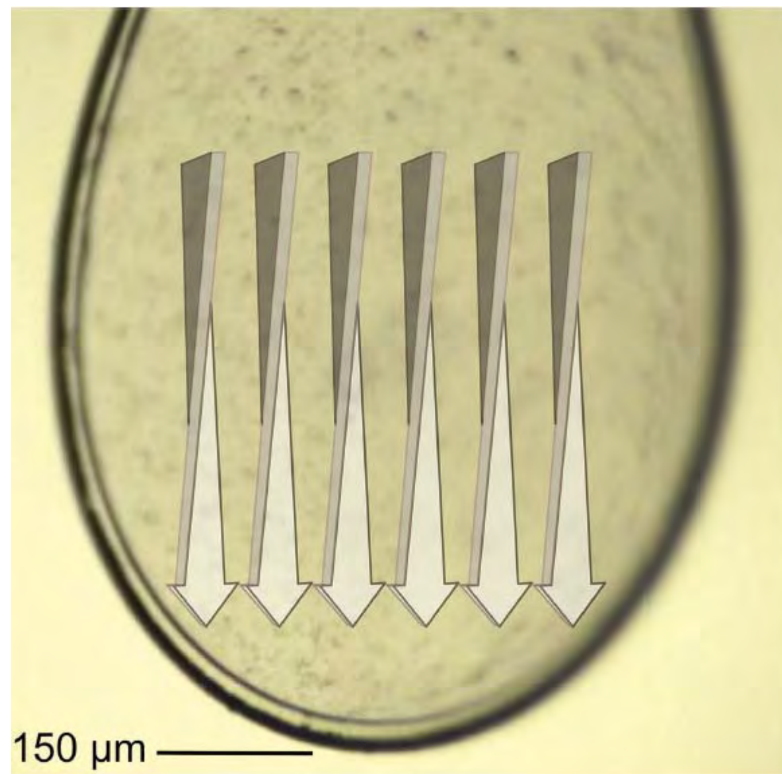
- Radiation damage

Slurry of cryocooled microcrystals on a loop

Serial crystallography on *in vivo* grown microcrystals using synchrotron radiation

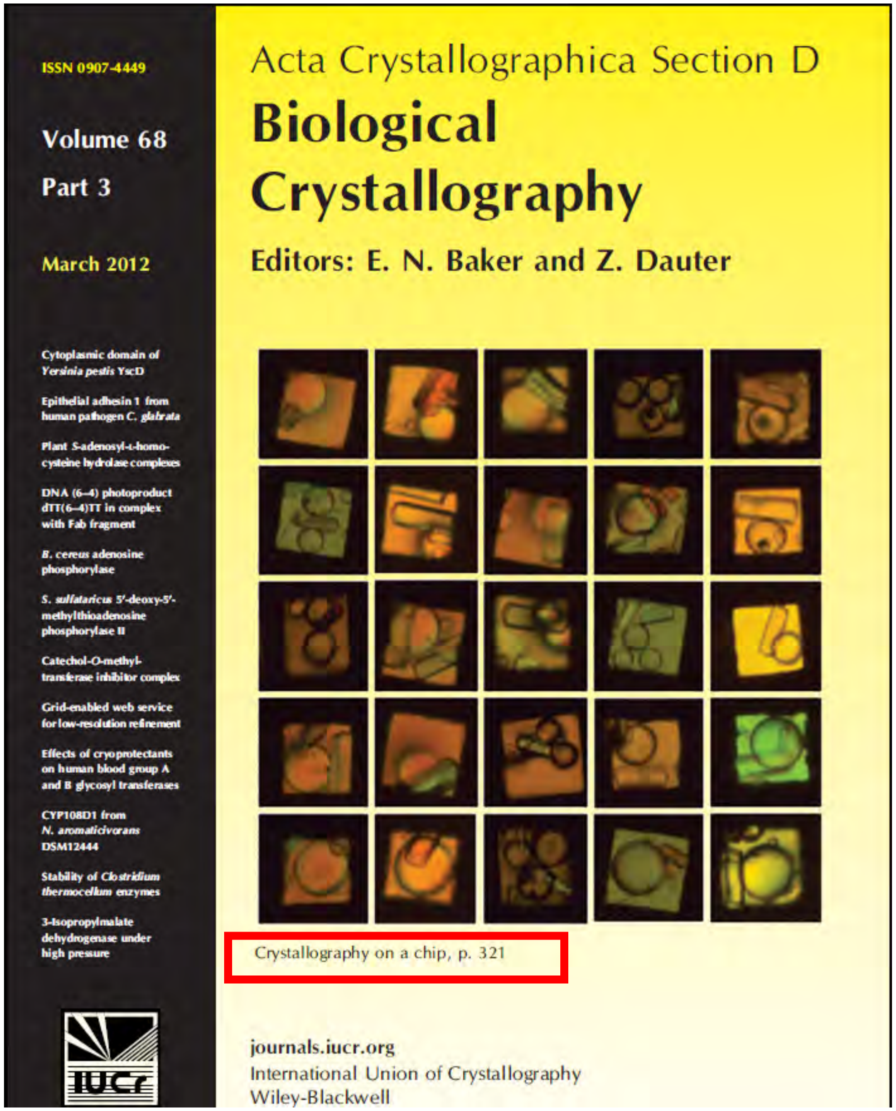
Cornelius Gati,[a]‡ Gleb Bourenkov,[b]‡ Marco Klinge,[c] Dirk Rehders,[c] Francesco Stellato,[a] Dominik Oberthür,[a,d] Oleksandr Yefanov,[a] Benjamin P. Sommer,[d,e] Stefan Mogk,[e] Michael Duszenko,[e] Christian Betzel,[d] Thomas R. Schneider,[b]* Henry N. Chapman[a,f]* and Lars Redecke[c]*





IUCRJ, 2014

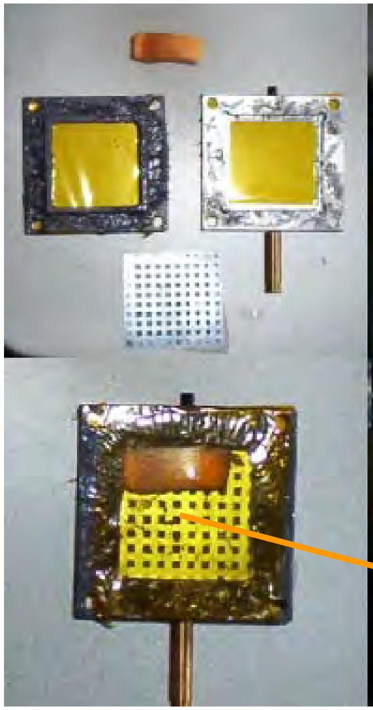
High throughput crystallography at room temperature

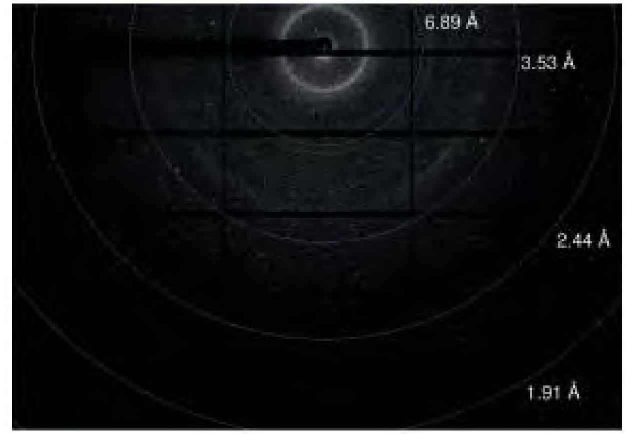


Chip mounts

- control over solution, all crystals can be handled (after appropriate surface chemistry is established)
- with or without bottoms
- diffraction images +/- laser for time resolved studies
- 100% hit rates, chip can be pre-scanned for occupied positions

Practical aspects of the chip





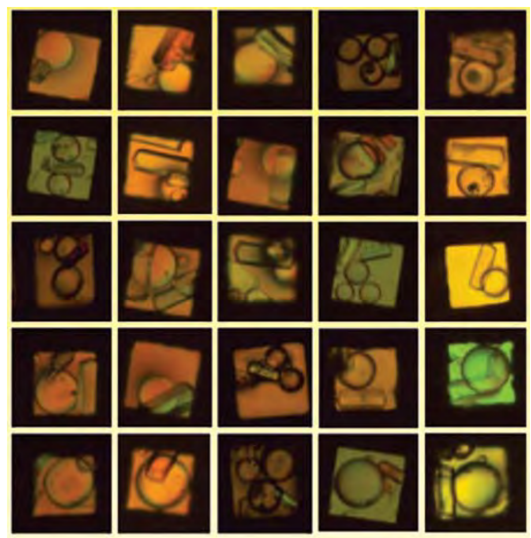
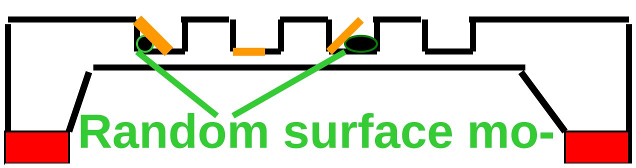


Plate like crystals with random orientations



Surface roughing of well-bottoms can induce random xtal orientations

Zarrine-Afsar et al Acta Cryst. D 68: 321-3 (2012)

Room temperature serial crystallography at the SLS using a high viscosity extrusion injector at atmospheric pressure

Data removed

Collection of still and rotation data possible

Different high viscosity media possible
accounting for required flow rate, crystal preference

Room temperature serial crystallography at the Swiss Light Source using a high viscosity extrusion injector at atmospheric pressure

Data removed

Serial crystallography

Serial collection of partial datasets of many many different crystals

- mitigate the effects of **radiation damage**
- **time-resolved** measurements

in high throughput fashion

to provide fast sample change to account for short lifetime of sample

ID29 (Phase II) 10^{14} photons/s/μm^2 Henderson limit 200 μs
XFELs 10^{12} photons/pulse, few fs exposures

Background
Radiation damage (mechanisms)

Jet@SLS, LCLS

Sabine Botha,
Thomas Barends
Karol Nass
Robert Shoeman
R. Bruce Doak
Lutz Foucar
Wolfgang Kabsch

ARIZONA STATE UNIVERSITY

Chip@SLS

Asrash Zarrine-Afsar
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Lukas Lomb
RJ Dwayne Miller (MPISD)

Florian Dworkowski
Meitian Wang

PAUL SCHERRER INSTITUT

Martin Fuchs

LCLS

Henry Chapman and group, in particular Thomas White, DESY
John Spence, Uwe Weierstall and group, ASU
Petra Fromme and group, ASU

Sebastien Boutet, Marc Messerschmidt, Garth Williams

SLAC NATIONAL ACCELERATOR LABORATORY