# X-ray Lasers for Structural and Dynamic Biology

MAX-PLANCK-GESELLSCHAFT

LCLS

UHH

SLAC NATIONAL ACCELERATOR LABORATORY

BERKELEY LAB

CFEL SCIENCE

Lawrence Livermore National Laboratory

UPPSALA UNIVERSITET

GÖTEBORGS UNIVERSITET

# The LCLS is the world's first hard X-ray laser

ASU Arizona State University

LCLS

SLAC NATIONAL ACCELERATOR LABORATORY

1.8keV - 9keV
10 - 300fs pulses
$10^{12}$ photons/pulse
10 micron beam diameter
120Hz

***132 m long undulator***

# X-ray free-electron lasers may enable atomic-resolution imaging of biological macromolecules

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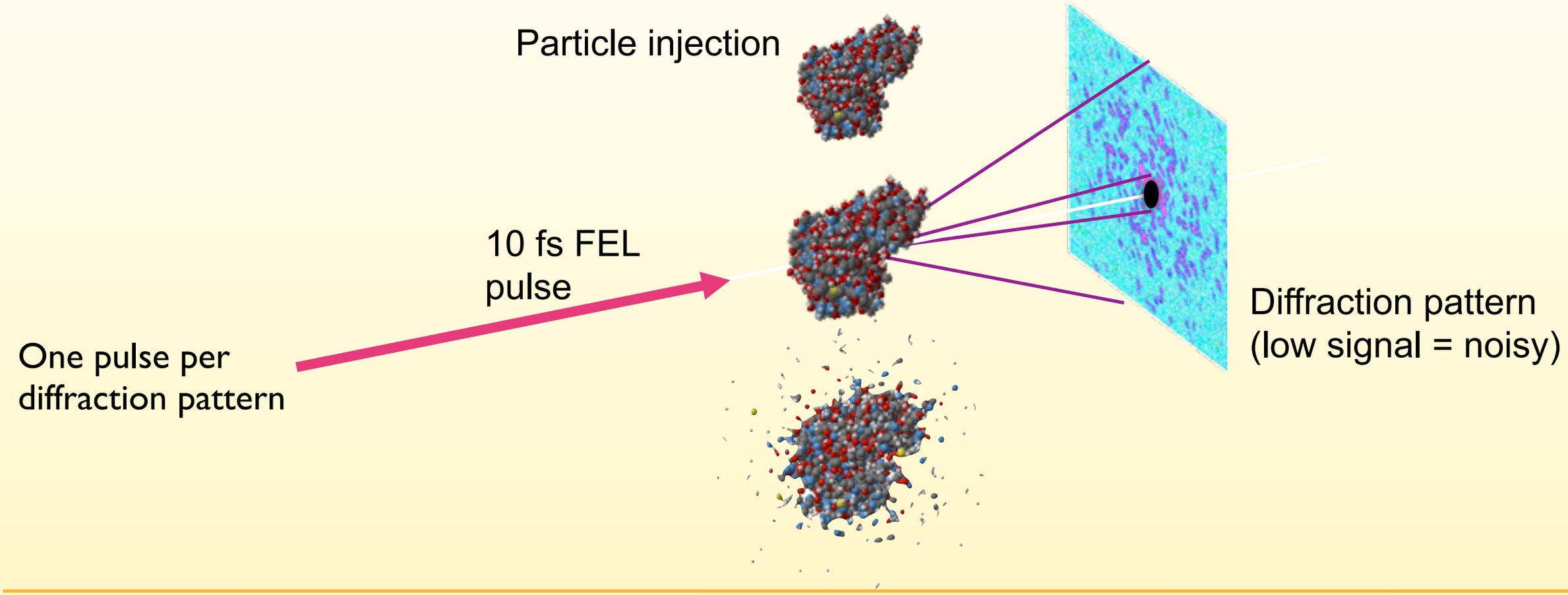
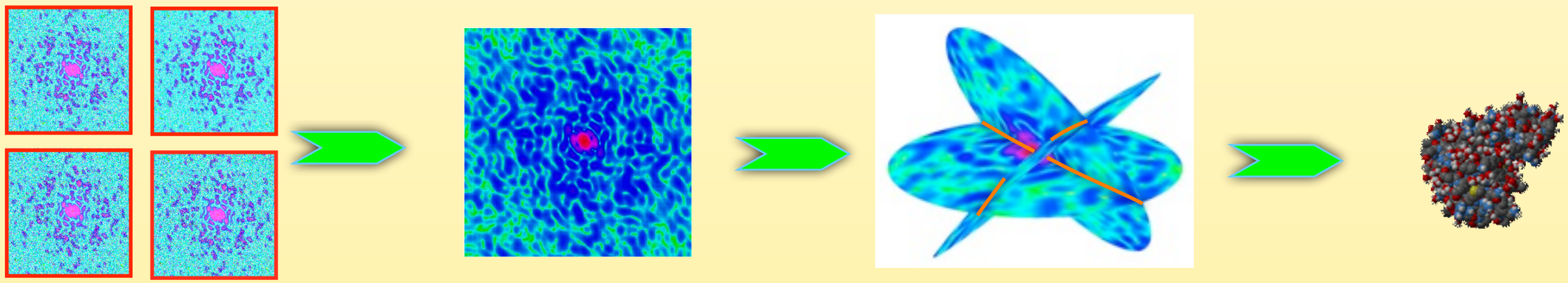
*R. Neutze, R. Wouts, D. van der Spoel, E. Weckert, J. Hajdu, Nature 406 (2000)*




Combine $10^5$-$10^7$ measurements



# Imaging *spatial* resolution is limited by radiation damage

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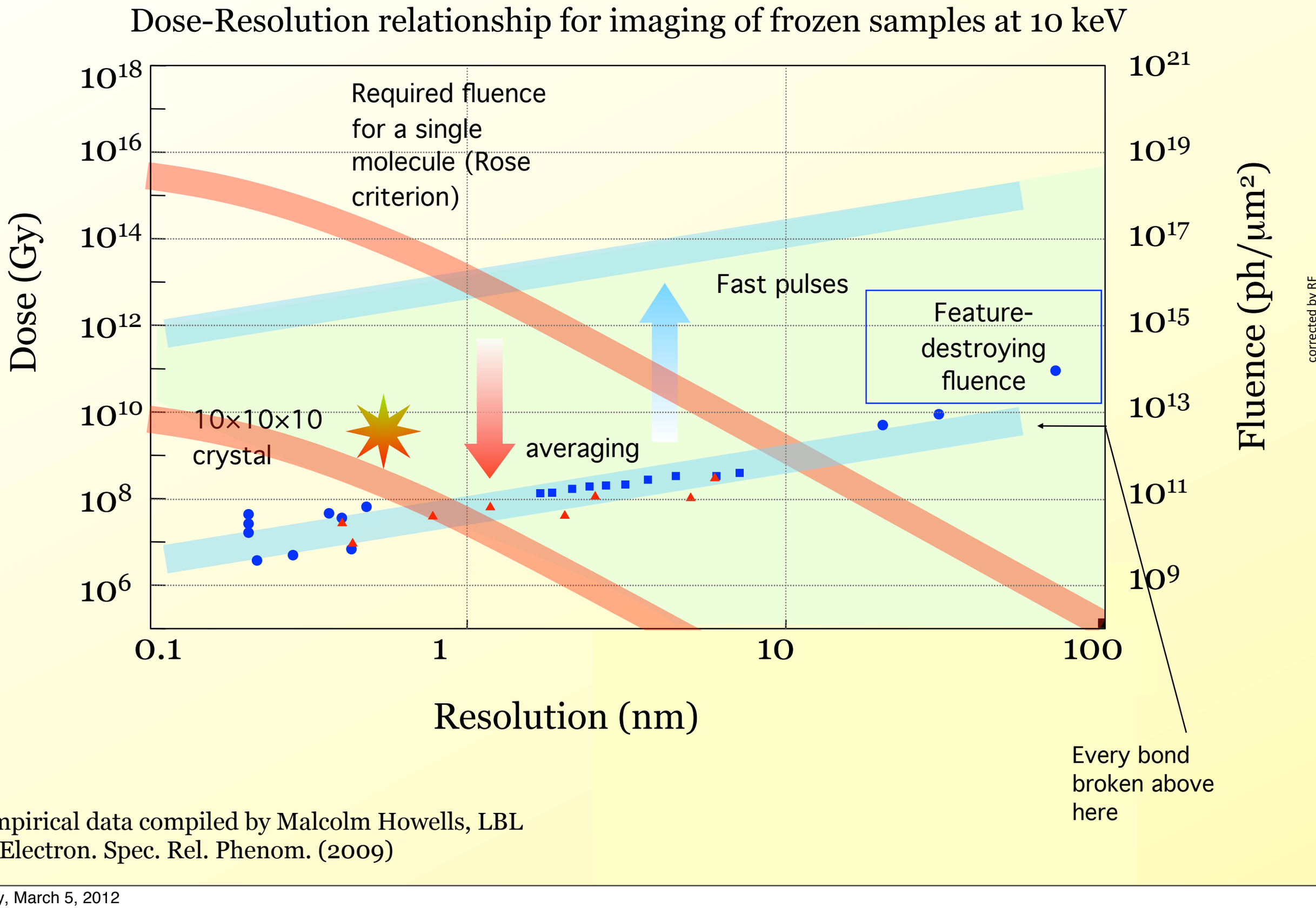
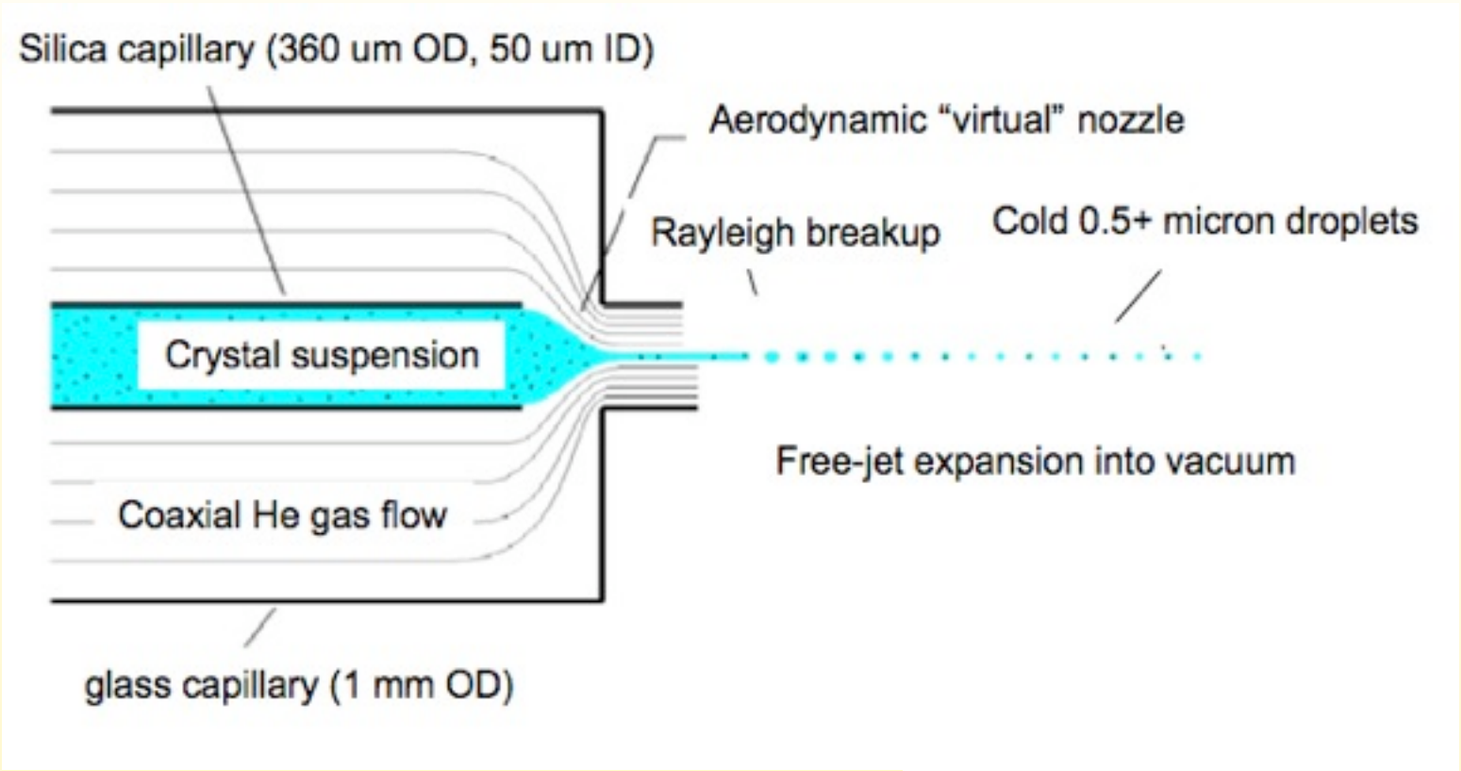


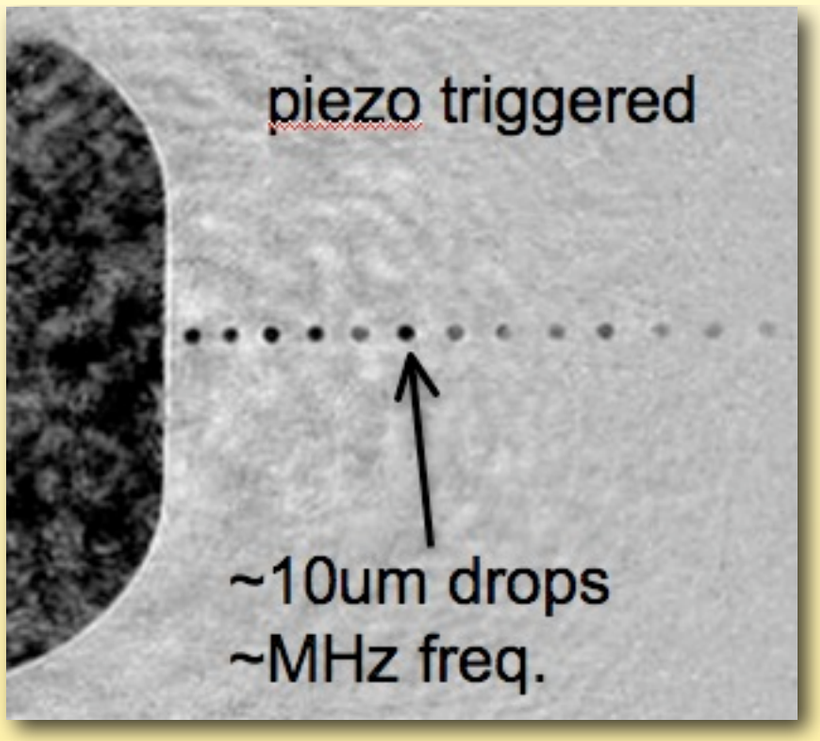

Empirical data compiled by Malcolm Howells, LBL
J. Electron. Spec. Rel. Phenom. (2009)

# Gas Dynamic Virtual Nozzle (GDVN)

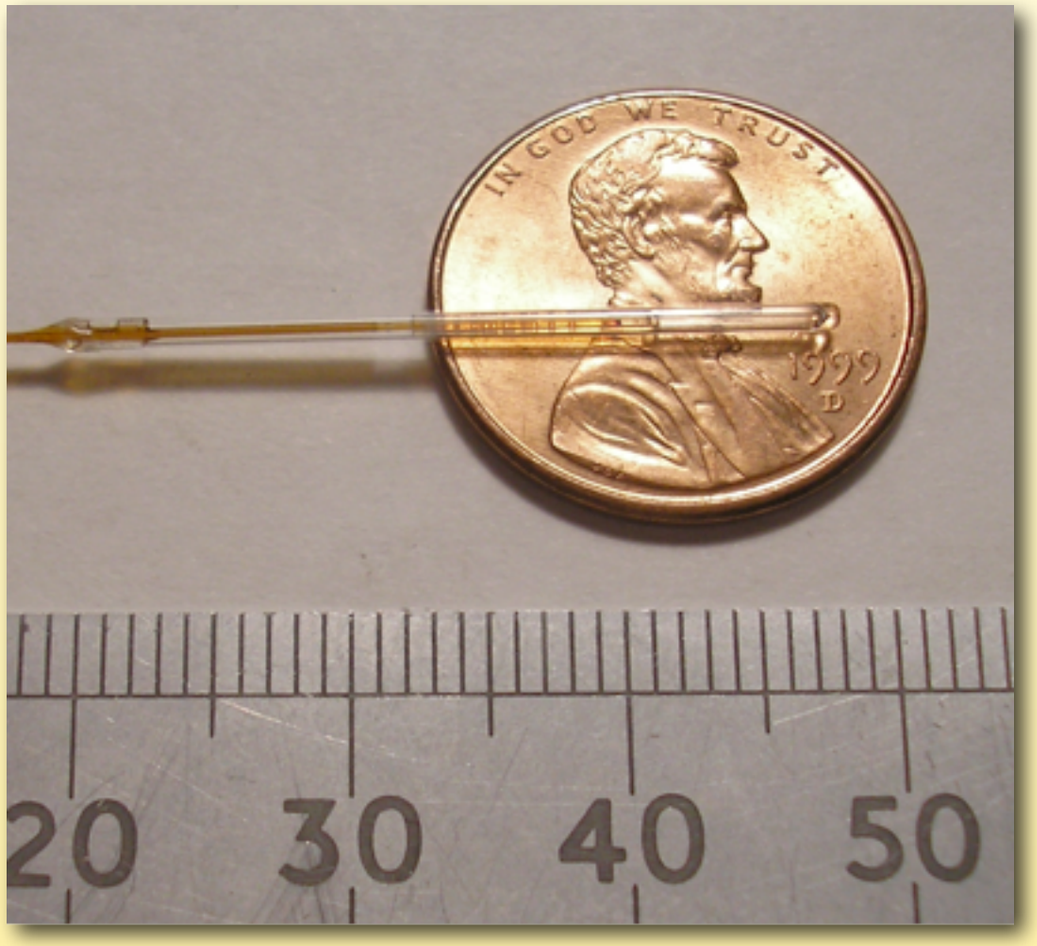



- Liquid velocity ~ 10m/s
- Flow rate ~ 10μL/m
- Jet diameter ~ 0.5-20 um
- Droplets cool at $10^6$ º/sec. in vacuum

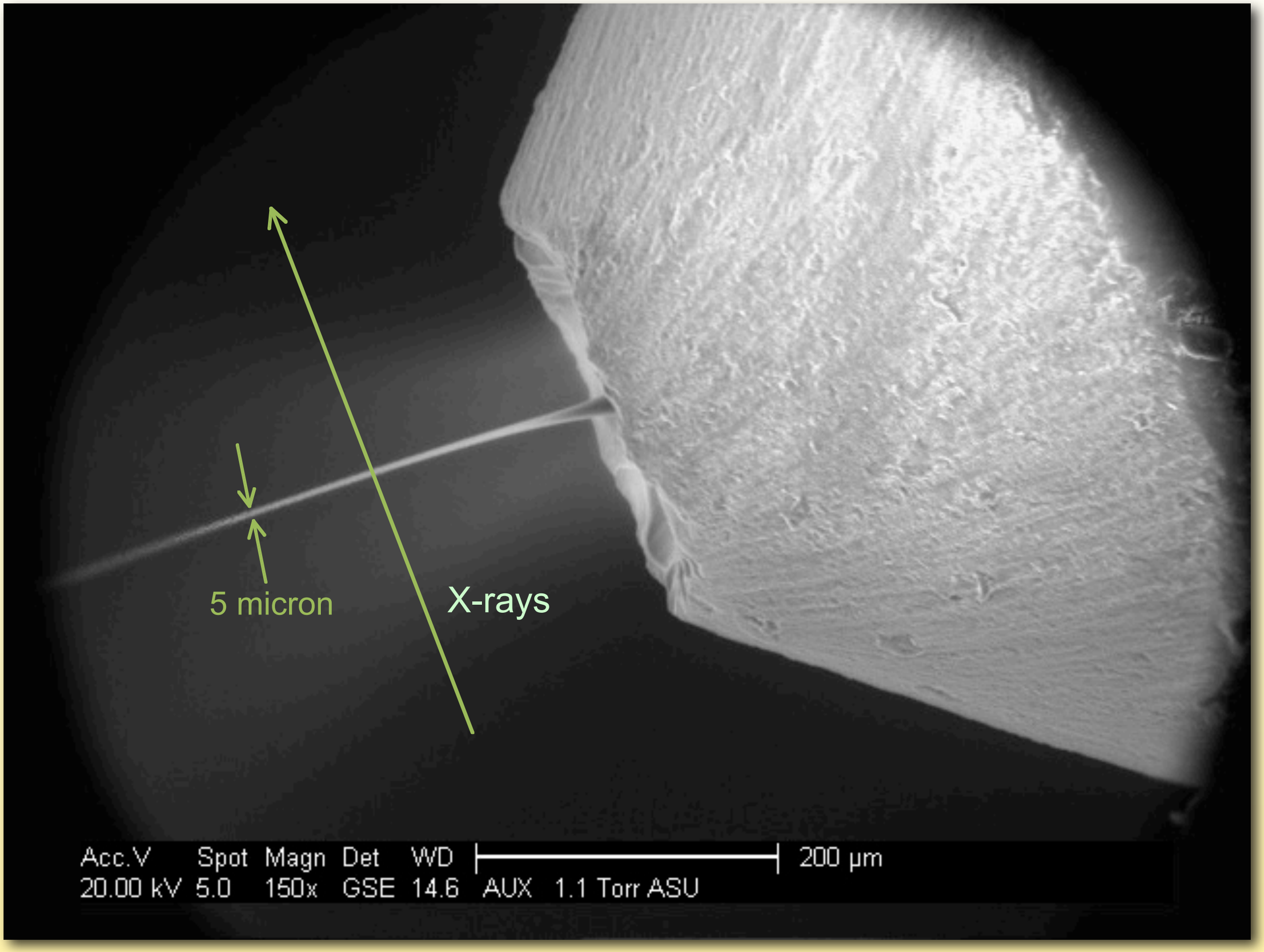
**No charging**
**No clogging**
**Low angular dispersion**
**Tunable in size**



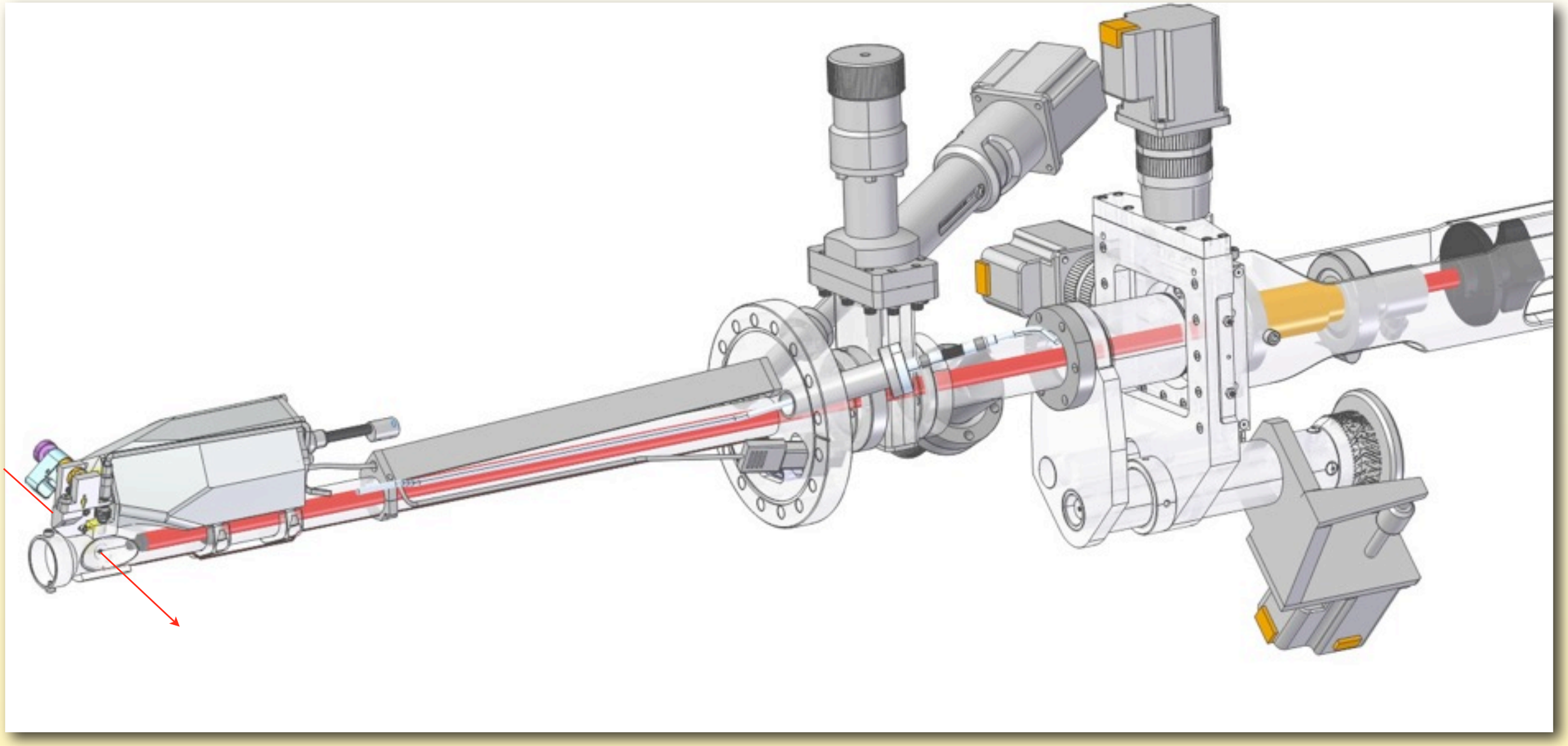

**droplet breakup can be triggered**



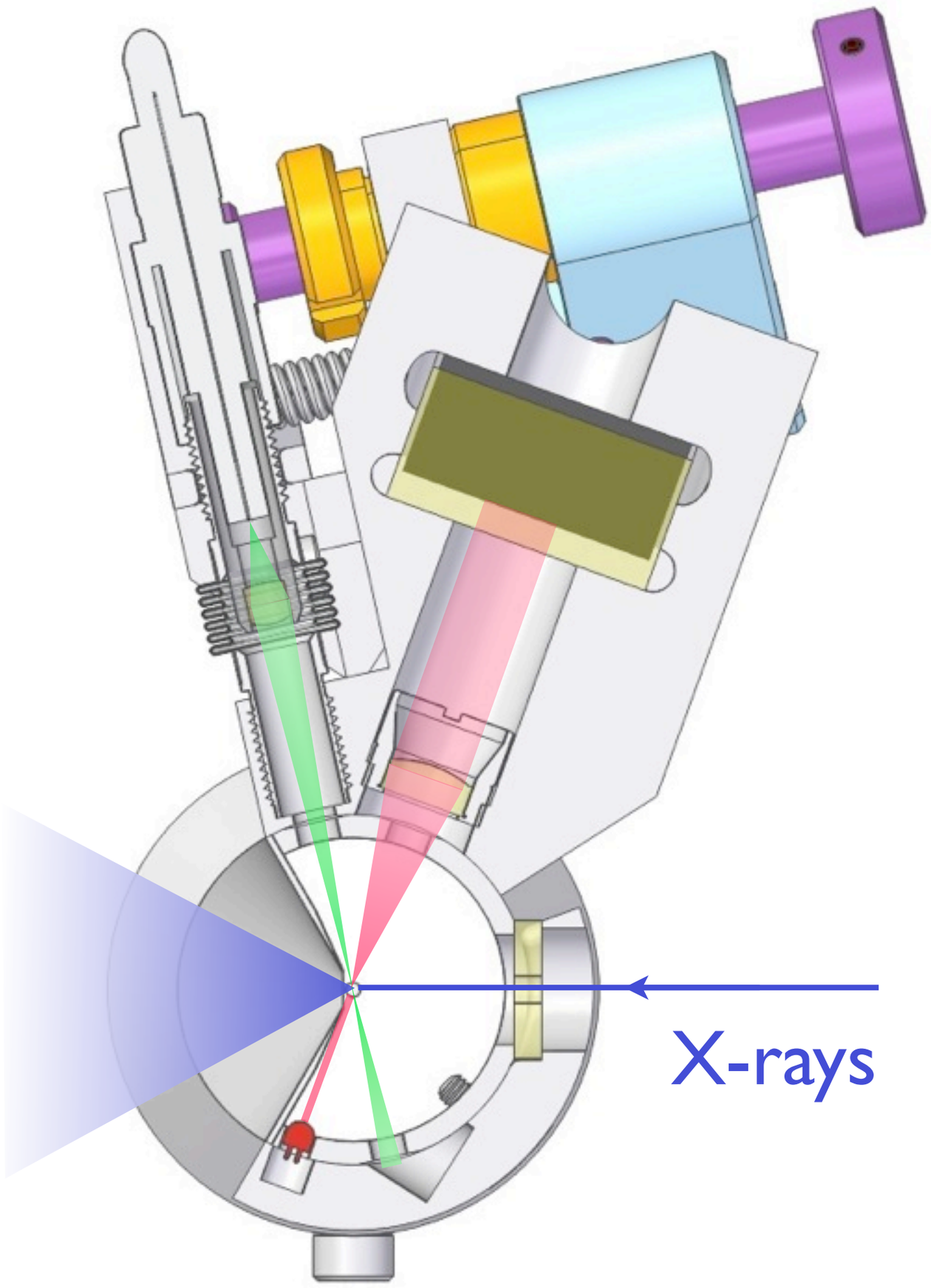
# ESEM image of GDVN in operation



# ASU injector shroud

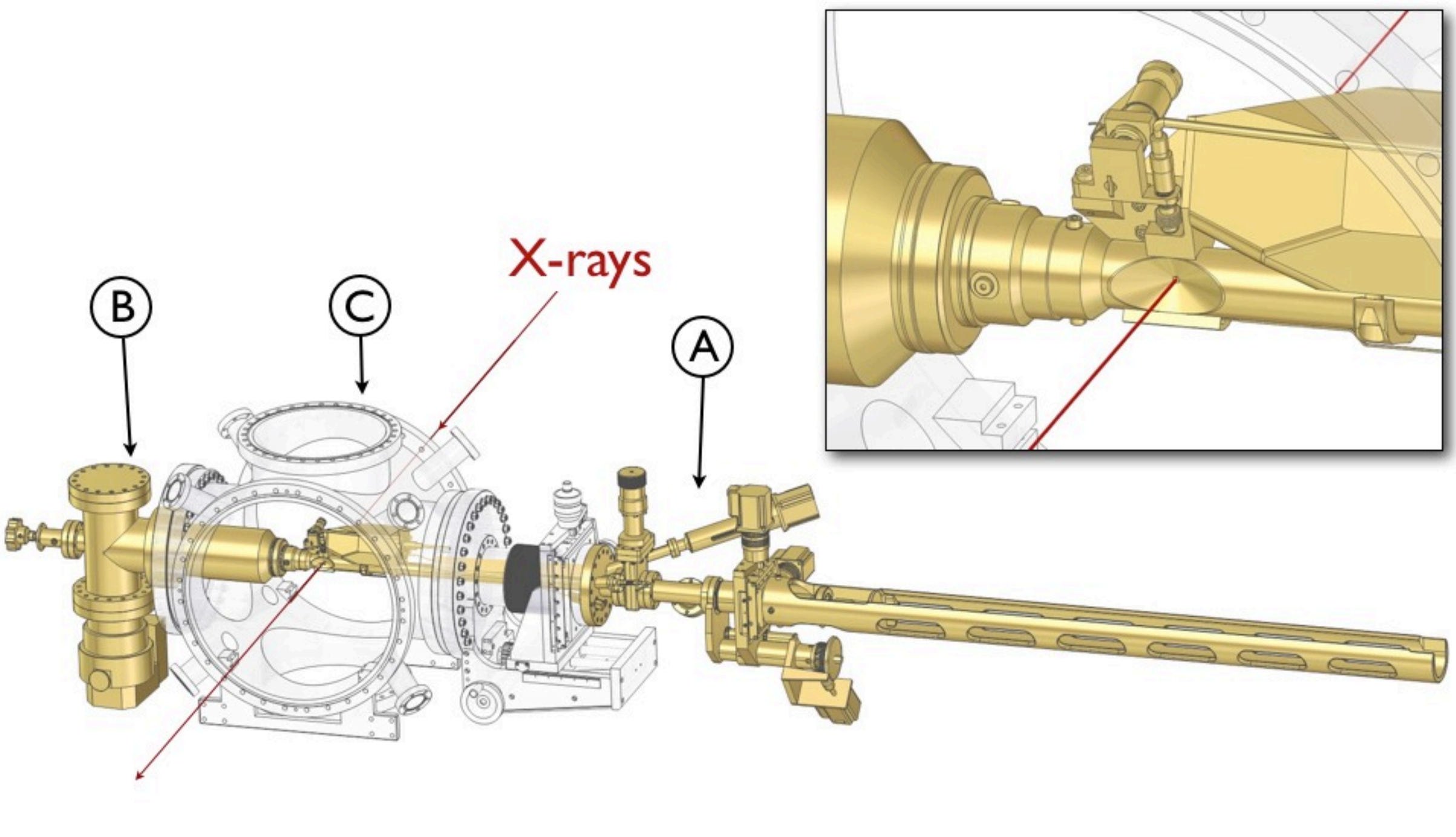


To operate the nozzle in high vacuum
we need differential pumping




**..... plus a pump laser for photoexcitation of the sample**

# Injector in Vacuum Chamber



# LCLS beam on liquid jet

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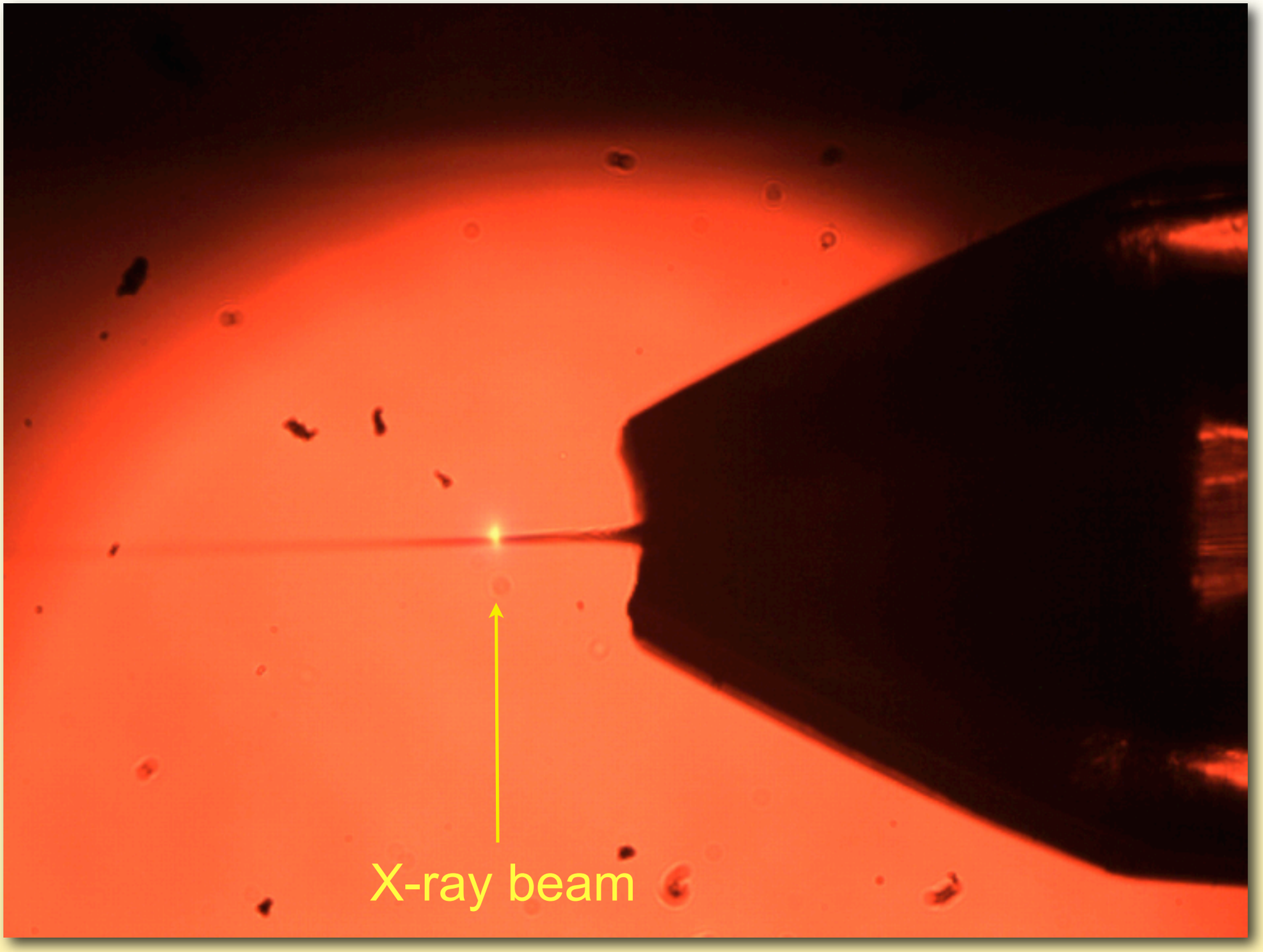
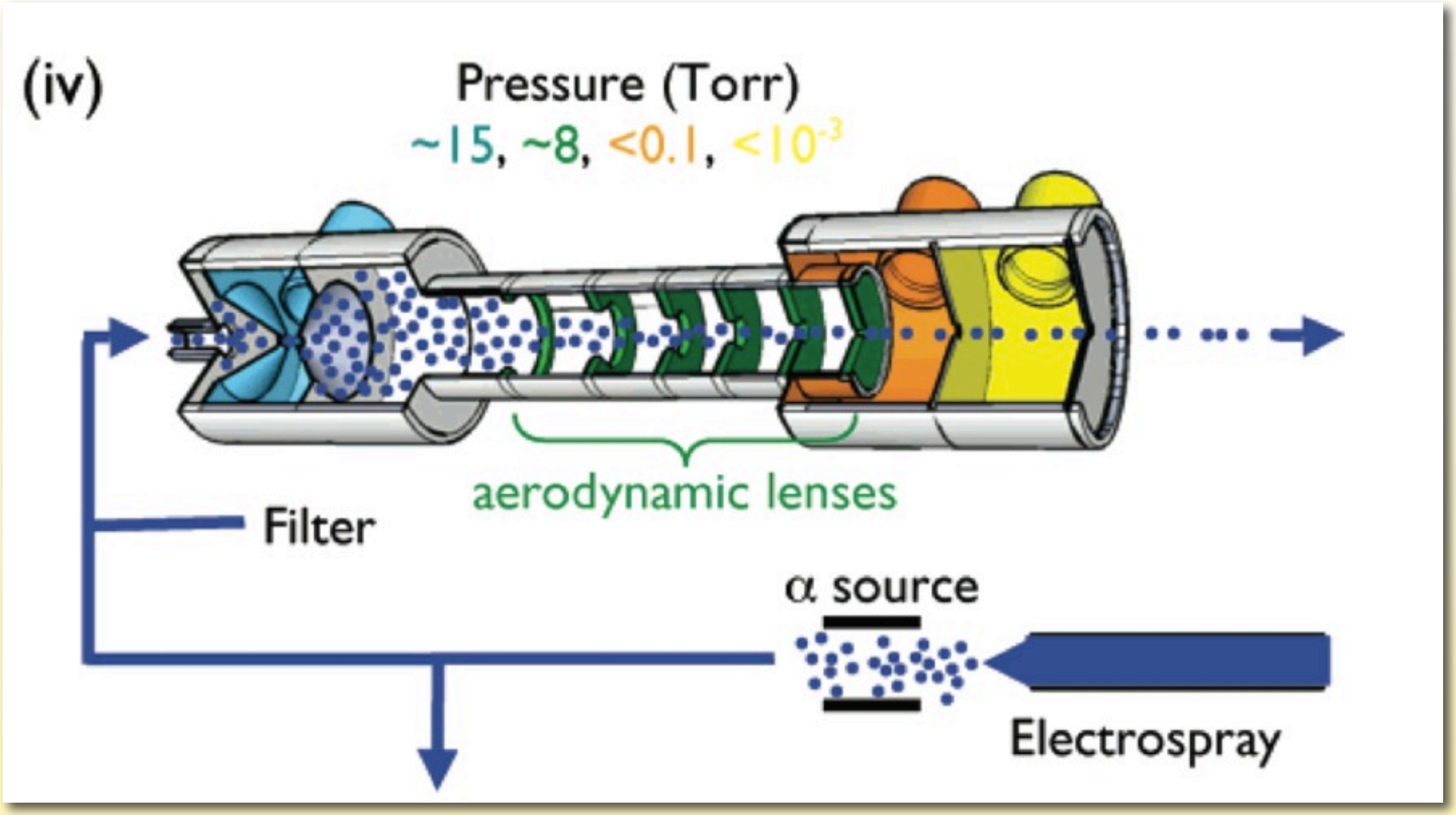



viewed with in vacuum microscope

# Aerosol Aerodynamic lens injector (LLNL and Uppsala)




developed at LLNL and Uppsala University
Uses Nebulizer or Electrospray to aerosolize particles
removes most of the water,
particle density at X-ray interaction region much lower.

# Mimi virus diffraction pattern (LCLS 2009)

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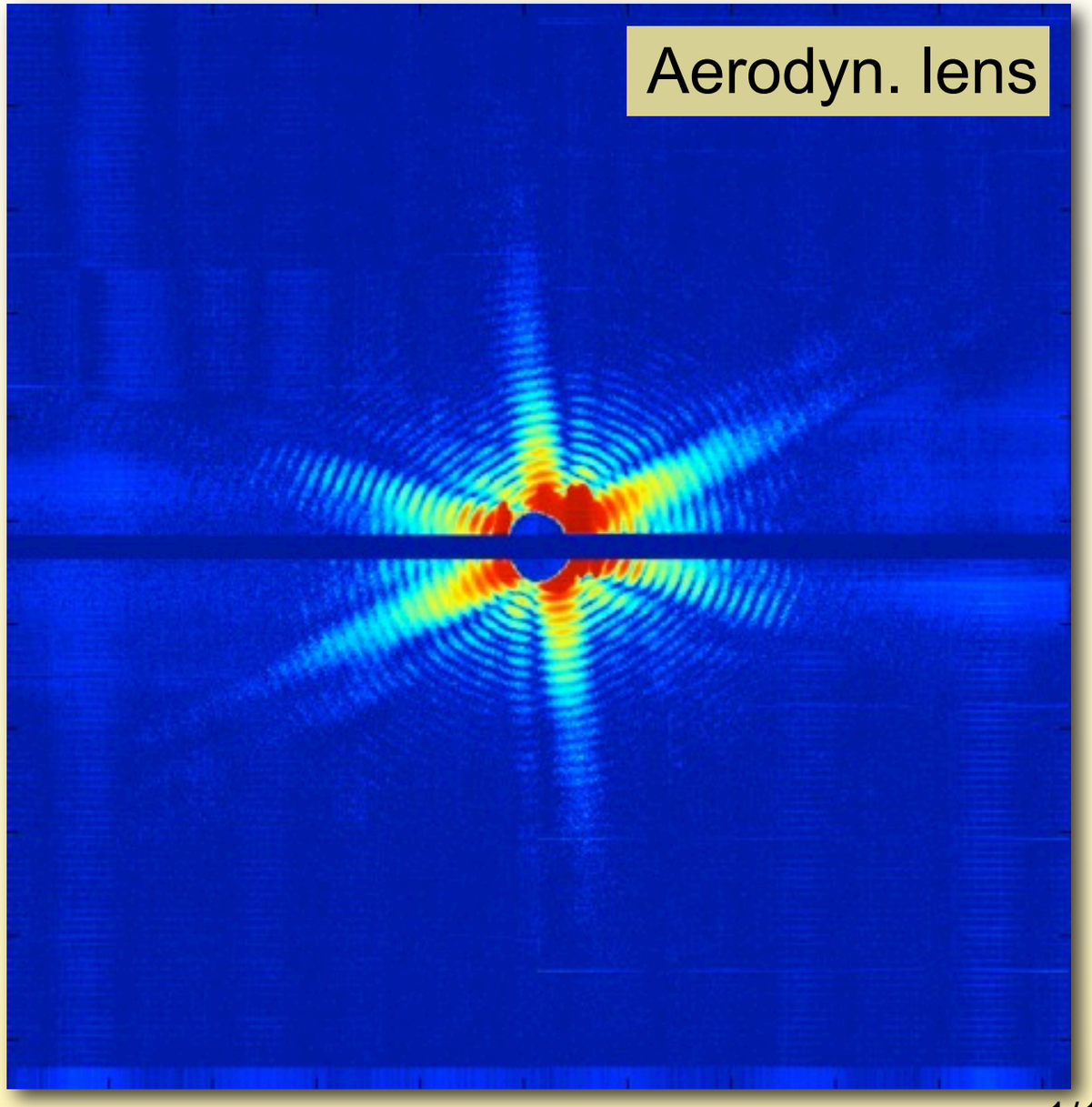
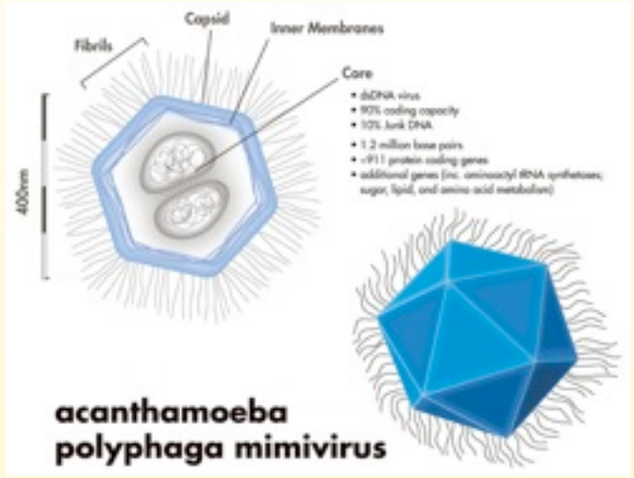



wavelength: 0.68 nm

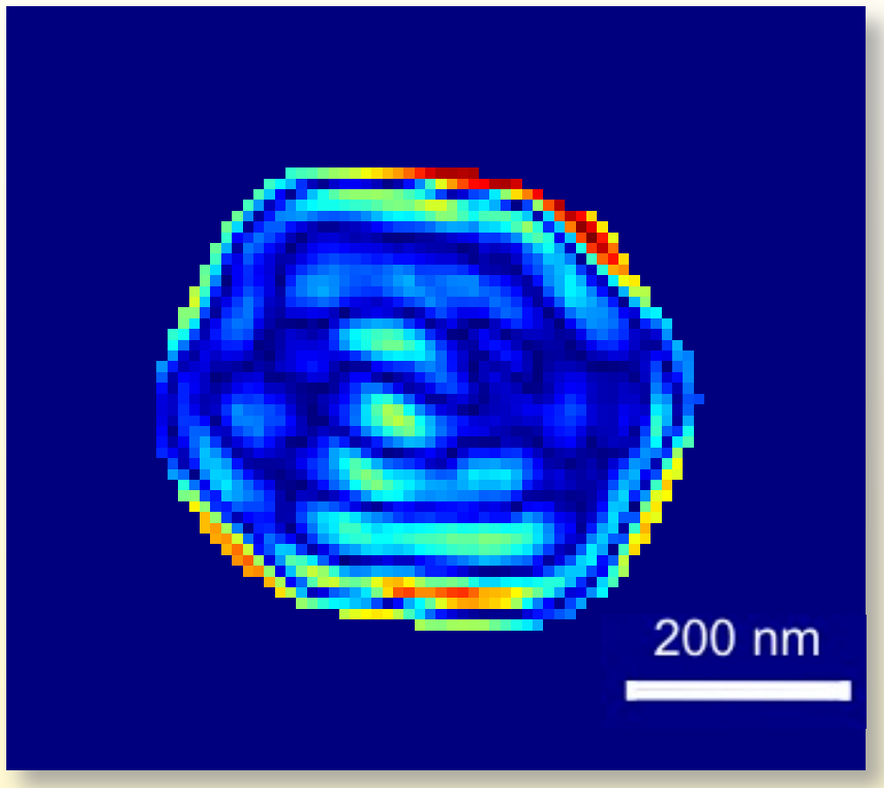
pulse duration: 70 fs

$6 \times 10^{12}$ photons per pulse

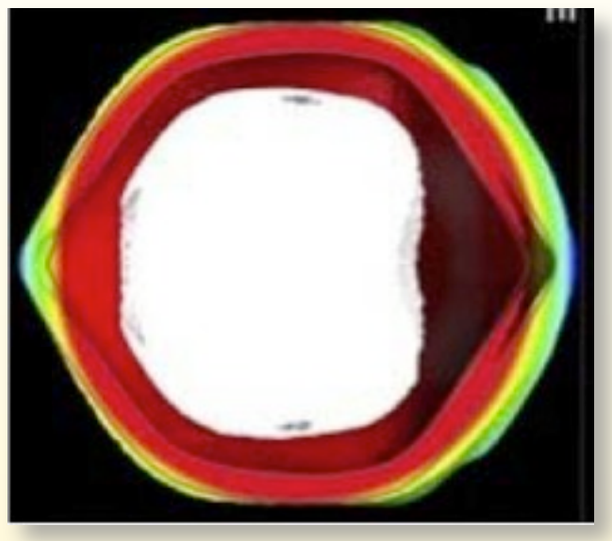
Aerosol injector

Samples Janos Hajdu, Uppsala University

# Single shot Mimi virus reconstruction



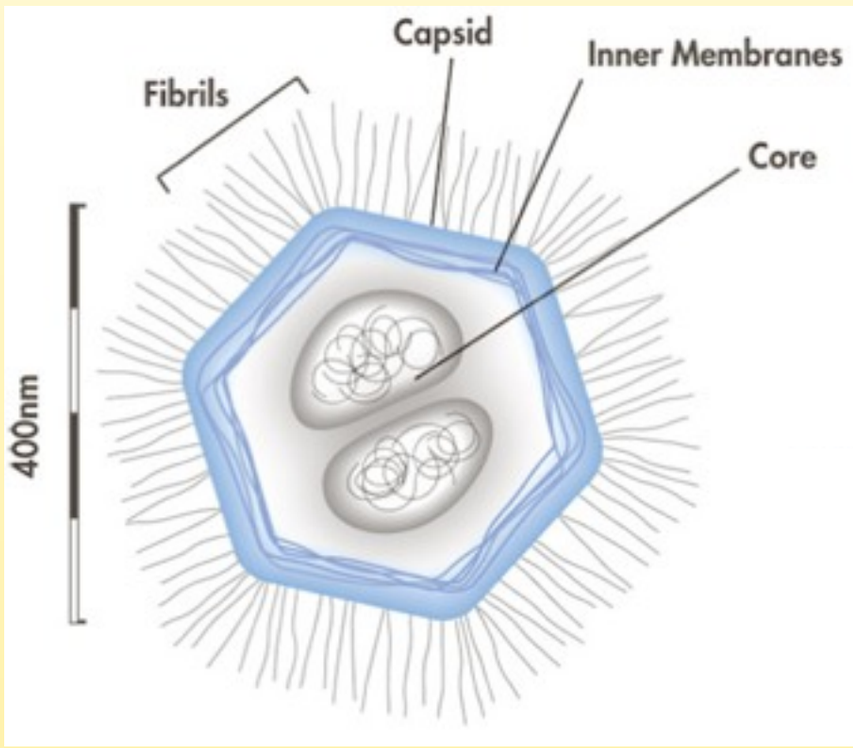

single particle reconstruction



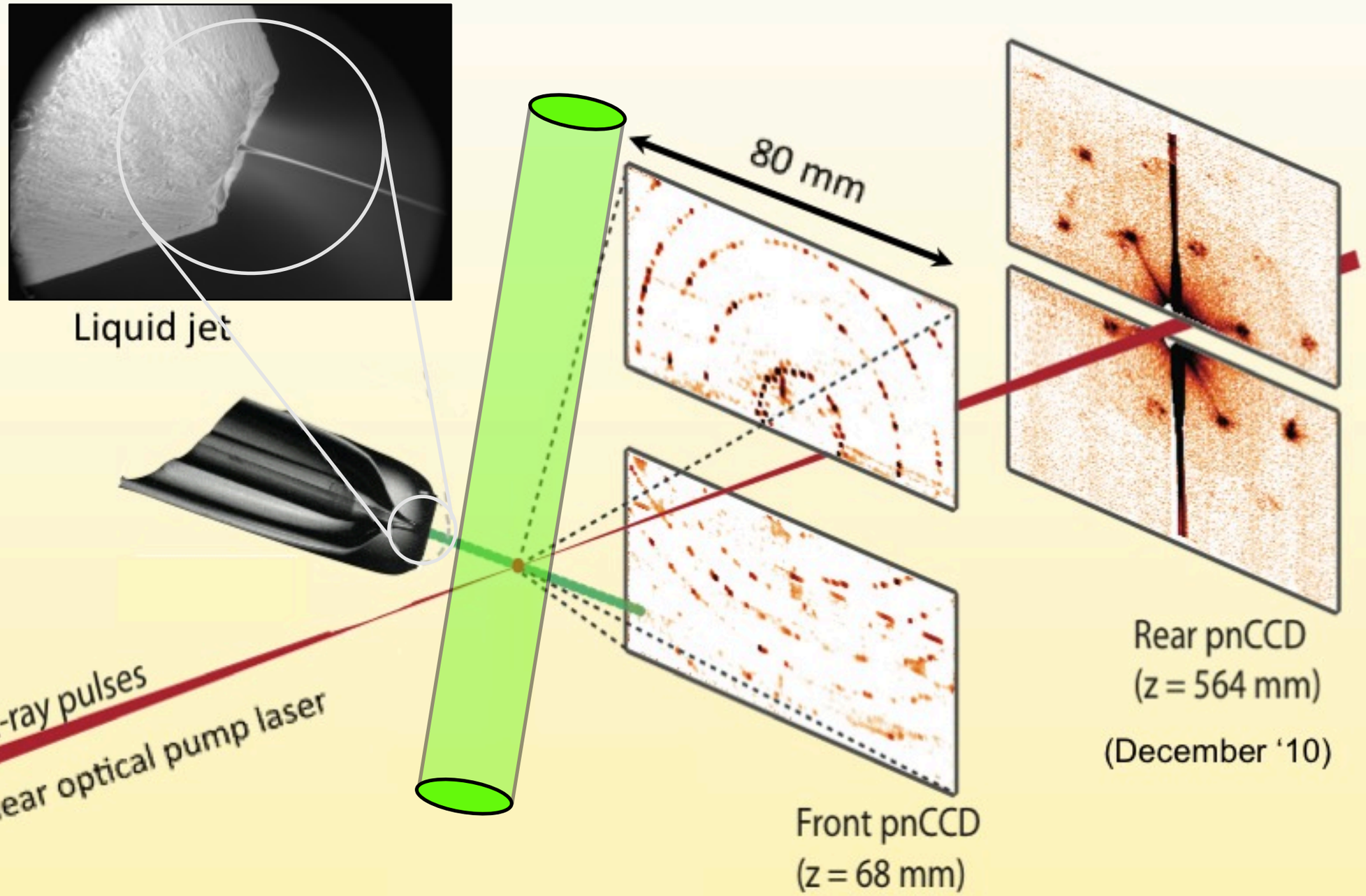
Cryo-EM:

30,000 images

C. Xiao et. al. PLoS Biology, 2009
vol. 7 (4) pp. 958-966



# Experiment geometry: nanocrystallography



# Why nanocrystals?

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**Growth of large crystals is the main bottleneck to structure determination by Protein Crystallography!**

**Solve more protein structures**

use showers of nanocrystals which may already be present in crystal growth screens, nanocrystals may be more perfect (Von Dreele)

**Dynamics**

irreversible transient conformational changes can be monitored

---> molecular movies

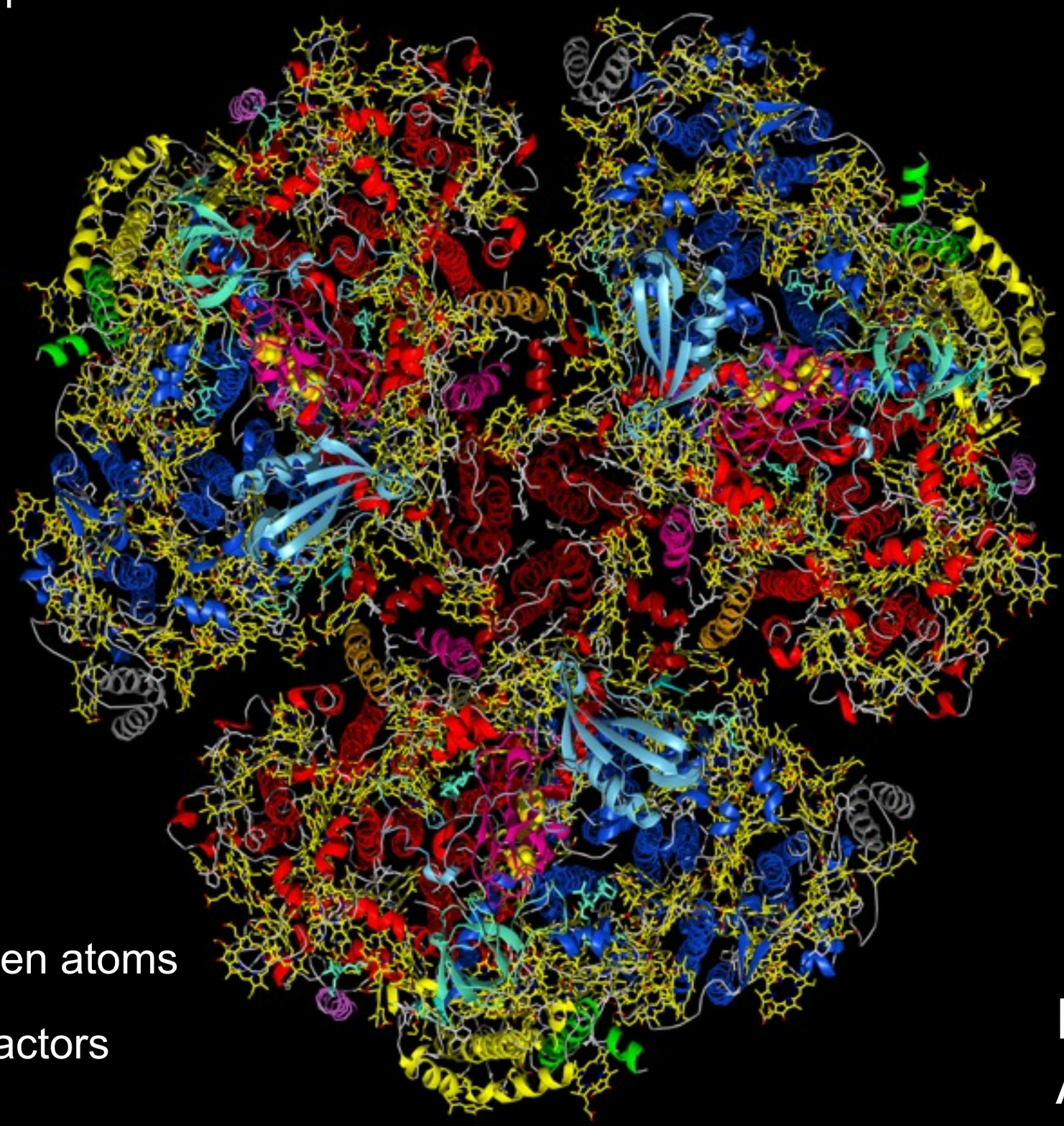
**New solutions to the phase problem**

- Nanocrystals allow sampling of diffracted intensity between Bragg reflections

# Nanocrystals of Photosystem I were produced in the Fromme Lab at ASU

ASU
Arizona State University

it took 13 years from first observation of microcrystals to atomic resolution structure



photosynthesis in plants and cyanobacteria

Photosystem I

~72000 non-hydrogen atoms

36 proteins 381 cofactors

Petra Fromme, ASU

# Photosystem I (PSI)

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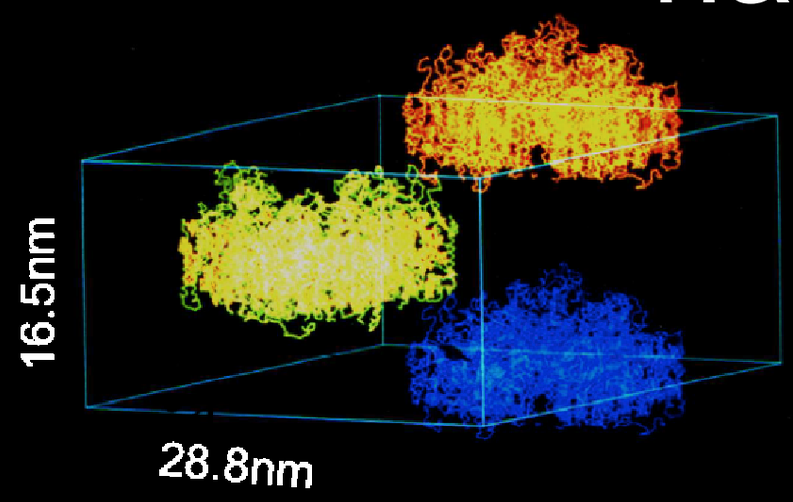
## PSI forms hexagonal nanocrystals

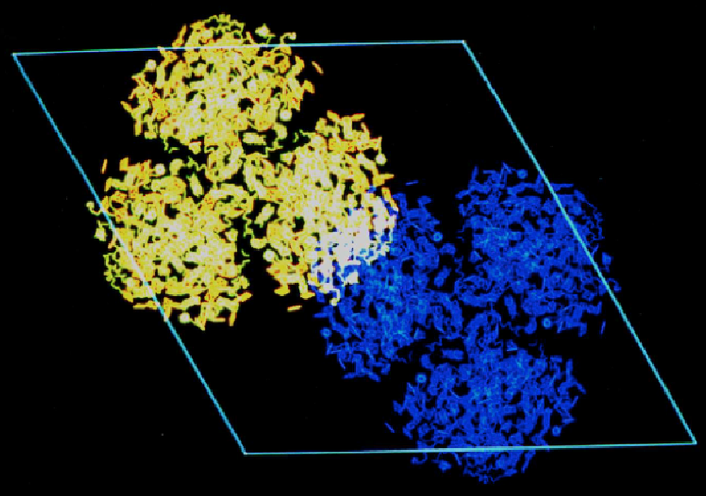



Photosystem I Protein was isolated from thermophilic cyanobacteria and crystallized at low ionic strength.

$P6_3$ space group

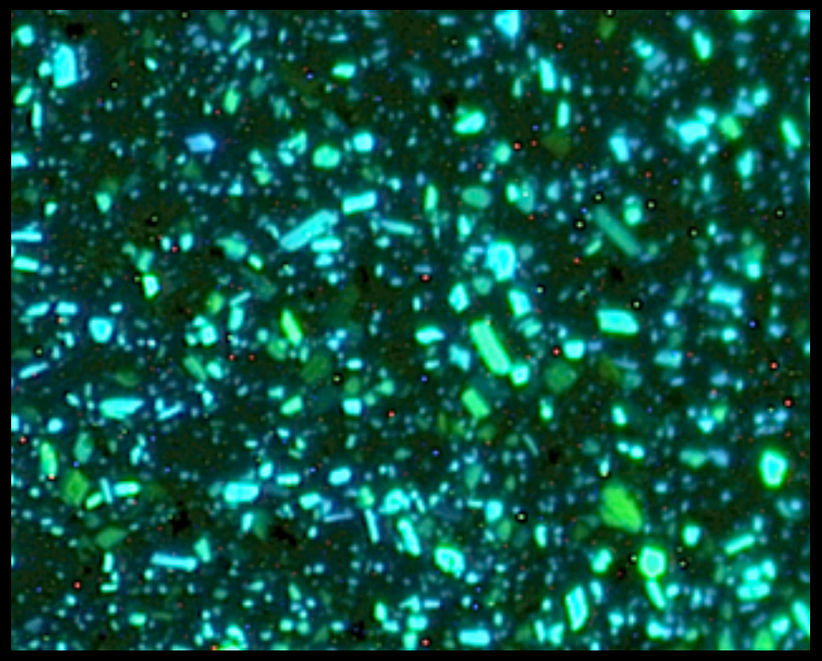
a=28.8, c=16.5nm

78% solvent



two trimers per cell

PSI microcrystals viewed through crossed-polarizers



# The crystals are sub-micron in size

14 fringes = 400 nm

cut through
shape transform

Lattice transform:

$$I(q) \sim \frac{\sin^2(Nq \cdot a)}{\sin^2(q \cdot a)}$$

**PSI at 1.8 keV**
**far detector**

9 fringes = 260 nm

# Shape transform can be inverted by iterative phasing

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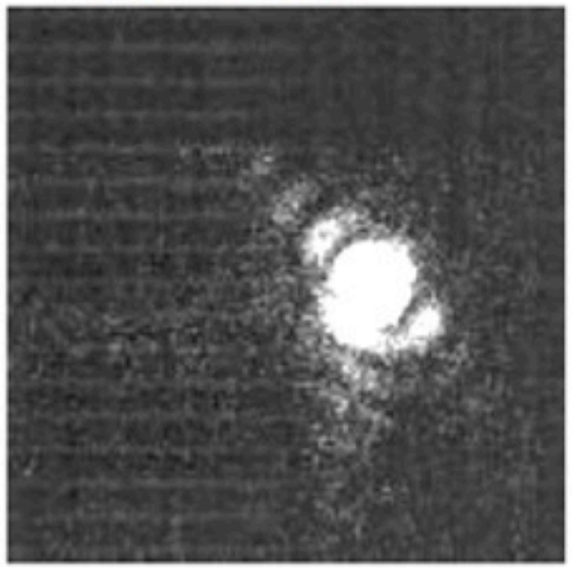
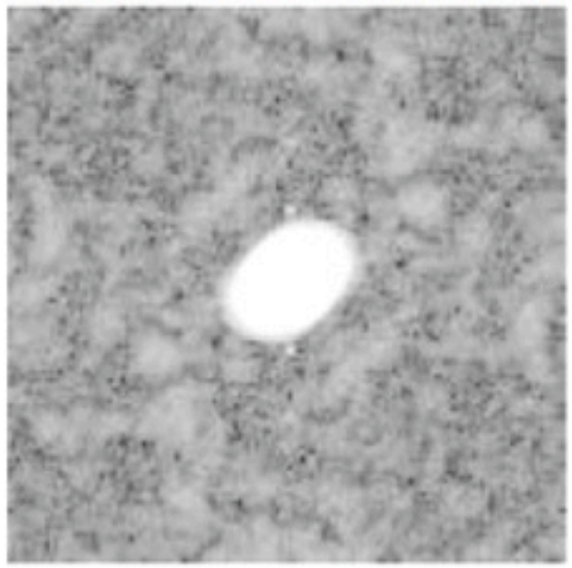
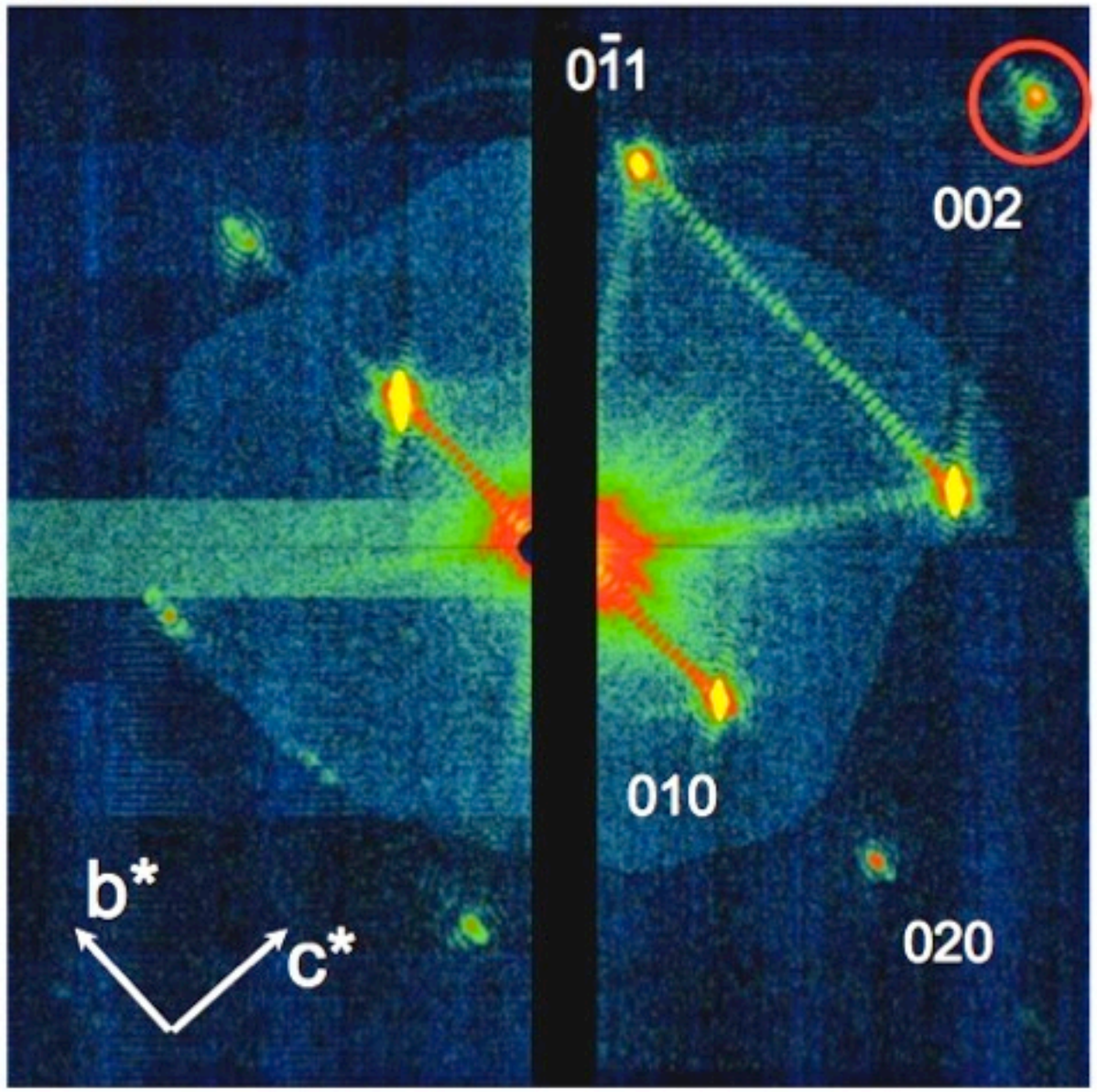
Original data

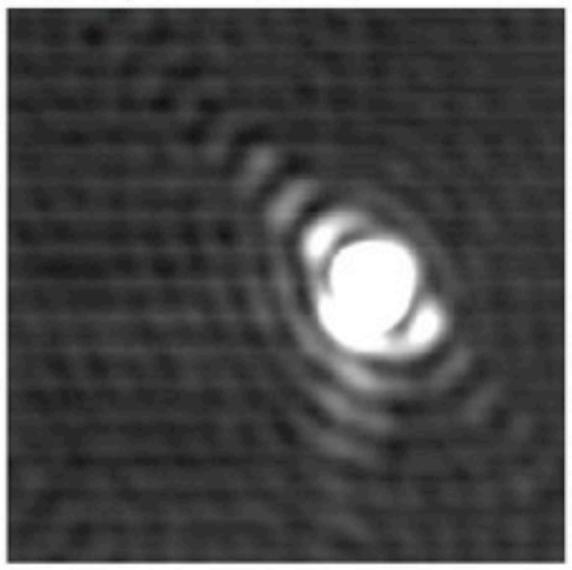


Autocorrelation intensity (log scale)

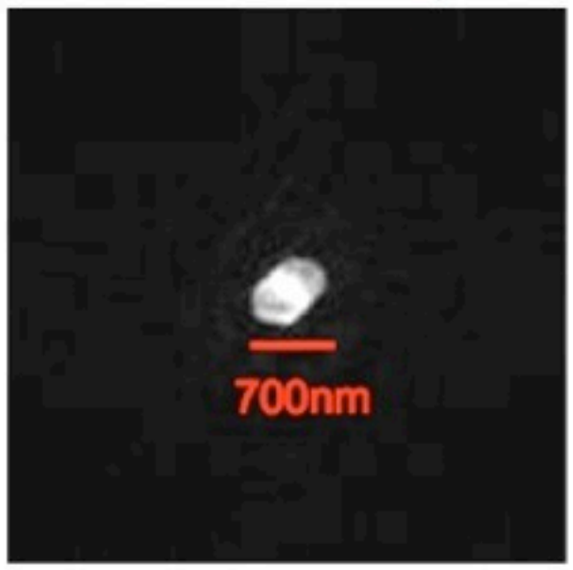





Low pass filtered

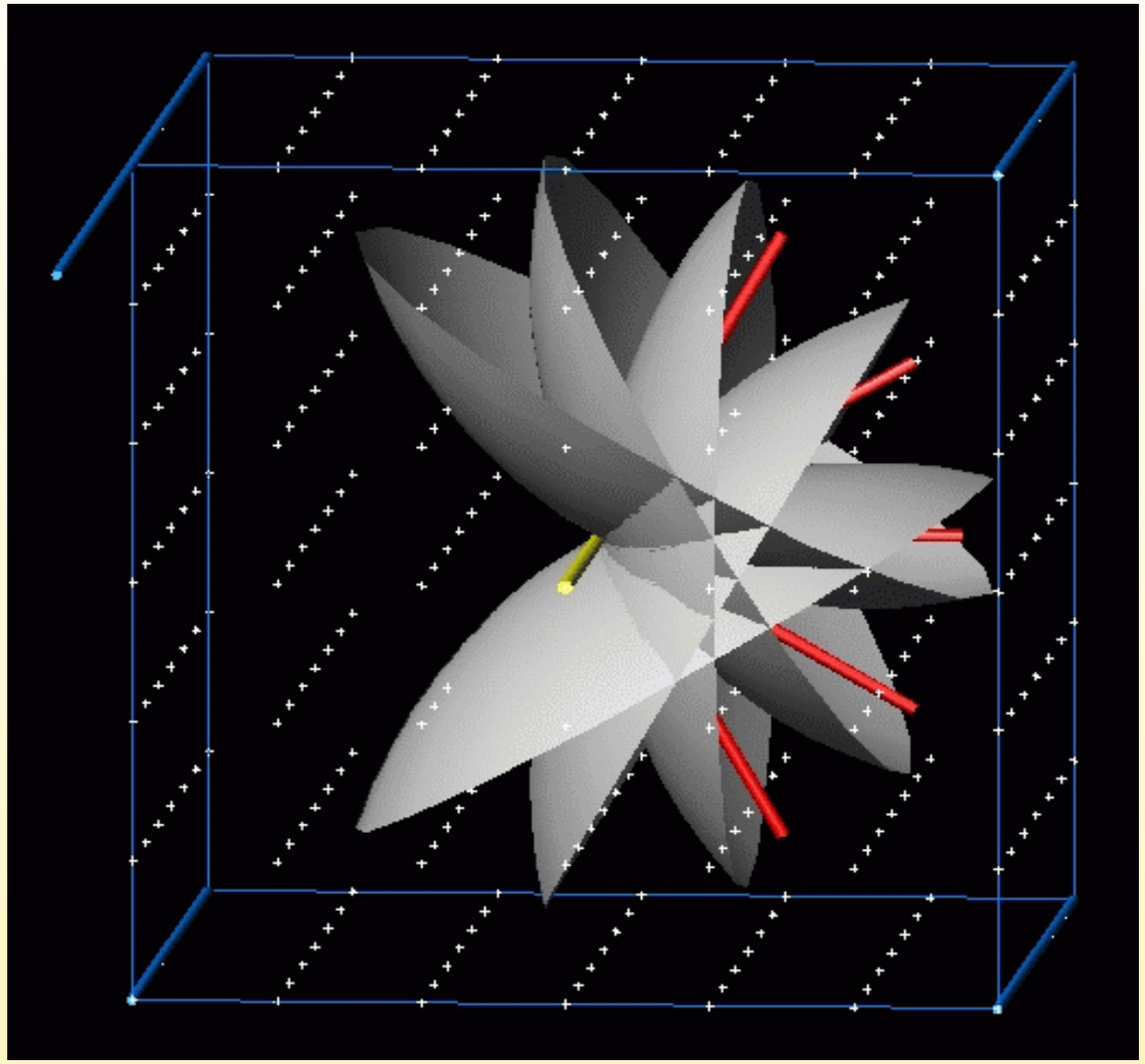


Reconstructed amplitude



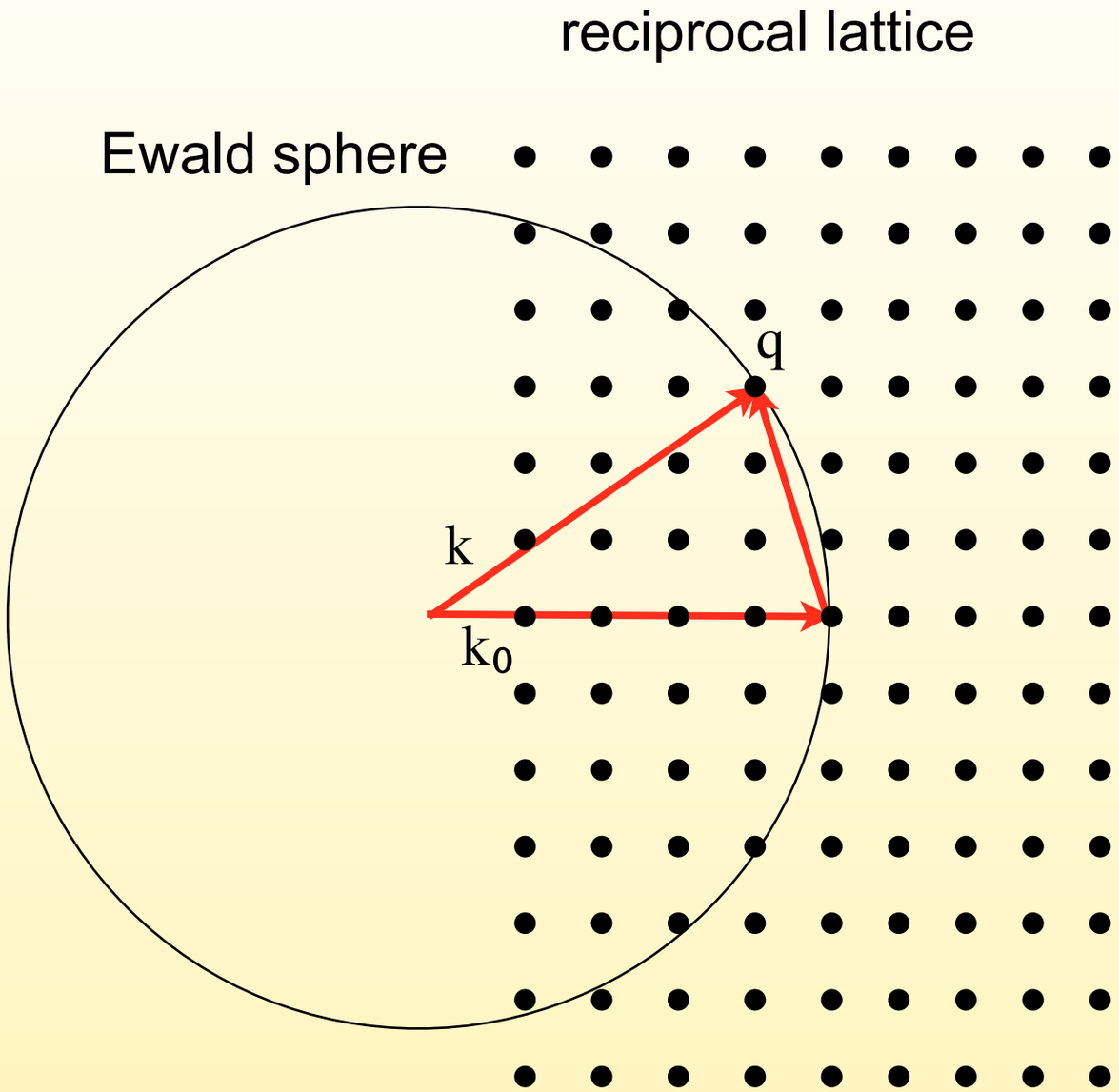

Andrew Martin, CFEL DESY

# Structure determination requires structure factor analysis



Conventional crystallography

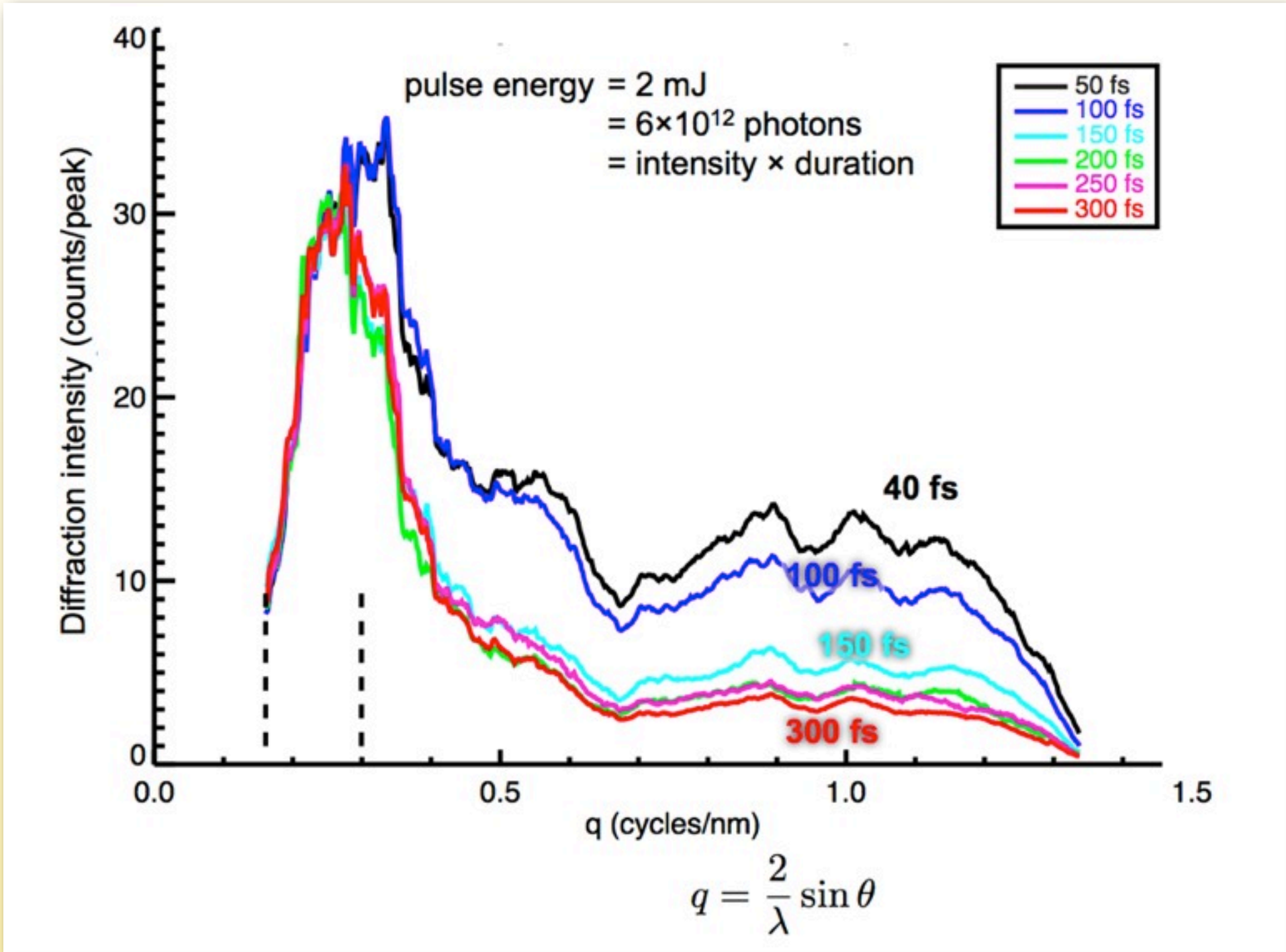
oscillation method integrates reflection intensities




Snapshot nanocrystallography

Random slices through shape-transformed reflections. Distribution of shape transforms due to crystal size distribution.

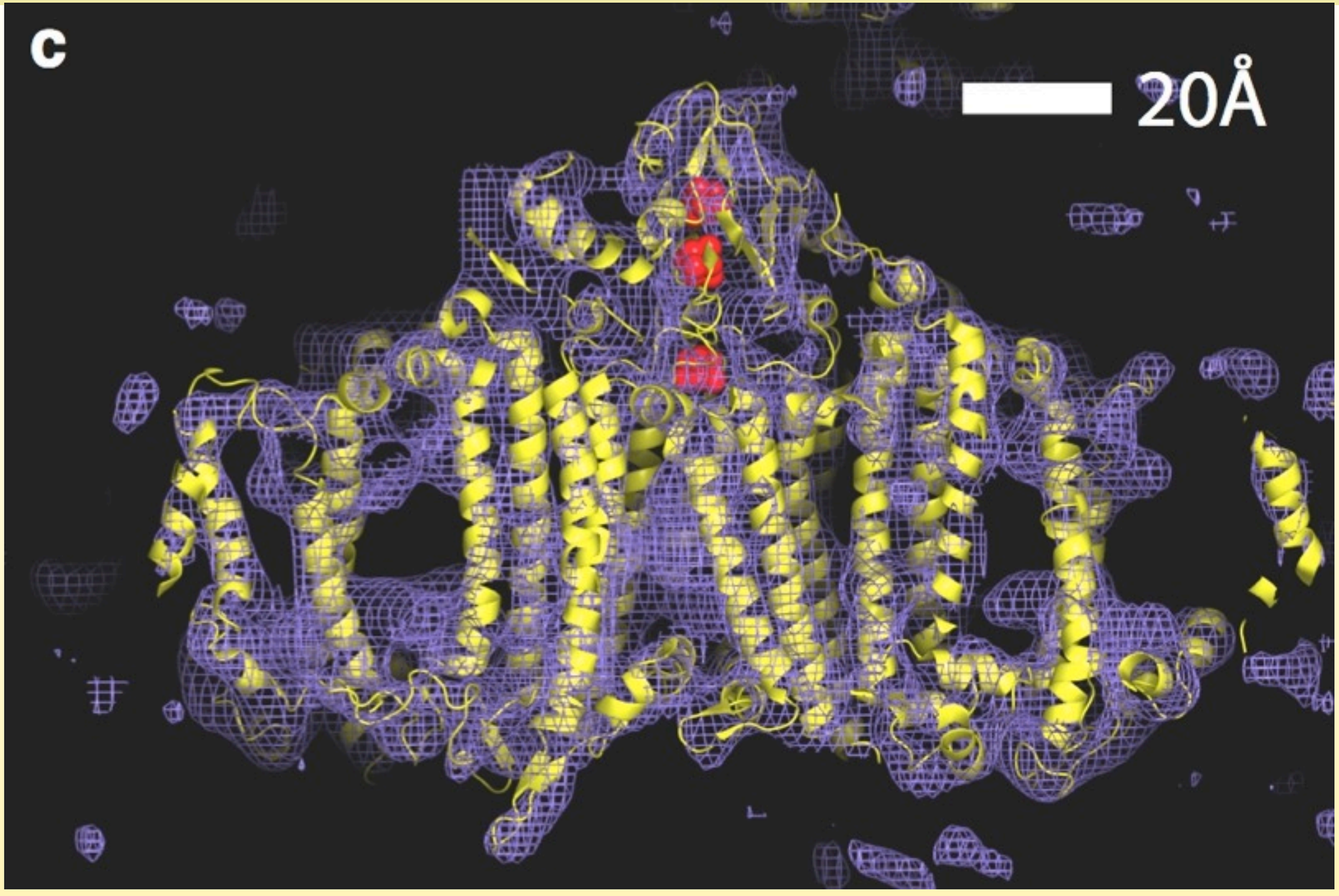
# Degradation of the sample at longer pulse duration




**Dose:**
**670 MGy**

**22 times more then considered safe in PX**

# Molecular replacement reconstructs the 9 Å structure

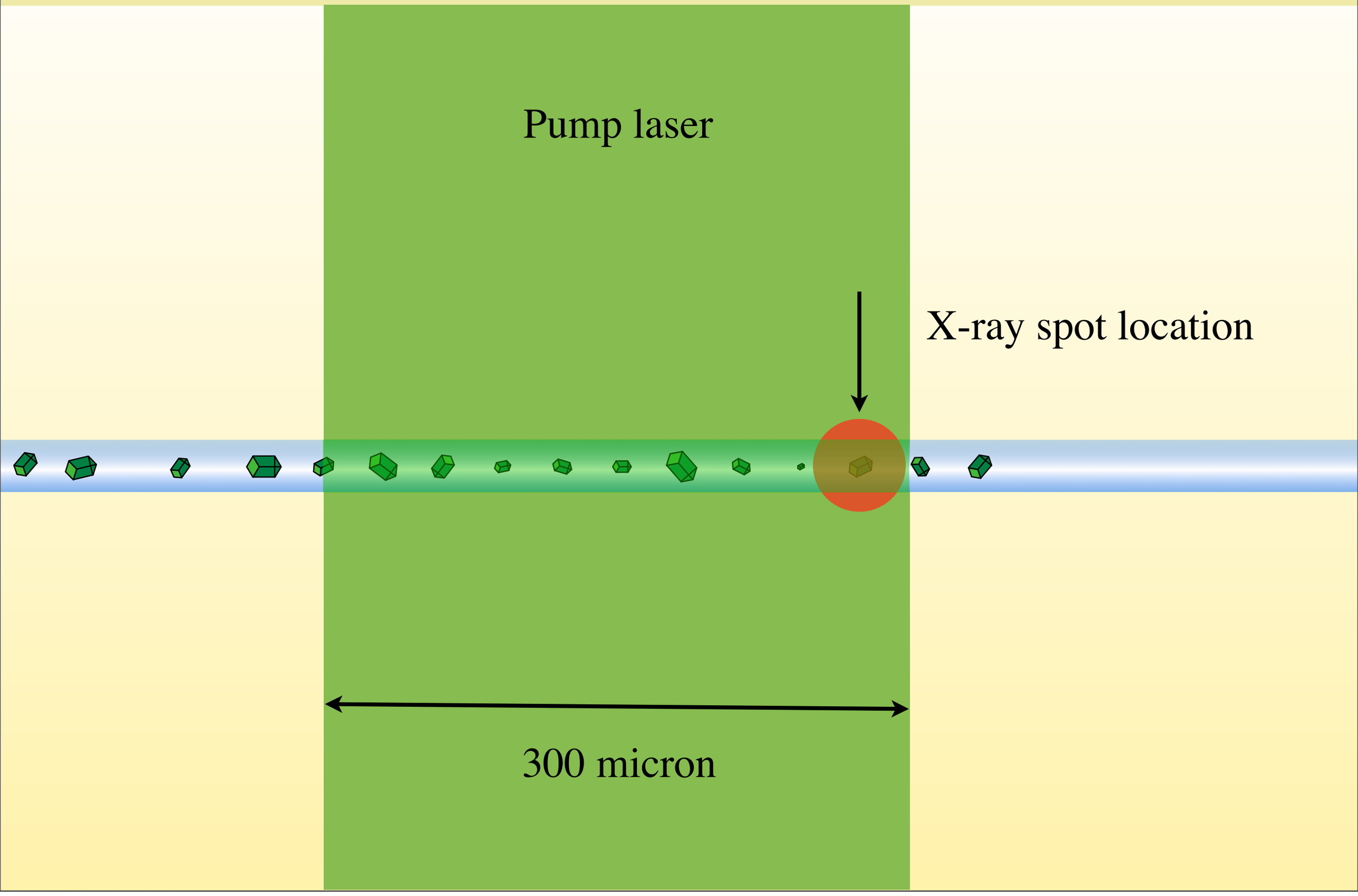



(c) Density map (purple) of PS I at 9 Angstroms resolution using 70 fs structure factors extracted from LCLS data in Dec 2010, and Molecular replacement for phases from PDB (1JB0).

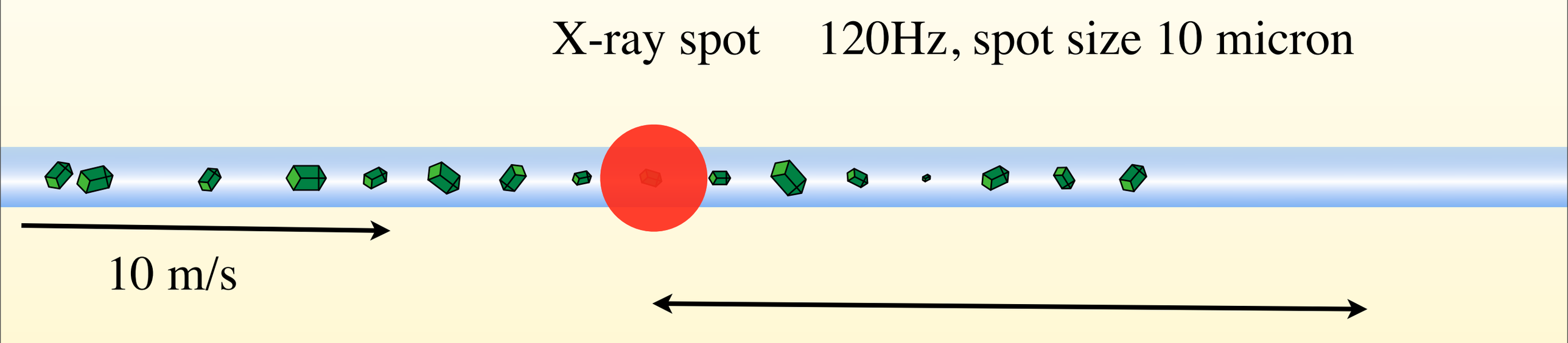
(d) An electron density map calculated from conventional synchrotron data truncated to 8.5 Å resolution, collected at a temperature of 100K.

Raimund Fromme, ASU, Tom White, CFEL, James Holton, LBLN

# Femtosecond time-resolved measurements of photoinduced dynamics



# Sample waste: nanocrystallography

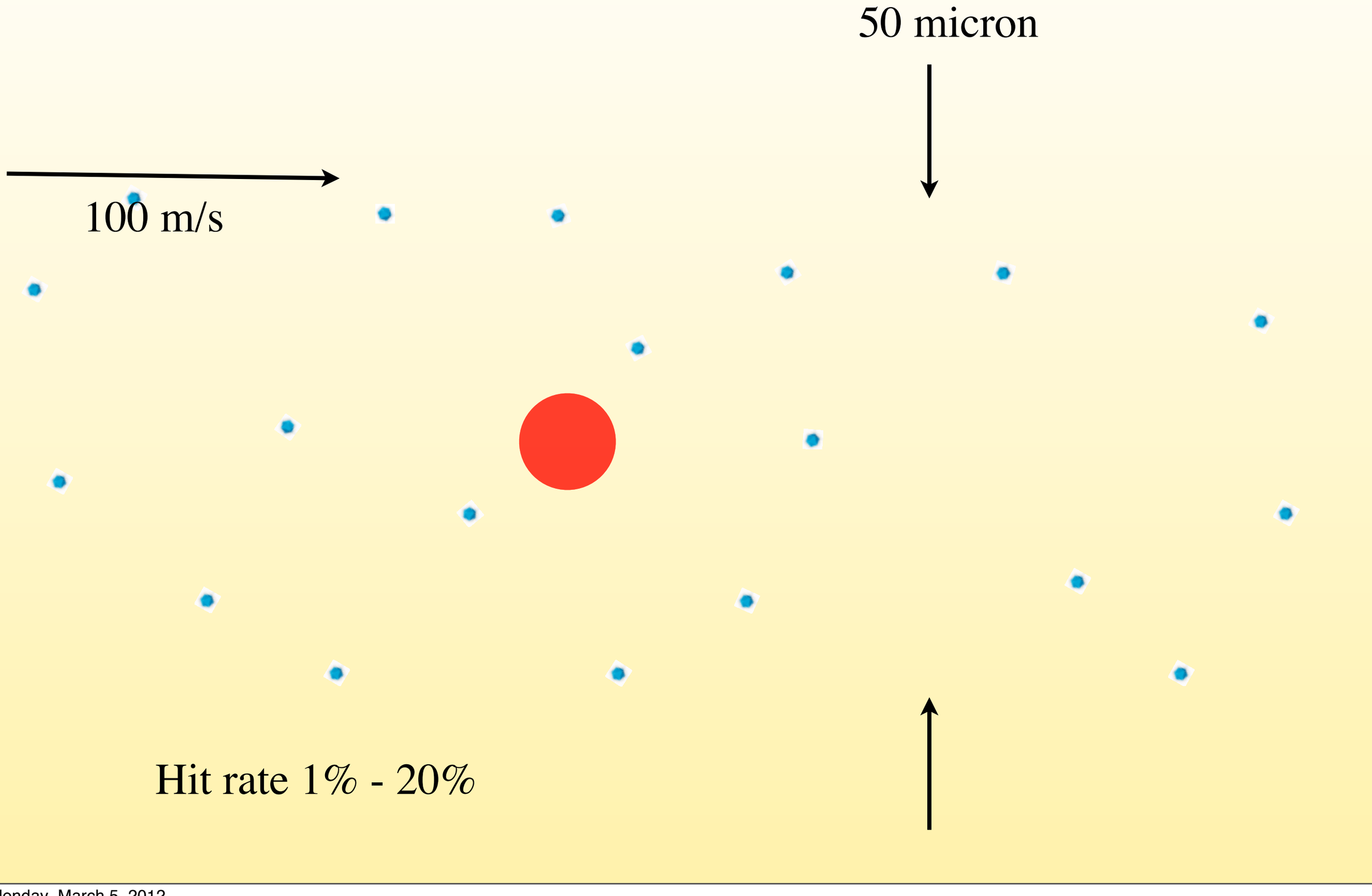



would need 1Mhz rep. rate to hit everything!

need:

- higher pulse repetition rate
- better intensity and spectral stability in the X- ray pulse.

# Sample waste: single particle



# single particle requirements: Intensity . . .

- resolution  for viruses is currently not limited by the X-ray wavelength or pulse duration, but  by structural inhomogeneity in the merged data,  by background noise, and by the limited dynamic range of detectors.

- total scattering intensity falls of as $q^{-4}$ for single particles.

- therefore need higher dynamic range of detectors, reduced background electronic and readout noise.

- The fact that we get good patterns from 10x10x10 molecule nanocrystals, which give 1000 times more total scattering than one molecule, suggests that we need an increase in fluence of 1000 to detect single shot, single molecule diffraction.

- higher hit rate (currently 1% - 20%) -- Injector improvements

# for non-reproducible particles: several views in one shot

ARIZONA STATE UNIVERSITY

PRL 101, 115507 (2008) PHYSICAL REVIEW LETTERS week ending 12 SEPTEMBER 2008

## Tomographic Femtosecond X-Ray Diffractive Imaging

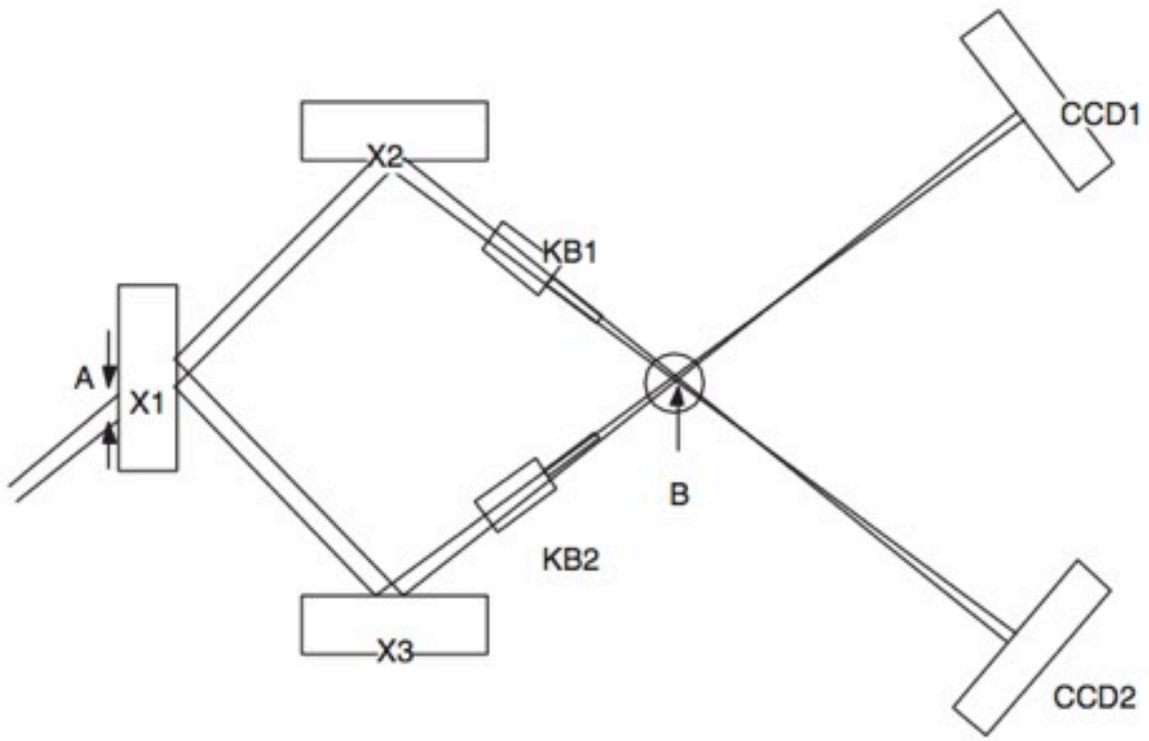



FIG. 1. Scheme for tomographic femtosecond diffraction, drawn for only two beams for simplicity. Beam splitter $X1$ is set to the dynamical 3-beam diffraction condition. Crystals $X2$ and $X3$ operate at the 2-beam dynamical condition. KB1 and KB2 are focusing optics for the target at $B$, with area detectors CCD1 and CCD2.

single cells are non-reproducible on a molecular level : only one shot possible

for identical particles:
3 views allow orientation determination for each particle.

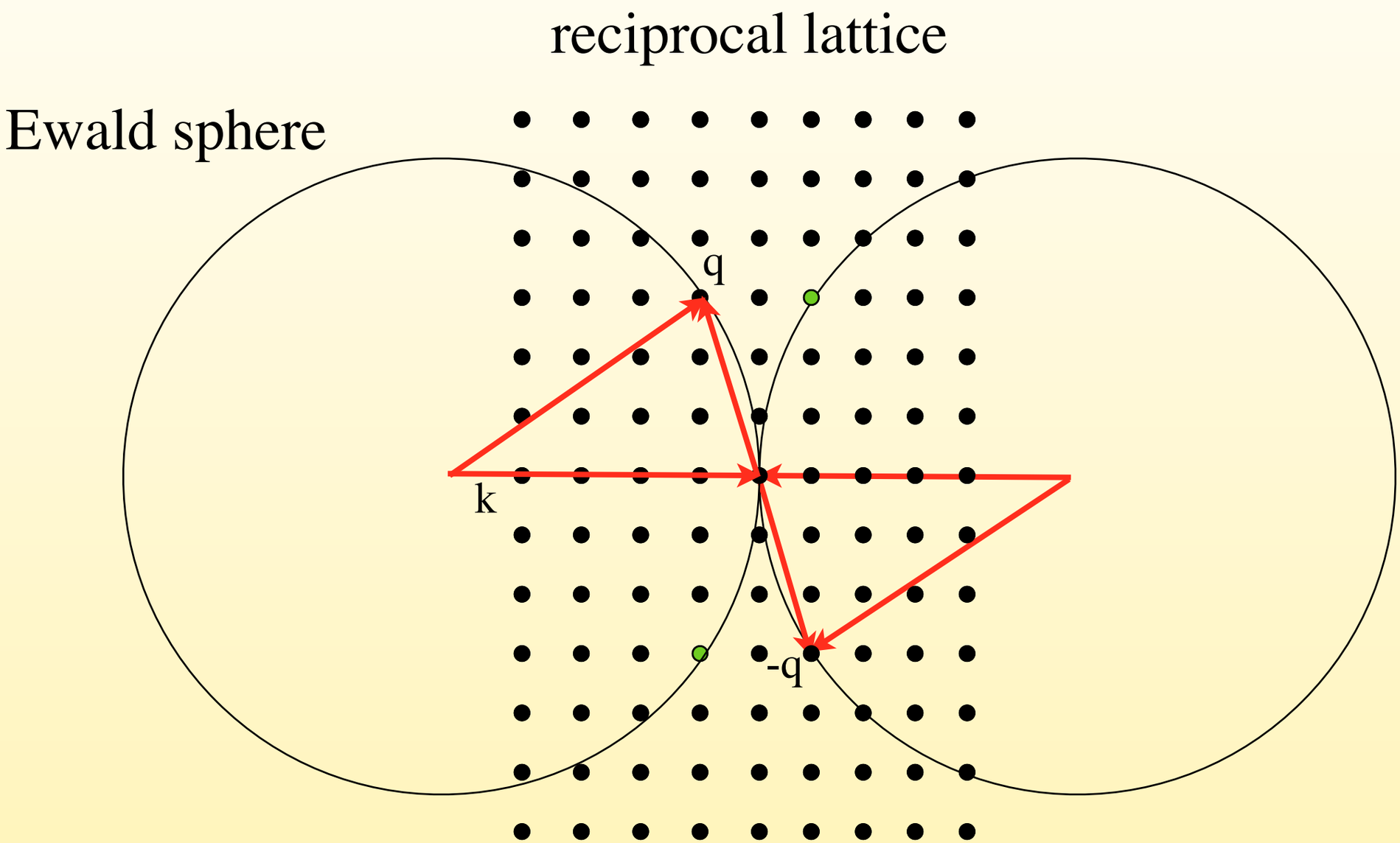
Delay line with beam splitter: Journal of Synchrotron Radiation, (2011) 18(3) p. 481–491

# nano-crystallography requirements:

- need higher dynamic range of detectors, reduced background electronic and readout noise, more pixels.

- need to reduce sample waste: Higher repetition rate of FEL (MHz)

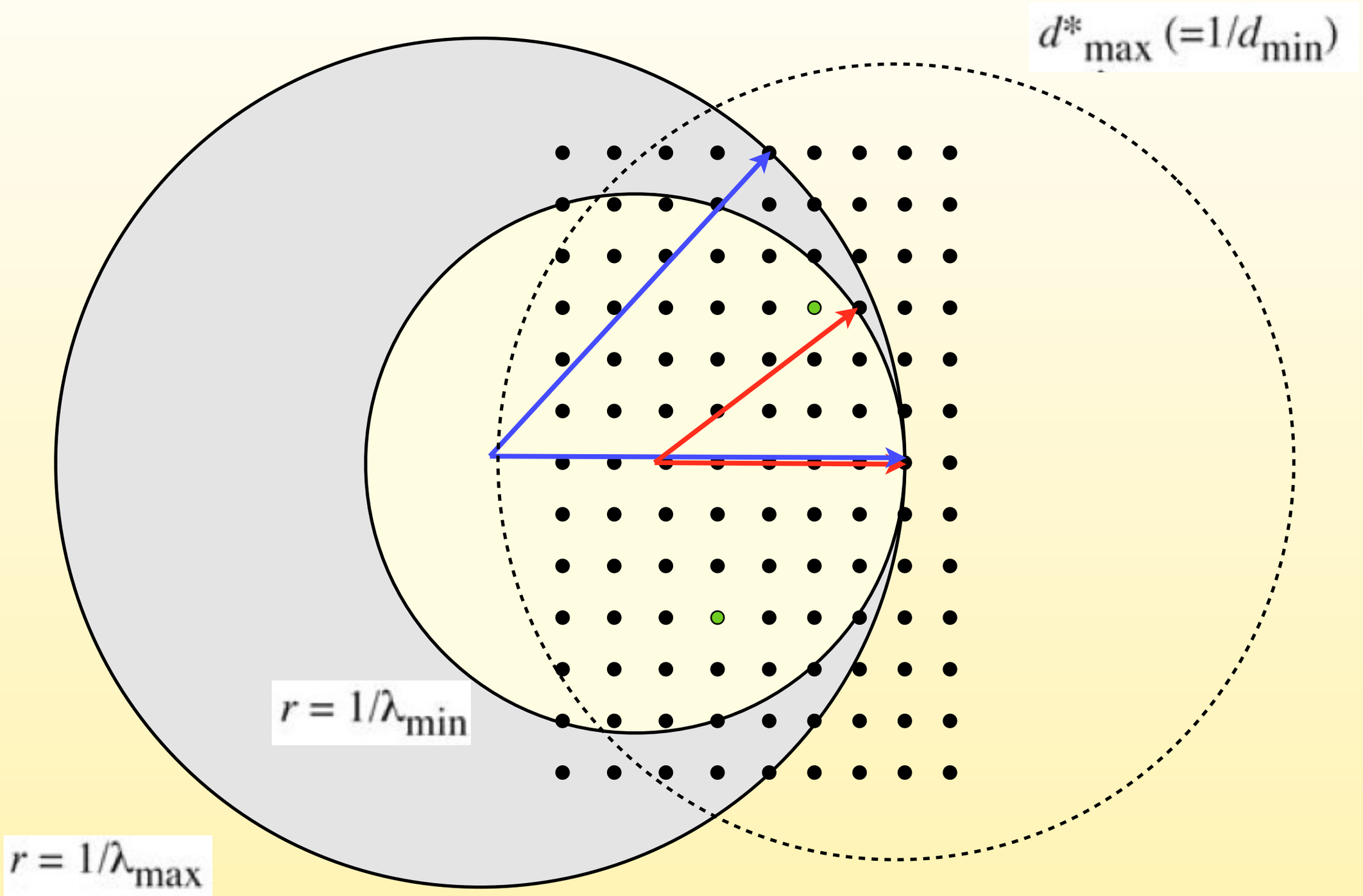
# MAD Friedel pairs in one shot?




two opposing beams from a beam splitter
hit the crystal at the same time

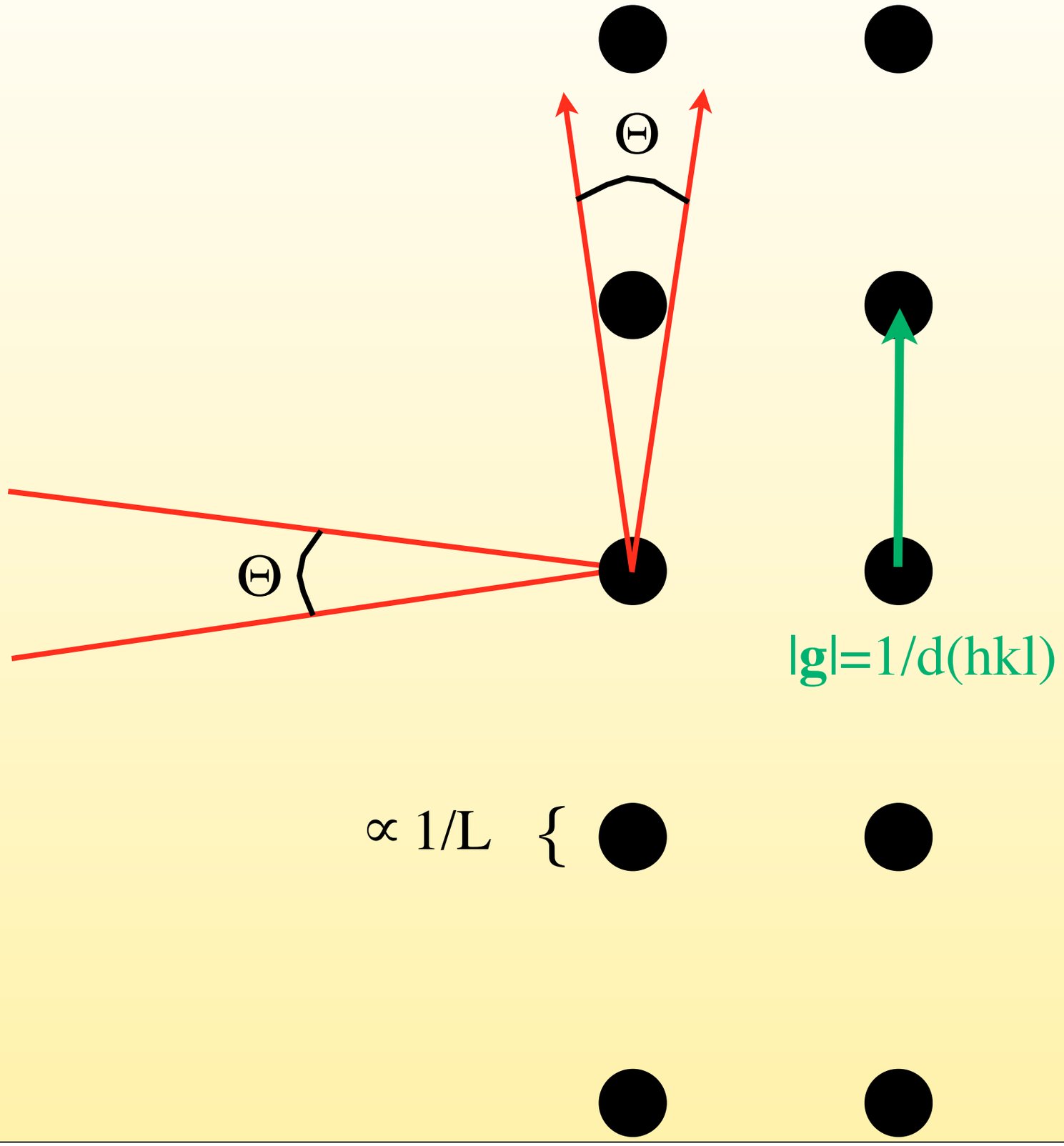
James Holton,
LBNL

# Full reflections in a single shot - Laue Diffraction




Cornaby et al. recently solved a structure using 5 Laue patterns from 5, 20μm crystals. ( Acta Cryst. D66, 2 (2010) )

# Will convergent-beam mode integrate partial reflections?




Beam Divergence needed:

$\Theta = (1/L)/g = d(hkl)/L$
$= 30nm/1000nm$
$= 30$ mrad

for PSI crystal with
L = 1 micron, first order

3 mrad for 10th order.

LCLS: 1 mrad

high orders are already CB!

# Summary

Diffract-before-destroy works at high resolution (0.3 nm) for delicate membrane proteins, fully hydrated. (Catepsin B, PSI, Reaction center, Lyzosyme)

Solve invisible xtals in mother liquor? Reduce crystallization bottleneck in protein crystallography. More perfect crystals for better resolution ?

Use short pulses instead of freezing to reduce damage, work at room temperature.

Dynamic studies - pump-probe for irreversible processes is possible.

**Wish list:**

Laue for time-resolved studies (XFEL with $\lesssim$ 30% Bandwidth)

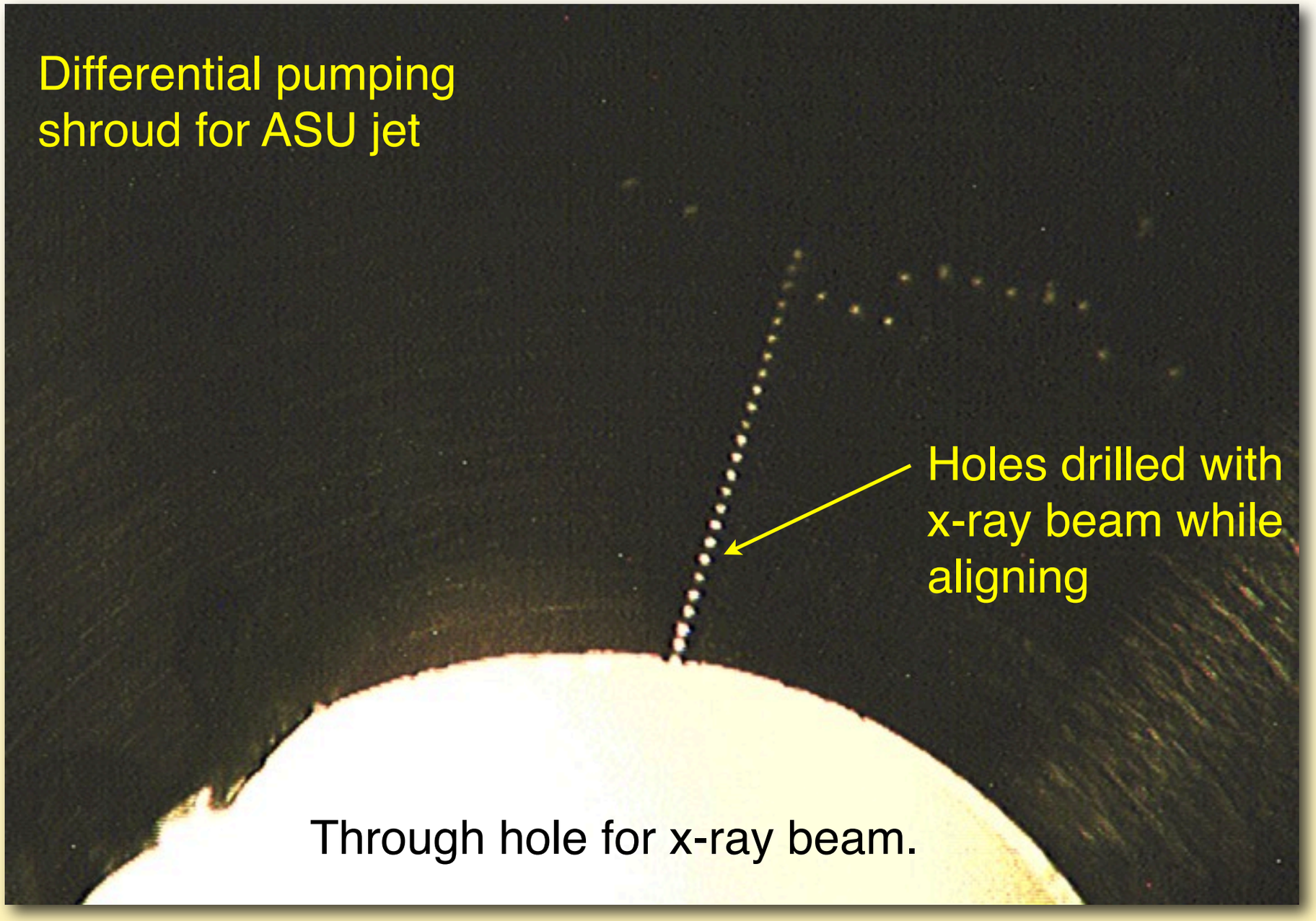
Convergent Beam to partially integrate Bragg intensities (Beam divergence smaller Bragg angle, e.g. $\lesssim$ 30mrad)

Multibeam schemes for MAD and single particles would be desirable.

More intensity (factor >1000 for single molecule) is needed.

Higher repetition rate for nano-crystallography (MHz) is needed.

# What you can do with the LCLS . . .



# The End