Imaging Molecular Structures using Short Intense X-ray Pulses

Henry Chapman
Center for Free-Electron Laser Science
DESY and University of Hamburg

Nobel Symposium, June 2015

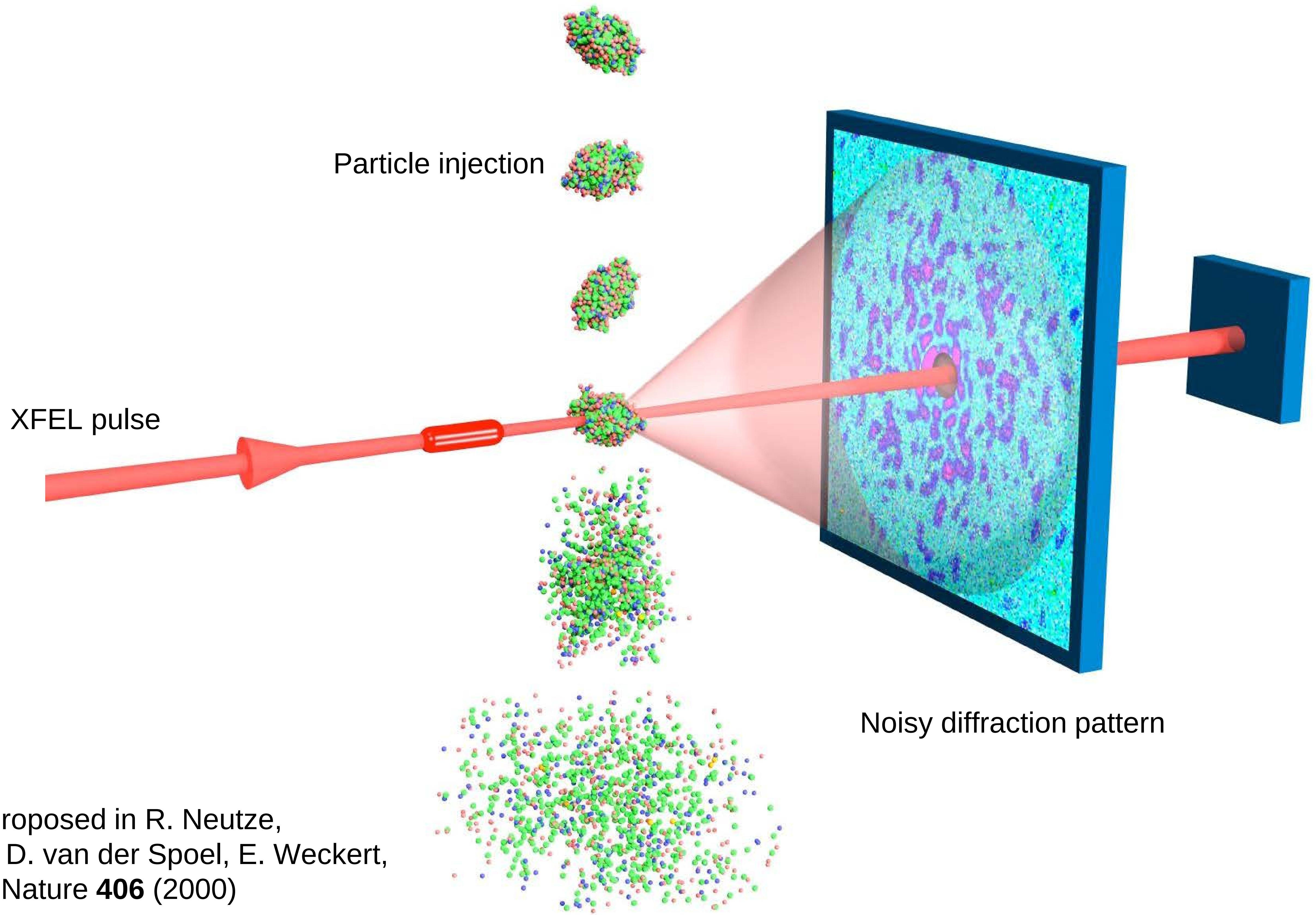
CUI THE HAMBURG CENTER FOR ULTRAFAST IMAGING

DESY

UHH

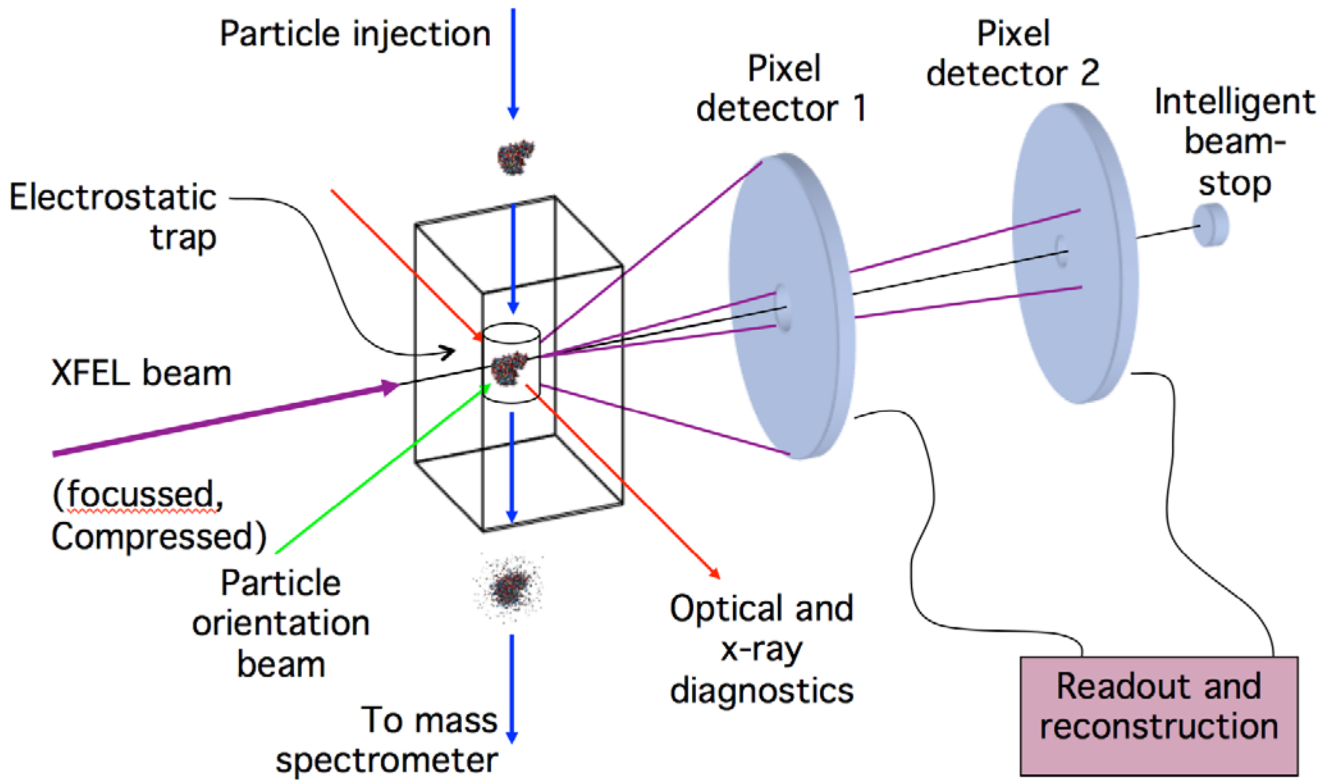
CFEL SCIENCE

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



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

Our first experiments set out to answer a number of open questions

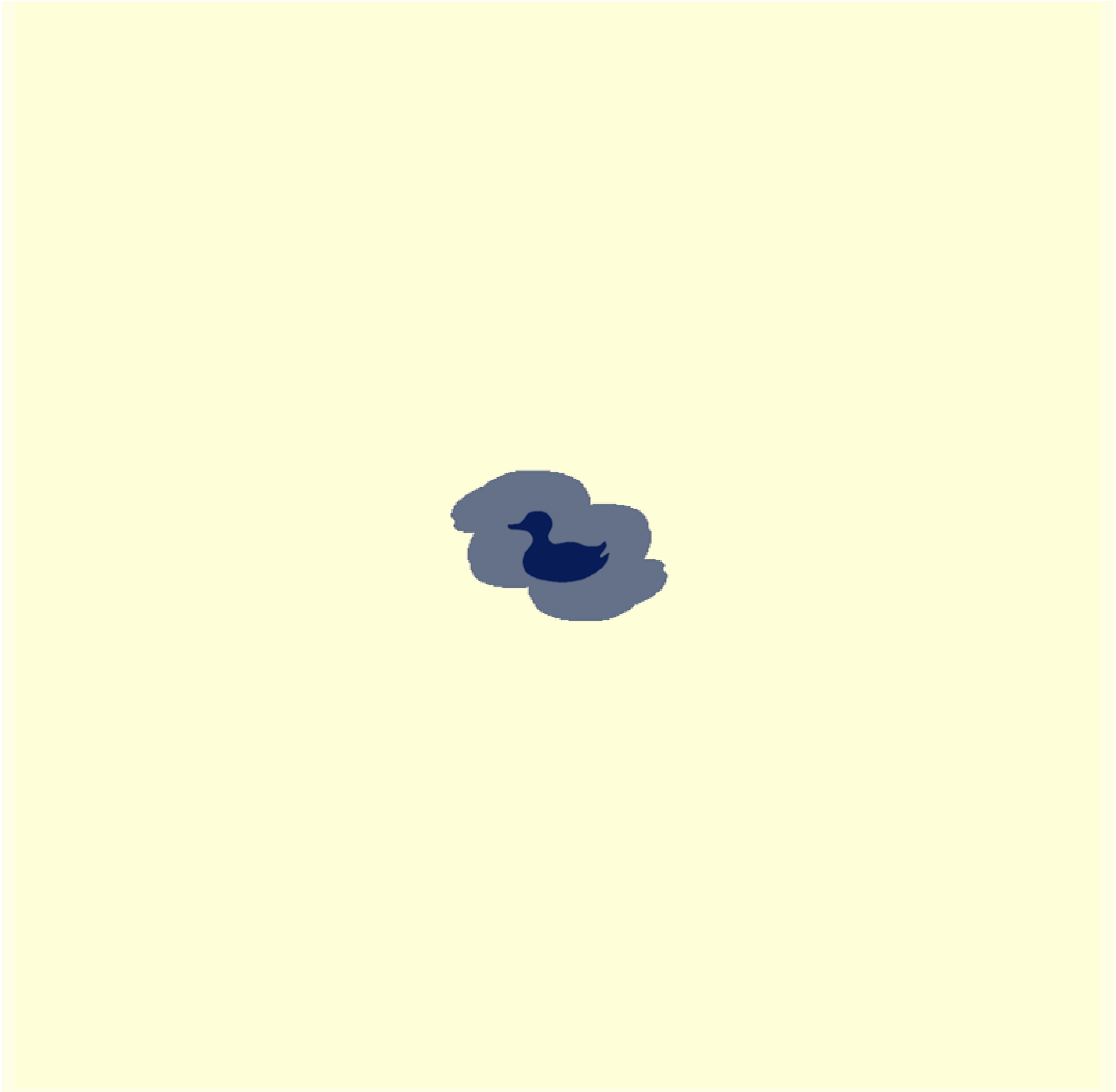


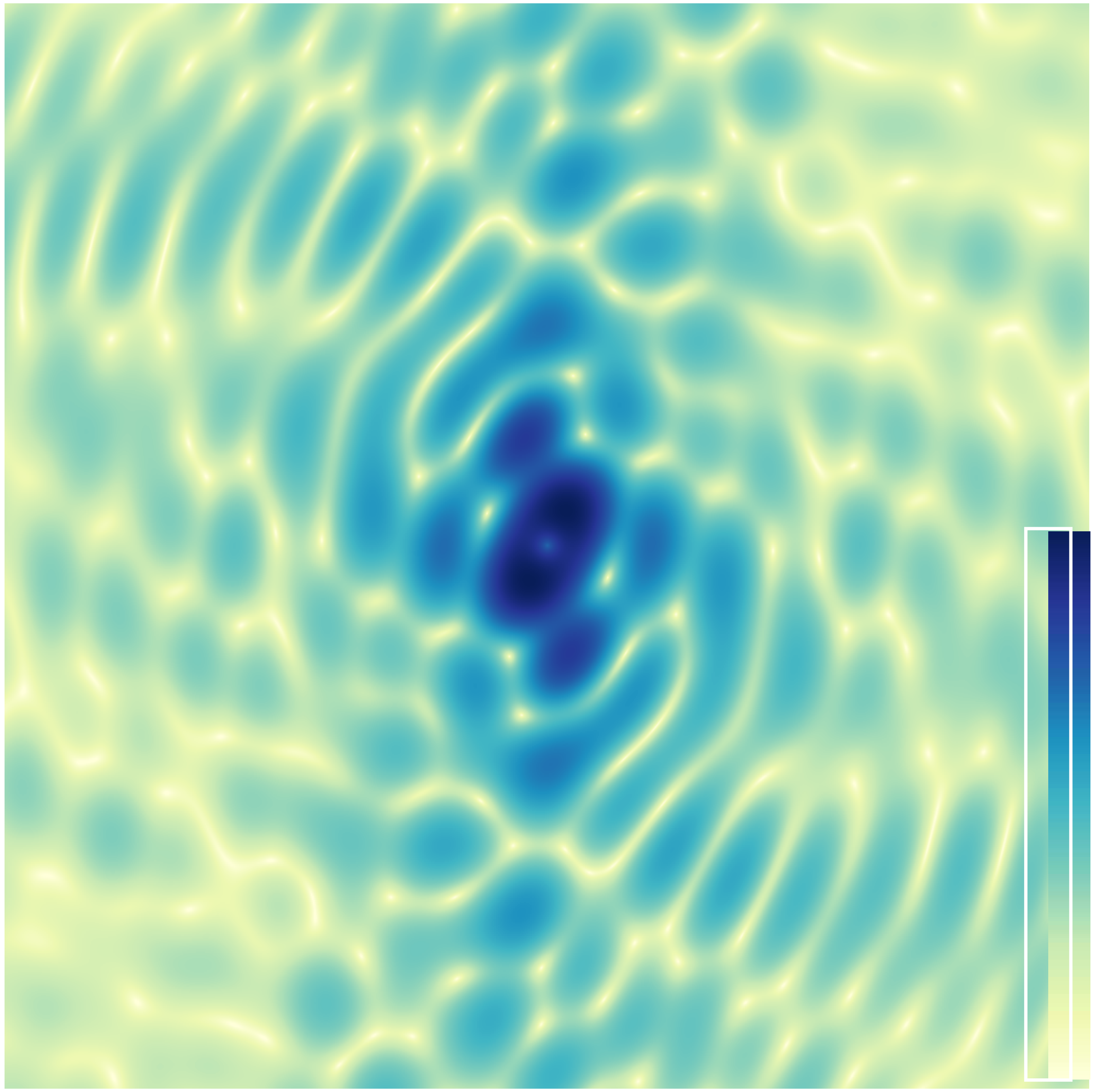
CBST - DOE Workshop
March 2004 at SLAC

Janos Hajdu
Keith Hodgson
Henry Chapman

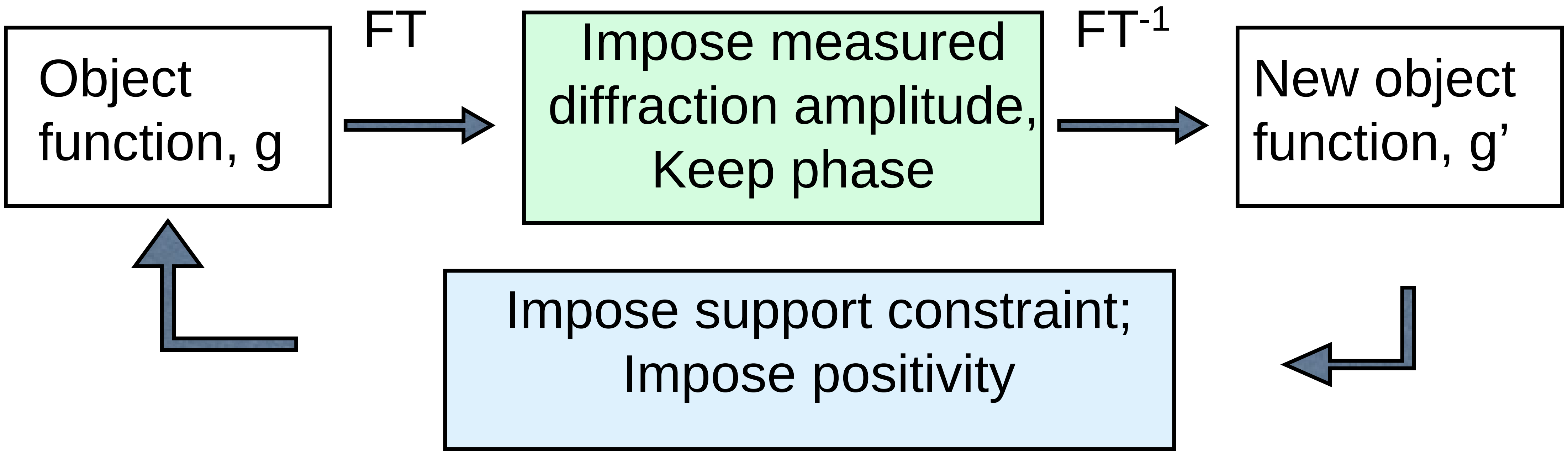
1. How short is short enough to outrun radiation damage?
2. How can you get single molecules into the X-ray beam?
3. How can you determine orientation from noisy diffraction?

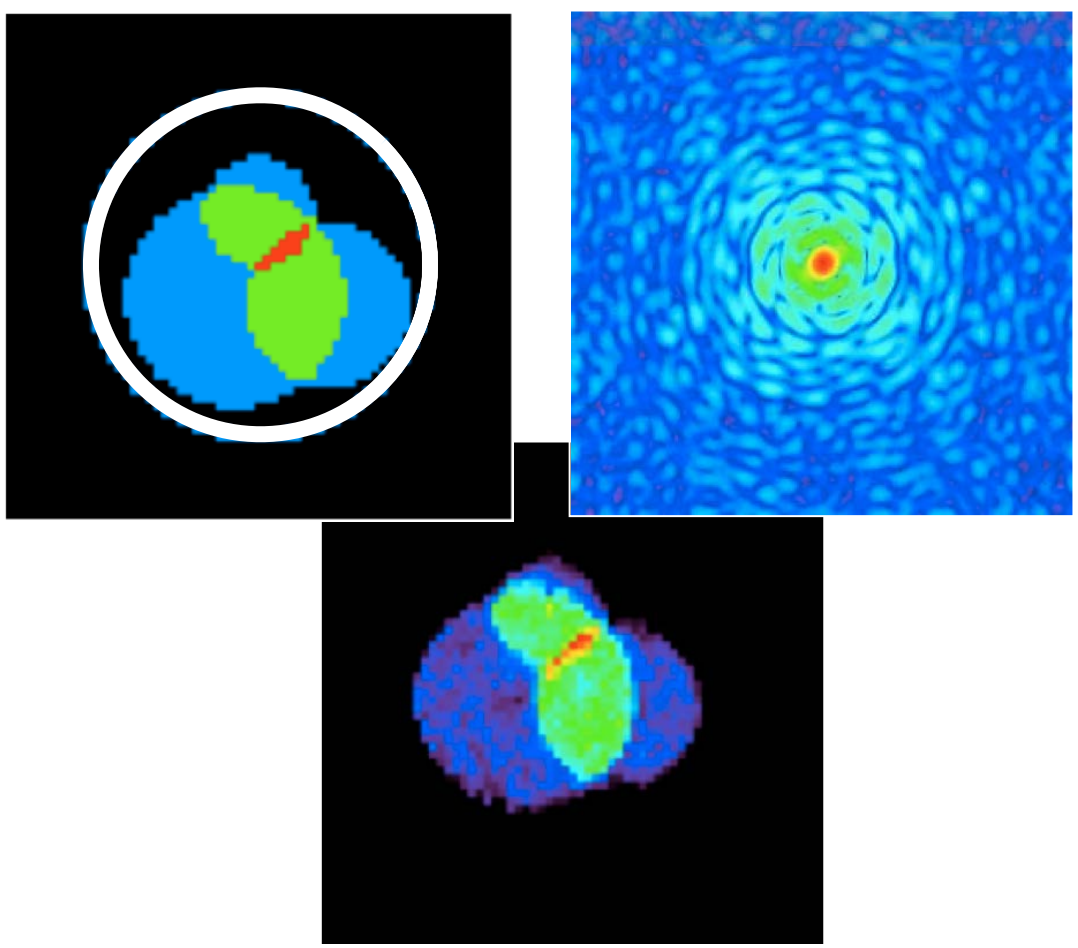
Single particles give rise to continuous diffraction patterns

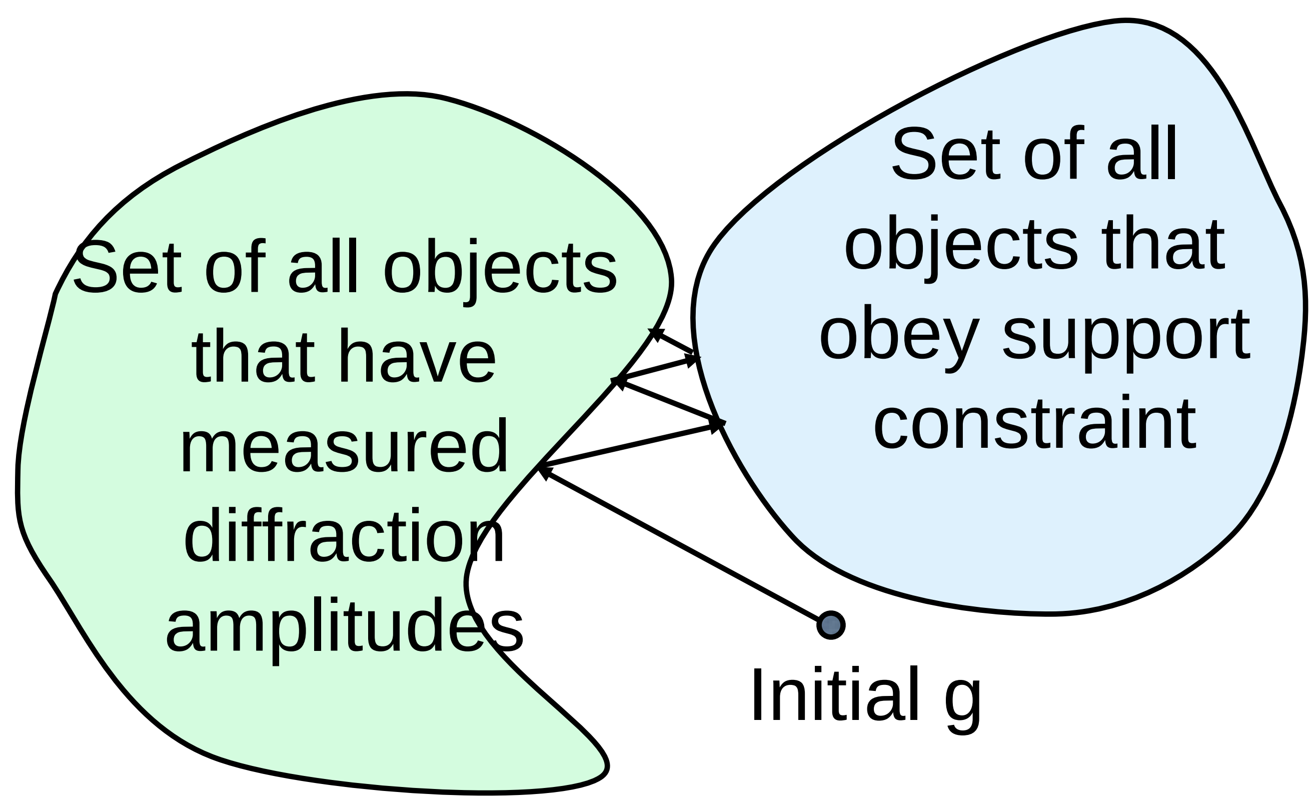




Phase retrieval can be accomplished with iterative transform algorithms







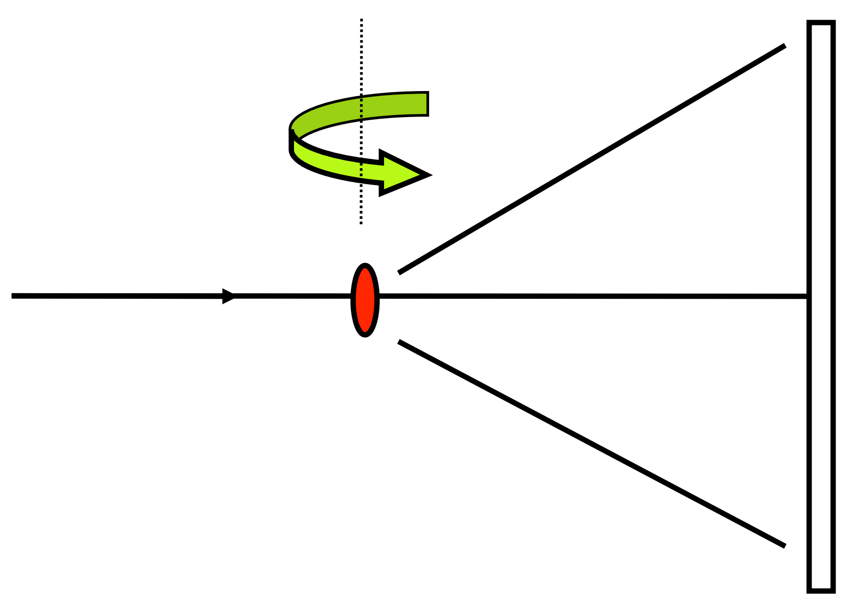
Fienup, Opt Lett 3 (1978)

We reconstructed a 3D X-ray image of a non-crystalline object at 10 nm resolution

Coherent X-ray diffraction data ⎣ =1.6 nm, from a sample of 50-nm gold spheres arranged on a pyramid on a ***synchrotron***

Complete image reconstruction achieved, without any prior knowledge, using our "**shrinkwrap**" algorithm, **parallelized** for 3D on 32-CPU cluster. Resolution = 10 nm

Coherent X-ray diffraction data, rotating the sample -70 to +70 degrees (5·10^8 data points)

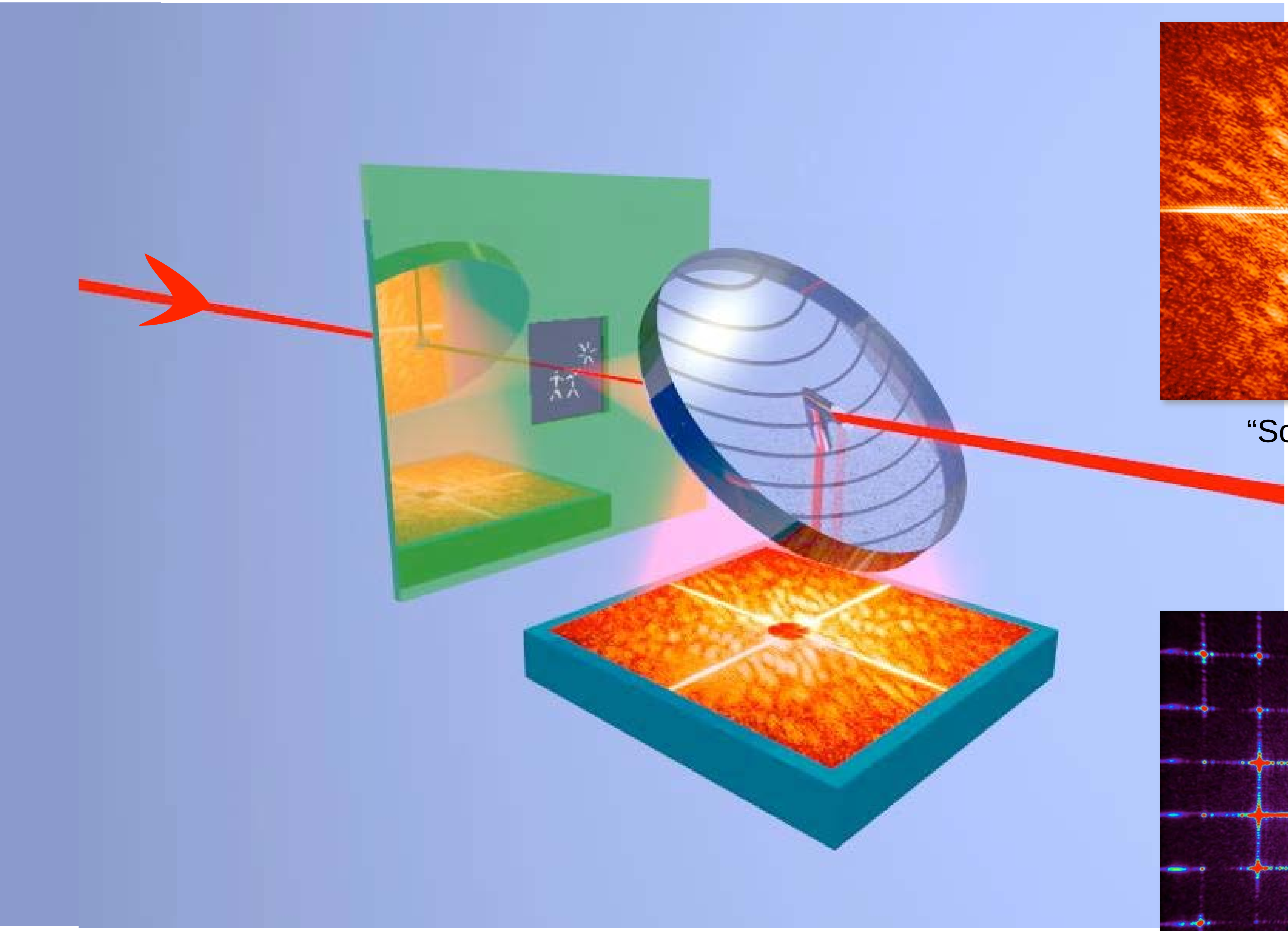


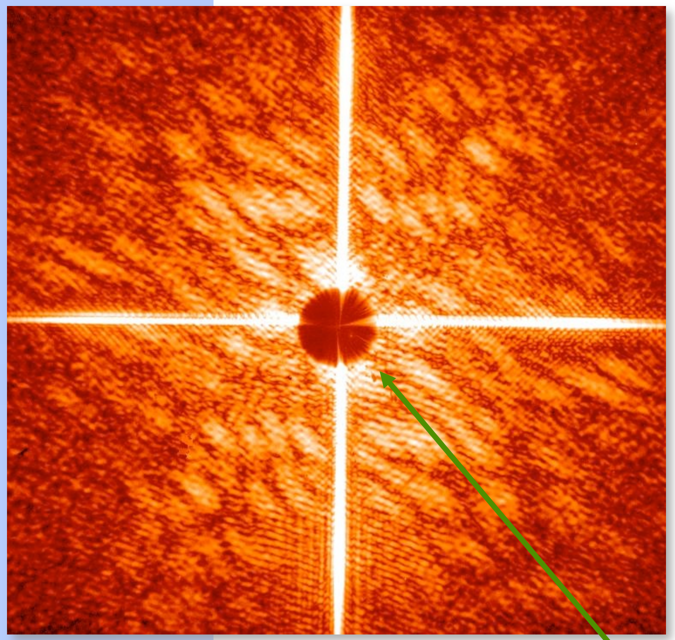
Anton Barty

Stefano Marchesini

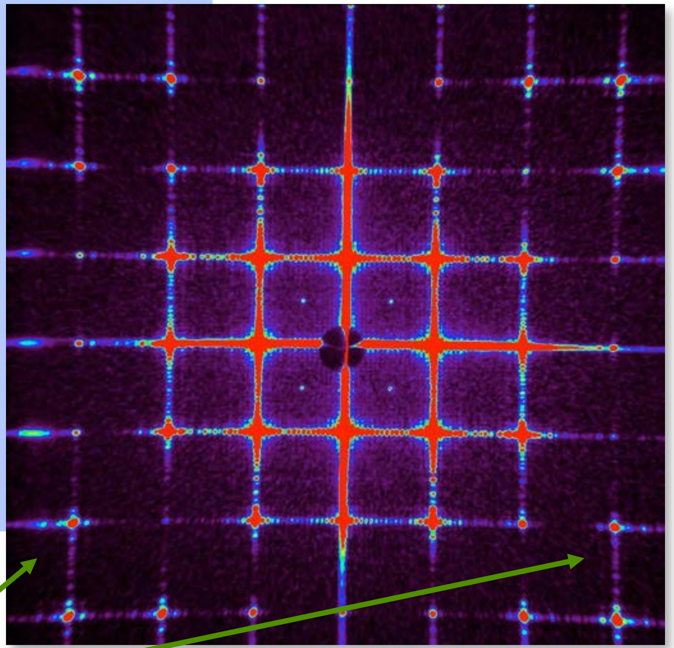
H. Chapman, et al., JOSA A 23 1169 (2006)

Our diffraction camera can measure forward scattering close to the direct soft-X-ray FEL beam



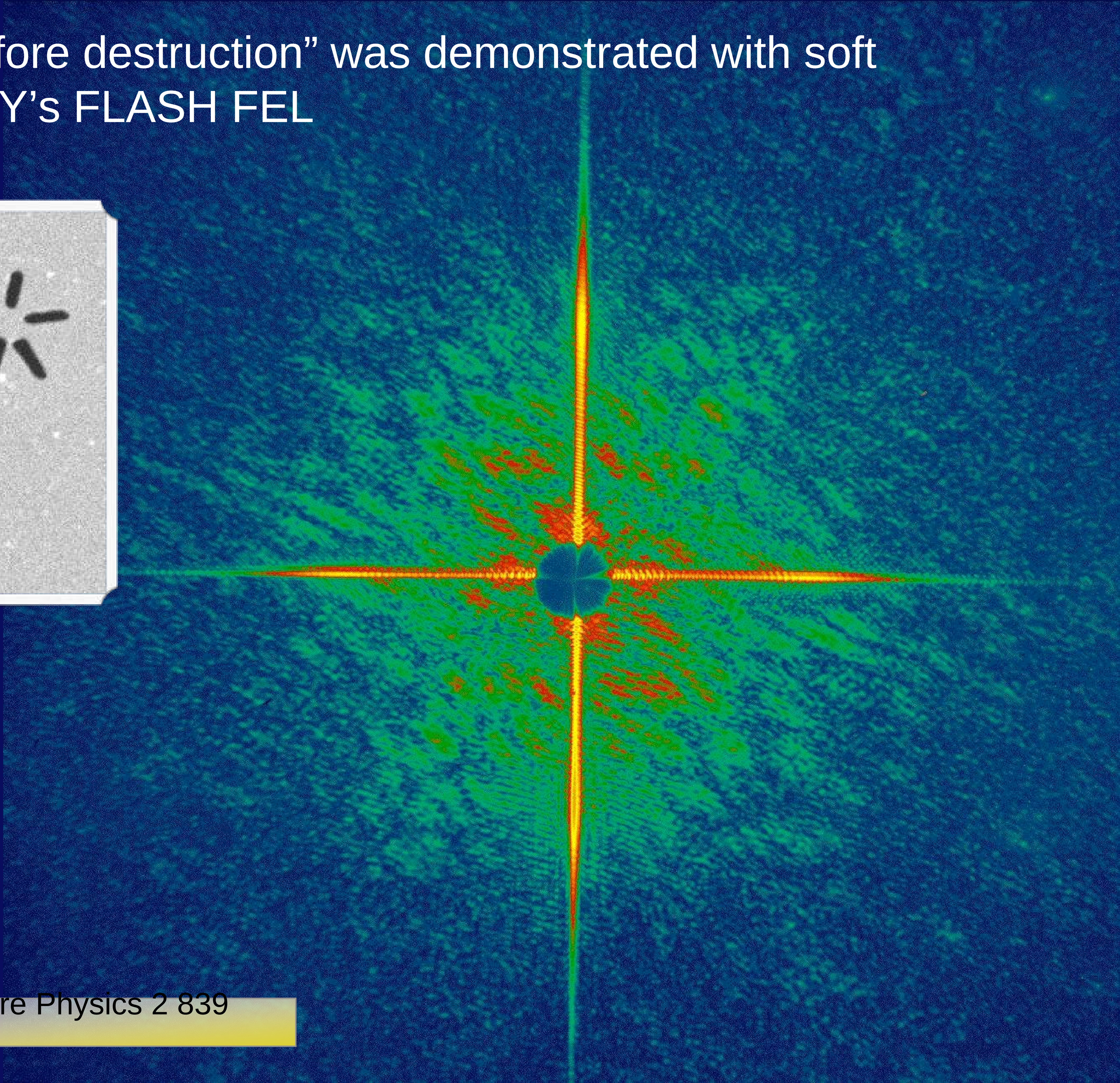


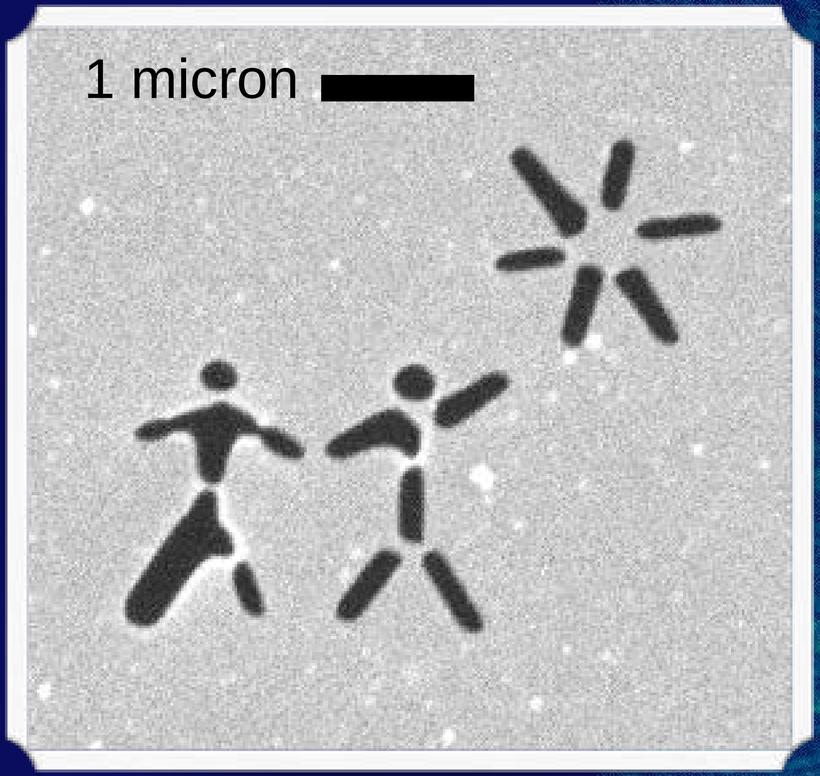
“Soft edge” prevents any scatter from the hole



Multilayer reflectivity is uniform across the 30° to 60° gradient

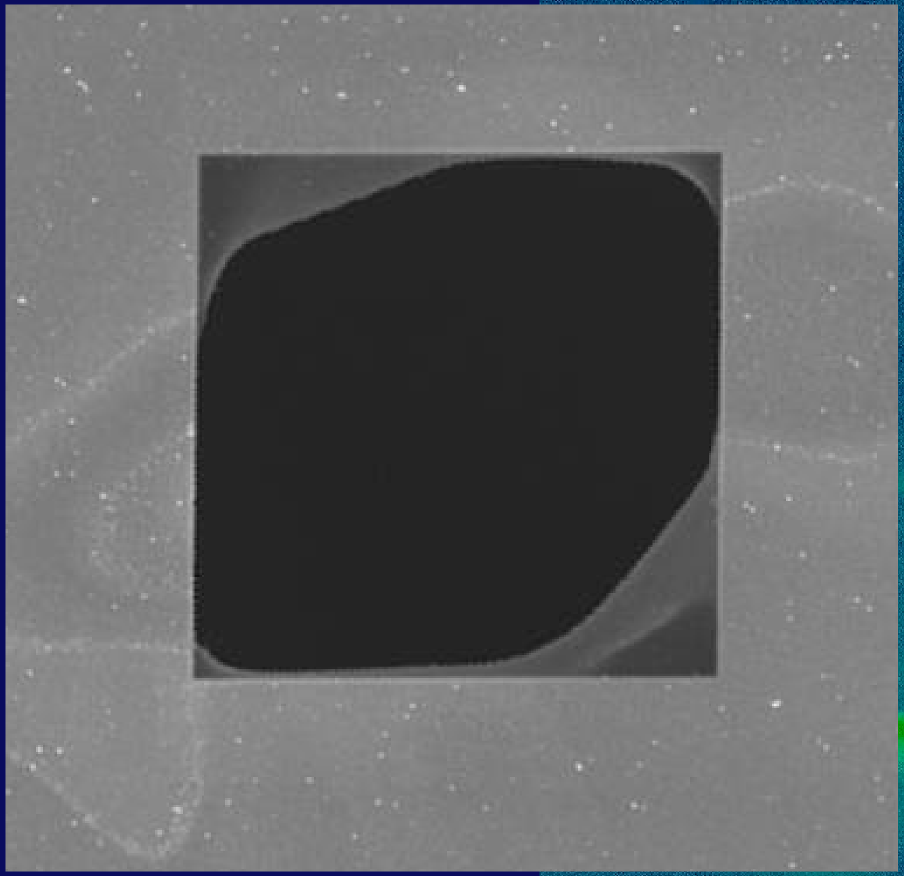
“Diffraction before destruction” was demonstrated with soft X-rays at DESY’s FLASH FEL

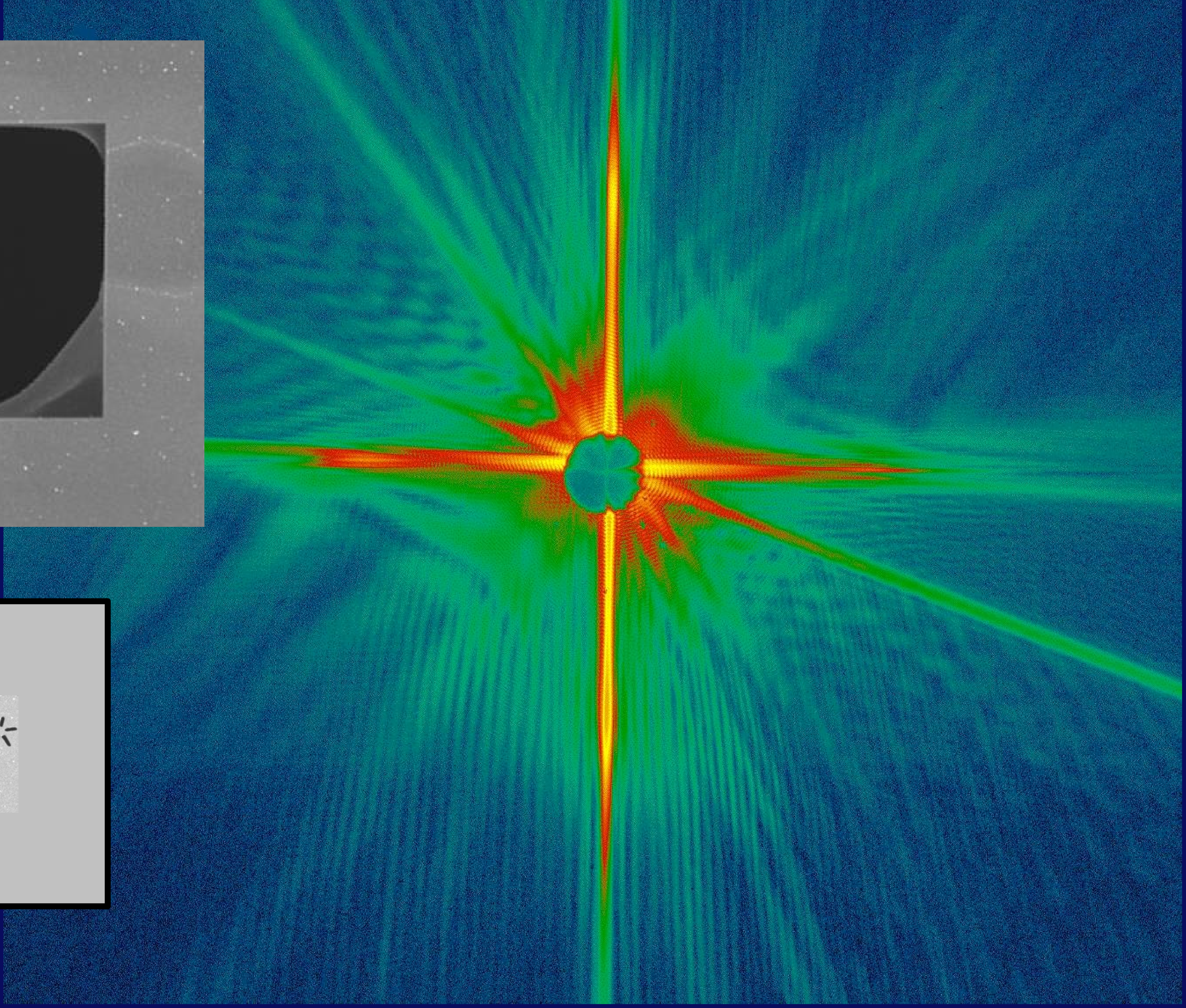




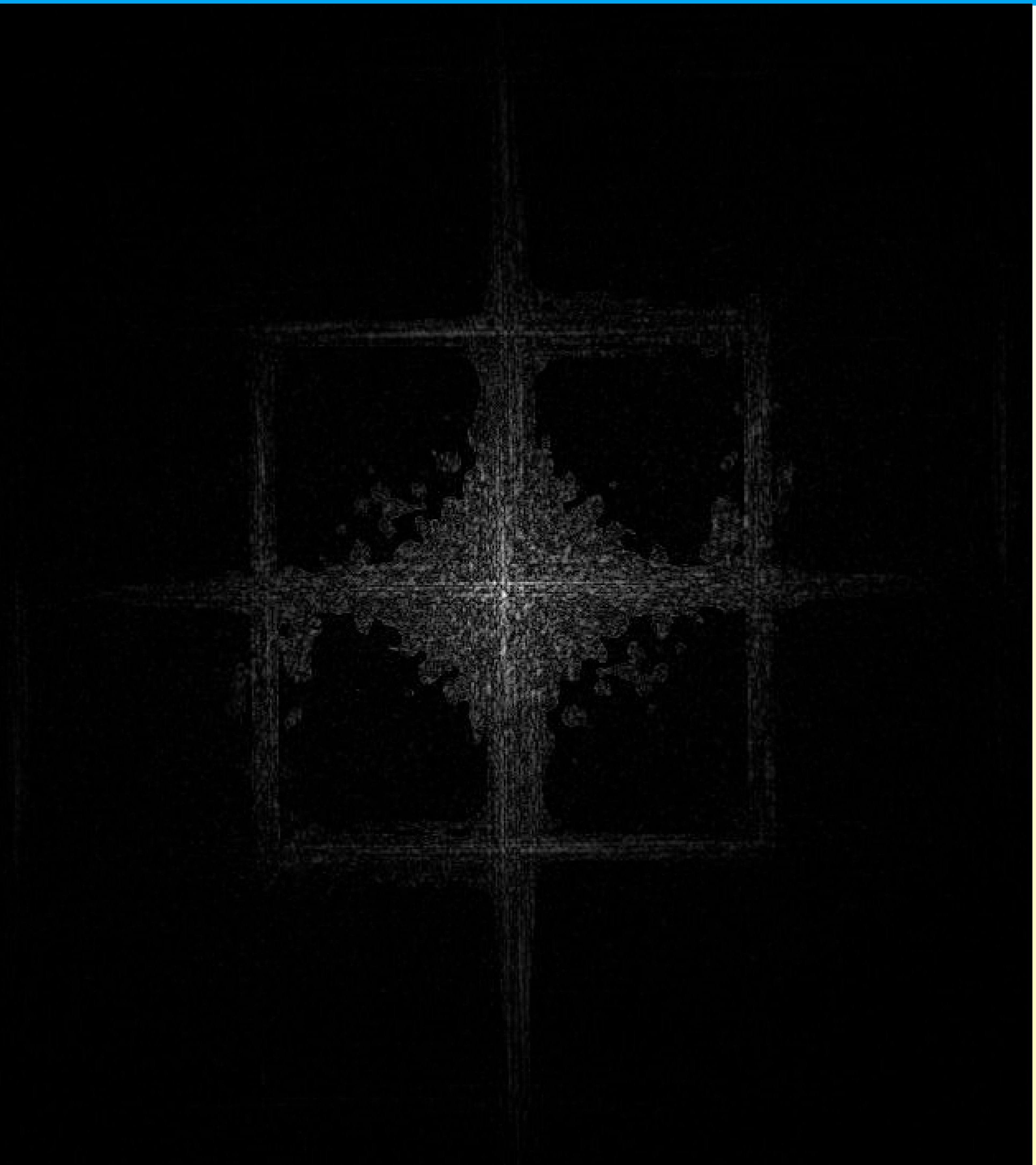
Chapman et al, Nature Physics 2 839 (2006)

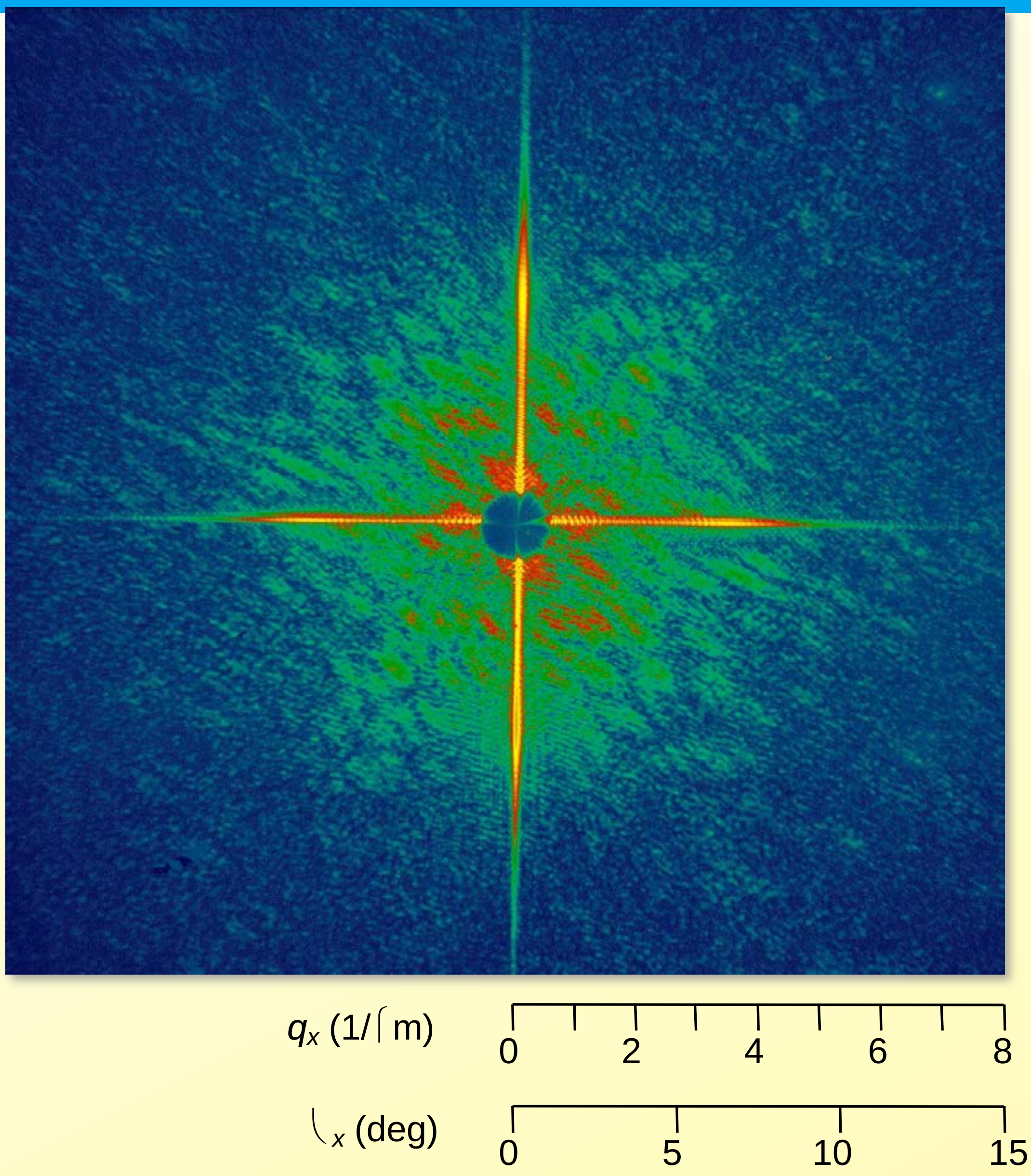
“Diffraction before destruction” was demonstrated with soft X-rays at DESY’s FLASH FEL





We perform ab initio image reconstruction with our “Shrinkwrap” algorithm

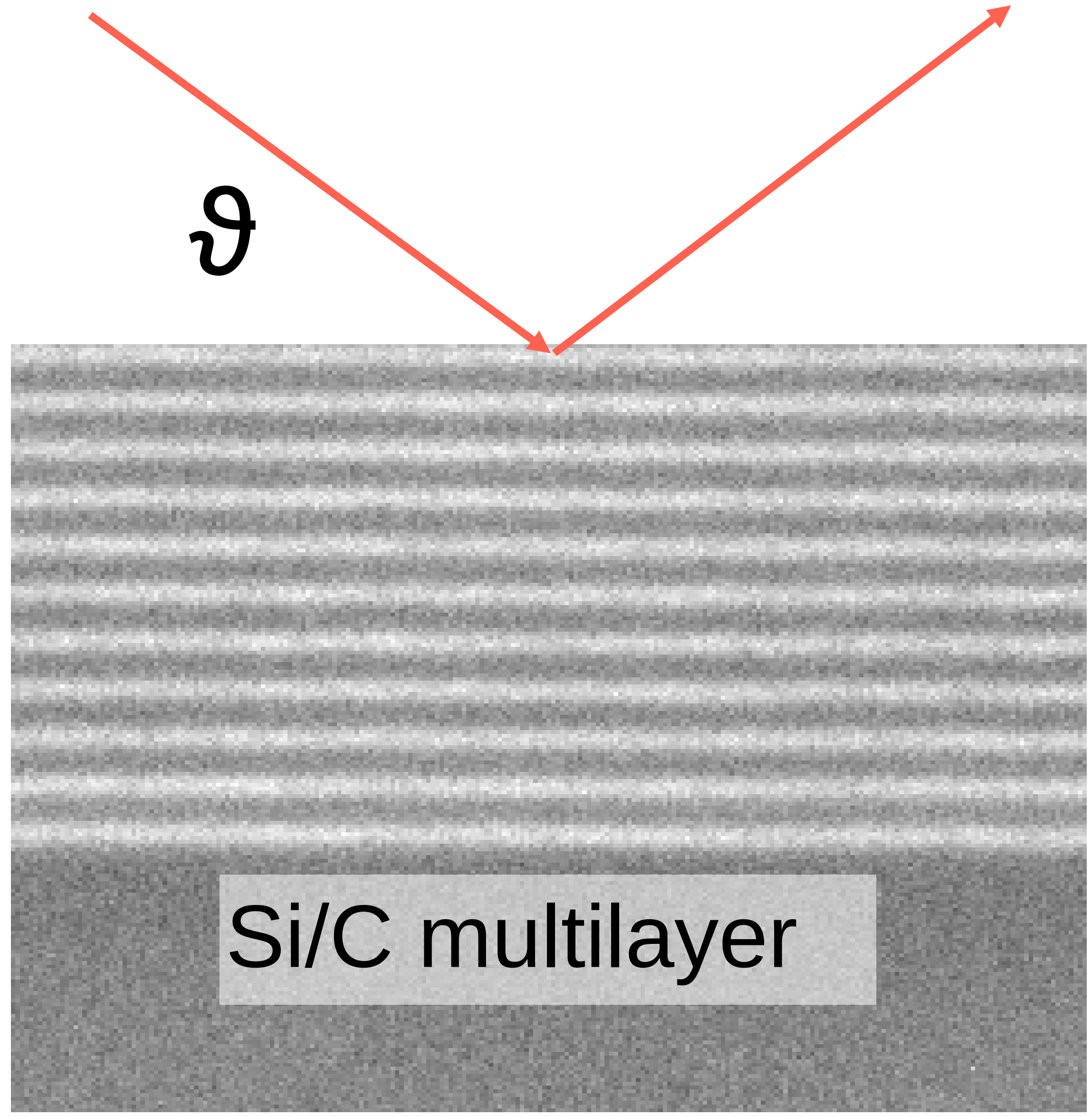




S. Marchesini et al. Phys Rev B 68 140101 (2003)

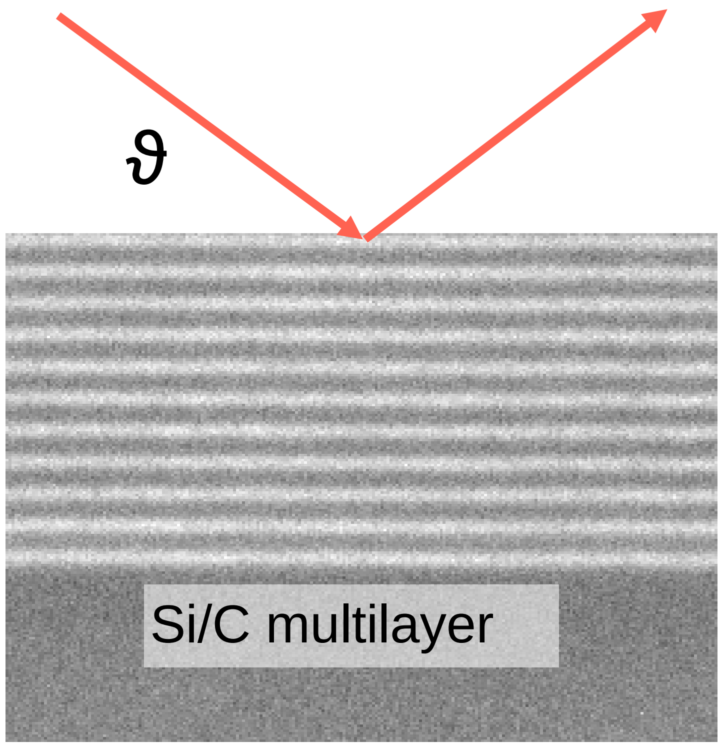
Our first experiment was actually a bit simpler…

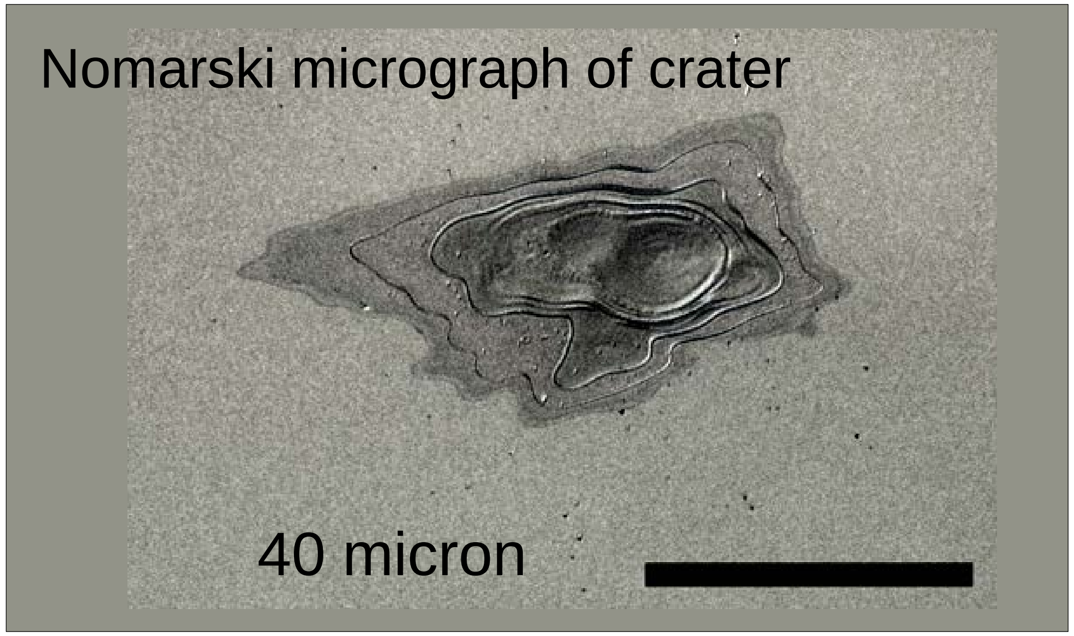
30 fs pulse (10^{14} W cm^{-2})
32 nm wavelength



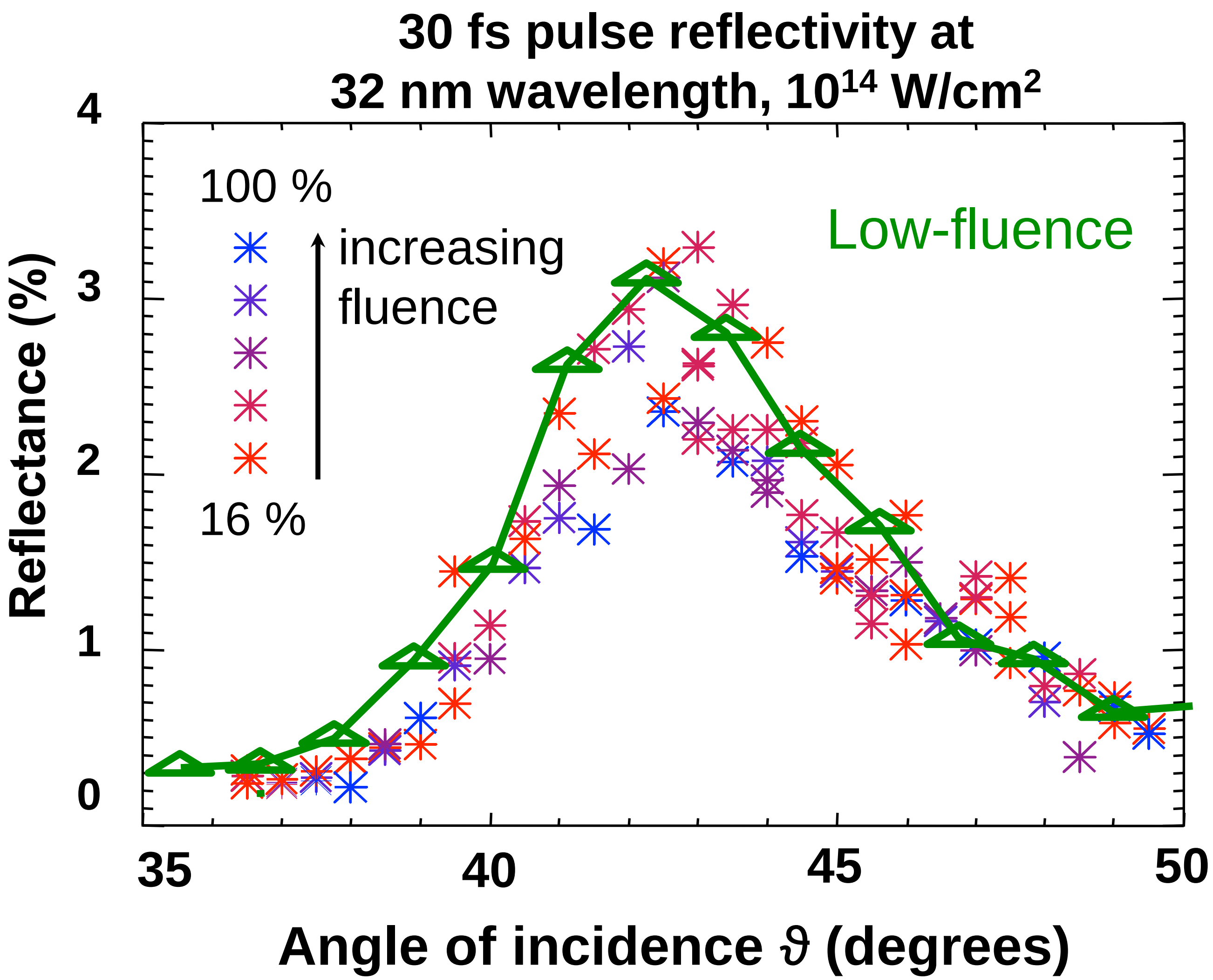
Saša Bajt

First EUV-FEL experiments show that structural information can be obtained before destruction



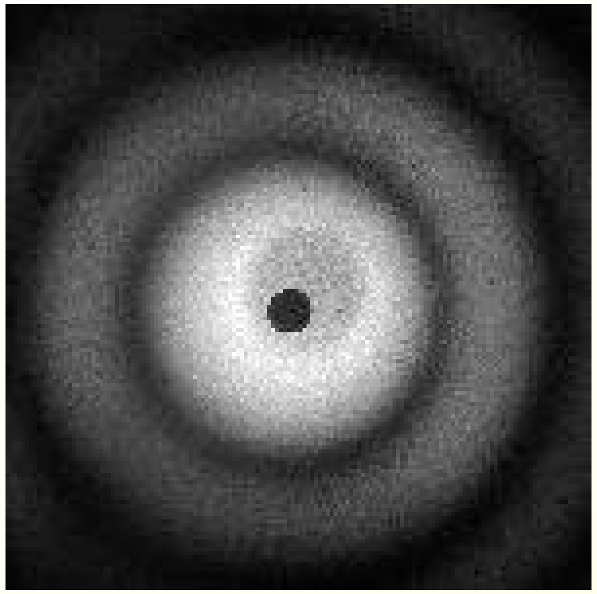


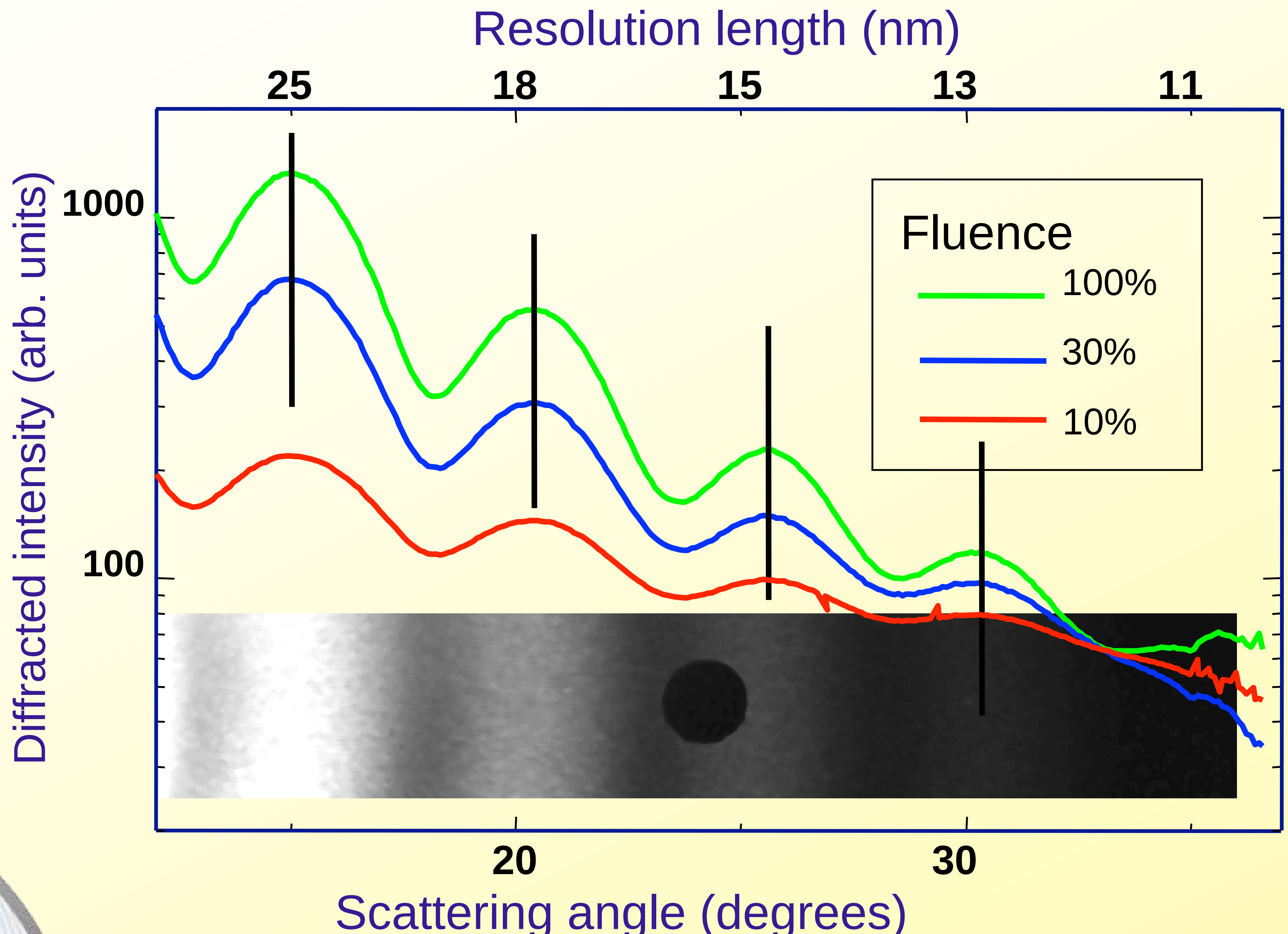
S. Hau-Riege et al PRL **98** (2007)

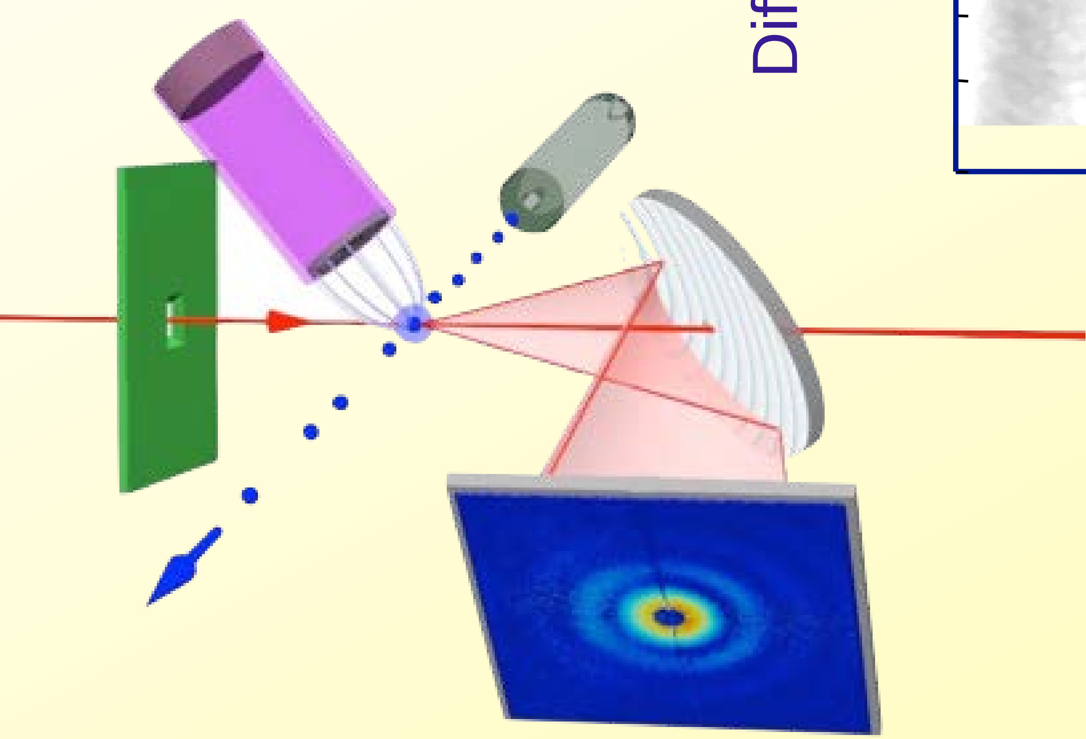


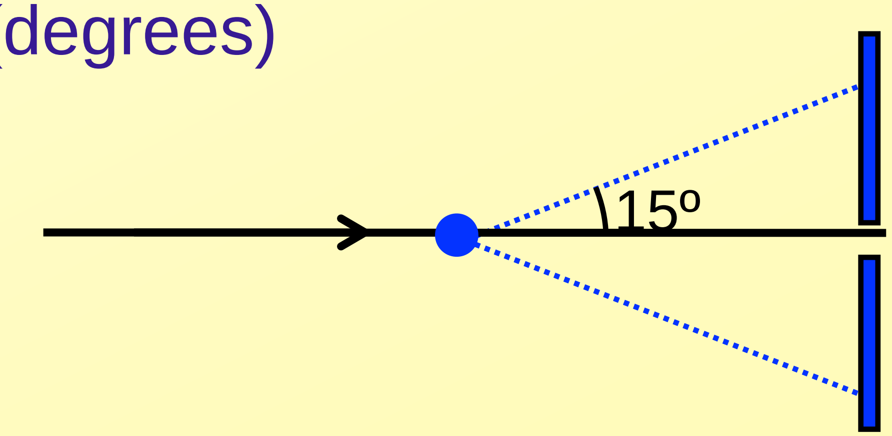
Reflectivity unchanged
Multilayer *d* spacing not changed by more than 0.3 nm

Initial high-angle diffraction shows no change in structure of particles greater than 12 nm



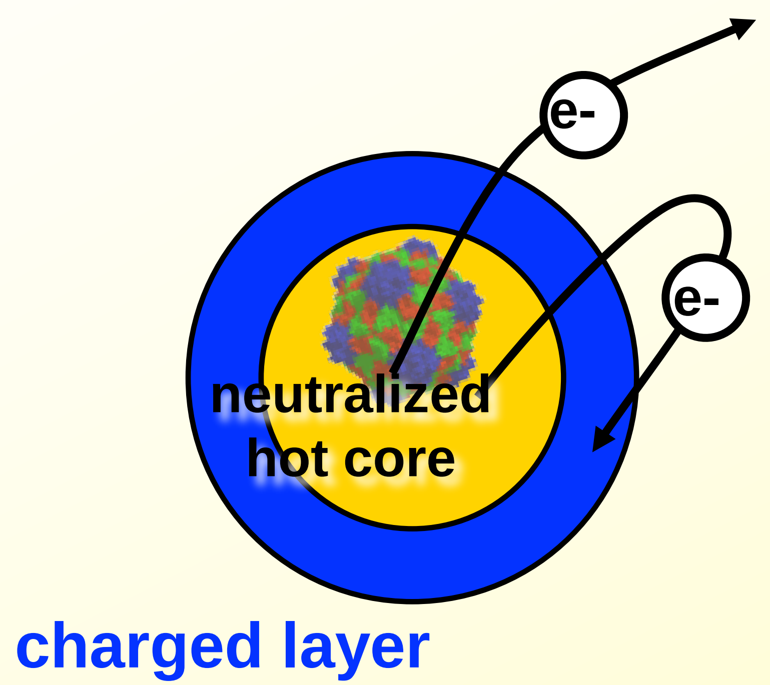


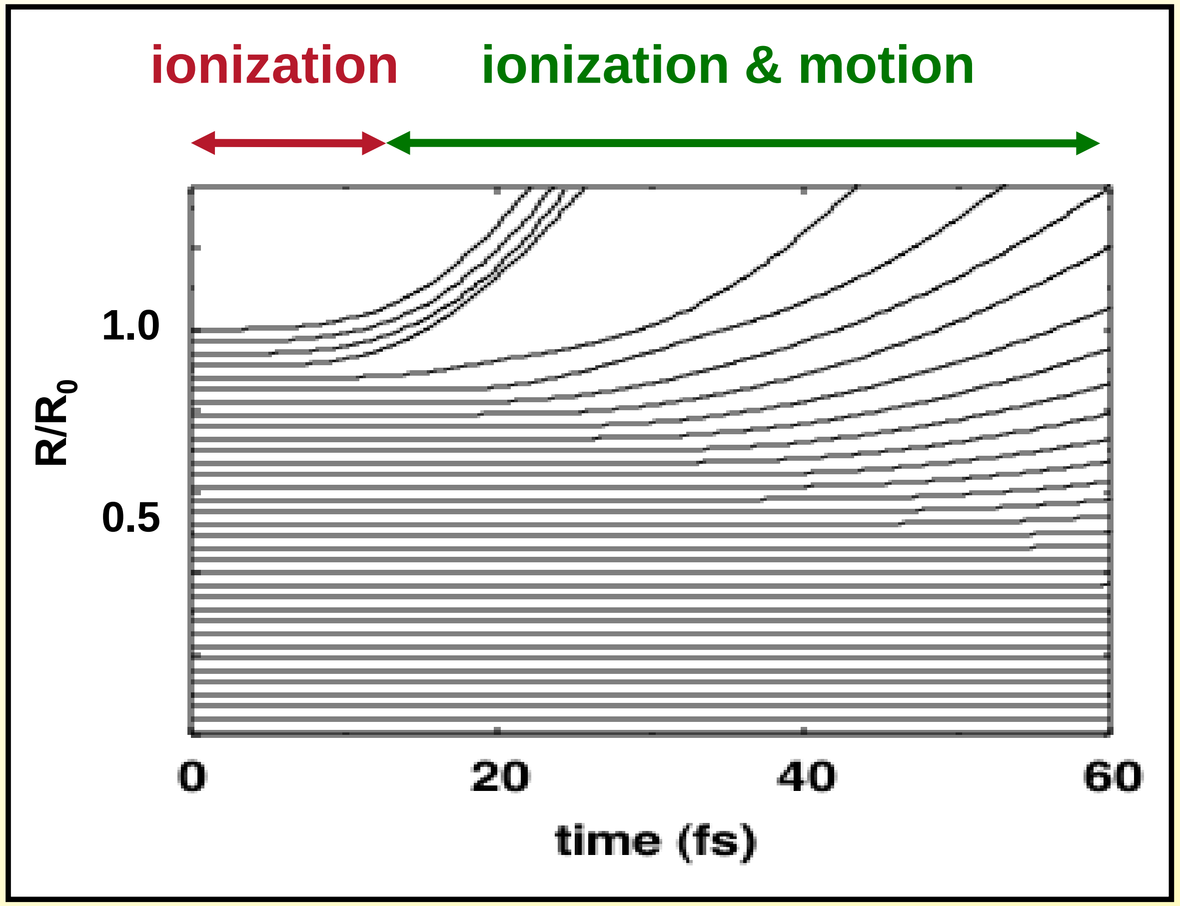




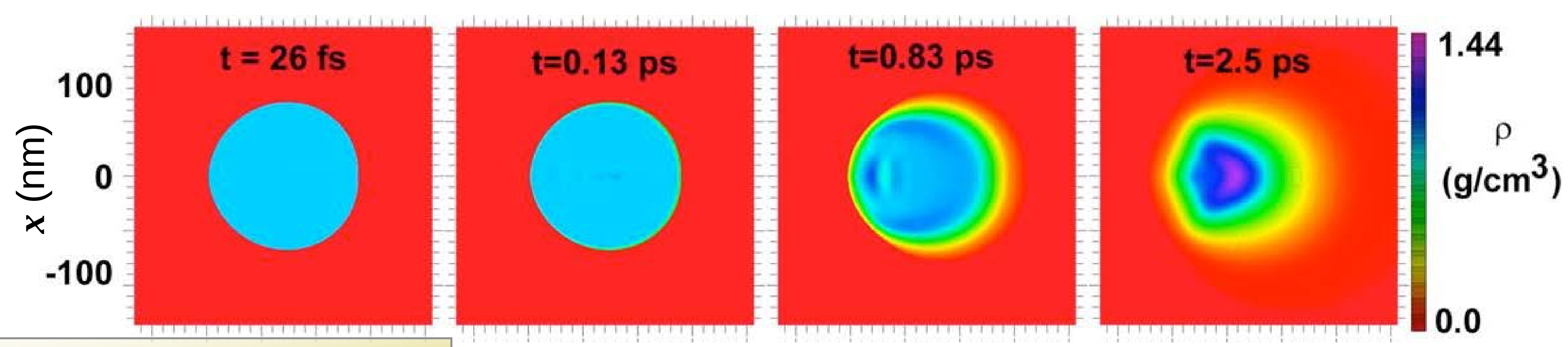
XFEL diffraction of molecules and clusters is modified (damaged) by photoionization and motion of atoms

- Photoionization (and subsequent collisional ionization) releases charges from the free molecule which lead to Coulomb explosion.
- After ~10 fs, charges are trapped and neutralize the core of the particle
- Nuclear motion occurs on outer layers first



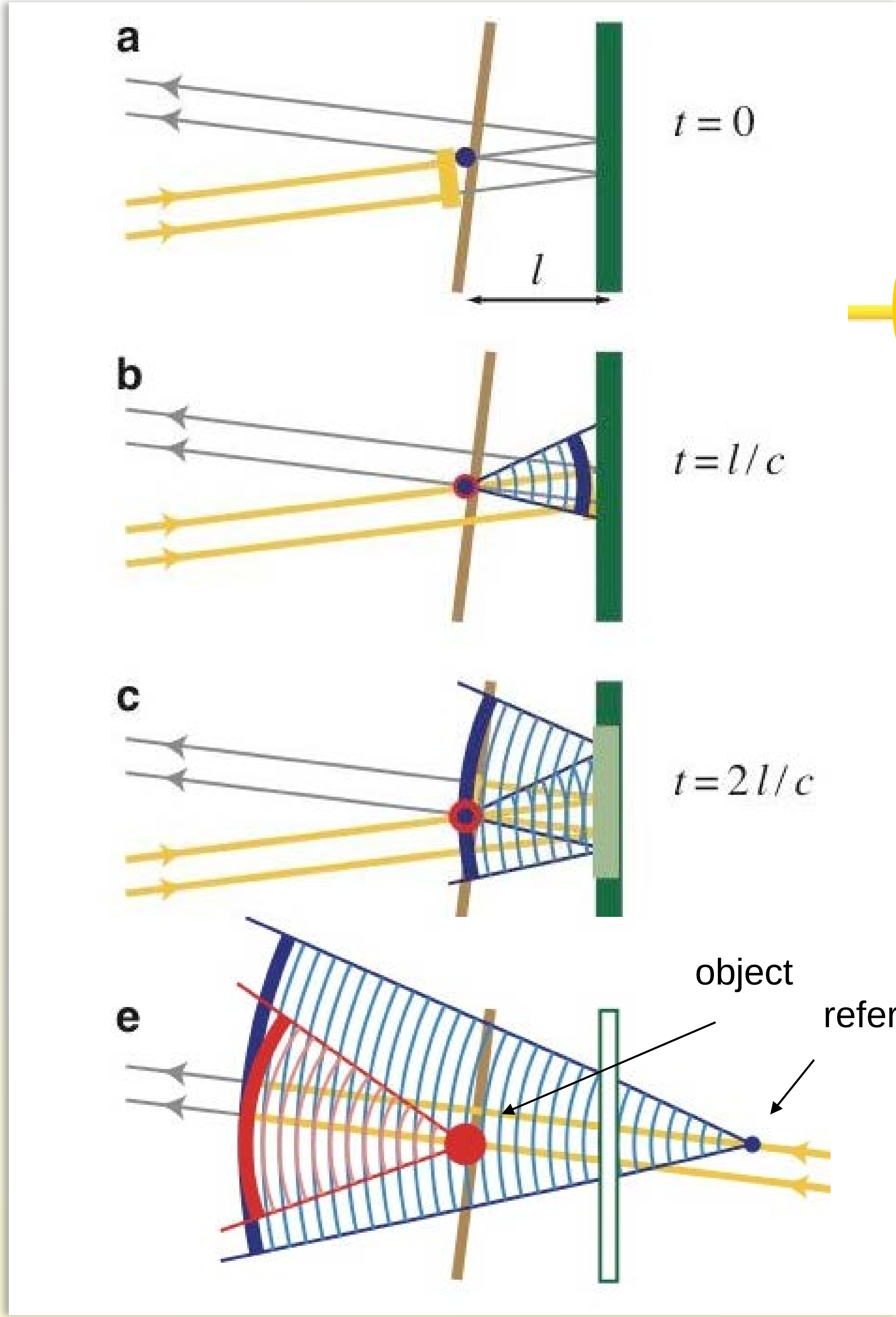


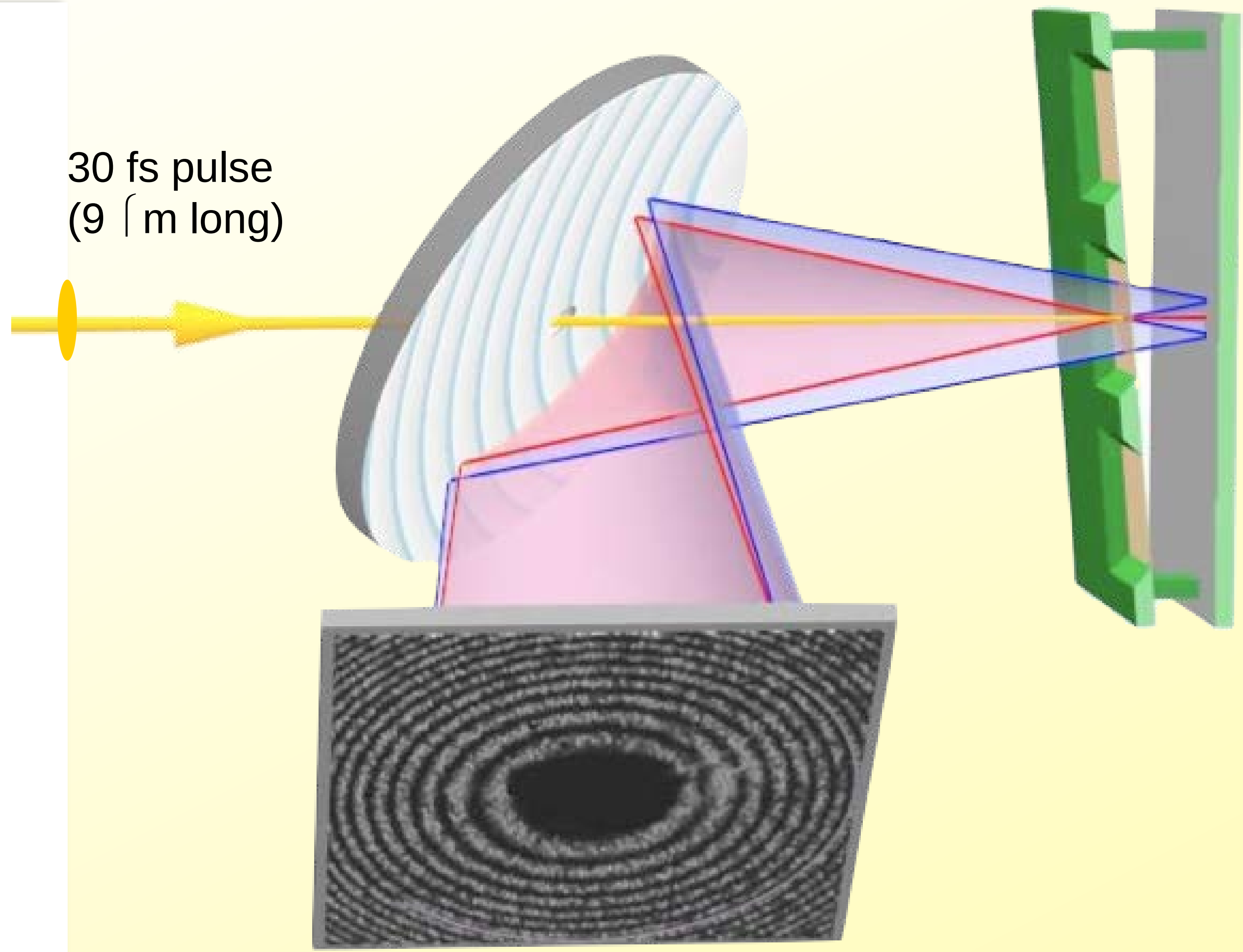
Hydrodynamic simulations of nanostructures



S. Hau-Riege et al, Phys Rev E 69, 051906 (2004)

We invented a new method called femtosecond time-delay holography





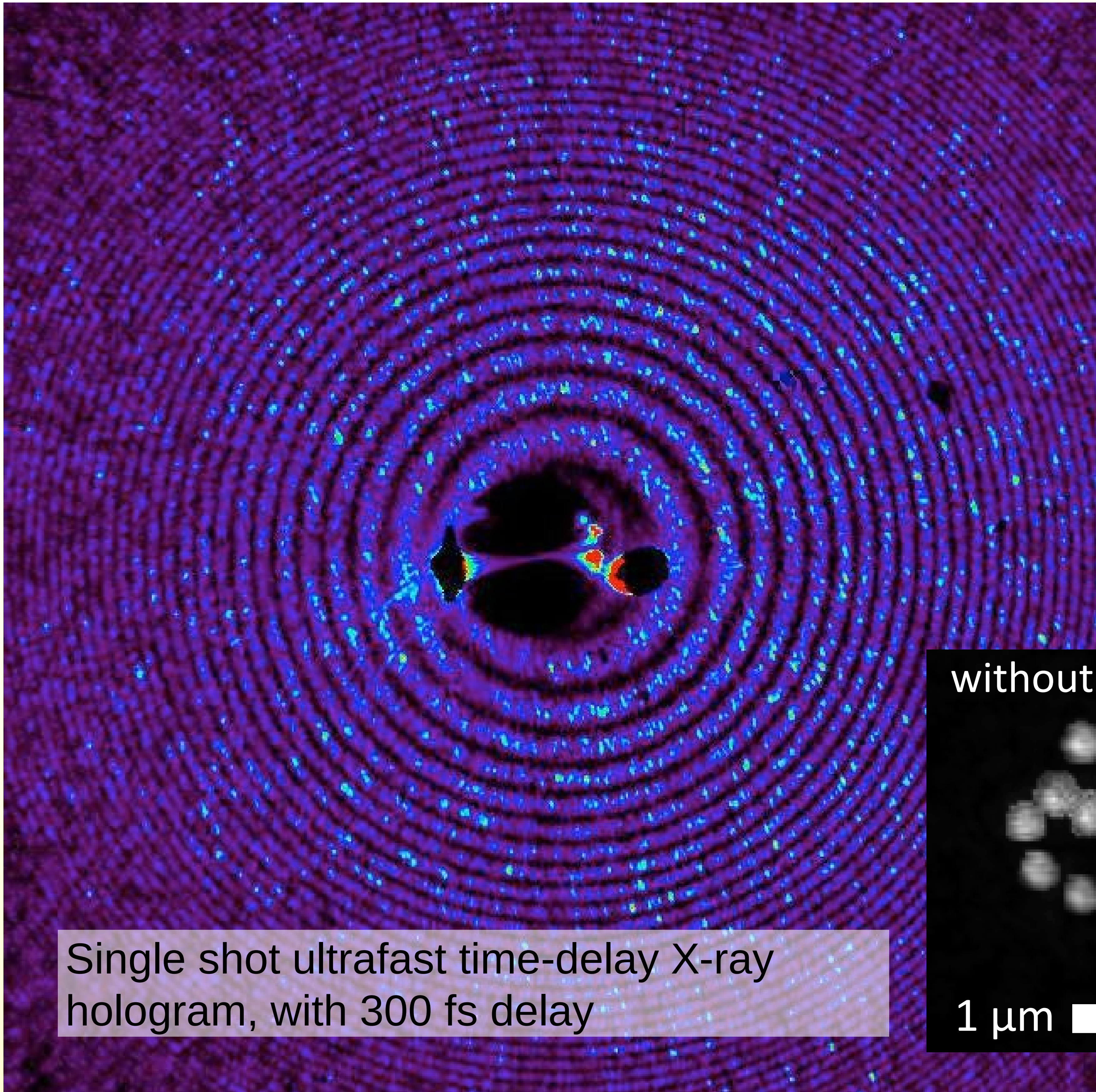
Prompt diffraction

Delayed diffraction

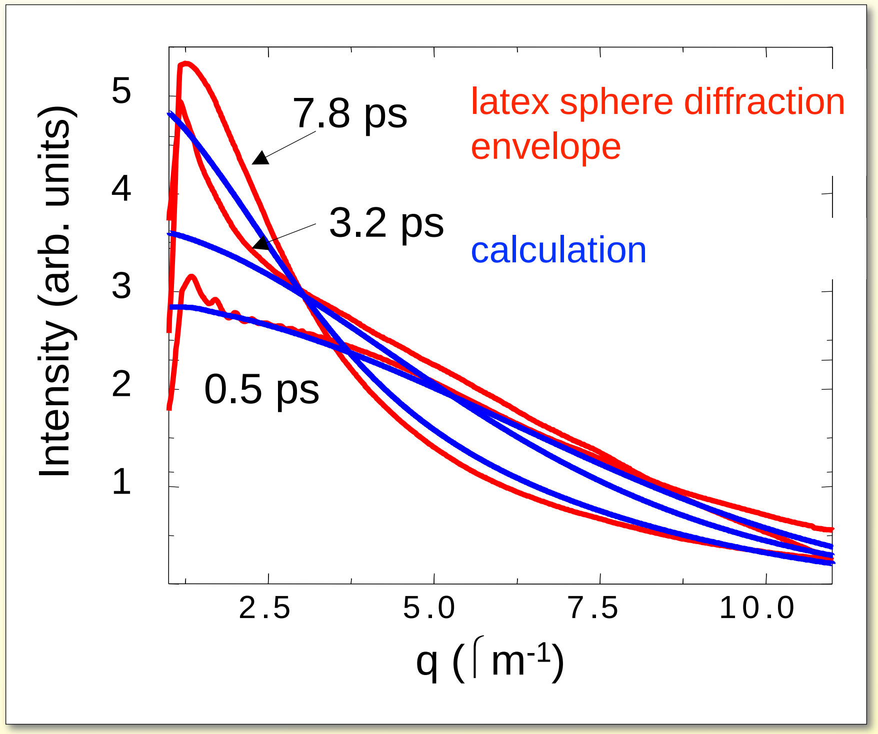
Time delay 2 *l* / c

H. Chapman et al., Nature 448 676 (2007)

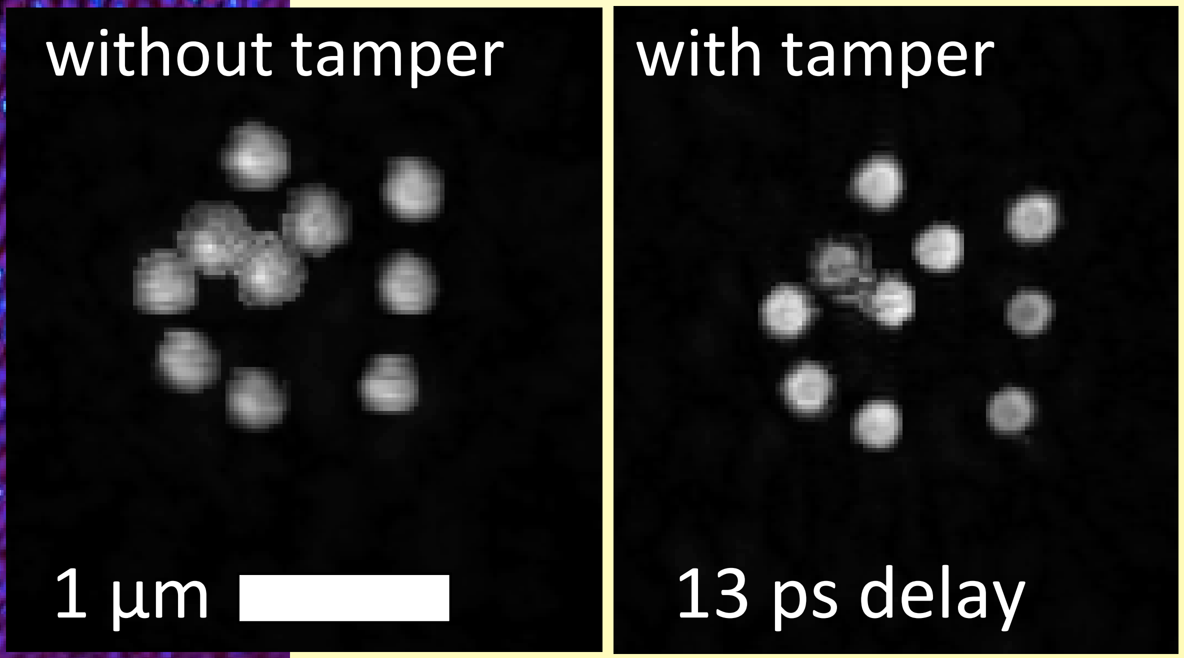
Time-delay holography with 3 fs time resolution indicates the particle explosion



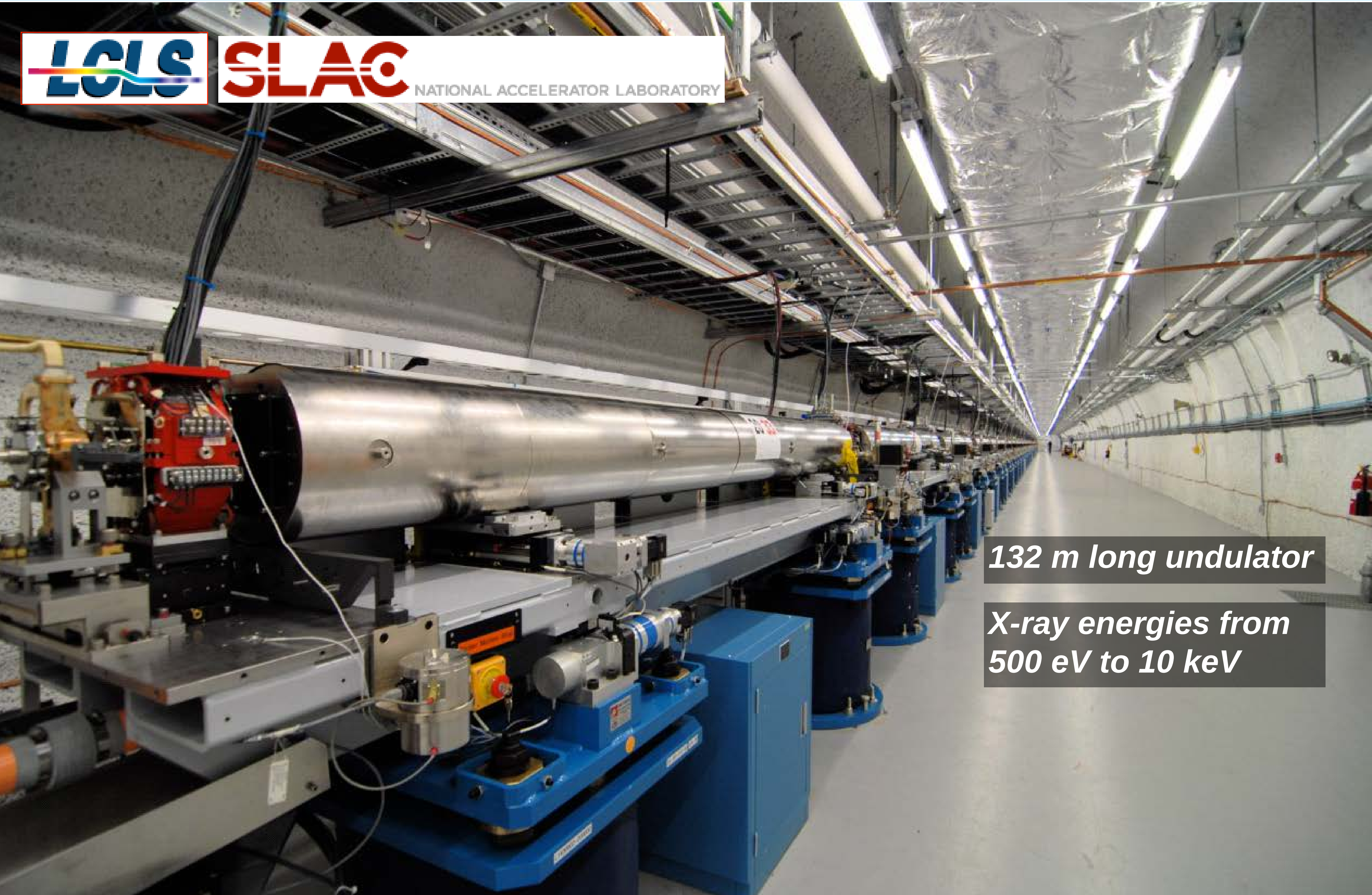
Single shot ultrafast time-delay X-ray hologram, with 300 fs delay



<4Å /(30 fs)

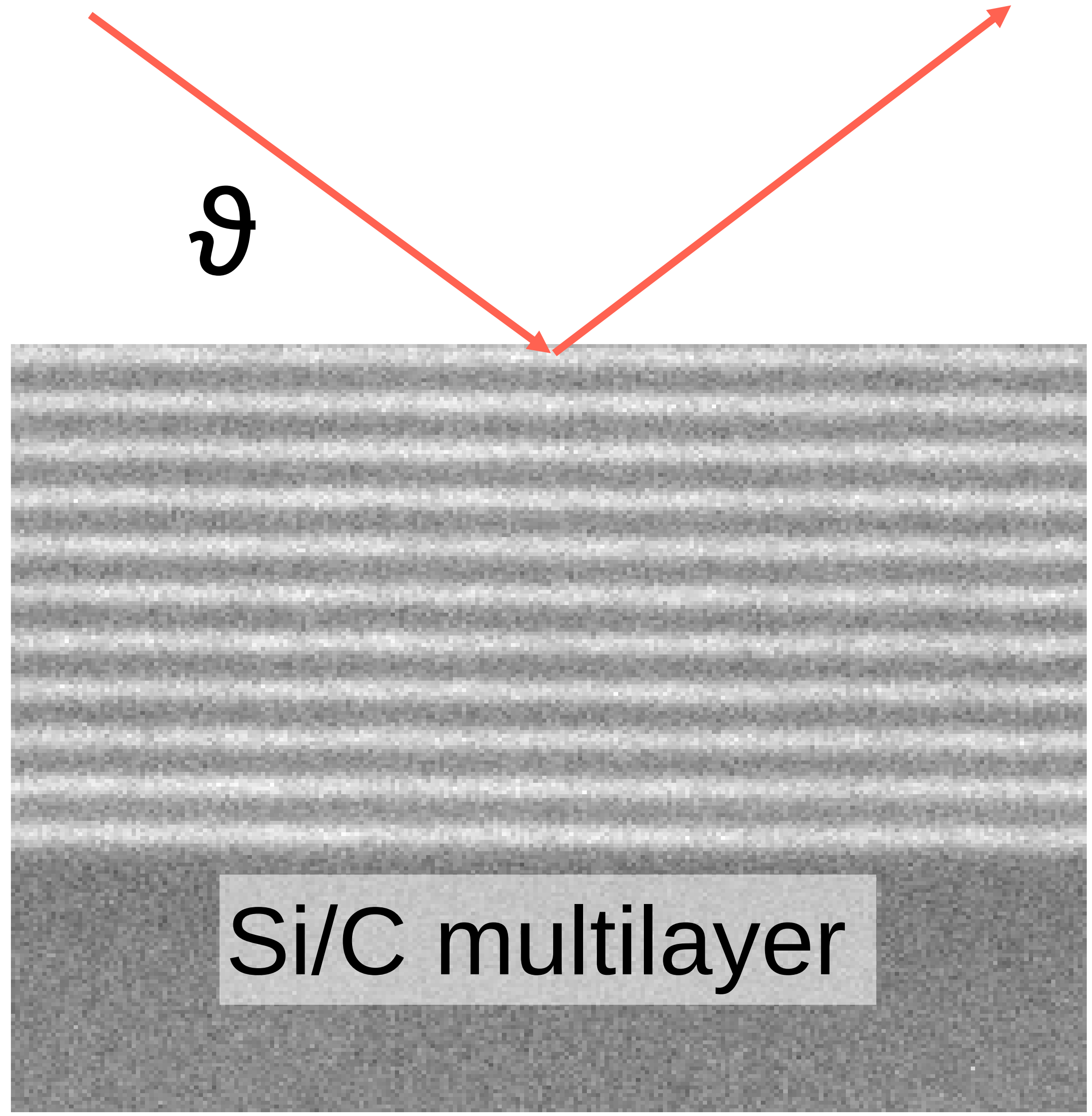


The Linac Coherent Light Source has been in operation since 2009



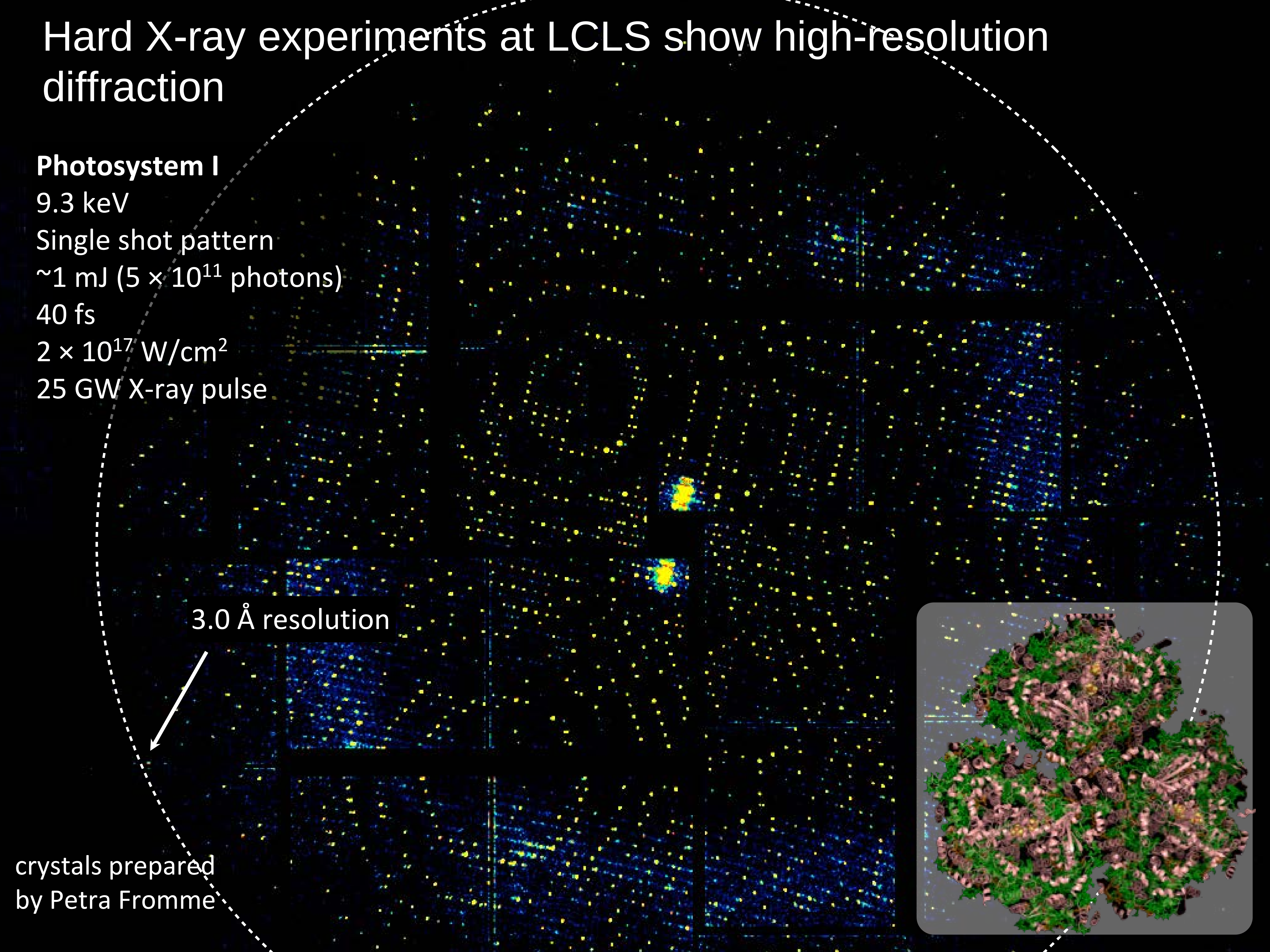
Bragg diffraction is a good indicator of structural integrity

30 fs pulse (10^{14} W cm^{-2})
32 nm wavelength



Hard X-ray experiments at LCLS show high-resolution diffraction

Photosystem I
9.3 keV
Single shot pattern
~1 mJ (5×10^{11} photons)
40 fs
2×10^{17} W/cm^2
25 GW X-ray pulse



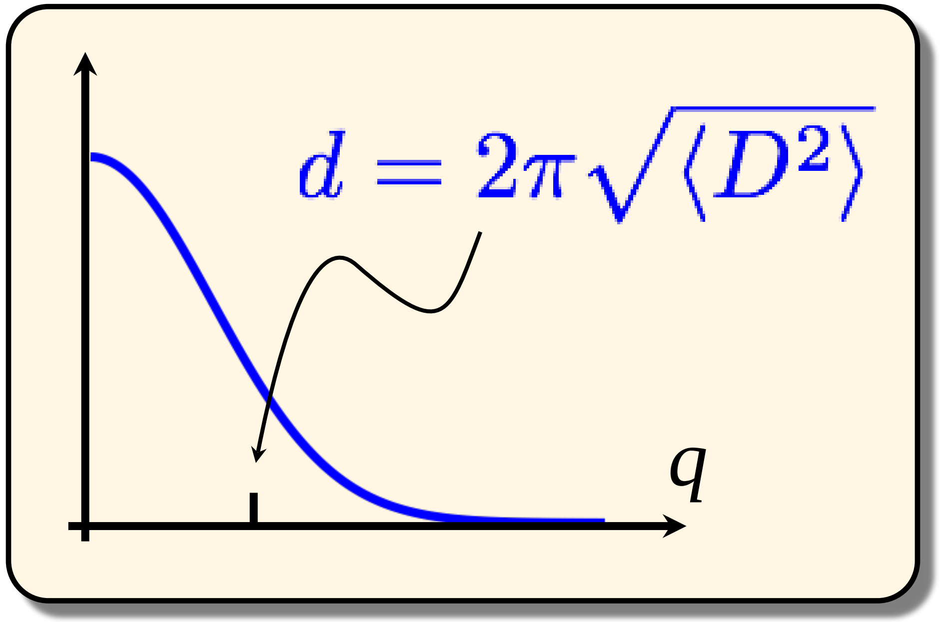
crystals prepared
by Petra Fromme

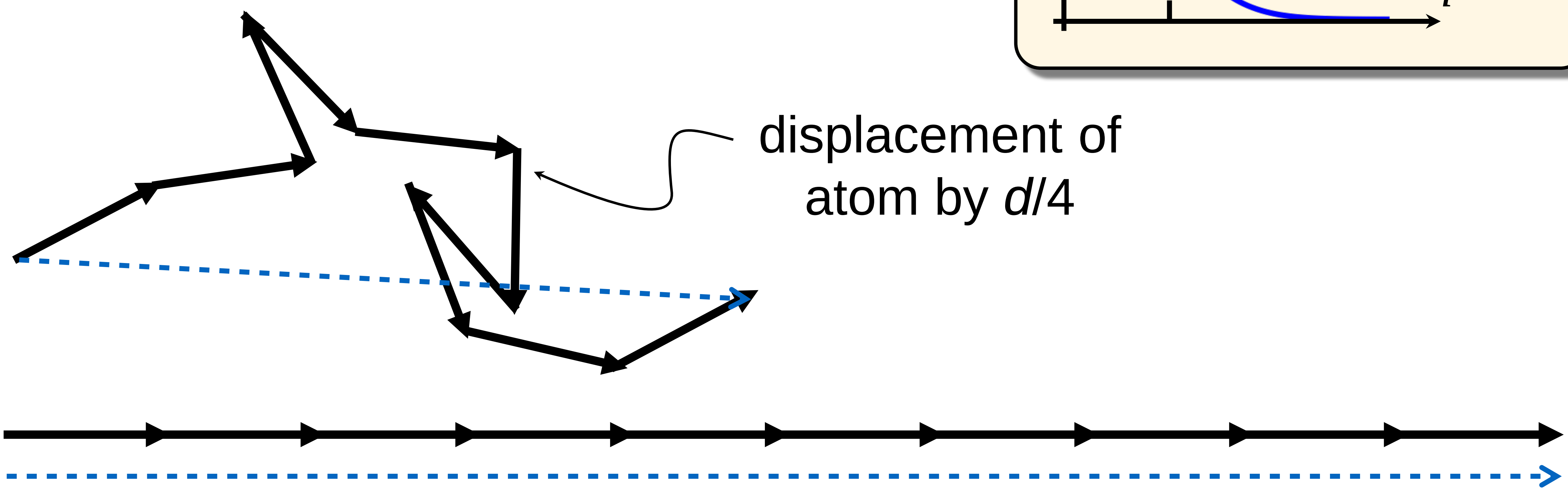
Crystal diffraction is sensitive to atomic displacements

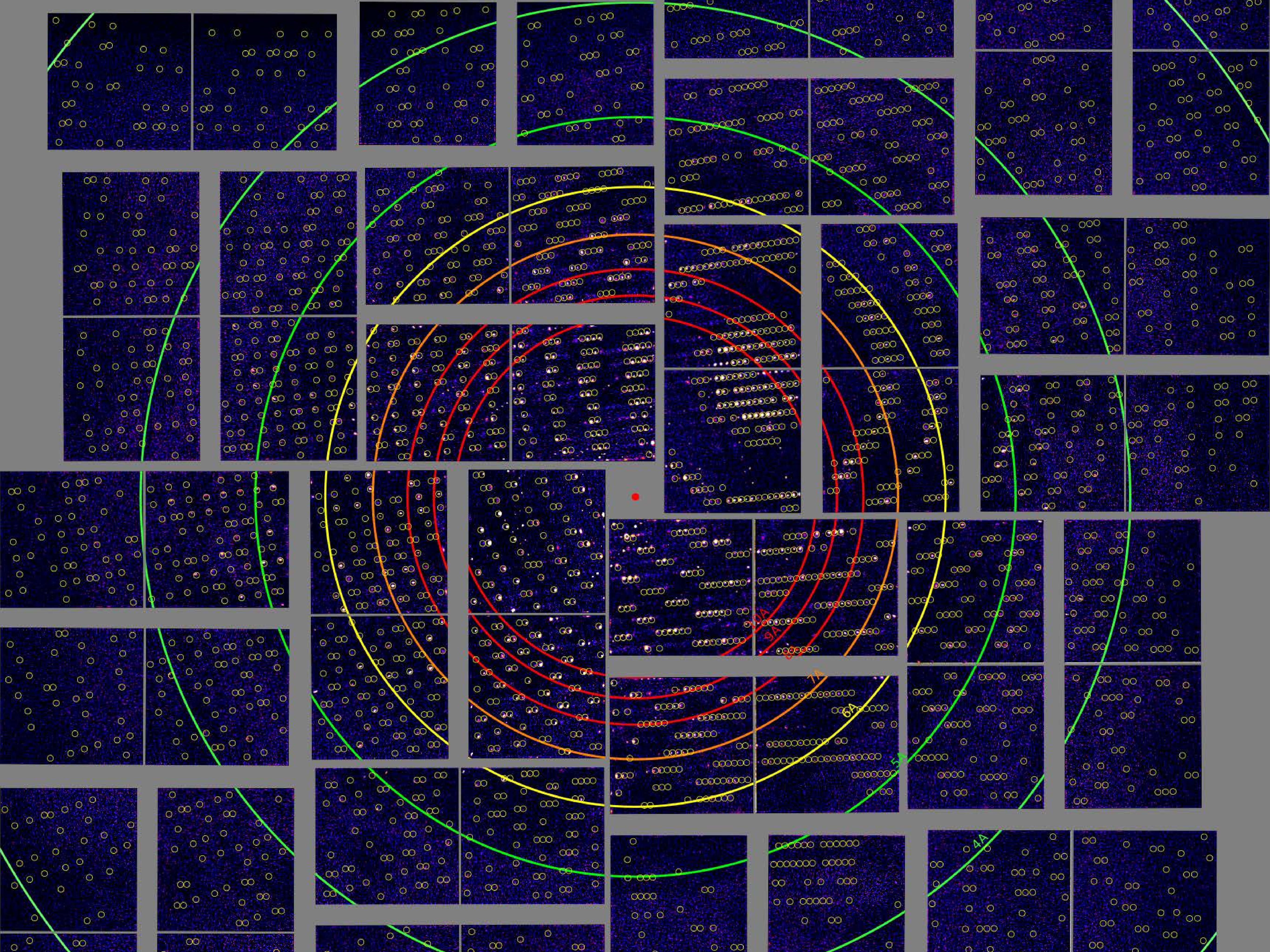
$$I(\mathbf{q}) = |f(\mathbf{q})|^2 = \left| \sum_i f_i \exp(i\mathbf{q}\cdot\mathbf{x}_i) \right|^2 \qquad |\mathbf{q}| = \frac{2\pi}{d}$$

$$\mathbf{x}_i \to \mathbf{x}_i + \mathbf{D}_i \qquad \langle \mathbf{D}_i \rangle = 0$$

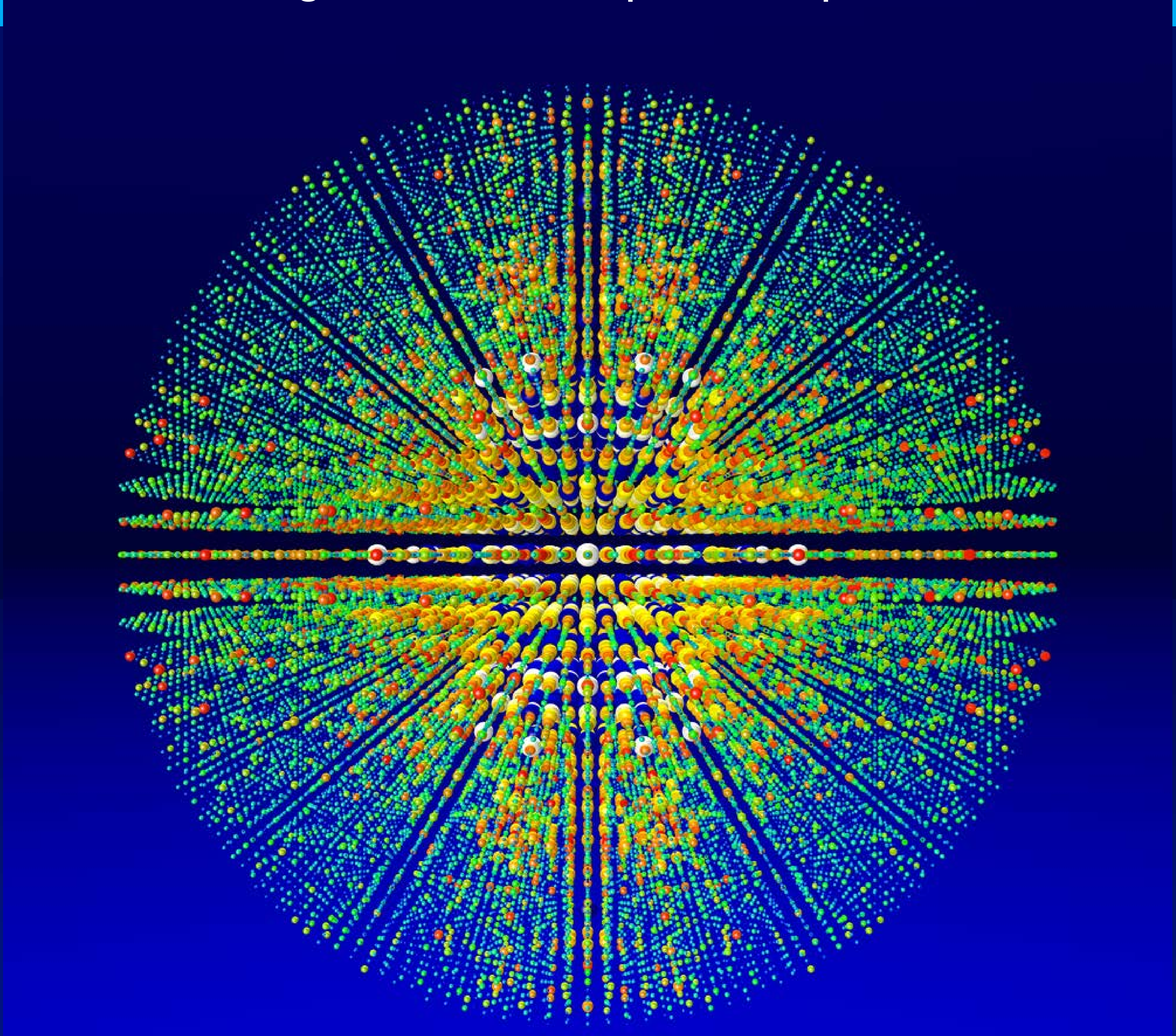
$$= |f(\mathbf{q})|^2 \exp(-q^2 \langle D^2 \rangle) + \sum_i f_i^2$$



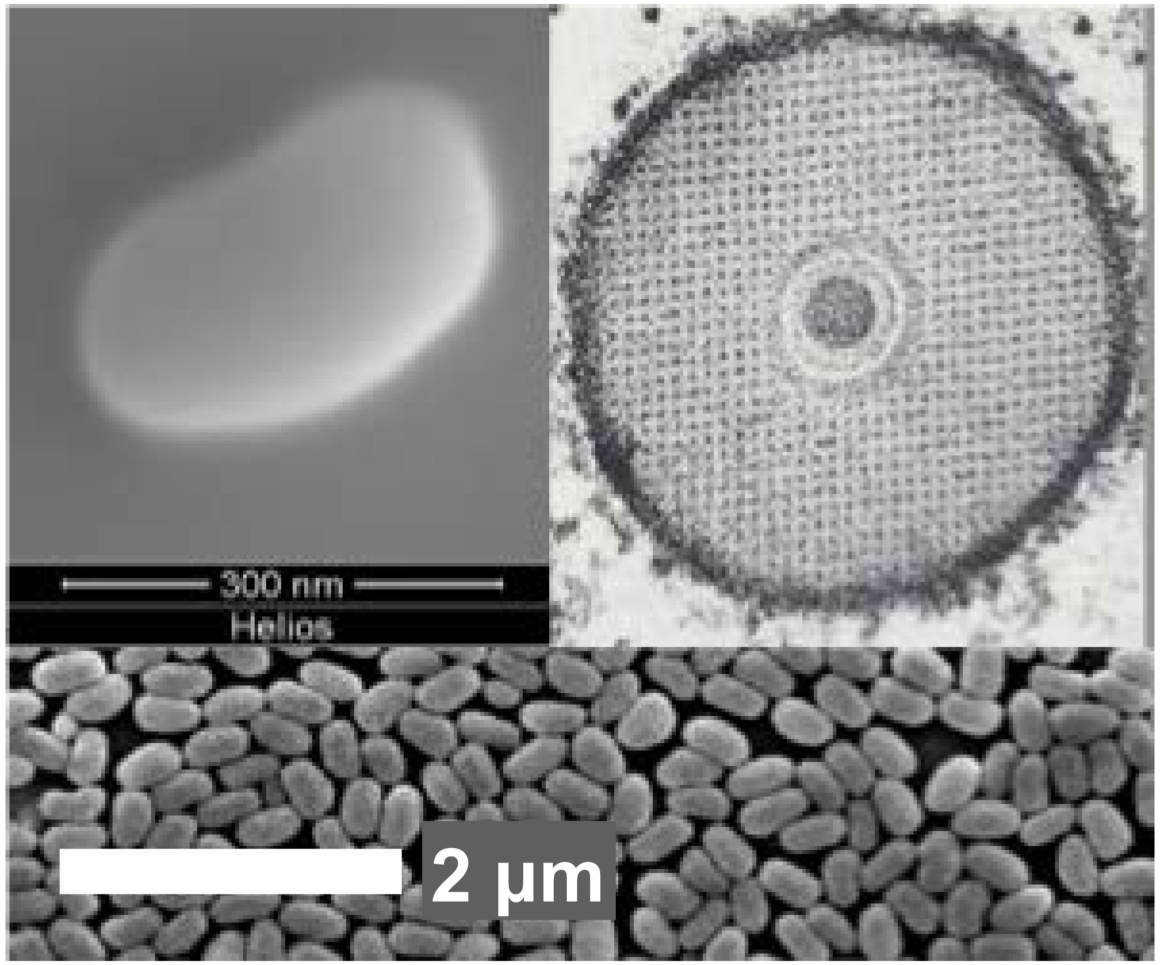


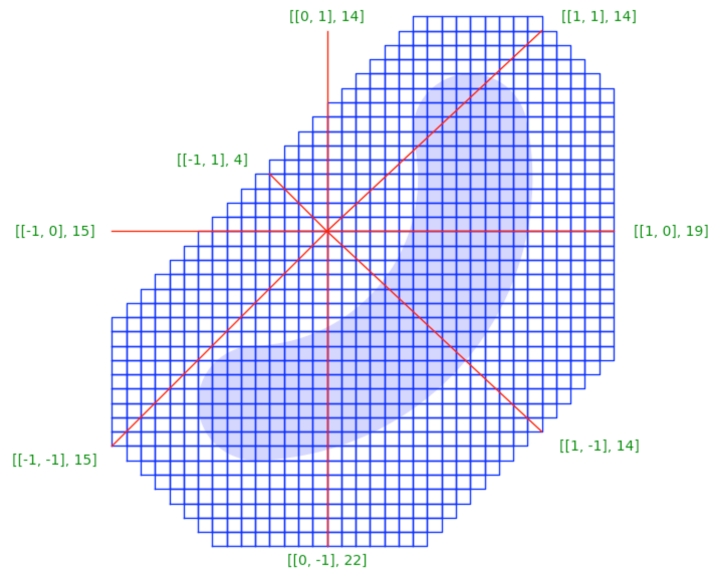

7A
6A
4A

Intensities are merged into a “3D powder” pattern



2.1 Å resolution structure of polyhedrin obtained from single Granulovirus particles

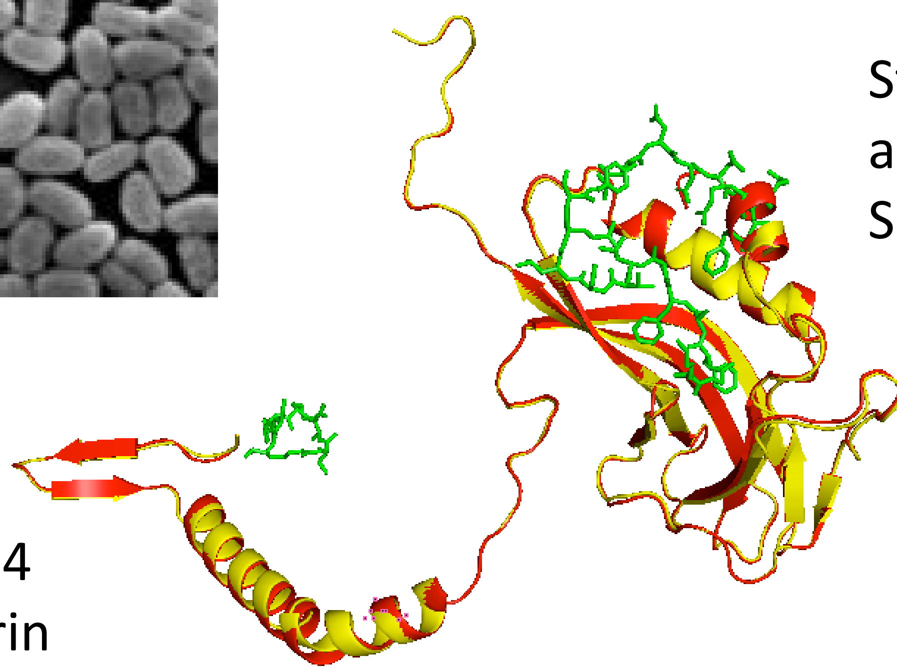


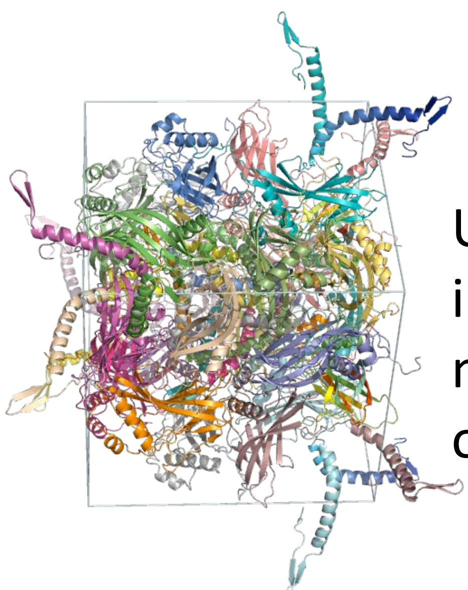


<u>Virus body</u>
8,000 unit cells,
a,b,c = 100Å
Volume of crystalline part: 0.008 μm^3

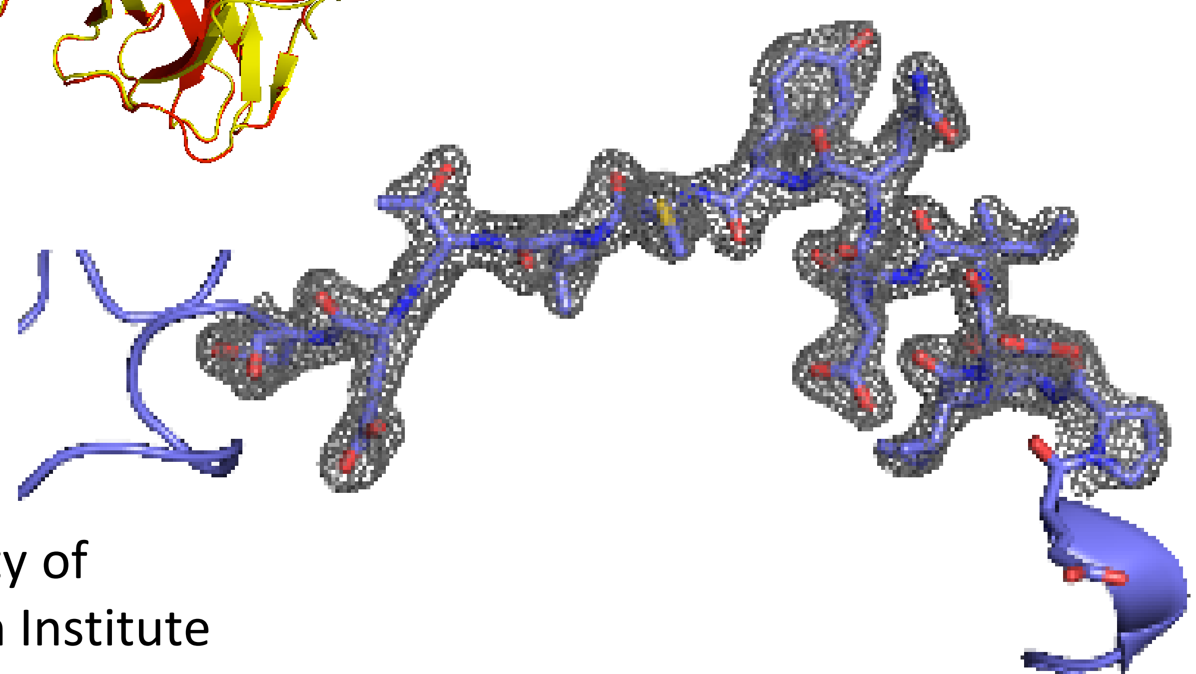
Dose: 2 GGy

Structure solved to 2.1 Å, by averaging over 100,000 patterns. Single-shot resolution about 4 Å



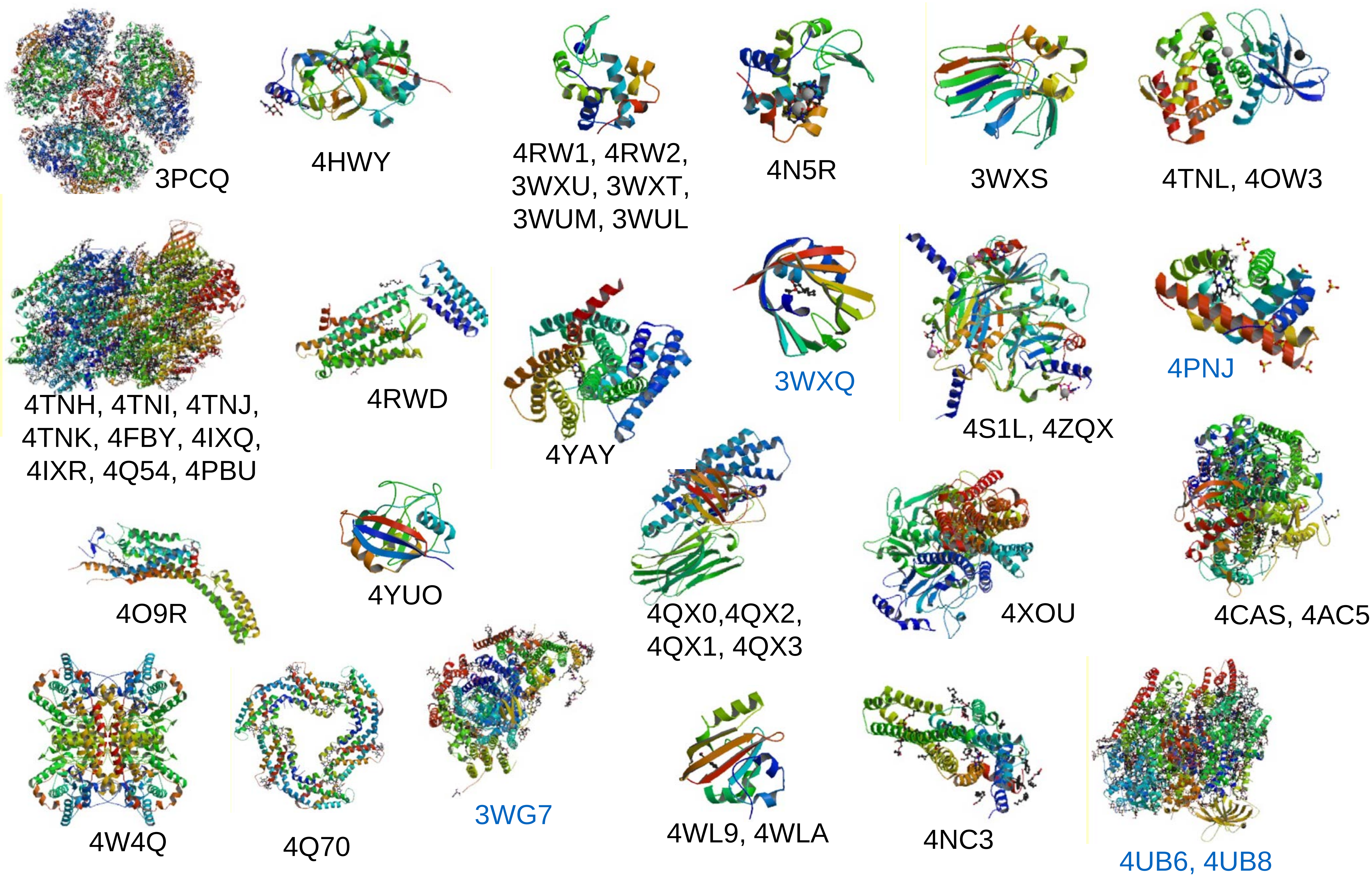


Unit cell consists of 24 interlocking polyhedrin molecules. Evolution optimised the crystal

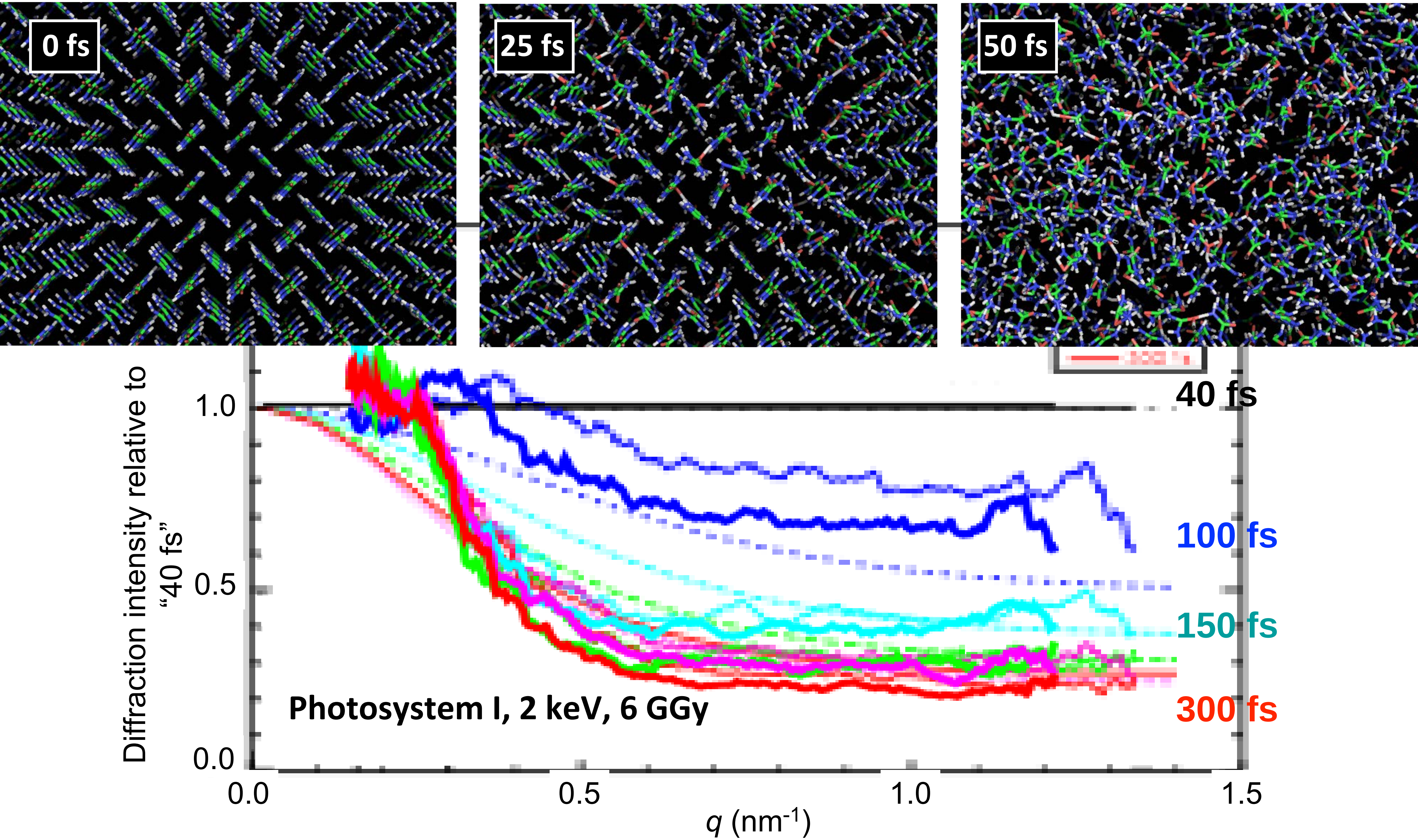


C. Gati, CFEL, with Peter Metcalf, University of Auckland and Johannes Jehle, Julius Kuehn Institute

There are now 44 FEL depositions in the protein databank

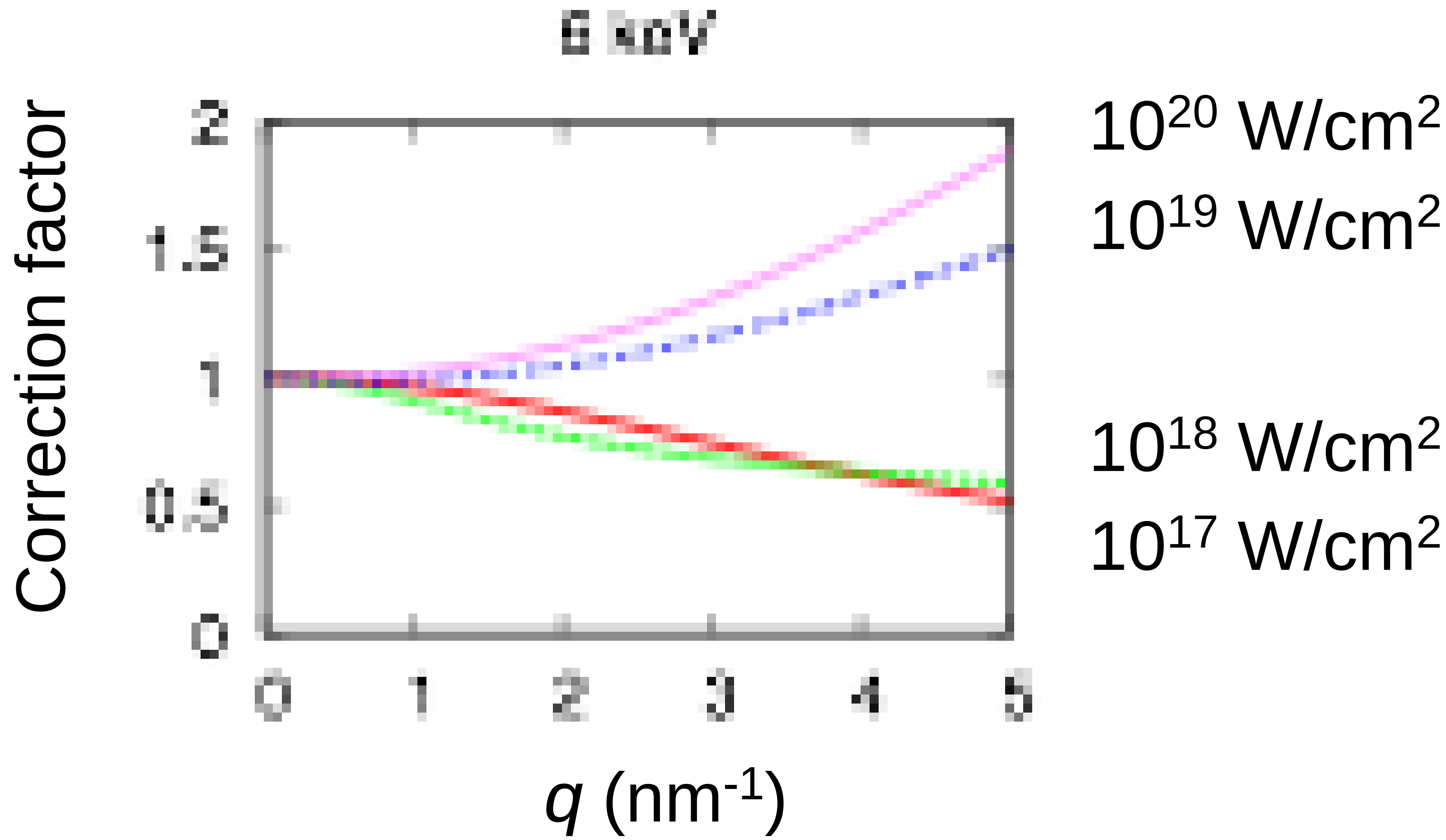


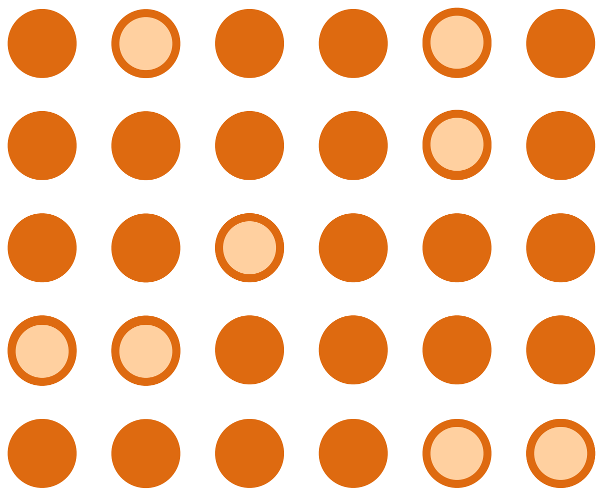
A crystal only gives Bragg diffraction when it is a crystal! Selecting Bragg peaks filters the data

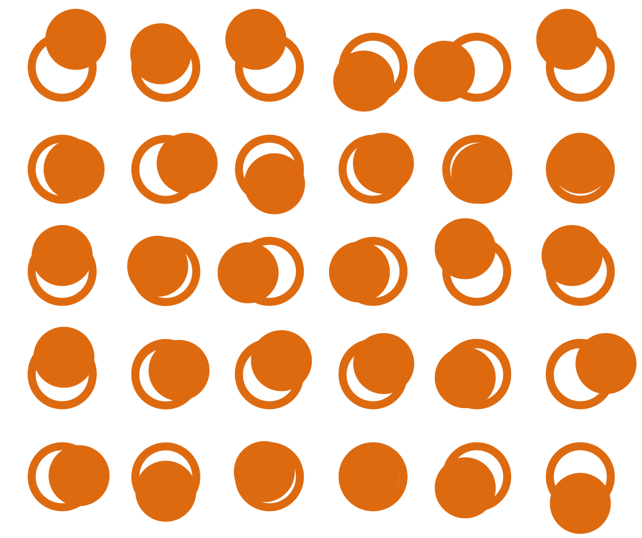


Barty *et al*. Nature Photon **6**, 35–40 (2012)

At fast timescales the ionisation gates the diffraction rather than nuclear motion

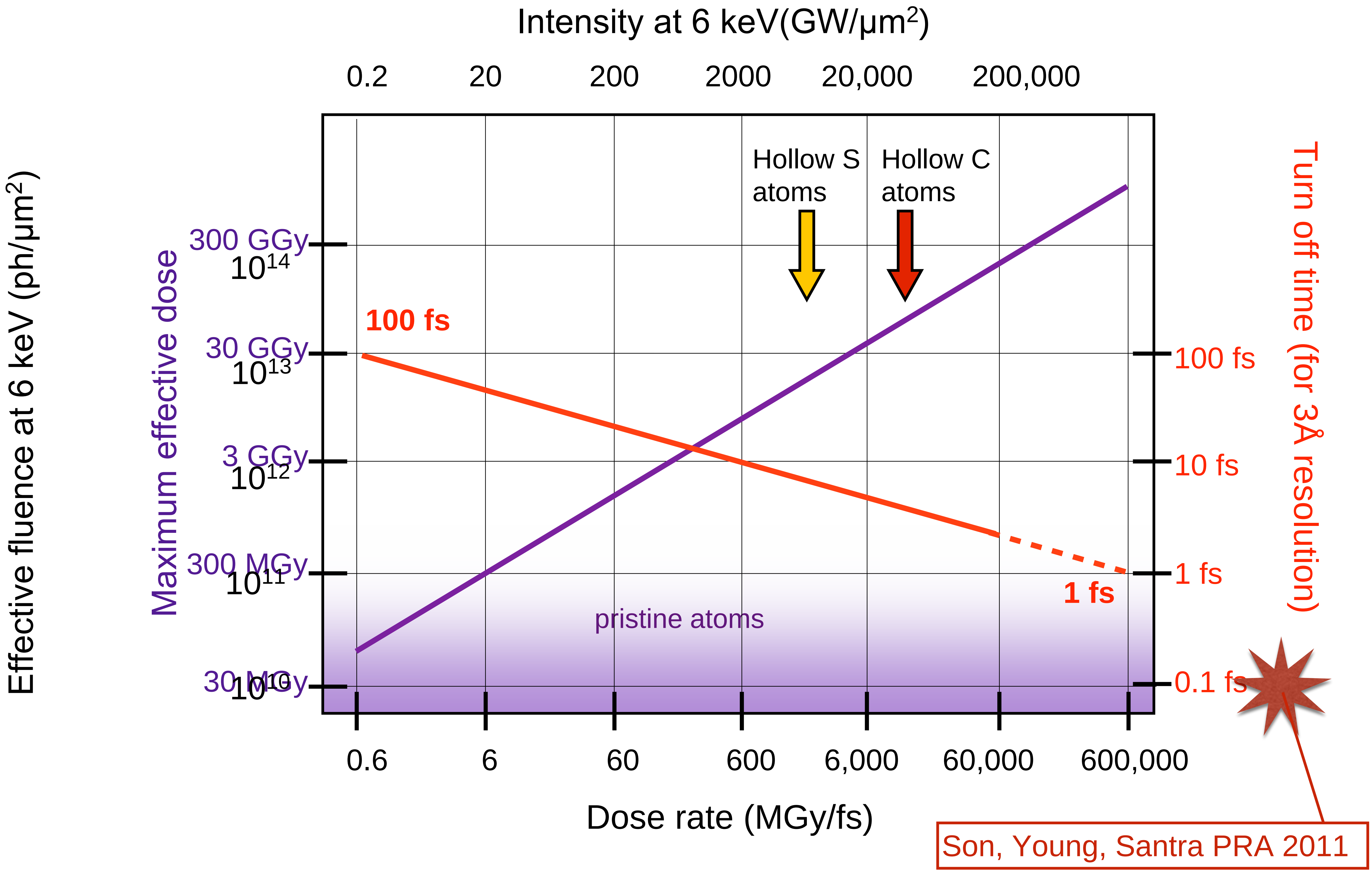




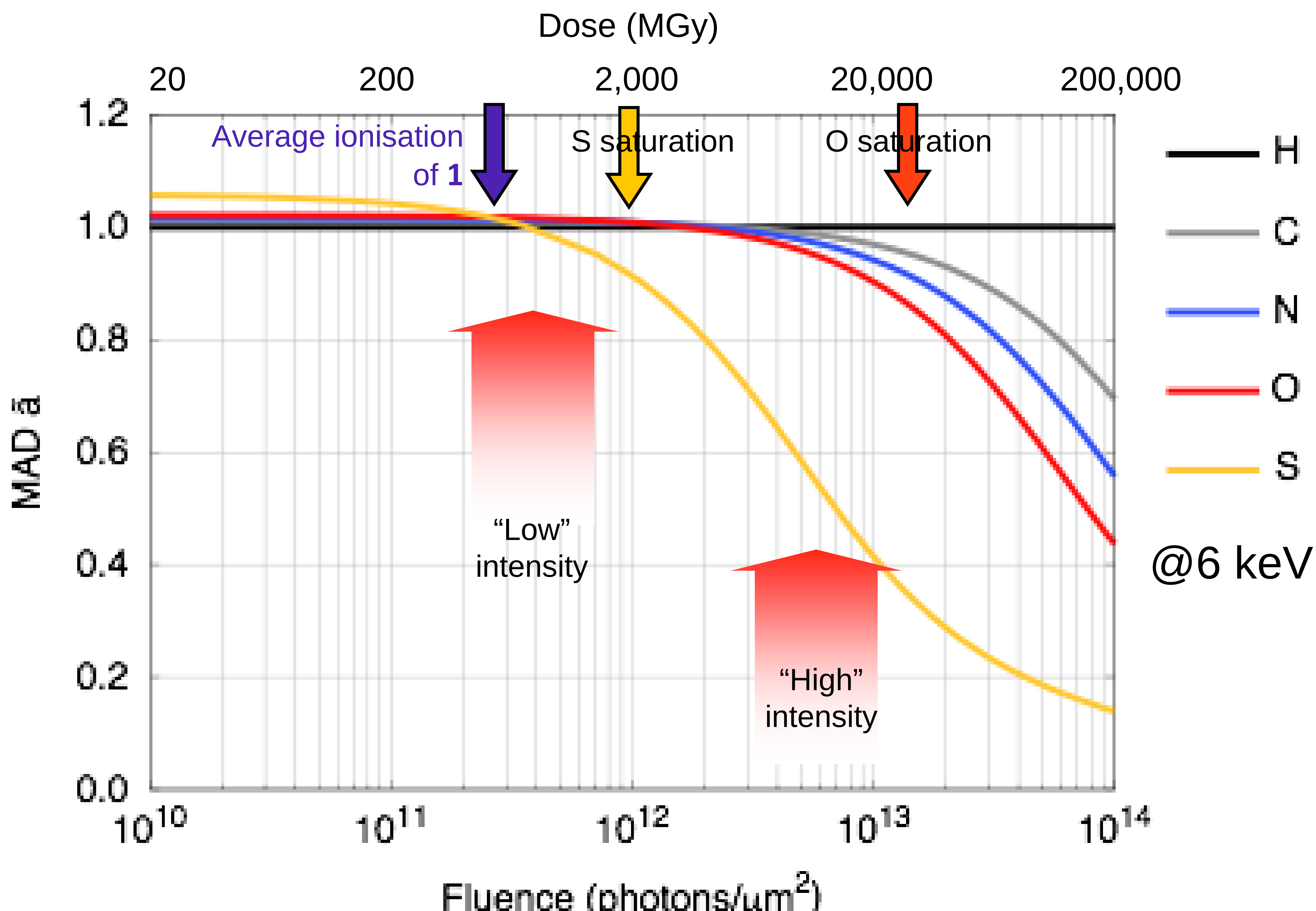


Caleman et al Opt Exp **23** 1213 (2015)

Higher dose rates (i.e. higher X-ray intensities) should give larger Bragg signals

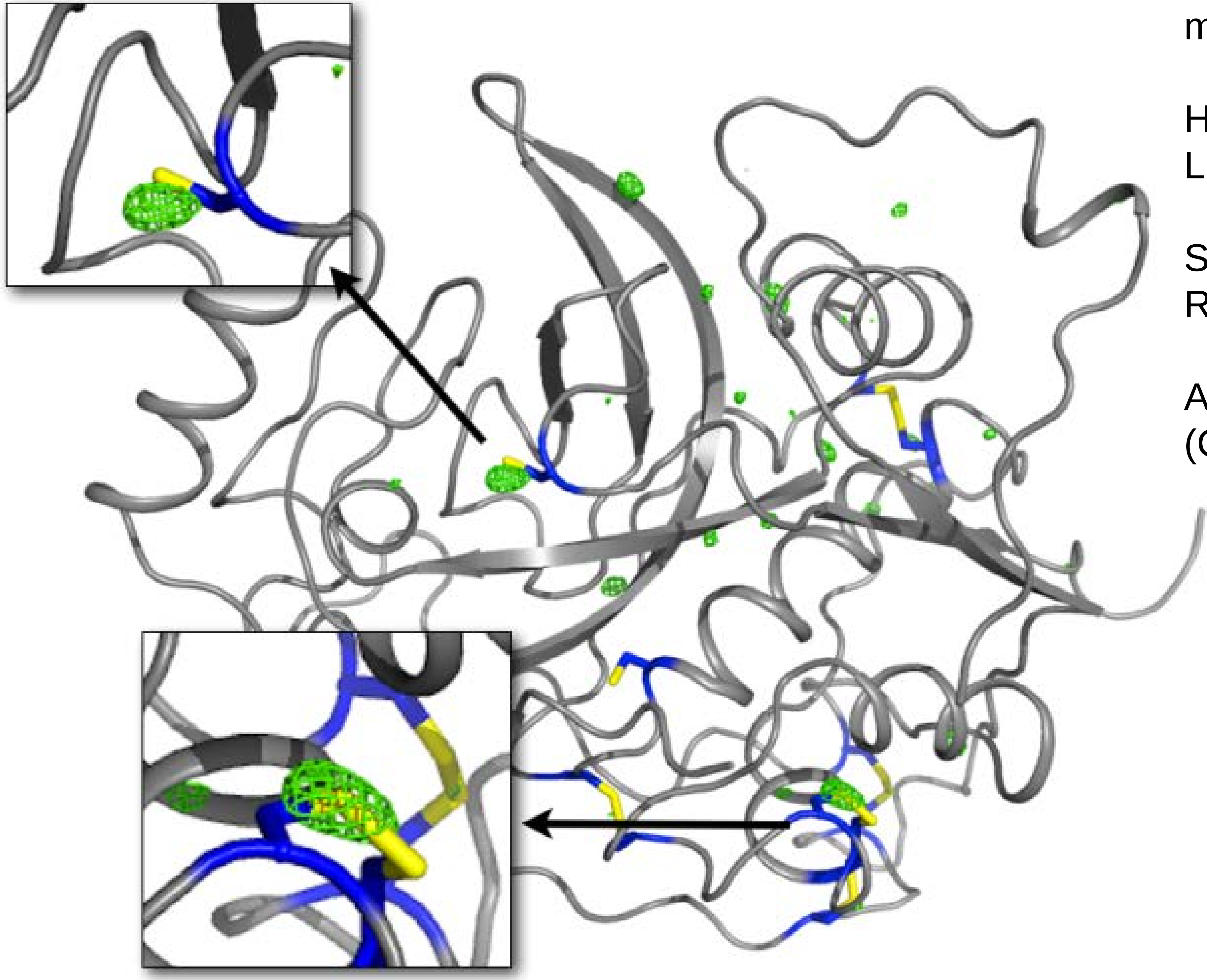


We propose to use the fluence dependence of heavier element scattering factors for phasing



S.K. Son, R. Santra CFEL

Difference electron density between high and low fluences reveals the positions of sulfur atoms



Difference map: High intensity minus low intensity

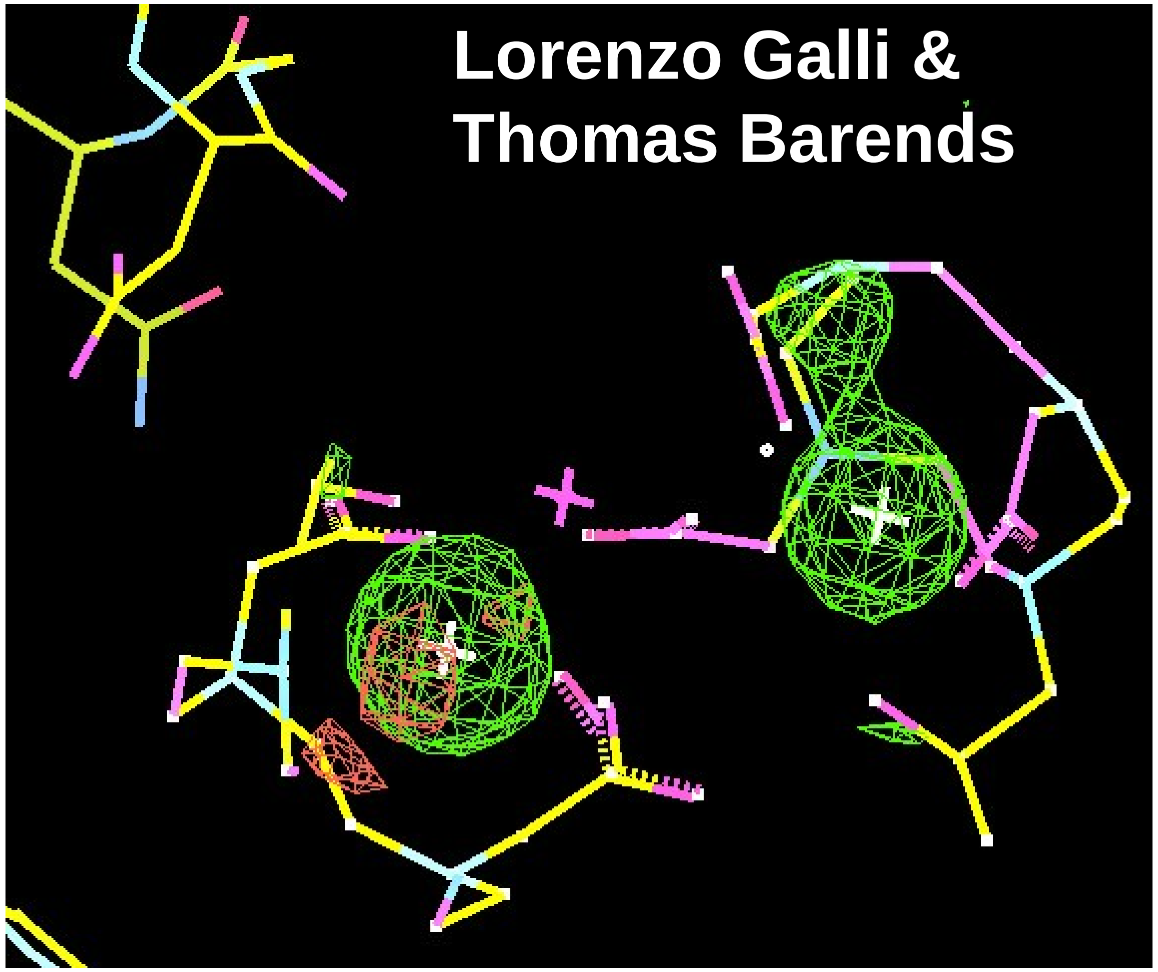
High intensity: ~20 GGy dose
Low intensity: ~2 GGy dose

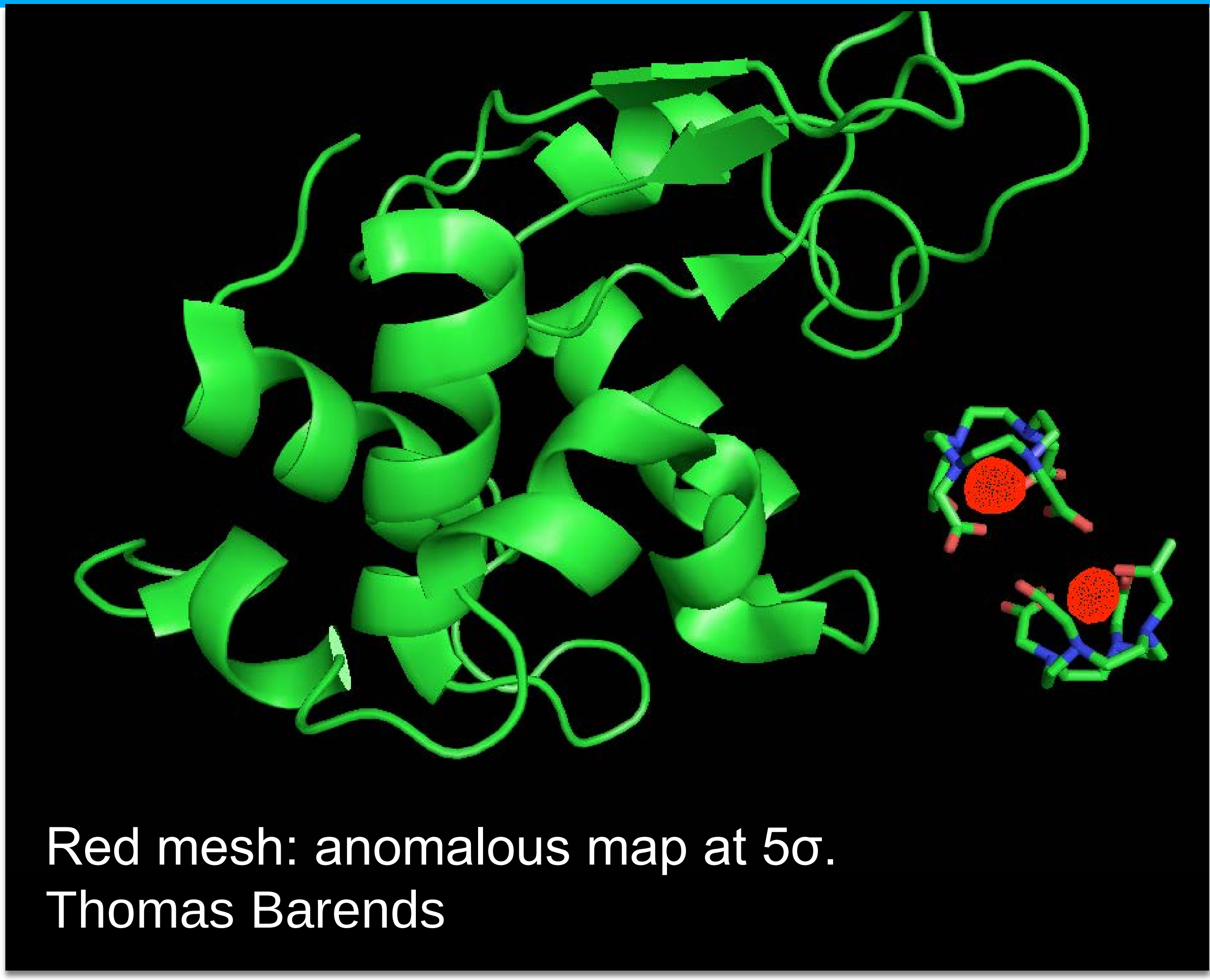
Sample: Cathepsin B (Lars Redecke)

Analysis: **Lorenzo Galli** (CFEL) & Max Nanao (ESRF)

CFEL SCIENCE

The Gd electron density is reduced at high fluence





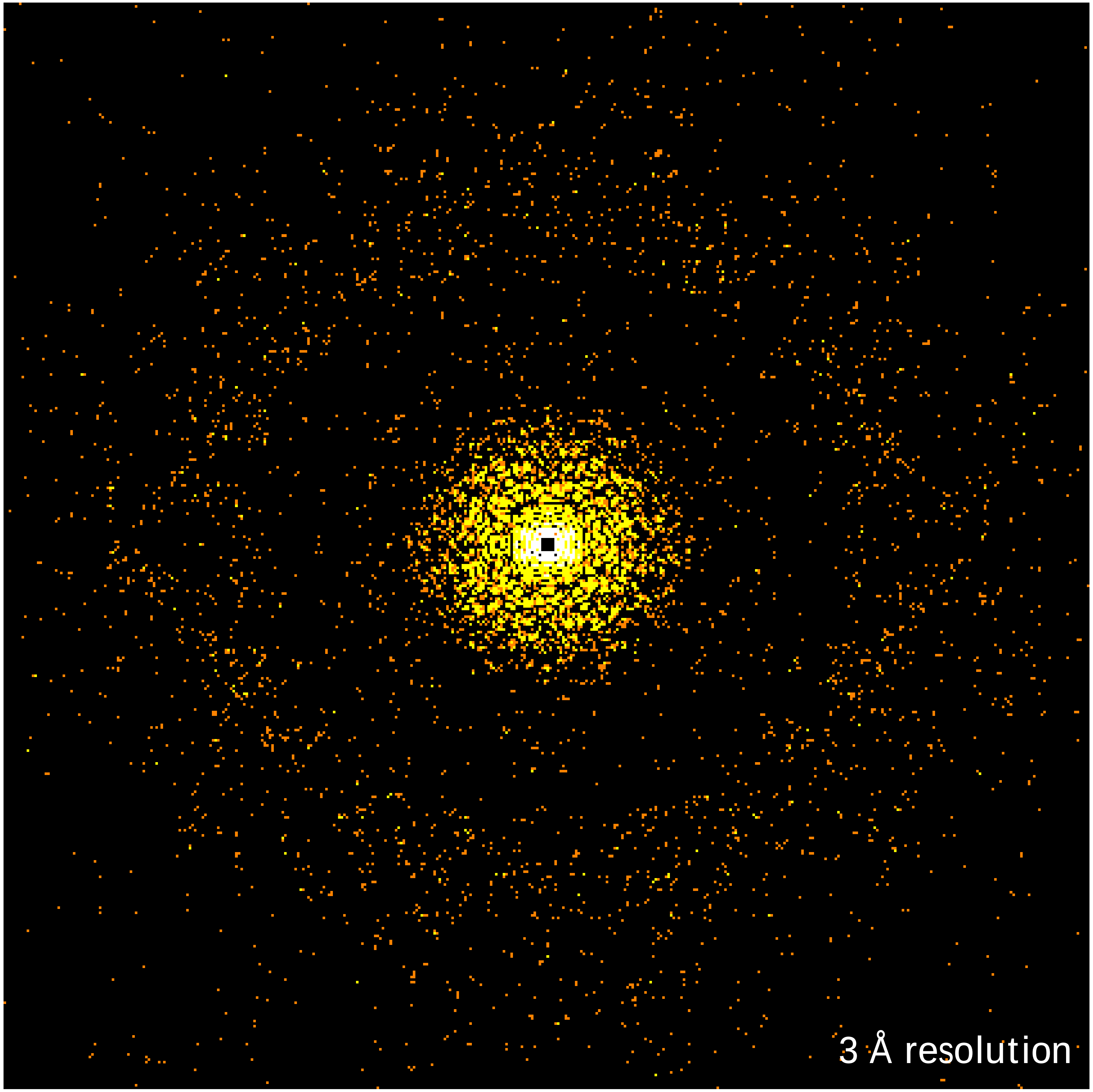
High fluence: 10^{13} ph/μm^2 (10 GGy)

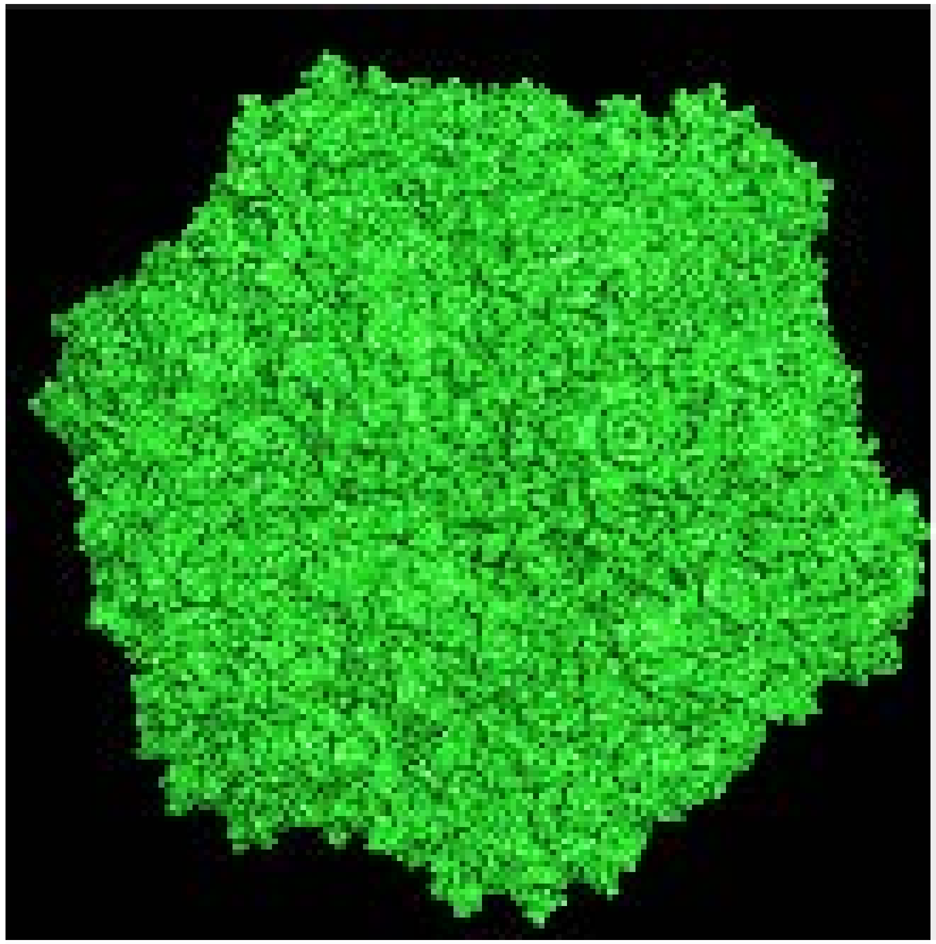
Low fluence: 2×10^{11} ph/μm^2 (200 MGy)

see also Barends et al Nature **505** (2014)

Difference: ~4 electrons in Gd (and surroundings)

Atomic-resolution diffraction from single particles should be possible with 10^{14} ph/μm^2



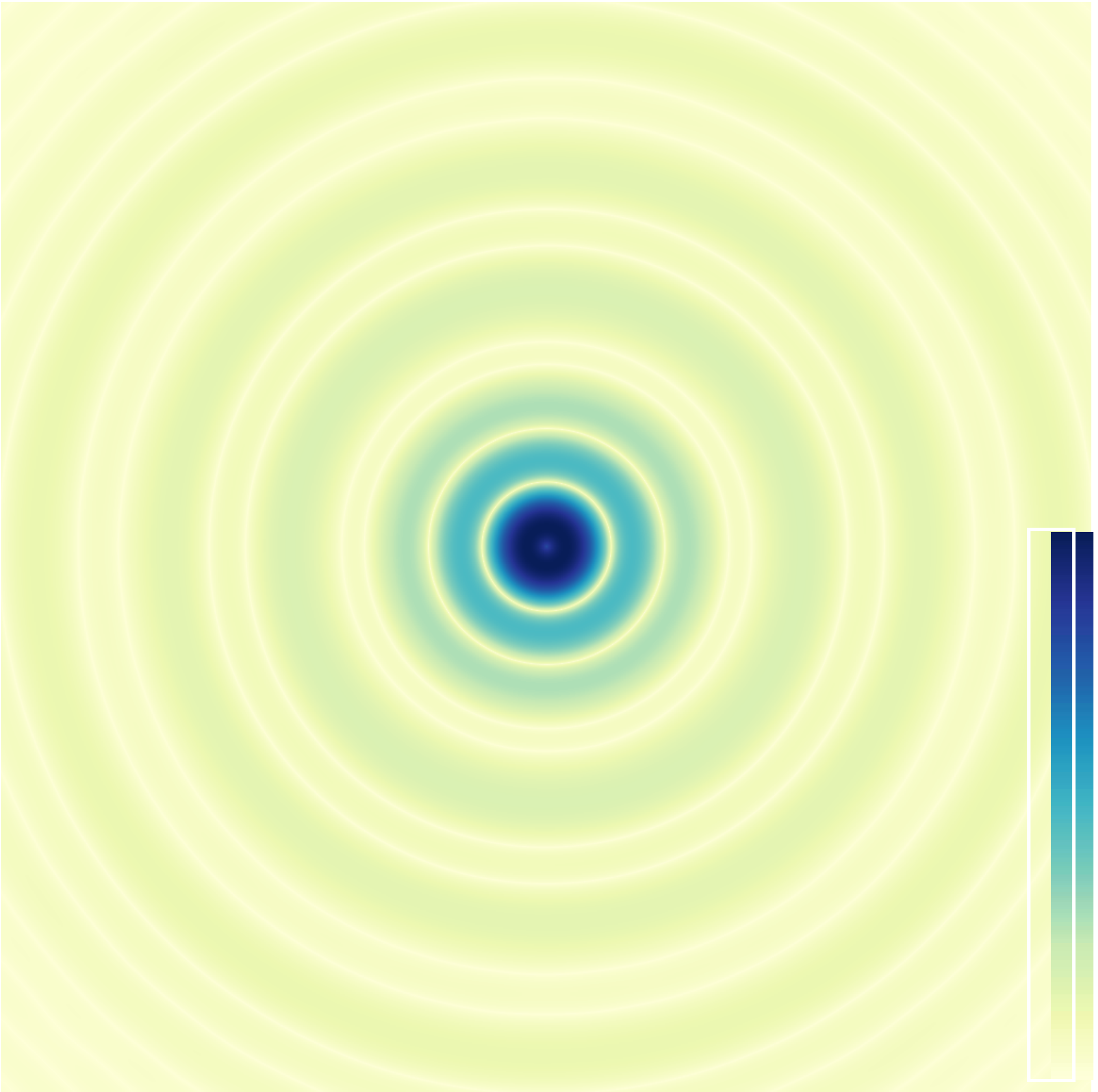


28 nm

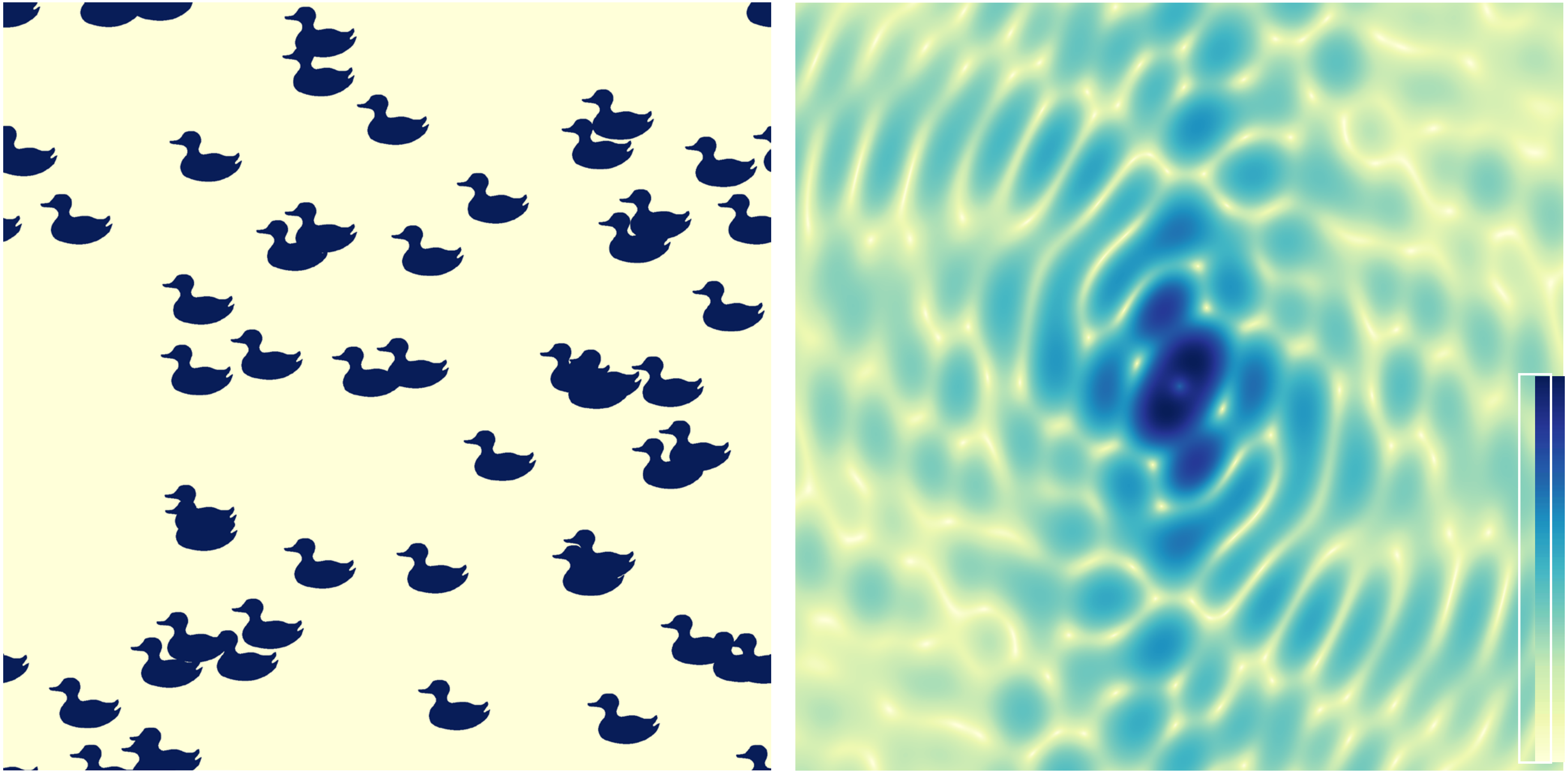
10^{14} ph/μm^2
60 GGy
6000 MGy/fs × 10 fs

RMS displacement: 0.5Å
half electrons ionized

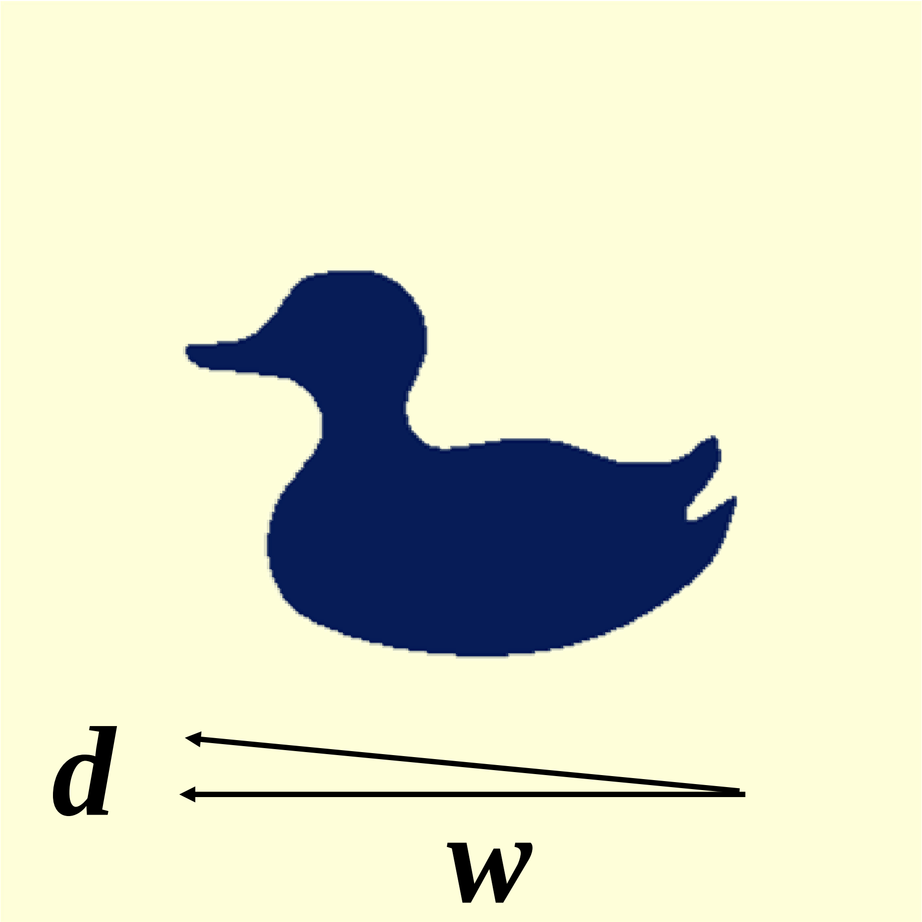
Solution scattering gives single-molecule diffraction, but orientationally averaged



Aligned molecules yield a single-molecule pattern

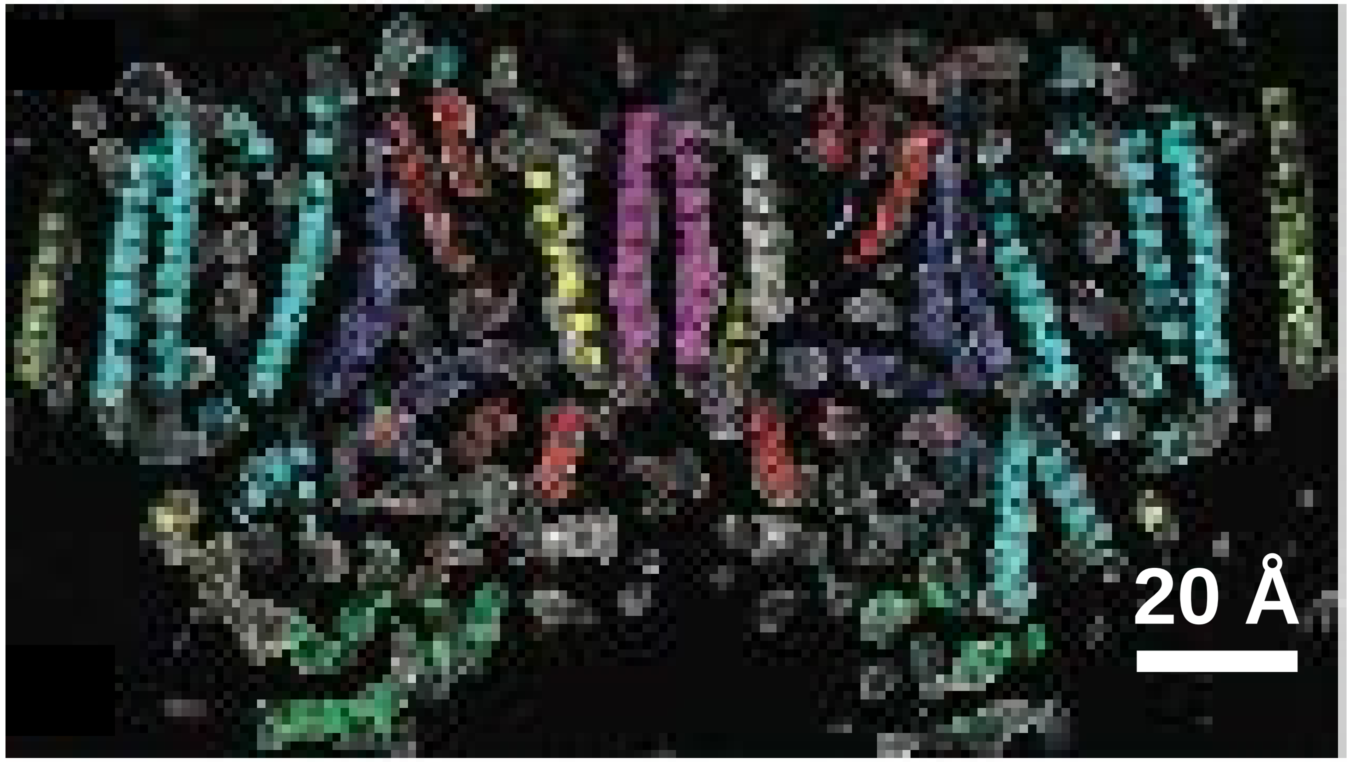


How well aligned do you need?



$$\Delta\phi = d/w$$

photosystem II



$$d = 3\text{Å}$$

$$w = 160\text{Å}$$

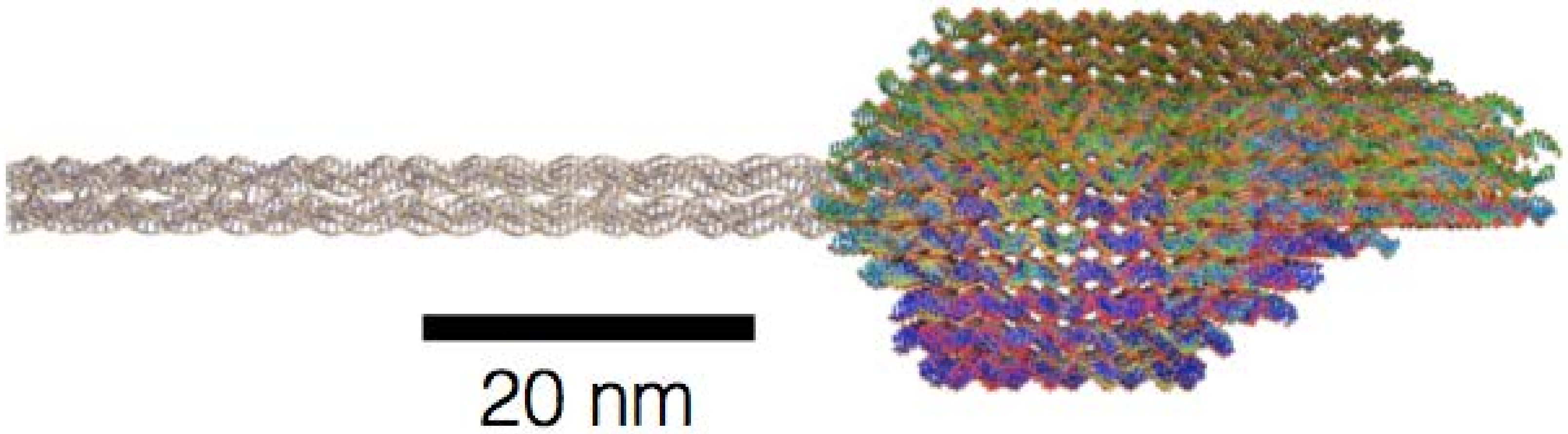
$$\Delta\phi = 1.1^\circ$$

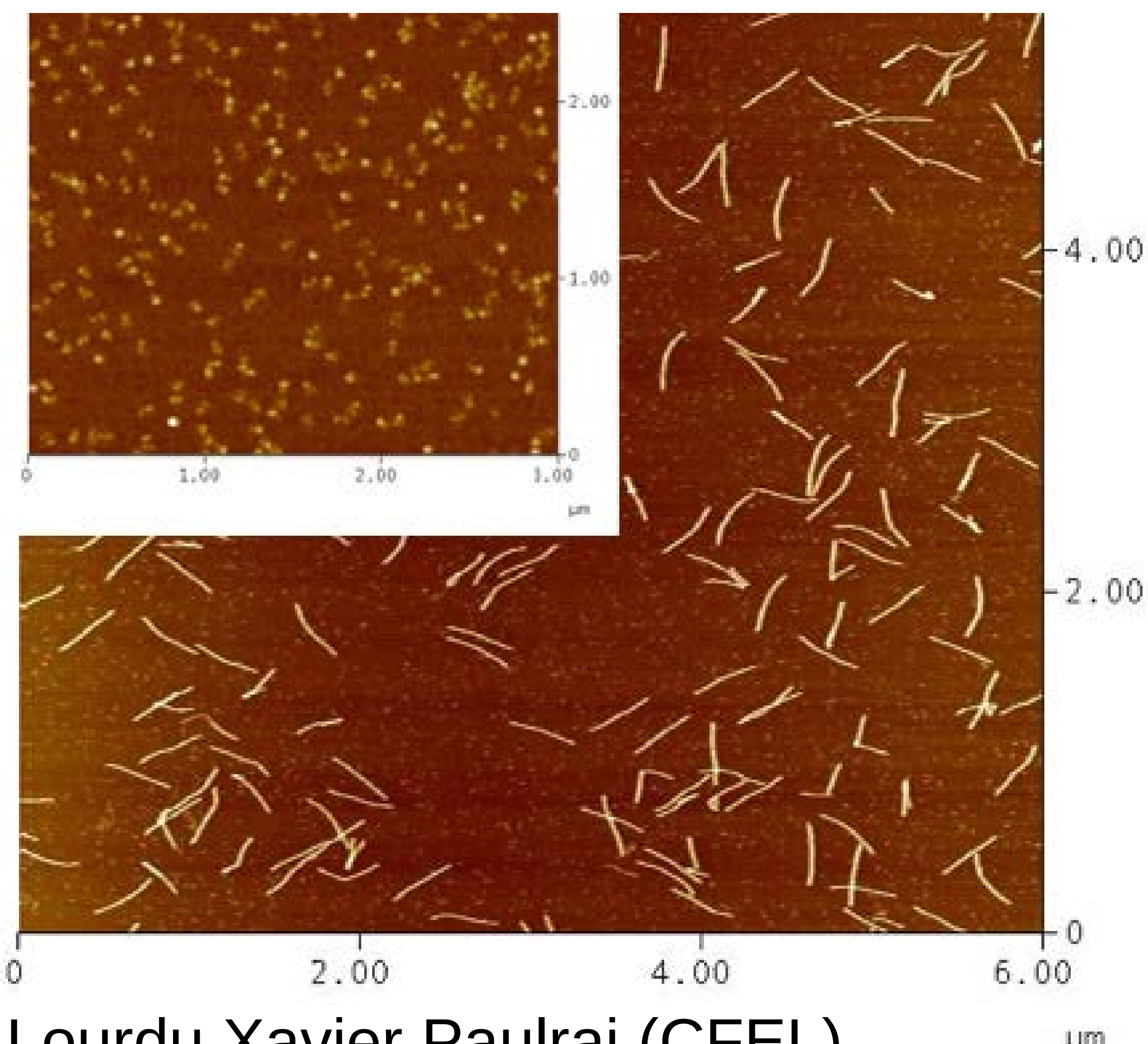
Needle-like objects align in the jet

Gas foc

Dan Deponte ASU

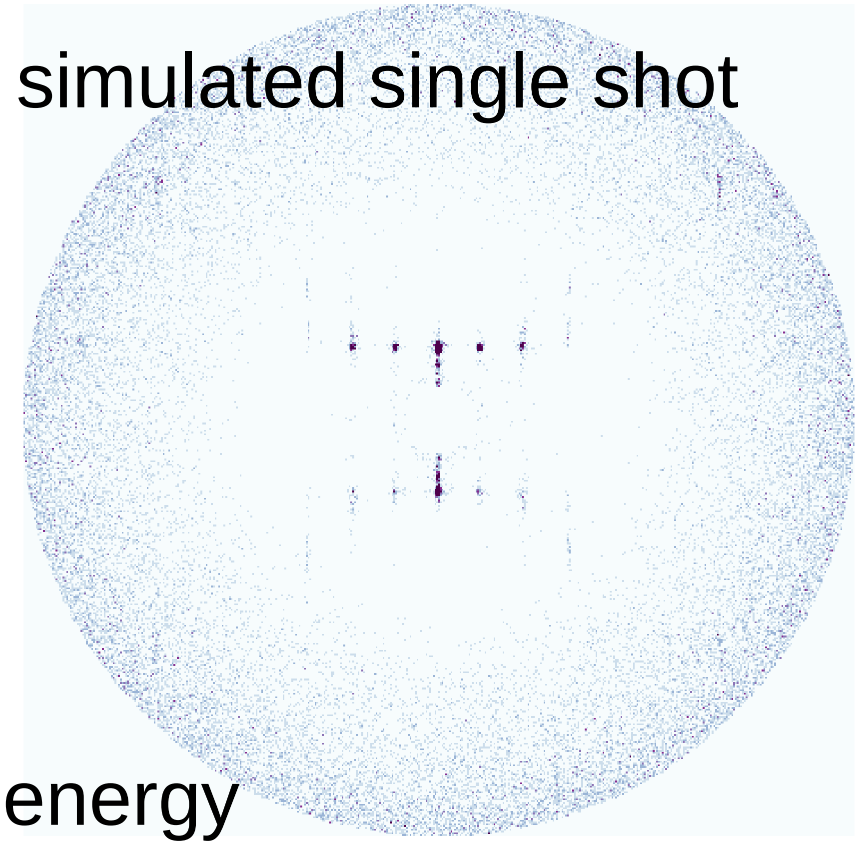
Adding DNA “kite tails” may align arbitrarily-shaped molecules





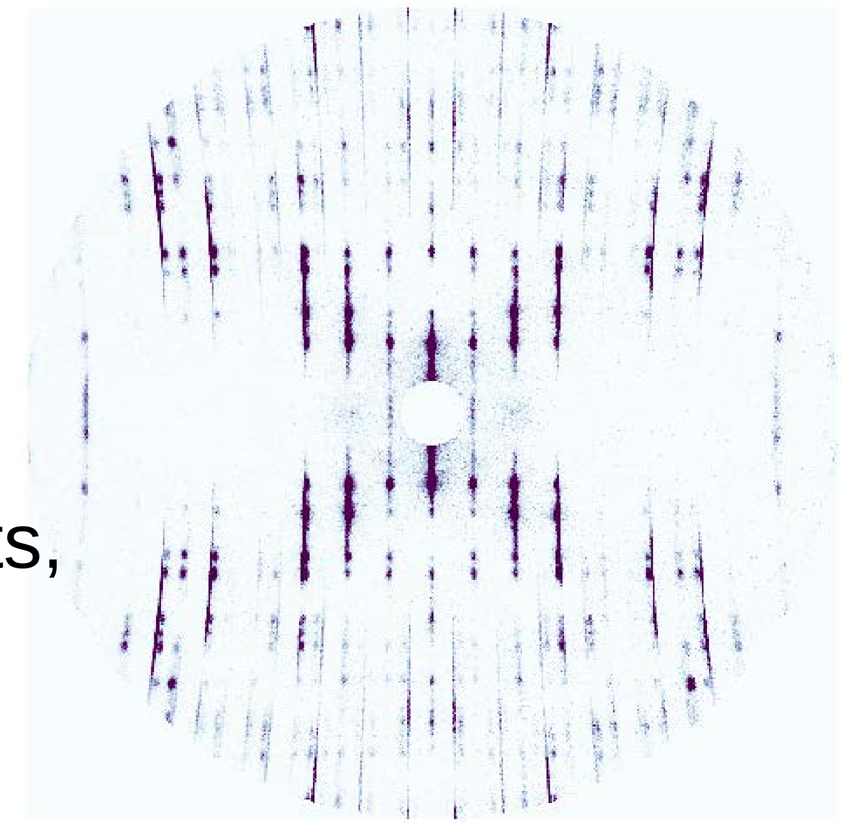
Lourdu Xavier Paulraj (CFEL)
Ned Seeman (NYU)

simulated single shot

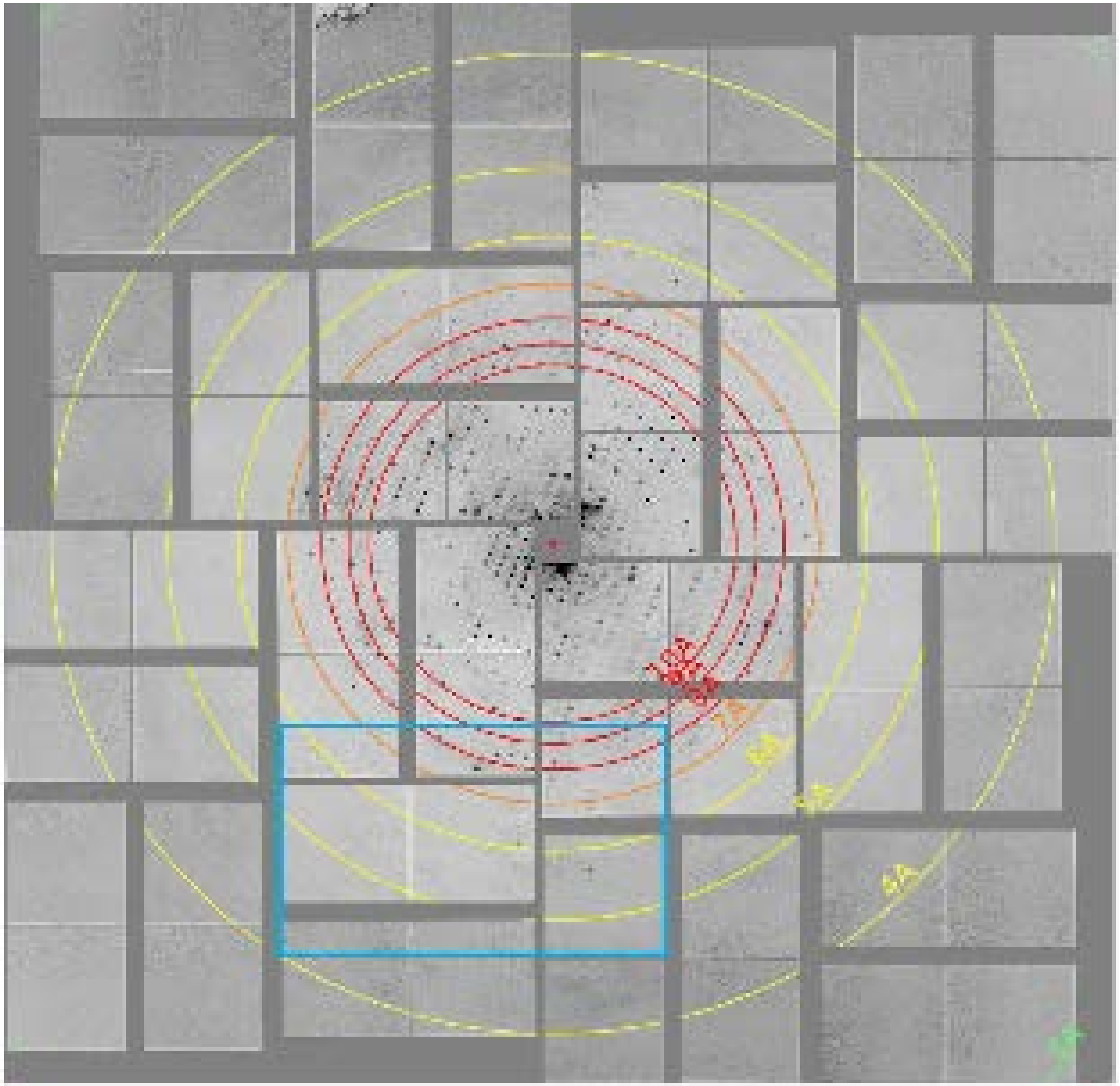


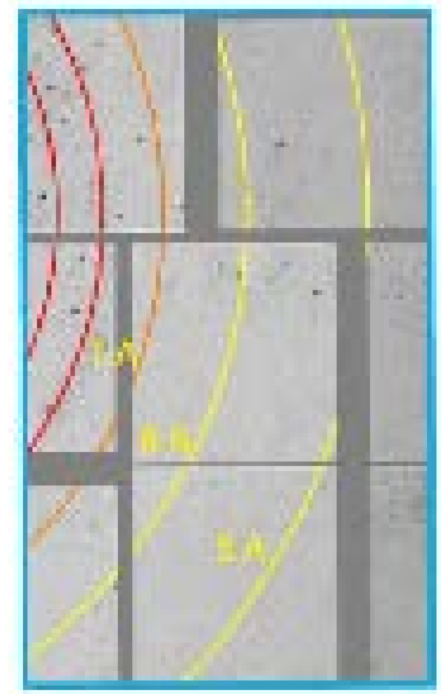
6 keV photon energy
10^{12} ph/µm^2
(1 GGy)

simulated
1000 shots,
axially
aligned



Even bad crystals should attain the required level of alignment





$$d = 2\pi\sqrt{\langle D^2\rangle}$$

$$d = 5\text{Å}$$

$$\sqrt{\langle D^2\rangle} = 0.8\text{Å}$$

Extended Data Figure 2 | Background corrected diffraction pattern of a photosystem II microcrystal

Kuptiz et al, Nature **513**, 261 (2014)

Acknowledgements

ALS: John Spence, Malcolm Howells, Stefano Marchesini, Anton Barty, Stefan Hau-Riege, Uwe Weierstall, David Shapiro, Haifeng He, Tobi Beetz, Chris Jacobsen

FLASH: Anton Barty, Mike Bogan, Sebastien Boutet, Matthias Frank, Stefan Hau-Riege, Stefano Marchesini, Bruce Woods, Saša Bajt, Henry Benner, Richard London, Elke Plonjes, Marion Kuhlmann, Rolf Treusch, Eberhard Spiller, Thomas Tschentscher, Thomas Möller, Christoph Bostedt, David Shapiro, Magnus Bergh, Carl Caleman, Gosta Huldt, Mavin Seibert, Filipe Maia, Dick Lee, Abraham Szoke, Nic Timneanu, Janos Hajdu

LCLS: Petra Fromme, Anton Barty, Thomas White, Rick Kirian, Andrew Aquila, Mark Hunter, Joachim Schulz, Daniel DePonte, Uwe Weierstall, R. Bruce Doak, Filipe Maia, Andrew Martin, Ilme Schlichting, Lukas Lomb, Nicola Coppola, Bob Shoeman, Sascha Epp, Robert Hartmann, Daniel Rolles, Artem Rudenko, Lutz Foucar, Nils Kimmel, Georg Weidenspointner, Peter Holl, Mengning Liang, Miriam Barthelmess, Carl Caleman, Sebastien Boutet, Mike Bogan, Jacek Krzywinski, Christoph Bostedt, Saša Bajt, Lars Gumprecht, Benedikt Rudek, Benjamin Erk, Carlo Schmidt, Andre Homke, Christian Reich, Daniel Pietschner, Lothar Struder, Gunter Hauser, Hubert Gorke, Joachim Ullrich, Sven Herrmann, Gerhard Schaller, Florian Schopper, Heike Soltau, Kai-Uwe Kuhnel, Marc Messerschmidt, John Bozek, Stefan Hau-Riege, Matthias Frank, Christina Hampton, Raymond Sierra, Dmitri Starodub, Garth Williams, Janos Hajdu, Nicusor Timneanu, Marvin Seibert, Jakob Andreasson, Andrea Rocker, Olof Jonsson, Martin Svenda, Stephan Stern, Karol Nass, Robert Andritschke, Claus-Dieter Schroter, Faton Krasniqi, Mario Bott, Kevin Schmidt, Xiaoyu Wang, Ingo Grotjohann, James Holton, Thomas Barends, Richard Neutze, Stefano Marchesini, Raimund Fromme, Sebastian Schorb, Daniela Rupp, Marcus Adolph, Tais Gorkhover, Inger Andersson, Helmut Hirsemann, Guillaume Potdevin, Heinz Graafsma, Bjorn Nilsson, Gergey Katona, Lars Redecke, Francseco Stellato, Nic Timneanu, Dingie Wang, Nadia Zatsepin, D. Schafer, J. Defever, P. A. Montanez, C. Kenney, R. Herbst, P. Hart, J. Pines, G. Haller, S. M. Gruner, H. T. Philipp, M. W. Tate, M. Hromalik, L. J. Koerner, N. van Bakel, J. Morse, W. Ghonsalves, John Spence
Trevor Forsyth, Estelle Mossou, Peter Metcalf, Ned Seeman, Christian Betzel, Ken Beyerlein, Dominik Oberthuer, Richard Bean, Chuck Yoon, Fenglin Wang, Lorenzo Galli, Oleksandr Yefanov, Cornelius Gati, Michael Heymann, C. Seuring, M. Metz, A. Morgan, V. Mariani, Robin Santra, Sang-Kil Son

Current Funding:

DESY

HELMHOLTZ ASSOCIATION

erc European Research Council Established by the European Commission

Bundesministerium für Bildung und Forschung

CUI THE HAMBURG CENTER FOR ULTRAFAST IMAGING

Acknowledgements - The phasers

Kartik Ayyer

Anton Barty

Oleksandr Yefanov

Dominik Oberthür

Lorenzo Galli

Valerio Mariani

Tom White

DESY

HELMHOLTZ | ASSOCIATION

erc
European Research Council
Established by the European Commission

Bundesministerium für Bildung und Forschung

CUI THE HAMBURG CENTER FOR ULTRAFAST IMAGING

Phasing diffraction of periodic structures is challenging because of the information deficit

David Sayre

Acta Cryst. (1952). **5**, 843

Some implications of a theorem due to Shannon. By D. Sayre, *Johnson Foundation for Medical Physics, University of Pennsylvania, Philadelphia 4, Pennsylvania, U. S. A.*

(*Received 3 July* 1952)

Shannon (1949), in the field of communication theory, has given the following theorem: If a function $d(x)$ is known to vanish outside the points $x = \pm a/2$, then its Fourier transform $F(X)$ is completely specified by the values which it assumes at the points $X = 0, \pm 1/a, \pm 2/a, \ldots$. In fact, the continuous $F(X)$ may be filled in merely by laying down the function $\sin \pi a X / \pi a X$ at each of the above points, with weight equal to the value of $F(X)$ at that point, and adding.

Now the electron-density function $d(x)$ describing a single unit cell of a crystal vanishes outside the points $x = \pm a/2$, where a is the length of the cell. The reciprocal-lattice points are at $X = 0, \pm 1/a, \pm 2/a, \ldots$, and hence the experimentally observable values of $F(X)$ would suffice, by the theorem, to determine $F(X)$ everywhere, if the phases were known. (In principle, the necessary points extend indefinitely in reciprocal space, but by using, say, Gaussian atoms both $d(x)$ and $F(X)$ can be effectively confined to the unit cell and the observable region, respectively.)

For centrosymmetrical structures, to be able to fill in the $|F|^2$ function would suffice to yield the structure, for sign changes could occur only at the points where $|F|^2$ vanishes. The structure corresponding to the $|F|^2$ function is the Patterson of a single unit cell. This has twice the width of the unit cell, and hence to fill in the $|F|^2$ function would require knowledge of $|F|^2$ at the half-integral, as well as the integral h's. This is equivalent to a statement made by Gay (1951).

I think the conclusions which may be stated at this point are:

1. Direct structure determination, for centrosymmetric structures, could be accomplished as well by finding the sizes of the $|F|^2$ at half-integral h as by the usual procedure of finding the signs of the F's at integral h.

2. In work like that of Boyes-Watson, Davidson & Perutz (1947) on haemoglobin, where $|F|^2$ was observed at non-integral h, it would suffice to have only the values at half-integral h.

The extension to three dimensions is obvious.

References

Boyes-Watson, J., Davidson, E. & Perutz, M. F. (1947). *Proc. Roy. Soc.* A, **191**, 83.

Gay, R. (1951). Paper presented at the Second International Congress of Crystallography, Stockholm.

Shannon, C. E. (1949). *Proc. Inst. Radio Engrs., N.Y.* **37**, 10.

Current phasing methods for crystal diffraction:

- Molecular replacement
- Anomalous diffraction
- Isomorphic replacement (and things like RIP)
- Direct methods (atomic resolution)
- Density modification