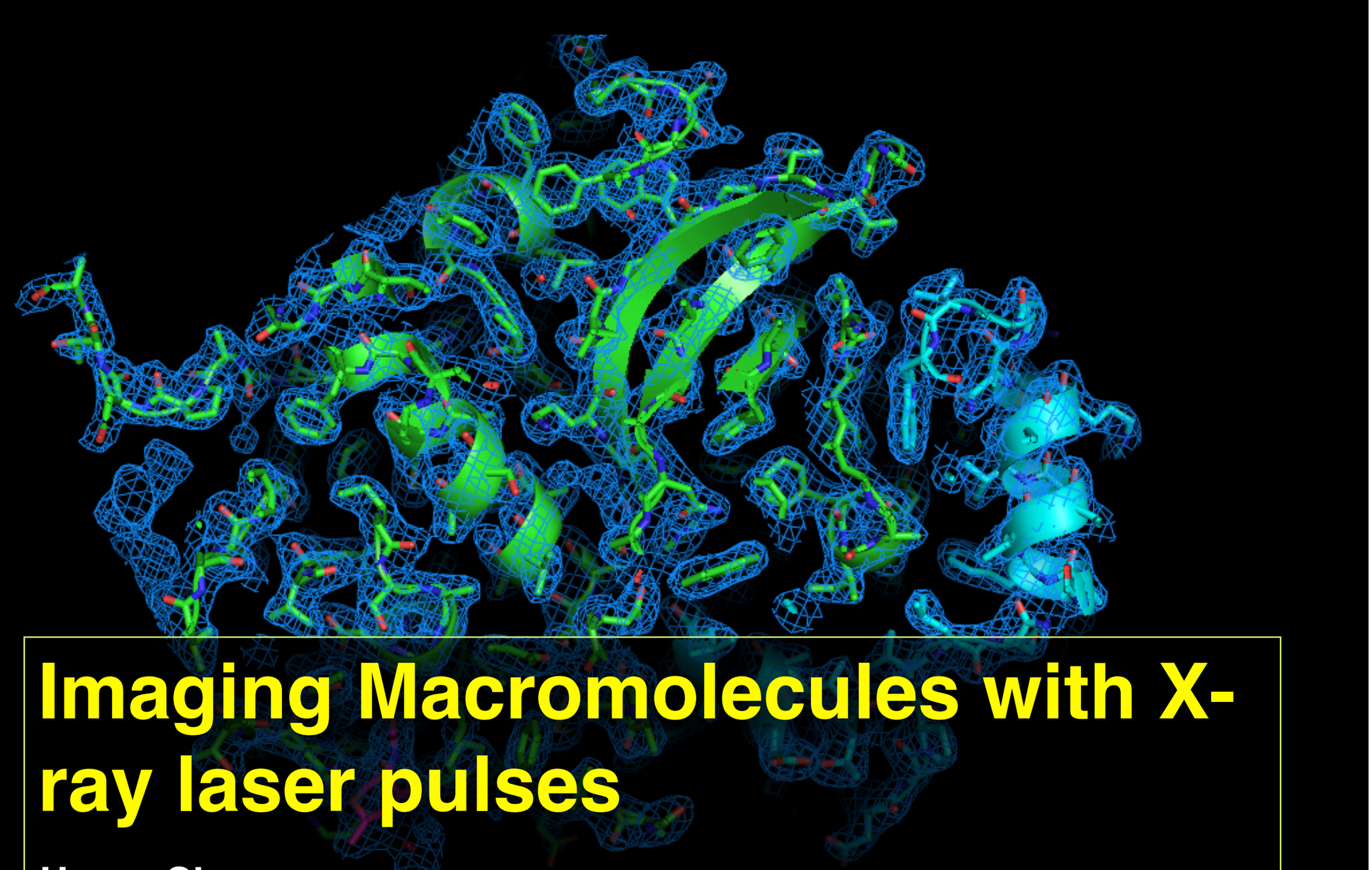


Imaging Macromolecules with X-ray laser pulses

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

CERN Colloquium - July 2017

DESY

UHH

CUI THE HAMBURG CENTER FOR ULTRAFAST IMAGING

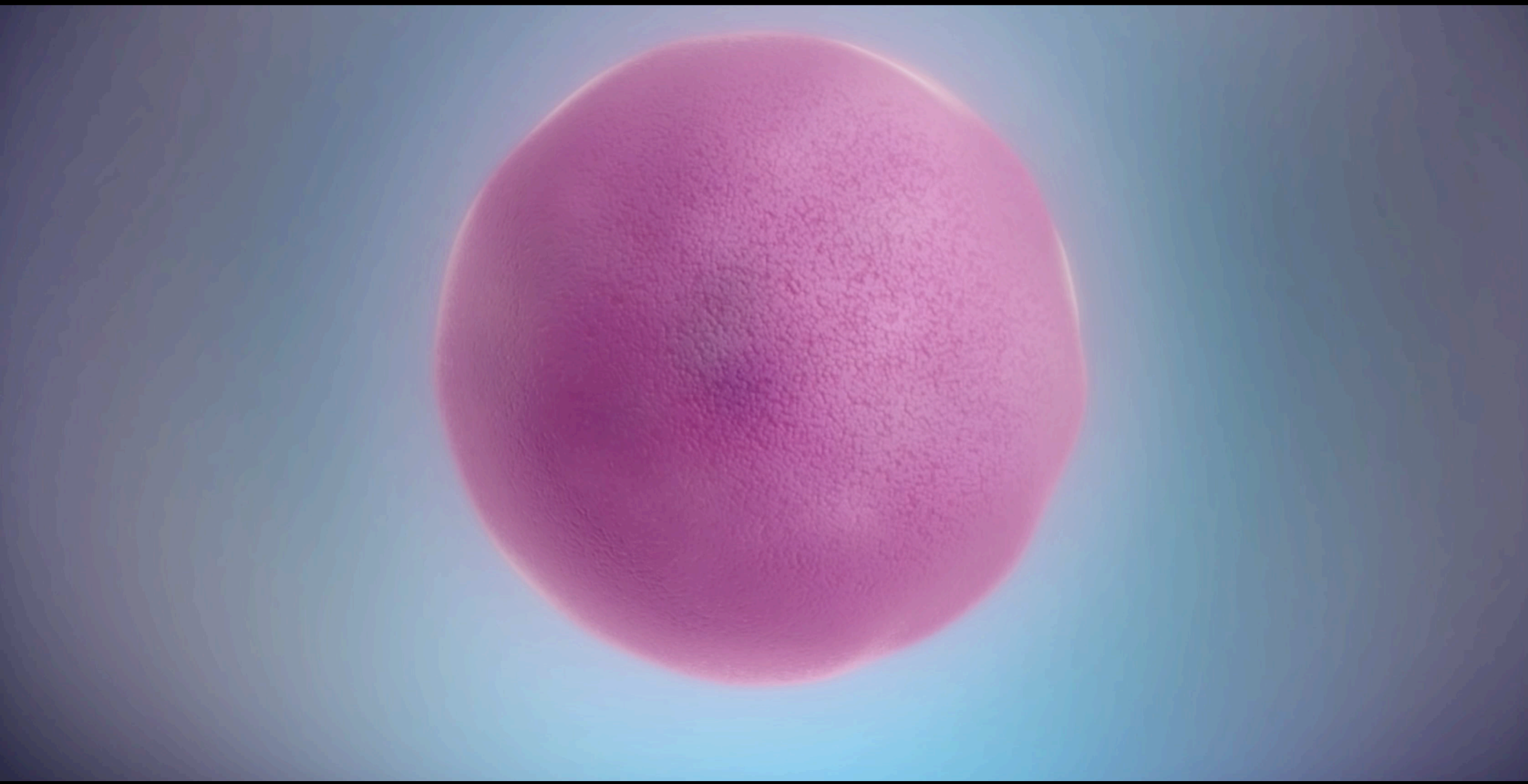
CFEL SCIENCE

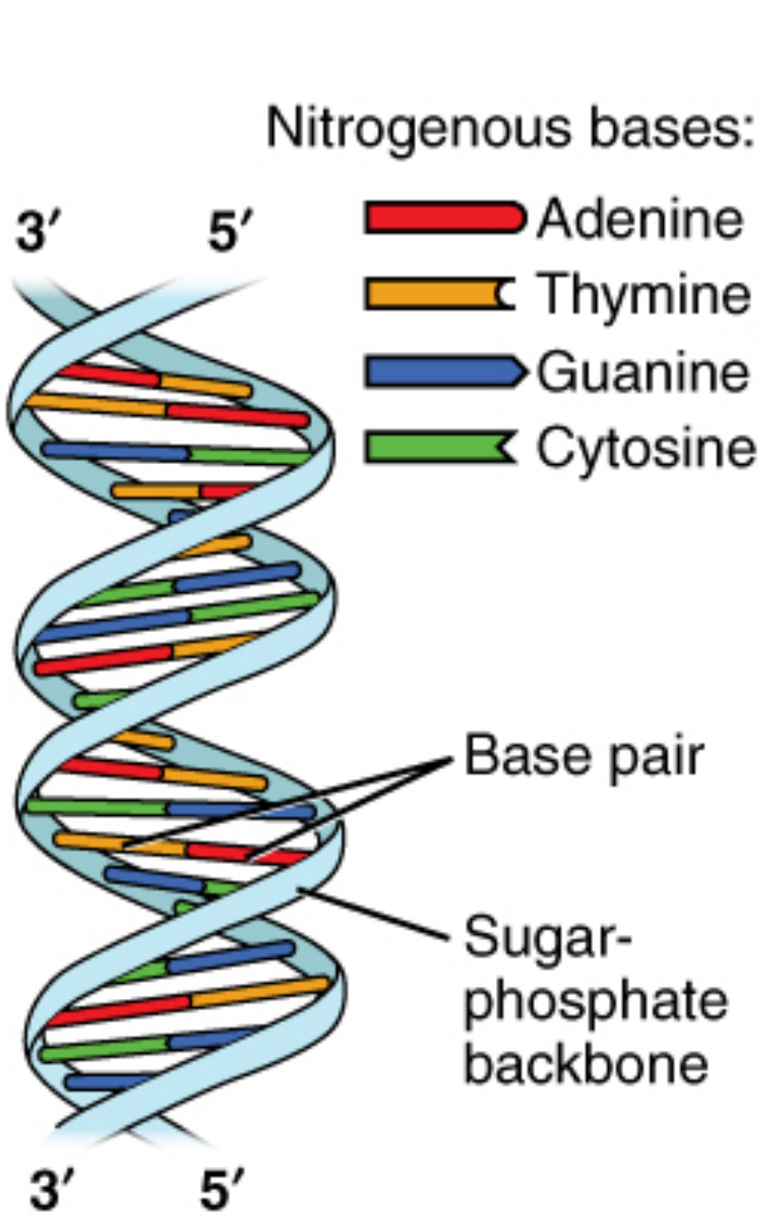
HELMHOLTZ ASSOCIATION

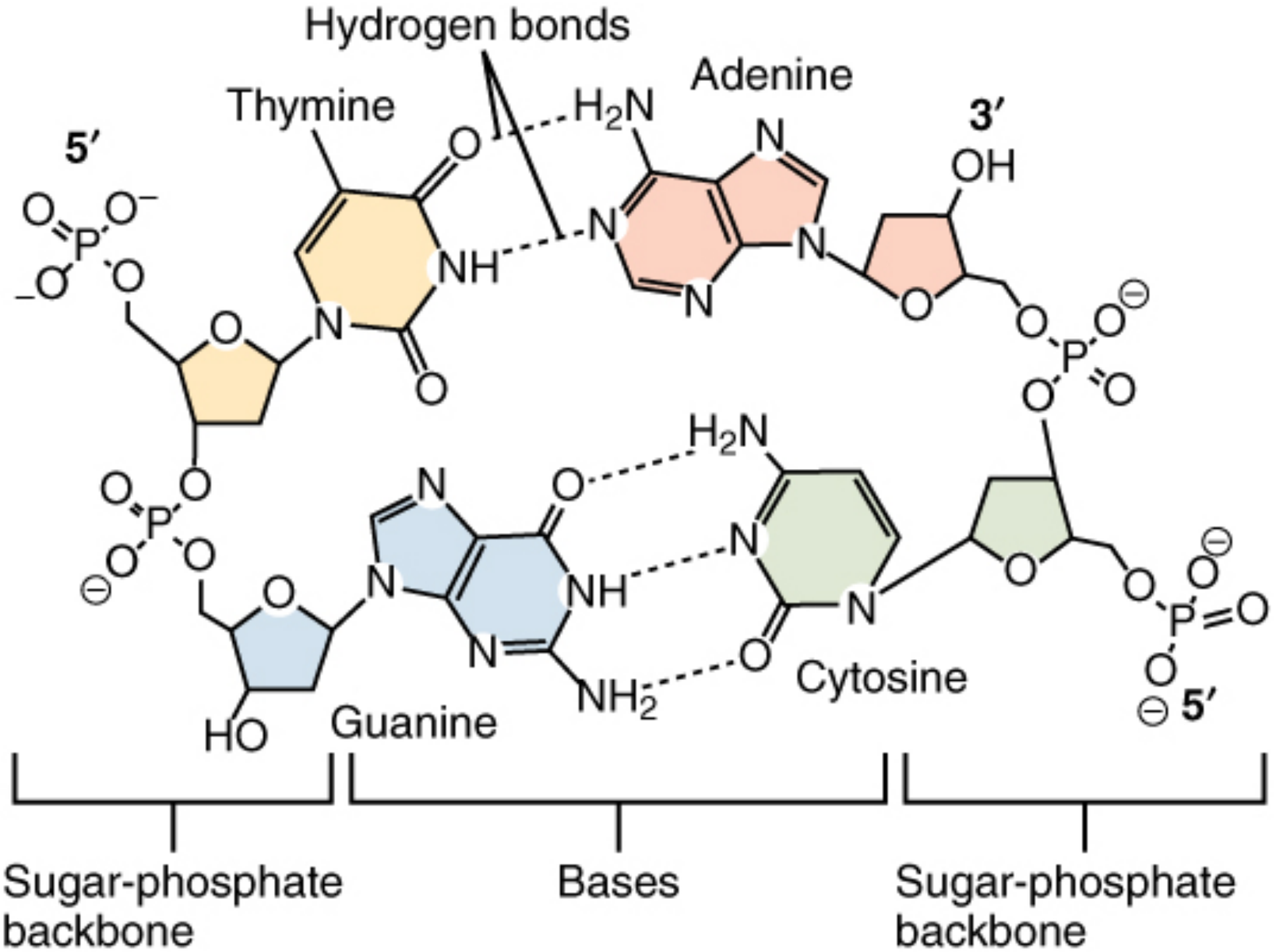
Bundesministerium für Bildung und Forschung

erc European Research Council Established by the European Commission

DFG

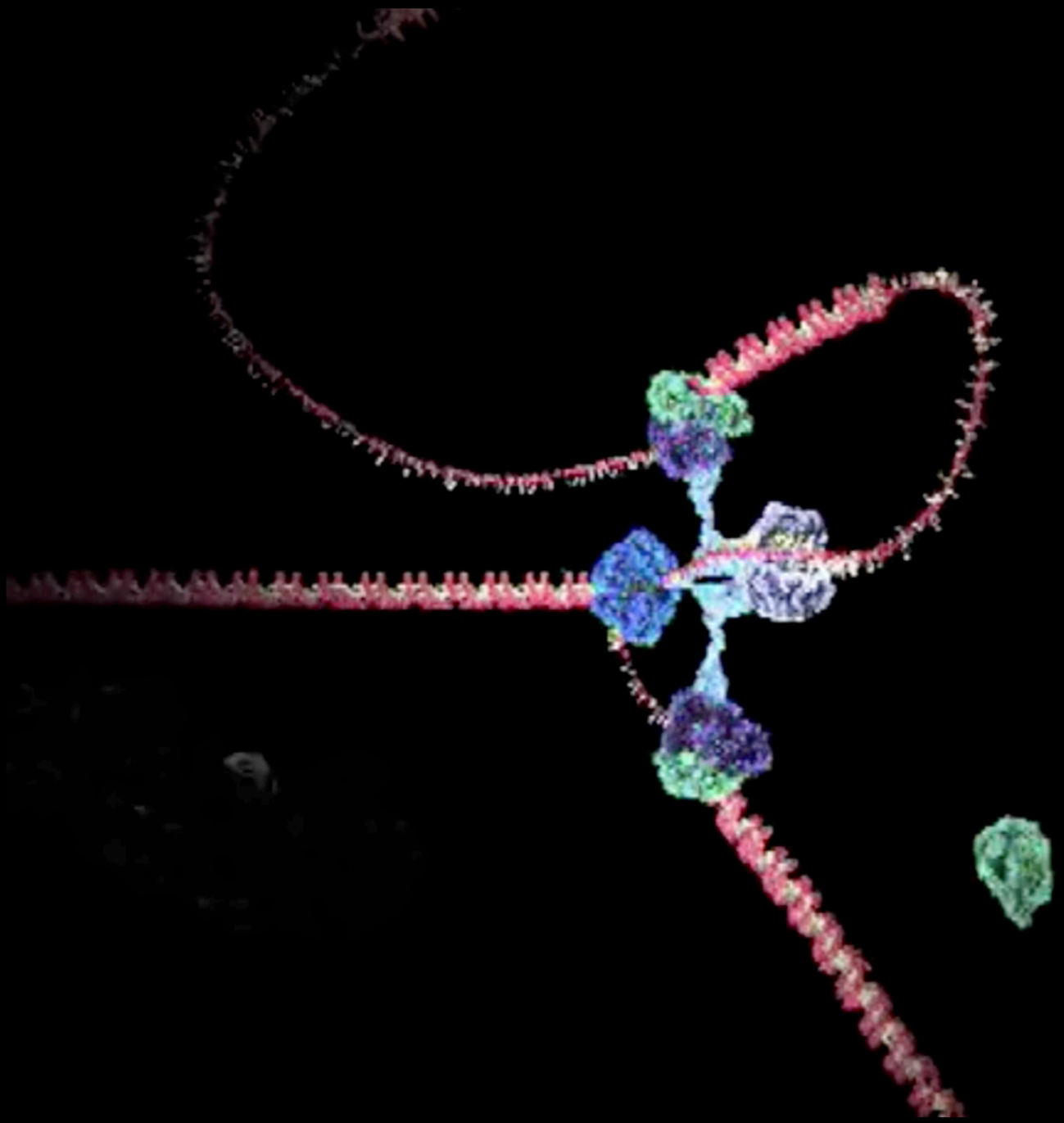






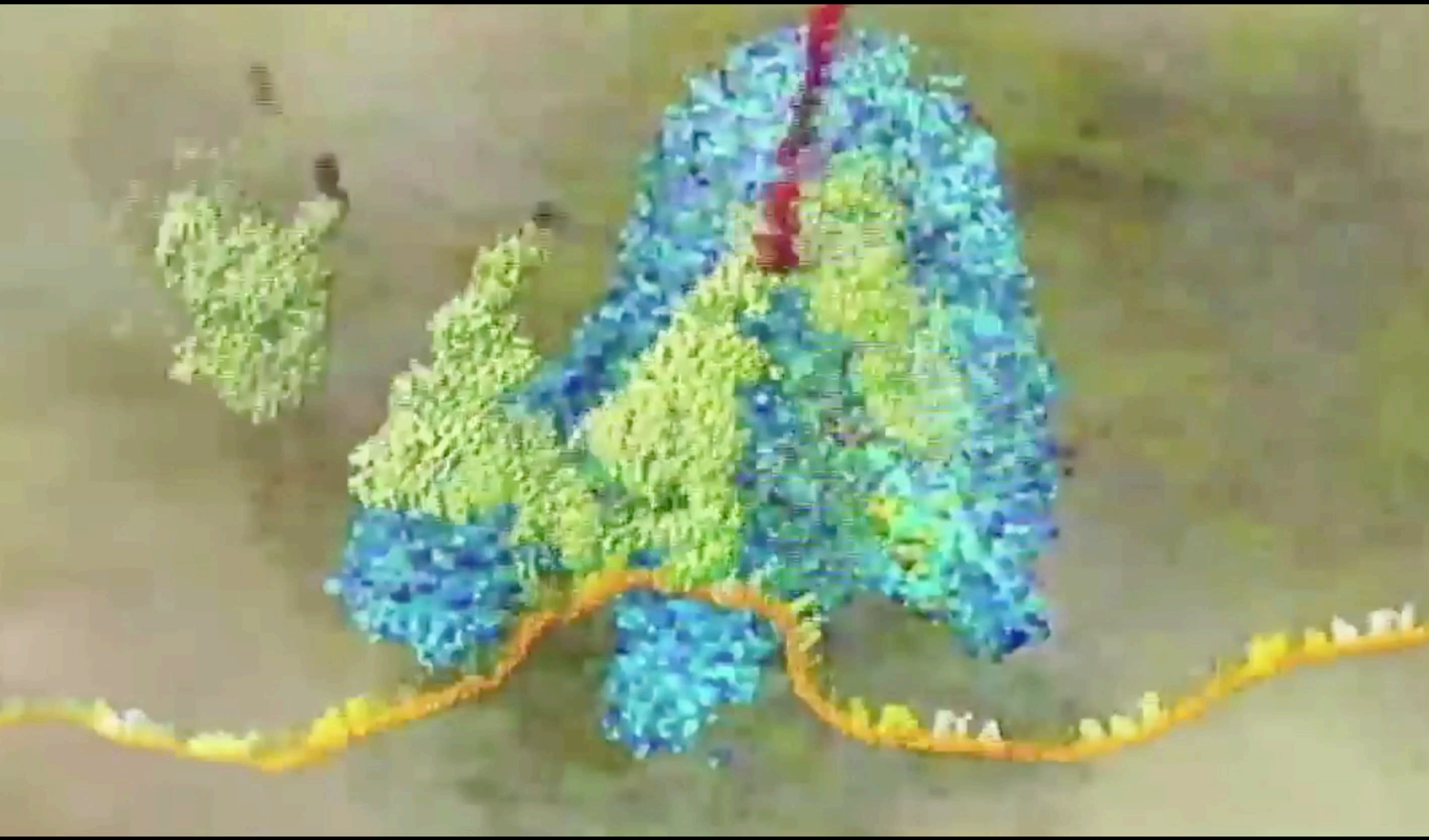
OpenStax Anatomy and Physiology

wehi.edu.au



Walter & Eliza Hall Institute

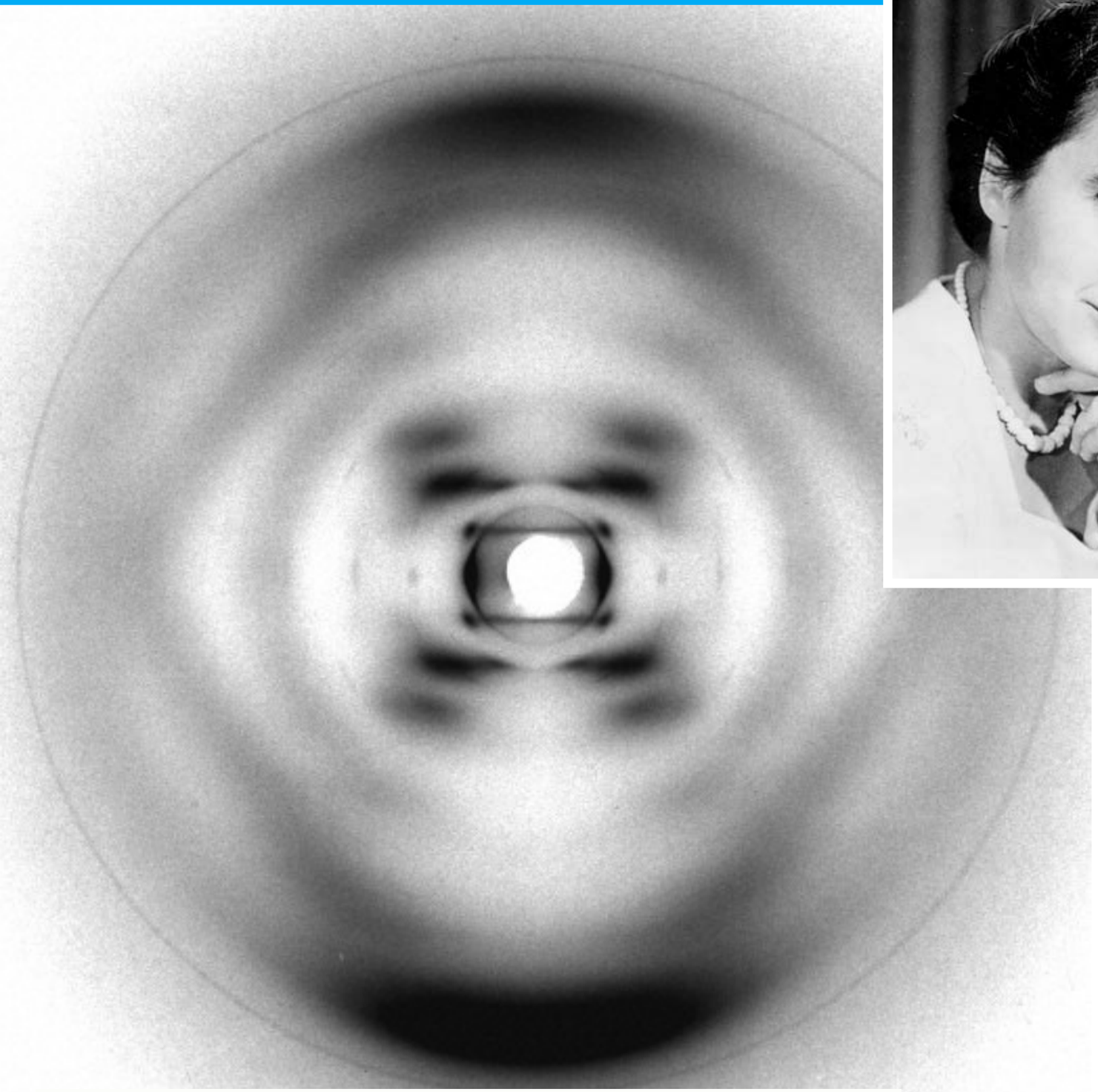
Walter & Eliza Hall Institute



Walter & Eliza Hall Institute

Yonath group, Weizmann Institute & Max Planck group Hamburg

X-ray diffraction led to the discovery of the double helix

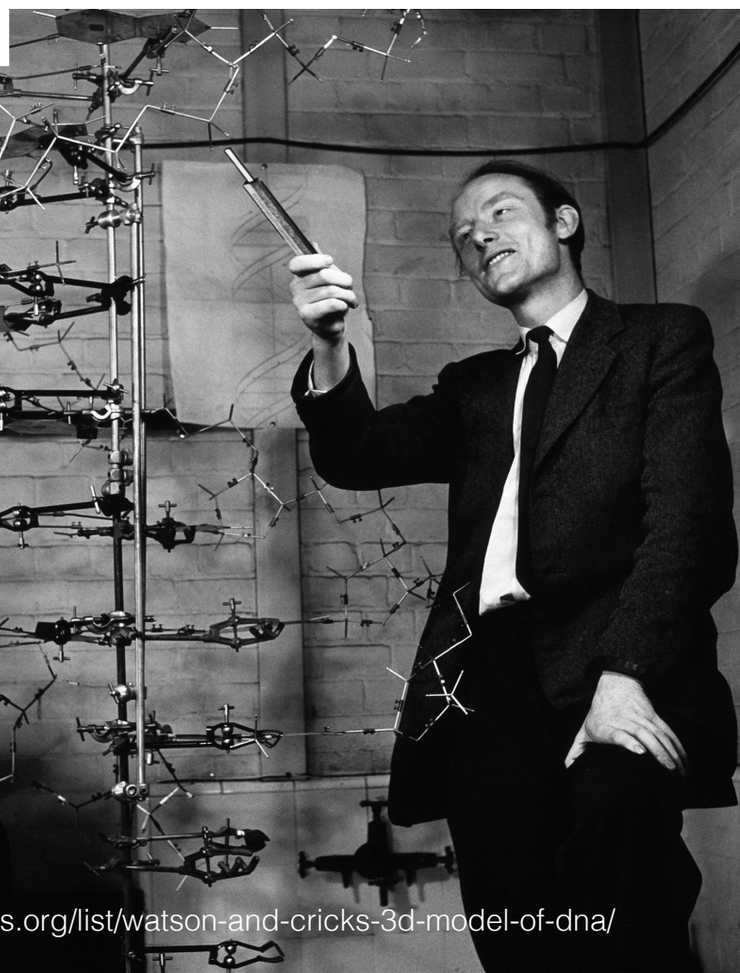


Photograph 51, the X-ray image produced by Rosalind Franklin and her PhD student Raymond Gosling in 1952. The cross pattern visible on the X-ray highlights the helical structure of DNA.

Wellcome Images

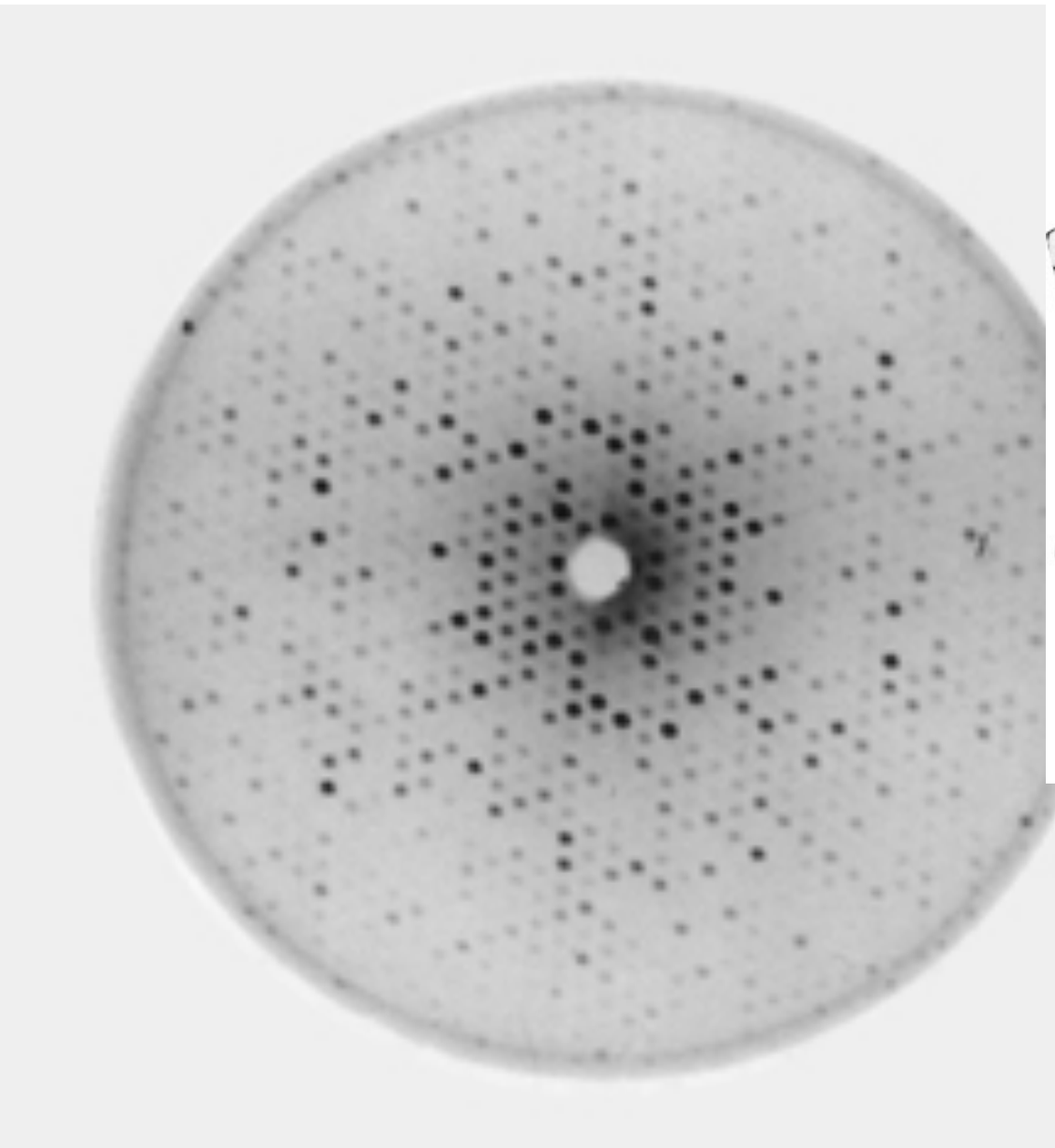
Rosalind Franklin

James Watson
& Francis Crick

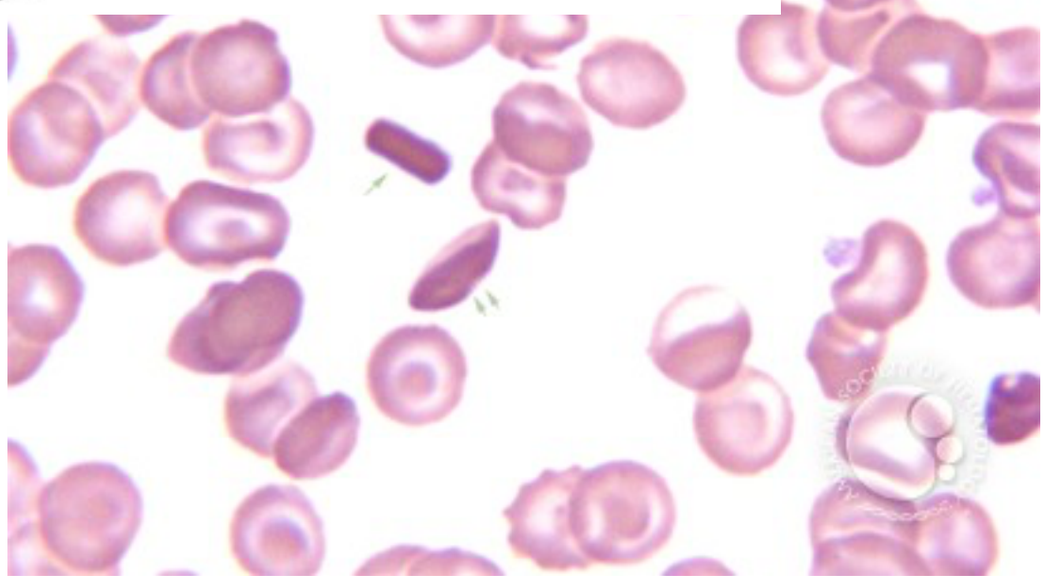


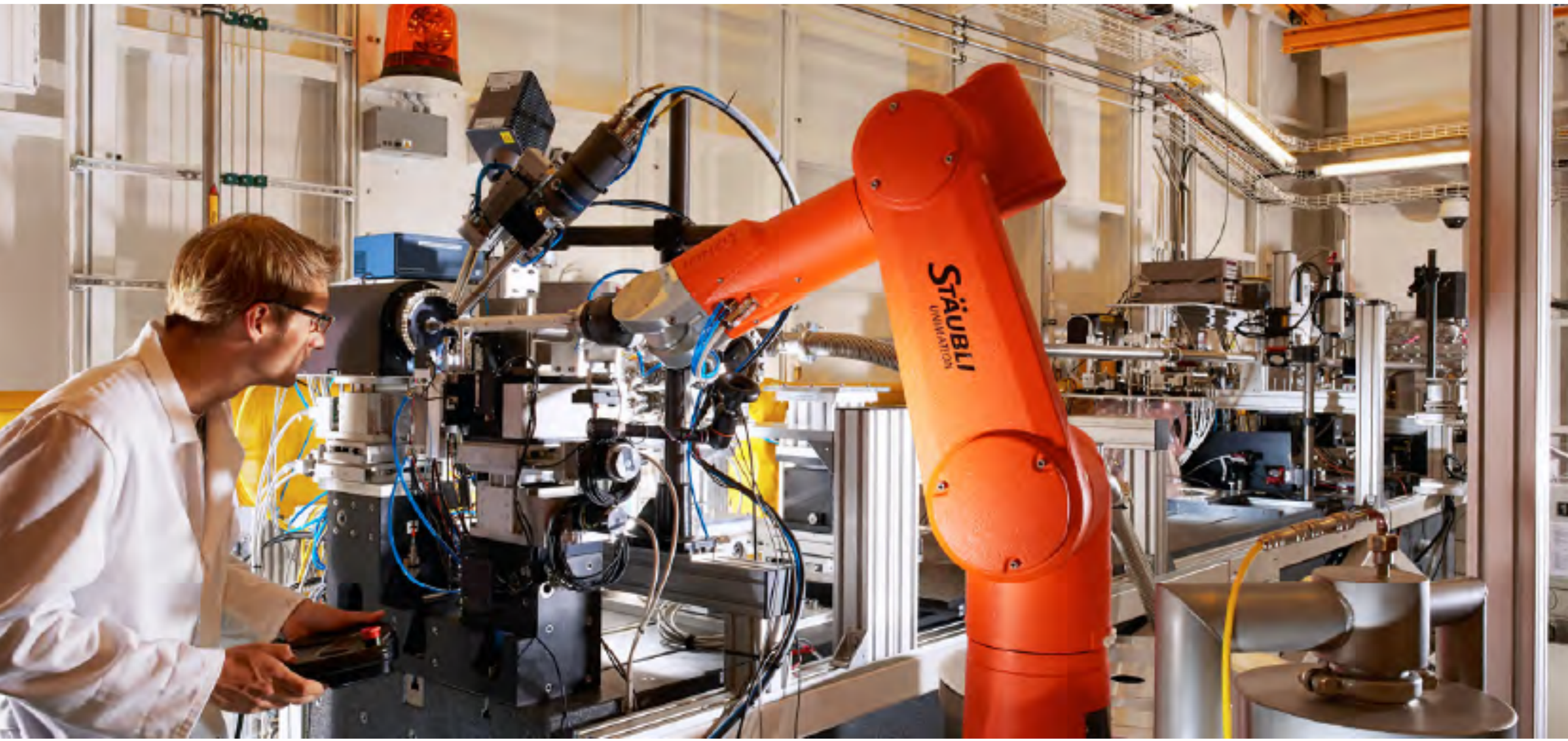
http://dataphys.org/list/watson-and-cricks-3d-model-of-dna/

The first protein structure to be determined was haemoglobin, in 1959



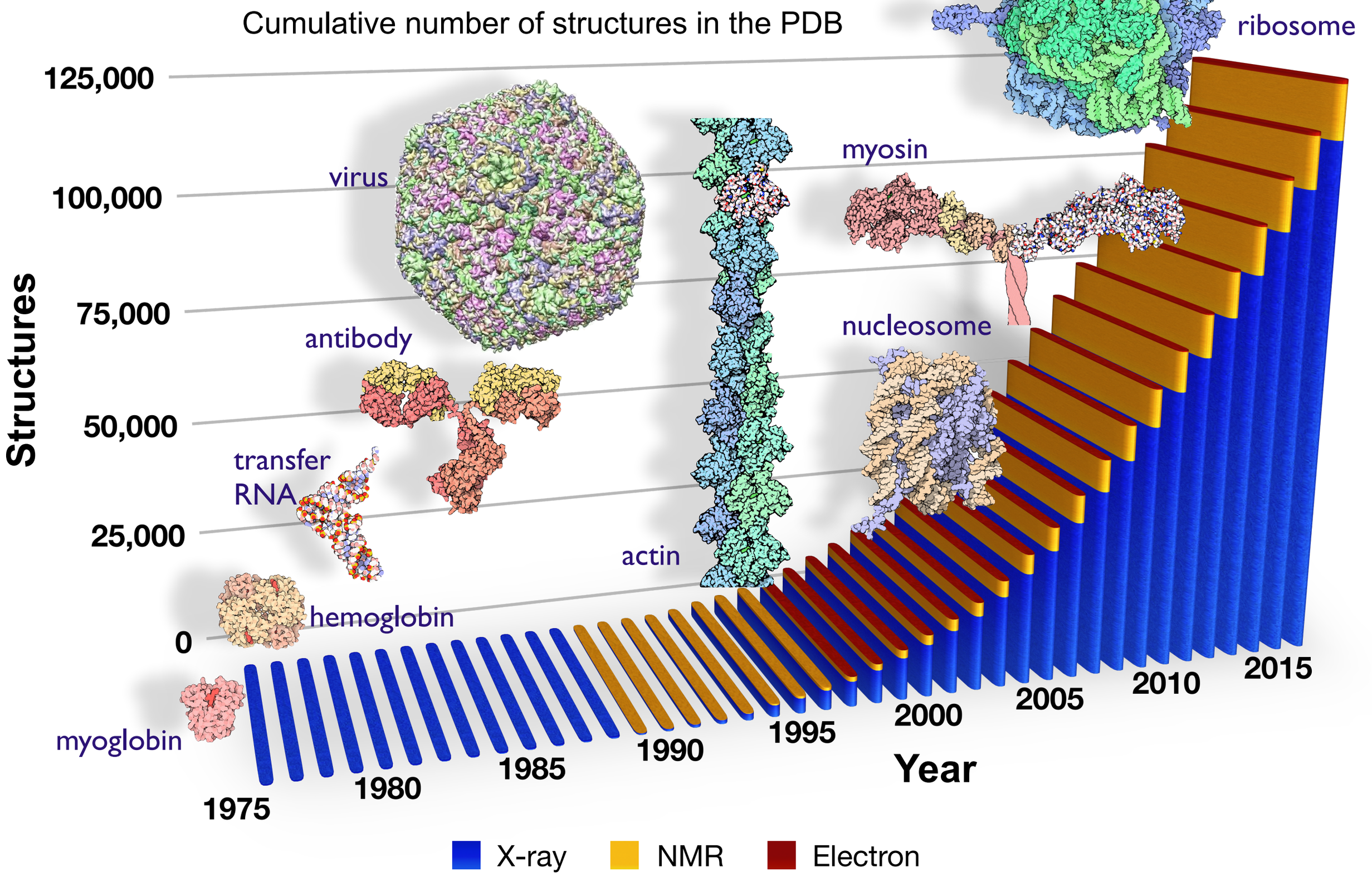
Max Perutz



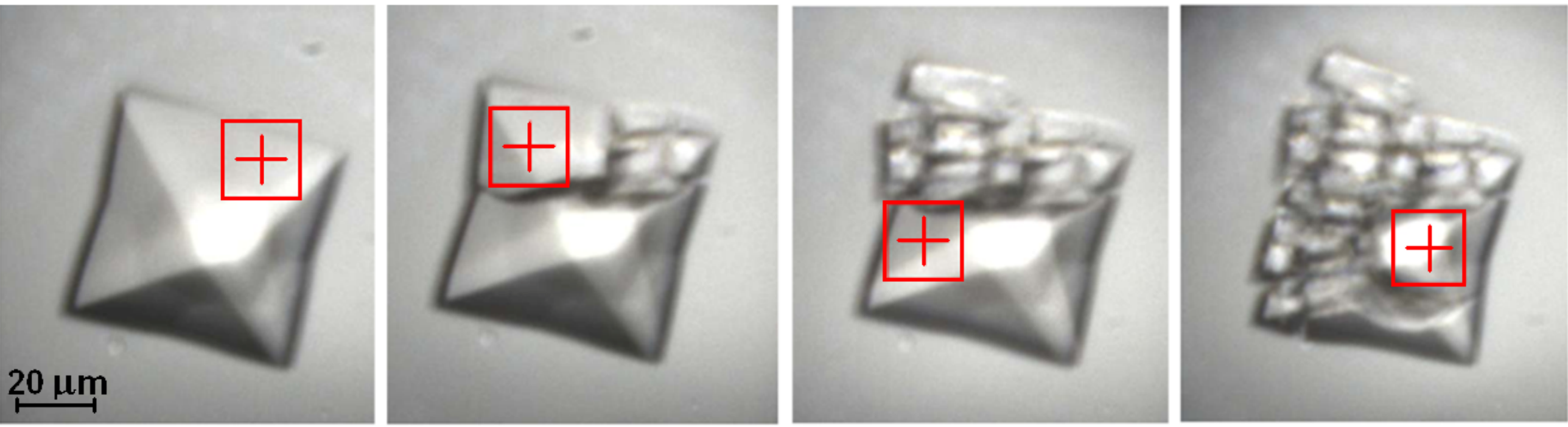

STÄUBLI
UNIMATION


PETRA III.
Max von Laue

Over 100,000 macromolecular structures have been solved using synchrotron sources



High radiation dose causes changes in molecular structure

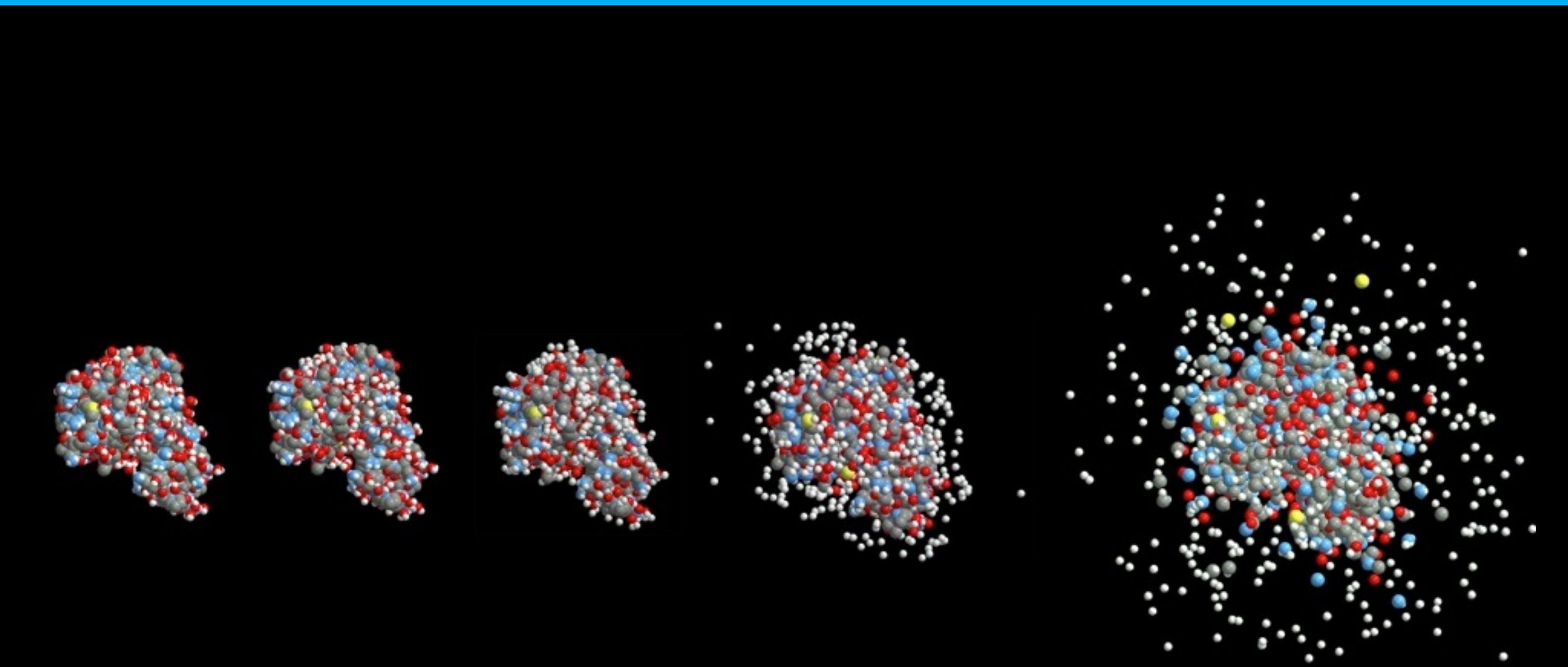


Crystal of Bovine enterovirus 2 (BEV2) after subsequent exposures of 0.5 s, 6 x 10^8 ph/µm^2
300 kGy dose
Room temperature

Cryogenic cooling gives 30 MGy tolerance

Axford et al. Acta Cryst. D68 592 (2012)
Diamond Light Source (courtesy Robin Owen & Elspeth Garman)

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



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

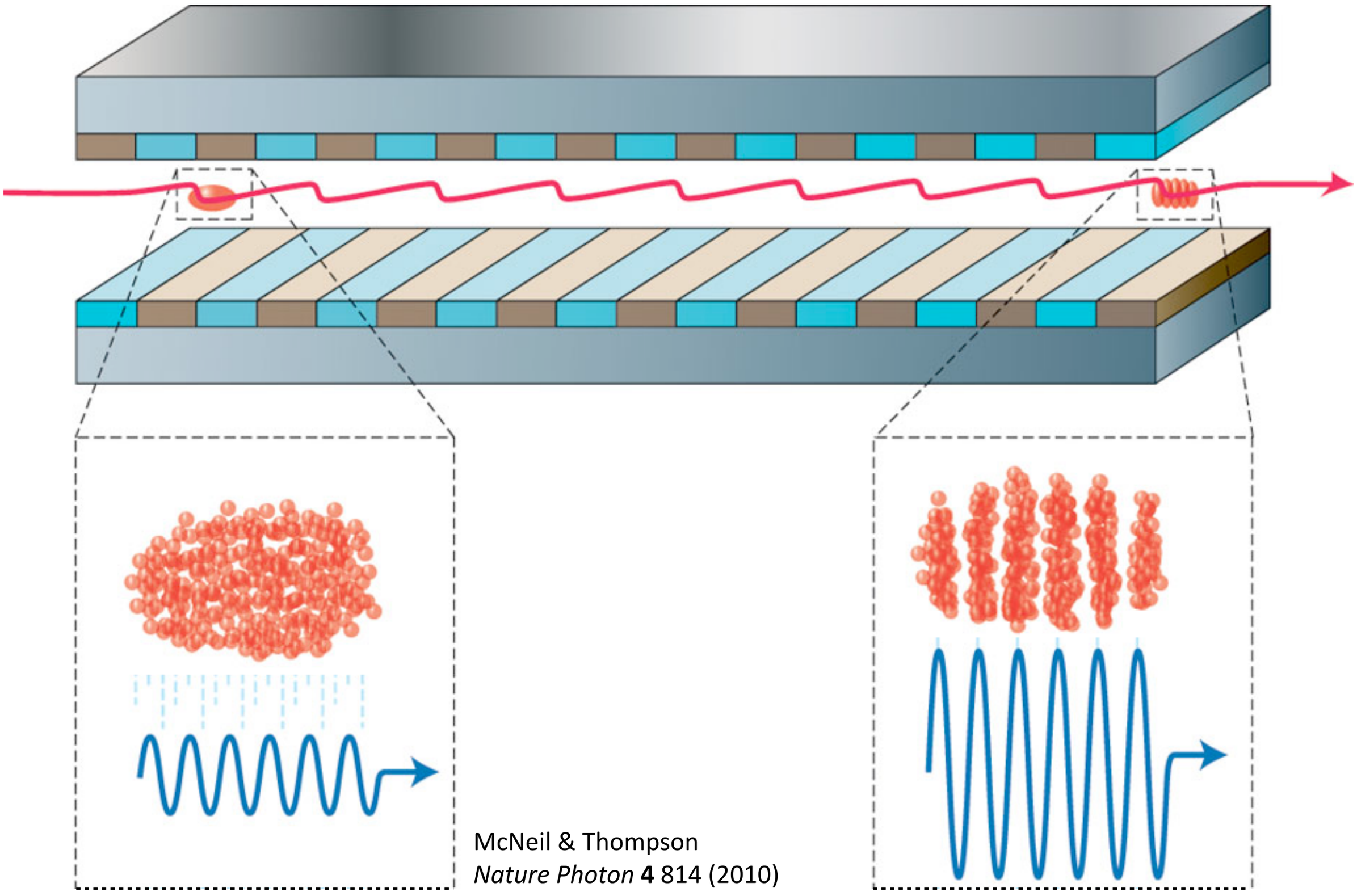
The European XFEL is located in Hamburg

The European XFEL in Hamburg

The European XFEL has just begun operations

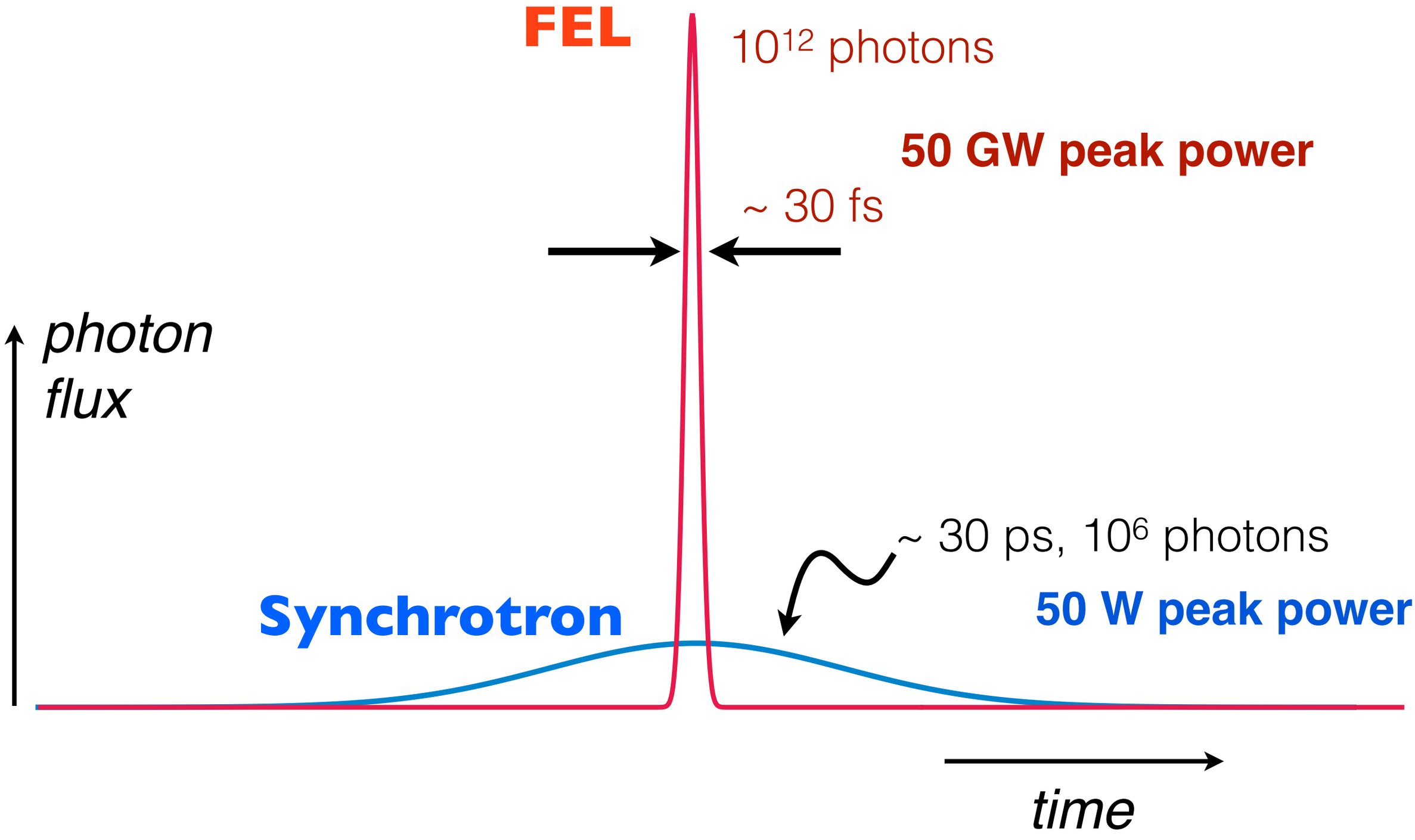
XFEL tunnel photo taken on 11 June 2015

X-rays are produced by a process called SASE

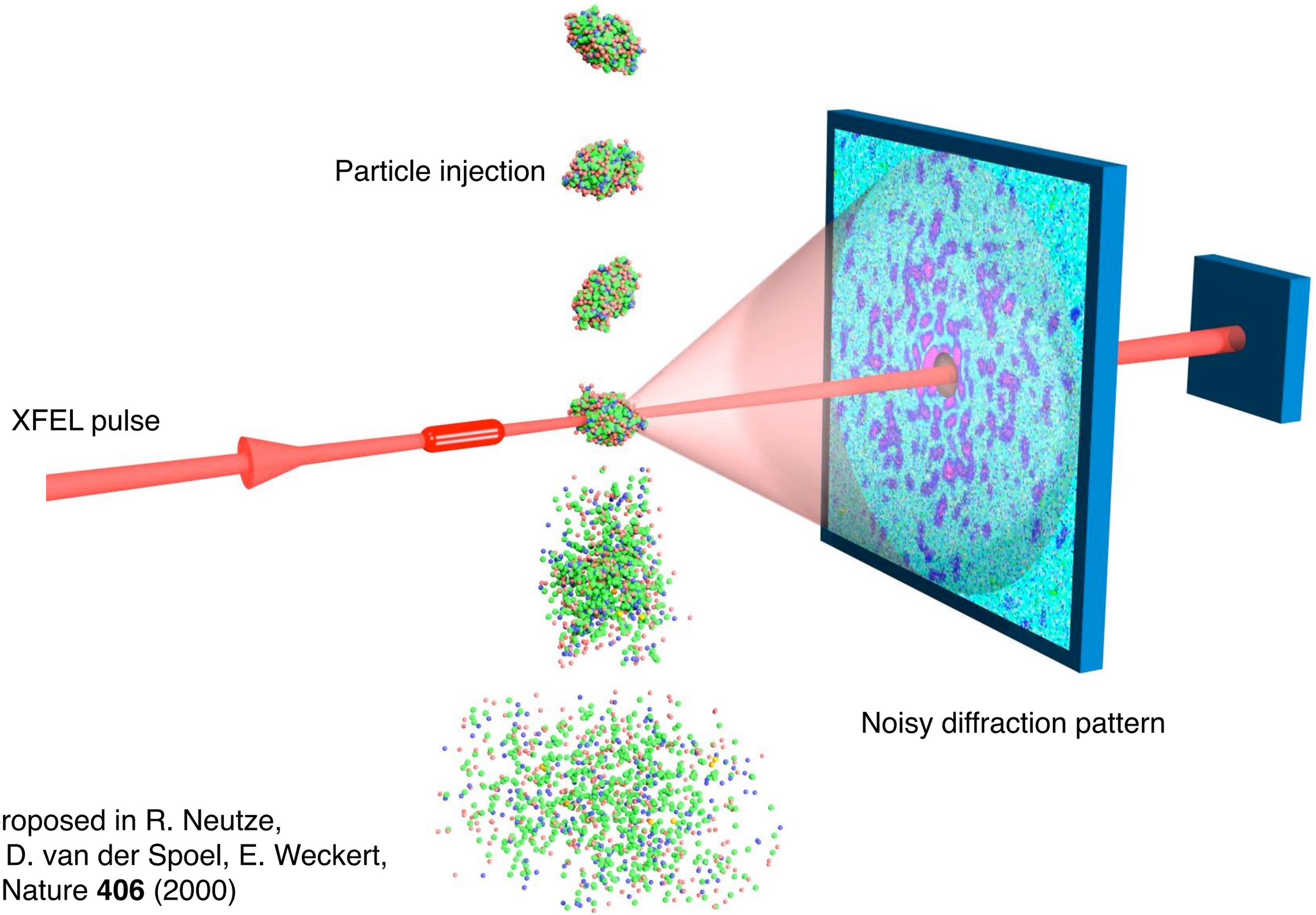


McNeil & Thompson
Nature Photon **4** 814 (2010)

X-ray FELs are a billion times brighter than synchrotrons

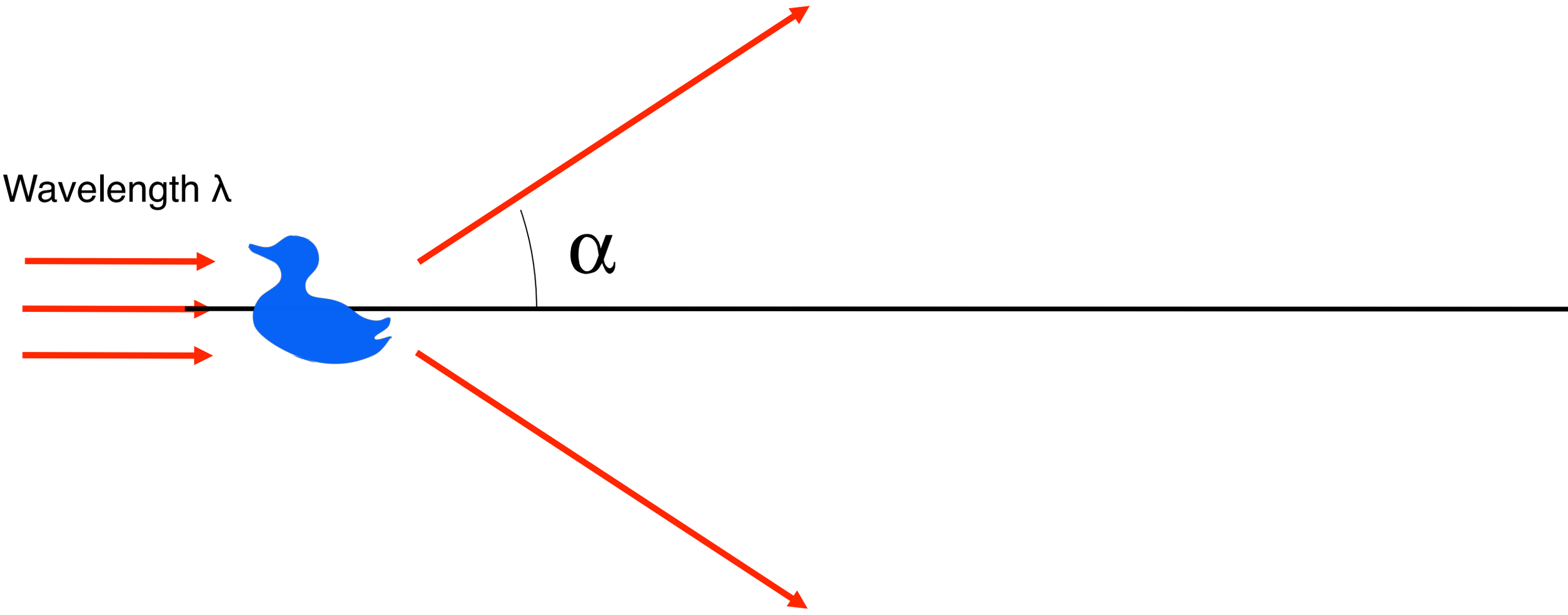


X-ray free-electron lasers may enable atomic-resolution imaging of biological macromolecules



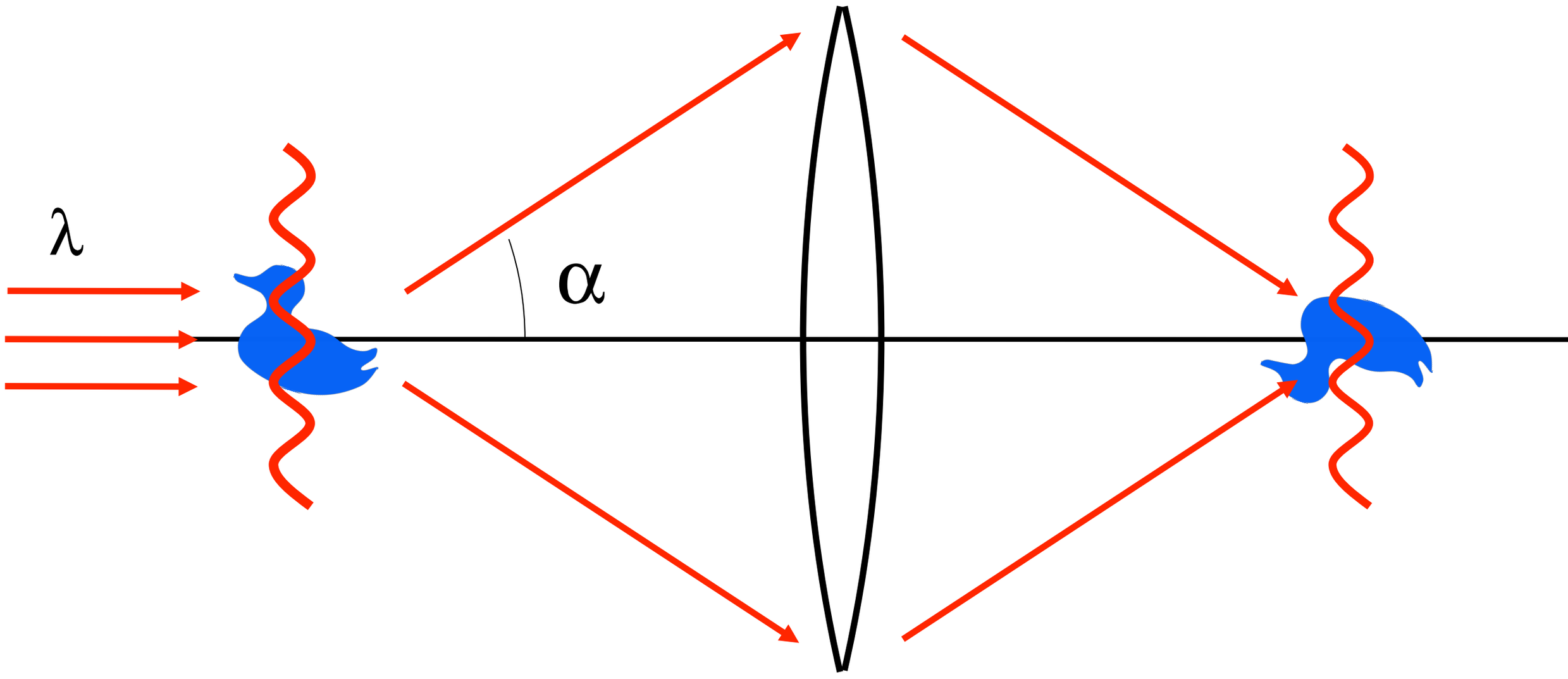
Scheme proposed in R. Neutze, R. Wouts, D. van der Spoel, E. Weckert, J. Hajdu, Nature **406** (2000)

Imaging can be achieved with a lens



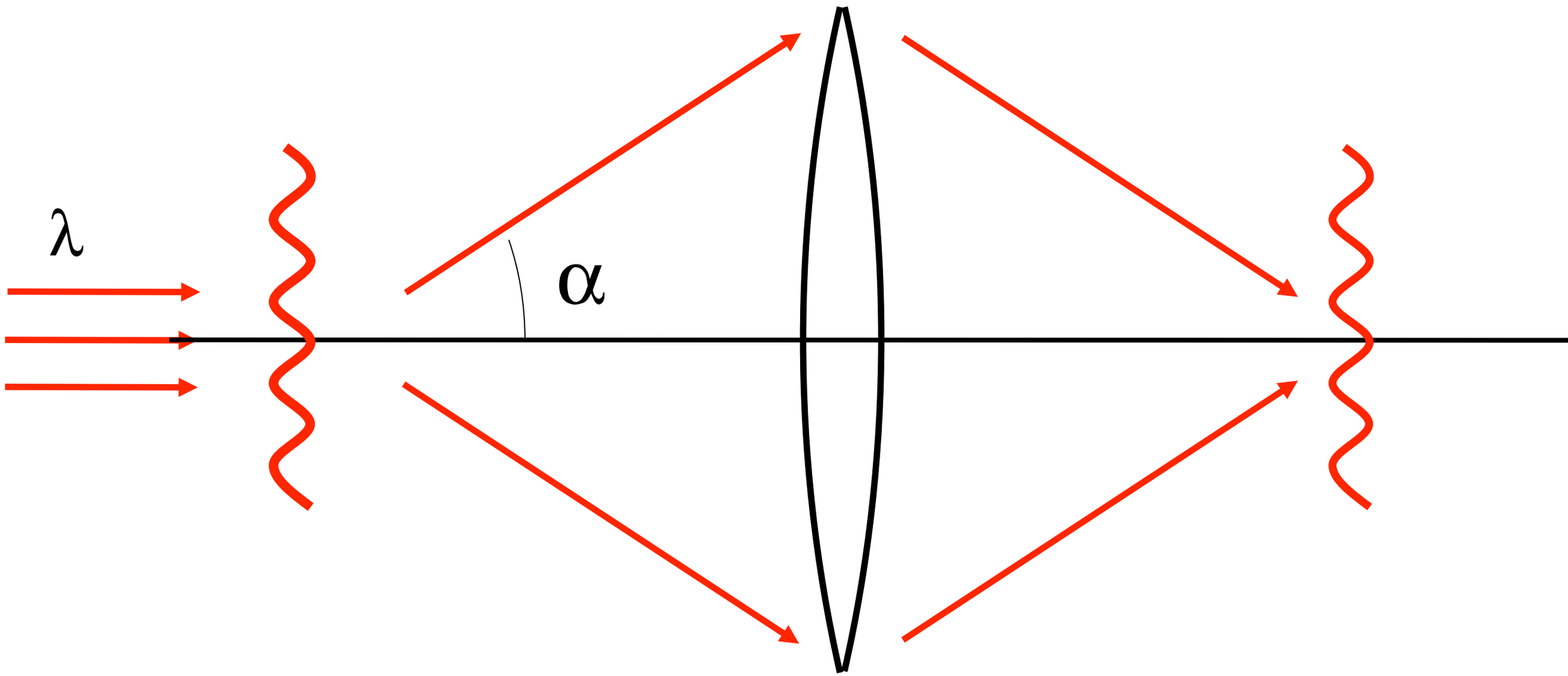
Resolution: $\delta = \lambda / \sin \alpha$

Imaging can be achieved with a lens



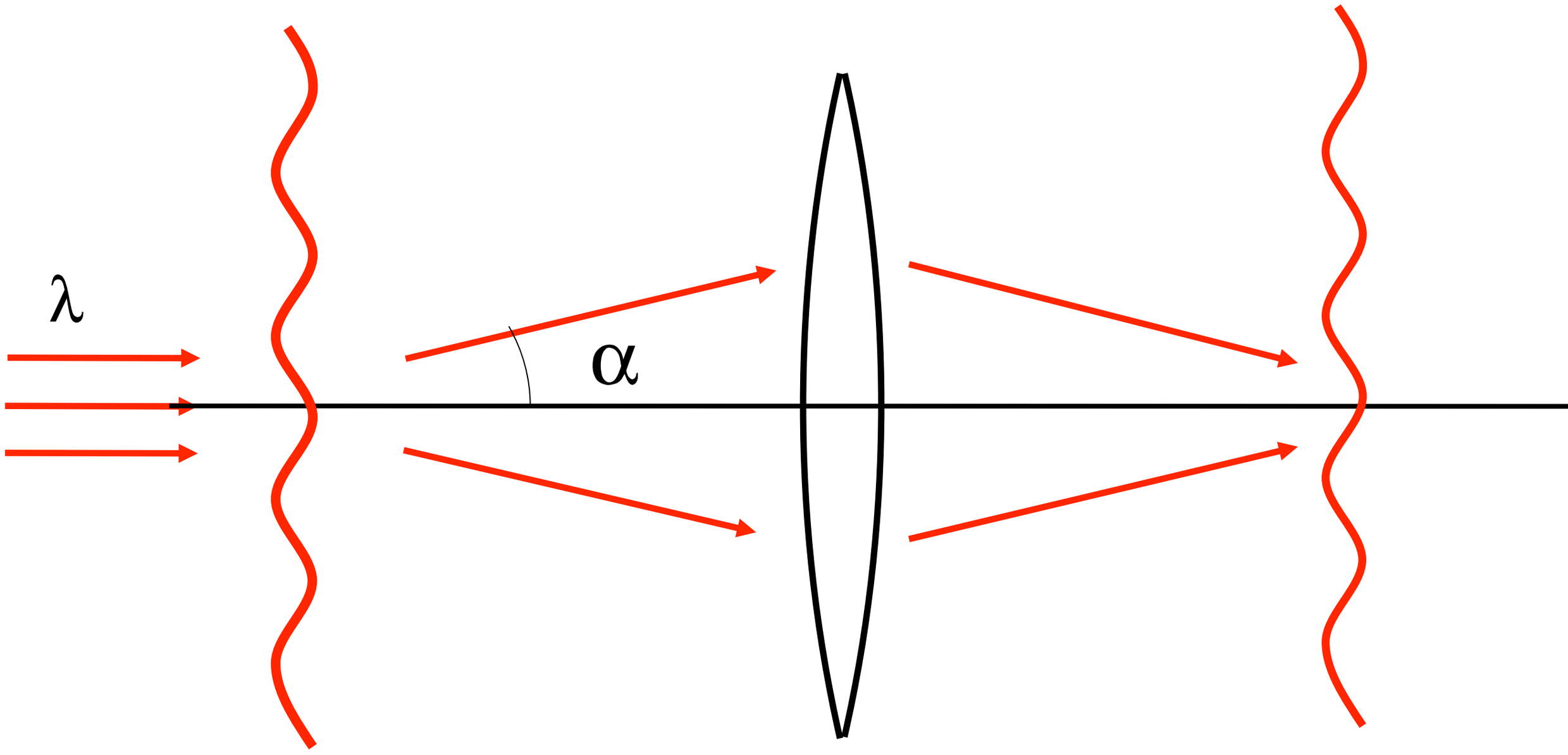
Resolution: $\delta = \lambda / \sin \alpha$

Imaging can be achieved with a lens



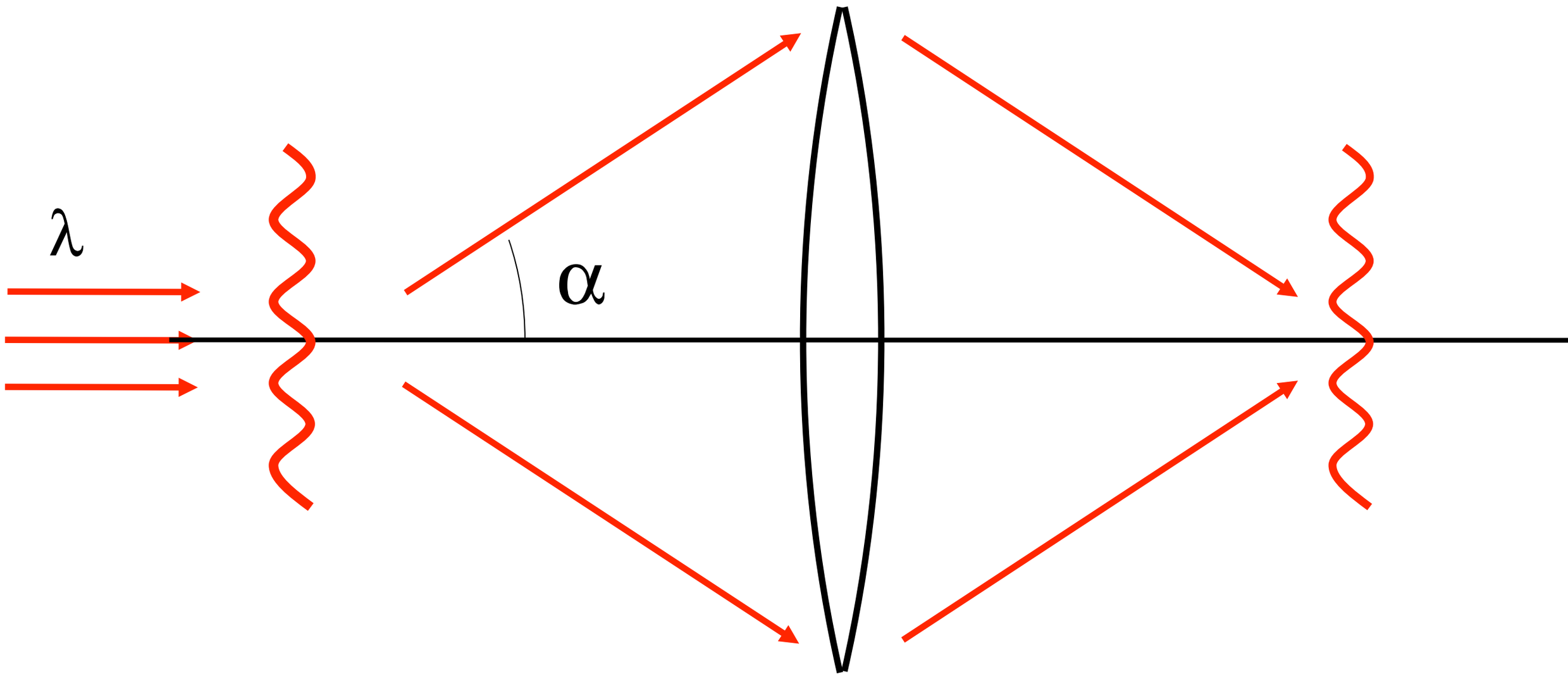
Resolution: $\delta = \lambda / \sin \alpha$

Imaging can be achieved with a lens



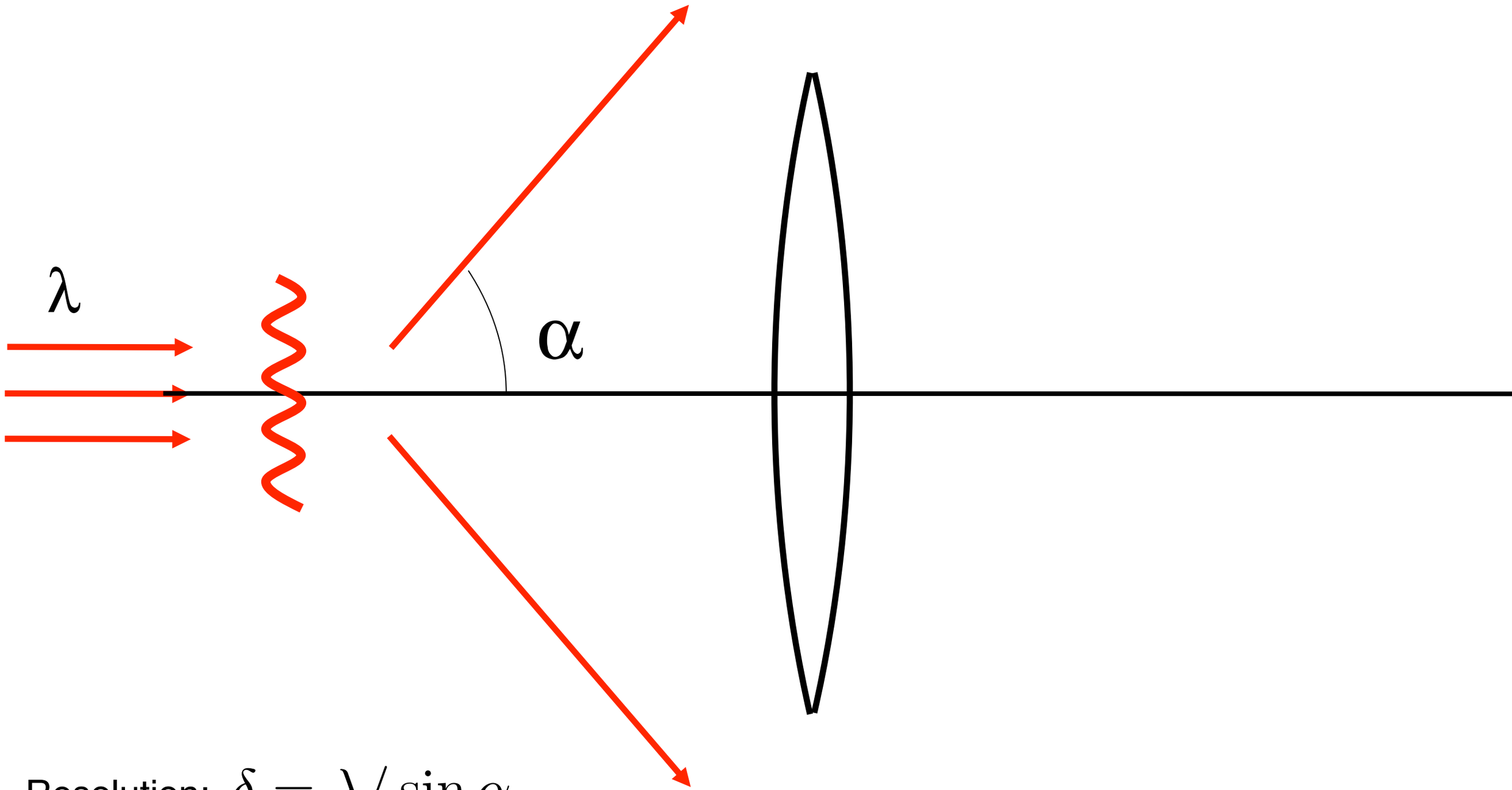
Resolution: $\delta = \lambda / \sin \alpha$

Imaging can be achieved with a lens



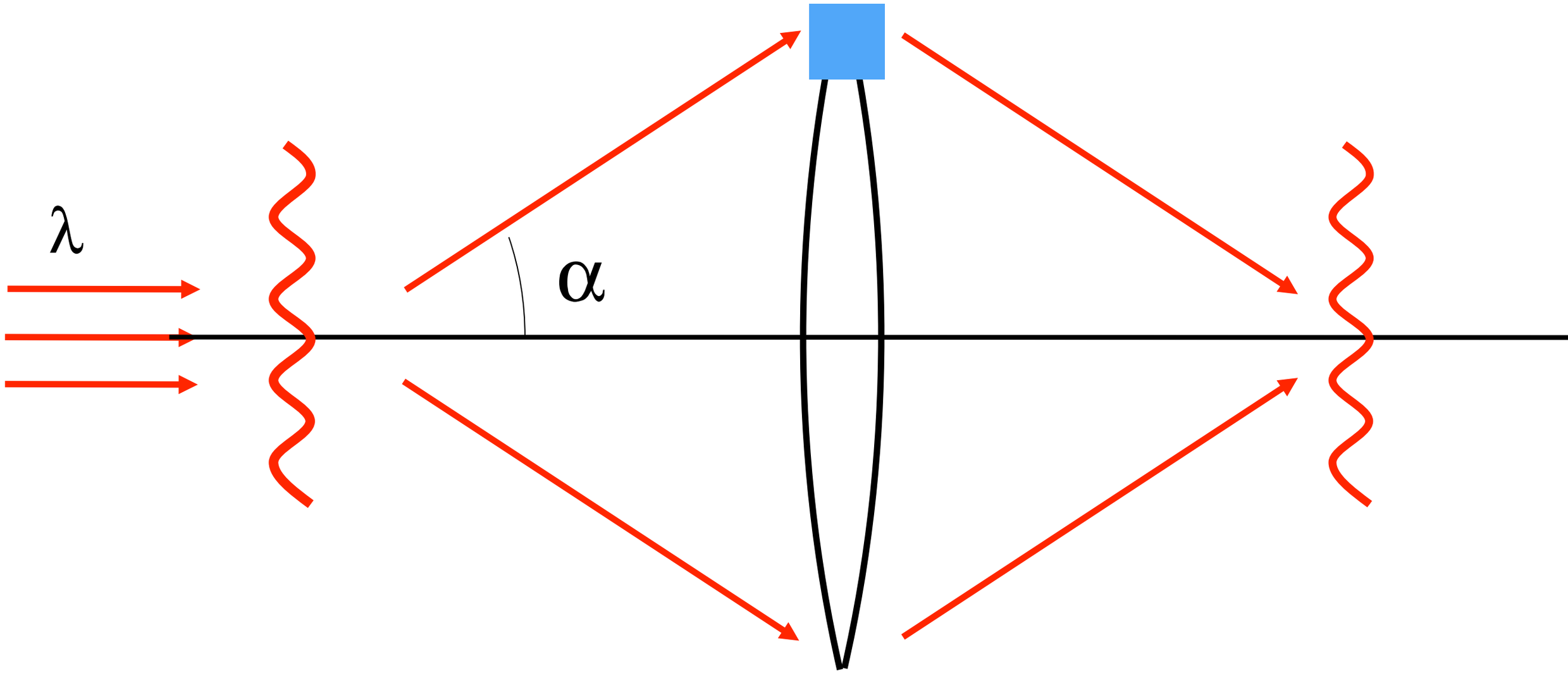
Resolution: $\delta = \lambda / \sin \alpha$

Imaging can be achieved with a lens



Resolution: $\delta = \lambda / \sin \alpha$

Imaging can be achieved with a lens

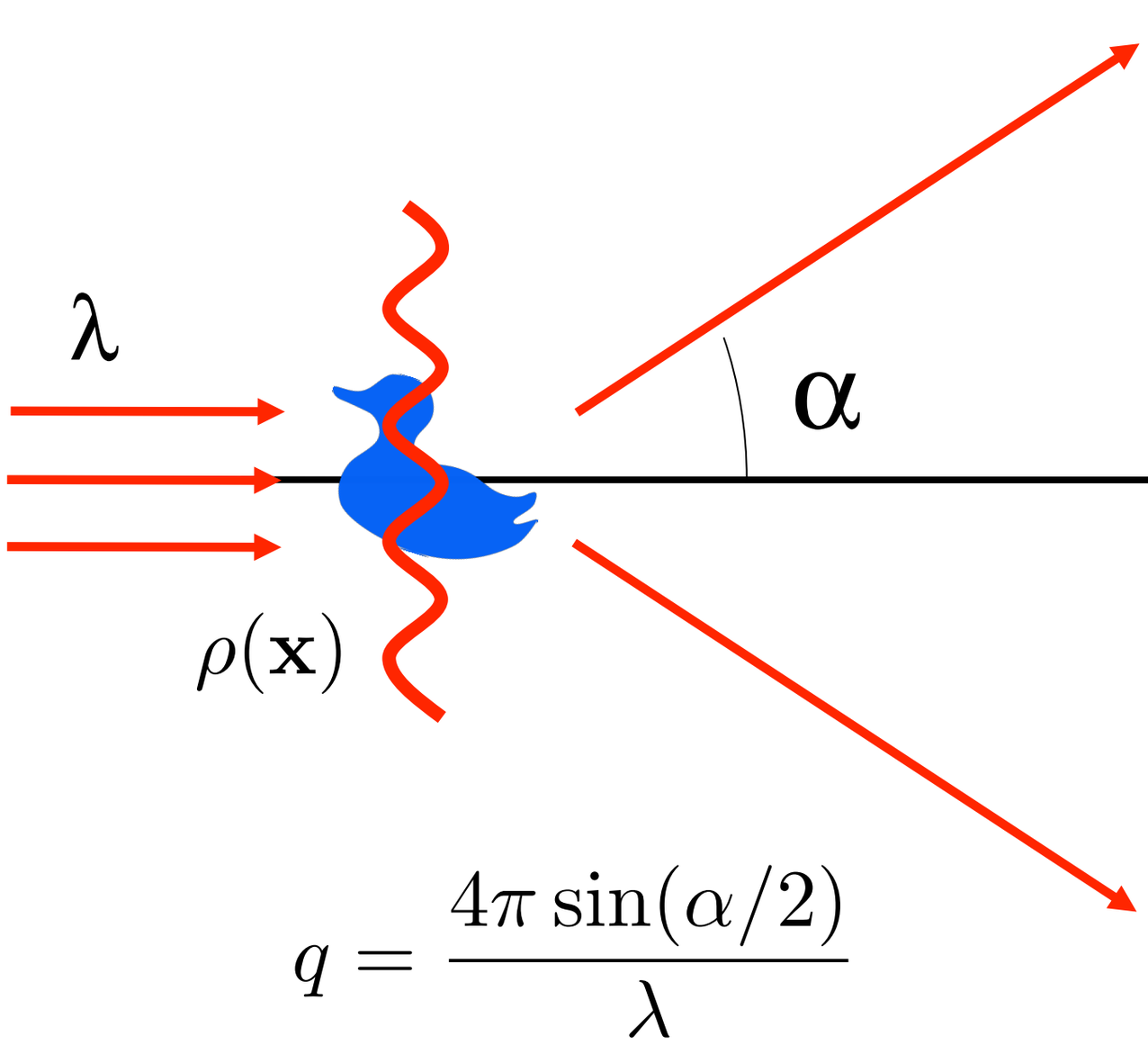


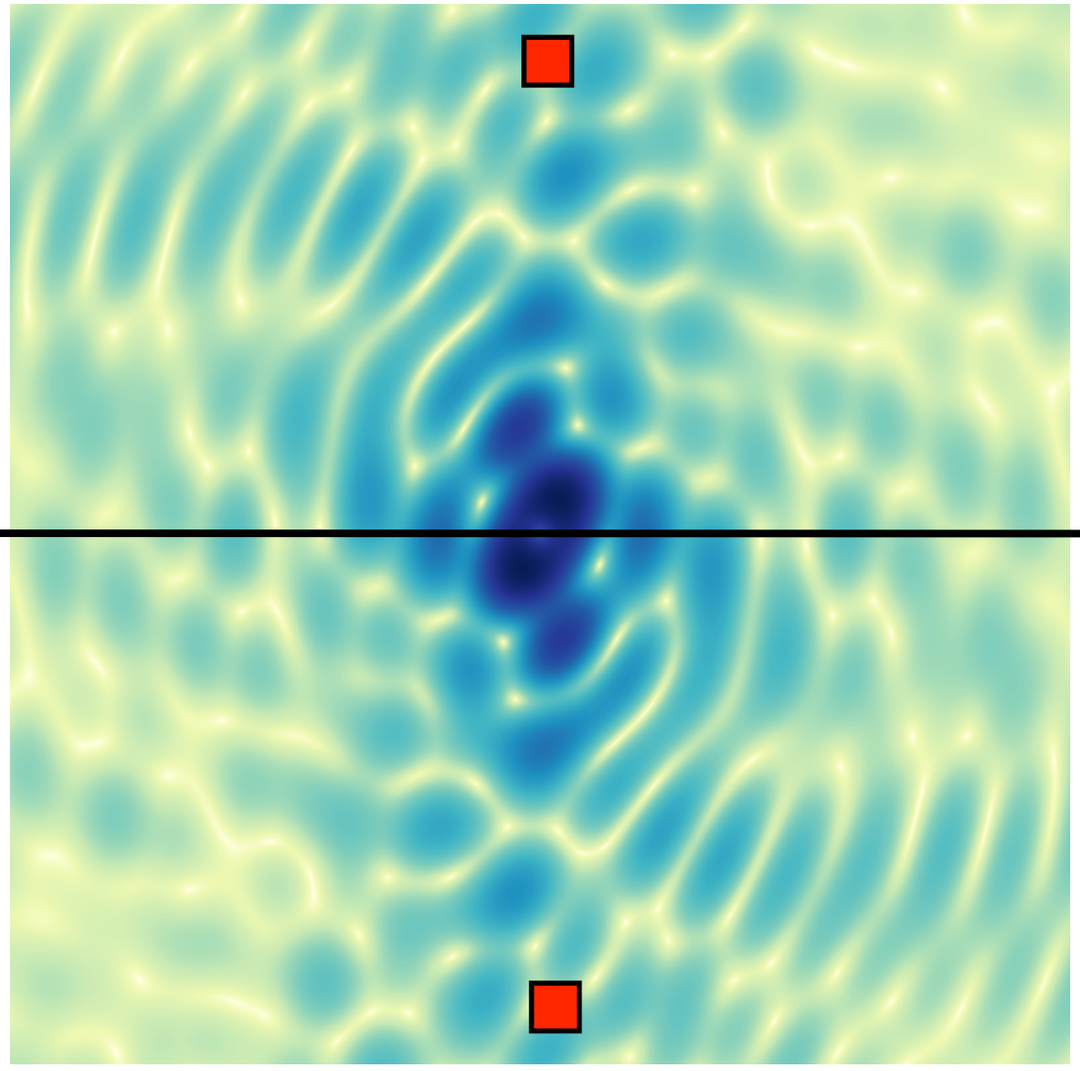
Resolution: $\delta = \lambda / \sin \alpha$

Imaging can be achieved with a lens

$$\hat{\rho}(\mathbf{q}) = -r_e \int \rho(\mathbf{x}) \exp(i\mathbf{q}\cdot\mathbf{x})\, d\mathbf{x}$$

$$I(\mathbf{q}) = |\hat{\rho}(\mathbf{q})|^2$$



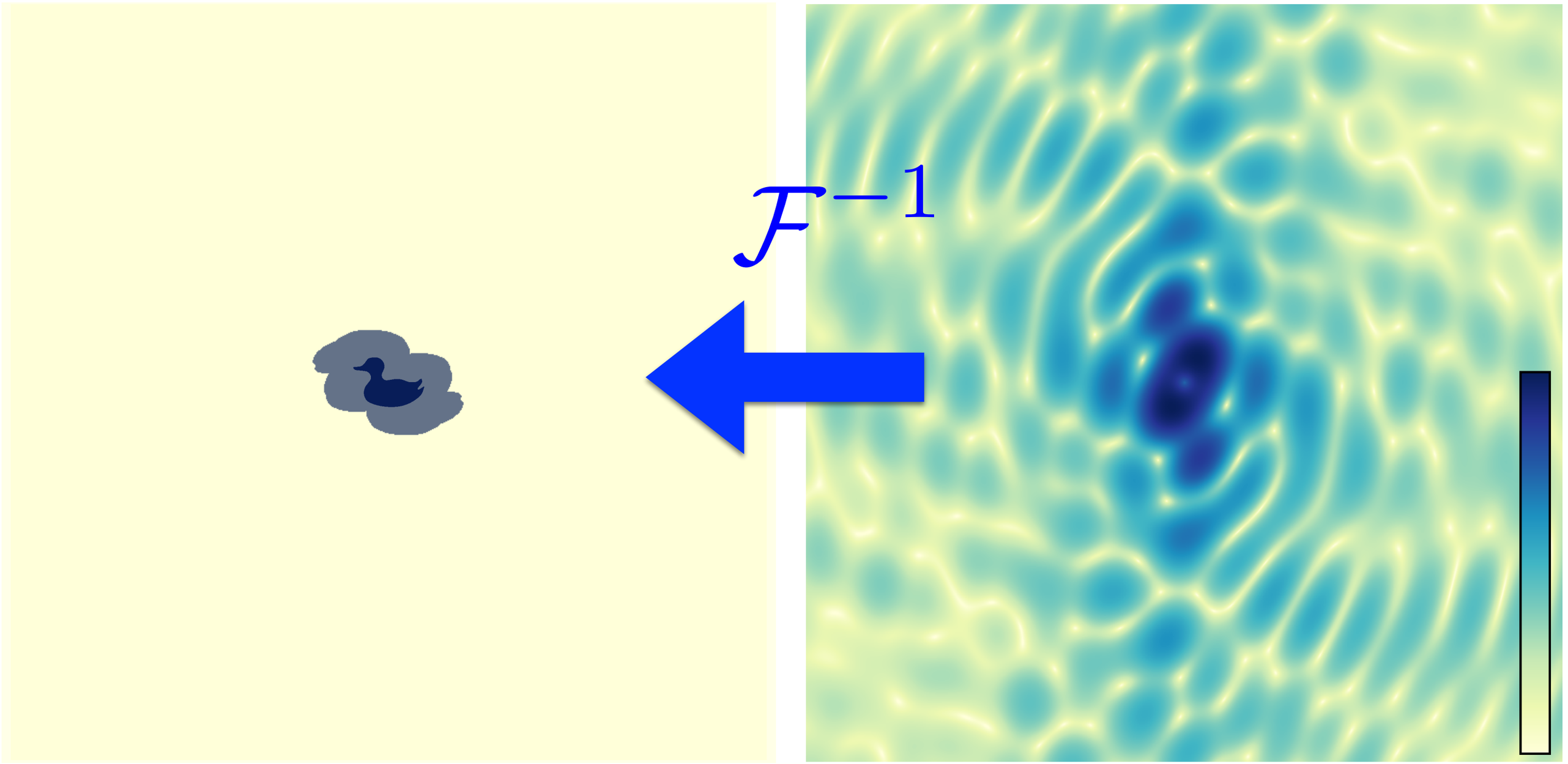


$$q = \frac{4\pi \sin(\alpha/2)}{\lambda}$$

Single particles give continuous diffraction patterns

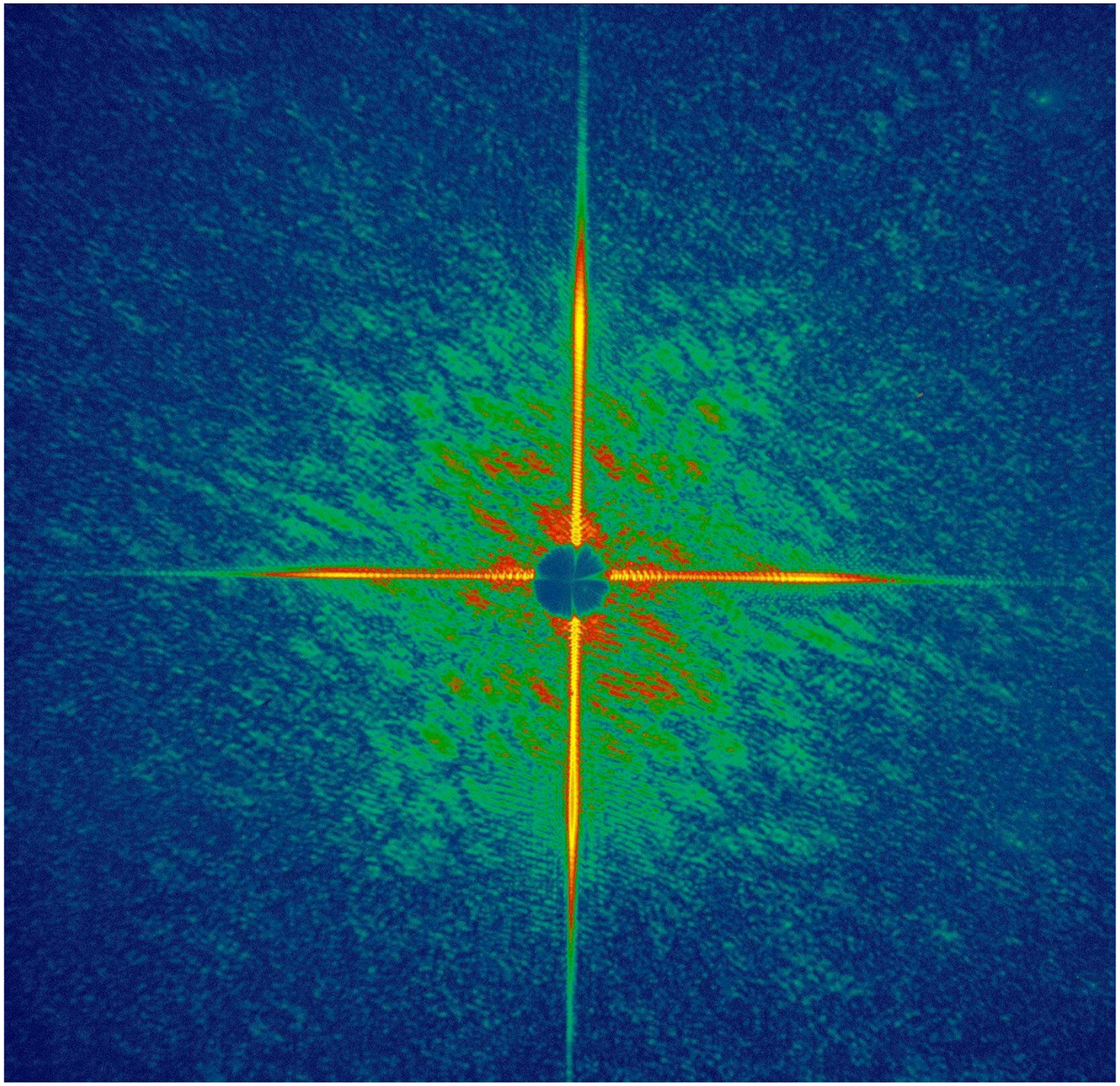
$$\mathcal{F}^{-1}\{I(\mathbf{q})\} = \rho(\mathbf{x}) \otimes \rho^*(-\mathbf{x})$$

$$I(\mathbf{q}) = |\hat{\rho}(\mathbf{q})|^2$$

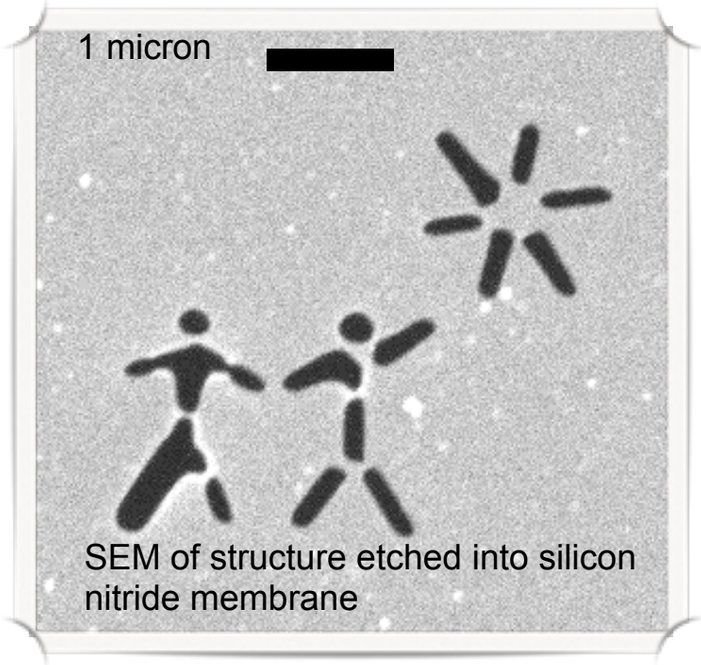


Over-constrained: more knowns than unknowns

Phasing is achieved using iterative algorithms

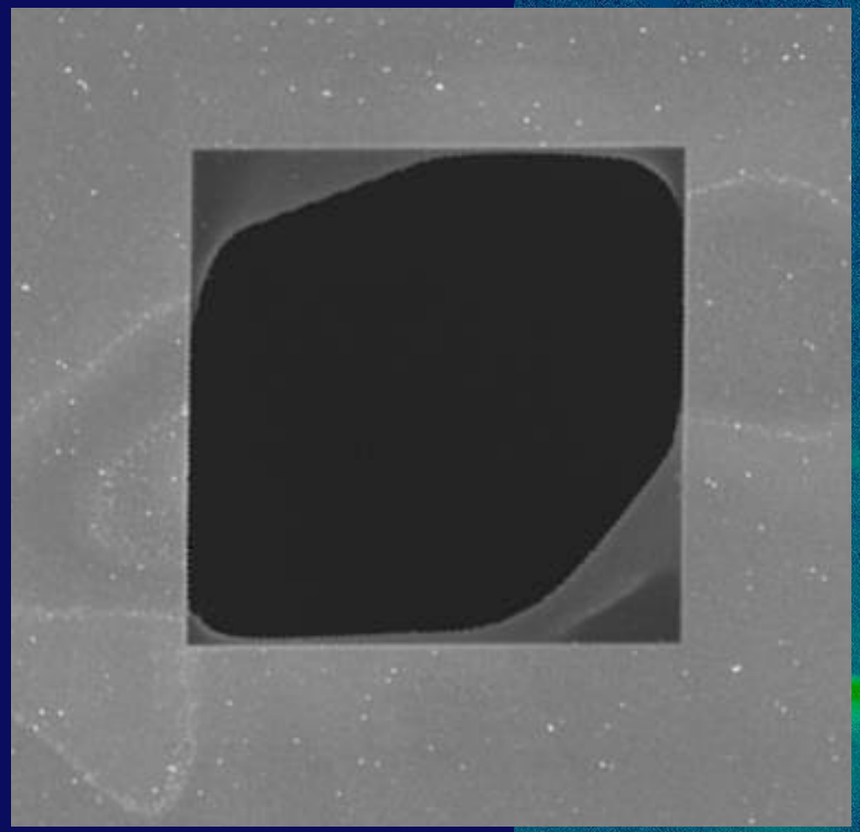


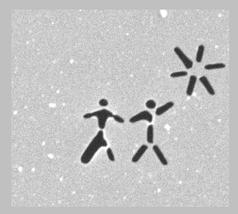
Chapman et al. Nature Physics 2 839 (2006)

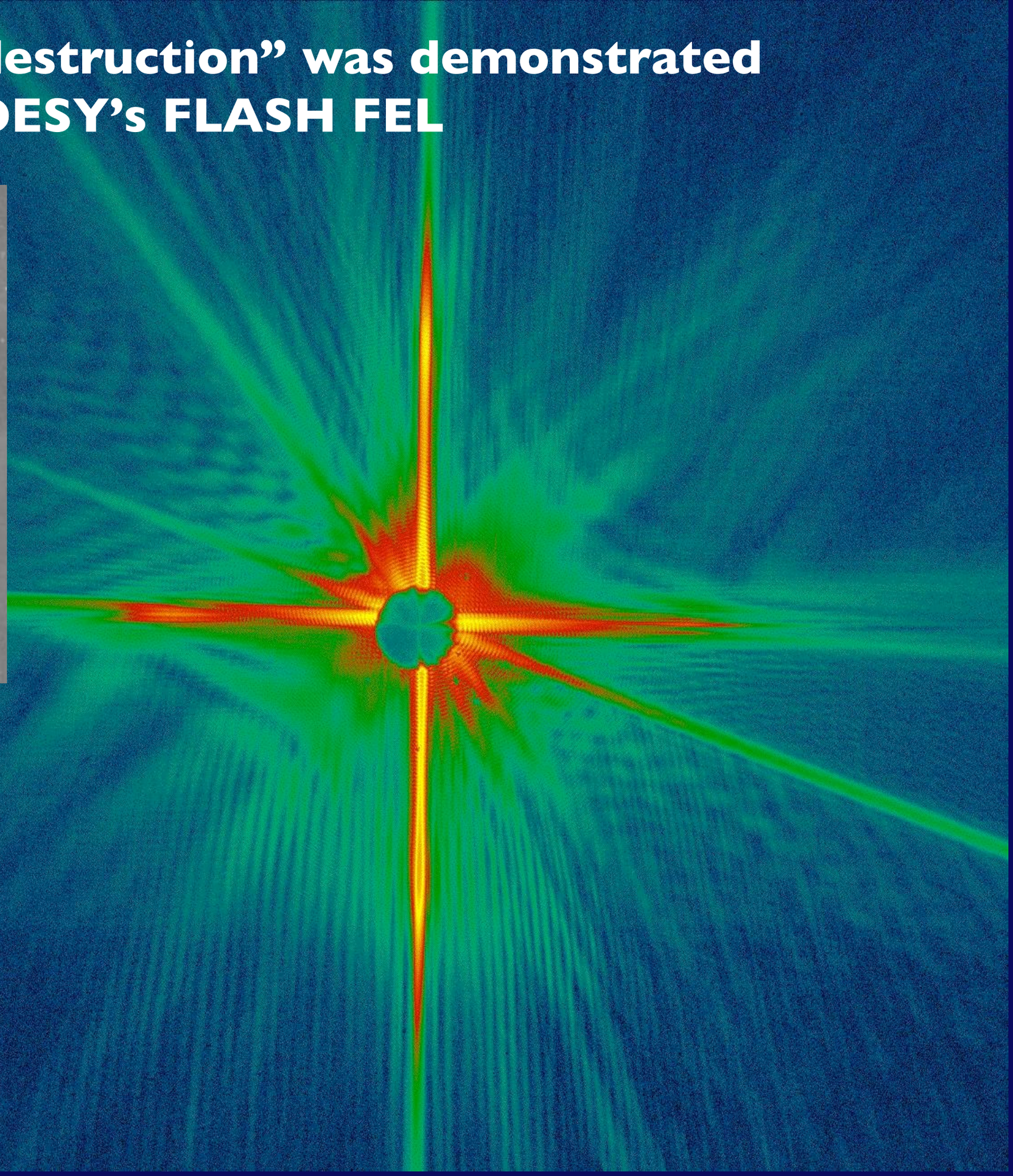


SEM of structure etched into silicon nitride membrane

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

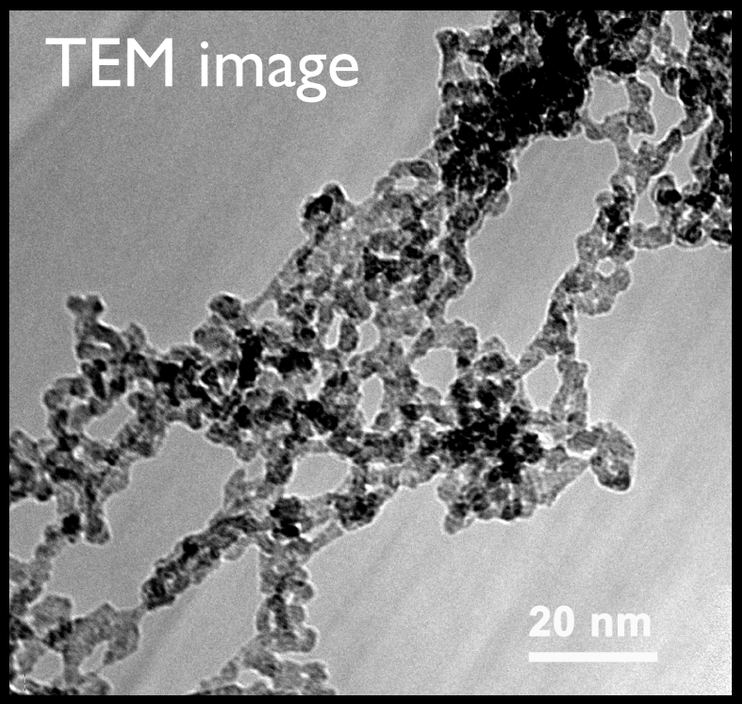


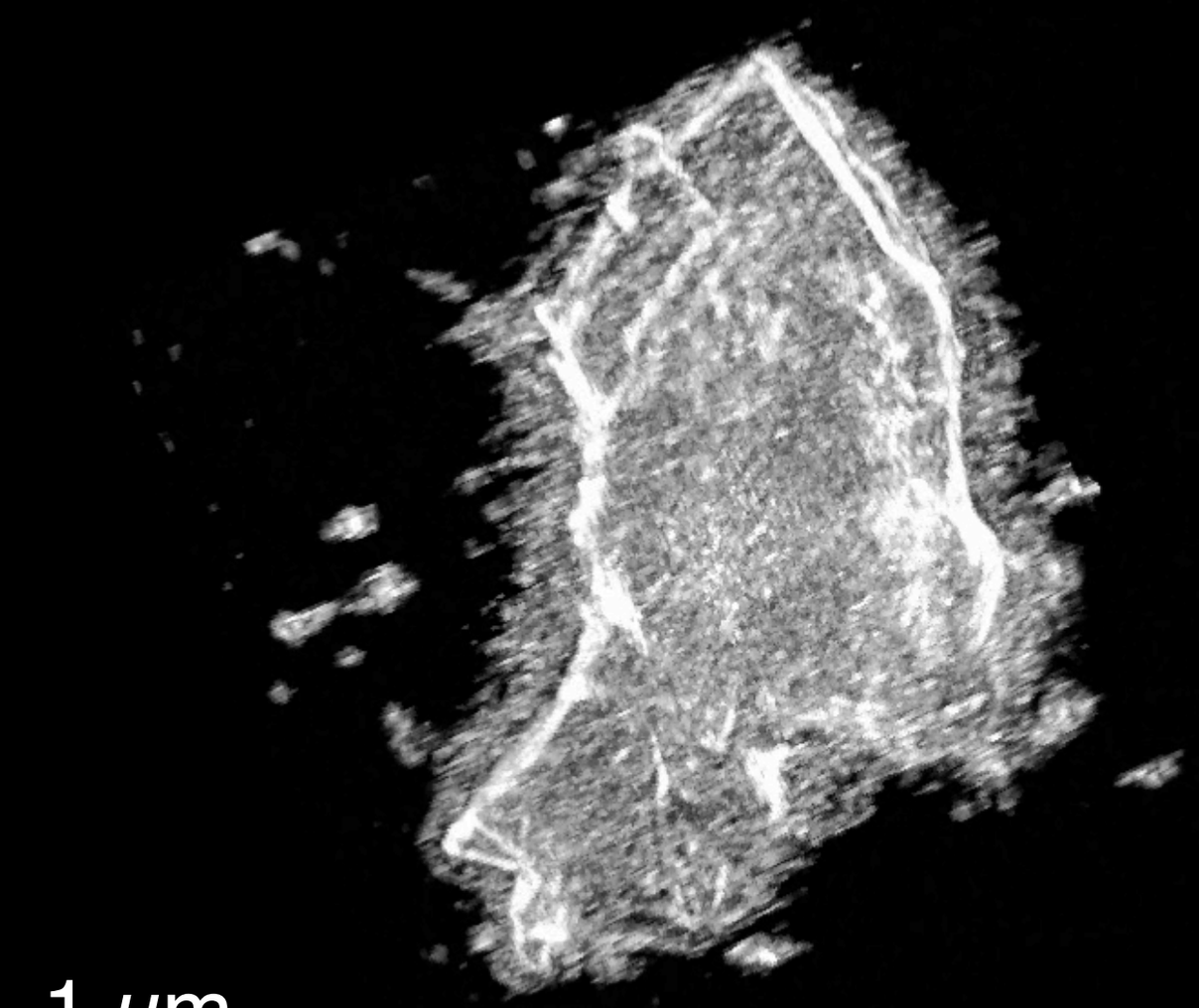




We have performed 3D X-ray imaging of Aerogel foam at 10 nm resolution

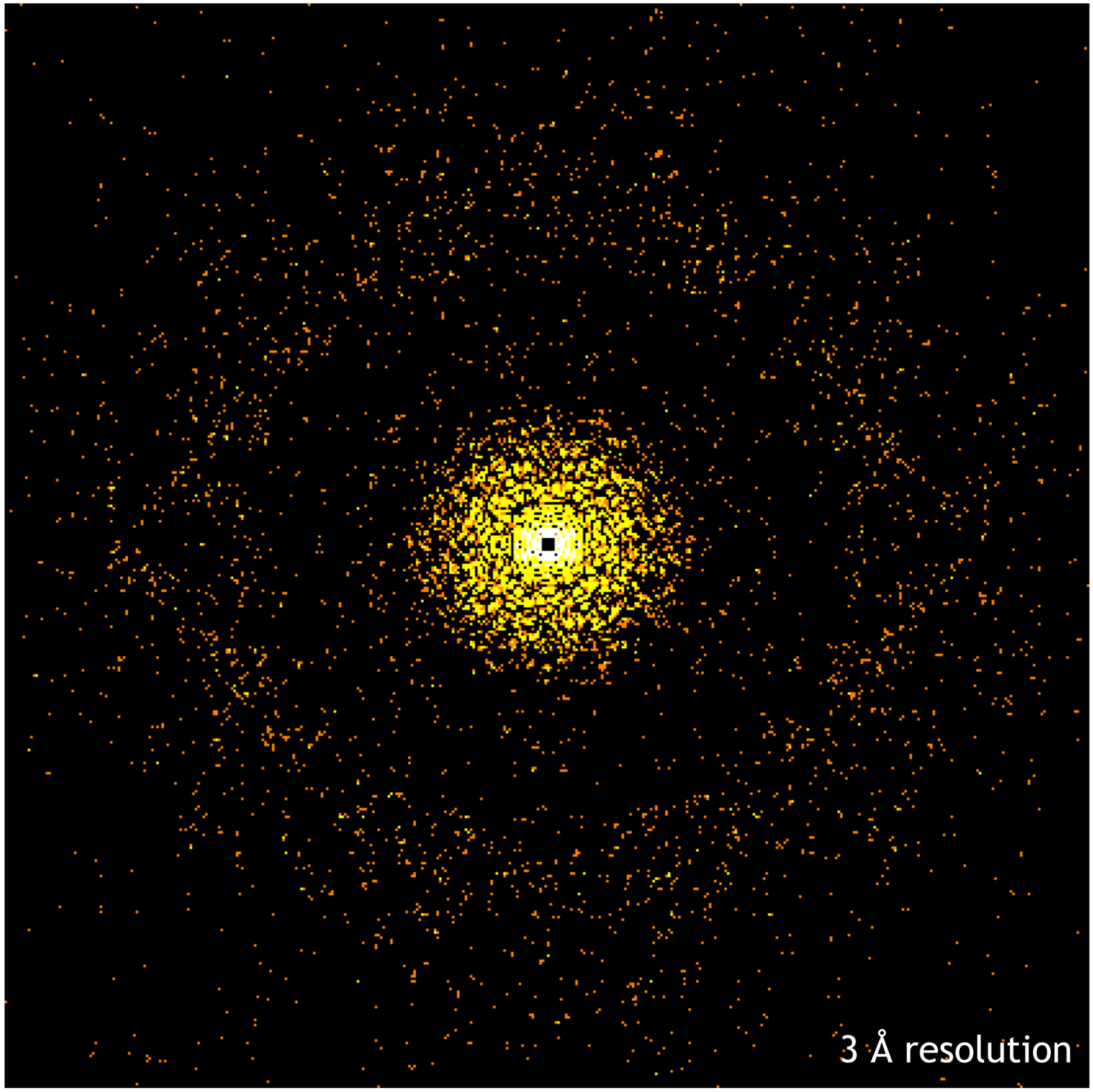
Analysis of the 3D image reveals anisotropy in the structure. Other characterization techniques (TEM, SAXS) could not reveal this

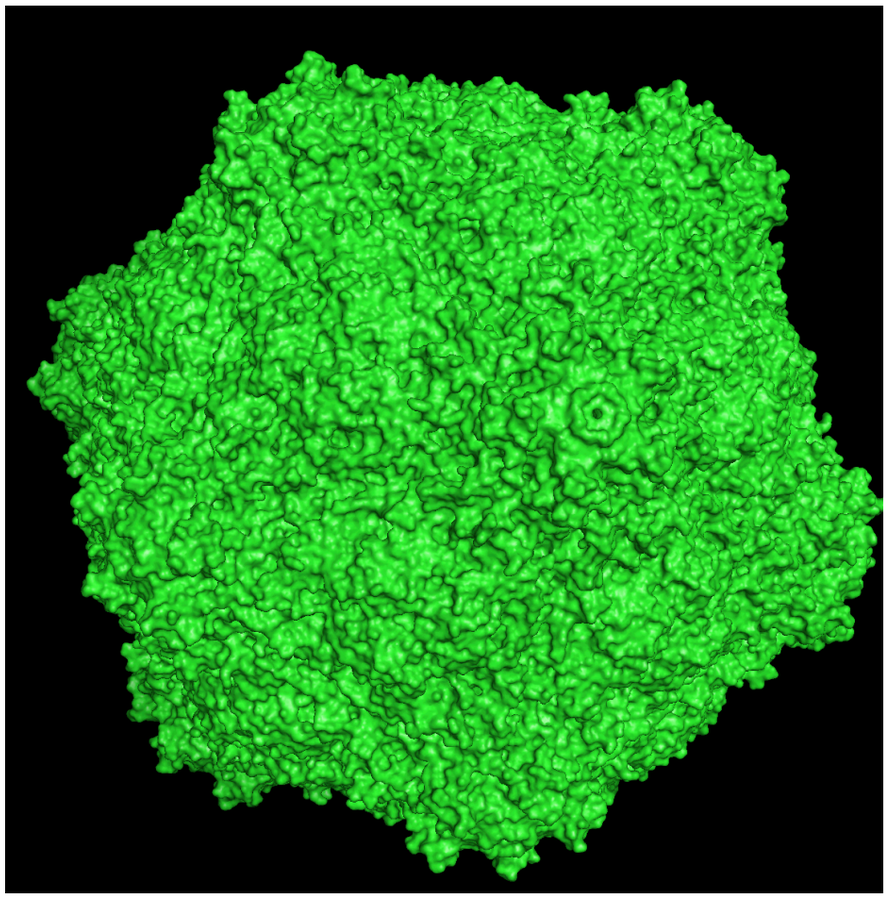




A. Barty, et al., PRL **101** 055501 (2008)

Atomic-resolution diffraction from single particles requires focused intensities of more than 10^{14} ph/μm^2





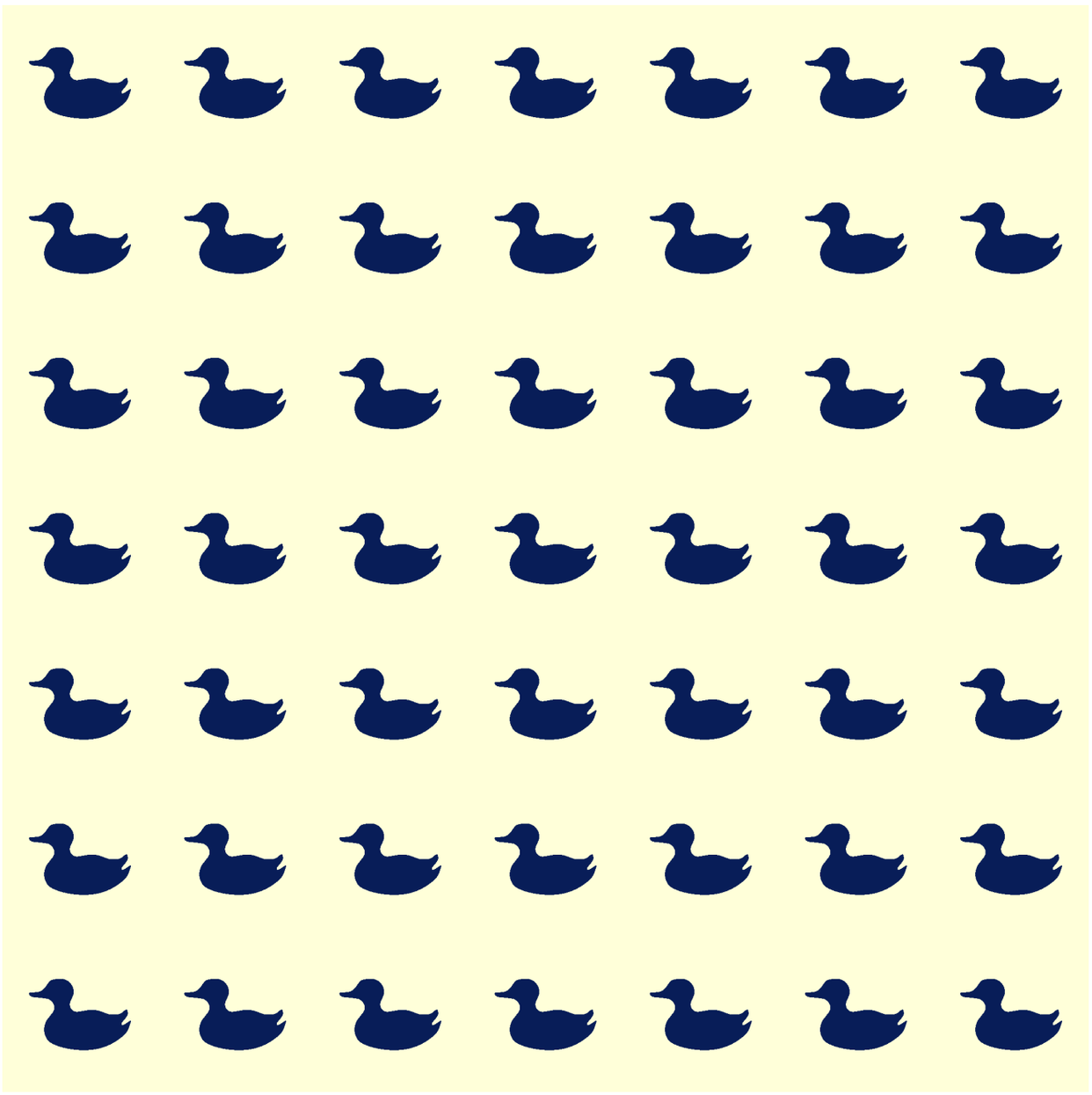
28 nm

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

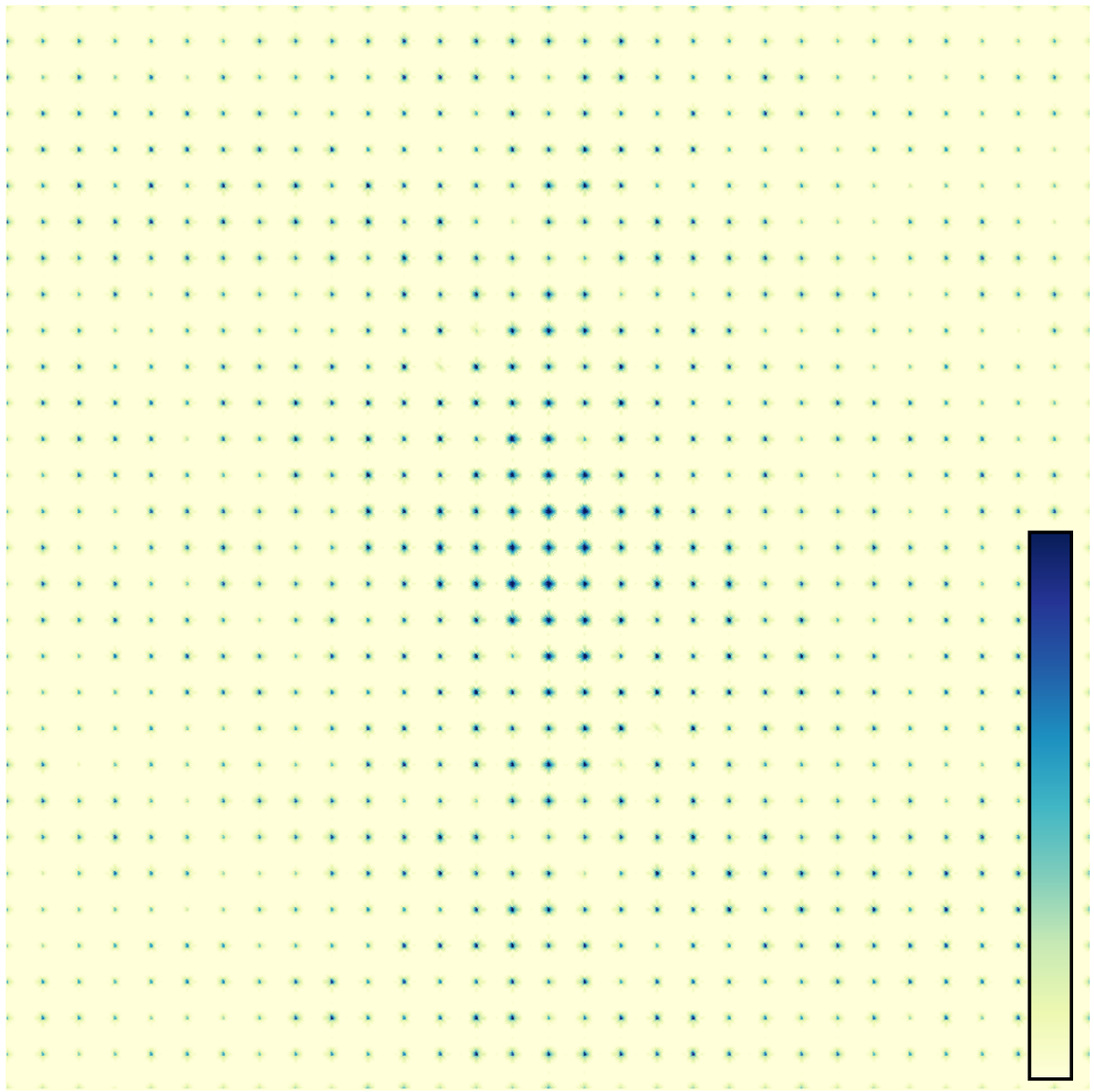
RMS displacement: 0.5Å
half electrons ionized

Crystals give Bragg spots

$\rho(\mathbf{x})$



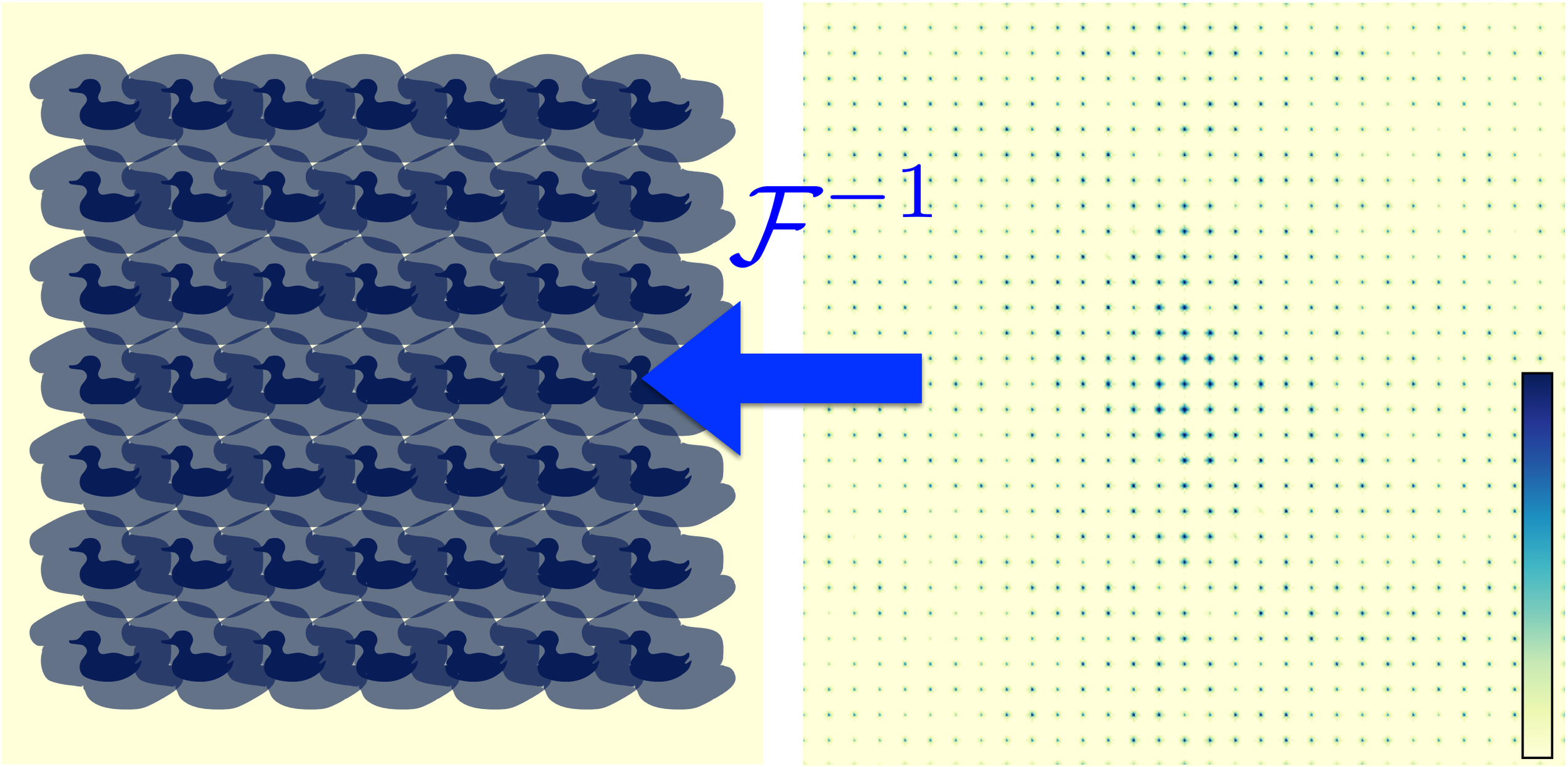
$I(\mathbf{q}) = |\hat{\rho}(\mathbf{q})|^2$



Crystals give Bragg spots

$$\mathcal{F}^{-1}\{I(\mathbf{q})\} = \rho(\mathbf{x}) \otimes \rho^*(-\mathbf{x})$$

$$I(\mathbf{q}) = |\hat{\rho}(\mathbf{q})|^2$$



Under-constrained: fewer knowns than unknowns

Recent hard X-ray experiments show high-resolution diffraction

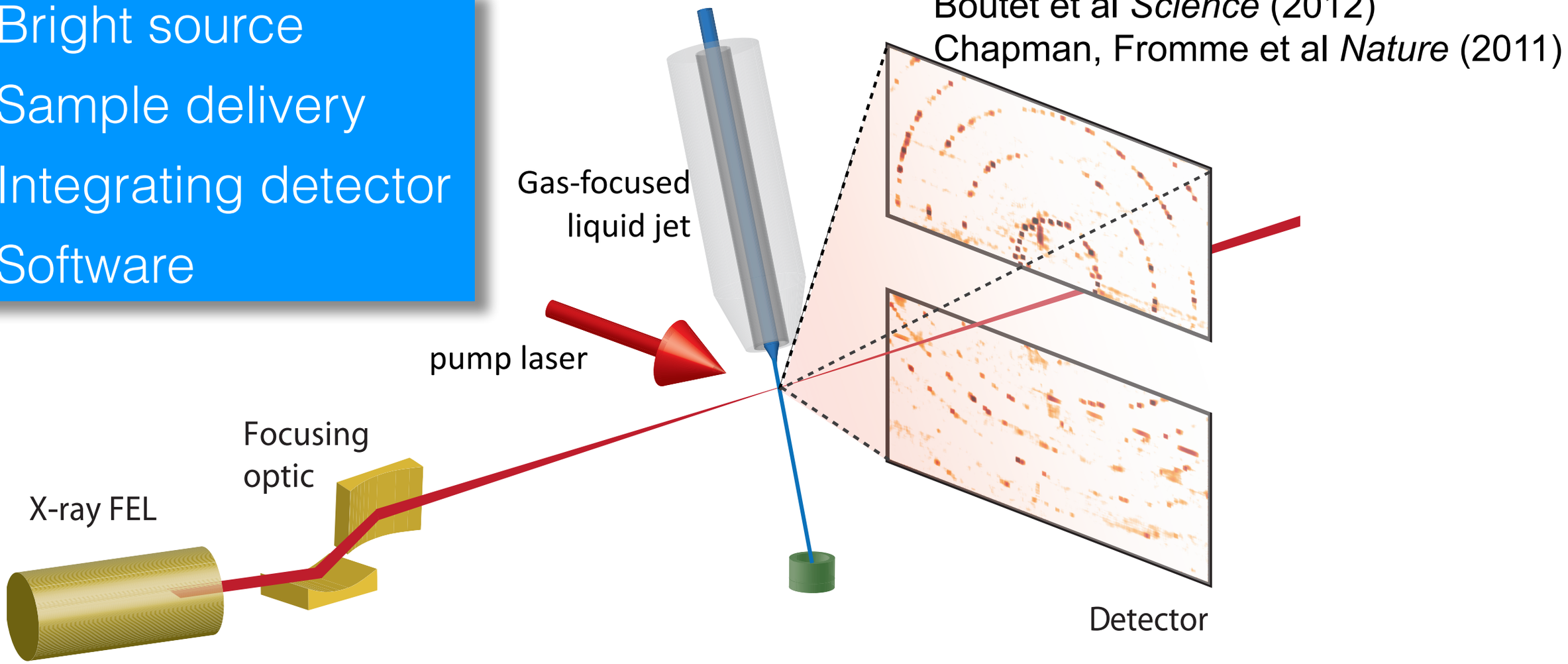
Photosystem I
9.3 keV
Single shot pattern
~1 mJ (5×10^{11} photons)
40 fs
2×10^{17} W/cm^2
25 GW X-ray pulse

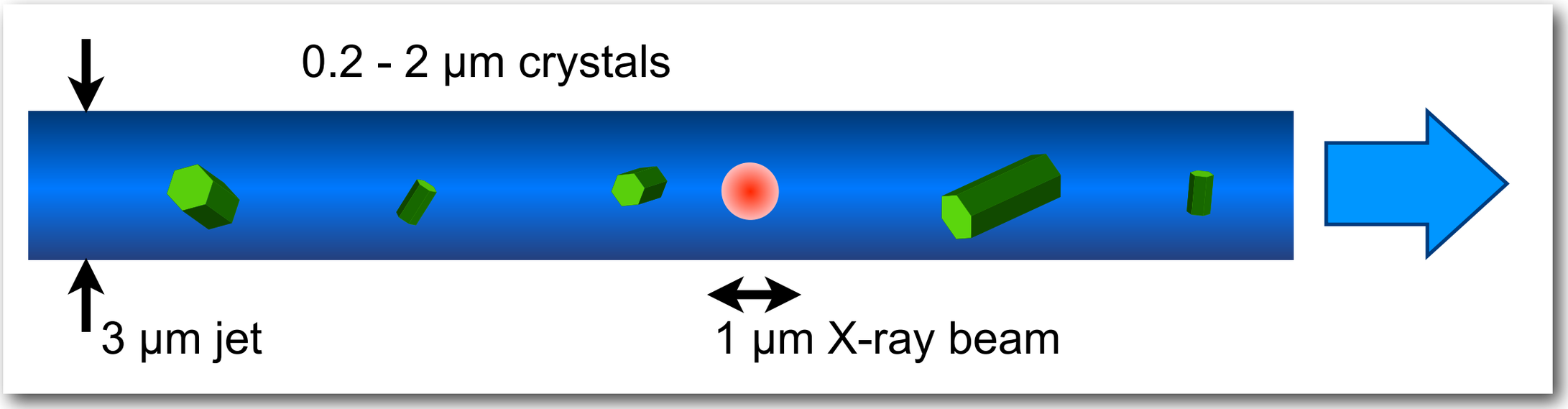
3.0 Å resolution

crystals by Petra Fromme

Serial crystallography is made possible by four key technologies

1. Bright source
2. Sample delivery
3. Integrating detector
4. Software



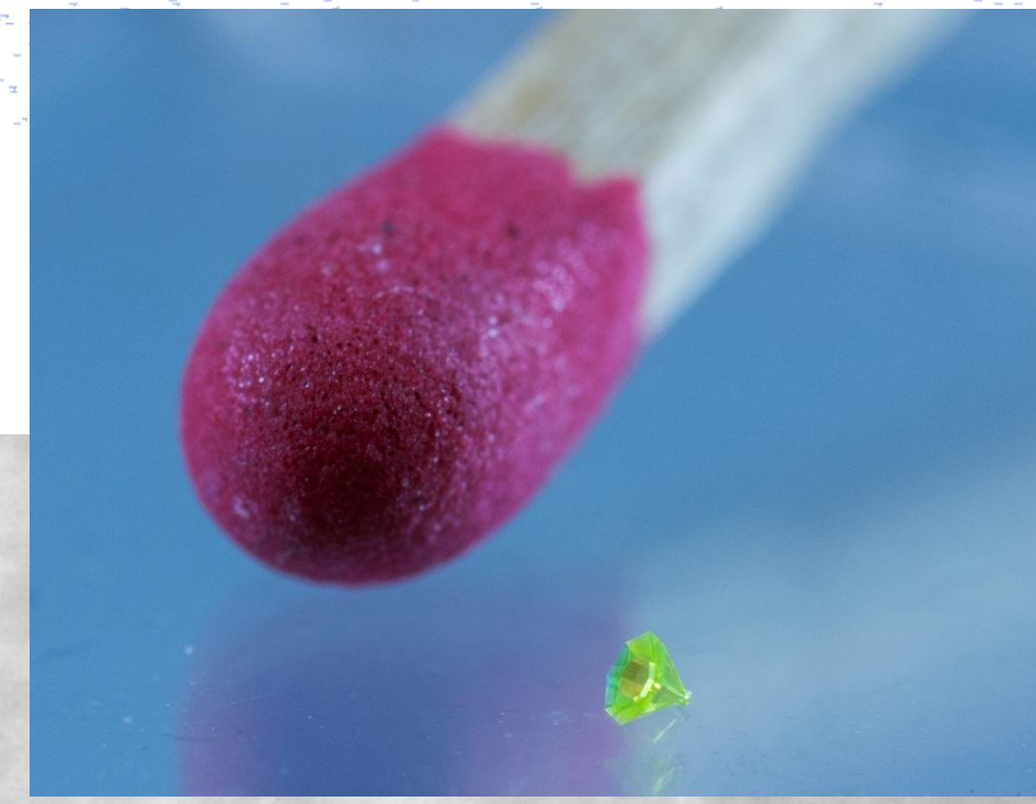


Micrometer-diameter jets are formed by fluid and gas focusing

Božidar Šarler

gas

100 μm

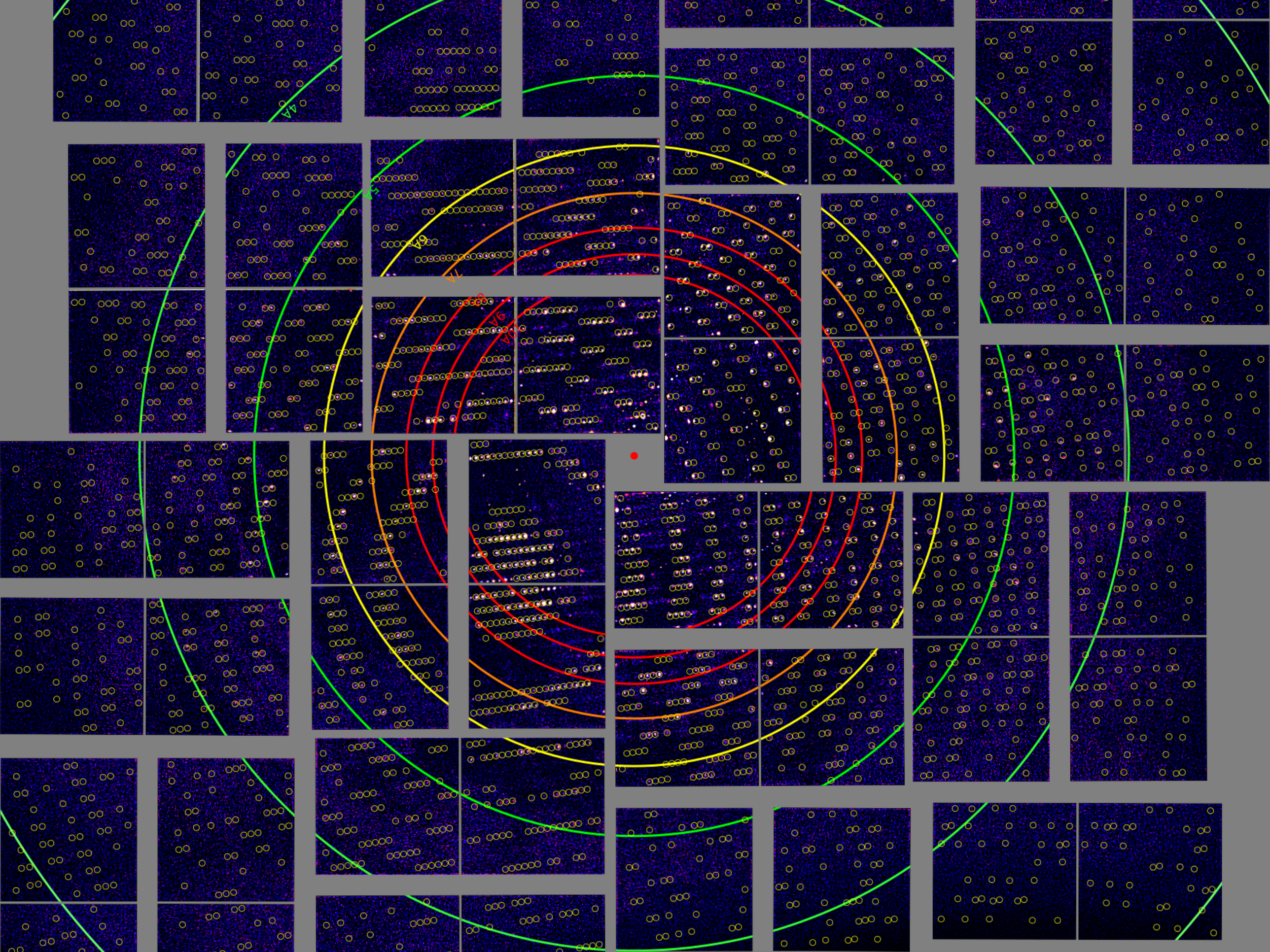


Juraj Knoška, Saša Bajt

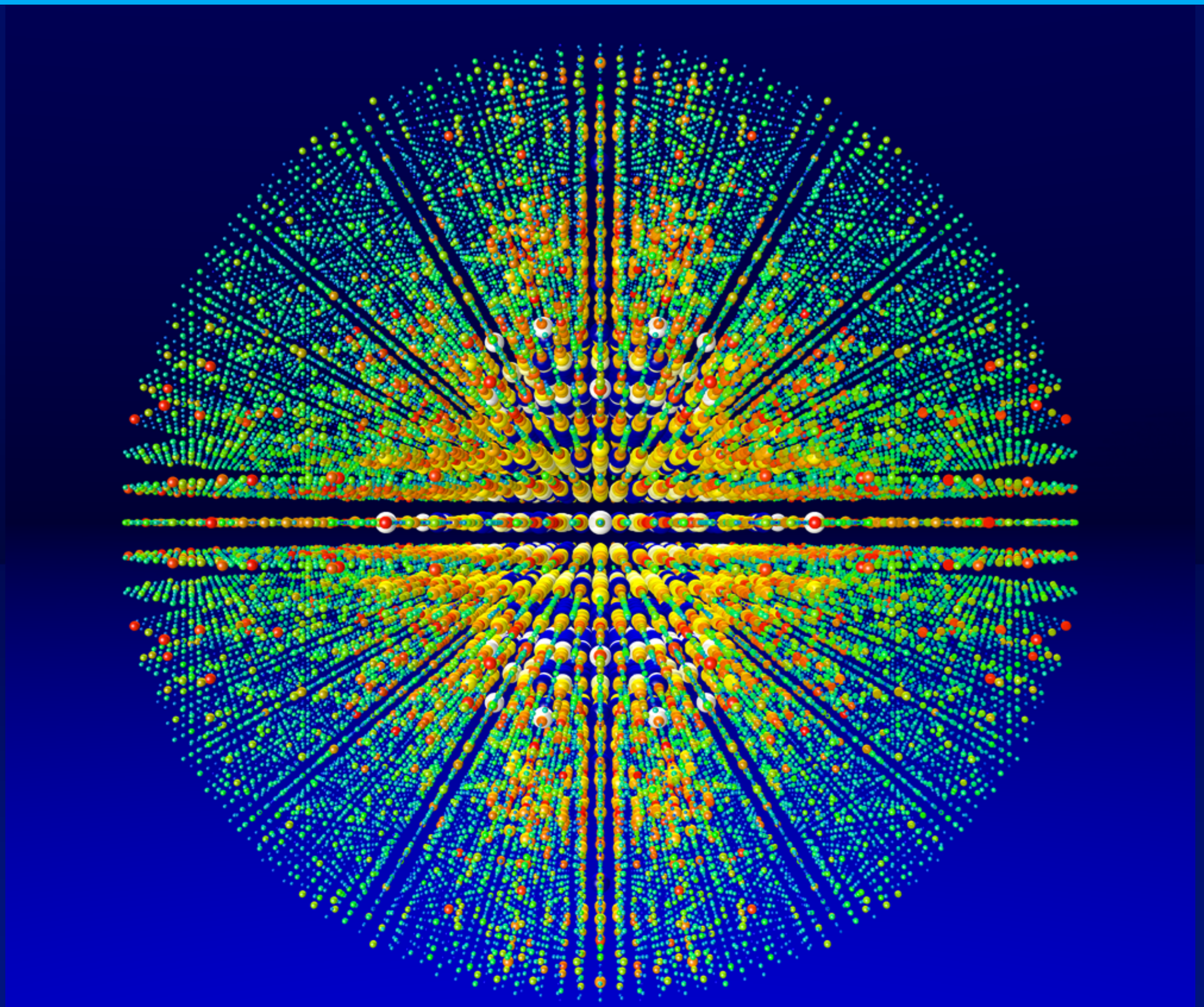
We can sum patterns to create a virtual powder pattern

Lysozyme crystals

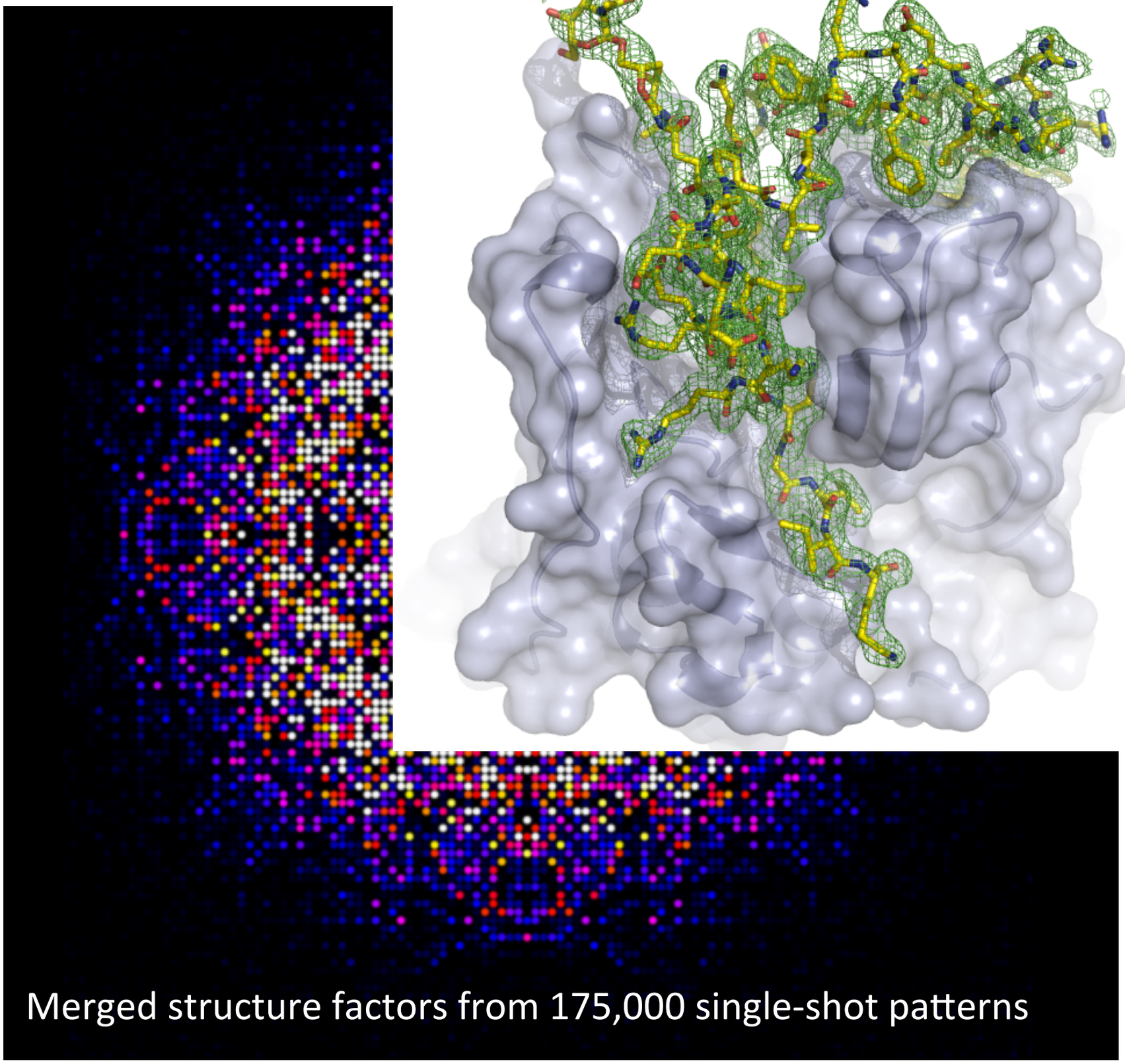
1.9 Å resolution



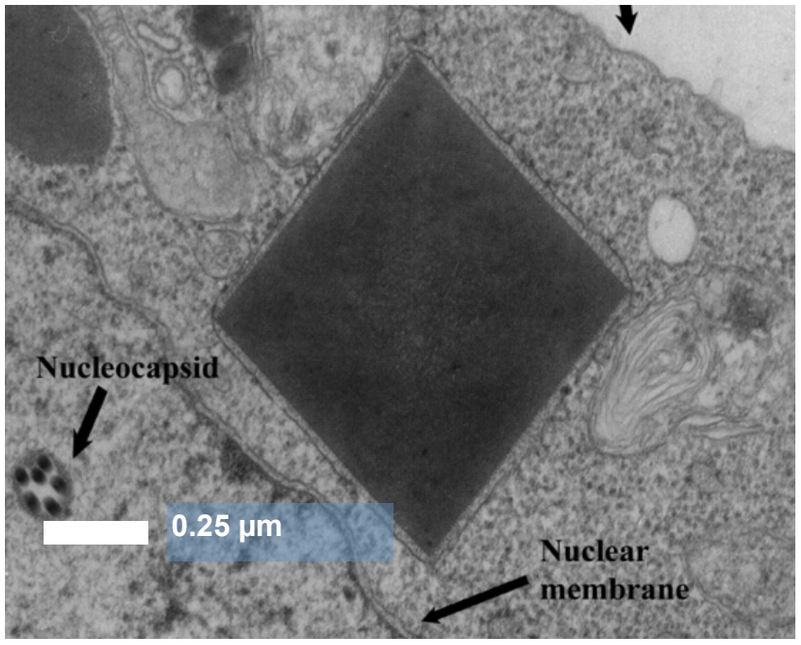
Intensities are merged into a “3D powder” pattern



Structures have been obtained by in vivo grown crystals

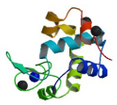


Trypanosoma brucei cathepsin B obtained from in vivo grown crystals



Redecke, Nass et al. Science (2013)

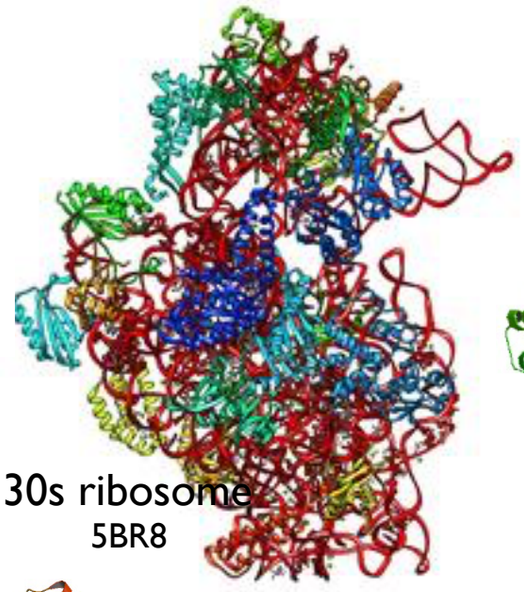
Over 100 XFEL structures have been solved



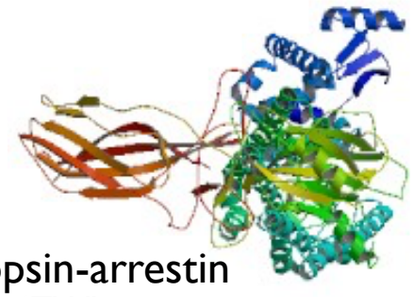
Lysozyme
4ZIX, 5C6I, 5C6J, 5C6L,
4RW1, 4RW2, 4N5R

Thermolysin
4OW3,
4TNL, 5DLH

ATPase
4XOU



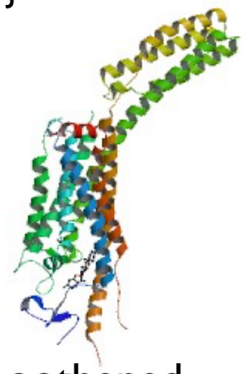
30s ribosome
5BR8



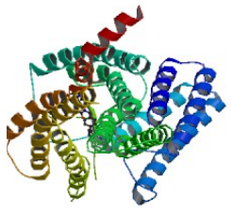
Rhodopsin-arrestin
4ZWJ

A2a
5K2D, 5K2C,
5K2B, 5K2A

5-HT2B
4NC3

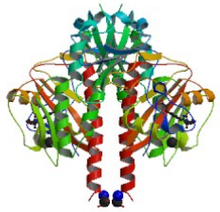


Smoothened
4O9R



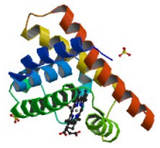
Angiotensin
4YAY

SNARE complex
5CCG



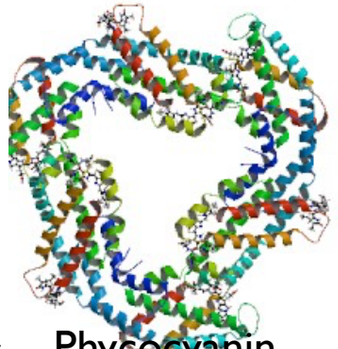
Phytochrome
5MG0, 5MG1, 5L8I

Cry toxin
4QX3,
4QX2,
4QX1, 4QX0

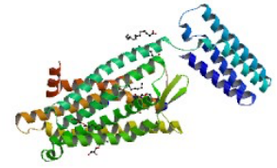


Myoglobin (x15)
4PNJ, 5CNG, 5CNF,
5CNE, 5CND, 5CNC
5CNB, 5CN9, 5CN8,
5CN7, 5CN6, 5CN5,
5CN4, 5CMV, 5JOM

BinAB
5FOY, 5FOZ, 5G37,

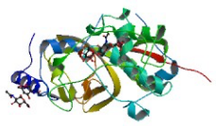


Phycocyanin
4Q70, 4Z8K, 4ZIZ



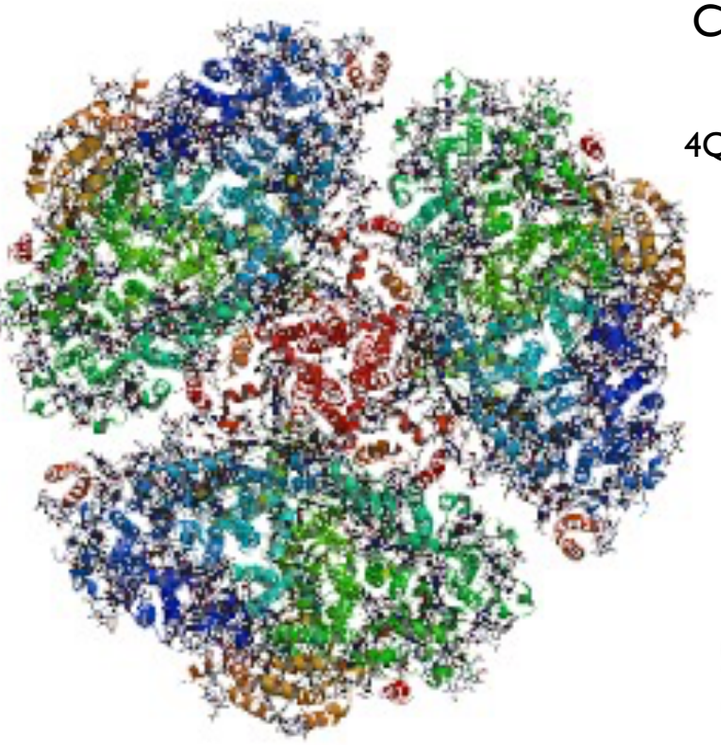
d-opioid
4RWD

DgkA
4UYO

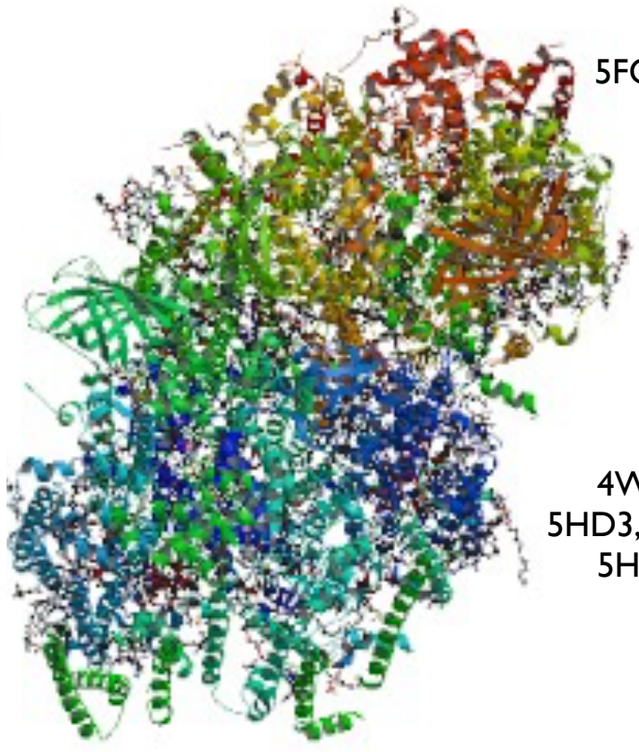


CathepsinB
4HWY

4ZQX

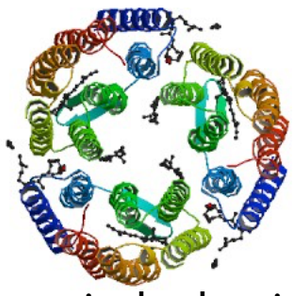


Photosystem I+II
3PCQ, 4FBY, 4IXR, 4IXQ,
4TNK, 4TNJ, 4TNI, 4TNH, 4PBU,
4RVY, 5E7C, 5TIS, 5KAI, 5KAF,



PYP
4WLA, 4WL9,
5HD3, 5HDS, 5HDD,
5HDC, 5HD5,

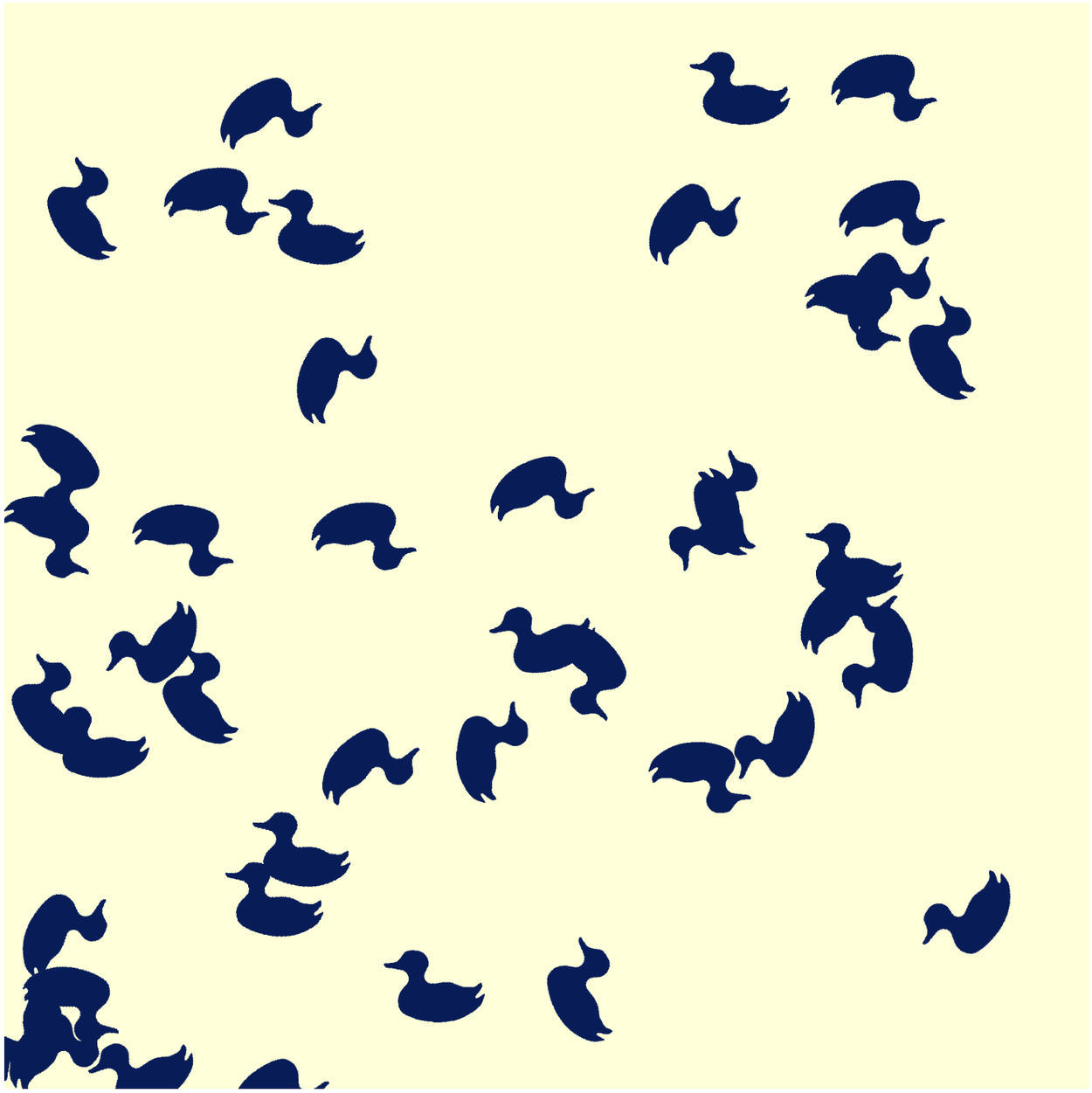
Reaction centre
4AC5, 4CAS,
5M7K, 5M7J

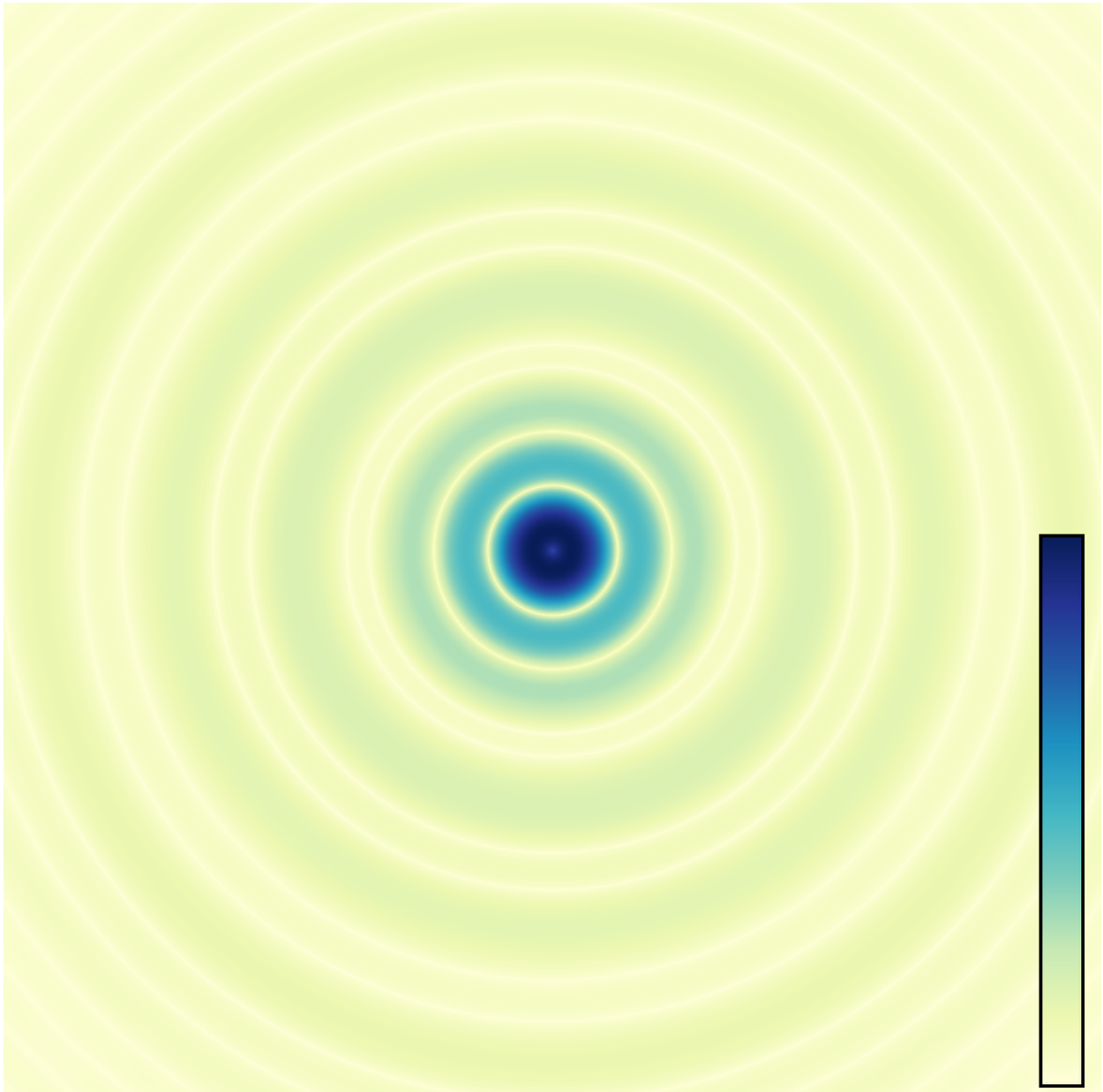


Bacteriorhodopsin
5J7A

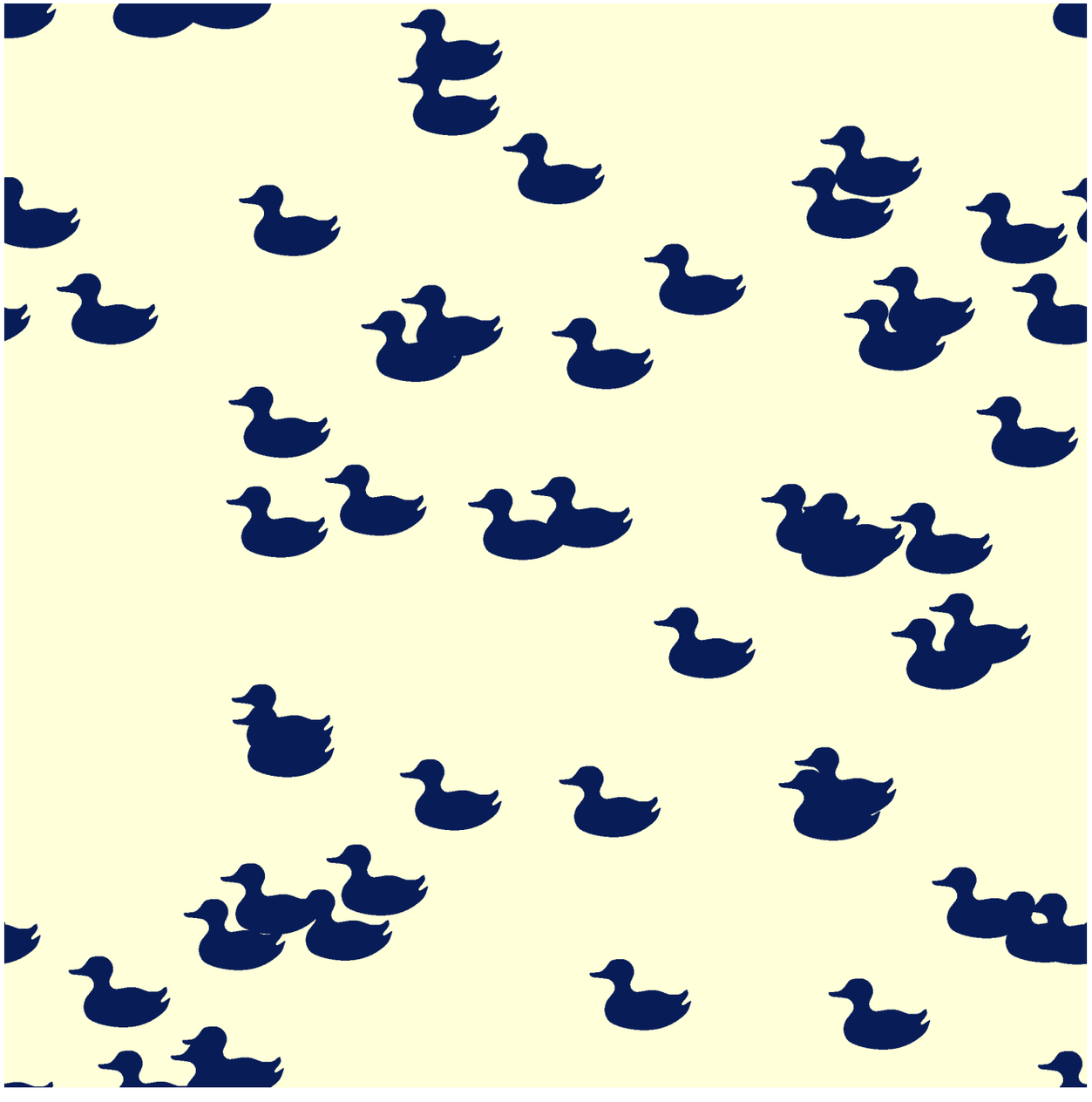
Riboswitch
5SWE, 5SWD, 5E54

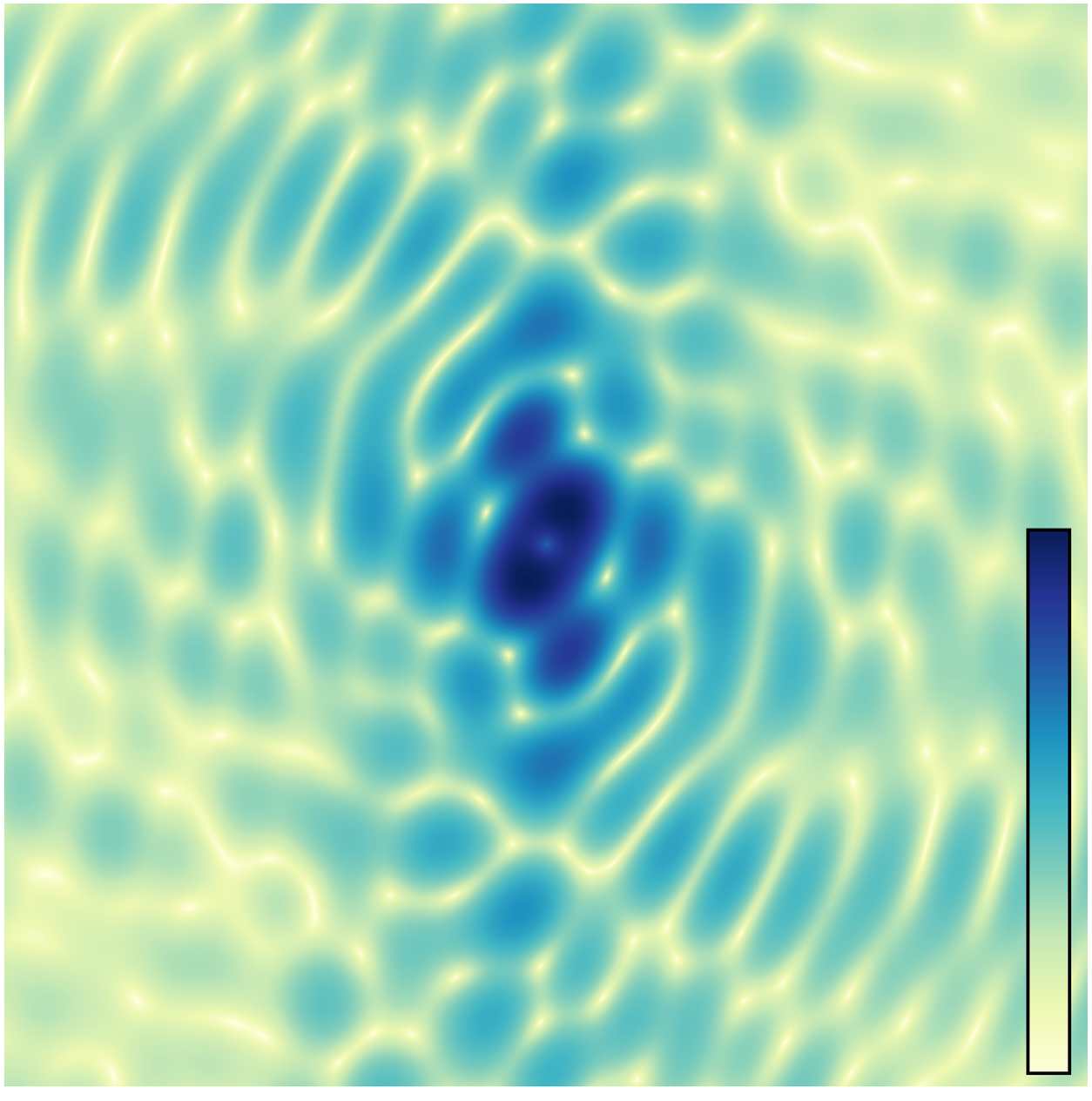
Solution scattering gives single-molecule diffraction, but orientationally averaged



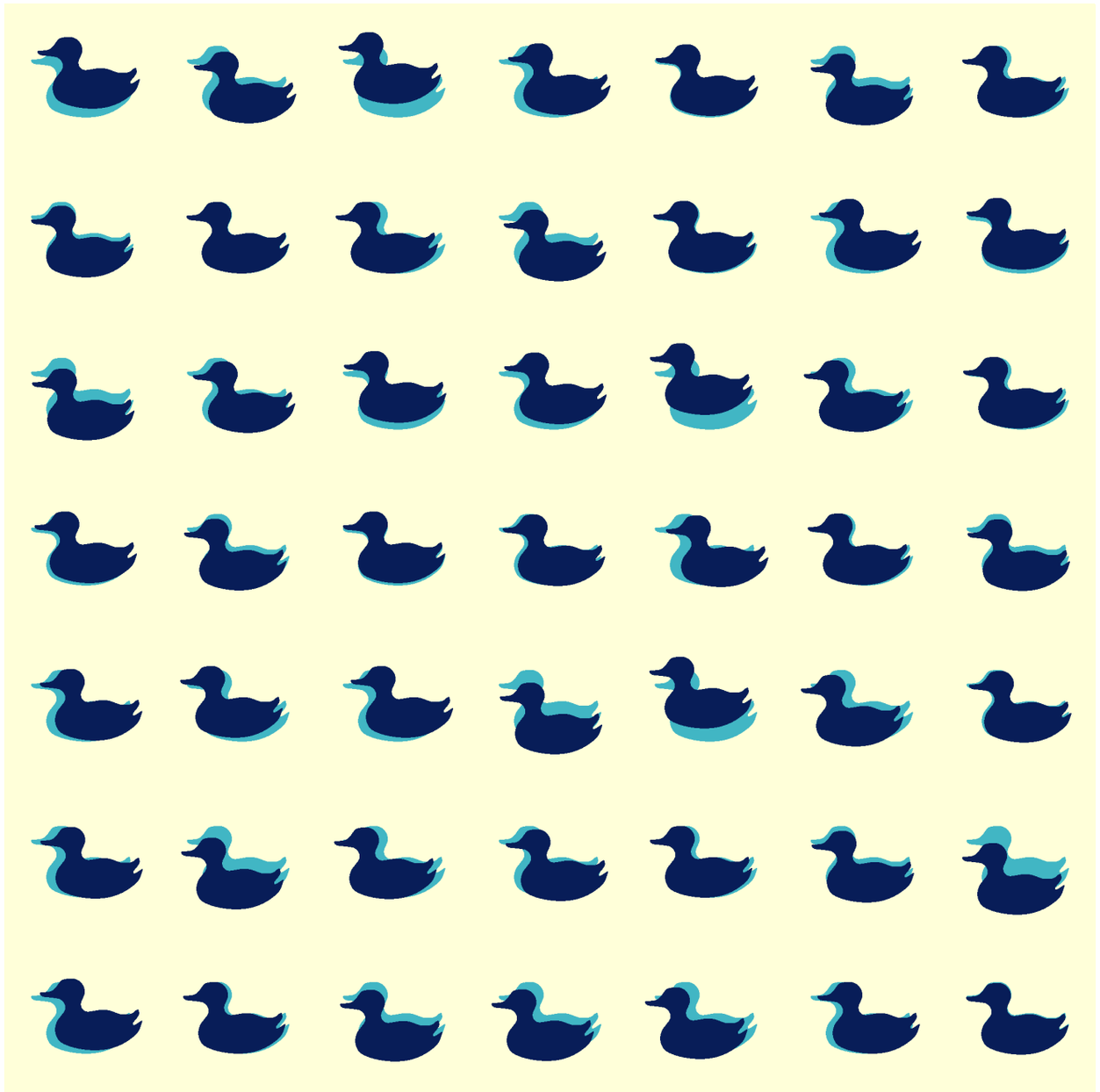


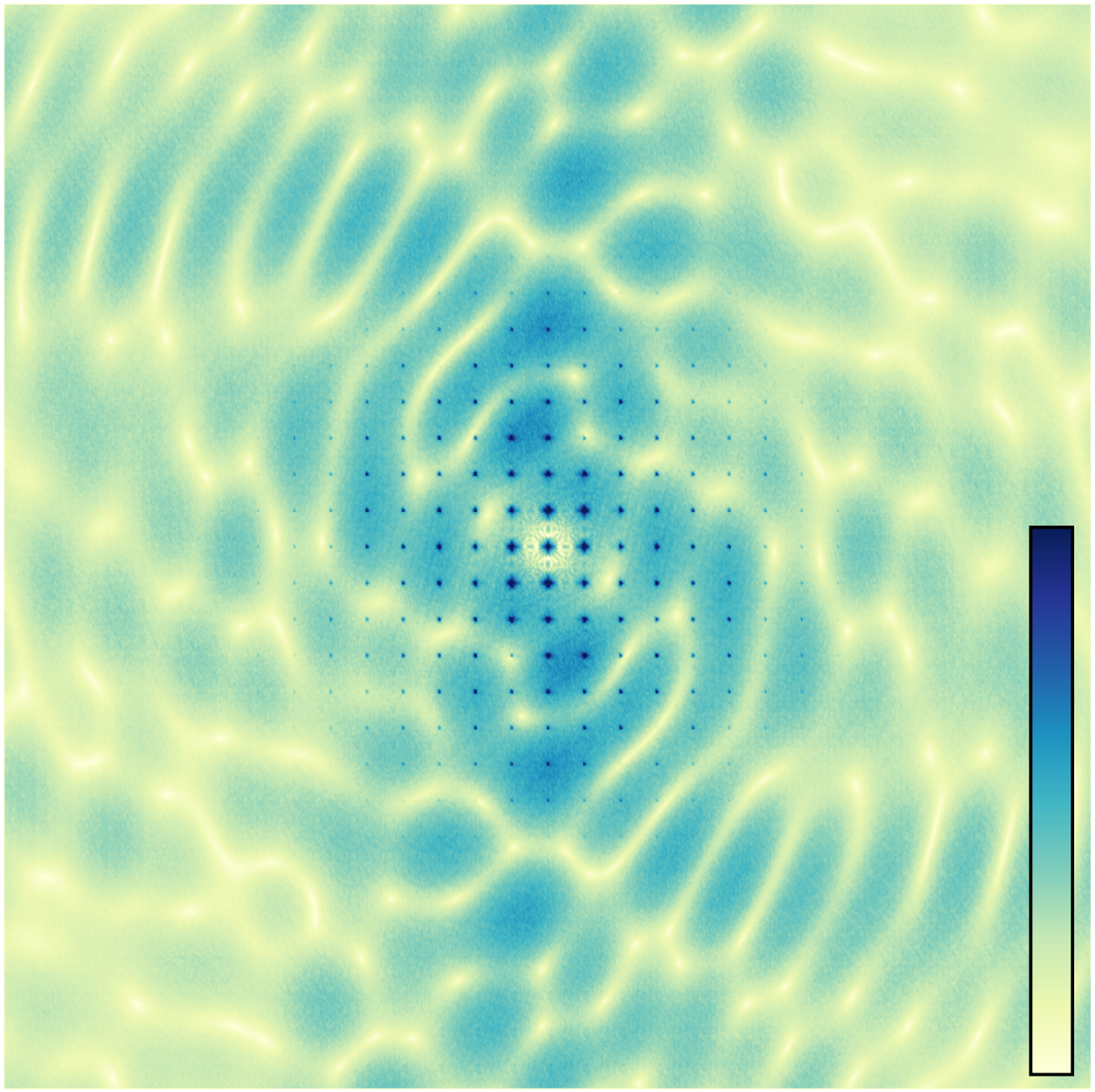
Aligned molecules yield a single-molecule pattern





Crystals provide a very high degree of alignment

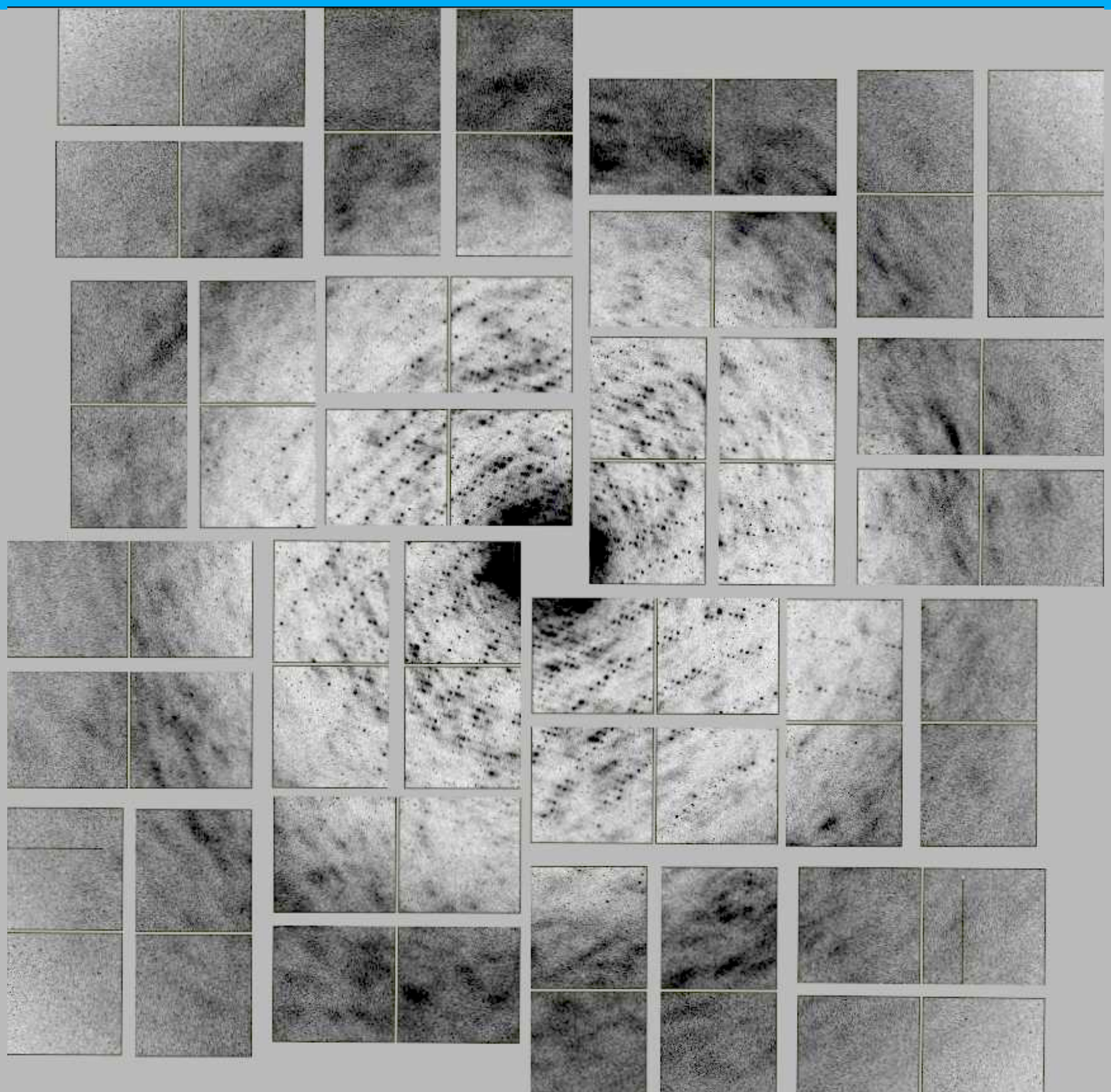


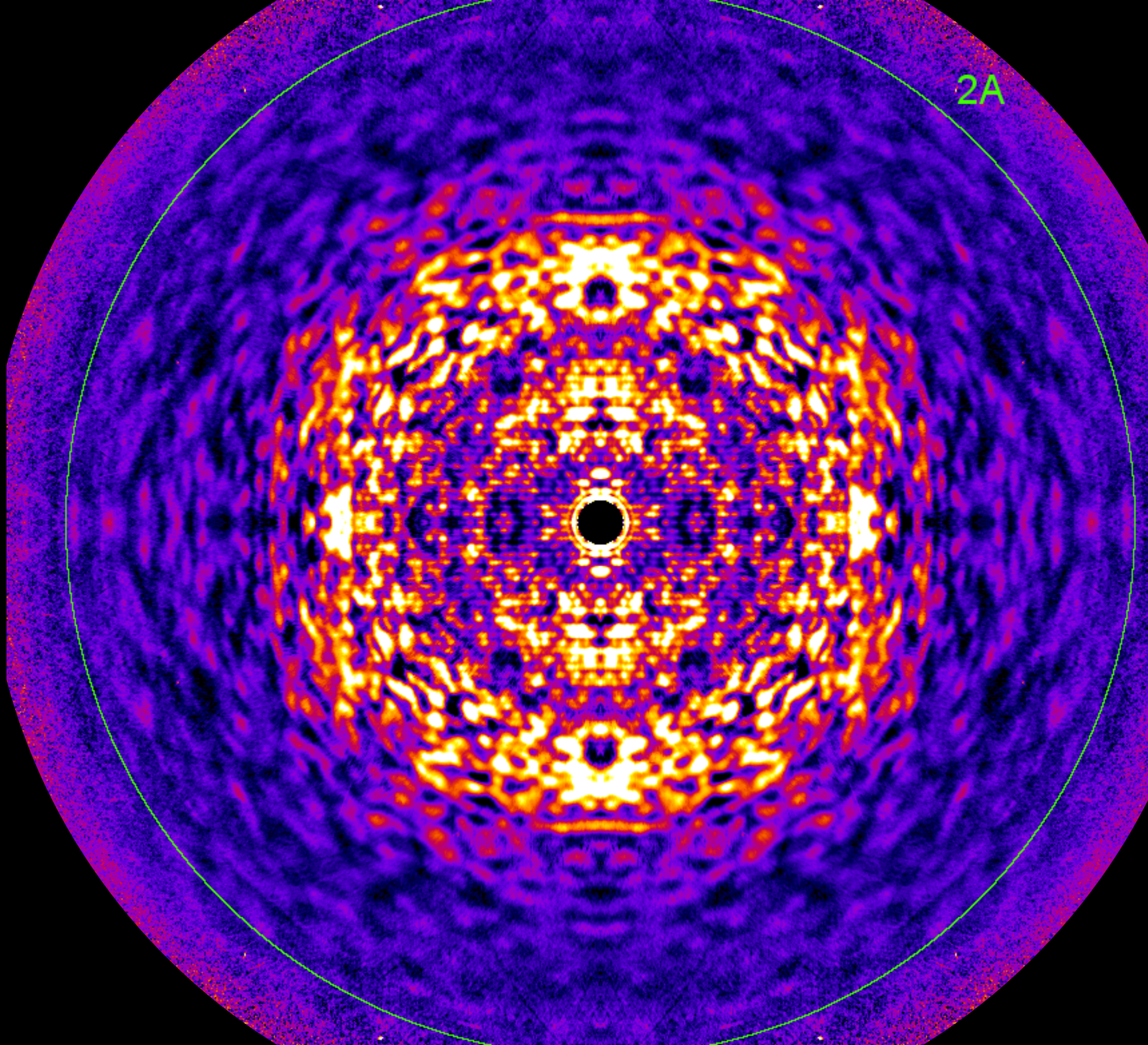


$$\langle I(\mathbf{q})\rangle = \left|\sum_i \hat{\rho}_i(\mathbf{q})\right|^2 \exp(-q^2\sigma^2) + \sum_i |\hat{\rho}_i(\mathbf{q})|^2(1-\exp(-q^2\sigma^2))$$

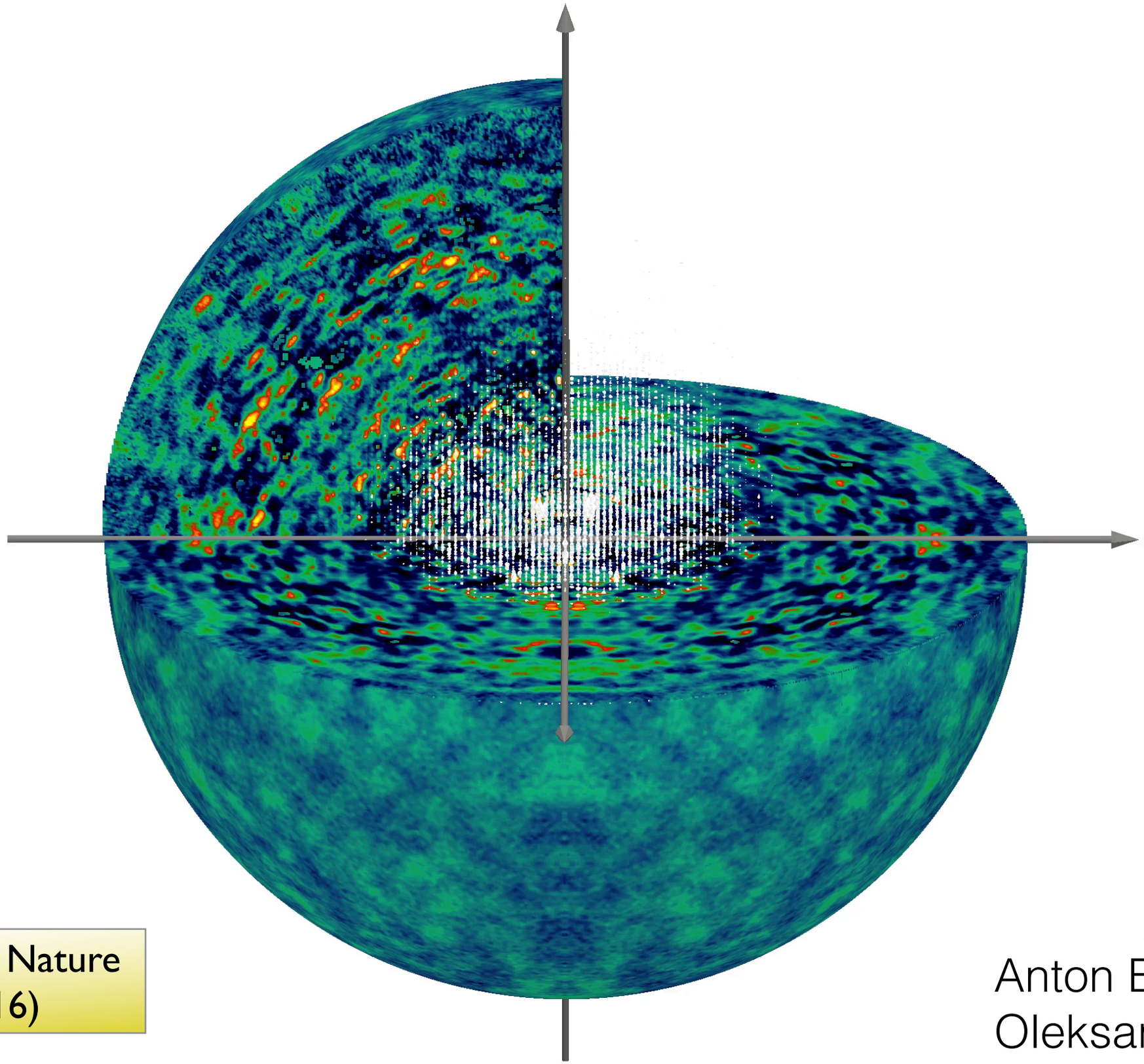
$$\sigma^2 = \langle D^2 \rangle$$

You can see a lot just by looking




2A

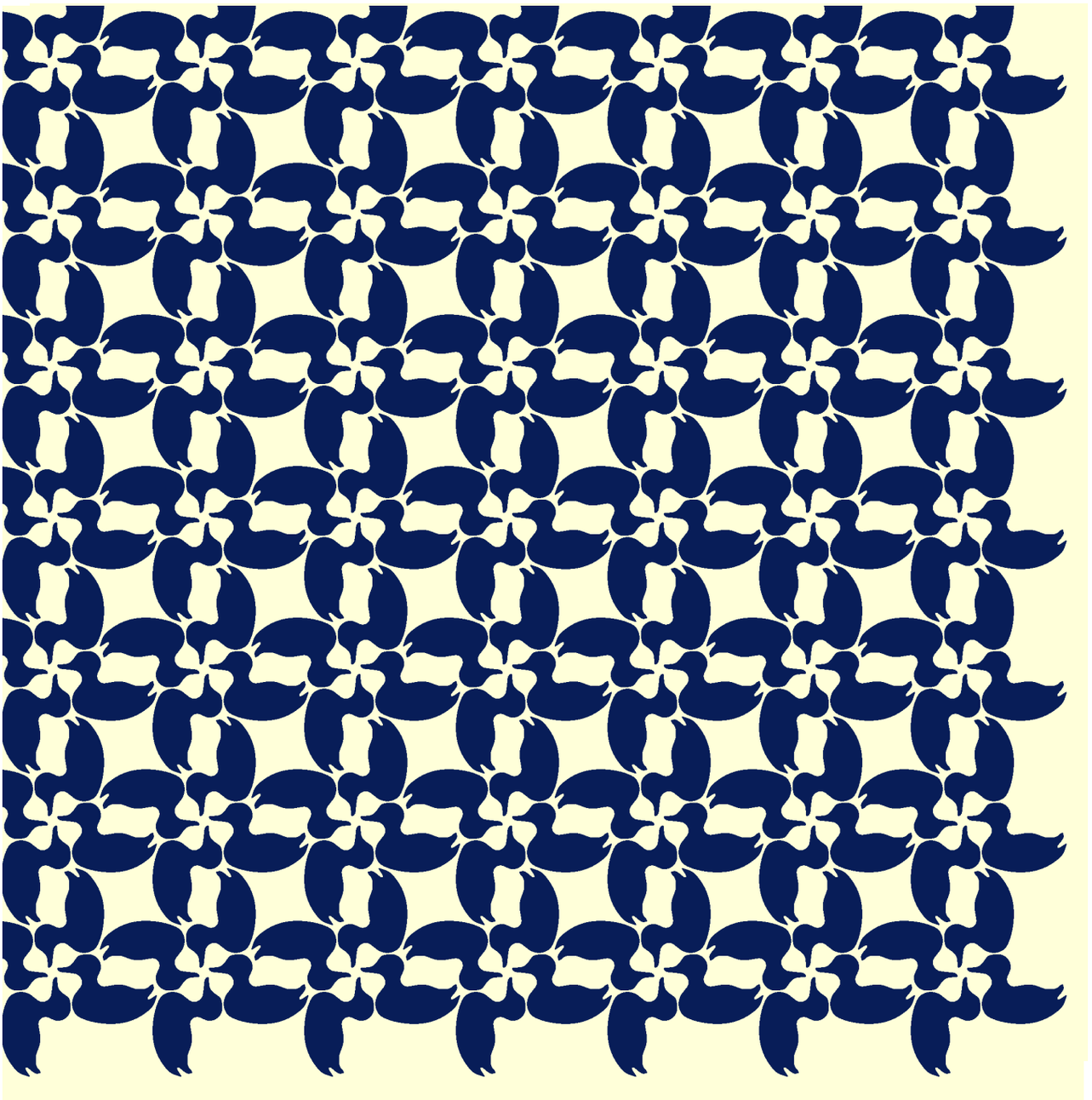
By averaging thousands of patterns a strong single molecule diffraction pattern emerges

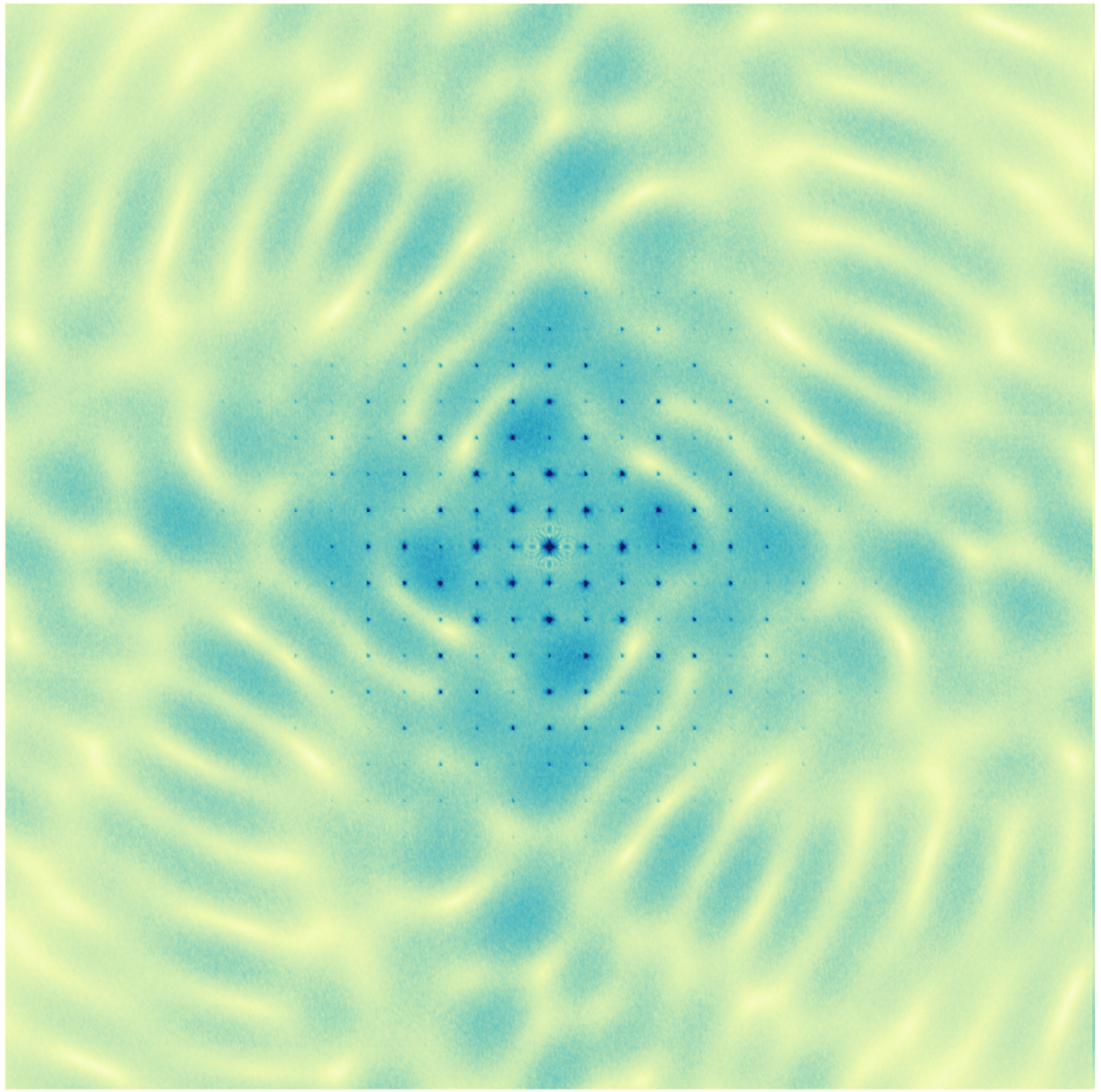


K. Ayyer et al. Nature **530**, 202 (2016)

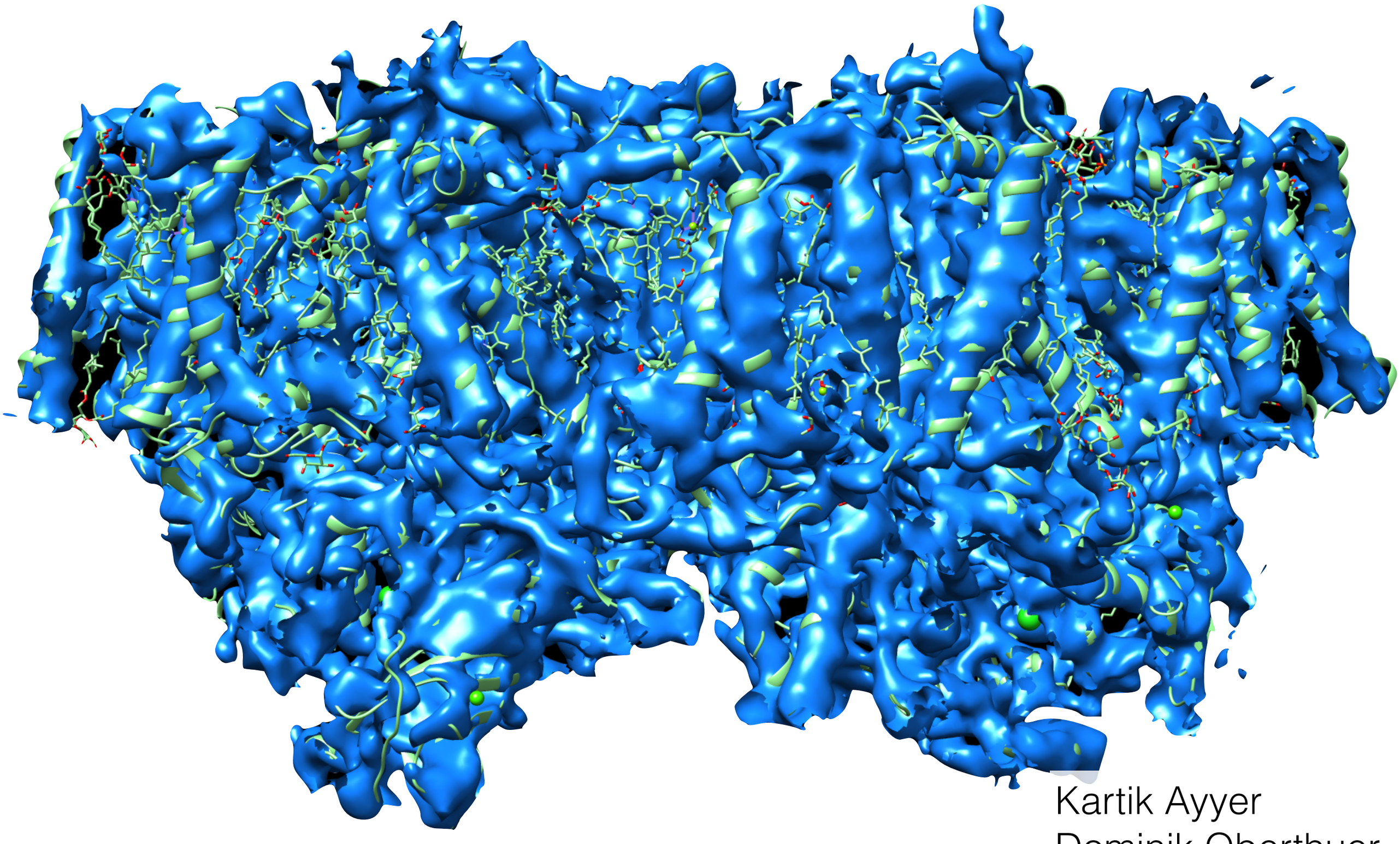
Anton Barty
Oleksandr Yefanov

The orientational symmetry of the crystal is preserved, but not the translational symmetry



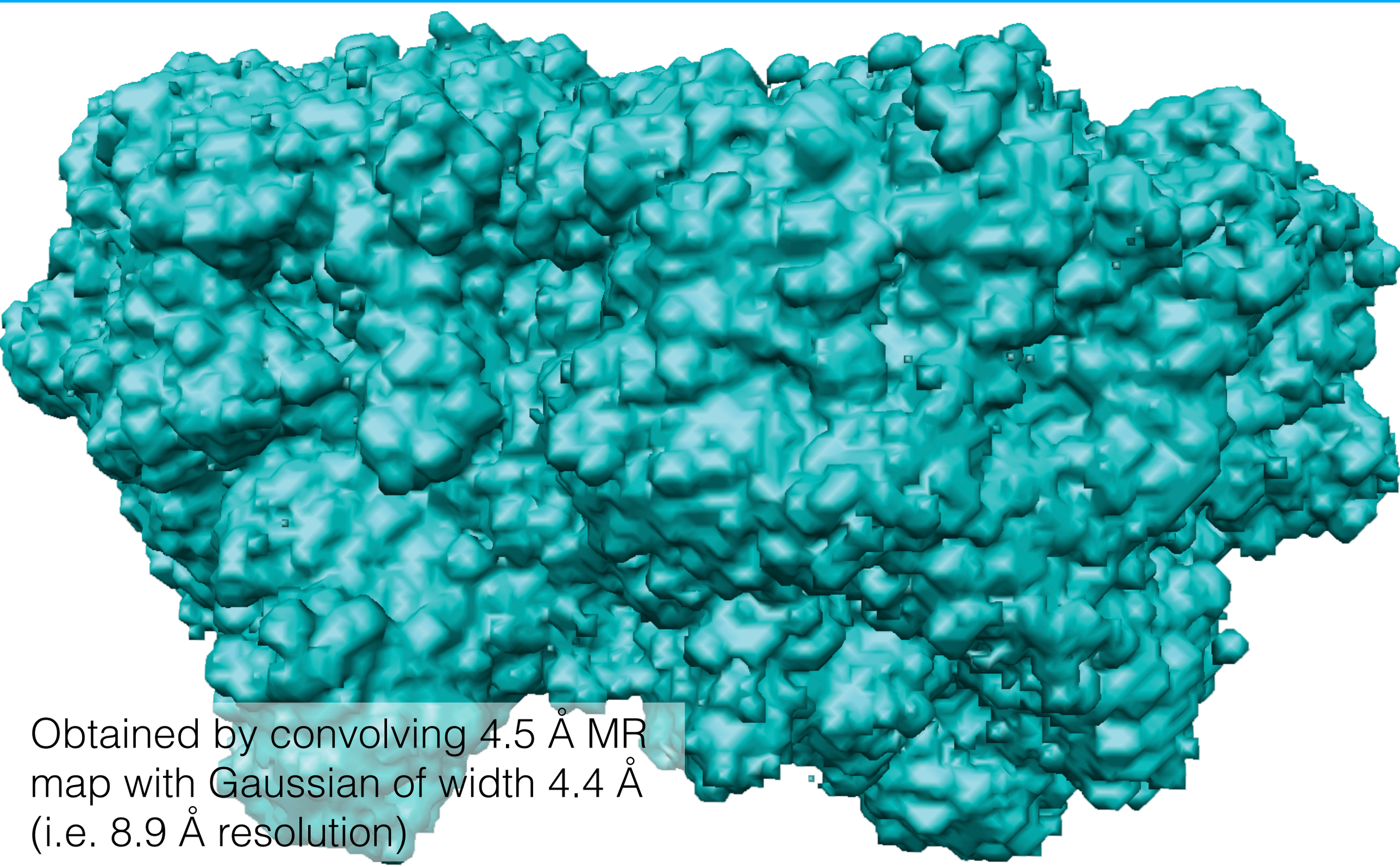


Electron density map from Bragg peaks alone (4.5 Å)



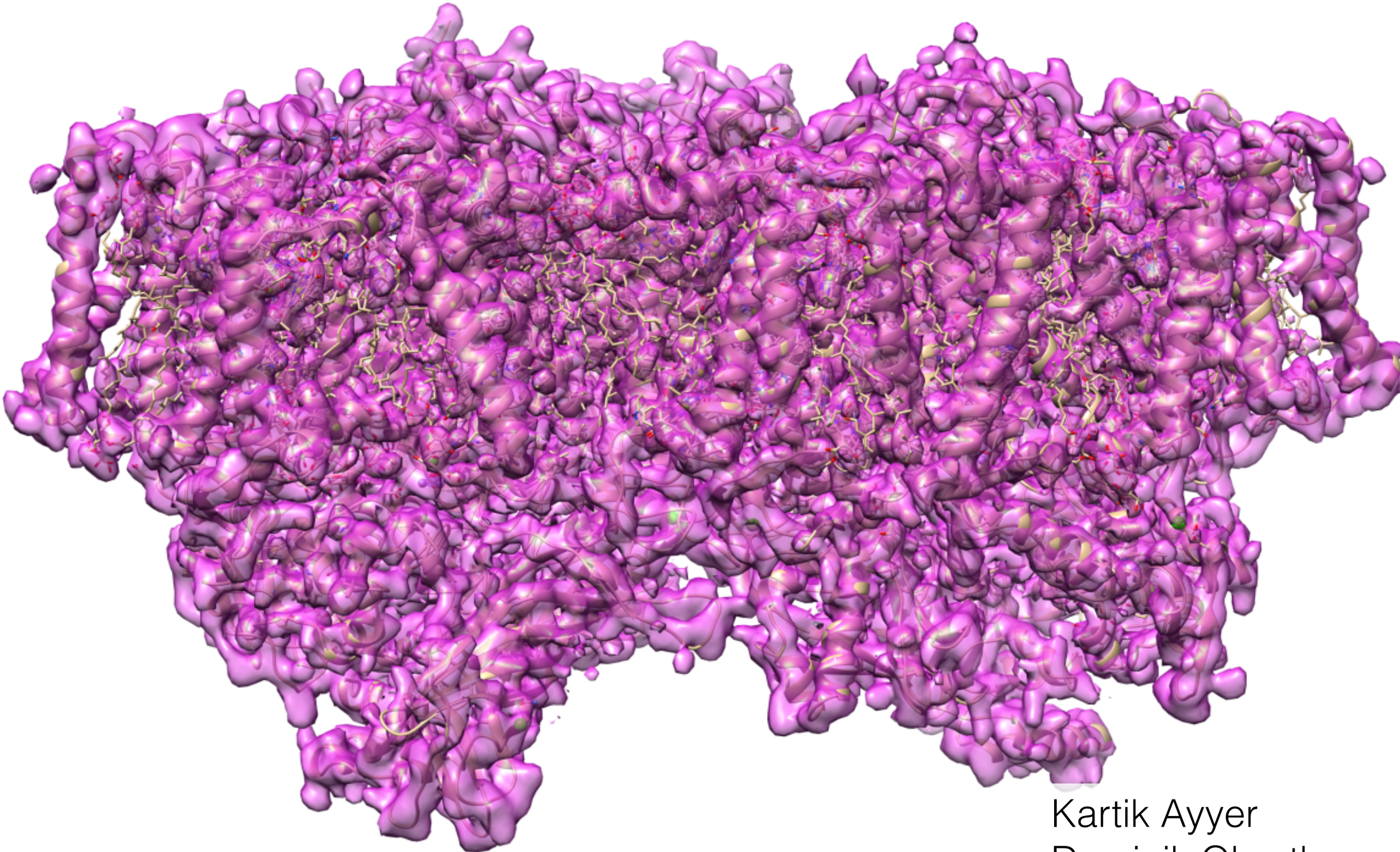
Kartik Ayyer
Dominik Oberthuer

The low-resolution support constrains the phases



Obtained by convolving 4.5 Å MR map with Gaussian of width 4.4 Å (i.e. 8.9 Å resolution)

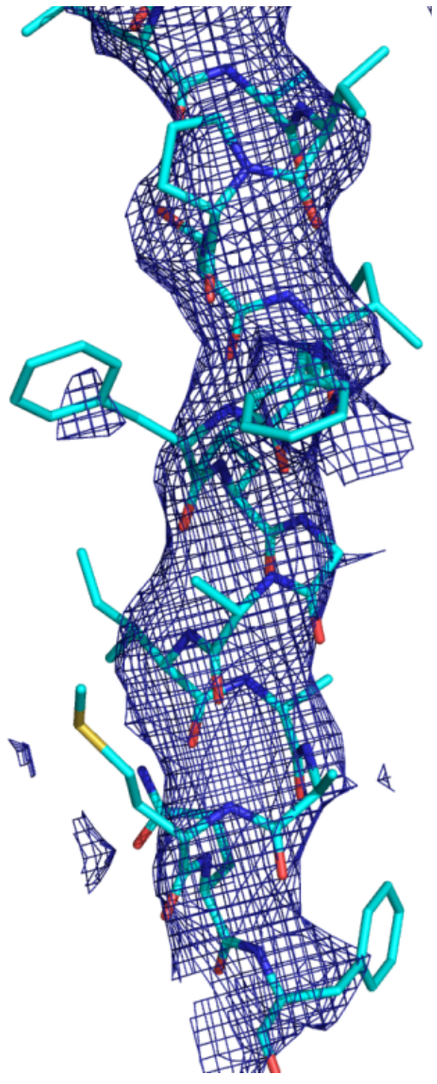
Electron density map including continuous diffraction



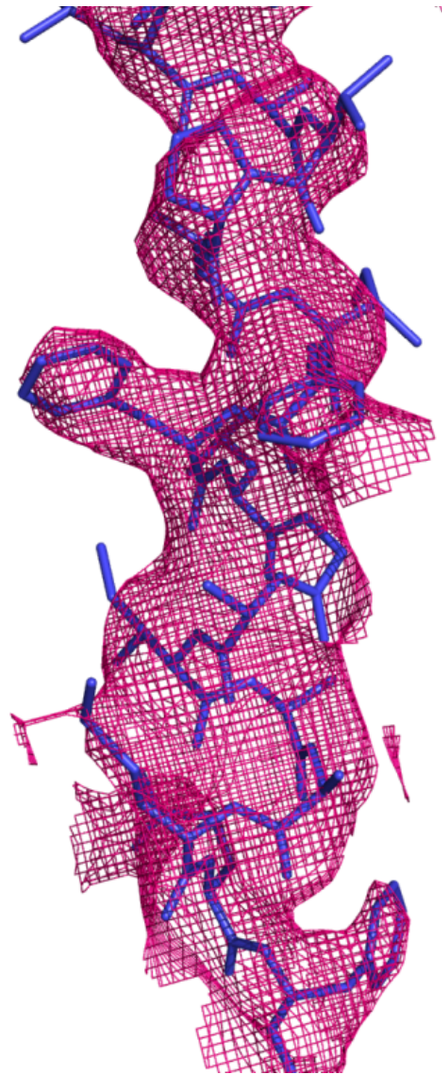
Kartik Ayyer
Dominik Oberthuer

The extended-resolution structure is superior

Bragg only
(4.5 Å)



Bragg and
continuous
(3.5 Å)



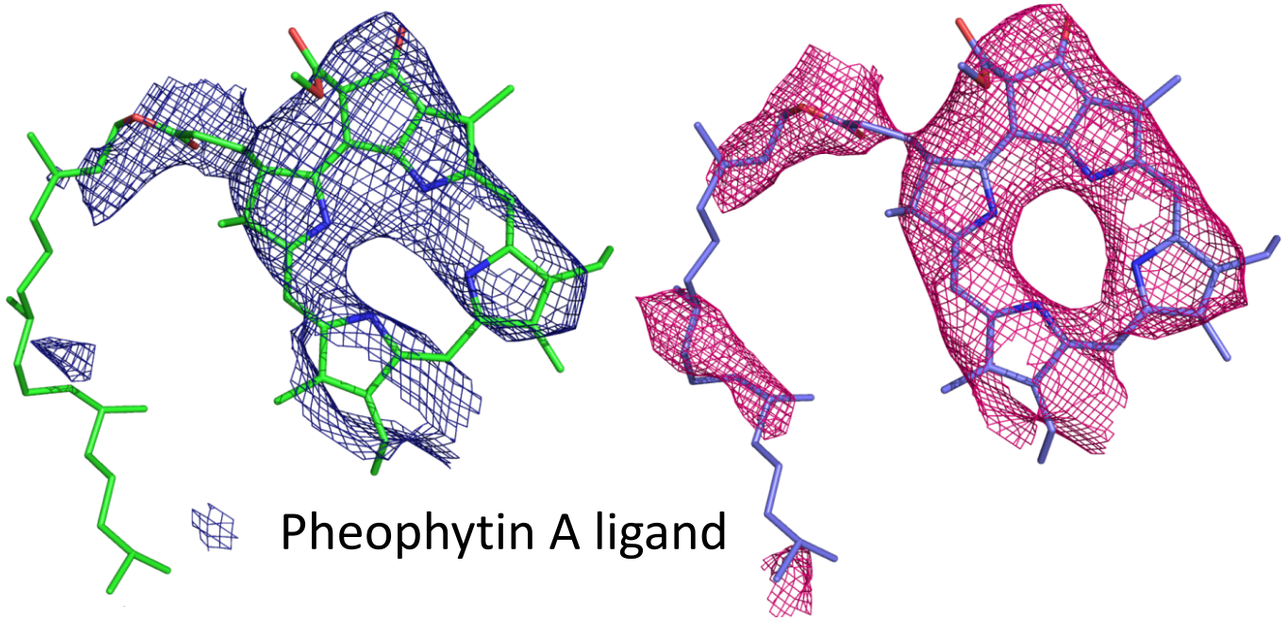
Higher diffraction sampling — model free phasing
— more reliable structure determination

Resolution not limited by the crystal, just detector extent and shots

Number of molecules per shot: 1 μm^3 x 4 / (9.2 x 10^6 $Å^3$) = 4 x10^5

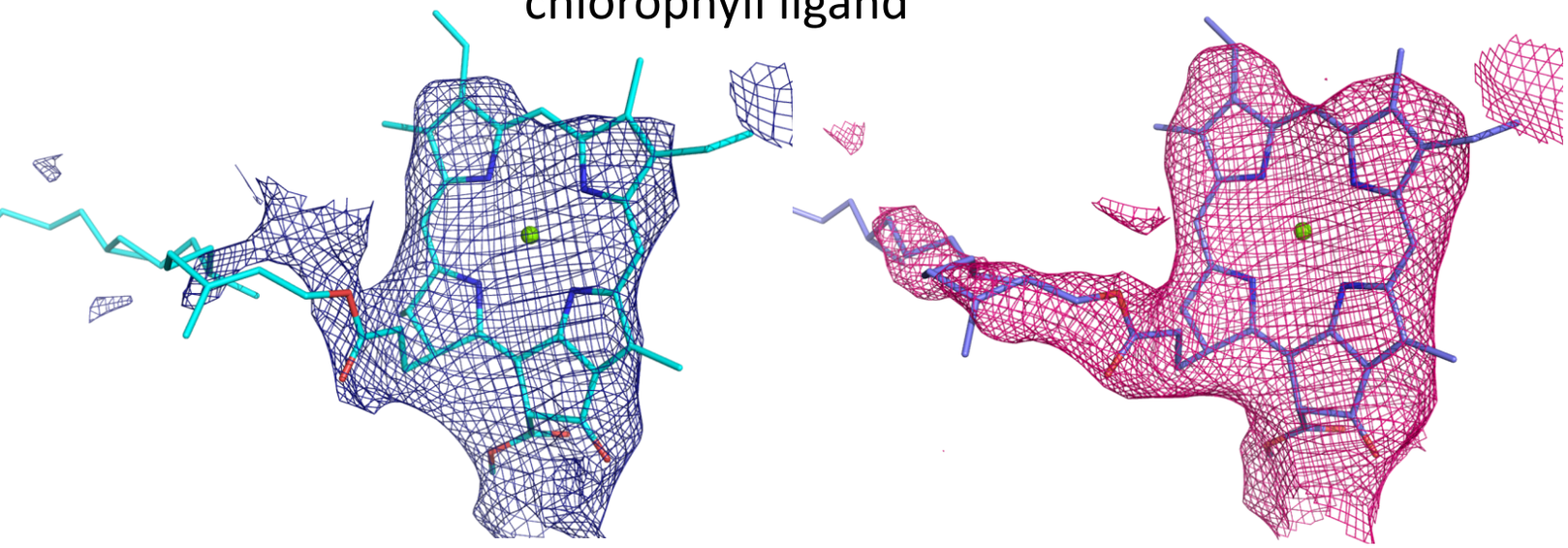
The extended-resolution structure is superior

Bragg only (4.5 Å)

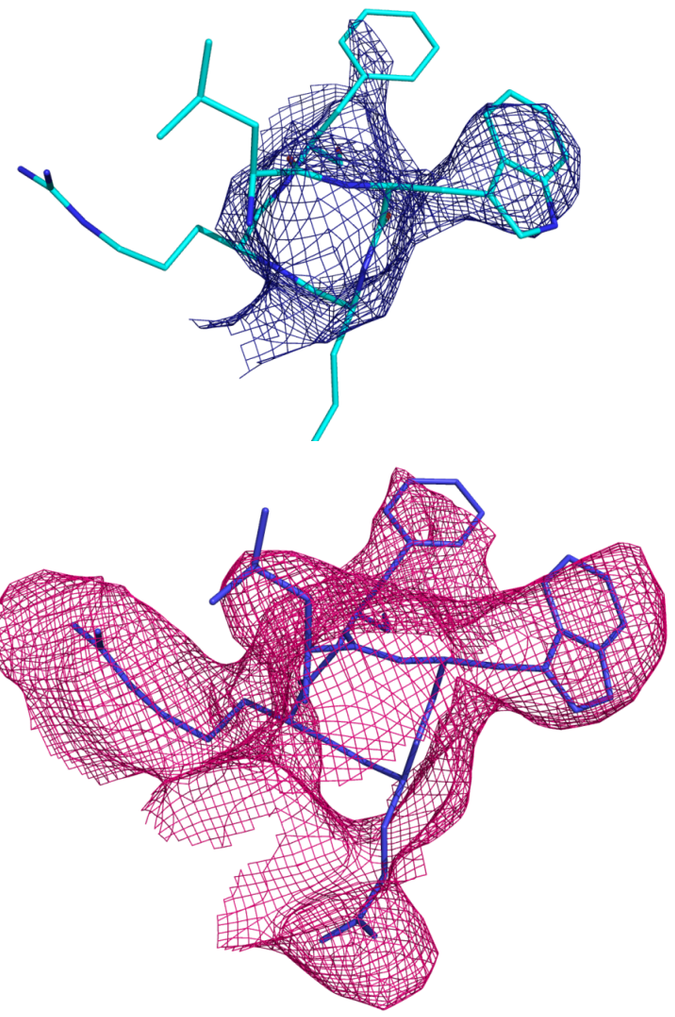


Pheophytin A ligand

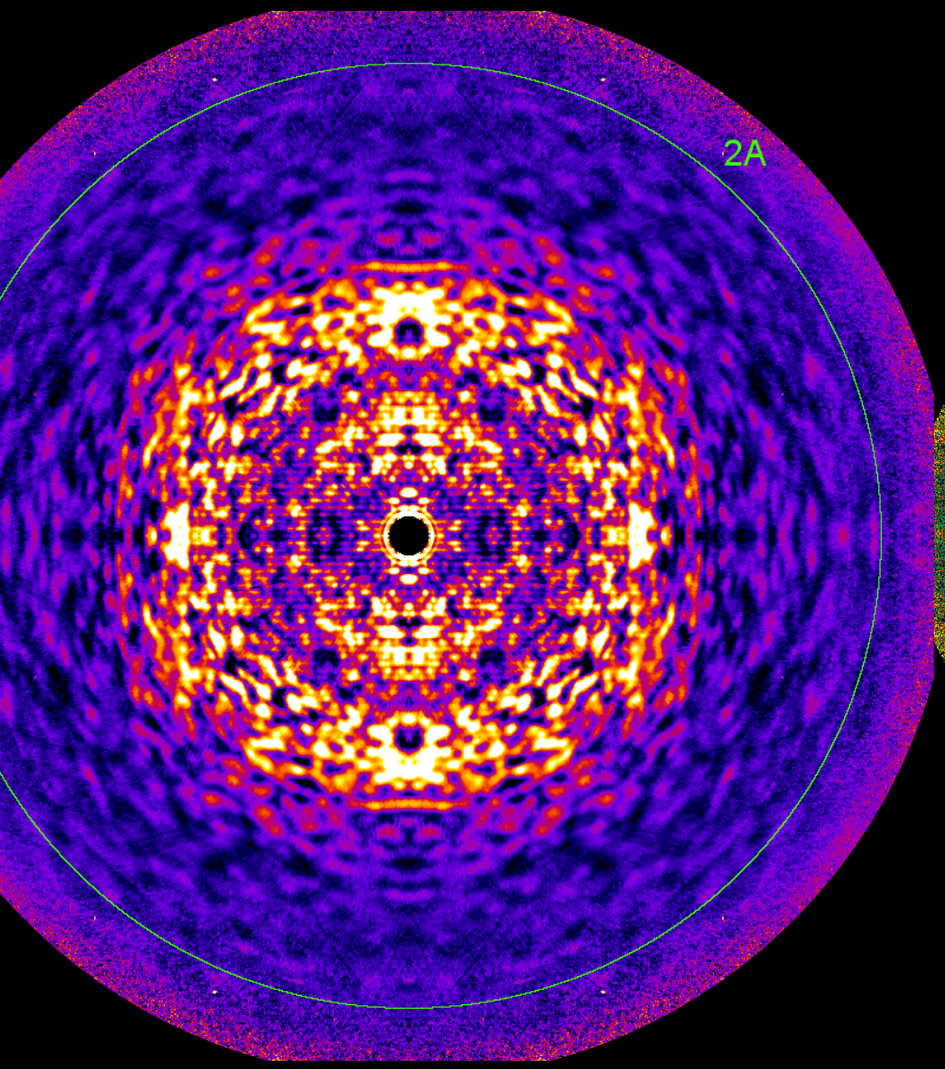
chlorophyll ligand

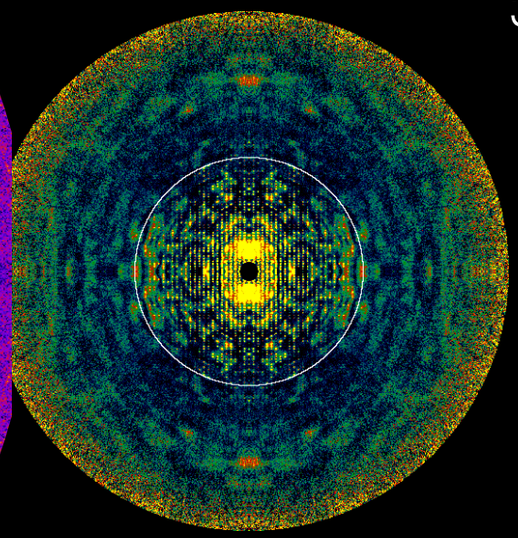


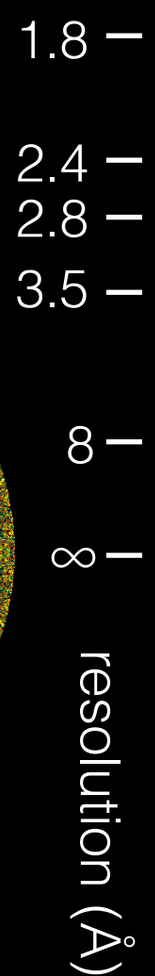
Bragg and continuous (3.5 Å)

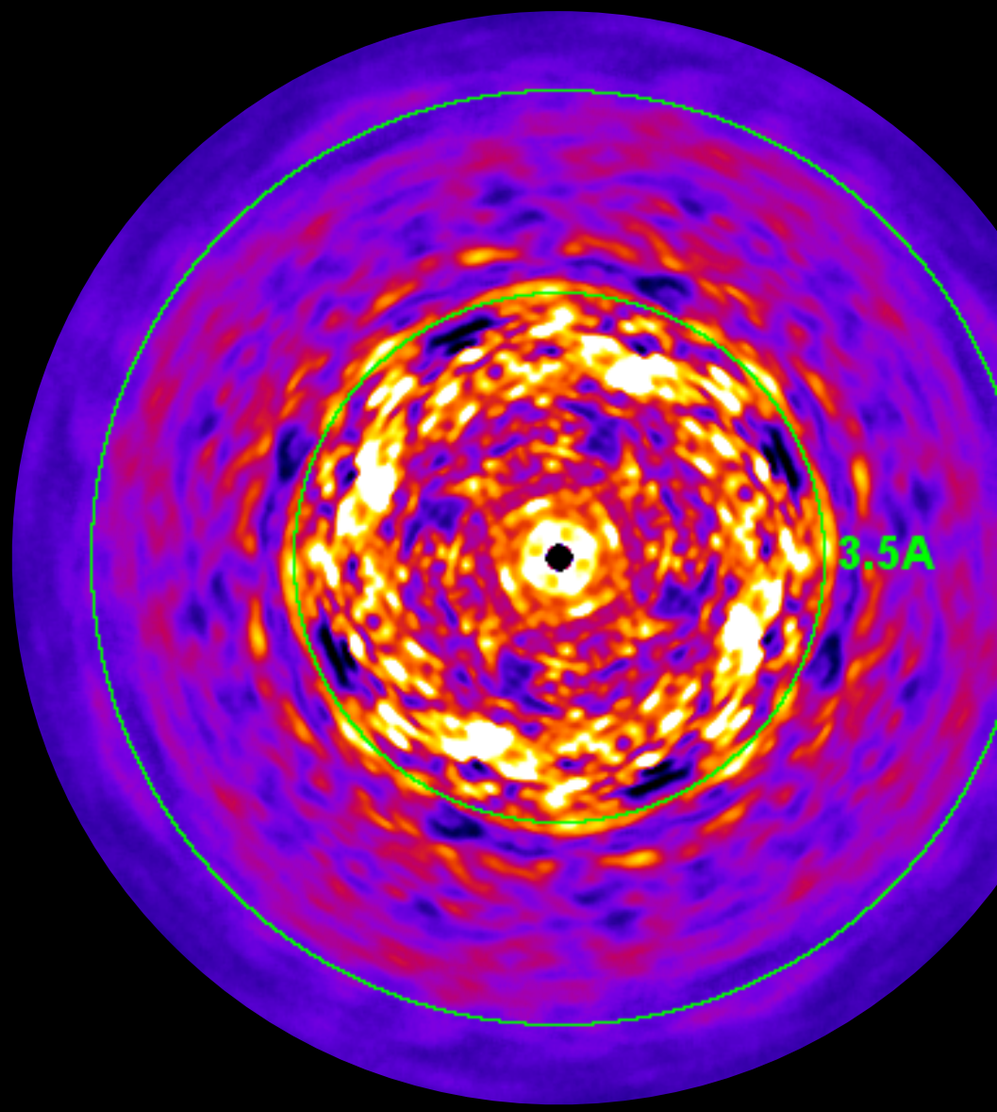


We observe continuous diffraction in other systems, and have extended observation of PS II to 1.9 Å







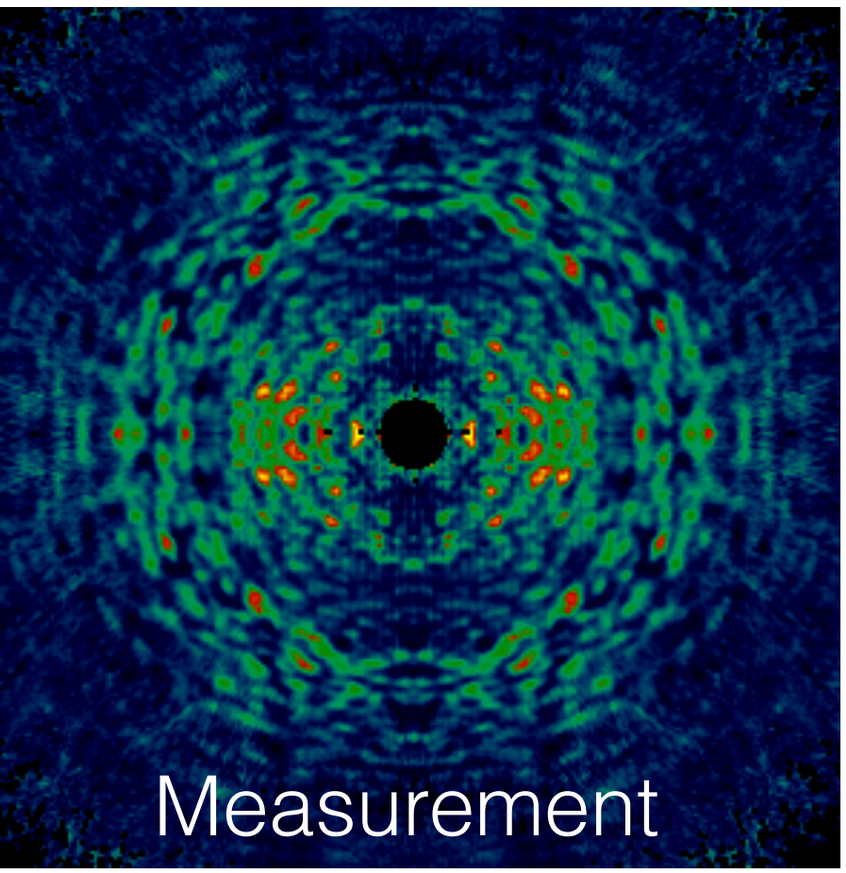


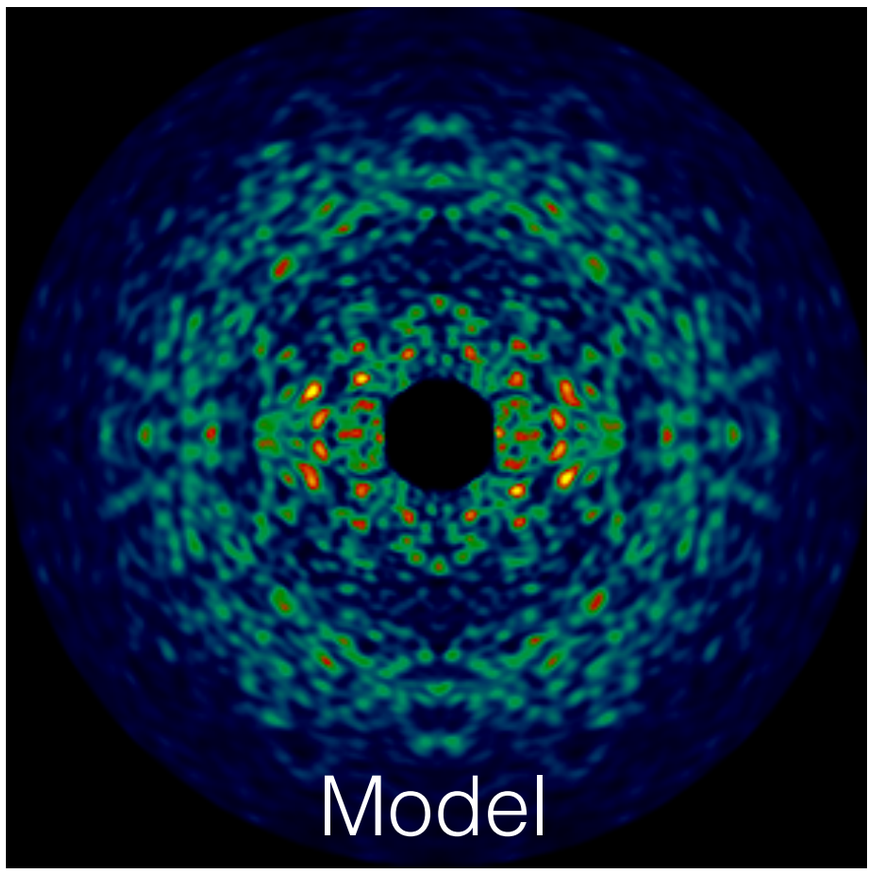
PS II

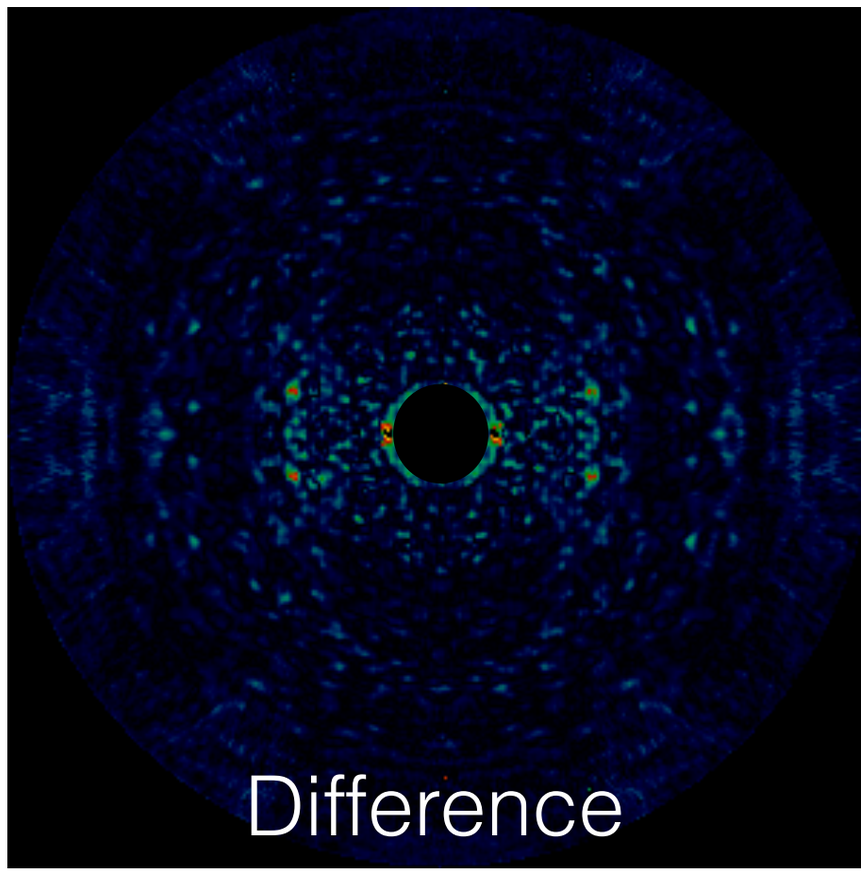
PS I:Fd

Aquaporin

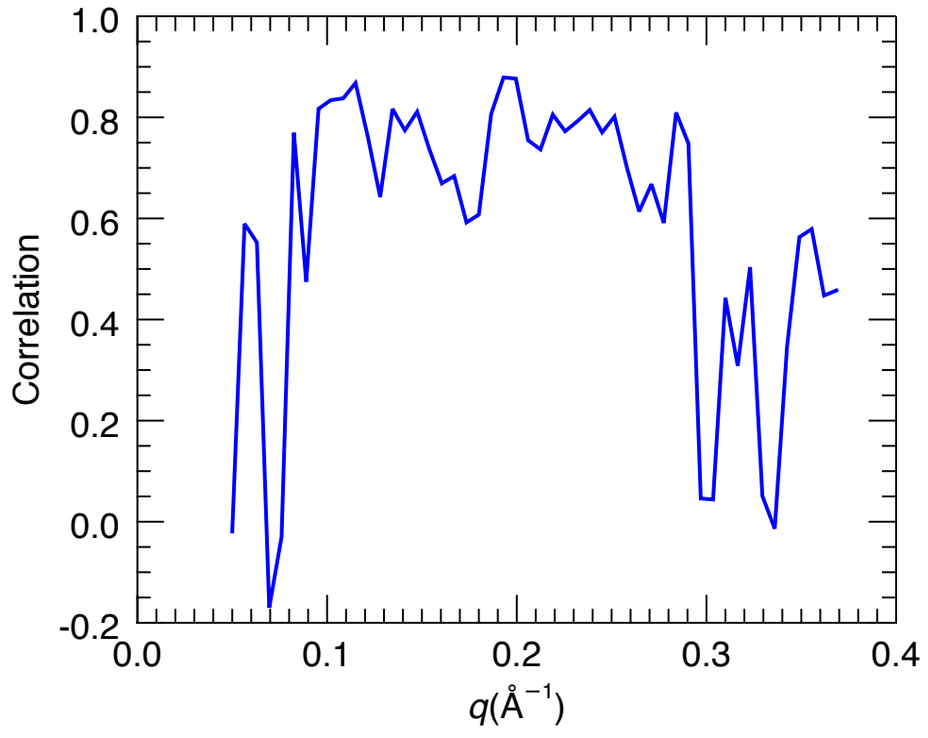
The continuous diffraction agrees with the simulated diffraction from the atomic model



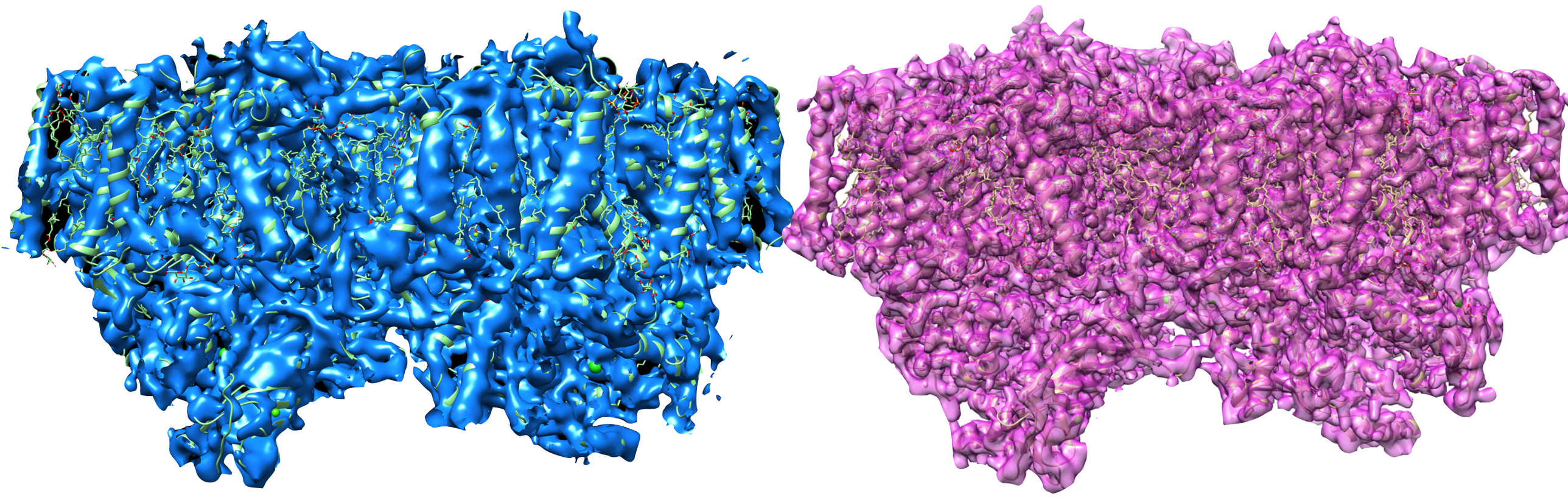




Cross Correlation = 75%



There are many opportunities for extending imaging concepts to X-ray diffraction at the atomic scale

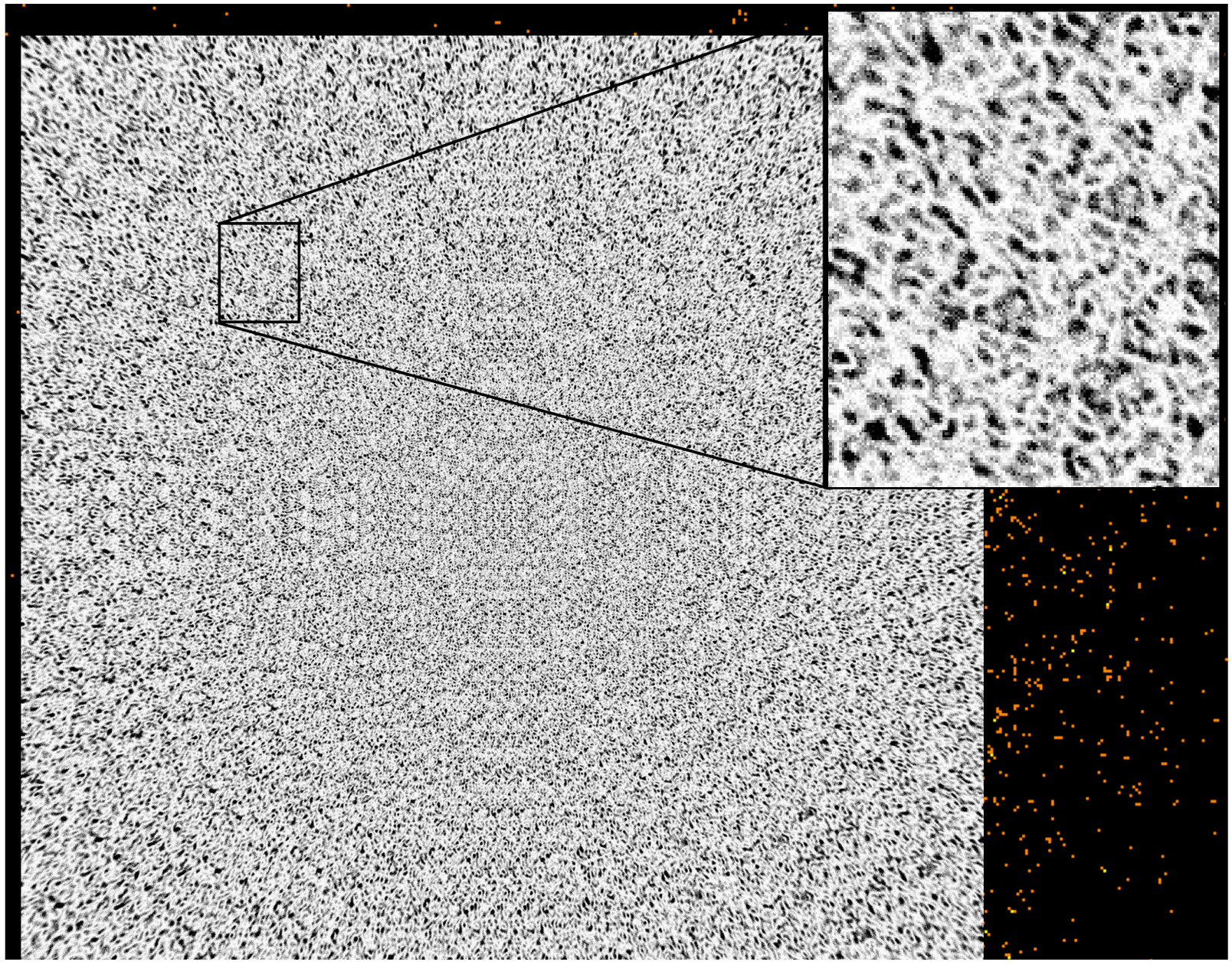


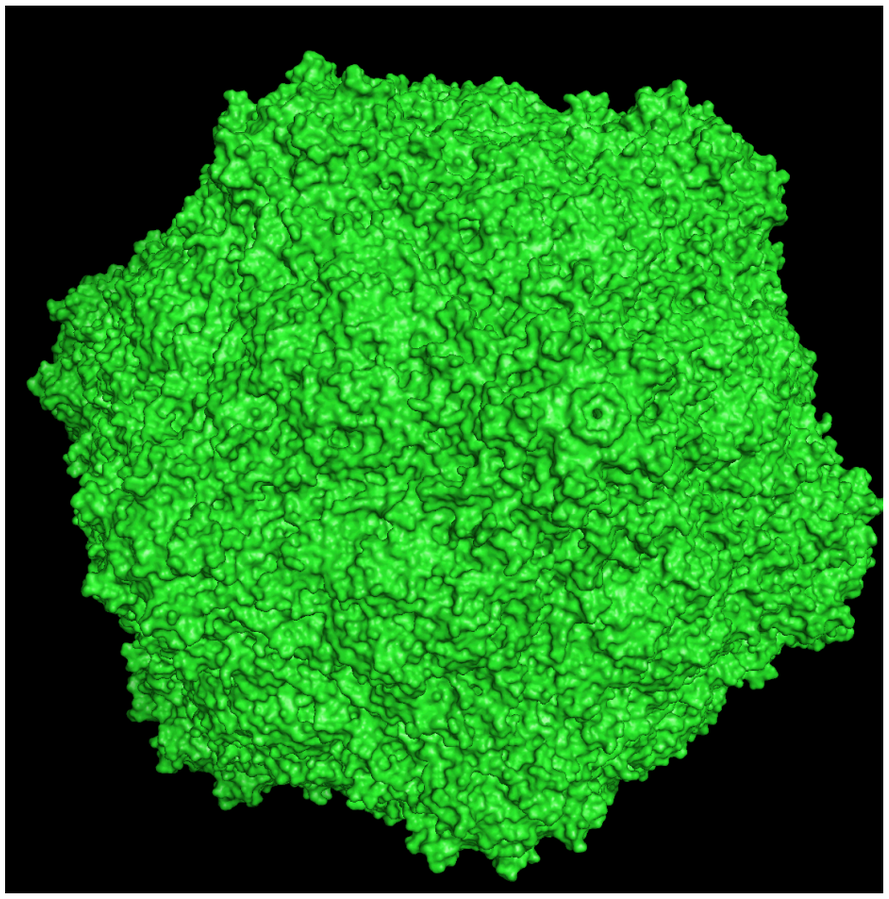
Measurements require care to eliminate background and record weak continuous diffraction

Poorly diffracting crystals are better!

- More information than required to describe the object
- model free phasing
- more reliable structure determination
- first new phasing since MAD
- resolution better than you think

Atomic-resolution diffraction from single particles requires focused intensities of more than 10^{14} ph/μm^2





A. Classen *et al* arXiv.org:abs/1705.08677

Measure interference of fluorescence during the coherence time

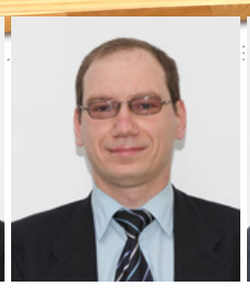
10^{14} ph/μm^2
60 GGy
6000 MGy/fs x 10 fs

RMS displacement: 0.5Å
half electrons ionized

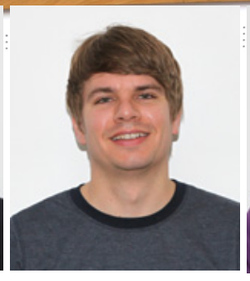
Coherent X-ray Imaging at CFEL

Kartik Ayyer

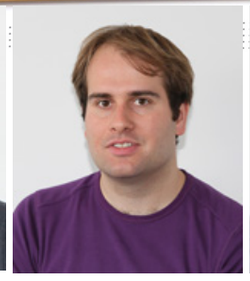
Anton Barty



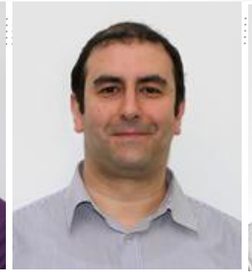
Oleksandr Yefanov



Dominik Oberthür



Tom White



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Lorenzo Galli

Kanupriya Pande

Andrew Morgan

Funding:

DESY

HELMHOLTZ ASSOCIATION

erc European Research Council Established by the European Commission

Bundesministerium für Bildung und Forschung

DFG

CUI THE HAMBURG CENTER FOR ULTRAFAST IMAGING

LCLS experiments and analyses are carried out as a large collaboration

CFEL-DESY/UHH	A. Barty, T. White, S. Stern, C. Caleman, K. Beyerlein, R. Bean, R. Kirian, F. Wang, H. Fleckenstein, L. Gumprecht, L. Galli, S. Bajt, M. Barthelmess, O. Yefanov, D. Oberthür, C. Gati, M. Heymann, C. Seuring, M. Metz, A. Morgan, V. Mariani, A. Tolstikova, J. Knoska, X. L. Pauraj, K. Ayyer
ASU	J. Spence, P. Fromme, U. Weierstall, B. Doak, X. Wang, I. Grotjohann, R. Fromme, N. Zatsepin, D. Wang, D. James, S. Basu, C. Kupitz, J. Coe, C. Conrad, K. Dörner, D. James, G. Nelson
MPG Med. Res.	I. Schlichting, R. Shoeman, L. Lomb, S. Kassemeyer, K. Nass, T. Barends, S. Botha
SLAC	S. Boutet, M. Liang, A. Aquila, G. Williams, C. Bostedt, J. Koglin, M. Messerschmidt, and many others
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Gotheburg	R. Neutze, L. Johansson, D. Arnlund
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U. Hamburg	C. Betzel, D. Redhers, D. Oberthür, M. Perbandt
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U. Milwalkee Wisconsin	M. Schmidt, J. Tenboer, K. Pande

Funding:

DESY

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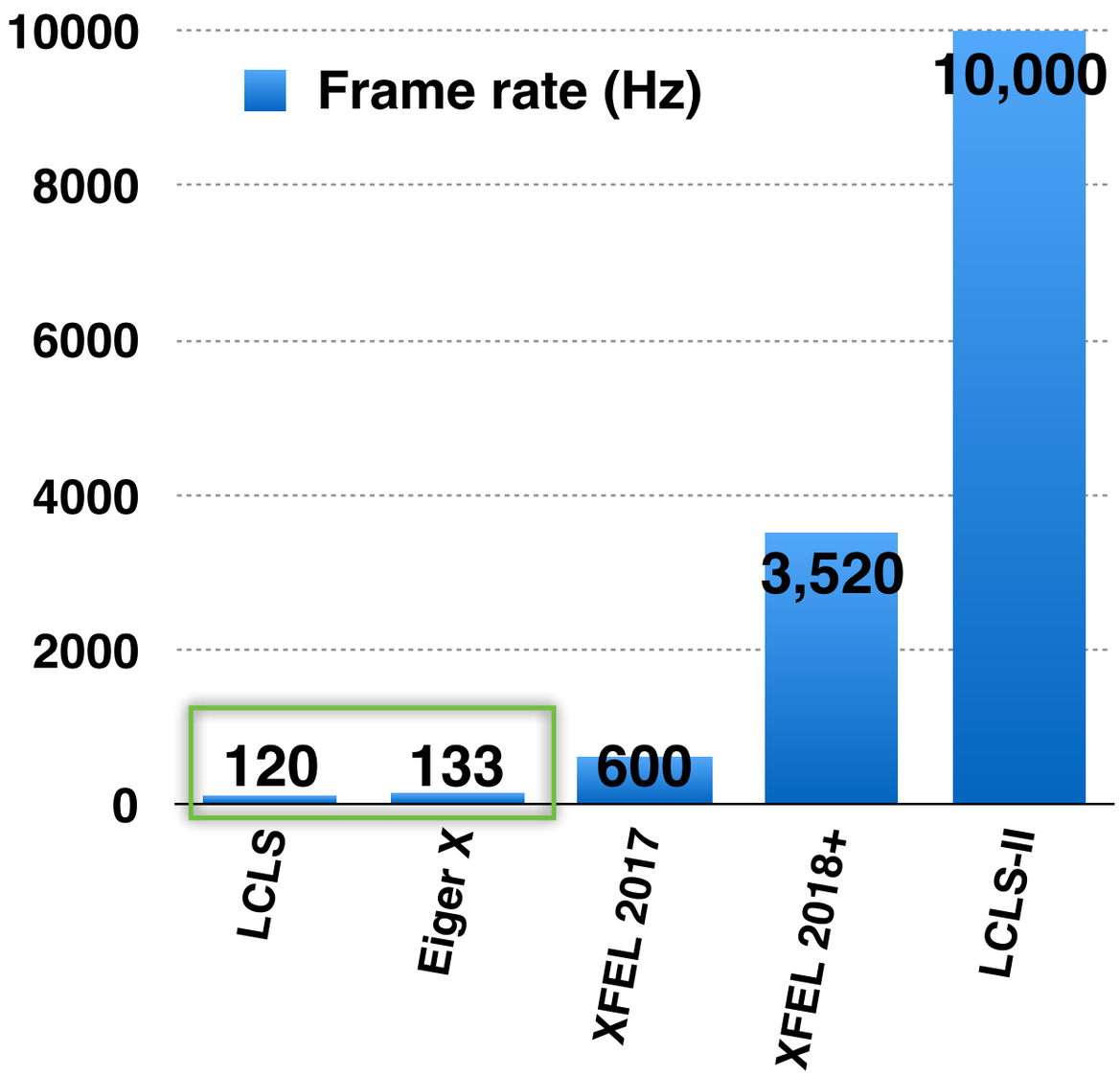
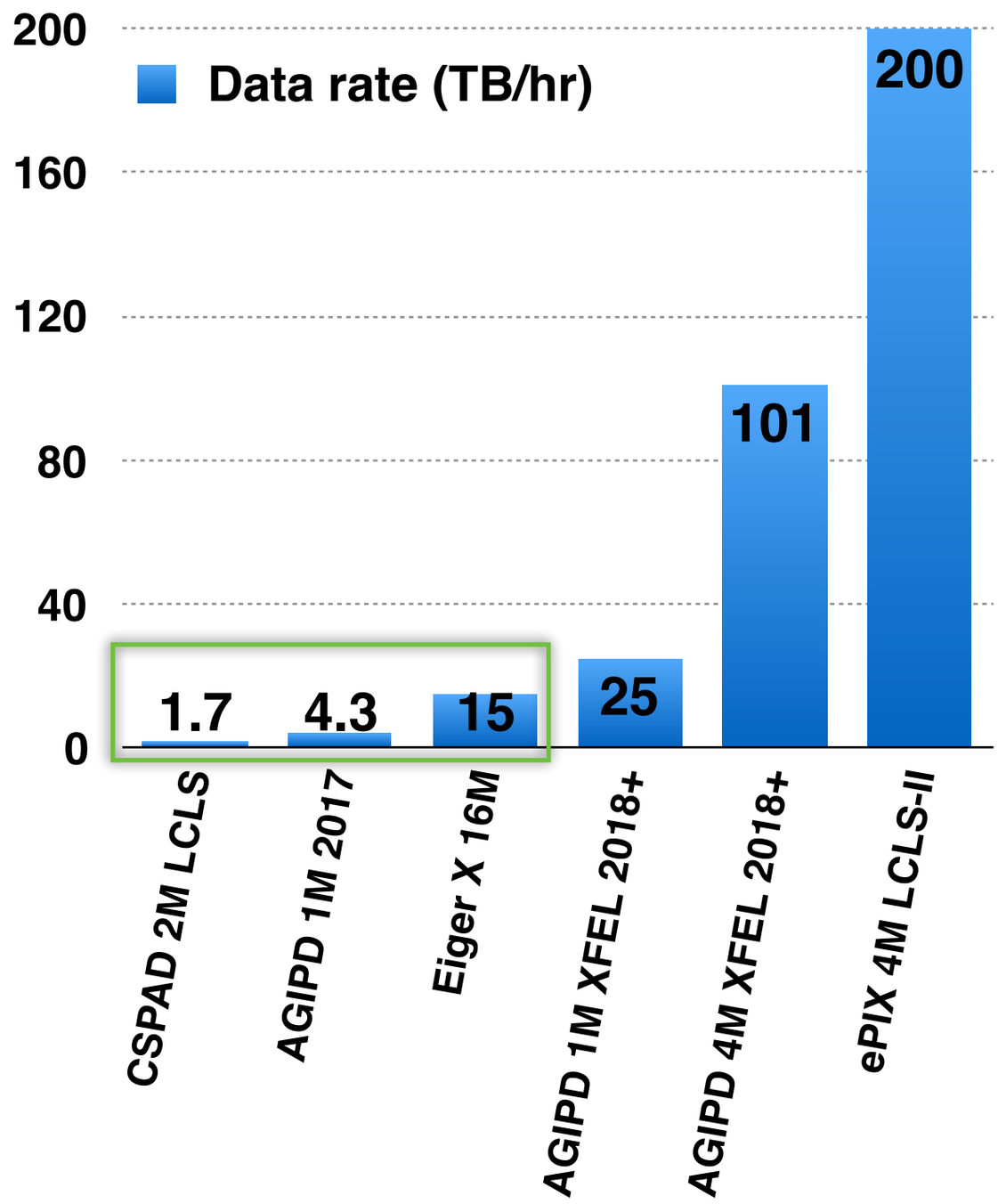
New sources and detectors are producing an explosion in experimental data volumes

1 kHz = 3.6M frames per hour



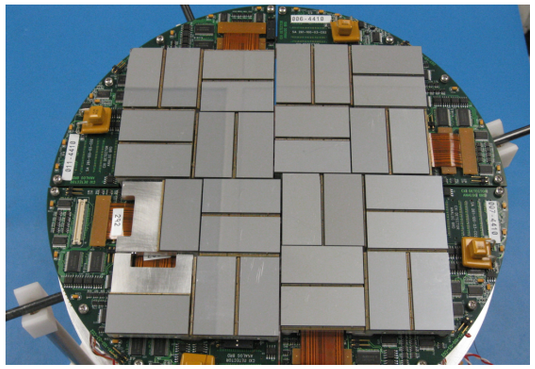
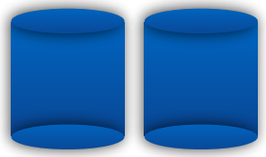
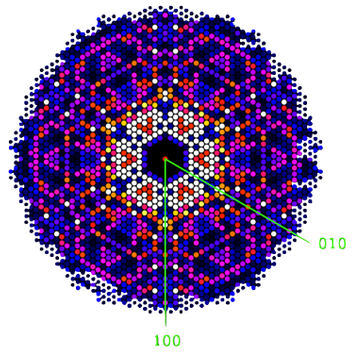
1 MPix at 1 kHz = 2000 MB/sec (16 bit)
~ 6.8 TB per hour



Anton Barty, CFEL

LCLS-II estimates a cost of over $250M to save all data, $35M to save two weeks' worth

Data processing is an exercise in data management and reduction

Detector	Storage	Offline reduction	Analysis
			
120 Hz 4 MB/event	Store all data (no corrections)	Retain only 'hits' (detector corrected)	Index and integrate
	> 4,000,000 frames	~ 400,000 hits	~ 300,000 indexed
1.8 TB/hr	50 TB / experiment	1-4 TB / experiment	1-50 GB / experiment

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Automated high throughput data processing is essential