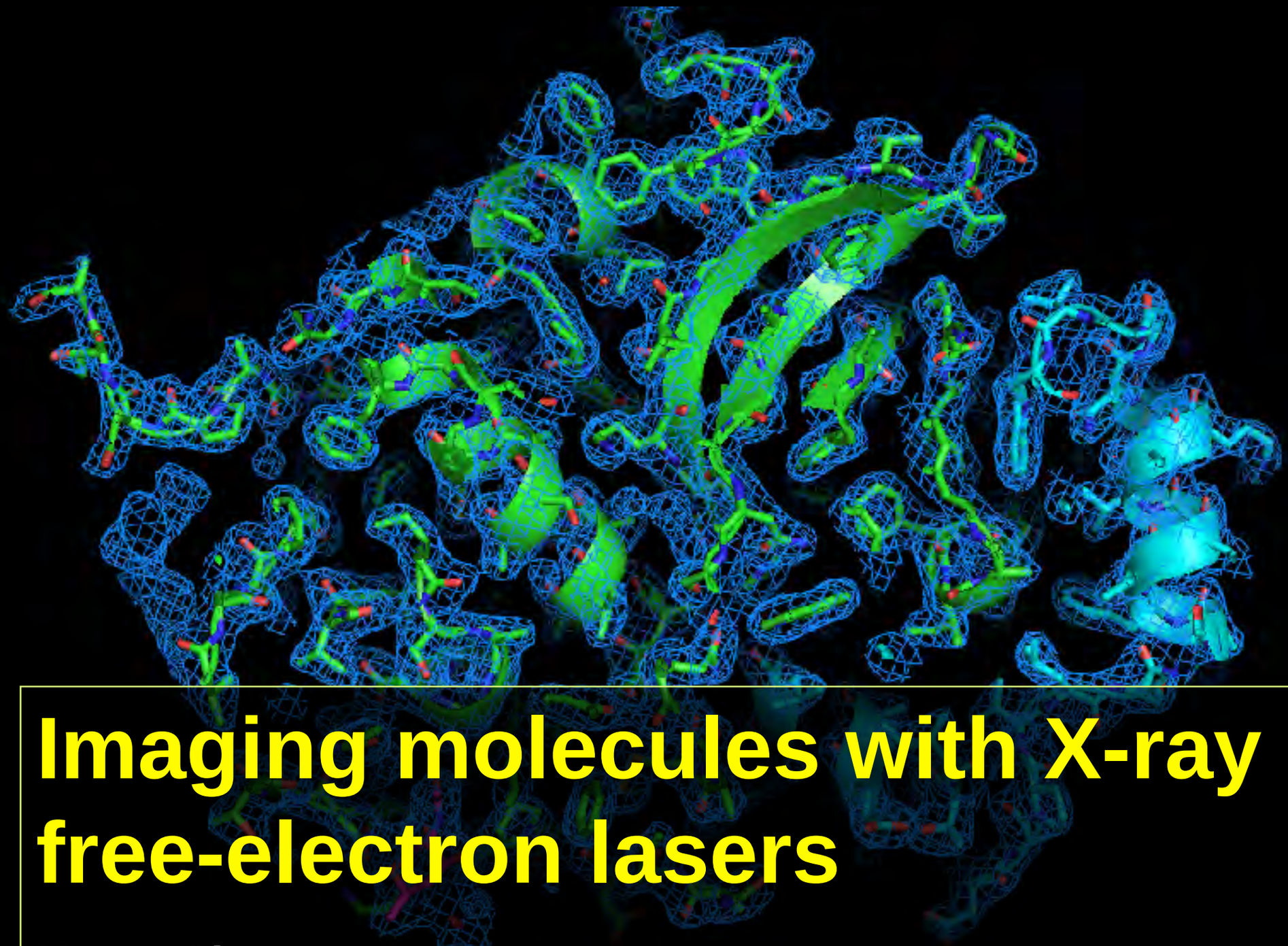
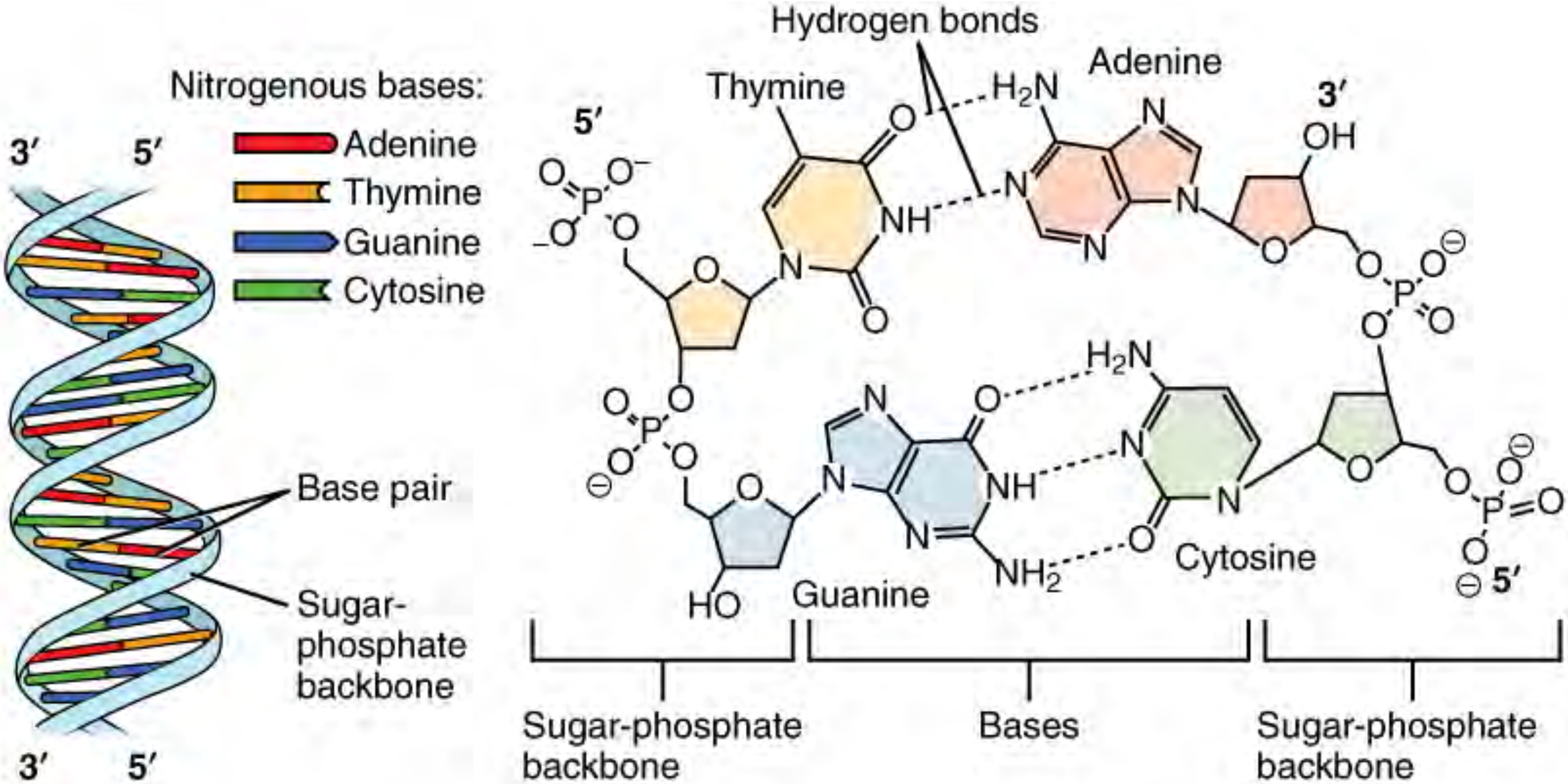


Imaging molecules with X-ray free-electron lasers

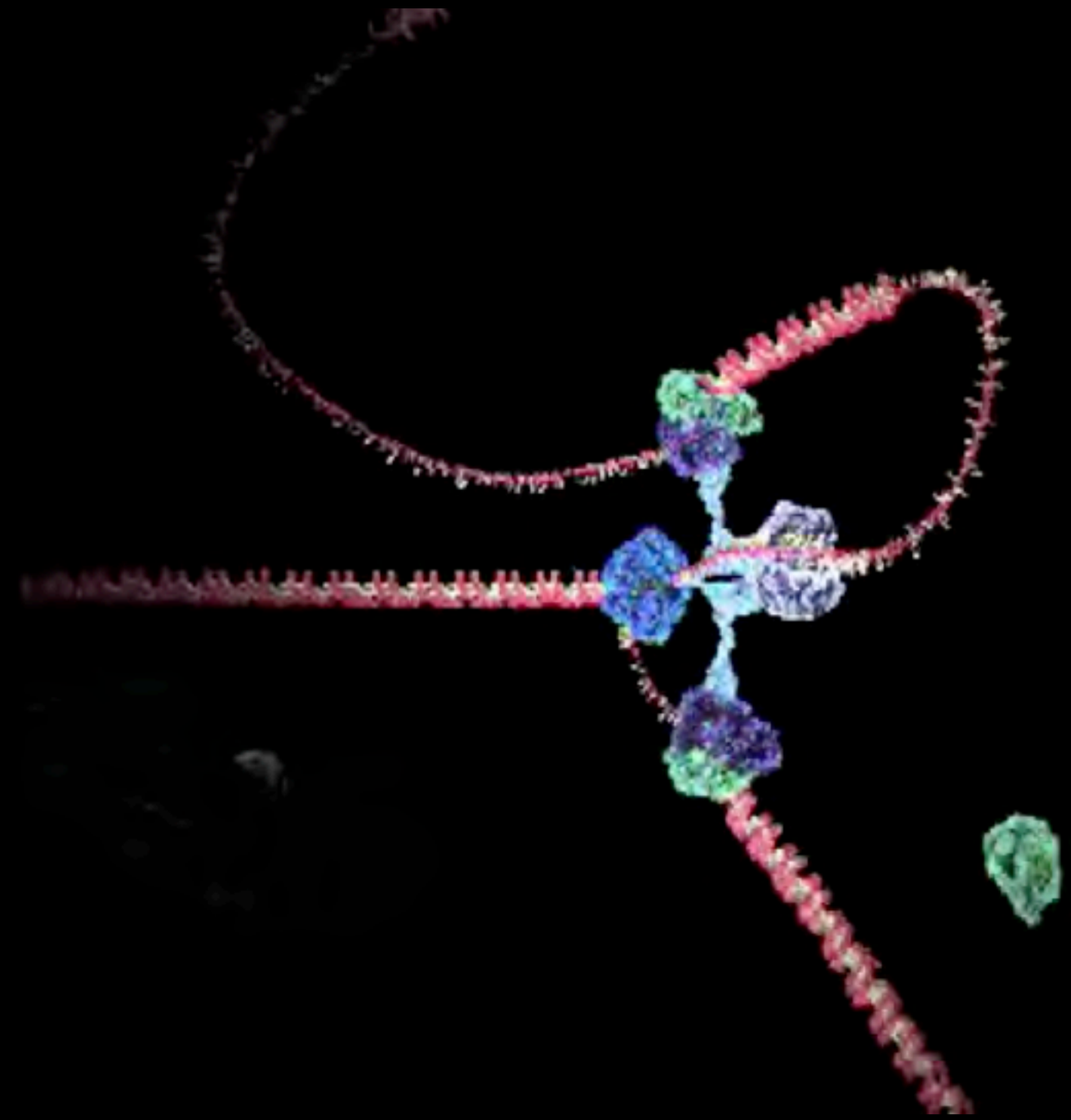
Henry Chapman
Center for Free-Electron Laser Science
DESY and University of Hamburg

DESY Physics Seminar, May 2017



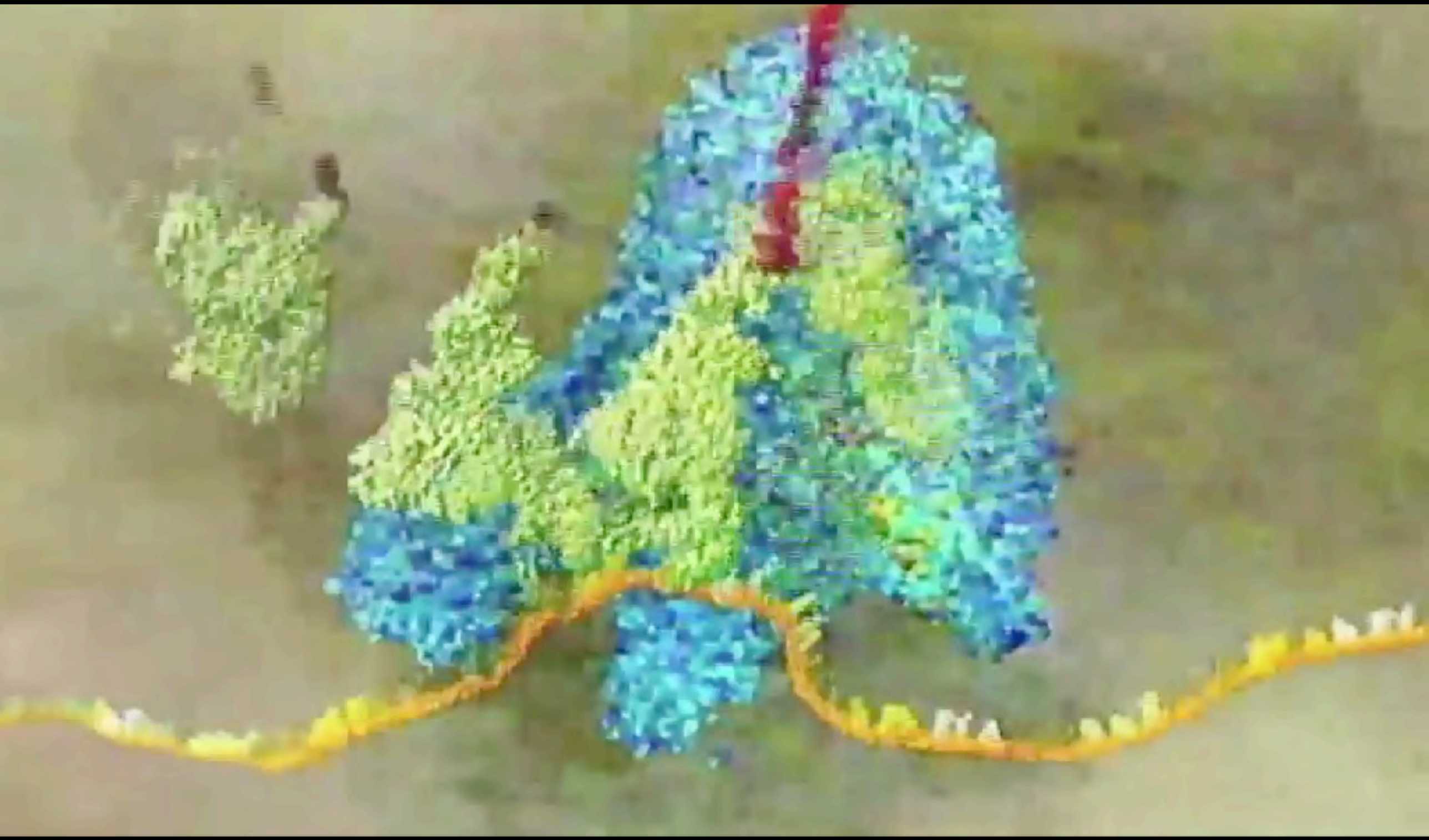
OpenStax Anatomy and Physiology

wehi.edu.au



Walter & Eliza Hall Institute

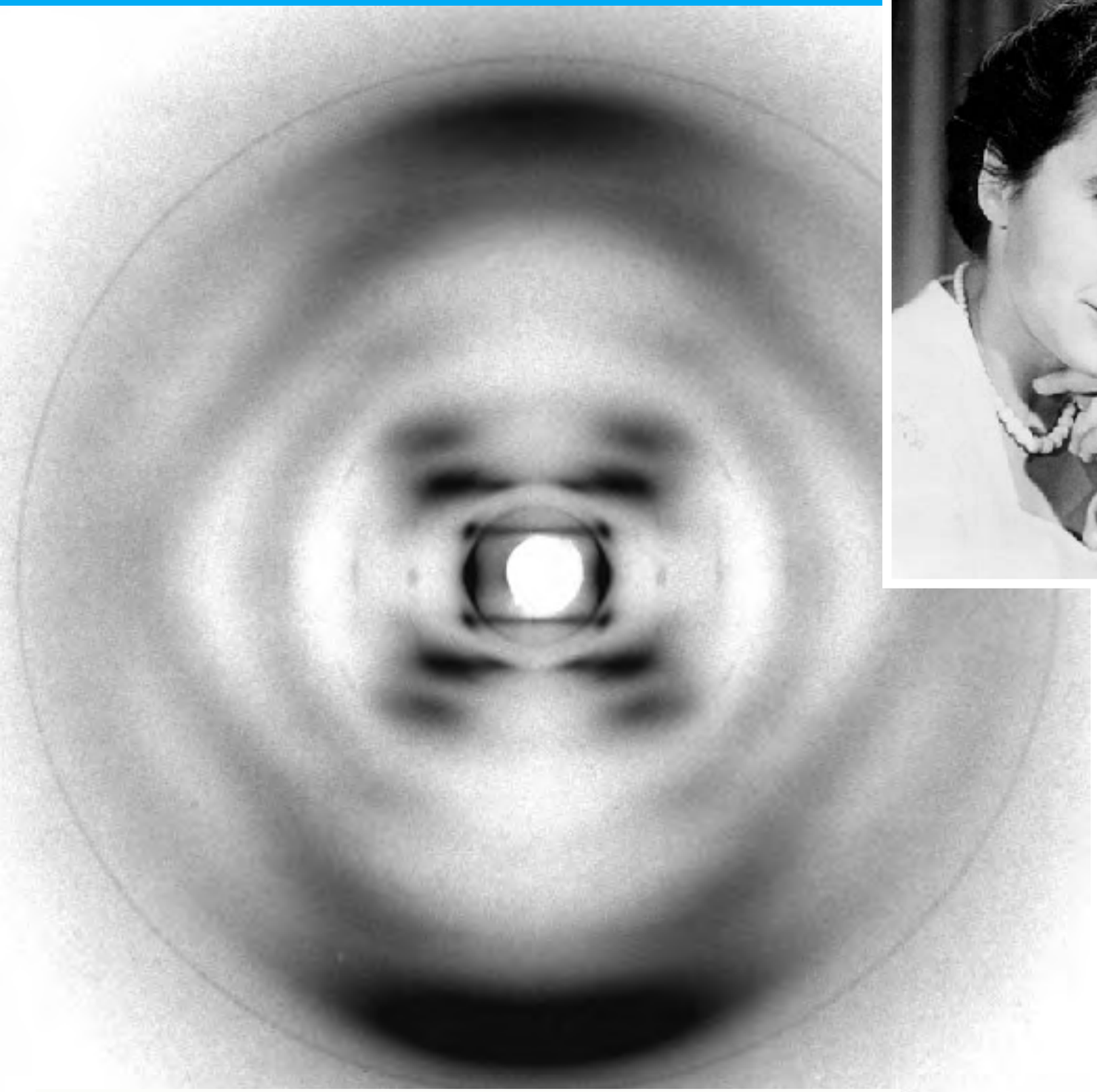
Walter & Eliza Hall Institute



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.

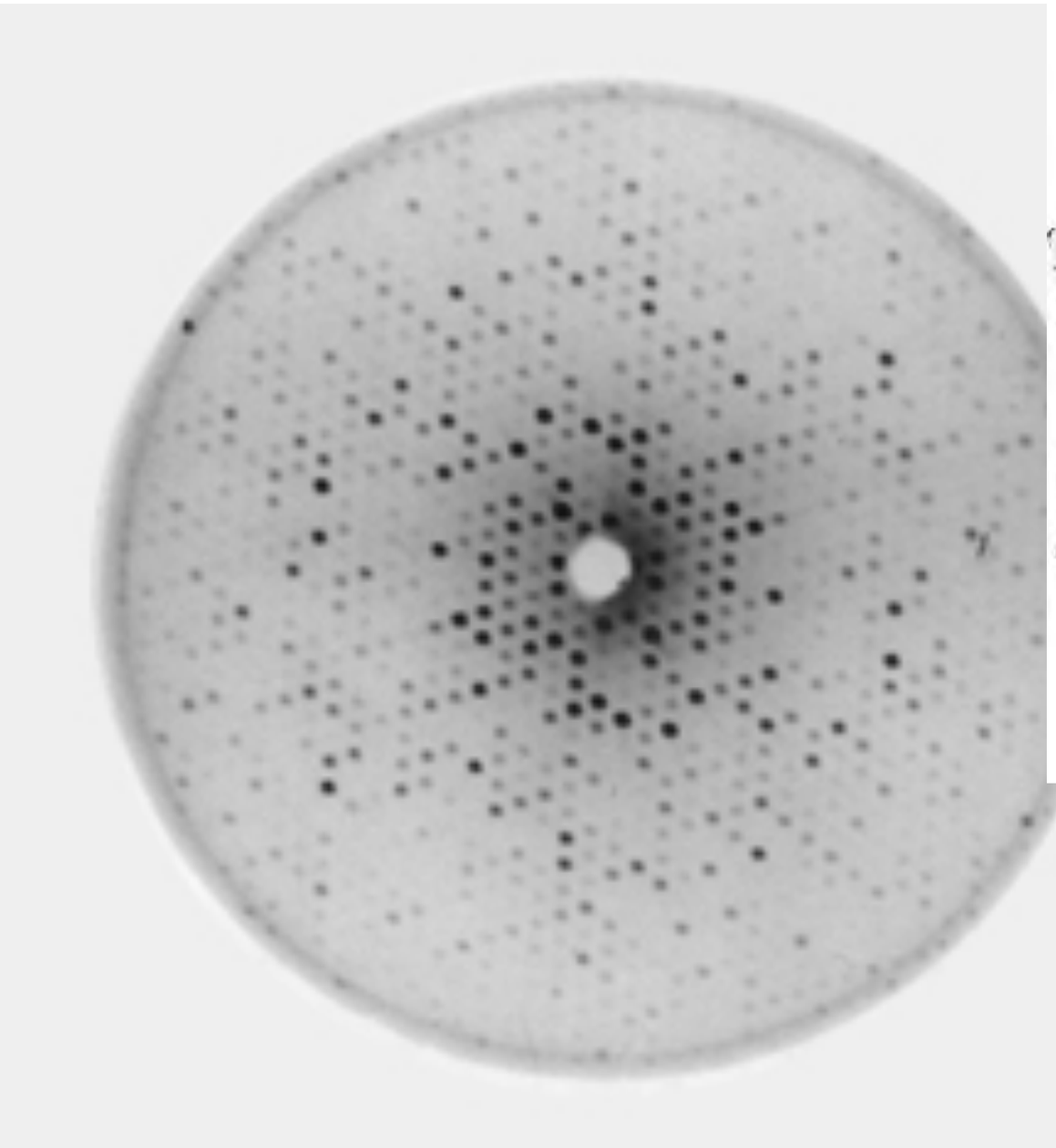
Wellcome Images

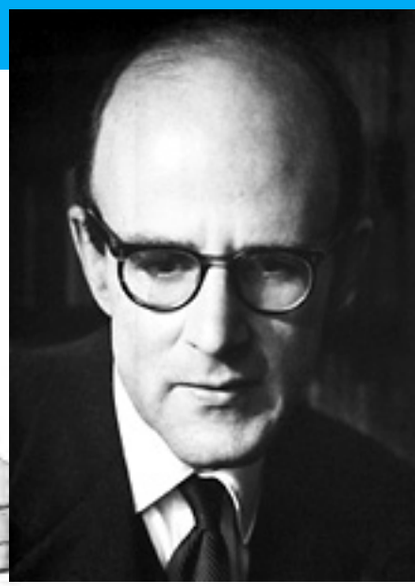
Rosalind Franklin

James Watson
& Francis Crick

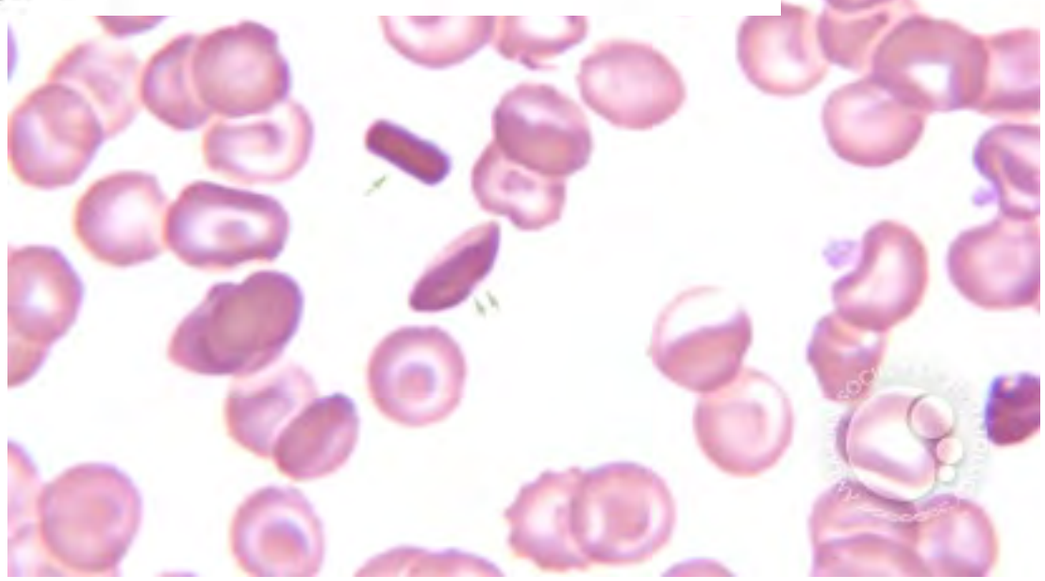
http://dataphys.org/list/watson-and-cricks-3d-model-of-dna/

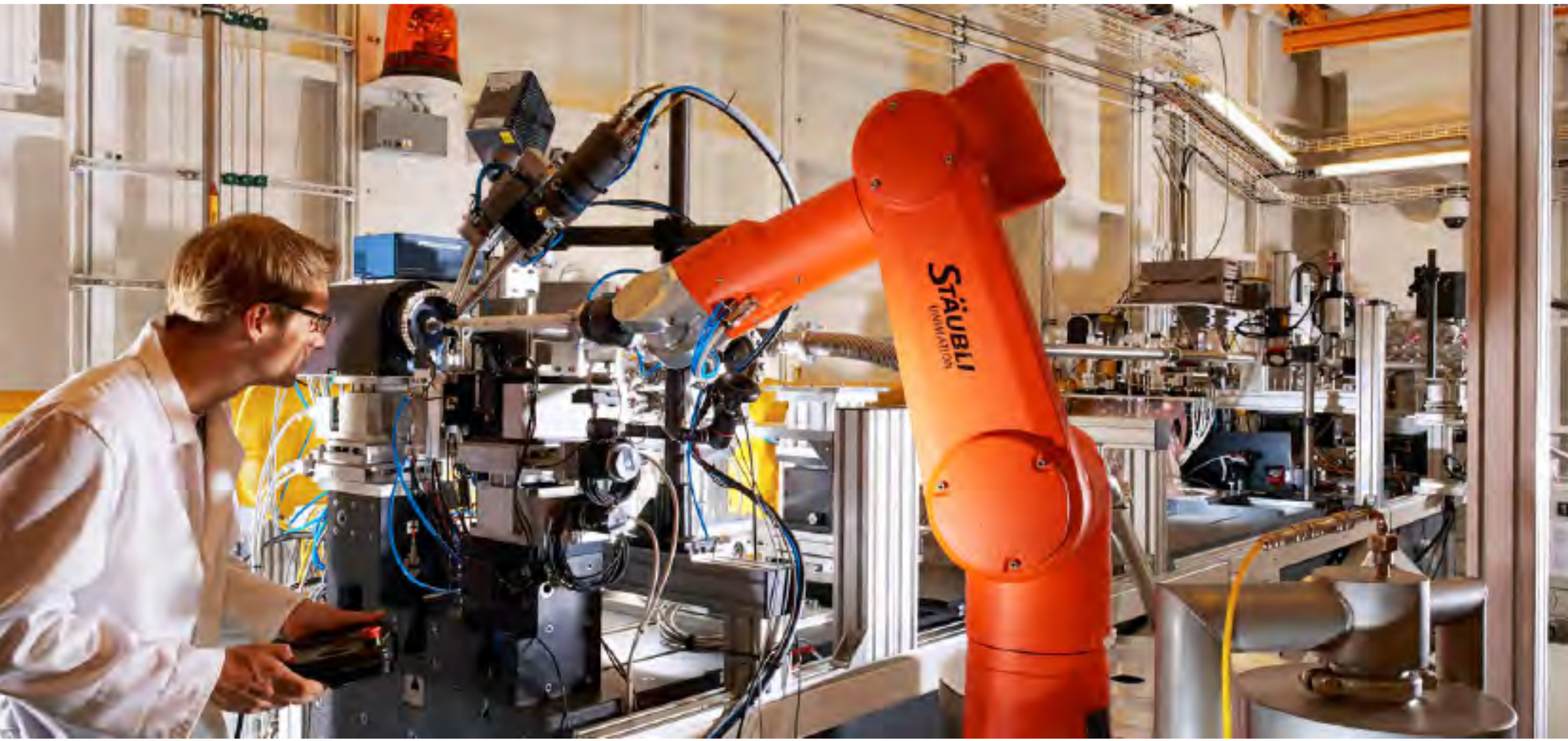
The first protein structure to be determined was haemoglobin, in 1959





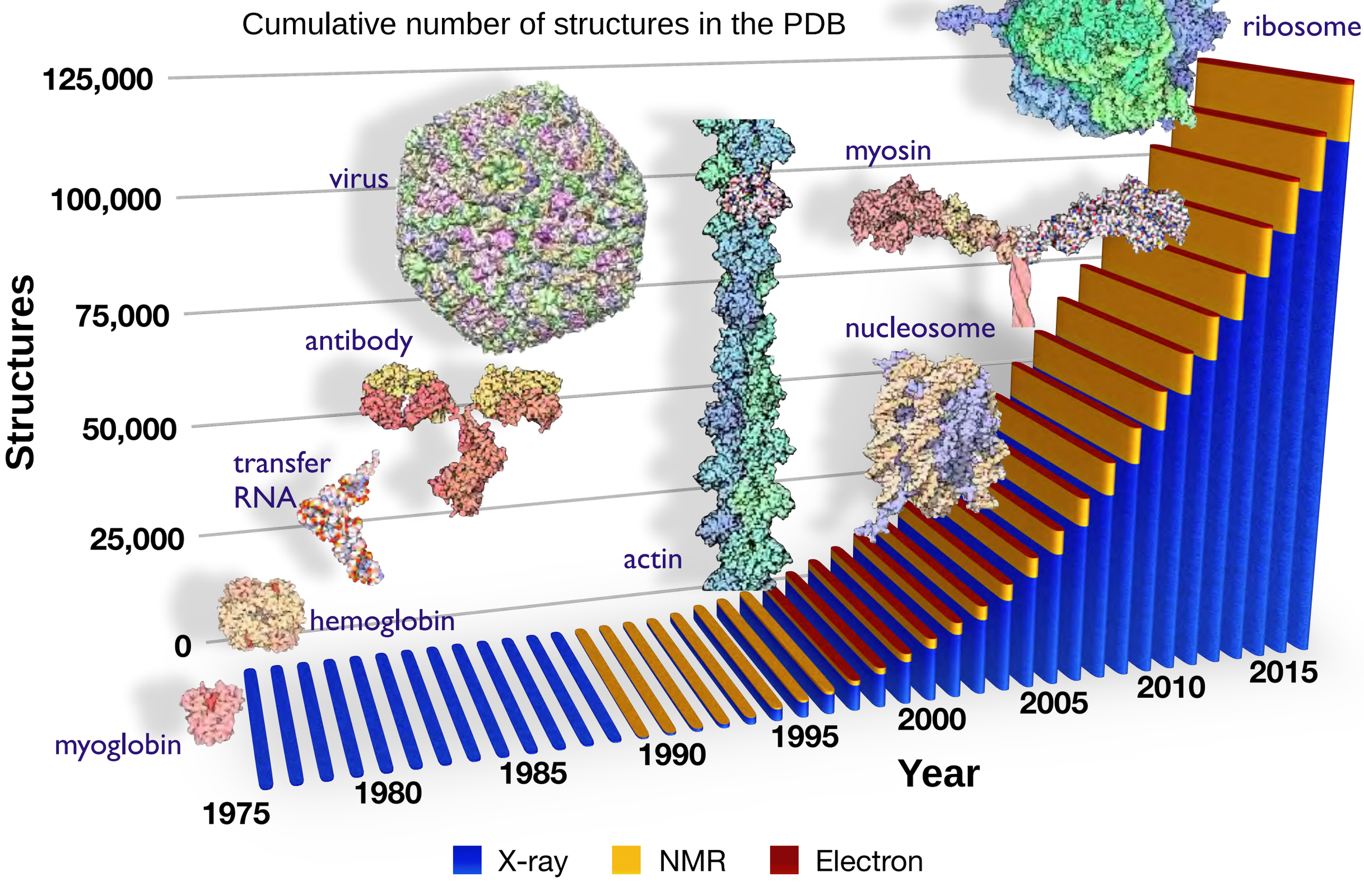
Max Perutz



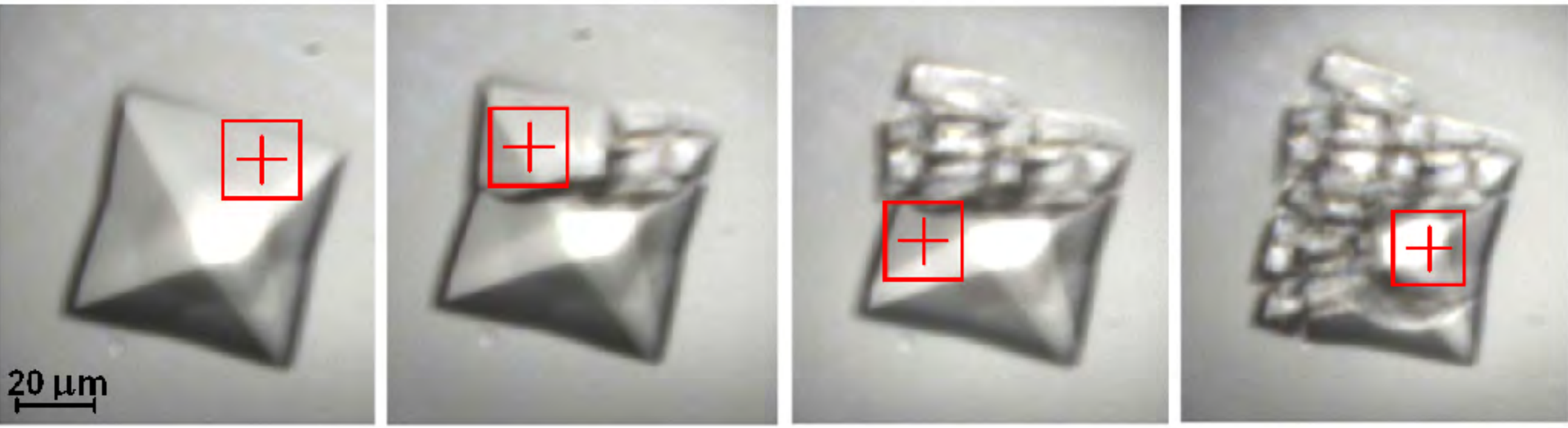

STÄUBLI
UNIMATION

PETRA III.
Max von Laue

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



High radiation dose causes changes in molecular structure

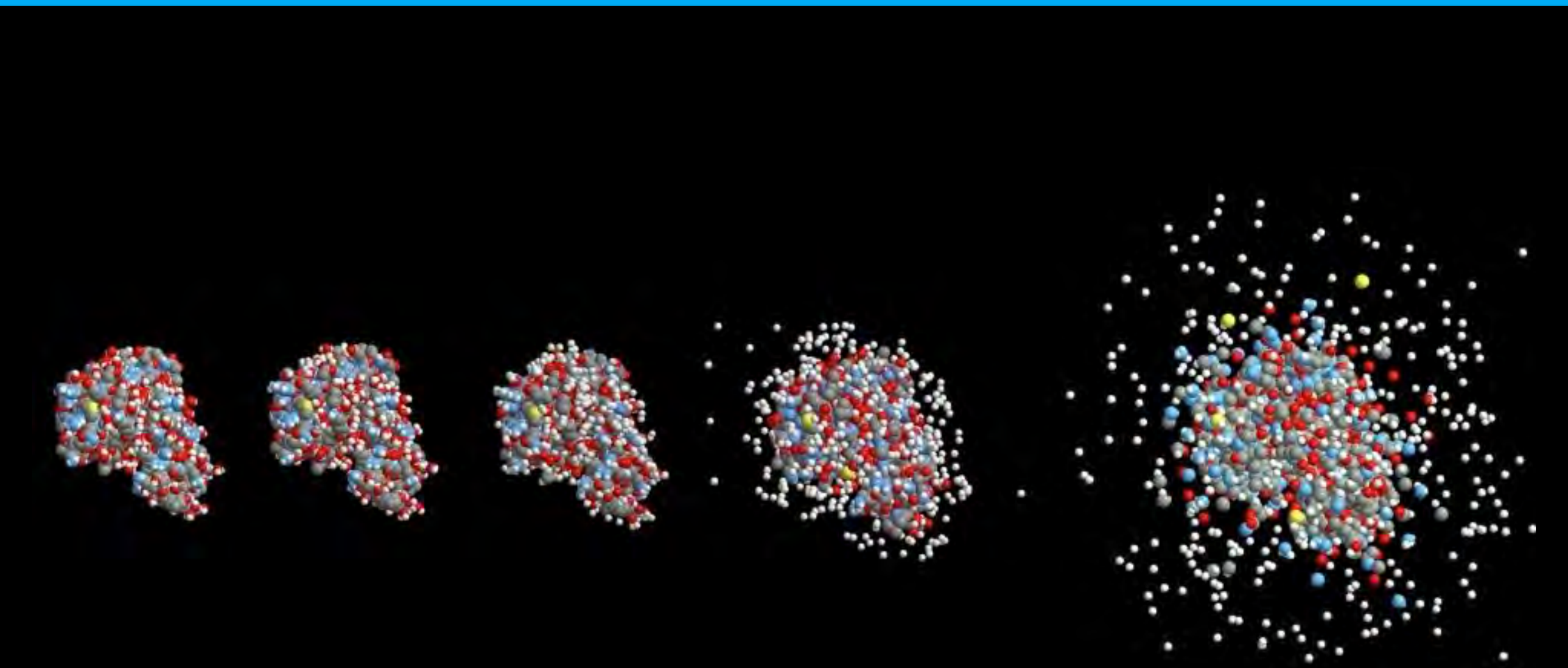


Crystal of Bovine enterovirus 2 (BEV2) after subsequent exposures of 0.5 s, 6 x 10^8 ph/µm^2
300 kGy dose
Room temperature

Cryogenic cooling gives 30 MGy tolerance

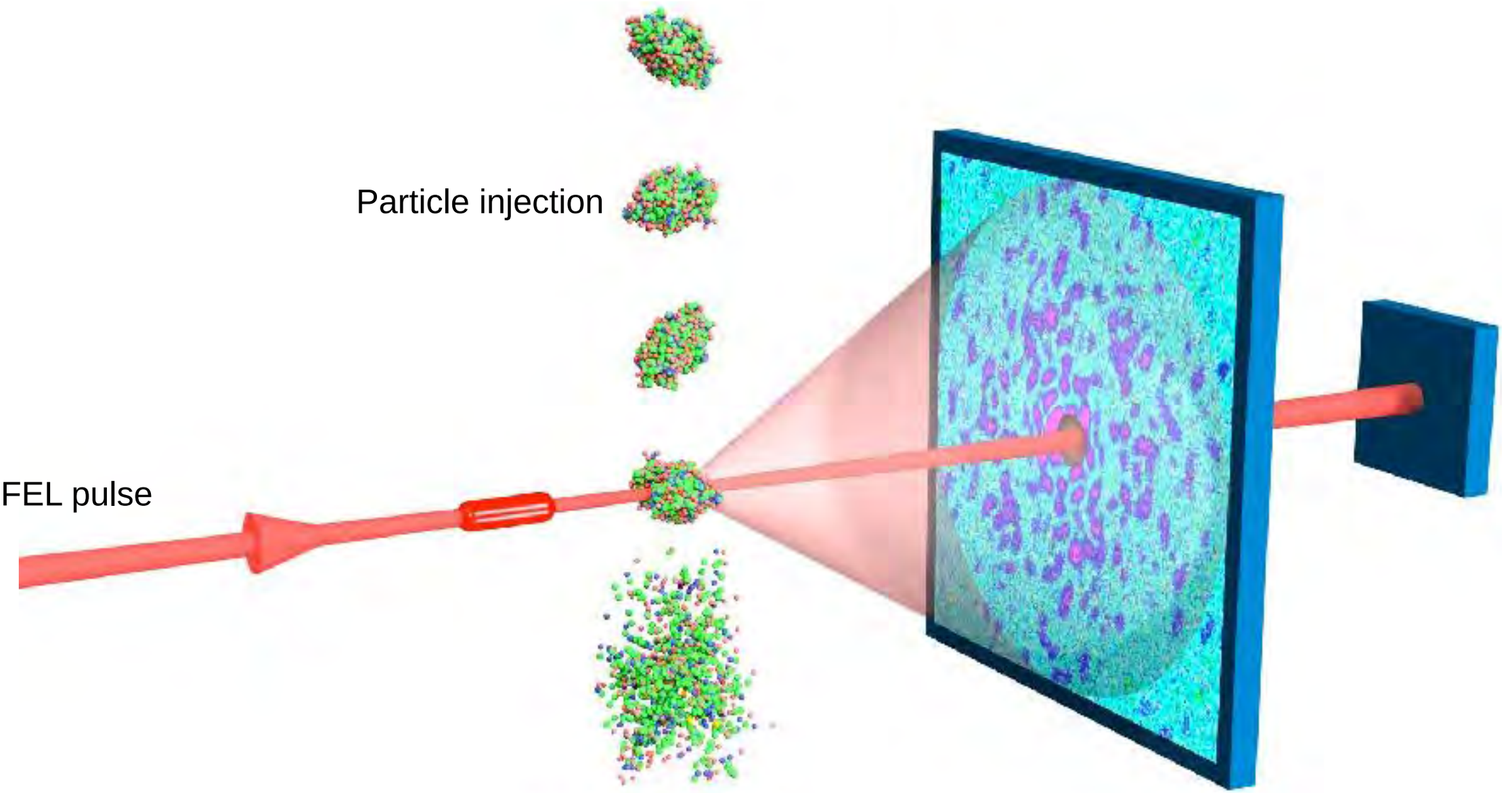
Axford et al. Acta Cryst. D68 592 (2012)
Diamond Light Source (courtesy Robin Owen & Elspeth Garman)

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



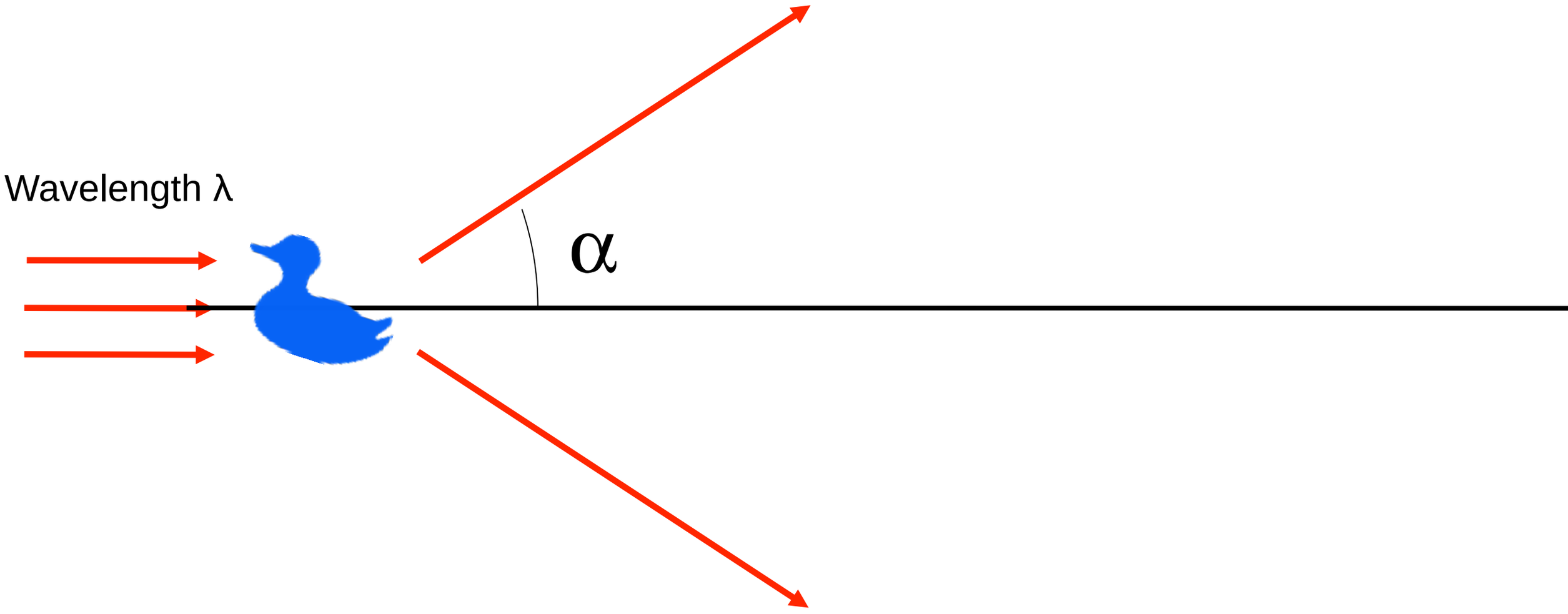
R. Neutze, R. Wouts, D. van der Spoel, E. Weckert, J. Hajdu, Nature **406** (2000)

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



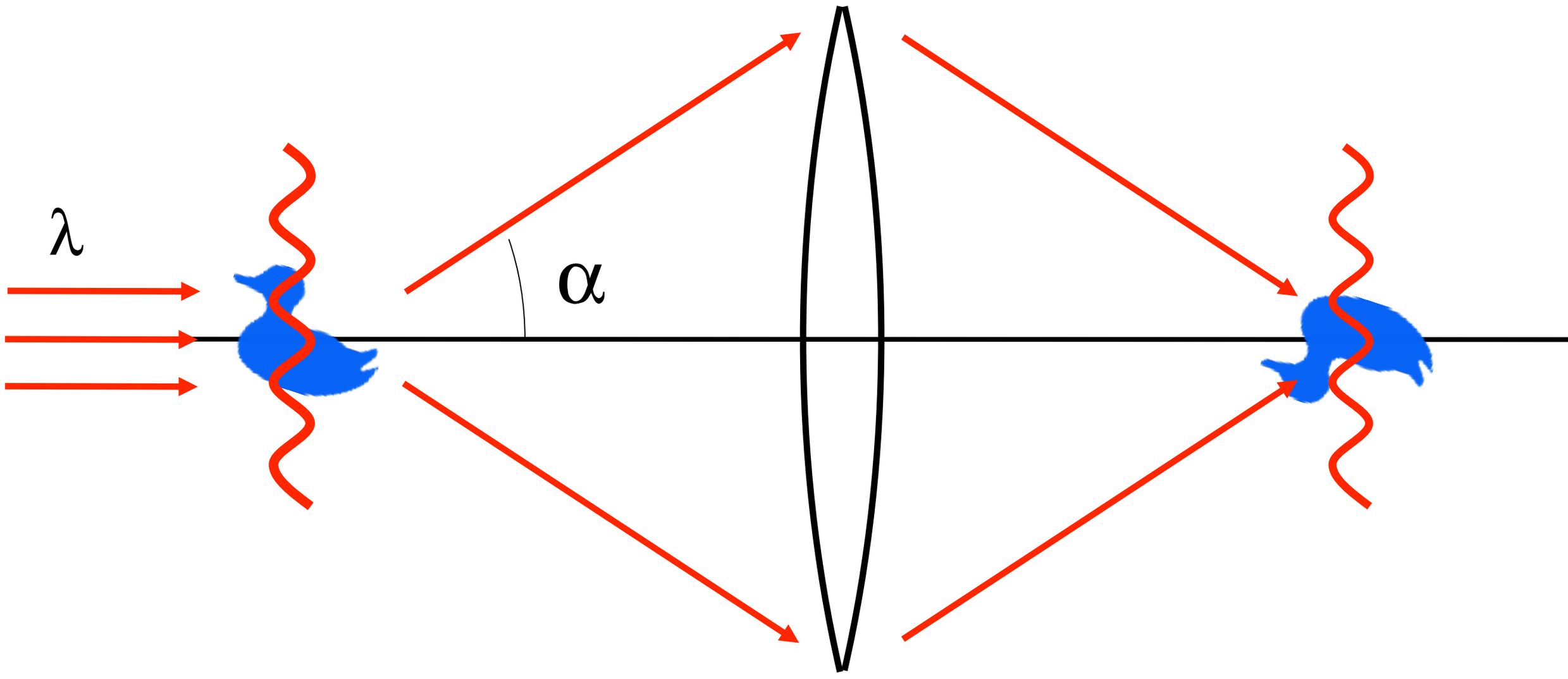
Scheme proposed in R. Neutze, R. Wouts, D. van der Spoel, E. Weckert, J. Hajdu, Nature **406** (2000)

Imaging can be achieved with a lens



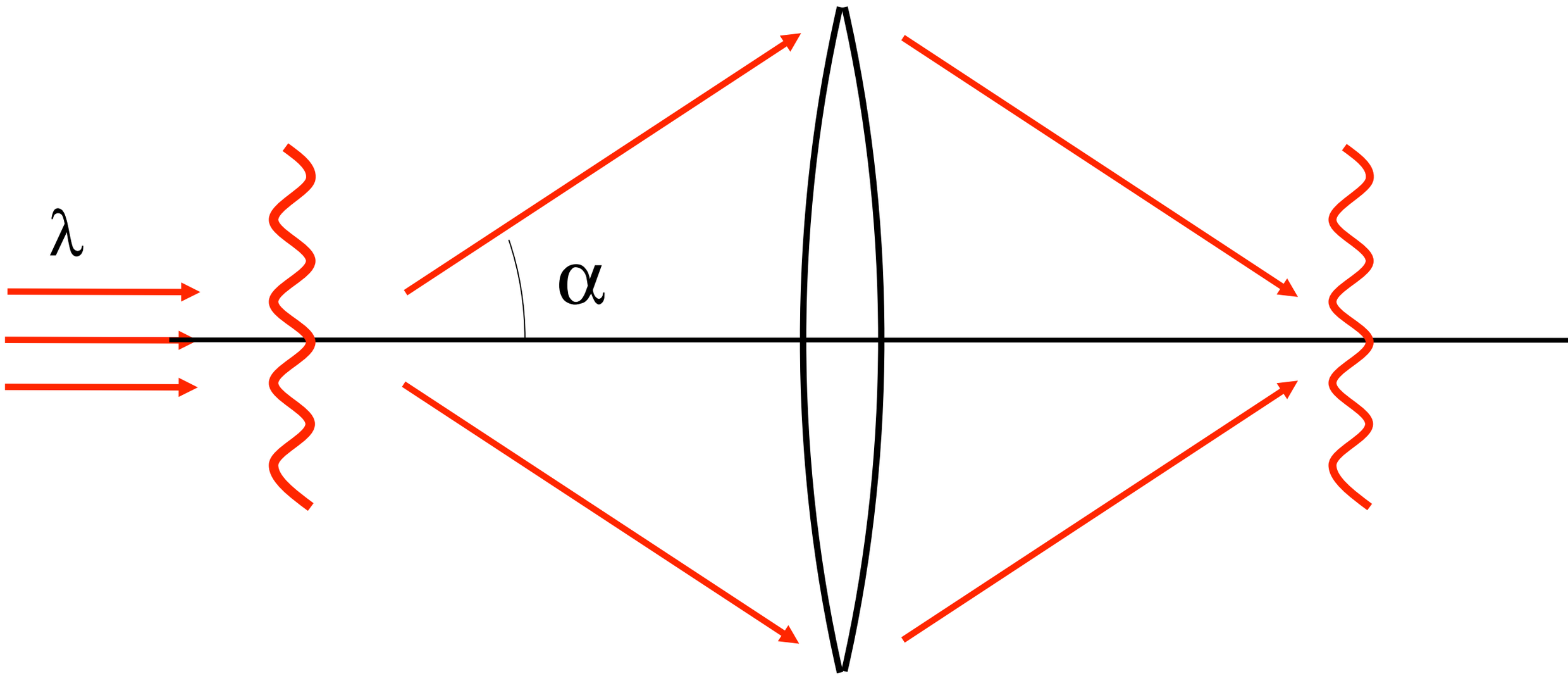
Resolution: $\delta = \lambda / \sin \alpha$

Imaging can be achieved with a lens



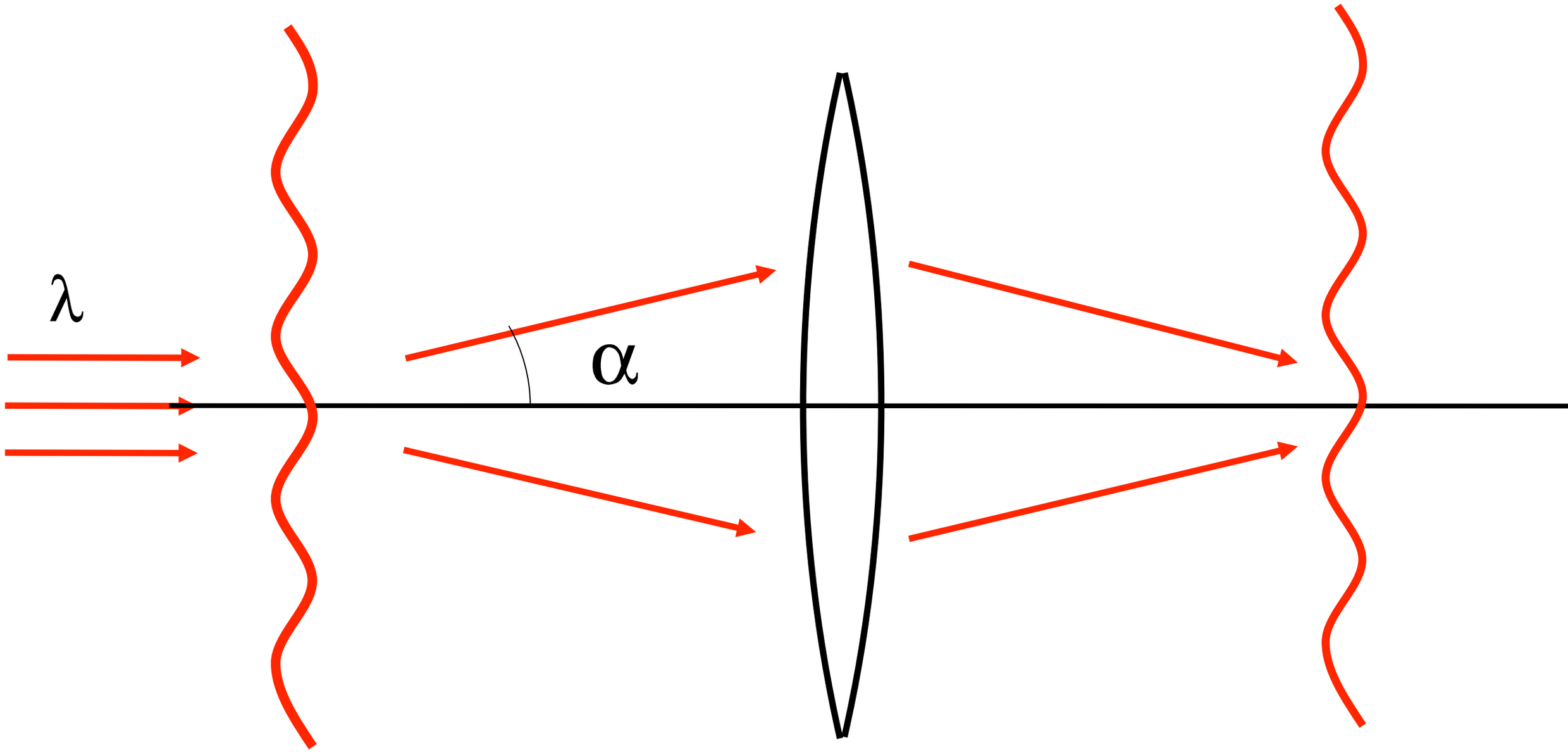
Resolution: $\delta = \lambda / \sin \alpha$

Imaging can be achieved with a lens



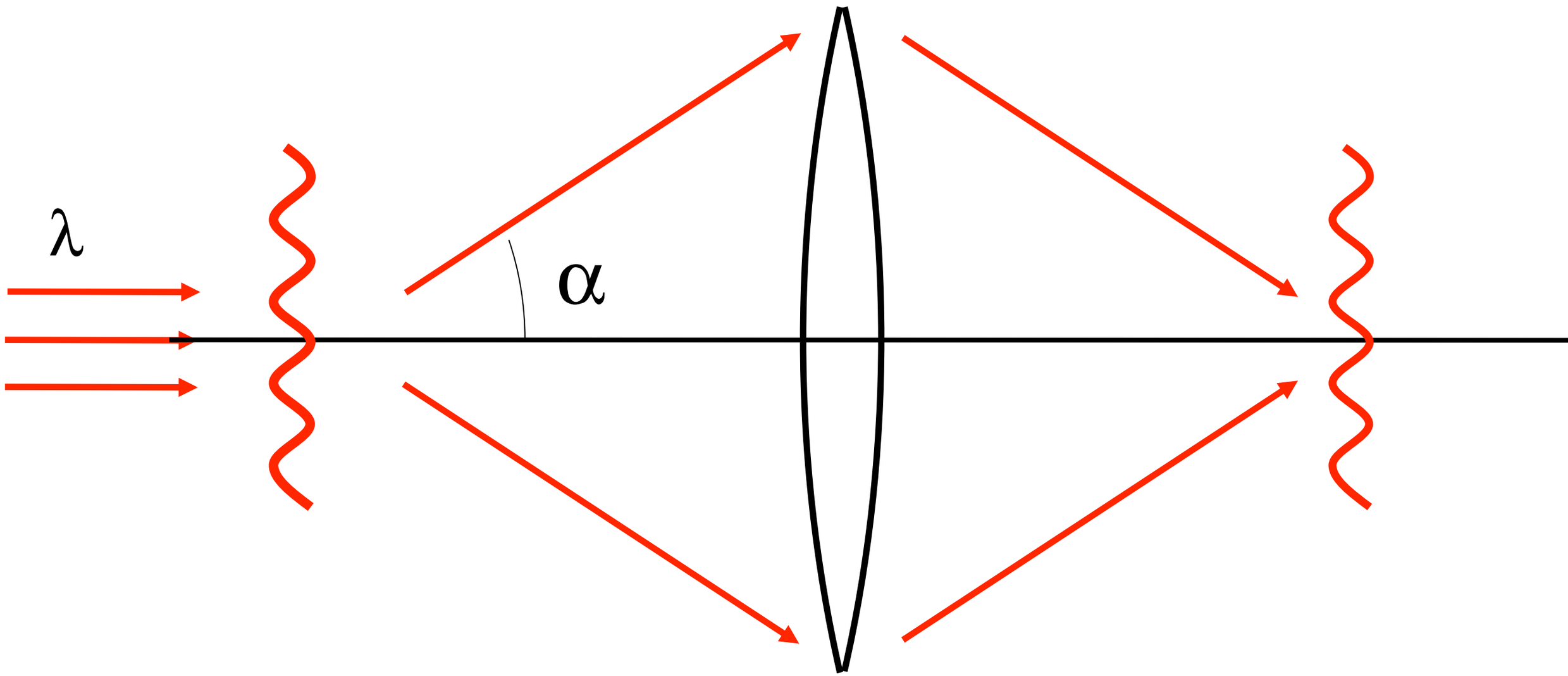
Resolution: $\delta = \lambda / \sin \alpha$

Imaging can be achieved with a lens



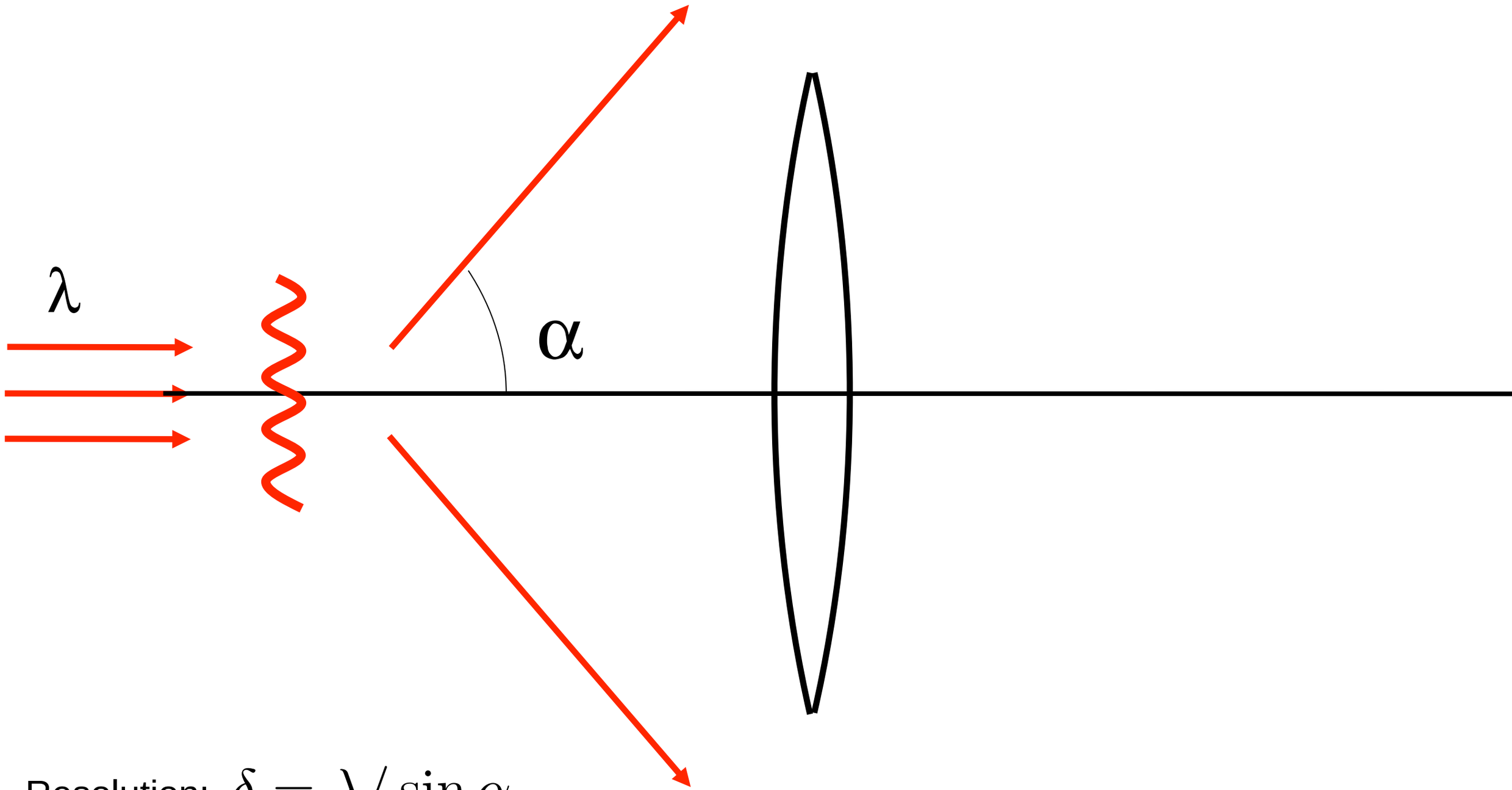
Resolution: $\delta = \lambda / \sin \alpha$

Imaging can be achieved with a lens



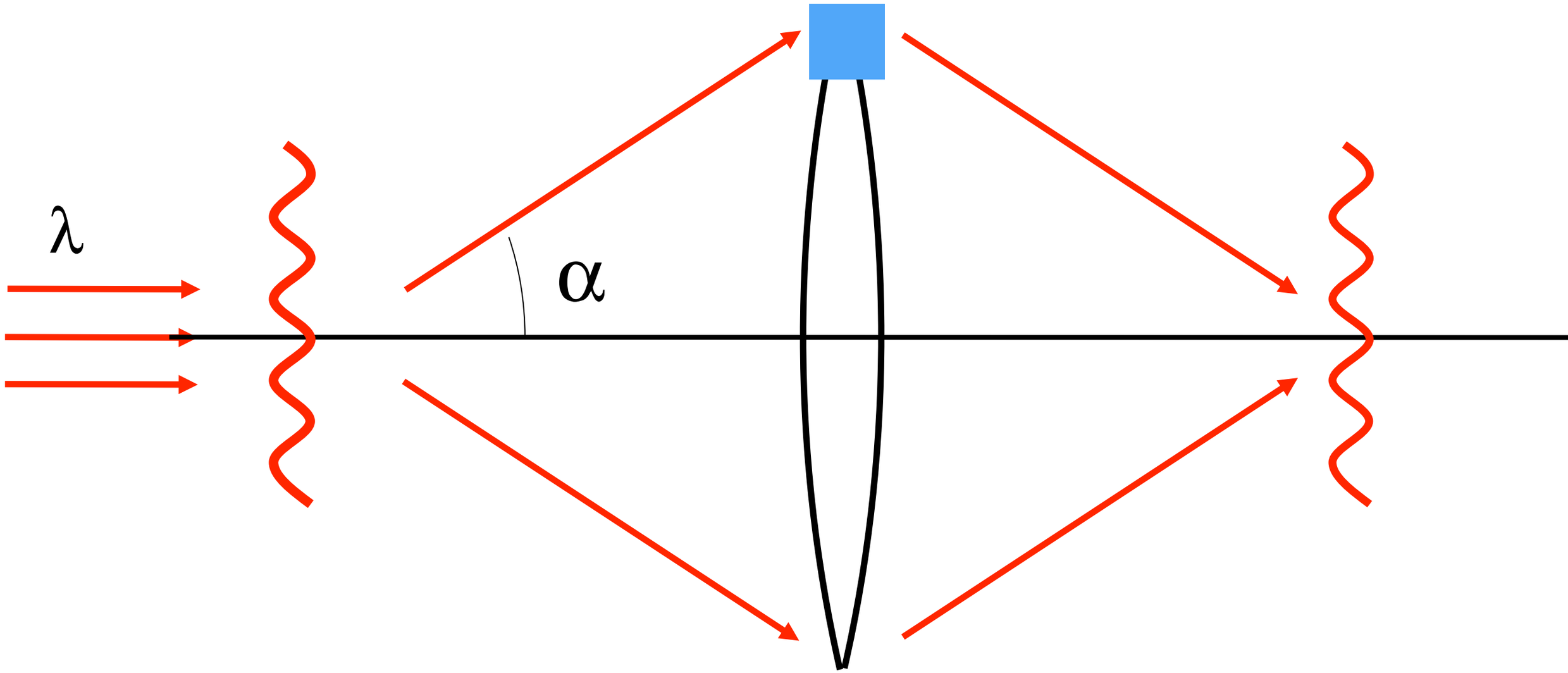
Resolution: $\delta = \lambda / \sin \alpha$

Imaging can be achieved with a lens



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

Imaging can be achieved with a lens

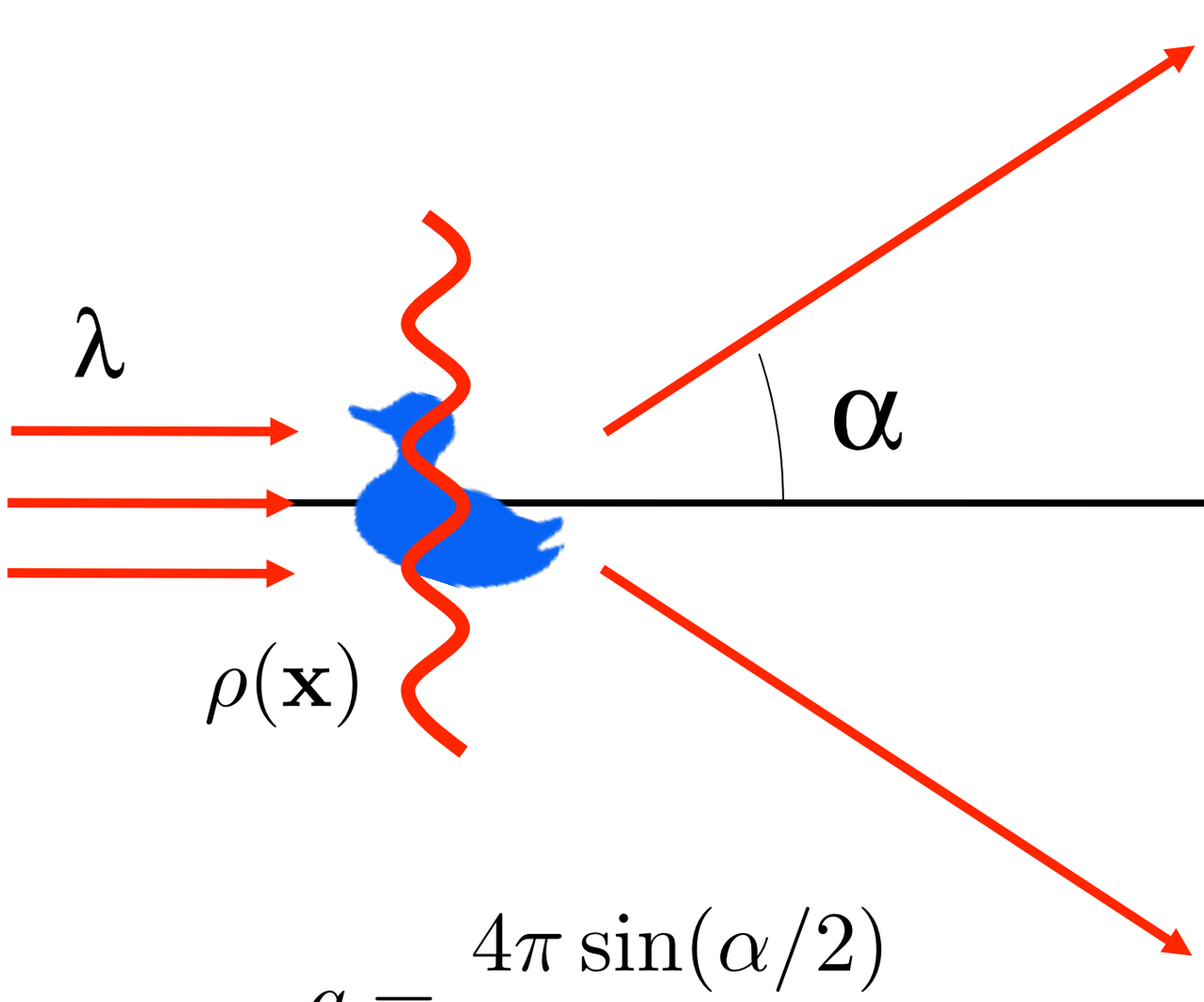


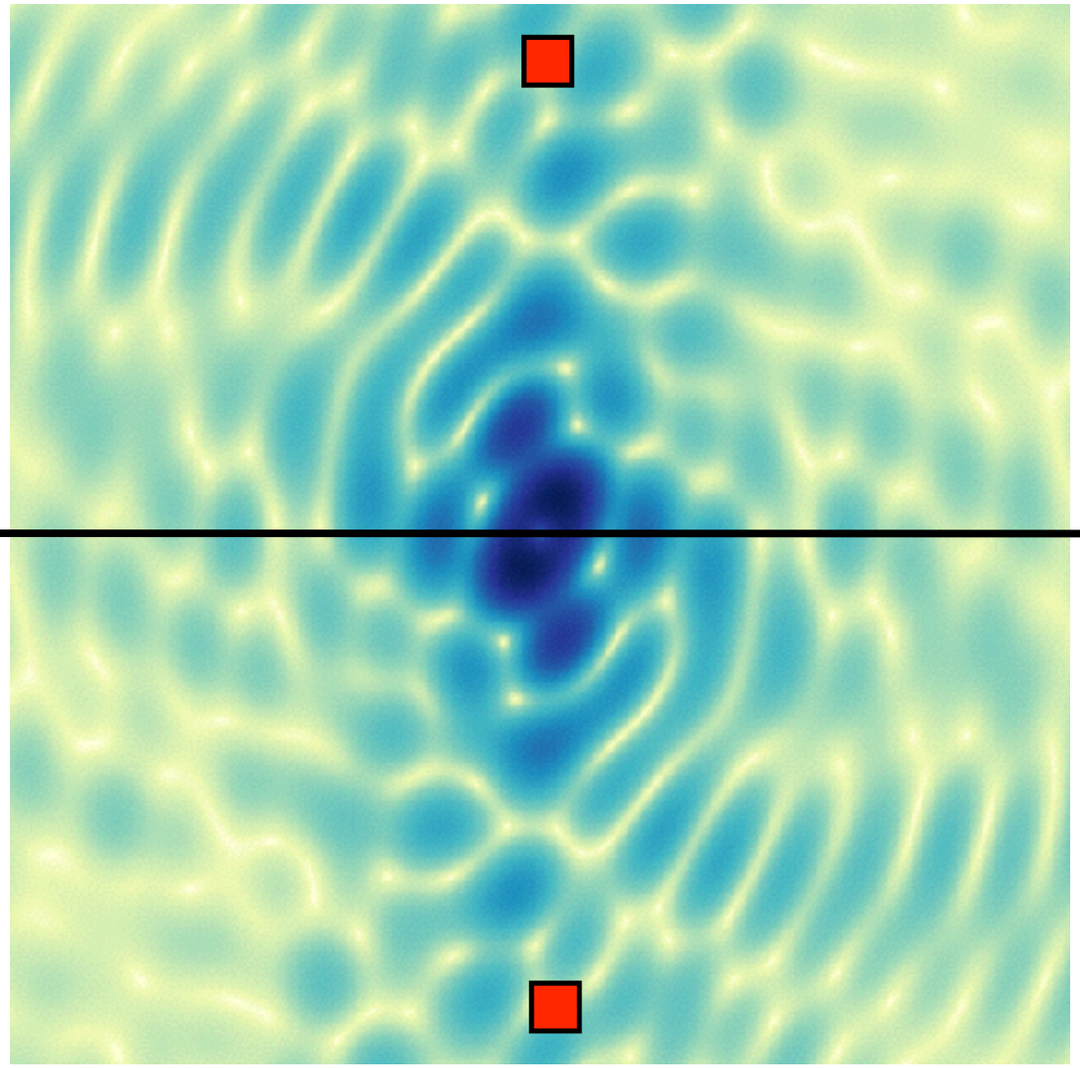
Resolution: $\delta = \lambda / \sin \alpha$

Imaging can be achieved with a lens

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

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



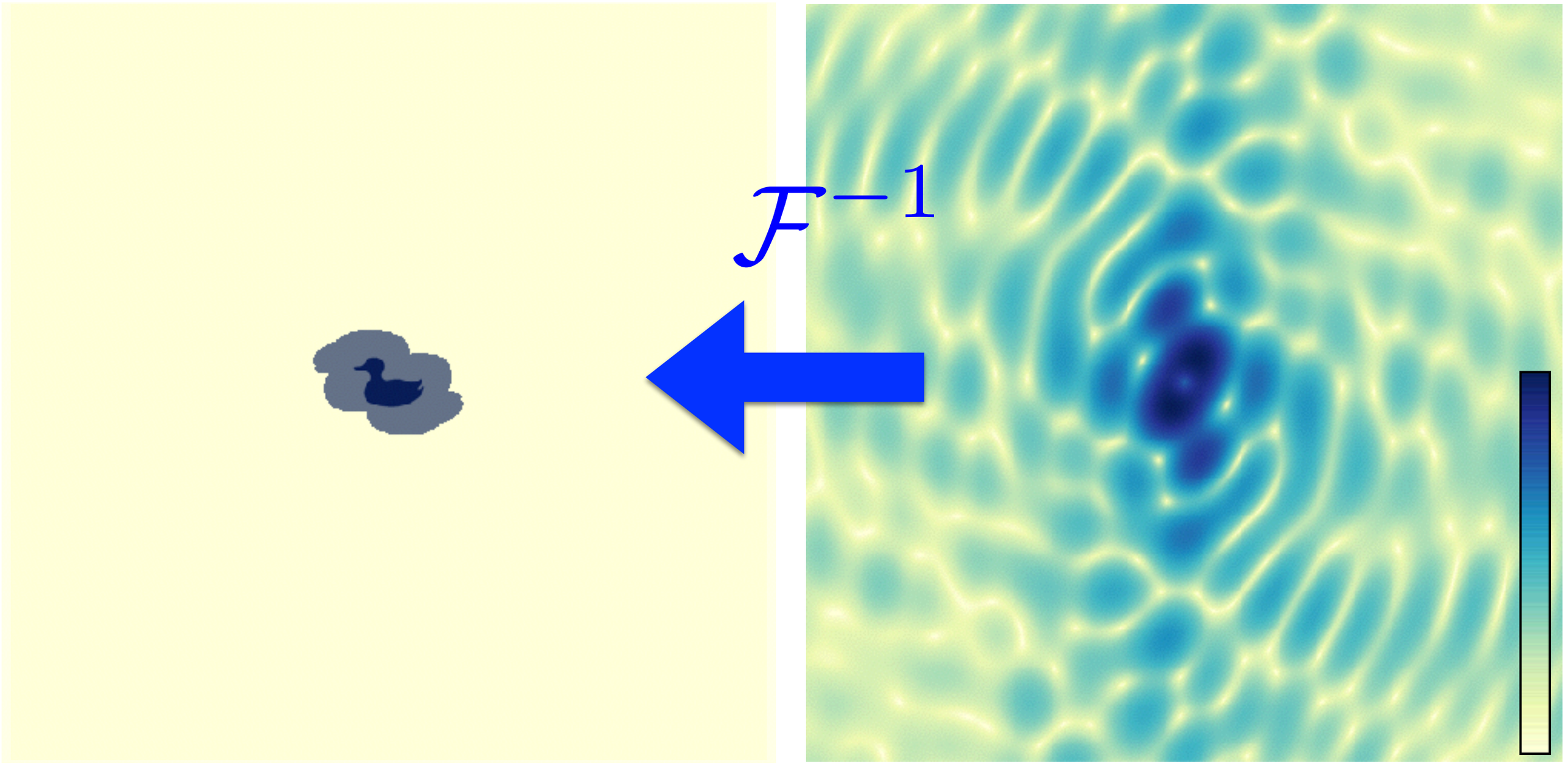


$$q = \frac{4\pi \sin(\alpha/2)}{\lambda}$$

Single particles give continuous diffraction patterns

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

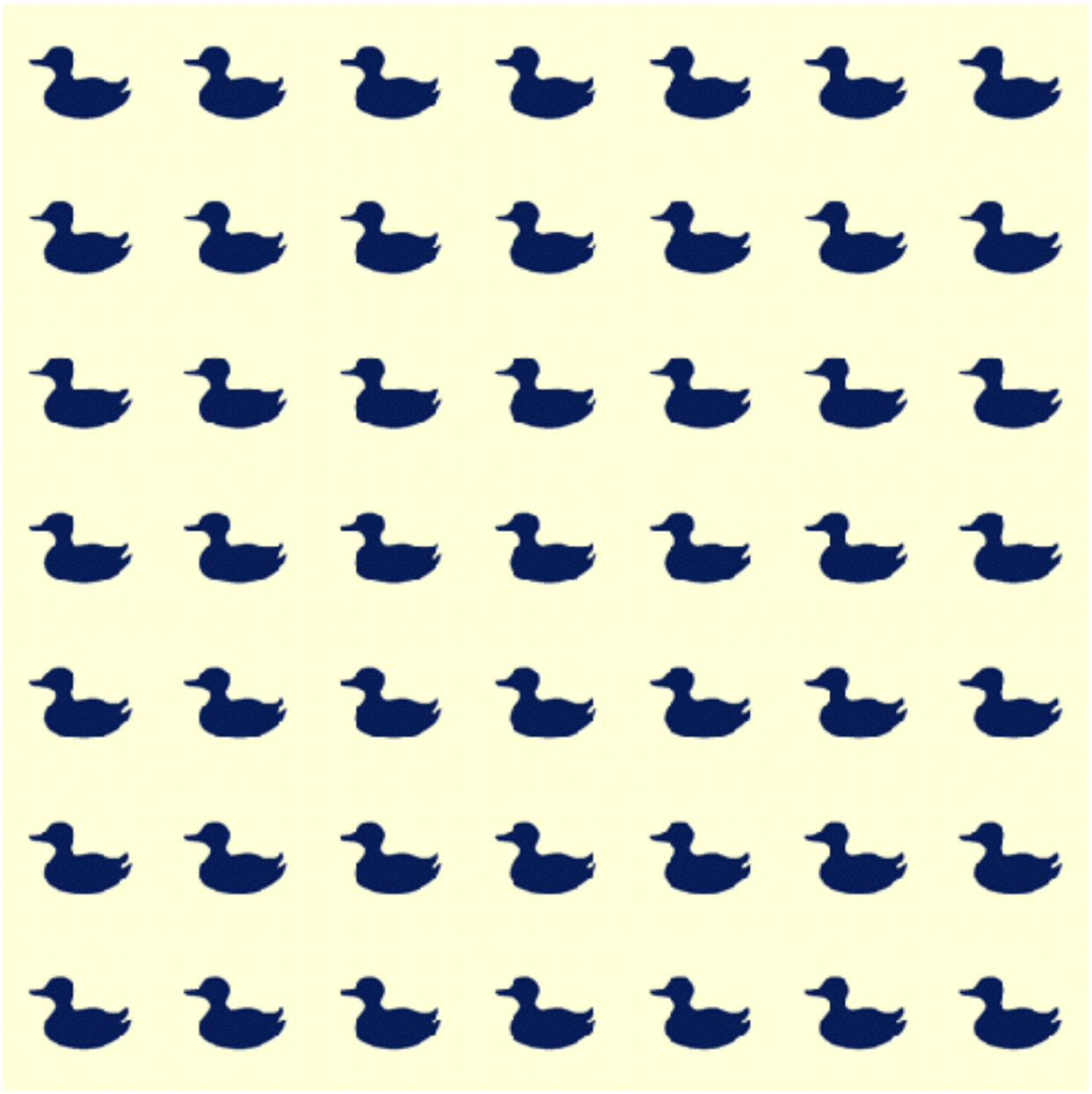
$$I(\mathbf{q}) = |\hat{\rho}(\mathbf{q})|^2$$



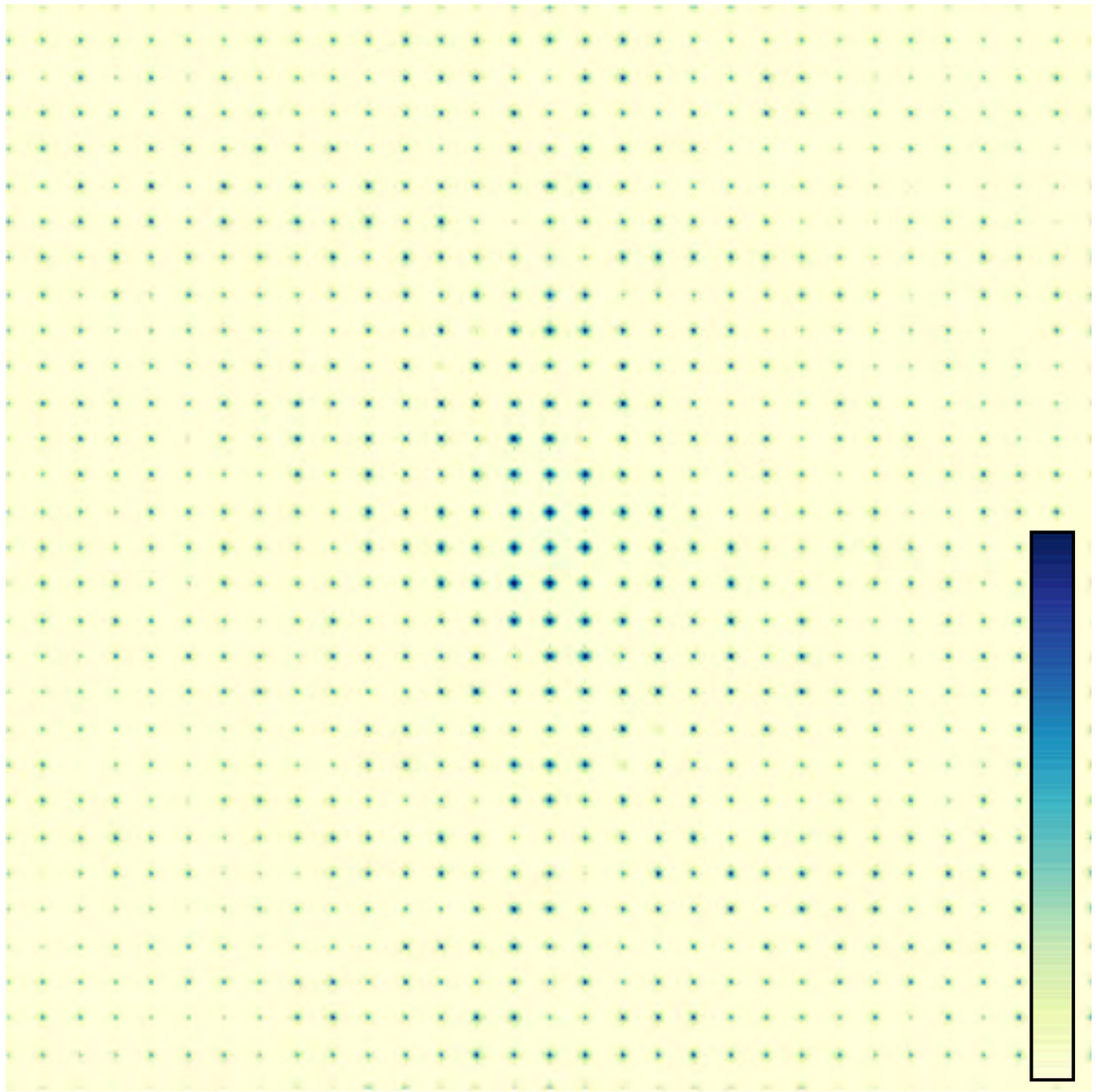
Over-constrained: more knowns than unknowns

Crystals give Bragg spots

$\rho(\mathbf{x})$



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



Crystals give Bragg spots

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

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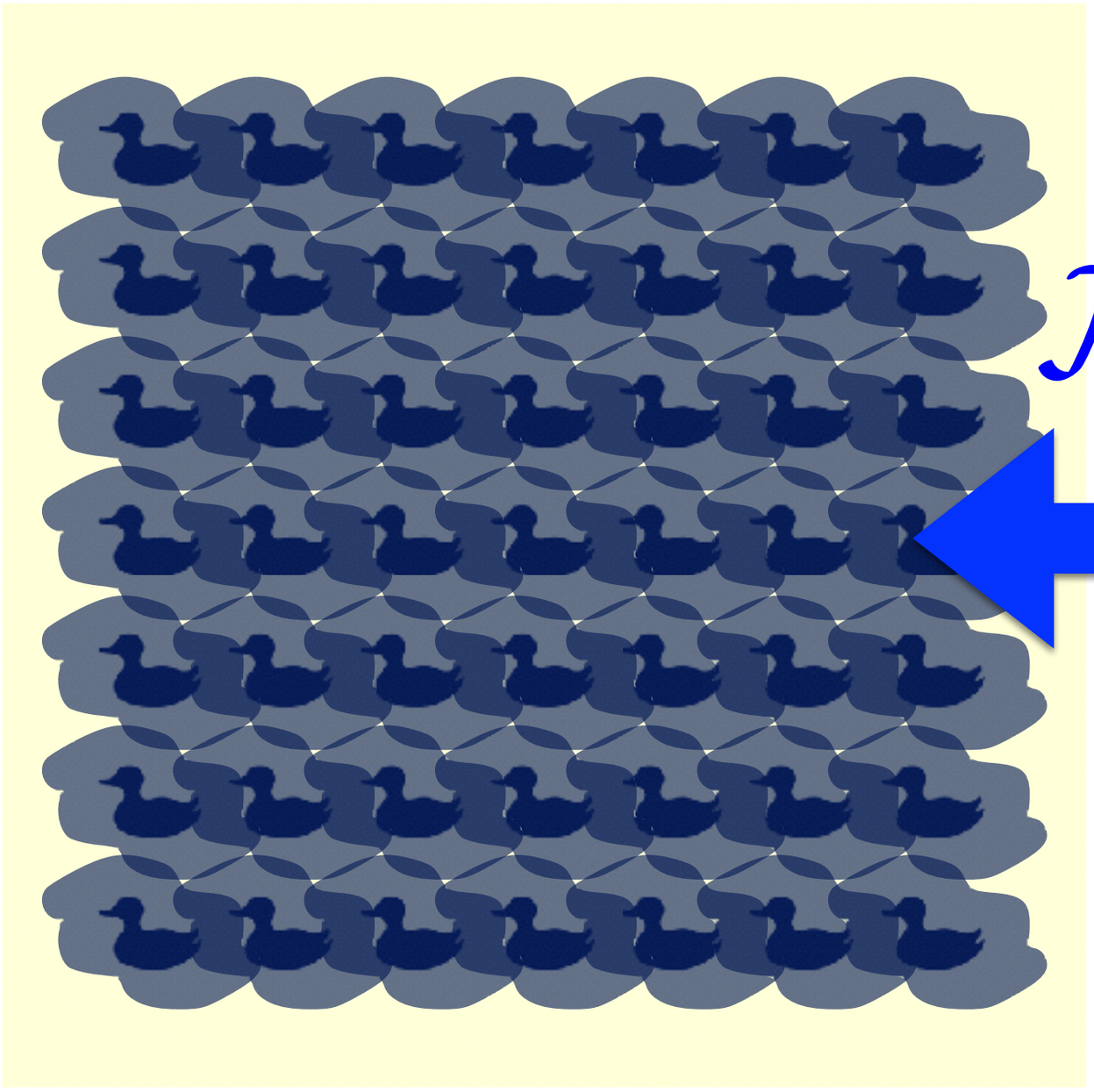
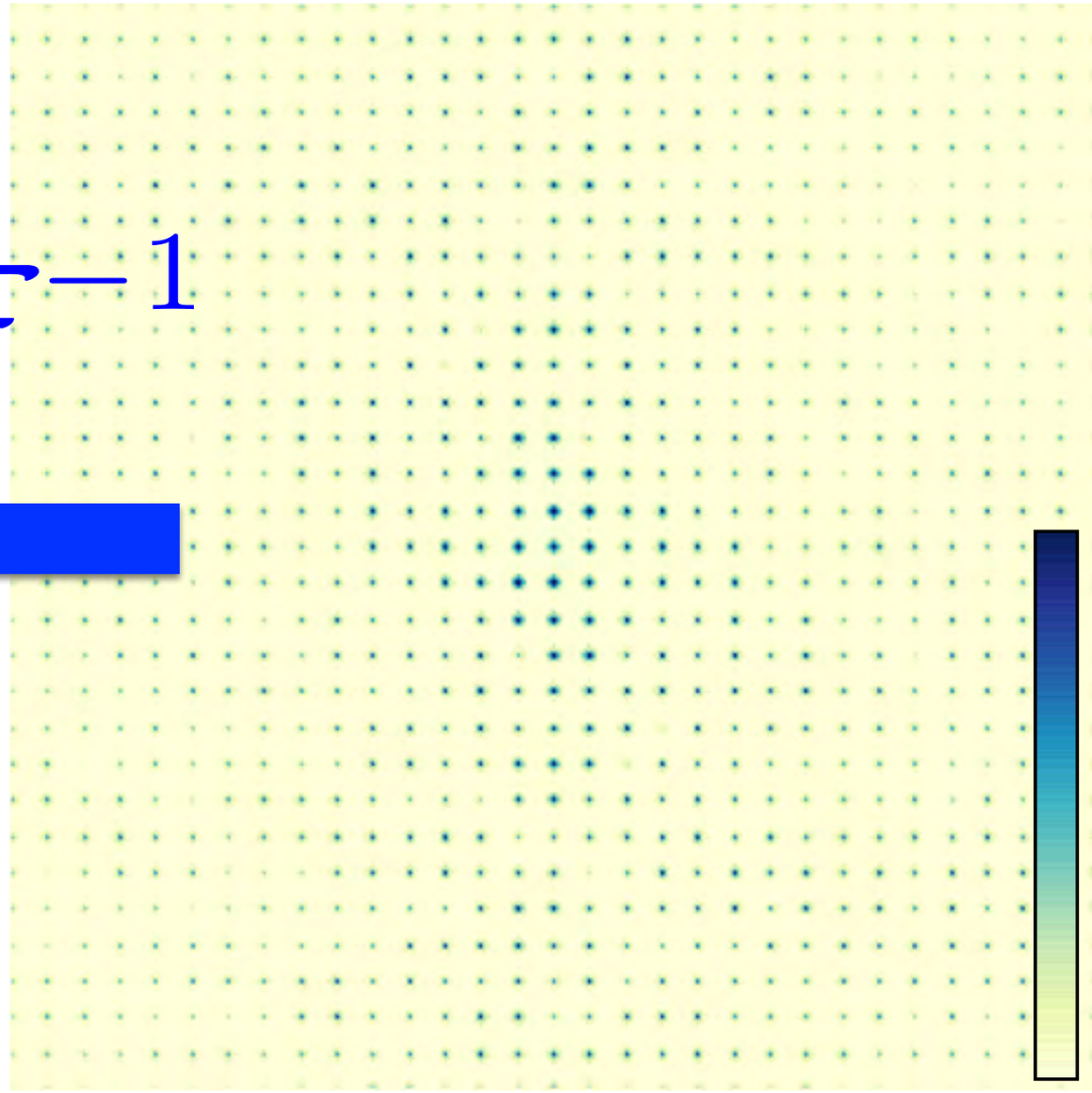
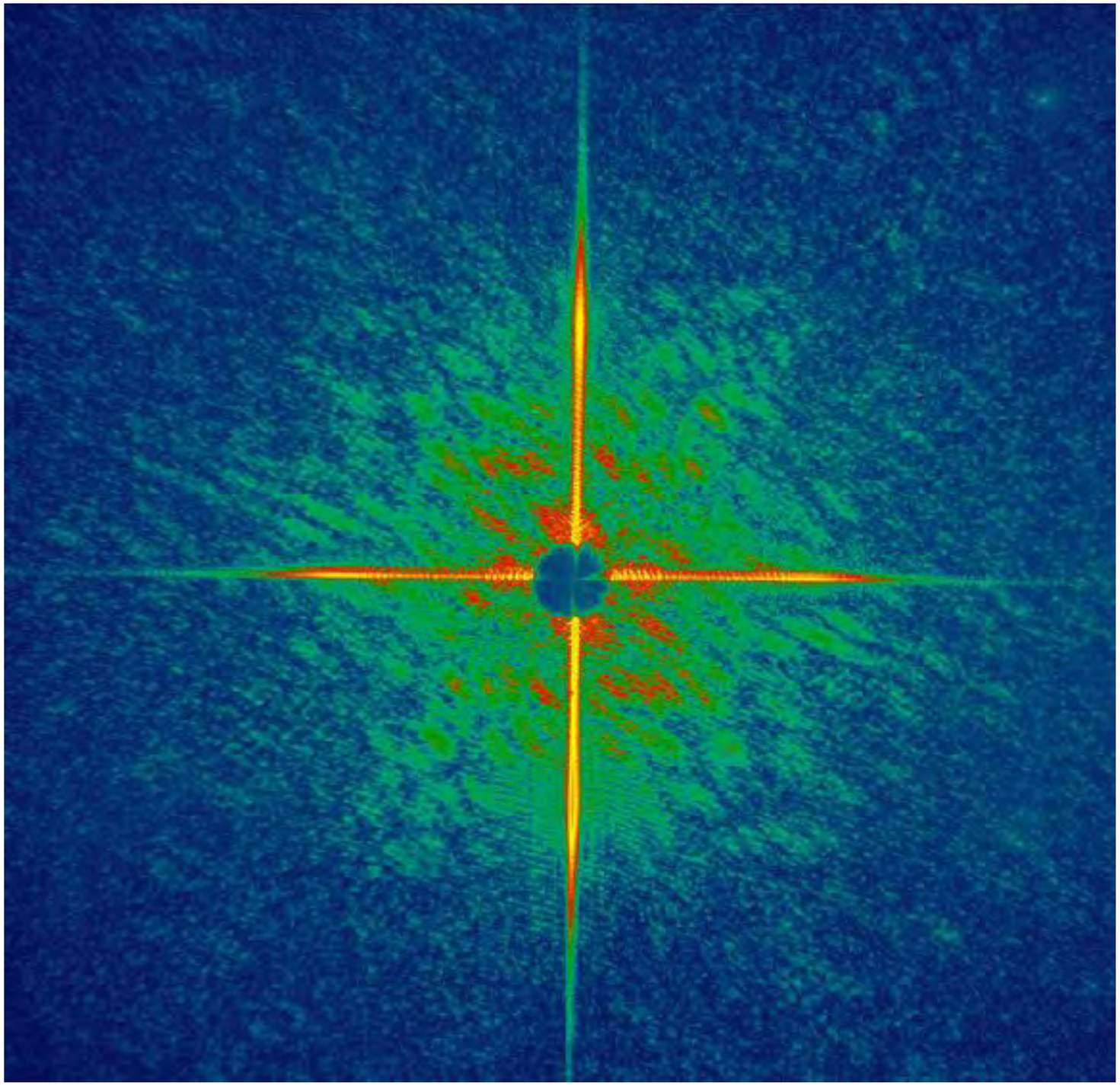


$\mathcal{F}^{-1}$

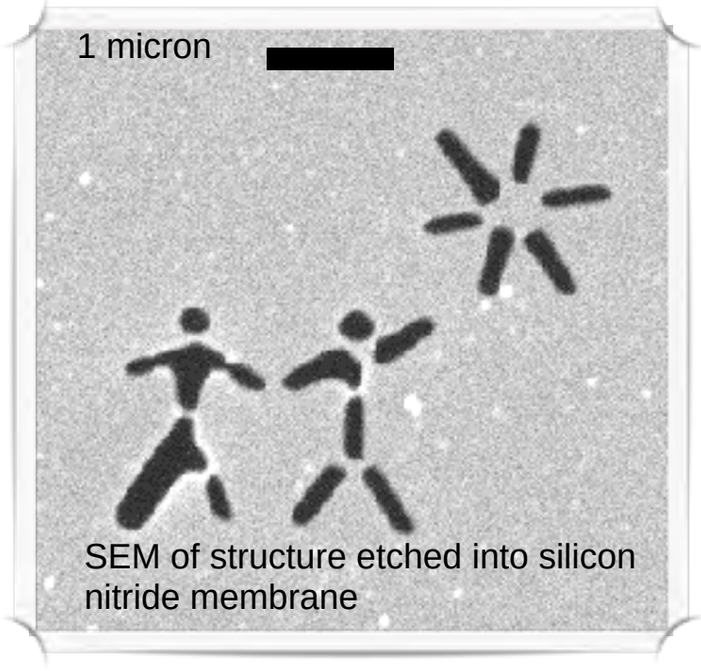


Under-constrained: fewer knowns than unknowns

Phasing is achieved using iterative algorithms



Chapman et al. Nature Physics 2 839 (2006)



SEM of structure etched into silicon nitride membrane

Recent hard X-ray experiments show high-resolution diffraction

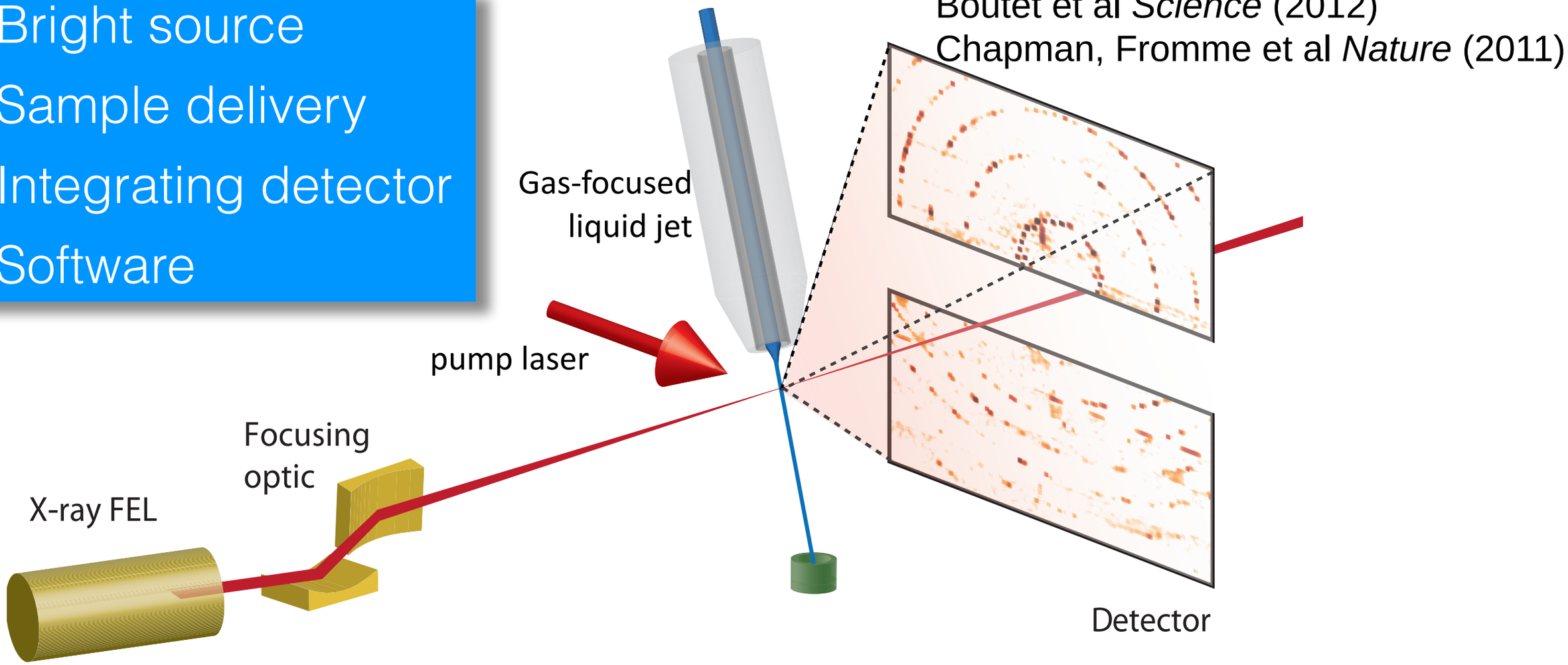
Photosystem I
9.3 keV
Single shot pattern
~1 mJ (5×10^{11} photons)
40 fs
2×10^{17} W/cm^2
25 GW X-ray pulse

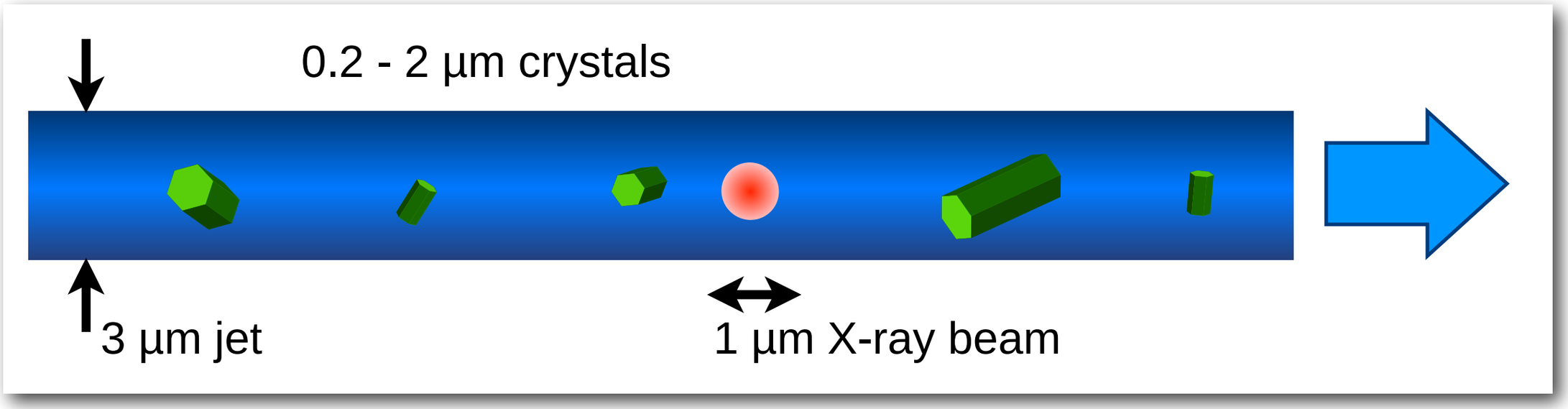
3.0 Å resolution

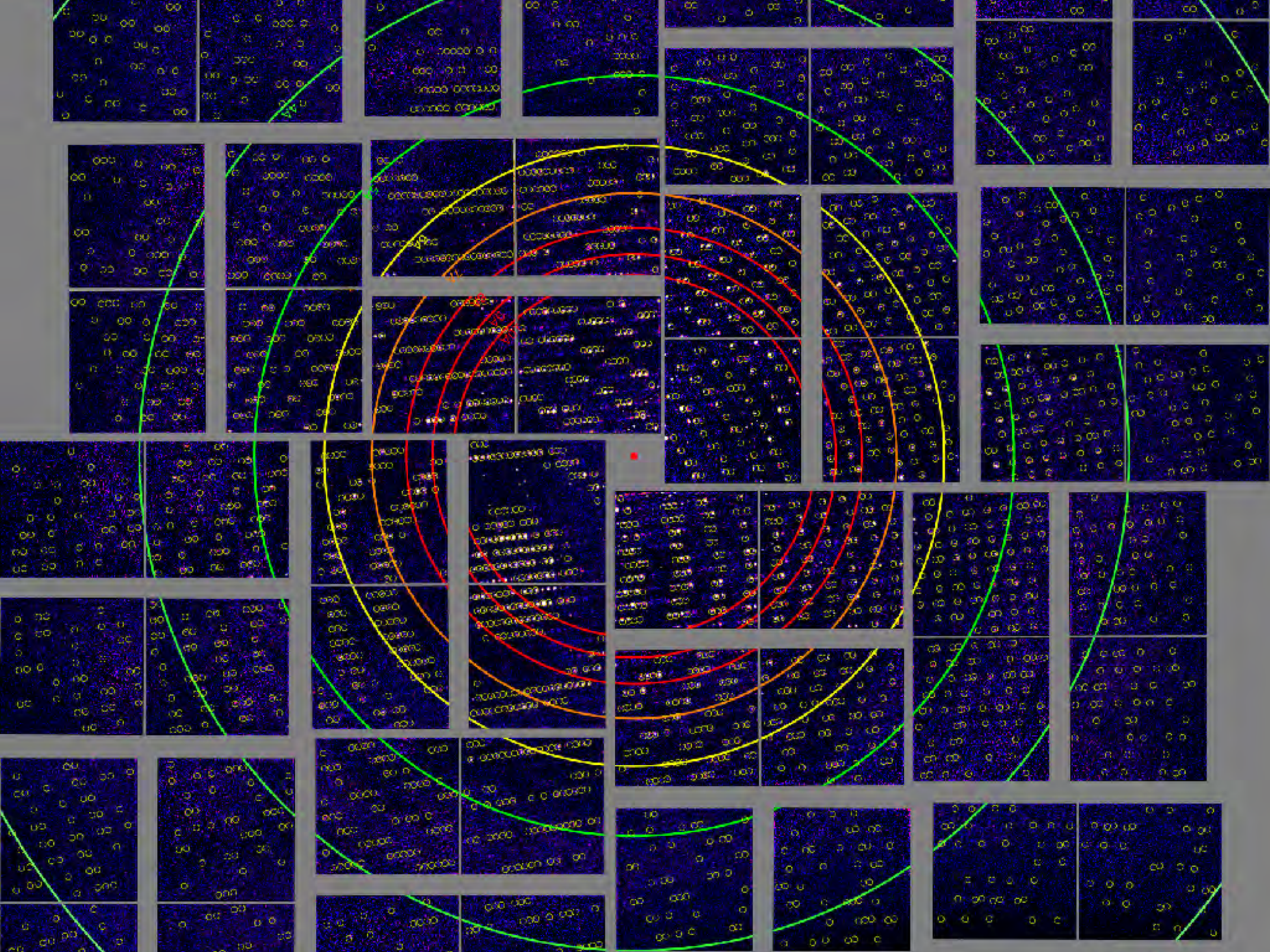
crystals by Petra Fromme

Serial crystallography is made possible by four key technologies

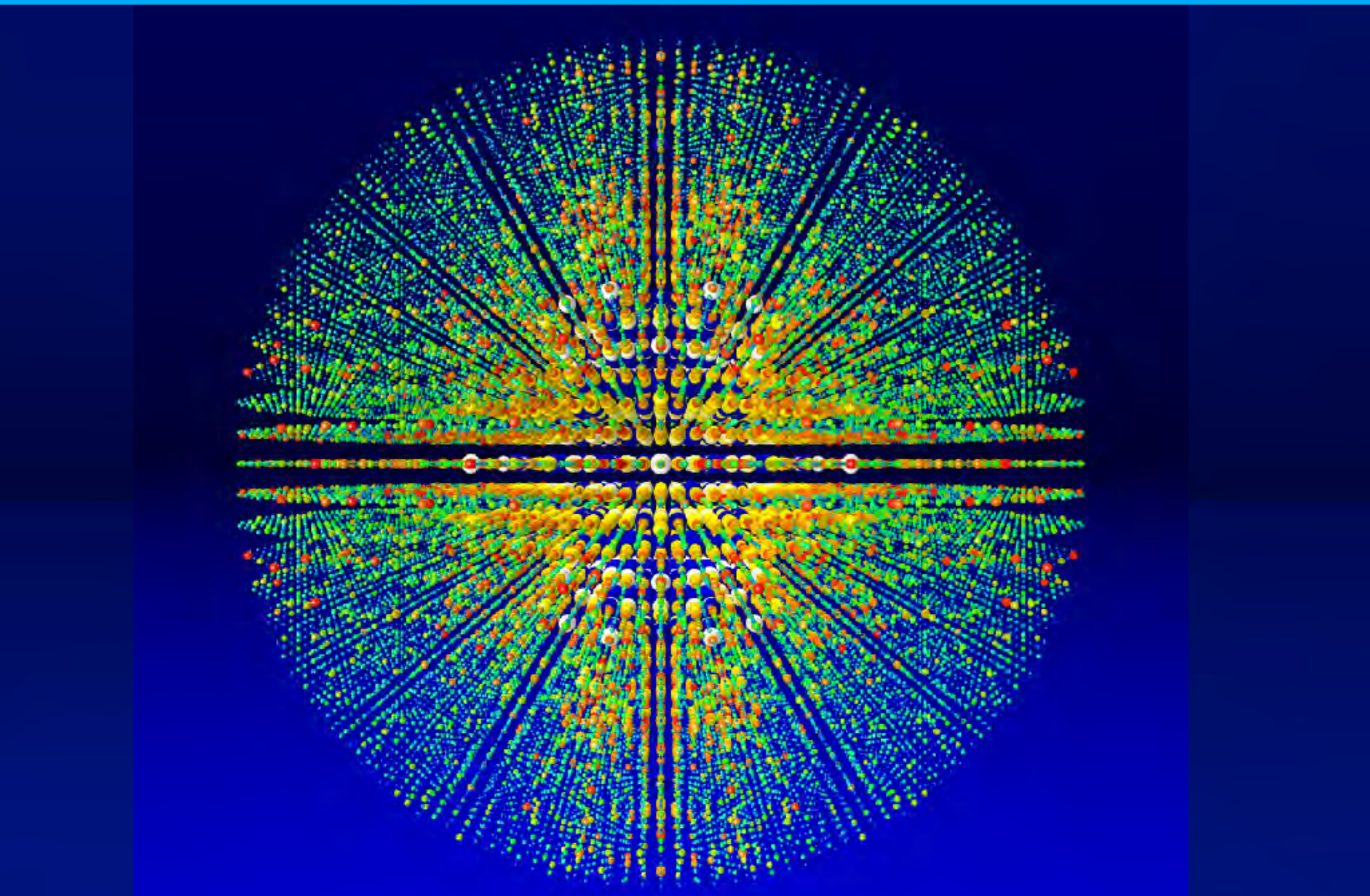
1. Bright source
2. Sample delivery
3. Integrating detector
4. Software



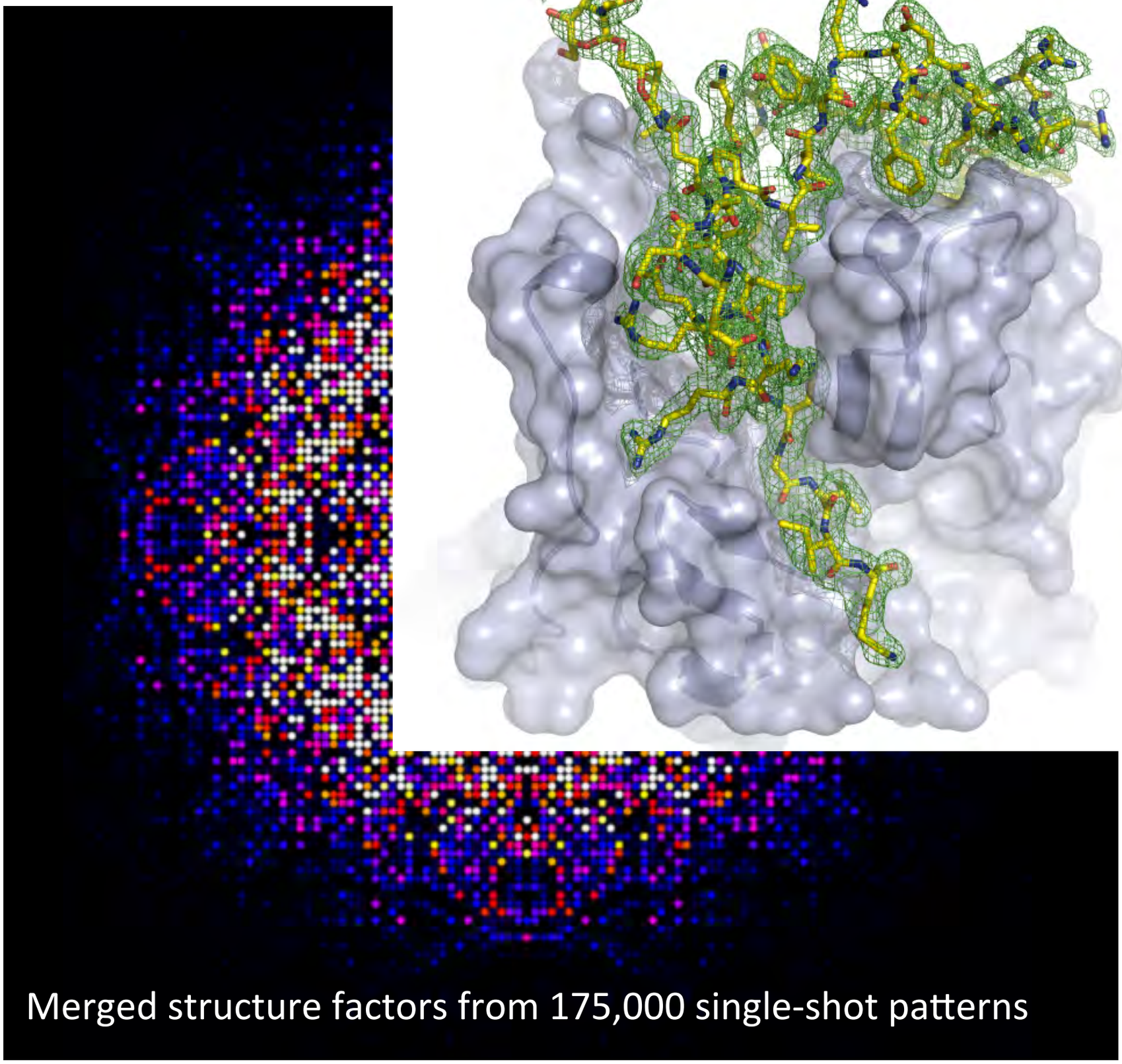




Intensities are merged into a “3D powder” pattern

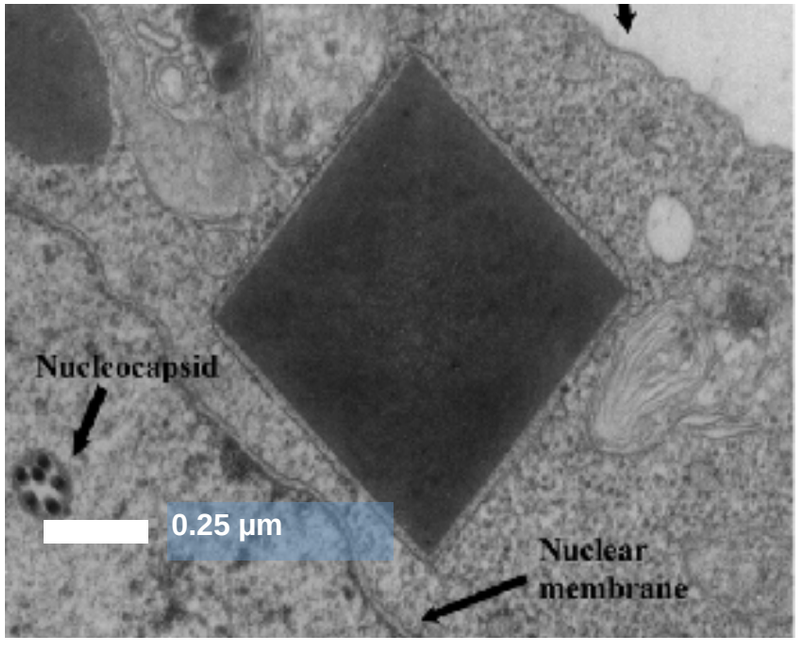


Structures have been obtained by in vivo grown crystals



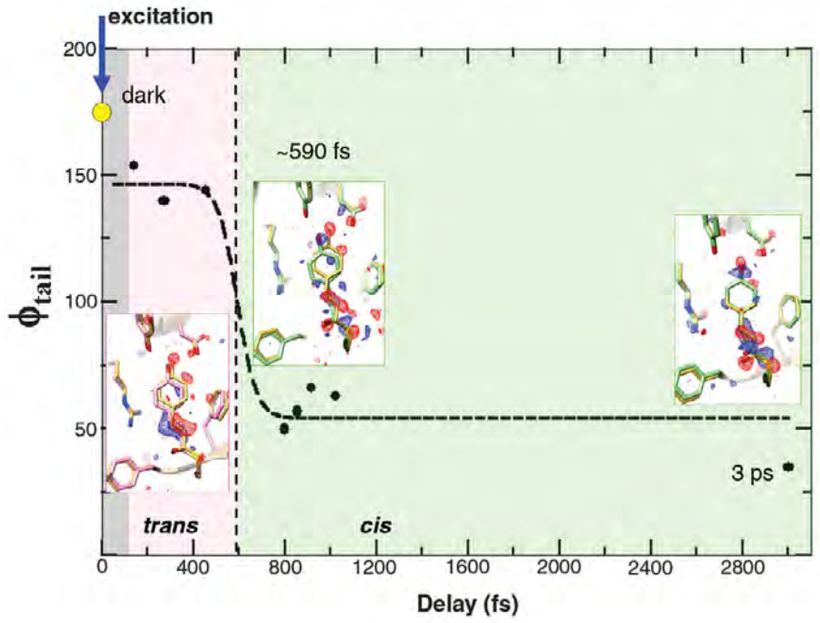
Merged structure factors from 175,000 single-shot patterns

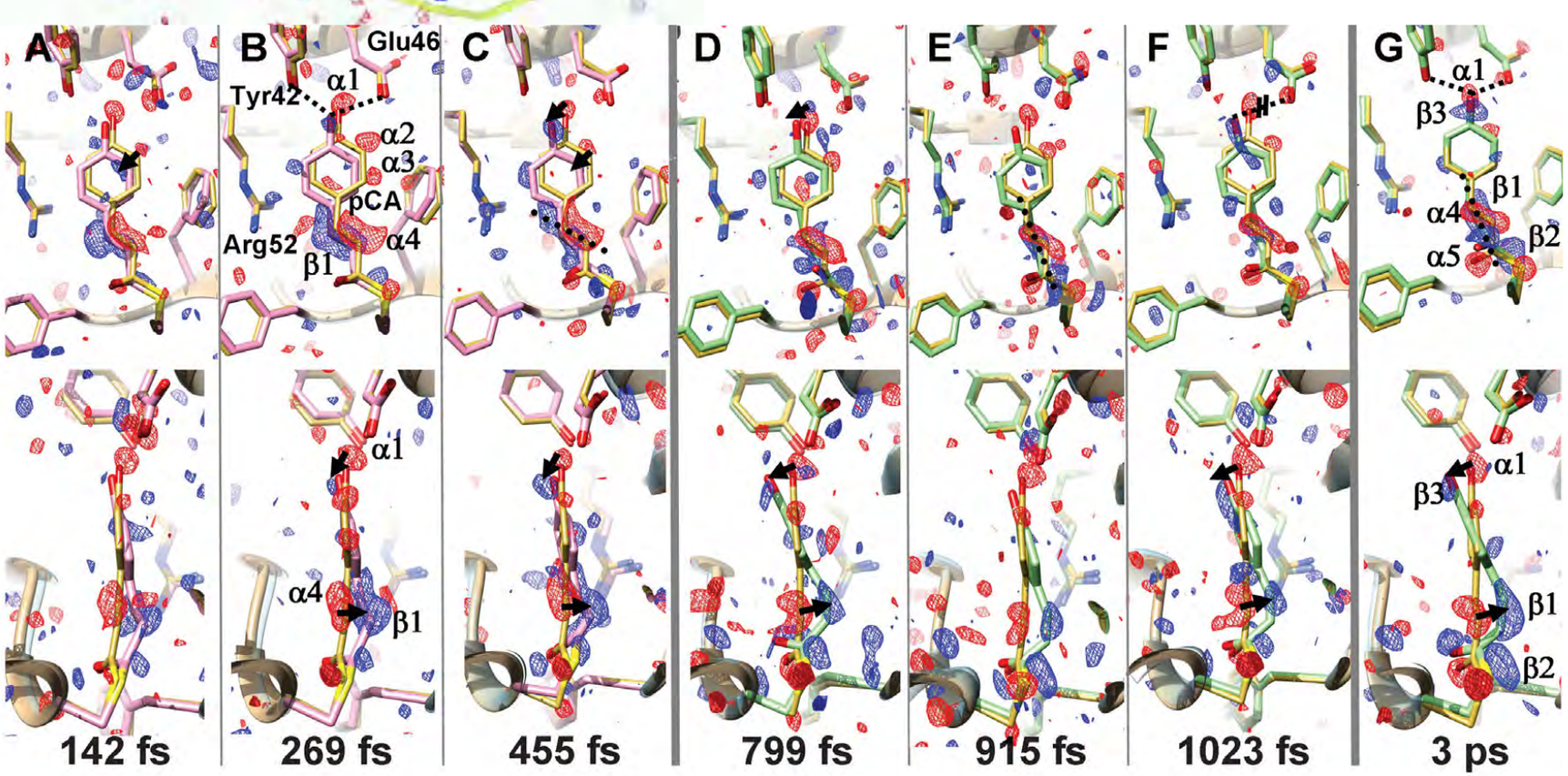
Trypanosoma brucei cathepsin B obtained from in vivo grown crystals



Redecke, Nass et al. Science (2013)

We have obtained time-resolved SFX structures of photoactive yellow protein (PYP)





Difference electron density map
1.6 Å resolution

> 250,000 patterns
R = 15 to 20%

Reaction initiaion: 40%
(18% pR_1, 22% pR_2)

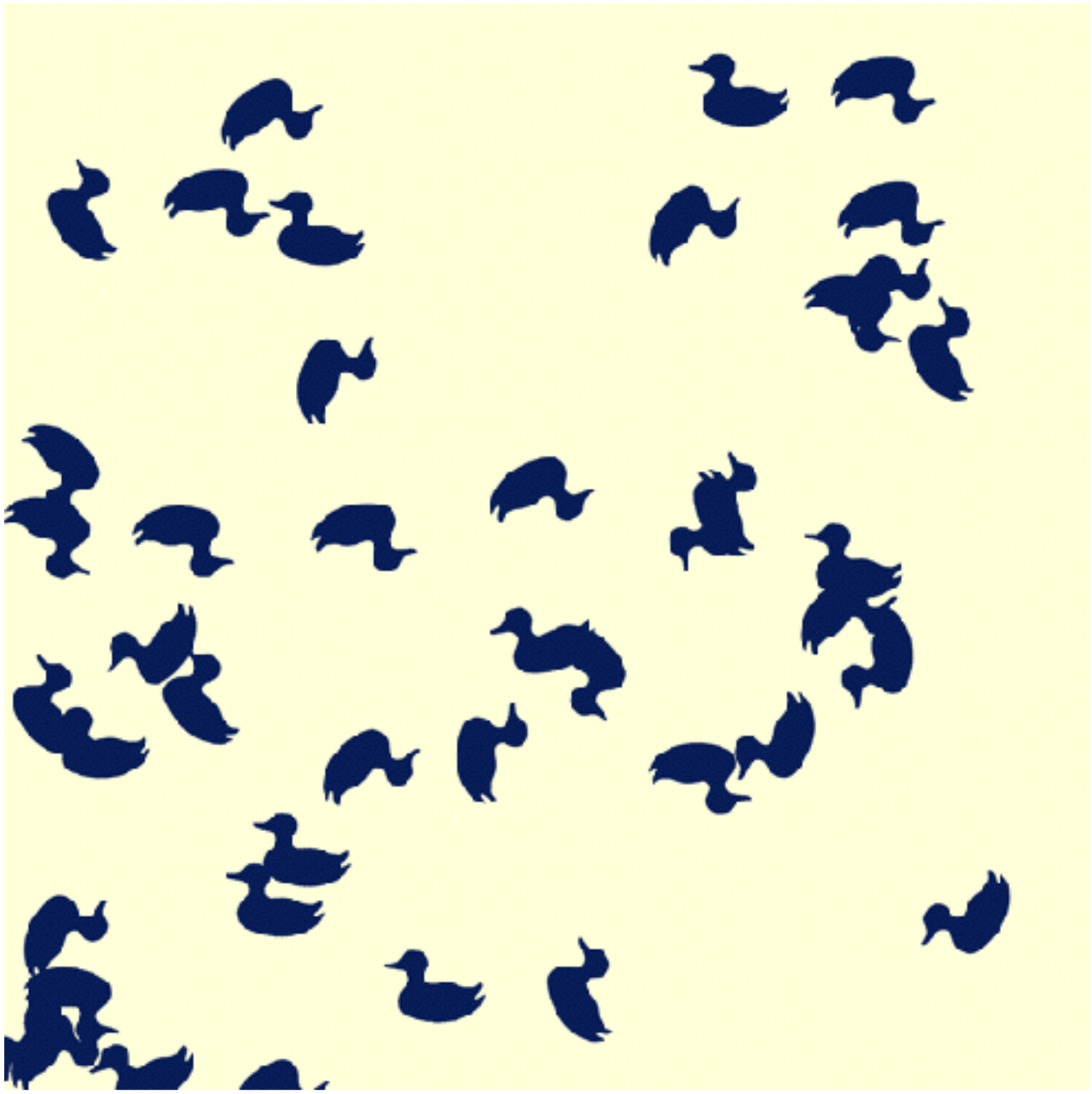
crystals <3 μm

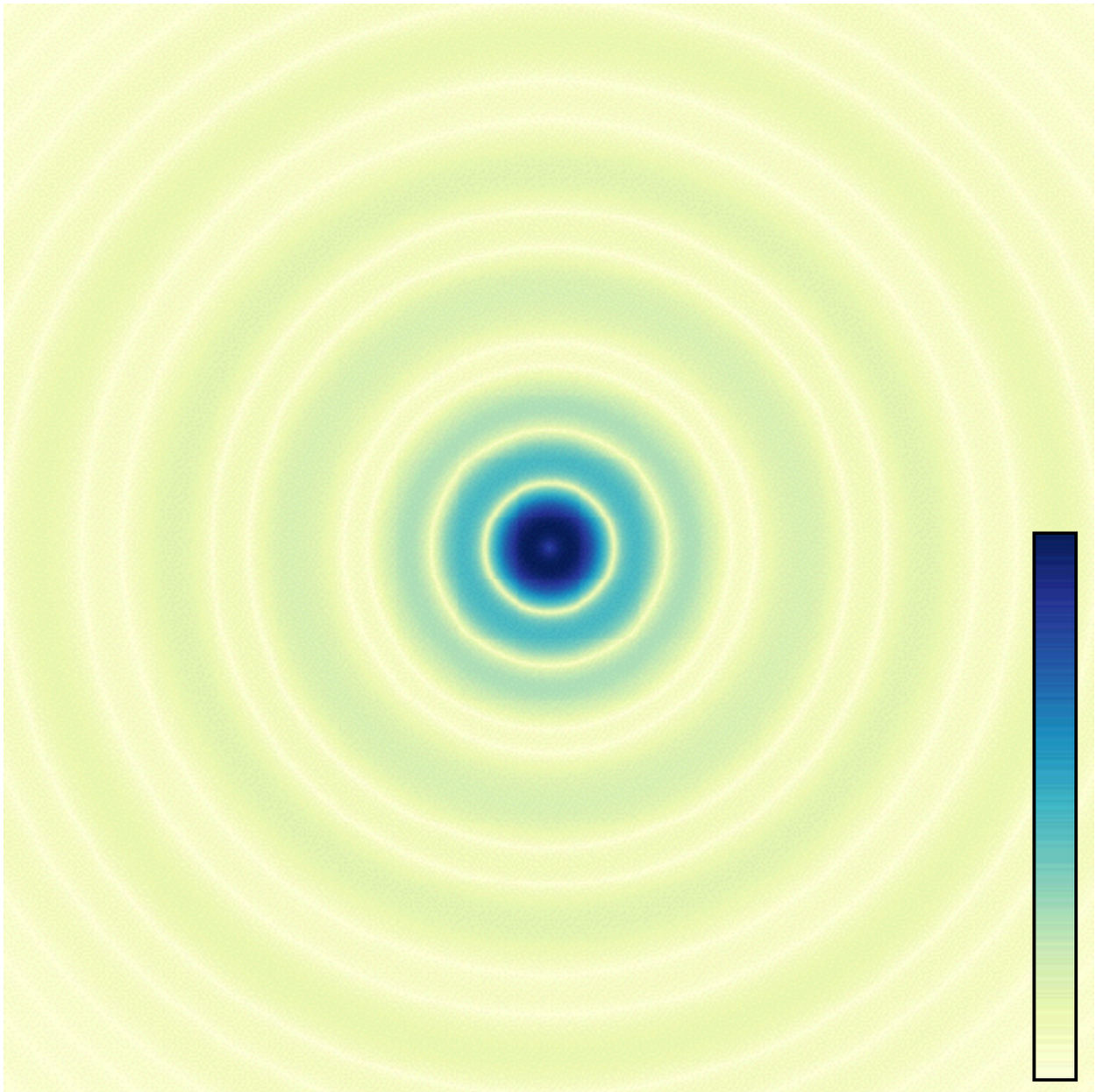
Experiments led by Marius Schmidt, U. Wisconsin

Tenboer et al *Science* **346** 1242 (2014)

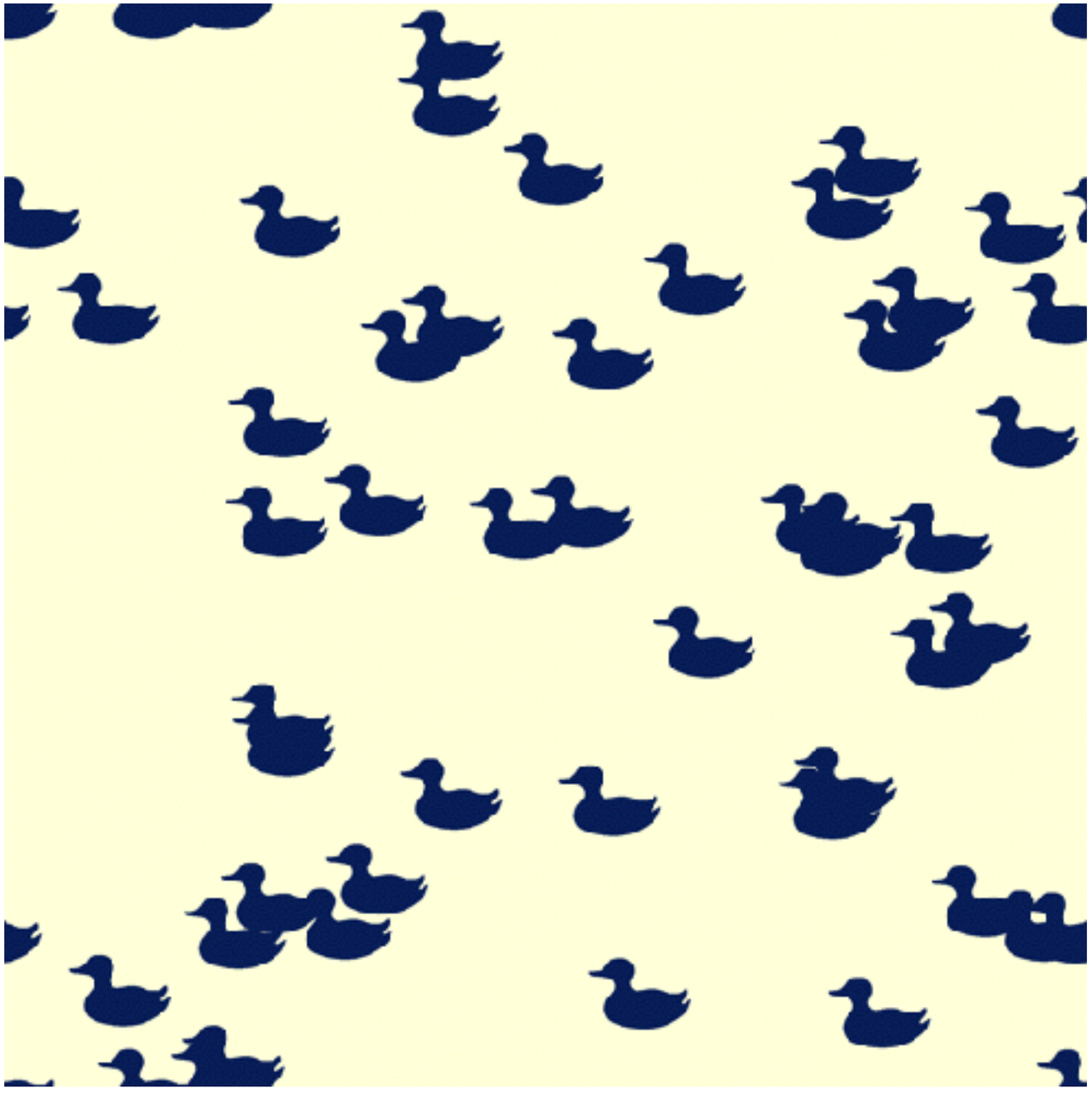
Pande et al *Science* **352** 725 (2016)

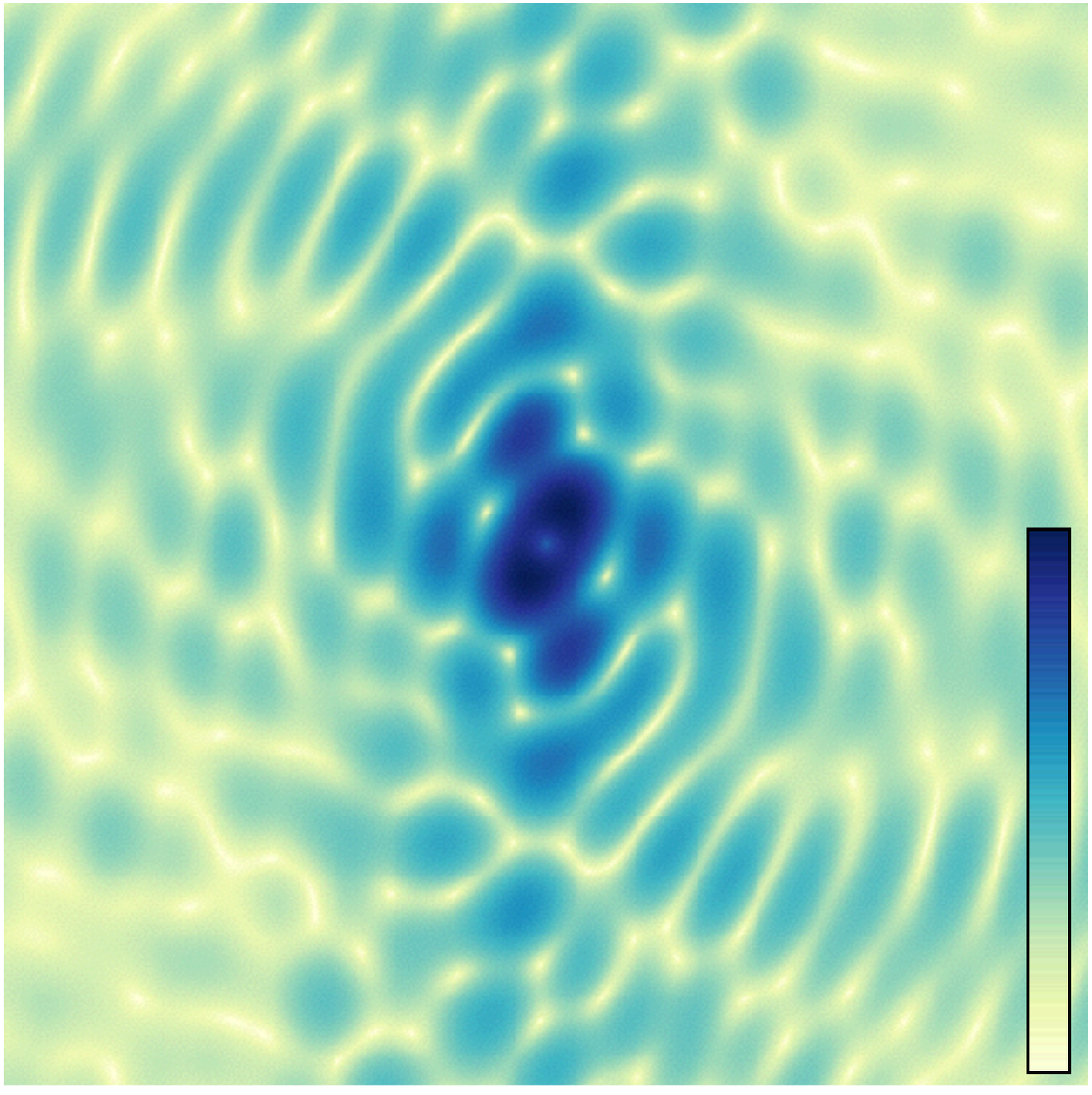
Solution scattering gives single-molecule diffraction, but orientationally averaged



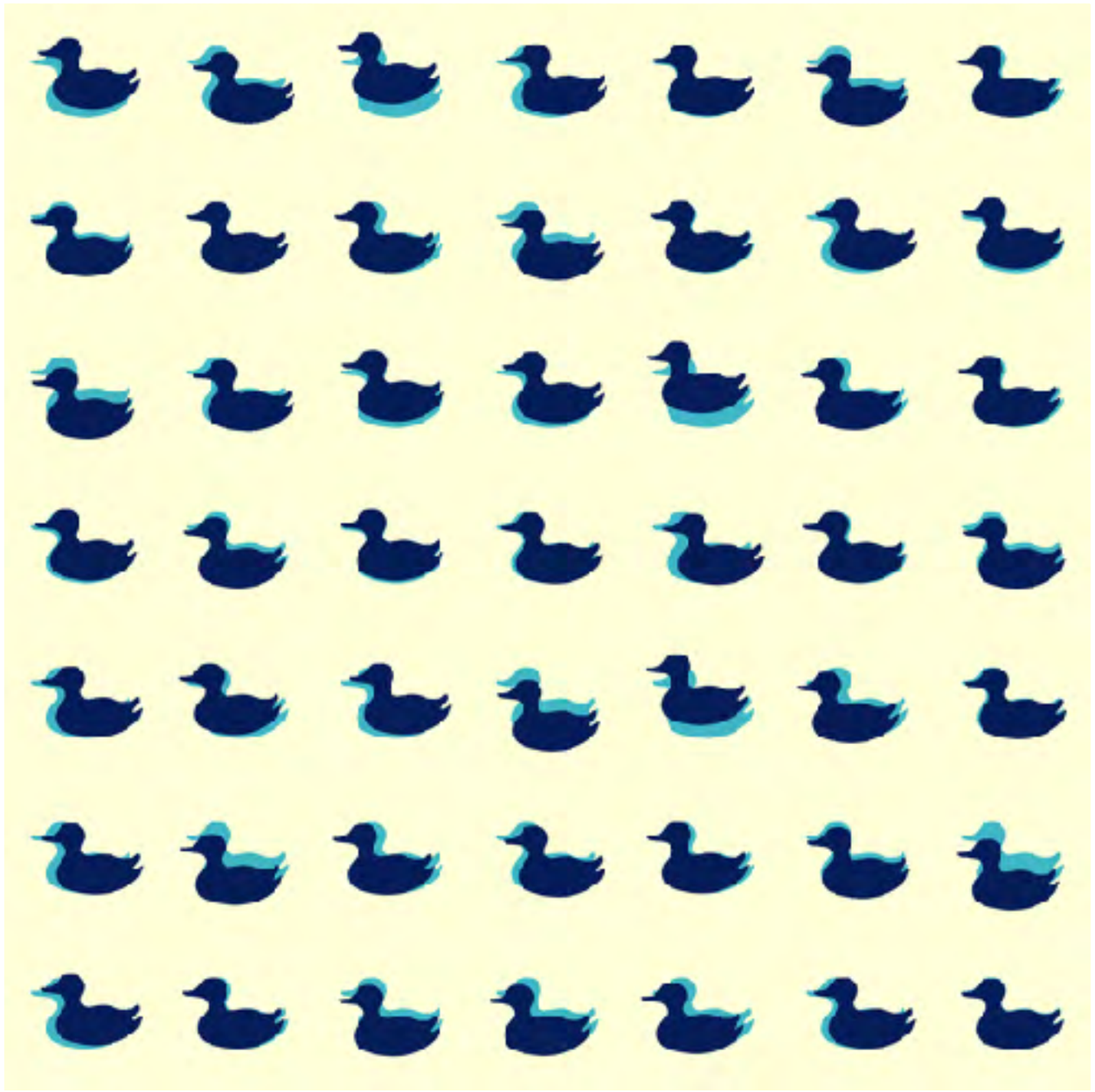


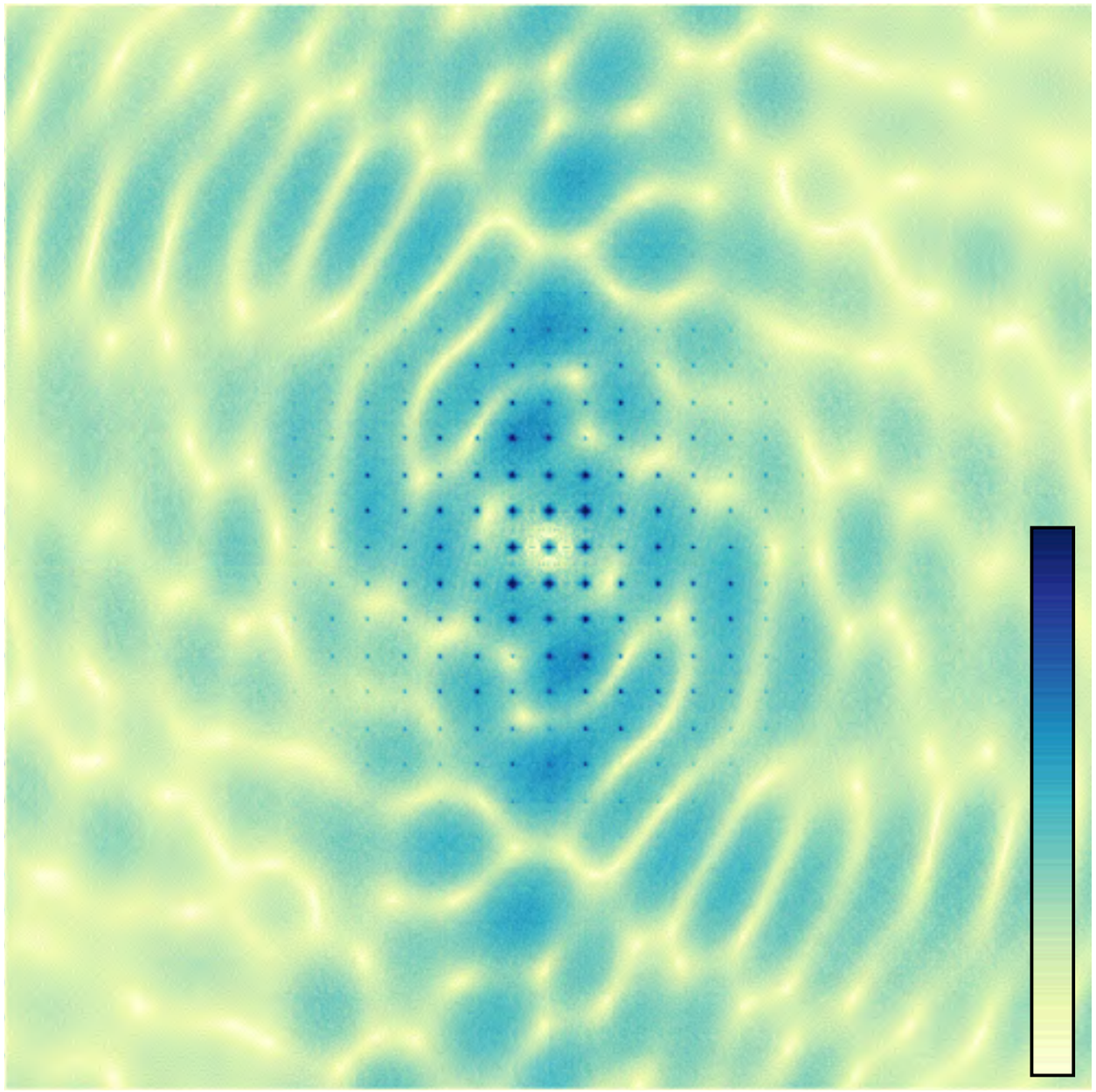
Aligned molecules yield a single-molecule pattern





Crystals provide a very high degree of alignment

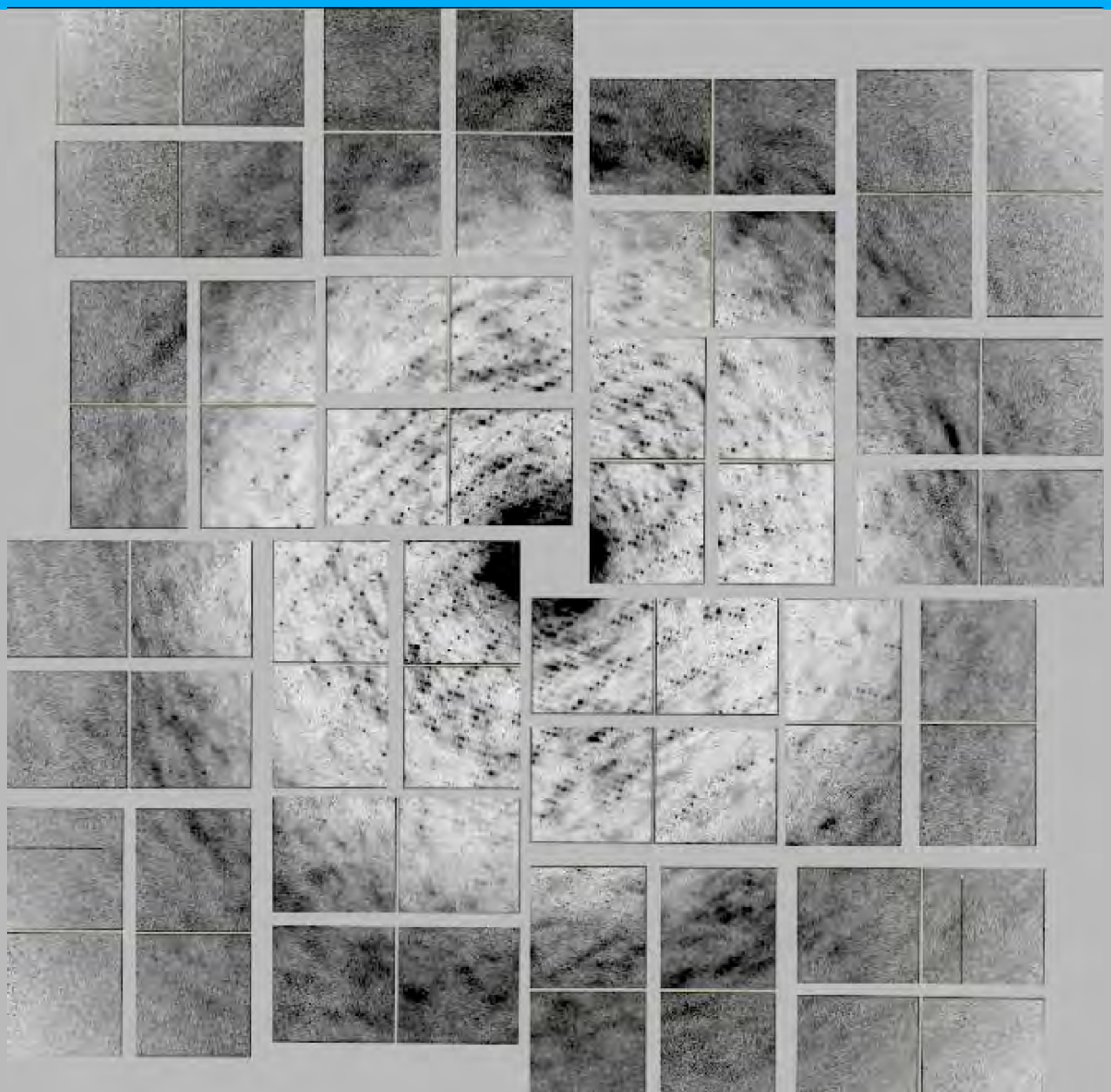


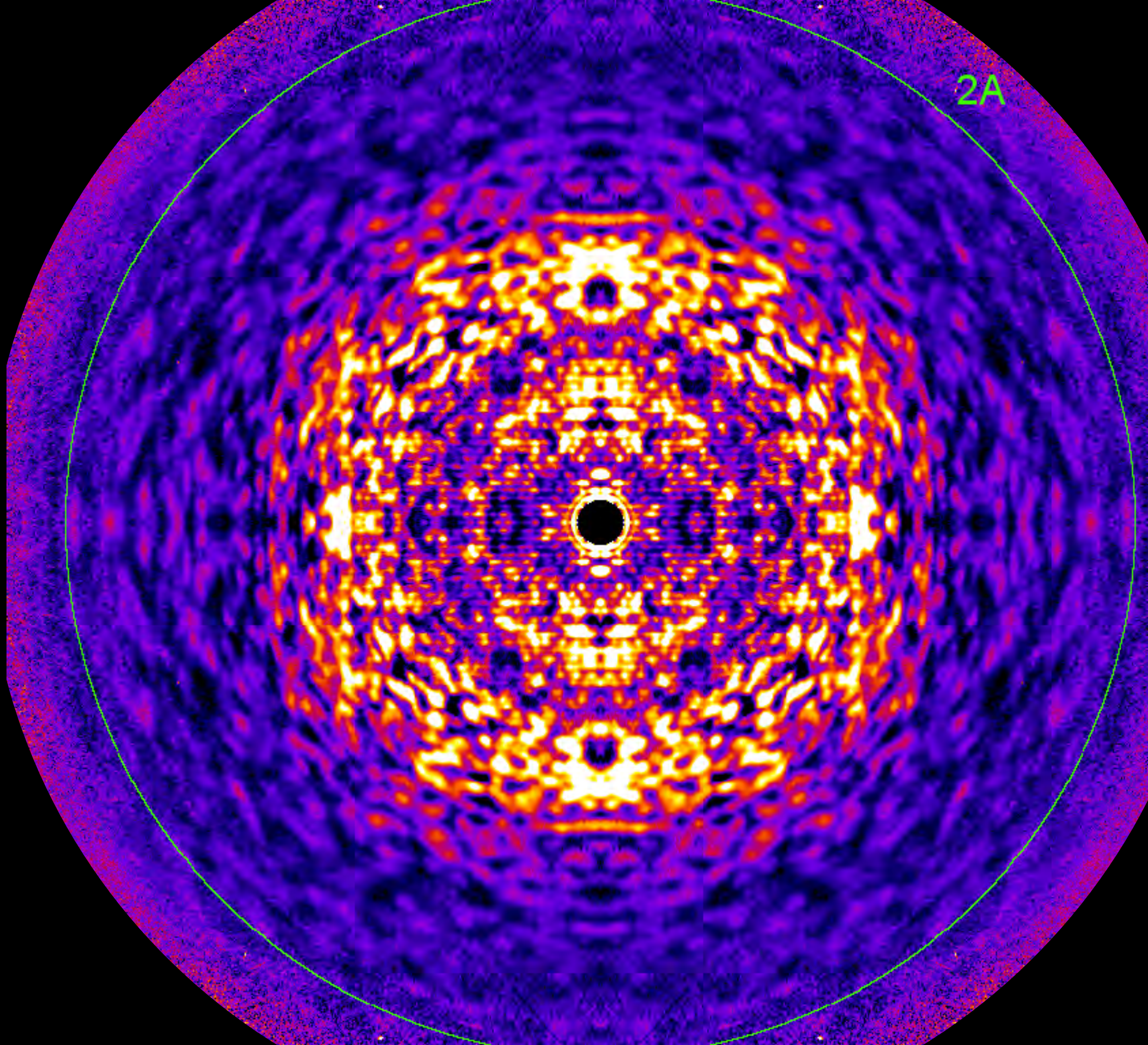


$$\langle I(\mathbf{q})\rangle = \left|\sum_i \hat{\rho}_i(\mathbf{q})\right|^2 \exp(-q^2\sigma^2) + \sum_i |\hat{\rho}_i(\mathbf{q})|^2(1-\exp(-q^2\sigma^2))$$

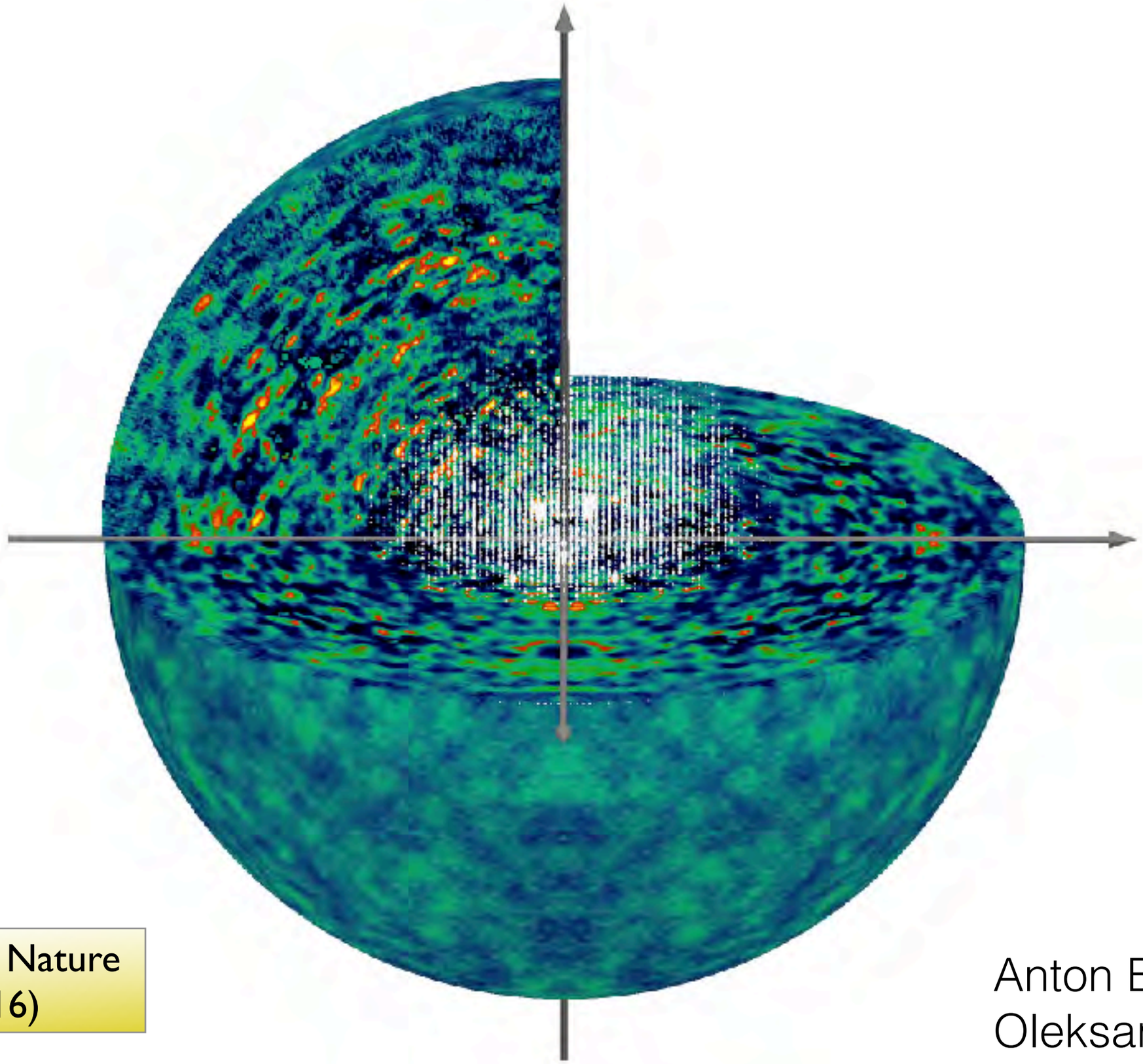
$$\sigma^2 = \langle D^2 \rangle$$

You can see a lot just by looking




2A

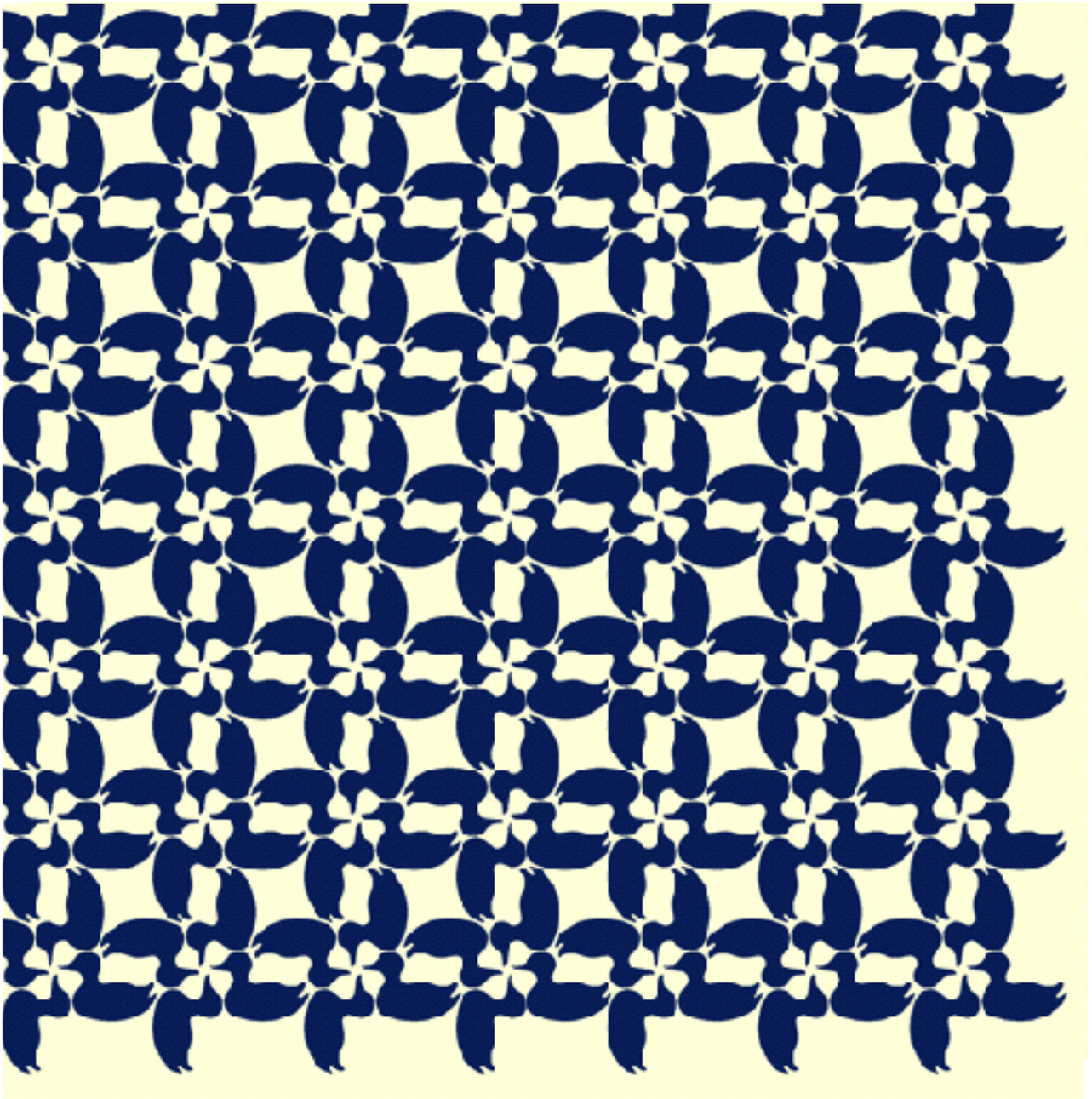
By averaging thousands of patterns a strong single molecule diffraction pattern emerges

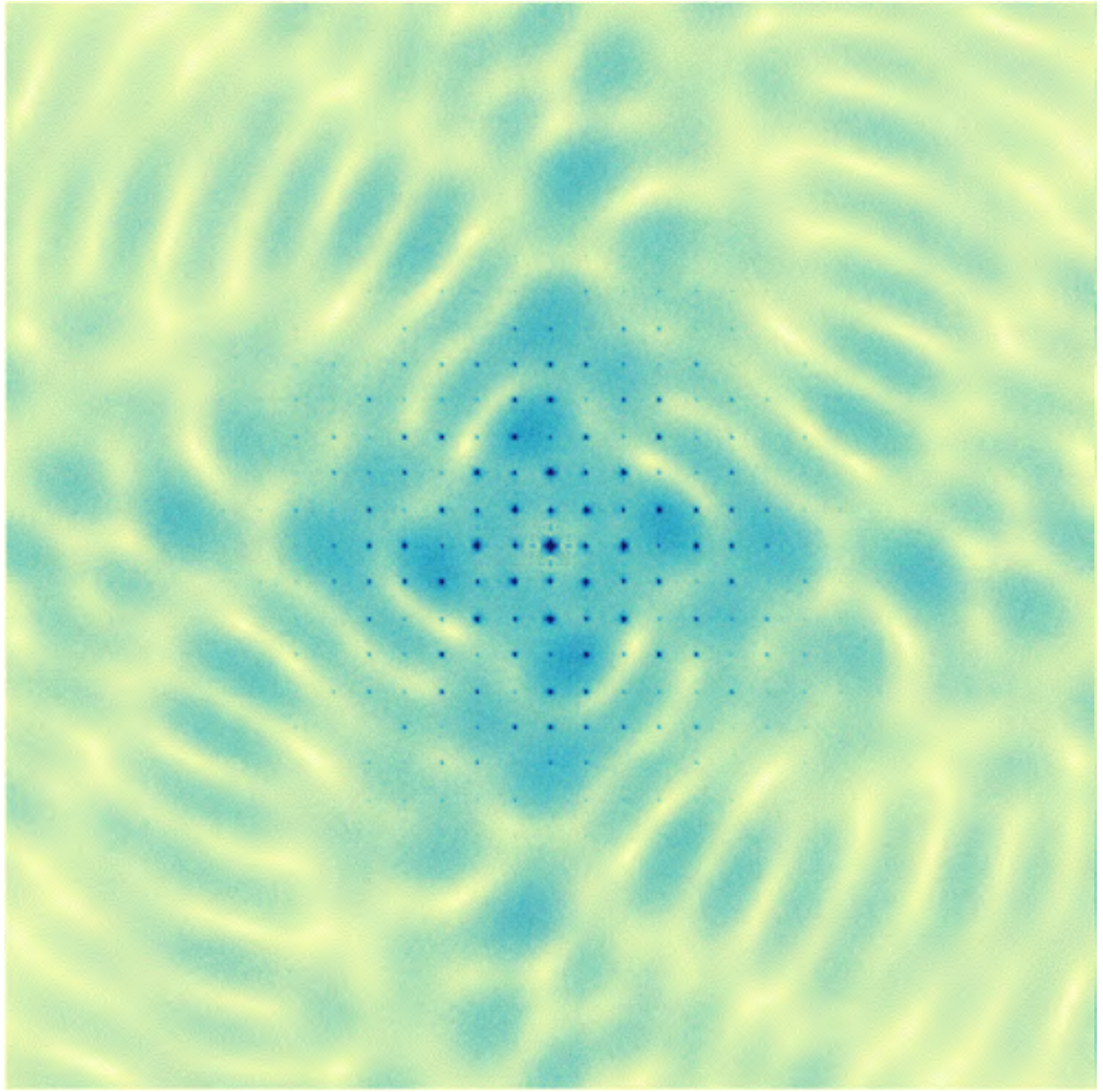


K. Ayyer et al. Nature **530**, 202 (2016)

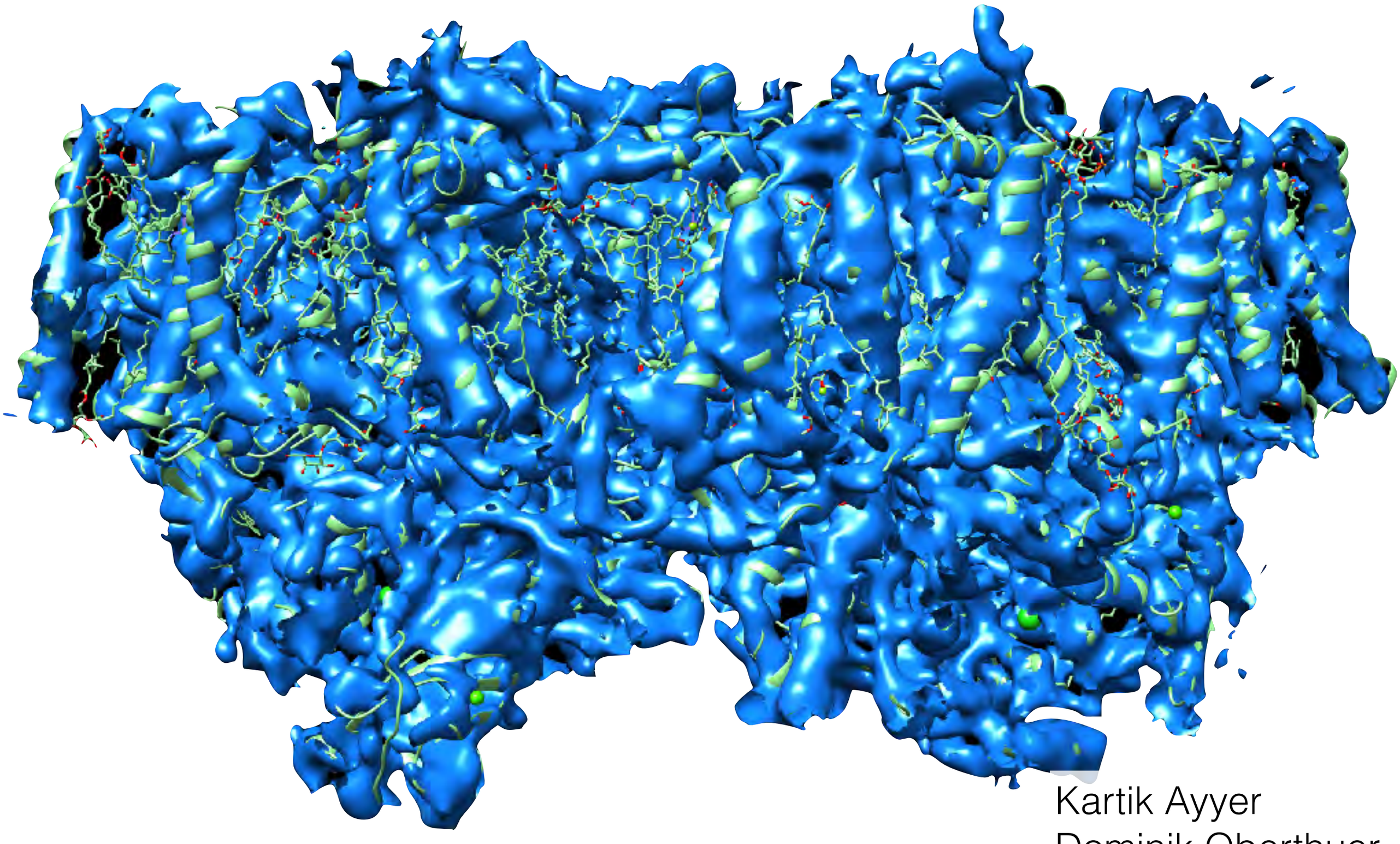
Anton Barty
Oleksandr Yefanov

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





Electron density map from Bragg peaks alone (4.5 Å)



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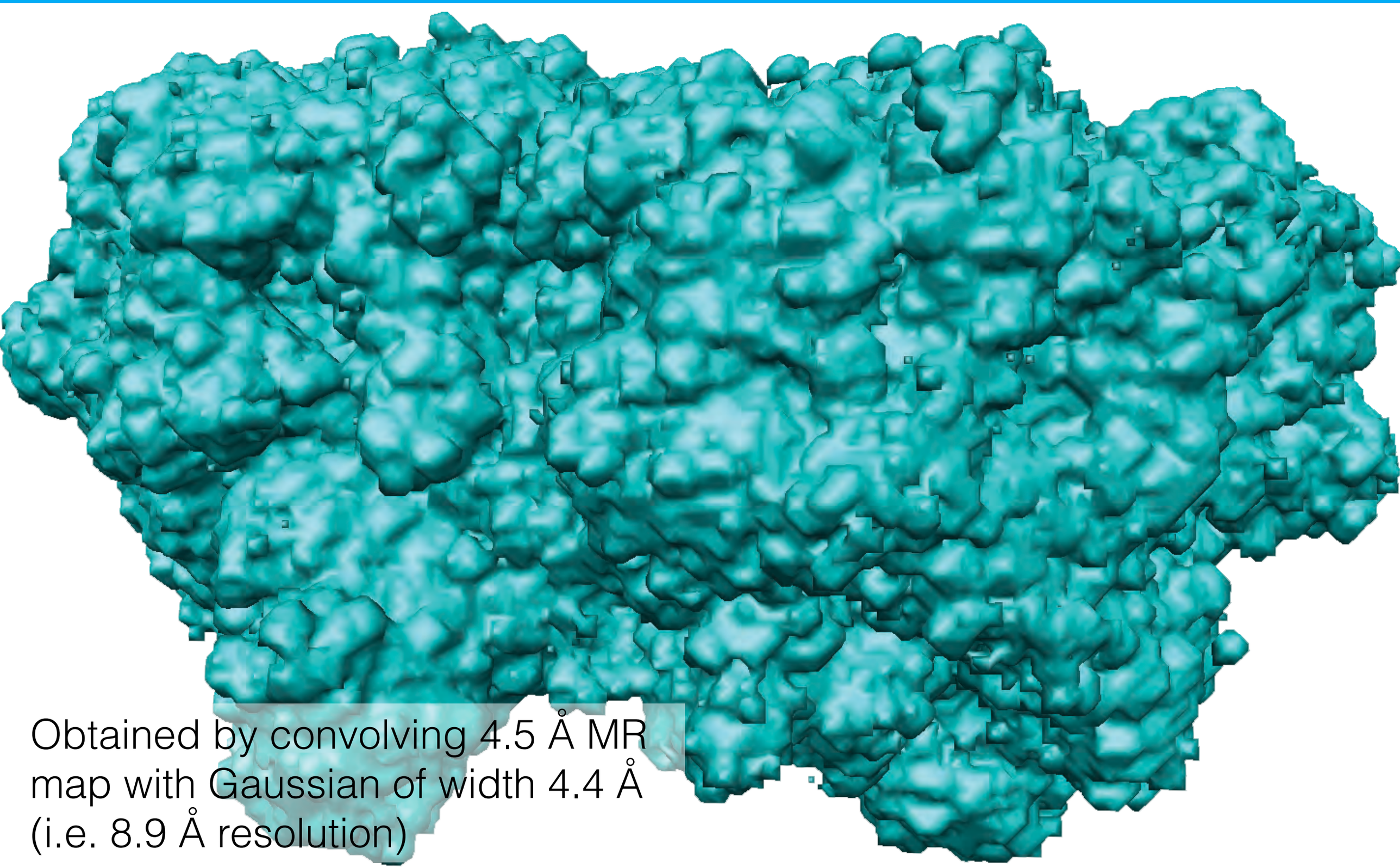
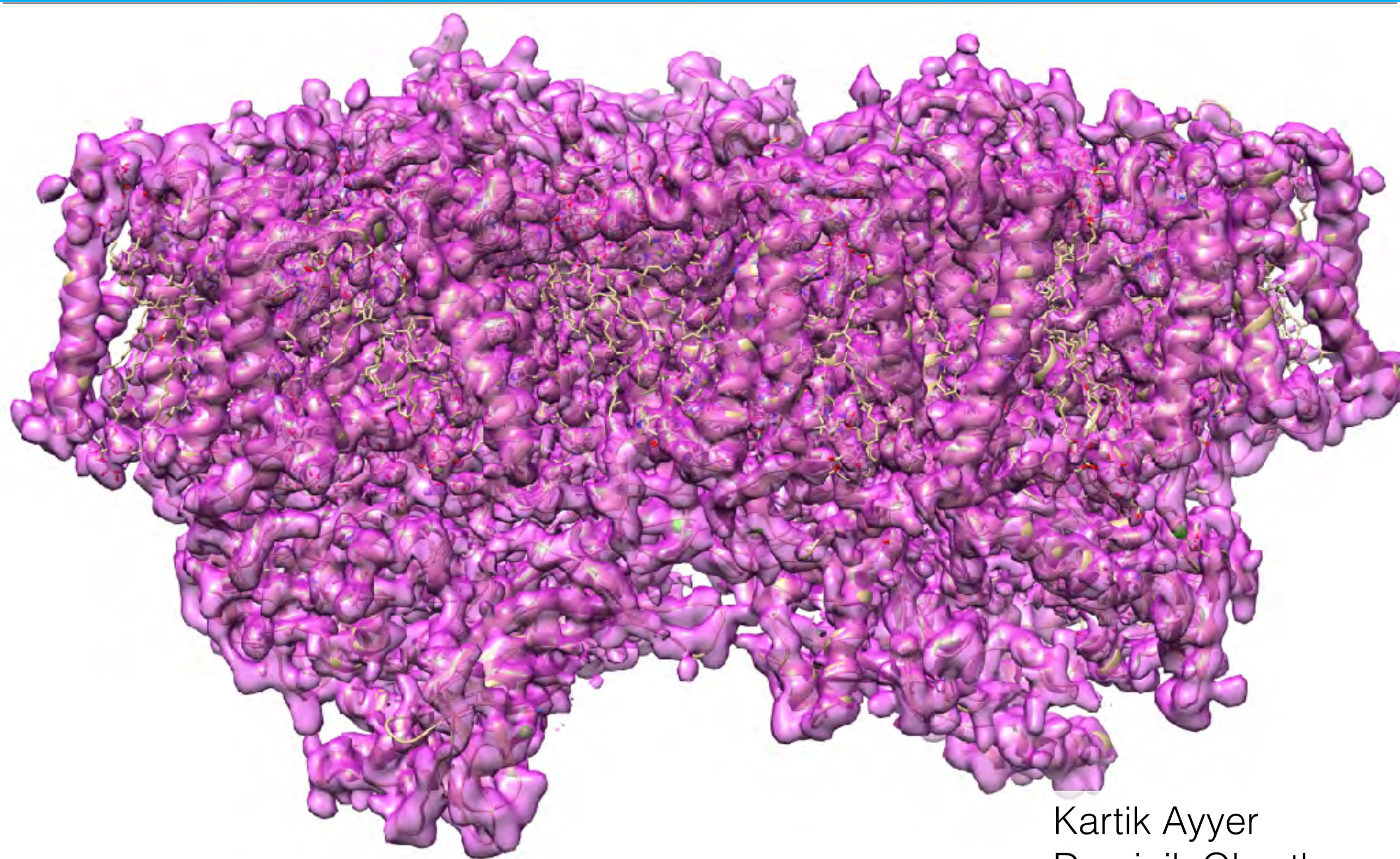
The low-resolution support constrains the phases



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



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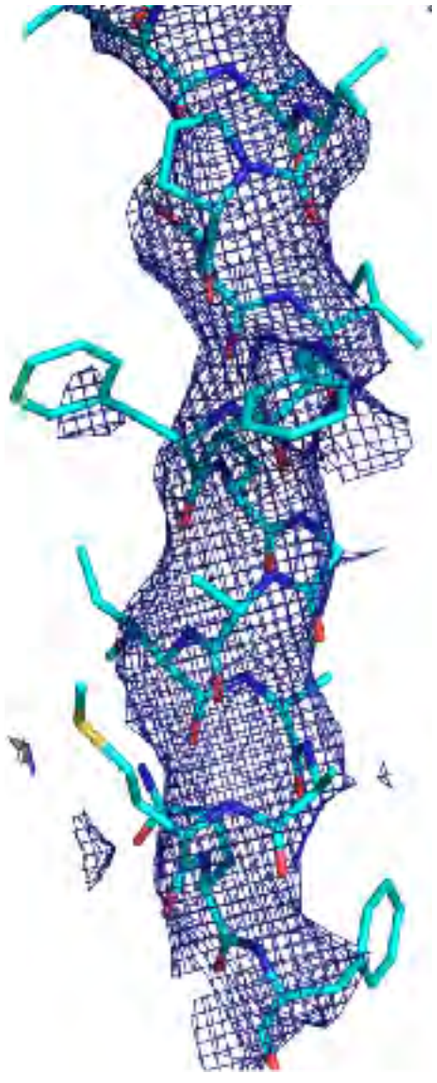
The extended-resolution structure is superior

Bragg only
(4.5 Å)



Bragg and
continuous
(3.5 Å)

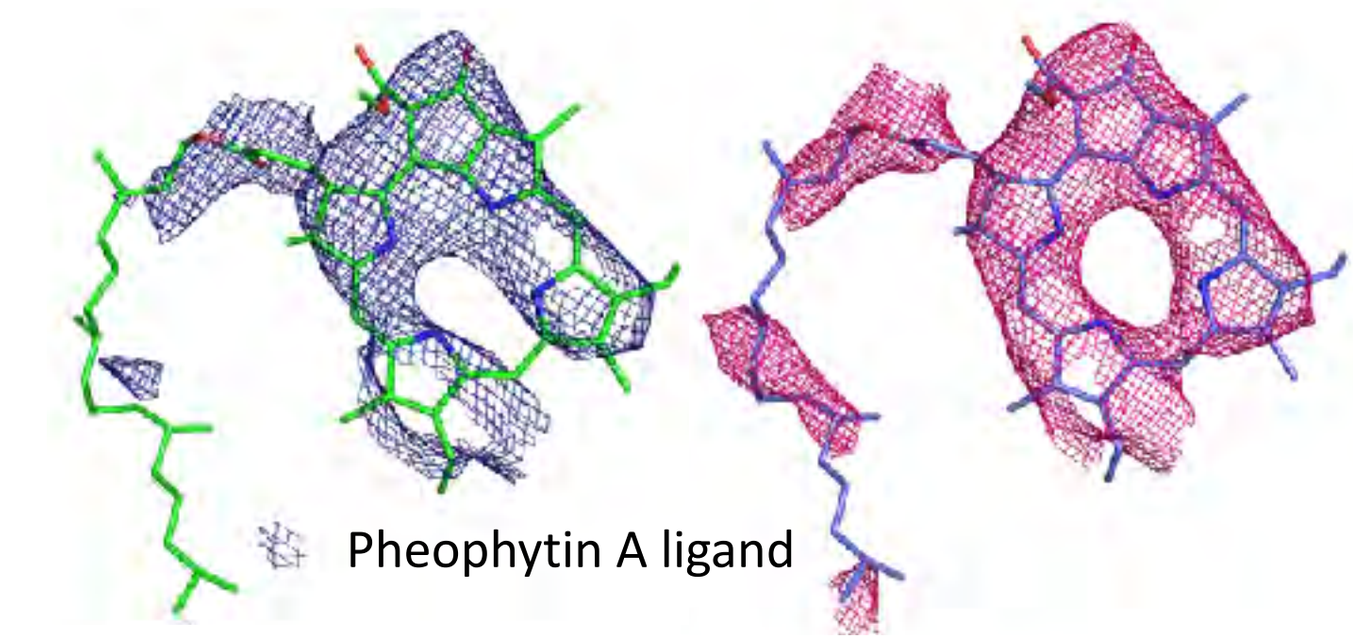
Higher diffraction sampling — model free phasing
— more reliable structure determination

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

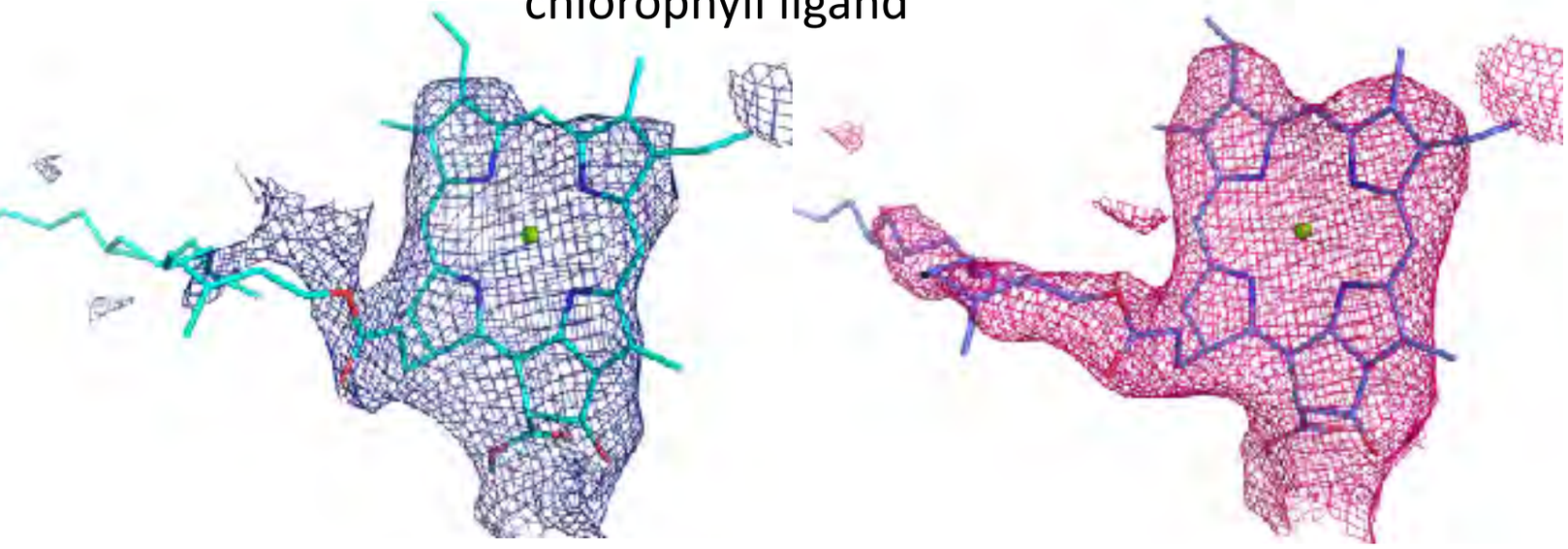
The extended-resolution structure is superior

Bragg only (4.5 Å)

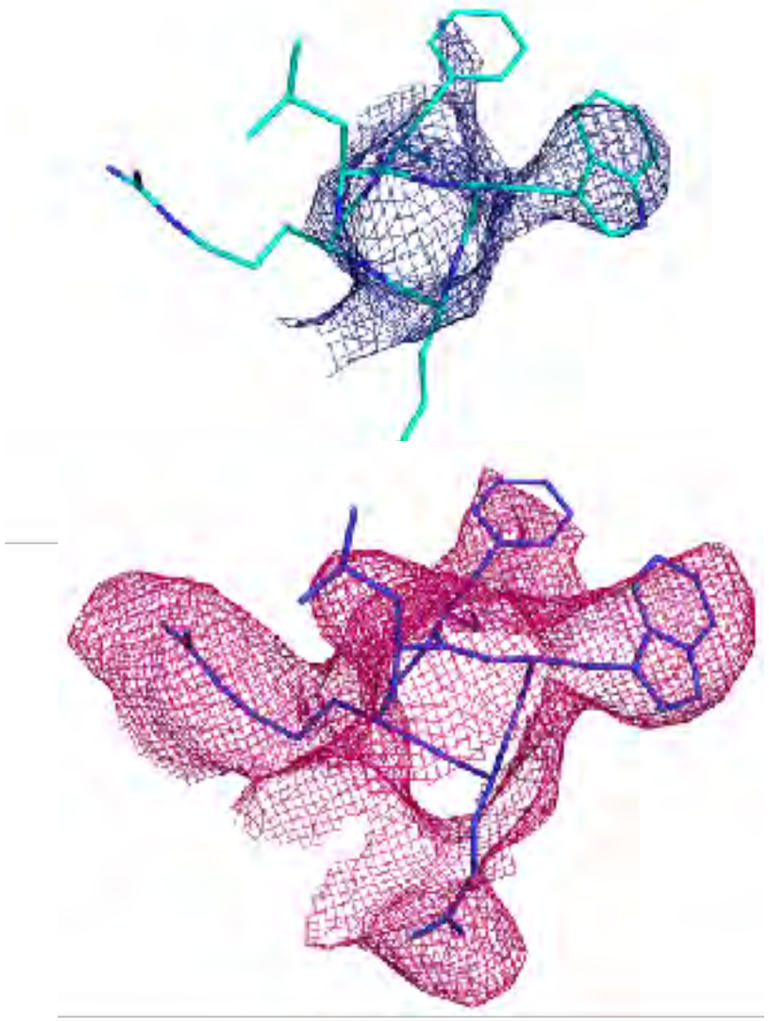


Pheophytin A ligand

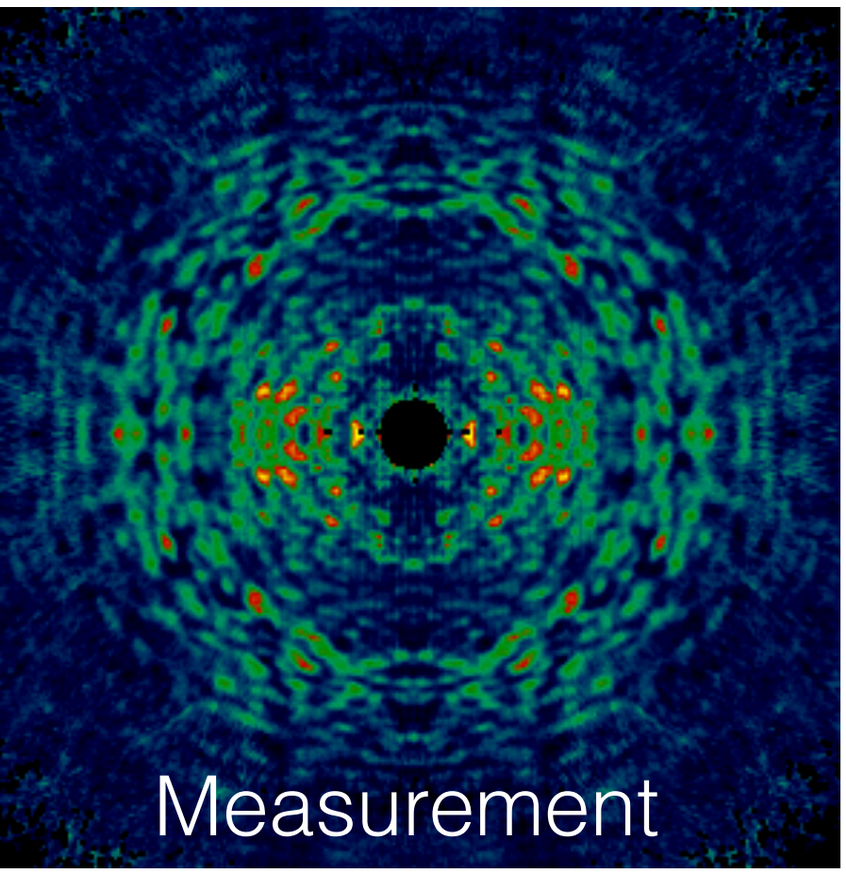
chlorophyll ligand

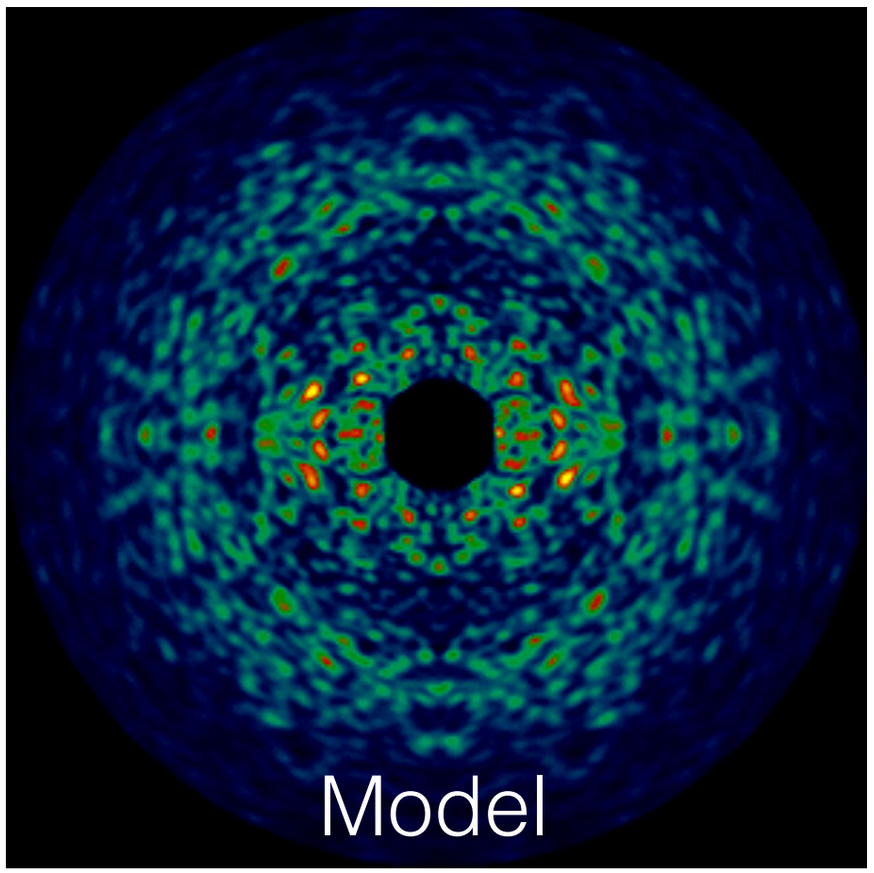


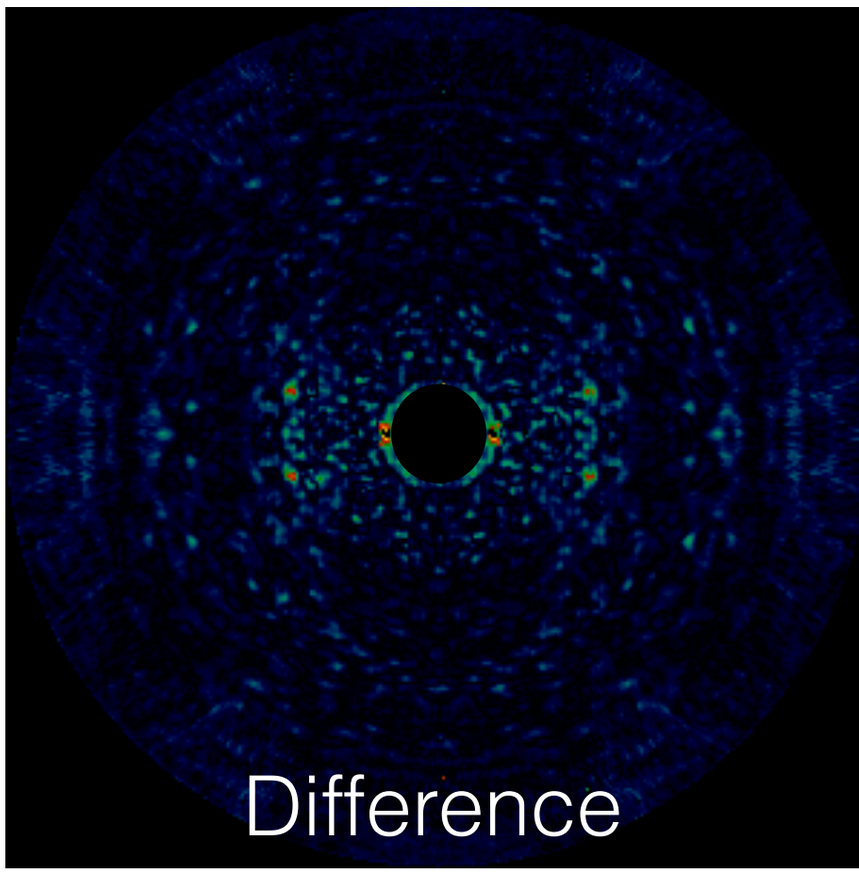
Bragg and continuous (3.5 Å)



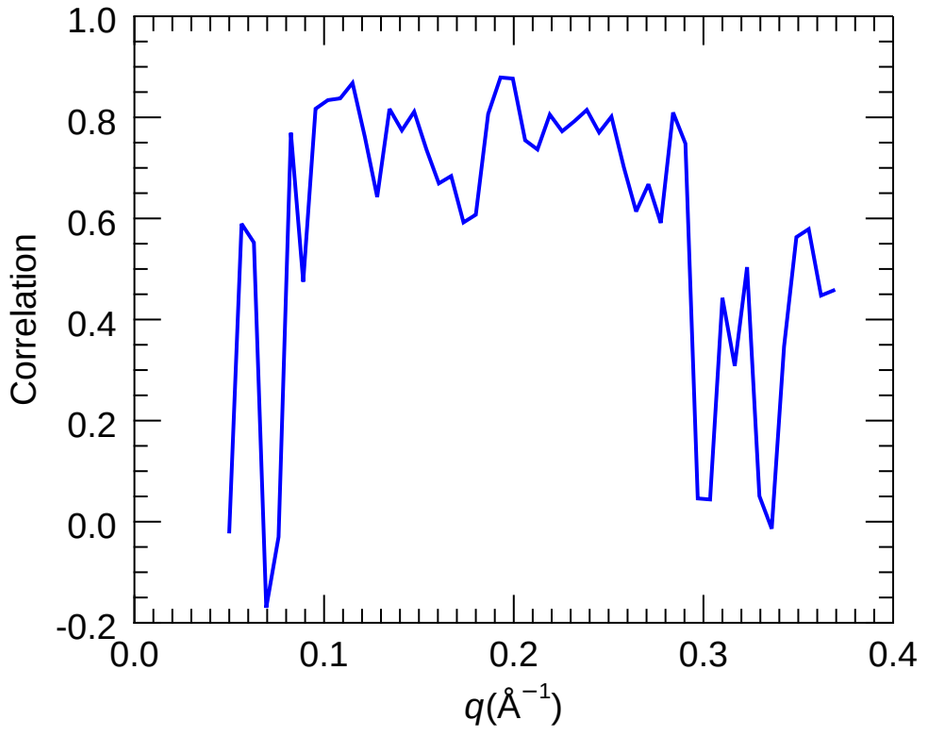
The continuous diffraction agrees with the simulated diffraction from the atomic model



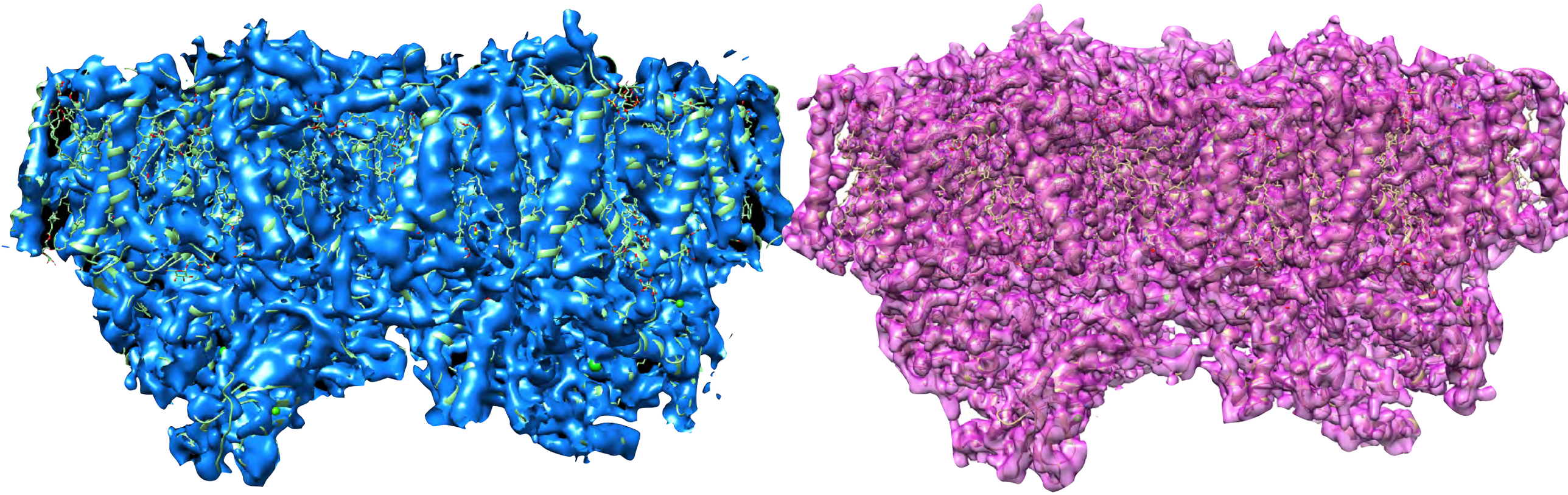




Cross Correlation = 75%



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



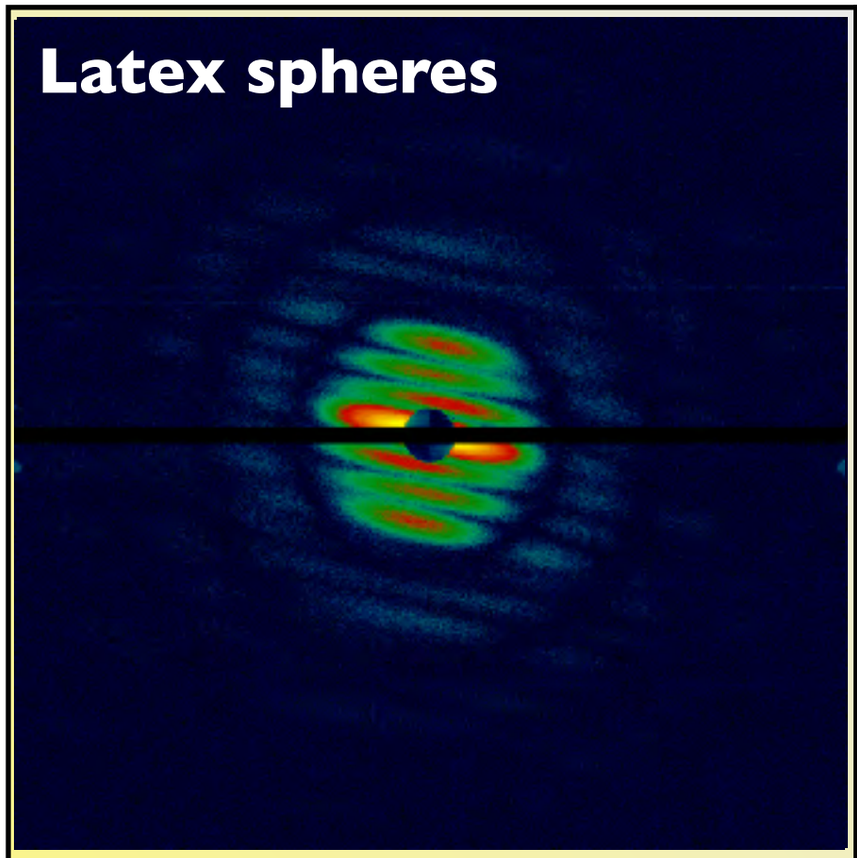
Measurements require care to eliminate background and record weak continuous diffraction

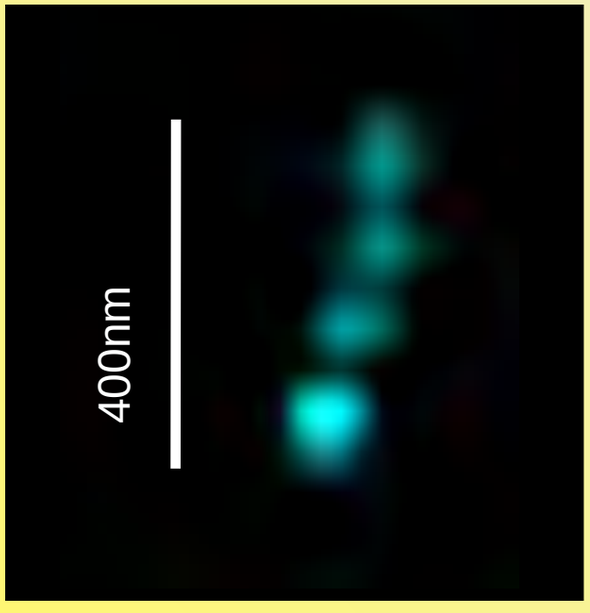
Poorly diffracting crystals are better!

- 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

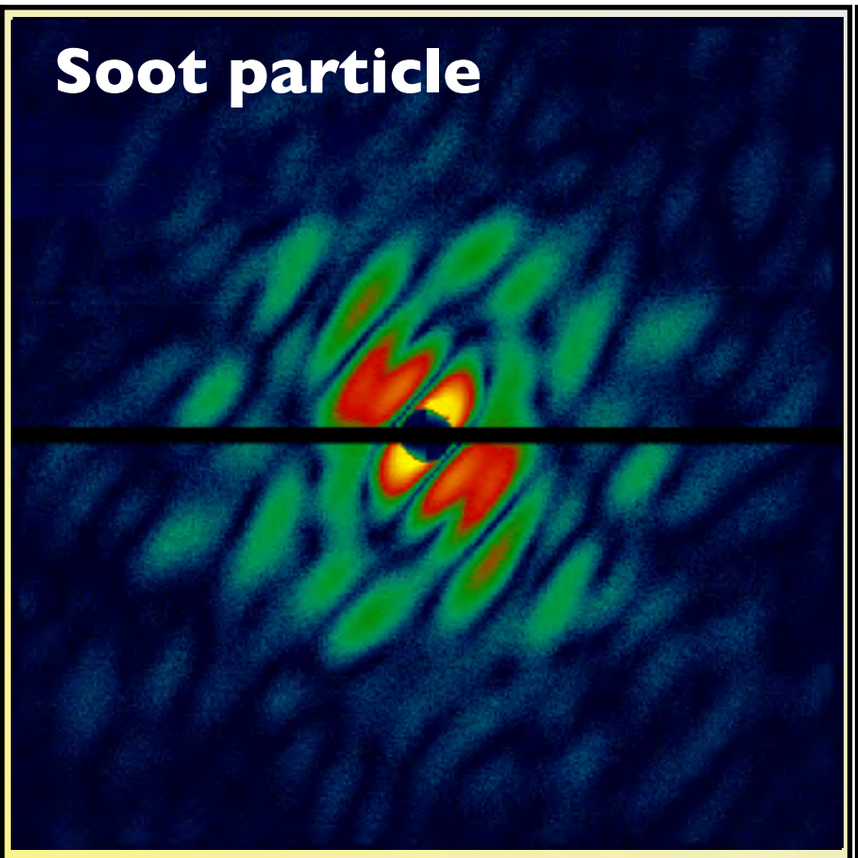
We can reconstruct images of soot, viruses, and nanoparticles

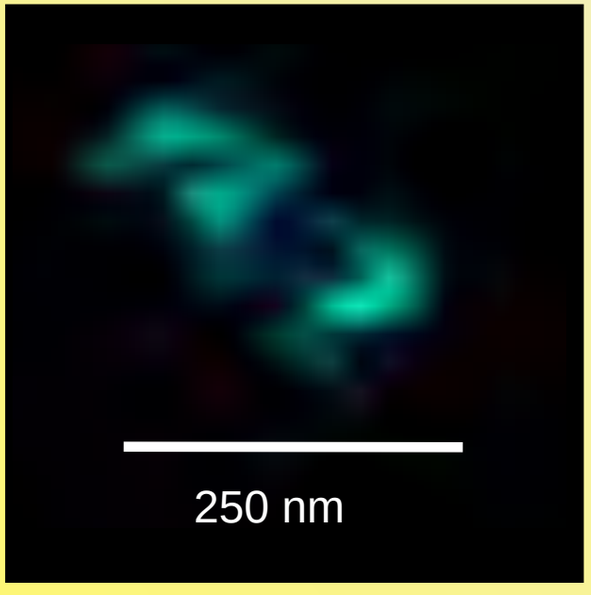
Latex spheres



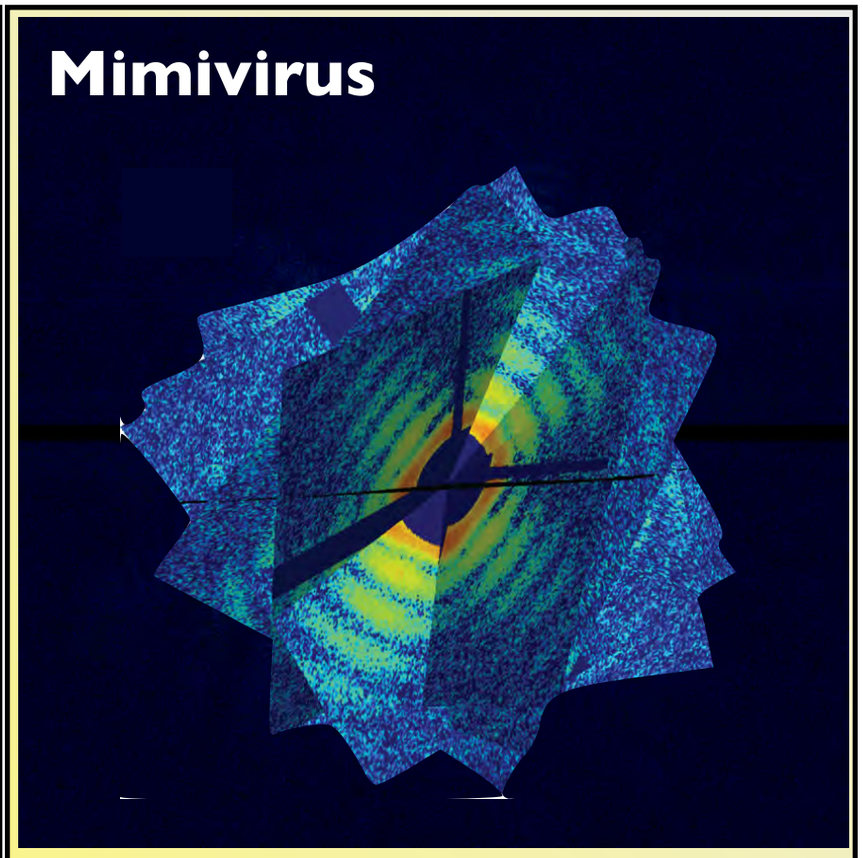


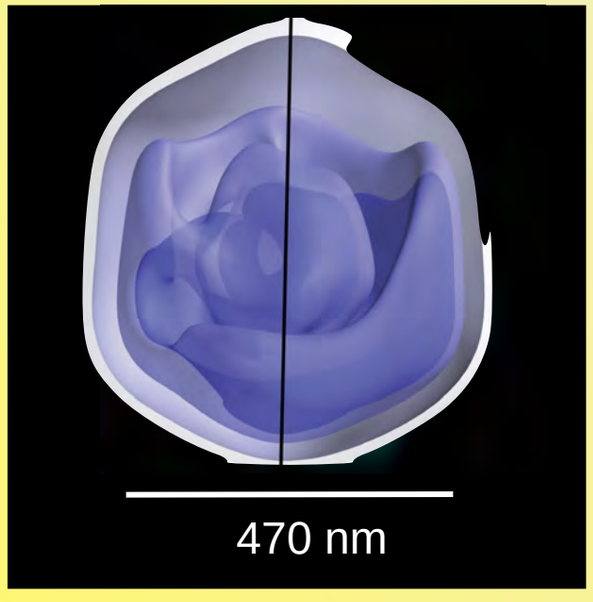
Soot particle





Mimivirus





Reconstructions: Andrew Martin

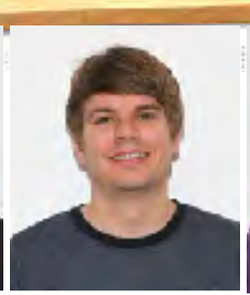
Ekeberg et al PRL **114** (2015)

Coherent X-ray Imaging at CFEL

Kartik Ayyer

Anton Barty

Oleksandr Yefanov



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