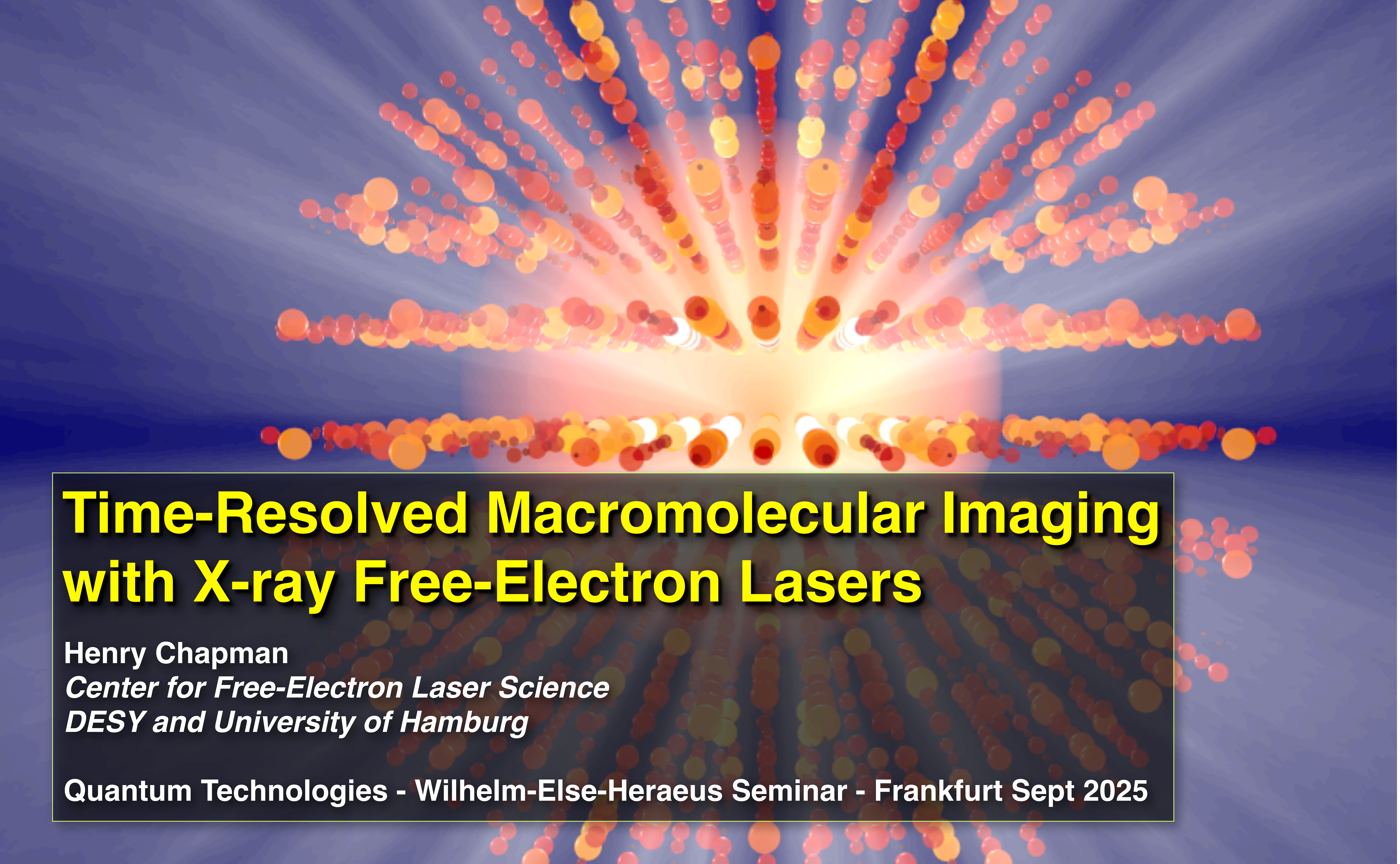


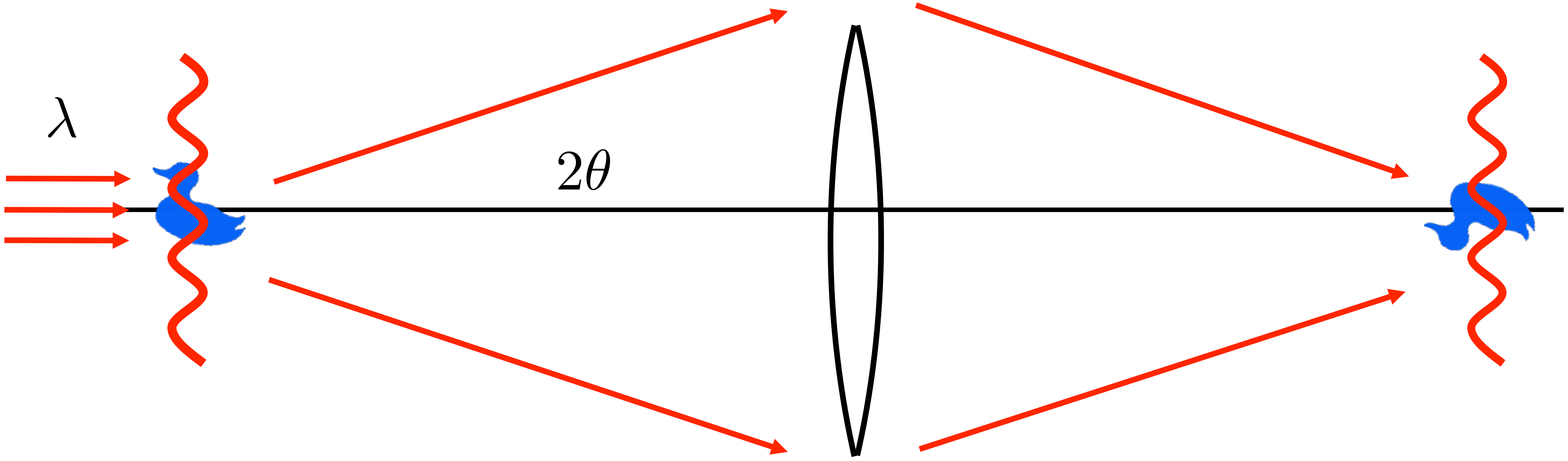
Time-Resolved Macromolecular Imaging with X-ray Free-Electron Lasers

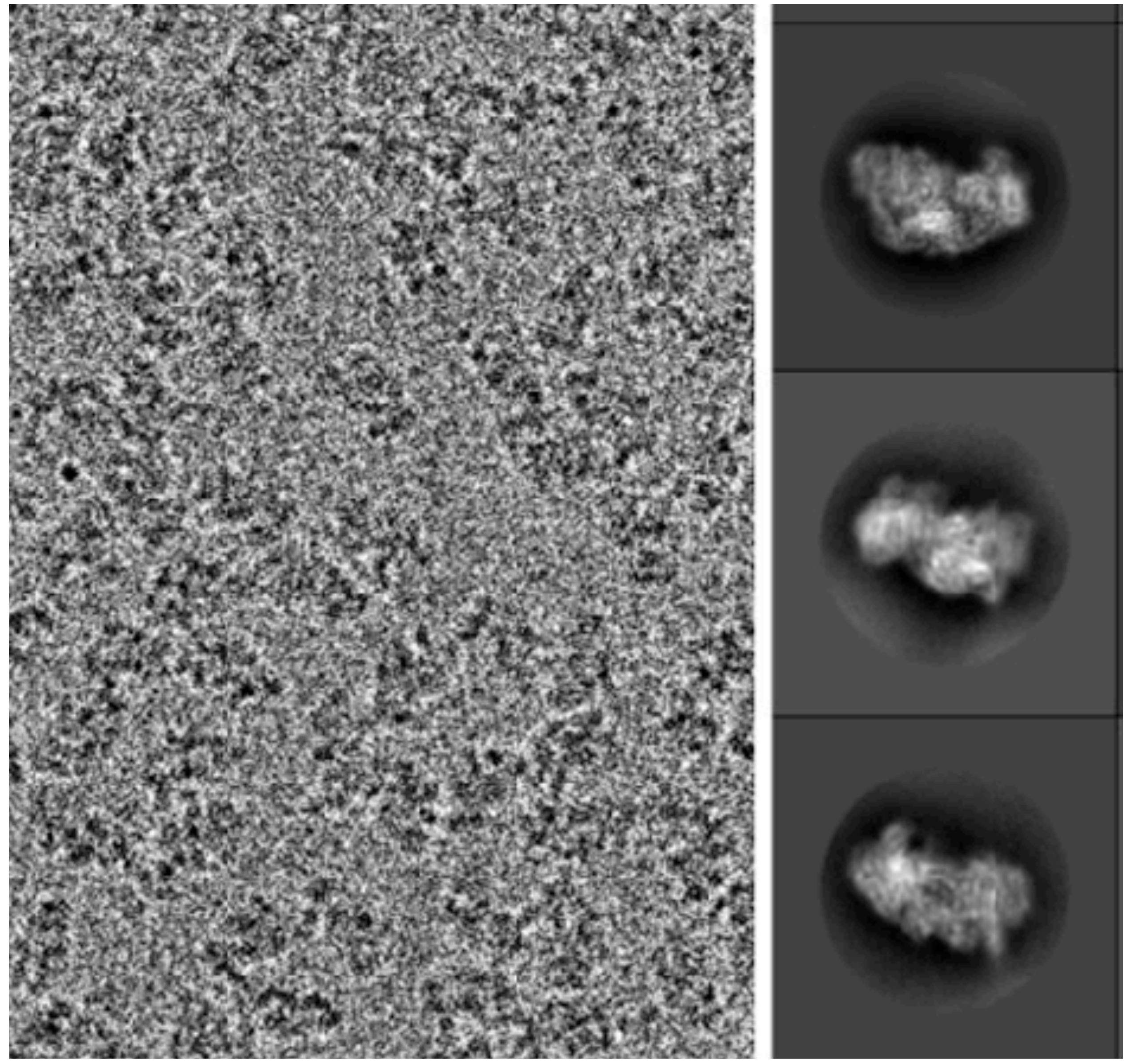
Henry Chapman
Center for Free-Electron Laser Science
DESY and University of Hamburg

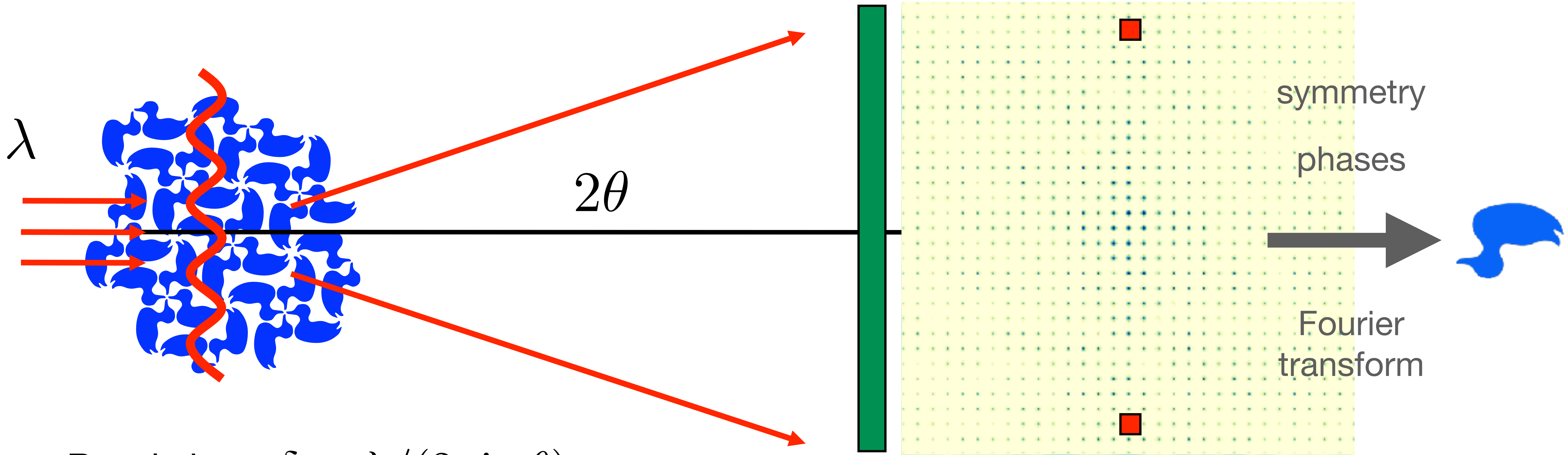
Quantum Technologies - Wilhelm-Else-Heraeus Seminar - Frankfurt Sept 2025

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

Imaging can be achieved with or without a lens



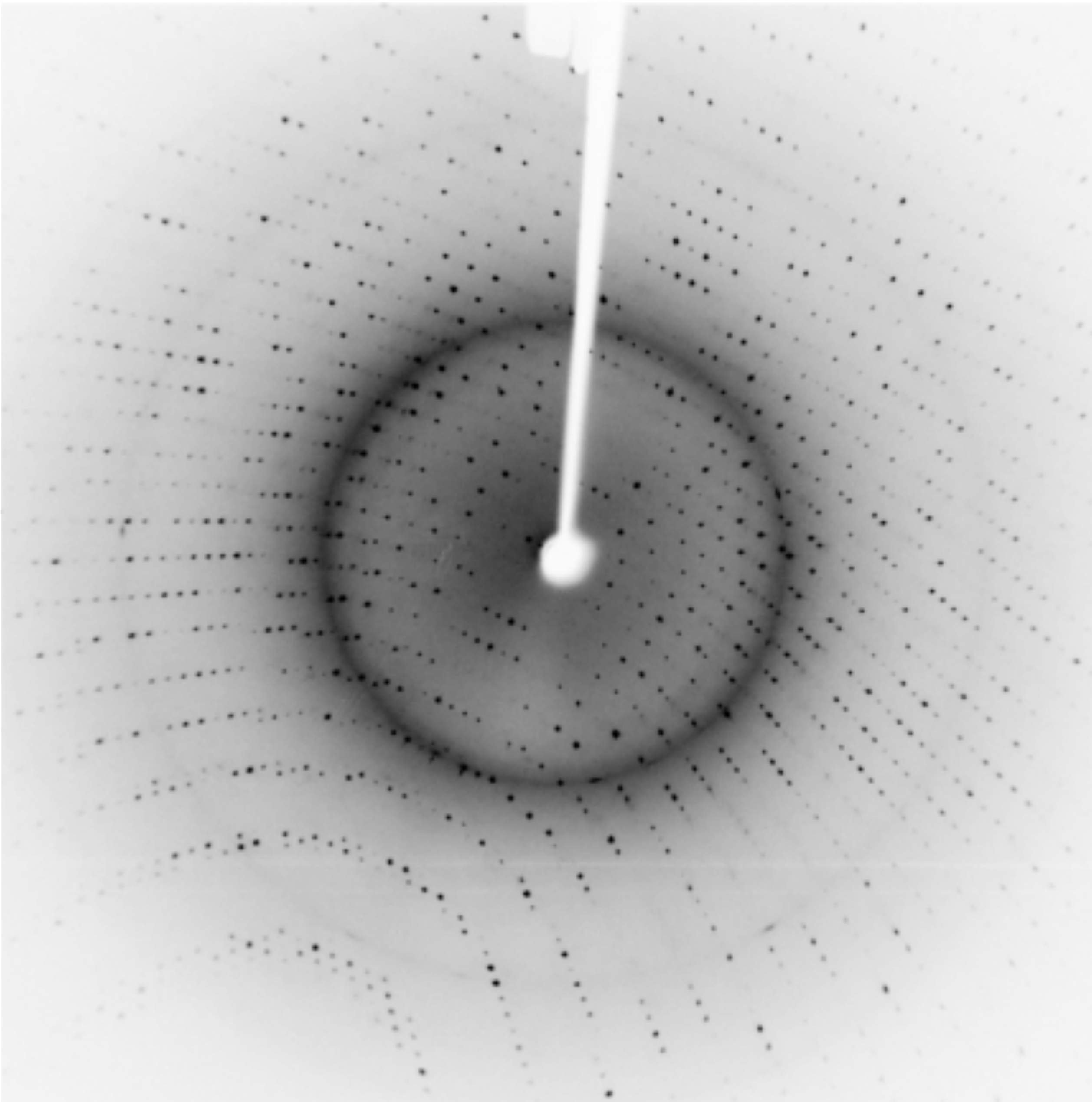




symmetry

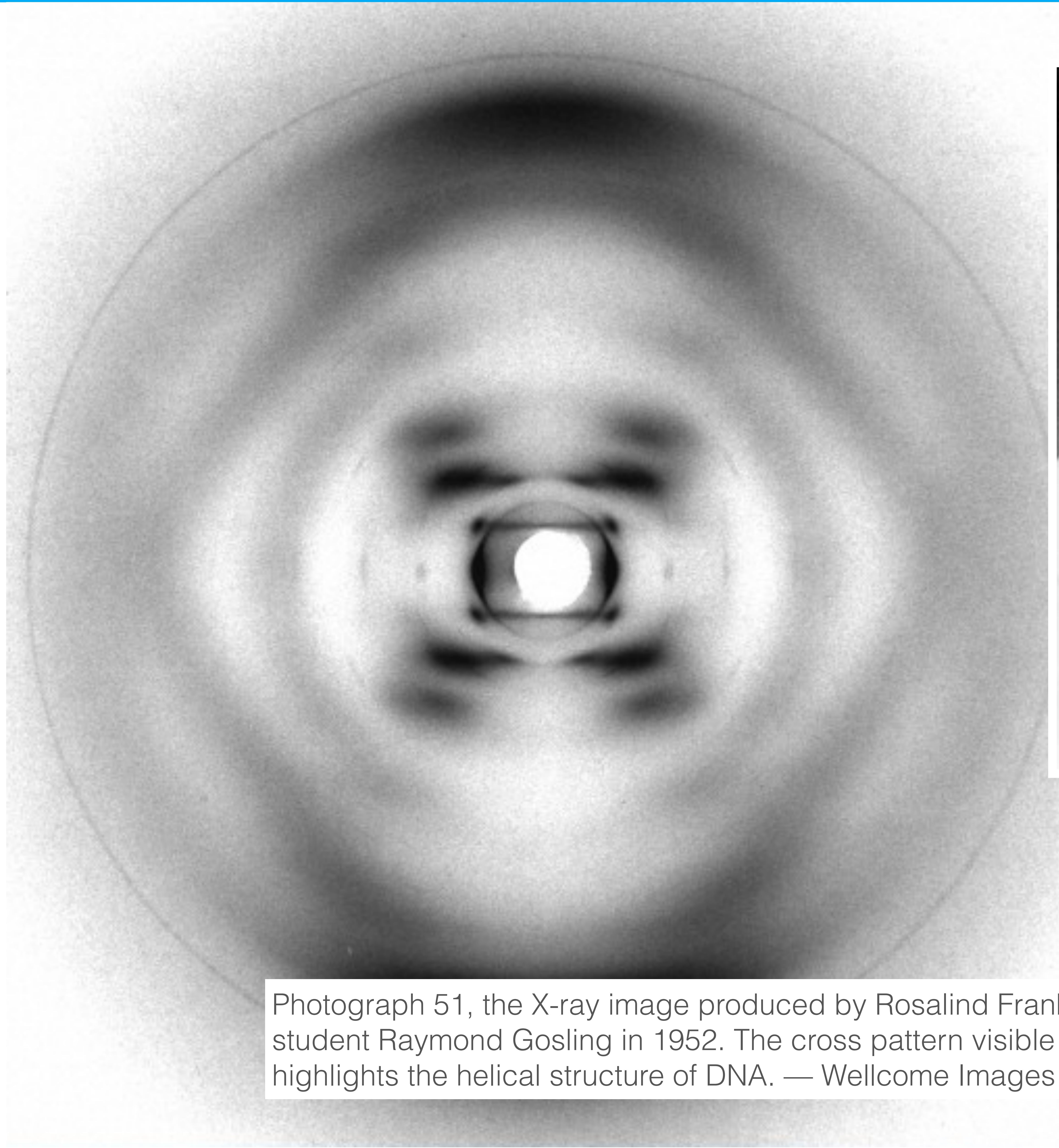
phases

Fourier transform



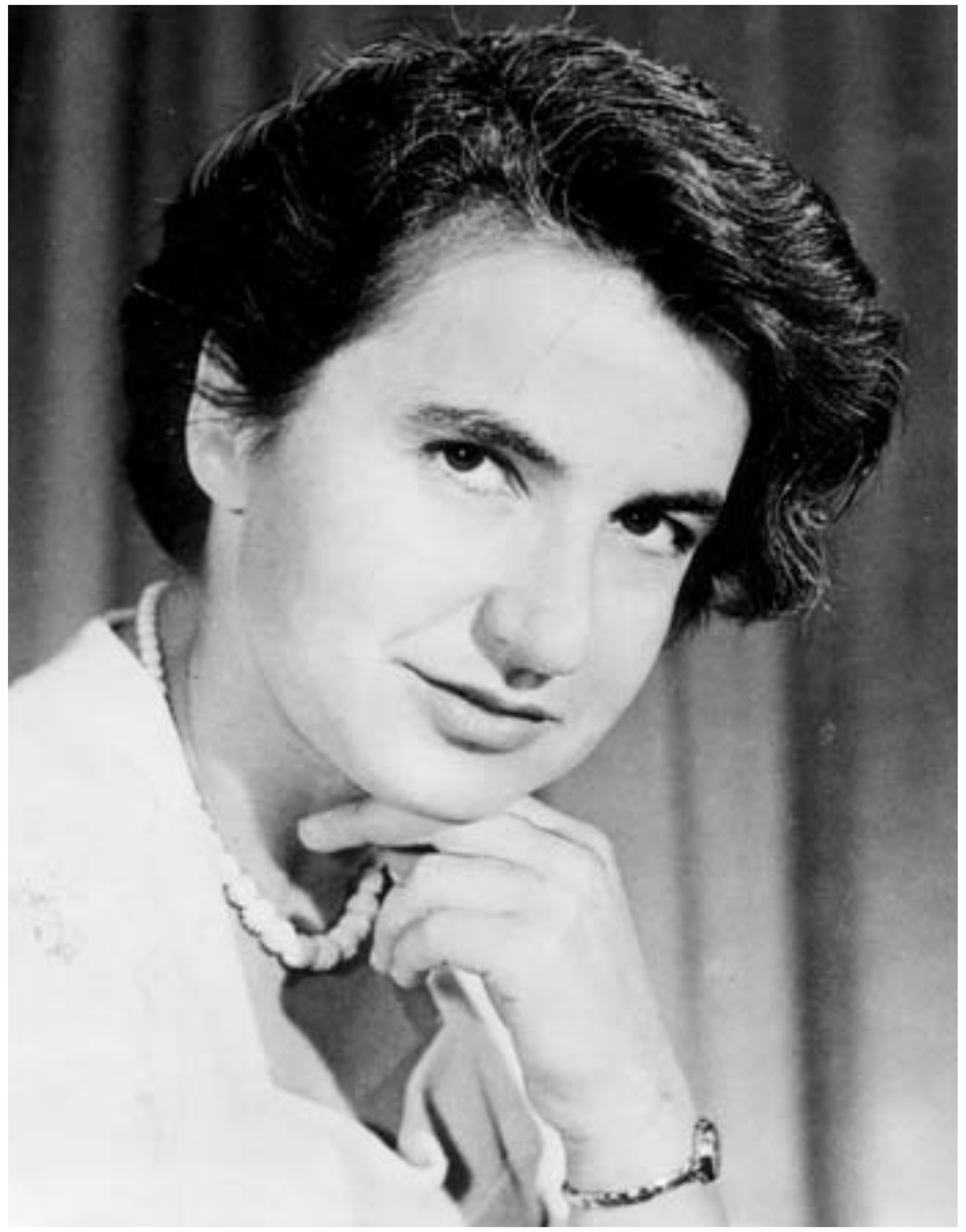
Resolution: $\delta = \lambda/(2\sin\theta)$

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

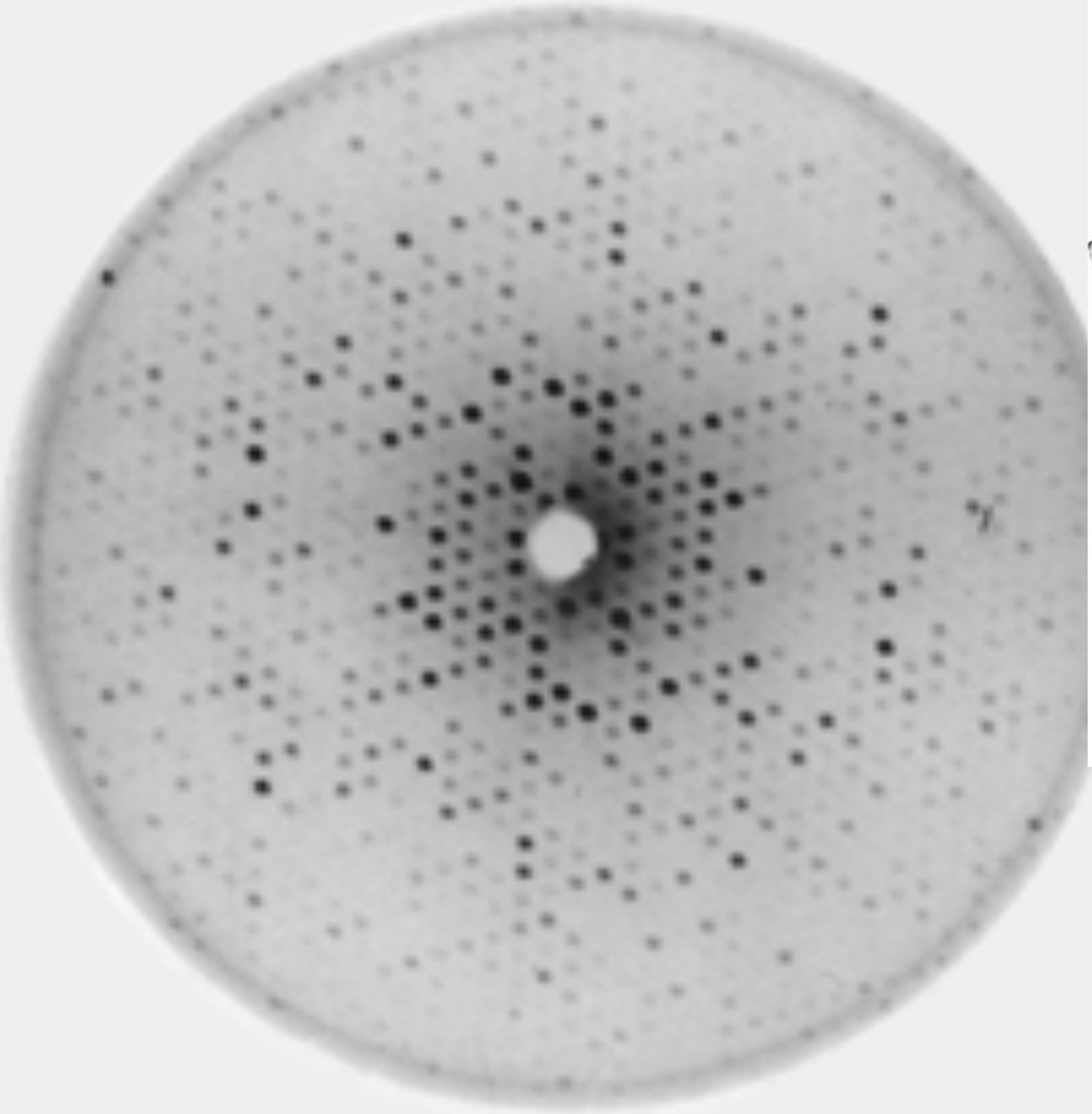
Rosalind Franklin

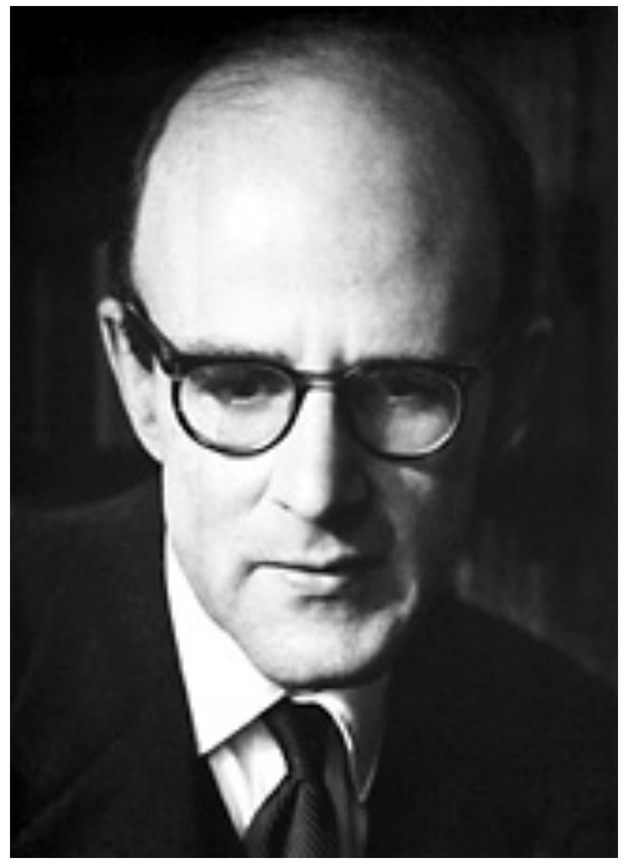


James Watson &
Francis Crick

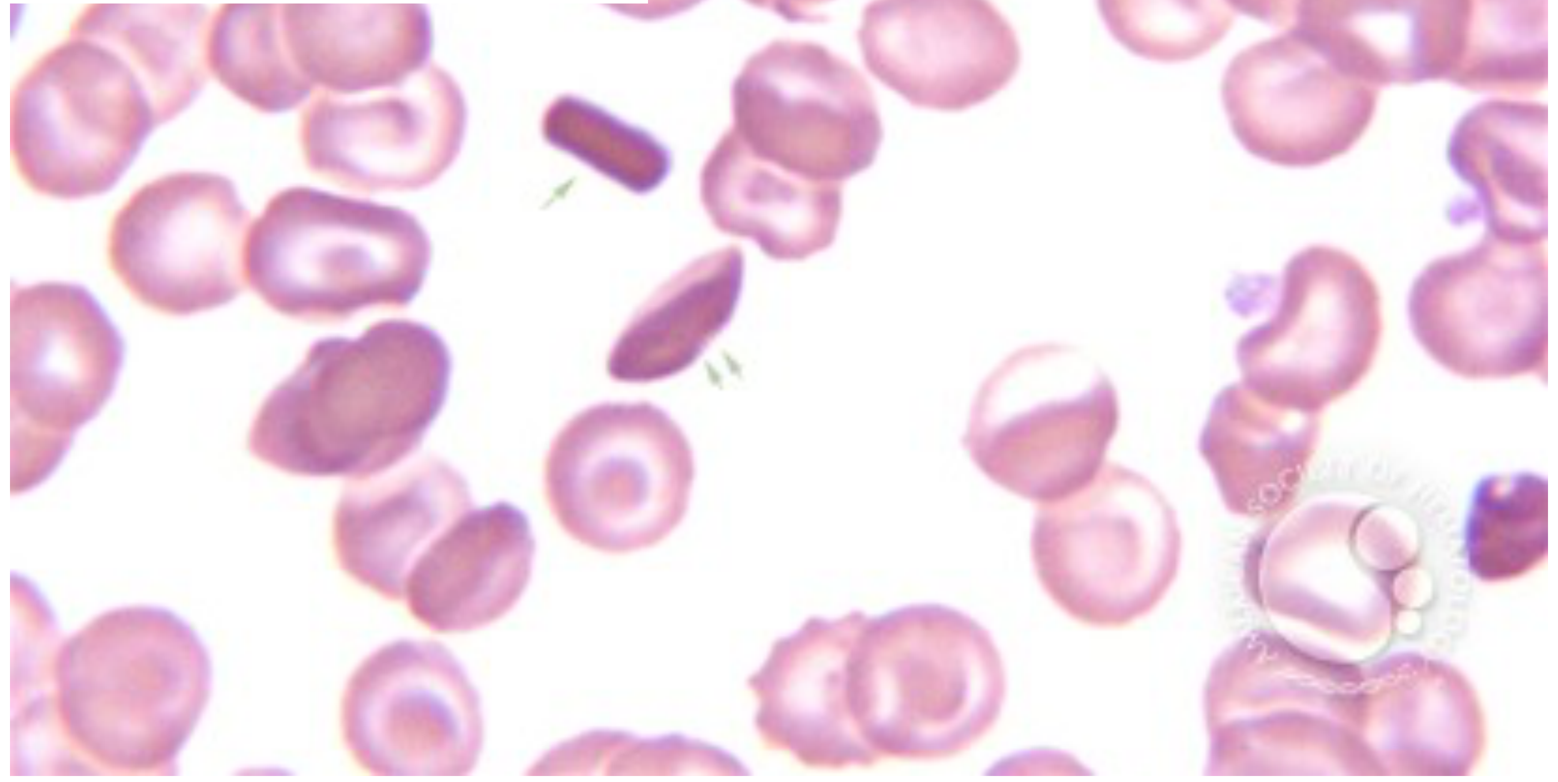
http://dataphys.org/list/watson-and-cricks-3d-model-of-dna/

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

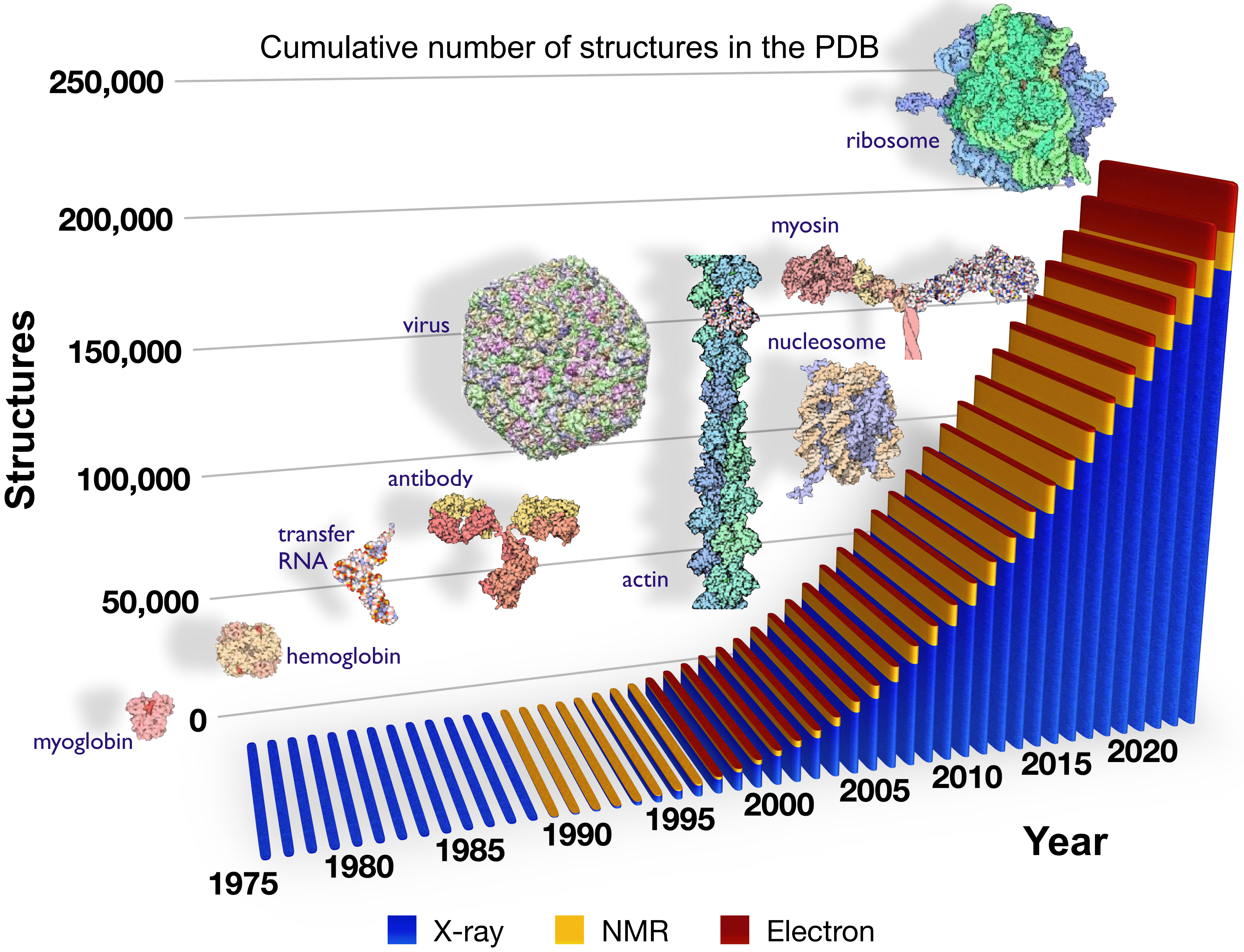




Max Perutz



Over 200,000 macromolecular structures have been deposited in the PDB, providing a database for deep learning



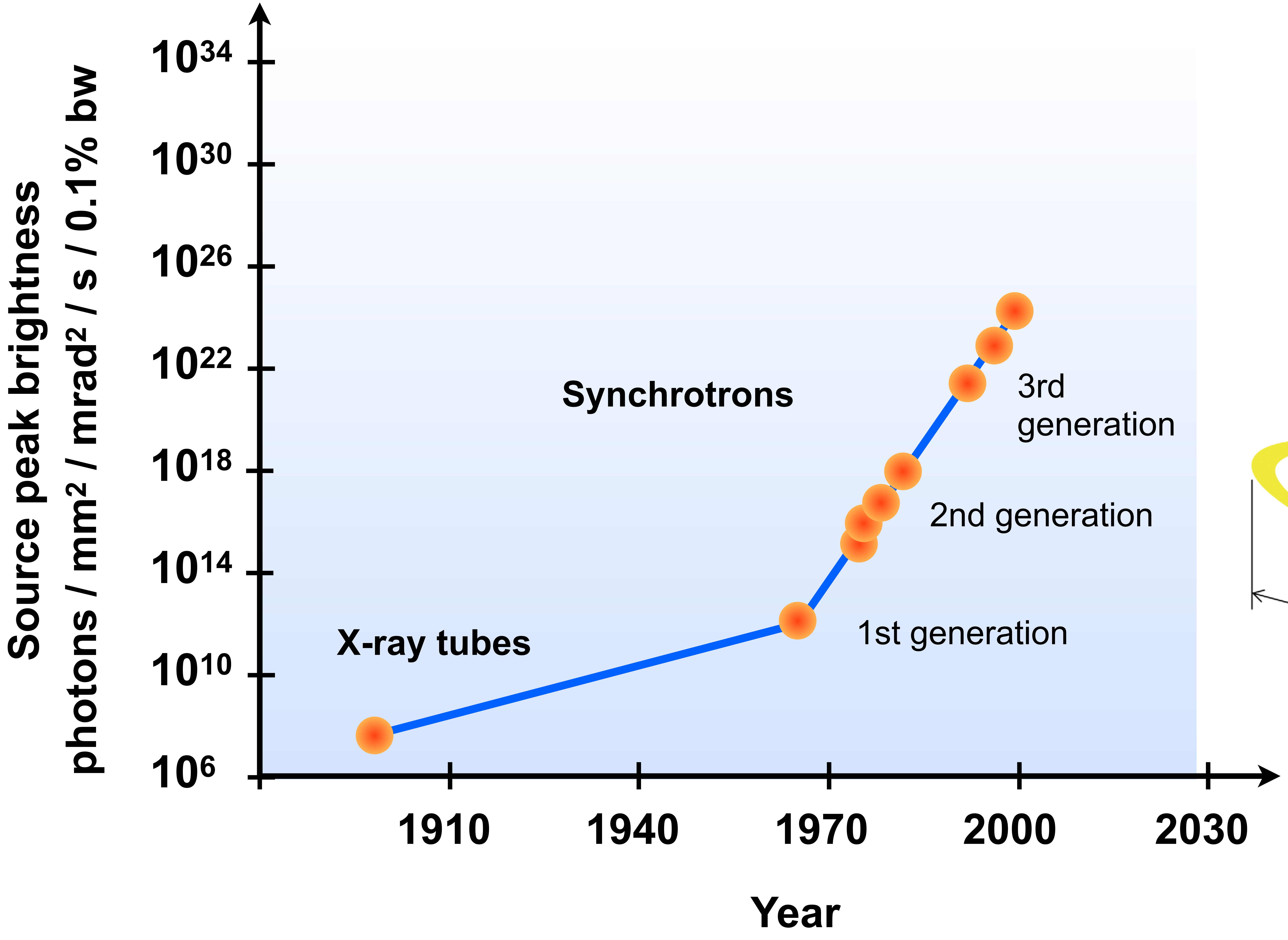
Q8W3K0: A potential plant disease resistance protein. Mean pLDDT 82.24.

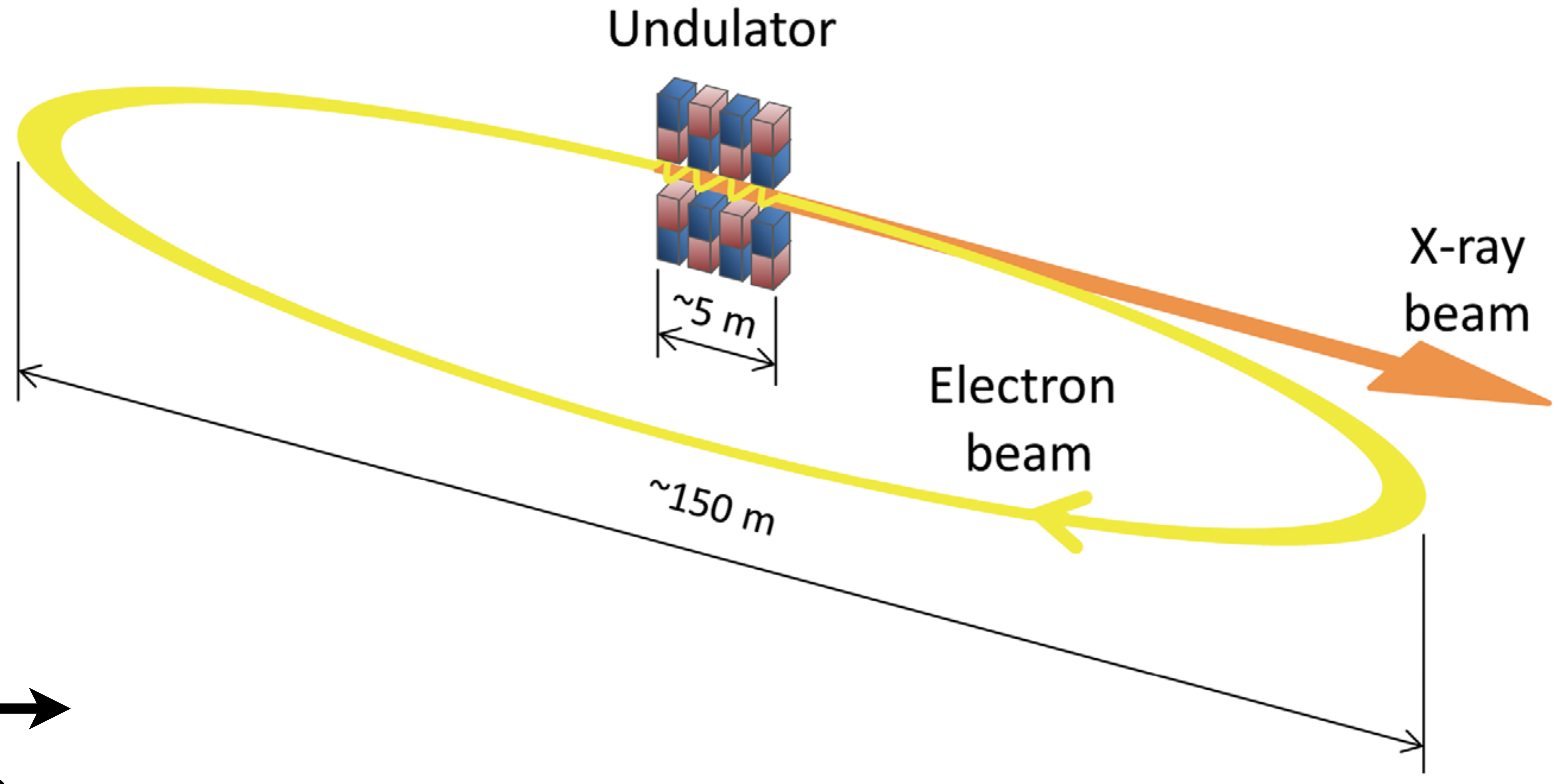
Google DeepMind

EMBL-EBI

J. Jumper at al
Nature **596** 583 (2021)

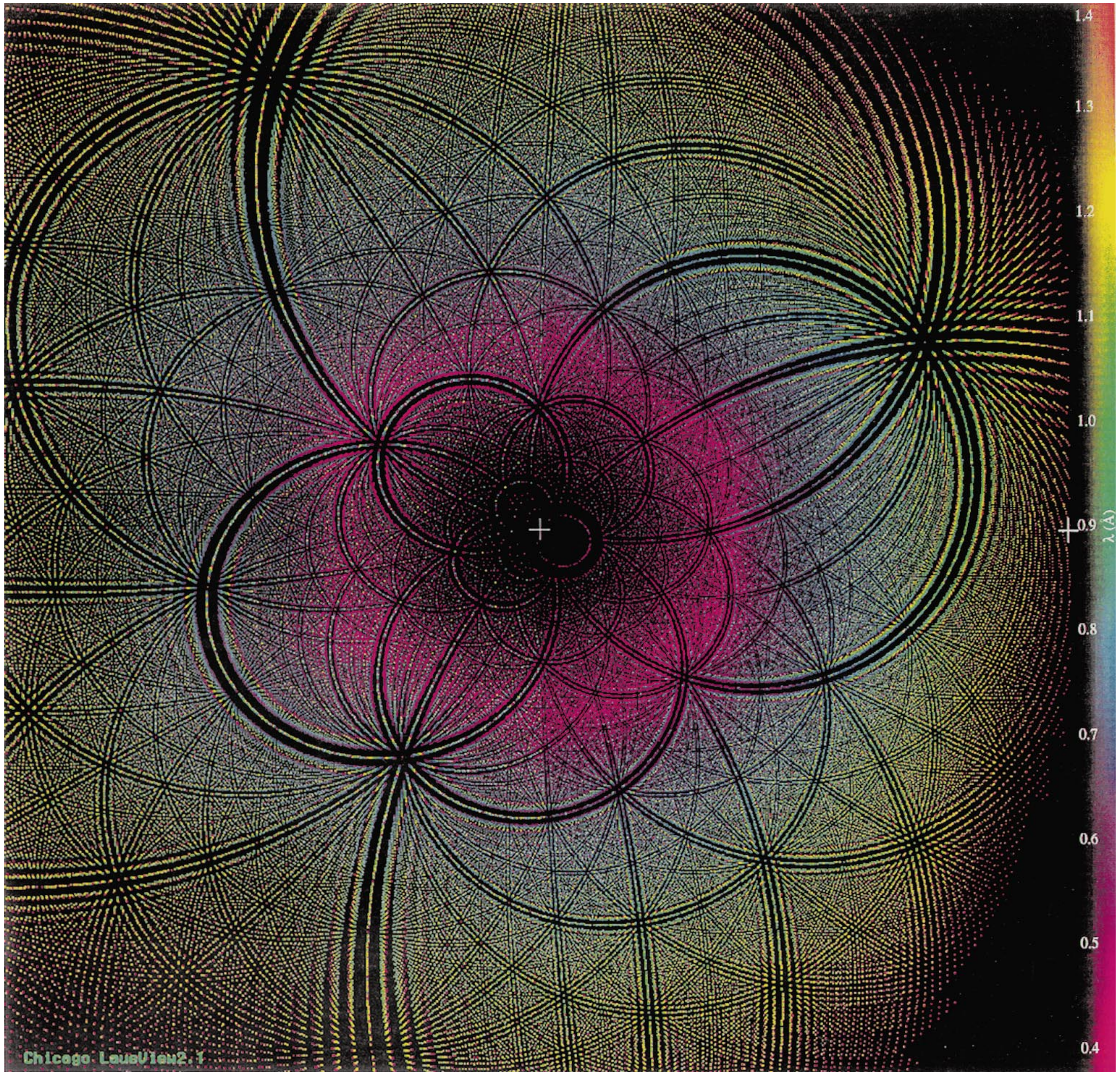
X-ray sources have developed at a staggering pace since their discovery in 1895

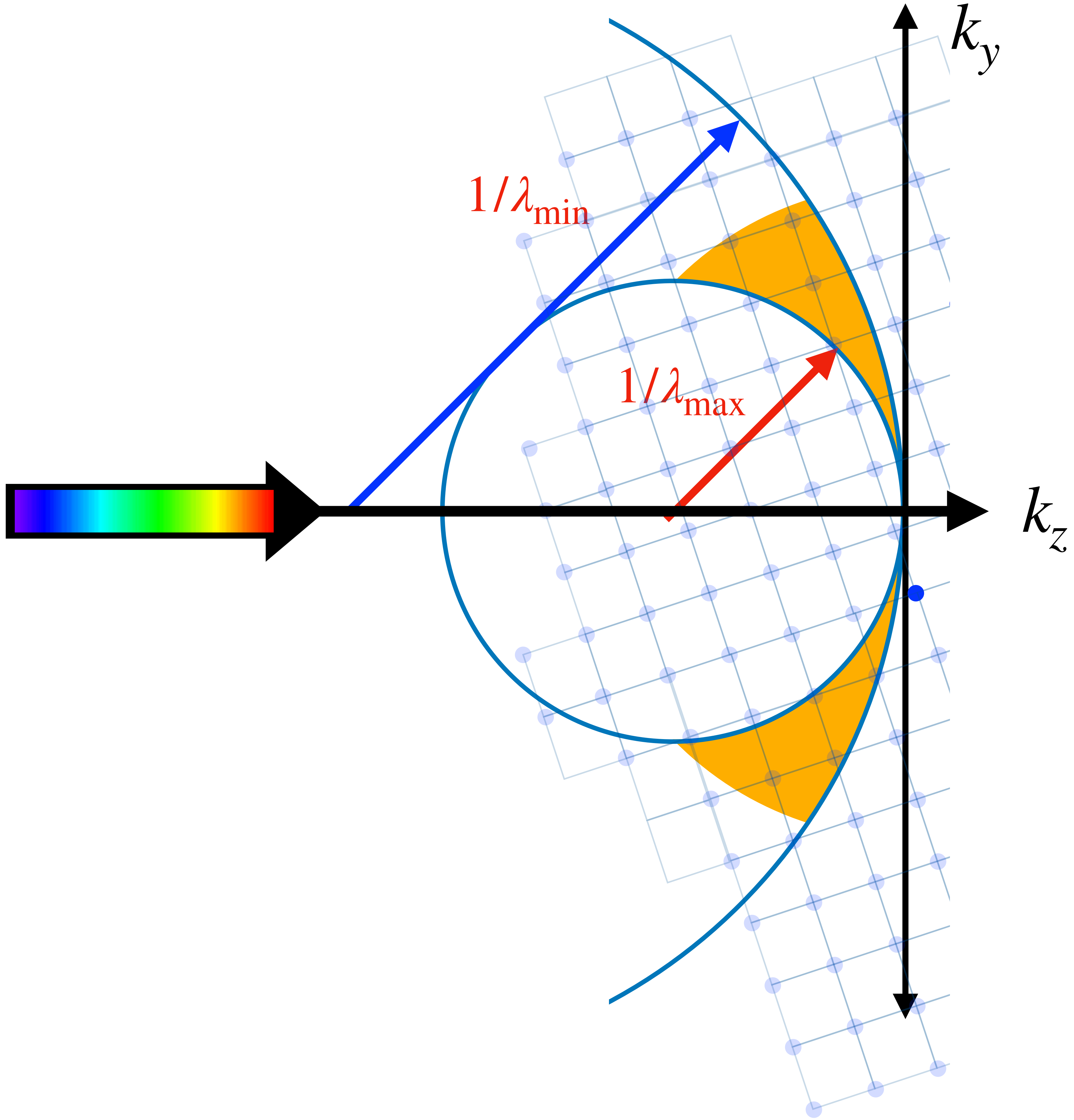




G. Branden and R. Neutze, Science 373 (2021)

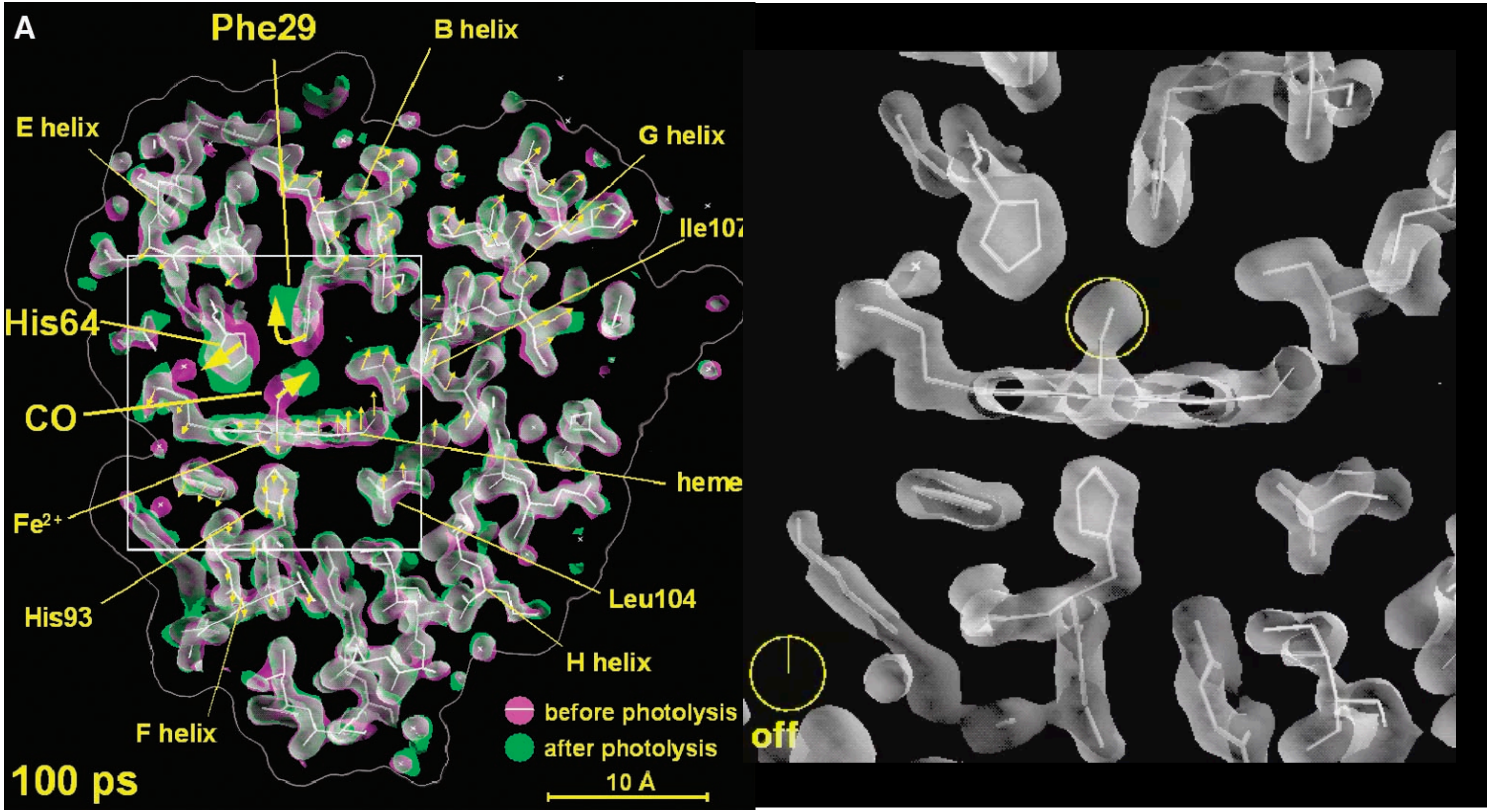
Polychromatic (Laue) diffraction gives 3D information in a single shot

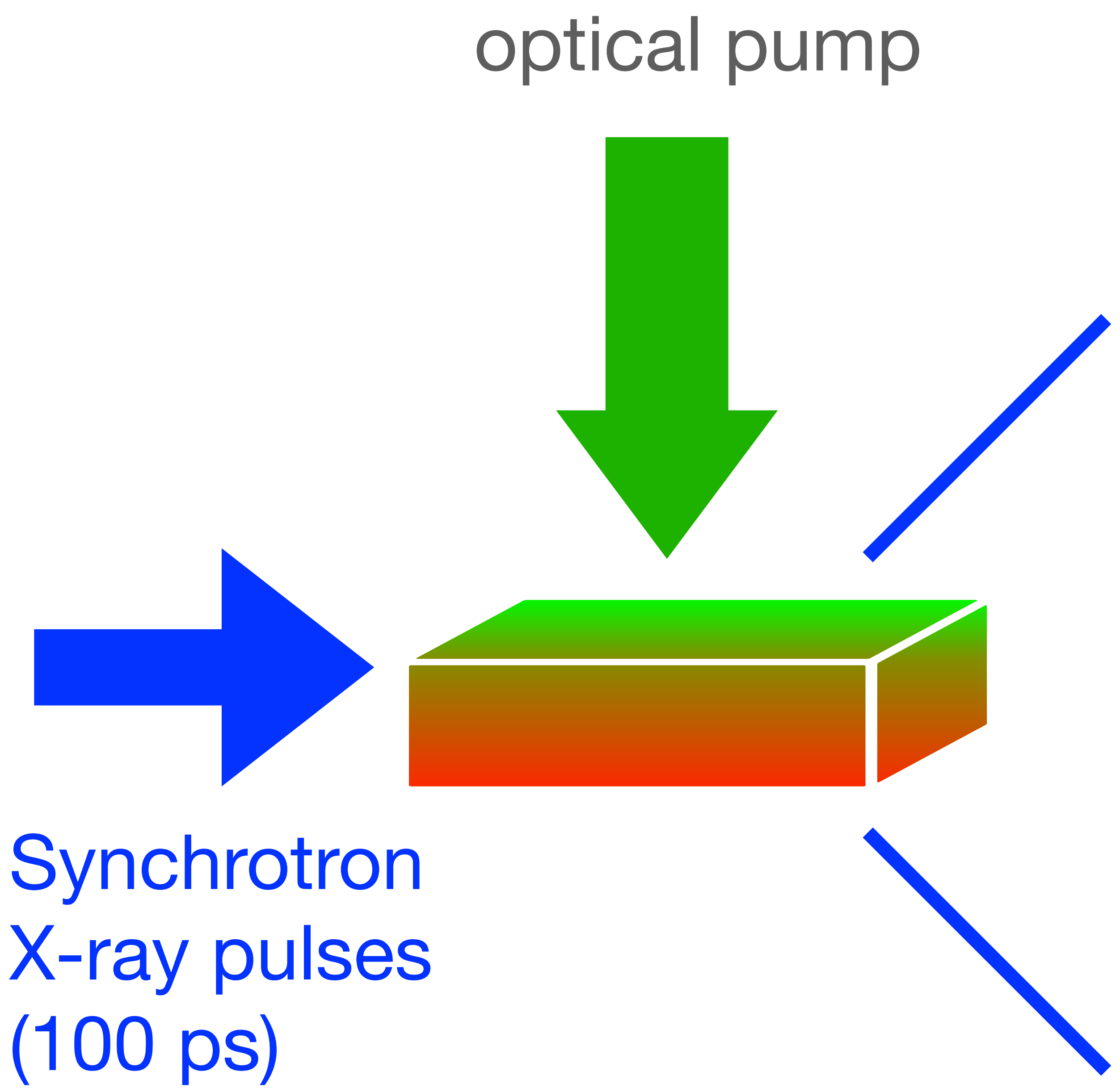




Keith Moffat, U. Chicago

Time-resolved structures let us see functioning proteins





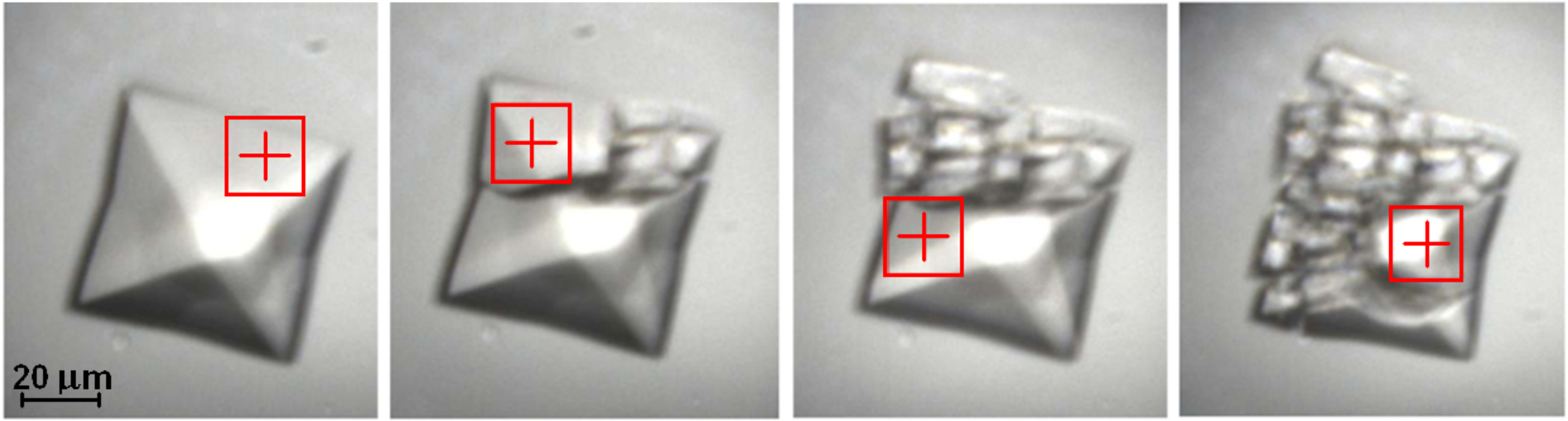
optical pump

Synchrotron
X-ray pulses
(100 ps)

- Polychromatic X-ray beam
- Large crystal to reduce radiation dose
- Crystal larger than optical extinction depth

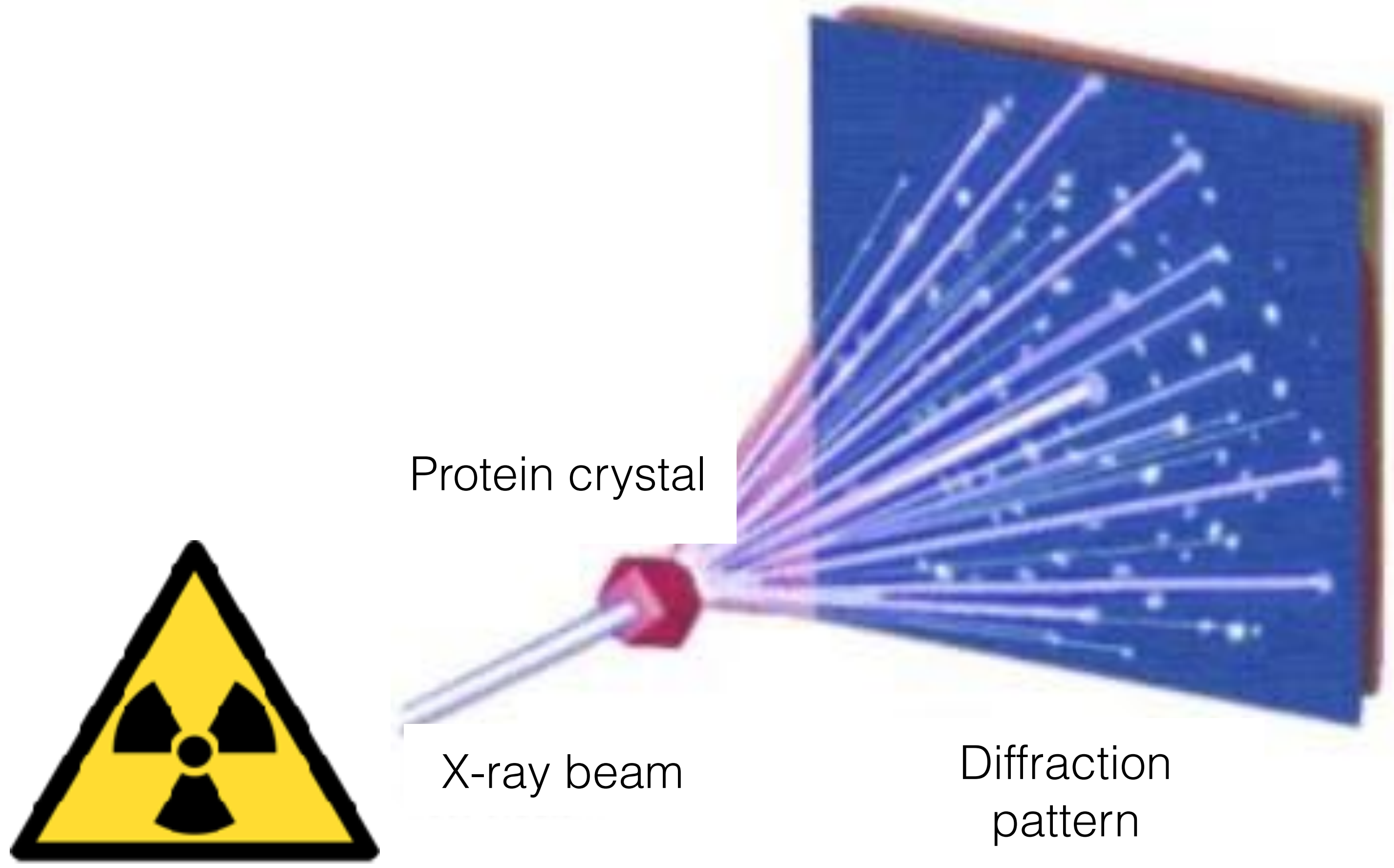
Schotte, et al et Anfinrud, Science **300** 1944 (2003)

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

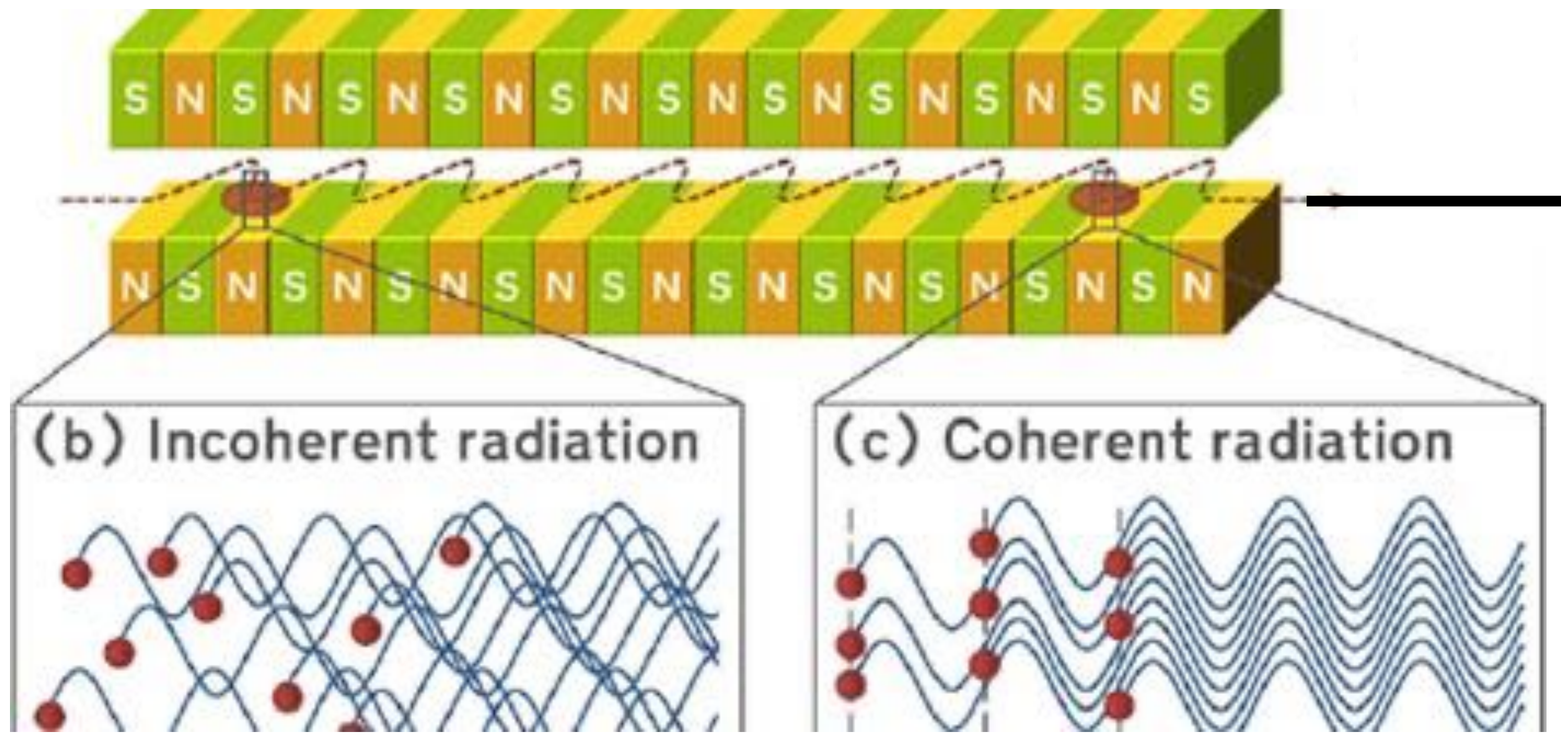
Cryogenic cooling gives 30 MGy tolerance

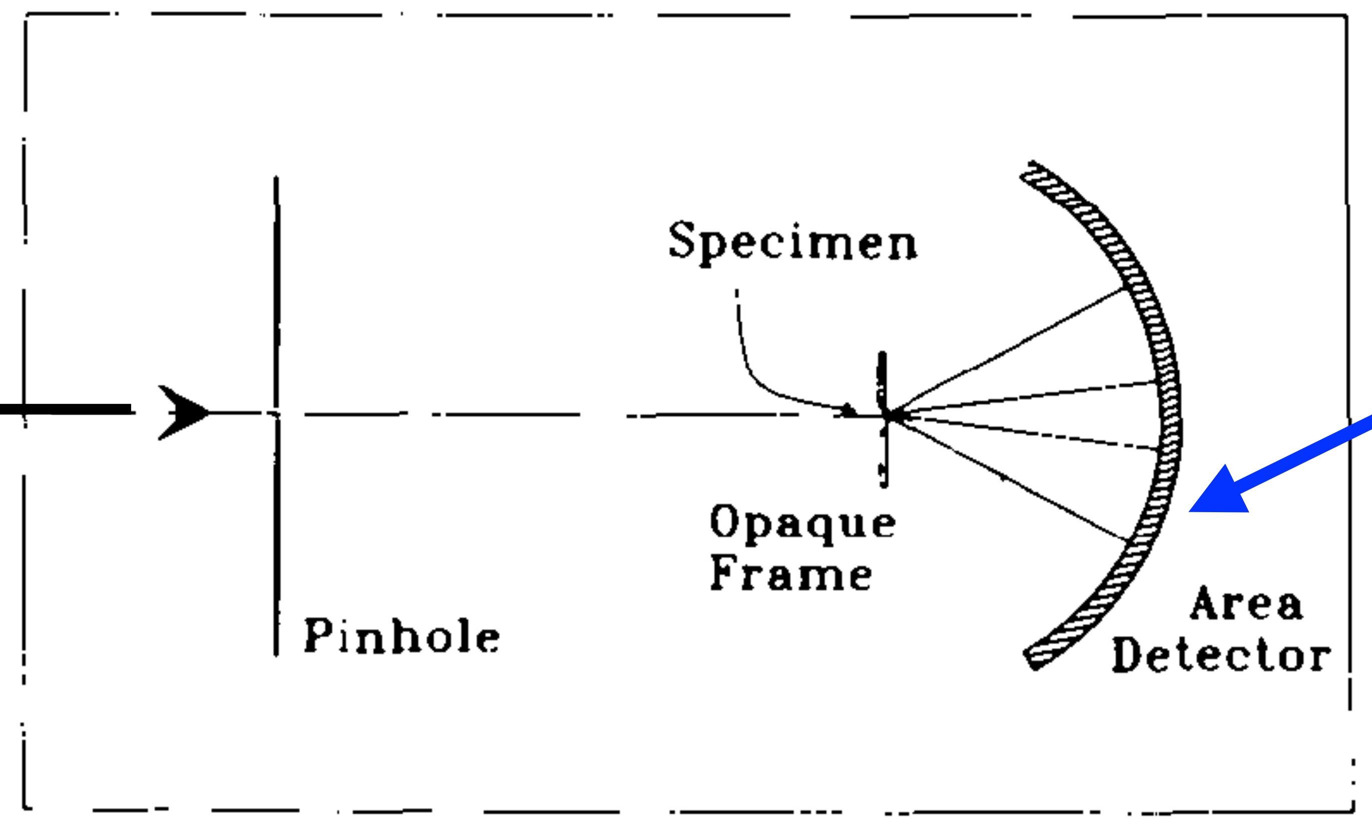


Axford et al. Acta Cryst. D68 592 (2012)
Diamond Light Source (courtesy Robin Owen & Elspeth Garman)

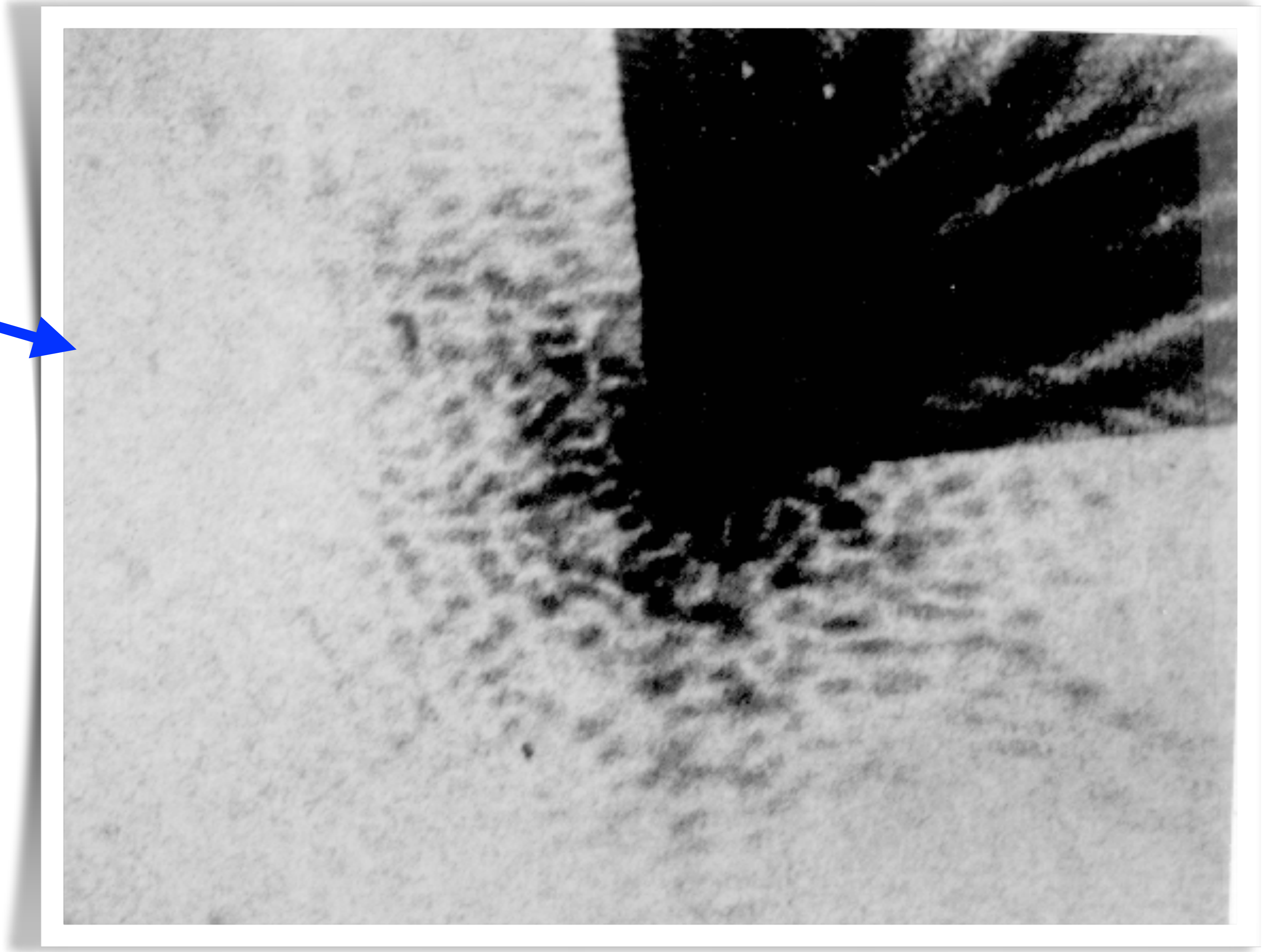
David Sayre proposed to use crystallographic phasing methods on general non-periodic objects

World’s first undulator, NSLS

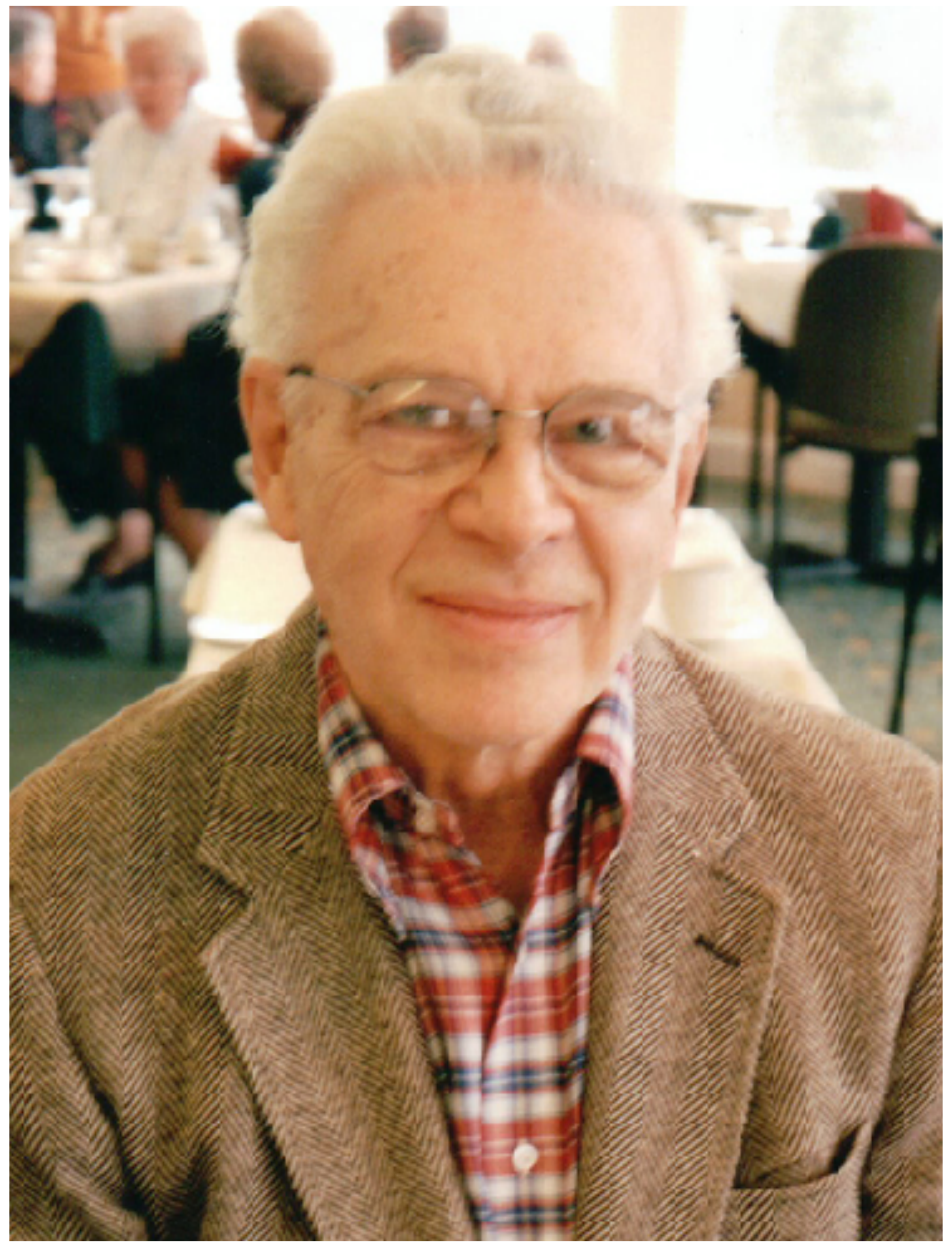




Film!

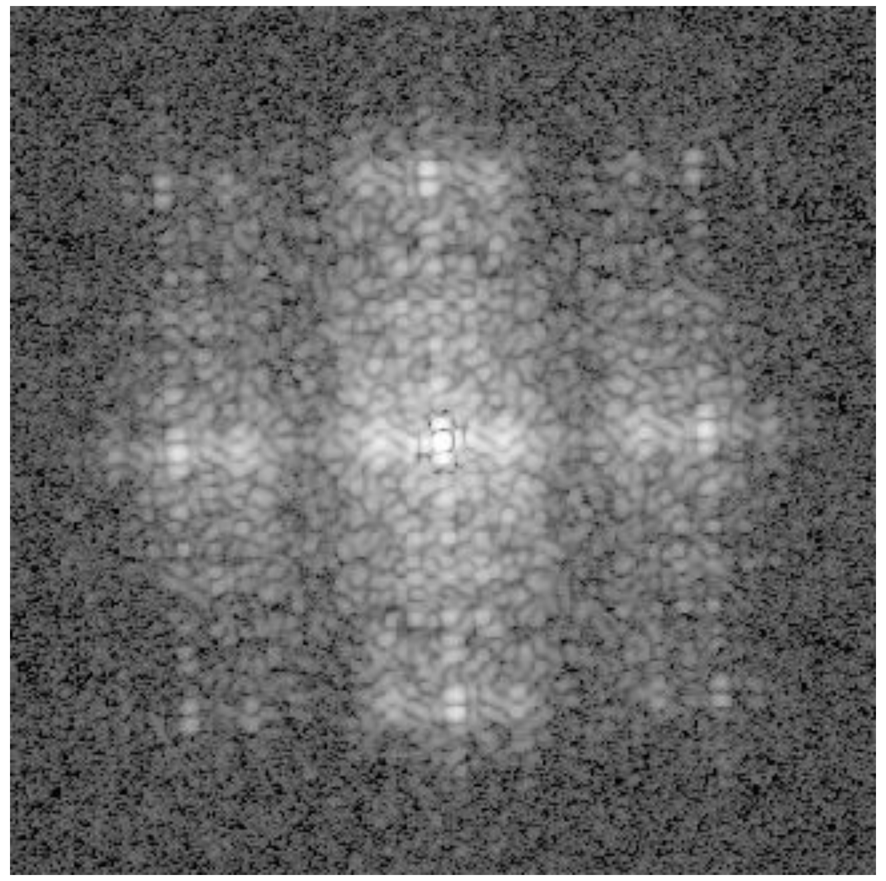


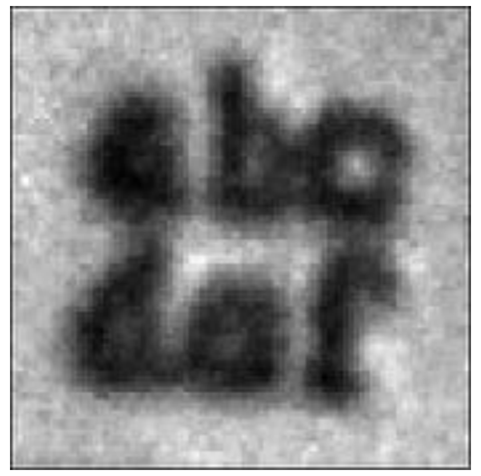
D. Sayre and H. N. Chapman. “X-ray microscopy” Acta Cryst A **51**, 237 (1995).



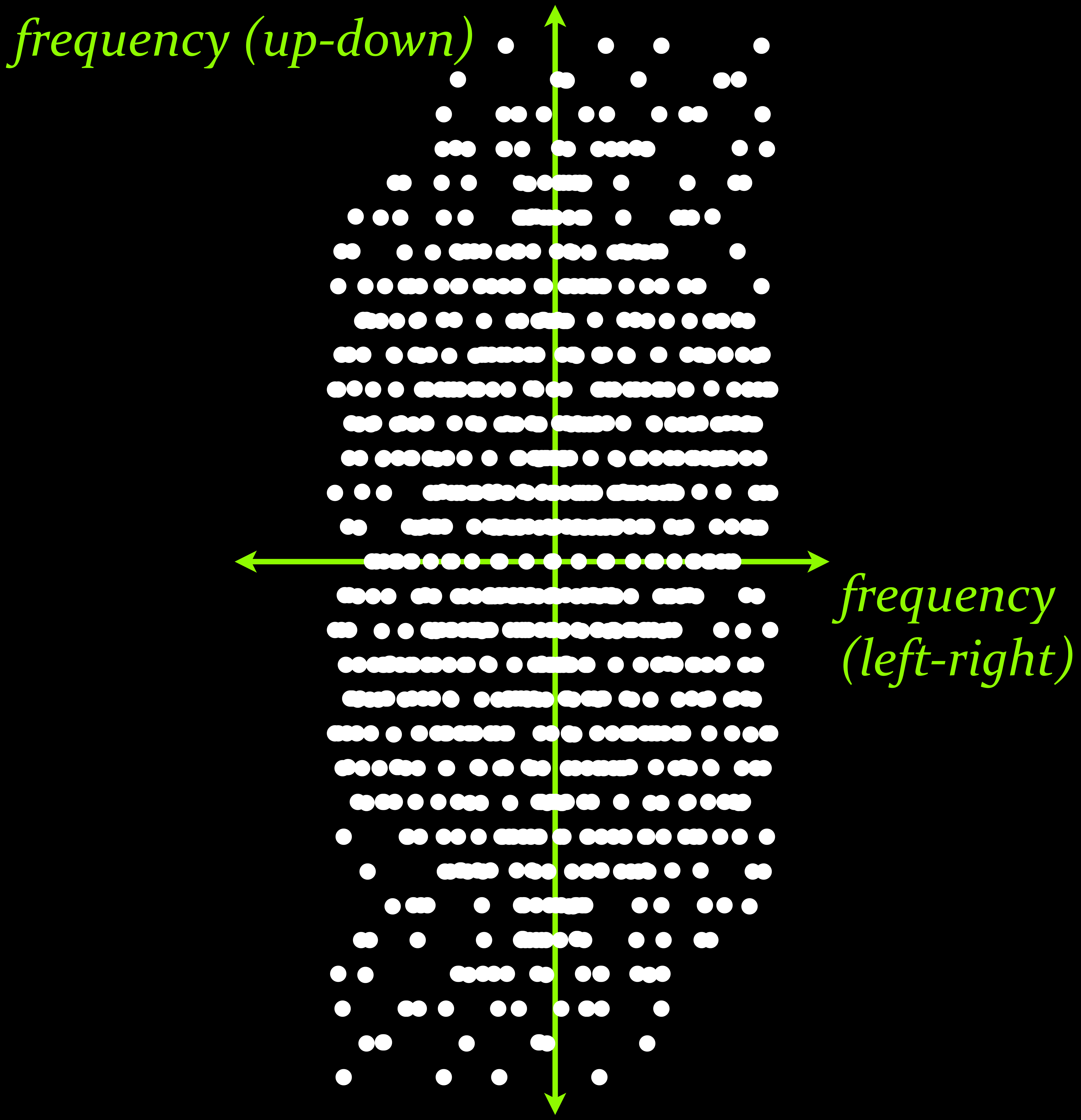
Successful reconstruction using Fienup’s hybrid input-output iterative phase retrieval algorithm

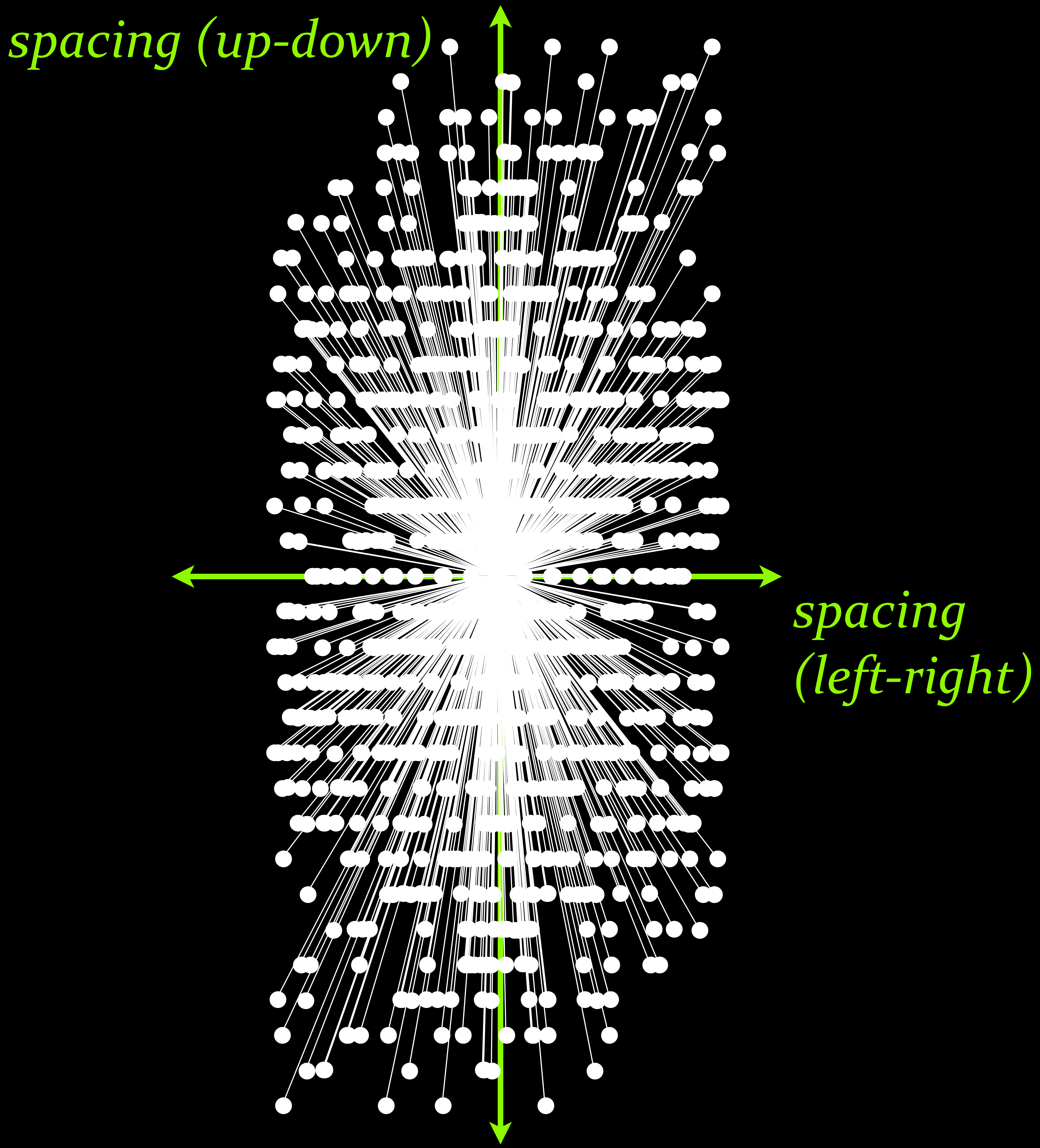
CCD!

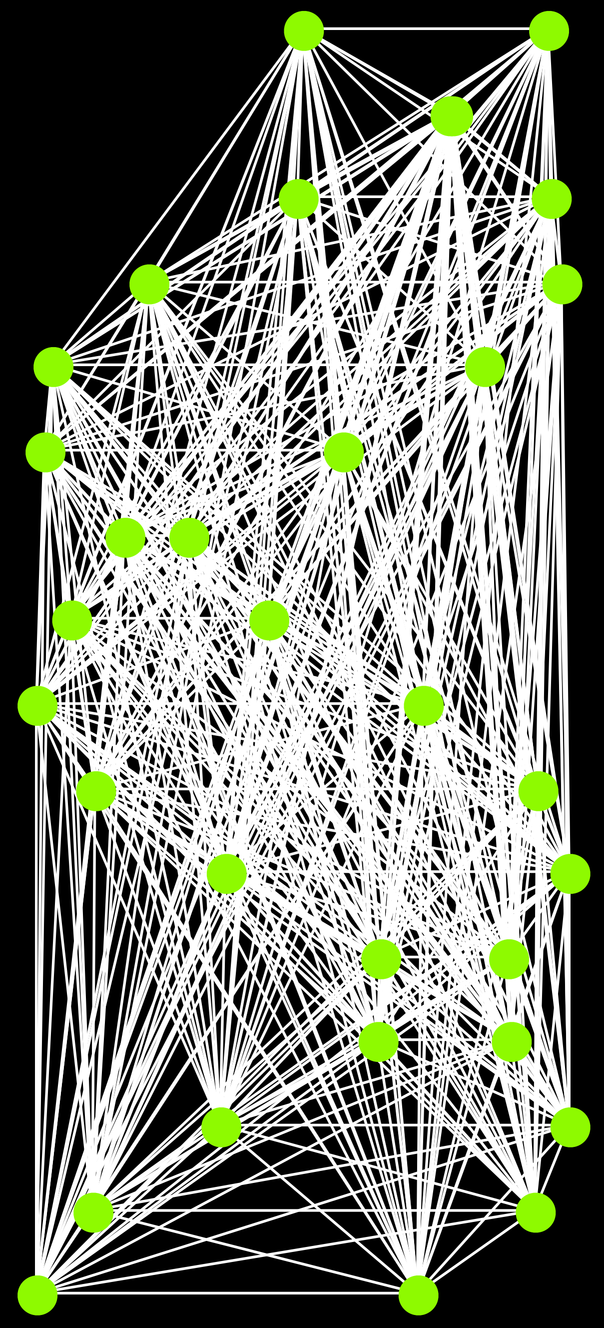


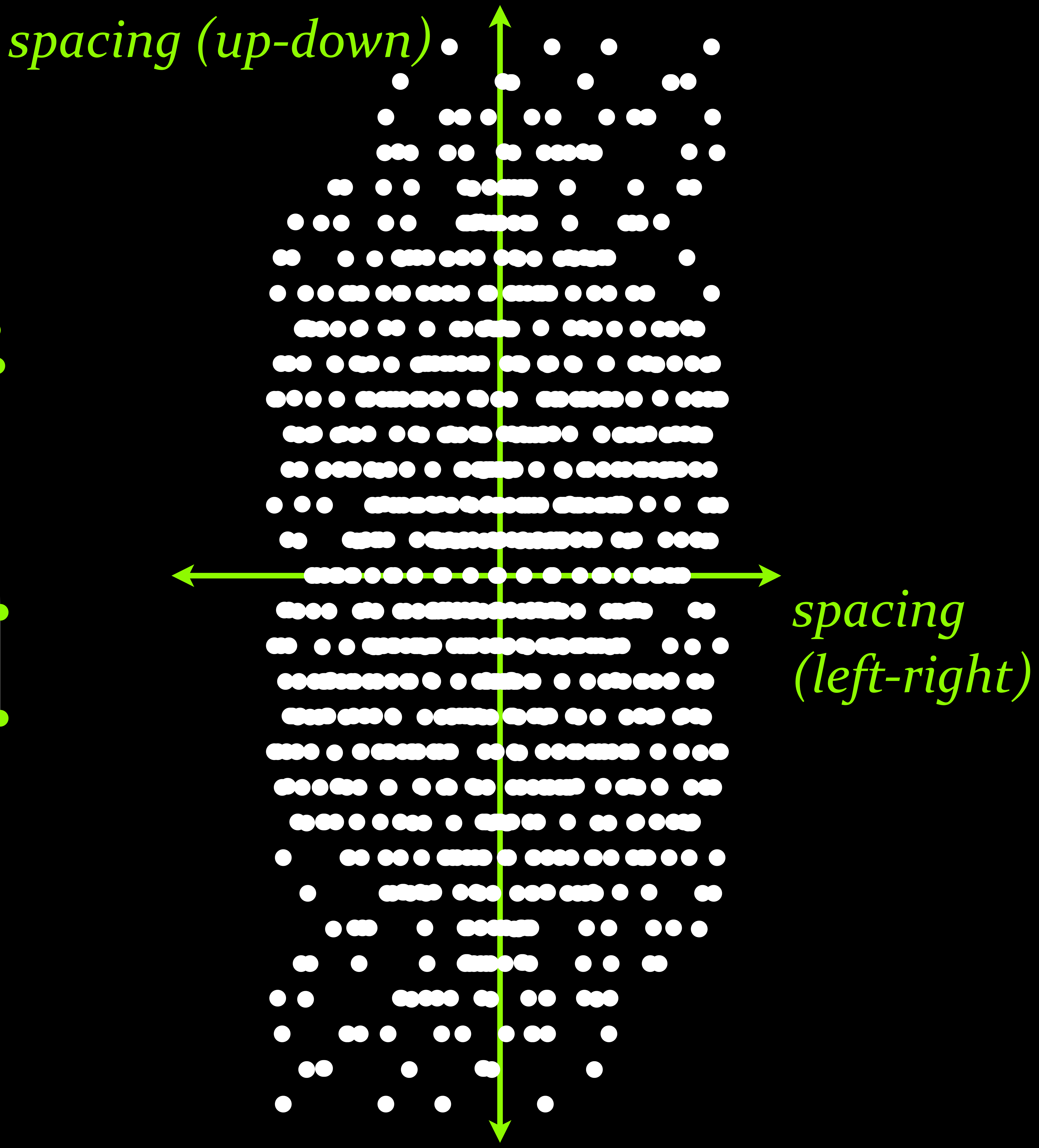


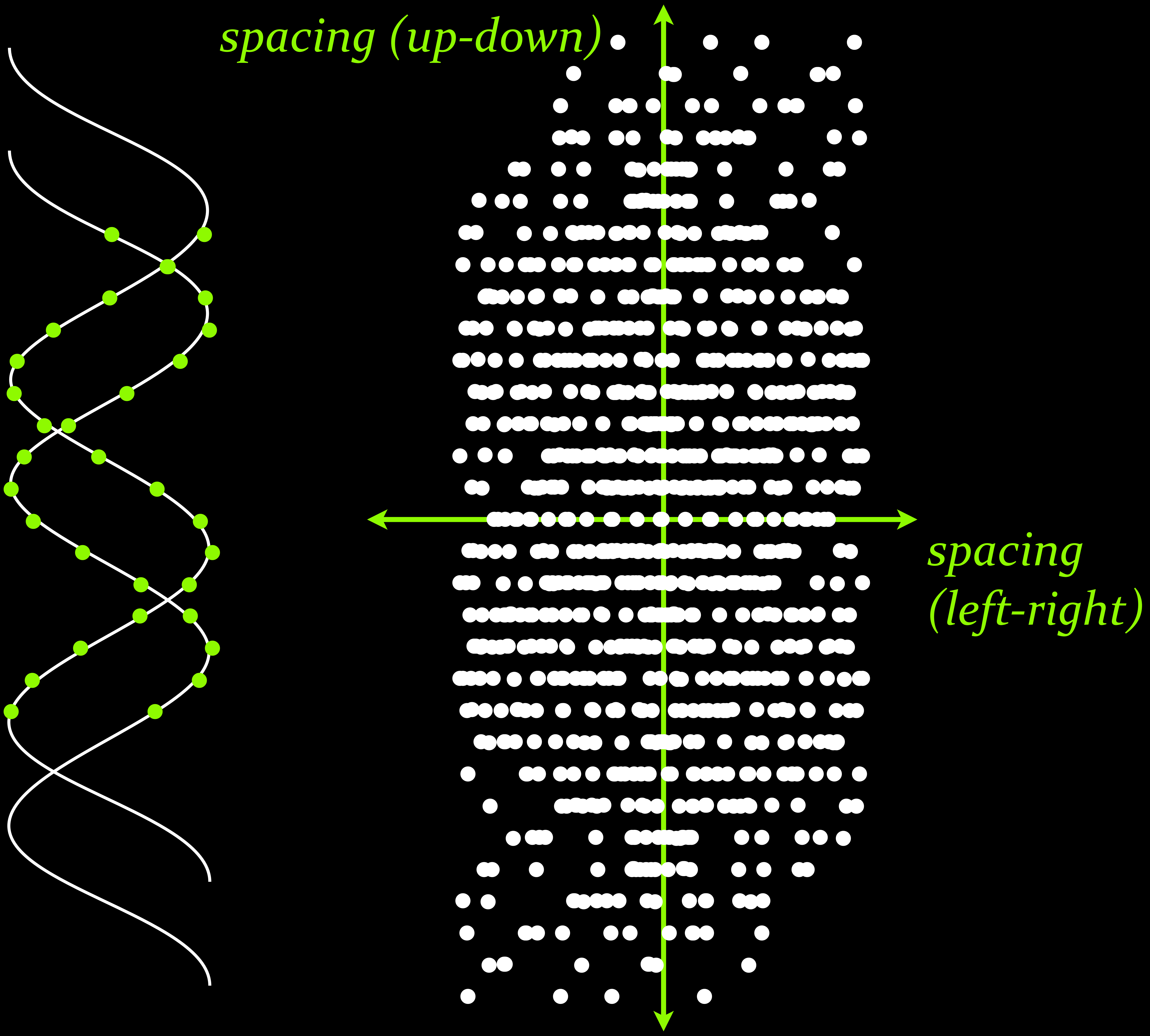
John Miao, P. Charalambous, J. Kirz and D. Sayre, Nature **400** (1999)


frequency (up-down)
frequency
(left-right)

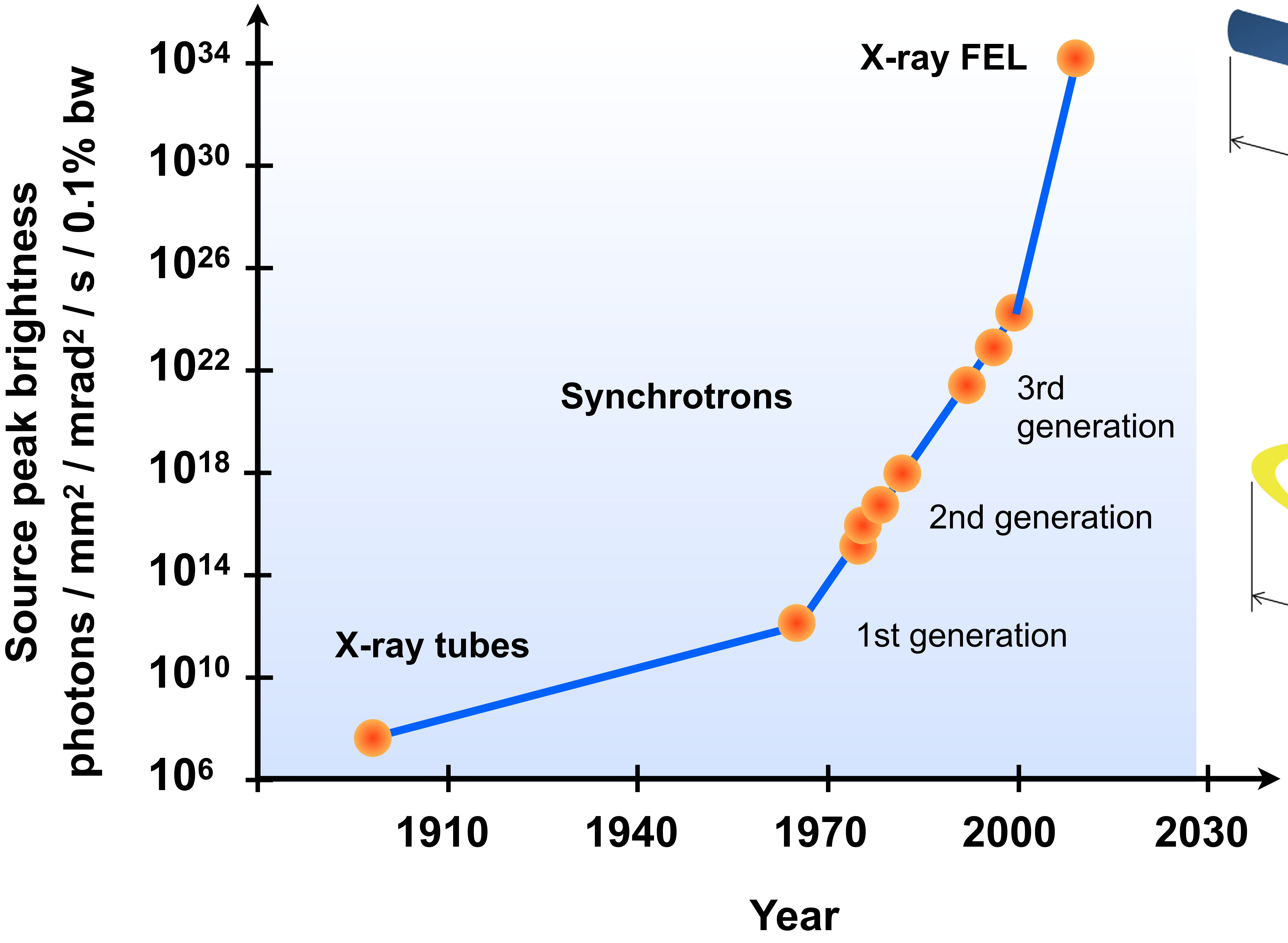

spacing (up-down)
spacing (left-right)

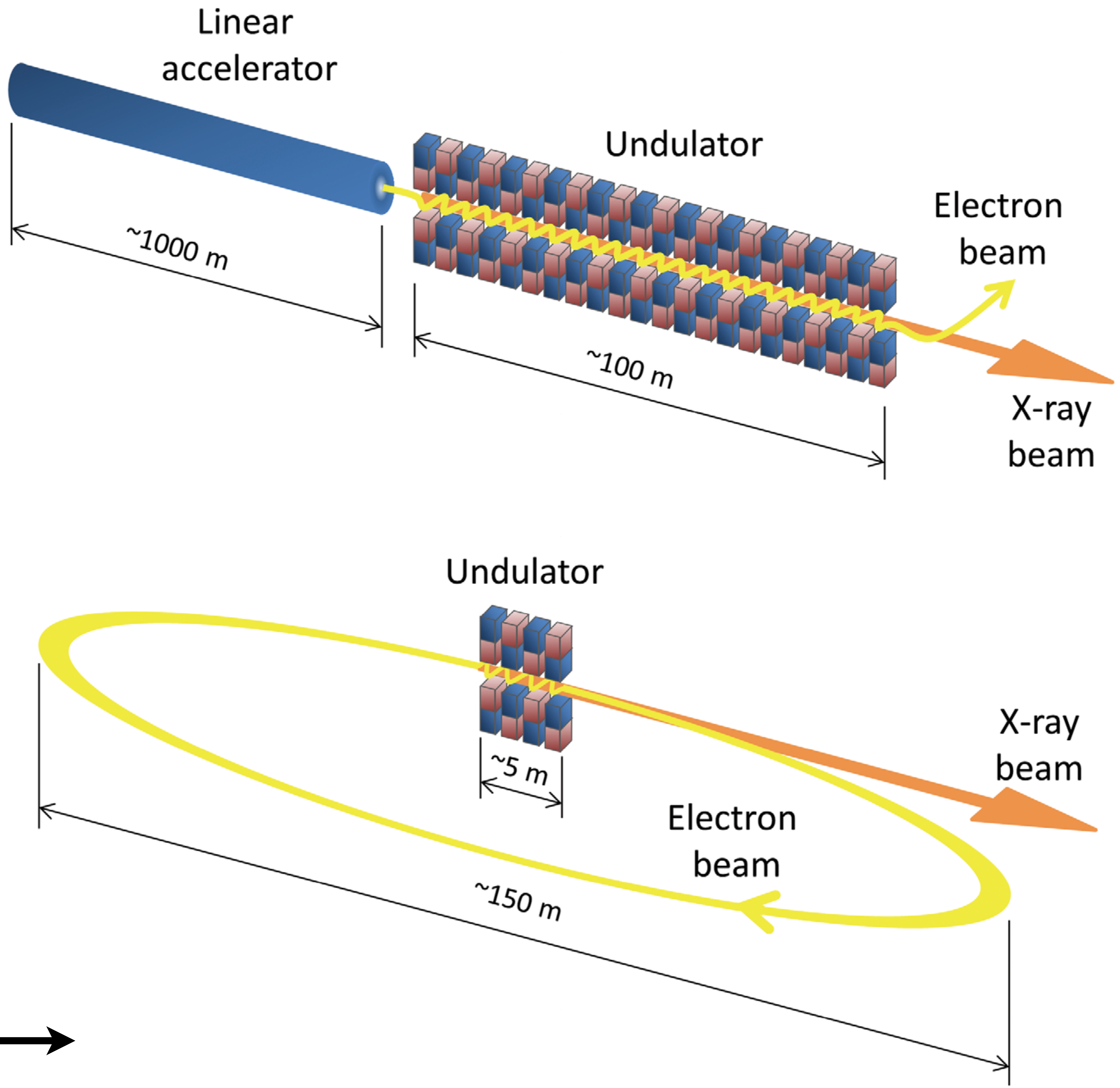



spacing (up-down)
spacing (left-right)


spacing (up-down)
spacing (left-right)

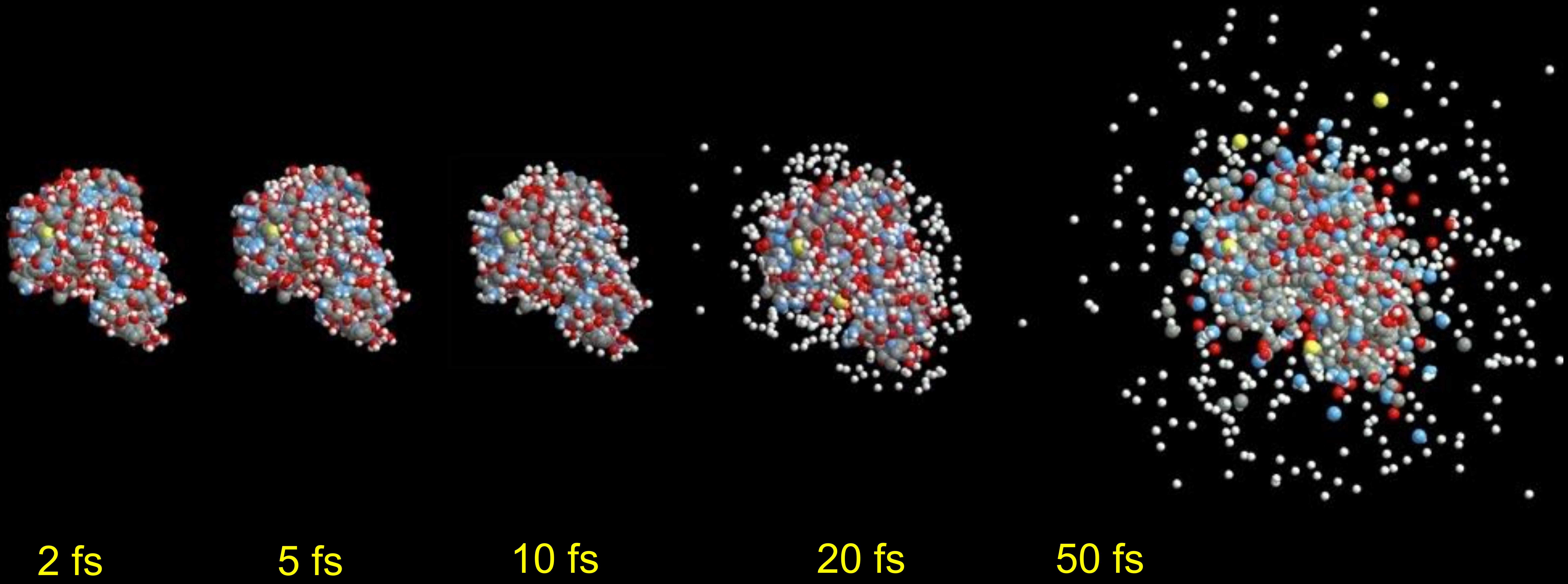
X-ray sources have developed at a staggering pace





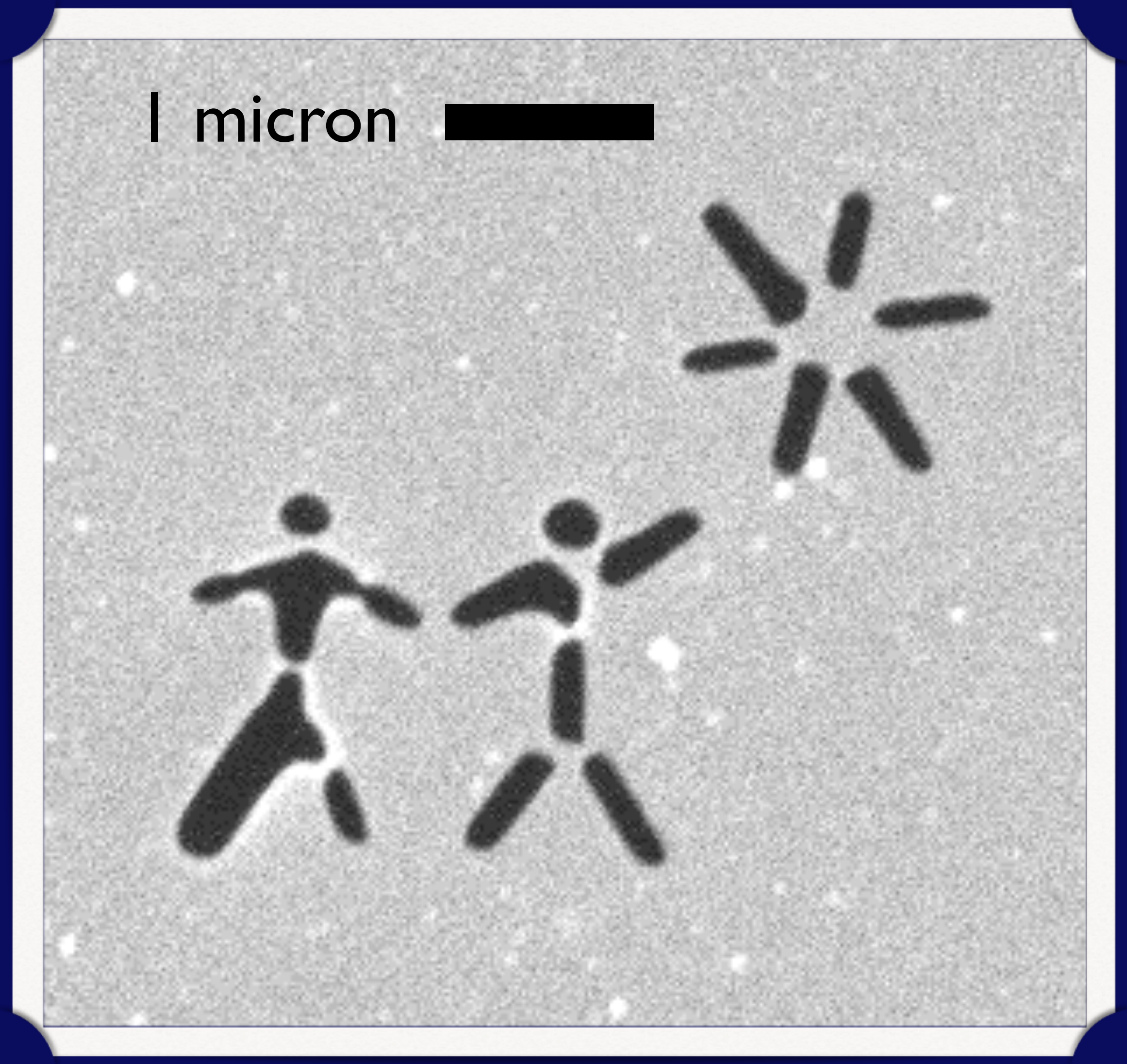
G. Branden and R. Neutze, Science 373 (2021)

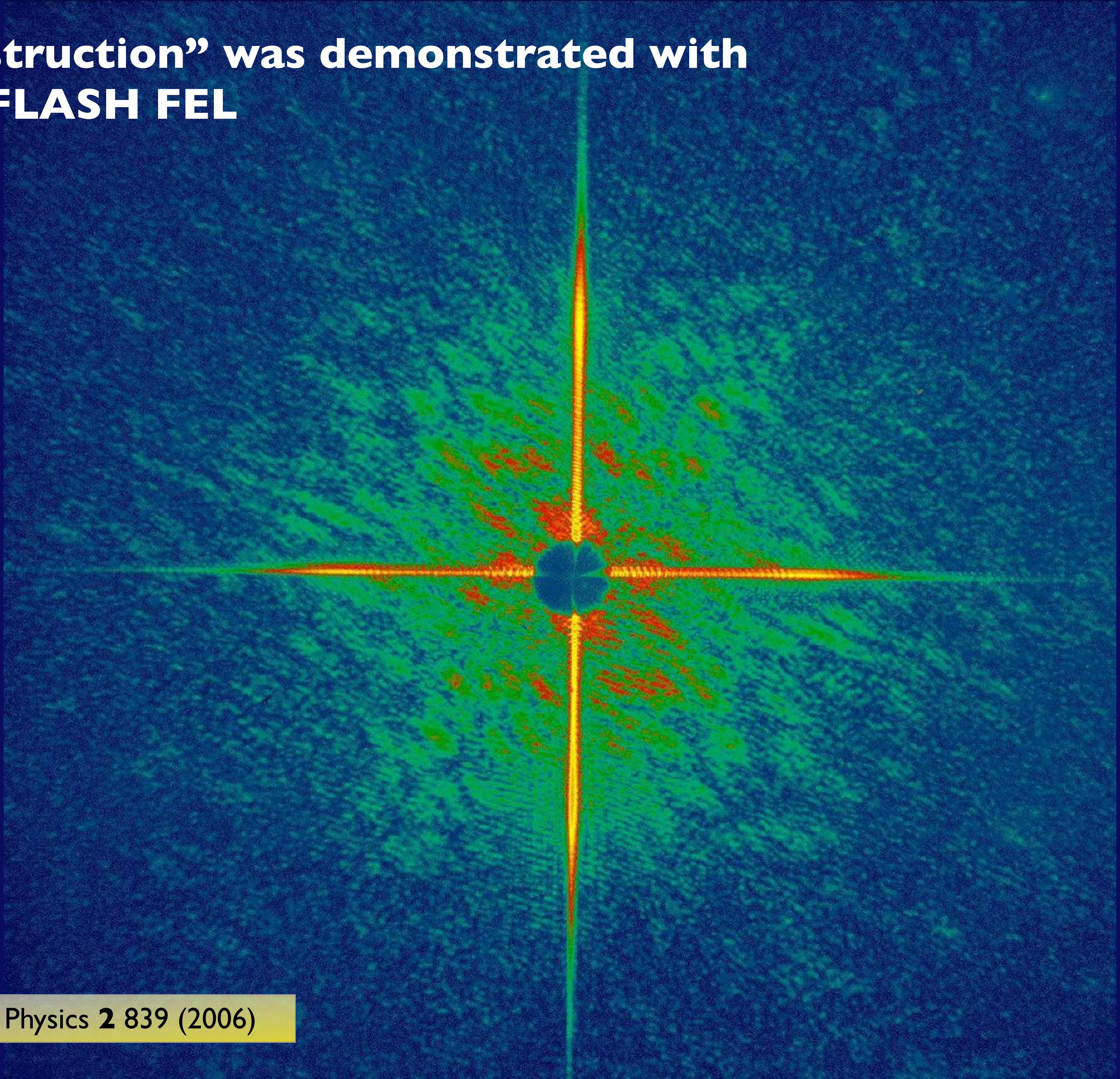
X-ray free-electron lasers may enable atomic-resolution imaging of single biological macromolecules



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

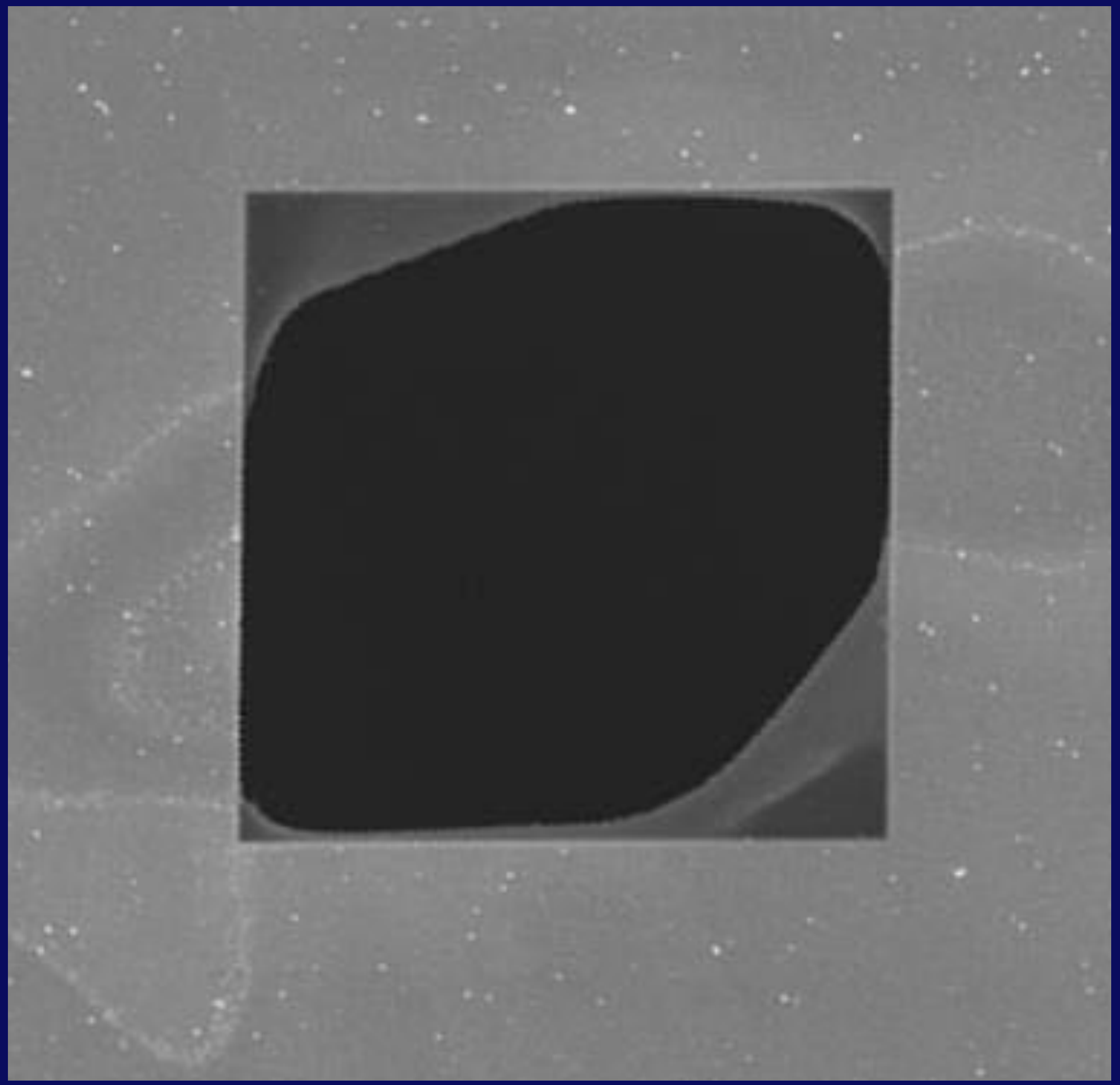
“Diffraction before destruction” was demonstrated with soft X-rays at DESY’s FLASH FEL

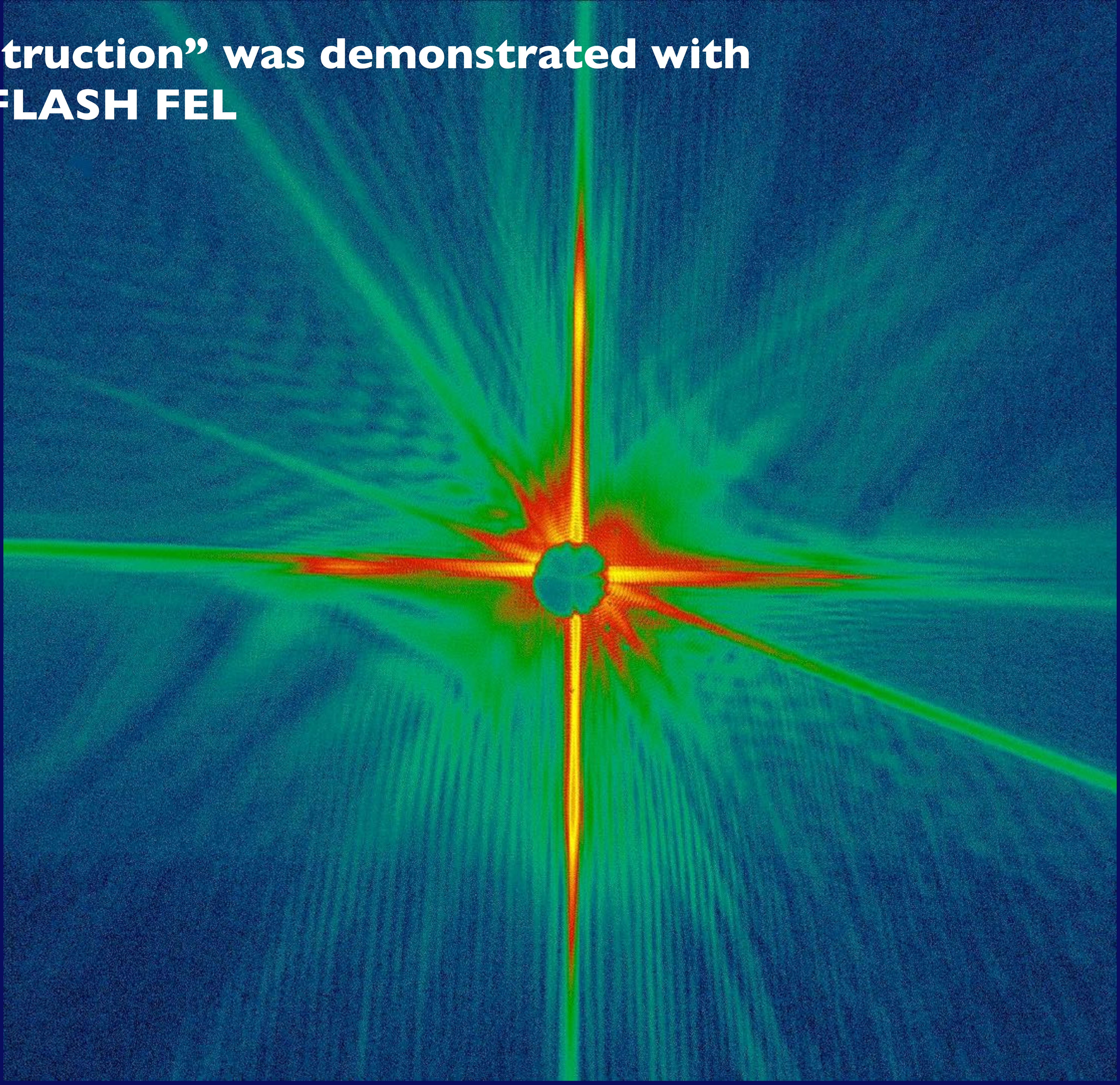




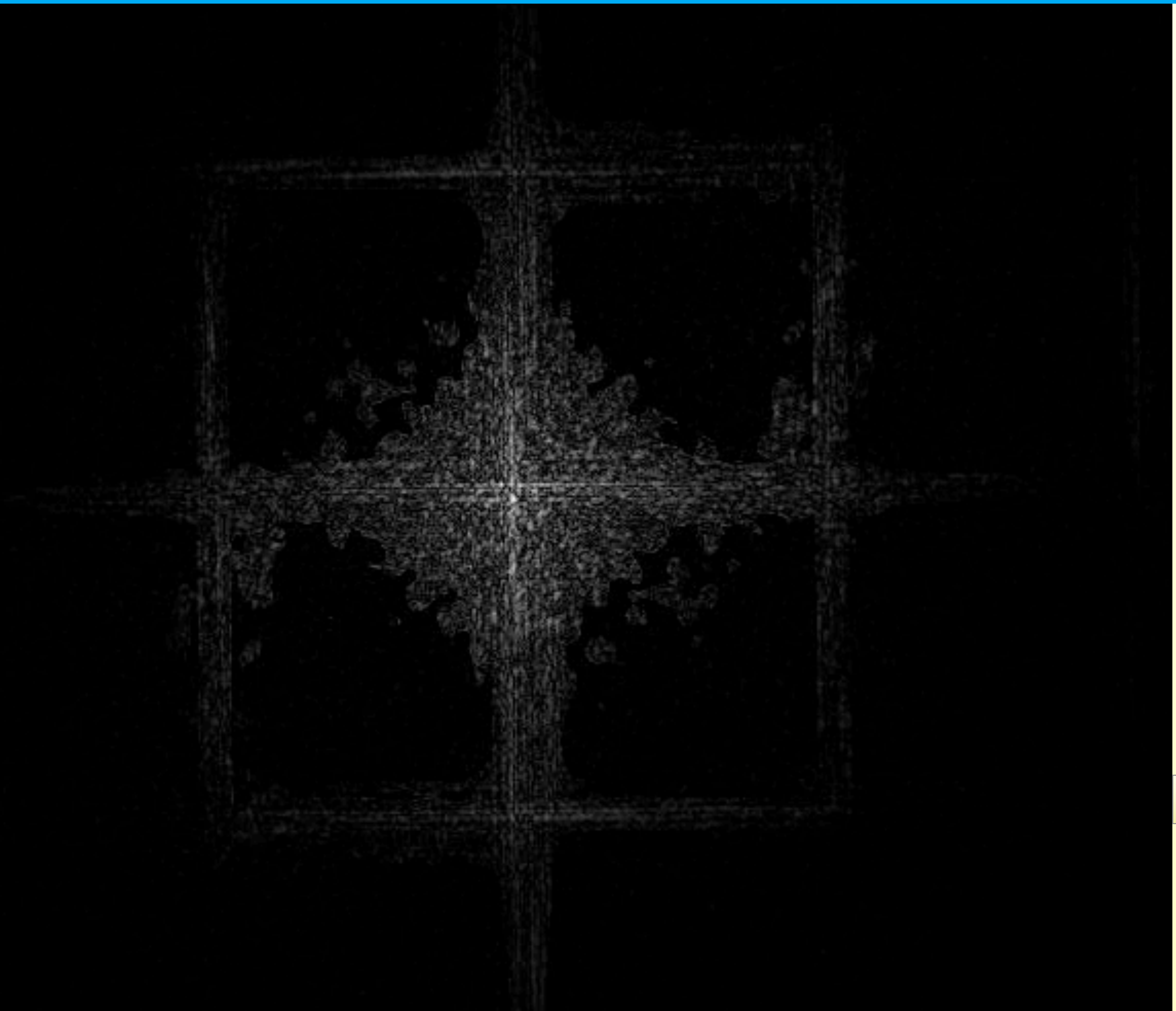
Chapman et al, Nature Physics **2** 839 (2006)

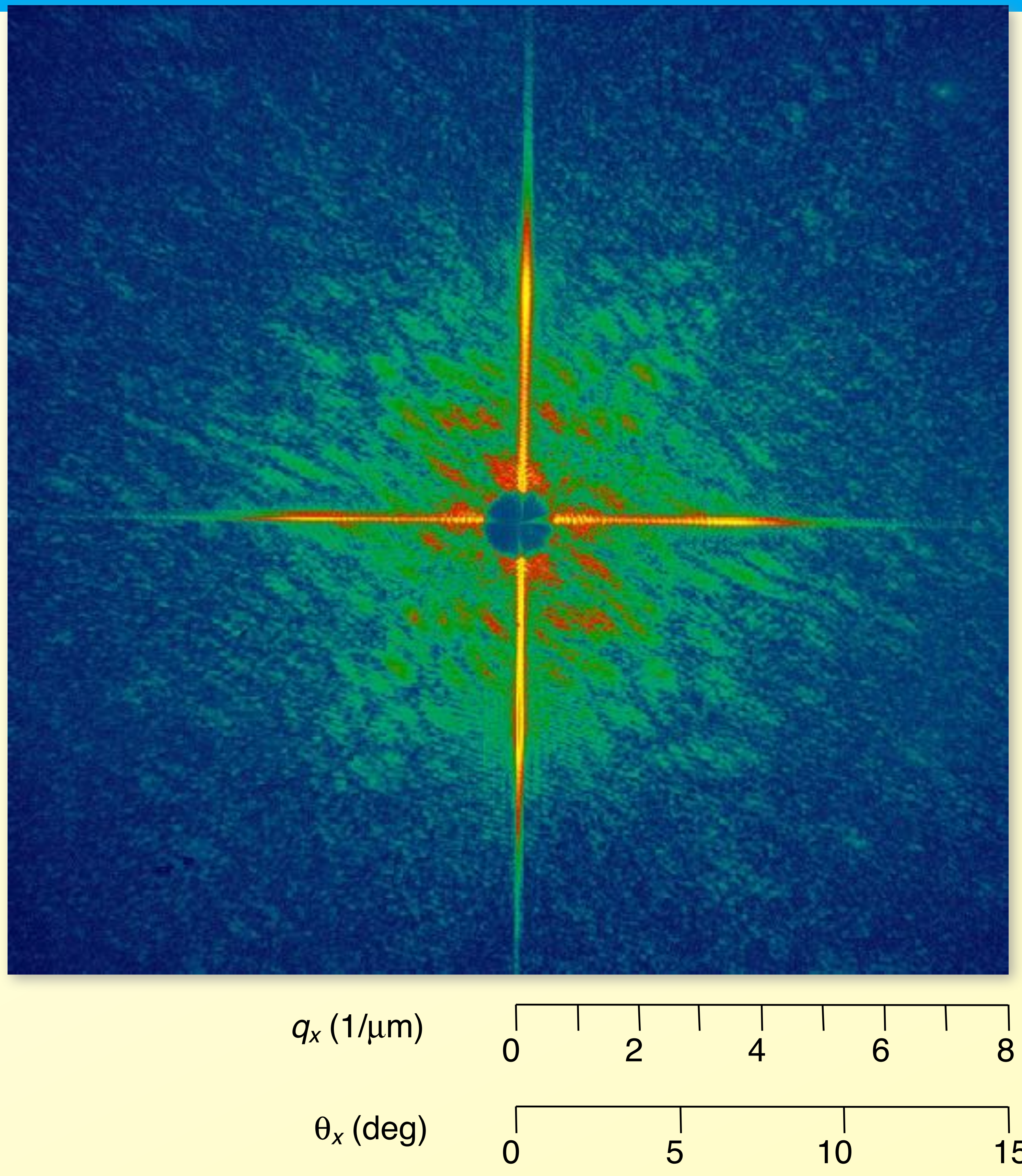
“Diffraction before destruction” was demonstrated with soft X-rays at DESY’s FLASH FEL





Images were reconstructed with our “Shrinkwrap” algorithm





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

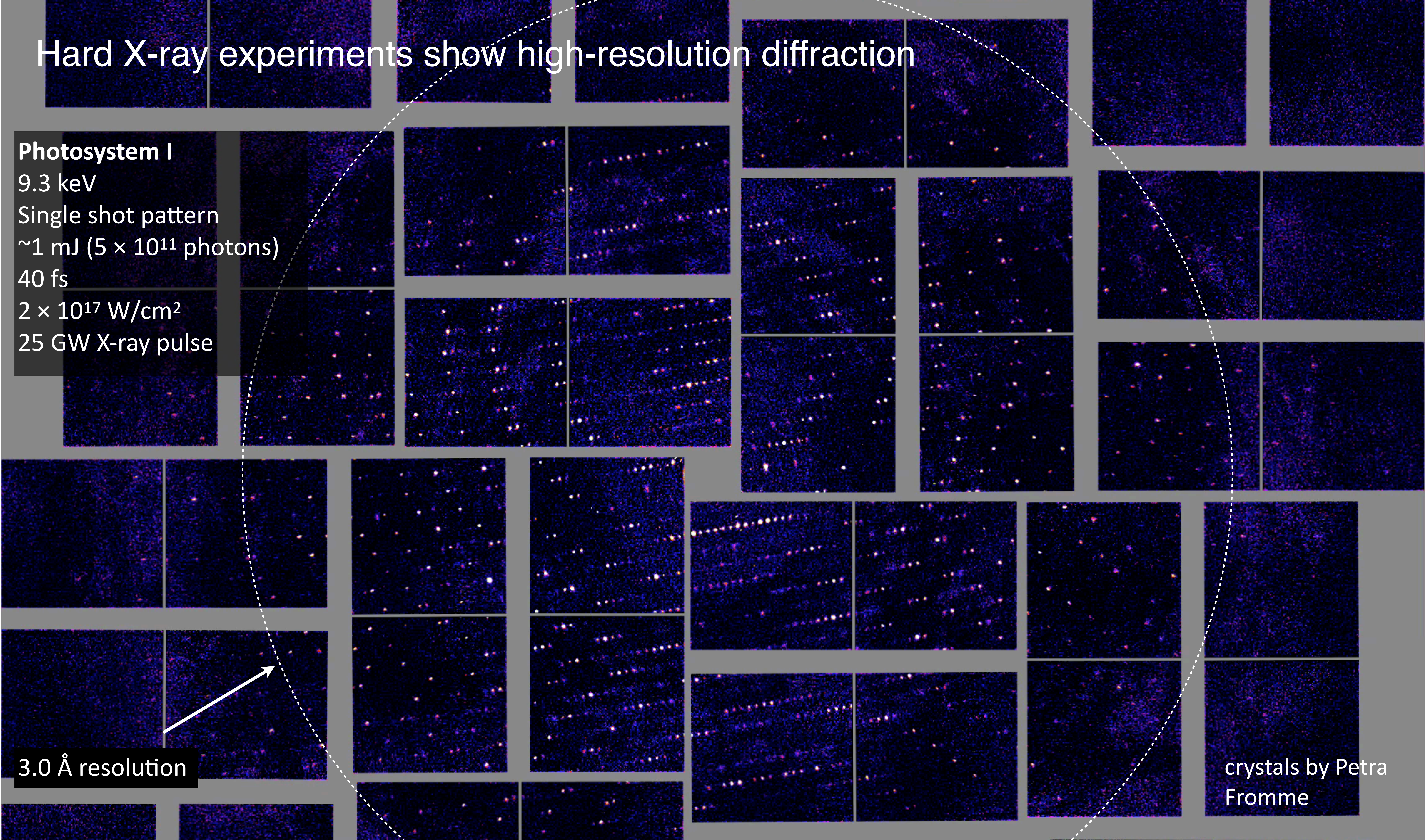
H. Chapman et al, Nature Physics **2** 839 (2006)

With femtosecond pulses, exposures can be increased by 1000's of times

Simulation by C. Caleman, using Cretin (H. Scott, LLNL)

100 fs
6 GGy
(60 MGy/fs)

Hard X-ray experiments show high-resolution diffraction



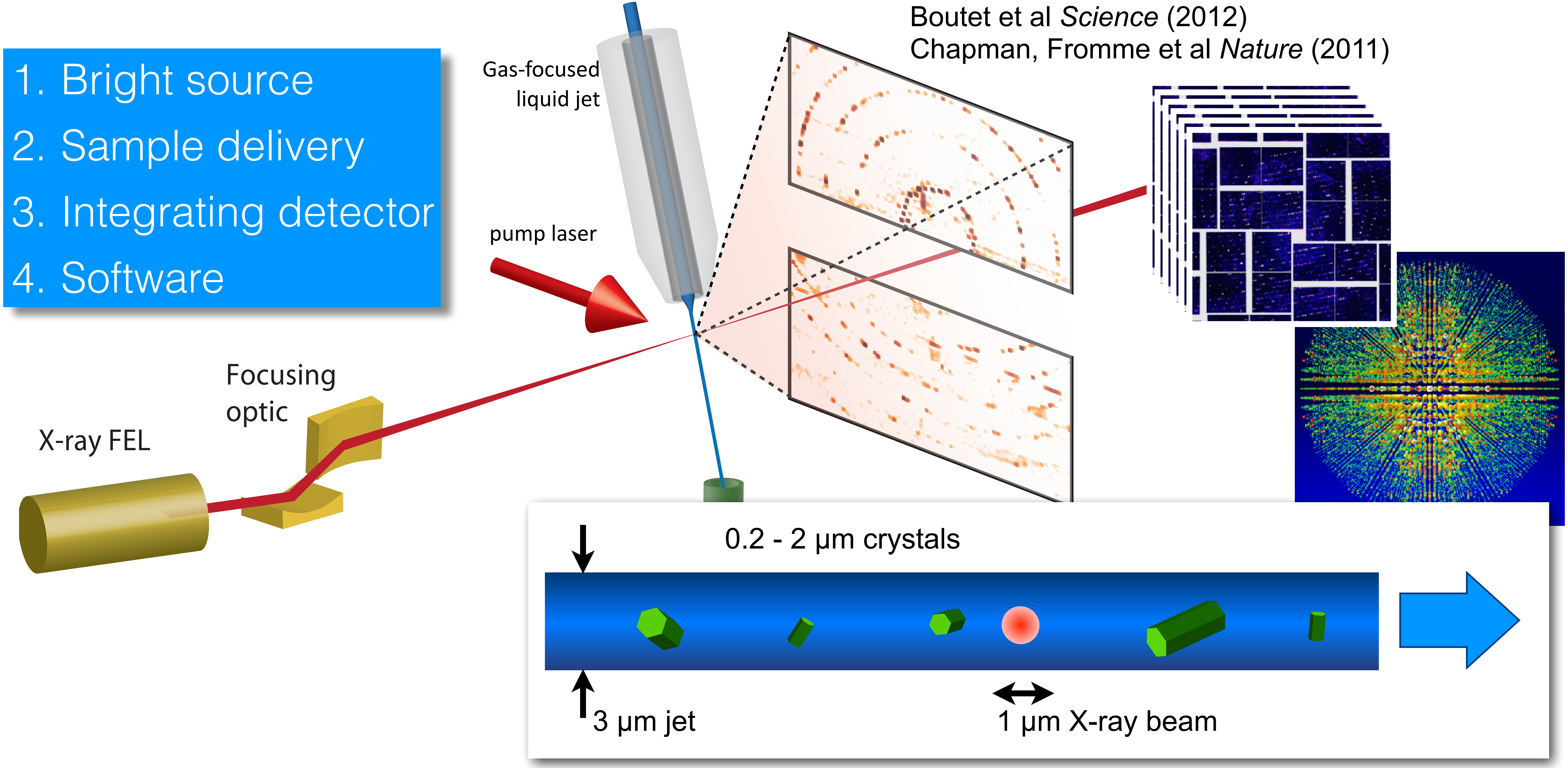
Photosystem I
9.3 keV
Single shot pattern
~1 mJ (5 × 10^{11} photons)
40 fs
2 × 10^{17} W/cm^2
25 GW X-ray pulse

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



Boutet et al *Science* (2012)
Chapman, Fromme et al *Nature* (2011)

Gas-focused liquid jet

pump laser

Focusing optic

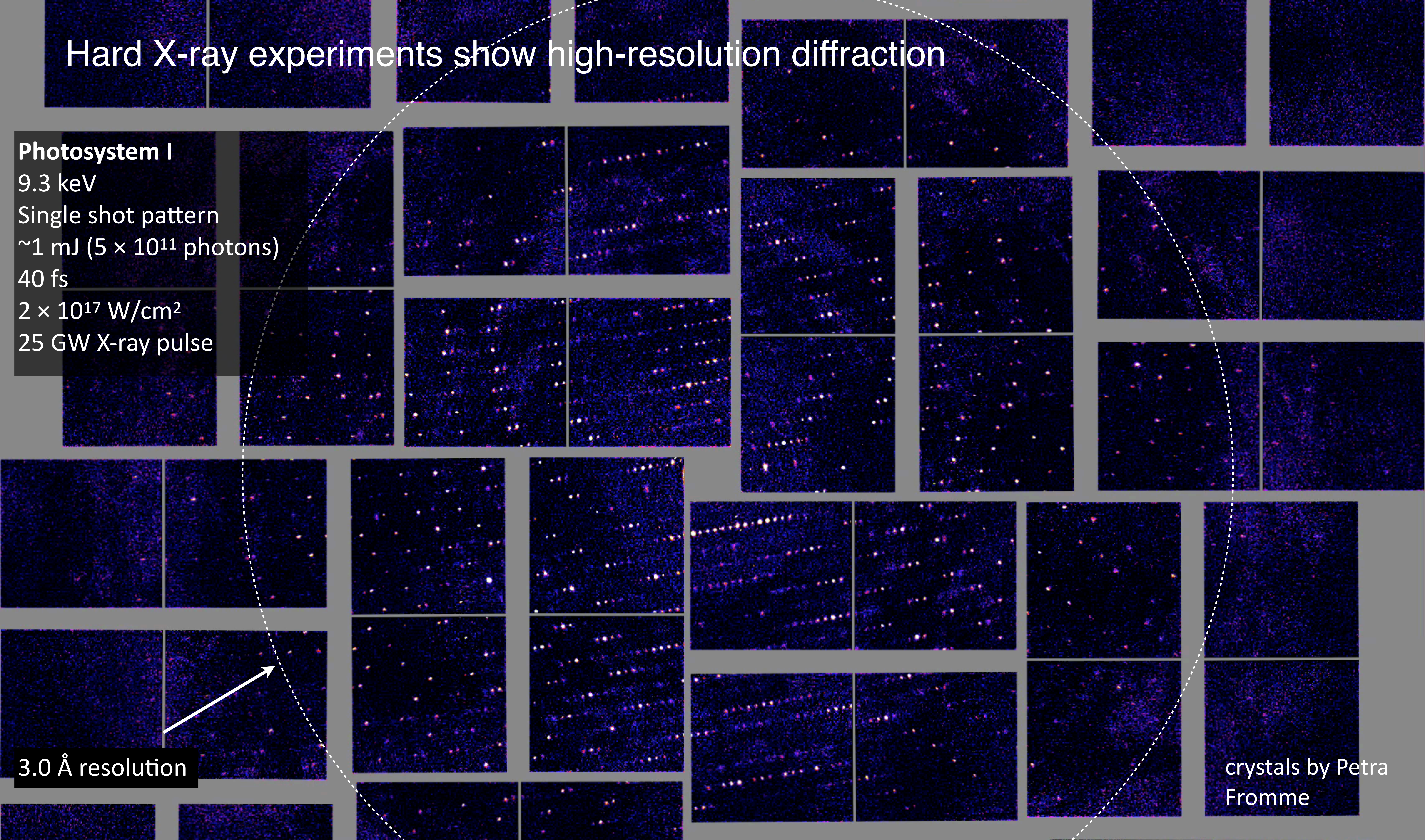
X-ray FEL

0.2 - 2 µm crystals

3 µm jet

1 µm X-ray beam

Hard X-ray experiments show high-resolution diffraction



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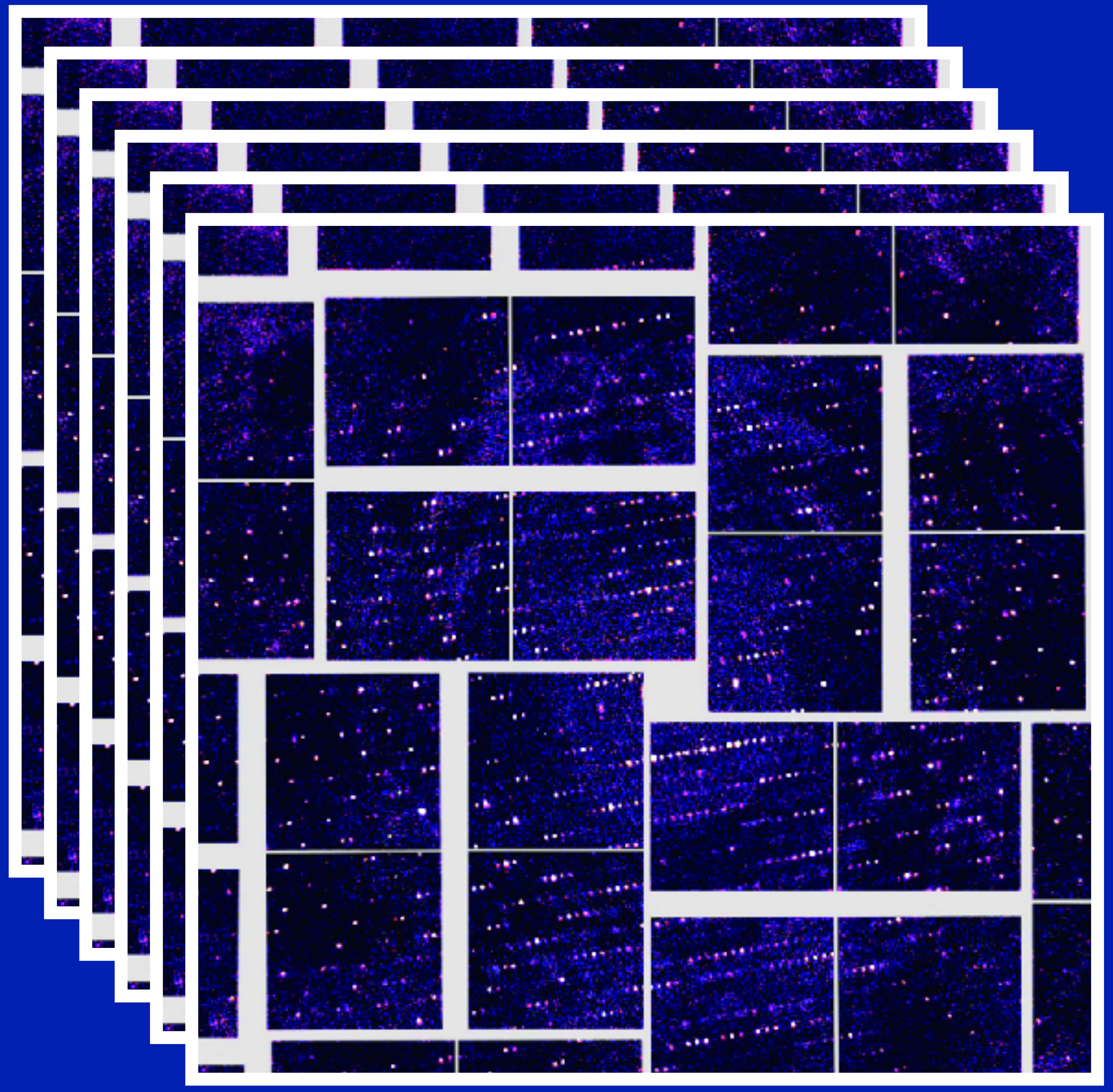
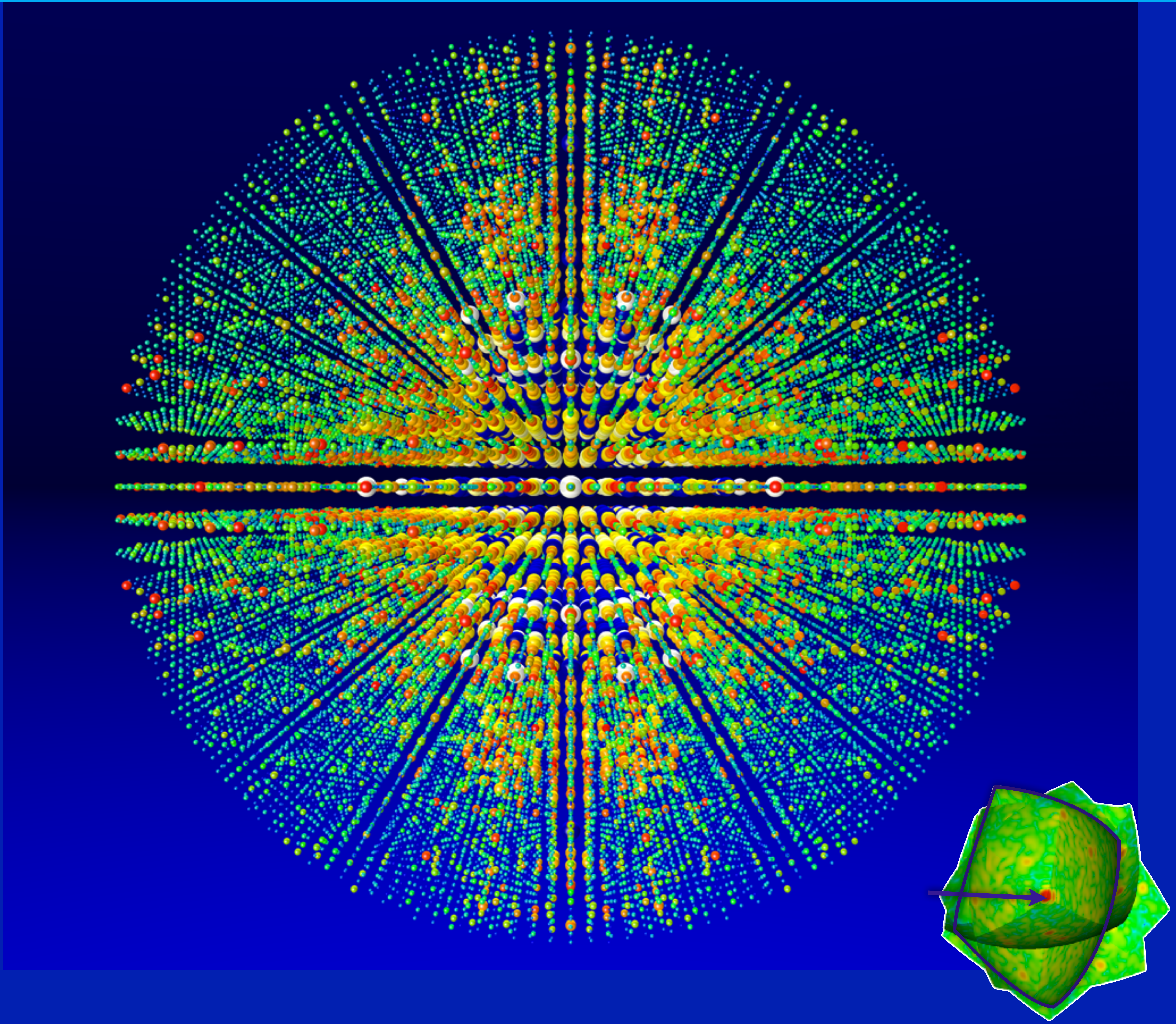
2 × 10^{17} W/cm^2
25 GW X-ray pulse

3.0 Å resolution

crystals by Petra Fromme

Snapshot intensities are merged in 3D using CrystFEL





CrystFEL
http://www.desy.de/~twhite/crystfel

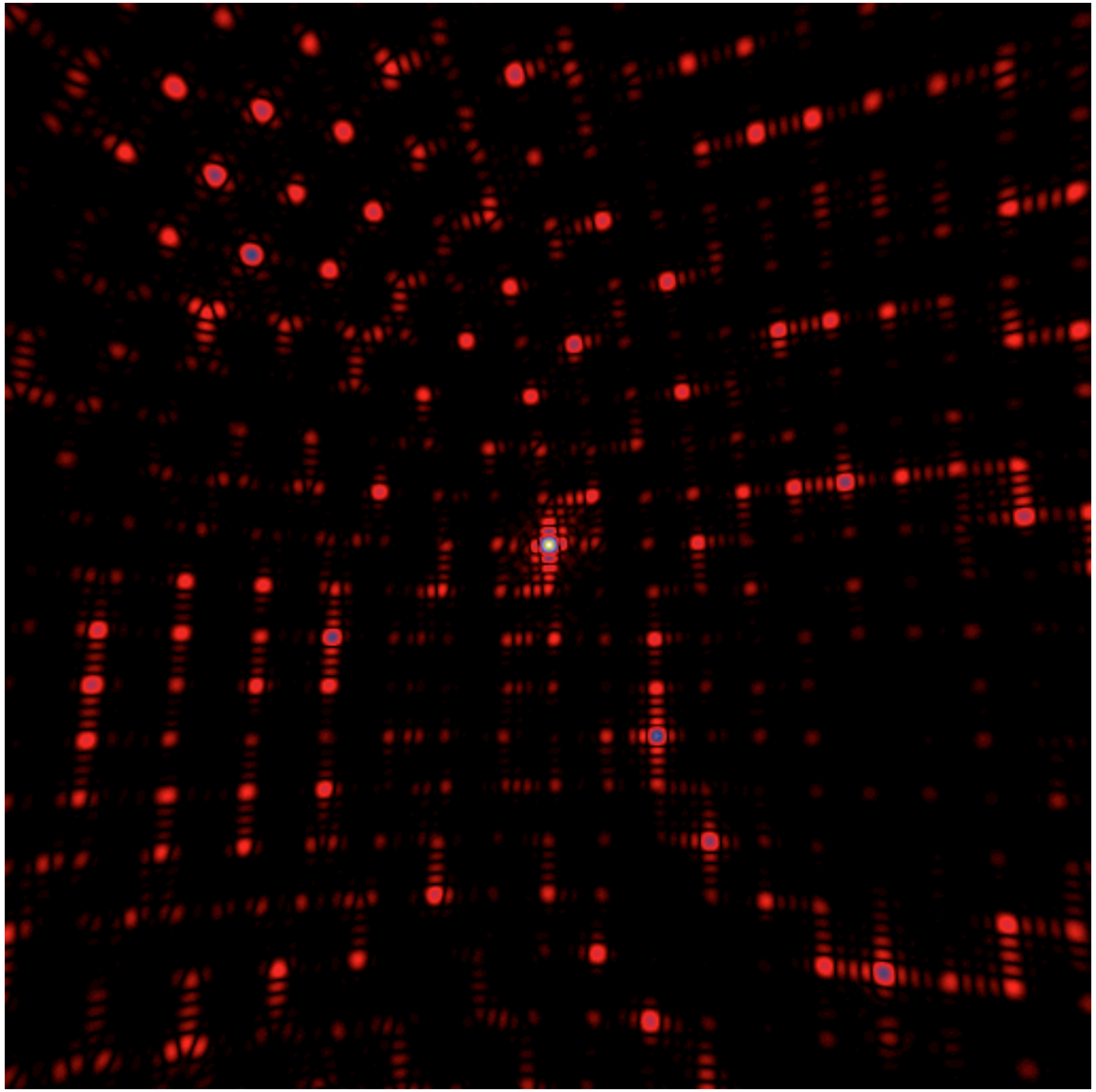
Free and open source (GNU GPLv3)
Documentation including a full tutorial online

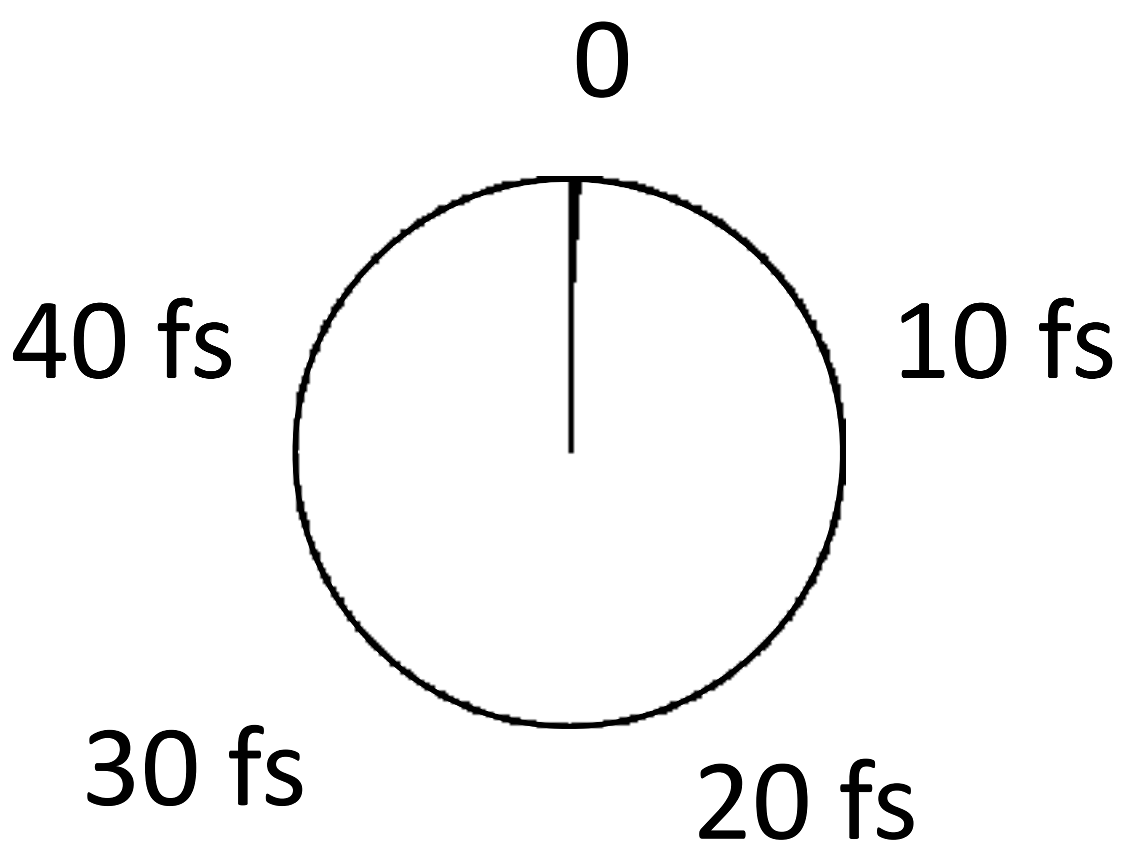
With femtosecond pulses, exposures can be increased by 1000’s of times

Simulation by C. Caleman, using Cretin (H. Scott, LLNL)

100 fs
6 GGy
(60 MGy/fs)

Bragg diffraction from a crystal gates the detection

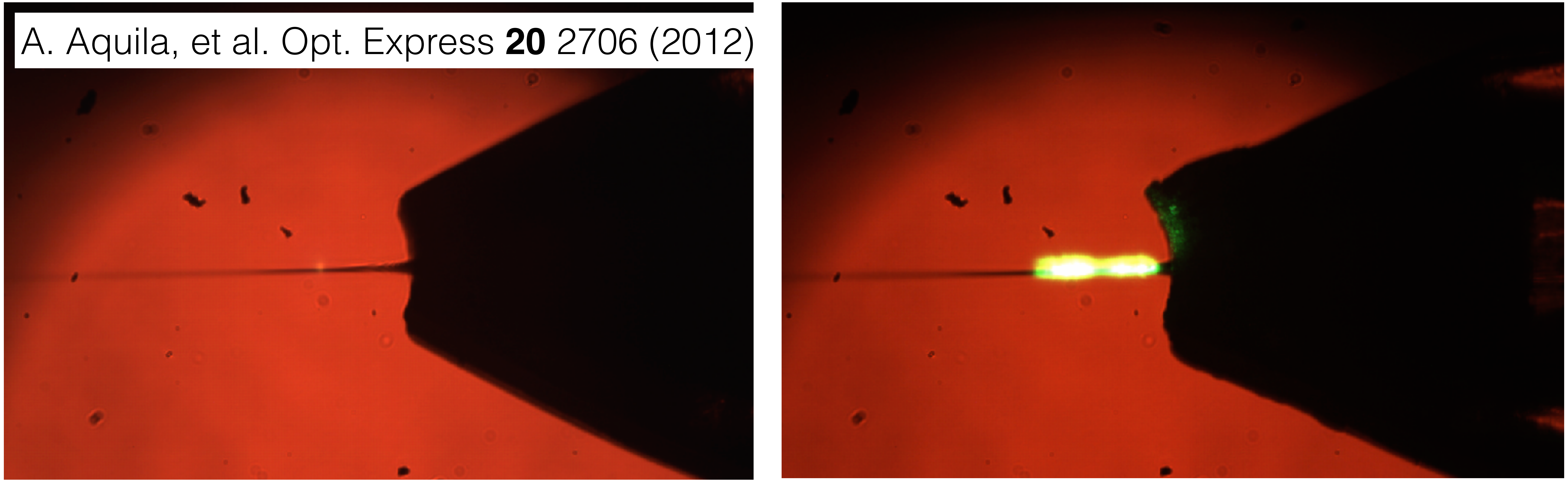


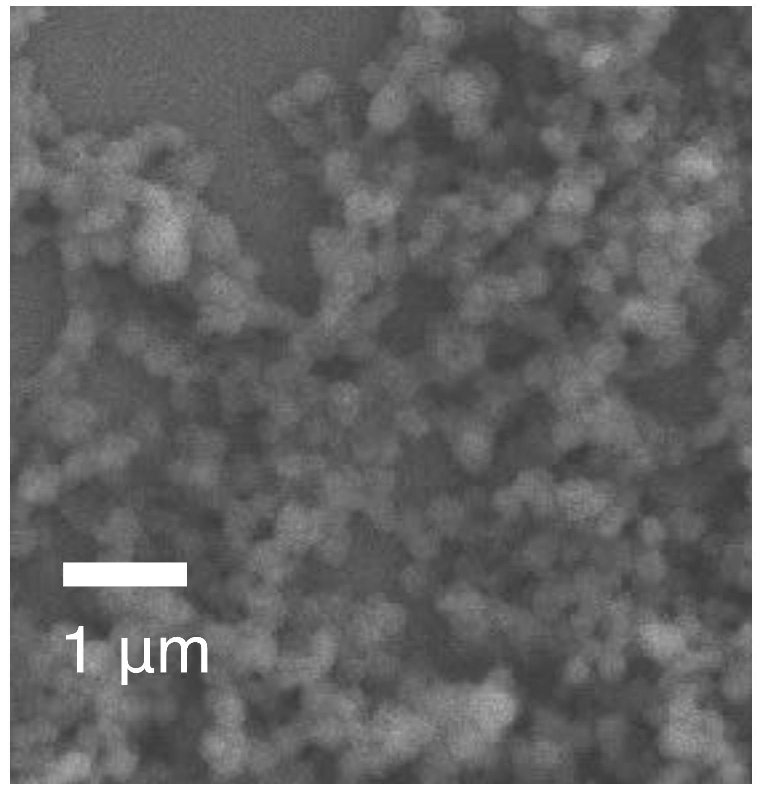


$$I(q) \propto |F(q)|^2 \int_0^T e^{-4\pi^2 q^2 \sigma^2(t)}\, dt$$

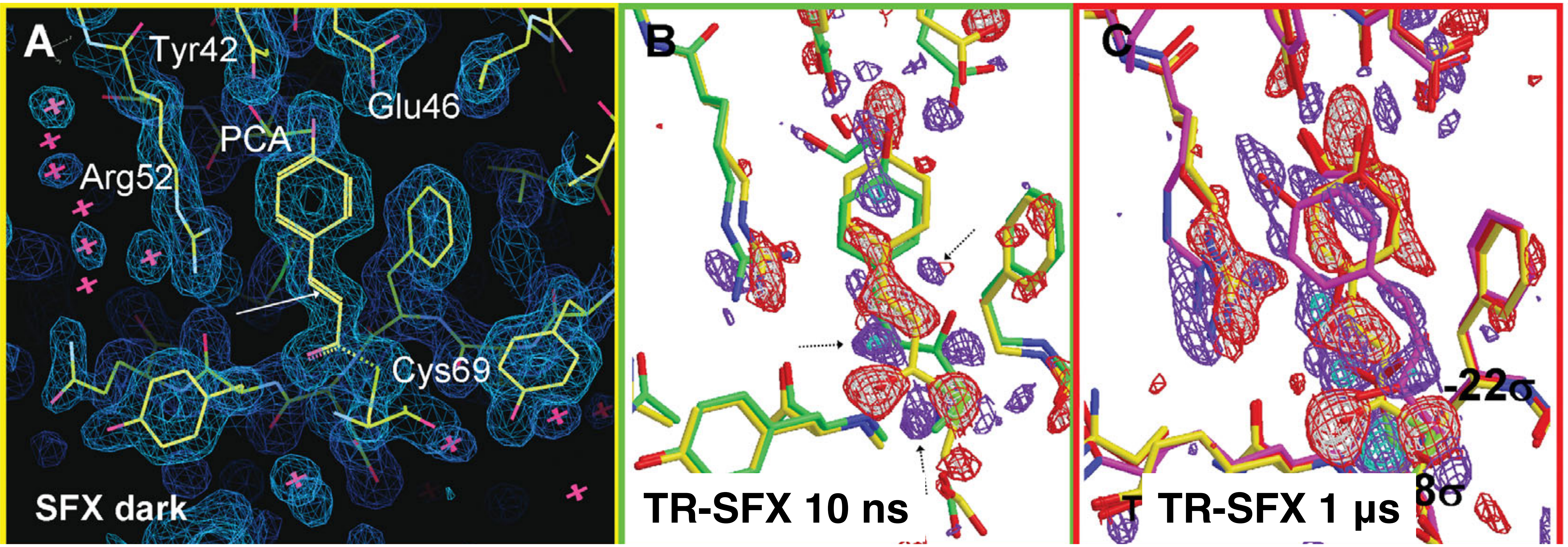
Integrated diffraction
rms displacement by end of pulse: 1 Å
Resolution: 2 Å

The serial approach can be applied to time-resolved measurements of irreversible reactions


A. Aquila, et al. Opt. Express **20** 2706 (2012)

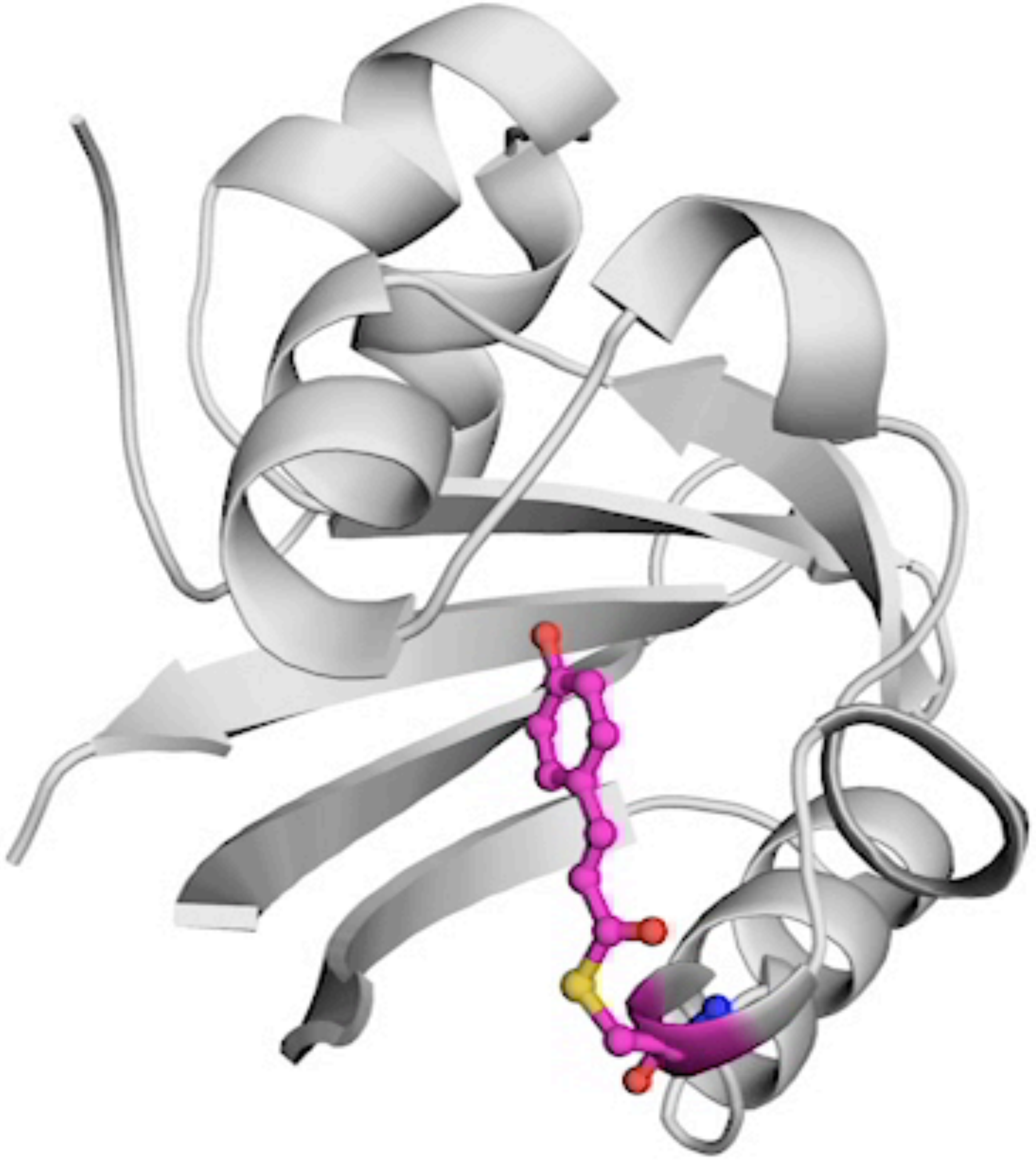


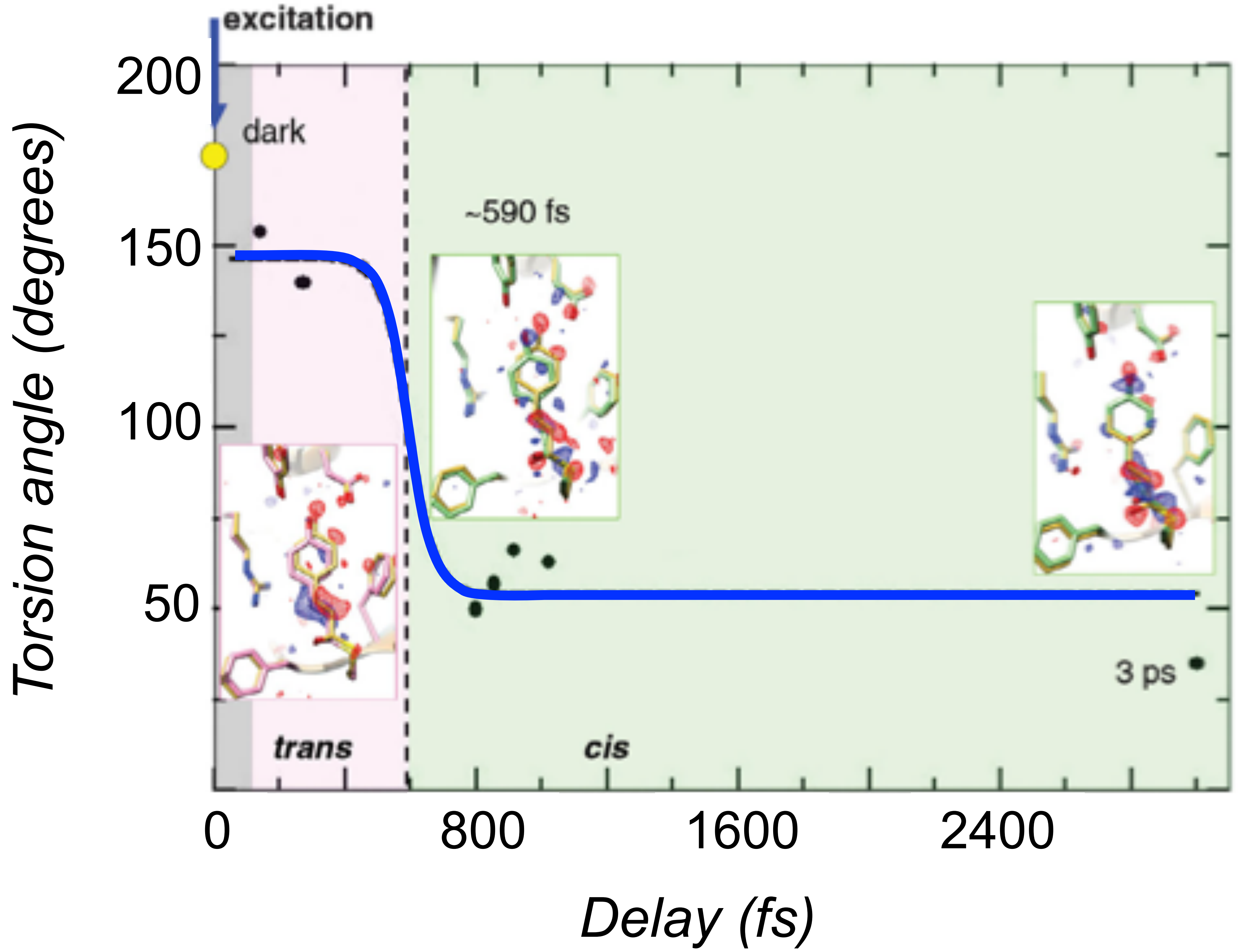
J. Tenboer et al
Science **346** 1242 (2014)



X-ray free-electron lasers enable us to determine how protein structures evolve, with femtosecond resolution

Photoactive Yellow Protein



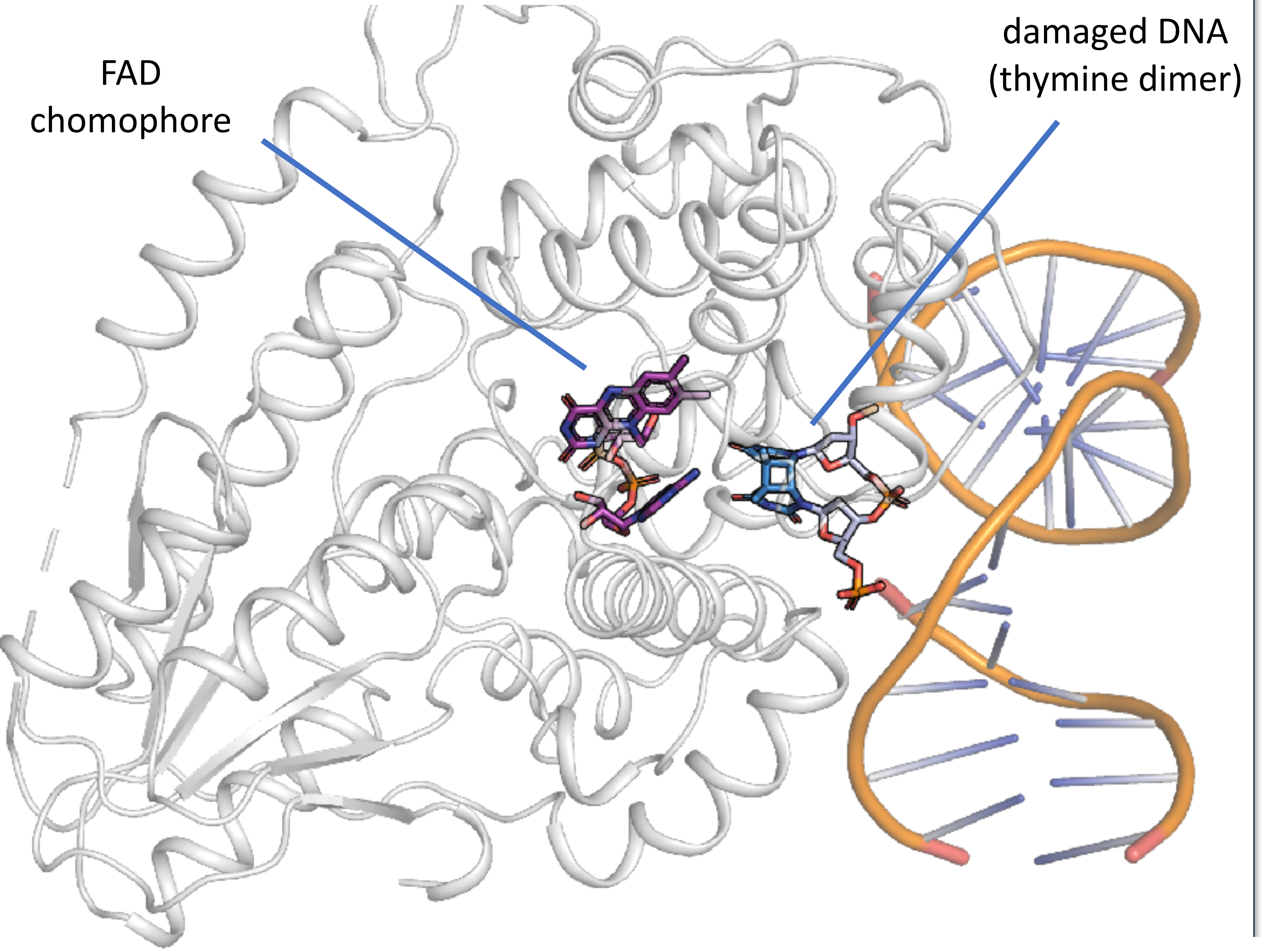


Experiments led by **Marius Schmidt**, U. Wisconsin

Tenboer et al *Science* **346** 1242 (2014)
Pande et al *Science* **352** 725 (2016)

Watching photolyase repair DNA: function from time-resolved serial crystallography

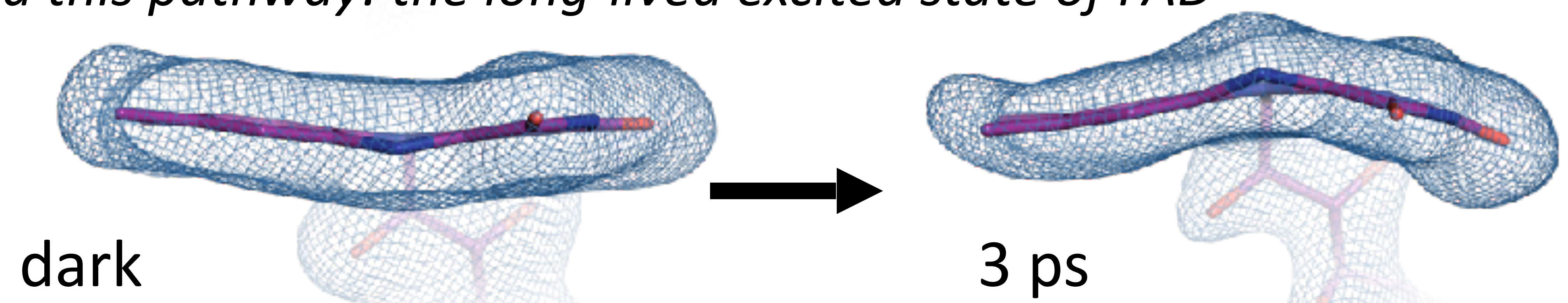
Photolyase repairs DNA damage caused by sunlight, using sunlight …

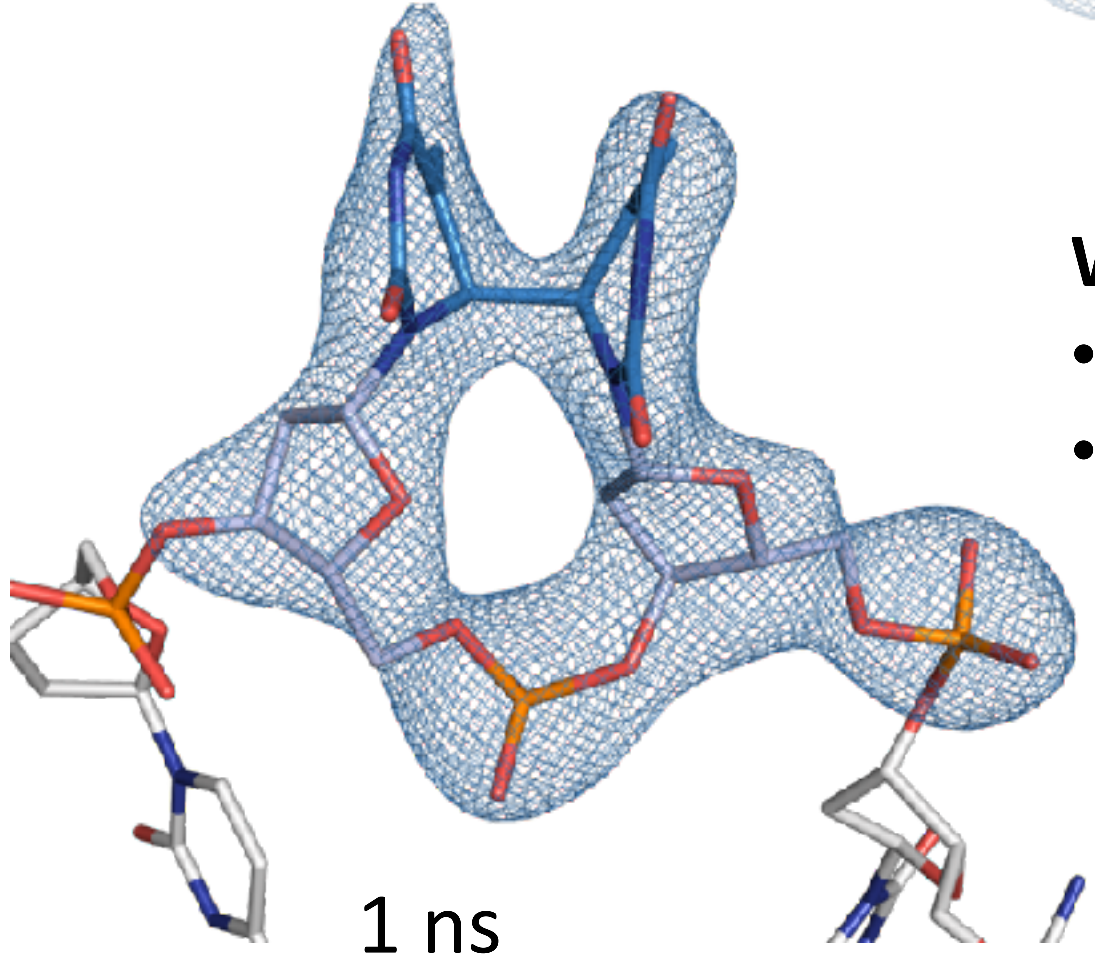


… our team captured the catalytic cycle of this enzyme (3 ps → 100 µs) using trSFX

How does photolyase productively use nearly all absorbed photons?

- FAD based models: < 5% efficient
- Photolyase: > 95% productive electron transfer
- *We characterized this pathway: the long-lived excited state of FAD*



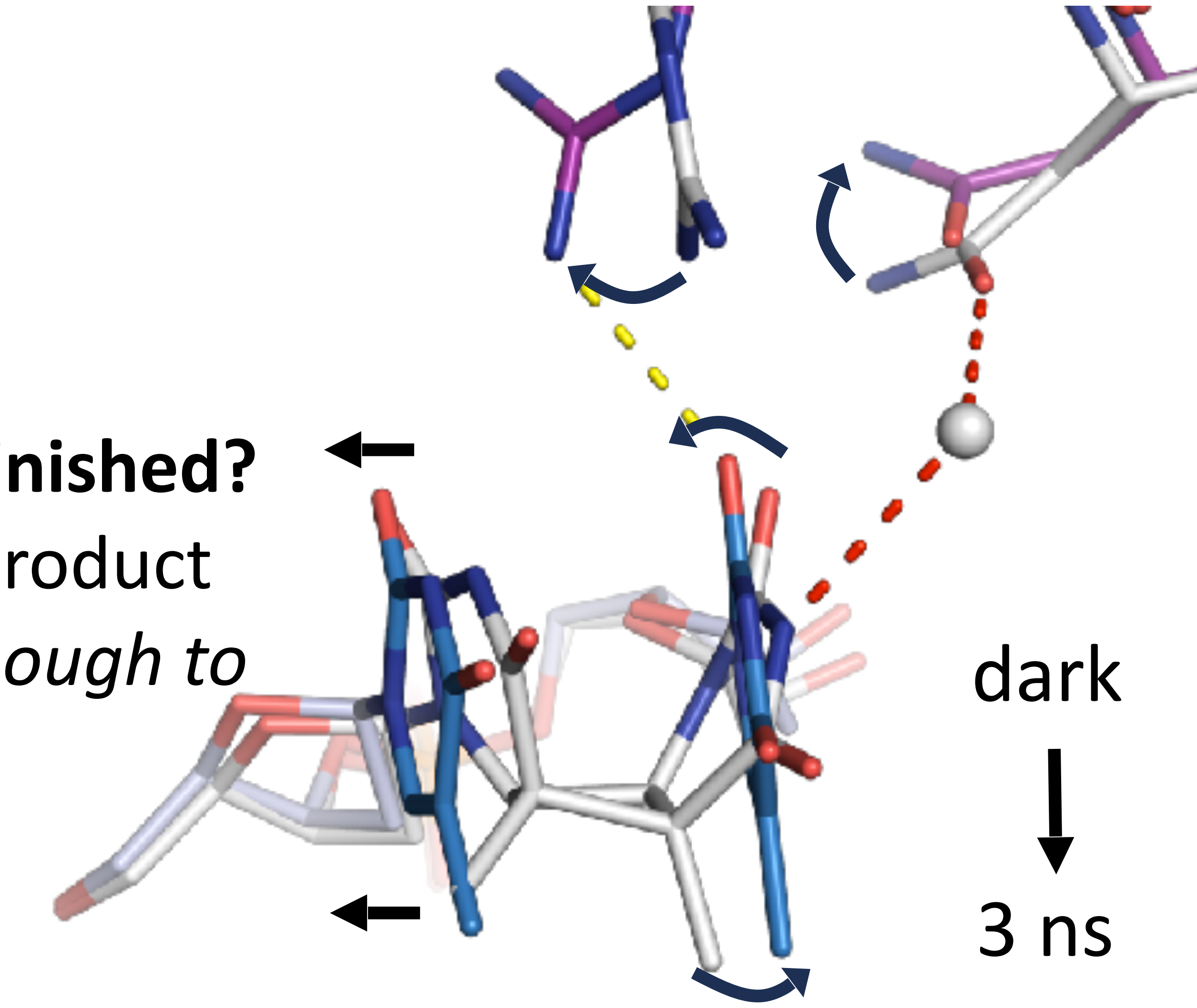


What is the mechanism of DNA repair?

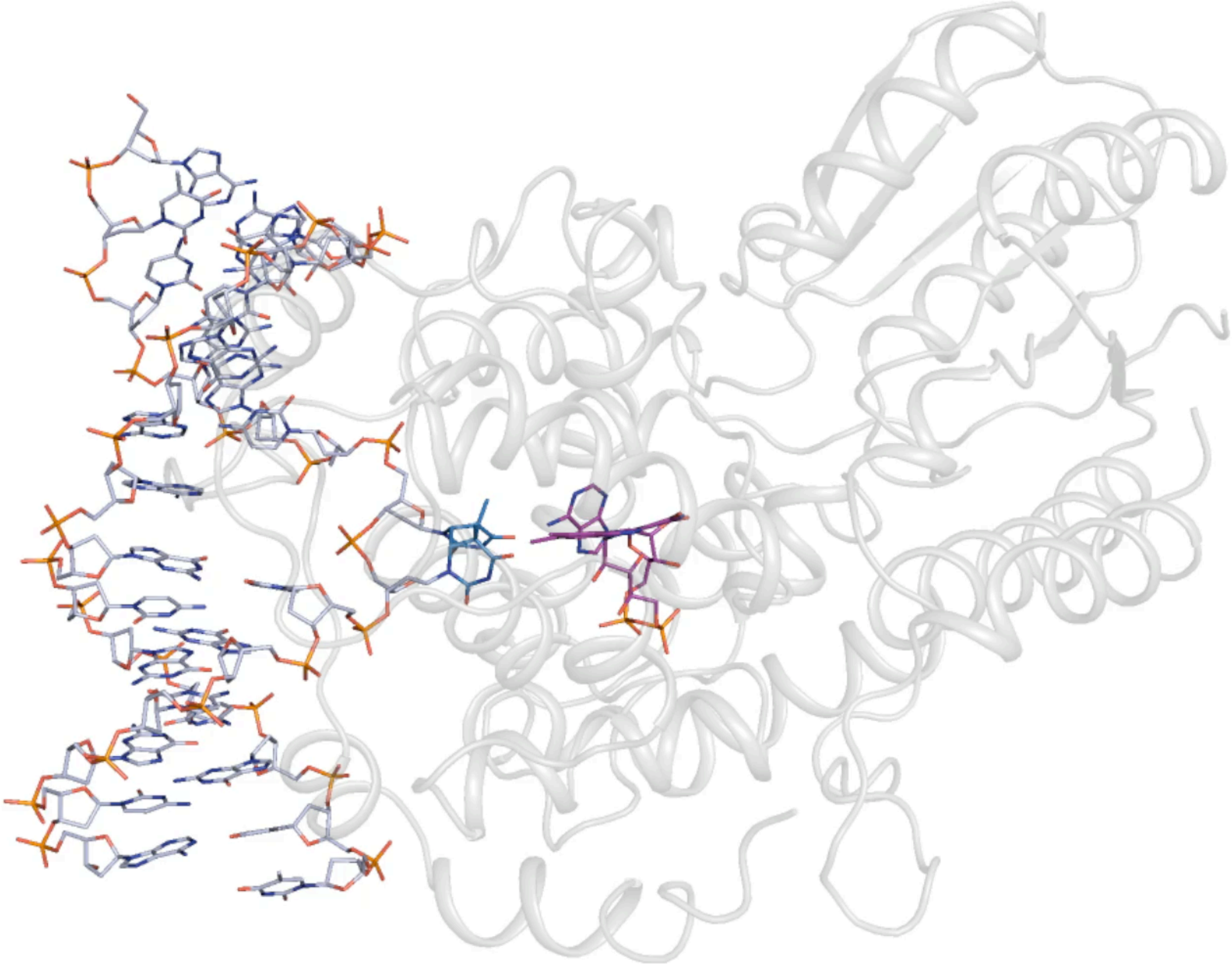
- Debated for decades: one bond or two
- *Our maps: unambiguous one-bond intermediate*

How does the enzyme know its job is finished?

- Much higher affinity for substrate *vs.* product
- *Big changes of DNA upon repair are enough to disrupt protein:product interactions*



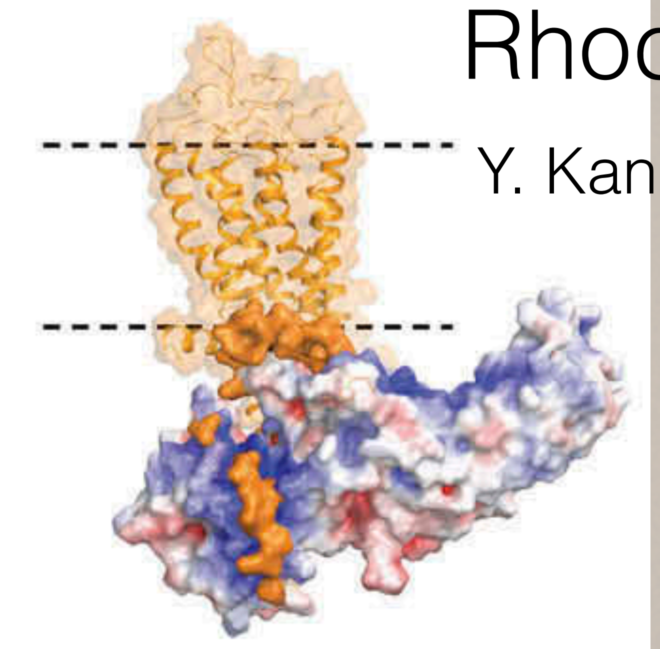
Nina Christou,et al, **TJ Lane**. *Science* **382**, 1015 (2023).



N.-E. Christou,.., T.J. Lane, *Science* 382, 1015 (2023)

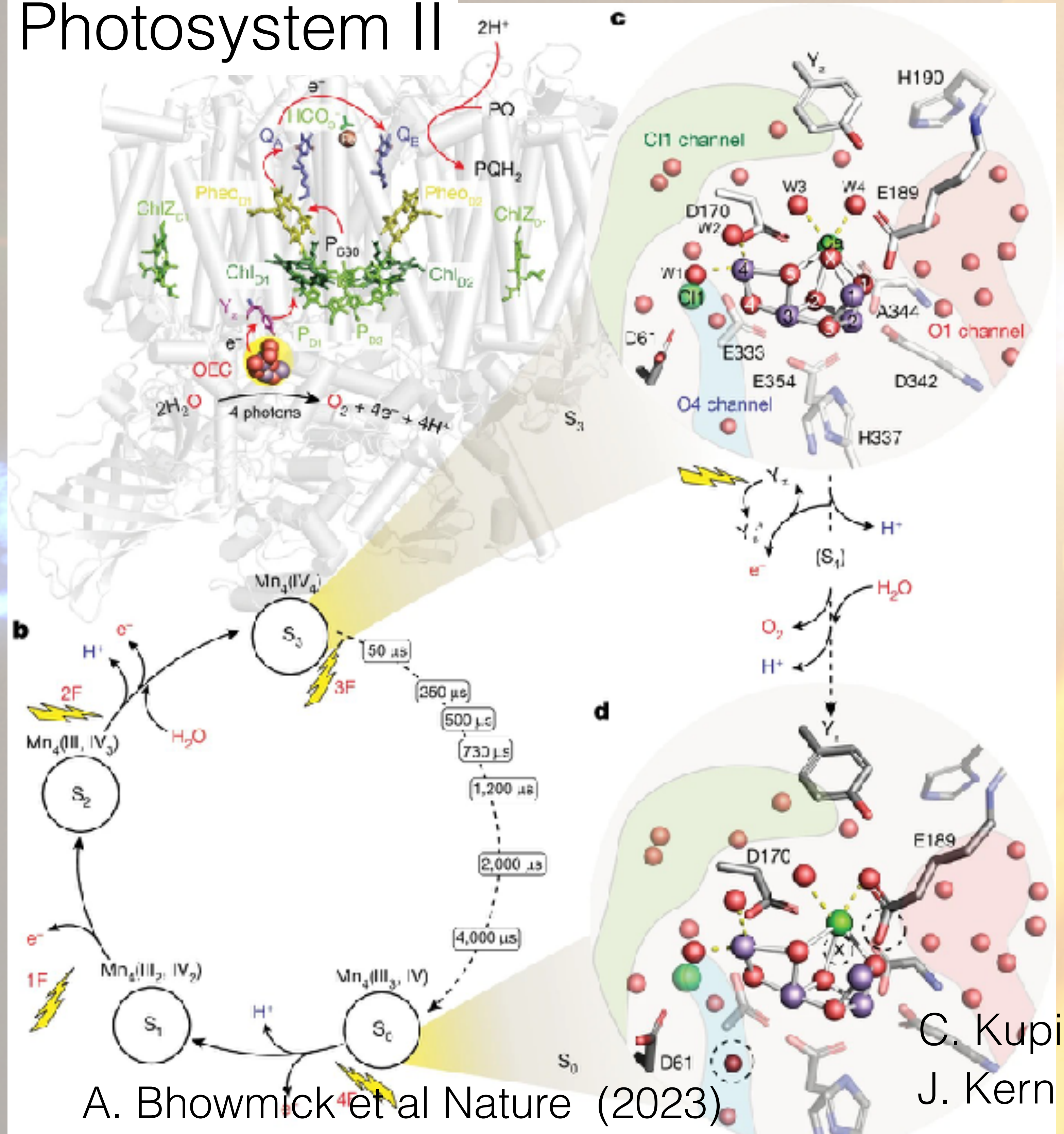
Proteins can be measured at physiological temperatures….in action

Rhodopsin-arrestin



Y. Kang et al Nature **523** (2015)

Photosystem II

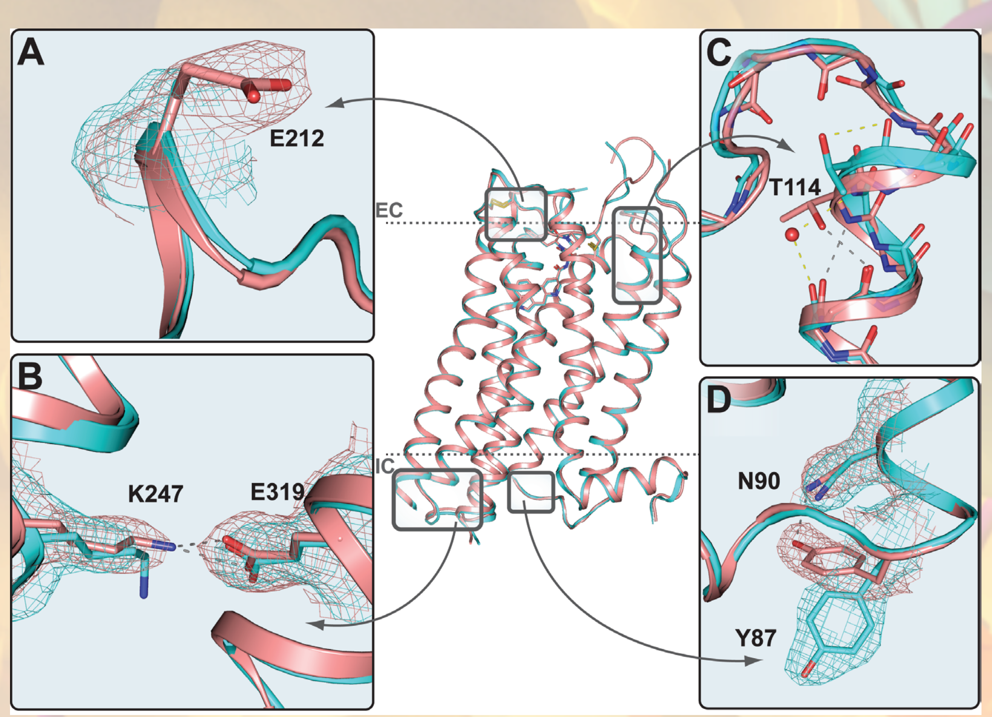


A. Bhowmick et al Nature (2023)

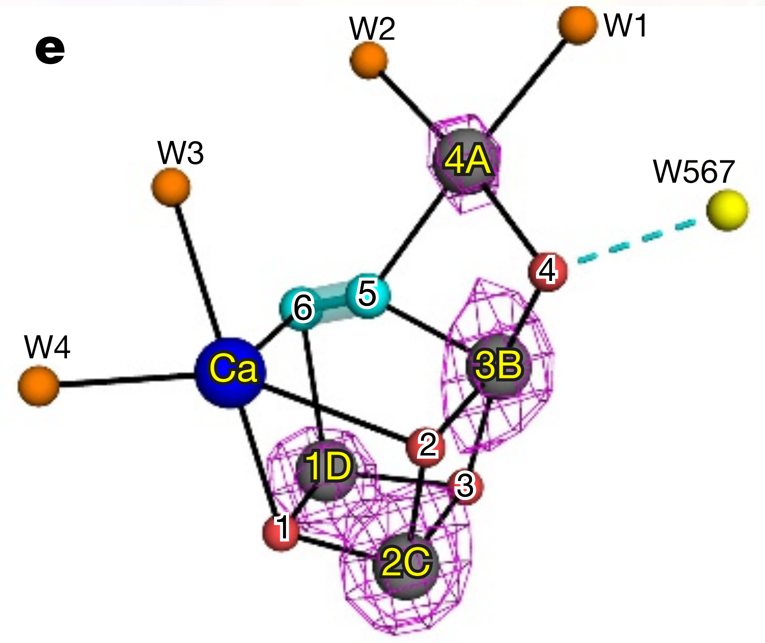
C. Kupitz et al Nature **513** (2014)

J. Kern et al Nature **563** (2018)

G-protein coupled receptor



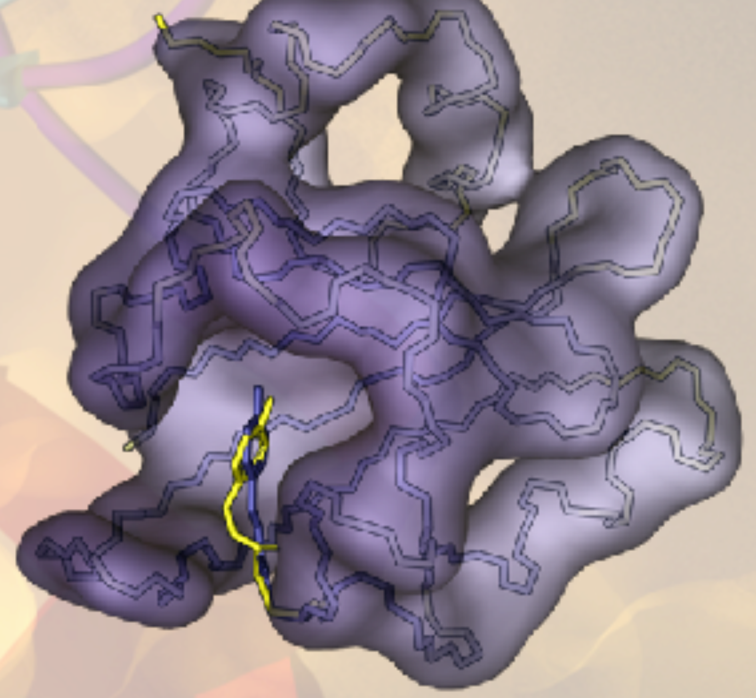
W. Liu et al Science **342** (2013)

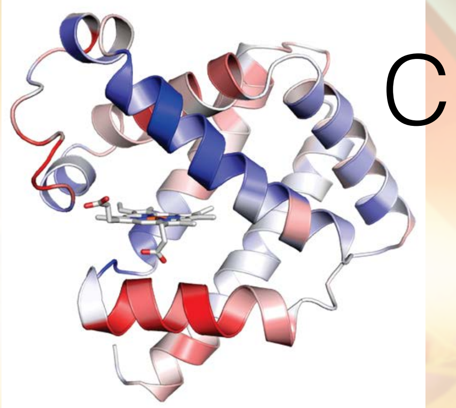


M. Suga et al
Nature **543**
(2017)

Photoactive Yellow Protein

J. Tenboer et al, Science **346** (2014)
K. Pande et al. Science **352** (2016)





CO myoglobin

T. Barends et al
Science **350**
(2015)

Gene switching

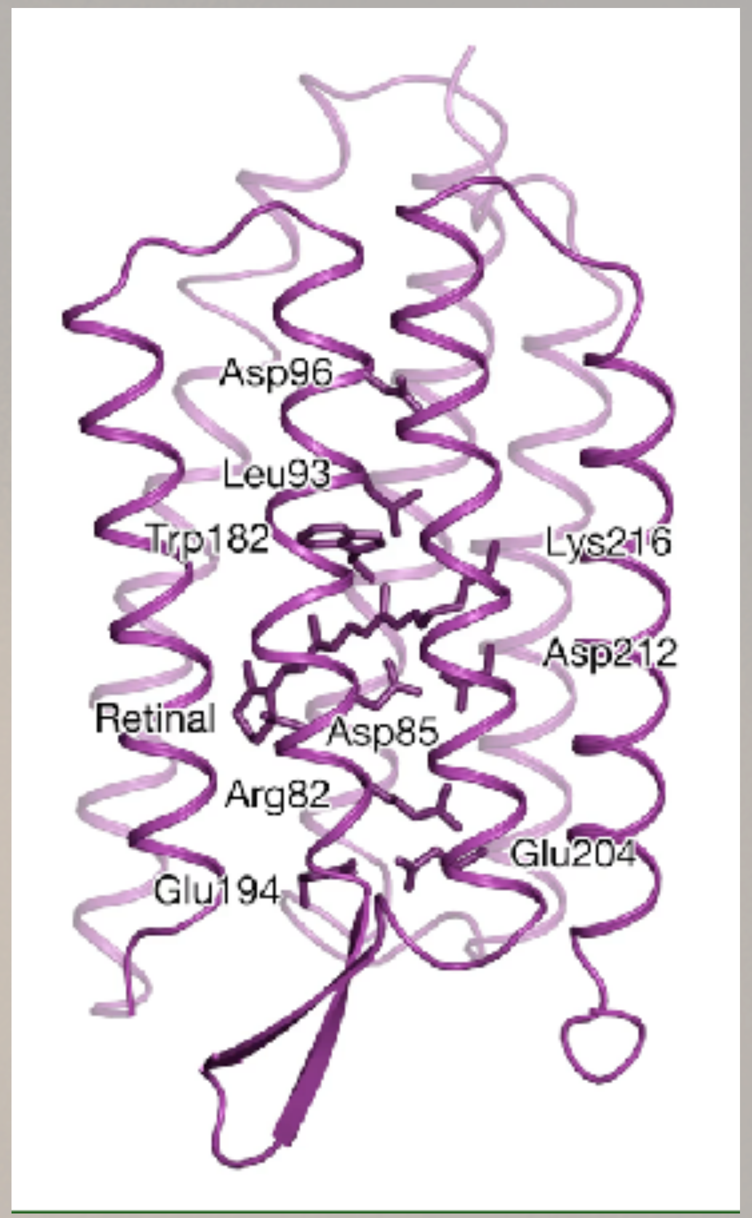
J. Stagno et al Nature **541** (2017)

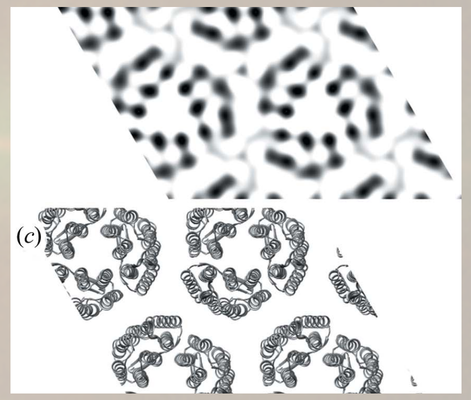
Bacterio-rhodopsin

E. Nango et al
Science **354**
(2016)

Rhodopsin

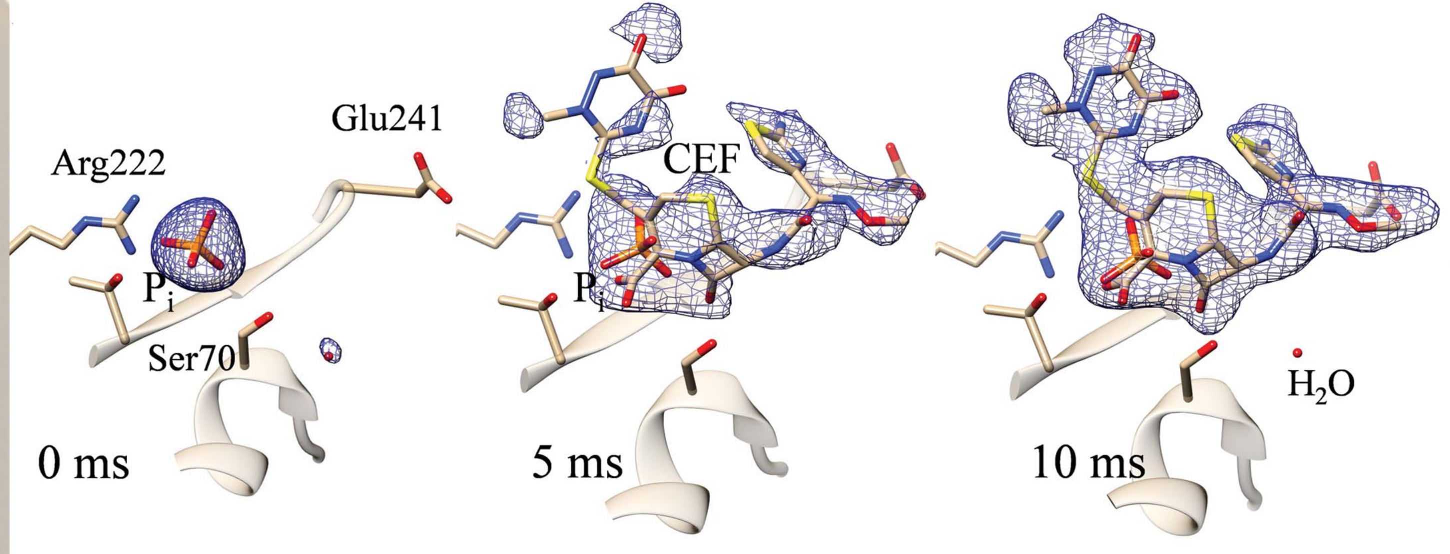
T. Gruhl et al
Nature **615** (2023)





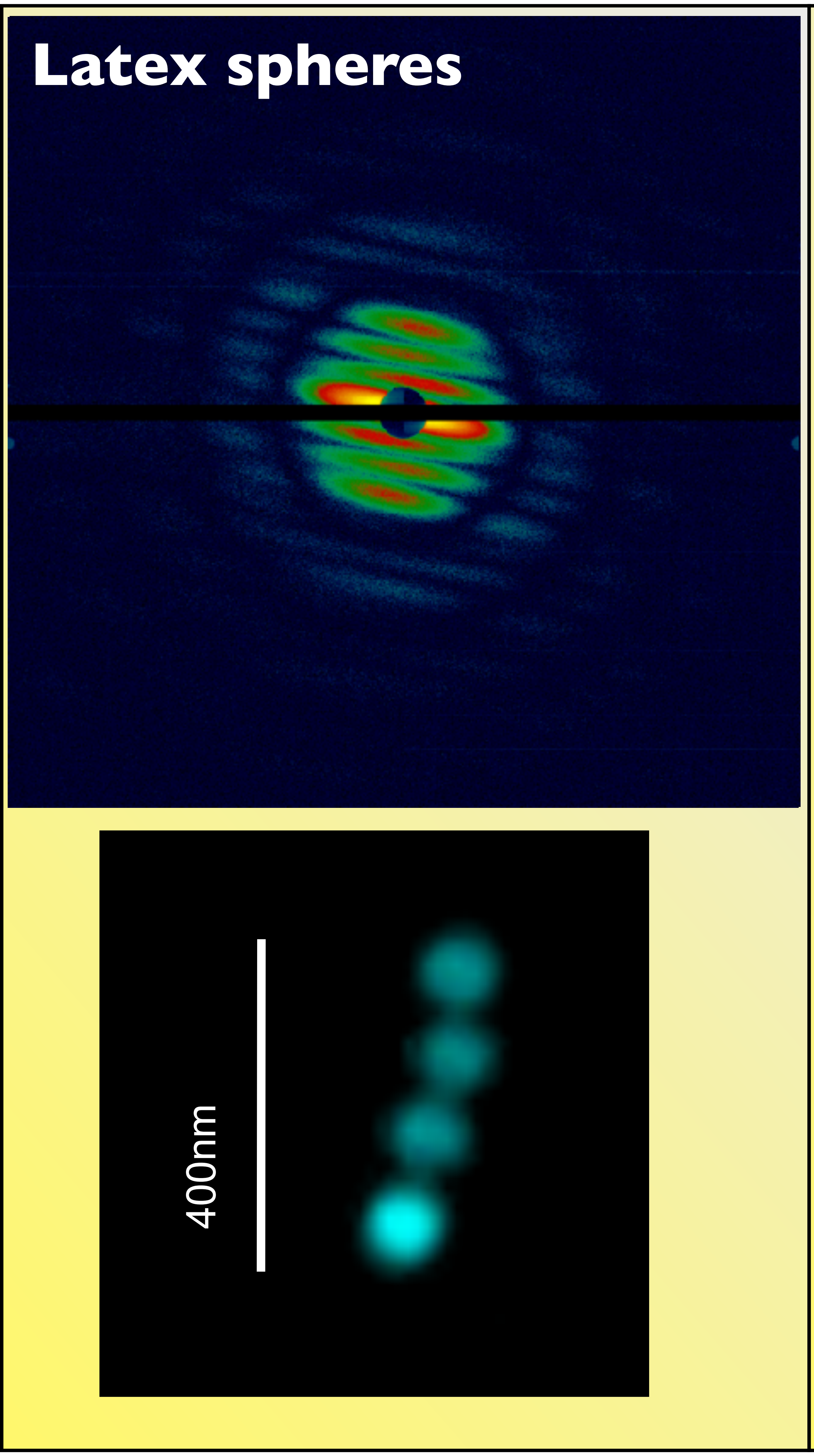
M. Frank et al,
IUCrJ **1** (2014)

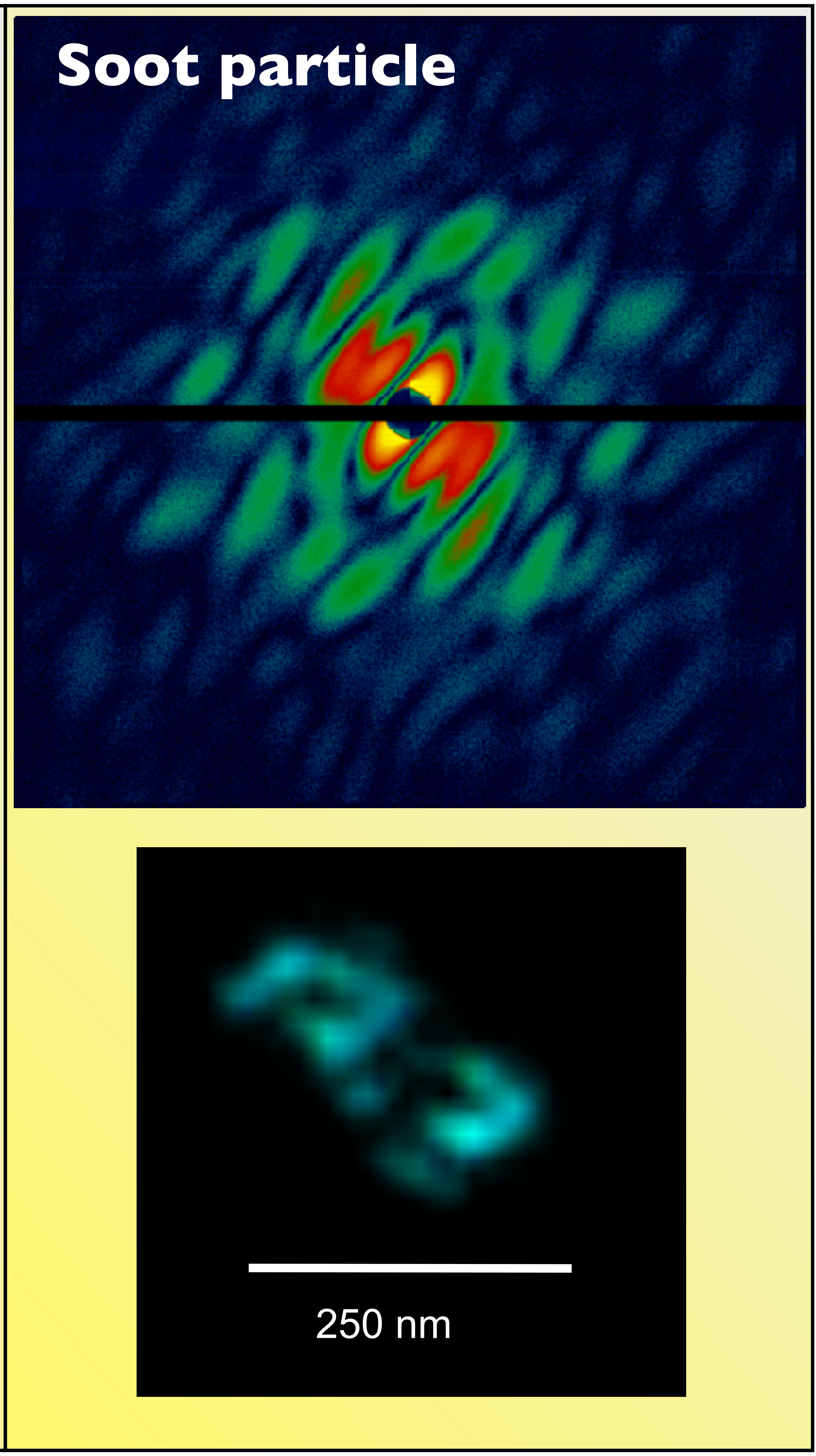
β lactamase

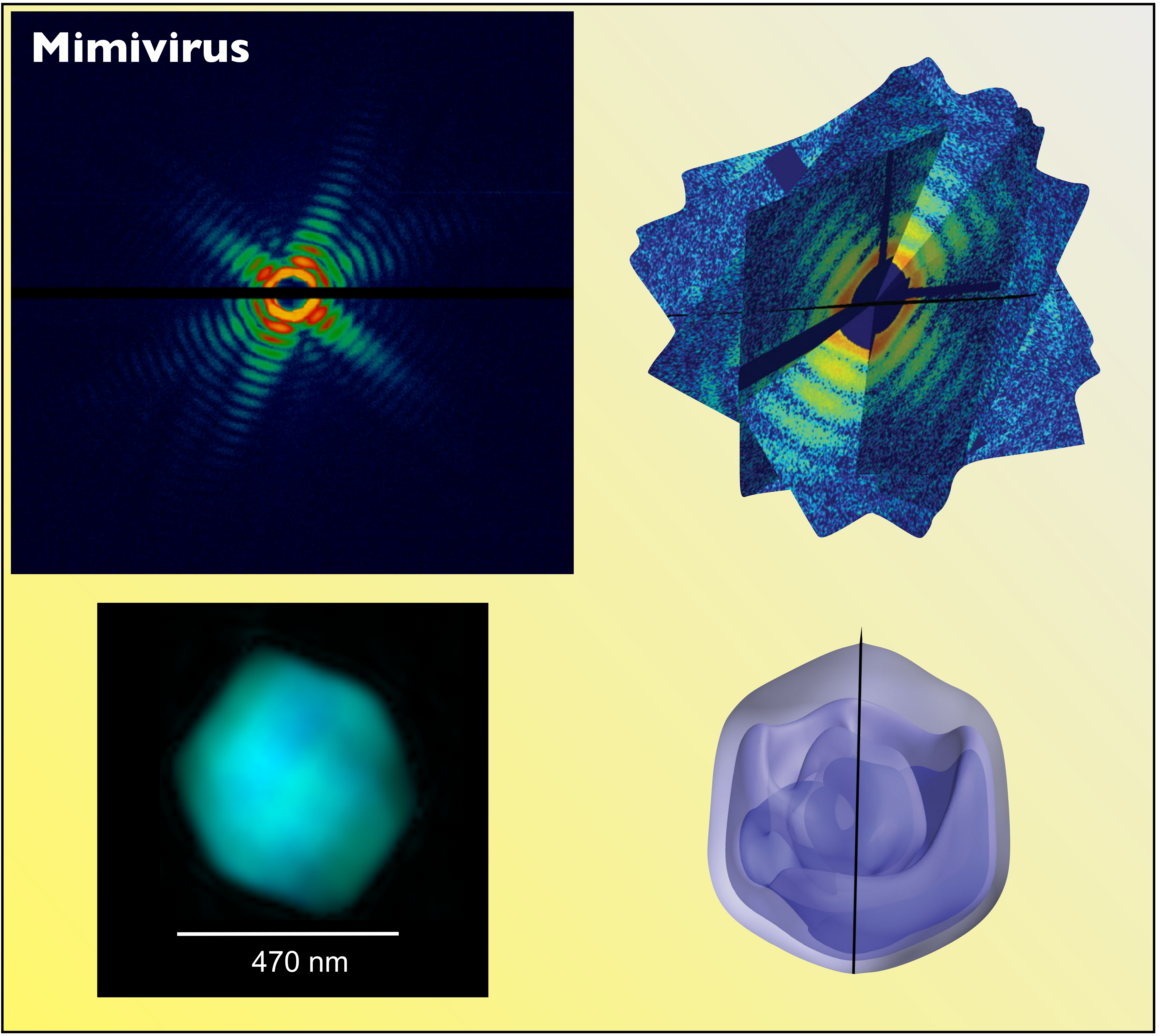


S. Pandey et al IUCrJ **8** (2021)

Non-crystalline objects can be imaged with FEL pulses by coherent diffraction

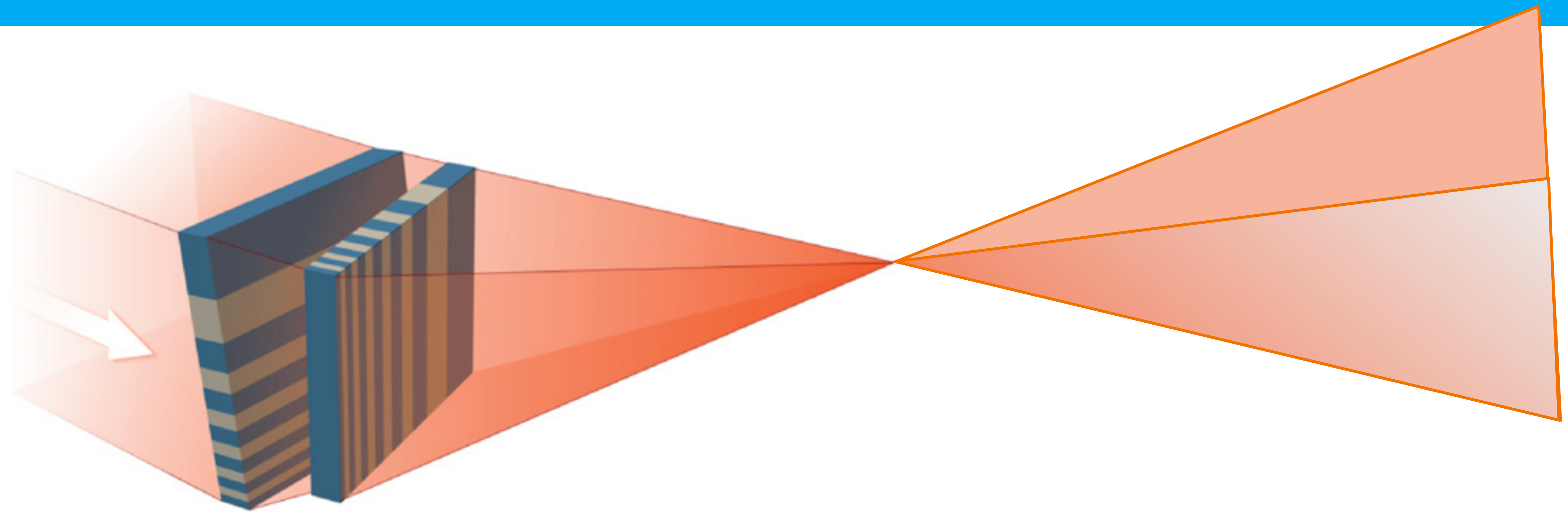

Latex spheres

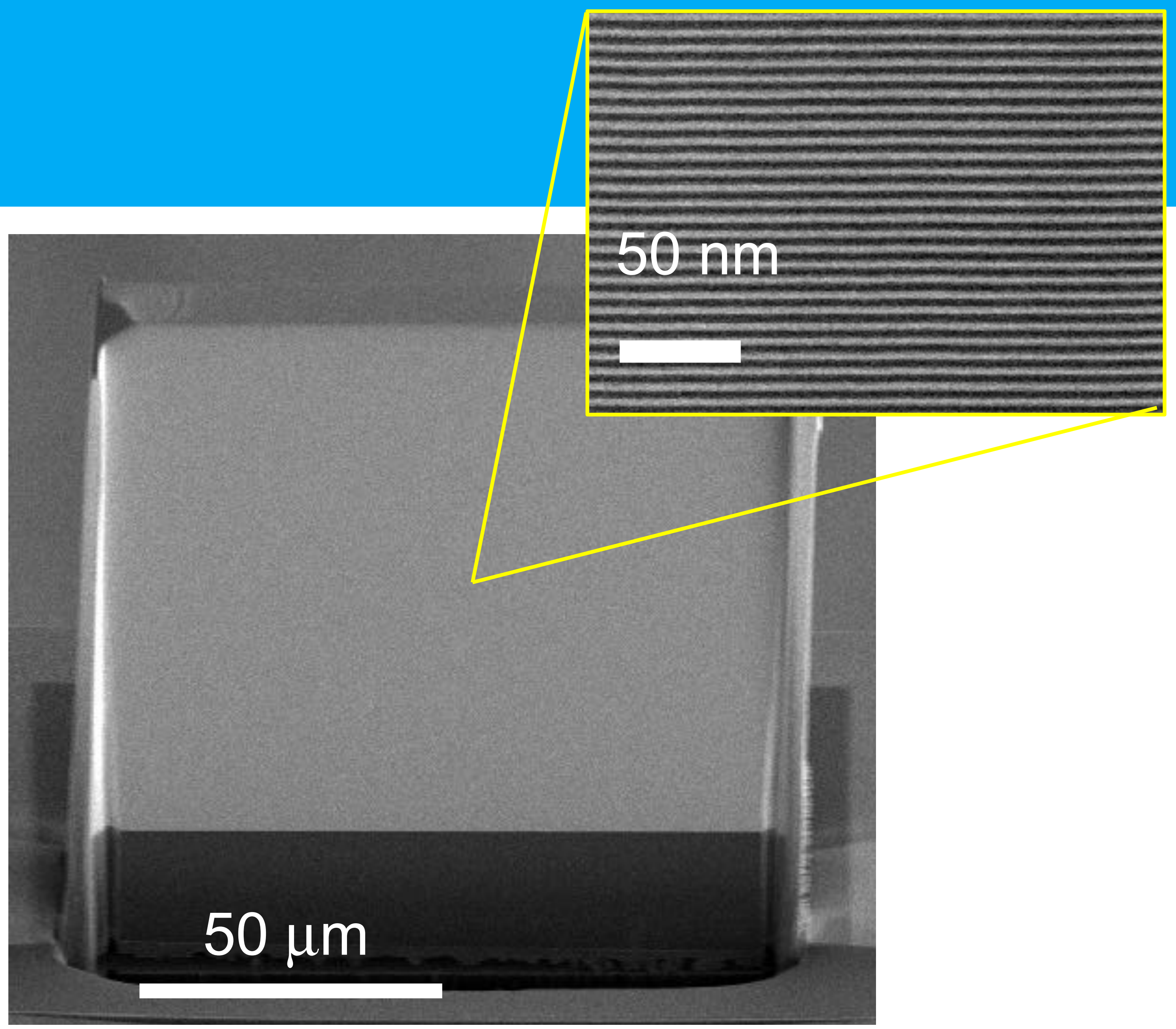

Soot particle

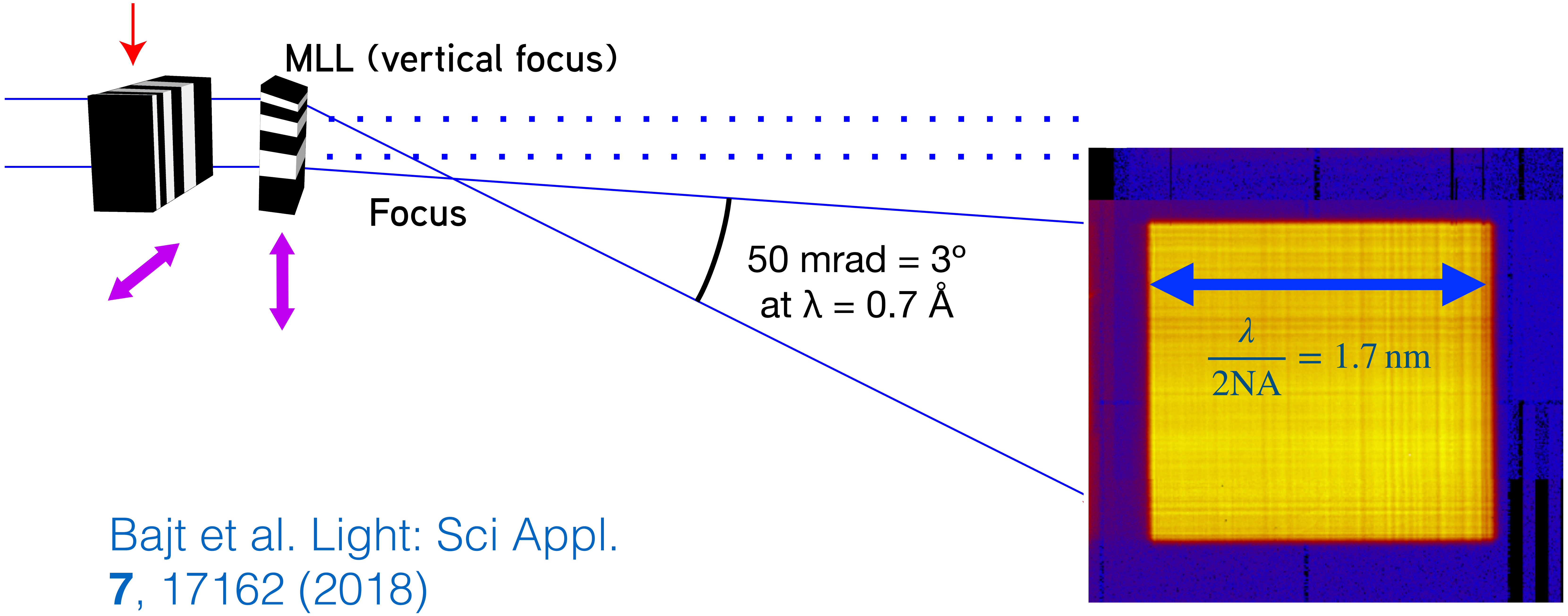

Mimivirus

Ekeberg et al PRL **114** (2105)

Multilayer Laue lenses produce highly convergent beams







MLL (horizontal focus)

MLL (vertical focus)

Focus

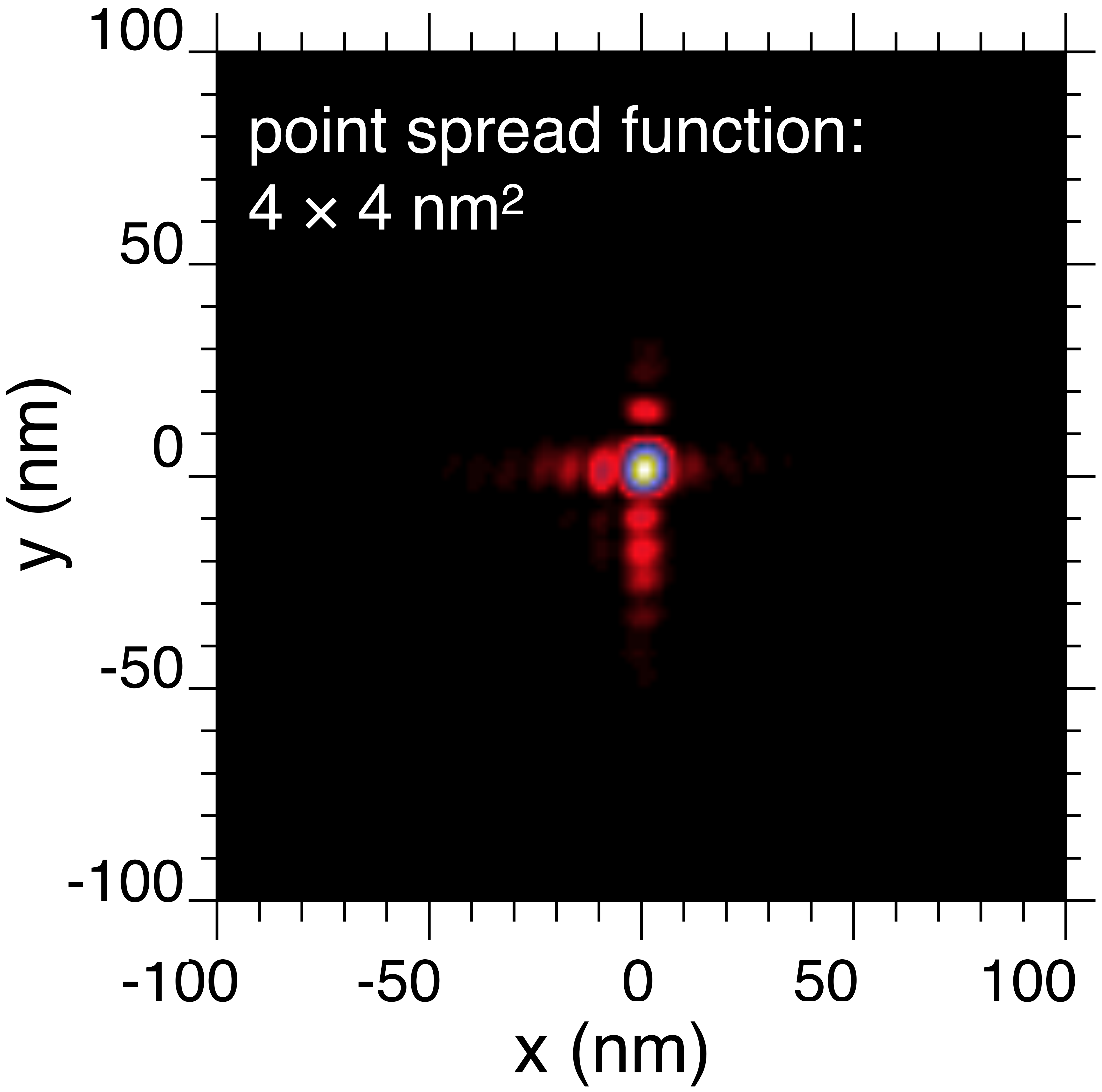
50 mrad = 3°
at λ = 0.7 Å

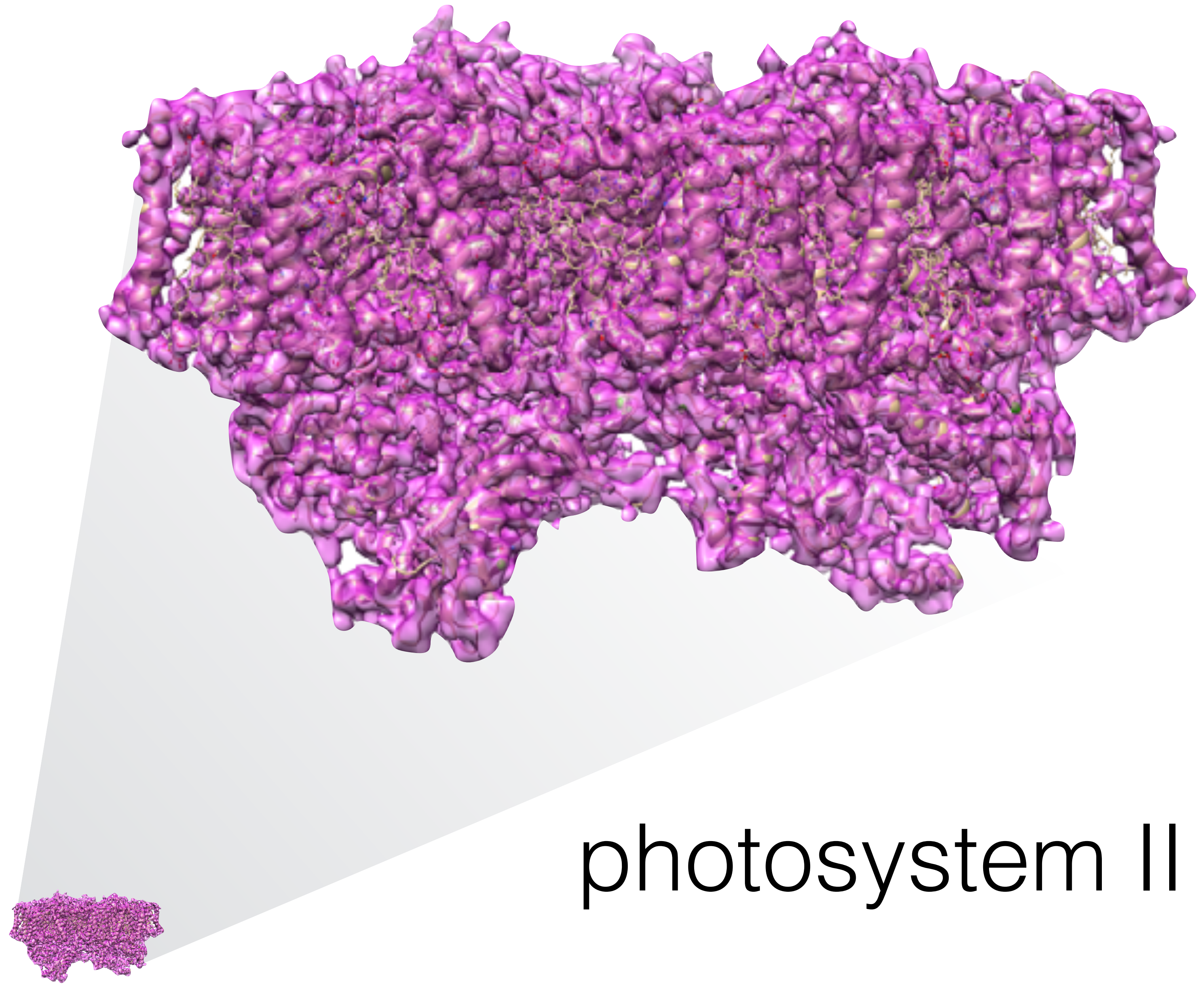
$$\frac{\lambda}{2\mathrm{NA}} = 1.7\ \mathrm{nm}$$

Microscopy TODAY
2018 Innovation Award

Bajt et al. Light: Sci Appl.
7, 17162 (2018)

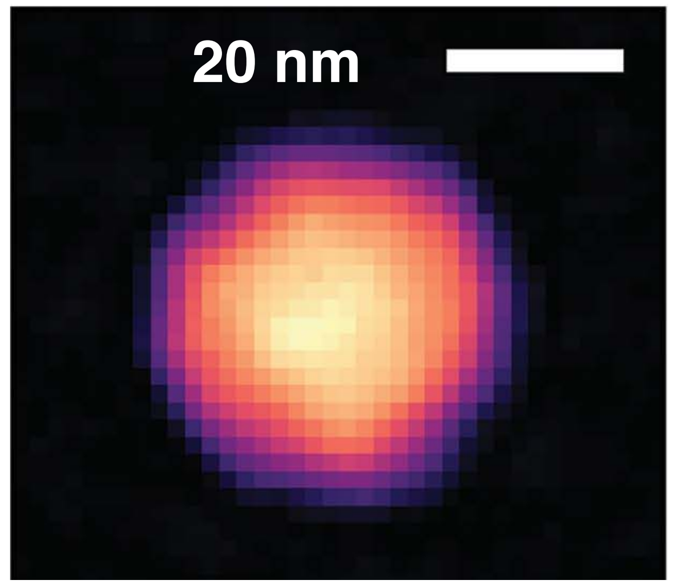
We can focus to molecular sizes





photosystem II

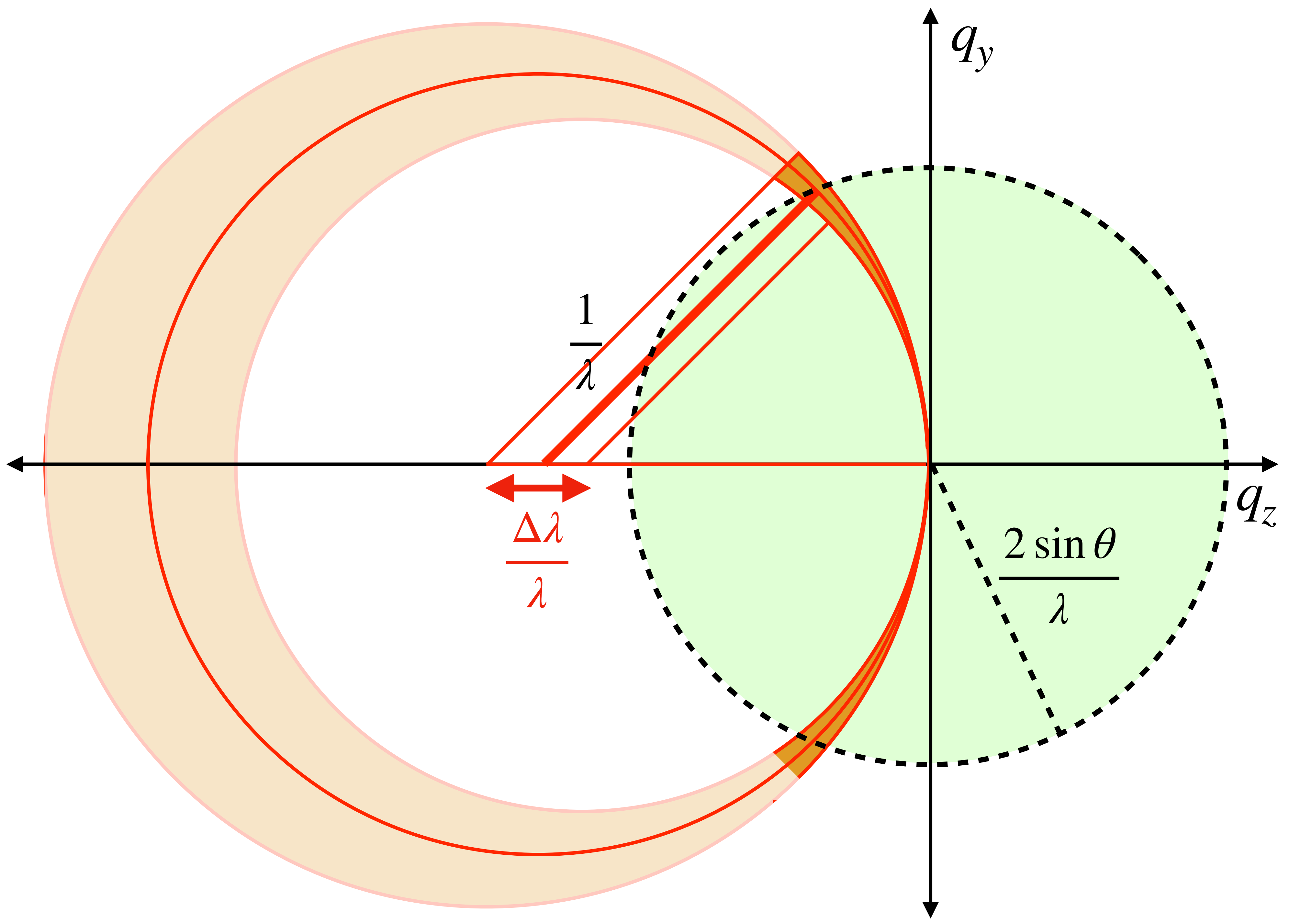
Omono River virus

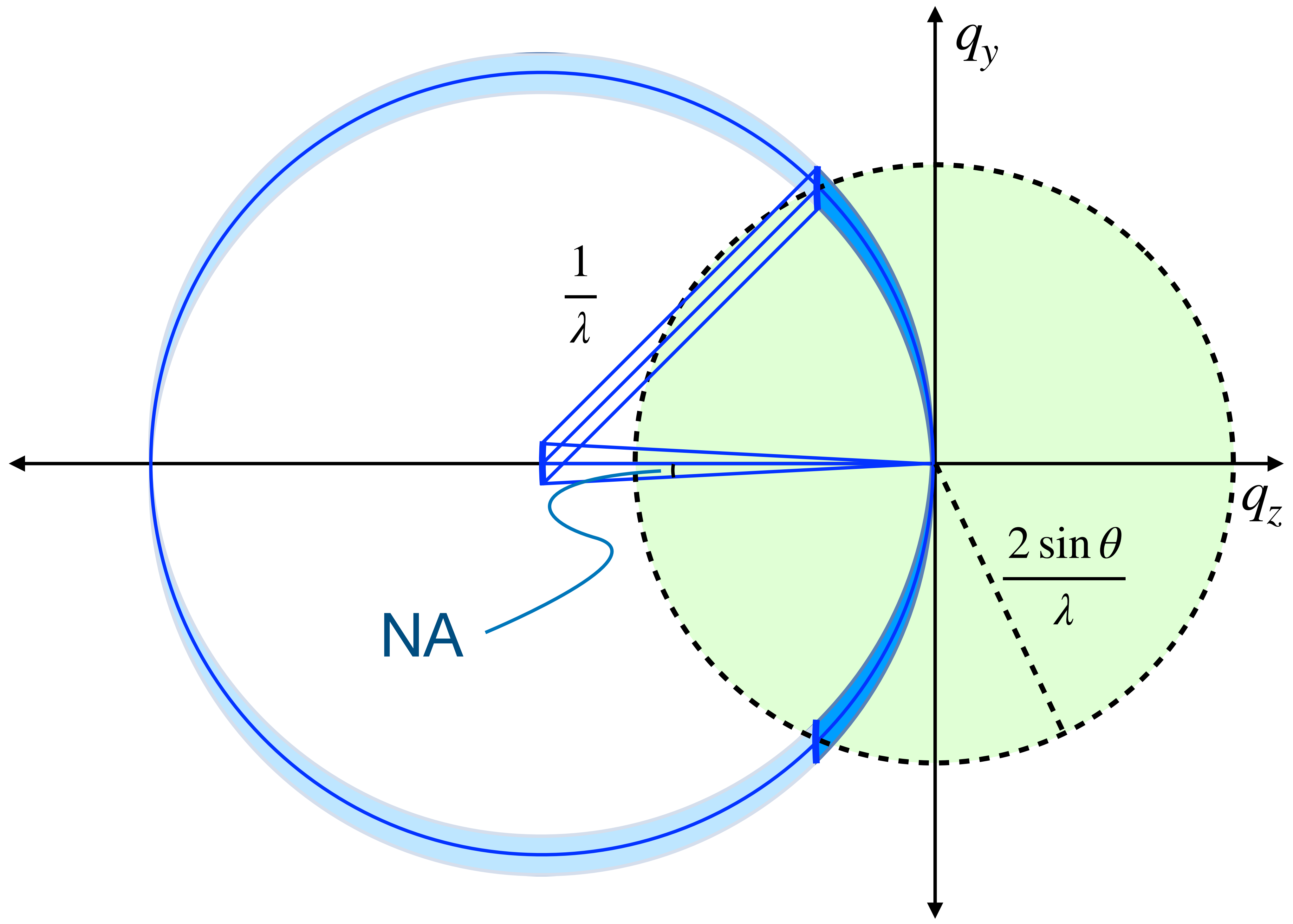


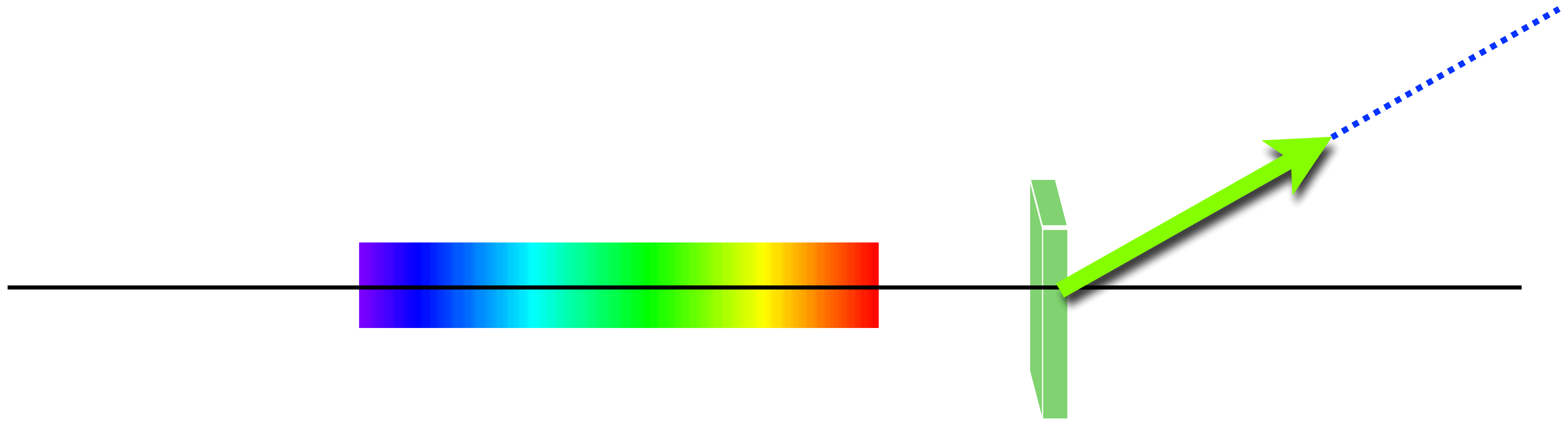
The 3D crystal selects the particular angle that obeys the Bragg condition

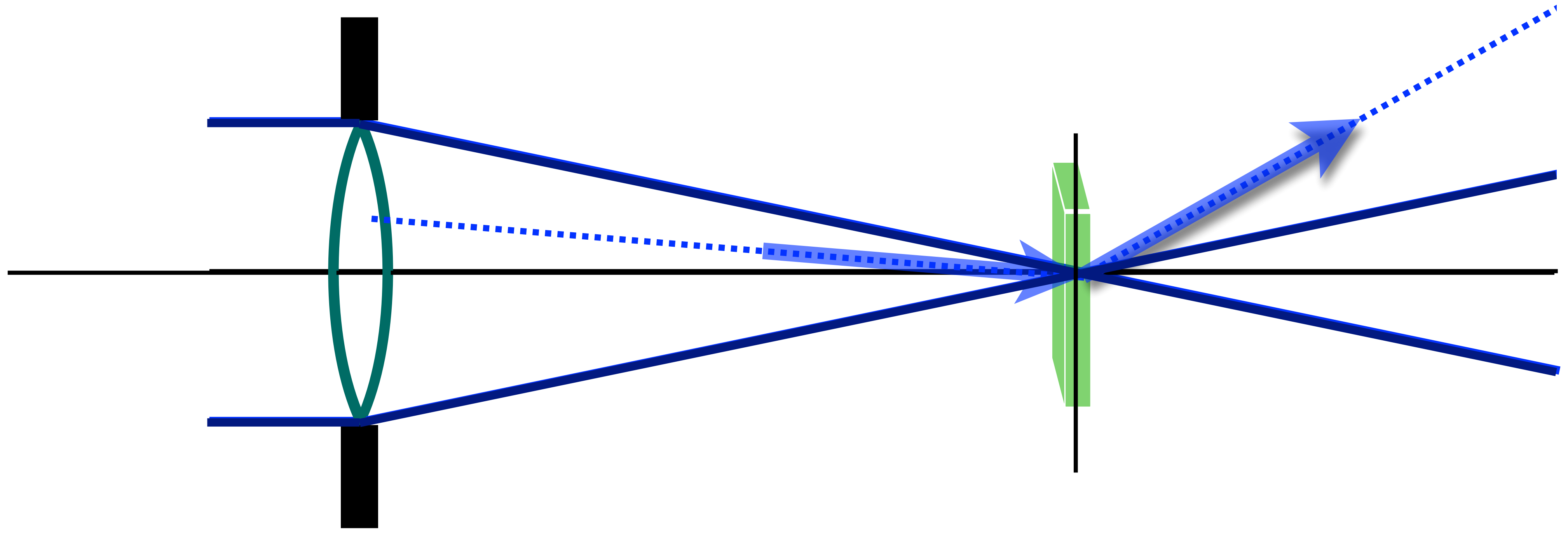
$$\lambda = 2d\sin\theta$$

Bragg's law, $\lambda = 2d\sin\theta$, has only three variables

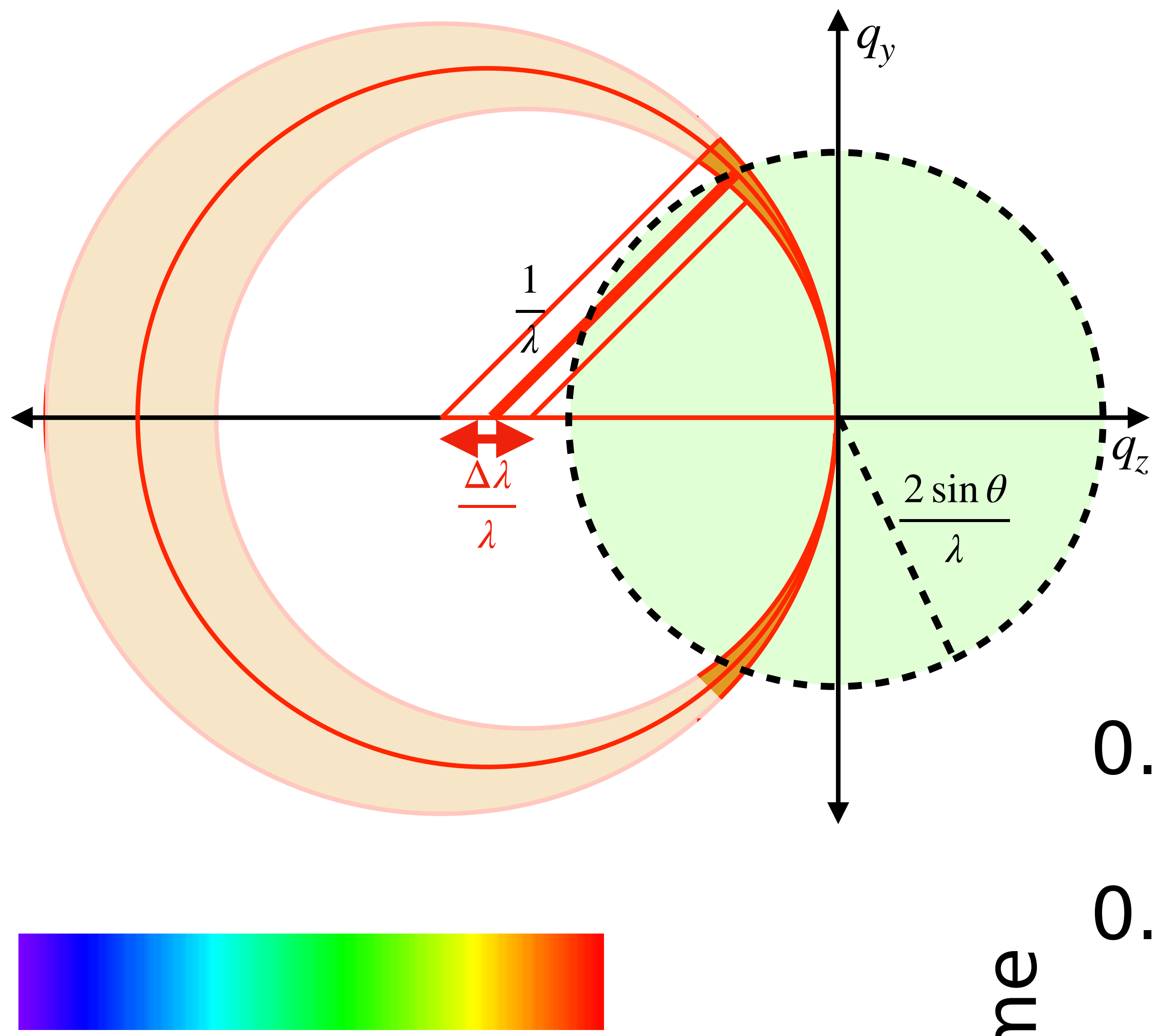


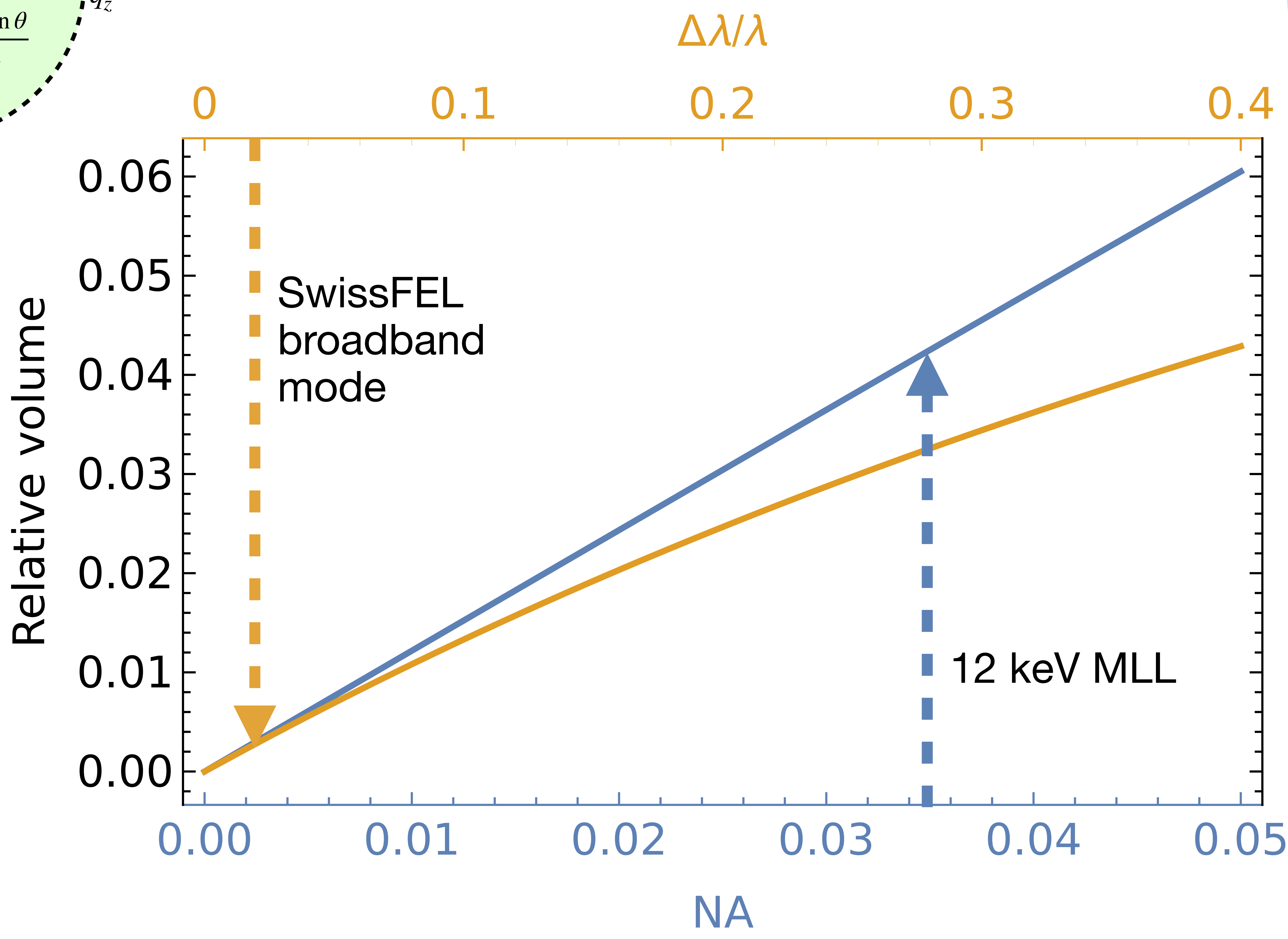




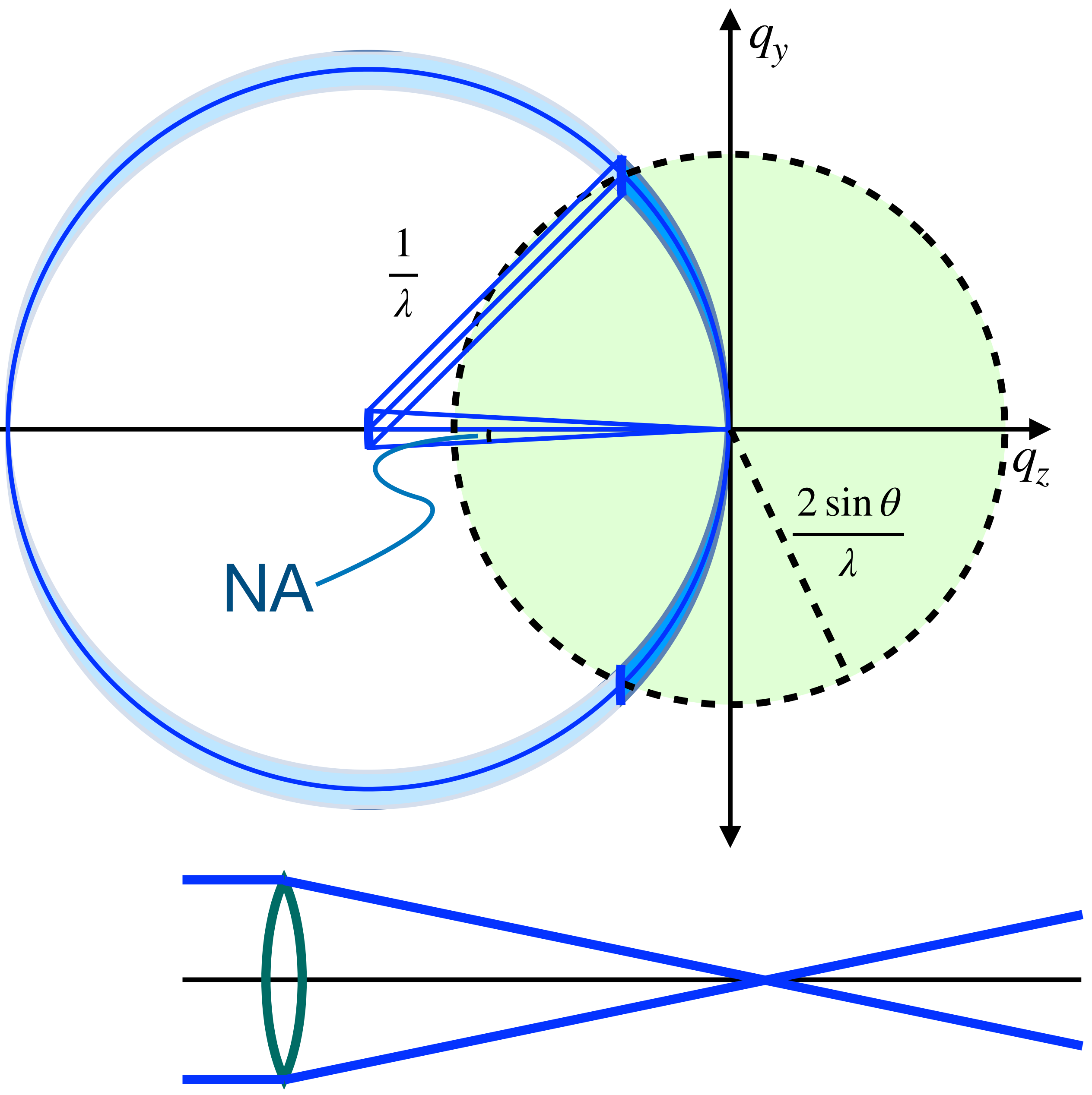


A convergence of 0.03 radian fills as much reciprocal space as 25% bandwidth

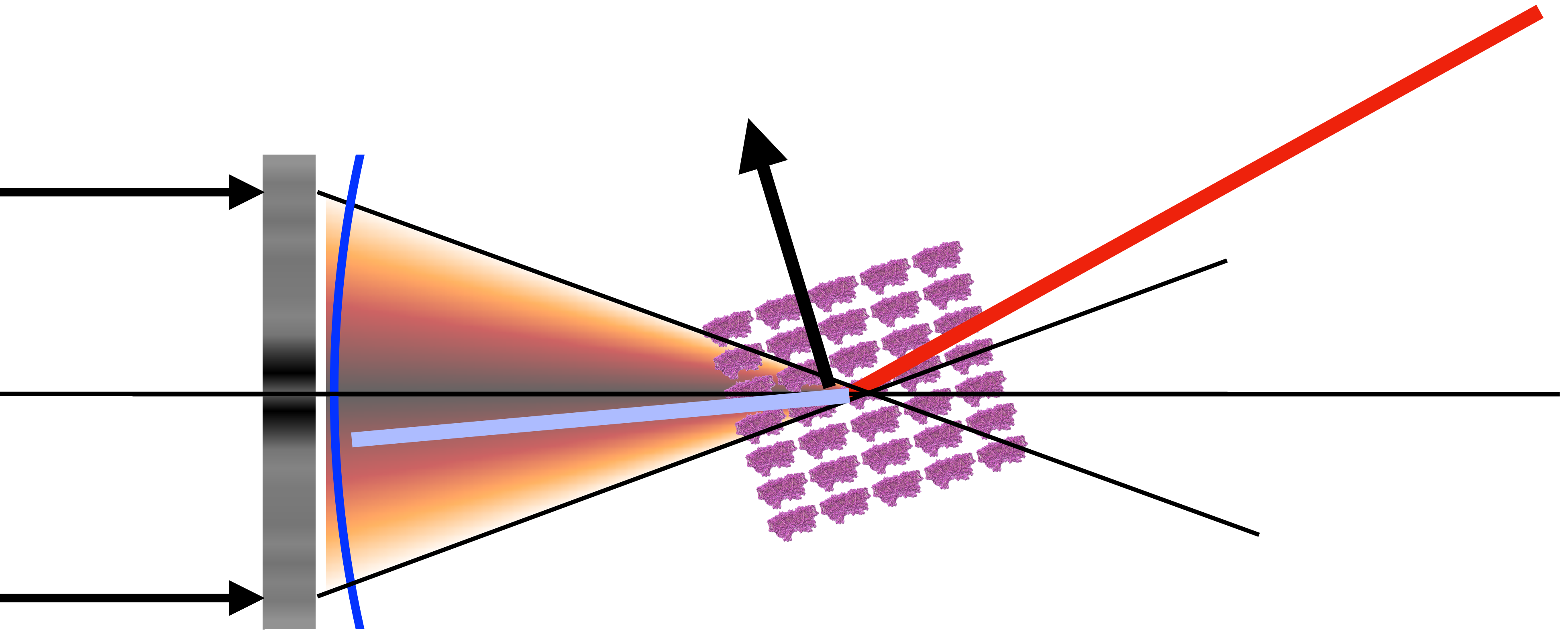


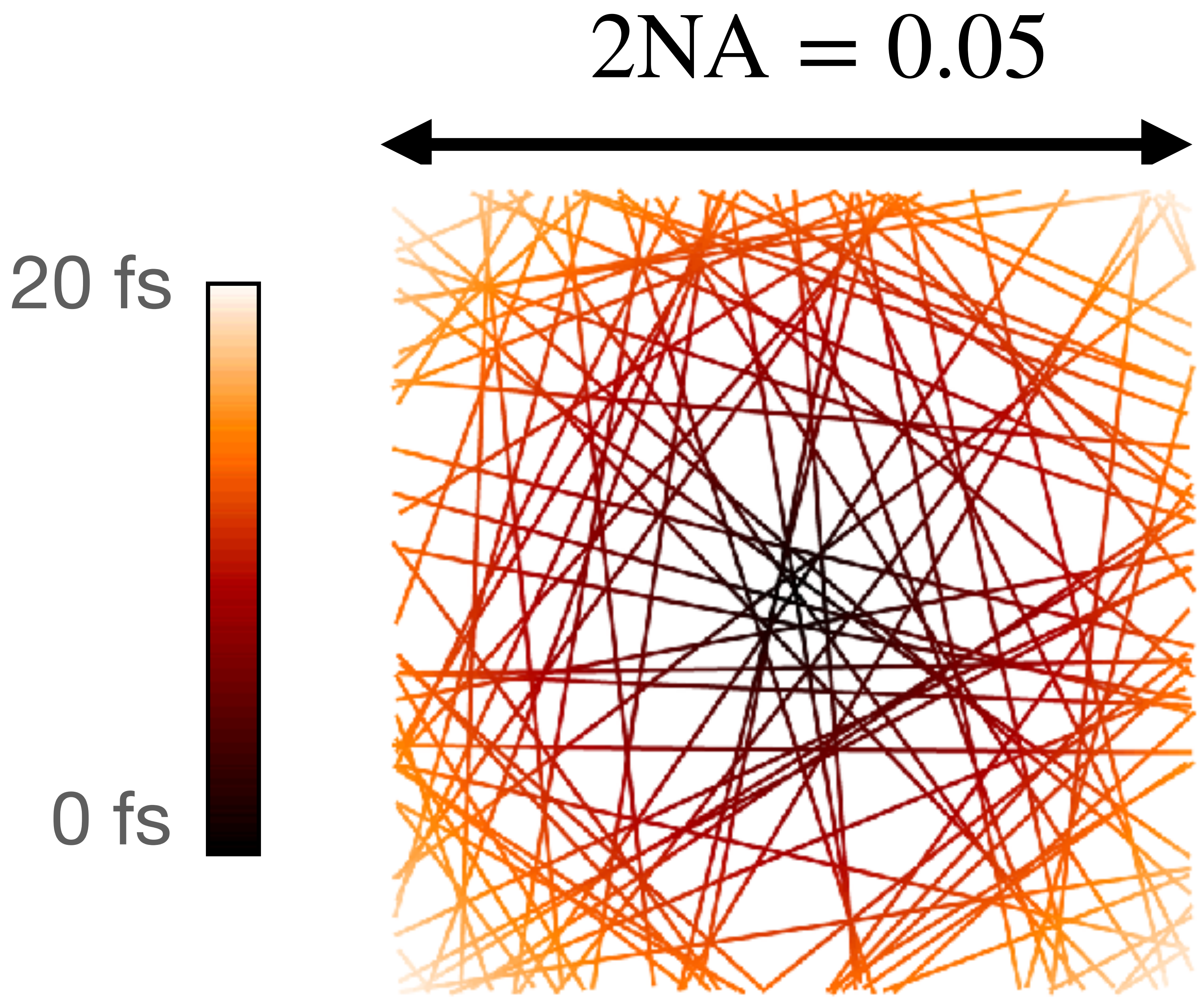


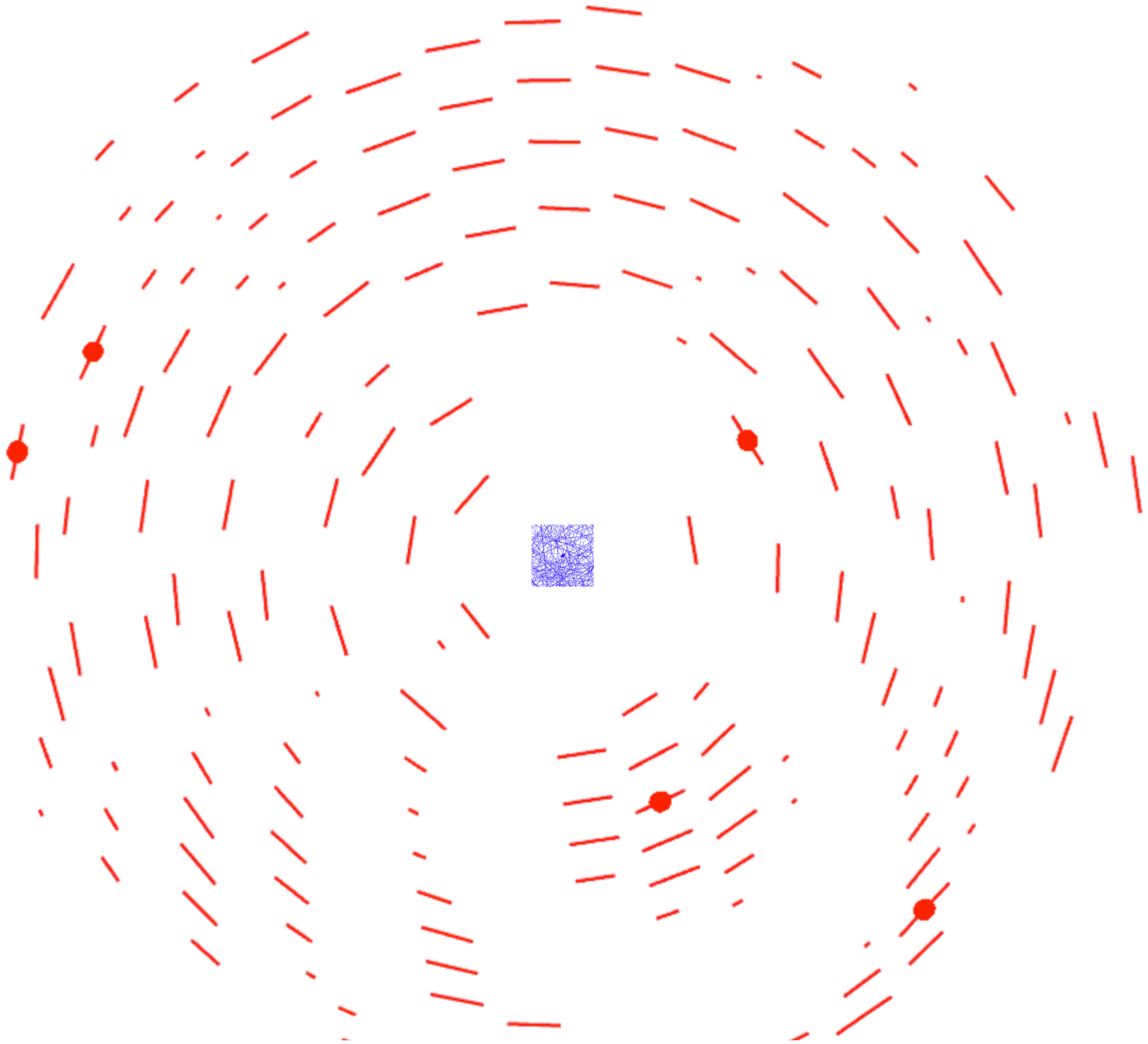
Fraction of reciprocal space covered up to 1Å resolution



Upon indexing, each streak can be mapped back to its incident component

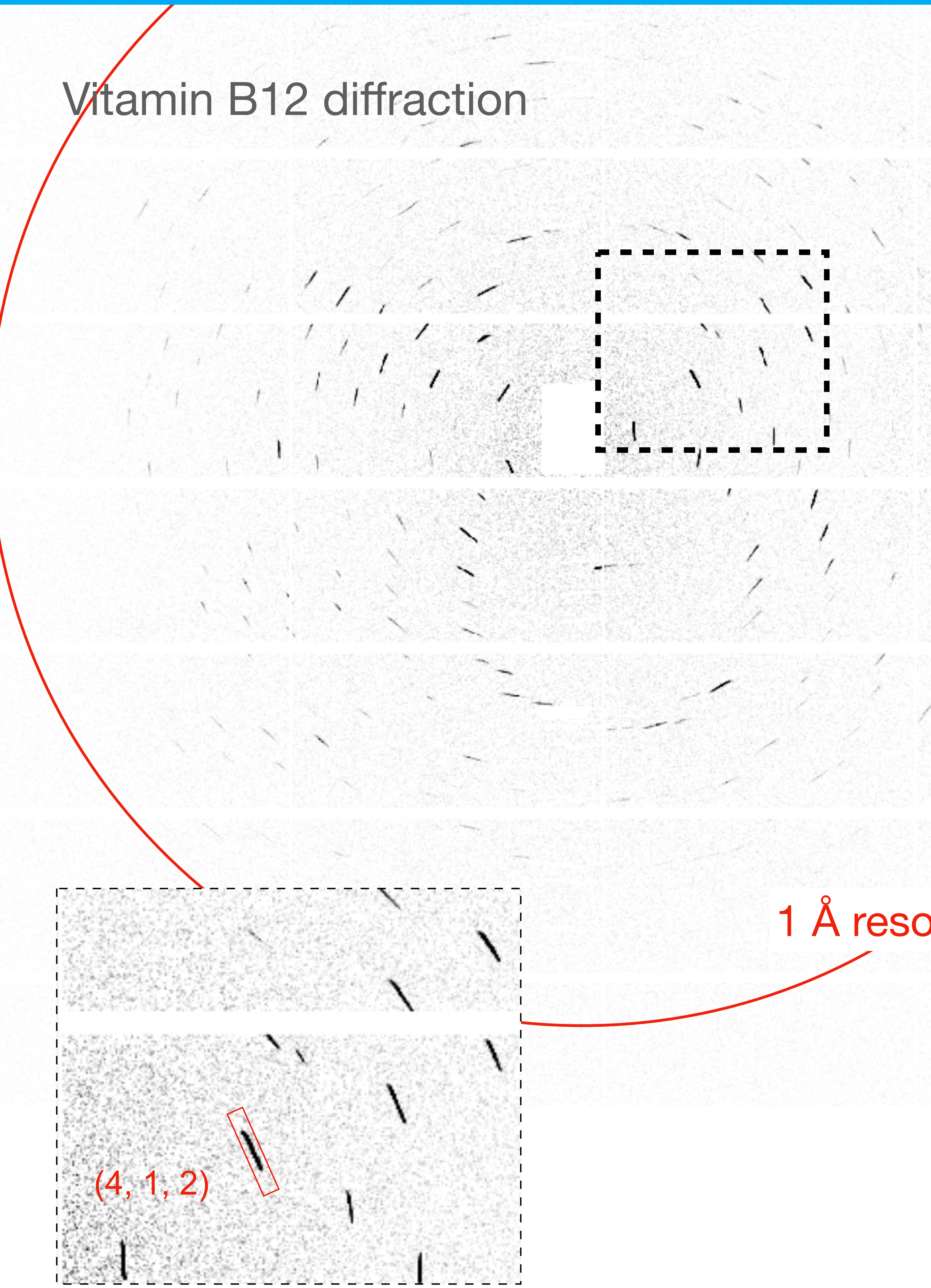


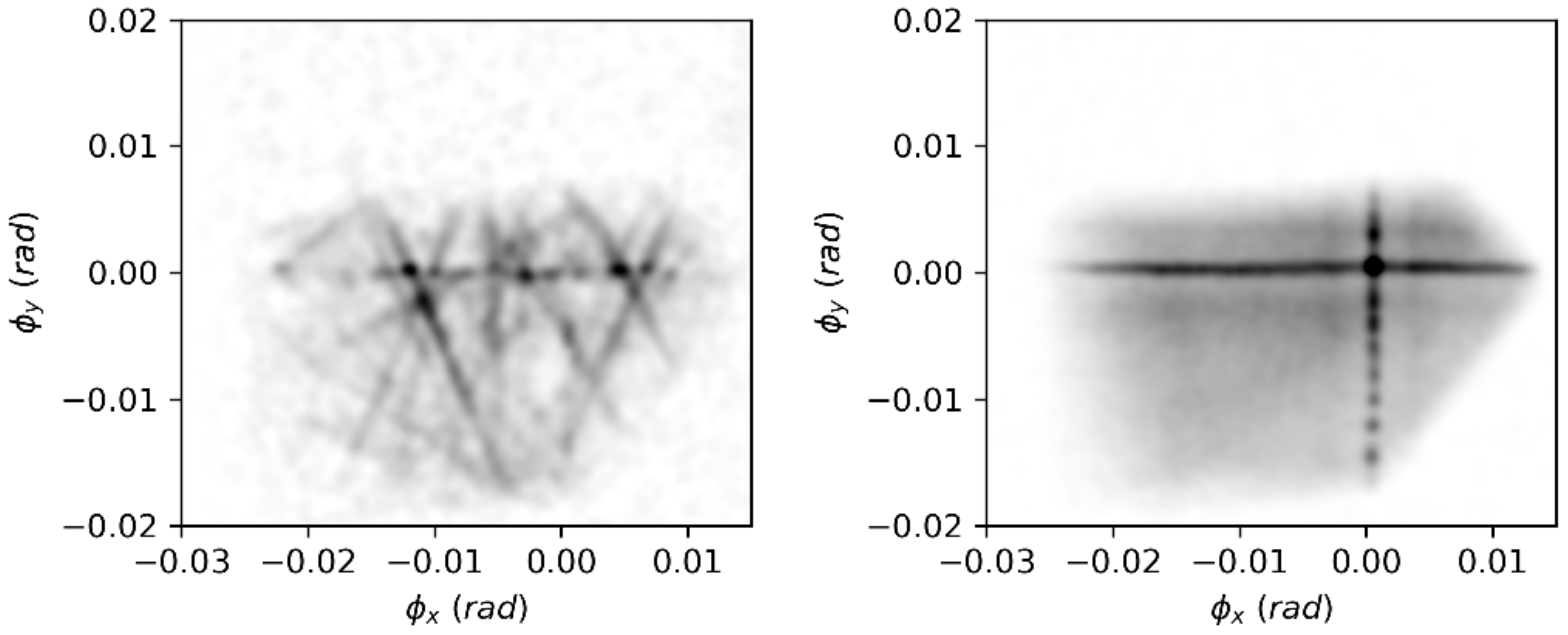


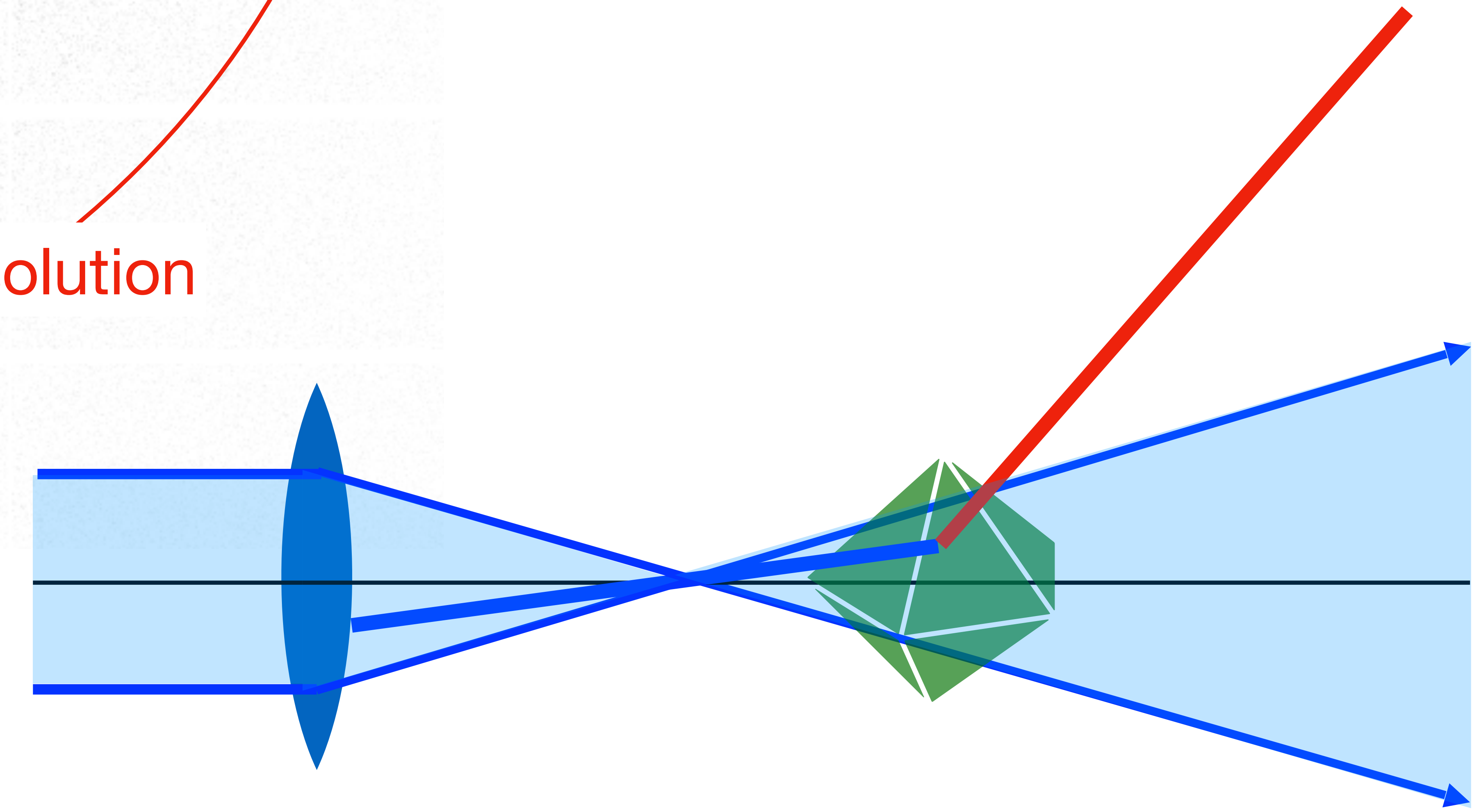


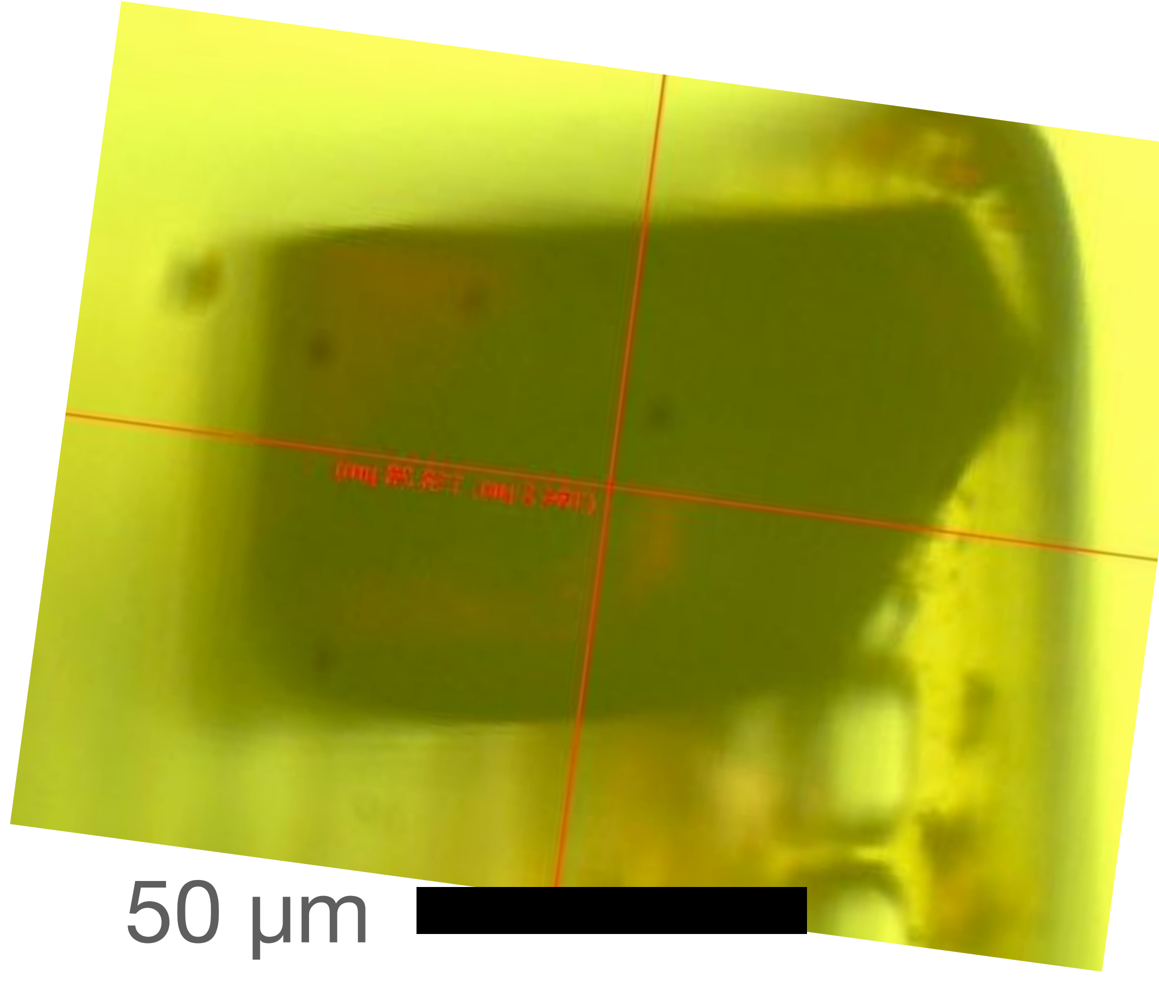
hkl and incident wavevector

Single shot patterns can be indexed and diffraction mapped back to a map of the diffraction efficiency of the crystal



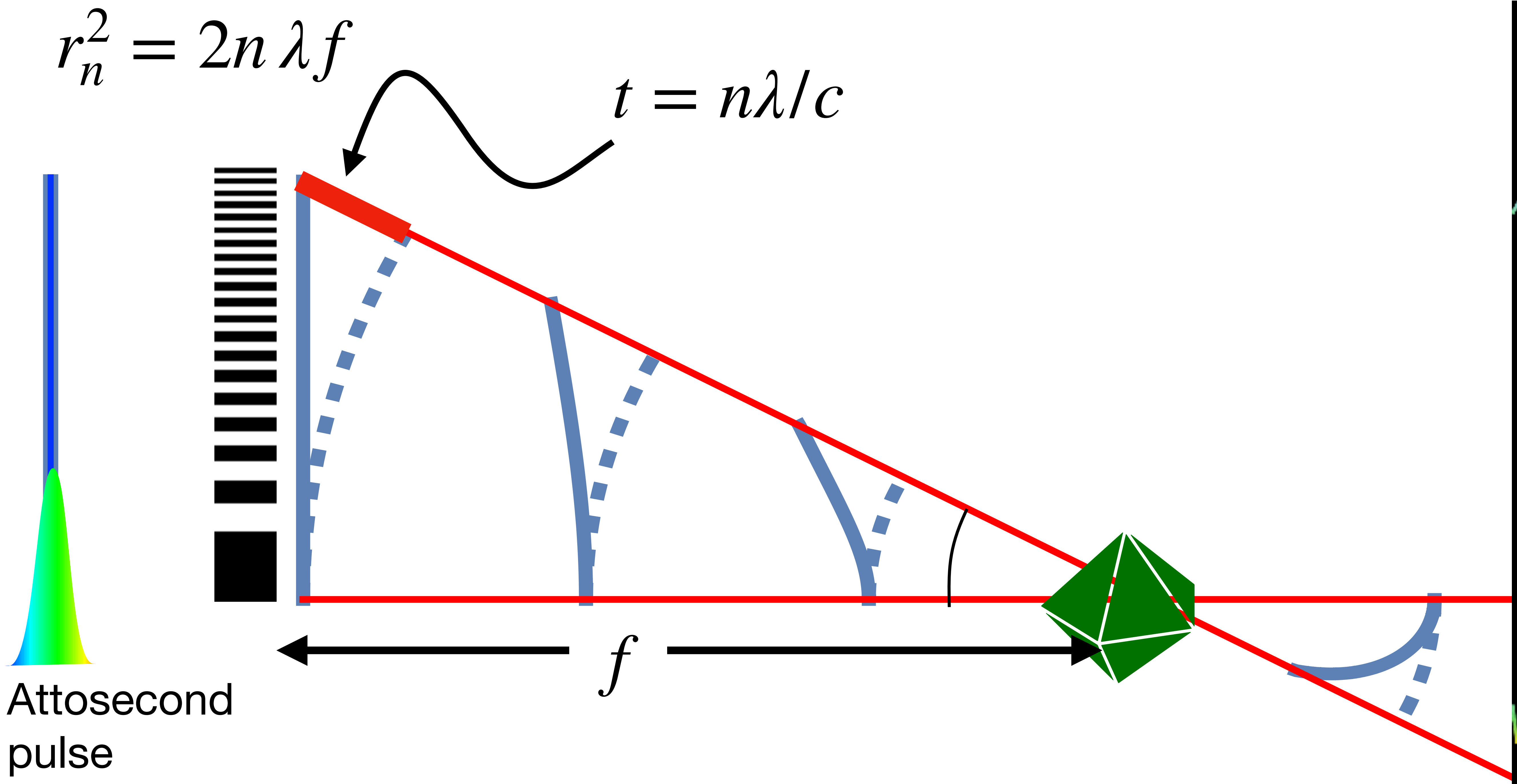






Vitamin B12 crystal

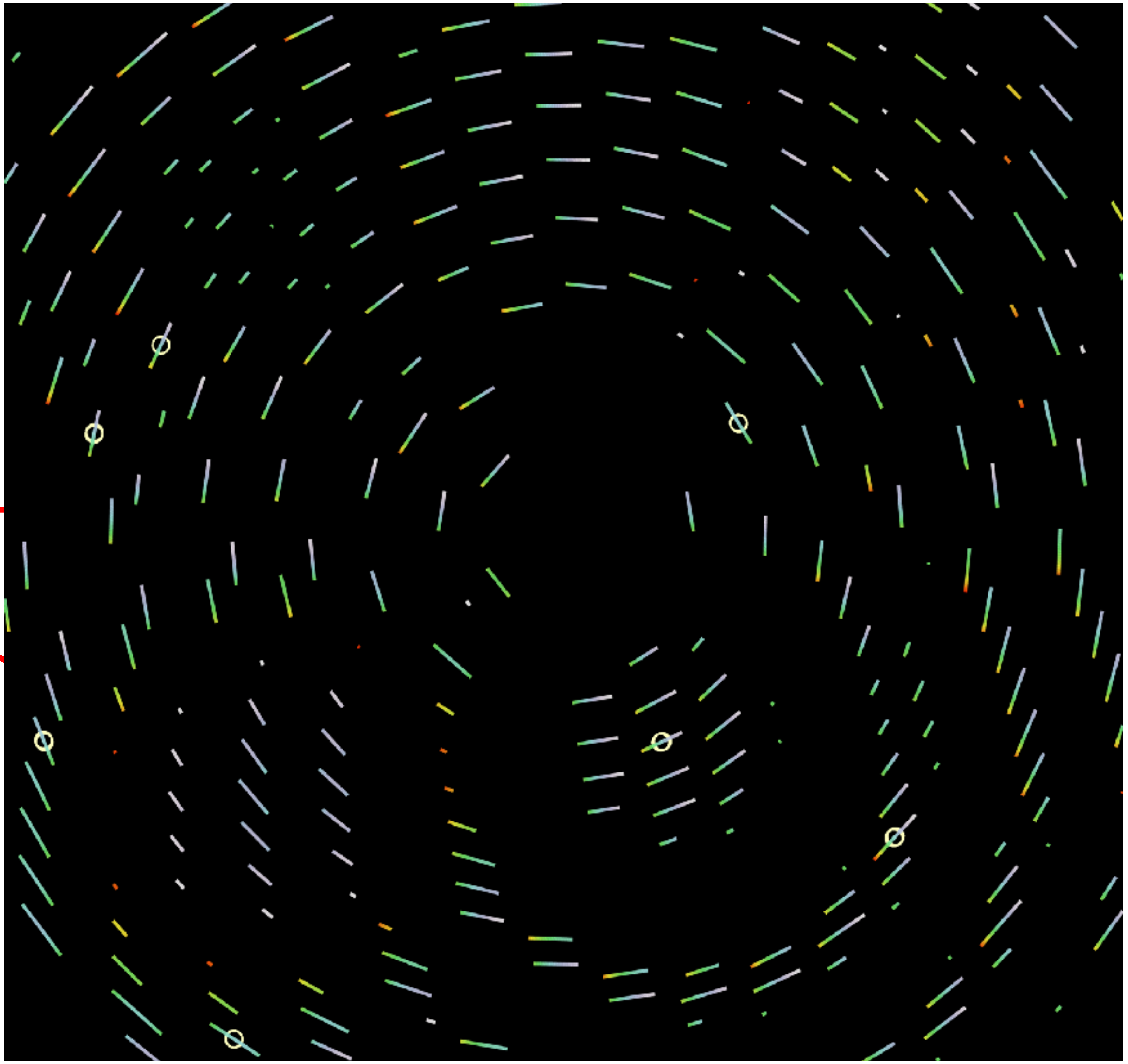
Each period of a zone plate adds one extra wavelength of path



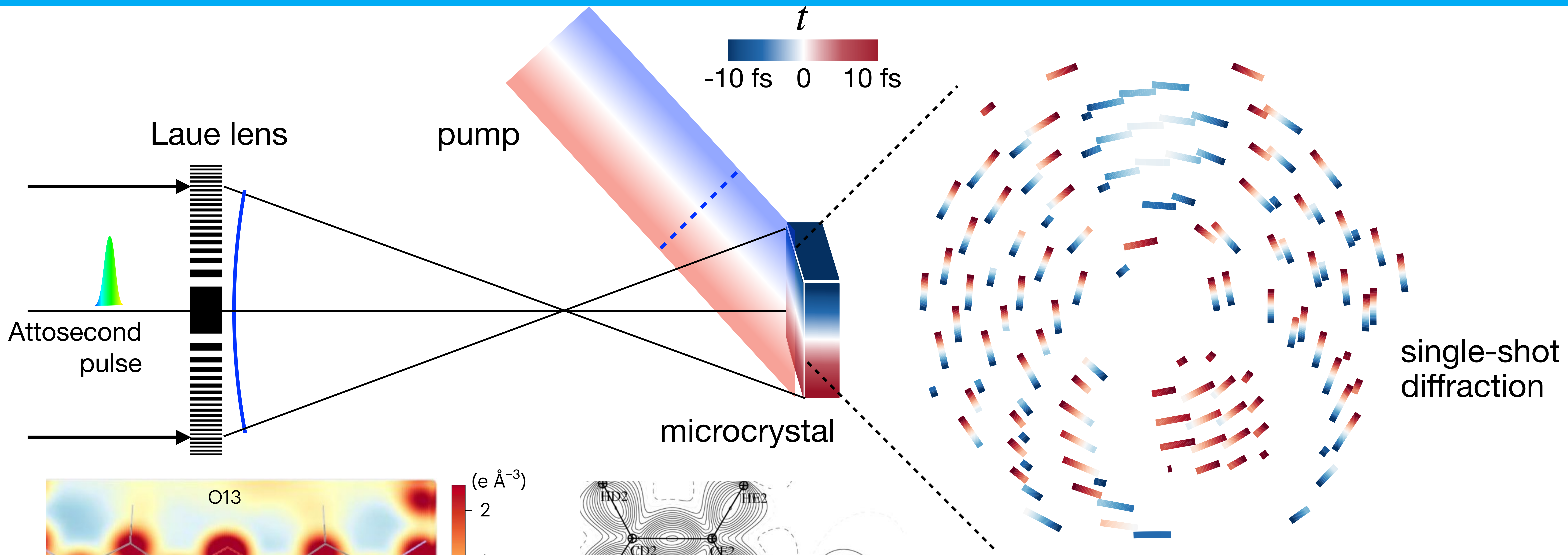
$$f = 2\,\mathrm{mm}$$

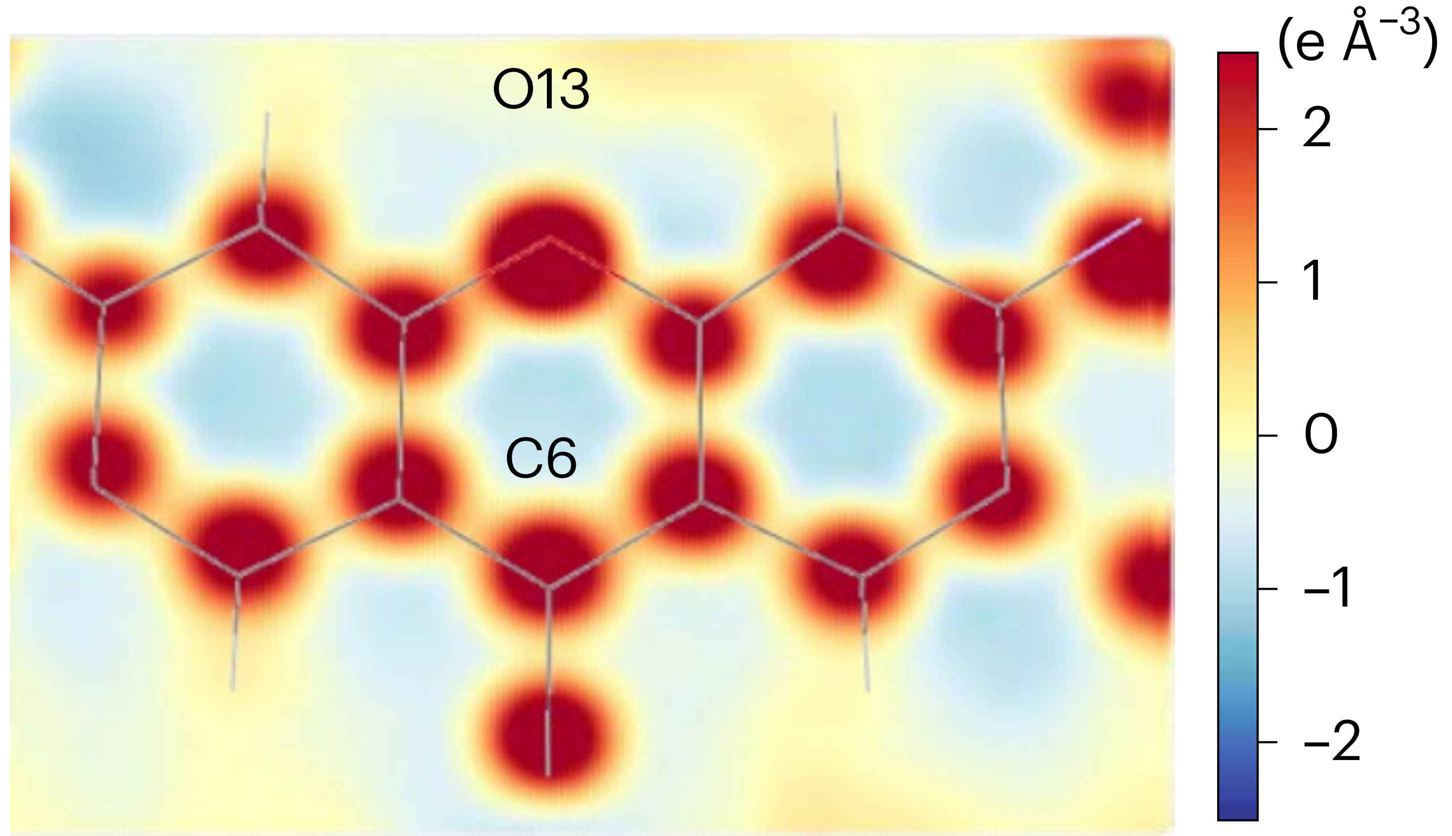
$$\theta_{\max} = 2\mathrm{NA} = 0.04$$

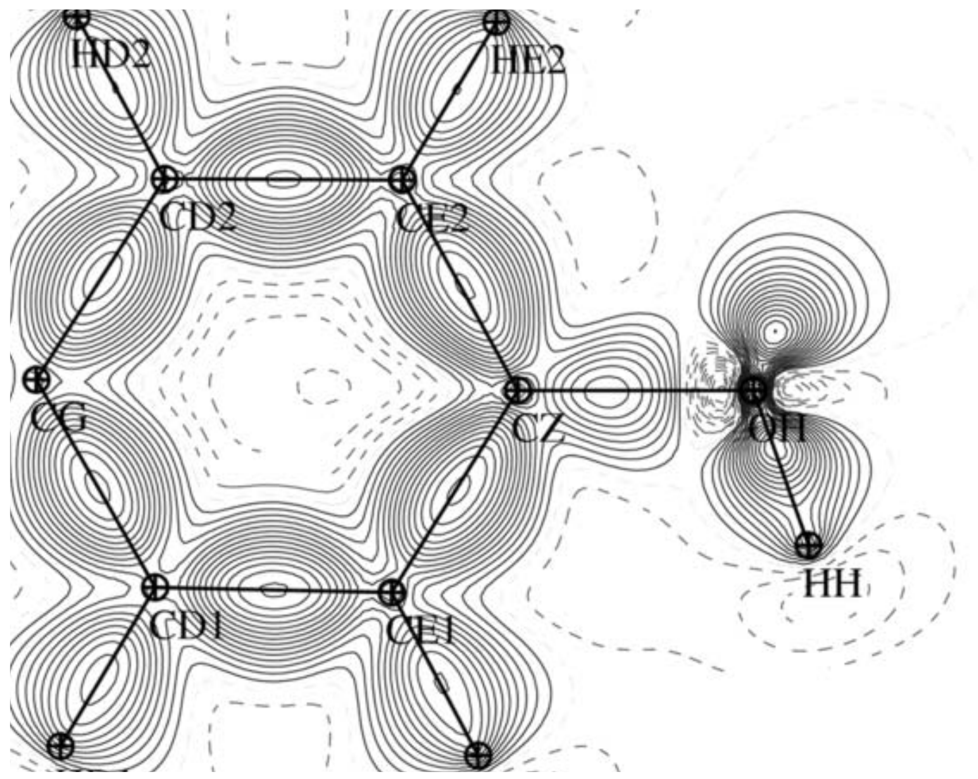
$$t_{\max} = 5.3\,\mathrm{fs}$$



Convergent-beam diffraction will be used for attosecond time-resolved crystallography







Collaboration partners:
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Francesca Calegari
Nina Rohringer

Chapman *et al*, Structural Dynamics **12** 014301 (2025)

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