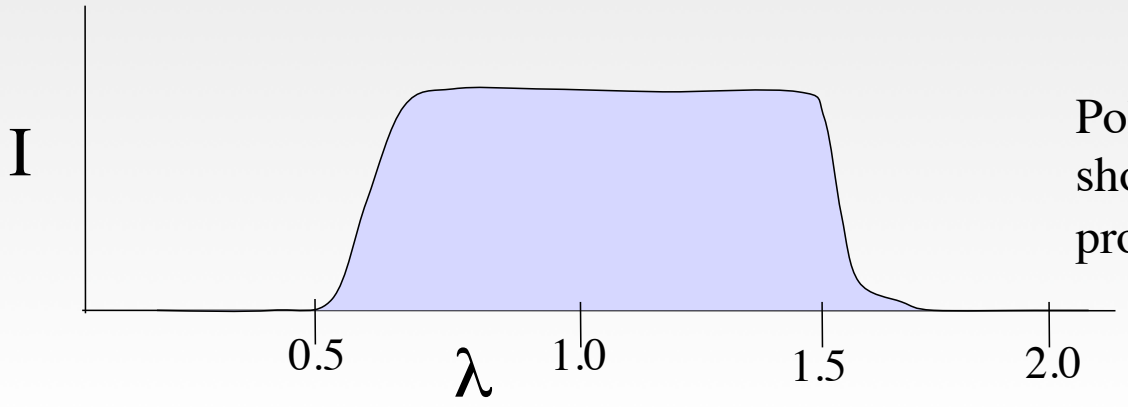
# PSD '18
# Xray lecture 12
*cutting-edge crystallography*

Laue photography,
X-ray laser,
Microcrystal Electron Diffraction

# Laue Method

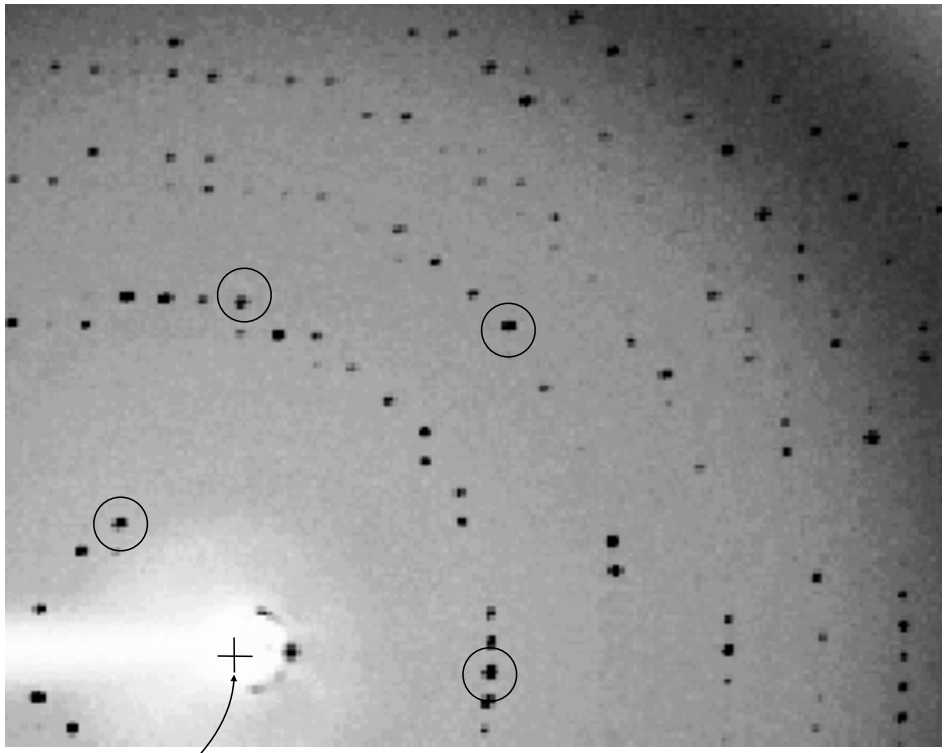
- Uses polychromatic ("white") X-rays. (generally $\lambda$<2.0Å)
- Allows data to be collected ultra-fast, leading to its application in "time-resolved" crystallography.
- Still photographs cover a wide range of reciprocal space. A whole dataset may be collected on a few films.
- Requires synchrotron radiation

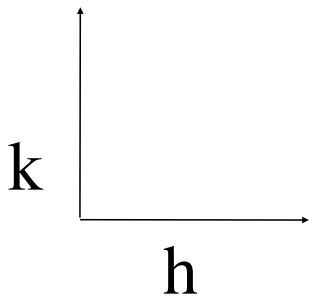



Polychromatic X-rays should have a flat intensity profile.

# Monochromatic diffraction

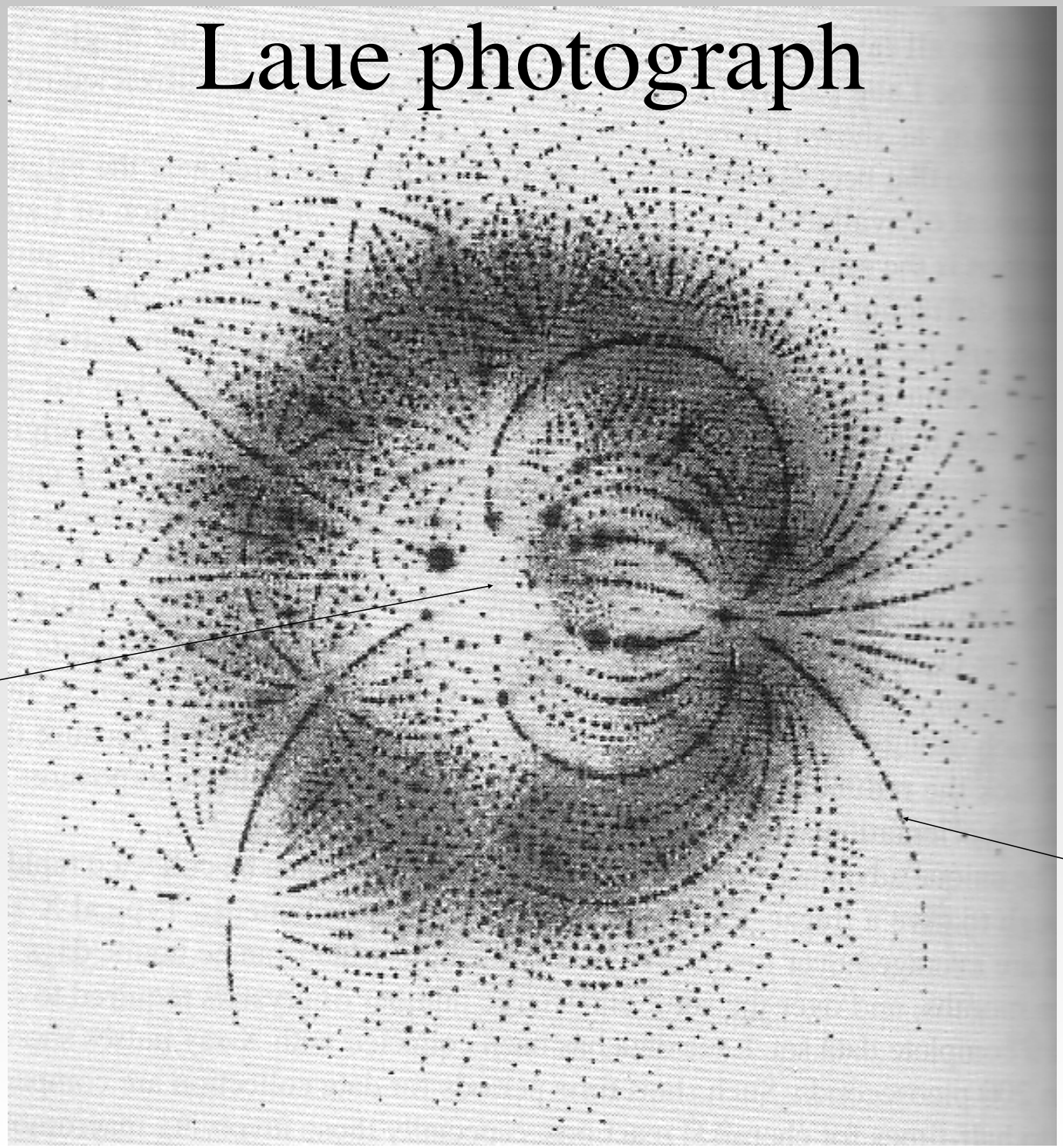
still image






beam

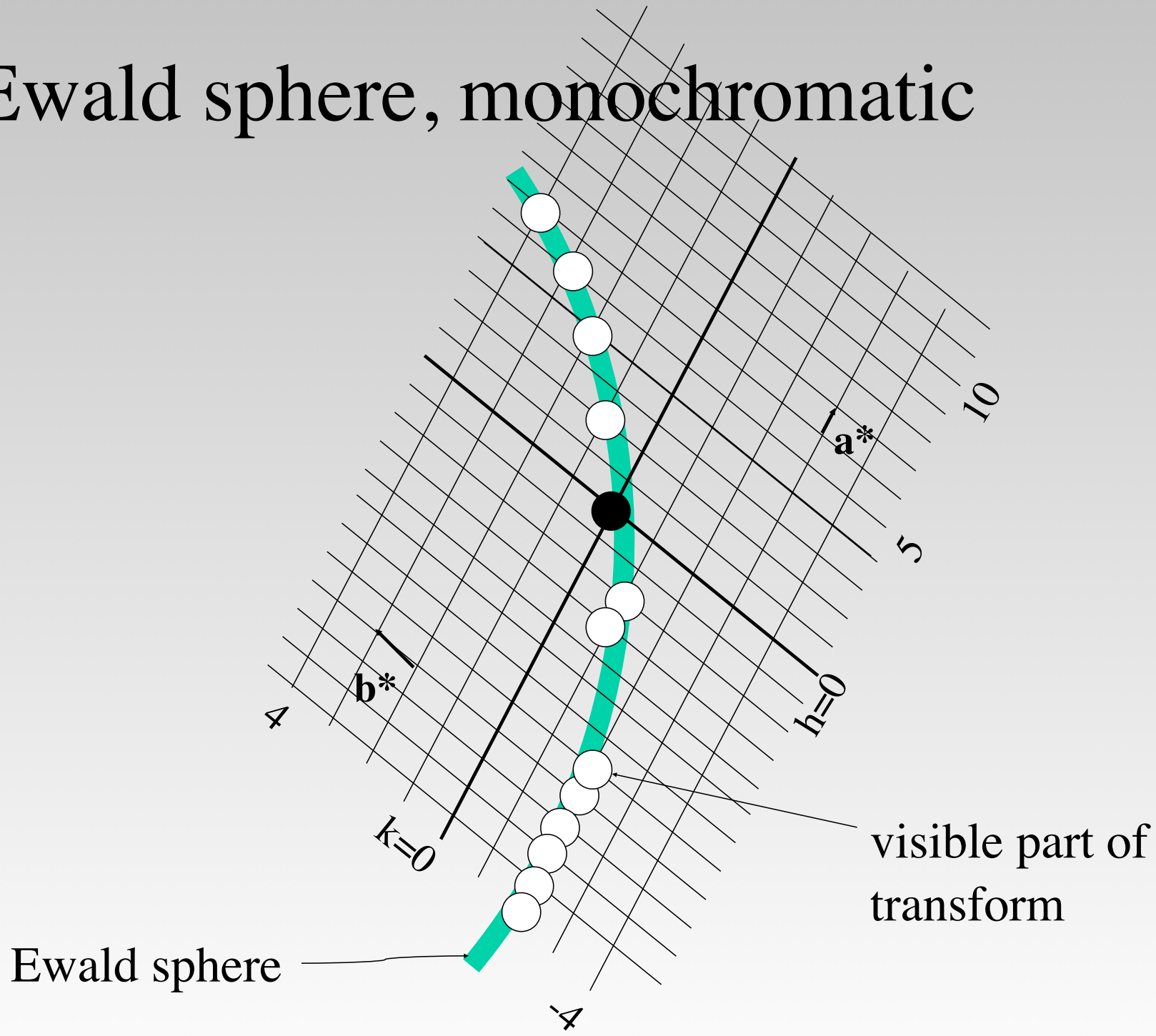
# Laue photograph



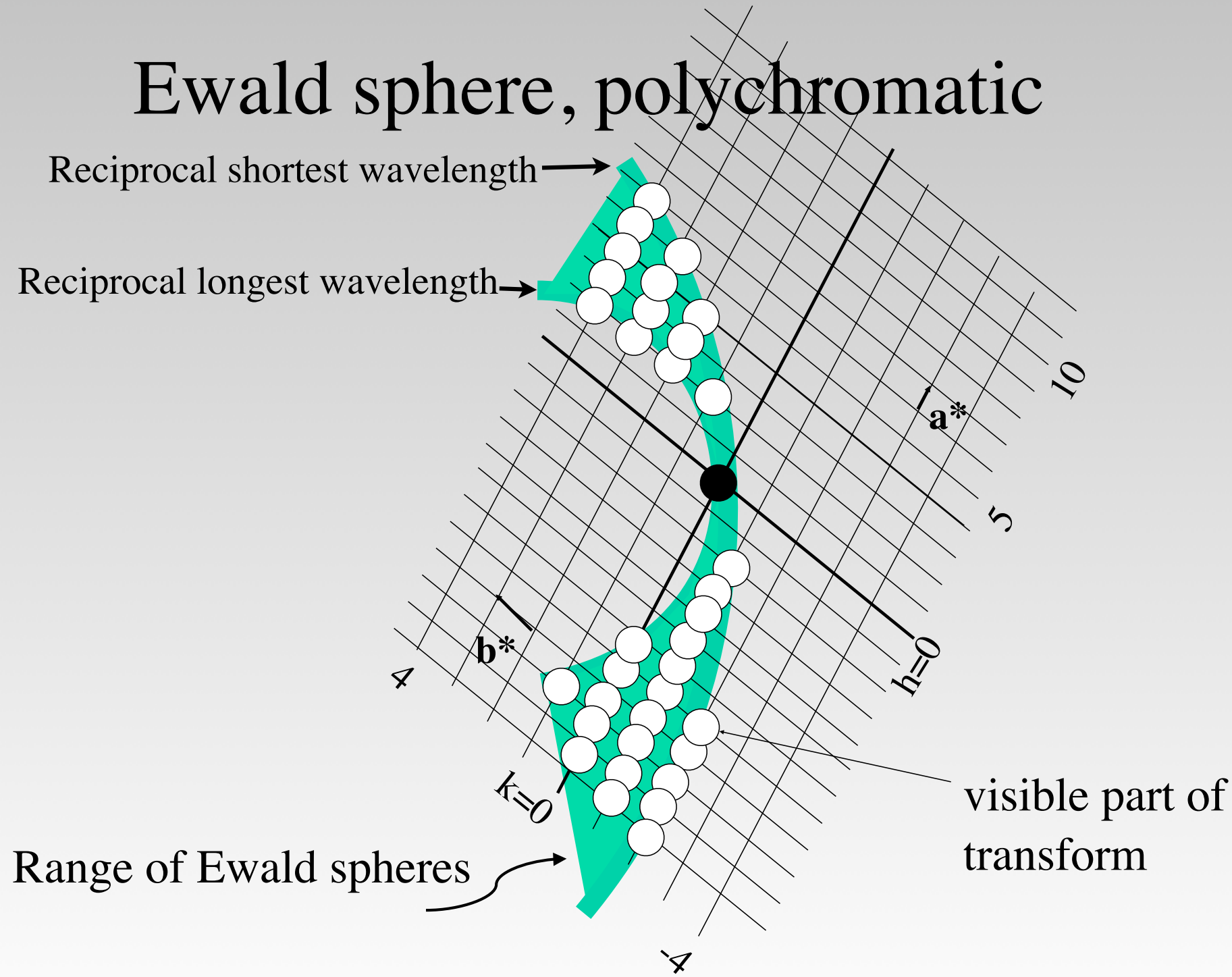
beam

**Note**: Very small separation between spots. This means the crystals must be small, the beam must be small, and the crystals must be well-ordered.

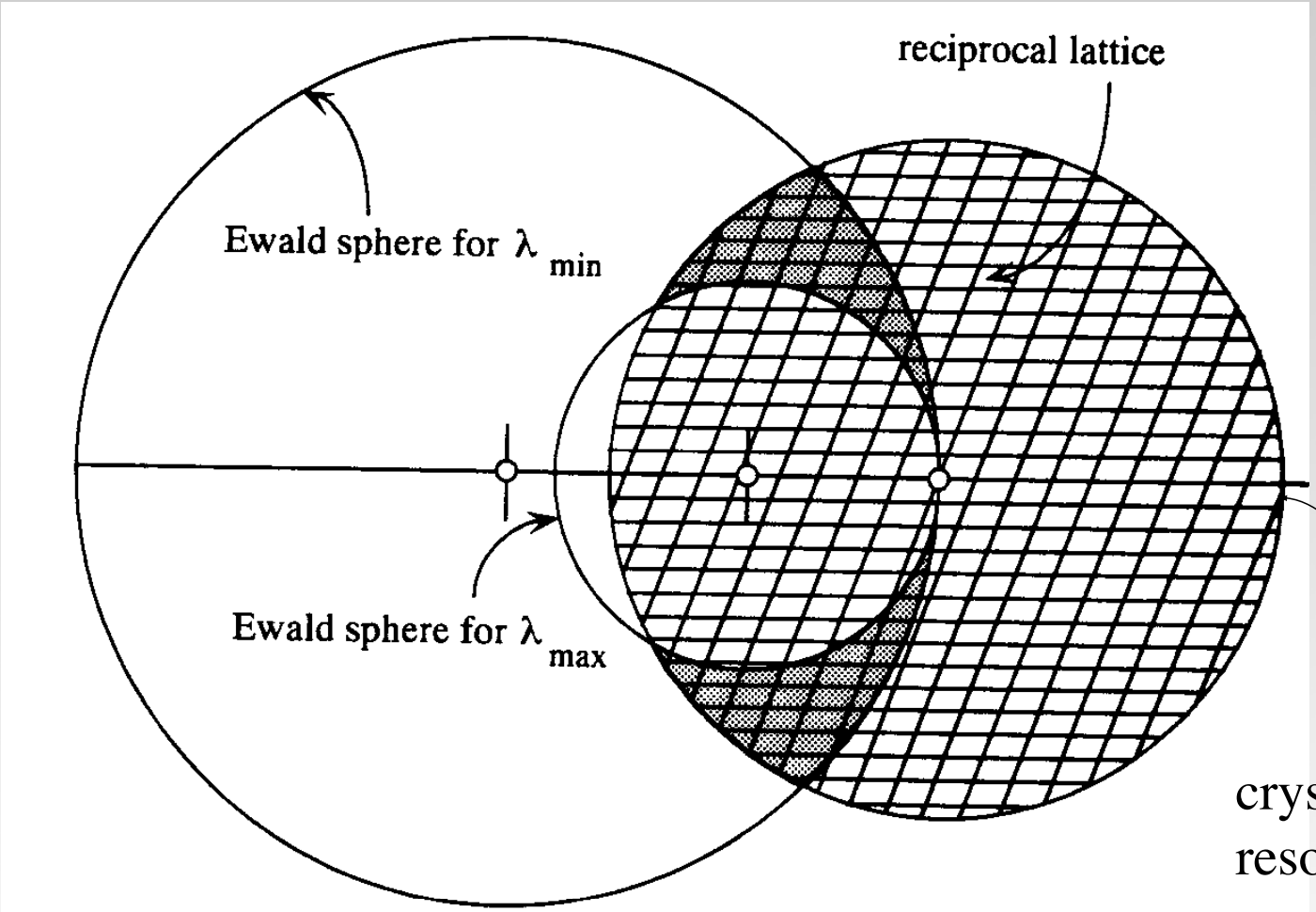
# Ewald sphere, monochromatic



# Ewald sphere, polychromatic

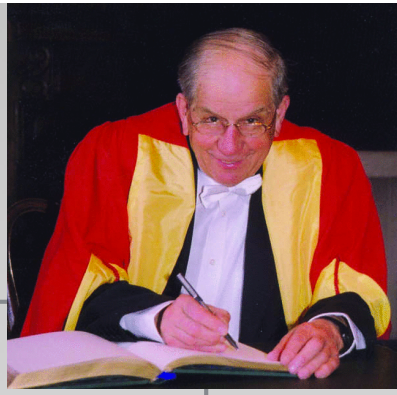


# The Ewald sphere(s), polychromatic




Ewald sphere has radius 1/λ. Longer wavelength X-rays cause the Ewald sphere to shrink, picking up a different part of the reciprocal lattice.

# Cruikshank's dilemma



In monochromatic crystallography:

one θ angle = one *d*.

In Laue crystallography,

one θ angle = a range of *d*'s.

Bragg's Law for monochromatic

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

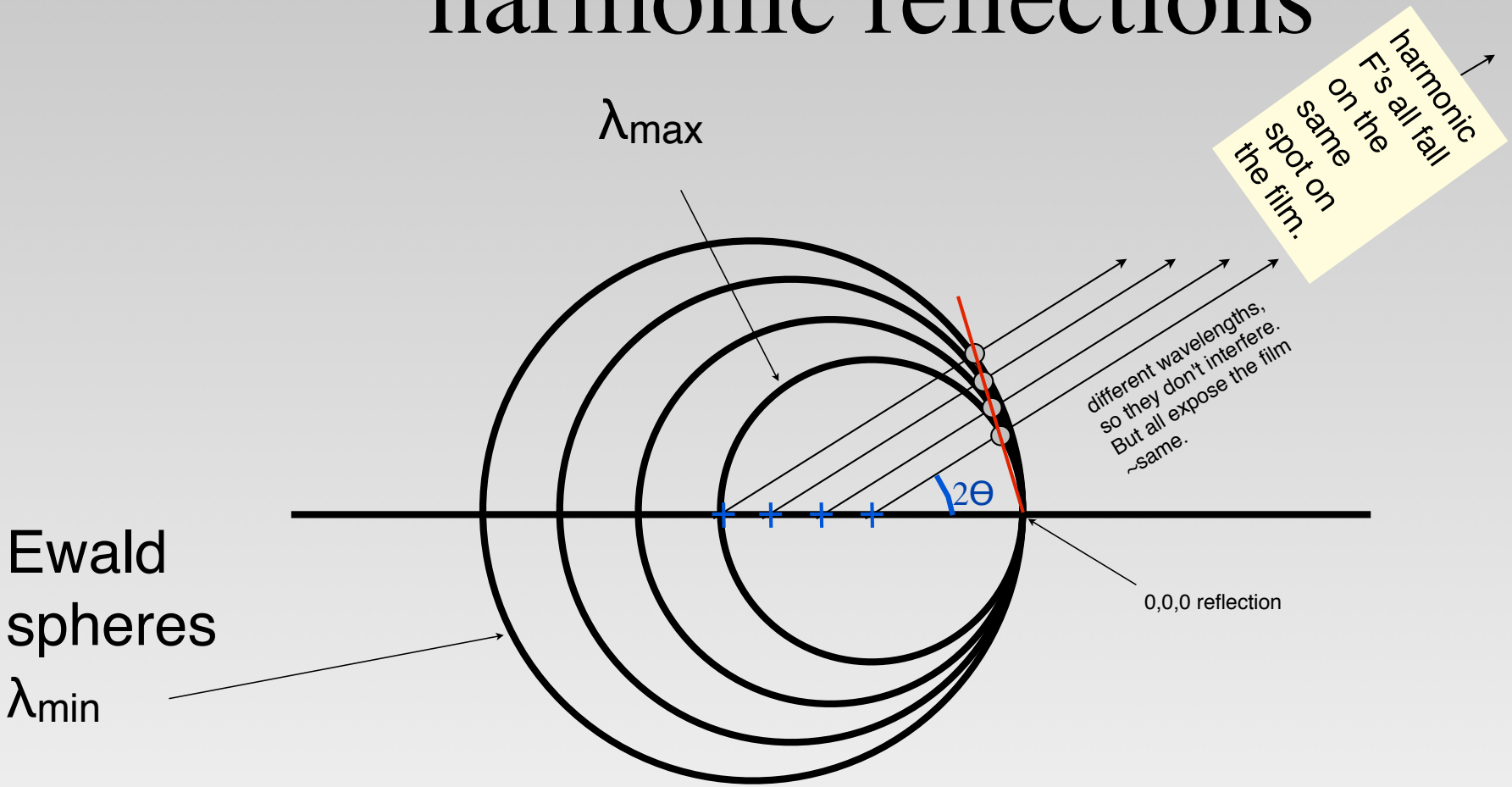
Bragg's Law(s) for Laue

$$n\lambda_{max} = 2d \sin \theta$$

$$= n\lambda_{max} /2 = 2(d/2) \sin \theta$$

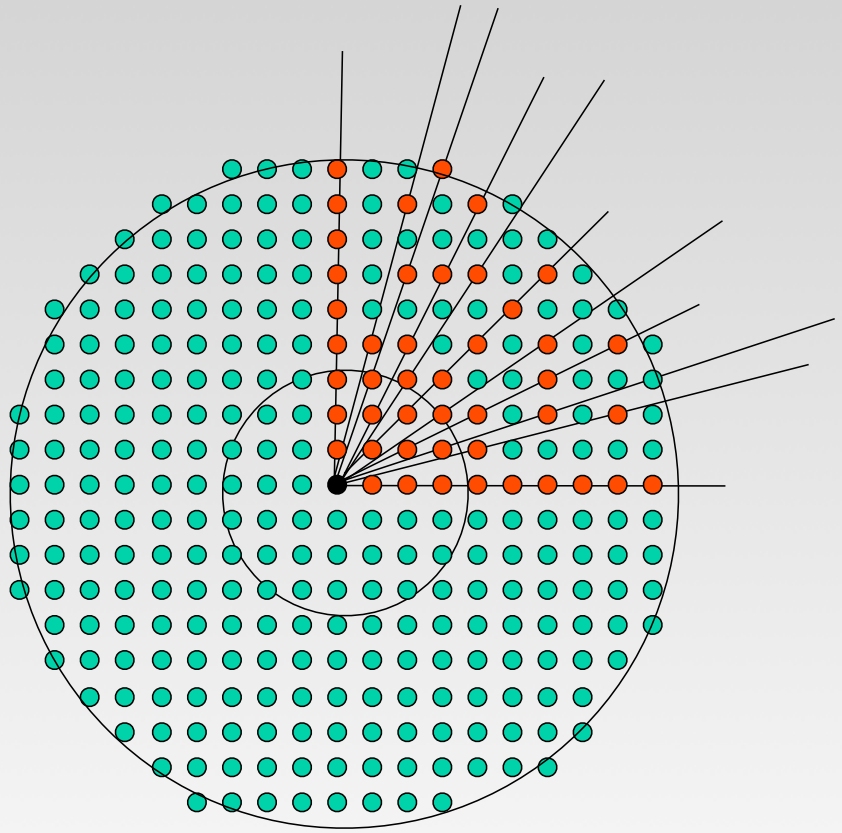
$$= n\lambda_{max} /3 = 2(d/3) \sin \theta$$

# harmonic reflections



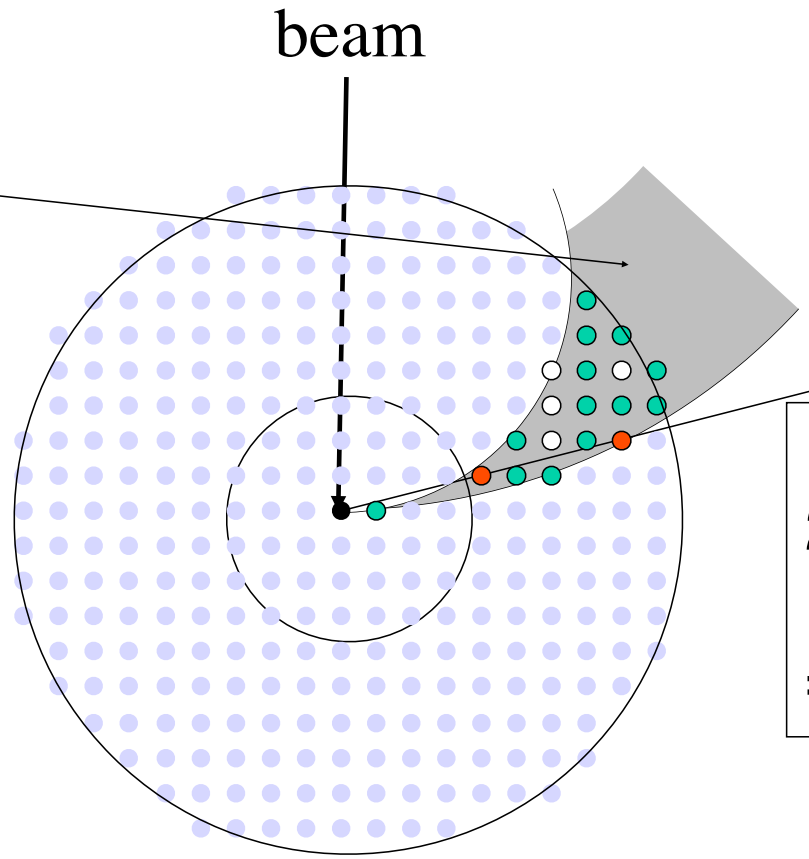

Harmonic reflections (nh,nk,nl) have the same *S* direction, but the length is inversely proportional to λ. So (h,k,l) at λ=2.0Å and (2h,2k,2l) at λ=1.0Å diffract to *exactly the same spot on the film*.

# Harmonics are reflections that lie along rays going out from the reciprocol space origin



73 refls
44 multiples
29 singles

# Not all harmonics fall within the Ewald zone (Cruikshank range)




range of Ewald spheres (fixed beam)

73 refls
44 multiples
29 singles

19 refl (18 spots)
2 multiples (1 spot)
17 singles
=89%

Now cut down all the trees except the ones that are on the Ewald sphere for one of the wavelengths (range $\lambda_{min}$-$\lambda_{max}$). Only the trees in the grey region remain. How many are multiples?

# Combining harmonics

$$I(nh, nk, nl) = \sum_{n} \frac{|F(nh, nk, nl)|^2}{f(\lambda(F))}$$

known total intensity for all harmonics

n=1,2,3 etc. within "Cruickshank range"

Scale factor for each. Accounts for beam intensity, polarization.

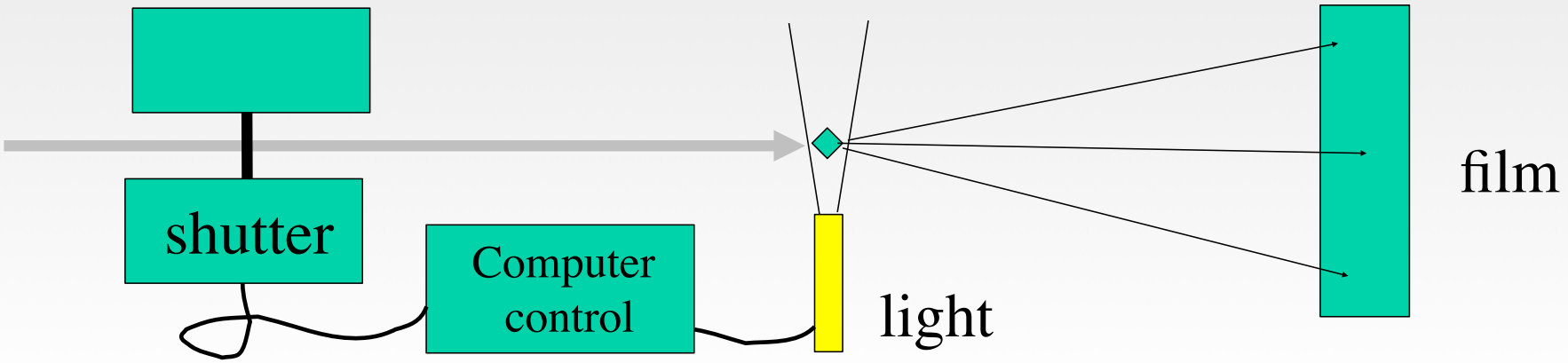
Unknown amplitudes

# Time-resolved crystallography!

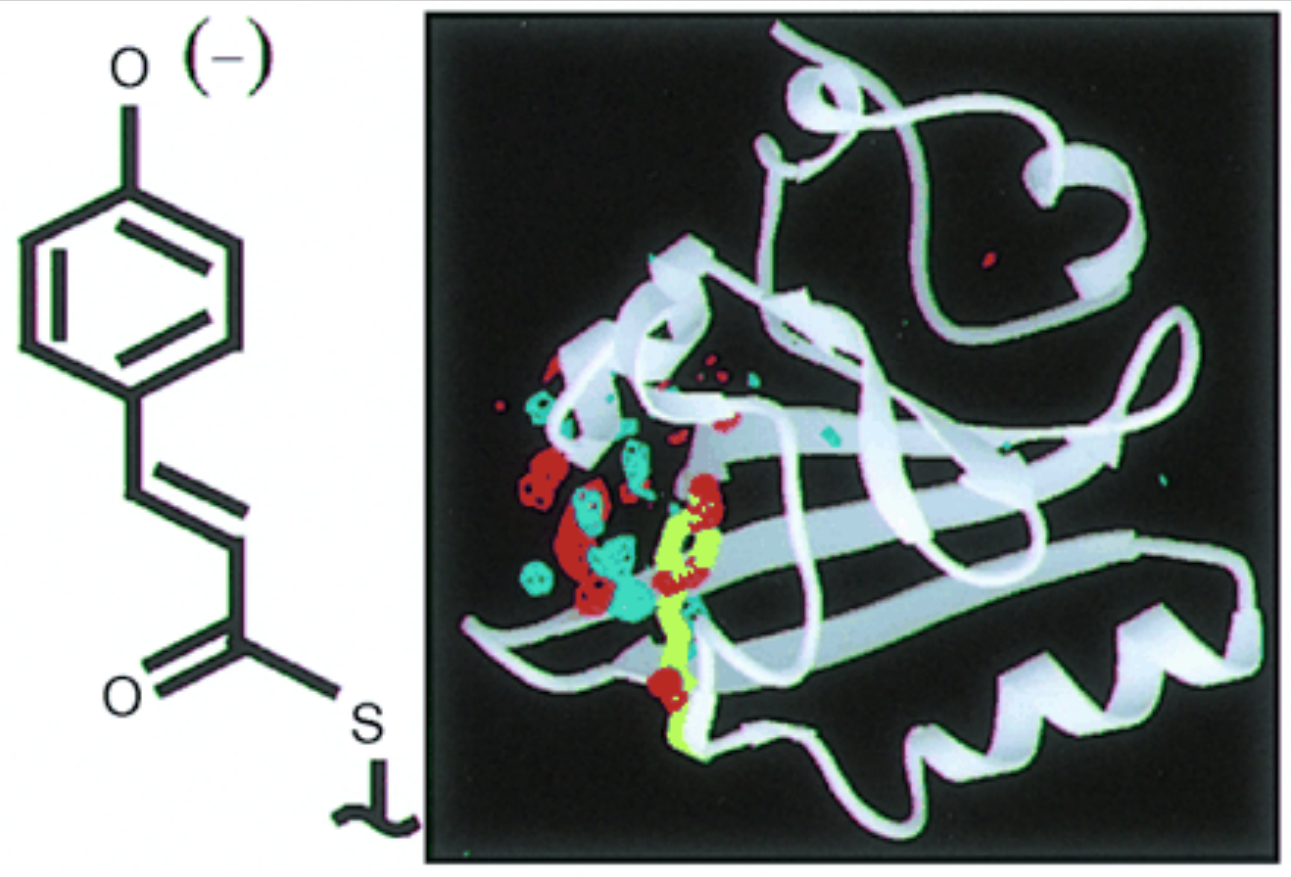
If a reaction can be initiated in a crystal, simultaneously throughout the crystal, then Laue photography can capture the structural changes at the *ns* ($10^{-9}$ *s*) to *ms* ($10^{-3}$ *s*) timescale.

Crystals must withstand ultrahigh fluxes of X-rays, or are destroyed in the process.

Light-initiated reactions can be studied using the Laue method.



# Photoactive yellow protein



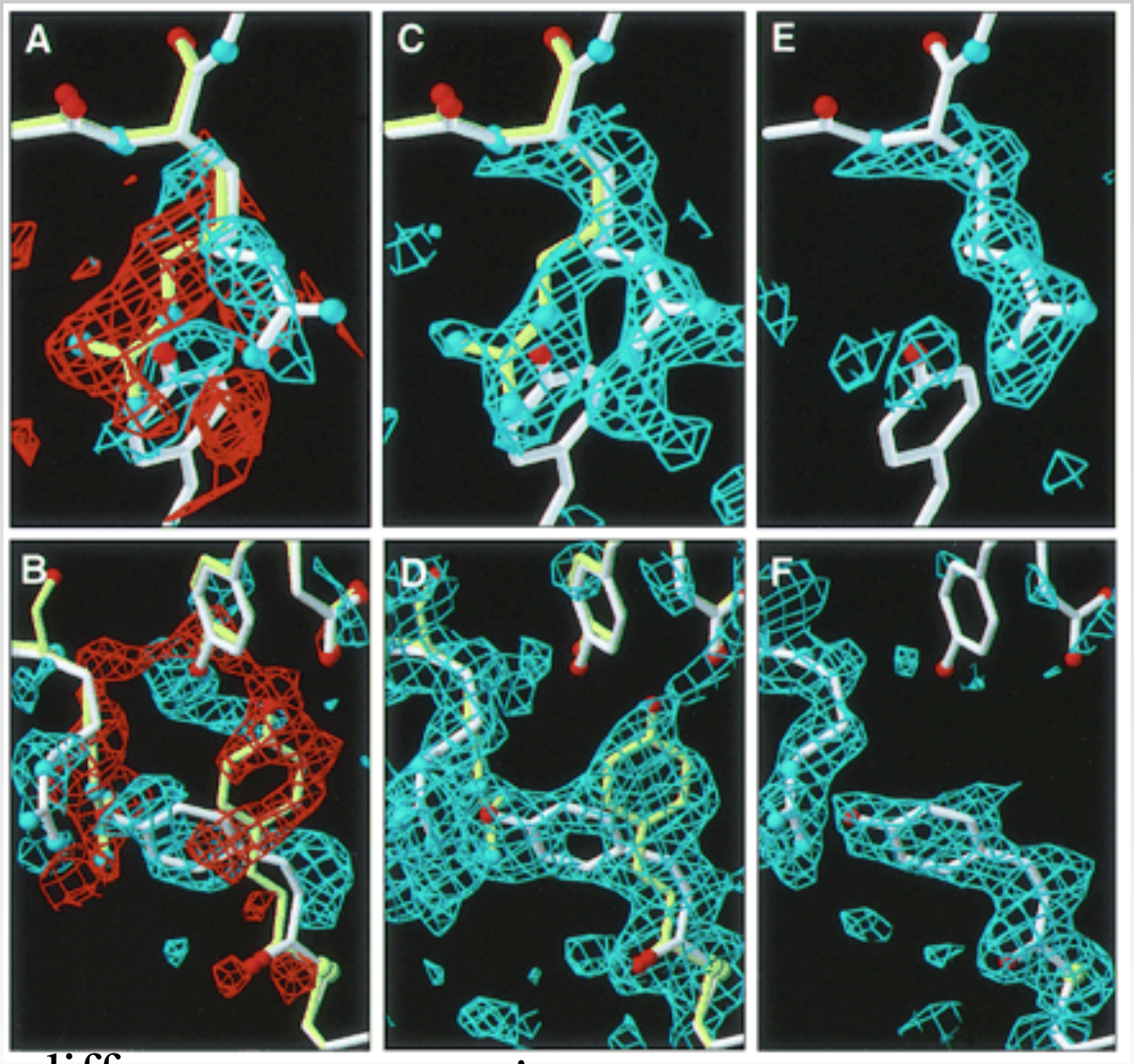

4-hydroxycinnamyl chromophore

Photoactive yellow protein (from the phototrophic bacterium Ectothiorhodospira halophila)

Genick *et al*. Science, 275 (5305): 1471

# Hybrid maps

$F_{bleached} - F_{dark}$



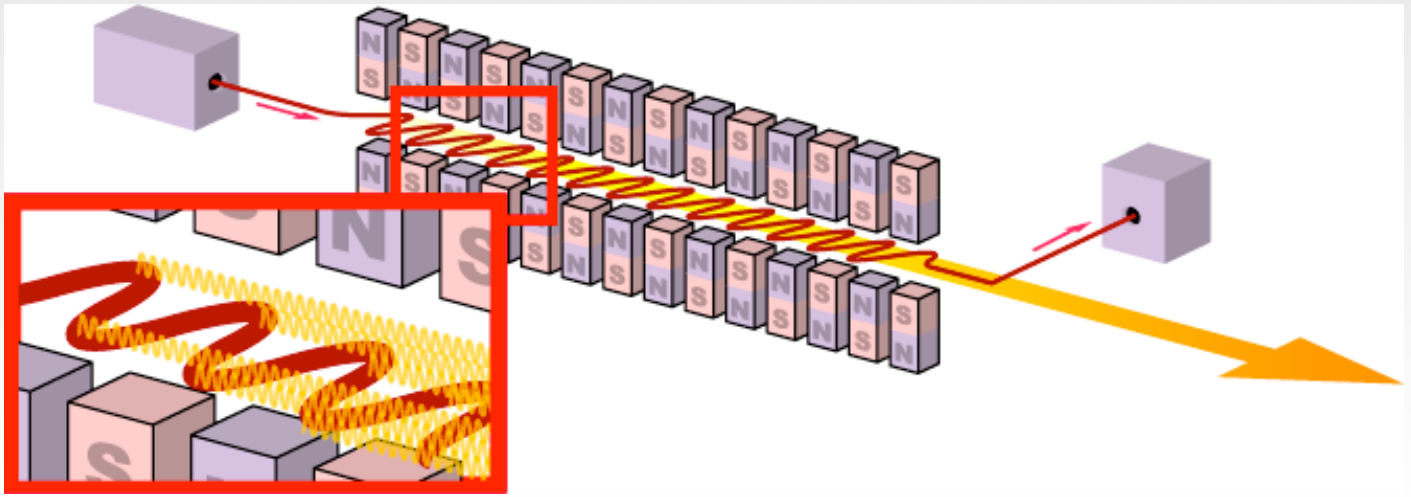

difference density

omit map

extrapolated density

# X-ray free electron laser (XFEL)

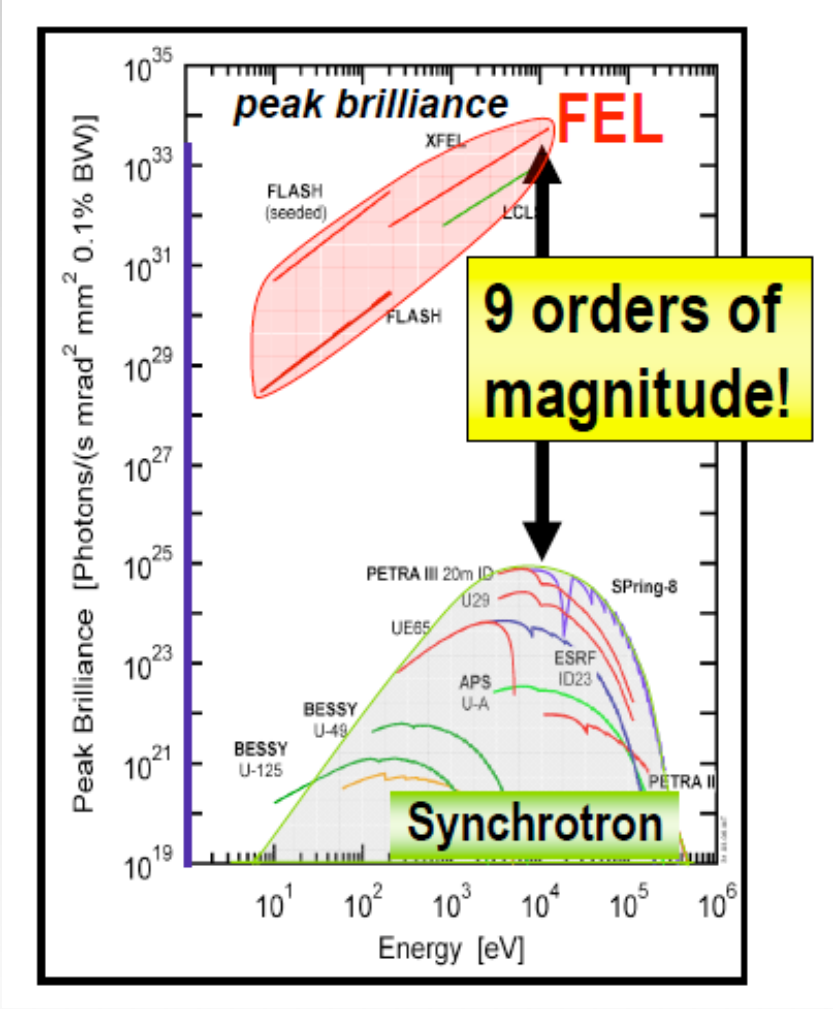
- First successful X-ray laser, published in July 2012.
- Generated by an electron beam which moves freely through a magnetic structure, under vacuum.
- Tunable to a wide range of frequencies.
- Billions of times more intense than conventional methods.



# European FEL construction site, Hamburg

# Free electron laser is quantum leap in brilliance

- $10^{12\text{-}13}$ photons: ~ 10 fs pulses
- repetition rate: now 120 Hz
- photon energies: 10 keV
- transversally: fully coherent

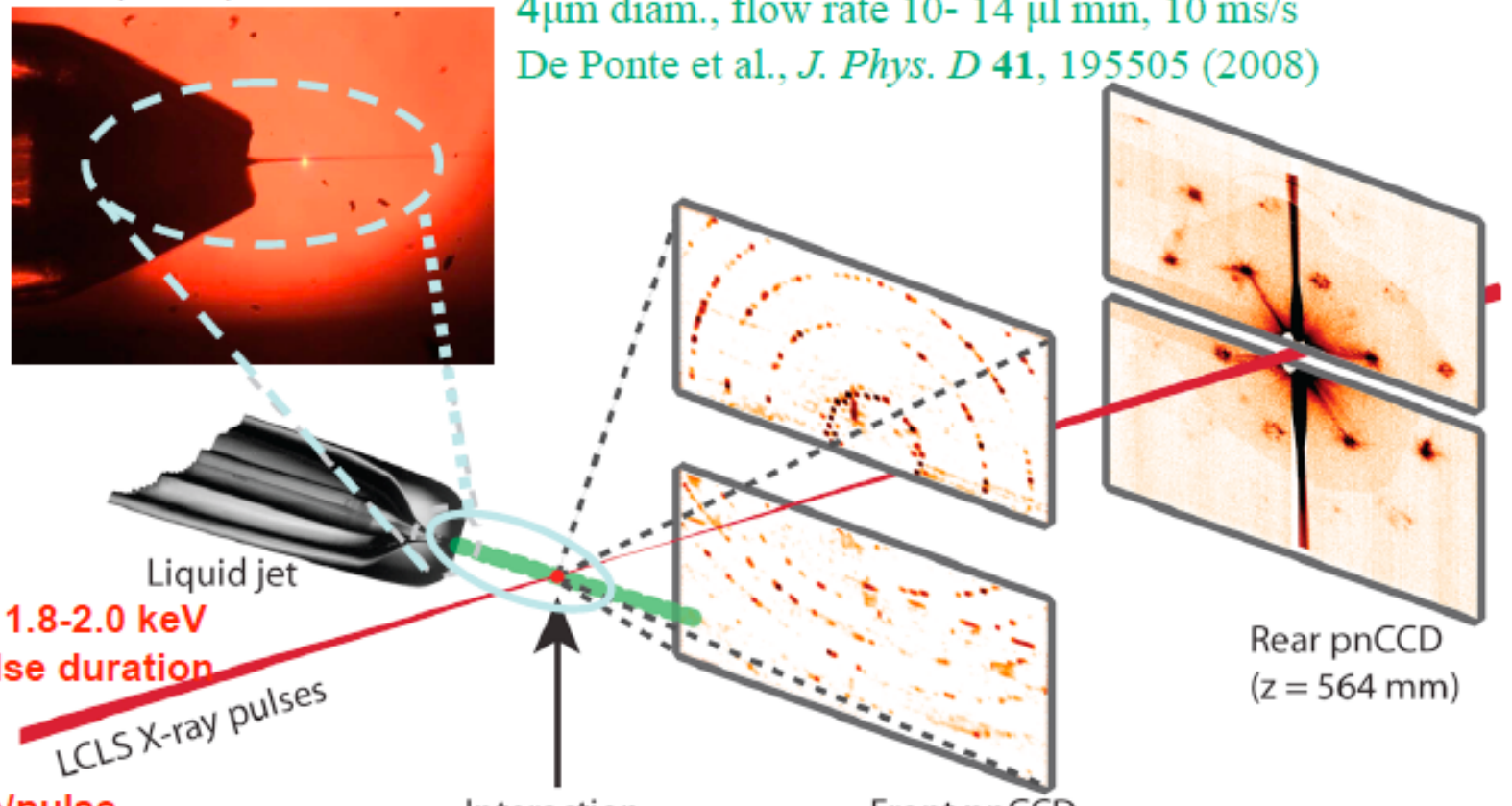



Images from Thomas Barends

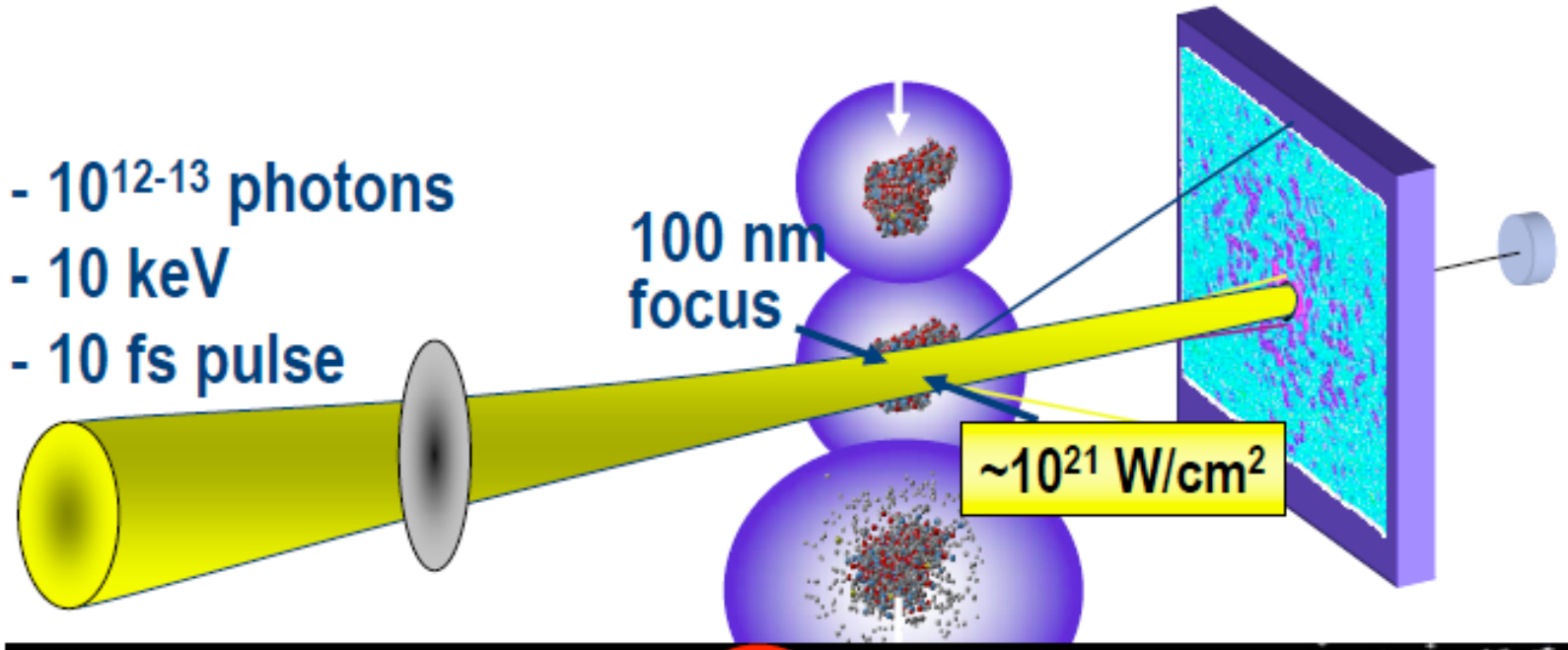
# First serial femtosecond crystallography experiments at LCLS/AMO/CAMP

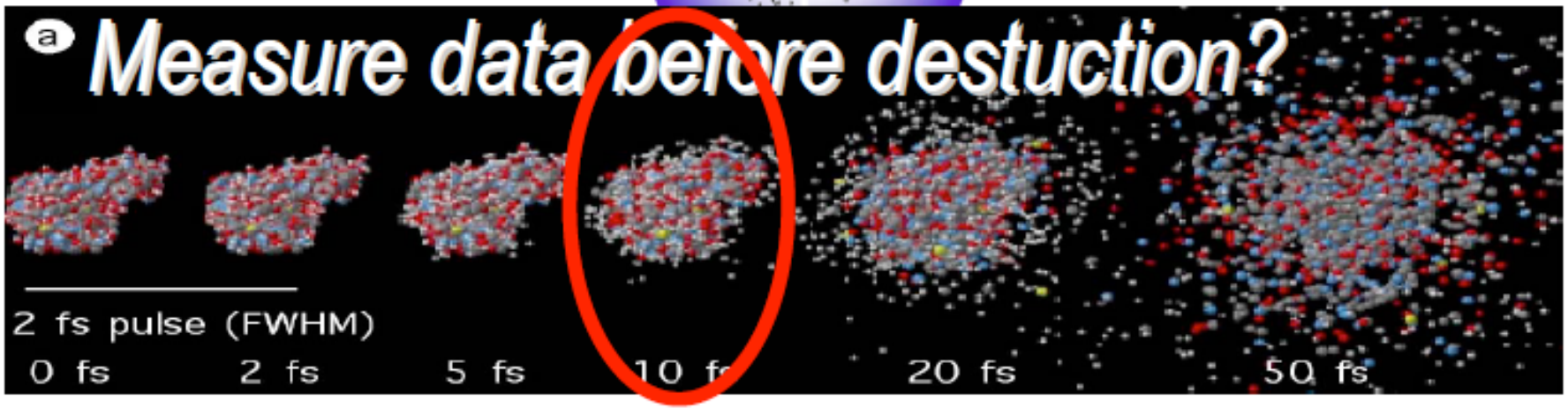
Chapman et al
Nature 470: 73 (2011)

**Gas focussed liquid jet:**
4μm diam., flow rate 10- 14 μl min, 10 ms/s
De Ponte et al., *J. Phys. D* **41**, 195505 (2008)



# Coherent Diffractive Imaging




Calculations in vacuum, Neutze et al., Nature 2000

# So what's the *bad* news?

- Hit rates are low, + only a fraction of hits indexable
- → the method needs:
  - 1-to-several ml of highly concentrated (yoghurt-like!) suspension of microcrystals (hit rates are low, for high resolution many 10,000s images needed)

*How do you make that much protein?*

(usual yields are in the 0.1-1mg range for membrane proteins..., very difficult to produce, not stable!)

*Can you make nanocrystals of it?*

(*how do you know you have them?*

*how do you know they are any good?*

testing them can only be done at the FEL...)

(*can you inject them?*

PEG/salts may clog the nozzle.....)

.....?

- $$$$$$

DROPLET-ON-DEMAND
TO SAVE SAMPLE ?
HIGH PULSE RATE?

# MicroED

- Microcrystal Electron Diffraction

  uses a cryo-electron microscope---->

- Proceed to Gonen2016.pdf

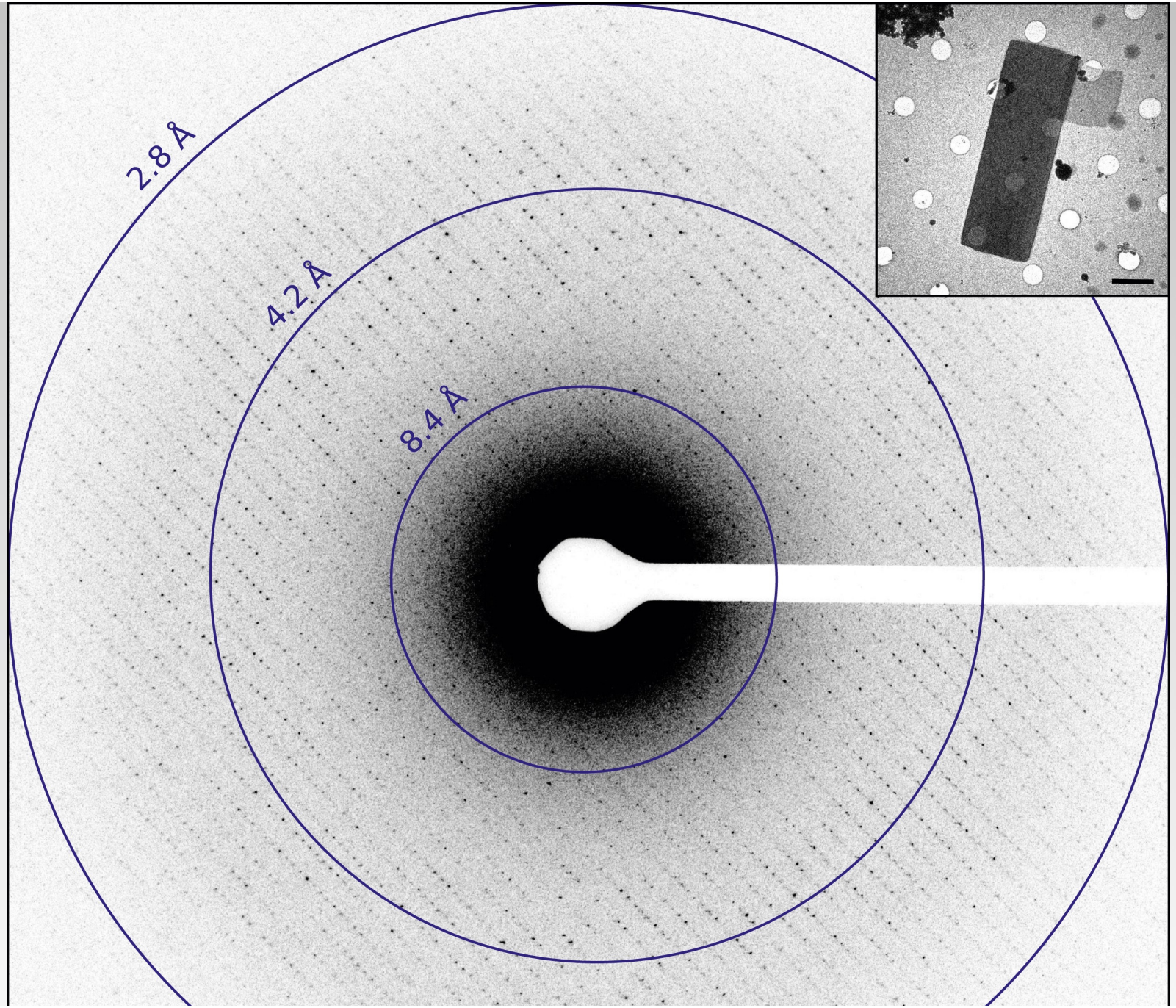
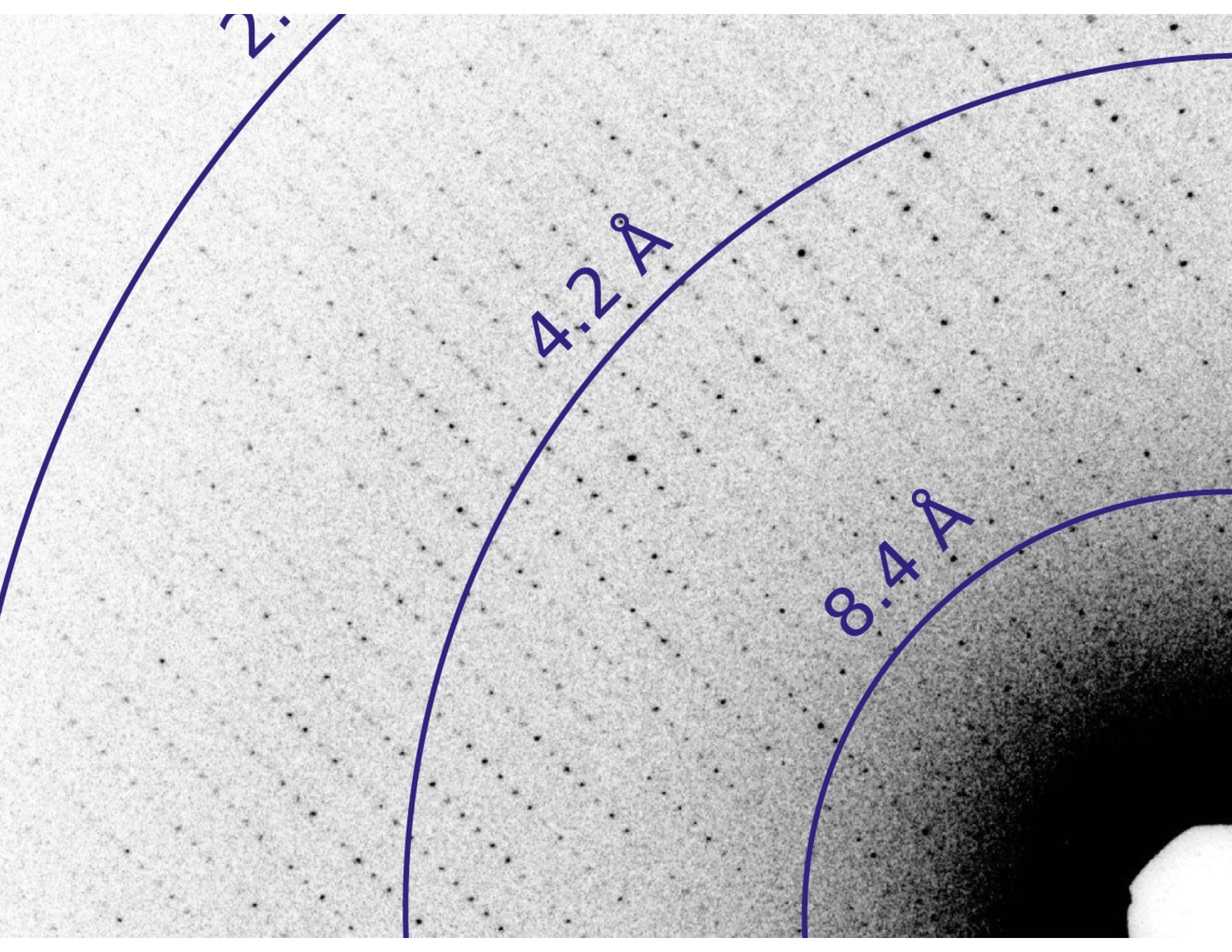
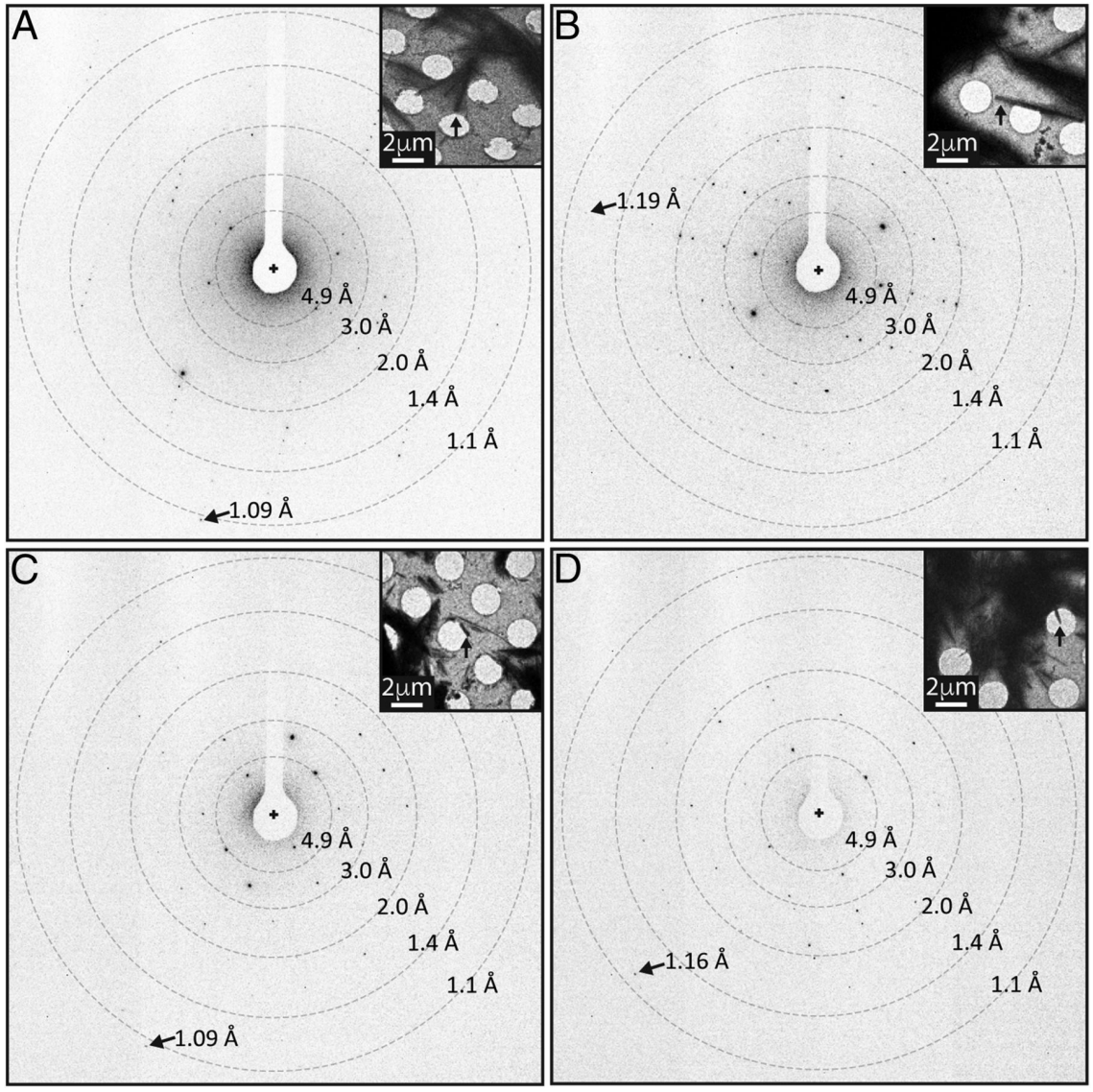



Figure 1.
Diffraction from catalase microcrystals.
Representative untilted still diffraction pattern of a catalase microcrystal that shows sharp reflections extending to approximately 3.0 Å. Crystals of this quality were used to collect a data set by continuous rotation. Inset shows an example catalase microcrystal as seen in over-focused diffraction mode. Scale bar is 2 $\mu$m. The dimensions of most microcrystals varied between 6 and 20 $\mu$m in length, 2 and 8 $\mu$m in width, and the thickness was approximately 100–200 nm, in agreement with previous catalase crystal sizes (Dorset and Parsons, 1975b).


4.2 Å
8.4 Å

# Atomic resolution electron diffraction from amyloid nanocrystals.




**Michael R. Sawaya et al. PNAS 2016;113:11232-11236**

Comparison of latest crystallography methods

| | XFEL | MicroED |
|---|---|---|
| **crystals** | micro/nano | nano |
| **cost** | $$$$ | $$ |
| **protein** | g | ng |
| **scattering** | e- | nucleus |

# Physics of Xray versus NMR

- In NMR *long*-wavelength light interacts with the ***nuclei***.
  In Xray, *short* wavelength light interacts with the ***electrons***.
- In NMR, light is an oscillating ***magnetic*** field.
  In Xray, light is an oscillating ***electric*** field.
- In NMR we Fourier transform from the ***time domain*** to the ***frequency domain*** (reciprocal time).
  In Xray we Fourier transform from the ***space domain*** to the ***reciprocal space domain***.
- In NMR we solve a protein structure by determining the ***internal*** coordinates (pairwise distances).
  In Xray we solve a structure by determining the ***Cartesian*** coordinates.