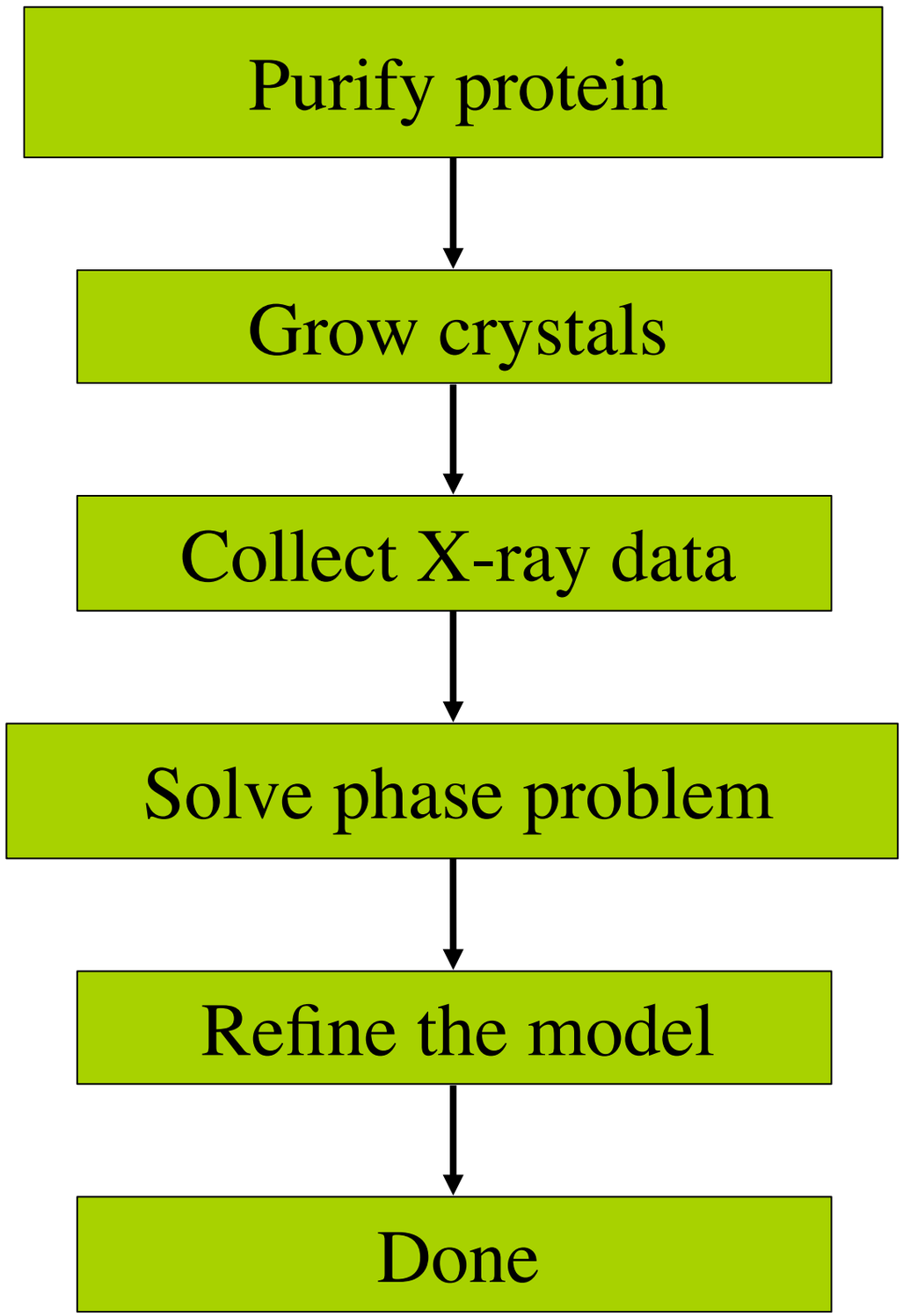
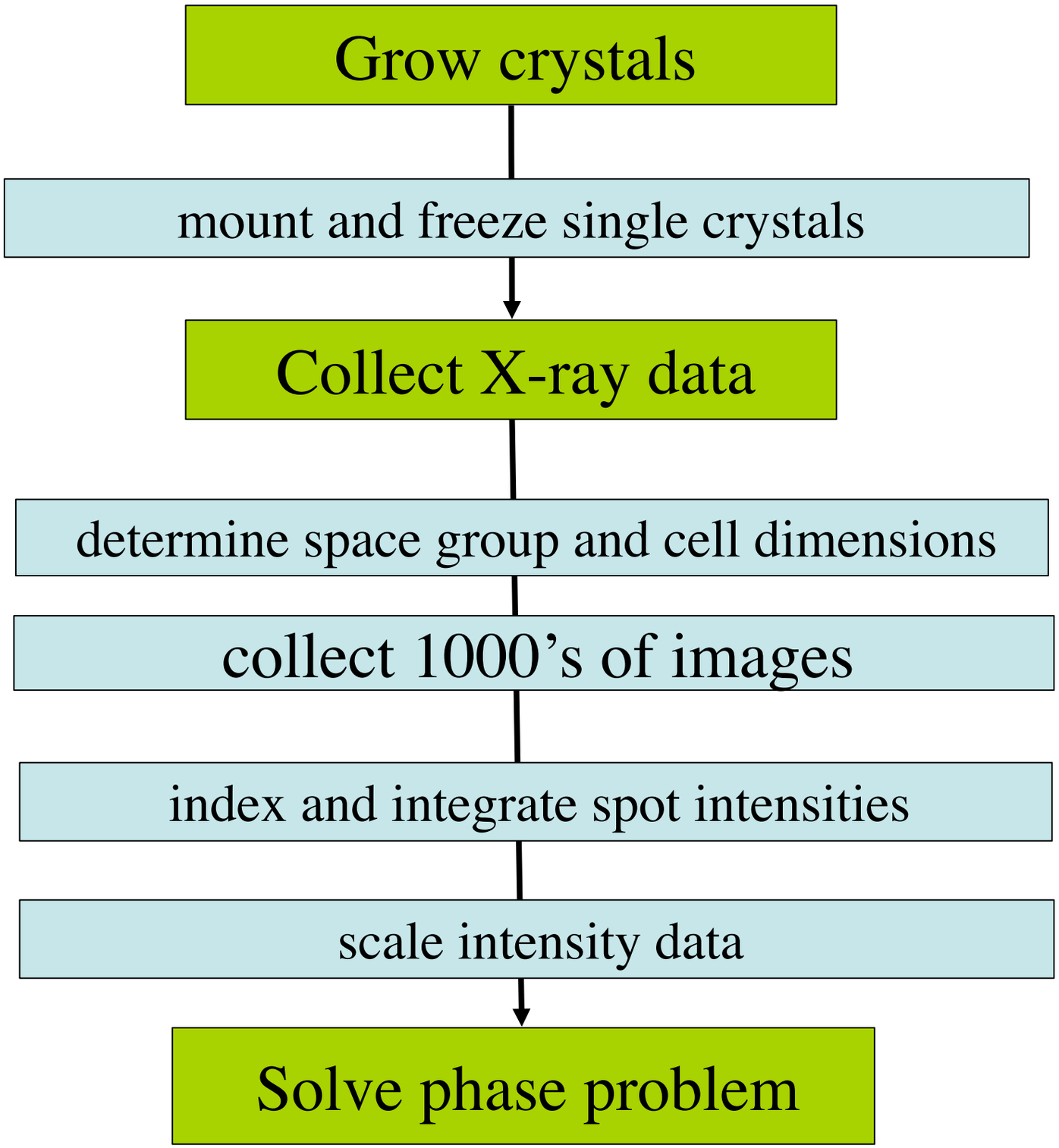
PSD
Xray lecture 13

Overview
Laue photography
X-ray laser femptosecond Laue photography

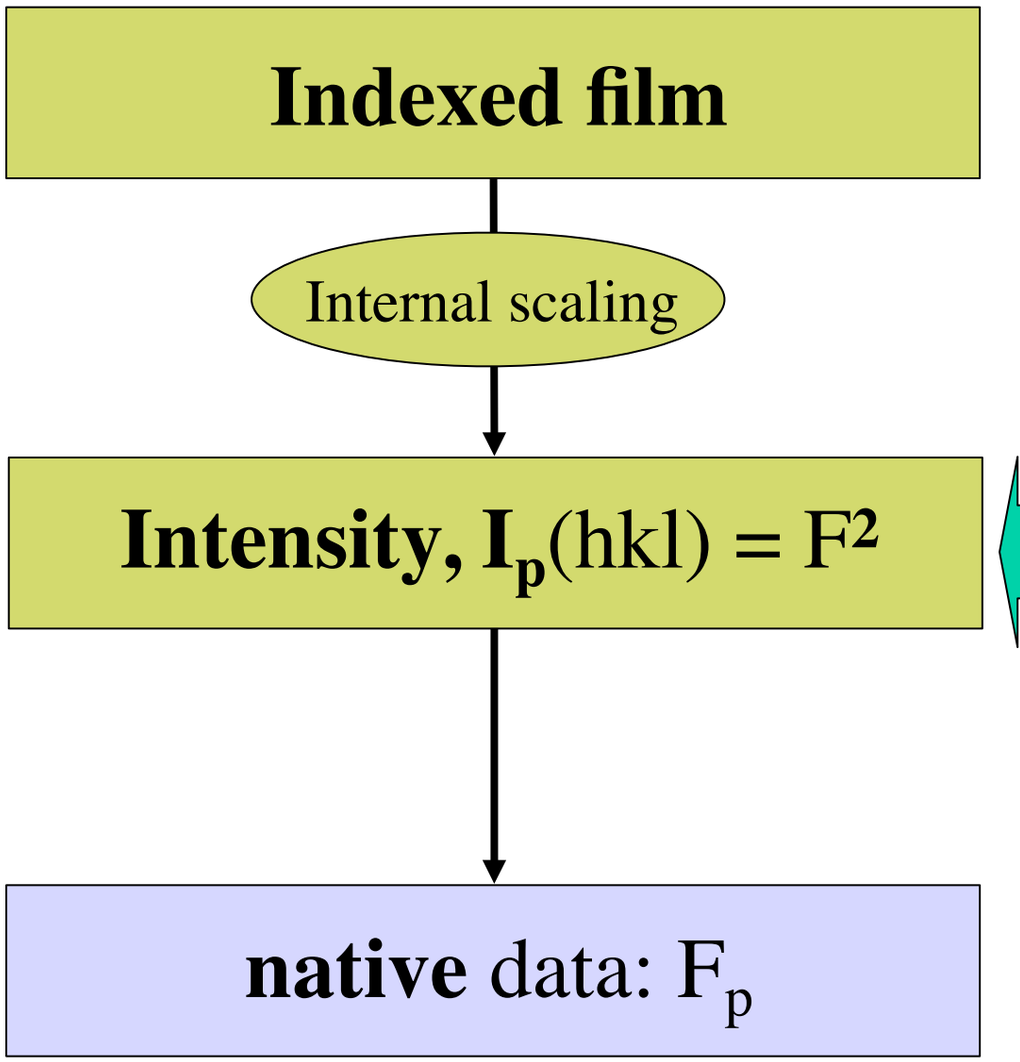
From protein to protein structure



From protein to phasing



From crystal to data



I is relative

Bigger crystal, higher I

Better crystal, higher I

Longer exposure, higher I

More intense Xrays, higher I

Because there is no absolute scale:

F_p and F_{ph} are on *different scales*

From data to Patterson map

native data: F_p

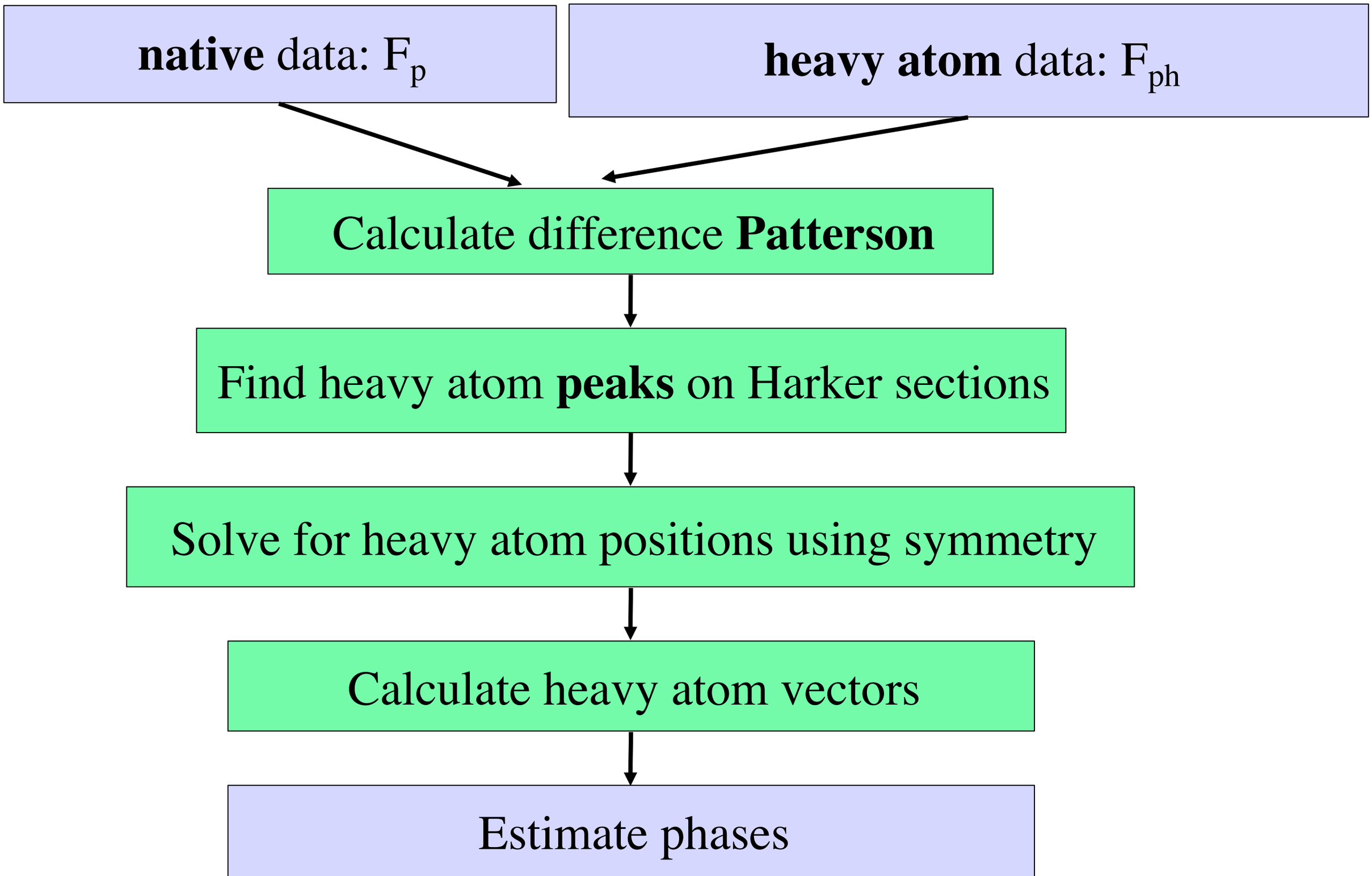
heavy atom data: F_{ph}

Find the *best scale factor*, **w**

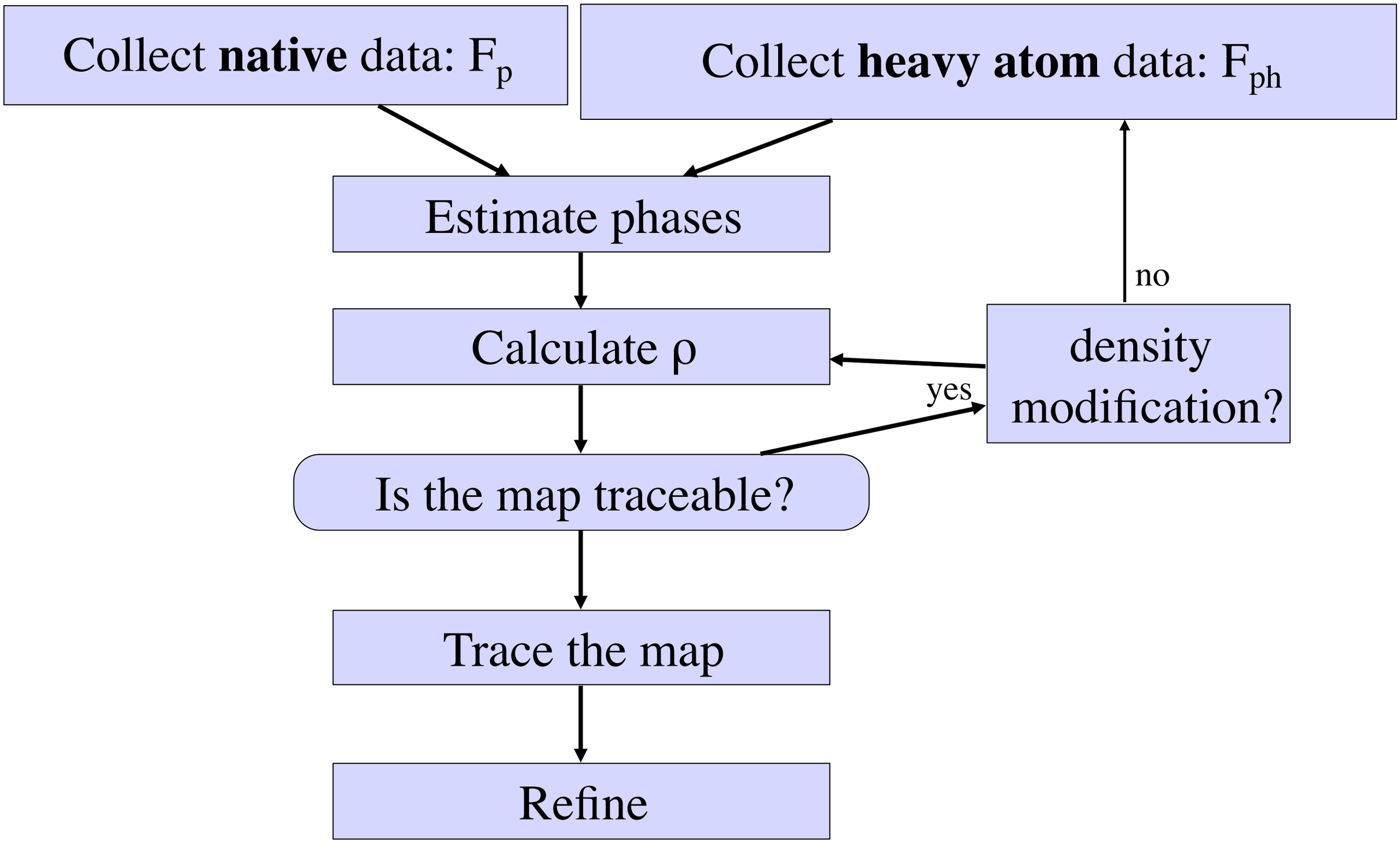
Calculate $F_{diff} = \mathbf{w}*|F_{ph}| - |F_p|$

Calculate difference **Patterson**

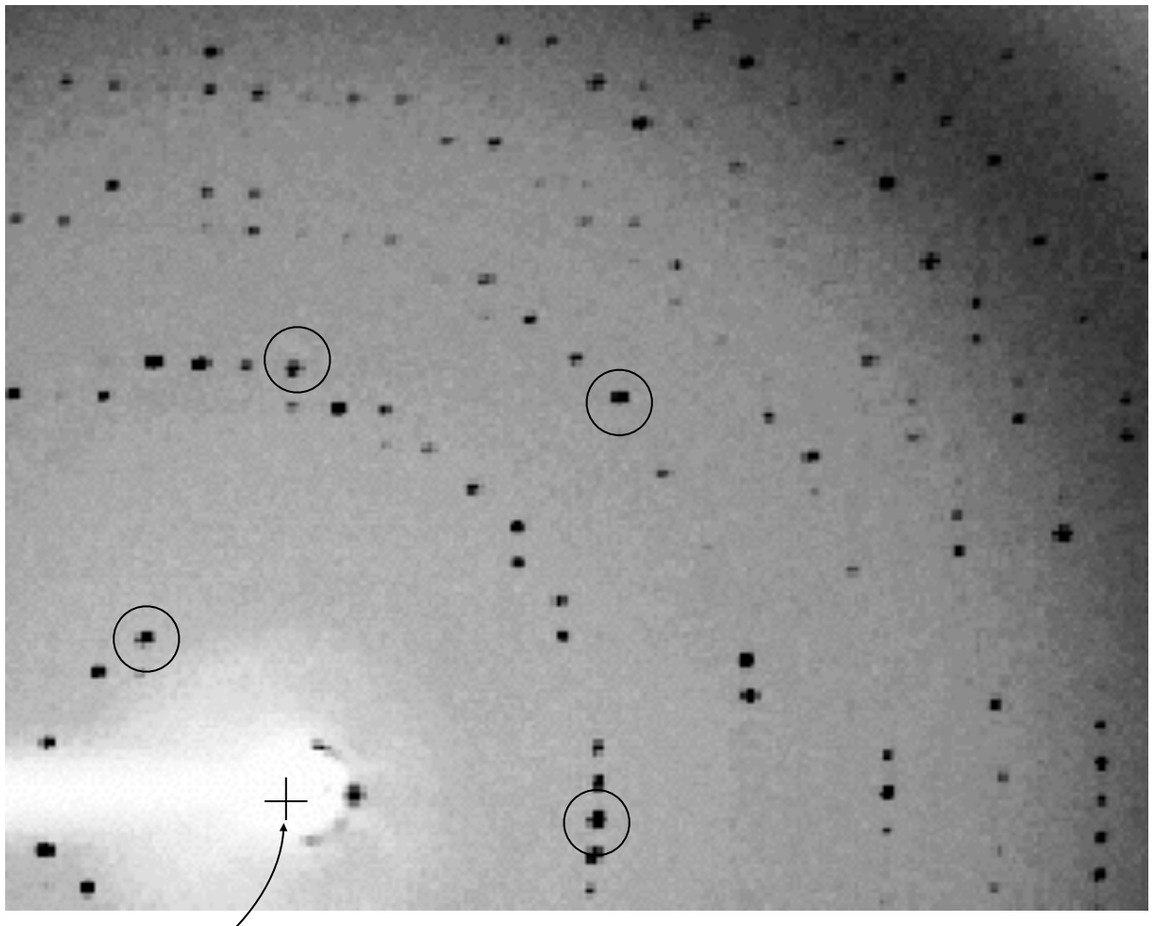
From data to phases

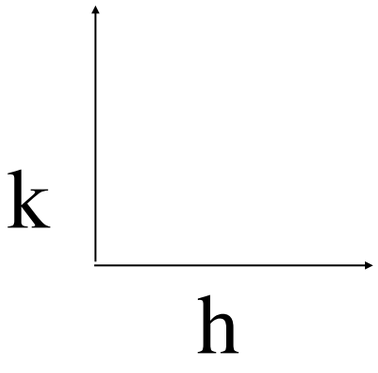


From data to model



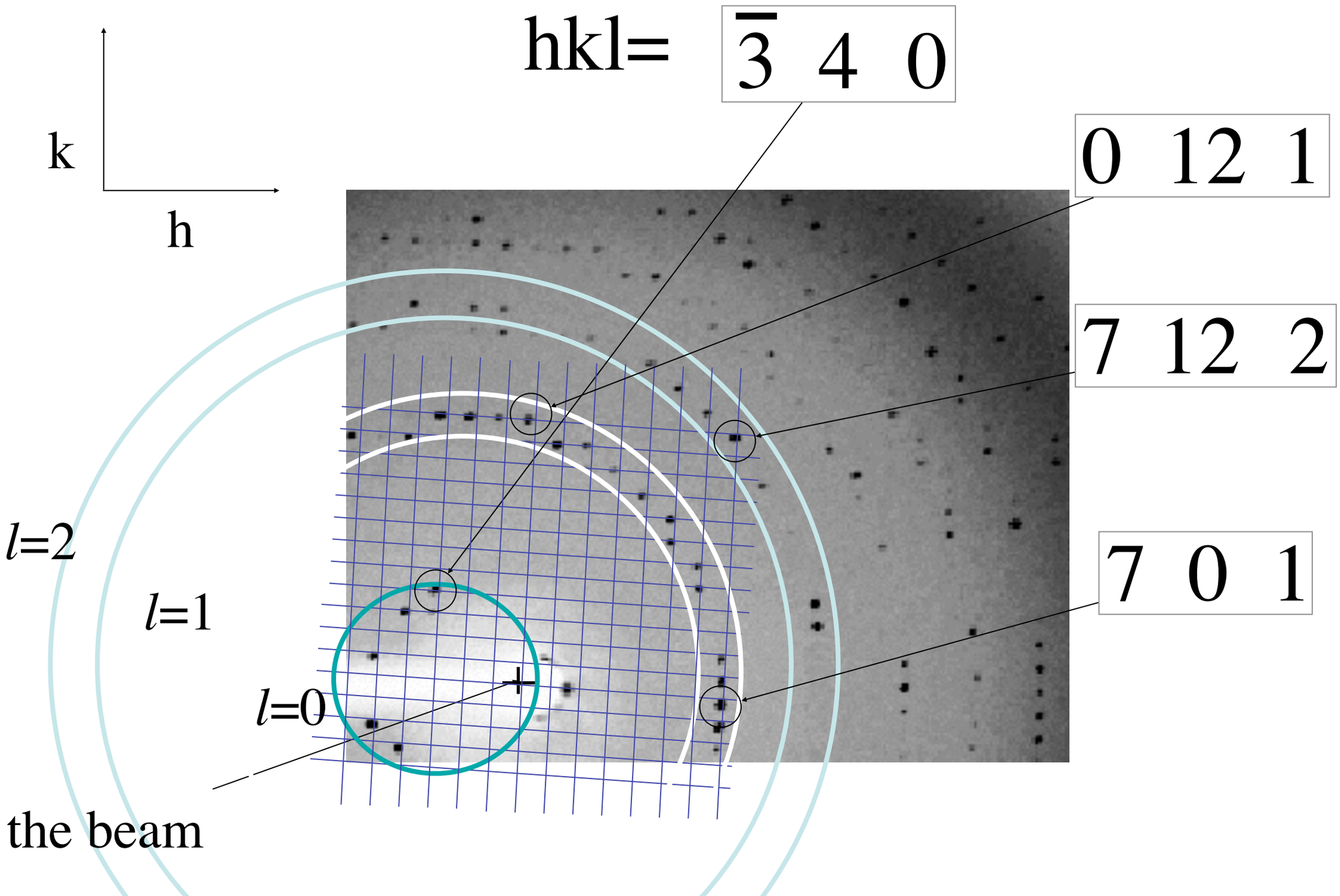
In class exercise: index these spots



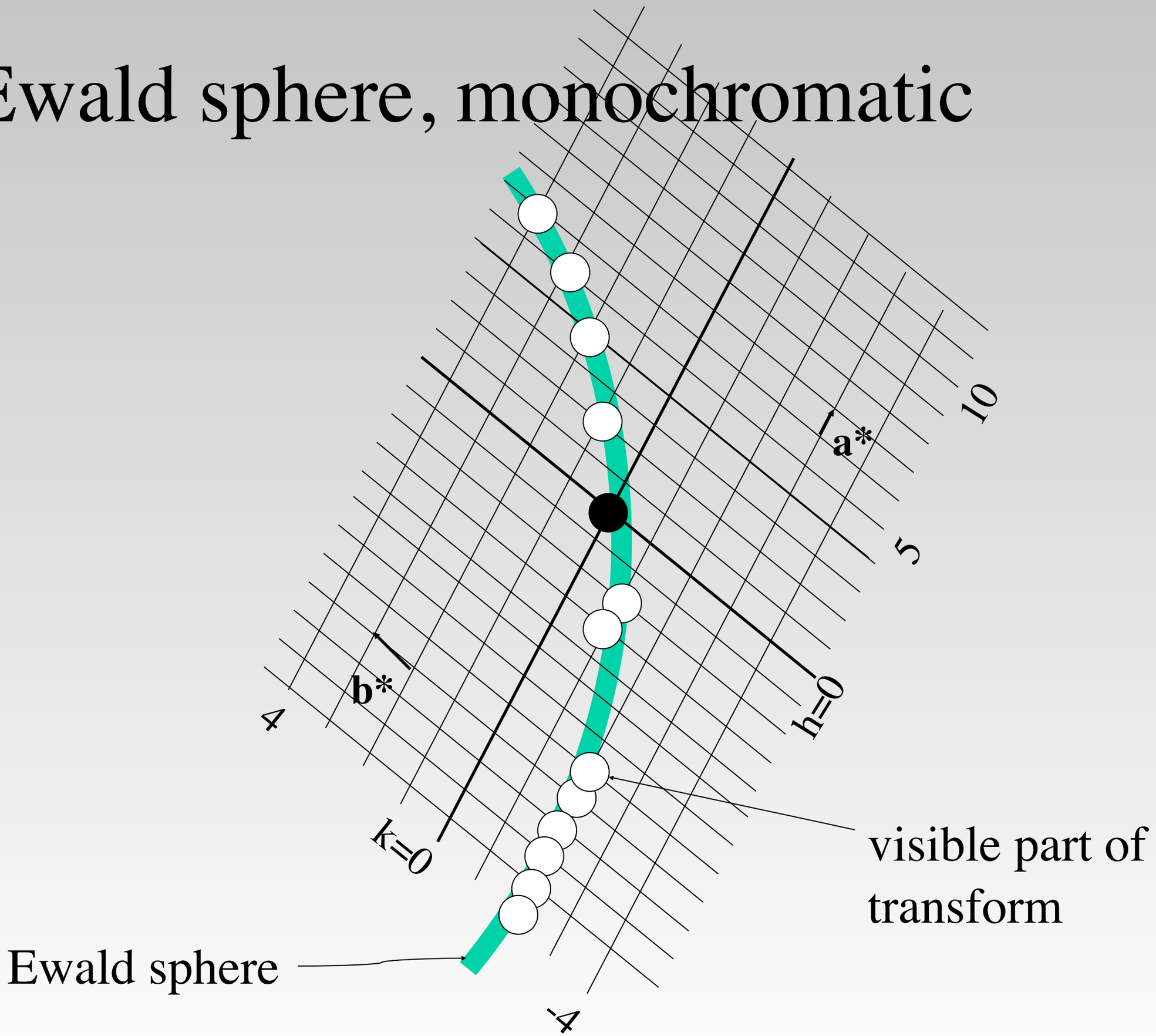


the beam

answer



Ewald sphere, monochromatic



Laue Photography

Mathematics

Structures

time-resolved crystallography

neutron crystallography

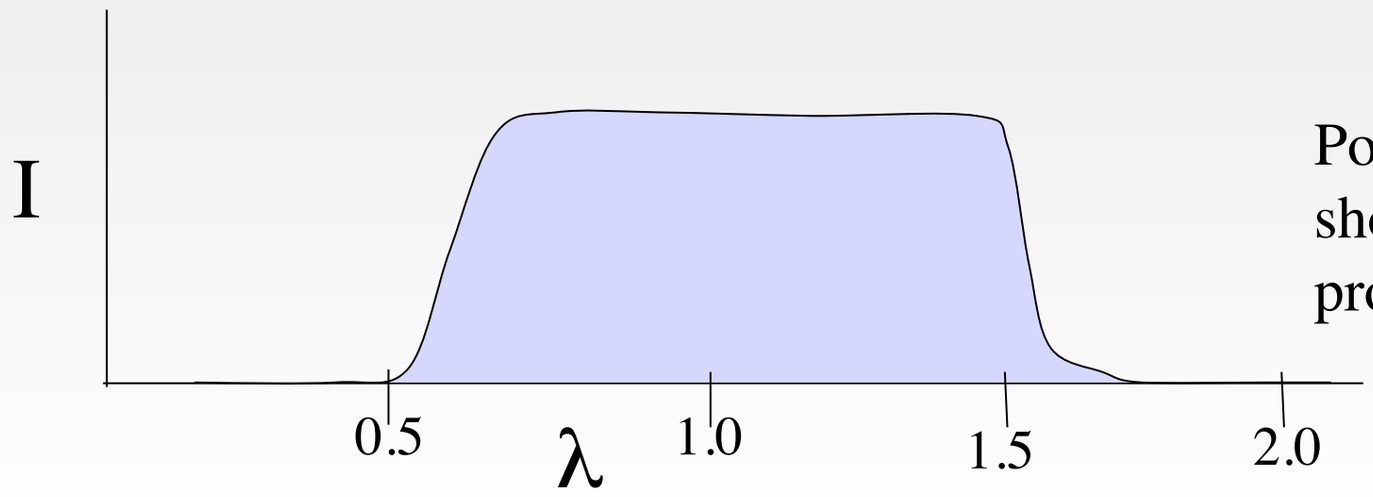
electron crystallography

X-ray free electron laser

nanocrystallography

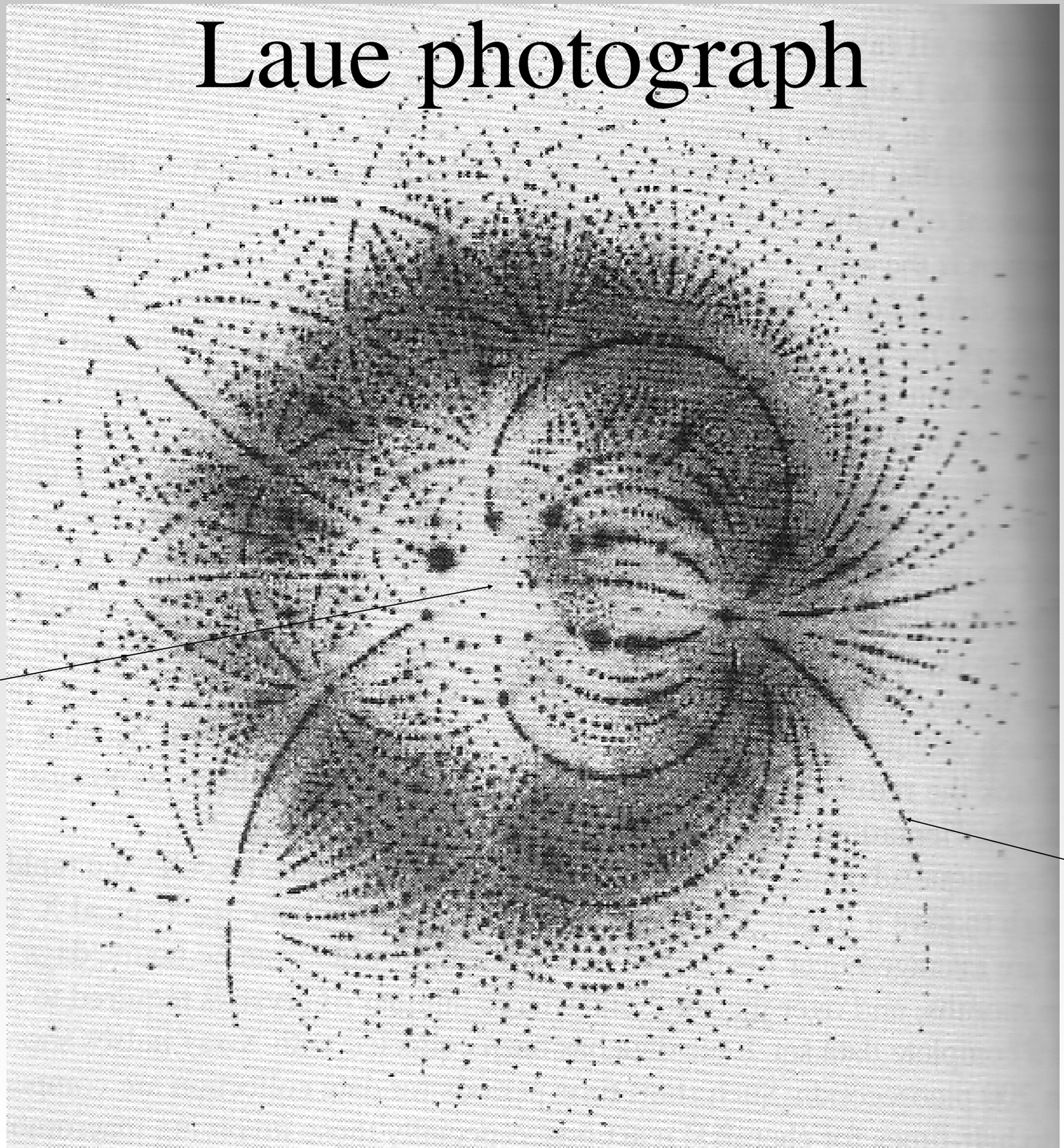
Laue Method

- Uses polychromatic ("white") X-rays. (generally λ<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.

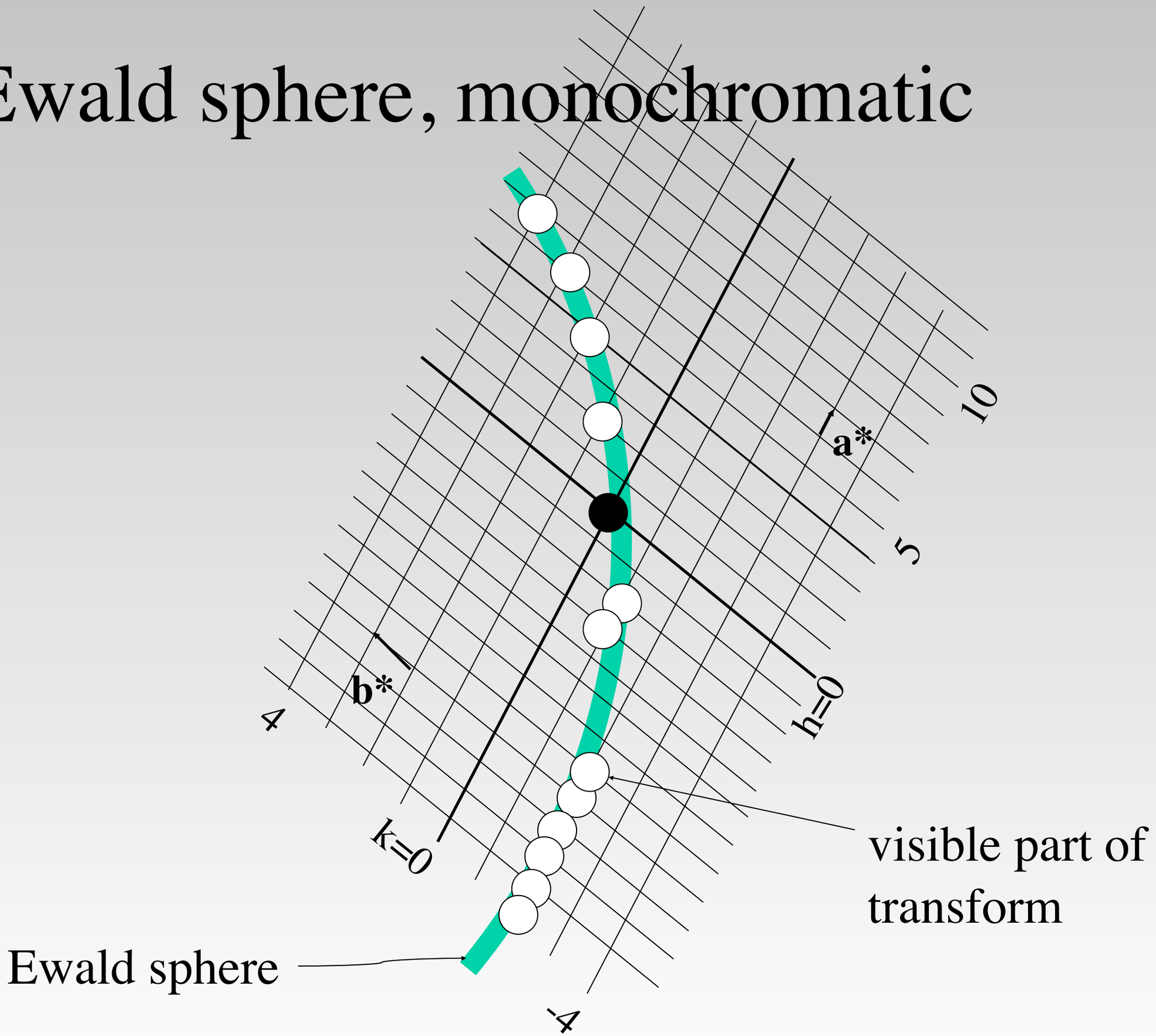
Laue photograph



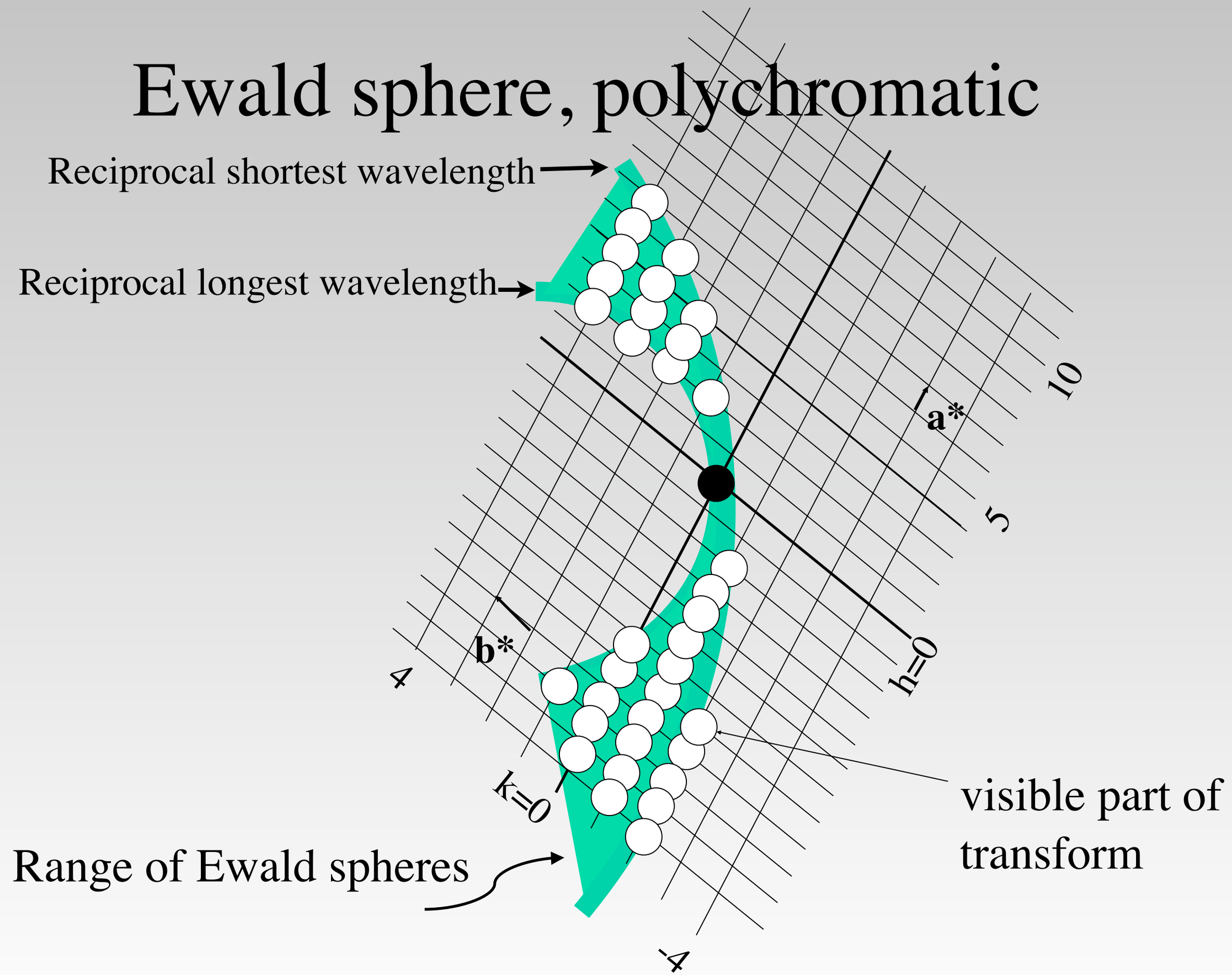
beam

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.

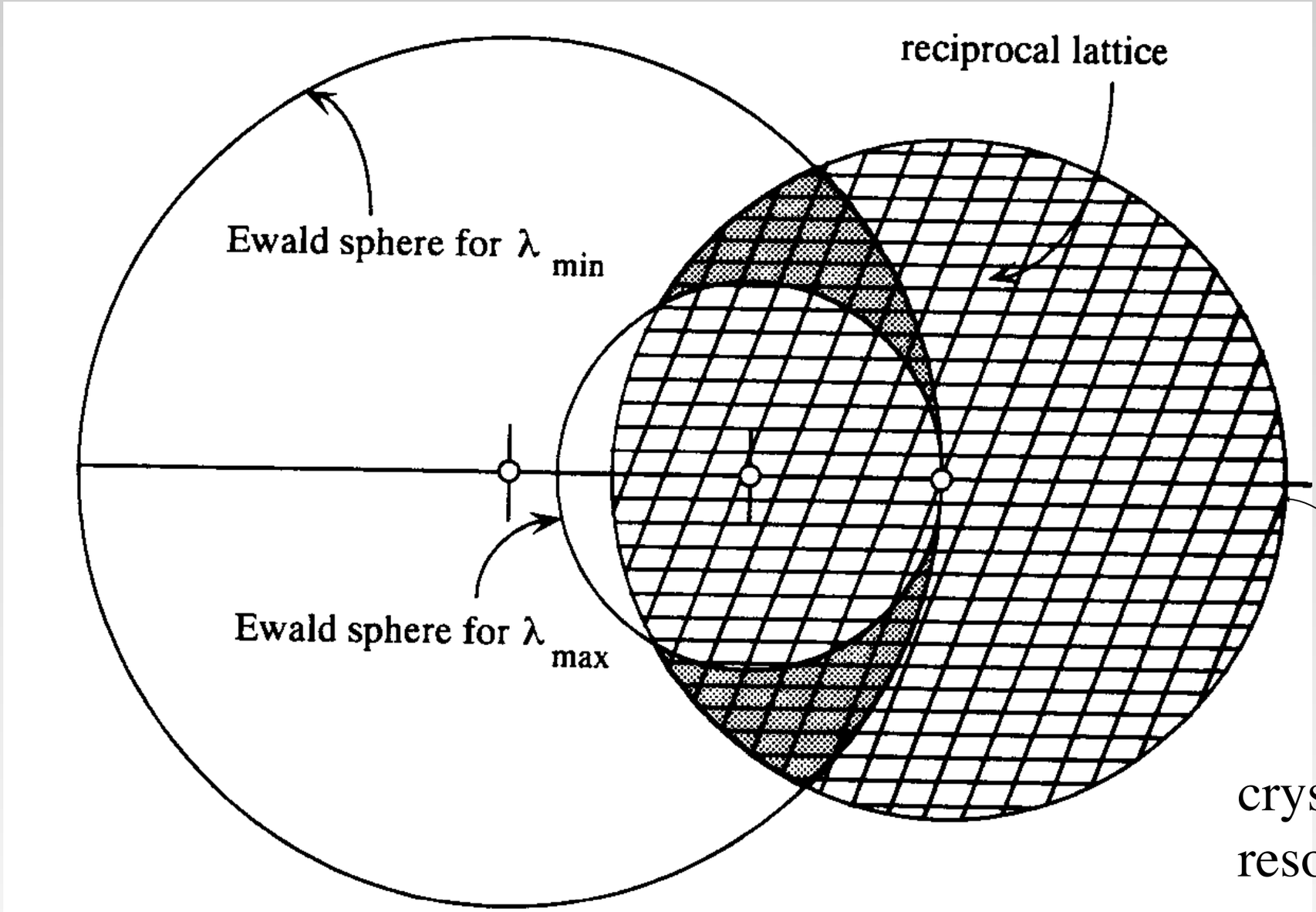
Ewald sphere, monochromatic



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

Bragg's Law for one wavelength

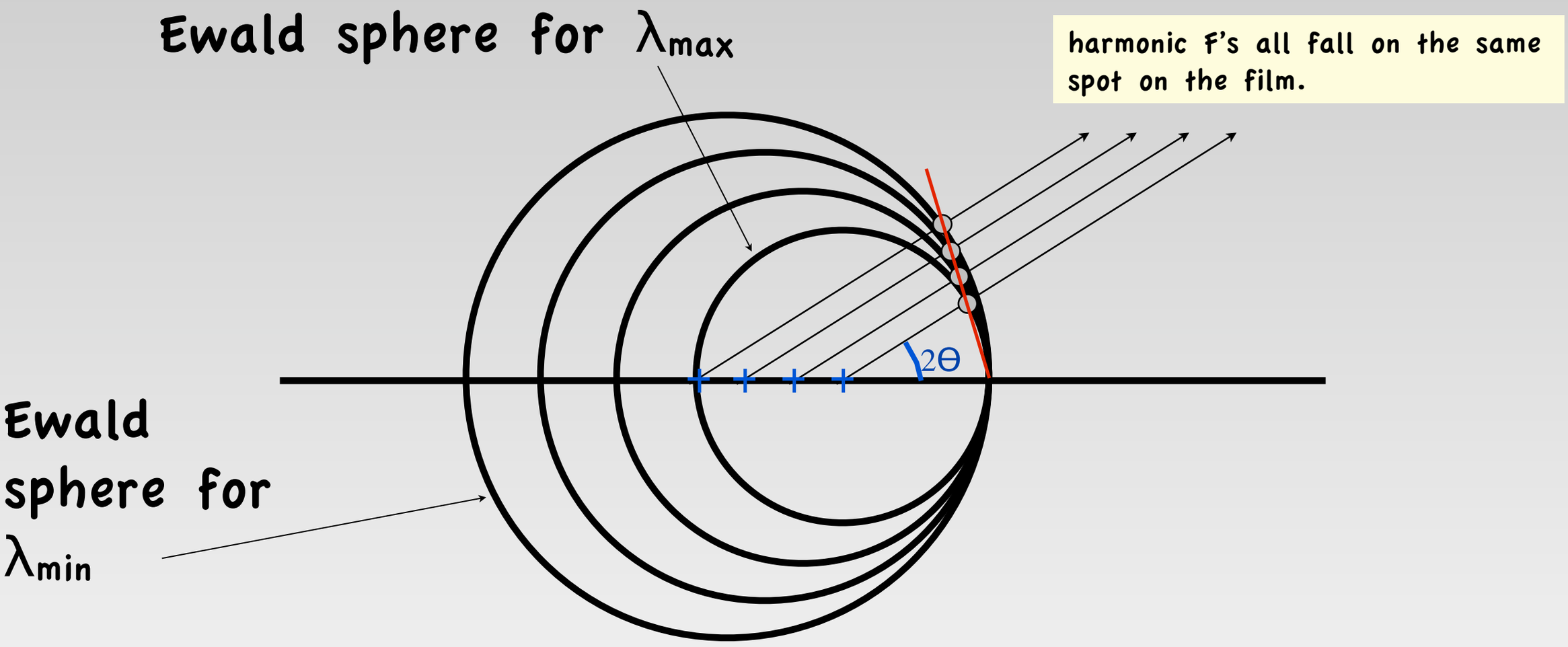
$$n\lambda = 2d \sin \theta$$

Bragg's Law for multiple wavelengths

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

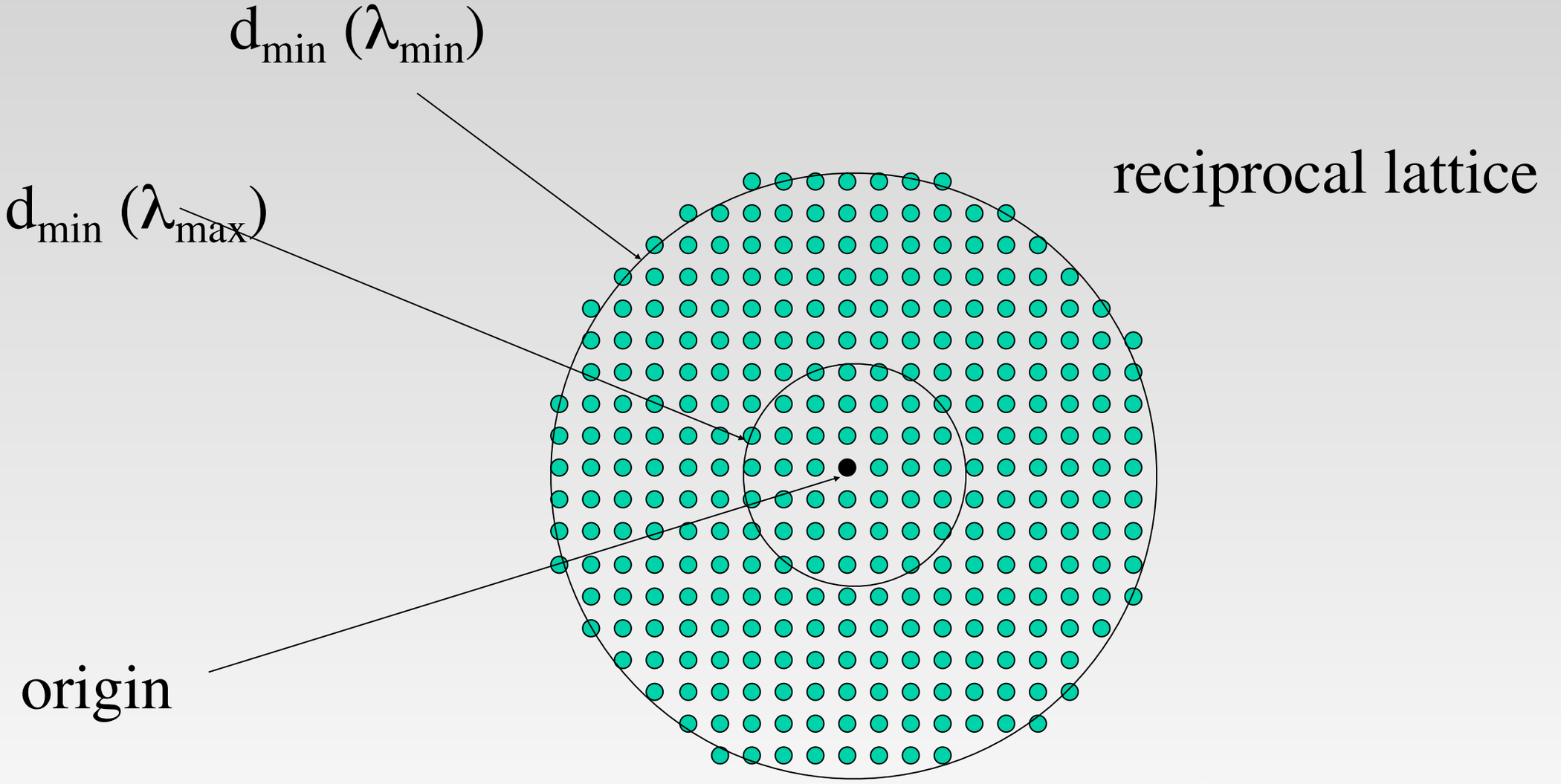
In monochromatic crystallography, one θ angle translates to one d (one resolution). In Laue crystallography, one θ angle translates a range of d. How do we know which wavelength produced the reflection??

harmonic reflections



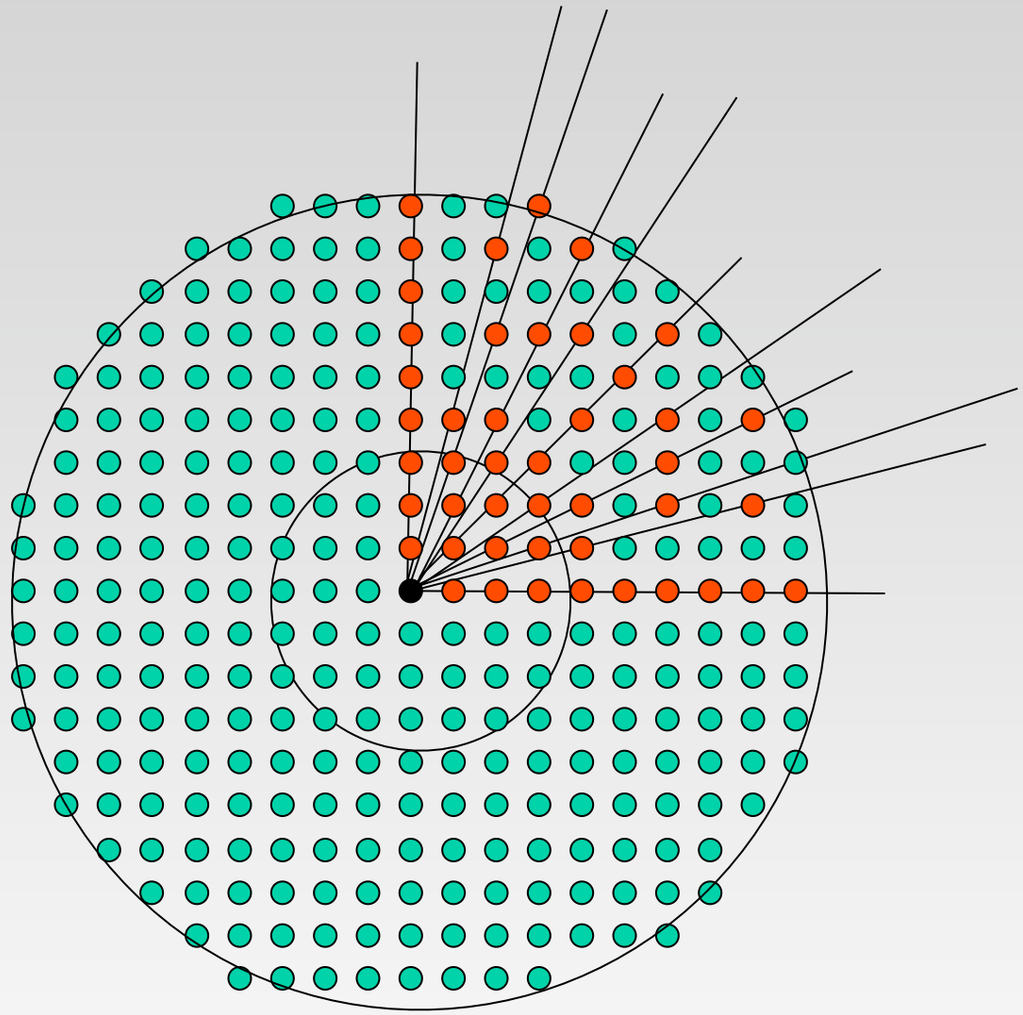
Harmonic reflections (nh,nk,nl) have the same S direction, but the length is inversely proportional to λ. So (h,k,l) at λ=2.0Å and (2h,2k,2l) at λ=1.0Å diffract to exactly the same spot on the film.

Which reflections are multiples? Analogy to trees in an orchard



Summing harmonics is like viewing a lattice from the origin.
Every line of sight represents one spot on the film.

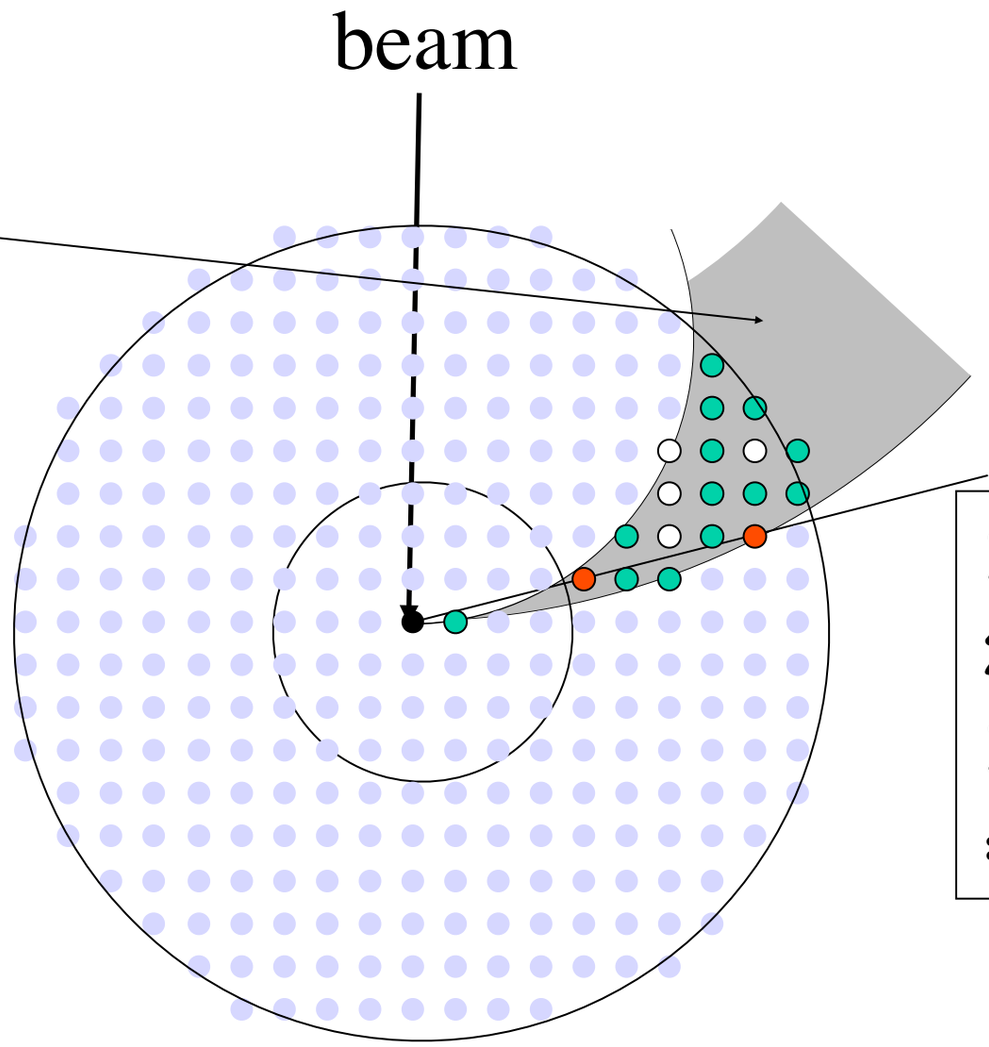
Which reflections are multiples? Analogy to trees in an orchard



73 refls
44 multiples
29 singles

Standing in the middle of a circular orchard, how many tree trunks you see are hiding other tree trunks (“multiples”, red)? And how many are not (“singles”, green)?

Which reflections are multiples? Analogy to trees in an orchard



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 λ_{min}-λ_{max}). Only the trees in the grey region remain. How many are multiples?

Solving for missing intensities (after the fact)

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

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

total intensity for all harmonics in range

Scale factors. A function of wavelength, polarization, etc.

Unknown amplitudes

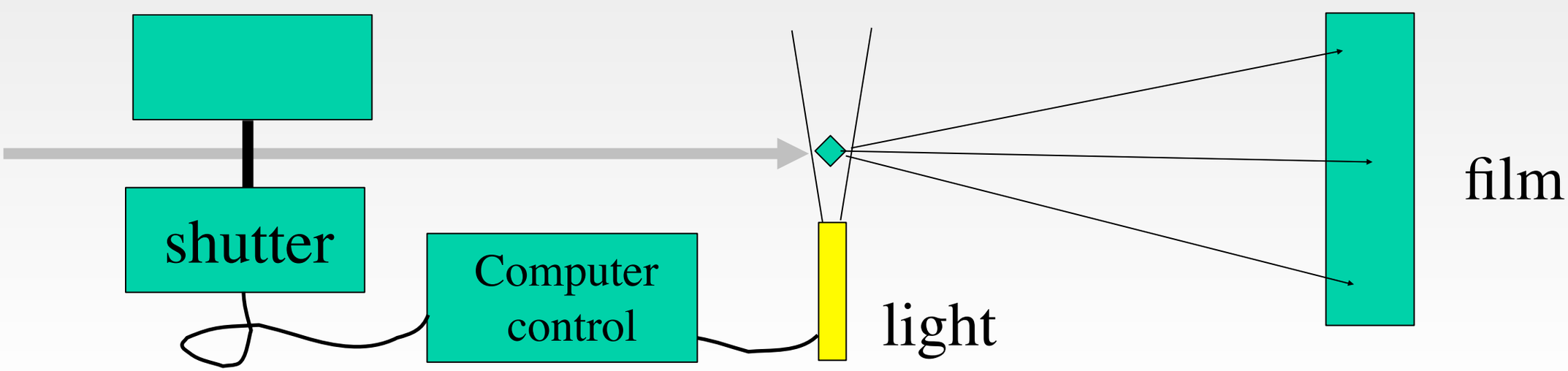
Scale factors (*f*) may be found for singles.
Then, each multiple is a linear equation of the unknown F^2's, which can be solved during *least squares refinement*.

Time-resolved crystallography

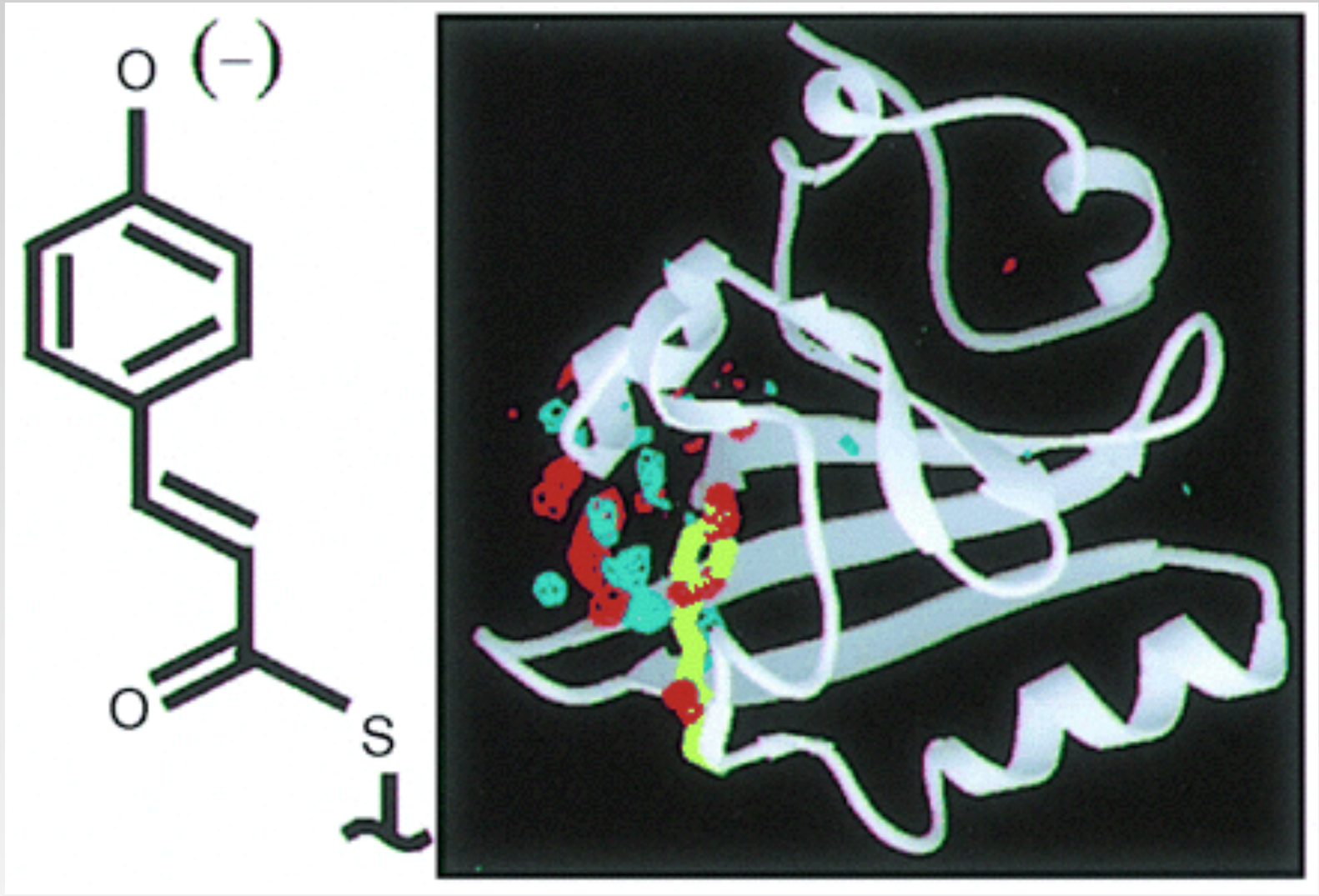
If a reaction can be initiated in a crystal, simultaneously throughout the crystal, then Laue photography can capture the structural changes at the ns (10^{-9} s) to ms (10^{-3} s) timescale.

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



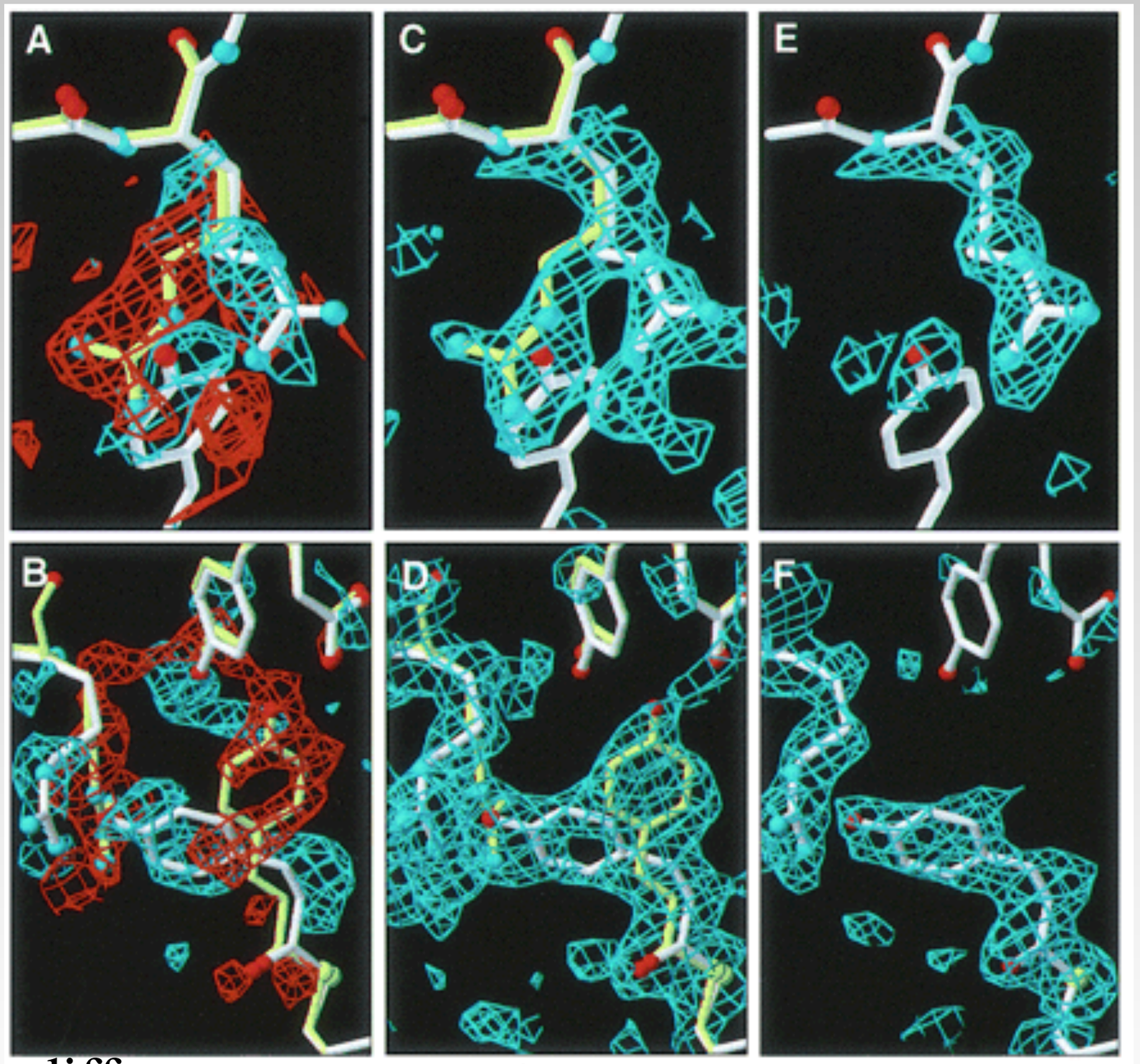
4-hydroxycinnamyl chromophore

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

Advantages of Laue method

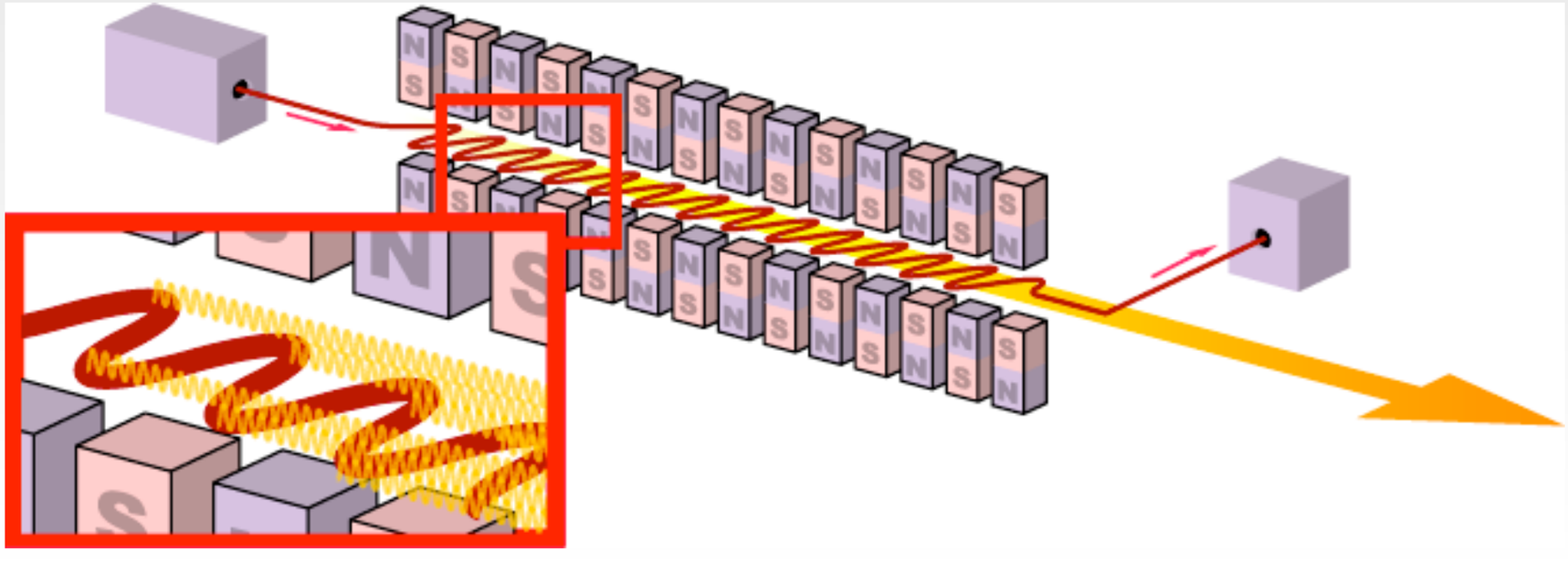
- Extremely short data collection time.
- Time-resolved.
- Just a few exposures covers reciprocal space, especially for high-symmetry space groups.

Problems with Laue method

- Unbalanced coverage of reciprocal space.
- Crystals must withstand intense short exposures, or many crystals must be used.
- Time-resolved study usually means crystals cannot be frozen, making them vulnerable to X-ray damage.
- Spacial overlap requires low mosaicity, small beam, crystal.

X-ray free electron laser (XFEL)

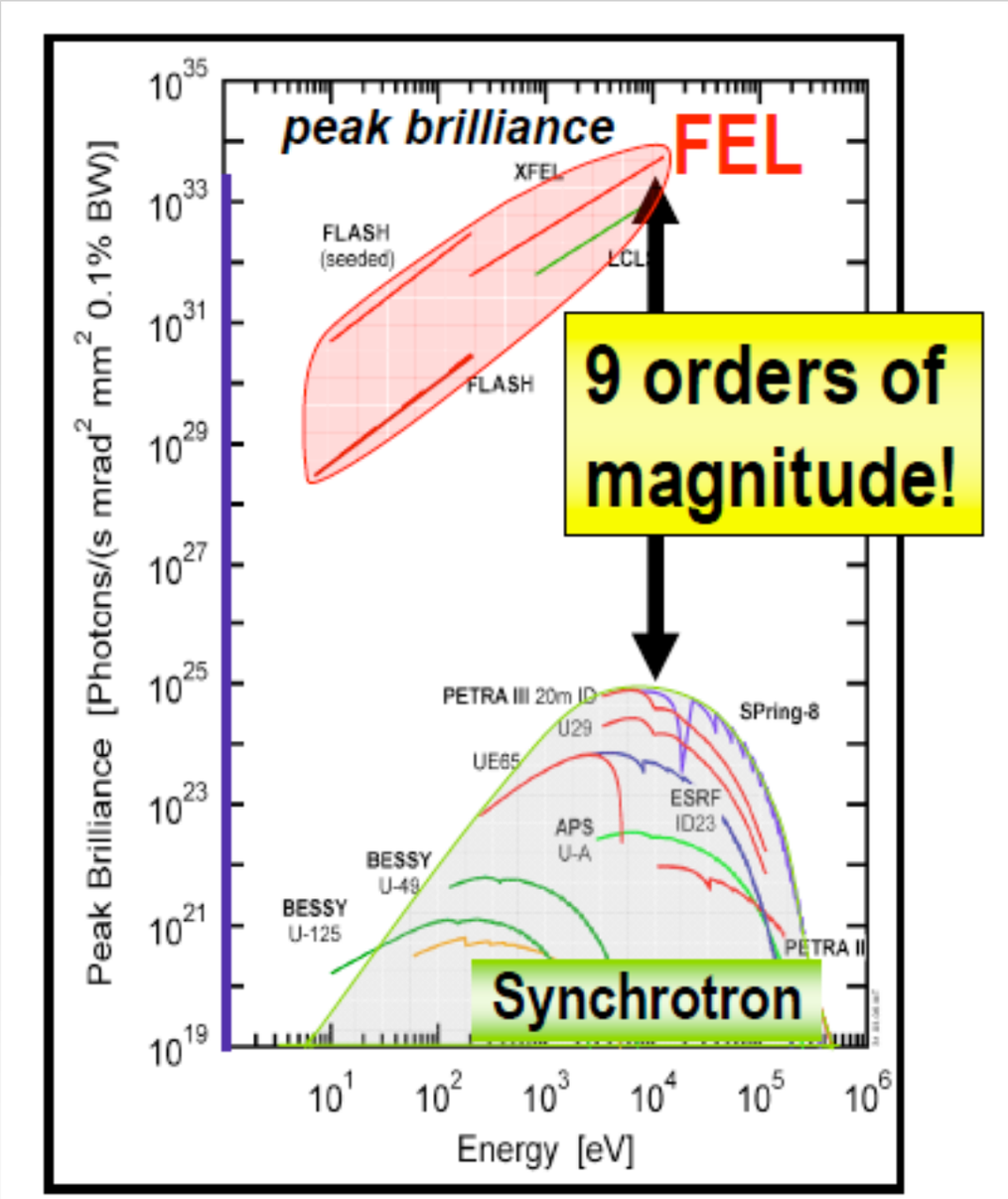
- First successful X-ray laser, published in July 2012.
- Generated by an electron beam which moves freely through a magnetic structure, under vacuum.
- Tunable to a wide range of frequencies.
- Billions of times more intense than conventional methods.



European FEL construction site, Hamburg

Free electron laser

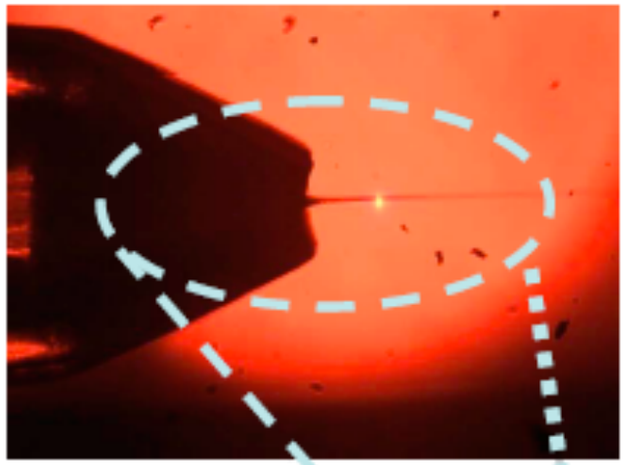
- $10^{12\text{-}13}$ photons: ~ 10 fs pulses
- repetition rate: now 120 Hz
- photon energies: 10 keV
- transversally: fully coherent



Images borrowed from Thomas Barends
google: Barends FELNanocrystallography

First serial femtosecond crystallography experiments at LCLS/AMO/CAMP

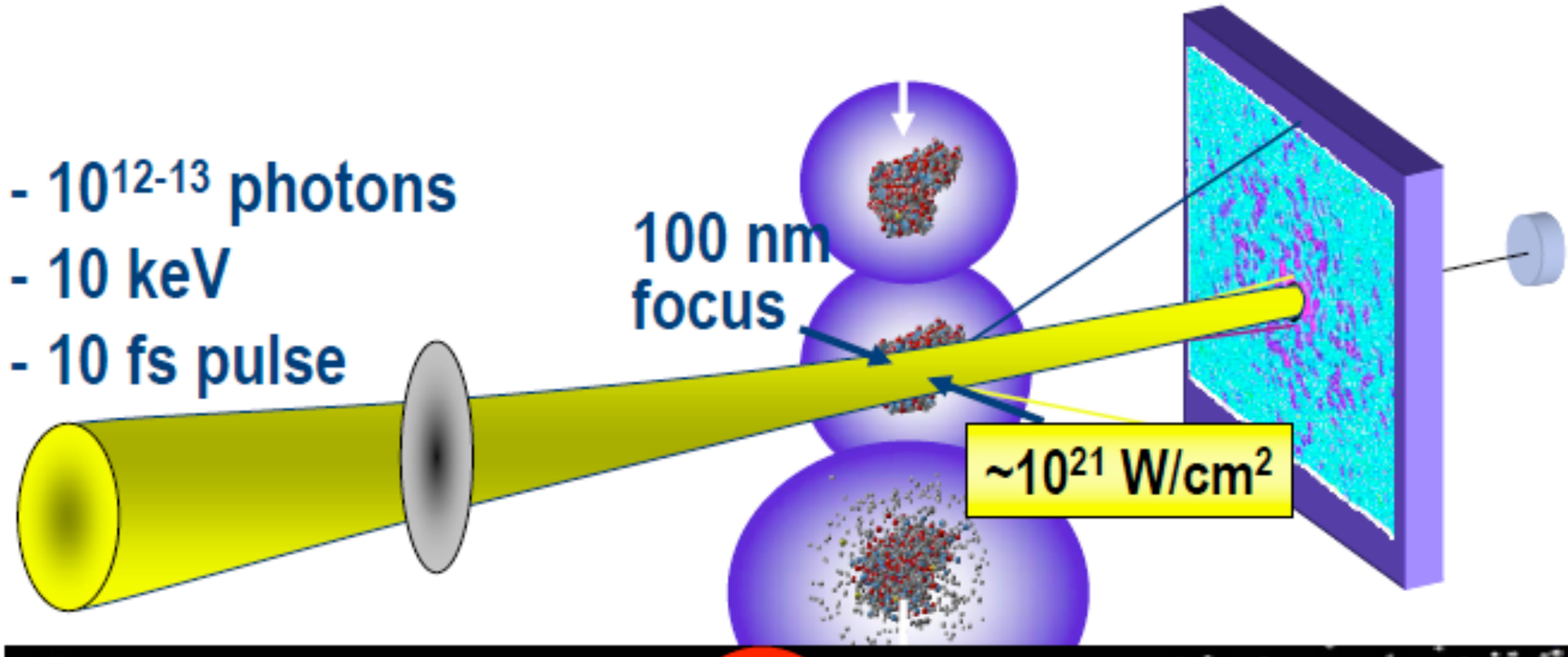
Chapman et al
Nature 470: 73 (2011)

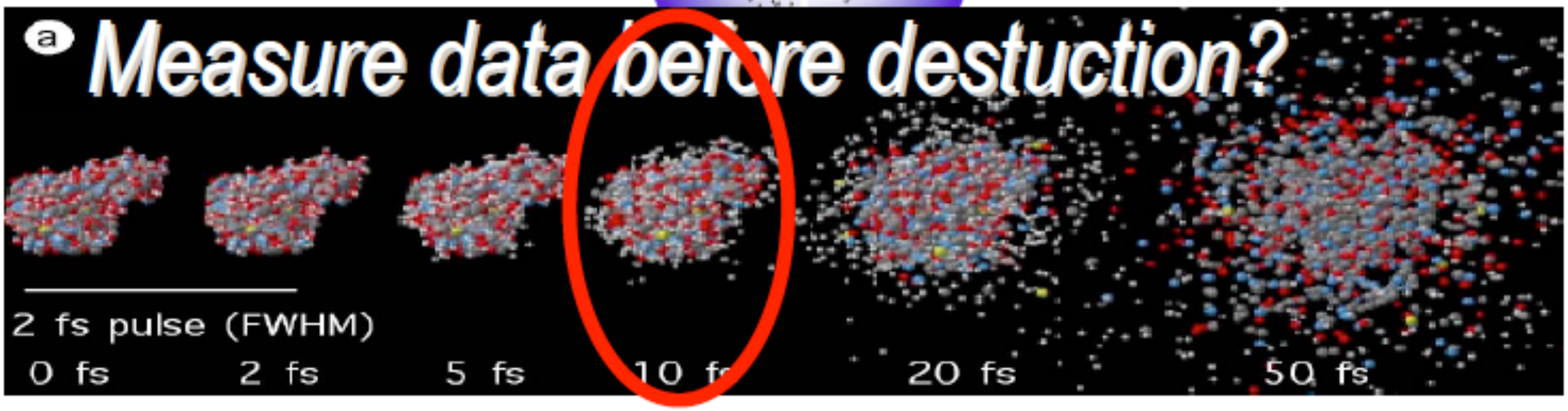


Gas focussed liquid jet:
4μm diam., flow rate 10- 14 μl min, 10 ms/s
De Ponte et al., *J. Phys. D* **41**, 195505 (2008)

Coherent Diffractive Imaging

- $10^{12\text{-}13}$ photons
- 10 keV
- 10 fs pulse



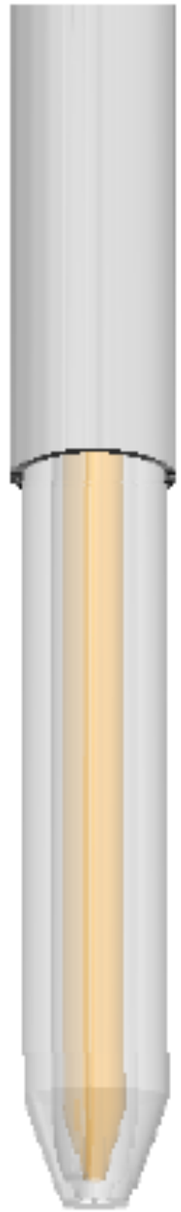


Calculations in vacuum, Neutze et al., Nature 2000

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?