Structural Analysis
Part III - X-ray tools

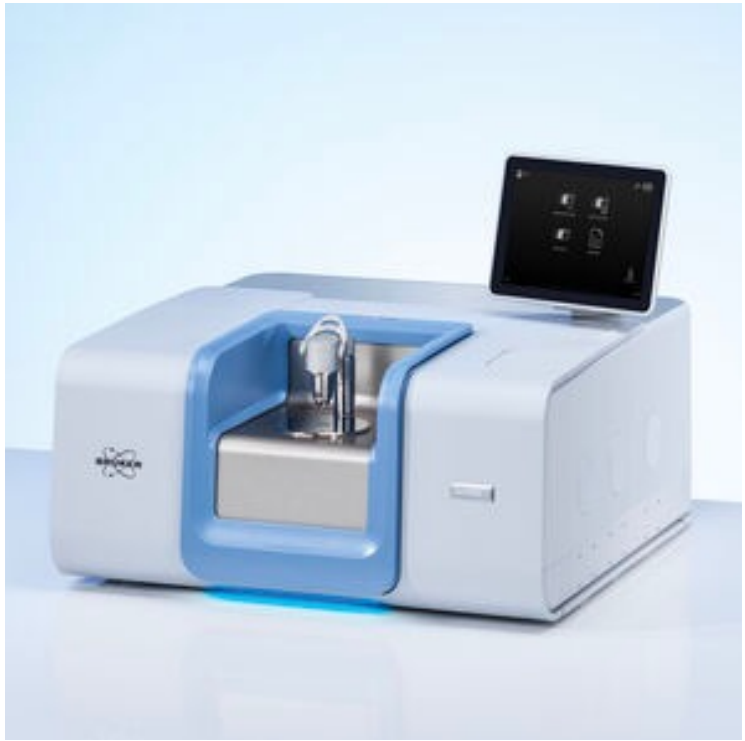
Session 3

X-ray scattering and diffraction

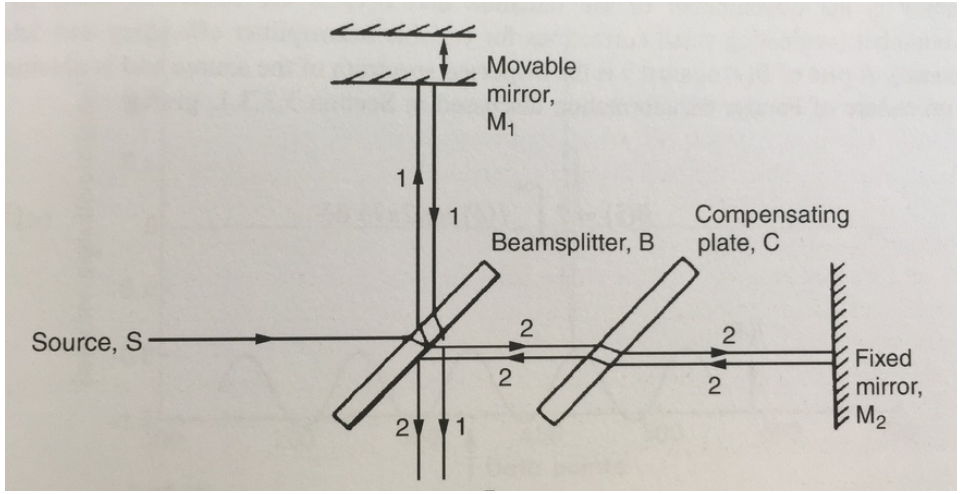
Intermezzo: Fourier Transform

Alternative approach: Fourier Transform (Infrared) Spectrometer)

- Not a dispersive measurement but based on interferometry
- Use all wavelength at the same time
- Manipulation in x - cm (real space) to get information in wave numbers x^{-1} – cm^{-1} (frequencies)
- Measure interferogram, Fourier transform into spectrum

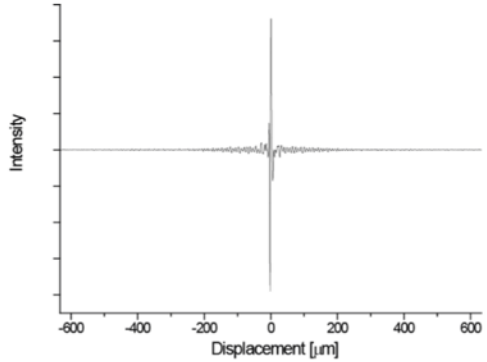


Measuring an interferogram



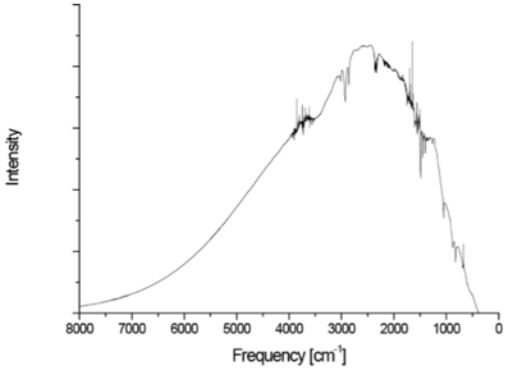
Sample

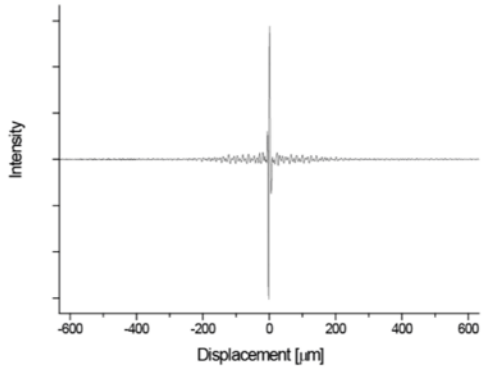
Detector



Empty Cell

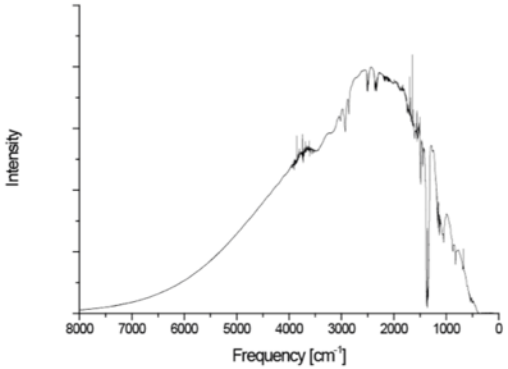
FT



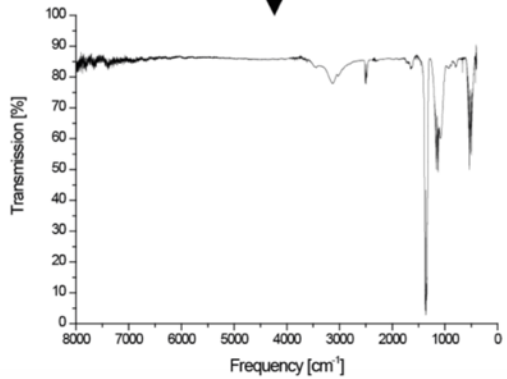


Filled Cell

FT



Divide



Prof. Emsley part of this class, 5 weeks ago!

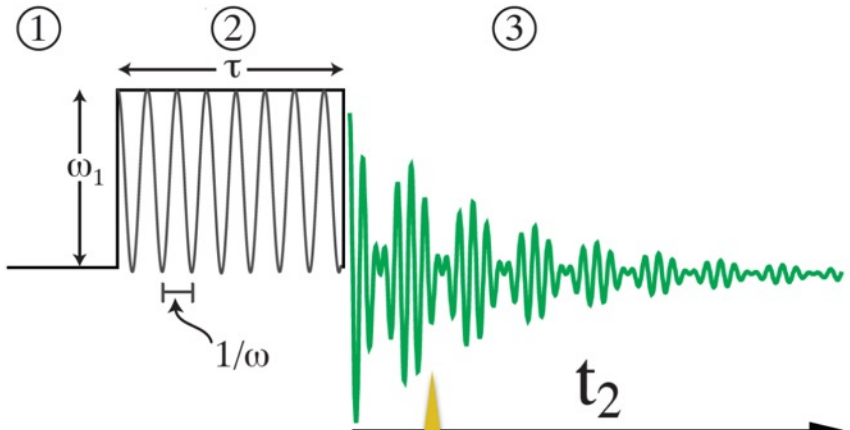
Pulsed FTNMR Spectroscopy

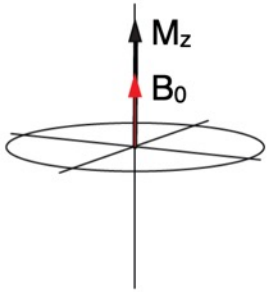
$\omega_1 = -\gamma B_1$

$\omega_1 \tau = \pi/2$

ω = carrier frequency, chosen by the operator to be near to the resonance frequencies

^{1}H





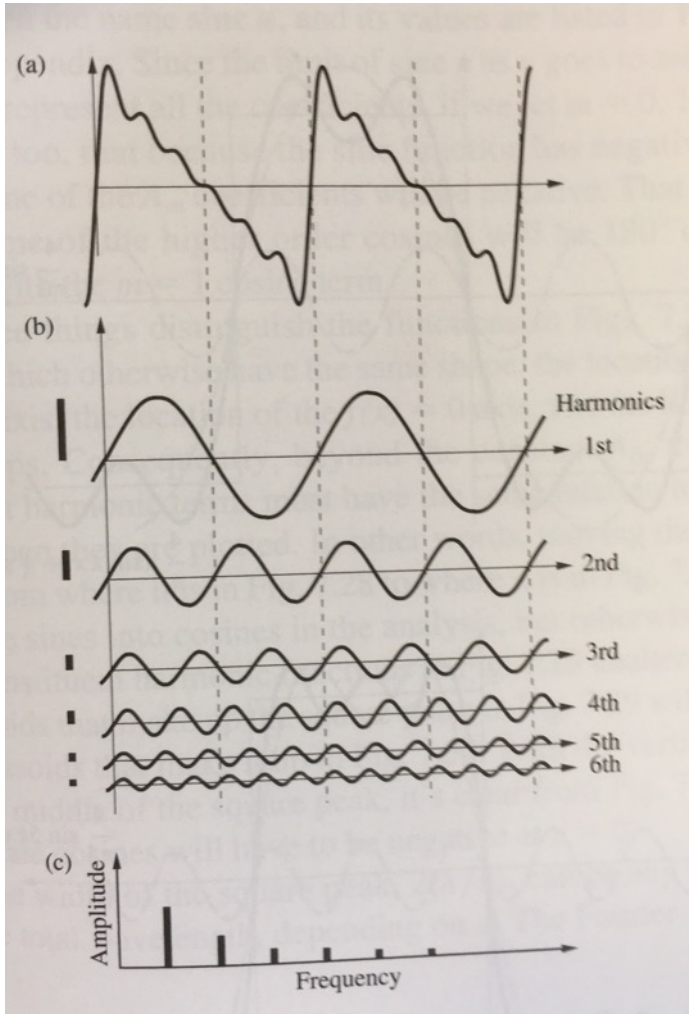
what does the signal actually look like?

1. Equilibrium. The net magnetization is aligned along the direction of the main field (z-axis).

2. A field is applied in the transverse plane. The magnetization of the ensemble precesses around the field.

3. The field is removed leaving a net transverse component of the ensemble magnetization. This ***coherence*** then starts to precess around the main field.

Principle idea



Mathematical description

- The principle idea of the Fourier analysis / transformation is that any function can be represented by an (infinite) series of harmonic functions.
- The Fourier transform decomposes a function into its constituent frequencies.

Thus we can write

$$f(x) = \frac{1}{2\pi}\int_{-\infty}^{+\infty} F(k)e^{-ikx}\,dk$$

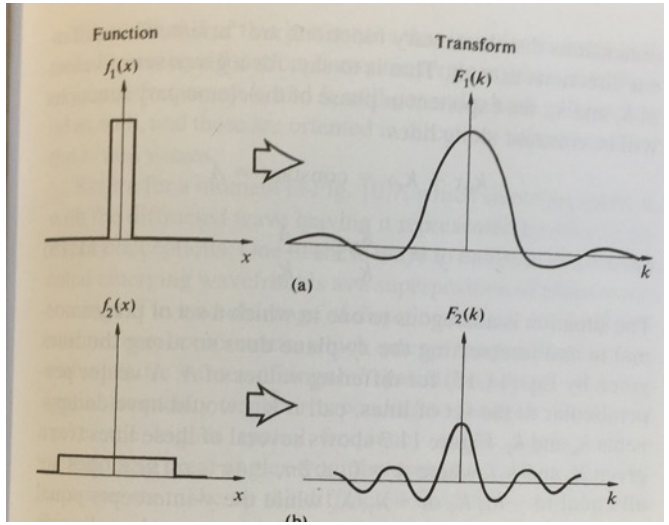
provided that

$$F(k) = \int_{-\infty}^{+\infty} f(x)e^{ikx}\,dx$$

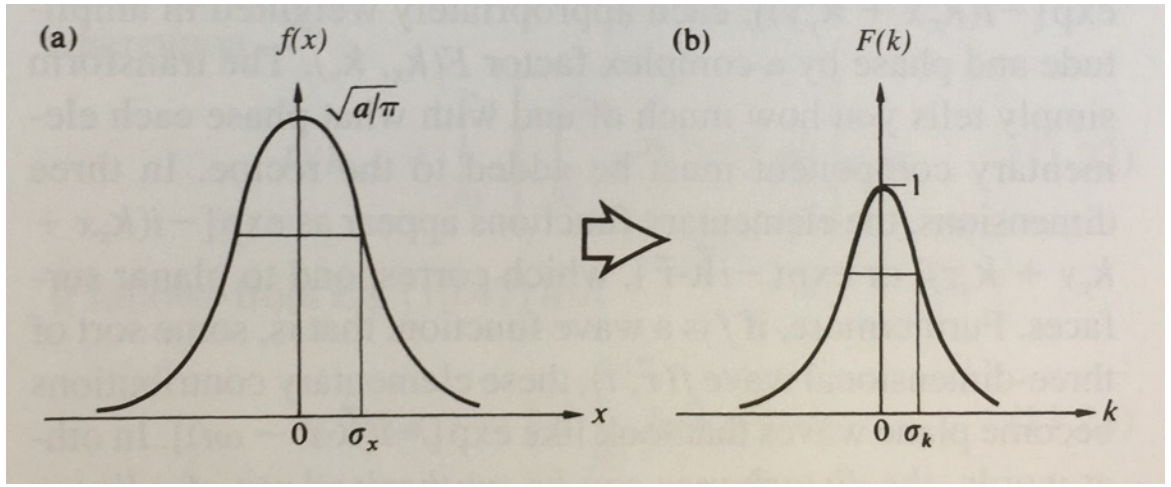
Hecht chapter 11

Examples

Square functions

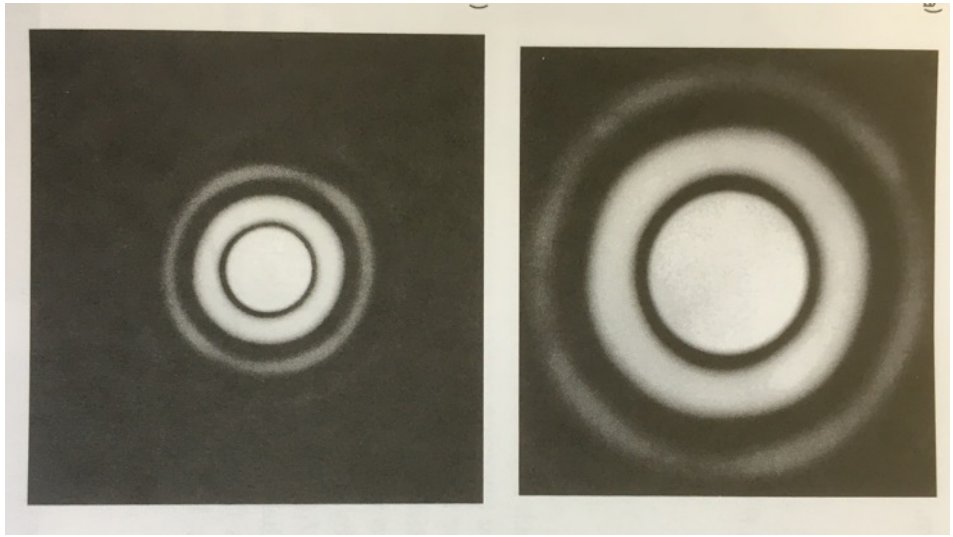


Gauss function

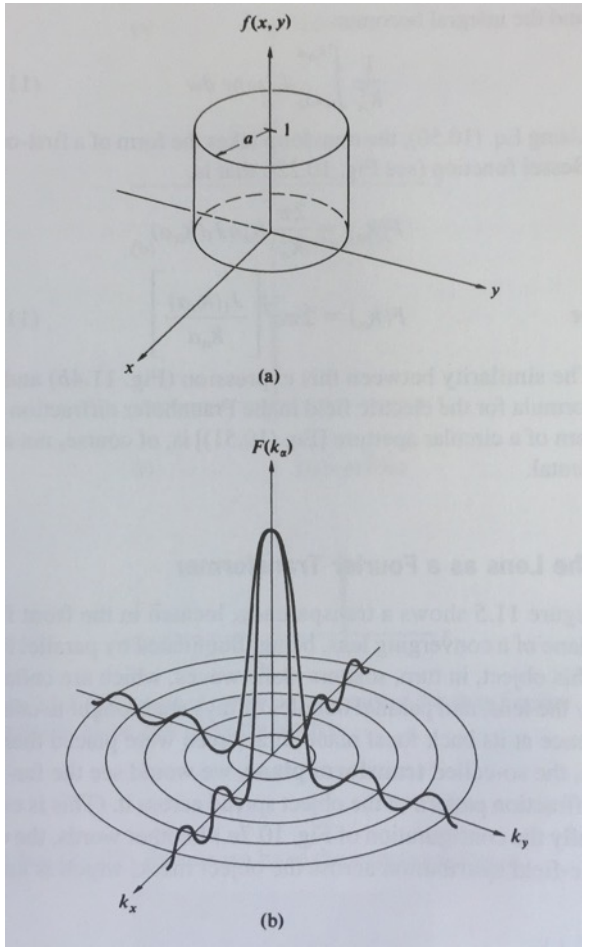


Fourier transform of round aperture and airy pattern

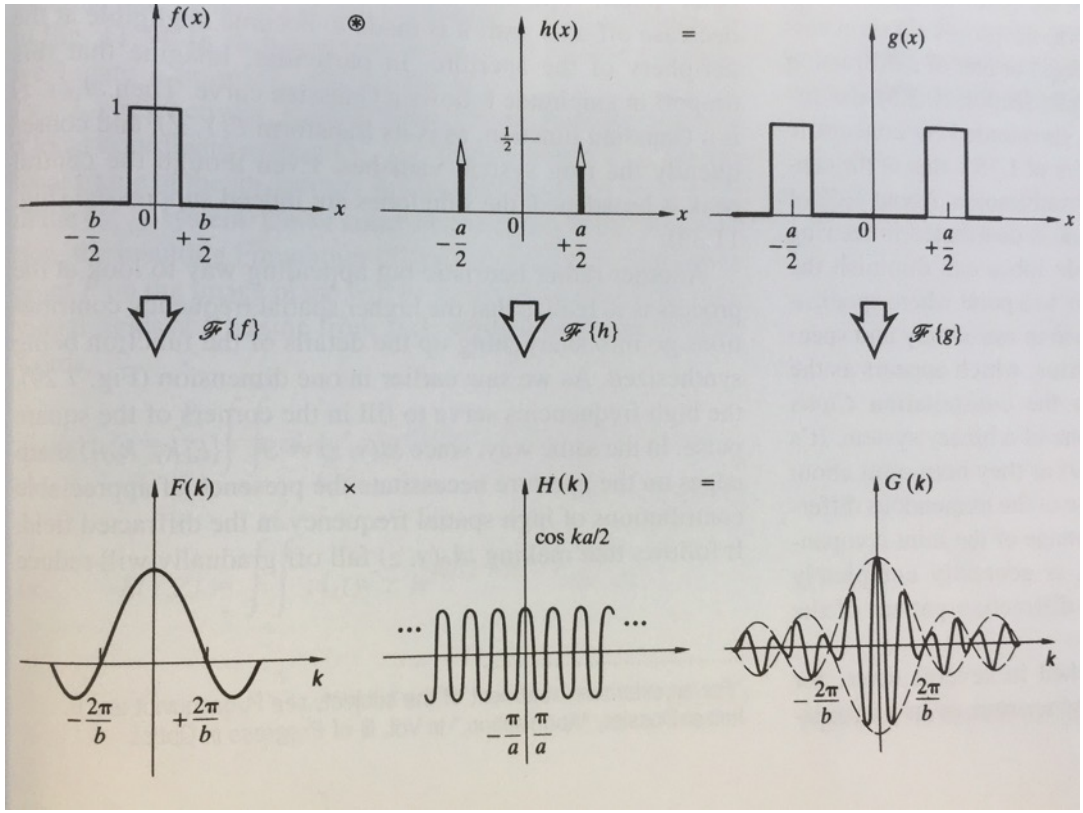
Experiment: Diffraction pattern of circular aperture, Airy pattern



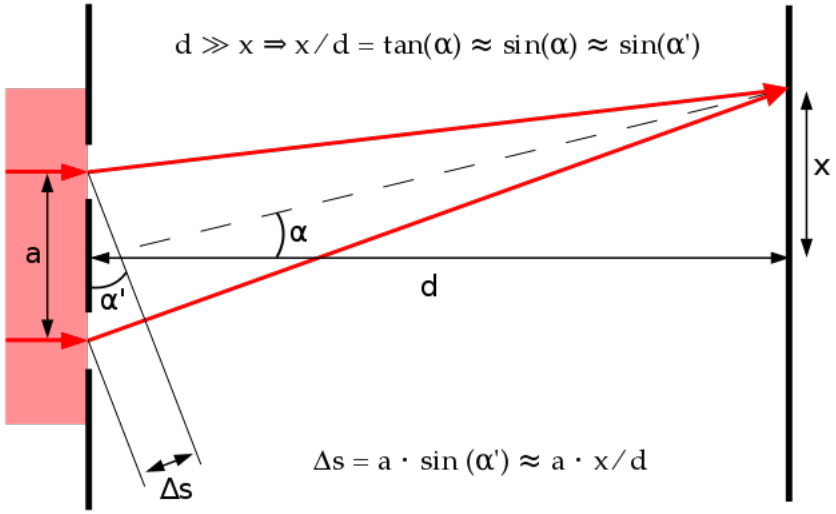
Theory: Fourier transform of cylinder or “top-hat” function



Diffraction as Fourier transform:



The double slit



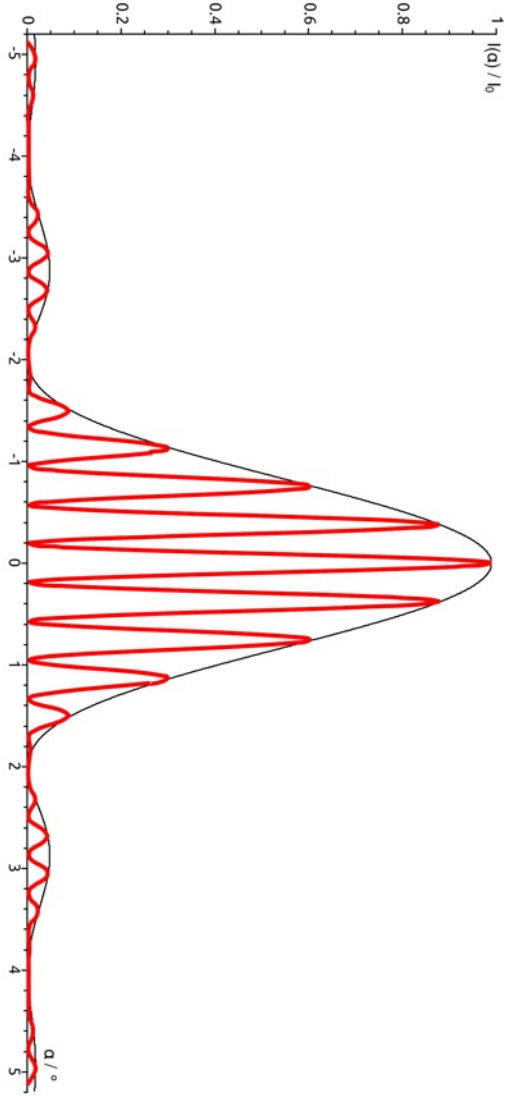
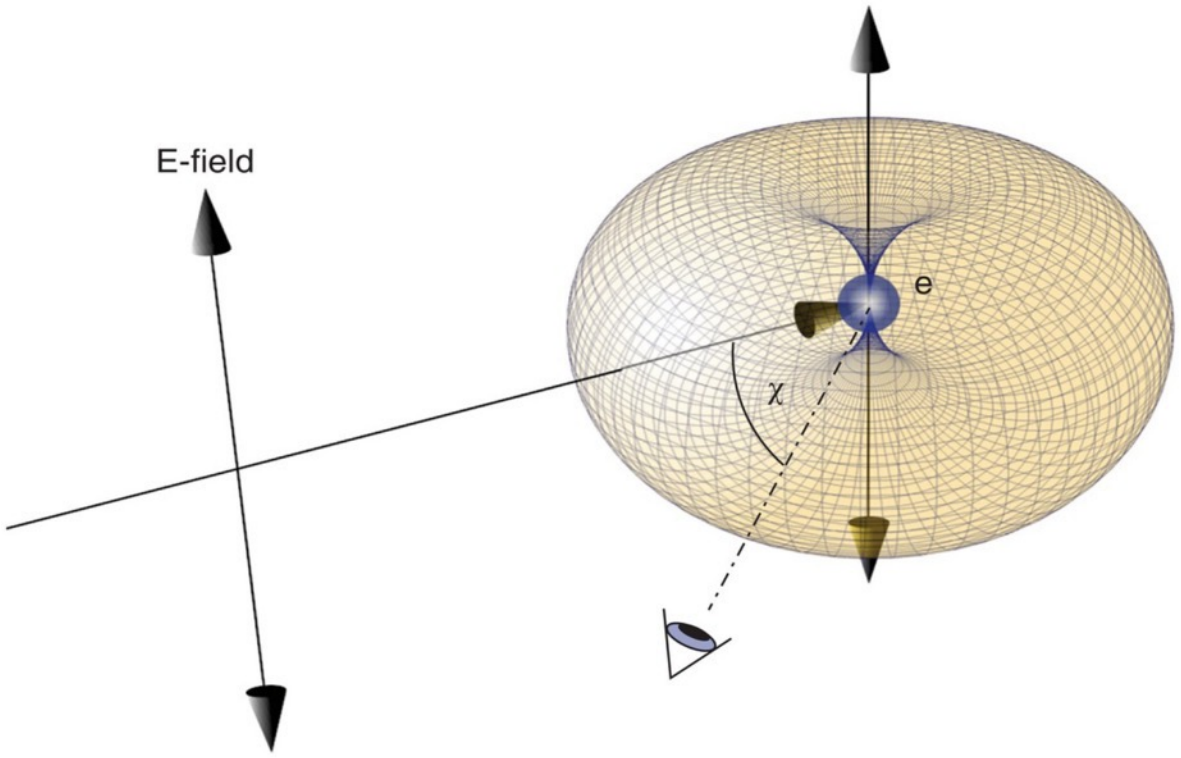


Image source: wikipedia

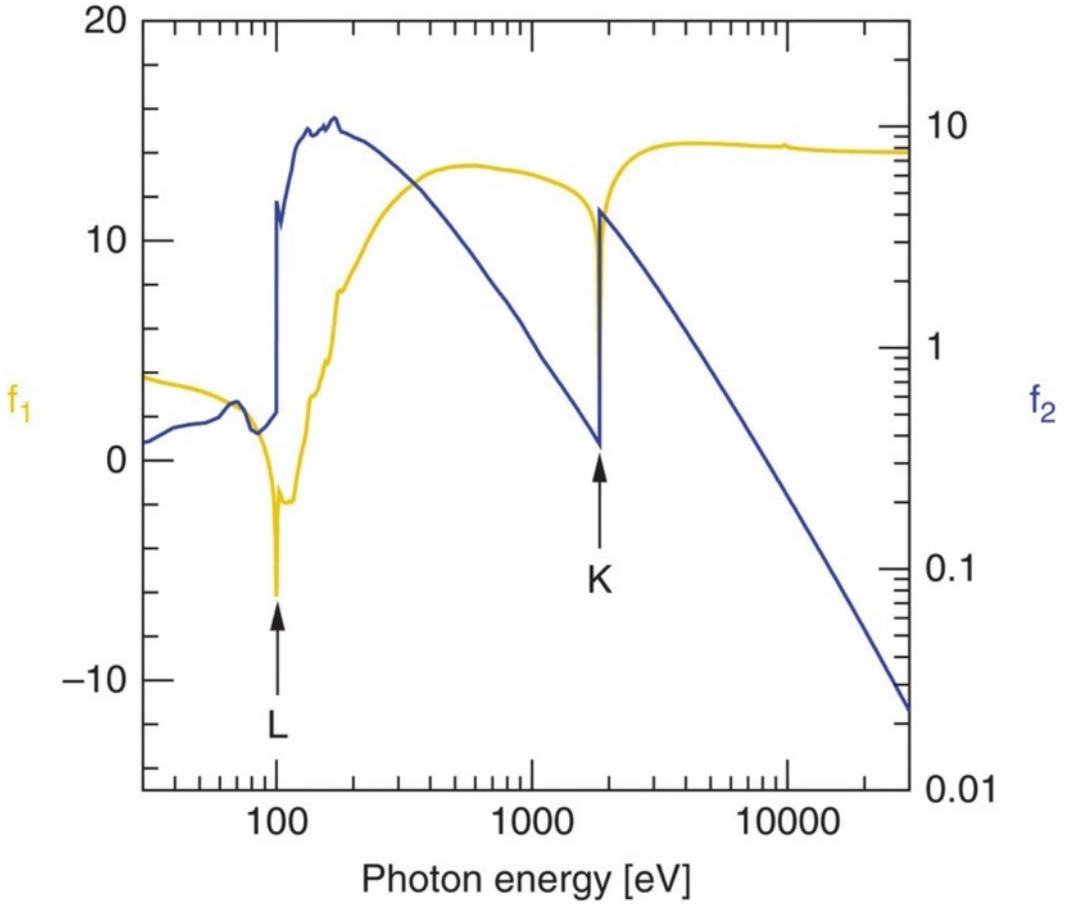
Fourier relationships – some examples

From Session 1

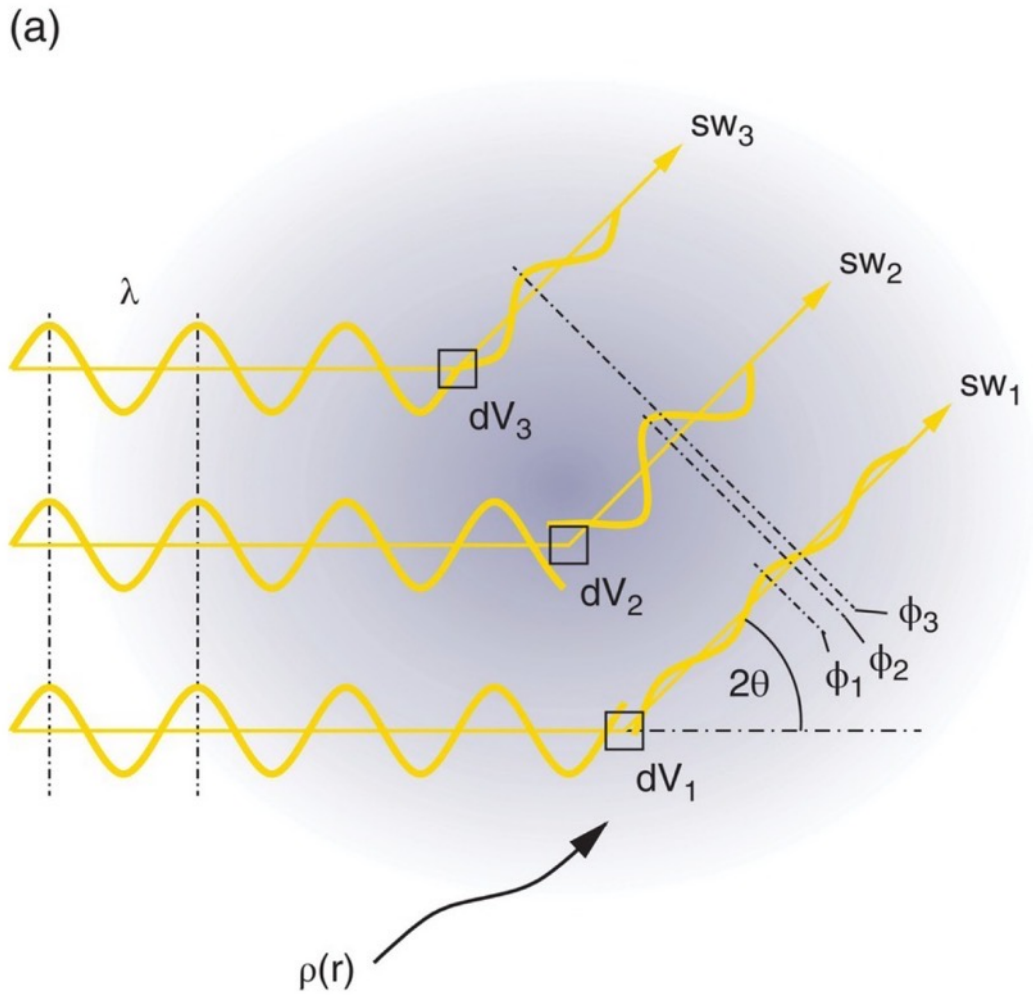
Thomson scattering from a single electron

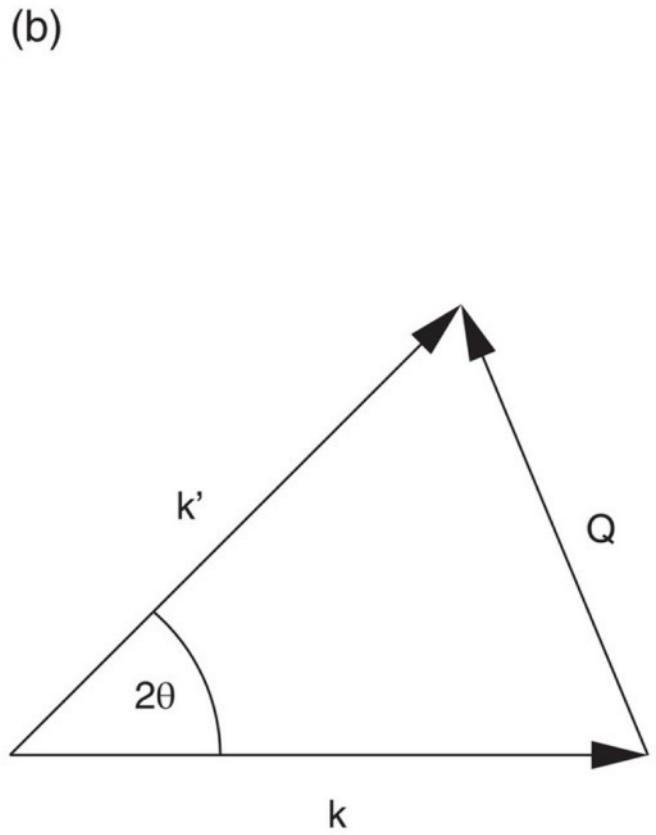


Atomic scattering factors and refractive index



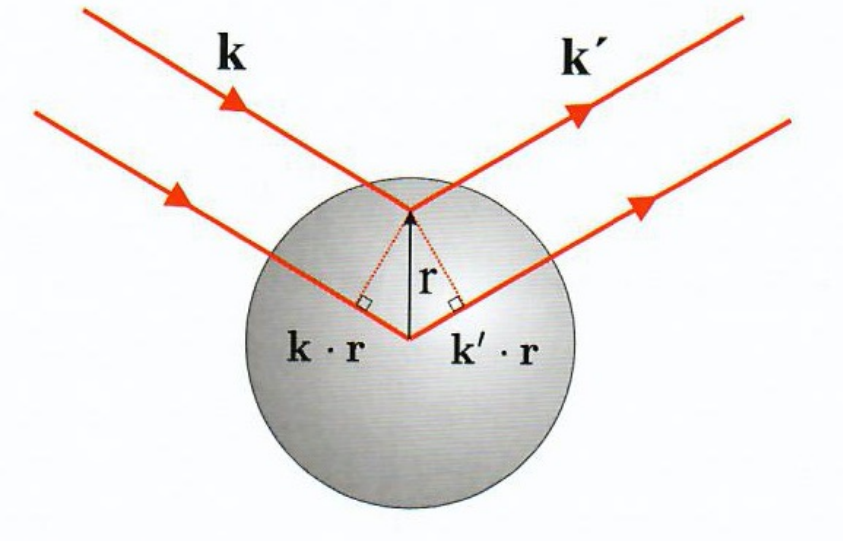
Definition of the scattering vector Q



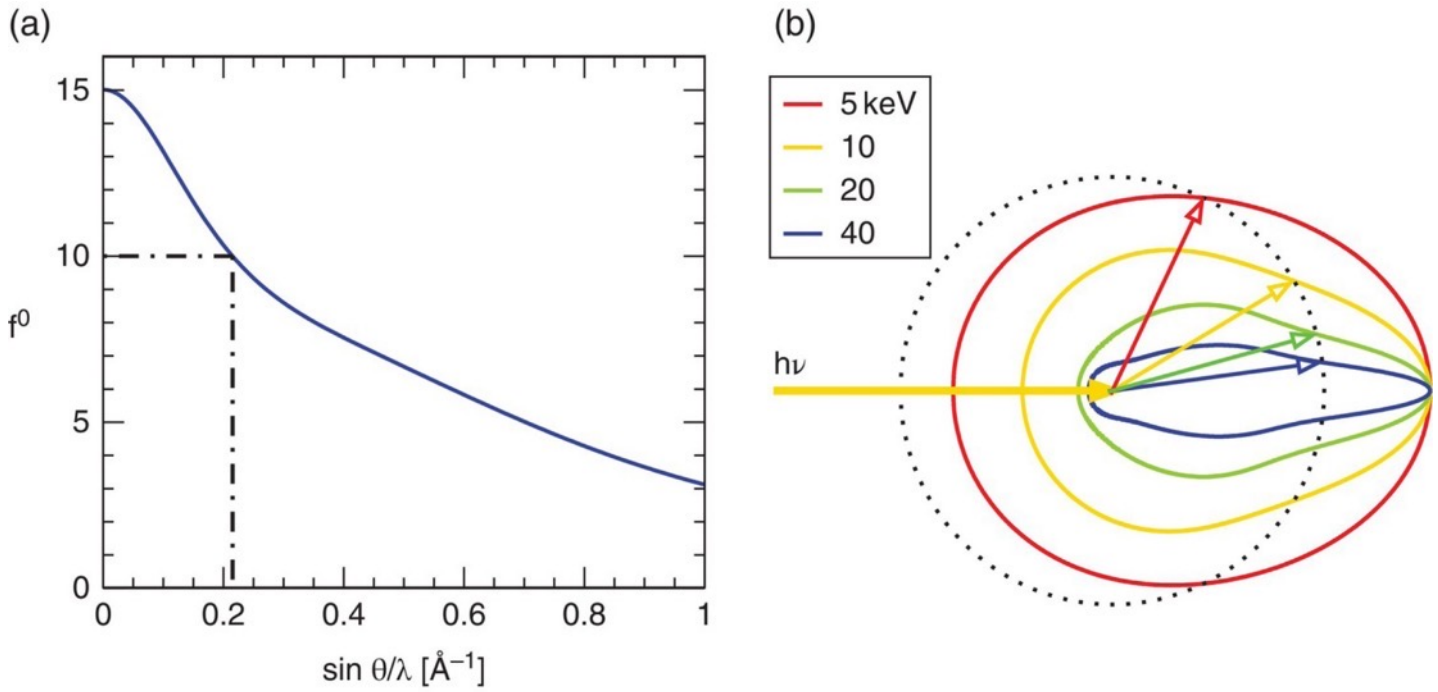


$$|k| = |k'| = 2\pi/\lambda$$

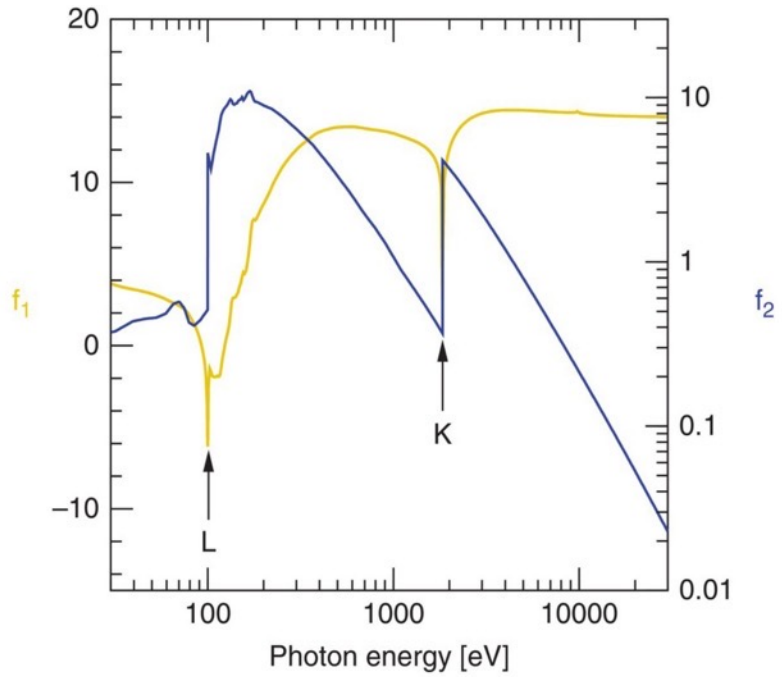
Scattering from an electron cloud

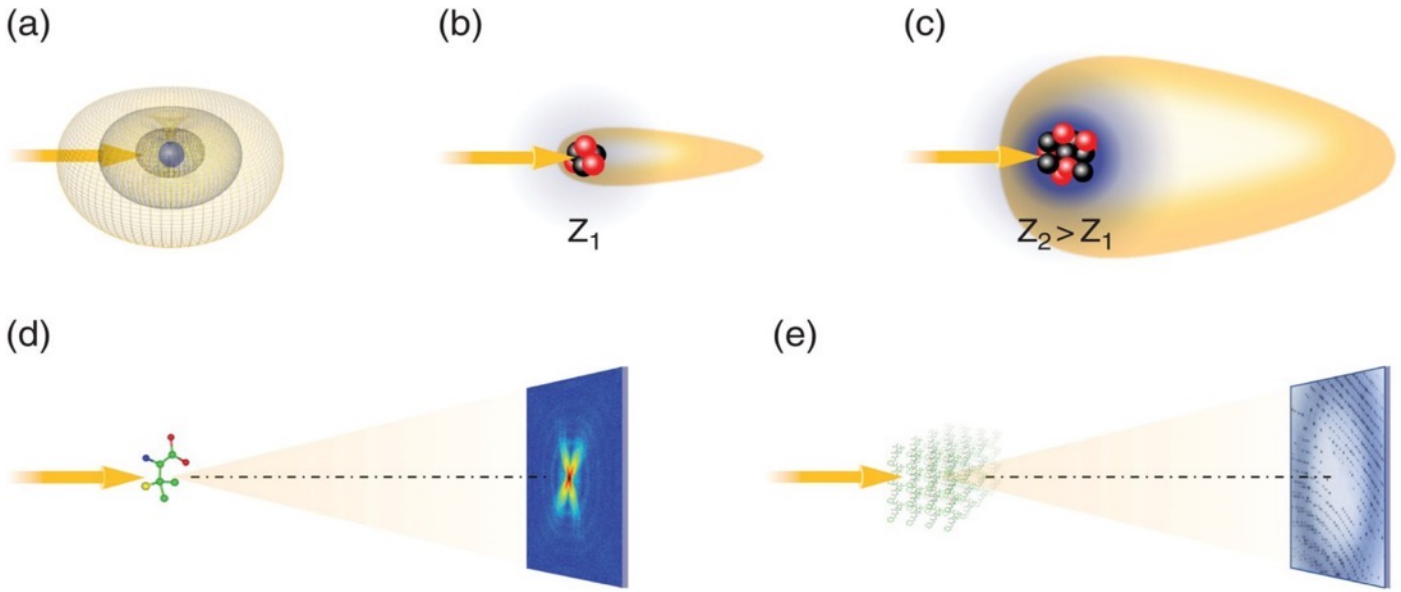


Now full cross section / atomic scattering factors



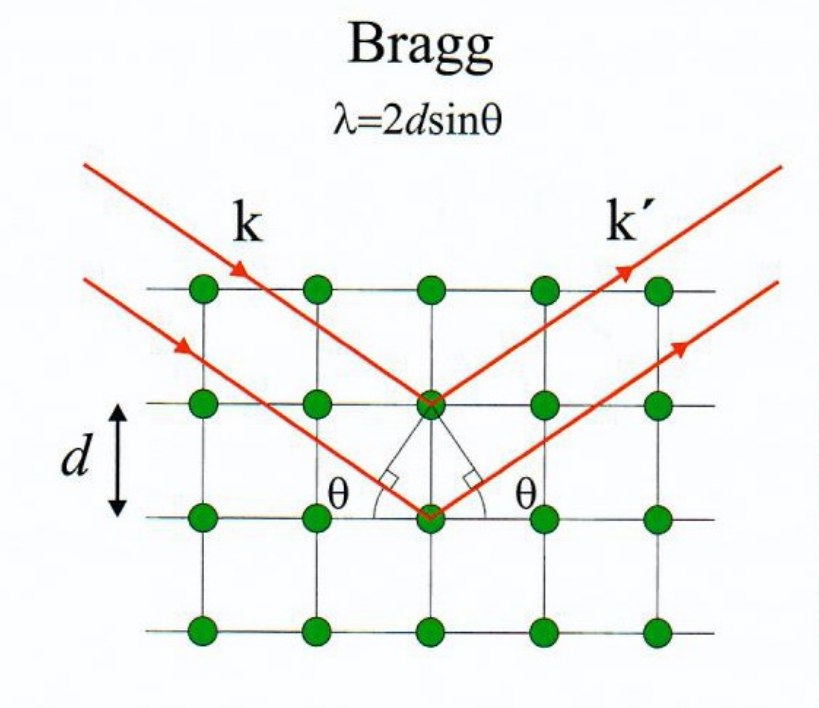
Atomic scattering factors again

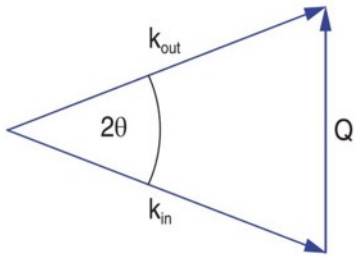




From Session 2

Bragg scattering





The Nobel Prize in Physics 1915

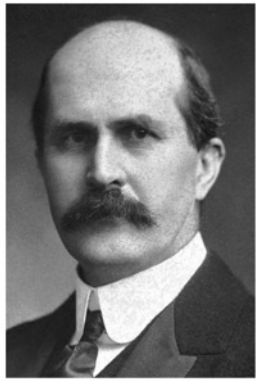


Photo from the Nobel Foundation archive.

Sir William Henry Bragg

Prize share: 1/2

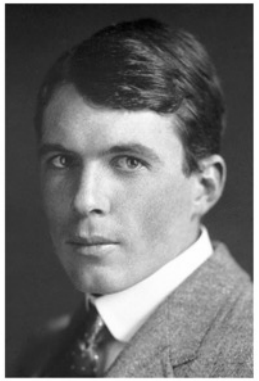
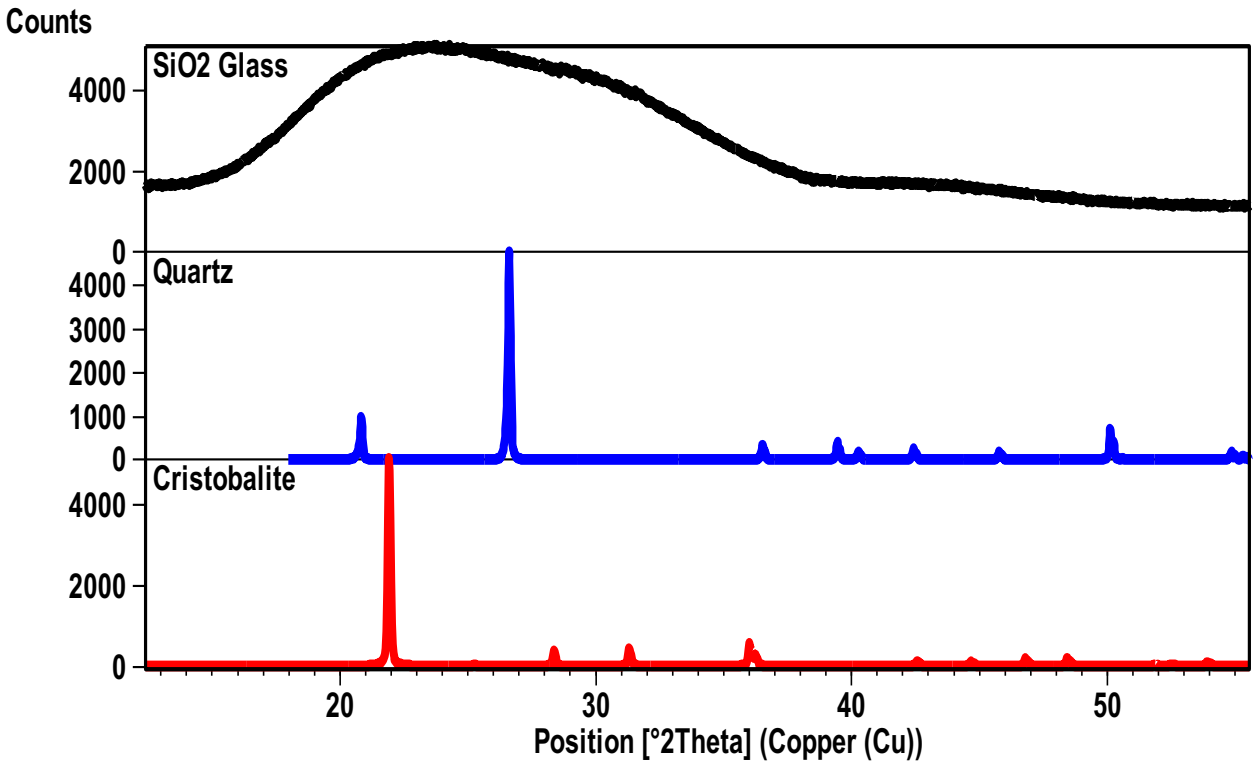


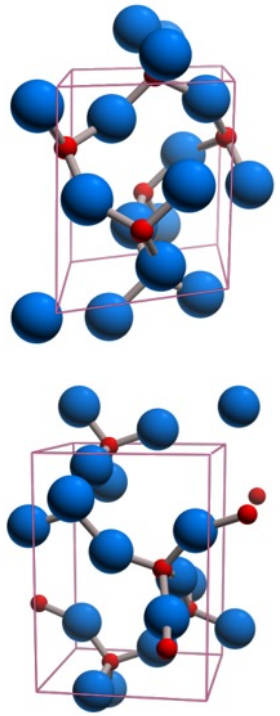
Photo from the Nobel Foundation archive.

William Lawrence Bragg

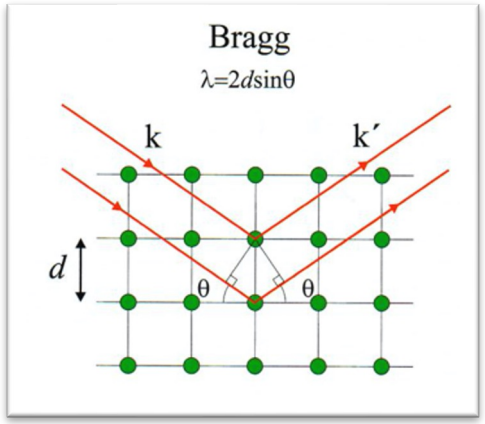
Prize share: 1/2

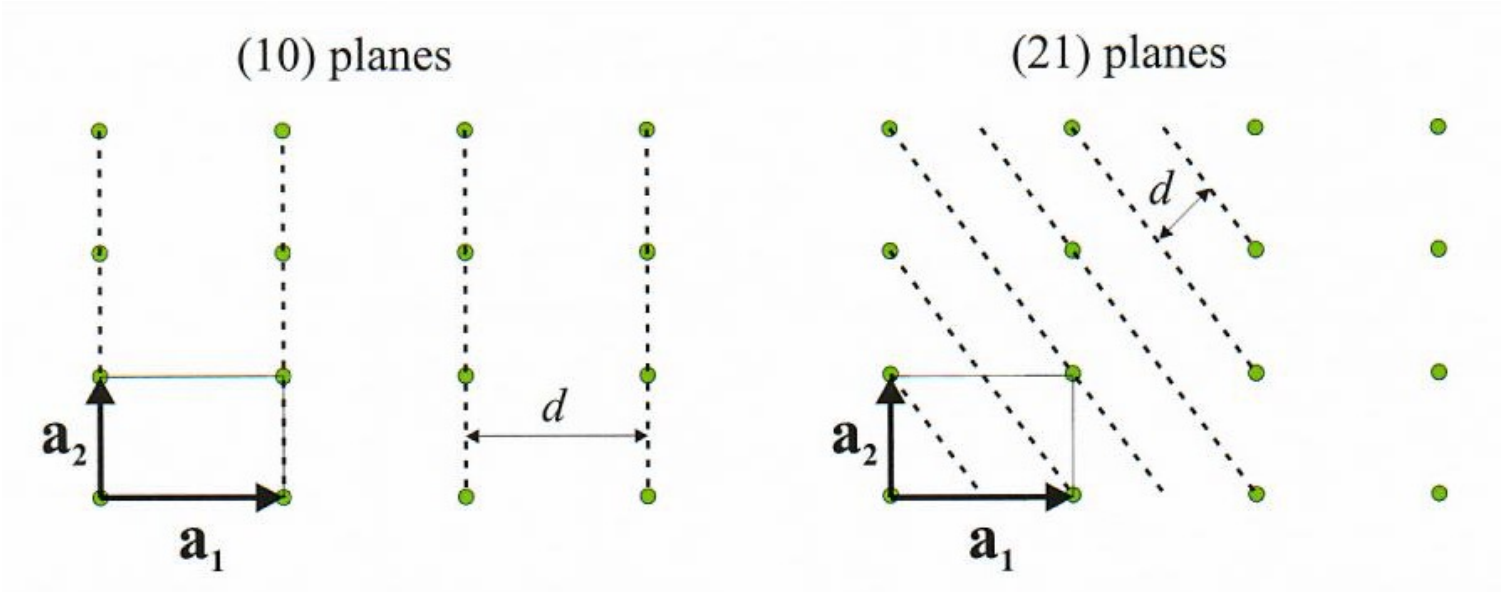
Characteristic signals from different – chemically identical – samples





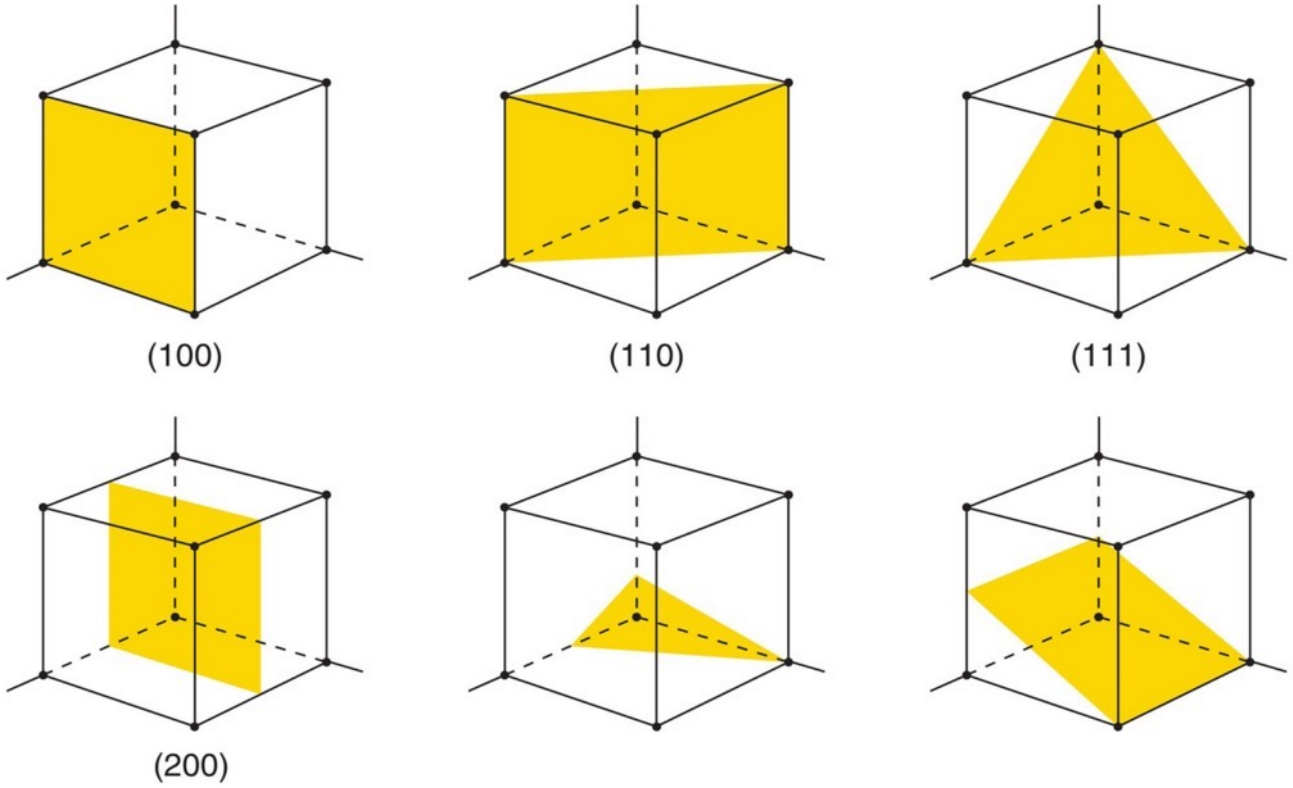
Lattice planes

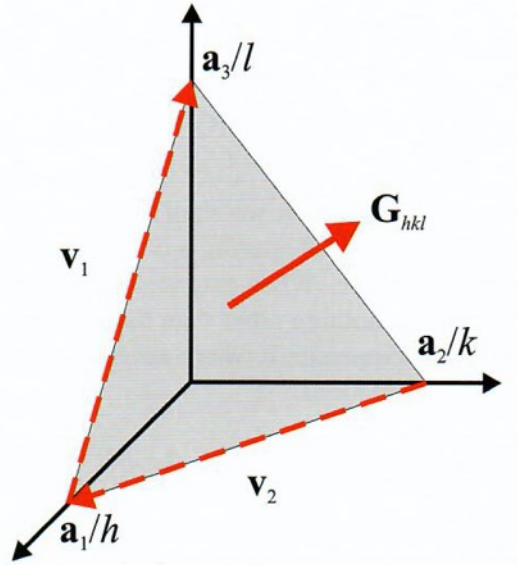




- Find the intercepts of the plane with the respective crystal axis
- Take the reciprocal of these numbers, reduce to smallest integer

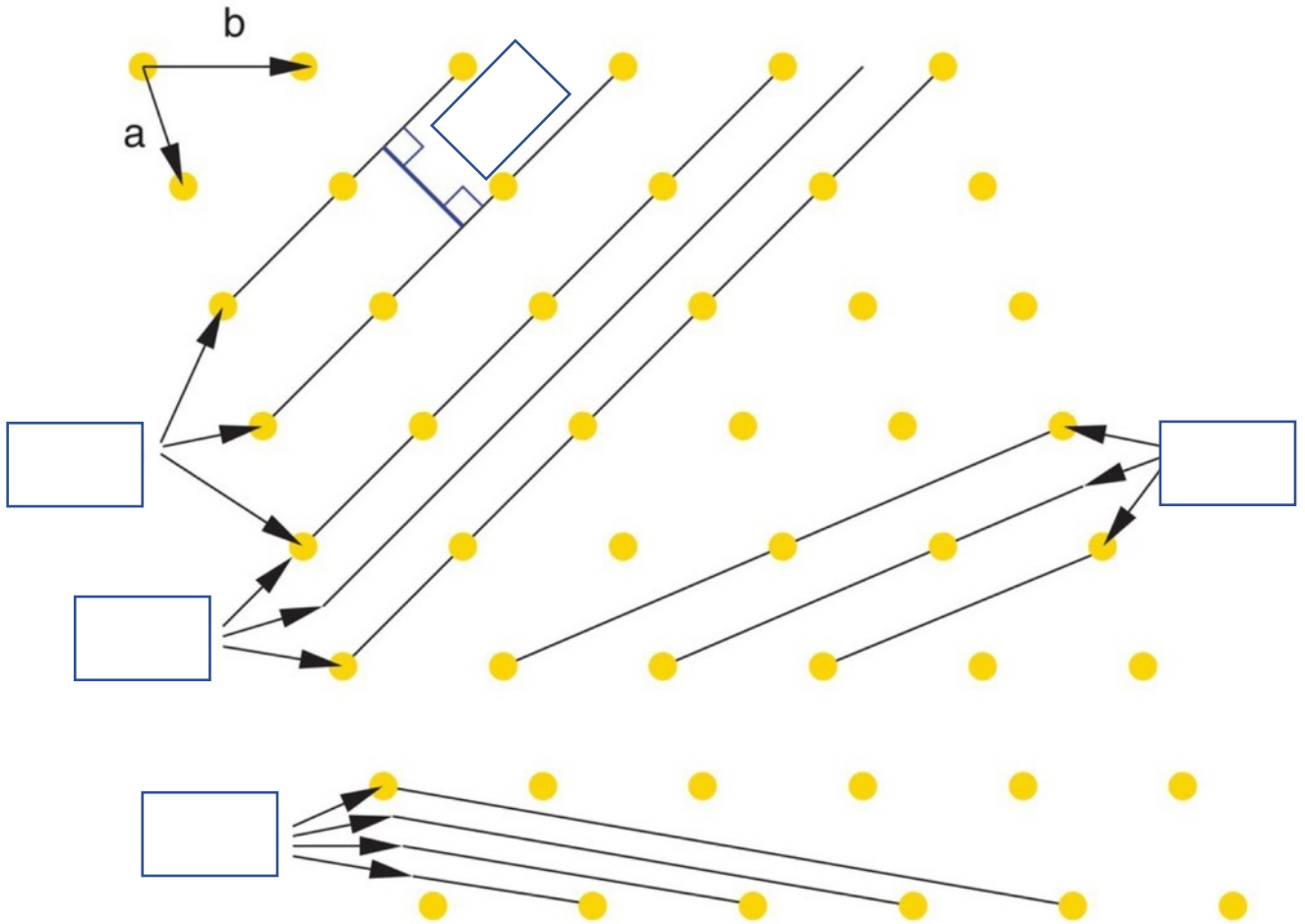
Miller indices





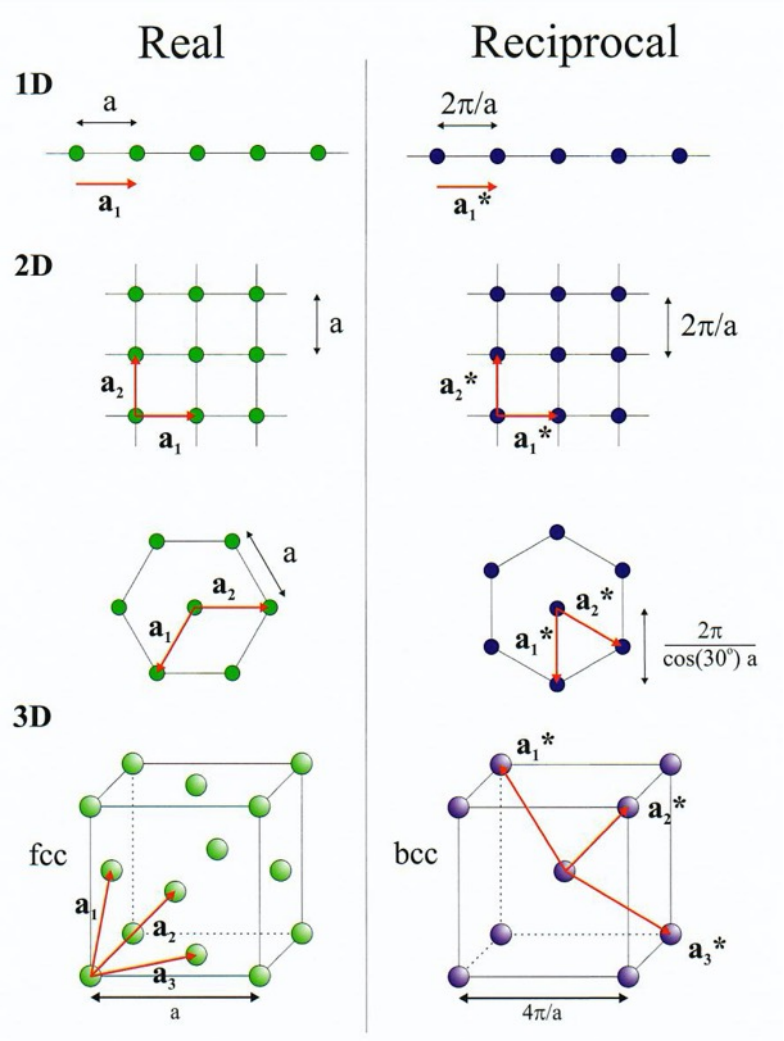
- Find the intercepts of the plane with the respective crystal axis
- Take the reciprocal of these numbers, reduce to smallest integer

Exercise: Identify lattice planes

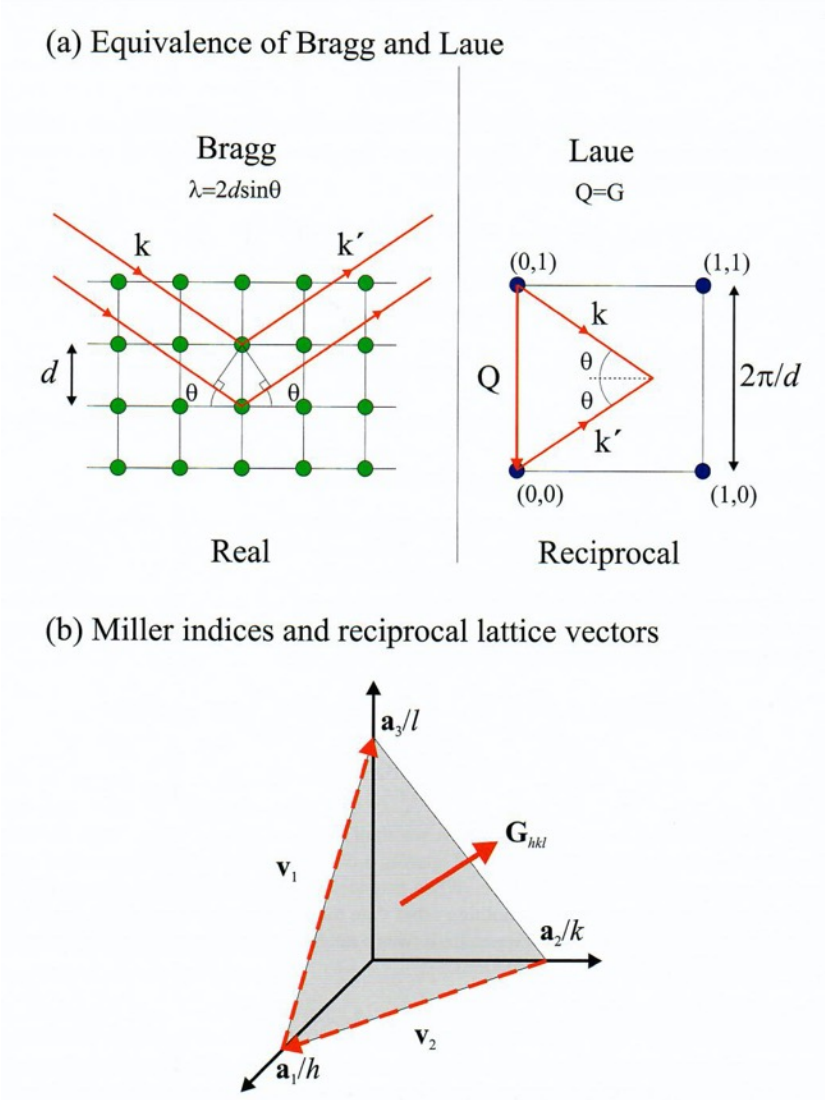


More on diffraction of single crystals

For each lattice a reciprocal lattice can be defined



Bargg and Laue conditions



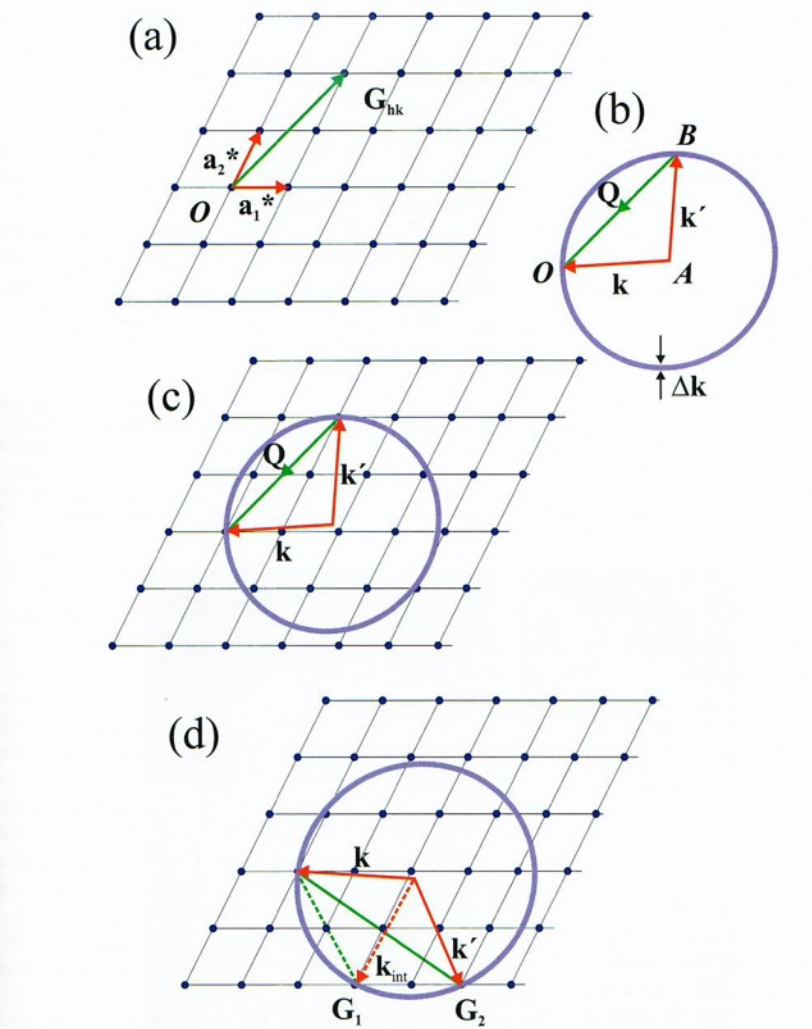
The Nobel Prize in Physics 1914

Photo from the Nobel Foundation archive.

Max von Laue

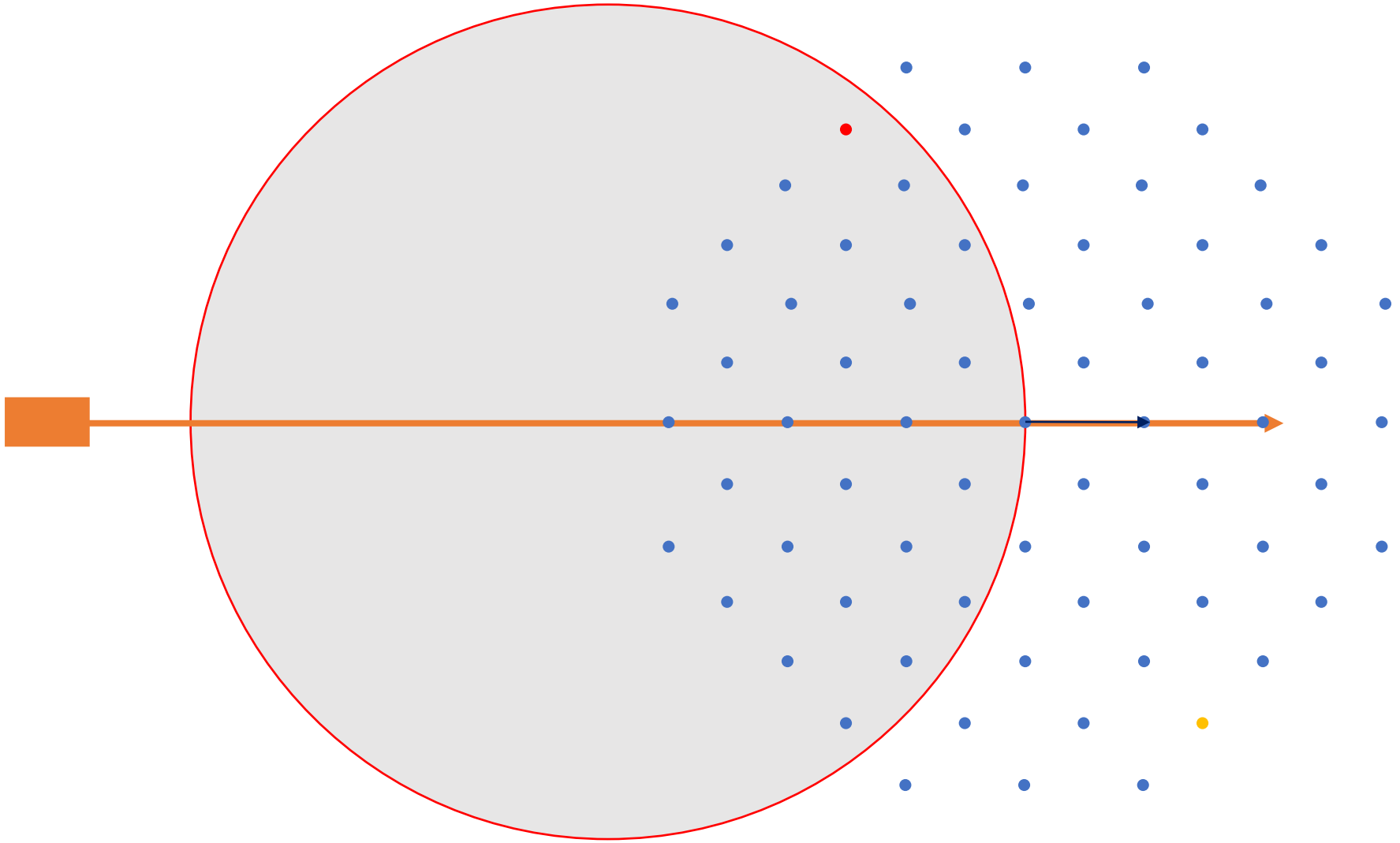
Prize share: 1/1

The Ewald Sphere

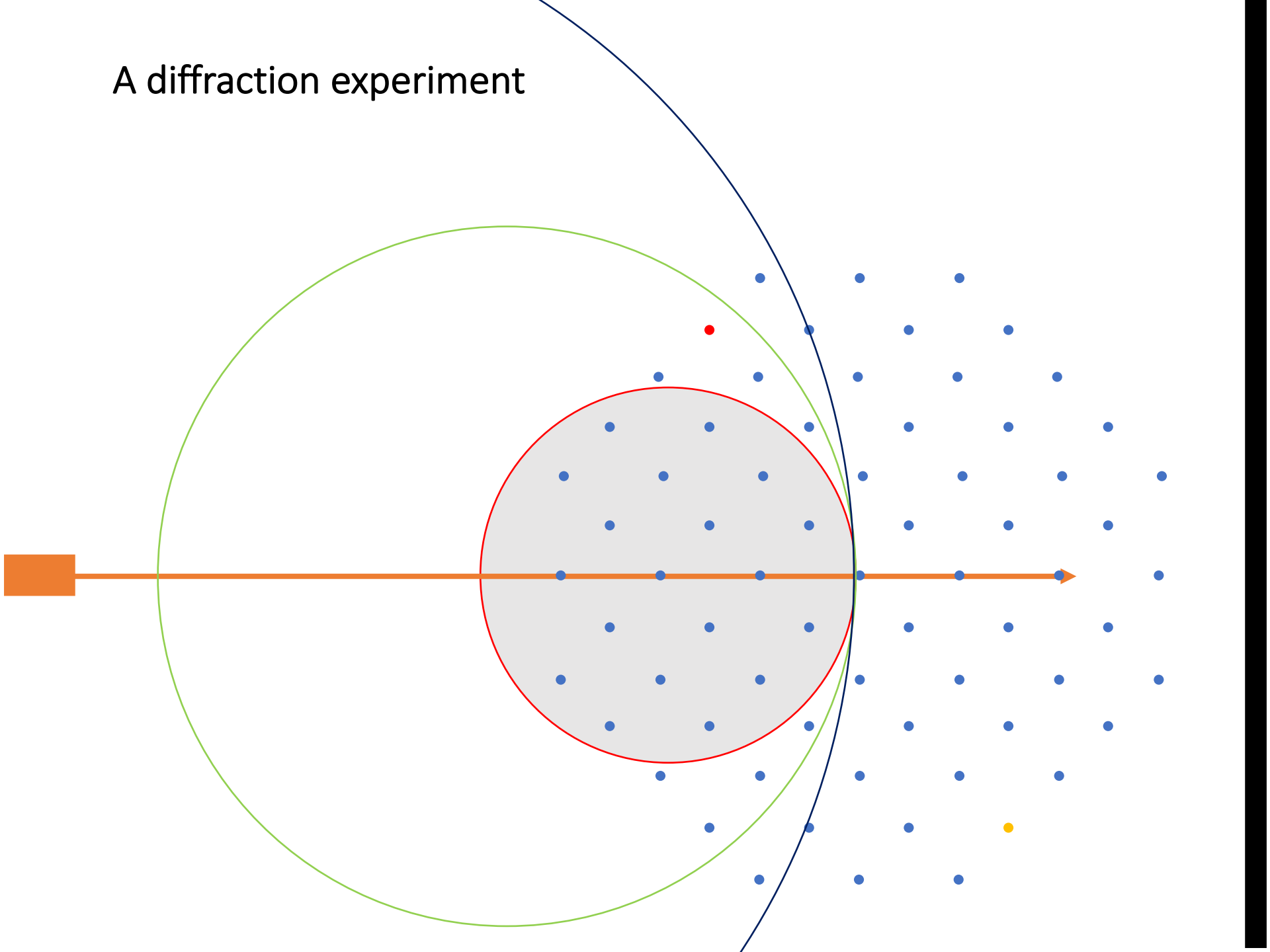


A Laue diffraction experiment

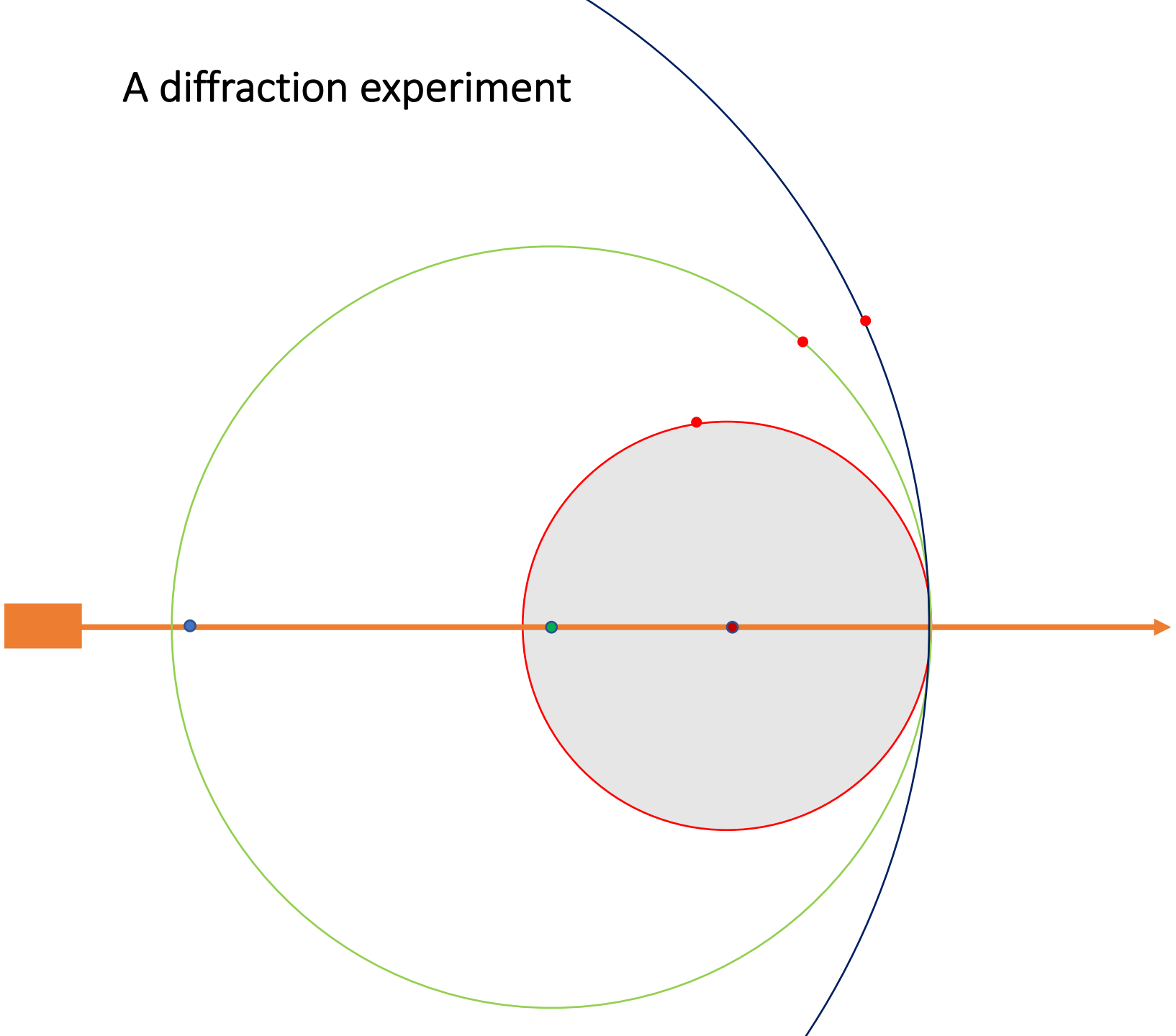
A diffraction experiment



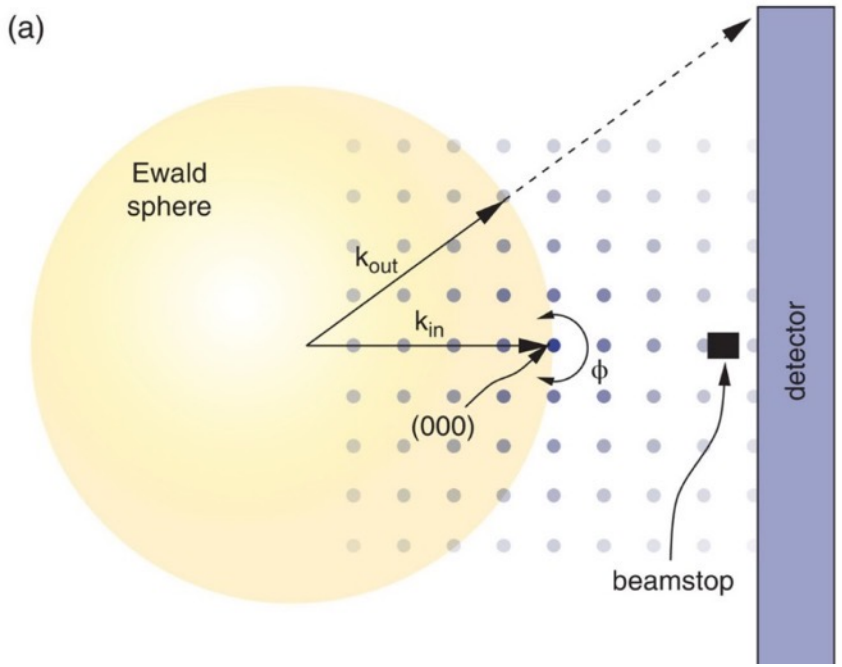
A diffraction experiment

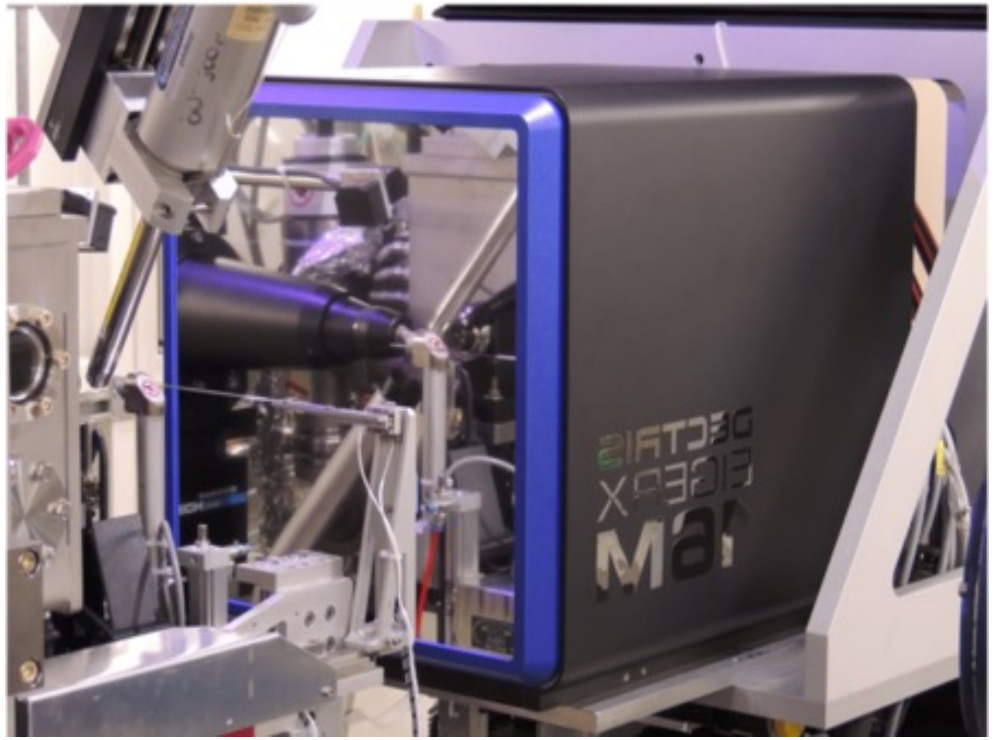


A diffraction experiment

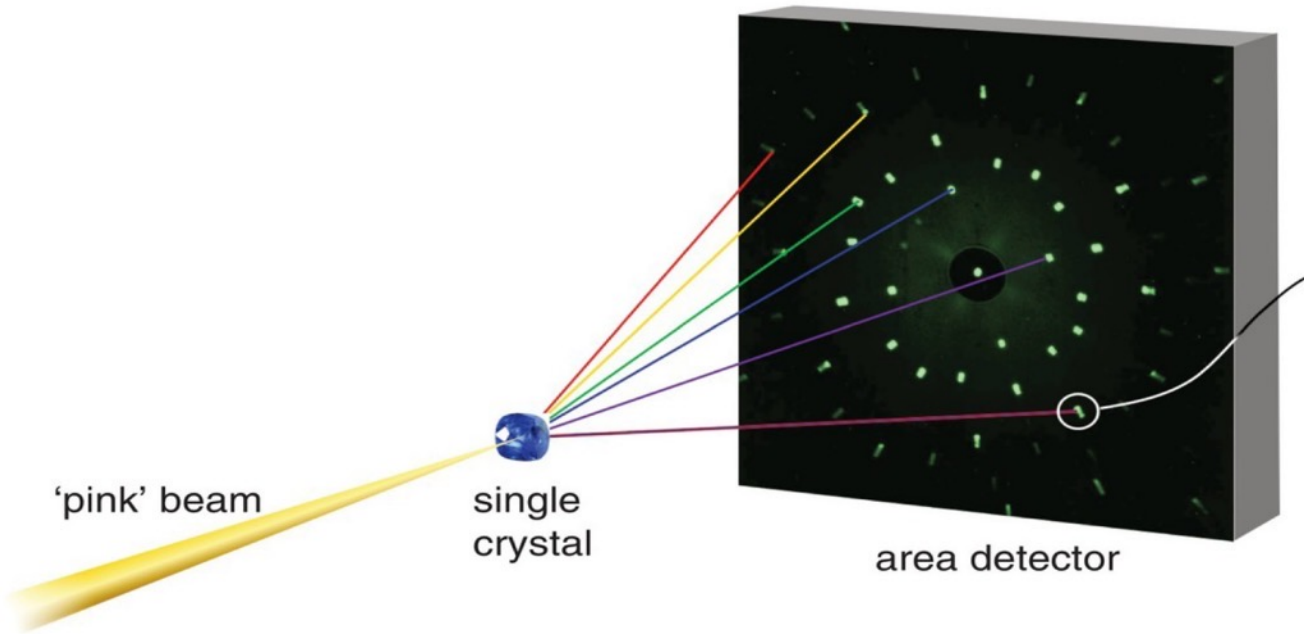


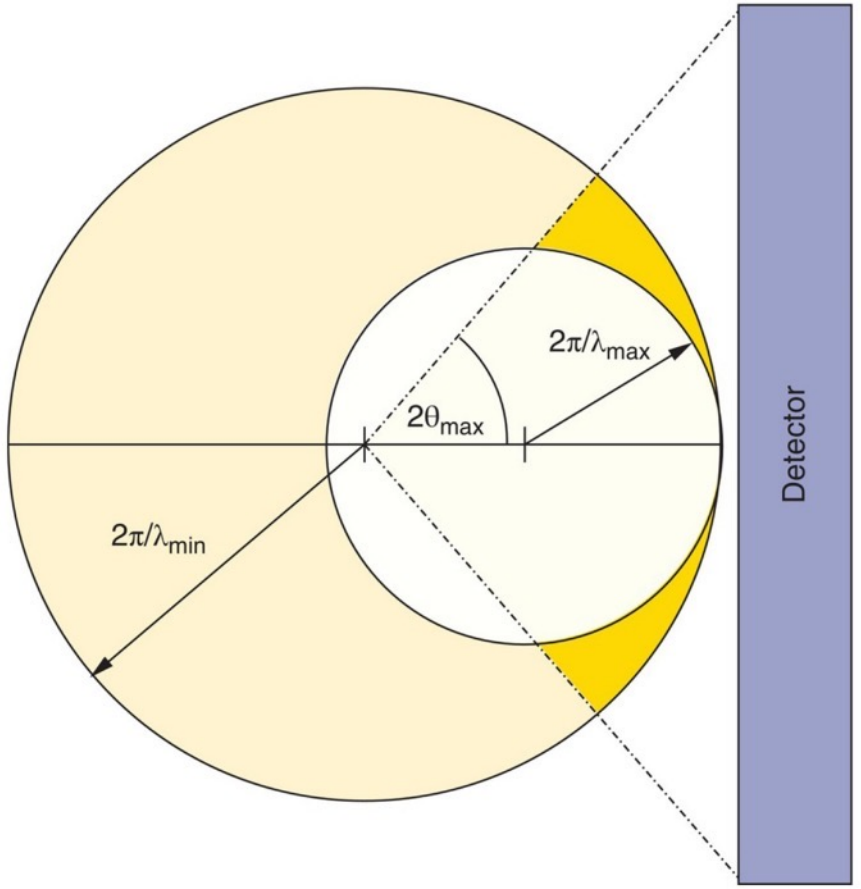
A diffraction experiment





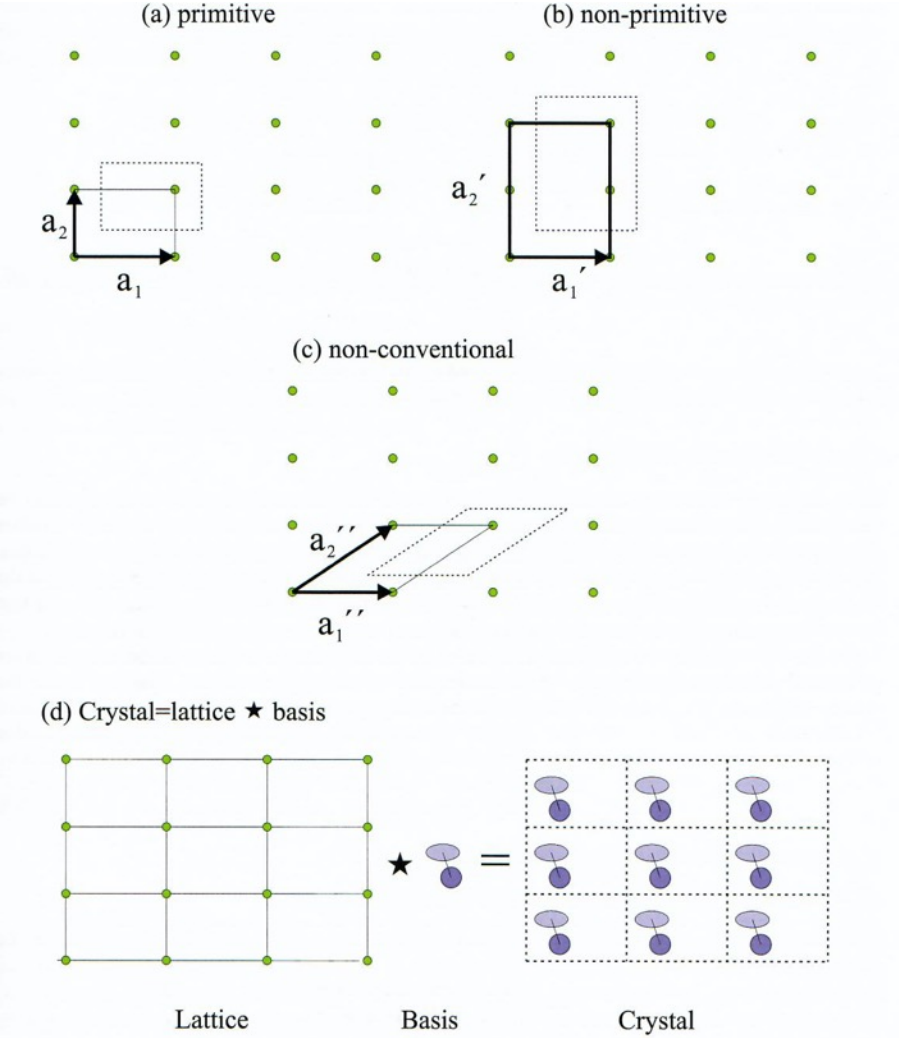
Laue method


'pink' beam
single
crystal
area detector

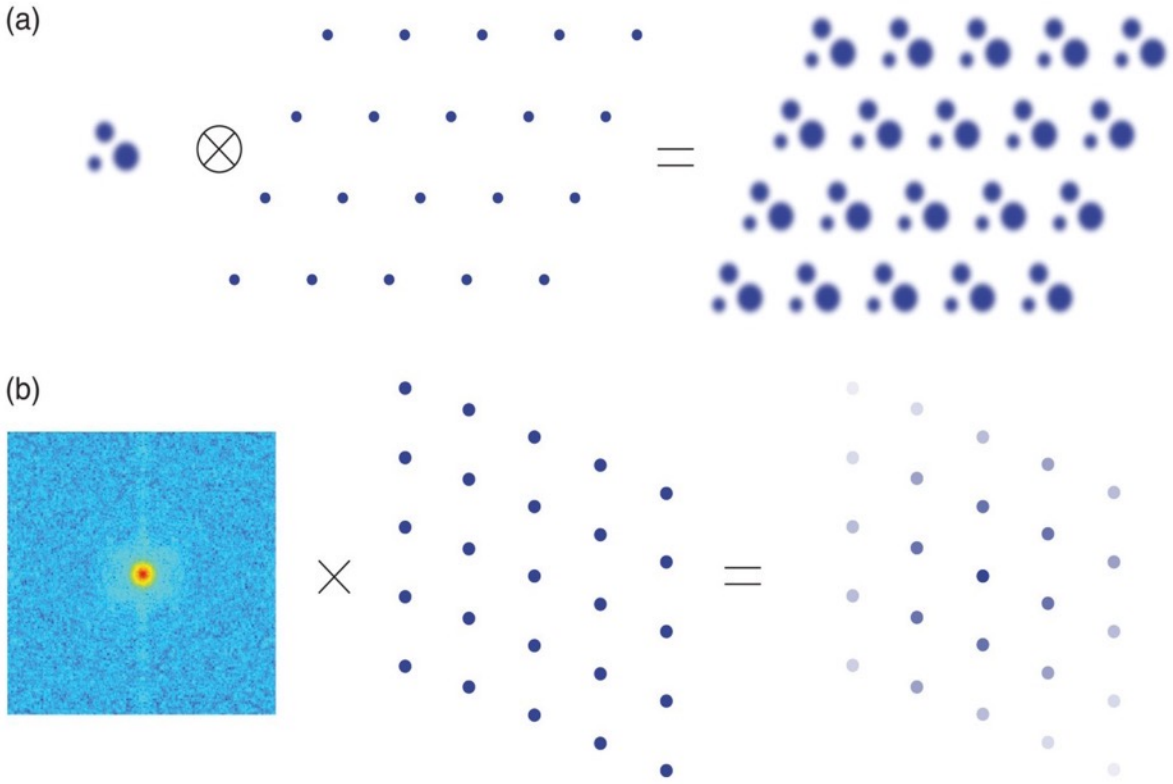

$2\pi/\lambda_{max}$
$2\theta_{max}$
$2\pi/\lambda_{min}$
Detector

More on Bragg peaks

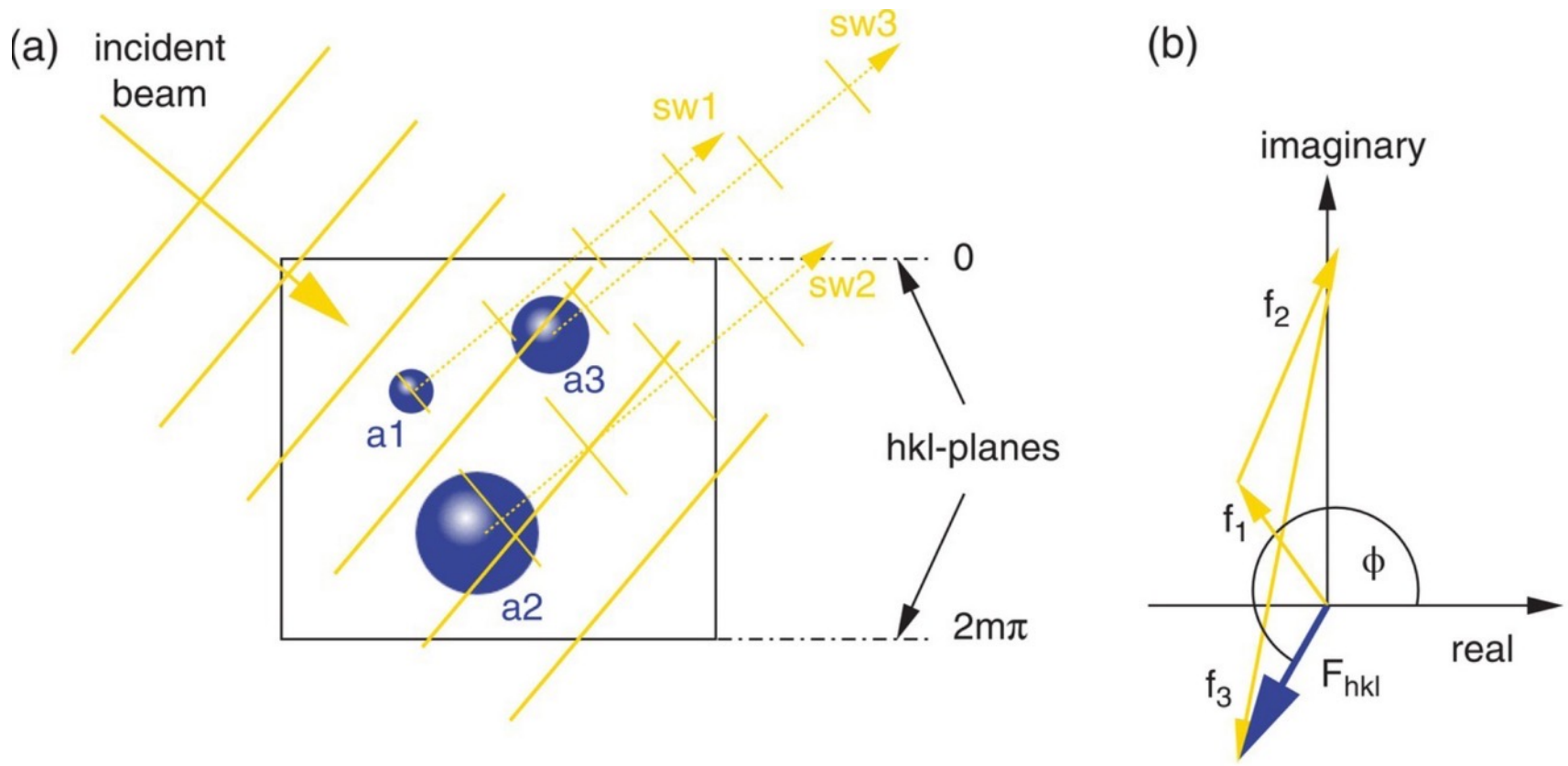
A crystal is defined by ist lattice and basis



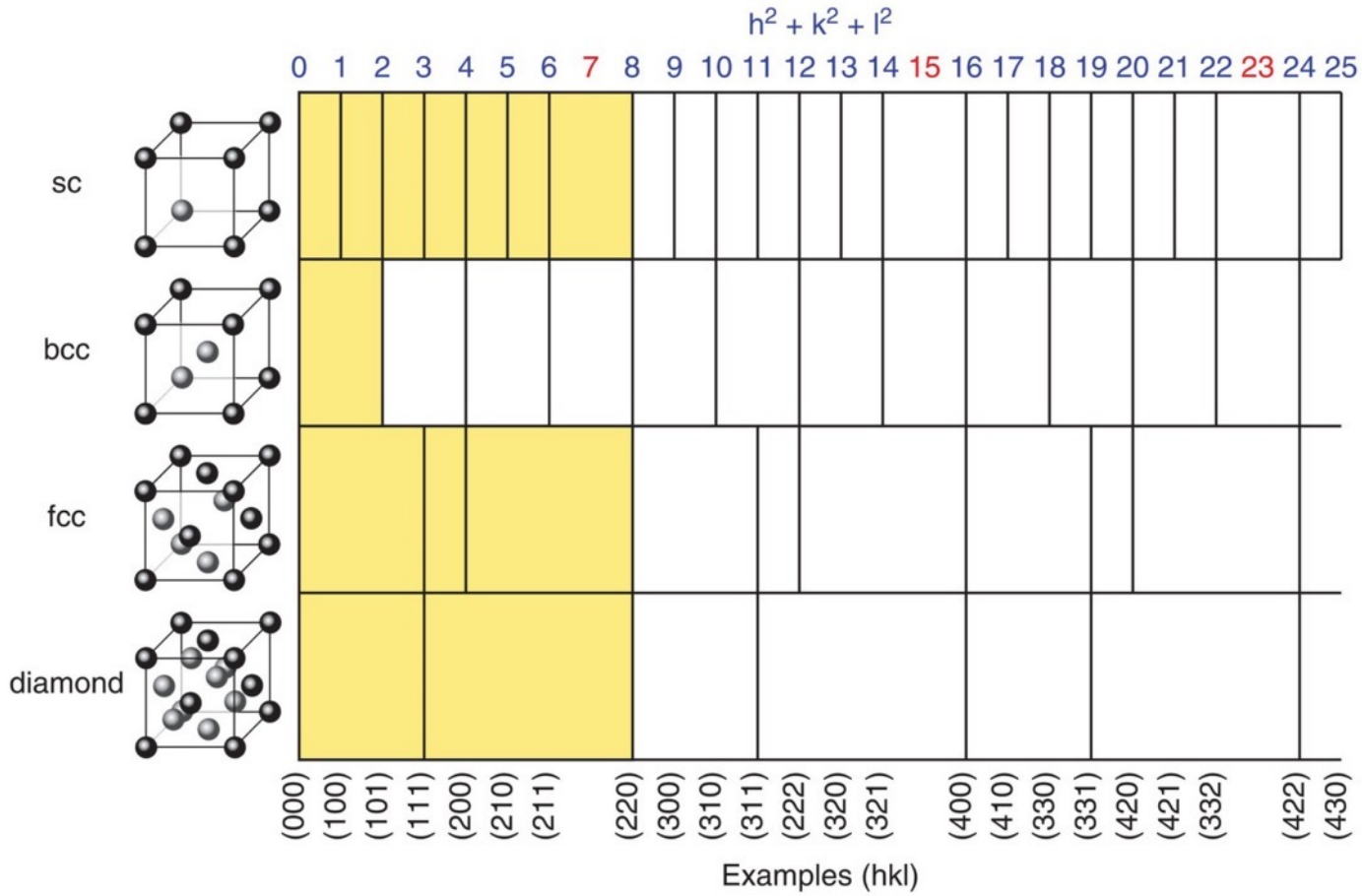
The structure factor



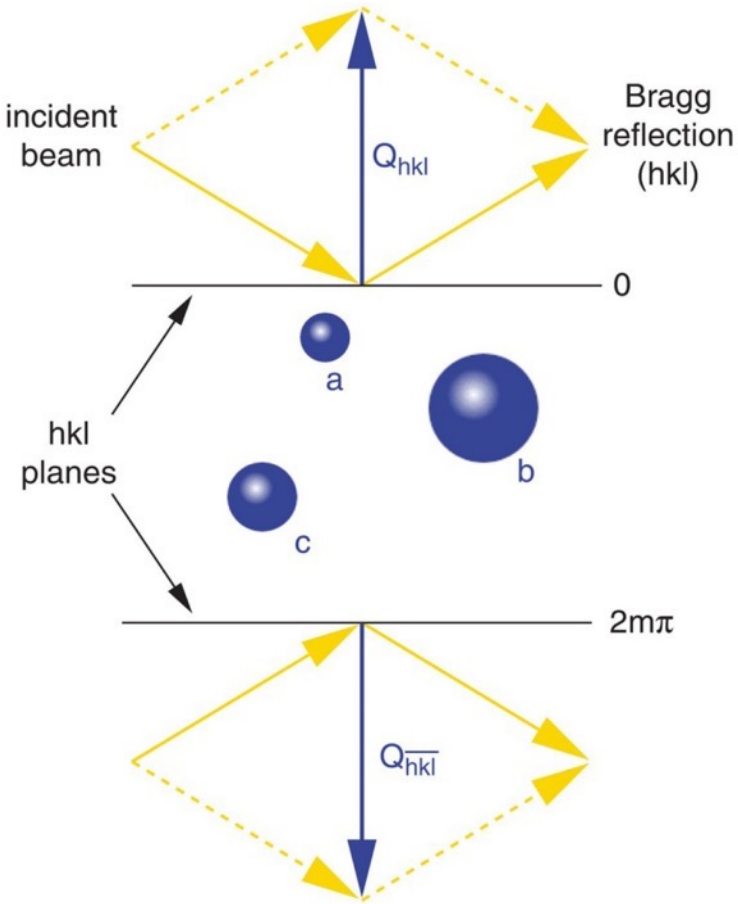
The structure factor

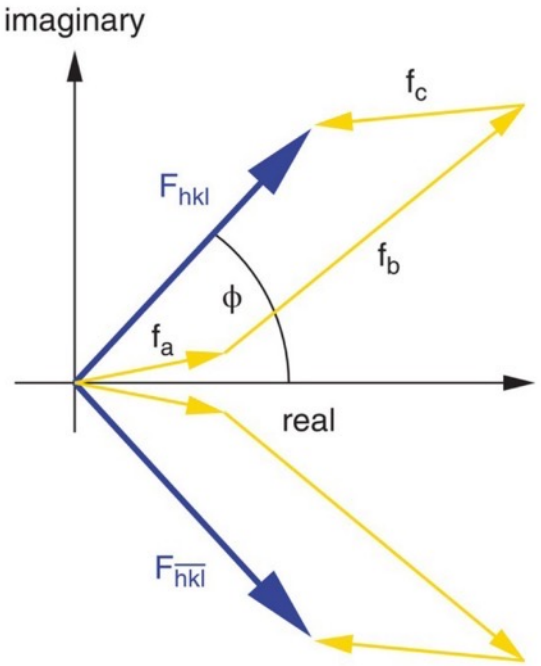


Allowed and forbidden reflections

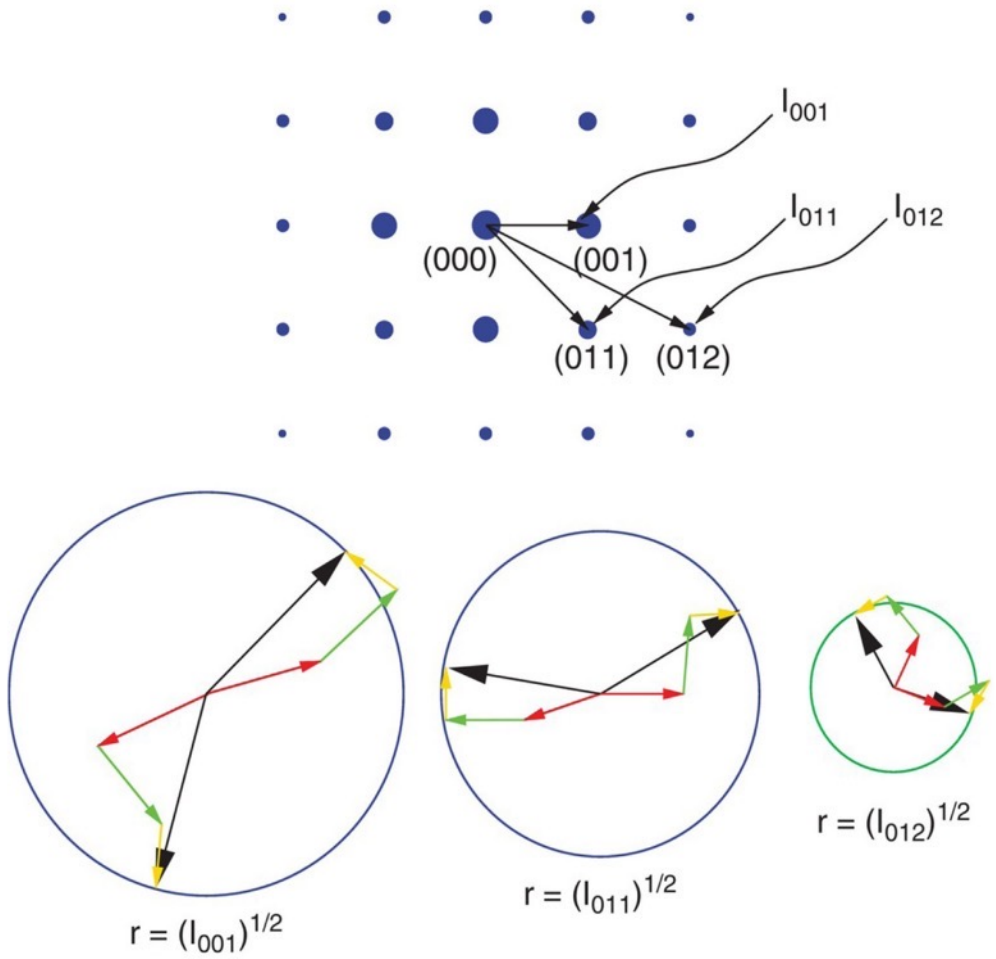


Friedels law

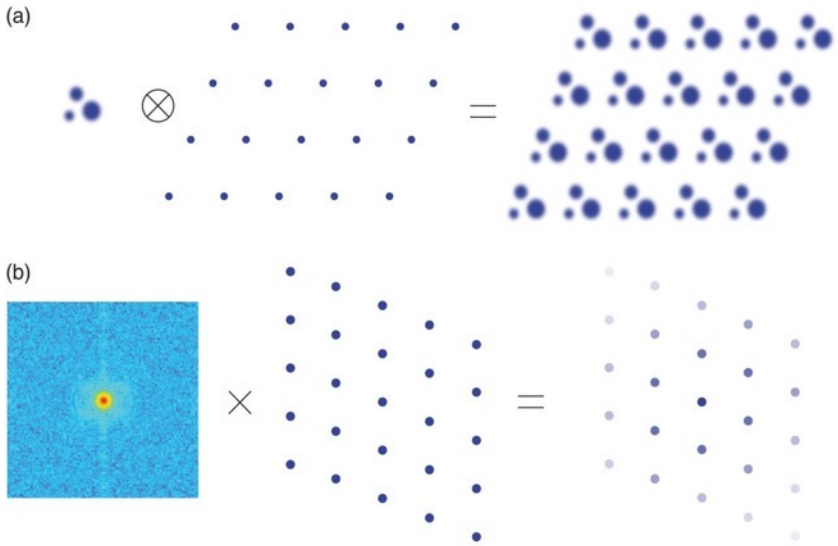


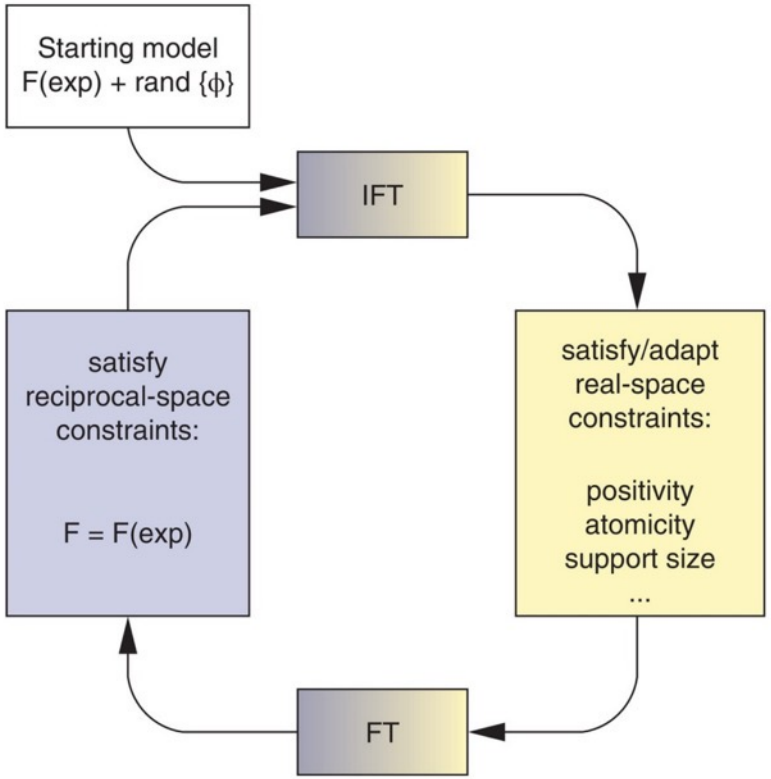


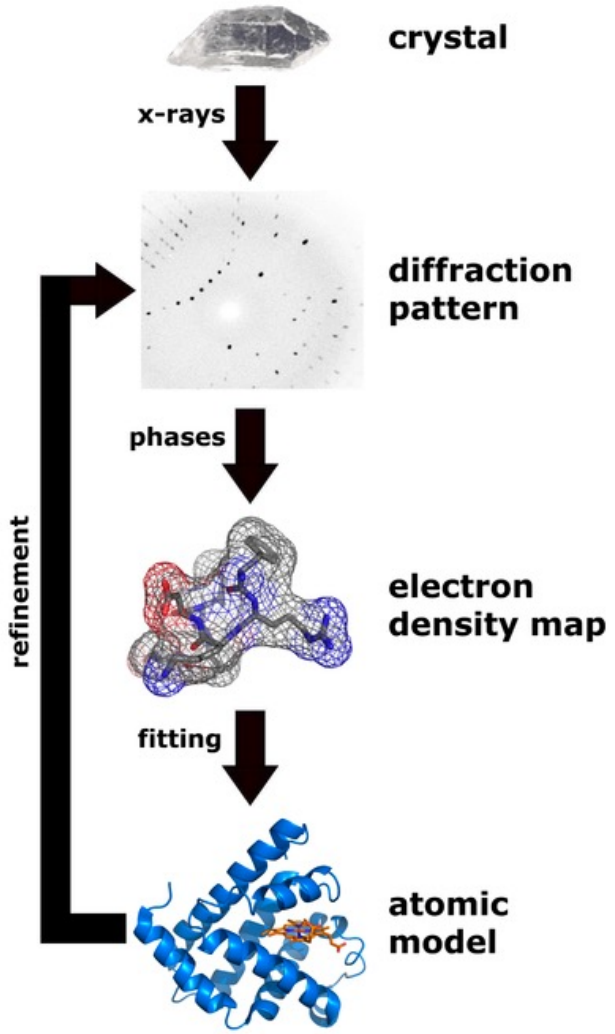
The phase problem



Solving a crystal structure







A few examples and case studies

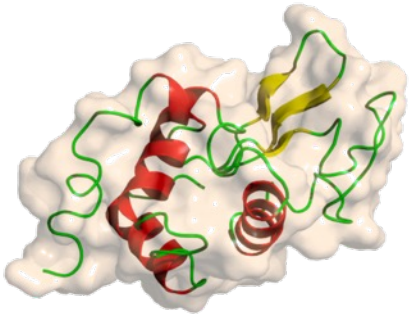
Remember last week example for diffaction of lysozyme crystal

Lysozym (Protein, Immunsystem)

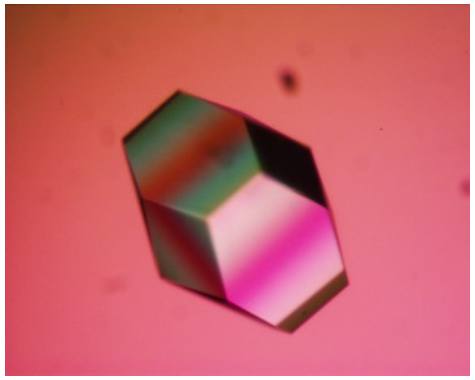
Stochiometric formula

$C_{125}H_{196}N_{40}O_{36}S_2$

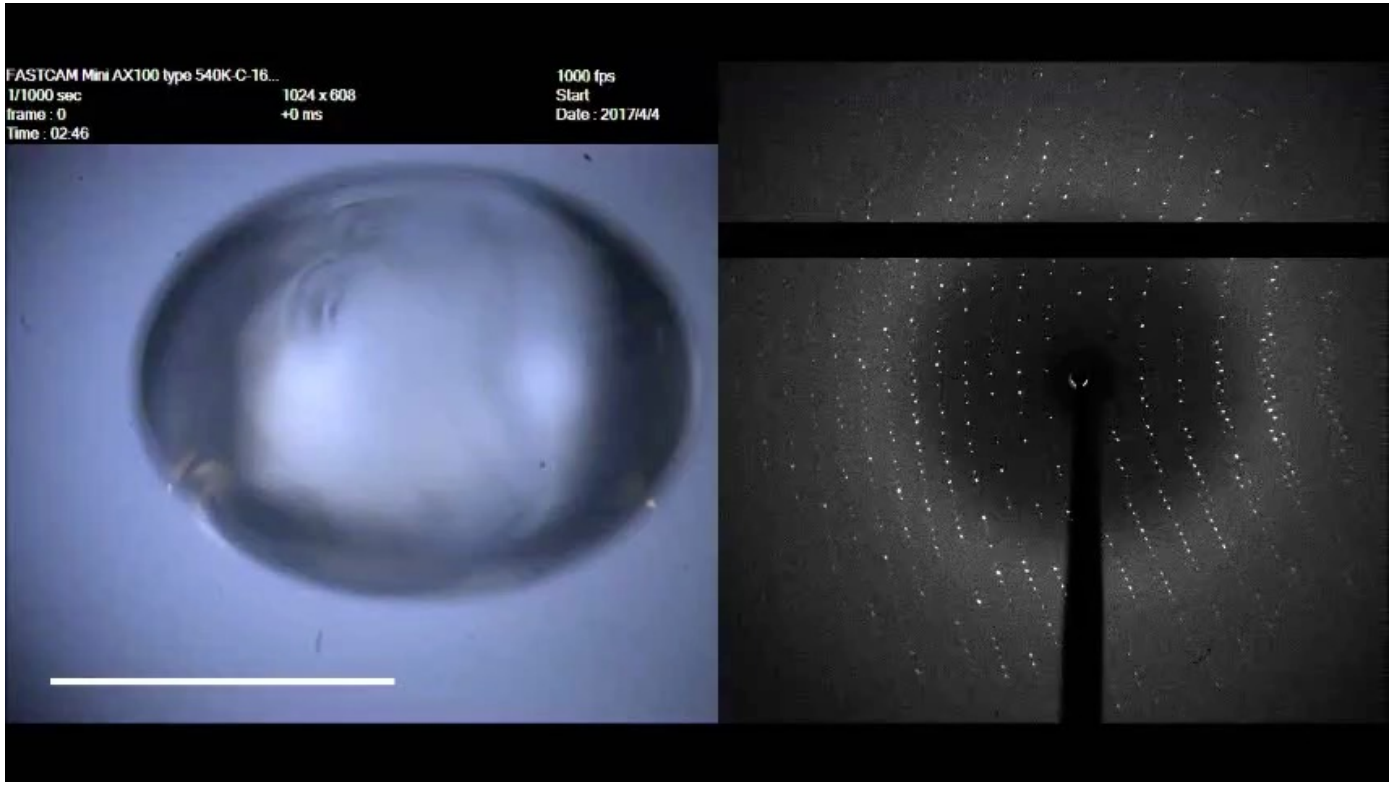
Structure



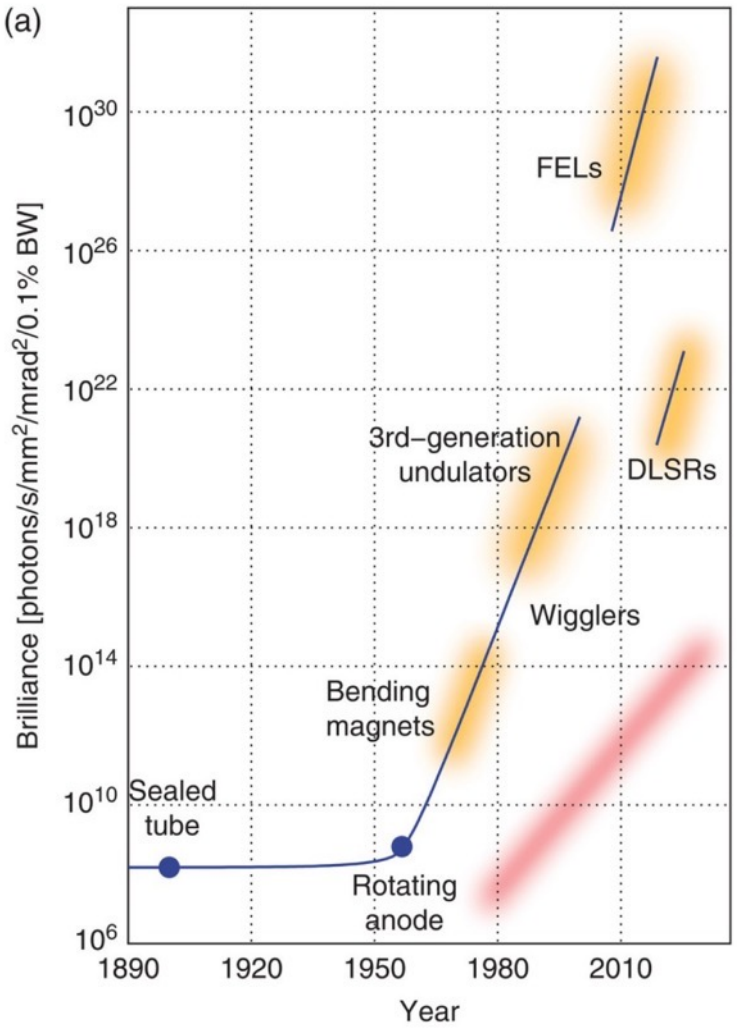
Crystal

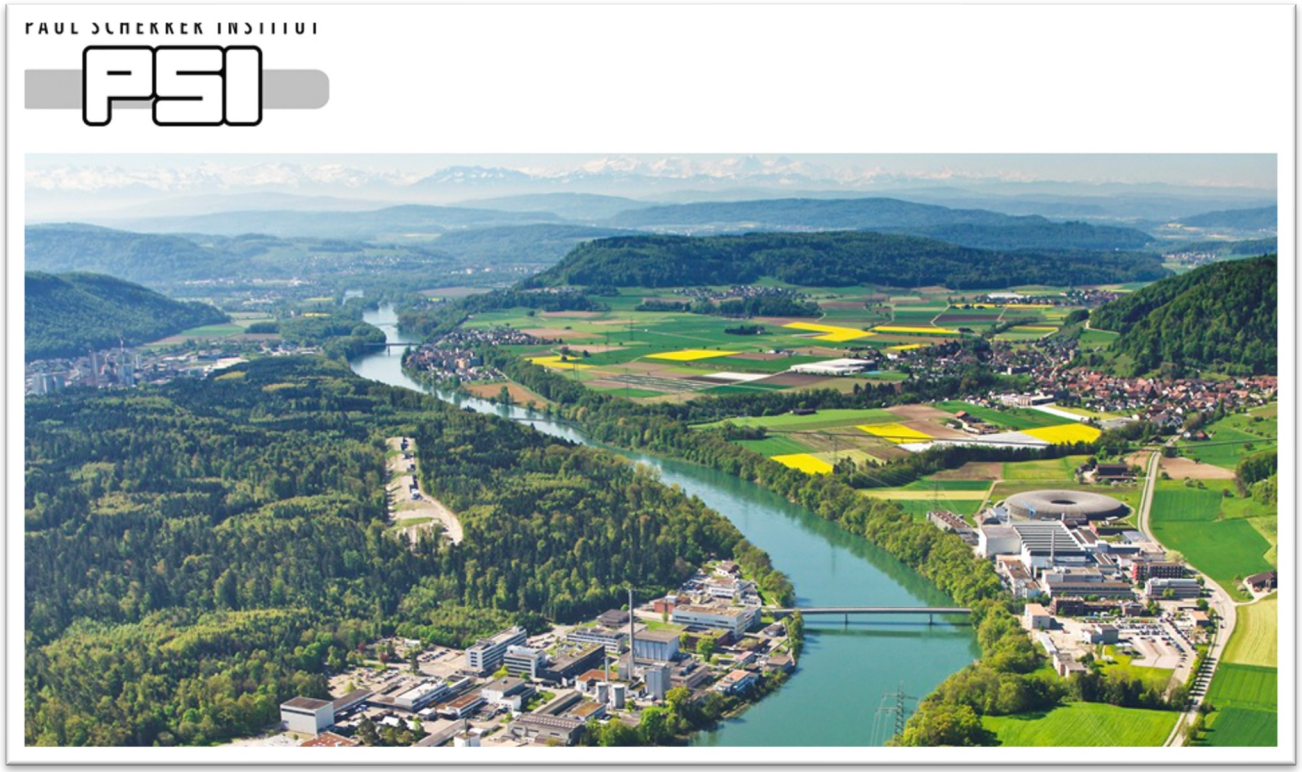


Experiment at the Swiss Light Source



Each X-ray source allows characteristic experiments. „Output“ varies by >20 orders of magnitude



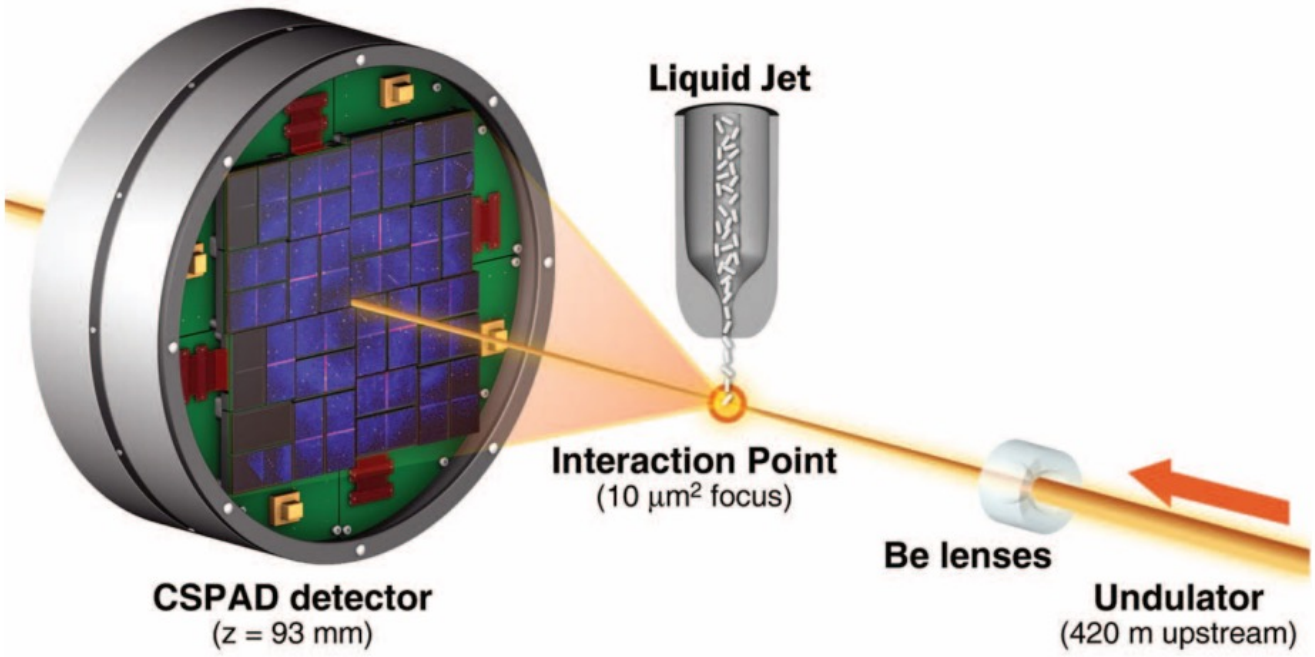


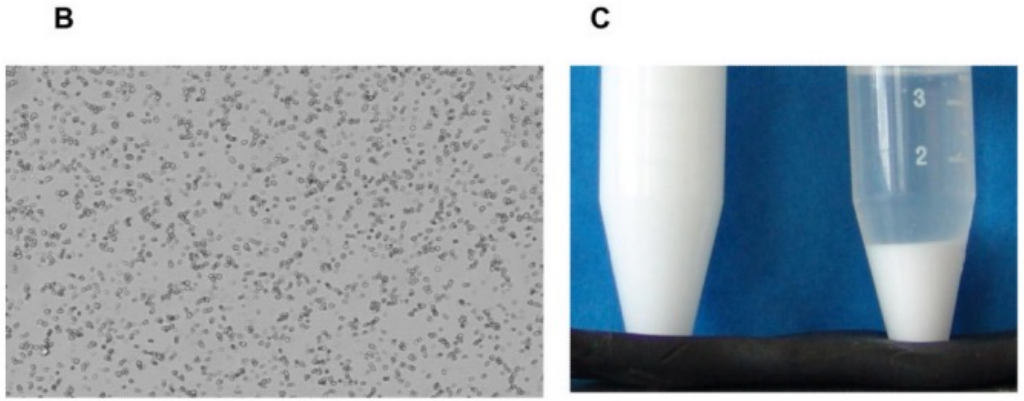
X-ray diffraction patterns from micro (nano) crystals

Science 337, page 363 (2012)

High-Resolution Protein Structure Determination by Serial Femtosecond Crystallography

Sébastien Boutet,[1]* Lukas Lomb,[2,3] Garth J. Williams,[1] Thomas R. M. Barends,[2,3] Andrew Aquila,[4] R. Bruce Doak,[5] Uwe Weierstall,[5] Daniel P. DePonte,[4] Jan Steinbrener,[2,3] Robert L. Shoeman,[2,3] Marc Messerschmidt,[1] Anton Barty,[4] Thomas A. White,[4] Stephan Kassemeyer,[2,3] Richard A. Kirian,[5] M. Marvin Seibert,[1] Paul A. Montanez,[1] Chris Kenney,[6] Ryan Herbst,[6] Philip Hart,[6] Jack Pines,[6] Gunther Haller,[6] Sol M. Gruner,[7,8] Hugh T. Philipp,[7] Mark W. Tate,[7] Marianne Hromalik,[9] Lucas J. Koerner,[10] Niels van Bakel,[11] John Morse,[12] Wilfred Ghonsalves,[1] David Arnlund,[13] Michael J. Bogan,[14] Carl Caleman,[4] Raimund Fromme,[15] Christina Y. Hampton,[14] Mark S. Hunter,[15] Linda C. Johansson,[13] Gergely Katona,[13] Christopher Kupitz,[15] Mengning Liang,[4] Andrew V. Martin,[4] Karol Nass,[16] Lars Redecke,[17,18] Francesco Stellato,[4] Nicusor Timneanu,[19] Dingjie Wang,[5] Nadia A. Zatsepin,[5] Donald Schafer,[1] James Defever,[1] Richard Neutze,[13] Petra Fromme,[15] John C. H. Spence,[5] Henry N. Chapman,[4,16] Ilme Schlichting[2,3]





Time-resolved x-ray diffraction

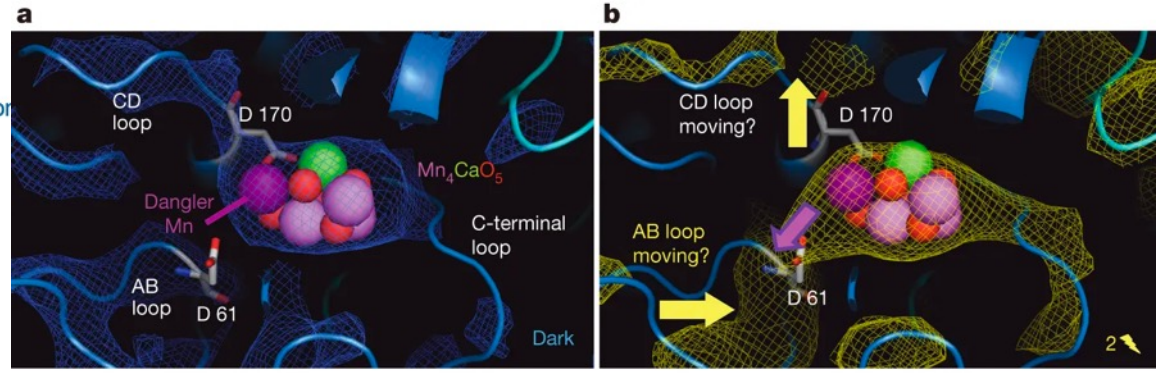
nature

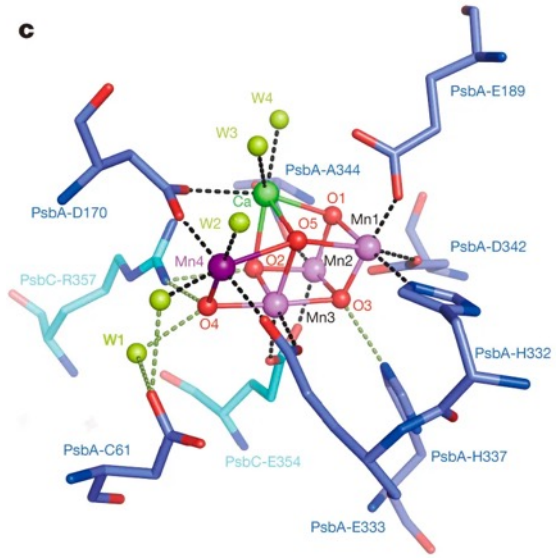
Letter | Published: 09 July 2014

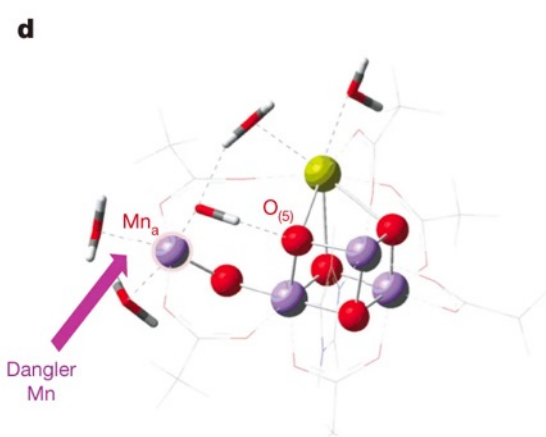
Serial time-resolved crystallography of photosystem II using a femtosecond X-ray laser

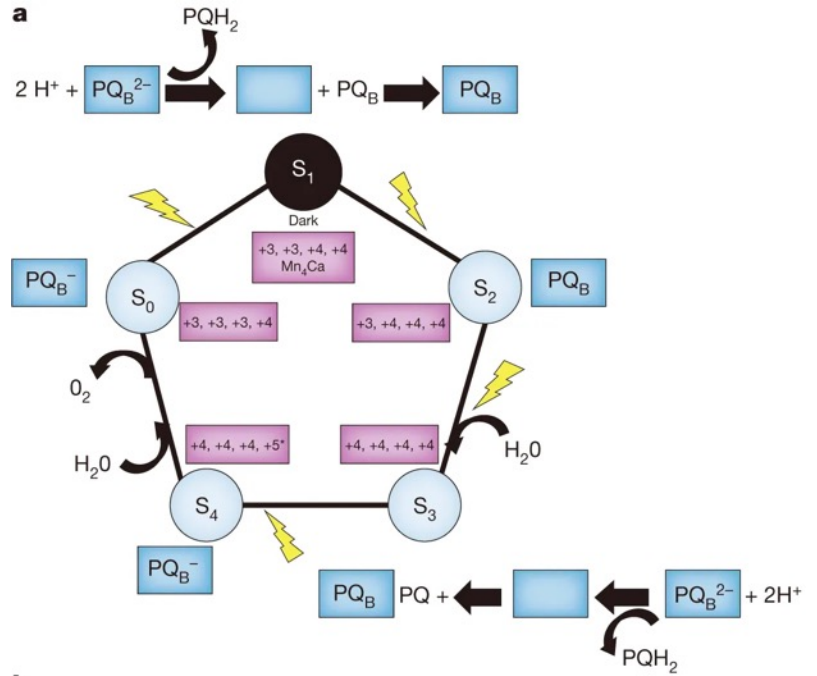
Christopher Kupitz, Shibom Basu, [...] Petra Fro

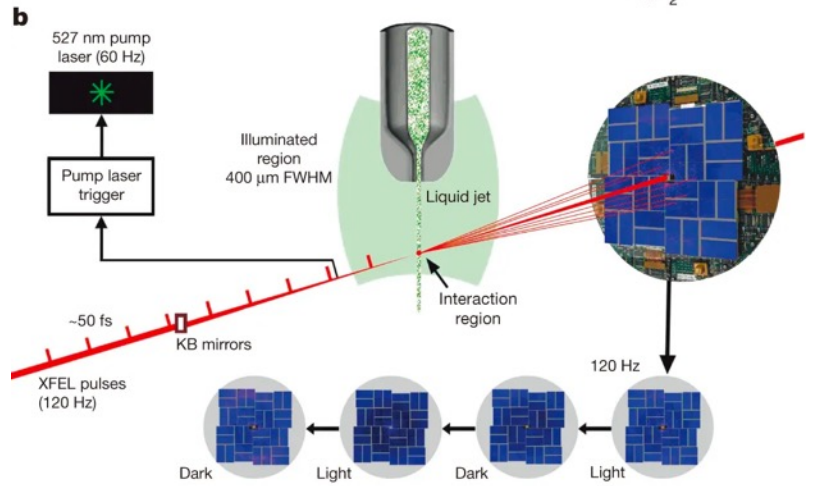
Nature **513**, 261–265(2014) | Cite this article

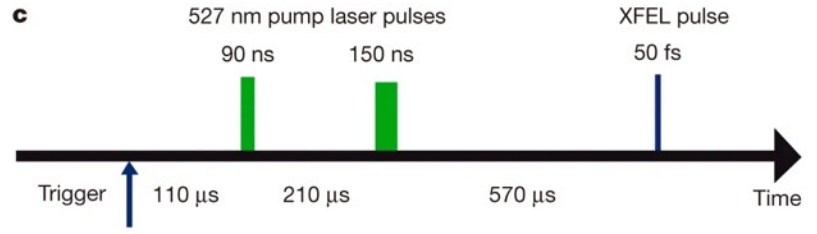












Recent results from SwissFEL

Article

Femtosecond-to-millisecond structural changes in a light-driven sodium pump

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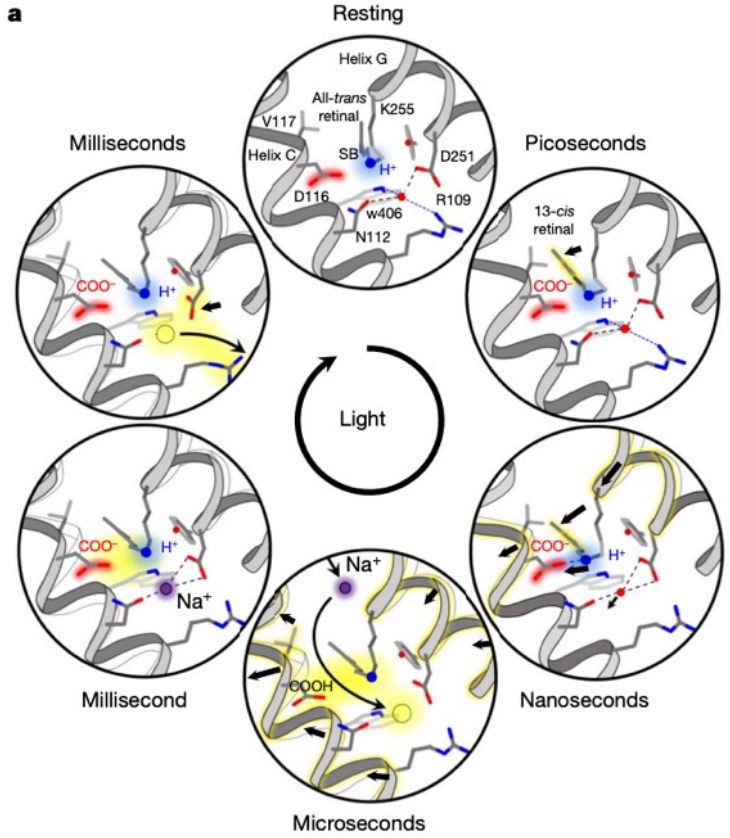
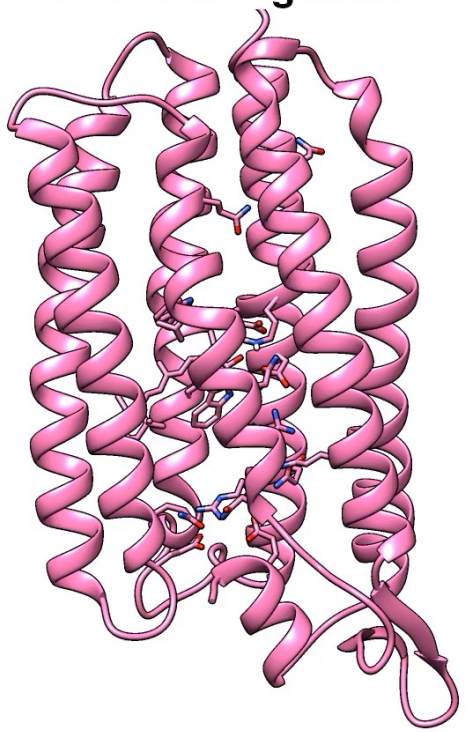
Petr Skopintsev[1], David Ehrenberg[2,13], Tobias Weinert[1,13], Daniel James[1], Rajiv K. Kar[3], Philip J. M. Johnson[4], Dmitry Ozerov[5], Antonia Furrer[1], Isabelle Martiel[6], Florian Dworkowski[6], Karol Nass[6,7], Gregor Knopp[7], Claudio Cirelli[7], Christopher Arrell[8], Dardan Gashi[1,7], Sandra Mous[9], Maximilian Wranik[1], Thomas Gruhl[1], Demet Kekilli[1], Steffen Brünle[1], Xavier Deupi[1,10], Gebhard F. X. Schertler[1,11], Roger M. Benoit[1,12], Valerie Panneels[1], Przemyslaw Nogly[9], Igor Schapiro[3], Christopher Milne[7], Joachim Heberle[2] & Jörg Standfuss[1]

Light-driven sodium pumps actively transport small cations across cellular membranes[1]. These pumps are used by microorganisms to convert light into membrane potential and have become useful optogenetic tools with applications in neuroscience. Although the resting state structures of the prototypical sodium pump *Krokinobacter eikastus* rhodopsin 2 (KR2) have been solved[2,3], it is unclear how structural alterations over time allow sodium to be translocated against a concentration gradient. Here, using the Swiss X-ray Free Electron Laser[4], we have collected serial crystallographic data at ten pump–probe delays from femtoseconds to milliseconds. High-resolution structural snapshots throughout the KR2 photocycle show how retinal isomerization is completed on the femtosecond timescale and changes the local structure of the binding pocket in the early nanoseconds. Subsequent rearrangements and deprotonation of the retinal Schiff base open an electrostatic gate in microseconds. Structural and spectroscopic data, in combination with quantum chemical calculations, indicate that a sodium ion binds transiently close to the retinal within one millisecond. In the last structural intermediate, at 20 milliseconds after activation, we identified a potential second sodium-binding site close to the extracellular exit. These results provide direct molecular insight into the dynamics of active cation transport across biological membranes.





The end