Introduction to Macromolecular Crystallography

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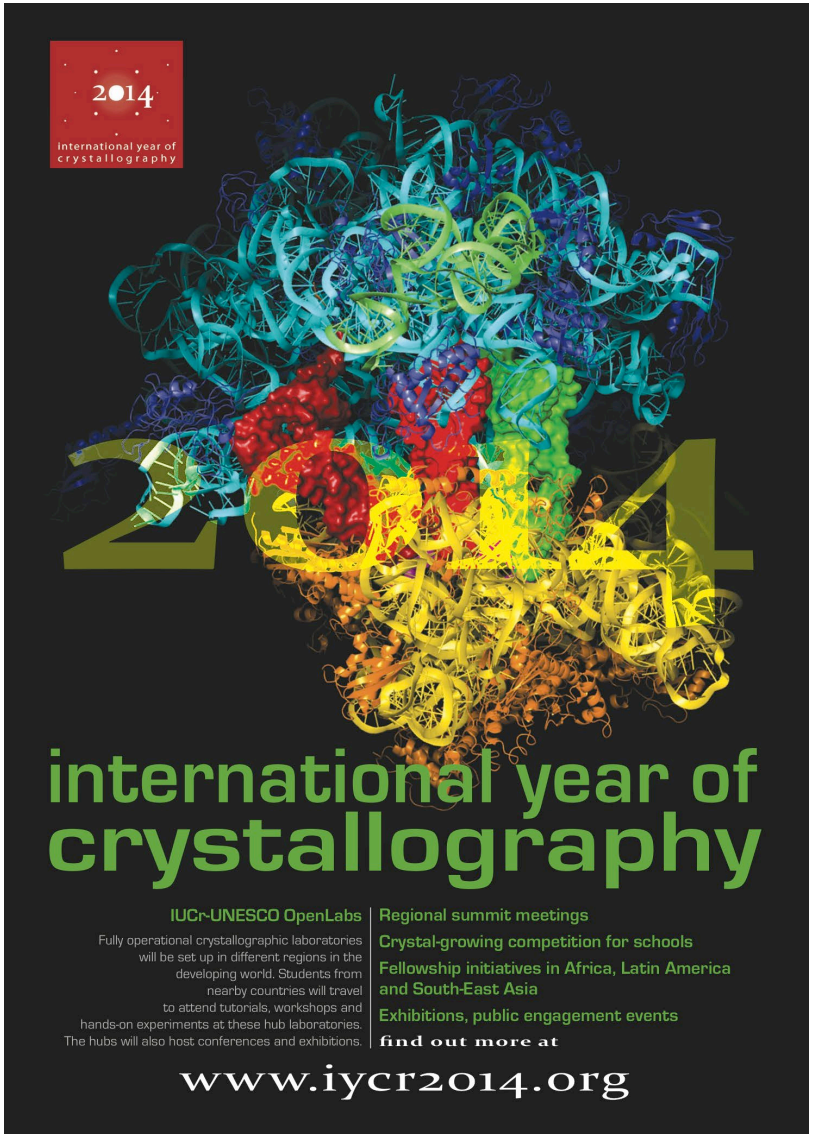
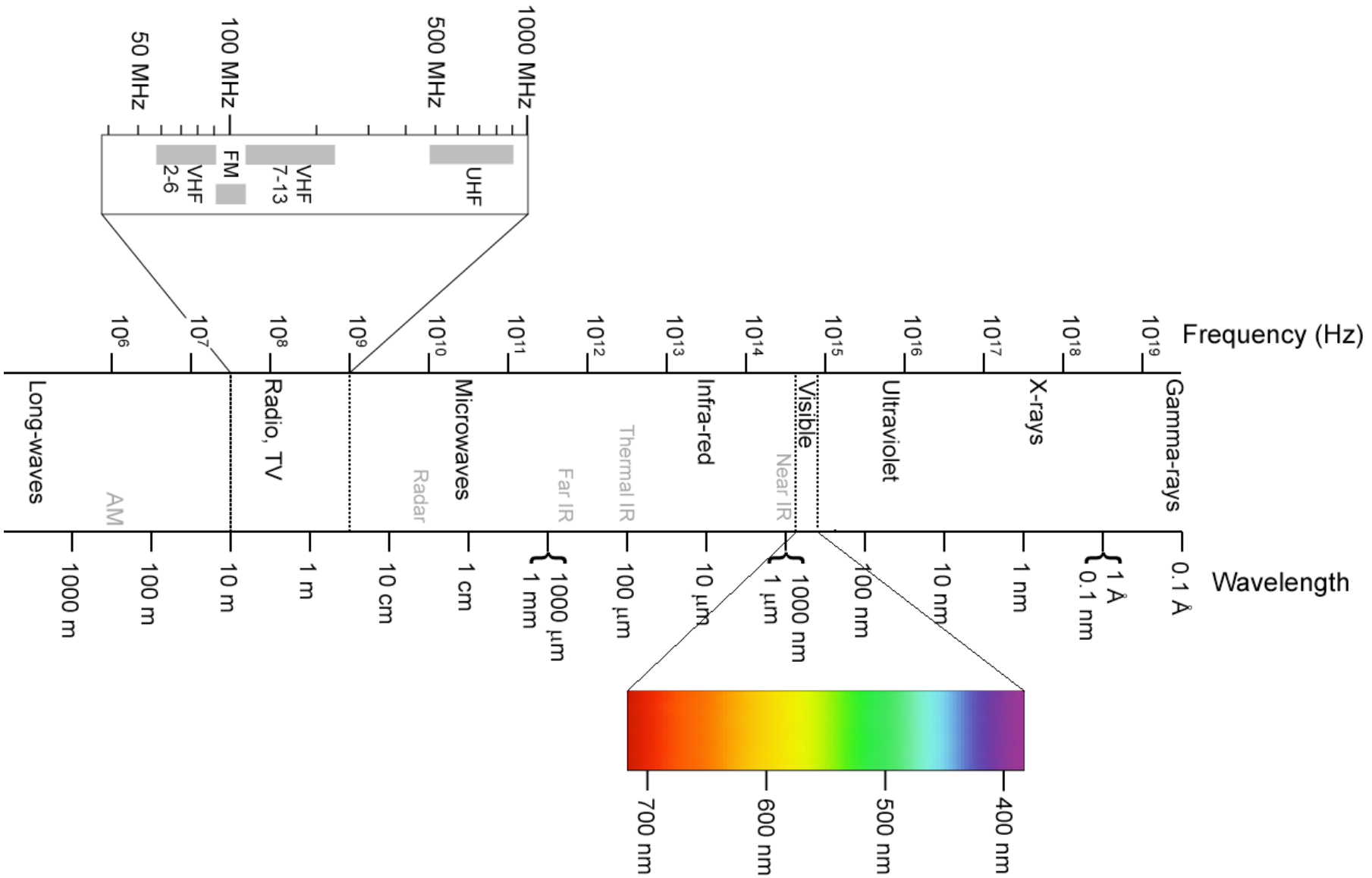
Integrative and Translational Bioimaging

2014 - International Year of Crystallography

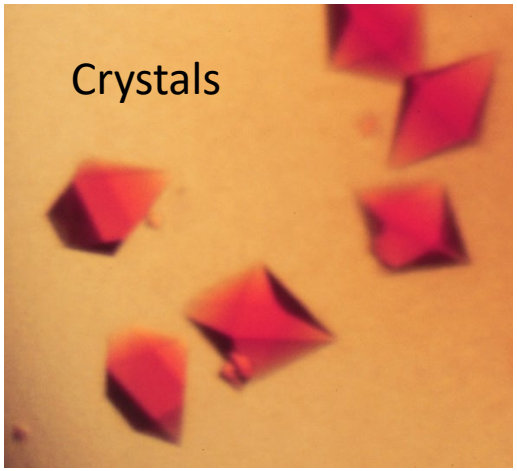


- 1895: Discovery of X-rays by Wilhelm Conrad Roentgen
- 1912: Discovery of diffraction of X-rays by crystals by Max von Laue, Paul Knipping and Walter Friedrich
- 1913: William Lawrence Bragg and William Henry Bragg propose Bragg's law

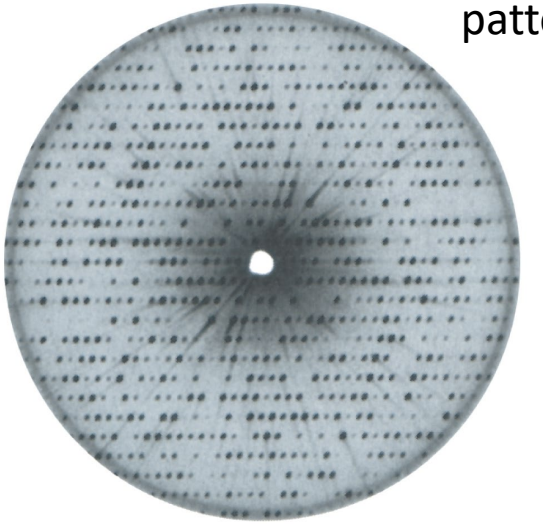
Electromagnetic Radiation

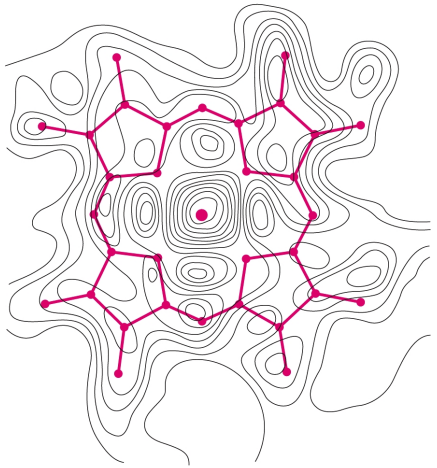


Protein Crystallography - Overview

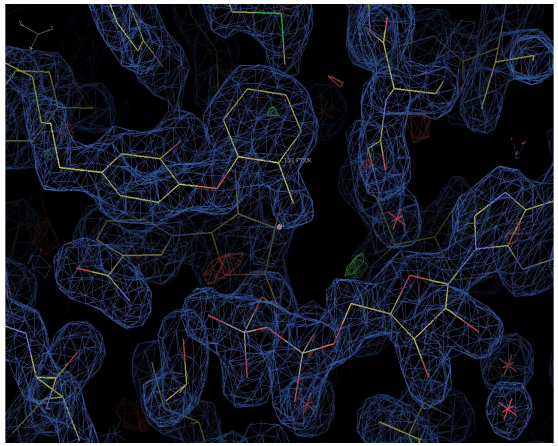


Diffraction pattern

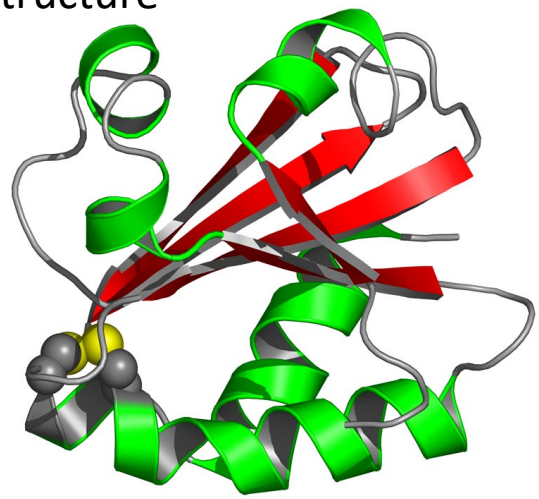




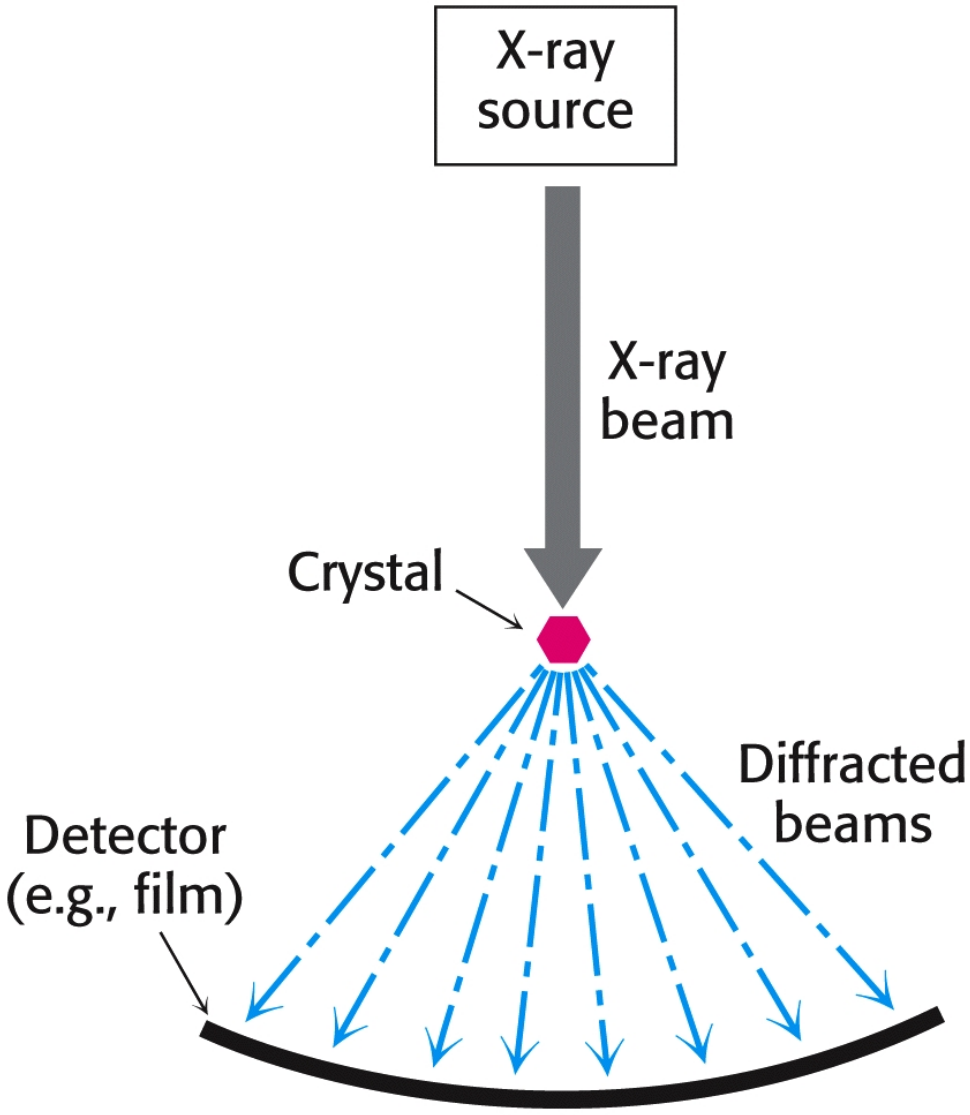
Electron density maps



Structure



Diffraction Experiment



X-rays: high energy electromagnetic radiation (wavelength ~ 1 Å)

Electrons scatter X-rays (amplitude and phase)

Crystals contain atoms in a defined arrangement

Scattered waves interfere (constructive vs. destructive interference)

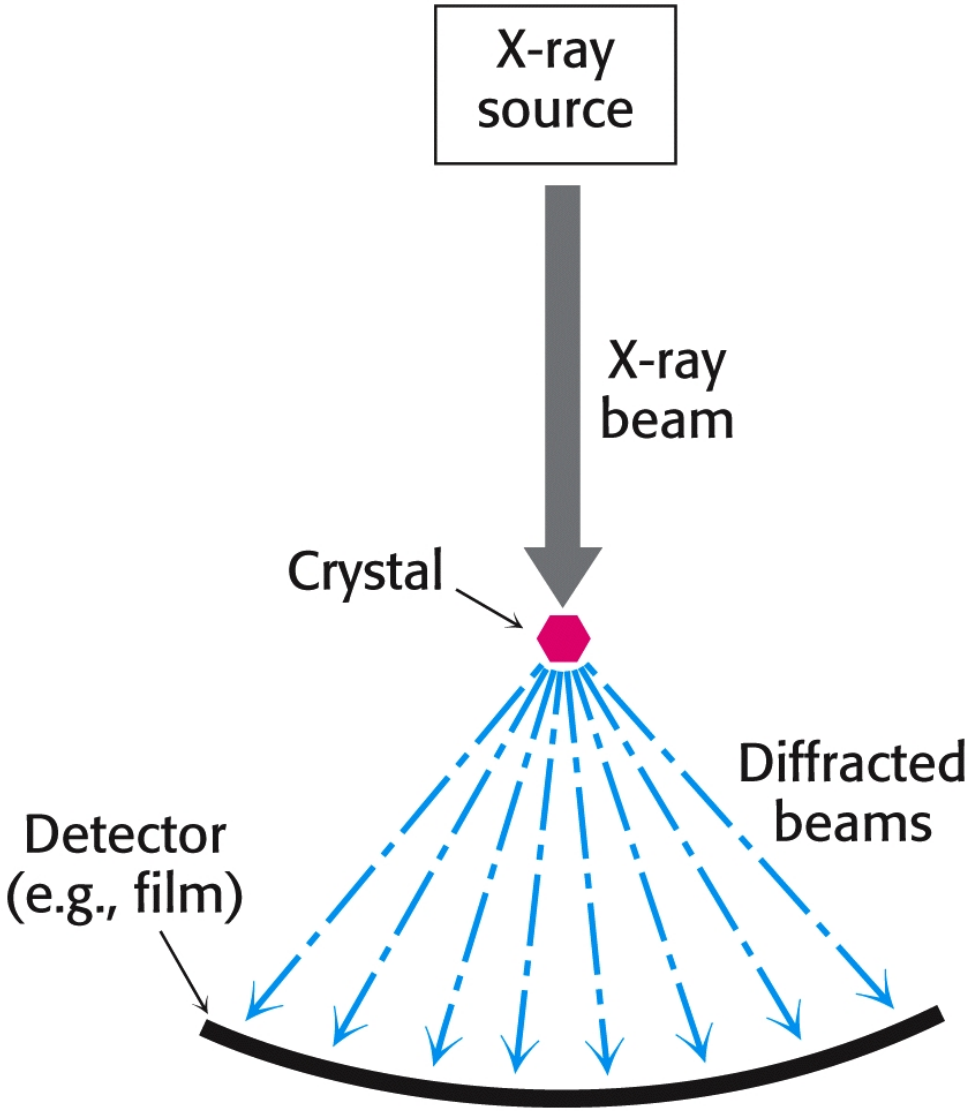
Interference pattern depends on arrangement of atoms

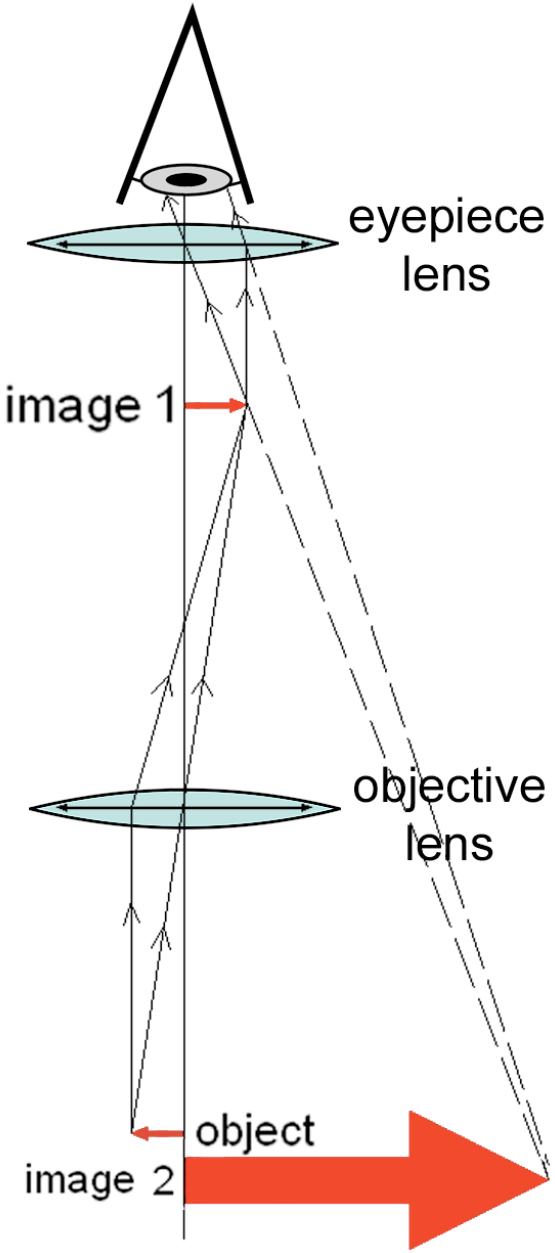
Resolution limit: up to 1 Å

Virtually no size limit

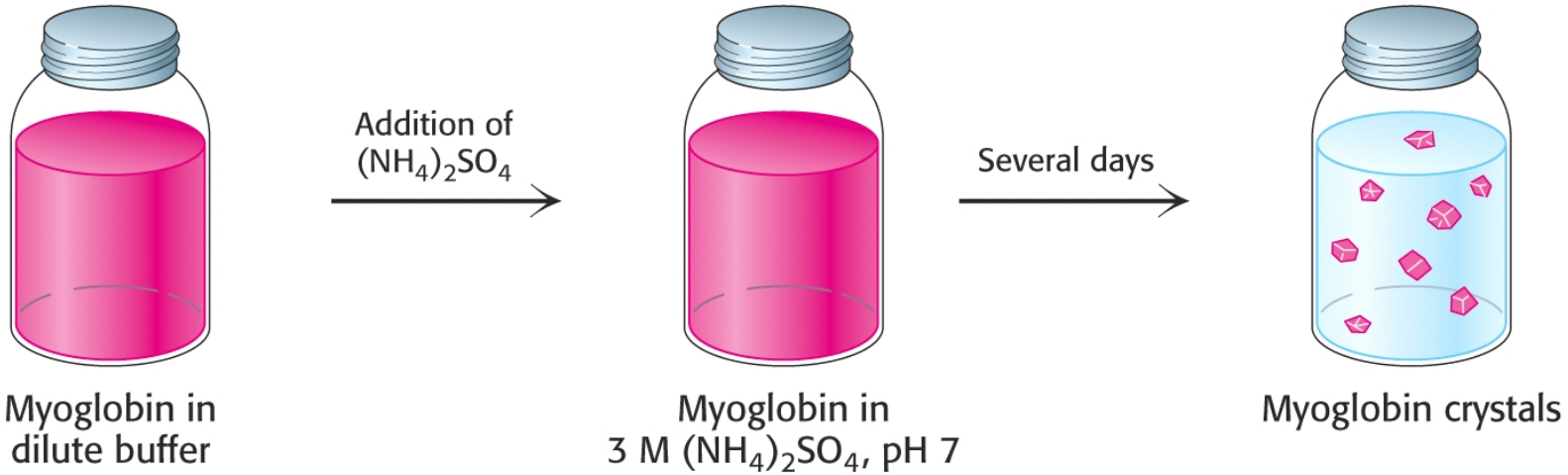
We cannot measure the phases of the diffracted X-rays!

Diffraction Experiment vs. Light Microscope



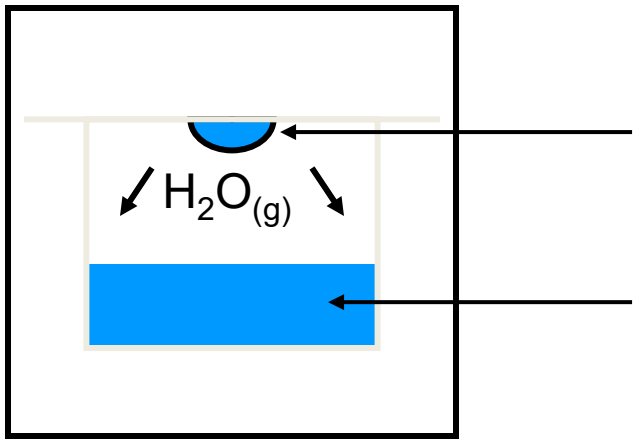


Crystal Growth



Salting out with $(NH_4)_2SO_4$: Solubility of myoglobin is reduced

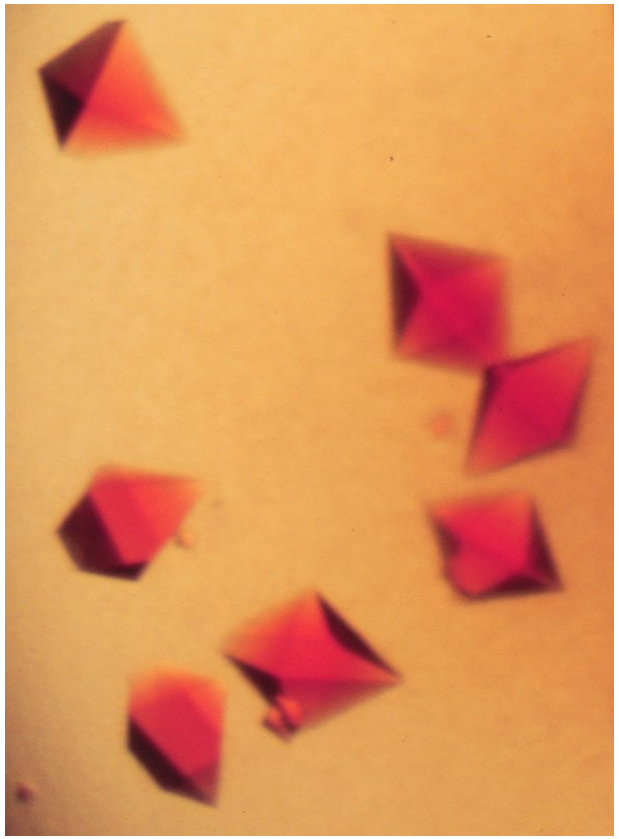
Trial and error process



~1 µl protein solution (c ~ 10 mg/ml)
+ ~1 µl reservoir solution

~1 ml reservoir solution (high concentration of salt or organic solvent)

Crystals



Colored due to heme prosthetic group (usually colorless)

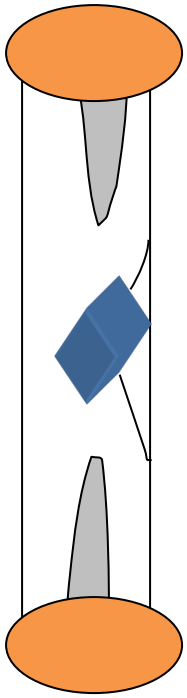
Size: > 0.1 mm in each direction

Fragile

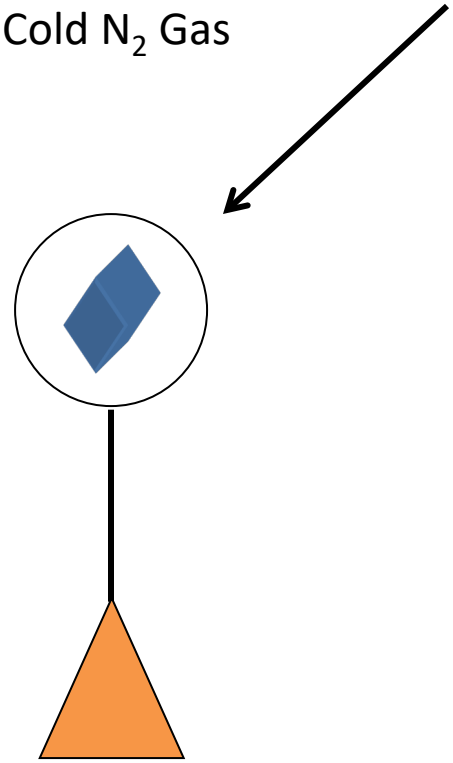
Prone to dehydration: 30%-80% solvent content

Soaking experiments

Crystal Mounting



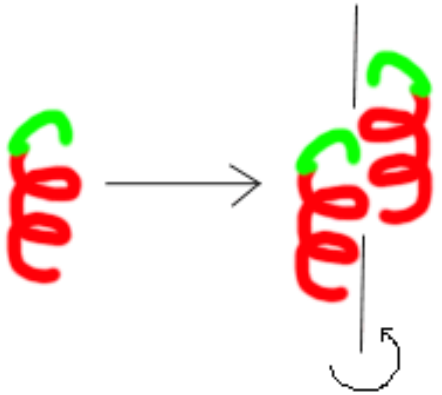
Capillary-mounted crystal for room temperature data collection

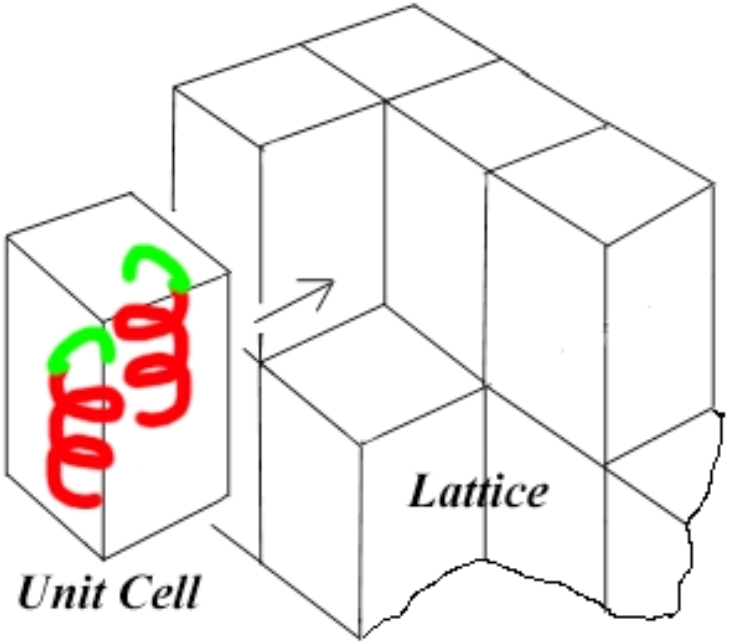


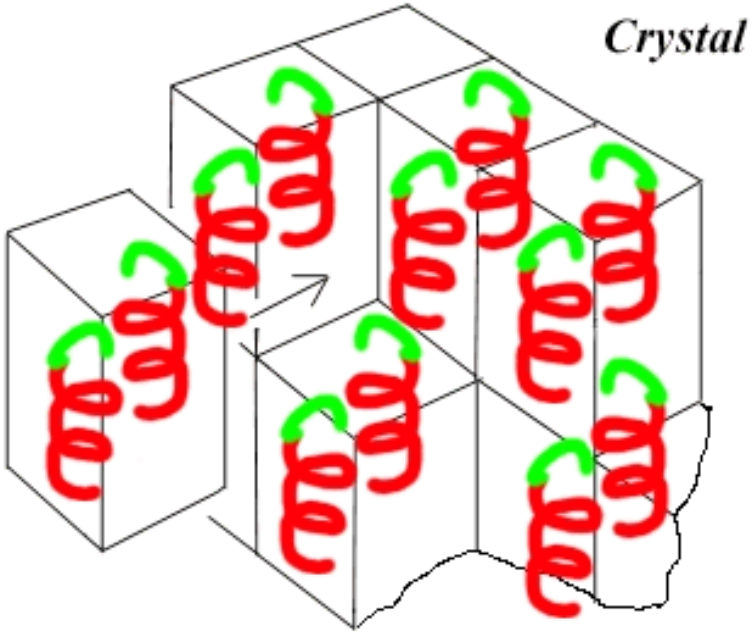
Loop-mounted crystal for cryogenic data collection

Asymmetric Unit, Unit Cell and Crystal

Motif





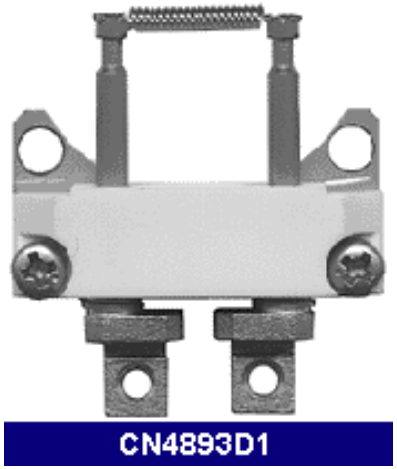


X-ray generator

Rotating anode generator

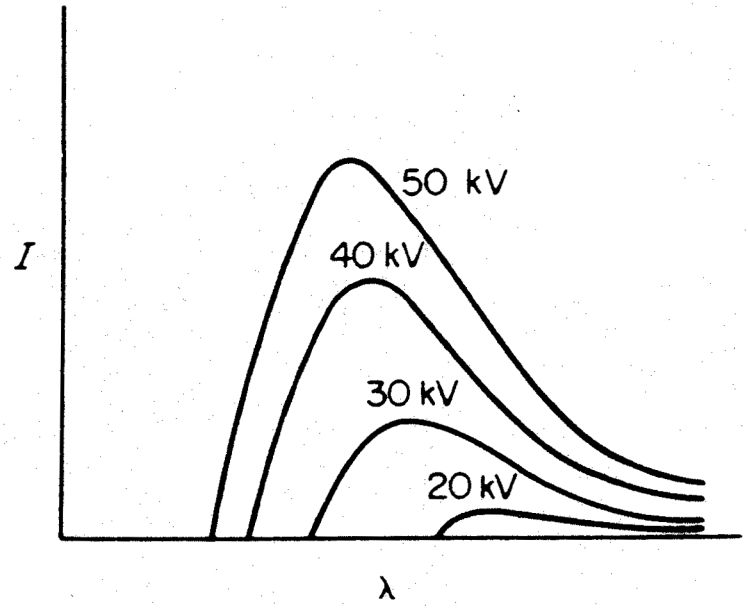
Micromax 007-HF
- 70 x 70 µm focal spot
- 9000 rpm
- 1.2 kW (40 kV and 30 mA)

Rotating Anode



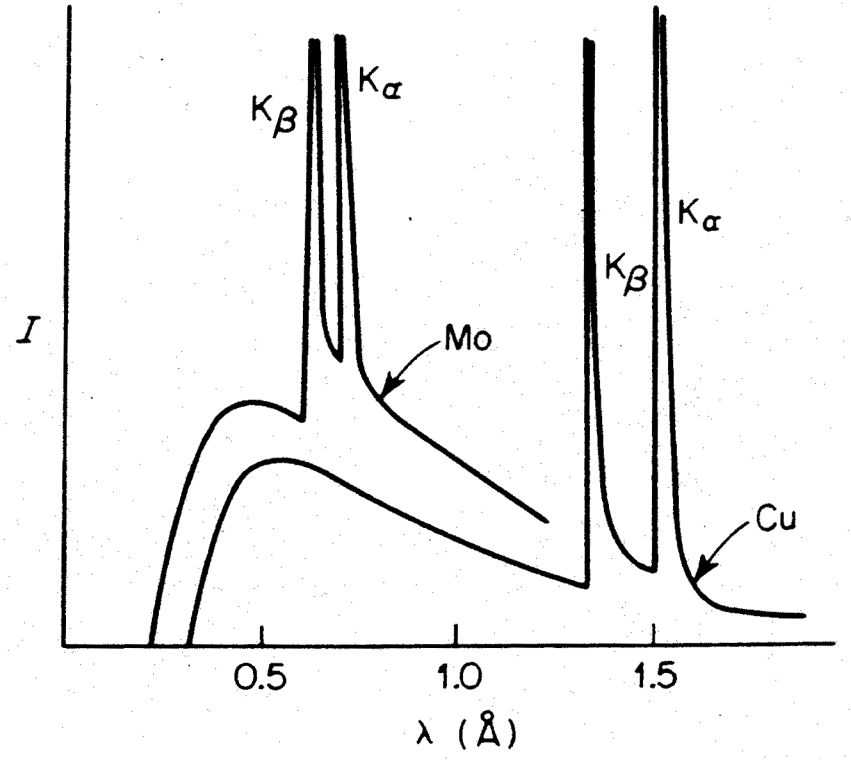
Filament

X-ray spectra



Continuous X-ray spectra as a function of accelerating voltage

$\lambda_{min} = 12{,}398 / V_{acc}$ (Å)



X-ray spectra with characteristic peaks (Mo at 50 kV and Cu at 35 kV)

$K_{\alpha 1}$, $K_{\alpha 2}$ $L \rightarrow K$

$K_{\beta 1}$, $K_{\beta 2}$ $M \rightarrow K$

Synchrotrons I

French Alps

Grenoble

Le Drac

L'Isère

Storage Ring

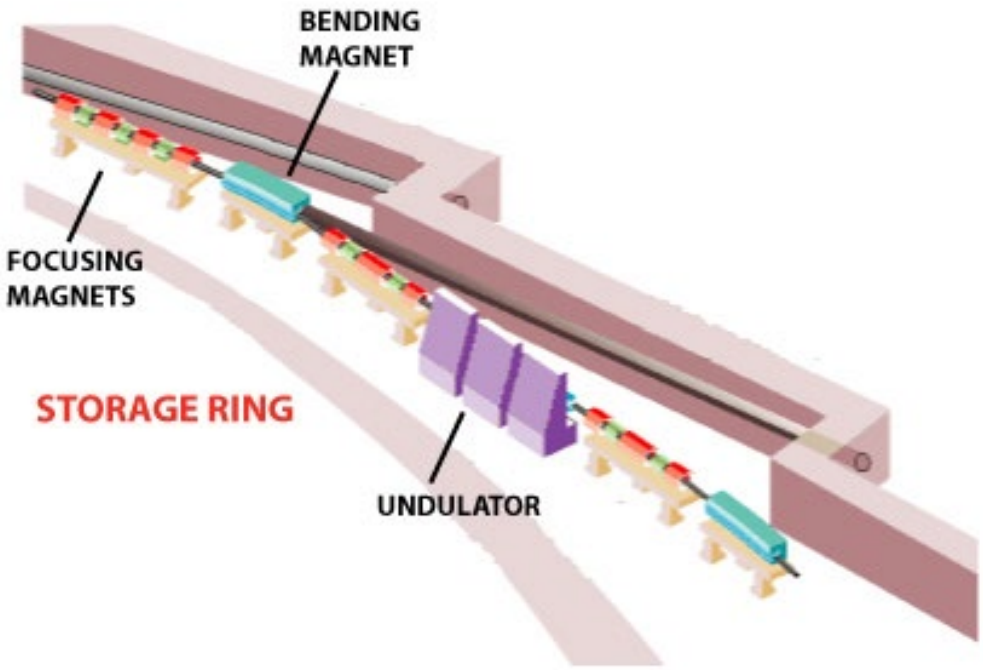
[How does it work?]

Main Advantages

Higher intensity
- Microfocus
- Time-resolved
- Laue crystallography

Tunability
- MAD/SAD

Synchrotrons II



Bending Magnet:

Electrons are deflected in BM from their straight path by several degrees

Change in direction causes electrons to emit synchrotron radiation

Undulators:

Complex array of small magnets

Force electrons to follow an undulating, or wavy, trajectory

Beams of radiation emitted from different bends overlap and interfere with each other to generate a much more intense beam

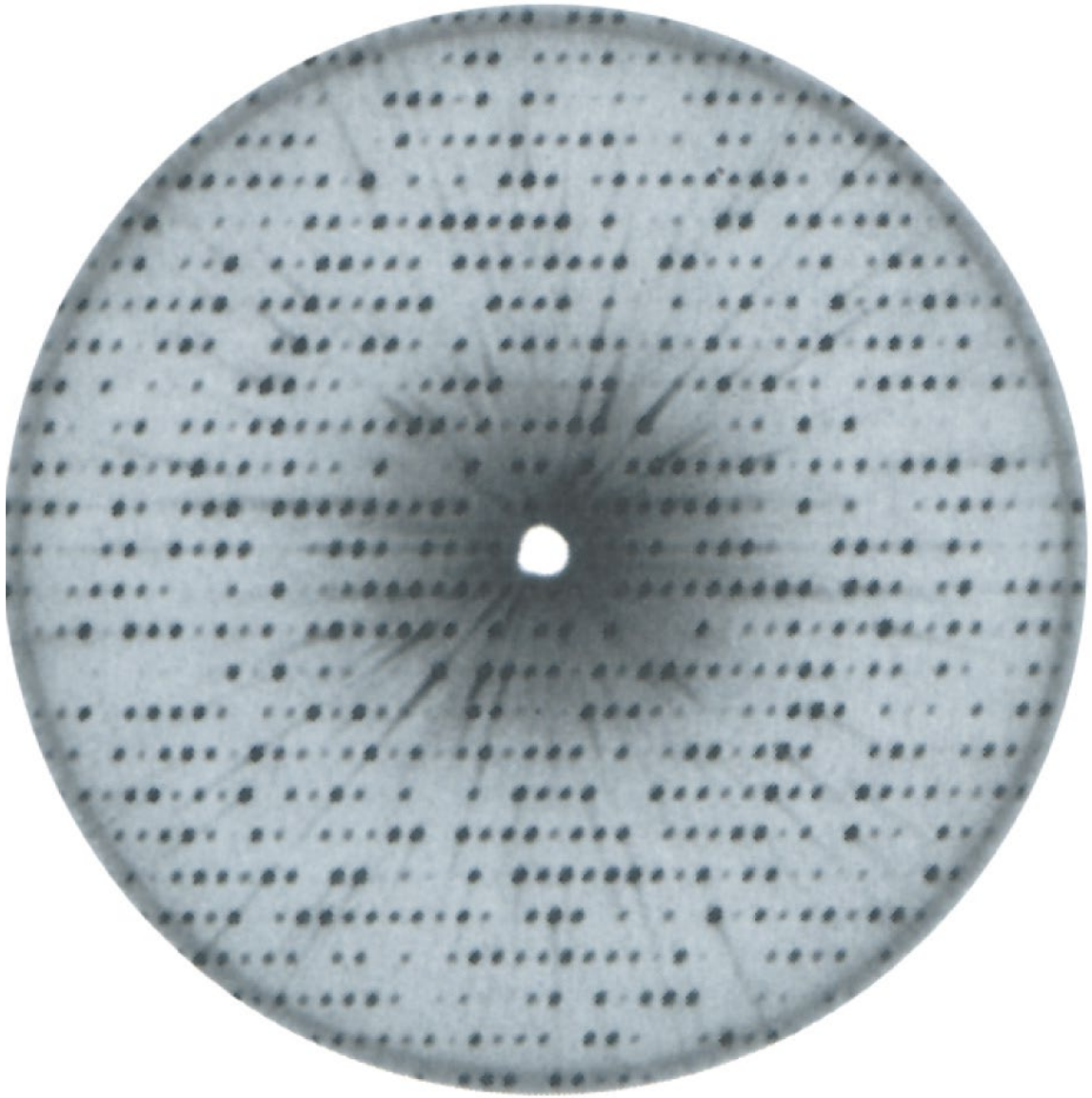
Focussing Magnet:

Placed in the straight sections of the storage ring

Focus electron beam to keep it small and well-defined

A small and well-defined electron beam will produce the very bright X-ray beam needed

Diffraction Pattern



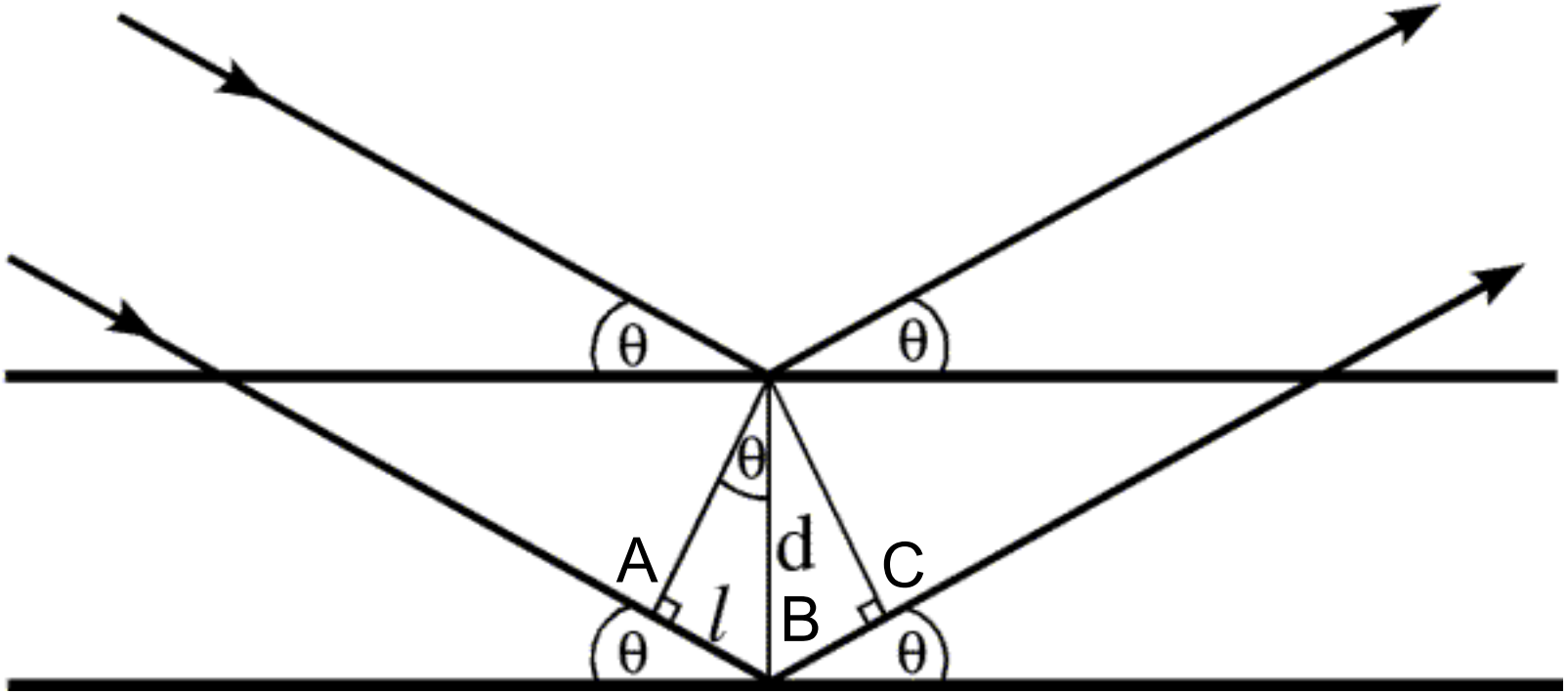
Recorded on hybrid pixel array detectors (old: X-ray sensitive film, imaging plates, CCD detectors)

Multiple images give a complete dataset

Crystal is exposed in different orientations

Consists of 10^4-10^6 spots, which differ in their intensity.

Bragg's Law



Constructive interference if extra distance is an integral (n) multiple of the wavelength (λ) :

$n\lambda = AB + BC = 2\,AB$

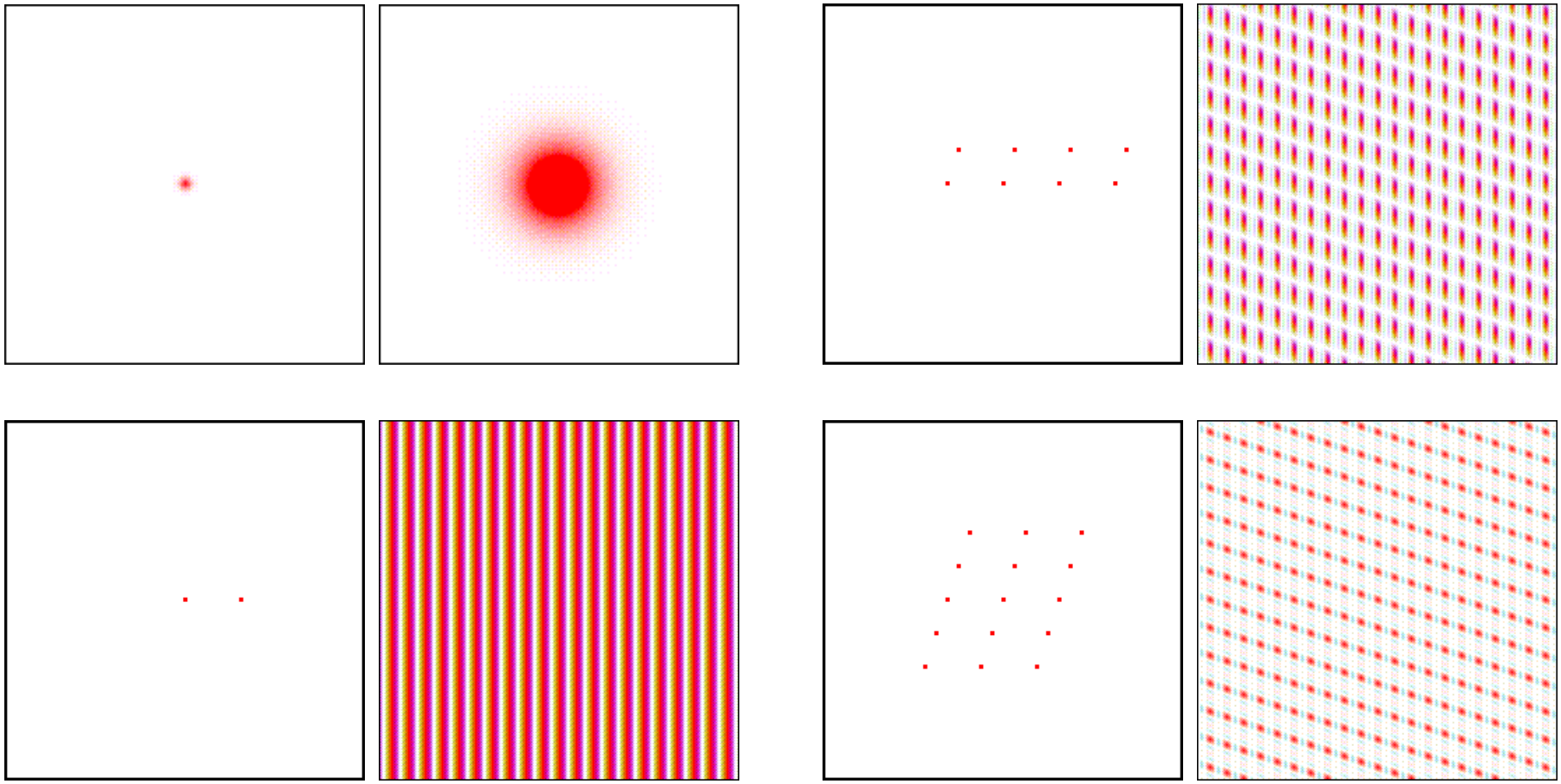
Remembering the definition of the sine function

$AB = d \sin\theta$

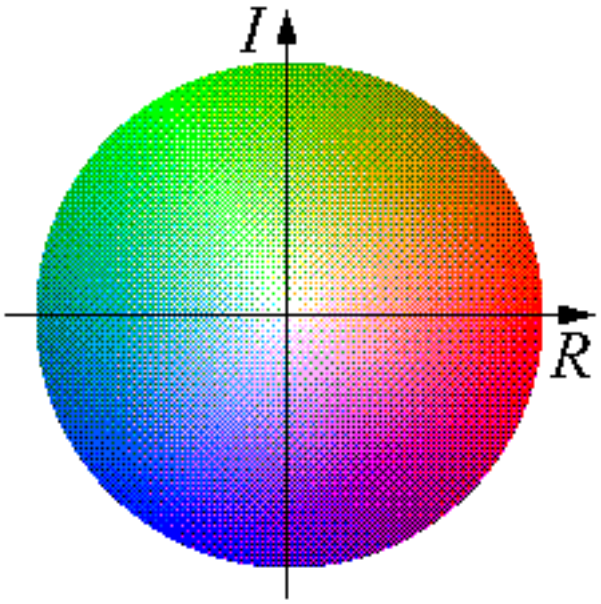
Combining the two equations yields Bragg's law:

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

A Lattice and its Diffraction Image



Representation of Phases and Amplitudes



Amplitude is represented by color saturation and brightness (small amplitudes: faded colors, large amplitudes: saturated colors)

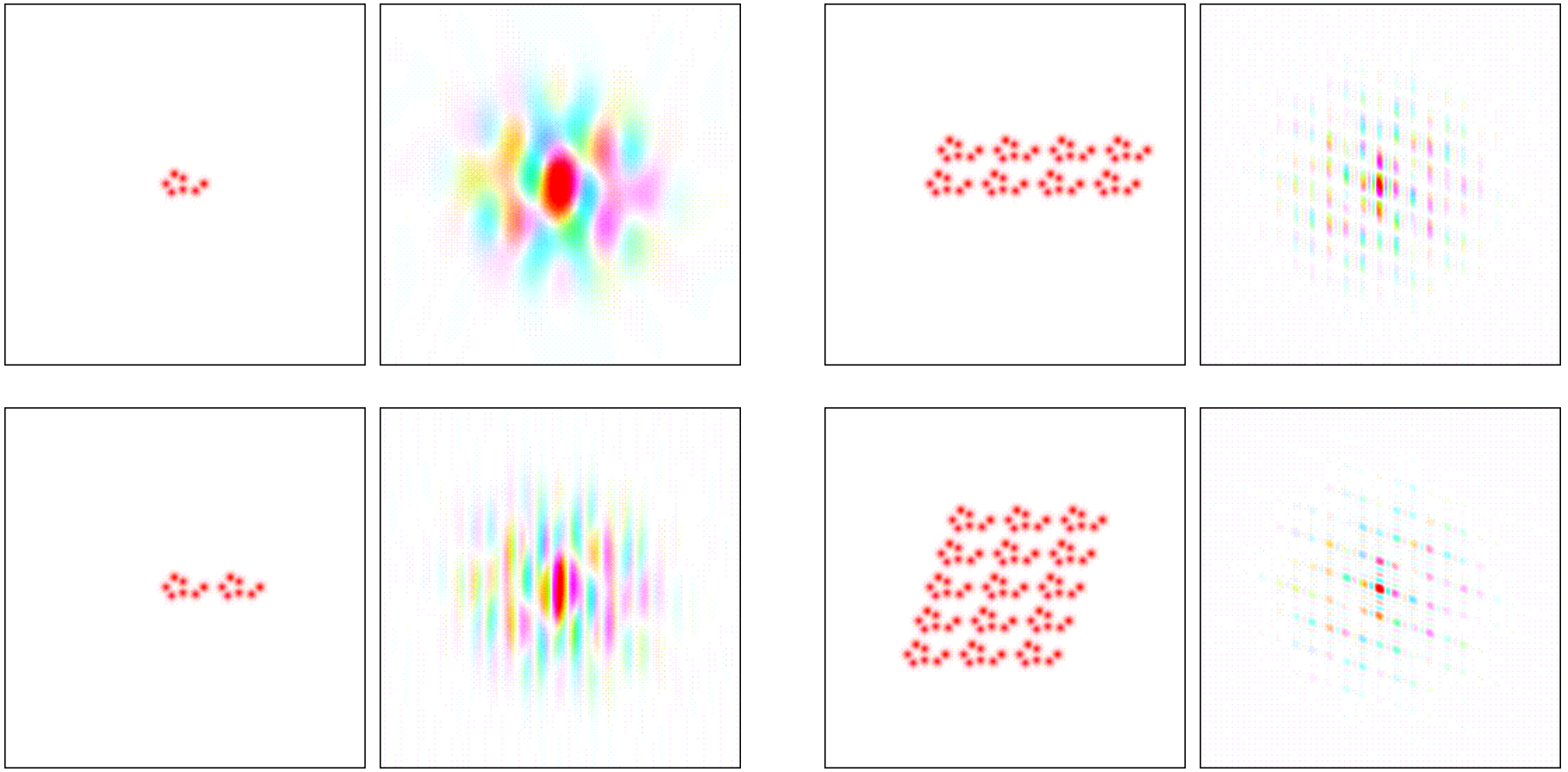
Phase is given by hue.

The positive real (horizontal) axis to the right represents a phase of 0.

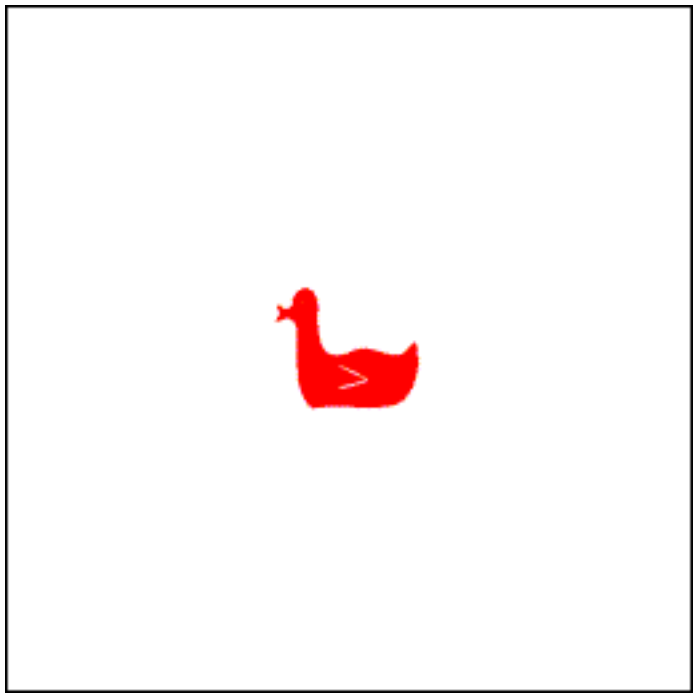
Phase increases anti-clockwise

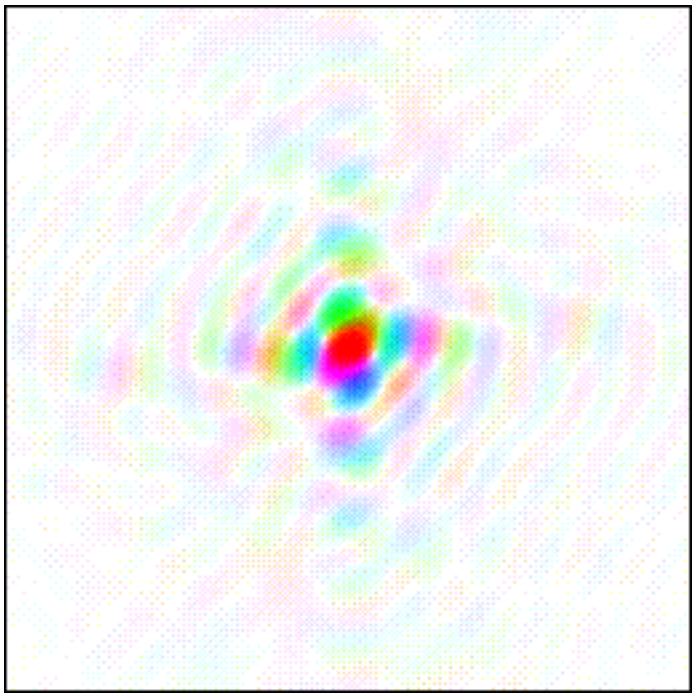
- 0 degrees is red
- 120 degrees is green
- 240 degrees is blue.

From Molecules to Crystals



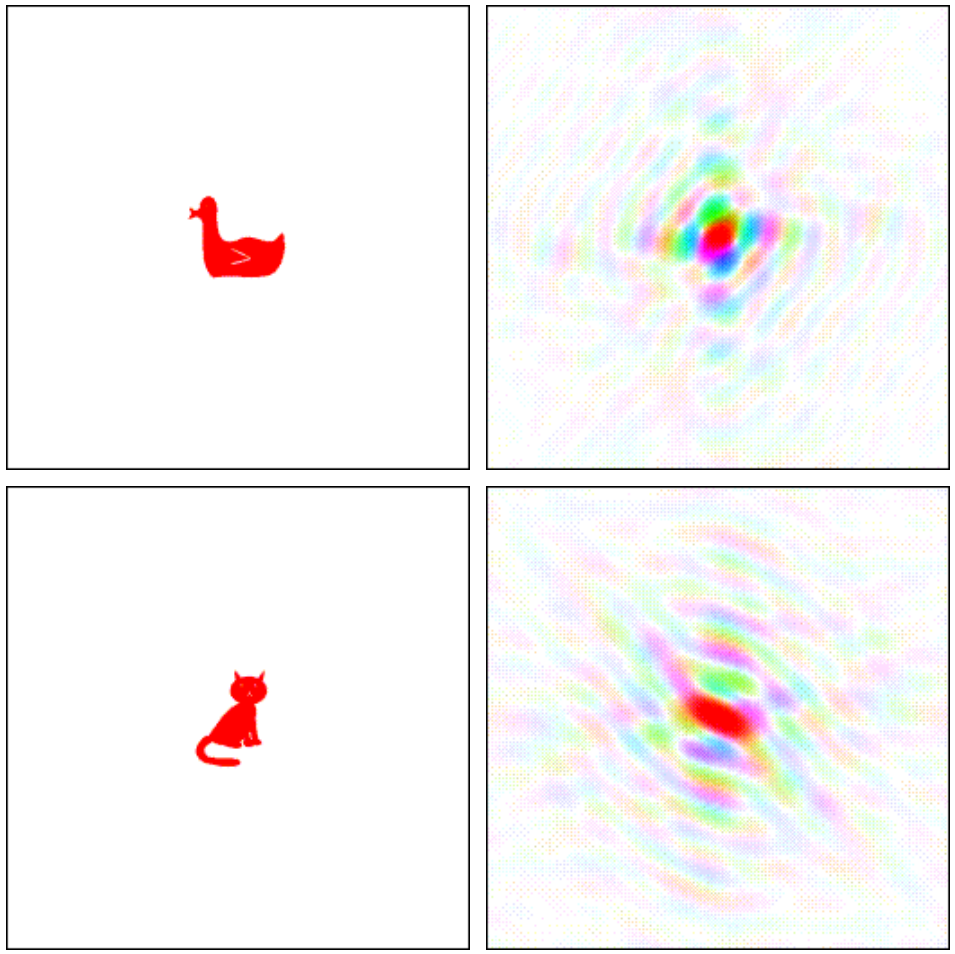
Duck Tales





The diffraction pattern of any object can be computed.

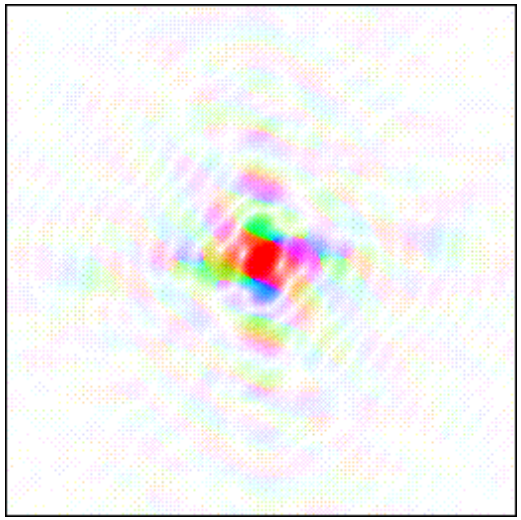
Animal Magic I

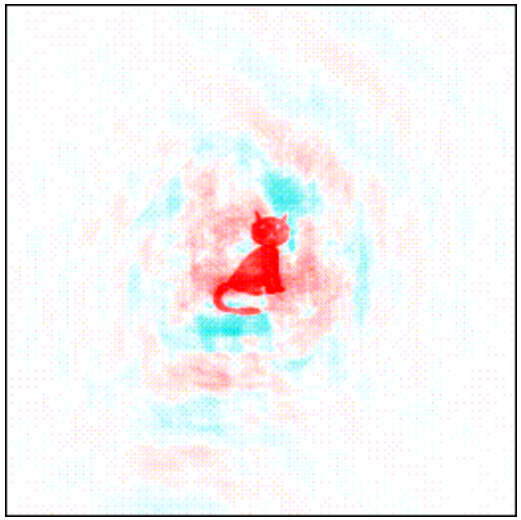


A duck and its Fourier Transform

A cat and its Fourier Transform

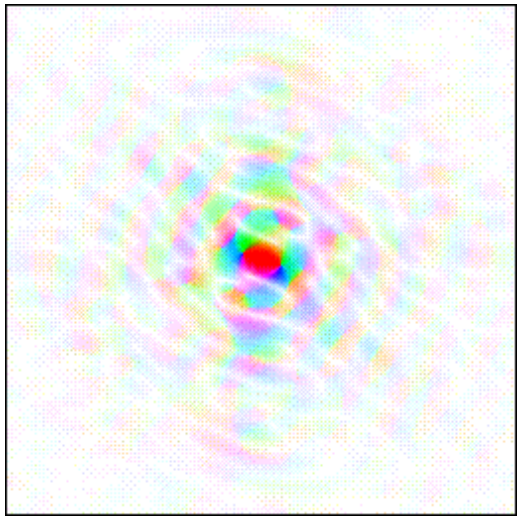
Animal Magic II

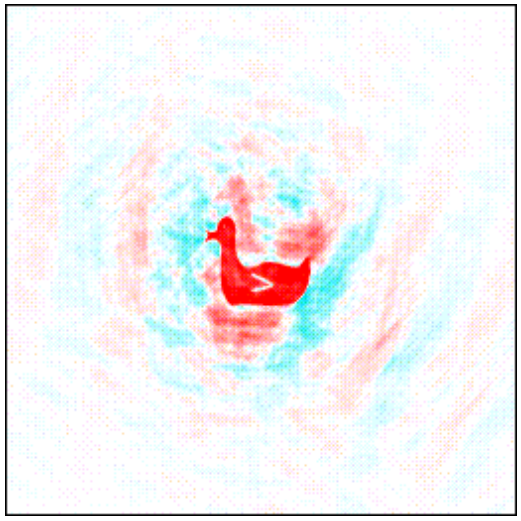




Duck magnitudes and cat phases reveal a:

Cat





Cat magnitudes and duck phases reveal a:

Duck

Structure Factor and Electron Density Equations

$$\mathbf{F}(\mathbf{h}) = \int_{cell} \rho(\mathbf{x}) \exp(2\pi i \mathbf{h}\cdot\mathbf{x}) d\mathbf{v} \qquad \rho(\mathbf{x}) = \frac{1}{V}\sum_{\mathbf{h}} \mathbf{F}(\mathbf{h}) \exp(-2\pi i \mathbf{h}\cdot\mathbf{x})$$

h is the vector (h,k,l) and **x** is the vector (x,y,z)
h•x = hx + ky + lz

Both operations are Fourier transformations, which are mathematical vehicles between real space (ρ(**x**)) and reciprocal space (**F**(**h**))

ρ(**x**) has units of electrons per Å^3 and **F**(**h**) of electrons. Specifically, F(000) is equal to the number of electrons in the unit cell. F(000) cannot be measured since it coincides with the primary beam

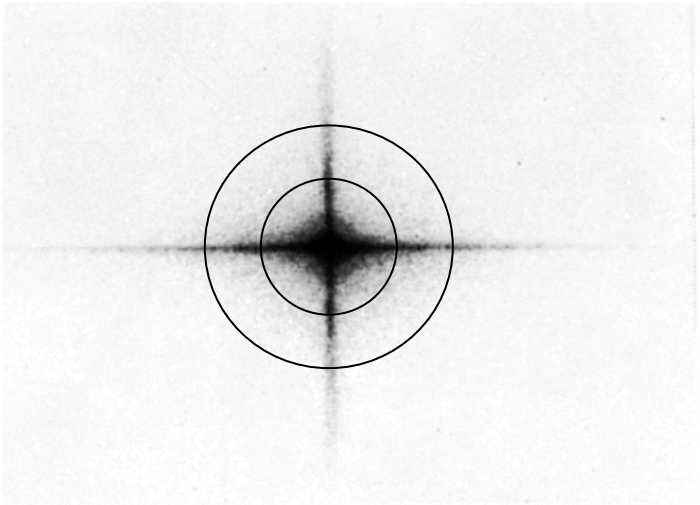
Fourier transformation of the electron density yields the structure factor and Fourier transformation of the structure factor yields the electron density

These operations are sometimes also referred to as Fourier synthesis and Fourier analysis

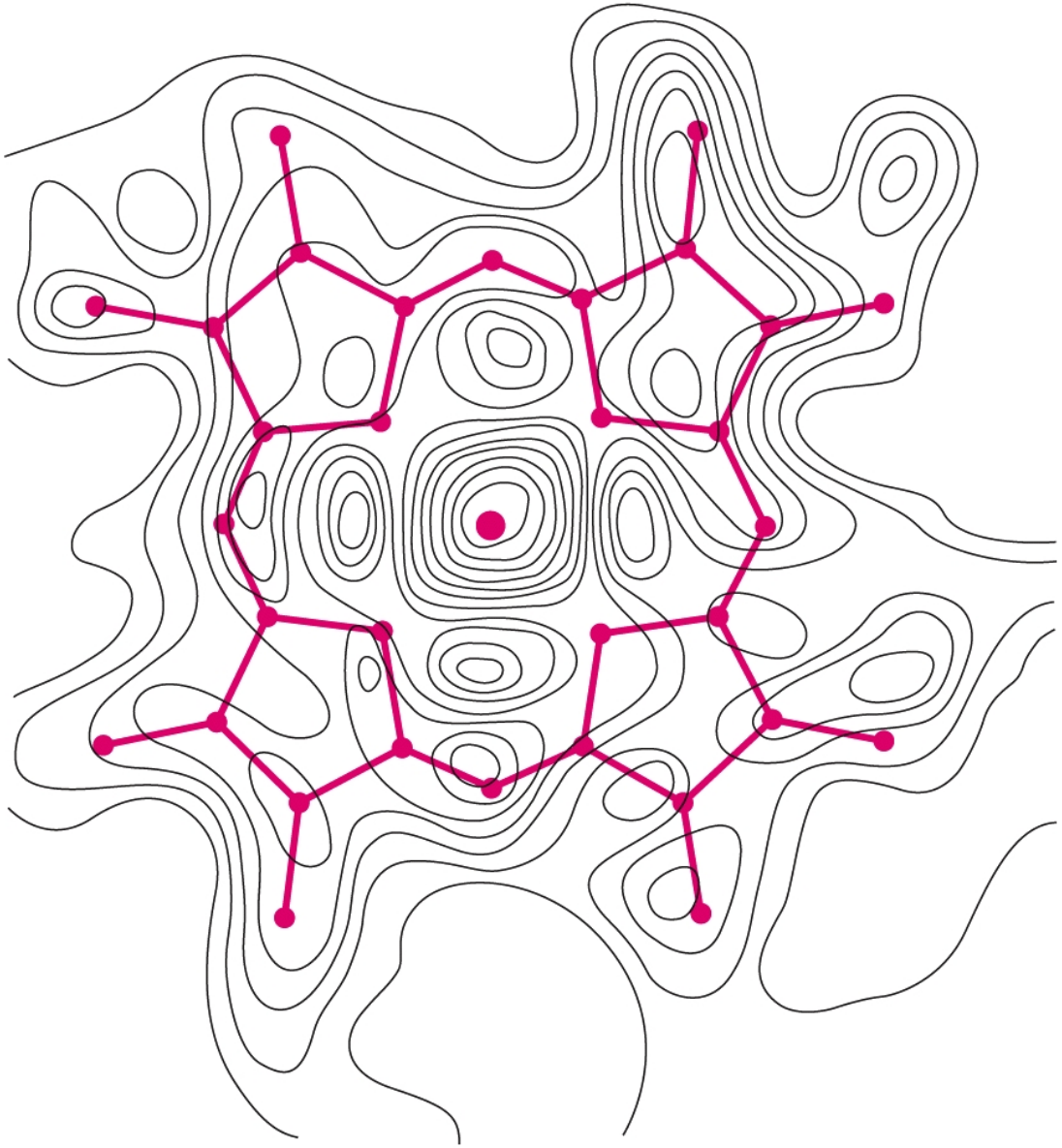
Resolution I

(A)

(B)



Electron Density Map



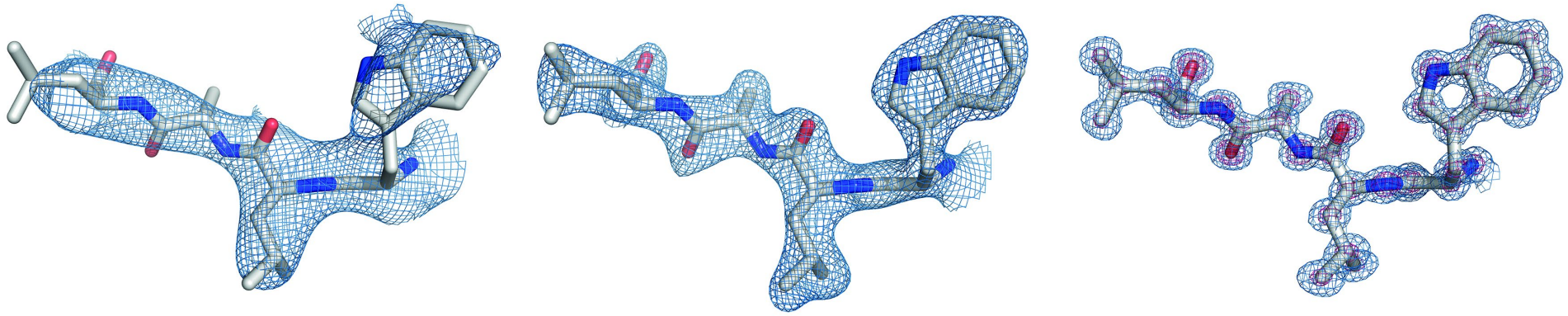
X-rays are diffracted by electrons

3D Map in general, here shown as a 2D slice

Higher contour levels for more electron dense atoms (Fe)

Atomic model has to be fitted to electron density map

Resolution II



Electron density maps at (A) 4 Å, (B) 2.5 Å and (C) 1 Å resolution. In the 1 Å map there is a low contour level in blue and a high contour level in red.

Quality of Refinement

Refinement progress has been traditionally monitored by the R-factor:

$$R = \sum_{hkl} | \, |F_o| - |F_c| \, | \; / \sum_{hkl} |F_o|$$

Typical R-values for macromolecular structures are ~0.2 (20%).

For a set of randomly distributed atoms in an acentric crystal: R = 0.586

R can be lowered artificially by refining additional parameters (B-factors when not appropriate, addition of spurious solvent molecules).

More recently the free R-factor (R_{free}) has been used, in which the R-factor is calculated for a subset of reflections which are never included in the refinement.

R_{free} is sensitive towards overfitting, i.e. the refinement of parameters not warranted at a given resolution.

Typical R_{free}-values for macromolecular structures are ~0.25-0.3 (25-30%).

The Final Model

Each crystal structure will result in a file containing the coordinates of all atoms together with their B-factor and occupancy.

Various graphic programs are available to display these models.

Two particularly useful programs are:

Swiss-PDBViewer: http://ca.expasy.org/spdbv/

Pymol: http://www.pymol.org/

The model will be analyzed to understand the biological function of the macromolecule.

Macromolecular structures are deposited in the Protein Data Bank:

http://www.rcsb.org or http://www.ebi.ac.uk/pdbe/

The Protein Data Bank can be searched to retrieve entries of interest.

Image Sources

J.A. Gatehouse

Jeremy M. Berg, John L. Tymoczko and Lubert Stryer “Biochemistry”, W.H. Freeman

Rigaku - X-ray Analytical Instrumentation
www.rigaku.com

European Synchrotron Radiation Facility (ESRF)
www.esrf.eu

Kevin Cowtan’s home page:
http://www.ysbl.york.ac.uk/~cowtan/fourier/fourier.html