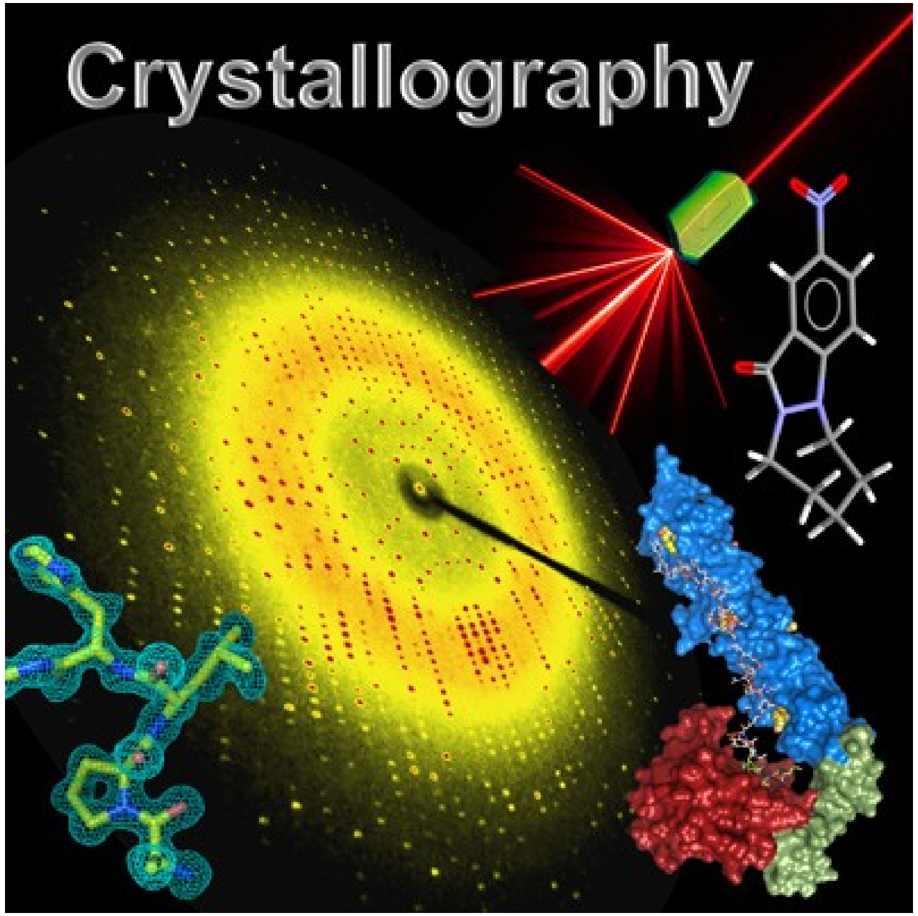
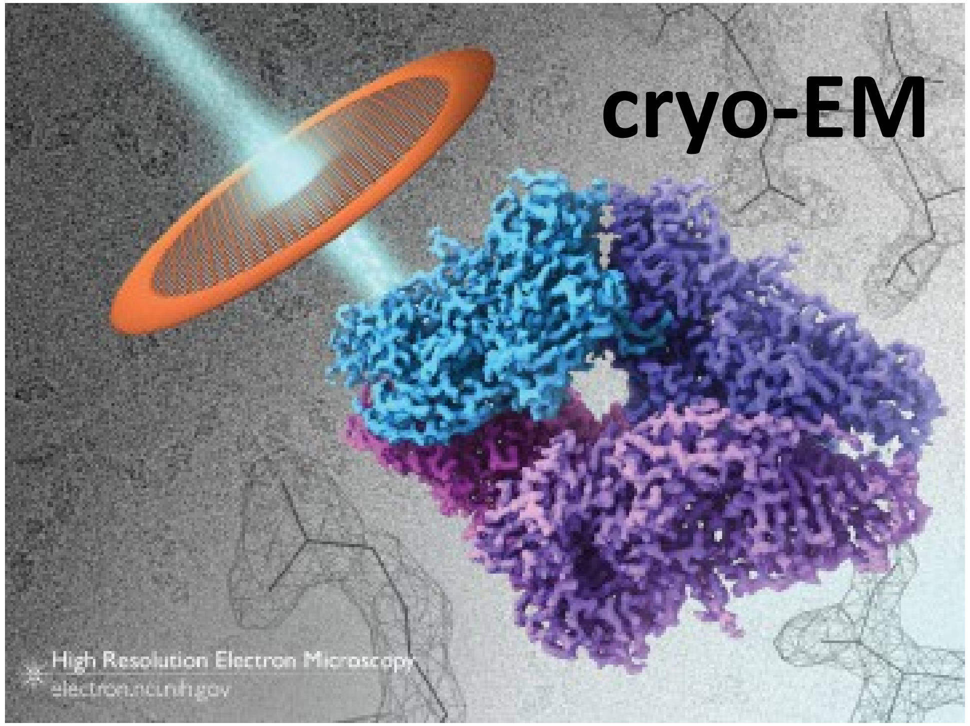
C7270

Biological X-Ray Crystallography and Cryo-Electron Microscopy

Fall 2022

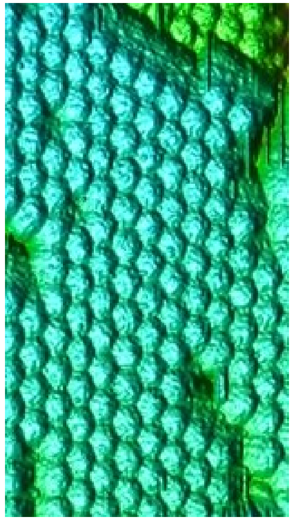
Pavel Plevka, Tibor Füzik, Jiří Nováček, Holger Stark,


Crystallography

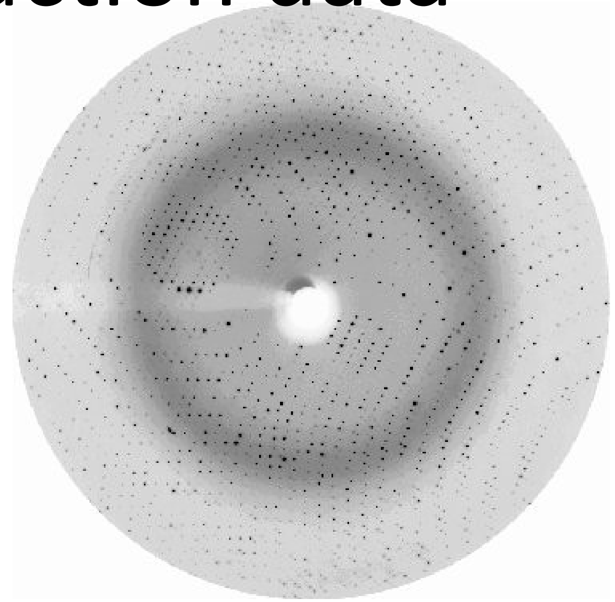

cryo-EM
High Resolution Electron Microscopy
electron.nci.nih.gov

1. Expression & purification

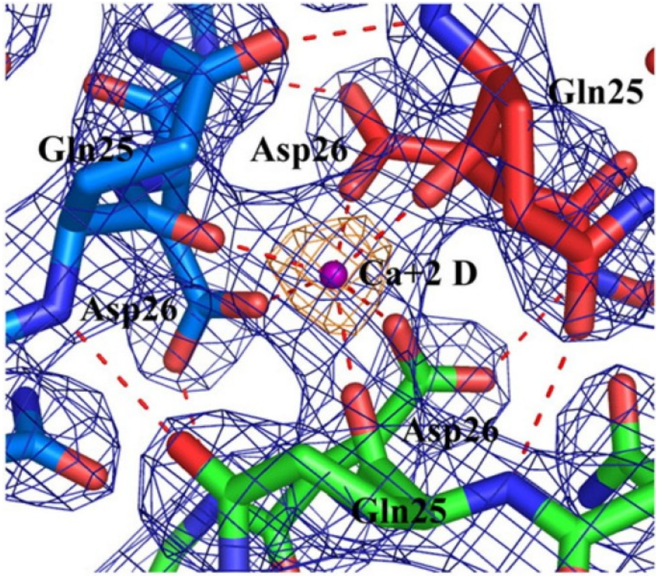
2. Crystallization



3. Diffraction data

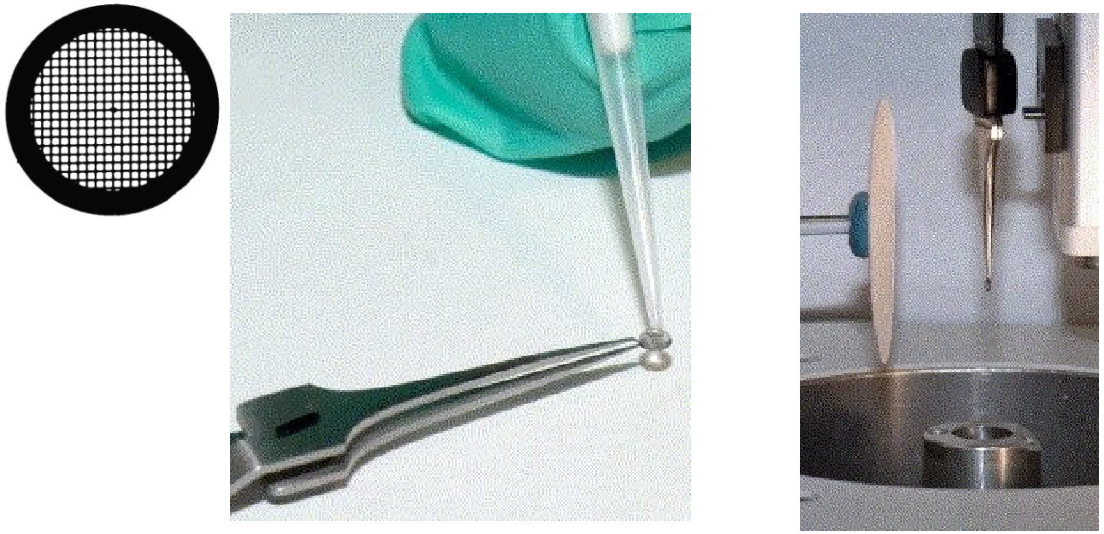


4. Solve structure

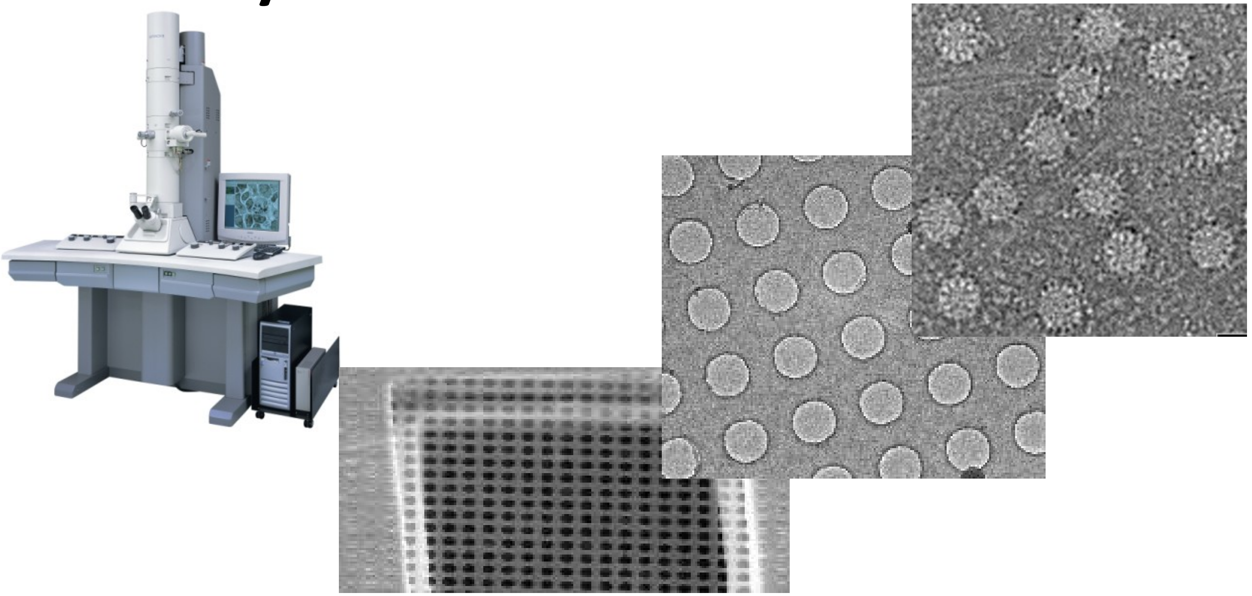


1. Expression & purification

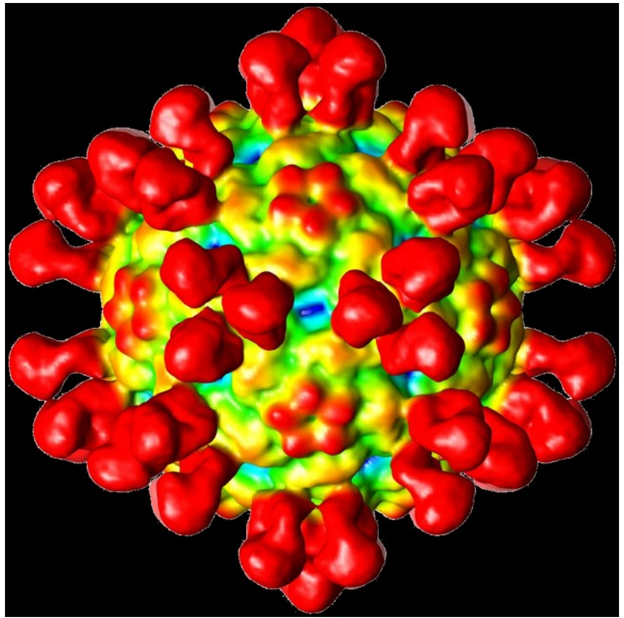
2. Grid preparation



3. cryo-EM data



4. Reconstruction



Aims of the course

- Diffraction of light
- Approaches to resolve phase problem in crystallography
- Use of electrons to display objects with high magnification and fine detail
- Calculation of three-dimensional reconstruction from two-dimensional projections

What is asked of you:

- Be present and awake
- Participate in discussions
- I am here to help, learning is up to you!

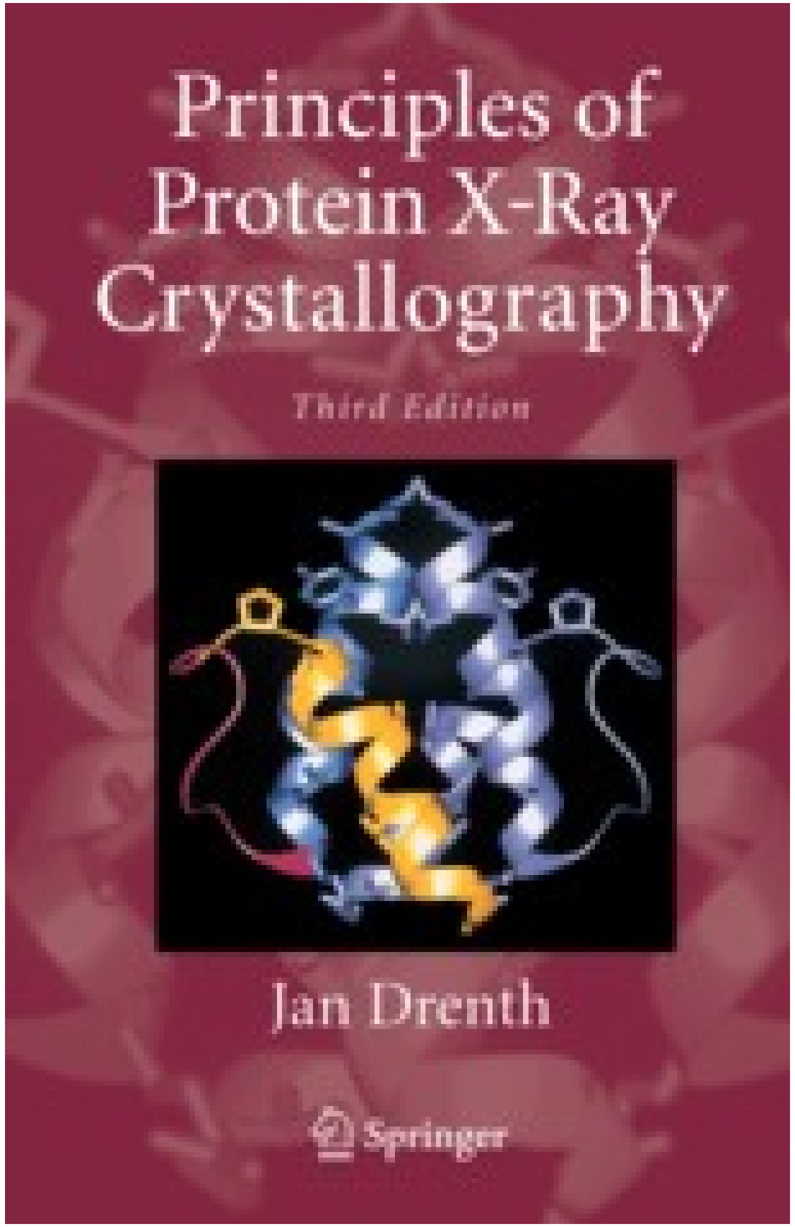
- Ask questions - it will help to clarify the issue not only for you but for your peers as well!
- In class discussions, be respectful of other students' opinions.

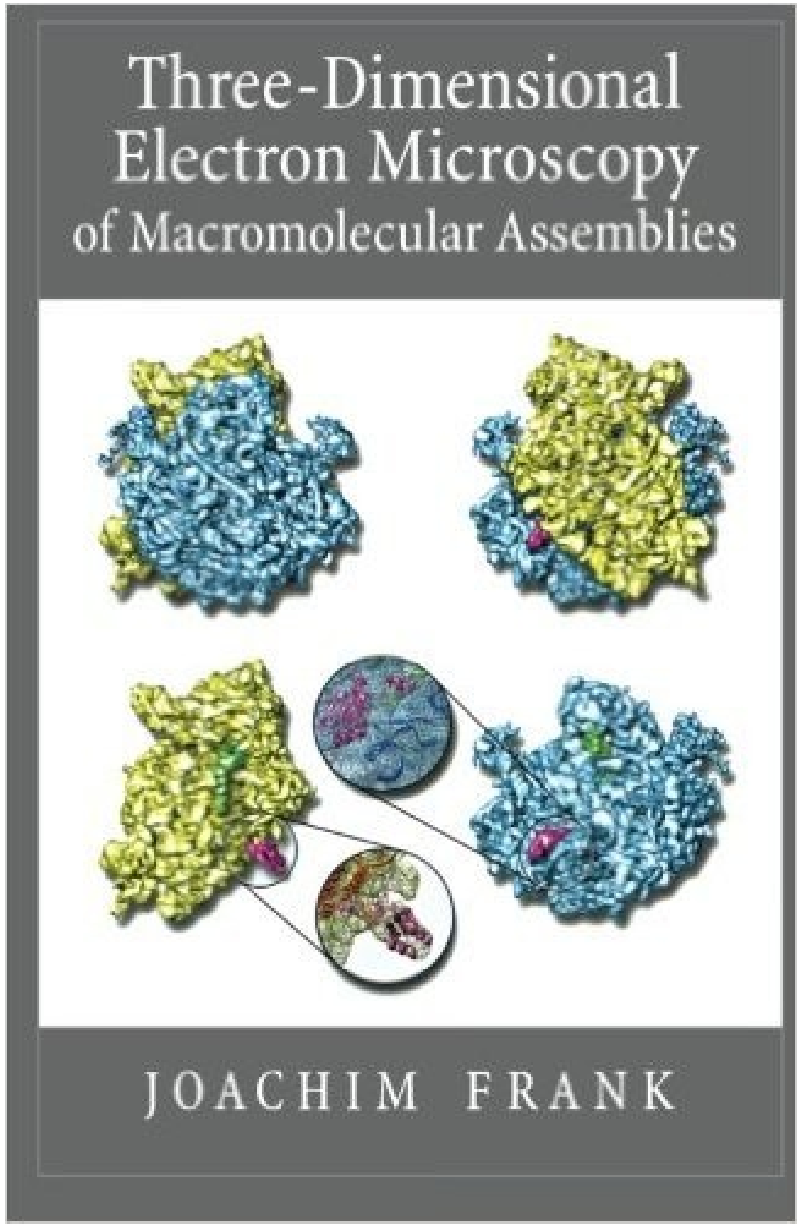
Not part of this course:

- Basic math – mental overload by dealing with simple equations. (Observed before.)

- Reserve time for thinking.

Course textbooks:



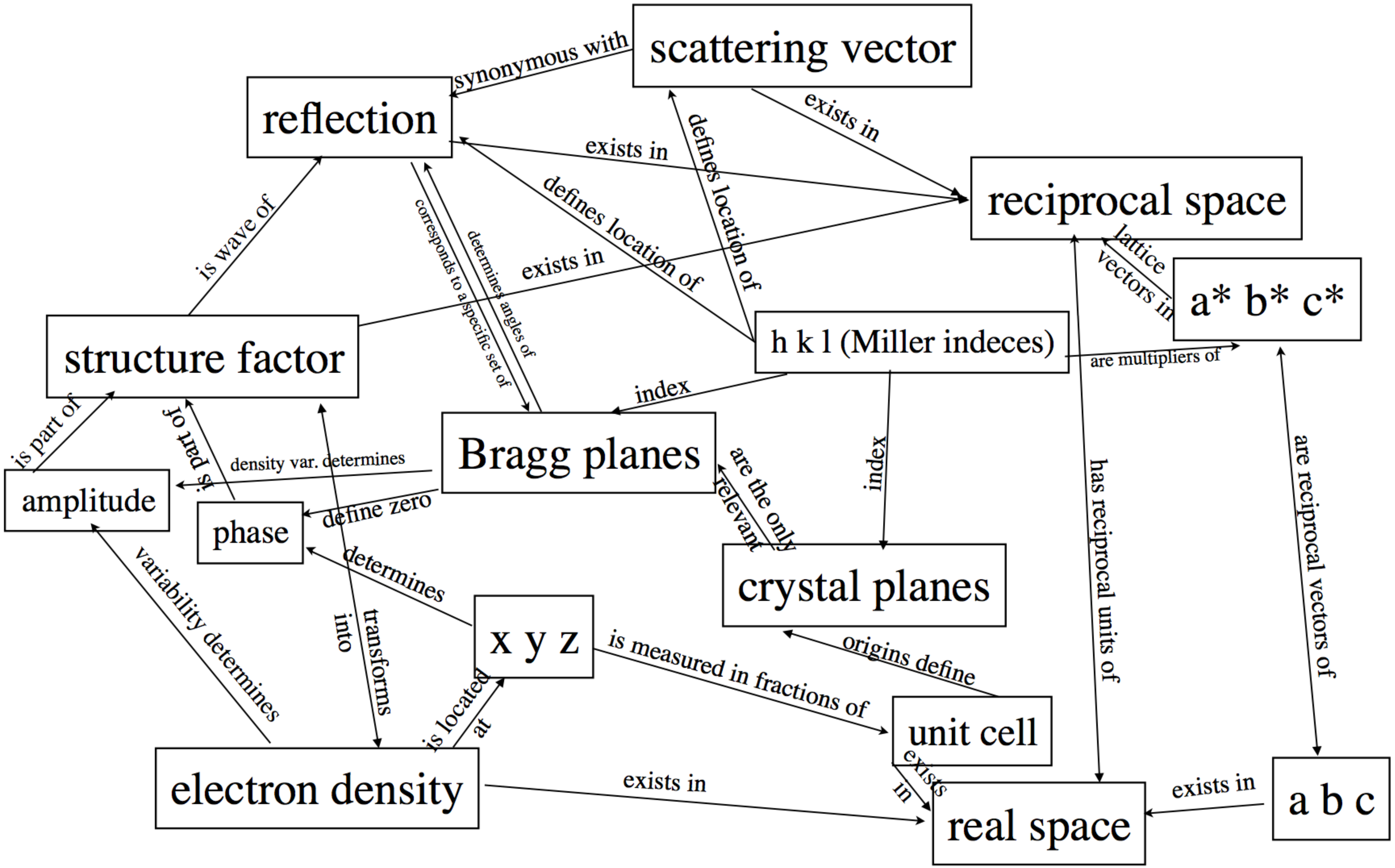


Course plan

L#	Date	Time	Lecturer	Topic	Chapter reading
1	13.9.	12:00-13:25	Pavel Plevka	Development of X-ray crystallography, crystallization of macromolecules, phase diagram, Crystal symmetry, symmetry operators, point groups, space groups.	Drenth: 1, 2, 3
2	20.9.	12:00-14:30	Pavel Plevka	Diffraction of light by electrons, atoms, unit cell, crystal. Bragg's law. Diffraction images and indexing.	Drenth: 4
	27.9.			No lecture	
3	4.10.		Pavel Plevka	Fourier transform, structure factor, intensity of diffraction spots.	Drenth: 4, 5
4	11.10.		Pavel Plevka	Solutions to phase problem in X-ray crystallography. Isomorphous replacement, SAD, MAD, Molecular replacement. Rotation and translation function. Model building and refinement.	Drenth: 7, 10
5	18.10.		Tibor Füzik	Electron microscope. Interaction of electrons with matter, electron imaging. Amplitude and phase contrast. Contrast transfer function.	Frank: 1, 2
6	25.10.		Tibor Füzik	Fourier transform and its properties, convolution, point spread function.	Frank: 2
7	1.11.		Jiří Nováček	Analysis of electron micrographs. 2D classification. Principal component analysis.	Frank: 3, 4
8	8.11.		Jiří Nováček	Three dimensional reconstruction - single particle reconstruction and tomogram calculation. 3D classification.	Frank: 5
9	15.11		Jiří Nováček	Improving cryo-EM reconstruction, particle polishing, Ewalds, sphere correction, per particle CTF, … Model building and refinement. Detection of errors, validation and detection of mistakes.	Frank: 6
10	TBD	TBD	Holger Stark	State-of-the-art cryo-EM of macromolecular complexes.	
11	TBD	TBD	Holger Stark	State-of-the-art cryo-EM of macromolecular complexes.	

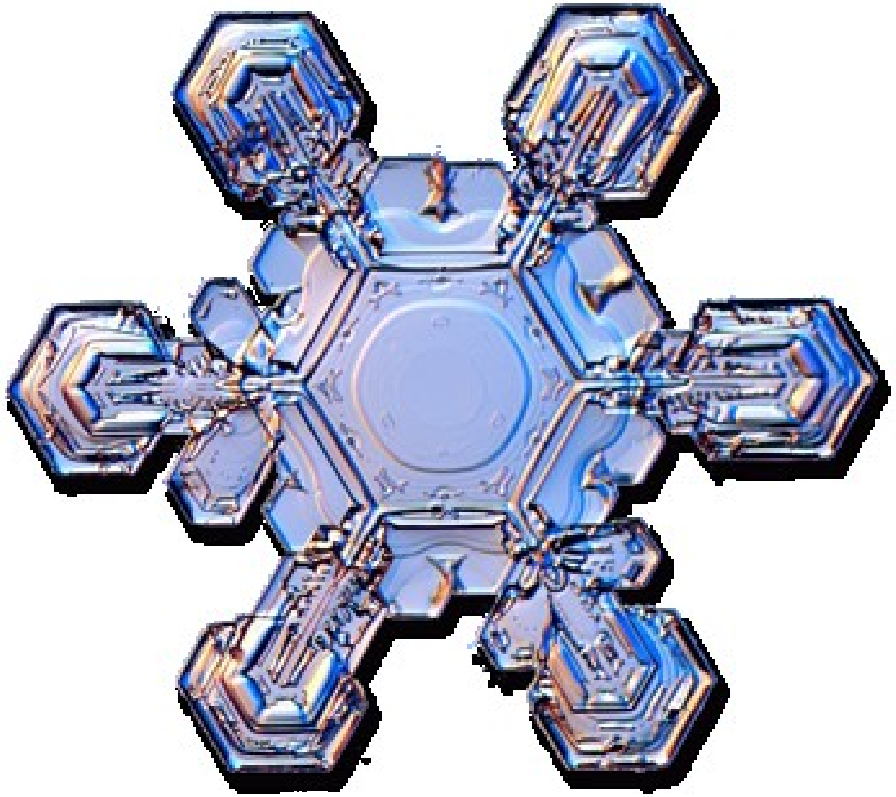
Can we start at 14:00 or 14:30?

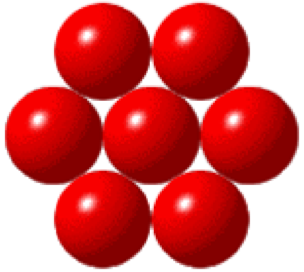
Biological X-Ray Crystallography


scattering vector
synonymous with
reflection
exists in
exists in
defines location of
defines location of
reciprocal space
lattice vectors in
a* b* c*
is wave of
corresponds to a specific set of
determines angles of
exists in
structure factor
h k l (Miller indeces)
are multipliers of
index
index
is part of
is part of
density var. determines
Bragg planes
amplitude
phase
define zero
are the only relevant
has reciprocal units of
are reciprocal vectors of
crystal planes
determines
variability determines
into
transforms
x y z
is measured in fractions of
origins define
unit cell
is located at
exists in
electron density
exists in
real space
exists in
a b c

Why do single snowflakes, before they become entangled with other snowflakes, always fall with six corners? Why do snowflakes not fall with five corners or with seven?

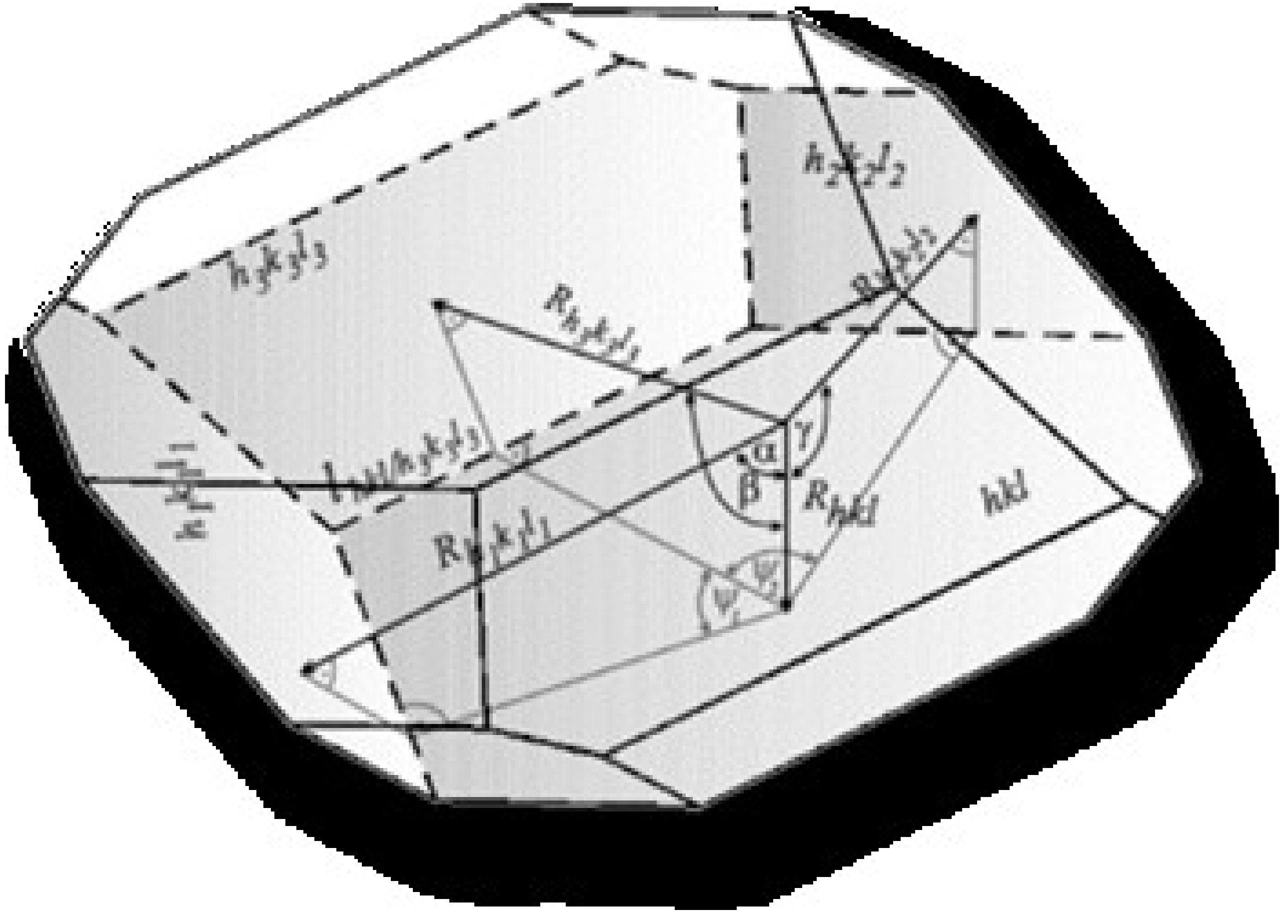
Johannes Kepler (1571-1630)



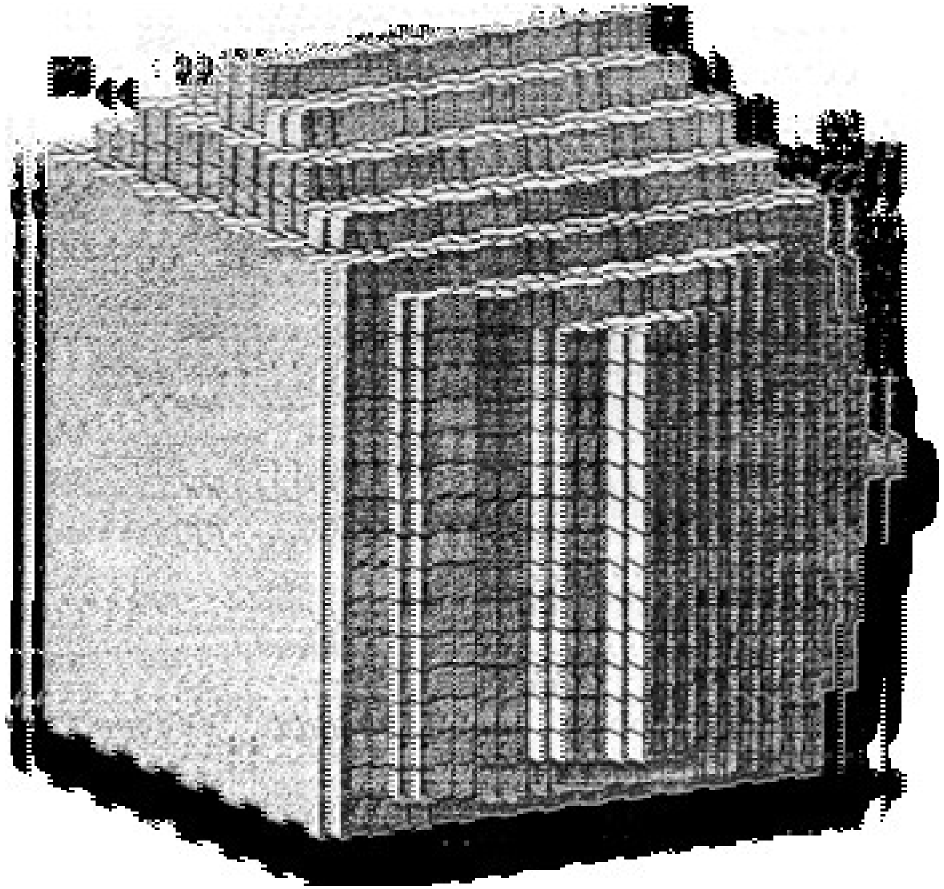


Although crystals of quartz and hematite appear in a great variety of shapes and sizes, the same interfacial angles persisted in every specimen. "*Law of Constancy of Angles*"

Niels Stensen (1638-1686)



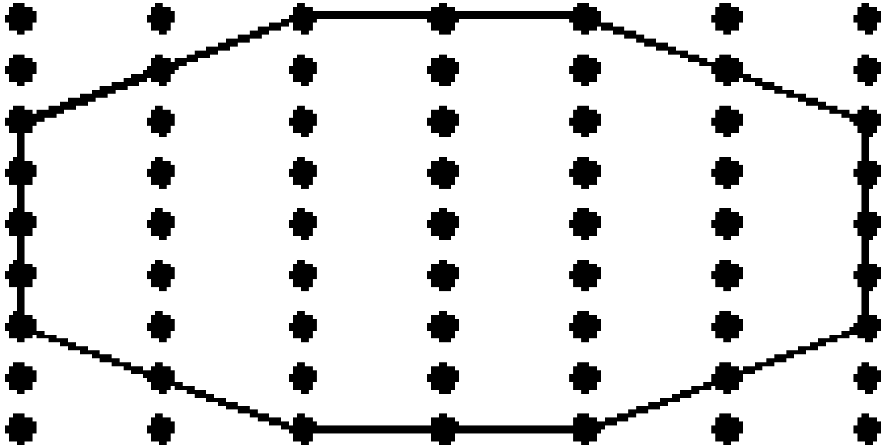
“Law of Constancy of Angles”

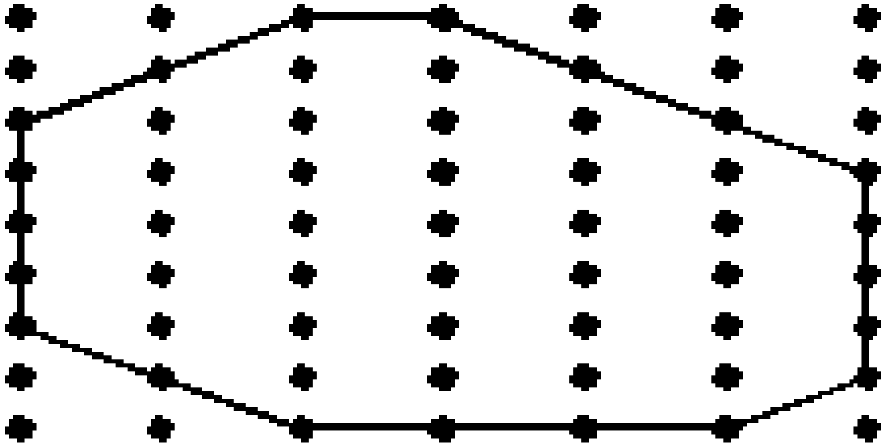


René Just Haüy (1743-1822)

“Law of Constancy of Angles”

René Just Haüy (1743-1822)



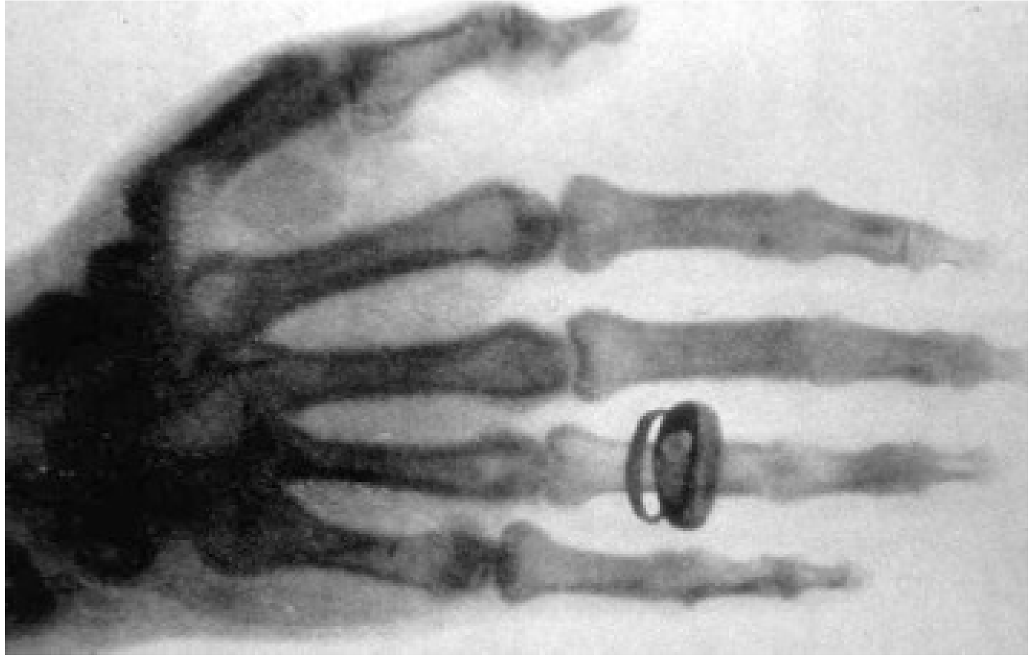


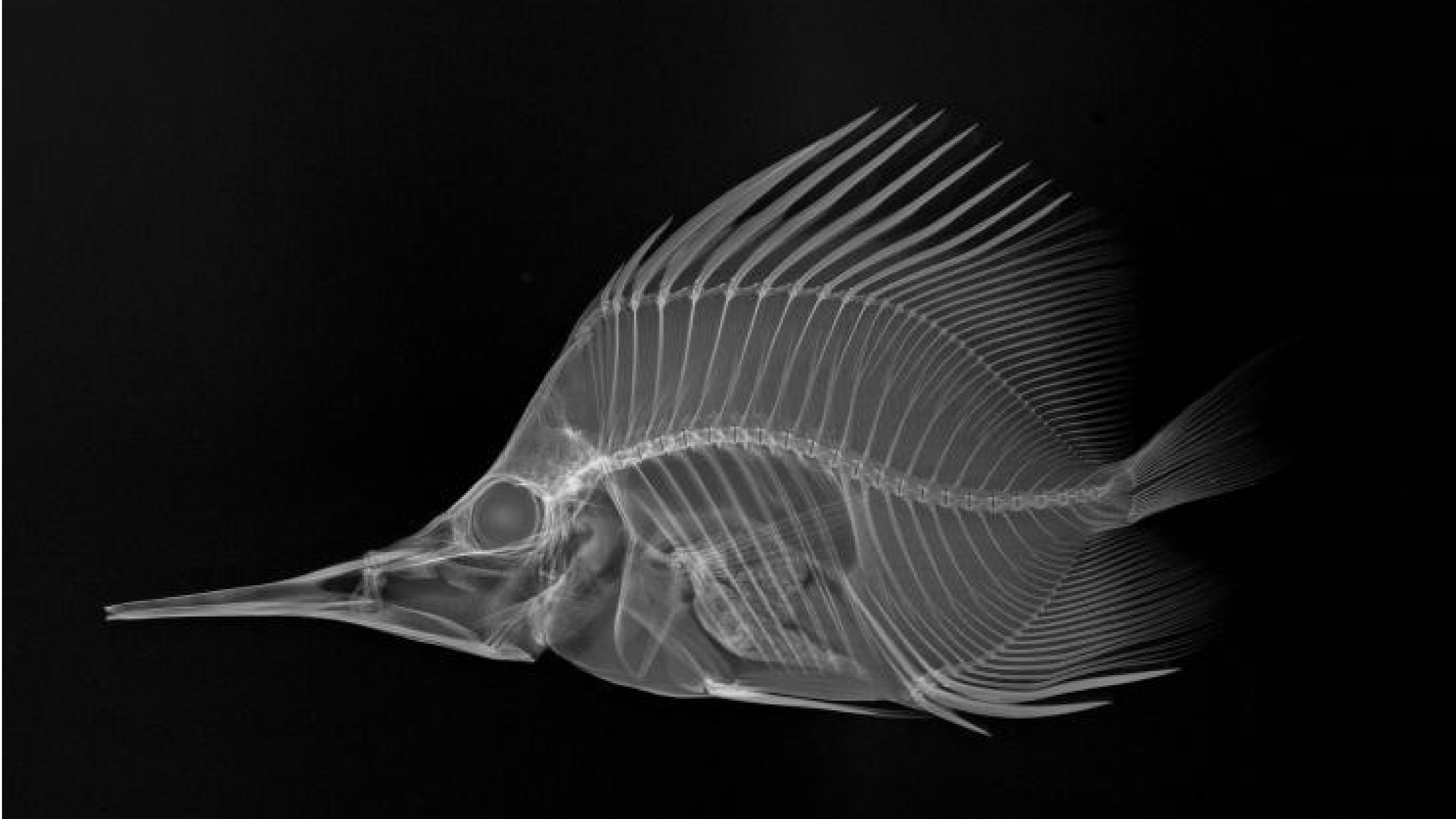
History of fundamental discoveries

WILHELM CONRAD RÖNTGEN (1845-1923)

- **1901 Nobel Laureate in Physics**

  discovery of the remarkable rays subsequently named after him

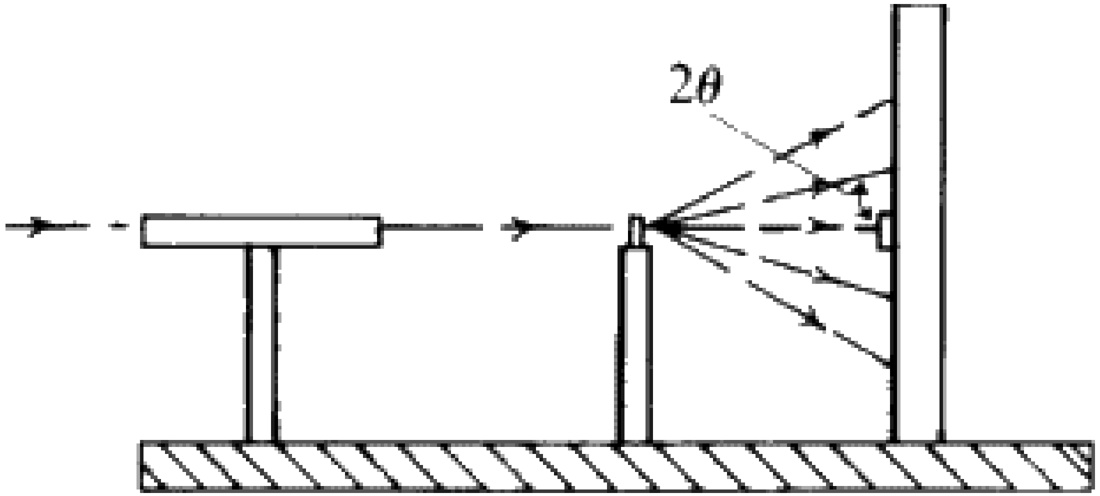




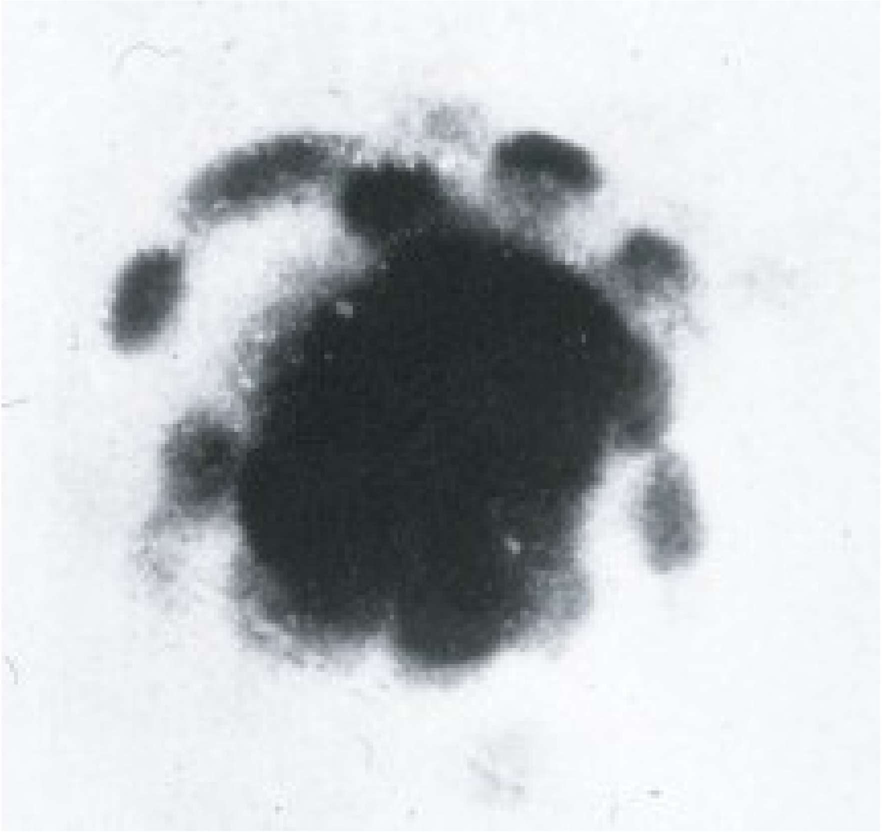
MAX VON LAUE (1879-1960)

- **1914 Nobel Laureate in Physics**

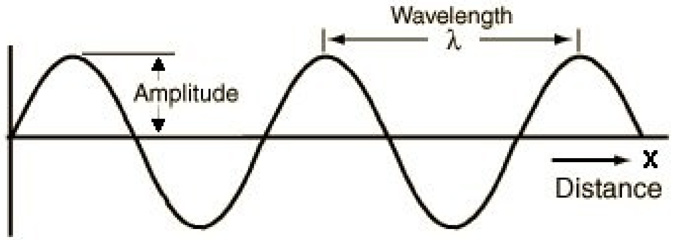
  for his discovery of the diffraction of X-rays by crystals

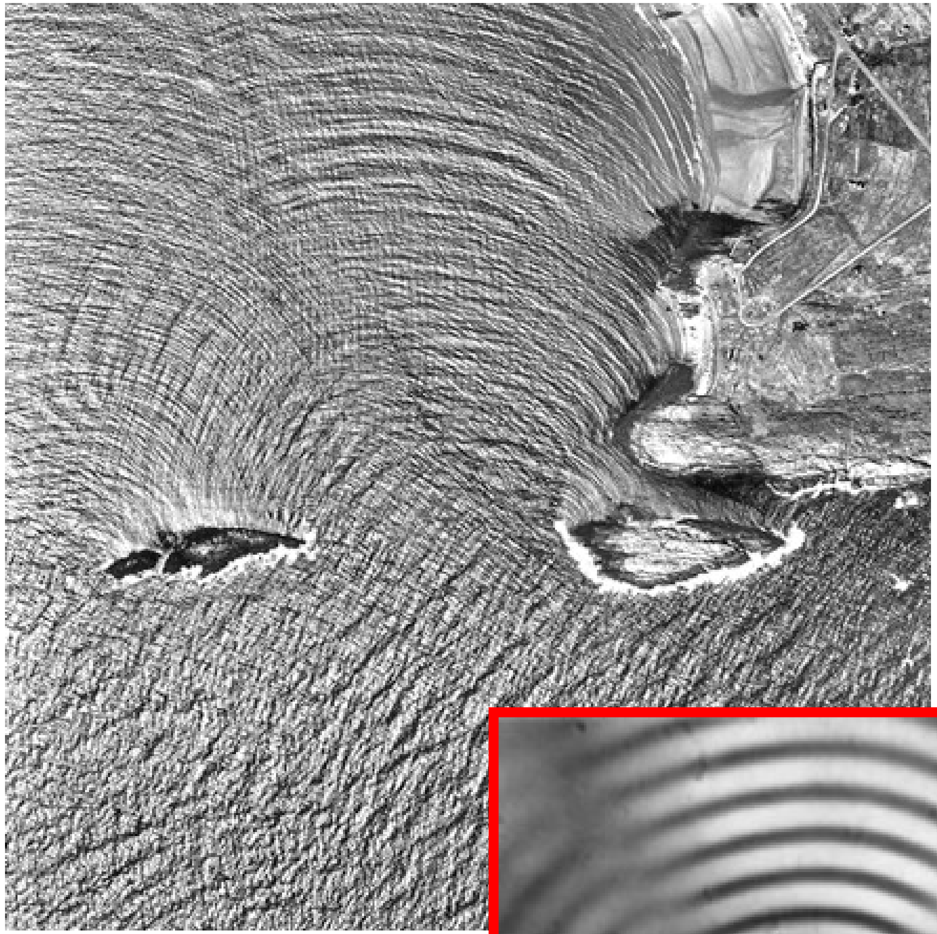


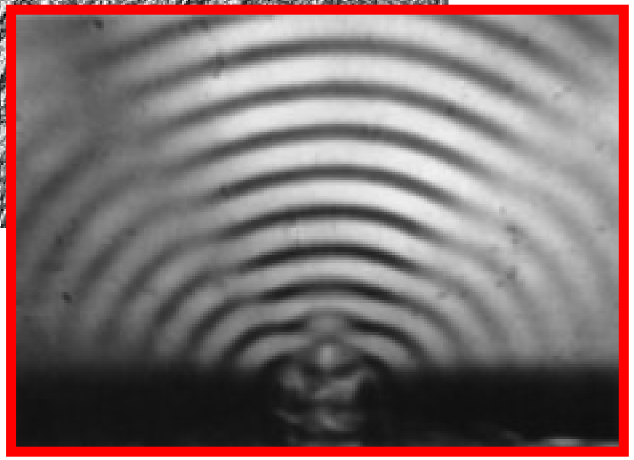
Friedrich and Knipping



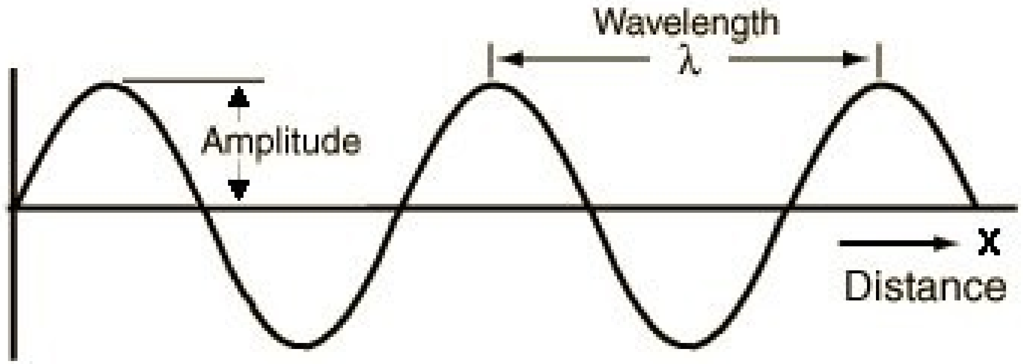
Wavelength and diffraction

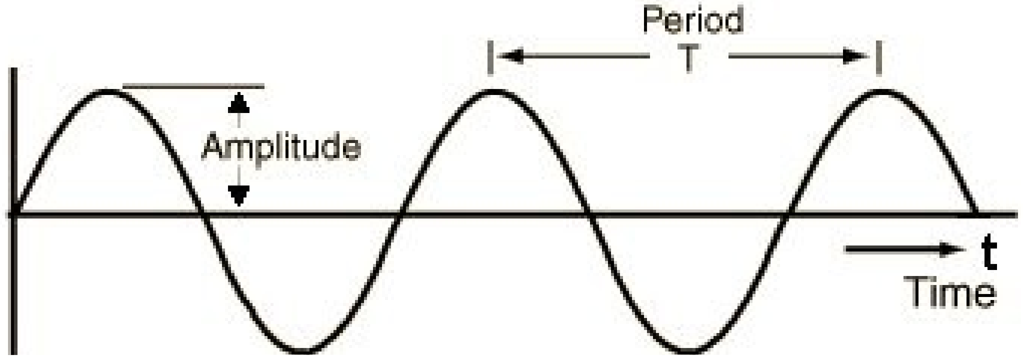




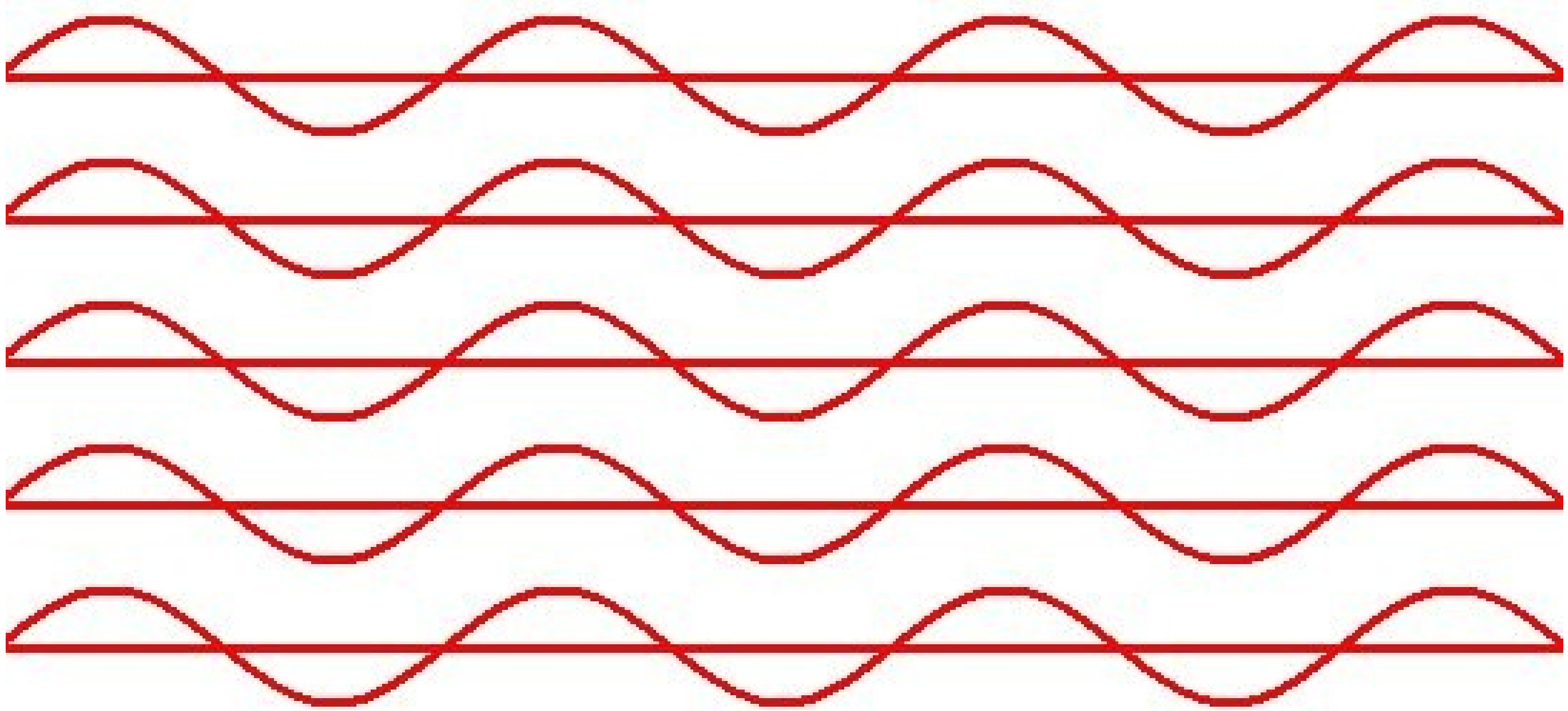


Waves

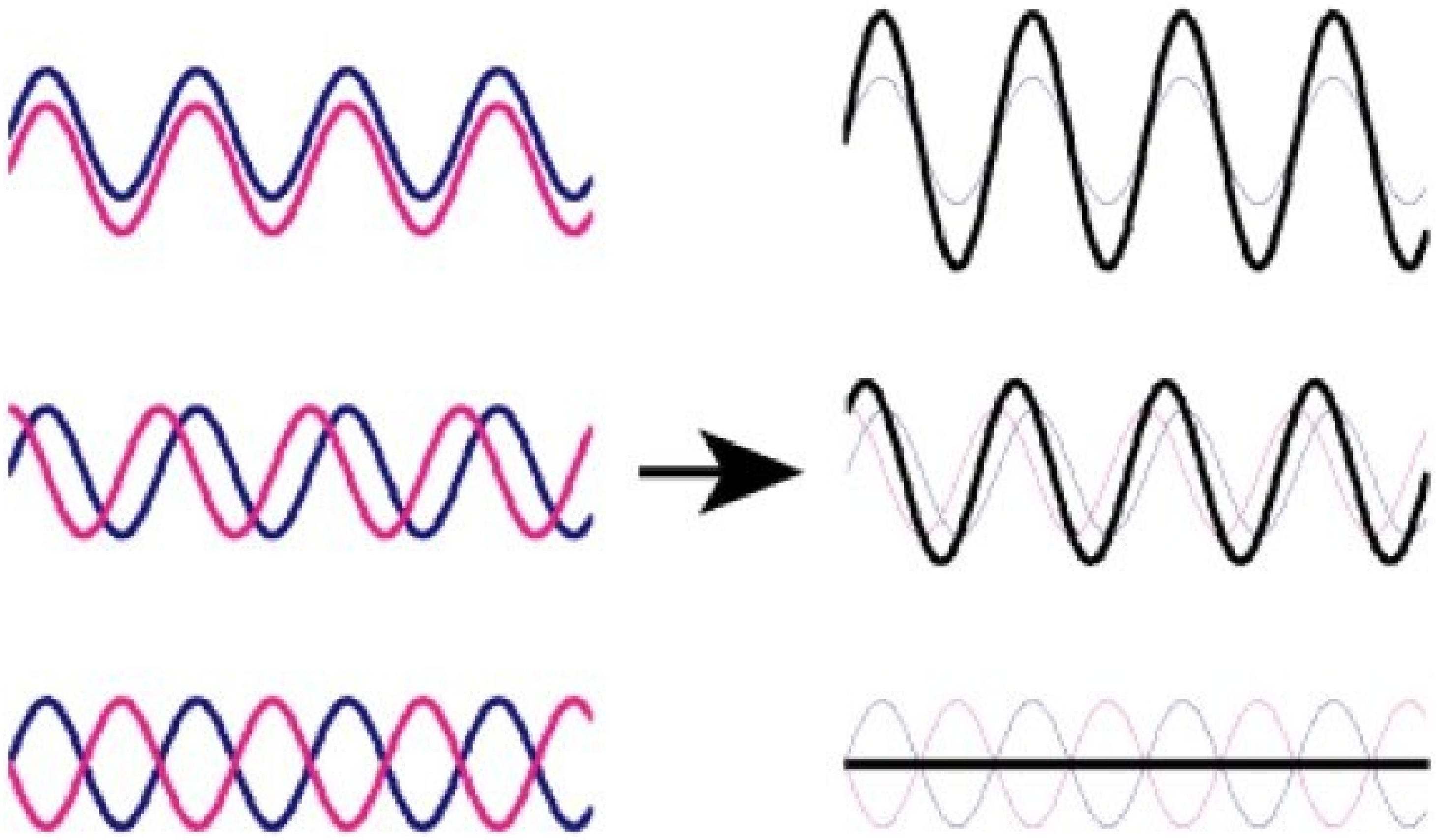

Wavelength
λ
Amplitude
x
Distance


Period
T
Amplitude
t
Time

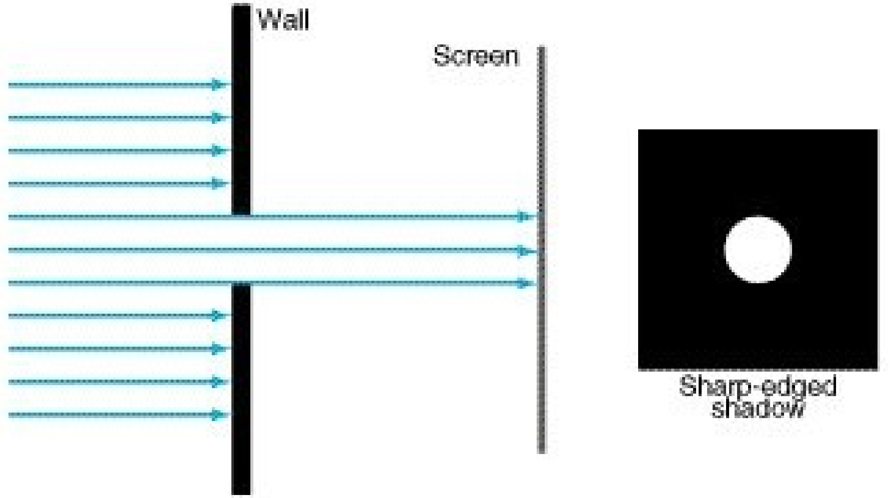
Coherent beam

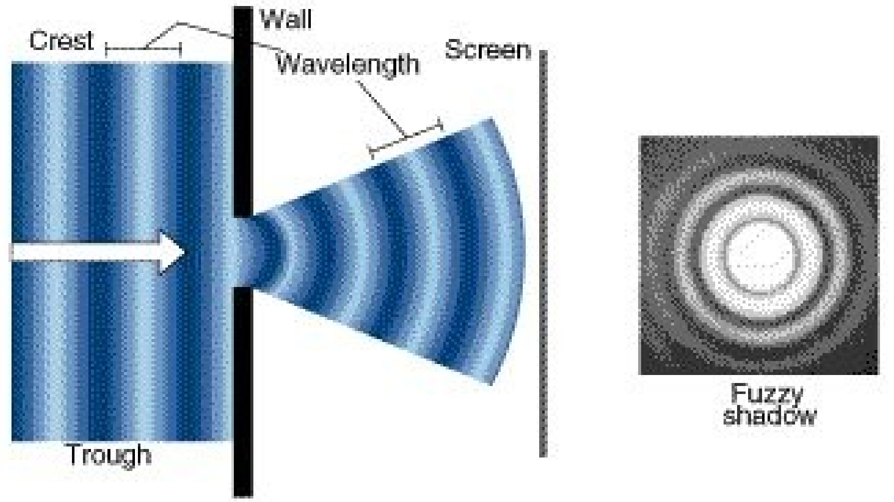


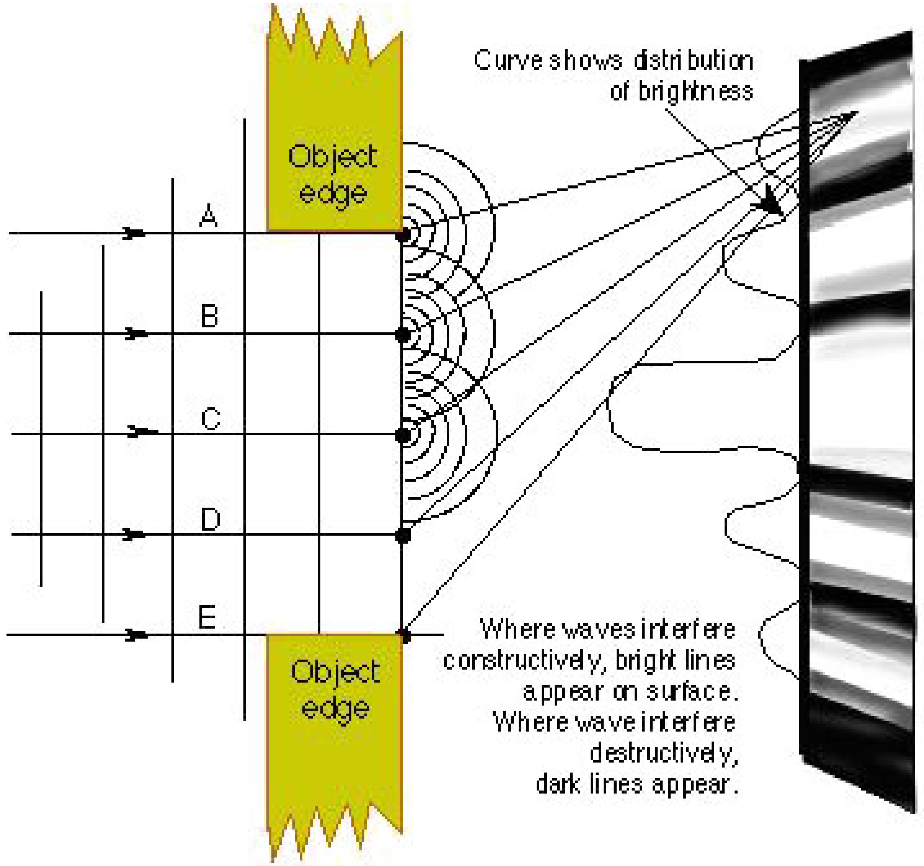
Addition of waves



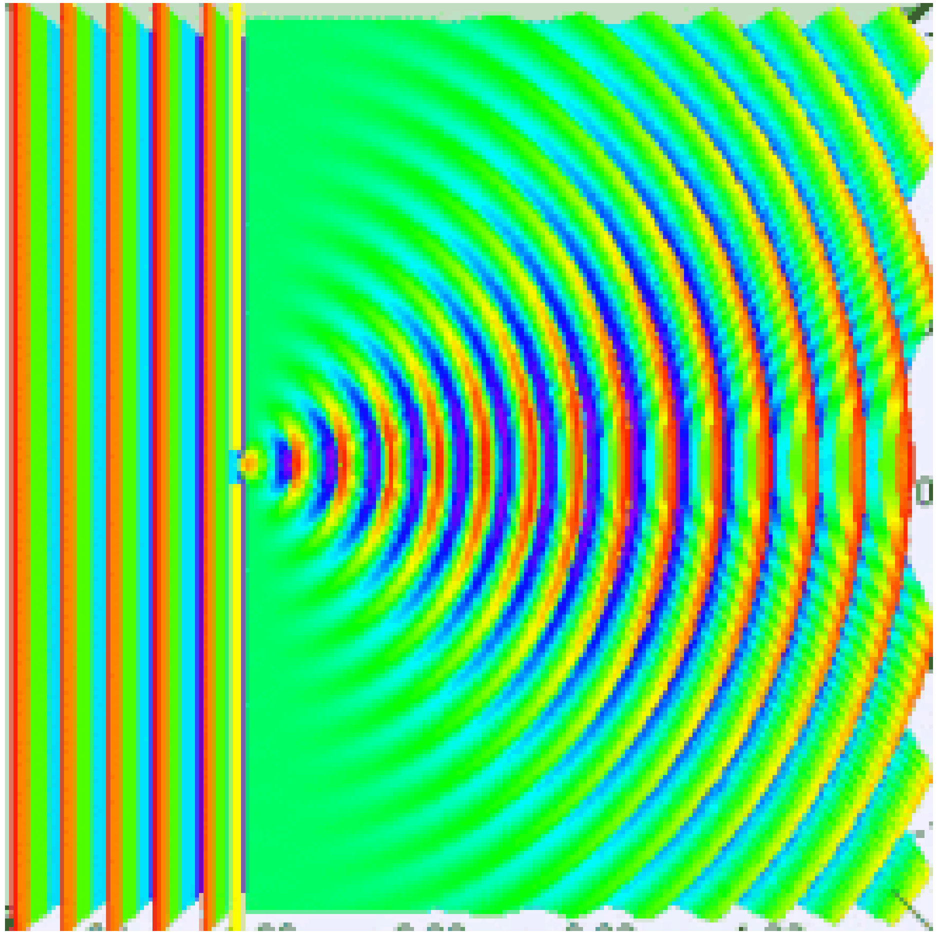
Particles & waves

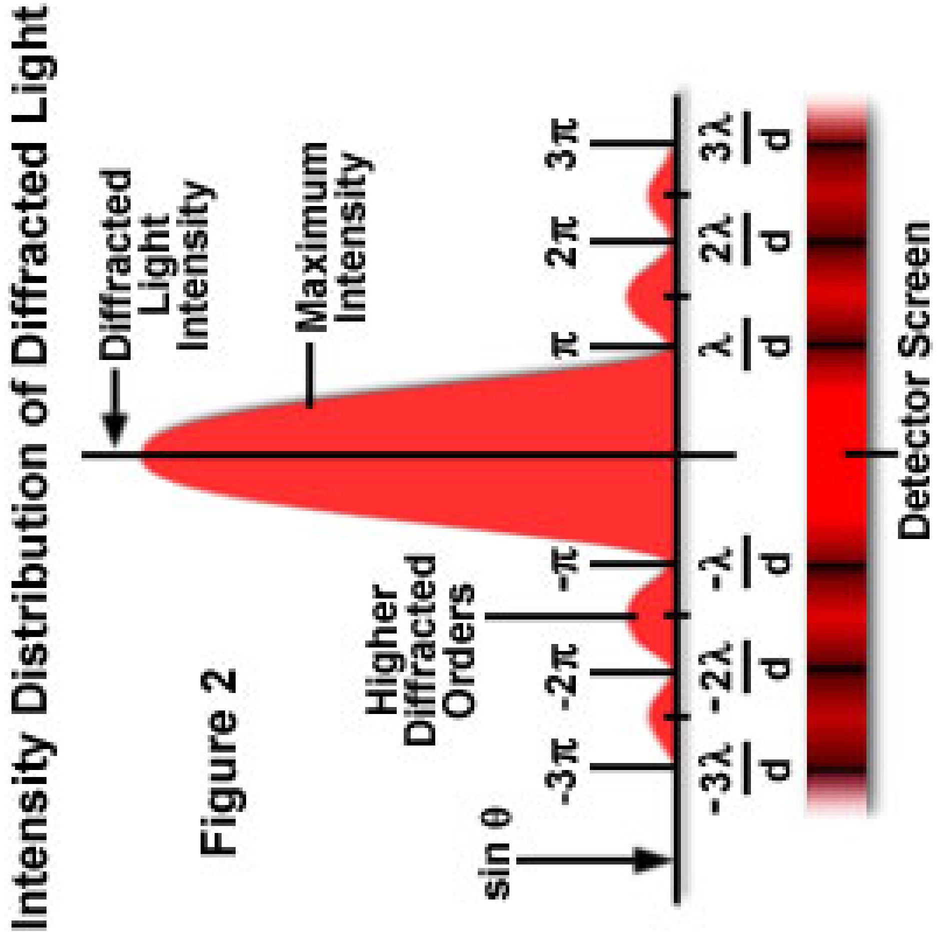




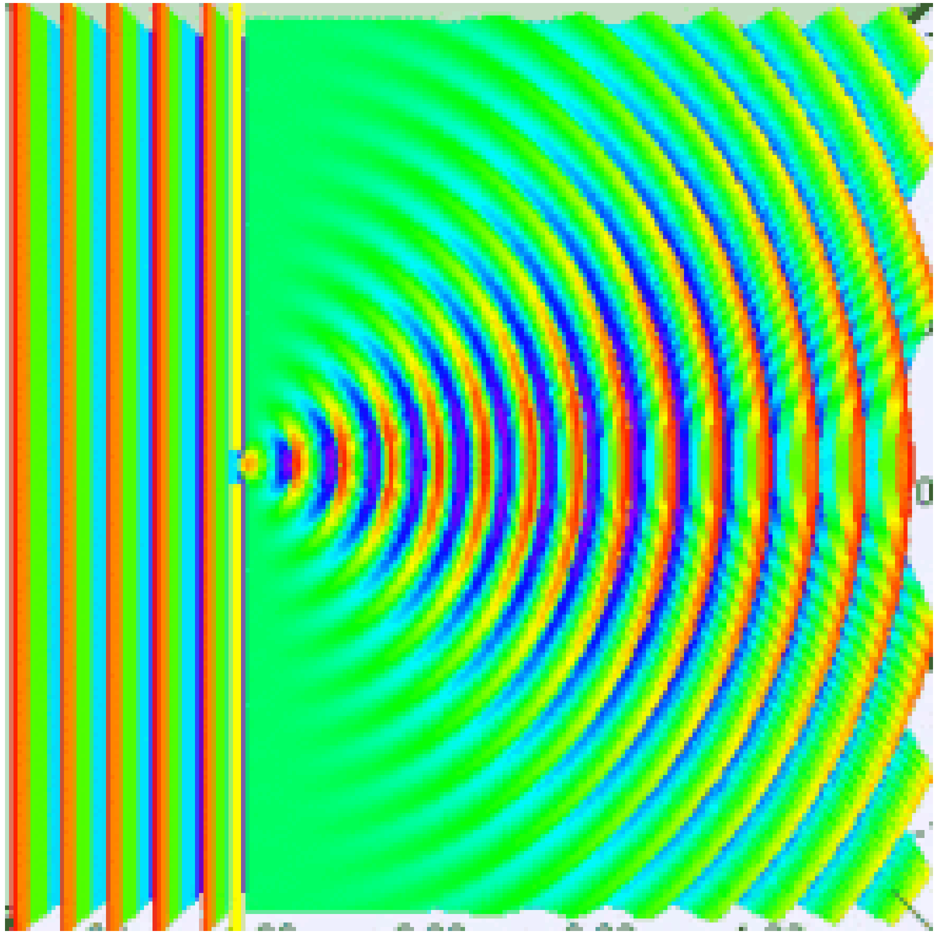


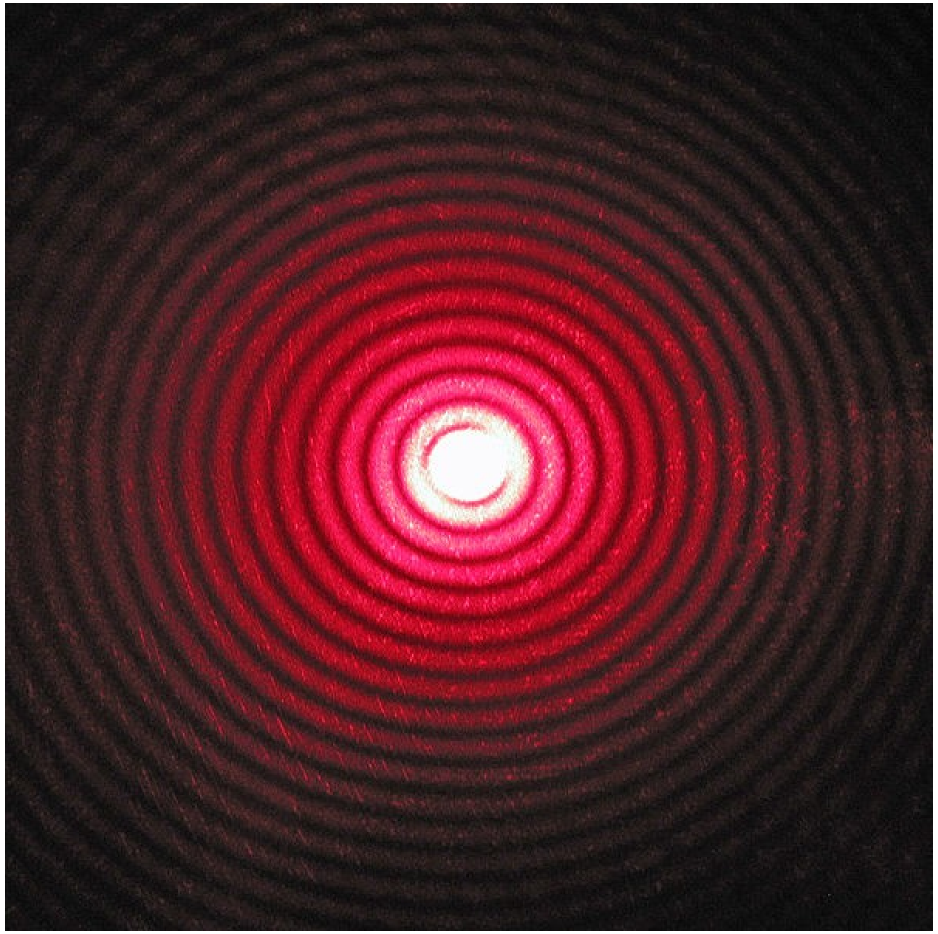
Diffraction of light



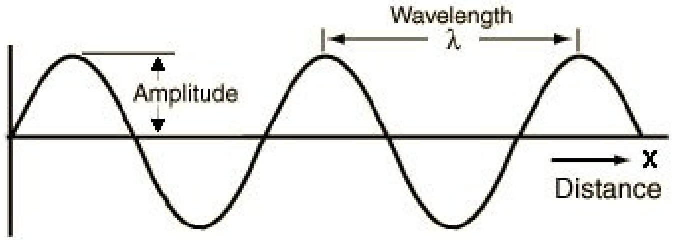


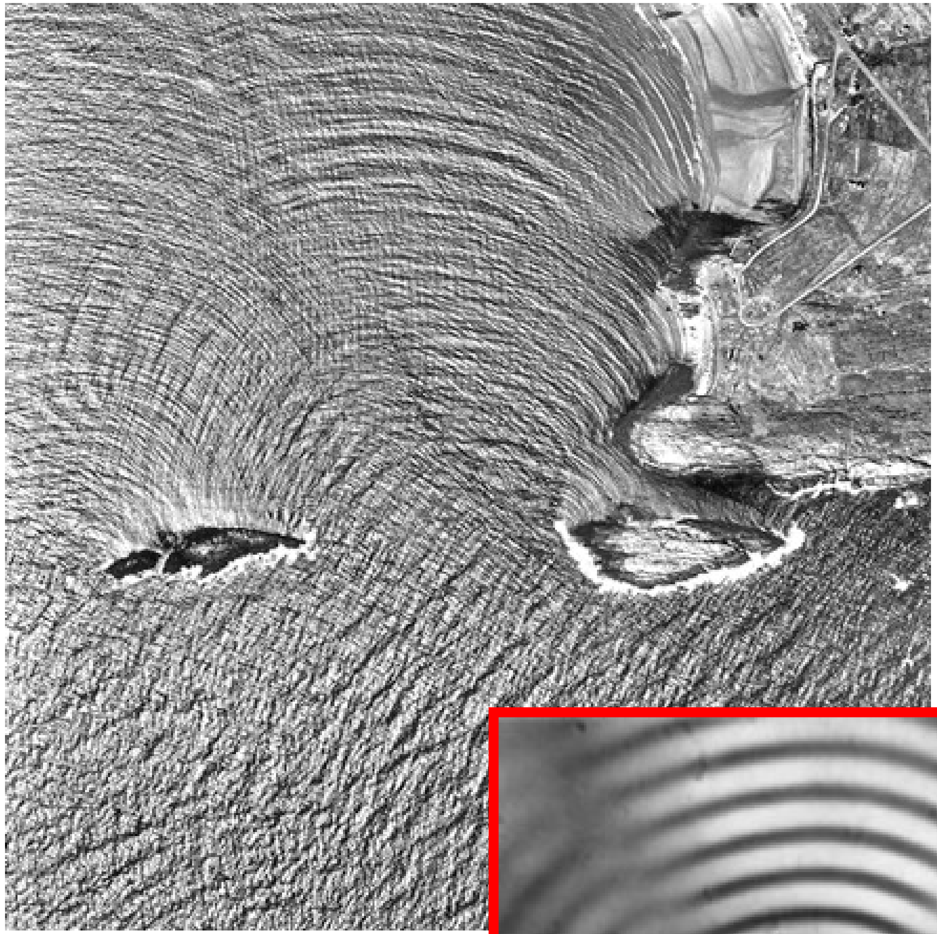
Diffraction of light

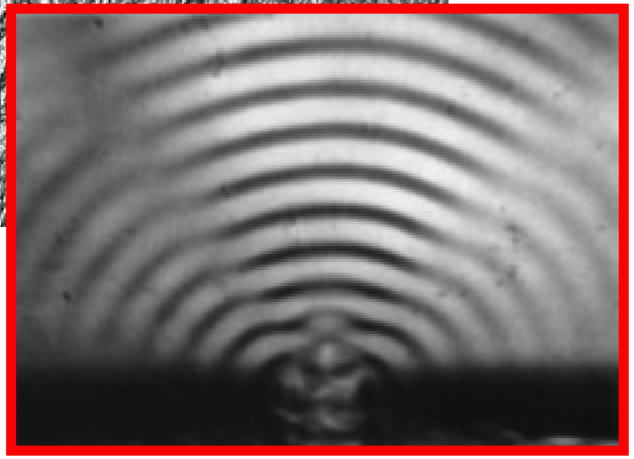




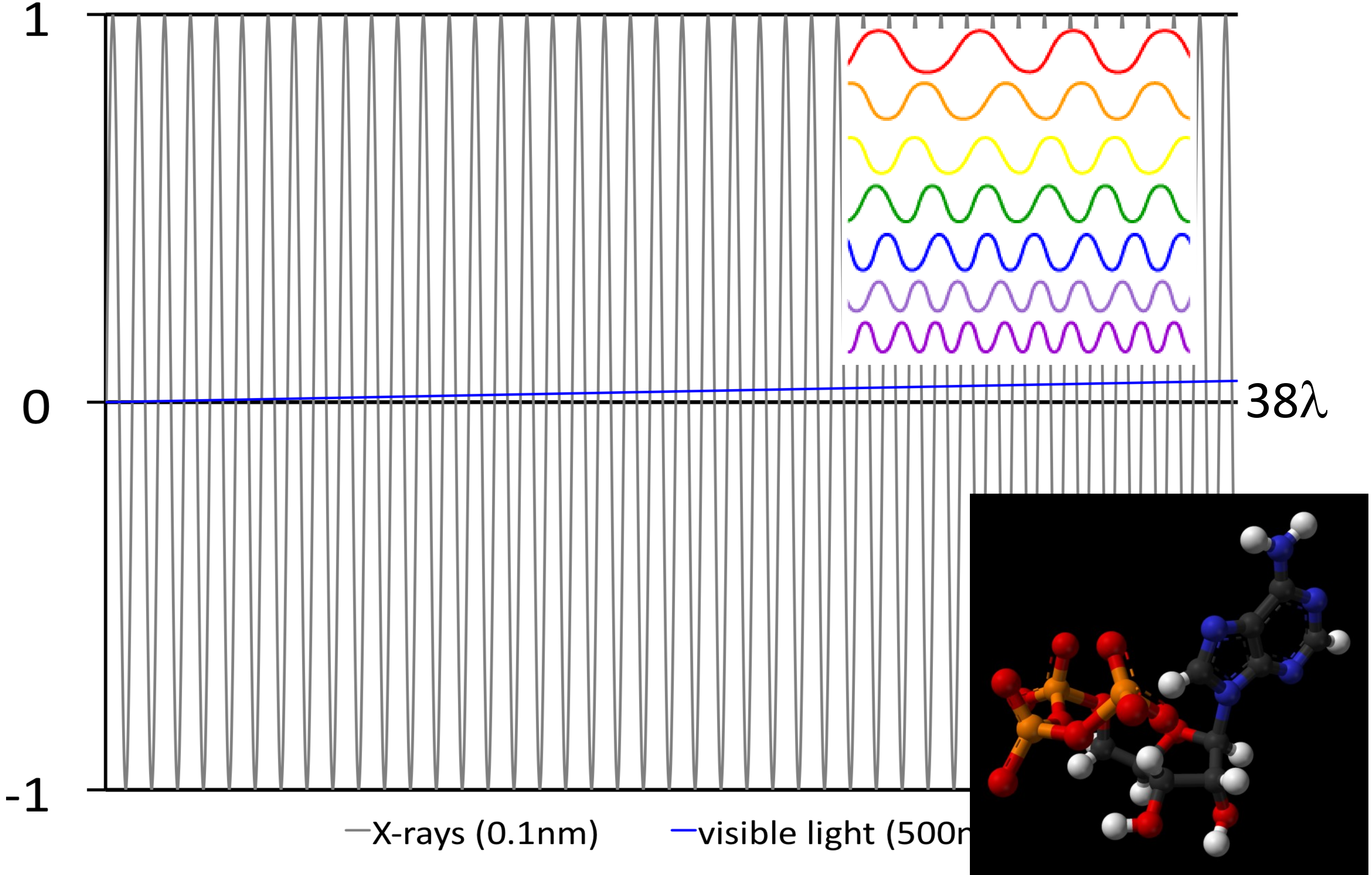
Wavelength and diffraction



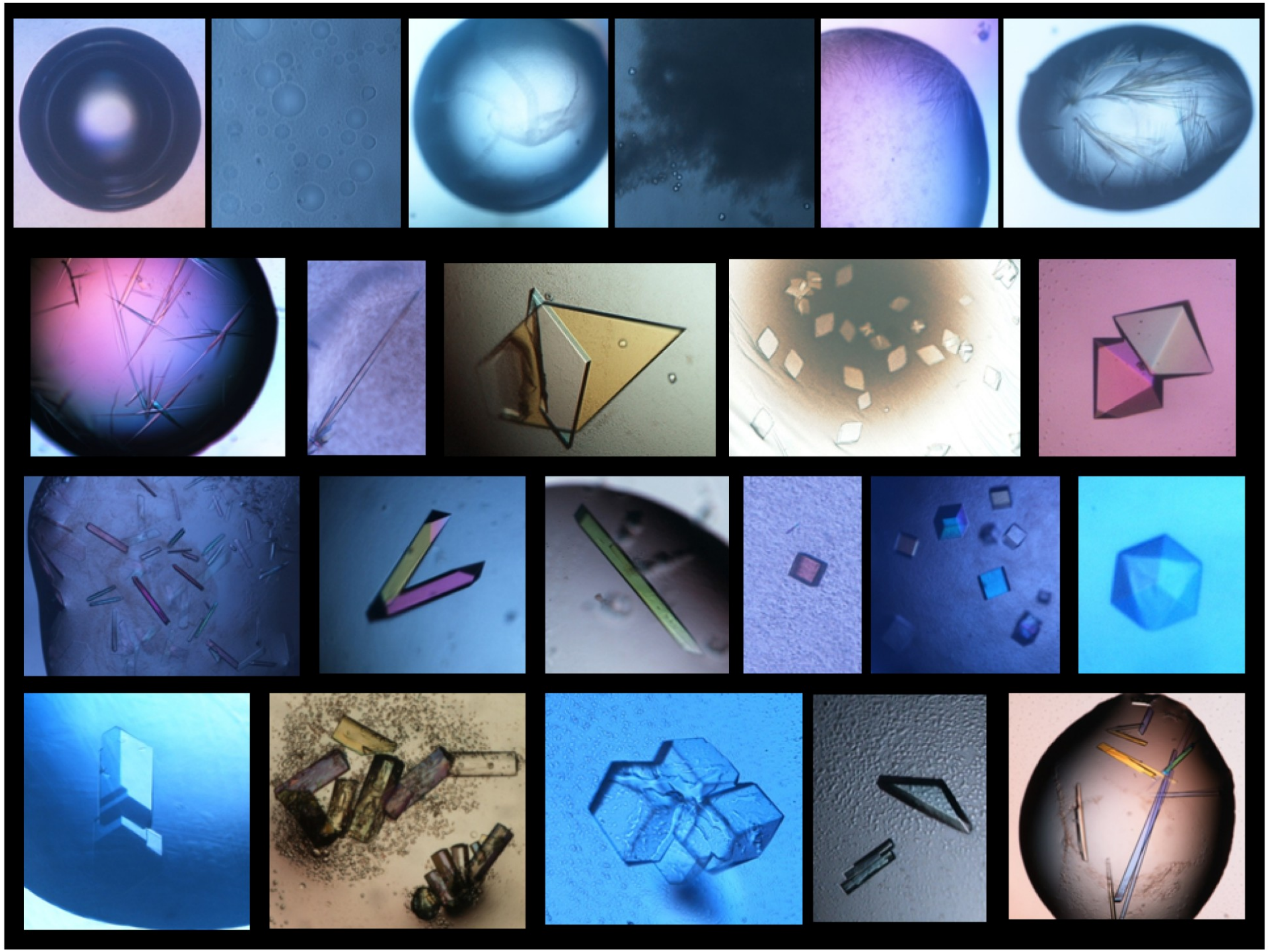




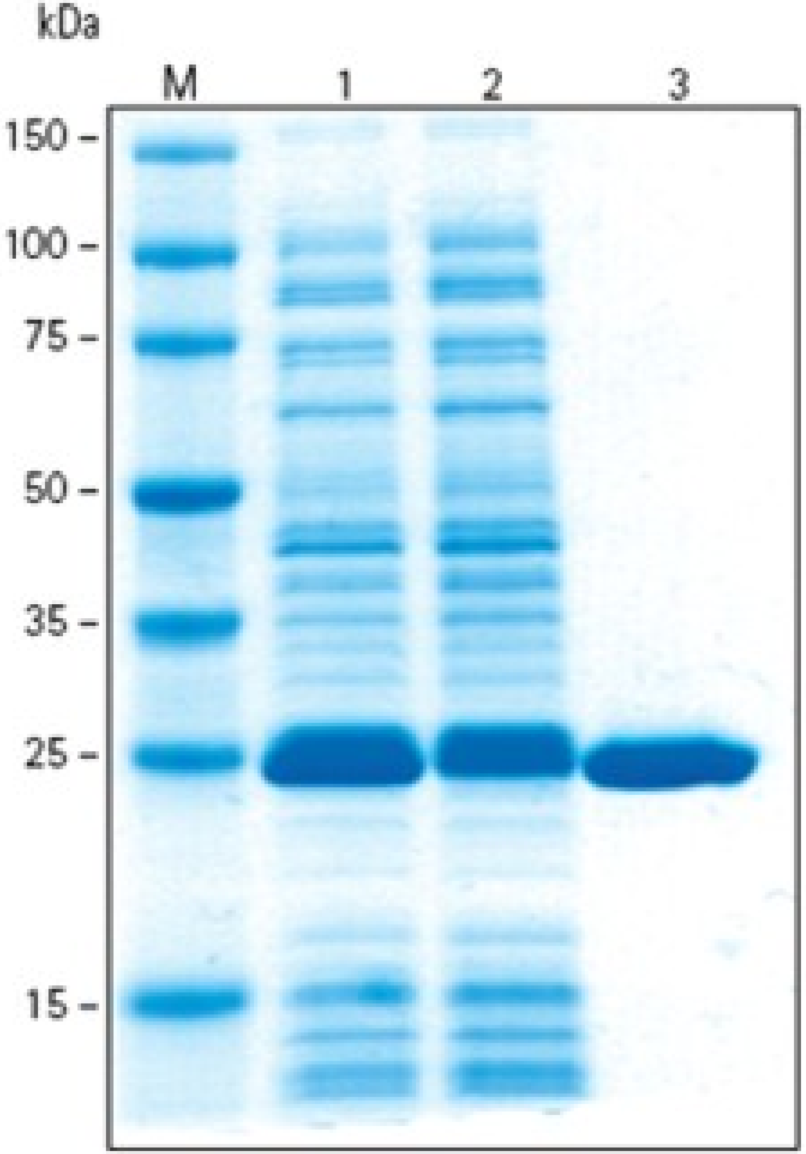
Wavelength comparison of X-rays and visible light



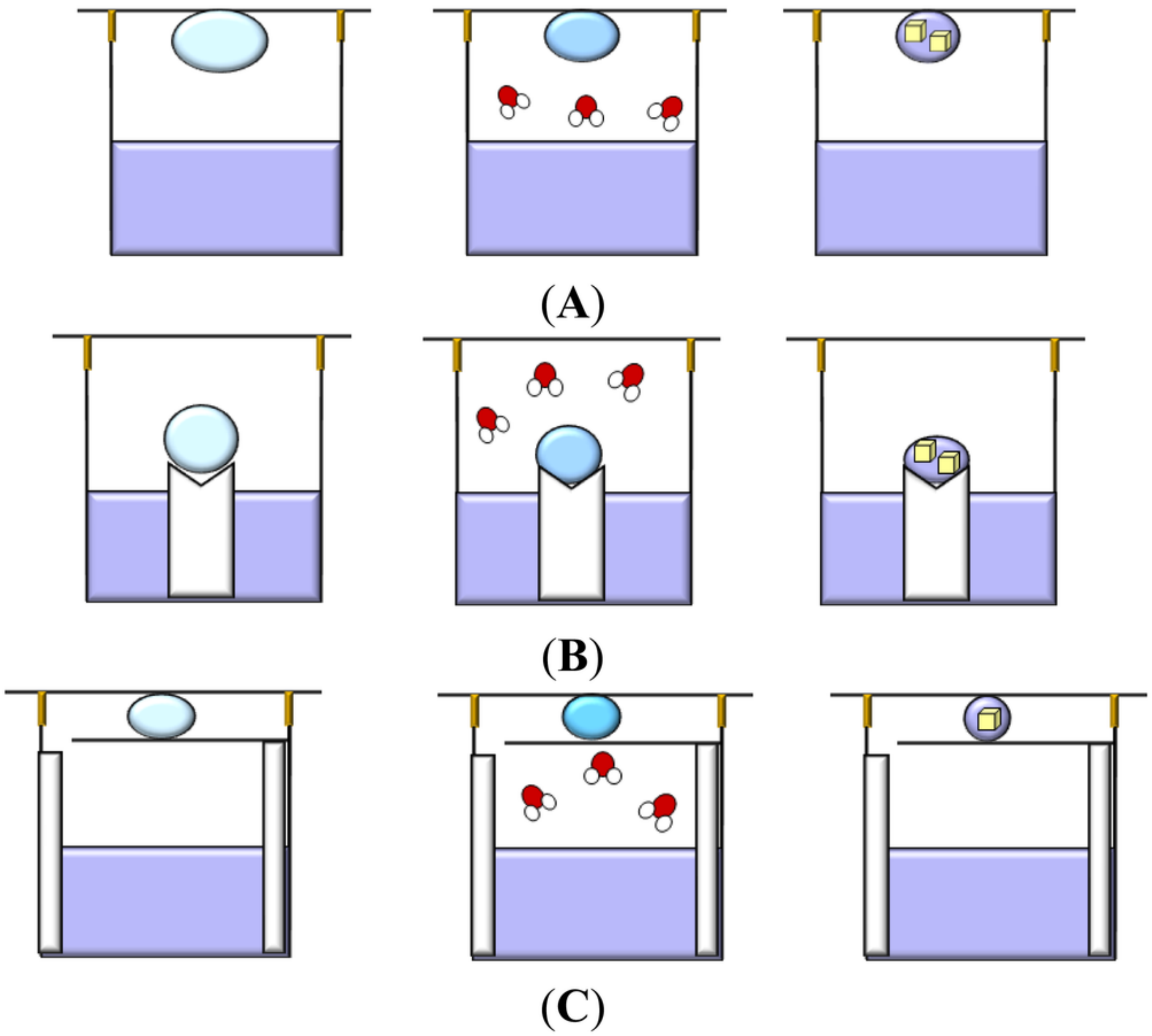
Crystallizing a Protein



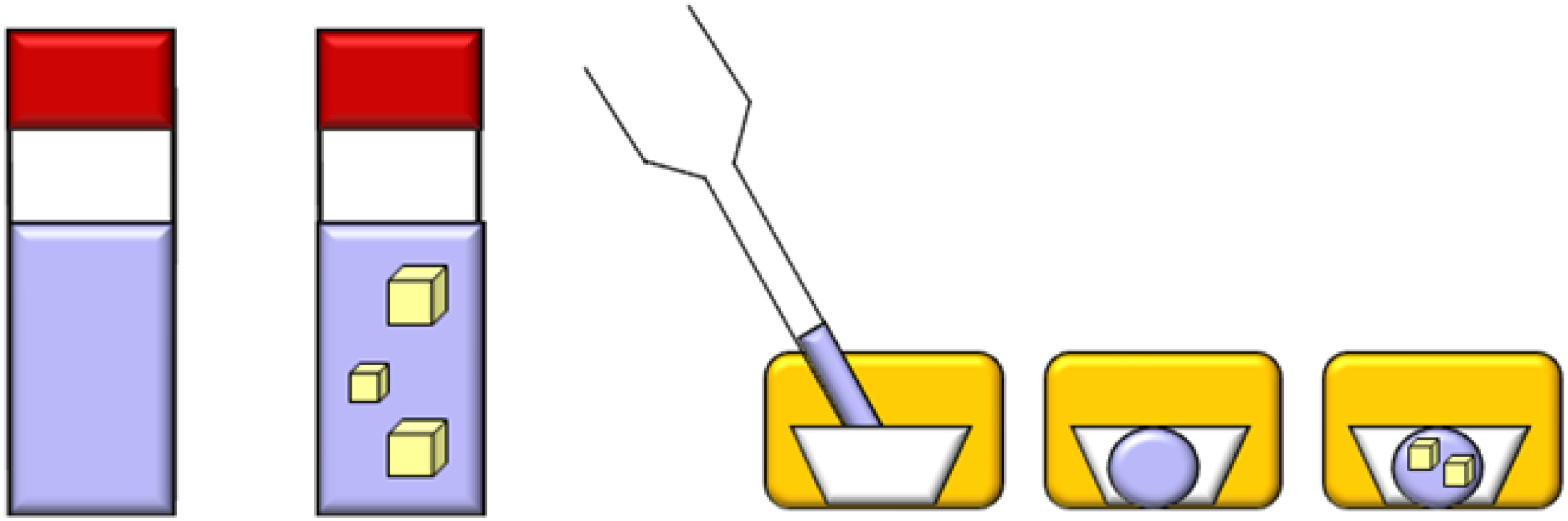
Protein expression and purification



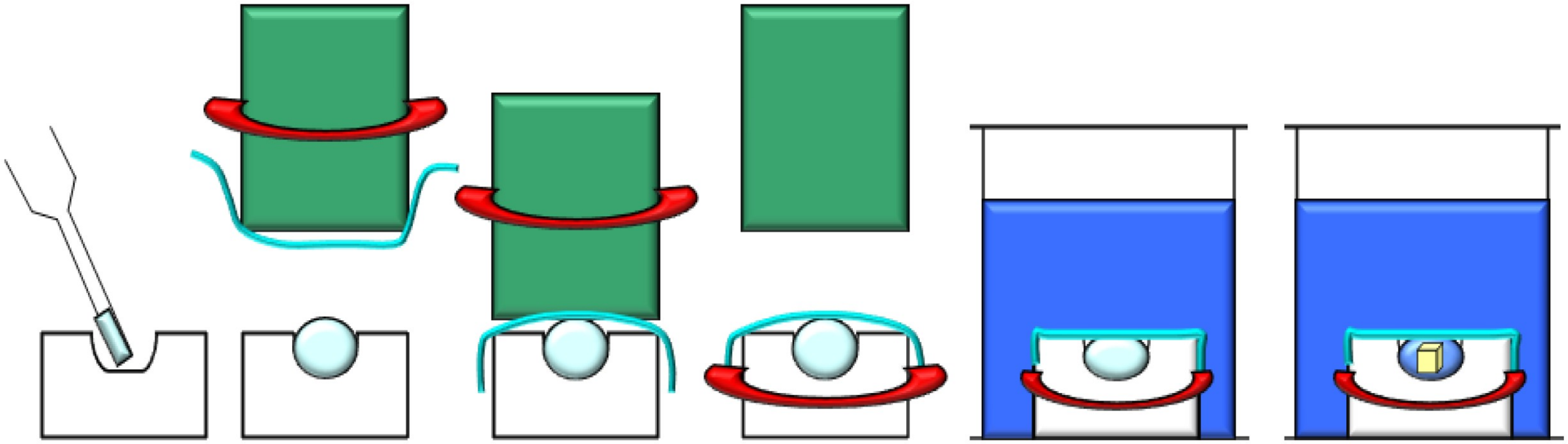
Vapor-diffusion



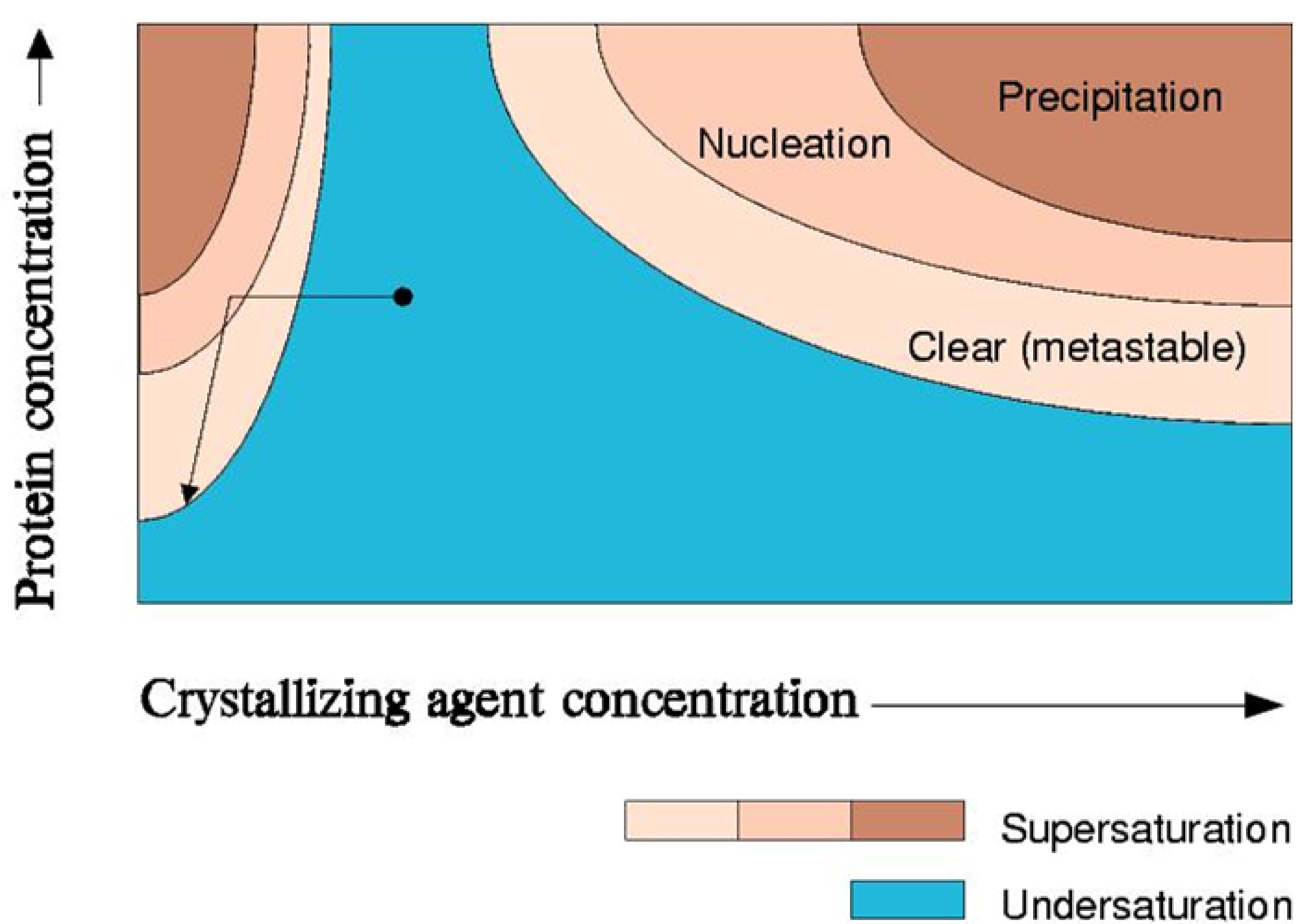
Batch and microbatch

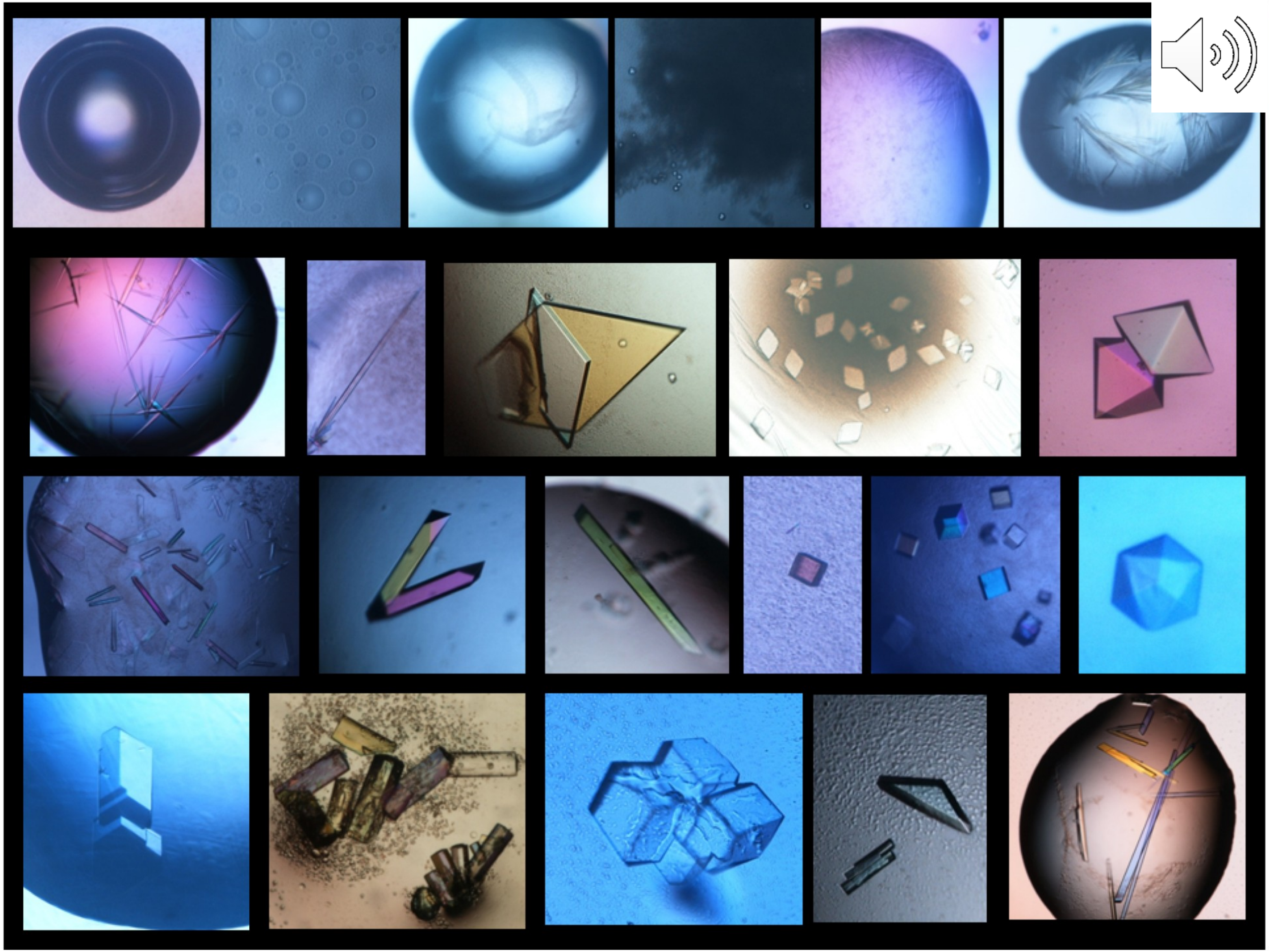


Microdialysis

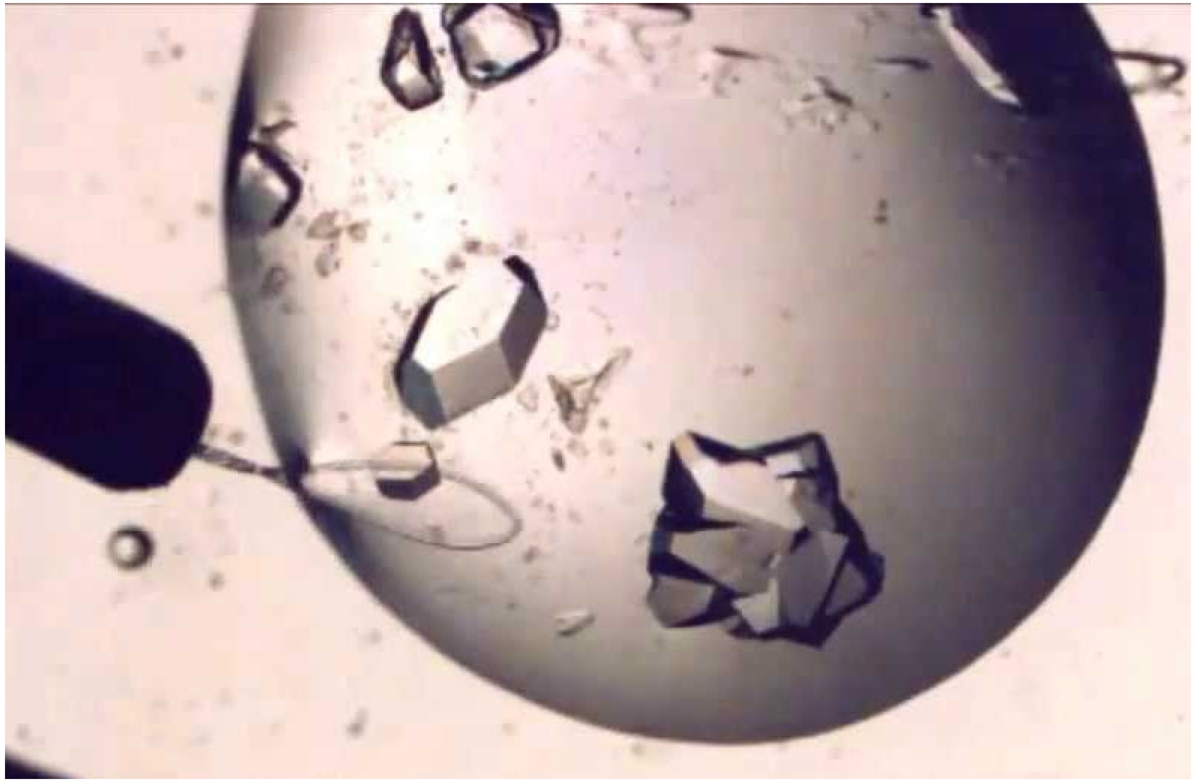


Protein crystallization phase diagram

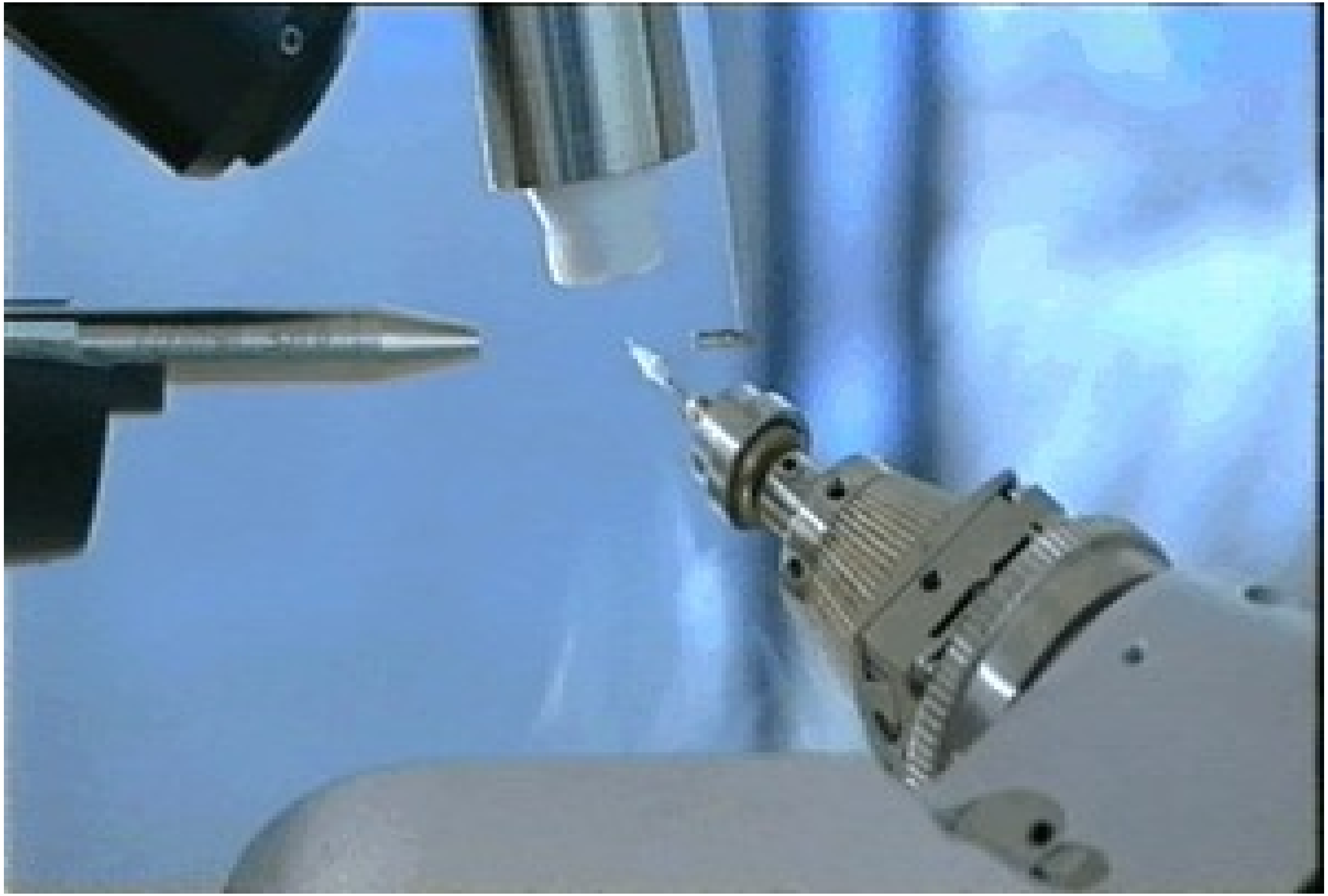




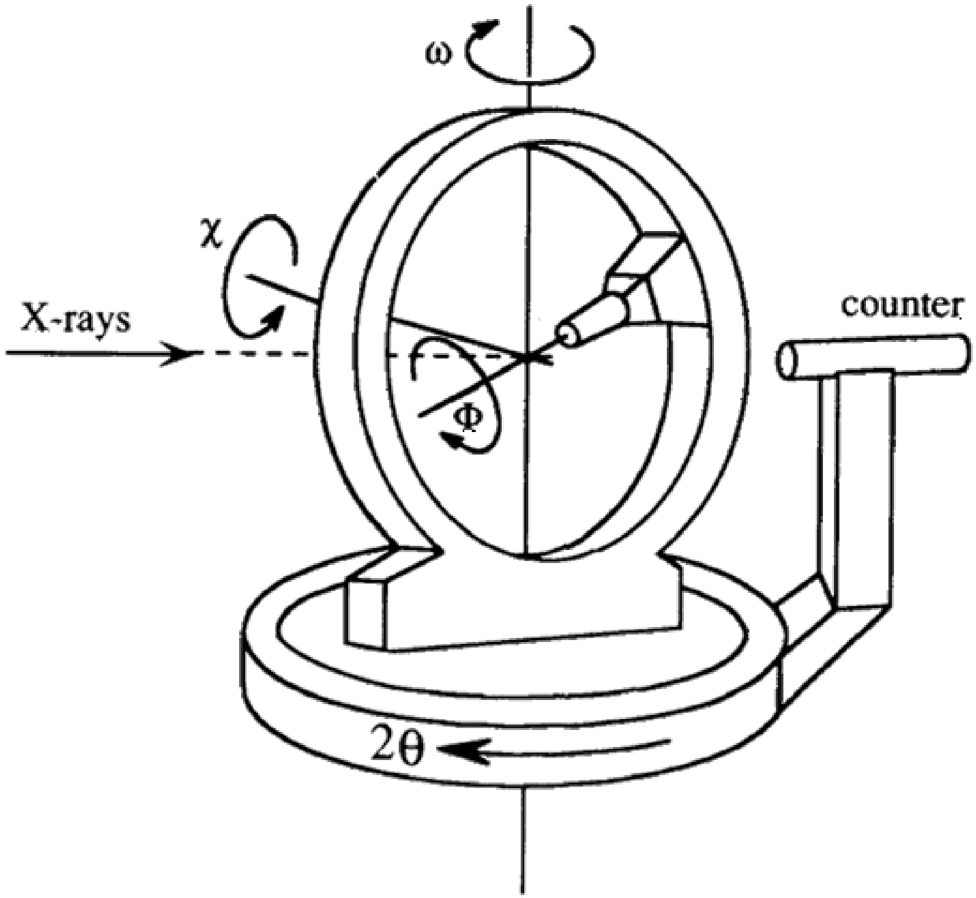
Preparing crystals for diffraction experiment

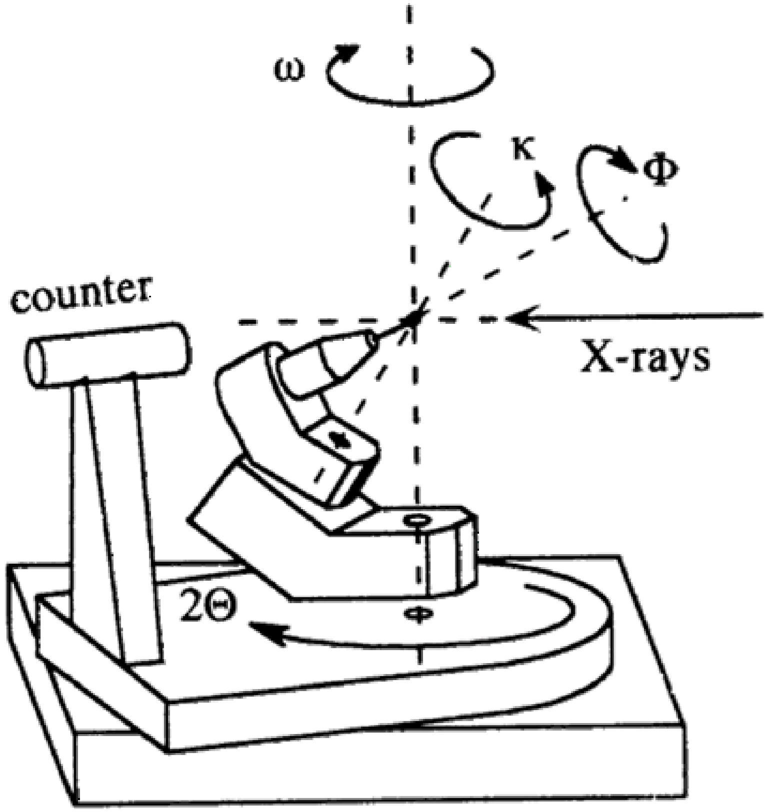


Diffractometer with goniometer

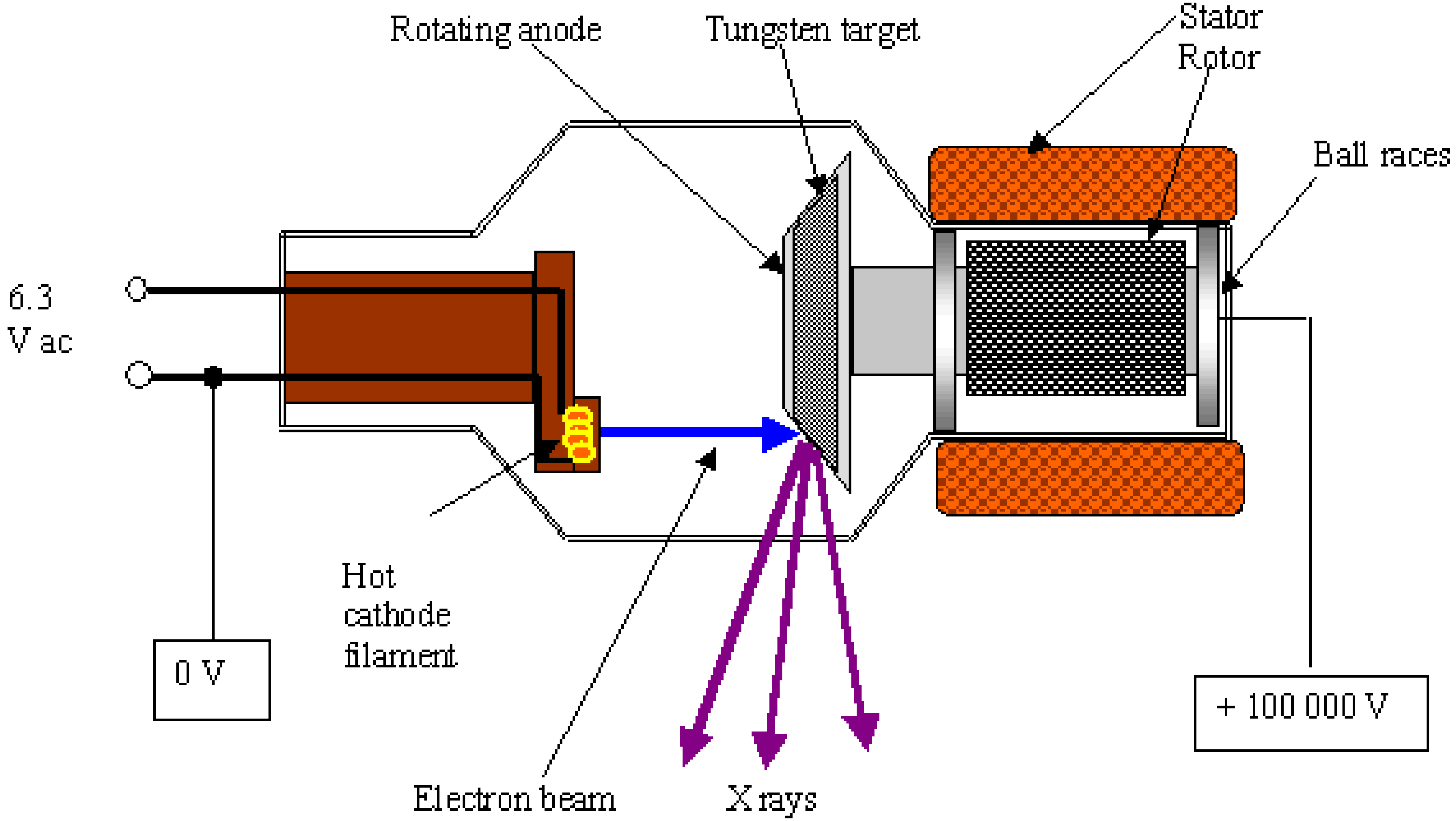


Diffractometer with goniometer

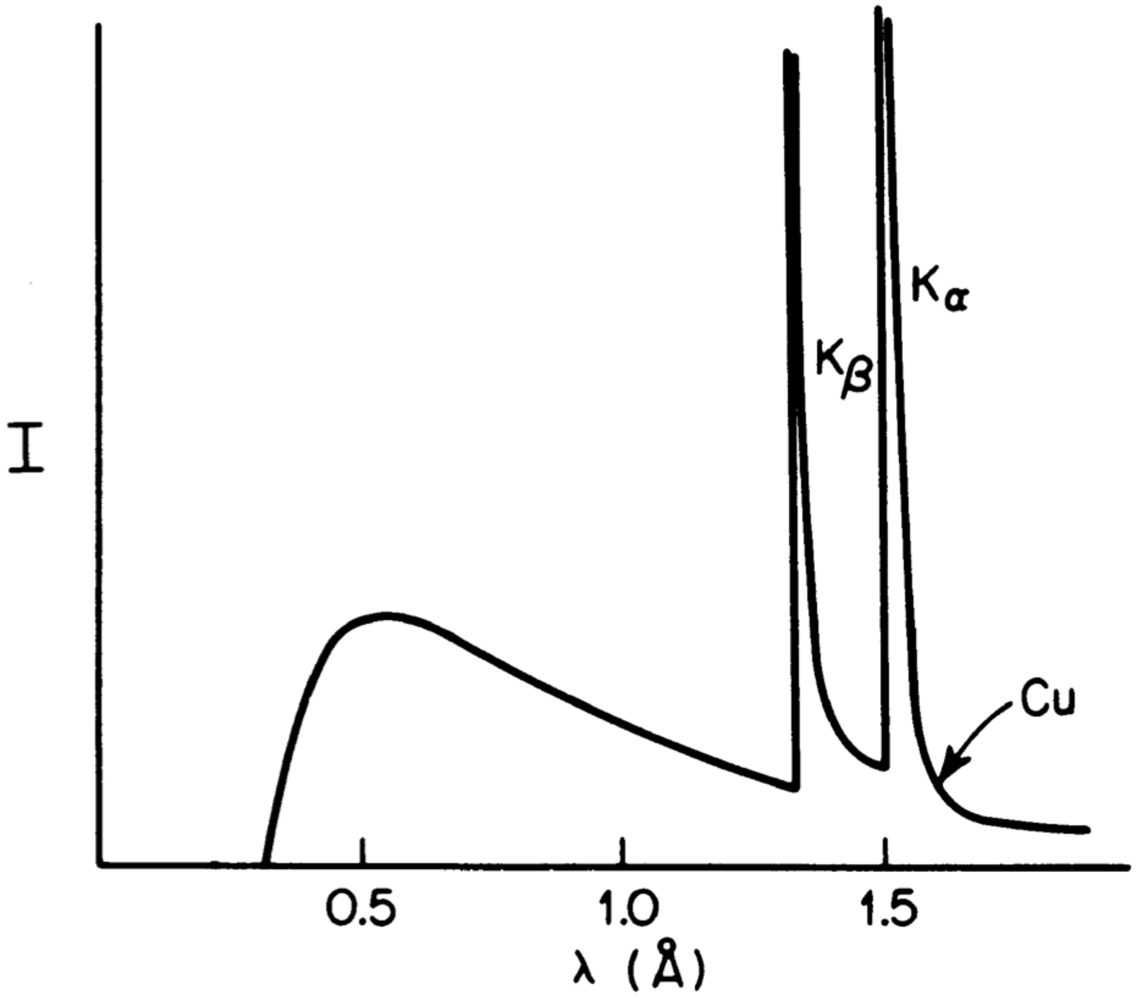




X-ray sources
- sealed X-ray tube



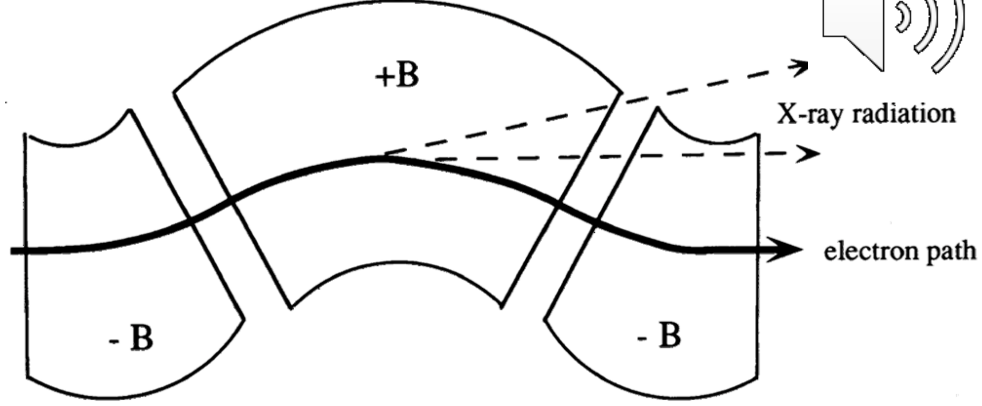
Spectrum of copper anode

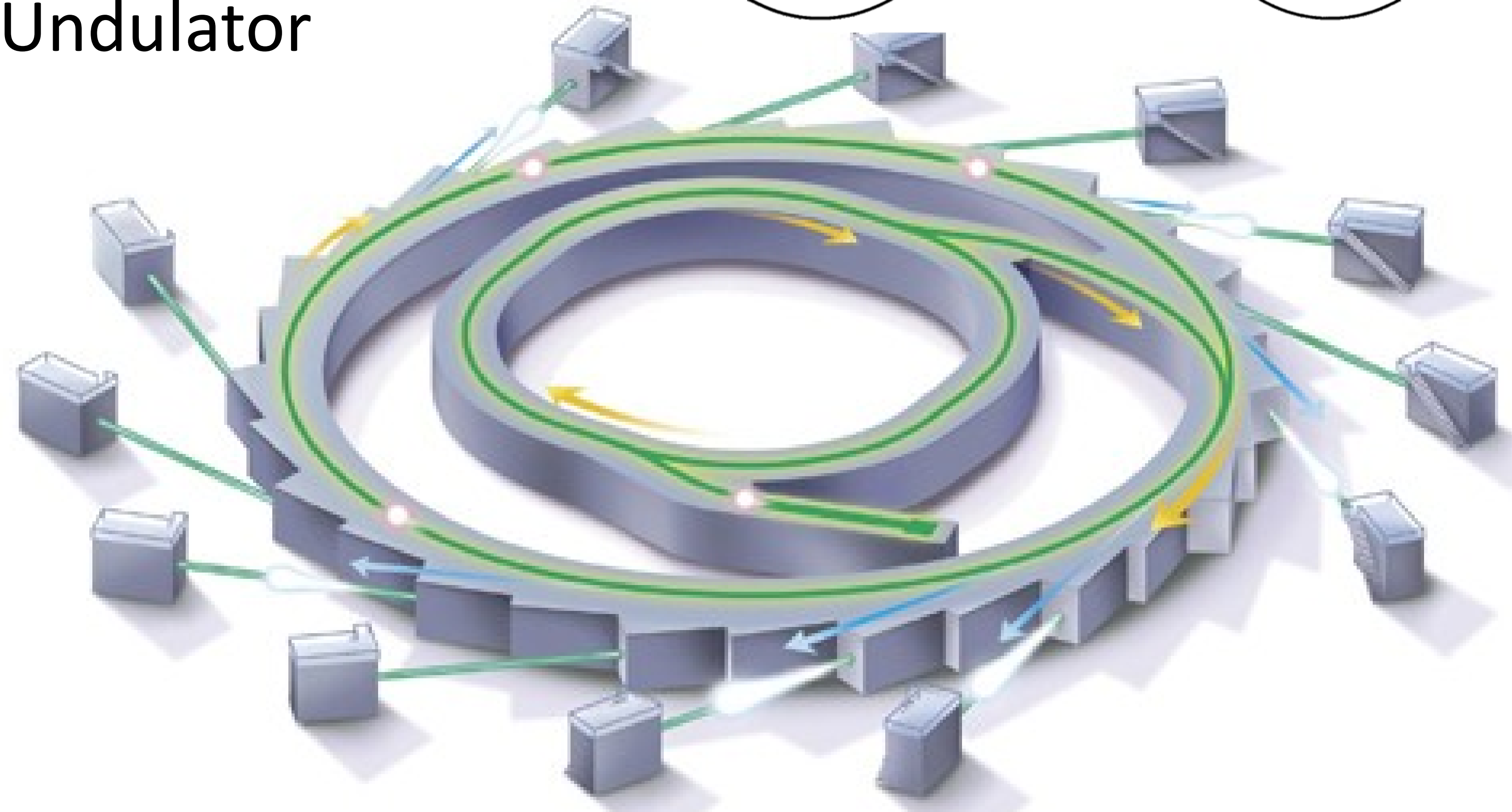


	λ (Å)	
$K_{\alpha}(1)$	1.54051	The weight average value for $K_{\alpha}(1)$ and $K_{\alpha}(2)$ is taken as 1.54178 Å
$K_{\alpha}(2)$	1.54433	because the intensity of $K_{\alpha}(1)$ is twice that of $K_{\alpha}(2)$
K_{β}	1.39217	

Synchrotron

- Bending magnet
- Wavelength shifter
- Wiggler
- Undulator





X-ray detectors

Single photon counter

Film

Image plates

Area detectors:

- CCDs
- Direct X-rays detectors - Pilatus

Crystals

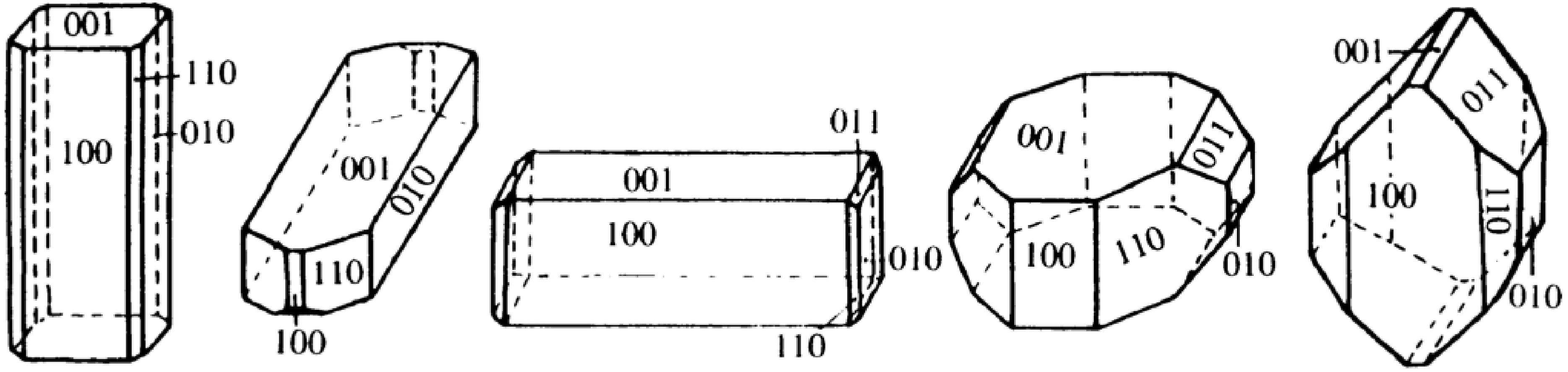


Figure 3.1. Crystals of trimethylammonium bromide belonging to the same crystal form but exhibiting a range of morphologies.

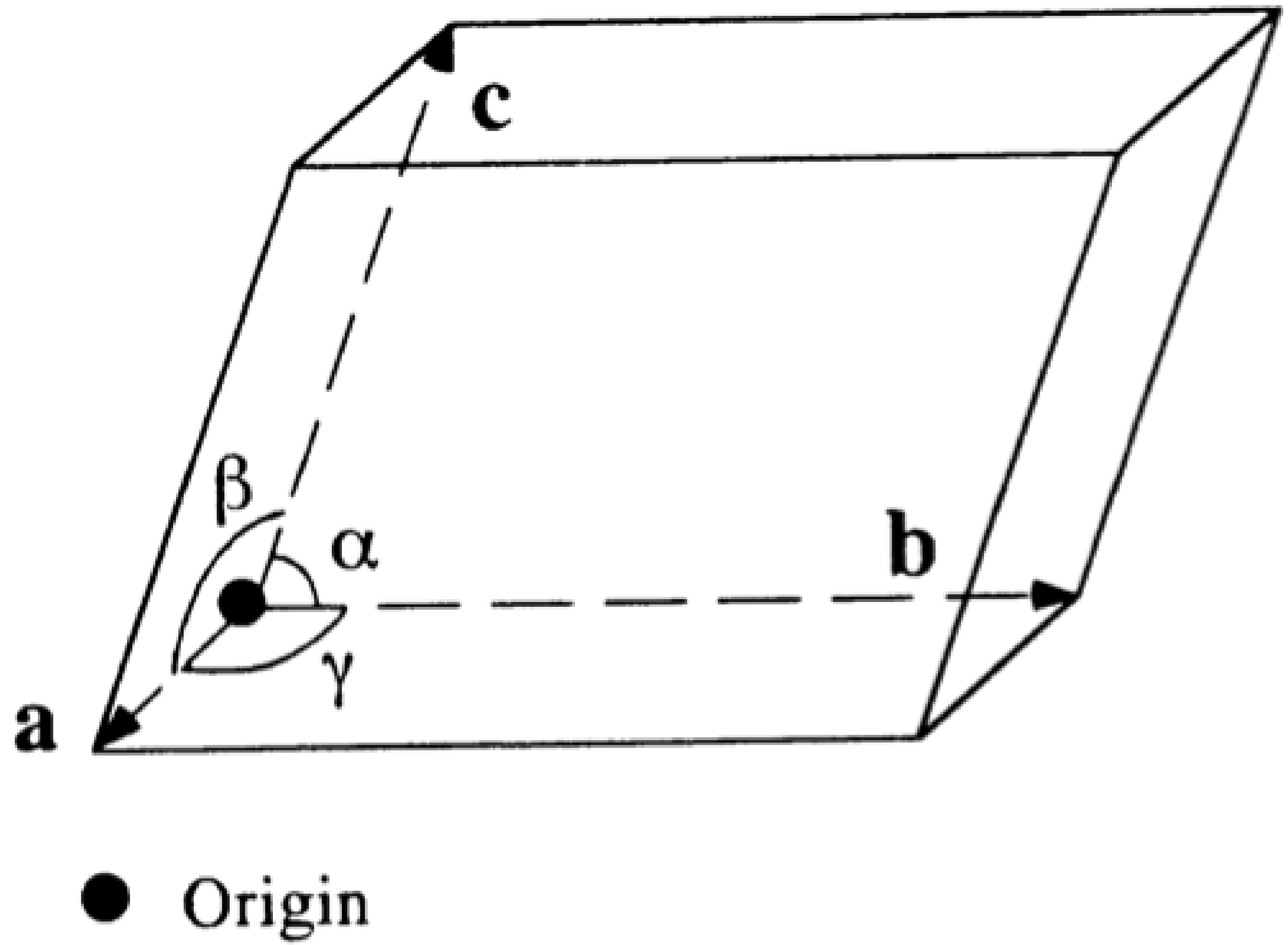


Figure 3.3. One unit cell in the crystal lattice.

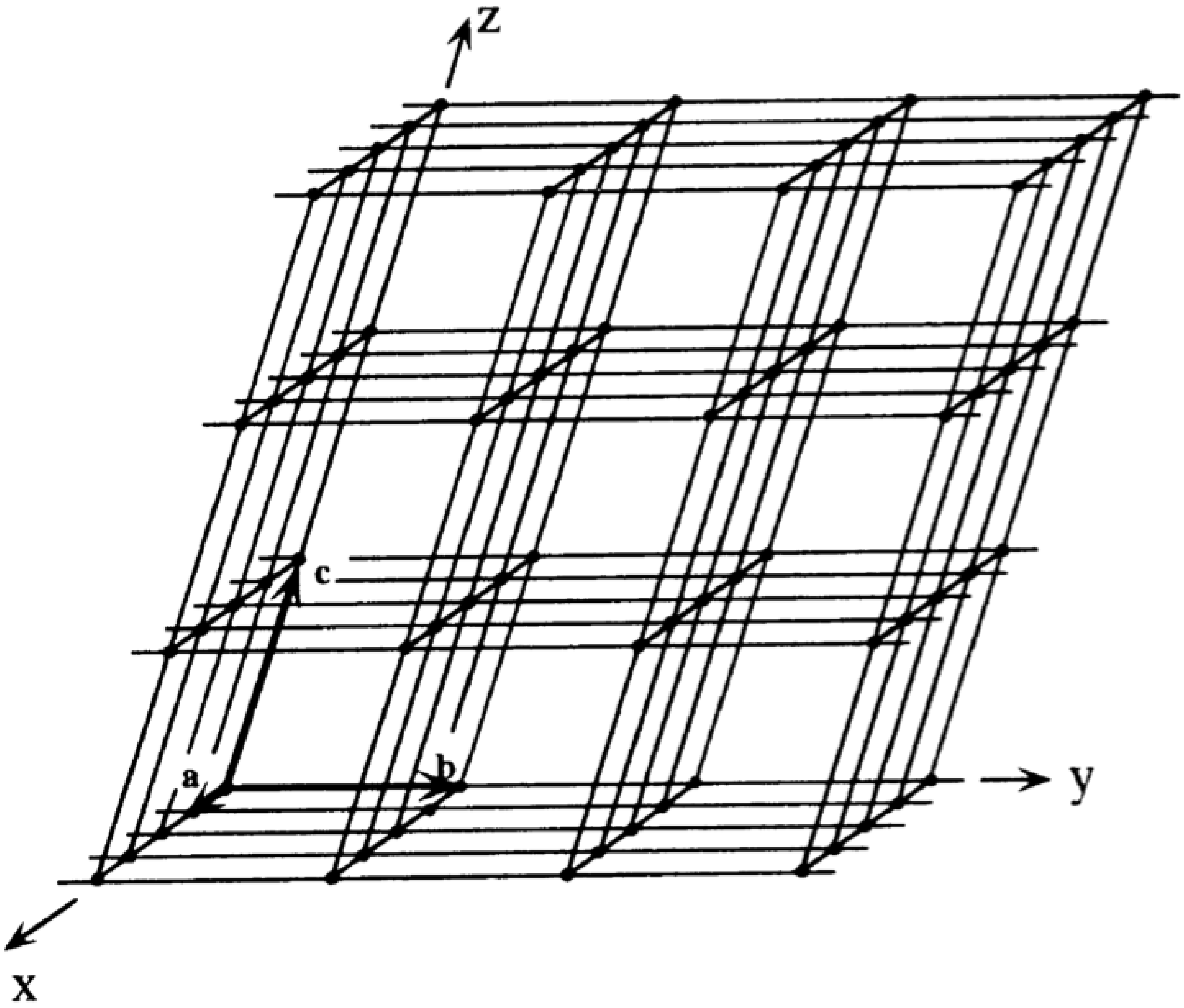
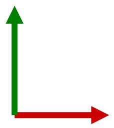


Figure 3.4. A crystal lattice is a three-dimensional stack of unit cells.

A 2D lattice



Lattice

Translationally periodic arrangement of **points**

Crystal

Translationally periodic arrangement of **motifs**

Crystal = Lattice + Motif

Lattice ➢ the underlying periodicity of the crystal

Motif ➢ atom or group of atoms associated with each lattice point

Lattice

Motif

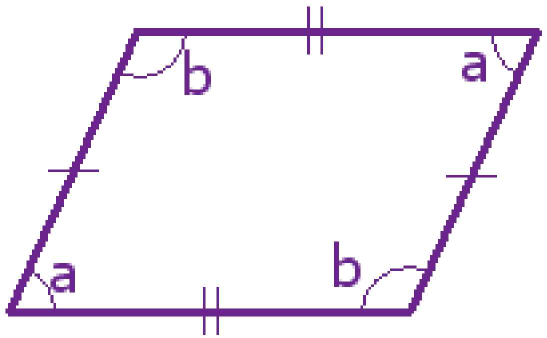
Crystal

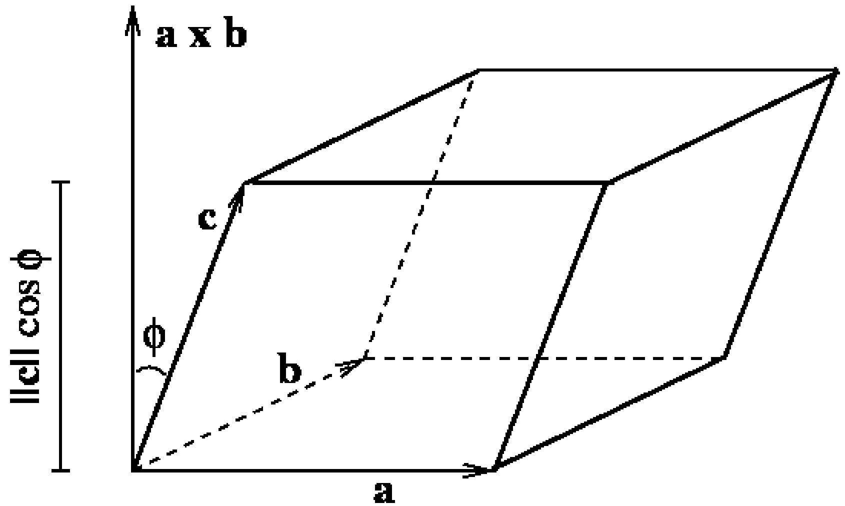
Courtesy Dr. Rajesh Prasad

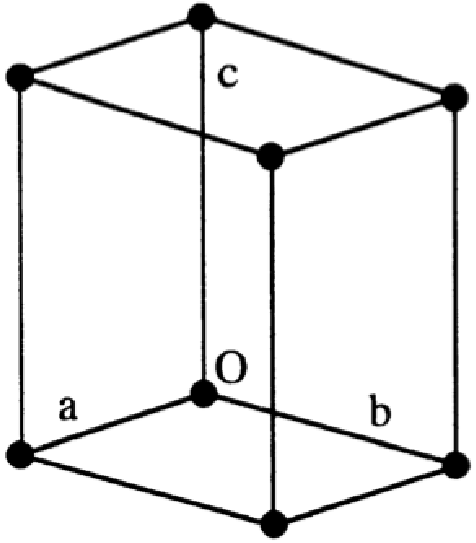
Unit cells

Instead of drawing the whole structure I can draw a representative part and specify the repetition pattern

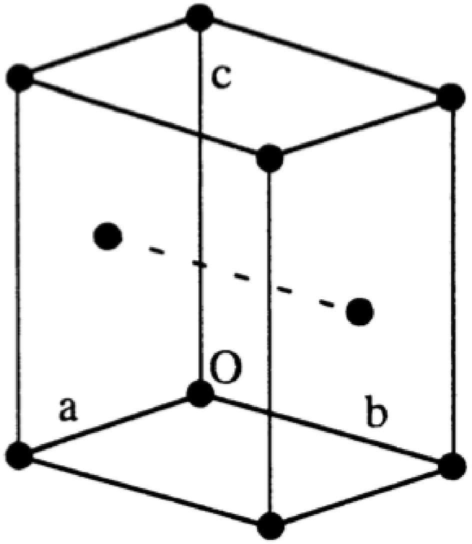
- A cell is a finite representation of the infinite lattice
- A cell is a parallelogram (2D) or a parallelopiped (3D) with lattice points at their corners.
- If the lattice points are only at the corners, the cell is primitive.
- If there are lattice points in the cell other than the corners, the cell is nonprimitive.



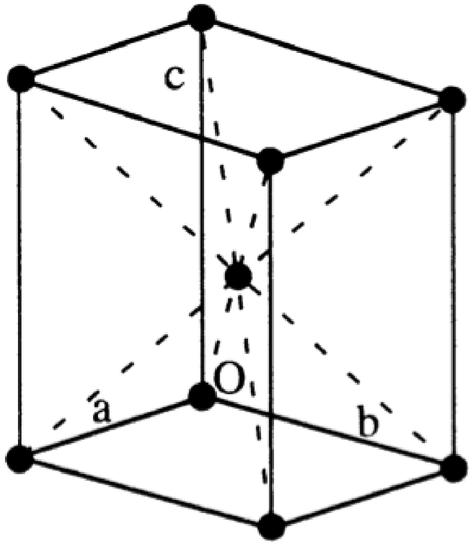




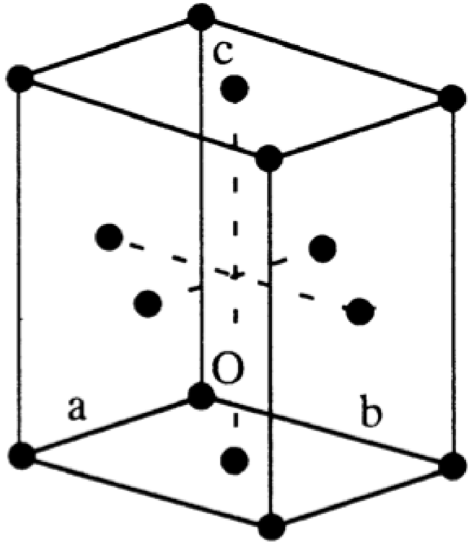
a primitive unit cell (P)



a unit cell centered in the (010) planes (B)



a body-centered unit cell (I)

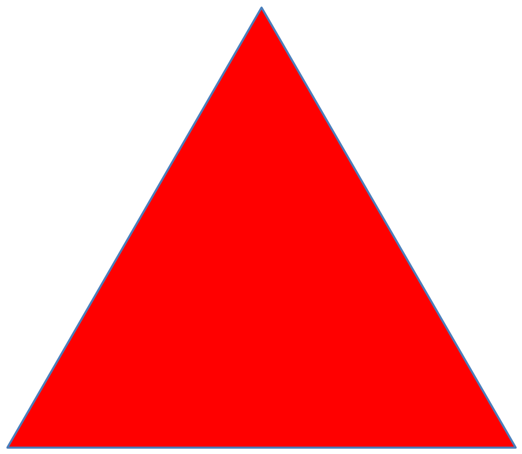


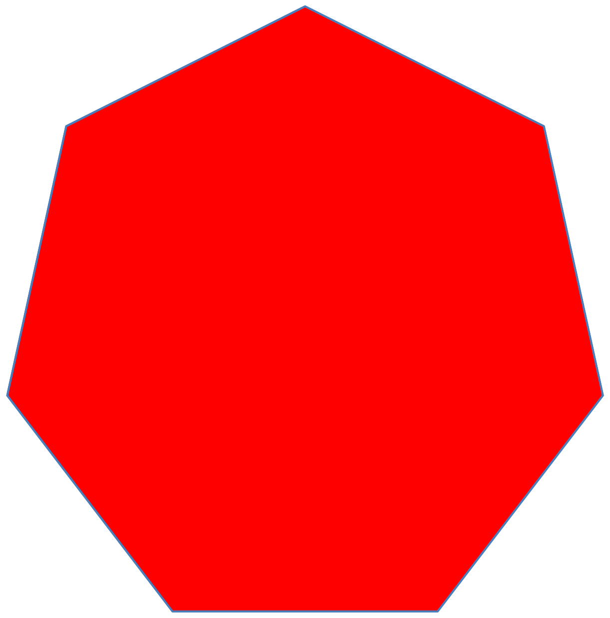
a face-centered unit cell (F)

Arrangement of lattice points in the unit cell
No. of Lattice points / cell

		Position of lattice points	Effective number of Lattice points / cell
1	P	8 Corners	= 8 x (1/8) = 1
2	I	8 Corners + 1 body centre	= 1 (for corners) + 1 (BC)
3	F	8 Corners + 6 face centres	= 1 (for corners) + 6 x (1/2) = 4
4	A/ B/ C	8 corners + 2 centres of opposite faces	= 1 (for corners) + 2x(1/2) = 2

SYMMETRY





If an object is brought into self-coincidence after some operation it said to possess symmetry with respect to that operation.

Bravais Lattice

A **lattice** is a set of points constructed by translating a single point in discrete steps by a set of *basis vectors*. In three dimensions, there are 14 unique **Bravais** lattices *(distinct from one another in that they have different space groups)* in three dimensions. All crystalline materials recognized till now fit in one of these arrangements.

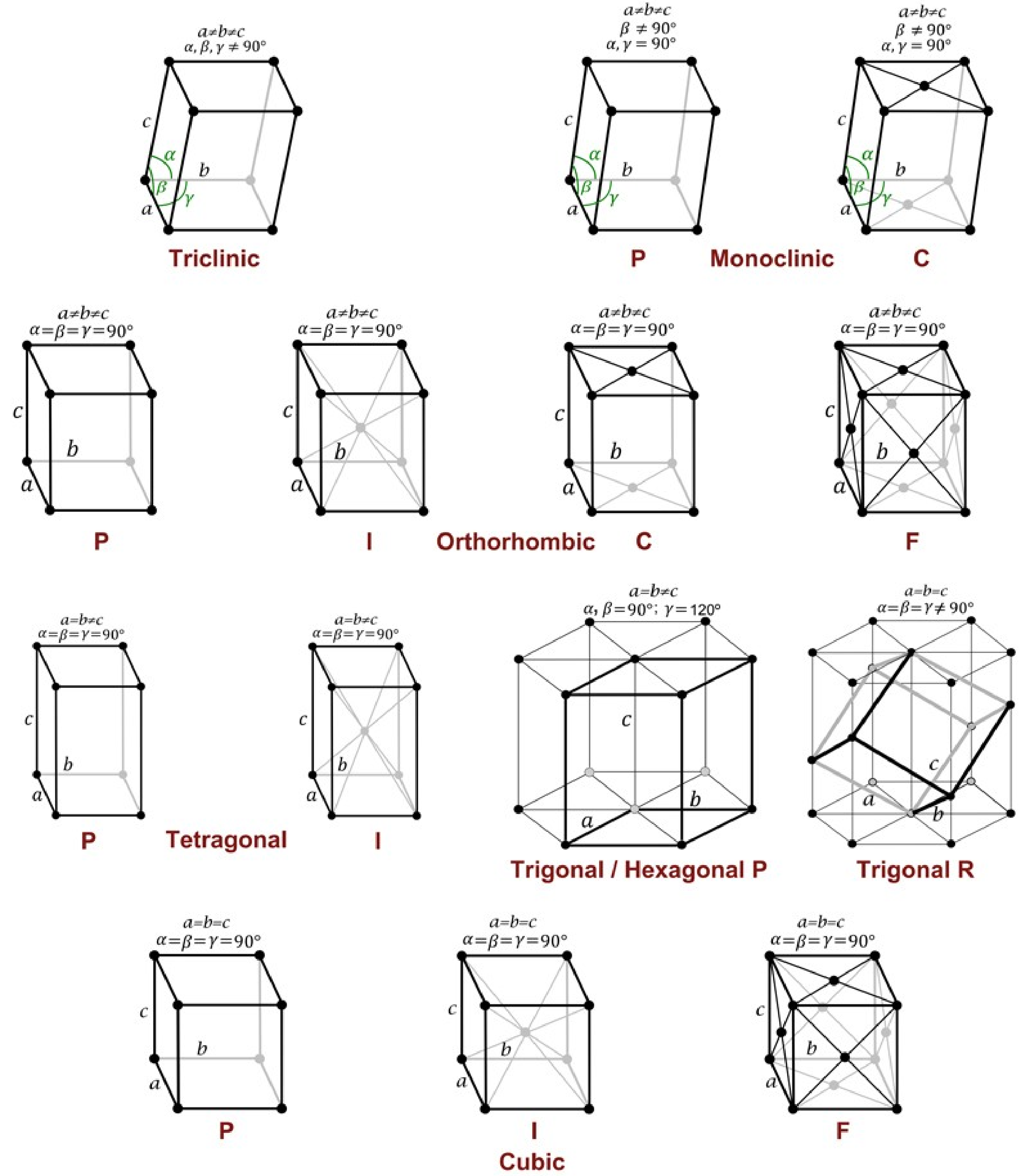

$a \neq b \neq c$
$\alpha, \beta, \gamma \neq 90°$
Triclinic
$a \neq b \neq c$
$\beta \neq 90°$
$\alpha, \gamma = 90°$
P
Monoclinic
C
$a \neq b \neq c$
$\alpha = \beta = \gamma = 90°$
P
I
Orthorhombic
C
F
$a = b \neq c$
$\alpha = \beta = \gamma = 90°$
P
Tetragonal
I
$a = b \neq c$
$\alpha, \beta = 90°;\ \gamma = 120°$
Trigonal / Hexagonal P
$a = b = c$
$\alpha = \beta = \gamma \neq 90°$
Trigonal R
$a = b = c$
$\alpha = \beta = \gamma = 90°$
P
I
F
Cubic
a
b
c
α
β
γ

Table 3.2. The Seven Crystal Systems

Crystal system	Conditions imposed on cell geometry	Minimum point group symmetry
Triclinic	None	1
Monoclinic	$\alpha = \gamma = 90^\circ$(b is the unique axis; for proteins this is a 2-fold axis or screw axis)	2
	or: $\alpha = \beta = 90^\circ$ (c is unique axis; for proteins this is a 2-fold axis or screw axis)	
Orthorhombic	$\alpha = \beta = \gamma = 90^\circ$	222
Tetragonal	$a = b$; $\alpha = \beta = \gamma = 90^\circ$	4
Trigonal	$a = b$; $\alpha = \beta = 90^\circ$; $\gamma = 120^\circ$ (hexagonal axes)	3
	or: $a = b = c$; $\alpha = \beta = \gamma$ (rhombohedral axes)	
Hexagonal	$a = b$; $\alpha = \beta = 90^\circ$; $\gamma = 120^\circ$	6
Cubic	$a = b = c$; $\alpha = \beta = \gamma = 90^\circ$	23

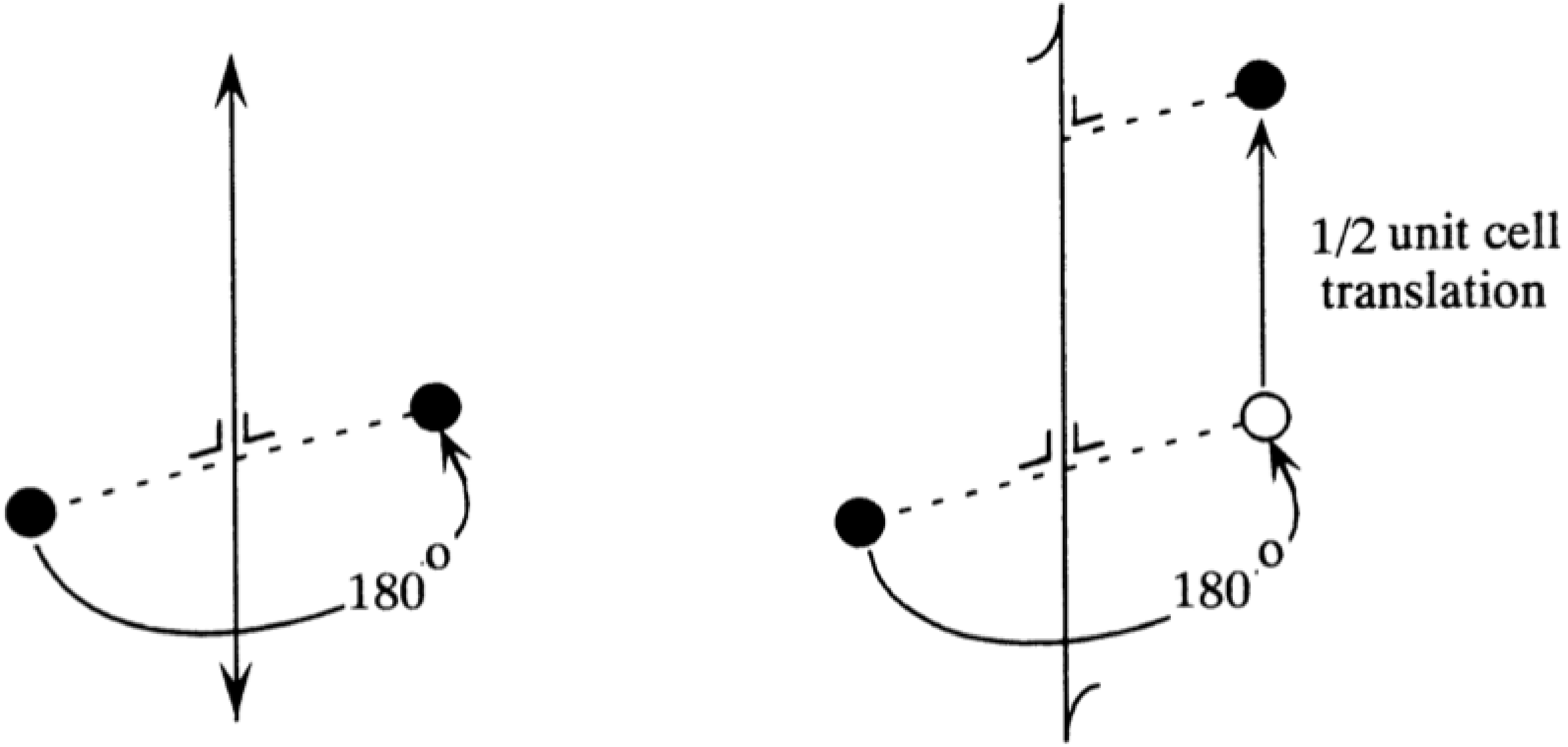


Figure 3.12. A 2-fold axis (left) and a 2-fold screw axis (right); the latter relates one molecule to another by a 180° rotation plus a translation over half of the unit cell.

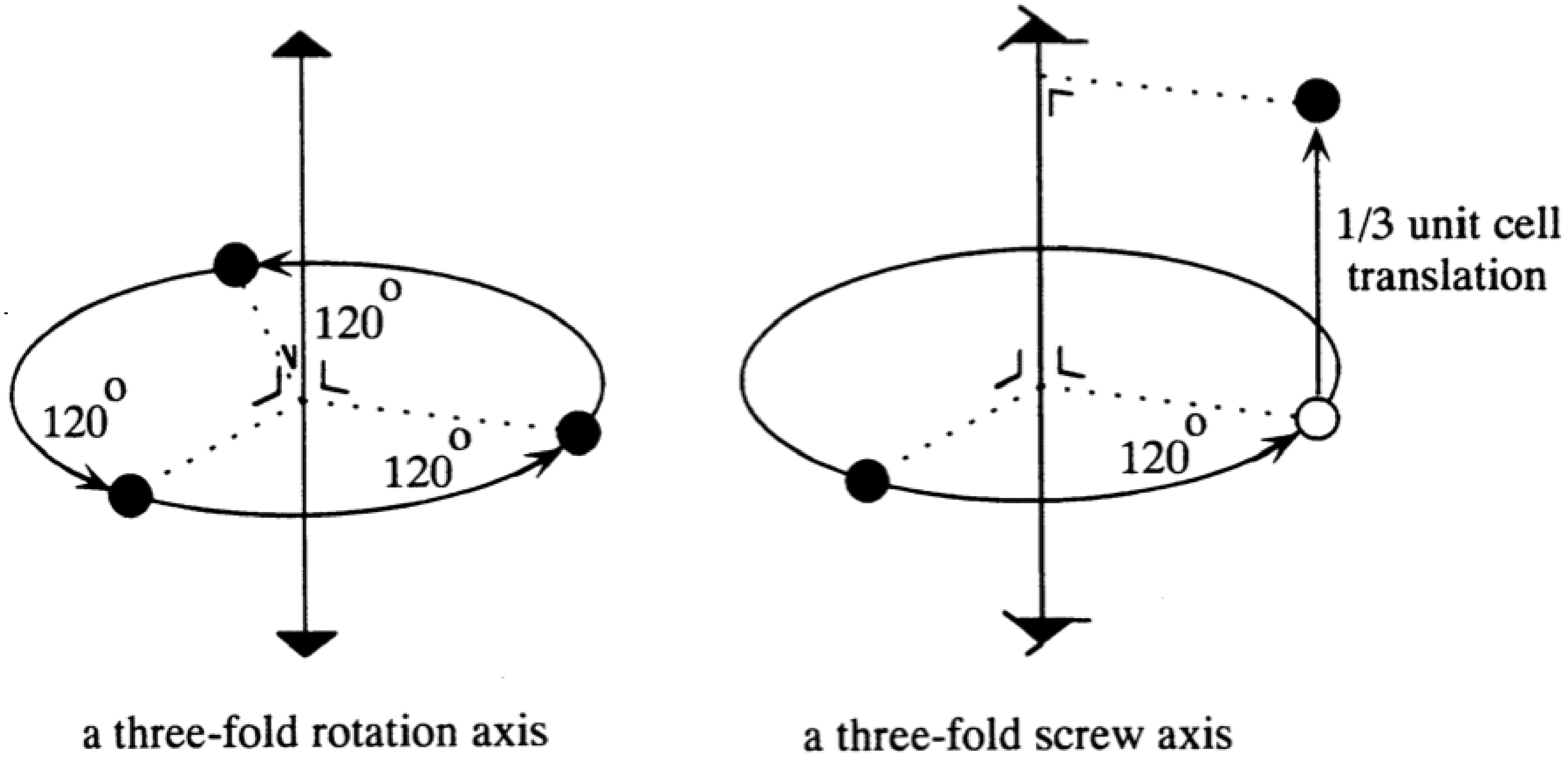


Figure 3.13. A 3-fold axis (left) and a 3-fold screw axis (right); the latter relates one molecule to another by a 120° rotation and a translation over one-third of the unit cell.

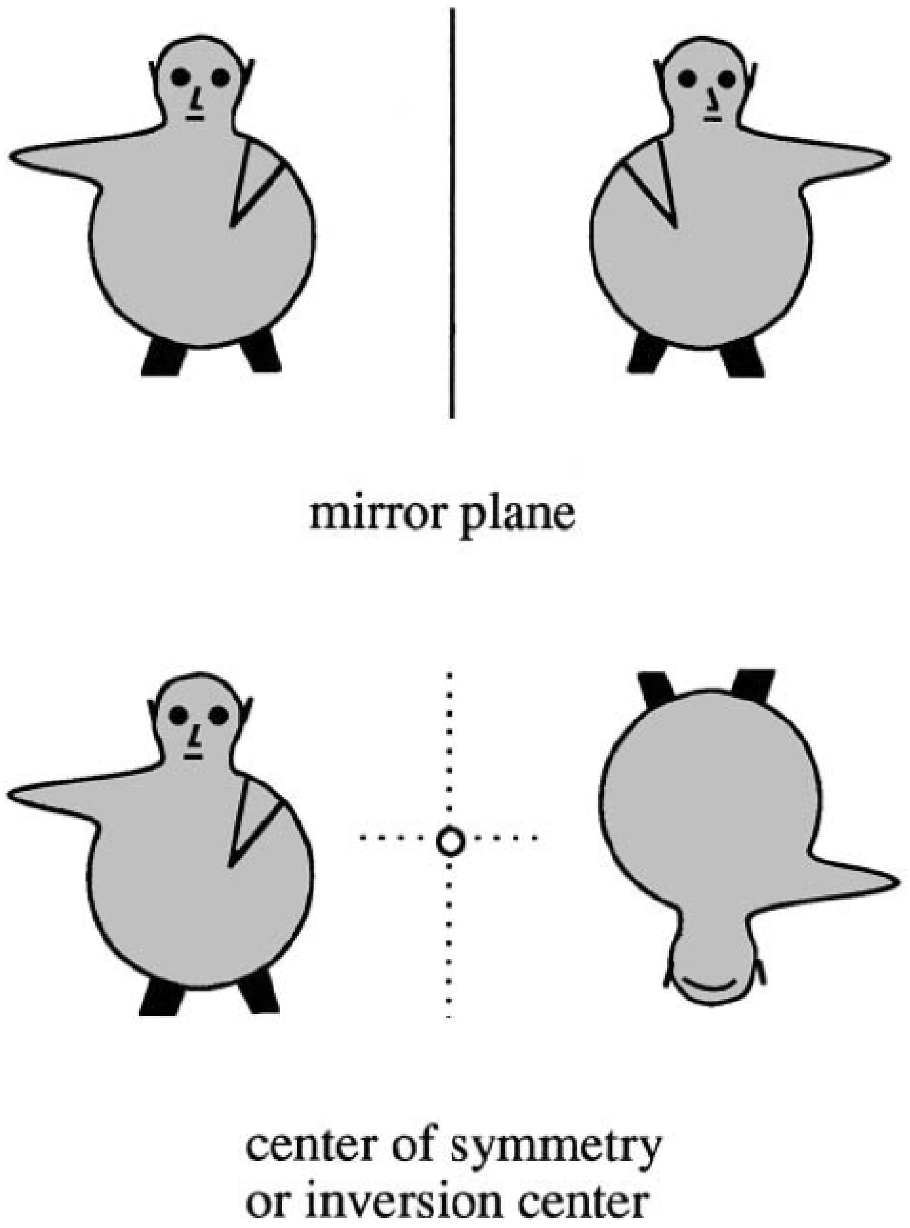


Figure 3.14. The effect of a mirror and of an inversion center.

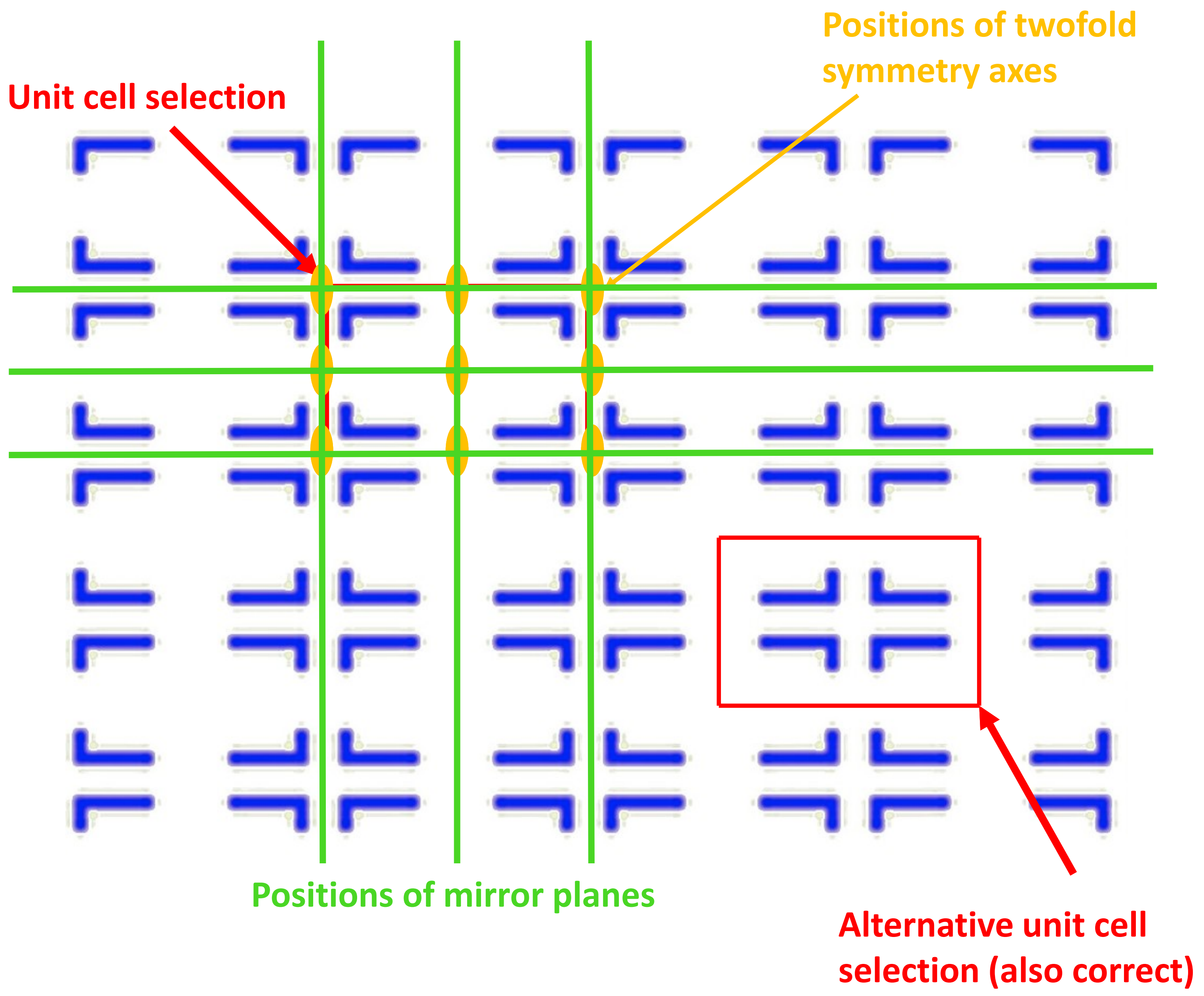
Guide to the recognising of wallpaper groups

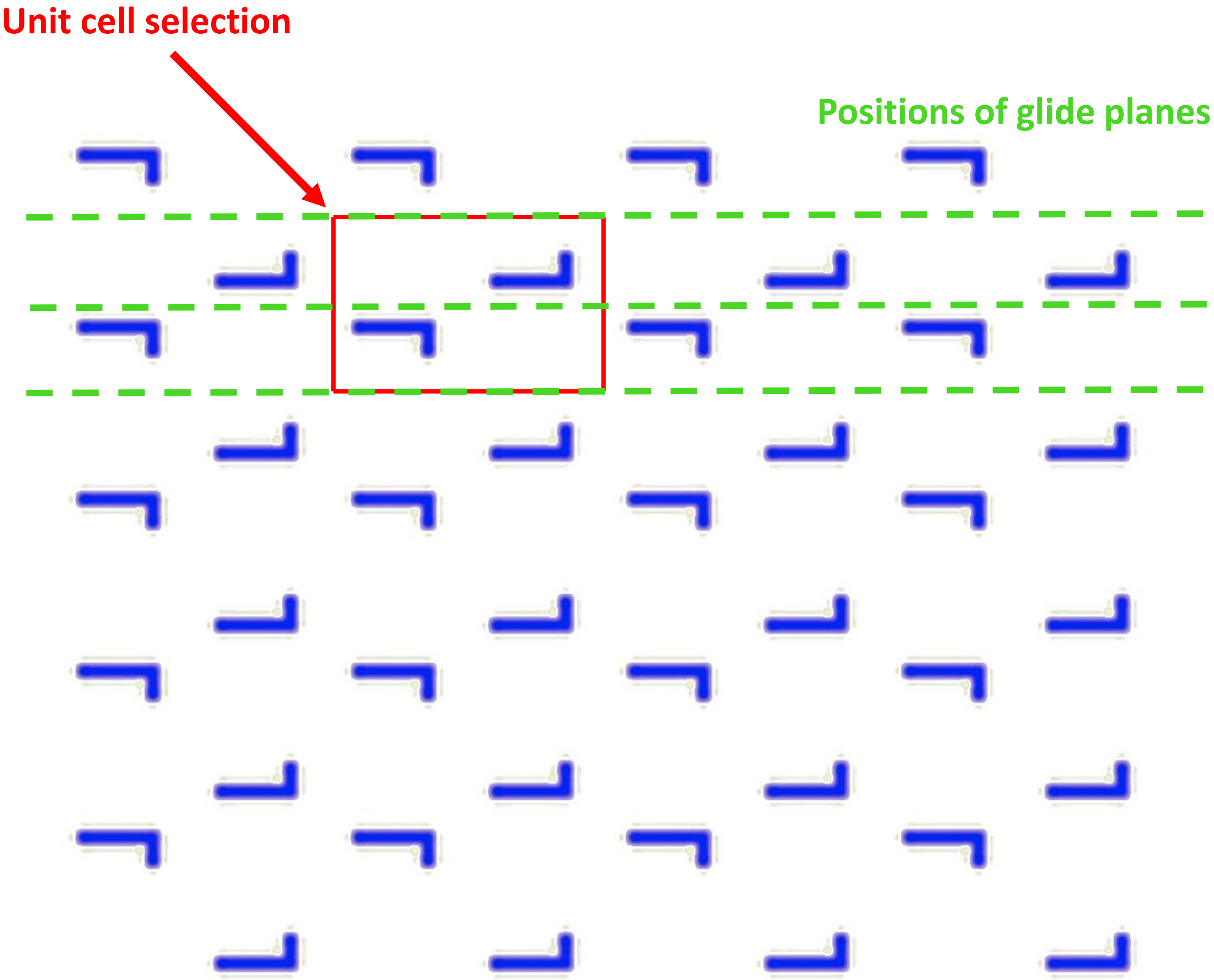
1. Identify the smallest unit cell that represents all the symmetry included in the pattern. (Be particularly careful in the case of centered symmetry. Use rhomb shaped cells for patterns with 3 and 6-fold rotation axes.)

2. Search for mirror and glide planes, mark rotation axes if any.

3. Use the following table to identify the wallpaper group:
   1. Find the least rotation.
   2. Are there mirror planes in the pattern?
   3. Answer the subsequent question(s) if there are any.

<table>
<tr><th rowspan="2">Least rotation</th><th colspan="2">Has the pattern mirror plane(s)?</th></tr>
<tr><th>Yes</th><th>No</th></tr>
<tr><td>60°</td><td>p6m</td><td>p6</td></tr>
<tr><td>90°</td><td>Do the 4-fold axes lie on mirrors?
yes - p4mm no - p4g</td><td>p4</td></tr>
<tr><td>120°</td><td>Is there at least one rotation centre not lying on mirrors?
yes - p31m no - p3m1</td><td>p3</td></tr>
<tr><td>180°</td><td>Are the mirrors perpendicular?
Yes: Is there at least one rotation centre not lying on mirrors? Yes - c2mm No - p2mm
No: p2mg</td><td>Has the pattern glide plane?
Yes - p2gg No - p2</td></tr>
<tr><td>360°</td><td>Has a glide plane not identical with mirror plane?
Yes - cm No - pm</td><td>Has the pattern glide plane?
Yes - pg No - p1</td></tr>
</table>


Positions of twofold symmetry axes
Unit cell selection
Positions of mirror planes
Alternative unit cell selection (also correct)


Unit cell selection
Positions of glide planes

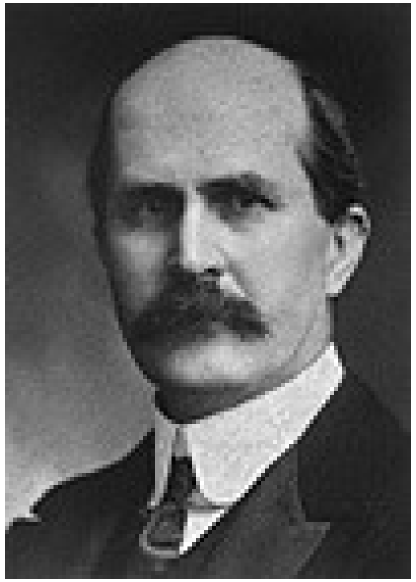
End of lecture #1 in 2022

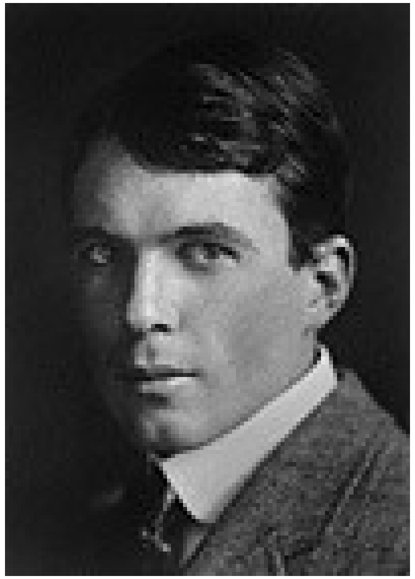
SIR WILLIAM HENRY BRAGG (1862-1942)
SIR WILLIAM LAWRENCE BRAGG (1890-1971)

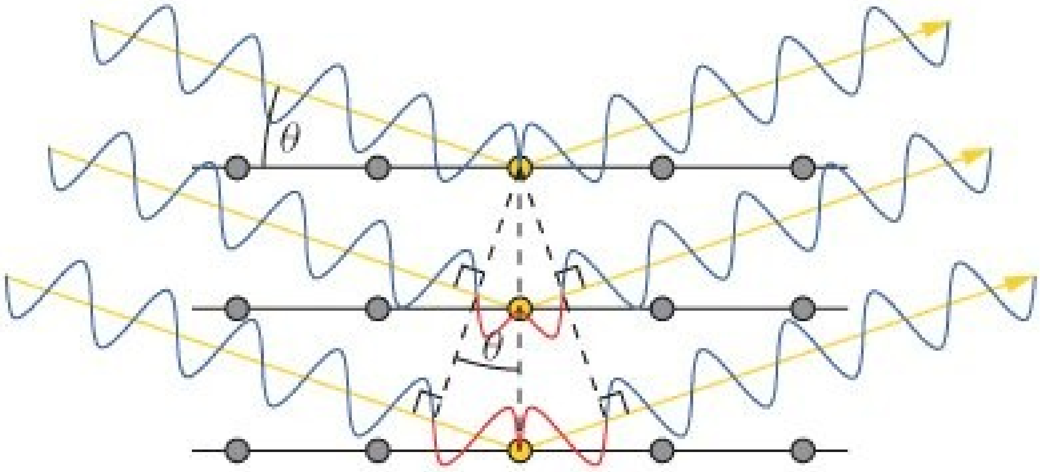
- **1915 Nobel Laureates in Physics**

for the analysis of crystal structure by means of X-rays

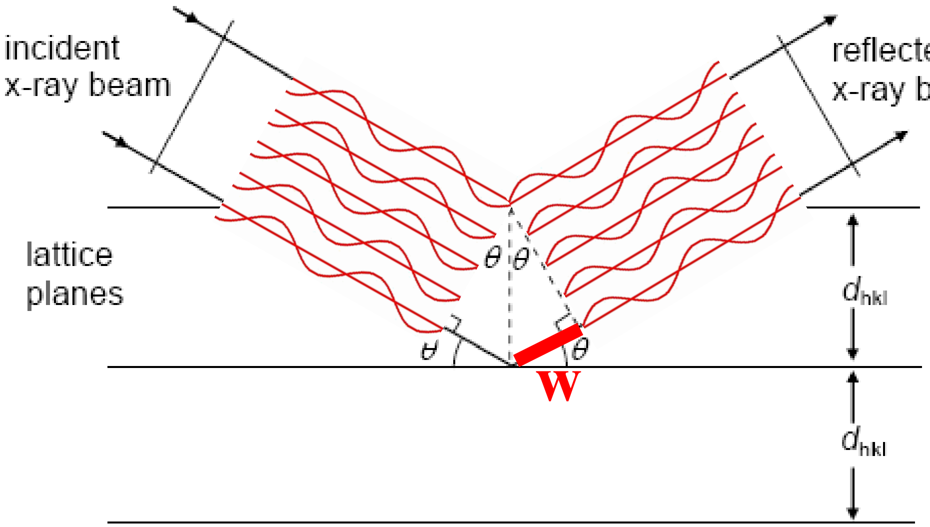
$$\boxed{n\lambda = 2d\sin\theta}$$





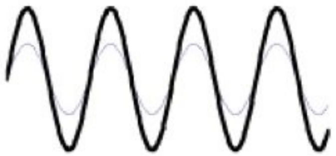


There is NO PHASE DIFFERENCE if the path differences are equal to whole number multiplies of wavelength (λ)



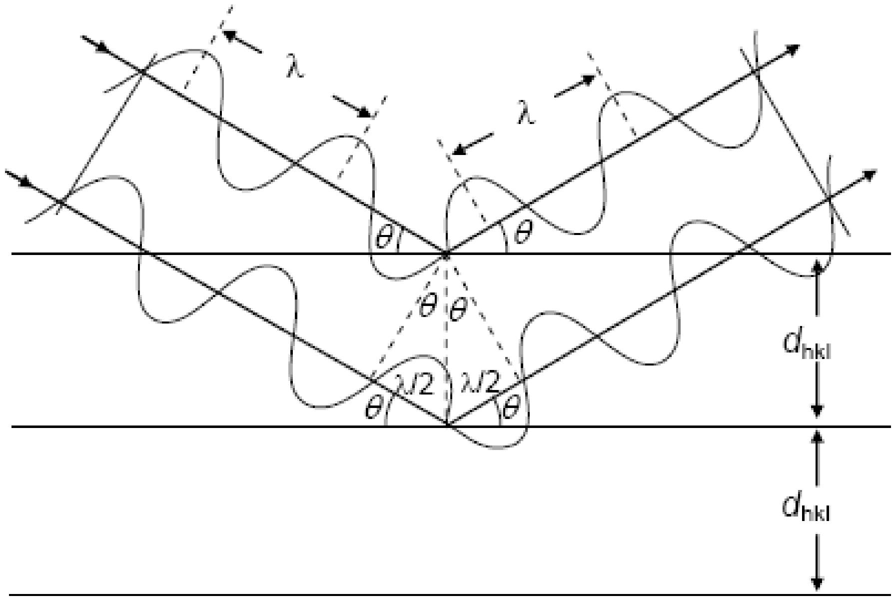
Bragg's law:

$$\boxed{n\lambda = 2d \sin\theta}$$

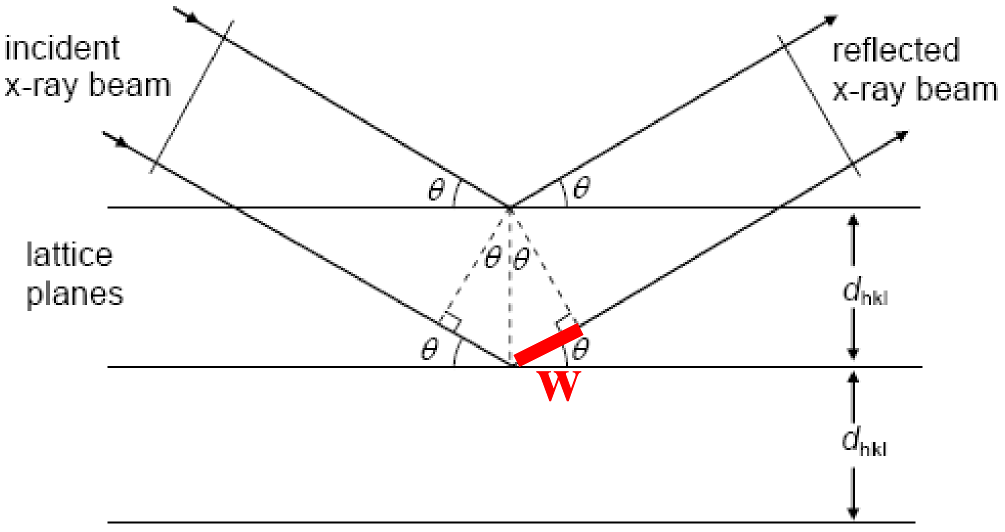


$\sin\theta = \mathbf{w}/d$

$2\mathbf{w} = n\lambda$

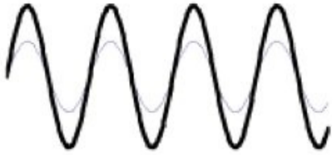


There is NO PHASE DIFFERENCE if the path differences are equal to prime number multiples of wavelength (λ)



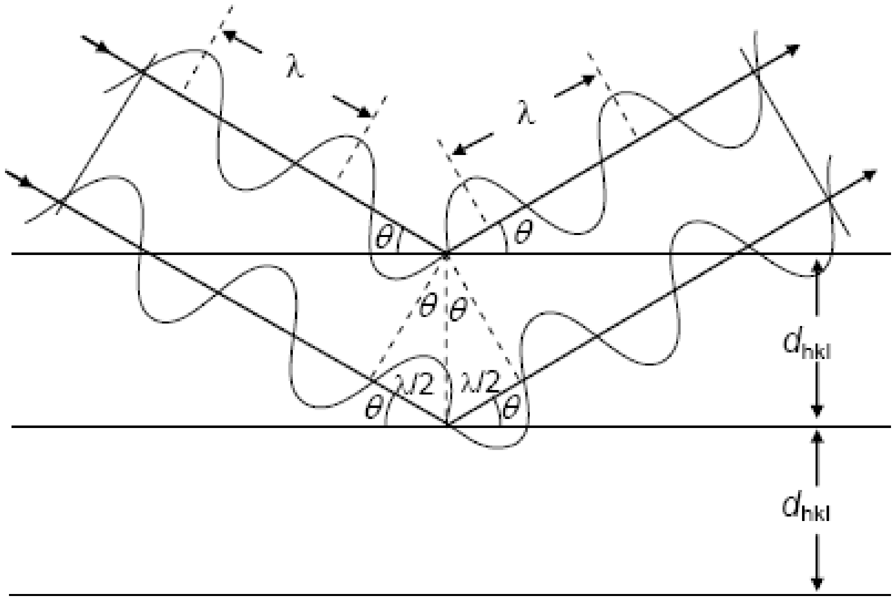
Bragg's law:

$$\boxed{n\lambda = 2d\sin\theta}$$



$\sin\theta = \mathbf{w}/d$

$2\mathbf{w} = n\lambda$



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