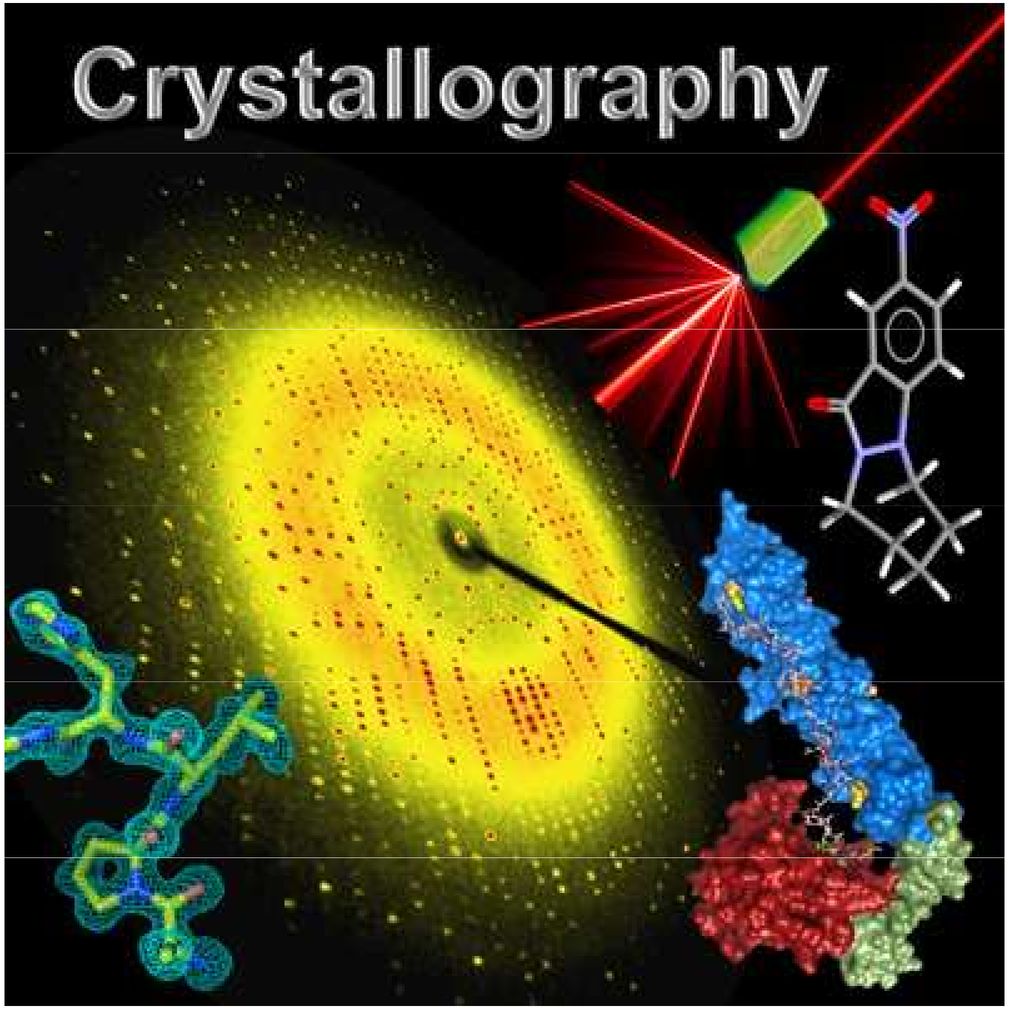
C7270
Biological X-Ray Crystallography and Cryo-Electron Microscopy

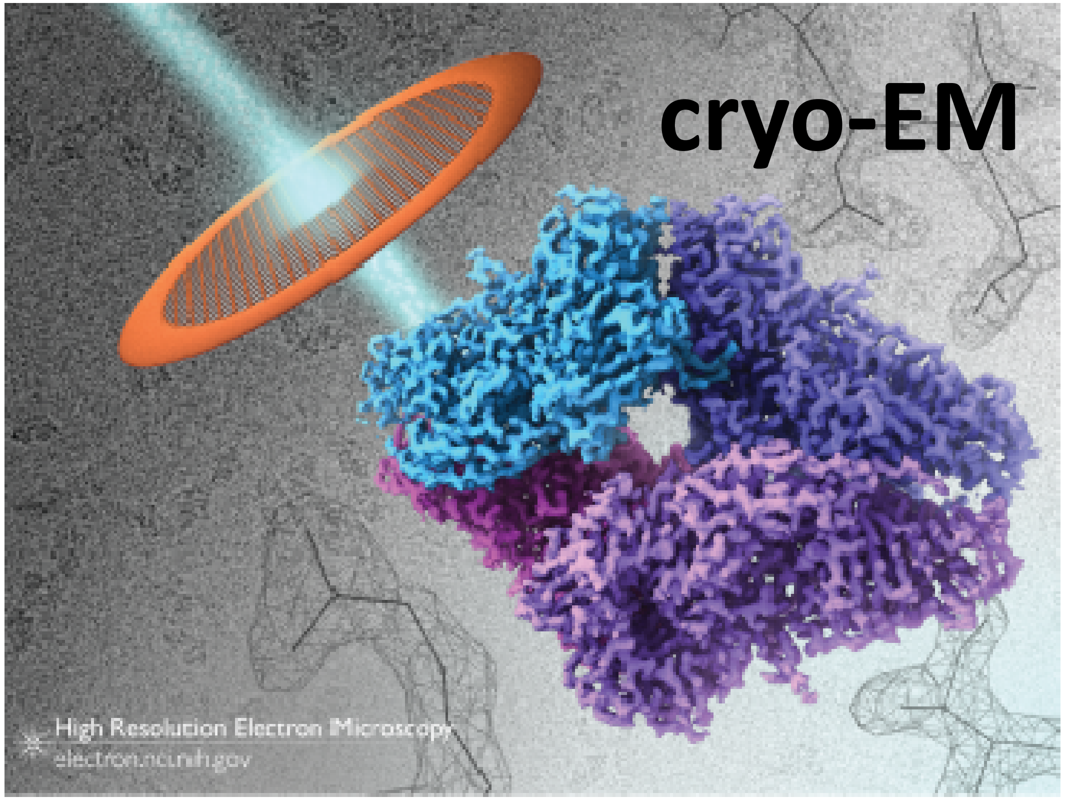
Fall 2020

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

Class rules

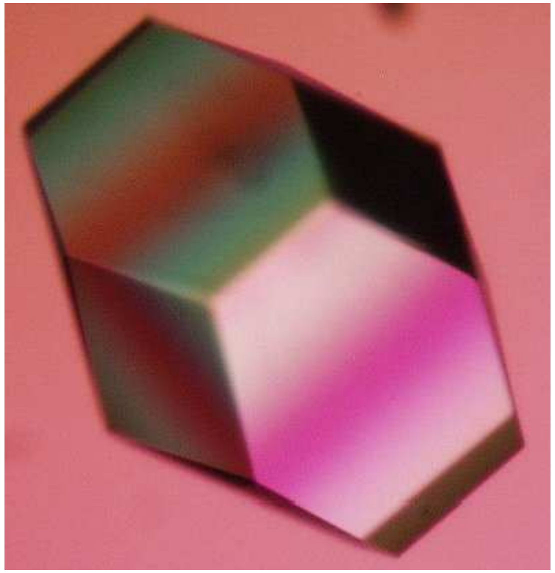
- Please keep your microphone muted.
- When you want to ask question or comment, please unmute your microphone and speak directly.
- I would appreciate if you keep your video on. It is not much fun to lecture to black boxes.
- Ask questions - it will help to clarify the issue not only for you but for your peers as well!
- In class discussions, be respectful of other students' opinions.

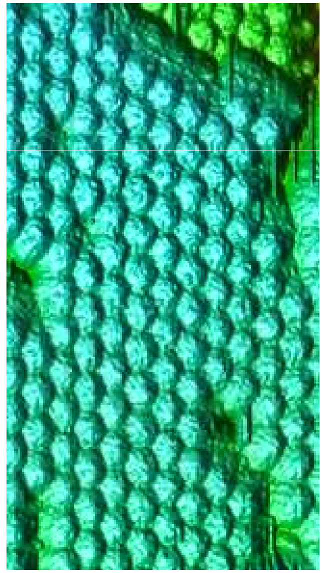

Crystallography


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

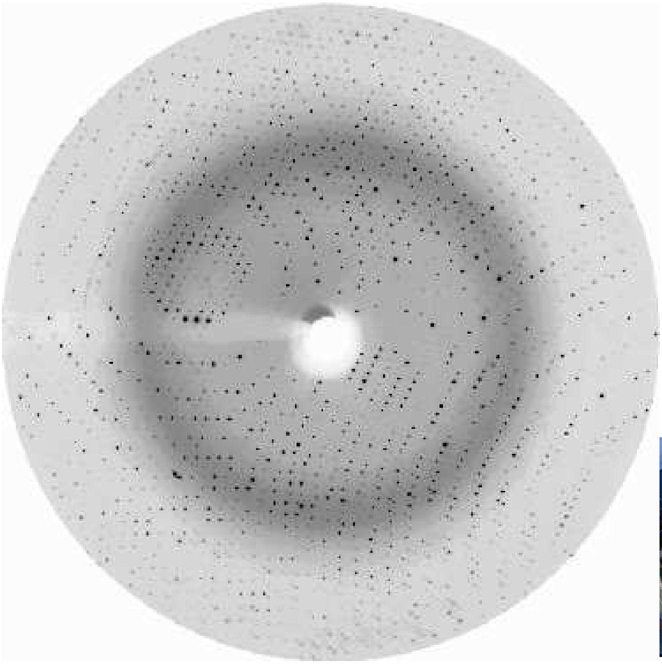
1. Expression & purification

2. Crystallization

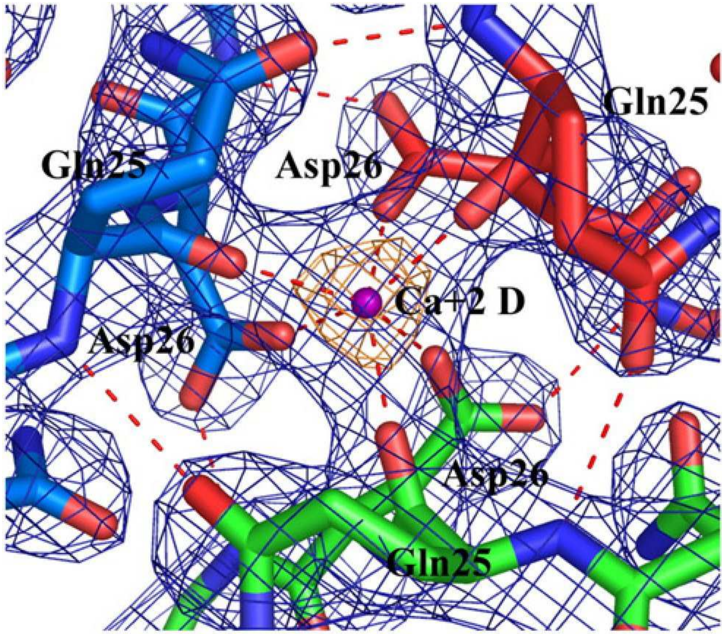




3. Diffraction data

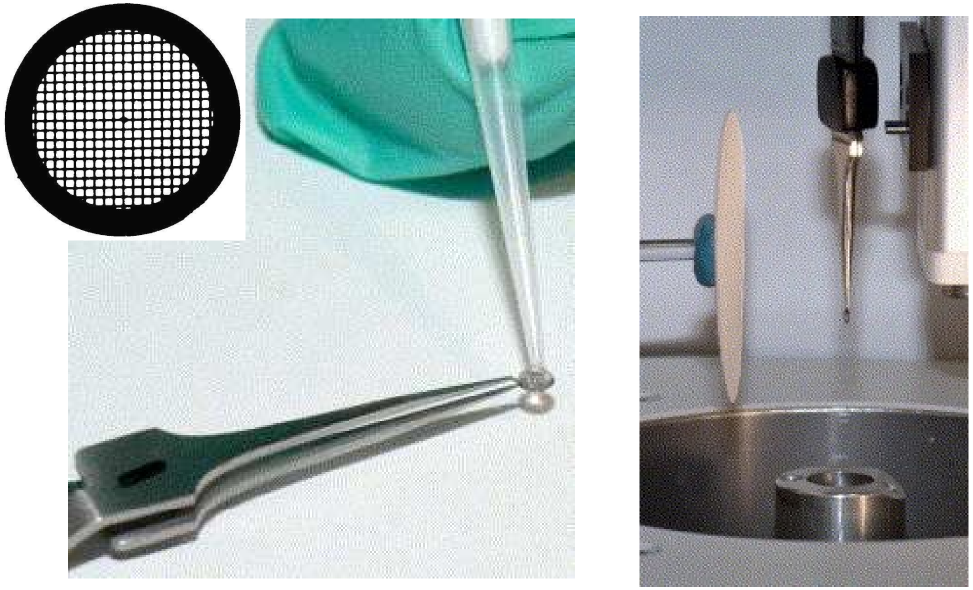


4. Solve structure

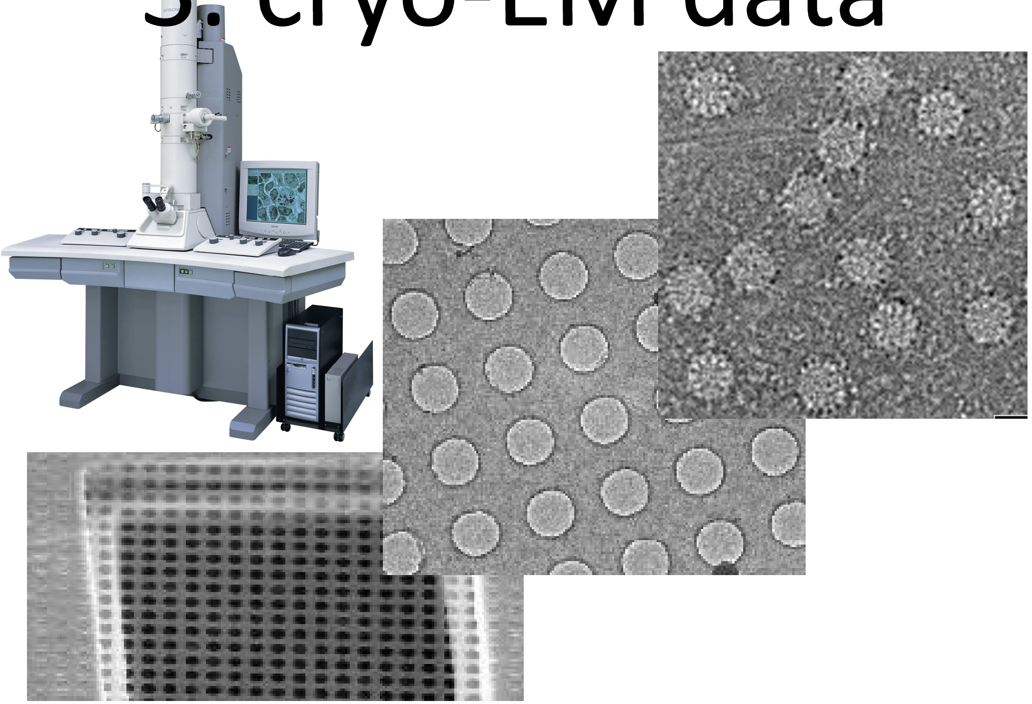


1. Expression & purification

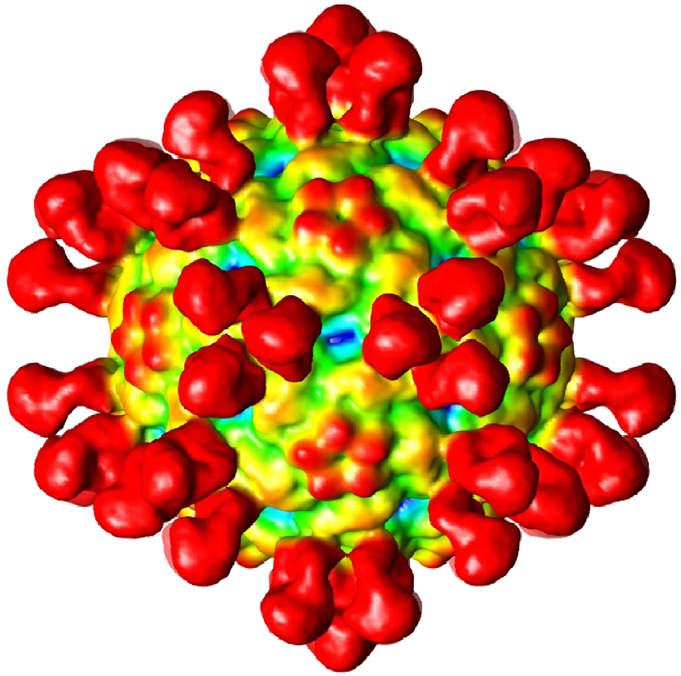
2. Grid preparation



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
- Do voluntary homeworks
- I am here to help, learning is up to you!

Levels of passing the course:

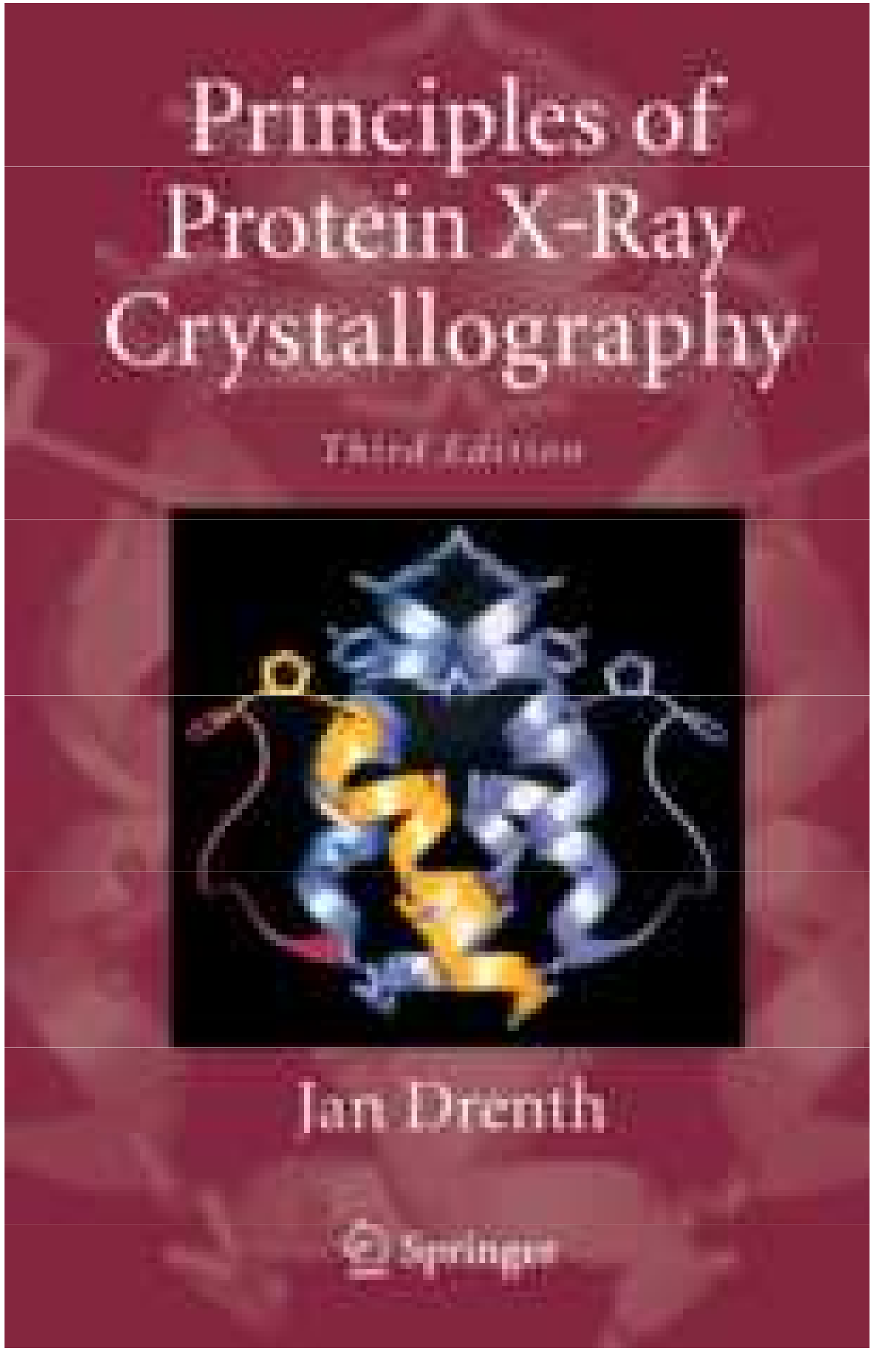
“Sitter” – hand in homework, participate in discussions => grade E

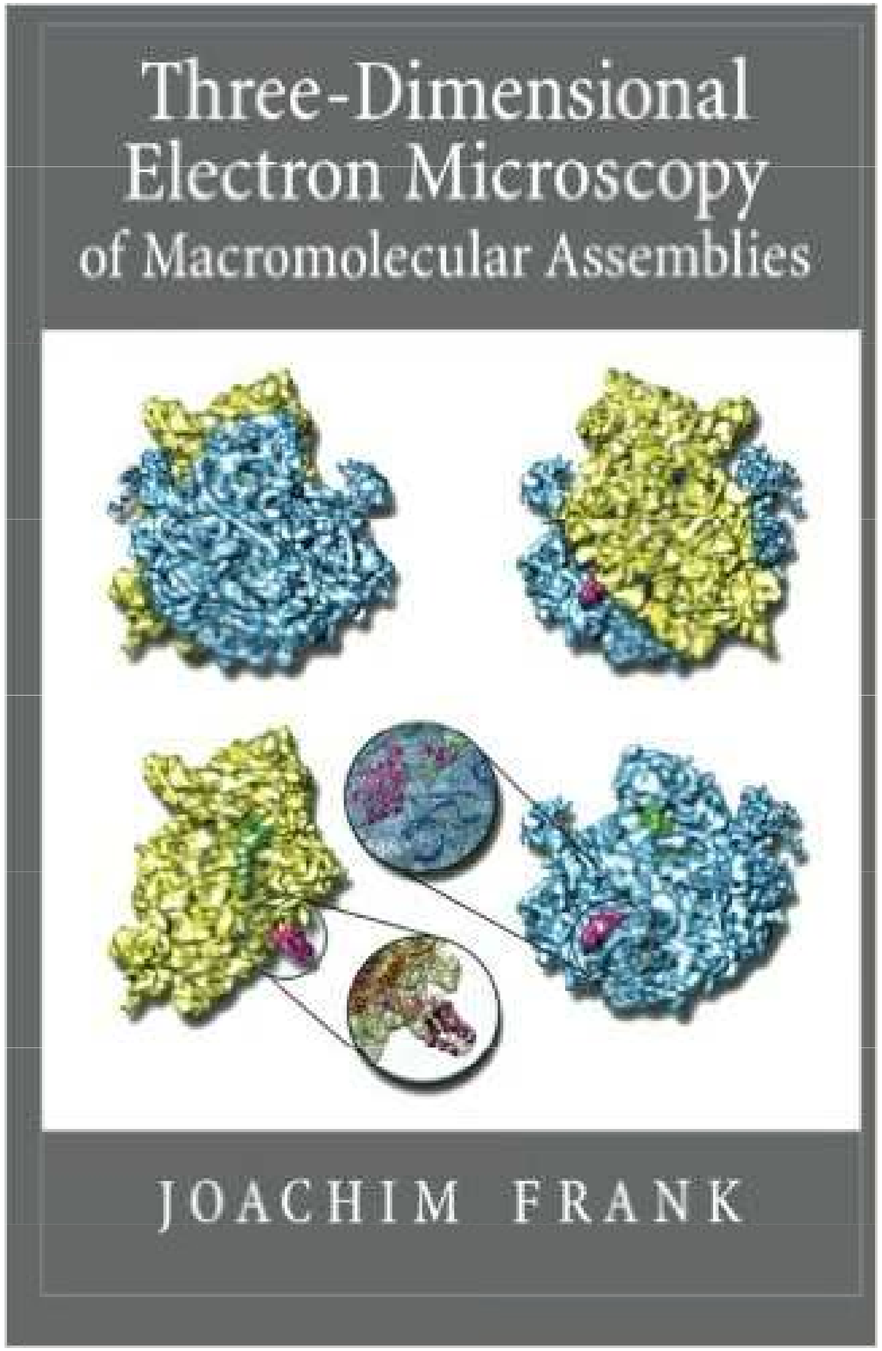
“Student” – sitter + take theoretical part of the exam (will include symmetry and equations)

Not part of this course:

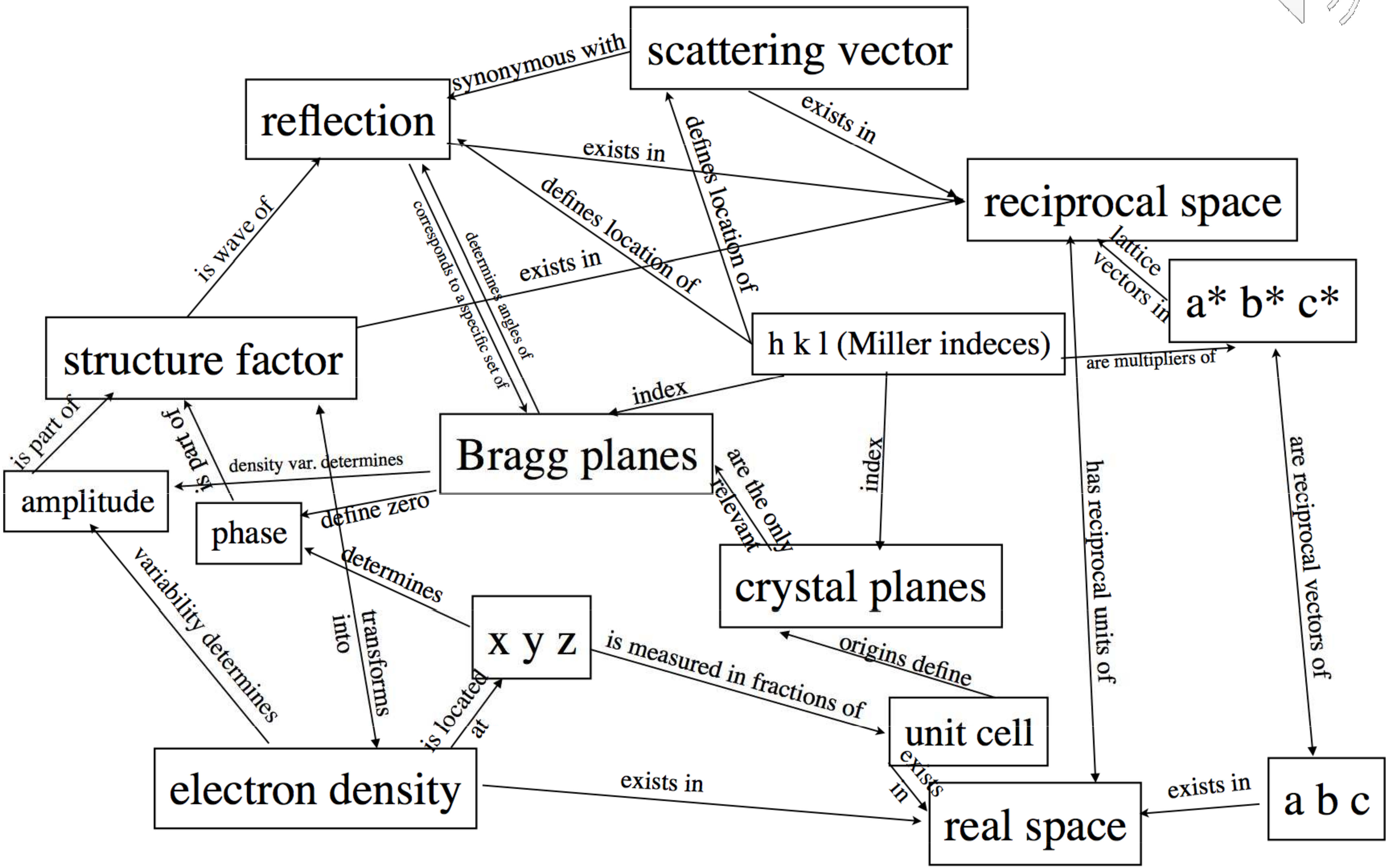
- Basic math – mental overload by dealing with simple equations. (Observed before.)
- Reserve time for thinking. You will never have more time than now.

Course textbooks:

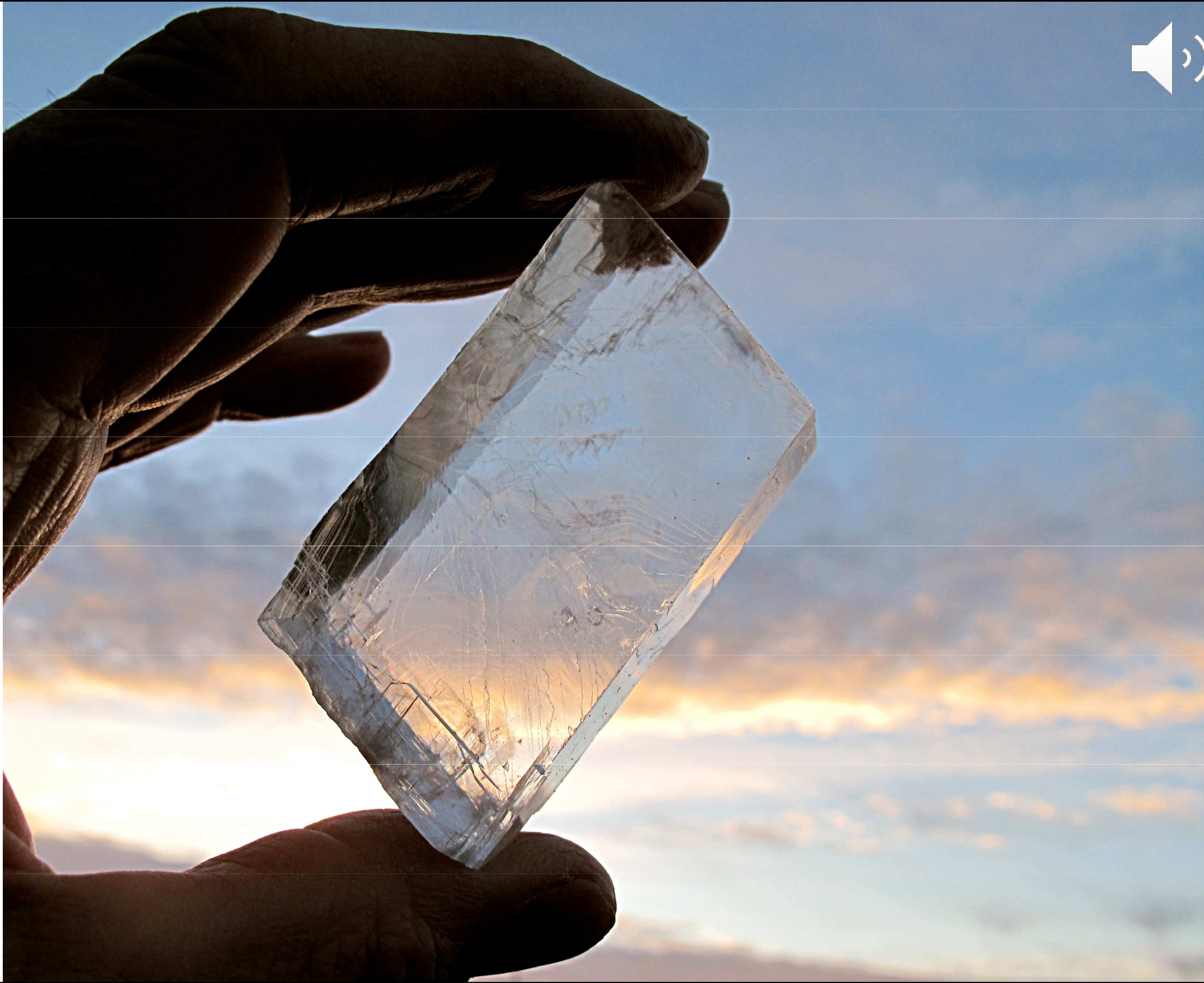




L#	Date	Time	Lecturer	Topic	Chapter reading
1	7.10.	14:00 - 16:30	Pavel Plevka	Development of X-ray crystallography, crystallization of macromolecules, phase diagram, Crystal symmetry, symmetry operators, point groups, space groups.	Drenth: 1, 2, 3
2	14.10.	14:00 - 16:30	Pavel Plevka	Diffraction of light by electrons, atoms, unit cell, crystal. Bragg's law. Diffraction images and indexing.	Drenth: 4
3	21.10.	14:00 - 16:30	Pavel Plevka	Fourier transform, structure factor, intensity of diffraction spots.	Drenth: 4, 5
4	28.10.	14:00 - 16:30	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	4.11.	14:00 - 16:30	Tibor Füzik	Electron microscope. Interaction of electrons with matter, electron imaging. Amplitude and phase contrast. Contrast transfer function.	Frank: 1, 2
6	11.11.	14:00 - 16:30	Tibor Füzik	Fourier transform and its properties, convolution, point spread function.	Frank: 2
7	18.11.	14:00 - 16:30	Jiří Nováček	Analysis of electron micrographs. 2D classification. Principal component analysis.	Frank: 3, 4
8	25.11.	14:00 - 16:30	Jiří Nováček	Three dimensional reconstruction - single particle reconstruction and tomogram calculation. 3D classification.	Frank: 5
9	2.12.	14:00 - 16:30	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.	

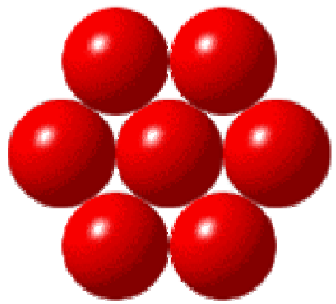


scattering vector
reflection
synonymous with
exists in
exists in
reciprocal space
defines location of
defines location of
is wave of
corresponds to a specific set of
determines angles of
exists in
lattice vectors in
a* b* c*
structure factor
h k l (Miller indeces)
are multipliers of
index
is part of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

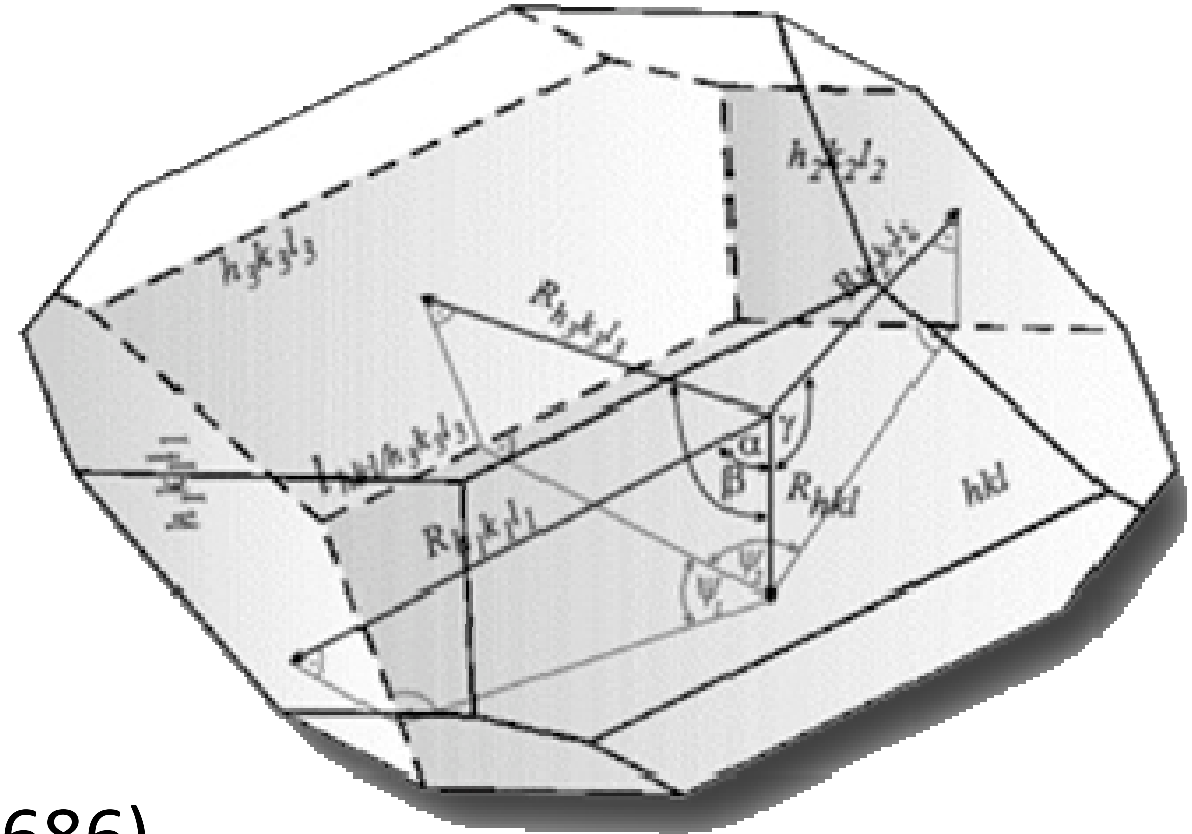


Why do single snowflakes, before they become entangled with other snowflakes, always fall with six corners? Why do snowflakes not fall with five corners or with seven?

Johannes Kepler (1571-1630)



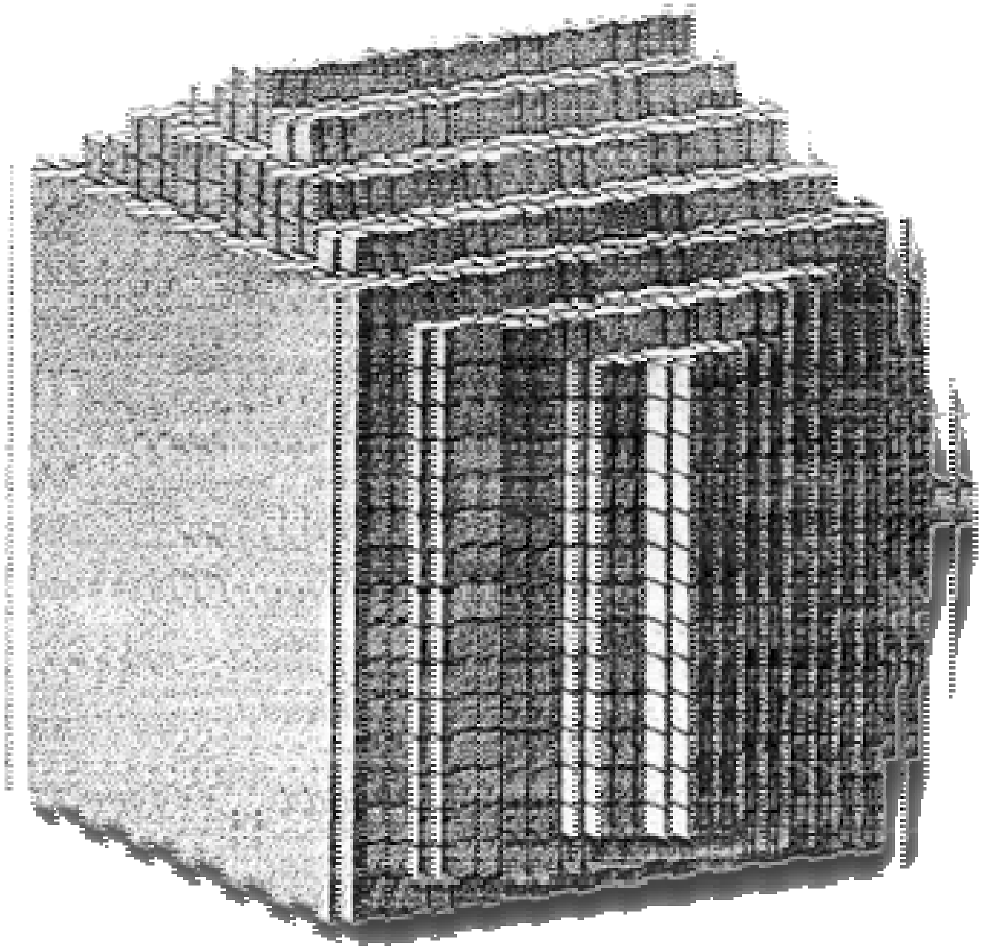
Although crystals of quartz and hematite appear in a great variety of shapes and sizes, the same interfacial angles persisted in every specimen. *“Law of Constancy of Angles”*



Niels Stensen (1638-1686)

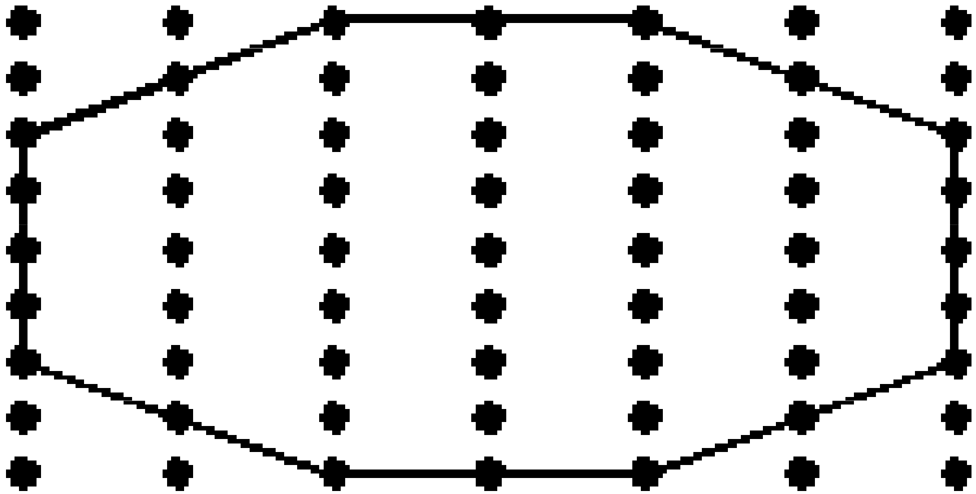
"Law of Constancy of Angles"

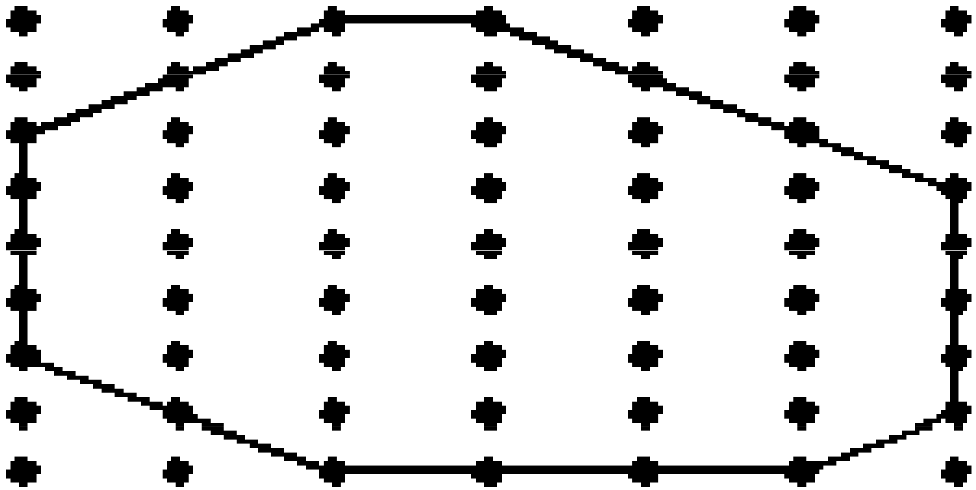
René Just Haüy (1743-1822)



"Law of Constancy of Angles"

René Just Haüy (1743-1822)



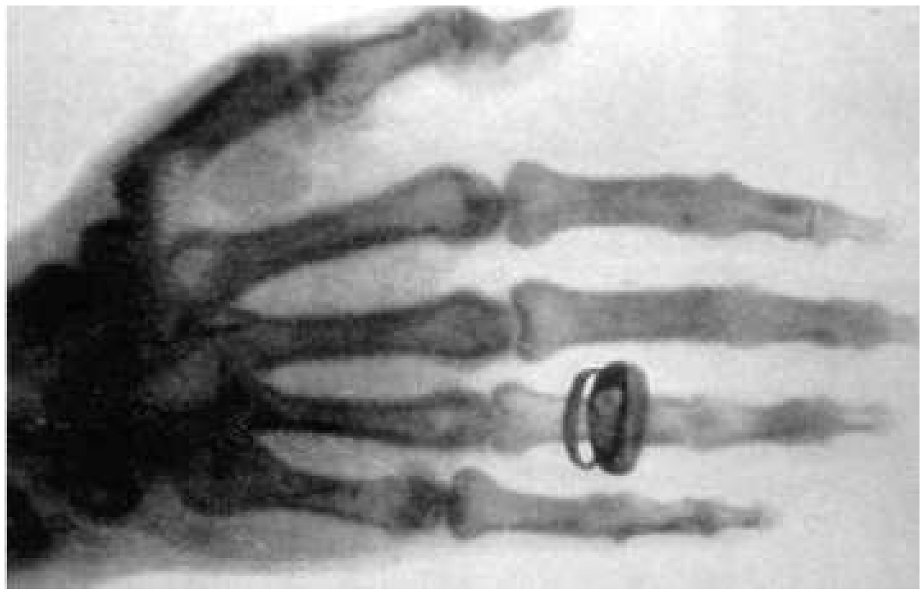


History of fundamental discoveries

WILHELM CONRAD RÖNTGEN (1845-1923)

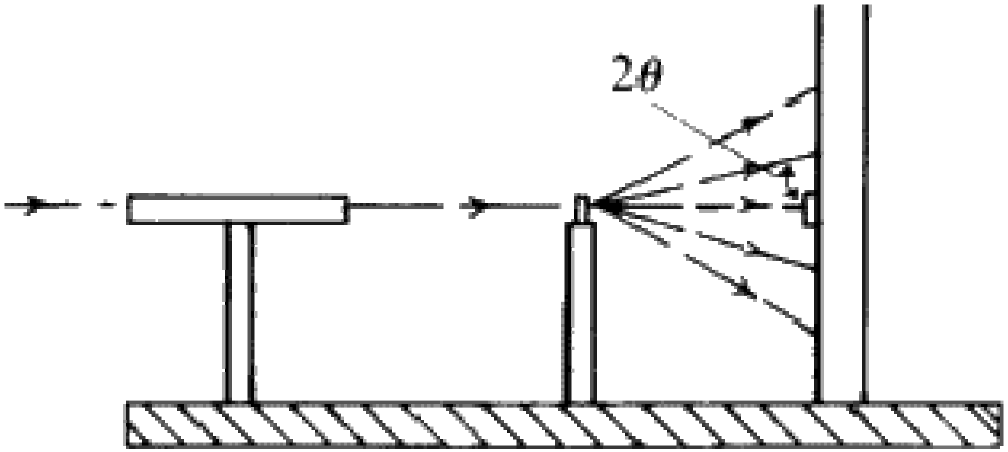
- **1901 Nobel Laureate in Physics**

  discovery of the remarkable rays subsequently named after him

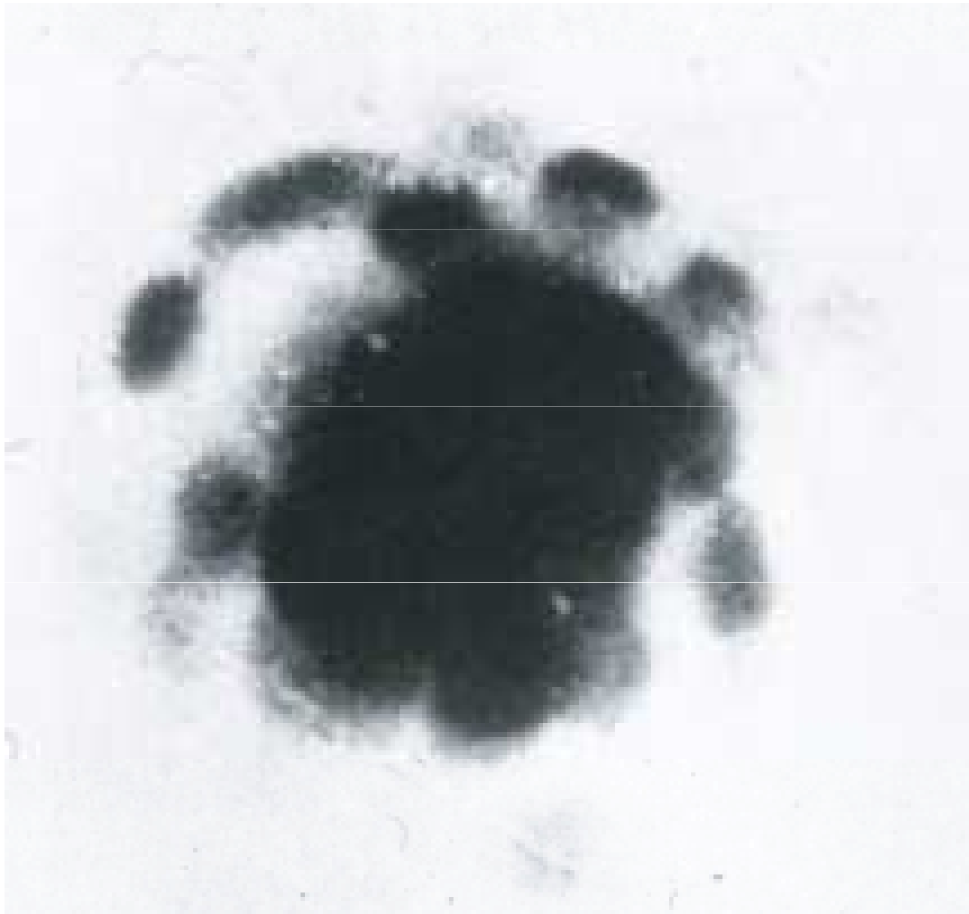


MAX VON LAUE
(1879-1960)

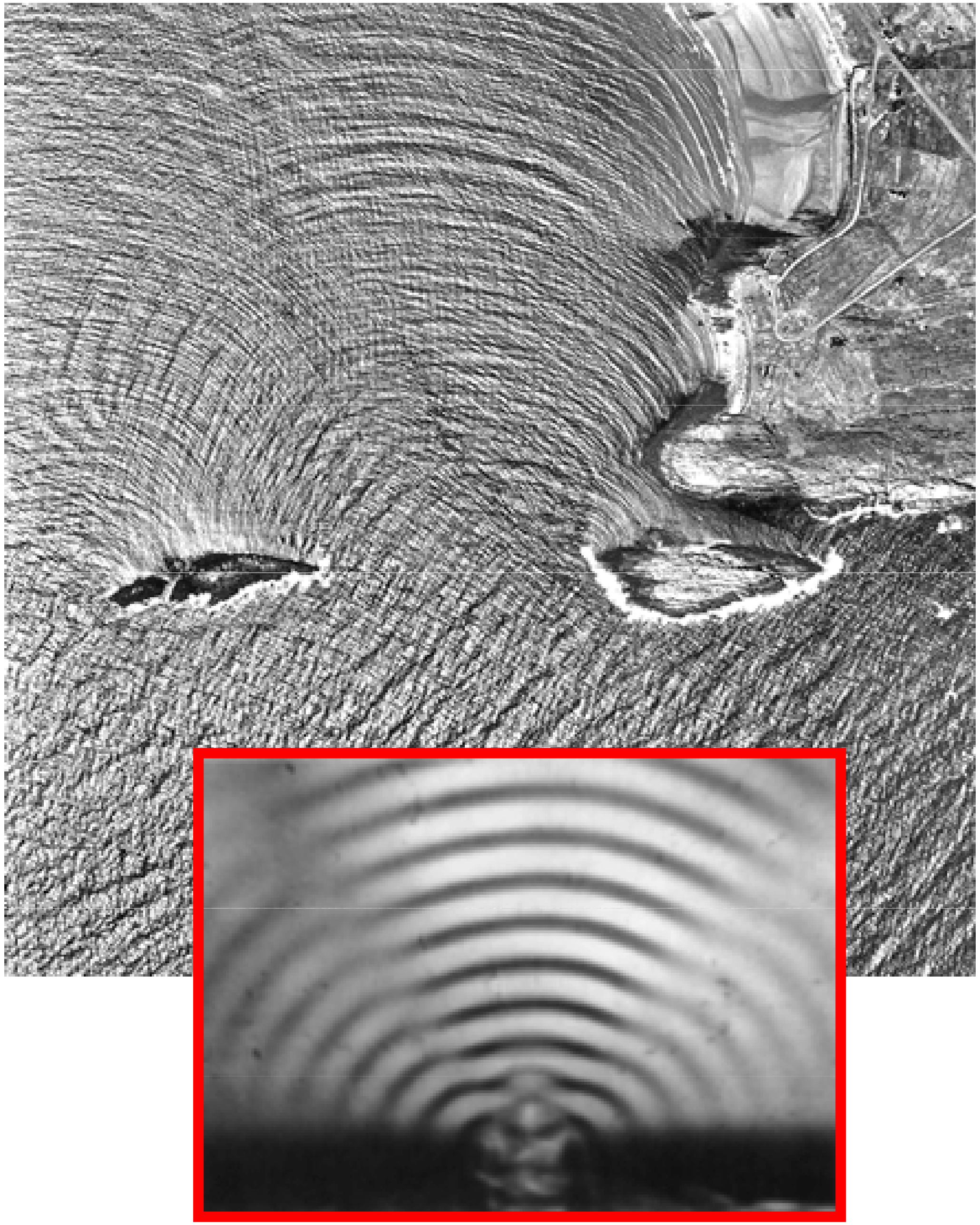
- **1914 Nobel Laureate in Physics**
  for his discovery of the diffraction of X-rays by crystals

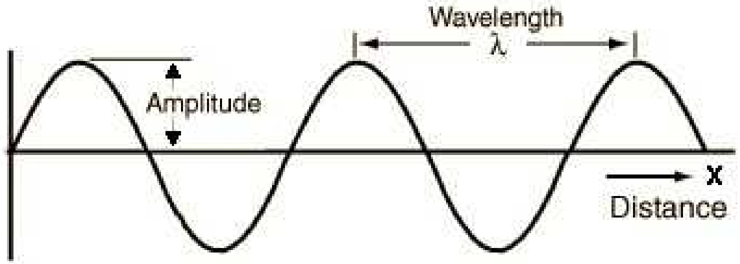


Friedrich and Knipping

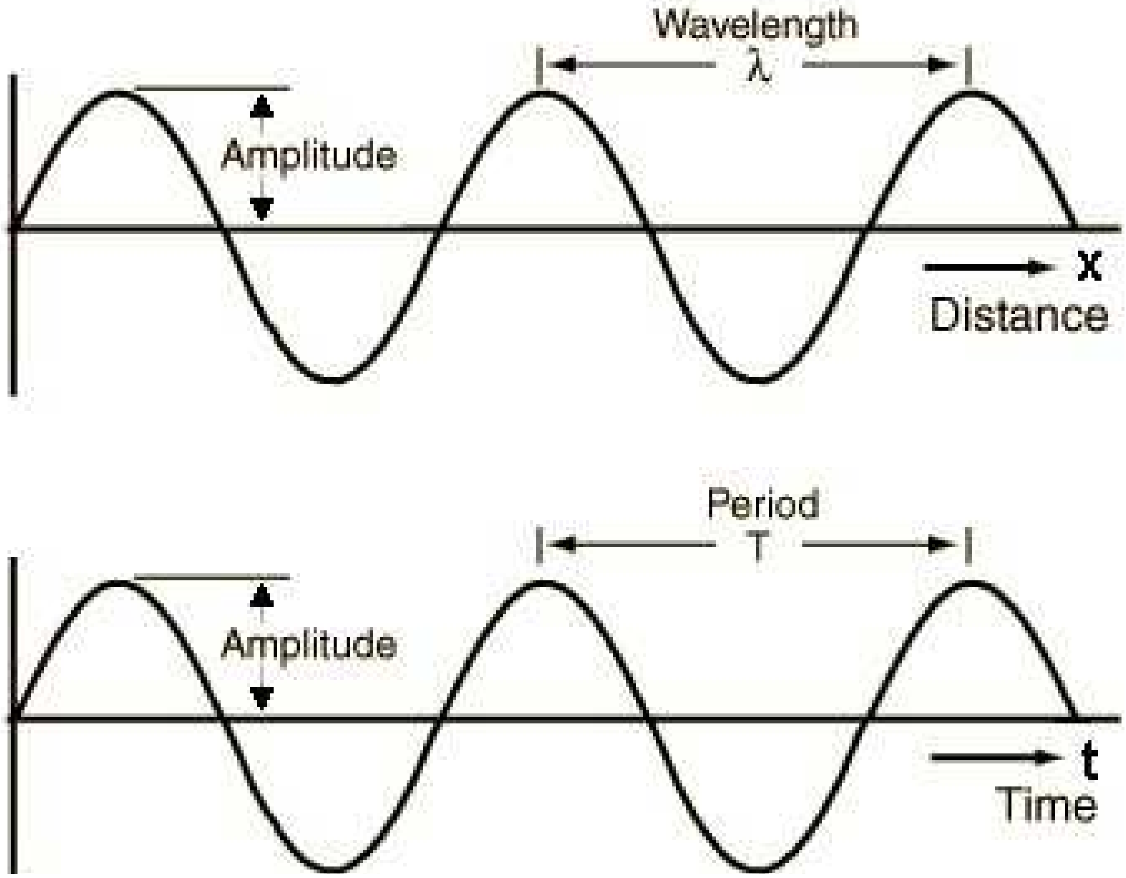


Wavelength and diffraction

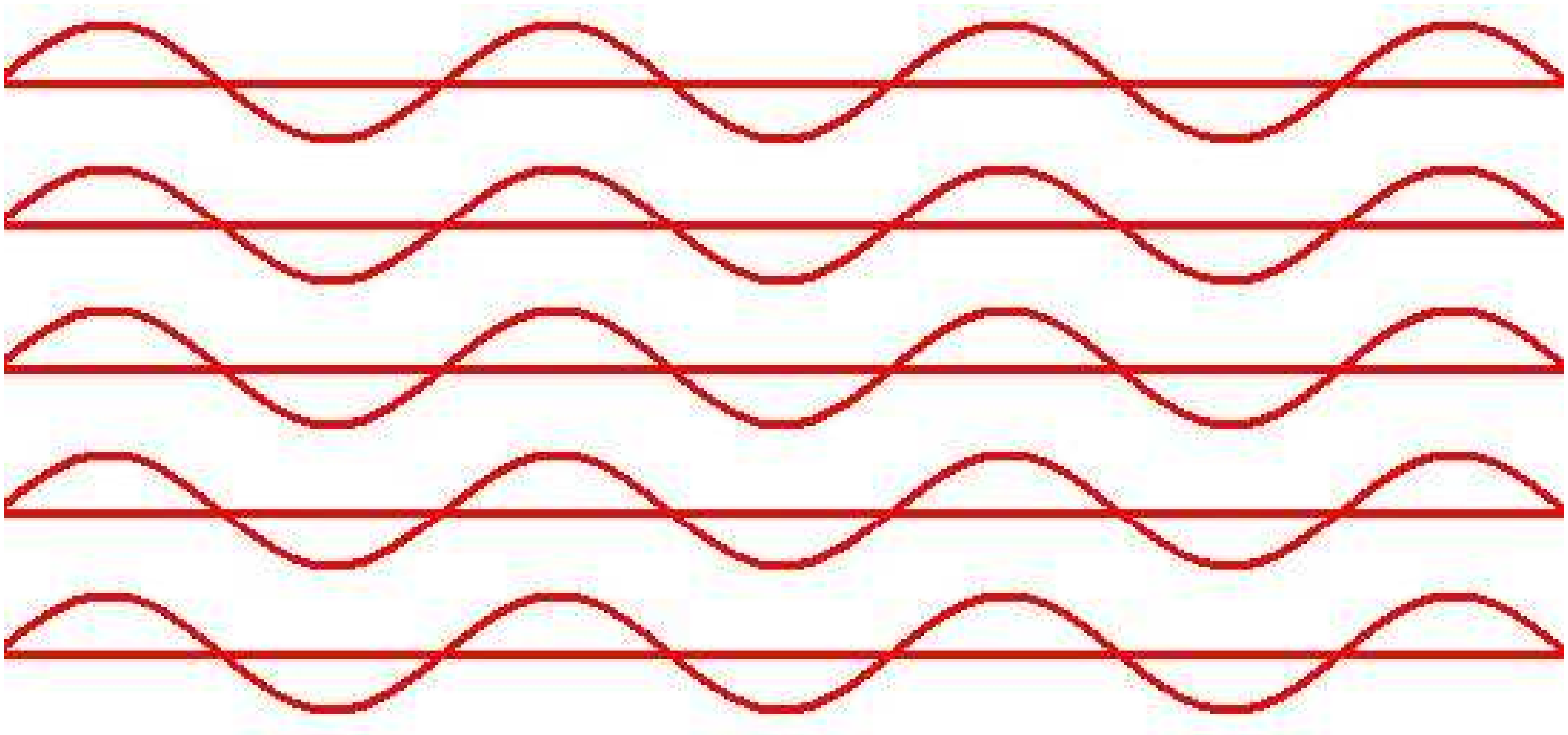




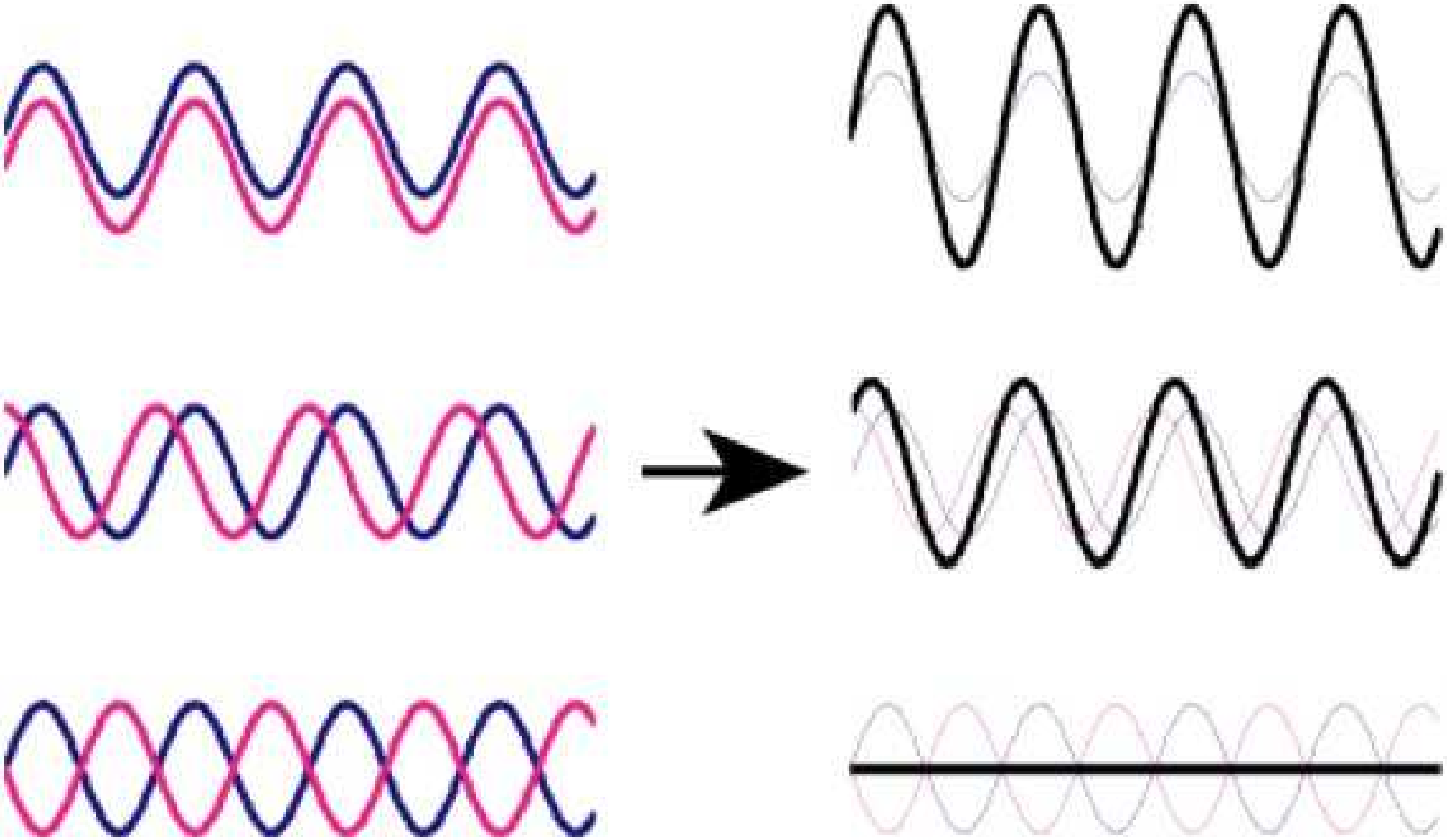
Waves


Wavelength
λ
Amplitude
x
Distance
Period
T
Amplitude
t
Time

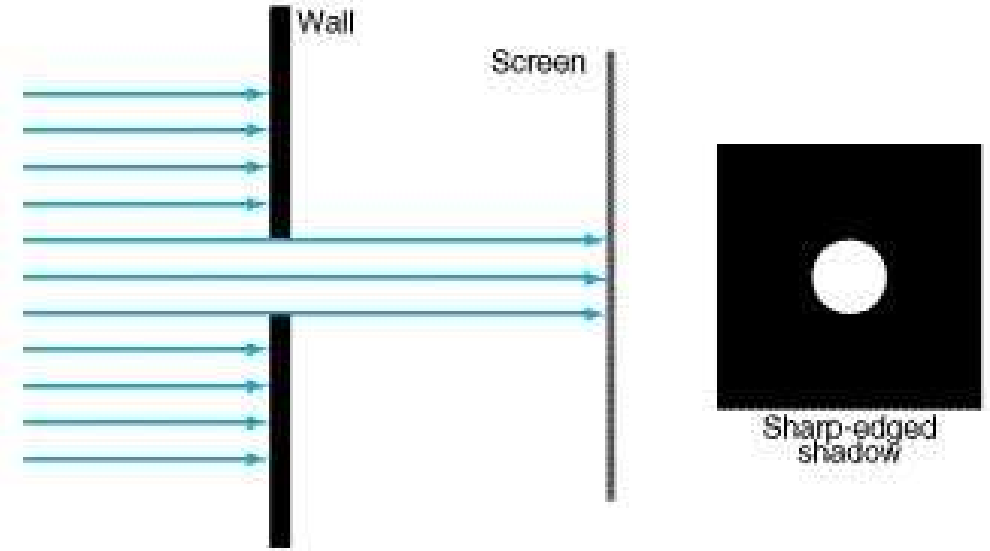
Coherent beam

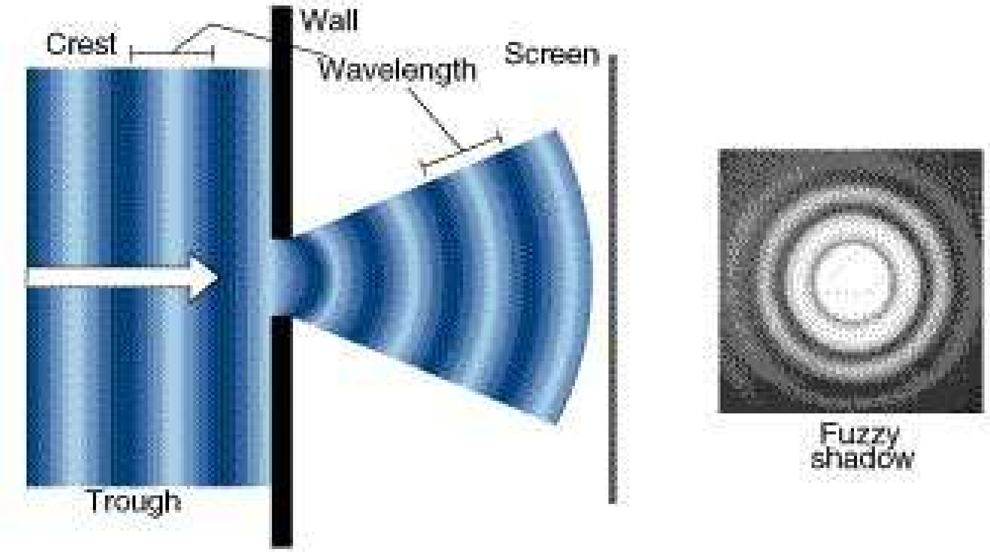


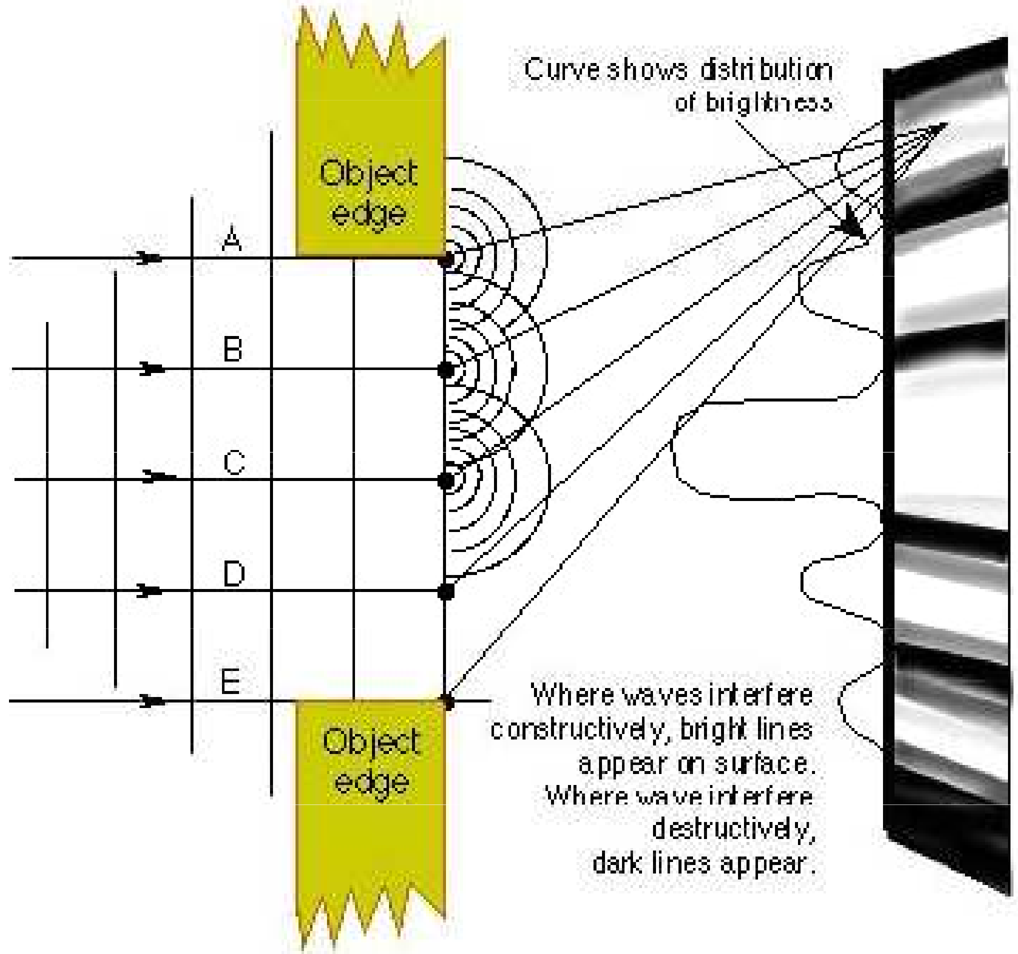
Addition of waves



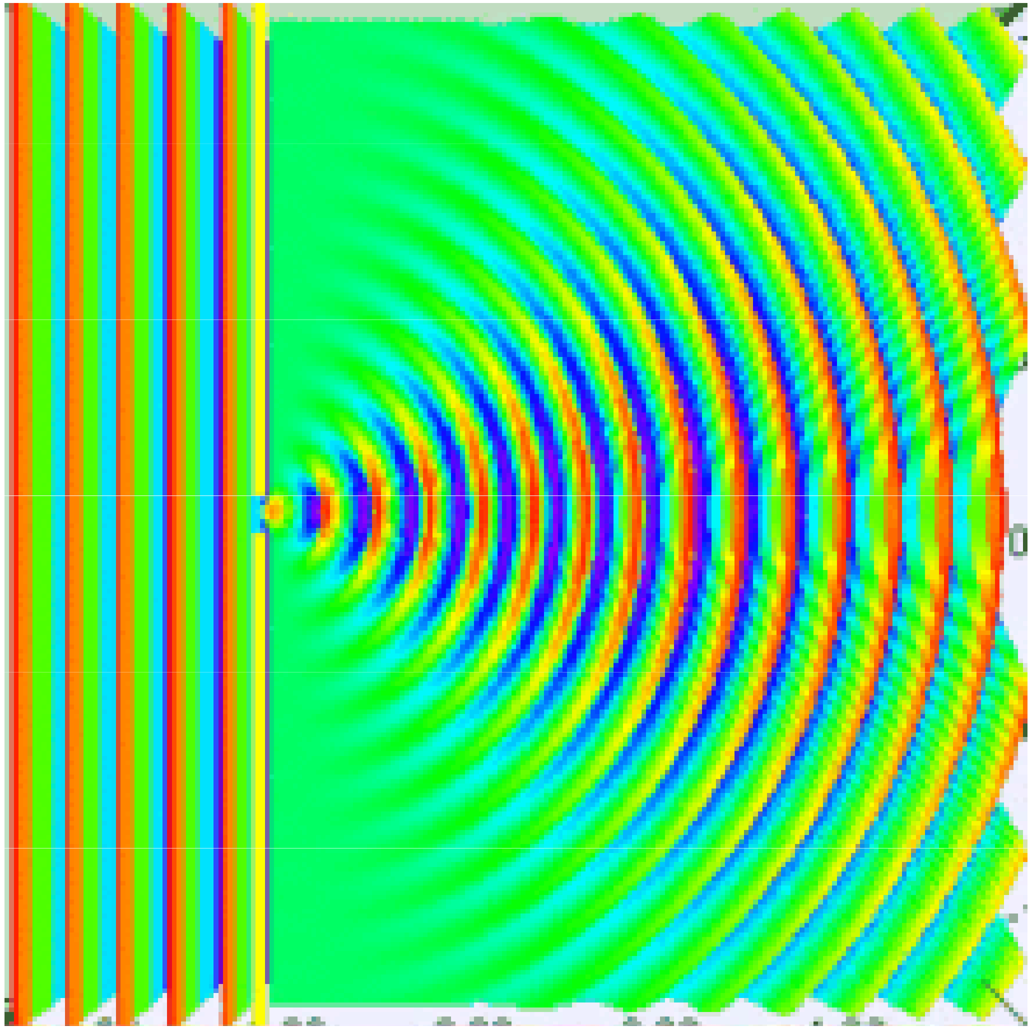
Particles & waves

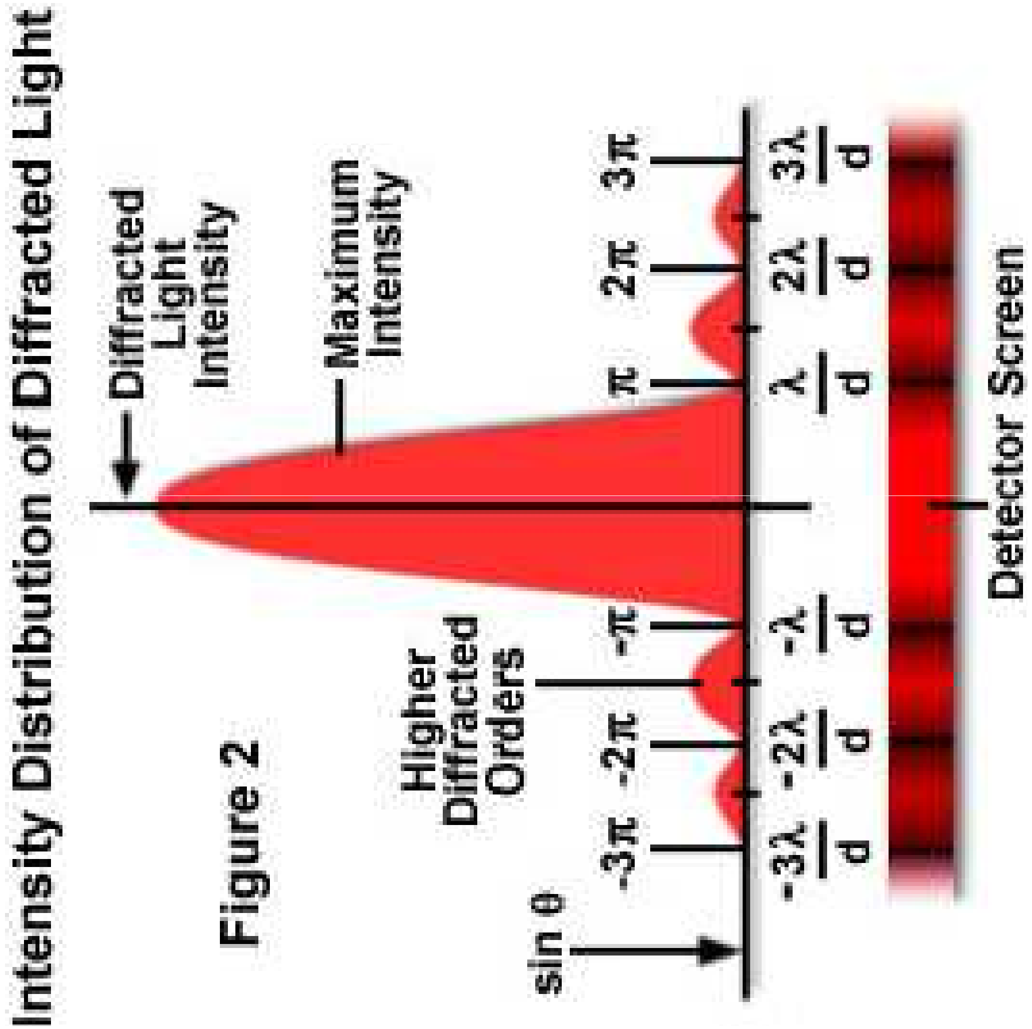




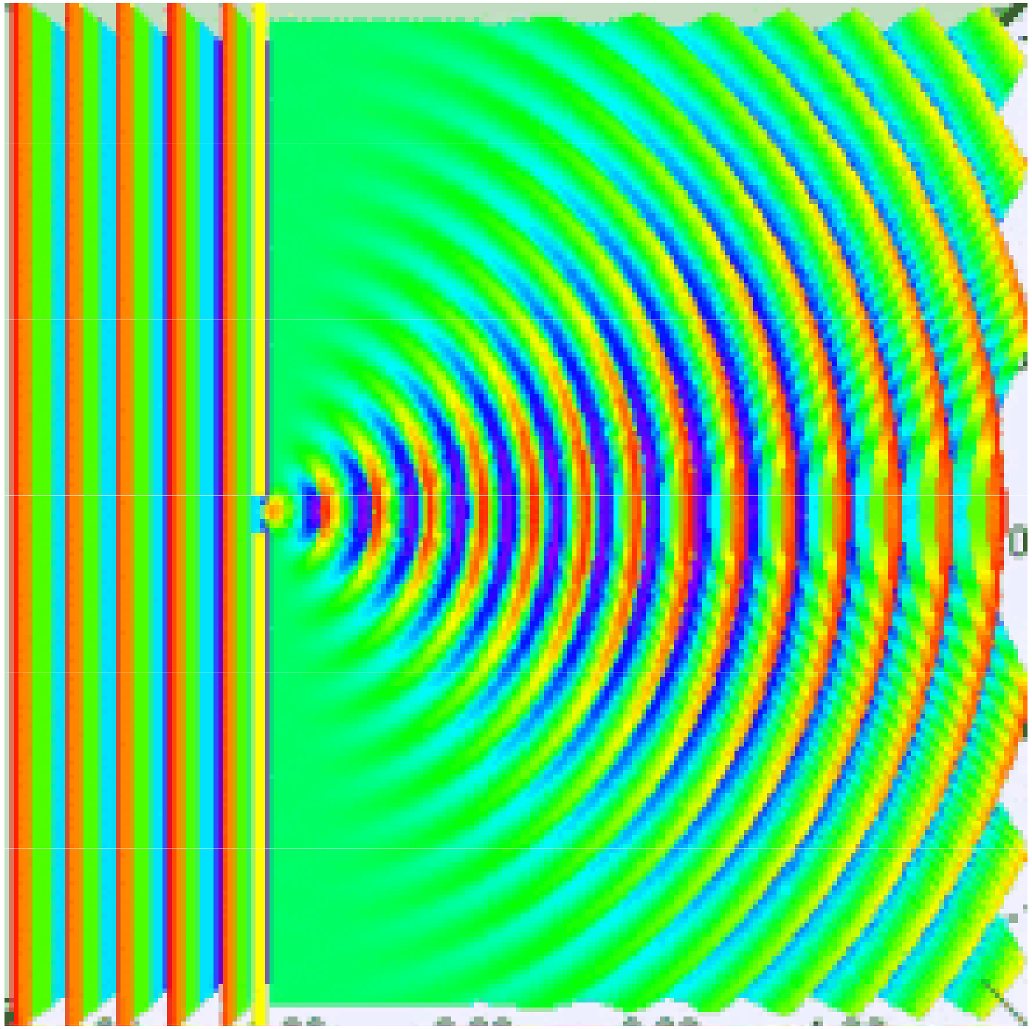


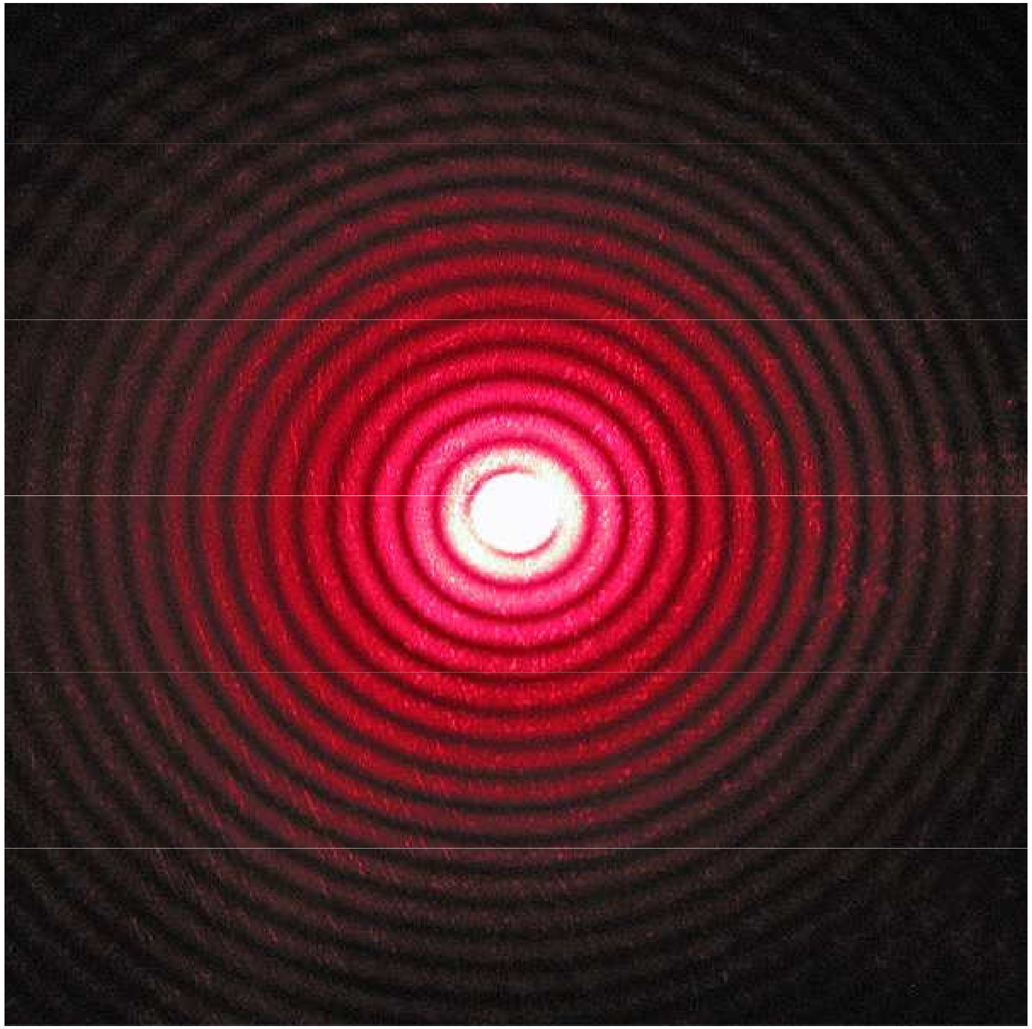
Diffraction of light



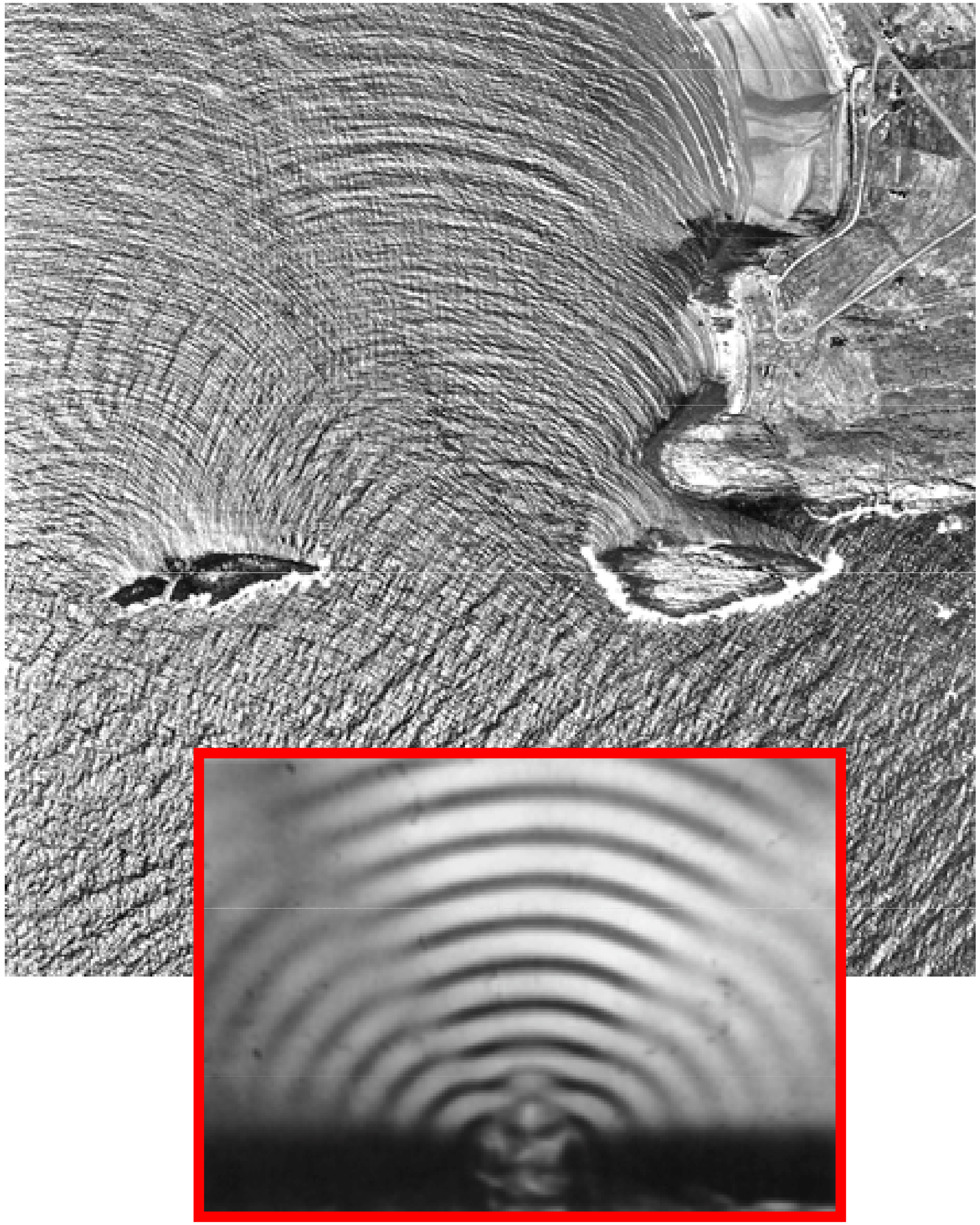


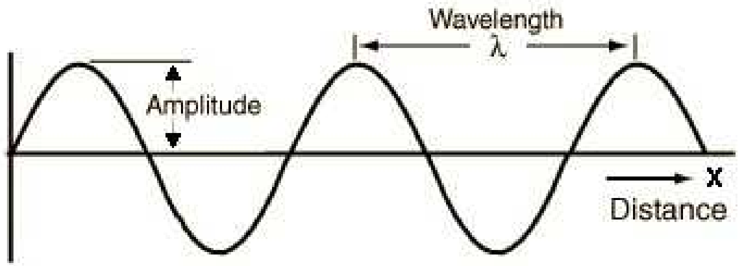
Diffraction of light



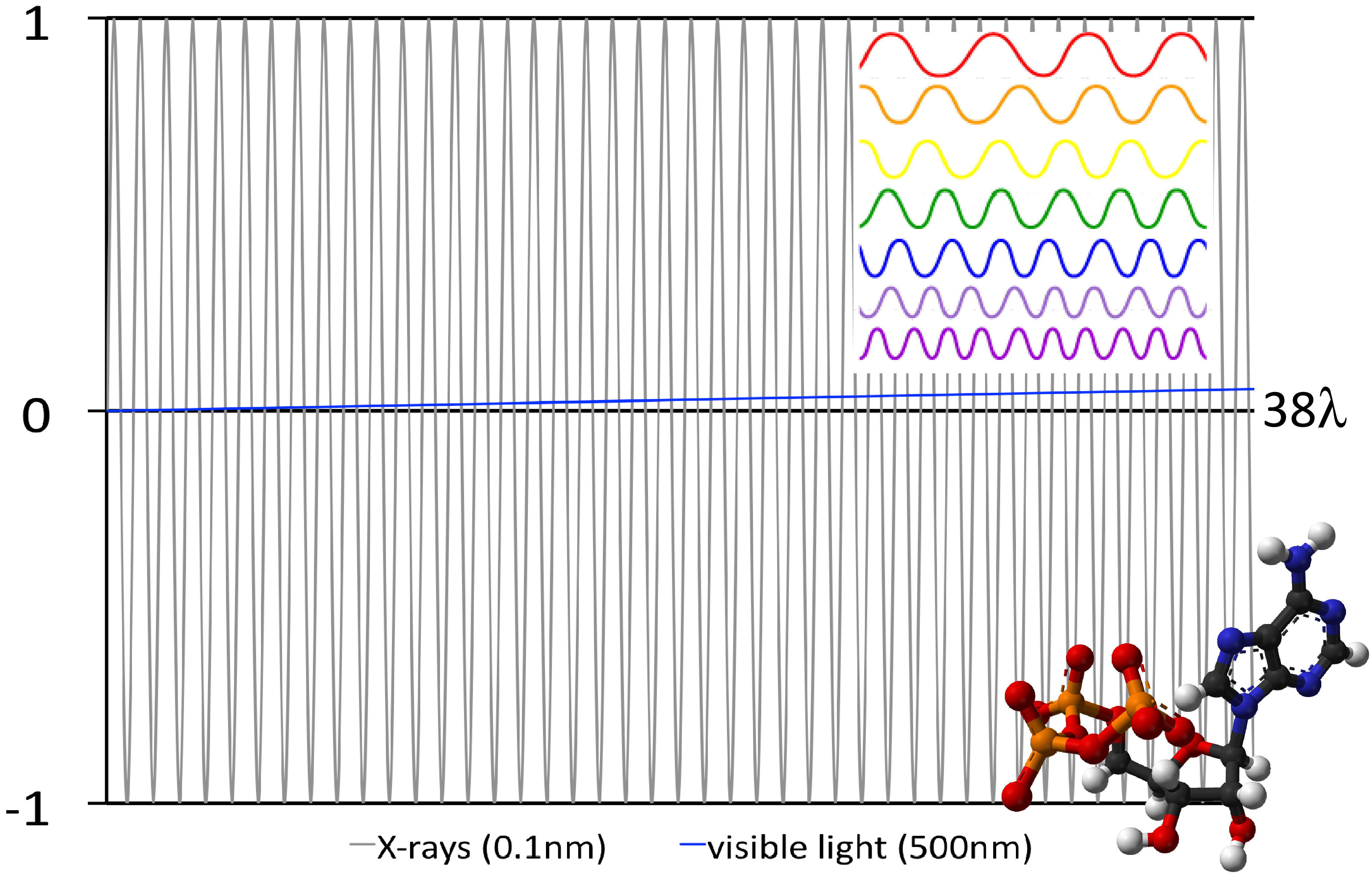


Wavelength and diffraction

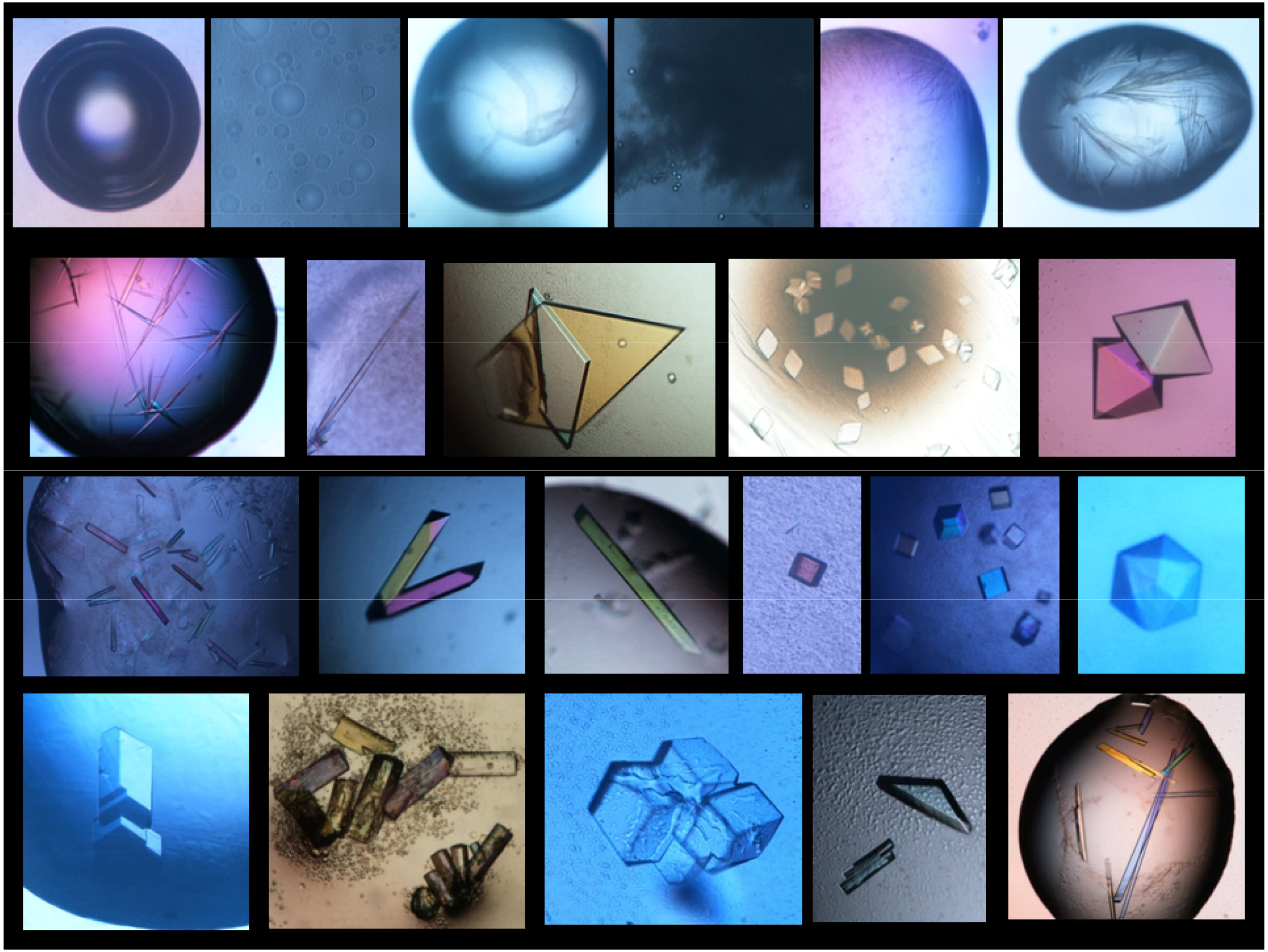




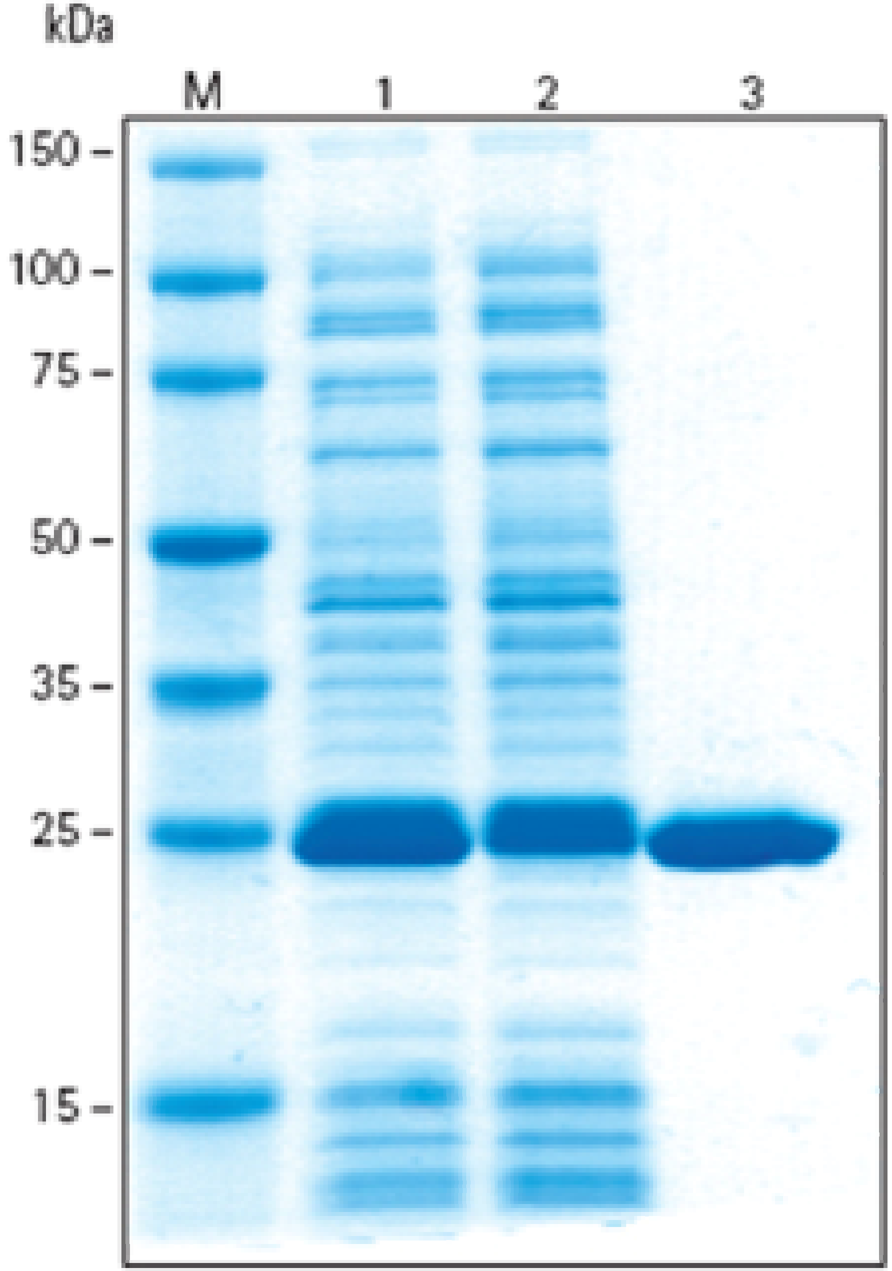
Wavelength comparison of X-rays and visible light



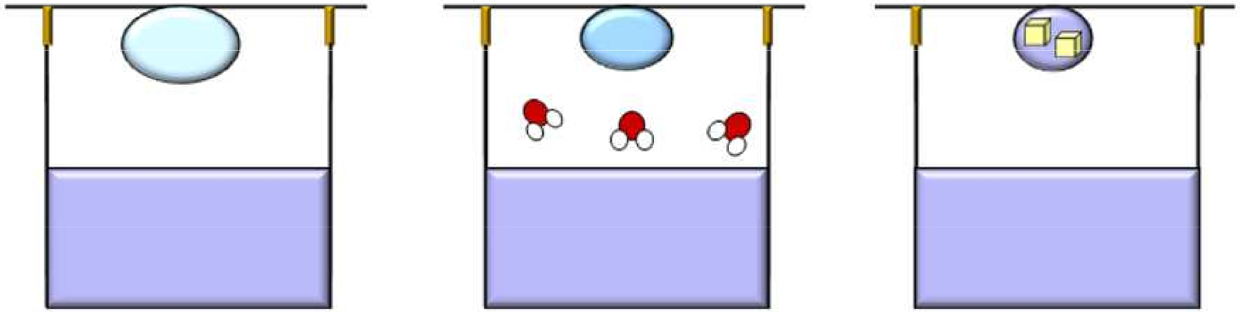
Crystallizing a Protein

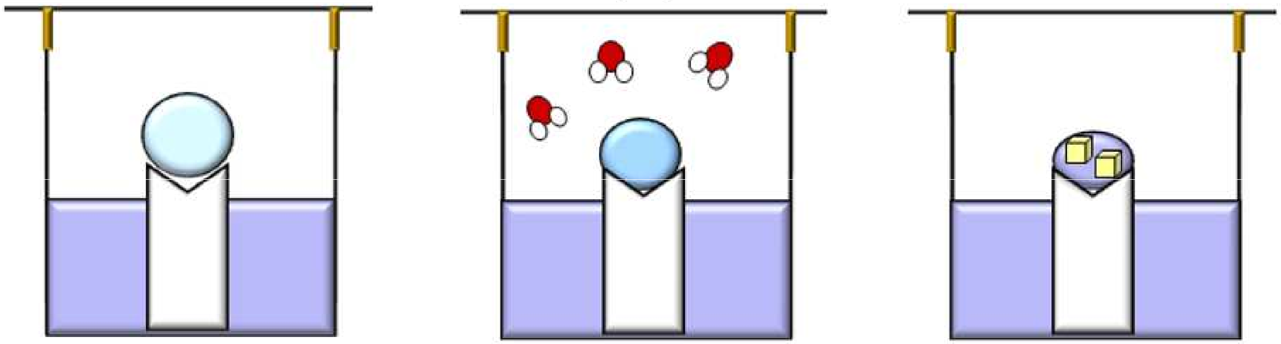


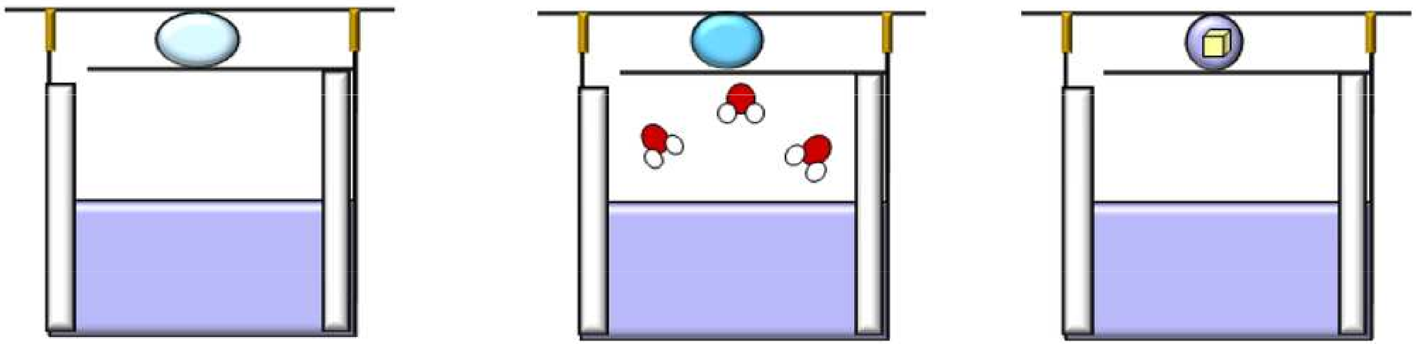
Protein expression and purification



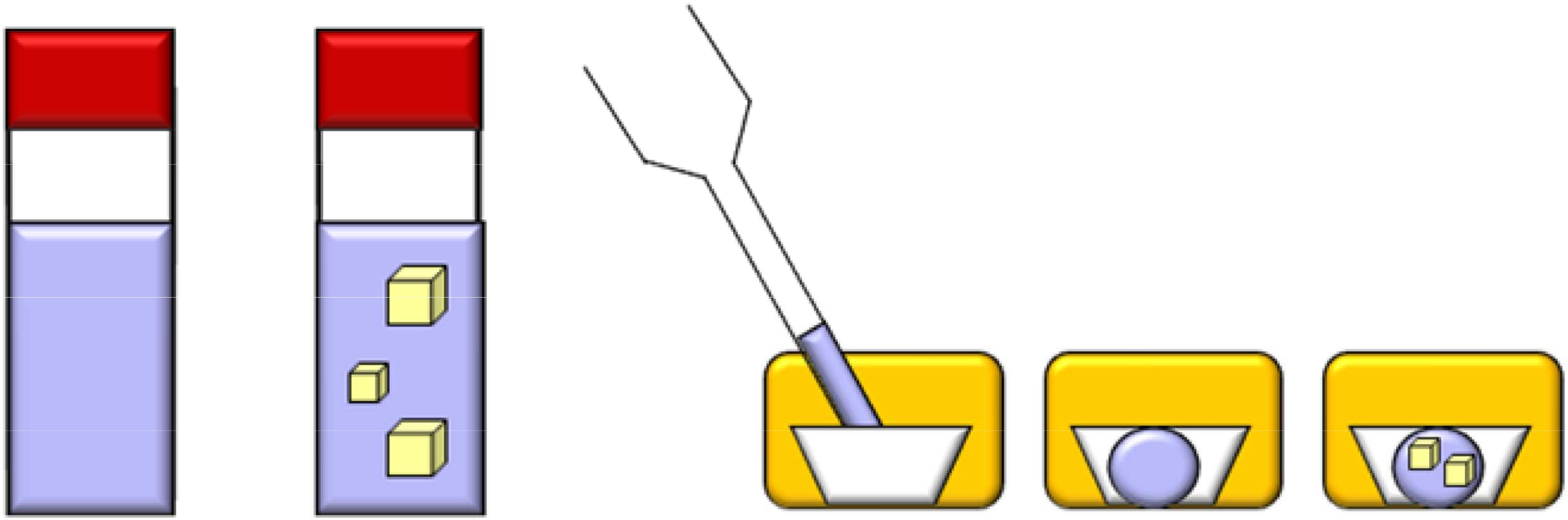
Vapor-diffusion


(A)

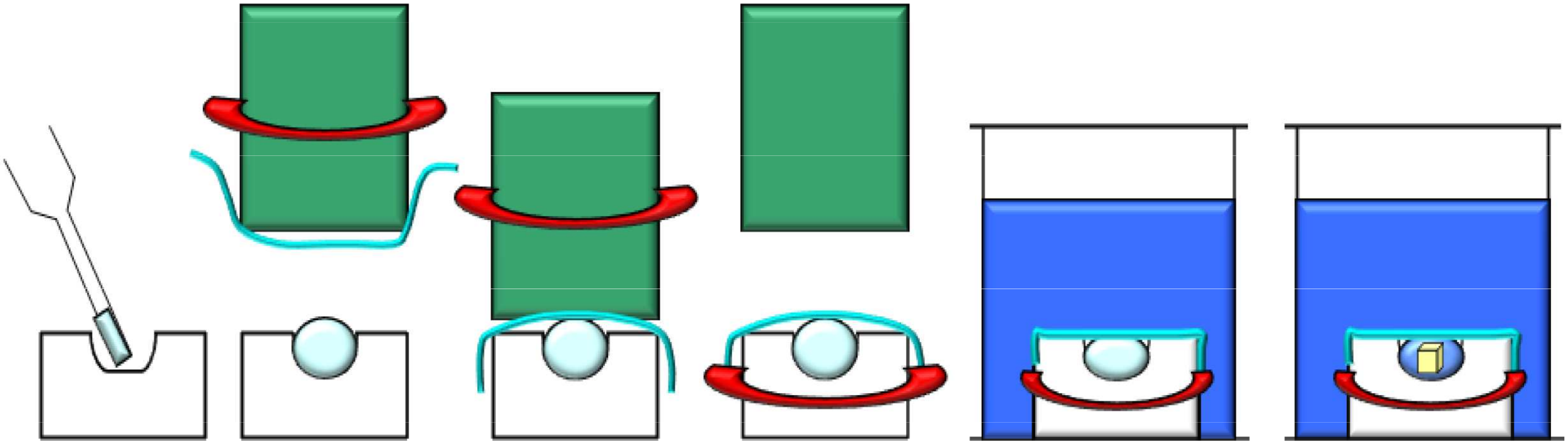

(B)


(C)

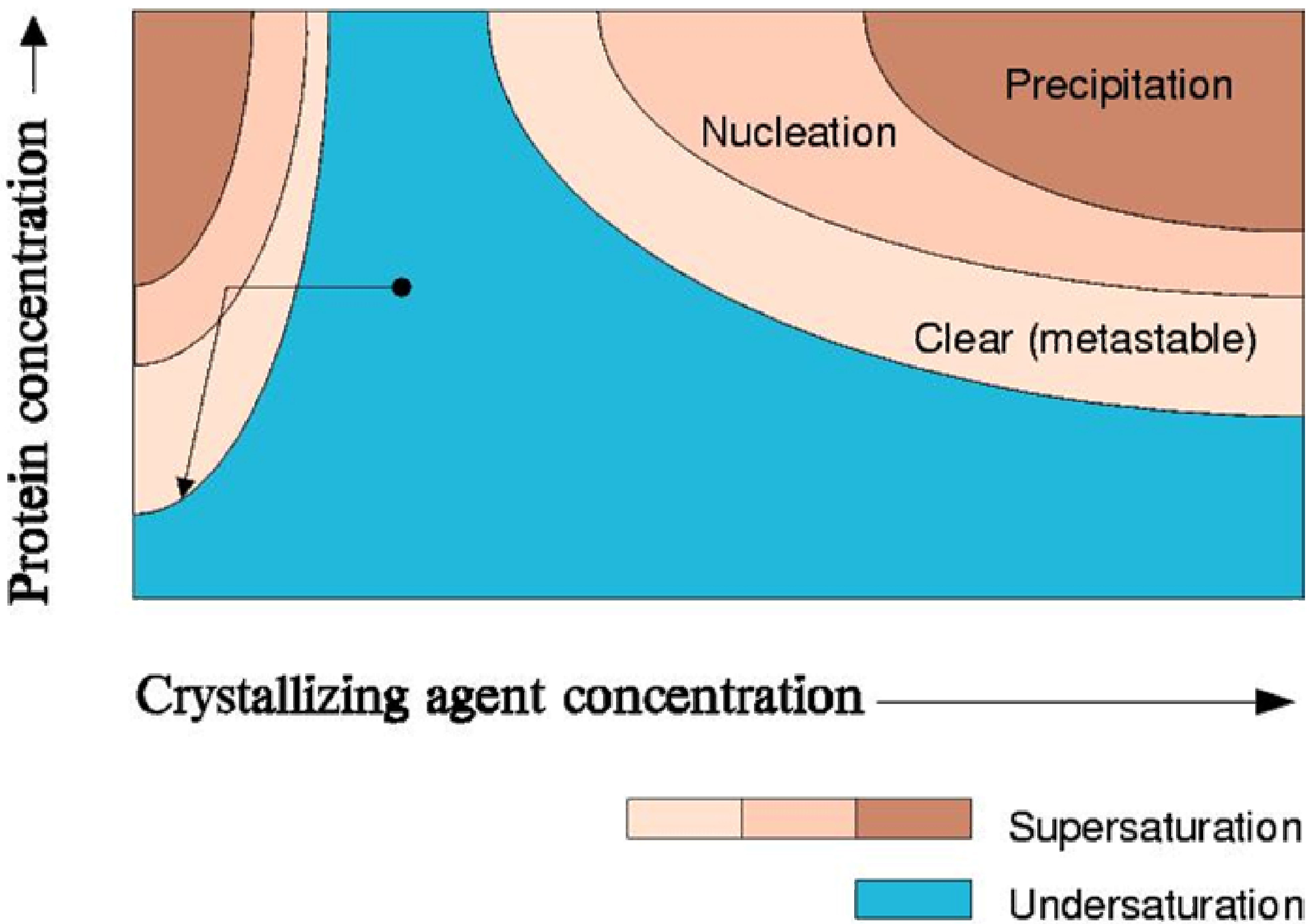
Batch and microbatch

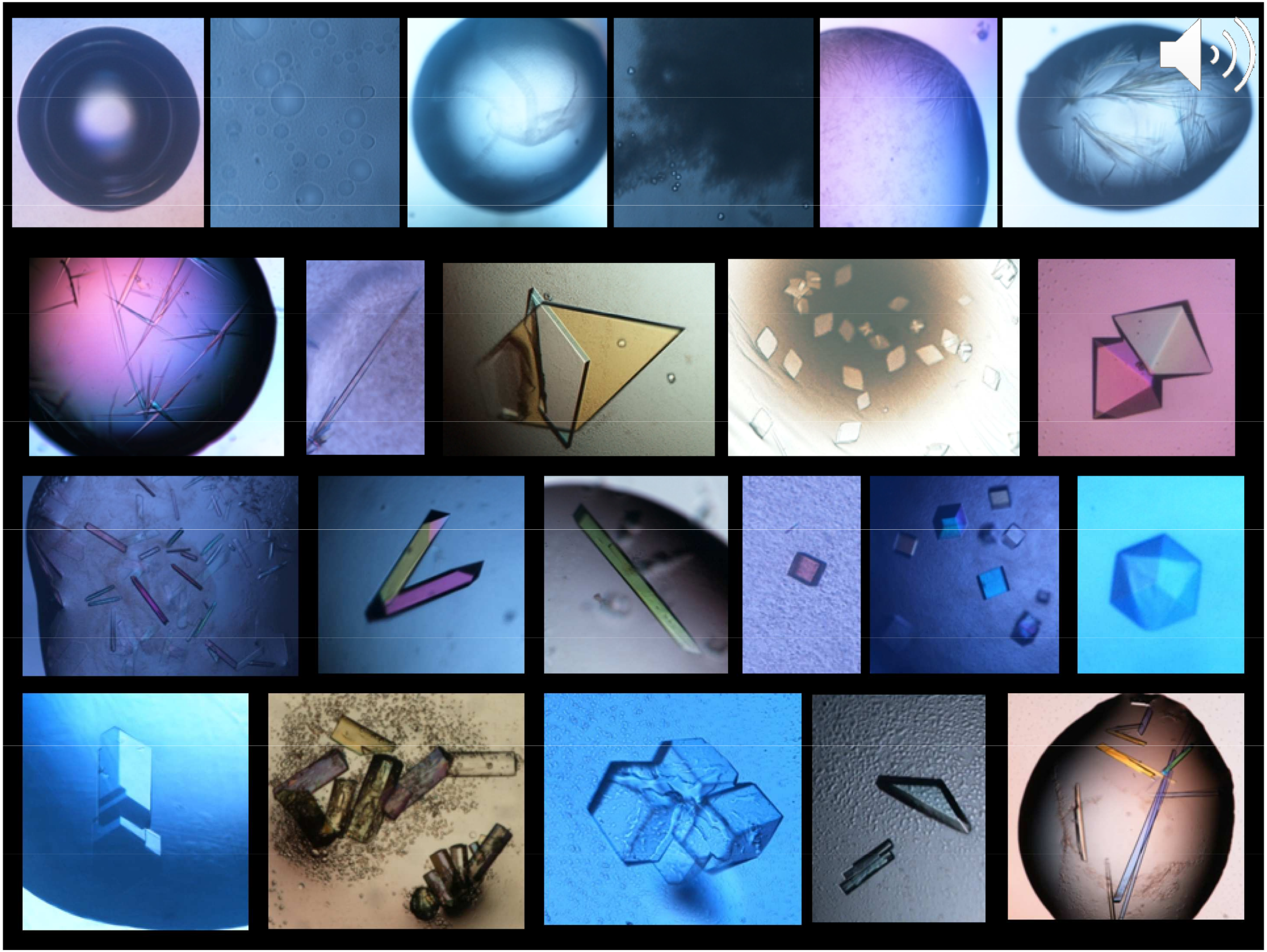


Microdialysis

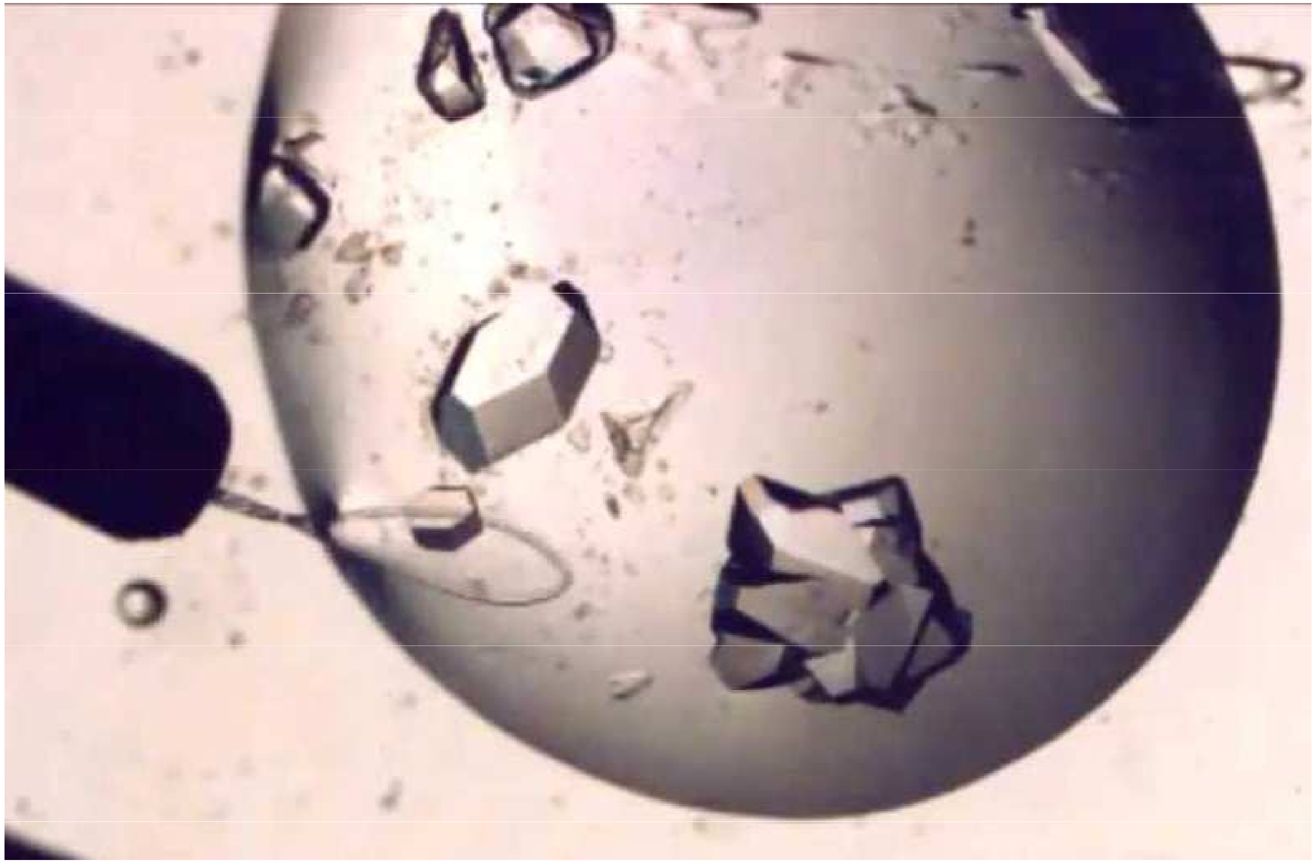


Protein crystallization phase diagram

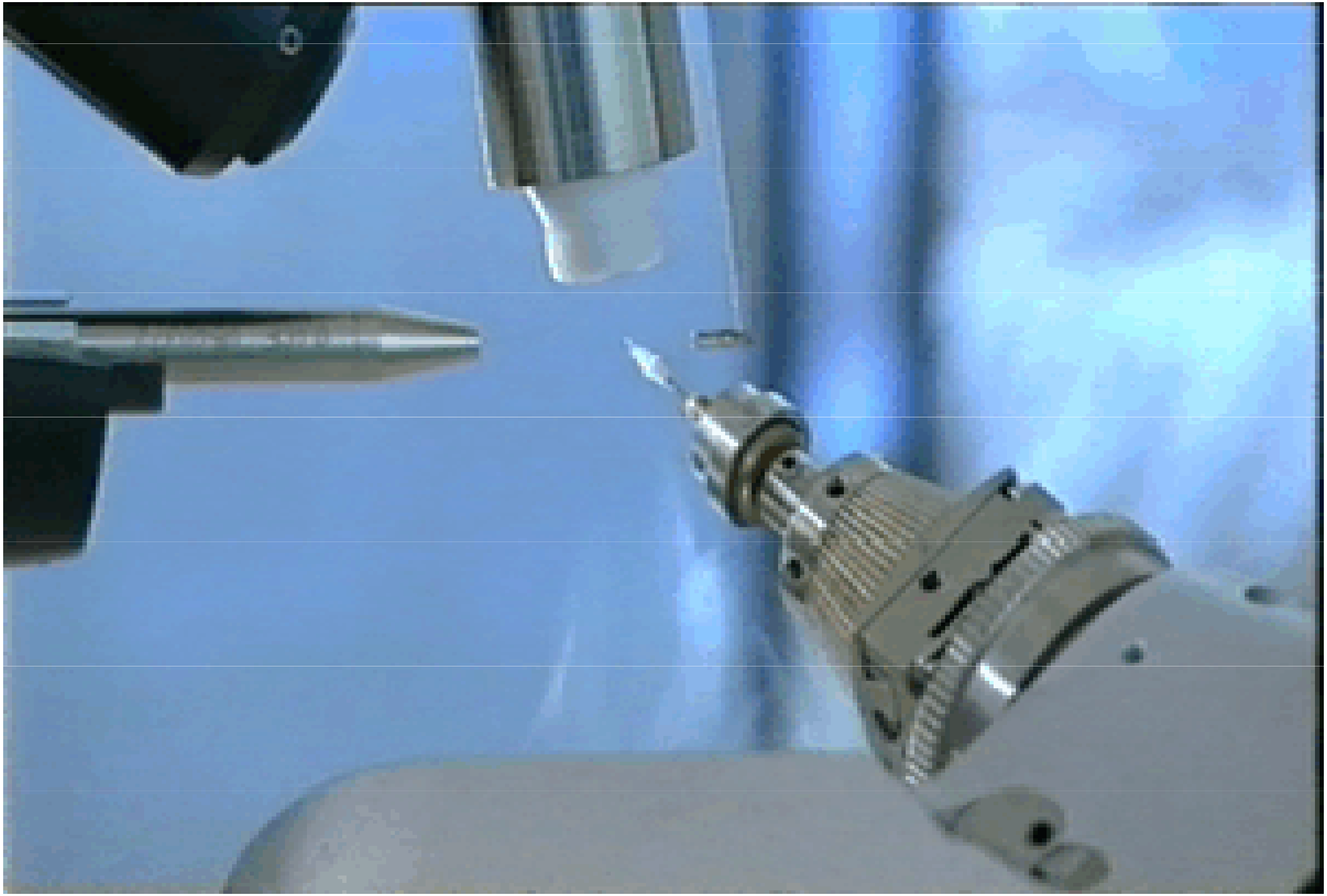




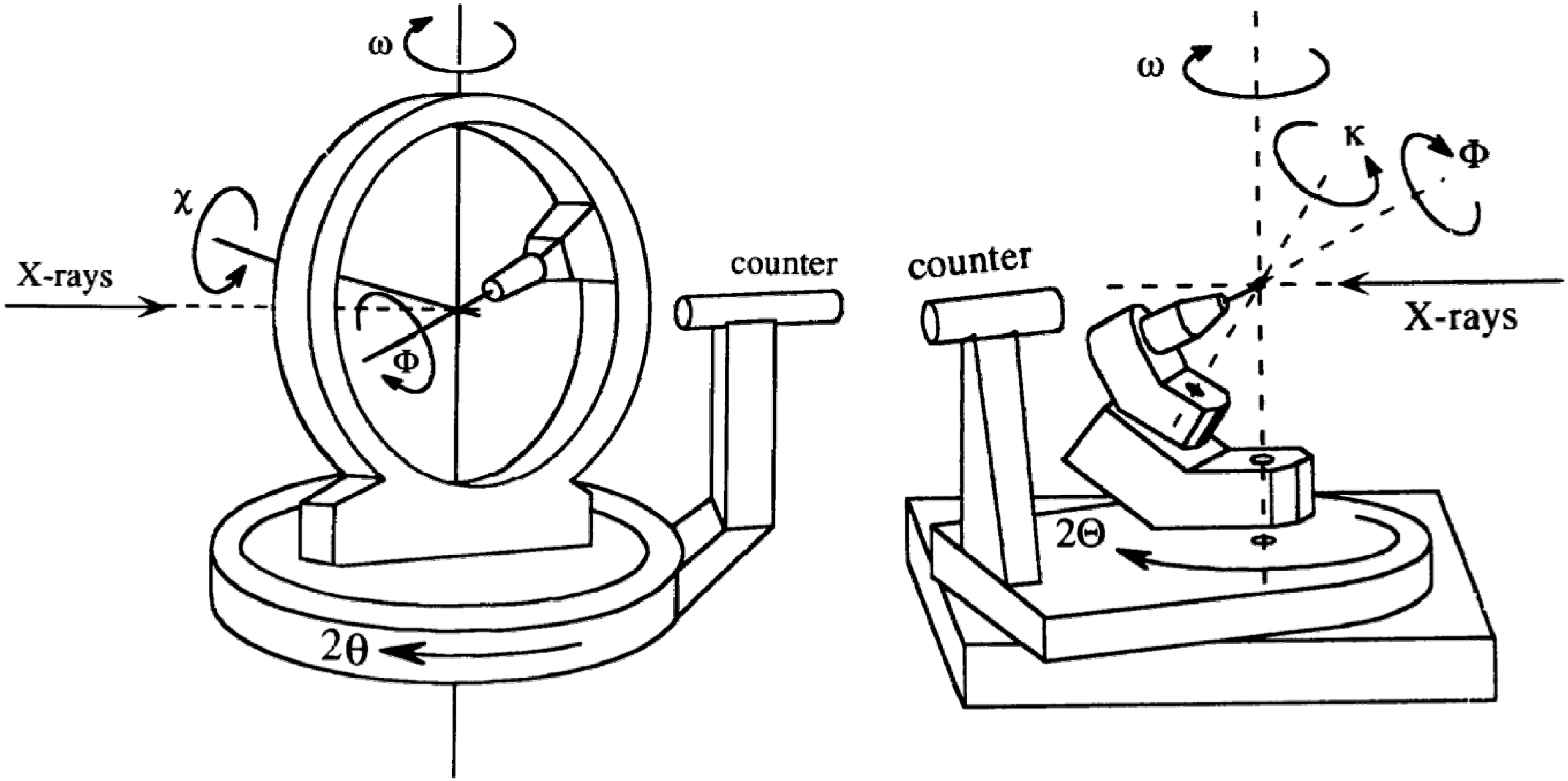
Preparing crystals for diffraction experiment



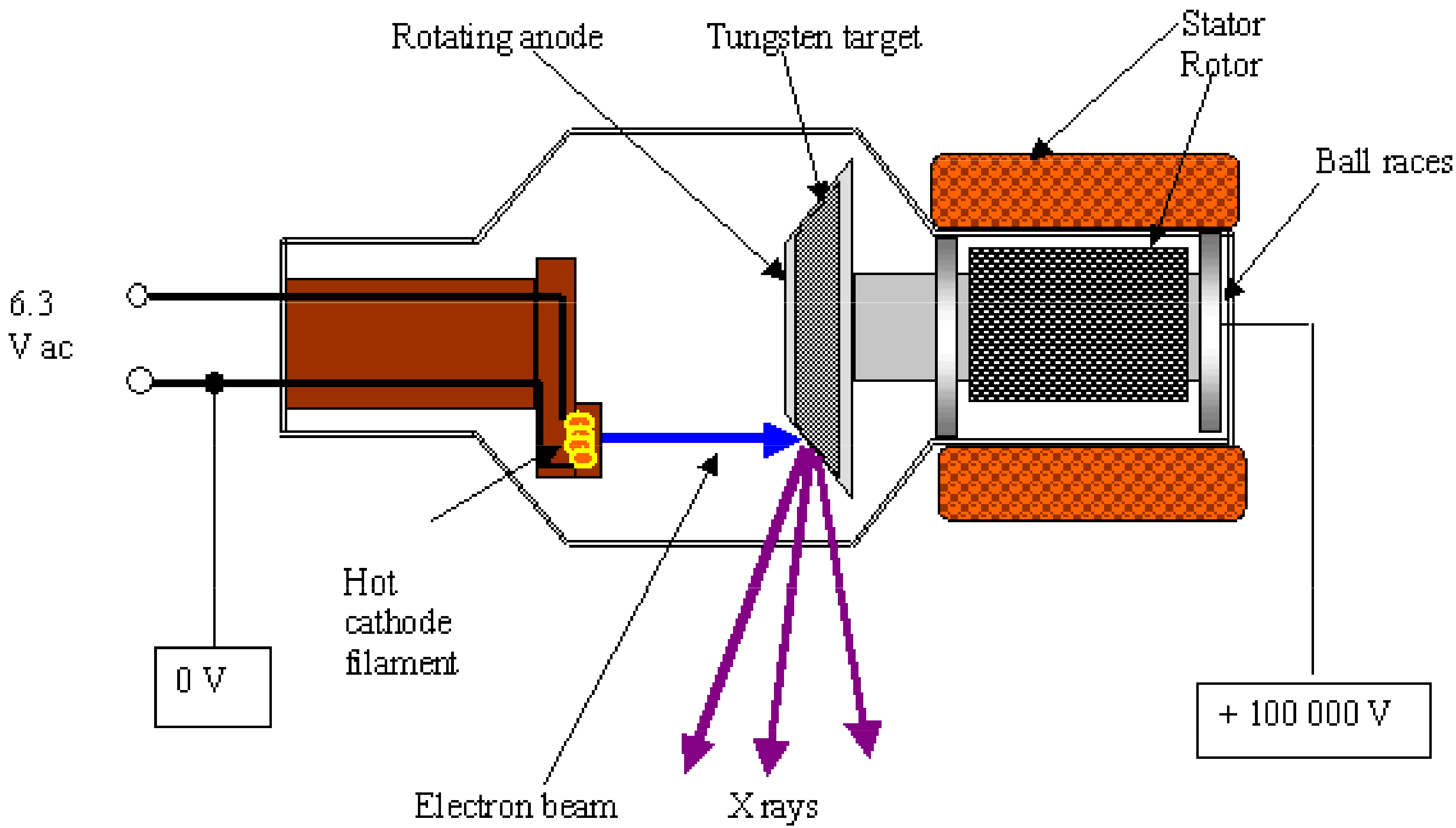
Diffractometer with goniometer



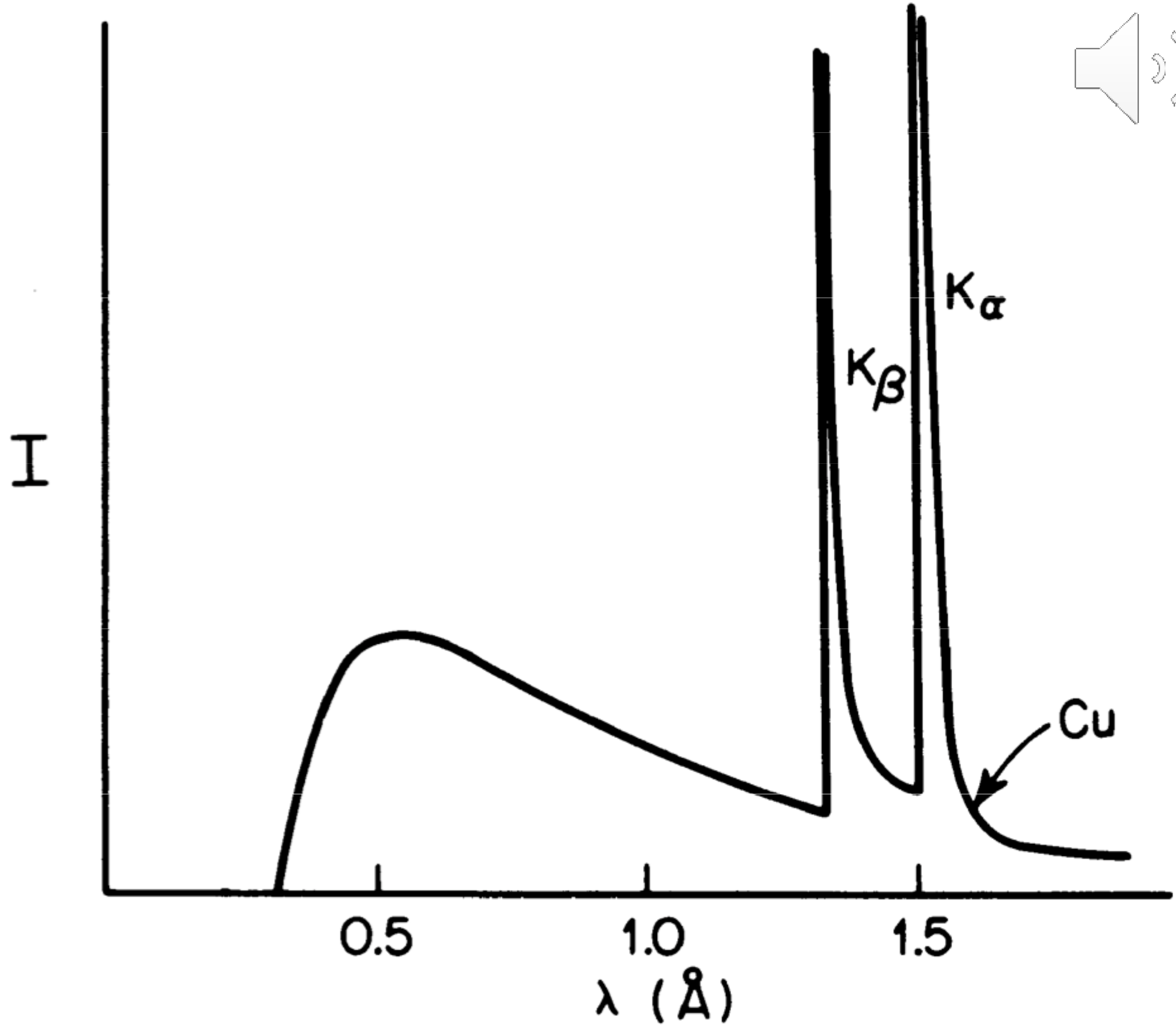
Diffractometer with goniometer



X-ray sources
- sealed X-ray tube



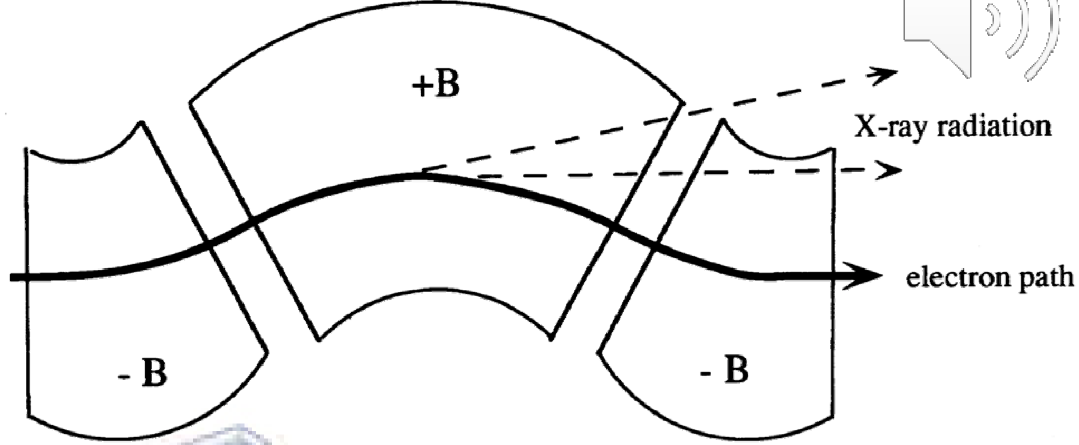
Spectrum of copper anode

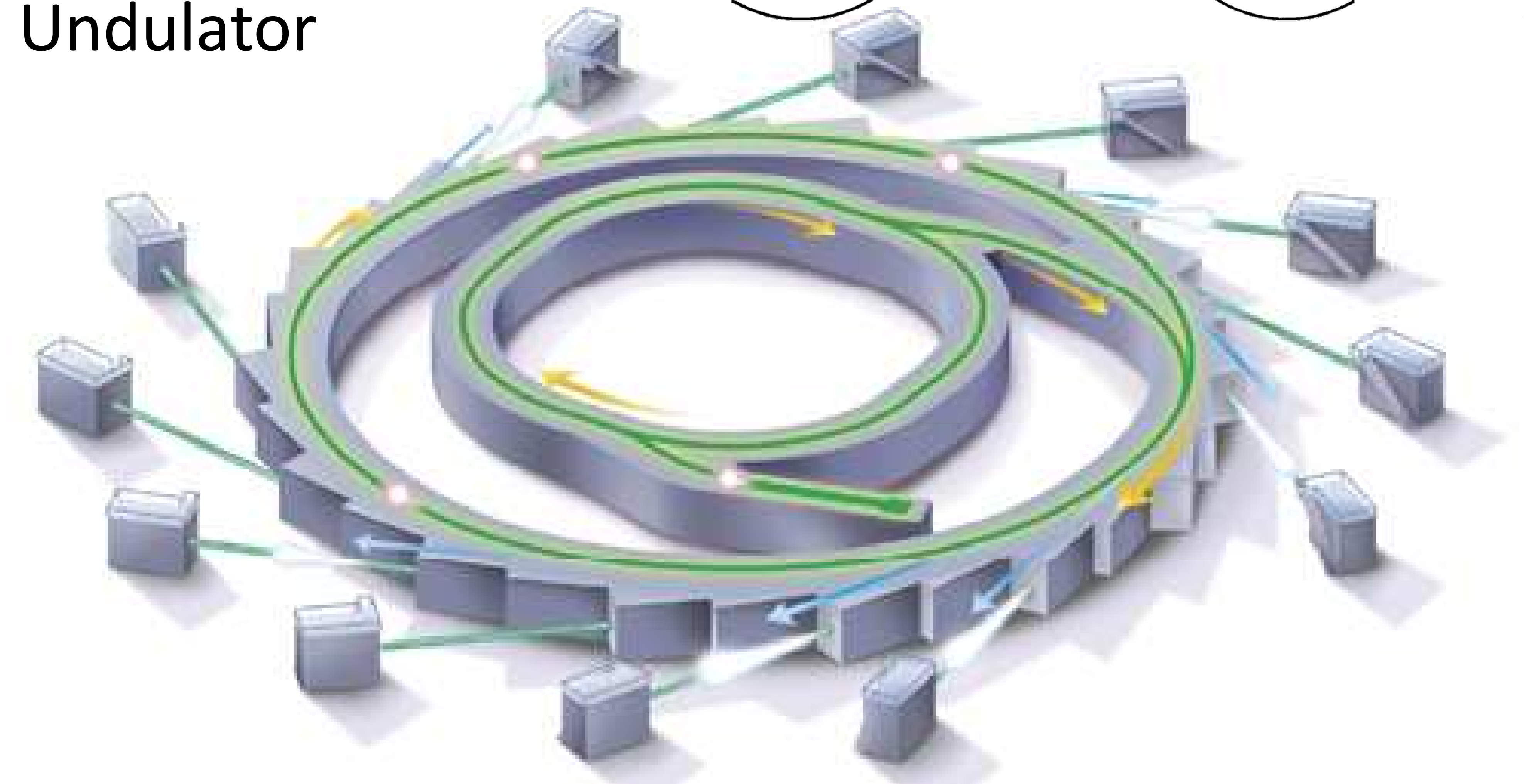


	λ (Å)	
$K_\alpha(1)$	1.54051	The weight average value for $K_\alpha(1)$ and $K_\alpha(2)$ is taken as 1.54178 Å
$K_\alpha(2)$	1.54433	because the intensity of $K_\alpha(1)$ is twice that of $K_\alpha(2)$
K_β	1.39217	

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

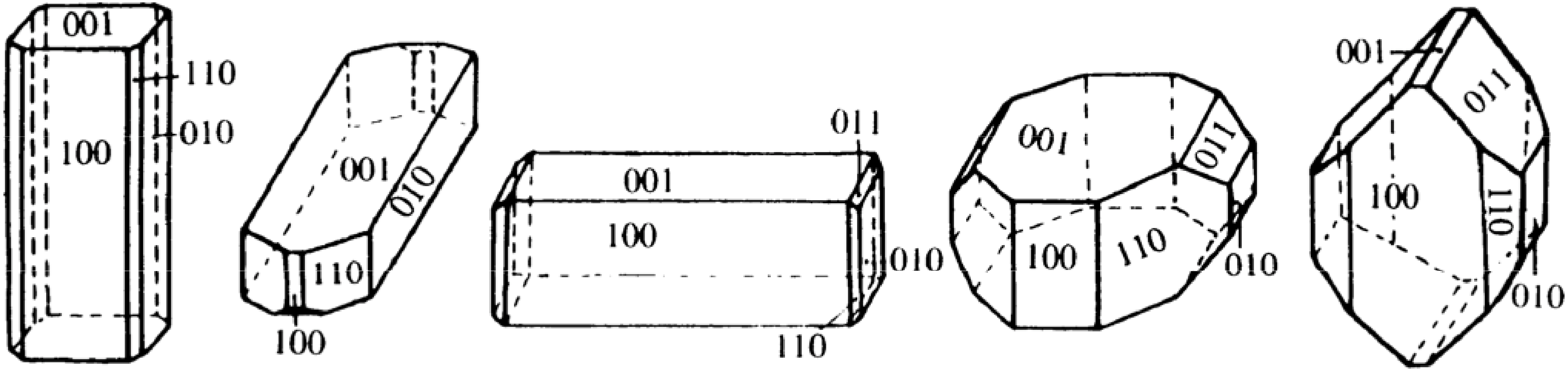


Figure 3.1. Crystals of trimethylammonium bromide belonging to the same crystal form but exhibiting a range of morphologies.

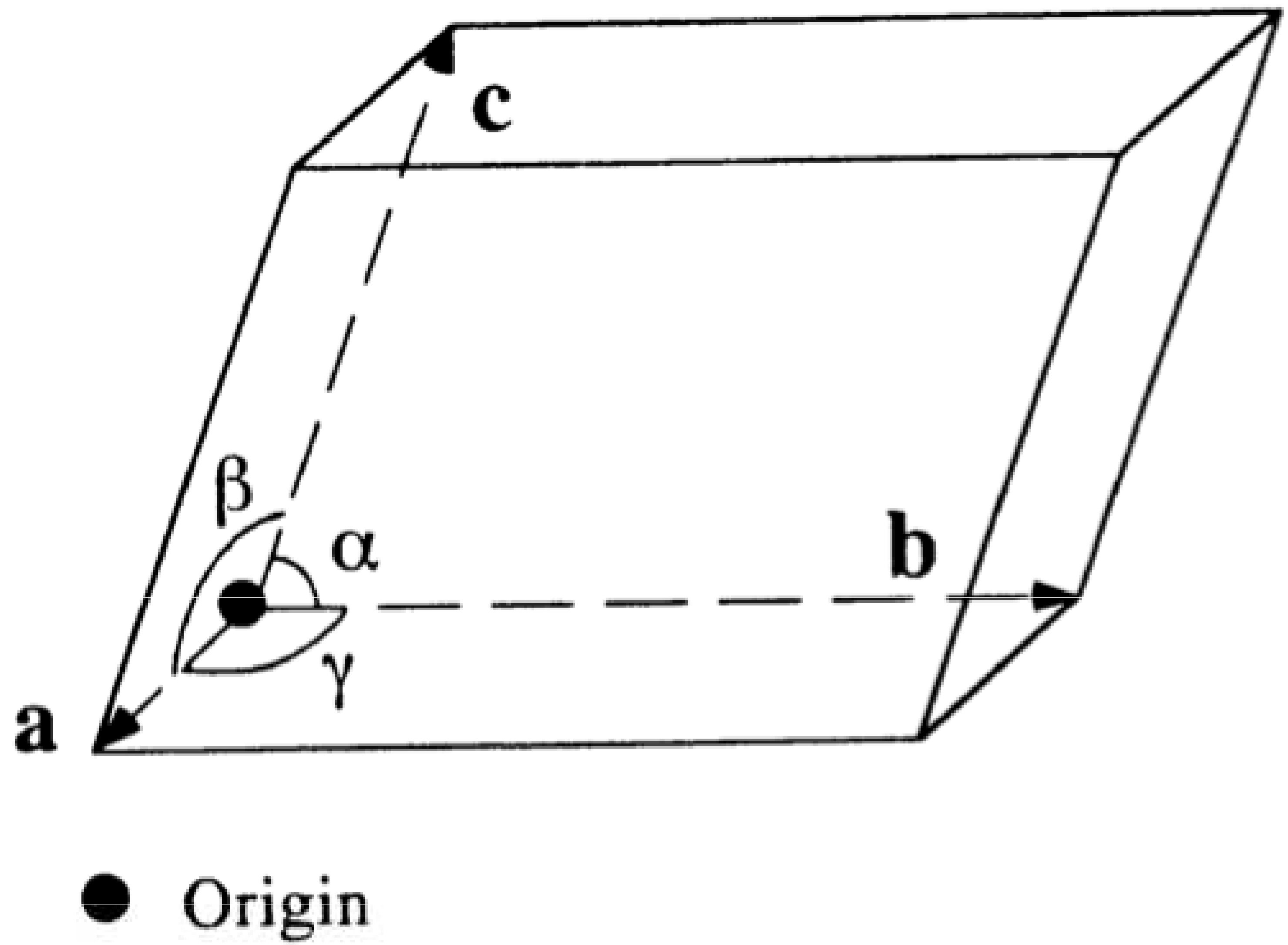


Figure 3.3. One unit cell in the crystal lattice.

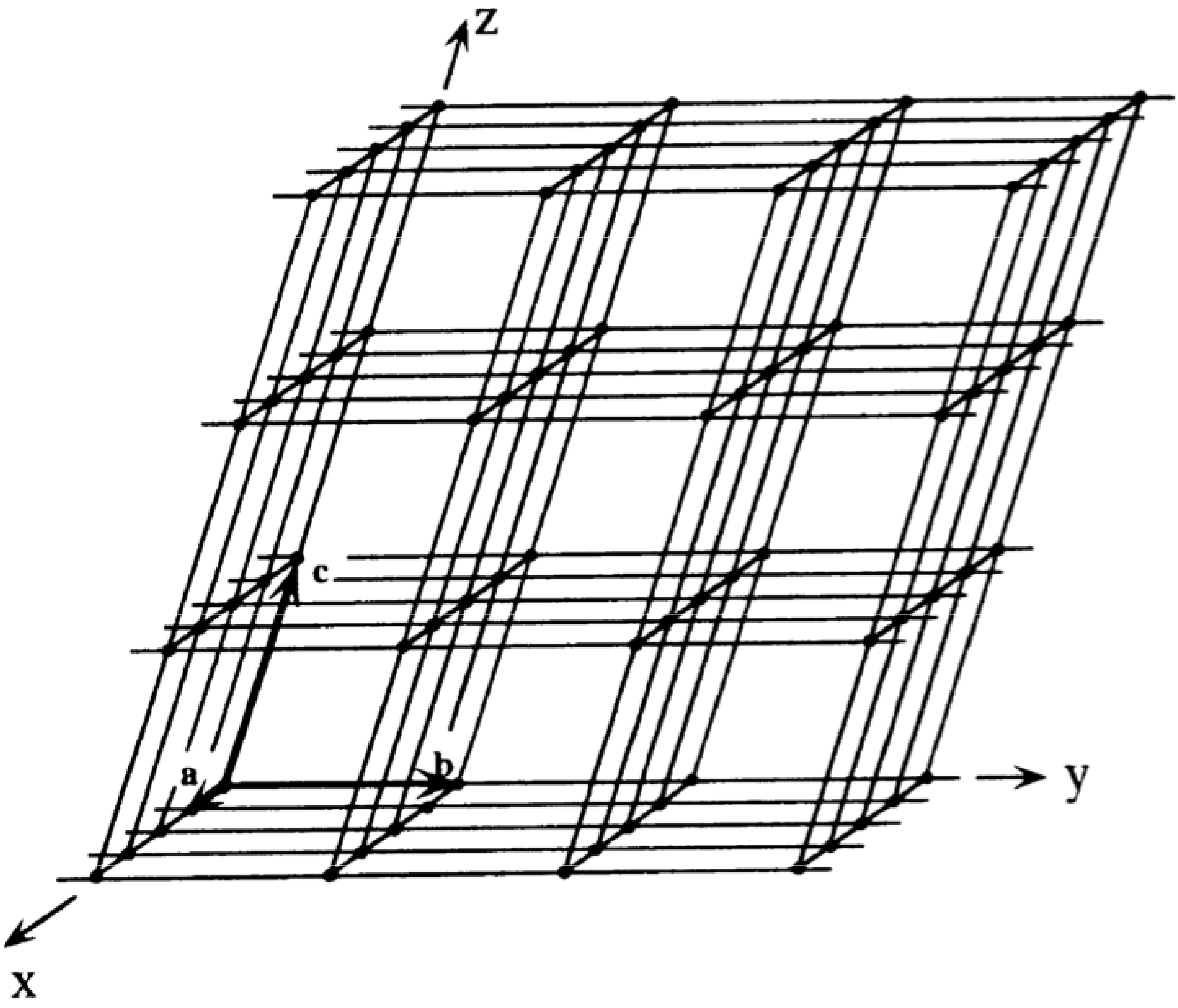
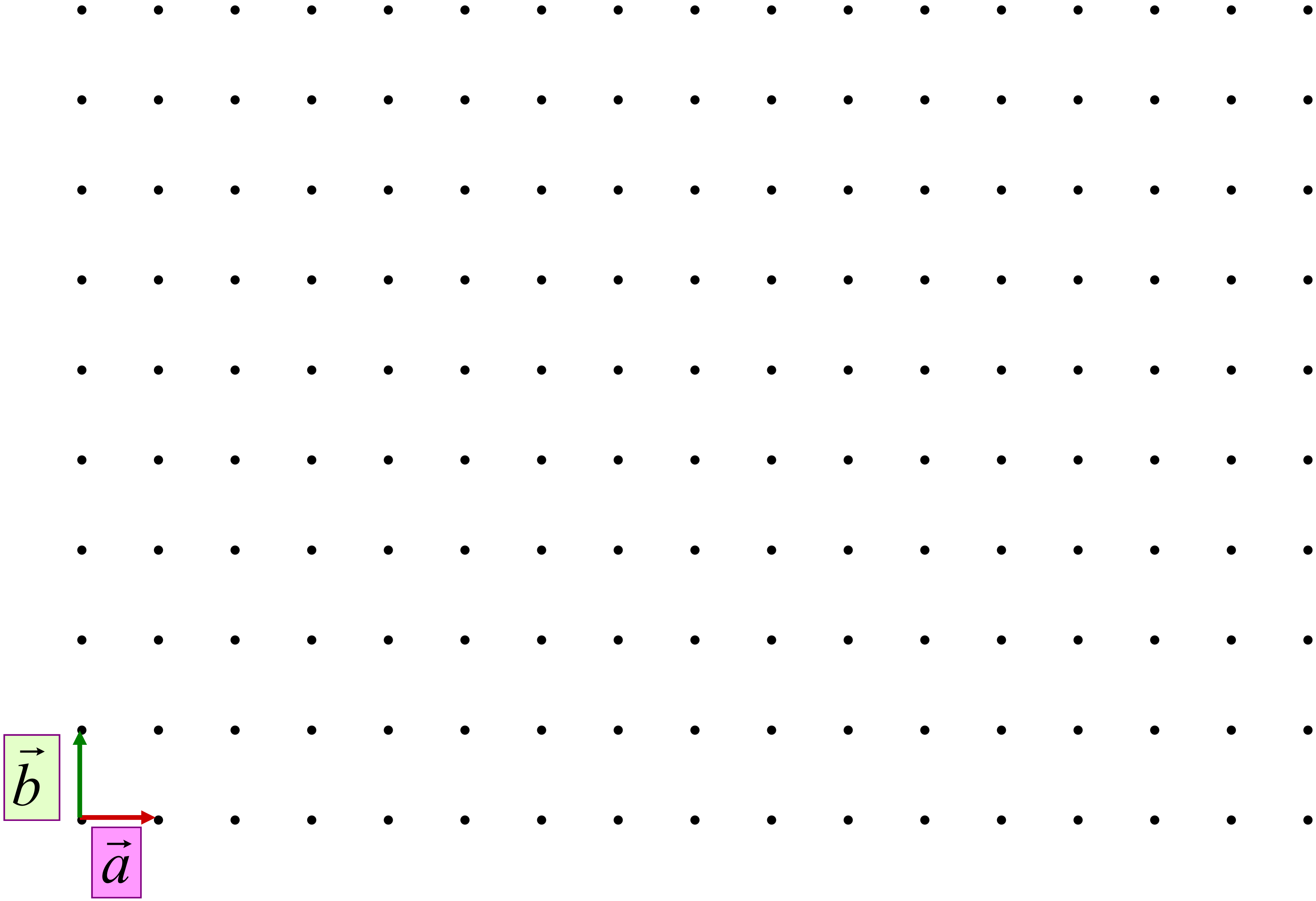


Figure 3.4. A crystal lattice is a three-dimensional stack of unit cells.

A 2D lattice



Lattice

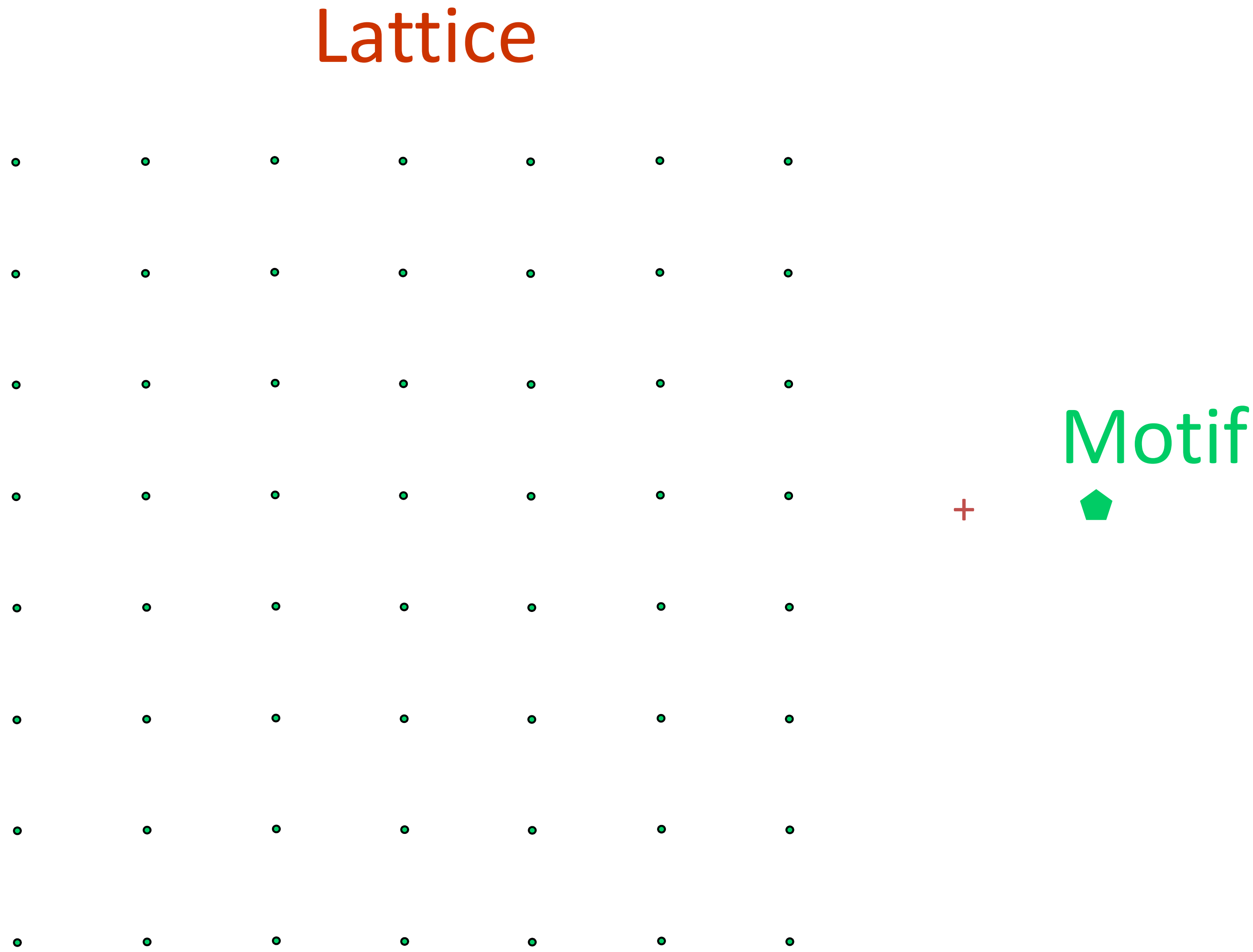
Translationally periodic arrangement of **points**

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

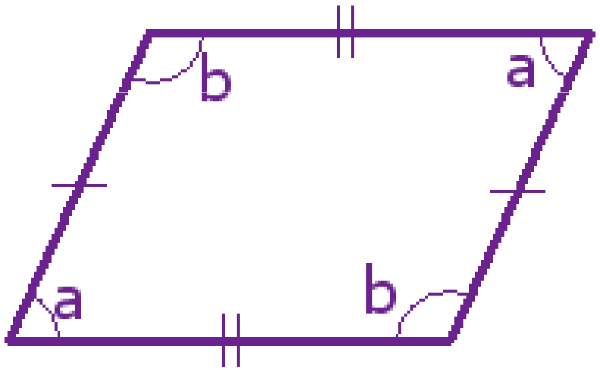
Crystal

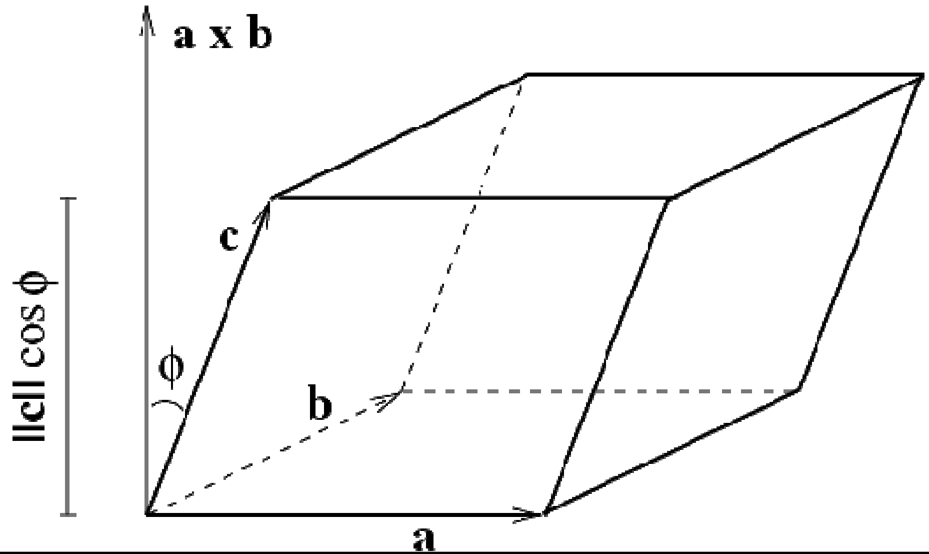
Courtesy Dr. Rajesh Prasad

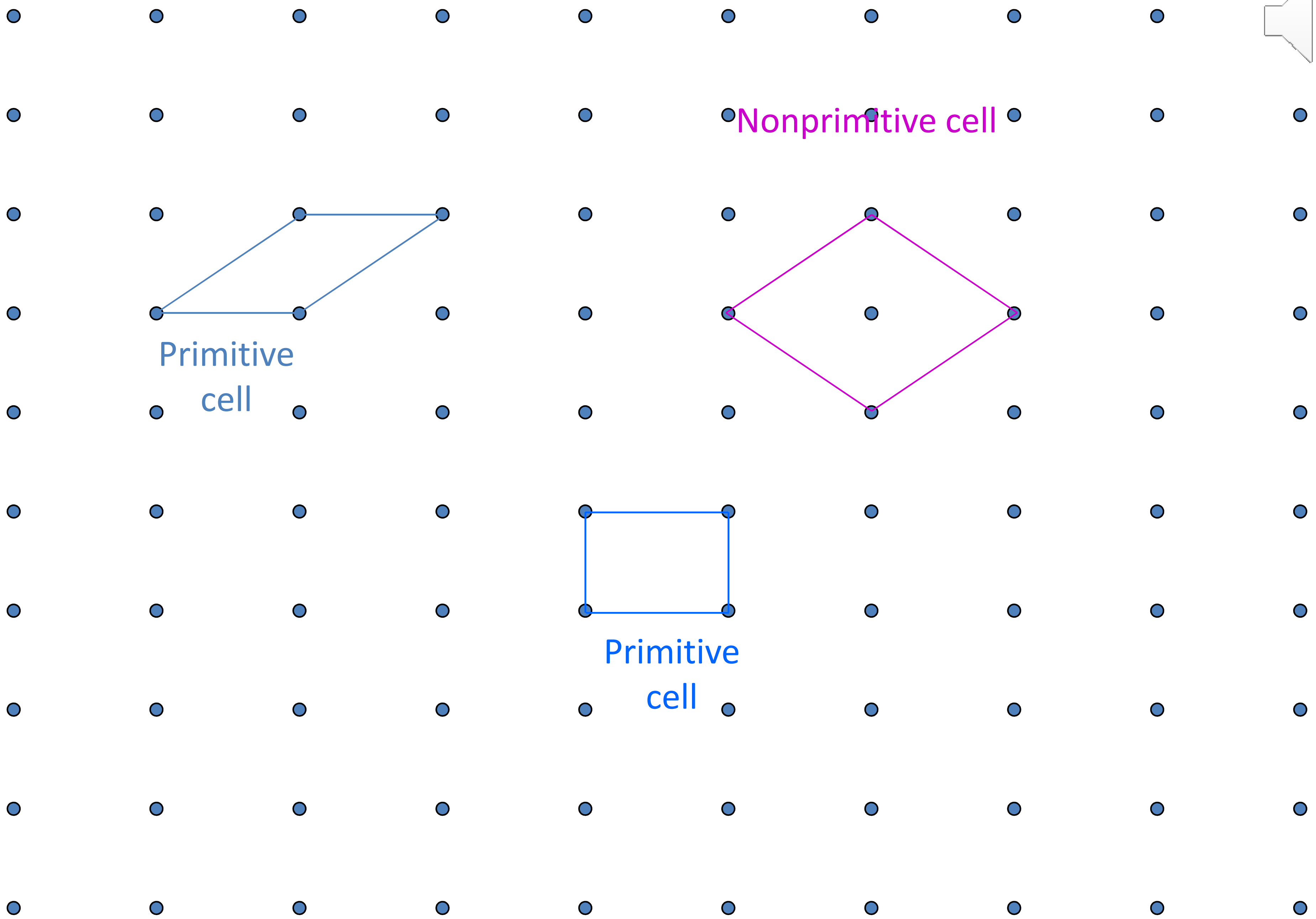
Unit cells

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

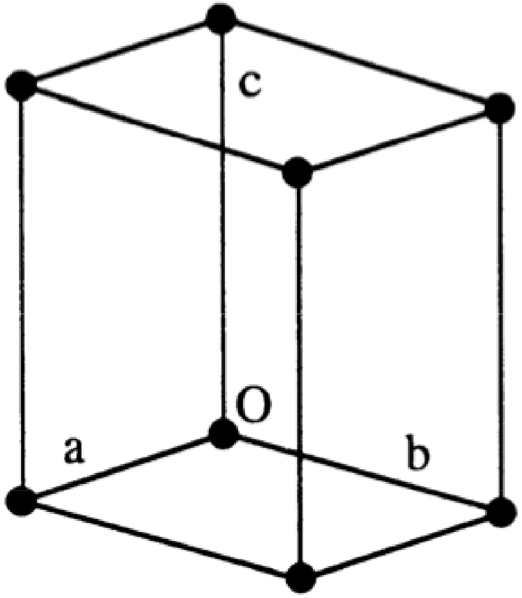
- A cell is a finite representation of the infinite lattice
- A cell is a parallelogram (2D) or a parallelopiped (3D) with lattice points at their corners.
- If the lattice points are only at the corners, the cell is primitive.
- If there are lattice points in the cell other than the corners, the cell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



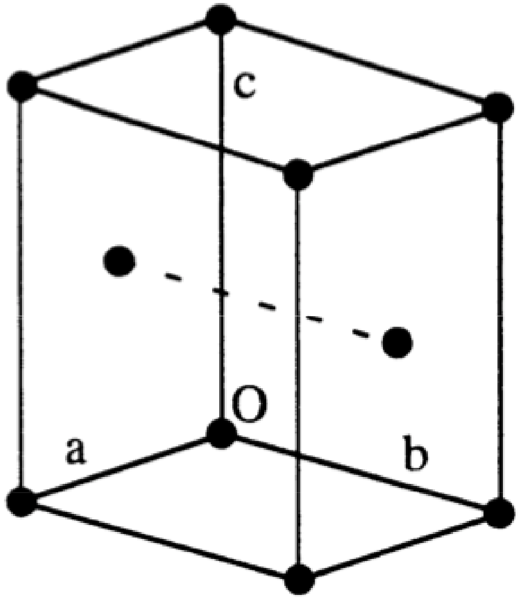




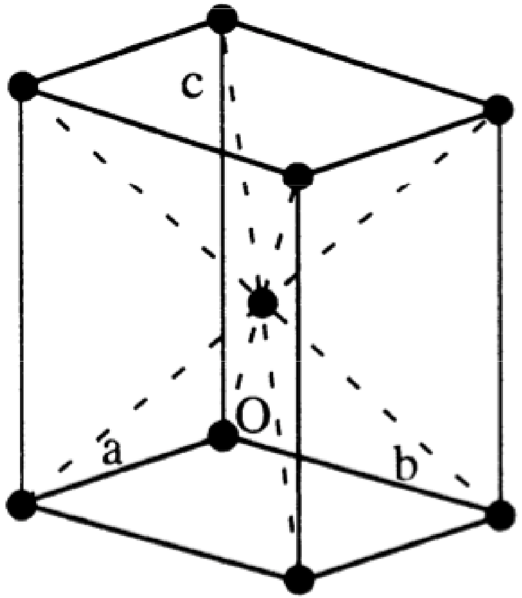
Courtesy Dr. Rajesh Prasad



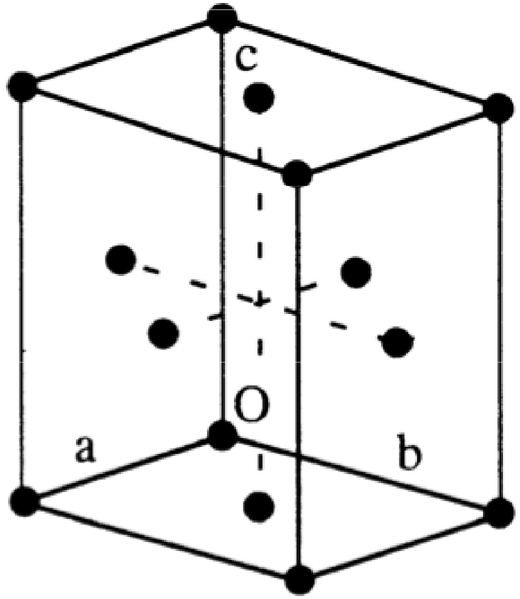
a primitive unit cell (P)



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



a body-centered unit cell (I)



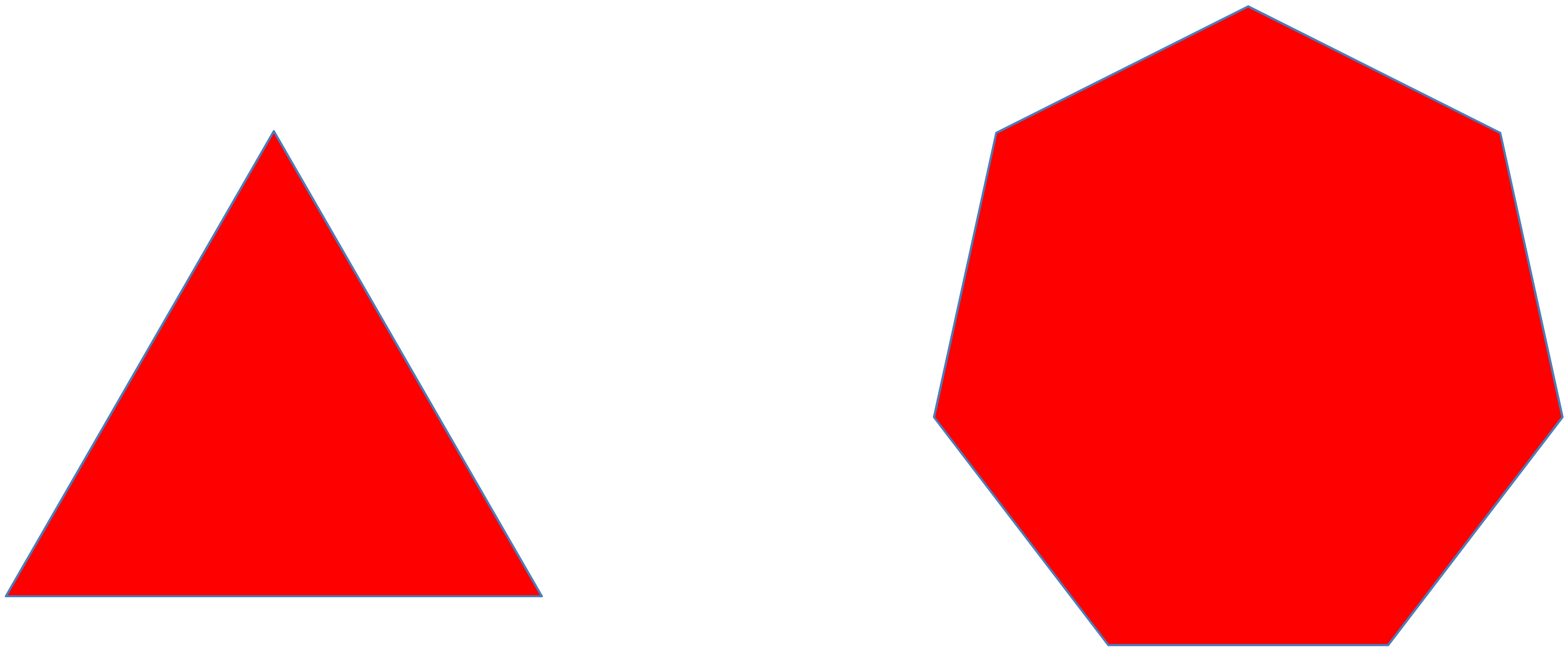
a face-centered unit cell (F)

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

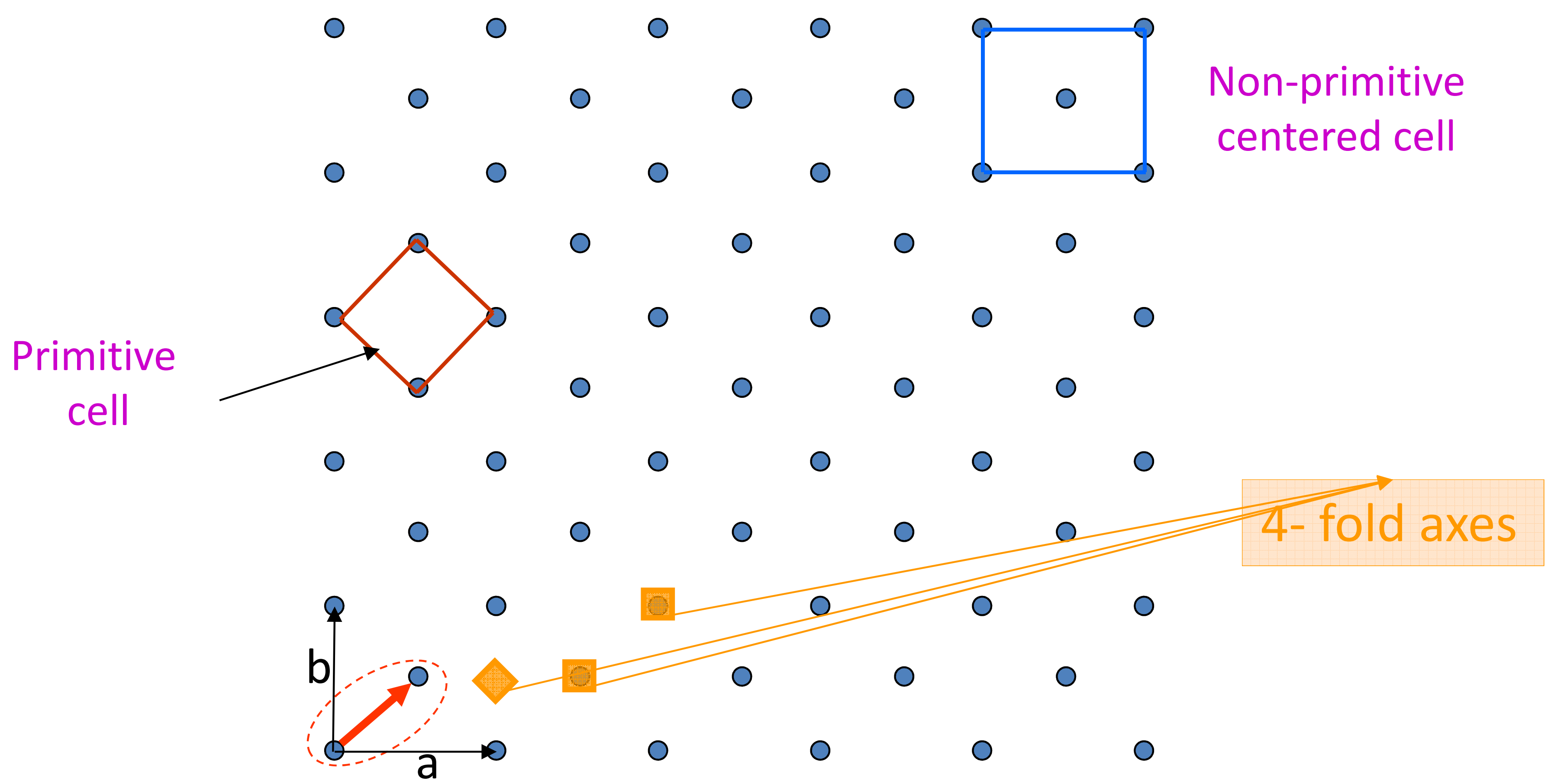
SYMMETRY



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

Primitive unit cell

For each crystal structure there is a *conventional unit cell*, usually chosen to make the resulting lattice as symmetric as possible. However, the conventional unit cell is not always the smallest possible choice. A **primitive unit cell** of a particular crystal structure is the smallest possible unit cell one can construct such that, when tiled, it completely fills space.



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.

14 Bravais lattices are divided into seven crystal systems

Crystal system	Bravais lattices			
1. Cubic	P	I	F	
2. Tetragonal	P	I		
3. Orthorhombic	P	I	F	C
4. Hexagonal	P			
5. Trigonal	P			
6. Monoclinic	P			C
7. Triclinic	P			

Courtesy Dr. Rajesh Prasad

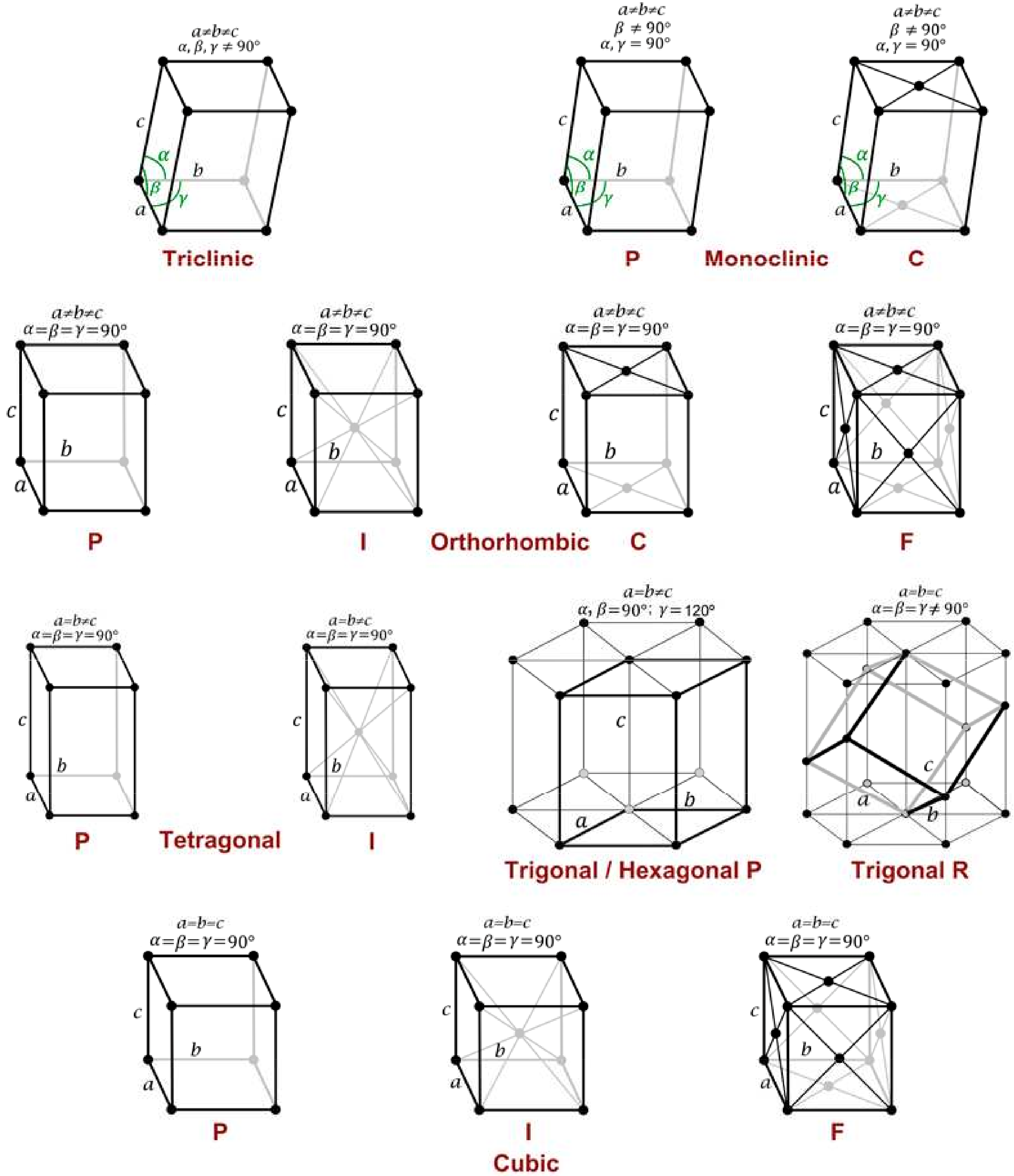
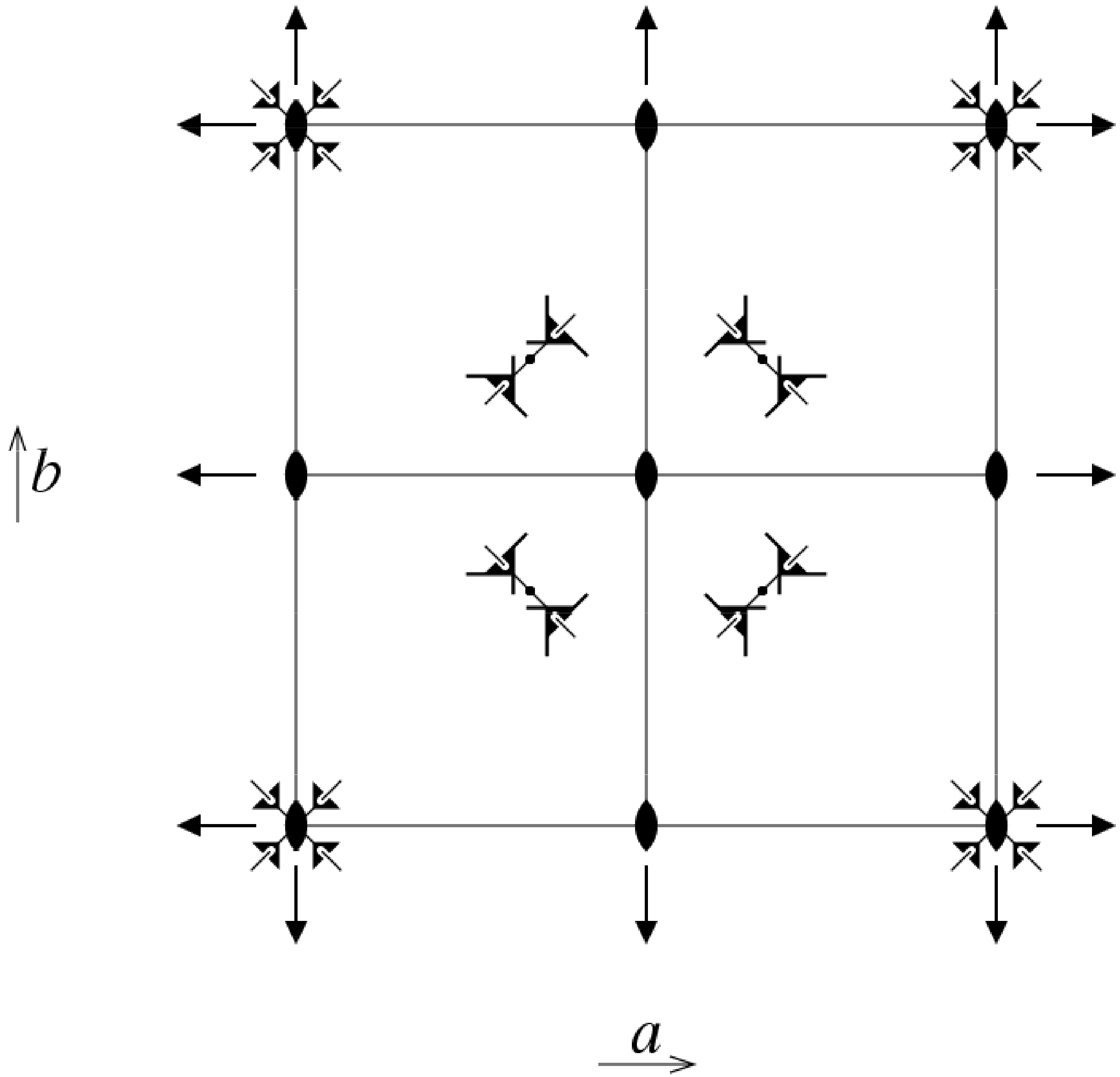

a≠b≠c
α, β, γ ≠ 90°
Triclinic
a≠b≠c
β ≠ 90°
α, γ = 90°
P
Monoclinic
C
a≠b≠c
α=β=γ=90°
P
I
Orthorhombic
C
F
a=b≠c
α=β=γ=90°
P
Tetragonal
I
a=b≠c
α, β=90°; γ=120°
Trigonal / Hexagonal P
a=b=c
α=β=γ≠ 90°
Trigonal R
a=b=c
α=β=γ=90°
P
I
Cubic
F

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

230 space groups

*P*23   *P* 2 3   23   No. 195



1 x, y, z

2 $x, \bar{y}, \bar{z}$

3 $\bar{x}, y, \bar{z}$

4 $\bar{x}, \bar{y}, z$

5 z, x, y

6 $\bar{z}, \bar{x}, y$

7 $z, \bar{x}, \bar{y}$

8 $\bar{z}, x, \bar{y}$

9 y, z, x

10 $\bar{y}, z, \bar{x}$

11 $\bar{y}, \bar{z}, x$

12 $y, \bar{z}, \bar{x}$

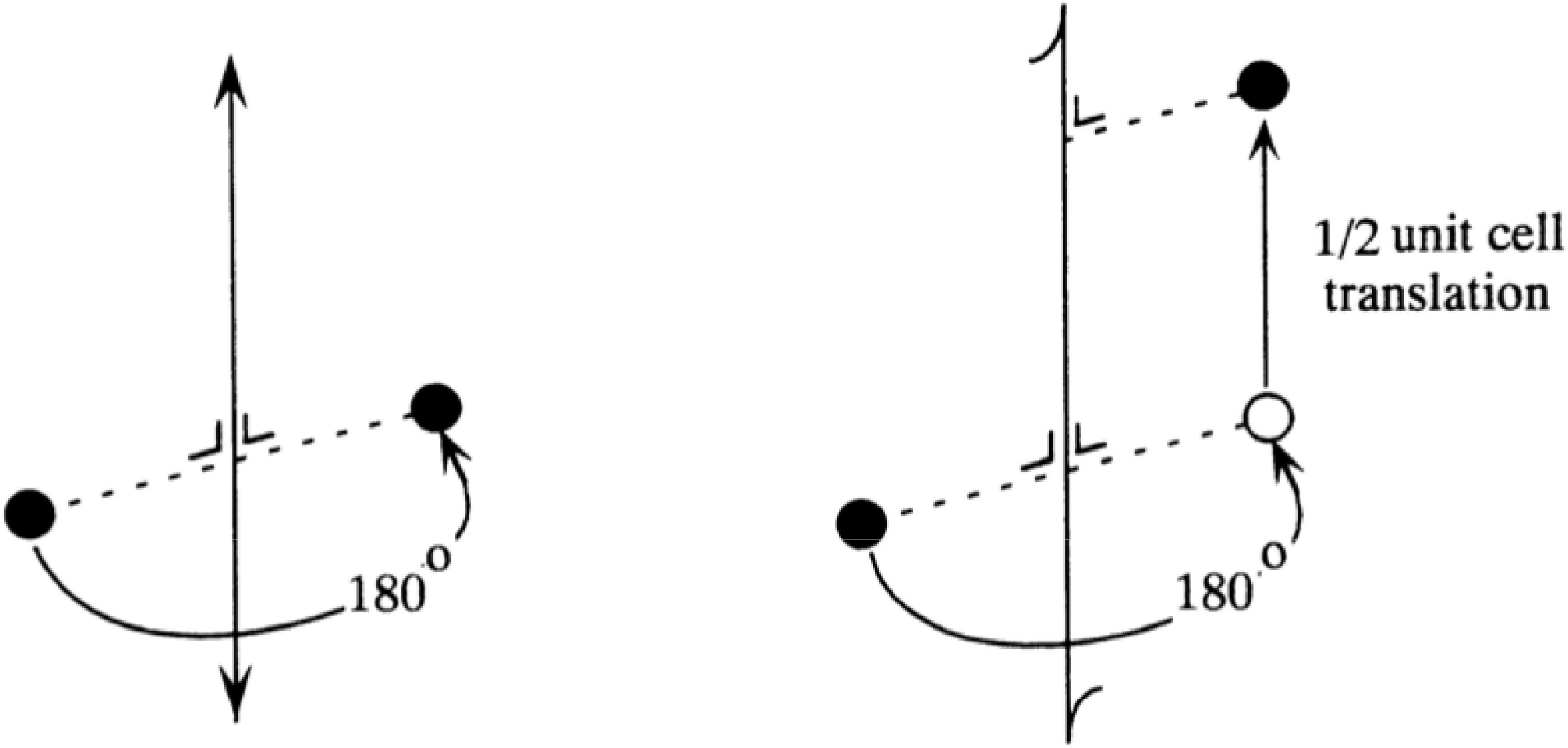


Figure 3.12. A 2-fold axis (left) and a 2-fold screw axis (right); the latter relates one molecule to another by a 180° rotation plus a translation over half of the unit cell.

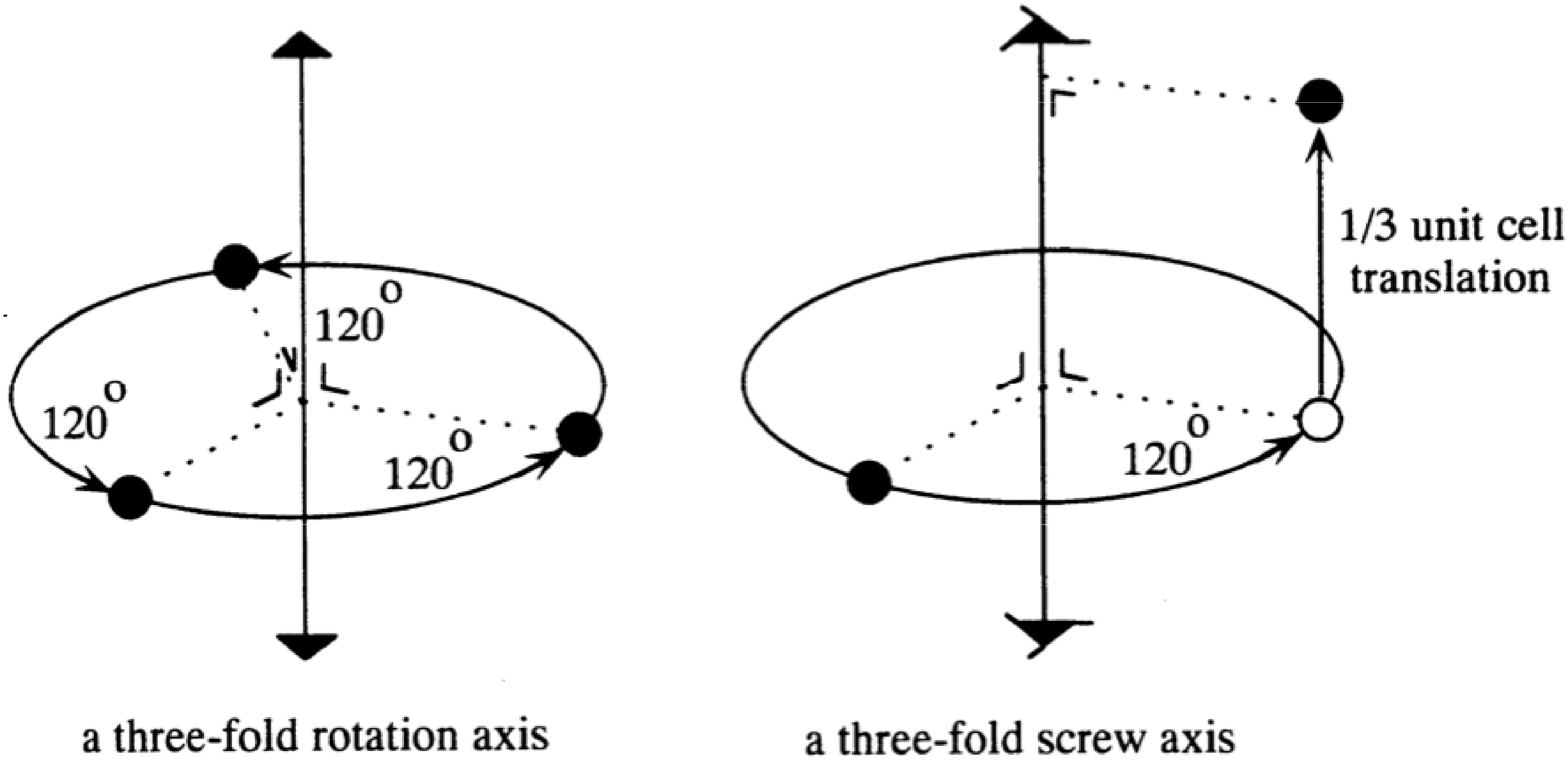


Figure 3.13. A 3-fold axis (left) and a 3-fold screw axis (right); the latter relates one molecule to another by a 120° rotation and a translation over one-third of the unit cell.

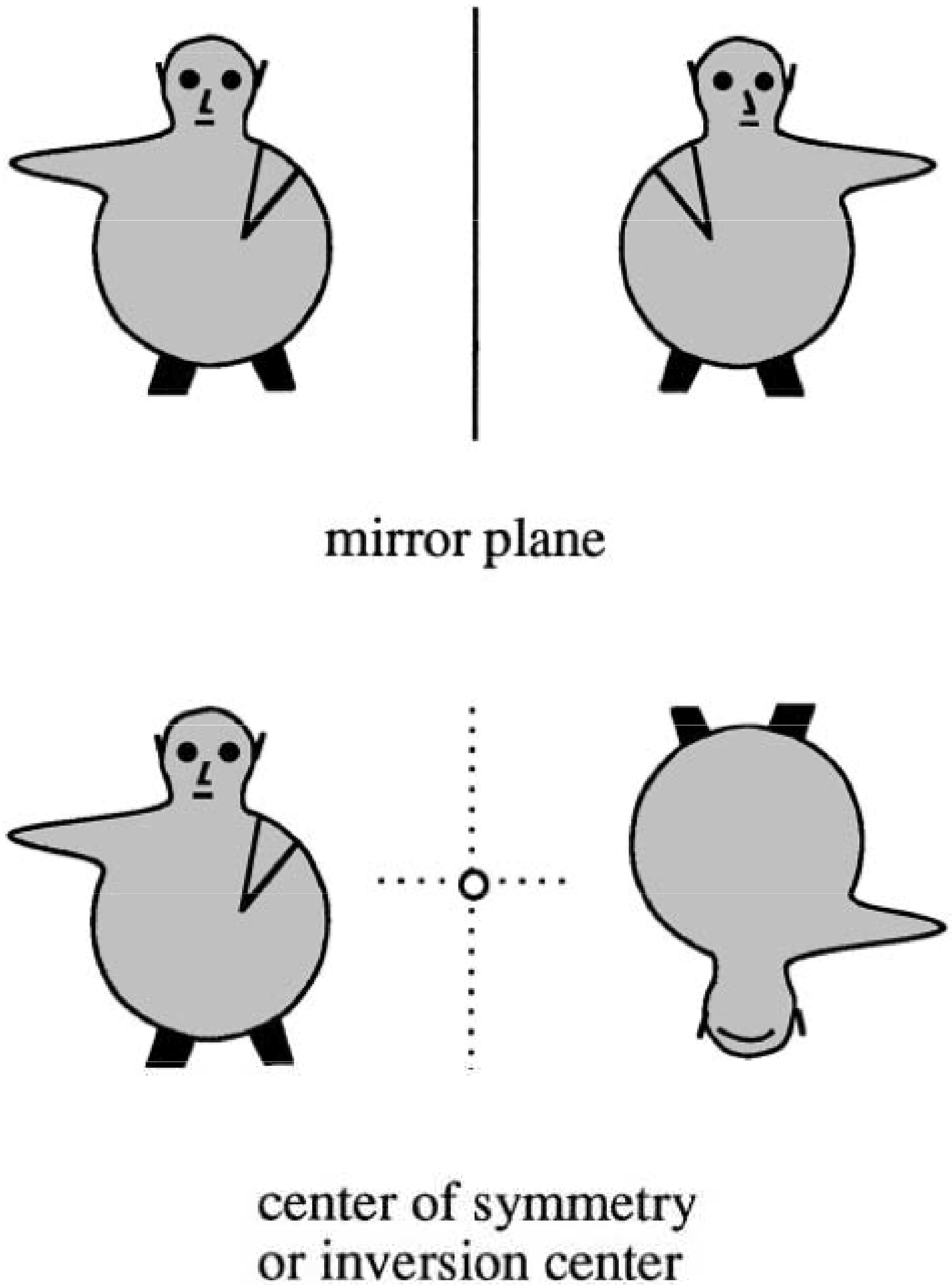


Figure 3.14. The effect of a mirror and of an inversion center.

Table 3.1. Graphic Symbols for Symmetry Elements

Symmetry axis or symmetry point	Graphic symbol	Screw vector of a right-handed screw rotation in units of the shortest lattice translation vector parallel to the axis	Printed symbol
Symmetry axes normal to the plane of projection (three dimensions) and symmetry points in the plane of the figure (two dimensions)			
Identity	None	None	1
Twofold rotation axis Twofold rotation point (two dimensions)		None	2
Twofold screw axis: “2 sub 1”		$\frac{1}{2}$	2_1
Threefold rotation axis Threefold rotation point (two dimensions)		None	3
Threefold screw axis: “3 sub 1”		$\frac{1}{3}$	3_1
Threefold screw axis: “3 sub 2”		$\frac{2}{3}$	3_2
Fourfold rotation axis Fourfold rotation point (two dimensions)		None	4
Fourfold screw axis: “4 sub 1”		$\frac{1}{4}$	4_1
Fourfold screw axis: “4 sub 2”		$\frac{1}{2}$	4_2
Fourfold screw axis: “4 sub 3”		$\frac{3}{4}$	4_3
Sixfold rotation axis Sixfold rotation point (two dimensions)		None	6
Sixfold screw axis: “6 sub 1”		$\frac{1}{6}$	6_1
Sixfold screw axis: “6 sub 2”		$\frac{1}{3}$	6_2
Sixfold screw axis: “6 sub 3”		$\frac{1}{2}$	6_3

(*cont.*)

Table 3.1. (*Continued*)

Symmetry axis or symmetry point	Graphic symbol	Screw vector of a right-handed screw rotation in units of the shortest lattice translation vector parallel to the axis	Printed symbol
Sixfold screw axis: “6 sub 4”		$\frac{2}{3}$	6_4
Sixfold screw axis: “6 sub 5”		$\frac{5}{6}$	6_5
Center of symmetry, inversion center: “1 bar” Reflection point, mirror point (one dimension)		None	$\bar{1}$
Twofold rotation axis with center of symmetry		None	$2/m$
Twofold screw axis with center of symmetry		$\frac{1}{2}$	$2_1/m$
Inversion axis: “3 bar”		None	$\bar{3}$
Inversion axis: “4 bar”		None	$\bar{4}$
Fourfold rotation axis with center of symmetry		None	$4/m$
“4 sub 2” screw axis with center of symmetry		$\frac{1}{2}$	$4_2/m$
Inversion axis: “6 bar”		None	$\bar{6}$
Sixfold rotation axis with center of symmetry		None	$6/m$
“6 sub 3” screw axis with center of symmetry		$\frac{1}{2}$	$6_3/m$

Symmetry axes parallel to the plane of projection

Twofold rotation axis		None	2
Twofold screw axis: “2 sub 1”		$\frac{1}{2}$	2_1
Fourfold rotation axis		None	4
Fourfold screw axis: “4 sub 1”		$\frac{1}{4}$	4_1
Fourfold screw axis: “4 sub 2”		$\frac{1}{2}$	4_2
Fourfold screw axis: “4 sub 3”		$\frac{3}{4}$	4_3
Inversion axis: “4 bar”		None	$\bar{4}$

Symmetry axes inclined to the plane of projection (in cubic space groups only)

Twofold rotation axis		None	2
Twofold screw axis: “2 sub 1”		$\frac{1}{2}$	2_1
Threefold rotation axis		None	3
Threefold screw axis: “3 sub 1”		$\frac{1}{3}$	3_1
Threefold screw axis: “3 sub 2”		$\frac{2}{3}$	3_2
Inversion axis: “3 bar”		None	$\bar{3}$

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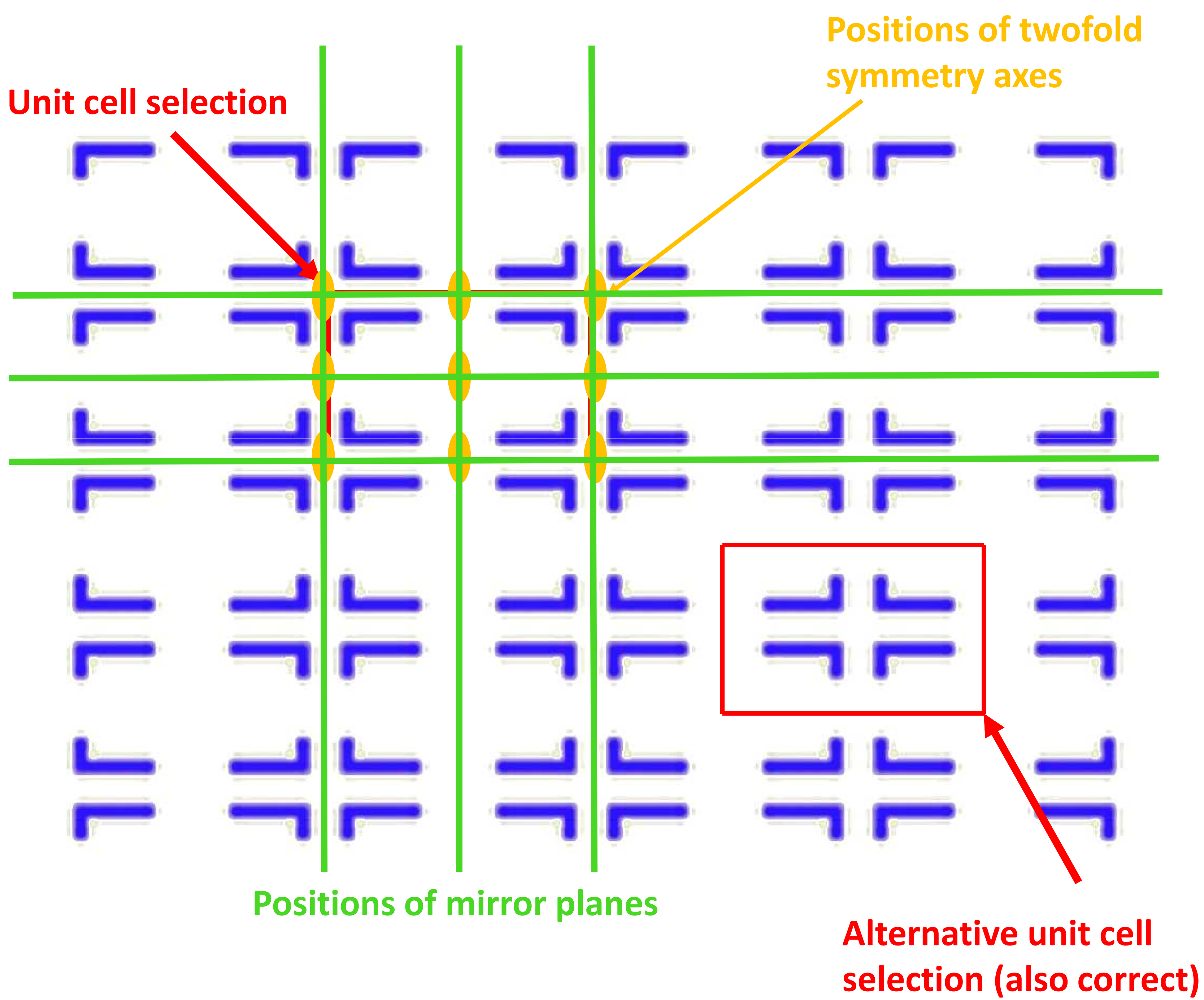
Guide to the recognising of wallpaper groups

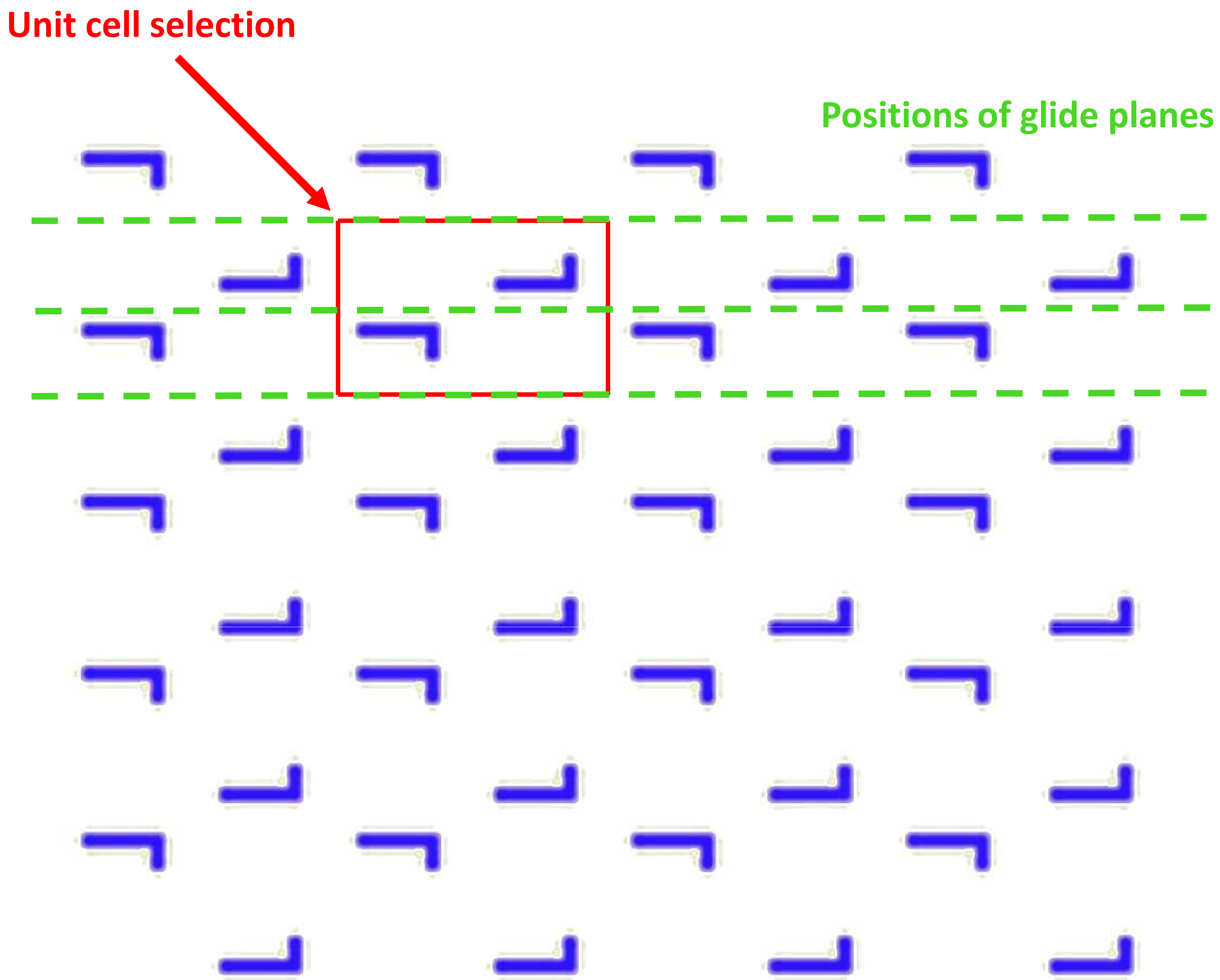
1. Identify the smallest unit cell that represents all the symmetry included in the pattern. (Be particularly careful in the case of centered symmetry. Use rhomb shaped cells for patterns with 3 and 6-fold rotation axes.)

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

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

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


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