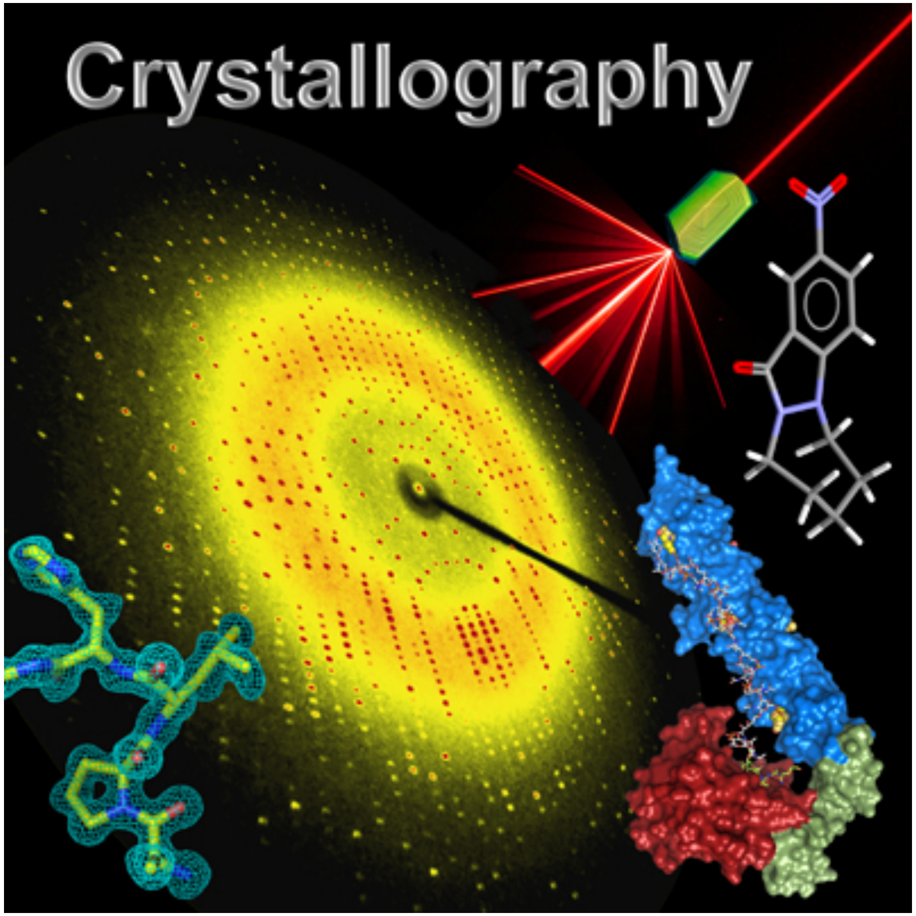
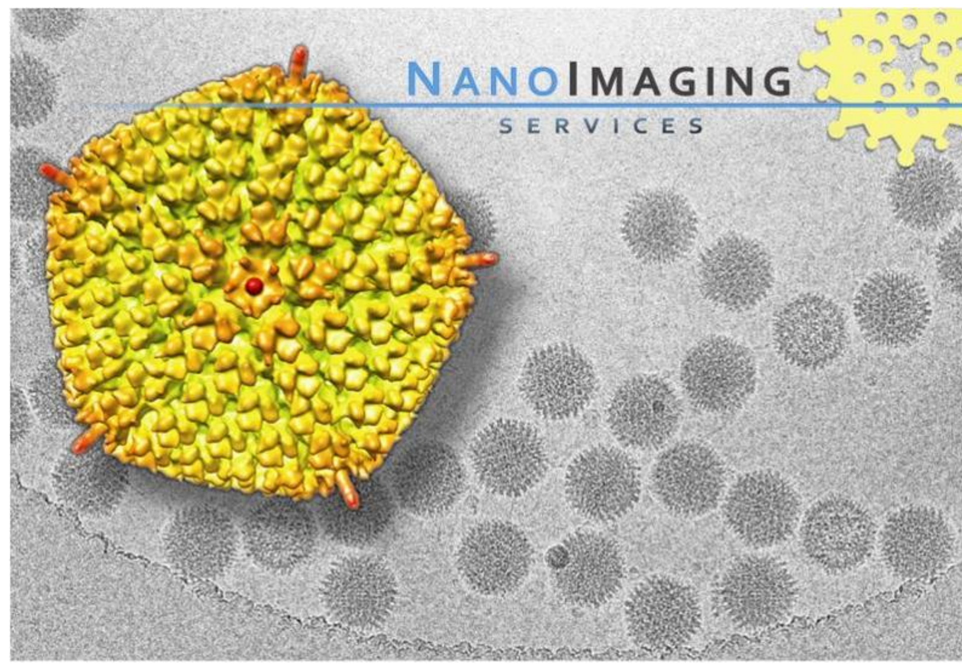
Structural Biology Methods

Fall 2015

Pavel Plevka



Electron microscopy
– crystallography without crystals



Aims of the course

1 Physical principles allowing the use of X-ray crystallography, cryo-EM, and AFM
2 Properties of X-ray radiation that make it suitable to study (macro)molecular structures
3 Diffraction of light
4 Crystallographic space group symmetries
5 Approaches to resolve phase problem in crystallography
6 Use of electrons to display objects with high magnification and fine detail
7 Calculation of three-dimensional reconstruction from two-dimensional projections

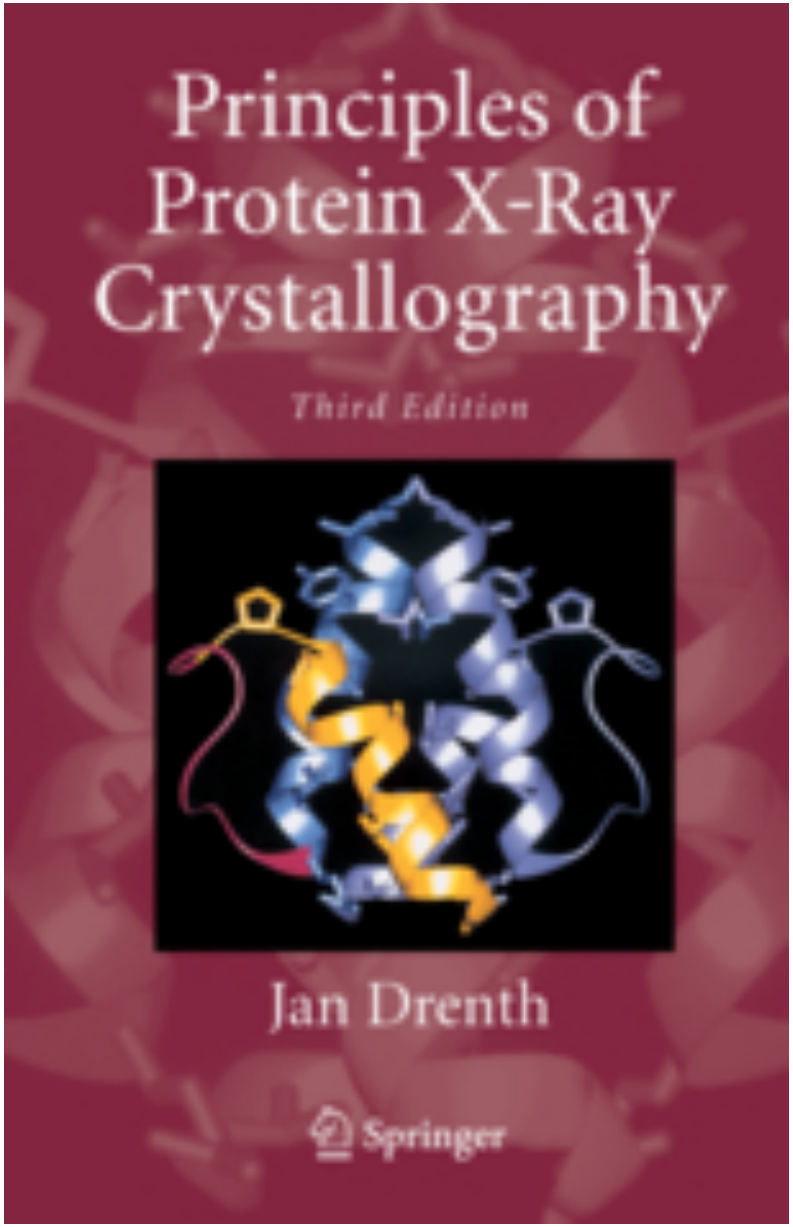
Course plan

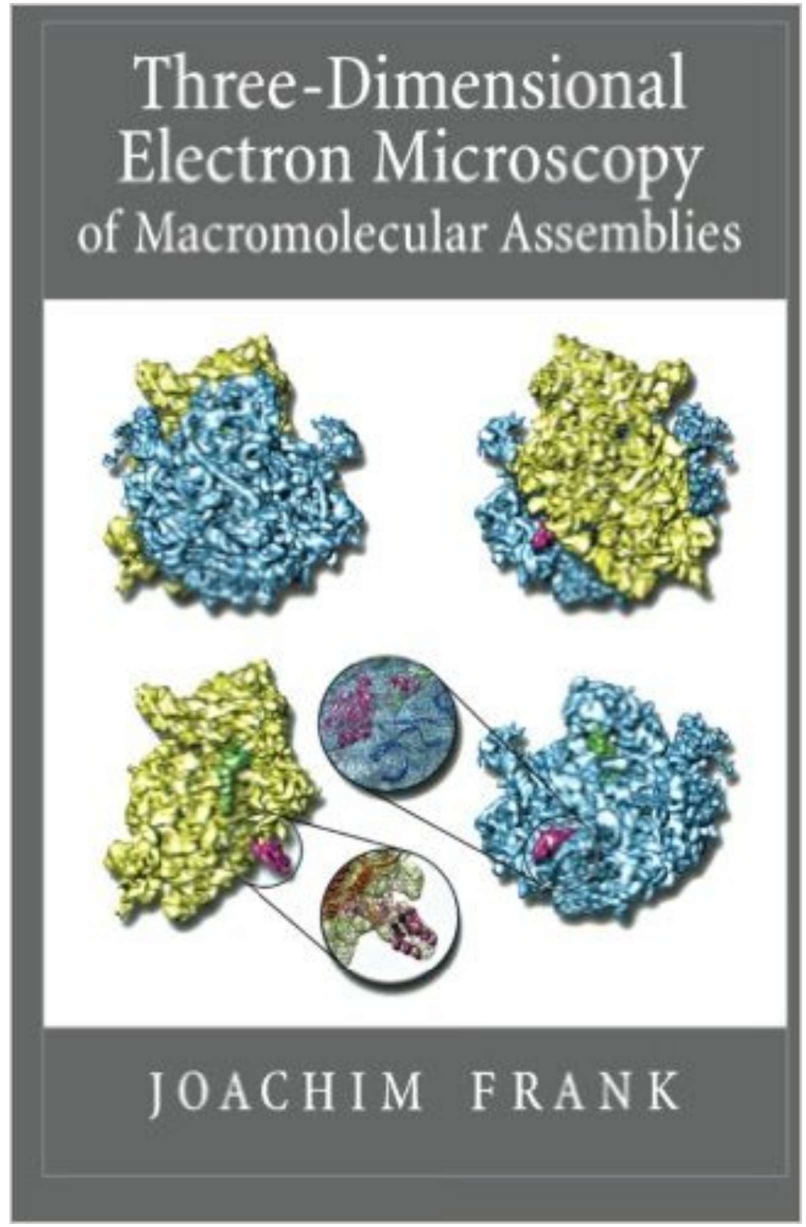
L#	Dt 2015	Topic	Chapter reading
1	21.9.	Introduction, crystals and symmetry I., and X-rays	1, 2, 3, 16
2	5.10.	Crystals and Symmetry II. (continued) and the Theory of X-Ray Diffraction	3, 4
3	12.10.	The Theory of X-Ray Diffraction by a Crystal I.	4
4	19.10.	Atomic Force Microscopy	2 - AFM
5	26.10.	The Theory of X-Ray Diffraction by a Crystal II.	4
6	2.11.	Average Reflection Intensity, Distribution of Structure Factor Data, Special Forms of the Structure Factor.	5, 6
7	9.11.	The Solution of the Phase Problem by the Isomorphous Replacement Method	7
8	16.11.	Phase Improvement	8
9	23.11.	Anomalous Scattering in the Determination of the Protein Phase Angles and the Absolute Configuration and Molecular Replacement I.	9, 10
10	30.11.	Molecular Replacement II., Laue Diffraction, Refinement of the Model Structure, The Combination of Phase Information, Checking for Gross Errors and Estimating the Accuracy of the Structural Model.	10, 12, 13, 14, 15
11	7.12.	Electron Microscopy of Macromolecular Assemblies	2 - EM
12	14.12.	Multivariate Data Analysis and Classification of Images	3, 4 - EM
13	21.12.	Three-Dimensional Reconstruction	5 - EM

Class rules

- Turn off anything that beeps or rings.
- Reading any material that is not related to the class, texting, or checking the internet during the class is rude and will not be tolerated.
- Please refrain from eating during class. Having something to drink is fine.
- 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.

Course textbooks:





What is asked of you:

- Read assigned texts **BEFORE** the day for which they are assigned
- Participate in discussions
- Do excercises and homeworks
- I am here to help, learning is up to you!

Levels of passing the course:

"Sitter" – do exercises, hand in homework, participate in discussions => grade E

"Theoretician" – "Sitter" + take theoretical part of the exam (will include symmetry and equations) => best possible grade B

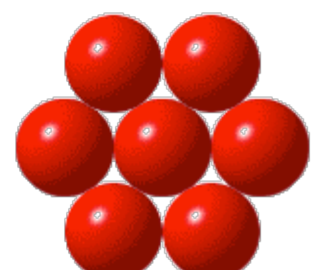
"Crystallographer" – "Theoretician" + extra part of exam that will include questions related to input to crystallographic and cryo-EM programs and interpretation of program outputs

Not part of this course:

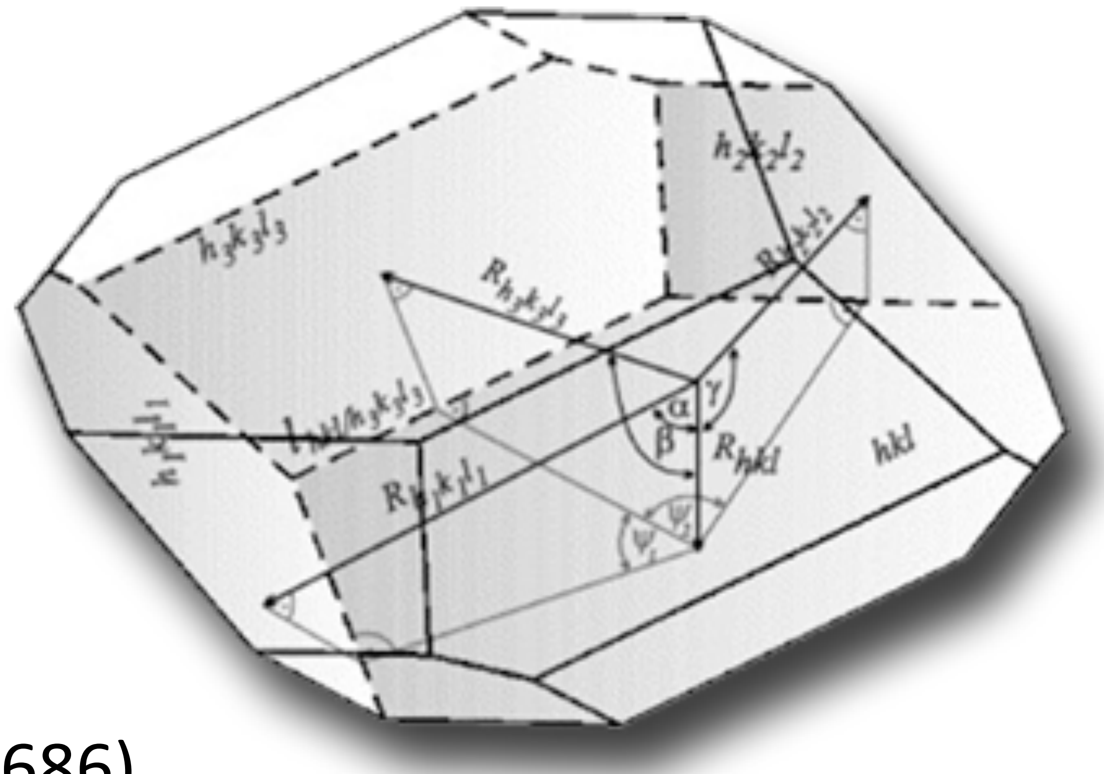
- Computer literacy (linux, terminal, shell environment) – mental overload by using computer. (Observed in my group.)
- Practical exercises will be demonstrations because of time constrains
- Install programs on your computer. Try solving structures. (You will never have more time than now.)

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)



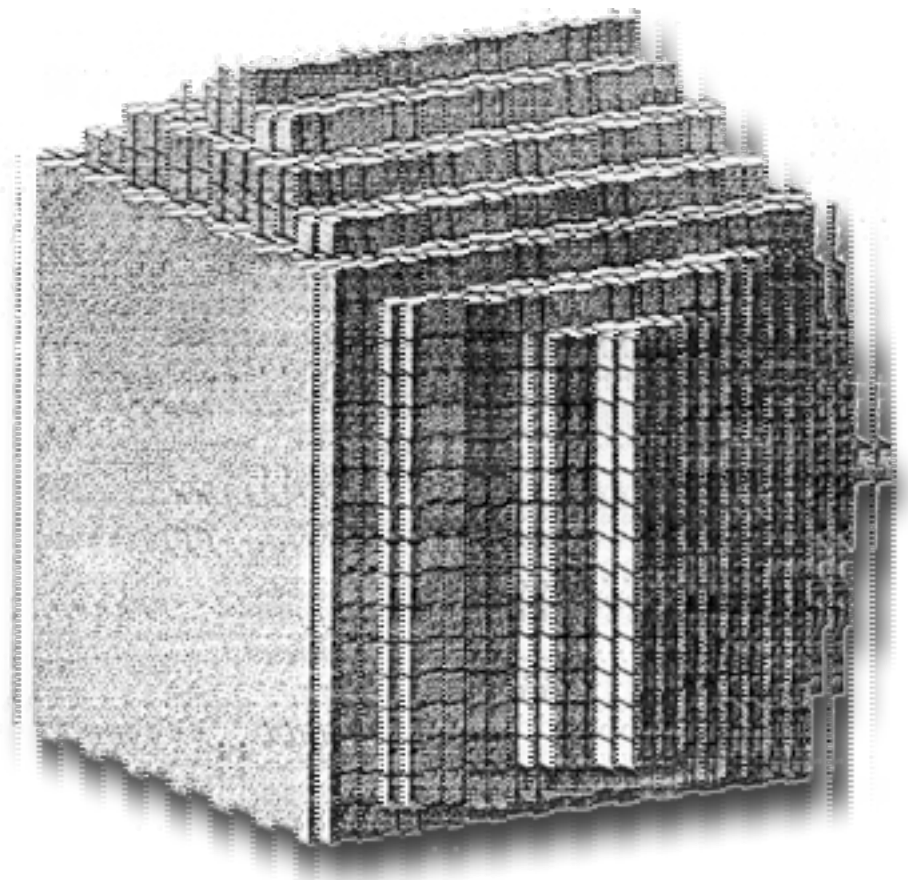
Although crystals of quartz and hematite appear in a great variety of shapes and sizes, the same interfacial angles persisted in every specimen. *"Law of Constancy of Angles"*



Niels Stensen (1638-1686)

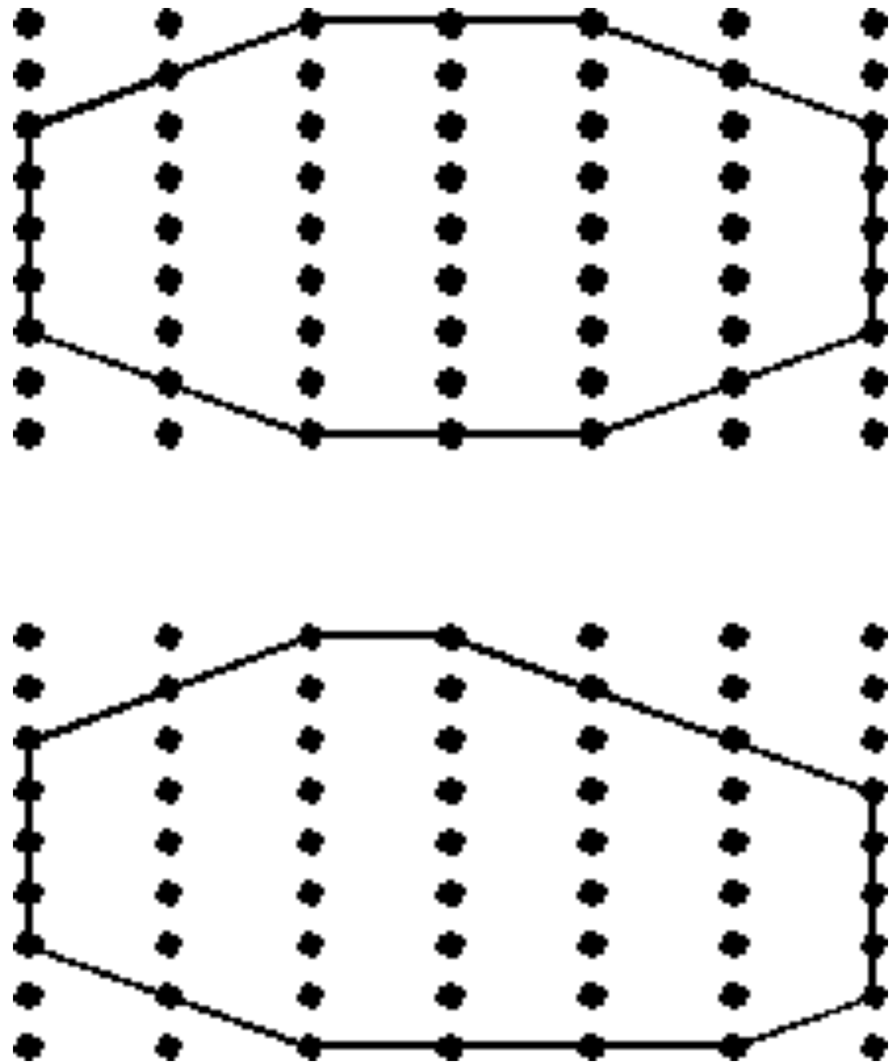
"Law of Constancy of Angles"

René Just Haüy (1743-1822)



"Law of Constancy of Angles"

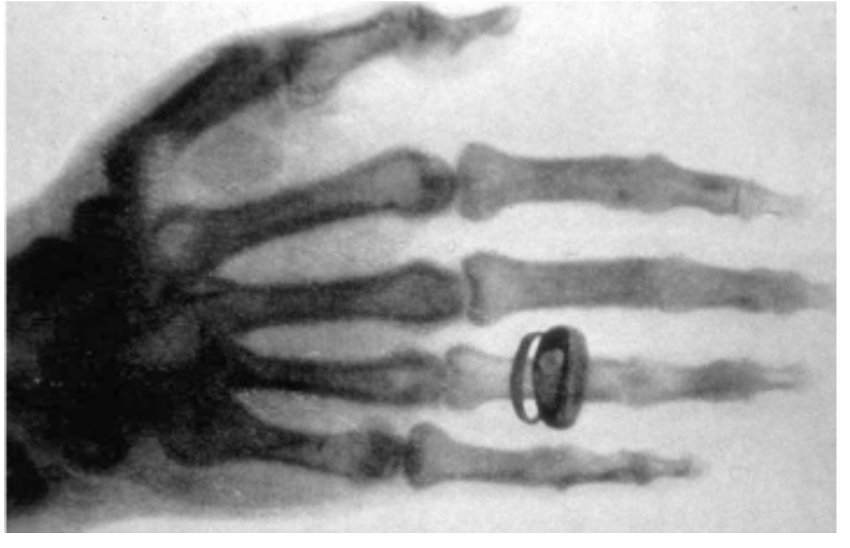
René Just Haüy (1743-1822)



History of fundamental discoveries

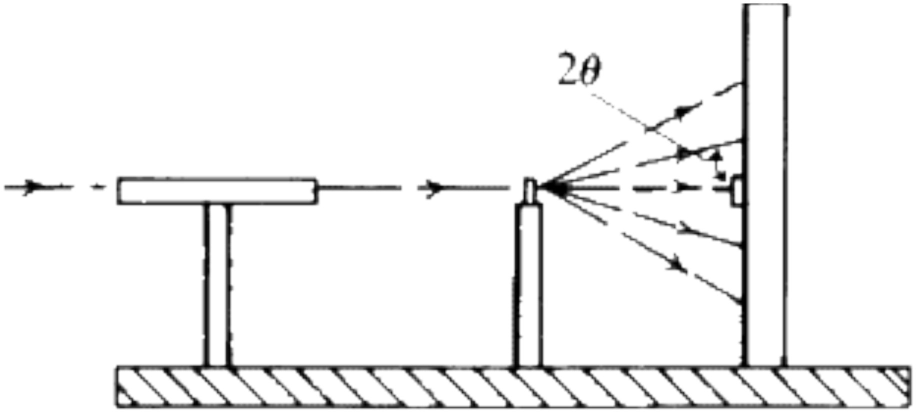
WILHELM CONRAD RÖNTGEN
(1845-1923)

- **1901 Nobel Laureate in Physics**
  discovery of the remarkable rays subsequently named after him

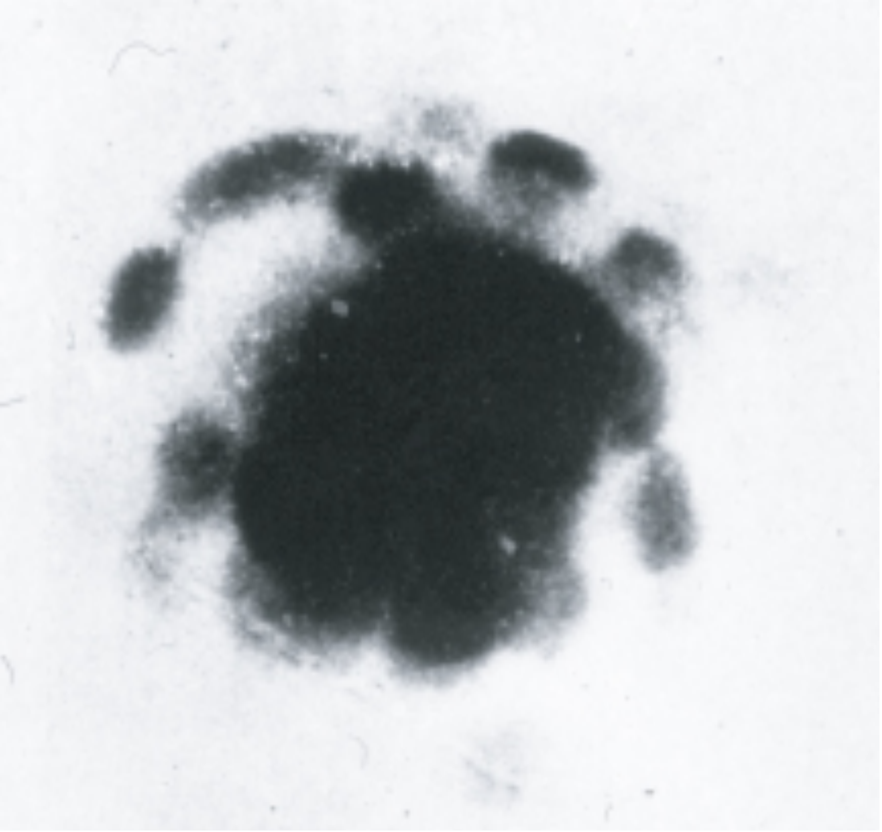


MAX VON LAUE
(1879-1960)

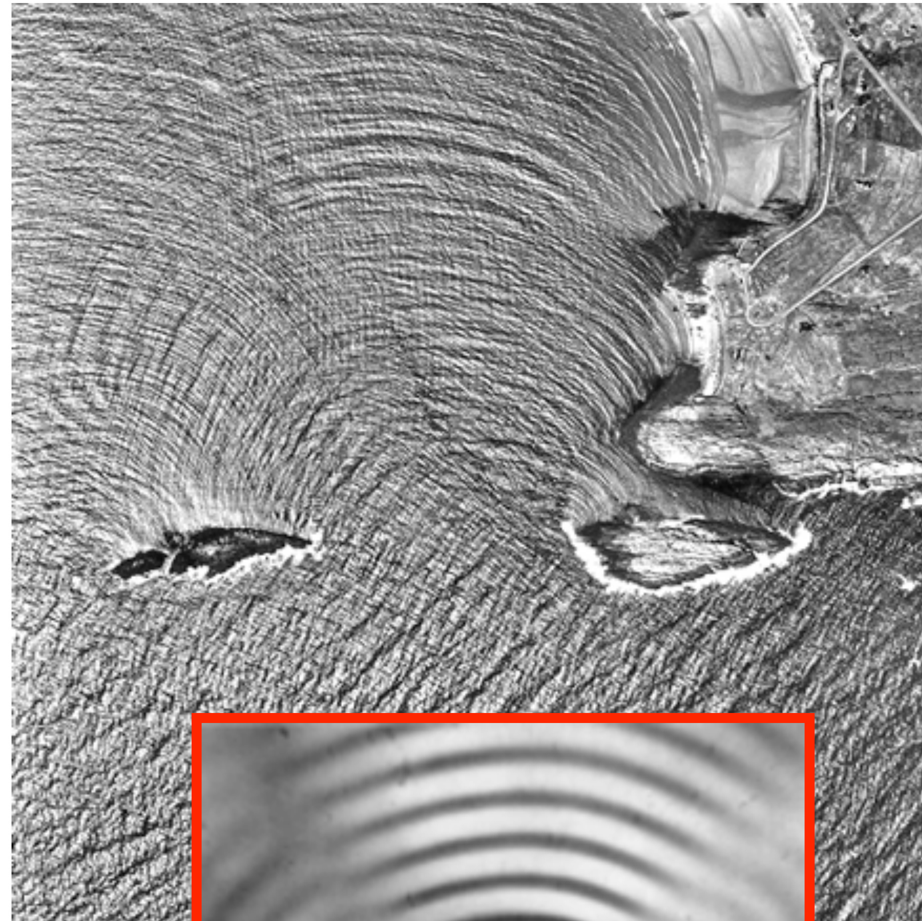
- **1914 Nobel Laureate in Physics**
  for his discovery of the diffraction of X-rays by crystals

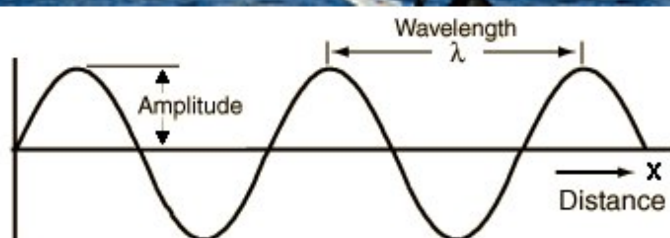


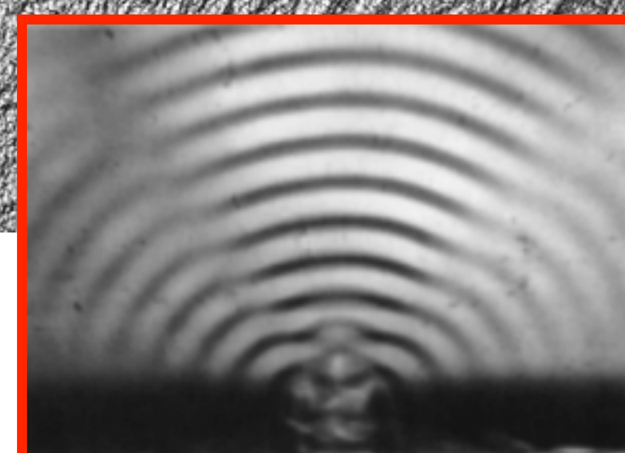
Friedrich and Knipping



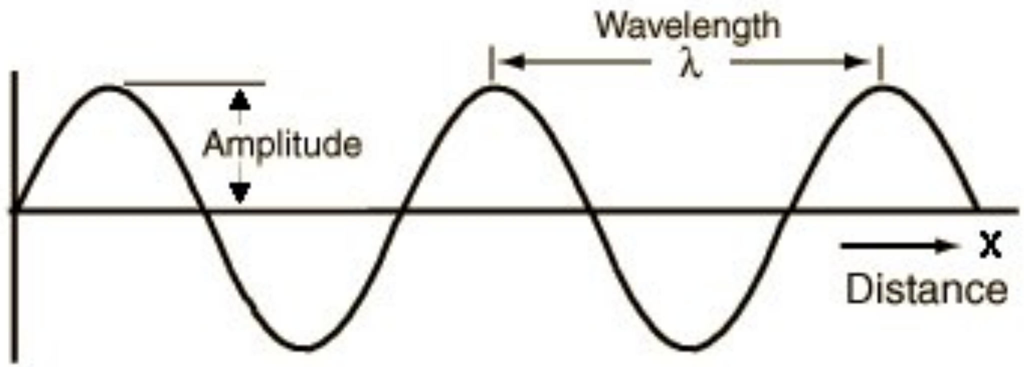
Wavelength and diffraction

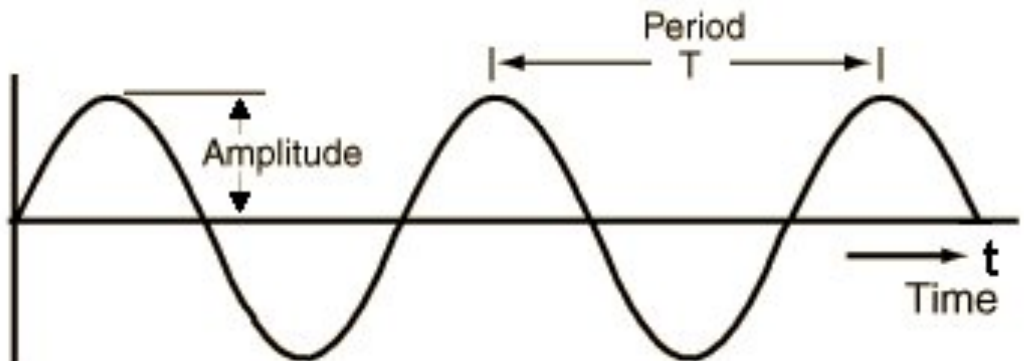




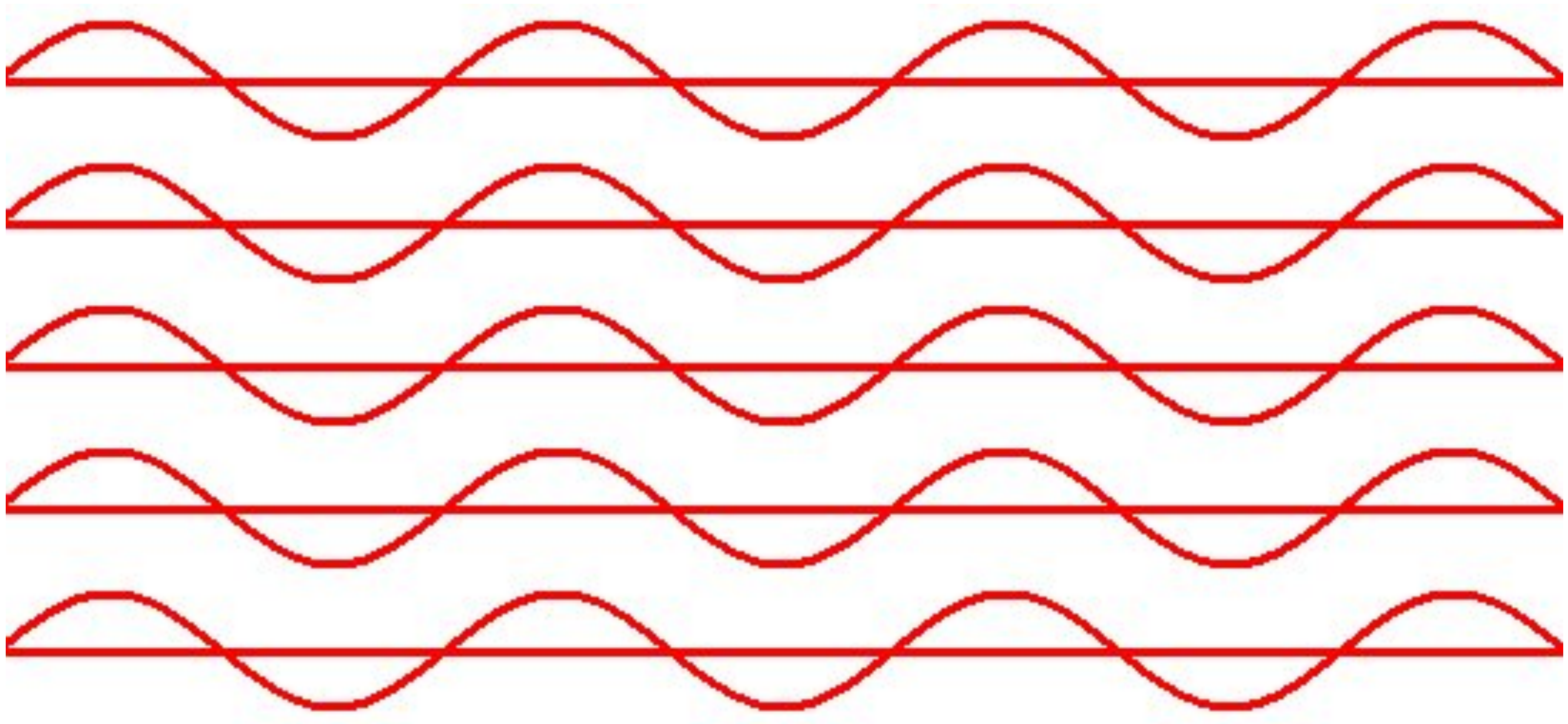


Waves

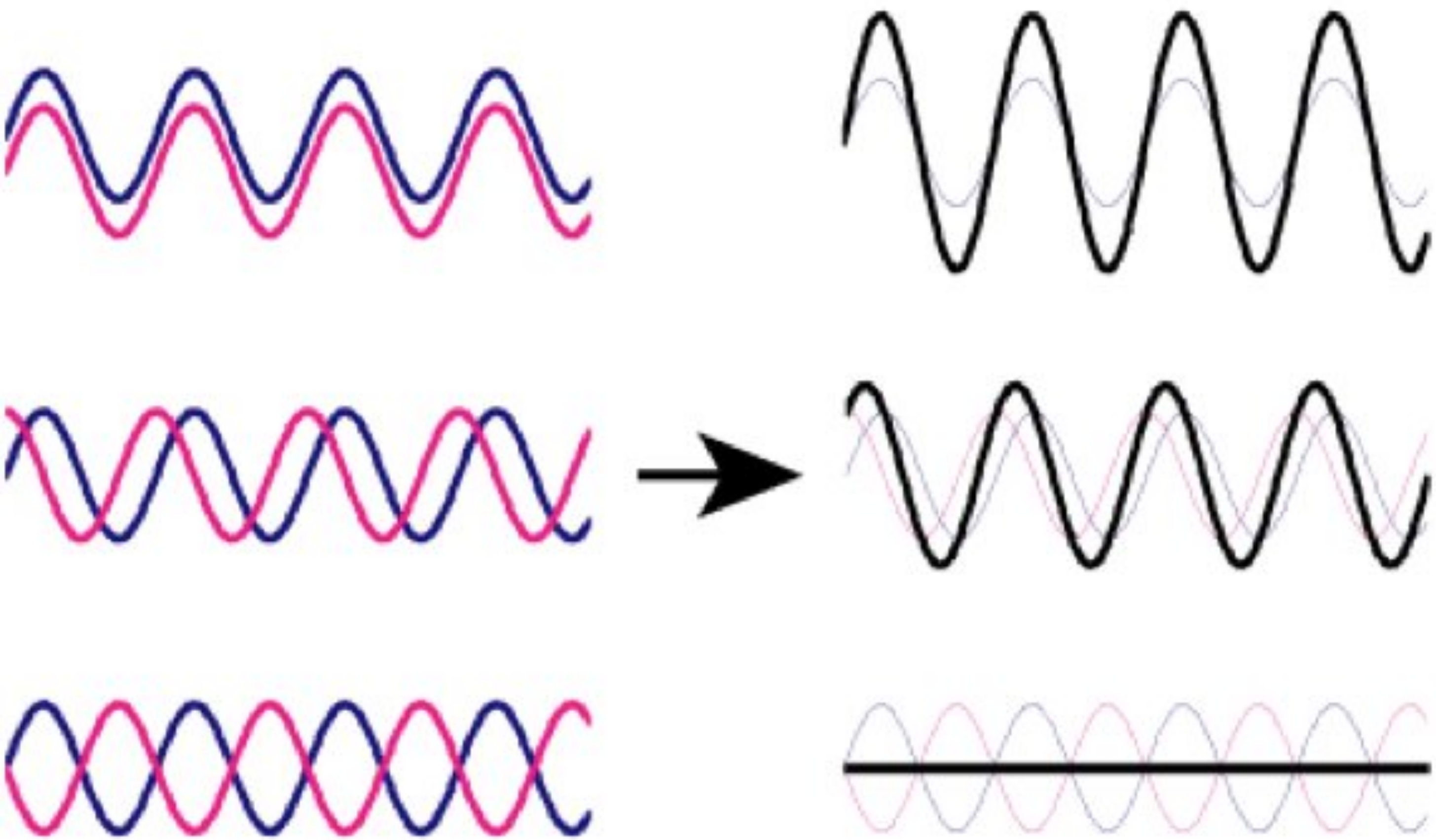

Wavelength
λ
Amplitude
x
Distance


Period
T
Amplitude
t
Time

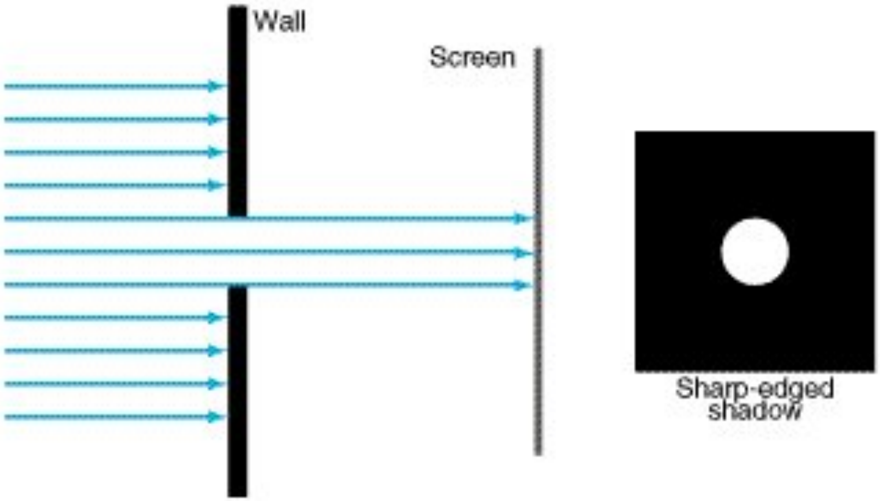
Coherent beam

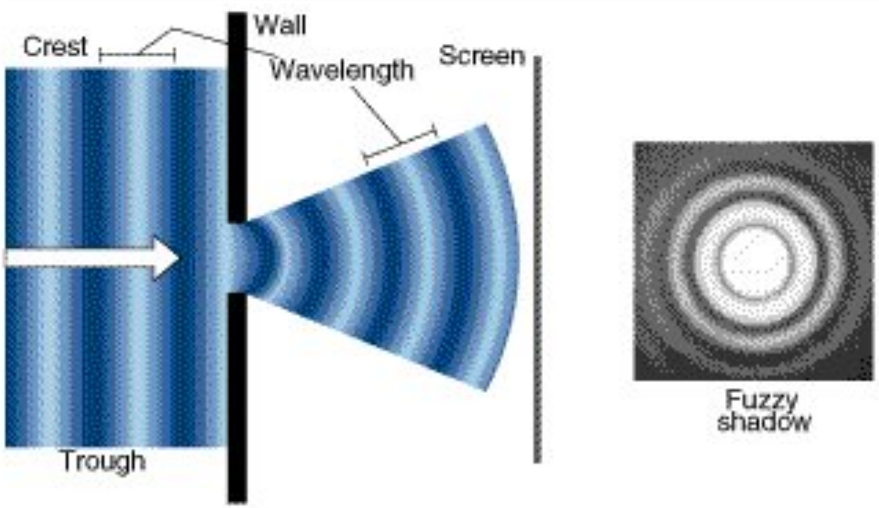


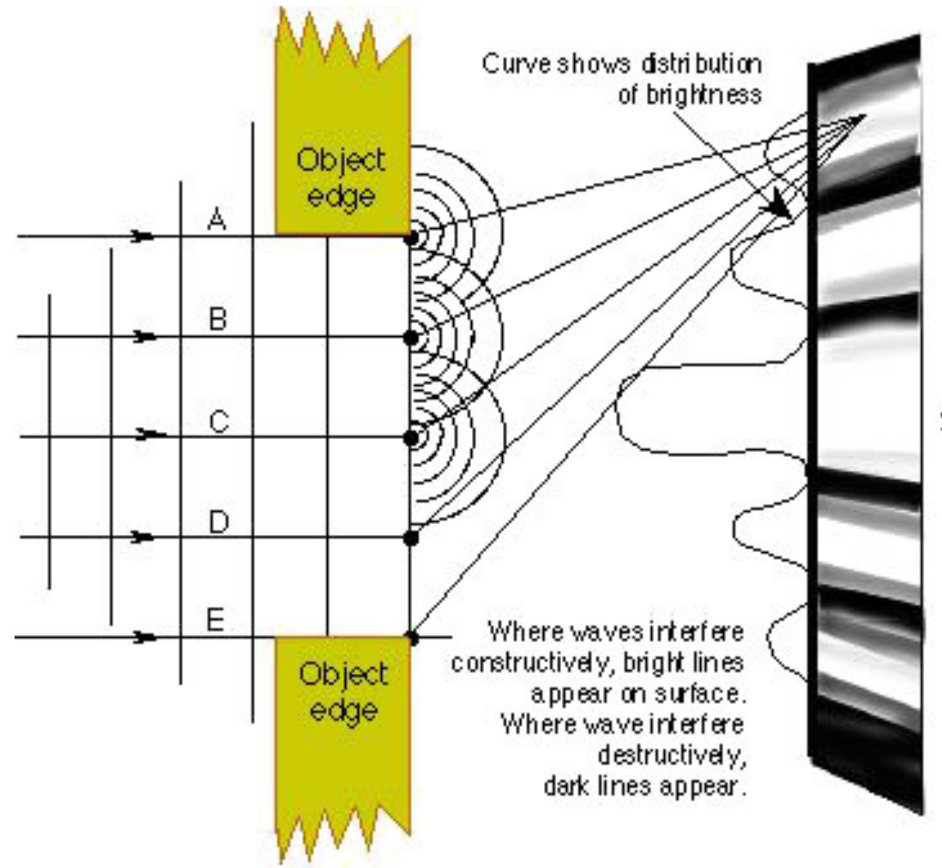
Addition of waves



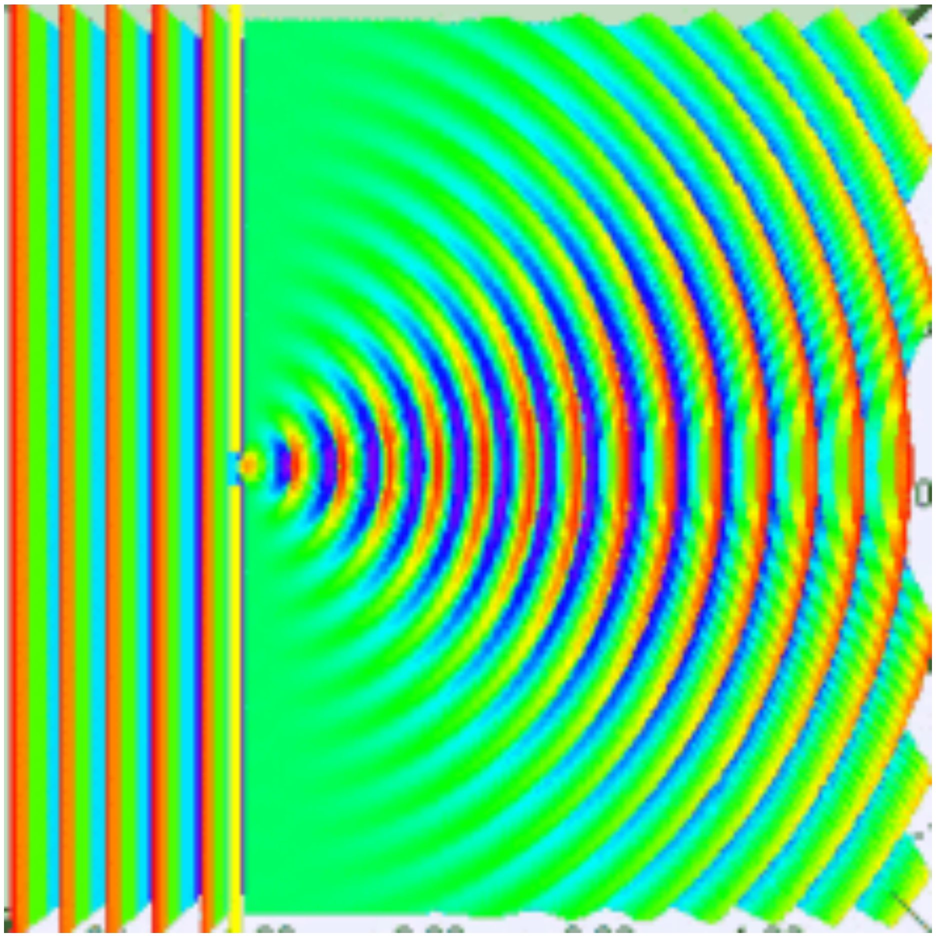
Particles & waves

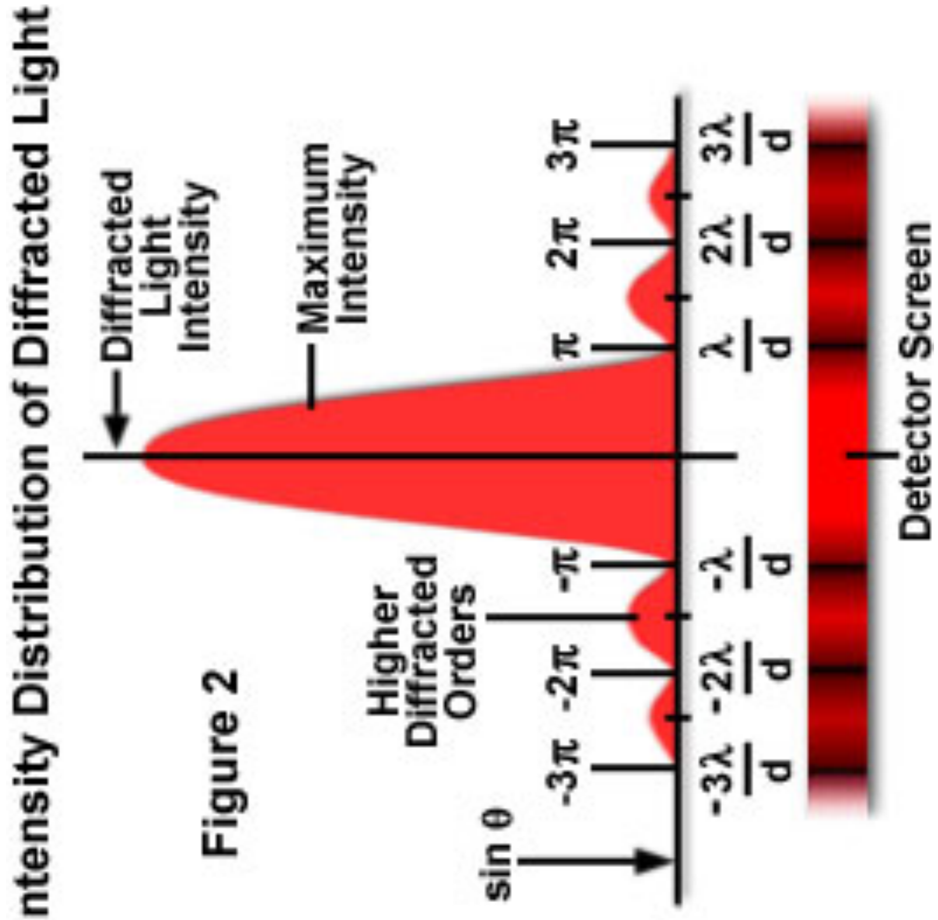




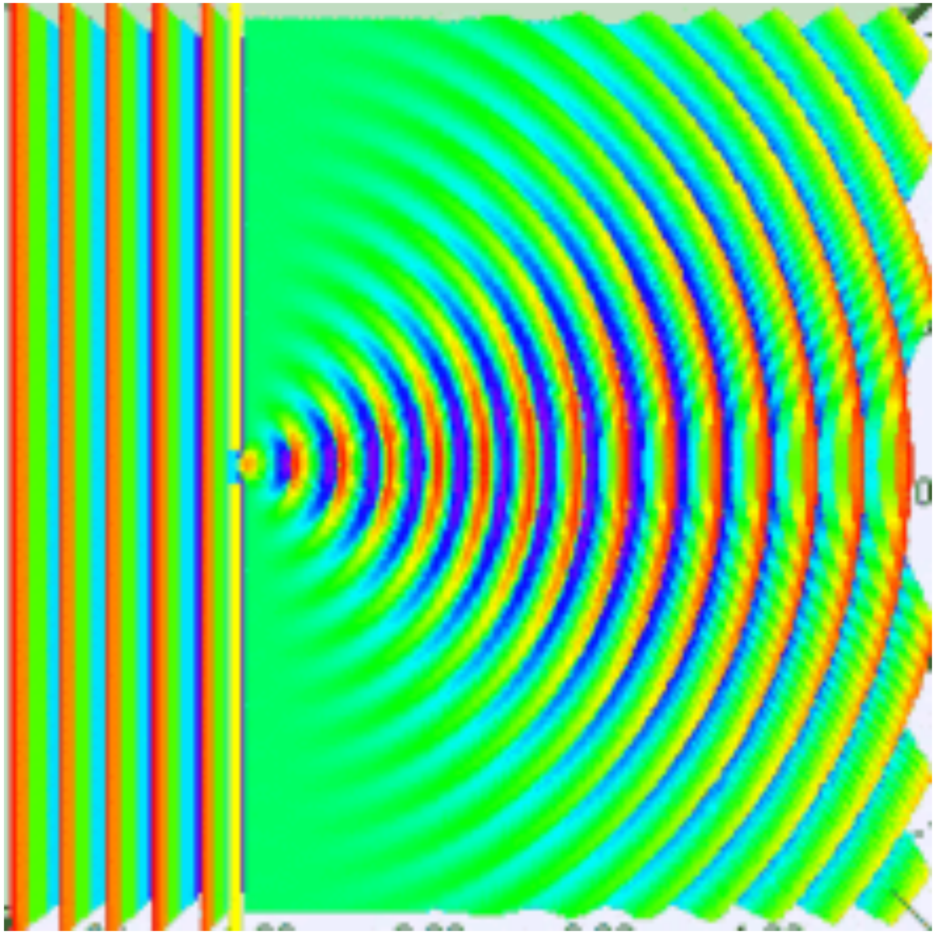


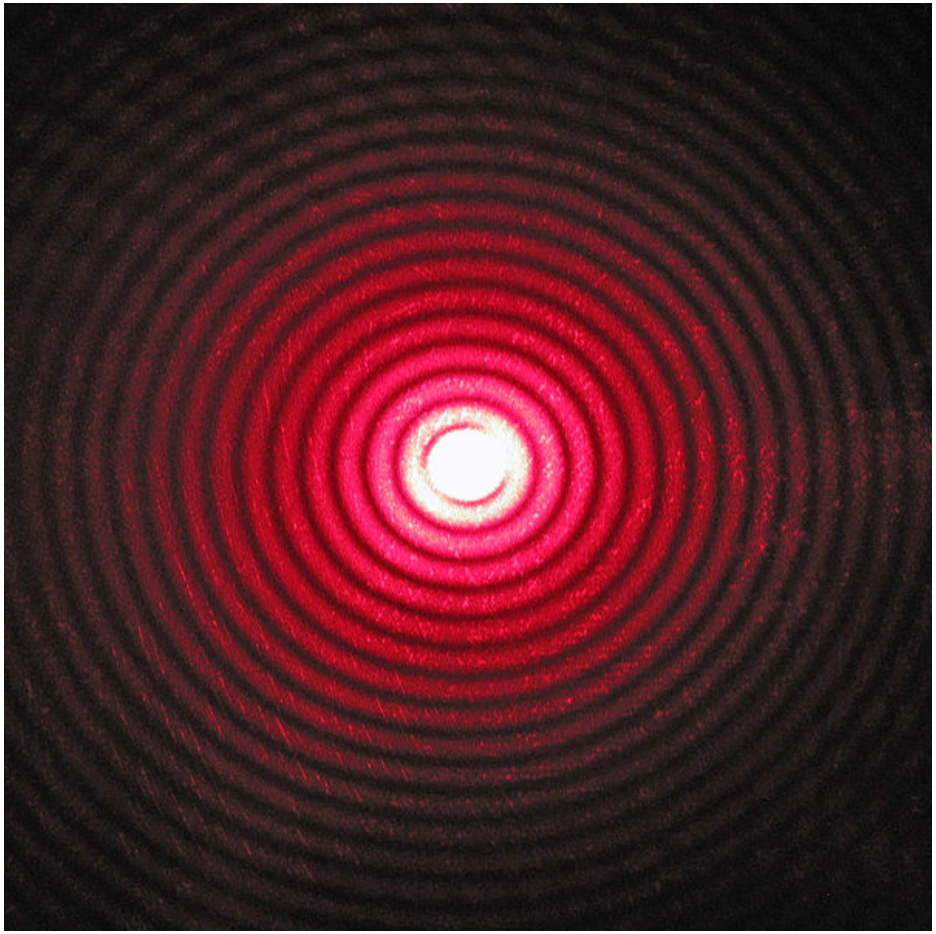
Diffraction of light



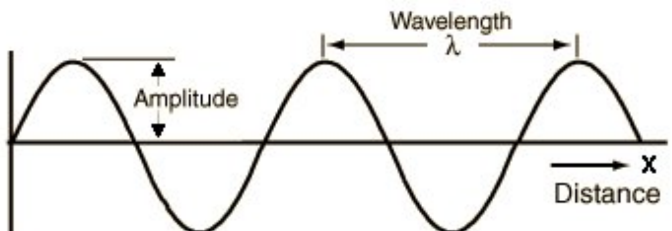


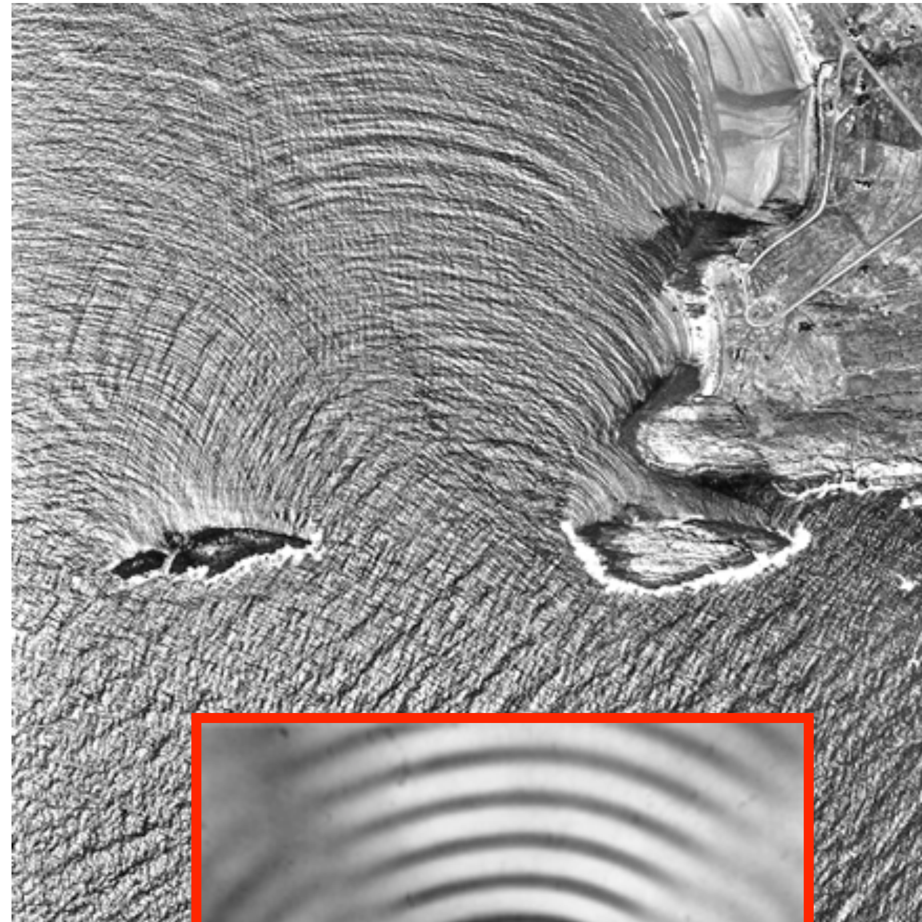
Diffraction of light

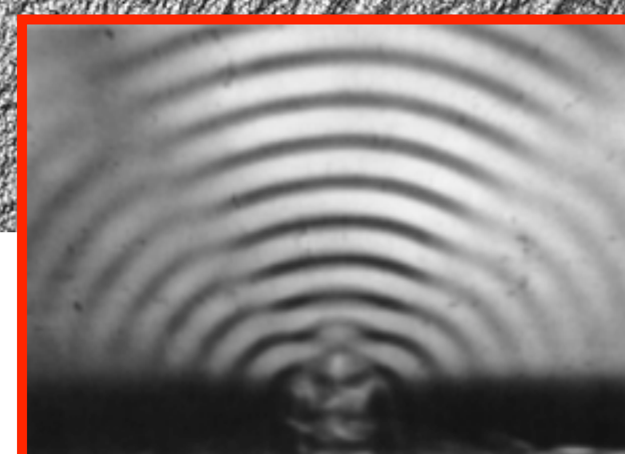




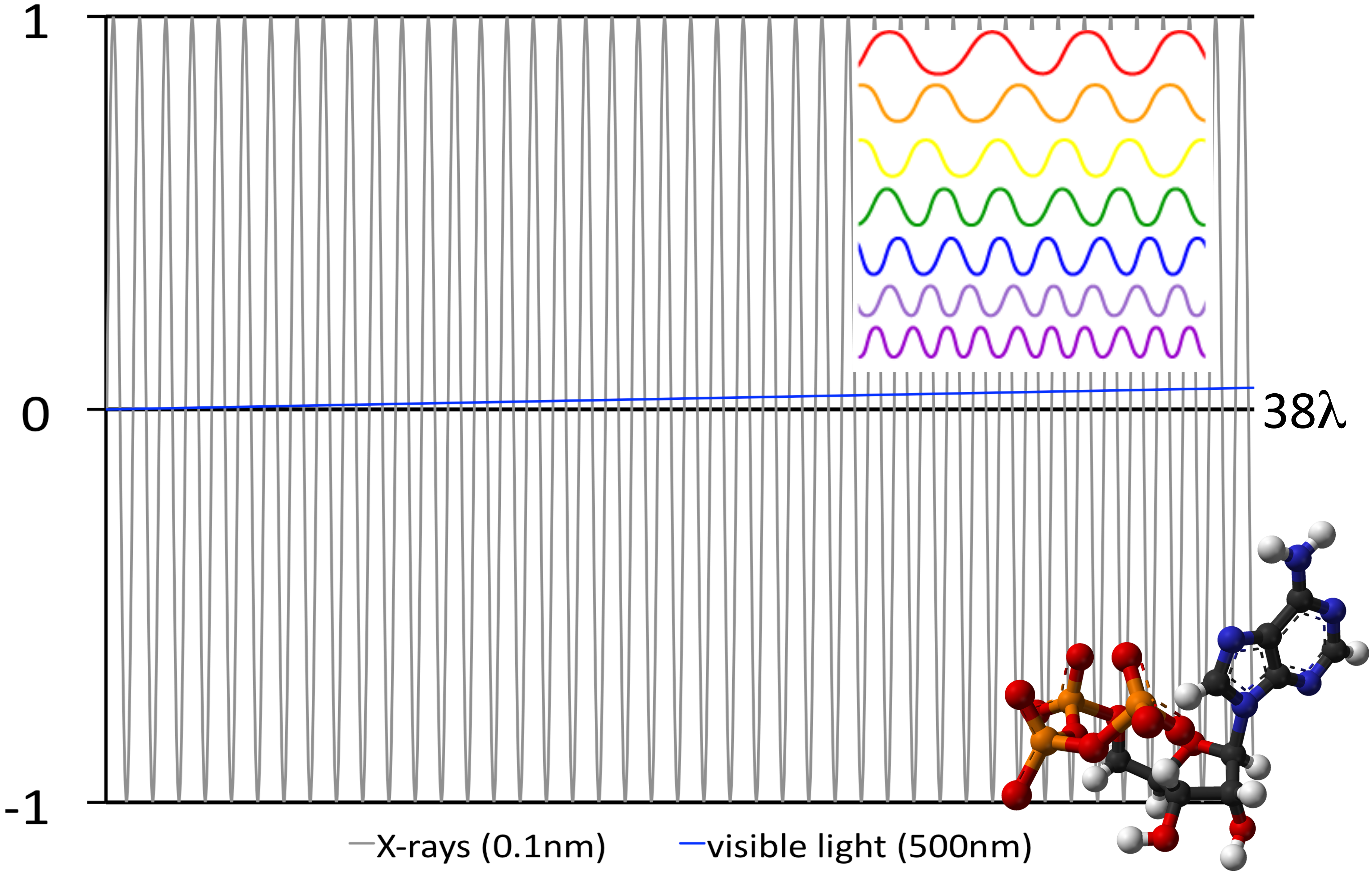
Wavelength and diffraction



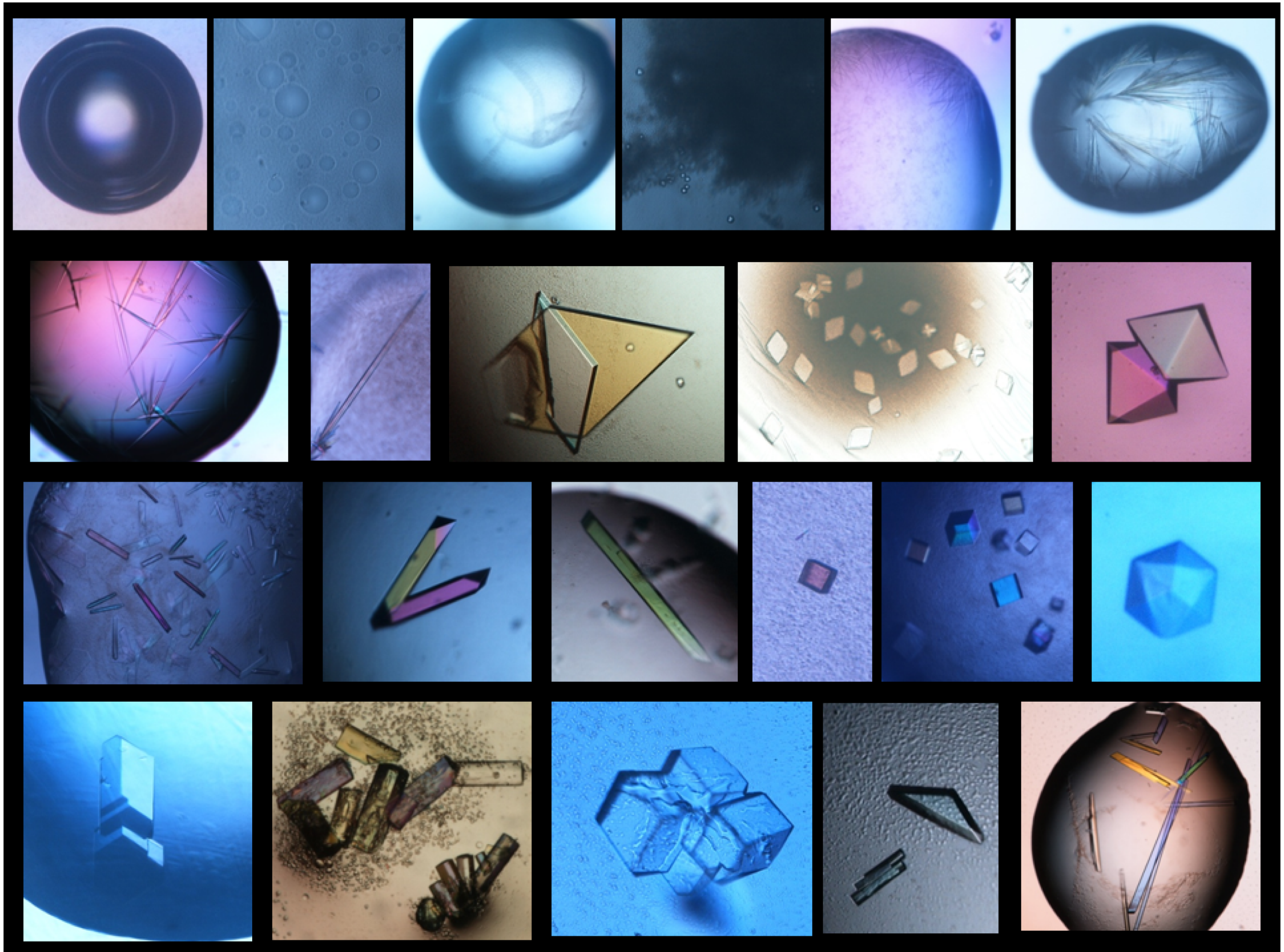




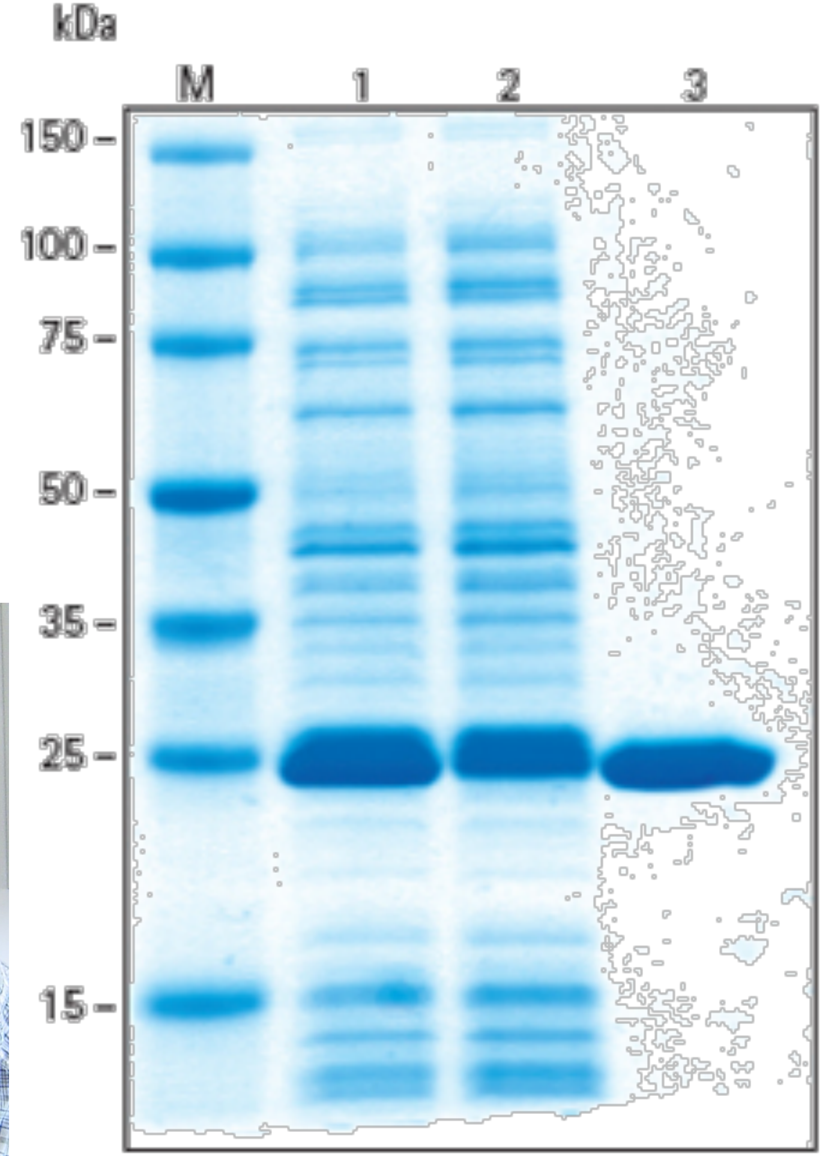
Wavelength comparison of X-rays and visible light



Crystallizing a Protein

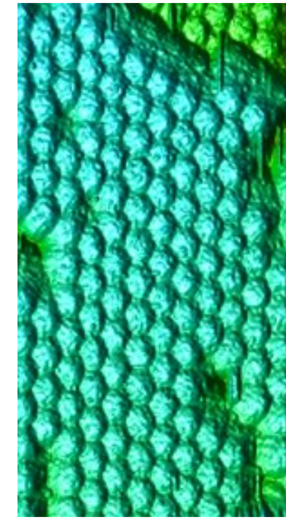


Protein expression and purification

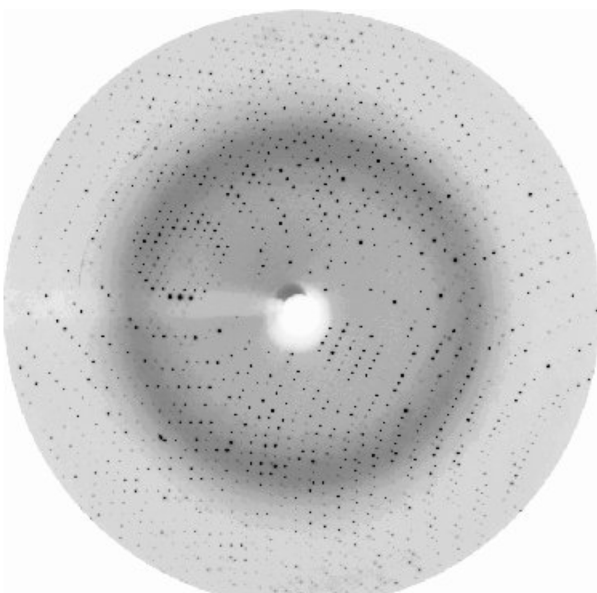


1. Expression & purification

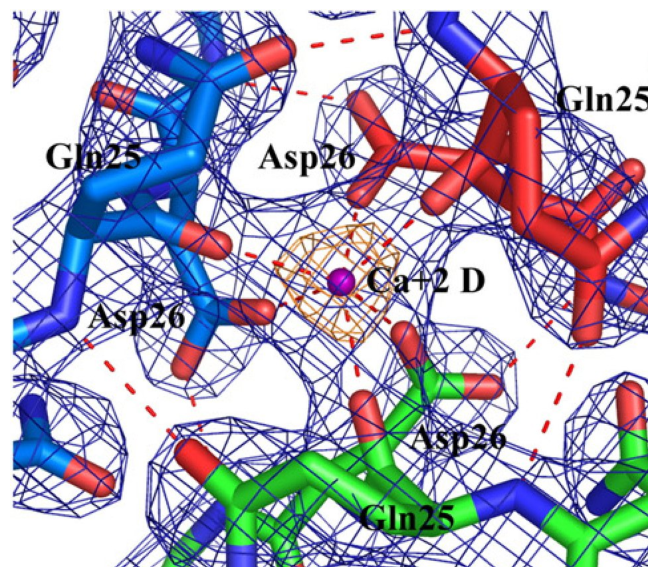
2. Crystallization

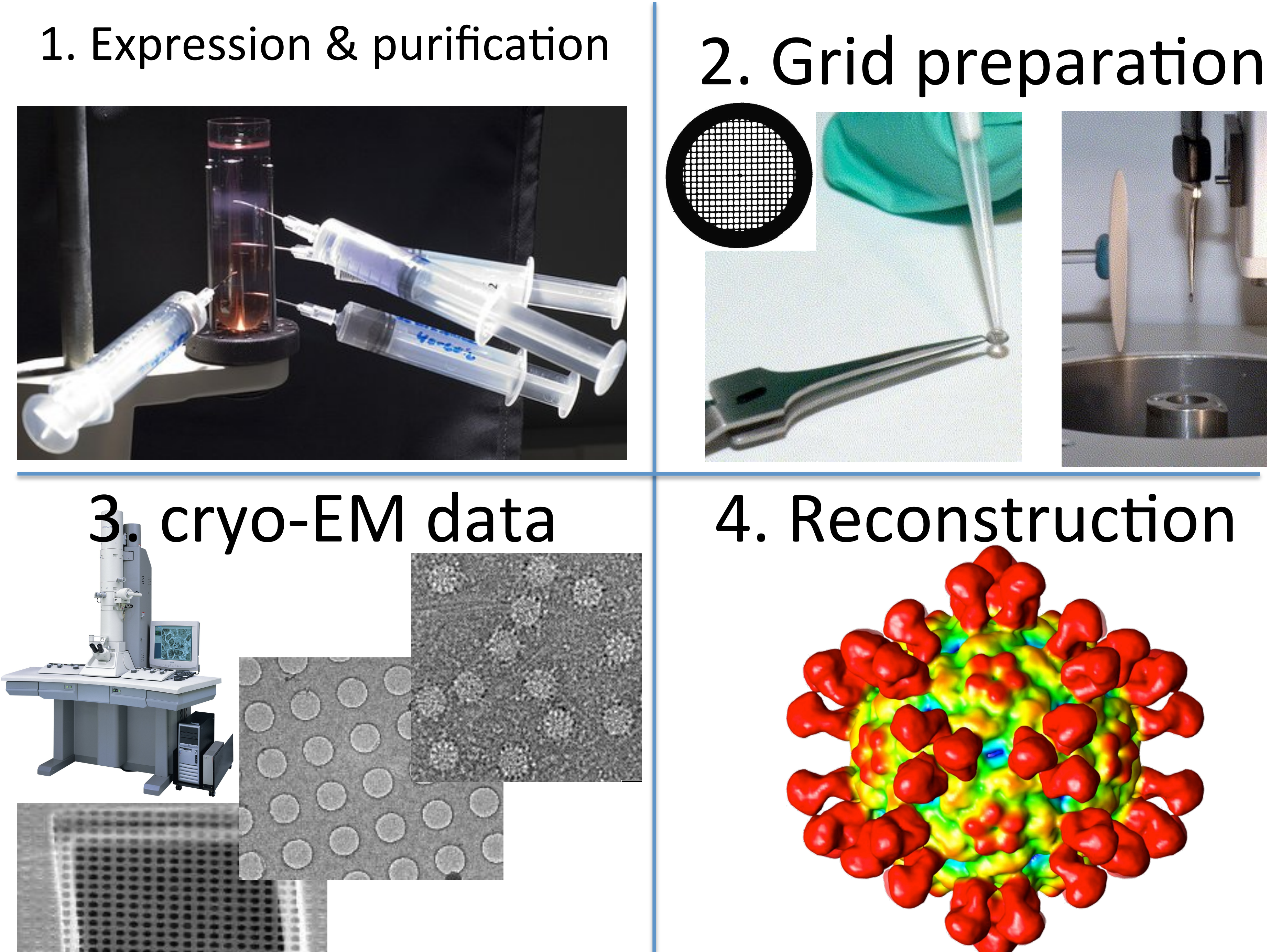


3. Diffraction data

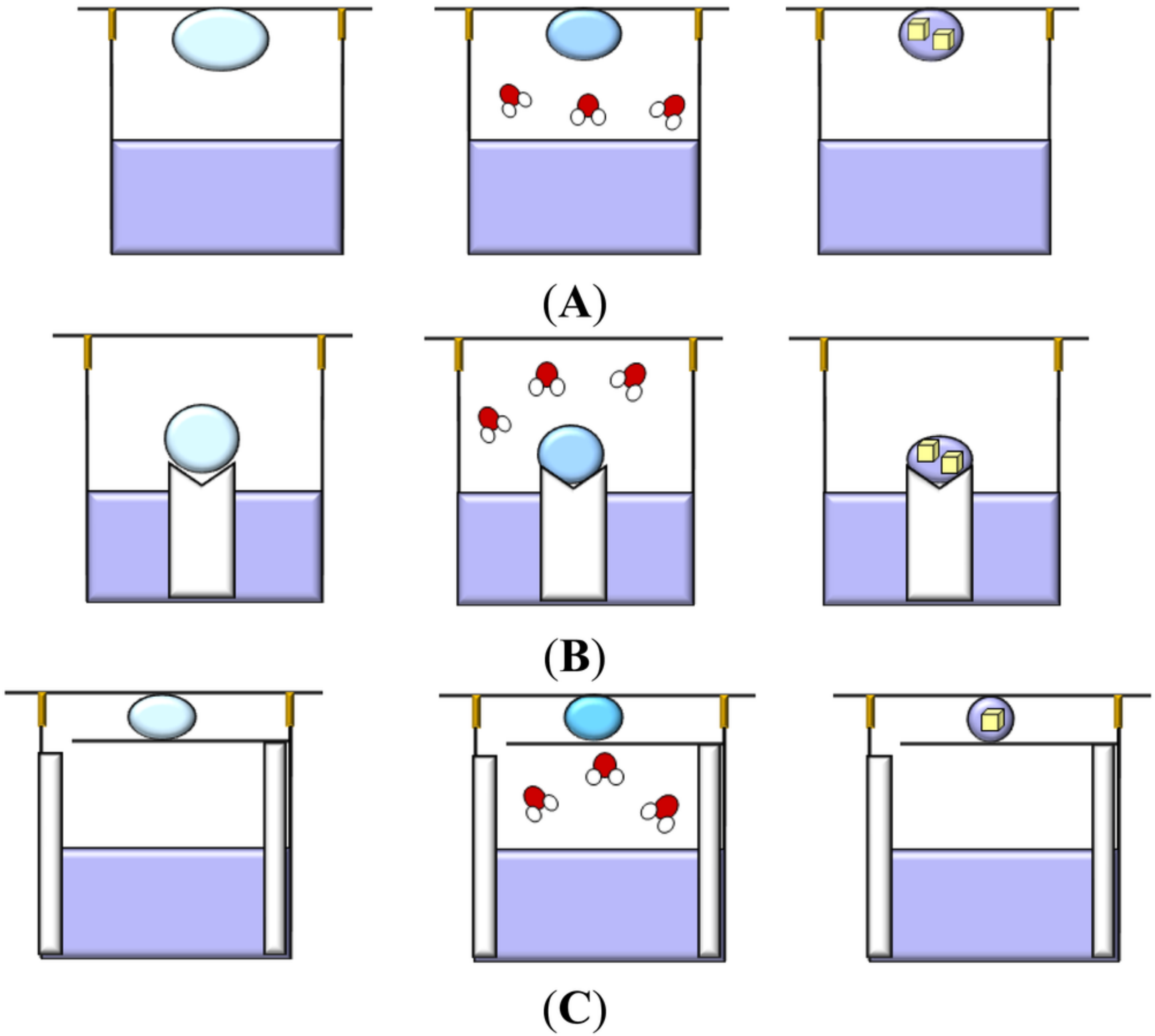


4. Solve structure

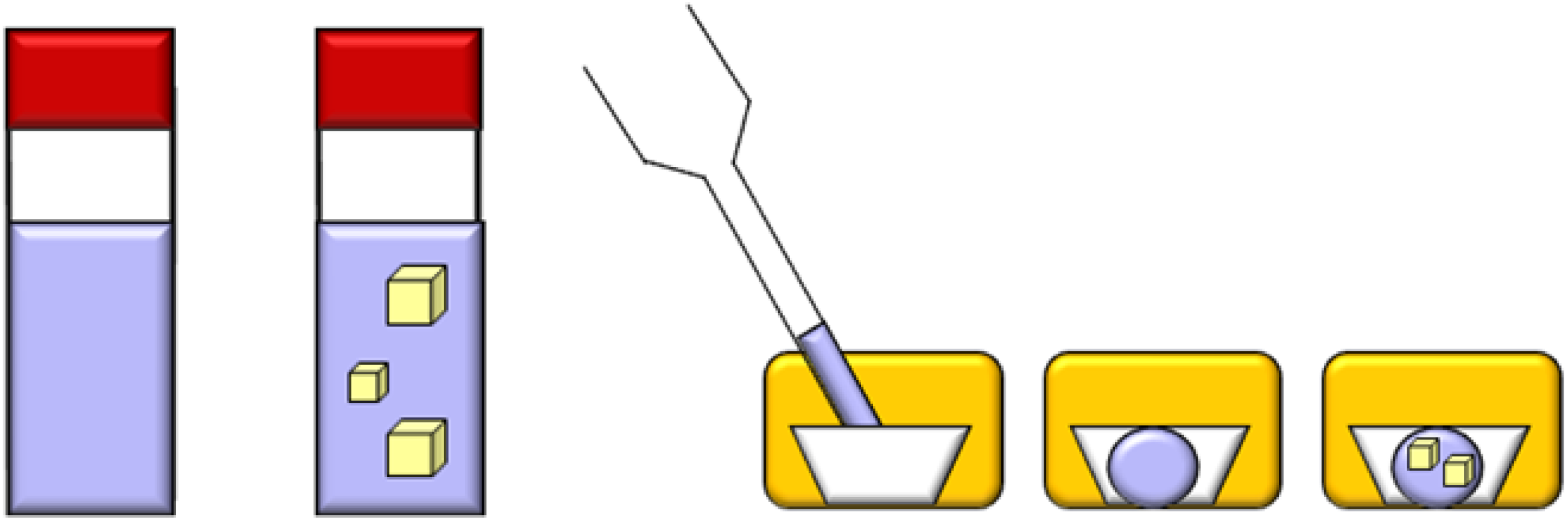



1. Expression & purification
2. Grid preparation
3. cryo-EM data
4. Reconstruction

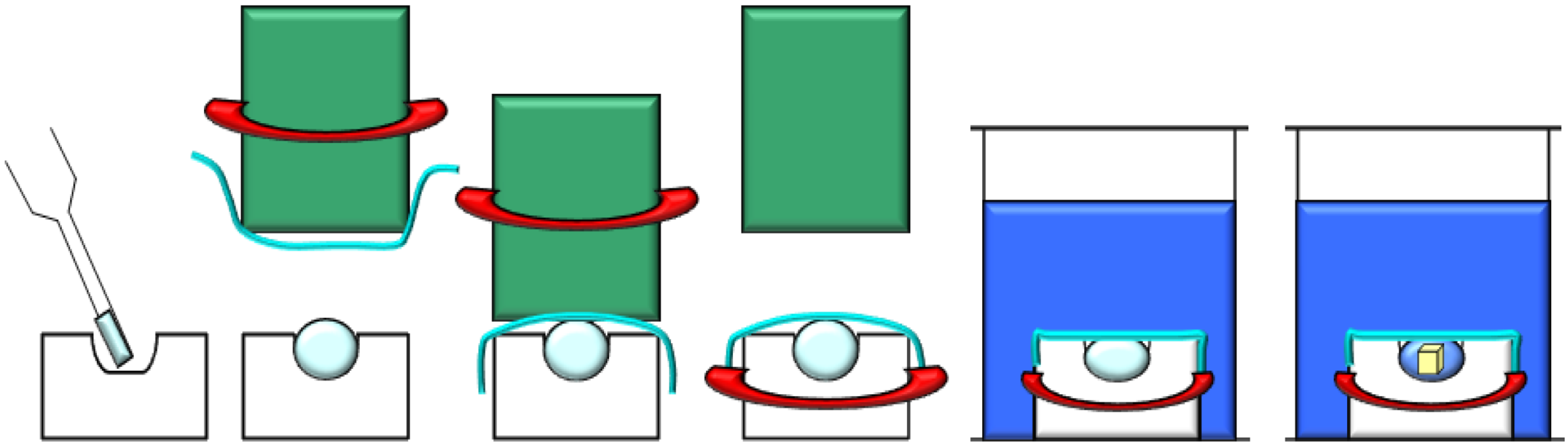
Vapor-diffusion



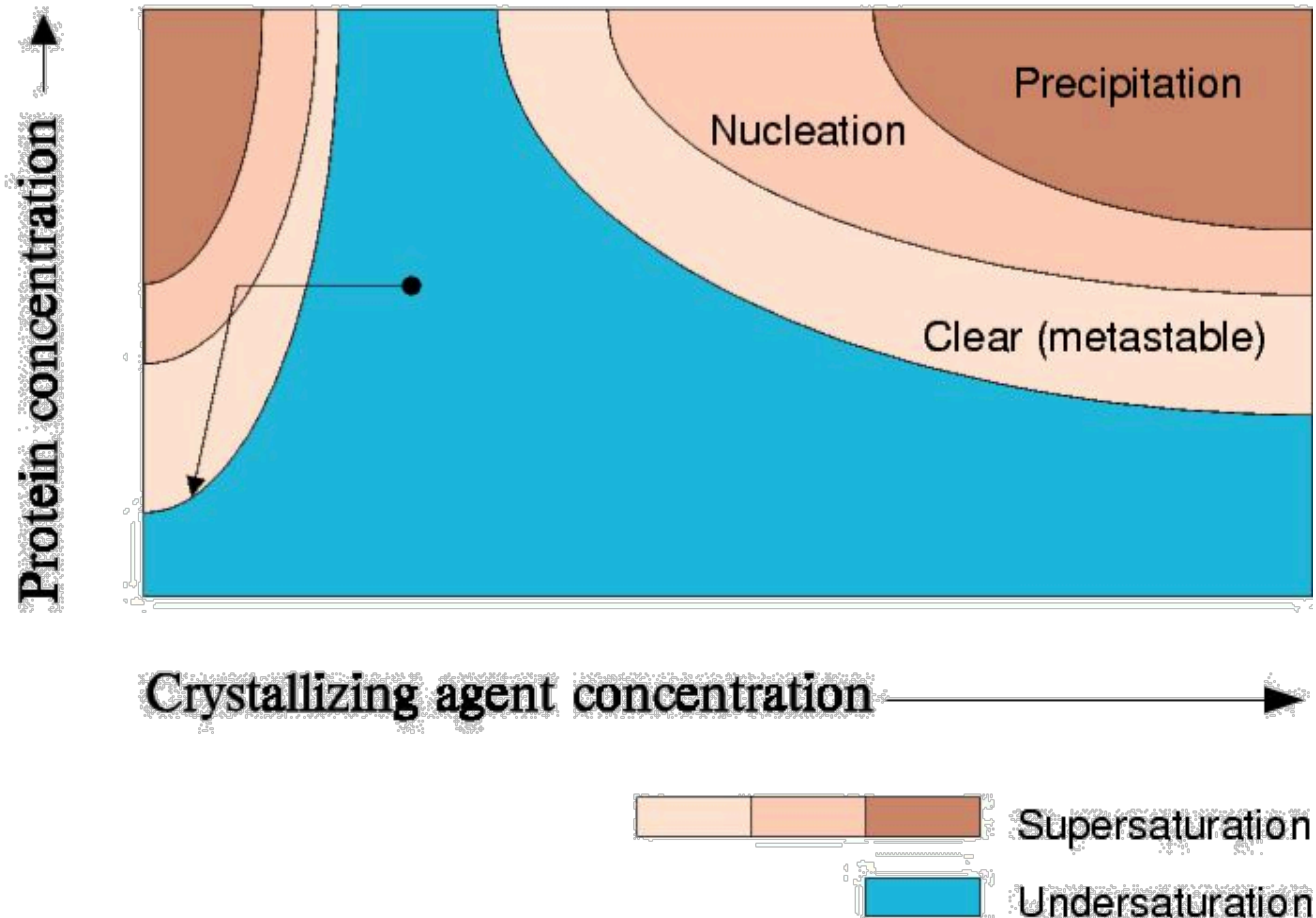
Batch and microbatch

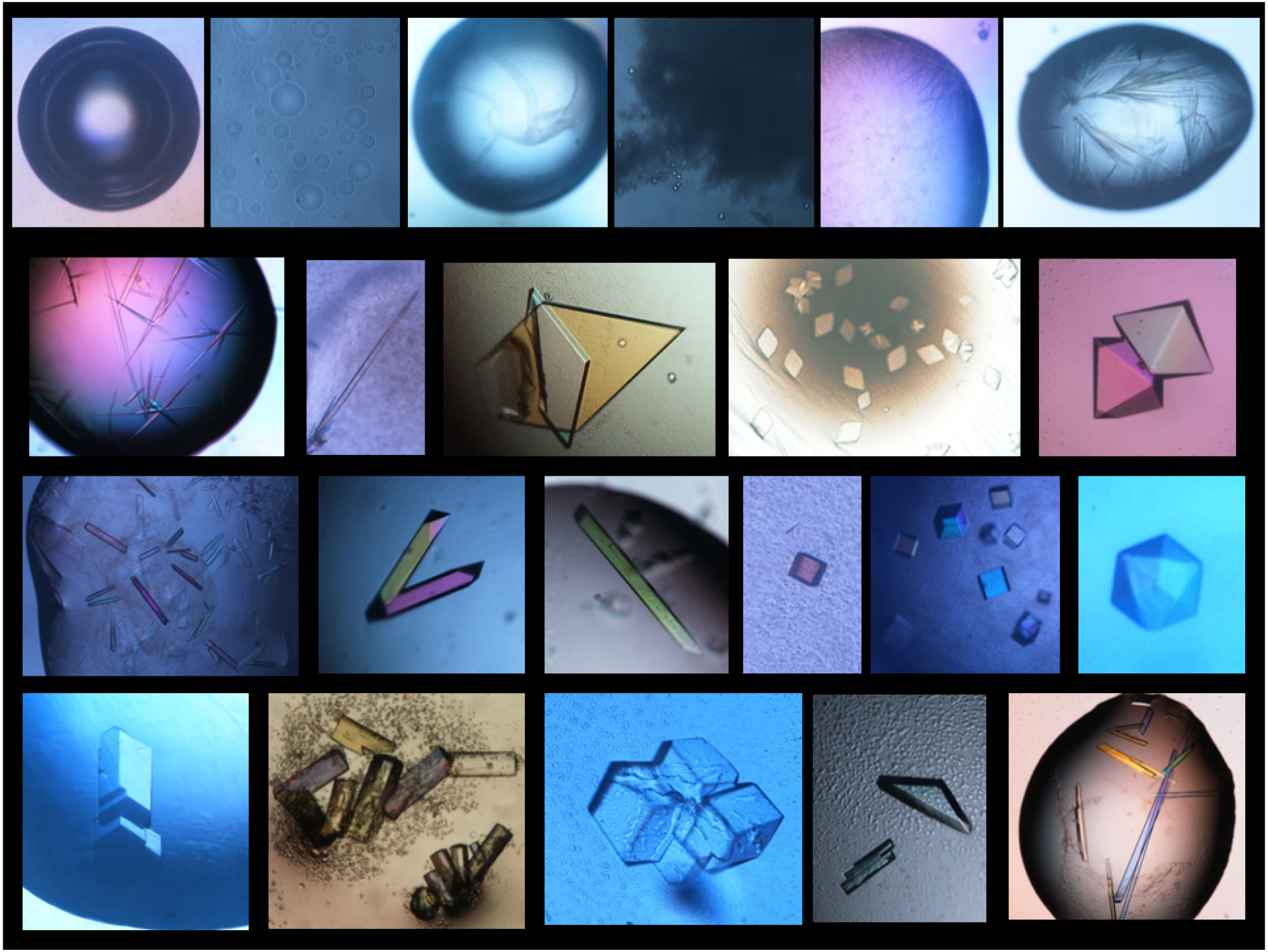


Microdialysis

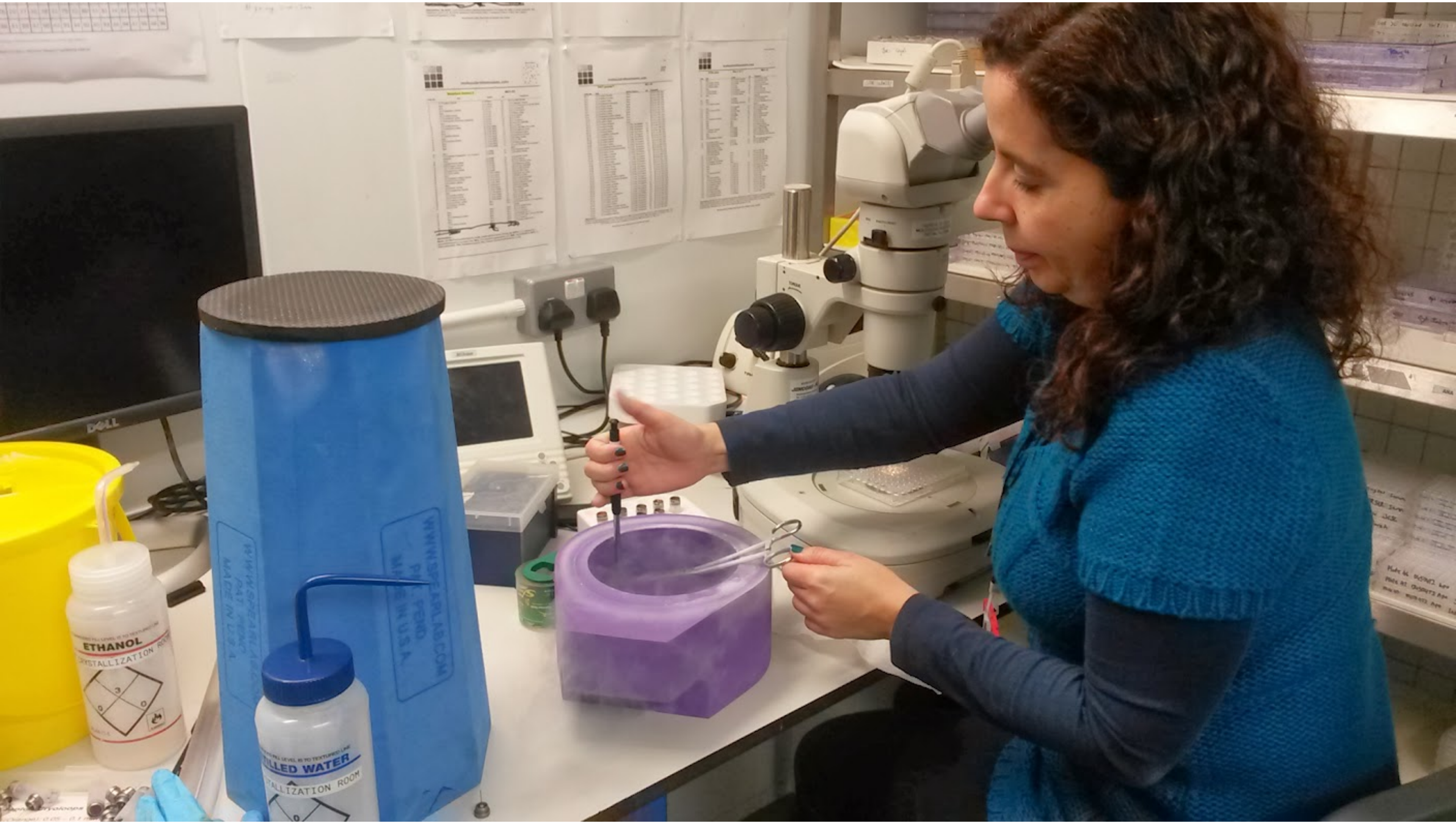


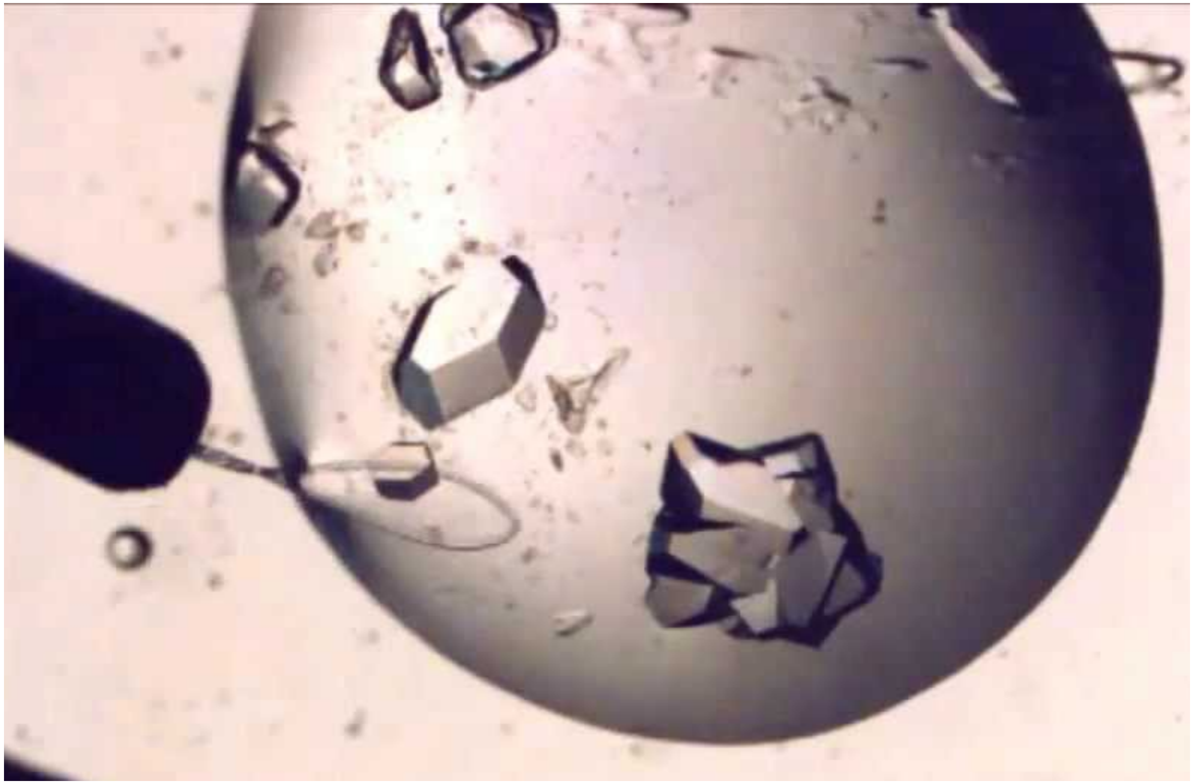
Protein crystallization phase diagram



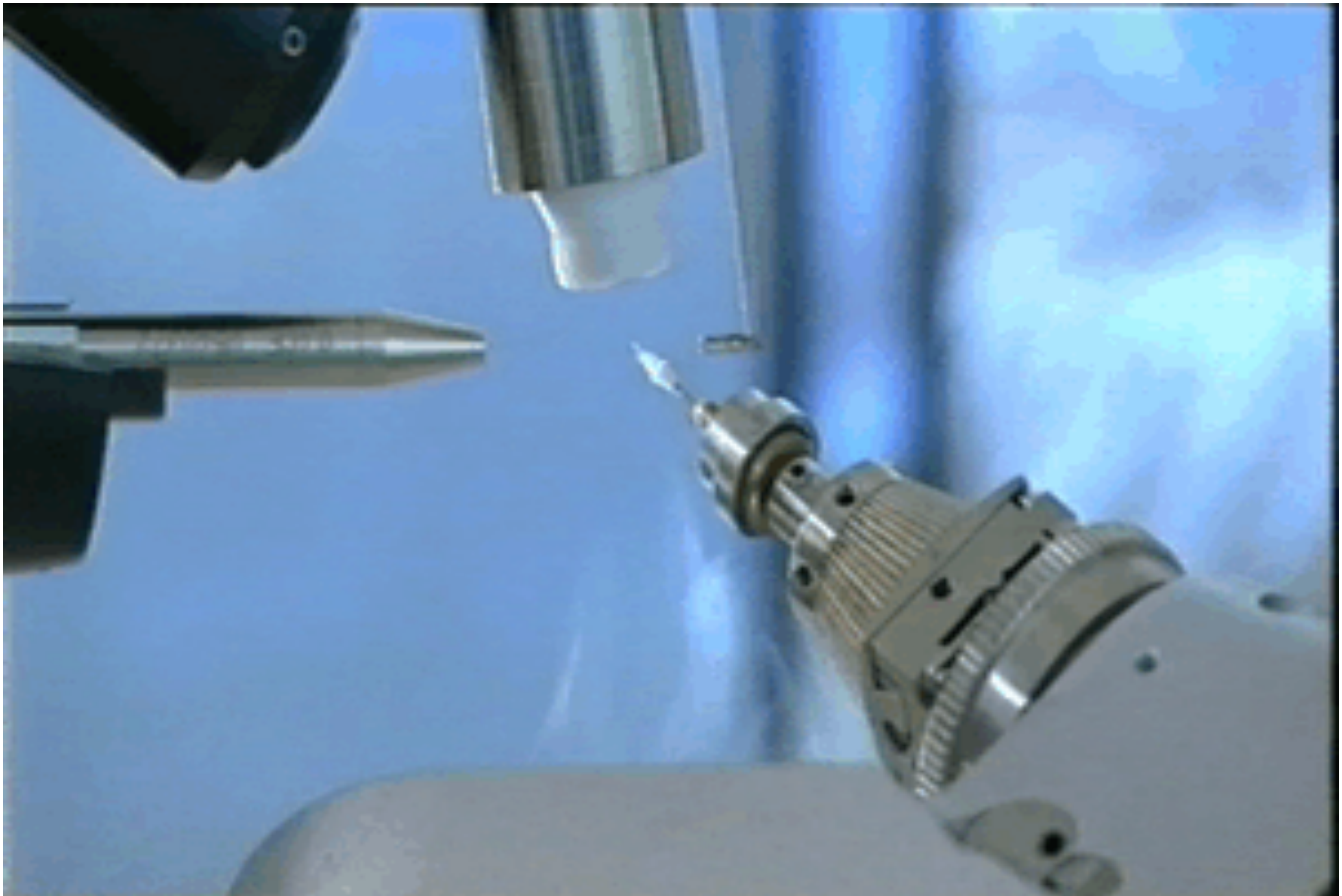


Preparing crystals for diffraction experiment

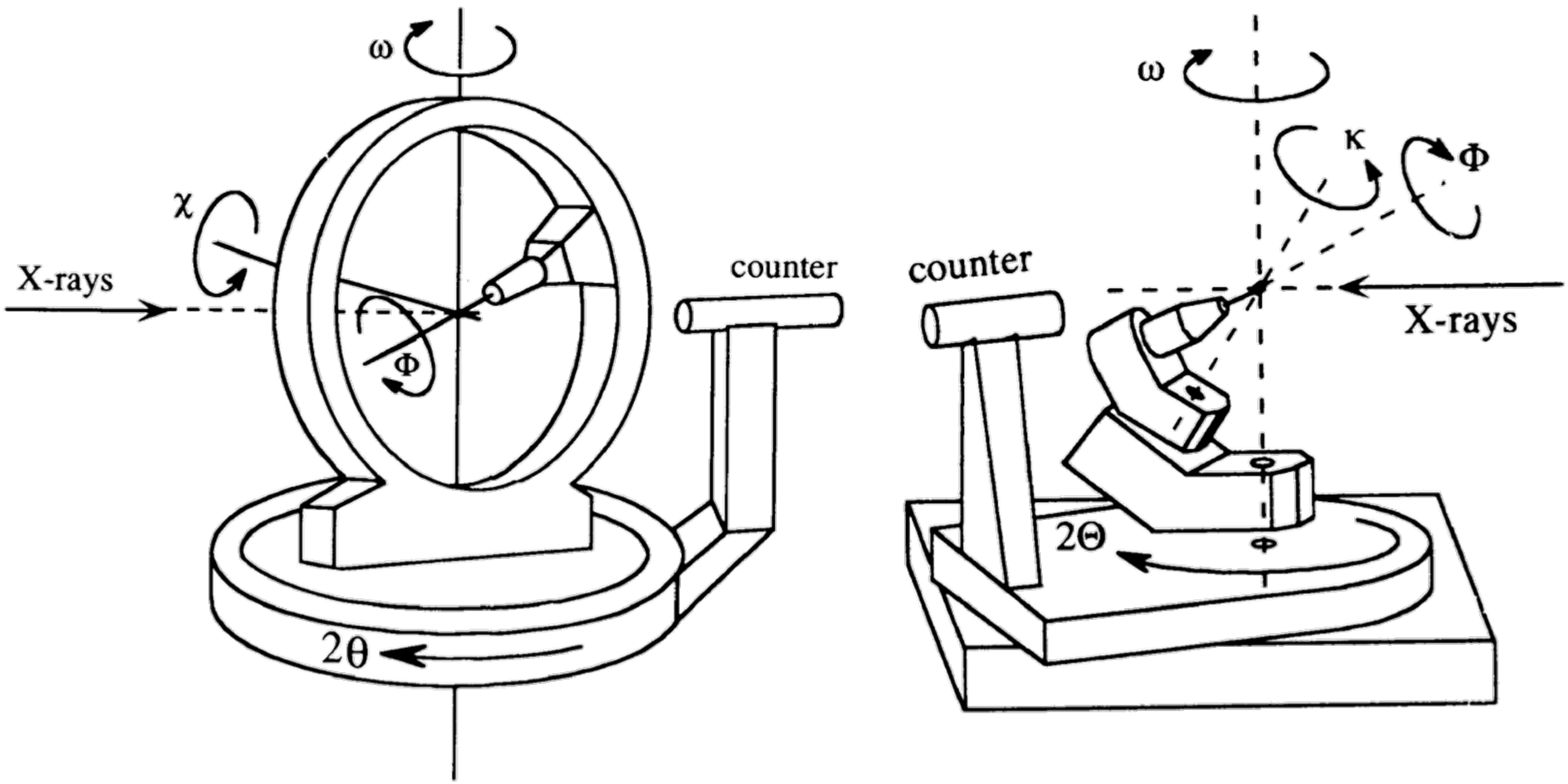




Diffractometer with goniometer



Diffractometer with goniometer

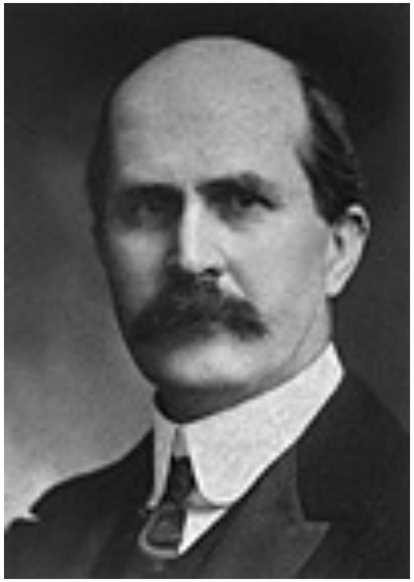


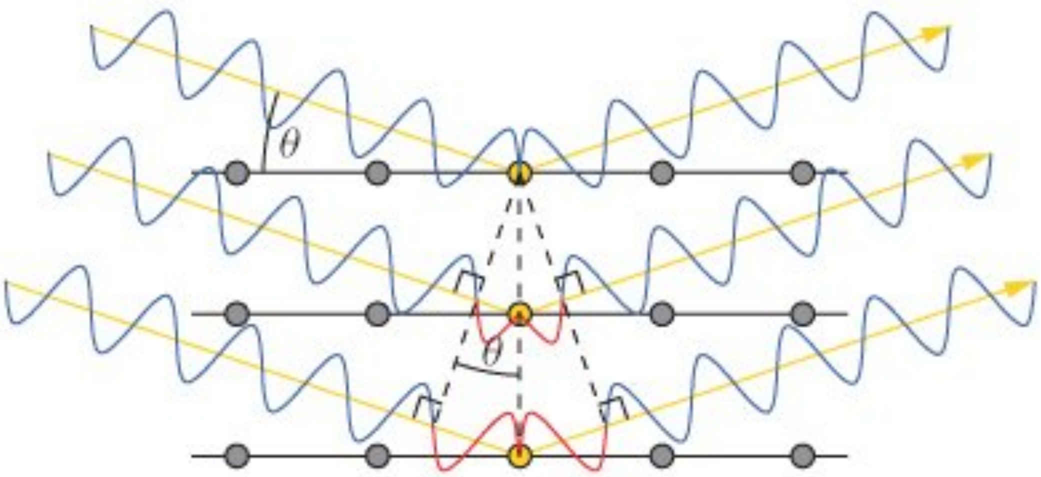
SIR WILLIAM HENRY BRAGG (1862-1942)
SIR WILLIAM LAWRENCE BRAGG (1890-1971)

- **1915 Nobel Laureates in Physics**

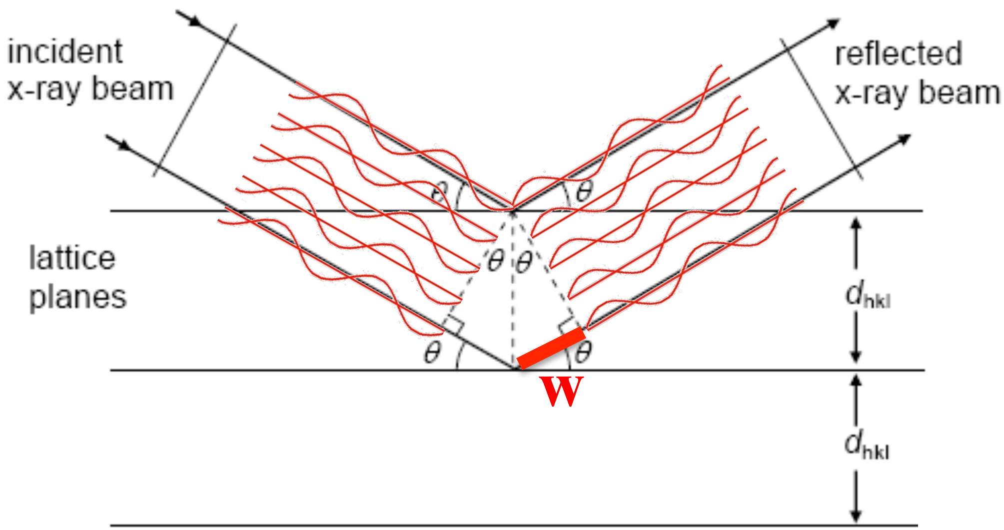
for the analysis of crystal structure by means of X-rays

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



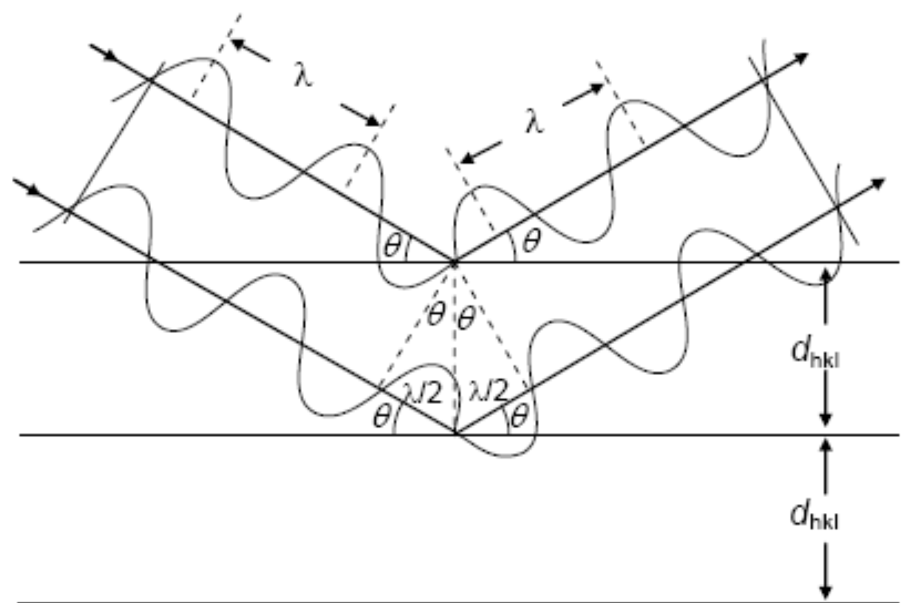


There is NO PHASE DIFFERENCE if the path differences are equal to whole number multiplies of wavelength (λ)



Bragg's law:

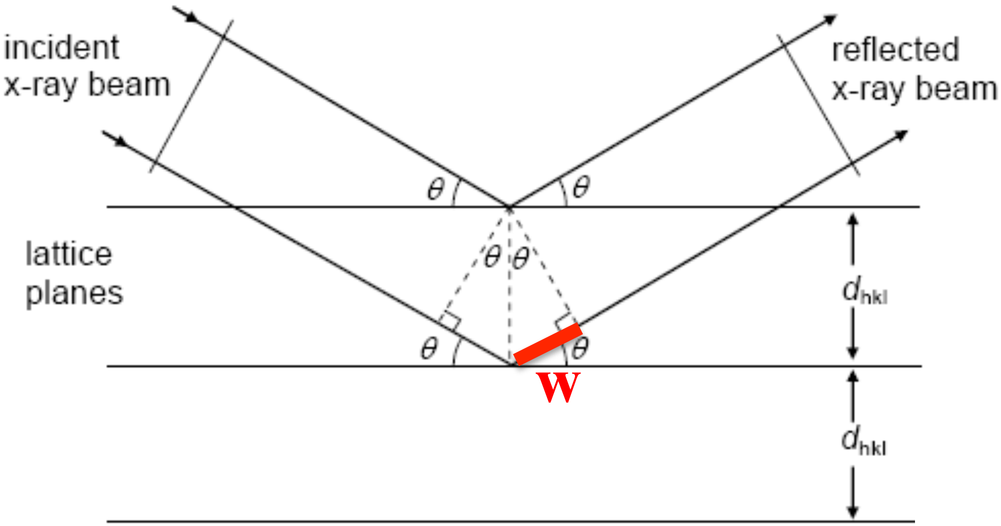
$$n\lambda = 2d\sin\theta$$



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

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

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

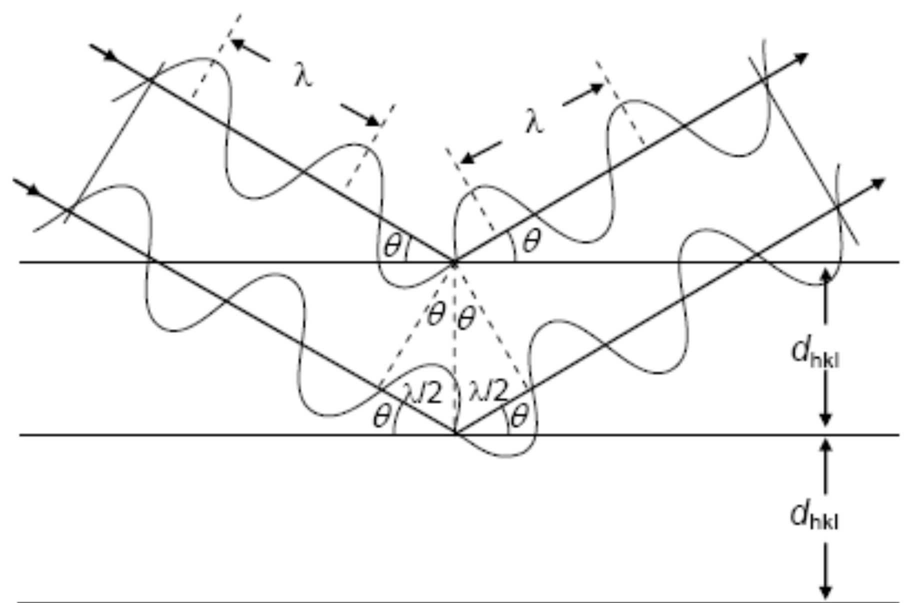


$\sin\theta$ = **w**/d

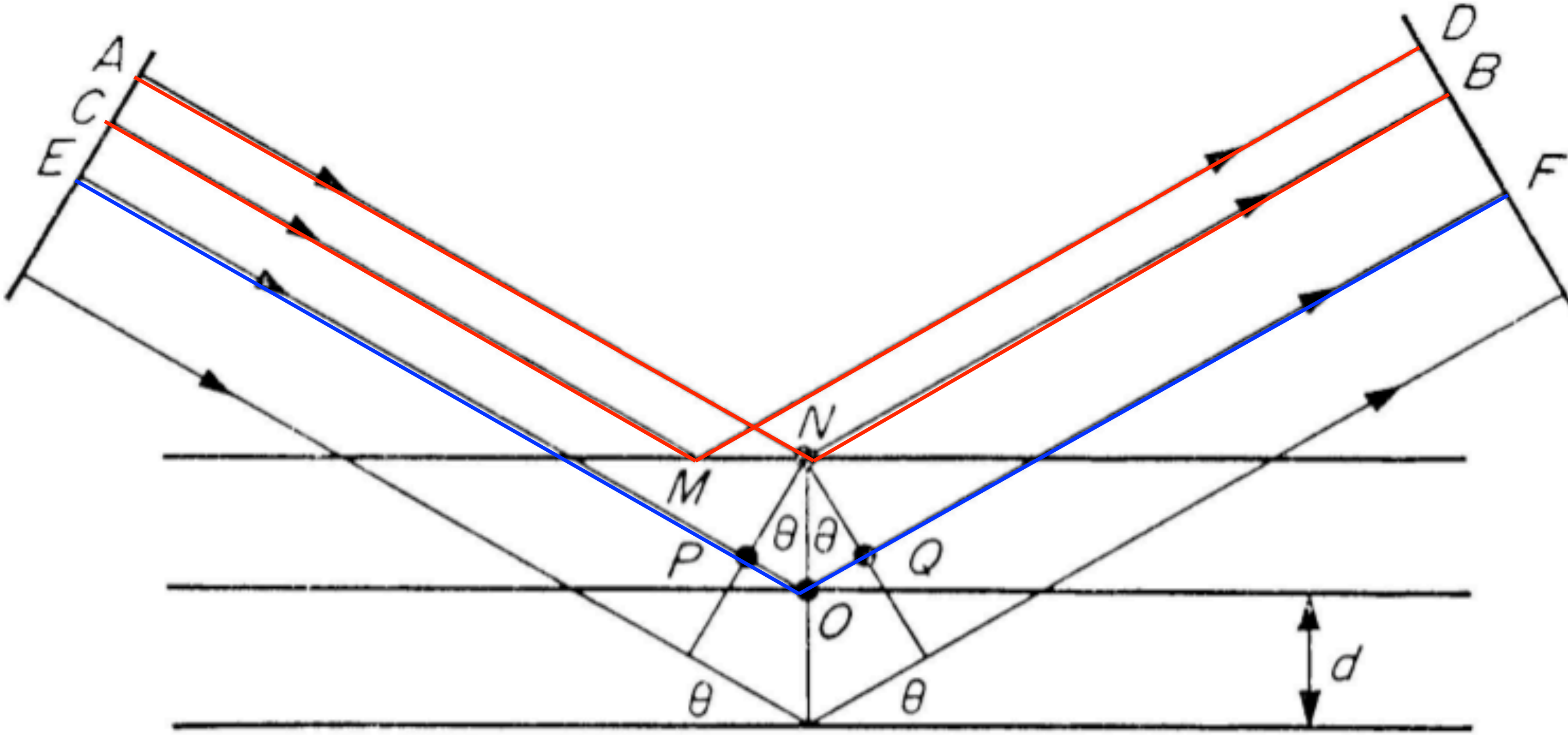
2**w** = $n\lambda$

Bragg's law:

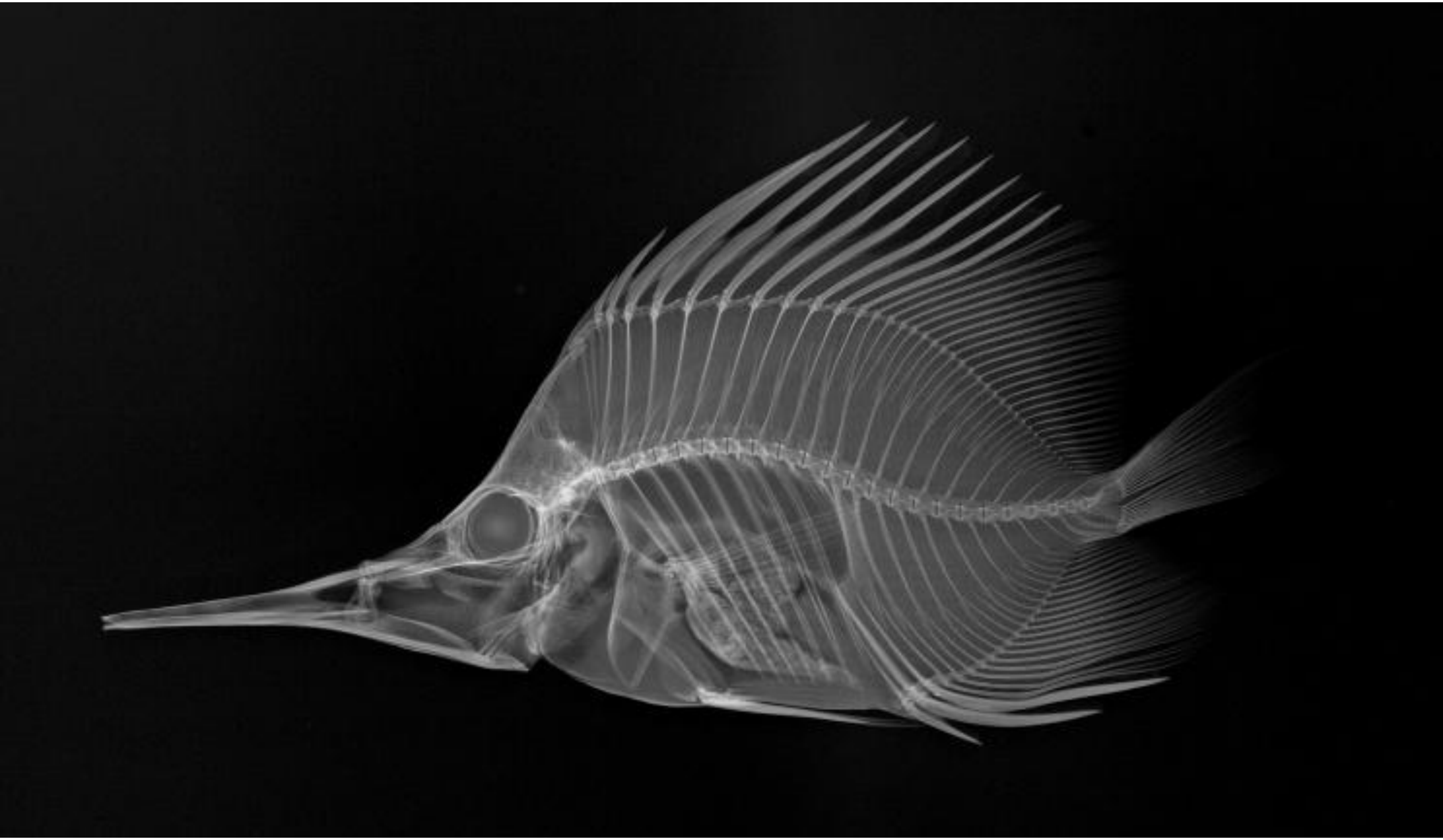
$$n\lambda = 2d \sin\theta$$



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

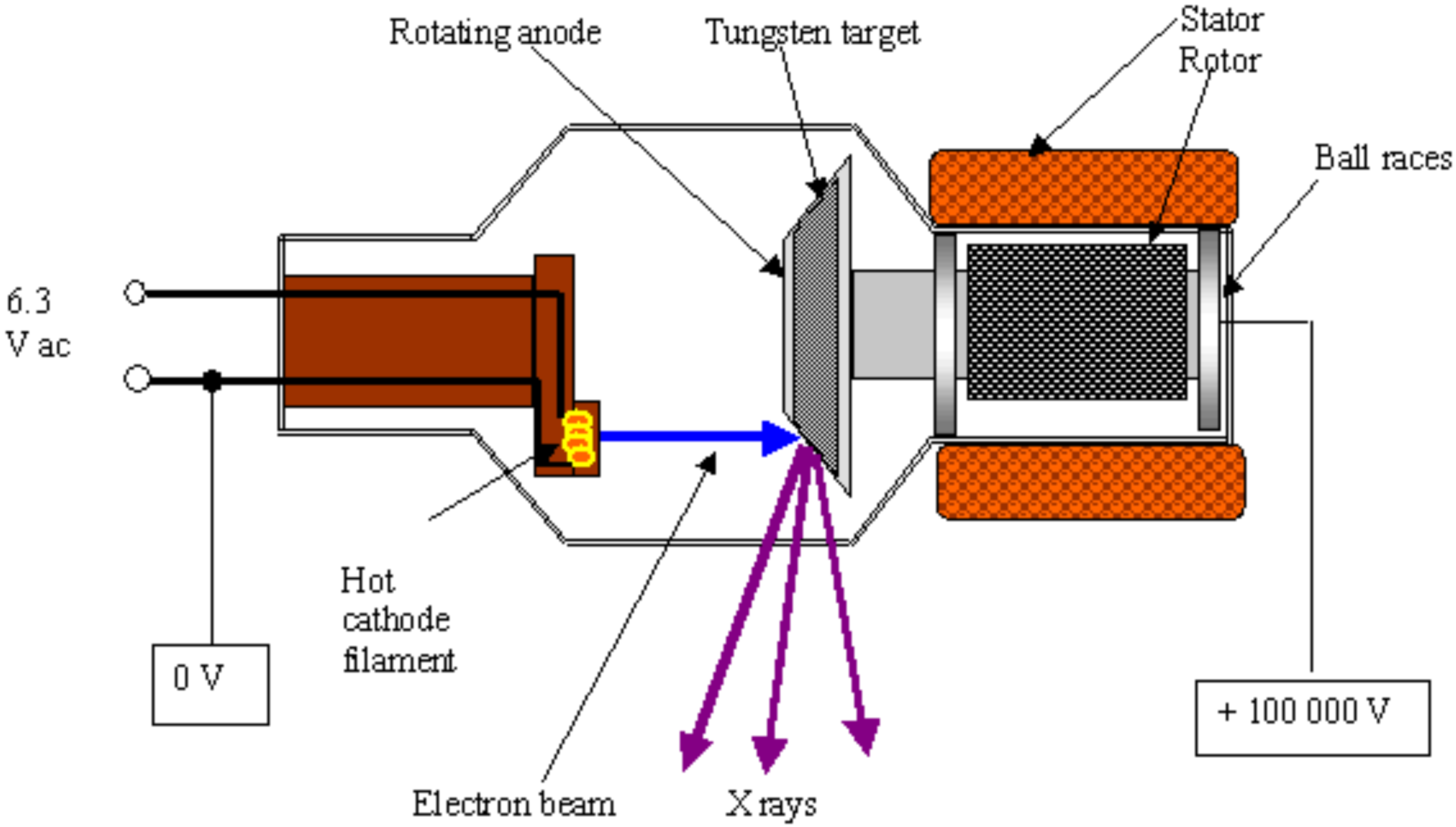


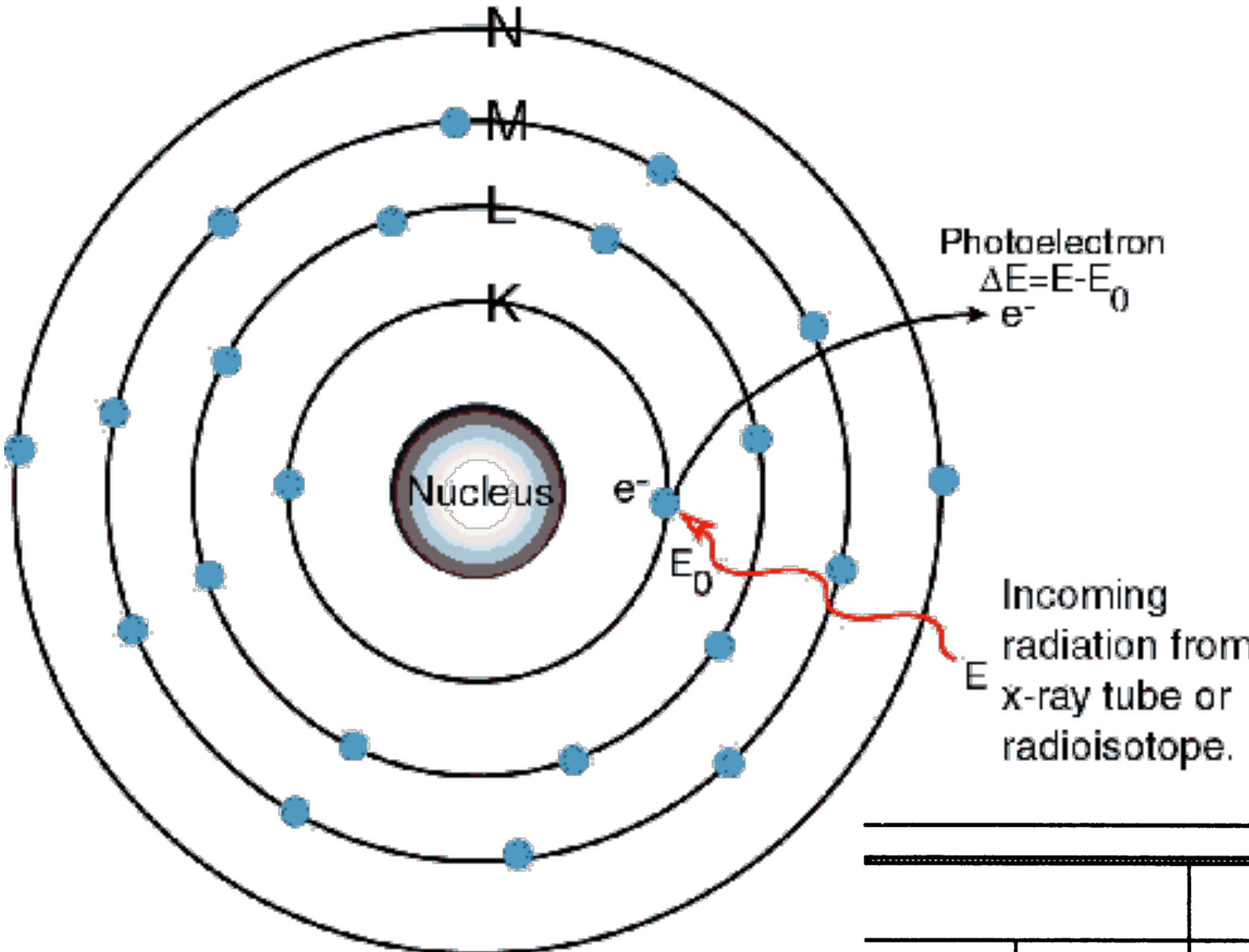
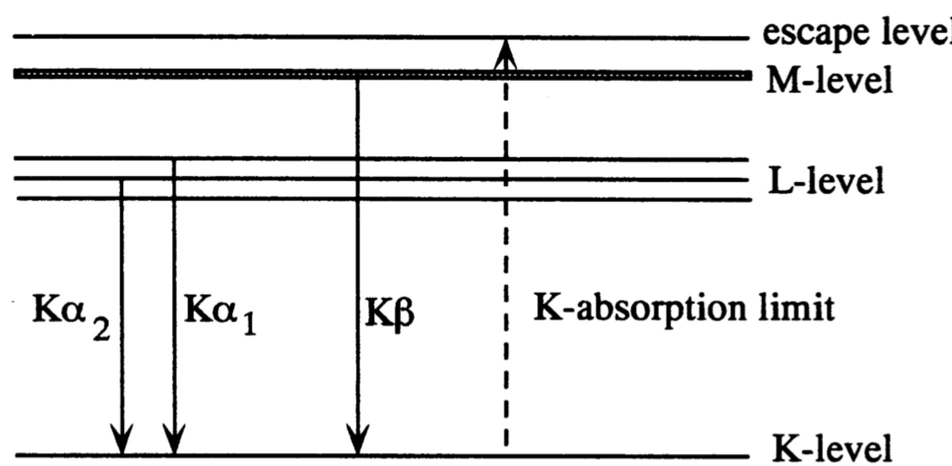
X-ray sources and detectors



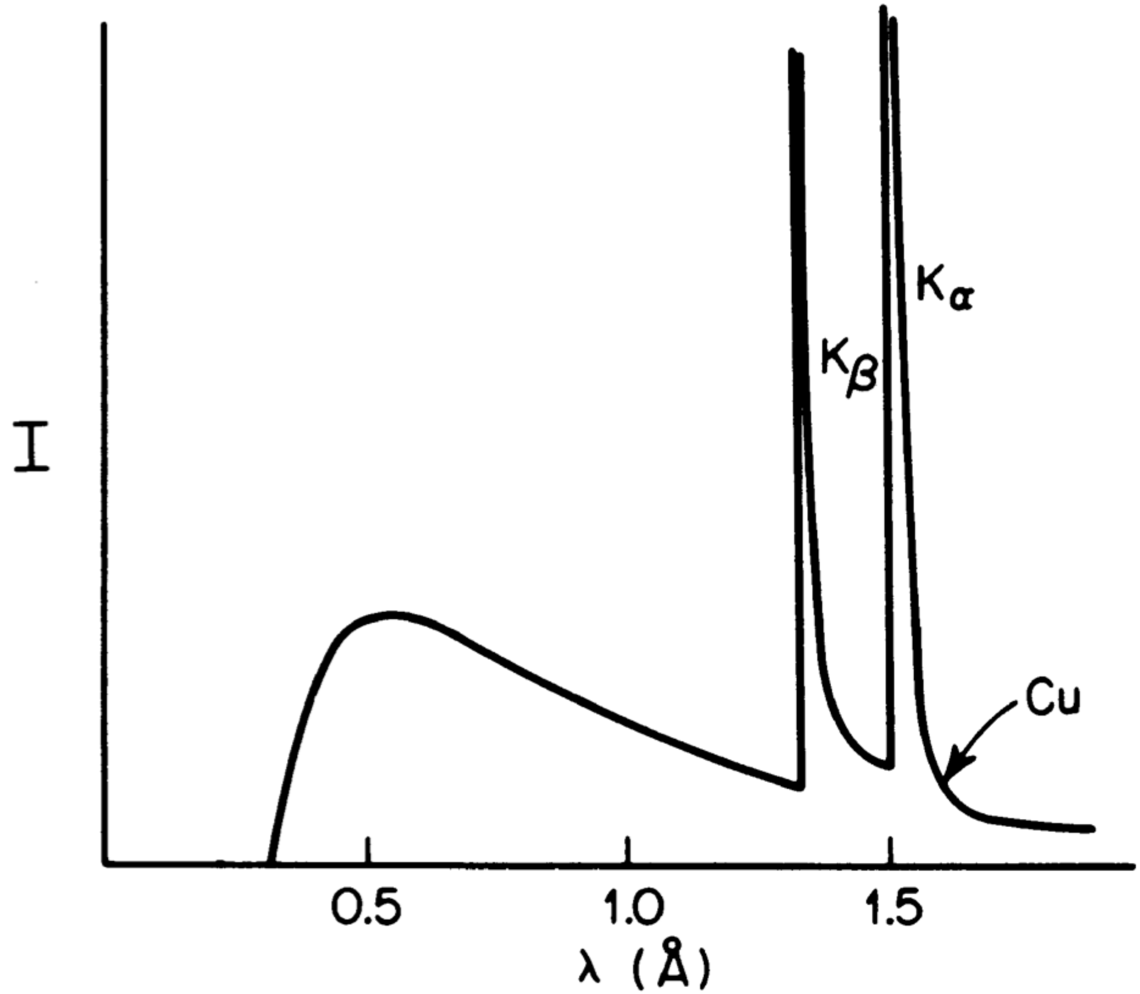
X-ray sources

- sealed X-ray tubes
- synchrotrons




N
M
L
K
Nucleus
e⁻
E_0
Photoelectron
$\Delta E = E - E_0$
e⁻
E
Incoming radiation from x-ray tube or radioisotope.

escape level
M-level
L-level
$K\alpha_2$
$K\alpha_1$
$K\beta$
K-absorption limit
K-level

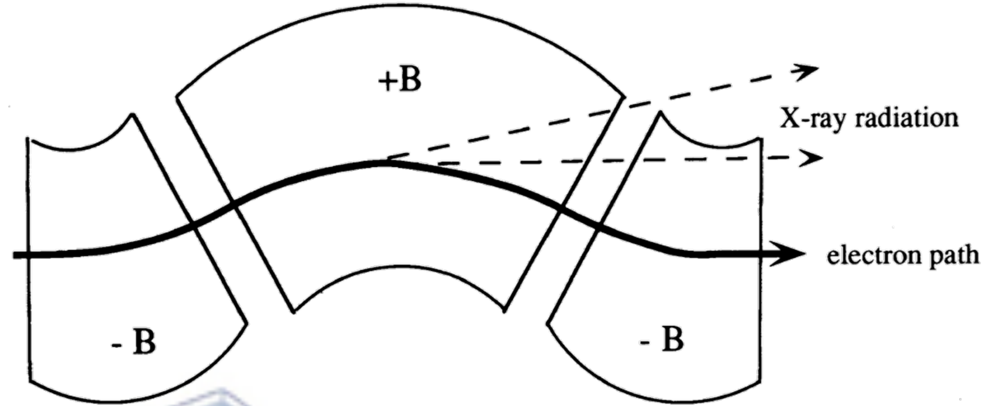
Spectrum of copper anode

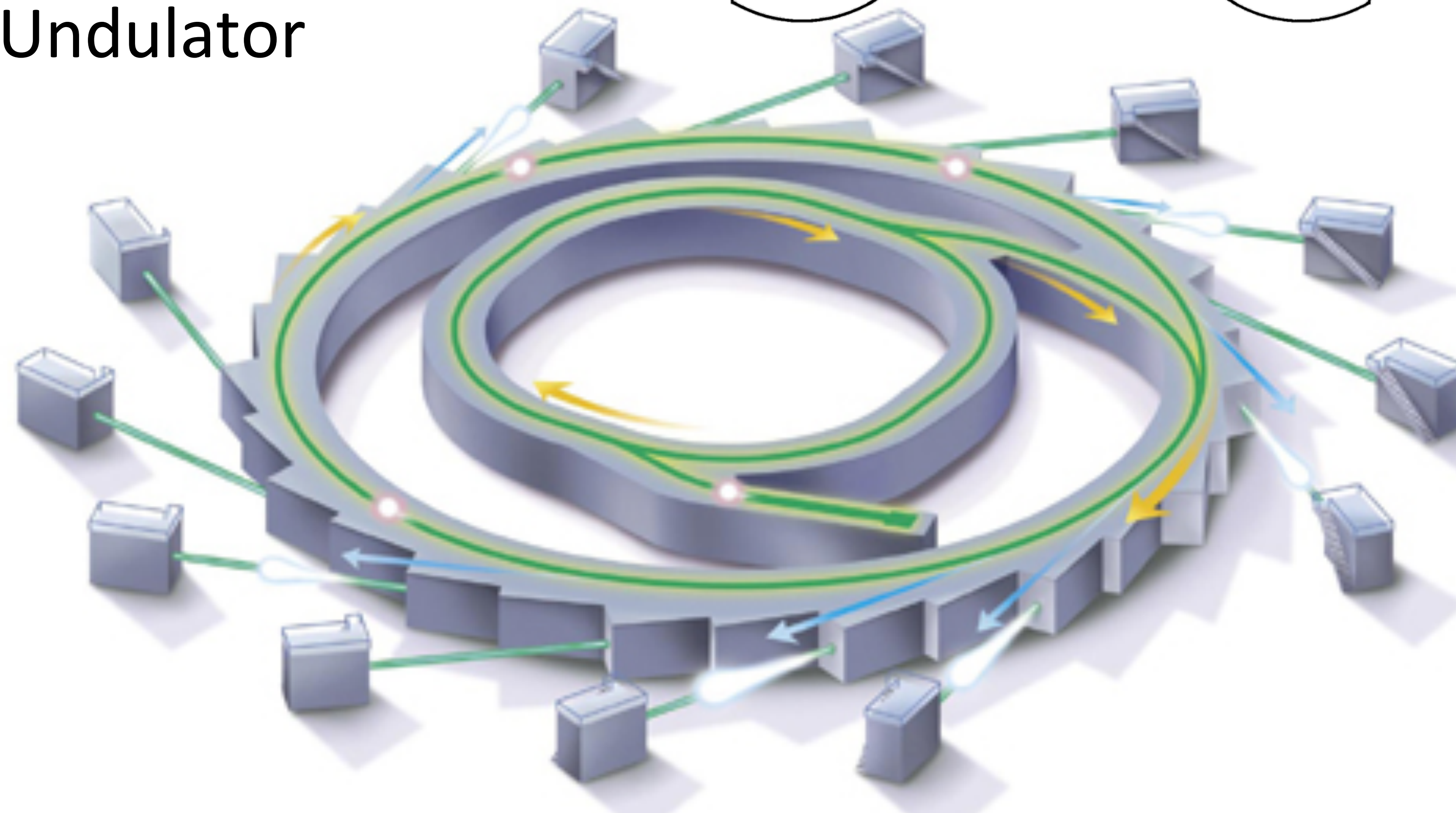


	λ (Å)	
$K_{\alpha}(1)$	1.54051	The weight average value for $K_{\alpha}(1)$ and $K_{\alpha}(2)$ is taken as 1.54178 Å
$K_{\alpha}(2)$	1.54433	because the intensity of $K_{\alpha}(1)$ is twice that of $K_{\alpha}(2)$
K_{β}	1.39217	

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

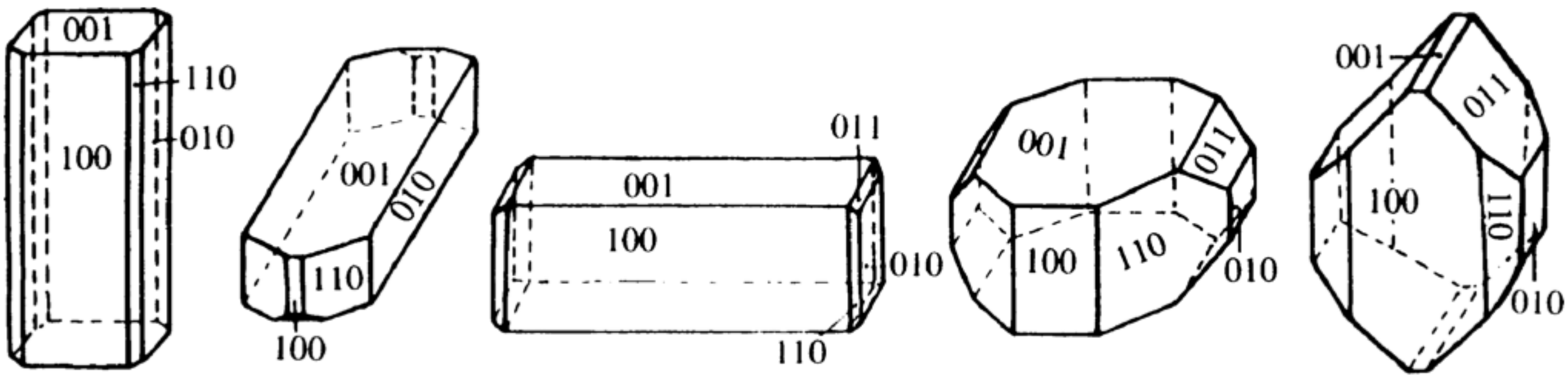


Figure 3.1. Crystals of trimethylammonium bromide belonging to the same crystal form but exhibiting a range of morphologies.

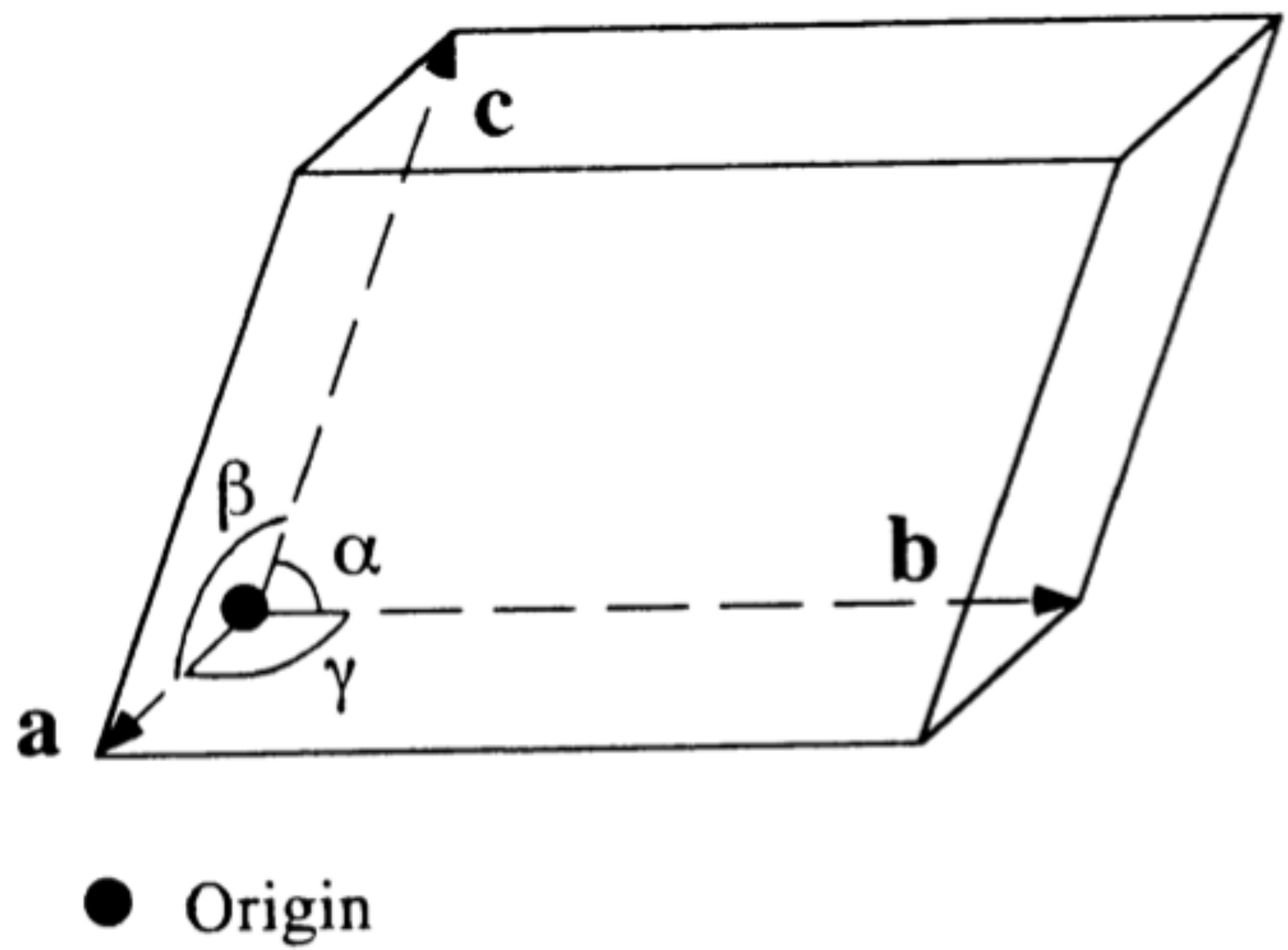


Figure 3.3. One unit cell in the crystal lattice.

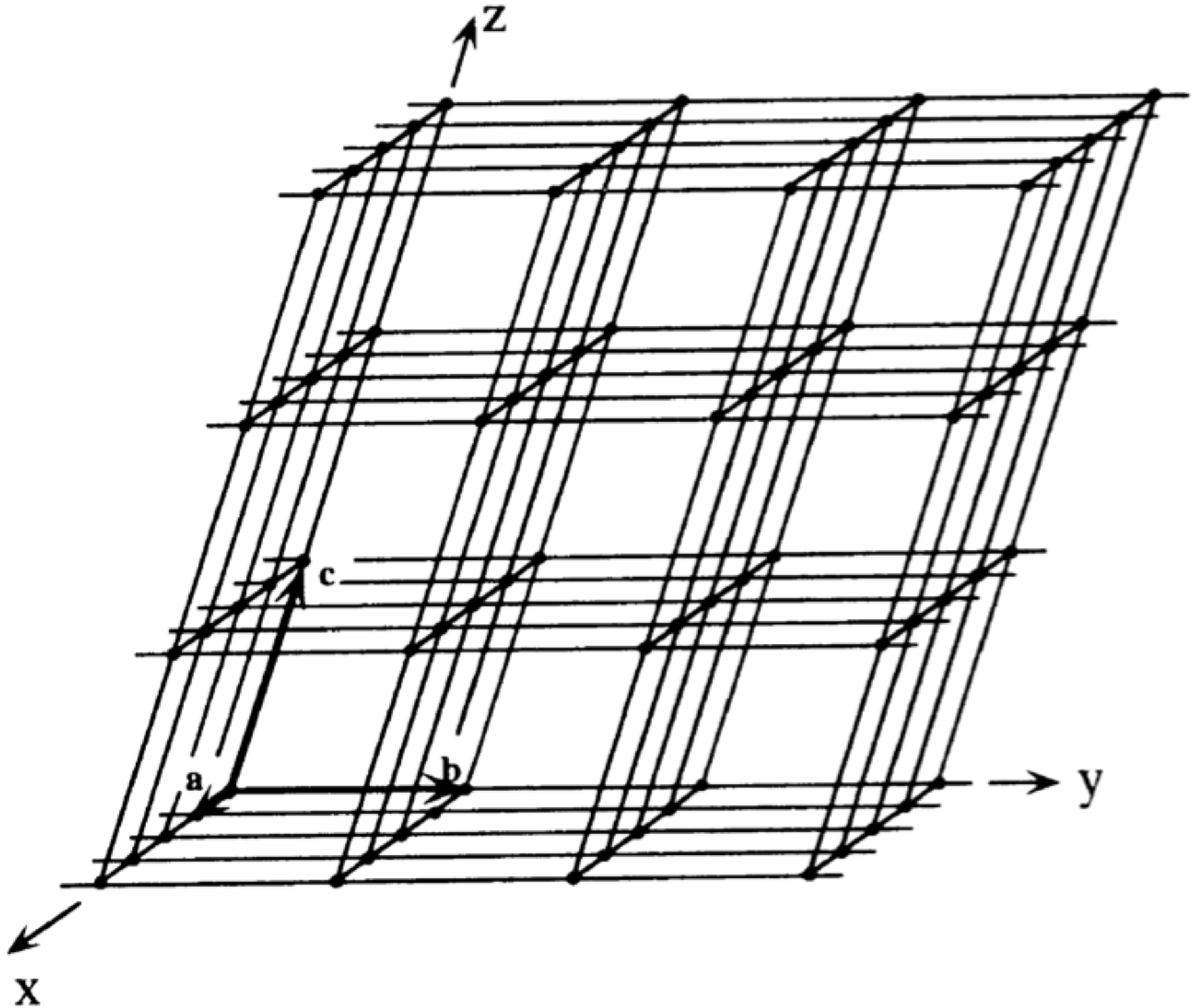
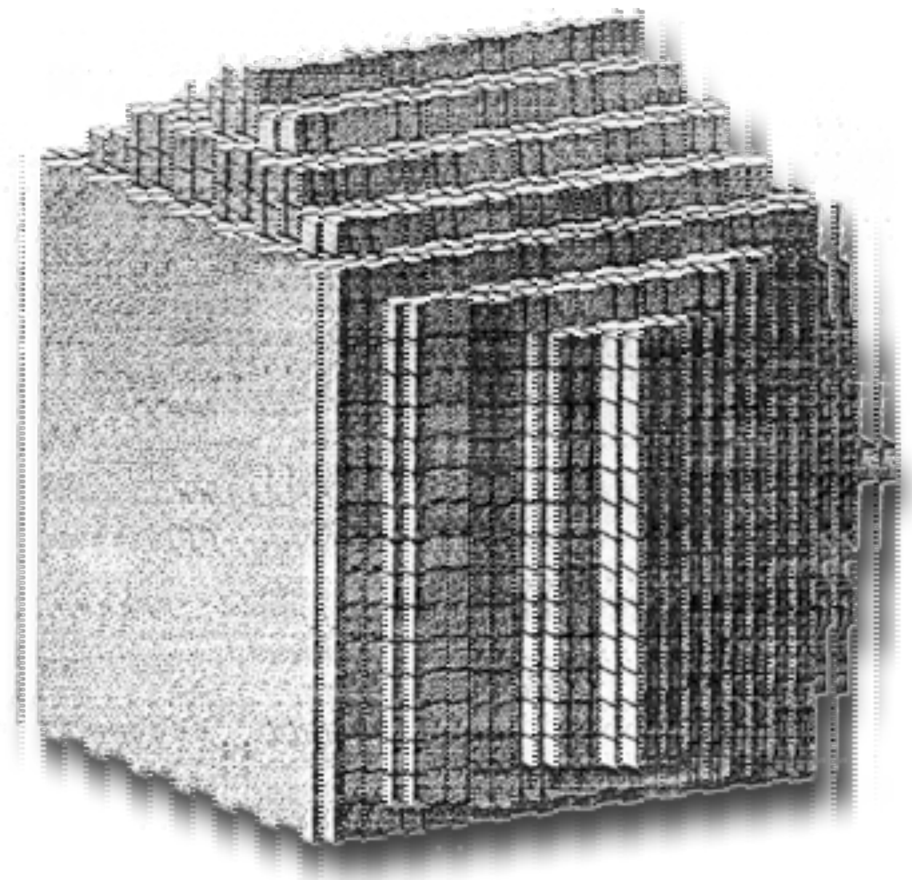


Figure 3.4. A crystal lattice is a three-dimensional stack of unit cells.

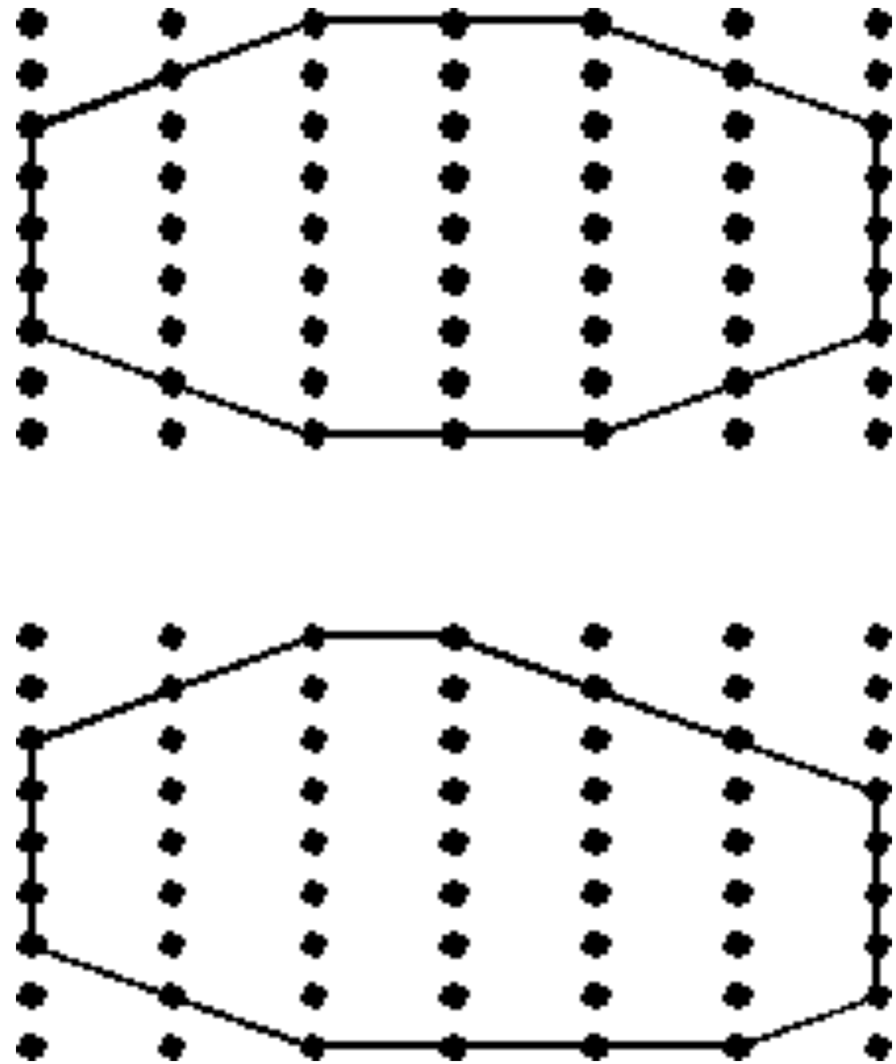
“Law of Constancy of Angles”

René Just Haüy (1743-1822)



"Law of Constancy of Angles"

René Just Haüy (1743-1822)



Make a drawing – sub divisions for different angles

Lattice planes and Indices

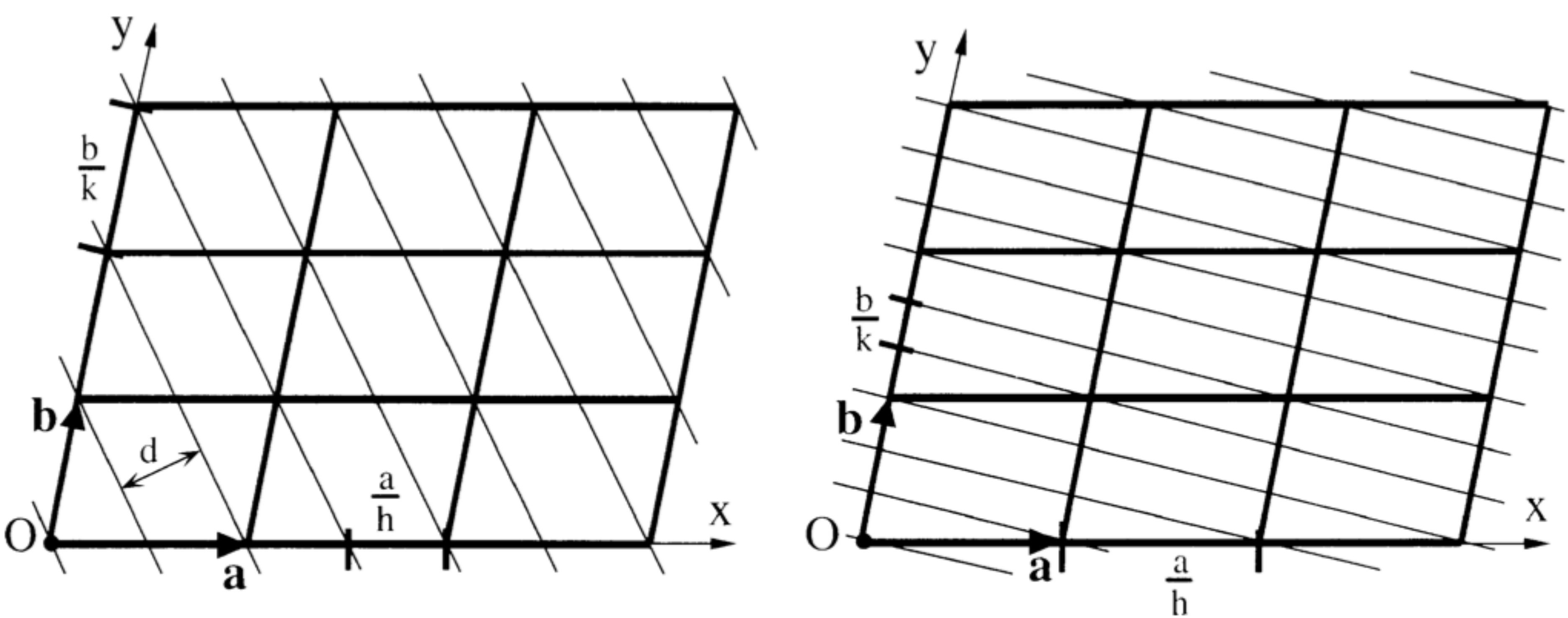
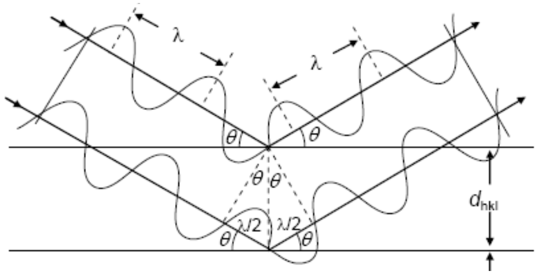
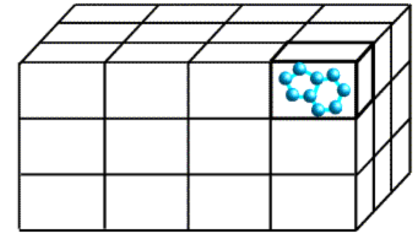
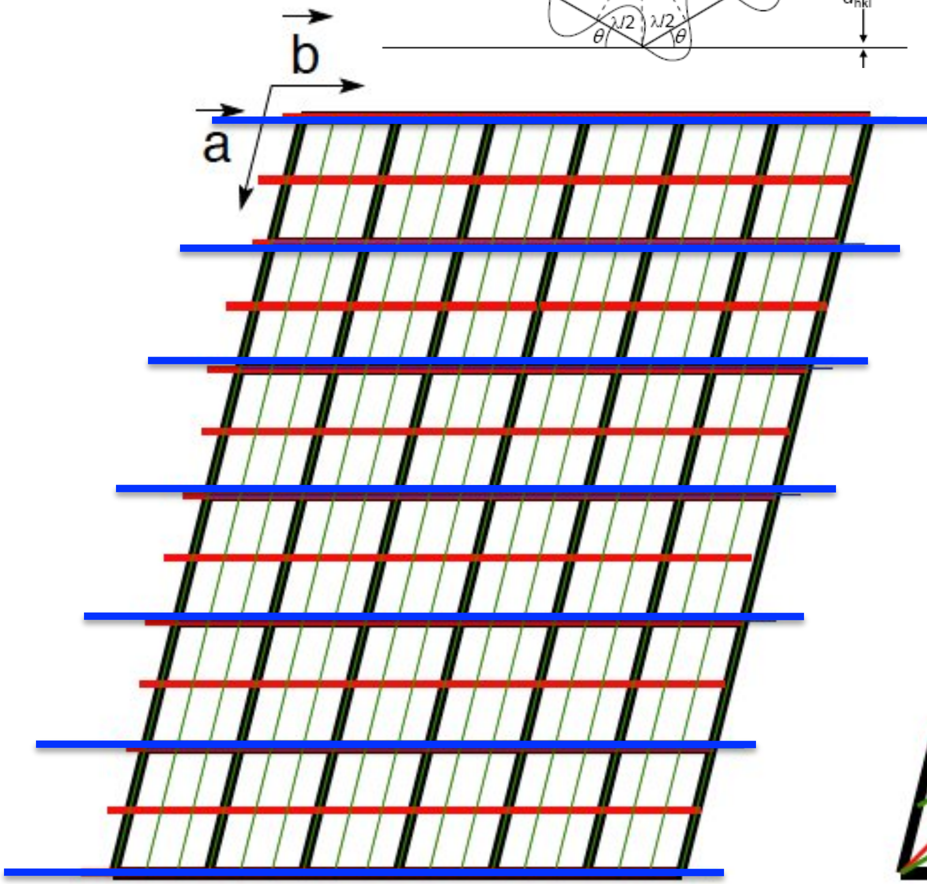


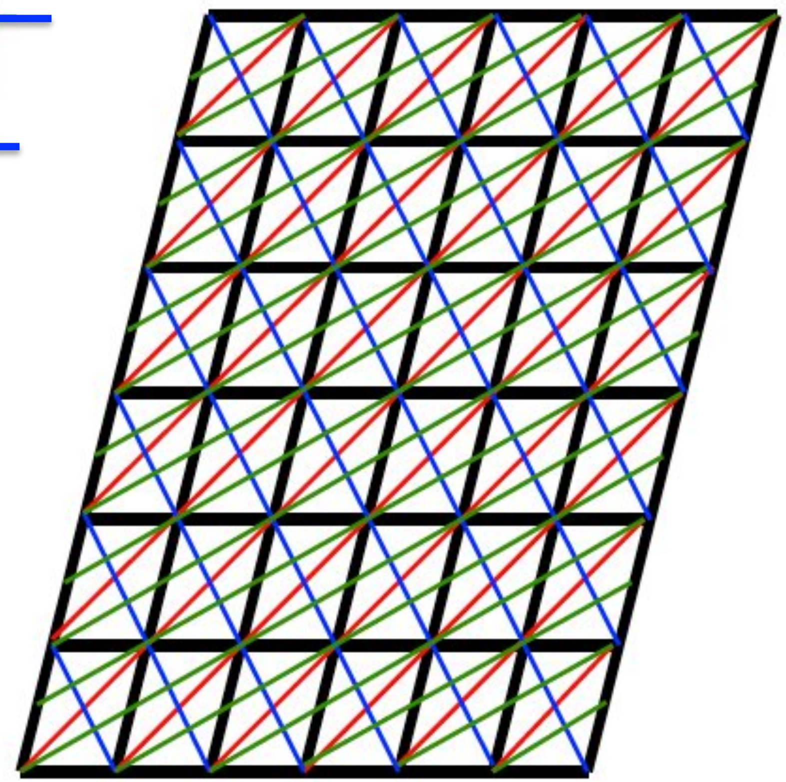
Figure 3.5. Lattice planes in a two-dimensional lattice. On the left, $h = 2$ and $k = 1$; on the right, $h = 1$ and $k = 3$.

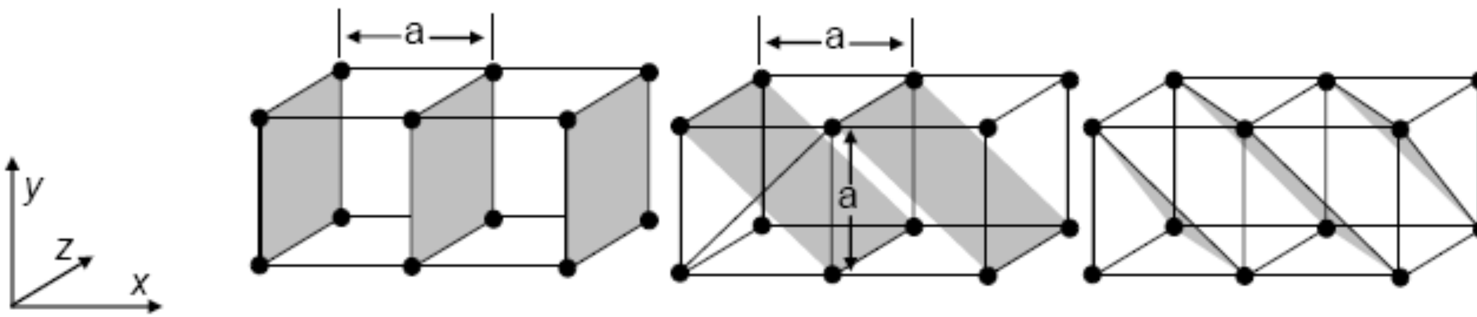
Lattice planes distance d











(h, k, l)

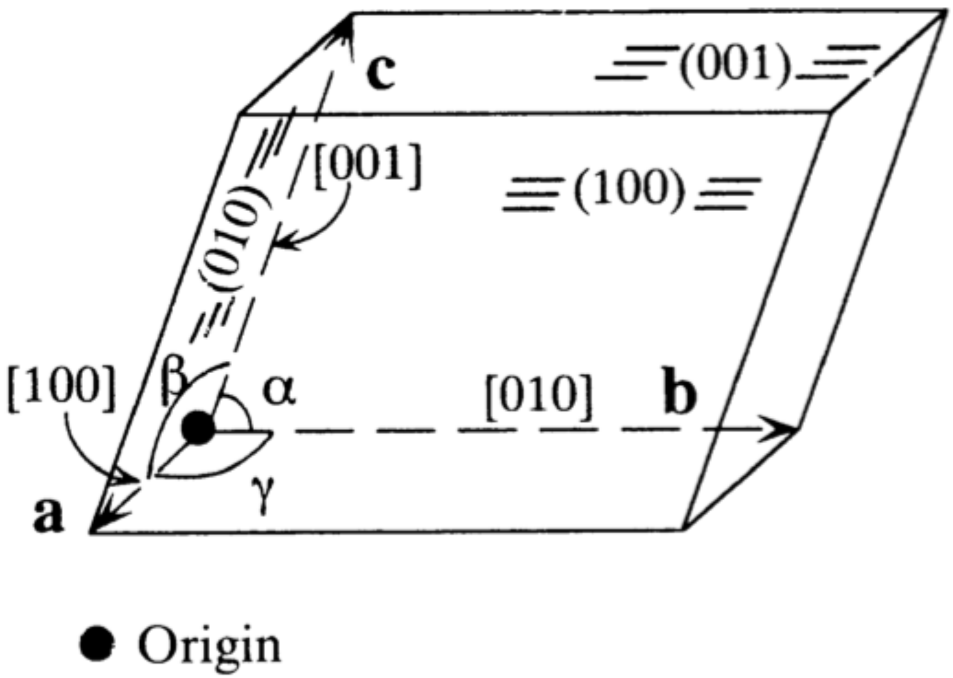


Figure 3.6. One unit cell bounded by the planes (100), (010), and (001). The directions along **a**, **b**, and **c** are indicated by [100], [010], and [001], respectively.

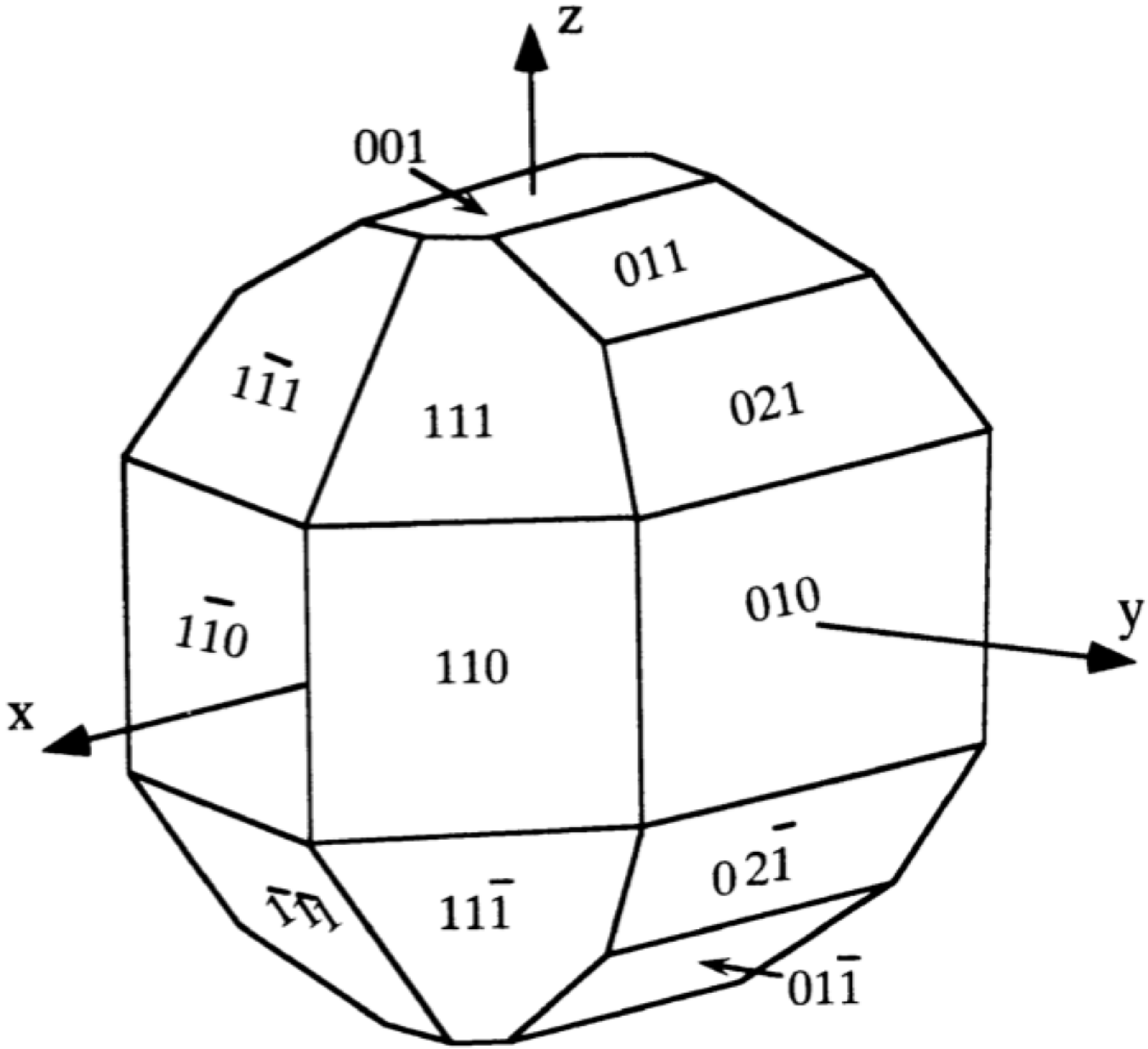
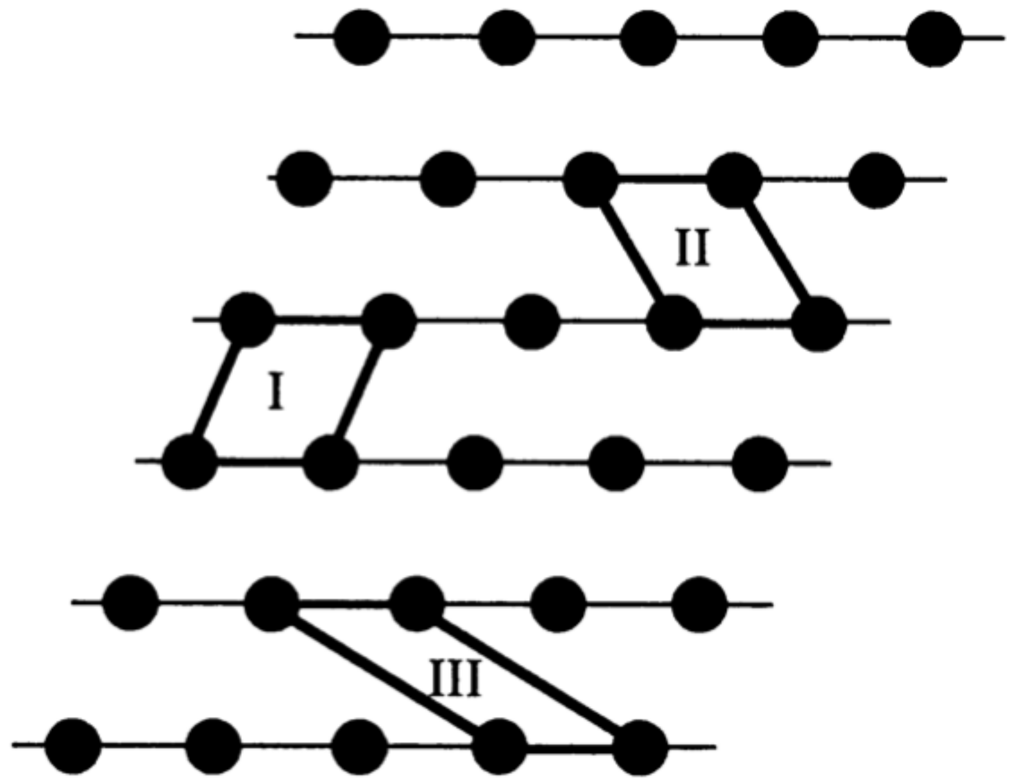


Figure 3.7. A crystal showing several faces.

Unit cell choice / selection

Figure 3.8. In this two-dimensional lattice, the unit cell can be chosen in different ways: as I, as II, or as III.



GEOMETRY OF CRYSTALS

- Space Lattices
- Crystal Structures
- Symmetry, Point Groups and Space Groups

Acknowledgments: Prof. Rajesh Prasad *for a lot of things*

The language of crystallography is one succinctness

Crystal = Lattice + Motif

Motif or basis:

an atom or a group of atoms associated with each lattice point

Space Lattice

An array of points such that every point has ***identical surroundings***

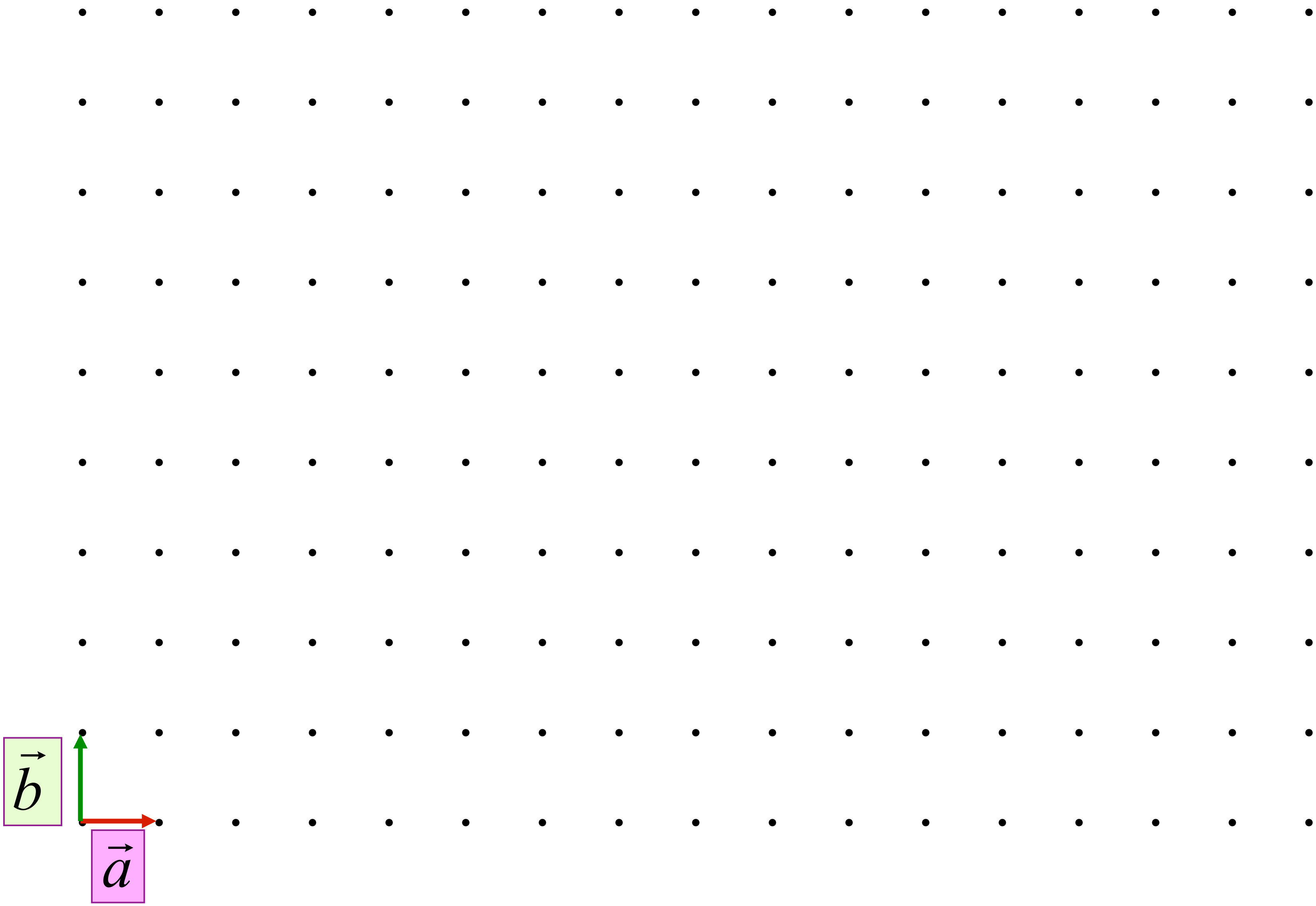
- **In Euclidean space $\Rightarrow$ infinite array**

- **We can have 1D, 2D or 3D arrays (lattices)**

or

Translationally periodic arrangement of points in space is called a lattice

A 2D lattice



Lattice

Translationally periodic arrangement of **points**

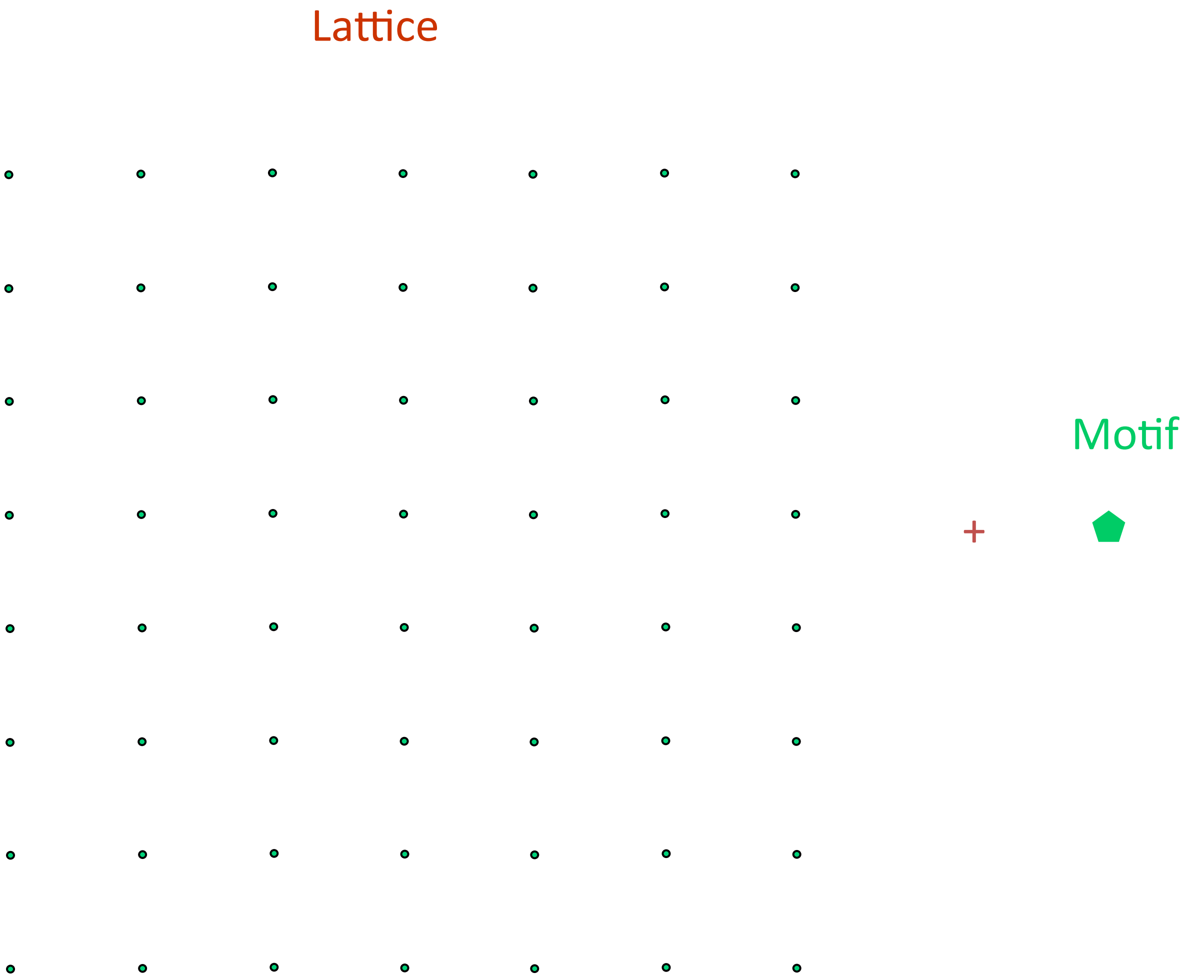
Crystal

Translationally periodic arrangement of **motifs**

Crystal = Lattice + Motif

- Lattice ➢ the underlying periodicity of the crystal
- Basis ➢ atom or group of atoms associated with each lattice points

- Lattice ➢ how to repeat
- Motif ➢ what to repeat


Lattice
+
Motif

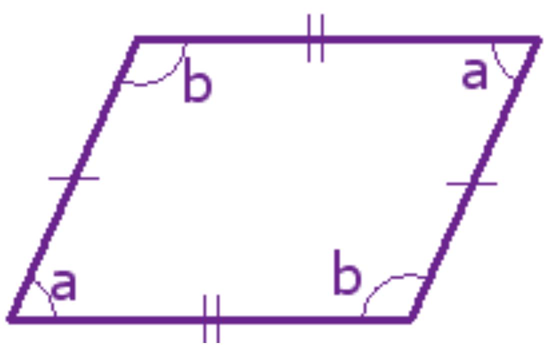
Crystal

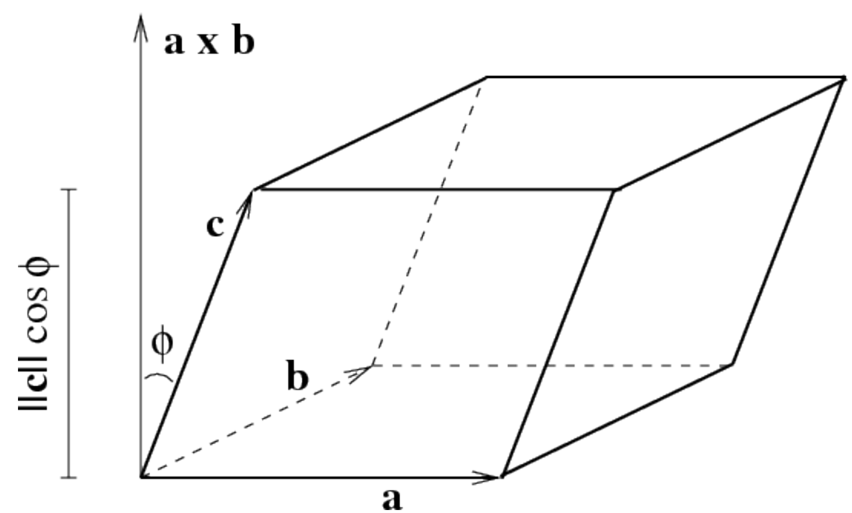
Courtesy Dr. Rajesh Prasad

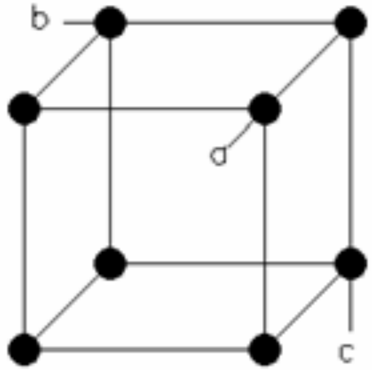
Cells

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

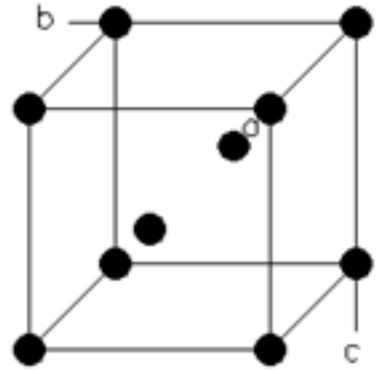
- A cell is a finite representation of the infinite lattice
- A cell is a parallelogram (2D) or a parallelopiped (3D) with lattice points at their corners.
- If the lattice points are only at the corners, the cell is primitive.
- If there are lattice points in the cell other than the corners, the cell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



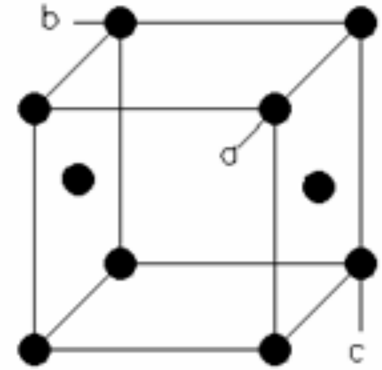




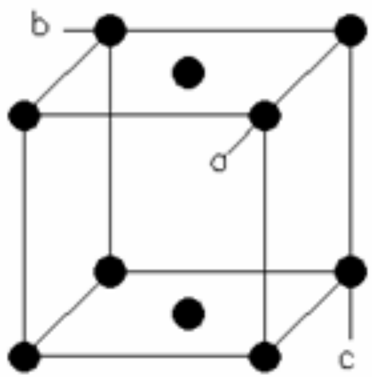
Primitive



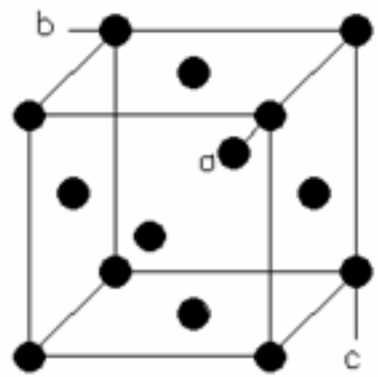
"A" centered



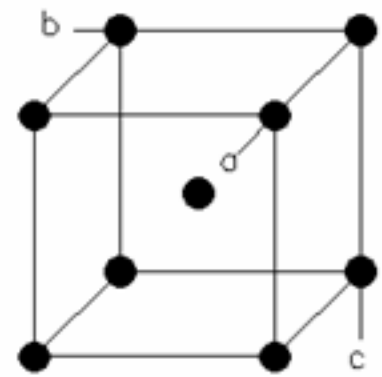
"B" centered



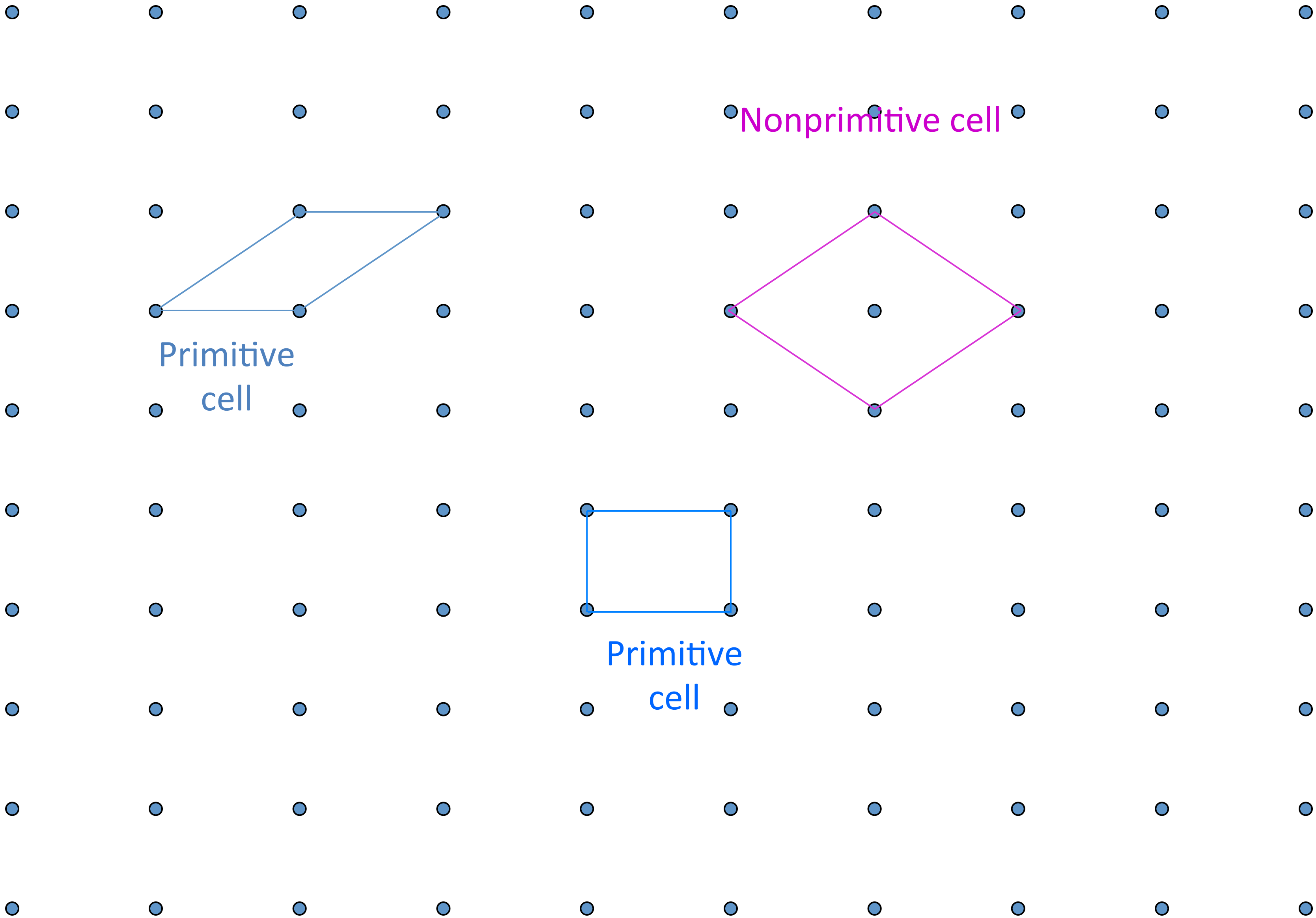
"C" centered



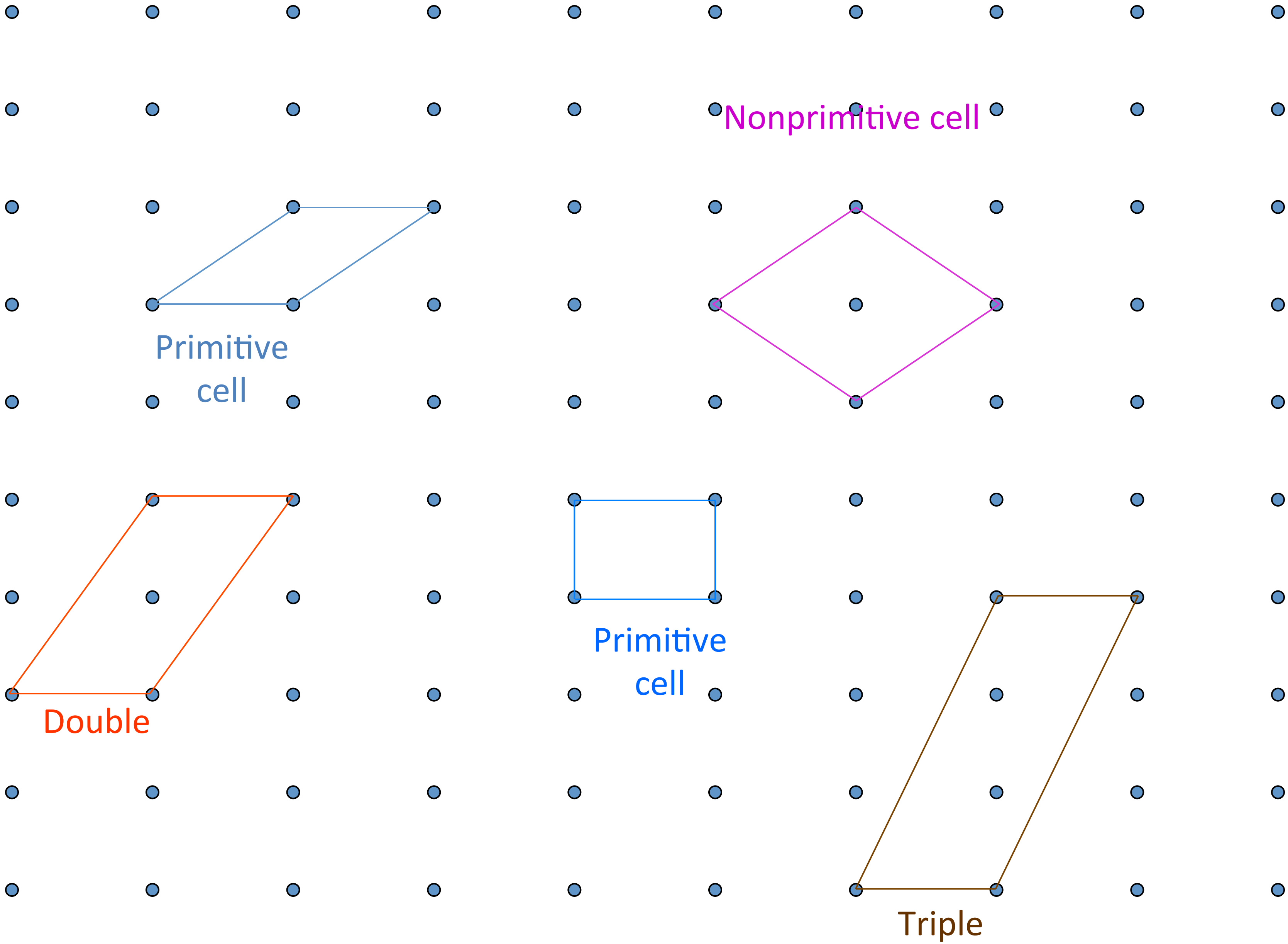
"F" (face) centered



"I" (body) centered

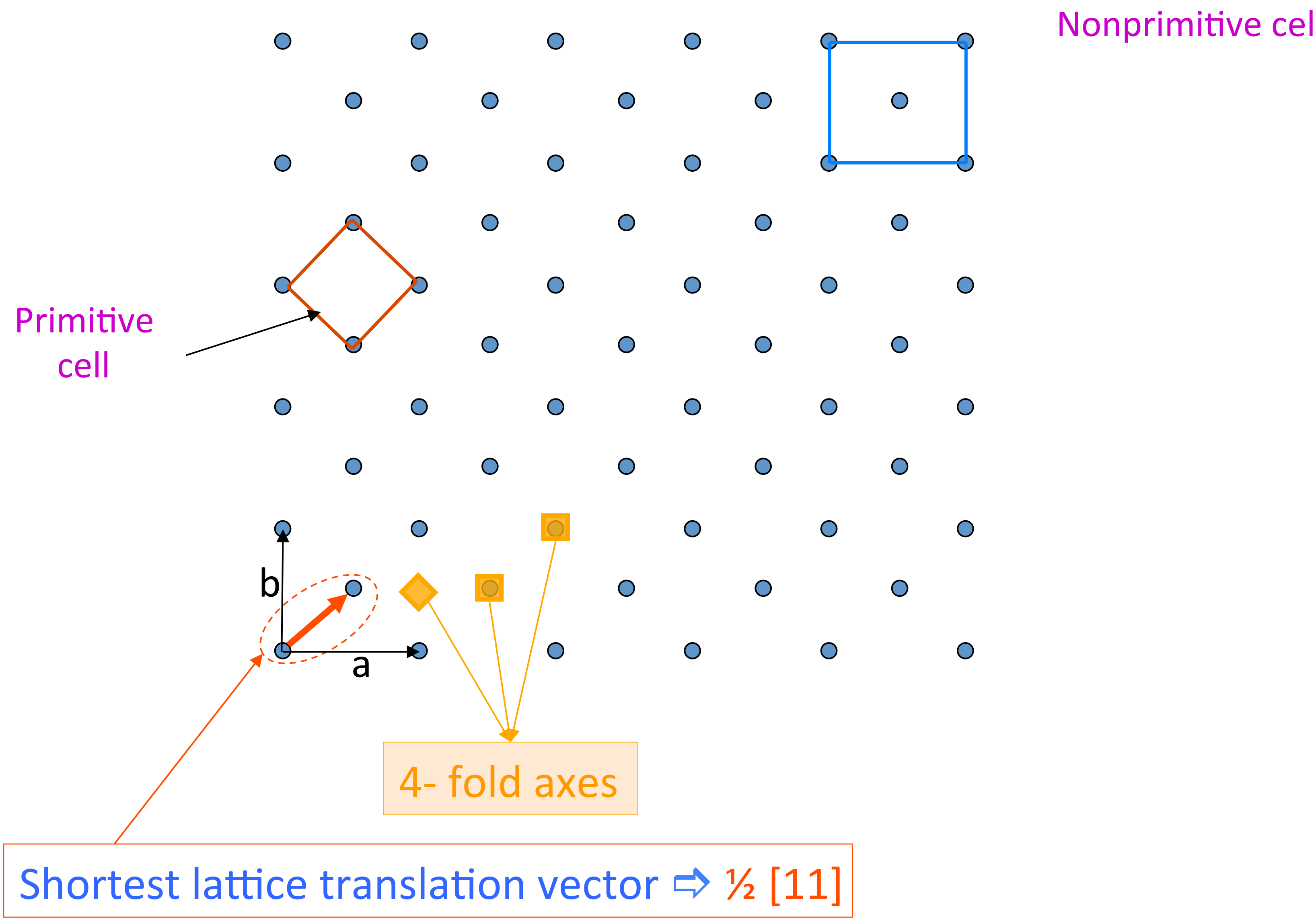


Courtesy Dr. Rajesh Prasad

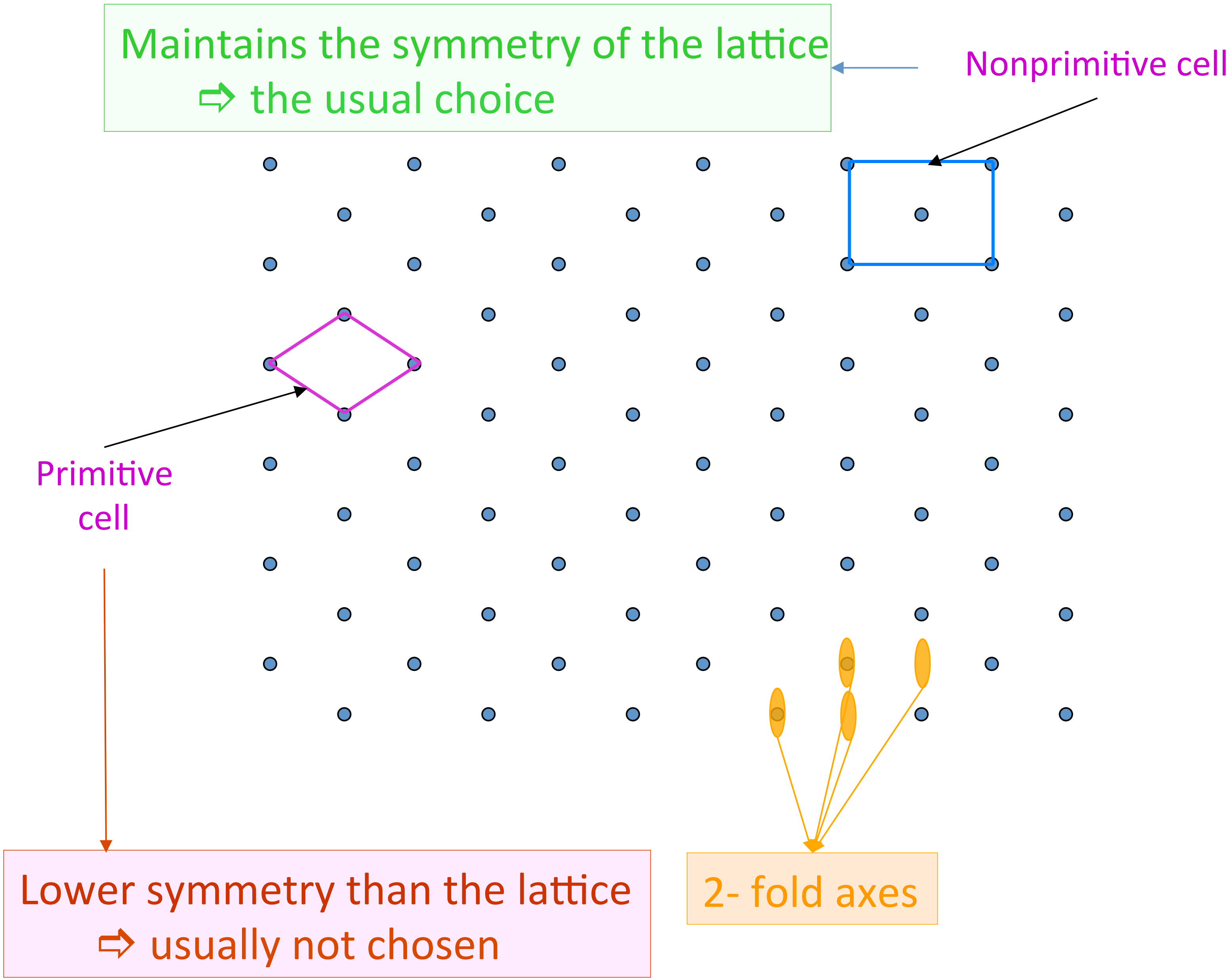


Symmetry of the Lattice or the crystal is not altered by our choice of unit cell!!

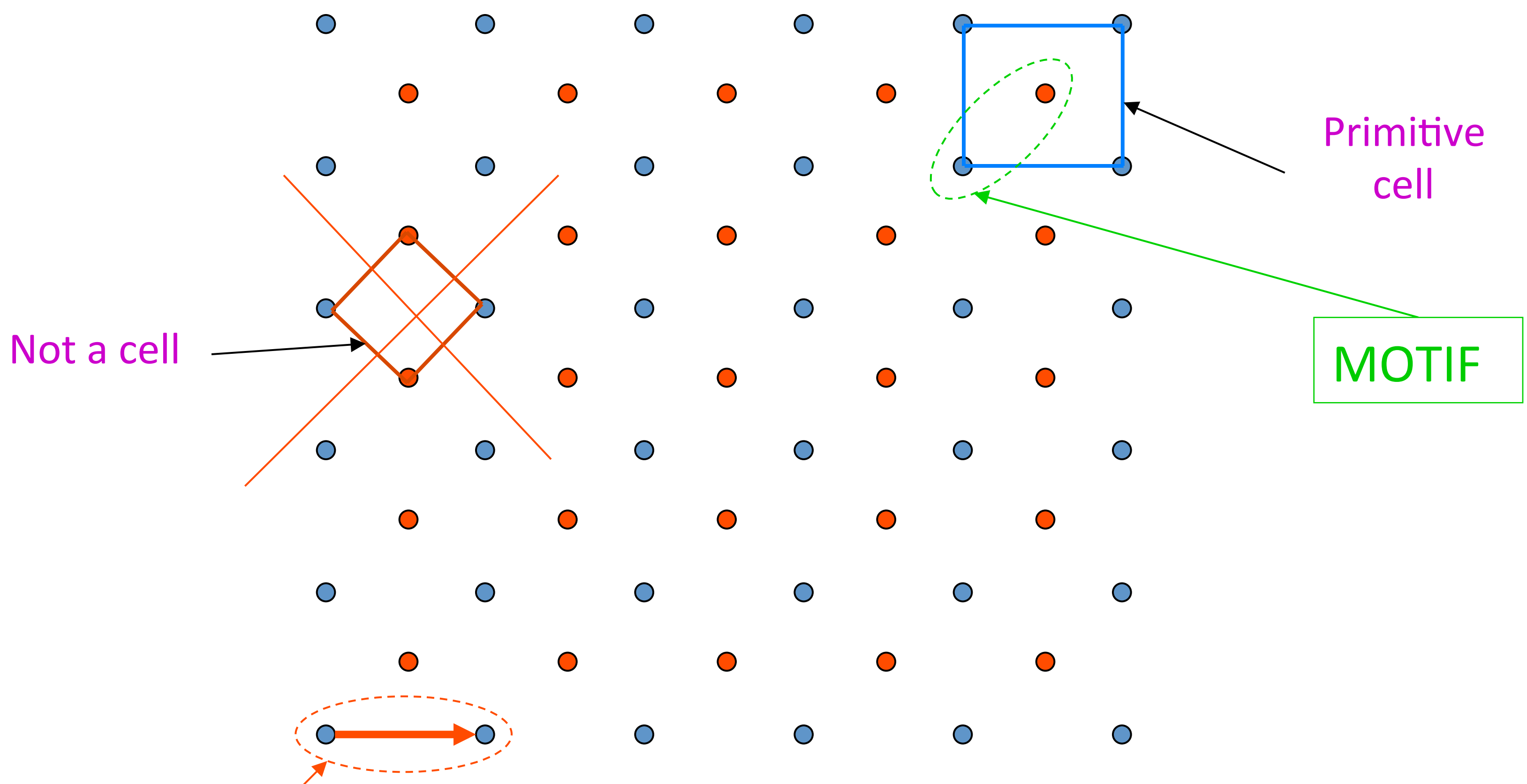
Centred square lattice = Simple/primitive square lattice



Centred rectangular lattice



Centred rectangular lattice → Simple rectangular Crystal



Shortest lattice translation vector ⇨ [10]

Courtesy Dr. Rajesh Prasad

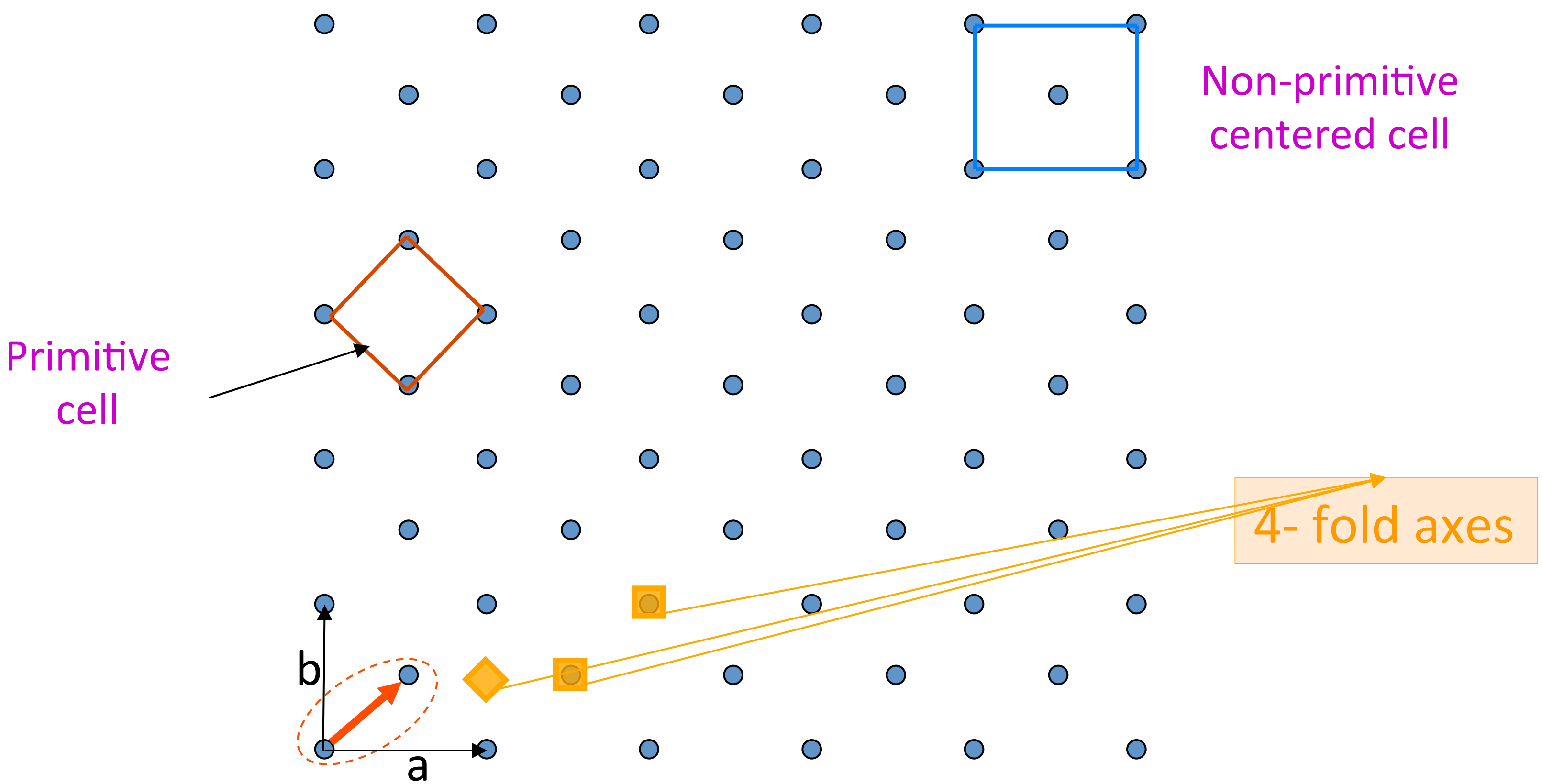
Cells- 3D

- In order to define translations in 3-d space, we need 3 non-coplanar vectors
- Conventionally, the fundamental translation vector is taken from one lattice point to the next in the chosen direction
- With the help of these three vectors, it is possible to construct a parallelopiped called a CELL

This was the end in 2015

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.

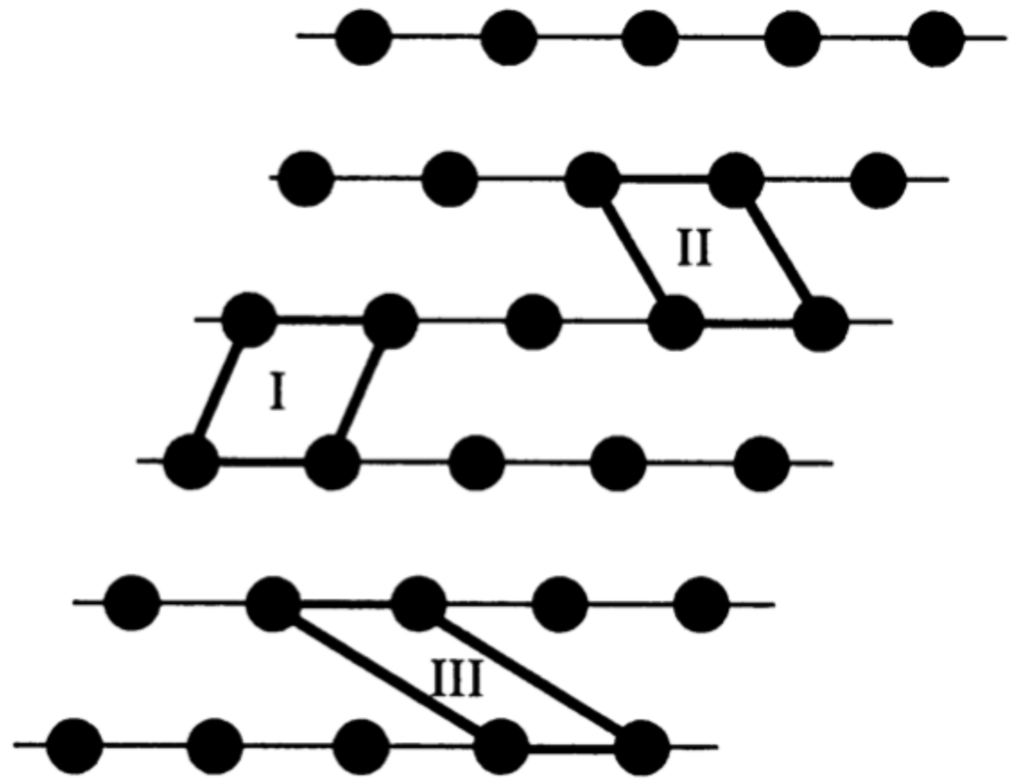


SYMMETRY

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

Unit cell choice / selection

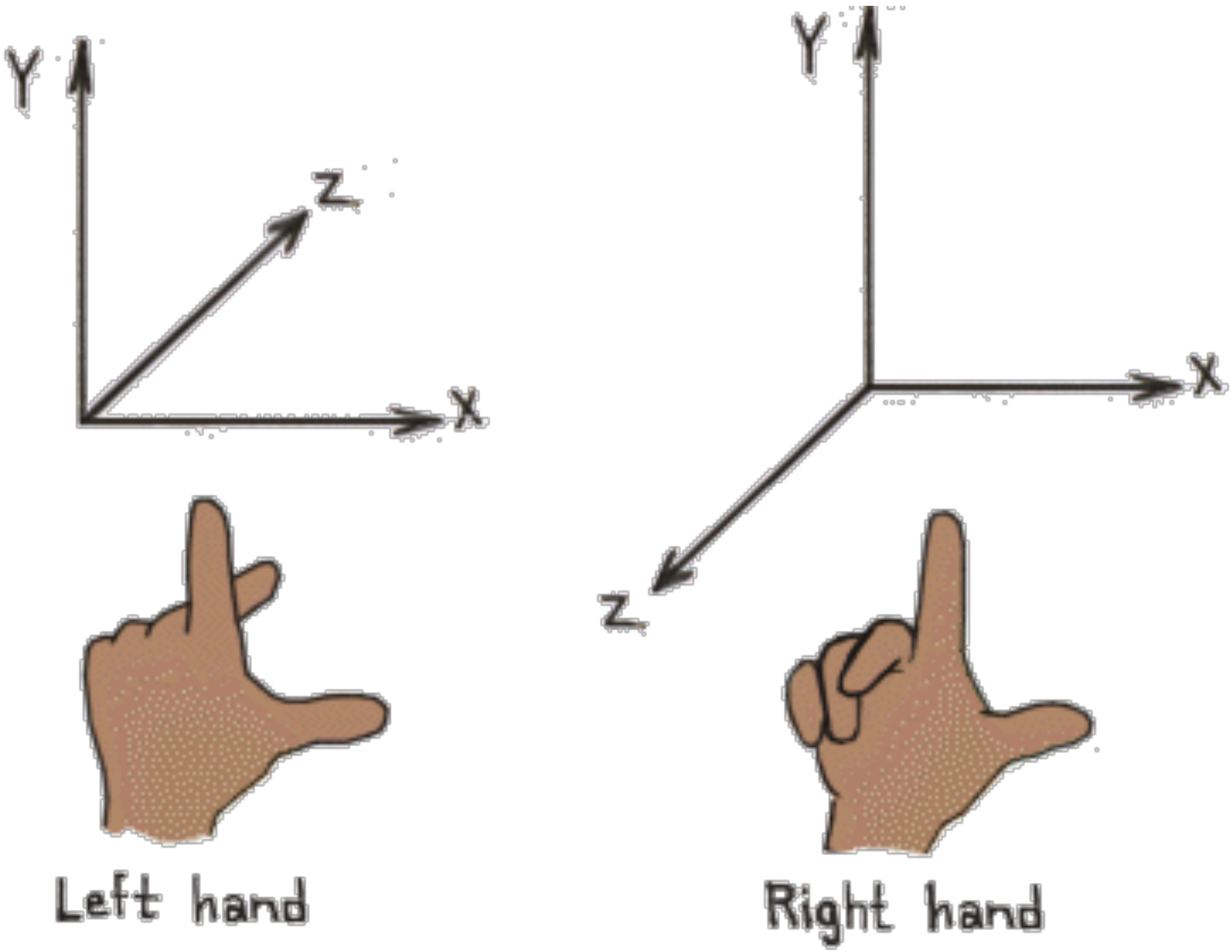
Figure 3.8. In this two-dimensional lattice, the unit cell can be chosen in different ways: as I, as II, or as III.



Unit cell selection

1. The axis system should be right-handed.
2. The basis vectors should coincide as much as possible with directions of highest symmetry (Section 3.2).
3. The cell taken should be the smallest one that satisfies condition 2. This condition sometimes leads to the preference of a face-centered (A, B, C, or F) or a body-centered (I) cell over a primitive (P) smallest cell (Figure 3.9). Primitive cells have only one lattice point per unit cell, whereas nonprimitive cells contain two or more lattice points per unit cell. These cells are designated A, B, or C if one of the faces of the cell is centered: It has extra lattice points on opposite faces of the unit cell, respectively, on the *bc* (A), *ac* (B), or *ab* (C) faces. If all faces are centered, the designation is F (Figure 3.9).
4. Of all lattice vectors, none is shorter than *a*.
5. Of those not directed along **a**, none is shorter than *b*.
6. Of those not lying in the *a*, *b* plane none is shorter than *c*.
7. The three angles between the basis vectors **a**, **b**, and **c** are either all acute ($<90^\circ$) or all obtuse ($\geq 90^\circ$).

Handedness of axis system



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.

or

In geometry and crystallography, a **Bravais lattice** is an infinite set of points generated by a set of discrete translation operations.

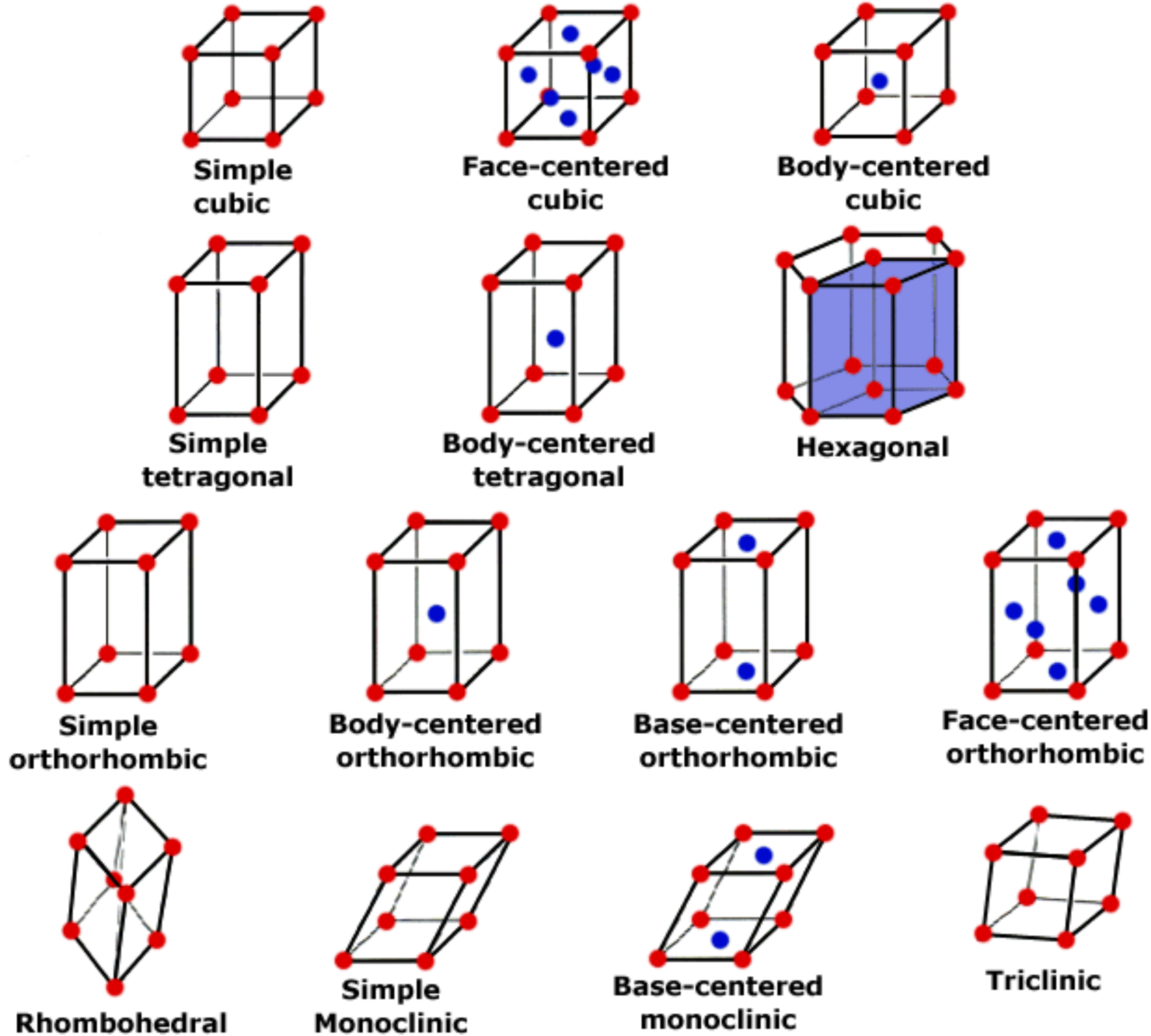
Arrangement of lattice points in the unit cell & No. of Lattice points / cell

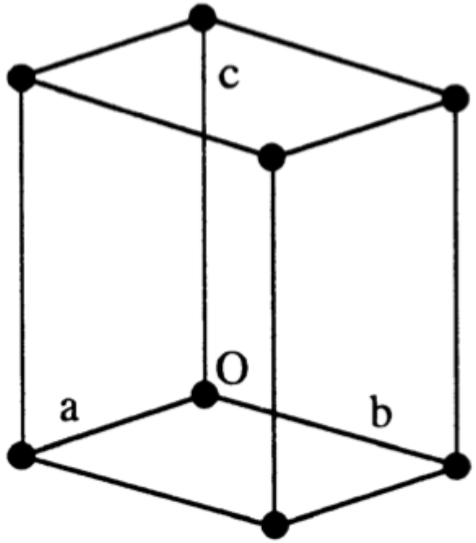
		Position of lattice points	Effective number of Lattice points / cell
1	P	8 Corners	= 8 x (1/8) = 1
2	I	8 Corners + 1 body centre	= 1 (for corners) + 1 (BC)
3	F	8 Corners + 6 face centres	= 1 (for corners) + 6 x (1/2) = 4
4	A/ B/ C	8 corners + 2 centres of opposite faces	= 1 (for corners) + 2x(1/2) =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

14 Bravais lattices divided into seven crystal systems

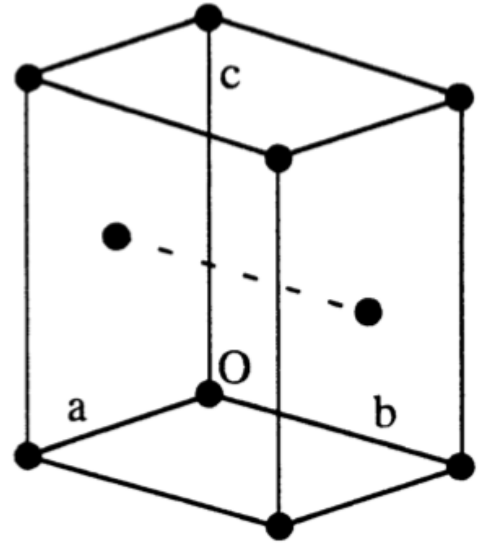
Crystal system	Bravais lattices			
1. Cubic	P	I	F	
2. Tetragonal	P	I		
3. Orthorhombic	P	I	F	C
4. Hexagonal	P			
5. Trigonal	P			
6. Monoclinic	P			C
7. Triclinic	P			

Courtesy Dr. Rajesh Prasad

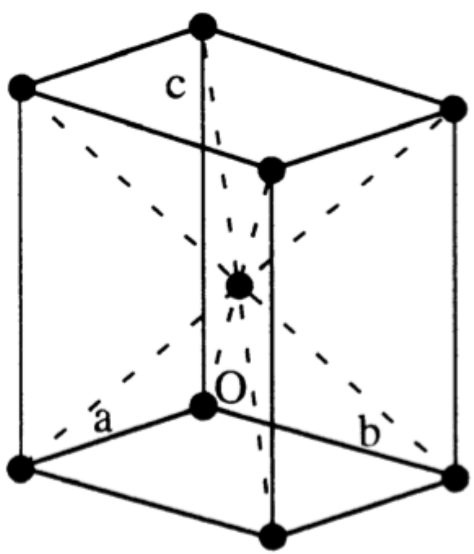

Simple cubic
Face-centered cubic
Body-centered cubic
Simple tetragonal
Body-centered tetragonal
Hexagonal
Simple orthorhombic
Body-centered orthorhombic
Base-centered orthorhombic
Face-centered orthorhombic
Rhombohedral
Simple Monoclinic
Base-centered monoclinic
Triclinic



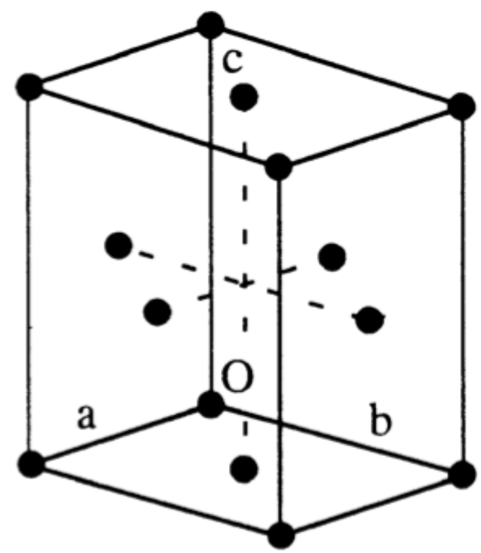
a primitive unit cell (P)



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



a body-centered unit cell (I)



a face-centered unit cell (F)

The following 4 things are different

Symmetry of the

- Lattice
- Motif
- Crystal
- Unit Cell

Eumorphic crystal (equilibrium shape and growth shape of the crystal)

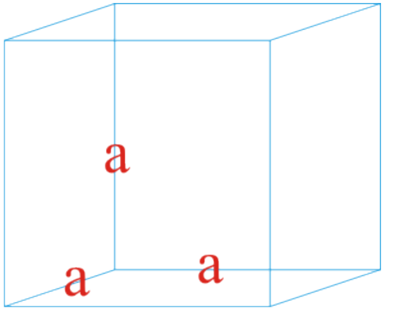
The shape of the crystal corresponds to the point group symmetry of the crystal

THE 7 CRYSTAL SYSTEMS

1. Cubic Crystals

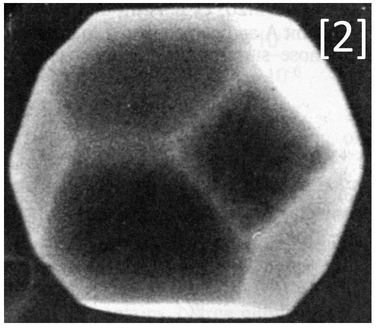
a = b= c

$\alpha = \beta = \gamma = 90º$



- Simple Cubic (P)
- Body Centred Cubic (I) – BCC
- Face Centred Cubic (F) - FCC

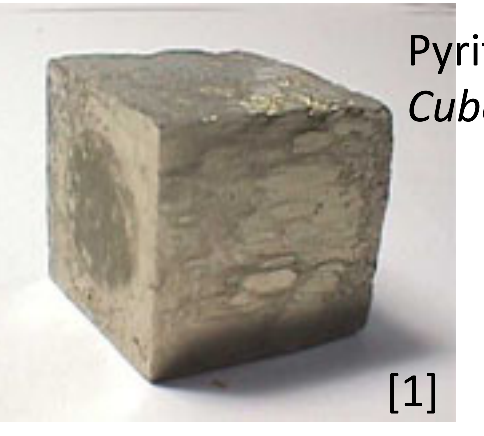
$$\text{Point groups} \Rightarrow 23,\ \bar{4}3m,\ m\bar{3},\ 432,\ \frac{4}{m}\bar{3}\frac{2}{m}$$



Vapor grown NiO crystal
Tetrakaidecahedron
(Truncated Octahedron)

Fluorite
Octahedron

Garnet
Dodecahedron



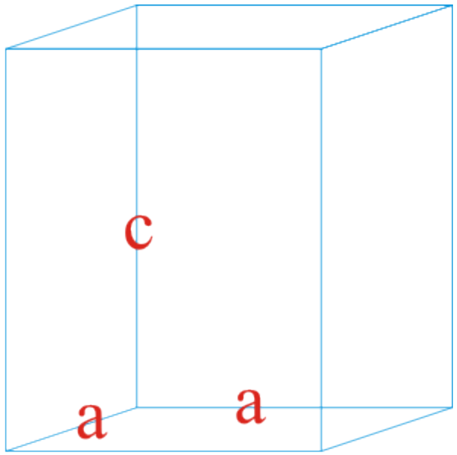
Pyrite
Cube

[1] http://www.yourgemologist.com/crystalsystems.html
[2] L.E. Muir, Interfacial Phenomenon in Metals, Addison-Wesley Publ. co.

2. Tetragonal Crystals

$a = b \neq c$

$\alpha = \beta = \gamma = 90º$



- Simple Tetragonal
- Body Centred Tetragonal

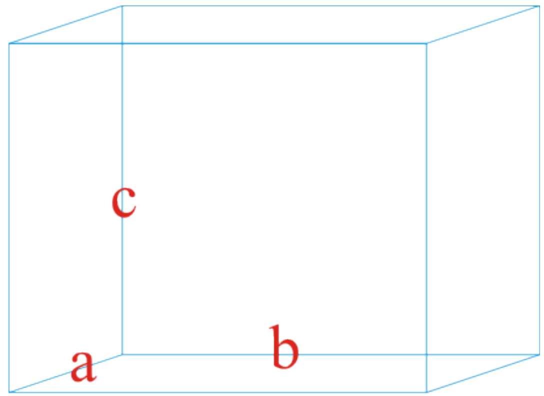
$$\text{Point groups} \Rightarrow 4,\ \bar{4},\ \frac{4}{m},\ 422,\ 4mm,\ \bar{4}2m,\ \frac{4}{m}\frac{2}{m}\frac{2}{m}$$

Zircon

[1] *http://www.yourgemologist.com/crystalsystems.html*

3. Orthorhombic Crystals

$a \neq b \neq c$
$\alpha = \beta = \gamma = 90º$



- Simple Orthorhombic
- Body Centred Orthorhombic
- Face Centred Orthorhombic
- End Centred Orthorhombic

$$\text{Point groups} \Rightarrow\ 222,\ 2mm,\ \frac{2}{m}\frac{2}{m}\frac{2}{m}$$

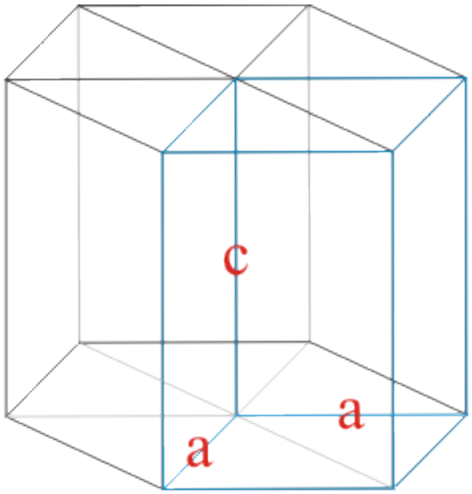
Topaz

[1] http://www.yourgemologist.com/crystalsystems.html

4. Hexagonal Crystals

$a = b \neq c$

$\alpha = \beta = 90º \quad \gamma = 120º$



- Simple Hexagonal

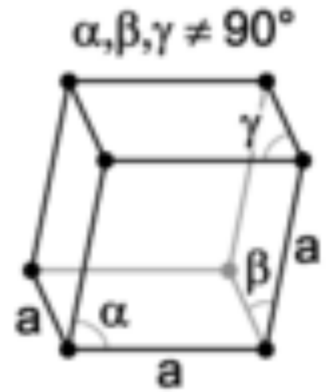
$$\text{Point groups} \Rightarrow 6,\ \bar{6},\ \frac{6}{m},\ 622,\ 6mm,\ \bar{6}m2,\ \frac{6}{m}\frac{2}{m}\frac{2}{m}$$

[1] *http://www.yourgemologist.com/crystalsystems.html*

5. Rhombohedral Crystals

$a = b = c$

$\alpha = \beta = \gamma \neq 90º$



- Rhombohedral (simple)

$$\text{Point groups} \Rightarrow 3,\ \bar{3},\ 32,\ 3m,\ \bar{3}\frac{2}{m}$$


[1]


[1]

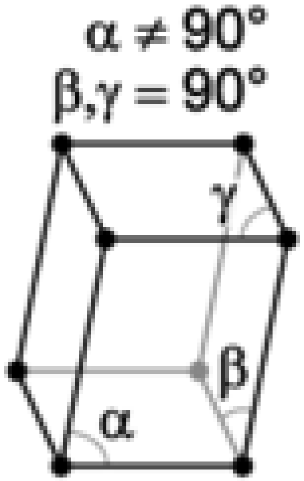
Tourmaline

[1] http://www.yourgemologist.com/crystalsystems.html

6. Monoclinic Crystals

$a \neq b \neq c$

$\alpha = \gamma = 90º \neq \beta$



- Simple Monoclinic
- End Centred (base centered) Monoclinic (A/C)

$$\text{Point groups} \Rightarrow 2,\ \bar{2},\ \frac{2}{m}$$

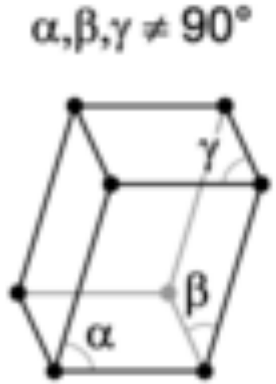
Kunzite

[1] *http://www.yourgemologist.com/crystalsystems.html*

7. Triclinic Crystals

$a \neq b \neq c$

$\alpha \neq \gamma \neq \beta$



- Simple Triclinic

$$\text{Point groups} \Rightarrow 1,\ \bar{1}$$

[1]

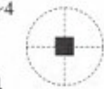
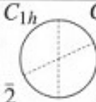
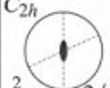
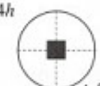
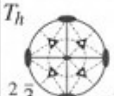
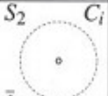
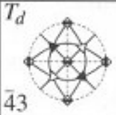
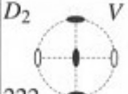
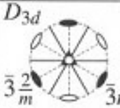
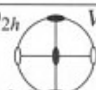
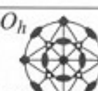
Amazonite

[1] http://www.yourgemologist.com/crystalsystems.html

Table 3.2. The Seven Crystal Systems

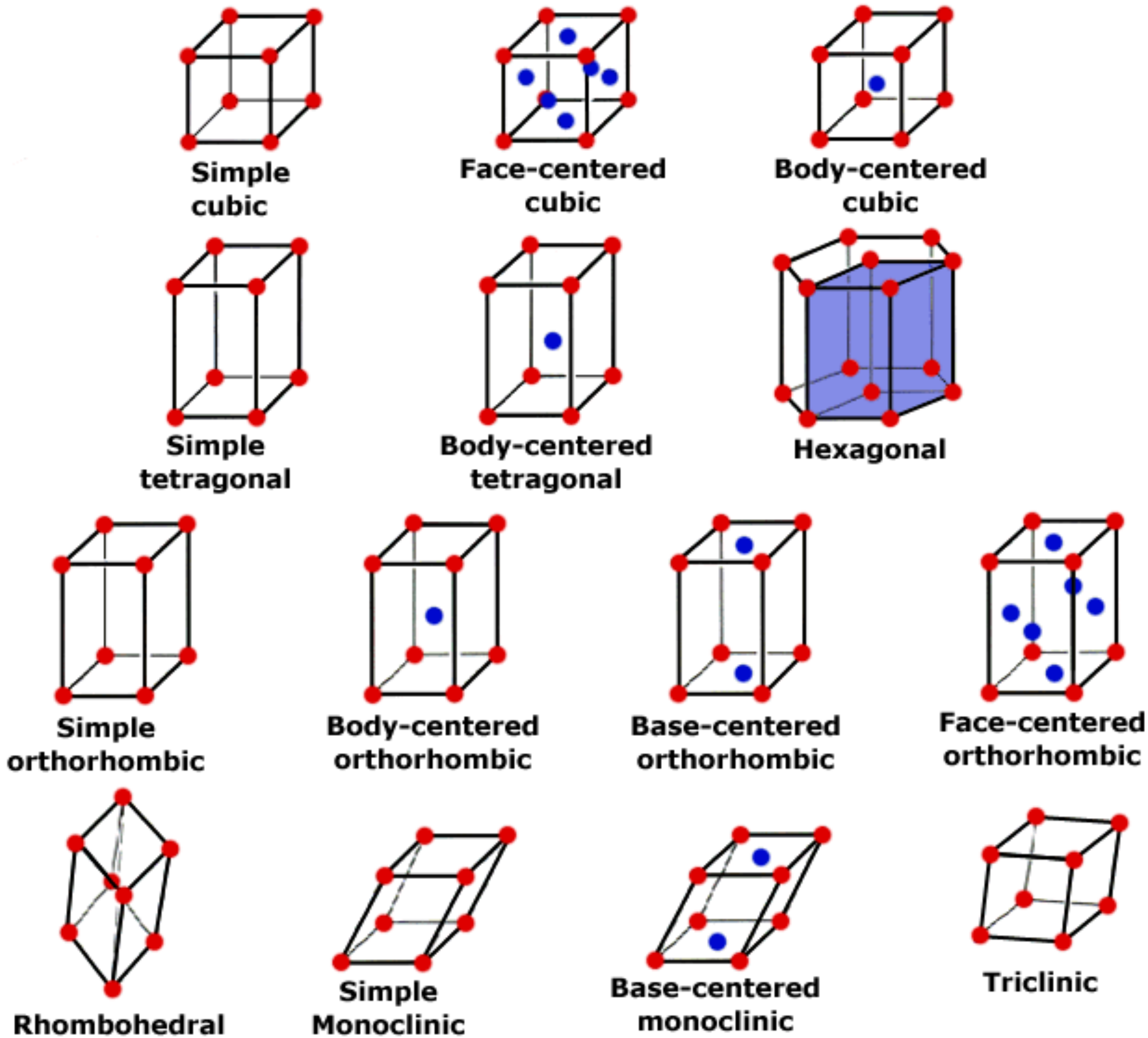
Crystal system	Conditions imposed on cell geometry	Minimum point group symmetry
Triclinic	None	1
Monoclinic	$\alpha = \gamma = 90^\circ$(b is the unique axis; for proteins this is a 2-fold axis or screw axis)	2
	or: $\alpha = \beta = 90^\circ$ (c is unique axis; for proteins this is a 2-fold axis or 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

Table 2.9. The 32 crystallographic point groups

Triclinic	Monoclinic	Orthorhombic	Trigonal	Tetragonal	Hexagonal	Cubic
C_1  1	C_2  2		C_3  3	C_4  4	C_6  6	T  23
	C_{1h} C_s  $\bar{2}$ m				C_{3h}  $\bar{6}$	
	C_{2h}  $\frac{2}{m}$ $2/m$			C_{4h}  $\frac{4}{m}$ $4/m$	C_{6h}  $\frac{6}{m}$ $6/m$	T_h  $\frac{2}{m}\bar{3}$ $m\bar{3}$
		C_{2v}  $2mm$ $mm2$	C_{3v}  $3m$	C_{4v}  $4mm$	C_{6v}  $6mm$	
S_2 C_i  $\bar{1}$			S_6 C_{3i}  $\bar{3}$	S_4  $\bar{4}$		T_d  $\bar{4}3$
		D_2 V  222	D_3  32	D_4  422	D_6  622	O  432
			D_{3d}  $\bar{3}\frac{2}{m}$ $\bar{3}m$	D_{2d} V_h  $\bar{4}2m$		
					D_{3h}  $\bar{6}m2$	
		D_{2h} V_h  $\frac{2}{m}\frac{2}{m}\frac{2}{m}$ mmm		D_{4h}  $\frac{4}{m}\frac{2}{m}\frac{2}{m}$ $4/mmm$	D_{6h}  $\frac{6}{m}\frac{2}{m}\frac{2}{m}$ $6/mmm$	O_h  $\frac{4}{m}\bar{3}\frac{2}{m}$ $m\bar{3}m$

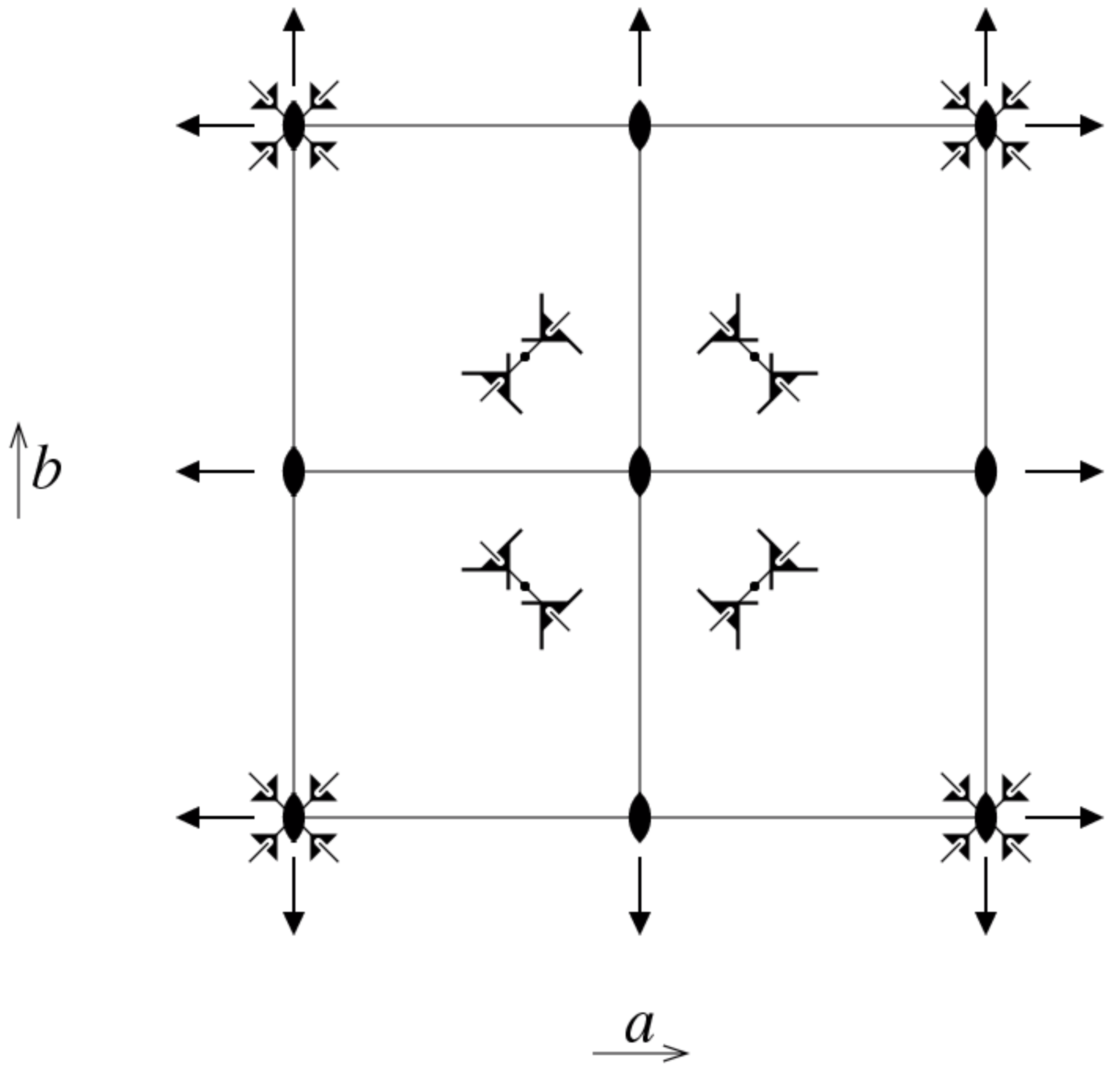
Point groups are illustrated by stereograms that display the symmetry axes of each group. The symmetry axes are described by the symbols defined in Table 2.10. A solid line always describes a mirror plane, while a dashed line refers to a plane about which mirror symmetry is *not* present.

14 Bravais lattices



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

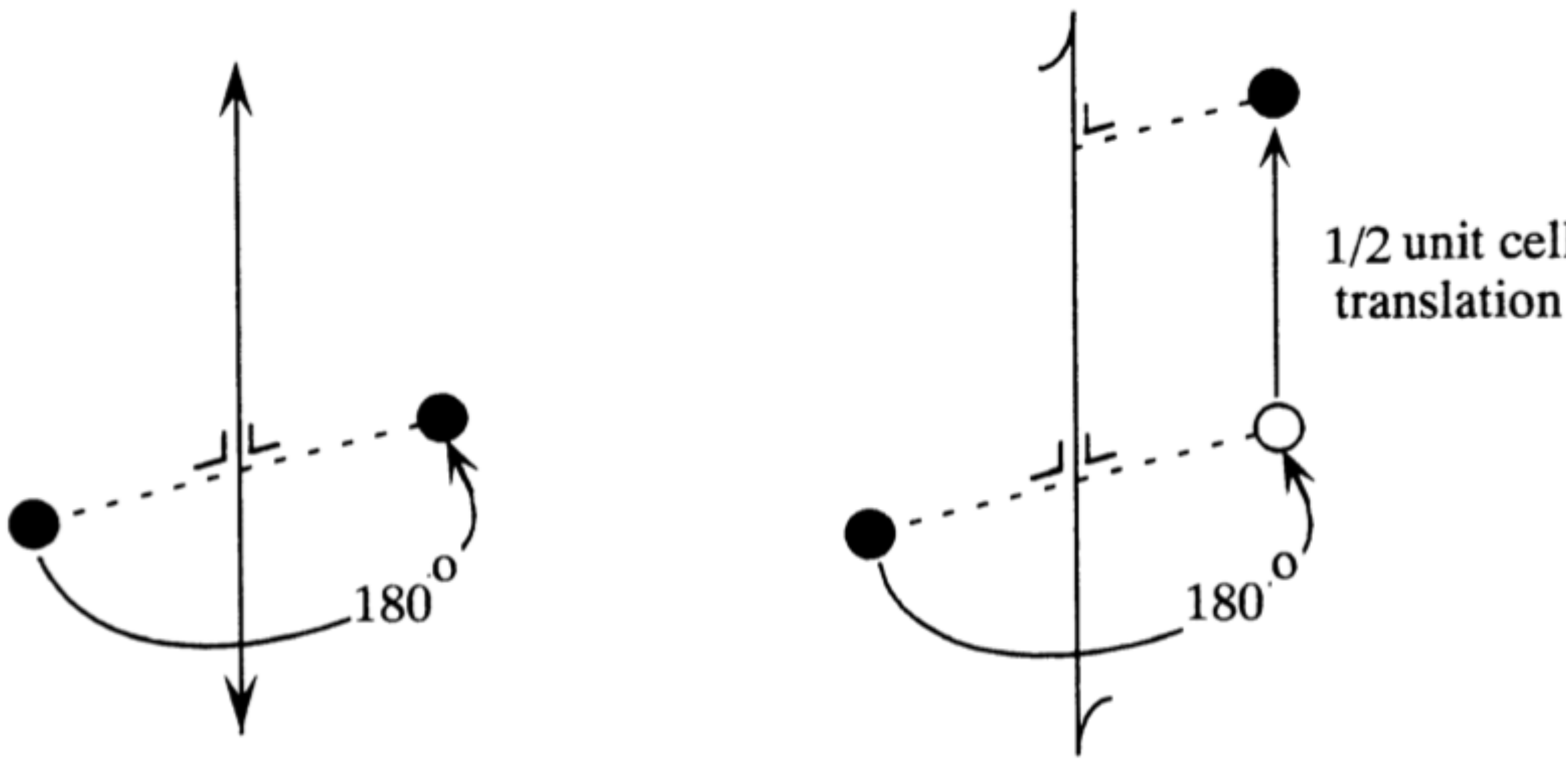


Figure 3.12. A 2-fold axis (left) and a 2-fold screw axis (right); the latter relates one molecule to another by a 180° rotation plus a translation over half of the unit cell.

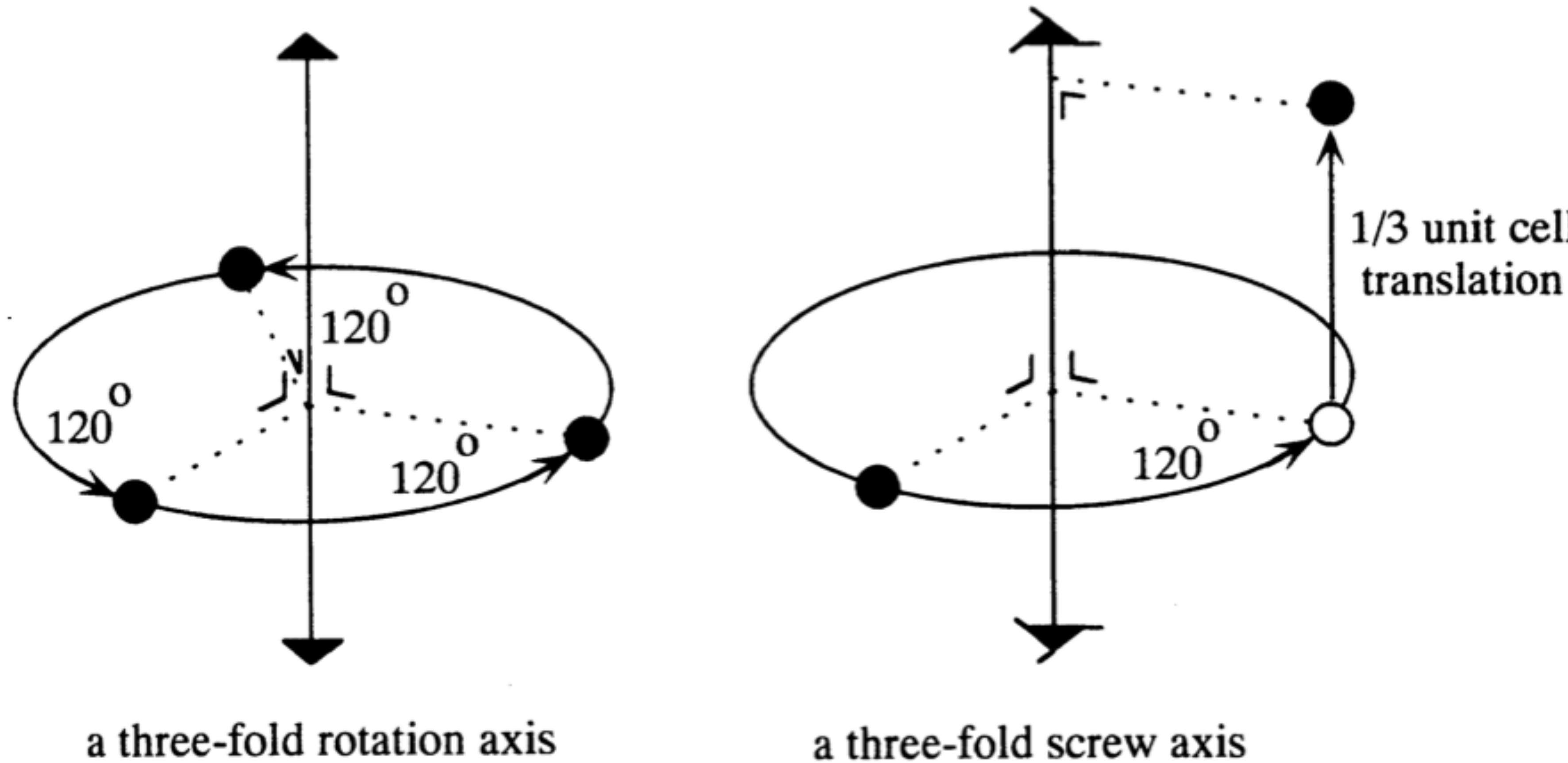


Figure 3.13. A 3-fold axis (left) and a 3-fold screw axis (right); the latter relates one molecule to another by a 120° rotation and a translation over one-third of the unit cell.

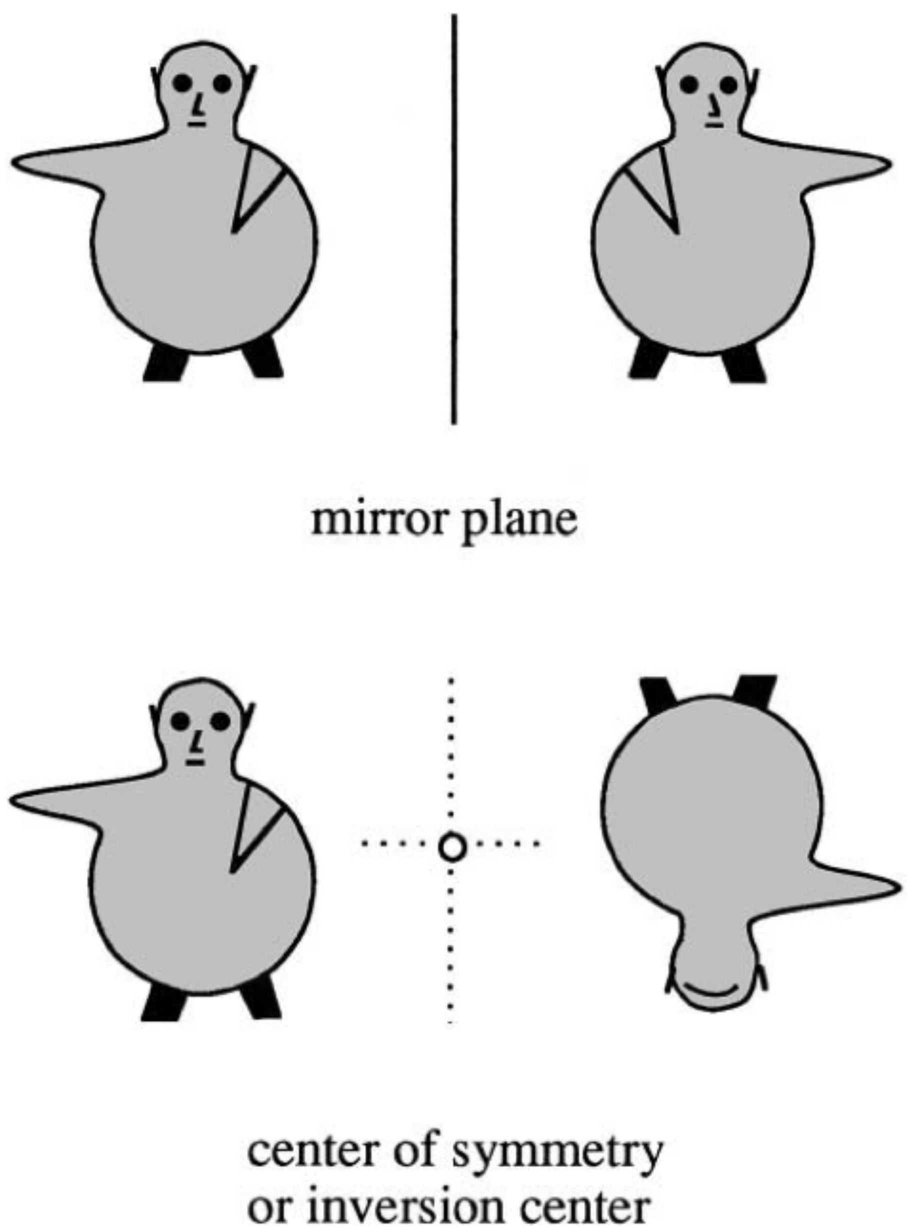


Figure 3.14. The effect of a mirror and of an inversion center.

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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Explain general and special position
Explain crystallographic asymmetric unit