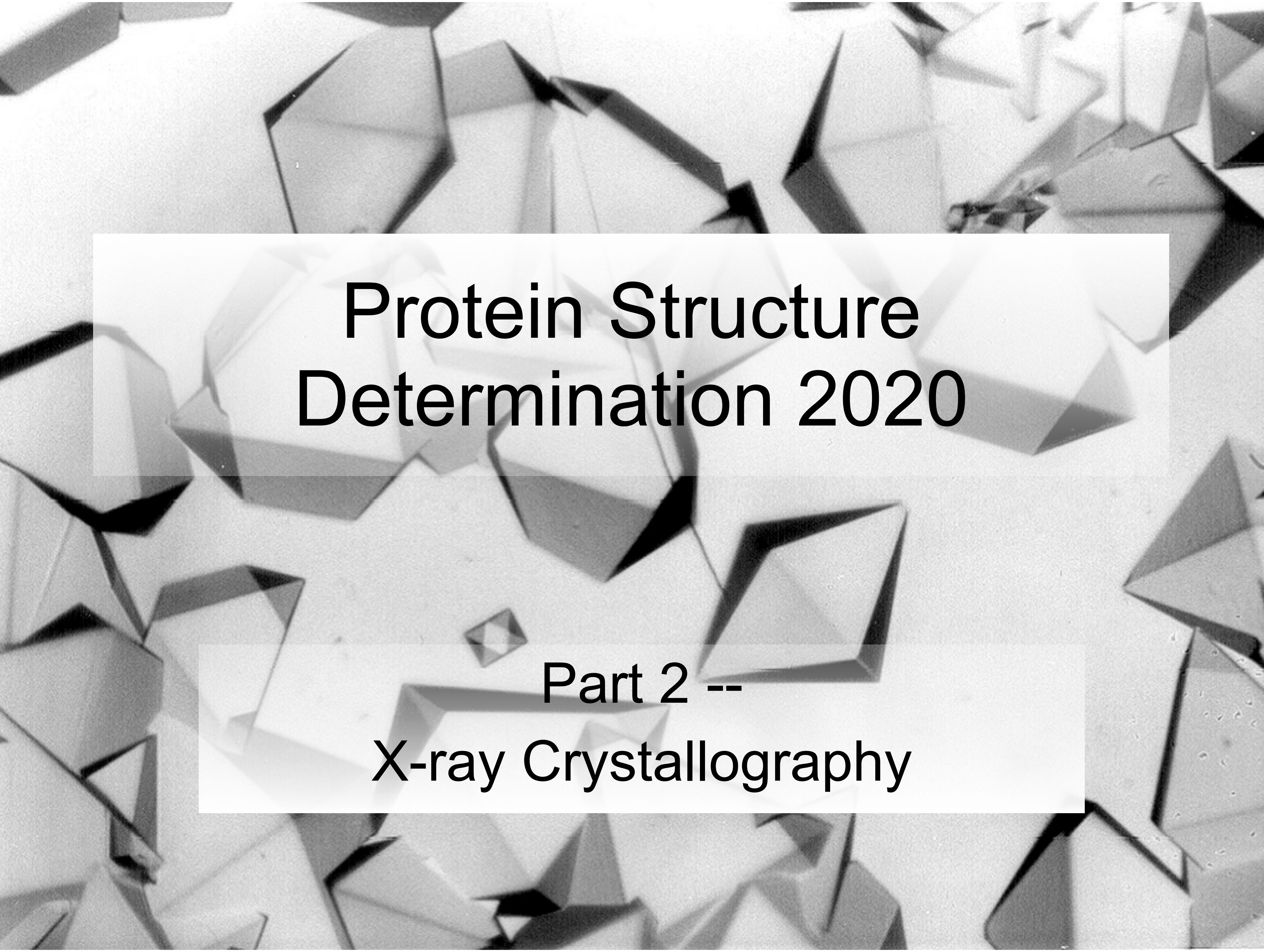
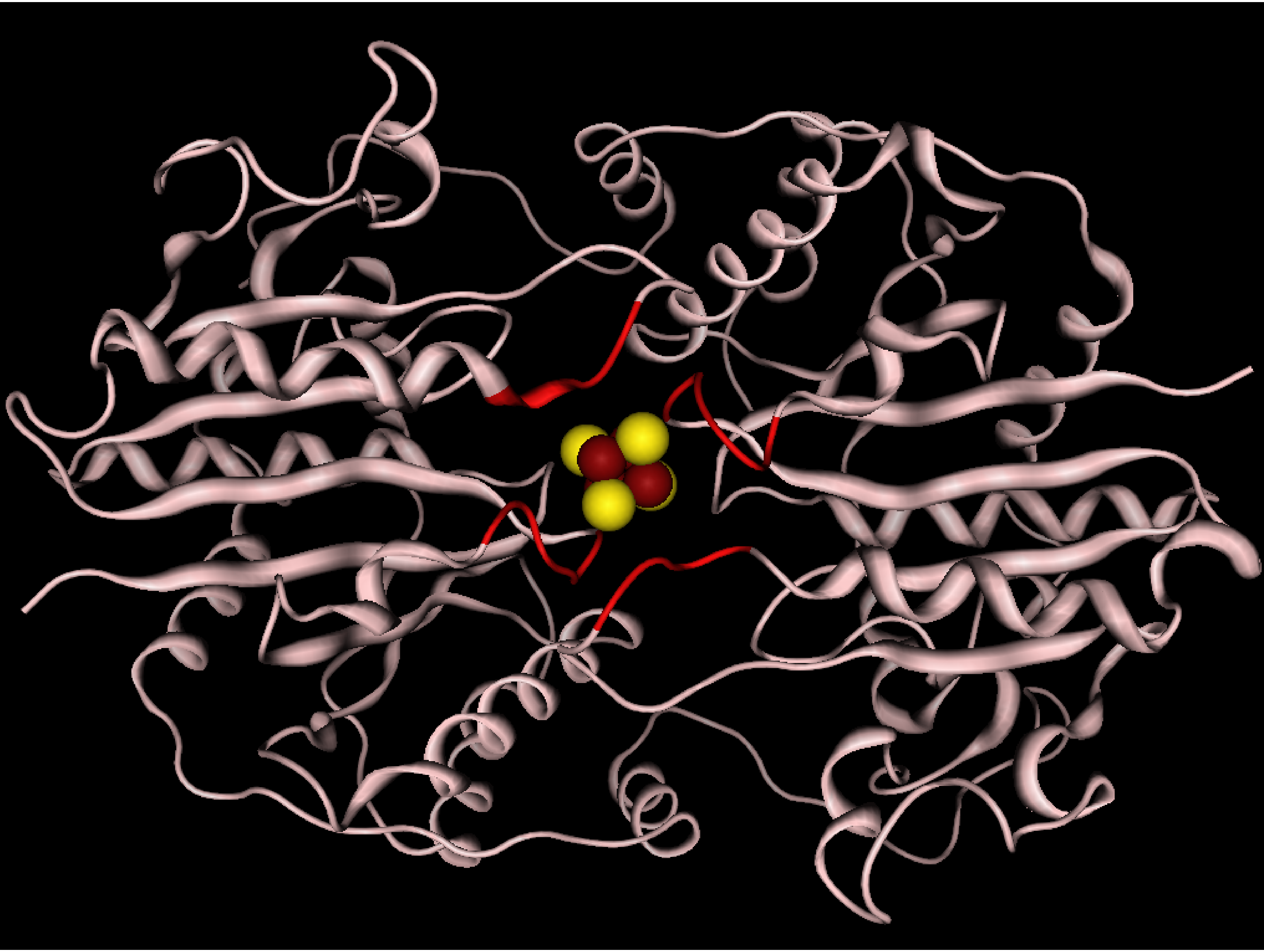


Protein Structure Determination 2020

Part 2 --
X-ray Crystallography



Topics covering in this 1/2 course

- Crystal growth
- Diffraction theory
- Symmetry
- Solving phases using heavy atoms
- Solving phases using a model
- Model building and refinement
- Errors and validation
- Navigating protein structures

“Theory” questions we will be able to answer by the end of this course

- Why do crystals diffract Xrays?
- What is a Fourier transform?
- What is the phase problem?

“Practice” questions we will know how to answer by the end of this course

- How do we grow crystals?
- How do collect Xray data?
- How do we solve the phase problem?
- How do we model electron density?

Equations you will learn to recognize

$$e^{i\alpha} = \cos\alpha + i\sin\alpha$$

Euler's theorem

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

Bragg's law

$$\vec{S} = \frac{\vec{s}_o - \vec{s}}{\lambda}$$

Reciprocal space

$$\vec{x}_{sym} = \underline{M}\vec{x} + \vec{v}$$

Symmetry operation

$$F(hkl) = \sum_{xyz} \rho(xyz) e^{2\pi i(hx+ky+lz)}$$

Fourier transform

$$\rho(xyz) = \sum_{hkl} F(hkl) e^{-2\pi i(hx+ky+lz)}$$

Inverse Fourier transform

Materials

Gale Rhodes "Crystallography Made Crystal Clear"

3rd Ed. Academic Press

graph paper

straight edge

protractor

compass

calculator w/trig functions

Software:

Phenix: www.phenix-online.org (not required)
Coot: https://www2.mrc-lmb.cam.ac.uk/personal/pemsley/coot/
Coot wiki: strucbio.biologie.uni-konstanz.de/ccp4wiki/index.php/COOT
XRayView: http://phillipslab.org/downloads

Course website:

http://www.bioinfo.rpi.edu/bystrc/courses/bcbp4870/index.html

Supplementary reading

Matrix algebra

"An Introduction to Matrices, Sets and Groups for Science Students" by G. Stephenson ($7.95)

Wave physics

"Physics for Scientists and Engineers" by Paul A. Tipler

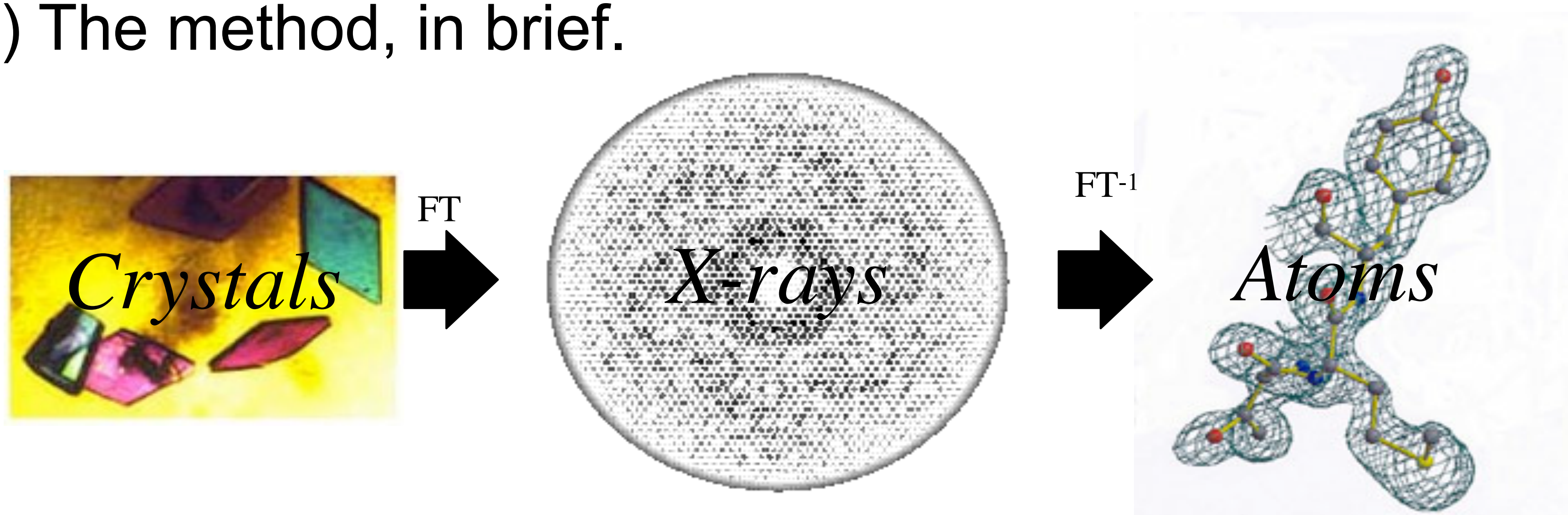
Protein structure

"Introduction to Protein Structure"-- by Carl-Ivar Branden and John Tooze

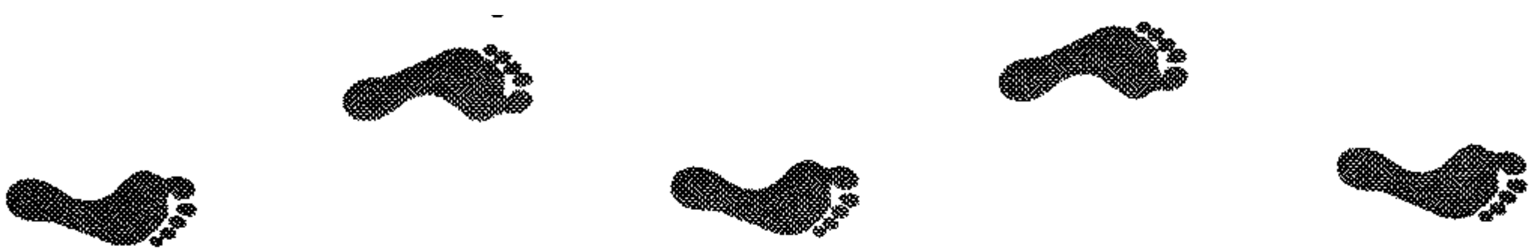
"Introduction to Protein Architecture : The Structural Biology of Proteins" -- by Arthur M. Lesk

Today's lecture

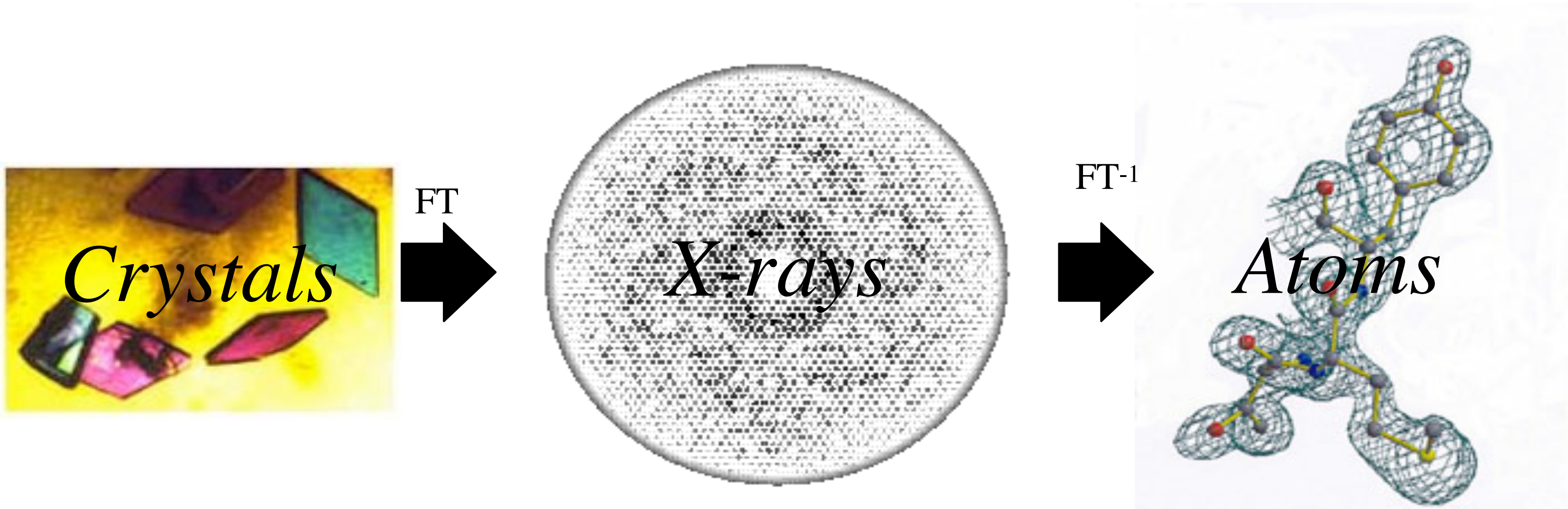
1) The method, in brief.



2) Symmetry.



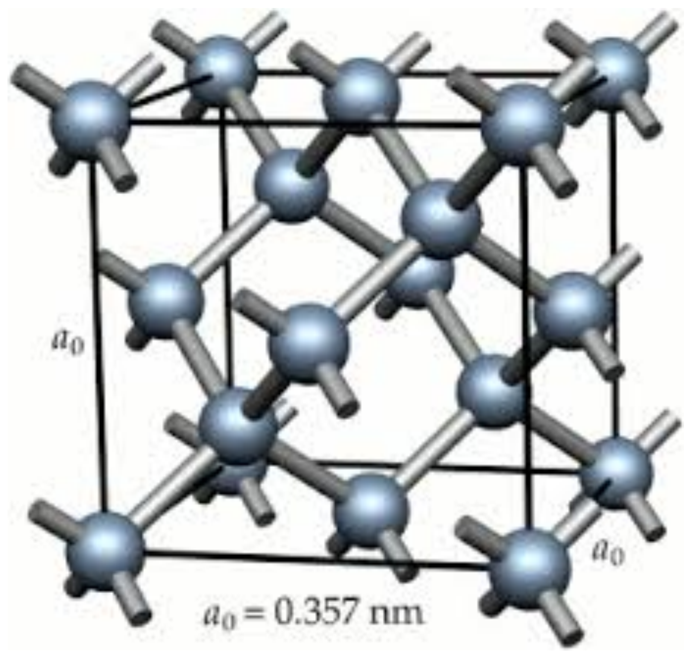
The method, in brief.



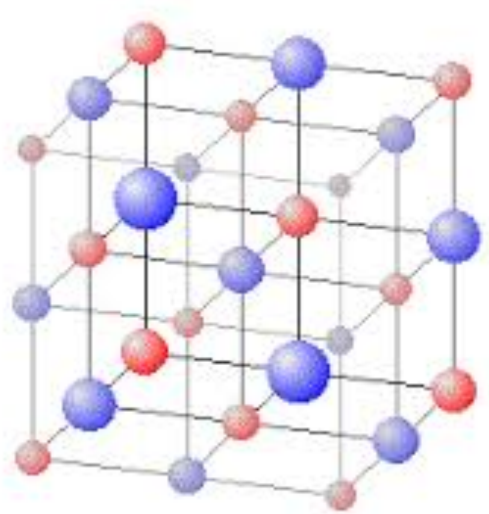
1)Purify protein
2)Grow crystals
3)Collect Xray data
4)Phase the data (solve the structure)
5)Fit the electron density
6)Refine.

What is a crystal?

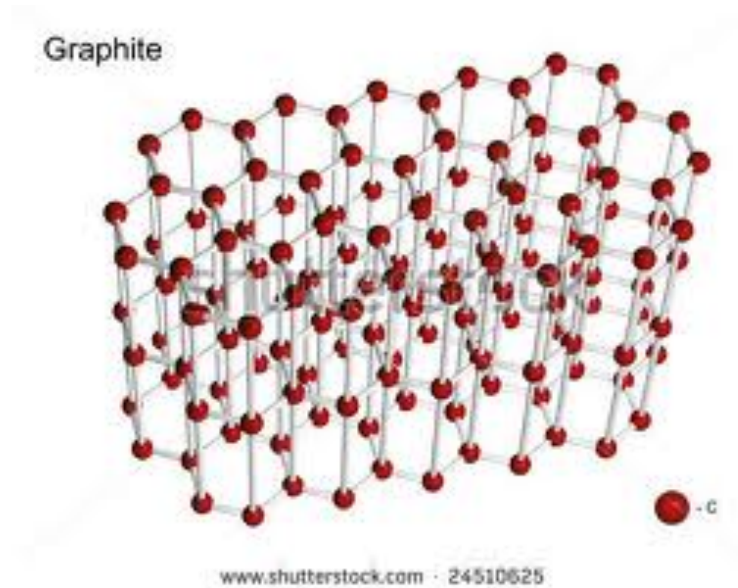
diamond



salt



Graphite



Molecules arranged in a 3D lattice, usually having *space group symmetry*.
One lattice unit is called a "unit cell".

What is symmetry?

An object or function is symmetrical if a spatial transformation of it looks identical to the original.

This is an X

This is an X rotated by 180°

X

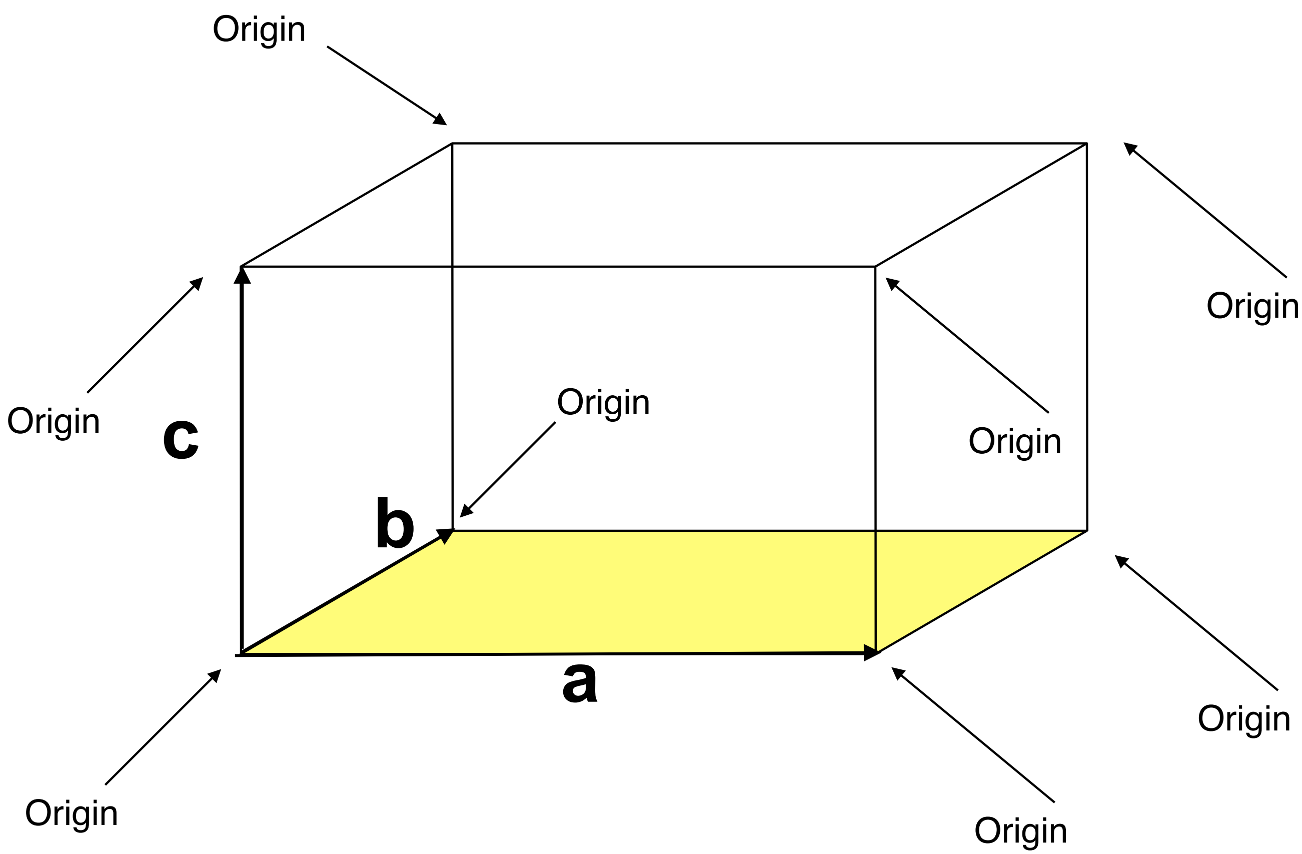
X

Can you see the difference? If not, then the letter is symmetric. If the difference are subtle, then it is pseudo-symmetric.

Why is symmetry essential in crystallography?

- Understanding crystal packing.
- Solving for where the heavy atoms are.
- Knowing the number and arrangement of molecules in the unit cell.
- Proper indexing of the Xray data.

Anatomy of a Unit Cell



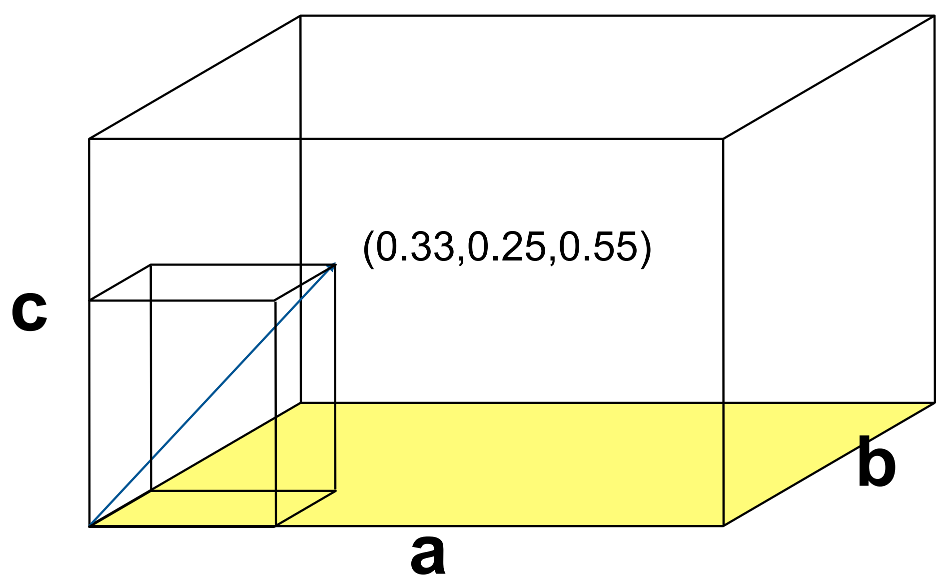
The coordinate system is composed of three vectors, **a, b** and **c**.

Not necessary orthogonal!

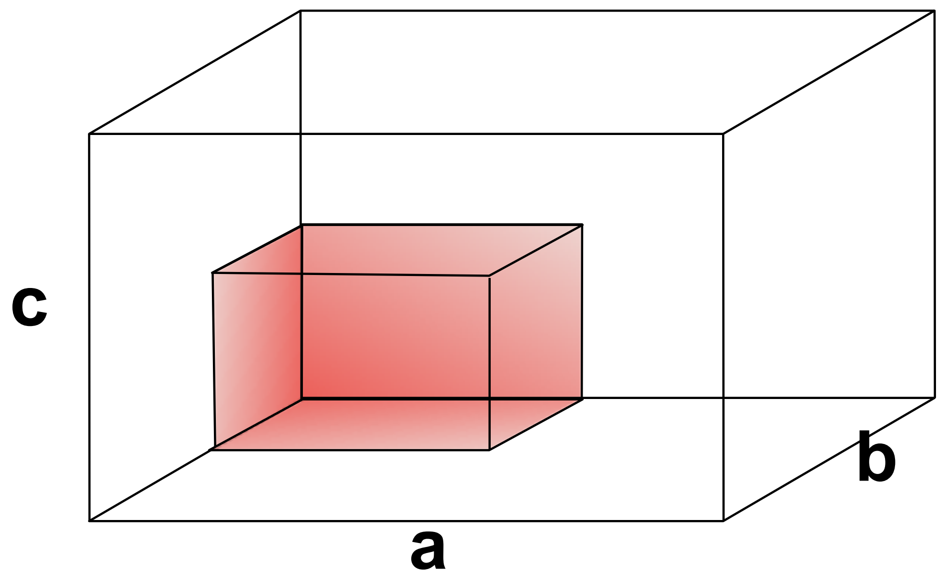
*The crystallographic coordinate system is called **fractional coordinates***

If (x,y,z) is a point in fractional coordinates, then the location in orthogonal Å coordinates (Cartesian) is

$\mathbf{p} = x\mathbf{a}+y\mathbf{b}+z\mathbf{c}$.



The part of the unit cell that has all unique contents is the ***asymmetric unit***



Applying symmetry to the asymmetric unit generates the unit cell.

Translational symmetry is ***vector addition***

Example: translation to center of unit cell

$$\begin{pmatrix} x' \\ y' \\ z' \end{pmatrix} = \begin{pmatrix} x \\ y \\ z \end{pmatrix} + \begin{pmatrix} 1/2 \\ 1/2 \\ 1/2 \end{pmatrix}$$

(0.1, 0.1, 0.0) is symmetry-equavalent of (0.6, 0.6, 0.5)

Lattice symmmetry is translational symmetry

...where the shifts are integers. For example: shifting by 1 in each direction

$$\begin{pmatrix} x' \\ y' \\ z' \end{pmatrix} = \begin{pmatrix} x \\ y \\ z \end{pmatrix} + \begin{pmatrix} 1 \\ 1 \\ 1 \end{pmatrix}$$

(1.1, 1.2, 1.3) is symmetry-equavalent of (0.1, 0.2, 0.3)

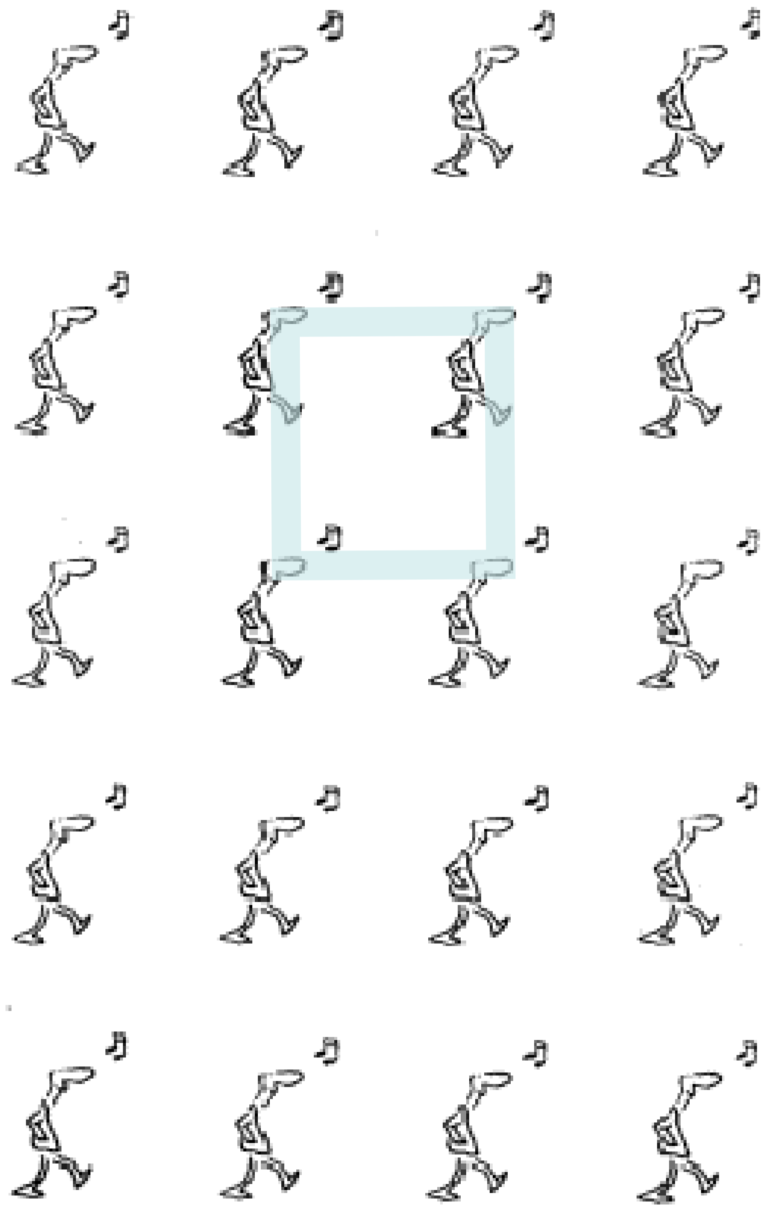
*General equation for **Lattice symmetry***

Every unit cell is shifted by a integer multiple of 1 in each direction

$$\begin{pmatrix} x' \\ y' \\ z' \end{pmatrix} = \begin{pmatrix} x \\ y \\ z \end{pmatrix} + \begin{pmatrix} t \\ u \\ v \end{pmatrix}$$

t, u, and v are integers. For example: (0.1, 0.2, -0.3) equivalent to (2.1, 24.2, 0.7)

Draw the unit cell.



Space group P1

*Rotational symmetry is **matrix multiplication***

Example: a 180° rotation. Remember to multiply "row times column"

$$\begin{pmatrix} -1 & 0 & 0 \\ 0 & -1 & 0 \\ 0 & 0 & 1 \end{pmatrix} \begin{pmatrix} x \\ y \\ z \end{pmatrix} = \begin{pmatrix} -x \\ -y \\ z \end{pmatrix}$$

(-x,-y,z) is rotated 180° around the origin.

Example: (1.50, 2.20, 5.00) and (-1.50, -2.20, 5.00)

What axis did I rotate around?

A general matrix for Z-axis rotation

α° rotation.

$$\begin{pmatrix} x' \\ y' \\ z' \end{pmatrix} = \begin{pmatrix} \cos\alpha & -\sin\alpha & 0 \\ \sin\alpha & \cos\alpha & 0 \\ 0 & 0 & 1 \end{pmatrix} \begin{pmatrix} x \\ y \\ z \end{pmatrix}$$

rotation is always right-handed.

A general matrix for X-axis rotation

$\alpha°$ rotation.

$$\begin{pmatrix} x' \\ y' \\ z' \end{pmatrix} = \begin{pmatrix} 1 & 0 & 0 \\ 0 & \cos\alpha & -\sin\alpha \\ 0 & \sin\alpha & \cos\alpha \end{pmatrix} \begin{pmatrix} x \\ y \\ z \end{pmatrix}$$

A general matrix for y-axis rotation

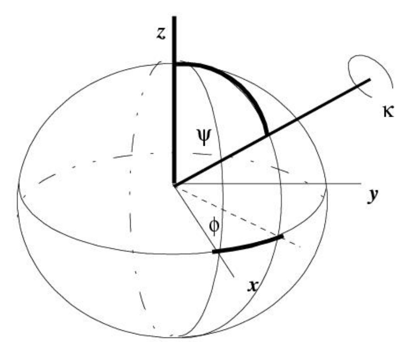
$\alpha°$ rotation.

$$\begin{pmatrix} x' \\ y' \\ z' \end{pmatrix} = \begin{pmatrix} \cos\alpha & 0 & \sin\alpha \\ 0 & 1 & 0 \\ -\sin\alpha & 0 & \cos\alpha \end{pmatrix} \begin{pmatrix} x \\ y \\ z \end{pmatrix}$$

A general matrix for rotation around axis (ϕ,ψ)

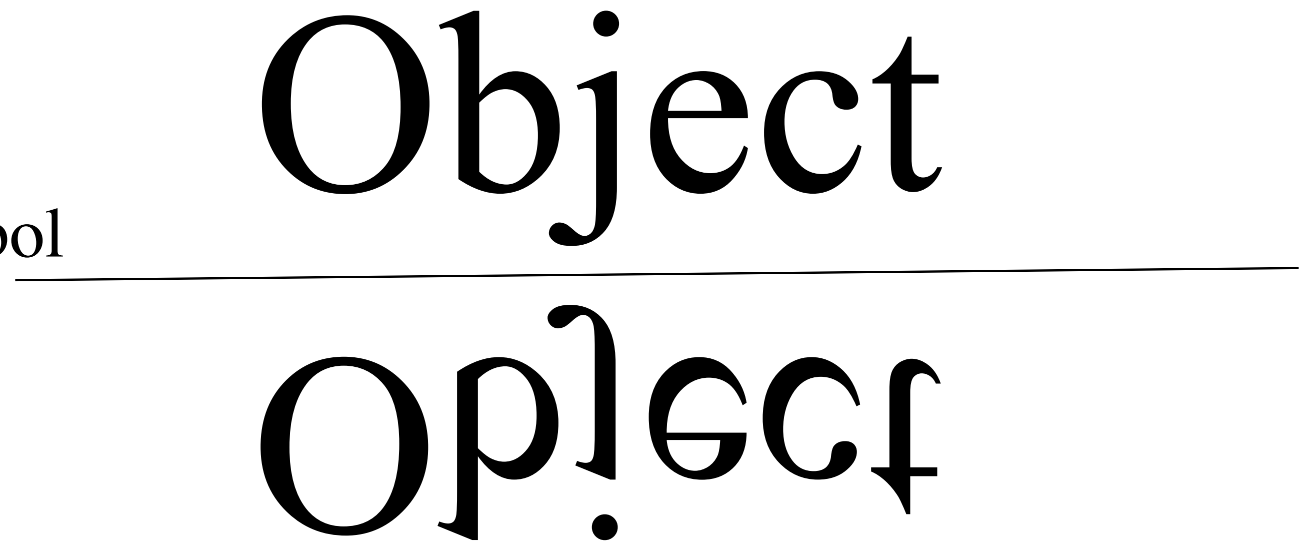
$\kappa°$ rotation.

$$\begin{pmatrix} x' \\ y' \\ z' \end{pmatrix} = \begin{pmatrix} \cos\phi & -\sin\phi & 0 \\ \sin\phi & \cos\phi & 0 \\ 0 & 0 & 1 \end{pmatrix} \begin{pmatrix} 1 & 0 & 0 \\ 0 & \cos\psi & -\sin\psi \\ 0 & \sin\psi & \cos\psi \end{pmatrix} \begin{pmatrix} \cos\kappa & -\sin\kappa & 0 \\ \sin\kappa & \cos\kappa & 0 \\ 0 & 0 & 1 \end{pmatrix} \begin{pmatrix} 1 & 0 & 0 \\ 0 & \cos\psi & \sin\psi \\ 0 & -\sin\psi & \cos\psi \end{pmatrix} \begin{pmatrix} \cos\phi & \sin\phi & 0 \\ -\sin\phi & \cos\phi & 0 \\ 0 & 0 & 1 \end{pmatrix} \begin{pmatrix} x \\ y \\ z \end{pmatrix}$$



mirror plane

$$\begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & -1 \end{pmatrix} \begin{pmatrix} x \\ y \\ z \end{pmatrix} = \begin{pmatrix} x \\ y \\ -z \end{pmatrix}$$

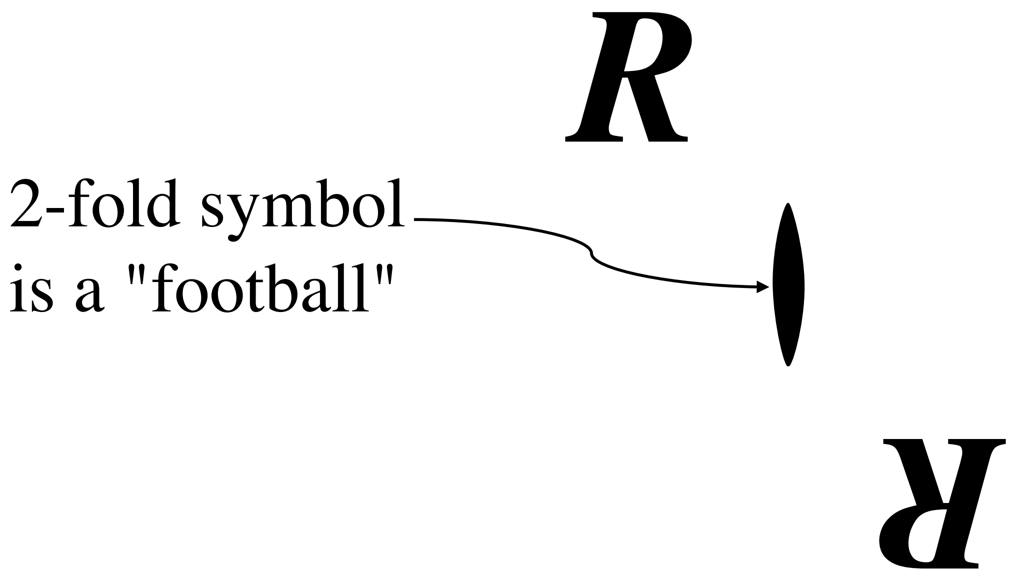


mirror symbol
is a line

centric symmetry

2-fold rotation

$$\begin{pmatrix} -1 & 0 & 0 \\ 0 & -1 & 0 \\ 0 & 0 & 1 \end{pmatrix} \begin{pmatrix} x \\ y \\ z \end{pmatrix} = \begin{pmatrix} -x \\ -y \\ z \end{pmatrix}$$

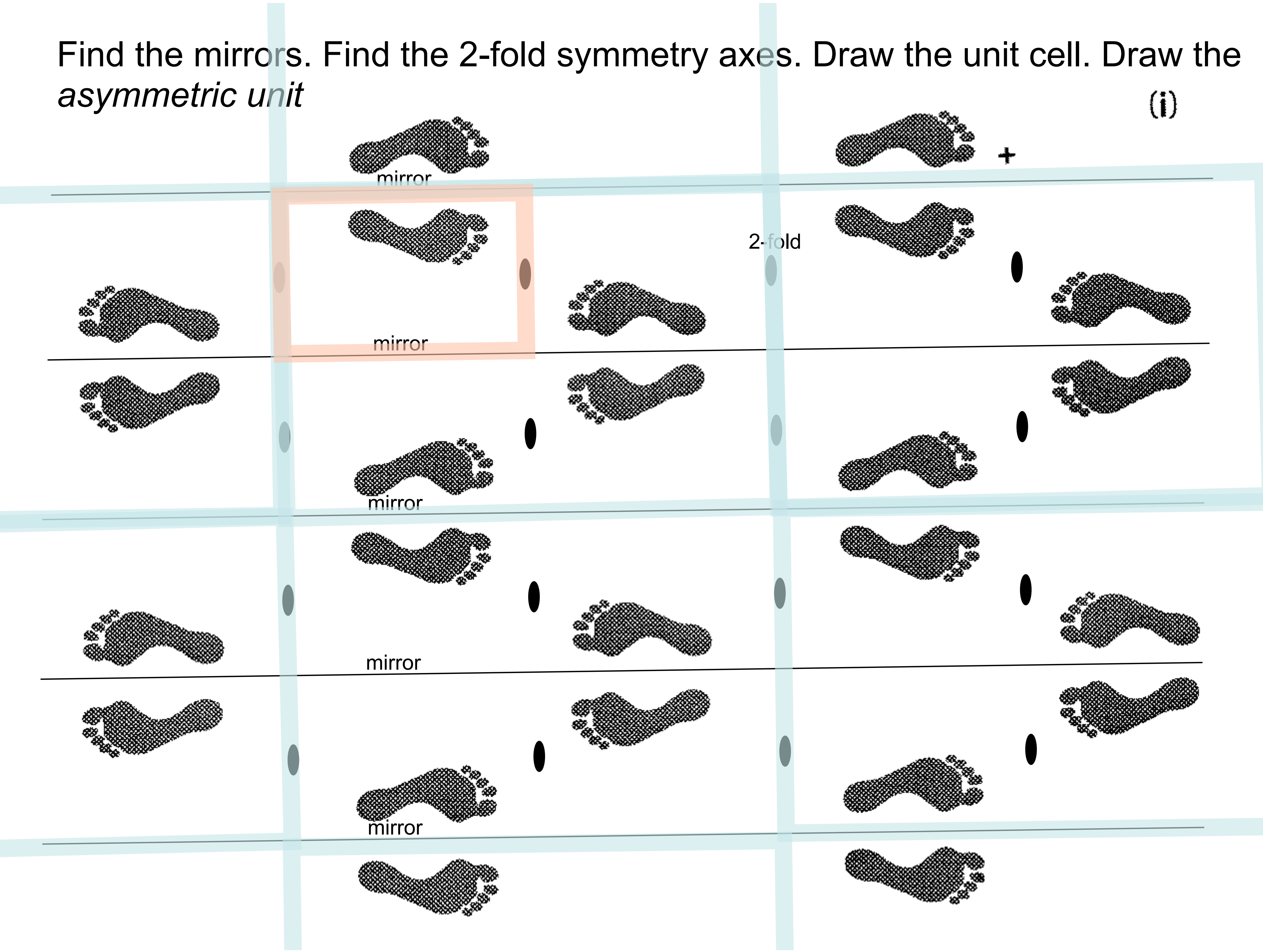


Equivalent positions:

x,y,z   -x,-y,z

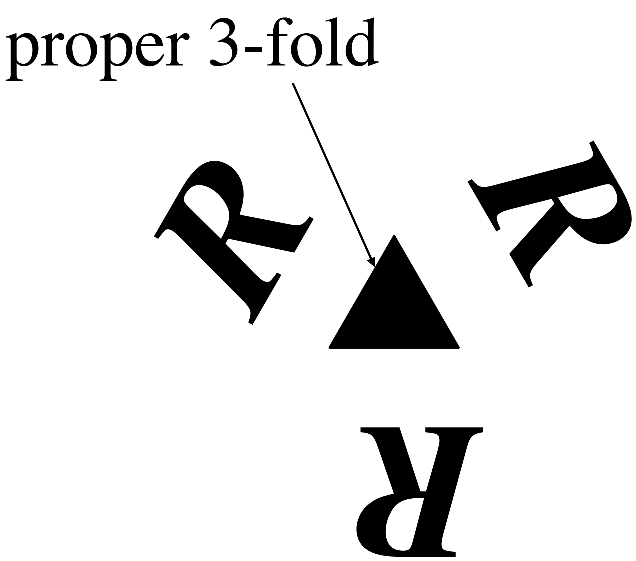
non-centric symmetry

Find the mirrors. Find the 2-fold symmetry axes. Draw the unit cell. Draw the *asymmetric unit*



3-fold rotation

$$\begin{pmatrix} 0 & -1 & 0 \\ 1 & -1 & 0 \\ 0 & 0 & 1 \end{pmatrix} \begin{pmatrix} x \\ y \\ z \end{pmatrix} = \begin{pmatrix} -y \\ x-y \\ z \end{pmatrix}$$

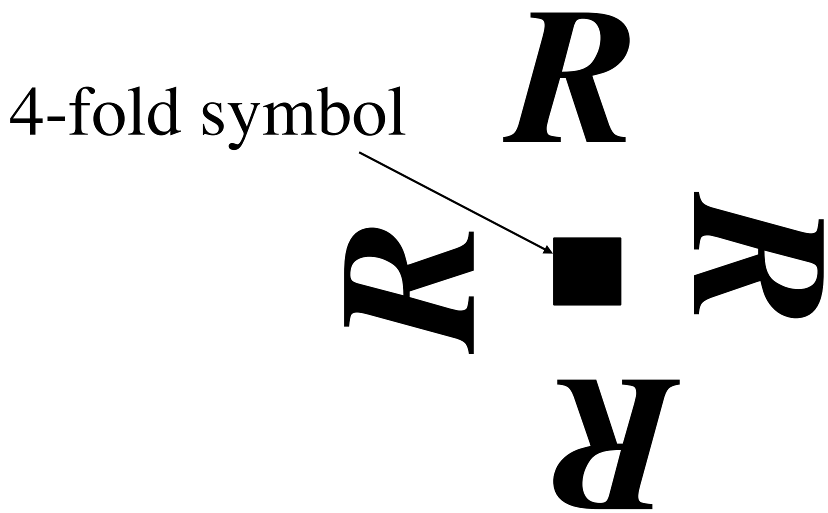


Equivalent positions :

x,y,z -y,x-y,z -x+y,-x,z

4-fold rotation

$$\begin{pmatrix} 0 & -1 & 0 \\ 1 & 0 & 0 \\ 0 & 0 & 1 \end{pmatrix}\begin{pmatrix} x \\ y \\ z \end{pmatrix} = \begin{pmatrix} -y \\ x \\ z \end{pmatrix}$$



Equivalent positions:

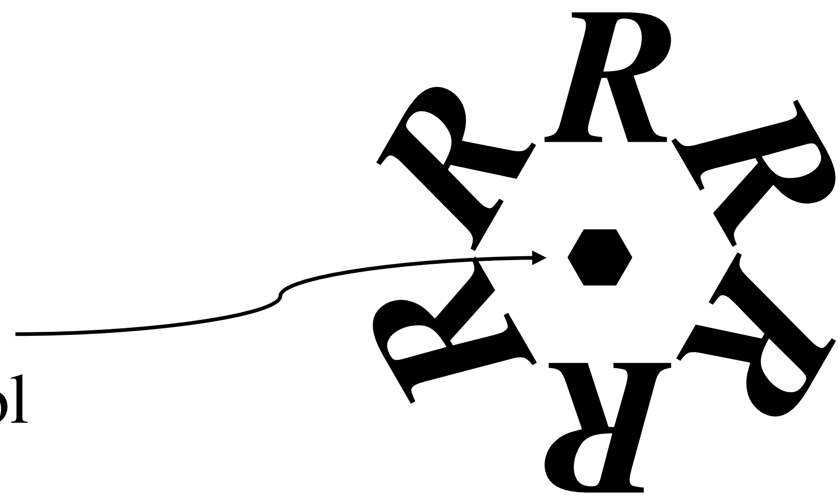
x,y,z   -x, -y,z

-y, x,z   y,-x,z

6-fold rotation

$$\begin{pmatrix} -1 & 1 & 0 \\ -1 & 0 & 0 \\ 0 & 0 & 1 \end{pmatrix} \begin{pmatrix} x \\ y \\ z \end{pmatrix} = \begin{pmatrix} -x + y \\ -x \\ z \end{pmatrix}$$

6-fold symbol

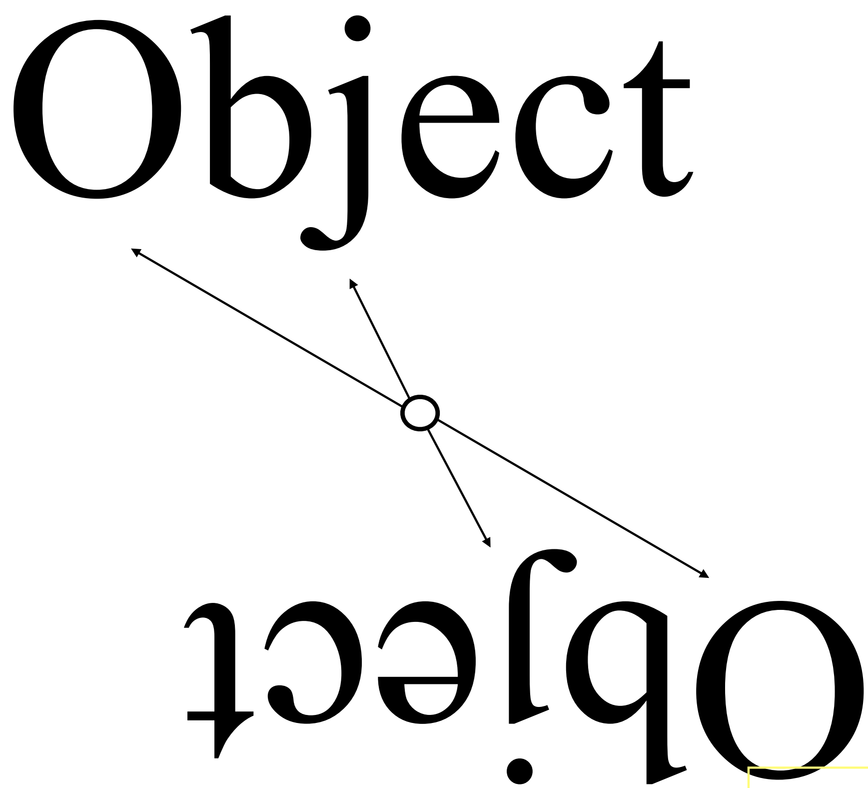


Equivalent positions:

x,y,z	-y,x-y,z	-x+y,-x,z
-x,-y,z	y,-x+y,z	x-y,x,z

point of inversion

$$\begin{pmatrix} -1 & 0 & 0 \\ 0 & -1 & 0 \\ 0 & 0 & -1 \end{pmatrix} \begin{pmatrix} x \\ y \\ z \end{pmatrix} = \begin{pmatrix} -x \\ -y \\ -z \end{pmatrix}$$

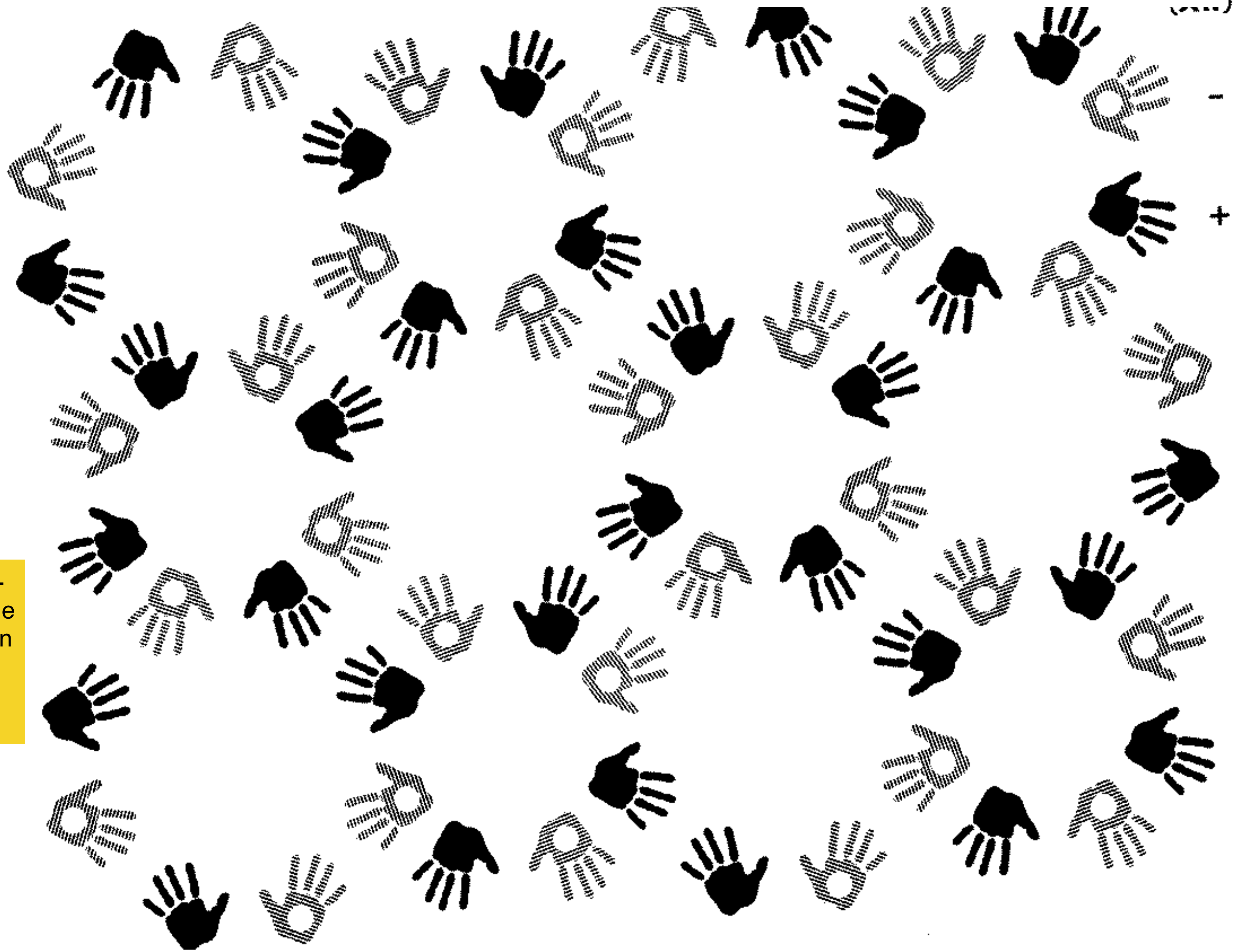


Equivalent positions:

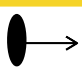
x,y,z   -x,-y,-z

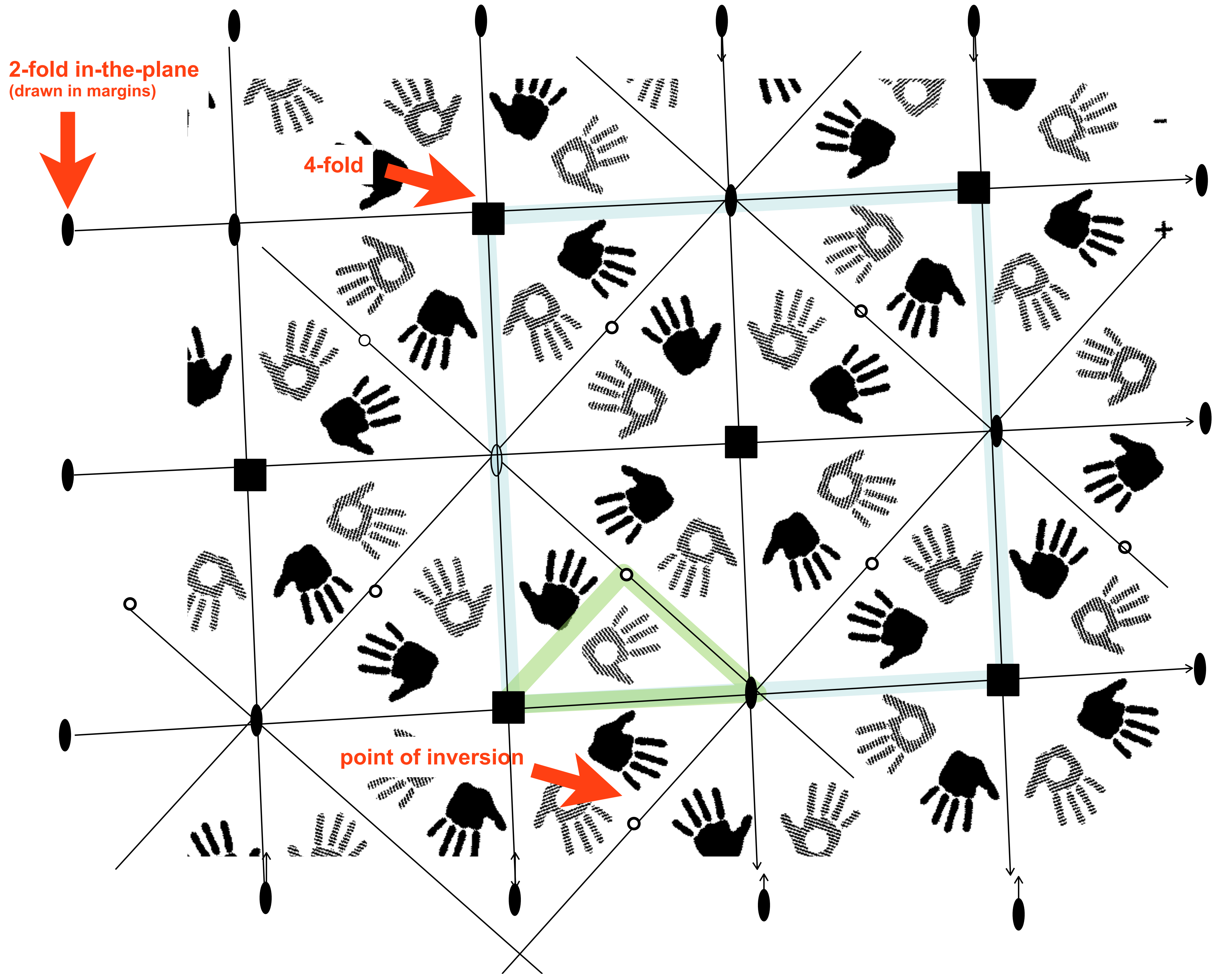
centric symmetry

Where are the mirrors, points of inversion, 2-folds, 4-folds, unit cell, asymetric unit?



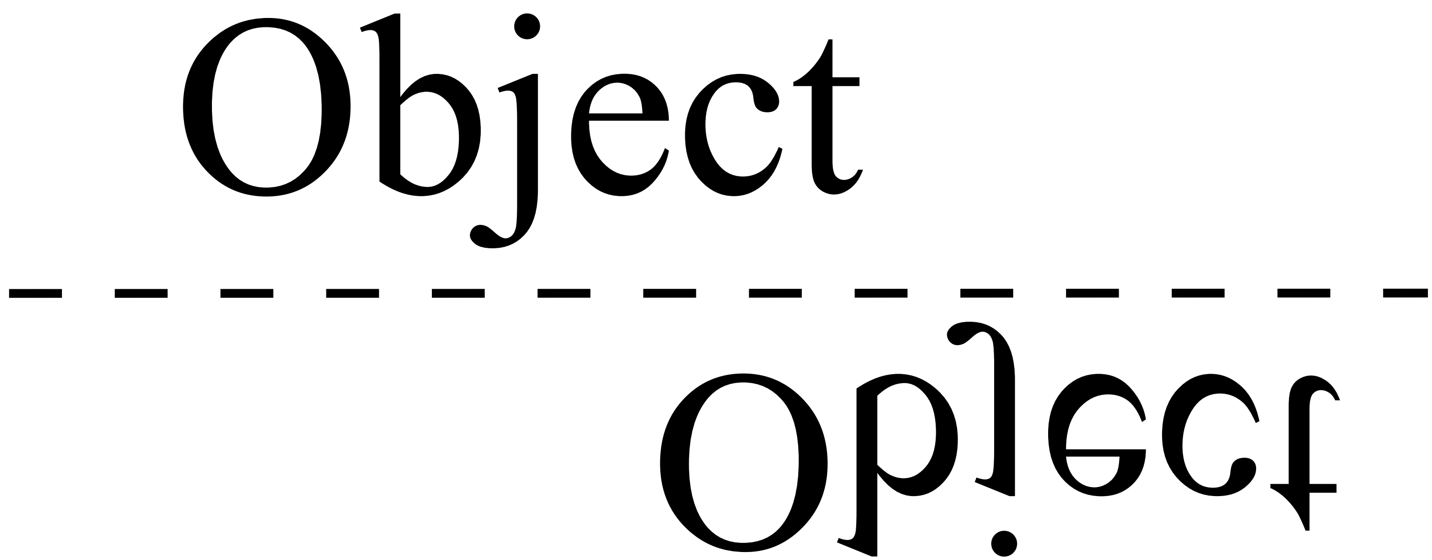
Draw in-the-plane 2-folds in the margins like this




2-fold in-the-plane
(drawn in margins)
4-fold
point of inversion

glide plane

$$\begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & -1 \end{pmatrix} \begin{pmatrix} x \\ y \\ z \end{pmatrix} + \begin{pmatrix} 1/2 \\ 0 \\ 0 \end{pmatrix} = \begin{pmatrix} x + 1/2 \\ y \\ -z \end{pmatrix}$$



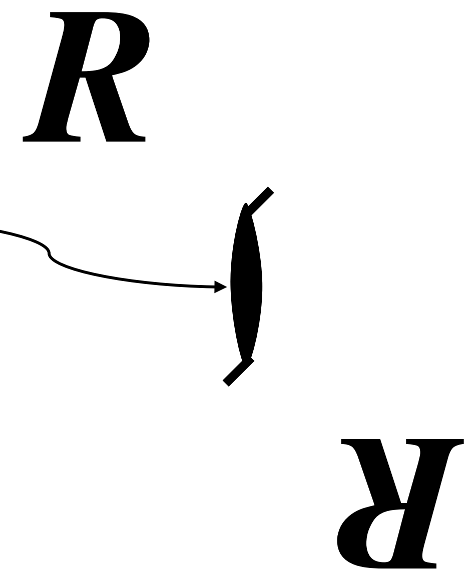
glide plane symbol
is dashed line

This is one example: mirror in xy, glide in x.

2-fold screw rotation

$$\begin{pmatrix} -1 & 0 & 0 \\ 0 & -1 & 0 \\ 0 & 0 & 1 \end{pmatrix} \begin{pmatrix} x \\ y \\ z \end{pmatrix} + \begin{pmatrix} 0 \\ 0 \\ 1/2 \end{pmatrix} = \begin{pmatrix} -x \\ -y \\ z+1/2 \end{pmatrix}$$

2-fold screw symbol is a "football with wings"



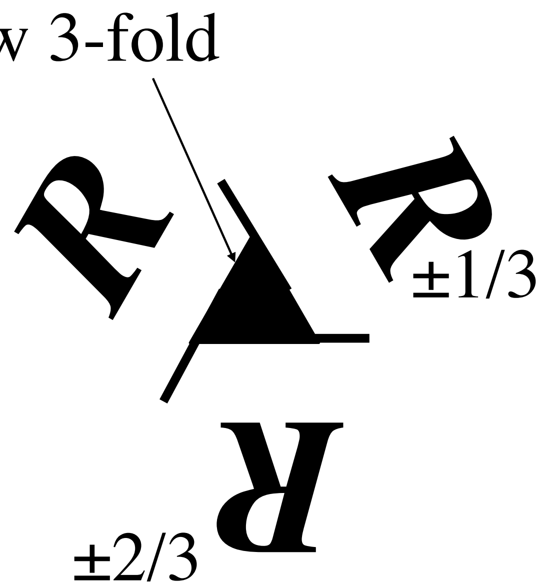
Equivalent positions:

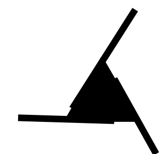
x,y,z -x,-y,z+1/2

non-centric symmetry

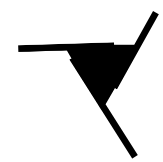
screw 3-fold rotation

$$\begin{pmatrix} 0 & -1 & 0 \\ 1 & -1 & 0 \\ 0 & 0 & 1 \end{pmatrix}\begin{pmatrix} x \\ y \\ z \end{pmatrix} + \begin{pmatrix} 0 \\ 0 \\ \pm 1/3 \end{pmatrix} = \begin{pmatrix} -y \\ x-y \\ z \pm 1/3 \end{pmatrix}$$





minus, L-handed
3-fold screw, 3_2



plus, R-handed
3-fold screw, 3_1

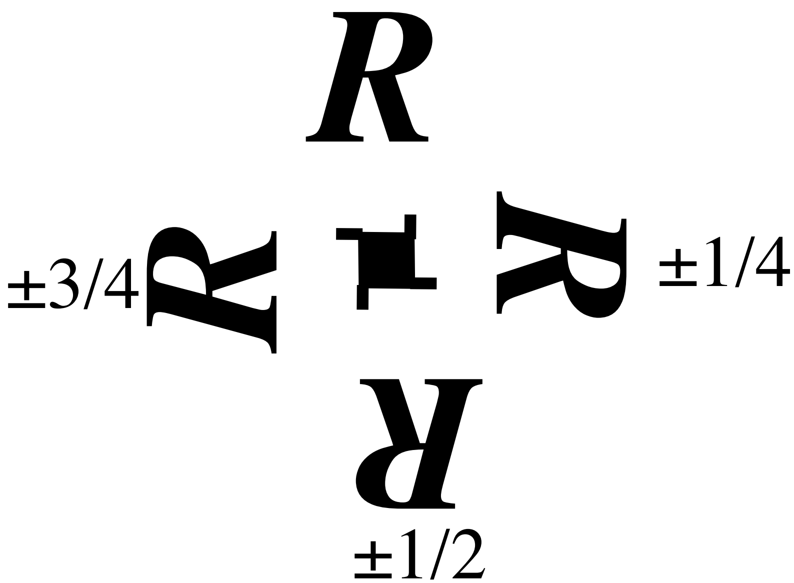
Equivalent positions :

x,y,z -y,x-y,z±1/3 -x+y,-x,z±2/3

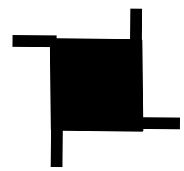
non-centric symmetry

screw 4-fold rotation

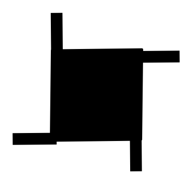
$$\begin{pmatrix} 0 & -1 & 0 \\ 1 & 0 & 0 \\ 0 & 0 & 1 \end{pmatrix} \begin{pmatrix} x \\ y \\ z \end{pmatrix} + \begin{pmatrix} 0 \\ 0 \\ \pm 1/4 \end{pmatrix} = \begin{pmatrix} -y \\ x \\ z \pm 1/4 \end{pmatrix}$$



screw 4-fold symbols



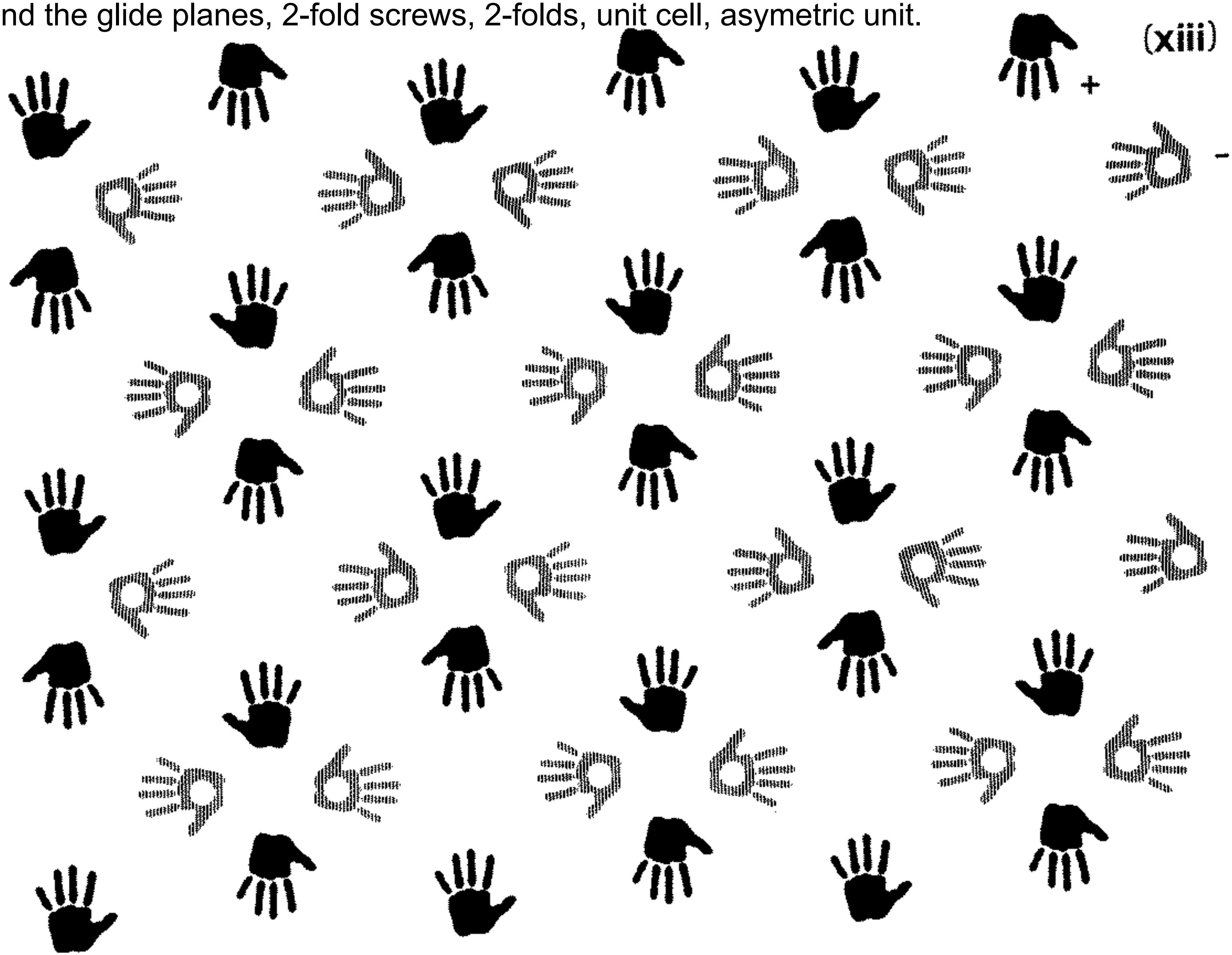
R-handed 4-fold screw 4_1

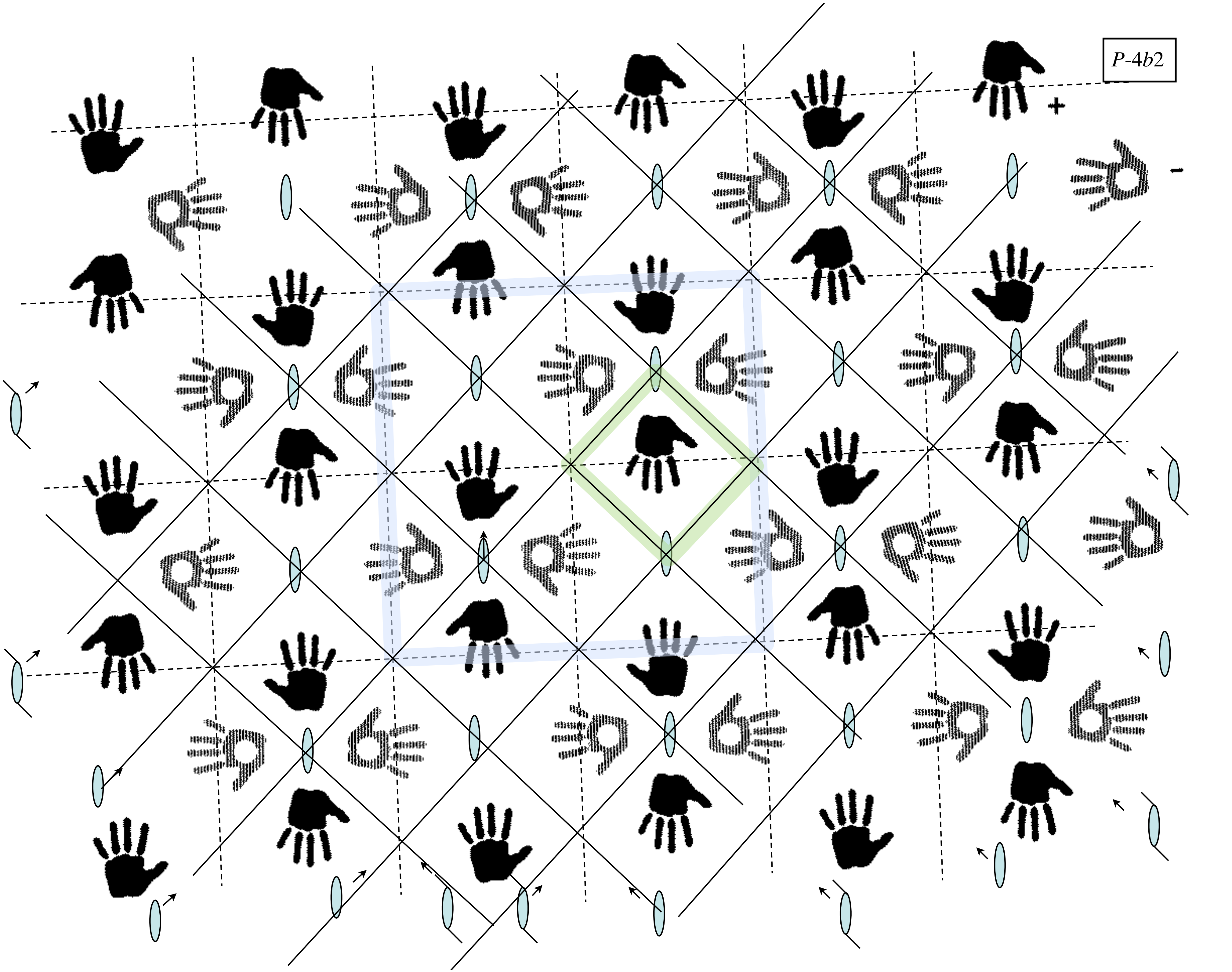


L-handed 4-fold screw 4_3

non-centric symmetry

Find the glide planes, 2-fold screws, 2-folds, unit cell, asymetric unit.





P-4b2
+
-

Exercise 1
*submit to homework server**
by Thurs. Oct 22

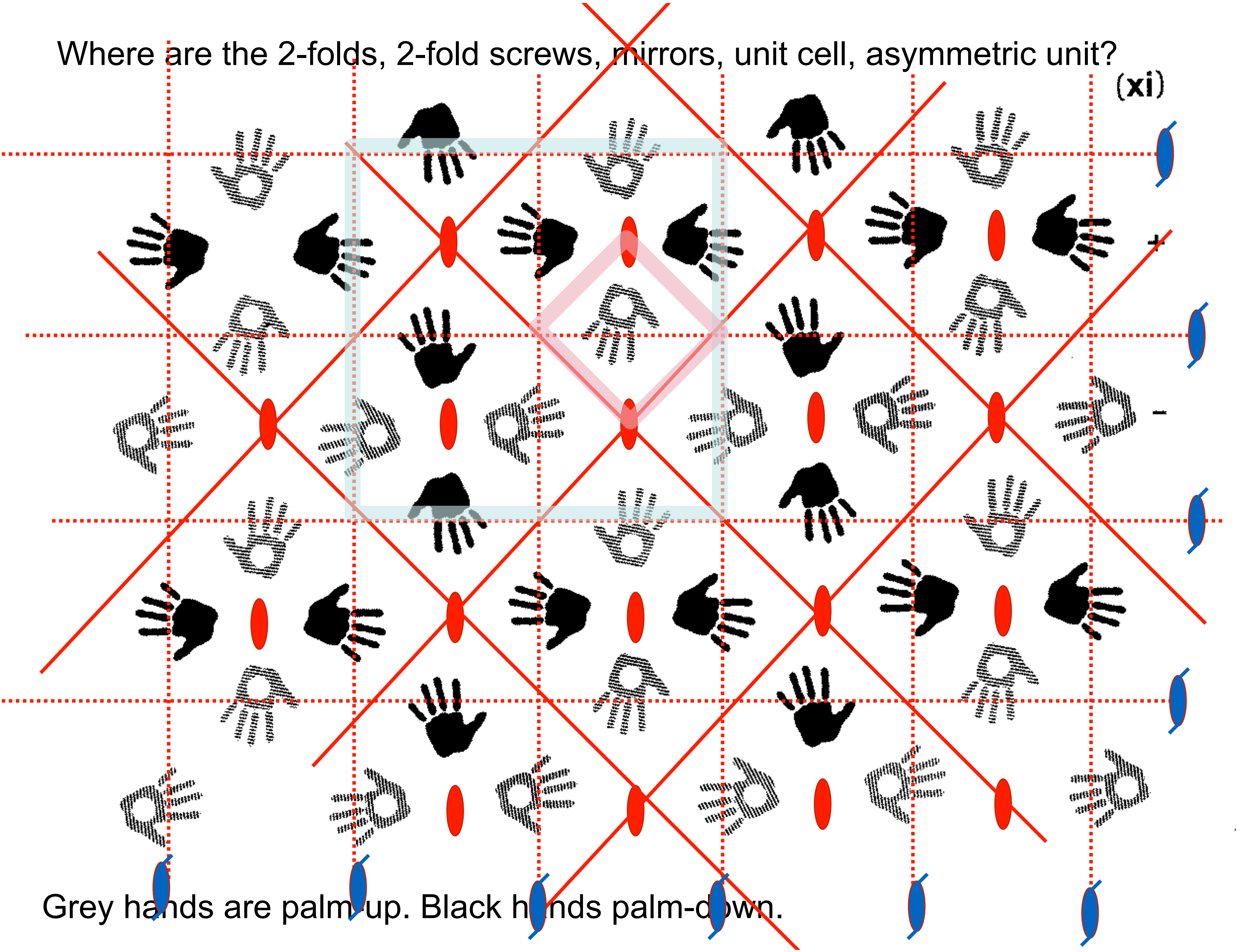
Upload the following pages into Powerpoint or KeyNote

Draw symmetry operators as requested.

Draw unit cell

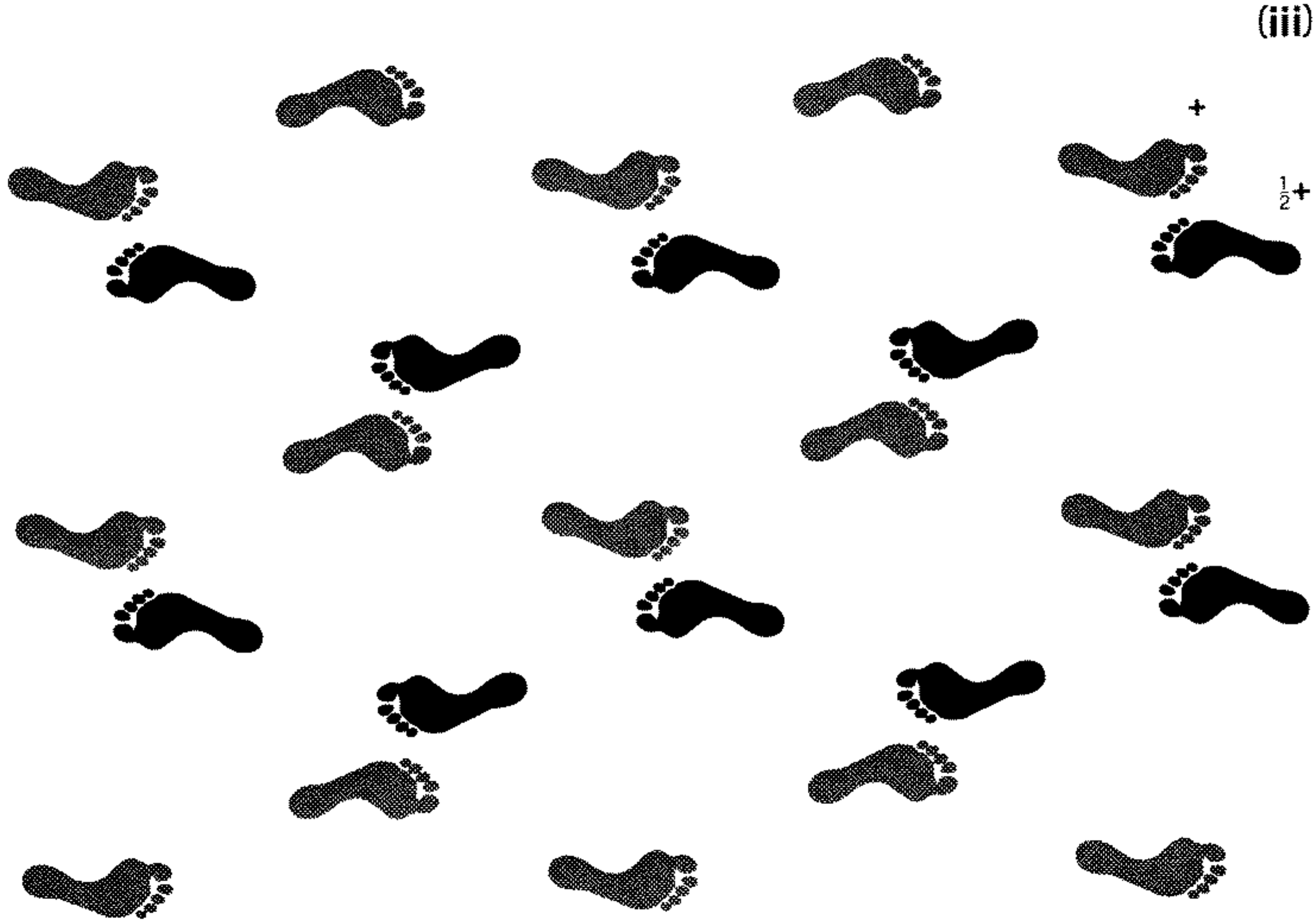
Draw asymmetric unit (using a different color)

Where are the 2-folds, 2-fold screws, mirrors, unit cell, asymmetric unit?



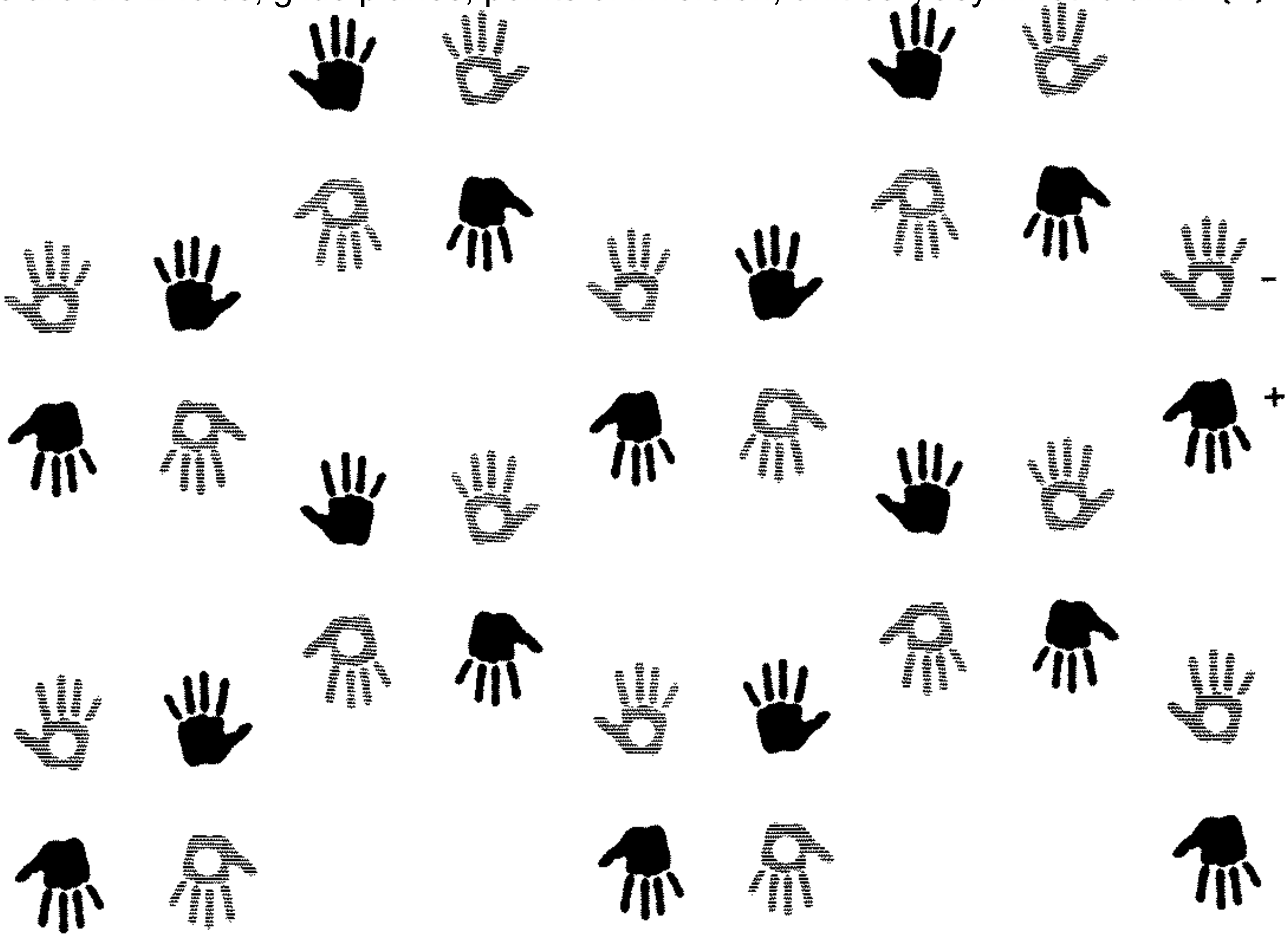
Grey hands are palm-up. Black hands palm-down.

Where are the glide planes, points of inversion, 2-fold screws, unit cell, asymmetric unit?



Grey feet are sole-up. Black feet sole-down.

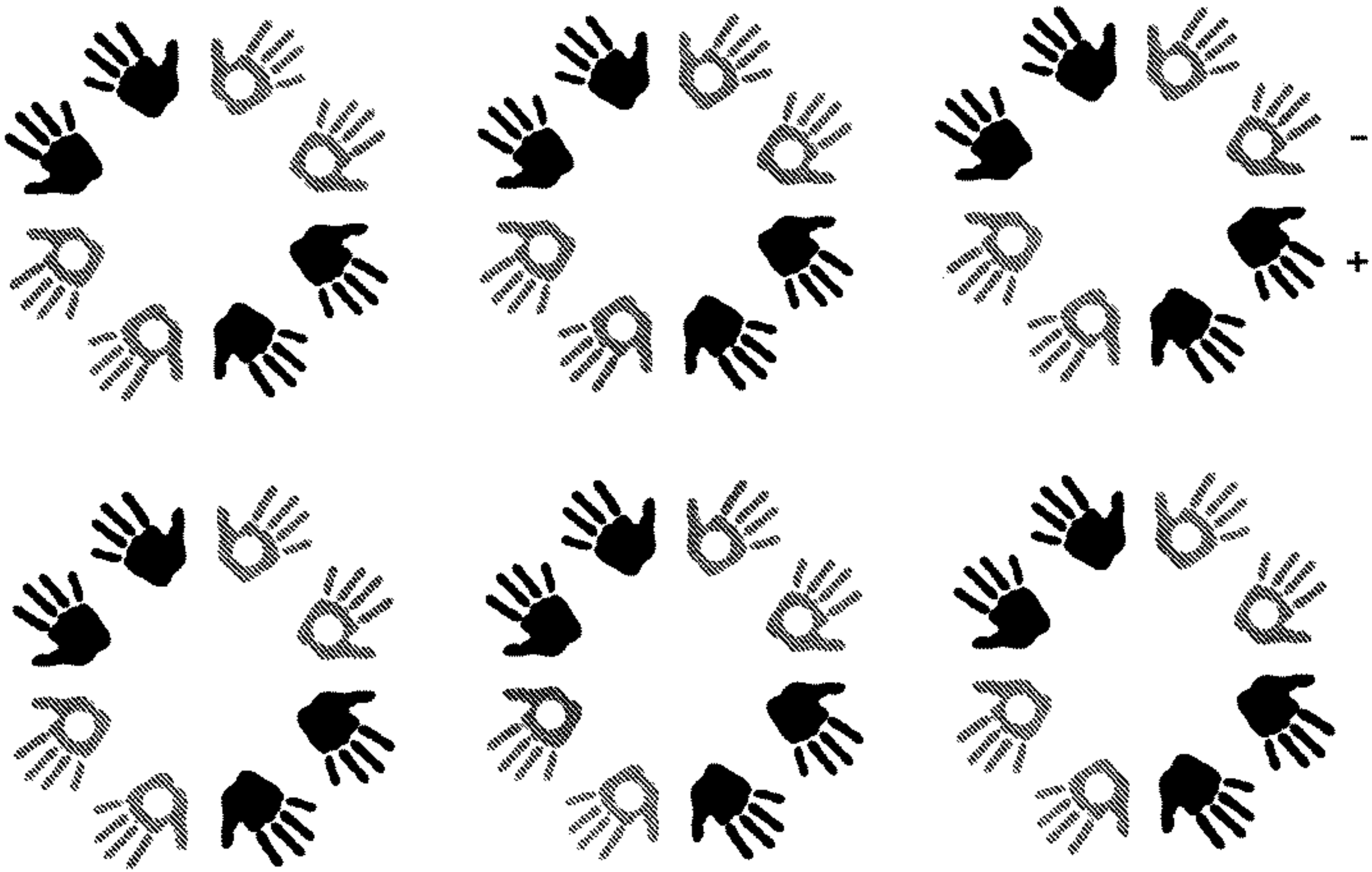
Where are the 2-folds, glide planes, points of inversion, unit cell, asymmetric unit? (x)



Grey hands are palm-up. Black hands palm-down.

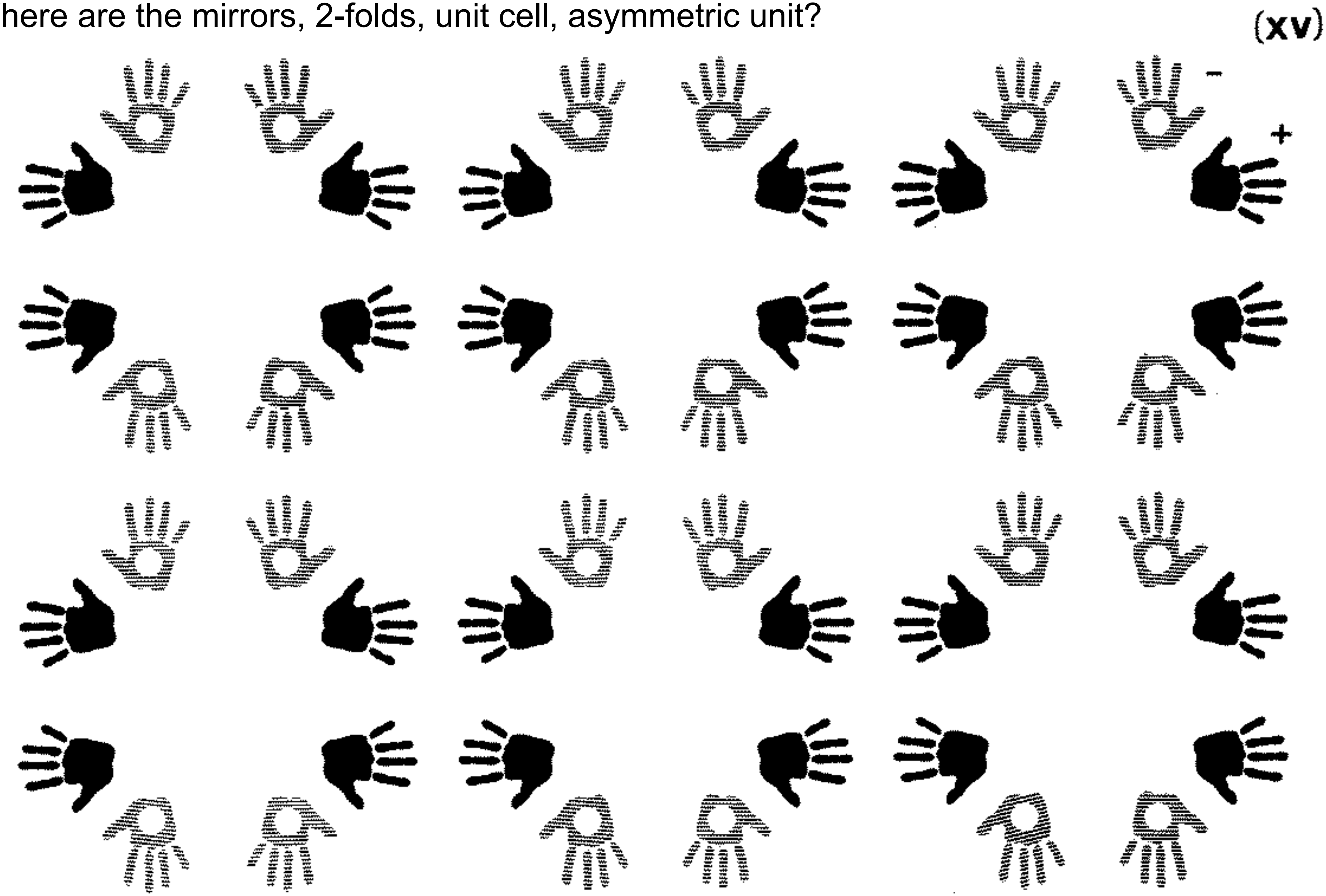
Where are the mirrors, 2-folds,, unit cell, asymmetric unit?

(xiv)



Grey hands are palm-up. Black hands palm-down.

Where are the mirrors, 2-folds, unit cell, asymmetric unit?



Grey hands are palm-up. Black hands palm-down.

centric symmetry

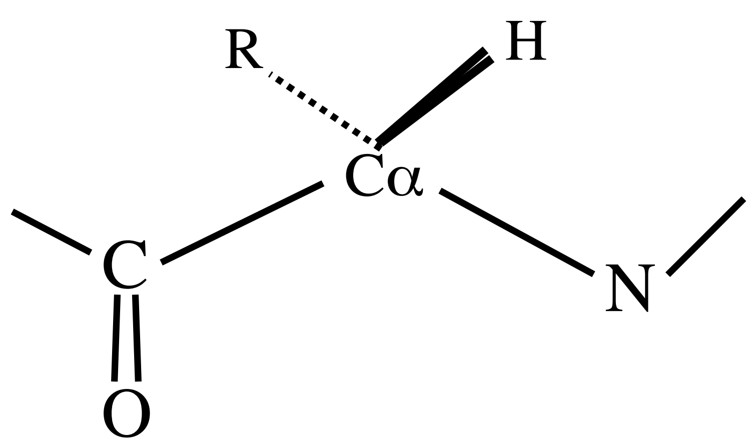
Protein crystals don't have it.

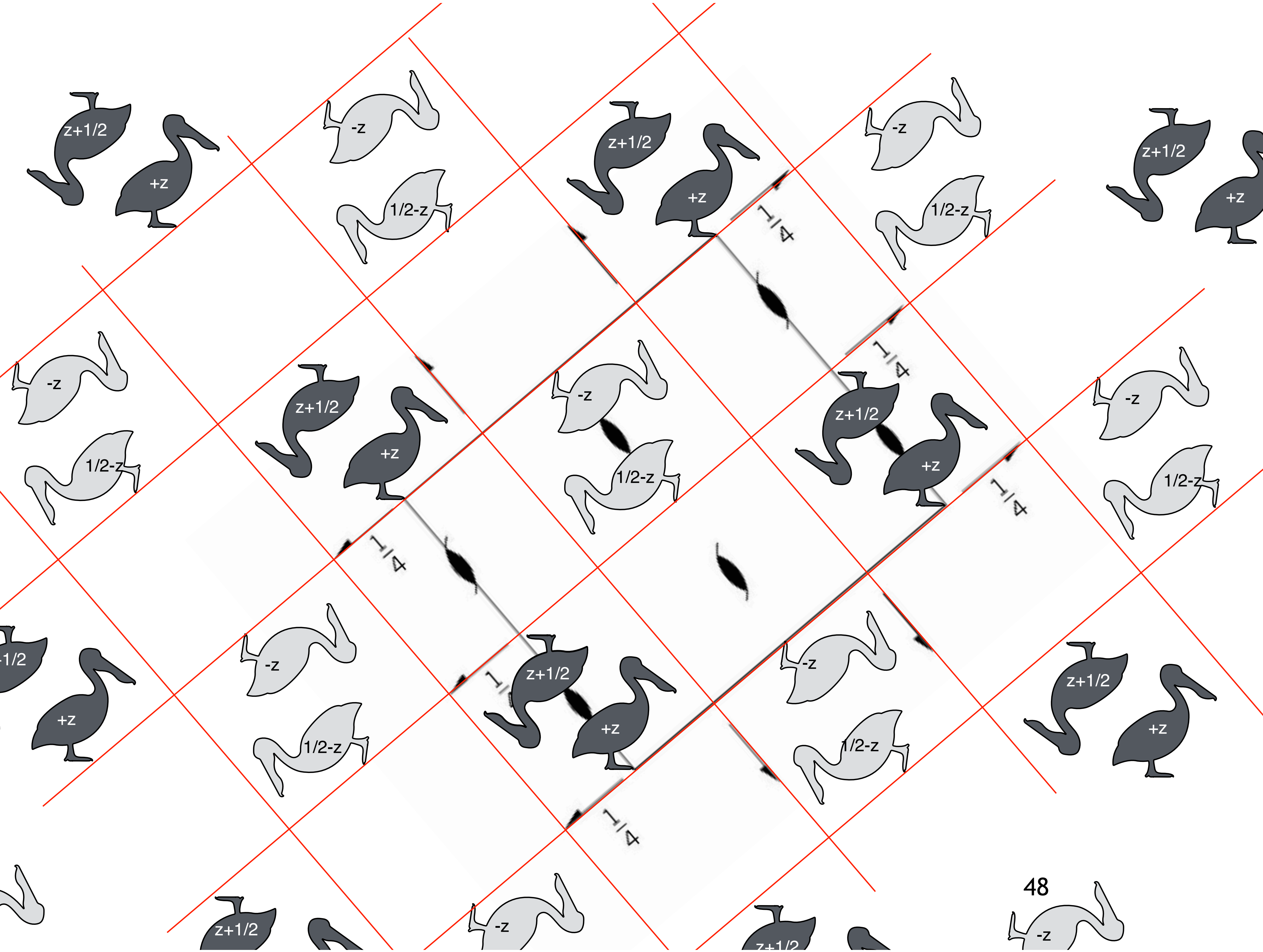
Centric symmetry operators invert the image of he object.
Examples of centric operators:

mirrors, **glide planes**, **points of inversion**

Inverted images cannot be created by pure rotations.

Centric operations would change the chirality of chiral centers such as the alpha-carbon of amino acids or the ribosal carbons of RNA or DNA.




z+1/2
+z
-z
1/2-z
1/4

z+1/2
+z
-z
1/2-z
z+1/2
+z
-z
1/2-z
z+1/2
+z
-z
1/2-z
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