

X-Ray Crystallography

"If a picture is worth a thousand words, then a macromolecular structure is priceless to a physical biochemist." – van Holde

Topics:

1. Protein Data Bank (PDB)

Data mining and Protein Structure Analysis Tools

2. Image Formation

Resolution / Wavelength (Amplitude, Phase) / Light Microscopy / EM / X-ray / (NMR)

3. X-Ray Crystallography (after NMR)



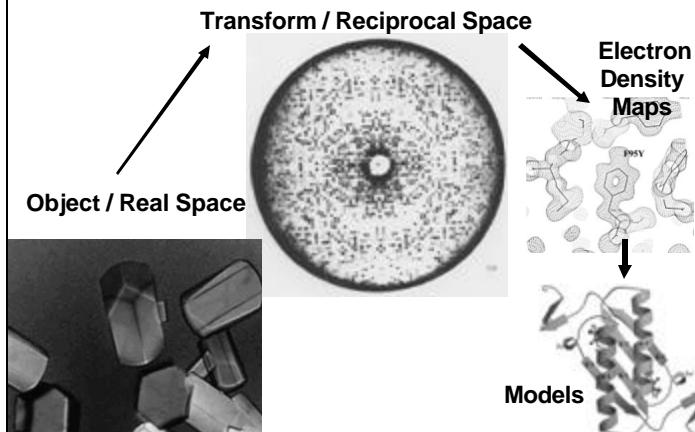
e) Data Collection – Methods / Detectors / Structure Factors

f) Structure Solution – Phase Problem: MIR / MR / MAD

h) Refinements and Models

i) Analysis and presentation of results

Object → Transform → Image



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b) Crystal Lattices - Lattice Constants / Space Groups / Asymmetric Unit

c) X-ray Sources – Sealed Tube / Rotation Anode / Synchrotron

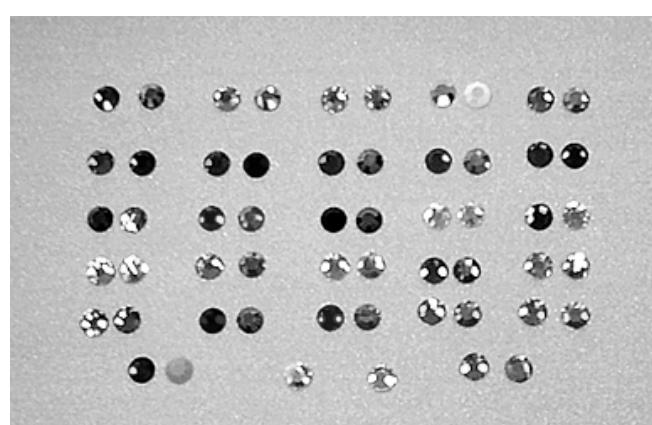
d) Theory of Diffraction – Bragg's Law / Reciprocal Space

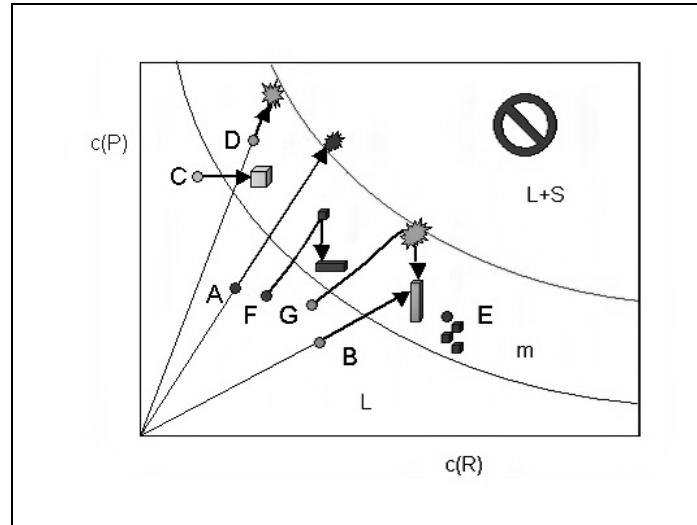
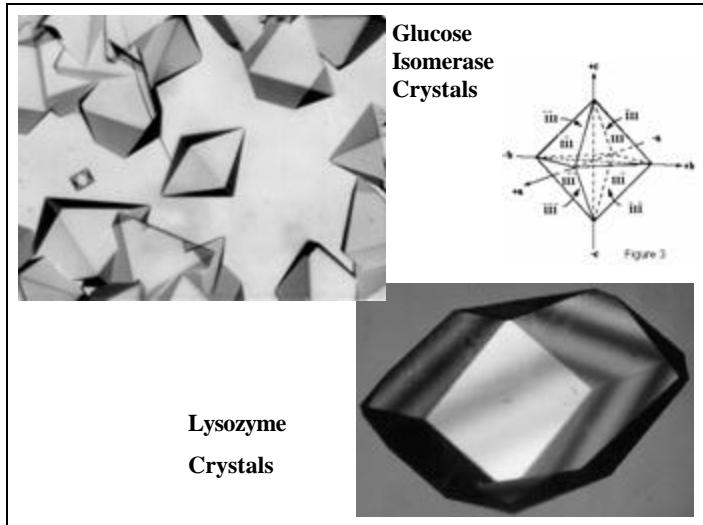
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Types of Crystals
Welcome to the Home Page of Terese Bergfors
"The Protein Crystallization Page" <http://x-ray.bmc.se/~terese/>

Beamsheets: These often begin as spherical droplets that begin to grow from a "seed". They are extremely thin, so they often appear as a single "dot". Often they appear "shiny". The white beam can easily penetrate them, so they are often used for growing from independent nucleation sites in the same drop.

Needles:

Tremendously fine needles growing from a single nucleation centre. Since these needles are much longer, I would not call them a "new world" any longer. Rather the large 3-D crystal growing in the same drop.

Needles: Not too many but at least they are single needles. The nucleation rate is too high which is why you have too many, and too thin. Try reducing the protein or salt concentration. If that does not help, then you may need to get a shape of well around the nucleation site in the droplet drop set up. (See Chapter 9, references here.) See also the Tutorial on needles.

Plates: Two-dimensional. Plates are usually considered an improvement over needles. There are growing from a single nucleation centre and are growing well, which is fine for systematic attempts to grow them as repeatedly and steadily.

A three dimensional crystal: (but check the diffraction before you get out the champagne)

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Tutorial 1. Appearances can be deceiving!

This first thing you should know is that the appearance (dots, morphology, etc.) of your crystals **MUST** when is important. Beautiful looking crystals may not affect secondary light scattering angle diffraction noticeably. The only diffraction proof of a "30000" crystal is its diffraction pattern on the 2D detector. Therefore, do not be fooled by the appearance of your crystals because it is misleading.

Beamsheets: In early stage drops.
Don't be fooled by appearance. These crystals may look very, but they don't affect.

Needles: These are ugly. In fact they are so ugly you probably wouldn't even bother to count them but they diffused to 0.

Plates: Much better... much easier to get the appearance of your crystals from the 2D diffraction patterns that creates.

With that said, you can now continue the tutorial on understanding your crystallization drop.

Variables that influence crystal growth

1. Nature of macromolecule – Purity and concentration of macromolecule
2. Nature and concentration of precipitant
3. pH / Temperature / Pressure
4. Level of reducing agent or oxidant
5. Substrates, coenzymes, and ligands / Metal ions
6. Preparation and storage of macromolecule / Proteolysis and fragmentation
7. Age of macromolecule / Degree of denaturation
8. Vibration and sound
9. Volume of crystallization sample
10. Seeding
11. Amorphous precipitate
12. Buffers
13. Cleanliness
14. Organism or species from which the macromolecule was isolated
15. Gravity, gradients and convection

Common Compounds used in Crystallization

Ammonium or sodium sulfate
Sodium or ammonium citrate
Sodium or ammonium acetate
Magnesium sulfate
Cetyltrimethyl ammonium salts
Polyethylene glycol 400, 1,000, 4,000, 6,000, 15,000 (now also 2,000, 8,000, etc.)

Methods for protein crystallization

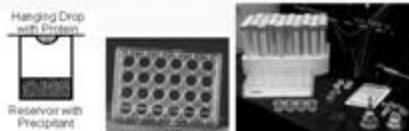
Batch crystallization (simply dump reagents together)
Liquid-liquid diffusion in a capillary tube
Vapor diffusion-the most successful method (hanging drop, sitting drop), typically using a Limbro plate. Equilibration occurs between the liquid and vapor phase.

Dialysis

Hanging Drop Method - Crystal Screening

The Experimental Setup

In order to obtain a crystal, the protein molecule must assemble into a periodic lattice. One way with a solution of the protein, with a fairly high concentration (20–30 mg/ml), and cold reagent that makes the solubility close to spontaneous precipitation. By slow dialysis concentration, and under conditions favorable for the formation of a few nucleation sites, small crystals may start to grow. Often, very many crystals have to be used to succeed. That is usually done by added assistance, followed by a systematical optimization of conditions. Crystallization should be for a few months at a time, in such dimensions as to be useful for diffraction experiments.



Right: The hanging drop technique. Create 24 such hanging drop experiments on one tip in a Latex plate. **Right:** A set of different screening solutions, a set up Latex plate, dialysis bottles and a water bath incubator behind a protective lead.

The next section, relating to glass-prism experiments, is for the **Ising-Bragg** technique. A few millilitres of prismsolutions are mixed with about one-third weight amount of reservoir solution containing the precipitate. A drop of this mixture is placed on a glass dish which contains the reservoir. As the prismsolution/precipitate mixture in the drop is heated (increasing the concentration of reservoir solution), we move the prism solution with the reservoir solution about 1 cm, water evaporates from the drop into the reservoir. As a result the concentration of both prisms and precipitate in the drop sharply increases, and crystals may form. This is a variety of other techniques enabled such as stirring drops, surface heating, and gel and nevertheless techniques. The drop needs to be heated for activation and optimization of crystallization conditions. We have implemented a work comprising series of three **Ising-Bragg** **TESTS**, an intensively efficient strategy arrives for crystallization conditions that you can customize.

Hampton Crystal Screen Solutions



Note :

A mini-screen can be set up from the most successful conditions. Those are indicated in the column labeled Miniscreen.

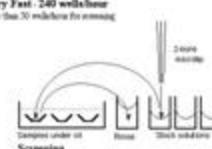
- a nice list of detergents commonly used in crystallization experiments
 - pretty pictures
 - CRYSTOOL efficient random screen made for you here on the WEB.

Tube #	SALT	BUFFER	Precipitant	Miniscreen	Tube #
1	0.02M Calcium Chloride	0.1M Na Acetate pH 4.6	30% w/v 2-methyl-2,4-pentanediol	Y	1
2	None	None	0.4M Na Tartrate tetrahydrate		2
3	None	None	0.4M Ammonium dihydrogen phosphate		3
4	None	0.1M Tris-HCl pH 8.5	2.0M Ammonium Sulfate	Y	4
5	0.2M tri-sodium citrate	0.1M Na HEPES pH 7.5	30% w/v 2-methyl-2,4-pentanediol		5
6	0.2M Magnesium chloride	0.1M Tris-HCl pH 8.5	30% w/v PEG 4000		6
7	None	0.1M Na Acetate pH 4.6	1.4M Lithium sulfate monohydrate		7
8	0.2M tri-sodium citrate	0.1M Na Citrate pH 6.5	30% w/v 2-propanol		8
9	0.2M Ammonium acetate	0.1M Na Citrate pH 5.6	30% w/v PEG 4000	Y	9
10	0.2M Ammonium acetate	0.1M Na Acetate pH 4.6	30% w/v PEG 4000	Y	10
11	None	0.1M Na Citrate pH 5.6	1.0M Ammonium dihydrogen phosphate		11
12	0.2M Magnesium chloride	0.1M Na HEPES pH 7.5	30% v/v 2-propanol		12
13	0.2M tri-sodium citrate	0.1M Tris-HCl pH 8.5	30% w/v PEG 400		13
14	0.2M Calcium Chloride	0.1M Na HEPES pH 7.5	28% w/v PEG 4000		14
15	0.2M Ammonium acetate	0.1M Na Citrate pH 6.5	20% w/v PEG 8000	Y(best)	
16	None	0.1M Na HEPES pH 7.5	1.5M Lithium sulfate monohydrate		16
17	0.2M Li sulfate	0.1M Tdts-HCl pH 8.5	30% w/v PEG 4000	Y(2nd best)	

Using Oryx 6 for Crystallization with Microbatch

Microbiarch operation is identical to IMPAX 1.

<http://www.douglas.co.uk/sry1.htm>



Microstretch suspension finds more traction

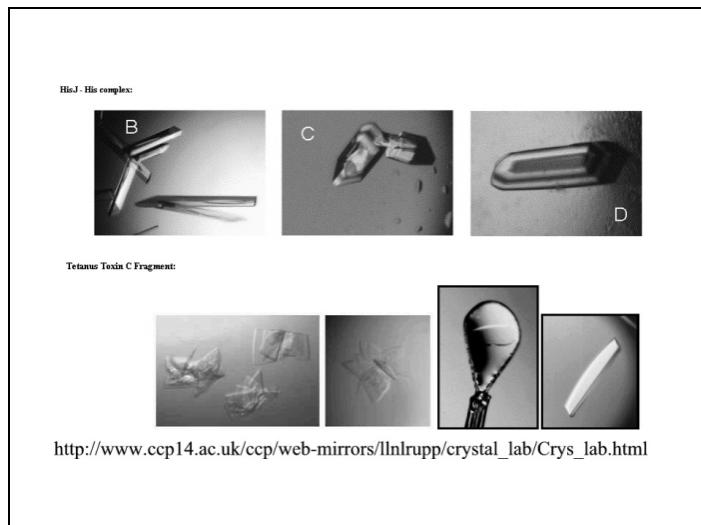
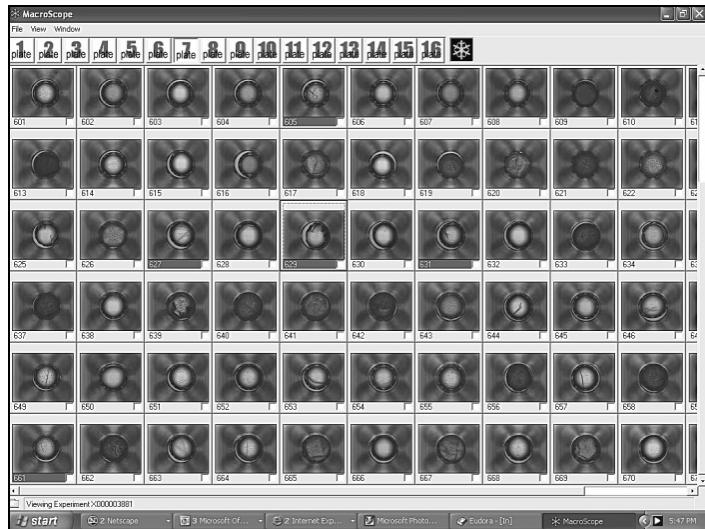
	Associated MS	Associated VS
Prostate removed	9	9
Blowering exhibited	40	40
Rate	1	1
Volume per void	2 ± 0.2	4 ± 0.2
Total volume voided	800 µl	1150 µl
Urination time	2 hr	20 hr
Oxytostimulation condition found	43	40
Oxyper condition	13	13



30 copies of the *luciferase* gene must be injected into 10 eggs which were isolated. Enders, J. *Proc. Am. Acad. Hypnototherapy*, 2, 12 (1978).



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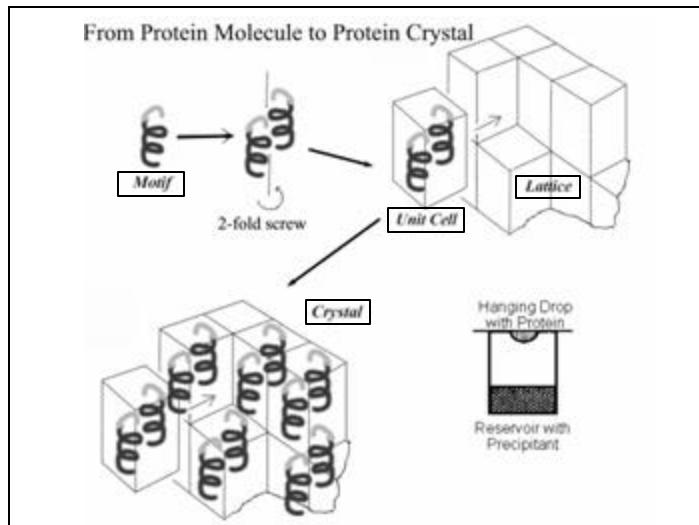


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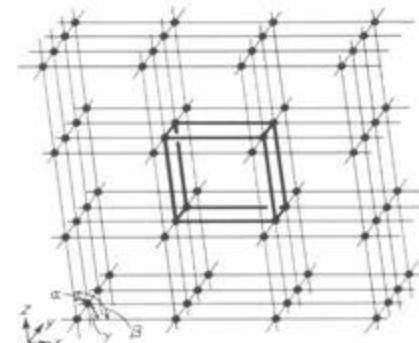
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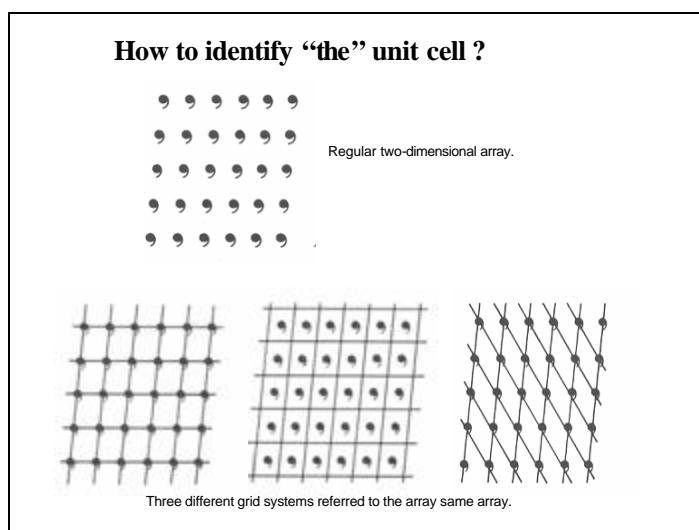


A unit cell is defined by its lattice constants:

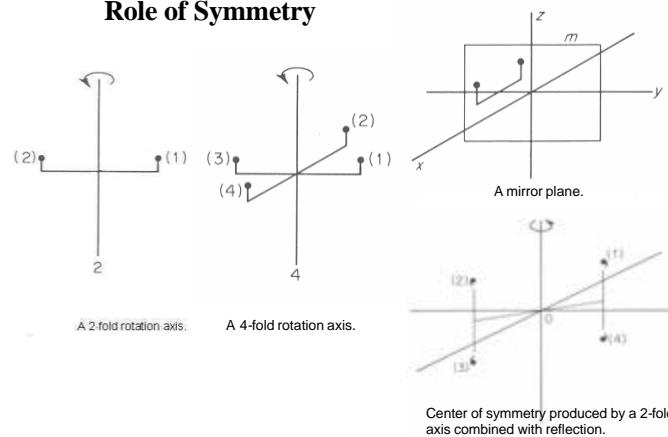
a, b, c and α, β, γ



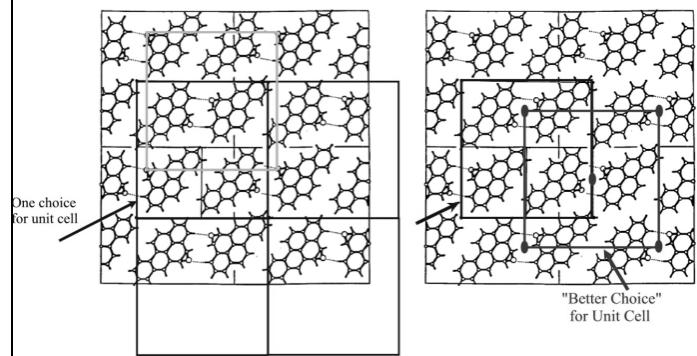
Three-dimensional lattice, showing unit cell (heavy lines).



How to identify “the” unit cell ?
Role of Symmetry



Unit Cell Selection is Based on Symmetry



The Fourteen Bravais Lattices

There are fourteen distinct space groups that a Bravais lattice can have. Thus, from the point of view of symmetry, there are fourteen different kinds of Bravais lattices. Auguste Bravais (1811–1863) was the first to count the categories correctly.



Crystal Systems

Crystal System	Bravais Type(s)	External Minimum Symmetry	Unit Cell Properties
Tetrahedral	P	None	$a, b, c < 4\ell, \alpha, \beta, \gamma$
Monoclinic	P, C	One 2-fold axis, parallel b-(b-tilde)	$a, b, c < 90^\circ, \alpha, \beta, \gamma$
Orthorhombic	P, I, F	Three perpendicular 2-folds	$a, b, c < 90^\circ, 90^\circ, 90^\circ$
Trigonal	P, I	One 3-fold axis, parallel c	$a, b, c < 90^\circ, 90^\circ, 90^\circ$
Trigonal	P, R	One 3-fold axis	$a, b, c < 90^\circ, 90^\circ, 120^\circ$
Hexagonal	P	One 6-fold axis	$a, b, c < 90^\circ, 90^\circ, 120^\circ$
Cubic	F, P, I	Four 3-folds along space diagonal	$a, b, c < 90^\circ, 90^\circ, 90^\circ$

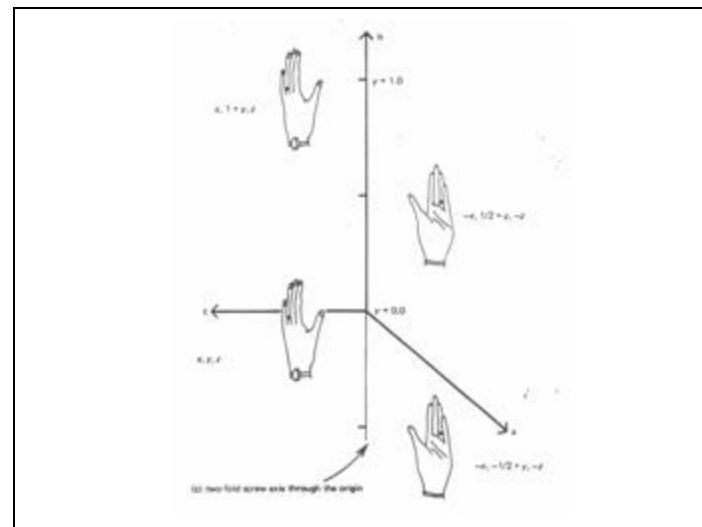
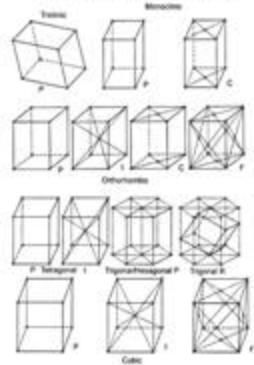
Symmetry operations: 1,2,3,4,6, -1,-2,-3,-4,-6,-m

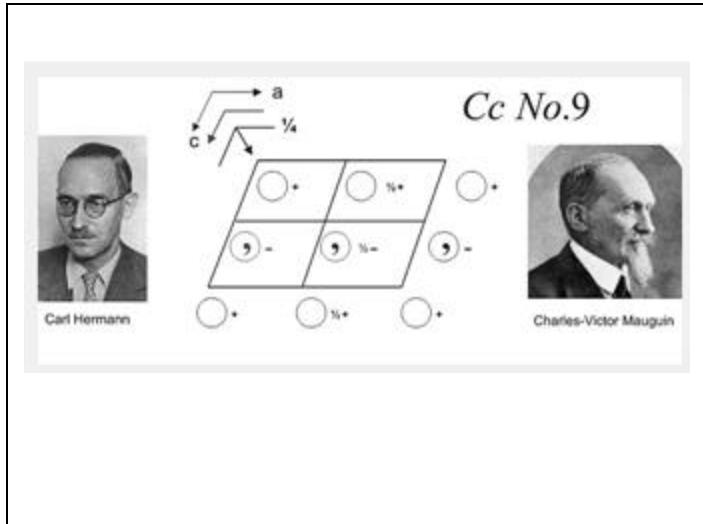
Crystal System	Point group	Lattice Class	Patterson Symmetry
Tetrahedral	1, -1	-1	P-1
Monoclinic	2, m, 2m	2m	P2/m, C2/m
Orthorhombic	222, mm2, mmm	mmm	Pmmm, Cmma, Fmmm, Tmmm
Trigonal	3, -3, 32, 3m, -3 -m	3, -3m	P-3, R-3, P-3m, P-31m, R-3m
Trigonal	3, 3, 32, 3m, -3 -m	3, -3m	P-3, R-3, P-3m, P-31m, R-3m
Hexagonal	6, 6, 6m, 622, 6mm, 62m, 6mm	6mm, 6m, 6mm	P6/m, P6/mmm
Cubic	23, m-3, 432, -3m, m-3m	m-3, m-3m	Pm-3, Im-3, F-3m, Fm-3m, Im-3m

Notes

- Lattice class corresponds to symmetry of reciprocal space (diffraction pattern)
- Patterson symmetry is Lattice class plus altered Bravais centering, i.e. centrosymmetric and synomorph

The 14 Bravais Lattices





Orthorhombic 222	$P\bar{2}_1\bar{2}_1\bar{2}_1$	No. 19	$P\bar{2}_1\bar{2}_1\bar{2}_1$ D_2^4
		Origin halfway between three pairs of non-intersecting screw axes	
Number of positions, Wyckoff notation, and point symmetry	Co-ordinates of equivalent positions	Conditions limiting possible reflections	
4 a 1 $x, y, z; \frac{1}{2} -x, y, \frac{1}{2} +z; \frac{1}{2} +x, \frac{1}{2} -y, z; x, \frac{1}{2} +y, \frac{1}{2} -z.$		$\begin{cases} hkl: & \\ 0kl: & \\ h0l: & \\ h00: & h=2n \\ 0k0: & k=2n \\ 00l: & l=2n \end{cases}$	
	Symmetry of special projections		
(001) $p\bar{gg}$; $a'=a, b'=b$	(100) $p\bar{gg}$; $b'=b, c'=c$	(010) $p\bar{gg}$; $c'=c, a'=a$	

FIGURE 7.2 Part of a Page from "International Tables for X-Ray Crystallography," Volume I.

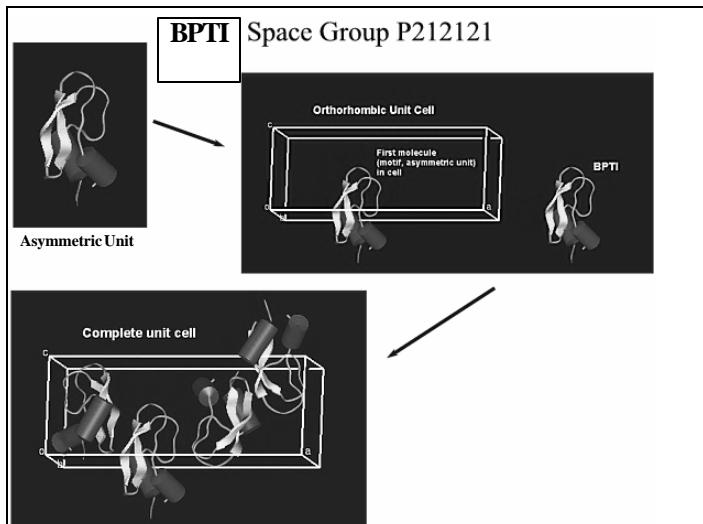


TABLE 7.6 Space Groups in Standard Orientation*						
Space Group	Point Group			Space Group		Enantiomeric
	$P\bar{1}$	$P\bar{2}_1$	$C\bar{2}$	$P\bar{2}_1$	$P\bar{2}_1\bar{2}_1$	
Monoclinic	$\bar{1}$	$\bar{2}_1$	$C\bar{2}$	$P\bar{2}_1$	$P\bar{2}_1\bar{2}_1$	112
$\bar{2}_1$	$\bar{2}_1$	$\bar{2}_1$	$C\bar{2}$	$P\bar{2}_1$	$P\bar{2}_1\bar{2}_1$	112
$\bar{2}/m$	$\bar{2}/m$	$\bar{2}/m$	$C\bar{2}/m$	$P\bar{2}_1$	$P\bar{2}_1\bar{2}_1$	112
$\bar{m}\bar{m}\bar{2}$	$\bar{m}\bar{m}\bar{2}$	$\bar{m}\bar{m}\bar{2}$	$C\bar{2}/m$	$P\bar{2}_1$	$P\bar{2}_1\bar{2}_1$	112
$\bar{m}\bar{2}\bar{m}$	$\bar{m}\bar{2}\bar{m}$	$\bar{m}\bar{2}\bar{m}$	$C\bar{2}/m$	$P\bar{2}_1$	$P\bar{2}_1\bar{2}_1$	112
$\bar{2}\bar{2}\bar{2}$	$\bar{2}\bar{2}\bar{2}$	$\bar{2}\bar{2}\bar{2}$	$C\bar{2}/m$	$P\bar{2}_1$	$P\bar{2}_1\bar{2}_1$	112
Orthorhombic	$\bar{1}\bar{1}\bar{1}$	$\bar{2}\bar{2}\bar{2}$	$\bar{2}\bar{2}\bar{2}$	$P\bar{2}_1\bar{2}_1\bar{2}_1$	$P\bar{2}_1\bar{2}_1\bar{2}_1$	112
$\bar{2}\bar{2}\bar{1}$	$\bar{2}\bar{2}\bar{1}$	$\bar{2}\bar{2}\bar{1}$	$\bar{2}\bar{2}\bar{1}$	$P\bar{2}_1\bar{2}_1\bar{1}$	$P\bar{2}_1\bar{2}_1\bar{1}$	112
$\bar{2}\bar{1}\bar{2}$	$\bar{2}\bar{1}\bar{2}$	$\bar{2}\bar{1}\bar{2}$	$\bar{2}\bar{1}\bar{2}$	$P\bar{2}_1\bar{1}\bar{2}$	$P\bar{2}_1\bar{1}\bar{2}$	112
$\bar{1}\bar{1}\bar{2}$	$\bar{1}\bar{1}\bar{2}$	$\bar{1}\bar{1}\bar{2}$	$\bar{1}\bar{1}\bar{2}$	$P\bar{1}\bar{1}\bar{2}$	$P\bar{1}\bar{1}\bar{2}$	112
$\bar{2}\bar{2}\bar{2}\bar{1}$	$\bar{2}\bar{2}\bar{2}\bar{1}$	$\bar{2}\bar{2}\bar{2}\bar{1}$	$\bar{2}\bar{2}\bar{2}\bar{1}$	$P\bar{2}_1\bar{2}_1\bar{2}_1\bar{1}$	$P\bar{2}_1\bar{2}_1\bar{2}_1\bar{1}$	112
$\bar{2}\bar{2}\bar{1}\bar{2}$	$\bar{2}\bar{2}\bar{1}\bar{2}$	$\bar{2}\bar{2}\bar{1}\bar{2}$	$\bar{2}\bar{2}\bar{1}\bar{2}$	$P\bar{2}_1\bar{2}_1\bar{1}\bar{2}$	$P\bar{2}_1\bar{2}_1\bar{1}\bar{2}$	112
$\bar{1}\bar{1}\bar{2}\bar{2}$	$\bar{1}\bar{1}\bar{2}\bar{2}$	$\bar{1}\bar{1}\bar{2}\bar{2}$	$\bar{1}\bar{1}\bar{2}\bar{2}$	$P\bar{1}\bar{1}\bar{2}\bar{2}$	$P\bar{1}\bar{1}\bar{2}\bar{2}$	112
Tetragonal	$\bar{1}\bar{1}\bar{1}\bar{1}$	$\bar{2}\bar{2}\bar{2}\bar{2}$	$\bar{2}\bar{2}\bar{2}\bar{2}$	$P\bar{2}_1\bar{2}_1\bar{2}_1\bar{2}_1$	$P\bar{2}_1\bar{2}_1\bar{2}_1\bar{2}_1$	112
$\bar{2}\bar{2}\bar{1}\bar{1}$	$\bar{2}\bar{2}\bar{1}\bar{1}$	$\bar{2}\bar{2}\bar{1}\bar{1}$	$\bar{2}\bar{2}\bar{1}\bar{1}$	$P\bar{2}_1\bar{2}_1\bar{1}\bar{1}$	$P\bar{2}_1\bar{2}_1\bar{1}\bar{1}$	112
$\bar{1}\bar{1}\bar{2}\bar{2}$	$\bar{1}\bar{1}\bar{2}\bar{2}$	$\bar{1}\bar{1}\bar{2}\bar{2}$	$\bar{1}\bar{1}\bar{2}\bar{2}$	$P\bar{1}\bar{1}\bar{2}\bar{2}$	$P\bar{1}\bar{1}\bar{2}\bar{2}$	112
$\bar{2}\bar{2}\bar{2}\bar{2}\bar{1}$	$\bar{2}\bar{2}\bar{2}\bar{2}\bar{1}$	$\bar{2}\bar{2}\bar{2}\bar{2}\bar{1}$	$\bar{2}\bar{2}\bar{2}\bar{2}\bar{1}$	$P\bar{2}_1\bar{2}_1\bar{2}_1\bar{2}_1\bar{1}$	$P\bar{2}_1\bar{2}_1\bar{2}_1\bar{2}_1\bar{1}$	112
$\bar{2}\bar{2}\bar{1}\bar{1}\bar{2}$	$\bar{2}\bar{2}\bar{1}\bar{1}\bar{2}$	$\bar{2}\bar{2}\bar{1}\bar{1}\bar{2}$	$\bar{2}\bar{2}\bar{1}\bar{1}\bar{2}$	$P\bar{2}_1\bar{2}_1\bar{1}\bar{1}\bar{2}$	$P\bar{2}_1\bar{2}_1\bar{1}\bar{1}\bar{2}$	112
$\bar{1}\bar{1}\bar{2}\bar{2}\bar{2}$	$\bar{1}\bar{1}\bar{2}\bar{2}\bar{2}$	$\bar{1}\bar{1}\bar{2}\bar{2}\bar{2}$	$\bar{1}\bar{1}\bar{2}\bar{2}\bar{2}$	$P\bar{1}\bar{1}\bar{2}\bar{2}\bar{2}$	$P\bar{1}\bar{1}\bar{2}\bar{2}\bar{2}$	112
Trigonal/Trigonal	$\bar{1}\bar{1}\bar{1}\bar{1}\bar{1}$	$\bar{2}\bar{2}\bar{2}\bar{2}\bar{2}$	$\bar{2}\bar{2}\bar{2}\bar{2}\bar{2}$	$P\bar{2}_1\bar{2}_1\bar{2}_1\bar{2}_1\bar{2}_1$	$P\bar{2}_1\bar{2}_1\bar{2}_1\bar{2}_1\bar{2}_1$	112
$\bar{2}\bar{2}\bar{1}\bar{1}\bar{1}$	$\bar{2}\bar{2}\bar{1}\bar{1}\bar{1}$	$\bar{2}\bar{2}\bar{1}\bar{1}\bar{1}$	$\bar{2}\bar{2}\bar{1}\bar{1}\bar{1}$	$P\bar{2}_1\bar{2}_1\bar{1}\bar{1}\bar{1}$	$P\bar{2}_1\bar{2}_1\bar{1}\bar{1}\bar{1}$	112
$\bar{1}\bar{1}\bar{2}\bar{2}\bar{2}$	$\bar{1}\bar{1}\bar{2}\bar{2}\bar{2}$	$\bar{1}\bar{1}\bar{2}\bar{2}\bar{2}$	$\bar{1}\bar{1}\bar{2}\bar{2}\bar{2}$	$P\bar{1}\bar{1}\bar{2}\bar{2}\bar{2}$	$P\bar{1}\bar{1}\bar{2}\bar{2}\bar{2}$	112
Hexagonal	$\bar{1}\bar{1}\bar{1}\bar{1}\bar{1}\bar{1}$	$\bar{2}\bar{2}\bar{2}\bar{2}\bar{2}\bar{2}$	$\bar{2}\bar{2}\bar{2}\bar{2}\bar{2}\bar{2}$	$P\bar{2}_1\bar{2}_1\bar{2}_1\bar{2}_1\bar{2}_1\bar{2}_1$	$P\bar{2}_1\bar{2}_1\bar{2}_1\bar{2}_1\bar{2}_1\bar{2}_1$	112
$\bar{2}\bar{2}\bar{1}\bar{1}\bar{1}\bar{1}$	$\bar{2}\bar{2}\bar{1}\bar{1}\bar{1}\bar{1}$	$\bar{2}\bar{2}\bar{1}\bar{1}\bar{1}\bar{1}$	$\bar{2}\bar{2}\bar{1}\bar{1}\bar{1}\bar{1}$	$P\bar{2}_1\bar{2}_1\bar{1}\bar{1}\bar{1}\bar{1}$	$P\bar{2}_1\bar{2}_1\bar{1}\bar{1}\bar{1}\bar{1}$	112
$\bar{1}\bar{1}\bar{2}\bar{2}\bar{2}\bar{2}$	$\bar{1}\bar{1}\bar{2}\bar{2}\bar{2}\bar{2}$	$\bar{1}\bar{1}\bar{2}\bar{2}\bar{2}\bar{2}$	$\bar{1}\bar{1}\bar{2}\bar{2}\bar{2}\bar{2}$	$P\bar{1}\bar{1}\bar{2}\bar{2}\bar{2}\bar{2}$	$P\bar{1}\bar{1}\bar{2}\bar{2}\bar{2}\bar{2}$	112
Cubic	$\bar{1}\bar{1}\bar{1}\bar{1}\bar{1}\bar{1}\bar{1}$	$\bar{2}\bar{2}\bar{2}\bar{2}\bar{2}\bar{2}\bar{2}$	$\bar{2}\bar{2}\bar{2}\bar{2}\bar{2}\bar{2}\bar{2}$	$P\bar{2}_1\bar{2}_1\bar{2}_1\bar{2}_1\bar{2}_1\bar{2}_1\bar{2}_1$	$P\bar{2}_1\bar{2}_1\bar{2}_1\bar{2}_1\bar{2}_1\bar{2}_1\bar{2}_1$	112
$\bar{2}\bar{2}\bar{1}\bar{1}\bar{1}\bar{1}\bar{1}$	$\bar{2}\bar{2}\bar{1}\bar{1}\bar{1}\bar{1}\bar{1}$	$\bar{2}\bar{2}\bar{1}\bar{1}\bar{1}\bar{1}\bar{1}$	$\bar{2}\bar{2}\bar{1}\bar{1}\bar{1}\bar{1}\bar{1}$	$P\bar{2}_1\bar{2}_1\bar{1}\bar{1}\bar{1}\bar{1}\bar{1}$	$P\bar{2}_1\bar{2}_1\bar{1}\bar{1}\bar{1}\bar{1}\bar{1}$	112
$\bar{1}\bar{1}\bar{2}\bar{2}\bar{2}\bar{2}\bar{2}$	$\bar{1}\bar{1}\bar{2}\bar{2}\bar{2}\bar{2}\bar{2}$	$\bar{1}\bar{1}\bar{2}\bar{2}\bar{2}\bar{2}\bar{2}$	$\bar{1}\bar{1}\bar{2}\bar{2}\bar{2}\bar{2}\bar{2}$	$P\bar{1}\bar{1}\bar{2}\bar{2}\bar{2}\bar{2}\bar{2}$	$P\bar{1}\bar{1}\bar{2}\bar{2}\bar{2}\bar{2}\bar{2}$	112

*For 112 least symmetries are assigned to horizontal lines.

TABLE 18-5 The 65 "Biological" Space Groups

CRYSTAL SYSTEM	LATTICE	MINIMUM SYMMETRY OF UNIT CELL	UNIT CELL EDGES AND ANGLES ^a	DIFFRACTION PATTERN PATTERN SPACER METRIN ^b	SPACE GROUPS ^c
Tetragonal	P	None	$a = b \neq c$ $\alpha = \beta = \gamma = 90^\circ$	1	P1
Monoclinic	P	3-fold axis parallel to b	$a = b \neq c$ $\alpha = \beta = \gamma = 90^\circ$	2m	P2, P2 ₁ , C2
	C		$a = c \neq b$ $\beta = 90^\circ$		
Orthorhombic	P	3 mutually perpendicular 2-fold axes	$a \neq b \neq c$ $\alpha = \beta = \gamma = 90^\circ$	mmm	P121, P123,2, P223,2, P131,2 C121, C223,2, C311,2 I123, I231,2, I312,2 F123
	C				
	I				
	F				
Tetragonal	P	4-fold axis parallel to c	$a = b = c$ $\alpha = \beta = \gamma = 90^\circ$	4m	P4, P4 ₁ , P4 ₂ , P4 ₃ , P4 ₁ ₂ , P4 ₂ ₂ , P4 ₃ ₂ , P4 ₁₃ , P4 ₂₁ , P4 ₃₁ , P4 ₁₂ , P4 ₂₃ , P4 ₃₂ , P4 ₁₃ ₂ , P4 ₂₁ ₂ , P4 ₃₁ ₂ , P4 ₁₂ ₂ , P4 ₂₃ ₂ , P4 ₃₂ ₂
	I				
Tetragonal orthorhombic	P ₁₃ P ₂₁	3-fold axis parallel to c	$a = b = c$ $\alpha = \beta = \gamma = 90^\circ$	3	R3
					P3 ₁ , P3 ₂ , P3 ₃
					m3
					P3 ₁₂ , P3 ₂₁ , P3 ₃₁ , P3 ₁₃ , P3 ₂₃ , P3 ₃₂
Hexagonal	P	6-fold axis parallel to c	$a = b \neq c$ $\alpha = \beta = 90^\circ$ $\gamma = 120^\circ$	6/m	P6, (P6) ₁ , P6 ₅ , (P6) ₂ , P6 ₄
					P6 ₃ (P6 ₂₁ , P6 ₂₂), P6 ₂₂ , (P6 ₂₂) ₂
Cubic	P	3-fold axes along cube diagonals	$a = b = c$ $\alpha = \beta = \gamma = 90^\circ$	m3	P23, P2 ₃ , {I23, I2 ₃ } F23
	I				P432, (P4 ₃₂ , P4 ₃₂) ₂
	F				P4 ₂₂ , I4 ₃₂ , F4 ₃₂ , F4 ₃₂

X-ray tubes: the “sealed” tube

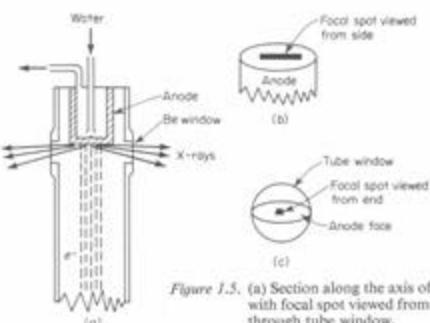


Figure 1.5. (a) Section along the axis of an X-ray tube, (b, Anode with focal spot viewed from side, (c) Focal spot viewed through tube window.

X-Ray Crystallography

"If a picture is worth a thousand words, then a macromolecular structure is priceless to a physical biochemist." – van Holde

Topics:

1. Protein Data Bank (PDB)

Data mining and Protein Structure Analysis Tools

2. Image Formation

Resolution / Wavelength (Amplitude, Phase) / Light Microscopy / EM / X-ray / (NMR)

3. X-Ray Crystallography (after NMR)

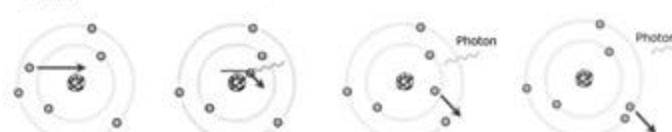
- a) Crystal Growth – Materials / Methods
 - b) Crystal Lattices - Lattice Constants / Space Groups / Asymmetric Unit
 - c) Data Processing – Structure Factor Calculations
 - d) Theory of Diffraction – Bragg's Law / Reciprocal Space
 - e) Data Collection – Methods / Detectors / Structure Factors
 - f) Structure Solution – Phase Problem: MIR / MR / MAD
 - g) Refinements and Models
 - h) Analysis and presentation of results

Origin of Non-characteristic X-rays

Bremsstrahlung X-rays

In an X-ray tube the electrons emitted from the anode are accelerated towards the metal target cathode by an accelerating voltage of typically 50 kV. The high energy electrons interact with the atoms in the metal target. Sometimes the electron comes very close to a nucleus in the target and is deviated by the electromagnetic interaction. In this process, which is called bremsstrahlung (braking radiation), the electron loses much energy and a photon (X-ray) is emitted. The energy of the emitted photon can take any value up to a maximum corresponding to the energy of the incident electron.

The electron (much lighter than the nucleus) comes very close to the nucleus and the electromagnetic interaction causes a deviation of the trajectory where the electron loses energy and an X-ray photon is emitted.



Origin of characteristic X-rays

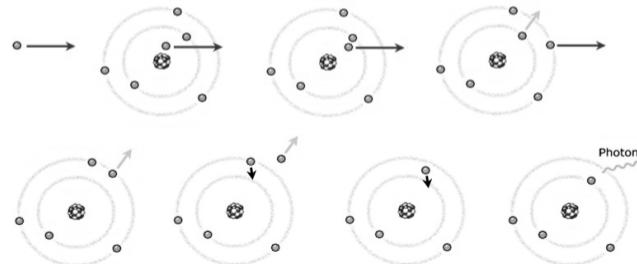
Related Laureate



The Nobel Prize in Physics 1917 - Charles Glover Barkla »

Characteristic X-ray Lines

The high energy electron can also cause an electron close to the nucleus in a metal atom to be knocked out from its place. This vacancy is filled by an electron further out from the nucleus. The well defined difference in binding energy, characteristic of the material, is emitted as a monoenergetic photon. When detected this X-ray photon gives rise to a characteristic X-ray line in the energy spectrum. C. Barkla observed these lines in 1908-09 and was given the 1917 Nobel Prize for this discovery. He also made the first experiments suggesting that the X-rays are electromagnetic waves.



Characteristic X-rays arise from electronic transitions

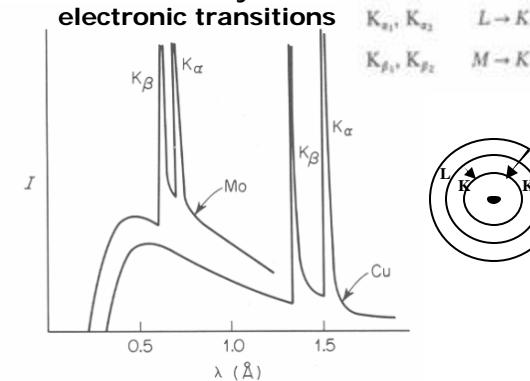


Figure 1.2. X-ray spectra with characteristic peaks: MoK α , 50 kV; CuK α , 35 kV.

Characteristic X-rays have defined λ

Table 1.1. Target Materials and Associated Constants

	Cr	Fe	Cu	Mo
Z	24	26	29	42
α_1 , Å	2.2896	1.9360	1.5405	0.70926
α_2 , Å	2.2935	1.9399	1.5443	0.71354
$\bar{\alpha}$, Å	2.2909	1.9373	1.5418	0.71069
β_1 , Å	2.0848	1.7565	1.3922	0.63225
β , filt.	V, 0.4 mil†	Mn, 0.4 mil	Ni, 0.6 mil	Nb, 3 mils
α , filt.	Ti	Cr	Co	Y
Resolution, Å	1.15	0.95	0.75	0.35
Critical potential, kV	5.99	7.11	8.98	20.0
Operating conditions, kV:	30-40	35-45	35-45	50-55
half- or full-wave-rectified, mA	10	10	20	20
constant potential, mA	7	7	14	14

* $\bar{\alpha}$ is the intensity-weighted average of α_1 and α_2 and is the figure usually used for the wavelength when the two lines are not resolved.

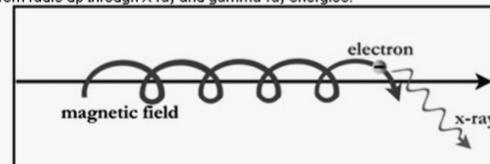
† 1 mil = 0.001 inch = 0.025 mm.

Another Source of "X-rays"

Synchrotron Radiation

X-ray photons can also be created under different conditions. When physicists were operating the first particle accelerators, they discovered that electrons can produce photons without colliding at all. This was possible because the magnetic field in the accelerators was causing the electrons to move in large spirals around magnetic field lines of force. This process is called synchrotron radiation.

In the cosmos particles such as electrons can be accelerated to high energies—near the speed of light—by electric and magnetic fields. These high-energy particles can produce synchrotron photons with wavelengths ranging from radio up through X-ray and gamma-ray energies.



Synchrotron Radiation: Electrons moving in magnetic field radiate photons.

"X-ray" Sources: Beyond X-ray tubes

The **brilliance** of a light source is defined as the number of photons emitted per second, per unit source size, per unit spectral range and for a bandwidth of 1/1000 of the photon energy.

The comparison between various sources of X-rays shows large differences in their brilliance:

X-ray tubes:

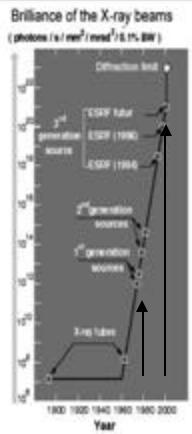
While Conrad Röntgen discovered X-rays in 1895 whilst working with cathode-ray tubes. Using the principle of hot electrons hitting a metal target, a first industrial gain in brilliance was not obtained until the introduction of rotating anode sources (~1920).

Synchrotron Radiation Facilities:

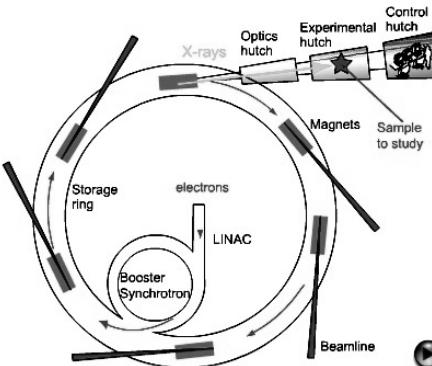
The progress of high energy physics, with the construction of powerful particle accelerators gave birth to what we now call **First generation synchrotron sources** (~1970). Using the deflection of high energy electrons by a magnetic field for the production of X-rays proved so promising that a number of dedicated **Second generation sources** were built (~1980). Building on the combination of needle thin electron beam and Injection Devices, **Third generation synchrotron sources** (~2005) are now resulting synchrotron X-ray beams that are a billion (10^{12}) times more brilliant than those produced by X-ray tubes.

Free Electron X-ray Lasers:

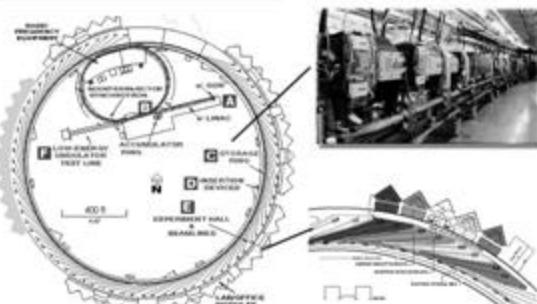
Coupling electron and X-ray beams together, the Free Electron X-ray Lasers currently on the drawing boards could be the **fourth generation** of X-ray sources. While they promise to achieve an increase in peak brilliance by another factor of a million, the first prototypes may be operational around the year 2015.



How synchrotron light is produced?



APS - Advanced Photon Source
Argonne National Laboratory



X-Ray Crystallography

"If a picture is worth a thousand words, then a macromolecular structure is priceless to a physical biochemist." – van Holde

Topics:

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Resolution / Wavelength (Amplitude, Phase) / Light Microscopy / EM / X-ray / (NMR)

3. X-Ray Crystallography (after NMR)

a) Crystal Growth – Materials / Methods

b) Crystal Lattices - Lattice Constants / Space Groups / Asymmetric Unit

c) X-ray Sources – Sealed Tube / Rotation Anode / Synchrotron

e) Data Collection – Methods / Detectors / Structure Factors

f) Structure Solution – Phase Problem: MIR / MR / MAD

h) Refinements and Models

i) Analysis and presentation of results

X-RAYS
X-rays: What Are They? 6/7

X-rays: What Are They? 7/7

More About the Bragg Formula

X-rays scattered from different layers of atoms can interfere with each other. The interference depends on the wavelength of the X-ray and on the distance between the atom layers. An X-ray with well-known wavelength can be used to explore the structure of the crystal. For a well-known crystal, the X-ray properties can be examined.

Incident wave
Scattered wave
 $d = 0.3 \text{ nm}$
 $n\lambda = 2d \sin\theta$

Crystal planes, in NaCl, ordinary salt. Other planes are also possible.

Related Laureates

The Nobel Prize in Physics 1915 - Sir William Henry Bragg
The Nobel Prize in Physics 1915 - William Lawrence Bragg

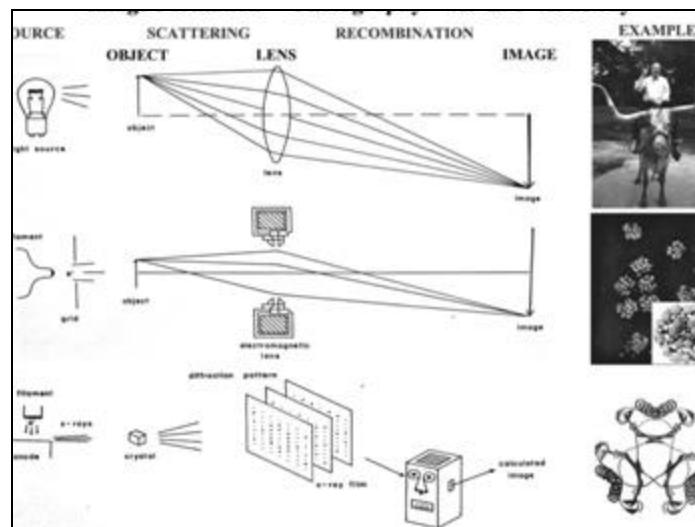
Related Laureates

	The Nobel Prize in Physics 1901 - Wilhelm Conrad Röntgen		The Nobel Prize in Physics 1914 - Max von Laue		The Nobel Prize in Physics 1915 - Sir William Henry Bragg
	The Nobel Prize in Physics 1915 - William Lawrence Bragg		The Nobel Prize in Physics 1917 - Charles Glover Barkla		The Nobel Prize in Physics 1927 - Arthur Holly Compton
	The Nobel Prize in Chemistry 1962 - John Cowdry Kossel		The Nobel Prize in Chemistry 1936 - Peter Josephus Wilhelmus Debye		The Nobel Prize in Chemistry 1962 - Max Ferdinand Perutz
	The Nobel Prize in Physiology or Medicine 1962 - Francis Harry Compton Crick		The Nobel Prize in Chemistry 1964 - Dorothy Crowfoot Hodgkin		The Nobel Prize in Physiology or Medicine 1962 - James Dewey Watson
	The Nobel Prize in Physiology or Medicine 1979 - Allan M. Cormack		The Nobel Prize in Physiology or Medicine 1979 - Godfrey H.ounsfeld		The Nobel Prize in Chemistry 1965 - Herbert A. Hauptman
	The Nobel Prize in Chemistry 1985 - Jerome Karle		The Nobel Prize in Chemistry 1982 - Robert Huber		The Nobel Prize in Chemistry 1980 - Hartmut Michel
	The Nobel Prize in Chemistry 1998 - Johann Deisenhofer				

"If a picture is worth a thousand words, then a macromolecular structure is priceless to a physical biochemist." – van Holde

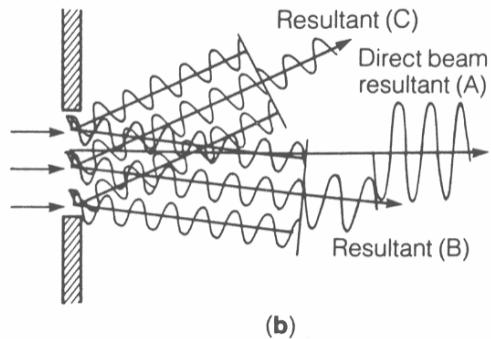
- Light Photography
 $1 \sim 400 - 700 \text{ nm}$
- Electron Microscopy
 $1 \sim 0.001 - 0.1 \text{ nm}$
- X-Ray or NMR
 $1 \sim 0.1 \text{ nm}$

Image Formation
Abbe (~1873):
Limit Res. $\sim \lambda/2$



Single Hole Scattering Experiment

Transforms / Reciprocal Space

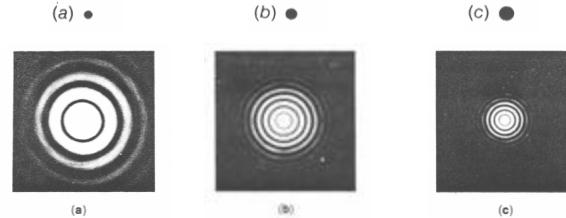


(b)

Single Hole Scattering Experiment

Transforms / Reciprocal Space

Different size holes



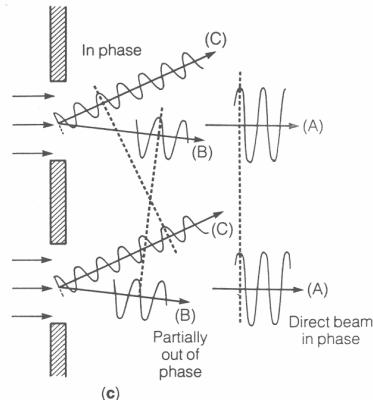
(a)

(b)

(c)

Effect of Multiple "Scatterers"

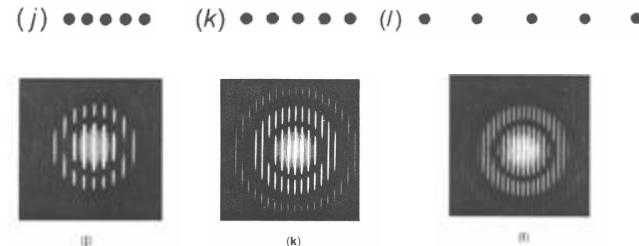
Transforms / Reciprocal Space



(c)

Transforms / Reciprocal Space

Five horizontal holes
with various spacings



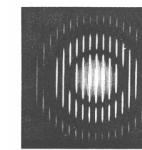
(j) ●●●●●

(k) ●●●●●

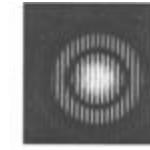
(l) ● ● ● ● ●



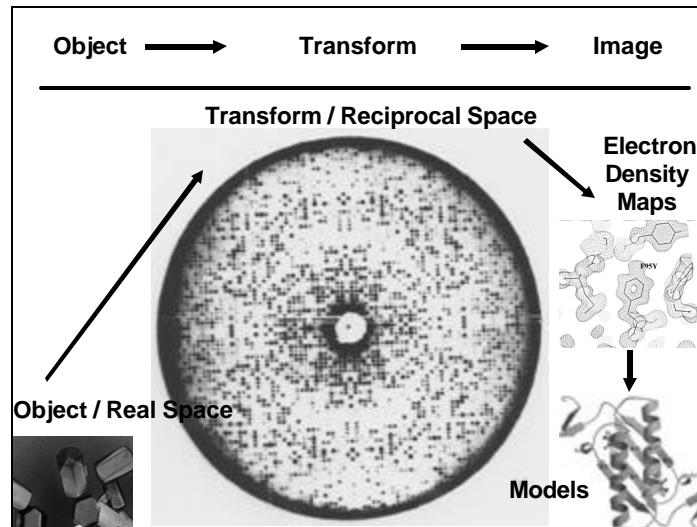
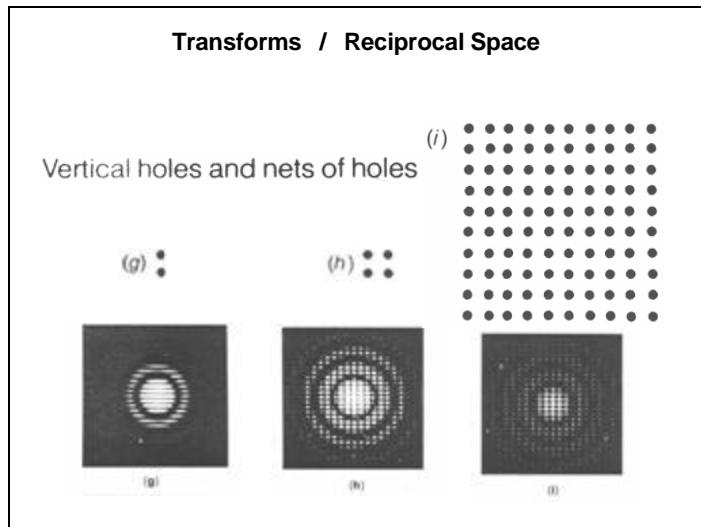
(j)



(k)



(l)

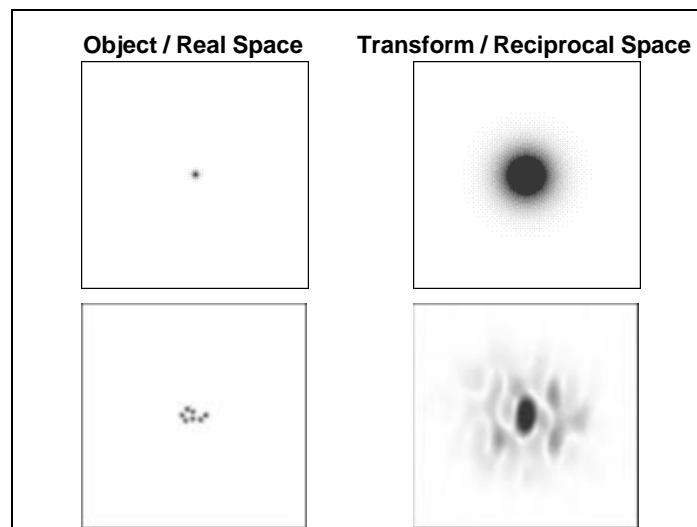


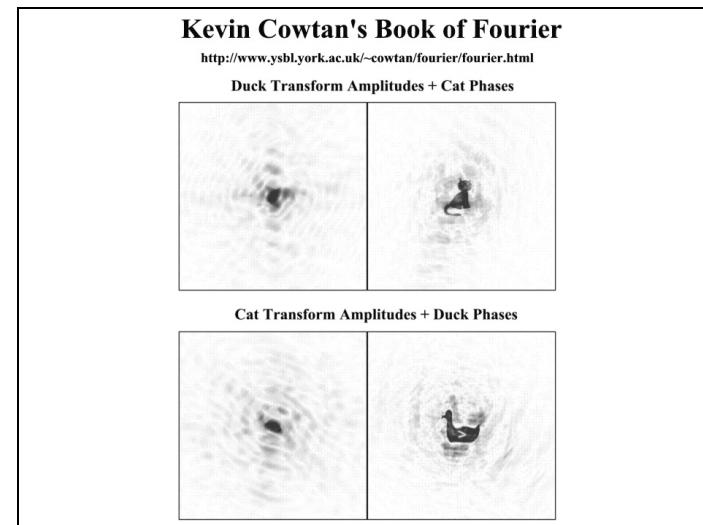
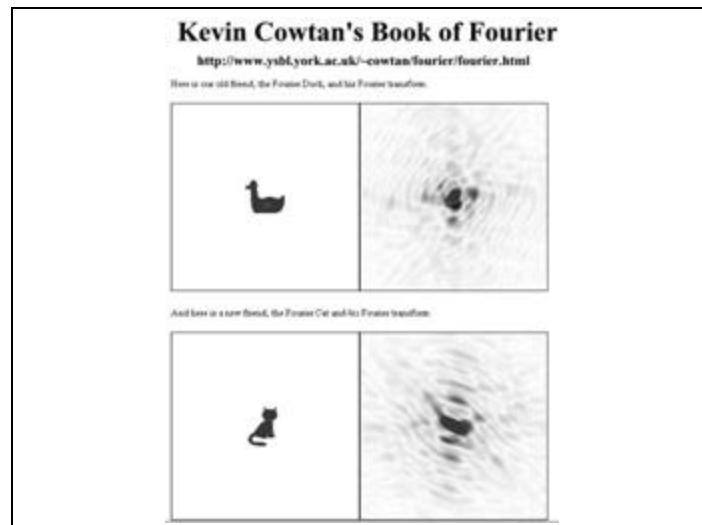
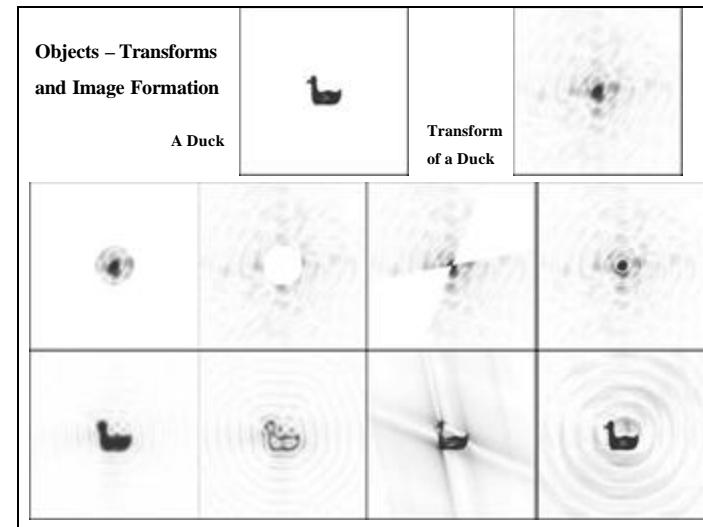
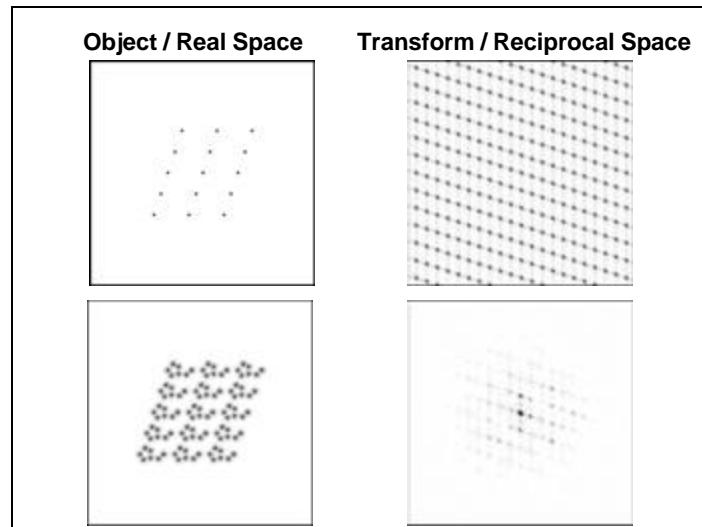
Kevin Cowtan's Picture Book of Fourier Transforms - Netscape

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Kevin Cowtan's Book of Fourier



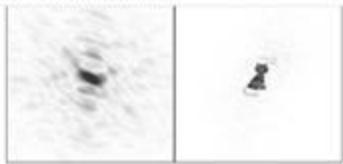


Kevin Cowtan's Book of Fourier

<http://www.york.ac.uk/~cowtan/fourier/fourier.html>



a) Cat - Cat Transform (Amplitudes only)
b) Manx (tailless) Cat - Manx Transform



c) Cat Amplitudes + Manx Phases
d) [2x(Cat Amplitudes) - Manx Amplitudes]
+ Manx Phases

