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

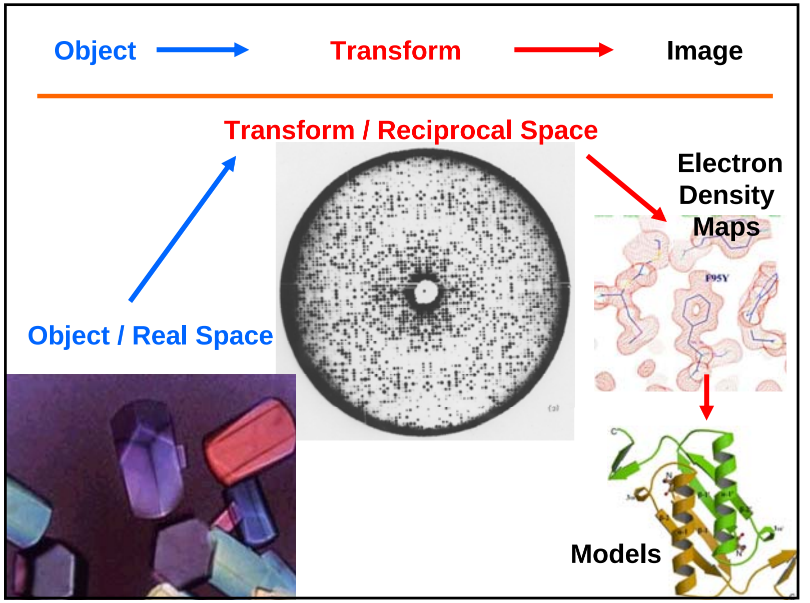
d)Theory of Diffraction – Bragg's Law / Reciprocal Space

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



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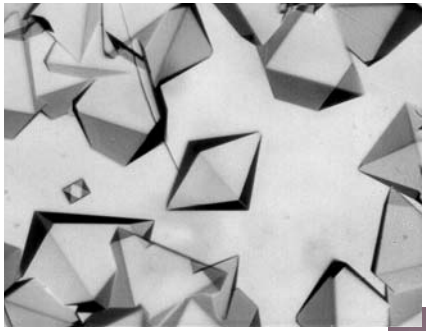
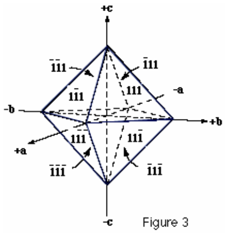
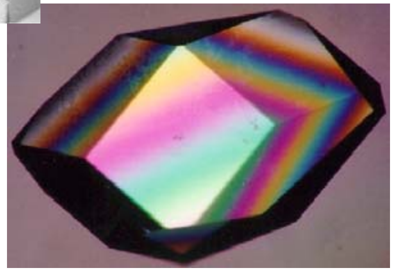
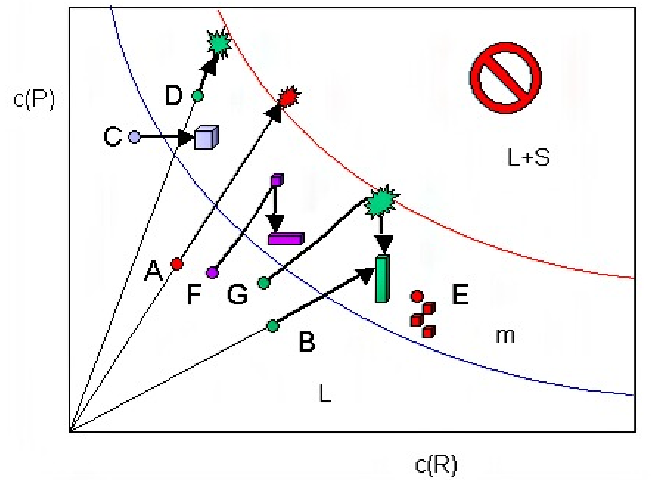


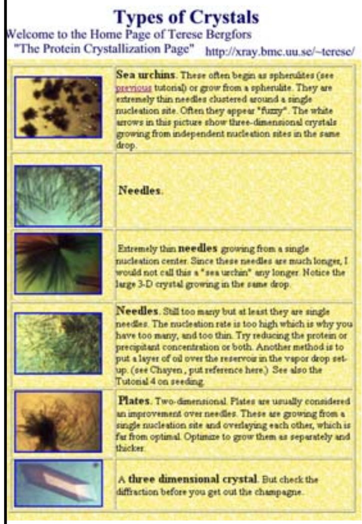
Glucose Isomerase Crystals



Lysozyme Crystals







Types of Crystals

Welcome to the Home Page of Terese Bergfors
"The Protein Crystallization Page" http://xray.bmc.uu.se/~terese/

Sea urchins. These often begin as spherulites (see previous tutorial) or grow from a spherulite. They are extremely thin needles clustered around a single nucleation site. Often they appear "fuzzy". The white arrows in this picture show three-dimensional crystals growing from independent nucleation sites in the same drop.

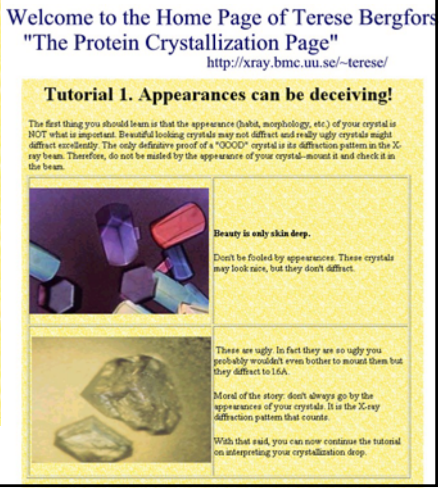
Needles.

Extremely thin **needles** growing from a single nucleation center. Since these needles are much longer, I would not call this a "sea urchin" any longer. Notice the large 3-D crystal growing in the same drop.

Needles. Still 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 precipitant concentration or both. Another method is to put a layer of oil over the reservoir in the vapor drop set-up. (see Chayen , put reference here.) See also the Tutorial 4 on seeding.

Plates. Two-dimensional. Plates are usually considered an improvement over needles. These are growing from a single nucleation site and overlaying each other, which is far from optimal. Optimize to grow them as separately and thicker.

A **three dimensional crystal**. But check the diffraction before you get out the champagne.



Welcome to the Home Page of Terese Bergfors
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Tutorial 1. Appearances can be deceiving!

The first thing you should learn is that the appearance (habit, morphology, etc.) of your crystal is NOT what is important. Beautiful looking crystals may not diffract and really ugly crystals might diffract excellently. The only definitive proof of a "GOOD" crystal is its diffraction pattern in the X-ray beam. Therefore, do not be misled by the appearance of your crystal--mount it and check it in the beam.

Beauty is only skin deep.

Don't be fooled by appearances. These crystals may look nice, but they don't diffract.

These are ugly. In fact they are so ugly you probably wouldn't even bother to mount them but they diffract to 1.6A.

Moral of the story: don't always go by the appearances of your crystals. It is the X-ray diffraction pattern that counts.

With that said, you can now continue the tutorial on interpreting 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

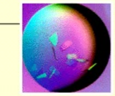
Common Compounds used in Crystallization

Ammonium or sodium sulfate
Sodium or ammonium citrate
Sodium or ammonium acetate
Magnesium sulfate
Cetyltrimethyl ammonium salts
Polyethylene glycol 400, 1000, 4000, 6000, 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

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.

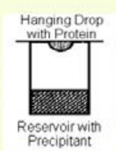
- a nice list of detergents commonly used in crystallization experiments.
- pretty pictures
- CRYSTOOL efficient random screen made for you here on the WEB.

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 K,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 Cacodylate pH 6.5	1.4M Sodium acetate trihydrate		7
8	0.2M tri-sodium citrate	0.1M Na Cacodylate pH 6.5	30% v/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% v/v PEG 400		13
14	0.2M Calcium Chloride	0.1M Na HEPES pH 7.5	28% v/v PEG 400	Y (best)	14
15	0.2M Ammonium acetate	0.1M Na Cacodylate pH 6.5	30% w/v PEG 8000		15
16	None	0.1M Na HEPES pH 7.5	1.5M Lithium sulfate monohydrate	Y	16
17	0.2M Lithium sulfate	0.1M Tris-HCl pH 8.5	30% w/v PEG 4000	Y (2nd best)	17

Hanging Drop Method - Crystal Screening

The Experimental Setup

In order to obtain a crystal, the protein molecules must assemble into a periodic lattice. One starts with a solution of the protein with a fairly high concentration (2-50 mg/ml) and adds reagents that reduce the solubility close to spontaneous precipitation. By slow further concentration, and under conditions suitable for the formation of a few nucleation sites, small crystals **may** start to grow. Often very many conditions have to be tried to succeed. This is usually done by initial screening, followed by a systematic optimization of conditions Crystals should to be a few tenth of a mm in each direction to be useful for diffraction experiments.



Right : The hanging drop technique. Center : 24 such hanging drop experiments are set up in a Linbro plate. Right : A kit of different screening solutions, a set-up Linbro plate, dialysis buttons and a micro batch plate behind a goniometer head.

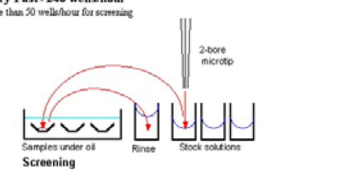
The most common setup to grow protein crystals is by the **hanging drop** technique : A few microliters of protein solution are mixed with an about equal amount of reservoir solution containing the precipitants. A drop of this mixture is put on a glass slide which covers the reservoir. As the protein/precipitant mixture in the drop is less concentrated than the reservoir solution (remember: we mixed the protein solution with the reservior solution about 1:1), water evaporates from the drop into the reservoir. As a result the concentration of both protein and precipitant in the drop slowly increases, and crystals may form. There is a variety of other techniques available such as sitting drops, dialysis buttons, and gel and microbatch techniques. Robots are useful for automatic screening and optimization of crystallization conditions. We have implemented a web computing service of Brent Segelke's CRYSTOOL, an inherently efficient random screen for crystallization conditions that you can customize. The main advantage is the small sample size a crystallization robot can handle reproducibly, but it needs some effort to set it up. Click here to learn more about the IMPAX-II robot.

Using Oryx 6 for Crystallization with Microbatch

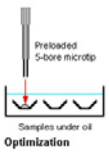
Microbatch operation is identical to IMPAX 1-5

http://www.douglas.co.uk/oryx.htm

Very Fast - 240 wells/hour
more than 50 wells/hour for screening



Uses little protein
0.1 - 1 µl per well

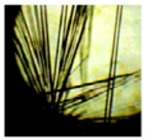


Microbatch screening finds more leads
than VD in a given time

	Automated MB	Manual VD
Proteins screened	6	6
Screening solutions	48	48
Runs	3	1
Volume per well	1 + 1µl	4 +4 µl
Total protein used	864 µl	1152µl
Operator time	3 hr	24 hr
Crystallization conditions found	43	41
Unique conditions	17	15

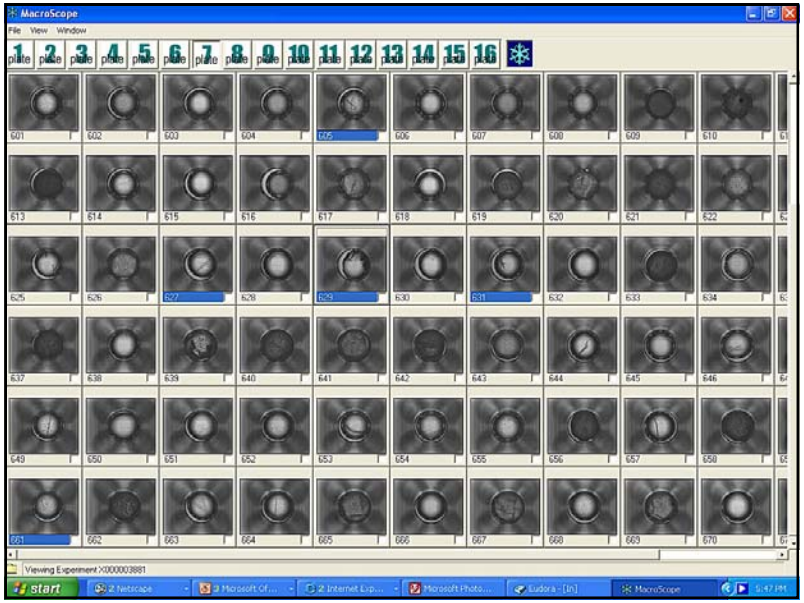
J. Crystal Growth. 168 (1996),pp 170-174. or: [illegible]

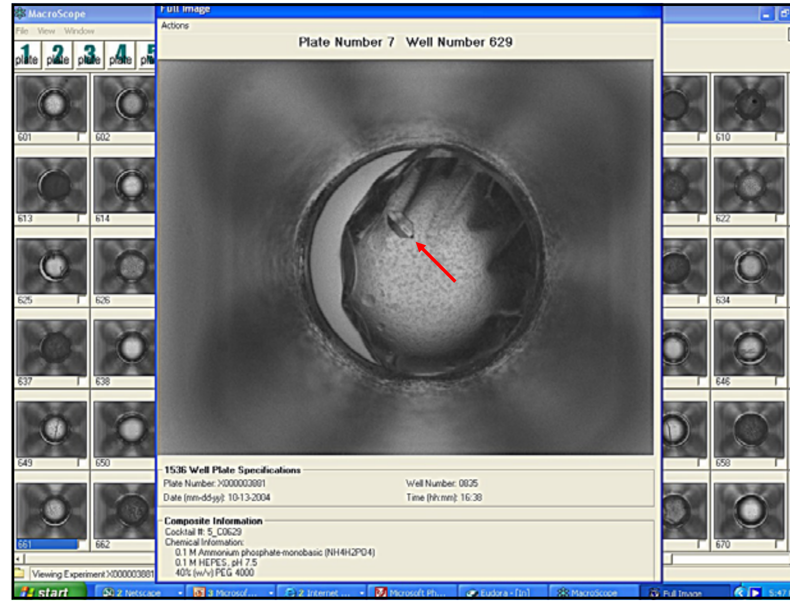
Large diffracting crystals



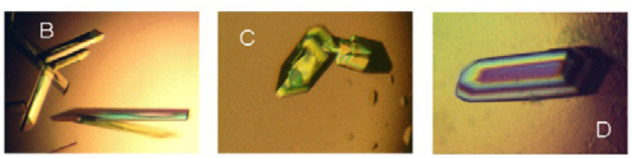
MB crystals of firefly luciferase. Data could not be collected from VD crystals which were unstable. Courtesy of E. Conti. Acta Crystallographica. D 52 (1996),4, pp [illegible]
[illegible]

MB crystal of alcohol dehydrogenase from a thermophilic bacterium. Courtesy of Y. Korkhin. Acta Crystallographica. D 52 (1996),pp [illegible]
[illegible]

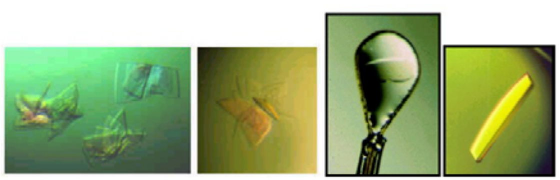




HisJ - His complex:



Tetanus Toxin C Fragment:



http://www.ccp14.ac.uk/ccp/web-mirrors/llnlrupp/crystal_lab/Crys_lab.html

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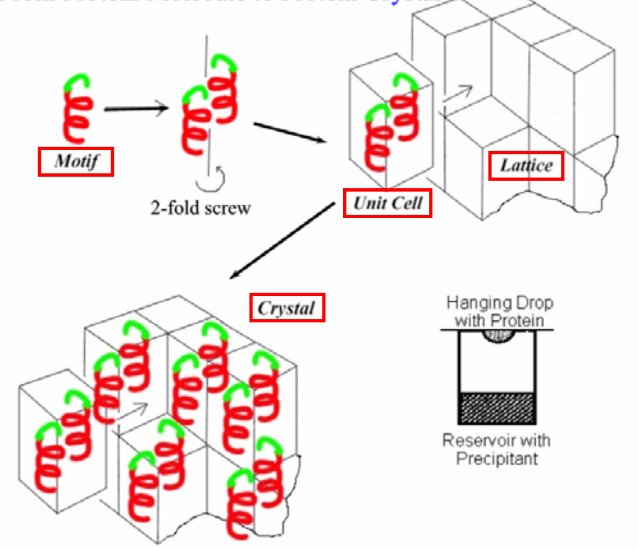
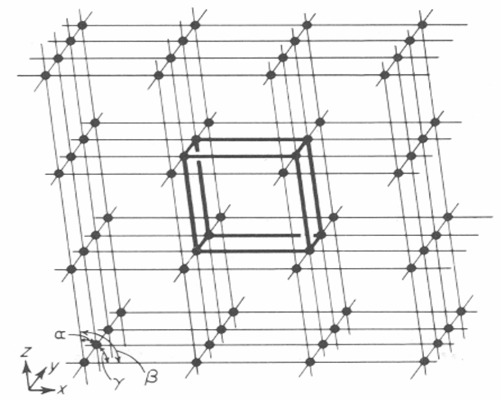
From Protein Molecule to Protein Crystal



A unit cell is defined by its lattice constants:

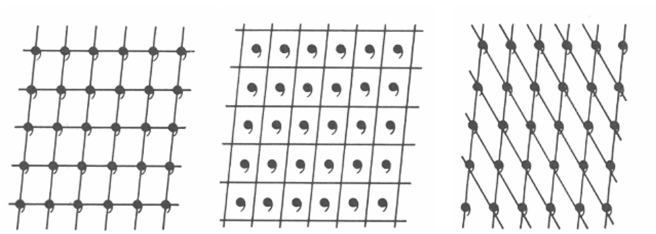
a, b, c and α, β, γ



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

How to identify "the" unit cell ?

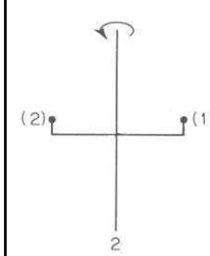
Regular two-dimensional array.



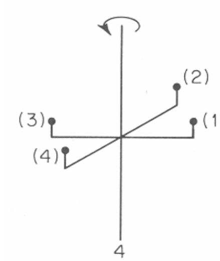
Three different grid systems referred to the array same array.

How to identify "the" unit cell ?

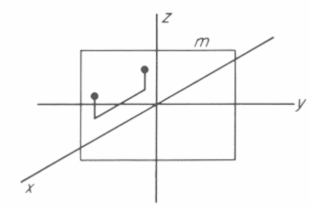
Role of Symmetry



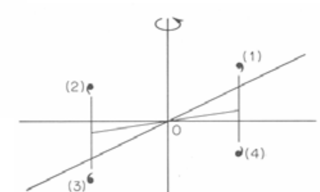
A 2-fold rotation axis.



A 4-fold rotation axis.

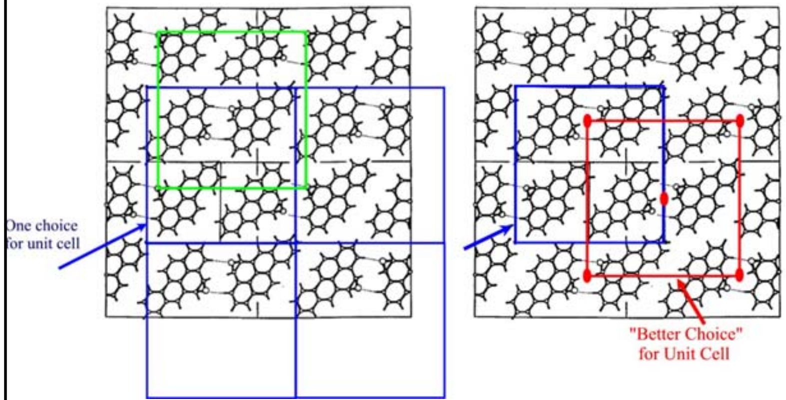


A mirror plane.



Center of symmetry produced by a 2-fold axis combined with reflection.

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
Triclinc	P	None	a, b, c, al, be, ga,
Monoclinic	P, C	One 2-fold axis, parallel b (b unique)	a, b, c, 90, be, 90
Orthorhombic	P, I, F	Three perpendicular 2-folds	a, b, c, 90, 90, 90
Tetragonal	P, I	One 4-fold axis, parallel c	a, a, c, 90, 90, 90
Trigonal	P, R	One 3-fold axis	a, a, c, 90, 90, 120
Hexagonal	P	One 6-fold axis	a, a, c, 90, 90, 120
Cubic	P, F, I	Four 3-folds along space diagonal	a, a, ,a, 90, 90, 90

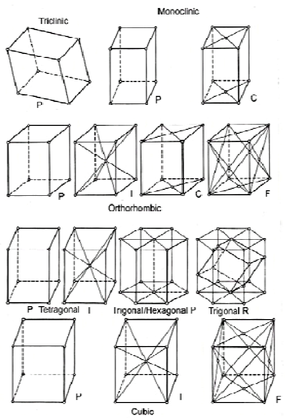
Symmetry operations : 1,2,3,4,6, -1,-2,-3,-4,-6, m

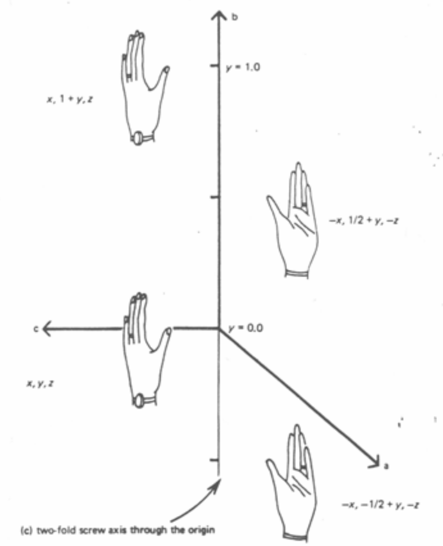
Crystal System	Point groups	Laue Class	Patterson Symmetry
Triclinc	1, -1	-1	P-1
Monoclinic	2, m, 2/m	2/m	P2/m, C2/m
Orthorhombic	222, mm2 , mmm	mmm	Pmmm, Cmmm, Fmmm, Immm
Tetragonal	4, -4, 4/m, 422, 4mm, -42m, 4/mmm	4/m, 4/mmm	P4/m, I4/m, P4/mmm, I4/mmm
Trigonal	3, -3, 32, 3m, -3 m	-3, -3m	P-3, R-3, P-3m1, P-31m, R-3m
Hexagonal	6, -6, 6/m, 622, 6mm, -62m, 6/mmm	6/m, 6/mmm	P6/m, P6/mmm
Cubic	23, m-3, 432, -43m, m3m	m-3, m-3m	Pm-3, Im-3, F-3m, Pm-3m, Fm-3m, Im-3m

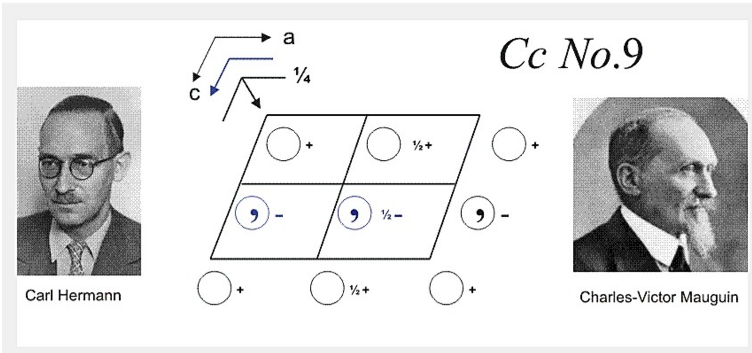
Notes

- Laue class corresponds to symmetry of reciprocal space (diffraction pattern)
- Patterson symmetry is Laue class plus allowed Bravais centering, i.e. centrosymmetric and symorphic

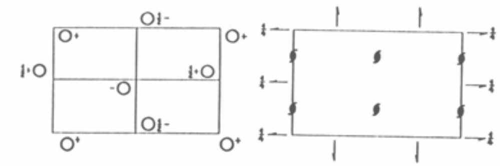
The 14 Bravais Lattices







Orthorhombic 222 $P\,2_1\,2_1\,2_1$ No. 19 $P\,2_1\,2_1\,2_1$ 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	$x,y,z;\ \tfrac{1}{2}-x,\bar{y},\tfrac{1}{2}+z;\ \tfrac{1}{2}+x,\tfrac{1}{2}-y,\bar{z};\ \bar{x},\tfrac{1}{2}+y,\tfrac{1}{2}-z.$	hkl: $0kl$: $h0l$: $hk0$: No conditions; $h00$: $h=2n$; $0k0$: $k=2n$; $00l$: $l=2n$

Symmetry of special projections

(001) pgg; $a'=a$, $b'=b$ (100) pgg; $b'=b$, $c'=c$ (010) pgg; $c'=c$, $a'=a$

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

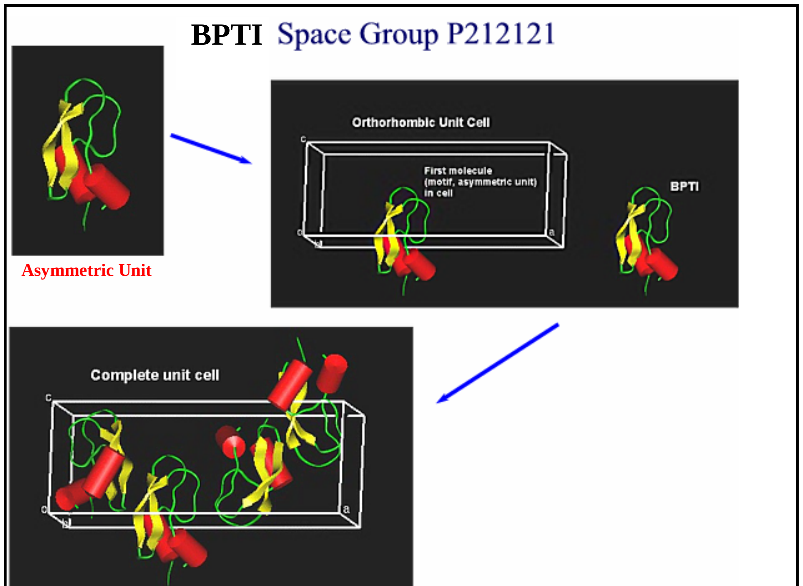


TABLE 3.4 Space Groups in Standard Orientations*

System	Point Group	Space Group						Fraction
Triclinic	1	$P1$						1/2
	$\bar{1}$	$P\bar{1}$						
Monoclinic	2	$P2$	$P2_1$	$C2$				1/4
	m	Pm	Pc	Cm	Cc			
	$2/m$	$P2/m$	$P2_1/m$	$C2/m$	$P2/c$	$P2_1/c$	$C2/c$	
Orthorhombic	222	$P222$	$P222_1$	$P2_12_12$	$P2_12_12_1$	$C222_1$	$C222$	1/8
		$F222$	$I222$	$I2_12_12_1$				
	$mm2$	$Pmm2$	$Pmc2_1$	$Pcc2$	$Pma2$	$Pca2_1$	$Pnc2$	
		$Pmn2_1$	$Pba2$	$Pna2_1$	$Pnn2$	$Cmm2$	$Cmc2_1$	
		$Ccc2$	$Amm2$	$Abm2$	$Ama2$	$Aba2$	$Fmm2$	
		$Fdd2$	$Imm2$	$Iba2$	$Ima2$			
	mmm	$Pmmm$	$Pnnn$	$Pccm$	$Pban$	$Pmma$	$Pnna$	
		$Pmna$	$Pcca$	$Pbam$	$Pccn$	$Pbcm$	$Pnnm$	
		$Pmmn$	$Pbcn$	$Pbca$	$Pnma$	$Cmcm$	$Cmca$	
		$Cmmm$	$Cccm$	$Cmma$	$Ccca$	$Fmmm$	$Fddd$	
		$Immm$	$Ibam$	$Ibca$	$Imma$			
Tetragonal	4	$P4$	$P4_1$	$P4_2$	$P4_3$	$I4$	$I4_1$	1/8
	$\bar{4}$	$P\bar{4}$	$I\bar{4}$					
	$4/m$	$P4/m$	$P4_2/m$	$P4/n$	$P4_2/n$	$I4/m$	$I4_1/a$	
	422	$P422$	$P42_12$	$P4_122$	$P4_12_12$	$P4_222$	$P4_22_12$	1/16
		$P4_322$	$P4_32_12$	$I422$	$I4_122$			
	$4mm$	$P4mm$	$P4bm$	$P4_2cm$	$P4_2nm$	$P4cc$	$P4nc$	
		$P4_2mc$	$P4_2bc$	$I4mm$	$I4cm$	$I4_1md$	$I4_1cd$	
	$\bar{4}2m$	$P\bar{4}2m$	$P\bar{4}2c$	$P\bar{4}2_1m$	$P\bar{4}2_1c$	$P\bar{4}m2$	$P\bar{4}c2$	
		$P\bar{4}b2$	$P\bar{4}n2$	$I\bar{4}m2$	$I\bar{4}c2$	$I\bar{4}2m$	$I\bar{4}2d$	
	$4/mmm$	$P4/mmm$	$P4/mcc$	$P4/nbm$	$P4/nnc$	$P4/mbm$	$P4/mnc$	
		$P4/nmm$	$P4/ncc$	$P4_2/mmc$	$P4_2/mcm$	$P4_2/nbc$	$P4_2/nnm$	
		$P4_2/mbc$	$P4_2/mnm$	$P4_2/nmc$	$P4_2/ncm$	$I4/mmm$	$I4/mcm$	
		$I4_1/amd$	$I4_1/acd$					
Trigonal/rhombohedral	3	$P3$	$P3_1$	$P3_2$	$R3$			1/6
	$\bar{3}$	$P\bar{3}$	$R\bar{3}$					
	32	$P312$	$P321$	$P3_112$	$P3_121$	$P3_212$	$P3_221$	1/12
		$R32$						
	$3m$	$P3m1$	$P31m$	$P3c1$	$P31c$	$R3m$	$R3c$	
	$\bar{3}m$	$P\bar{3}1m$	$P\bar{3}1c$	$P\bar{3}m1$	$P\bar{3}c1$	$R\bar{3}m$	$R\bar{3}c$	
Hexagonal	6	$P6$	$P6_1$	$P6_5$	$P6_2$	$P6_4$	$P6_3$	1/12
	$\bar{6}$	$P\bar{6}$						
	$6/m$	$P6/m$	$P6_3/m$					
	622	$P622$	$P6_122$	$P6_522$	$P6_222$	$P6_422$	$P6_322$	1/24
	$6mm$	$P6mm$	$P6cc$	$P6_3cm$	$P6_3mc$			
	$\bar{6}m2$	$P\bar{6}m2$	$P\bar{6}c2$	$P\bar{6}2m$	$P\bar{6}2c$			
	$6/mmm$	$P6/mmm$	$P6/mcc$	$P6_3/mcm$	$P6_3/mmc$			
Cubic	23	$P23$	$F23$	$I23$	$P2_13$	$I2_13$		1/24
	$m3$	$Pm3$	$Pn3$	$Fm3$	$Fd3$	$Im3$	$Pa3$	
		$Ia3$						
	432	$P432$	$P4_232$	$F432$	$F4_132$	$I432$	$P4_332$	1/48
		$P4_132$	$I4_132$					
	$\bar{4}3m$	$P\bar{4}3m$	$F\bar{4}3m$	$I\bar{4}3m$	$P\bar{4}3n$	$F\bar{4}3c$	$I\bar{4}3d$	
	$m3m$	$Pm3m$	$Pn3n$	$Pm3n$	$Pn3m$	$Fm3m$	$Fm3c$	
		$Fd3m$	$Fd3c$	$Im3m$	$Ia3d$			

*The 11 Laue symmetries are separated by horizontal lines.

TABLE 16-5 *The 65 "Biological" Space Groups*

CRYSTAL SYSTEM	LATTICE	MINIMUM SYMMETRY OF UNIT CELL	UNIT CELL EDGES AND ANGLES[a]	DIFFRACTION PATTERN SYMMETRY[b]	SPACE GROUPS[c]
Triclinic	*P*	None	$a \neq b \neq c$ $\alpha \neq \beta \neq \gamma$	$\bar{1}$	*P*1
Monoclinic	*P* *C*	2-fold axis parallel to **b**	$a \neq b \neq c$ $\alpha = \gamma = 90°$ $\beta \neq 90°$	2/*m*	$P2$, $P2_1$ $C2$
Orthorhombic	*P* *C* *I* *F*	3 mutually perpendicular 2-fold axes	$a \neq b \neq c$ $\alpha = \beta = \gamma = 90°$	*mmm*	$P222$, $P2_12_12_1$, $P222_1$, $P2_12_12$ $C222$, $C222_1$ [$I222$, $I2_12_12_1$] $F222$
Tetragonal	*P* *I*	4-fold axis parallel to **c**	$a = b \neq c$ $\alpha = \beta = \gamma = 90°$	4/*m* 4/*mmm*	$P4$, ($P4_1$, $P4_3$), $P4_2$ $I4$, $I4_1$ $P422$, ($P4_122$, $P4_322$), $P4_222$ $P42_12$, ($P4_12_12$, $P4_32_12$), $P4_22_12$ $I422$, $I4_122$
Trigonal/rhombohedral	*R*[d] *P*[d]	3-fold axis parallel to **c**	$a = b = c$ $\alpha = \beta = \gamma \neq 90°$	$\bar{3}$ $\bar{3}m$	$R3$ $P3$, ($P3_1$, $P3_2$) $R32$ [$P321$, $P312$] [($P3_121$, $P3_221$), ($P3_112$, $P3_212$)]
Hexagonal	*P*	6-fold axis parallel to **c**	$a = b \neq c$ $\alpha = \beta = 90°$ $\gamma = 120°$	6/*m* 6/*mmm*	$P6$, ($P6_1$, $P6_5$) $P6_3$, ($P6_2$, $P6_4$) $P622$, ($P6_122$, $P6_522$) $P6_322$, ($P6_222$, $P6_422$)
Cubic	*P* *I* *F*	3-fold axes along cube diagonals	$a = b = c$ $\alpha = \beta = \gamma = 90°$	*m*3 *m*3*m*	$P23$ $P2_13$ [$I23$, $I2_13$] $F23$ $P432$, ($P4_132$, $P4_332$) $P4_232$ $I432$, $I4_132$ $F432$, $F4_132$

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**
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   - **c) X-ray Sources – Sealed Tube / Rotation Anode / Synchrotron**
   - **d)Theory of Diffraction – Bragg's Law / Reciprocal Space**
   - **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-ray tubes: the "sealed" tube

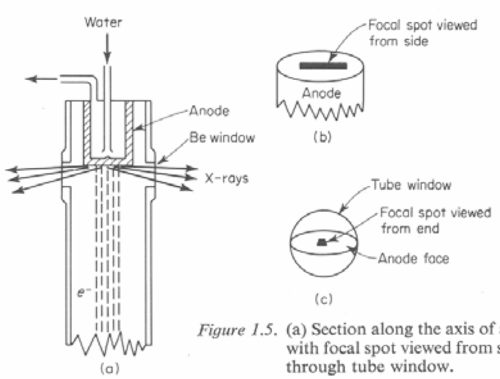
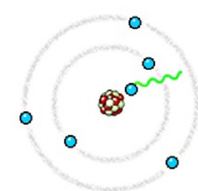


Figure 1.5. (a) Section along the axis of an X-ray tube. (b, Anode with focal spot viewed from side. (c) Focal spot viewed through tube window.

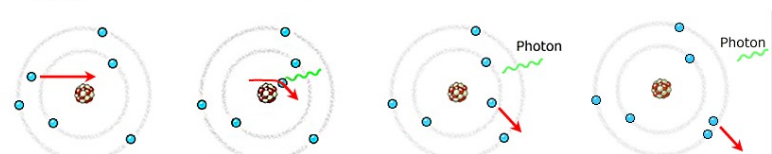
Origin of Non-characteristic X-rays



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 looses energy and an X-ray photon is emitted.

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 looses 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.



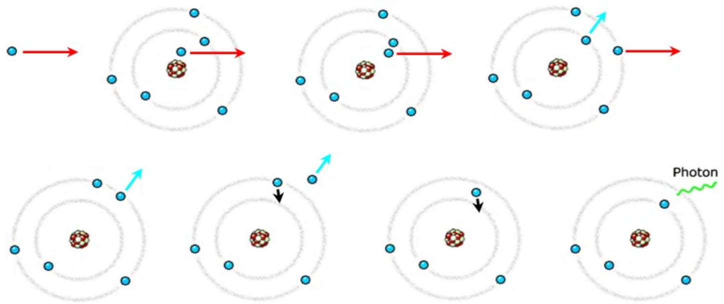
Origin of characteristic X-rays

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.

Related Laureate

The Nobel Prize in Physics 1917 - Charles Glover Barkla »



Characteristic X-rays arise from electronic transitions

$K_{\alpha_1}, K_{\alpha_2}$ $L \rightarrow K$

K_{β_1}, K_{β_2} $M \rightarrow K$

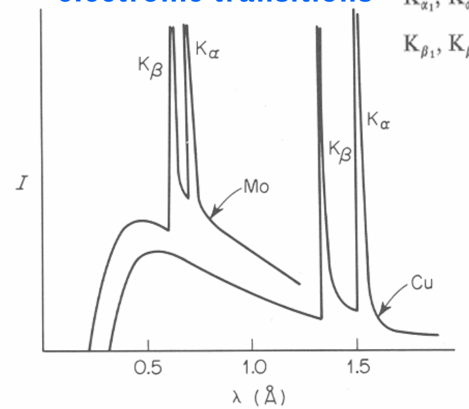
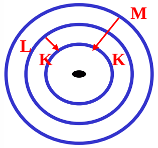


Figure 1.2. X-ray spectra with characteristic peaks: MoKα, 50 Kv; CuKα, 35 Kv.



Characteristic X-rays have defined λ

Table 1.1. Target Materials and Associated Constants

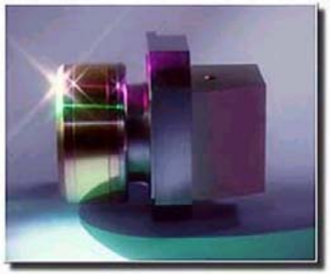
	Cr	Fe	Cu	Mo
Z	24	26	29	42
α_1, Å	2.2896	1.9360	1.5405	0.70926
α_2, Å	2.2935	1.9399	1.5443	0.71354
$\bar{\alpha}$,* Å	2.2909	1.9373	1.5418	0.71069
β_1, Å	2.0848	1.7565	1.3922	0.63225
β, filt.	V, 0.4 mil†	Mn, 0.4 mil	Ni, 0.6 mil	Nb, 3 mils
α, filt.	Ti	Cr	Co	Y
Resolution, Å	1.15	0.95	0.75	0.35
Critical 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.

X-ray Generators

FR591



FR591 Rotating Anode X-ray Generator

The Nonius' FR591 rotating anode X-ray generator now has dramatically improved the performance of the anode, by a complete redesign.
We now have a static shaft and a rotating anode, instead of rotating both. The cooling water flow has also been redesigned to give much higher throughput, higher flow and higher turbulence, which results in better heat transfer and hence better cooling capacity.

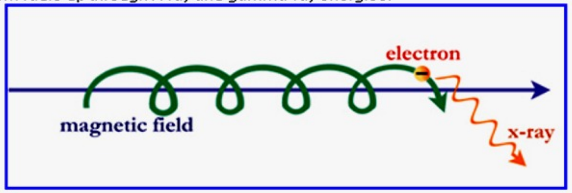
Now with the new ULTRA anode you can get 6 kW on a 0.3mm focus!

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

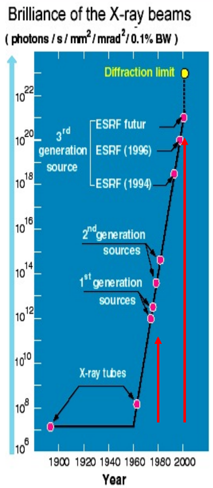
The **brilliance** of a light source is defined as the number of photons emitted per second, per unit source size, per unit space angle and for a bandwidth of 1/1000 of the photon energy

The Comparison between various sources of X-rays shows large differences in their brilliance.

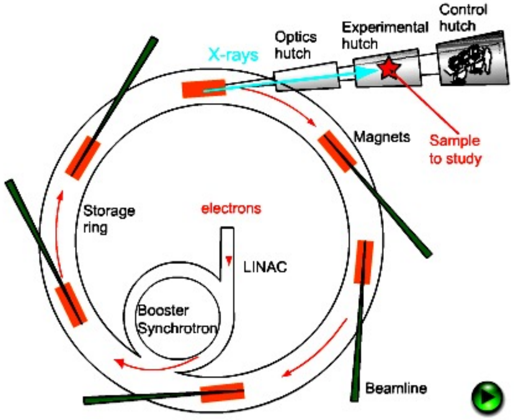
X-ray tubes:
Wilhelm Conrad Röntgen discovered X-rays in 1895 whilst working with cathode-ray tubes. Using the principle of fast electrons hitting a metallic target, a first substantial gain in brilliance was not obtained until the introduction of rotating anode sources (~1960).

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). Relying on the combination of needle thin electron beams and Insertion Devices, *Third* generation synchrotron sources (~1995) are now emitting synchrotron X-ray beams that are a trillion (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 next generation of X-ray sources. While they promise to achieve an increase in peak brilliance by another factor of a trillion, the first prototypes may be operational around the year 2010.

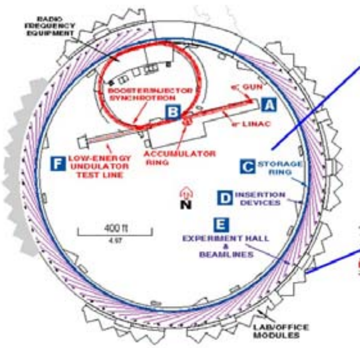


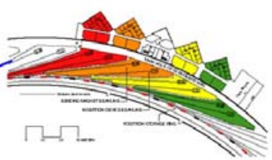
How synchrotron light is produced?



APS - Advanced Photon Source

Argonne National Laboratory





X-Ray Crystallography

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2. **Image Formation**

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   h) Refinements and Models

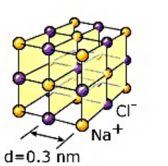
   i) Analysis and presentation of results

X-RAYS

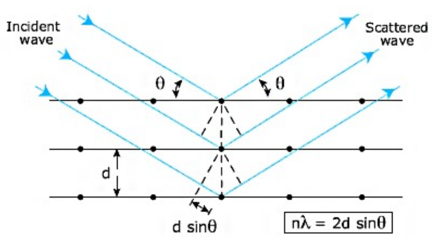
X-rays, What Are They? 6:7 X-rays, What Are They? 7:7 »

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.

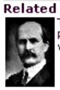


Crystal planes, in NaCl, ordinary salt. Other planes are also possible.



X-ray scattering from three crystal planes, separated by the distance d. For constructive interference in a direction θ the path difference must be an even number of wavelengths.

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 »

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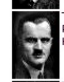
 The Nobel Prize in Physics 1915 - William Lawrence Bragg »

 The Nobel Prize in Physics 1917 - Charles Glover Barkla »

 The Nobel Prize in Physics 1924 - Karl Manne Georg Siegbahn »

 The Nobel Prize in Physics 1927 - Arthur Holly Compton »

 The Nobel Prize in Chemistry 1936 - Petrus (Peter) Josephus Wilhelmus Debye »

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 The Nobel Prize in Chemistry 1962 - John Cowdery Kendrew »

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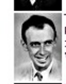 The Nobel Prize in Physiology or Medicine 1962 - James Dewey Watson »

 The Nobel Prize in Physiology or Medicine 1962 - Maurice Hugh Frederick Wilkins »

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 The Nobel Prize in Physiology or Medicine 1979 - Allan M. Cormack »

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 The Nobel Prize in Physics 1981 - Kai M. Siegbahn »

 The Nobel Prize in Chemistry 1985 - Herbert A. Hauptman »

 The Nobel Prize in Chemistry 1985 - Jerome Karle »

 The Nobel Prize in Chemistry 1988 - Johann Deisenhofer »

 The Nobel Prize in Chemistry 1988 - Robert Huber »

 The Nobel Prize in Chemistry 1988 - Hartmut Michel »

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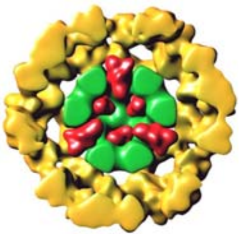
- Light Photography
  λ ~ 400 - 700 nm
- Electron Microscopy
  λ ~ 0.001 - 0.1 nm



- X-Ray or NMR
  λ ~ 0.1 nm

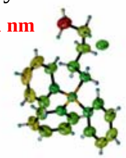
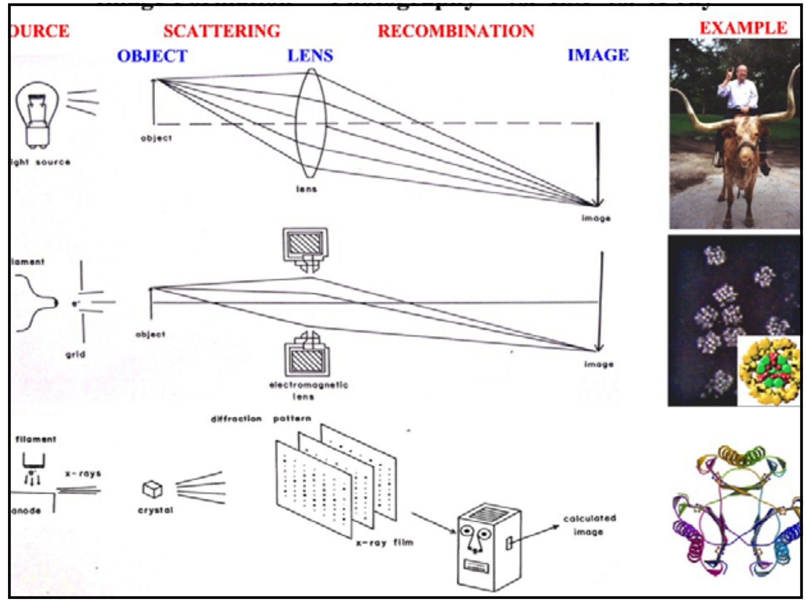


Image Formation

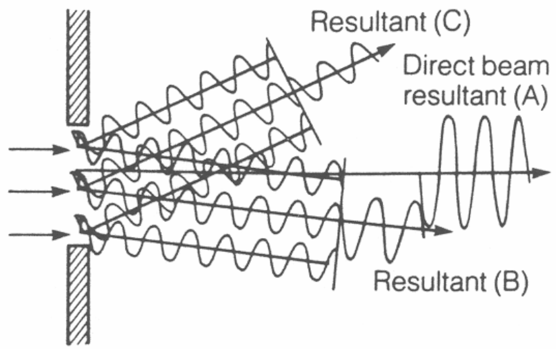
Abbe (~1873):

Limit Res. ~ λ/2



Single Hole Scattering Experiment

Transforms / Reciprocal Space

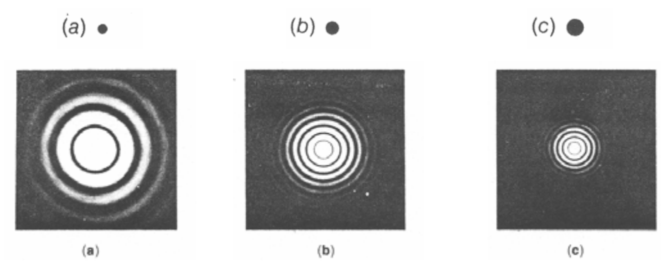


(b)

Single Hole Scattering Experiment

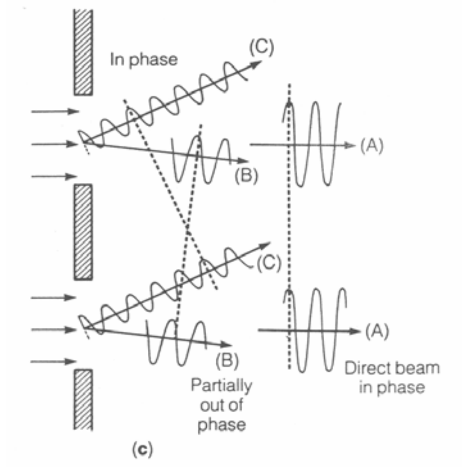
Transforms / Reciprocal Space

Different size holes



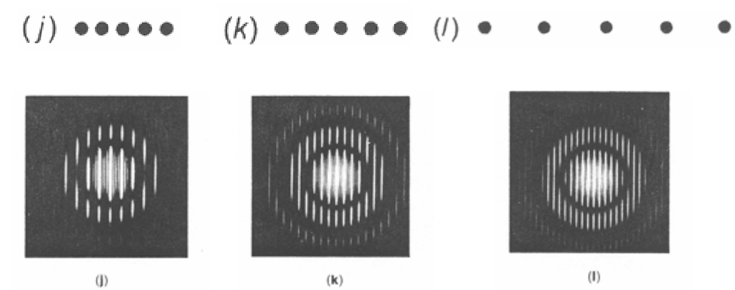
Effect of Multiple "Scatterers"

Transforms / Reciprocal Space



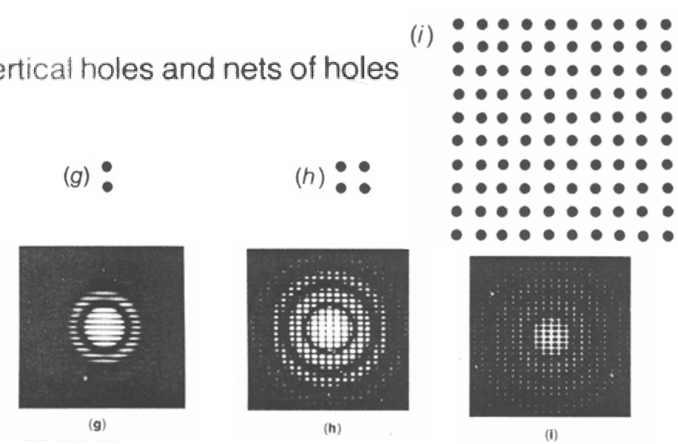
Transforms / Reciprocal Space

Five horizontal holes with various spacings

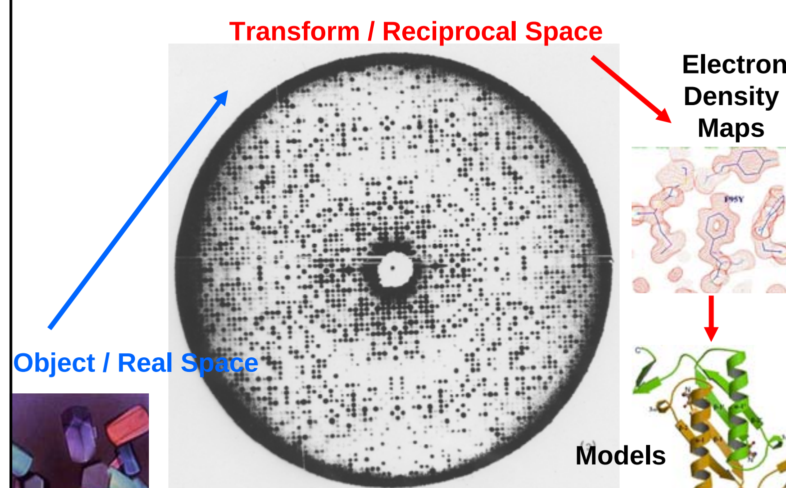


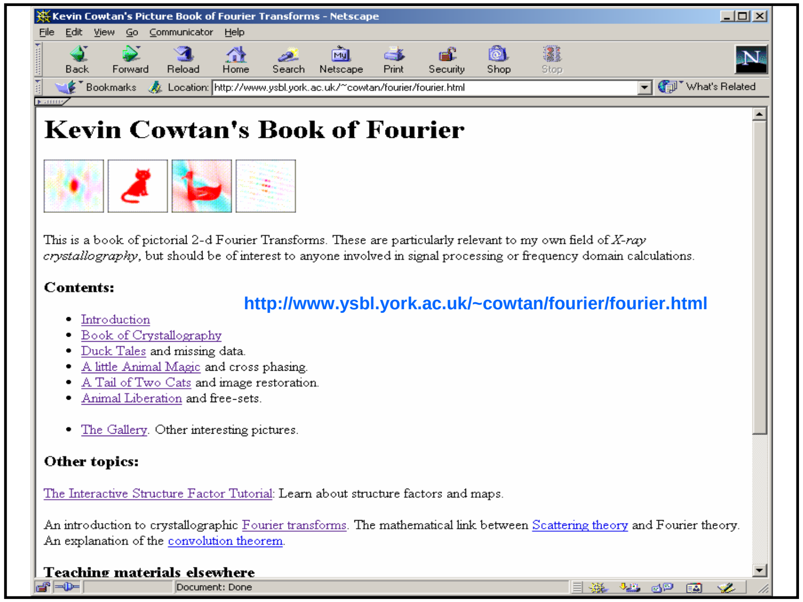
Transforms / Reciprocal Space

Vertical holes and nets of holes



Object → Transform → Image

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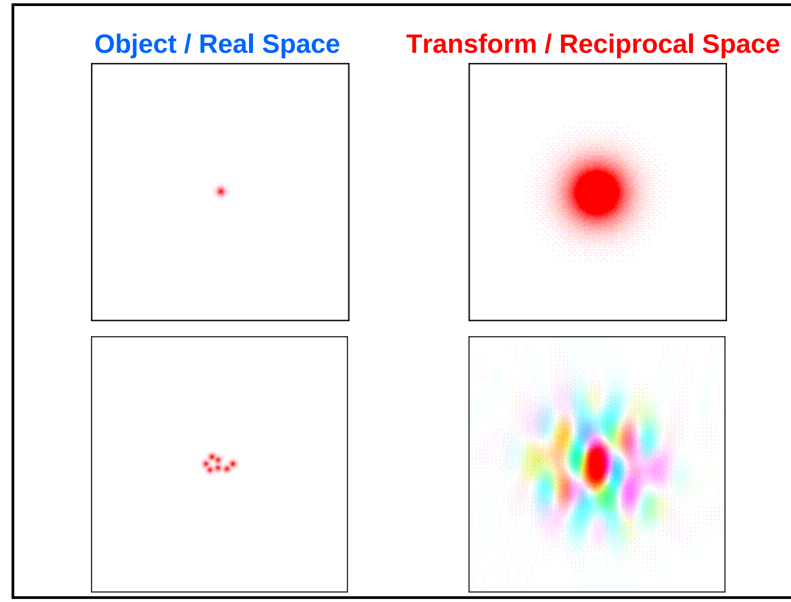
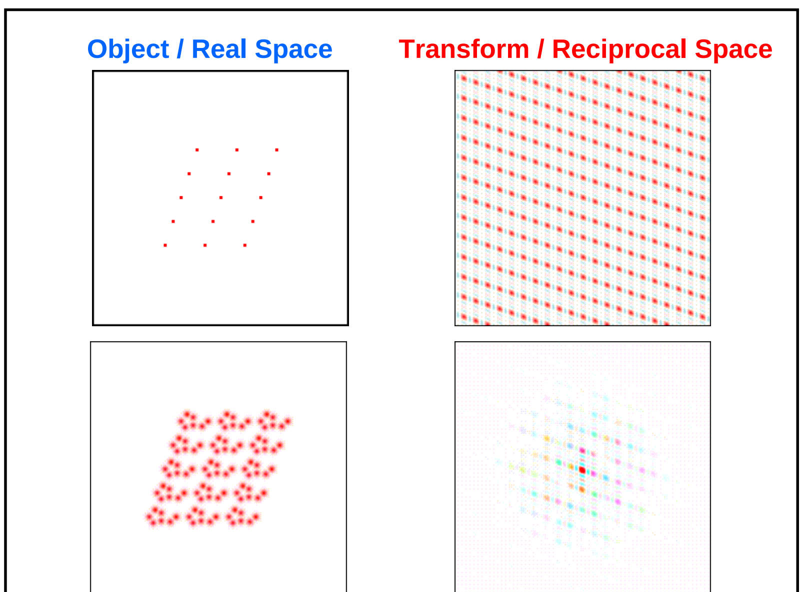
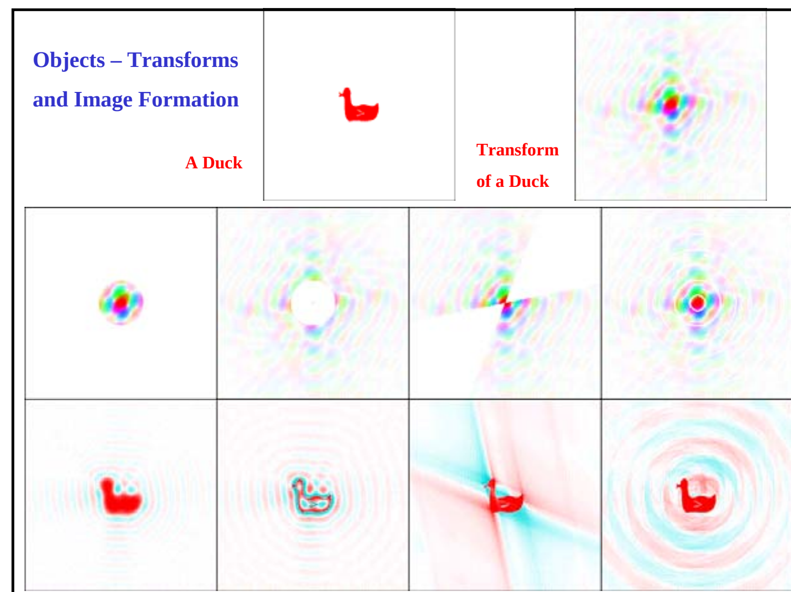
Back Forward Reload Home Search Netscape Print Security Shop Stop
Bookmarks Location: http://www.ysbl.york.ac.uk/~cowtan/fourier/fourier.html What's Related
Kevin Cowtan's Book of Fourier
This is a book of pictorial 2-d Fourier Transforms. These are particularly relevant to my own field of X-ray crystallography, but should be of interest to anyone involved in signal processing or frequency domain calculations.
Contents:
http://www.ysbl.york.ac.uk/~cowtan/fourier/fourier.html
Introduction
Book of Crystallography
Duck Tales and missing data.
A little Animal Magic and cross phasing.
A Tail of Two Cats and image restoration.
Animal Liberation and free-sets.
The Gallery. Other interesting pictures.
Other topics:
The Interactive Structure Factor Tutorial: Learn about structure factors and maps.
An introduction to crystallographic Fourier transforms. The mathematical link between Scattering theory and Fourier theory. An explanation of the convolution theorem.
Teaching materials elsewhere
Document: Done


Object / Real Space
Transform / Reciprocal Space

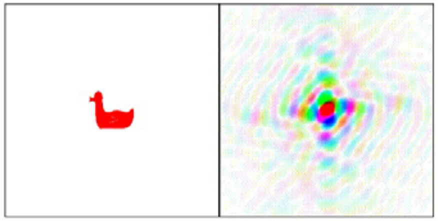

Object / Real Space
Transform / Reciprocal Space


Objects – Transforms
and Image Formation
A Duck
Transform
of a Duck

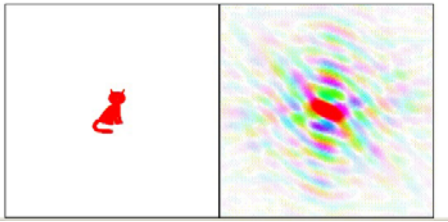
Kevin Cowtan's Book of Fourier

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

Here is our old friend, the Fourier Duck, and his Fourier transform:



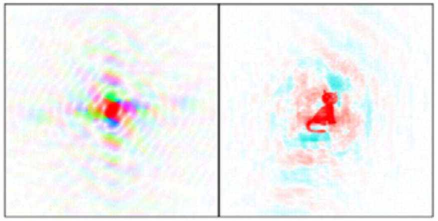
And here is a new friend, the Fourier Cat and *his* Fourier transform:



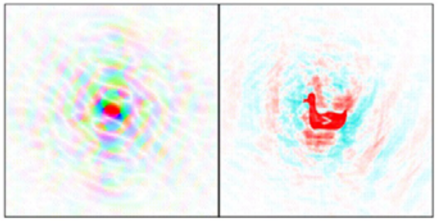
Kevin Cowtan's Book of Fourier

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

Duck Transform Amplitudes + Cat Phases



Cat Transform Amplitudes + Duck Phases



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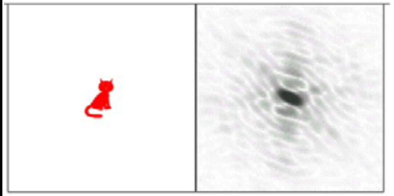
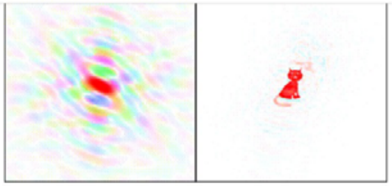
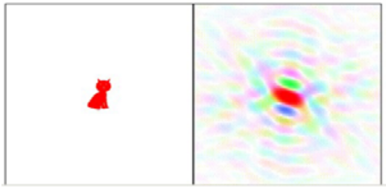
http://www.ysbl.york.ac.uk/~cowtan/fourier/fourier.html

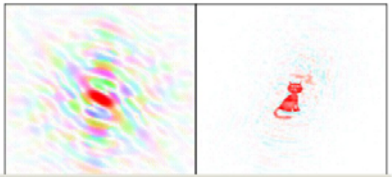




a) Cat - Cat Transform (Amplitudes only)
b) Manx (tailless) Cat - Manx Transform

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





X-Ray Crystallography

Quiz questions:

1. Crystal Growth – Materials / Methods

What is the single most important factor that determines crystal growth?

What are the two most common precipitating agents for growing protein crystals?

2. Crystal Lattices - Lattice Constants / Space Groups / Asymmetric Unit

Identify the unit cell, asymmetric unit and symmetry present in the pattern shown.

3. X-ray Sources – Sealed Tube / Rotation Anode / Synchrotron

What is responsible for “characteristic” X-rays?

What are the major advantages of using synchrotron radiation?

4. Theory of Diffraction – Bragg’s Law / Reciprocal Space

When collecting an X-ray data set, what is being measured and how is that data useful?