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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Data mining and Protein Structure Analysis Tools

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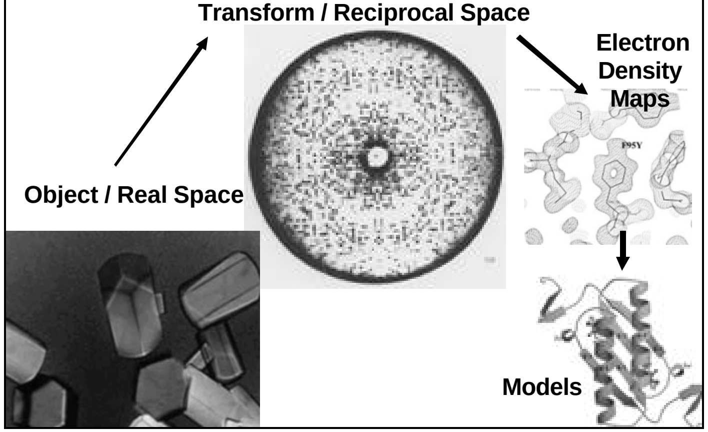
e) Data Collection – Methods / Detectors / Structure Factors

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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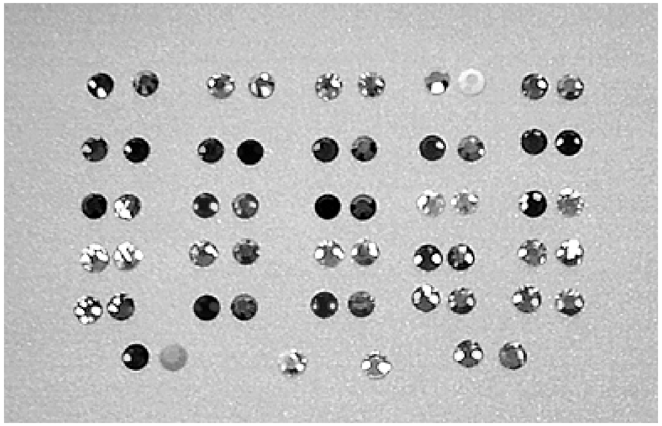
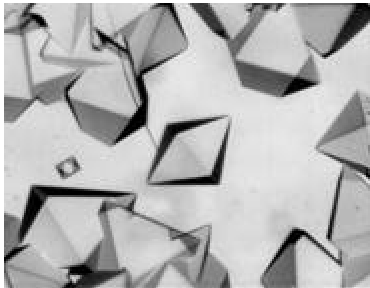
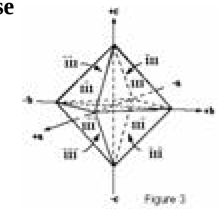
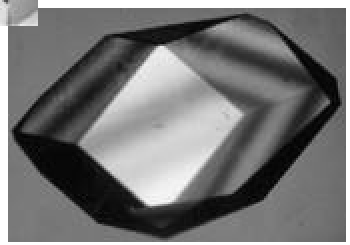


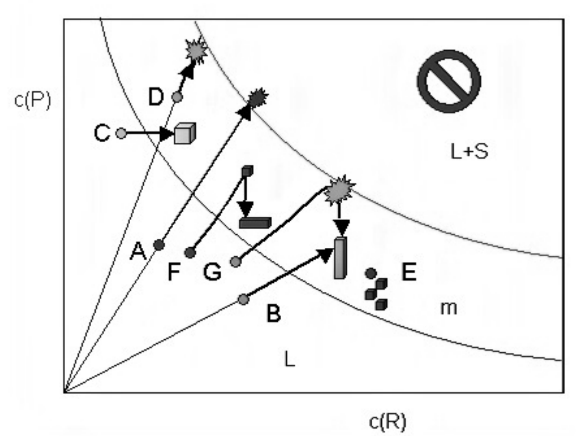
Glucose Isomerase Crystals

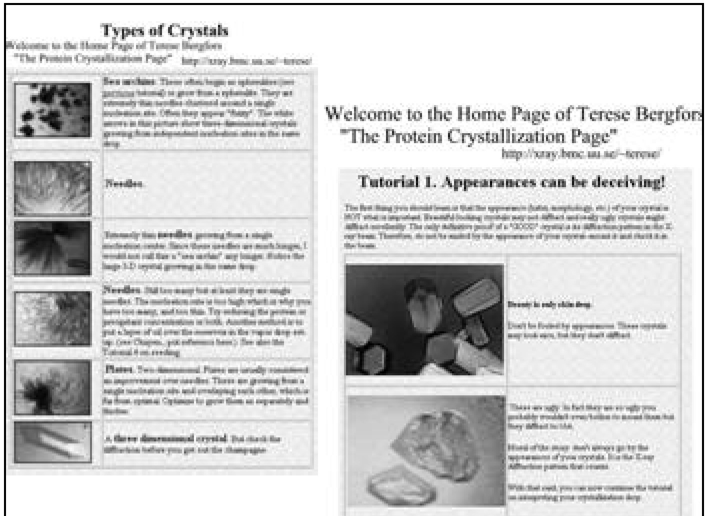




Lysozyme Crystals







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

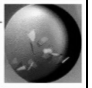
Common Compounds used in Crystallization

- 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.

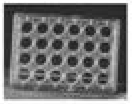
- a nice list of detergents commonly used in crystallization experiments.
- pretty pictures
- CRYSTOOL efficient random screen made for you here on the WEB.

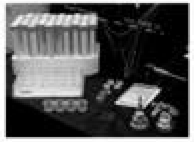
Tube #	SALT	BUFFER	Precipitant	Miniscreen	Tube #
1	0.02M Calcium Chloride	0.1M Na Acetate pH 4.6	30% w/v 2-methyl-2,4-pentanediol	Y	**1**
2	None	None	0.4M K,Na Tartrate tetrahydrate		**2**
3	None	None	0.4M Ammonium dihydrogen phosphate		**3**
4	None	0.1M Tris-HCl pH 8.5	2.0M Ammonium Sulfate	Y	**4**
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

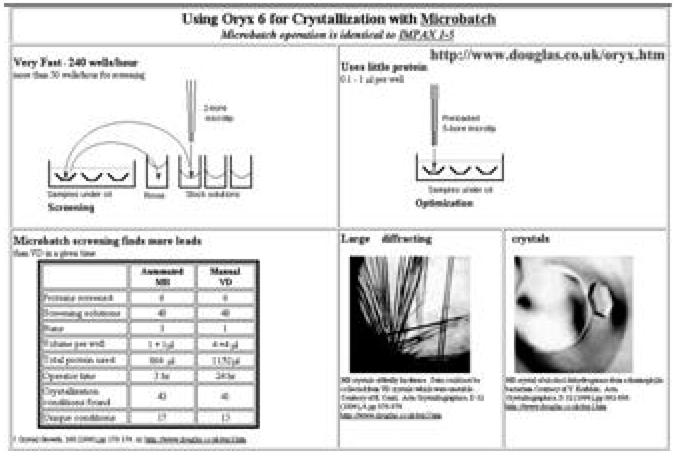
[illegible]

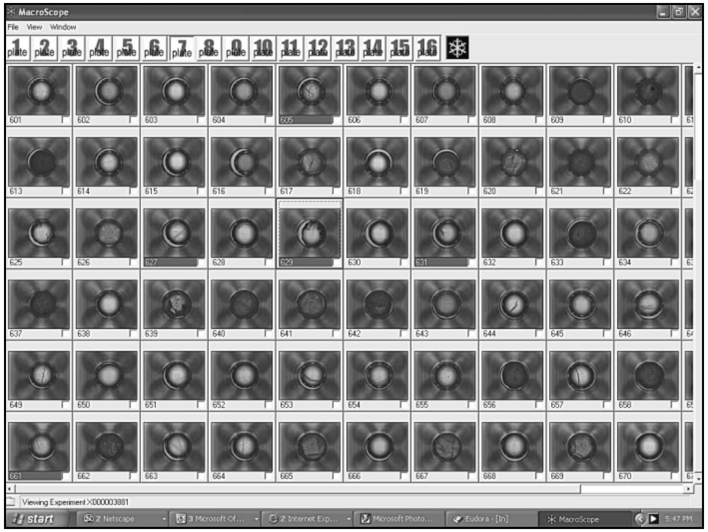


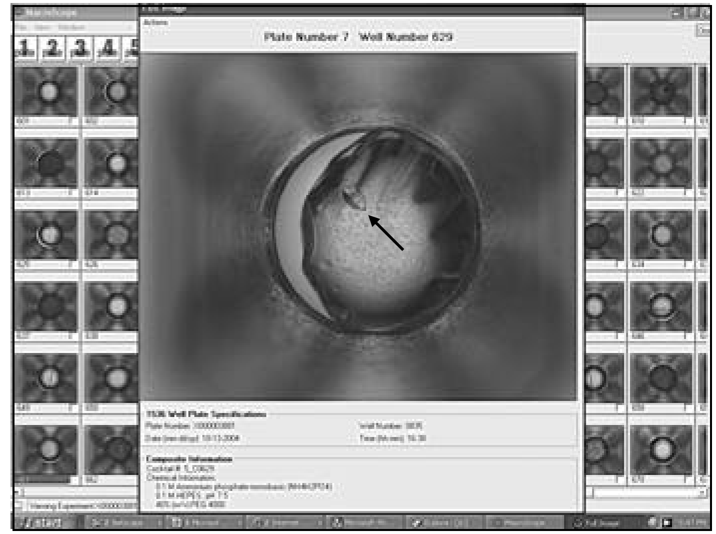


[illegible]

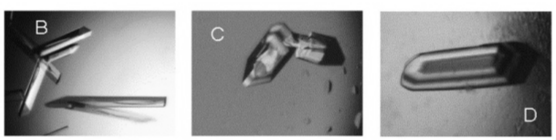
[illegible]



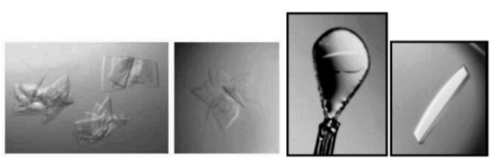




HisJ - His complex:



Tetanus Toxin C Fragment:



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

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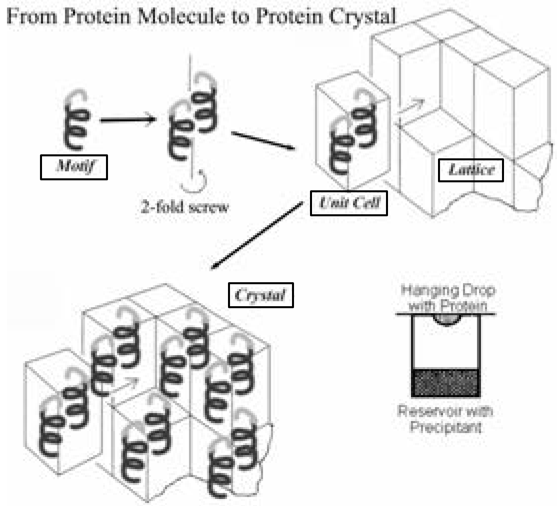
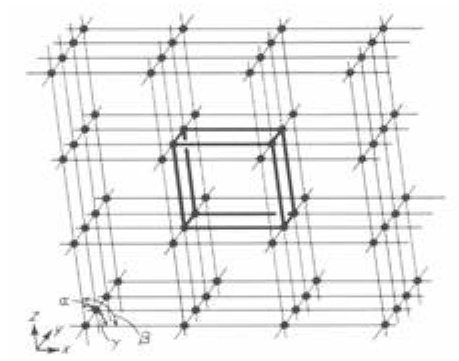


A unit cell is defined by its lattice constants:

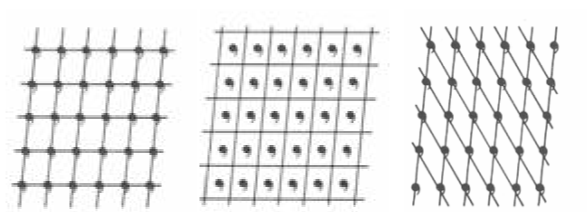
a, b, c and a, b, g



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

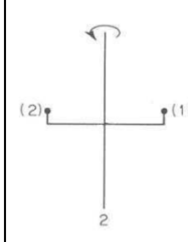
How to identify "the" unit cell ?

Regular two-dimensional array.

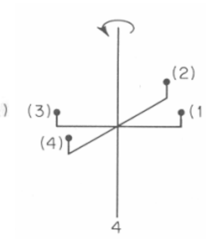


Three different grid systems referred to the array same array.

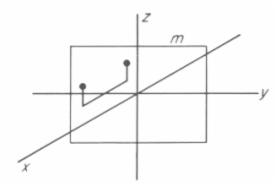
How to identify "the" unit cell ?
Role of Symmetry



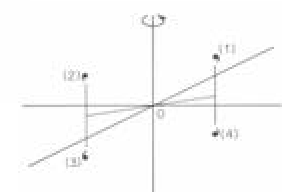
A 2-fold rotation axis.



A 4-fold rotation axis.

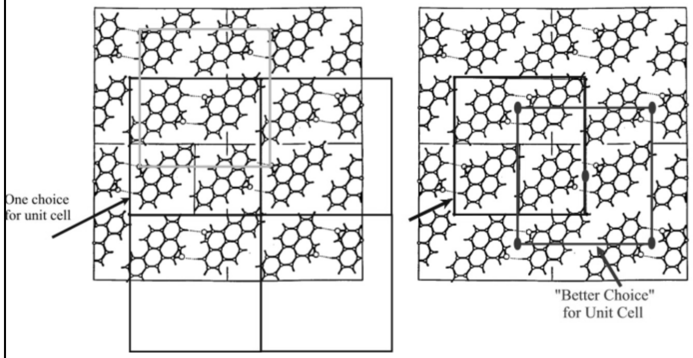


A mirror plane.



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

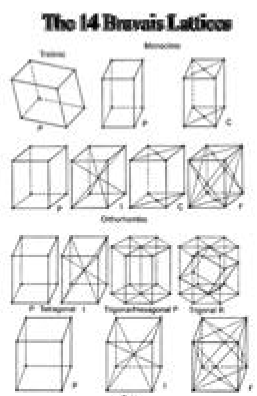
Crystal System	Bravais Type(s)	External Minimum Symmetry	Unit Cell Properties
Triclinic	P	None	a, b, c, al, be, ga,
Monoclinic	P, C	One 2-fold axis, parallel b (b unique)	a, b, c, 90, be, 90
Orthorhombic	P, I, F	Three perpendicular 2-folds	a, b, c, 90, 90, 90
Tetragonal	P, I	One 4-fold axis, parallel 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

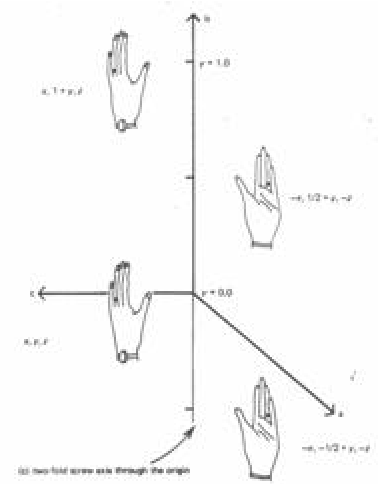
Symmetry operations : 1,2,3,4,6, -1,-2,-3,-4,-6, m

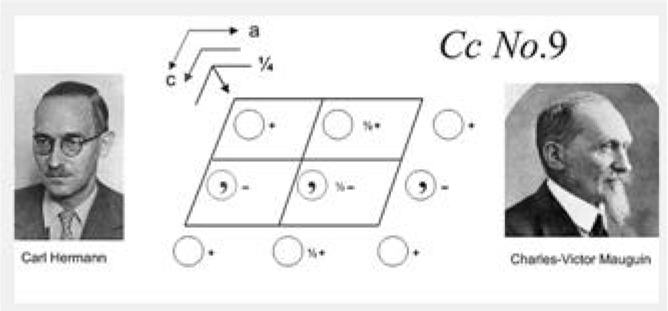
Crystal System	Point groups	Laue Class	Patterson Symmetry
Triclinic	1, -1	-1	P-1
Monoclinic	2, m, 2/m	2/m	P2/m, C2/m
Orthorhombic	222, mm2 , mmm	mmm	Pmmm, Cmmm, Fmmm, Immm
Tetragonal	4, -4, 4/m, 422, 4mm, -42m, 4/mmm	4/m, 4/mmm	P4/m, I4/m, P4/mmm, I4/mmm
Trigonal	3, -3, 32, 3m, -3 m	-3, -3m	P-3, R-3, 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

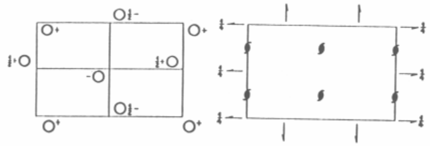
- Laue class corresponds to symmetry of reciprocal space (diffraction pattern)
- Patterson symmetry is Laue class plus allowed Bravais centering, i.e. centrosymmetric and symorphic







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	$x,y,z;\ \tfrac12-x,\bar y,\tfrac12+z;\ \tfrac12+x,\tfrac12-y,\bar z;\ \bar x,\tfrac12+y,\tfrac12-z.$	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.*

BPTI Space Group P212121

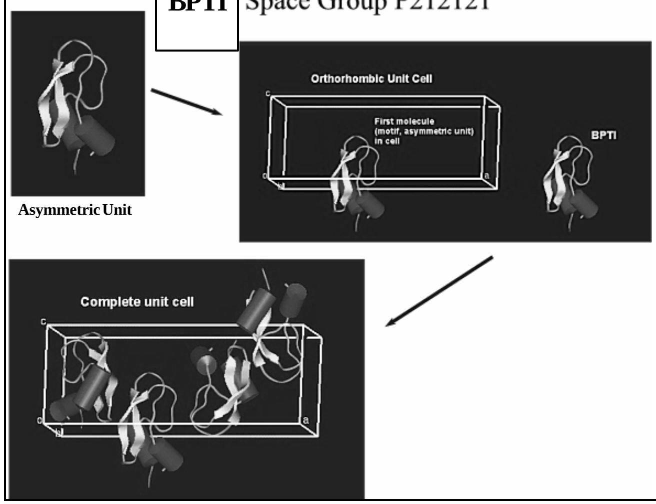


TABLE 18-5 *The 65 "Biological" Space Groups*

CRYSTAL SYSTEM	LATTICE	MINIMUM SYMMETRY OF UNIT CELL	UNIT CELL EDGES AND ANGLES[a]	DIFFRACTION PATTERN SYMMETRY[b]	SPACE GROUPS[c]
Triclinic	P	None	$a \neq b \neq c$, $\alpha \neq \beta \neq \gamma$	$\bar{1}$	$P1$
Monoclinic	P	2-fold axis parallel to b	$a \neq b \neq c$, $\alpha = \gamma = 90°$, $\beta \neq 90°$	$2/m$	$P2$, $P2_1$
	C				$C2$
Orthorhombic	P	3 mutually perpendicular 2-fold axes	$a \neq b \neq c$, $\alpha = \beta = \gamma = 90°$	mmm	$P222$, $P2_12_12_1$, $P222_1$, $P2_12_12$
	C				$C222$, $C222_1$
	I				$[I222, I2_12_12_1]$
	F				$F222$
Tetragonal	P	4-fold axis parallel to c	$a = b \neq c$, $\alpha = \beta = \gamma = 90°$	$4/m$	$P4$, $(P4_1, P4_3)$, $P4_2$
	I				$I4$, $I4_1$
				$4/mmm$	$P422$, $(P4_122, P4_322)$, $P4_222$
					$P42_12$, $(P4_12_12, P4_32_12)$, $P4_22_12$
					$I422$, $I4_122$
Trigonal/rhombohedral	R*	3-fold axis parallel to c	$a = b = c$, $\alpha = \beta = \gamma \neq 90°$	$\bar{3}$	$R3$
	P*				$P3$, $(P3_1, P3_2)$
				$\bar{3}m$	$R32$
					$[P321, P312]$
					$[(P3_121, P3_221), (P3_112, P3_212)]$
Hexagonal	P	6-fold axis parallel to c	$a = b \neq c$, $\alpha = \beta = 90°$, $\gamma = 120°$	$6/m$	$P6$, $(P6_1, P6_5)$
					$P6_3$, $(P6_2, P6_4)$
				$6/mmm$	$P622$, $(P6_122, P6_522)$
					$P6_322$, $(P6_222, P6_422)$
Cubic	P	3-fold axes along cube diagonals	$a = b = c$, $\alpha = \beta = \gamma = 90°$	$m3$	$P23$
					$P2_13$
	I				$[I23, I2_13]$
	F				$F23$
				$m3m$	$P432$, $(P4_132, P4_332)$
					$P4_222$
					$I432$, $I4_132$
					$F432$, $F4_132$

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X-ray tubes: the "sealed" tube

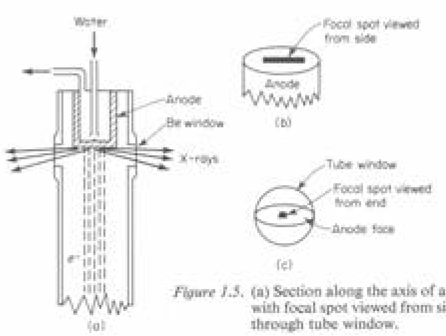
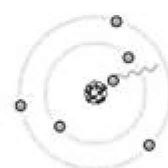


Figure 1.5. (a) Section along the axis of an X-ray tube. (b, Anode with focal spot viewed from side. (c) Focal spot viewed through tube window.

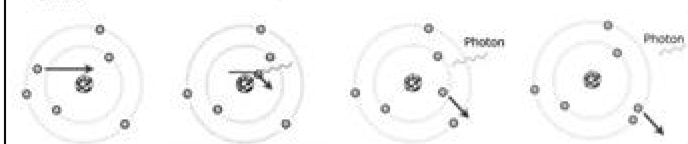
Origin of Non-characteristic X-rays



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.

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.



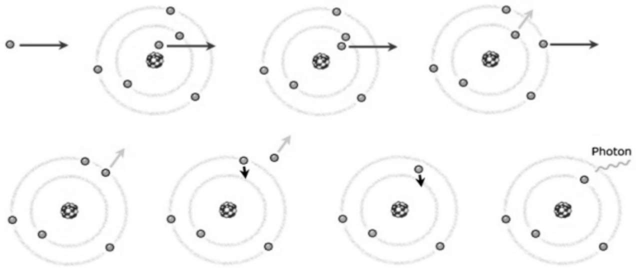
Origin of characteristic X-rays

Related Laureate

The Nobel Prize in Physics 1917 - Charles Glover Barkla »

Characteristic X-ray Lines

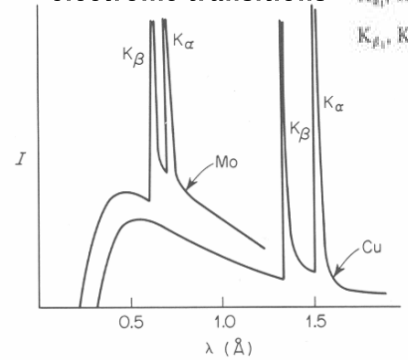
The high energy electron can also cause an electron close to the nucleus in a metal atom to be knocked out from its place. This vacancy is filled by an electron further out from the nucleus. The well defined difference in binding energy, characteristic of the material, is emitted as a monoenergetic photon. 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

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

K_{β_1}, K_{β_2} $M \rightarrow K$



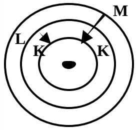


Figure 1.2. X-ray spectra with characteristic peaks: MoKα, 50 Kv; CuKα, 35 Kv.

Characteristic X-rays have defined l

Table 1.1. Target Materials and Associated Constants

	Cr	Fe	Cu	Mo
Z	24	26	29	42
α_1, Å	2.2896	1.9360	$\boxed{1.5405}$	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.

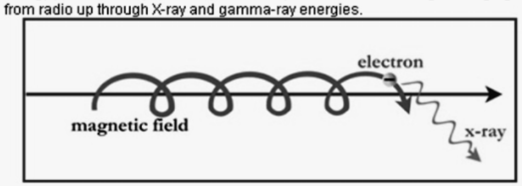
† 1 mil = 0.001 inch = 0.025 mm.

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 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.

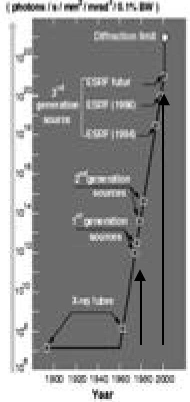
X-ray tubes:
Wilhelm Conrad Röntgen discovered X-rays in 1895 whilst working with cathode-ray tubes. Using the principle of fast electrons hitting a metallic target, a first substantial gain in brilliance was not obtained until the introduction of rotating anode sources (~1960).

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.

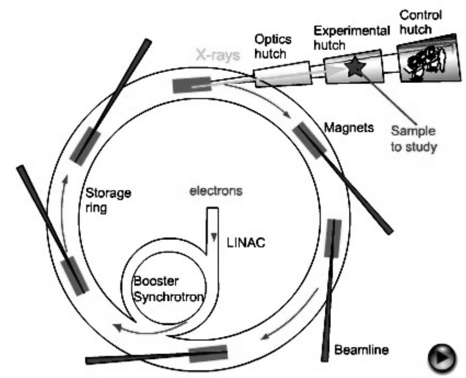
Free Electron X-ray Lasers:
Coupling electron and X-ray beams together, the *Free Electron X-ray Lasers* currently on the drawing boards could be the next generation of X-ray sources. While they promise to achieve an increase in peak brilliance by another factor of a trillion, the first prototypes may be operational around the year 2010.

Brilliance of the X-ray beams

(photons / s / mm² / mrad² / 0.1% BW)

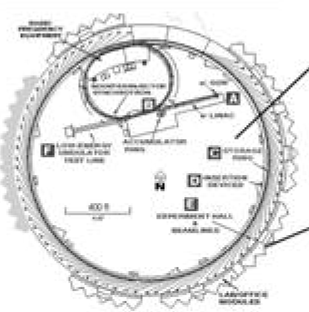


How synchrotron light is produced?



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:

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

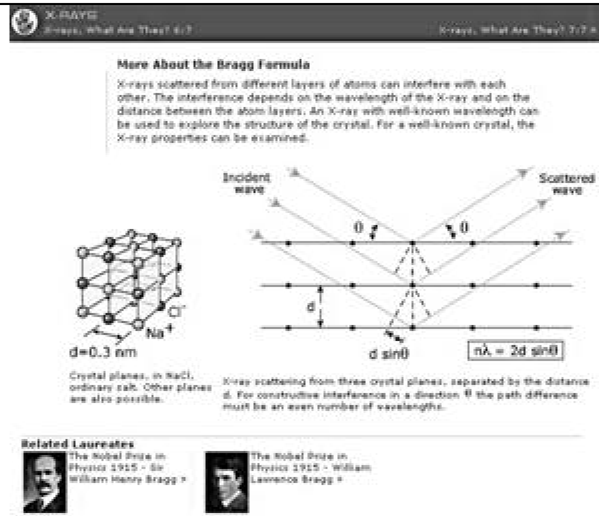
c) X-ray Sources – Sealed Tube / Rotation Anode / Synchrotron

e) Data Collection – Methods / Detectors / Structure Factors

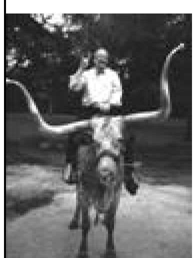
f) Structure Solution – Phase Problem: MIR / MR / MAD

h) Refinements and Models

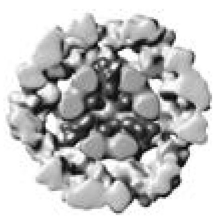
i) Analysis and presentation of results



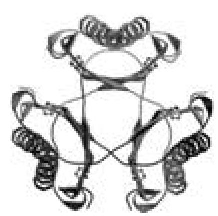
"If a picture is worth a thousand words, then a macromolecular structure is priceless to a physical biochemist." – van Holde



- Light Photography
  l ~ **400 - 700 nm**



- Electron Microscopy
  l ~ **0.001 - 0.1 nm**



- X-Ray or NMR
  l ~ **0.1 nm**

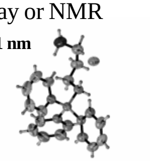
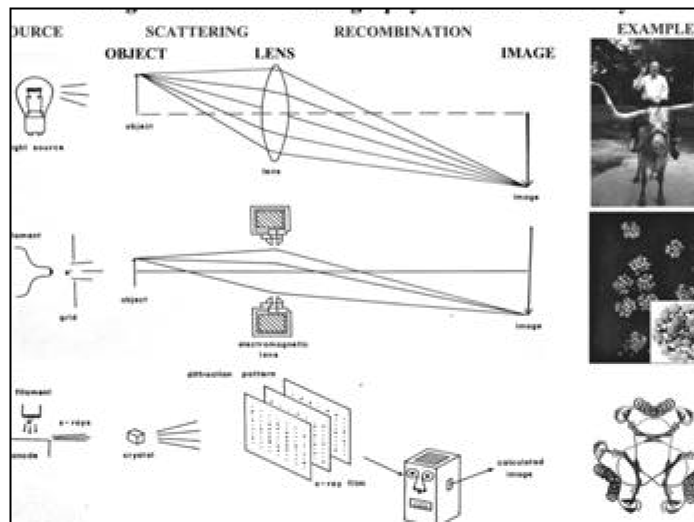


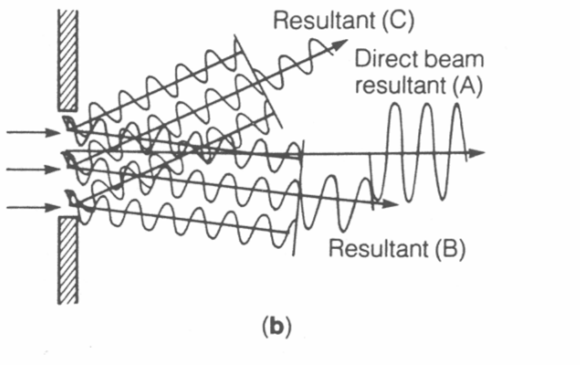
Image Formation
Abbe (~1873):

Limit Res. ~ $\lambda/2$



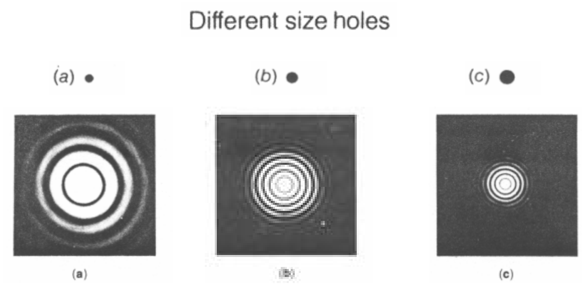
Single Hole Scattering Experiment

Transforms / Reciprocal Space



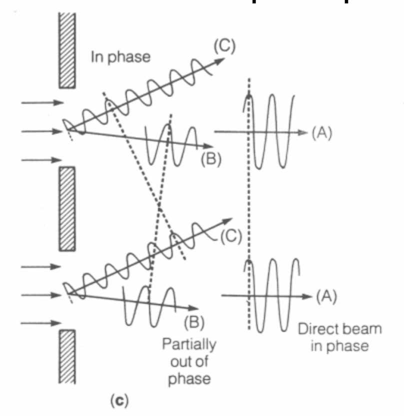
Single Hole Scattering Experiment

Transforms / Reciprocal Space

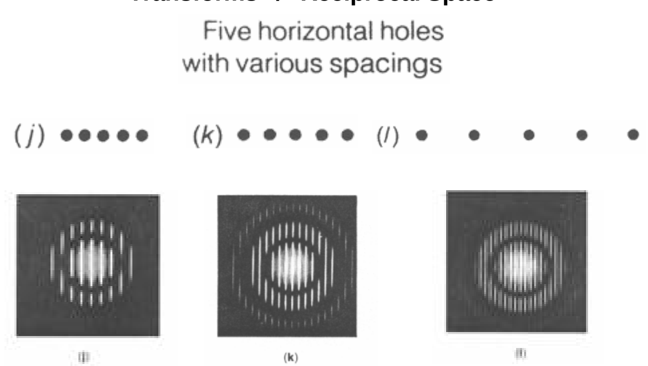


Effect of Multiple "Scatterers"

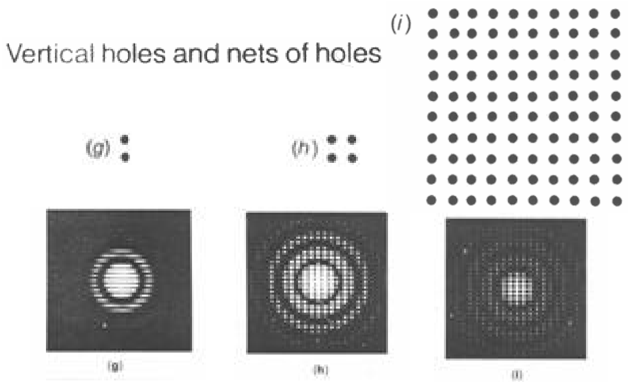
Transforms / Reciprocal Space



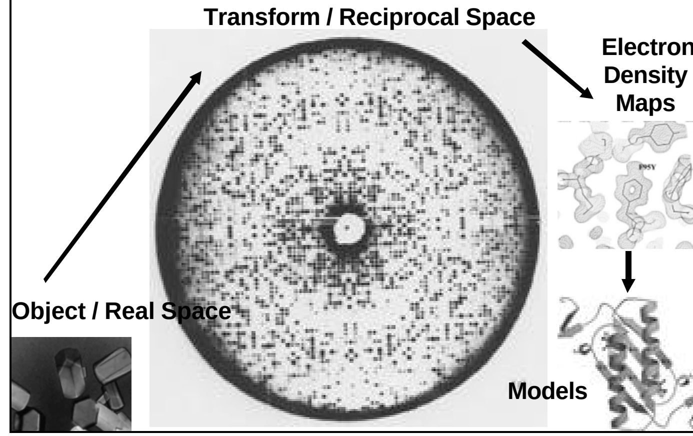
Transforms / Reciprocal Space



Transforms / Reciprocal Space



Object → Transform → Image



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:

- 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.

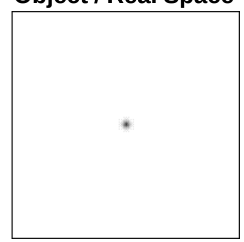
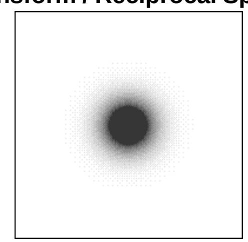
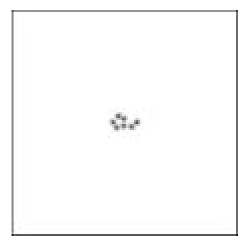
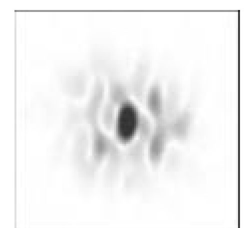
Other topics:

The Interactive Structure Factor Tutorial Learn about structure factors and maps.

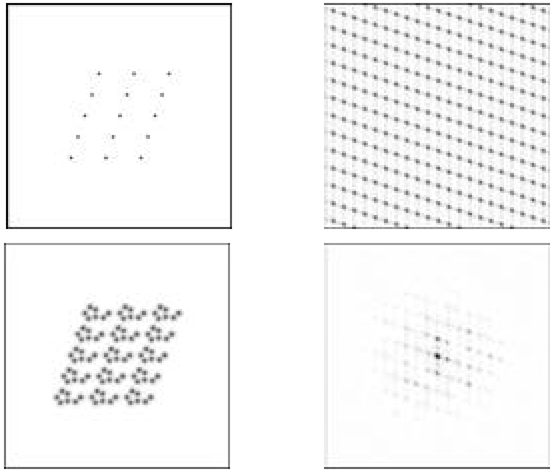
An introduction to crystallographic Fourier transforms. The mathematical link between Scattering theory and Fourier theory. An explanation of the convolution theorem.

Teaching materials elsewhere

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

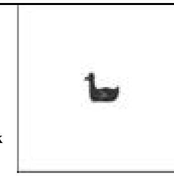
Object / Real Space	Transform / Reciprocal Space
	
	

Object / Real Space — Transform / Reciprocal Space

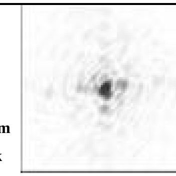


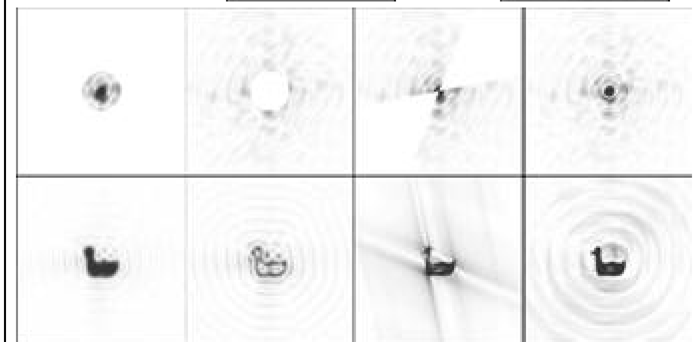
Objects – Transforms and Image Formation

A Duck



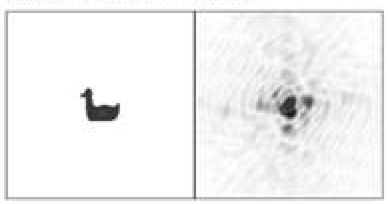
Transform of a Duck

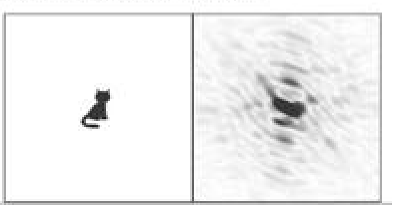




Kevin Cowtan's Book of Fourier

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

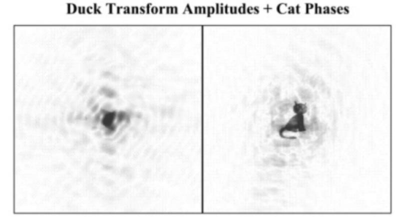




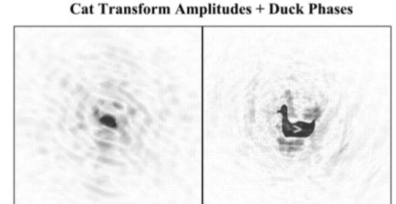
Kevin Cowtan's Book of Fourier

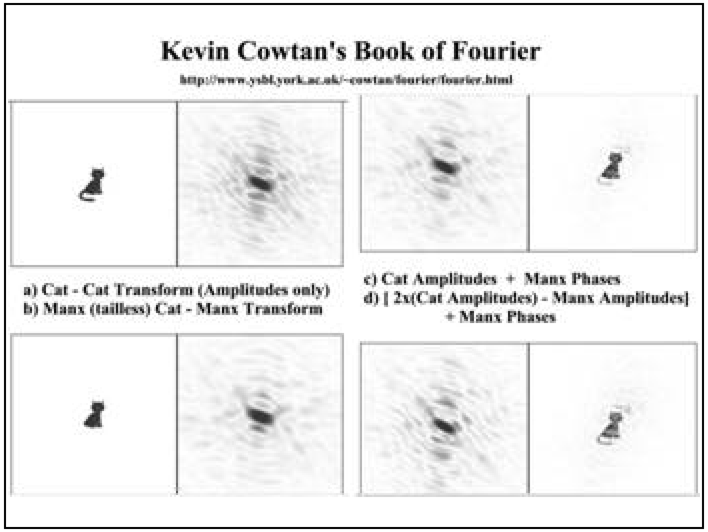
http://www.ysbl.york.ac.uk/~cowtan/fourier/fourier.html

Duck Transform Amplitudes + Cat Phases



Cat Transform Amplitudes + Duck Phases




Kevin Cowtan's Book of Fourier
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