X-Ray Crystallography

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

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X-ray tubes: the "sealed" tube

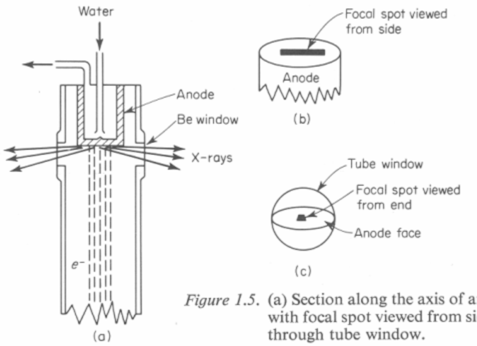
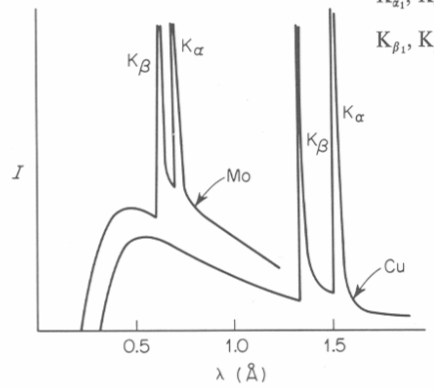


Figure 1.5. (a) Section along the axis of an X-ray tube. (b, Anode with focal spot viewed from side. (c) Focal spot viewed through tube window.

Characteristic X-rays arise from electronic transitions

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

K_{β_1}, K_{β_2} $M \rightarrow K$



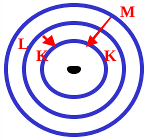


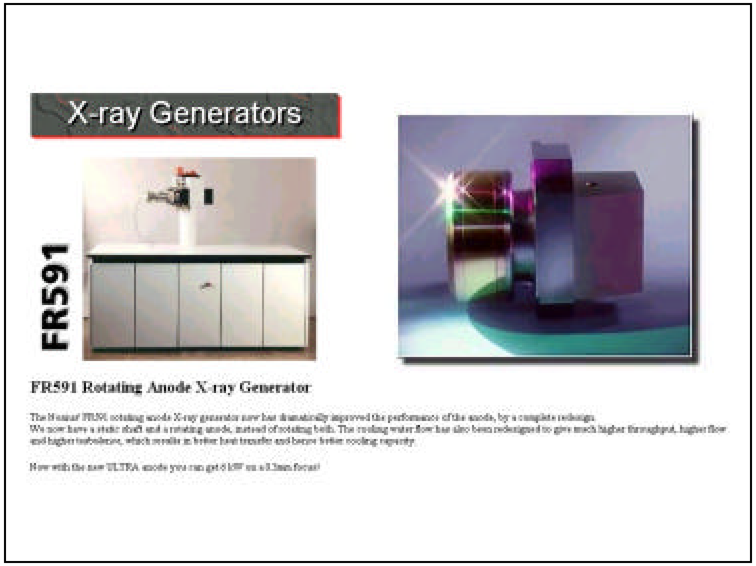
Figure 1.2. X-ray spectra with characteristic peaks: MoKα, 50 Kv; CuKα, 35 Kv.

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	$\boxed{1.5418}$	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.

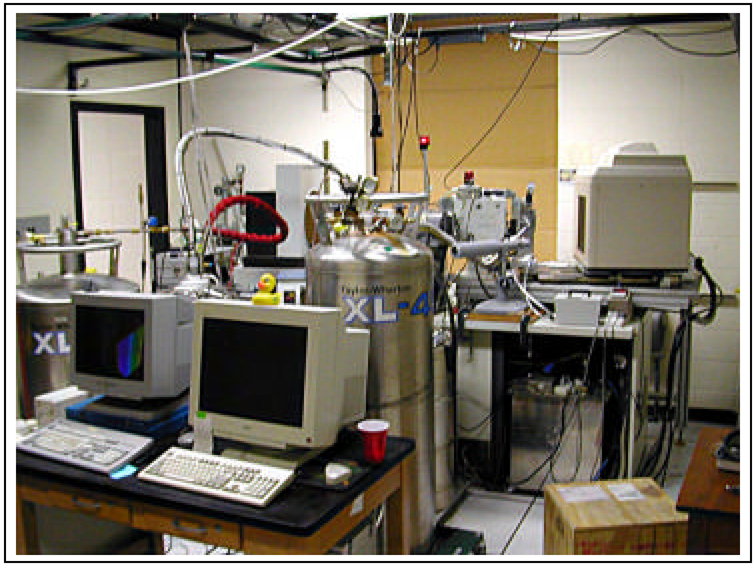


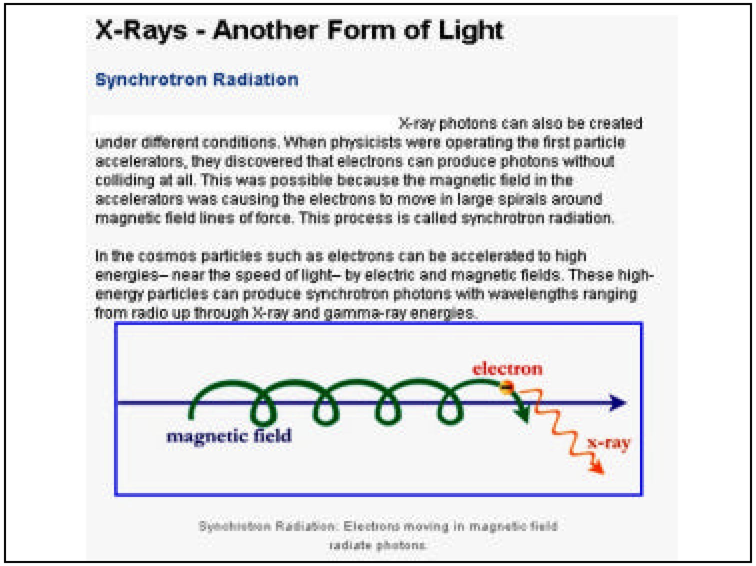
X-ray Generators

FR591 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!





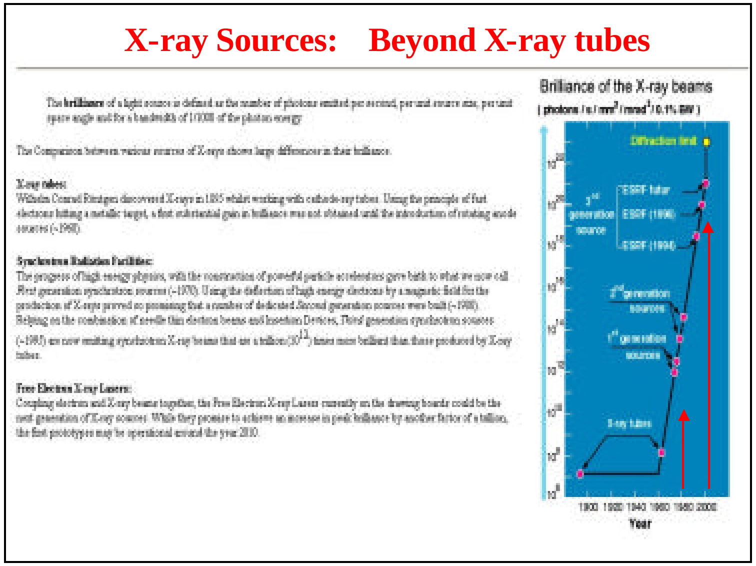
X-Rays - Another Form of Light

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.

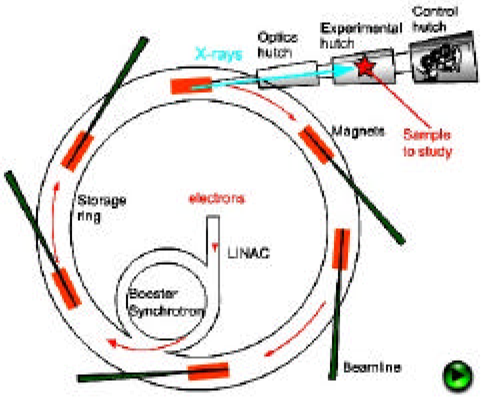
The Comparison between various sources of X-rays shows large differences in their brilliance.

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.

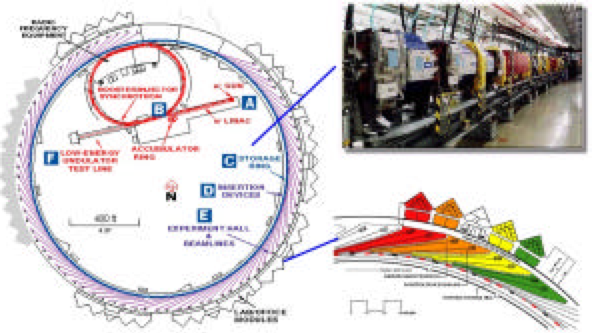
Free Electron X-ray Lasers:
Coupling electrons and X-ray beams together, the Free Electron X-ray Lasers currently on the drawing boards could be the next generation of X-ray sources. While they promise to achieve an increase in peak brilliance by another factor of a billion, the first prototypes may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

How synchrotron light is produced?



APS - Advanced Photon Source

Argonne National Laboratory



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Diffraction: Scattering from "atoms"

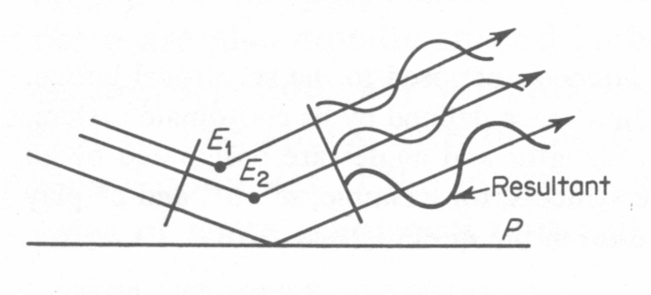
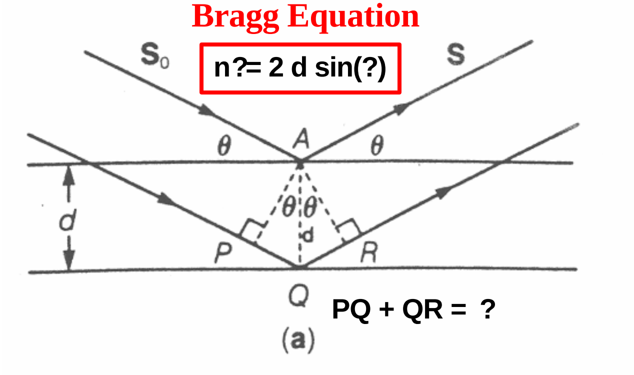


Figure 2.10. Diffraction from E_1 and E_2 as if reflected from plane P.

Crystals: Scattering from “planes”

Bragg Equation



n?= 2 d sin(?)

PQ + QR = ?

Bragg Planes

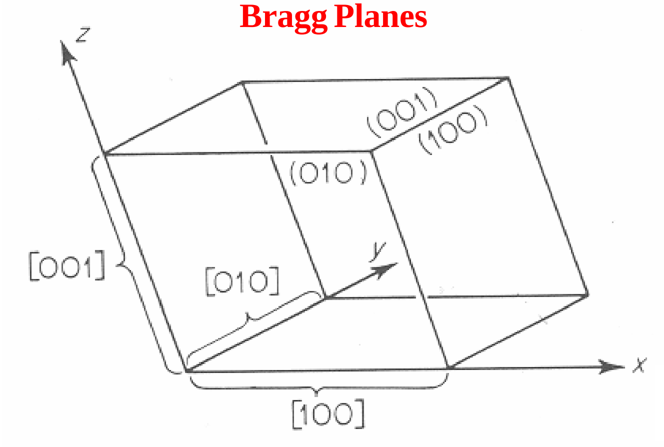


Figure 2.7. Unit cell showing bounding planes and edges.

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1 1 0 **1 3 0** **-2 1 0**

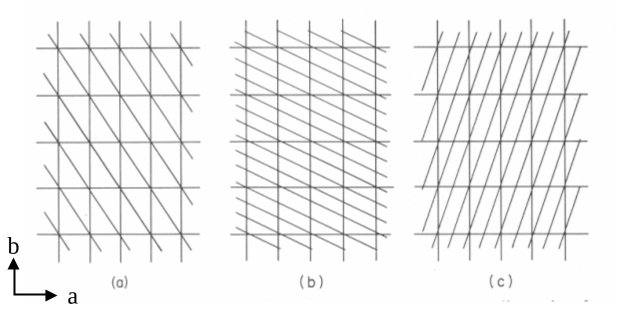


Figure 2.5. Three families of lattice "planes" in a two-dimensional lattice.

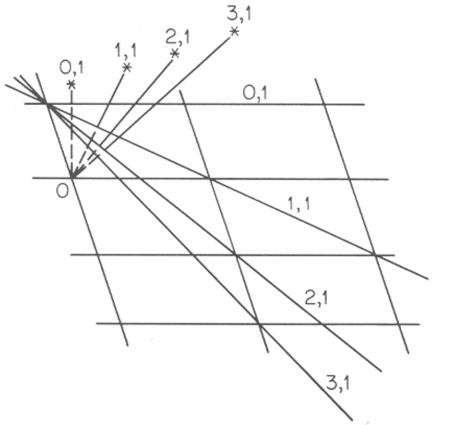
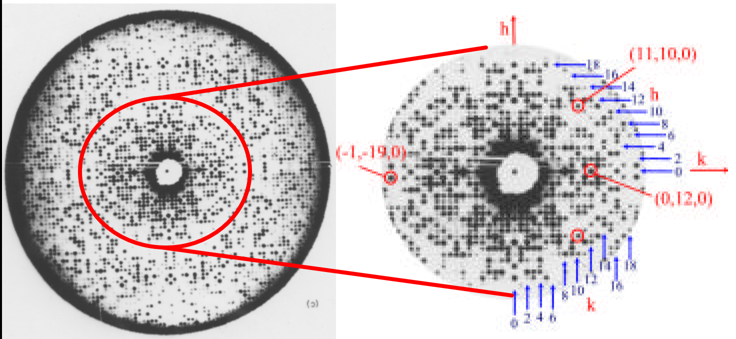


Figure 2.11. Planes in direct space represented by points in reciprocal space.

Electron Density Function

$$\rho(X,Y,Z) = \frac{1}{V}\sum_h\sum_k\sum_l \underline{F(hkl)}\ \underline{\exp[i\alpha(hkl)]}\exp[-2\pi i(hX + kY + lZ)]$$



Measure thousands of **Amplitudes** - $[F_{hkl}]$'s - ?? How do we obtain **Phases** α_{hkl} ??

→ **Phase Problem**

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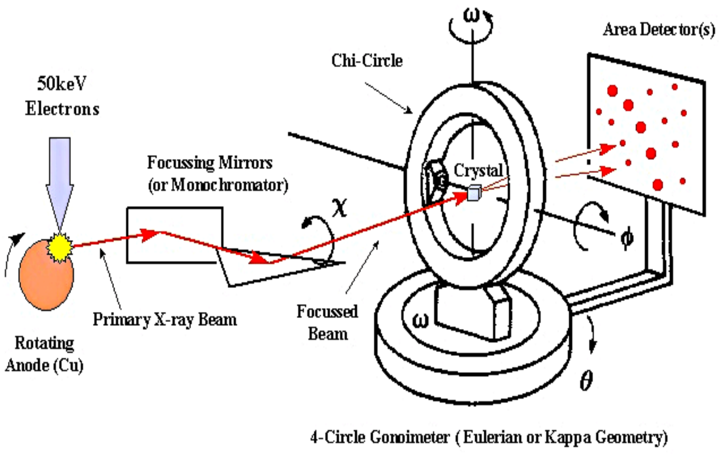
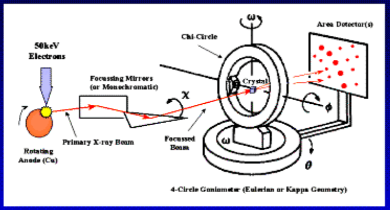
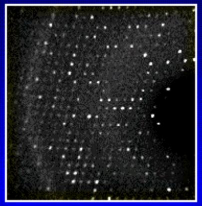


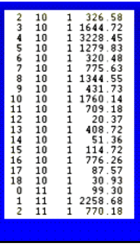
Advanced Methods in Modern Biomolecular Crystallography

The information we get from a single diffraction experiment…..

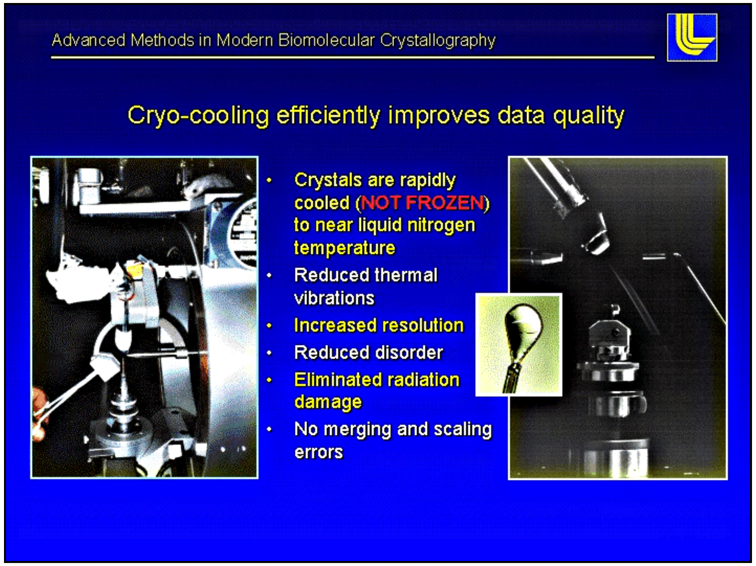




The reflections are indexed (consistent assignment of reciprocal cell indices h,k,l) and all we get for the money is a long list of intensities from several ten thousand reflections



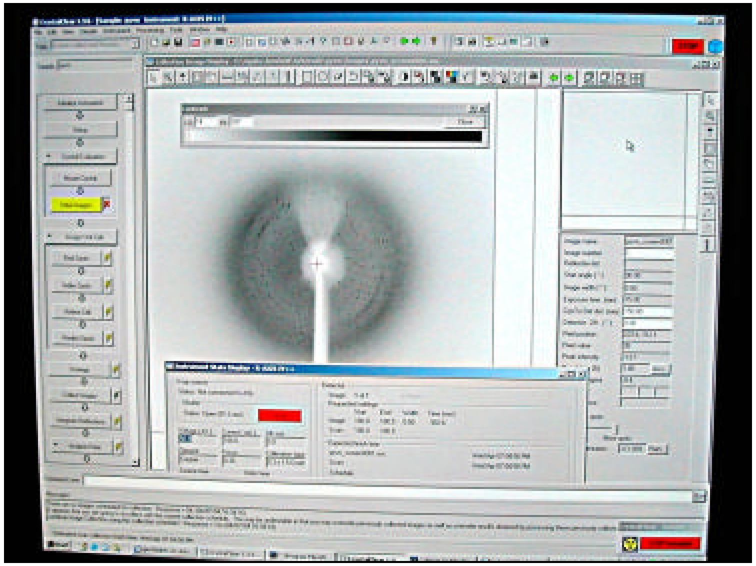
```
  2  10    1   326.58
  3  10    1  1644.72
  4  10    1  3228.45
  5  10    1  1279.83
  6  10    1   320.48
  7  10    1   775.63
  8  10    1  1344.55
  9  10    1   431.73
 10  10    1  1760.14
 11  10    1   709.18
 12  10    1    20.37
 13  10    1   408.72
 14  10    1    51.36
 15  10    1   114.72
 16  10    1   776.26
 17  10    1    87.57
 18  10    1    30.93
  0  11    1    99.30
  1  11    1  2258.68
  2  11    1   770.18
```



Advanced Methods in Modern Biomolecular Crystallography

Cryo-cooling efficiently improves data quality

- Crystals are rapidly cooled (NOT FROZEN) to near liquid nitrogen temperature
- Reduced thermal vibrations
- Increased resolution
- Reduced disorder
- Eliminated radiation damage
- No merging and scaling errors



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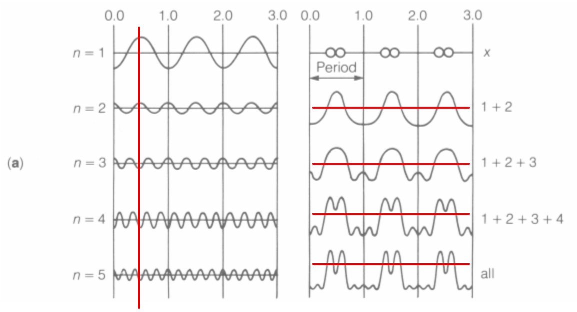
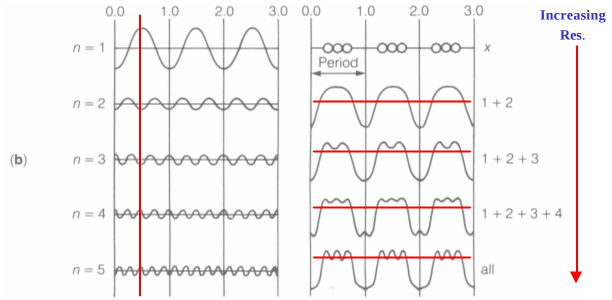
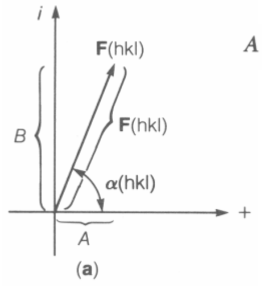


Representation of the electron density of a one-dimensional "crystal" by a superposition of waves. The crystal is formed by a periodic repetition of a diatomic molecule, as shown at the top of the right-hand column. The component waves, each with proper phase and amplitude, are on the left. The curves on the right show the successive superposition of the five waves on the left. (From Waser, 1968.)



Representation of another one-dimensional crystal, this one containing a triatomic molecule. Note that this crystal is built up from the same waves as the crystal of (a) ; only the amplitudes and phases have been changed. (From Waser, 1968.)

$$\mathbf{F} = A + iB$$



$$A = F \cos \alpha \quad \text{and} \quad B = F \sin \alpha$$

$$F = |\mathbf{F}| = \sqrt{A^2 + B^2} = \sqrt{\mathbf{FF}^*}$$

$$\alpha = \tan^{-1} \frac{B}{A}$$

The structure factor magnitude F(hkl) is represented by the length of a vector in the complex plane.

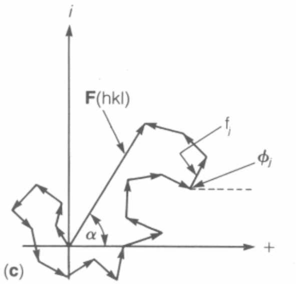
The phase angle α(hkl) is given by the angle. measured counterclockwise, between the positive real axis and the vector F.

F(*hkl*) = SQRT [cI(*hkl*)]

Experimental

Calculated

$$\mathbf{F}(hkl) = F(hkl)e^{i\alpha(hkl)} = \sum_{j=1}^{N'} \mathbf{f}_j(hkl) = \sum_{j=1}^{N'} f_j(hkl)e^{i\phi_j(hkl)}$$



The structure factor for a reflection may be thought of as the vector sum of the x-ray scattering contributions from many atoms.

Each of the j contributions may be represented as a vector in the complex plane, with amplitude f_j and phase phi_j.