SESAME/JSPS School

Protein Crystallography (PX)

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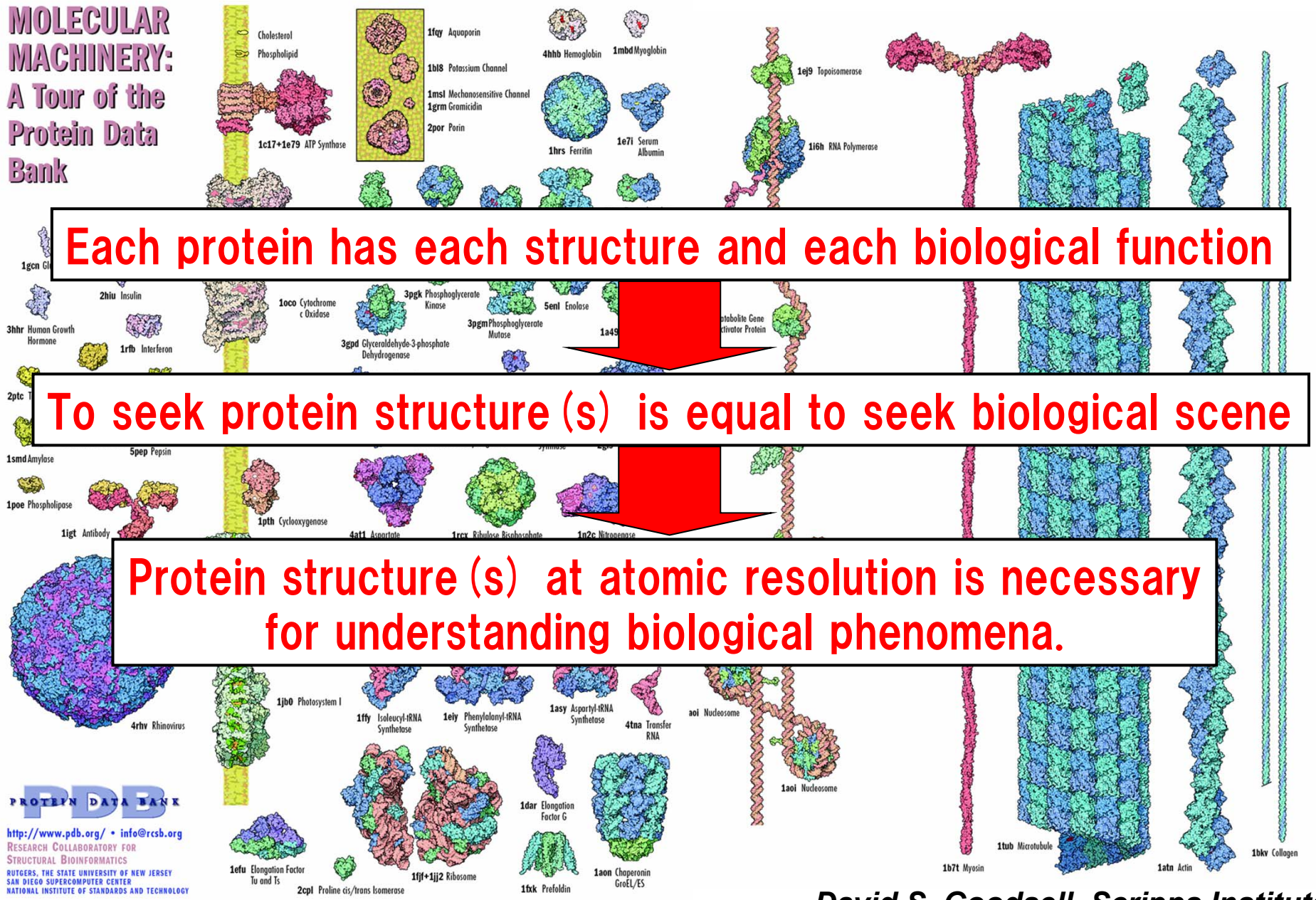
(Photon Factory, IMSS, KEK, Japan)

Amman(Jordan) on Nov 14, 2011

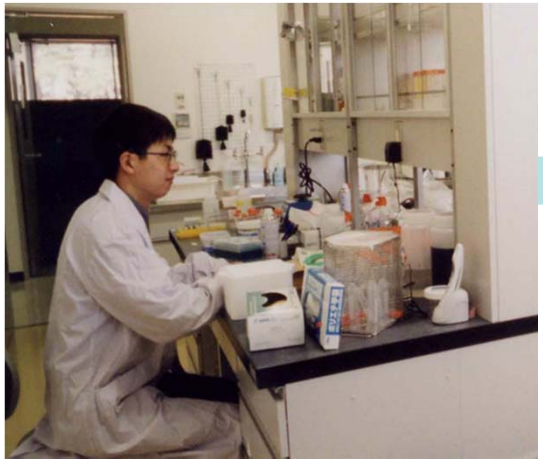
The Worldwide Protein Data Bank (wwPDB)



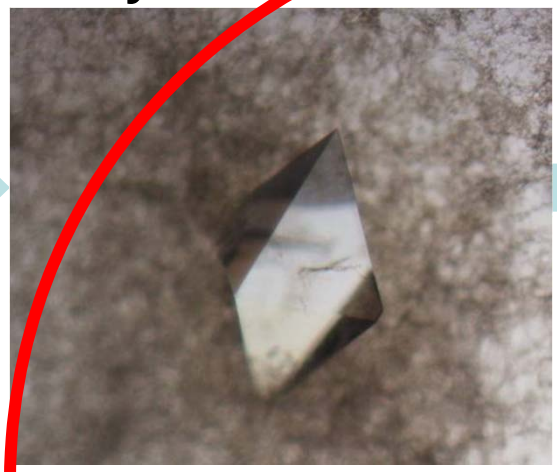
David S. Goodsell, Scripps Institute

Scheme of protein crystallography

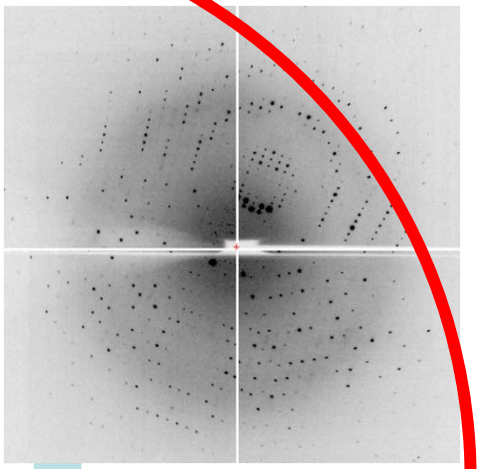
Expression &
Purification



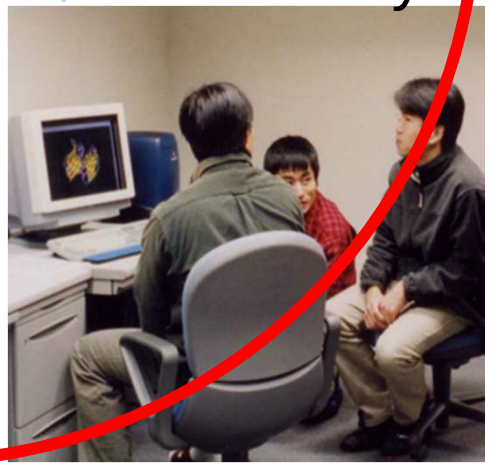
Crystallization



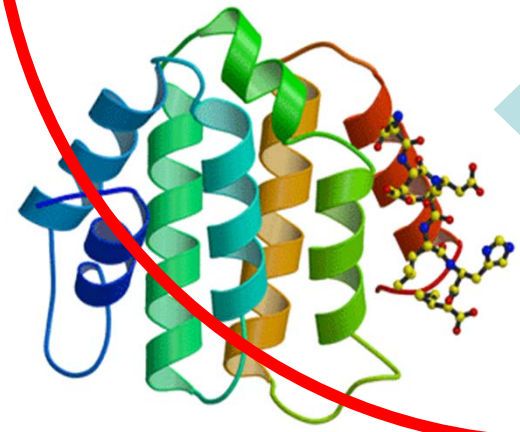
Data collection



Data analysis

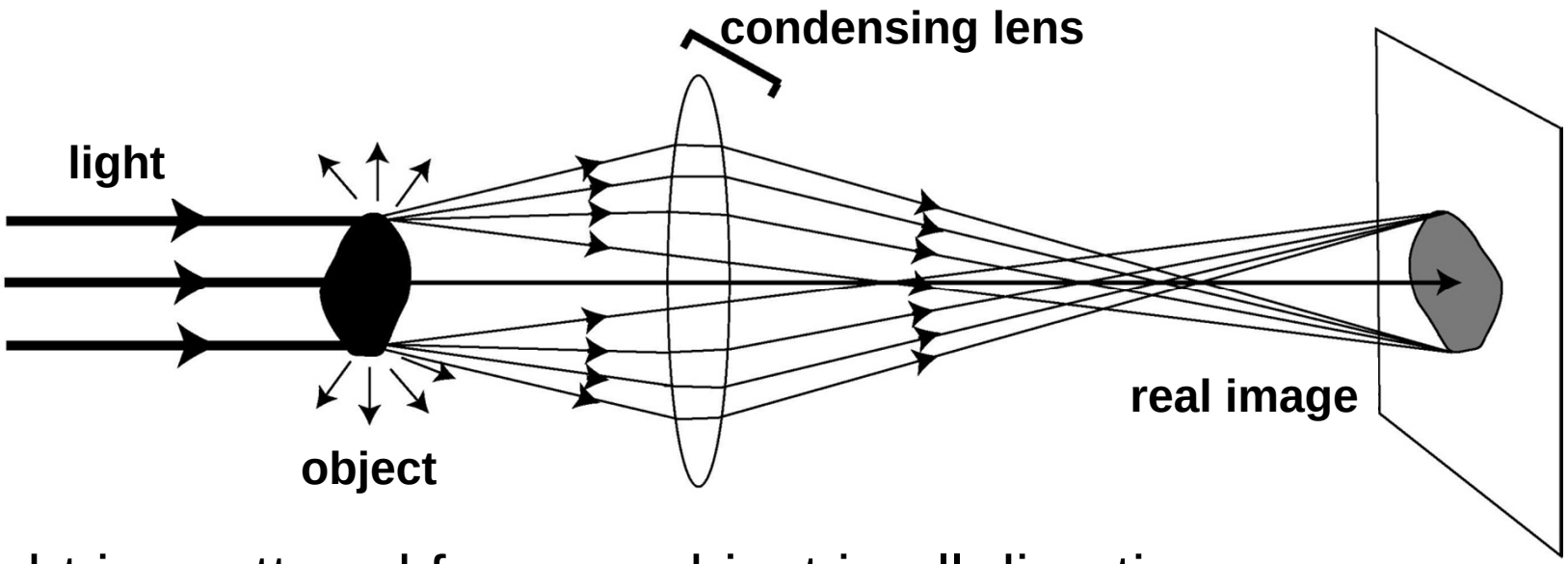


Structure



Functional
analysis

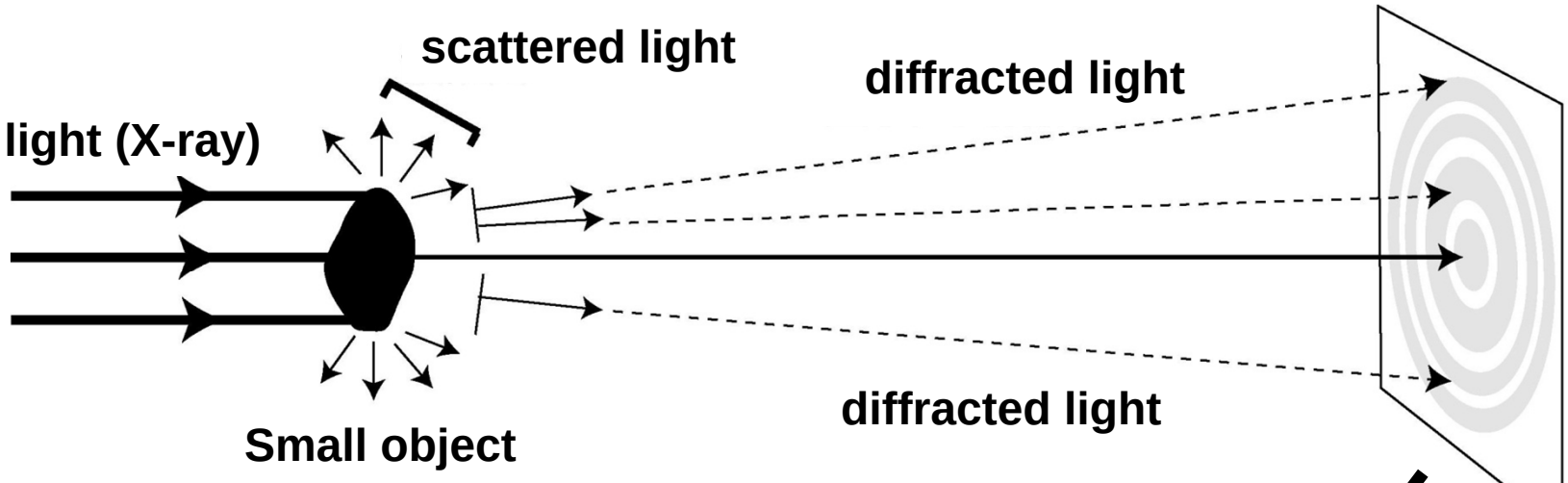
To See Objects, ….



Light is scattered from an object in all directions.
A condensing lens (e.g., eye lens, camera, microscope, or telescope) collects the scattered light and focuses it on a screen (the retina in the case of human being).
This creates an image of the object.

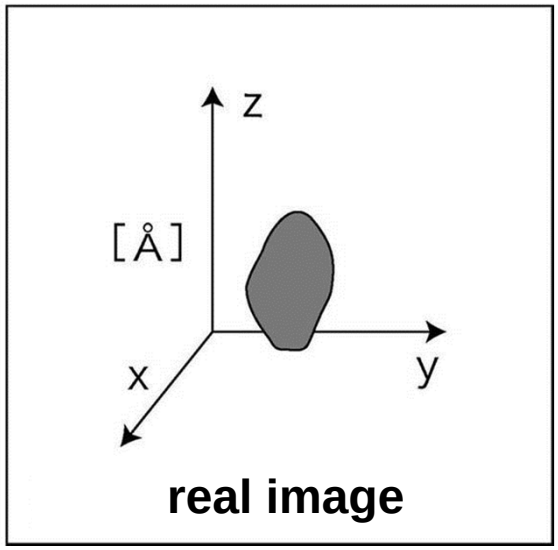
X-rays are necessary for smaller objects (at atomic resolution).
However, lenses for X-rays do not exist, therefore one cannot create an X-ray image.

Scattering and Diffraction



diffraction image

calculation by computer (Fourier transformation)

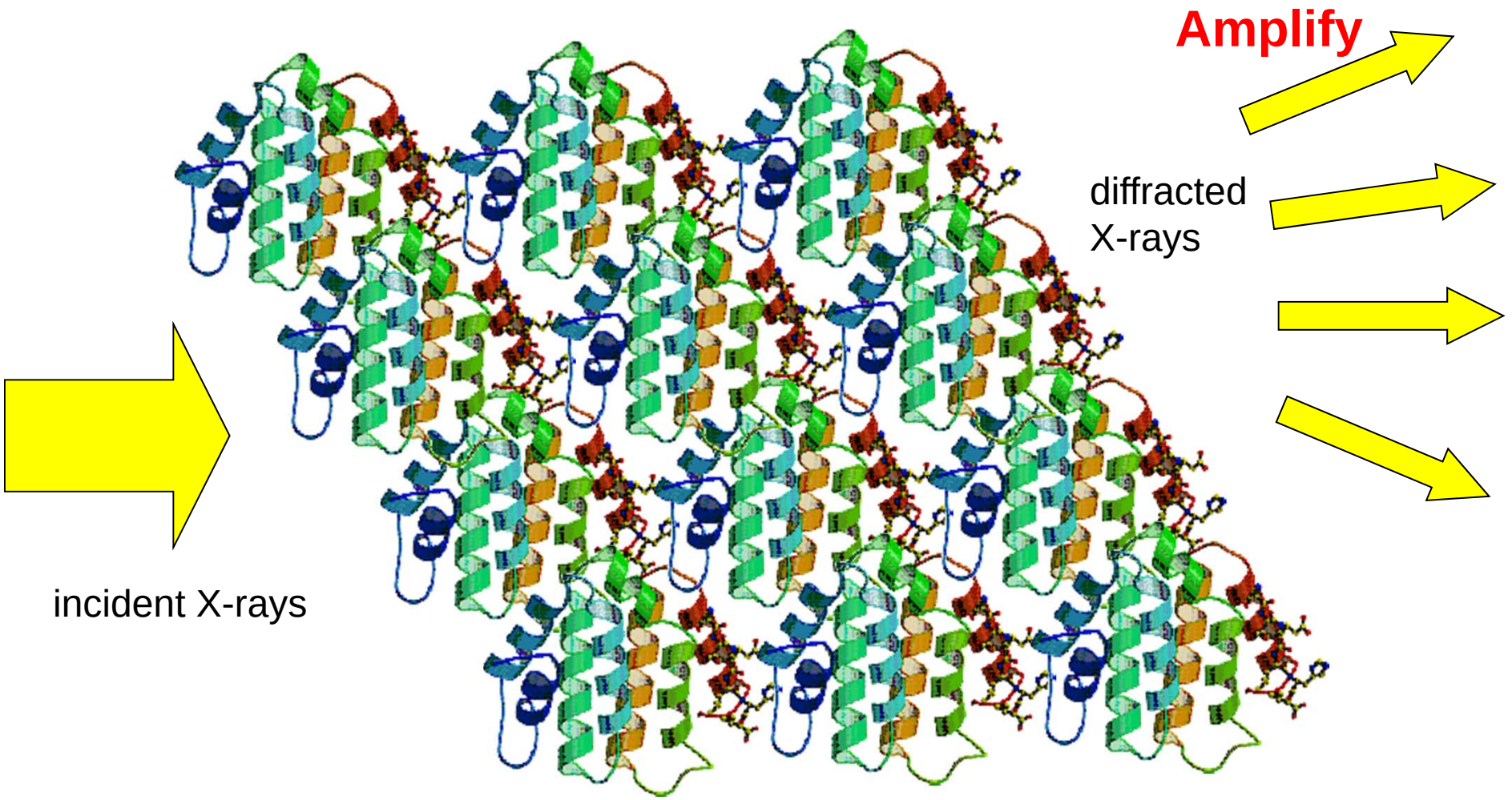


However, the signals are too week to detect the diffraction image.

Needs amplify the signal!

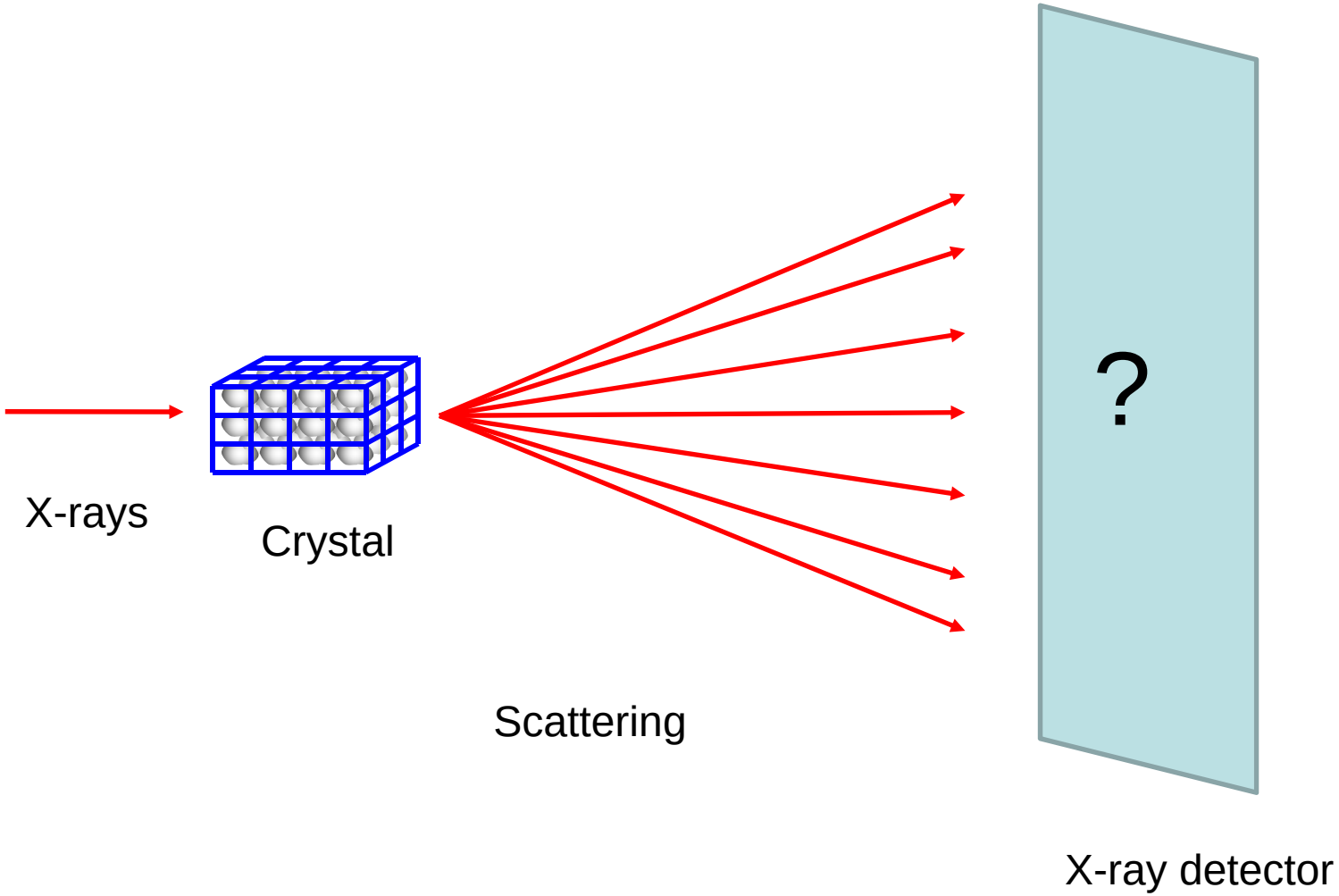
Instead condensing lens

Periodic formation of protein molecules

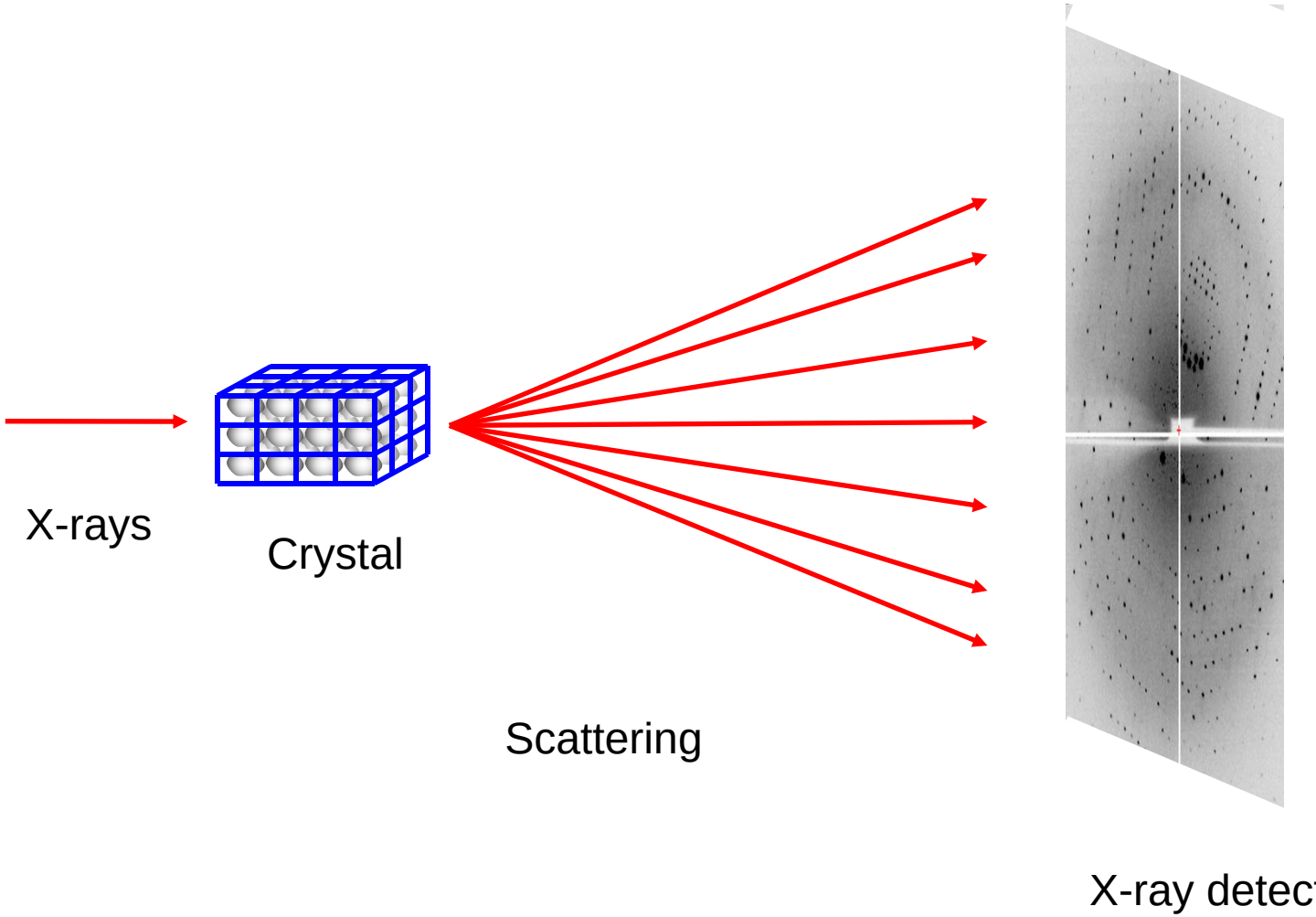


This is two-dimensional arrangement.
If this formation is three-dimensional, it is CRYSTAL.

What kind of images come from a periodic structure ?

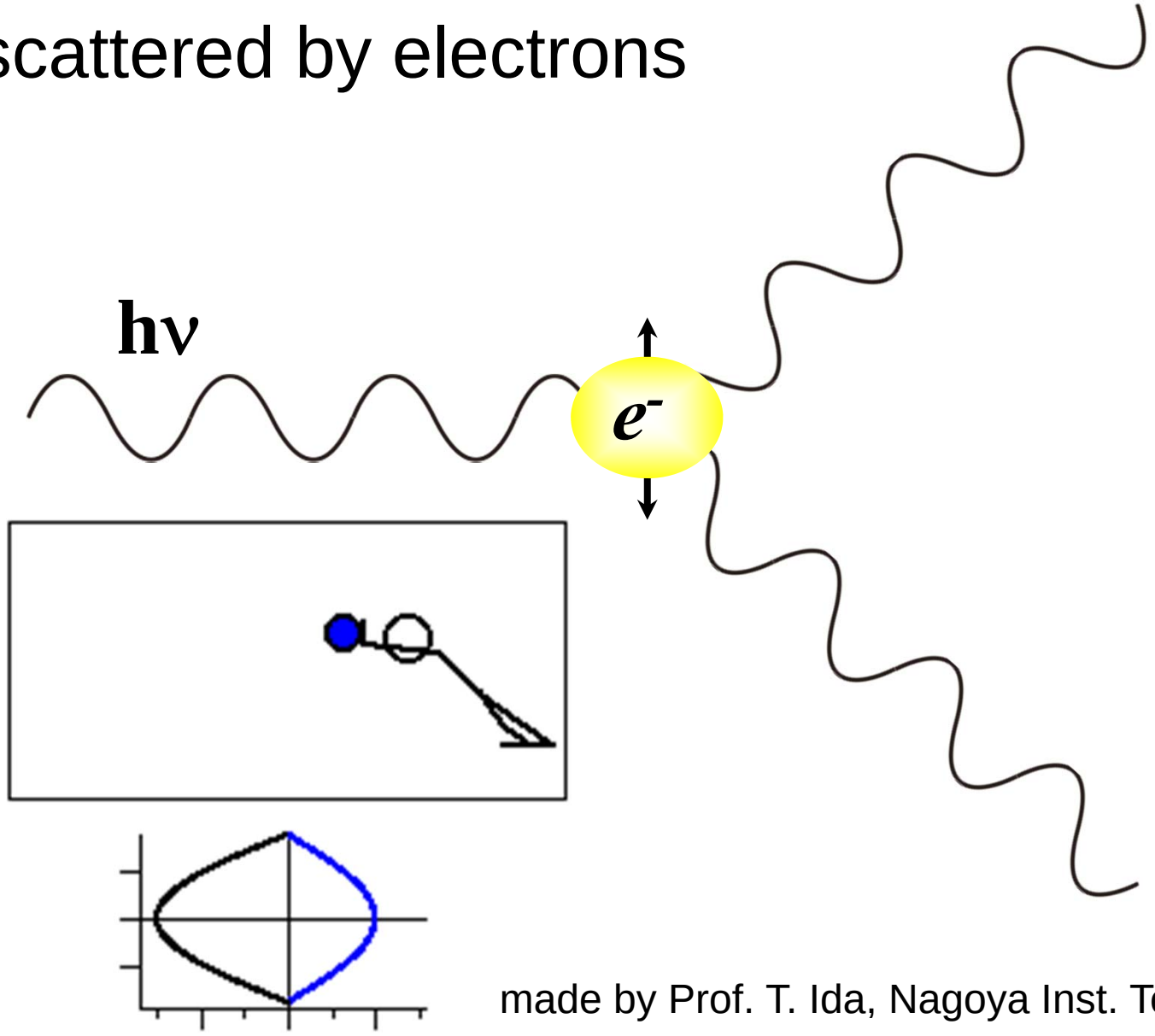


What kind of images come from a periodic structure ?



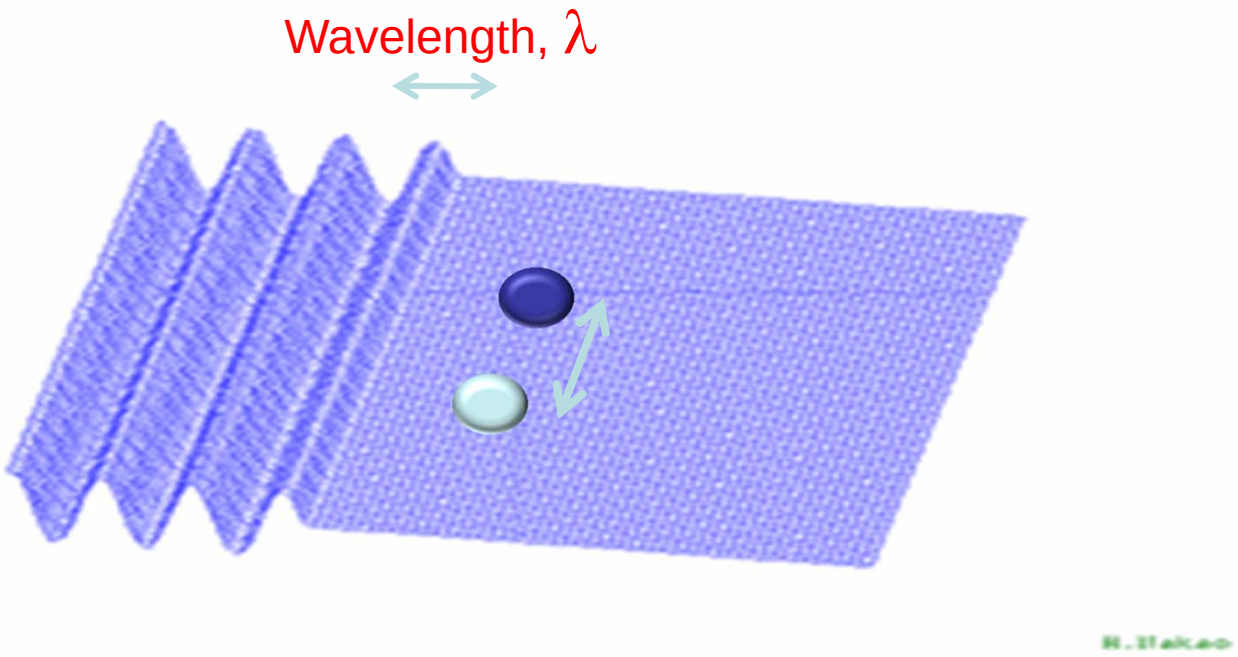
Periodic structure gives periodic diffraction pattern.

X-rays have wave characteristics and are scattered by electrons



made by Prof. T. Ida, Nagoya Inst. Tech

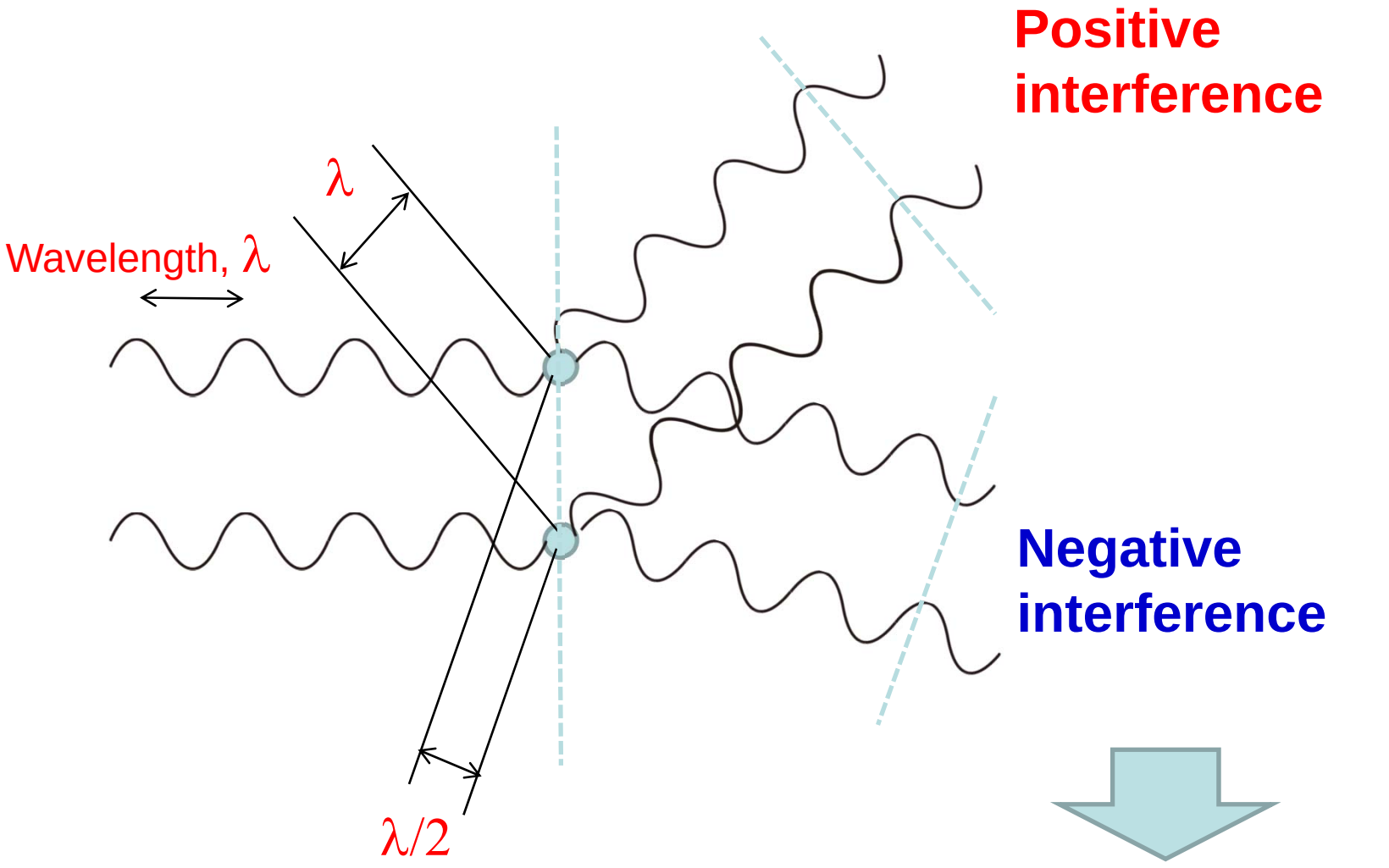
Elastic scattering by two elements



Electromagnetic field vibrates electrons in a material, which give scattering with same wavelength.

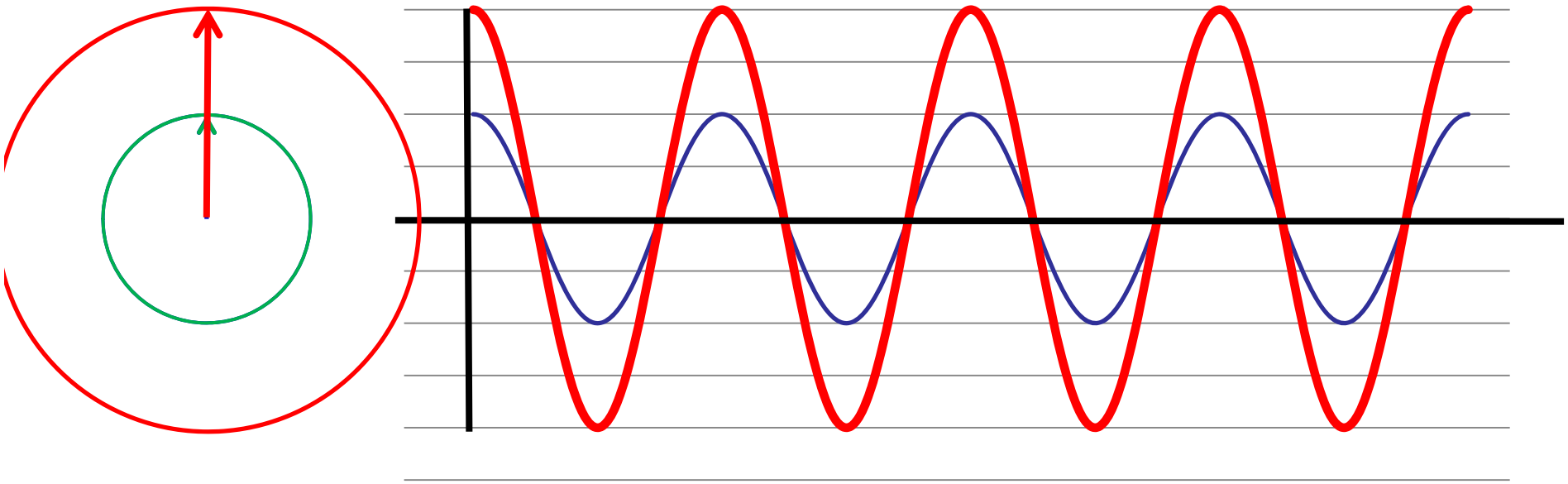
→ Diffraction

Scattering by two elements

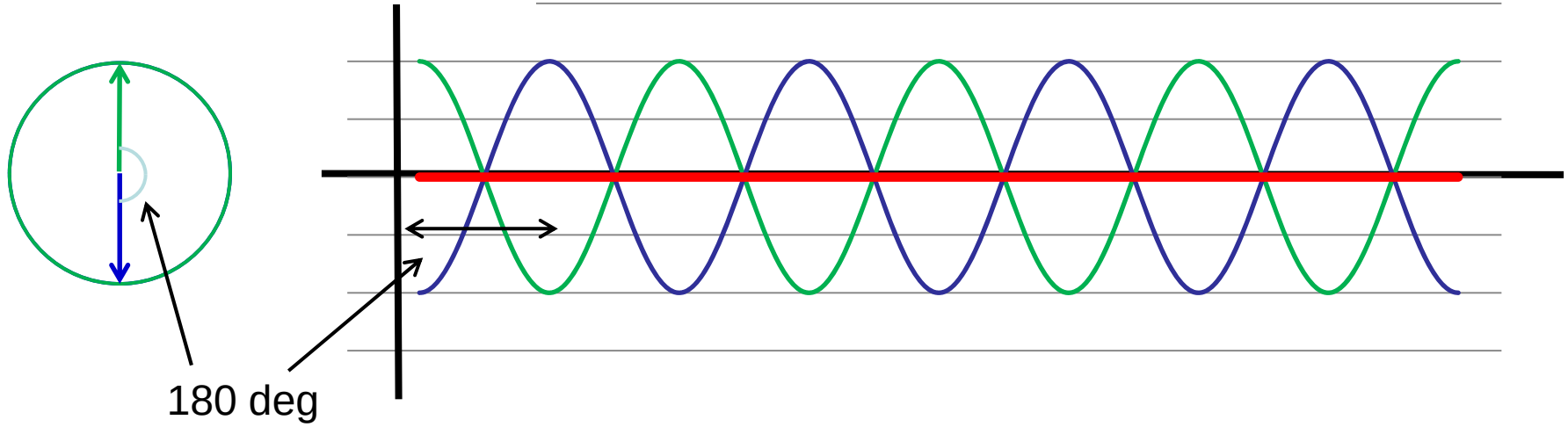


Wave can be expressed on a complex plane

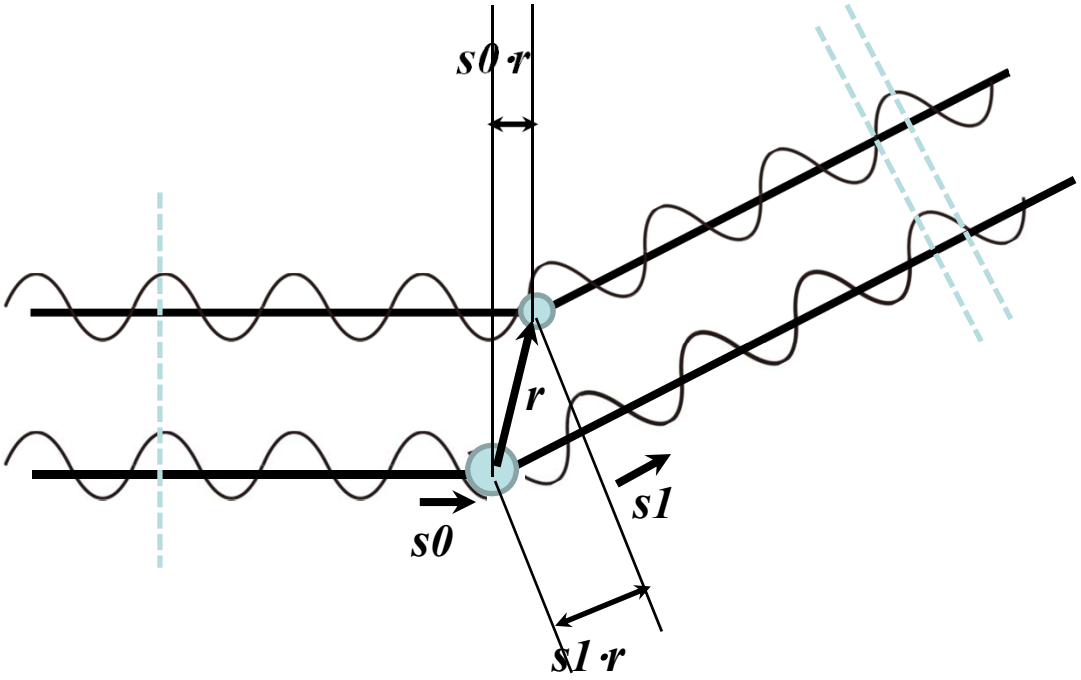
Positive interference



Negative interference



Scattering by two elements



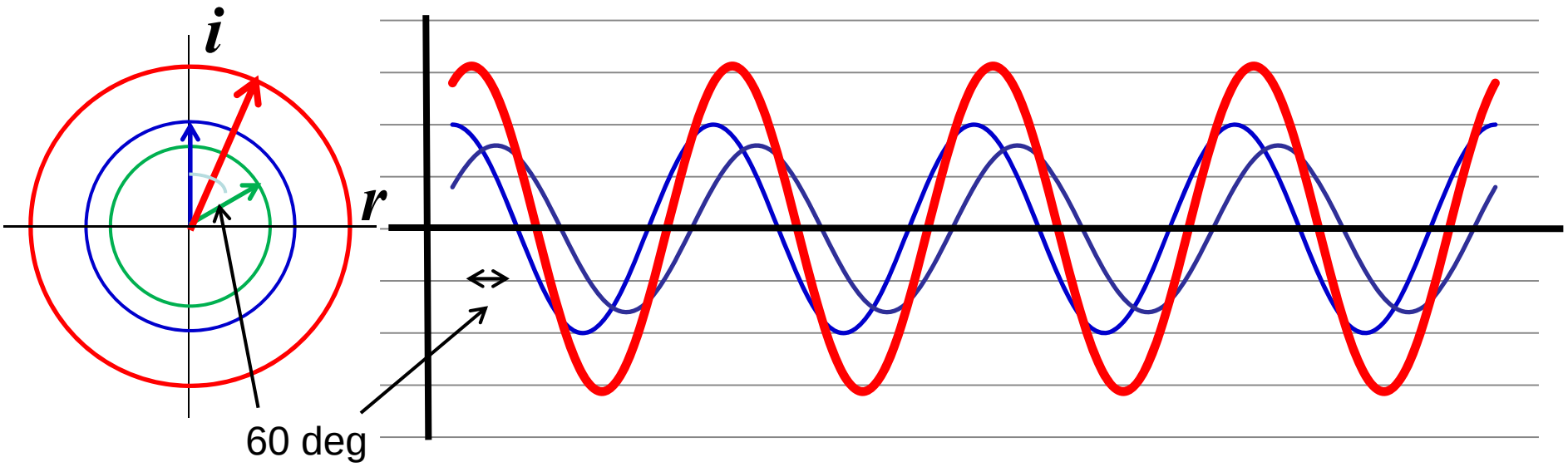
Optical Path difference= $\boldsymbol{s}_1 \cdot \boldsymbol{r} - \boldsymbol{s}_0 \cdot \boldsymbol{r} = (\boldsymbol{s}_1 - \boldsymbol{s}_0) \cdot \boldsymbol{r}$

Phase difference = $2\pi\,(\boldsymbol{s}_1 - \boldsymbol{s}_0) \cdot \boldsymbol{r} / \lambda$

$= 2\pi\,(\boldsymbol{k}_1 - \boldsymbol{k}_0) \cdot \boldsymbol{r}$

($\boldsymbol{s}_i/\lambda = \boldsymbol{k}_i$, $\boldsymbol{s}_i$ is an unit vector, λ is wavelength)

Equation of wave function and sum of waves



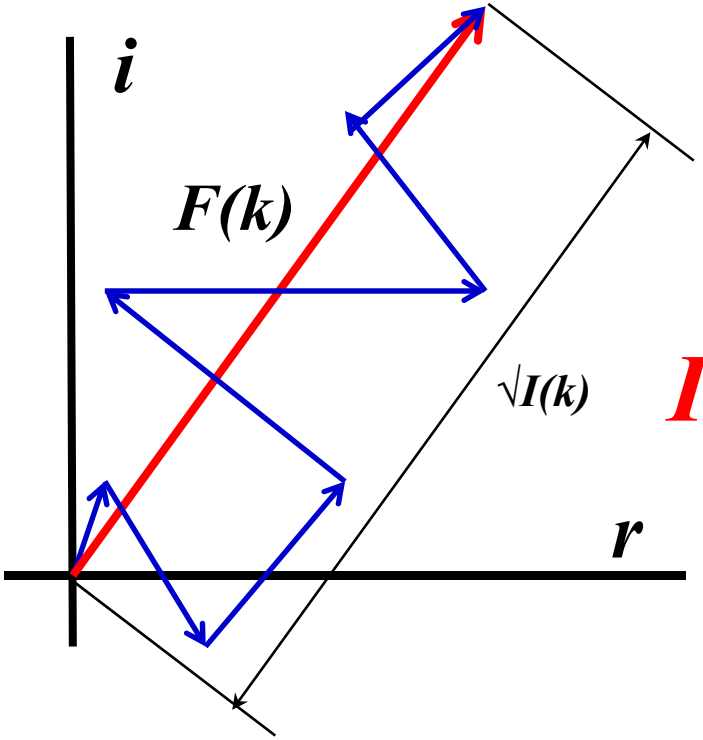
$$A(k) = \rho(r_1) \cdot e^{2\pi i\, k \cdot r1} + \rho(r_2) \cdot e^{2\pi i\, k \cdot r2}$$

Electron density

Wave from two elements can be explained by multiply of two vectors on a complex plane

Phase differences = distance between elements from a direction

Multiply of waves



$$I(k)=F(k)F(k)^*$$

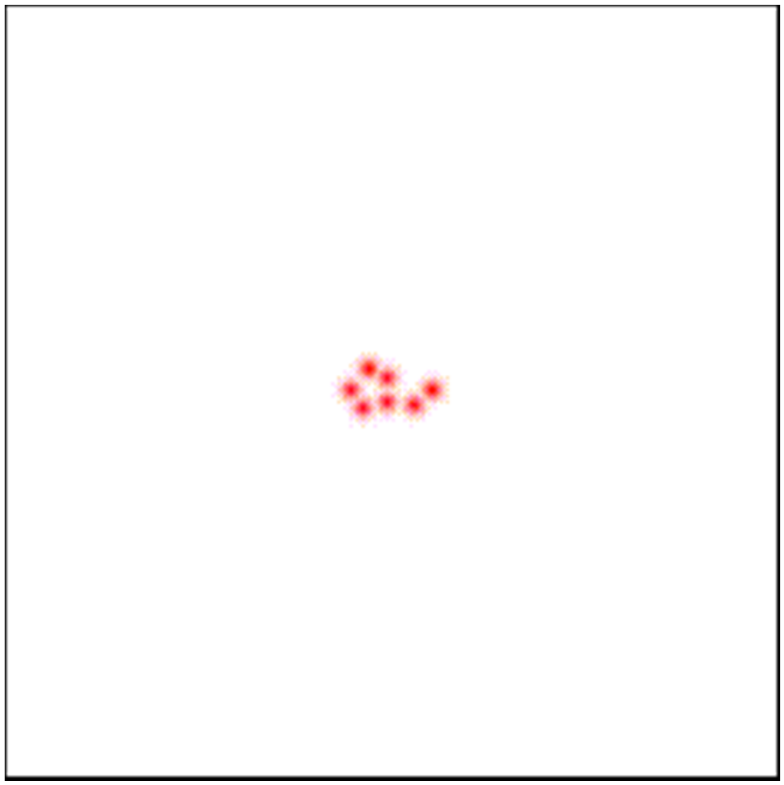
Multiplied wave from all molecular elements is expressed as,

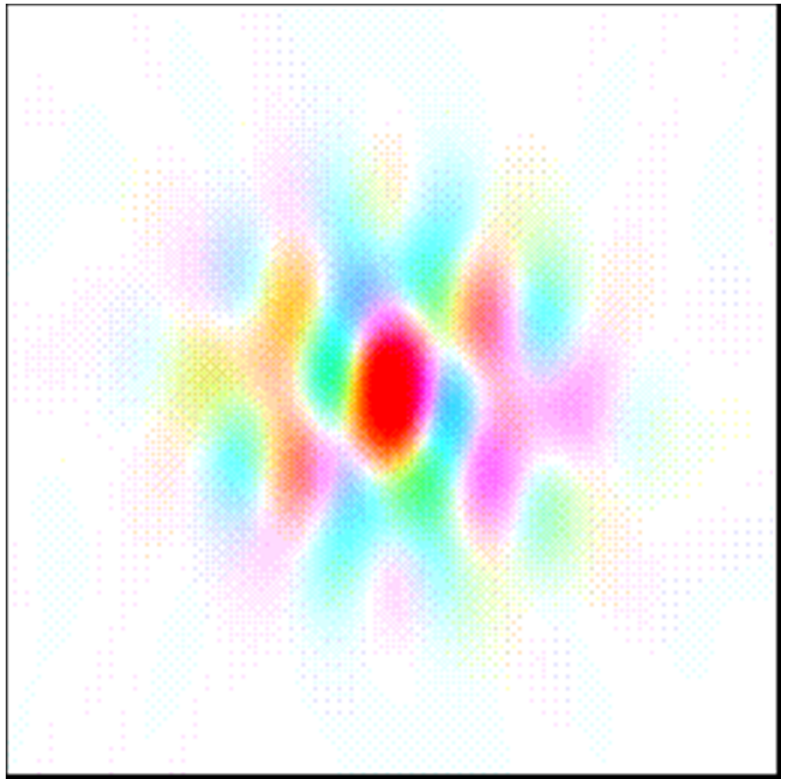
$$F(k) = \Sigma\, \rho\,(r_i) \cdot e^{2\pi i\, k \cdot ri}$$

Structure factor

Diffraction image is a convolution of scattering pattern of a motif and periodic function.

Single motif

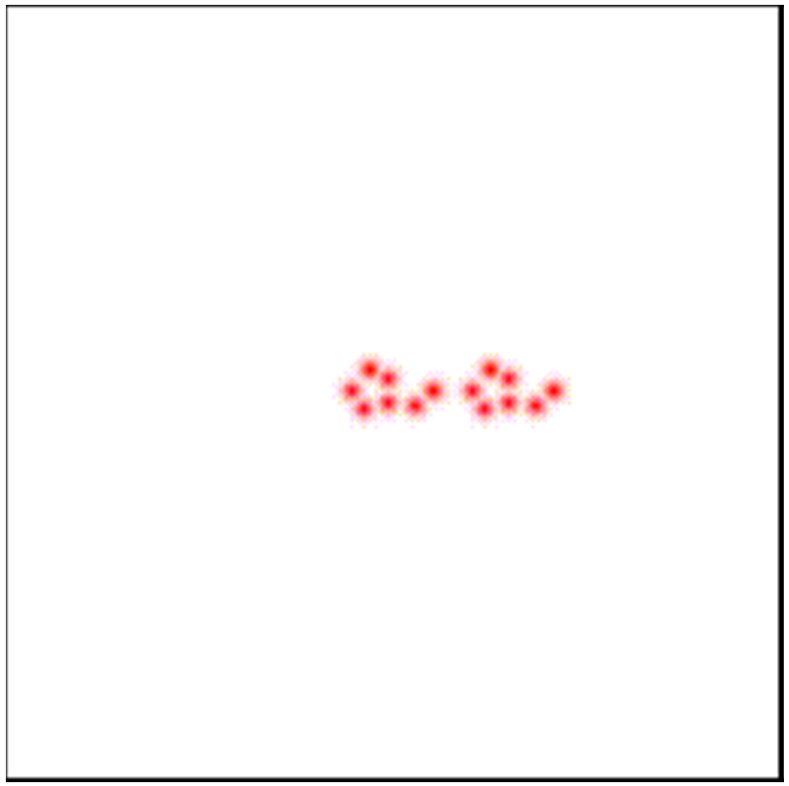


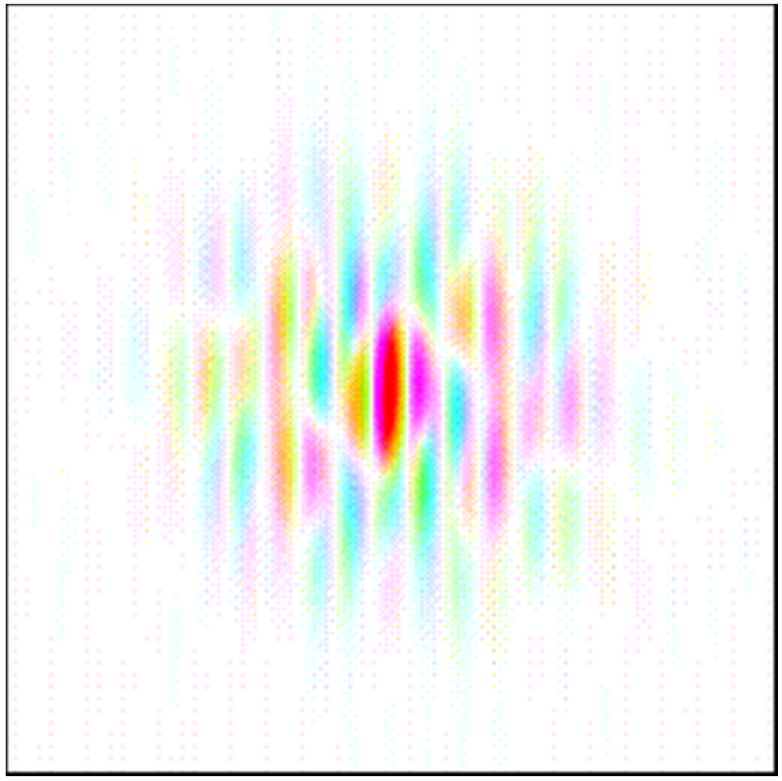


http://www.ysbl.york.ac.uk/~cowtan/fourier/fourier.html

Diffraction image is a convolution of scattering pattern of a motif and periodic function.

Two translated motifs

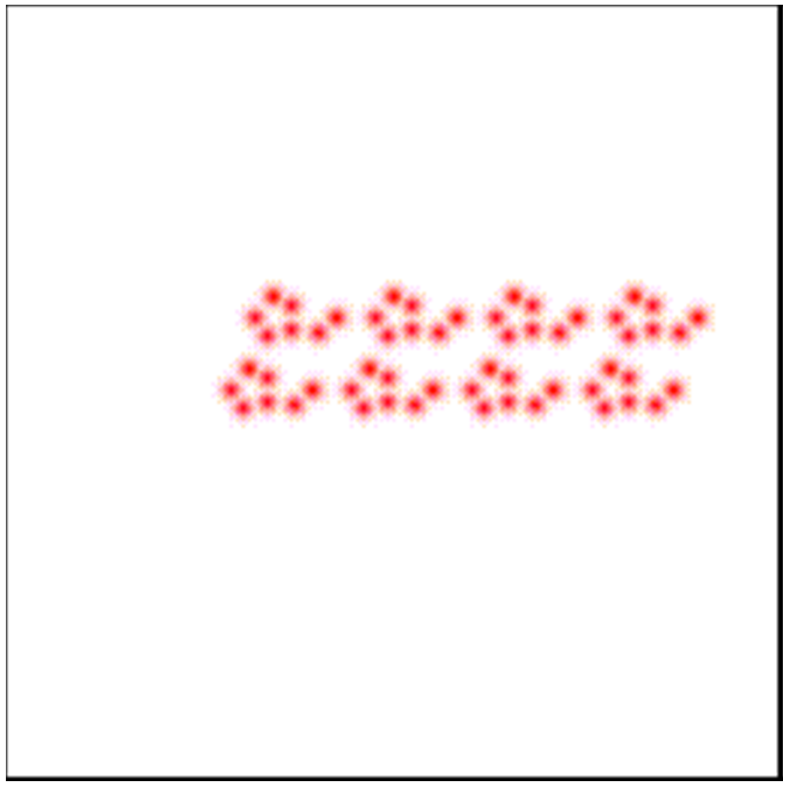


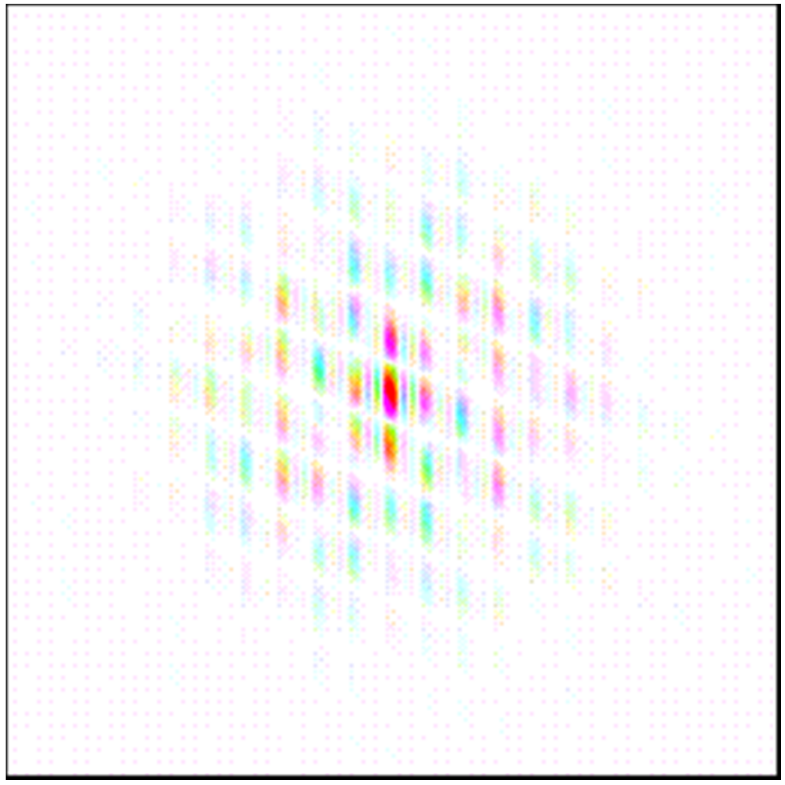


http://www.ysbl.york.ac.uk/~cowtan/fourier/fourier.html

Diffraction image is a convolution of scattering pattern of a motif and periodic function.

2x4 translated motifs

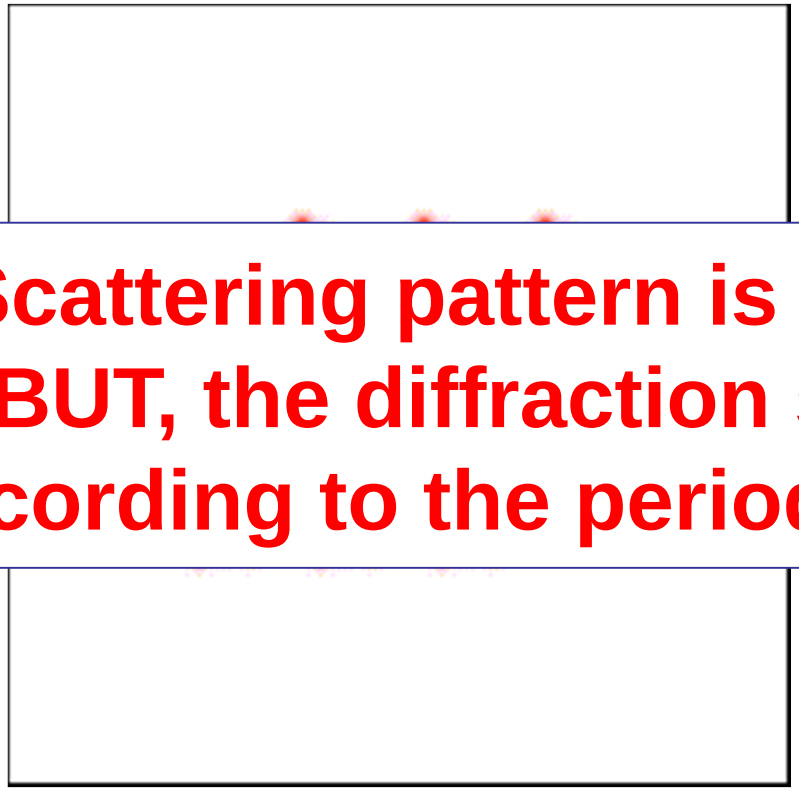


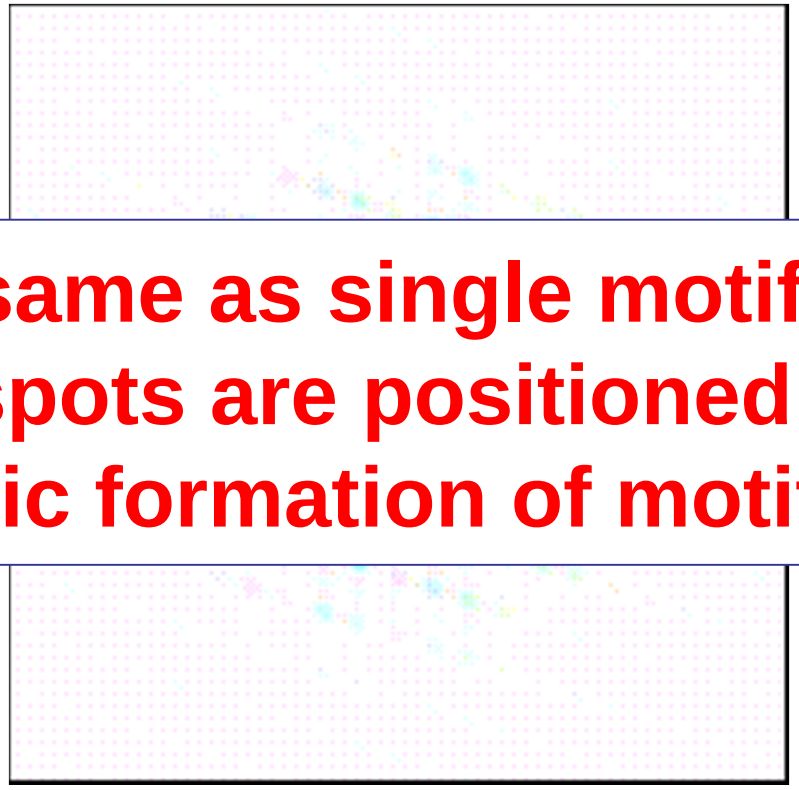


http://www.ysbl.york.ac.uk/~cowtan/fourier/fourier.html

Diffraction image is a convolution of scattering pattern of a motif and periodic function.

5x3 translated motifs

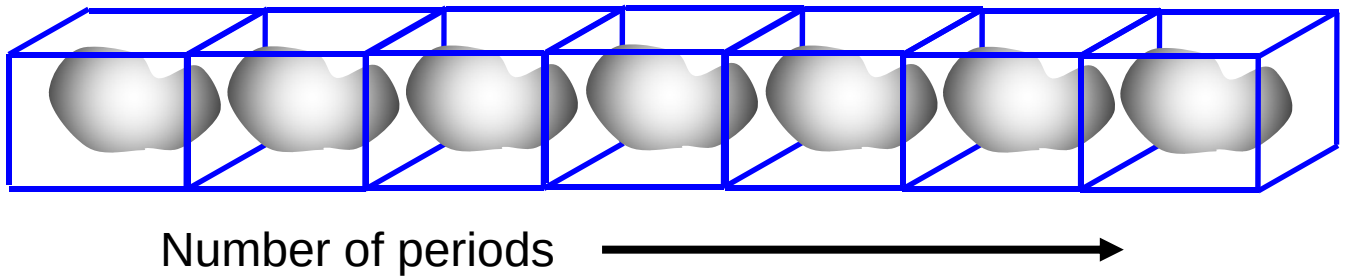


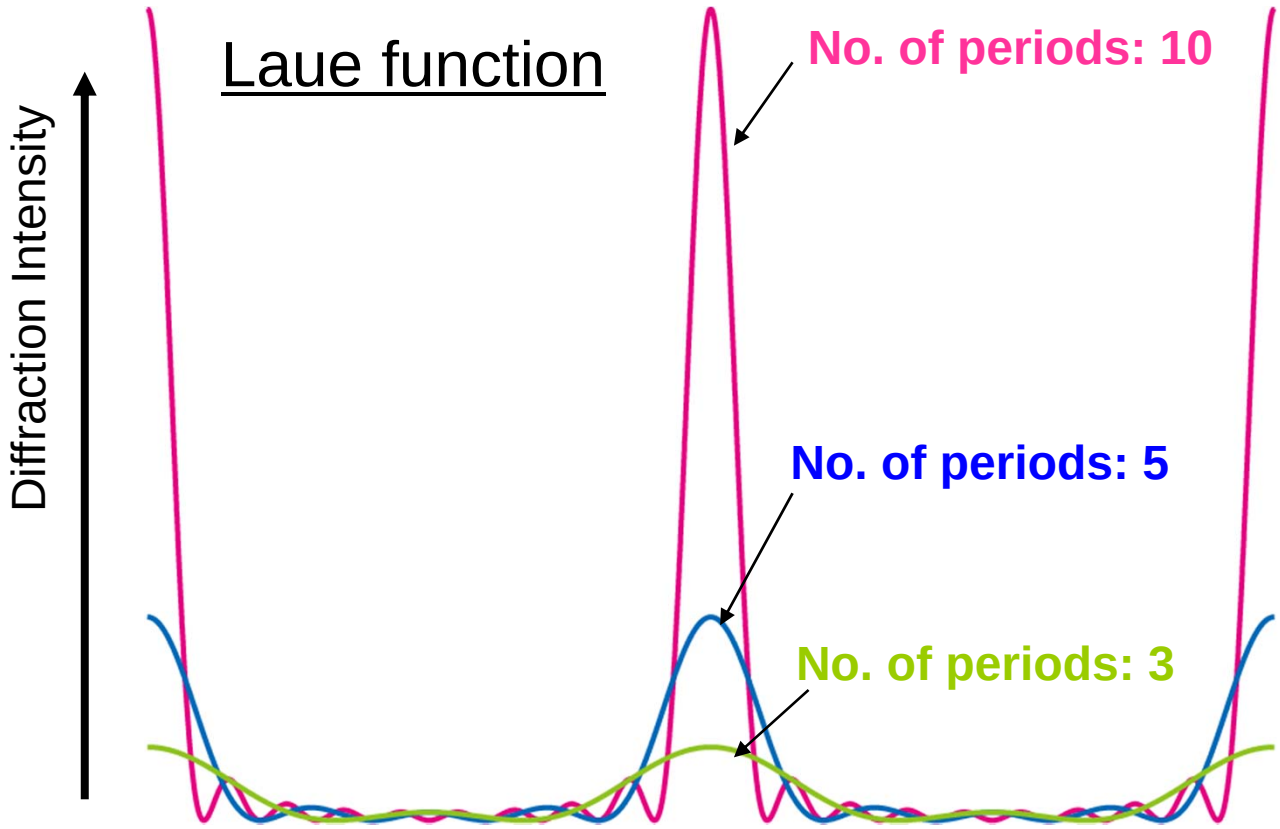


Scattering pattern is same as single motif. BUT, the diffraction spots are positioned according to the periodic formation of motifs.

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

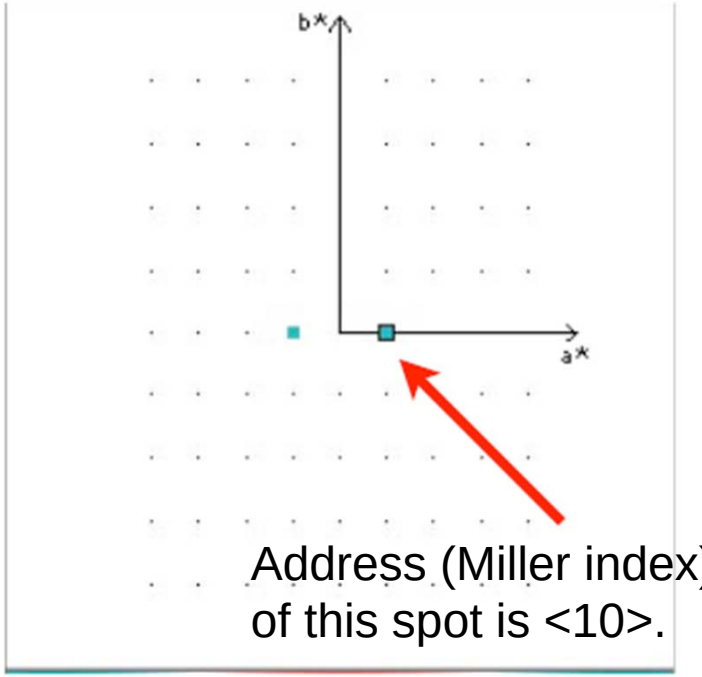
Periodic number and Laue function

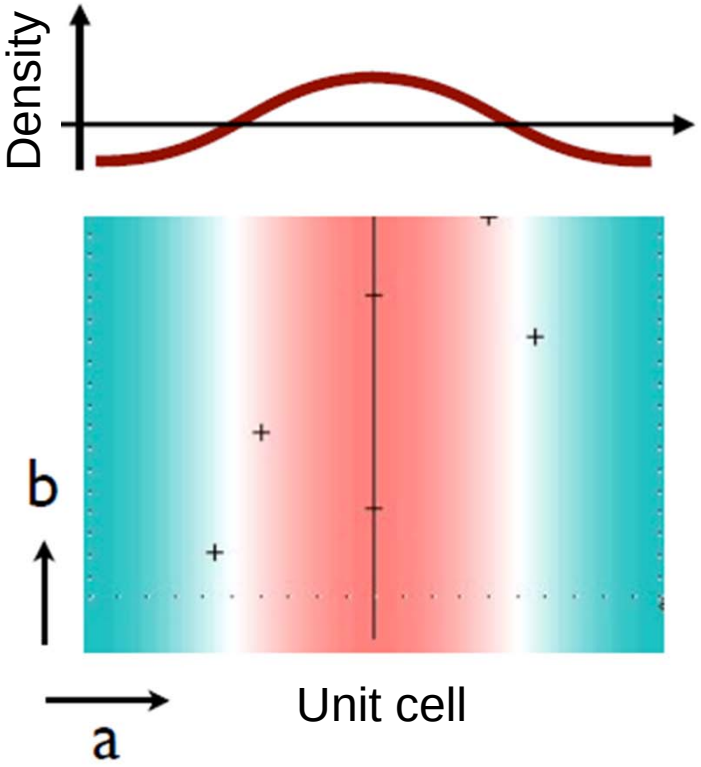




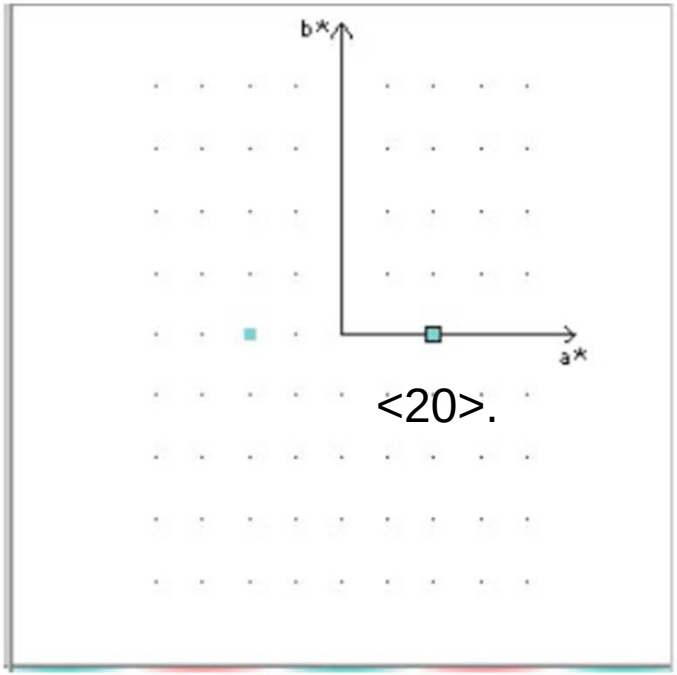
Increase of the No. of periods makes the diffraction intensity stronger.

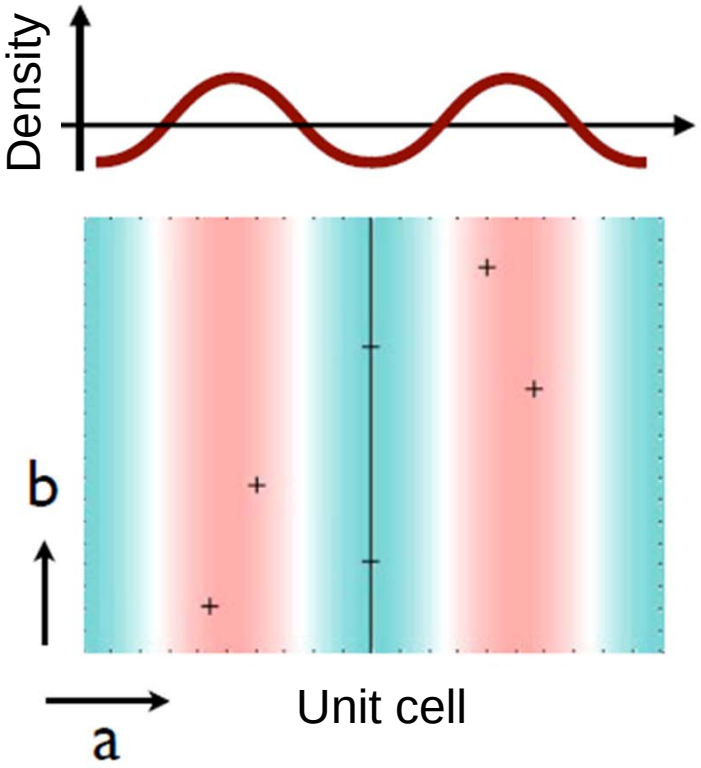
diffraction pattern & Fourier component



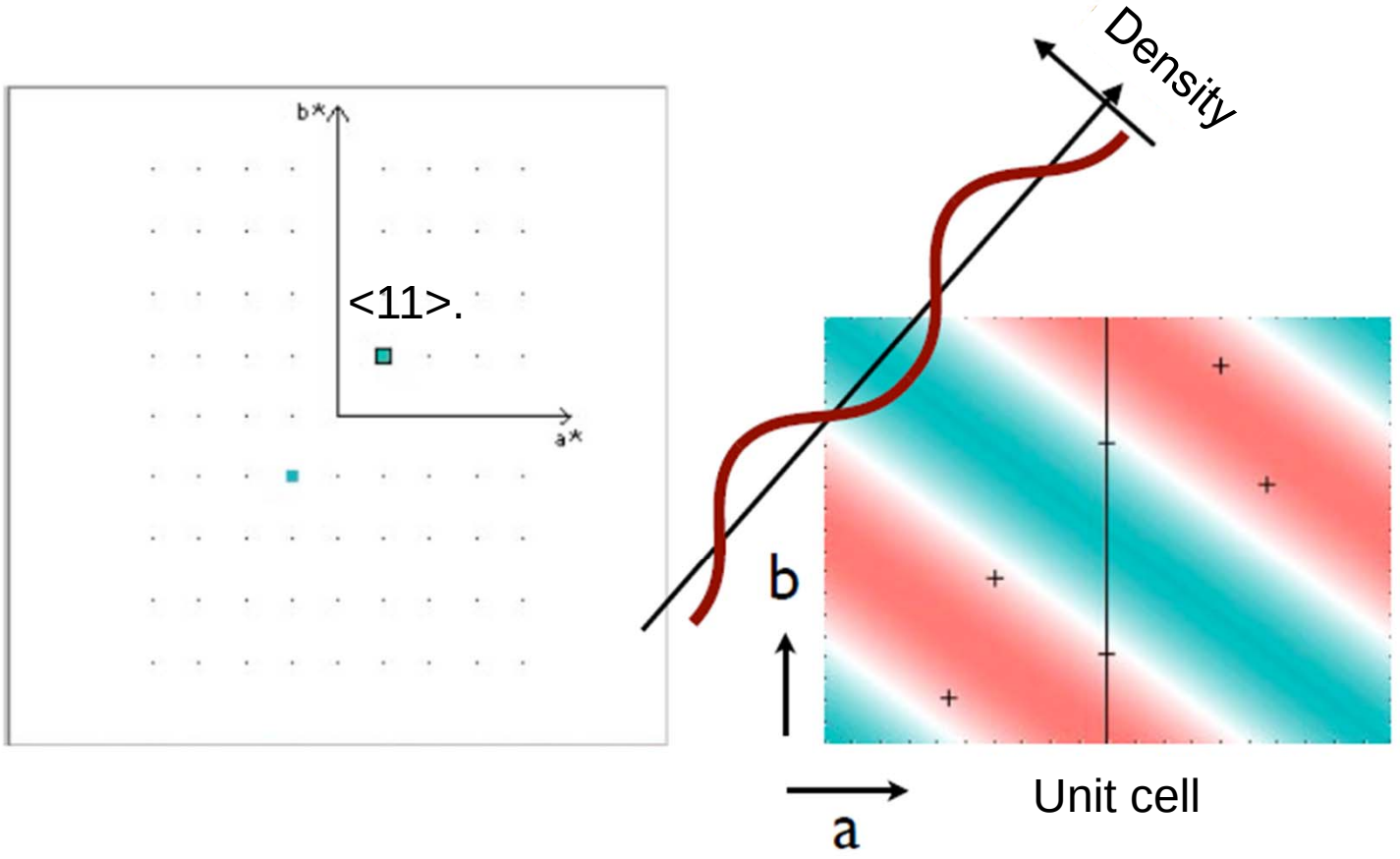


diffraction pattern & Fourier component

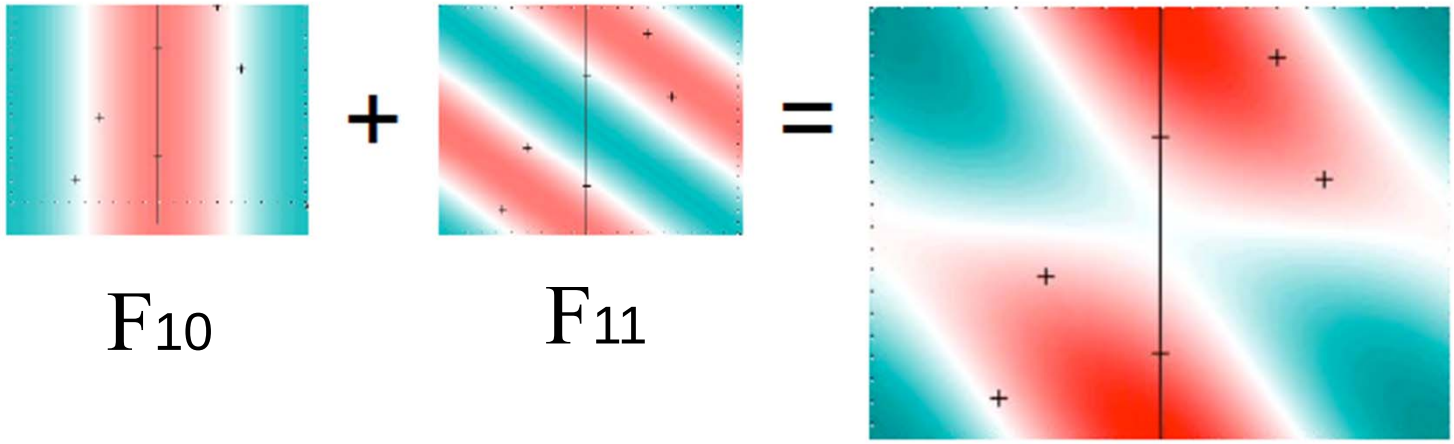




diffraction pattern & Fourier component

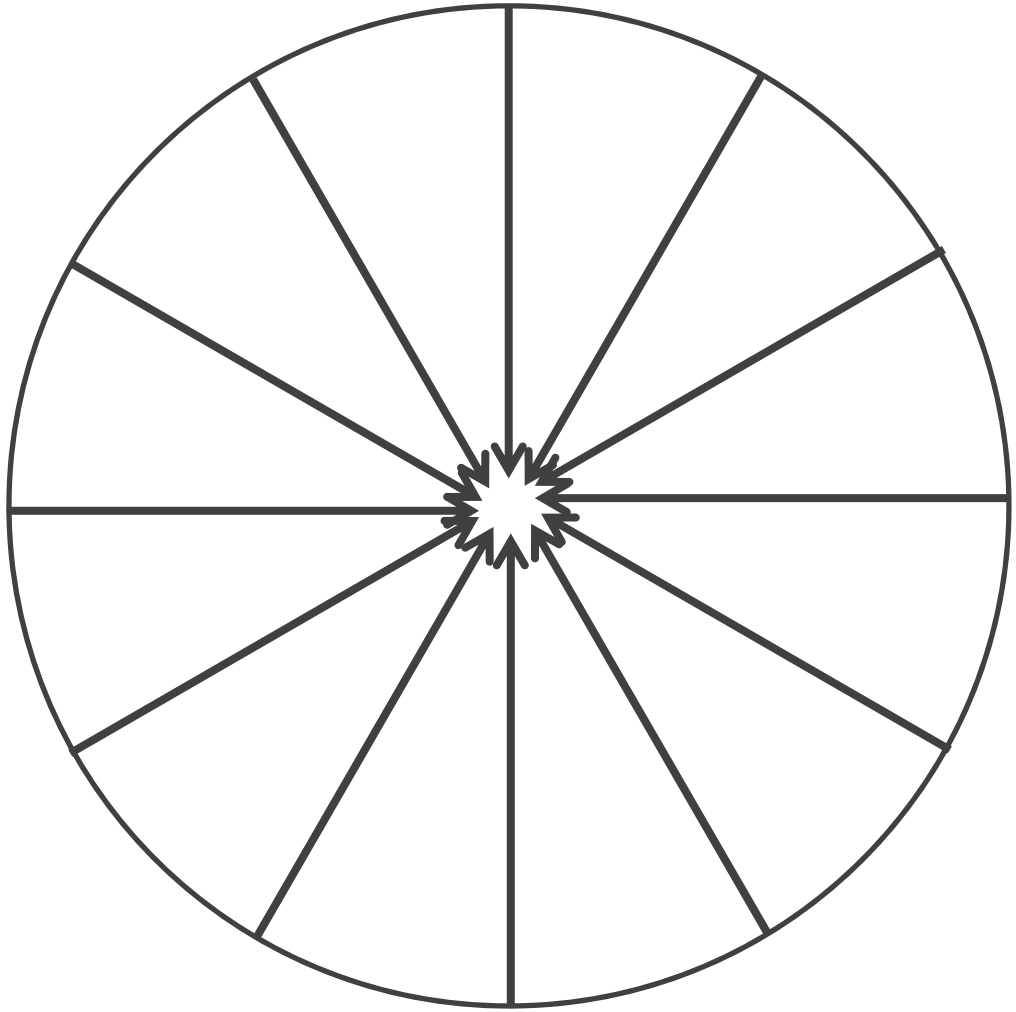


Fourier synthesis



F_{10} F_{11}

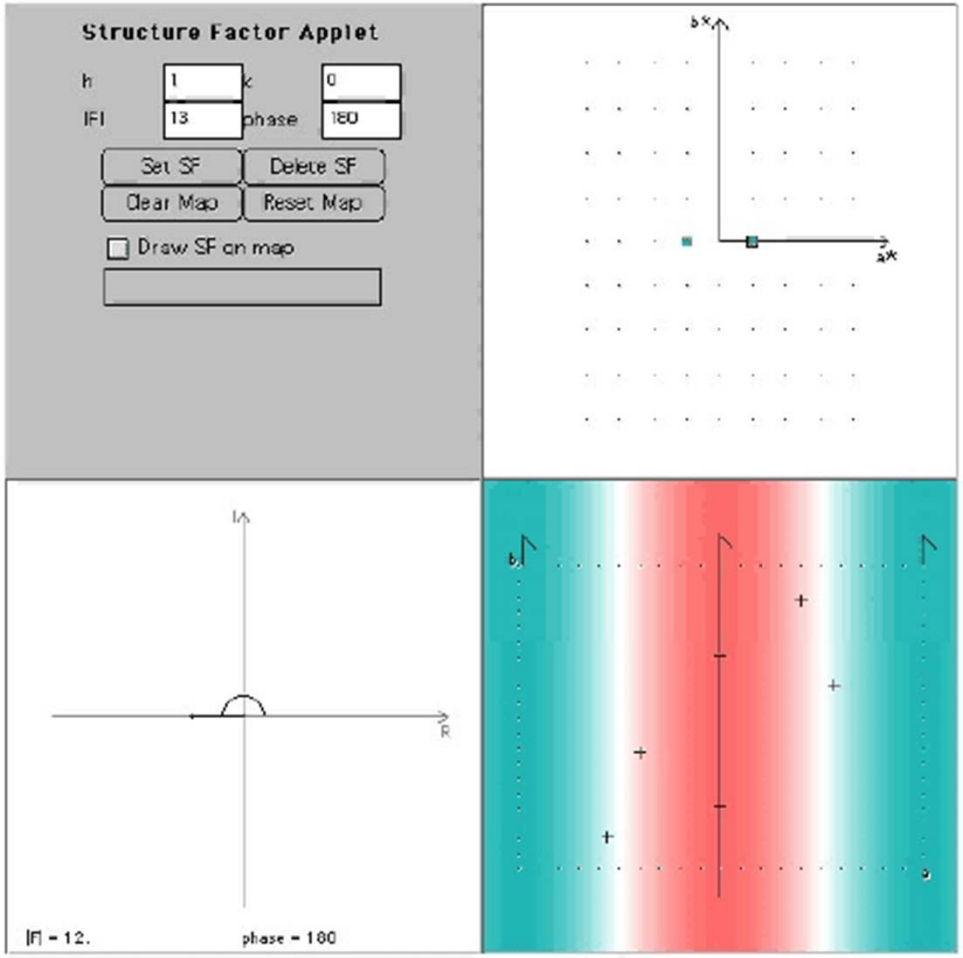
Fourier synthesis



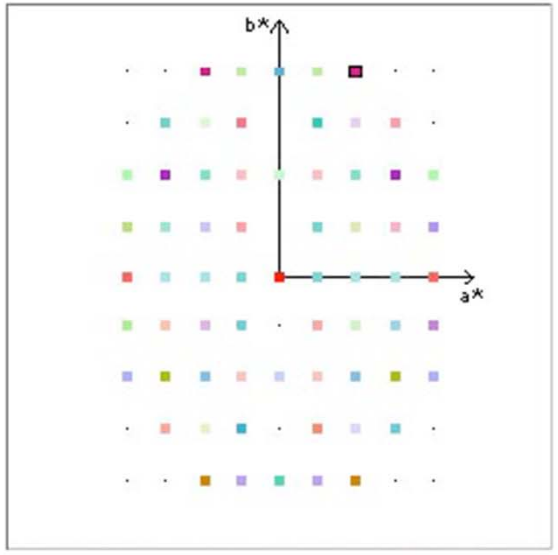
Summation of all waves (Fourier synthesis) can reconstruct the object, like computer tomography (CT)

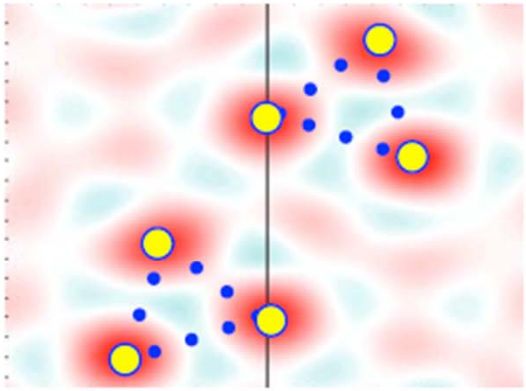
Fourier synthesis

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



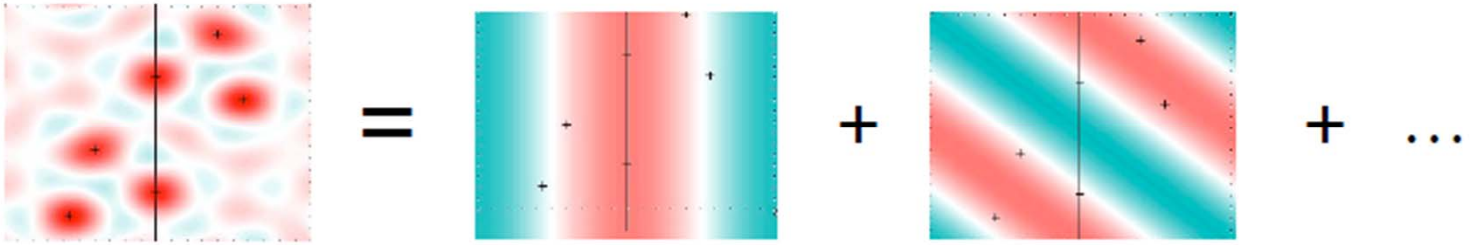
Fourier synthesis





Fourier synthesis

$$\rho(x,y,z) = \sum_h \sum_k \sum_l \mathbb{F}_{hkl} e^{-2\pi i(hx+ky+lz)}$$



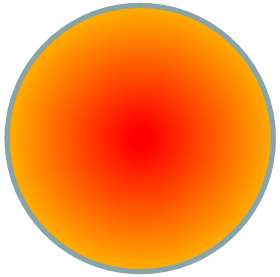
$\mathbb{F}_{10}$ $\mathbb{F}_{11}$

Structure factor $$\boldsymbol{F(k)} = \int \rho(\boldsymbol{r})\, e^{2\pi i\, \boldsymbol{k}\cdot\boldsymbol{r}}\, d\boldsymbol{r}$$

Electron density $$\rho(r) = \int \boldsymbol{F(k)}\, e^{-2\pi i\, \boldsymbol{k}\cdot\boldsymbol{r}}\, d\boldsymbol{k}$$

F(k) and *ρ(r)* have a relationship of Fourier transformation.

Atomic scattering factor $$\boldsymbol{f(k)} = \int_{\text{atomic}} \rho(\boldsymbol{r})\, e^{2\pi i\, \boldsymbol{k}\cdot\boldsymbol{r}}\, d\boldsymbol{r}$$

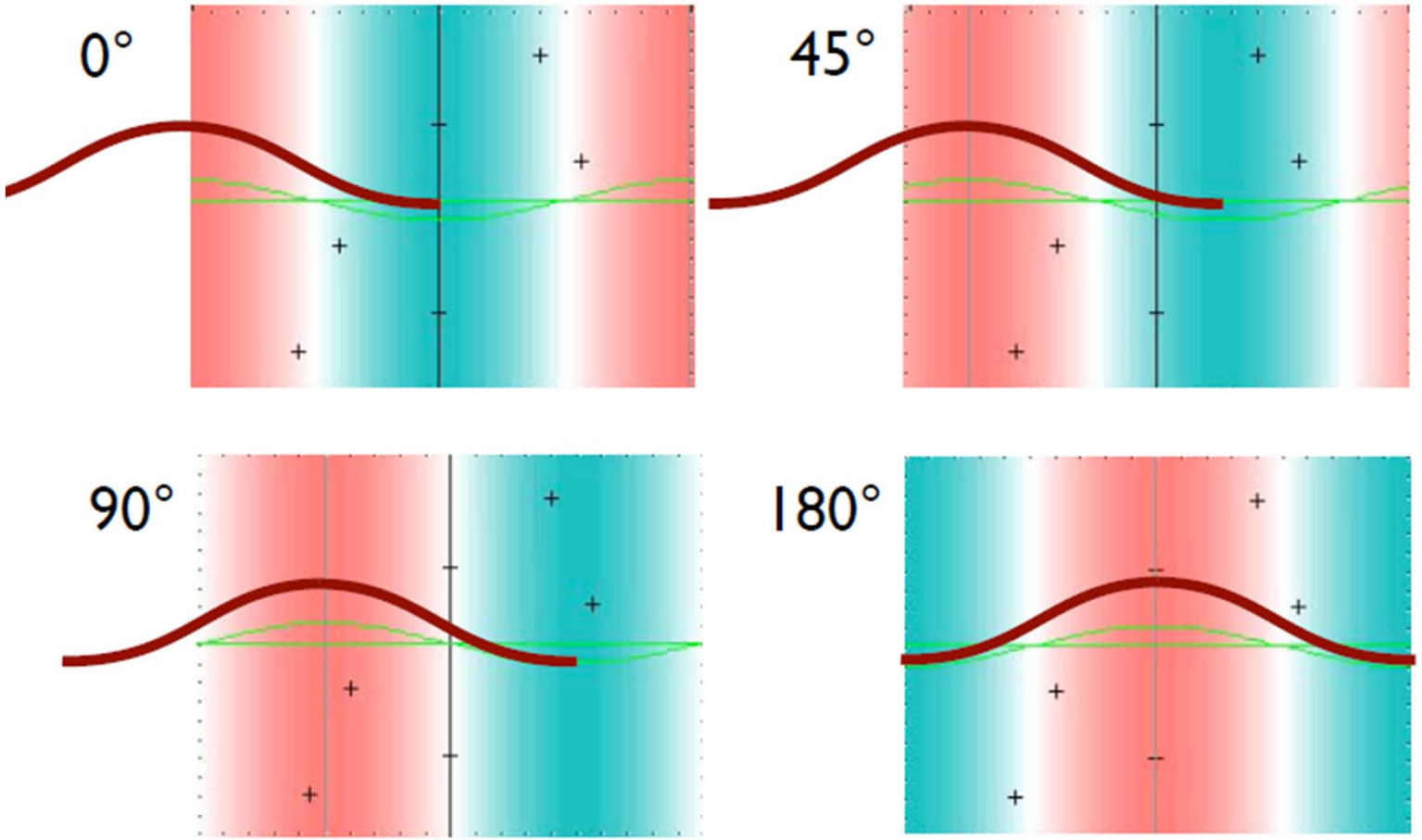


Using this,

Structure factor $$\boldsymbol{F(k)} = \sum_j f_j\, e^{2\pi i\, \boldsymbol{k}\cdot\boldsymbol{r}_j}$$

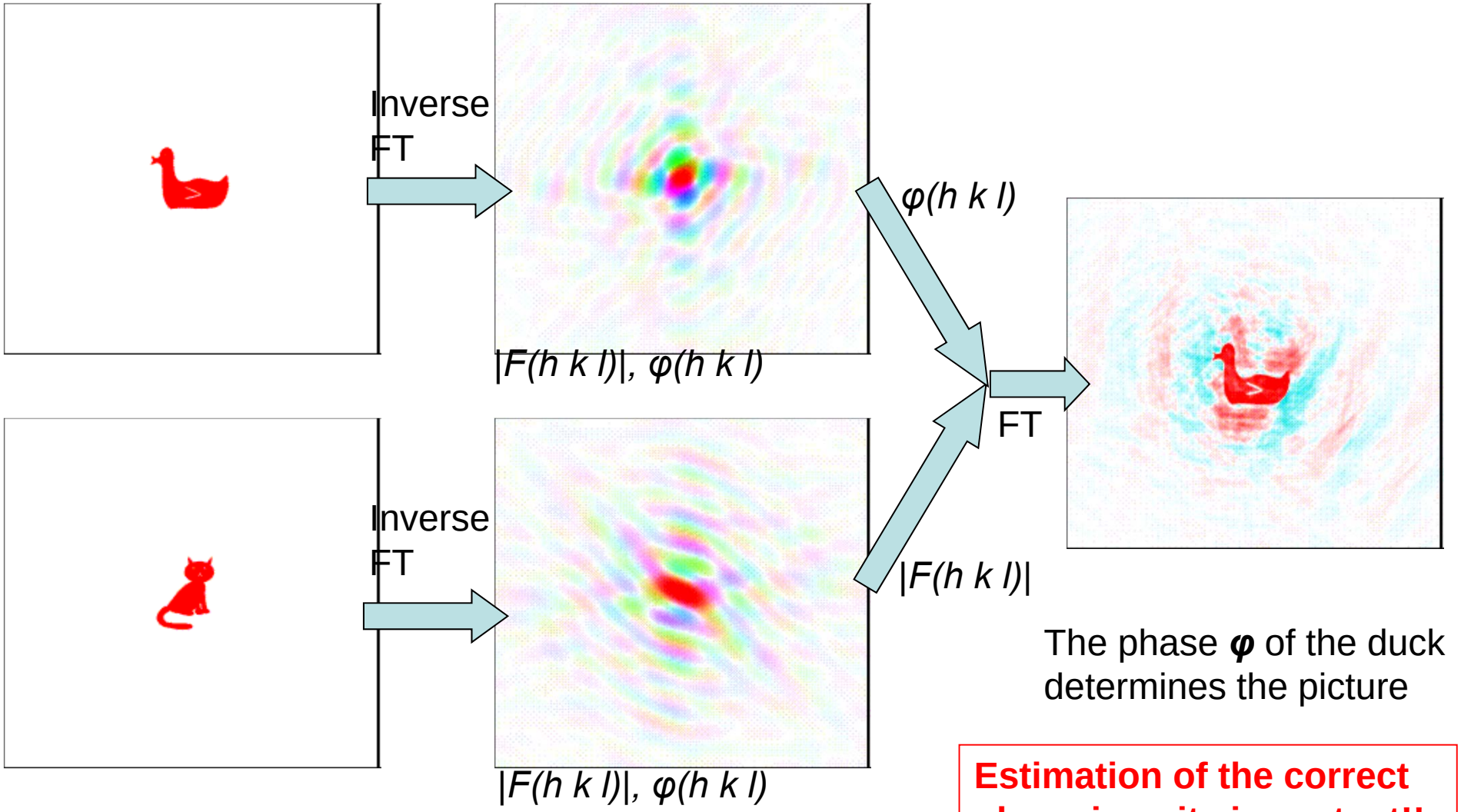
The structure factors can be calculated by molecular coordinates. Consistency between the calculated F (Fcalc) and the observed F (Fobs) indicates a reliability of the structure.

phase



Importance of Phase

The phase of the structure factor contains the main information about the shape of the molecule.

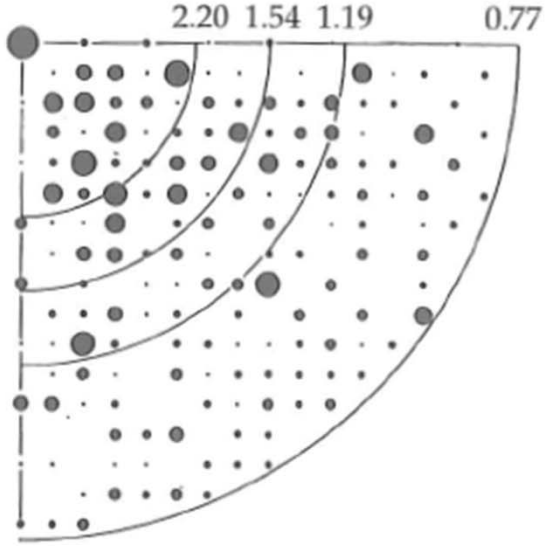


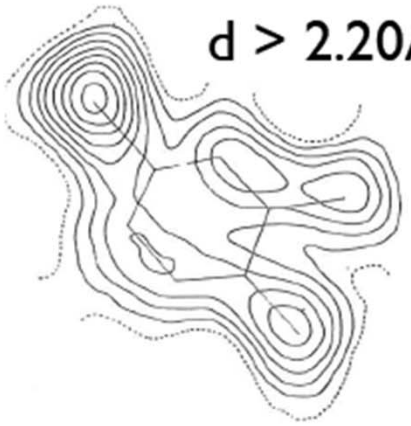
The phase $\boldsymbol{\varphi}$ of the duck determines the picture

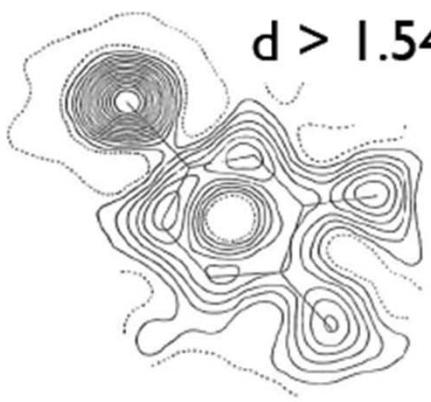
Estimation of the correct phase is quite important!!

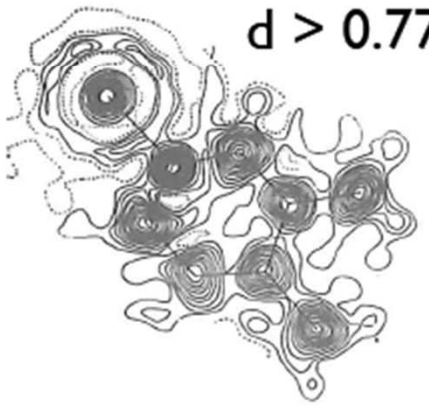
http://www.ysbl.york.ac.uk/~cowtan/fourier/fourier.html

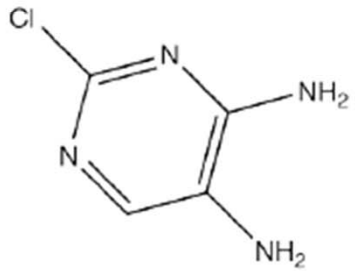
resolution & electron density map





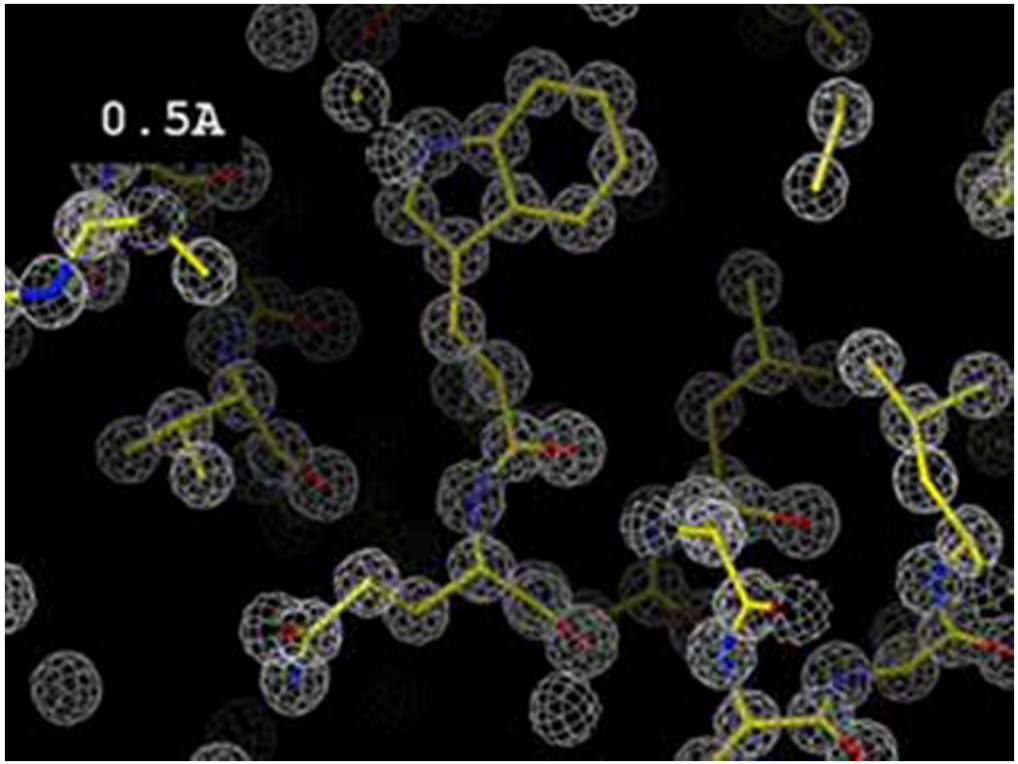






4,5-diamino-2-chloropyrimidine

Resolution and electron density map



Movie from http://ucxray.berkeley.edu/~jamesh/movies/

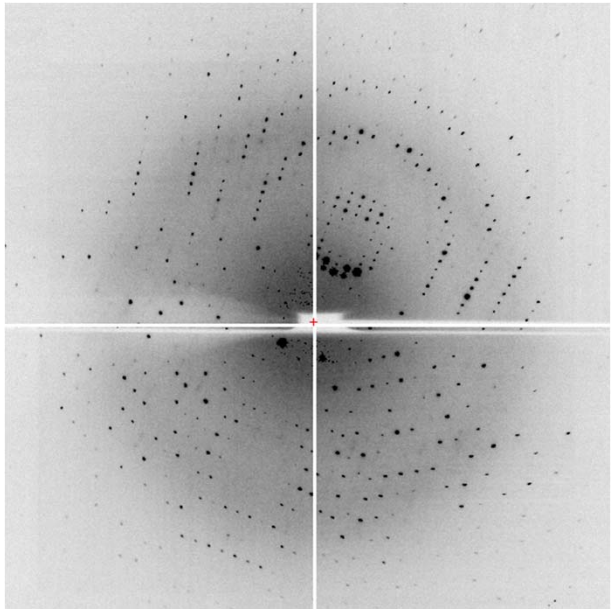
< 1.0 A: Hydrogen atom can be detected.
< 2.5 A: Side chains can be easily detected.
> 4.0 A: Detection of side chains is very difficult.

X-ray crystal structure analysis

protein crystal

X-ray ⟶

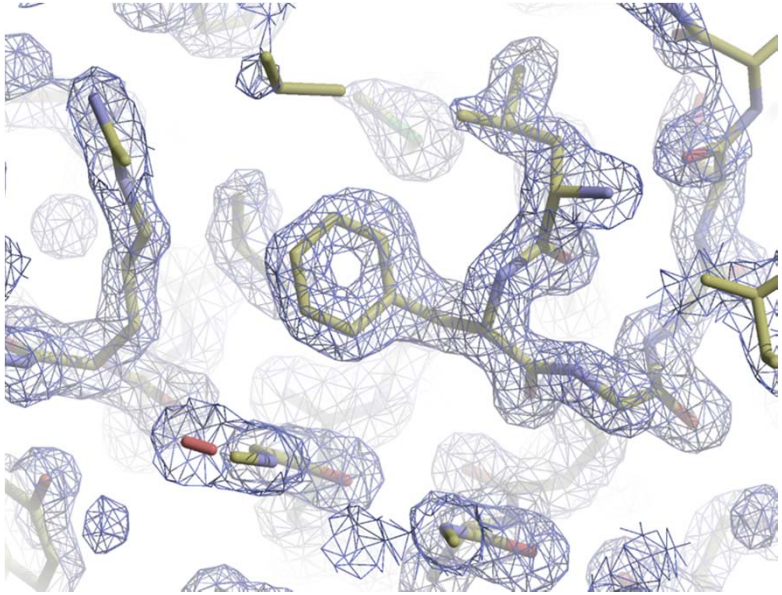
diffraction data



measure positions and intensities of all diffraction spots

Fourier transformation (requires phase information)

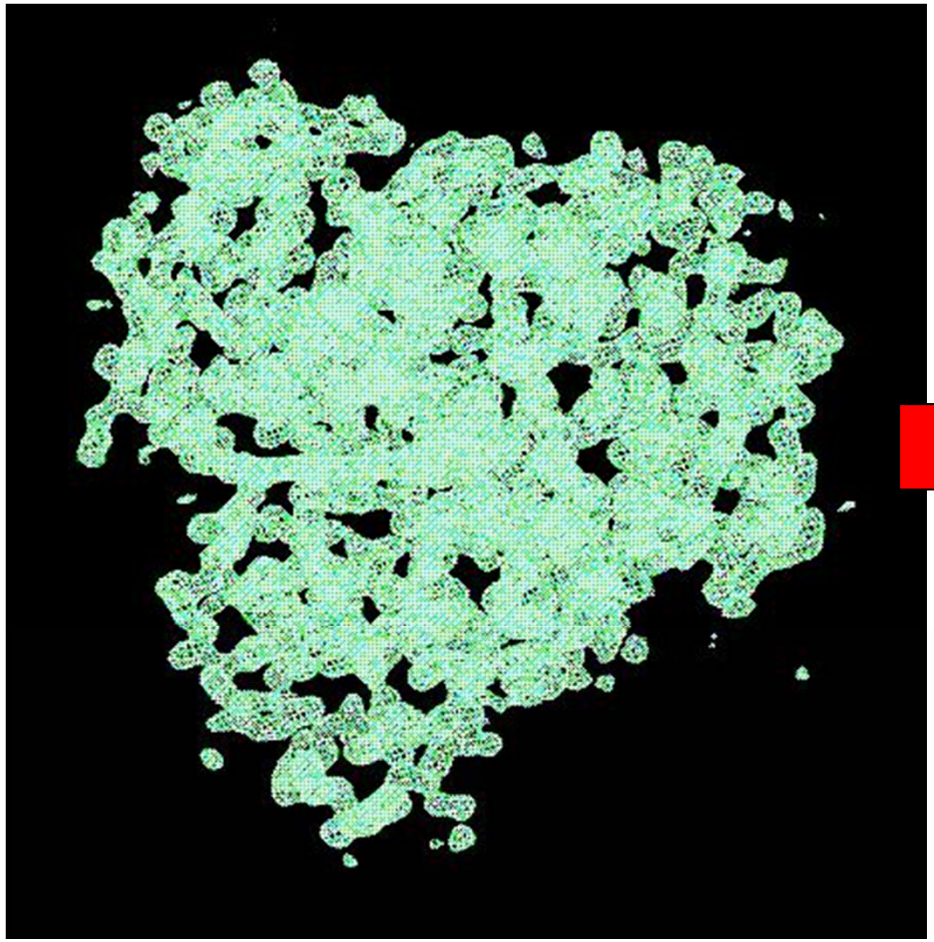
electron density map

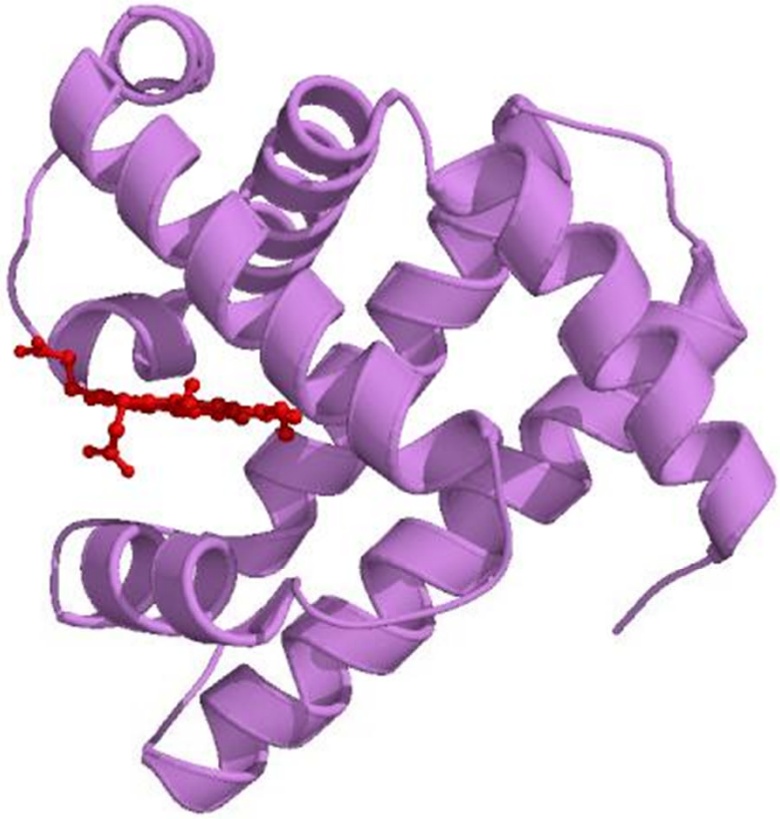


Electron density and molecular model

We can obtain only electron density map from diffraction experiments because X-rays are diffracted by electrons but not by atomic nucleus.

Based on the electron density map, we can build a molecular model.

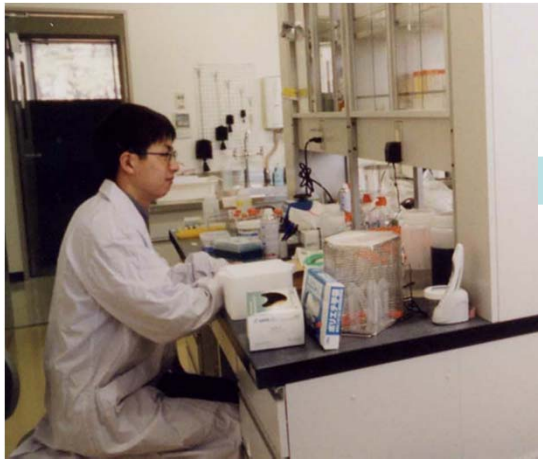




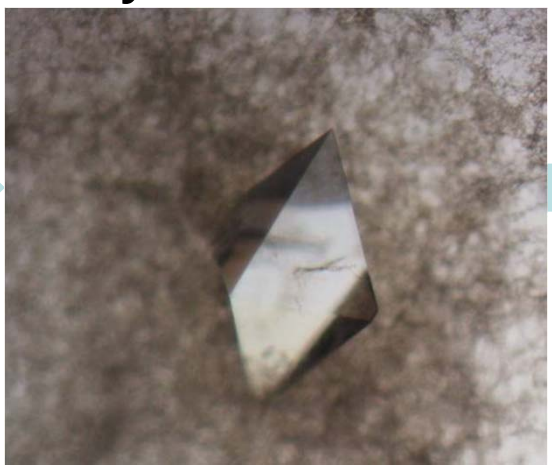
ribbon model of Myoglobin

Scheme of macromolecular crystallography

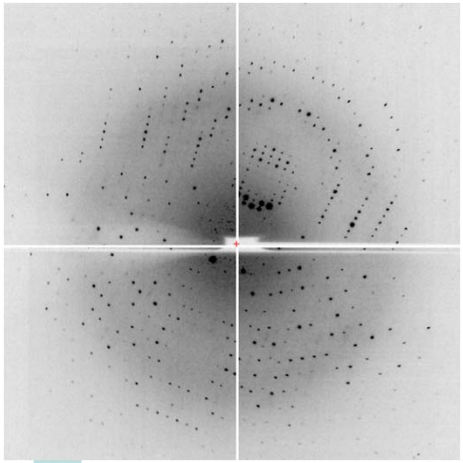
Expression & Purification



Crystallization

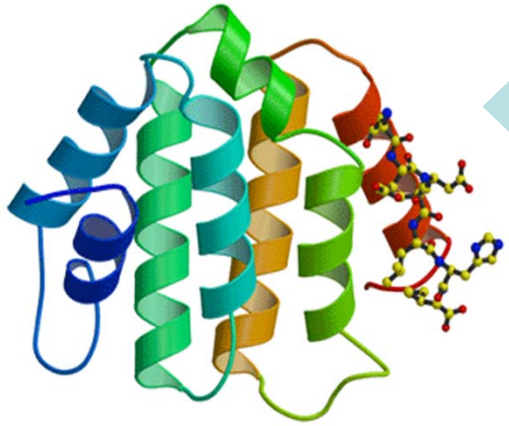


Data collection



Data analysis

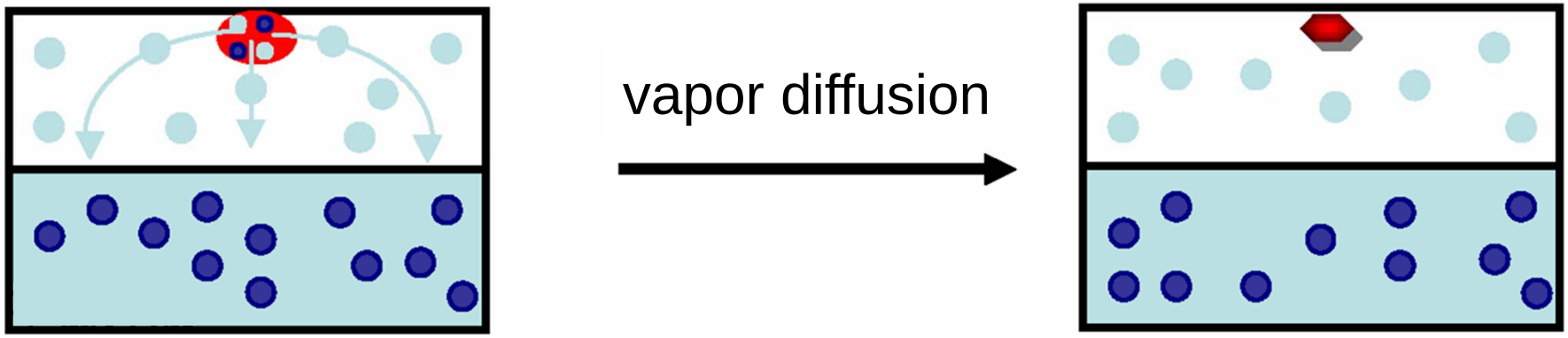
Structure



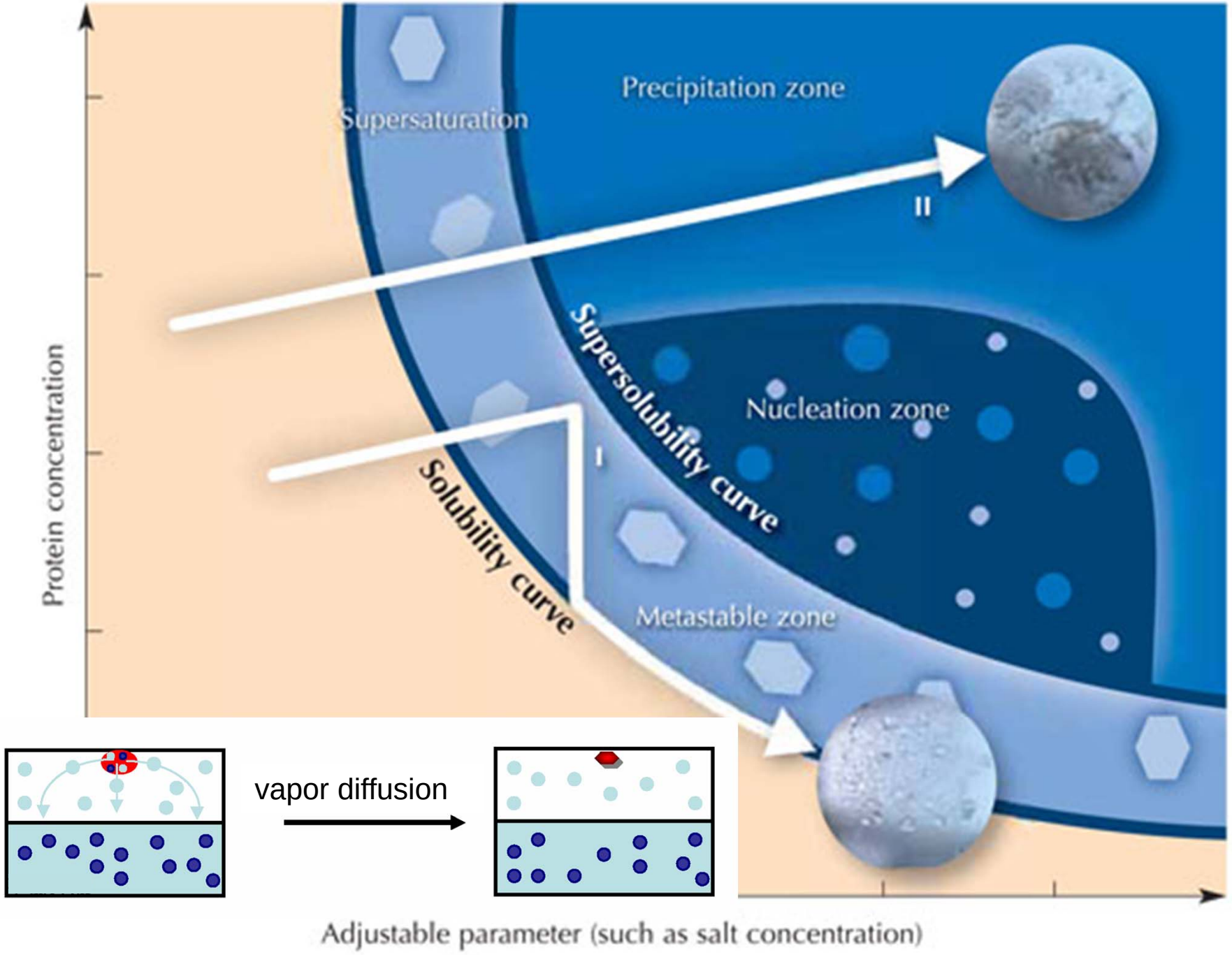
Functional analysis

Crystallization of Proteins

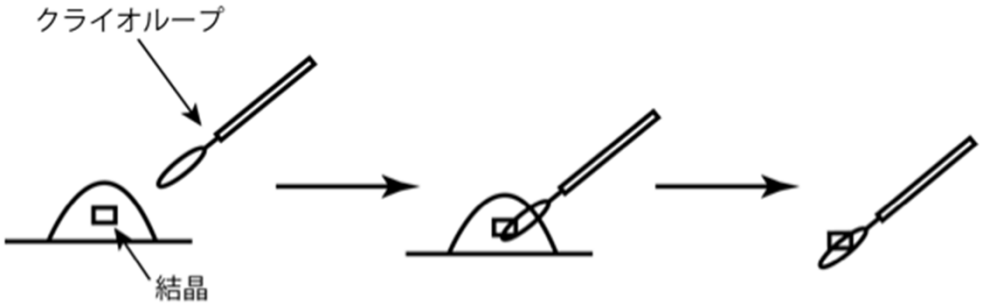
(vapor diffusion method)



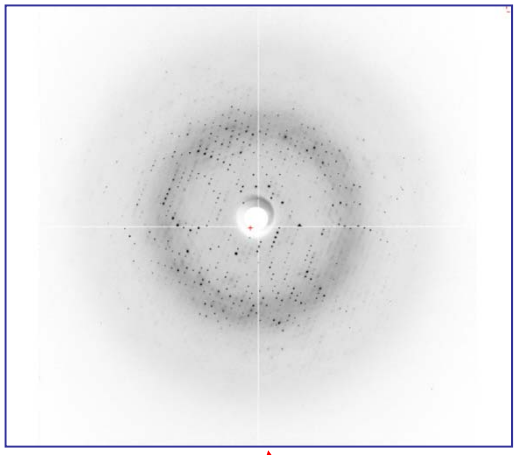
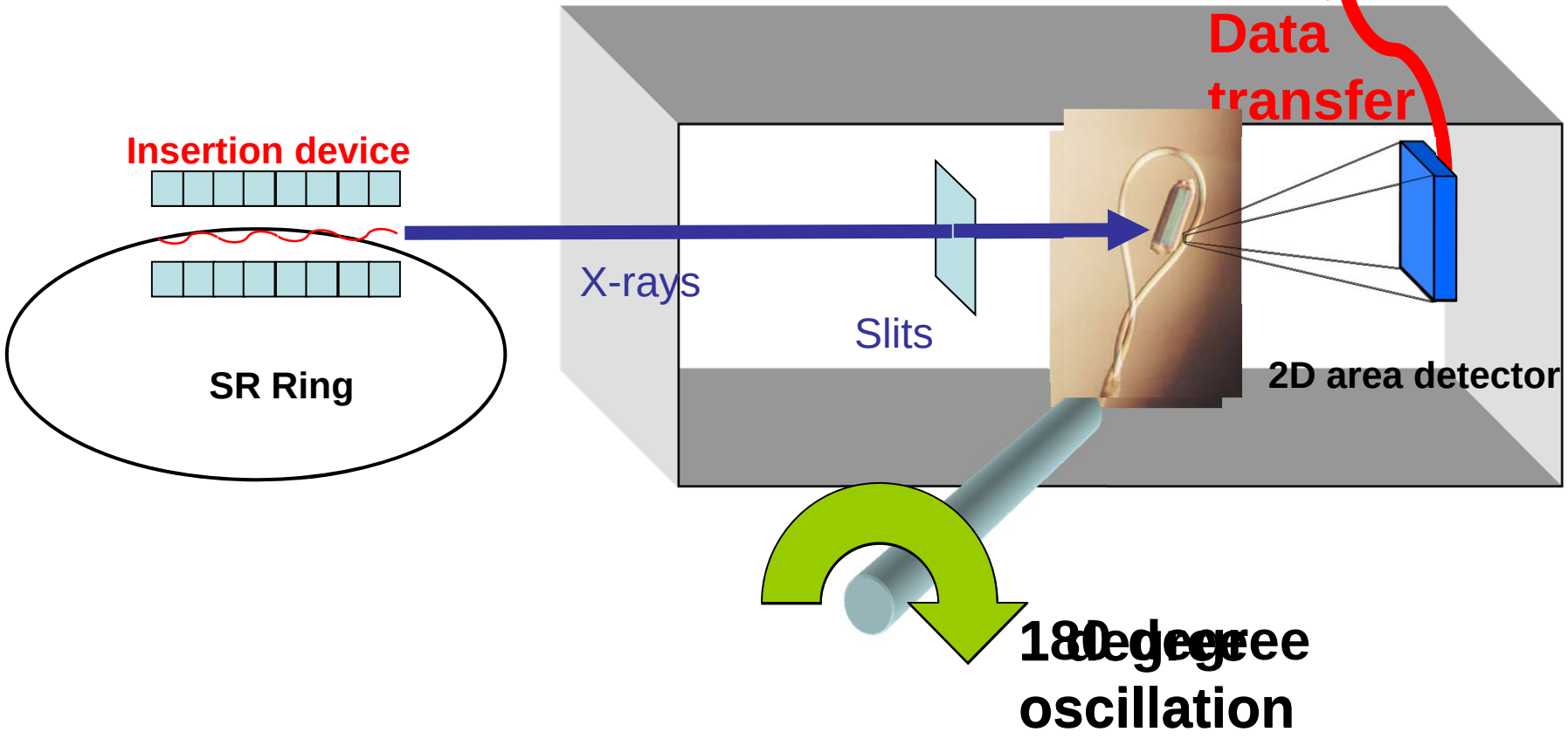
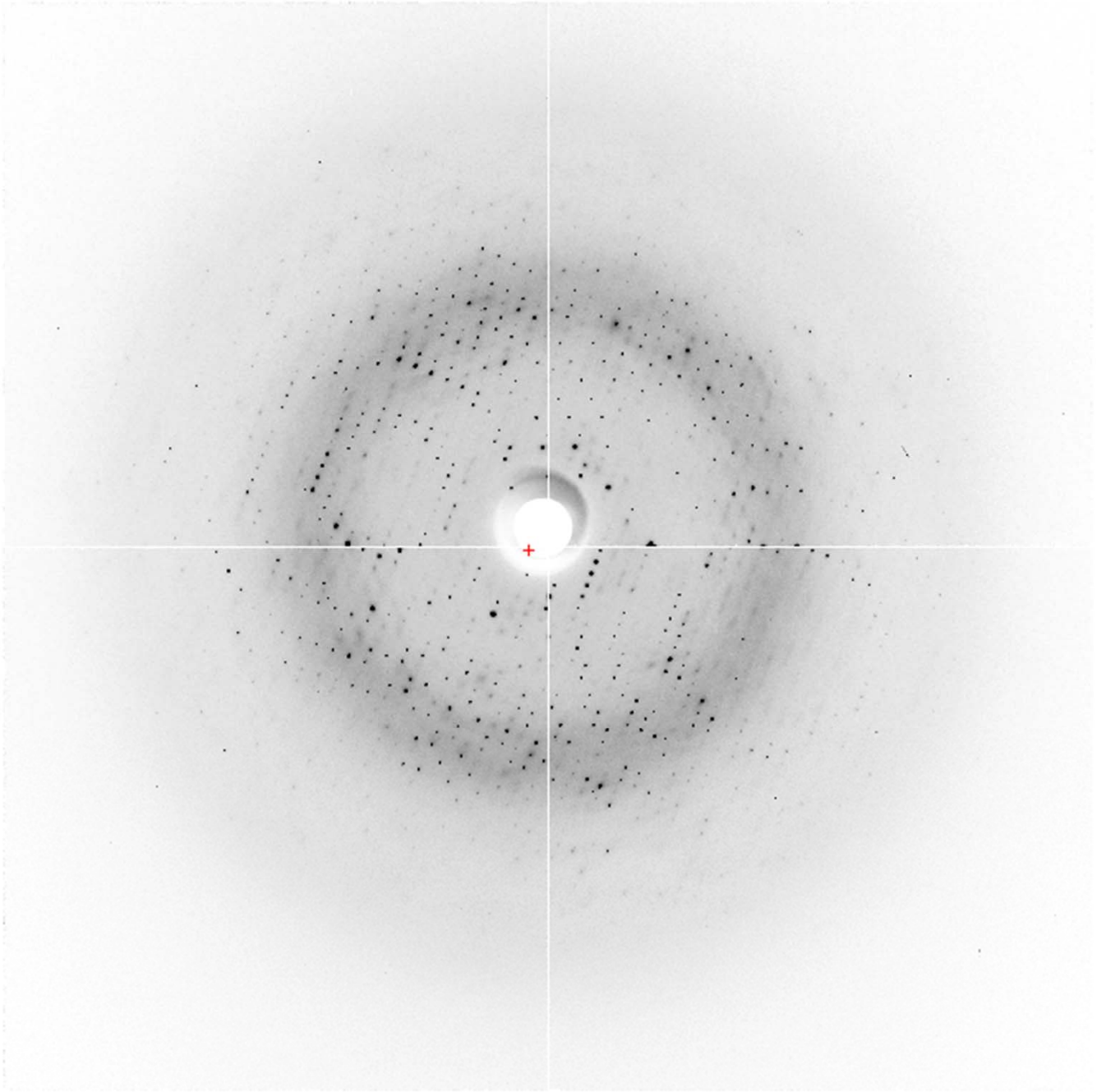
- sample drop (protein solution + precipitant)
- precipitant molecules
- water molecules
- protein crystals


Precipitation zone
Supersaturation
II
Supersolubility curve
Nucleation zone
I
Solubility curve
Metastable zone
Protein concentration
vapor diffusion
Adjustable parameter (such as salt concentration)

結晶のすくい方



実体顕微鏡を覗きながら、ループですくいます。あまり、倍率を上げ過ぎないのがコツ。
倍率を上げ過ぎると、指先の震えが大きく見えるので、かえって操作が難しくなる。

Data collection

（1 degree oscillation X 180 frames）