THE UNIVERSITY *of* TEXAS
SCHOOL OF HEALTH INFORMATION SCIENCES AT HOUSTON

X-Ray Crystallography Pt. I

For students of HI 6001-125
"Computational Structural Biology"

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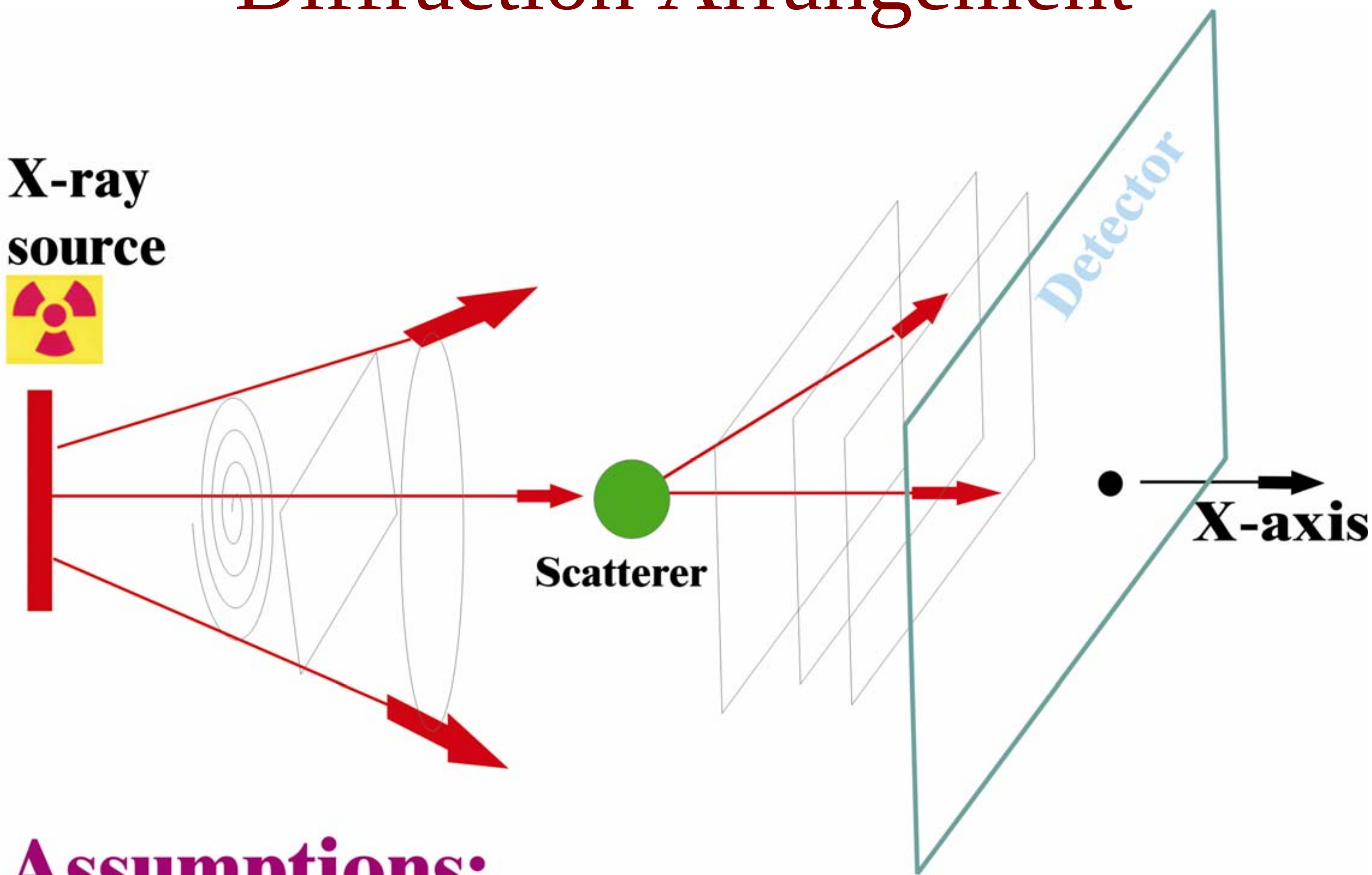
http://biomachina.org/courses/structures/02.html

Diffraction Arrangement



Assumptions:

- **Incident and scattered plane waves**
- **Elastic scattering: No change in wavelength**

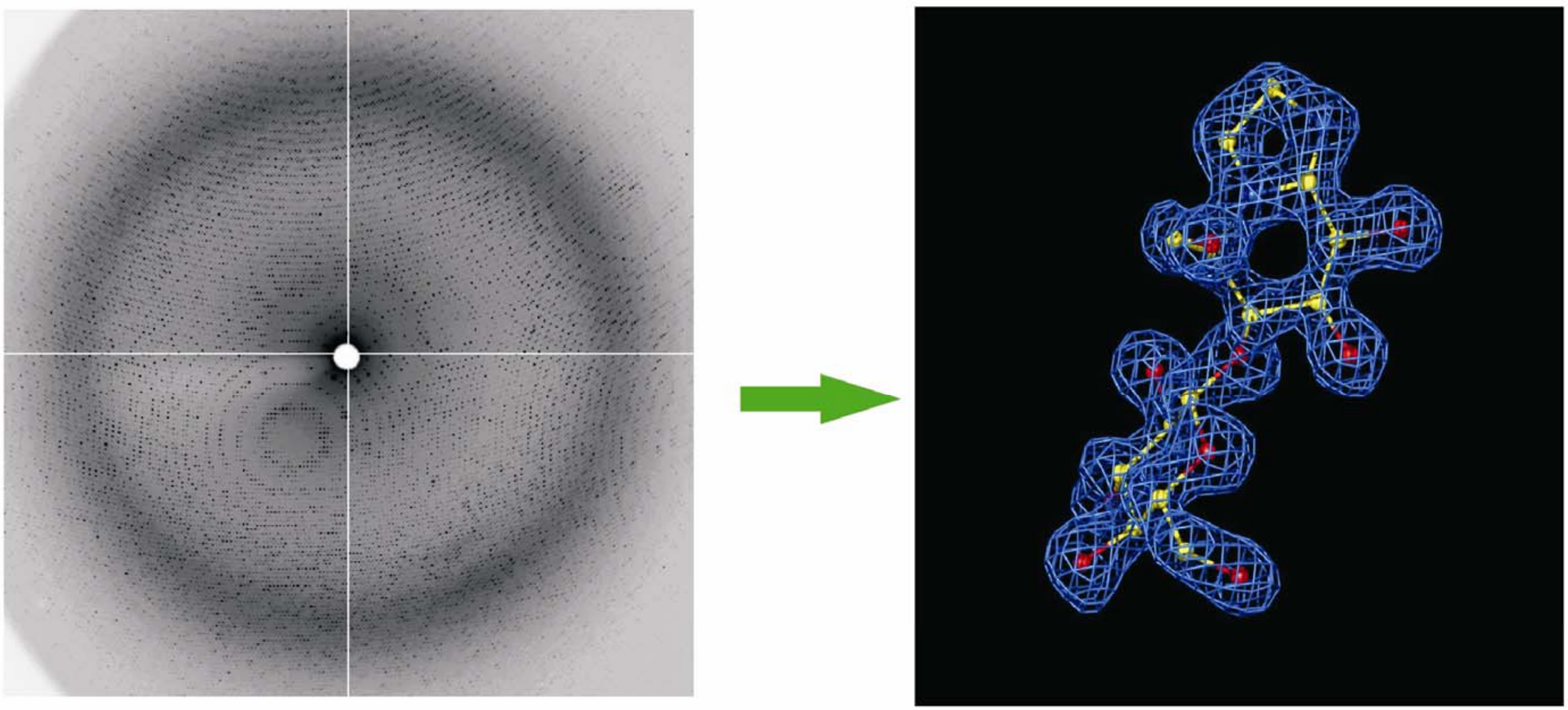
Probing Macromolecular Atomic Structures

- Crystalline sample

- Probe should be chosen such that its wavelength is of atomic dimensions: X-rays

- Suitable detection systems

From Diffraction Spots to Atomic Models



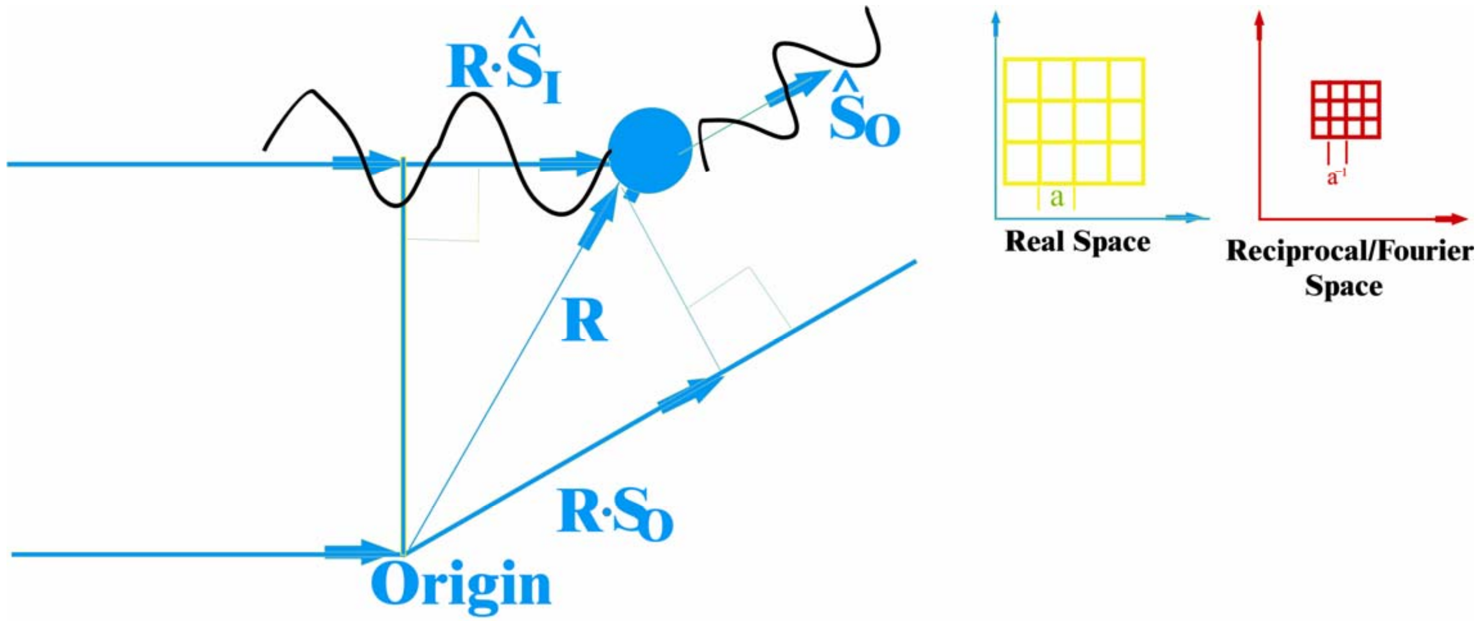
Scattering from Regular Atomic Arrays

- Diffraction pattern may be understood from principles of optical diffraction on gratings

- Scattering from single atom

- Scattering from an atomic array

Path and Phase Difference



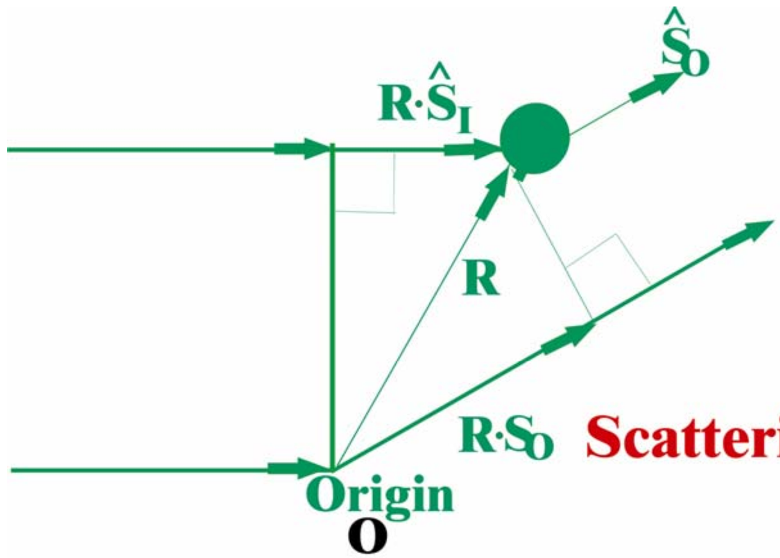
Path difference

$$\mathbf{R \cdot S_O - R \cdot S_I}$$

Phase difference

$$\frac{2\pi}{\lambda}\left[\mathbf{R \cdot S_O - R \cdot S_I}\right]$$

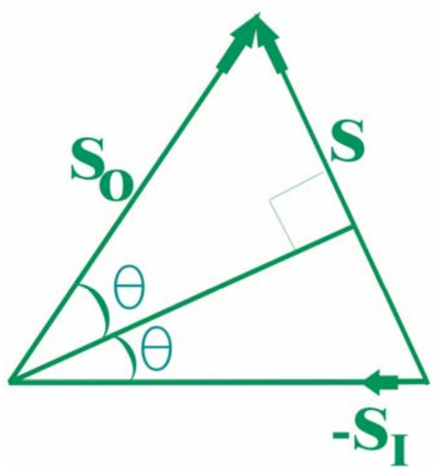
Scattering from a Single Atom



Path difference

$$\mathbf{R \cdot S_O - R \cdot S_I}$$

Scattering vector, $\hat{S}$: $\hat{S}_O/\lambda$ - $\hat{S}_I/\lambda$

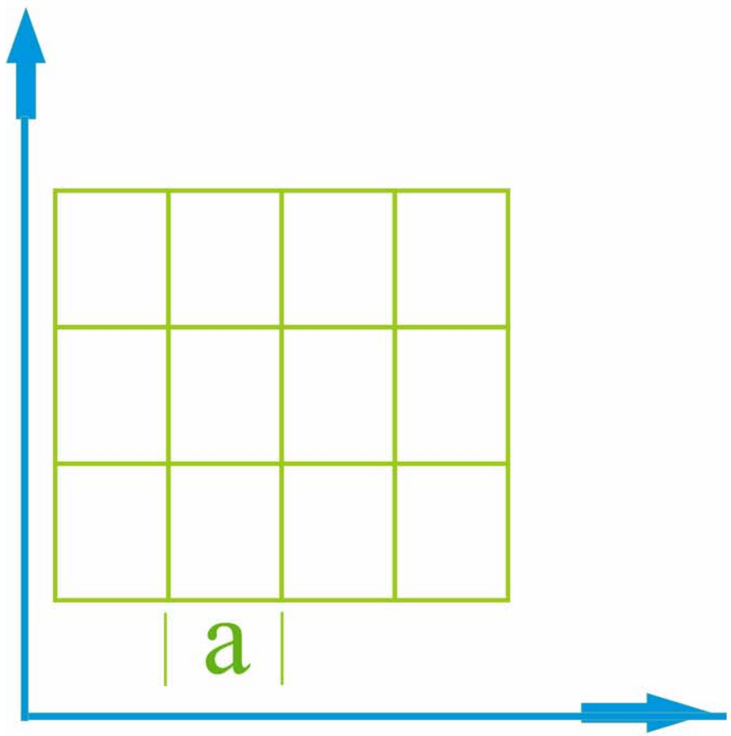


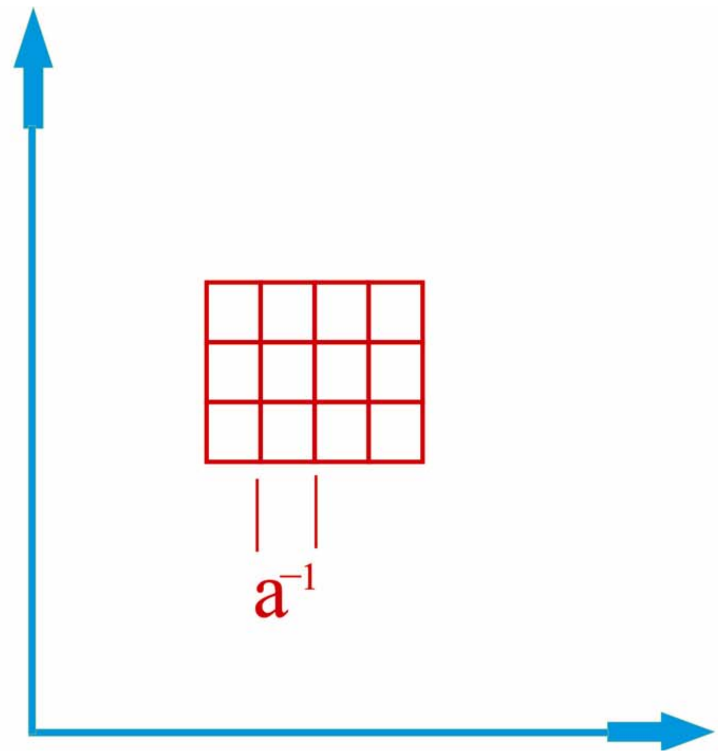
$$\mathbf{S \cdot S = 4 \sin^2\theta / \lambda^2}$$

$$|\mathbf{S}| = \mathbf{2}|\mathbf{sin}\theta| / \lambda$$

$$0 \leq |S| \leq 2/\lambda$$

Real and Reciprocal Space





real space/
direct space/
Cartesian space

reciprocal space/
frequency space/
Fourier space

Phase Difference and Scattering Power

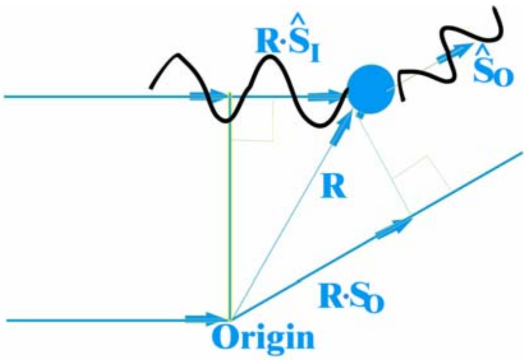
Real Space | **Reciprocal space**

Path difference

$R \cdot S_O - R \cdot S_I$

Phase difference

$\frac{2\pi}{\lambda}[R \cdot S_O - R \cdot S_I]$



If electron at origin has a scattering power E(S) along direction S,

Scattering power of an electron at R is

$E(S)\, e^{2\pi i S.R}$

Structure factor: $e^{2\pi i S.R}$

(Geometrical part of the scattering)

SF for Electron Density Distribution

Structure factor for a unit volume: $e^{2\pi i S.r}$

For a volume element dr

Structure factor: $\rho(r)e^{2\pi i S.r}dr$

For a continuous electron density distribution,

Structure factor:

$$\boxed{F(S) = \int \rho(r)e^{2\pi i S.r}dr}$$

Plane Waves and Fourier Transforms

$$\boxed{\mathbf{F(S)} = \int \rho(\mathbf{r}) e^{2\pi i \mathbf{S}.\mathbf{r}} d\mathbf{r}}$$

Structure factor is the Fourier Transform of the electron density distribution

Conversely, the electron density distribution is the inverse Fourier transform of the structure factor

$$\boxed{\rho(\mathrm{r}) = \frac{1}{\mathrm{V}} \int \mathbf{F(S)} e^{-2\pi i \mathbf{S.r}} d\mathbf{S}}$$

Phase Problem in Crystallography

The structure factor F(S) for a given direction S is a complex quantity.

$$\mathbf{F(S)} = |\mathbf{F(S)}|\, e^{i\phi}$$

$|\mathbf{F(S)}|$: **Structure factor amplitudes**

ϕ : **Phases**

The measured intensity I(S) is

$$\mathbf{I(S)} = \mathbf{F(S)} * \mathbf{F(S)} = |\mathbf{F(S)}|^{2}$$

Thus the phases ϕ are lost during measurement.

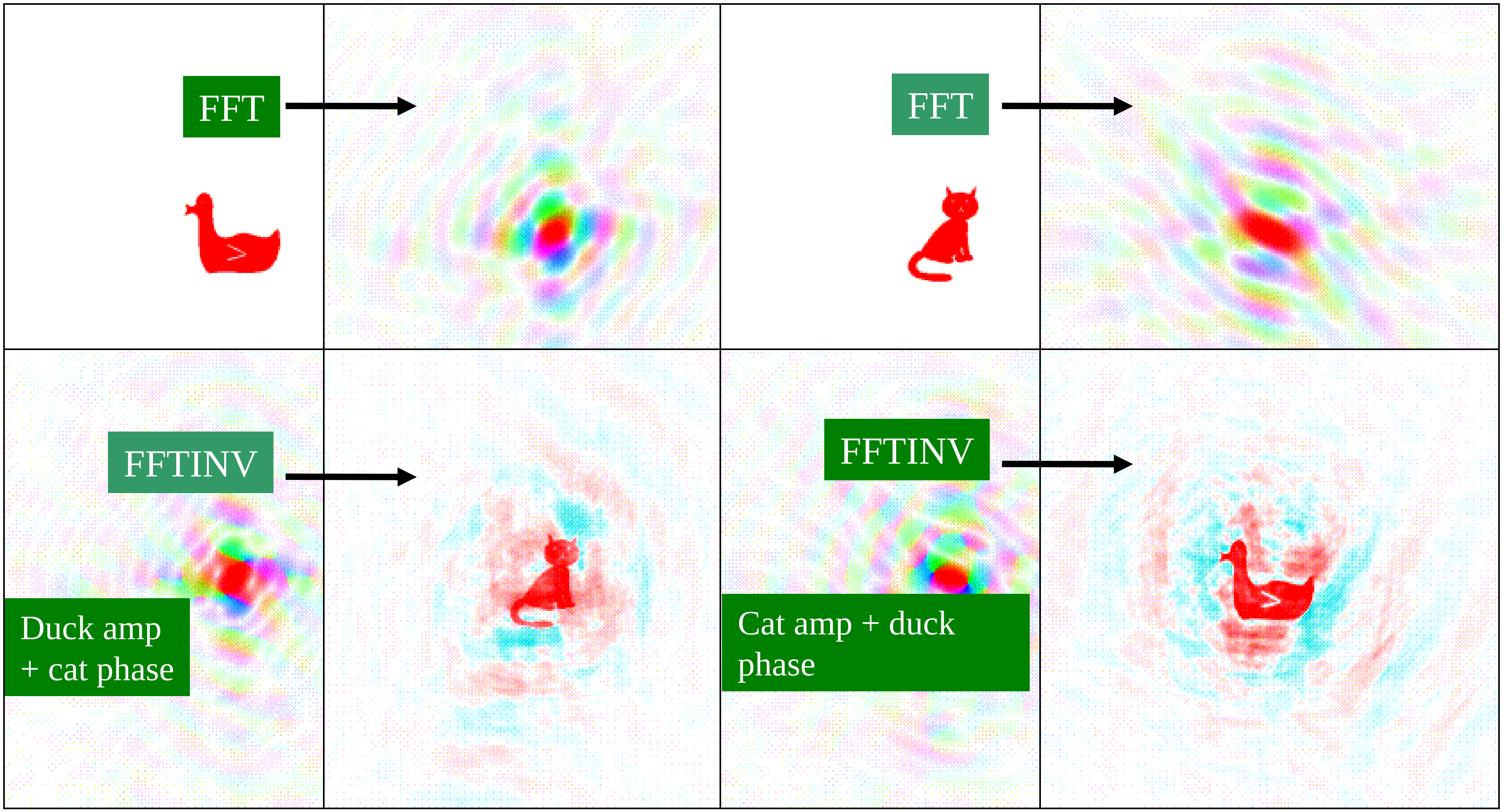
Phases Needed to Reconstruct Images

Scattered X-rays carry phase information

But no X-ray lens to recombine the amplitudes and the phases to reconstruct the original image!

Which is more important: Amplitudes or phases?

Phases more Important than Amplitudes



Phase Problem in Crystallography

How to estimate the phases that give the best electron density $\rho(\mathbf{r})$ from the structure factors F(S)

Uniform Sample Cannot Deflect X-rays

Atoms must have heterogenous electron density distribution to scatter X-rays.

Assume a uniform electron density distribution:

$$\rho(\mathbf{r}) = \rho$$

$$\mathbf{F(S)} = \int \rho(\mathbf{r}) e^{2\pi i \mathbf{S}.\mathbf{r}} d\mathbf{r}$$

$$= \rho \int e^{2\pi i \mathbf{S}.\mathbf{r}} d\mathbf{r}$$

$$= \rho\, \delta(\mathbf{S} - 0)$$

$$= \mathbf{F(0)}$$

Uniform Sample Cannot Deflect X-rays

$$\mathbf{F(S)} = \mathbf{F(0)} \quad \textbf{if} \quad \rho(\mathrm{r}) = \rho$$

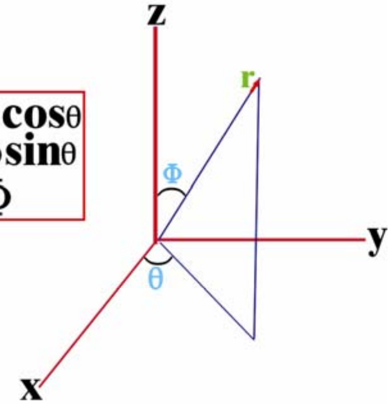
Thus, a uniform electron density gets scattered only in the forward direction.

Spatial or temporal heterogeneity that creates a contrast between the electrons and the background, is a must to scatter X-rays in all directions.

X-ray Scattering from a Single Atom

Assume a spherically symmetric electron density distribution

$$x = r\sin\Phi\cos\theta$$
$$y = r\sin\Phi\sin\theta$$
$$z = r\cos\Phi$$



$$F(\mathbf{S}) = \int \rho(\mathbf{r})\, e^{2\pi i \mathbf{S}.\mathbf{r}}\, d\mathbf{r}$$

$$F(\mathbf{S}) = \int_0^{2\pi} d\phi \int_0^{\pi} \sin\theta\, d\theta \int_0^{\infty} dr\, \rho(r)\, r^2\, e^{2\pi i \mathbf{S}.\mathbf{r}}$$

$$= 2\pi \int_0^{\infty} dr\, \rho(r)\, r^2 \int_0^{\pi} d\theta\, \sin\theta\, e^{2\pi i S r \cos\theta}$$

X-ray Scattering from a Single Atom

$$F(S) = 2\pi \int_0^{\infty} dr\,\rho(r)\,r^2 \int_0^{\pi} d\theta \sin\theta\, e^{2\pi i S r \cos\theta}$$

Substitute $x = r\cos\theta$

$$F(S) = \boxed{4\pi \int_0^{\infty} dr\,\rho(r)\,r^2 \left[\frac{\sin 2\pi S r}{2\pi S r}\right]}$$

f(S): Atomic form factor

Finite Atomic Size & Form Factor

Assuming a tailed electronic distribution

$\rho(r) = Zne^{-kr^2}$ **where Z is the atomic number**

it can be shown that $f(S) = Ze^{\left(\frac{-\pi^2}{k}\right)S^2}$

Thus, finite size of atom introduces a resolution dependent fall-off of the atomic form factor.

This is due to the destructive interference of the scattered radiation from electrons.

Resources

- Cantor, R.C. & Schimmel, P.R. (1980). *Biophysical Chemistry Part II: Techniques for the study of biological structure and function*. New York; W.H. Freeman & Co, Chapter 13.
- Blundell, T.L. & Johnson, L.N. (1976). *Protein Crystallography*. London; Academic Press.
- Stout, G.H. & Jensen, L.M. (1968). *X-ray Structure Determination: A* Practical Guide. New York: Macmillan