I: In introduction to molecular electron microscopy
- Imaging macromolecular assemblies

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2/28/2014 - Introduction of Molecular Microscopy: electron scattering; electron optics; image formation; contrast transfer function; cryoEM; Low-dose imaging technique; principle of 3D reconstruction;

3/3/2014 - Averaging; single particle EM;
paper discussion:

3/5/2014 - how to carry out a single particle EM study;
paper discussion:

3/7/2014 - Current status and future of the cryoEM;
paper discussion:
Cao, et. al. "TRPV1 structures in distinct conformations reveal activation mechanisms. Nature, 504, 113-118

Related interesting papers:
Resolution limit of an optical microscope system

Rayleigh criterion:

\[ \sin \theta = 1.22 \frac{\lambda}{D} \quad \text{or} \quad \Delta l = 1.22 \frac{\lambda}{D} \]

\( \theta \) is the angular resolution, \( \lambda \) is the wavelength and \( D \) is the diameter of the lens aperture.

or: \( \Delta l \) is the spatial resolution, \( f \) is the focal length of an ideal lens.

Thus: The resolution of an light microscope system is limited by the wavelength of the light used. One of the ways to improve the resolution is to use light with shorter wavelength.

Wave-particle duality of electron

It all started with the De Broglie’s hypothesis:

\[ \lambda = \frac{h}{p} \]

\( \lambda \) is wavelength, \( h \) is Planck’s constant, and \( p \) is momentum.

The original motivation of building an electron microscope came from the shorter wavelength of the electron.

Electron wavelength

Applying the principle of energy conservation to an electron (-e) traveled in voltage \( E_0 \):

\[ eE_0 = \frac{h^2}{2m\lambda^2} \]

\[ \lambda = \frac{h}{\sqrt{2meE_0}} \]

\( E_0 \) = acceleration voltage

\( \lambda \) = wavelength

\( m \) = electron mass

\( e \) = electron charge

\( h \) = Planck’s constant
Electron wavelength

Take the relativity into consideration, the wave length is:

$$\lambda = \frac{h}{\sqrt{2m_0eE_r}} \quad E_r = E_0 + \left(\frac{e}{2m_0c^2}\right)E_0^2$$

$$\lambda = \frac{1.22639}{\sqrt{E_0 + 0.97845 \times 10^{-6} E_0^2}}$$

120kV $\lambda = 0.033\text{Å}$; 200kV $\lambda = 0.025\text{Å}$; 300kV $\lambda = 0.020\text{Å}$;

Note that these wavelength is considerably shorter than that used in X-ray crystallography, which is $\sim$Å.

Atomic Scattering Factor for Electrons

Mott formula:

$$f_s(\theta) = \frac{me^2}{2\hbar^2} \left(\frac{\lambda}{\sin \frac{\theta}{2}}\right)^2 \left[Z - f_s(\theta)\right]$$

$$|g| = \frac{2\sin \frac{\theta}{2}}{\lambda} = 2|k|\sin \frac{\theta}{2}$$

Figure 4.6. Atomic scattering amplitudes as a function of $\sin \frac{\theta}{2}/\lambda$ for Al ($Z = 13$), Cu ($Z = 29$), Ag ($Z = 47$) and Au ($Z = 79$).

Electron v.s. X-ray

As particles:
- Electrons interact with the potential field of an atom, including shell electrons and nucleus, X-rays interact with only shell electrons;
- Electrons have much larger scattering cross-sections than X-rays; multiple scattering is severer in electron scatterings than in X-ray diffraction; For biological sample, radiation damage is also severer than X-ray diffraction.

As wave:
- Electrons can be focused by electromagnetic lens, X-ray cannot be focused by lens;
**Instrumentation of EM**

A schematic drawing of an electron microscope.

**Electron source**

- Tungsten filament
- LaB6 crystal
- Thermionic source

**Electron source**

- Field emission
- A field emission tip
- A Schottky field emission tip

Field emission source
specimen holder

Field emission source

Transmission Electron microscopes

Electromagnetic lens

* The focal length of a electromagnetic lens can be easily adjusted by changing the lens current.
Image formation

The image formation in the electron microscope can be treated as two separate processes:

1) The interaction of the incident beam with the specimen, described by the weak-phase object approximation, which is the theory used mostly to describe the image formation of thin specimen with light elements, such as a biological sample.
2) The propagation of the electron beam from exit plane of the specimen to the back image plane of the focus lens.

Weak-phase object approximation

This is a highly simplified theory based on the so-call weak-phase object, which is a very thin specimen formed mostly by low- and medium-weight molecules.

Suppose: 1) the specimen is very thin so that $\Phi(\vec{r},z)$ can be approximated by $\Phi(\vec{r})$; 2) both in-coming and exiting beams are parallel beams;

$$\Phi(\vec{r},z) \quad \Phi(\vec{r}) = \int \Phi(\vec{r},z) \, dz \quad \psi_{ex} = \psi_{in} \exp(i\Phi(\vec{r}))$$
\[
\psi_{ex} = \psi_{in} \exp(i\Phi(\vec{r})) \tag{2}
\]

\[
\psi_{ex}' = \psi_{in} \left[ 1 + i\Phi(\vec{r}) - \frac{1}{2}\Phi^2(\vec{r}) + \ldots \right] \tag{3}
\]

The first term in (3) represents the central unscattered beam, the second term the kinematically scattered beam and the higher terms are for the dynamical scattering. The weak phase object approximation assumes that \(\Phi(\vec{r}) \ll 1\), the phase shift is so small that the following approximation will work:

\[
\psi_{ex}' = \psi_{in} \left[ 1 + i\Phi(\vec{r}) \right] \tag{4}
\]

Taking absorption into consideration:

\[
\psi_{ex} = \psi_{in} \exp(i\Phi(\vec{r}) - \mu(\vec{r})) \tag{5}
\]

\[
\psi_{ex}' = \psi_{in} \left[ 1 - \mu(\vec{r}) + i\Phi(\vec{r}) \right] \tag{6}
\]

\[
I_{ex} = |\psi_{ex}'|^2 = |\psi_{in}|^2 = I_{in}
\]

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**Image formation**

At exit plane of specimen:

\[
\psi_{ex}'(\vec{r}) = \psi_{in}[1 + i\Phi(\vec{r})]
\]

At back focal plane:

\[
\Psi_{bf}(\vec{k}) = F[\psi_{ex}'(\vec{r})]
\]

At back image plane:

\[
\psi_{im}(\vec{r}) = F^{-1}[\Psi_{bf}(\vec{k})]
\]

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**Image formation**

At exit plane of specimen: \(\psi_{ex}'(\vec{r}) = \psi_{in}[1 + i\Phi(\vec{r})]\)

At back focal plane: \(\Psi_{bf}(\vec{k}) = F[\psi_{ex}'(\vec{r})]\)

At back image plane: \(\psi_{im}(\vec{r}) = F^{-1}[\Psi_{bf}(\vec{k})]\)
Image formation

The plane wave $\psi'$ of exit-beam travel through objective lens to the back focal plane. The wave function at back focal plane of the objective lens is the Fourier transform of the exit wave:

$$\Psi_{bf}(\vec{k}) = F(\psi'_{ex}(\vec{r})) = F[1 + i\Phi(\vec{r})] = \delta(\vec{k}) + iF(\Phi(\vec{r}))$$  \hspace{1cm} (7)

However the lens aberration and defocusing generate an extra phase shift to the scattered beam:

$$\gamma(\vec{k}) = 2\pi\chi_{\vec{k}}$$
$$\chi(k,\varphi) = \frac{1}{2} \lambda \left[ \Delta z + \frac{1}{2} \sin(2(\varphi - \varphi_0)) \right] k^2 + \frac{1}{2} \lambda^2 C \varphi^4$$  \hspace{1cm} (8)

Together with the aperture function $A(\vec{k})$ the wave function at back focal plane will become:

$$\Psi_{bf}(\vec{k}) = F(\psi'_{ex})A(\vec{k}) \exp(2\pi i\chi_{\vec{k}})$$  \hspace{1cm} (9)

Then, the wave function in the back image plane of the lens is the reverse Fourier transform of the wave function at back focal plane ($\otimes$ is for convolution):

$$\psi_{im}(\vec{r}) = \mathcal{F}^{-1}\{F(\psi'_{ex})A(\vec{k}) \exp(2\pi i\chi_{\vec{k}})\}$$
$$= 1 + i\Phi(-\vec{r}) \otimes J_0(\vec{r}) \otimes \mathcal{F}^{-1}[\exp(2\pi i\chi_{\vec{k}})]$$  \hspace{1cm} (10)

The observed intensity in the image is then:

$$I_b(\vec{r}) = \psi_{im}(\vec{r}) \psi_{im}^*$$
$$= 1 + 2\Phi(-\vec{r}) \otimes J_0(\vec{r}) \otimes \mathcal{F}^{-1}[\sin(2\pi\chi_{\vec{k}})]$$  \hspace{1cm} (11)

Contrast Transfer Function (CTF)

$$CTF = \sin(2\pi\chi_{\vec{k}})$$

The intensity of a recorded image is directly related to the projection of specimen (good!) but modified by the FT of CTF (bad!).

$$I_b(\vec{r}) = \psi_{im}(\vec{r}) \psi_{im}^*$$
$$= 1 + 2\Phi(-\vec{r}) \otimes J_0(\vec{r}) \otimes \mathcal{F}^{-1}(CTF)$$  \hspace{1cm} (12)
What is this CTF thing anyway and why do I care?

Distortions of CTF to the image are:
1) Contrast reversal of large area; 2) diminished contrast in large area; 3) edge enhancement and 4) appearance of fringes along the borders.

From Joachim Frank
Defocus +1 µm

Defocus +2 µm

Determine CTF

$E = 120 \text{ kV, } \Delta f = 21000 \text{ Å, } C_s = 2 \text{ mm, } A = 0.15$
Molecular electron microscopy of biological sample

Strong electron scattering power means two things:
1. high vacuum of microscope column;
2. strong scattering with protein sample;

Problems:
1. dehydration of biological sample;
2. radiation damage by high energy beam;

Frozen hydration preserve the biological sample


Taylor and Glaeser (2008) “Retrospective on the early development of cryoelectron microscopy of macromolecules and a prospective on opportunities for the future” Journal of Structural Biology

“Probably the best electron diffraction pattern of frozen hydrated catalase obtained in 1970's.”
Frozen hydrated specimen preparation


Equipment for cryo-electron microscopy

Cryo specimen holder

- cryo plunger for rapid freezing;
- cryo-holder and cryo-transfer station;

Oxford style cryo-holder and transfer station
working temperature: < -180°C
Cryo-electron microscopy

Against dehydration:
*glucose/trehalose embedding*: using glucose to substitute water, thus maintain hydration in the high vacuum. Only used for 2D crystal;
Frozen hydration: using plunge freezing to avoid crystal ice. Mostly for single particle;

• Against radiation damage:
  Low-temperature: LN2 (~80K) or LHe (~10K); Challenges to the instrumentations;
  Low-electron dose: Low-dose imaging; Results in extremely noisy images, challenges for the data processing;

Low-dose imaging technique

To record a good image, one needs to find the sample (search), adjust imaging condition (focus) and record image (exposure).

Low-dose imaging divide these steps into three different modes with different beam setting:

* SEARCH: extremely low-dose, \(\sim 10^{-3} \text{e}/\text{Å}^2/\text{sec}\);
* FOCUS: high magnification, away from the imaging area;
* Exposure: 10 ~ 30 e/Å\(^2\) dose rate to record image;

Three different modes in low dose

* Search: lowest possible beam intensity;
* Focus: off-exposure area, high magnification;
* Exposure: desired magnification and beam intensity;
Electron optics of Low-Dose imaging

Single particle cryo-electron microscopy of human transferrin receptor-transferrin complex

An image is the projection of a 3D object
**Fourier Central section theorem**

Central Section Theorem:
Fourier transform of a 2D projection equals the central section through its 3D Fourier transform perpendicular to the direction of projection.

DeRosier, D. and Klug, A. (1968) "Reconstruction of three dimensional structures from electron micrographs" *Nature 217* 130-134

Hart, R.G. (1968) "Electron microscopy of unstained biological material: the polytropic montage" *Science 159* 1464-1467

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**Cryo-EM techniques**

- Electron crystallography: for membrane protein resolution achieved: 2.5Å bacteriorhodopsin, Aquaporin-0 (recently: 1.9Å aquaporin-0) averaging of molecules which form 2D crystal;

- Single particle cryo-electron microscopy: protein in soluble form; resolution achieved: intermediate 20 Å ~ 3 Å averaging of many single molecules with the same structure;

- Cryo-electron tomography: large cellular organelle, whole cell etc. Resolution achieved: > 3nm no averaging, 3D reconstruction of single biological object;