Early TEM and Biology

1906 - 1988
Nobel Prize in Physics 1986

Early design, 1931

Bacterial culture in negative stain. Friedrich Krause, 1937
Decades of Development

Negative stain: Brenner & Horne (1959)

Quantitative molecular electron microscopy: Klug & DeRosier (1966); DeRosier & Klug (1968)

Cryo-EM: Taylor & Glaeser (1974); Dubochet & McDowell (1981)


"Movie" mode from fast-readout, direct electron detectors: Campbell et al & Grigorieff (2012); Li et al & Cheng (2013)

Software to implement powerful image analysis algorithms: Scheres (2012); Lyumkis et al & Grigorieff (2013)

Courtesy of Steve Harrison
Why Cryo?

• Sample is frozen in aqueous solution → native conditions
• Frozen state prevents dehydration in microscope vacuum
• Low temperature delays effects of radiation damage
(Cryo) Applications Today

**Tomography**

- Thin sections & organelles
  - Endoplasmic reticulum
    - Medalia et al. 2002

**Crystallography**

- 2D & small 3D crystals
  - Purple membrane
    - Grigorieff 1995
- Filaments
  - FtsZ filaments
    - Li et al. 2007
- Molecules/complexes
  - Nuclear pore
    - Kosinski et al. 2016

**Helical reconstruction**

- Acetylcholine receptor
  - Miyazawa et al. 2003

**Single-particle averaging**

- HIV capsid protein
  - Schur et al. 2016
- MloK1 channel
  - Scherer et al. 2014
- Microtubule
  - Alushin et al. 2014
- TRPV1 channel
  - Liao et al. 2013
Radiation Damage

Tobacco Mosaic Virus

20 e⁻/Å² exposure at 300 kV

60 e⁻/Å² exposure at 300 kV
Radiation Damage

Rotavirus VP6

5 e/Å²
200 kV
Catalase Thin 3D Crystals

\[ F(k, N) = F(k, 0)q(k, N) \]

\[ q(k, N) = e^{-\frac{N}{2N_e(k)}} \]

\( N_e \): critical dose

Baker et al. 2010 200 kV
Preservation of biological samples in vitrified ice: Jacques Dubochet and colleagues
EMBL, 1980’s

Bacteriorhodopsin 2D crystals (purple membrane) at 200 kV:

Protection factor = 1
Protection factor = 5 - 9
Protection factor = 10 - 20

Stark et al. 1996
Cryo Sample Preparation

1. Purified protein
2. Solution on holey carbon film
3. Liquid ethane
4. Image with particles in random projections
5. Electron microscopy
Cryo Plunger

Adapted from Subramaniam et al. 1993
Cryo Holder

Gatan 626 holder

High-end microscopes have a built-in cryo stage.
Data collection scheme (usually automated)

A. **Low mag montage:** low intensity, low magnification (50x), entire grid

B. **Medium mag montage:** low intensity, 4k mag, one grid square

C. **Focus:** high intensity, high magnification (230k), small area

D. **Exposure:** medium magnification (30k - 60k), beam size adjusted to cover entire detector (CMOS)

Dose used: 10 – 40 e⁻/Å²

Resch et al. 2011
Low-dose: 10 electrons/Å$^2$

Low signal-to-noise ratio: 0.3% of incident electrons are scattered (assuming 50 Å sample thickness)

$\Rightarrow$ SNR $\approx 1/20$ (1 pixel = 2 Å$^2$) (assuming perfect imaging)

Low resolution, unless many images are averaged
Low-Dose Microscopy

Purple membrane

SNR $\approx 1/60$
High-Resolution Cryo-EM

Purple membrane

2.6 Å resolution

Grigorieff 1995
Detecting Single Unit Cells

Purple membrane

~100 kDa
What is the smallest particle that can be aligned and reconstructed by cryo-EM?

Theoretical prediction (Henderson, Glaeser):

20 – 40 kDa

Also: a few thousand images of molecules should be sufficient to reach 3 Å resolution.
Cryo Images

Viral polymerase, 240 kDa

500Å

Rotavirus DLP, 70000 kDa
**Phase Contrast**

**Ferritin on Carbon**

\[ E = 100 \text{ kV} \]

$E = 300 \text{ kV}, \Delta f = 7900 \text{ Å}, C_s = 2.7 \text{ mm}, \text{amp. contrast} = 0.1$
Sample: Structure factor $F$

Microscope: Observe structure factor $O_i$ from image $i$

$$O_i = CTF_i \times F$$

Computer: Reconstruct original structure factor $F$

$$F = \frac{\sum_i CTF_i \times O_i}{w + \sum_i CTF_i^2}$$

$$w = \frac{1}{SNR}$$

(Wiener filter constant)
In a tomography experiment, the projection directions are known. In a single-particle experiment, the projection directions have to be determined through computational image alignment.

Baumeister et al. 1999
Aligning Particles

5 parameters to determine

Carbon

Ice

100Å
Iterative Refinement

Aligned Particles → Reference → High-resolution structure → Reference → Aligned Particles

Reference

Low-resolution structure
Example: VSV Polymerase

VSV = vesicular stomatitis virus
Infests cattle, horses and pigs (non-lethal)
Similar to rabies virus, Ebola virus, respiratory syncytial virus

Ge et al. 2010

Liang et al. 2015
Example: Papillomavirus

Data collected on film at 300 kV, 59k mag, 1.8 – 2.9 µm underfocus, 25 e⁻/Å²

3D reconstruction
3.6 Å resolution

Wolf et al. 2010
Example: Papillomavirus

3.6 Å resolution

1.5 million subunits averaged!
Henderson, Glaeser: a few thousand should suffice.

Wolf et al. 2010
Beam-Induced Movement

12 e⁻/Å²

After 200 e⁻/Å²

Graphite

Solution: Movie data collection
Dynamic range: $10^4$
Effective pixel size: 15 µm
Readout: 1 frame/5 sec
4k x 4k pixels

Dynamic range: $10^3$
Pixel size: 5-14 µm
Readout: 40 frames/sec
(internally 400 frames/sec)
4k x 4k pixels
The high-energy electrons scatter in the scintillator and generate light in several neighboring pixels:

- blurring of the image
- reduction of amplitudes in a Fourier transform
- counts depend on random trajectories: **noise**
Thin Detectors
Reduced Noise

Improved detector performance (DQE) by reducing the chance of backscattering and making trajectories less random.

McMullan & Henderson, 2009
Electron Counting

1. Electron enters detector

2. Signal is scattered

3. Charge collects in each pixel

4a. Events are reduced to the highest charge pixels
Detective Quantum Efficiency

**FindDQE**

\[
DQE(k) = \frac{SNR(k)_{\text{out}}}{SNR(k)_{\text{in}}}
\]

Ruskin et al. 2013
Heterogeneity and Biology

Translocation, Brilot et al 2013

Gluatmate receptor, Dürr et al 2014

Spliceosome, Wahl et al 2009

GroEL/GroES ATP cycle
Clare et al 2012

Kinesin power stroke
Sindelar & Downing 2010
Example: $F_1$-$F_0$ ATPase

Zhou, Rohou et al. and Rubinstein 2015

Figure: Lehninger Biochemistry
Example: $F_1$-$F_0$ ATPase

Zhou, Rohou et al. and Rubinstein 2015
Example: $F_1$-$F_0$ ATPase

State 1a/1b  
State 2a/2b  
State 3a/3b  
State 2b/2c

25 Å

Zhou, Rohou et al. and Rubinstein 2015
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