WET LAB WORKSHOP

“Molecular cryo-EM: Structural Biology without Crystals”

Topic:
The 3-hour workshop is a practical introduction to the cryo-electron microscopy (cryo-EM) to determine macromolecular structures at atomic/near-atomic resolution. The workshop will include: a brief background of cryo-EM in protein structural biology and some step-by-step procedures and things to consider before doing cryo-EM. We will cover on single-particle analysis (SPA) and a little microcrystal electron diffraction (MicroED). Common workflows and some limitations will be highlighted.

Instructor:
Jyh-Yeuan (Eric) Lee, PhD, Assistant Professor
Department of Biochemistry, Microbiology and Immunology
Faculty of Medicine, University of Ottawa

Oct. 7th, RGN 4161

2:00-5:00 PM
Limited spots available; tickets must be obtained on Eventbrite:
https://www.eventbrite.ca/e/wetlab-workshop-cryo-em-oct-7th-tickets-72382521069

Wet Lab Workshop
"Molecular cryo-EM: Structural Biology without Crystals

Jyh-Yeuan (Eric) Lee, Assistant Professor
Department of Biochemistry, Microbiology and Immunology

October 7th, 2019 @ Roger Guindon Hall 4161, 2-5 PM

Part 0

INTRODUCTION

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Menu

- Pt I: Cryo-EM as a branch in macromolecular structural biology
  - Background and history
  - Basics in Optics
- Pt II: From samples to structures
  - Sample preparations
  - Data collection
  - Image process
- Pt III: Challenges and opportunities

References

- Materials in this work are based on published literatures (as cited accordingly) and lectures/protocols from the following resources:

  - EMBO Cryo-EM Courses
  - EMAN Workshops
  - ACA Cryo-EM Workshops
  - FEMR online protocols (McGill University)

Part I

CRYO-EM AS A BRANCH IN MACROMOLECULAR STRUCTURAL BIOLOGY

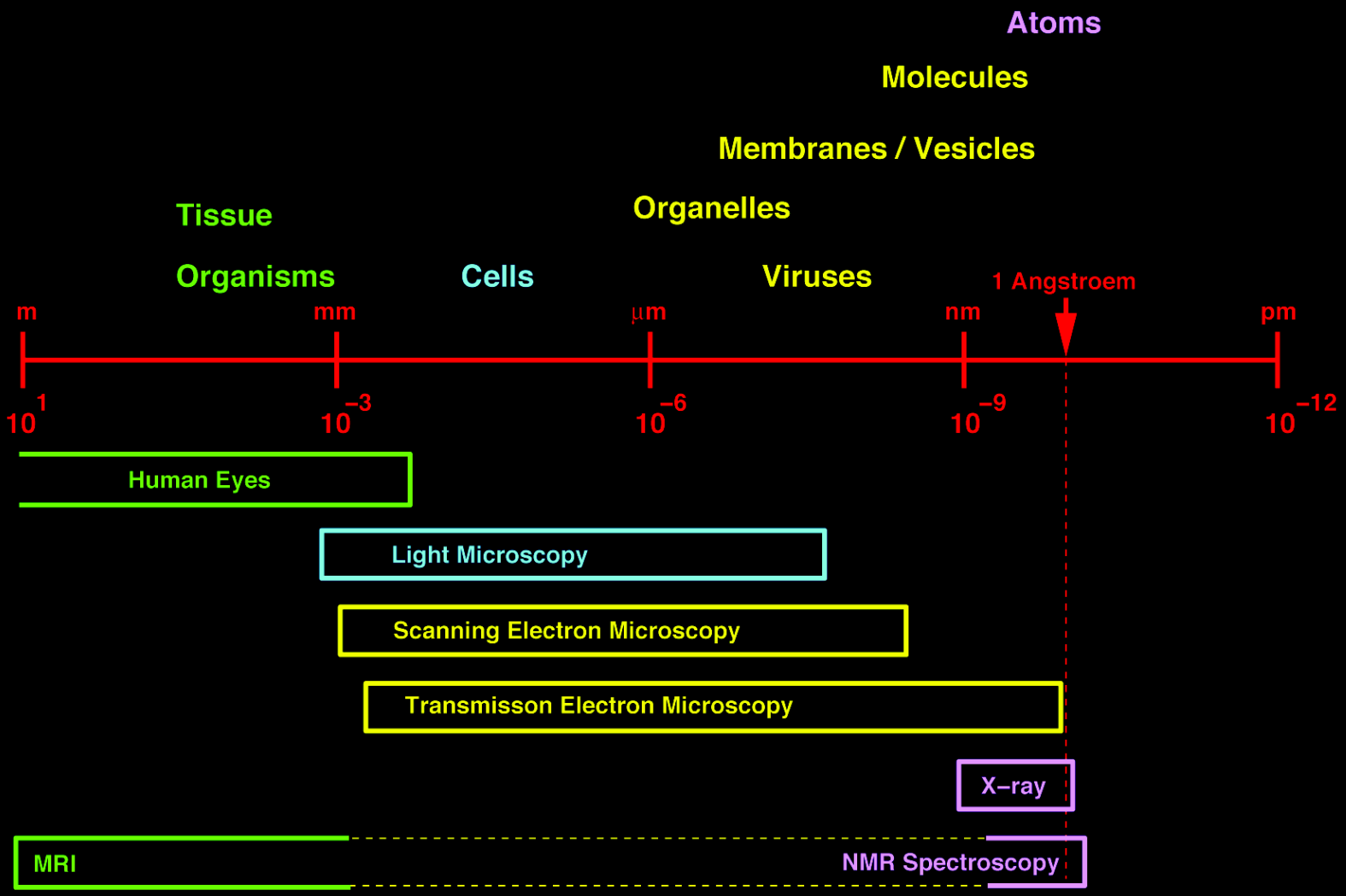
Wet Lab Workshop
"Molecular cryo-EM: Structural Biology without Crystals

Part I: Cryo-EM as a branch in macromolecular structural biology

Jyh-Yeuan (Eric) Lee, Assistant Professor, BMI

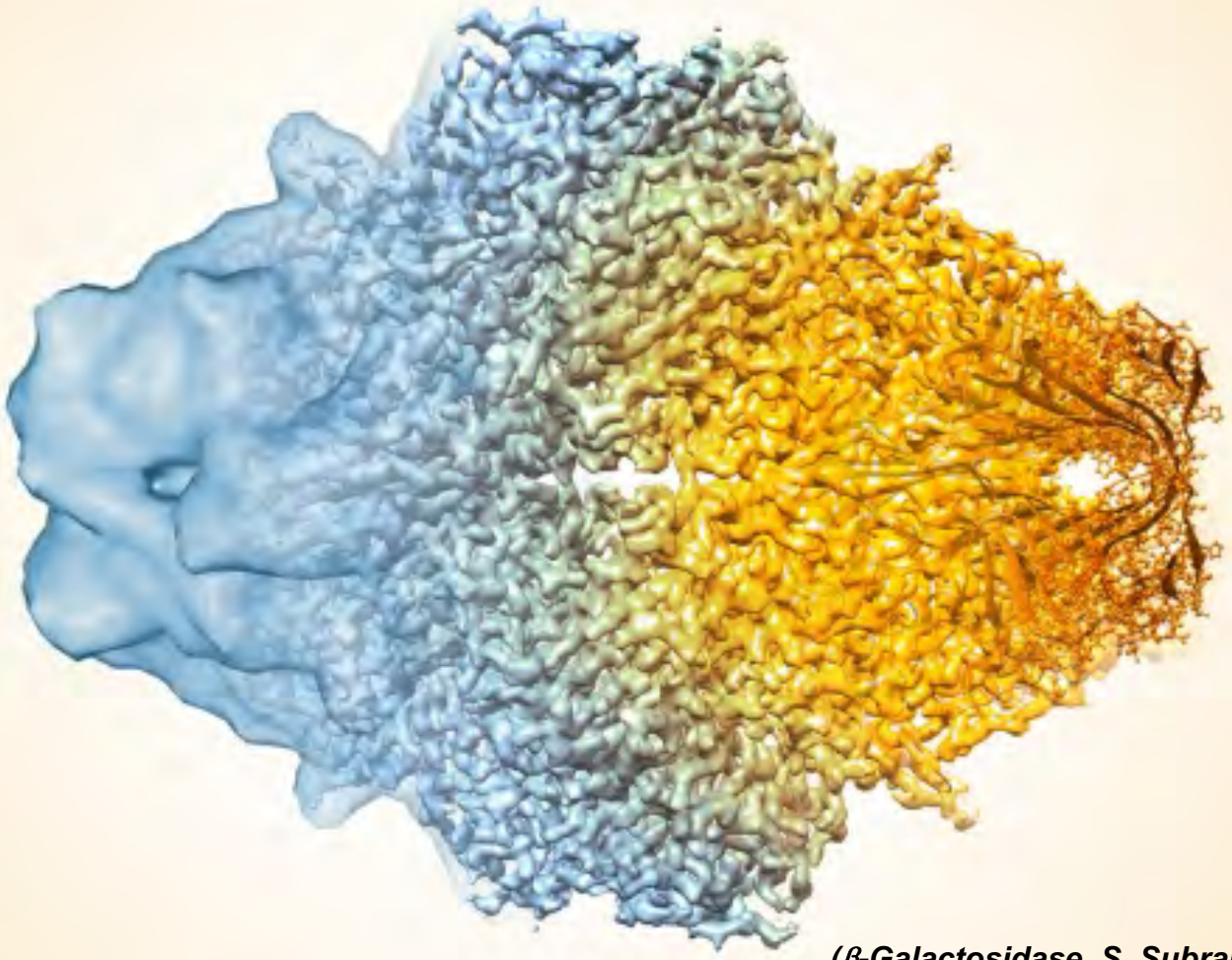
October 7th, 2019

How "Tiny" Can We See?



Outline

- **Cryo-EM: a new poster child**
  (previously under-appreciated)
- Instrumentation
- Optics and imaging
- Sub-branches of molecular cryo-EM
- Contrast transfer function (CTF)



(β-Galactosidase, S. Subramaniam, UBC)

Making Structural Biology Possible

1915: X-ray Crystallography

The Nobel Prize in Physics 1915

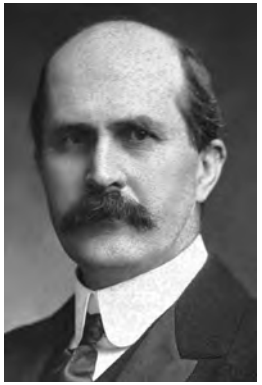


Photo from the Nobel Foundation archive.

Sir William Henry Bragg

Prize share: 1/2

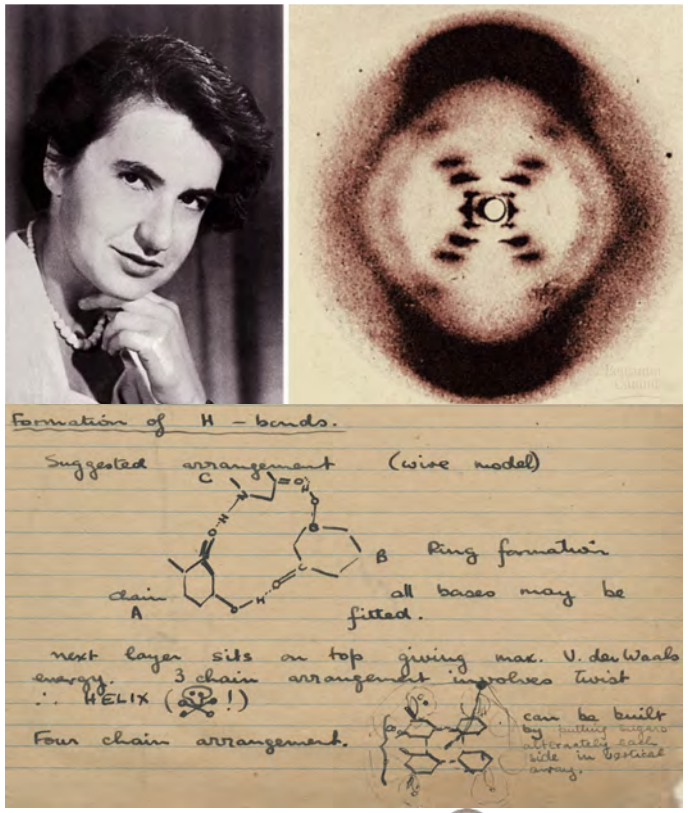
Photo from the Nobel Foundation archive.

William Lawrence Bragg

Prize share: 1/2

1952: DNA Double-Helix Structure

Rosalind Franklin



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Making Structural Biology Possible

2002: NMR / Mass Spectrometry

The Nobel Prize in Chemistry 2002

Photo from the Nobel Foundation archive.

John B. Fenn

Prize share: 1/4

Photo from the Nobel Foundation archive.

Koichi Tanaka

Prize share: 1/4

Photo from the Nobel Foundation archive.

Kurt Wüthrich

Prize share: 1/2

2010s: NMR for Bigger Proteins

Lewis Kay

Making Structural Biology Possible

2017: Cryo-Electron Microscopy (cryo-EM)

From left: Jacques Dubochet, Joachim Frank and Richard Henderson developed cryo-electron microscopy.

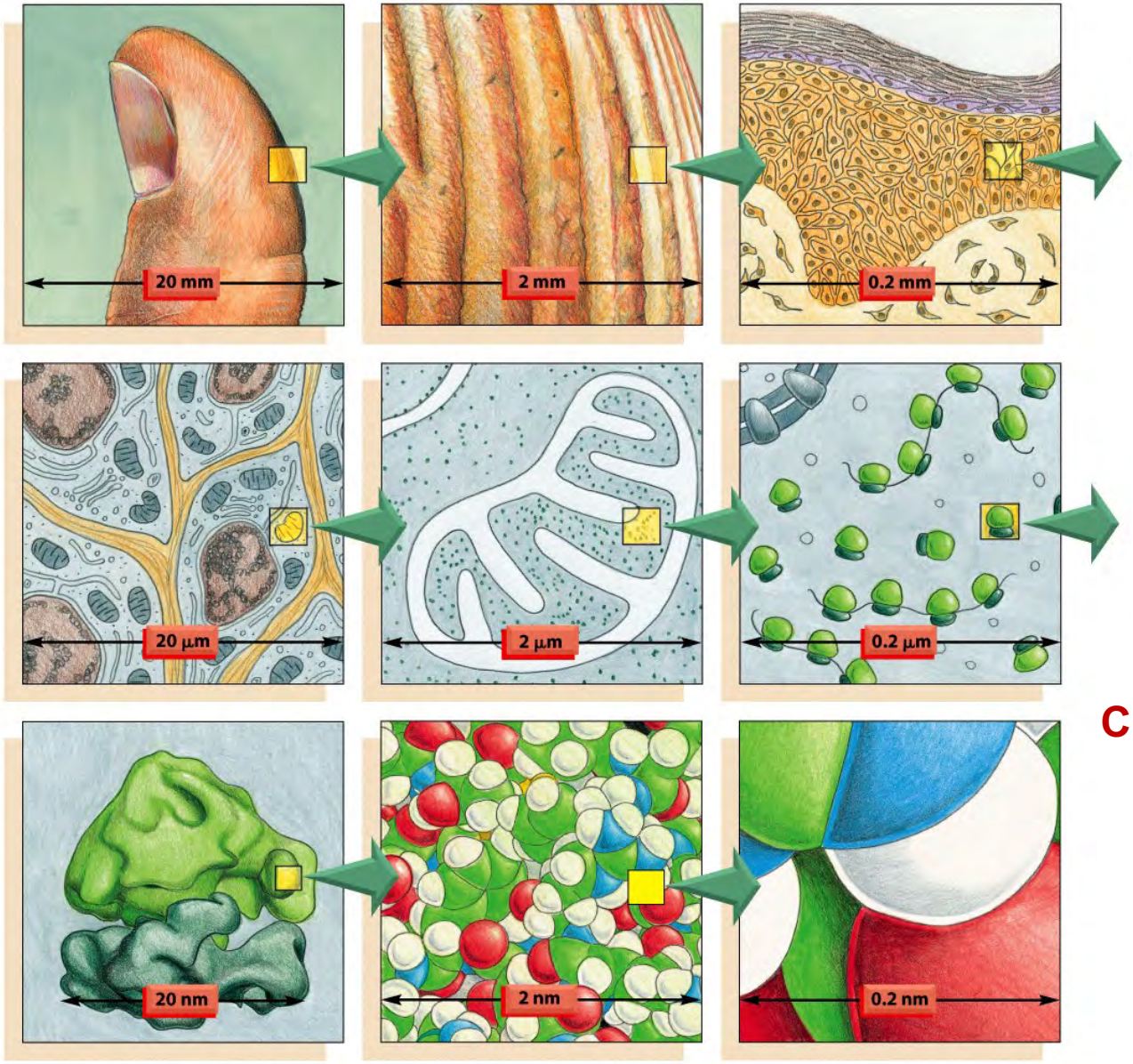
AWARDS

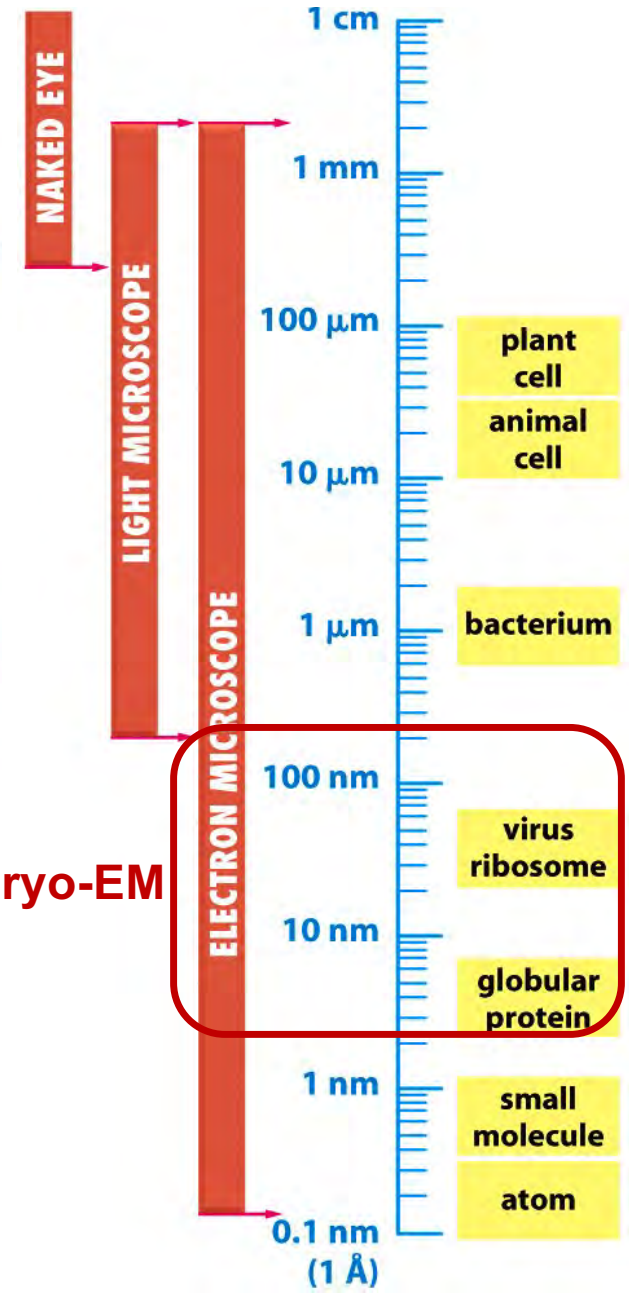
Molecular-imaging pioneers scoop Nobel

Chemistry prize hails work on cryo-electron microscopy.

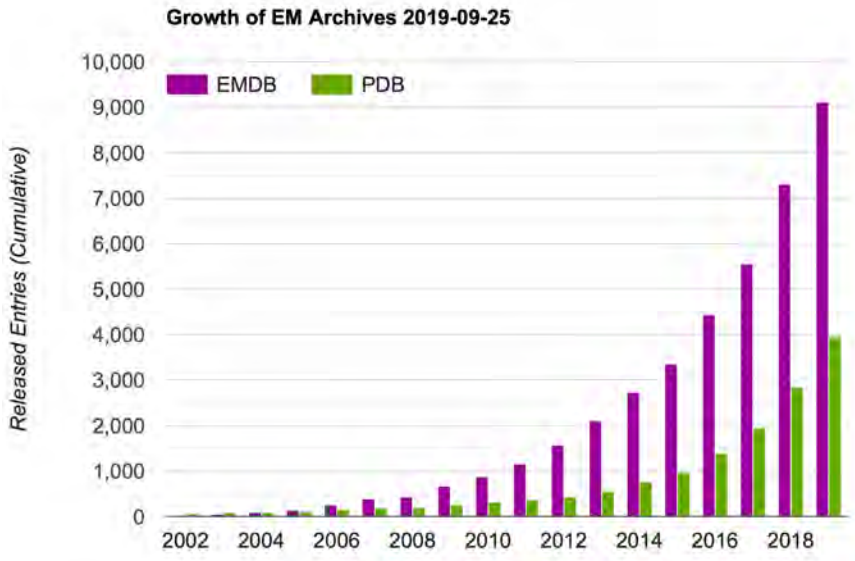
(Cressey & Callaway, Nature, 2017)

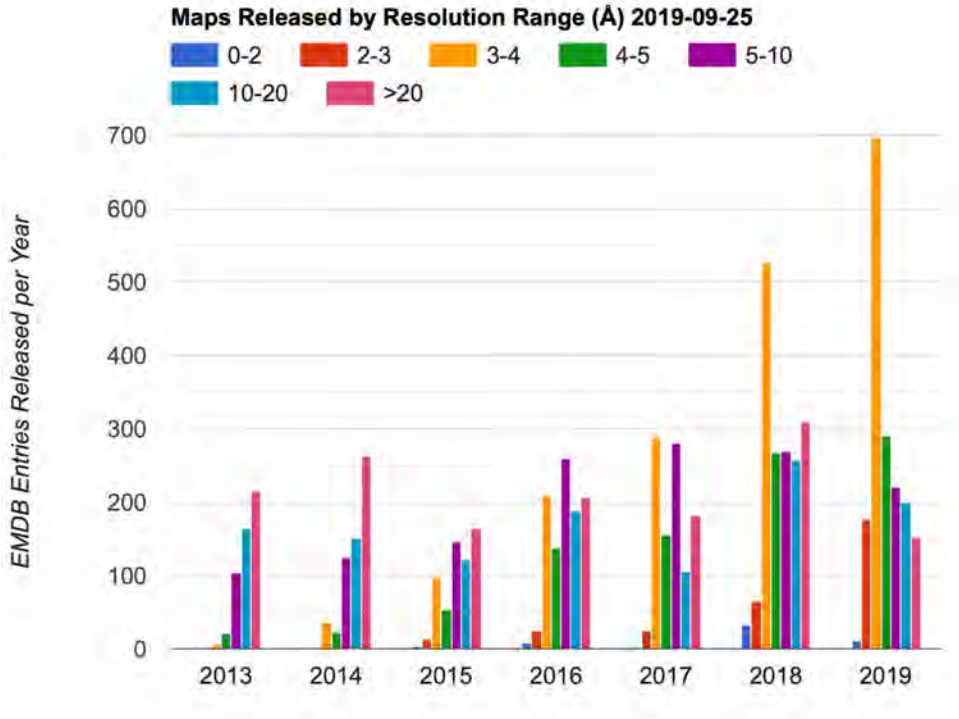
Cryo-EM: Method of the Year 2015


20 mm
2 mm
0.2 mm
20 µm
2 µm
0.2 µm
20 nm
2 nm
0.2 nm


NAKED EYE
LIGHT MICROSCOPE
ELECTRON MICROSCOPE
1 cm
1 mm
100 µm
10 µm
1 µm
100 nm
10 nm
1 nm
0.1 nm
(1 Å)
plant cell
animal cell
bacterium
virus
ribosome
globular protein
small molecule
atom
Cryo-EM

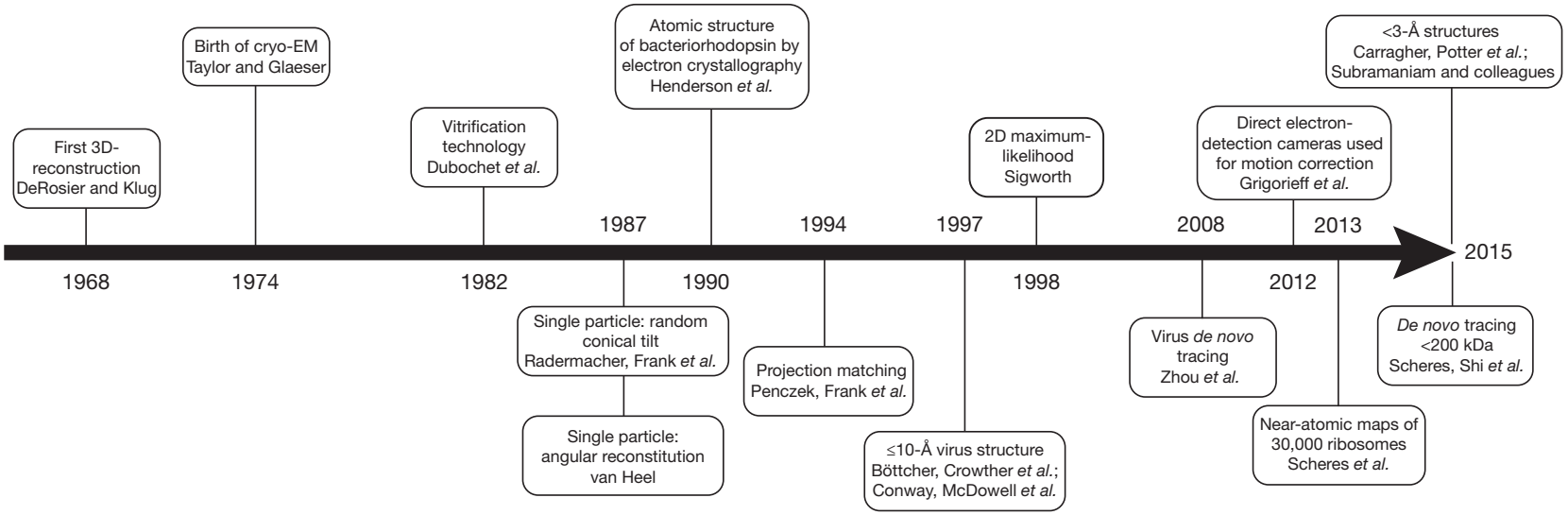
Statistics





(EMDB: Electron Microscopy Data Bank; PDB: Protein Data Bank)

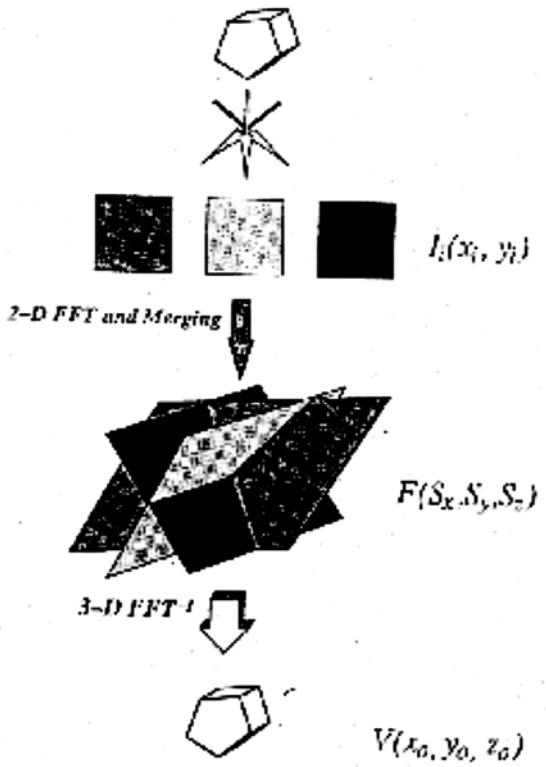
Milestones: Timeline



(Eva Nogales, Nat Methods, 2016)

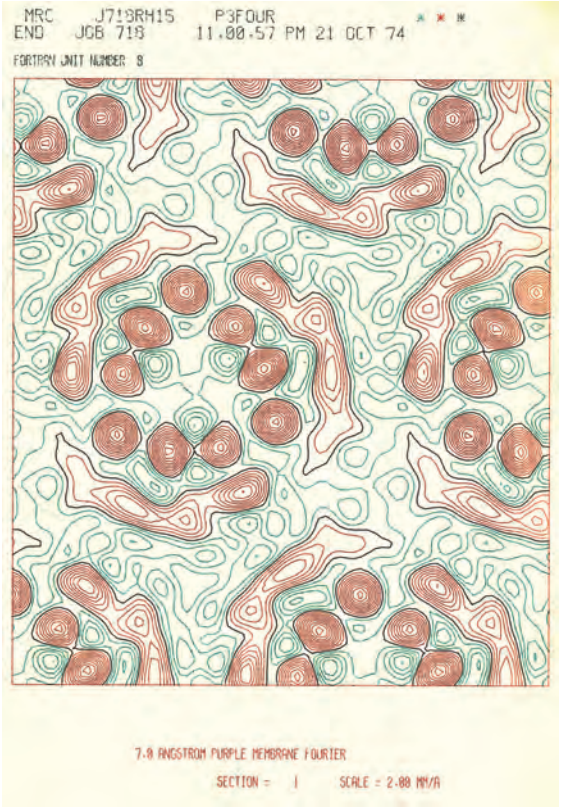
Milestones: 3-D Reconstruction

Principle



(Amos et al, Prog Biophys Mol Biol, 1983)

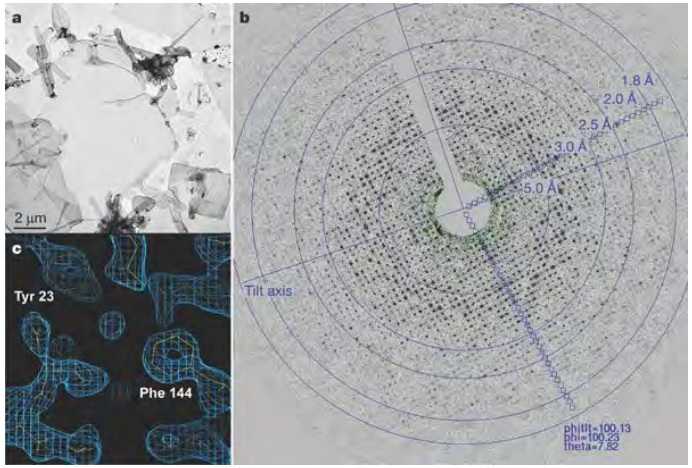
First Report (7 Å, 1974)



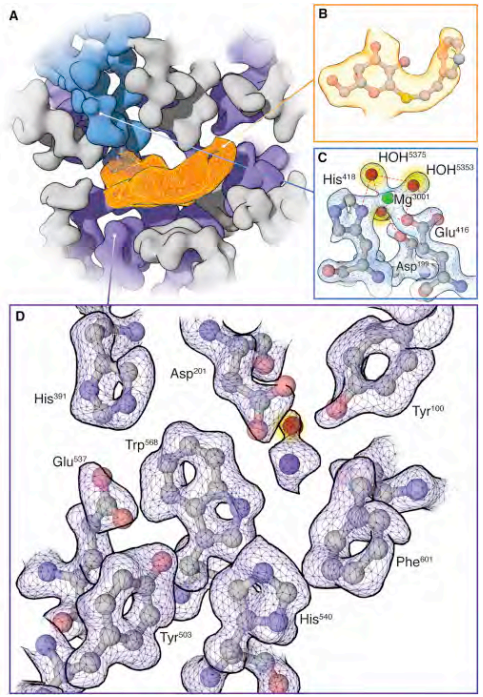
(Unwin & Henderson, J Mol Biol, 1975)

Milestones: 3-D Reconstruction

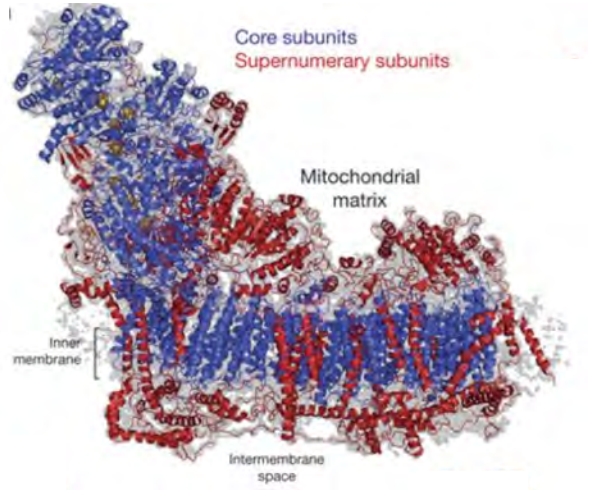
Higher Resolution (~2Å) and Bigger Molecules/Complexes



Aquaporin (1.9 Å)
(Gonen et al, Nature, 2005)



β-Galactosidase (1.5 Å)
(Bartesaghi et al, Structure, 2018)



Mitochondrial Complex I (4.2 Å)
(Zhu et al, Nature, 2016)

Cryo-EM Landscape in Canada

uOttawa

Outline

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  (previously under-appreciated)
- **Instrumentation**

- Optics and imaging

- Sub-branches of molecular cryo-EM

- Contrast transfer function (CTF)

Transmission Electron Microscopy

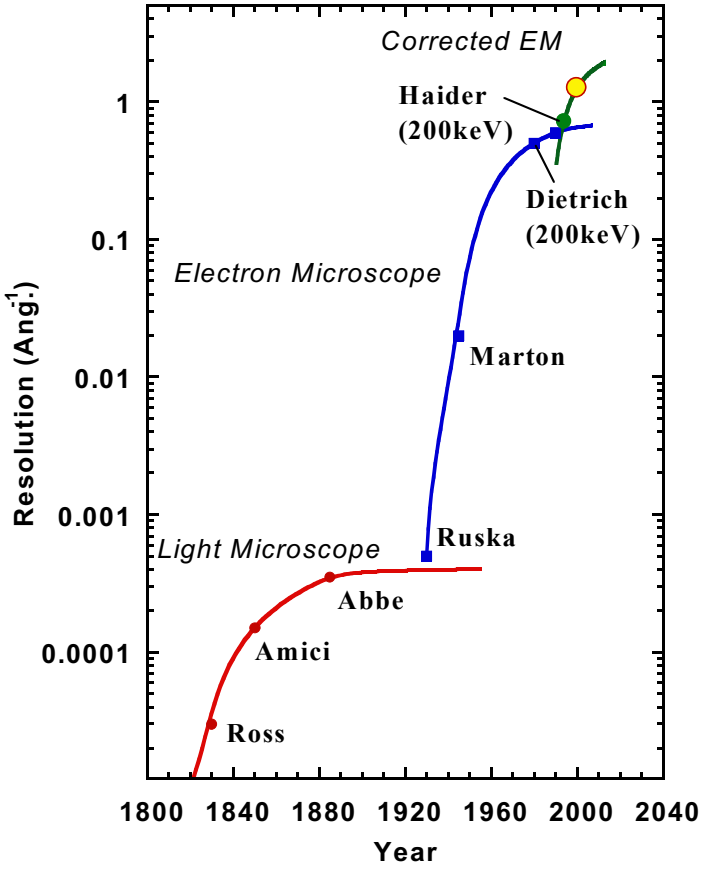
Ruska's First Electron Microscope, 1931

3 MV TEM-Toulouse, France, 1960

FEI Titan Krios, 2008

JEOL JEM-Z300FSC (CRYO ARM 300), 2017

Transmission Electron Microscopy



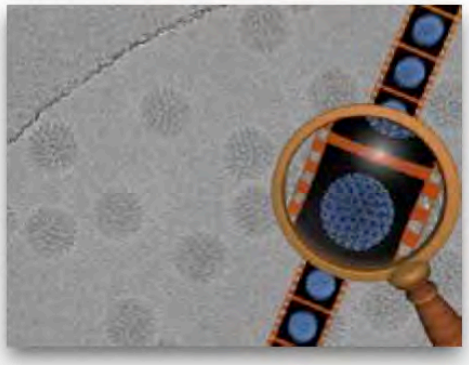
1897	J. J. Thompson	Discovers the electron
1924	Louis deBroglie	Identifies a wavelength to moving electrons (λ = h/mv)
1926	H. Busch	Magnetic or electric fields act as lenses for electrons
1929	E. Ruska	Ph. D thesis on magnetic lenses
1931	Knoll & Ruska	First electron microscope built
1931	Davisson & Calbrick	Properties of electrostatic lenses
1934	Driest & Muller	Surpass resolution of the LM
1938	von Borries & Ruska	First practical EM (Siemens) - 10 nm resolution
1940	RCA	Commercial EM with 2.4 nm resolution
1941	1.0 nm resolution	
~1970	HRTEM with resolution better than 4 Å	
1982	Nobel prize for A. Klug	
1986	Nobel prize for E. Ruska	
2003	Sub-Å resolution with aberration correction, monochromators	
2017	Nobel prize for Dubochet/Frank/ Henderson	

Pushing the Resolution Boundary

Hardware

Microscopes

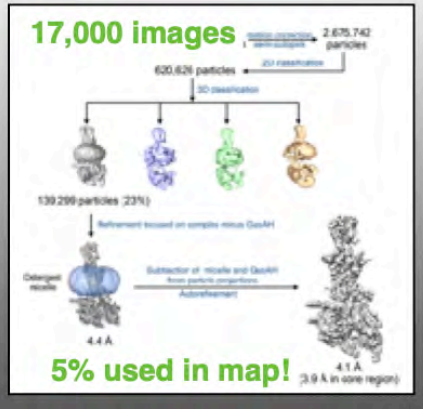
Direct Detectors



Computers

2012->2017
Cost reduced by 100x

Software



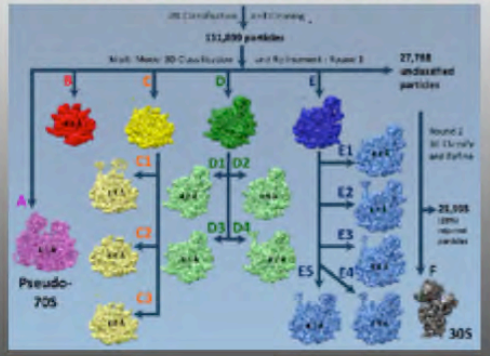
Leginon / SerialEM / EPU, …

MotionCorr2, Unblur, …

RELION, FREALIGN/cisTEM, cryoSPARC
EMAN, Sparx, SPHIRE, XMIPP, …

- **Automation**
- **Motion correction**
- **Image reconstruction algorithms**

14 independent structures



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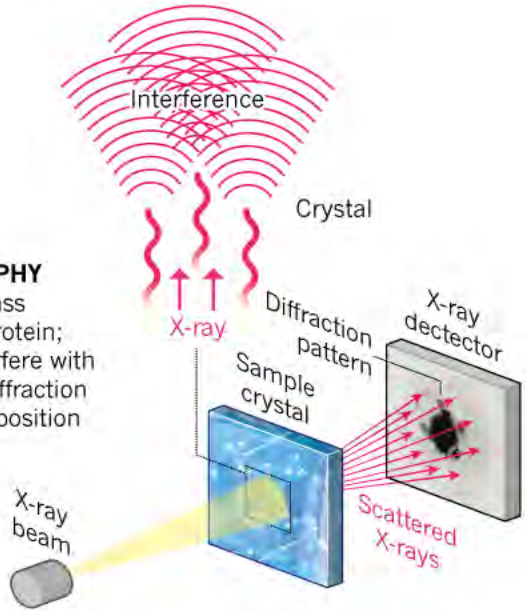
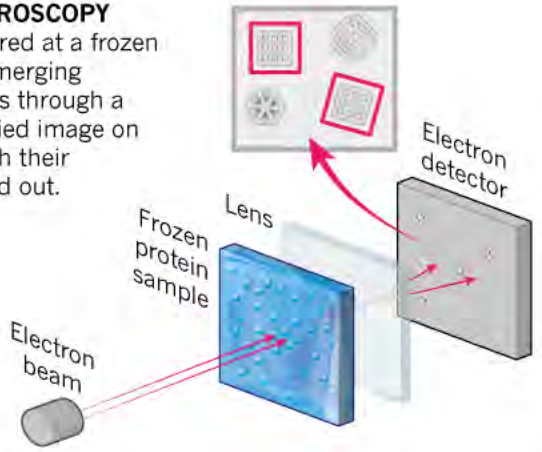
Optical & X-ray Diffractions

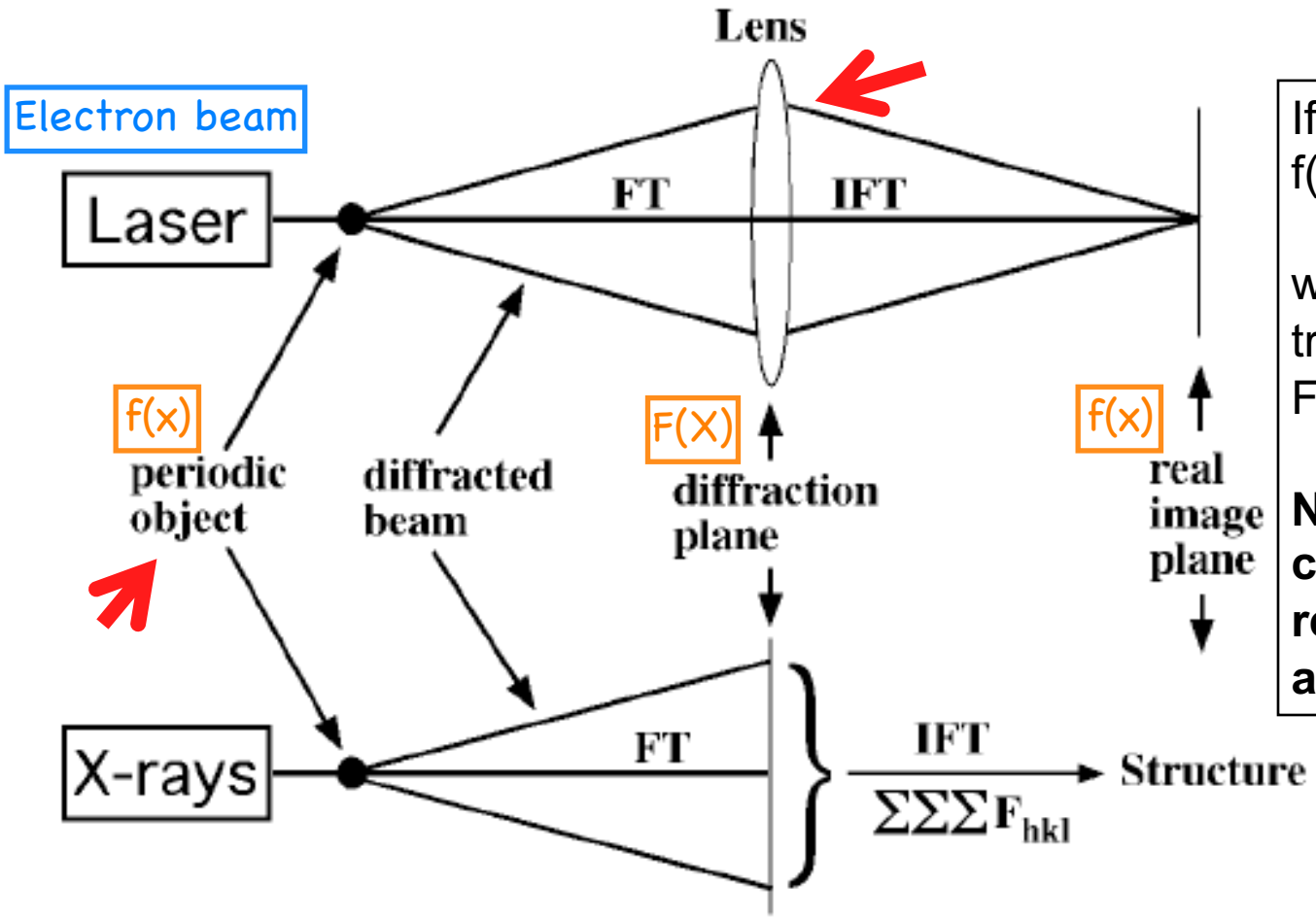
X-RAY CRYSTALLOGRAPHY
X-rays scatter as they pass through a crystallized protein; the resulting waves interfere with each other, creating a diffraction pattern from which the position of atoms is deduced.



CRYO-ELECTRON MICROSCOPY
A beam of electron is fired at a frozen protein solution. The emerging scattered electrons pass through a lens to create a magnified image on the detector, from which their structure can be worked out.



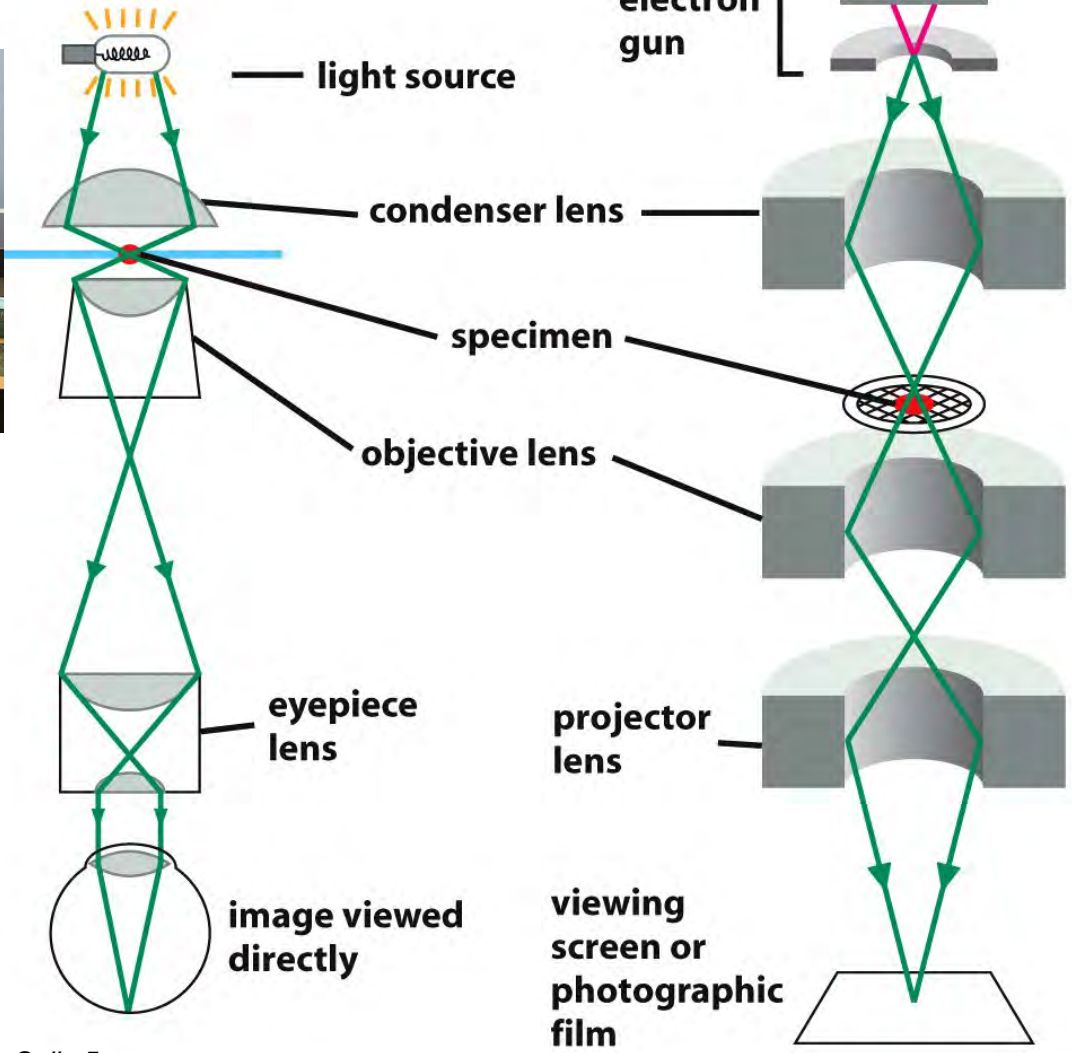
Optical & X-ray Diffractions



If F(X)=FT[f(x)], then f(x)=IFT[F(X)]

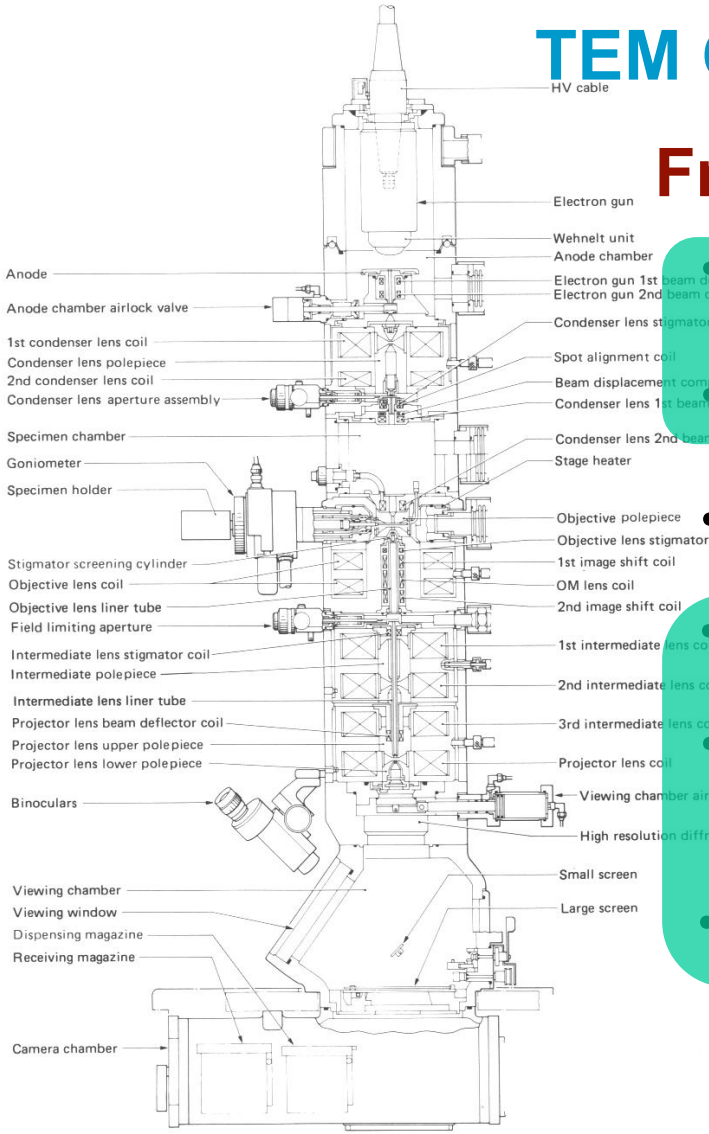
where FT=Fourier transform & IFT=Inverse Fourier transform.

Note: important in X-ray crystallography and 3D reconstruction algorisms.


light source
condenser lens
specimen
objective lens
eyepiece lens
image viewed directly
electron gun
projector lens
viewing screen or photographic film

Why do we need to know optics?

TEM Column



From Top to Bottom:

- Electron gun
- Condenser lens(es)

illumination system

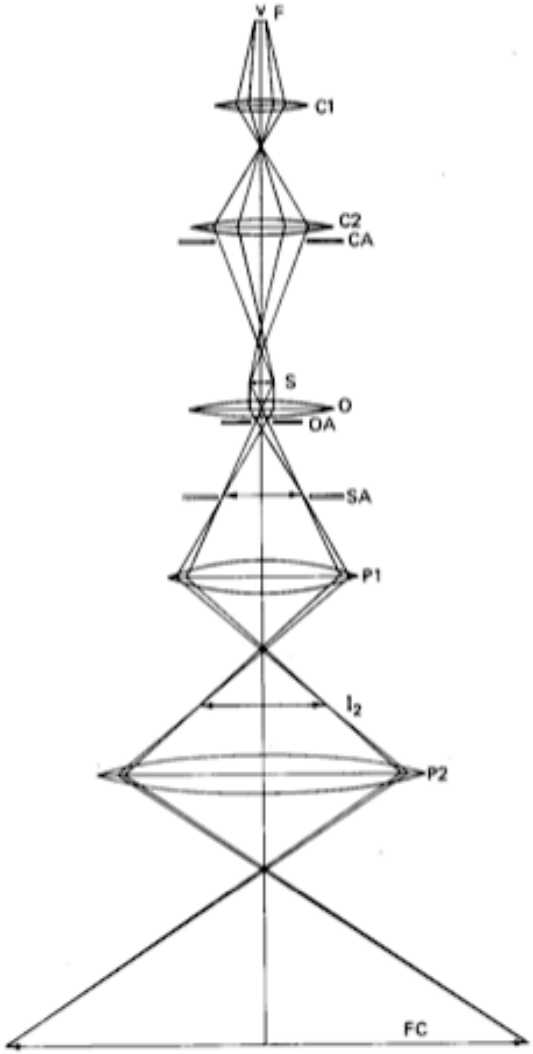
- Specimen stage
- Objective lens
- Intermediate/ Projector lenses

imaging system

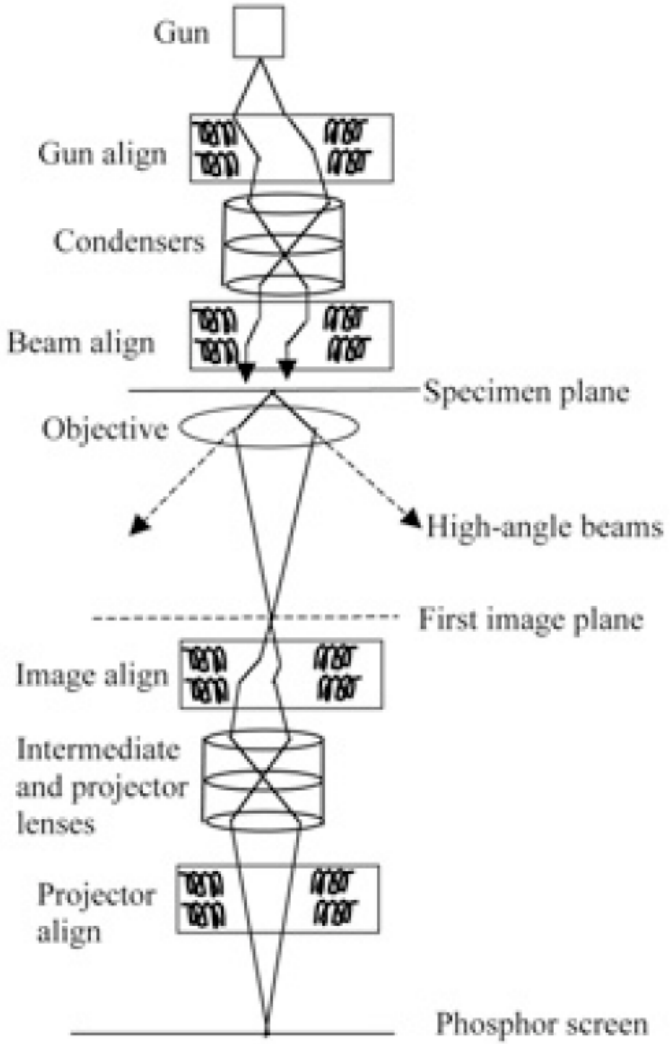
- Camera and viewing system

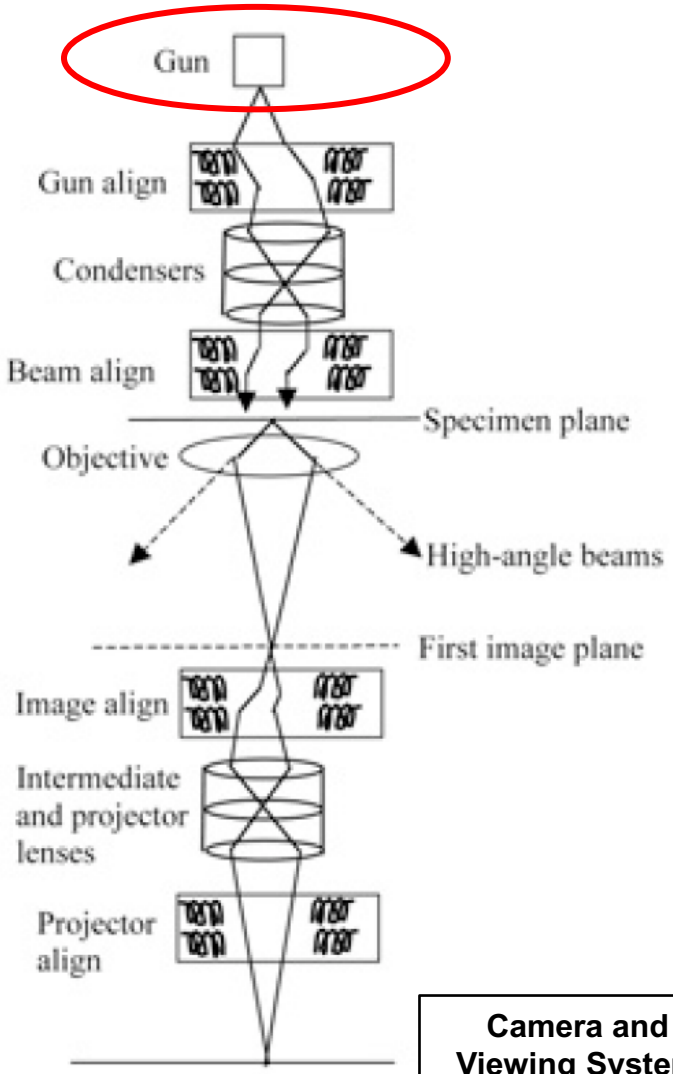
Why do we need to know optics?

Ideal instrument

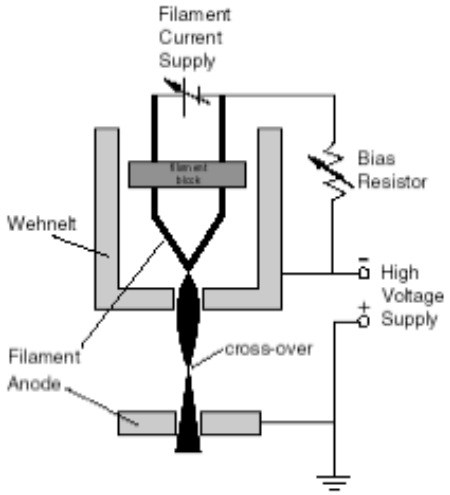


Real instrument



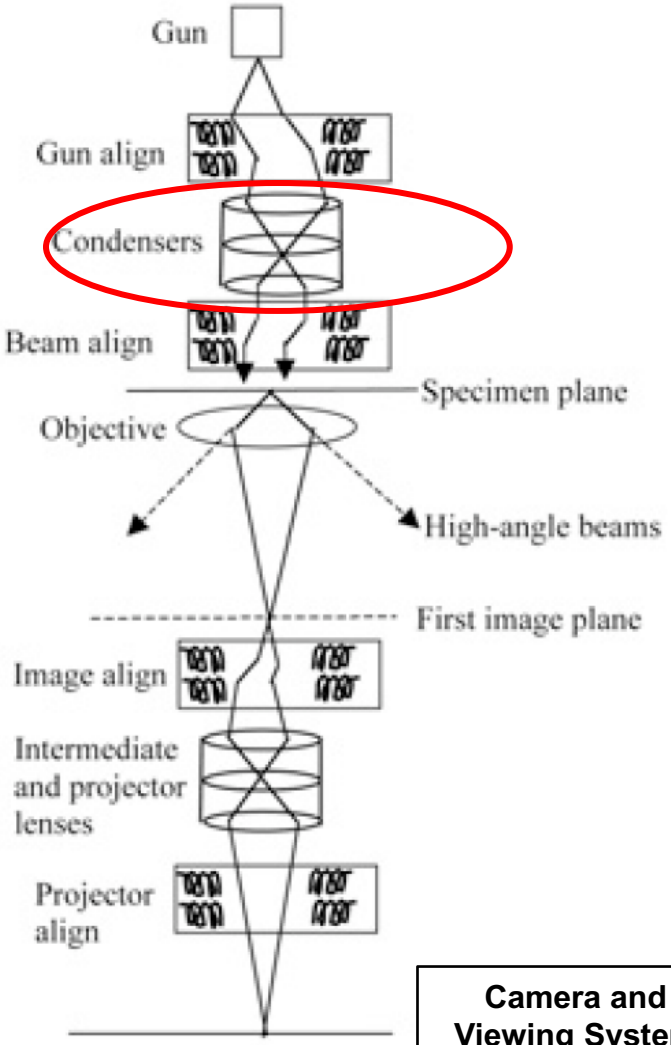


Electron Gun



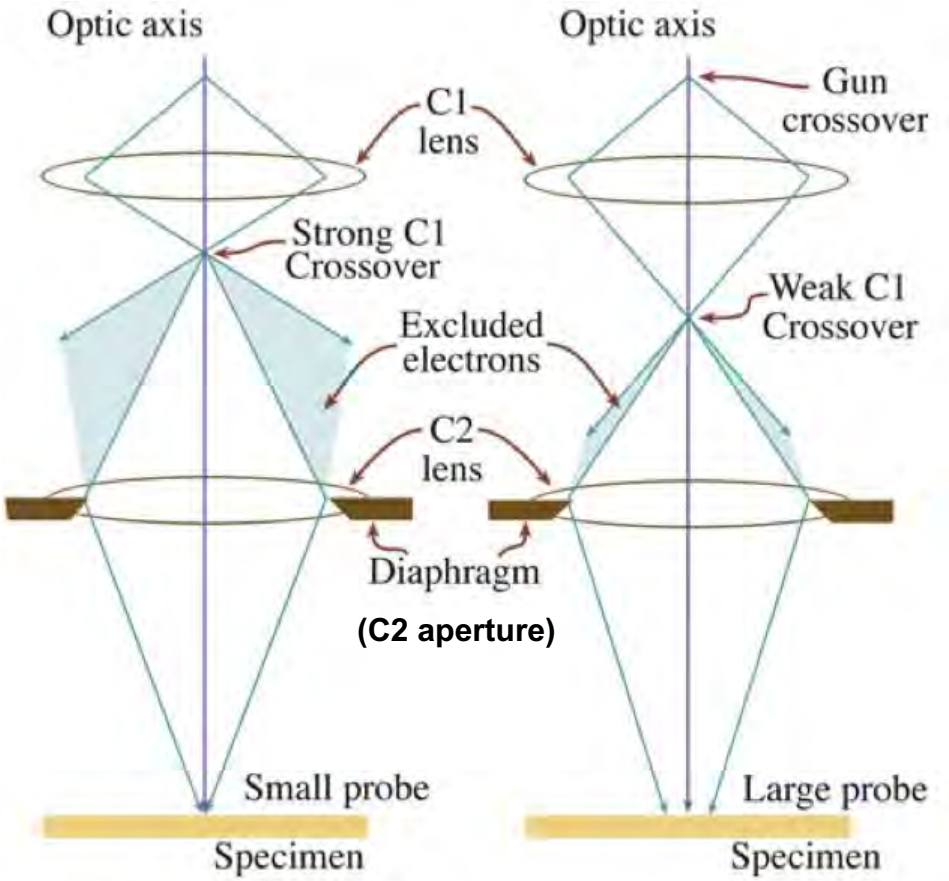
- Thermionic Emission Gun
  - Electrons are emitted from the heated filament.
  - Lanthanum Hexaboride (LaB6) Single crystal: ~1900K
  - Hairpin Tungsten (W) filament: ~2600K

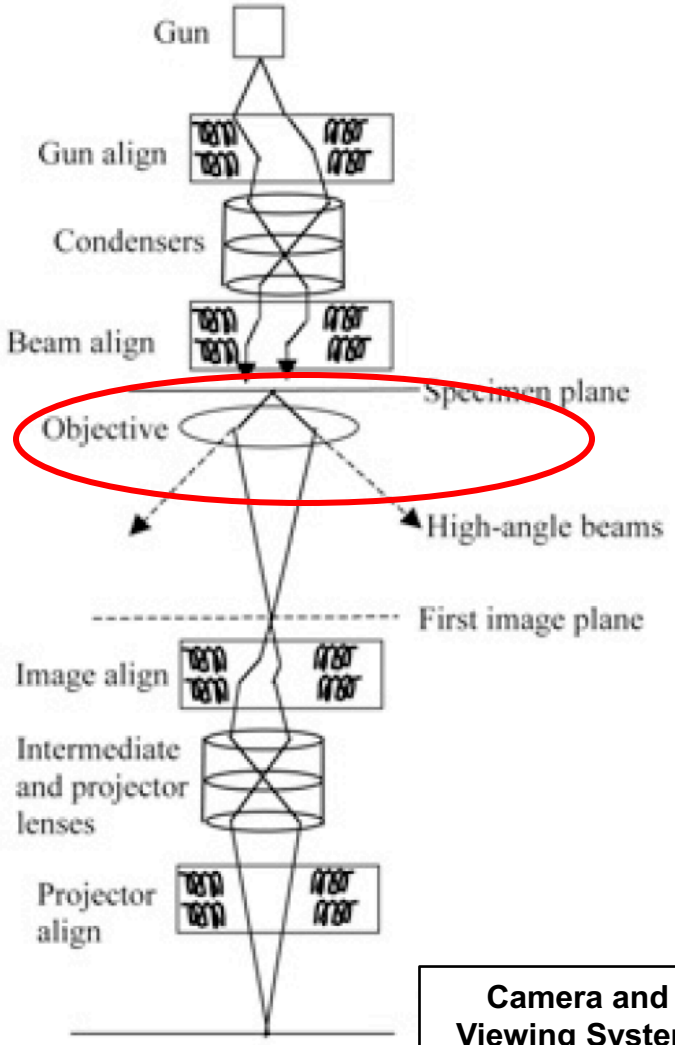
- Field Emission Gun **(high coherency)**
  - Electrons are extracted from the W emitter tip surface by the high electric field ~10V/nm.
  - Cold cathode type: room temperature
  - Schottky type: ~1800K



Camera and Viewing System

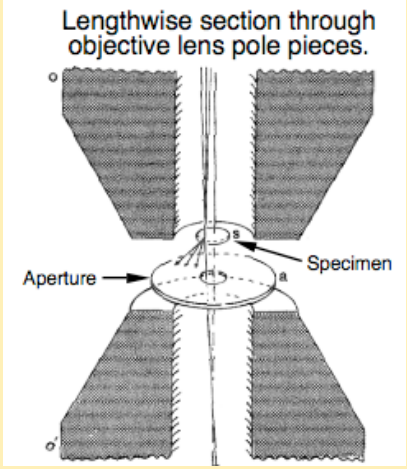
Condenser Lens



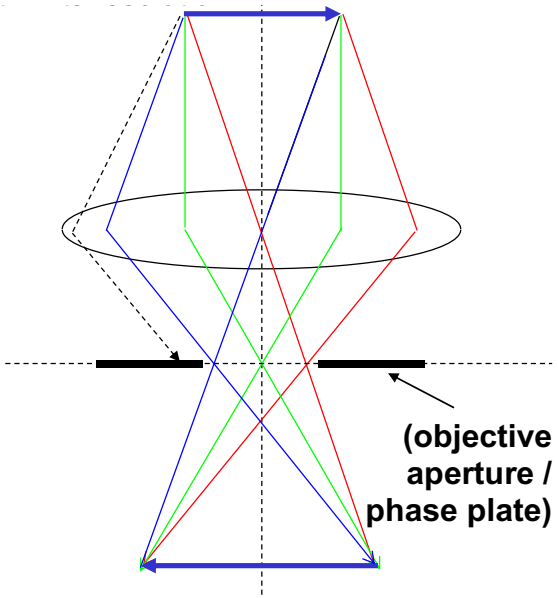


Camera and Viewing System

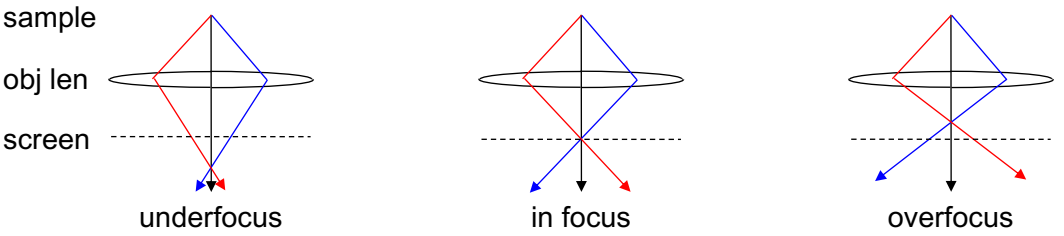
Objective Lens

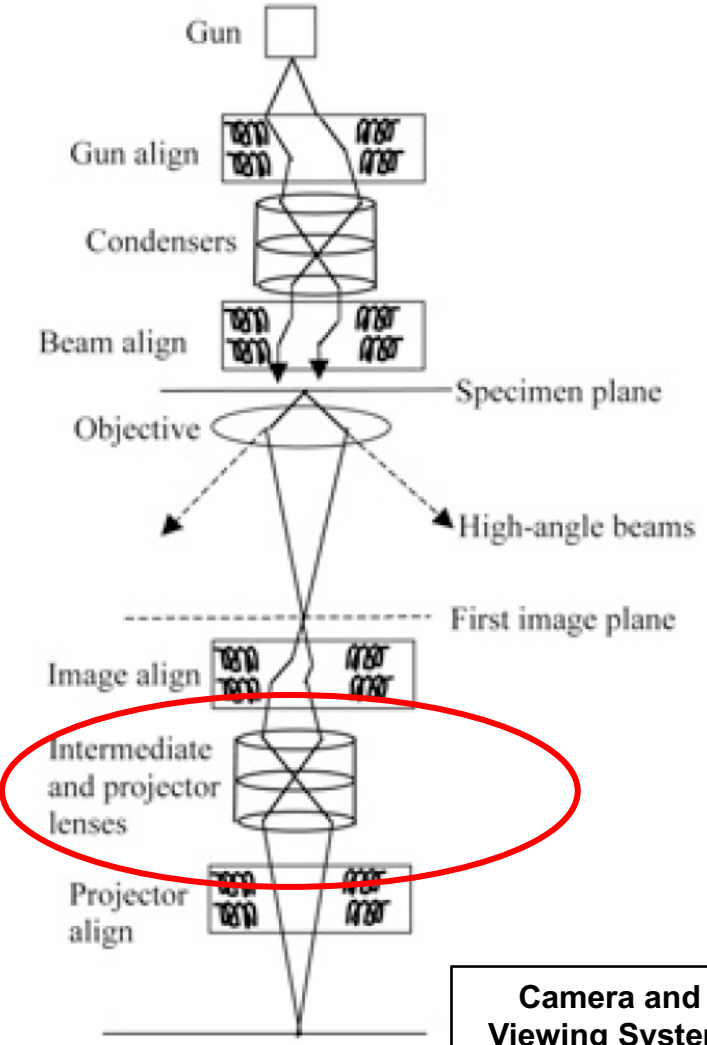


Wischnitzer 2nd ed., Fig. 51, p.60



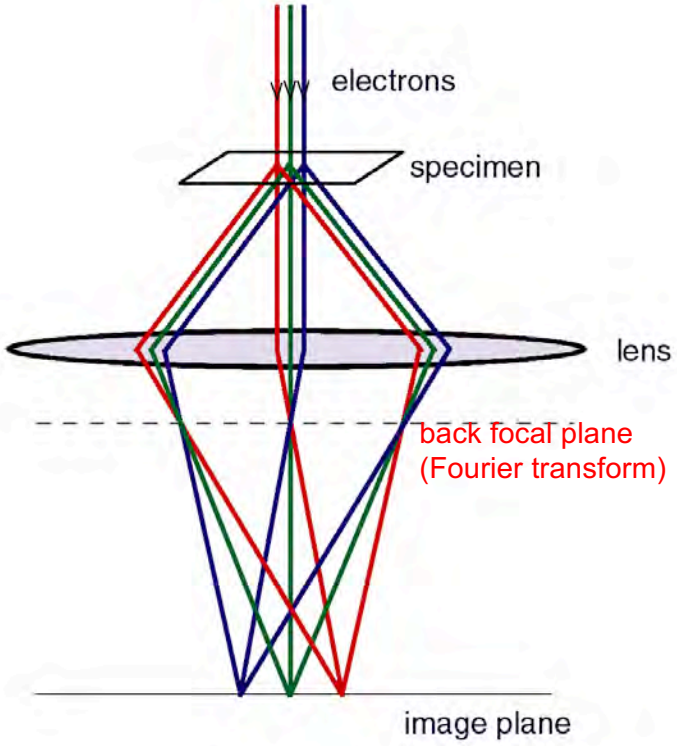
(objective aperture / phase plate)





Camera and Viewing System

Projector Lens (Fourier-transform lens)

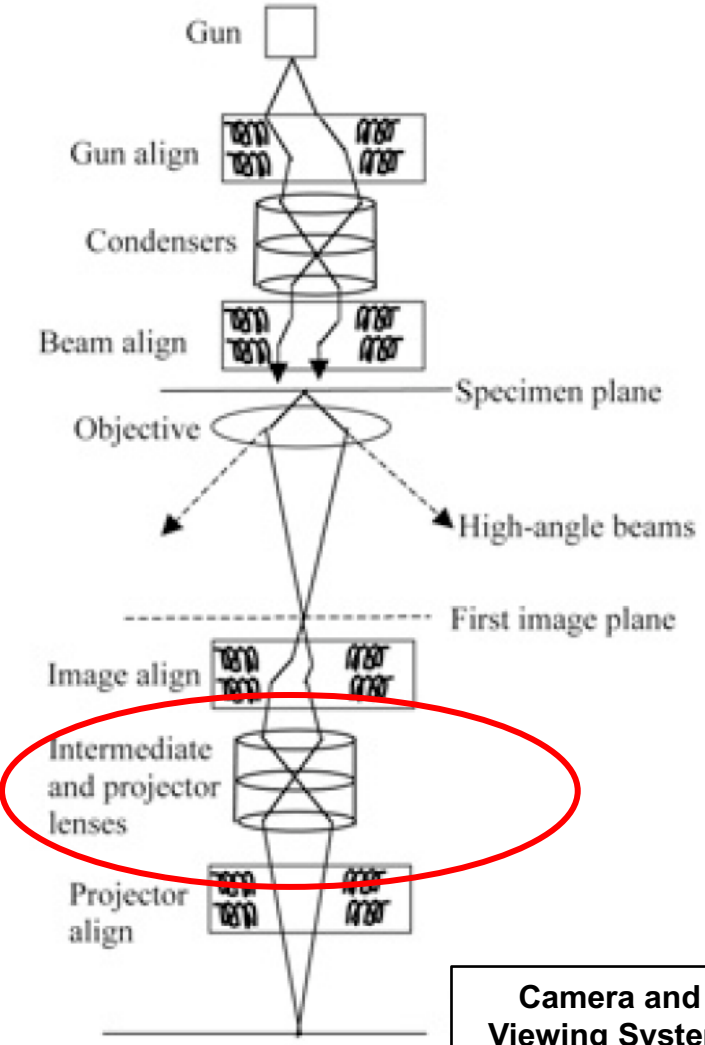


Diffraction = Fourier Transform

$$F(k_x, k_y) = \int_{-\infty}^{\infty}\int_{-\infty}^{\infty} f(x,y)e^{-j(k_x x + k_y y)}dxdy$$

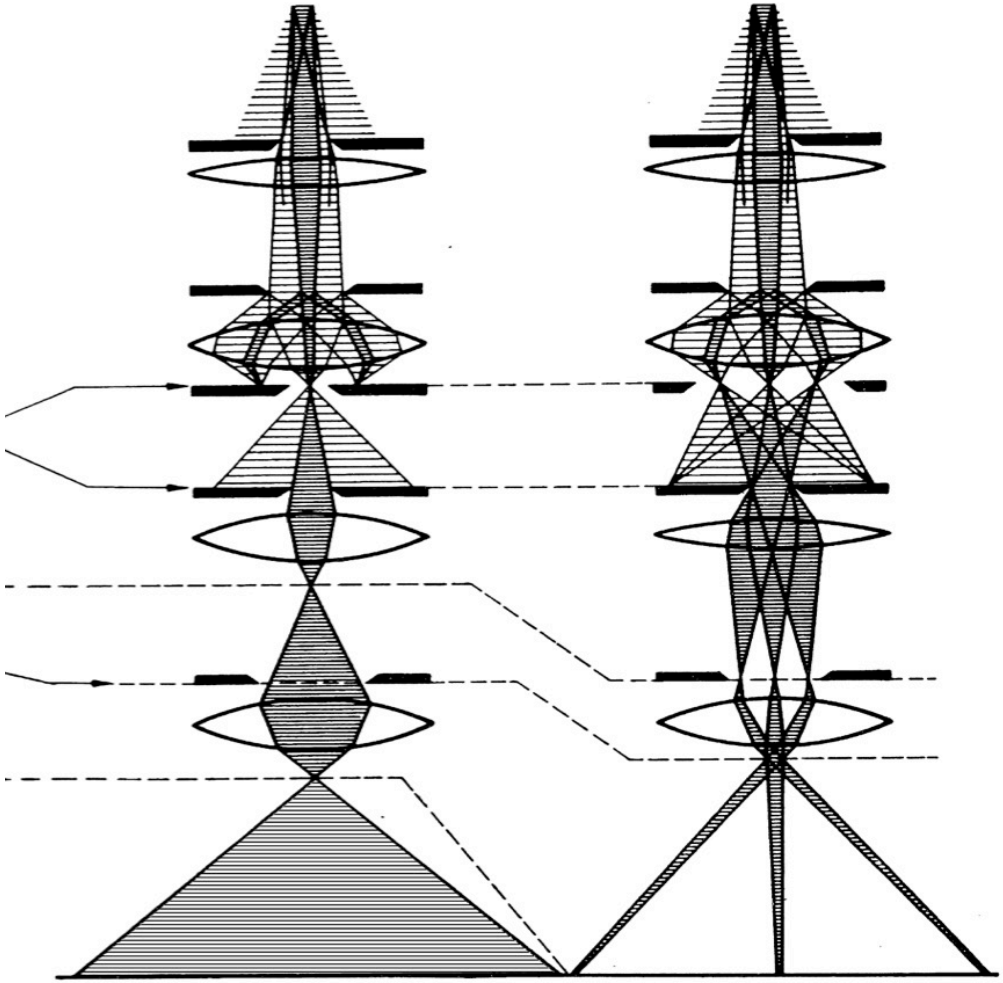
Inverse Fourier Transform

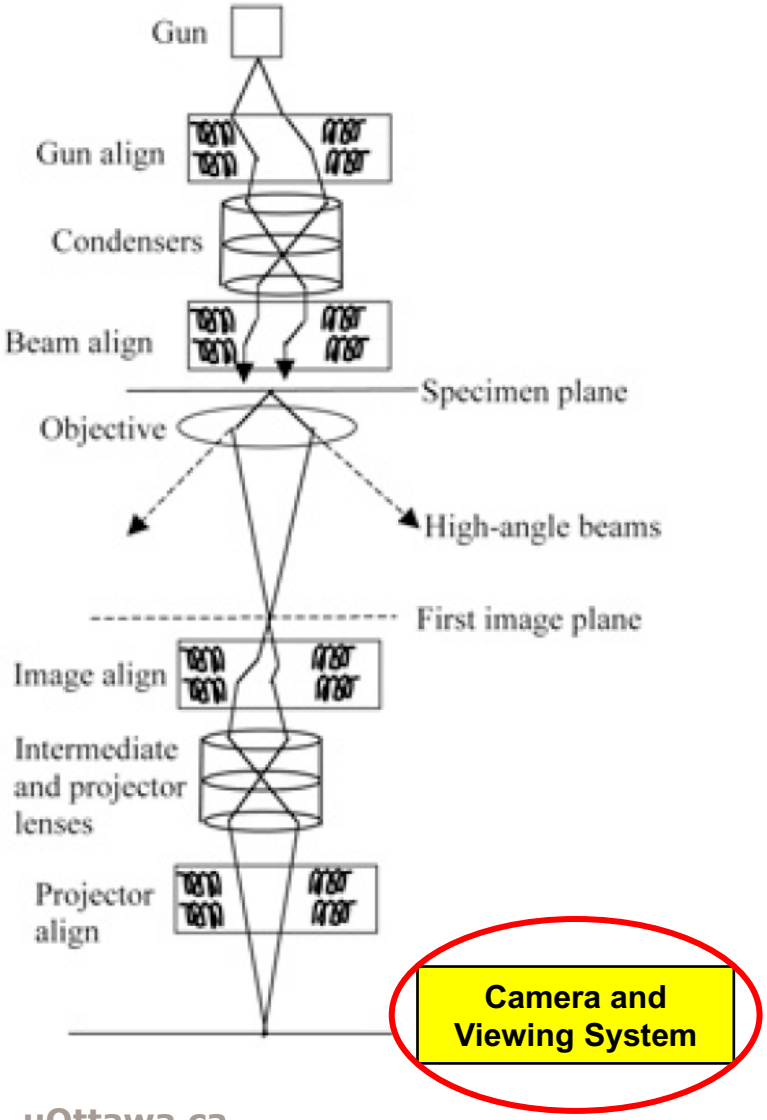
$$f(x,y) = \frac{1}{(2\pi)^2}\int_{-\infty}^{\infty}\int_{-\infty}^{\infty} F(k_x,k_y)e^{j(k_x x + k_y y)}dk_x dk_y$$



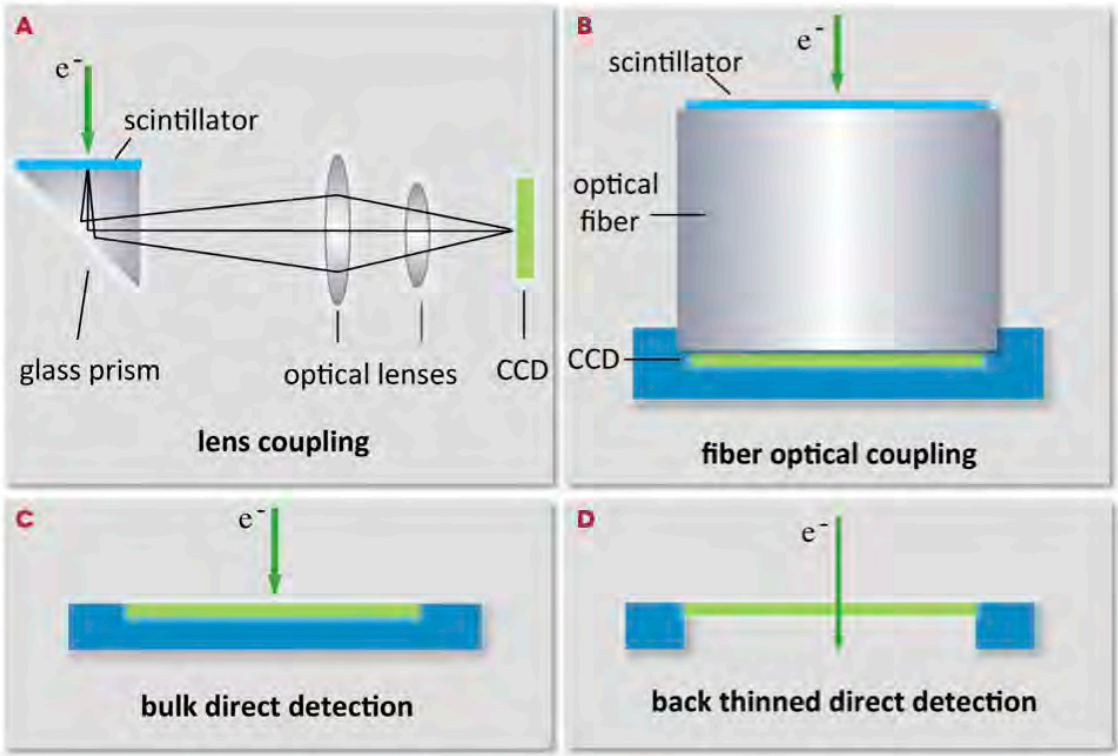
Camera and Viewing System

Electron Diffraction and Bright-field Imaging





Phosphor Screen or Digital Detectors



Direct Electron Detectors

$$\mathrm{DQE}(u) = \frac{\mathrm{SNR}^2_{out}(u)}{\mathrm{SNR}^2_{in}(u)}.$$

Direct Electron Detection

- Type: Gatan (K2/K3), Direct Electron (DE20/64), FEI (Falcon II/III) are the major vendors
- Size: 4k x 4k → 8k x 8k
- Advantages:
  - High quality signal (equal or better than film).
  - Integration mode → counting mode
  - Fast readout (25 to 1000 frames per second)
- Disadvantages:
  - Expensive ($200k to $600k)
  - Radiation damage (i.e. limited life time)

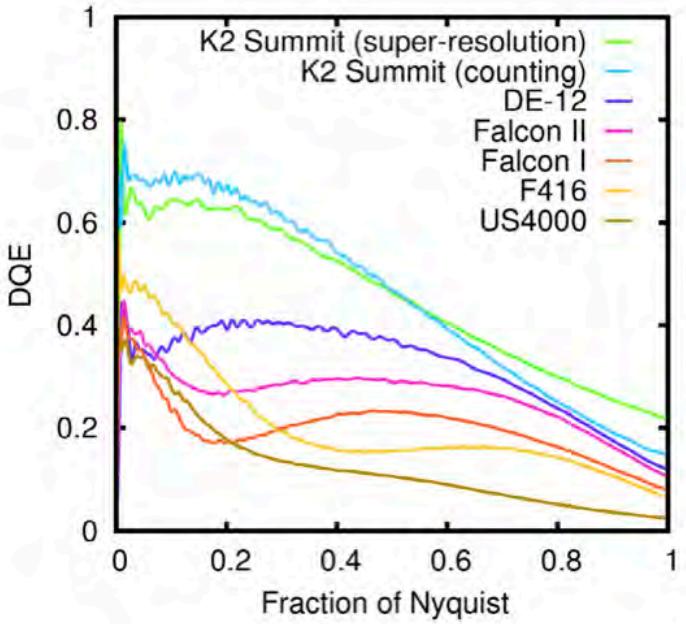
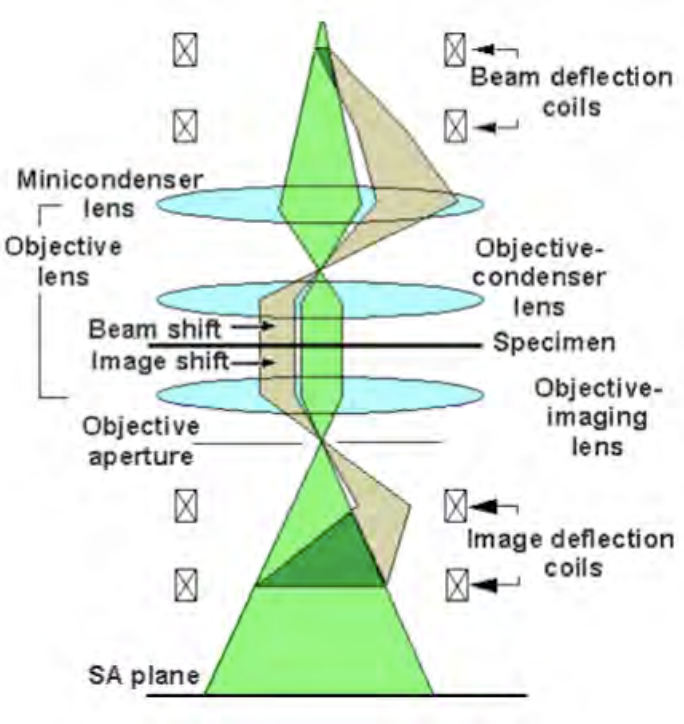
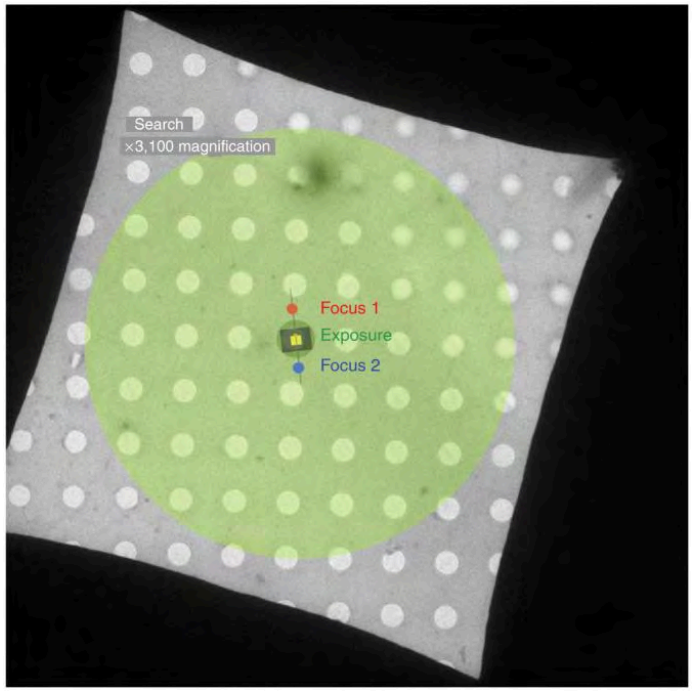


Fig.3. DQE of detectors at 200 kV. The DEDs outperform scintillator-based detectors. The dose rates used were: K2 Summit in super-resolution mode – 4 electrons/pixel/s (this value refers to physical pixels); K2 Summit in simple counting mode – 3 electrons/pixel/s; DE-12 – 13 electrons/pixel/s with a frame rate of 25 frames/s; Falcon I (Brandeis) – 6 electrons/pixel/s; Falcon II (Brandeis) – 10 electrons/pixel/s; F416 – 50 electrons/pixel/s; US4000 – 40 electrons/pixel/s.

(Ruskin et al, J Struct Biol, 2013)

Low-Dose Imaging





Grassucci … Frank. Nat Protoc 2008;3(2):330–339

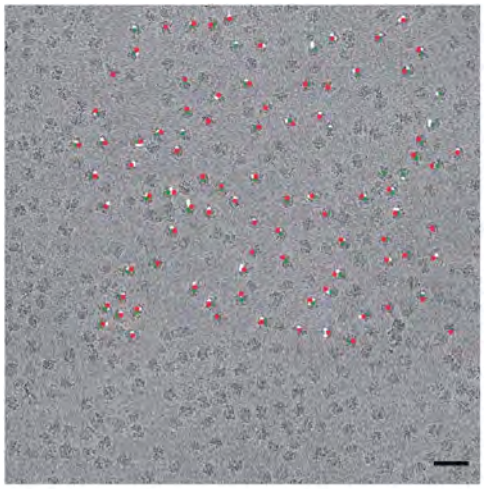
- Search Mode: Low mag, low dose rate 10^{-3} e/Å^2/sec
- Focus Mode: High dose in an adjacent area at chosen magnification
- Photo Mode: Defocus at the desirable value for an anticipated resolution. Use a total dose of ~20-50 e/Å^2

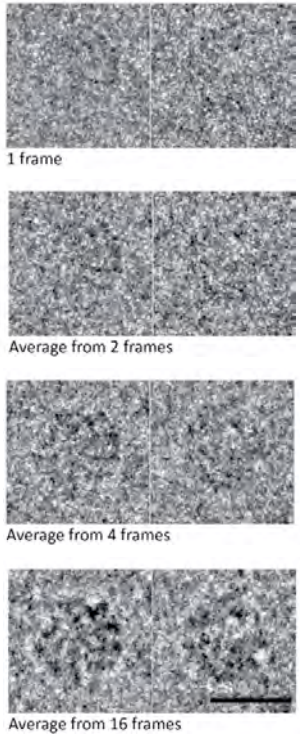
Outline

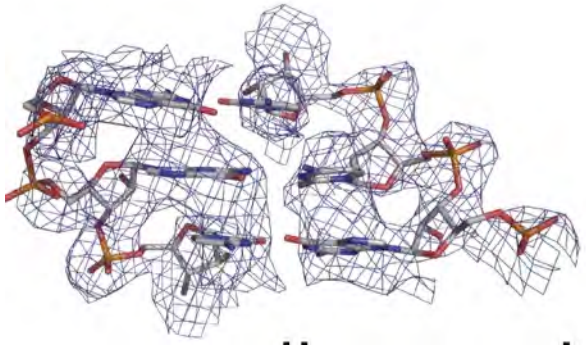
- Cryo-EM: a new poster child
  (previously under-appreciated)
- Instrumentation
- Optics and imaging
- **Sub-branches of molecular cryo-EM**
- Contrast transfer function (CTF)

Single-Particle Analysis (SPA)

Samples



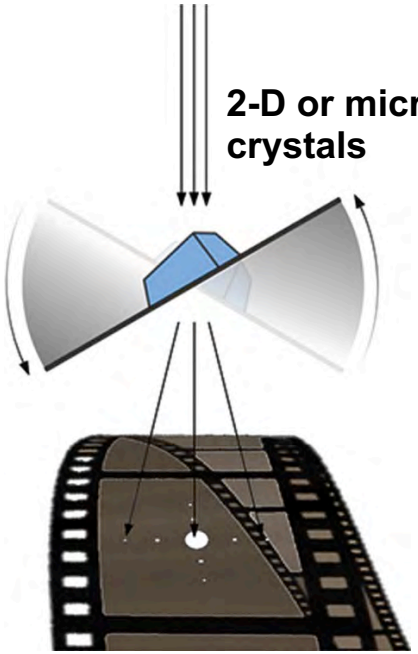


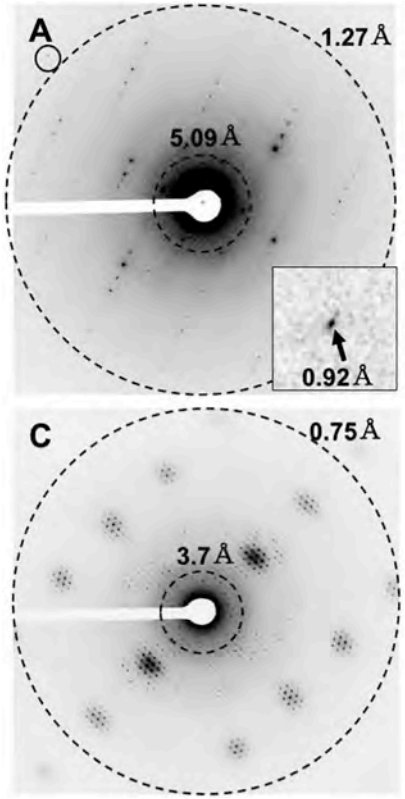


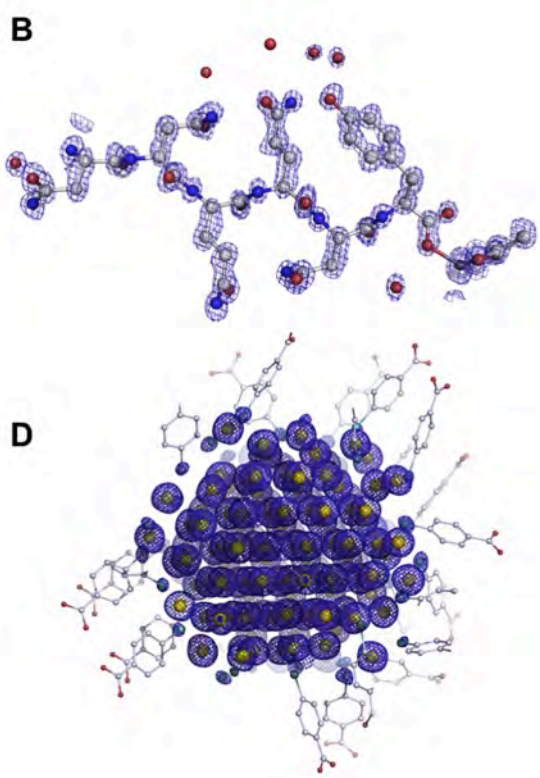
(Bai et al, eLife, 2013)

Microelectron Diffraction (MicroED) & Electron Crystallography

Samples

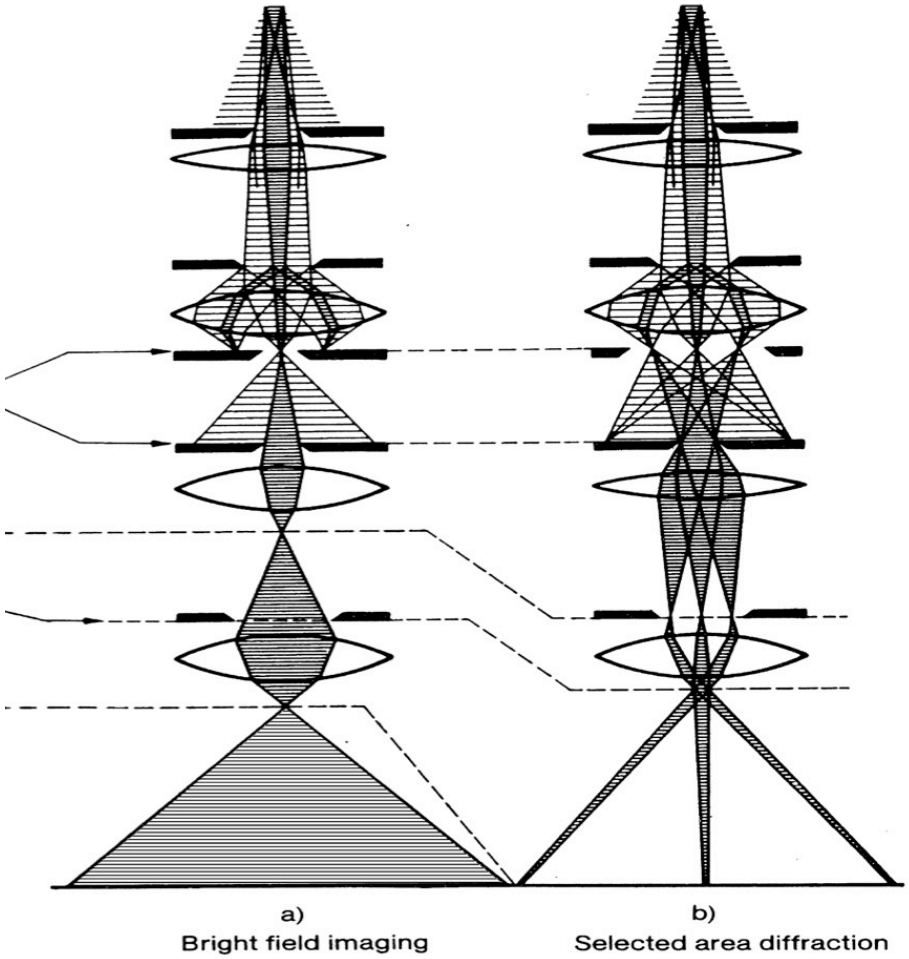






(Martynowycz & Gonen, Curr Opin Colloid Interf Sci, 2018)

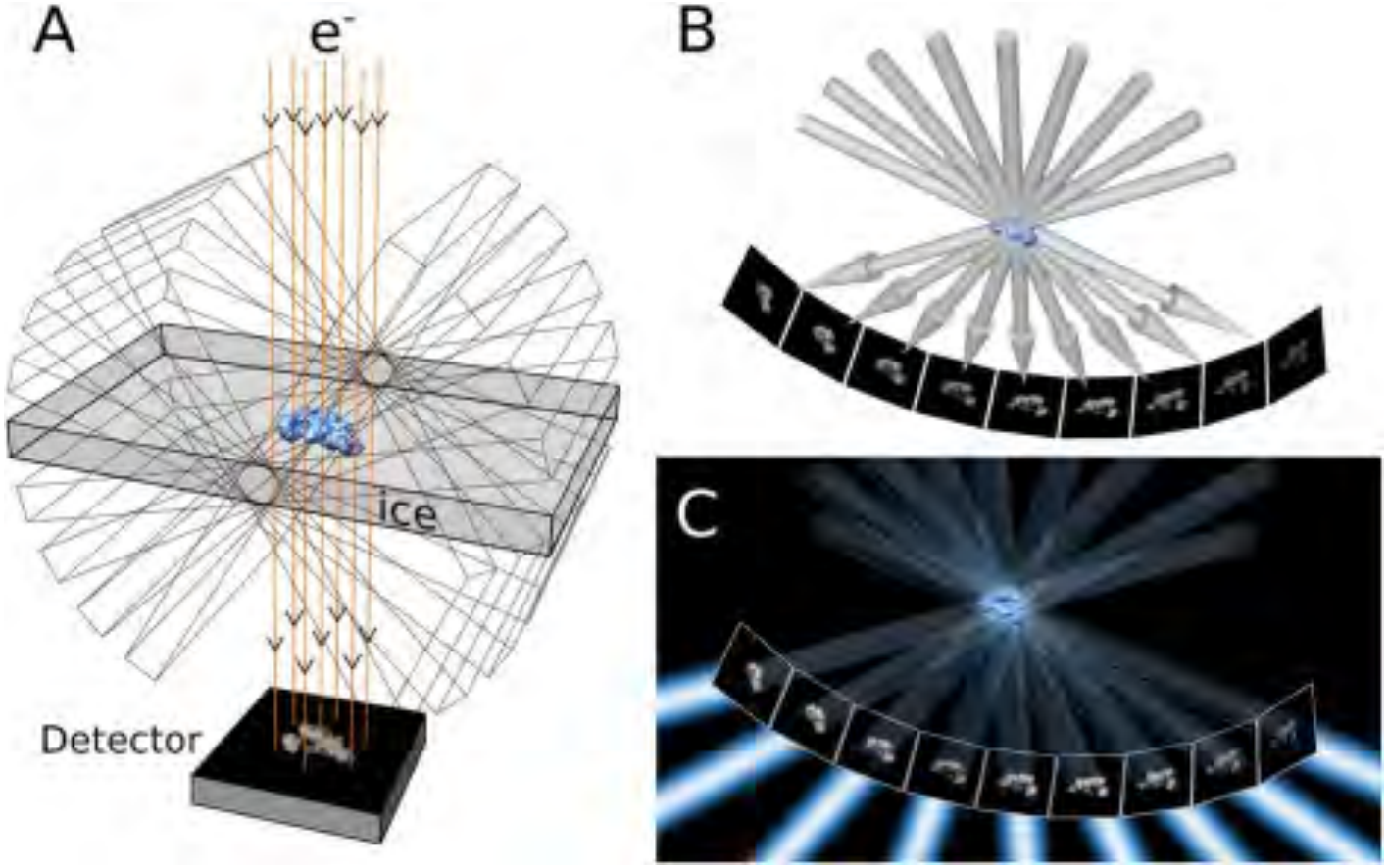
Microelectron Diffraction (MicroED) & Electron Crystallography



MicroED: Runner-up (Science's Breakthrough of the Year 2017)

Cryo-Electron Tomography (cryo-ET)

Samples



(Koning et al, Ann Anat, 2018)

Outline

- Cryo-EM: a new poster child
  (previously under-appreciated)
- Instrumentation
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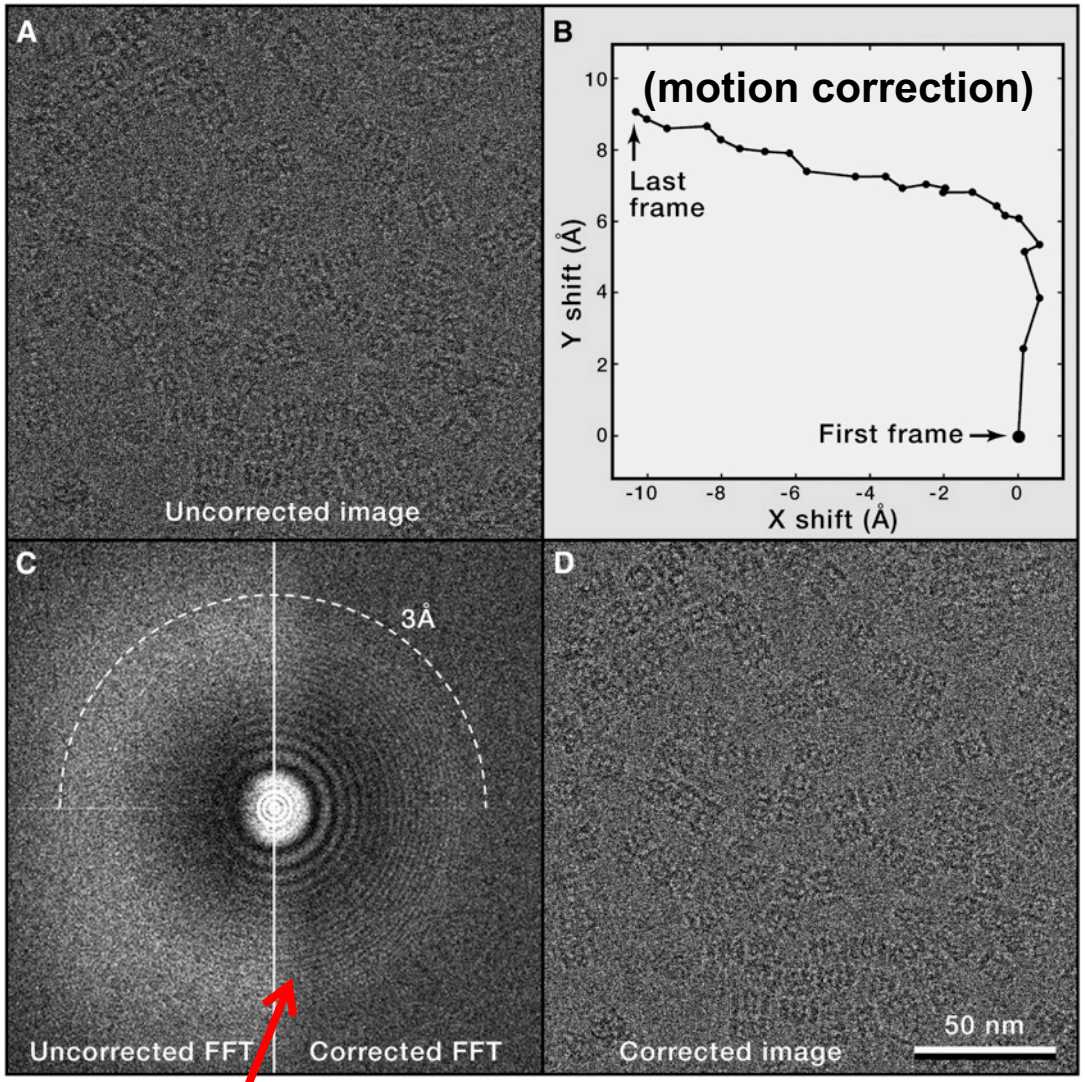
- **Contrast transfer function (CTF)**

Image film

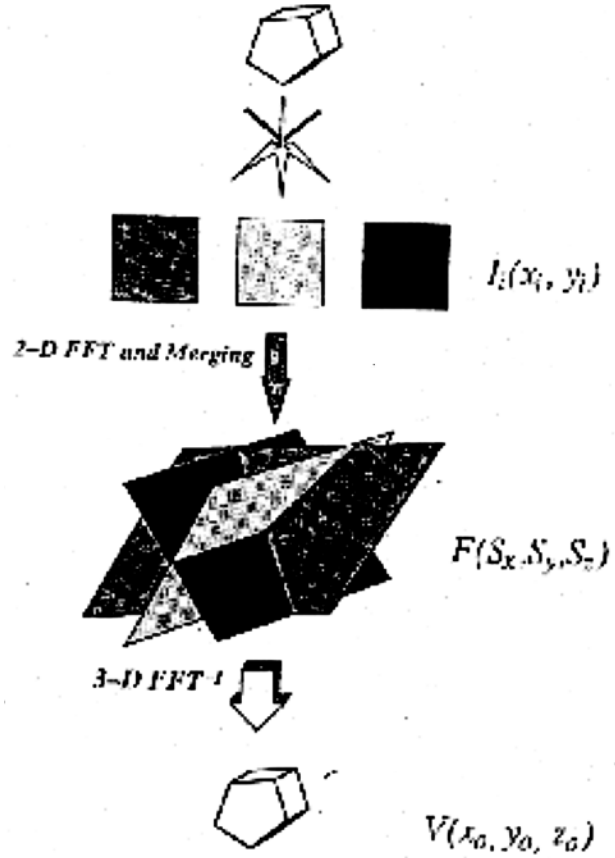
Power spectrum (Computed diffraction)

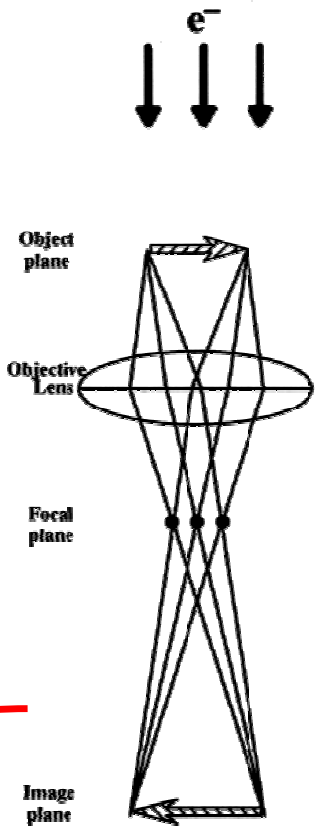


(Cheng et al, Cell, 2015)

What's this?

Image Formation





Object Coulomb potential function $V(x_o, y_o, z_o)$

Object transmitted wave function $\Psi_o(x_o, y_o)$

$$\Psi_o(x_o, y_o) \simeq 1 + i\sigma v(x_o, y_o)$$

$$v(x_o, y_o) = \int V(x_o, y_o, z_o)\, dz_o$$

Phase shift $\gamma(S)$ *introduced by objective lens*

$$\gamma(S) = 2\pi\left(\tfrac{1}{4} C_s \lambda^3 S^4 - \tfrac{1}{2} \Delta Z \lambda S^2\right)$$

Diffraction wave function $\Psi_d(S_x, S_y)$

$$\Psi_d(S_x, S_y) = F(S_x, S_y) \exp\left(i\gamma(S)\right)$$

$$F(S_x, S_y) = \mathcal{F}\left[\Psi_o(x_o, y_o)\right]$$

Diffraction intensity $I_d(S_x, S_y) = \Psi_d(S_x, S_y)\ \Psi_d^*(S_x, S_y)$

Image wave function $\Psi_i(x_i, y_i)$

$$\Psi_i(x_i, y_i) = \mathcal{F}^{-1}\left[\Psi_d(S_x, S_y)\right]$$

Image intensity $I_i(x_i, y_i)$

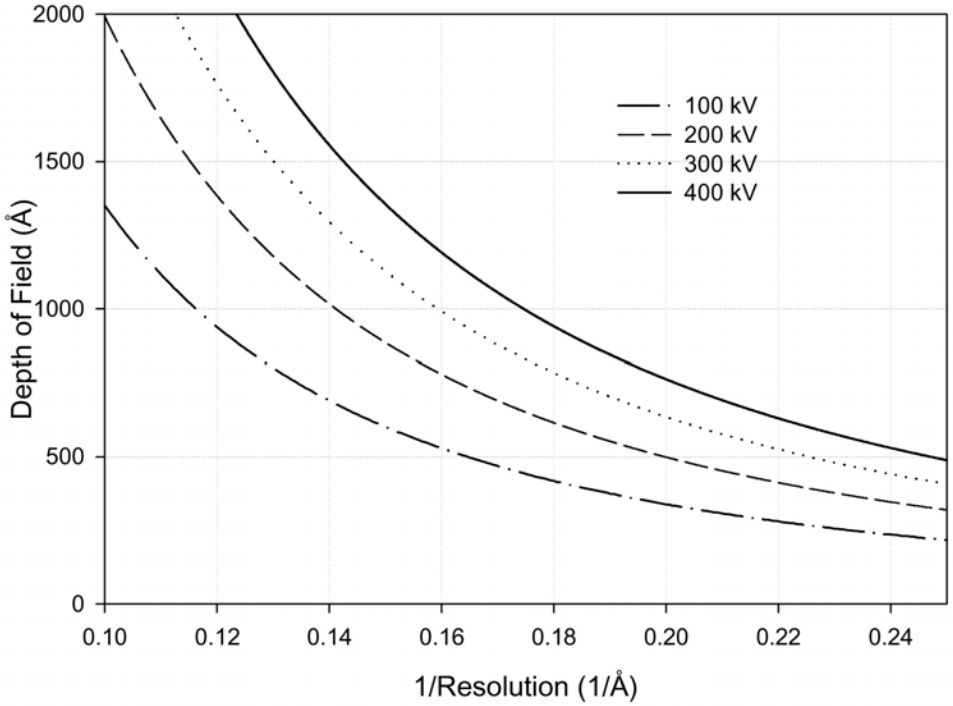
$$I_i(x_i, y_i) = \delta(0,0) - 2\sigma v(x_i, y_i) * \mathcal{F}^{-1}\left[\sin \gamma(S)\right]$$

Computed diffraction wave function $T(S_x, S_y)$

$$T(S_x, S_y) = \mathcal{F}\left[I_i(x_i, y_i)\right]$$
$$= \delta(0,0) - 2\, F(S_x, S_y) \sin \gamma(S)$$

Image Contract Theory

- Object is not too thick: the allowable thickness is resolution-dependent.
- Images are 2-D projections of the 3-D object with the same focus.
- Only the elastically scattered electrons form the images.



(Zhou & Chiu, Adv Prot Chem, 2003)

Computed Diffraction Pattern

$$F^2(s)\ CTF^2(s)\ Env^2(s) + N^2(s)$$

Structural Factor

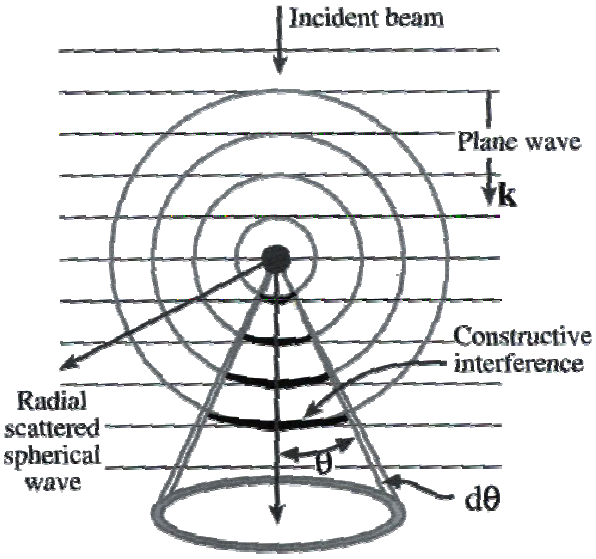
Contrast Transfer Function

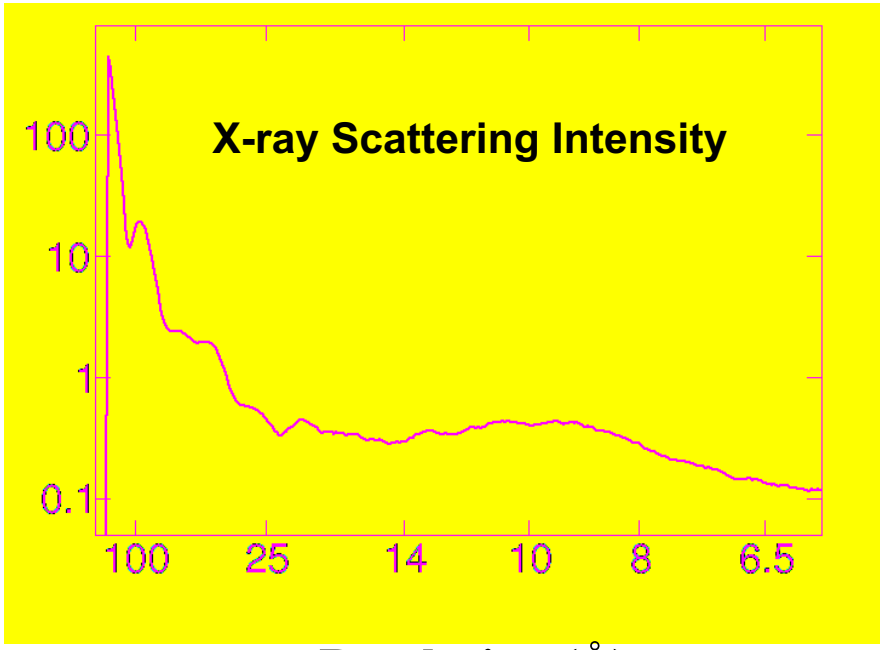
Envelope Function

Background Noise

Computed Diffraction Pattern

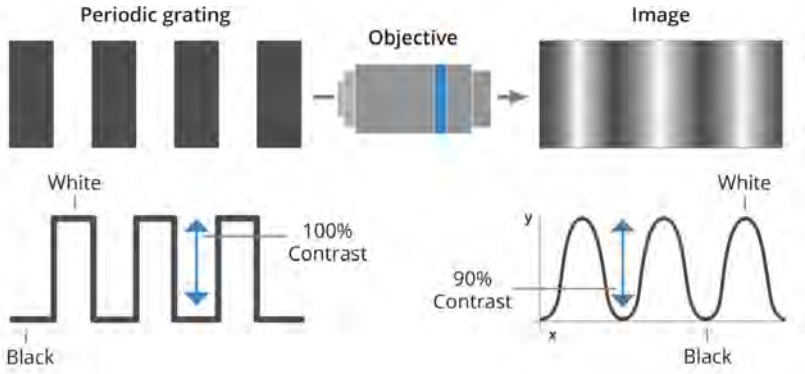
$$F^2(s)\ CTF^2(s)\ Env^2(s) + N^2(s)$$

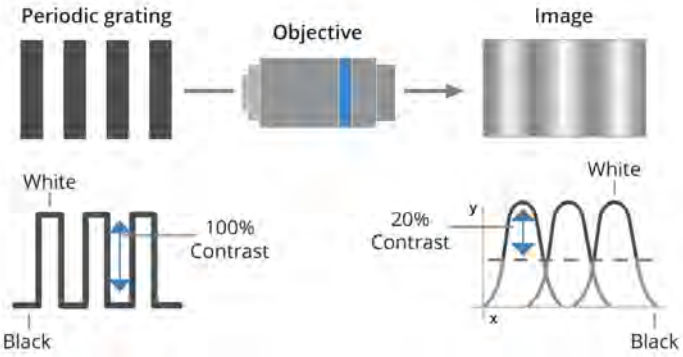


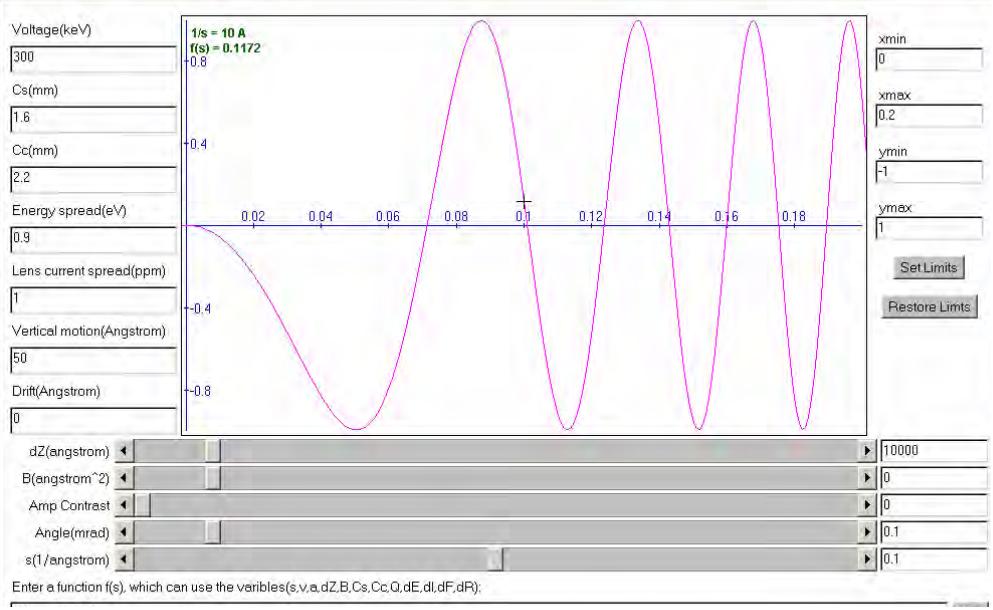


Computed Diffraction Pattern

$$F^2(s)\ CTF^2(s)\ Env^2(s) + N^2(s)$$







uOttawa

Computed Diffraction Pattern

$F^2(s)\ CTF^2(s)\ Env^2(s) + N^2(s)$

The CTF for biological samples can be described by the formula

$$Phase\ CTF = -2\sin\left[\pi\left(\Delta f \lambda q^2 - C_s \lambda^3 q^4 / 2\right)\right],$$

Phase CTF C_s = spherical aberration constant; Δf = defocus; q = spatial frequency; λ = electron wavelength. The spherical aberration coefficient and the electron wavelength are the only constants, and these values remain fixed for each electron microscope [52].

(Costa et al, Meth Mol Biol, 2017)

Computed Diffraction Pattern

$$F^2(s)\,CTF^2(s)\,Env^2(s) + N^2(s)$$

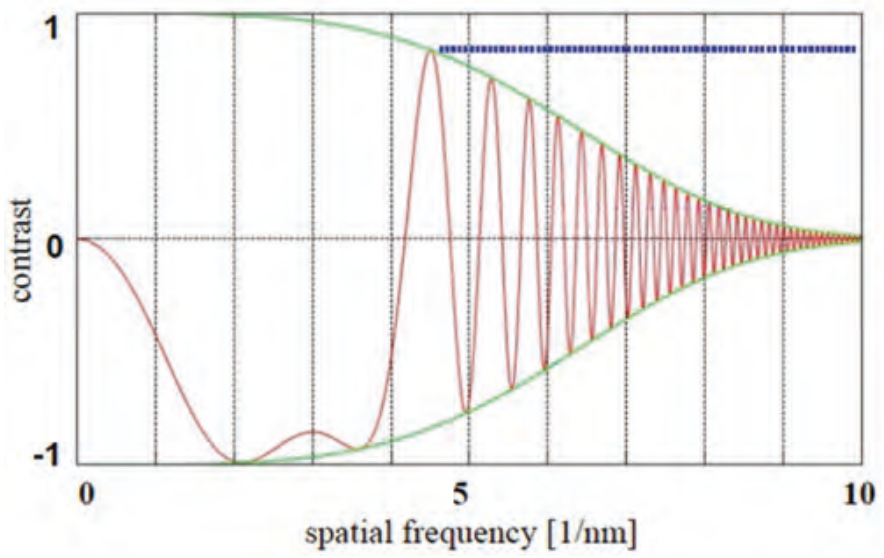
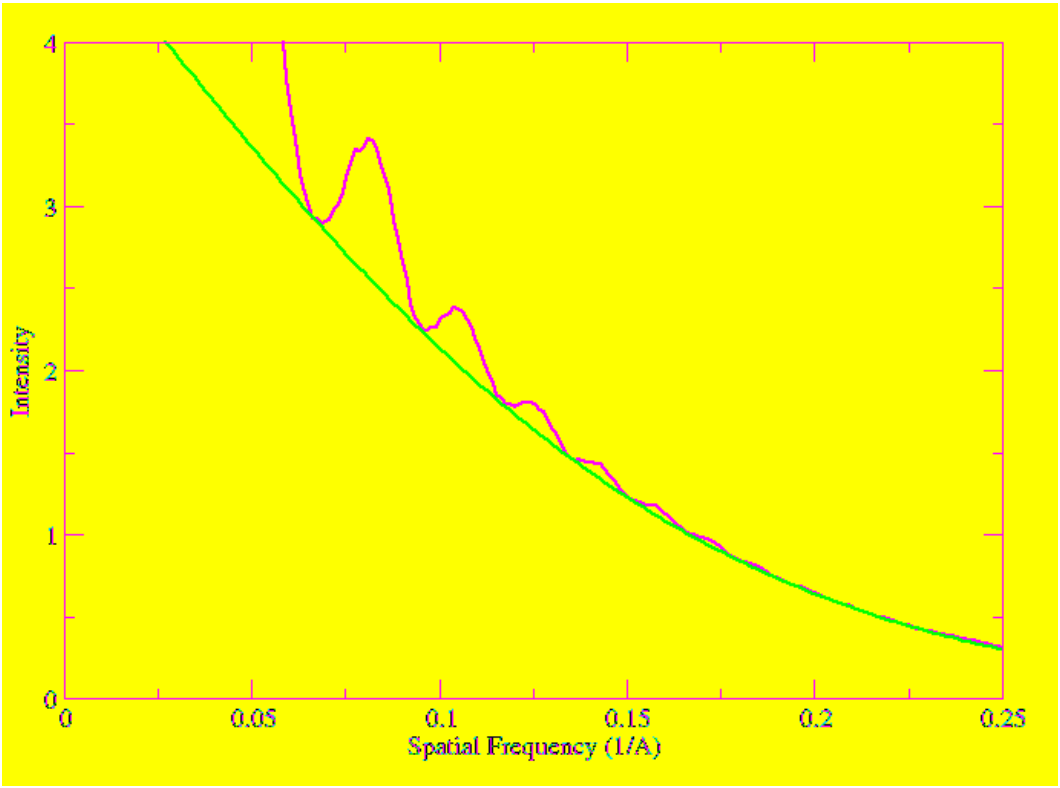


Fig. 7 CTF with envelope function. *Dotted blue line* : amplitude of all frequencies in perfect microscope; *green line*: effect of envelope function on CTF (*red*) resulting in suppression of high spatial frequencies

(Costa et al, Meth Mol Biol, 2017)

Computed Diffraction Pattern

$$F^2(s)\,CTF^2(s)\,Env^2(s) + N^2(s)$$



Contract Formation

$$\text{Contract} = (F^2\ CTF^2\ Env^2) / N^2$$

CTF Correction

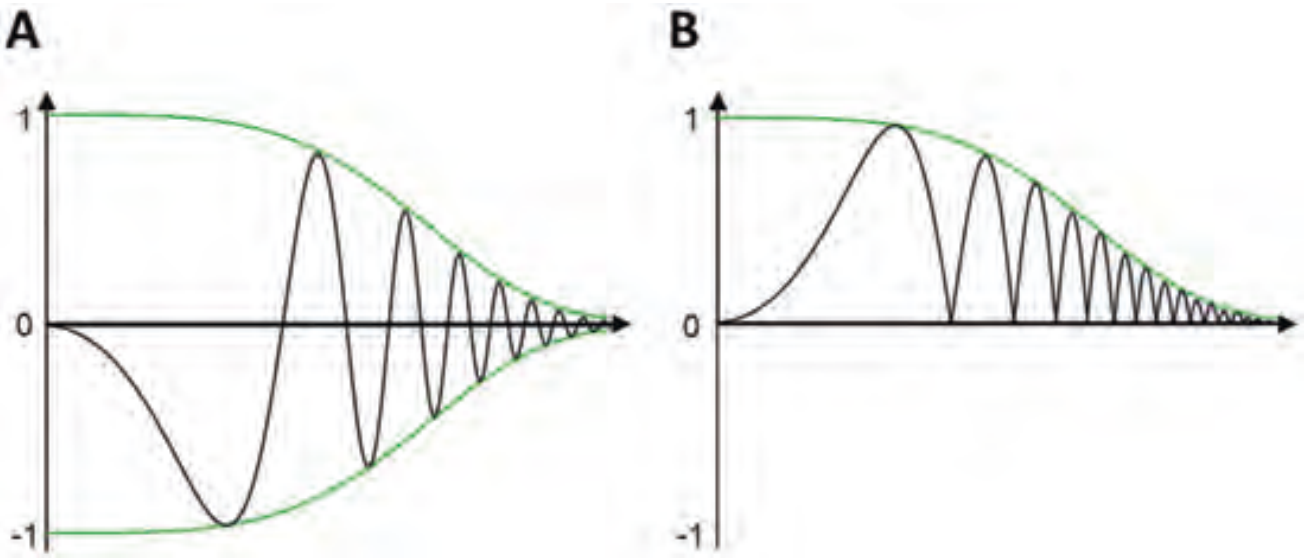


Fig. 9 CTF correction. (**a**) CTF oscillates changing contrast from negative to positive depending on frequencies. Information is lost only where CTF crosses zero line. (**b**) Negative lobes of uncorrected CTF are flipped over to positive (correction of CTF by phase flipping). The missing information can be recovered by collecting images at different defocus levels which fill these zero regions with information

(Costa et al, Meth Mol Biol, 2017)