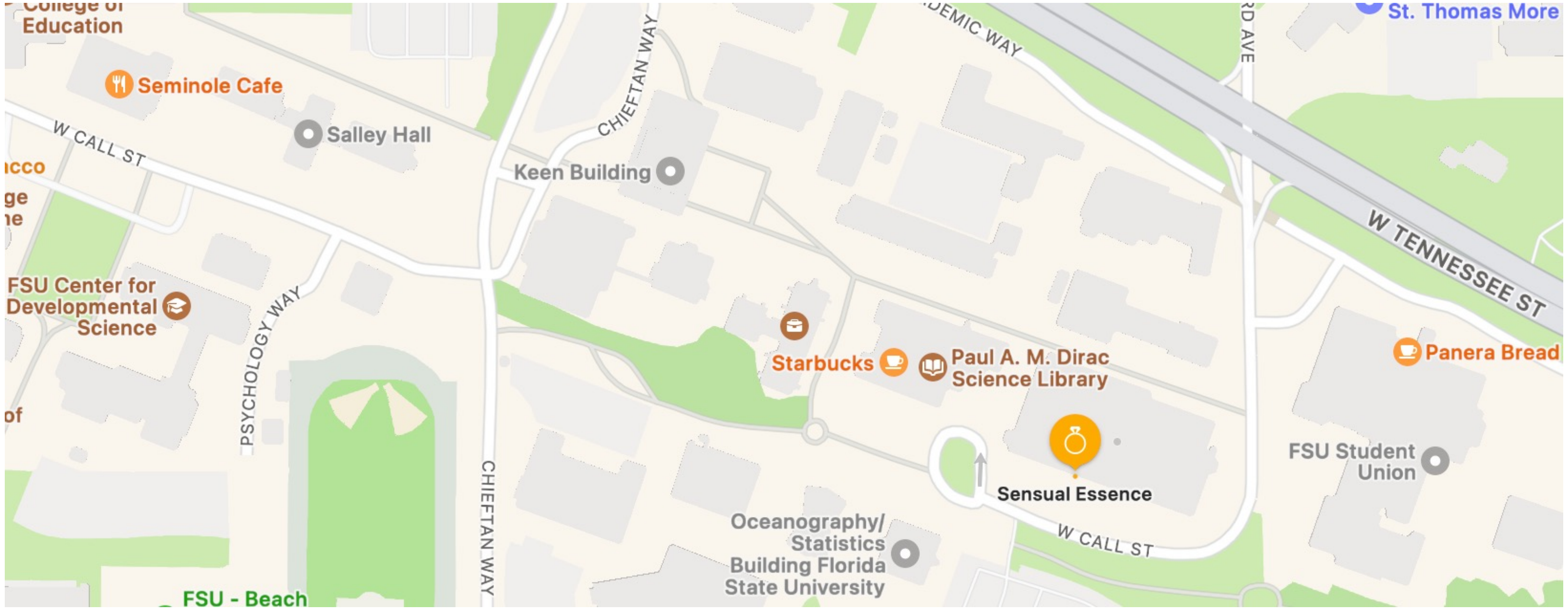
SECM^4 Data Processing Workshop

Scott Stagg

Introduction

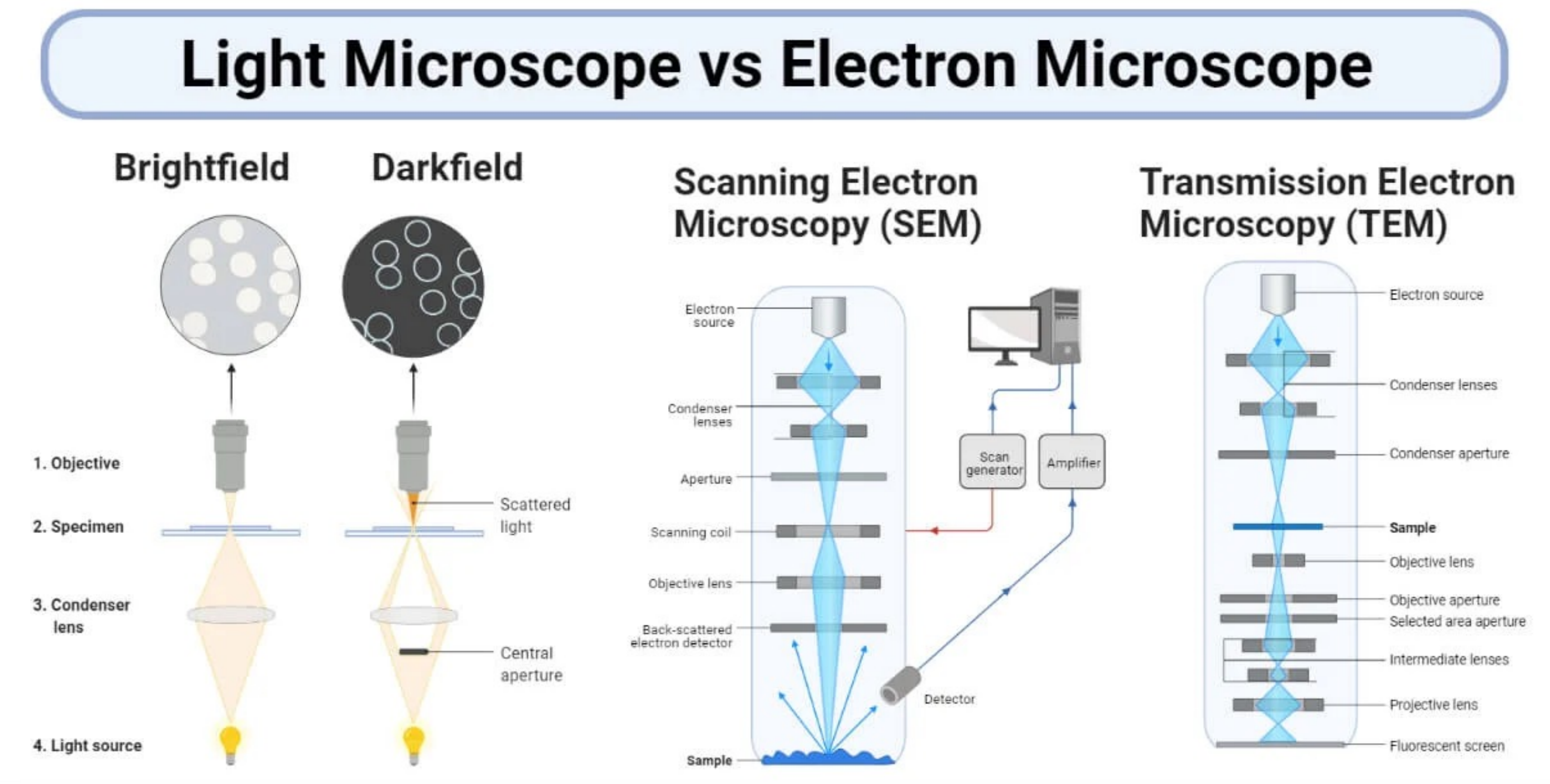
- Schedule
  - https://www.secm4.org/processing-workshop-agenda
- Food/Coffee
  - Seminole Cafe
  - FSU Student Union
- Instructors
  - Scott Stagg
  - Nebojša (Nash) Bogdanović
- Students



For a detailed introduction to cryo-EM practice and theory visit Grant Jensen's YouTube lectures

https://www.youtube.com/playlist?list=PLhiuGaXlZZenm7lu5qv_A59zEWkRKkBn5

Types of Microscopes

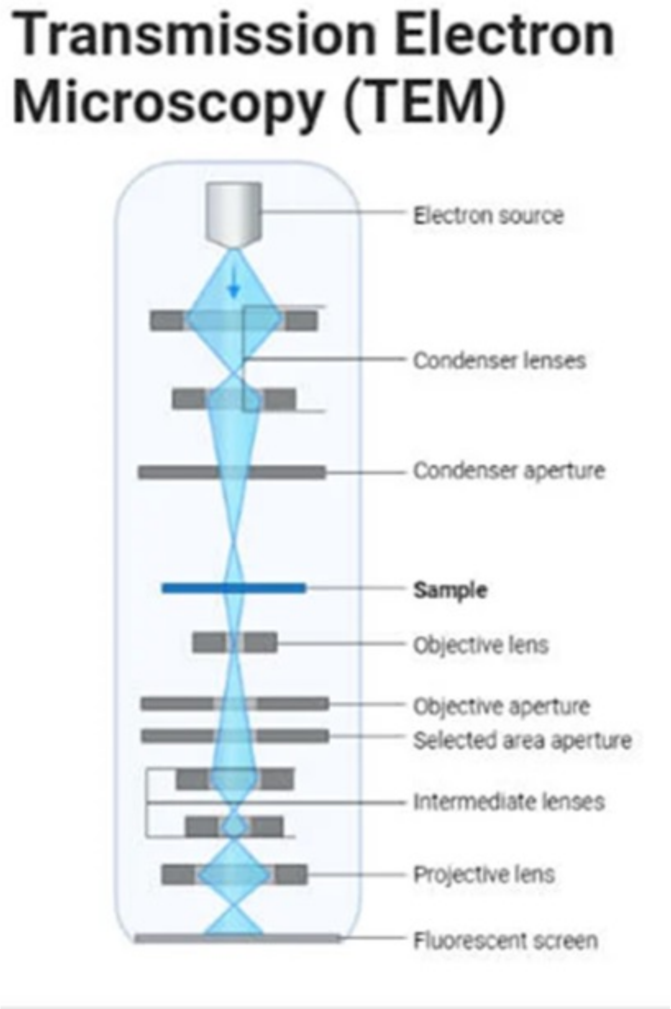


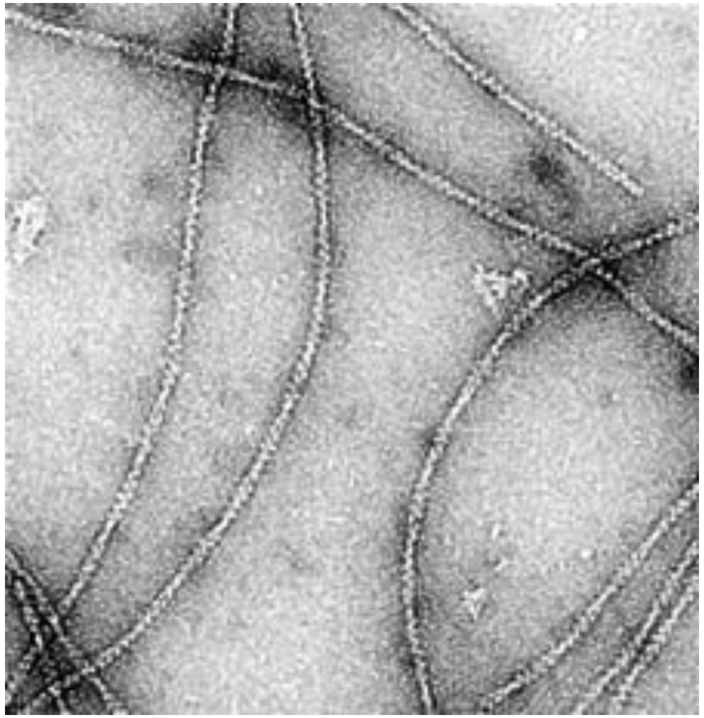
How Does a TEM Work?

- TEM uses electron beams for high-resolution imaging.

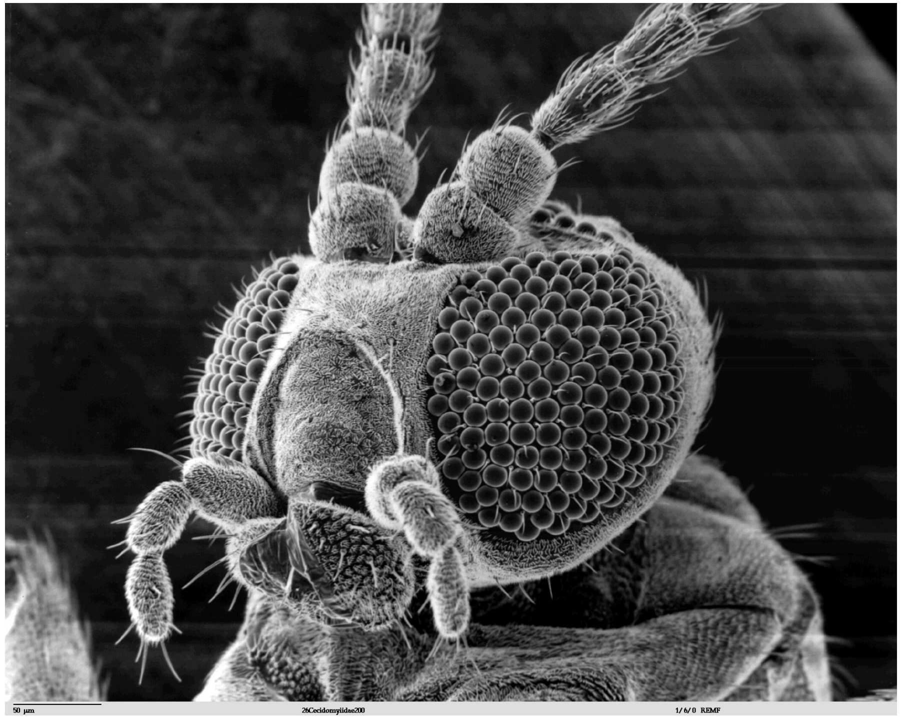
- **Principle**: Electrons pass through a thin specimen, creating an image.
- **Electron Source**: High-voltage gun emits the electron beam
- **Imaging**: Detected electrons form a magnified image on a screen
- **Applications**: Used in biology, materials science, and nanotechnology

TEM images are projection images





TEM projection through entire volume



SEM resolves only surface features

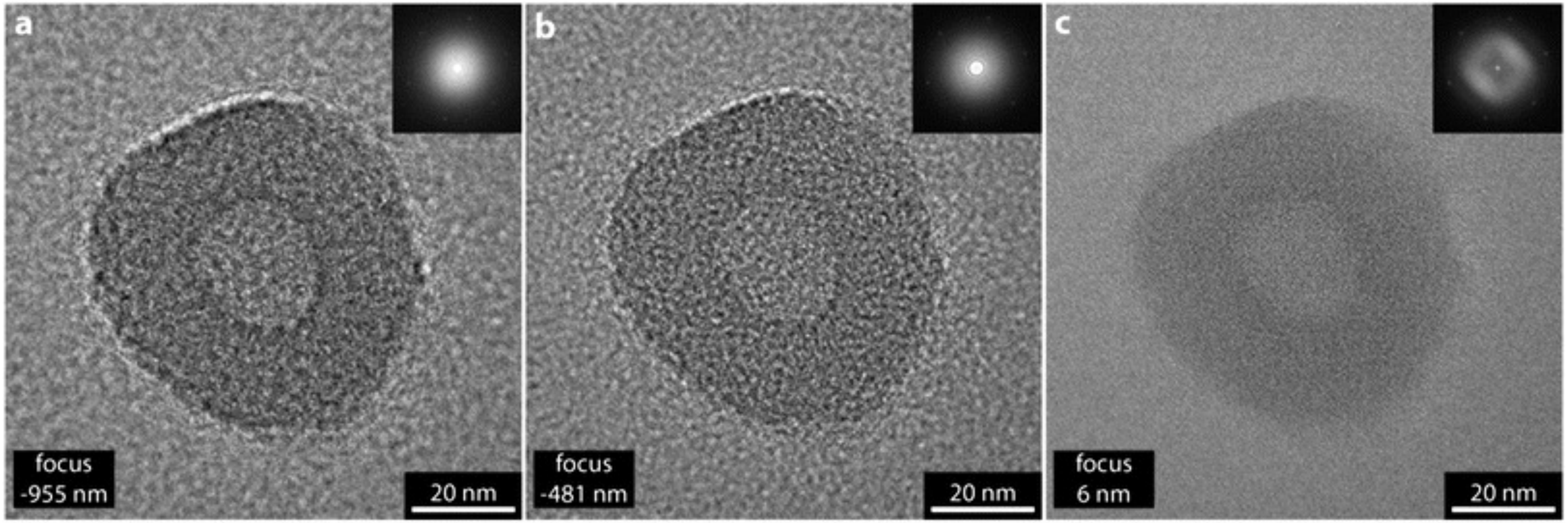
Electron micrographs are projection images

Projection of a solid sphere

Projection of a hollow sphere

Contrast Mechanism

- Contrast differentiates specimen from background.



Contrast in TEM is enhanced by **defocusing** the objective lens

Amplitude and phase contrast

- **Amplitude contrast** is produced by the loss of amplitude (i.e. electrons) from the beam
- **Phase contrast** originates from shifts in the relative phases of the portions of the beam which contribute to the image

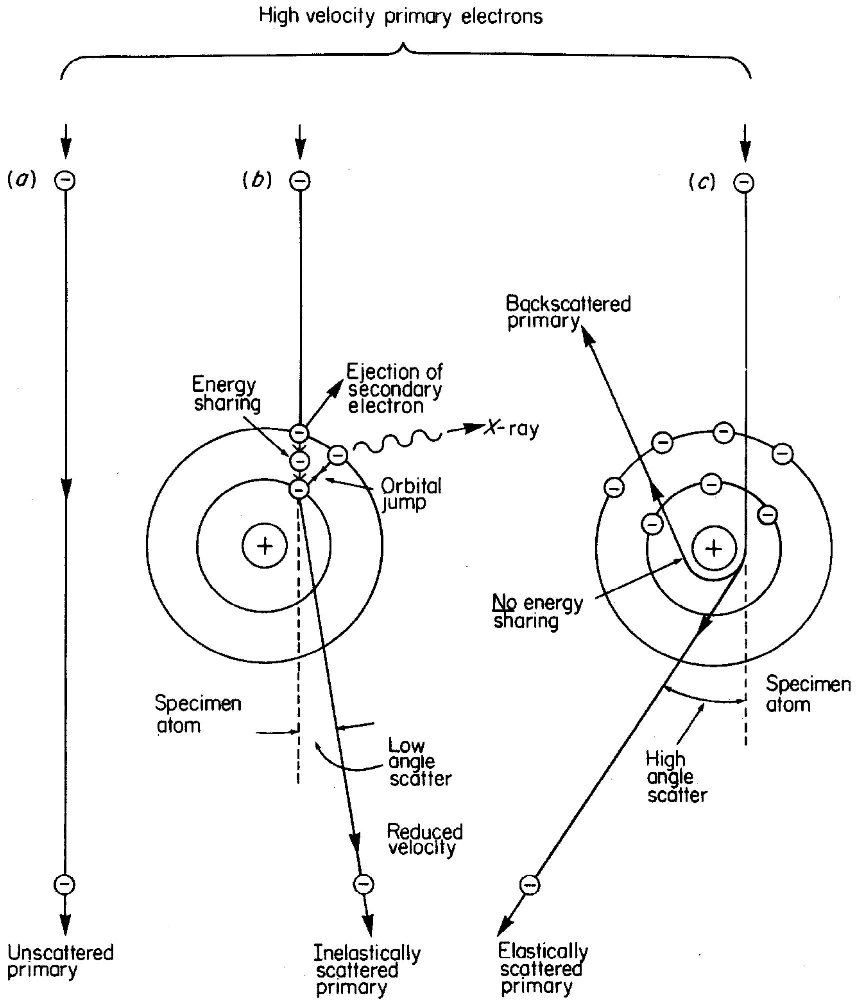
Contrast continued

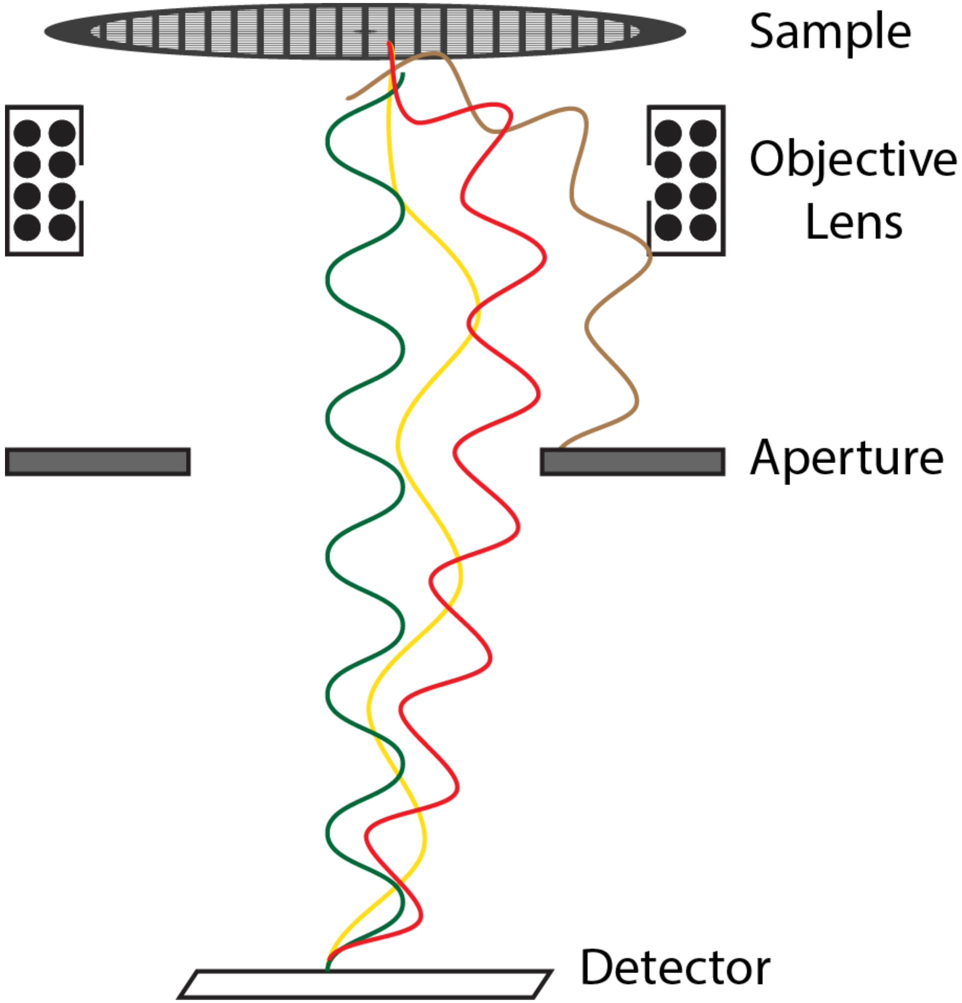
$$Percent\ contrast = 100 \times \frac{|I_o - I_b|}{I_b}$$

where I_o = intensity of the object point
I_b = intensity of the background adjacent to the object point

- In TEM, 7-10% of overall contrast comes from amplitude contrast
- 90-93% of contrast comes from phase contrast

Electron scattering generates contrast





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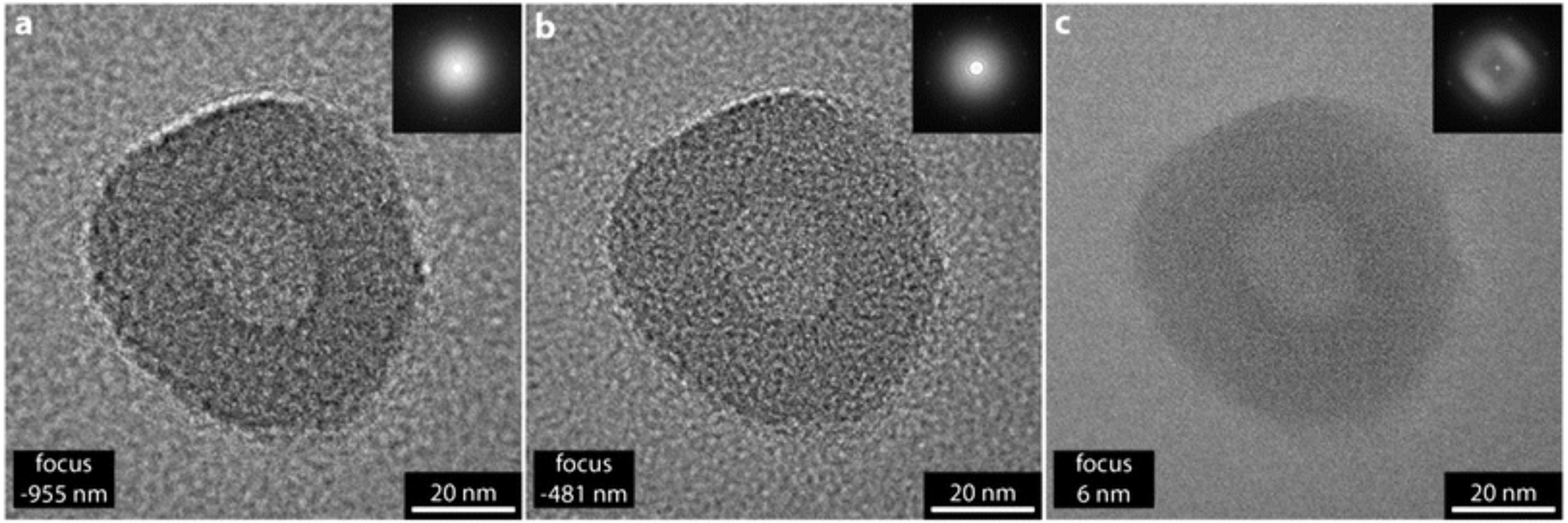

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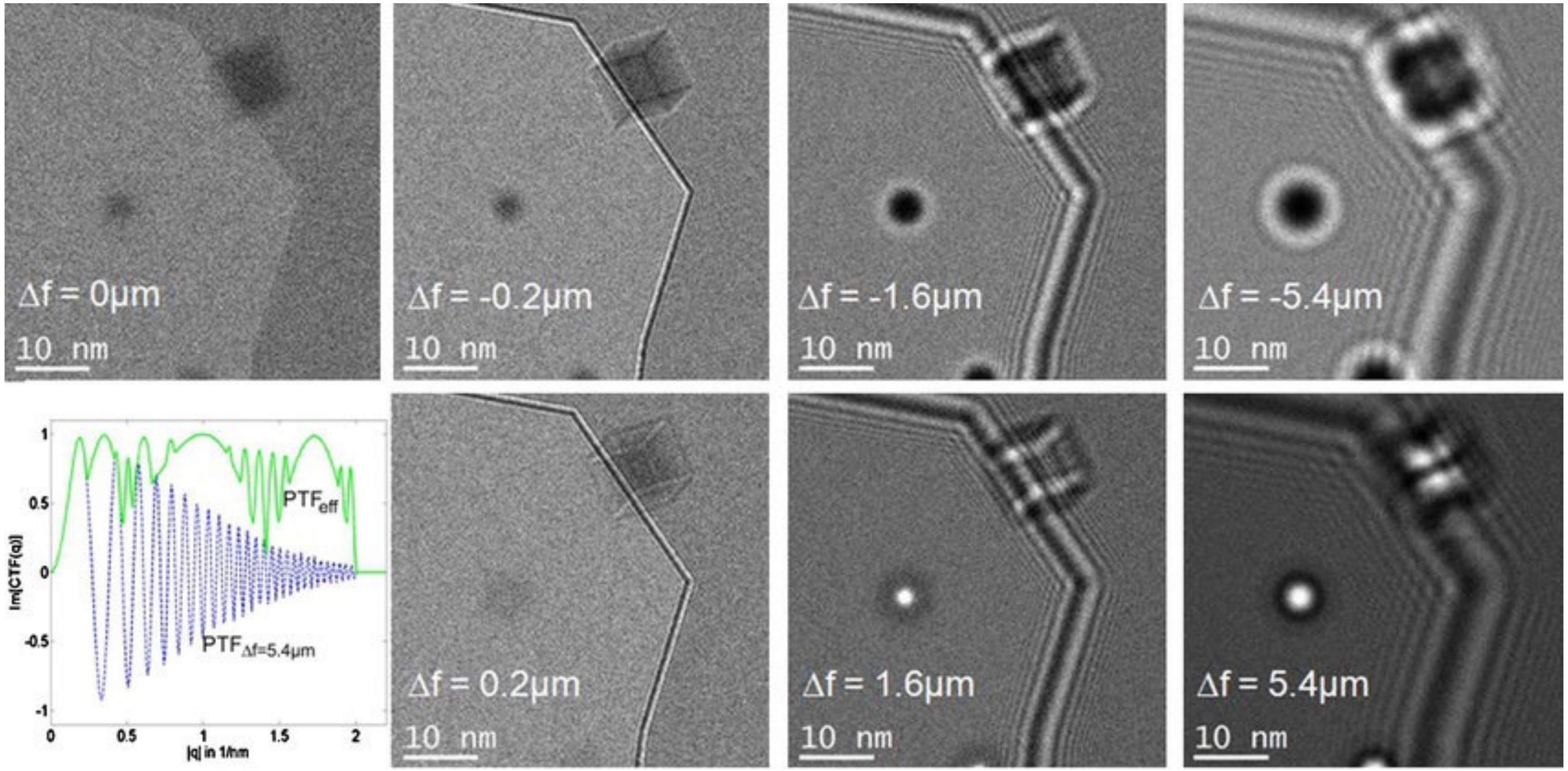
CTF (Contrast Transfer Function)

- **CTF Defined:** CTF (Contrast Transfer Function) describes how an imaging system modulates contrast at different spatial frequencies (resolutions)
- **Role in Microscopy:** CTF affects how microscopes interpret and display specimen details
- **Correction Importance:** Without CTF correction, images can have artifacts or missing information
- **Application:** Crucial in electron microscopy to achieve accurate, high-resolution images
- **Significance:** Understanding and correcting CTF ensures reliable and clear microscopic observations

Effects of CTF

Defocus:

- It refers to the extent to which an imaging system is out of focus. In electron microscopy, it's the distance between the plane where the microscope is focused and the actual plane of the specimen.



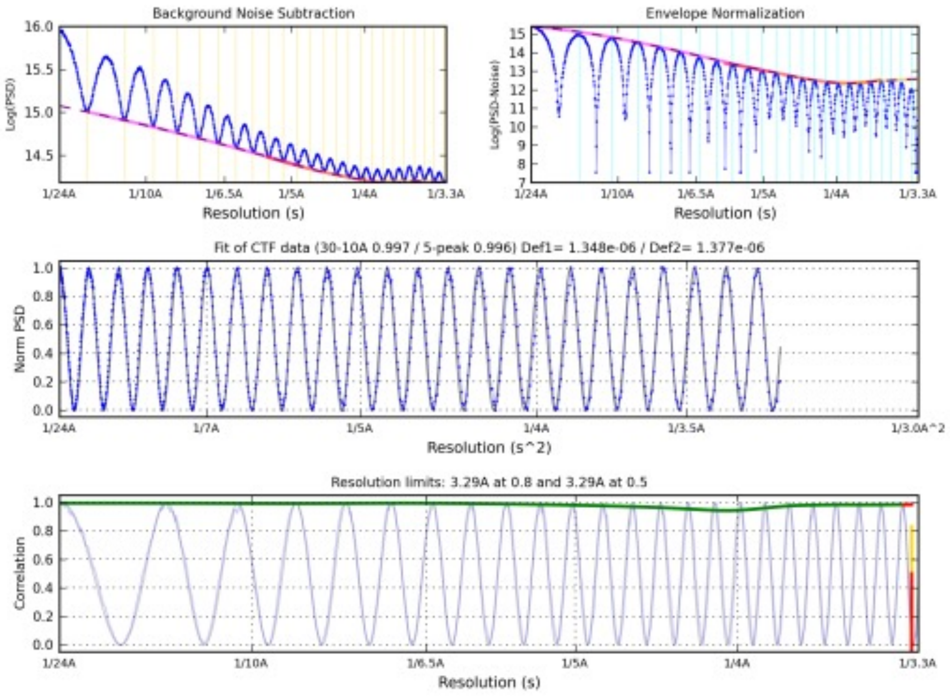
CTF image correction

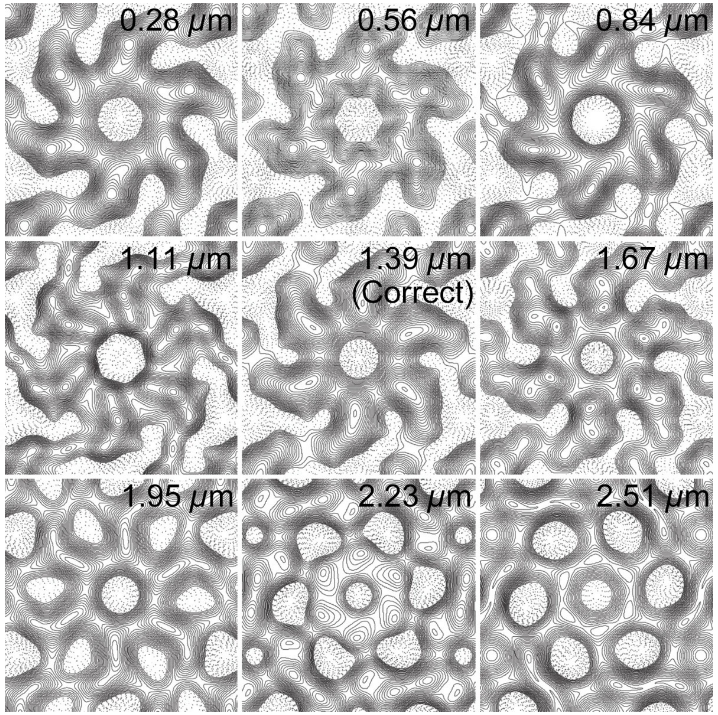
Image clarity: CTF correction ensures that the images produced by TEM are sharp and clear, free from distortions.

Accurate representation: Without CTF correction, the TEM images might not truly represent the specimen's actual structure.

Eliminate artifacts: CTF can introduce unwanted visual artifacts. Correcting it removes these, ensuring genuine observations.

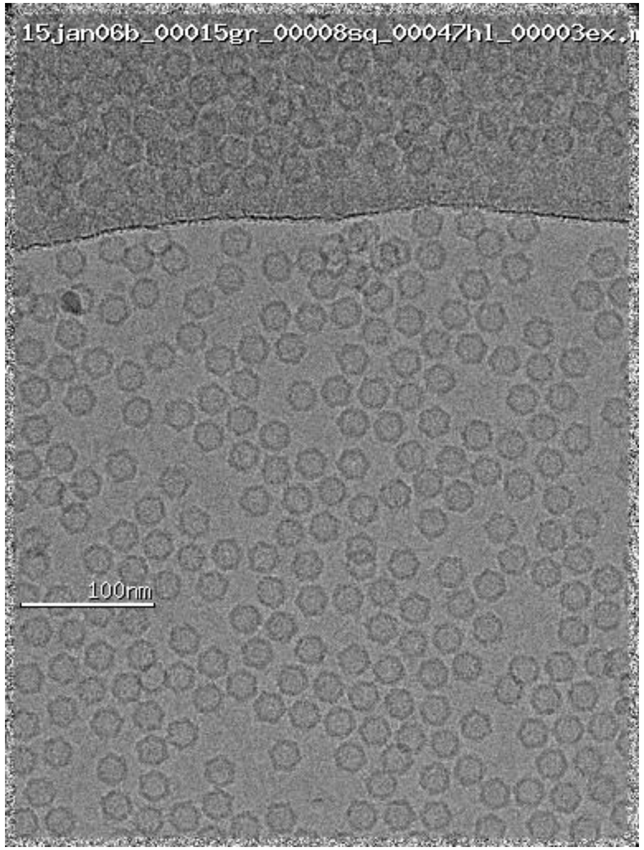
High-resolution imaging: For TEM to achieve its potential in high-resolution imaging, CTF correction is essential.





Jeong, HS.,. et al.. J Anal Sci Technol 4, 14 (2013)

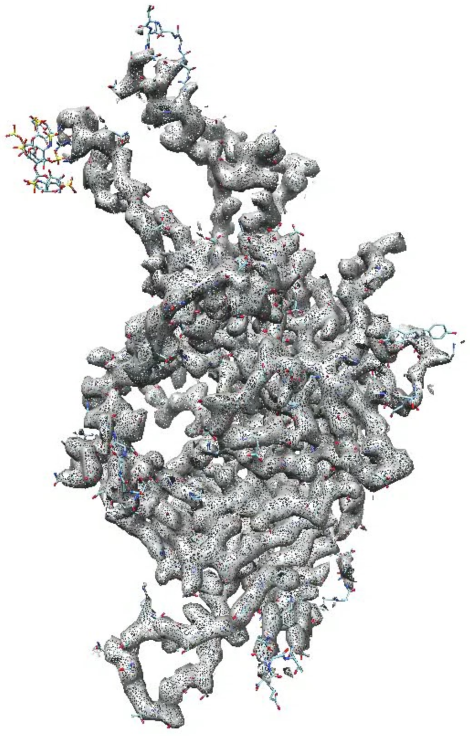
Single particle analysis



- ~1000 images (inherently atomic resolution)
- 10^4-10^6 particles

Alignment,
Classification,
Angular assignment

→

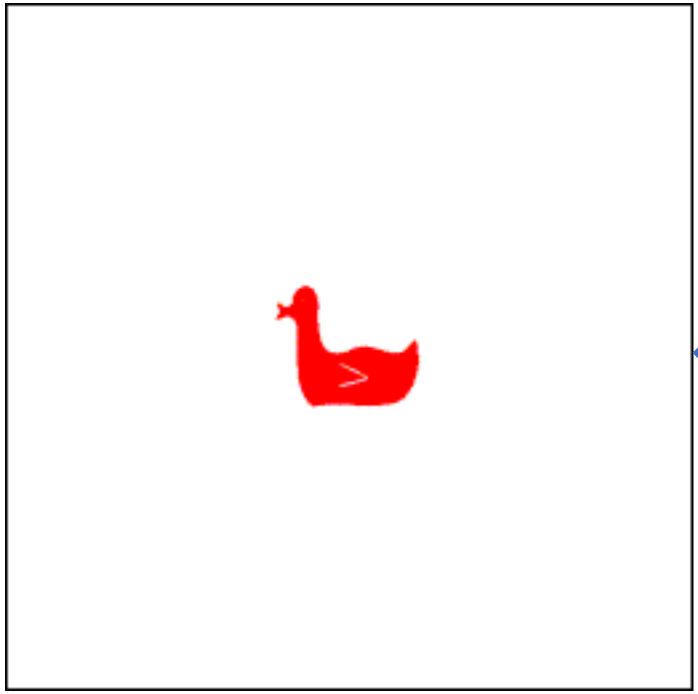


3D reconstruction

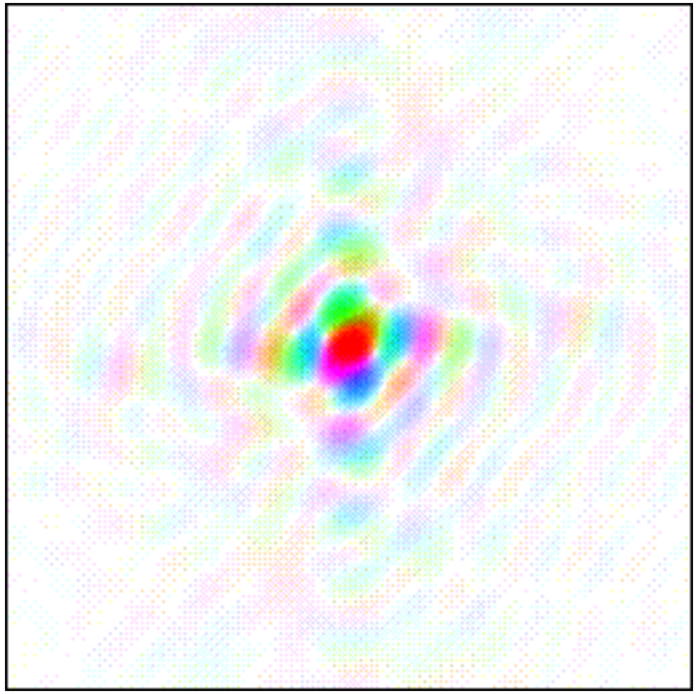
Steps in a single particle reconstruction

- Collect data
- Align frames
- Estimate CTF
- Pick particles
- 2D Classification
- Generate initial model
- Refine data against initial model
- Estimate resolution

Fourier Stuff



Real space image

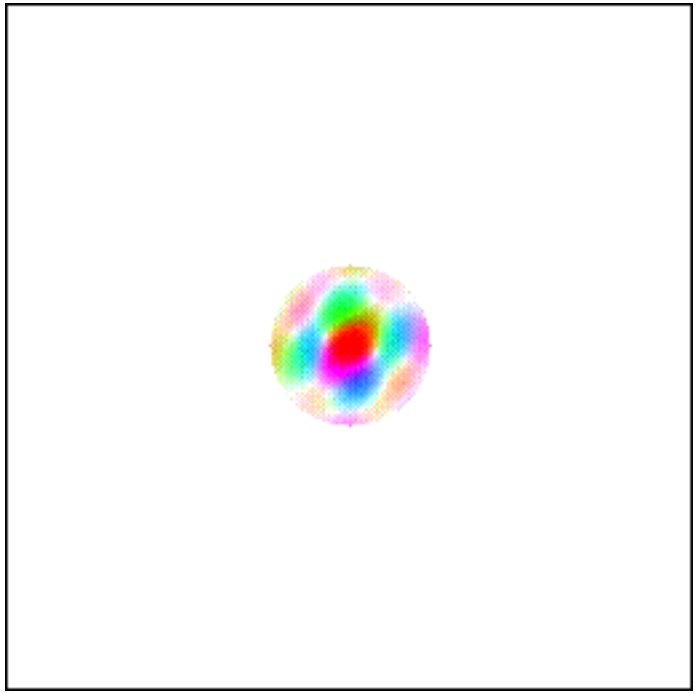


Fourier transform

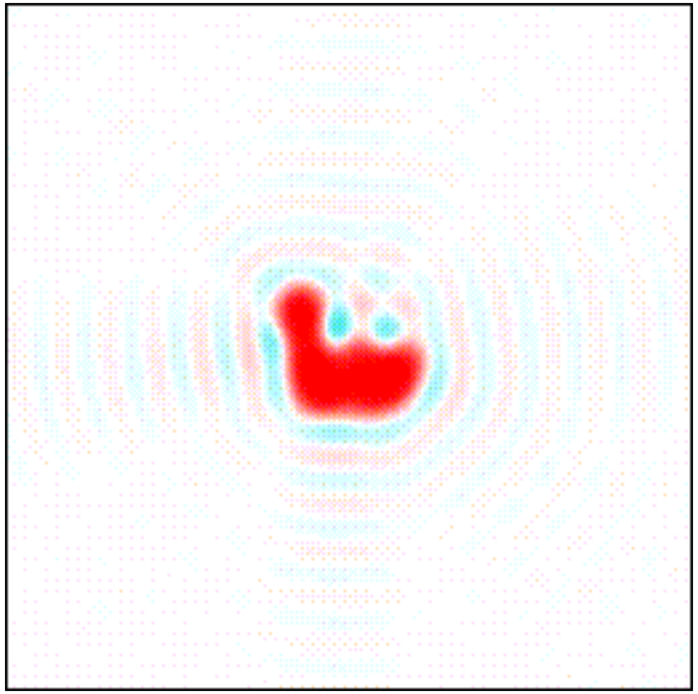
Brightness is amplitude,
colors are phases

From Kevin Cowtan's Picture Book of Fourier Transforms

Fourier Stuff



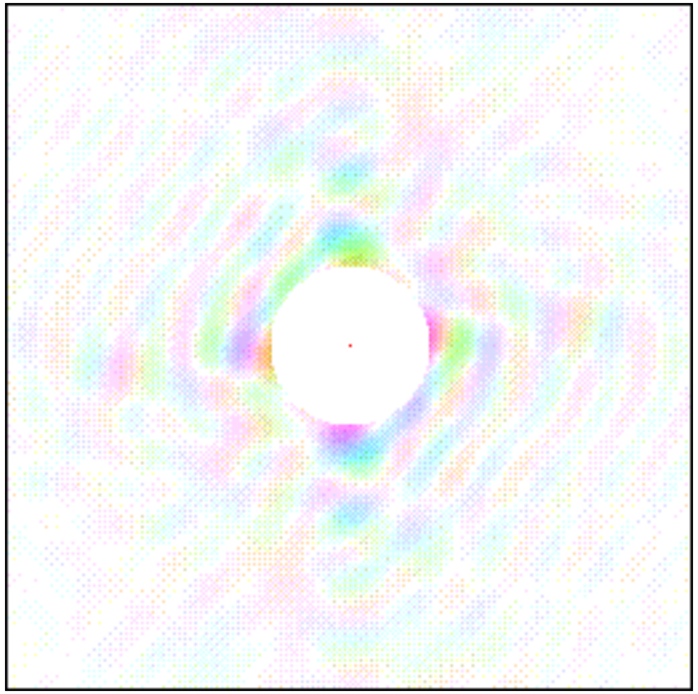
Low pass filter



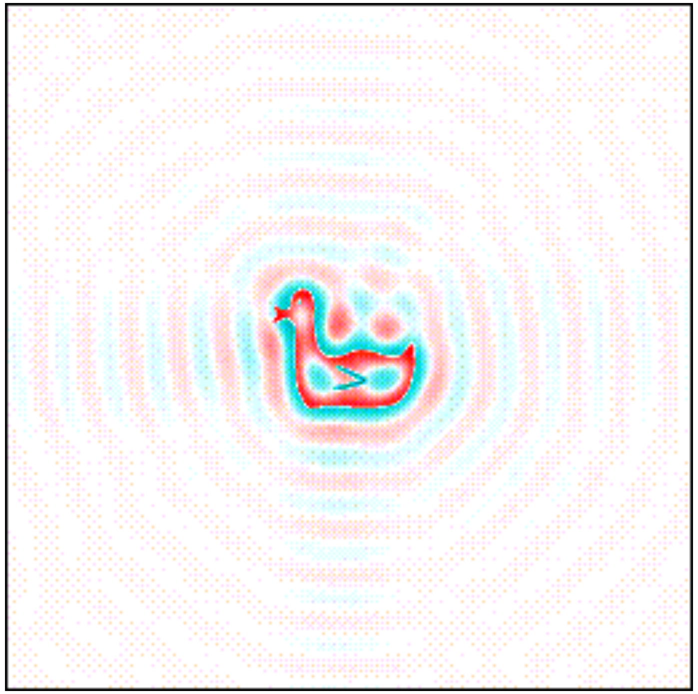
Reverse transform

From Kevin Cowtan's Picture Book of Fourier Transforms

Fourier Stuff



High-pass filter



Reverse transform

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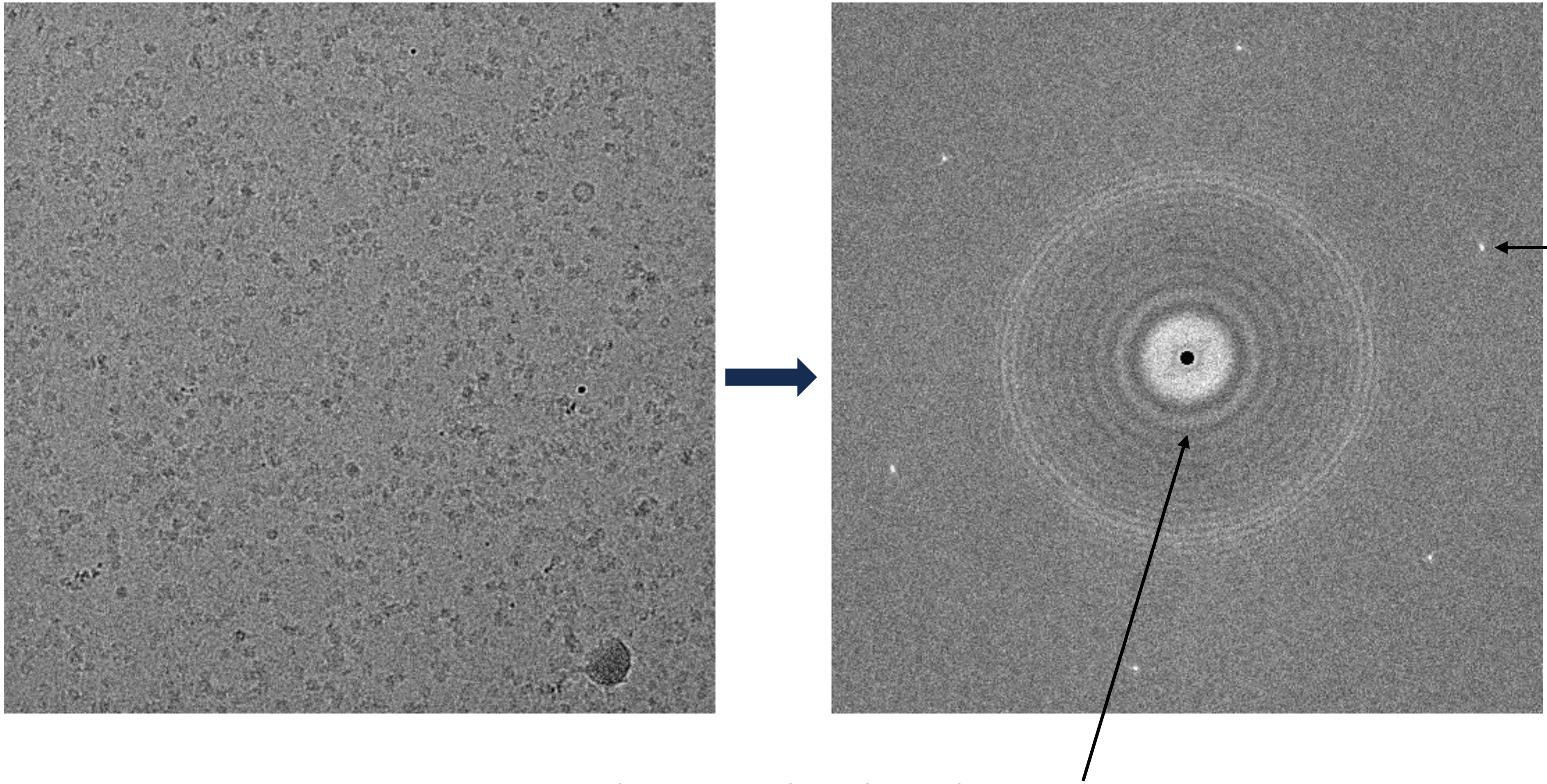
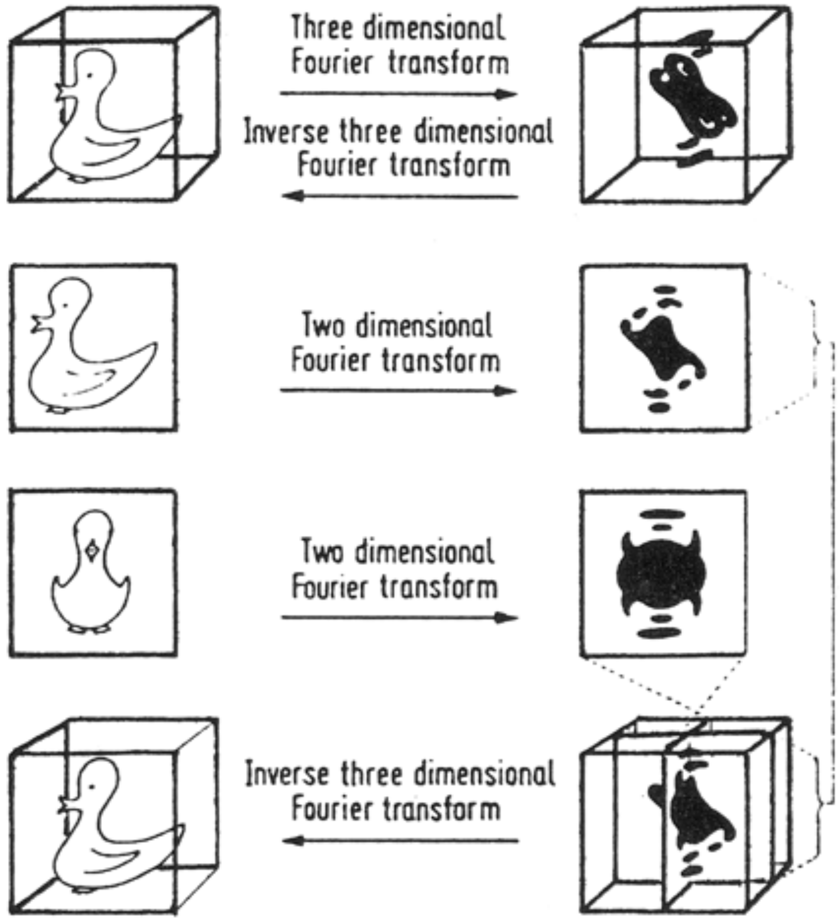
Fourier transform of a micrograph

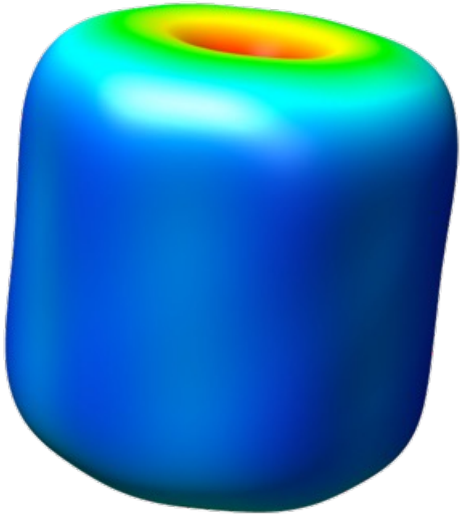


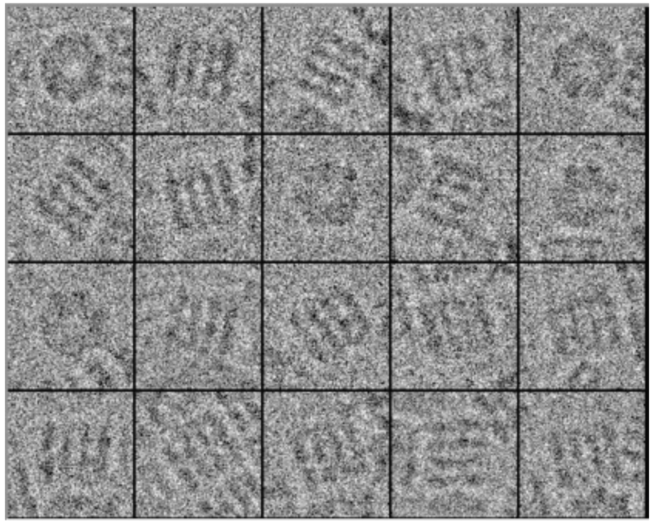
Principle of 3D reconstruction

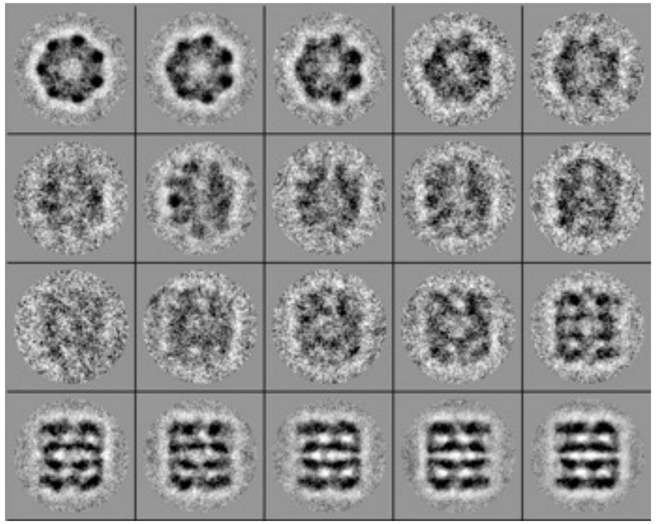
- The projection theorem says that each projection of an object is a central section in Fourier space
- A 3-D reconstruction can be obtained by measuring a sufficiently large number of these projections covering as much of 3-D Fourier space as possible.



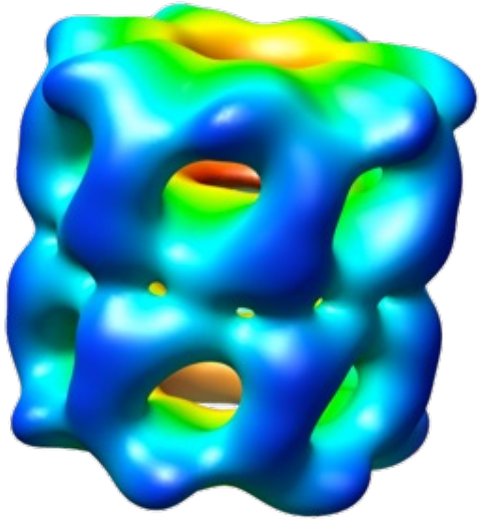
Classification and averaging

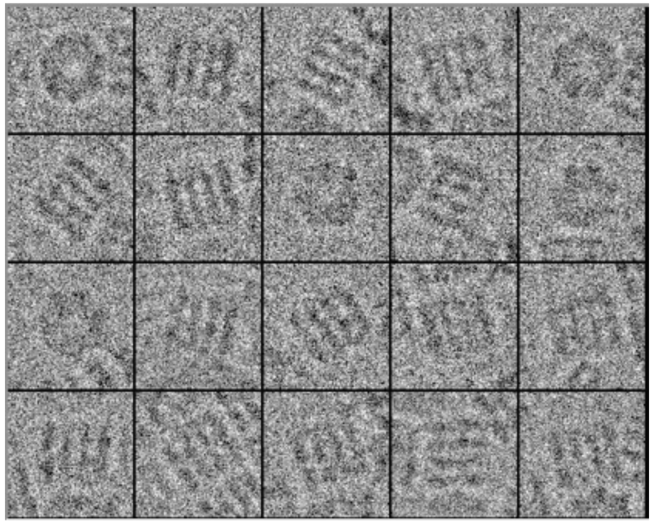


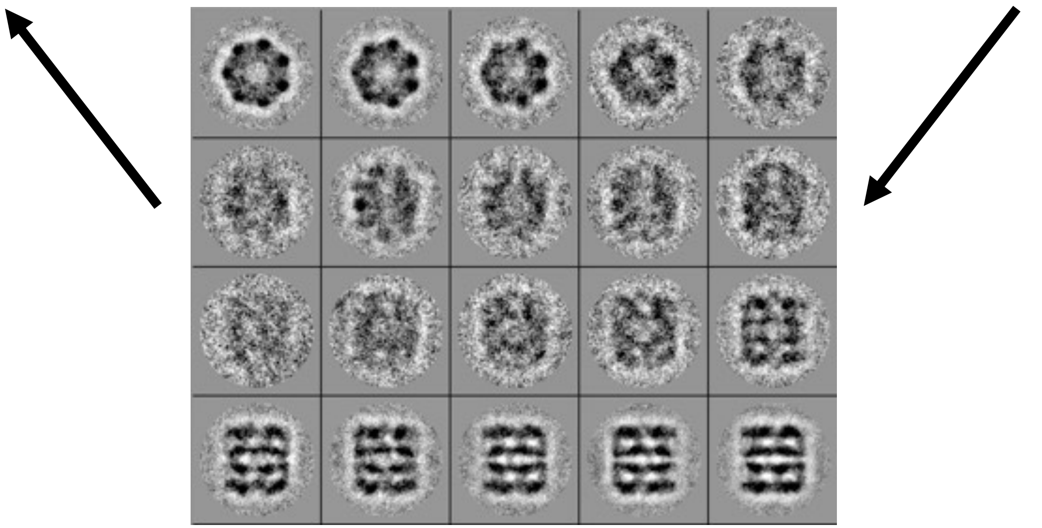




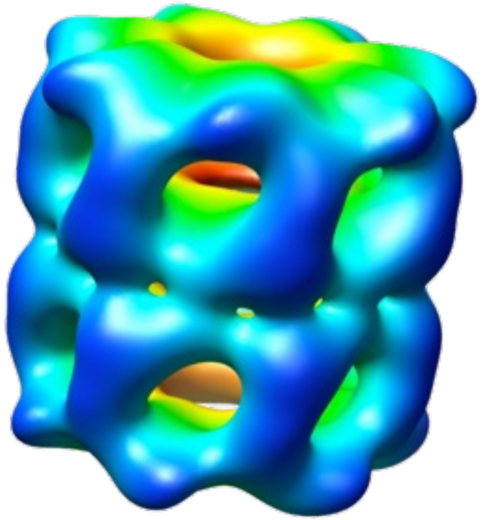
Backprojection yields new 3D model

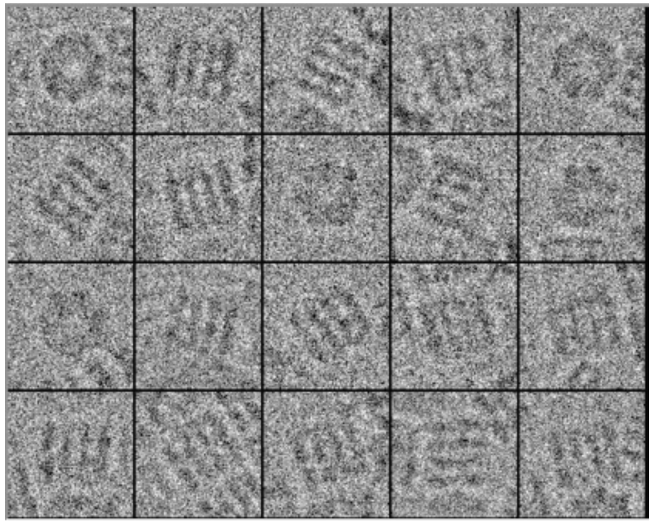


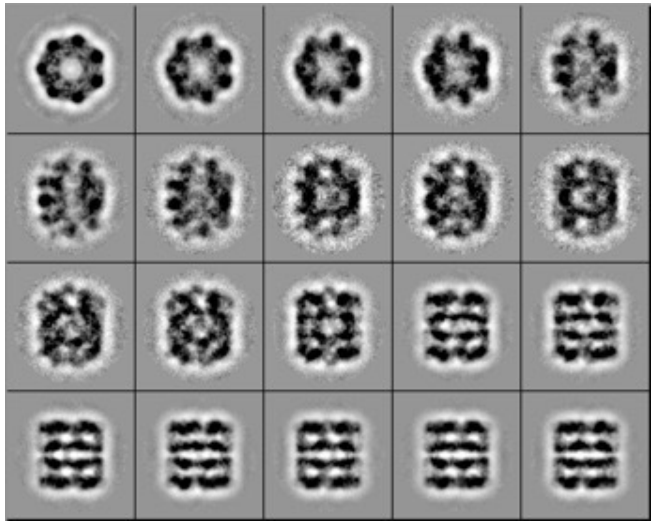




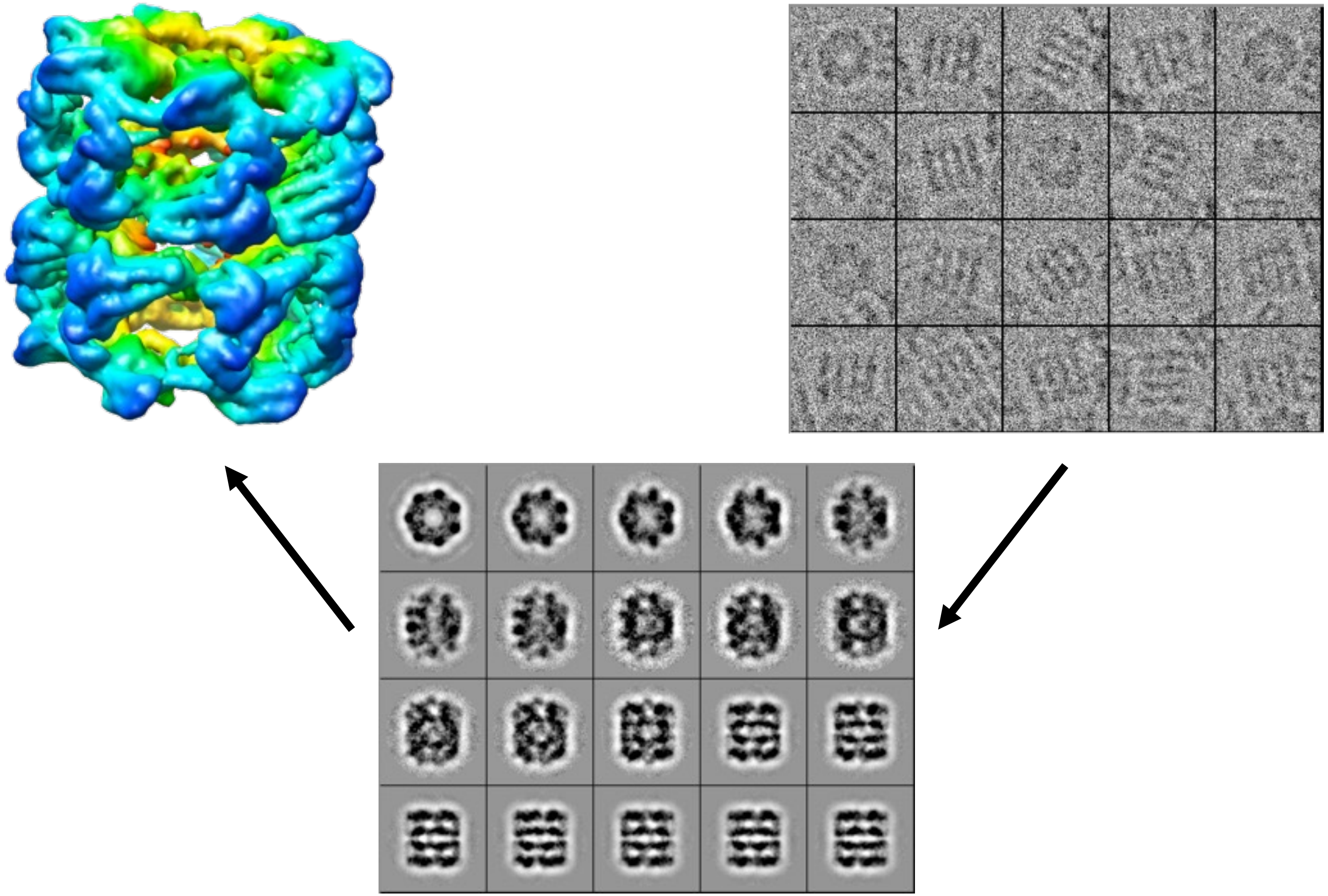
Higher resolution model yields better classification







Refinement converges on high resolution reconstruction



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