CS286: AI for Science and Engineering

Lecture 13: AI in Cryo EM (Part 1)

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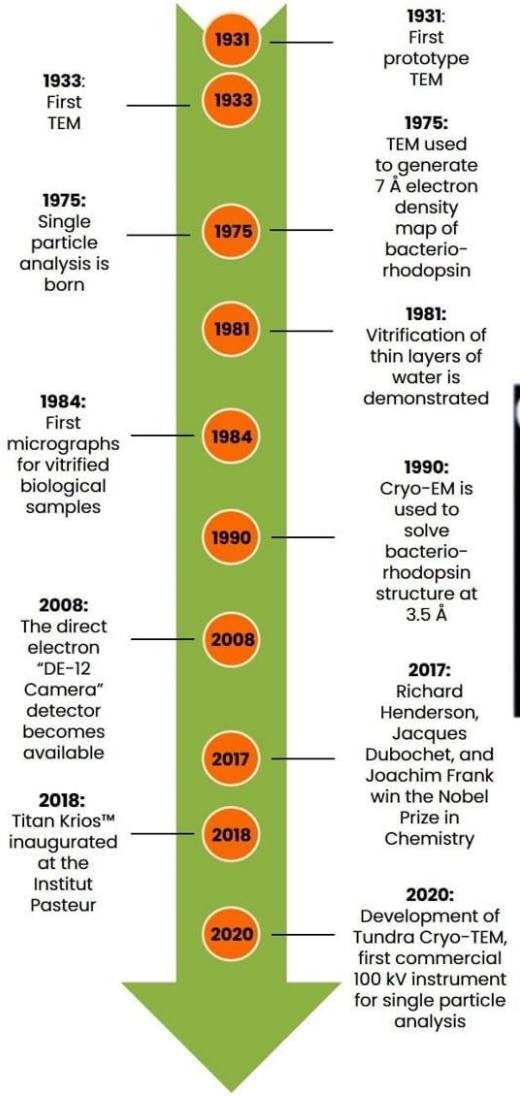
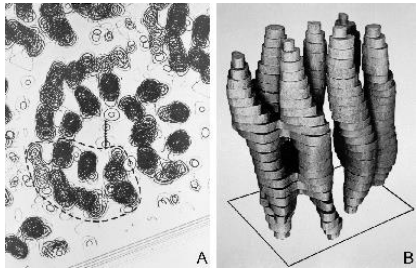
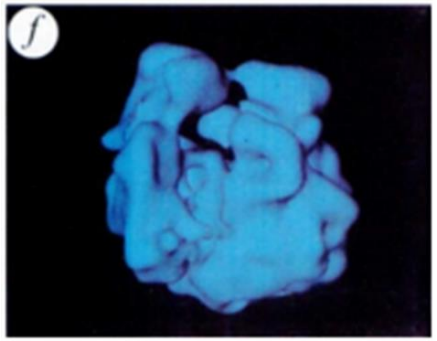
Outline

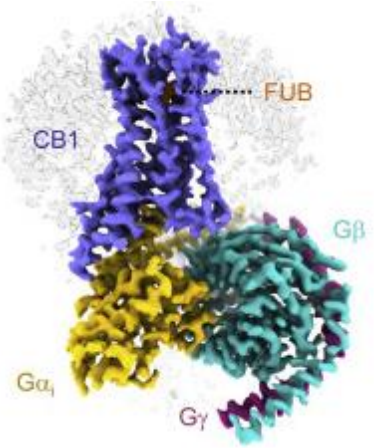
- A brief History of Cryo EM
- Principle of Cryo EM
- Short brief of Cryo ET
- What AI could do in Cryo EM

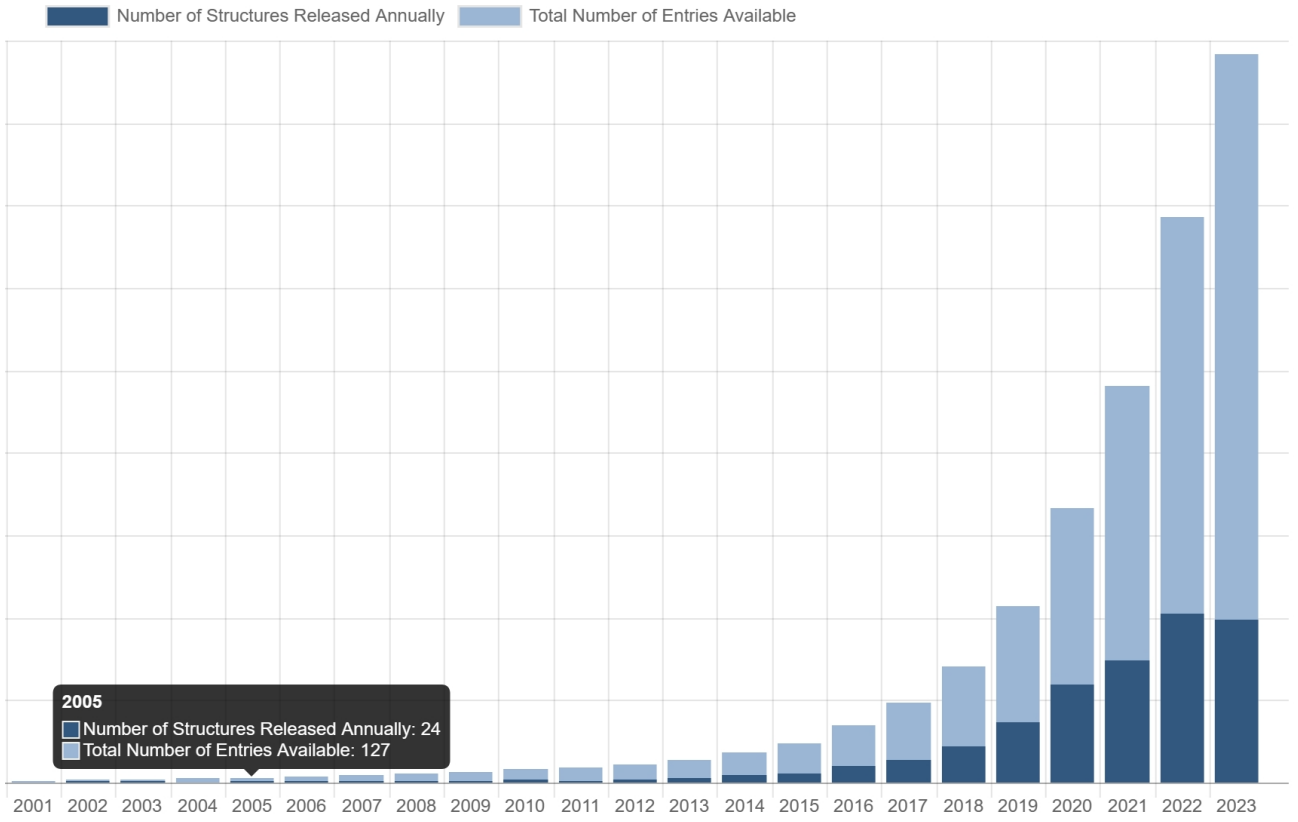
Time Stamp











Number of Structures solved by CryoEM grows annually

Inconvenience of TEM on sample of biology

- Strong interaction of electron beam with samples
- High level radiation damage
- Weak signal that could be detected

Why is difficult to solve CryoEM data

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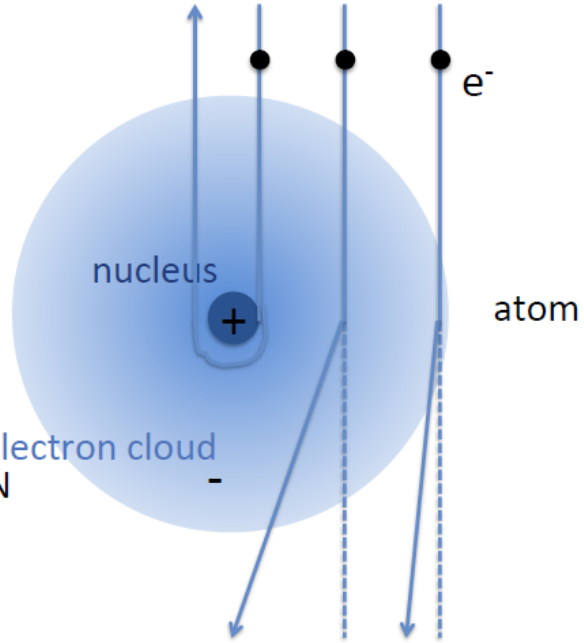
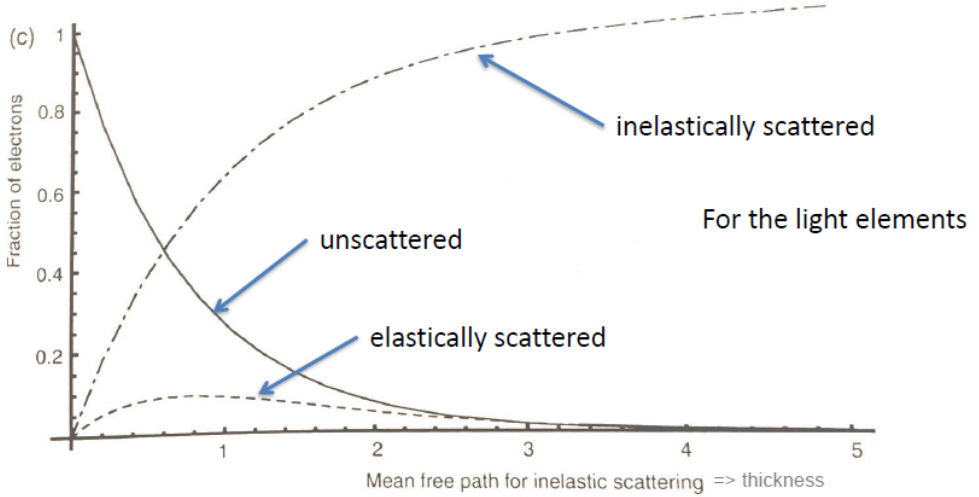
Particles: electron scattering



Maintain energy?
Elastic scattering
-> IMAGE FORMATION

Lose energy?
Inelastic scattering
-> deposited energy-> RADIATION DAMAGE!

The probability that an electron is scattered is a function of **sample thickness**.
Thinner is better.



For the light elements

fraction of unscattered electrons

Electron energy (keV)	10 nm	20 nm	30 nm	50 nm	100 nm	200 nm	300 nm
100	0.90	0.82	0.74	0.61	0.37	0.14	0.05
300	0.95	0.90	0.86	0.78	0.61	0.37	0.22

Mean free path is the distance in which fraction of unscattered electrons is = e^{-1} = 0.37

Electron Crystallography of Biological Molecules, Glaeser, Downing DeRosier, Chiu, Frank

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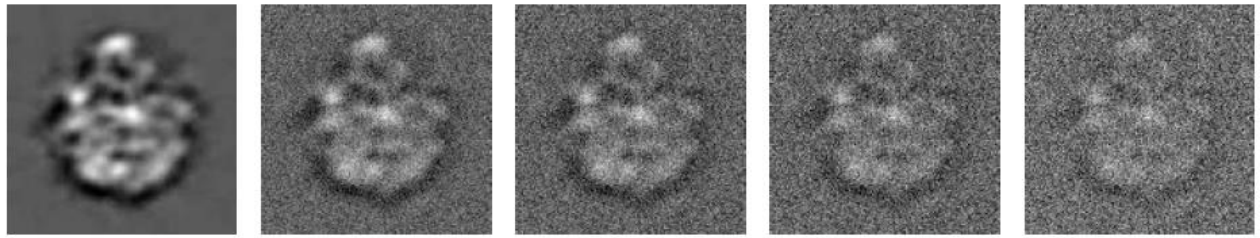
Signals of Cryo EM

$\sigma = 0$, $\sigma = 0.2$, and $\sigma = 1.2$.



(a) Clean (b) SNR=2^{0} (c) SNR=2^{-1} (d) SNR=2^{-2} (e) SNR=2^{-3}

(f) SNR=2^{-4} (g) SNR=2^{-5} (h) SNR=2^{-6} (i) SNR=2^{-7} (j) SNR=2^{-8}

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Nobel Prize in Chemistry in 2017

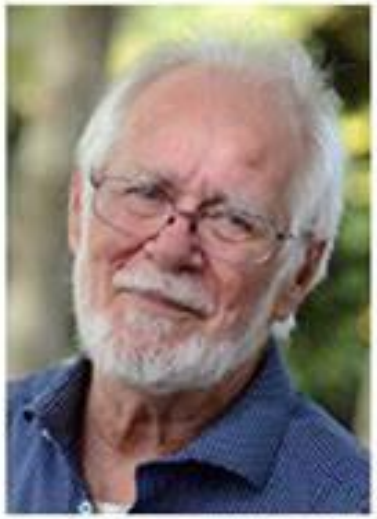

Photo: Félix Imhof © UNIL [CC BY-SA 4.0]

Jacques Dubochet

Prize share: 1/3

Photo: B. Winkowski © Columbia University Medical Center

Joachim Frank

Prize share: 1/3

Photo: MRC Laboratory of Molecular Biology

Richard Henderson

Prize share: 1/3

From left: Jacques Dubochet, Joachim Frank and Richard Henderson

Shared the Nobel Prize in Chemistry in 2017

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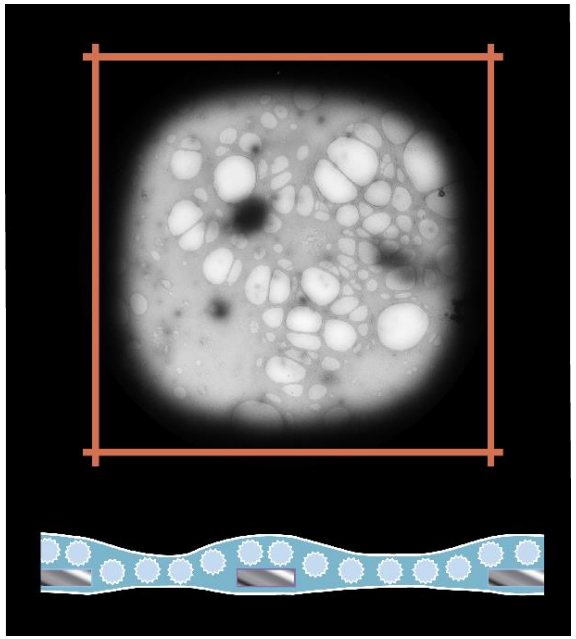
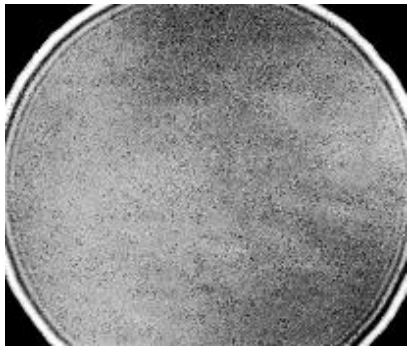
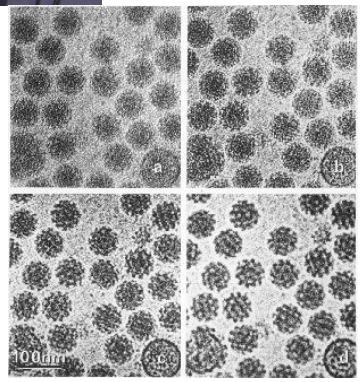
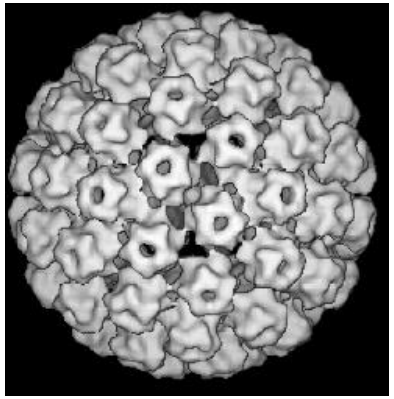
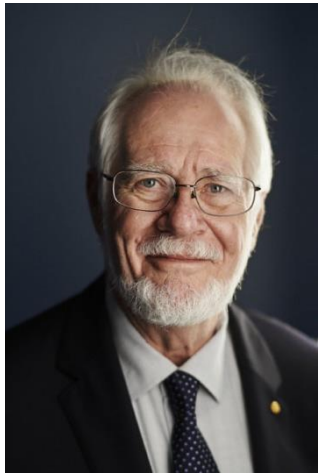
Method of Sample preparation





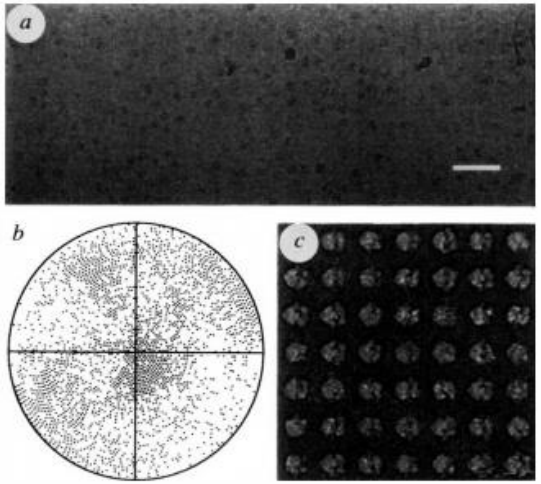


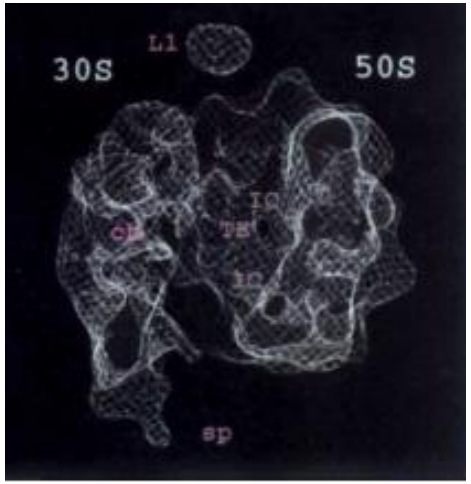


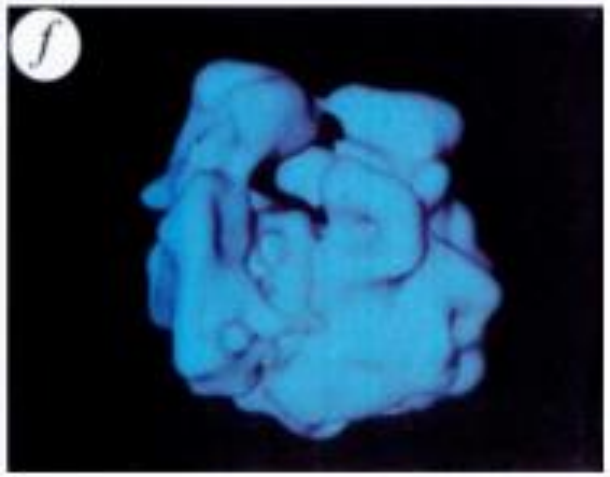


Dubochet

Method of single particle reconstruction



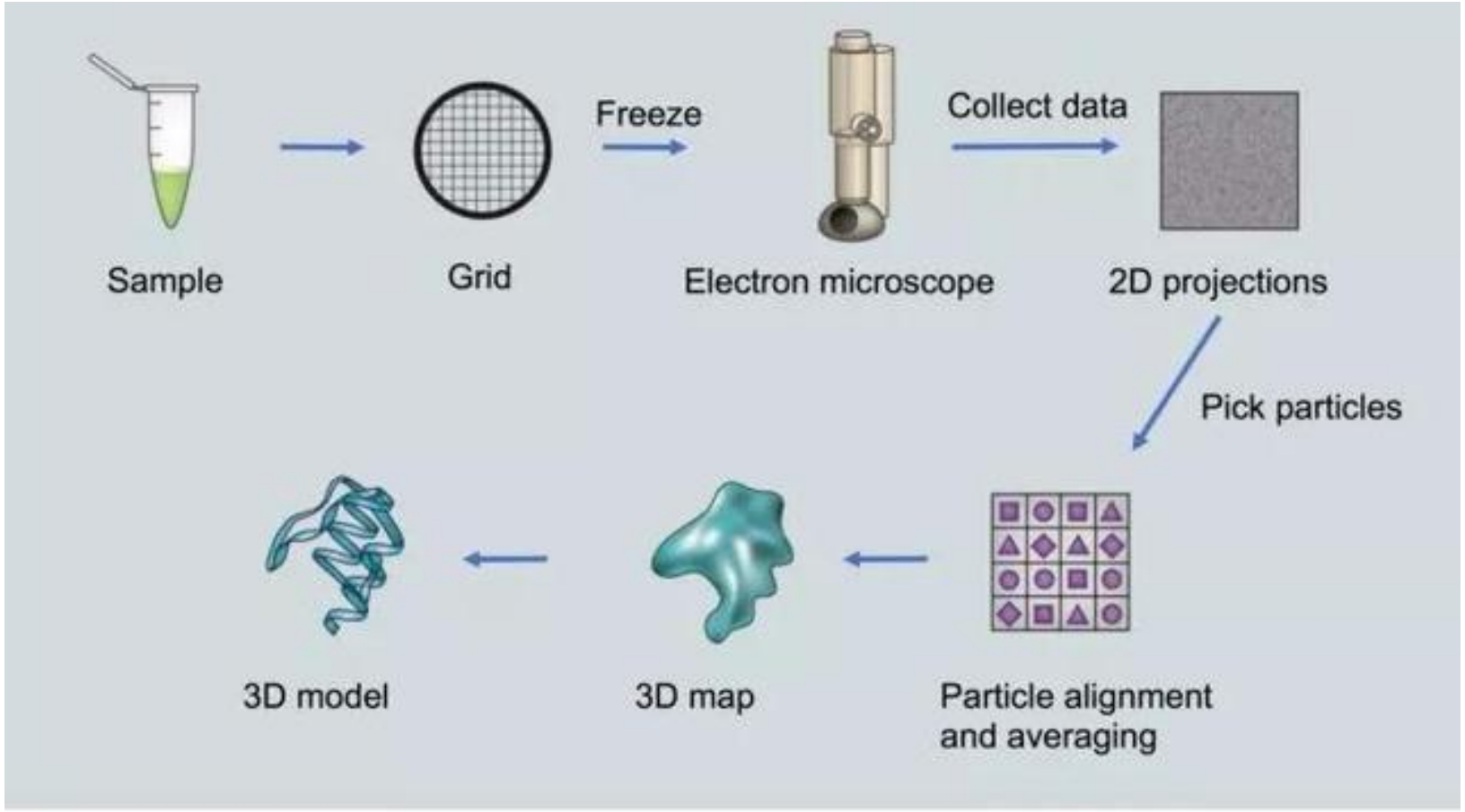


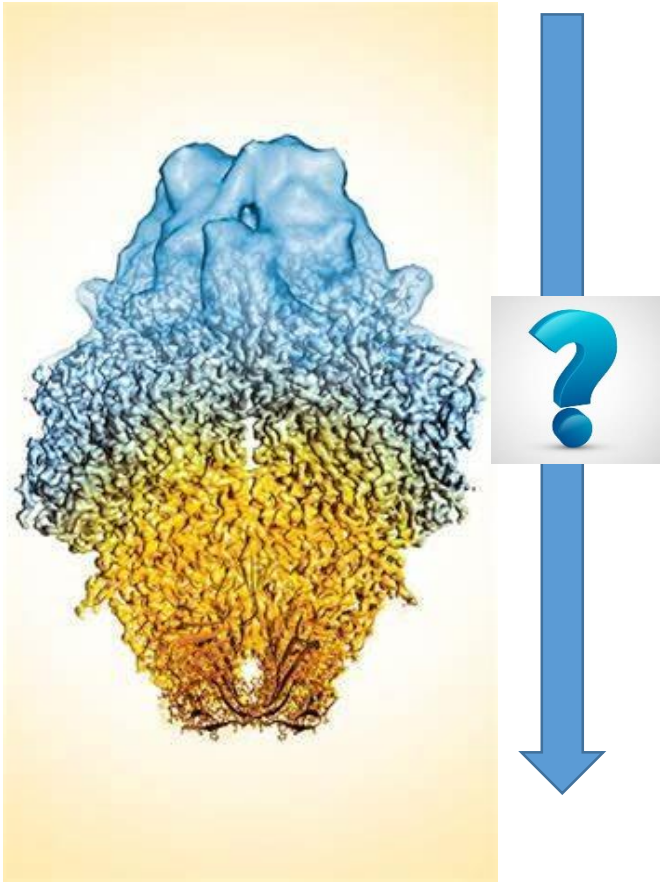


Frank et al Nature 1995

Joachim Frank

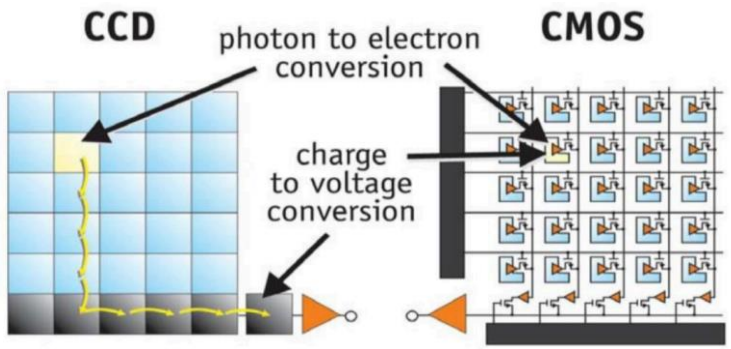
Processing of Cryo EM





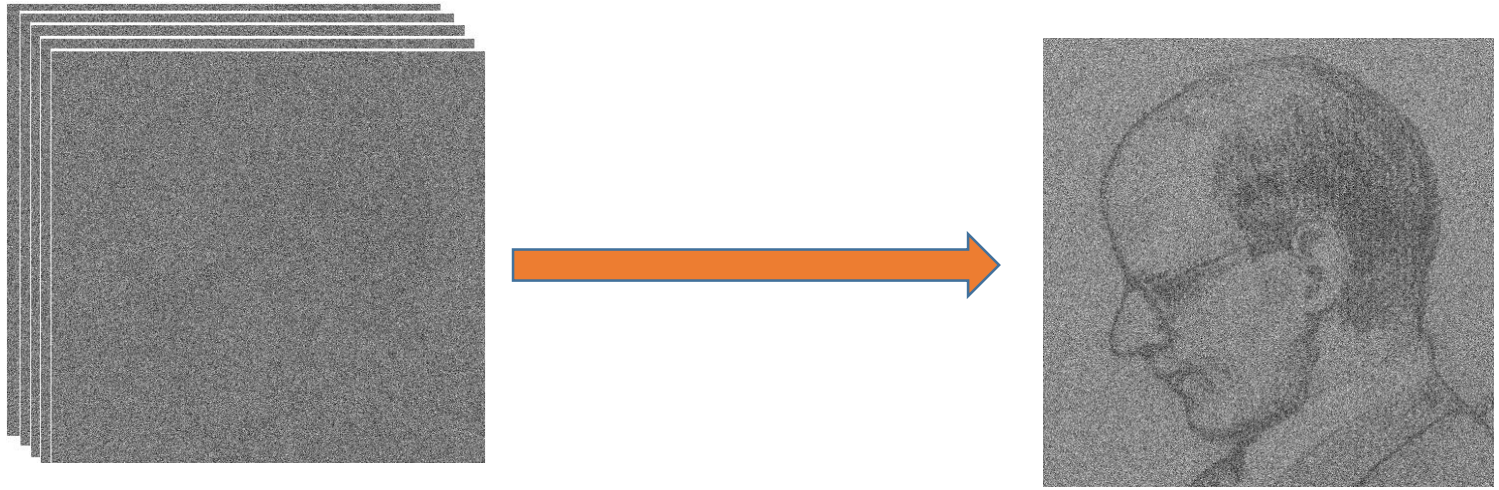
Processing of the method of Cryo EM to solve a structure

Direct electron detection camera



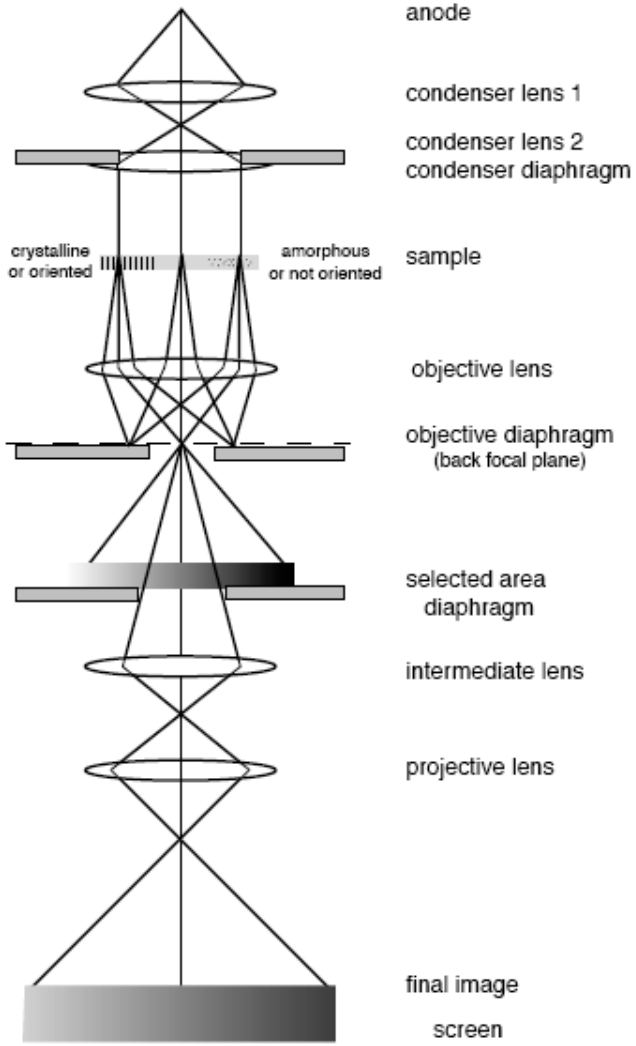
Unmatched DQE (detective quantum efficiency) up to 80%
Used for ground-breaking structural cryo-EM discoveries
Best resolution and contrast
High resolution imaging below 500 kDa
Resolve differences with heterogeneous samples
High quality cryo-tomography
4x larger area in each image, with super-resolution mode

Richard Henderson

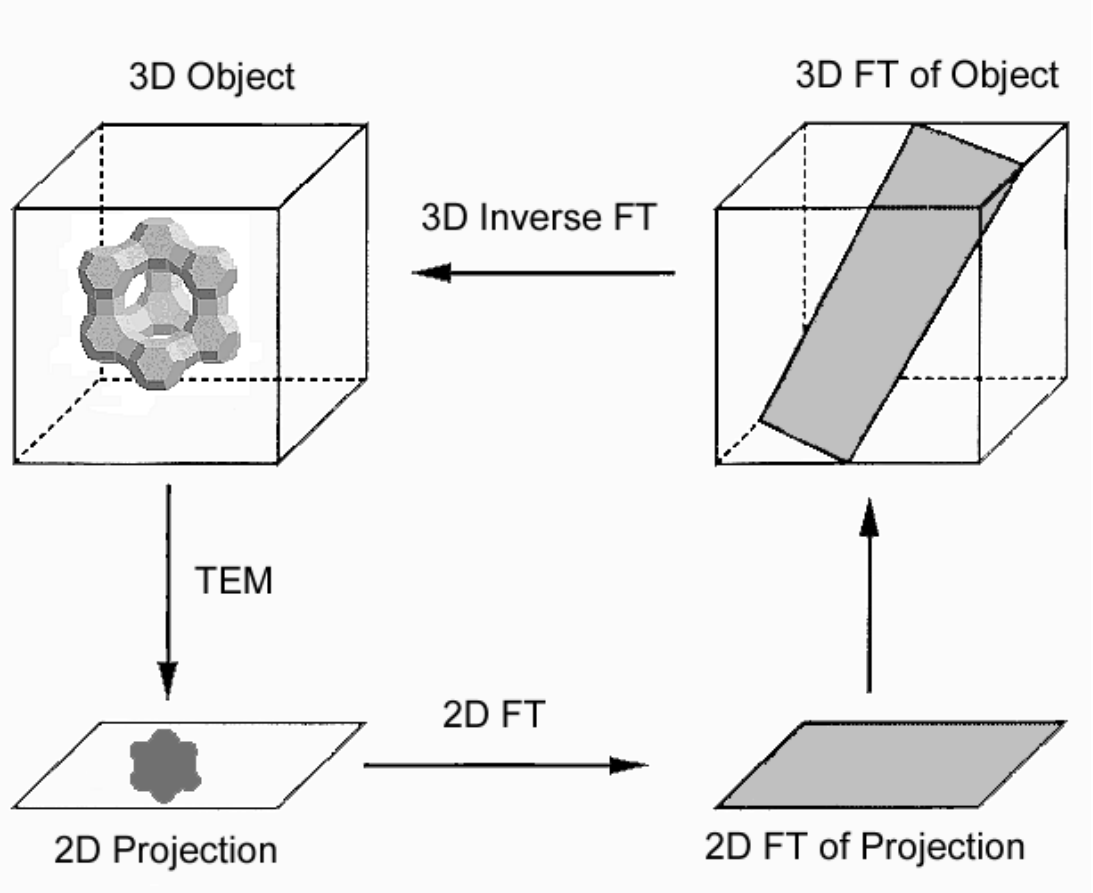


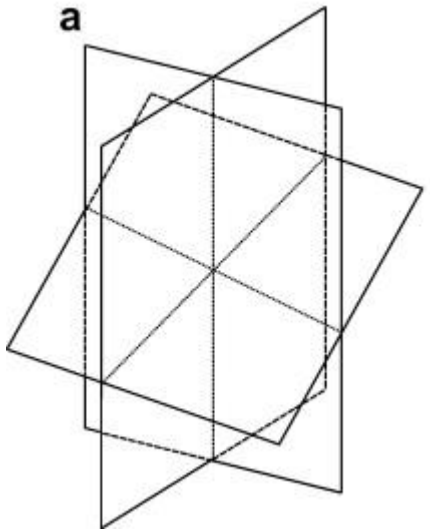
Principle of Cryo EM

The equipment



Principle of Cryo-EM



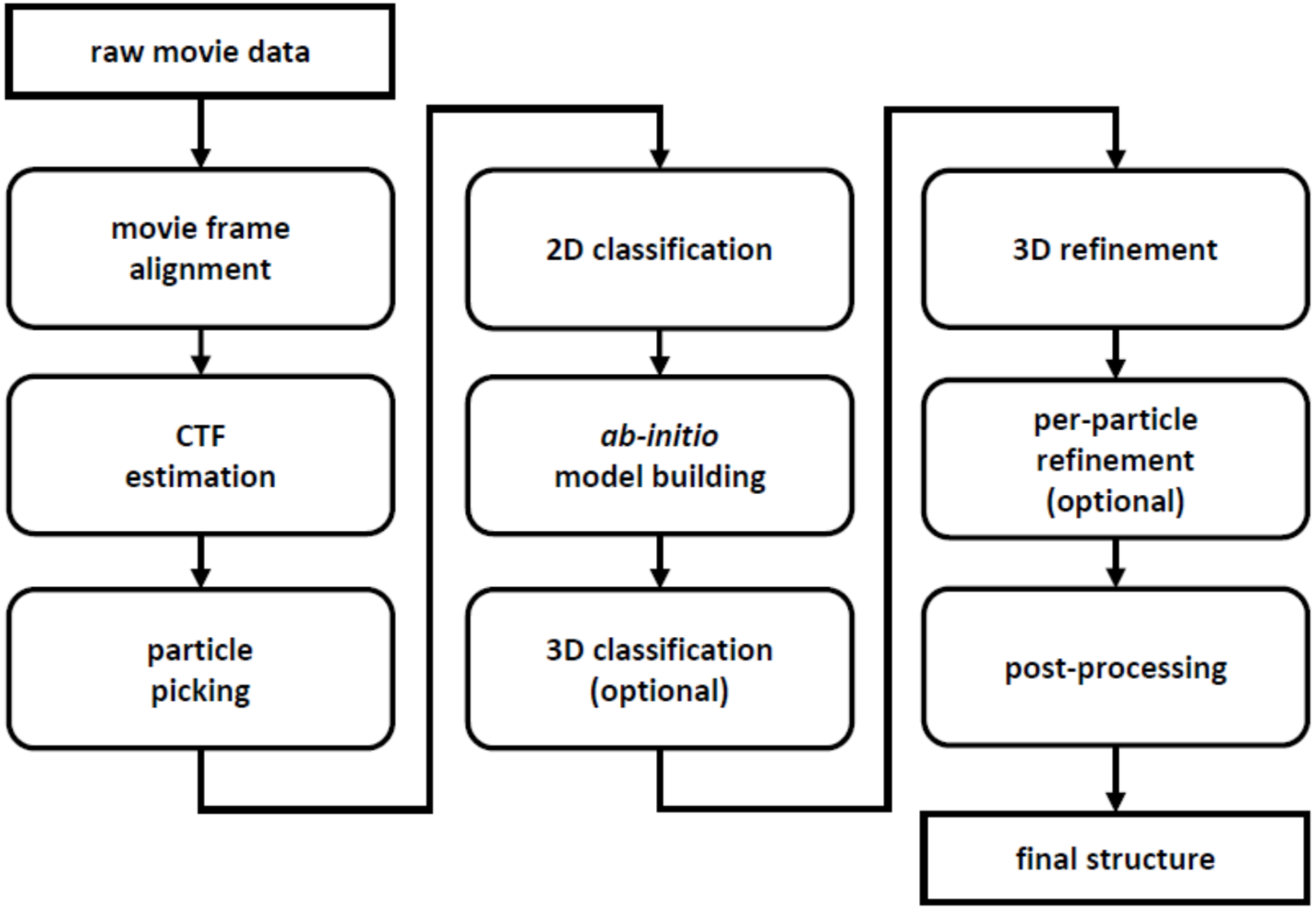


$(x, y, \theta, \psi, \phi)$

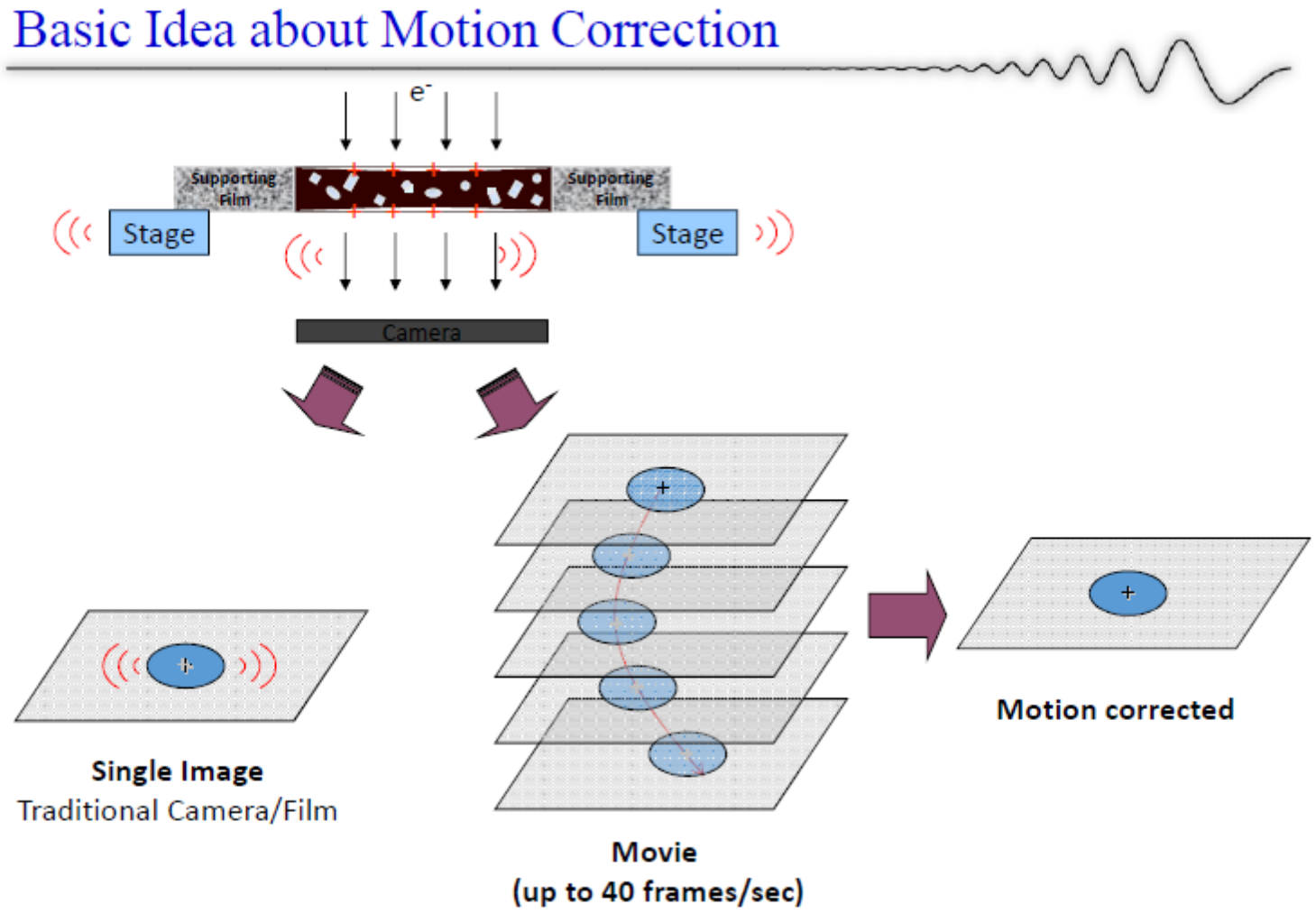
Projection--slice theorem

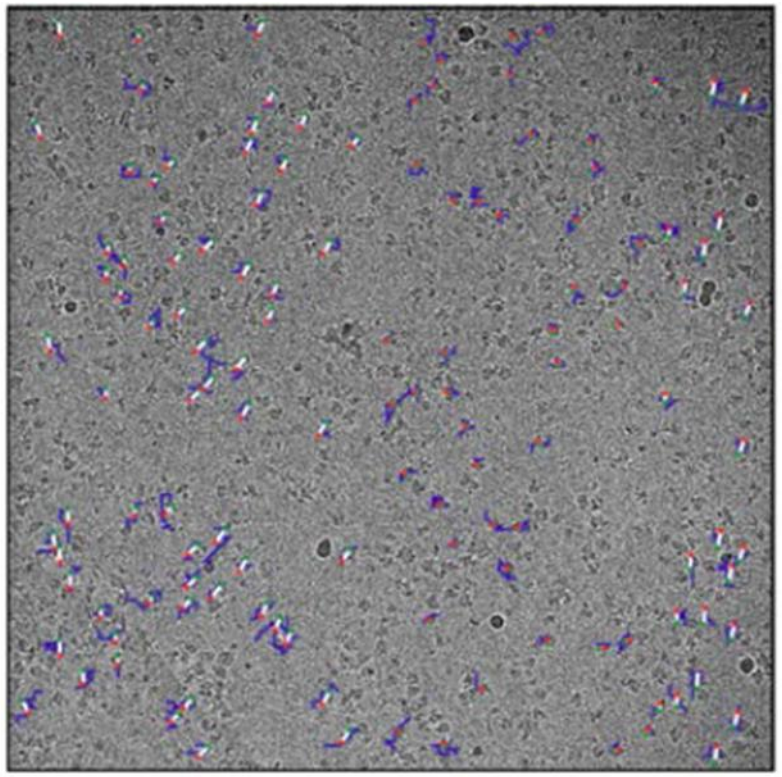
The F.T. of a 2D projection of a 3D object is a central slice through the 3D F.T. of that object

Flowchart of Single Particle Reconstruction

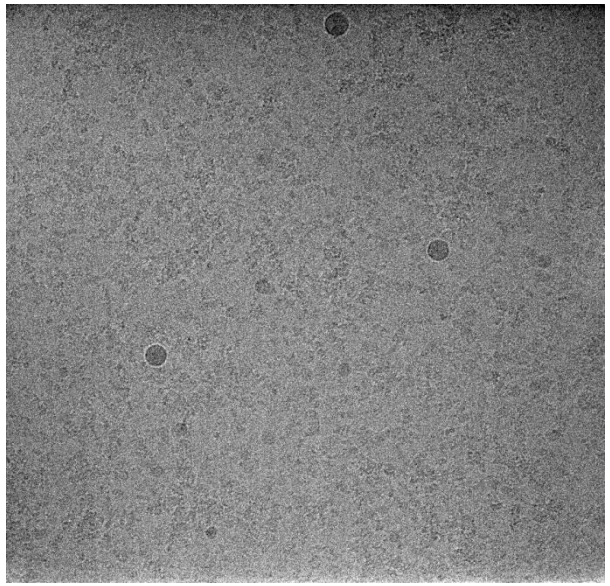


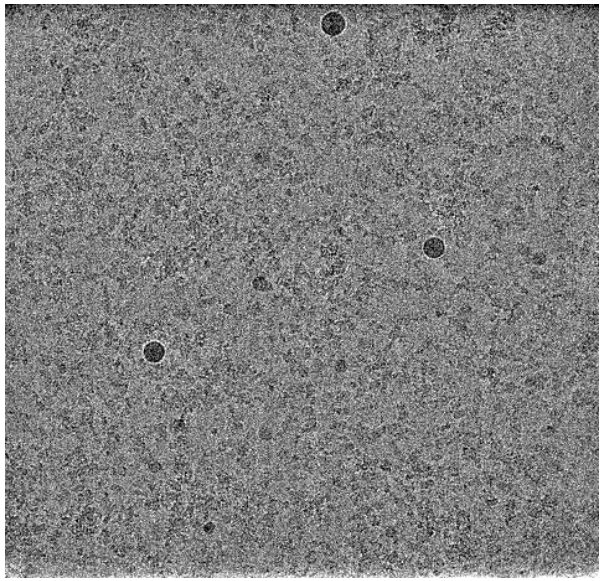
Motion Correction

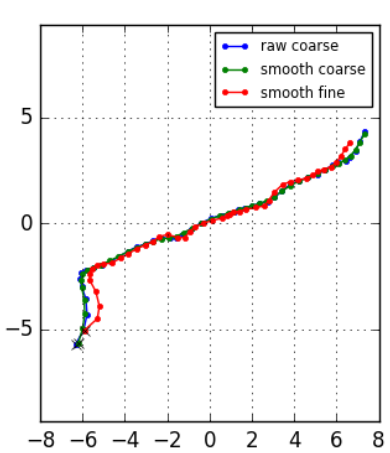


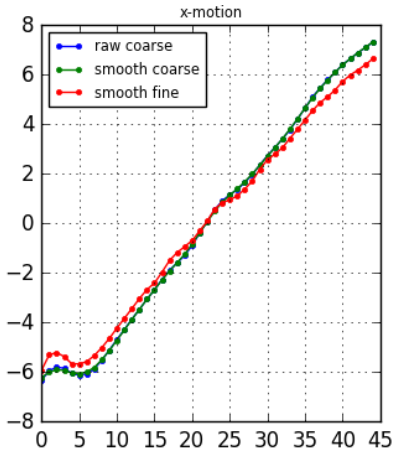


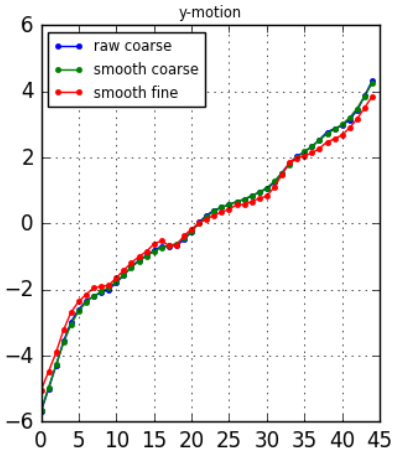
Motion Correction in Cryosparc

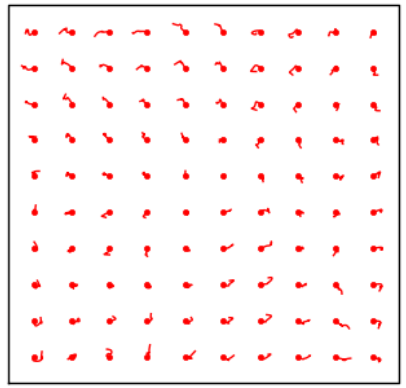








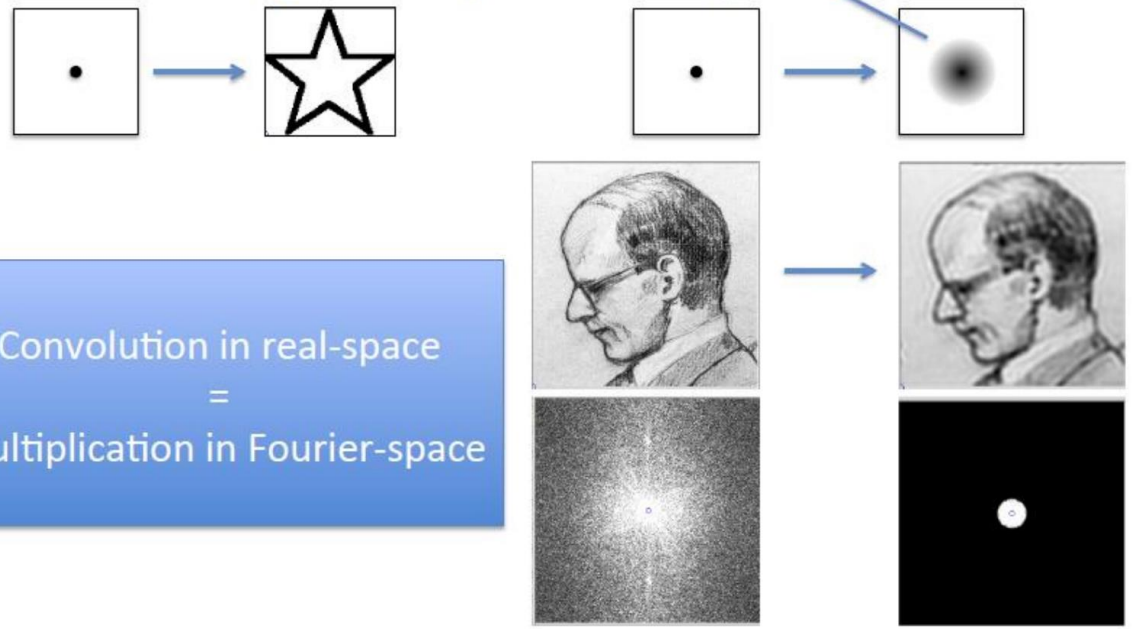




CTF Refinement

Point Spread Function

- Imperfect imaging:
  - Perfect signal -> Imperfect image



Convolution in real-space
=
Multiplication in Fourier-space

CTF equations...

s: frequency

A: Amplitude contrast: 4-7% in cryo-EM

$$\mathrm{CTF}(\vec{s}) = \sqrt{1 - A^2} \cdot \sin\left(\gamma(\vec{s})\right) + A \cdot \cos\left(\gamma(\vec{s})\right)$$

$$\gamma(\vec{s}) = \gamma(s,\theta) = -\frac{\pi}{2} C_s \lambda^3 s^4 + \pi \lambda z(\theta) s^2$$

REMEMBER!
CTF ≈ a sine function that varies with frequency and defocus

C_s: spherical aberration
λ: wavelength electrons
z: defocus

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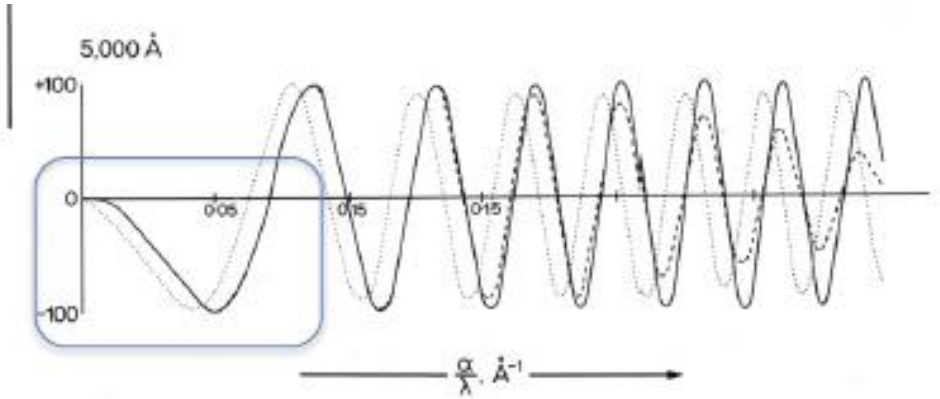
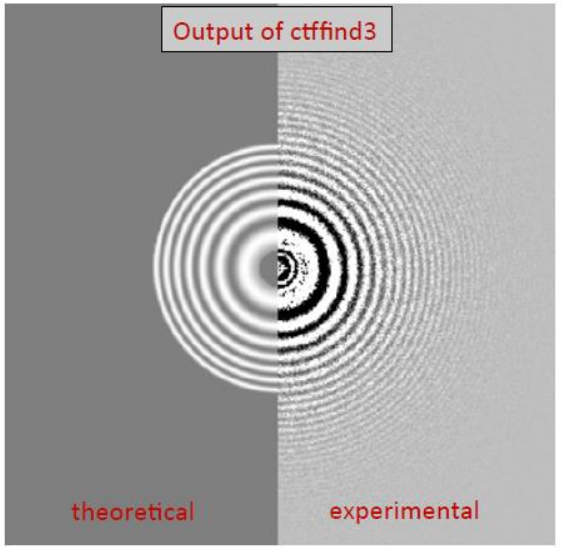
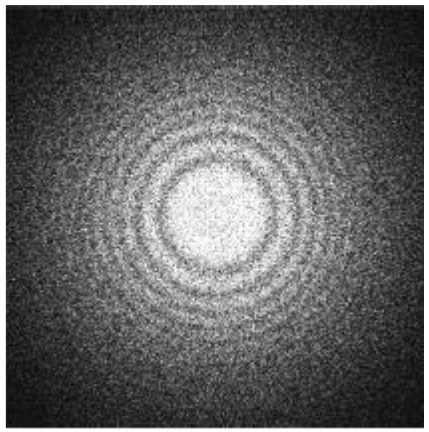
CTF Refienment

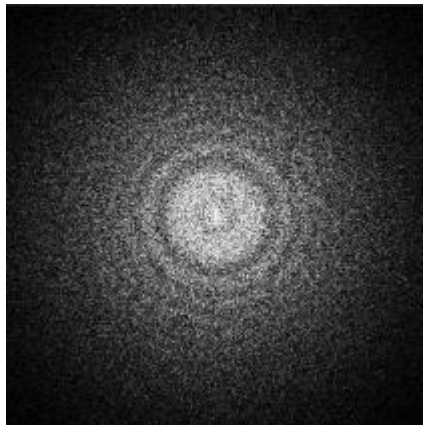
Starts negative...



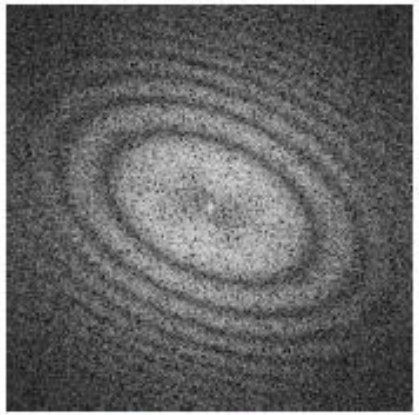




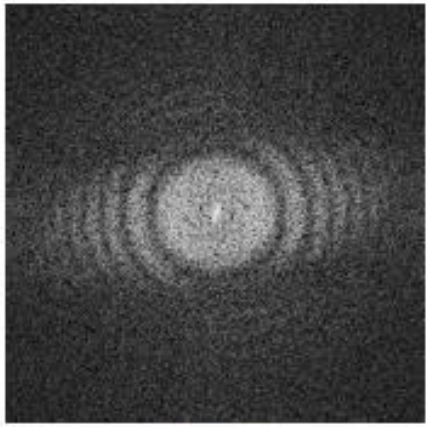
good



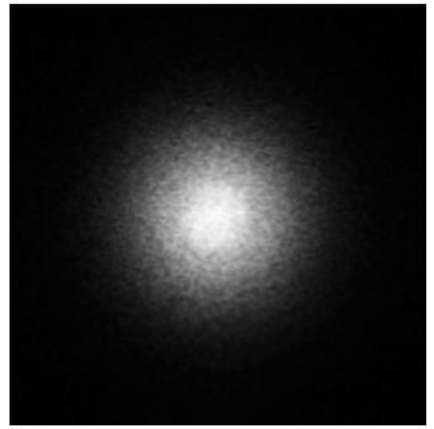
ice too thick



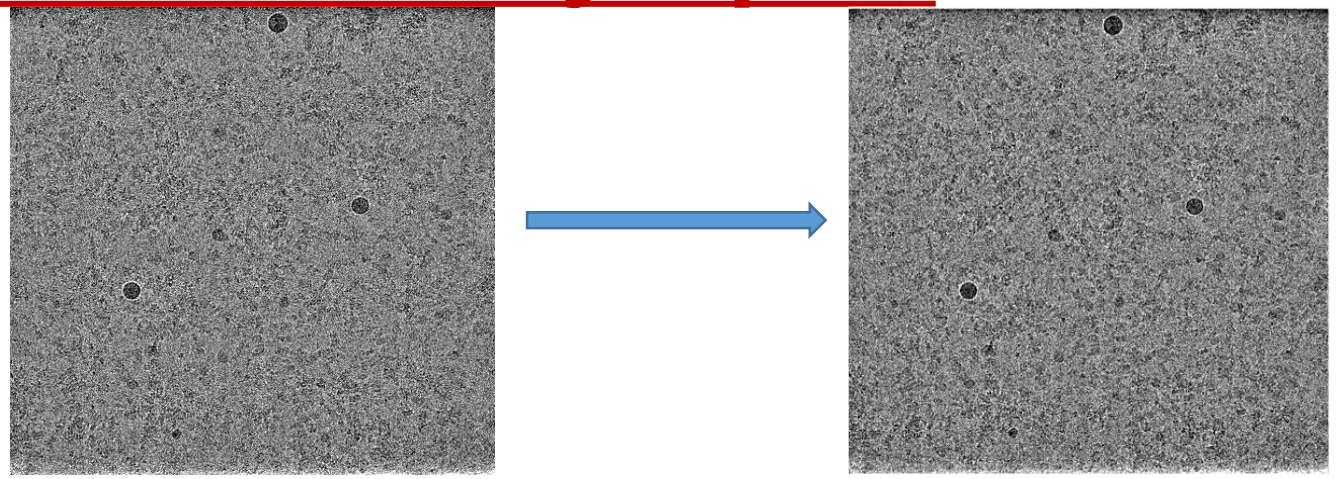
astigmatism

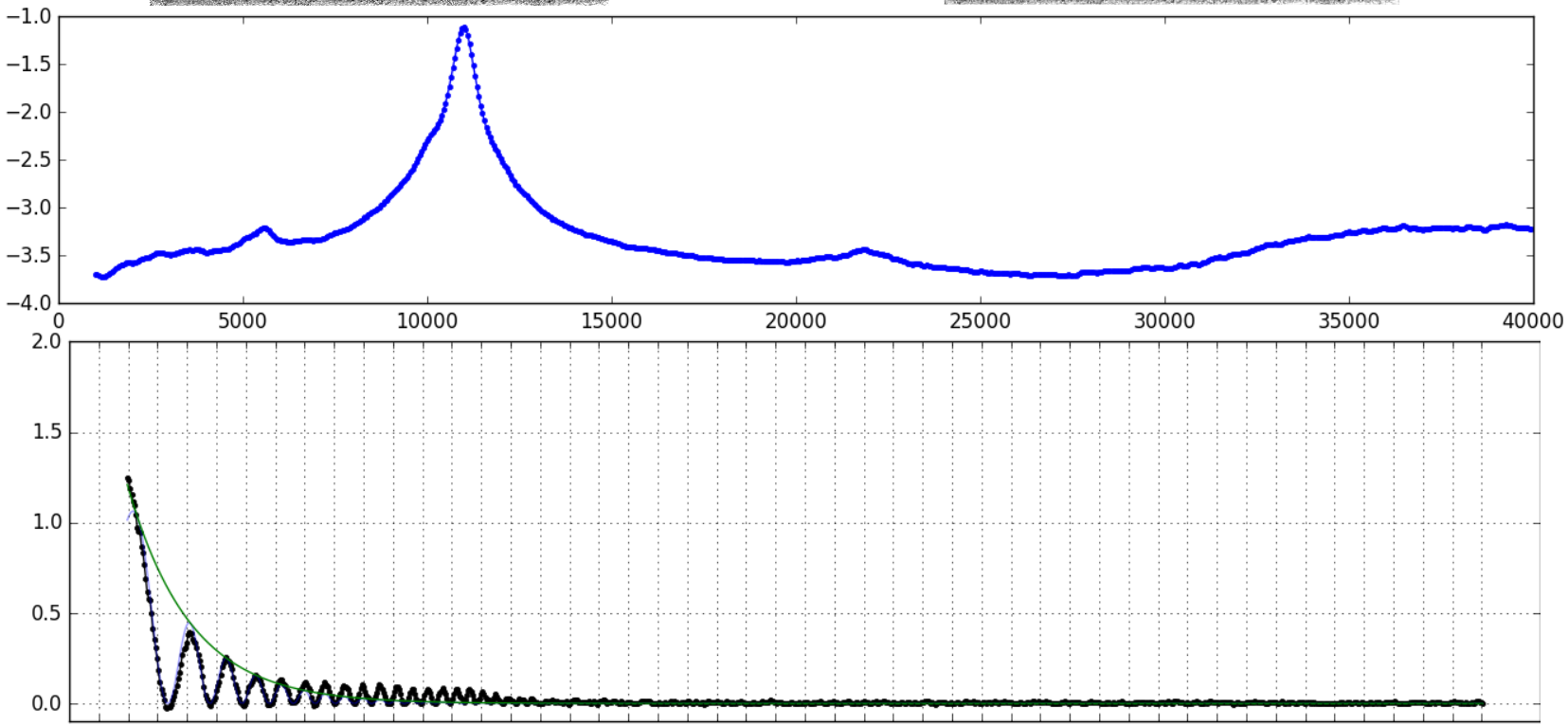


drift



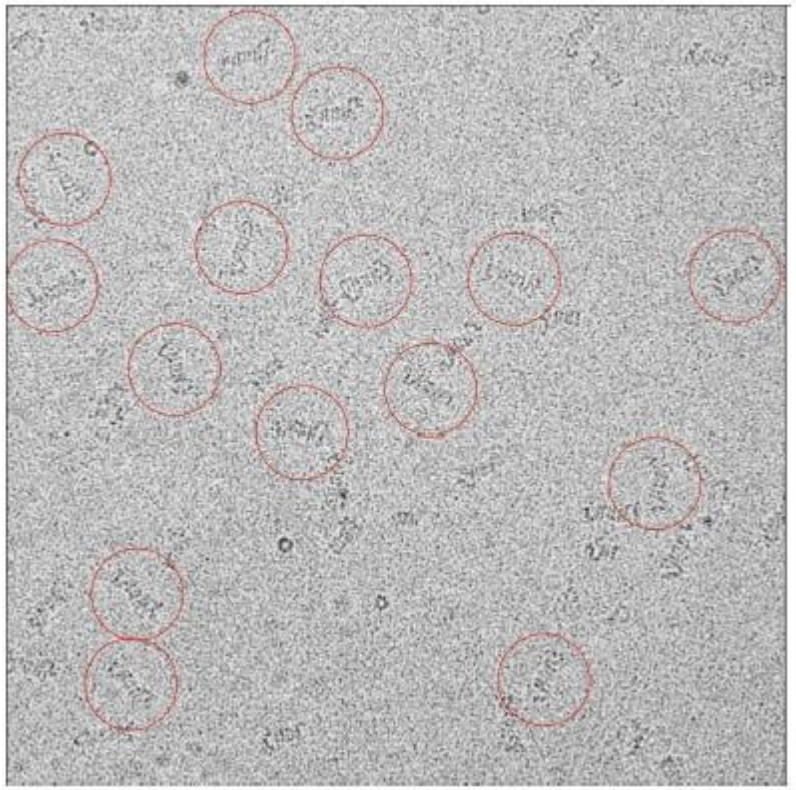
CTF refinement in Cryosparc





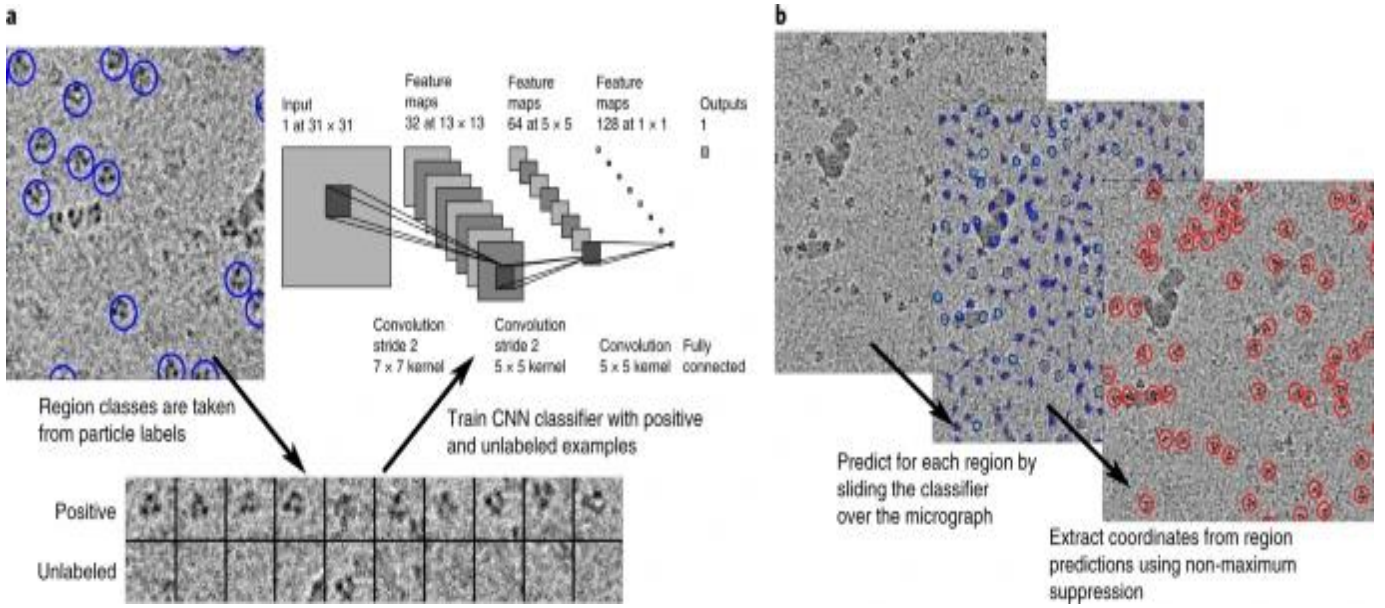
Particle picking

Particle picking



- it can be done manually -

Topaz (deep learning picking)



Positive-unlabeled convolutional neural networks for particle picking in cryo-electron micrographs. Tristan Bepler, Andrew Morin, Micah Rapp, Julia Brasch, Lawrence Shapiro, Alex J. Noble & Bonnie Berger Nature Methods volume 16, pages1153–1160(2019)

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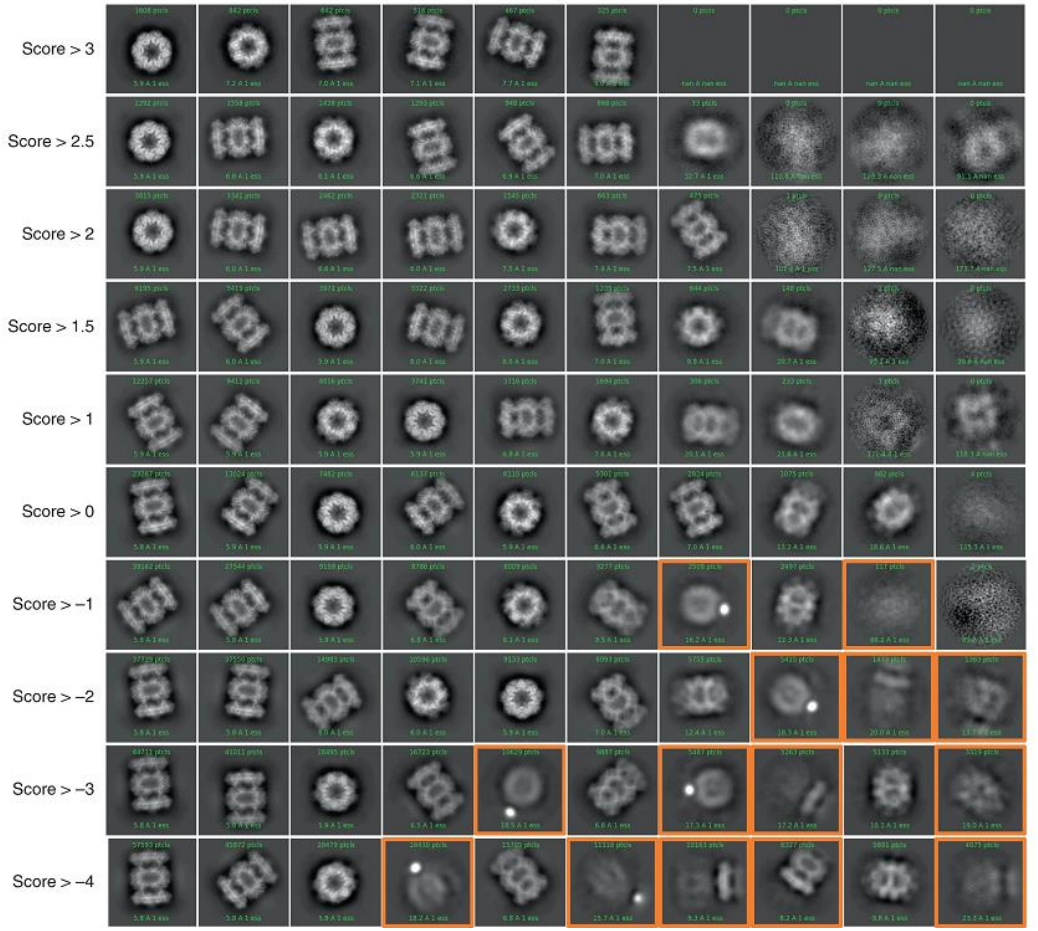
2D alignment and classification

2D Classification

- Reject noisy particles
- 2D images classification
  - 2D images alignment
  - 2D images average



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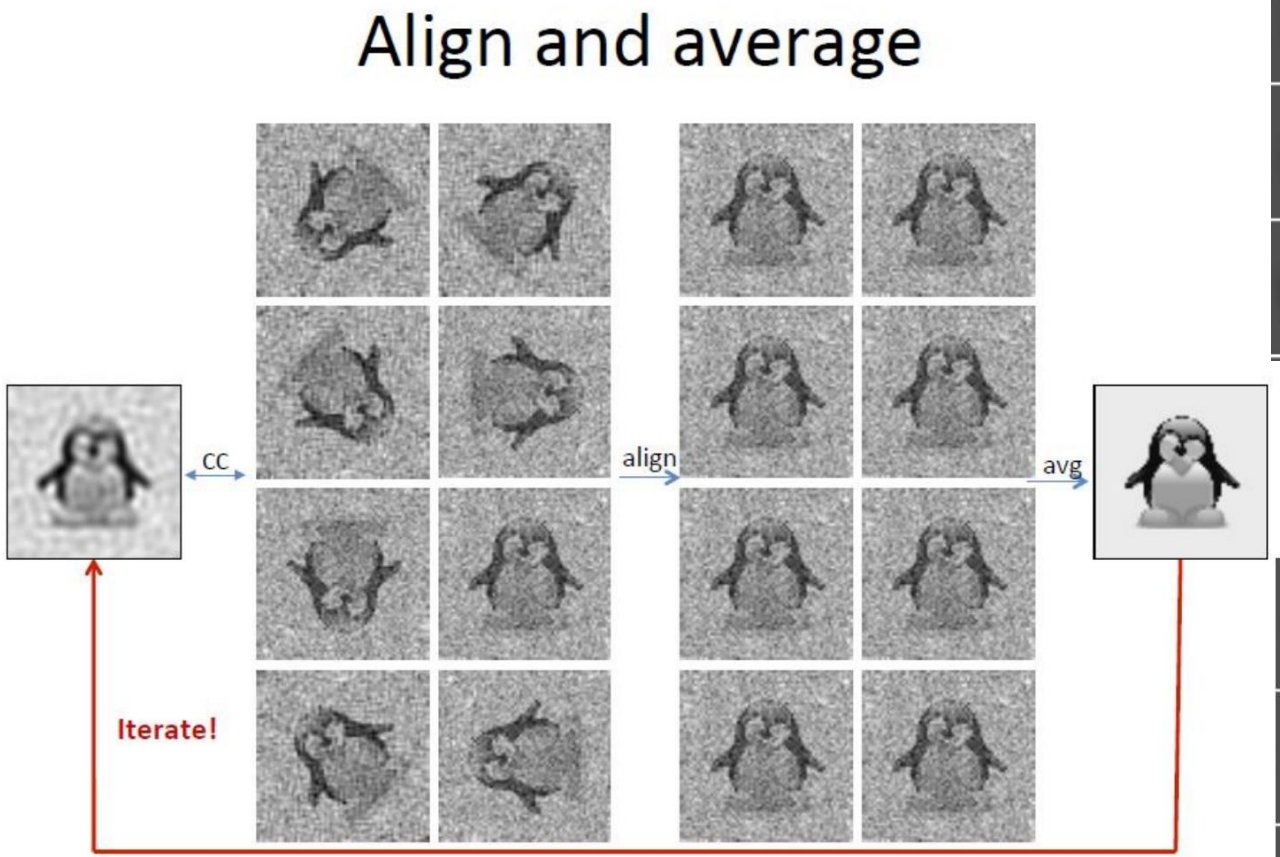
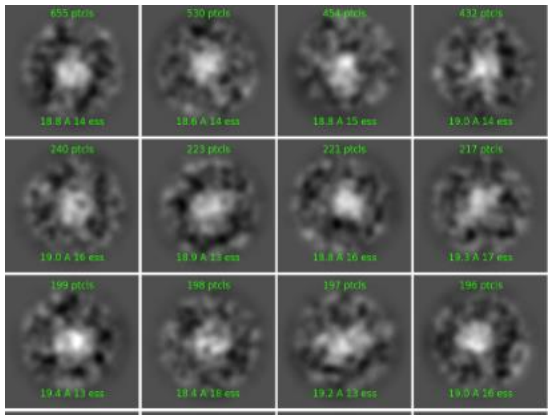
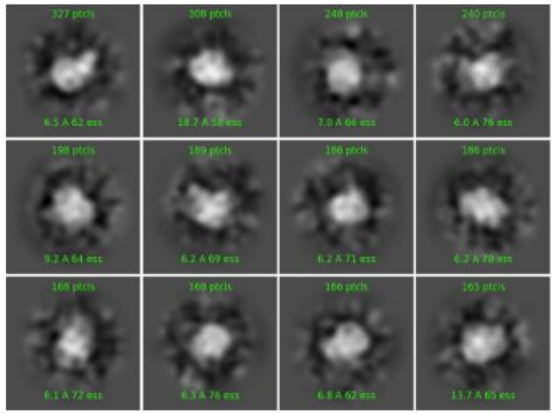
2D alignment

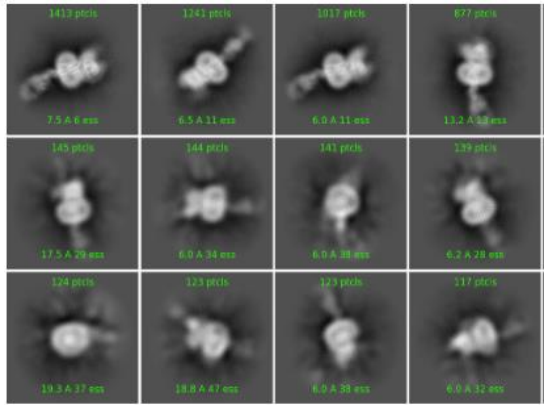




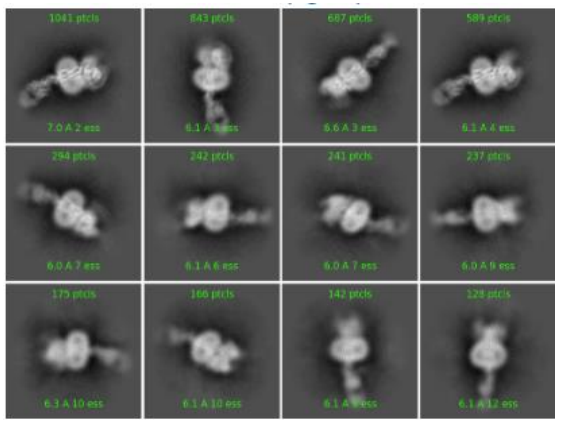
Iteration 0



Iteration 1



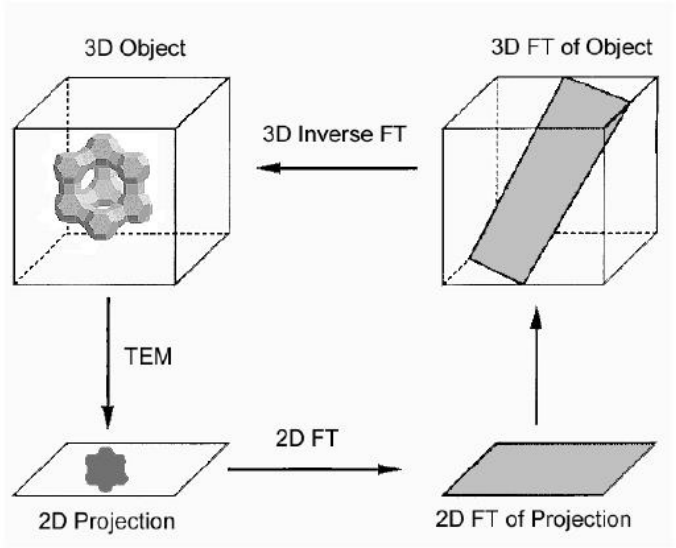
Iteration 5



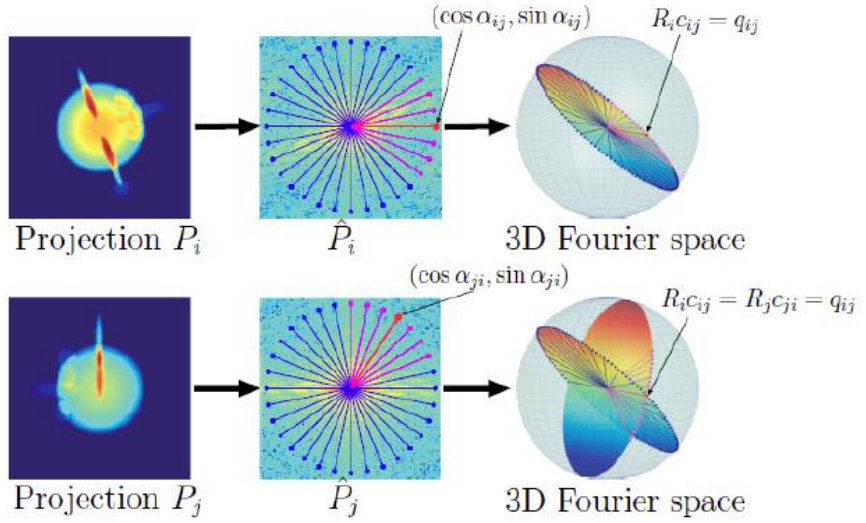
Iteration 10

Initial volume reconstruction

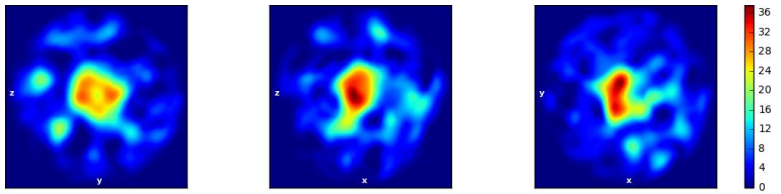
Option 5: Central Slice Theorem



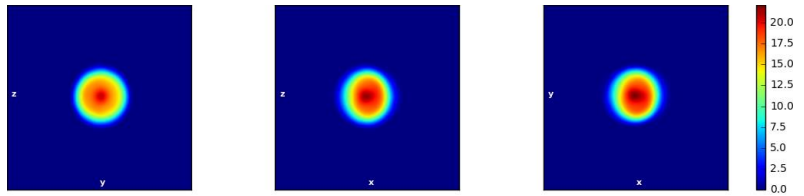
Option 5: Common lines



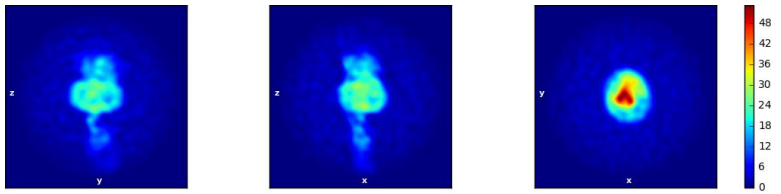
Initial volume reconstruction in Cryosparc



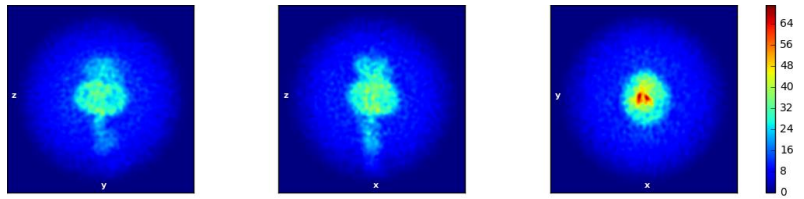
Start Model



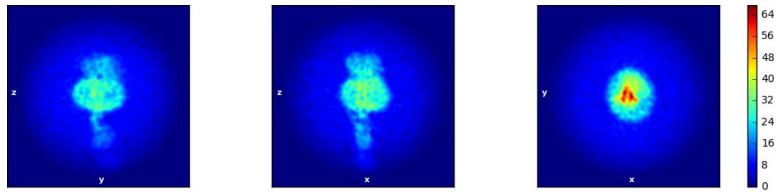
Cycle 100



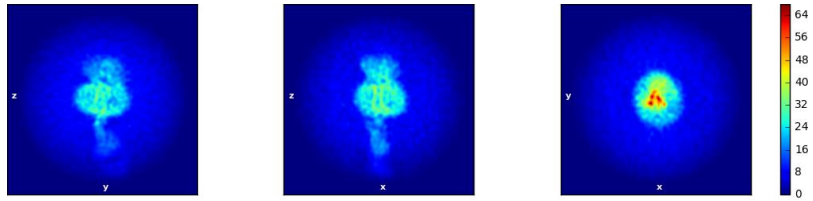
Cycle 500



Cycle 1000



Cycle 1500



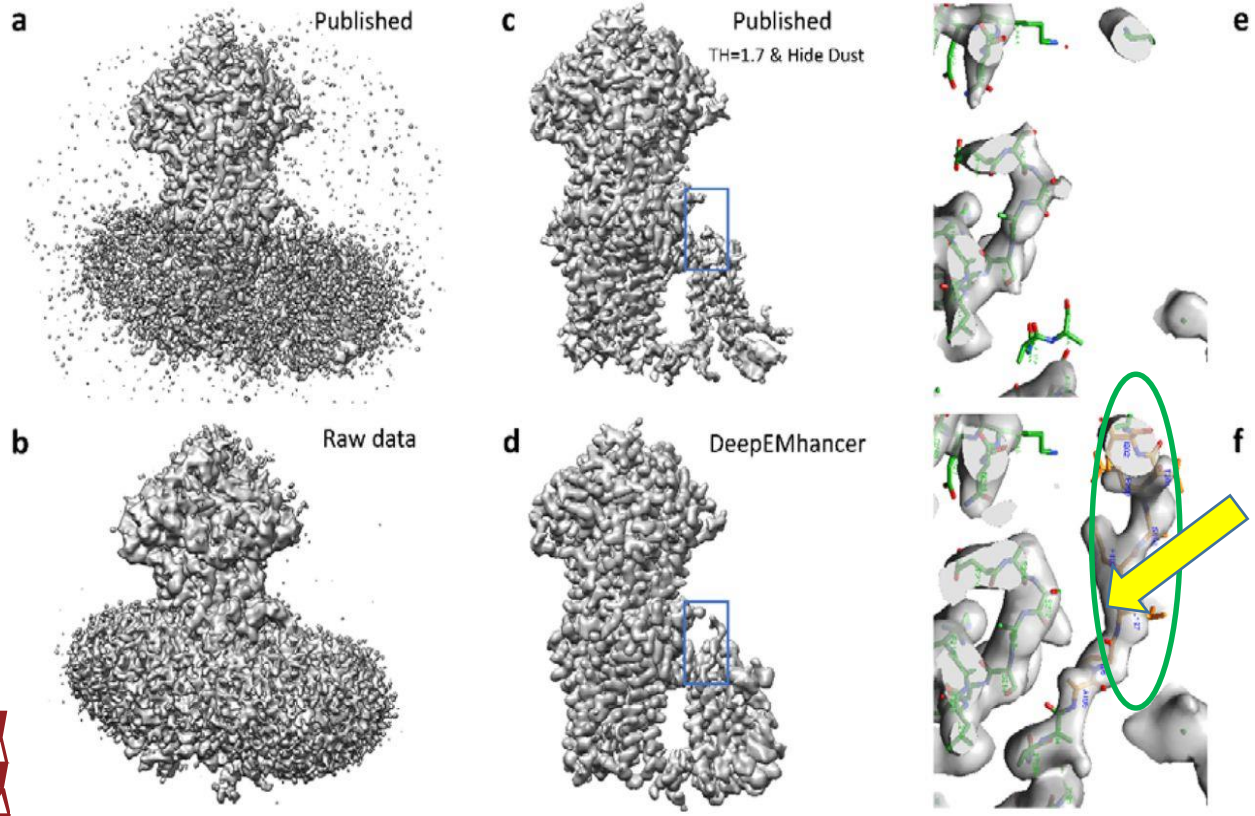
Final Model

- Small Sets of Particles.
- Low resolution map.
- Need further refinement.

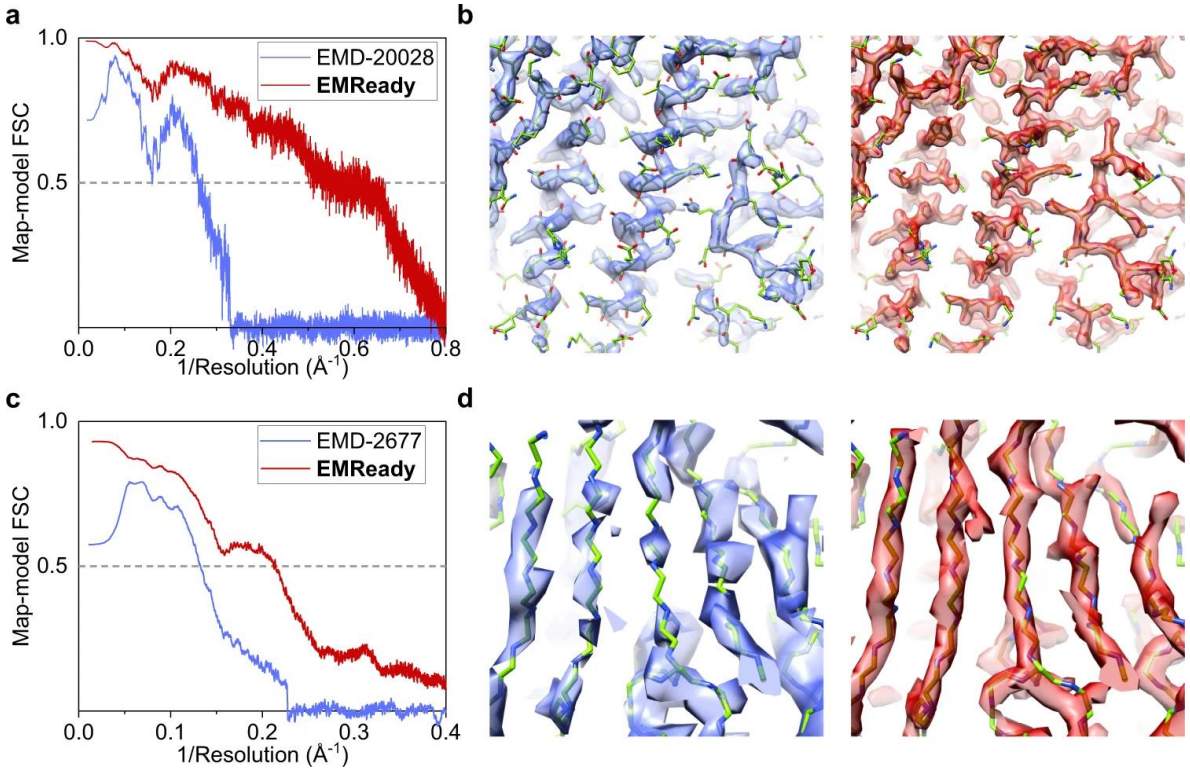
Post processing (B sharping)

上海科技大学 ShanghaiTech University

DeepEMhancer



EM Ready



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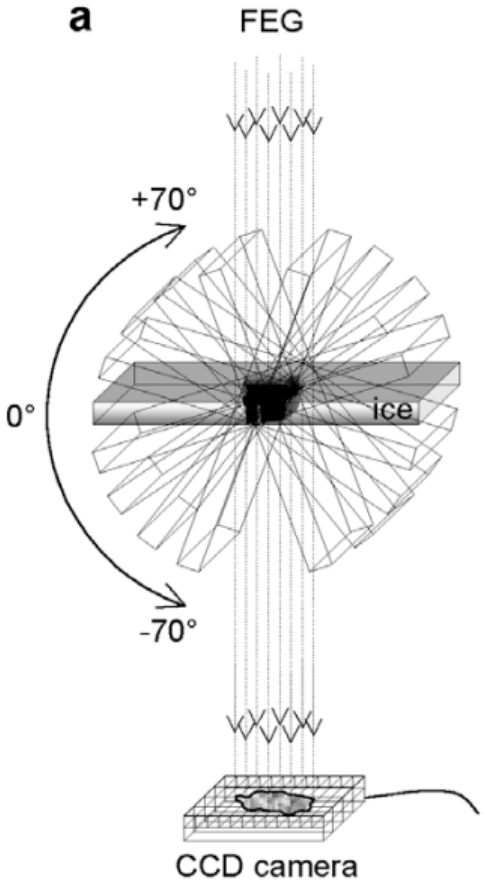
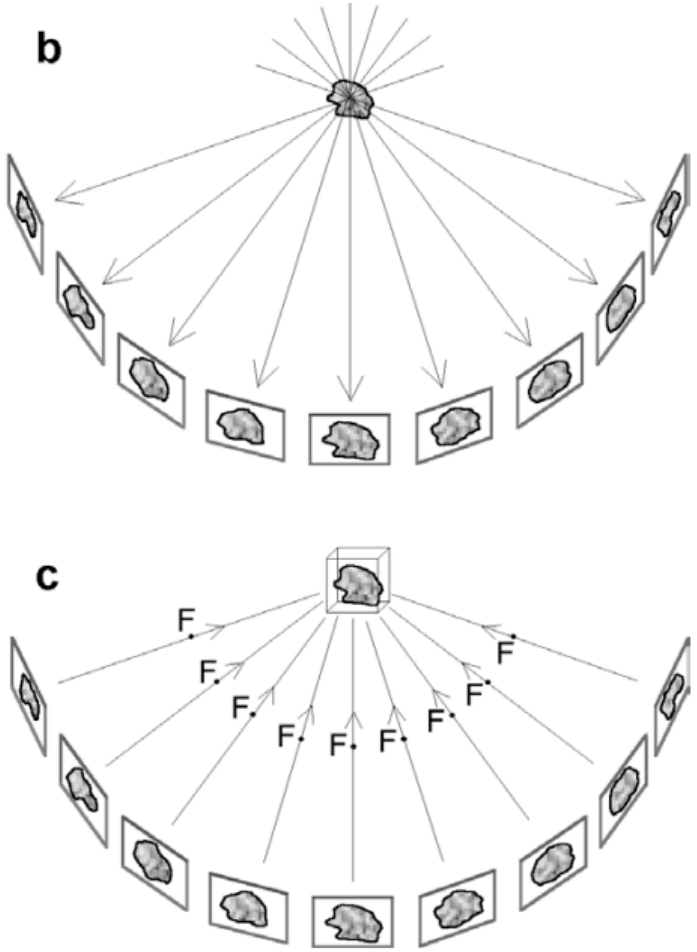
Principle of Cryo ET

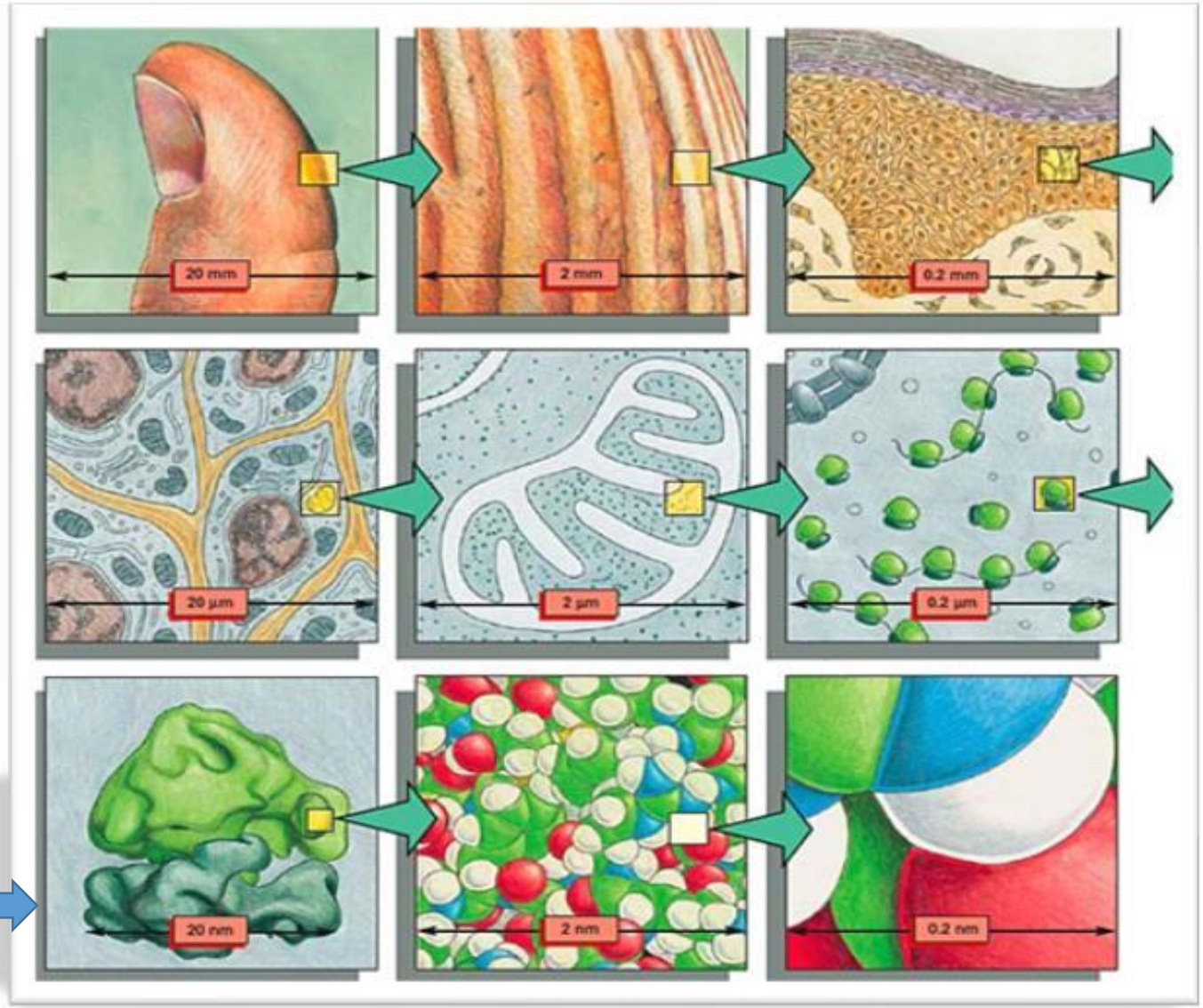




- In site experiment
- Single Particle

- Low dose
- Low resolution

Electron cryotomography



CryoET is here

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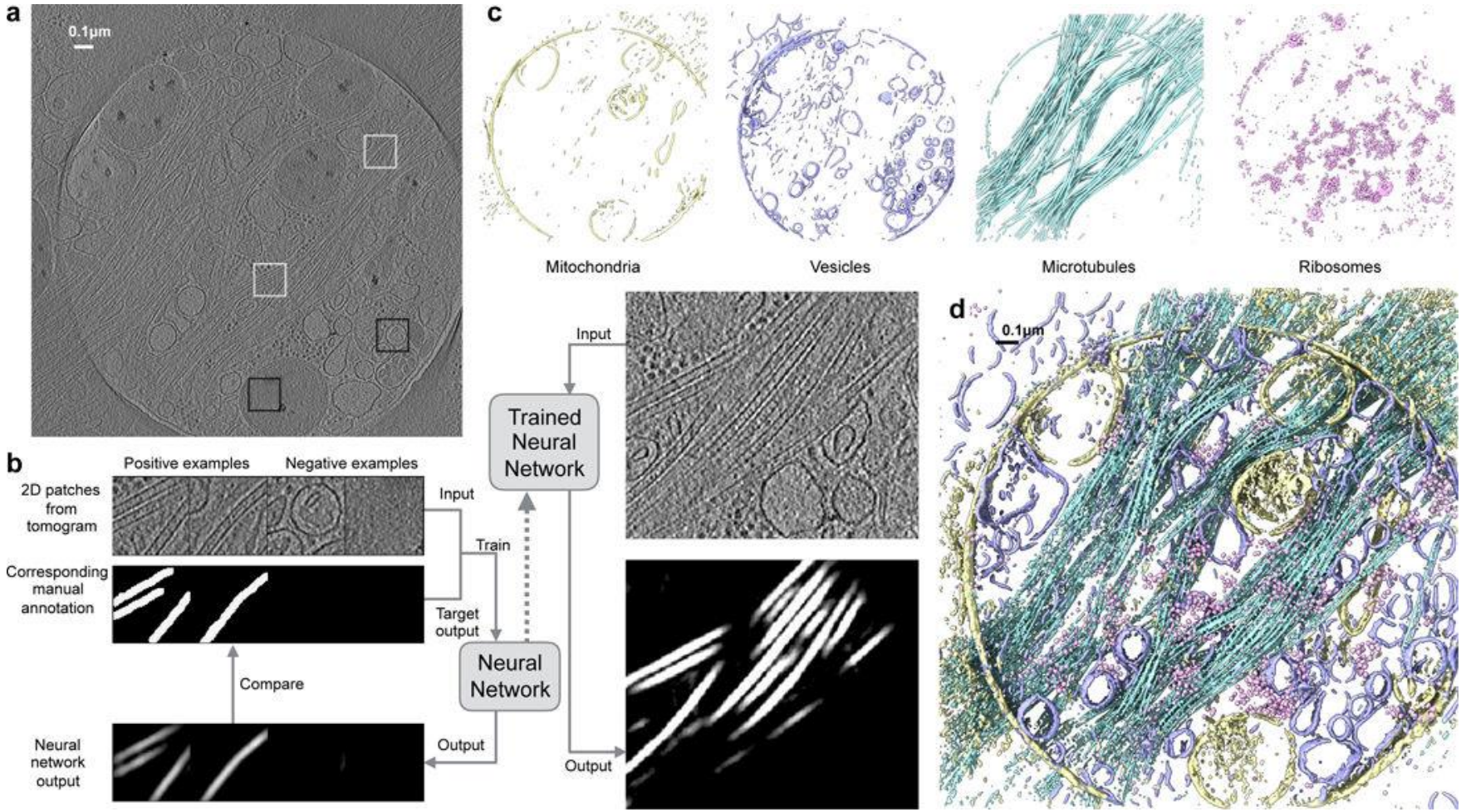
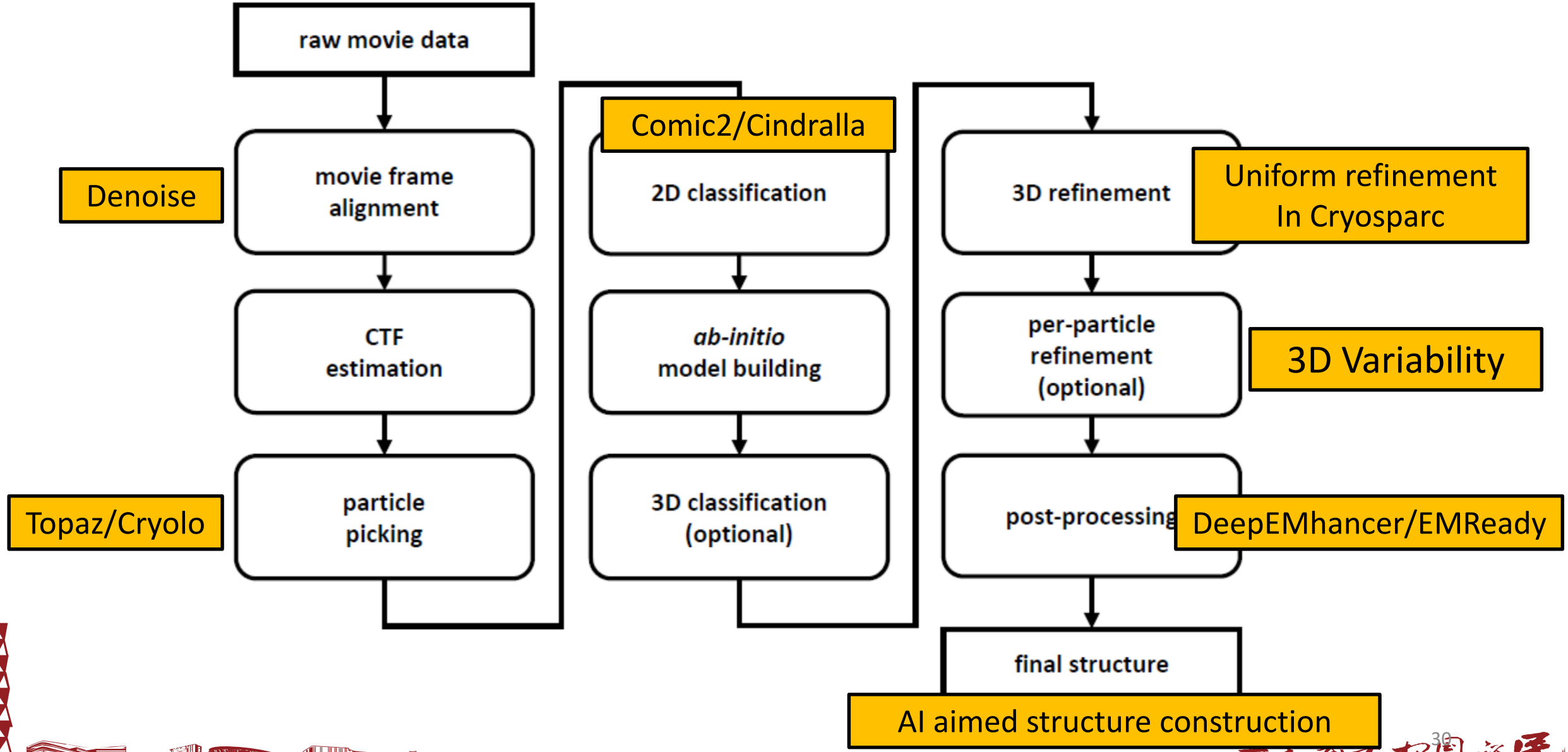
AI in electron cryotomography

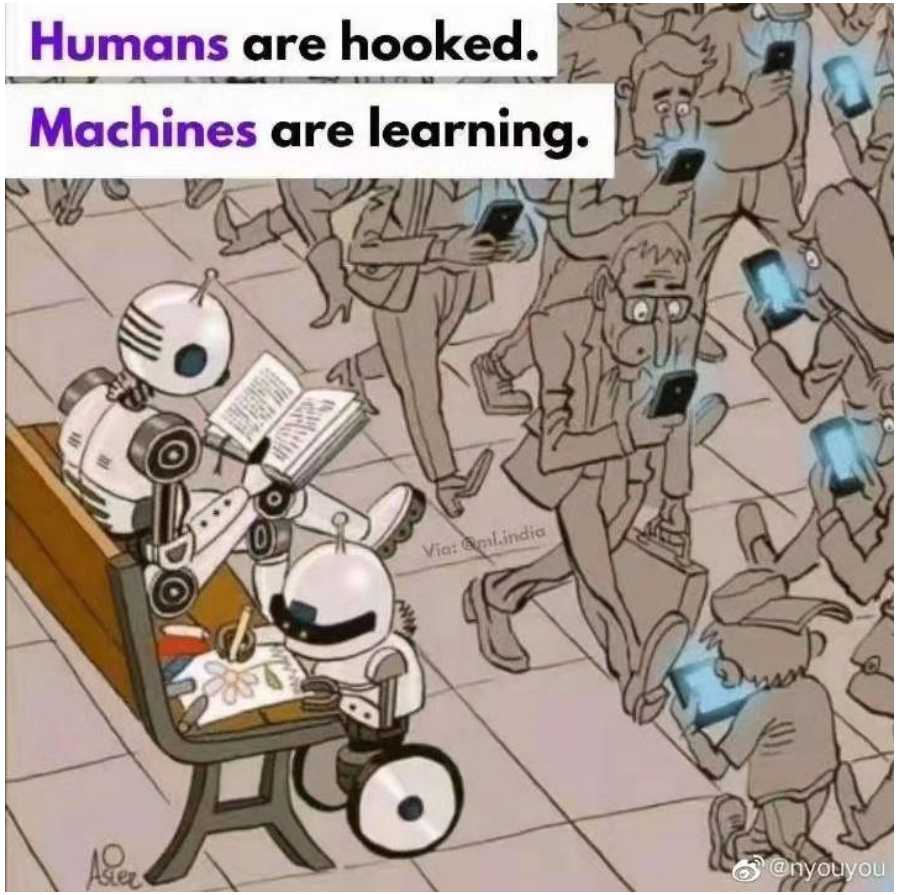


Nat Methods. Author manuscript; available in PMC 2018 February 28.

Method of AI in CryoEM



Thank you!


Humans are hooked.
Machines are learning.
Via: @ml.india
@nyouyou