



Science and
Technology
Facilities Council

Cryo-EM: 3D Electron Microscopy

Colin Palmer

DLS-CCP4 MX workshop, December 2019





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Facilities Council

What is CCP-EM?

What is CCP-EM?

Collaborative **C**omputational **P**roject for **E**lectron
cryo-**M**icroscopy

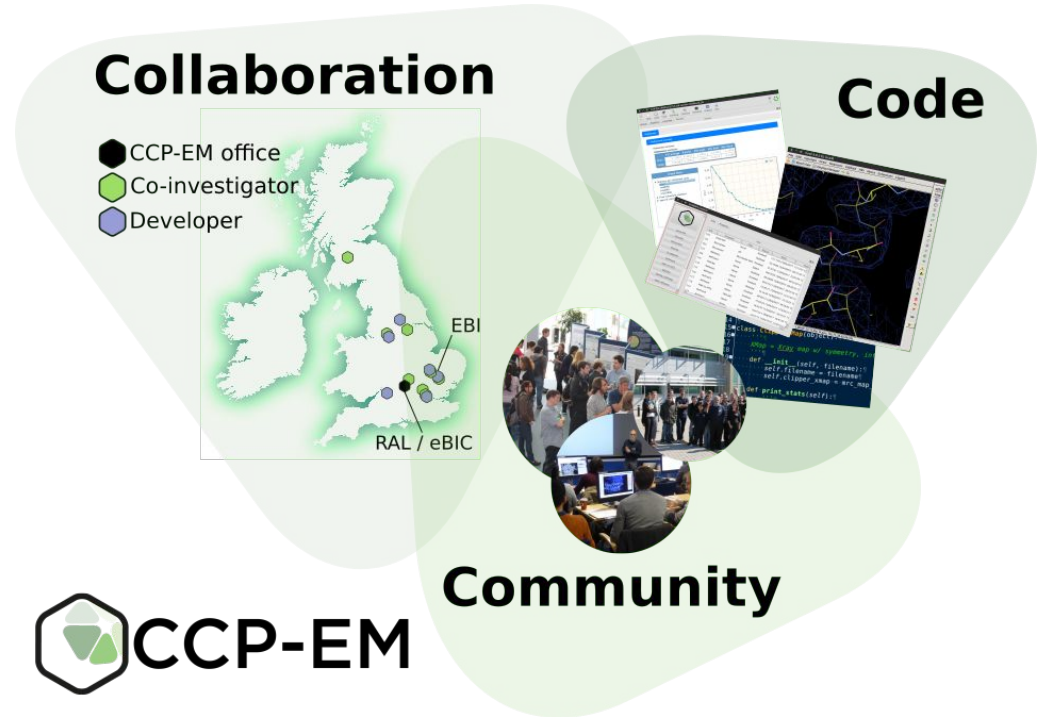
Cryo-EM equivalent of CCP4

(see also CCPN, CCPBioSim, CCP-SAS and others)

Support users and developers in computational
aspects of biological EM

1. Build UK EM community
2. Support software users
3. Support developers

Started in 2012



CCP-EM software suite

Same concept as CCP4

Suite of utilities for EM data processing

Common Python framework

Uses some CCP4 programs

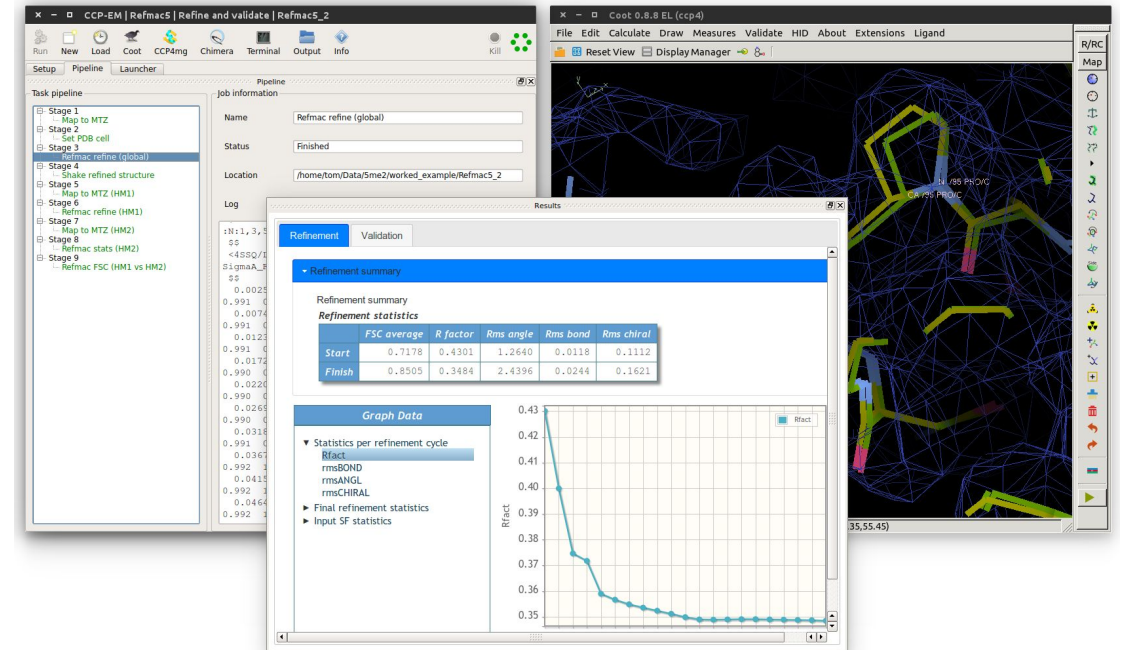
Download from ccpem.ac.uk

Linux & Mac

Free for academic use, fee for commercial

Bugs & requests:

ccpem@stfc.ac.uk



The screenshot shows the CCP-EM website at www.ccpem.ac.uk/download.php. The header includes the CCP-EM logo, the text 'Collaborative Computational Project for Electron cryo-Microscopy', and the MRC Medical Research Council logo. The navigation bar lists: Home, About CCP-EM, CCP-EM Projects, Downloads, Resources / Documentation, Workshops / Courses, and Symposium. The main heading is 'CCP-EM downloads'. The content area features the CCP-EM software suite logo, the latest release information, and links to download the software for Linux and Mac, along with release notes and an overview.

CCP-EM software suite

Latest release

[CCP-EM v1.3.0 Linux](#) (updated 12 April 2019)

[CCP-EM v1.3.0 Mac](#) (updated 12 April 2019)

[Release notes](#). See below for installation instructions.

Overview

CCP-EM Spring Symposium

National UK cryo-EM conference

“Study weekend for cryo-EM”

Next edition: 27–29 April 2020, University of Nottingham

Confirmed speakers include:

- Gabriel Lander
- Chun-wa Chung
- Gaia Pigino
- Chris Russo
- and many more...

Registration opening soon!

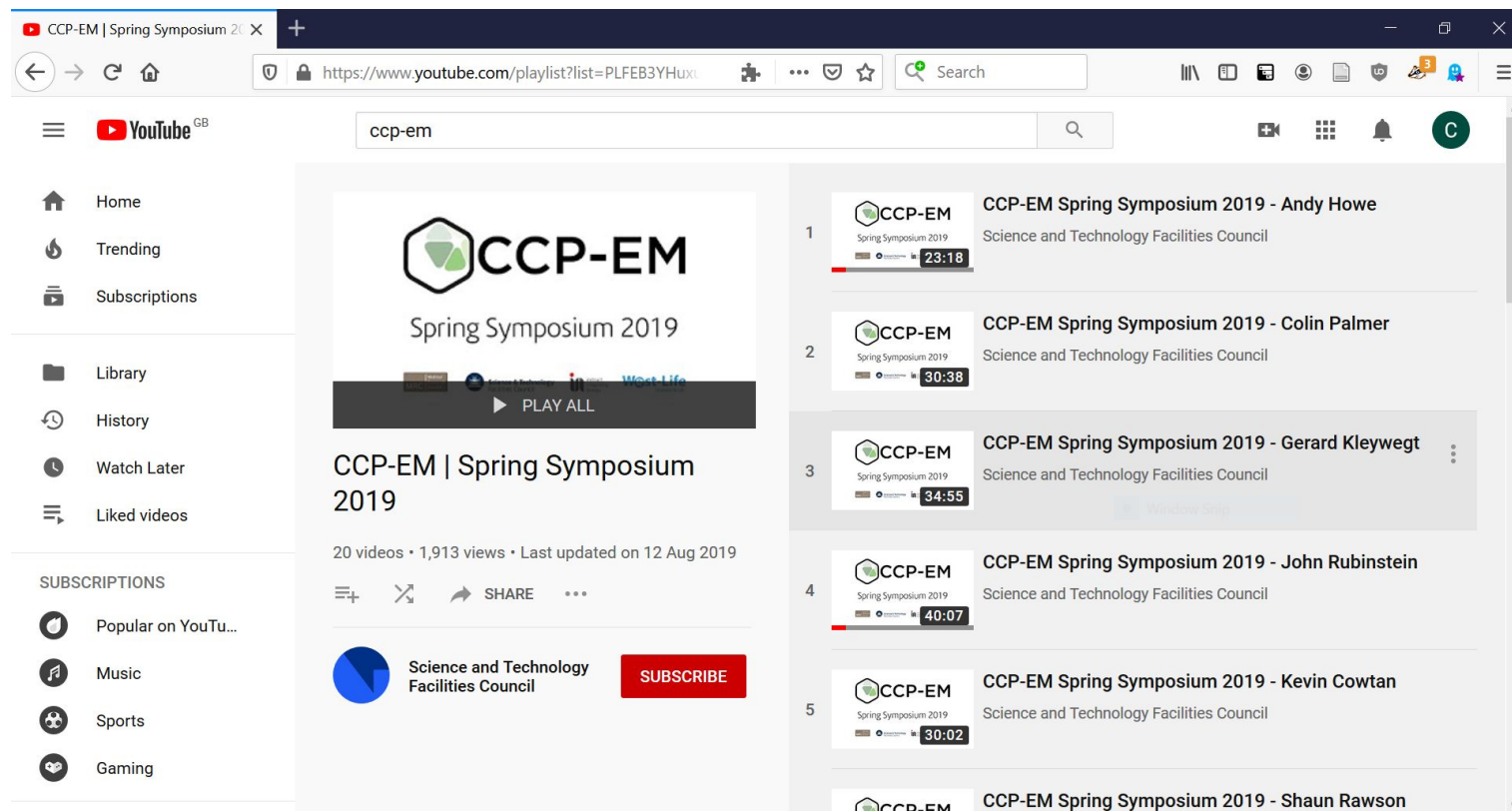
See CCP-EM mailing list for announcements



CCP-EM Spring Symposium

Talks from previous years:

- Schedules on www.ccpem.ac.uk
- Talks on YouTube (search “CCP-EM”)



The screenshot shows a web browser window displaying a YouTube playlist titled "CCP-EM | Spring Symposium 2019". The browser's address bar shows the URL <https://www.youtube.com/playlist?list=PLFEB3YHuxi>. The YouTube interface includes a left sidebar with navigation options like Home, Trending, Subscriptions, Library, History, Watch Later, and Liked videos. The main content area features the CCP-EM logo, the title "Spring Symposium 2019", and a "PLAY ALL" button. Below this, it states "CCP-EM | Spring Symposium 2019" with "20 videos • 1,913 views • Last updated on 12 Aug 2019". The channel name "Science and Technology Facilities Council" is visible with a "SUBSCRIBE" button. On the right, a list of five video thumbnails is shown, each with the CCP-EM logo, the title "CCP-EM Spring Symposium 2019 - [Speaker Name]", and the duration. The speakers listed are Andy Howe (23:18), Colin Palmer (30:38), Gerard Kleywegt (34:55), John Rubinstein (40:07), and Kevin Cowtan (30:02). A sixth video by Shaun Rawson is partially visible at the bottom.

Video Number	Speaker	Duration
1	CCP-EM Spring Symposium 2019 - Andy Howe	23:18
2	CCP-EM Spring Symposium 2019 - Colin Palmer	30:38
3	CCP-EM Spring Symposium 2019 - Gerard Kleywegt	34:55
4	CCP-EM Spring Symposium 2019 - John Rubinstein	40:07
5	CCP-EM Spring Symposium 2019 - Kevin Cowtan	30:02
6	CCP-EM Spring Symposium 2019 - Shaun Rawson	-

CCP-EM Spring Symposium

Talks from previous years:

- Schedules on www.ccpem.ac.uk
- Talks on YouTube (search “CCP-EM”)
- Proceedings published in *Acta Cryst. D*

The screenshot displays the Acta Crystallographica Section D website. The header includes the journal title, ISSN (2059-7983), and navigation links. The main content area features the 'June 2018 issue' titled 'Proceedings of the third CCP-EM Spring Symposium', edited by Tom Burnley, Paula da Fonseca, and Randy Read. A cover illustration shows a 3D reconstruction of a biological specimen. The text below the illustration explains the challenges of cryo-EM sample preparation and the effects of preferred orientation. The right sidebar contains links to 'Tweets by @ActaCrystD', 'Special issues', and 'Meetings', including the Macromolecular Crystallography School 2019 and the 3rd Missouri Symposium in Molecular Biophysics.

Acta Crystallographica Section D
STRUCTURAL BIOLOGY

ISSN: 2059-7983

June 2018 issue
Proceedings of the third **CCP-EM** Spring Symposium
Edited by Tom Burnley, Paula da Fonseca and Randy Read

Cover illustration: Cryo-EM can now be used to obtain high-resolution structural information on a diverse range of biological specimens. However, for many single particle cryo-EM projects, a major bottleneck remains at the sample preparation stage, where issues such as specimen aggregation, preferred orientation and affinity for the support film prevent the collection of high quality cryo-EM data (see Drulyte *et al.*, p. 560, for details). This cover image highlights the effects of the sample displaying a preferred orientation (right) which results in an under-sampling of some Fourier components and the reduction of overall resolution within the final reconstruction, compared with a more evenly orientated specimen (left) producing a final reconstruction with higher resolution.

introduction

Acta Cryst. (2018). D74, 490-491
<https://doi.org/10.1107/S2059798318008239>

OPEN ACCESS

Other cryo-EM resources

Lecture courses and talks:

- Caltech: <http://cryo-em-course.caltech.edu/> and <https://em-learning.com/>
- MRC-LMB: <ftp://ftp.mrc-lmb.cam.ac.uk/pub/scheres/EM-course/>

Many review papers:

- Cryo-EM: A unique tool for the visualization of macromolecular complexity. Nogales & Scheres, 2015. Mol Cell 58:677-689
- Single-particle cryo-EM—How did it get here and where will it go. Cheng, 2018. Science 361:876-880
- Plenty more, easy to find on Google or PubMed



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Cryo-EM

What is cryo-EM?

“It is very easy to answer many fundamental biological questions; you just look at the thing!”

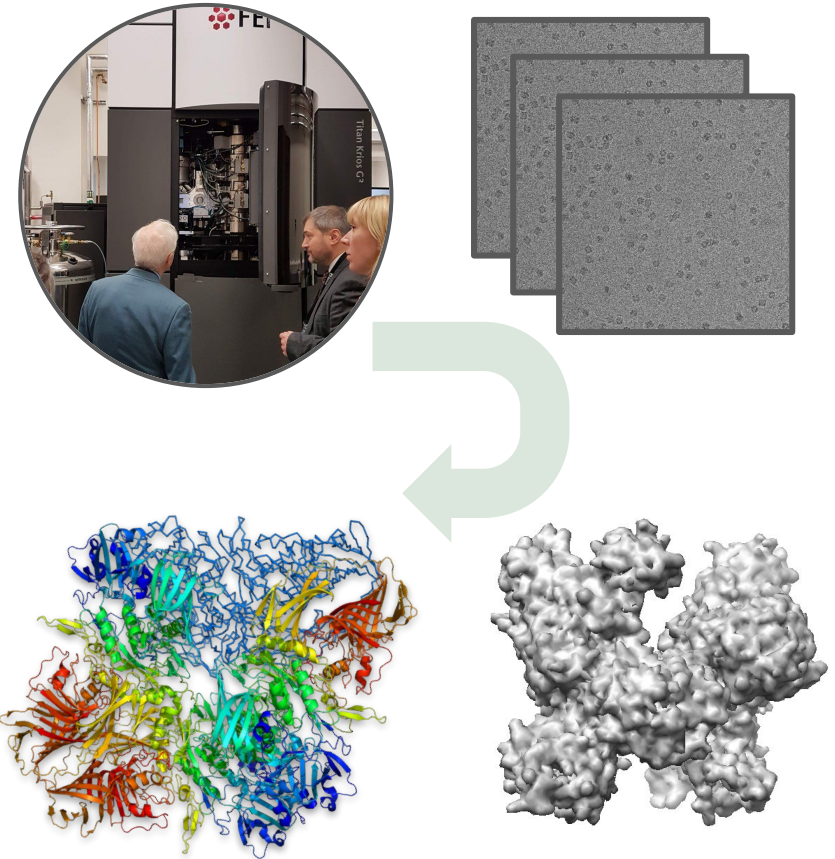
Richard Feynman, 1959

Very simple principle: look directly at the molecules we want to understand

Hard to do in practice!

Main limitation is radiation damage

Images have very low signal to noise $\Rightarrow$ need to use averaging to recover signal

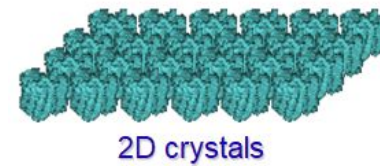


What is cryo-EM?

A family of methods, not a single technique

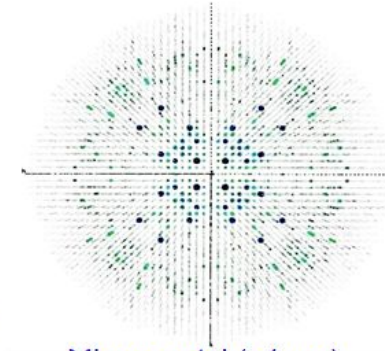
- Single Particle Reconstruction (SPR or SPA)
- Helical reconstruction
- Tomography (cryo-ET or ECT)
- Sub-tomogram averaging
- Electron crystallography
 - 2D and 3D (“microED”)

(But normally, “cryo-EM” means single particle reconstruction)



2D crystals

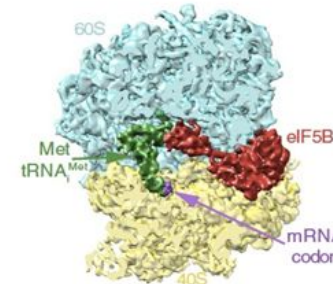
Electron crystallography
(views at different tilts)



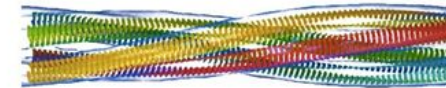
Microcrystal (<1 μm)
electron diffraction



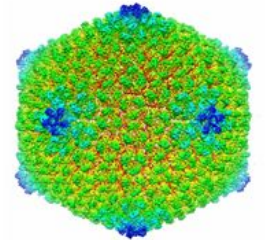
Whole cells or organelles
(**tomography** of unique objects,
cumulative irradiation)



Asymmetric **single particles**



Helical assemblies



Icosahedral viruses

Tools for structural biology



X-ray Crystallography

Atomic resolution (1-4Å)
Needs crystals
Conformation may be affected
by crystal lattice
Phase problem



NMR Spectroscopy

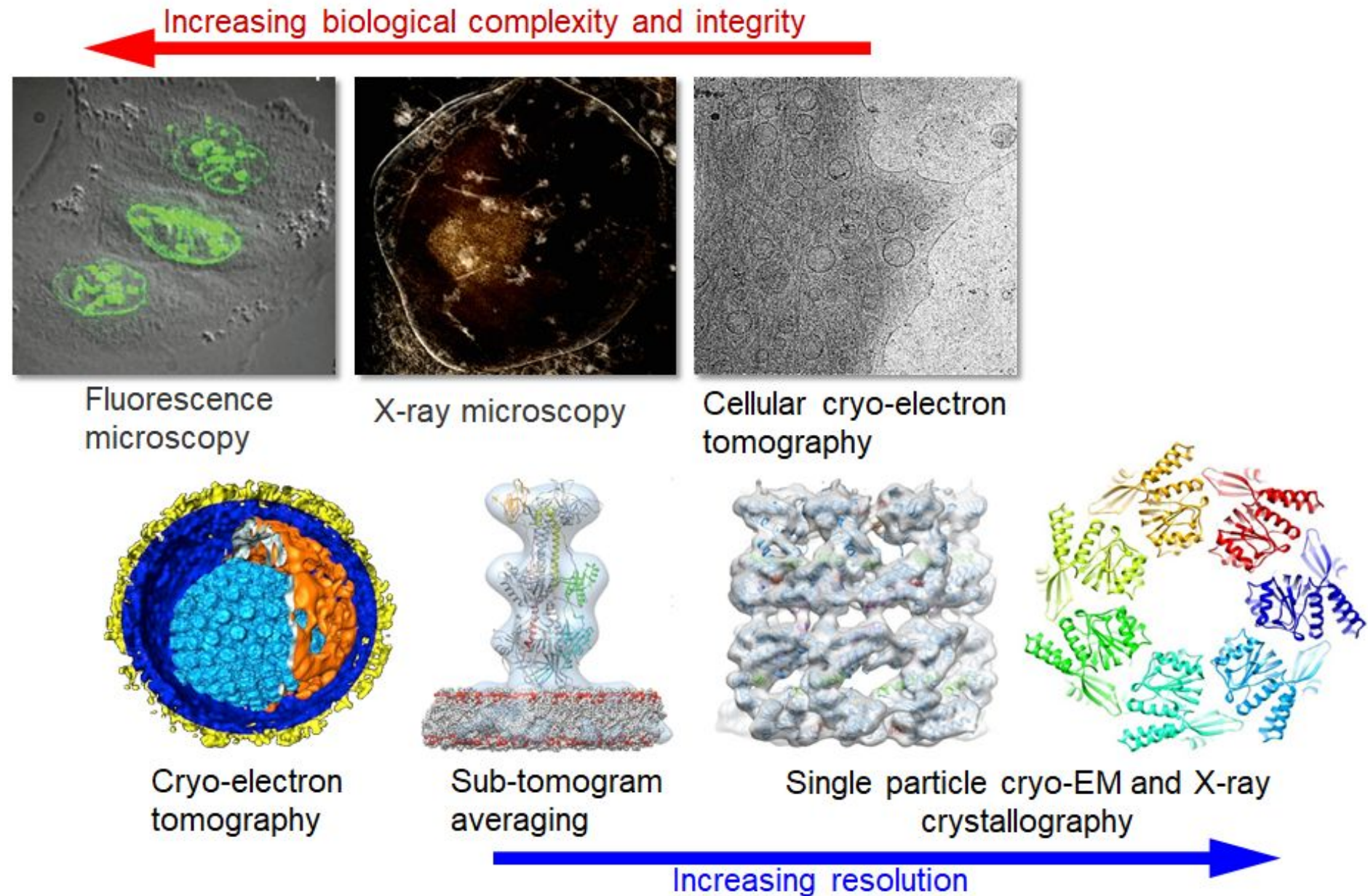
Provides distance restraints
Give near atomic ensemble
structures (~2-4Å)
Dynamic processes
Small proteins by solution NMR
Larger complexes by selective
labelling, solid state



Cryo-EM

Resolution 1.5-20Å
Ordered assemblies or isolated
particles
Can trap transient states and sort
heterogeneity
*Does not require crystals or
labelling*
Suitable for very large complexes

Structural biology from cells to molecules





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Single Particle EM



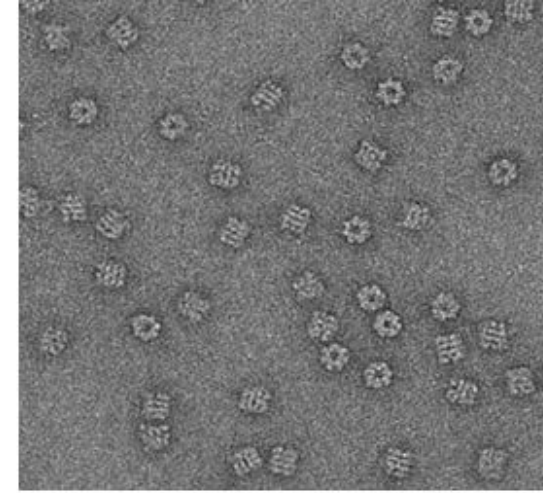
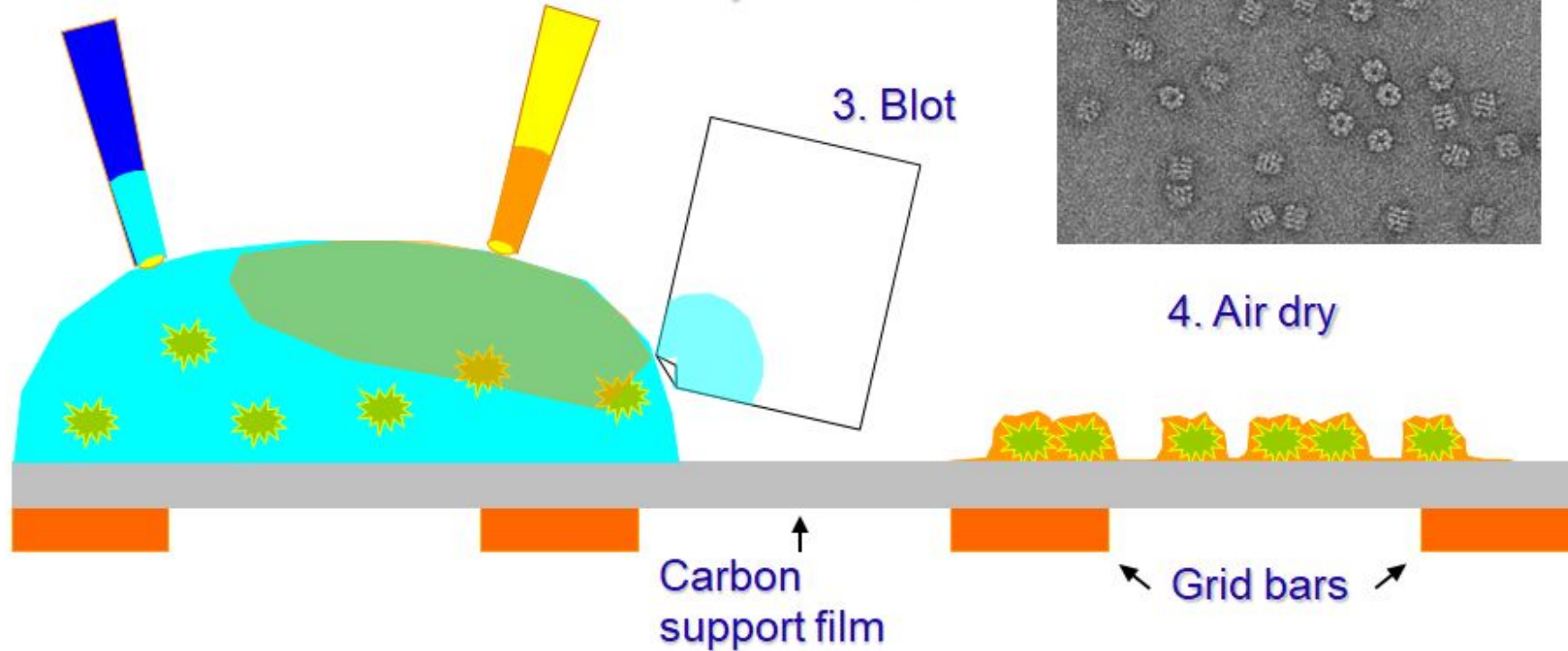
A quick detour: negative stain

1. Add protein in buffer

2. Add heavy metal stain

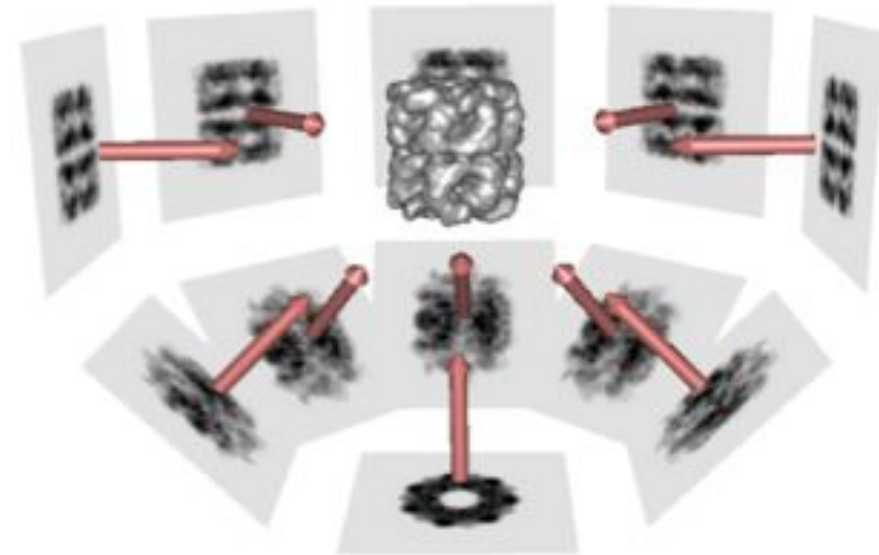
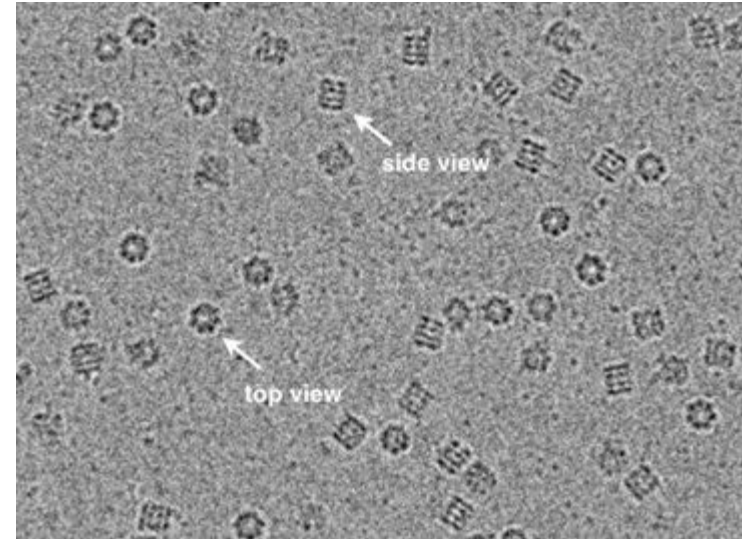
3. Blot

4. Air dry



Single particle cryo-EM overview

- **Collect images** of macromolecules frozen in ice
- **Identify** and **extract** particles from images
- **Orient** particles
- **Reconstruct** 3D volume
- Averaging technique:
 - More particles $\approx$ higher resolution (1.5–5 Å)



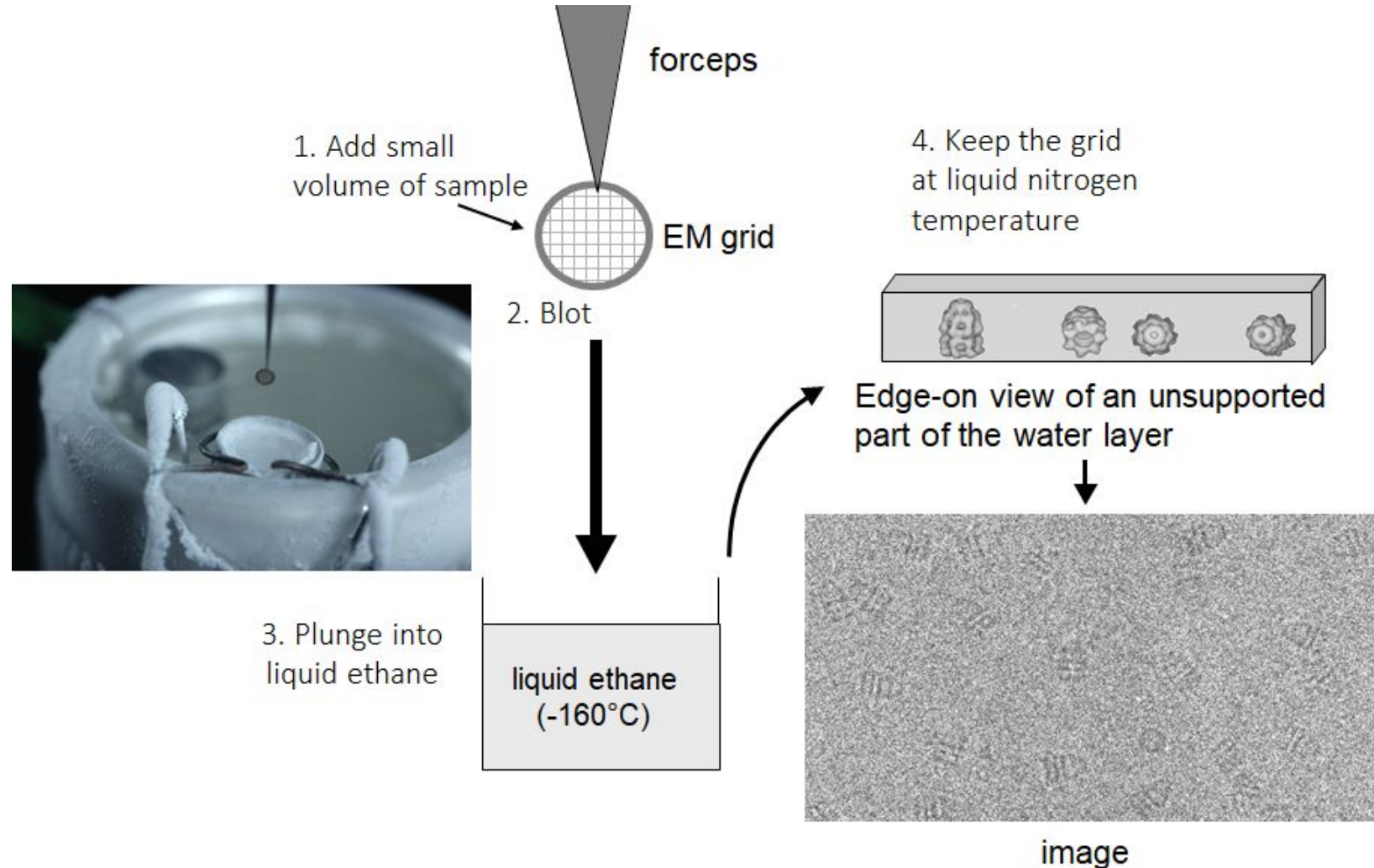
Cryo-EM sample preparation

No requirement for protein crystals...

...but must ensure no ice crystals either

Plunge freeze into liquid ethane

Very fast freezing: ice becomes vitreous instead of crystalline



Data acquisition

Equipment:

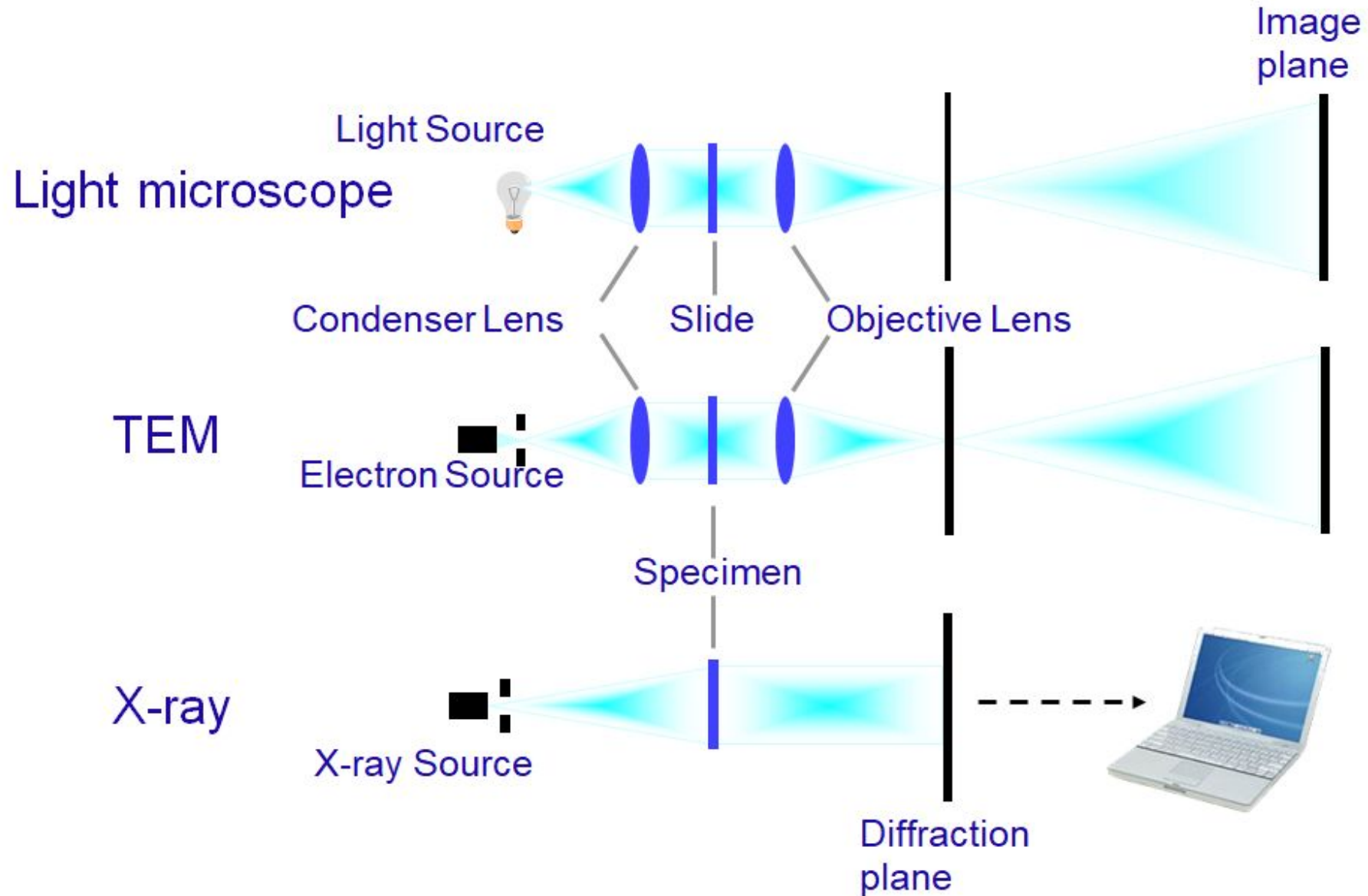
- Microscope (cryo-TEM)
- Cryo stage
- Detector

Available at Diamond via eBIC
(electron Bio-Imaging Centre)

Peer-reviewed free access,
much like MX beamlines



Imaging and diffraction



Cryo-EM images are noisy

Biological material is radiation-sensitive

High resolution information is lost first

Electron dose is very limited

Images have extremely low signal to noise ratio

Modern direct electron detectors help a lot

- Electron counting
- Motion correction

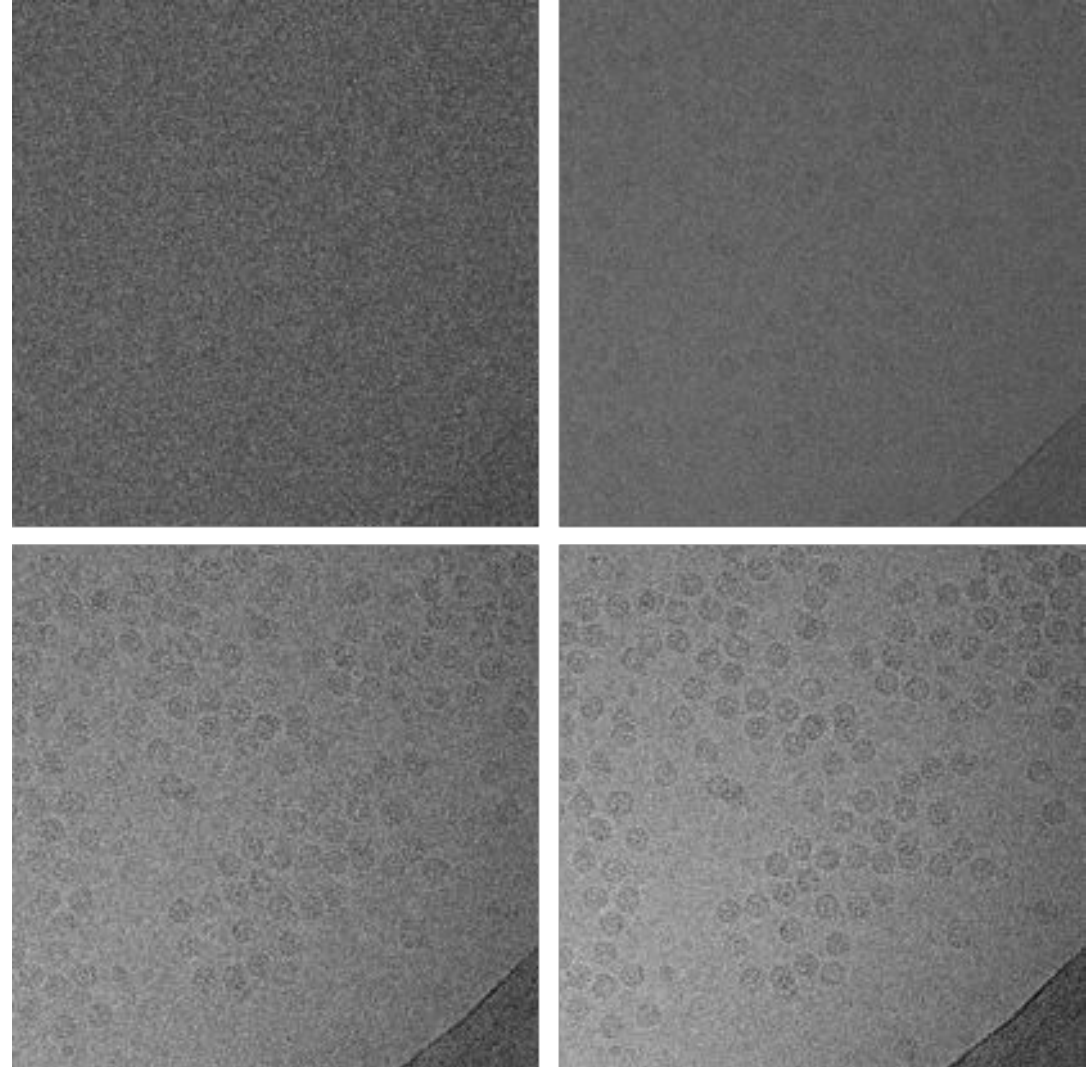
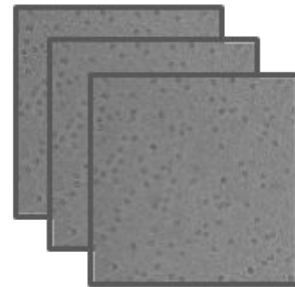


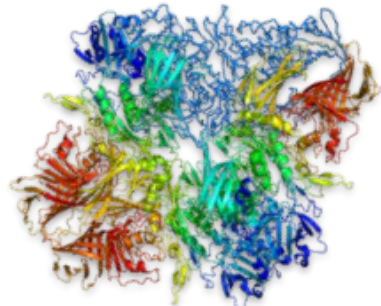
Image processing overview



Micrographs



3D map



Atomic Model

Image processing

Particle picking

2D classification

3D classification

3D map refinement



Map optimisation

Model docking

Model building

Model refinement

Ligand fitting

Map & model validation



Movie acquisition

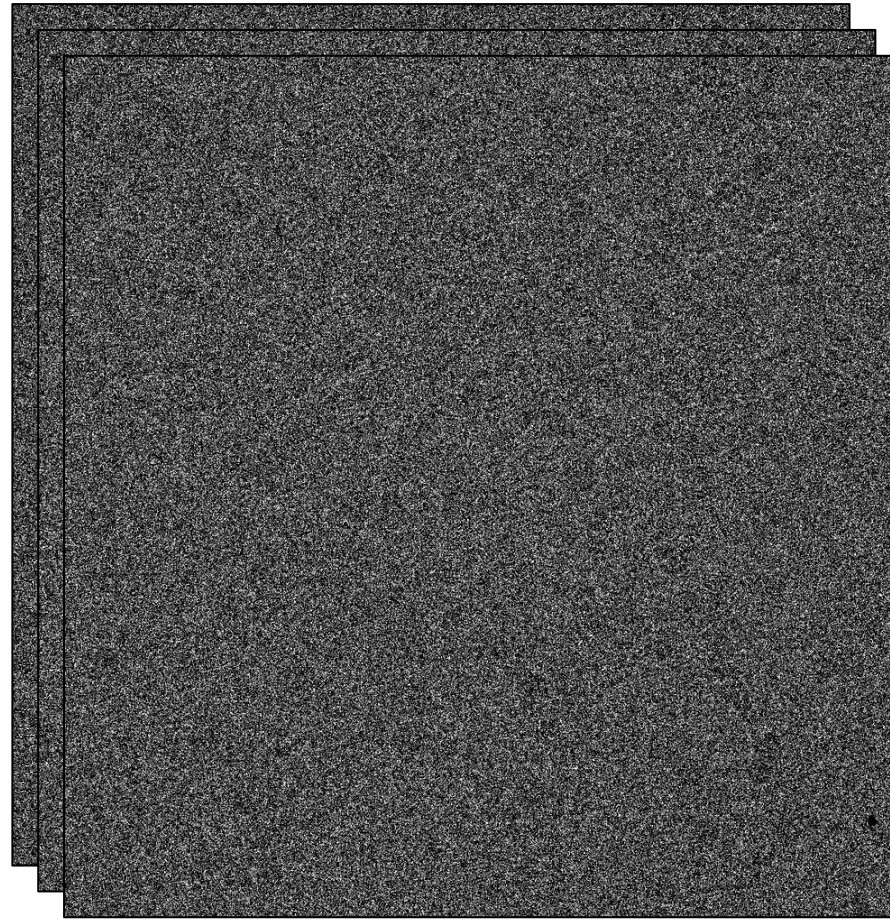
Direct electron detectors collect high speed movies (10s – 100s fps)

Typically 100s or 1000s of movies per session

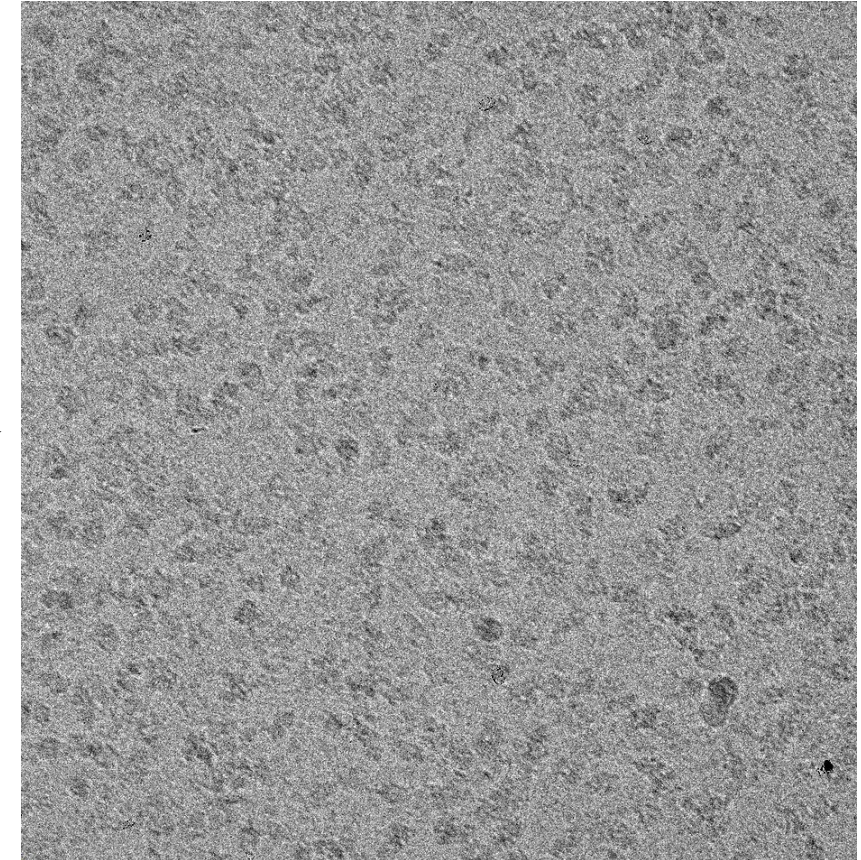
Electron counting

Very low dose

Sample moves in the electron beam



Raw movie frames

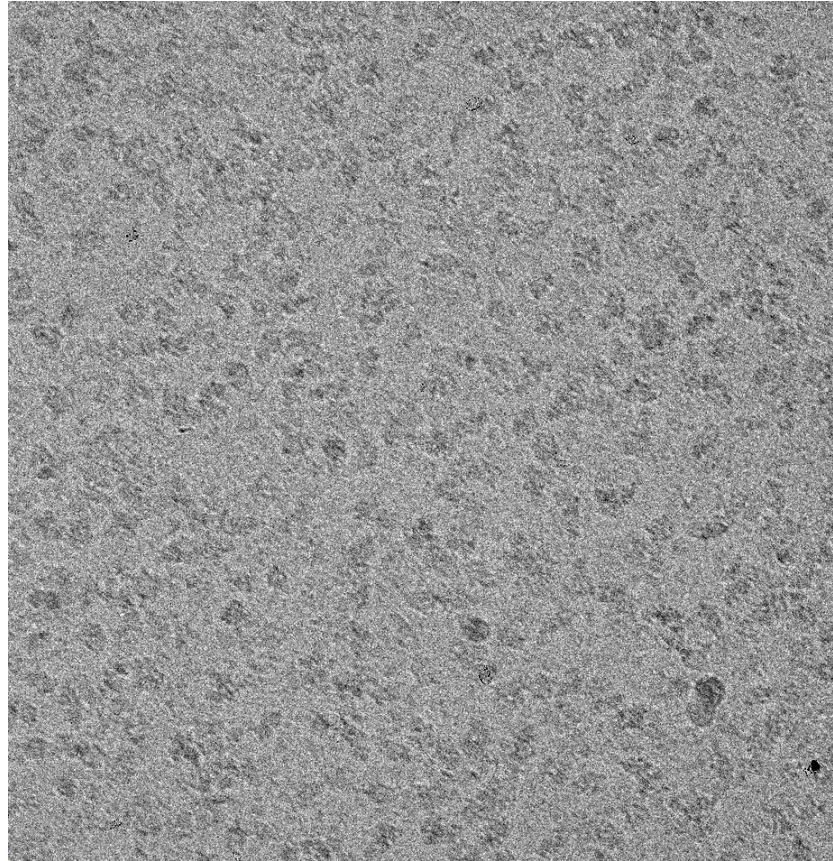


Summed micrograph

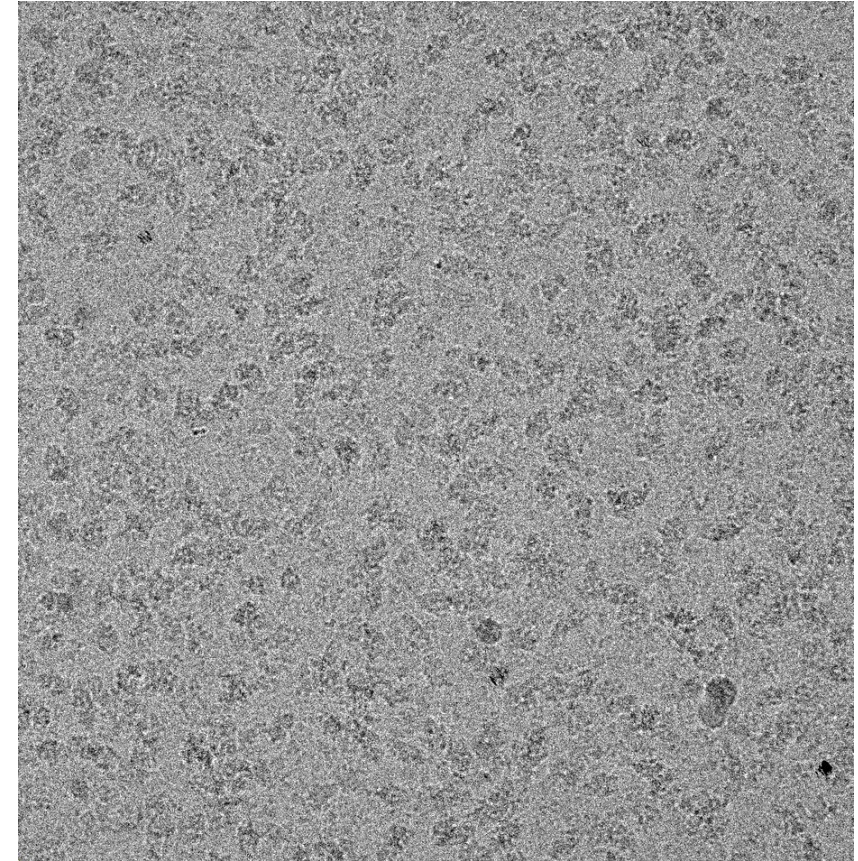
Motion correction

Sample motion can
be corrected by
aligning movie frames

Big improvement
compared to CCD
detectors or film



Summed micrograph



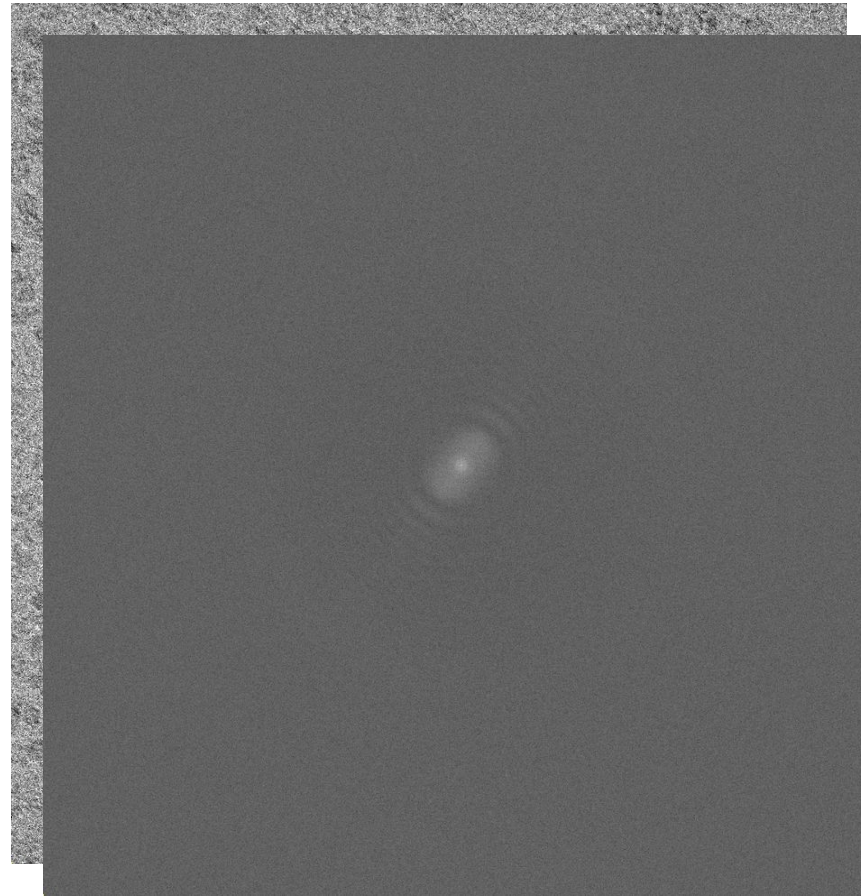
Motion corrected

Motion correction

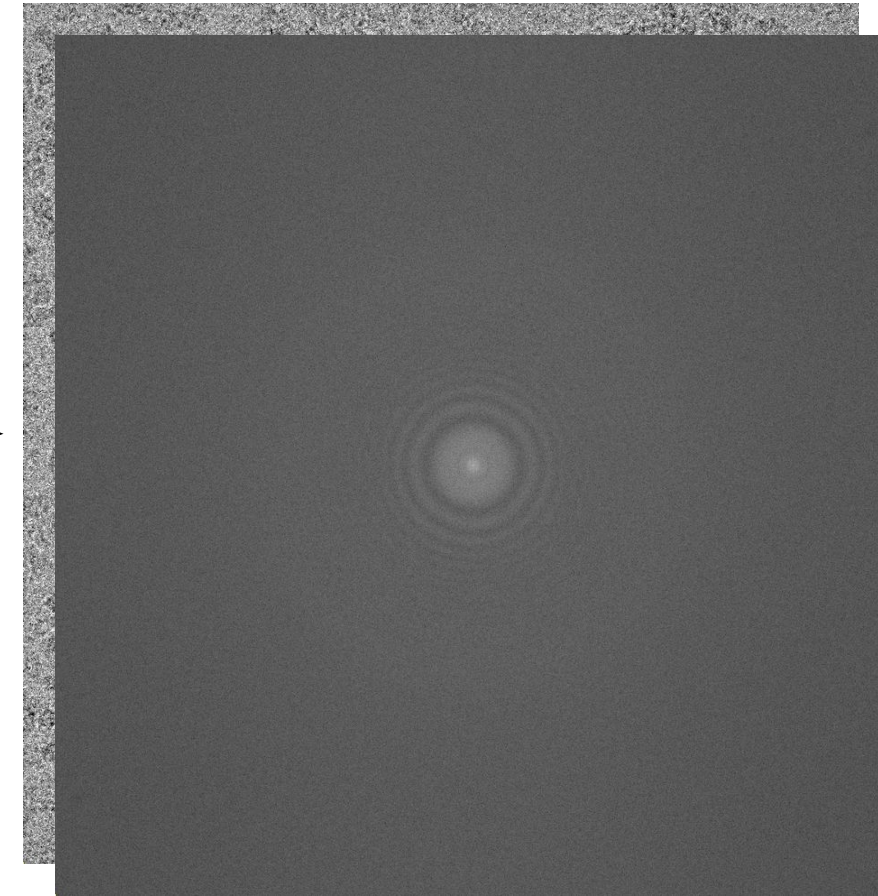
Sample motion can be corrected by aligning movie frames

Big improvement compared to CCD detectors or film

Power spectrum shows information recovery



Summed micrograph
power spectrum



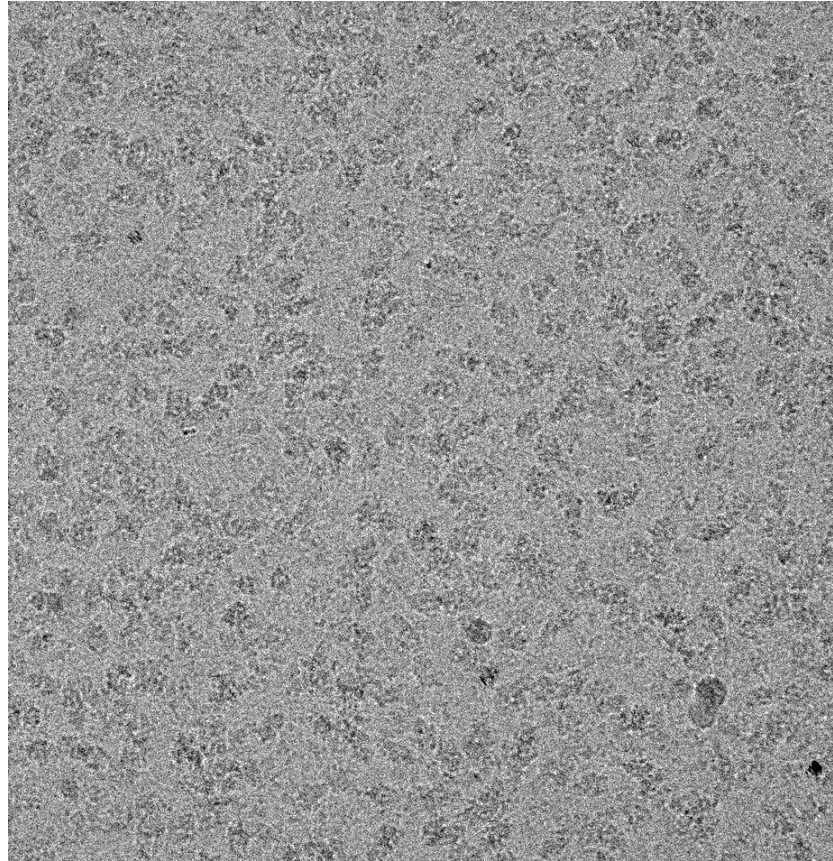
Motion corrected
power spectrum

CTF estimation

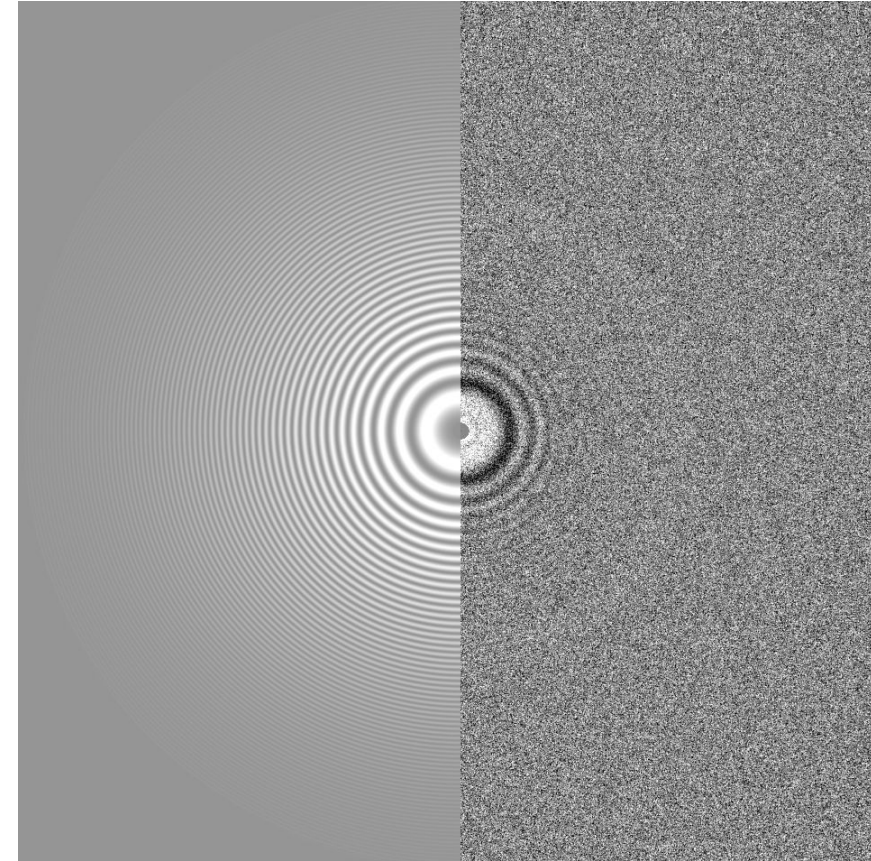
Electron optics cause phase oscillation and attenuation of high frequencies (and more complicated effects)

Phase contrast comes from defocus

Need to estimate and correct for the Contrast Transfer Function



Motion corrected micrograph



CTF estimate and power spectrum

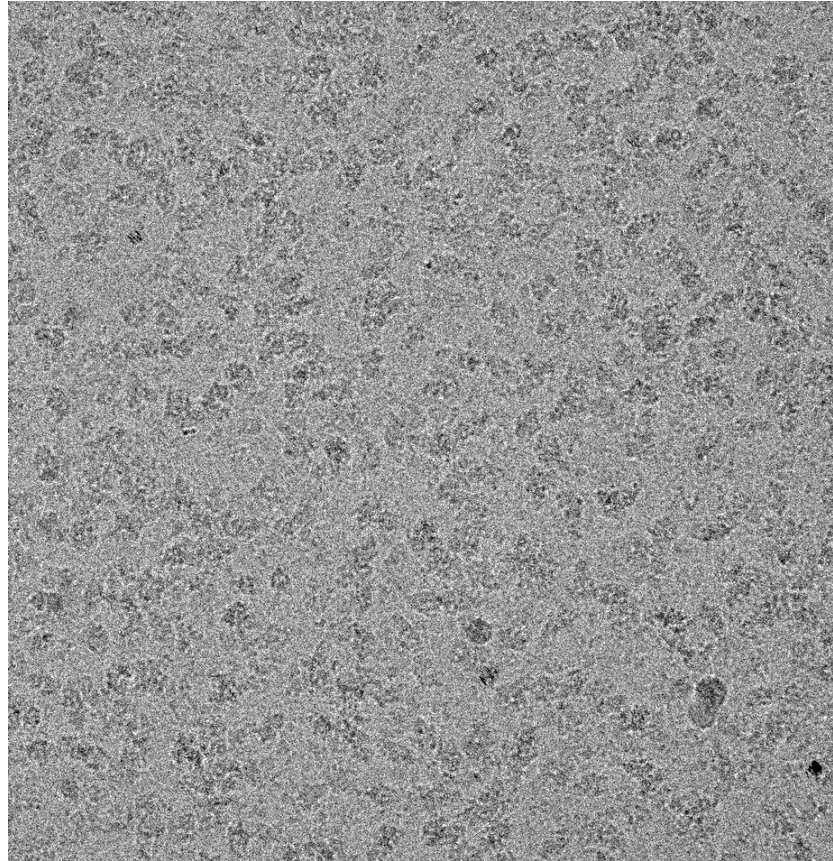
Particle picking

Need to identify
particle positions

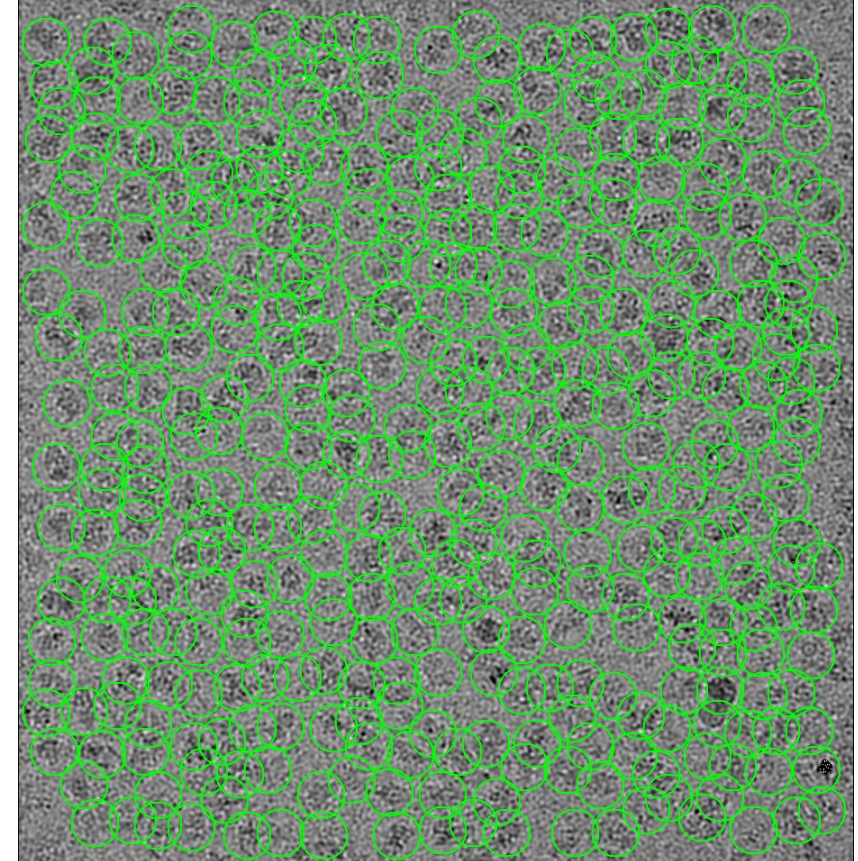
Used to be manual,
now usually automatic

Reference-based or
reference-free

Increasing use of
deep learning
methods



Motion corrected micrograph

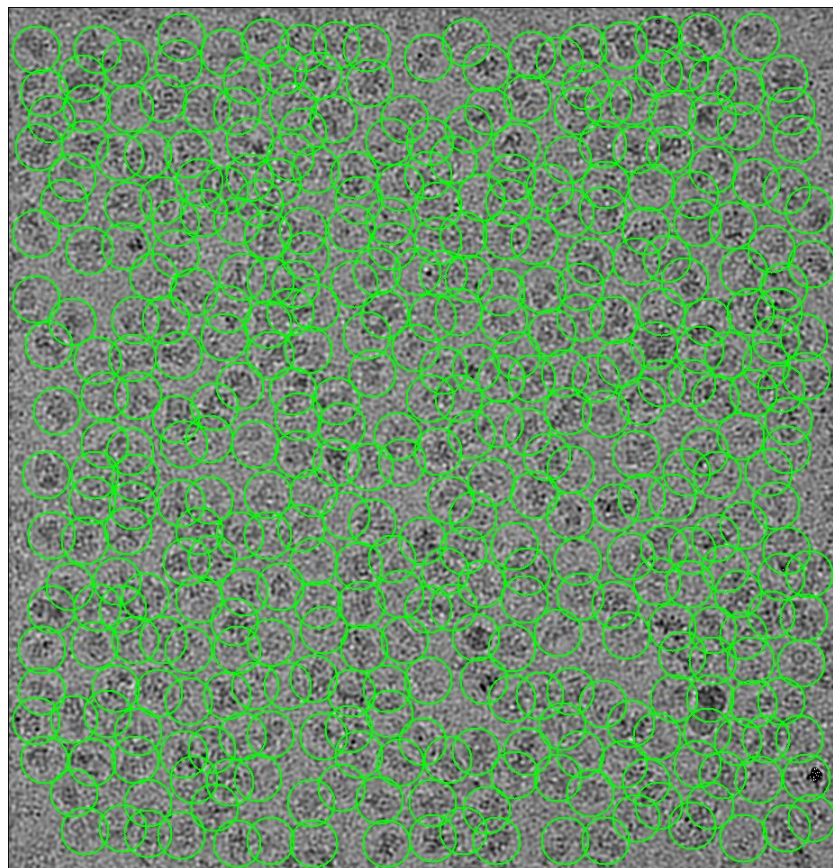


Picked particle positions

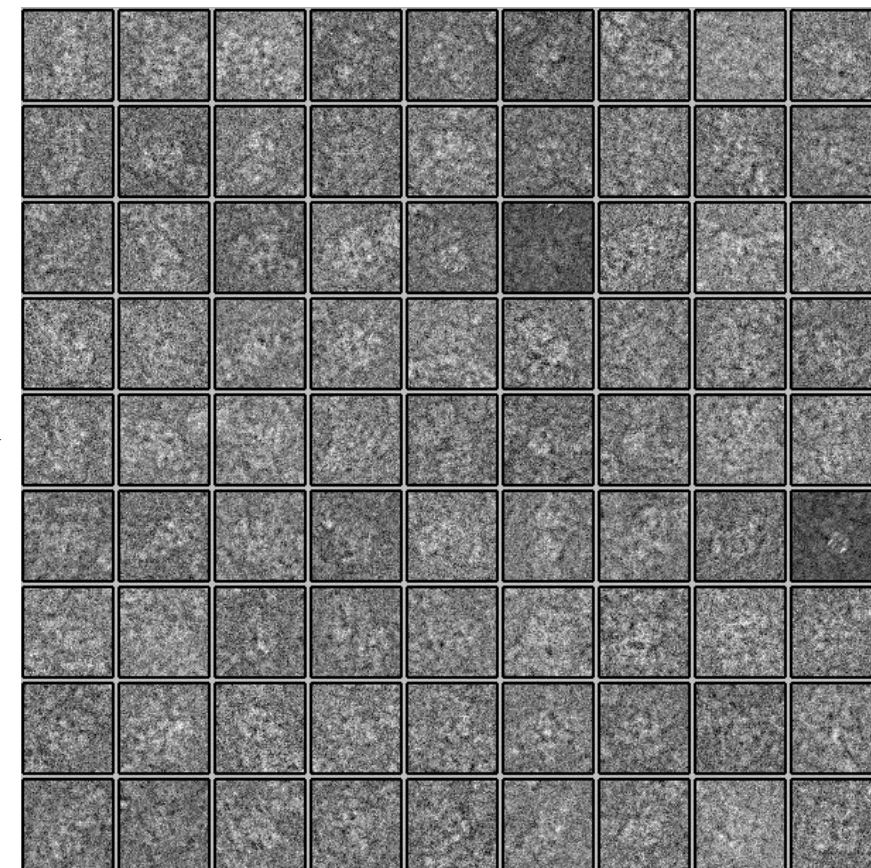
Particle extraction

Extract particle images (and metadata) from micrographs

Typically between 10,000 – 2,000,000 particles per dataset



Picked particle positions



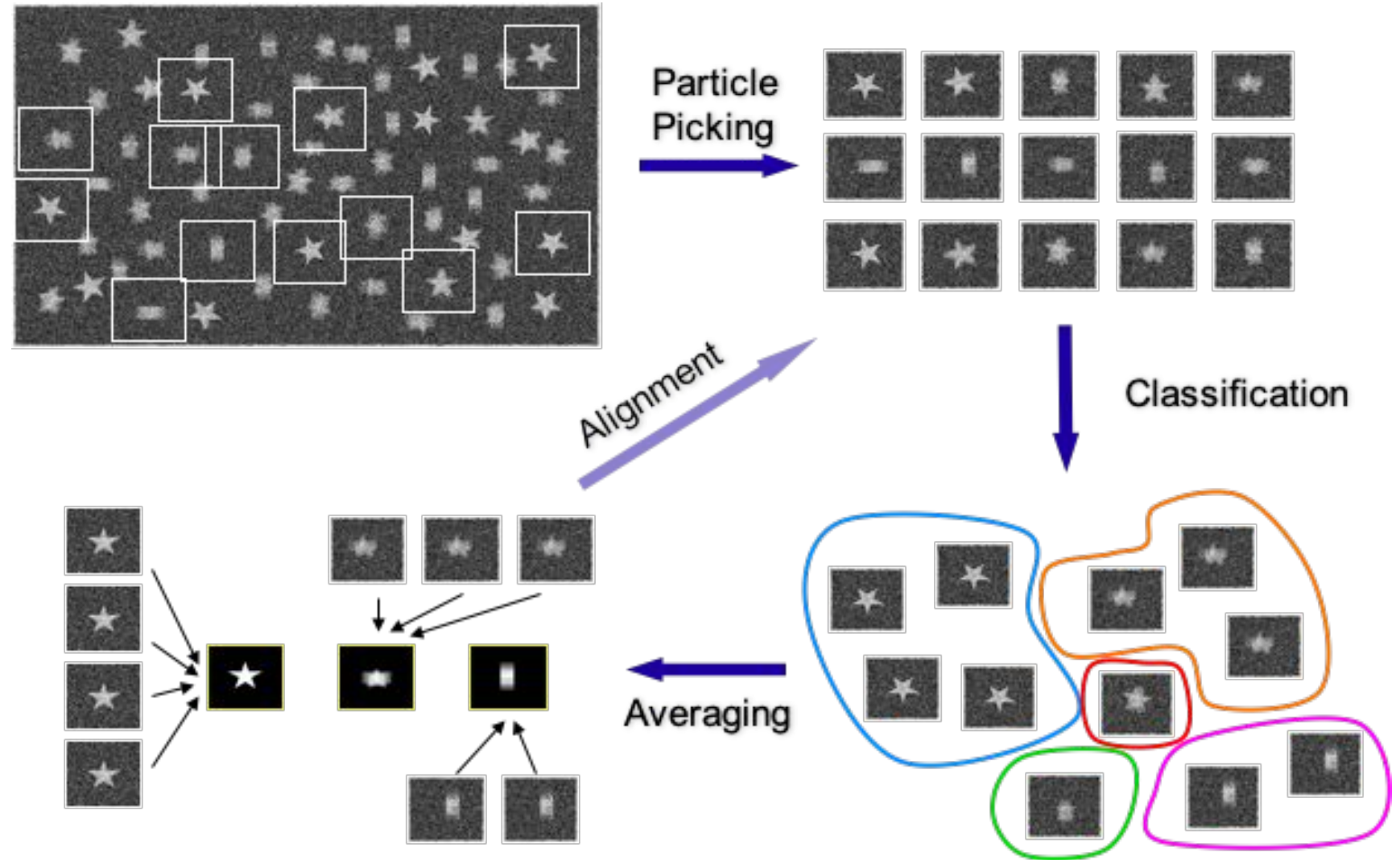
Individual particle images

2D classification

Reference-free
identification of similar
views

Iterative process:

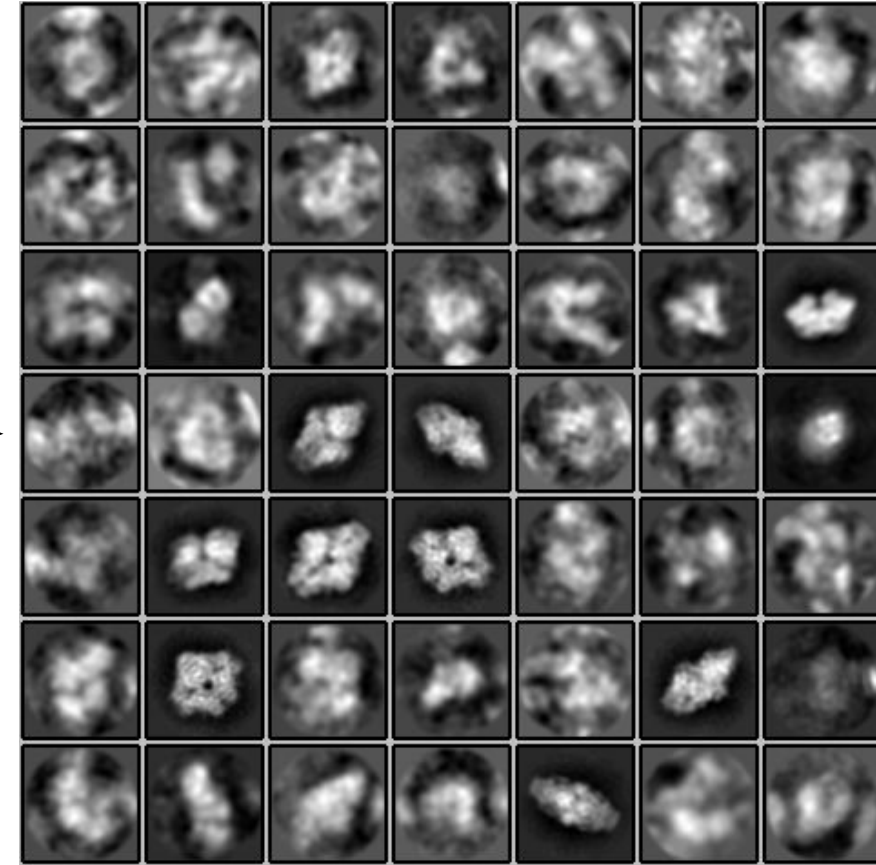
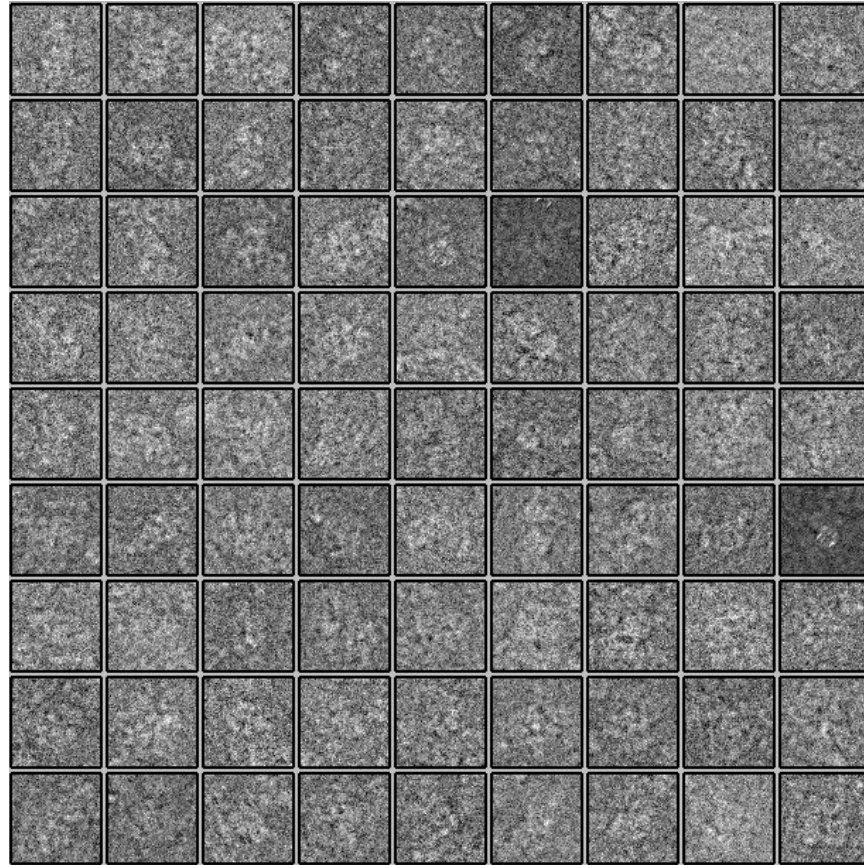
- Classify
- Average
- Align
- Repeat



2D classification

Good indicator of data quality

Use to discard bad particles



Individual particle images

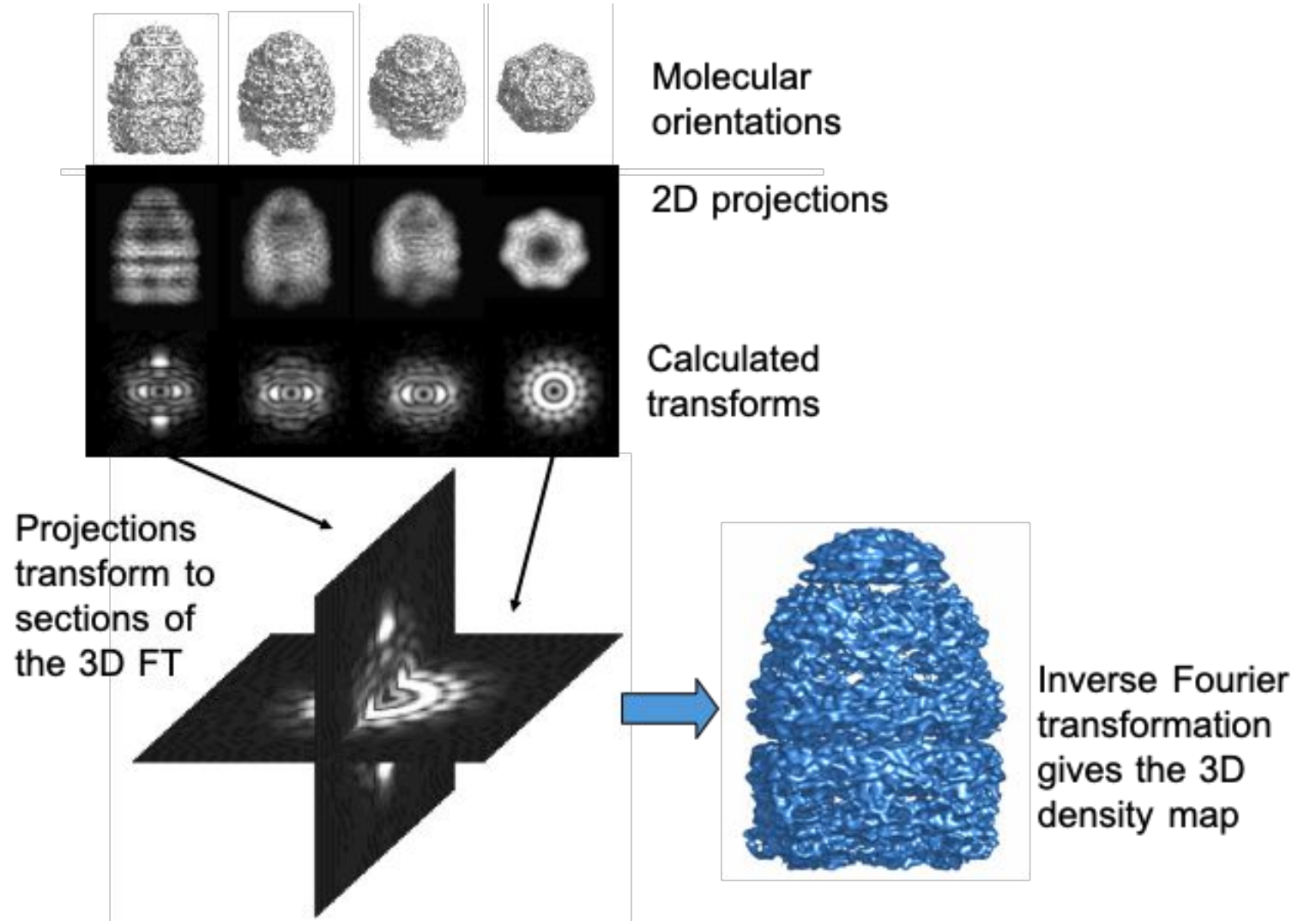
2D class averages

3D reconstruction

3D volume reconstructed from 2D images

Normally done in reciprocal space

Relies on Fourier slice theorem

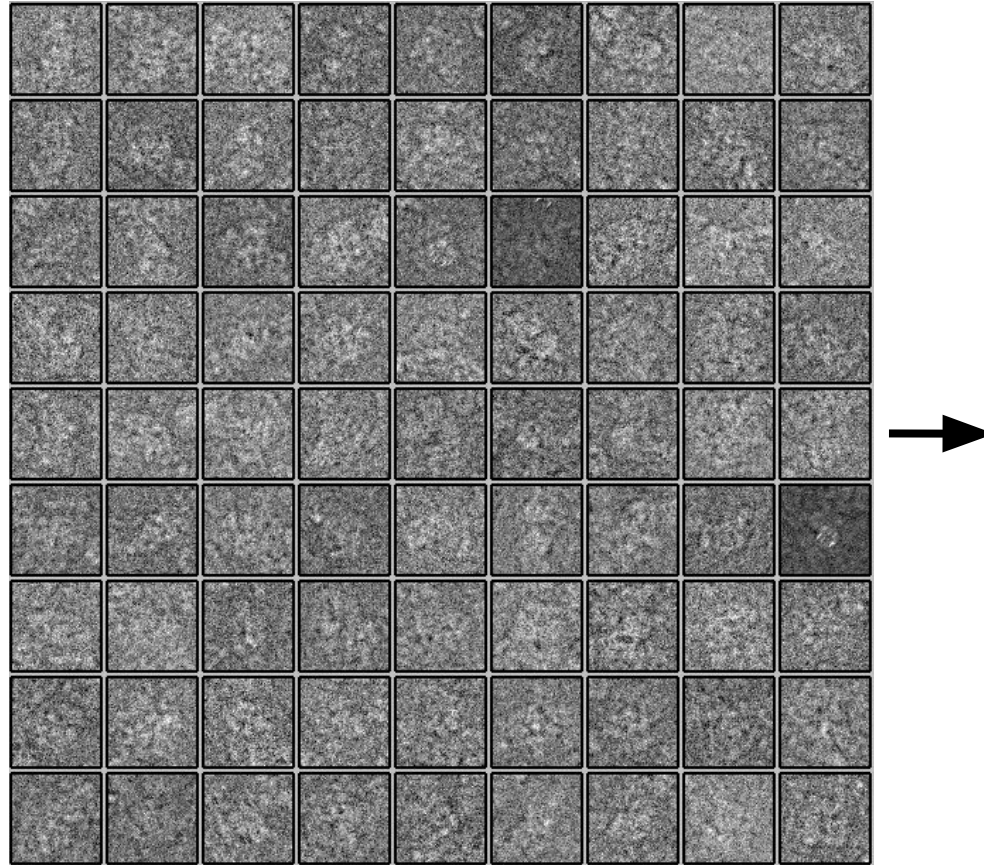


Initial model generation

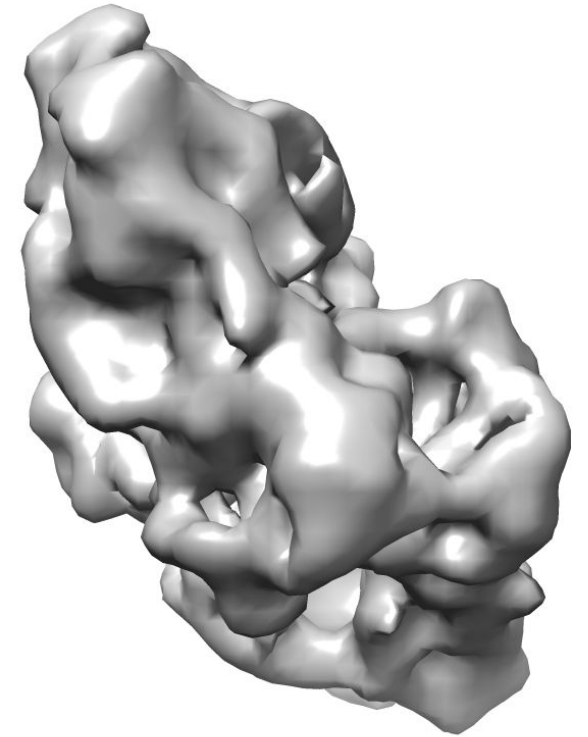
Used to be very
challenging

Much improved by
modern algorithms

Now reference-free



Individual particle images



3D initial model
(10–20 Å resolution)

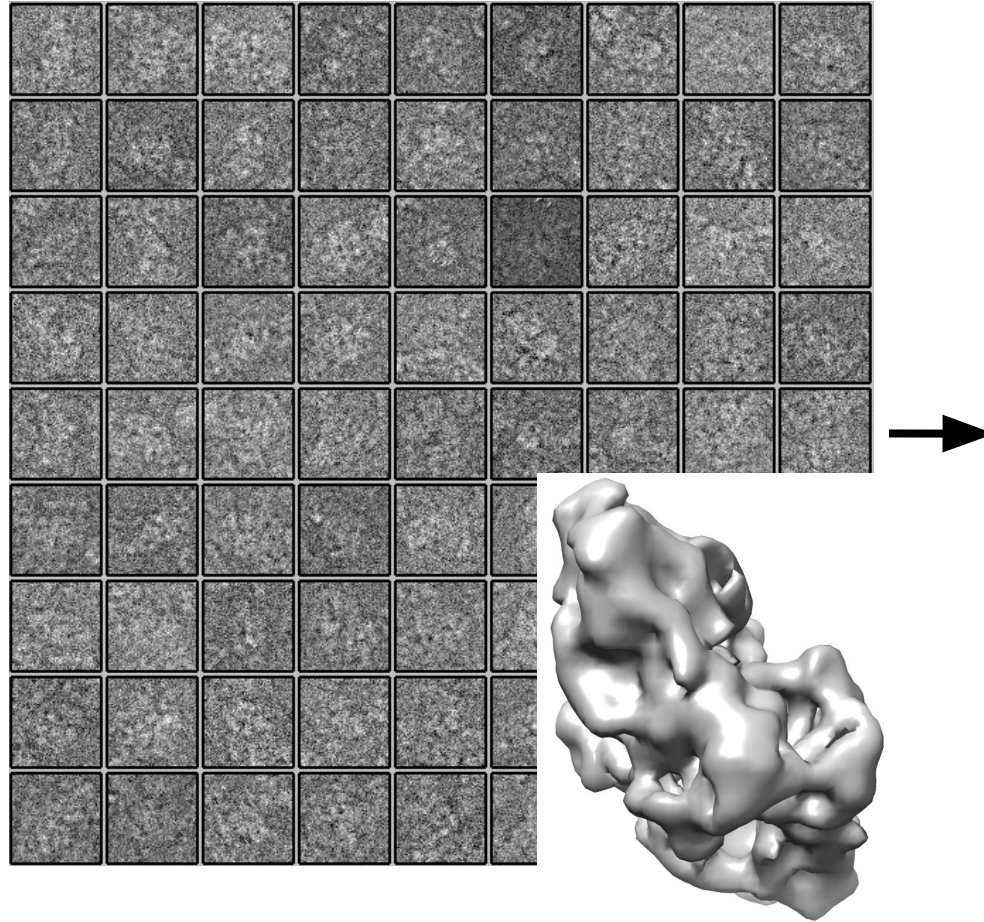
3D classification

Similar to 2D
classification

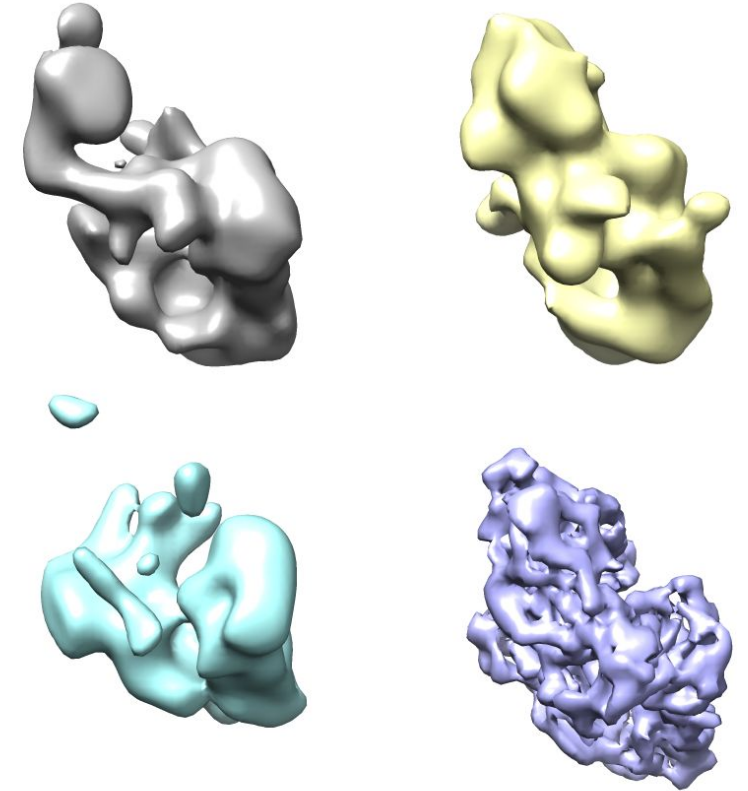
Iterative classification,
alignment and
averaging

Alignment by
projection matching

Can use to discard
bad particles, much
like 2D classification



Individual particle images
+ 3D initial model



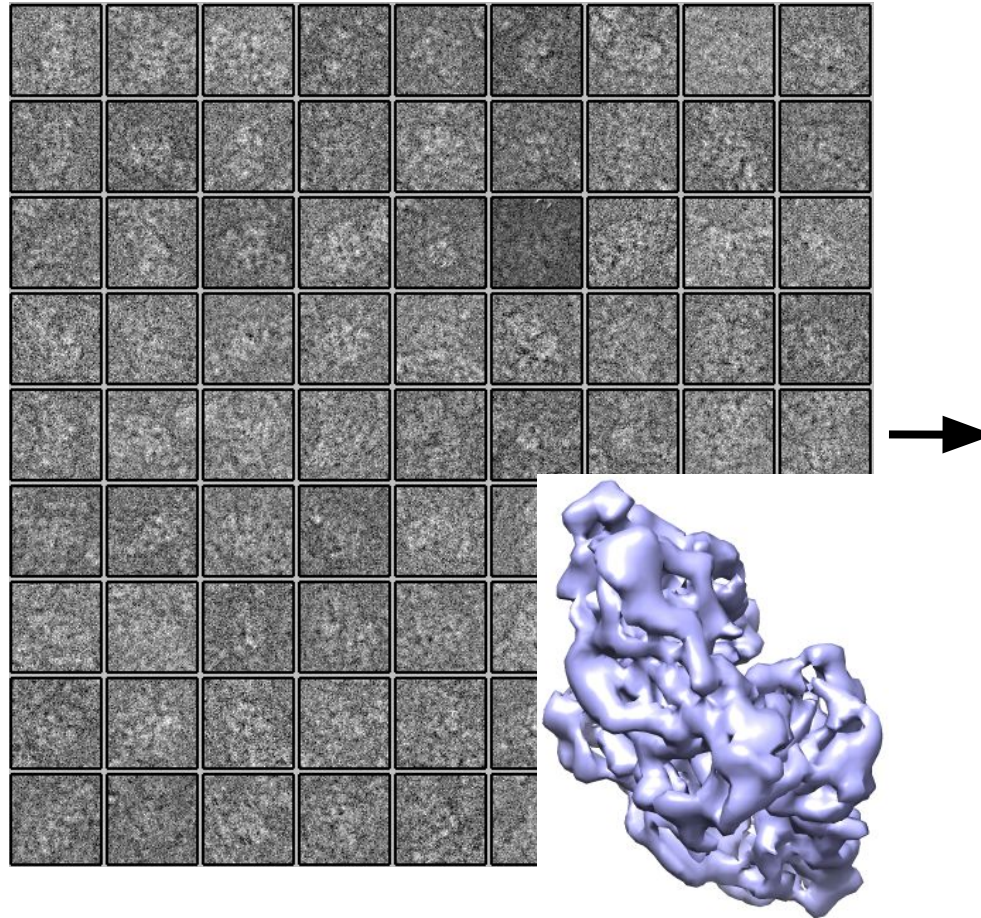
3D class averages
(up to ~ 4 Å resolution)

3D refinement

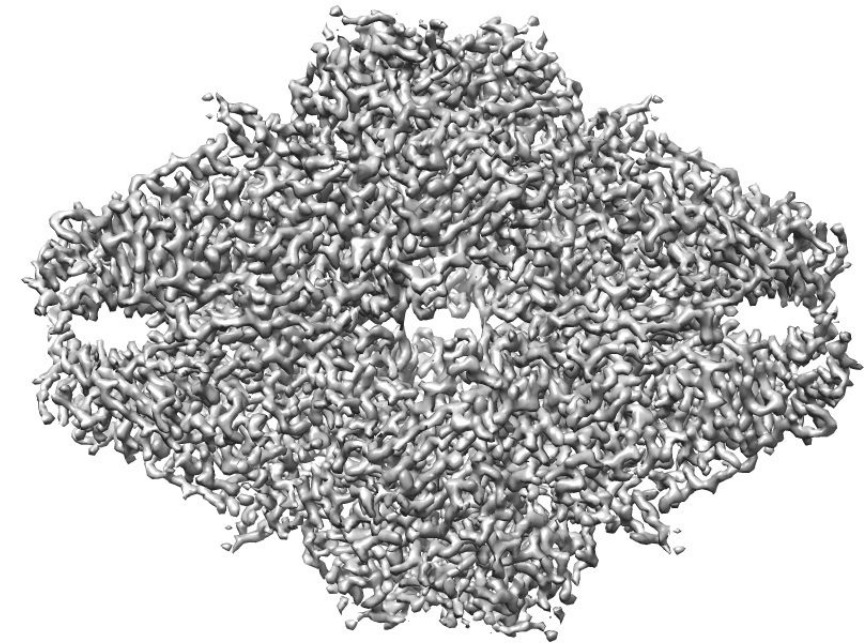
Iterative alignment and averaging to high resolution

Often better with fewer, higher-quality particles (10s of thousands)

Much improved by modern algorithms using Bayesian methods



Individual particle images
+ 3D class average



Refined 3D map
(up to 1.5 Å resolution)

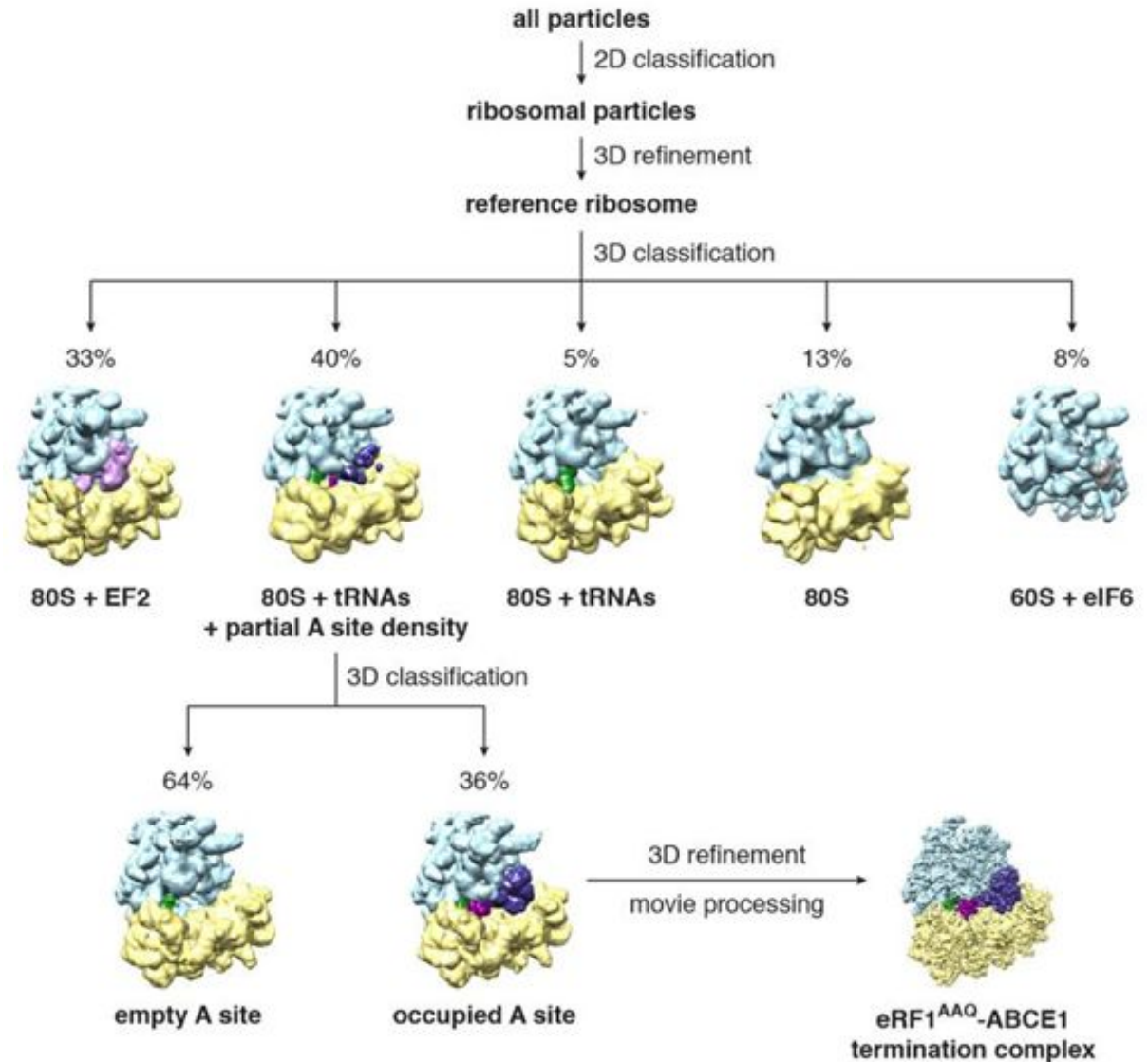
3D classification

Classification can resolve heterogeneity

“Purification in the computer”

Resolve snapshots of compositional or conformational states from a single sample

A major strength of cryo-EM



3D classification

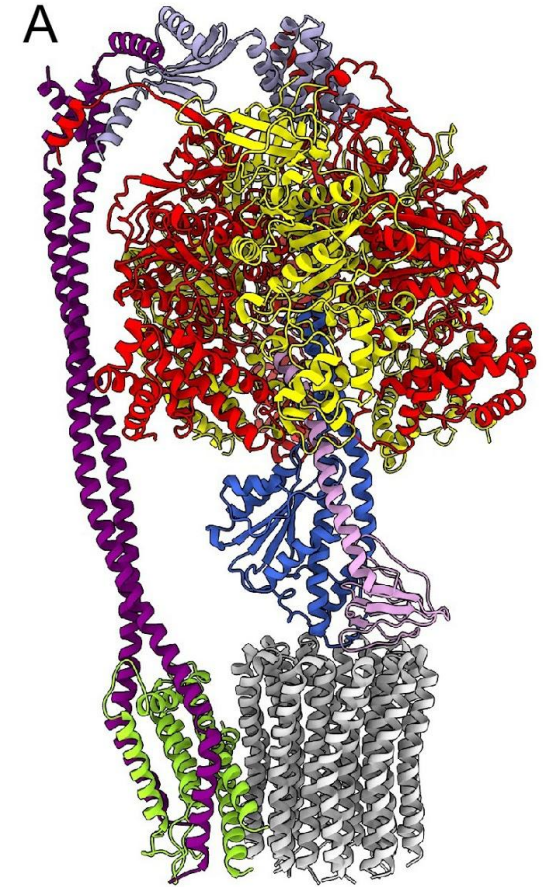
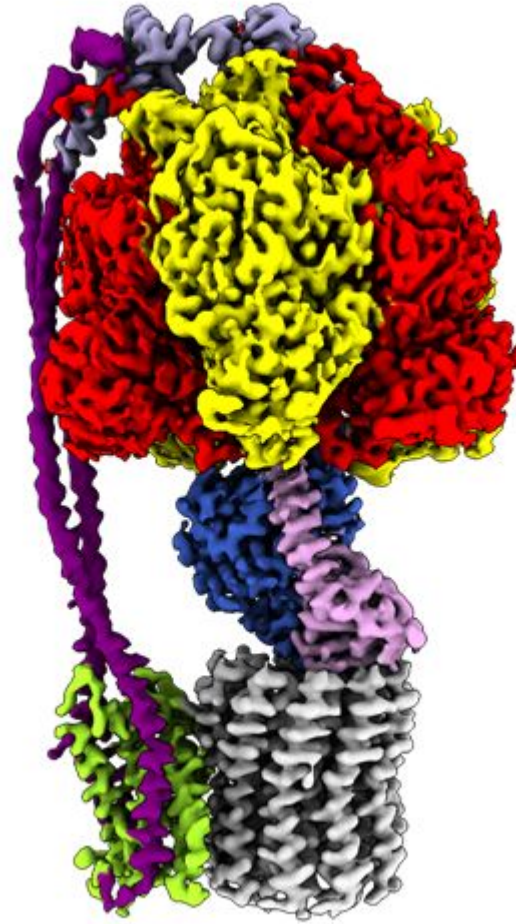
Classification can resolve heterogeneity

“Purification in the computer”

Resolve snapshots of compositional or conformational states from a single sample

A major strength of cryo-EM

States can indicate structural dynamics



See video at

<https://doi.org/10.7554/eLife.43128.009>

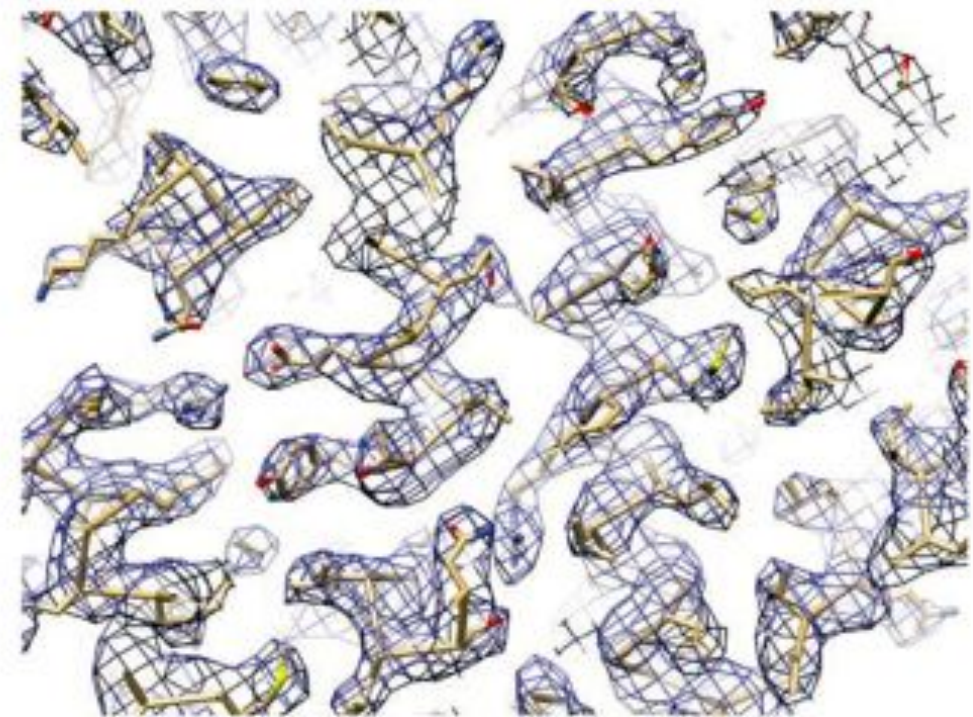
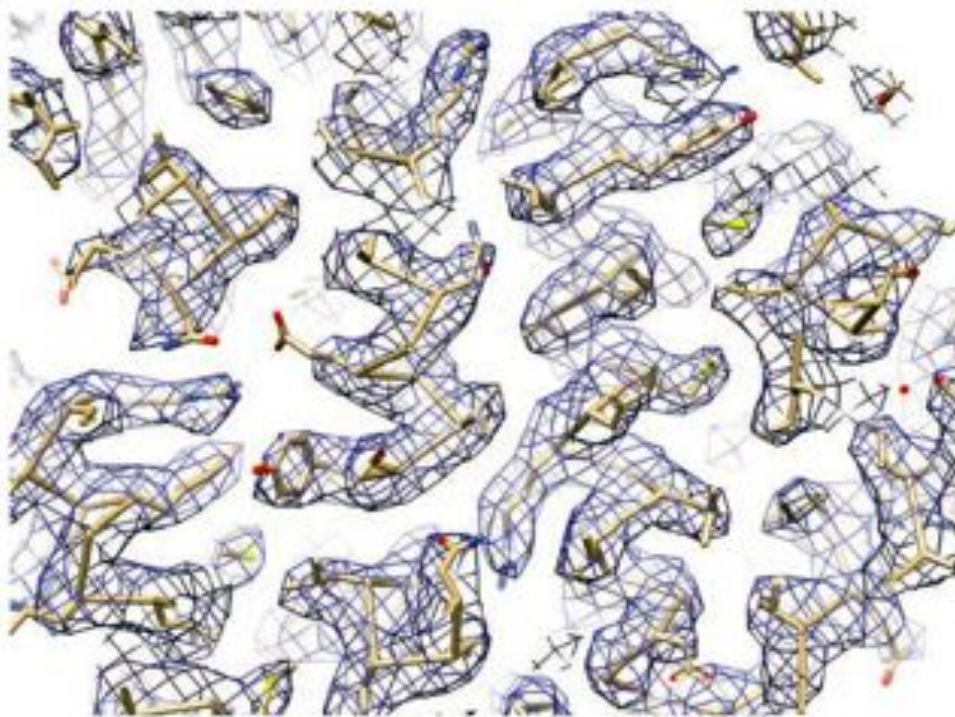


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Cryo-EM vs MX

Maps from cryo-EM look like maps from X-ray crystallography...

Cryo-EM
3.3 Å



MX
3.4 Å

20S proteasome

EM maps are different

Maps from cryo-EM look like maps from X-ray crystallography...

...but some important differences:

- Map is phased!
 - Better quality initially
 - Doesn't change during model building
- Densities represent ***Coulomb potential***, not ***electron density***
- Box size is arbitrary
- Local resolution can be highly variable
- Map sharpening affects refinement

Crystallography software can often handle cryo-EM maps, but needs care

Validation in cryo-EM

Single particle reconstruction is an ill-posed problem

Many parameters, noisy observations

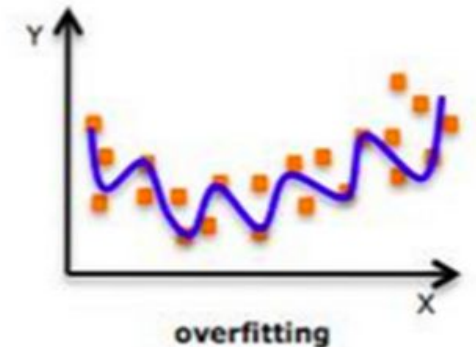
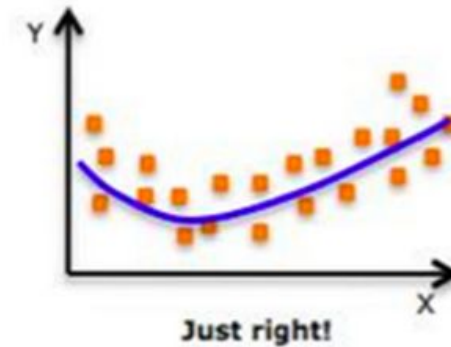
Prone to over-fitting in three areas:

- Particle selection (“Einstein from noise”)
- Particle alignment, orientation and masking
- Model fitting and refinement

Validation for cryo-EM is developing rapidly

Modern algorithms avoid severe over-fitting but still room for improvement

Take care with PDB models from cryo-EM: check any important details yourself!



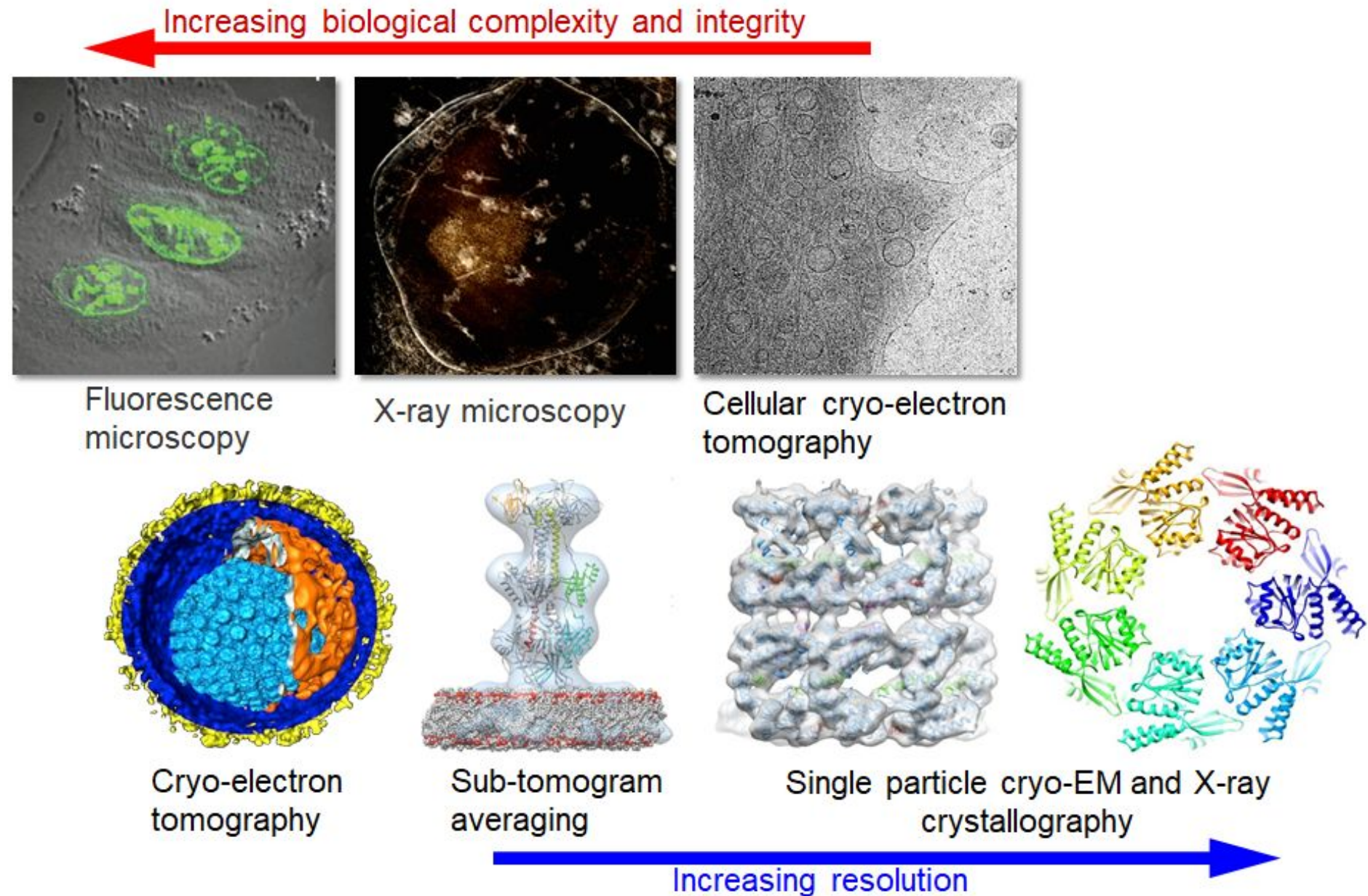


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Electron Tomography



Structural biology from cells to molecules



Electron cryo-tomography

Collect images of a single object from many angles

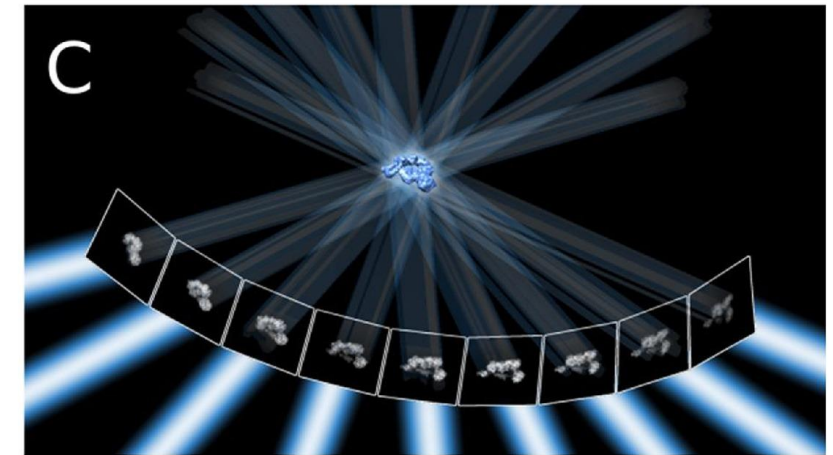
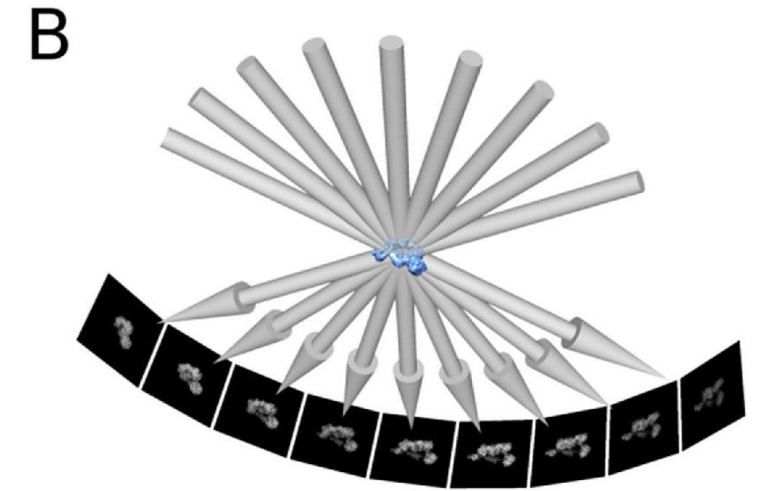
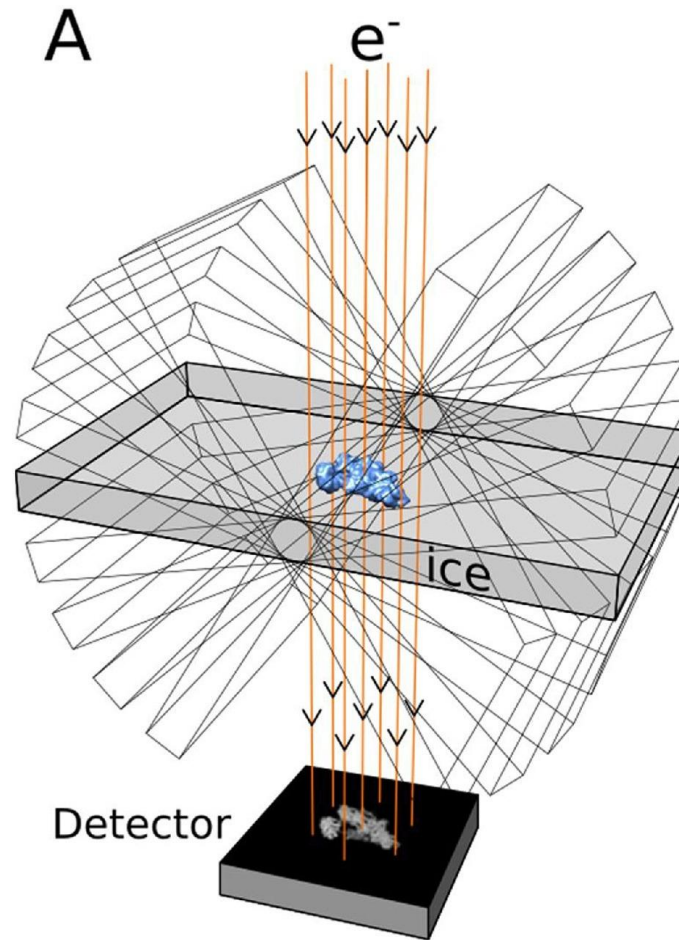
Object can be unique (unlike SPA)

Can view molecules inside cells

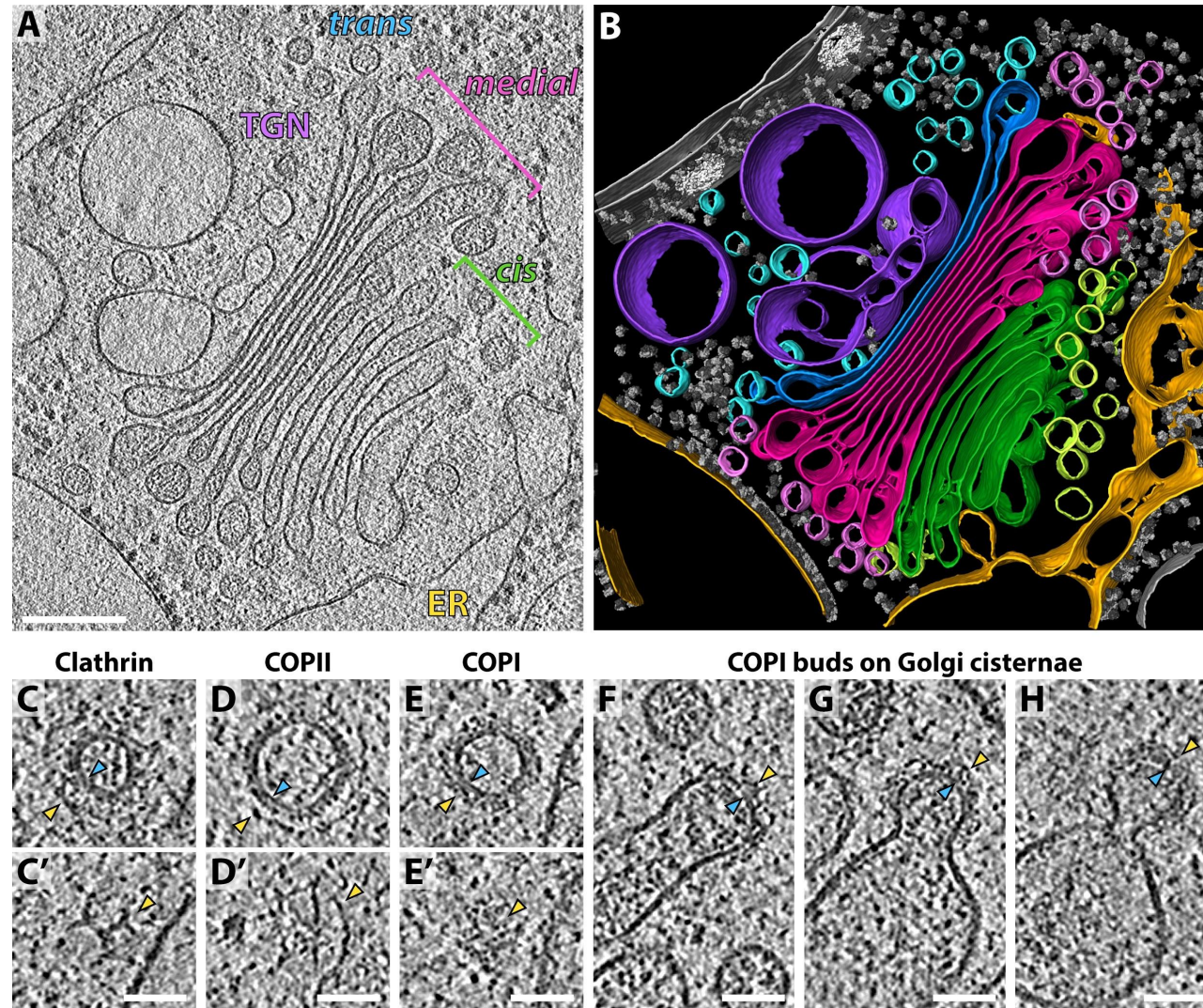
Enables structural biology *in situ*

Resolution 1–3 nm

Thickness limit $\sim 0.5\text{--}1\ \mu\text{m}$



Electron cryo-tomography

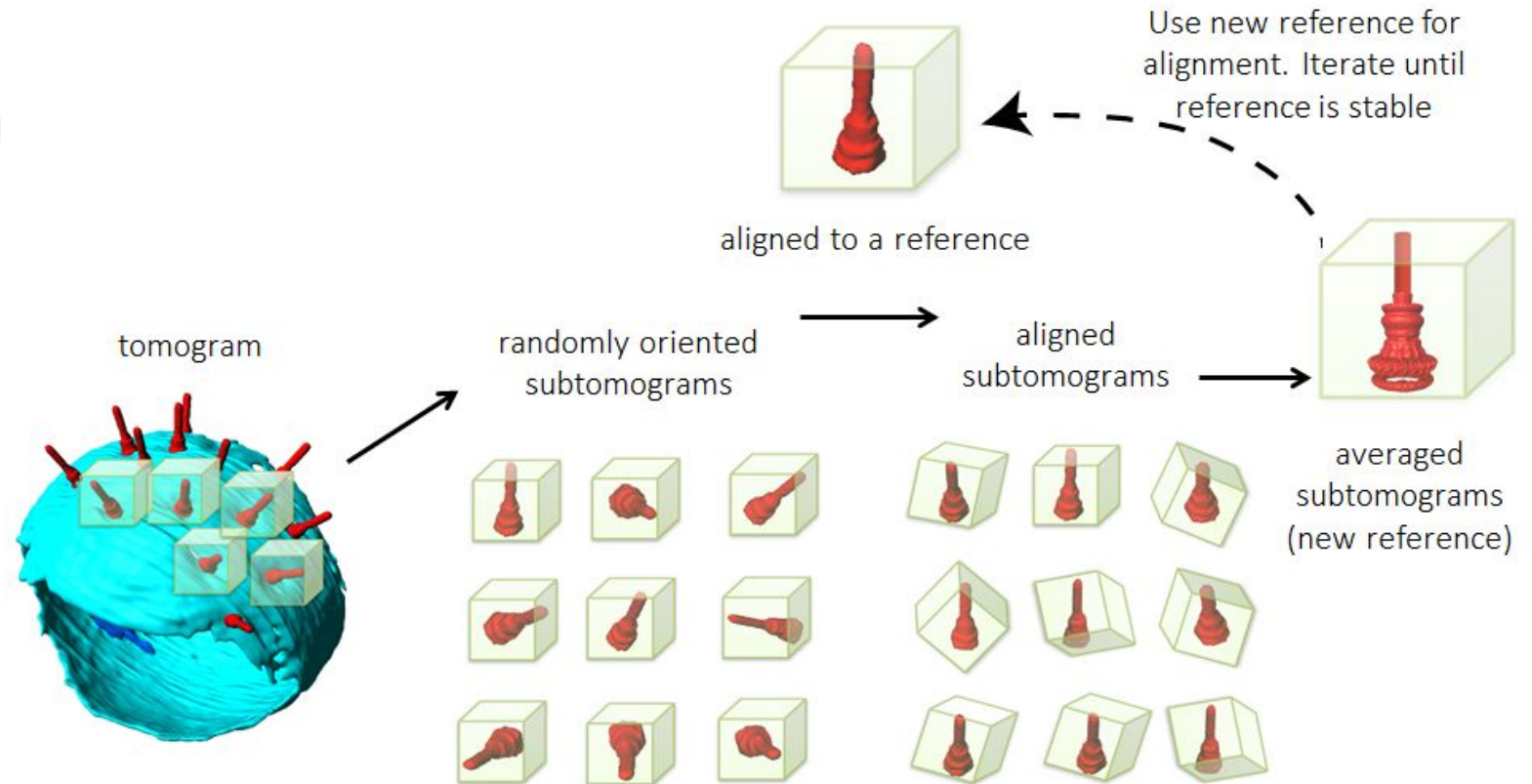


Sub-tomogram averaging

Single particle analysis in 3D

Similar process: particle picking,
iterative alignment and averaging

Typical resolution 8–20 Å



Sub-tomogram averaging

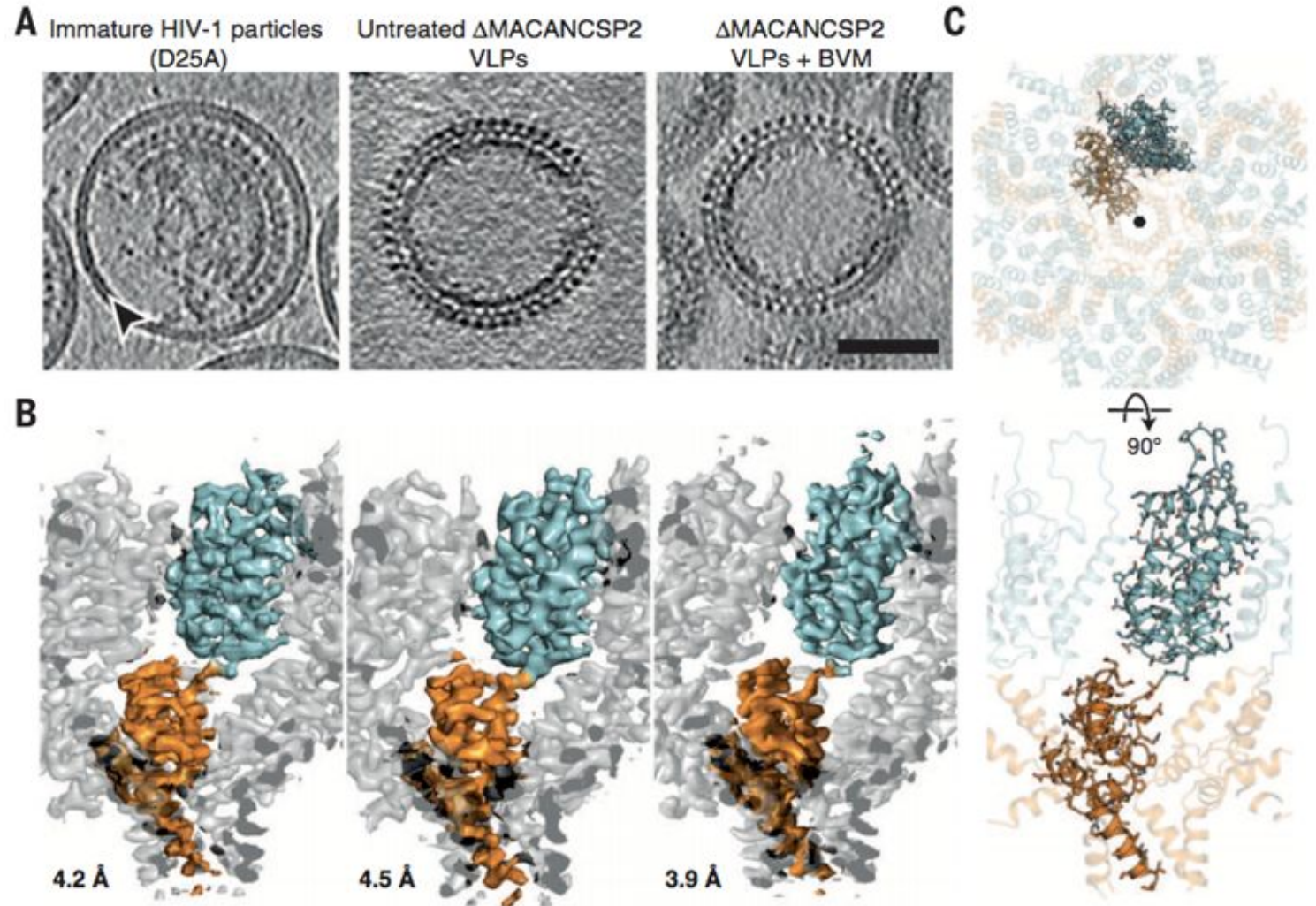
Single particle analysis in 3D

Similar process: particle picking, iterative alignment and averaging

Typical resolution 8–20 Å

Best example now reaches 3.1 Å

Shows the potential of the technique



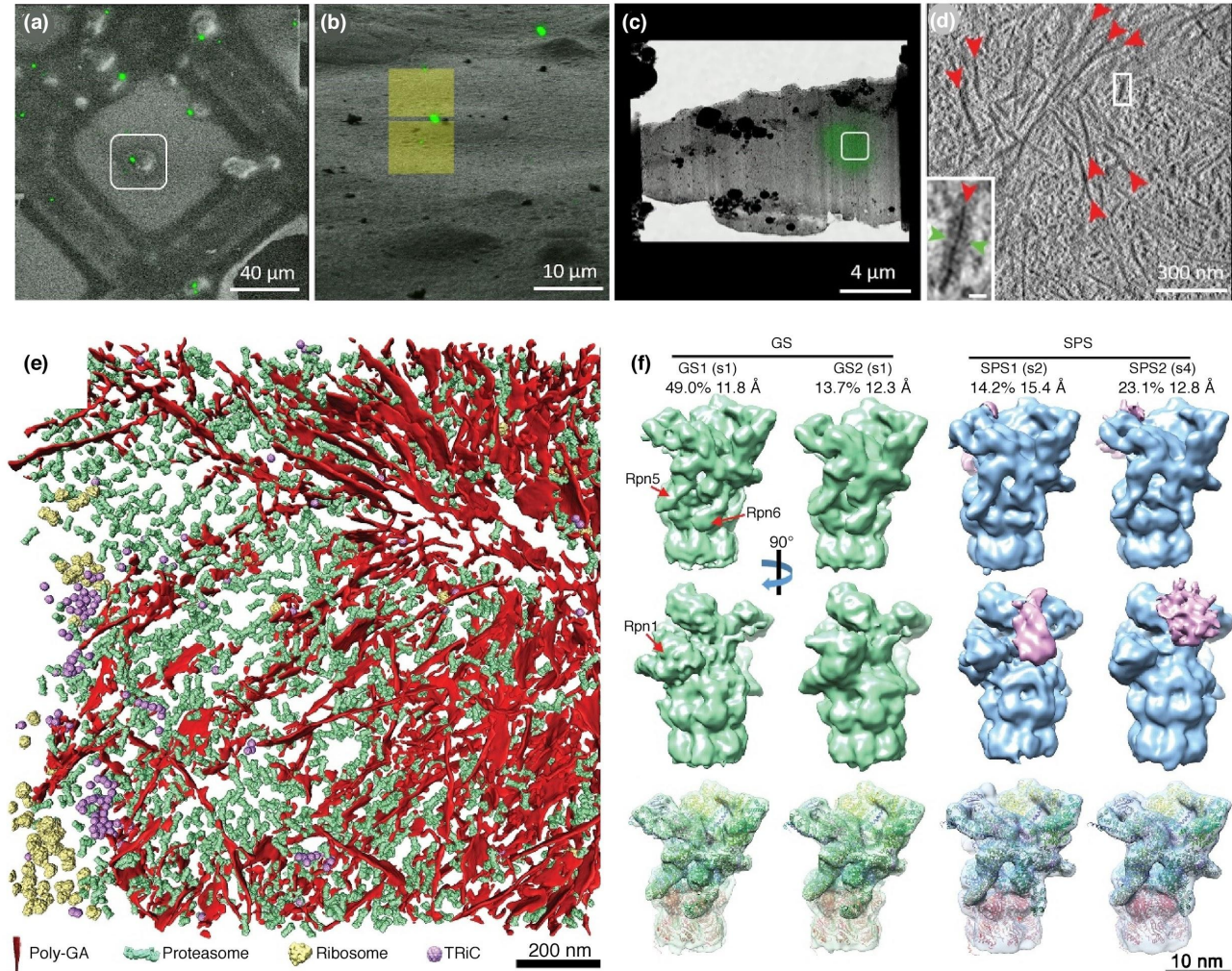
Multi-scale imaging

Correlative microscopy

Typical workflow:

1. Cryo-fluorescence microscopy to identify and locate target
2. FIB-SEM to extract region of interest
3. Cryo-ET to get cellular context
4. Sub-tomogram averaging and classification for higher resolution and quantification
5. Docking of models from MX or SPA for structural interpretation

Expect much more of this in future!



Current Opinion in Structural Biology

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CCP-EM papers:

Collaborative Computational Project for Electron cryo-Microscopy. Acta Cryst. D71:123–126 (2015)

Recent developments in the CCP-EM software suite. Acta Cryst. D73:469–477 (2017)

