# Cryogenic Electron Microscopy (cryo-EM)

CS 279 / BIOE 279 / BIOPHYS 279 / BMDS 245 / CME 279

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# THE REVOLUTION WILL NOT BE CRYSTALLIZED

**MOVE OVER X-RAY CRYSTALLOGRAPHY. CRYO-ELECTRON MICROSCOPY IS KICKING UP A STORM IN STRUCTURAL BIOLOGY BY REVEALING THE HIDDEN MACHINERY OF THE CELL.**

BY EWEN CALLAWAY

In a basement room, deep in the bowels of a steel-clad building in Cambridge, a major insurgency is under way.

A hulking metal box, some three metres tall, is quietly beaming terabytes' worth of data through thick orange cables that disappear off through the ceiling. It is one of the world's most advanced cryo-electron microscopes: a device that uses electron beams to photograph frozen biological molecules and lay bare their molecular shapes. The microscope is so sensitive that a shout can ruin an experiment, says Sjors Scheres, a structural biologist at the UK Medical Research Council Laboratory of Molecular Biology (LMB), as he stands dwarfed beside the £5-million (US$7.7-million) piece of equipment. "The UK needs many more of these, because there's going to be a boom," he predicts.

In labs around the world, cryo-electron microscopes such as this one are sending tremors through the field of structural biology. In the past three years, they have revealed exquisite details of protein-making ribosomes, quivering membrane proteins and other key cell molecules,

ILLUSTRATION BY VIKTOR KOEN


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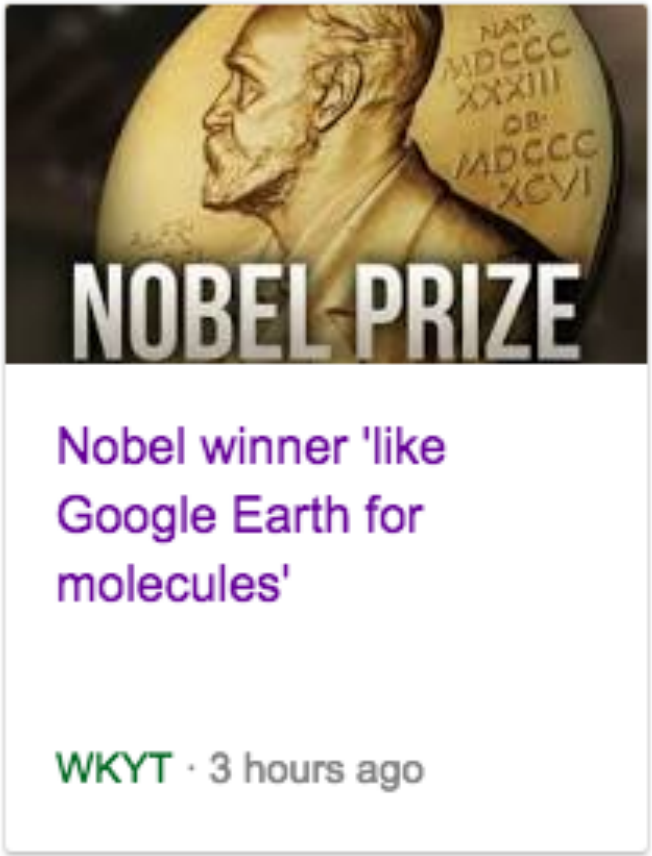
*Nature*, 2015

# 2017 Nobel Prize

Awarded to Jacques Dubochet, Joachim Frank and Richard Henderson, "For developing cryo-electron microscopy for the high-resolution structure determination of biomolecules in solution"



# Outline

- Overview of cryogenic electron microscopy (cryo-EM)
- Cryo-EM images are *projections*
- Computational reconstruction methods
  - 2D image analysis
    - Image preprocessing
    - Particle picking
    - Image clustering and class averaging
  - 3D reconstruction
    - Reconstruction with known view angles
    - Structure refinement with unknown view angles
    - Calculating an initial structure
    - Capturing multiple conformations
  - Building at atomic-level structural model

# Overview of cryogenic electron microscopy (cryo-EM)

# The basic idea

- We want the structure of a “particle”: a molecule (e.g., protein) or a well-defined complex composed of many molecules (e.g., a ribosome)
- We spread identical particles out on a film, and image them using an electron microscope
- The images are two-dimensional (2D), and each particle is positioned with a different, unknown orientation
- Given enough 2D images of particles, we can computationally reconstruct the 3D shape of the particle

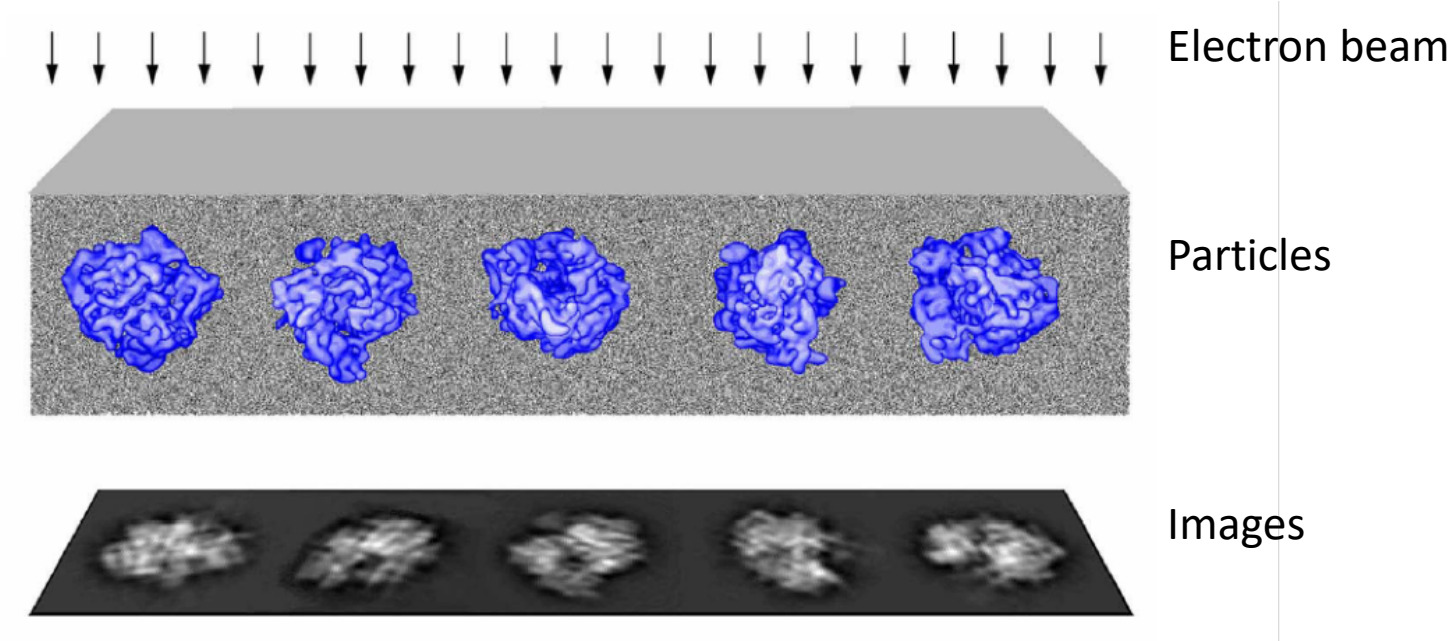





Image from Joachim Frank
http://biomachina.org/courses/structures/091.pdf




A high-end cryo-electron microscope



# Dramatic recent improvements

- Cryo-EM has been around for decades, but it has improved *dramatically* in recent years due to:
  - Invention of better cameras
    - Until around 2008, electrons were detected either by photographic film, or by scintillator-based digital cameras that converted electrons to photons for detection
    - New “direct-electron detectors” can detect electrons directly, substantially improving image resolution and quality
  - Better computational reconstruction techniques
- Cryo-EM is thus coming into much wider use, and is on track to become the dominant experimental method for determining molecular structure.

# Comparison to x-ray crystallography

- Cryo-EM's major advantage over crystallography is that it does not require formation of a crystal
  - Particularly advantageous for large complexes and for membrane proteins, as these are usually difficult to crystallize
  - Also avoids structural artifacts due to packing in a crystal lattice. In EM, particles are in a more natural environment.
- On the other hand:
  - Cryo-EM's resolution is usually worse than that of crystallography (but is improving)
  - Reconstructing structures of small proteins from EM images is difficult, because images from different orientations look similar (i.e., "a blob")

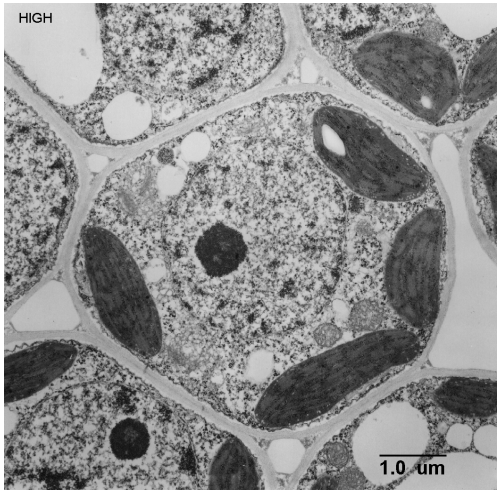
- Bottom line: Cryo-EM is particularly advantageous for larger complexes/molecules, because:
  - They tend to be harder to crystallize
  - The computational reconstruction problem in cryo-EM is usually easier to solve for large, asymmetric particles than for small ones

Cryo-EM images are *projections*

# Cryo-EM uses *transmission* electron microscopy

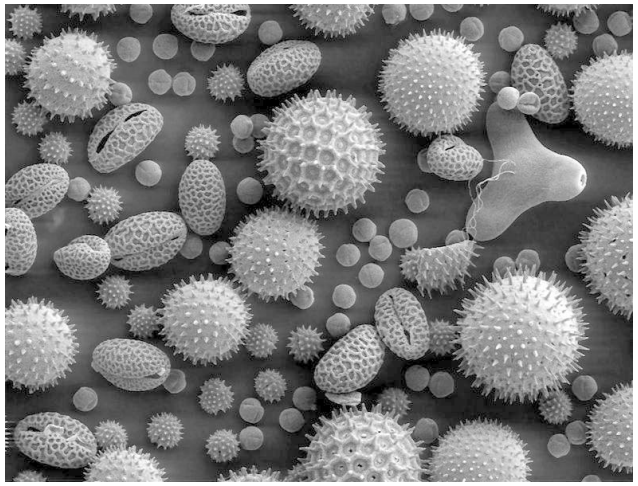
- In transmission electron microscopy, a beam of electrons passes through a thin sample before forming an image

Transmission electron microscopy




http://www.cas.miamioh.edu/~meicenrd/ANATOMY/Ch2_Ultrastructure/Tempcell.htm

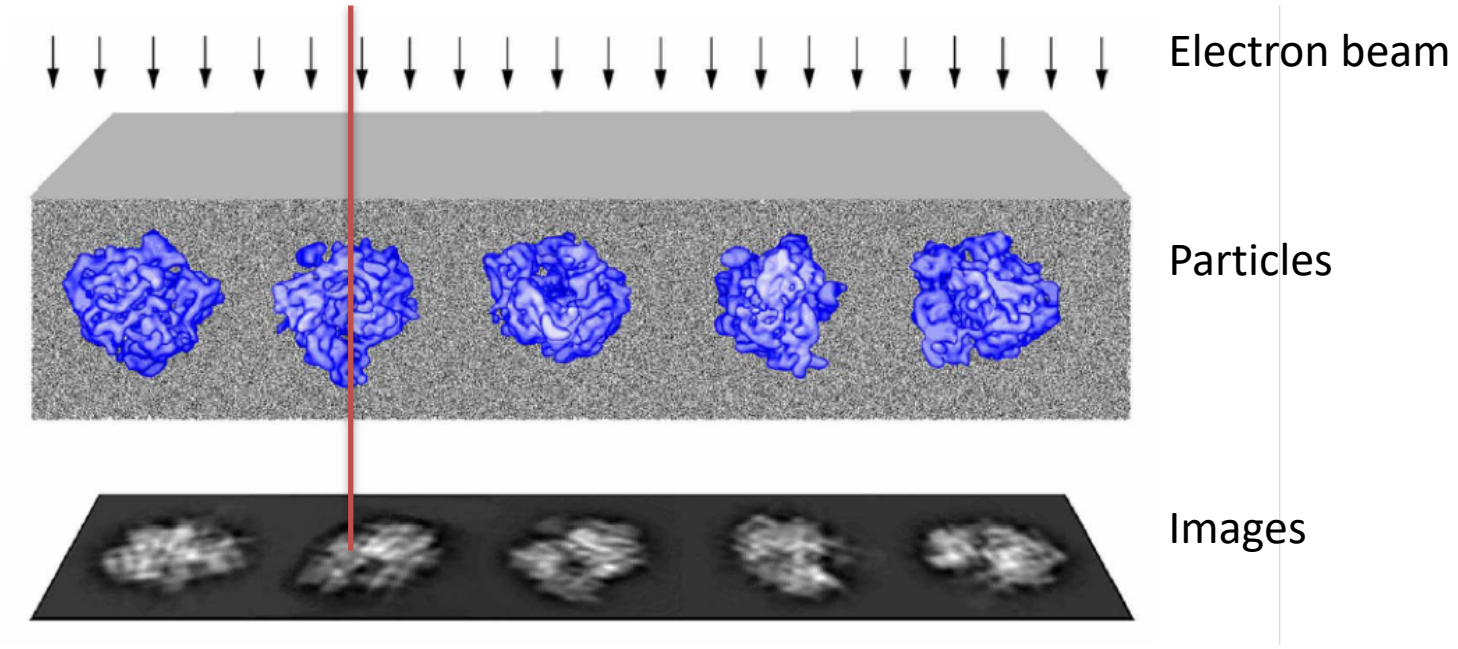
Scanning electron microsopy

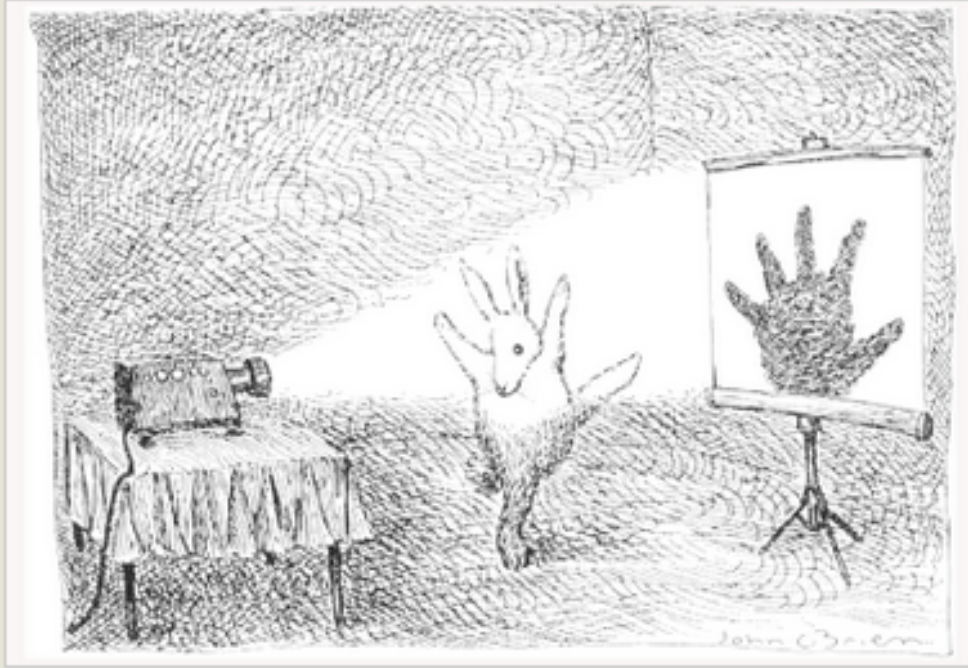


http://www.newscientist.com/data/images/ns/cms/dn14136/dn14136-1_788.jpg

# Cryo-EM images are projections

- Each recorded 2D image is thus a projection of the 3D shape (density) we want to reconstruct
  - That is, we can think of each pixel value in the 2D image as a sum of the values along a line through the 3D sample (in the direction of the electron beam)





From Joachim Frank, *Three-dimensional electron microscopy of macromolecular assemblies: Visualization of biological molecules in their native state*, 2006

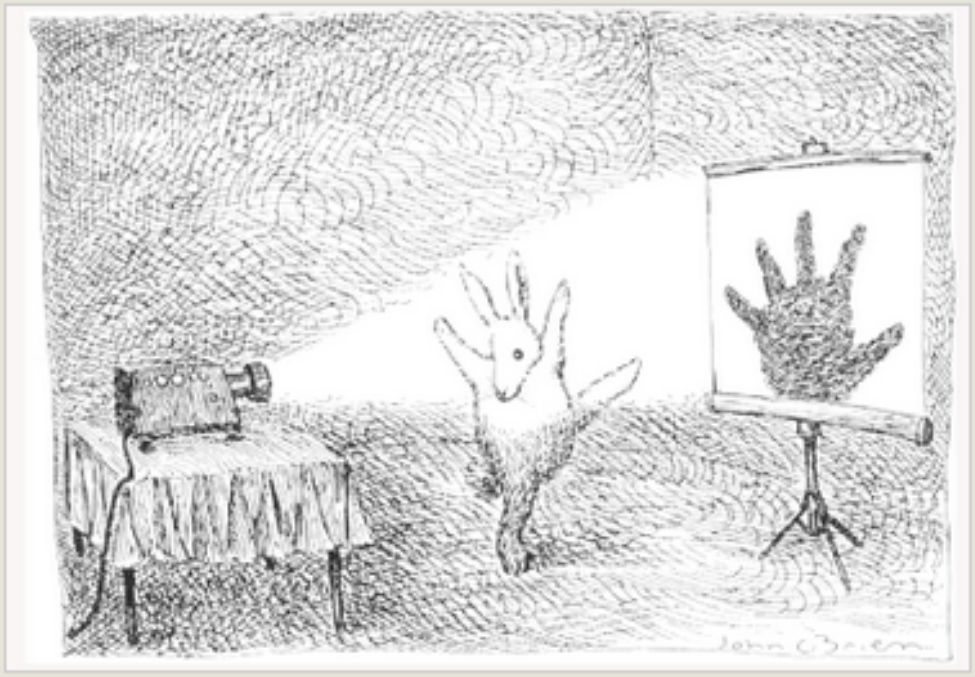


*Figure 5.1* A single projection image is plainly insufficient to infer the structure of an object. (Note, though, that TEM projections do not merely give the outline, as in this drawing, but internal features, too—the bones and internal organs of the rabbit, which we would see if the projector were to emit X-rays.) (Drawing by John O'Brien; © 1991 *The New Yorker*.)

In transmission EM, the image would look more like an "x-ray" of the bunny than a shadow of the bunny

From Joachim Frank, *Three-dimensional electron microscopy of macromolecular assemblies: Visualization of biological molecules in their native state*, 2006

# Vitrification

- To survive in the electron microscope (in a vacuum, under electron bombardment), particles are embedded in ice
- The sample is cooled extremely quickly (“flash frozen”), so the ice is “vitreous” (i.e., not crystalline)
- High-resolution single-particle EM relies on this “vitrification” process and is thus referred to as cryogenic electron microscopy (cryo-EM)



# Computational reconstruction methods

# Overview of computational methods

- **2D image analysis**: First, go from raw image data to higher-resolution 2D projections
  - Image preprocessing
  - Particle picking
  - Image clustering and class averaging
- **3D reconstruction**: Then use these higher-resolution projections to build a 3D density map
  - Background: Reconstruction with known view angles
  - Structure refinement with unknown view angles
  - Calculating an initial structure
  - Capturing multiple conformations
- **Model building**: Build an atomic-level structure into the density map

# Computational reconstruction methods

## 2D image analysis

# The raw images don’t look so good

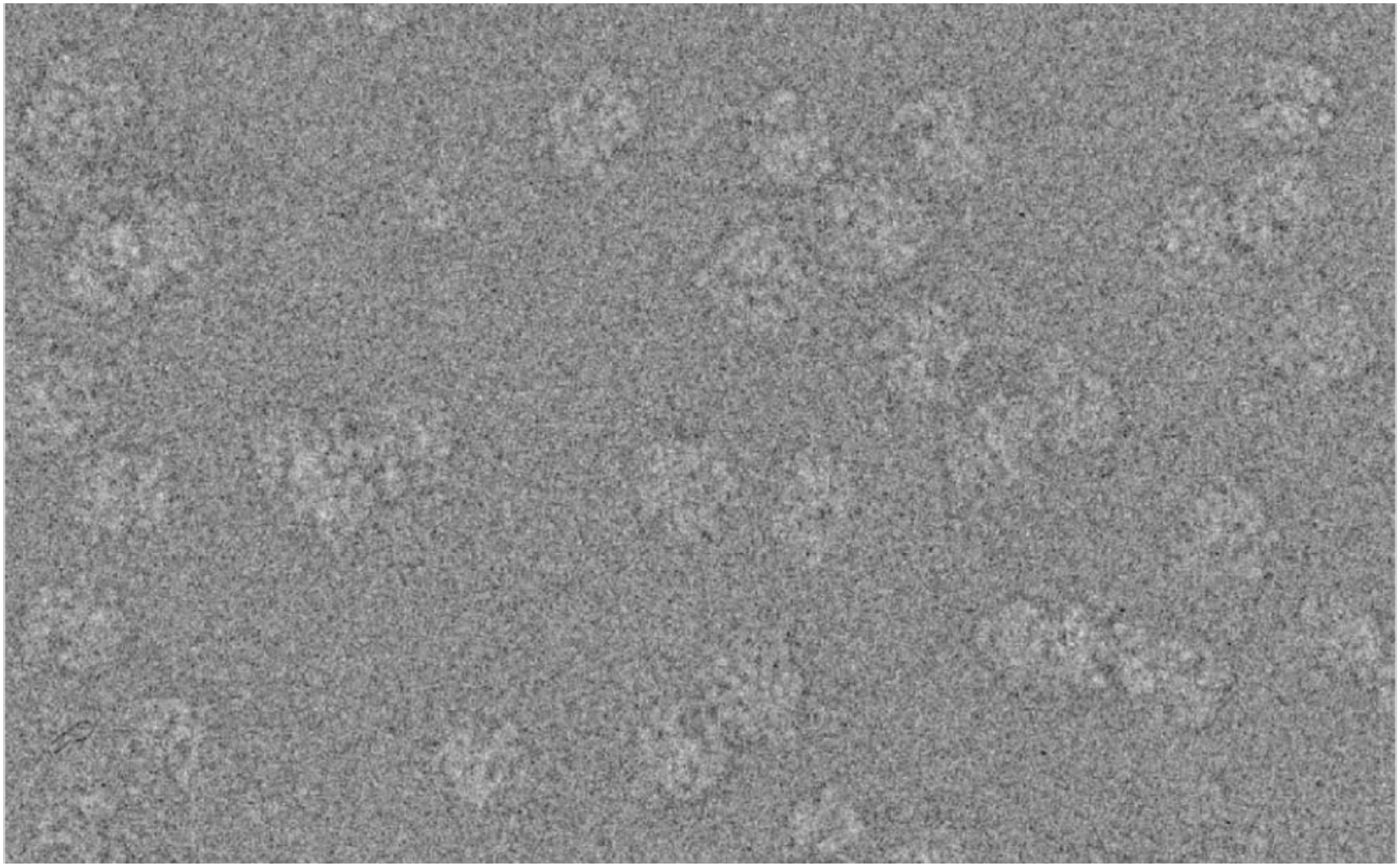


Image from Joachim Frank
http://biomachina.org/courses/structures/091.pdf



Before attempting any 3D reconstruction, we do several types of processing on the images

Computational reconstruction methods

2D image analysis

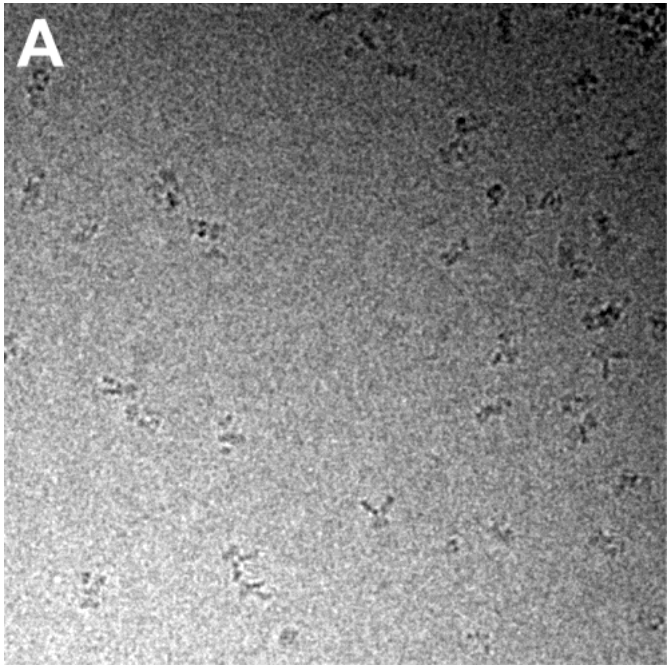
Image preprocessing

# Image preprocessing

- **Problem 1**: The sample tends to move slightly during imaging, blurring the image
- **Solution**
  - Direct electron detectors are fast enough to record a movie instead of a single image
  - Align the movie frames computationally, then average them together

# Image preprocessing

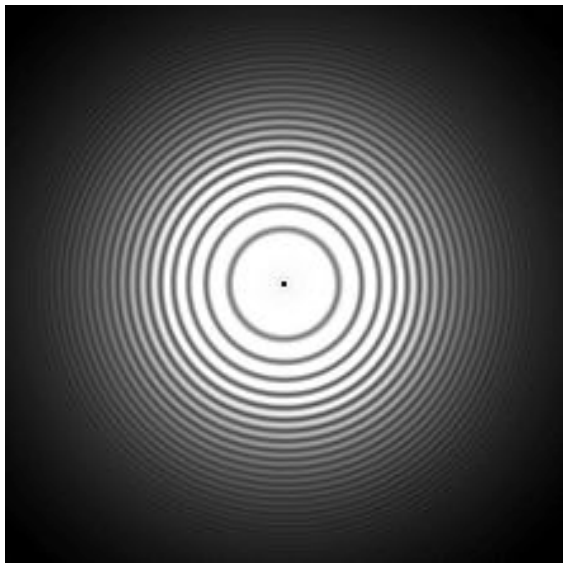
- **Problem 2**: Overall brightness is often non-uniform (due to uneven illumination or sample thickness)
- **Solution**: high-pass filter the image




Cheng et al., *Cell* 161:438 (2015)

# Image preprocessing

- **Problem 3**: The optics cause the recorded image to be a blurred version of the ideal image
  - This blurring is a convolution, and can thus be expressed as a multiplication in the frequency domain, where the ideal image is multiplied by the “contrast transfer function”
- **Solution**: Estimate parameters of the contrast transfer function, then correct for it
  - Some of the parameters are known (from the optics), while others are estimated from the images
  - Correction is generally done in the frequency domain



A typical contrast transfer function, in the frequency domain (zero frequency at the center)

https://en.wikipedia.org/wiki/Contrast_transfer_function

Computational reconstruction methods

2D image analysis

Particle picking

# Pick out the particles in the 2D images

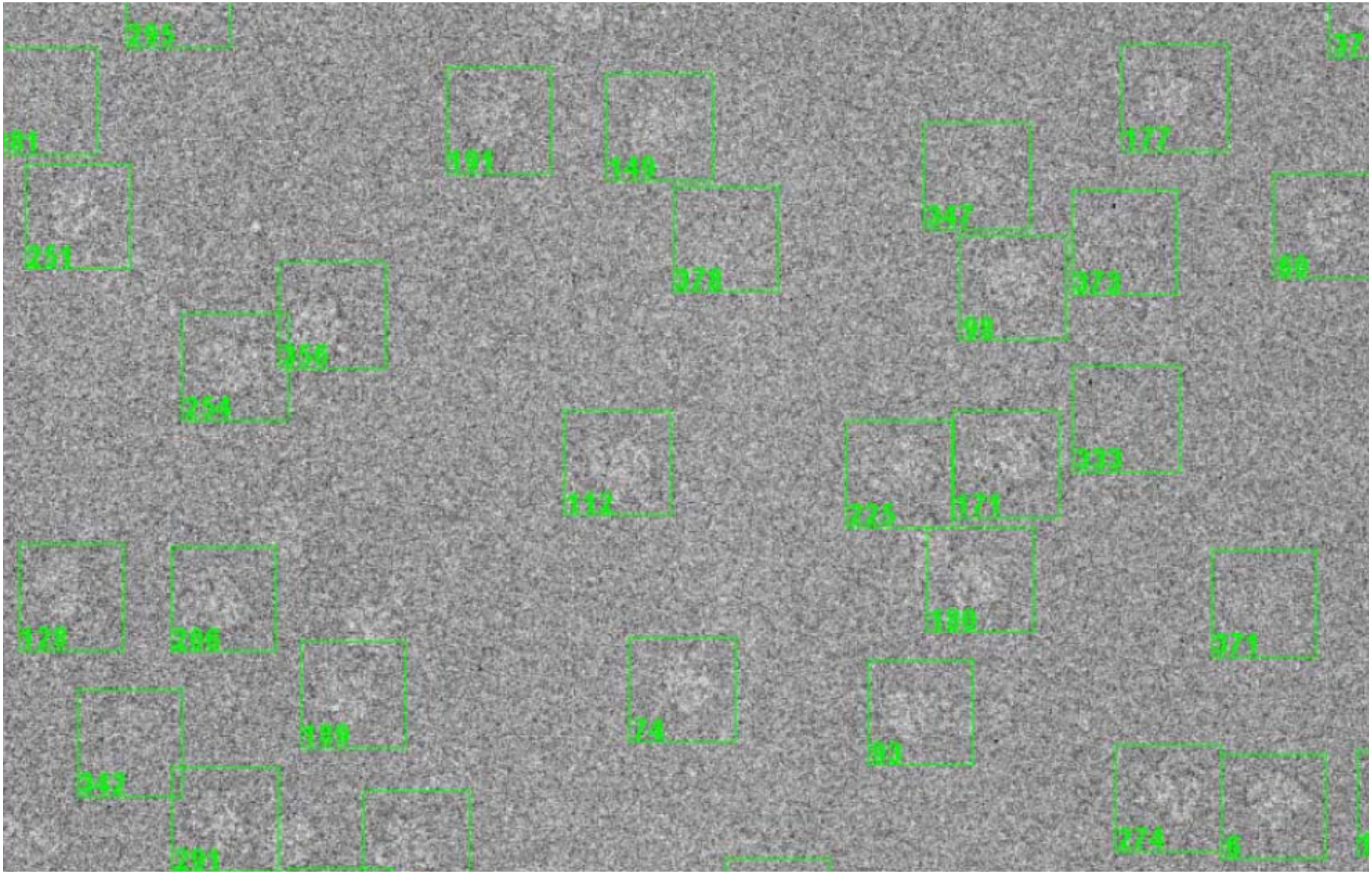


Image from Joachim Frank
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# Particle picking results

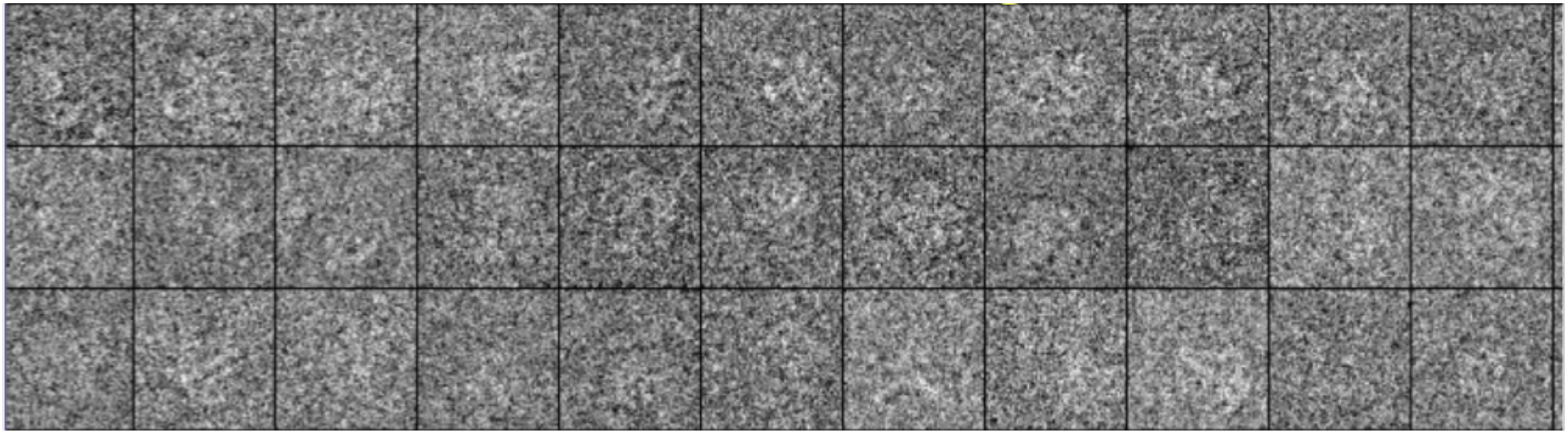

Image from Joachim Frank
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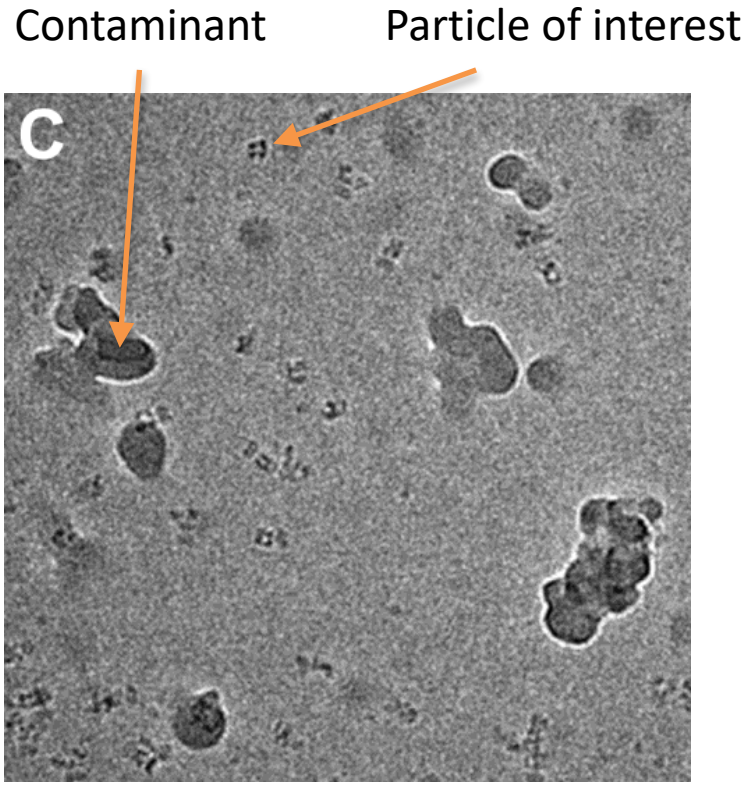
# Particle picking methods

- Particle picking can be difficult, because the images are low-contrast and noisy
  - Images may also have contaminants that should be ignored
- A variety of automated and semi-automated methods have been developed
  - The dominant methods use machine learning (neural networks)
  - Some particles are often still picked manually to provide training data specific to a particular sample




Cheng et al., *Cell* 2015