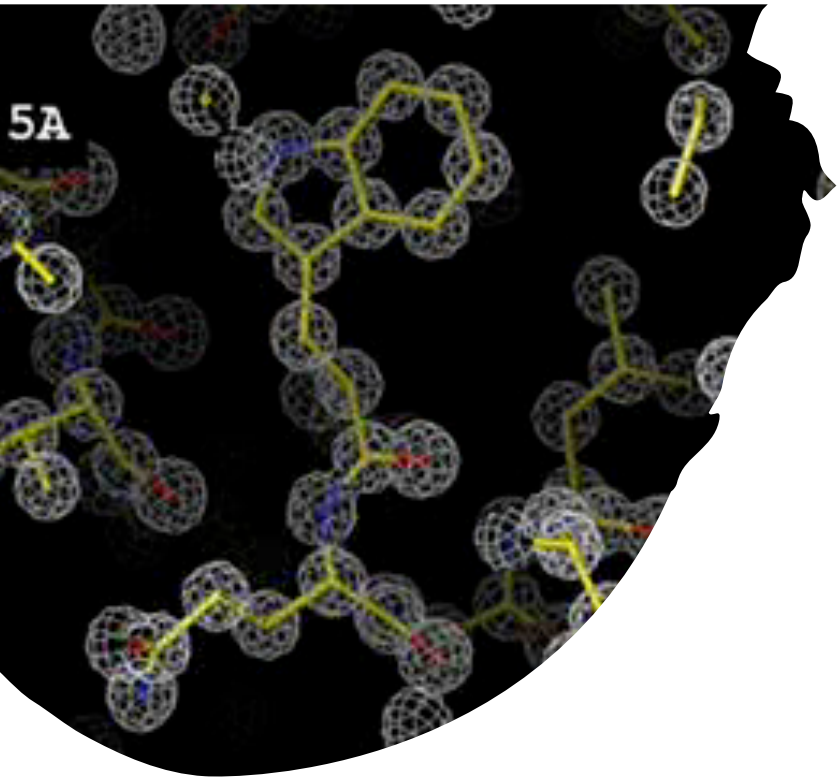
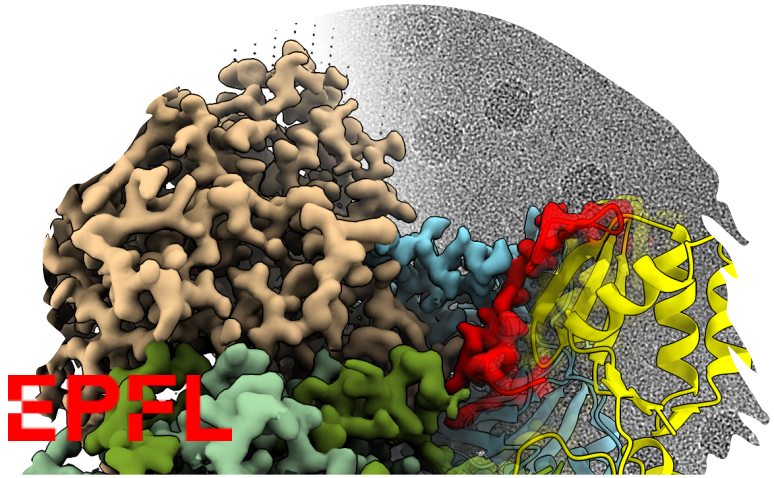




EDMS BIO-643: Integrative Structural Biology for Life Sciences

- Luciano Abriata
- Kelvin Lau
- Yoan Duhoo
- Anna-Sophia Krebs
- Jonathan Schneider
- Florence Pojer
- Guest: Prof. Henning Stahlberg

Protein Production and Structure Core Facility, SV-PTPSP

Fall Semester 2025

	PROS	CONS
X-ray crystallography	✓ Provide very detailed atomic information ✓ Easy to perform ✓ Not expensive ✓ Software free and user friendly ✓ No size limitation ✓ Synchrotron facilities around the world	✓ Need to form crystals ✓ High protein quantity ✓ Difficult for membrane proteins ✓ Difficult for flexible domains
BioNMR	✓ Small flexible proteins ✓ In solution ✓ Info on dynamics ✓ Info on ligand binding	✓ Not for big complexes (samples<40kDa) ✓ Low through-put ✓ High expertise ✓ High protein quantity, labeled ✓ Expensive
Single-particle EM	✓ Big complex, membrane proteins ✓ Not much protein needed (10 times less than crystallography) ✓ Achieve atomic resolution	✓ Still challenging for small protein <60kDa ✓ High expertise ✓ Low Throughput ✓ High-end equipment ✓ Expensive

EDMS BIO-643

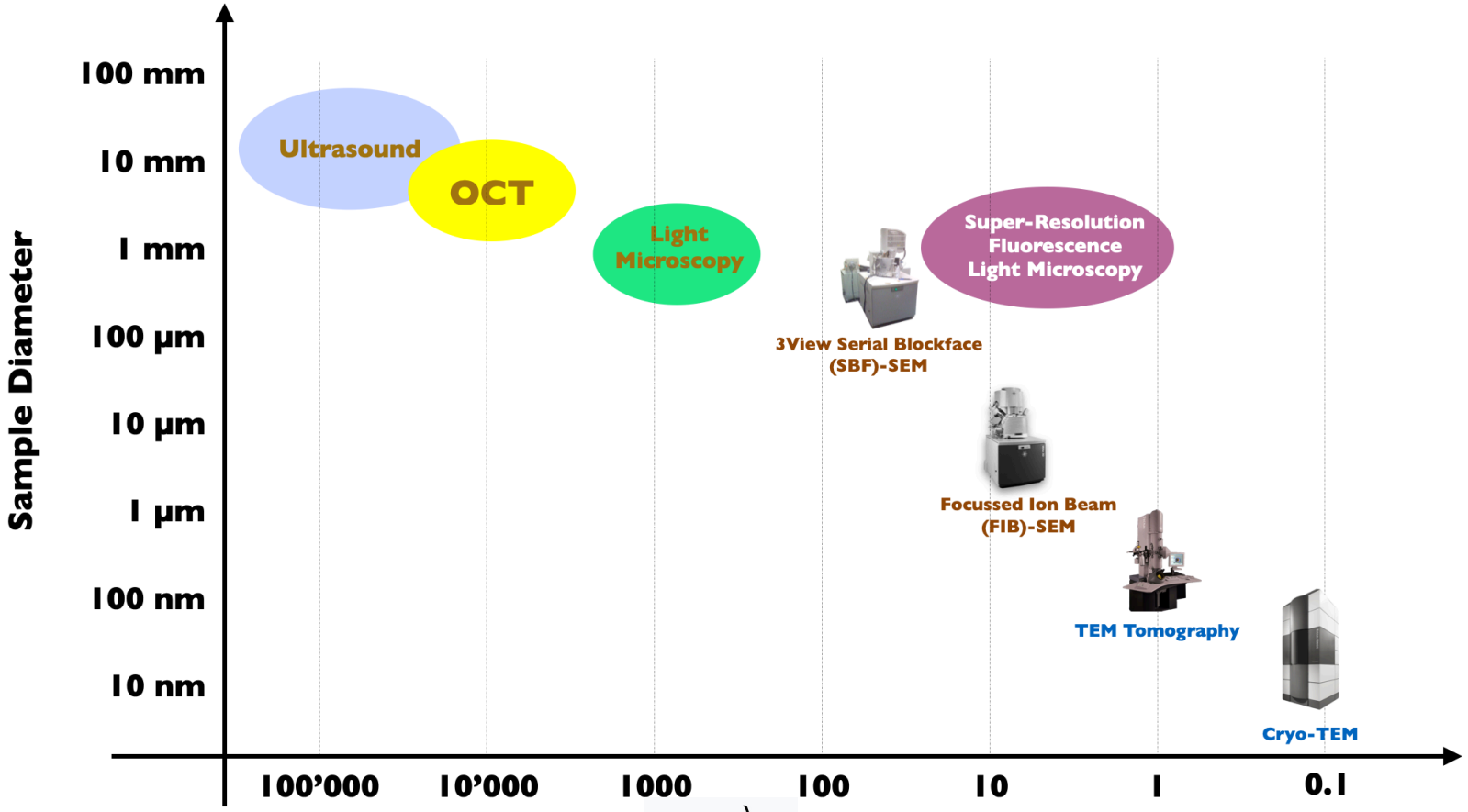
Integrative structural biology for Life sciences

Fall semester 2025

Date/Time: Thursdays 3:15 to 5pm
Location: AI 3142

Date	Topics	Speakers
Sept 11th	Course introduction	All teachers
Sept 18th	Modeling tools	Luciano
Sept 25th	Modeling tools -visualization in ChimeraX	Luciano, Yoan, Kelvin
Oct 2nd	X-ray crystallography theory + software	Florence/Kelvin
Oct 9th	no class	
Oct 16th	X-ray software	Kelvin/Florence
Oct 23rd	no class (EPFL break)	
Oct 30th	X-ray software	Kelvin/Florence
Nov 6th	**cryoEM theory**	**Henning**
Nov 13th	cryoEM software	Yoan, Anna-Sophia, Jonathan
Nov 20th	cryoEM-ET software	Yoan, Anna-Sophia, Jonathan
Nov 27th	cryoEM-ET software + visit DCI	Yoan, Anna-Sophia, Jonathan
Dec 4th	Bio-NMR theory + software + visit	Luciano, Kelvin
Dec 11th	Students presentations	All teachers
dec 18th	Students presentations	All teachers

No single instrument can cover all scales



$$d = \frac{\lambda}{2n \sin\theta}$$

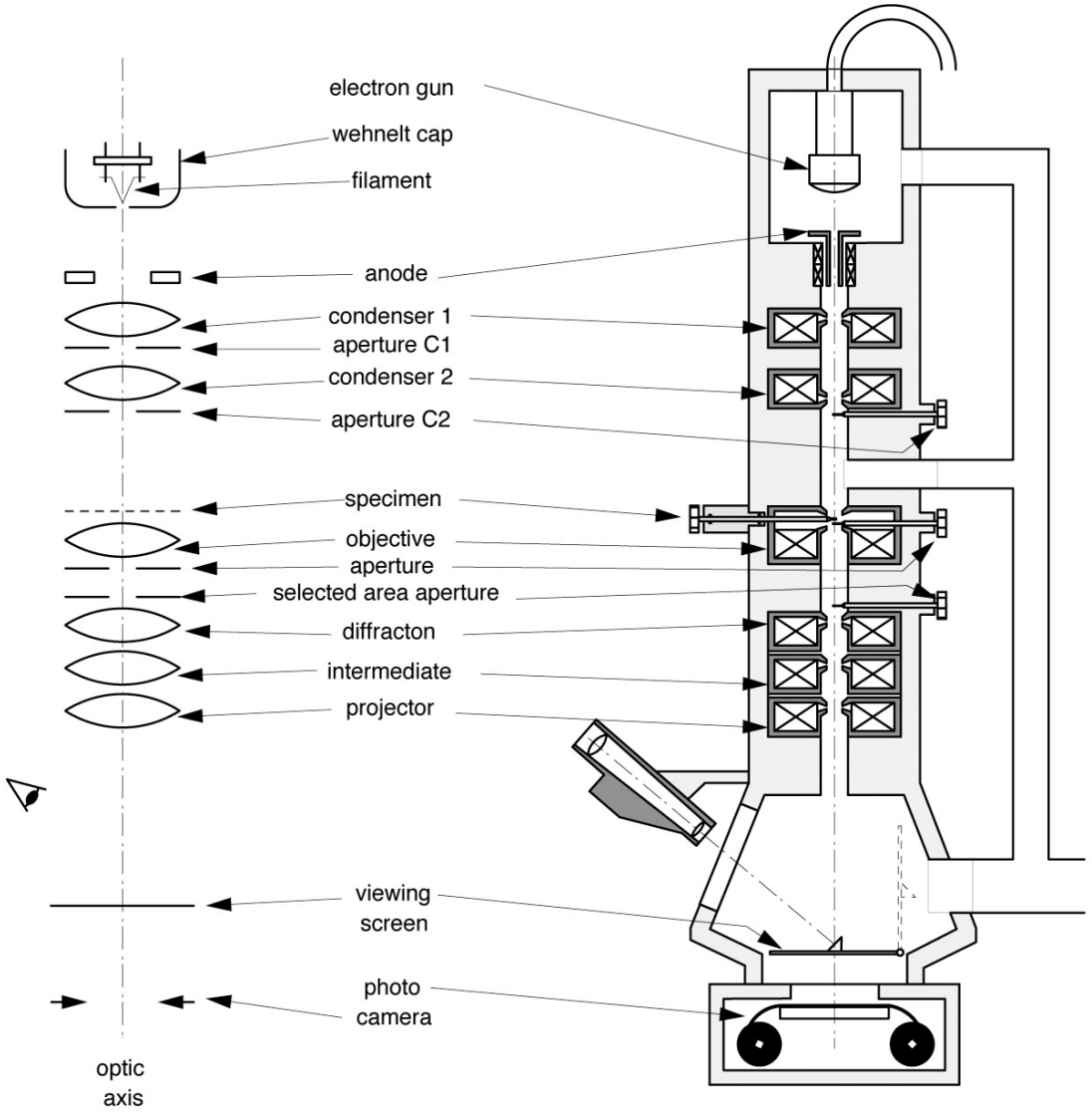
Ernst Abbe

History of TEM

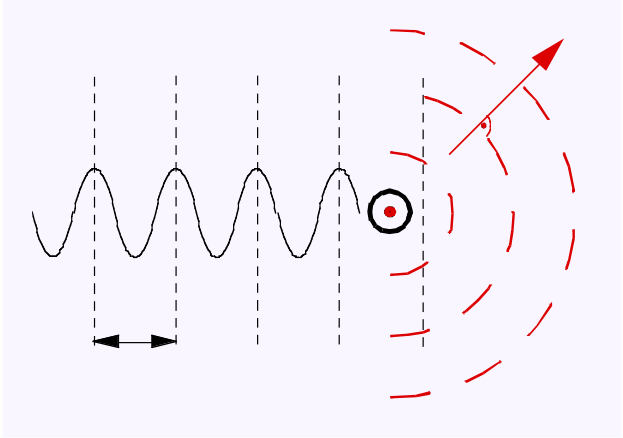
Ernst Ruska was awarded the Nobel prize 1986 for his construction of the first transmission electron microscope, together with Gerd Binning and Heinrich Rohrer for their invention of the scanning tunnelling microscope. As students, **Ernst Ruska** and **B. v. Borries** were in 1928 members of the group of **M. Knoll**, who was a group leader in the laboratory for high-tension technology and electric devices of Prof. **A. Matthias** at the Technische Hochschule (TH) Berlin, which later became the Technische Universität (TU) after the second world war. Ernst Ruska worked on a focusing coil for cathode beams. After his diploma thesis in the year 1930, he was unemployed, but he was allowed to continue his work unpaid. In April 1931 he managed to construct a magnifying device with 16 times magnification, using an electron beam. He and Knoll discussed at that time, if it would be possible to construct a high resolving microscope with an electron beam and the "lens" of Ruska. They imagined electrons as infinitely small particles, which would not limit the resolution. Only later they considered the possibility of a limitation to the resolution due to the "matter-wavelength" of an electron according to the definition of **Louis de Broglie** 1924/25. On July 4, 1931, Knoll presented their results at the public Cranz-colloquium in the TH Berlin. Their first publication using the word "electron microscope" was submitted on the 10th of September 1931.

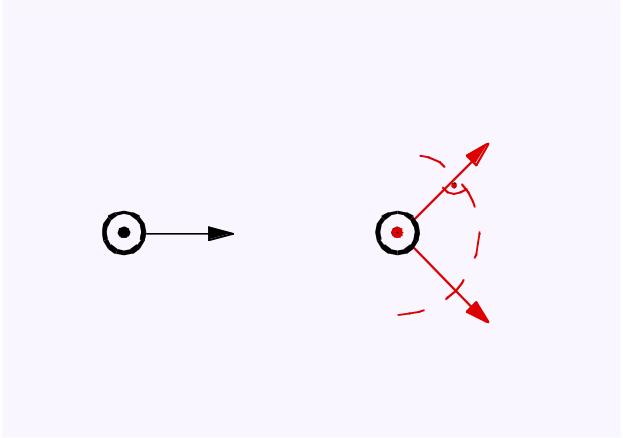
The first patent application, however, was submitted on May 30, 1931, by Prof. **Reinhold Rüdenberg**, who was at that time head of the scientific department of the Siemens-Schuckert-Werke in Berlin. Rüdenberg had been informed about the advances of Ernst Ruska by his assistant Dr. **Max Steenbeck**, who had visited the laboratory of Knoll and Ruska. This application resulted in three patents (DBP 895 635, 889 660 and 906 737), that were accepted only in the years 1953 and 1954. No participation of any kind in the development or the construction of an electron microscope by Rüdenberg has ever been documented.

The Transmission Electron Microscope



Wave-Particle Dualism





Waves <=====> **Particles**

momentum p=h/ λ

wavelength: λ

frequency: ν

velocity: v= $\lambda * \nu$

(e.g., wavefront, dif fraction, ...)

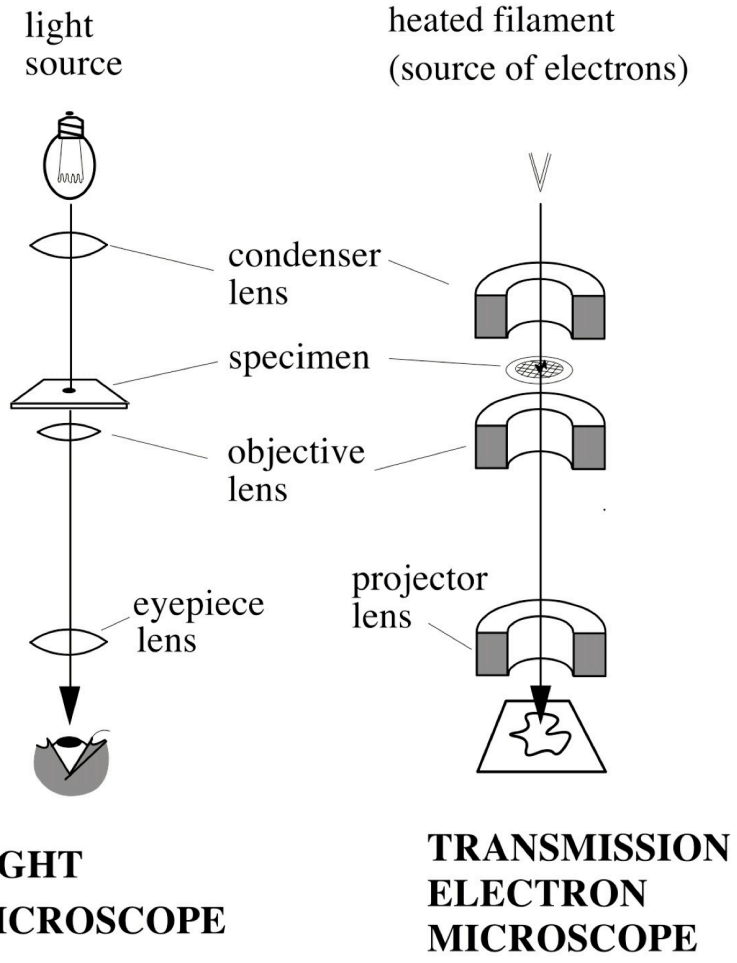
mass: $m = m_0 / \sqrt{1-(v/c)^2}$

velocity: v

momentum p=m*v

(e.g., trajectories, scattering, ...)

The electron beam



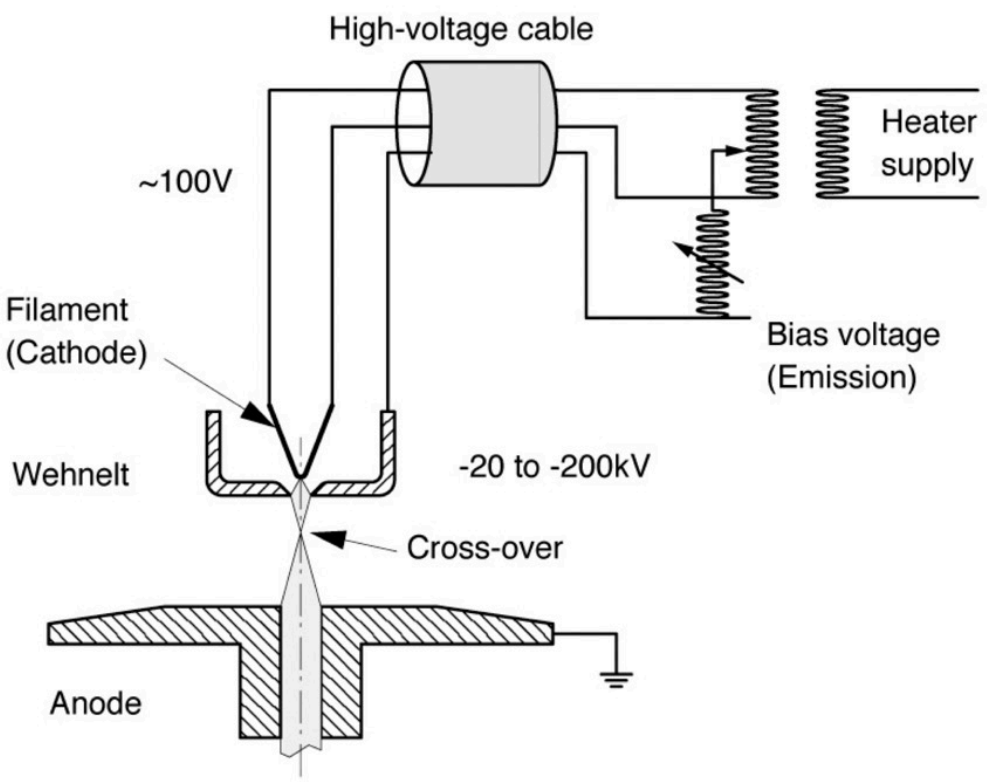
Type	Energy	Wavelength
Infrared	0.1 – 1 eV	10-1 µm
Visible	1-3 eV	1-0.3 µm
Ultraviolett	3-500 eV	0.3-0.01 µm
X-rays, γ	≥ 5000 eV	≤ 2 Å
Electrons	1 keV	0.4 Å
	10 keV	0.12 Å
	100 keV	0.037 Å
	1000 keV	0.009 Å

$$E = h \bullet v \qquad \lambda = \frac{c}{v}$$

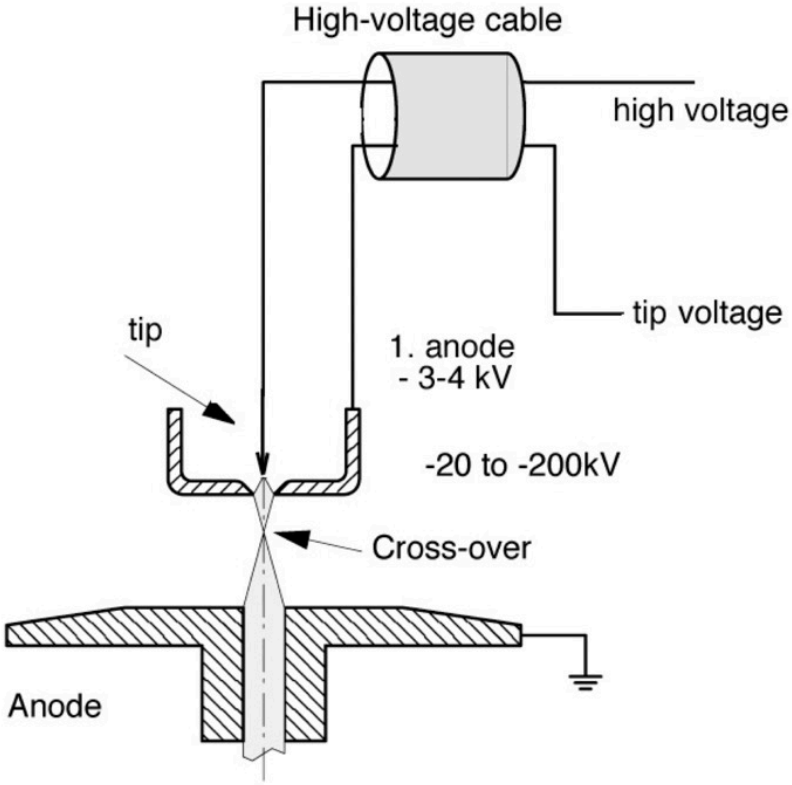
$$d = \frac{\lambda}{2NA}$$

1Å = 0.1 nm

Electron Sources

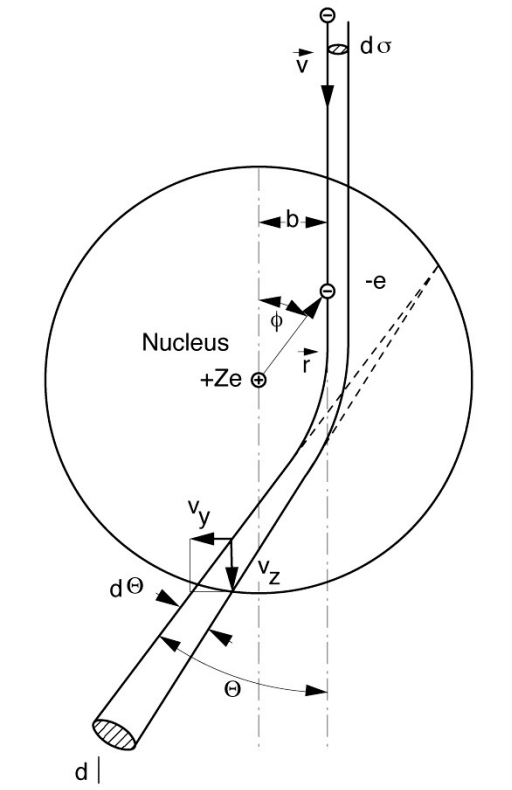


Heated Filament



Field Emission Gun (FEG)

Interaction Electron / Specimen Atom



The Primary Electron has 300'000 eV energy.

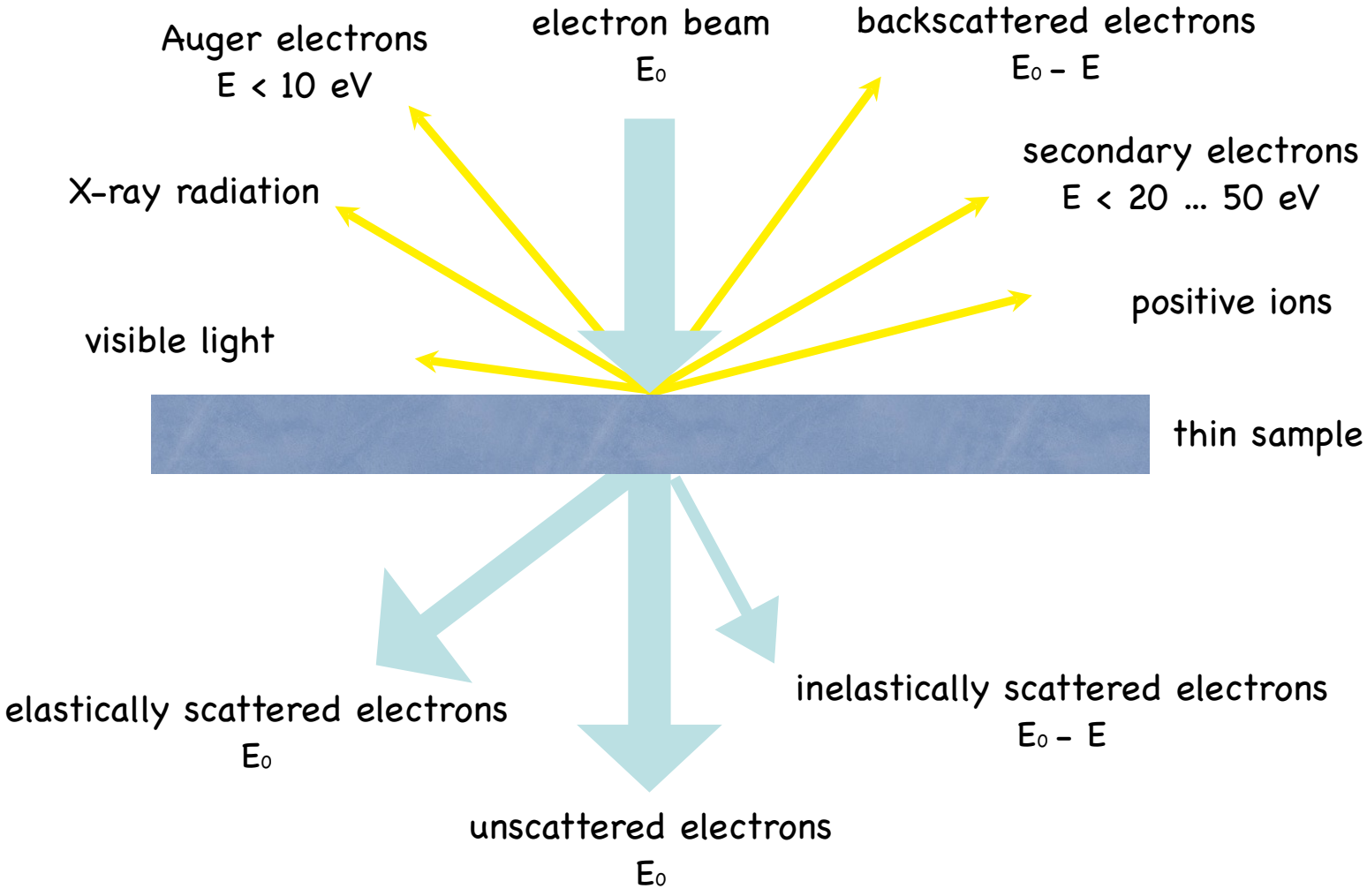
Bond Type	Approx. Energy value
Salt bridge (charge charge interaction)	1 eV
Hydrogen bond	0.1 eV
Van der Waals interaction	0.01 eV
Hydrophobic interaction	0.05 eV
Covalent bond	5 eV

An inelastic scattering causing a plasmon "steals" about 27 eV from the primary electron. That dos rarely stop it.

Think of a "Sniper Bullet" encountering a pile of chicken eggs….

Electrons are **scattered**, not absorbed by the atoms of the sample.

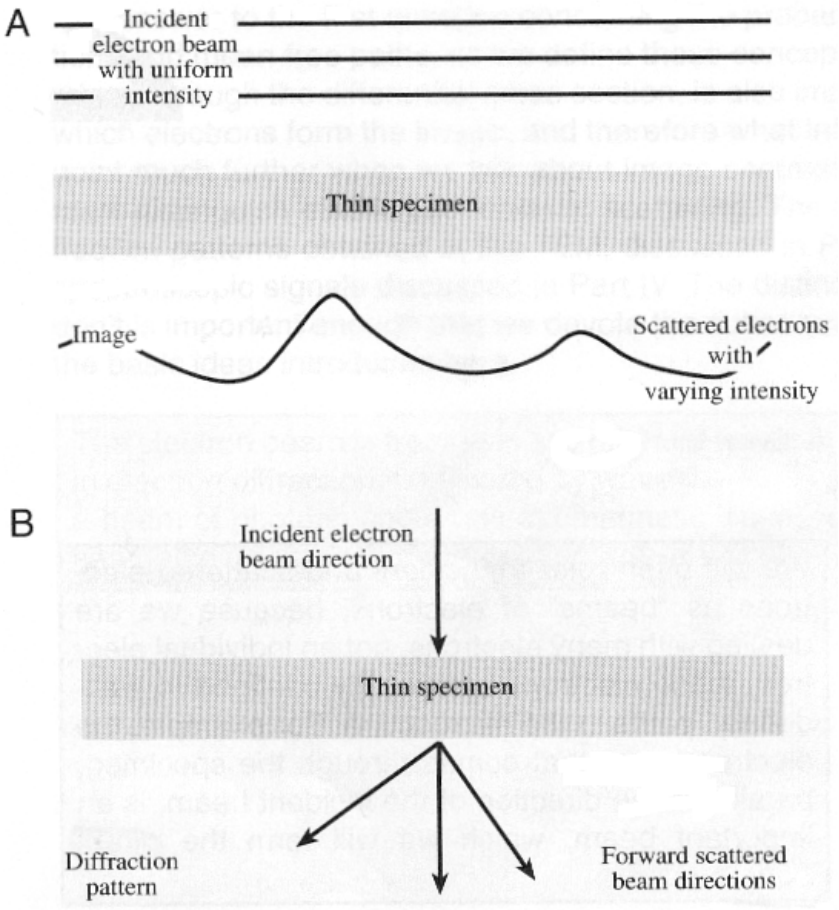
Electron Sample Interaction



Electron beam / sample interaction

What is an electron beam?

Wave Model

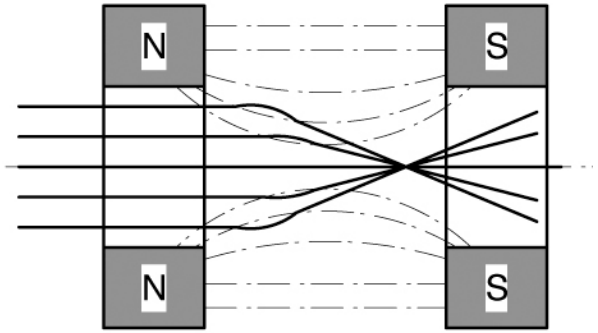


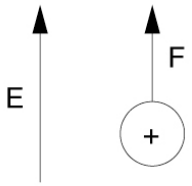
Particle Model

Both angular and spatial distribution to scattering

Diffraction patterns and images

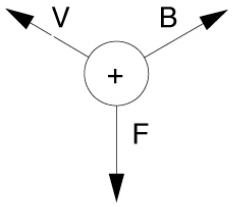
Electrons are focused by magnetic fields





$\vec{F} = q \cdot \vec{E}$

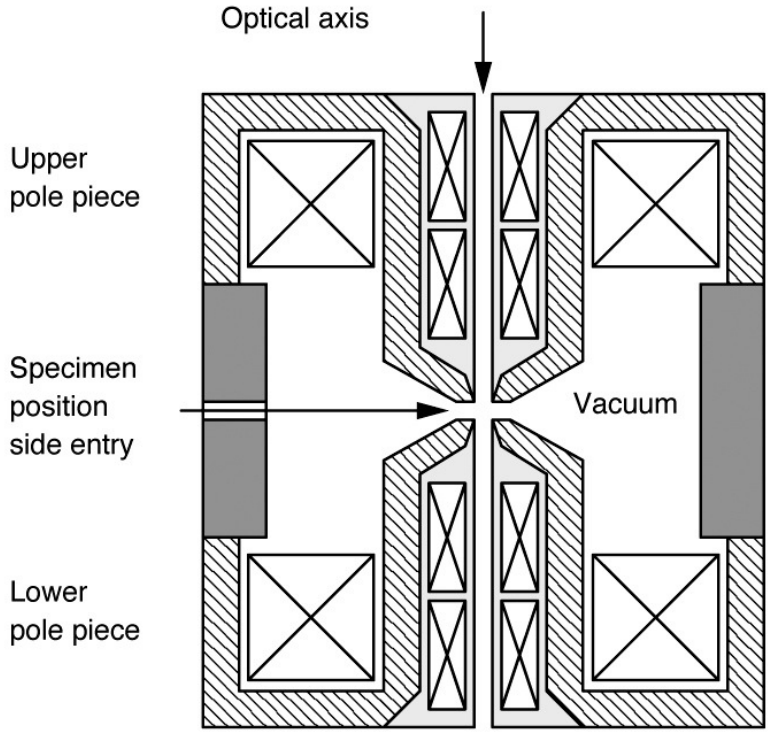
Electrostatic Force



$\vec{F} = q \cdot \vec{v} \times \vec{B}$

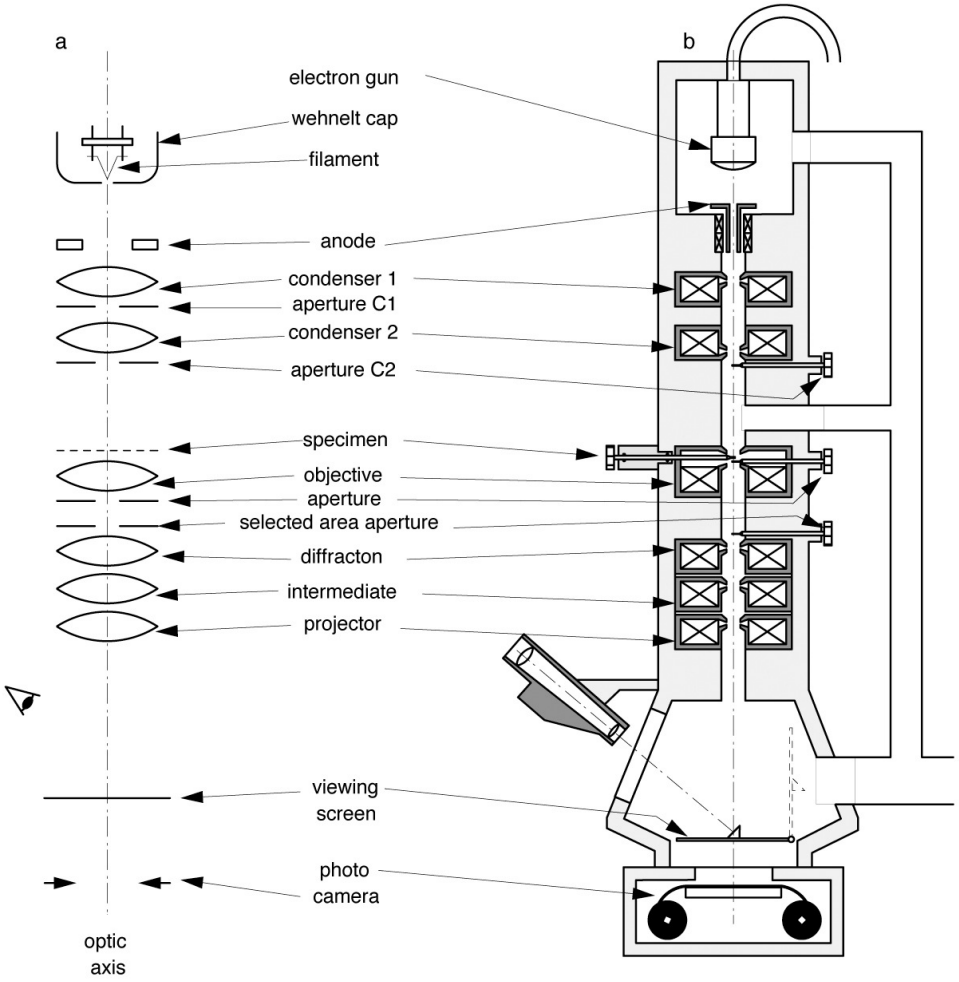
Lorentz Force

(Left-hand rule for negative electrons)

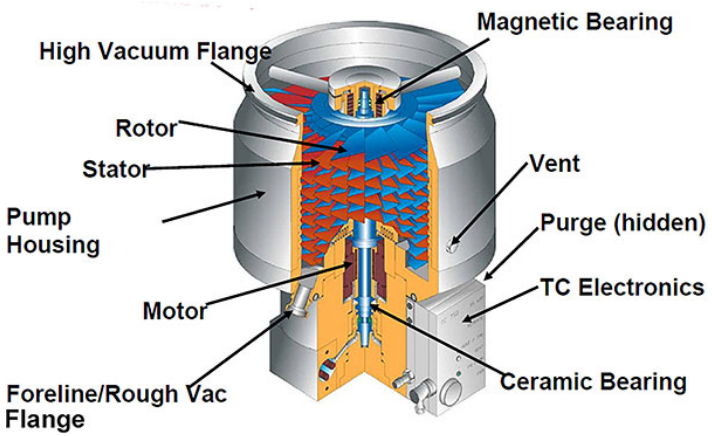


The magnetic electron lens

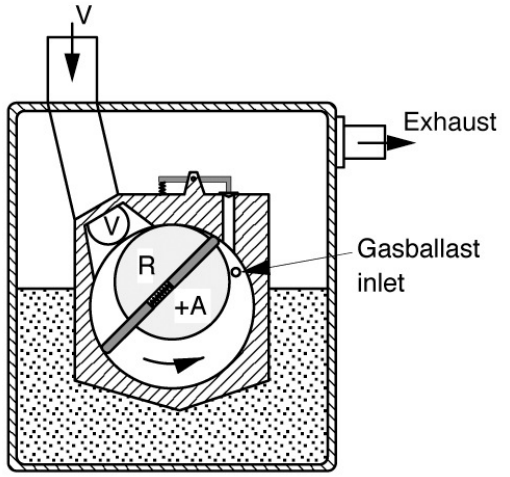
Flying Electrons require Vacuum



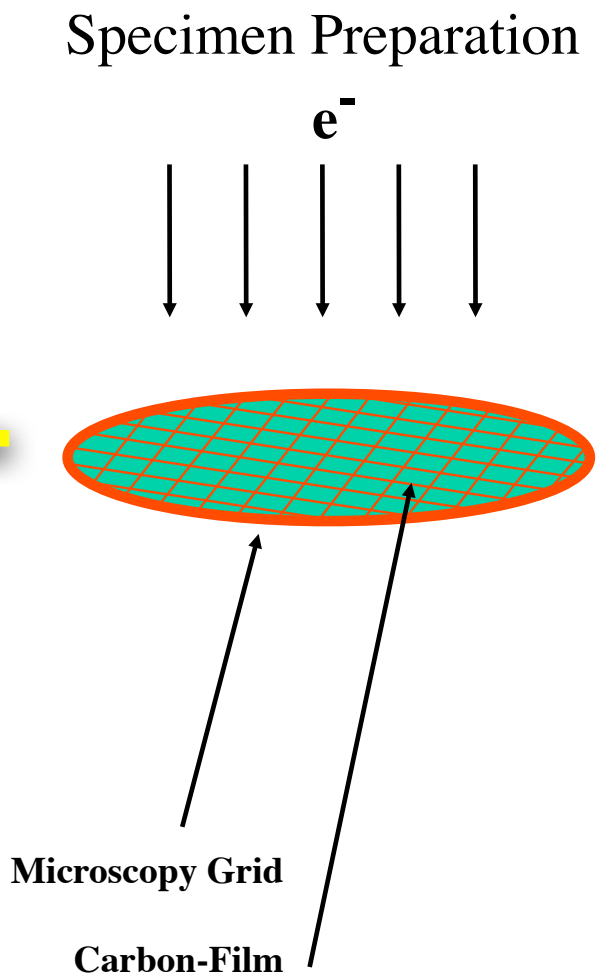
Turbo pump:
Can only start, once 10^{-2} mbar are reached,
and can reach 10^{-6} mbar.



Rotary pump:
Can work with any pressure,
but only reaches 10^{-3} mbar.



Transmission Electron Microscopy



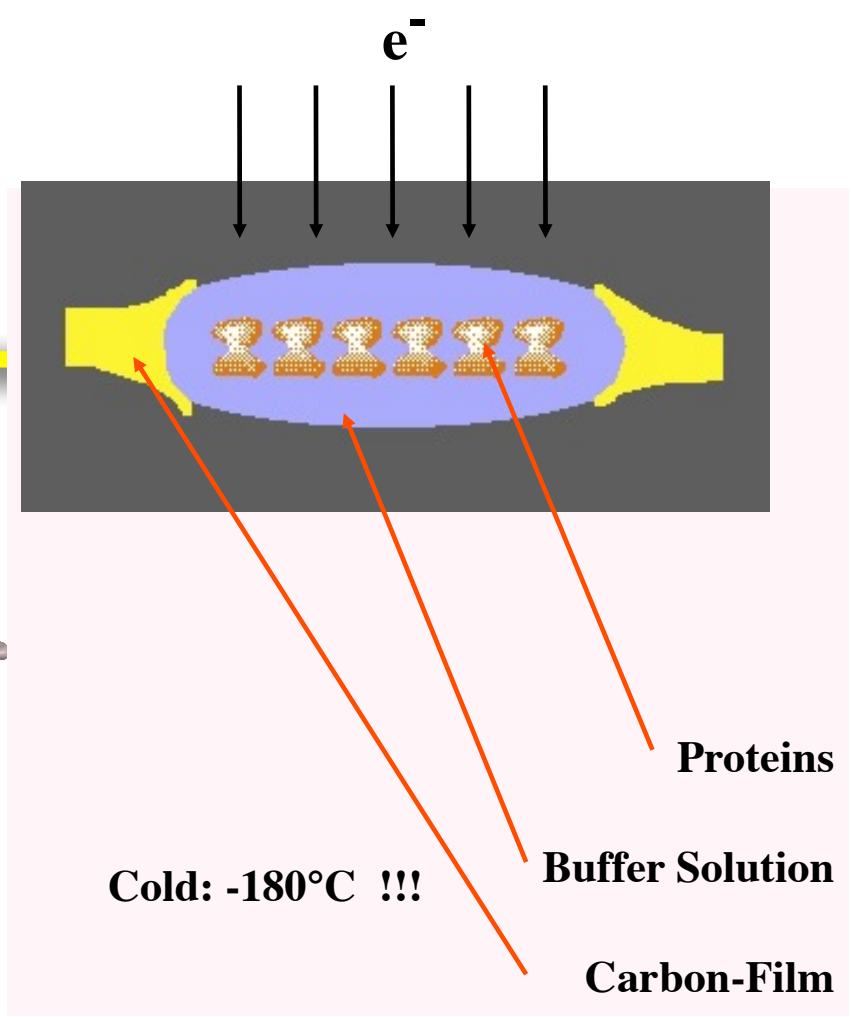
Transmission Electron Microscopy

Negative Stain TEM

Transmission Electron Microscopy

Cryo-EM

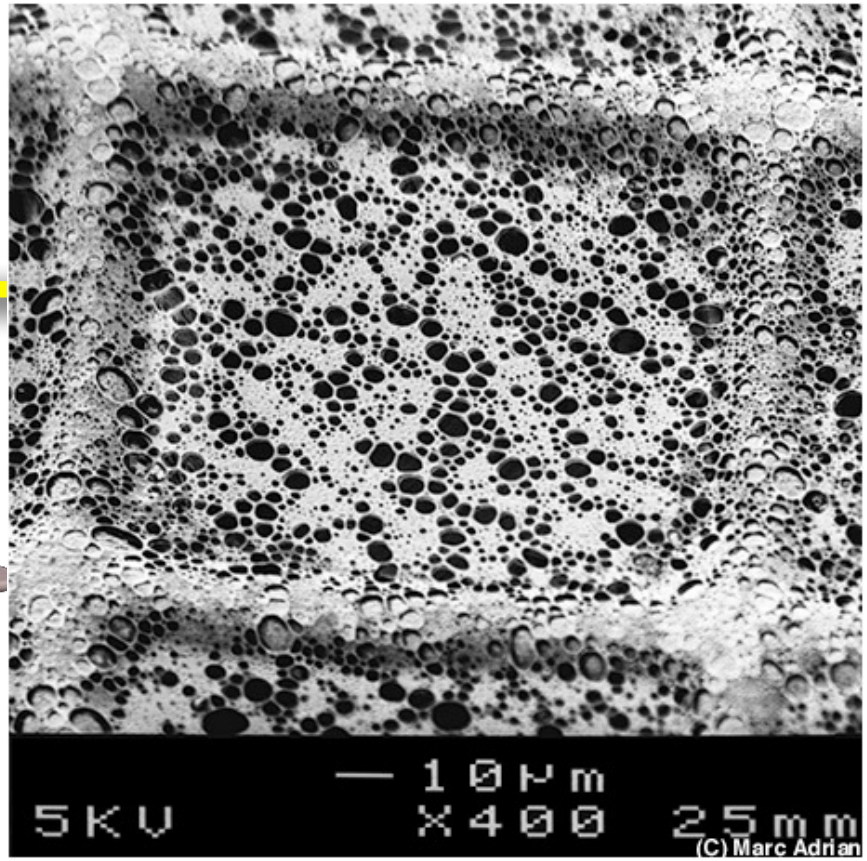
Specimen Preparation



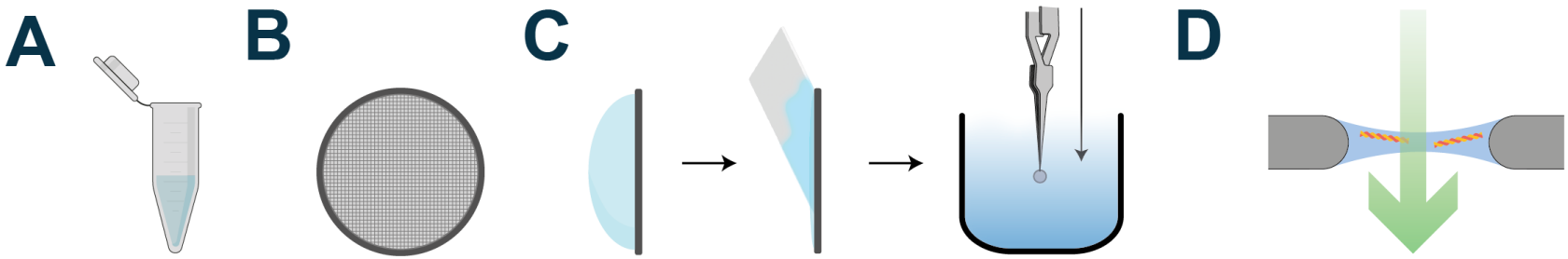
Transmission Electron Microscopy

Cryo-EM

Holey Carbon Film



Cryo-EM: principle workflow



- Homogenous sample (purified)
- Small sample aliquots (3µl) on a copper/gold grid
- Blot excess liquid (≈ 99%)
- Rapid plunge freezing (in liquid ethane @ -180°C)

➢Sample in a close to native state frozen in vitreous ice

➢Acquisition of projection images

https://cryoem101.org/


Transmission Electron Microscopy
Cryo-EM
3 µl

Cryo-Electron Microscopy

Infection of bacteria
(here liposomes with FhuA)
by the Bacteriophage T5

Animation by Rossmann group (Purdue),
based on data by Peter Leiman et al. (now EPFL)

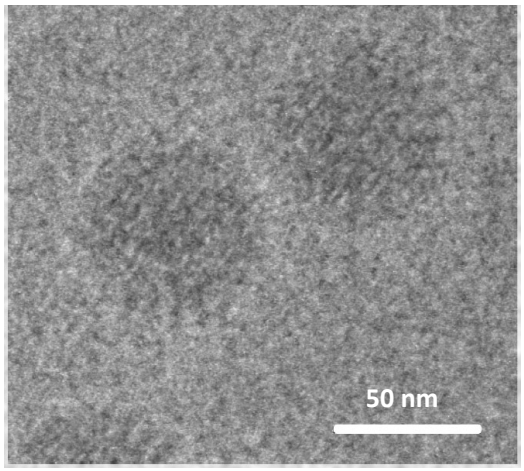
Electron microscopy

100 nm

Lambert et al. *Mol Microbiol 1998*

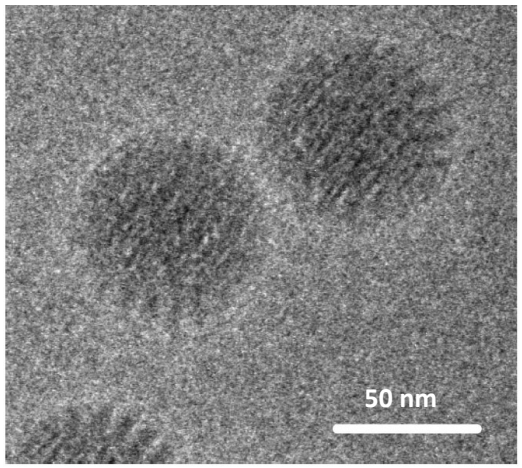
Particles move under the electron beam

< 2012
Imaged on a CCD camera or on film



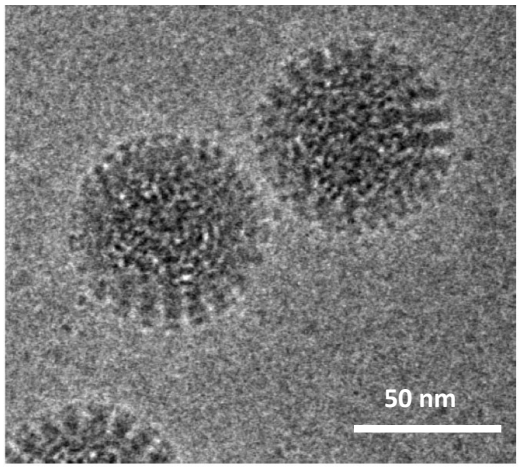
Typical Parameters:
Exposure Time: 1 second
Dose: 10 electrons / Å^2

> 2013
Imaged on a direct electron detector



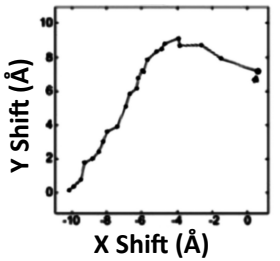
Typical Parameters:
Exposure Time: 1 second
Dose: 20 electrons / Å^2

Imaged on a direct electron detector with dose-fractionation and drift correction



Typical Parameters:
Sub-Frames: 100
Frame Exposure Time: 0.2 seconds
Total Exposure Time: 20 seconds
Total Dose: 100 electrons / Å^2

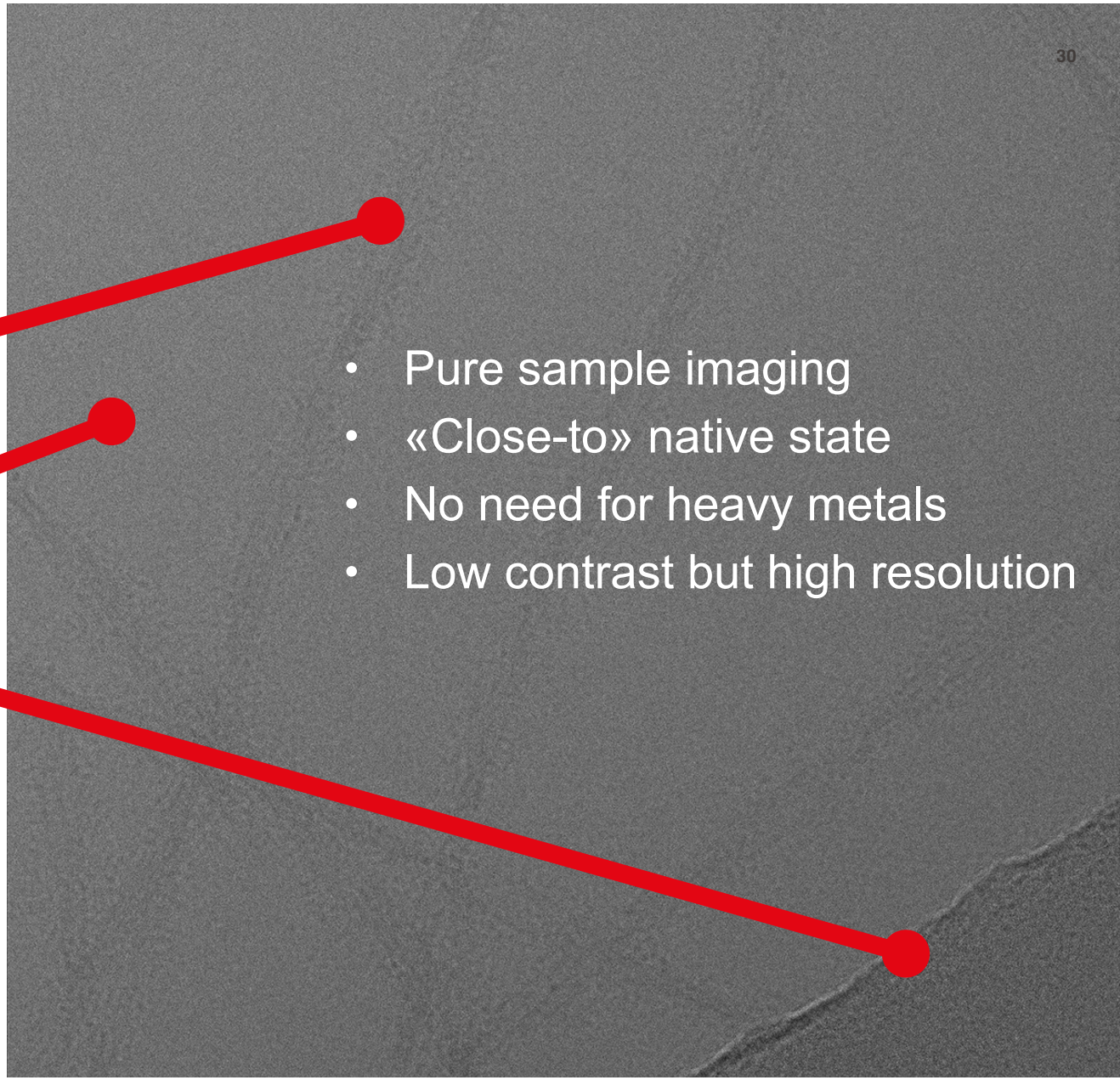
Sub-Frames are aligned and averaged by software.



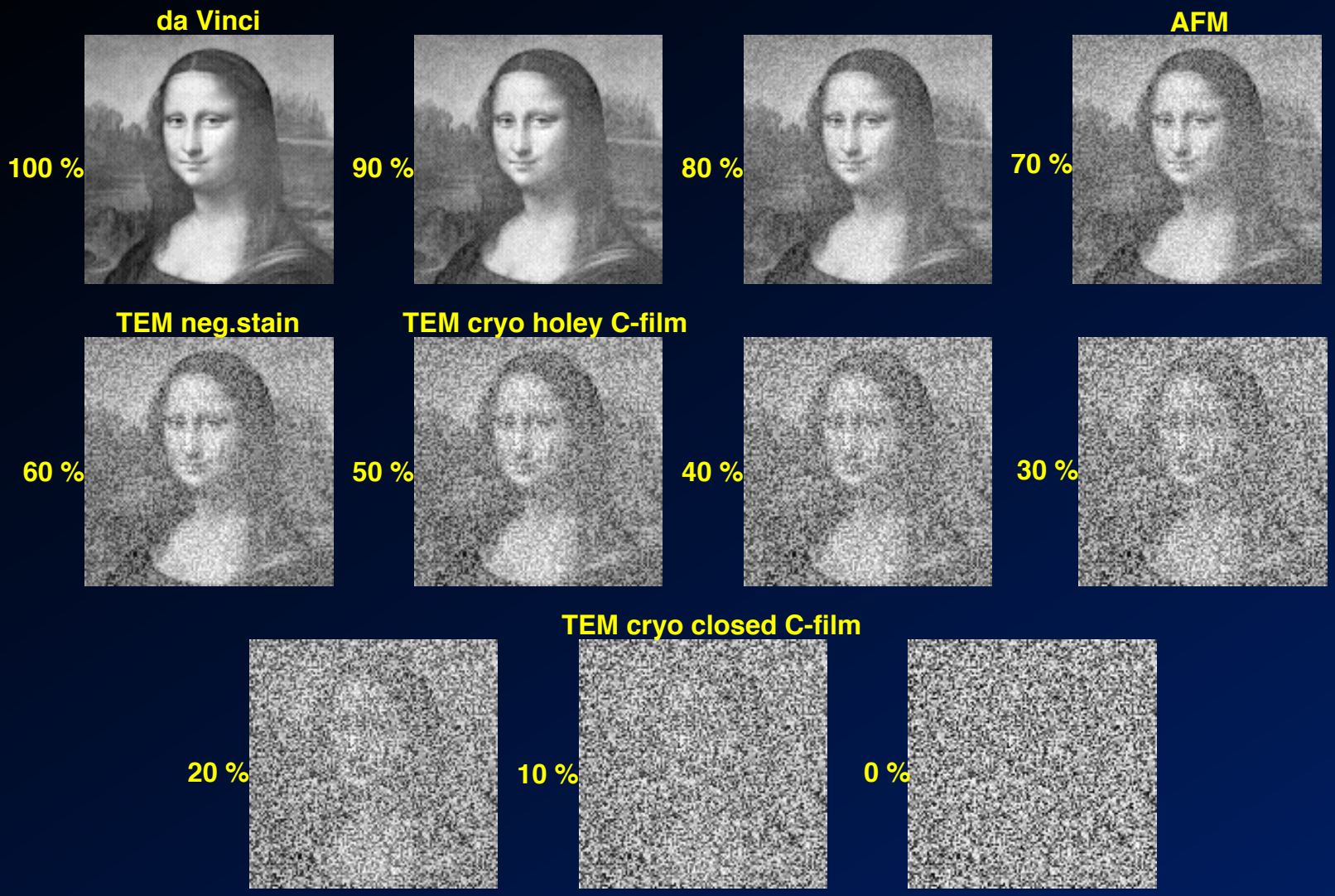
Rotavirus, mockup images adapted from Grigorieff, *eLife*, 2013;2:e00573.

Cryo-electron microscopy

- Protein in solution
- Buffer / vitreous
- Carbon edge



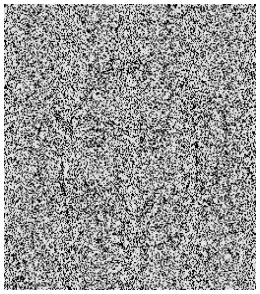
- Pure sample imaging
- «Close-to» native state
- No need for heavy metals
- Low contrast but high resolution


da Vinci
AFM
100 %
90 %
80 %
70 %
TEM neg.stain
TEM cryo holey C-film
60 %
50 %
40 %
30 %
TEM cryo closed C-film
20 %
10 %
0 %

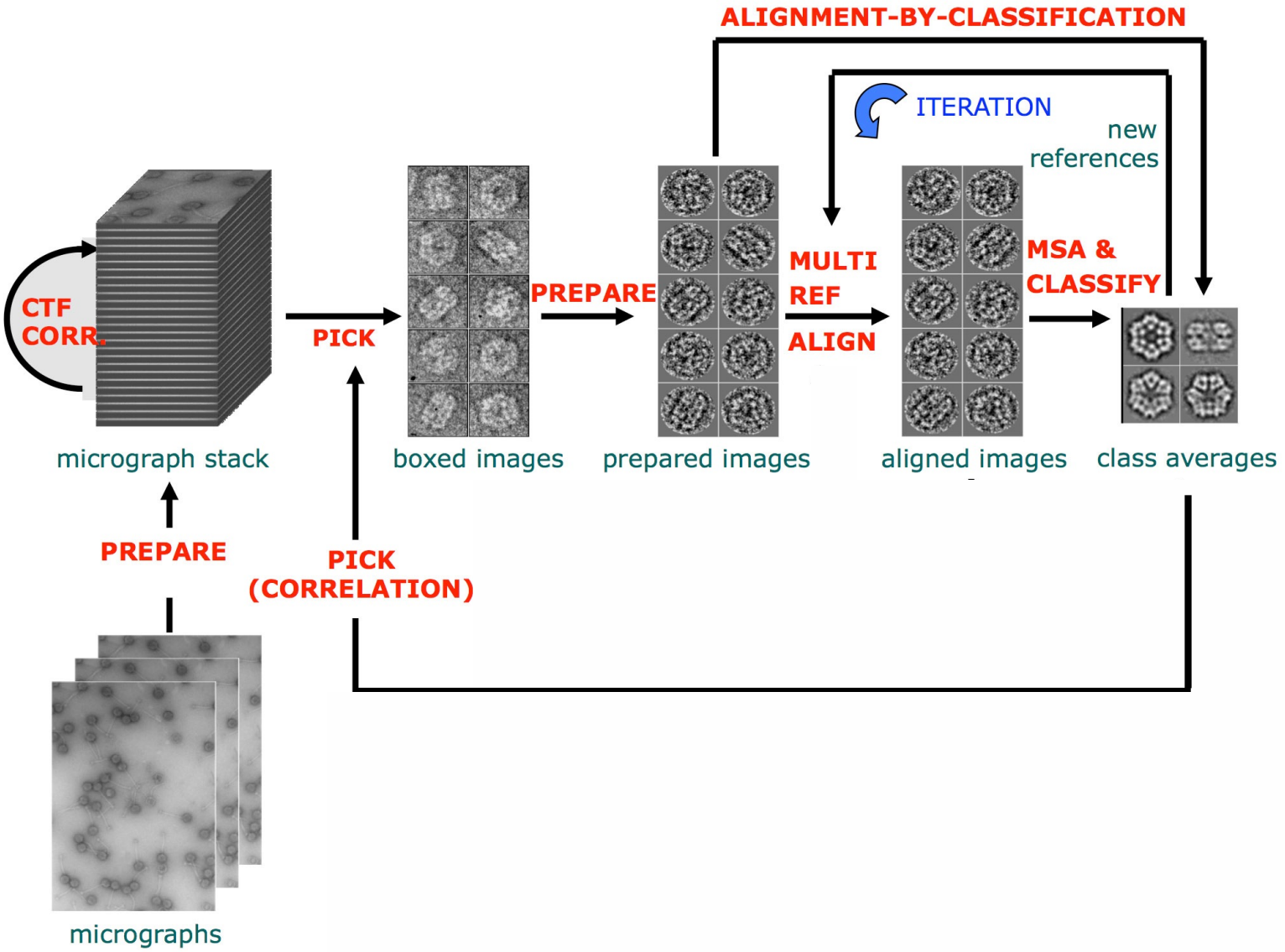
Single particle cryo-EM image processing

Noisy images can still contain the high-resolution structure.

The challenge is
- to find the particles,
- to recognise their orientation, and
- to average their image signals.



Single Particle Cryo-EM:
The general workflow


ALIGNMENT-BY-CLASSIFICATION
ITERATION
new
references
CTF
CORR.
PICK
PREPARE
MULTI
REF
ALIGN
MSA &
CLASSIFY
micrograph stack
boxed images
prepared images
aligned images
class averages
PREPARE
PICK
(CORRELATION)
micrographs

Eigenimage Analysis

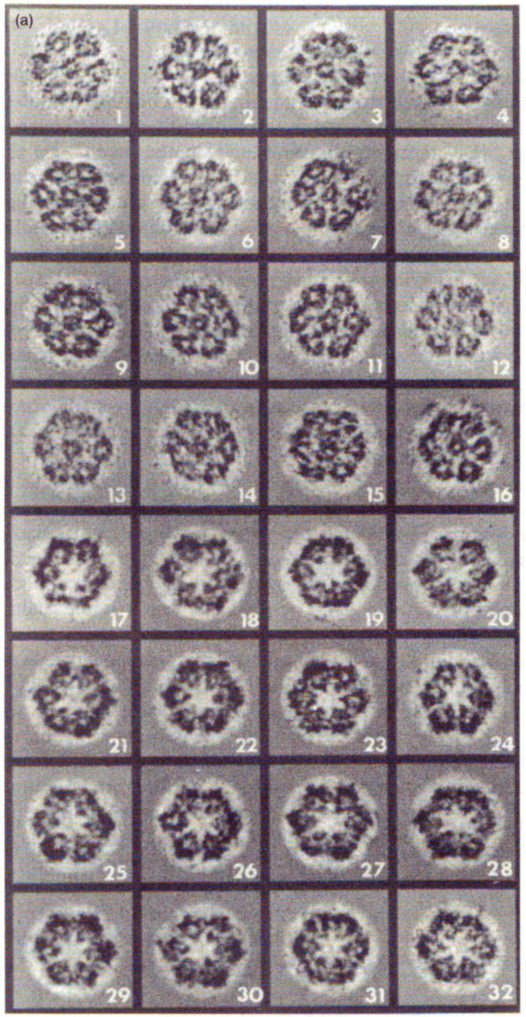
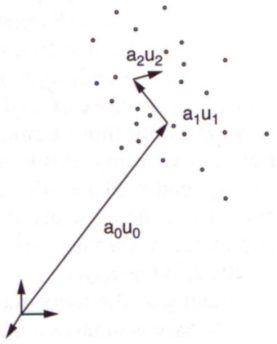


Figure 4.4 Demonstration of correspondence analysis for a set of macromolecules that vary strongly in one feature. (a) Set of worm hemoglobin molecules with (1–16) and without (17–32) extra central subunit. (b) Factor map (1 versus 2) separates images (represented by their ID numbers) unambiguously into two clusters along factor 1, the most prominent factor. Insets show images obtained by averaging over the respective clusters. From Frank and van Heel (1982b), reproduced with permission of Deutsche Gesellschaft für Elektronenmikroskopie, e.V.

Figure 4.5 Principle of reconstitution. Each image, represented by a vector in the space $\mathbf{R}^J$, can be approximated by successive addition of vectors pointing in the direction of the eigenvectors u_1, u_2, etc., to the vector $a_0\mathbf{u}_0$ representing the average image, which is centered in the data cloud. Only a few of these additional vectors are needed to recreate the distinguishing features of the image.



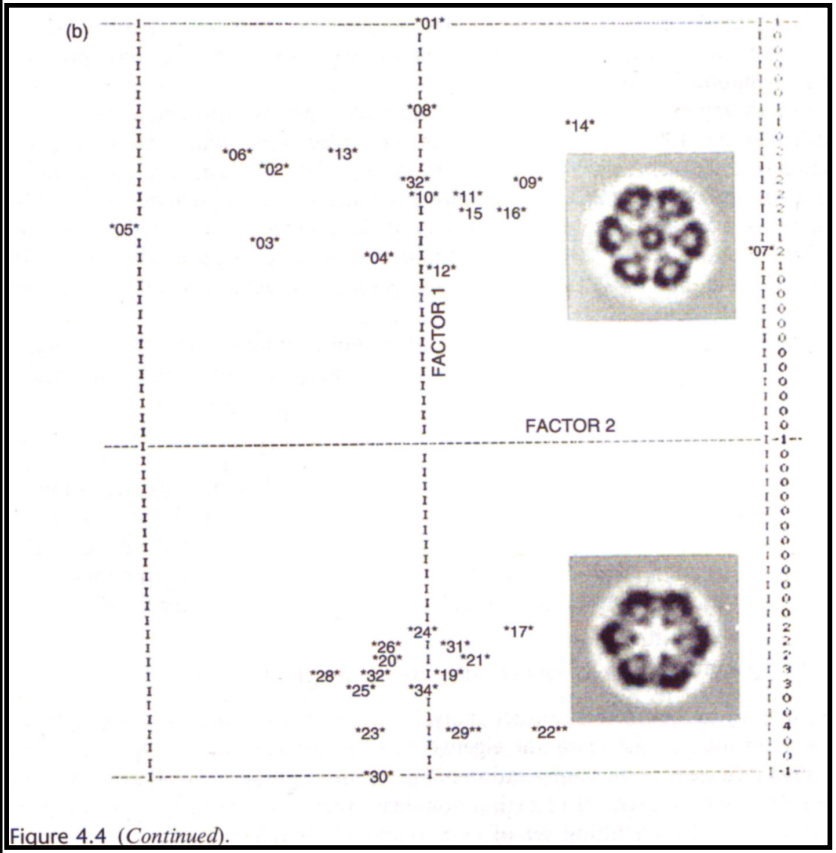


Figure 4.4 (*Continued*).

Each image is interpreted as a “point” in a space that has as many dimensions as the image has pixels.

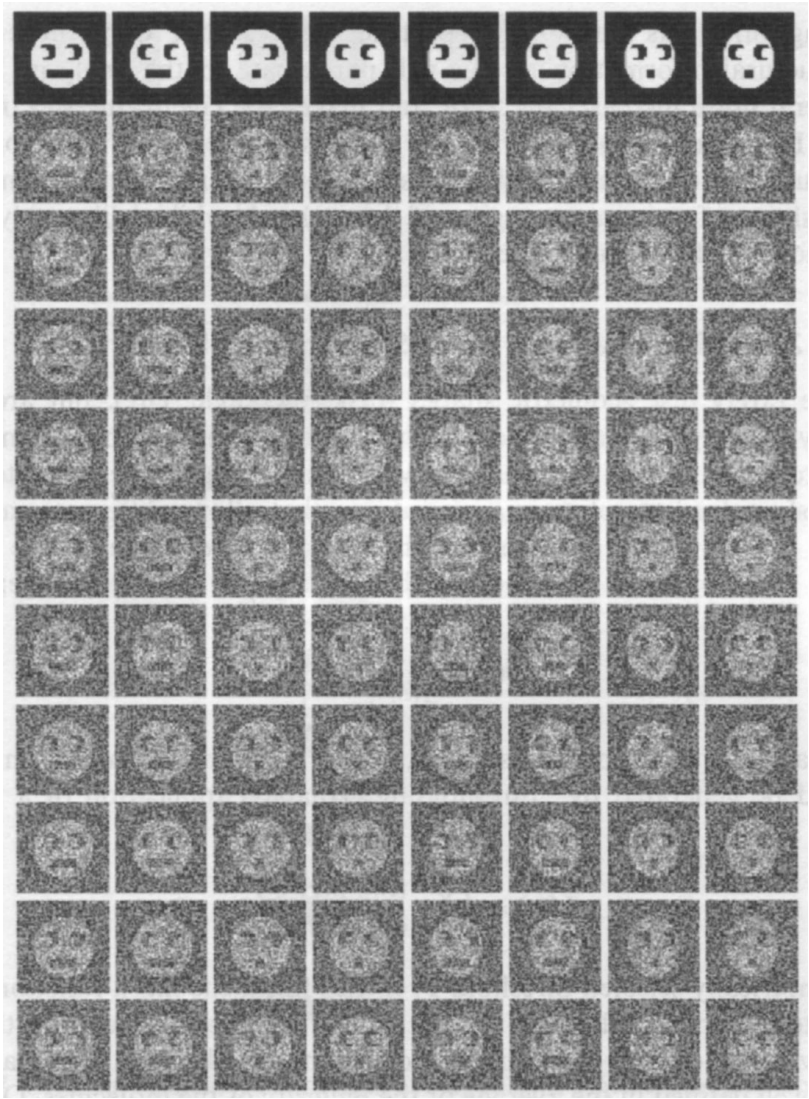
An image of 100x100 pixels is a point in a 10’000-dimensional space.

In that space, many images result in a cloud of points.

That could has a center (the average image).

The cloud has a direction of the longest diameter (= the main difference between the images).

That longest direction is called “Eigenvector 1”.
The diameter of the cloud in that direction is called “Eigenvalue 1”.



Eigenvalue Bar Plot

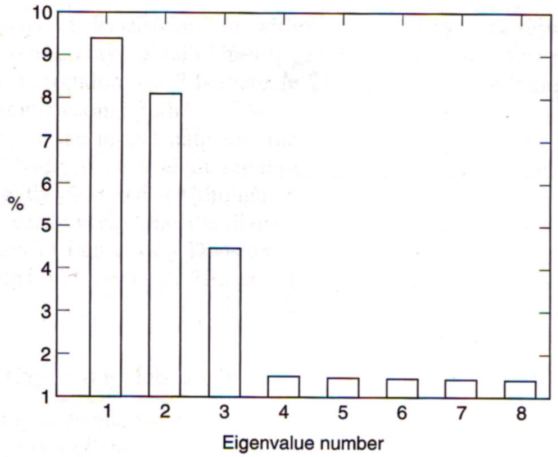
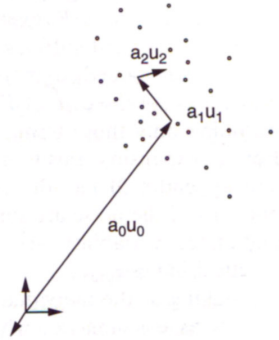


Figure 4.8 Eigenvalue histogram of the face data set. Three eigenvalues stand out clearly from the rest, indicating that the significant variations are concentrated in a three-dimensional factor space. Beyond factor 3, the histogram trails off, displaying the typical behavior noise. From this histogram, the decision would be reached that a display of factor maps 1 versus 2, 1 versus 3, and 2 versus 3 would be sufficient to gauge the distribution of the data, and the possible existence of clusters.



Factor Maps

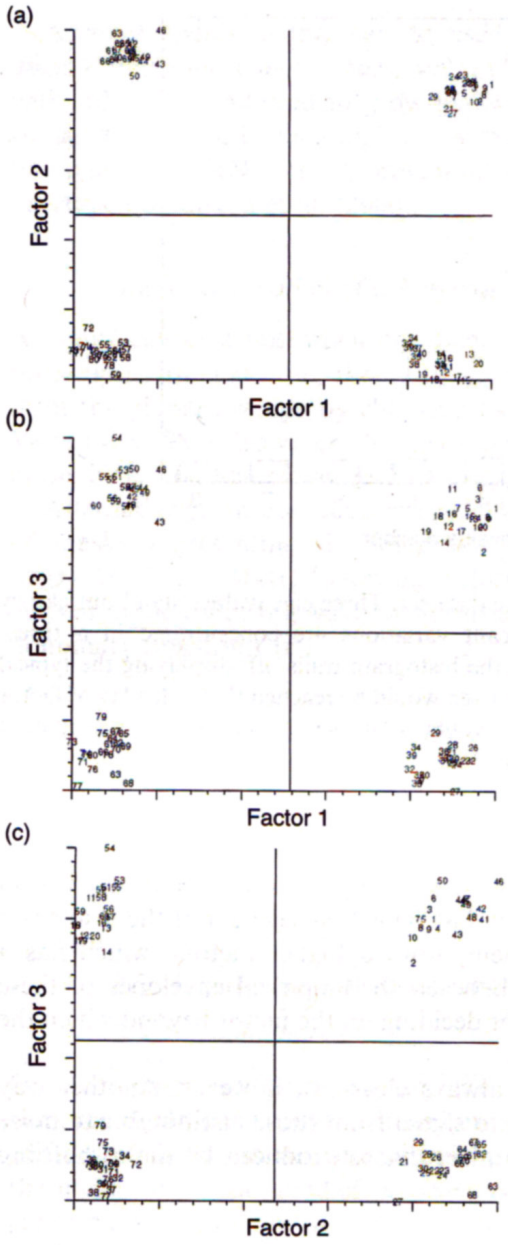


Figure 4.9 Factor maps of the face data set. Each of the factor maps, 1 versus 2 (a), 1 versus 3 (b), and 2 versus 3 (c), show very clear clustering into four clusters. This indicates that exactly eight clusters are present, sitting in the eight corners of a cubic space. A check of the numbers reveals that images are clustered according to their origin in figure 4.6.

Eigenimages

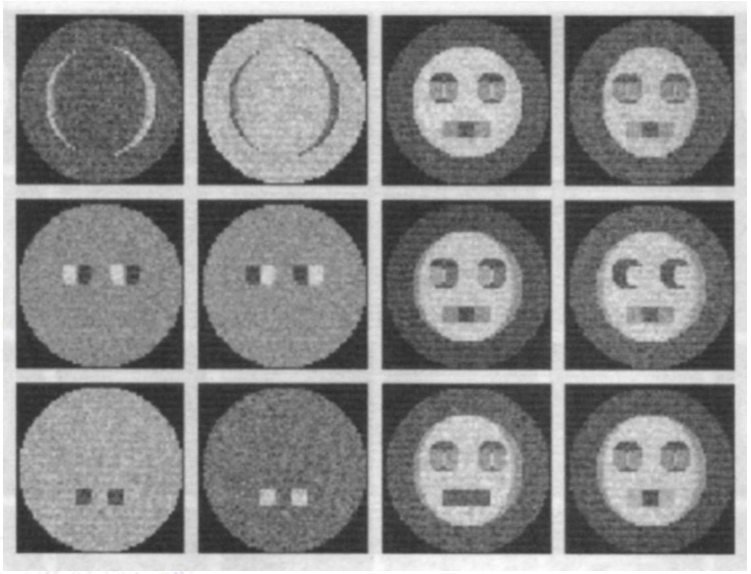
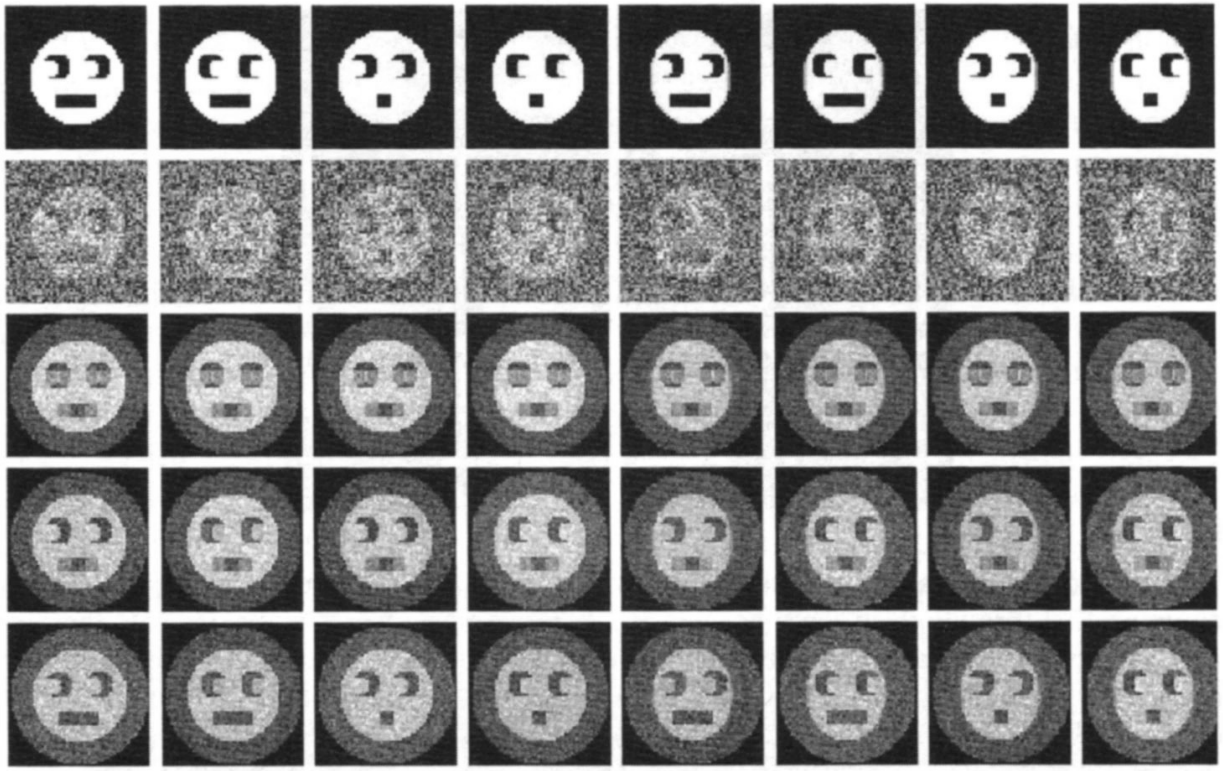


Image Reconstitution from Eigenimages



Original

Noise Added

Avg + 1st Eigenvector

+ 2nd Eigenvector

+ 3rd Eigenvector

Figure 4.16 Stepwise reconstitution of noisy images (second row of figure 4.10) and stopping after the third factor leads to virtually noise-free images that are very similar to the originals (shown for comparison in first row); second row: average image plus eigenvector #1; third row: same as second row, with eigenvector #2 added; 4th row: same as third row, with eigenvector #3 added.

Particle Classification
by
Multivariate Statistical Analysis

Classification by the K-means algorithm

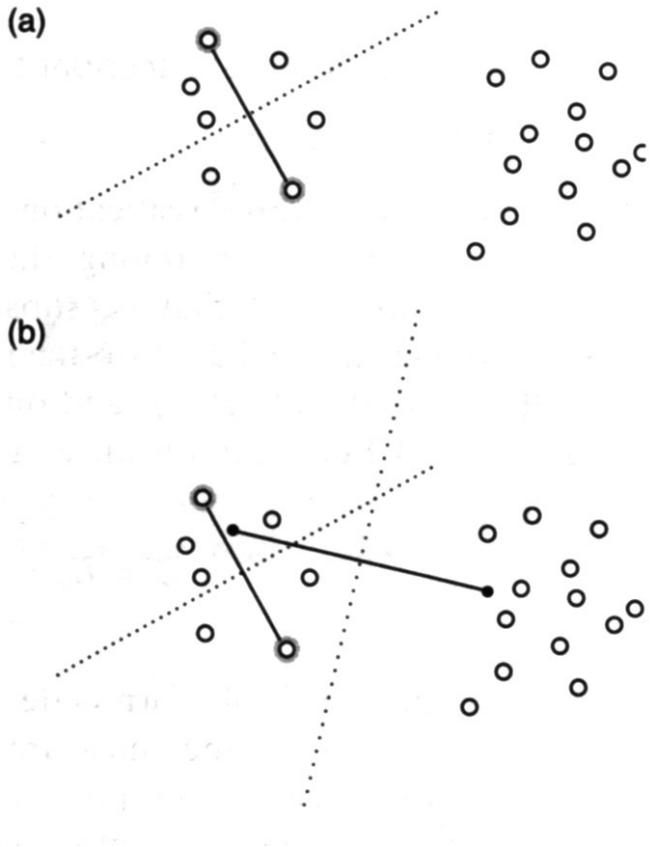


Figure 4.17 The *K*-means method of clustering, following an iterative procedure, illustrated here for the choice of $K=2$ clusters. (a) From among the images, represented by points in a high-dimensional space, two are picked at random as "seeds" (marked with concentric circles). They define a first partition of the set. (b) Next, the centers of gravity (filled dots) are computed for the two subsets created by the partition, and these centers define an improved partition, taking the place of the seeds, etc. In our example, the two clusters are already separated in the second iteration of this procedure.

Classification by Hirarchical Ascendant Classification (HAC)

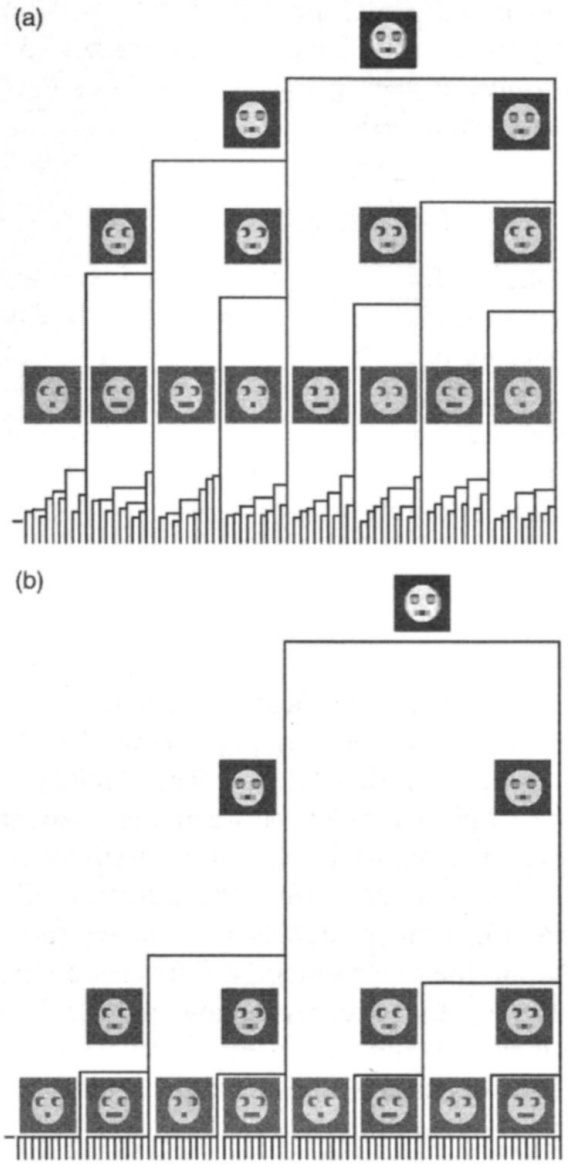


Figure 4.18 Hierachical ascendant classification (HAC) of the "face" test data set introduced in figure 4.6: (a) complete linkage; (b) Ward's criterion. Classes obtained by the different cutting levels are represented by their averages. At the very top is the average of all images, in which all three features are blurred. For the uppermost and middle cutting levels, two or one features appear blurred, respectively. Only at the lowest cutting level, all eight original classes are retrieved, as is seen from the sharp appearance of all three features in the class averages. Both HAC options perform equally well in retrieving the correct classes. However, the much higher level at which the eight classes branch off in the complete linkage case indicates more robustness against noise than in the case of Ward's criterion.

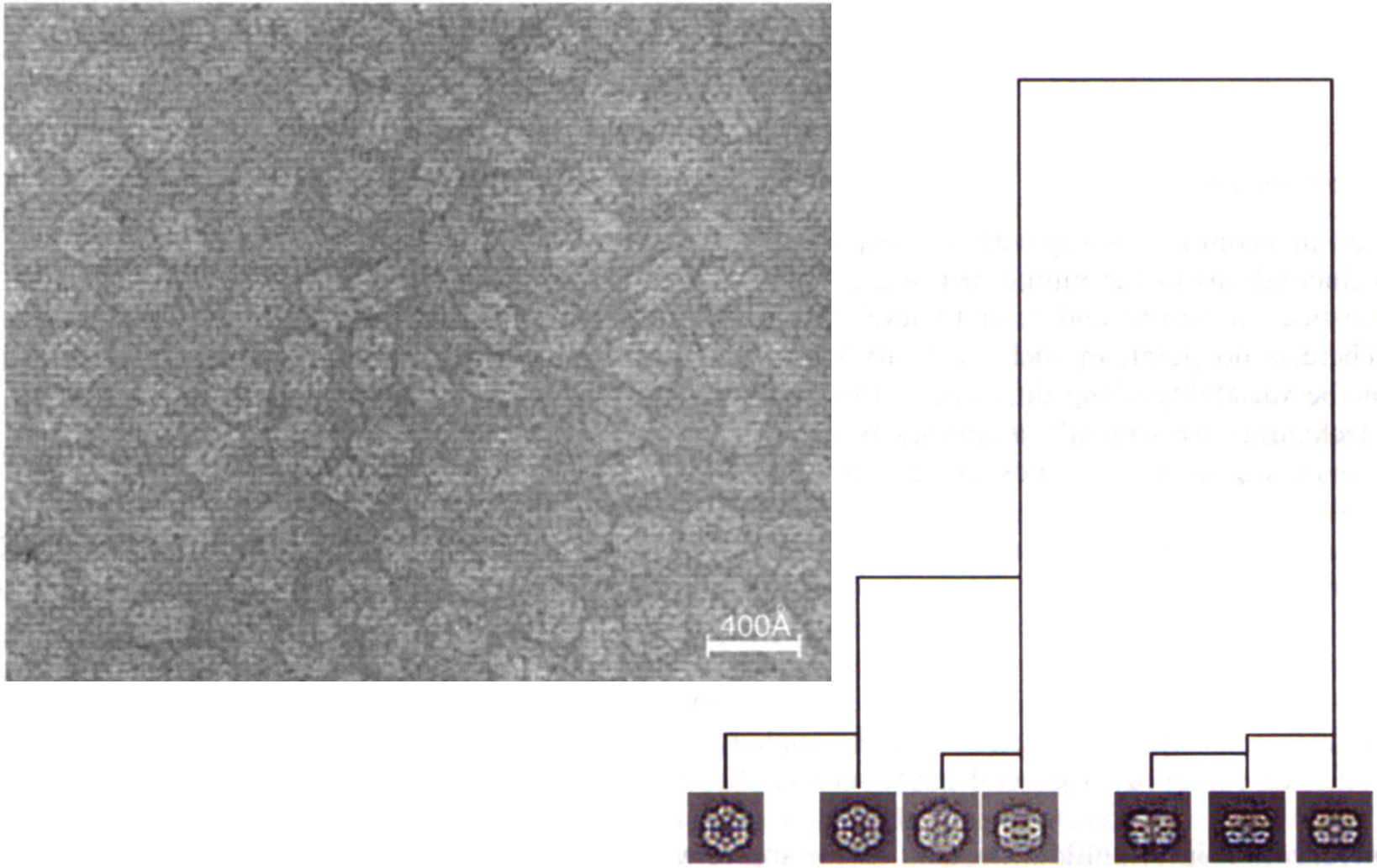


Figure 4.19 HAC with Ward's criterion applied to 2450 cryo-EM images of *Lumbricus terrestris* (earthworm) hemocyanin. (a) Appearance of a typical micrograph; (b) HAC dendrogram and class averages. The classes have (from left to right) 313, 420, 310, 311, 418, 355, and 323 images. (a) From Mouche et al. (2001), reproduced with permission of Academic Press; (b) kindly provided by N. Boisset (unpublished lecture material.)

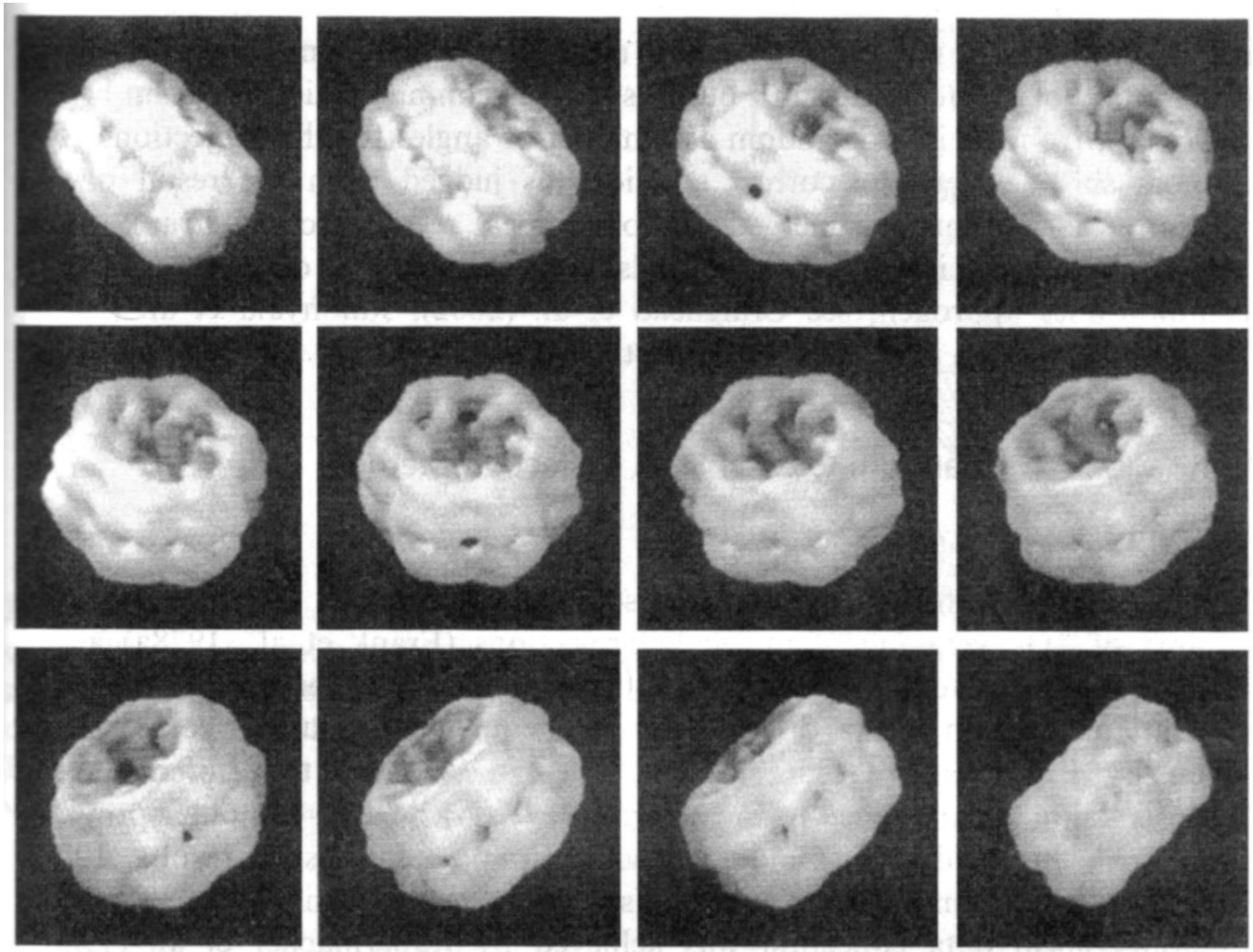
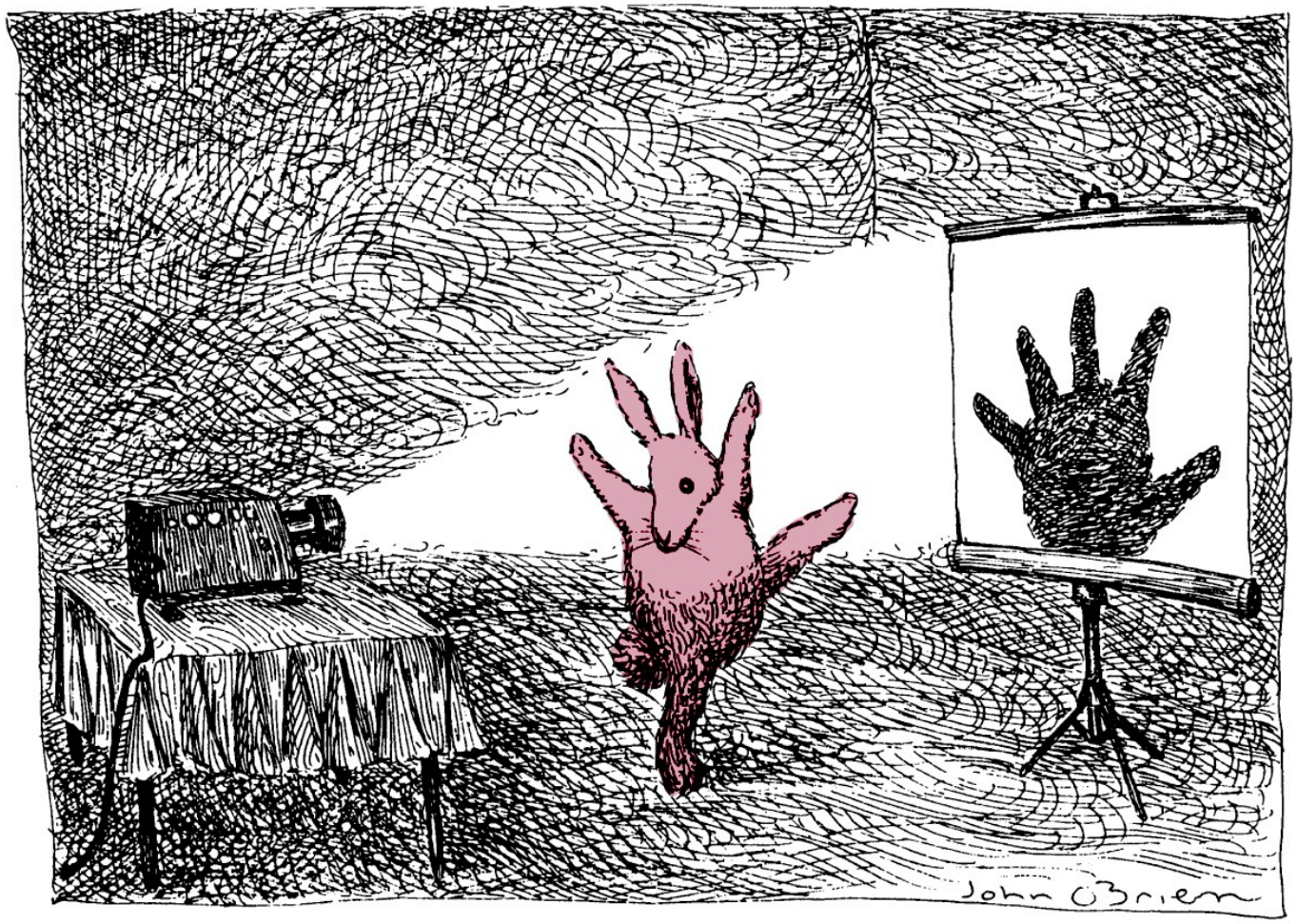


Figure 5.11 Three-dimensional reconstruction of *Lumbricus terrestris* erythrocruorin embedded in ice from class averages shown in figure 5.10a. In part, the angles were assigned based on the technique of "angular reconstitution" (see also Schatz et al., 1994). From Schatz (1992), reproduced with permission of the author.

3D Reconstruction

2D -> 3D

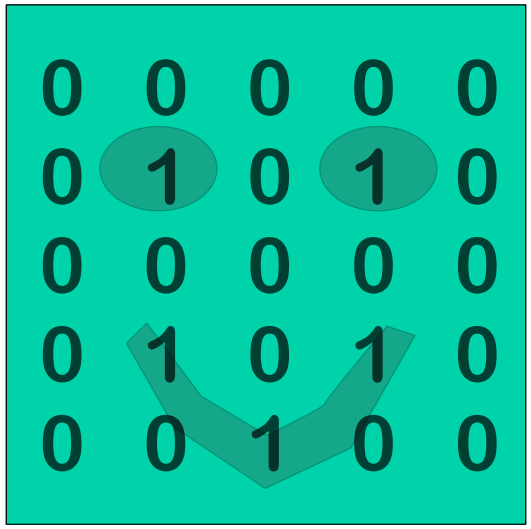


Drawing by John O'Brien; © 1991 The New Yorker Magazine

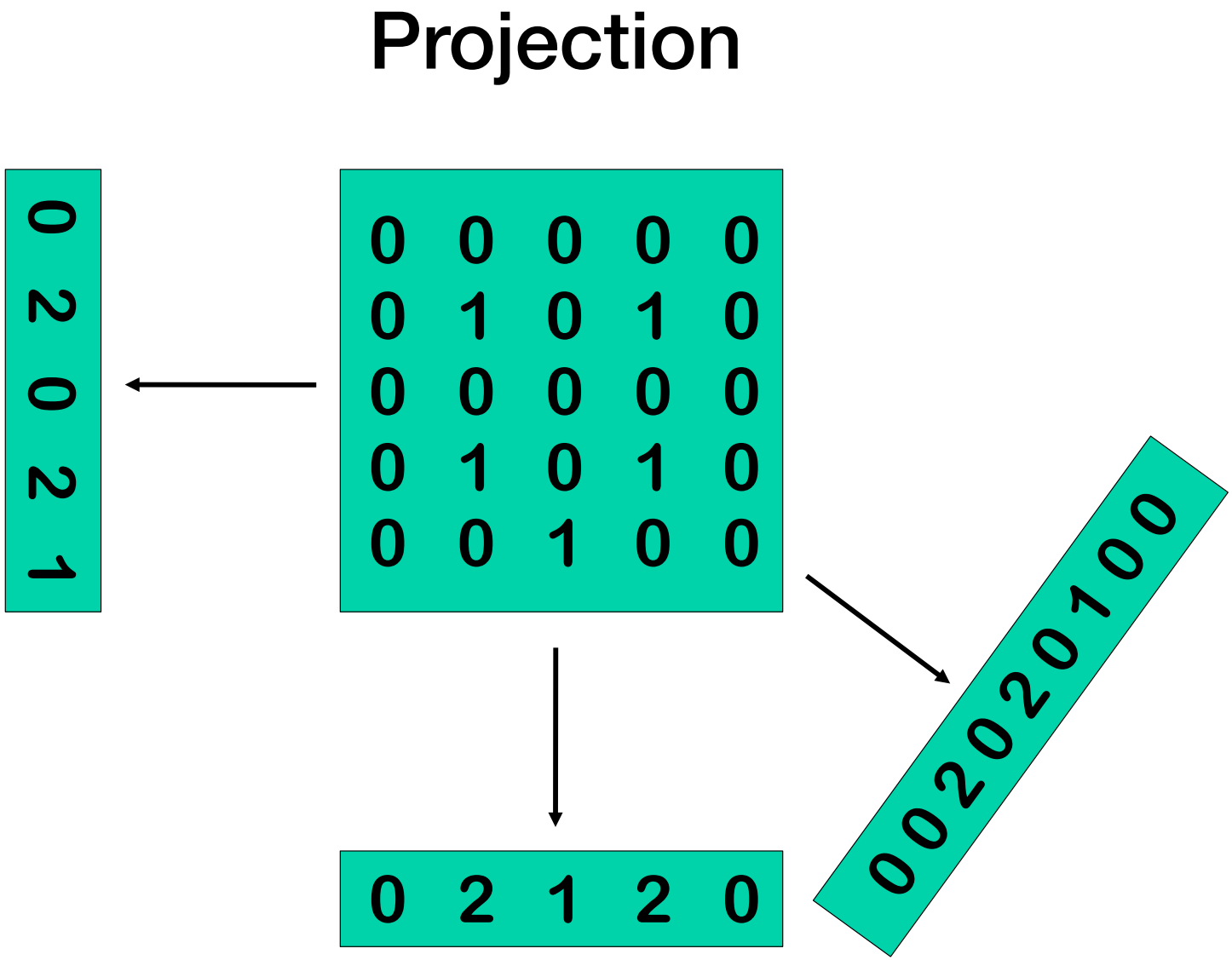
Projection

Real-Space Method

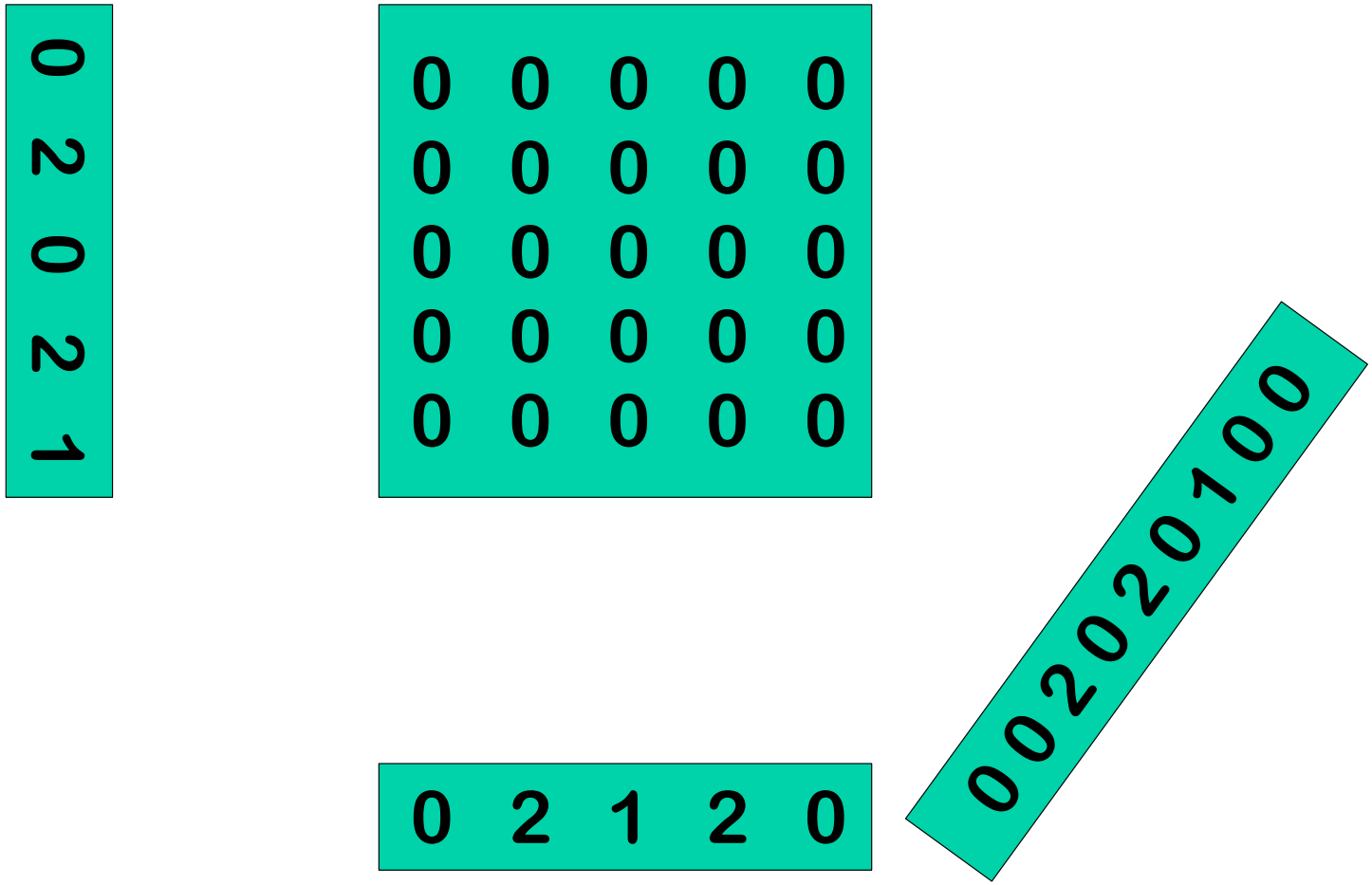
Projection



Real-Space Method

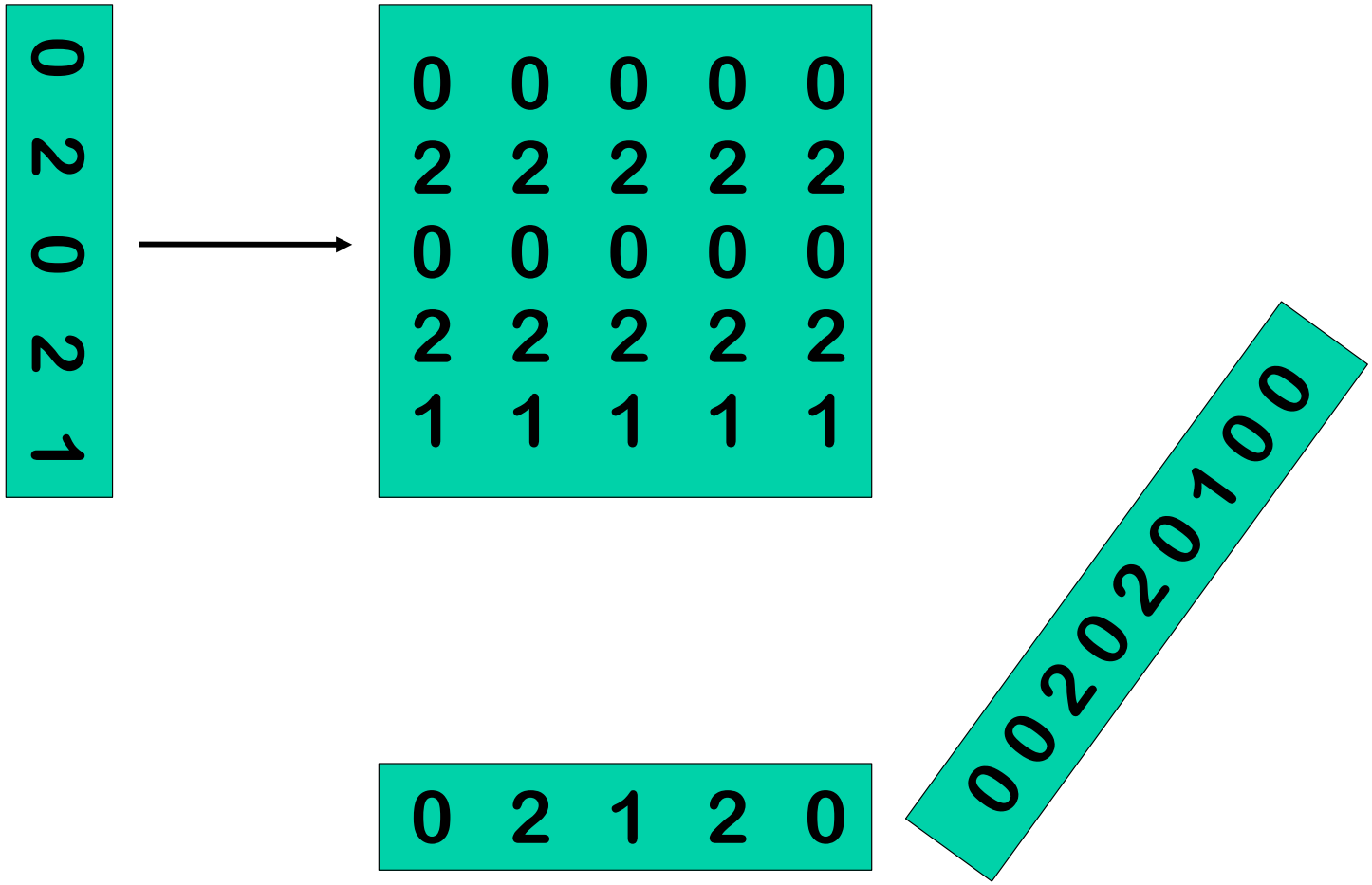

Projection
0 2 0 2 1
0 0 0 0 0
0 1 0 1 0
0 0 0 0 0
0 1 0 1 0
0 0 1 0 0
0 2 1 2 0
0 0 2 0 2 0 1 0 0
Real-Space Method

Backprojection



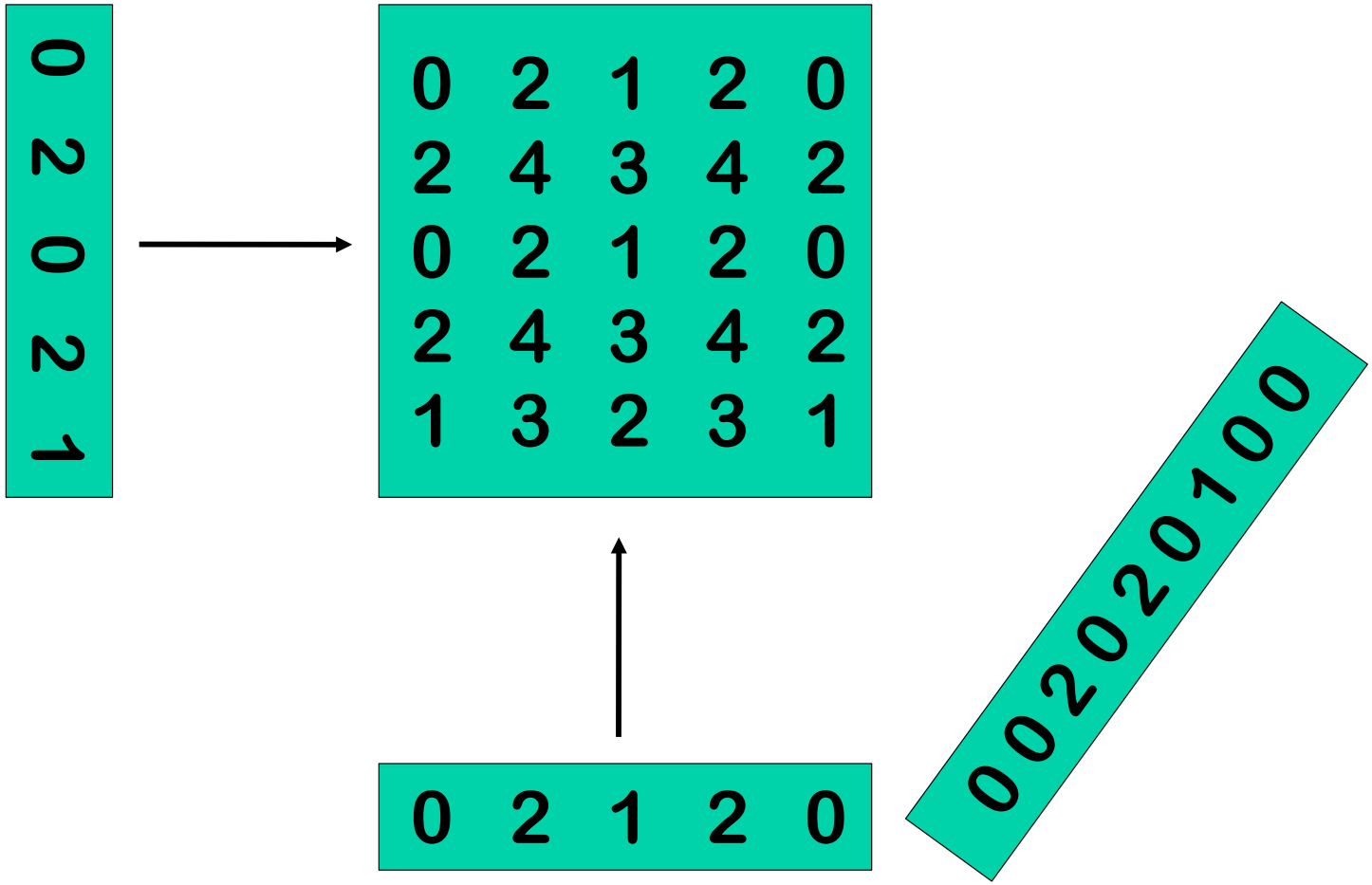
Real-Space Method

Backprojection



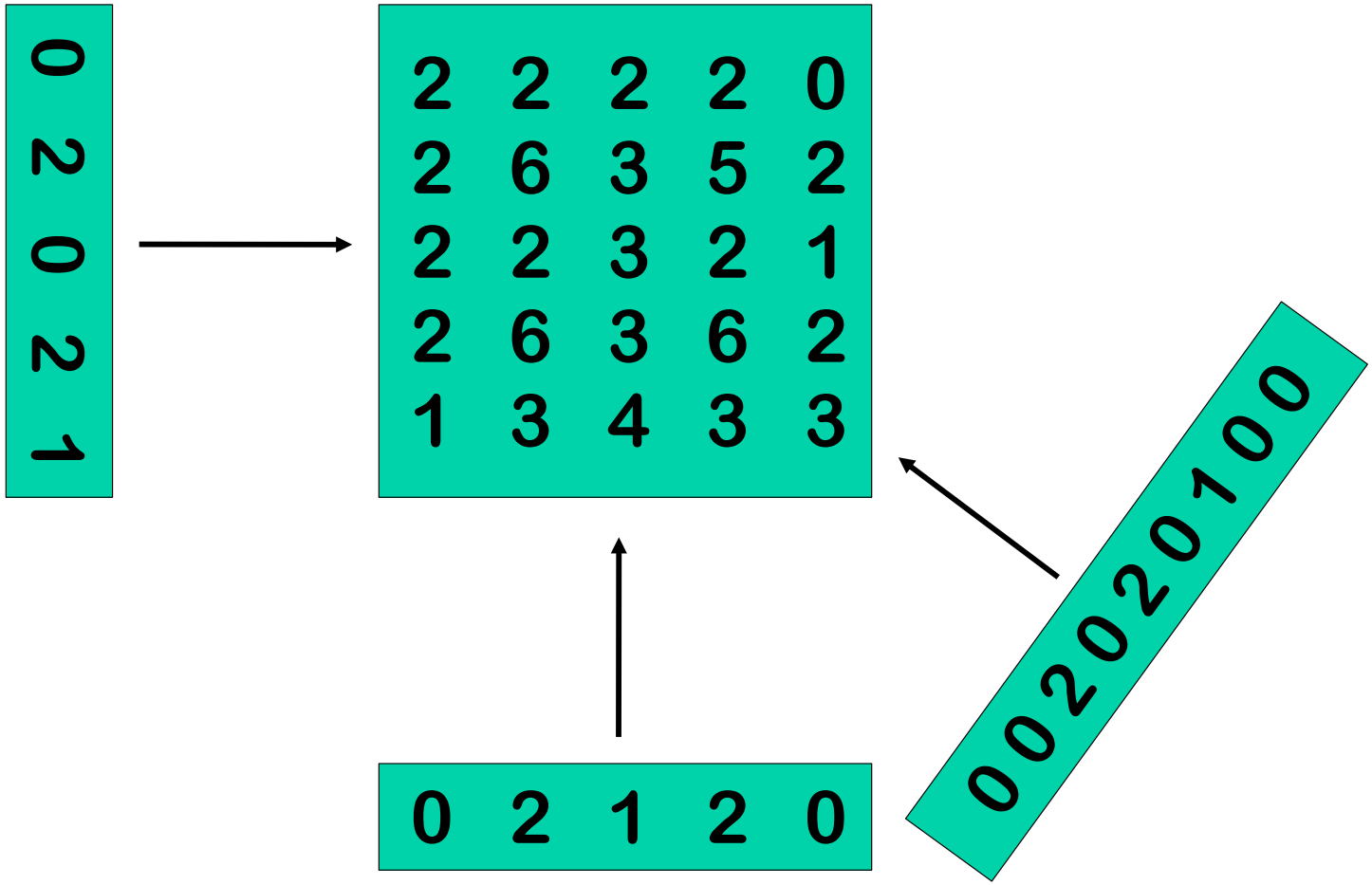
Real-Space Method

Backprojection



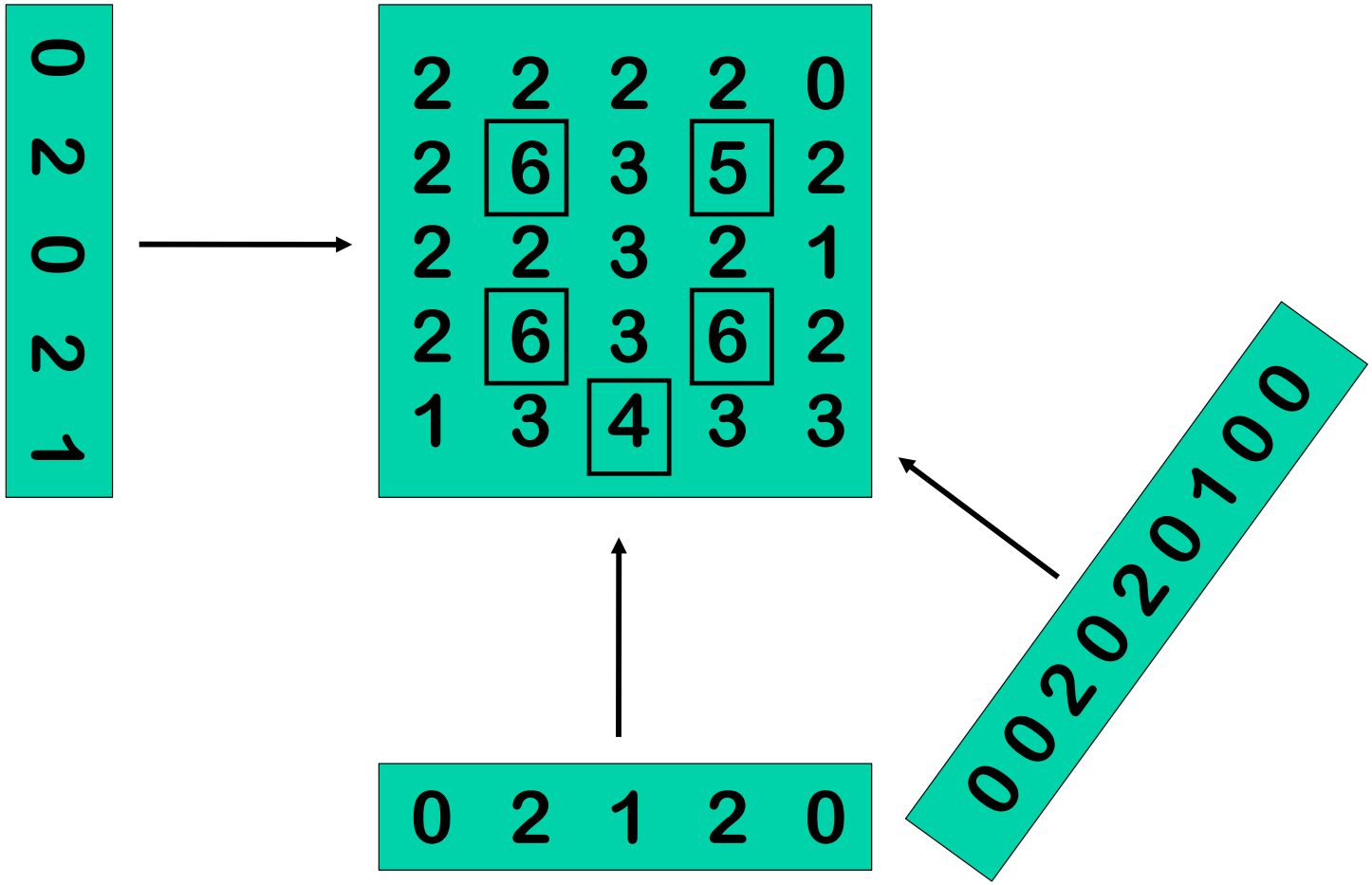
Real-Space Method

Backprojection



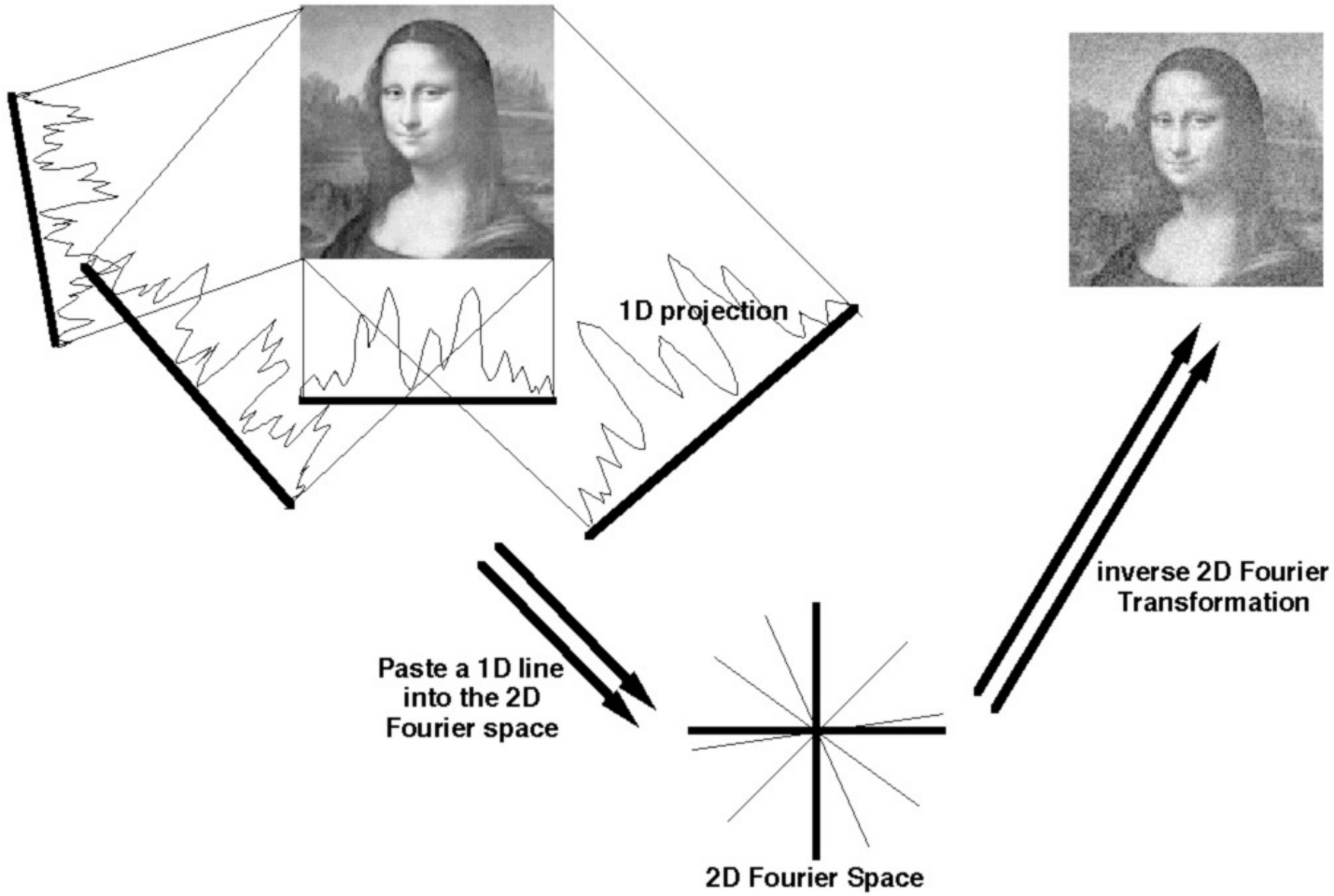
Real-Space Method

Backprojection



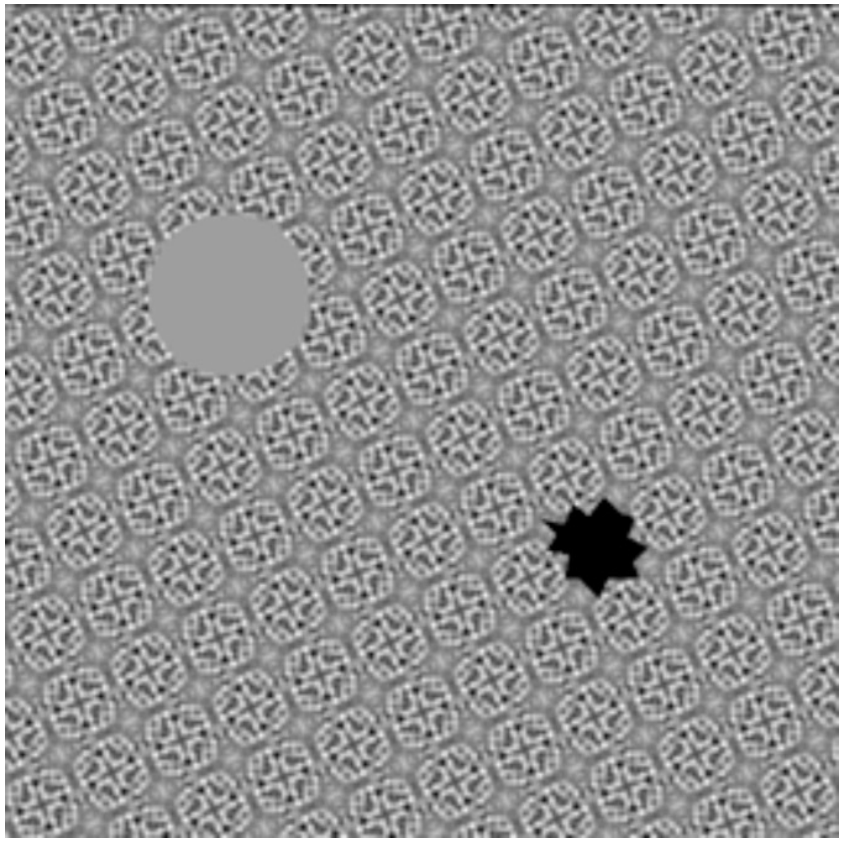
Real-Space Method

The Radon projection theorem for 3D reconstructions

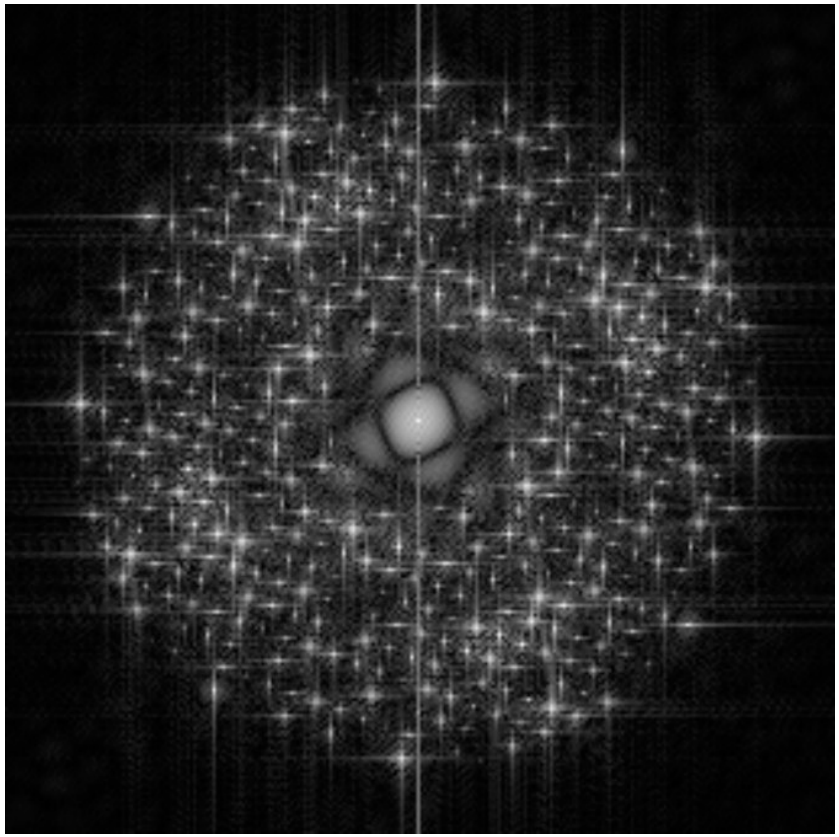


Fourier-Space Method

Real Space

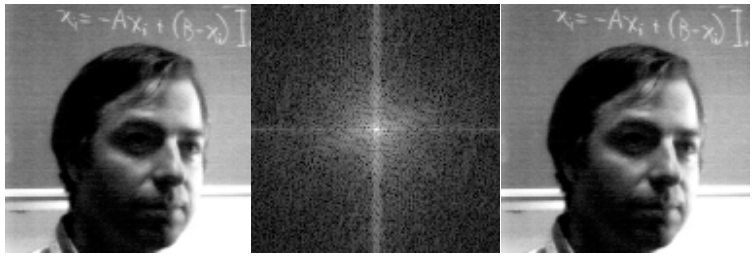


Fourier Transform

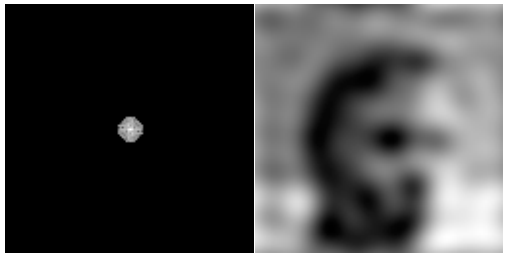


Fourier filters of images

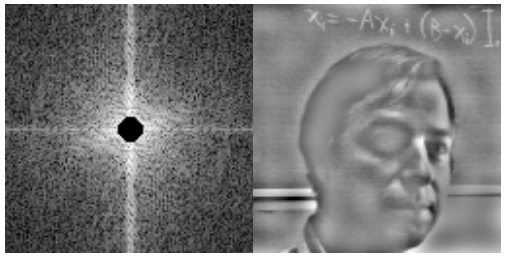
Real —> Fourier —> Real



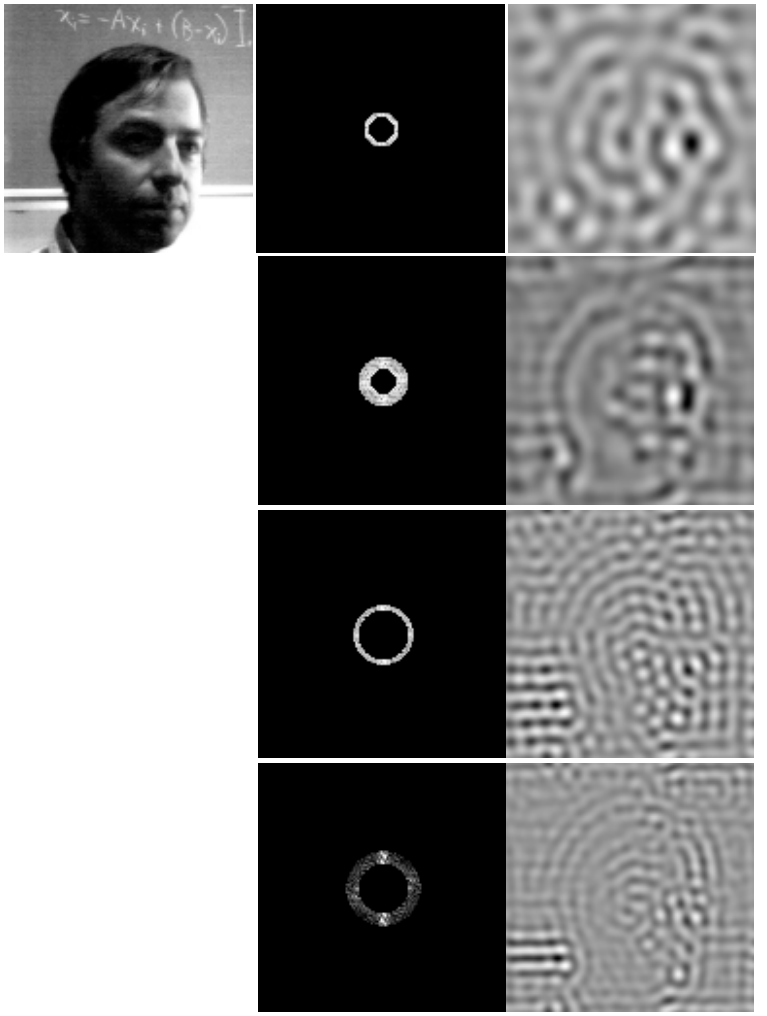
Low-pass filter



High-pass filter



Band-pass



http://sharp.bu.edu/~slehar/fourier/fourier.html

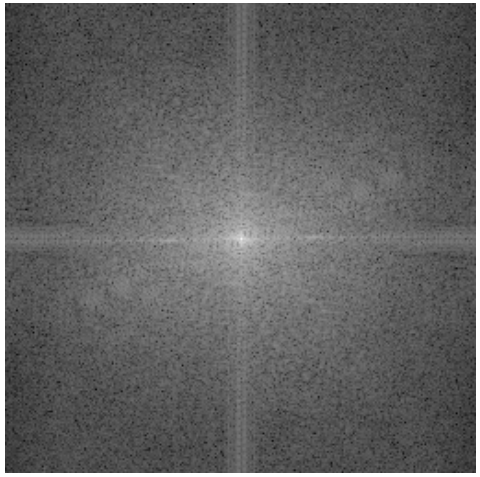
Real-space image <=> Fourier Transform

Real space

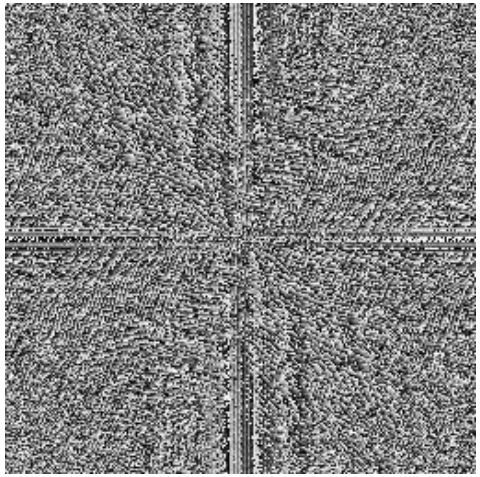
FT

FT^{-1}

Fourier space



Amplitudes



Phases

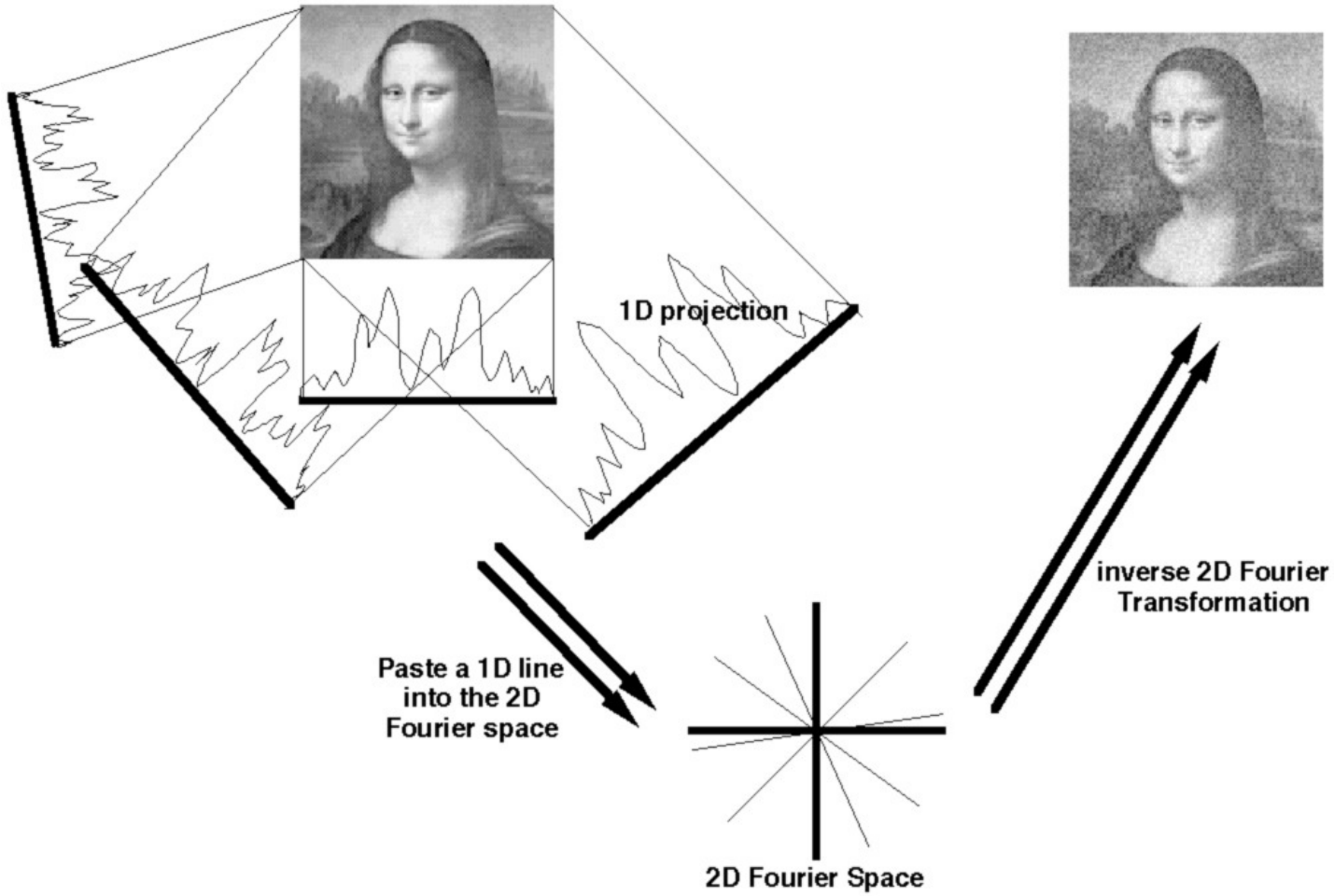
- Low resolution in the center
- High resolution on the edges

- The highest horizontal and vertical resolution is called "**Nyquist frequency**".

- Fourier space is "**Friedel symmetric**":
  - When mirrored across the center, amplitudes are the same and phases have 180º shift (complex conjugate).

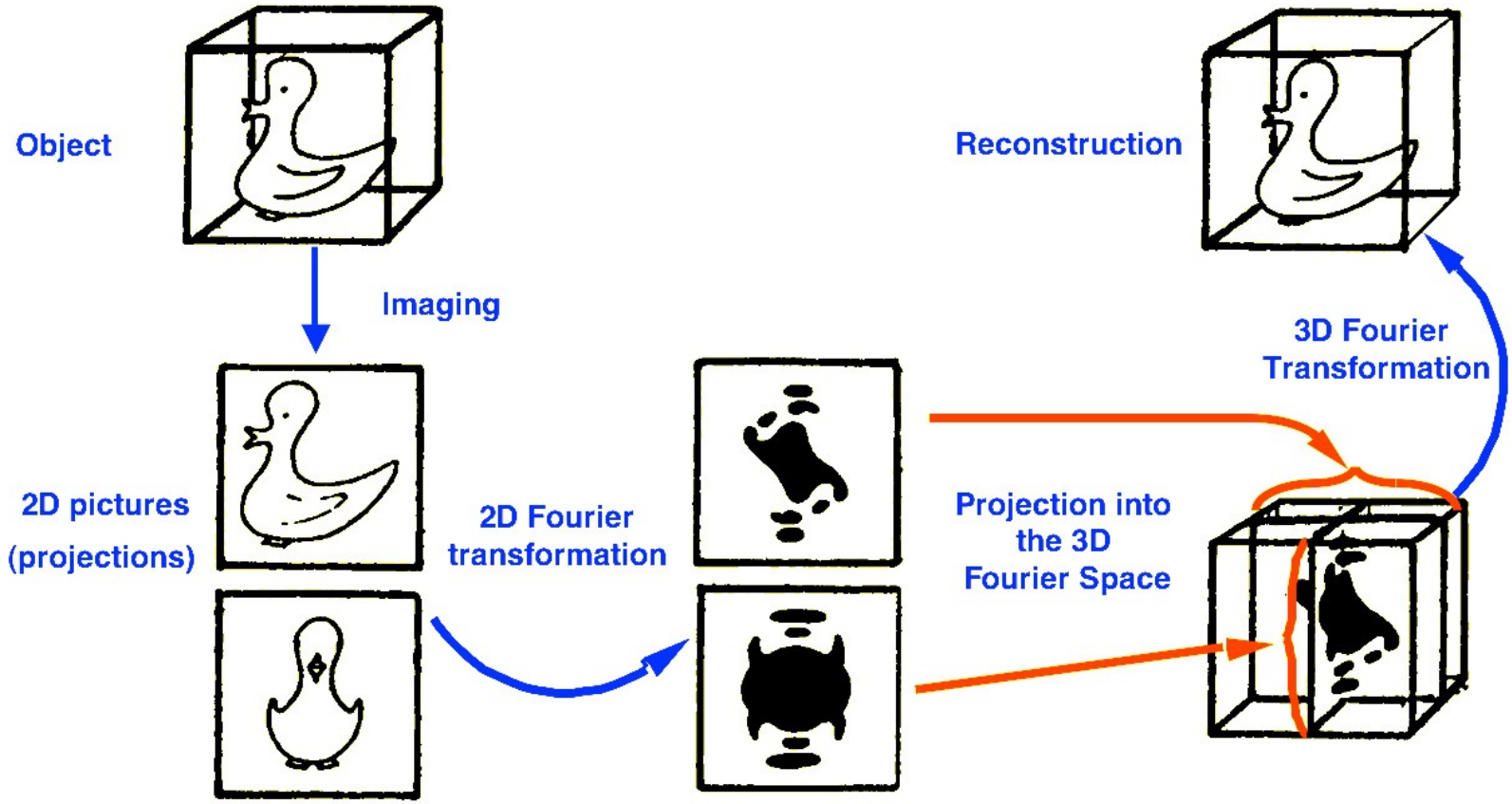
http://homepages.inf.ed.ac.uk/rbf/HIPR2/fourier.htm

The Radon projection theorem for 3D reconstructions



Fourier-Space Method

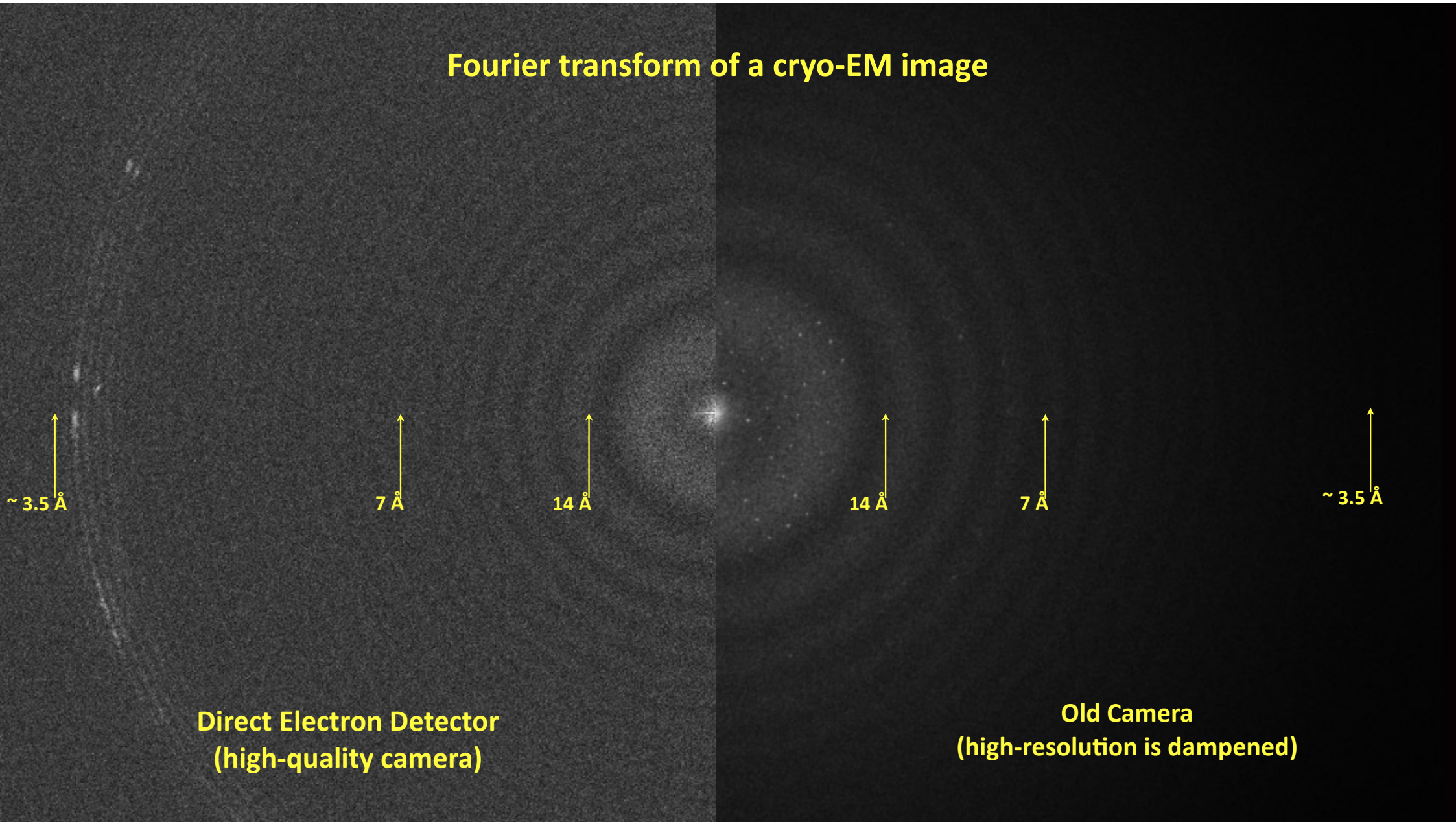
The Radon projection theorem for 3D reconstructions



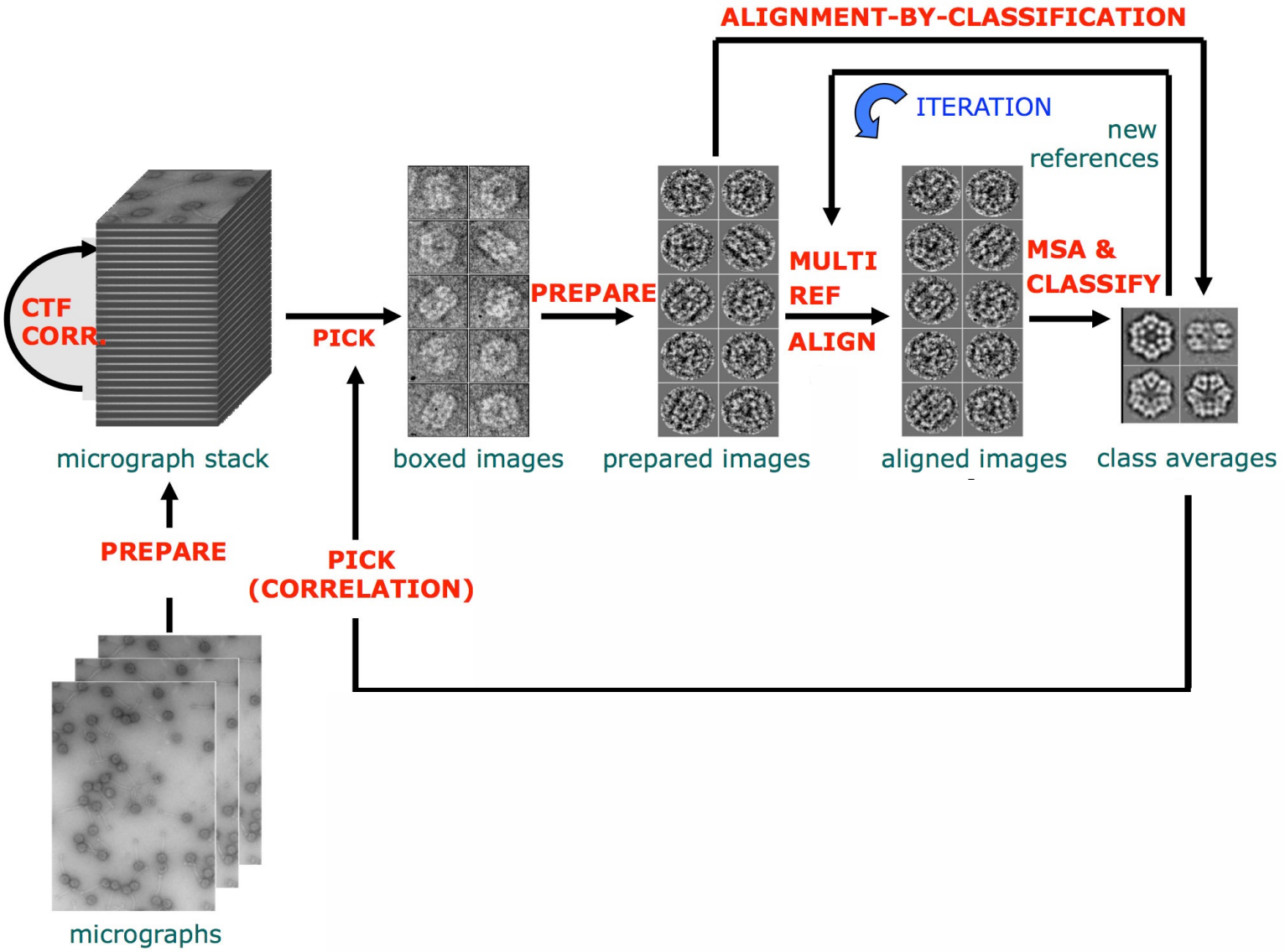
Lake, J. (1971). In "Optical Transforms", (H. Lipson, Ed.), Academic Press, London.

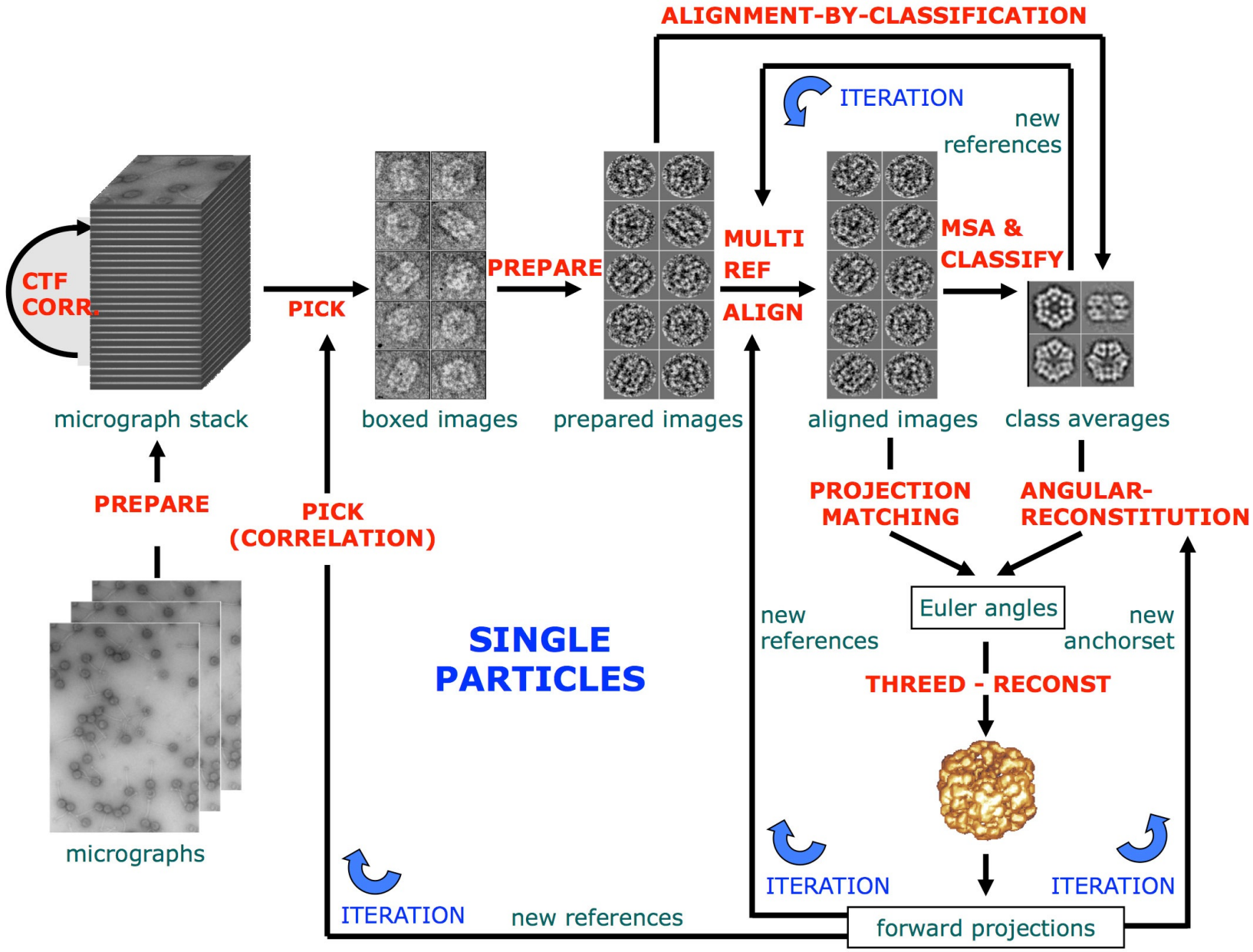
Fourier-Space Method

The quality of the camera is important


Fourier transform of a cryo-EM image
~ 3.5 Å
7 Å
14 Å
14 Å
7 Å
~ 3.5 Å
Direct Electron Detector
(high-quality camera)
Old Camera
(high-resolution is dampened)

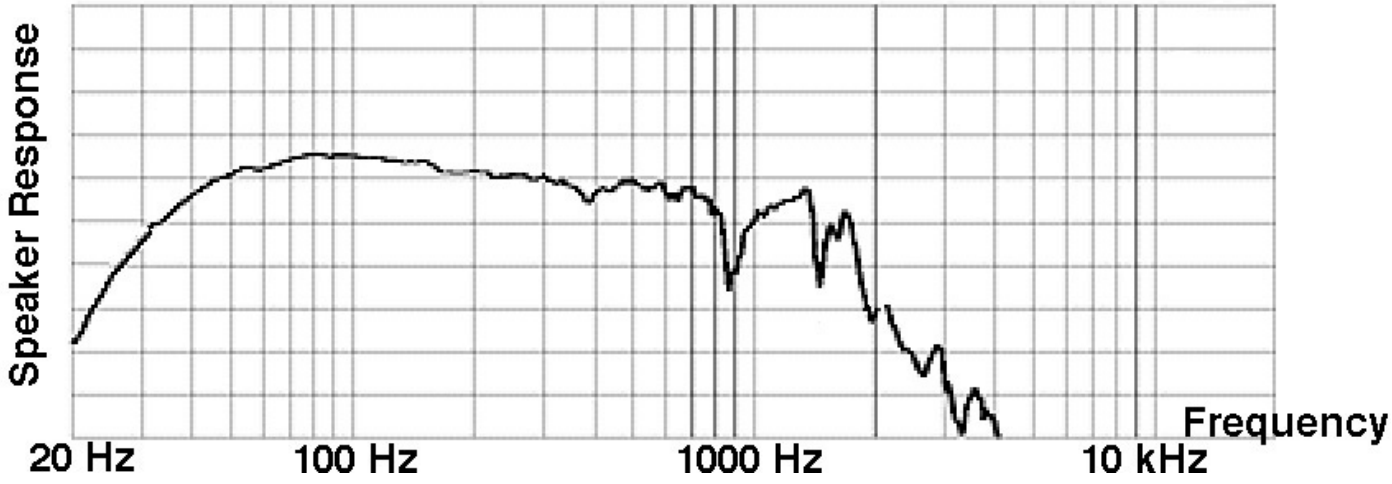
Single Particle Cryo-EM:
The general workflow


ALIGNMENT-BY-CLASSIFICATION
ITERATION
new
references
CTF
CORR.
PICK
PREPARE
MULTI
REF
ALIGN
MSA &
CLASSIFY
micrograph stack
boxed images
prepared images
aligned images
class averages
PREPARE
PICK
(CORRELATION)
micrographs

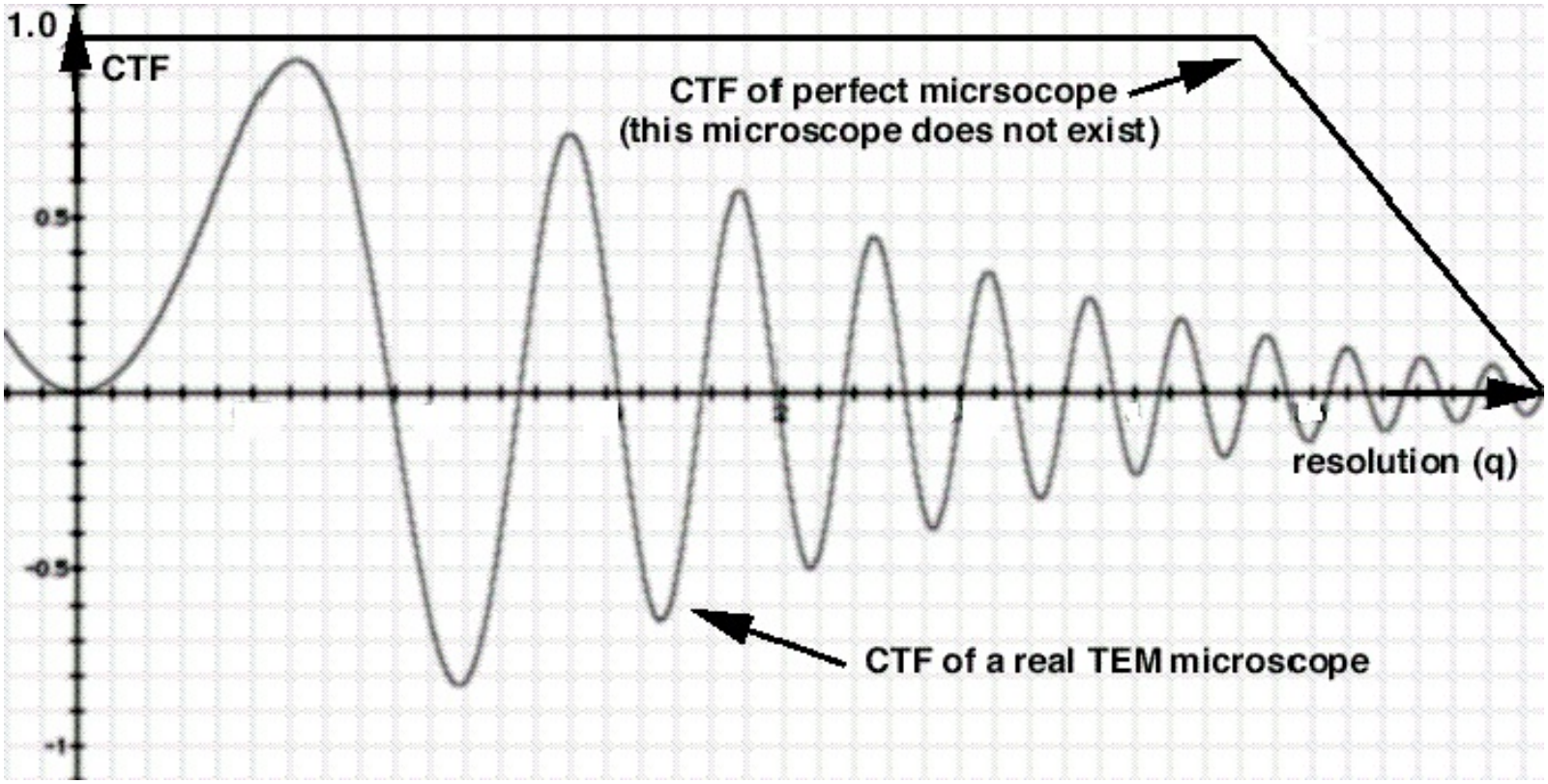

ALIGNMENT-BY-CLASSIFICATION
ITERATION
new references
CTF CORR.
PICK
PREPARE
MULTI REF ALIGN
MSA & CLASSIFY
micrograph stack
boxed images
prepared images
aligned images
class averages
PREPARE
PICK (CORRELATION)
PROJECTION MATCHING
ANGULAR-RECONSTITUTION
Euler angles
new references
new anchorset
SINGLE PARTICLES
THREED - RECONST
micrographs
ITERATION
new references
ITERATION
ITERATION
forward projections

Single Particle Cryo-EM:
The Contrast Transfer Function (CTF)

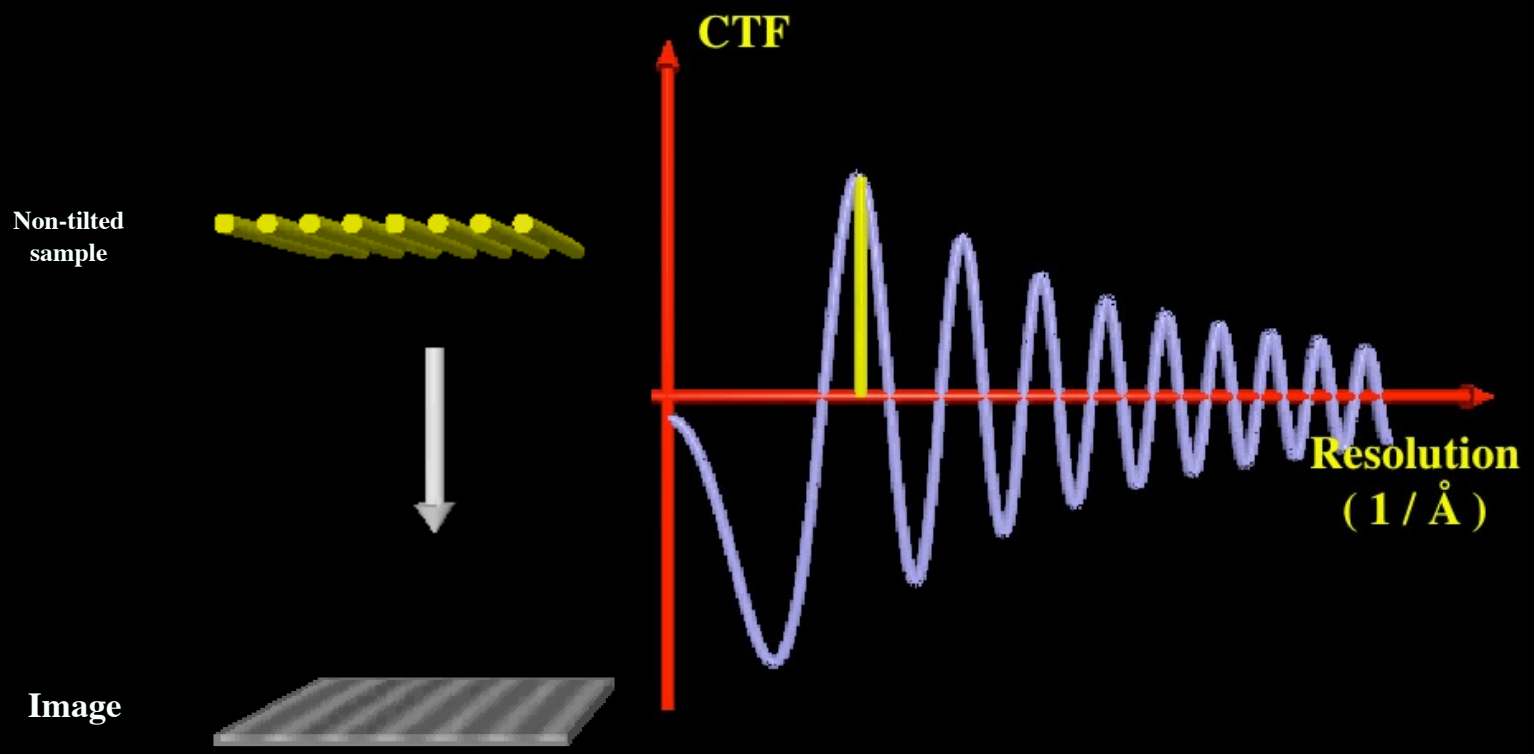
Transfer Function of a Loudspeaker



Transfer Function of a TEM

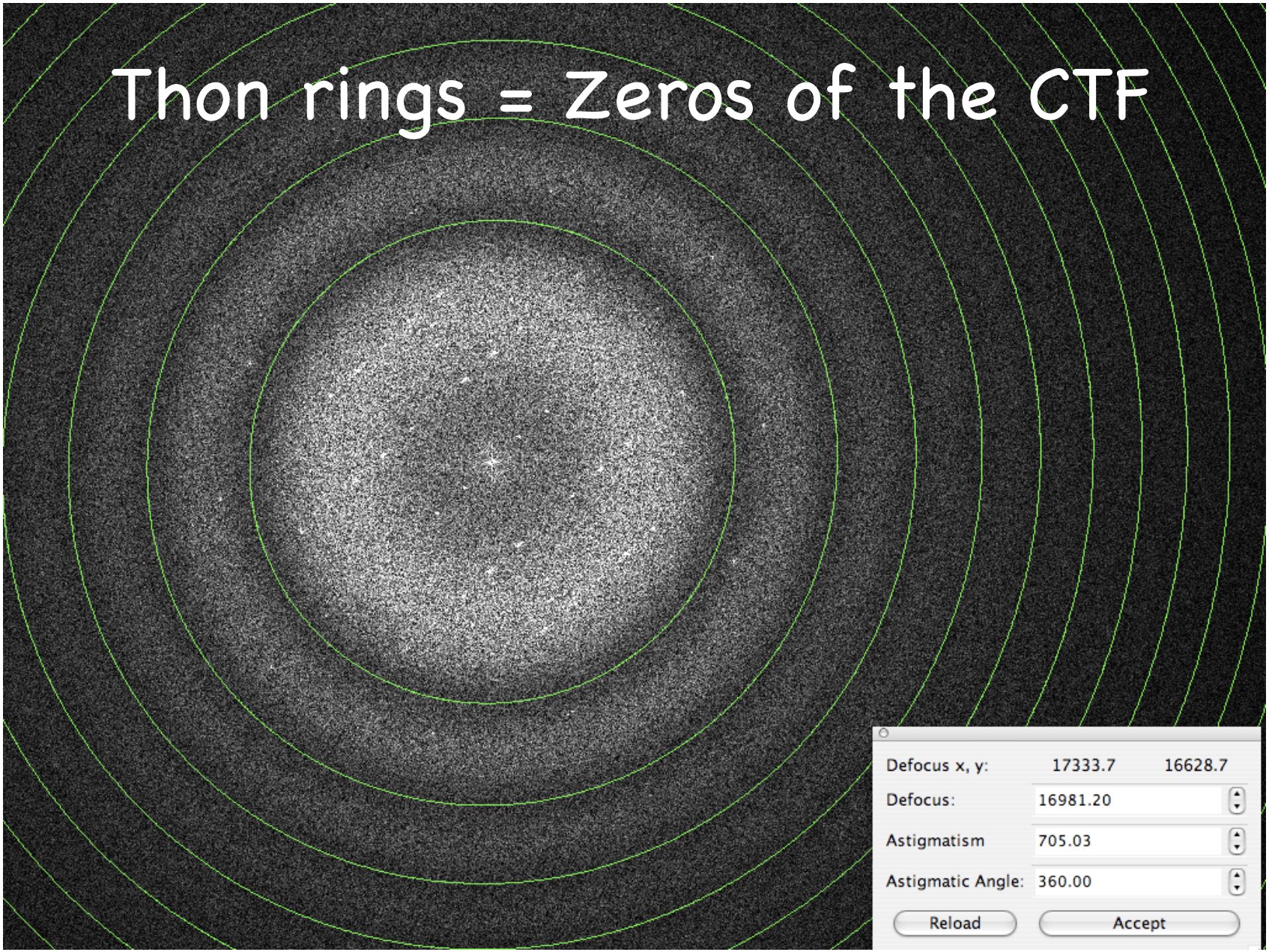


Contrast Transfer Function



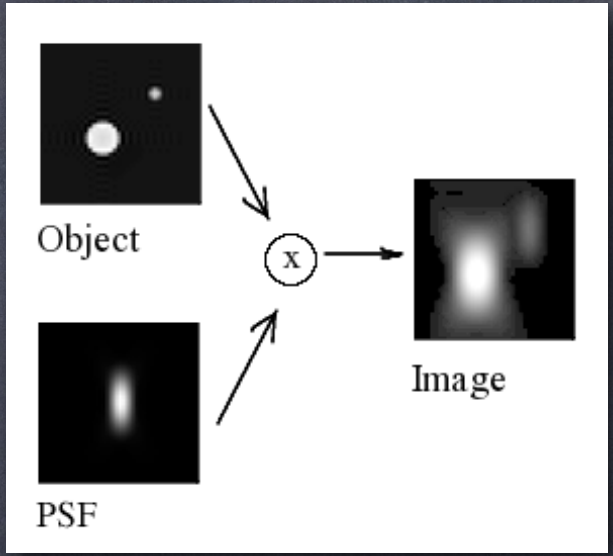
Continuously changing defocus

Thon rings = Zeros of the CTF



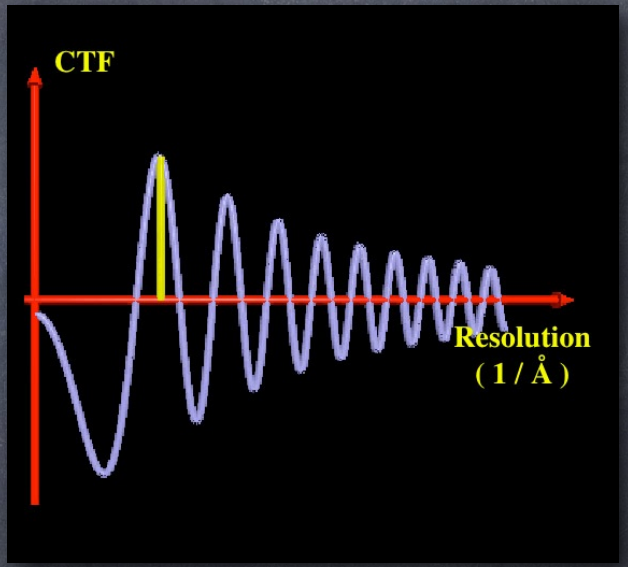
Real Space:
Point Spread Function

Object ⊗ PSF = Image



Fourier Space:
Contrast Transfer Function

FT(Object) • CTF = FT(Image)



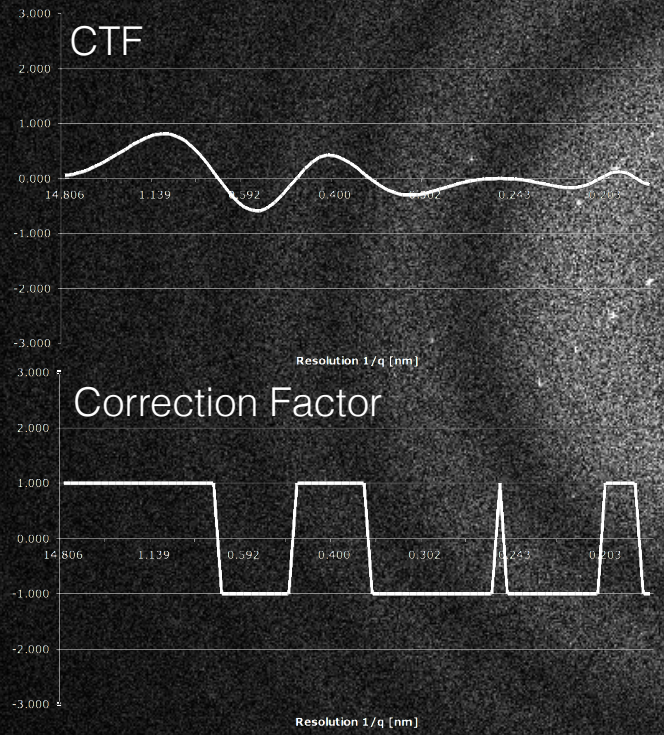
FT(PSF) ≈ CTF

PSF ≈ FT(CTF)

Can we correct the CTF ?

CTF correction: Phase flipping

$$NewImage = FT^{-1} \{ FT(Image) \bullet sign(CTF) \}$$



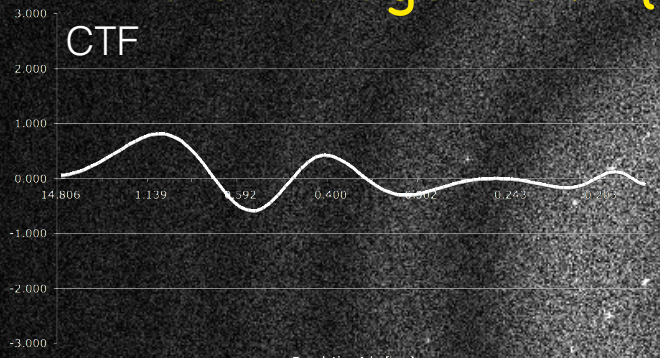
+ − + − + − +

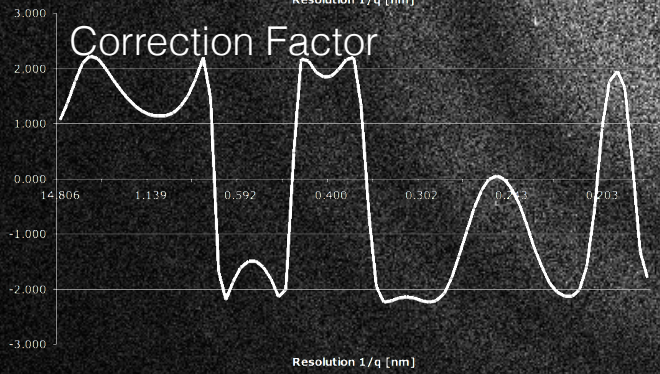
Shifts the phase by 180º behind every second Thon ring

CTF correction: Wiener Filter

"Divide by the CTF (but don't divide by zero...)"

$$\text{NewImage} = FT^{-1}\left\{ FT(\text{Image}) \bullet \frac{CTF}{CTF^2 + (N/S)^2} \right\}$$



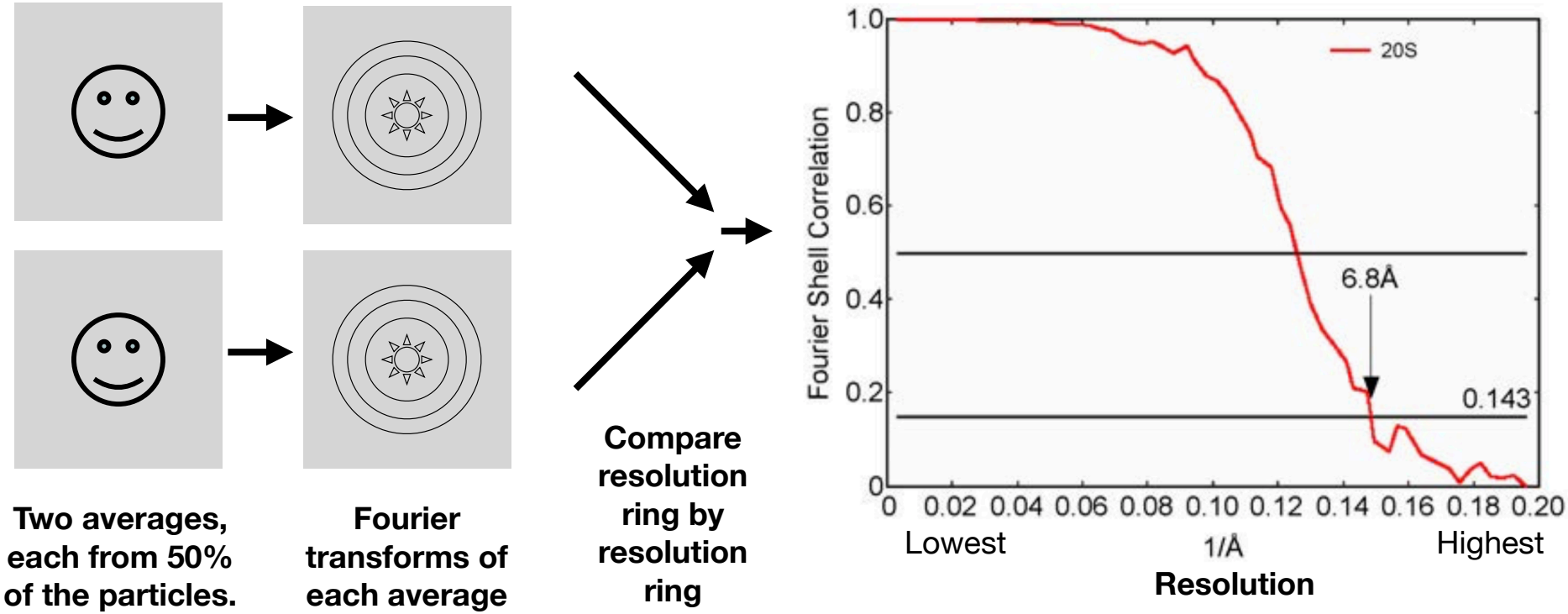


+ − + − + − +

Gives a result closest to the true structure.

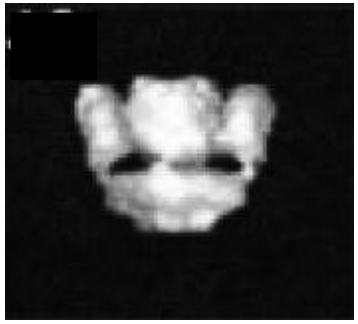
Single Particle Cryo-EM: Resolution Determination

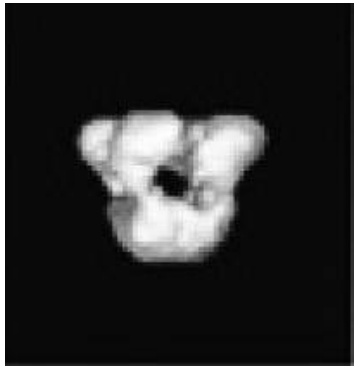
Resolution: Fourier shell correlation (FSC)

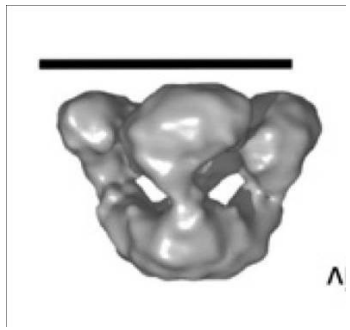


Single Particle Cryo-EM:
The “Einstein from Noise” Problem

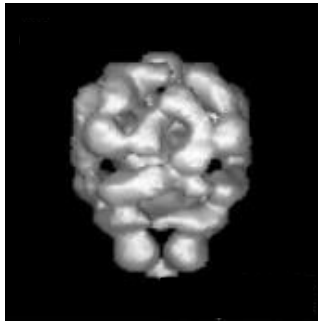
da Fonseca, 2003
Morris, 2010
neg. stain, 30Å



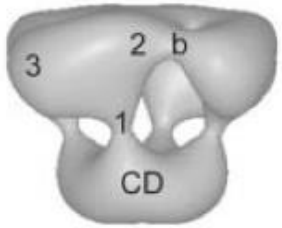




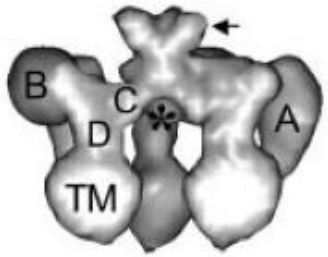
Sato, 2004
cryoEM 15Å



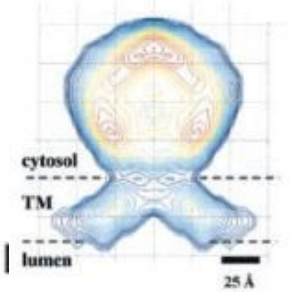
Hamada, 2003
neg. stain 34Å



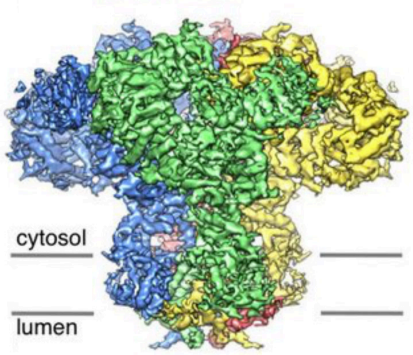
Serysheva, 2003
cryoEM, 30Å



Sigworth, 2002
cryoEM, 24Å

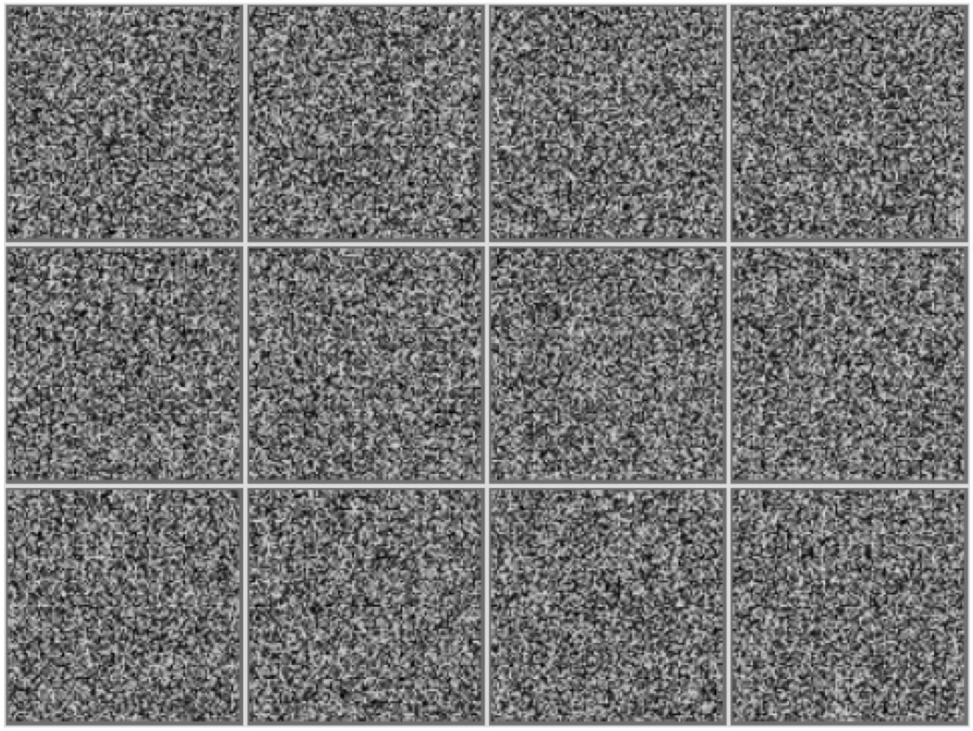


IP3 receptor, 1.3 MDa

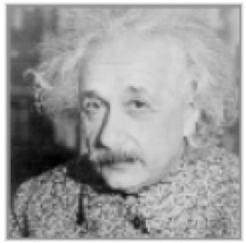


Fan et al., Nature 2015 (4.7Å)

Reference Bias: A dangerous phenomenon.



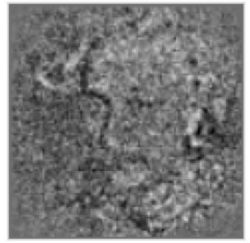
Random noise



Reference

ALIGN &

AVERAGE

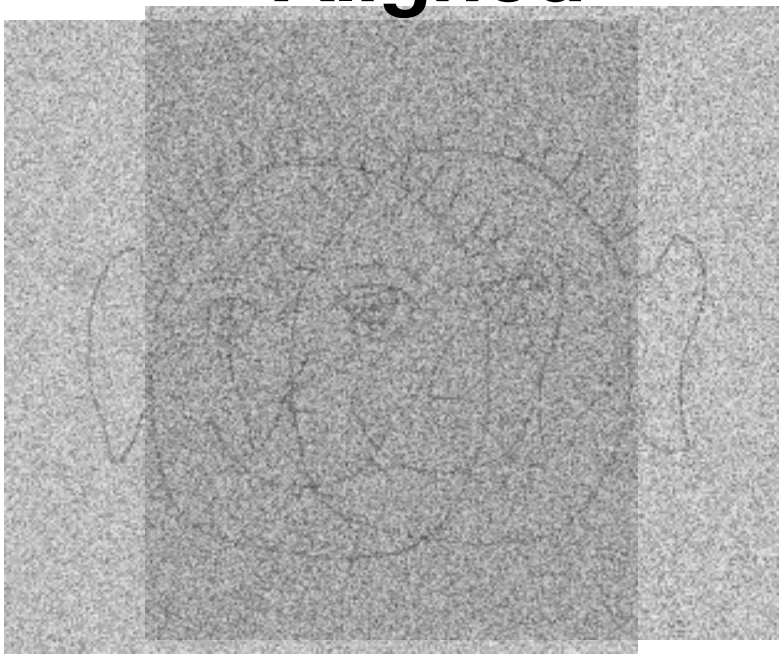


Average

Attention: Reference Bias in Particle Selection or in Particle Alignment can reproduce the reference feature, even if it was not present in the dataset.

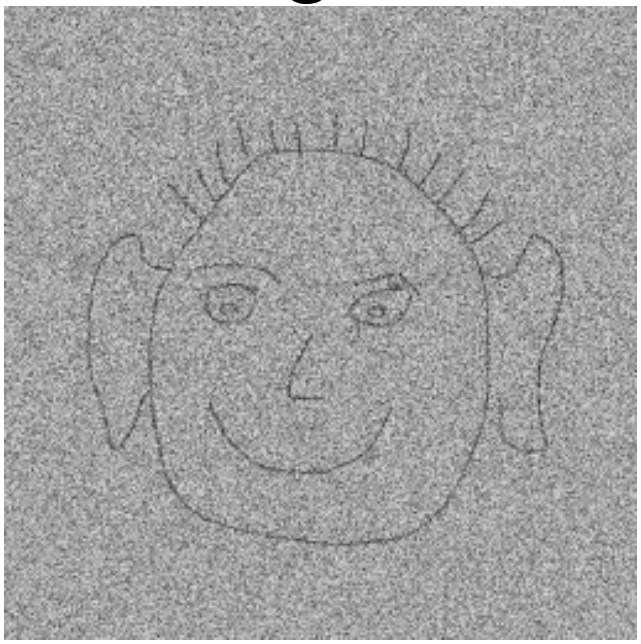
REFERENCE BIAS

Wrongly Aligned



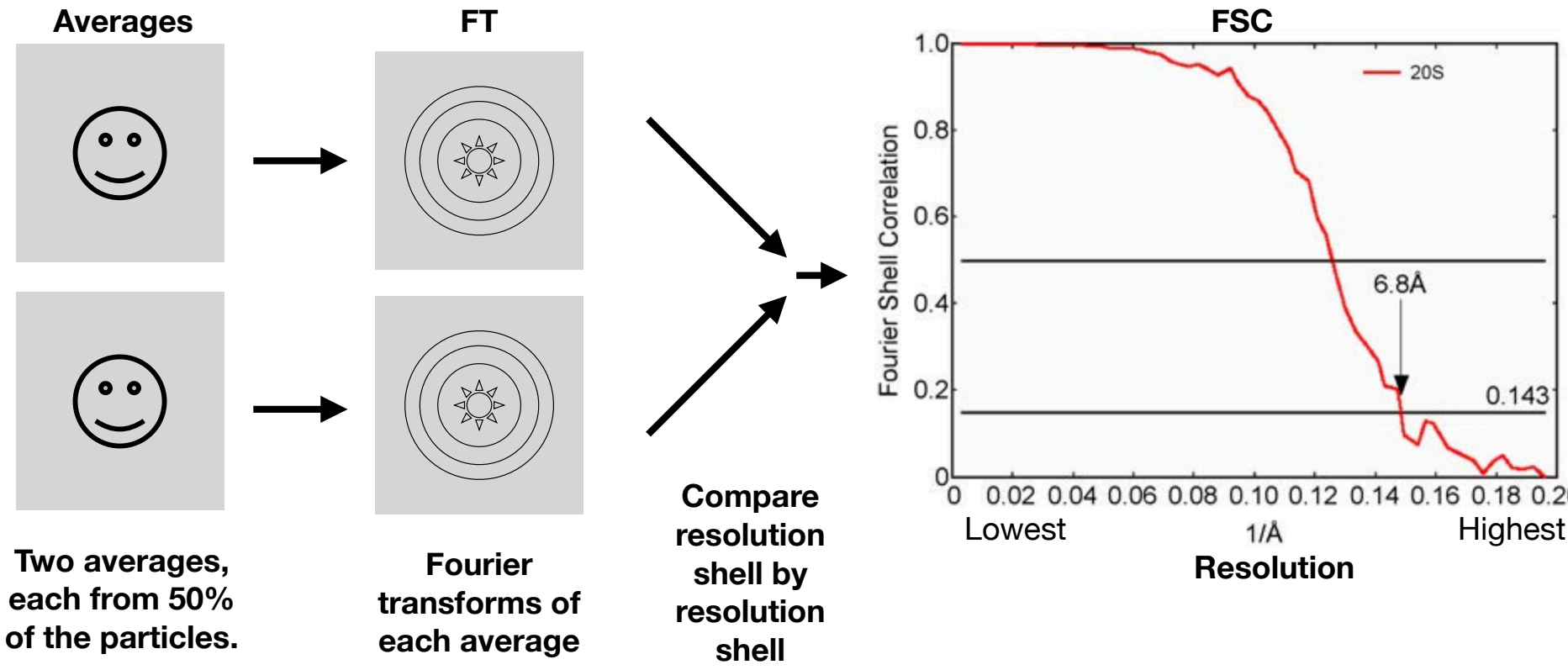
Three eyes

Correctly Aligned

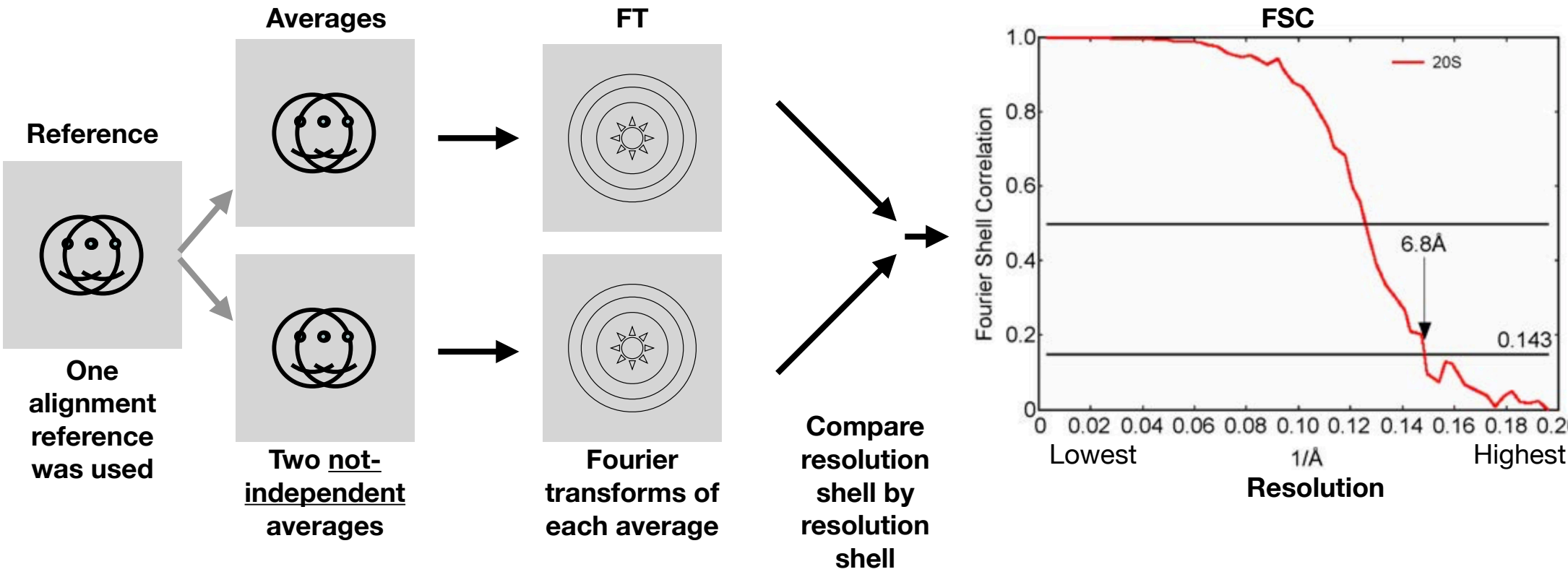


Two eyes

Resolution: Fourier shell correlation (FSC)



Reference Bias can lead to apparently high resolution

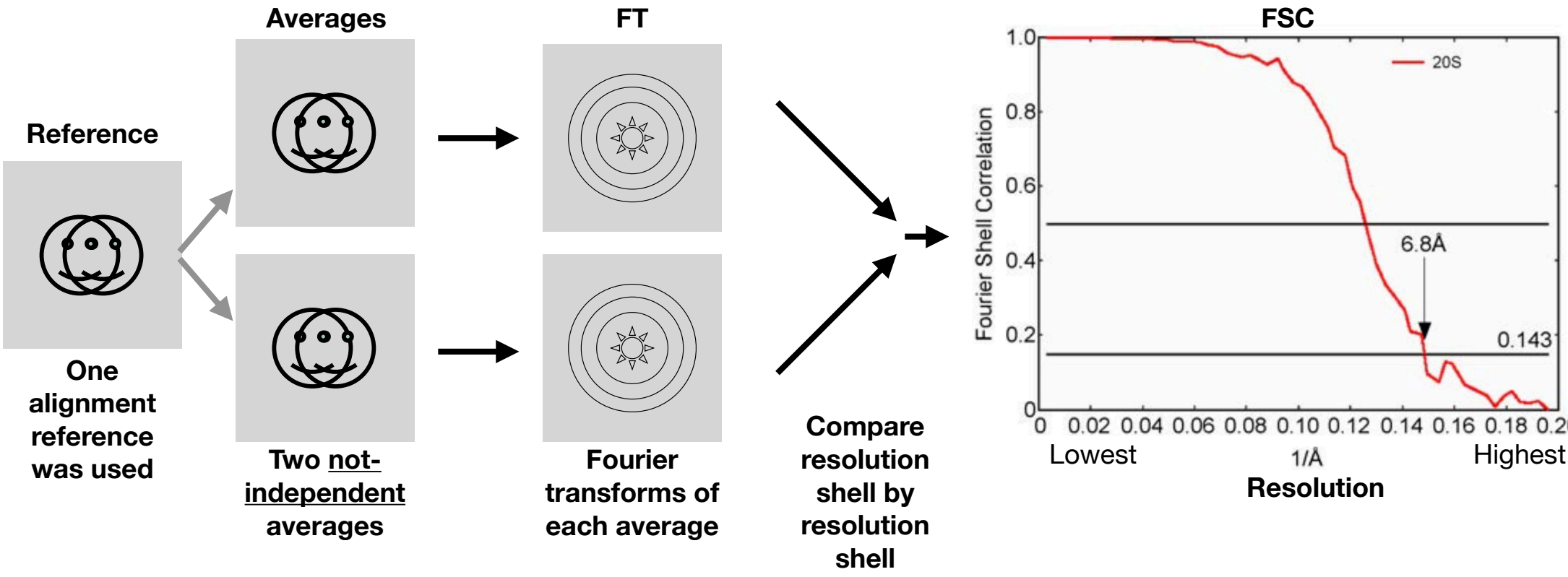


Solutions to the reference bias problem:

1. Publish artefacts. (was sometimes done in the past. At that time, people used *SEMPER, SPIDER*, *IMAGIC*)

2. Use two separate references. You need twice as many particles. (often used in: *EMAN2, SPARX, SPHIRE, CryoSPARC, BSOFT)*

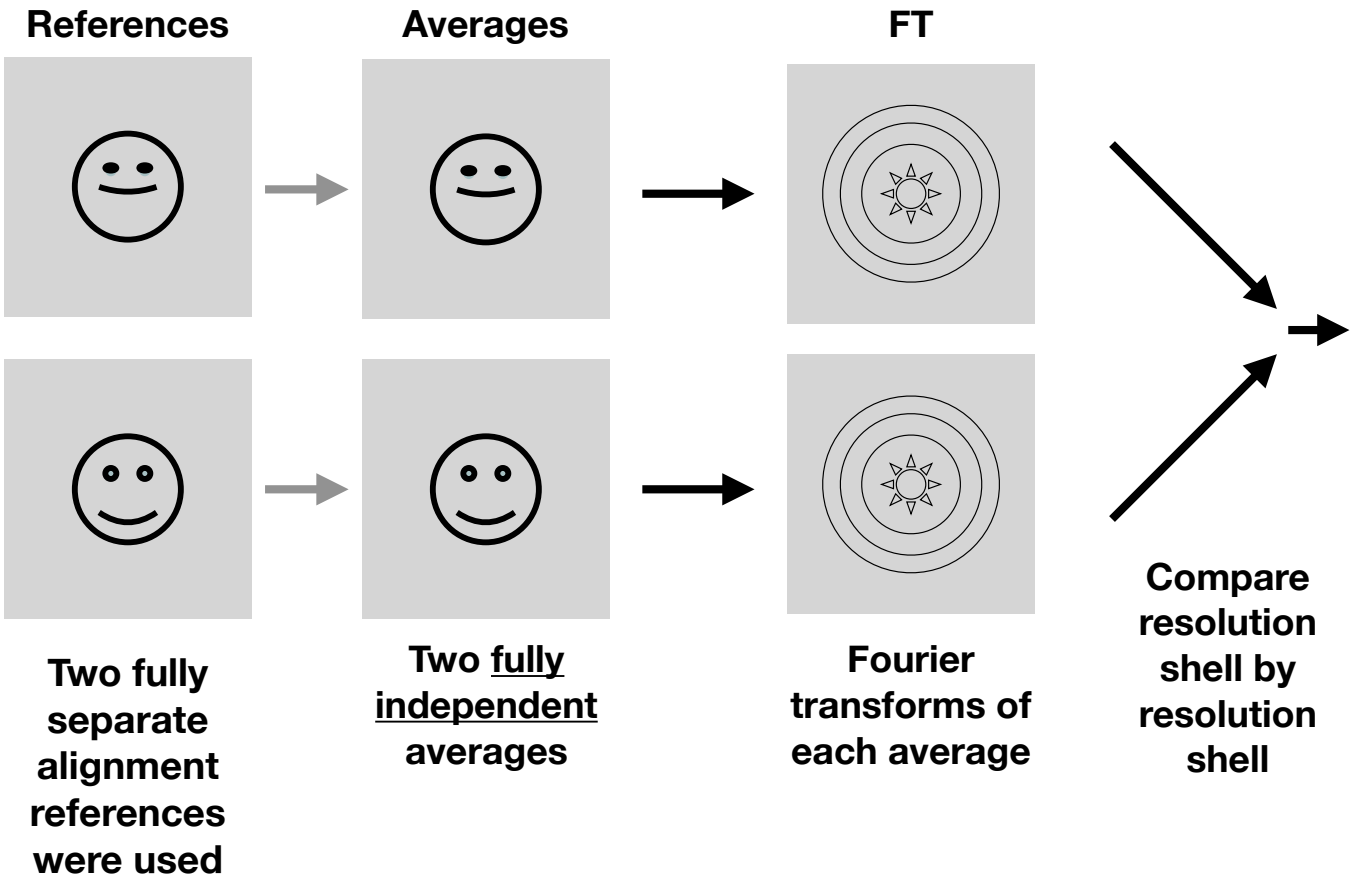
Reference Bias can lead to apparently high resolution

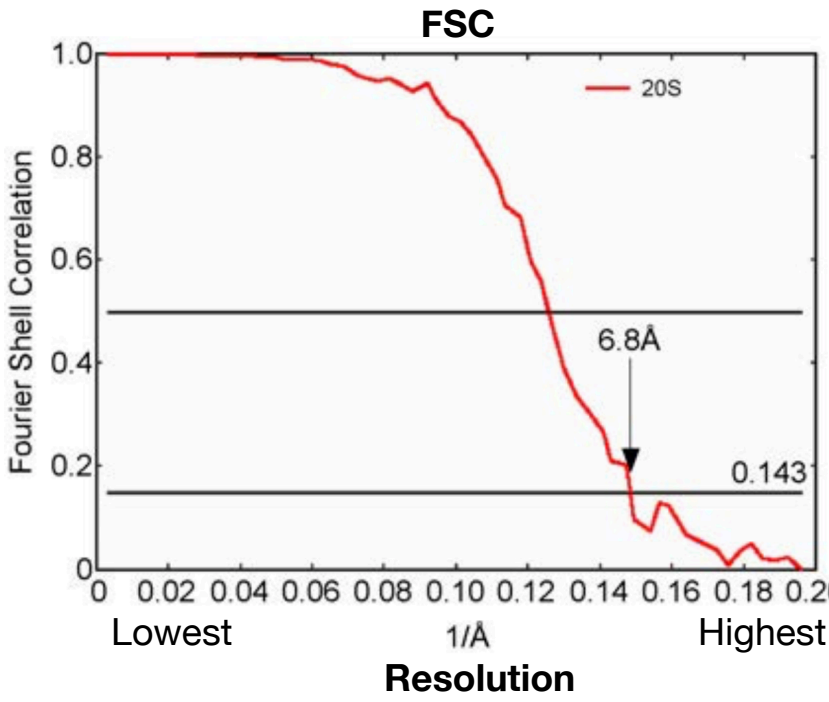


So-called “Gold Standard” gsFSC Method

Work with two half-sets of the particles, fully separated from A-Z.

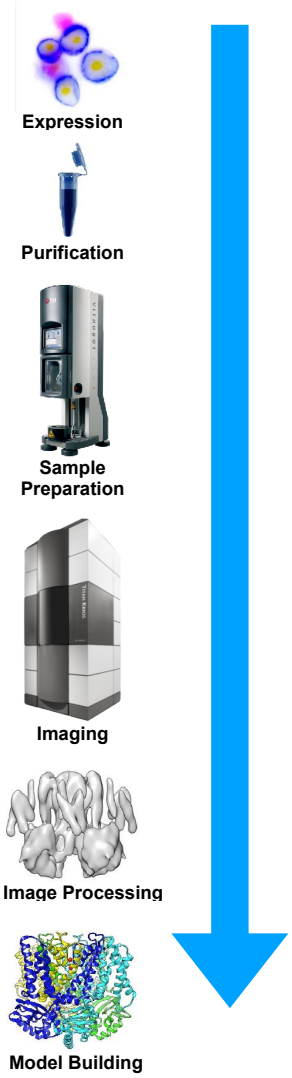
(You will need twice as many particles)



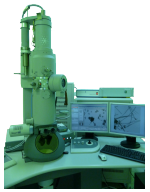


EMAN2, RELION

Single Particle Cryo-EM

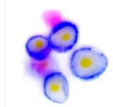


- **Stage 1: Is my sample suitable (pure, homogeneous, stable)?**
  - Preparation of negatively stained grids
  - TEM imaging @ 120kV
  - Computer analysis for sample homogeneity control



- **Stage 2: Can my sample be frozen well?**
  - Preparation of frozen grids
  - Cryo-EM imaging @ 200kV
  - Computer analysis for sample quality control

- **Stage 3: Get the high-resolution structure**
  - Identification of the optimal, frozen grid
  - Cryo-EM imaging @ 300kV
  - Computer analysis for 3D structure reconstruction



Expression

Purification

Sample Preparation

Imaging

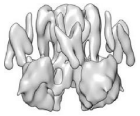
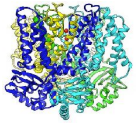
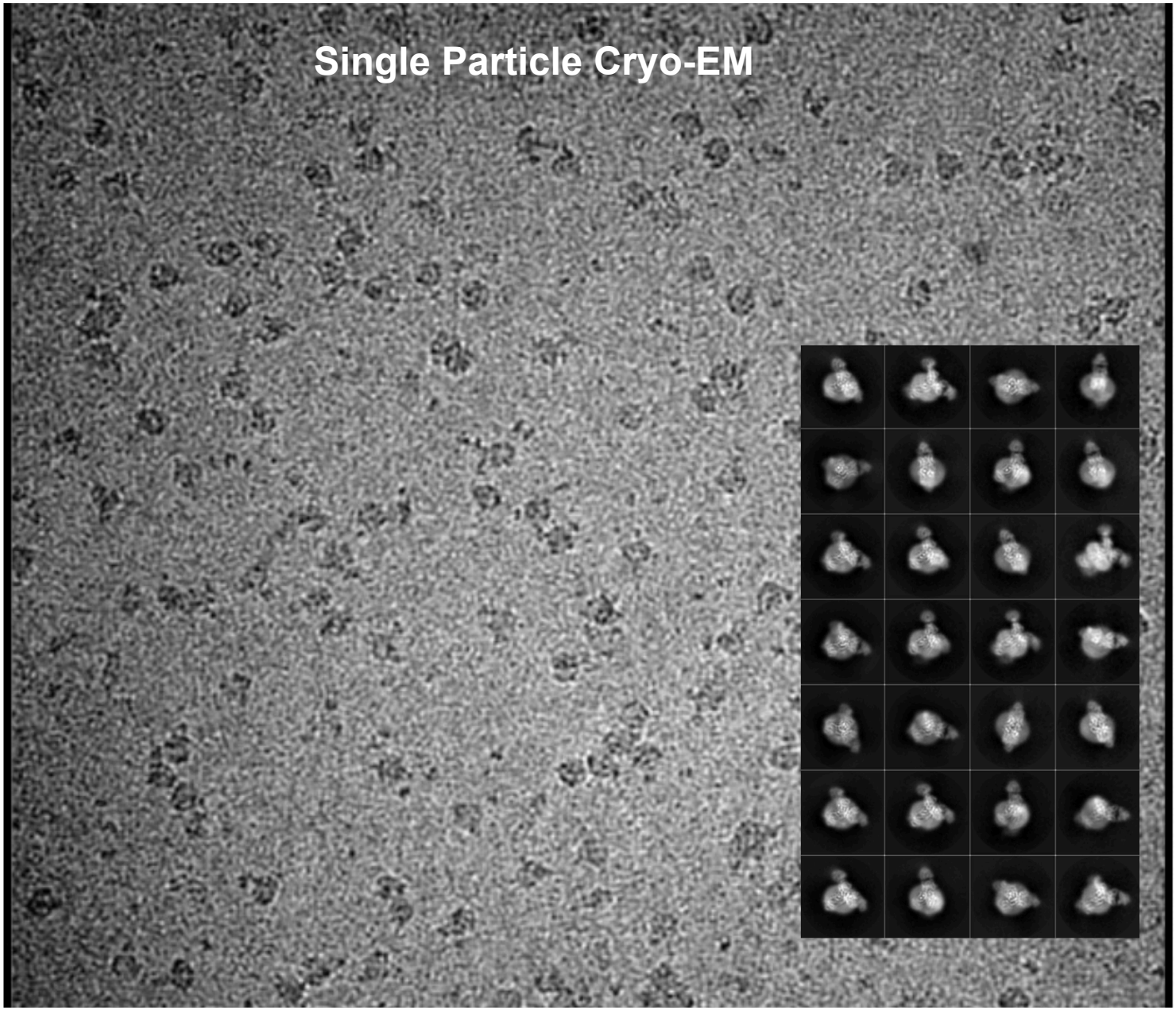


Image Processing



Model Building



Cryo-EM of the human ABC transporter ABCG2

Expression

Purification

Sample Preparation

Imaging

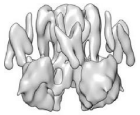
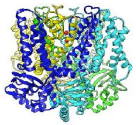
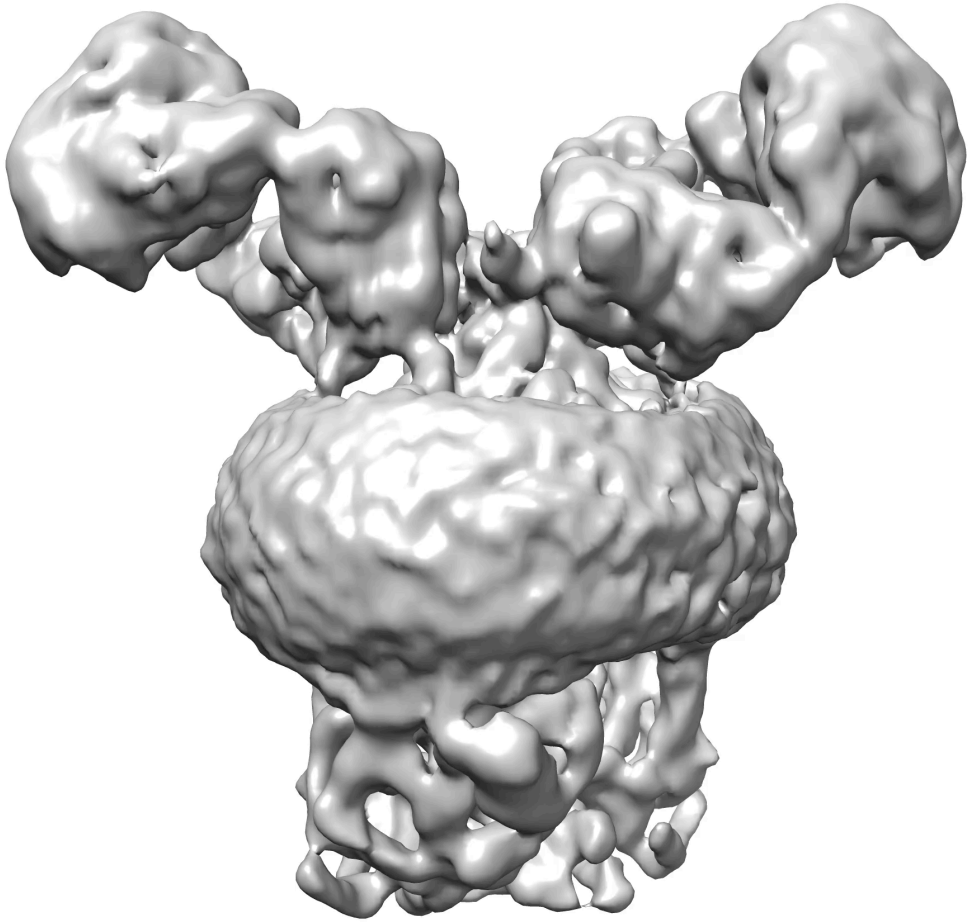


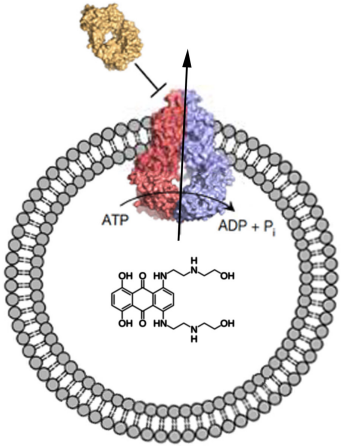
Image Processing



Model Building



Collaboration with Kaspar Locher, ETHZ

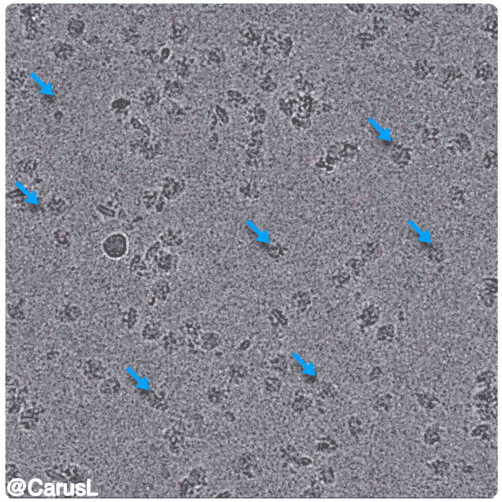


Membrane protein
in a lipid nanodisk.
Data collection:
3 days on C-CINA Titan,
1702 movies,
232’608 particles
97’612 particles in final map.
Final resolution 3.8Å

Cryo-Electron Microscopy

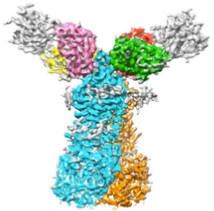
Frozen sample goes into microscope

10’000 Images (> 10 TB raw data)

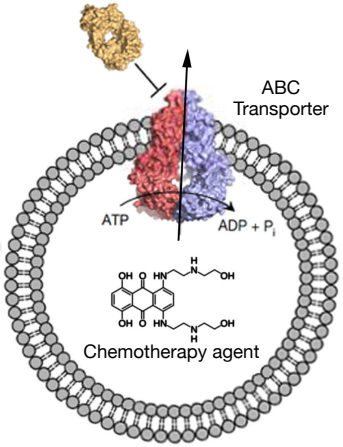


(1’000’000 particle images are needed)

Computer 3D Reconstruction



Atomic Model

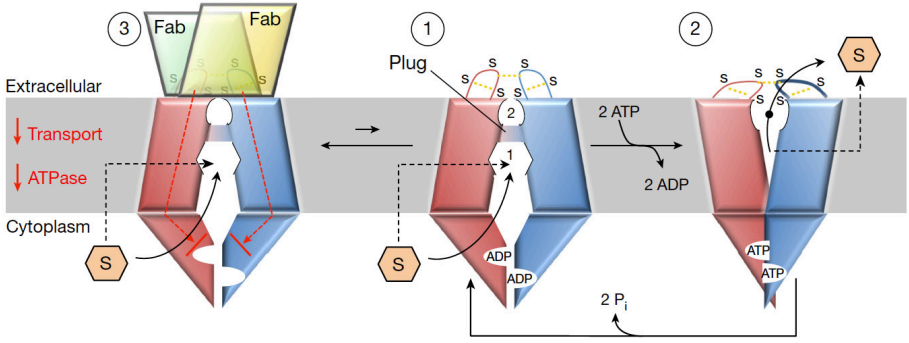


Understanding the mechanism of chemotherapy resistance

Cancer cells defend themselves from mitoxantrone chemotherapy with the protein ABCG2.

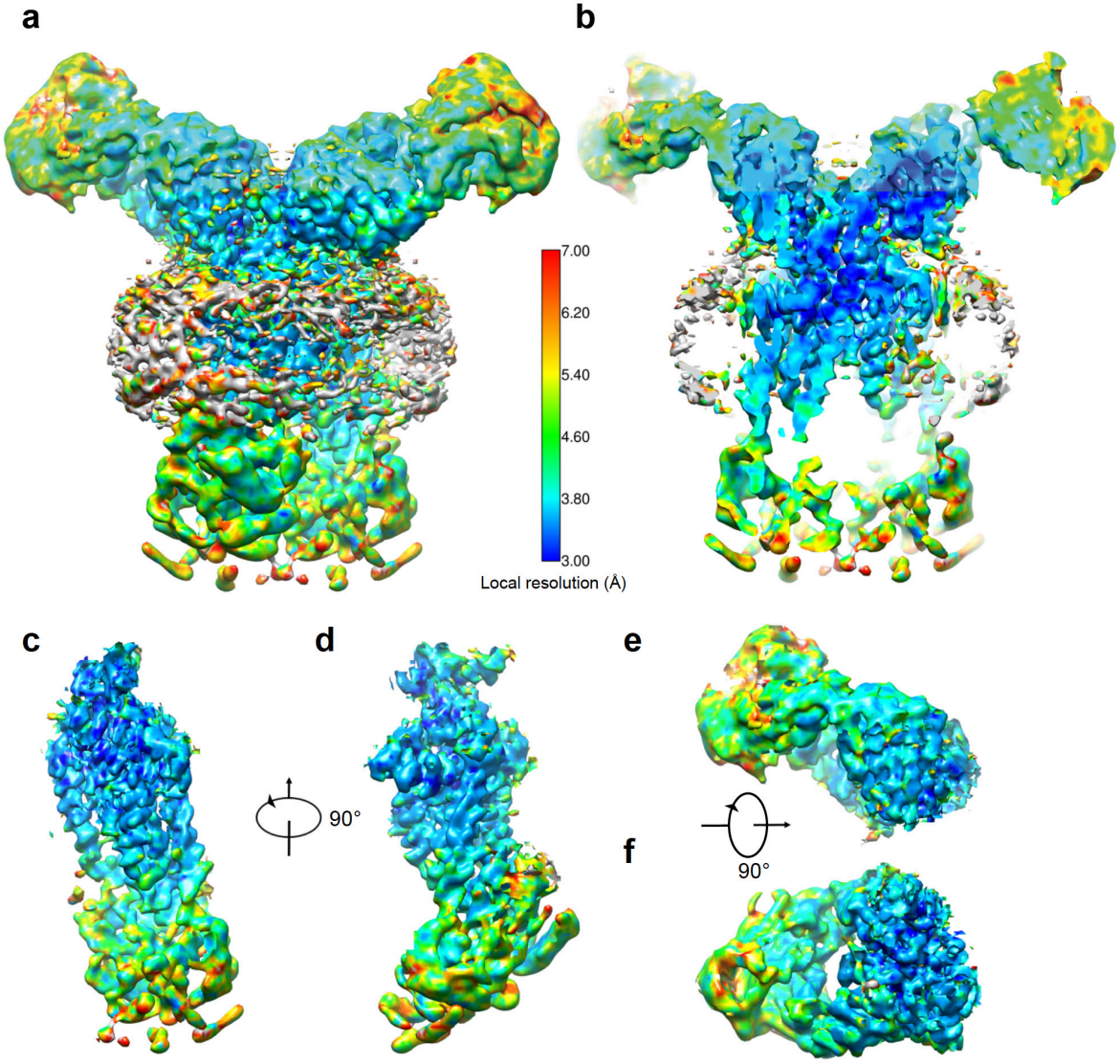
Blocking ABCG2 with a small *Fab* during chemotherapy kills the tumor.

<u>Understanding and fighting cancer resistance.</u>



Taylor et al., Nature (2017). With Kaspar Locher lab, ETHZ

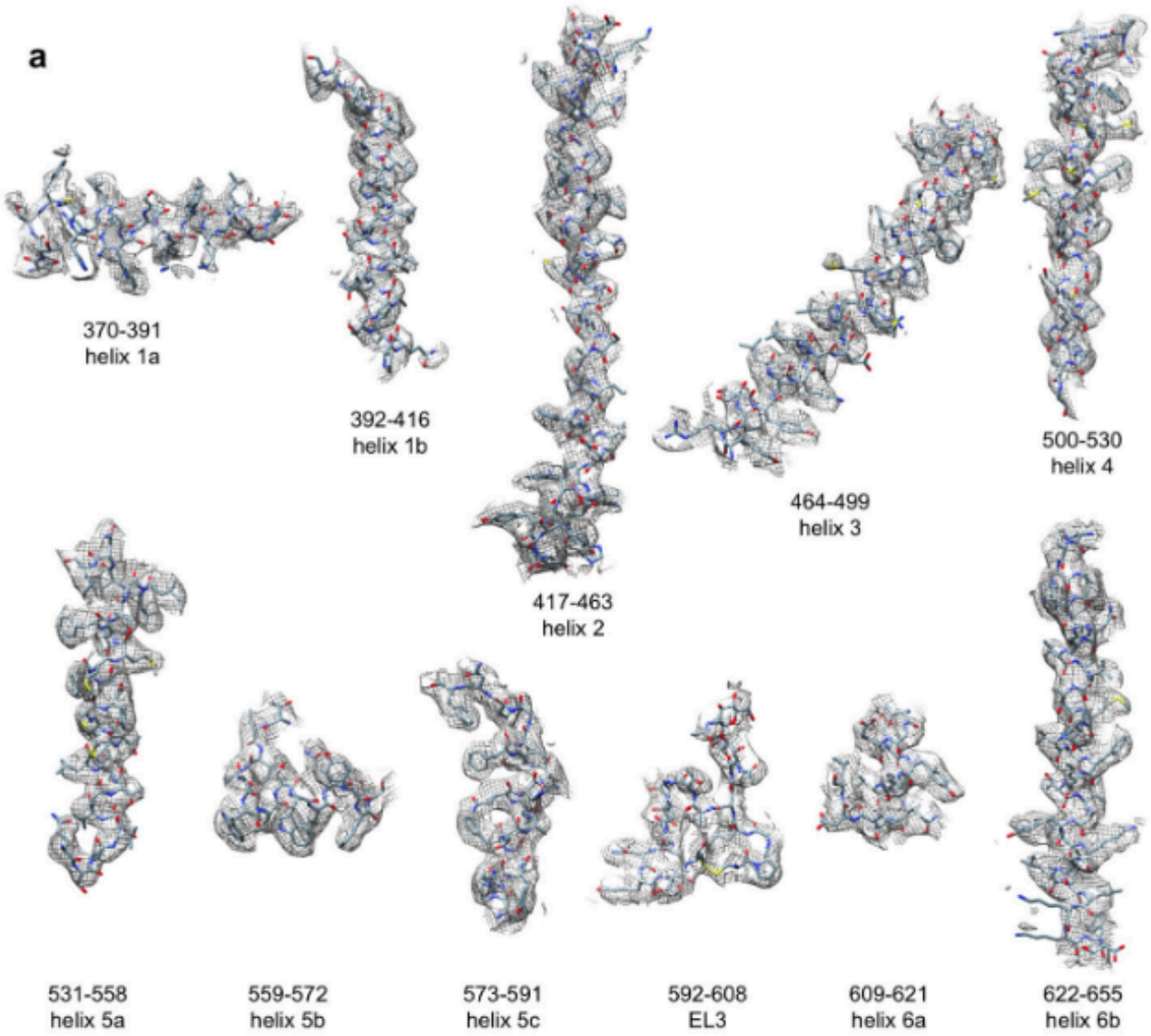
Cryo-EM of the human ABC transporter ABCG2



This plot shows the "local resolution" of the final 3D map, which ranges from 3.0 to 7.0 Å, on average 3.8Å.

Structure of the human multidrug transporter ABCG2

Nicholas M. I. Taylor[1]*, Ioannis Manolaridis[2]*, Scott M. Jackson[2]*, Julia Kowal[2], Henning Stahlberg[1] & Kaspar P. Locher[2]



Membrane protein
in a lipid nanodisk.
Data collection:
3 days on C-CINA Titan,
1702 movies,
232’608 particles
97’612 particles in final map.
Final resolution 3.8Å

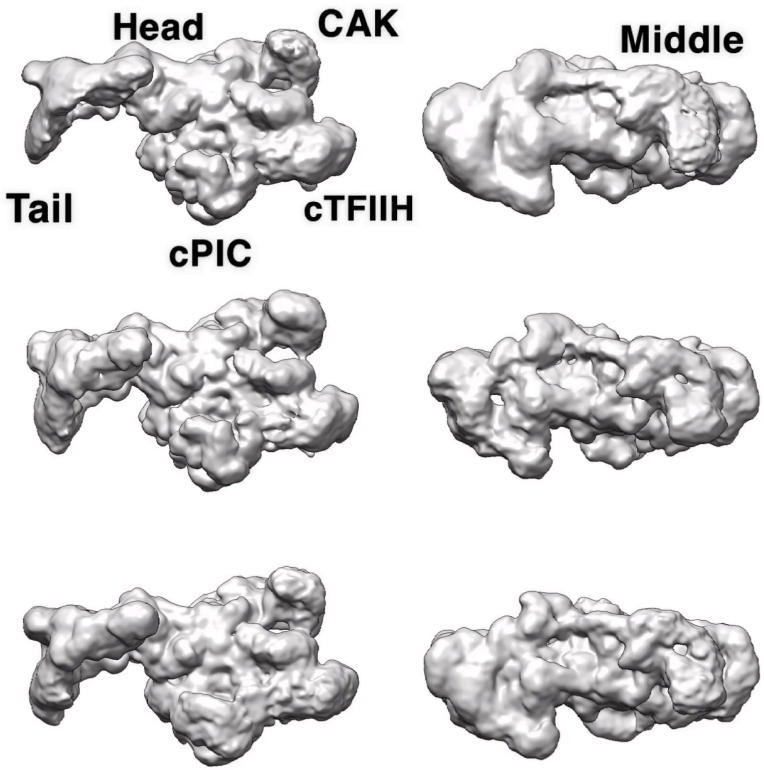
Nicholas Taylor et al., Nature (2017), with Kaspar Locher

Single Particle Cryo-EM Example: human Mediator-bound transcription preinitiation complex

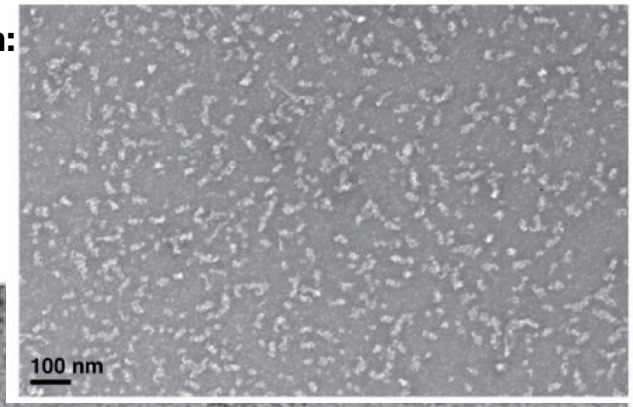
Particles are flexible

3D Variability Analysis
of the top three principal components

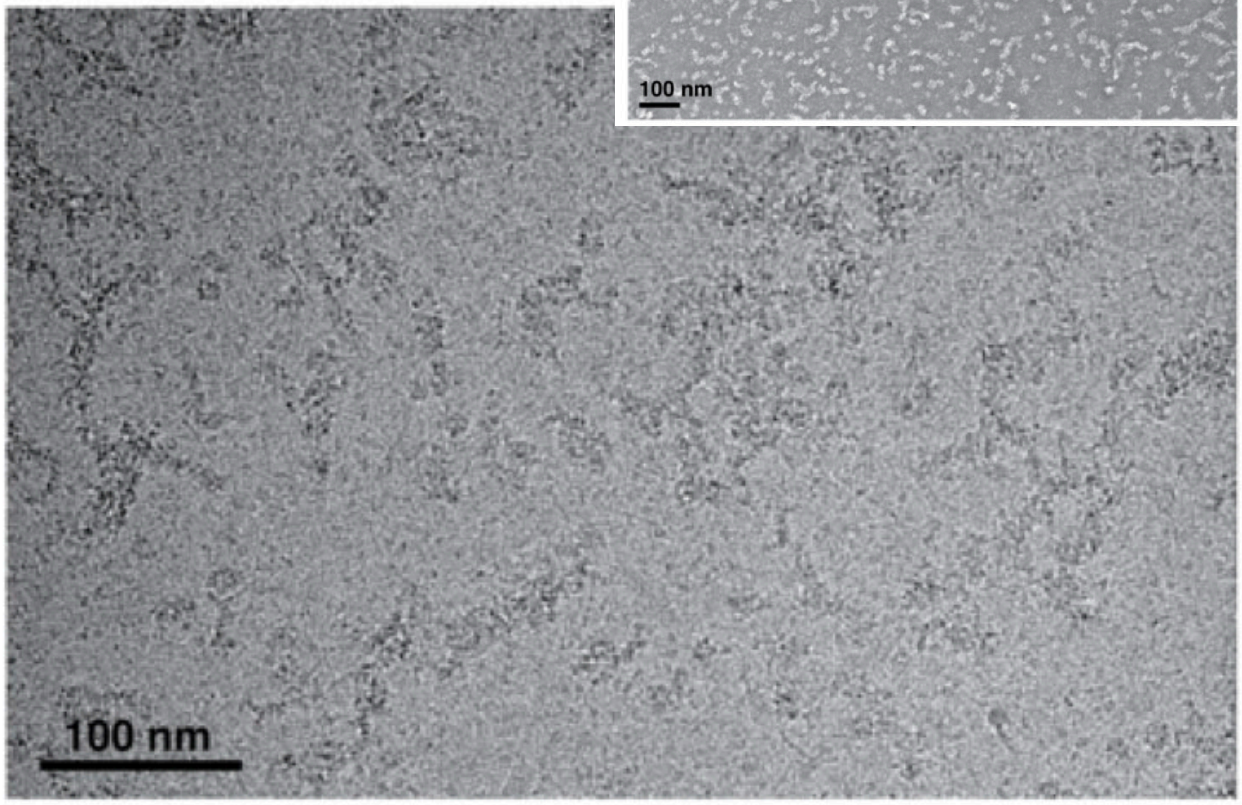
Side view Top view



Neg. Stain:



Cryo-EM:



Assembly of Med-PIC. A) SDS PAGE gel of purified Med-PIC factors. Lanes were rearranged for clarity. B-C) Representative negative stained (B) and cryogenic (C) electron micrograph and class averages show intact Med-PIC complexes with multiple views.

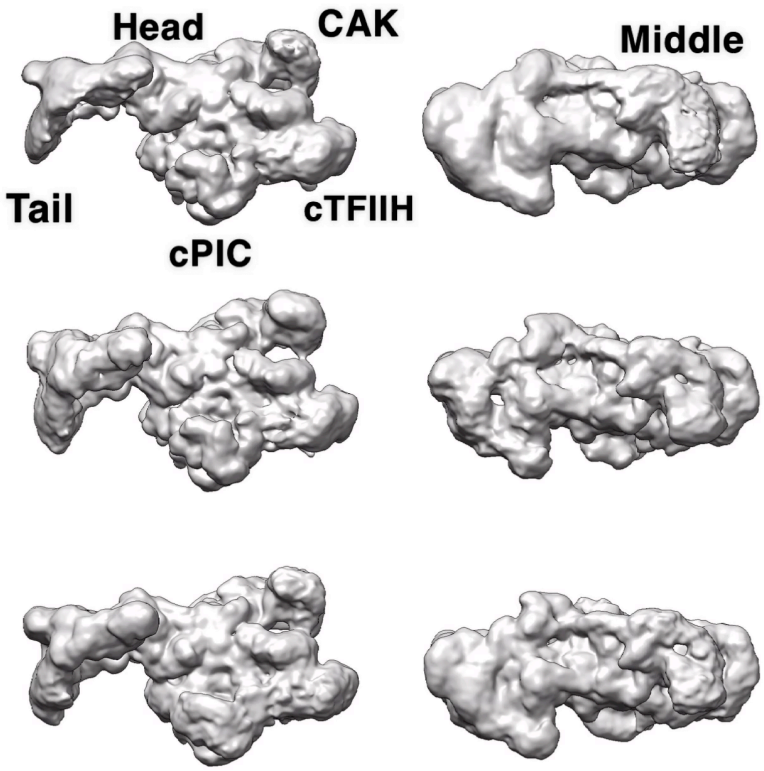
Abdella et al., *Science,* March 11, 2021

Single Particle Cryo-EM Example: human Mediator-bound transcription preinitiation complex

Particles are flexible

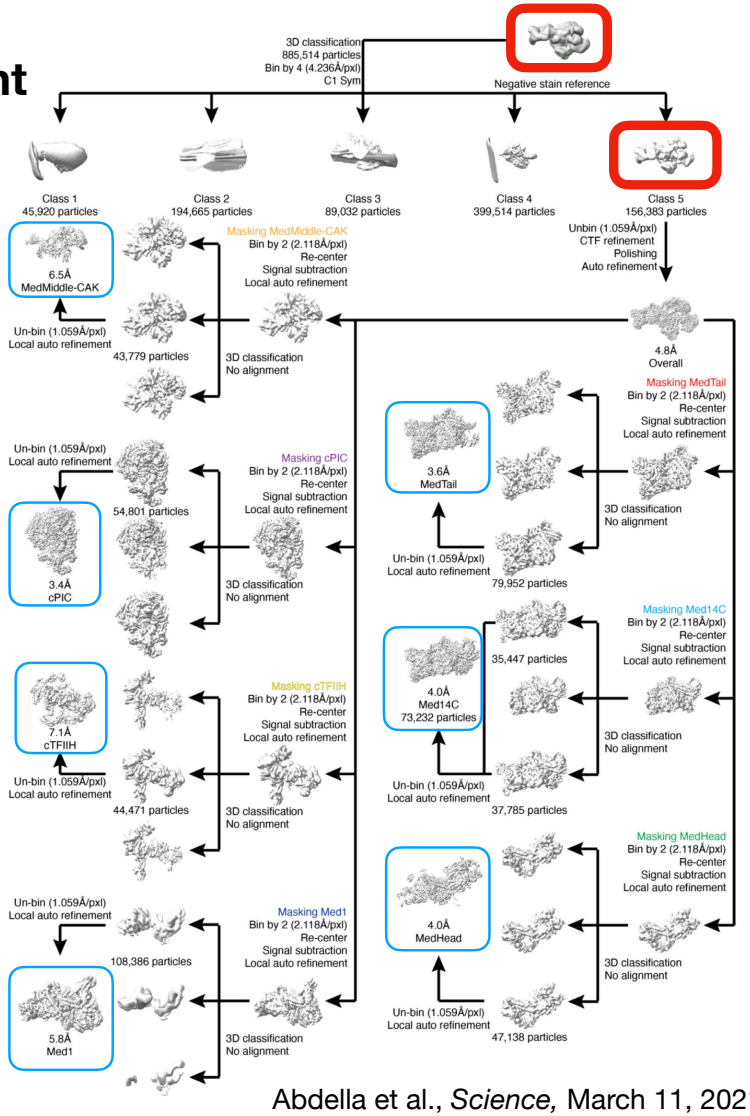
3D Variability Analysis
of the top three principal components

Side view Top view



3D Classification, Focused Refinement

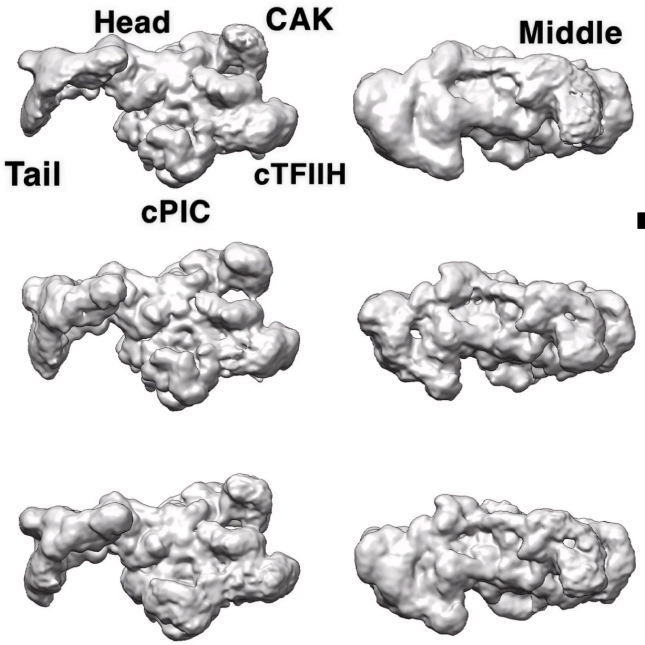
(Needs biological knowledge of the components)



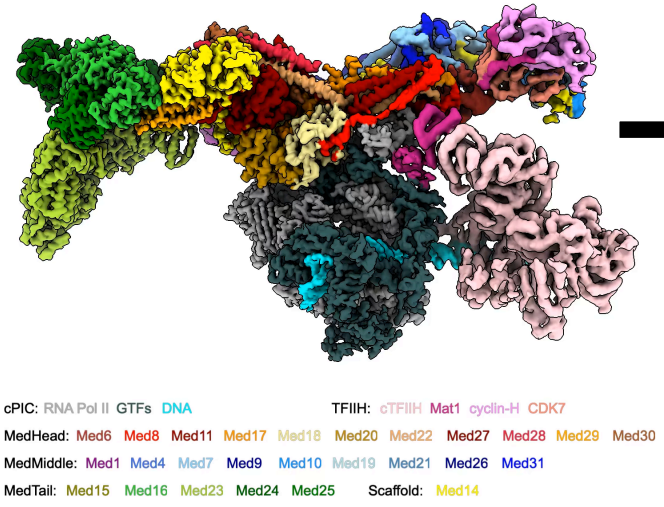
Abdella et al., *Science,* March 11, 2021

Single Particle Cryo-EM Example: human Mediator-bound transcription preinitiation complex

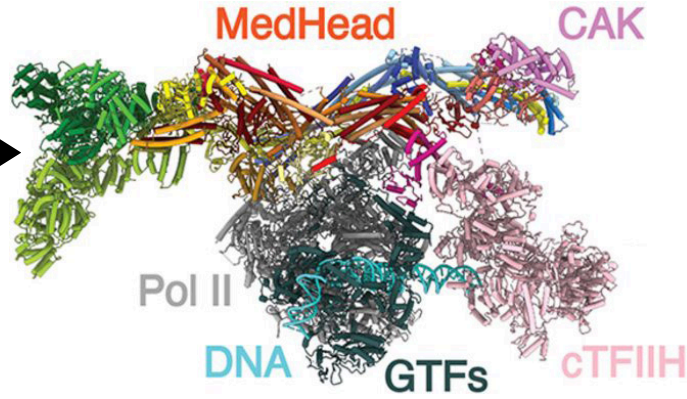
Original Map
(Calculated during data collection)



Focused refinement
(needs knowledge of the biology)

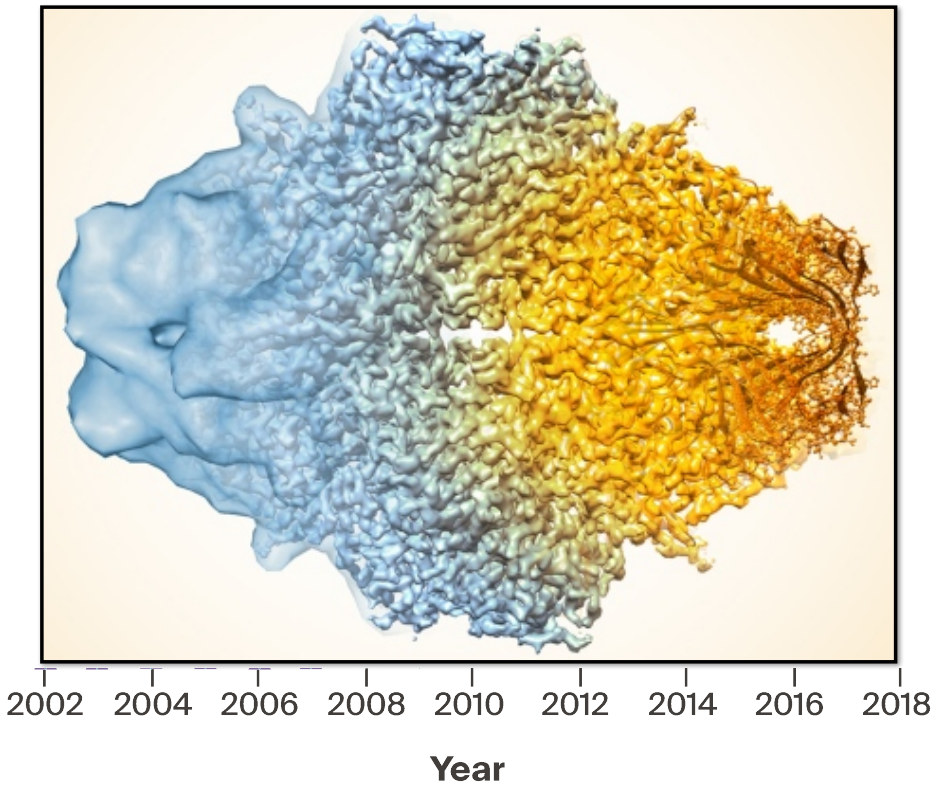


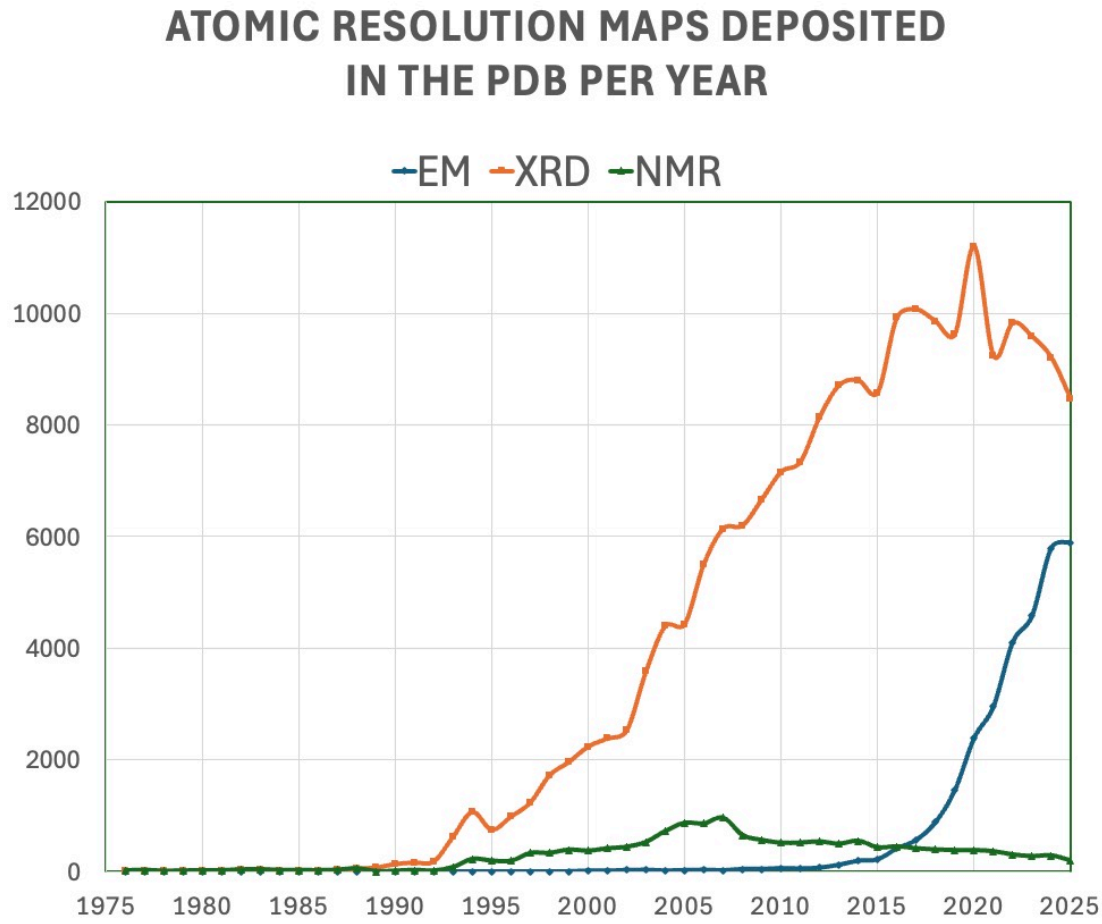
Atomic Model
(Model building needs chemistry knowledge)



Abdella et al., *Science,* March 11, 2021

The Resolution Revolution in Cryo-EM

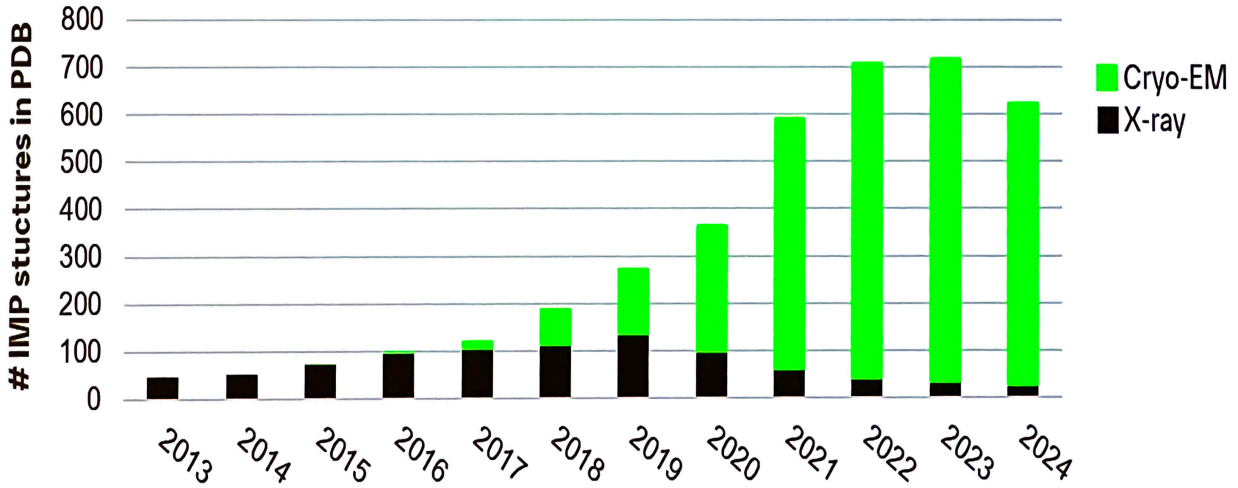




Novel technologies for structure determination

- Advanced in detector technologies and data processing software let to the "**resolution revolution**" in the single particle cryo-EM field.
- Cryo-EM allows to investigate large and more flexible complexes and is well suited for IMPs.

Number of membrane protein structures deposited in the PDB by method (human, <4 Å)



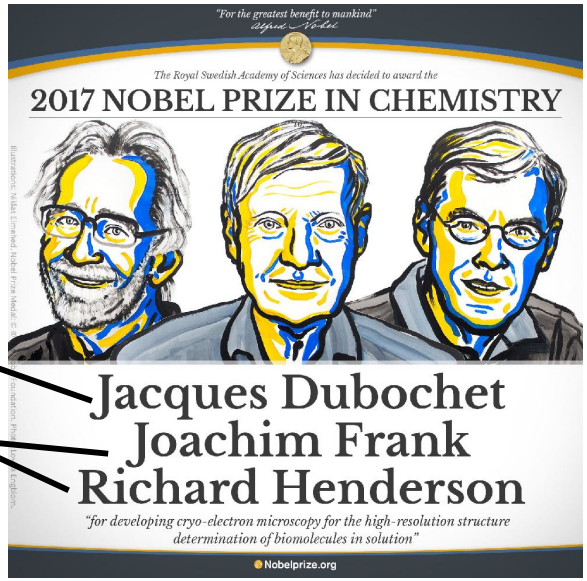
Boehringer Ingelheim

Advances in the cryo-EM field increased the success rates for IMP structure determination

Slide by Boehringer Ingelheim, shown at the PSI on a symposium, 27.10.2025

Cryo-EM as of today

What	*Time*	*Resource*
Expression, Purification	years	Pure, homogeneous, stable
Cryo-EM grid optimization	2 weeks	200kV Cryo-EM
Cryo-EM imaging	2 days	300kV high-end Cryo-EM
Image processing	1 month	500 CPUs or 10 GPUs



Frontiers in Single Particle Cryo-EM

Resolution:

3.0 Å resolution is routine	=>	Backbone, larger side chains
2.0 Å resolution is difficult	=>	ligands, inhibitors, ions, drug design
< 1.0 Å resolution is possible with the Titan	=>	Limitation by sample and software

Sample Size:

> 150 kDa is ok
100 kDa is difficult
45 kDa is current world record (only with phase plate)

Sample Homogeneity:

< 10 discrete conformations ok
Continuous variations are difficult to process.

Speed:

Today: 1 day on 300kV microscope, 1 month on computer
Desired: 30 min on microscope, processing in real time.

Sample Quantity:

> 3 µl per cryo-EM grid	=> mostly lost in filter paper
3 nl per cryo-EM grid	=> needed for imaging

Cryo-EM application: Multi-Scale Microscopy

Single Particle Cryo-Electron Microscopy

Atomic structures of proteins and protein complexes

Atomistic function of enzymes

Method Development:
- Automation
- Sample preparation
- Acceleration

Related:
- Protein production
- Biophysics
- XRD, NMR, MS, AFM
- Modeling

Tissue Cryo-Electron Tomography

Cellular and multi-cellular structure

Biological organization, structure

Method Development:
- cryo-FIB-SEM lamella
- 3D reconstruction
- 3D segmentation

Related:
- FIB-SEM Slice and View

Correlative Light and Electron Microscopy

Cellular and multi-cellular organization

Biological function

Method Development:
- Label technology
- Coordinate transform
- Super-resolution LM

Related:
- Correlated FIB-SEM