Image processing workflows for Tomography

Winter School on Structural Cell Biology

Julio Ortiz
Wolfgang Baumeister
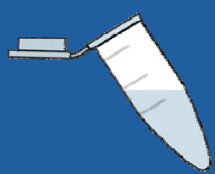MPI of Biochemistry
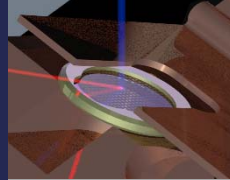Dept. of Structural Molecular Biology
Martinsried, Germany

Brno,12th February 2015

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Summary/Outlook

Sample Preparation

In vitro

In situ

Plunge freezing -180 °C

FIB

Instrumentation

FEI Quanta 3D

FEI Titan Krios

Direct Electron Detectors

Phase Plate

Electron Cryotomography

Single-axis tilt

Dual-axis tilt
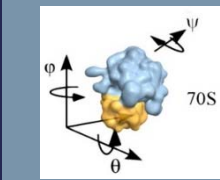
TM

Software
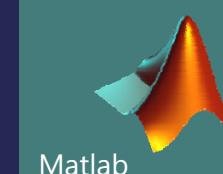
Matlab toolbox

PyTom

Amira
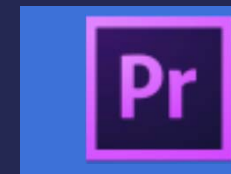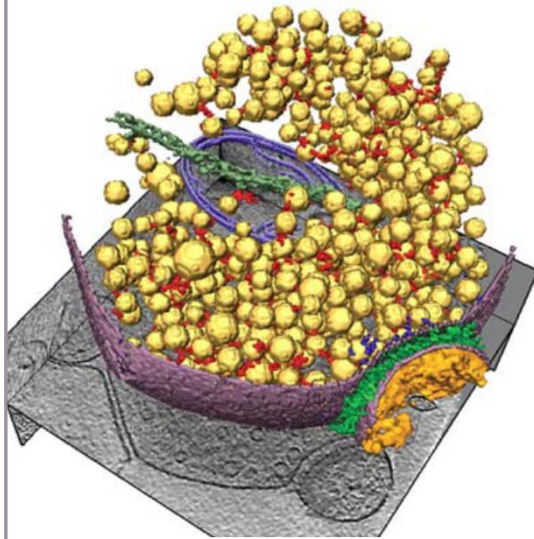

Chimera
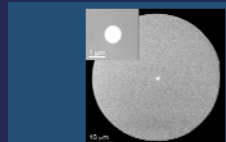
3ds Max

Adobe CS6

CET combines the power of 3-D imaging with a close-to-life preservation of cellular structures and a **allows to study macromolecular structures *in situ***.
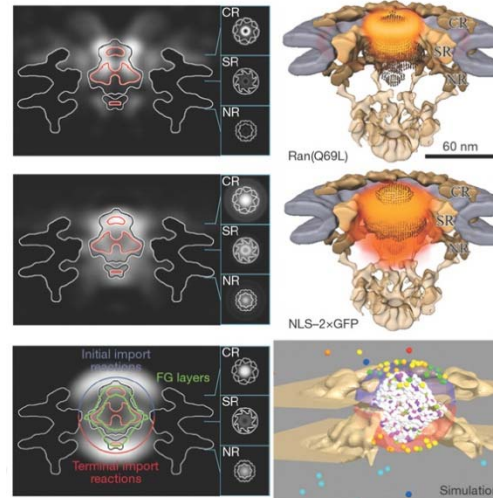
Cryo-electron tomography (CET)

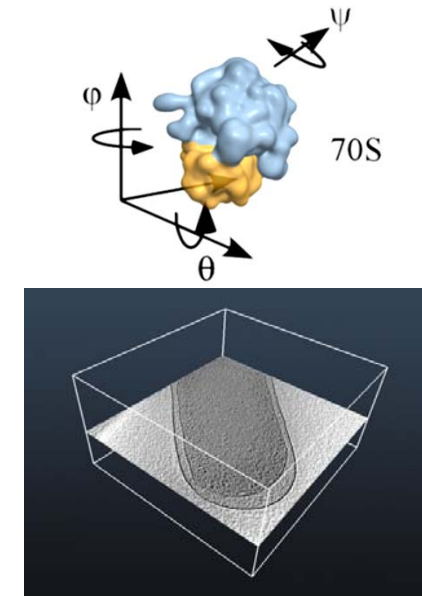# Low-level Techniques for Data Analysis in Tomography

Segmentation

Presynaptic vesicles
Fernández-Busnadiego, R. et al.,
J. Cell. Biol. (2010)

Electron-dense labeling

NPC substrate localization
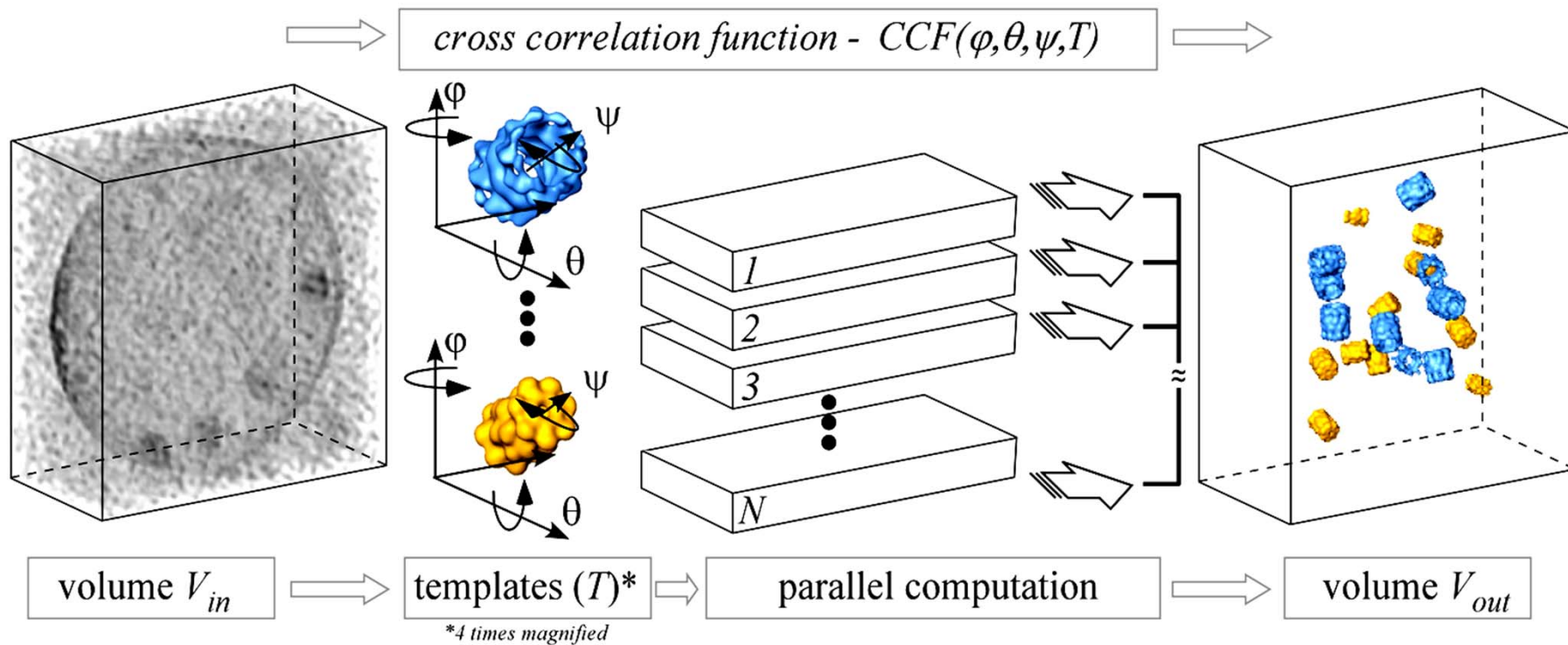Beck, M., et al., *Nature* (2007)

Template Matching


Boehm, J. et al. *Proc Natl Acad Sci* U S A. (2000)

Detection and identification strategy

cross correlation function - CCF(φ,θ,ψ,T)

volume V_{in} → templates (T)* → parallel computation → volume V_{out}
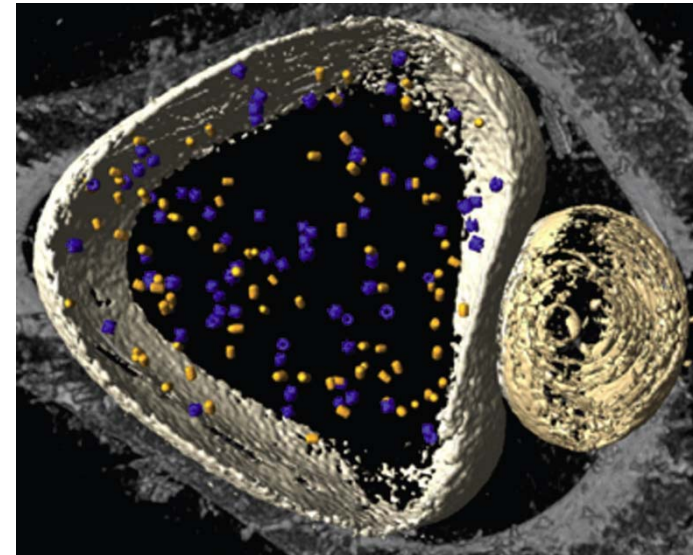
*4 times magnified

A.S. Frangakis, J. Böhm, F. Förster, S. Nickell, D. Nicastro, D. Typke, R. Hegerl, W. Baumeister:
Proc Natl Acad Sci USA 99 (2002) 14153-14158

Identification of macromolecular complexes in cryoelectron tomograms of phantom cells
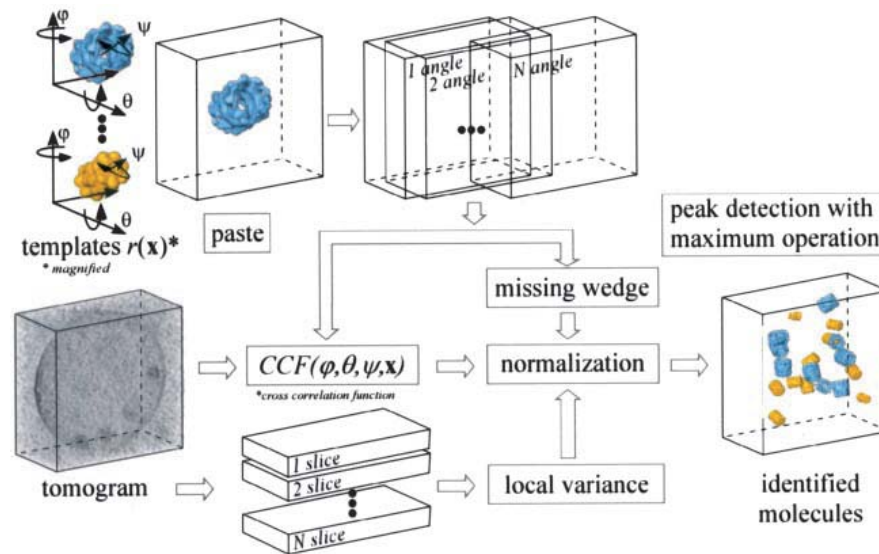
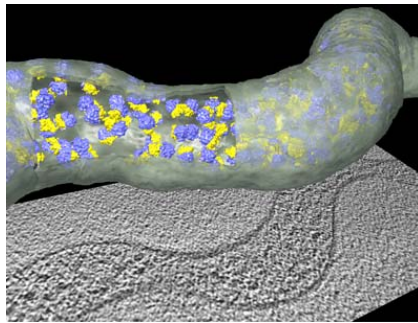Schematic flow diagram showing the detection and identification strategy

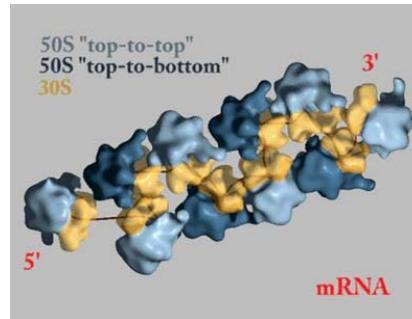Thermosomes and 20S proteasomes encapsulated in liposomes
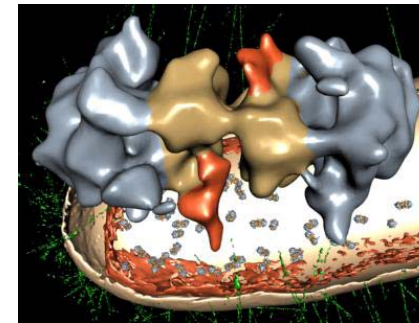
Frangakis, A. et al., *PNAS* (2002)
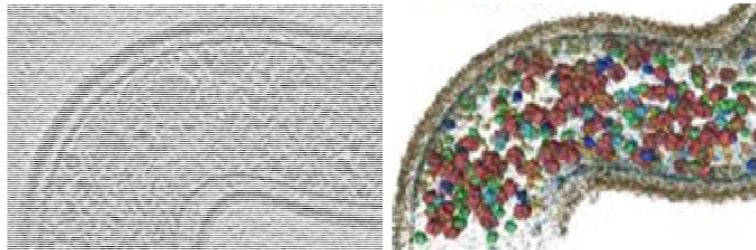
2002-2012: Template Matching Achievements

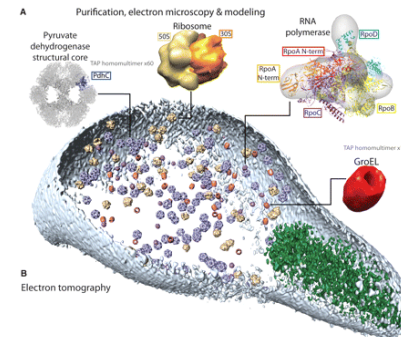*Spiroplasma*
Ortiz, J., et al. *J. Struct. Biol.* (2006)

Polysomes *in vitro*
Brandt, F., et al. *Cell.* (2009)

Hibernating ribosomes *(in vitro & in vivo)*
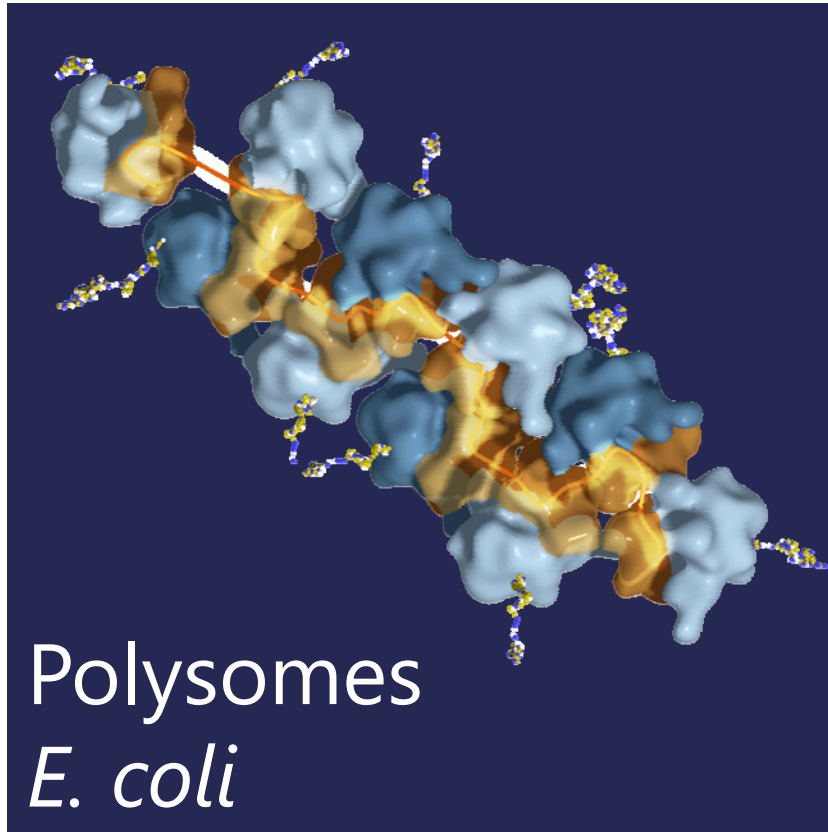Ortiz, J., et al. *J. Cell. Biol.* (2010)

Visual Proteomics - *Leptospira*
Beck, M., et al. *Nat. Methods* (2009)

Visual Proteomics - *Mycoplasma*
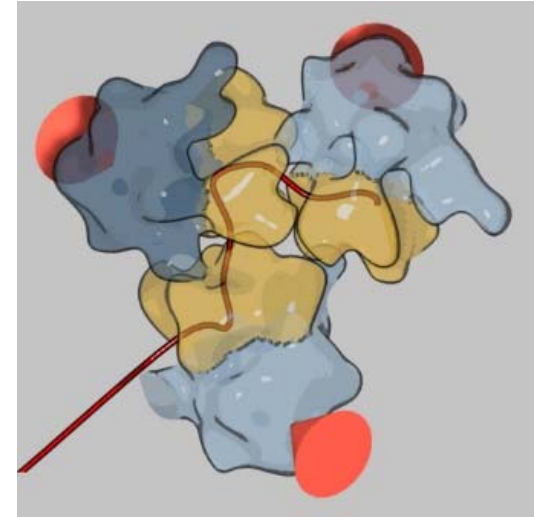Kuerner, S., et al. *Science* (2009)

Polysomes *in situ*

Polysomes
E. coli

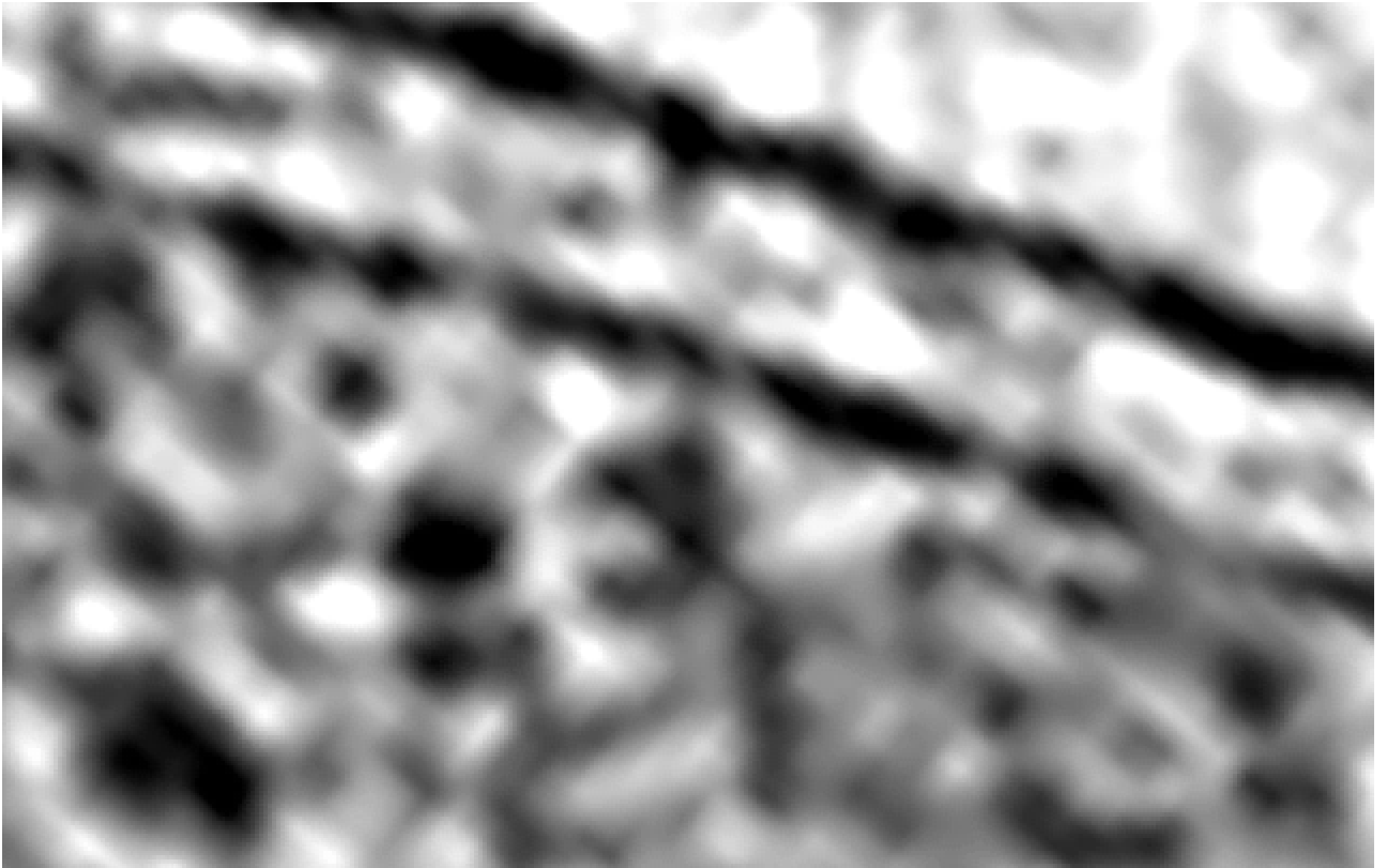Brandt, F. et al. *Cell.* 2009 **23**:261-71

E. coli

Aim:

To detect ribosomal supramolecular arrangements in the cytoplasm of fast growing *E. coli* cells.
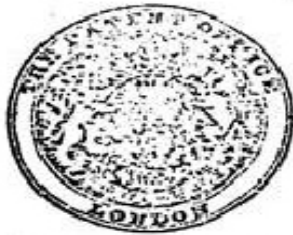
Tomogram of thin whole *E. coli* cell
(slow growing cell; CCD- camera)

Cryo-ultramicrotomy- Diamond Knife

PATENT SPECIFICATION 799,498

Date of Application and filing Complete Specification: Sept. 2, 1955.
No. 10682/57.
Application made in Sweden on Sept. 4, 1954.
(Divided out of No. 799,497).
Complete Specification Published: Aug. 6, 1958.

Index at acceptance:—Class 60, D1(D4X: H17), D2(A15: A20: K).
International Classification:—B24b.

COMPLETE SPECIFICATION

Improvements in or relating to a method of Polishing a Cutting Edge of a Diamond for a Cutting Tool

I, HUMBERTO FERNÁNDEZ-MORÁN, of Instituto Venezolano de Neurologia e Investigaciones Cerebrales, Ministerio de Sanidad, Caracas, Venezuela, a National of Venezuela, do hereby declare the invention, for which I pray that a patent may be granted to me, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The advantageous effect of the grain size chosen has been found in practice and may perhaps be explained as follows:—

By using a paste comprising diamond powder of a grain size between 0.002 and 0.005 micron, the frictional effects on the diamond being polished are such as to produce a high temperature having a burning-off effect on surface irregularities, rather than a wearing-
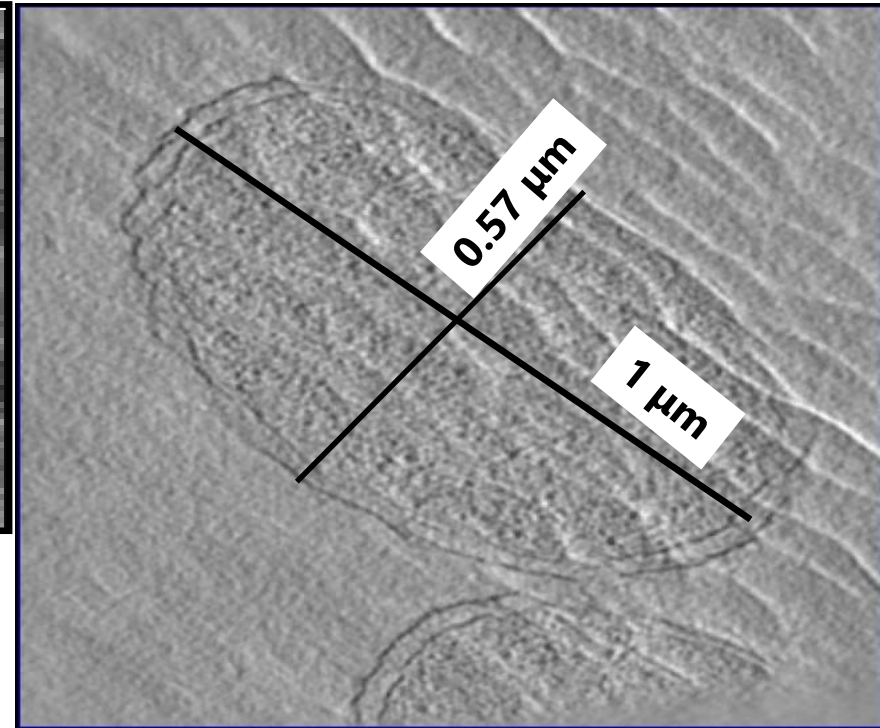
Cryo-ultramicrotomy

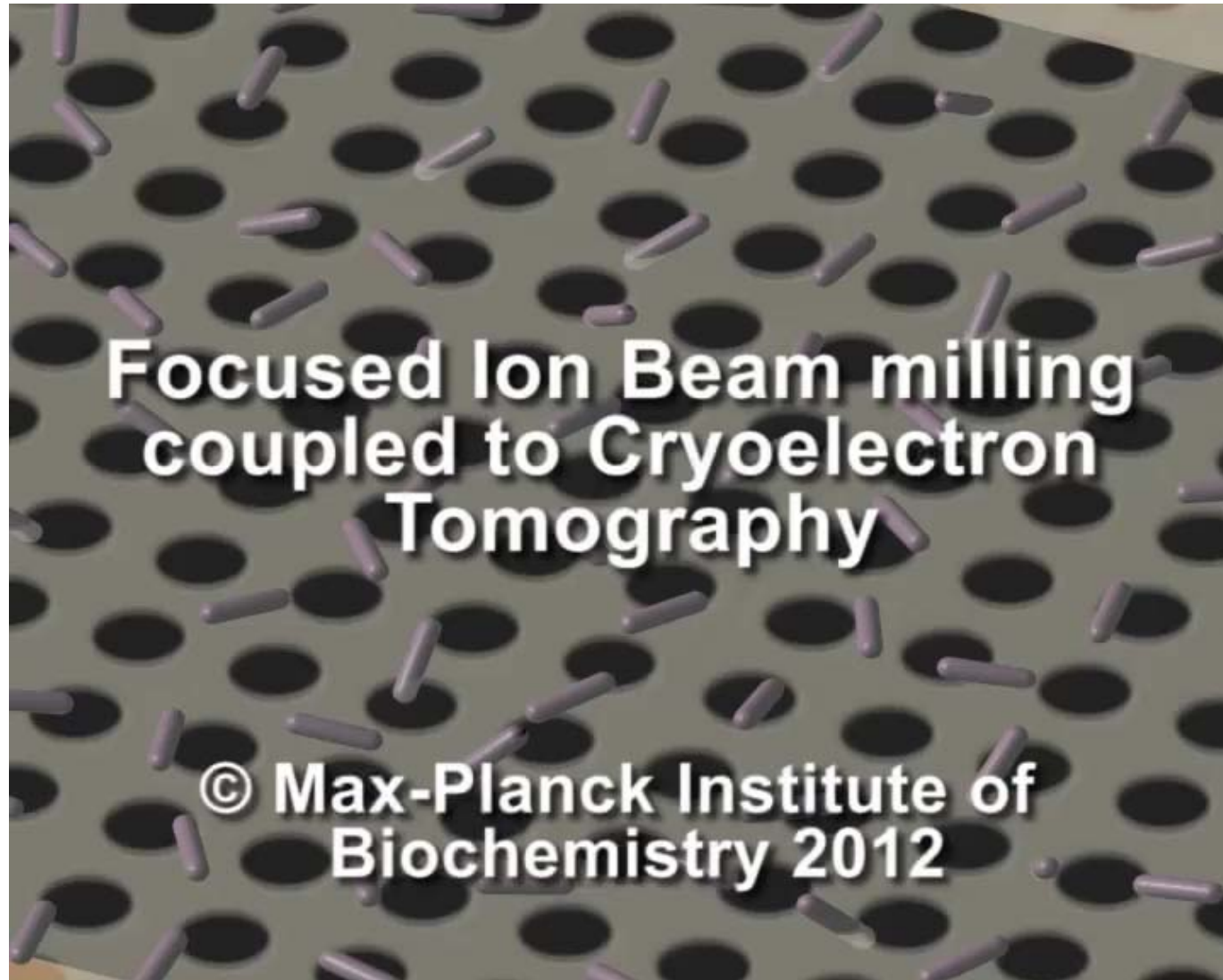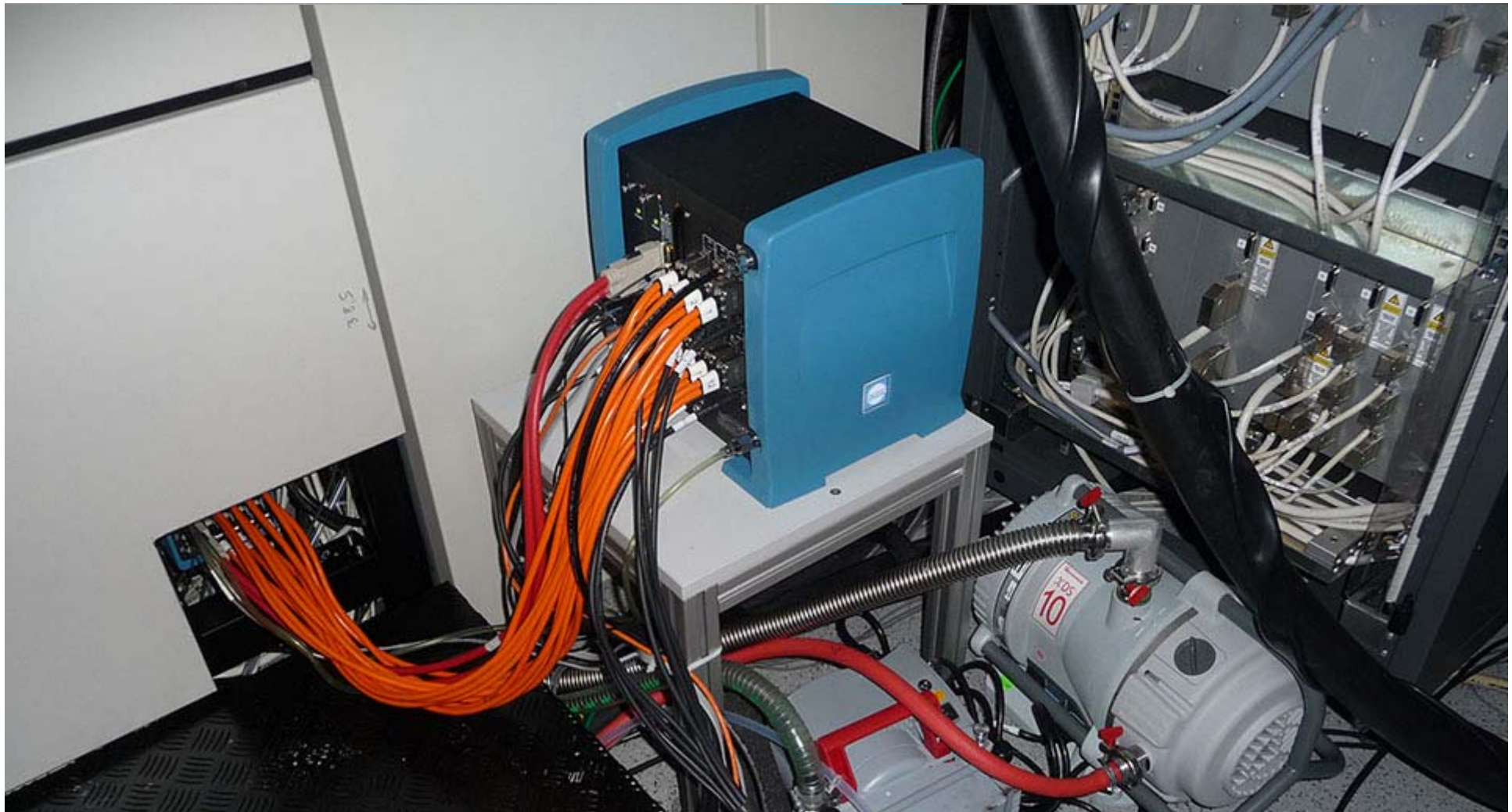## Cryo-section
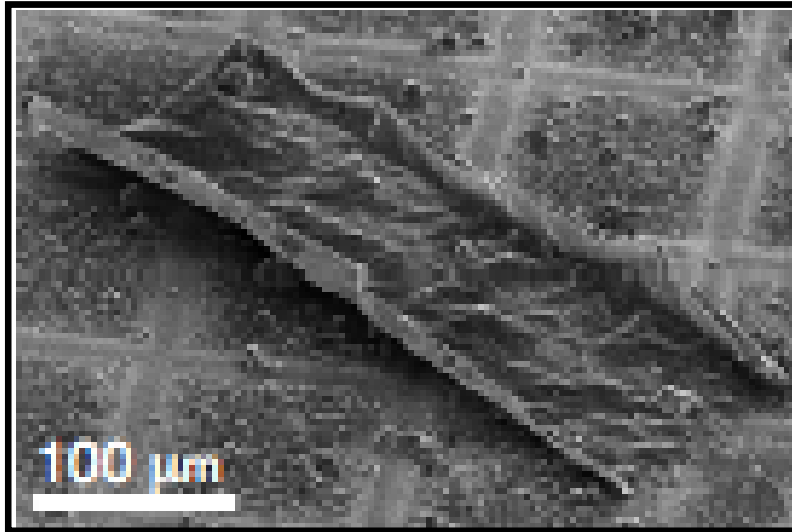
E. coli cell

NAD filtered slice from tomogram
~ 30 % compression

Polara 300 KV, Mag: 55 KX, Pixel size: 0.55 nm/pixel, Def: -14 nm, Thickness: ~100 nm

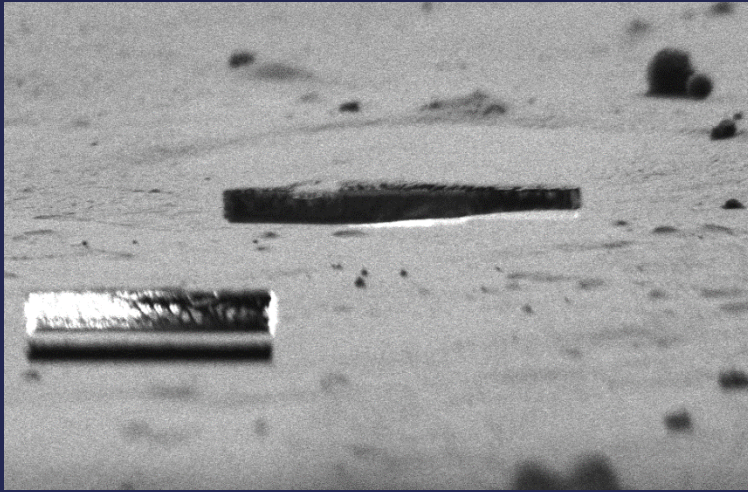# Focused Ion Beam milling coupled to CET
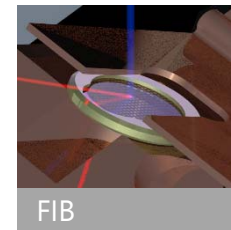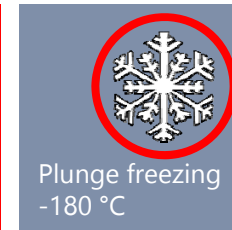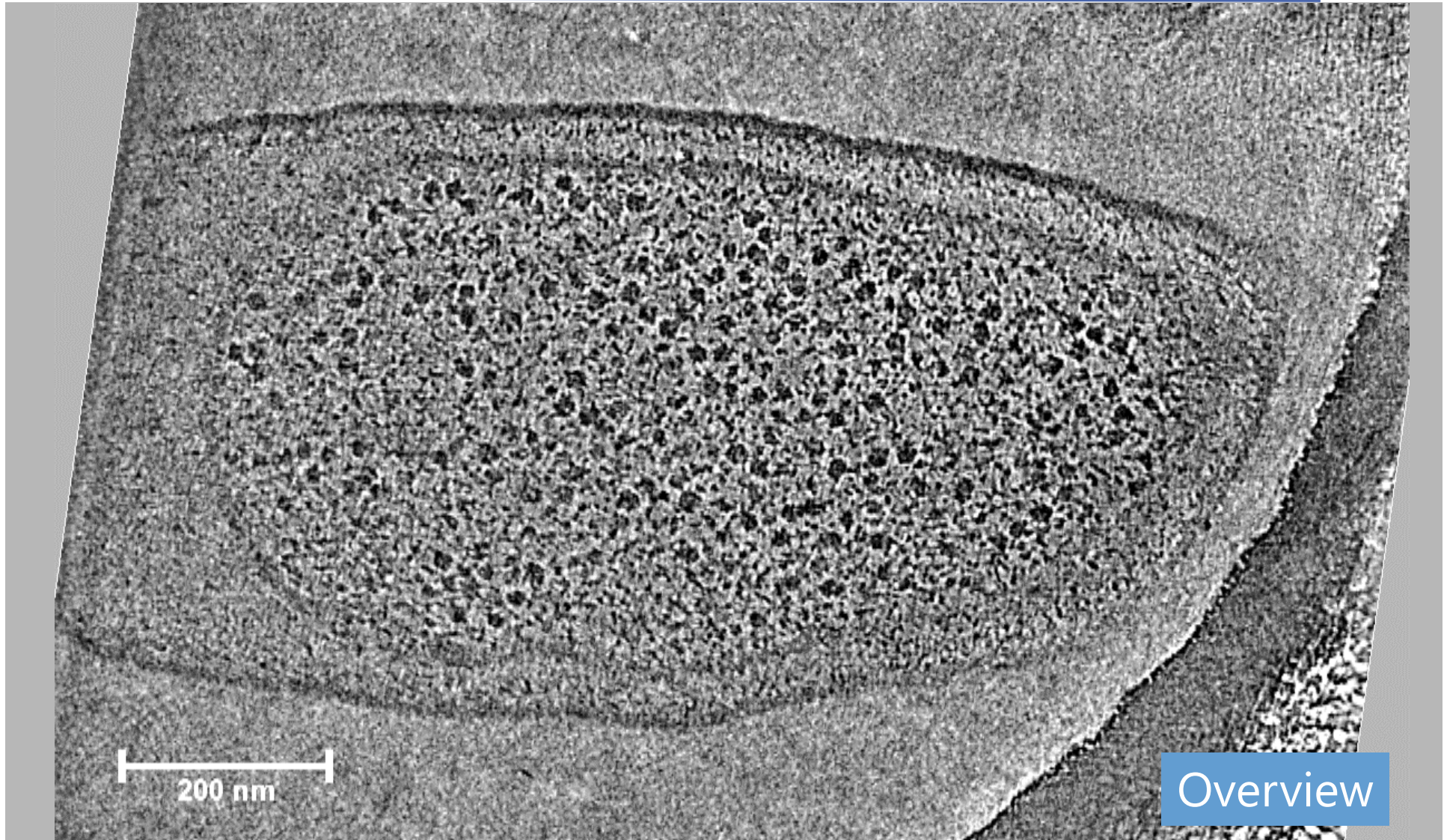
Advanced Transmission Electron Microscopy
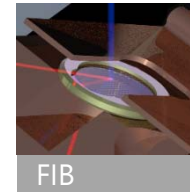
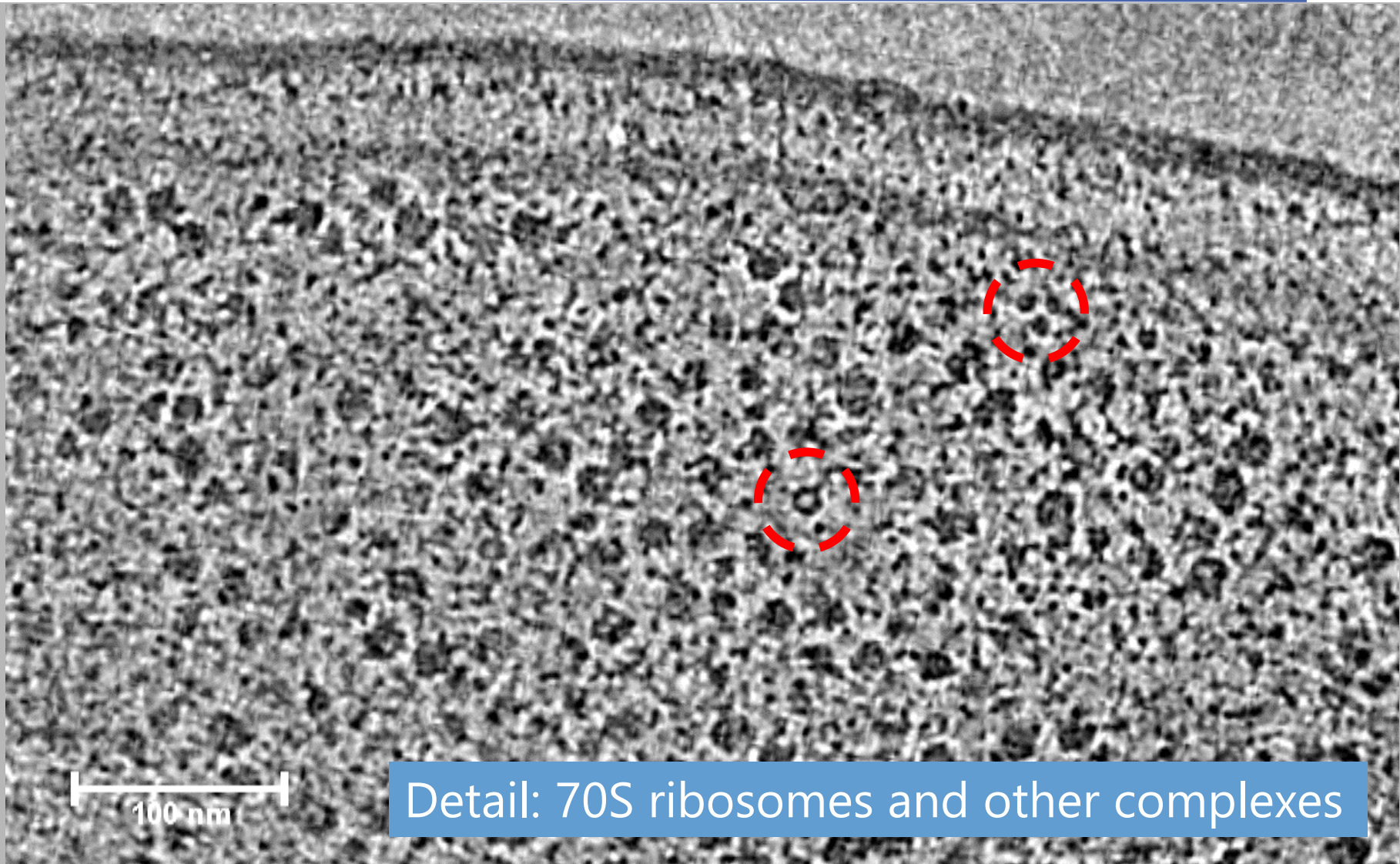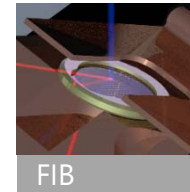Dual-tilt axis CET with K2 camera and Energy filter

Polysomes *in situ*

Polysomes
E. coli

In situ

Plunge freezing
−180 °C

FIB

200 nm

Overview

Detail: 70S ribosomes and other complexes

100 nm

Methods

Lysates or Intact cells

Cryoelectron Tomography (CET)

Template Matching

70S

Positions
(x,y,z)
Orientation
(φ,θ,ψ)
for putative
ribosomes

Classification

3D averaging

Maximum-Likelihood

Constrained Correlation

Relative Orientation
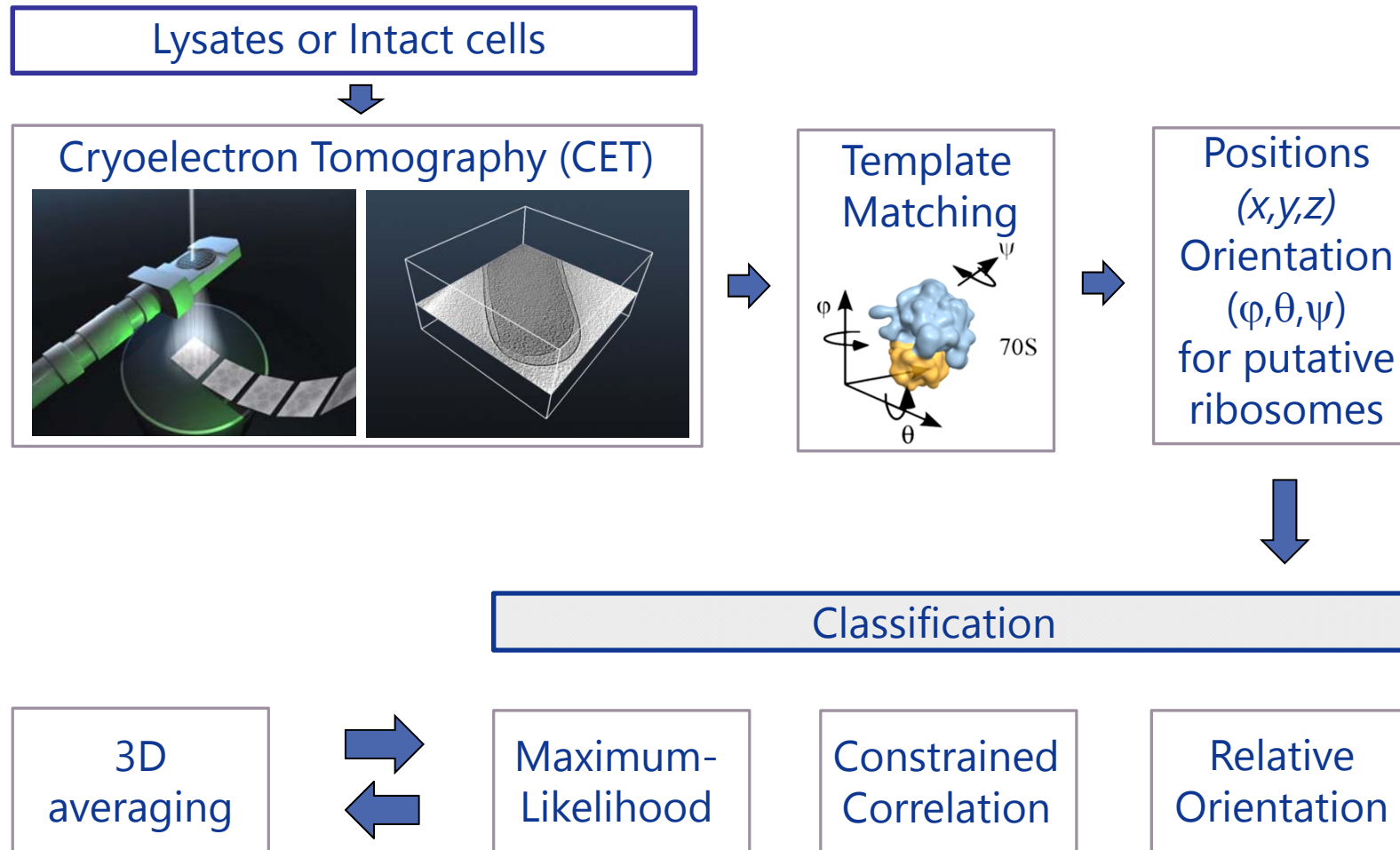
The art of averaging: Missing Wedge effect
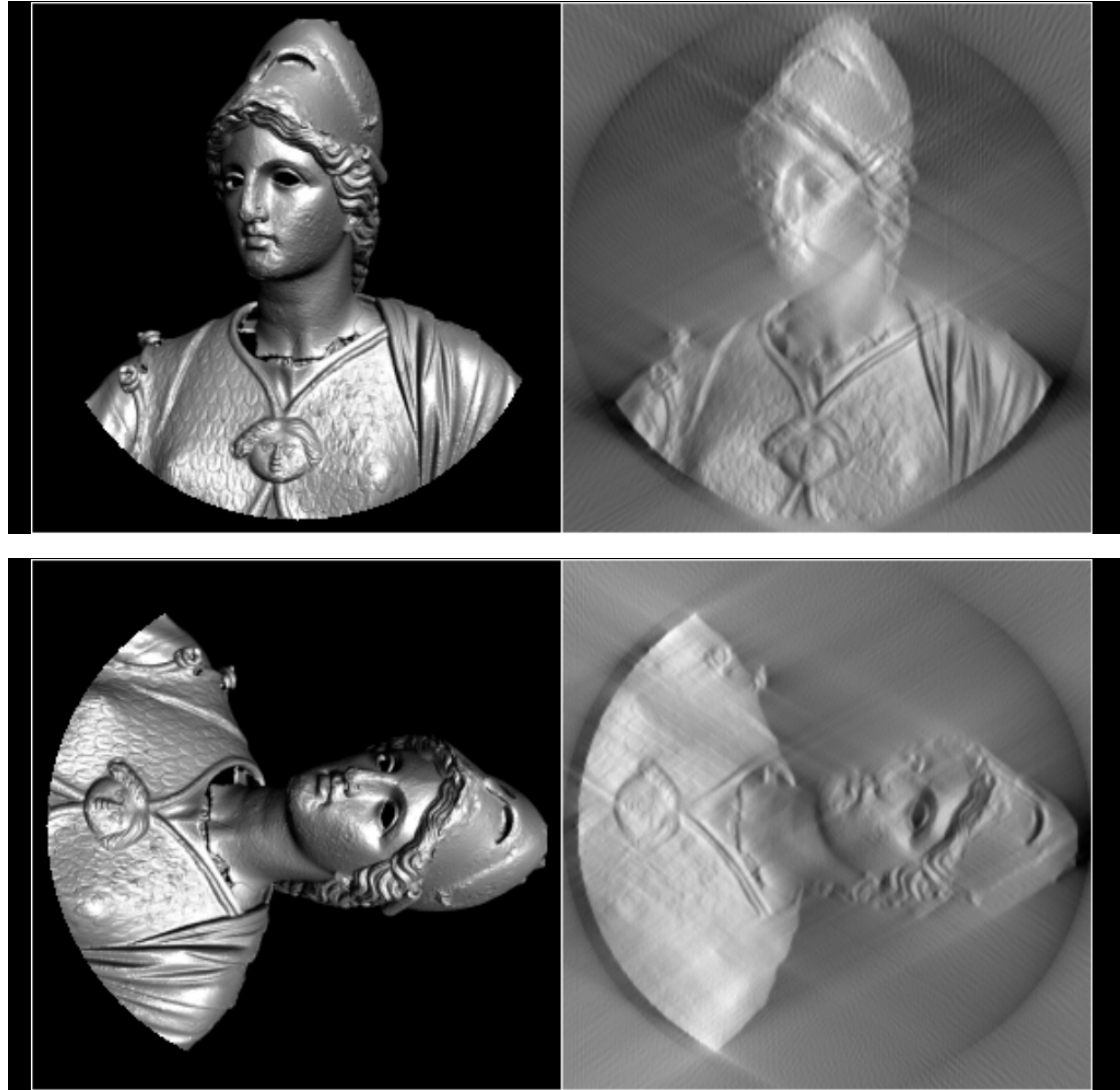
Combining reconstruction by averaging

2D Model
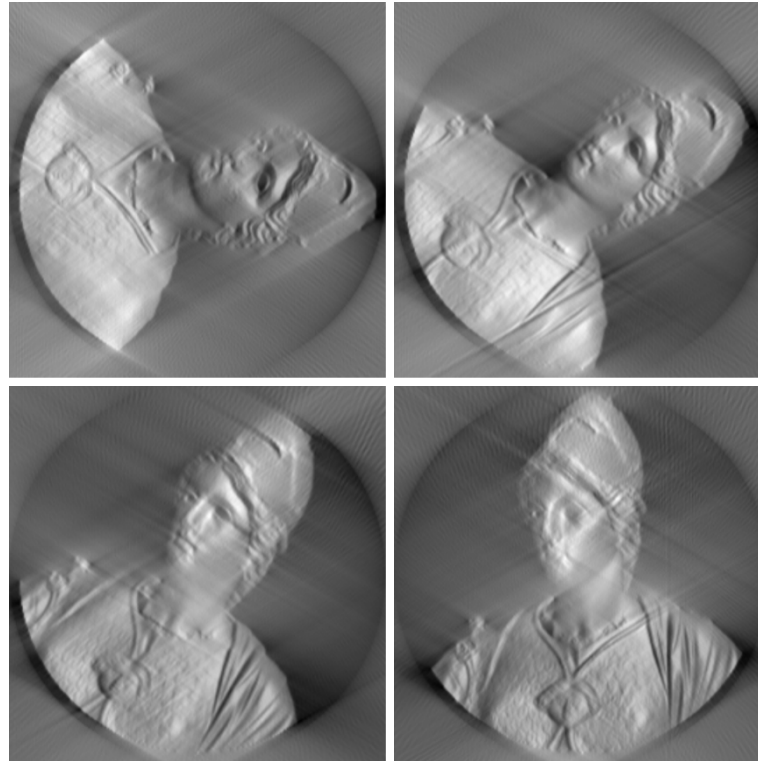
Reconstructions from identical objects
in different orientations

Combined reconstruction

The art of averaging

Combining objects in different orientations...
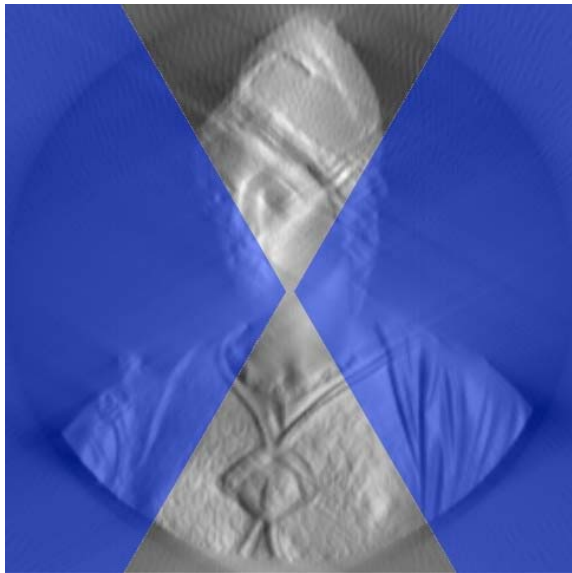
The art of averaging:
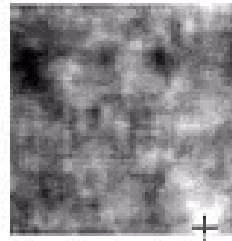# The "Mona Lisa" effect – Bias caused by reference
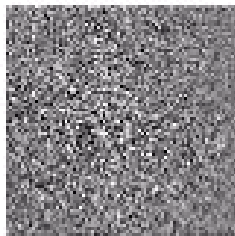

template
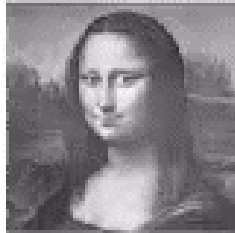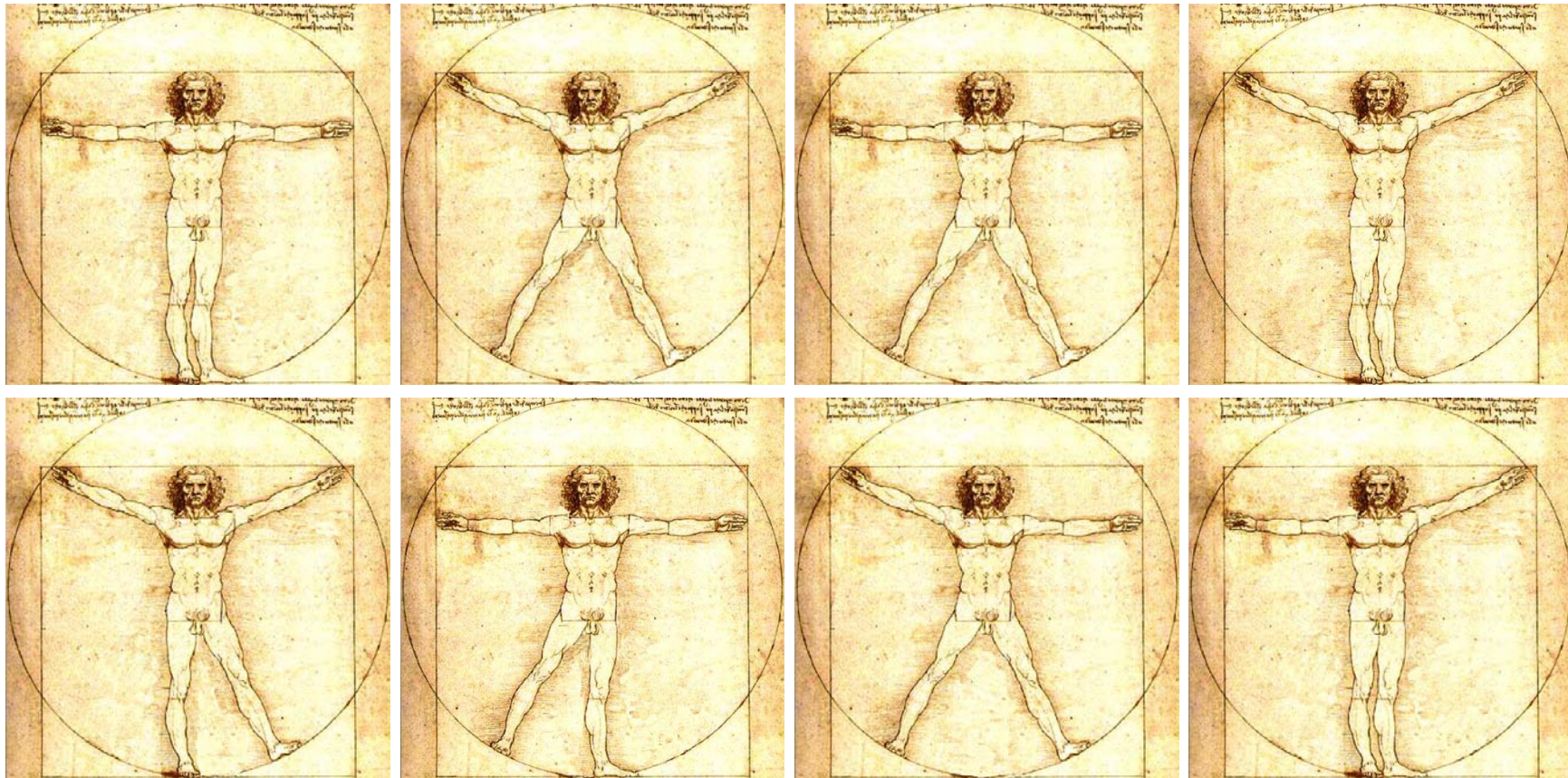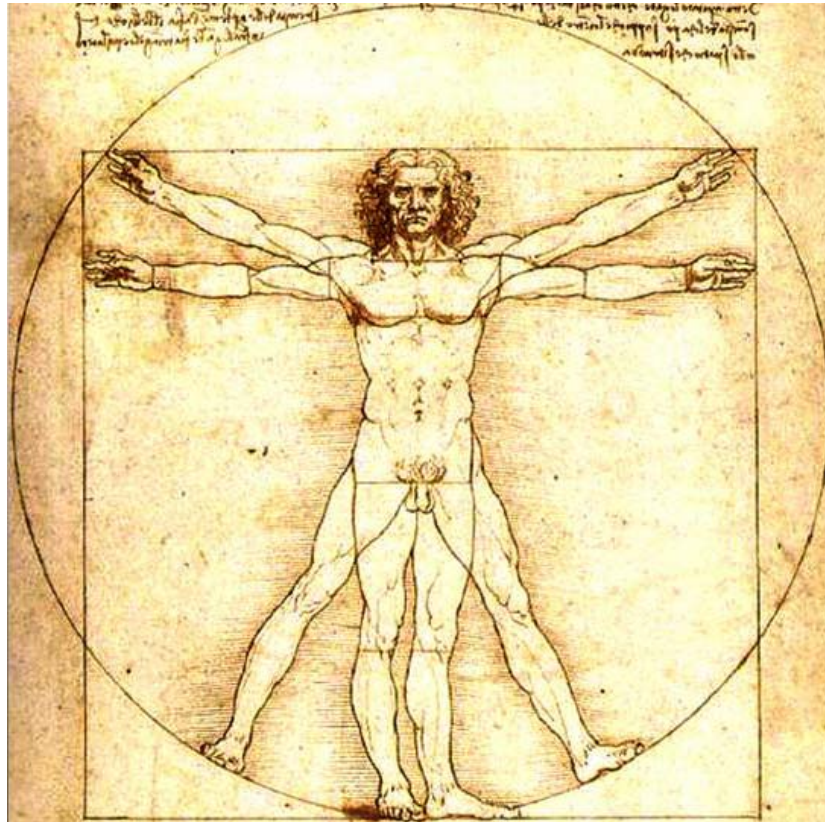
data
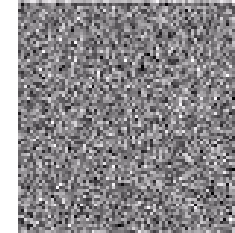
data aligned

cross correlation

aligned average

average
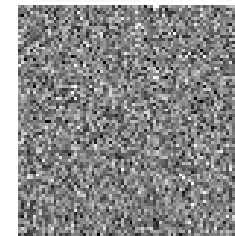
The art of averaging

The "Vitruvian man" effect – Flexibility and occupancy problems
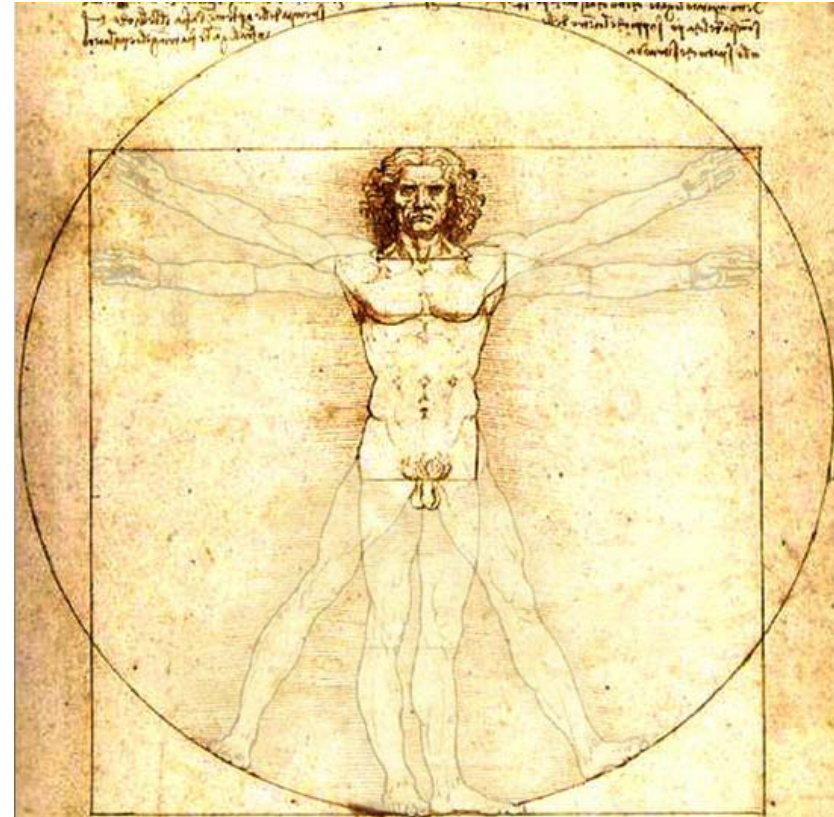
The art of averaging

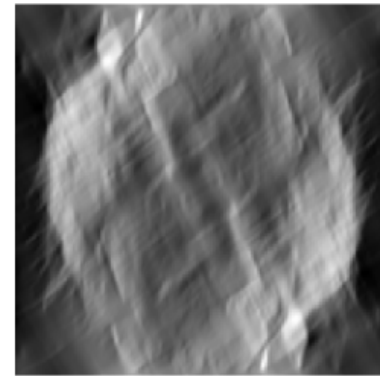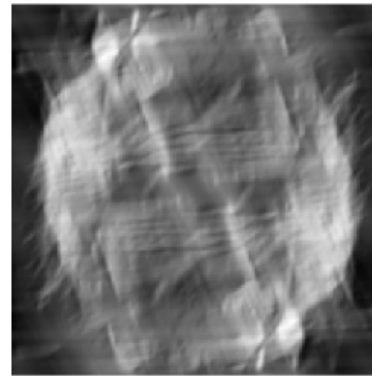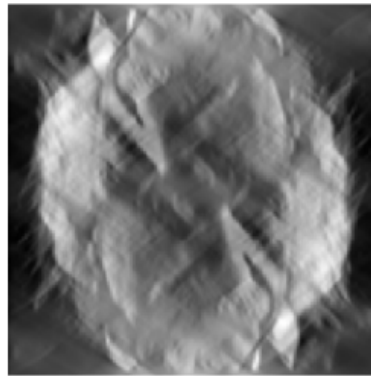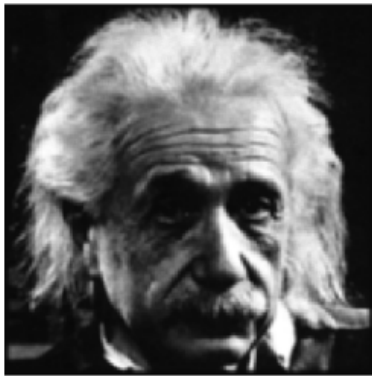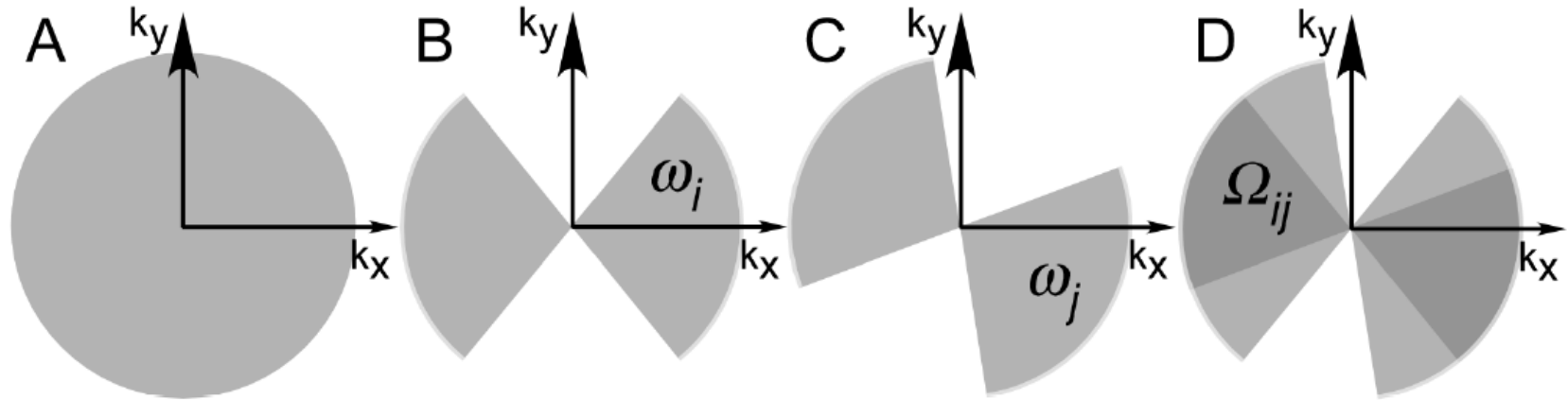The "Vitruvian man" effect – Flexibility and occupancy problems
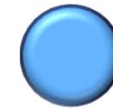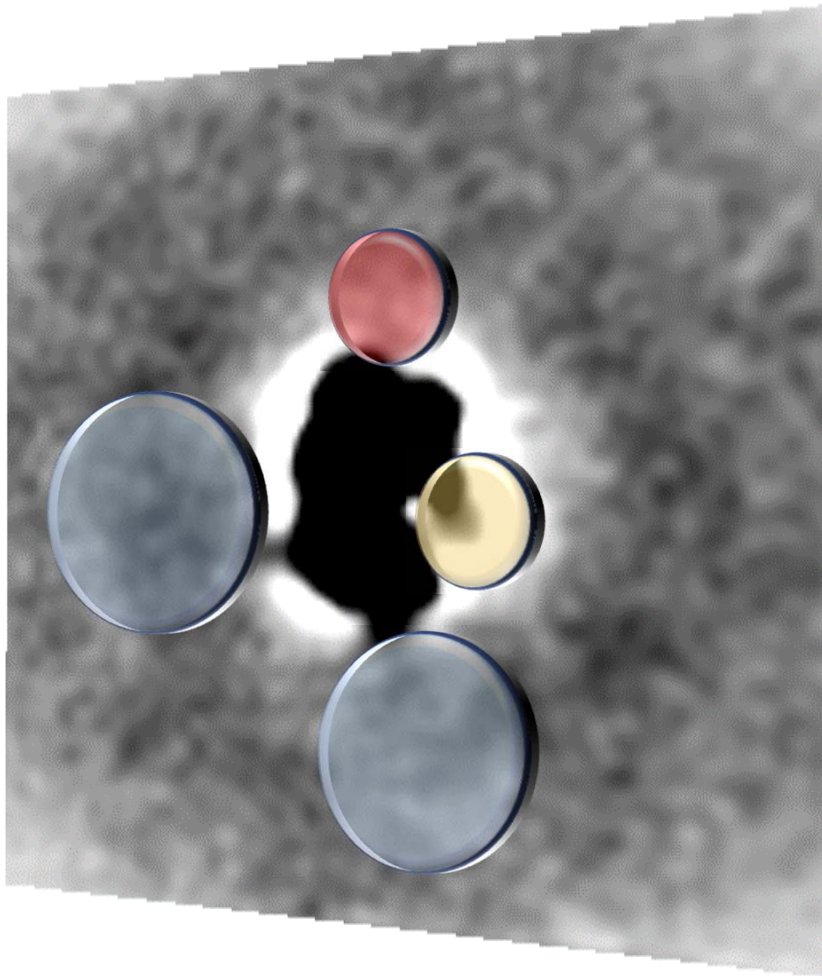

"Vitruvian man" – Leonardo Da Vinci

Average of 8 different images

Constrained Correlation Classification

Förster F., et al. (2007) J. Struct. Biol.

Constrained Correlation Classification
(3D-average selected ribosomes)

70S ribosome
Average of ~ 6000 part.
selected by
30S subunit similarity
resolution: ~2.6 nm

Areas with associated densities:
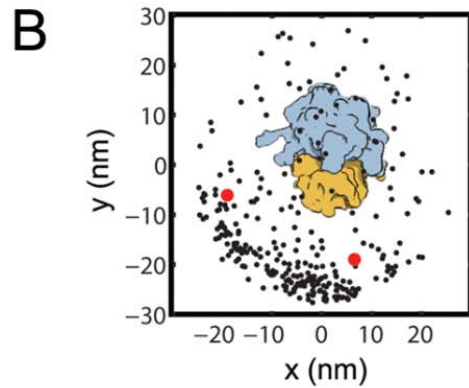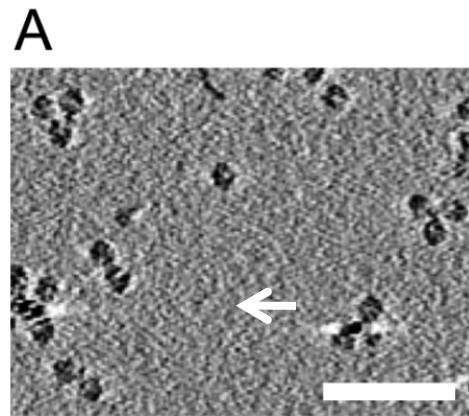
mRNA entry and
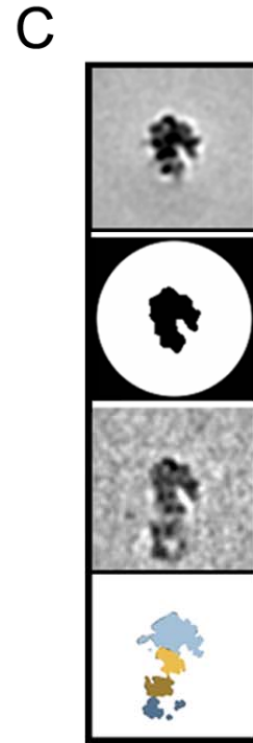exit site
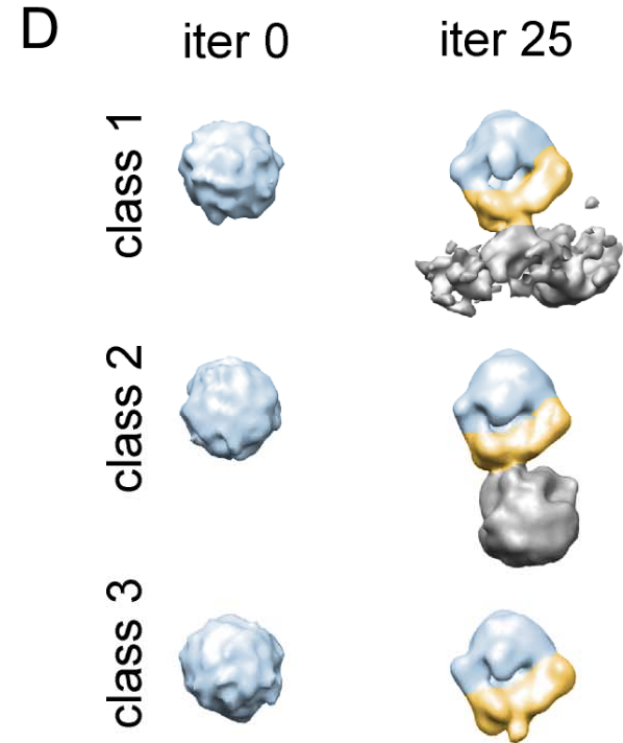
Elongation factor
binding sites

Nascent chain
exit site

Hibernating Ribosomes in Lysates from Starved *E. coli* Cells

3D Alignment and Averaging
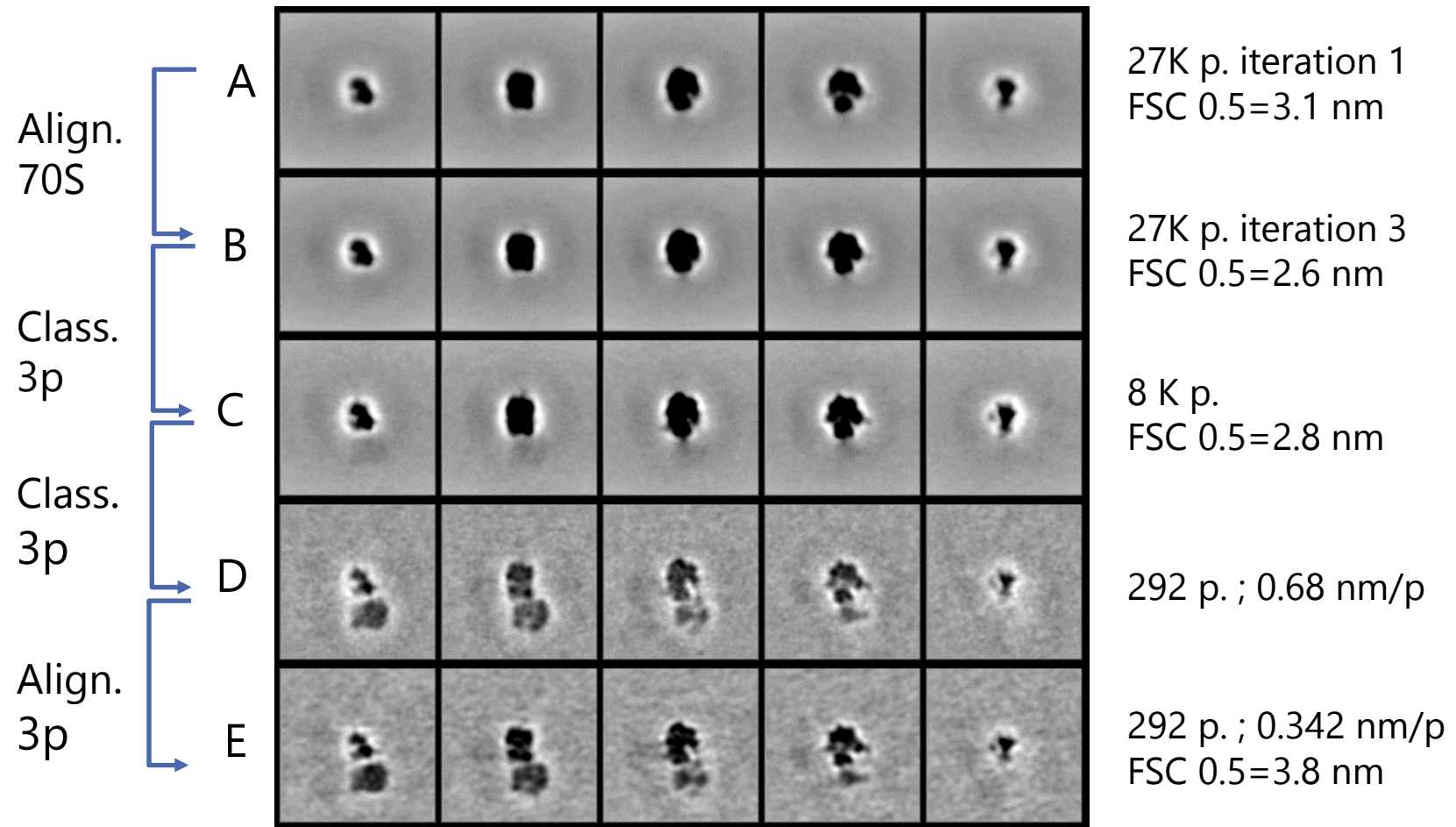

Align. 70S

Class. 3p

Class. 3p

Align. 3p

A — 27K p. iteration 1 FSC 0.5=3.1 nm

B — 27K p. iteration 3 FSC 0.5=2.6 nm

C — 8 K p. FSC 0.5=2.8 nm

D — 292 p. ; 0.68 nm/p

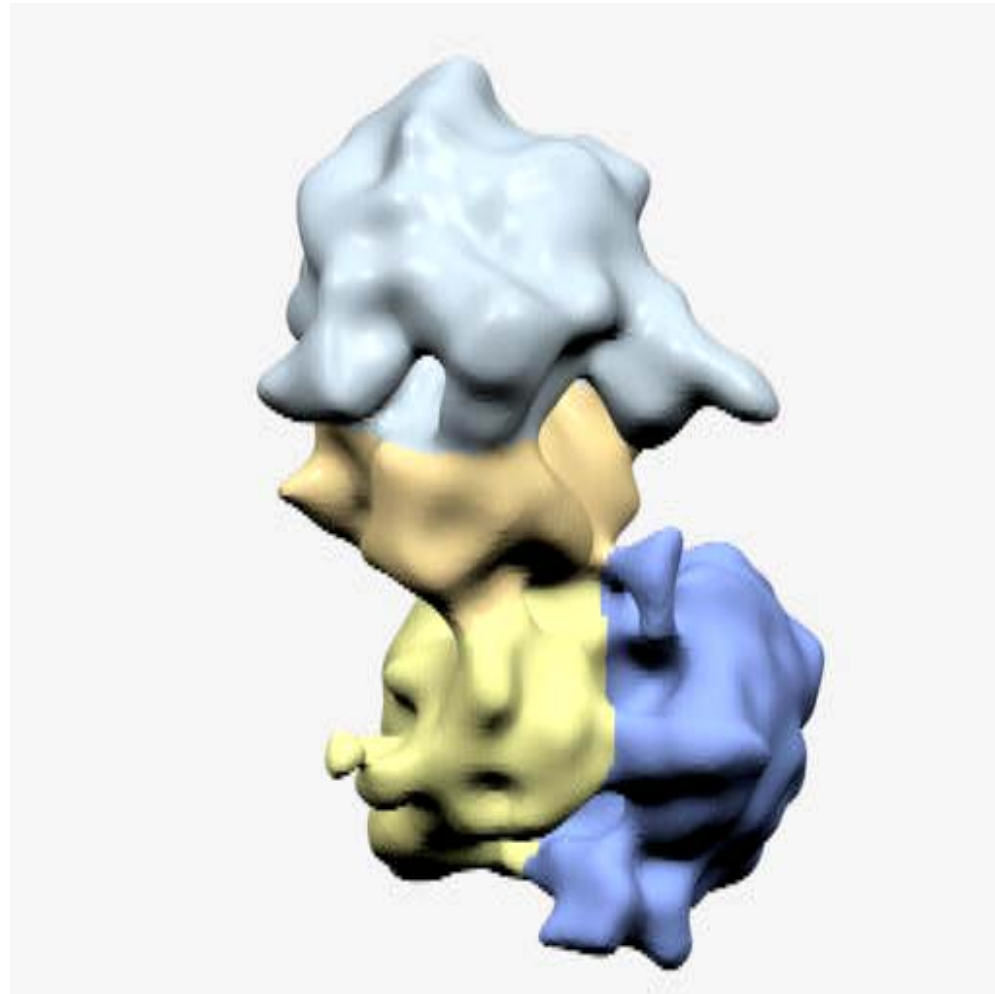E — 292 p. ; 0.342 nm/p FSC 0.5=3.8 nm

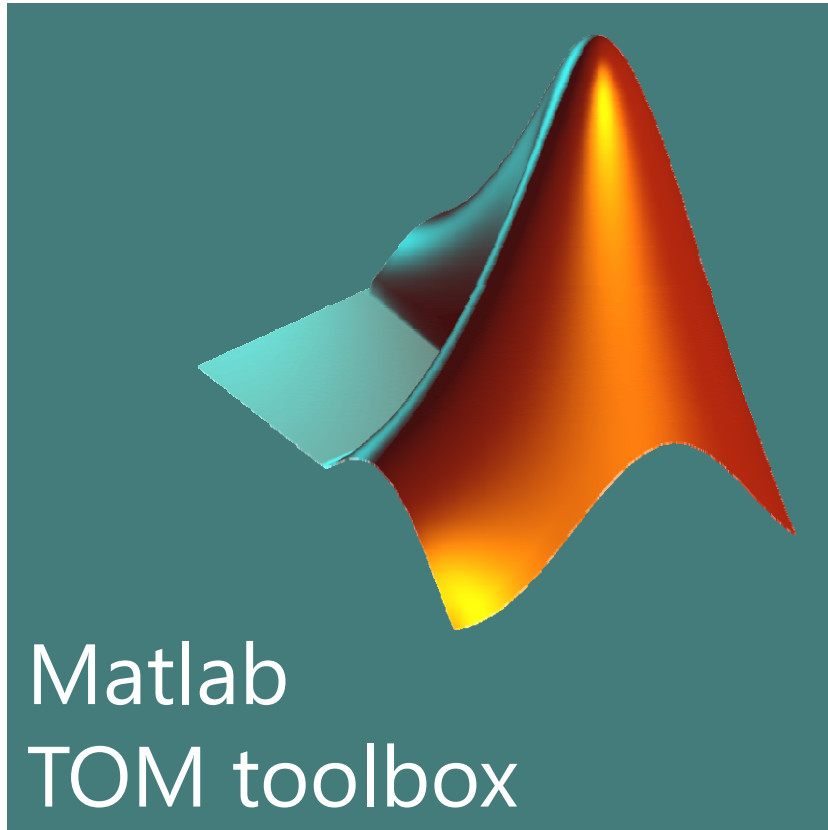3'-neighbor ribosome attached in ca. 1% of analyzed particles

Polysomal organization *in situ:* 3D-Average "top-to-top"

- 103 particles
- $(256)^3$p, 0,342 nm/p
- Average filtered at 3nm res.
- Particles present in all analyzed tomograms

□ 50S - central ribosome

□ 30S - central ribosome

□ 50S – neighbor ribosome

□ 30S – neighbor ribosome

Methods: Software

Matlab
TOM toolbox

Matlab TOM toolbox

The toolbox supports a wide range of functions for tomography that extend the capability of the MATLAB® numeric computing environment.

Aim:
Customable procedures for tomogram reconstruction and analysis.

Nickell. S. et al. (2005) *J. Struct. Biol.* **149**:227-34

Methods: Software

PyTom

PyTom

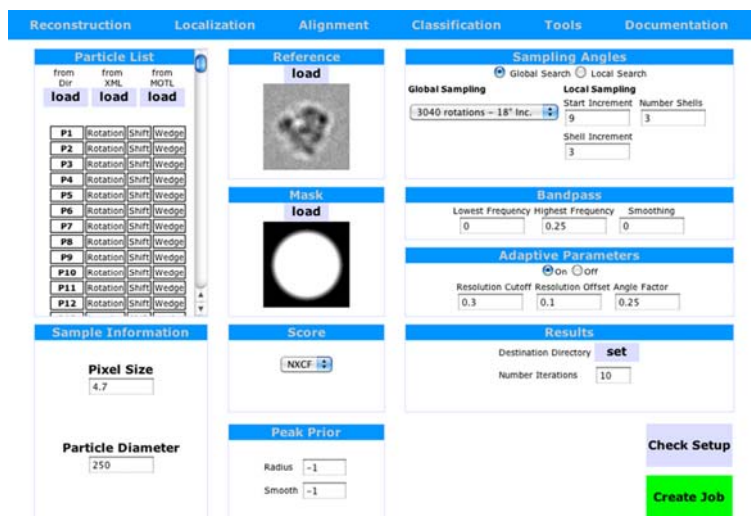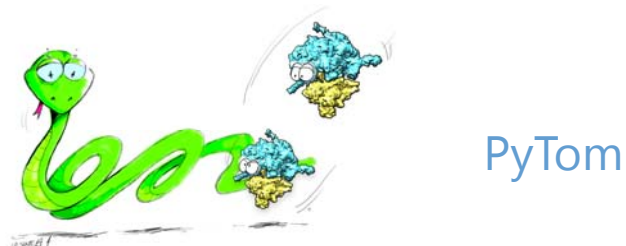Open-source platform that unifies standard tomogram processing steps in a single python-based toolbox.

Aim:
3D alignment, averaging and classification of subtomograms. Implementation of new algorithms for image processing in ECT.

Hrabe, T., et al. (2012) JSB 178: 177-188

Recent Improvements in Data Processing Workflow


PyTom

1. Reconstruction
   - Phase Flipping ('CTF correction')
   - Projection Alignment
     with Tilt-Specific Image Rotation + Magnification

2. Localization
   - Template Matching (+Support Vector Machines)
   - Parallel processing on LINUX cluster
   - Better angular sampling

3. Subtomogram Alignment
   - Parallel processing on LINUX cluster
   - Resolution-dependent lowpass: low bias
   - Resolution-dependent sampling
   - Direct reconstruction from projections

4. Classification
   - Multi-reference correlation with simulated annealing

Hrabe, T., et al. *J. Struct. Biol.* 2012

CET: Direct detection and Phase Plates

Asano, S. *et al.* (2014) *Science* 23:347

Sample: Hippocampal primary cultured neuronal cell
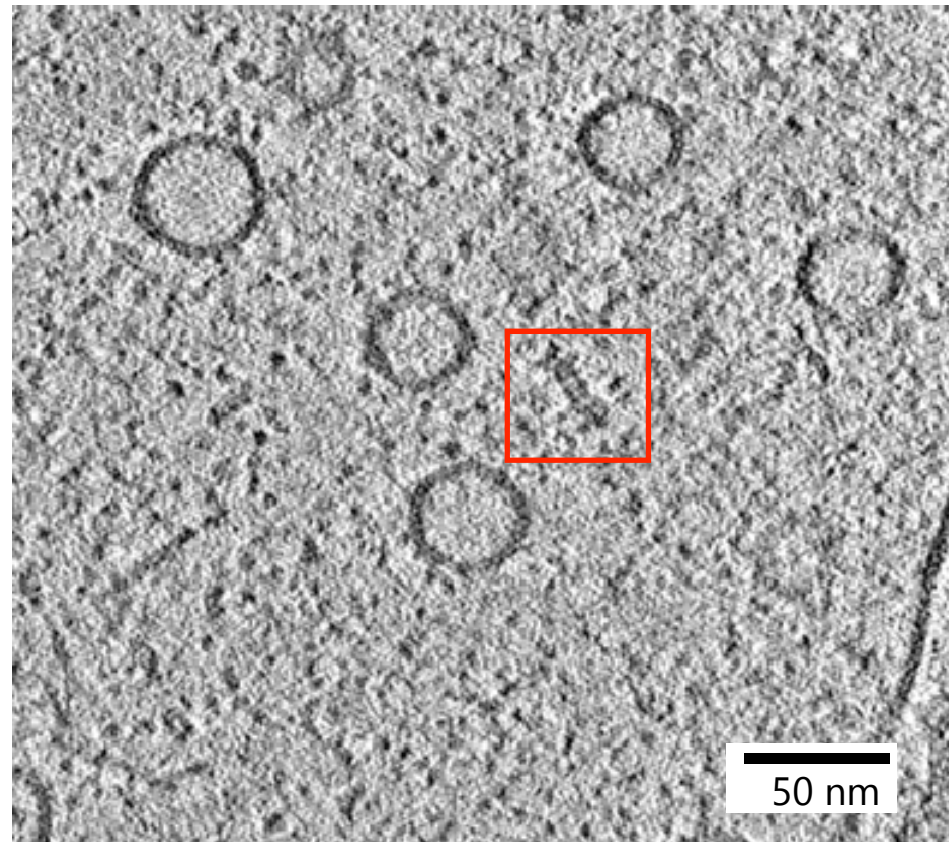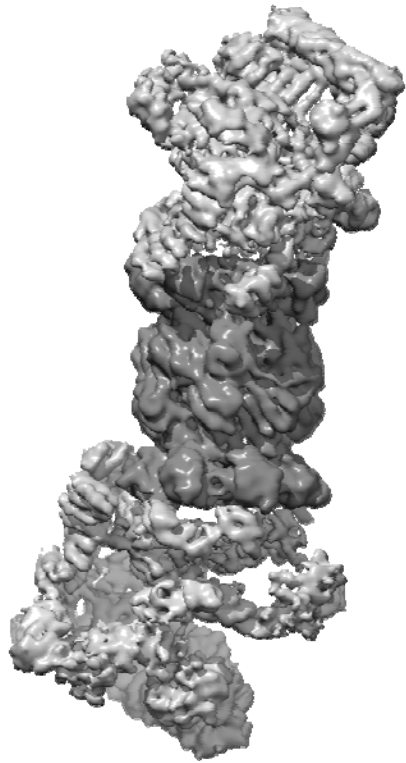
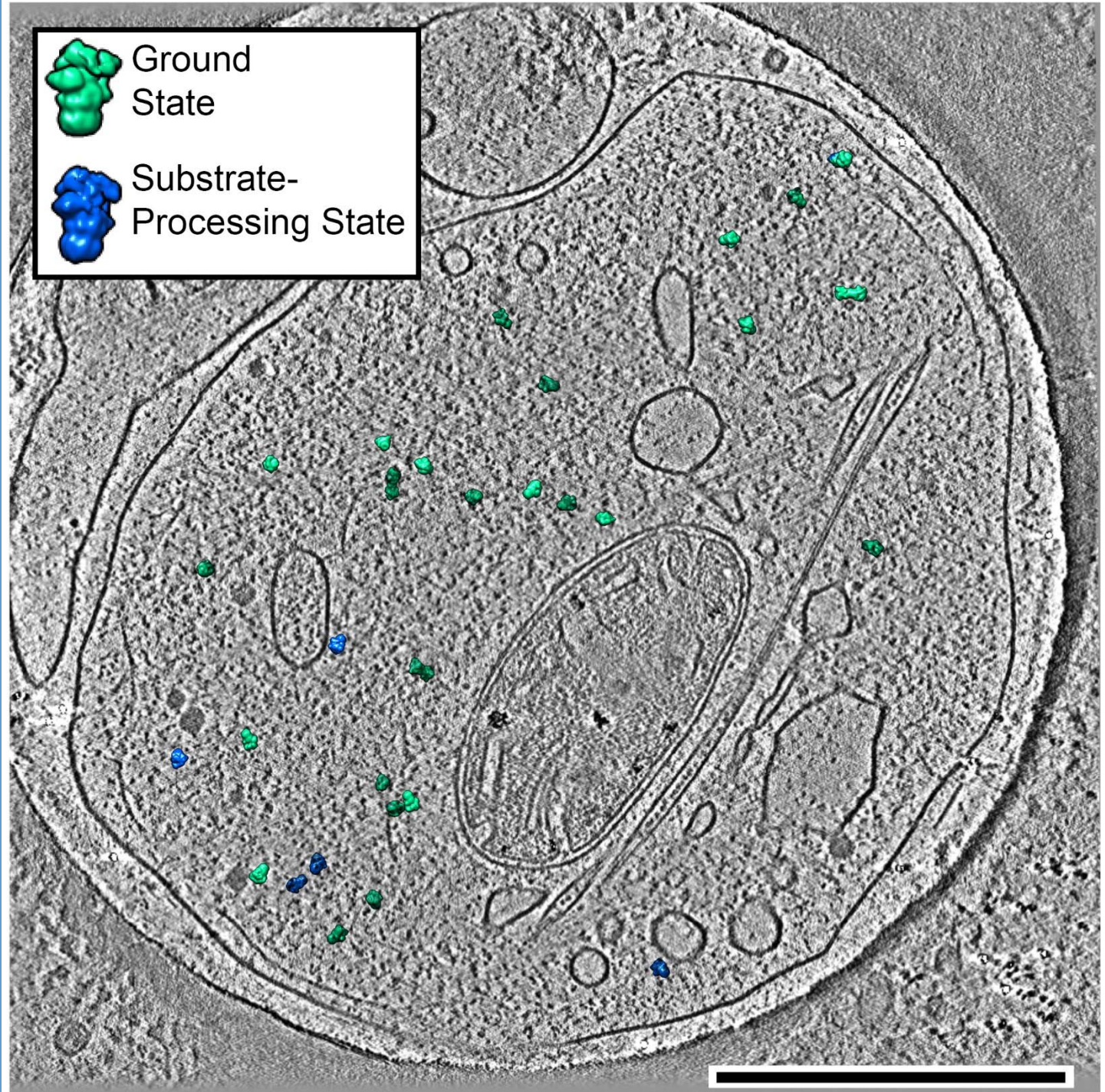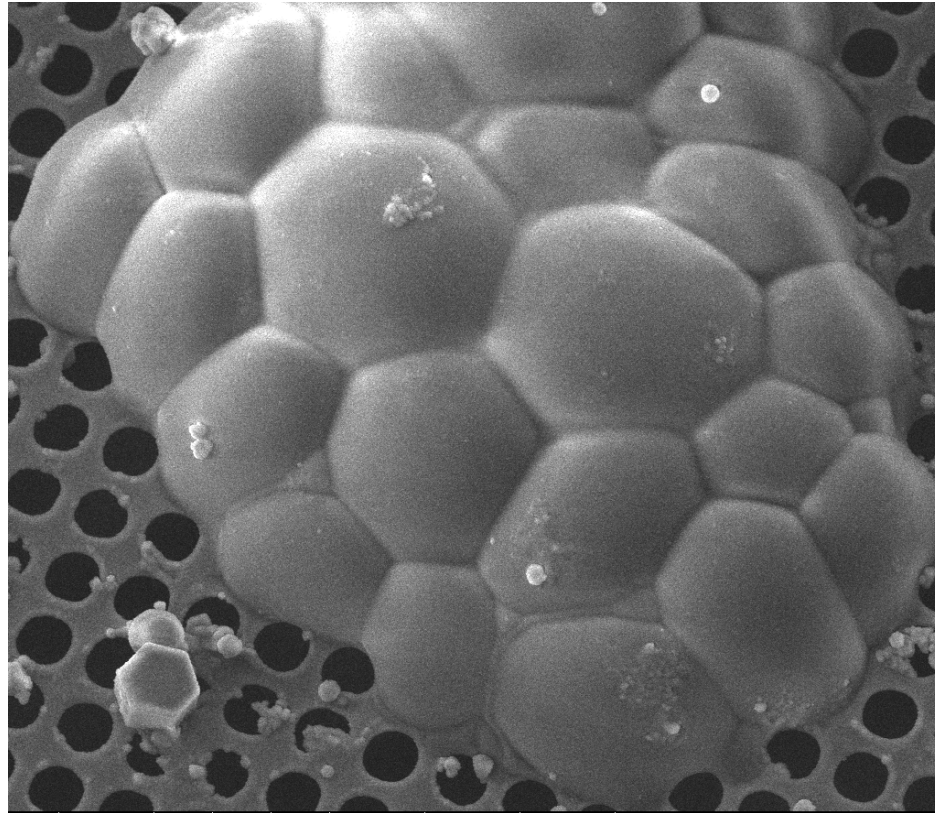26S Proteasome *in situ*!
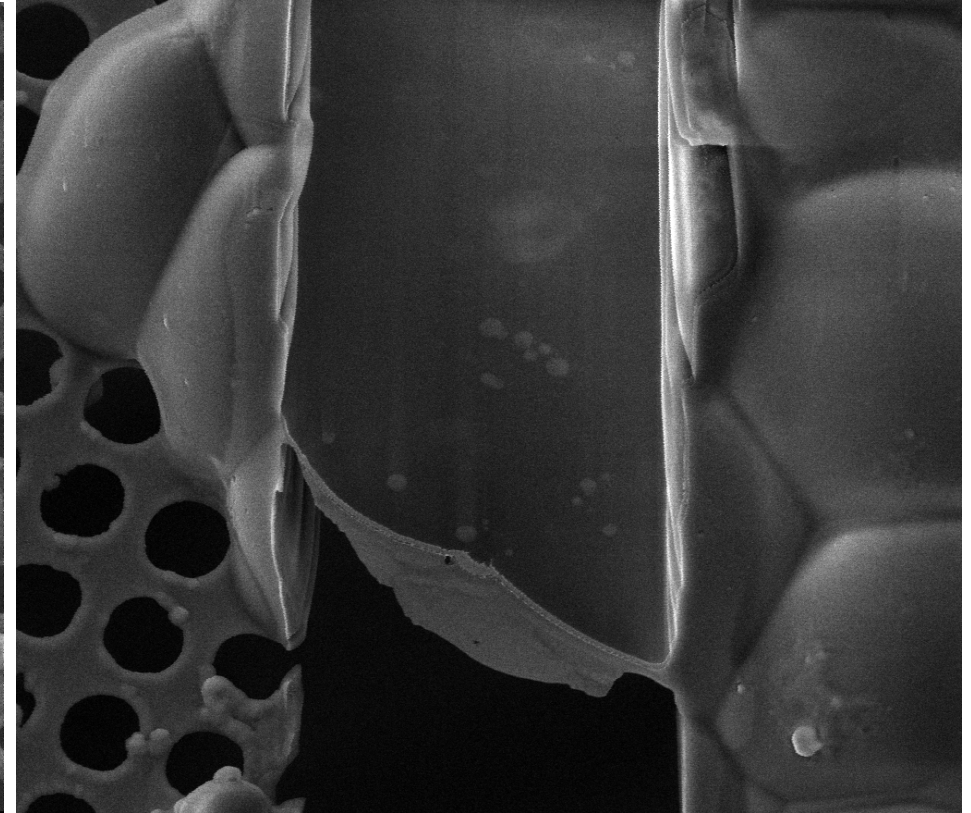
Proteasomes in neurons cytoplasm

Ground State

Substrate-Processing State

Asano, S. *et al.* (2015) *Science* 23:347

Cryo-FIB: Chlamydomonas reinhardtii

40 µm

10 µm

Engel, BD. *et al.* (2015) *Elife 13;4*

Cryo-FIB: Chlamydomonas

Chloroplast (thylakoids)
Pyrenoid (RuBisCO)
Starch
ER
Golgi
Lipid Droplets
Nucleus
Contractile Vacuoles
Centrioles
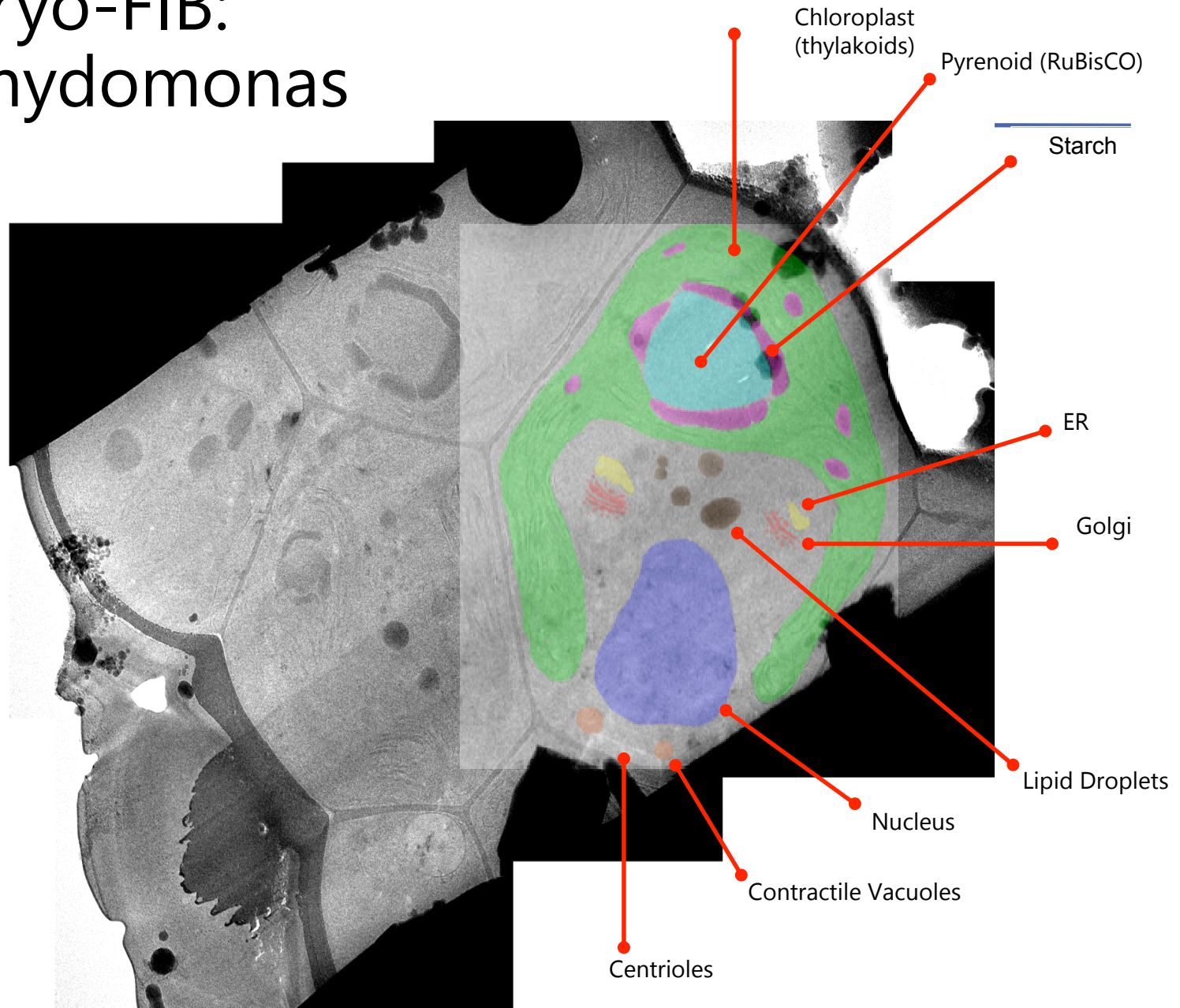
Cellular CET: 'fibbed' Chlamydomonas
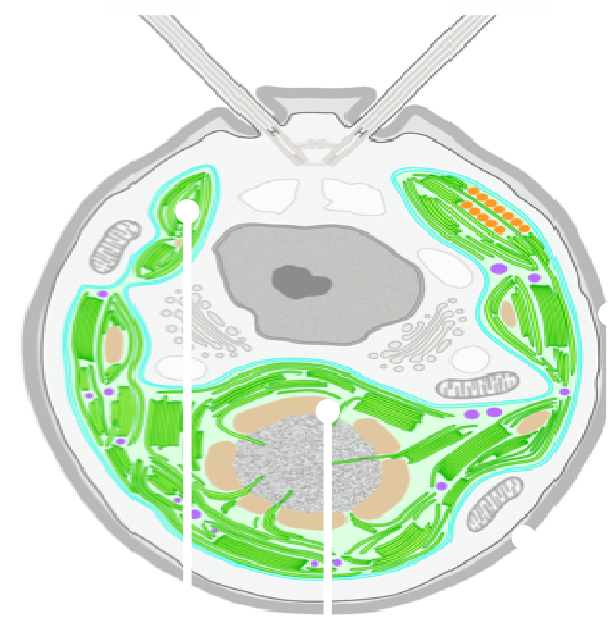
Starch

Globules

Pyrenoid

Thylakoids

Engel, BD. *et al.* (2015) *Elife 13;4*

Cryo-FIB: Chlamydomonas reinhardtii

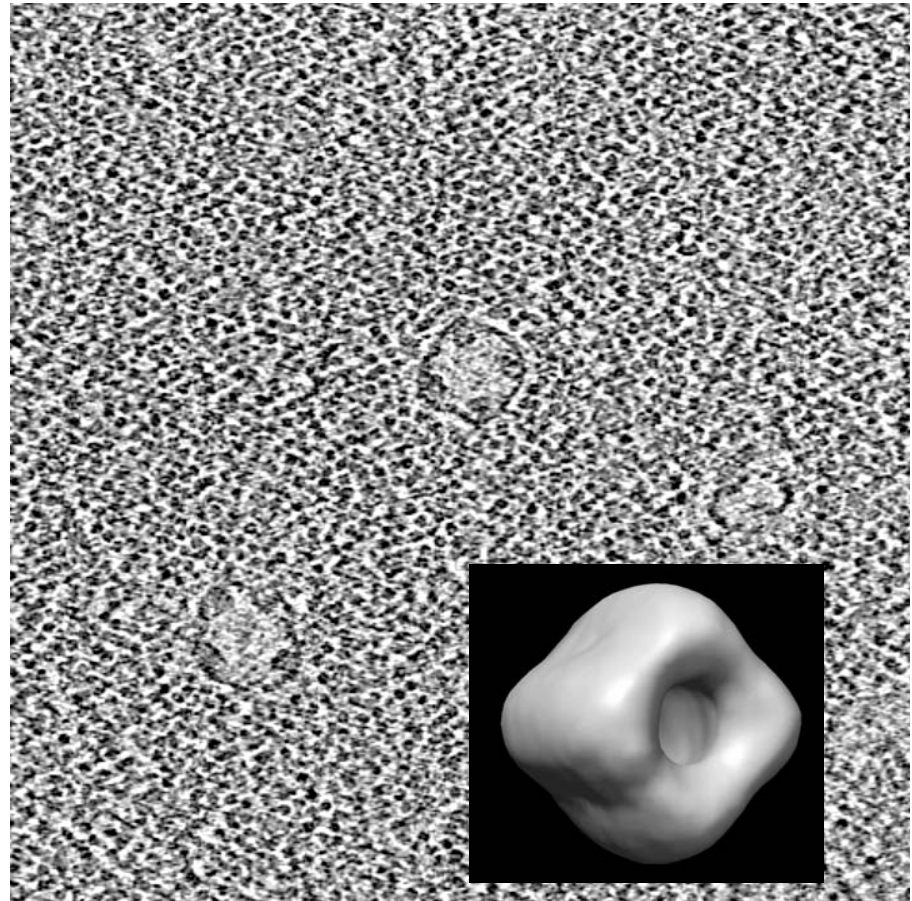Engel, BD. *et al.* (2015) *Elife 13;4*

RuBisCO

CCD

Pyrenoid

CRYO-FIB: Chlamydomonas reinhardtii

RuBisCO

CCD

Pyrenoid

K2 Summit

Robust membrane segmentation based on tensor voting

Original tomogram

Tensor voting

Filtered tomogram

Segmentation by thresholding

Volume-based labeling

Materials

Martinez-Sanchez, A., et al., (2014) *J. Struct. Biol. in press*

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Martinez-Sanchez, A., et al., (2014) *J. Struct. Biol. in press*

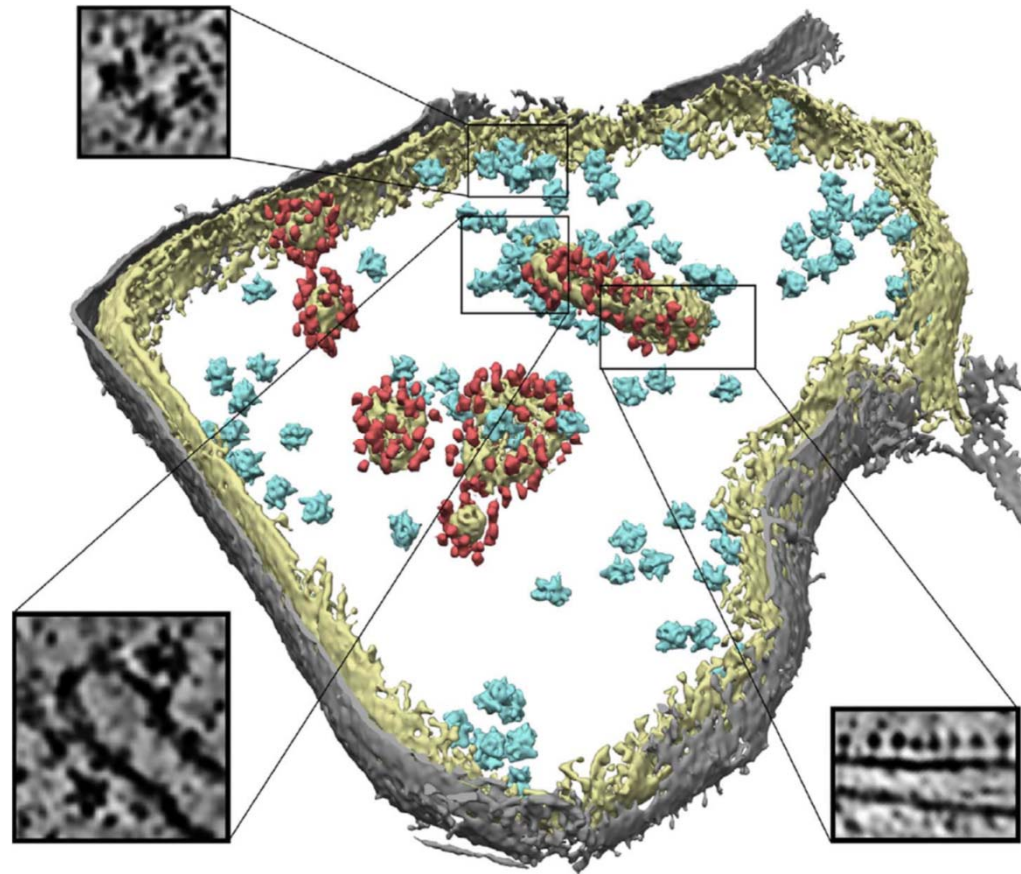# Robust membrane segmentation based on tensor voting

■ segmented outher membrane   ■ segmented inner membrane

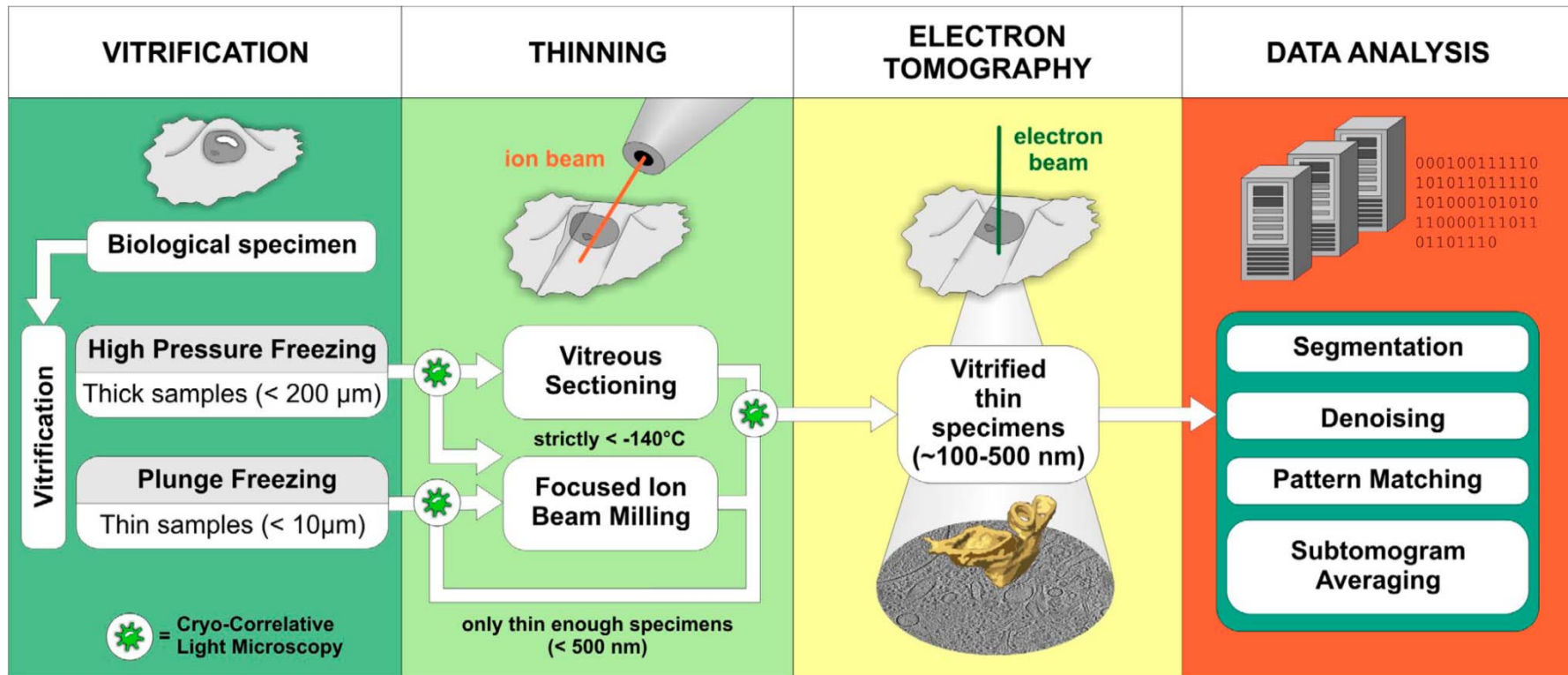# Organization of the mitochondrial translation machinery studied *in situ* by cryoelectron tomography

Distribution of mitoribosomes in translation-competent yeast mitochondria.

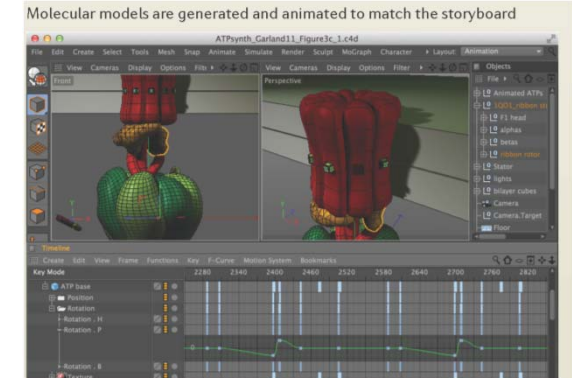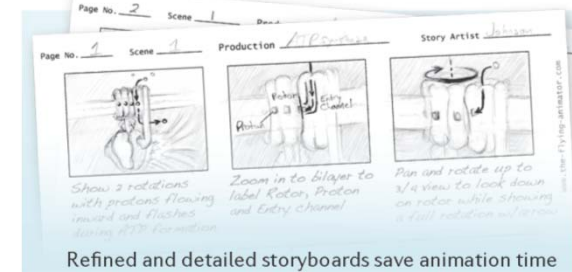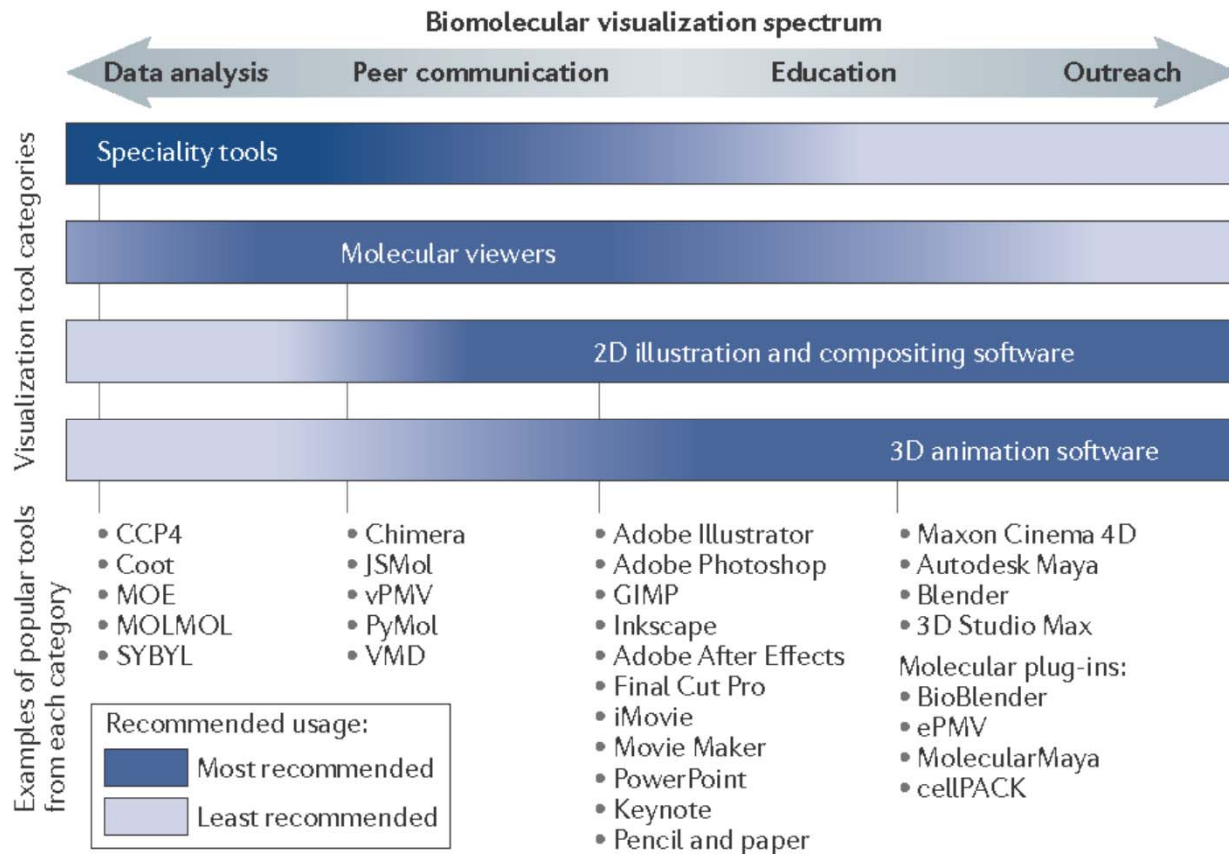Pfeffer, S. et al., (2015)
Nature Communications

Schematic representation of the cryo-ET workflow

Lučić, V. et al. *JCB* (2014)

Biomolecular Visualization Tools

Johnson,G and Hertig, S. *Nat Rev Mol Cell Biol.* (2014) 10:690-8.

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