



Max Planck Institute
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Martinsried, Germany

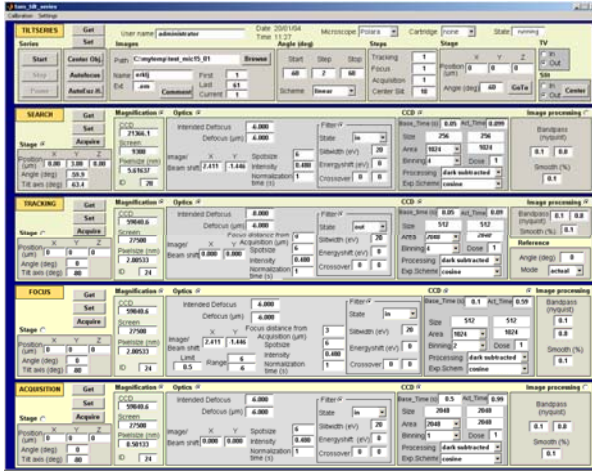


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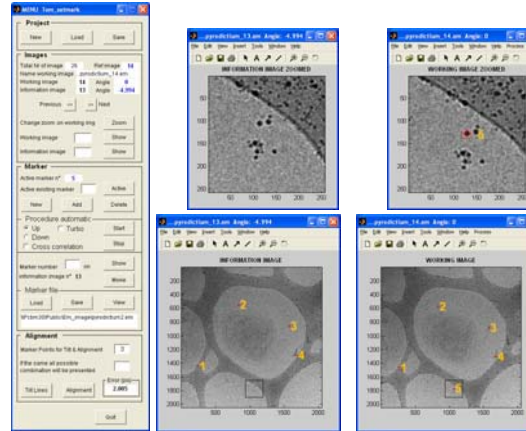
TOM
*A toolbox for Cryo-Electron
Tomography and Single Particle
Analysis*

Stefan Bohn

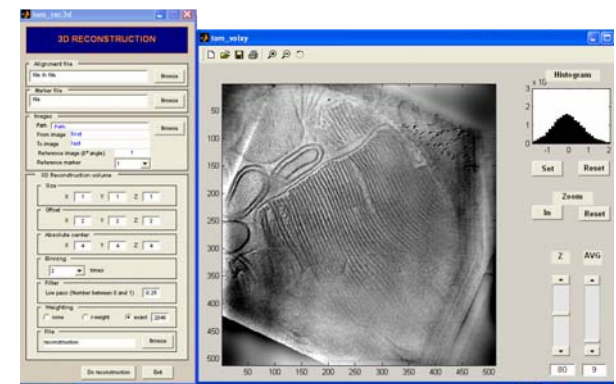
Aquisition



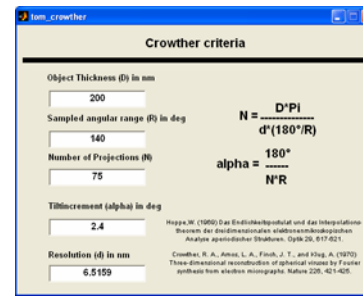
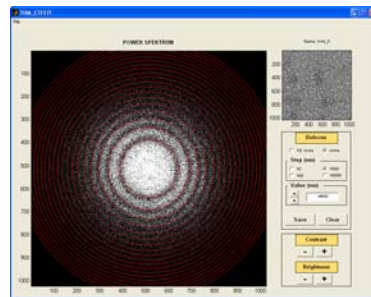
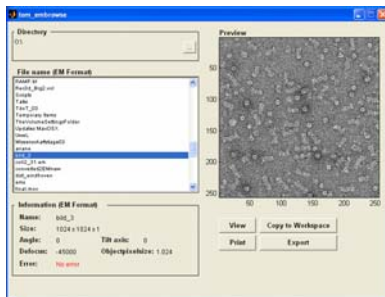
Alignment



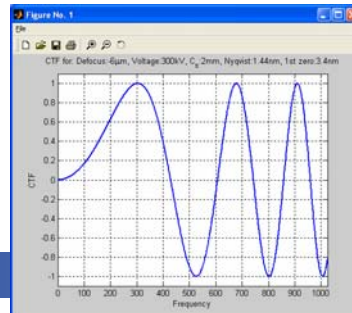
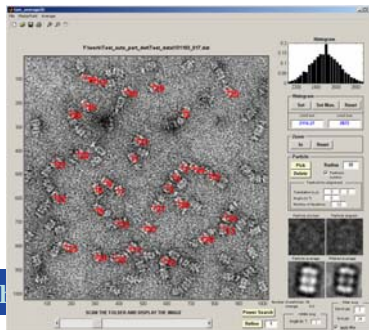
Reconstruction



Analysis

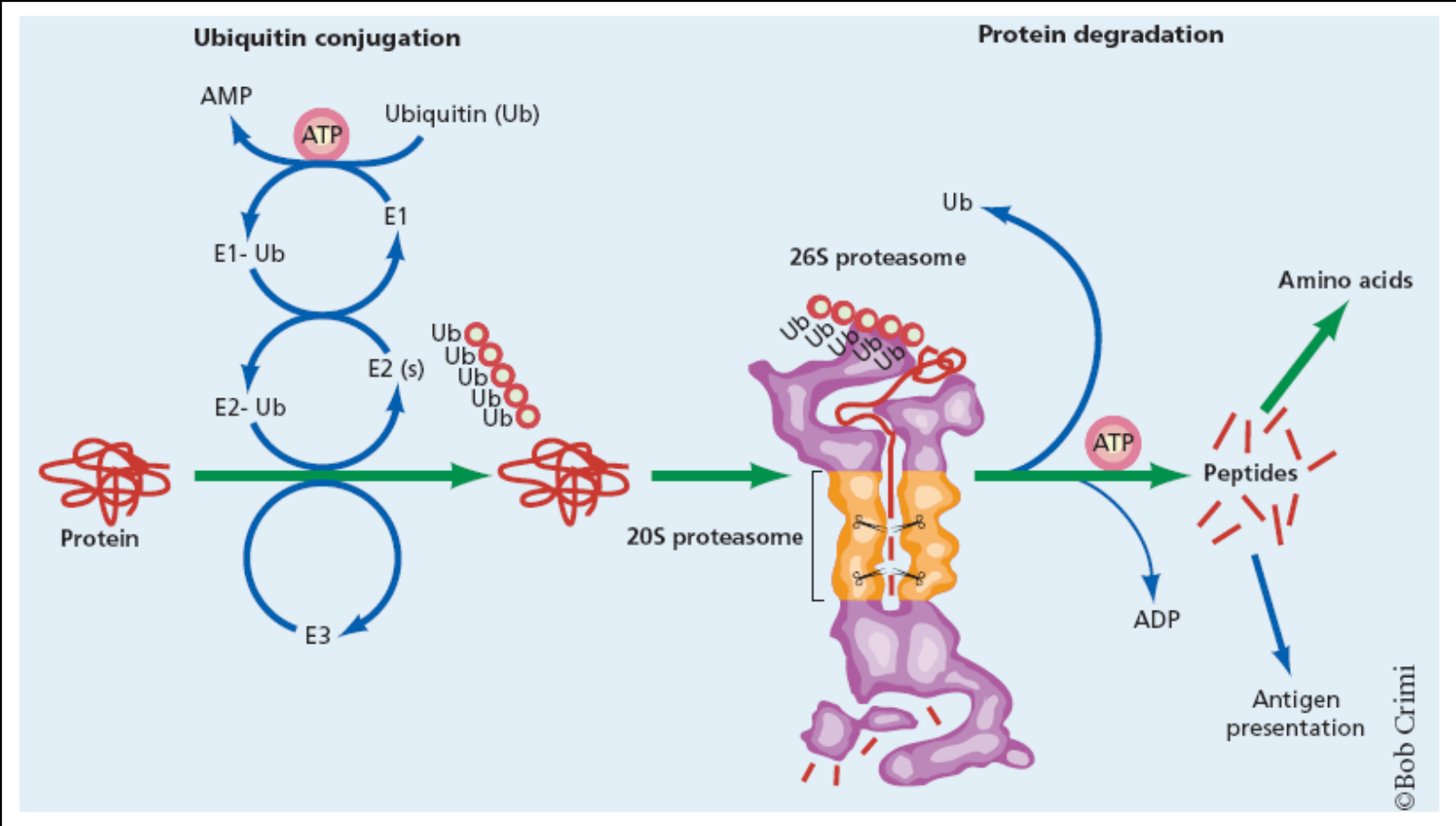


...more than 200 programs
 ...more than 150 labs use it
 worldwide

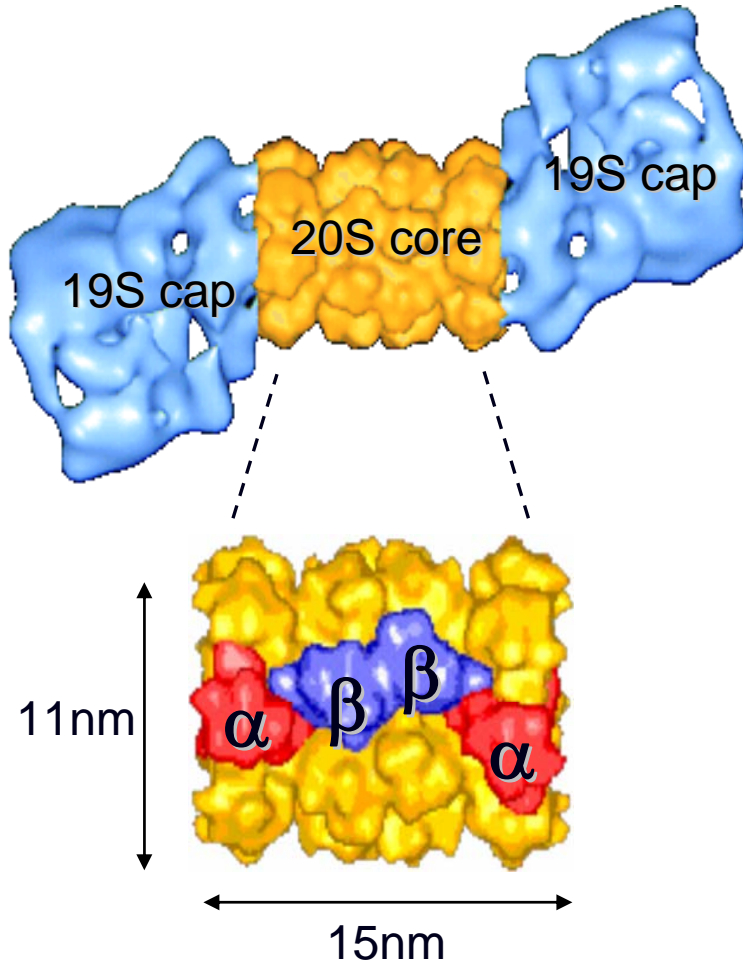


www.biochem.mpg.de/tom

Protein breakdown by the ubiquitin-proteasome pathway



Co-star: the 26S and 20S proteasome



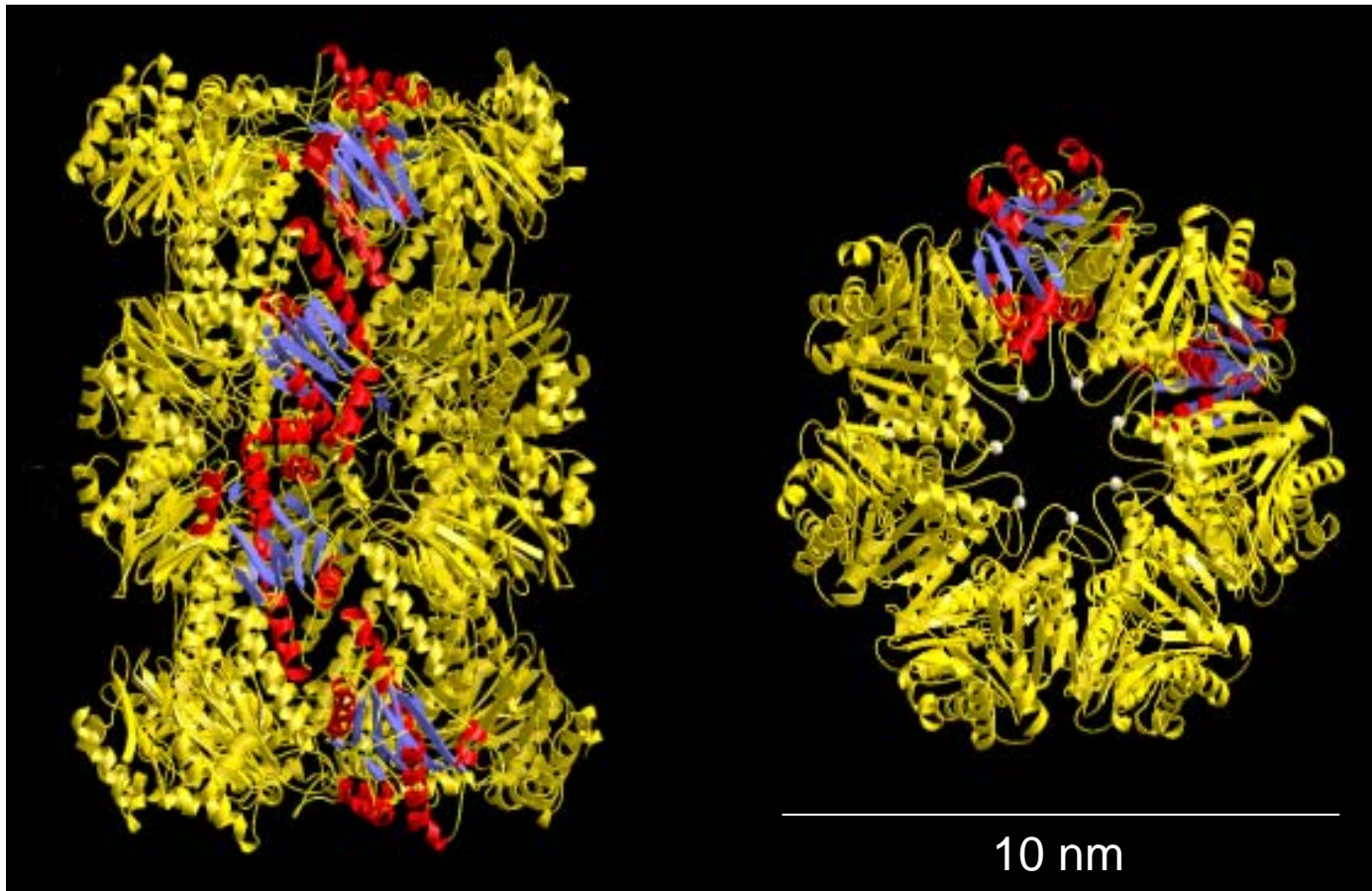
26S Proteasome

- A multi-subunit (>32) complex
- 2.5 MDa
- Degrades ubiquitinated substrates in an ATP-dependent manner

20S Proteasome

- The proteolytic core complex
- Conserved architecture from archaea to humans

The 20S proteasome of T. acidophilum



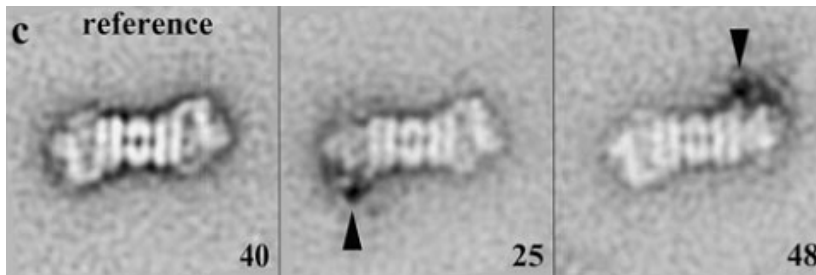
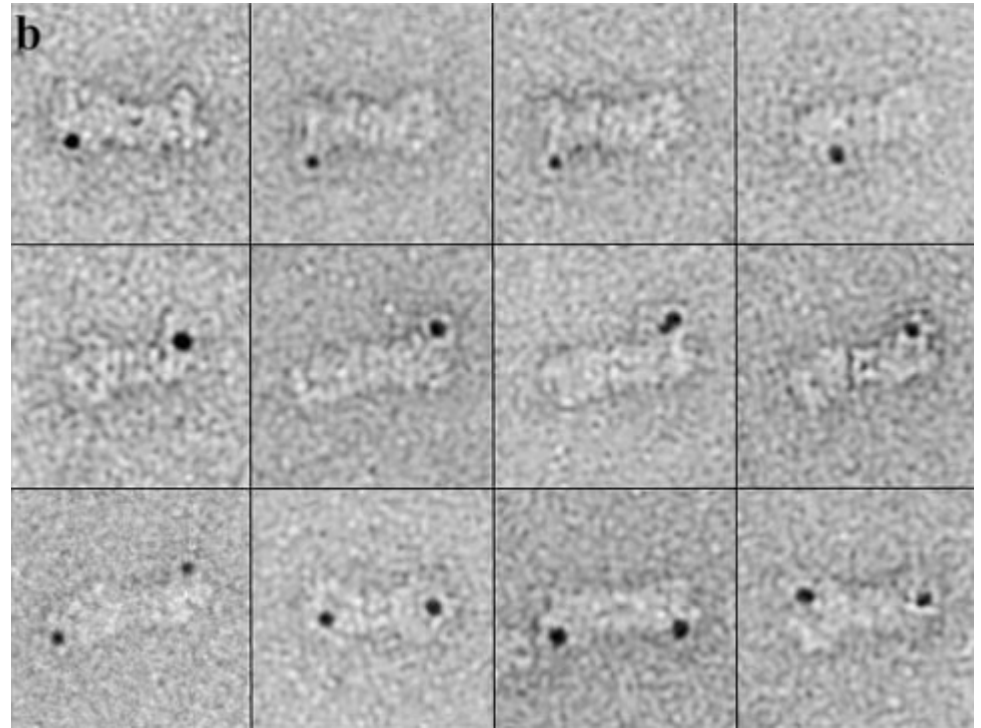
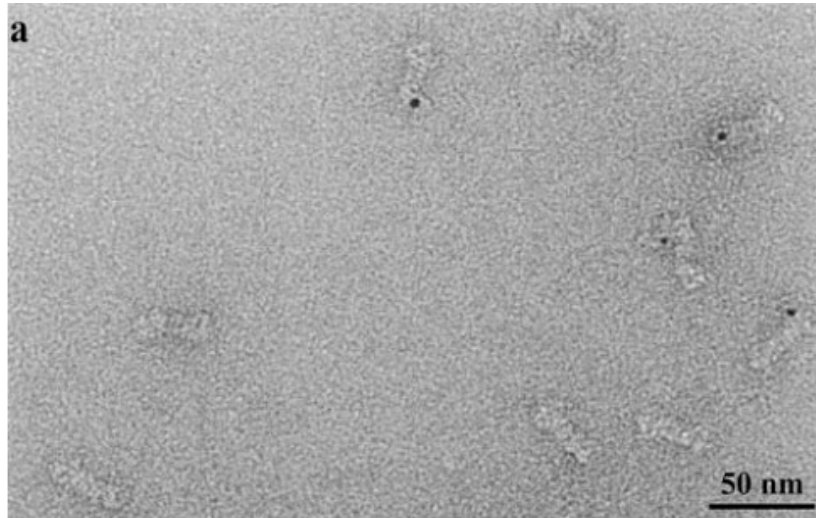
side view

top view

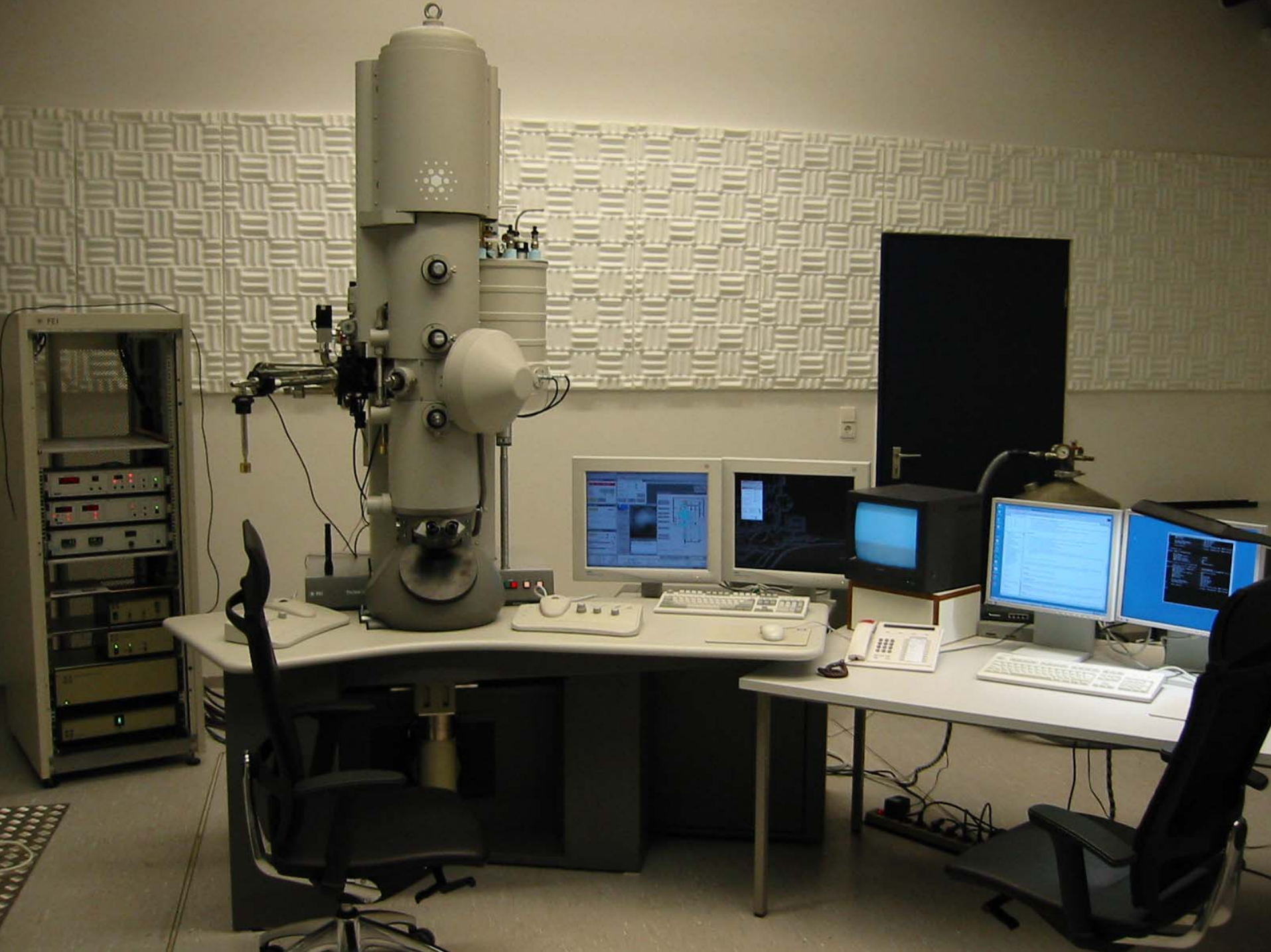
Loewe et. al., *Science* **268** (1995)



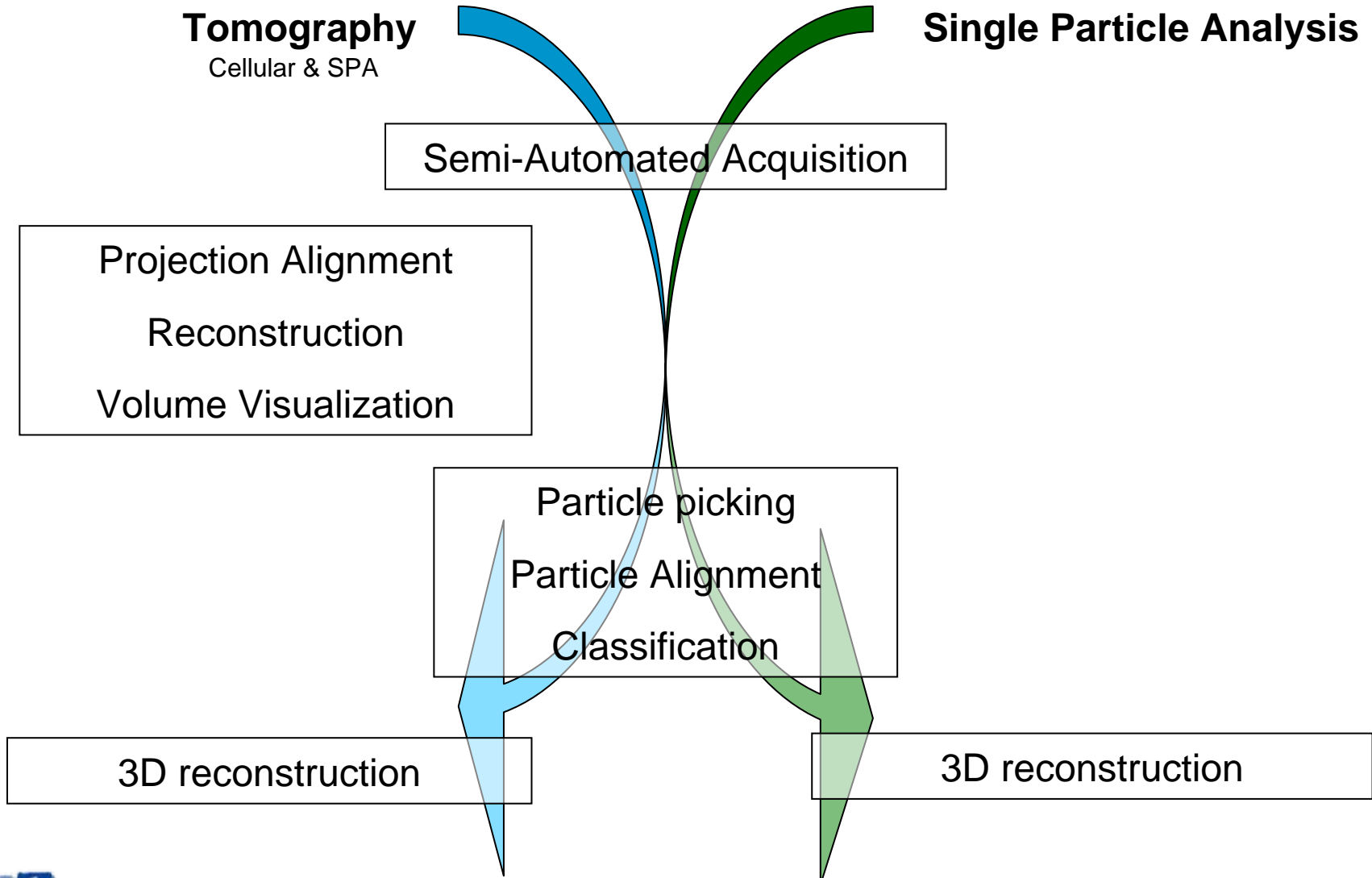
Gold labelled Ubiquitin-aldehyde binds 26S specifically



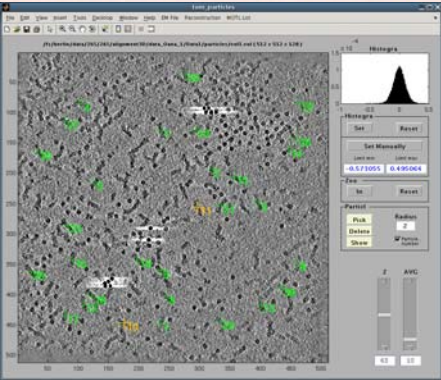
Hölzl et al., 2000



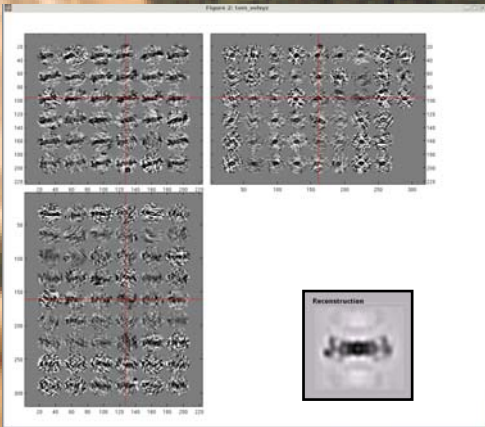
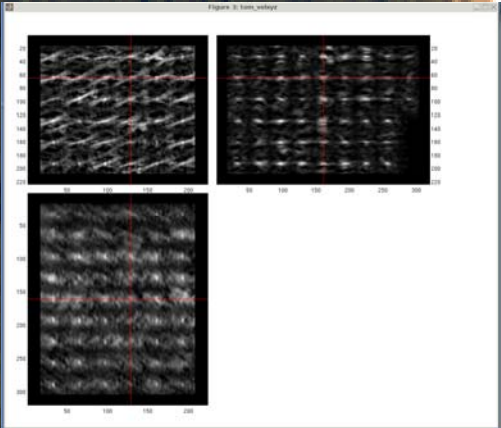
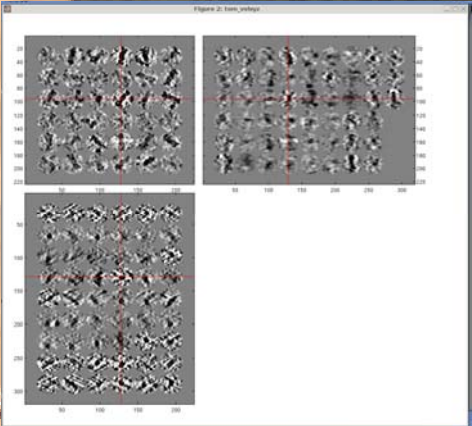
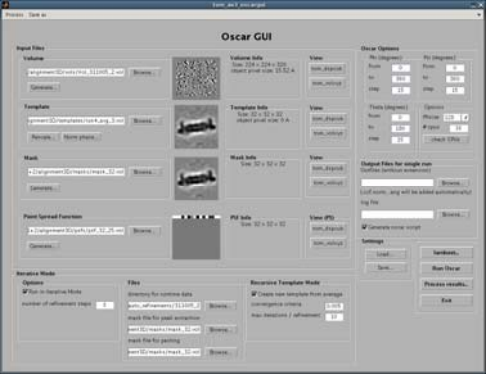
Integration of tomography and SPA in TOM



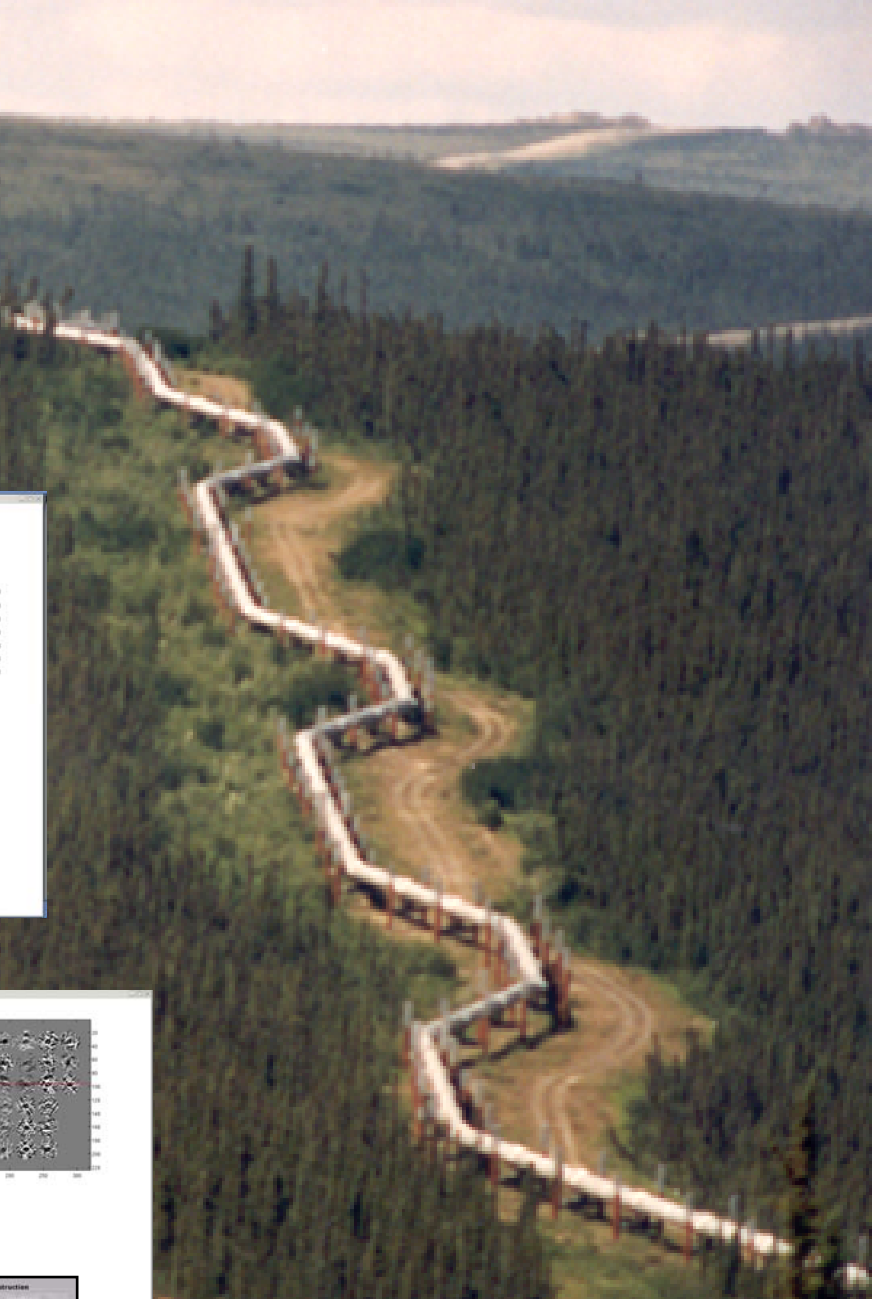
Picking



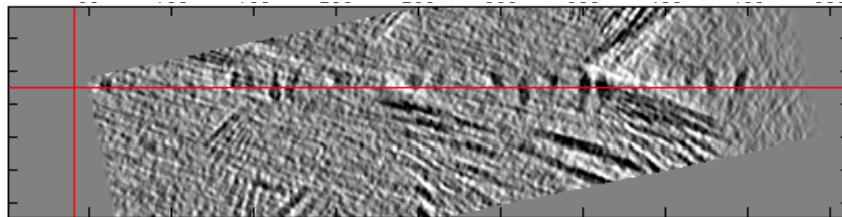
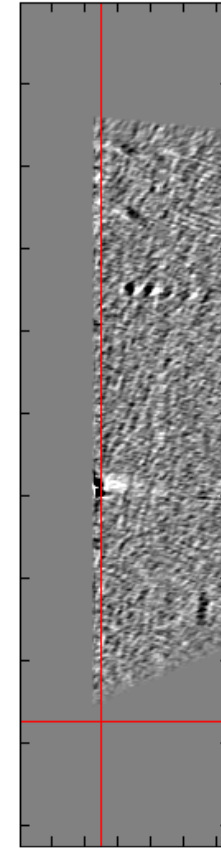
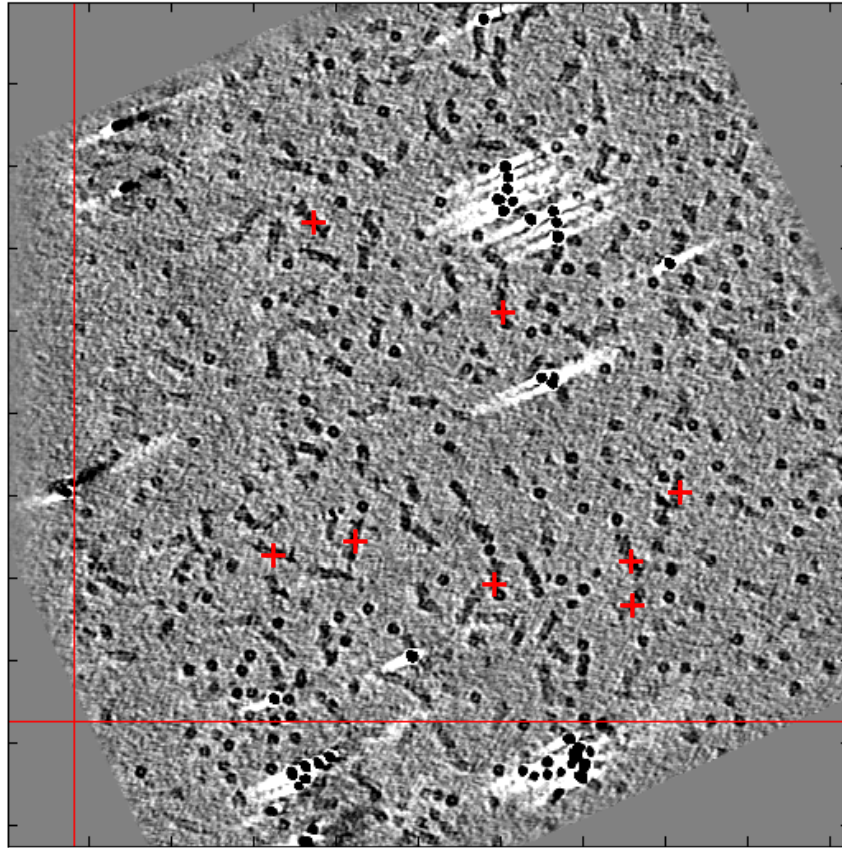
3D alignment



Averaging

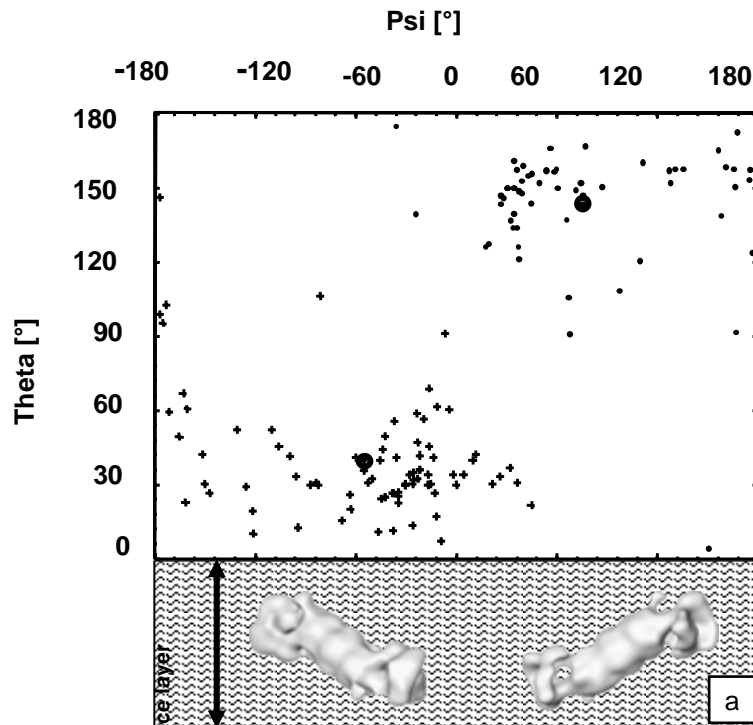


Tomographic reconstruction of 26S proteasomes

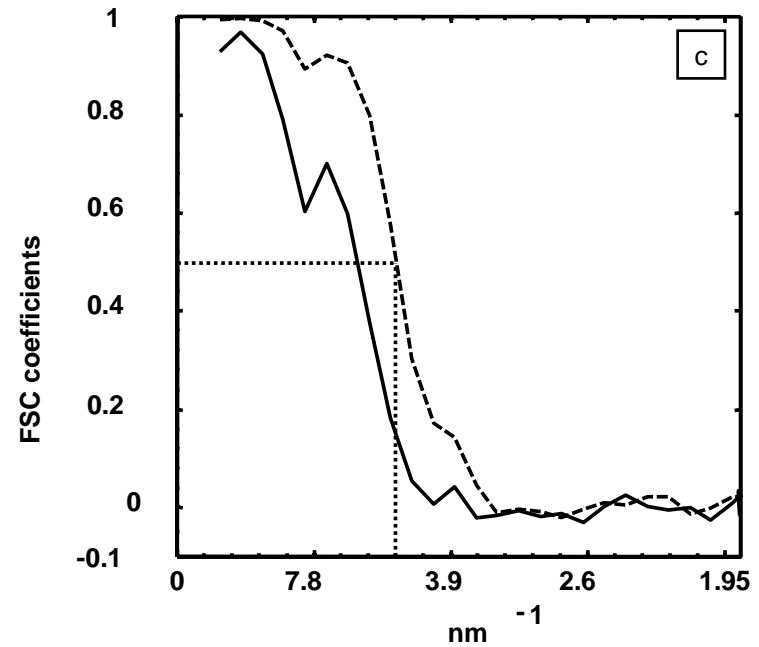


central slice

Non-Random Distribution of 26S in ice



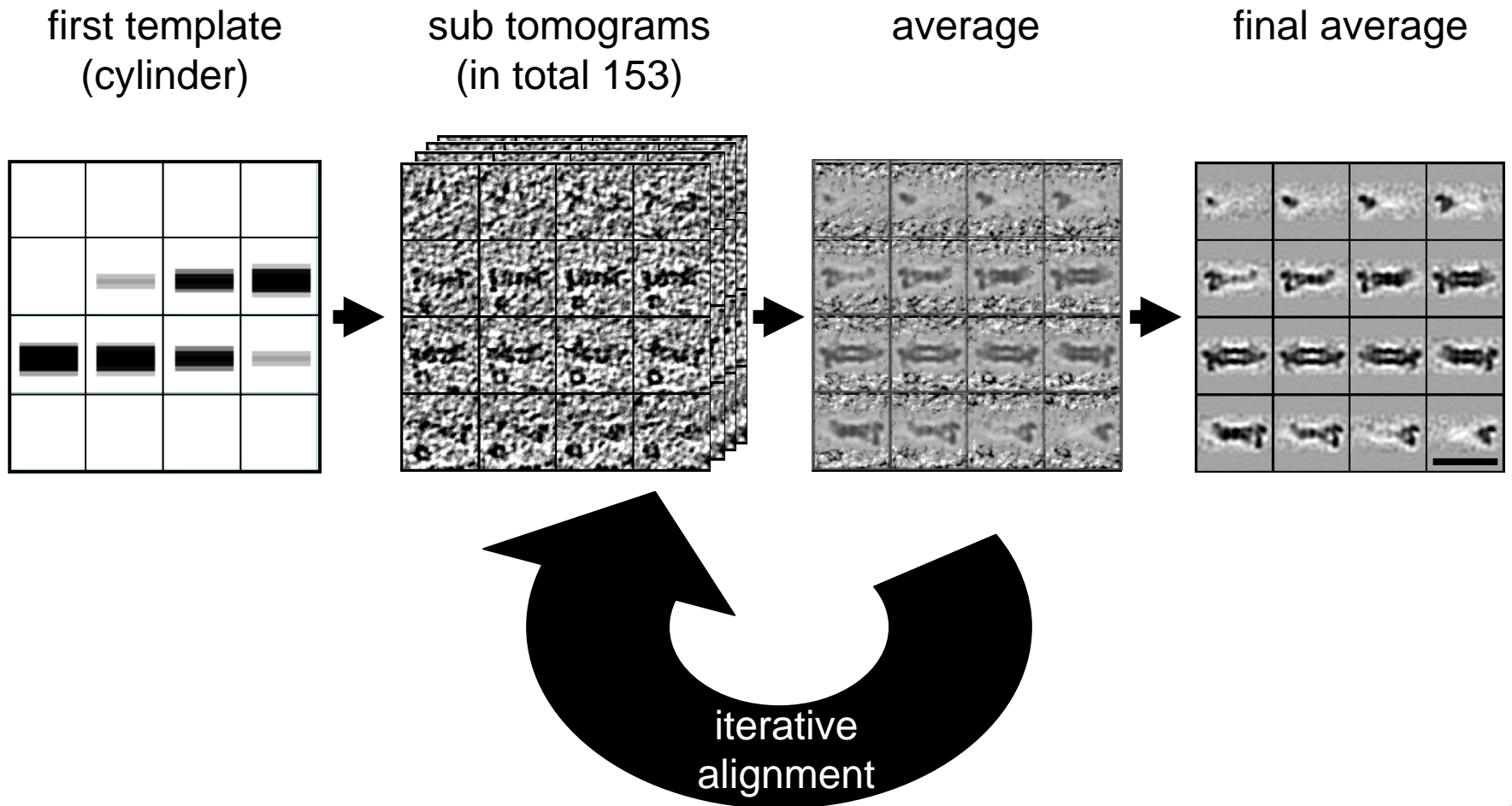
Angular distribution of 26S

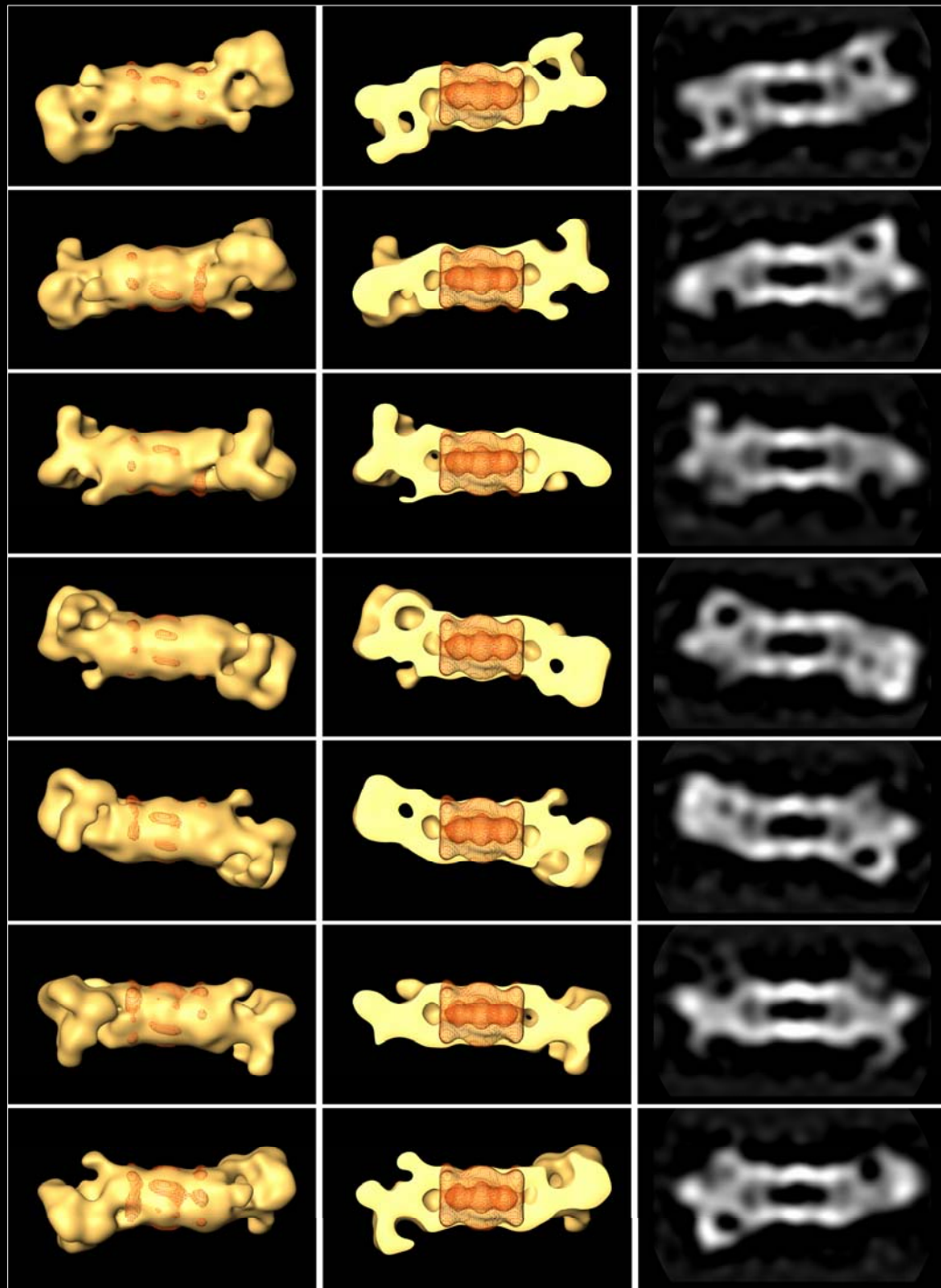


Resolution FRCC

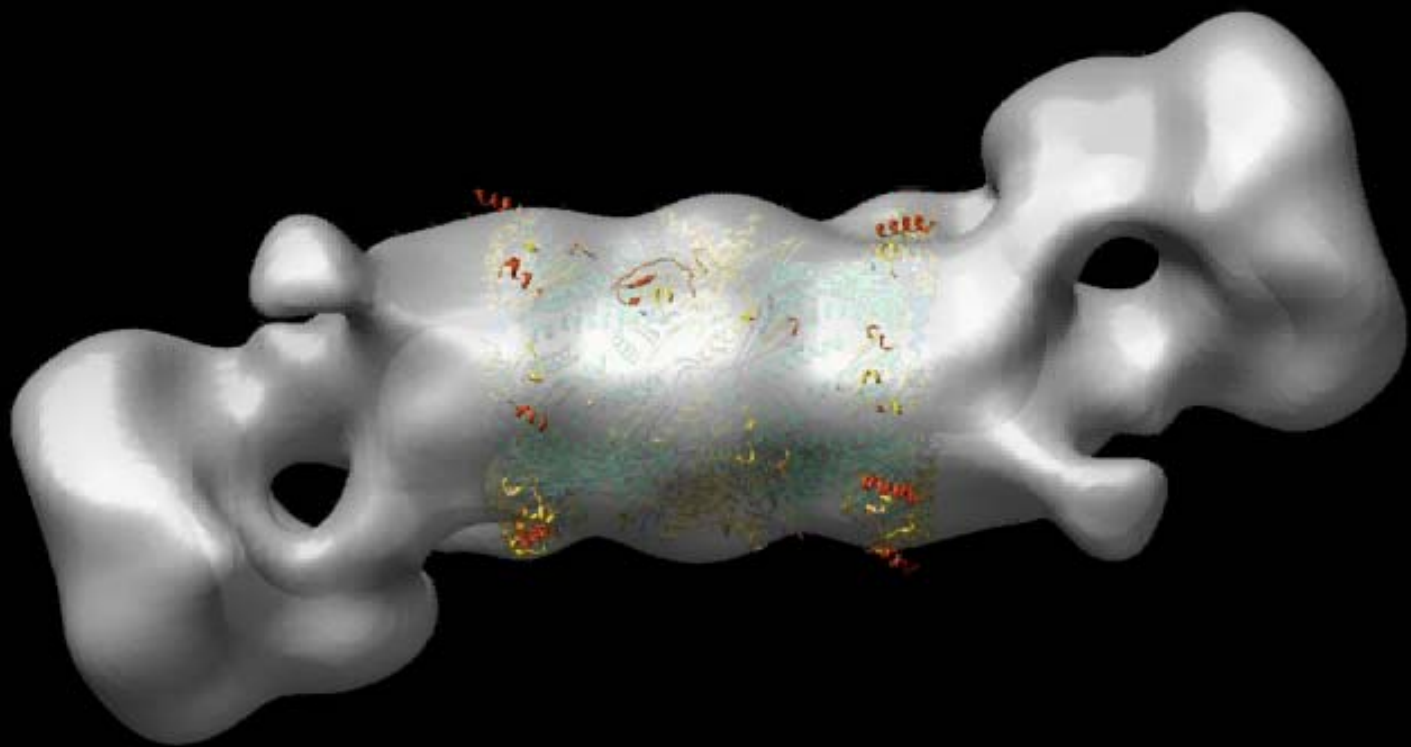
Nickell et al., BBRC (2007)

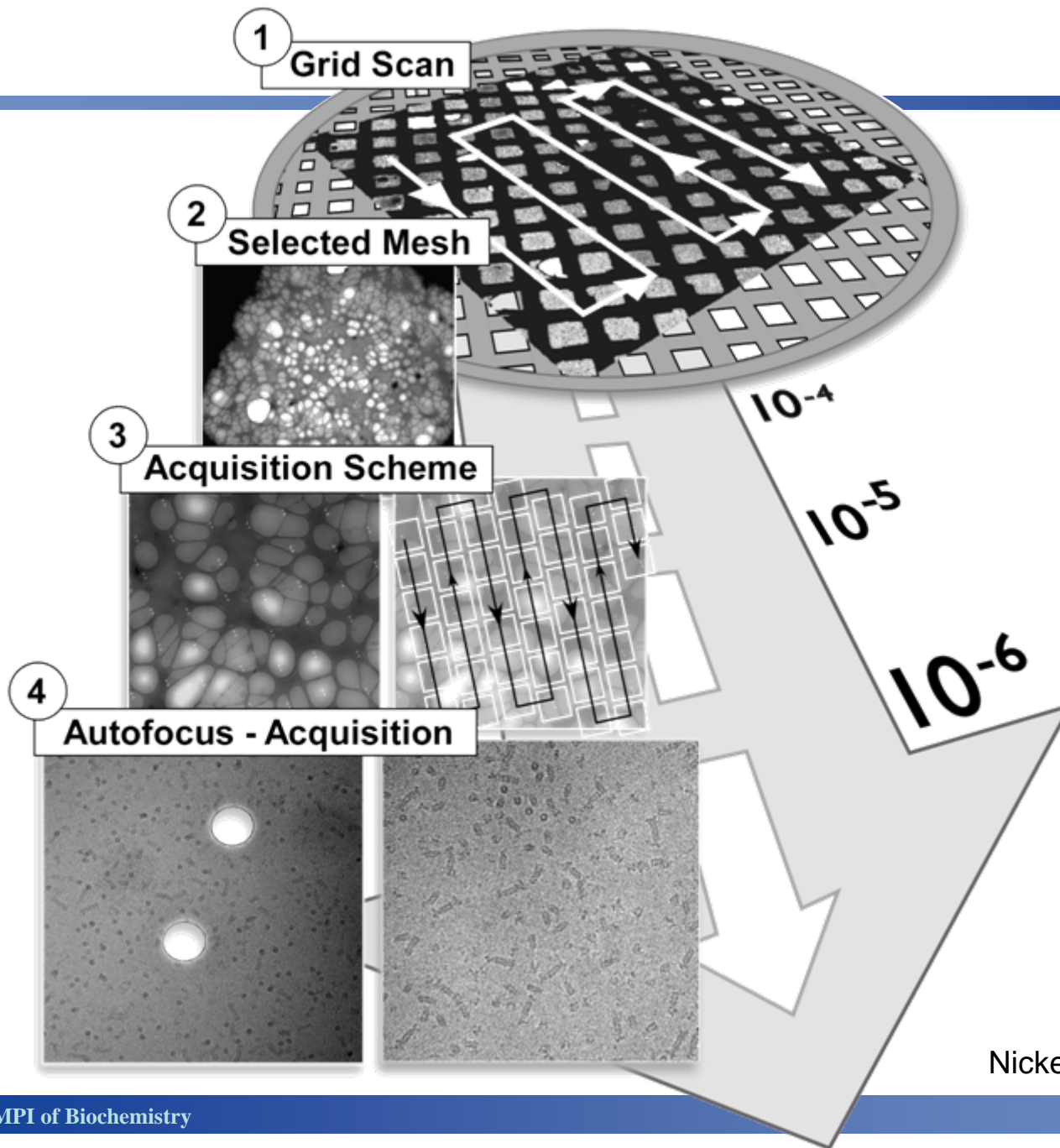
Averaging of tomographic reconstructions of the 26S proteasome





Nickell et al.,
BBRC (2007)

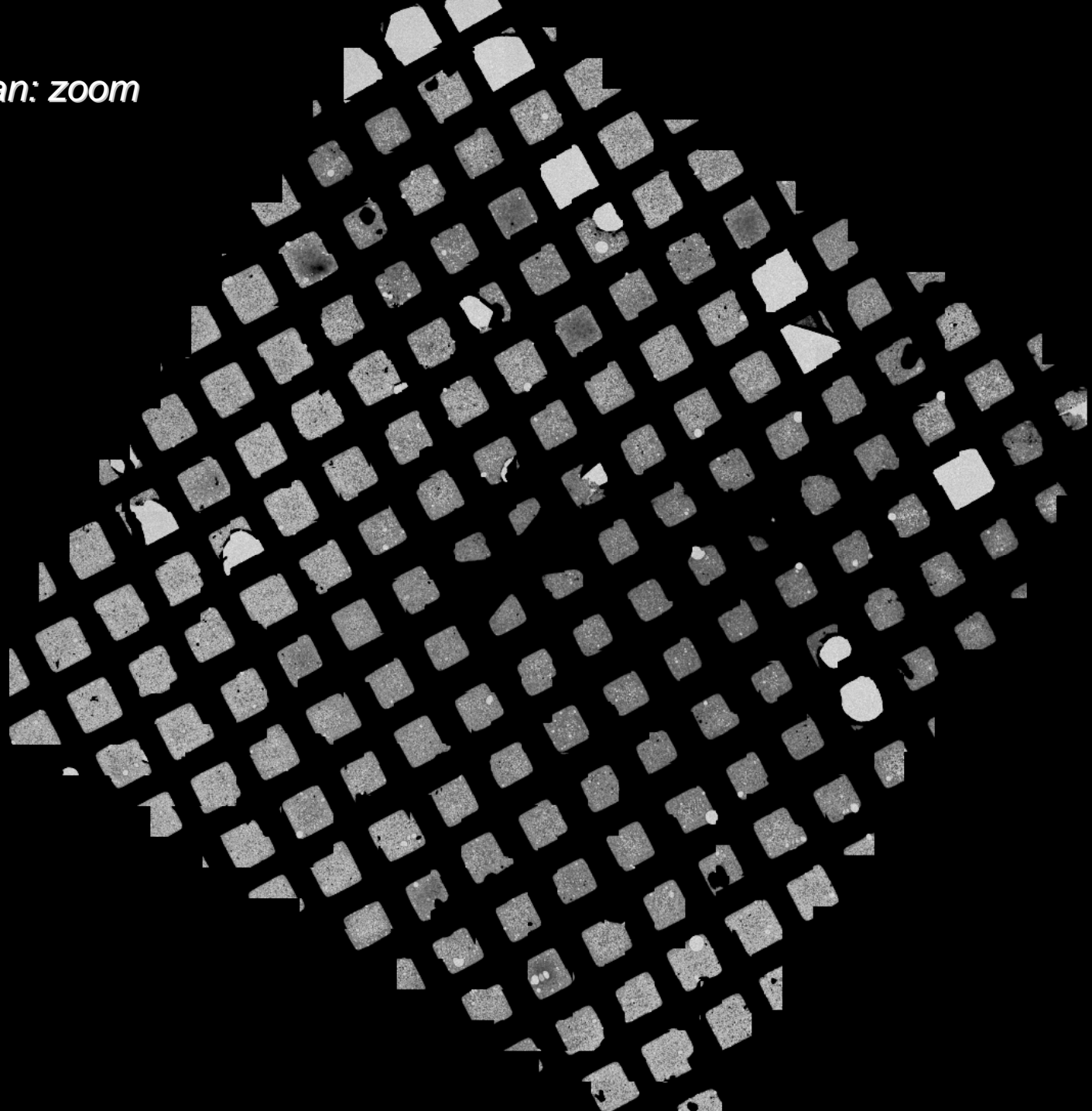


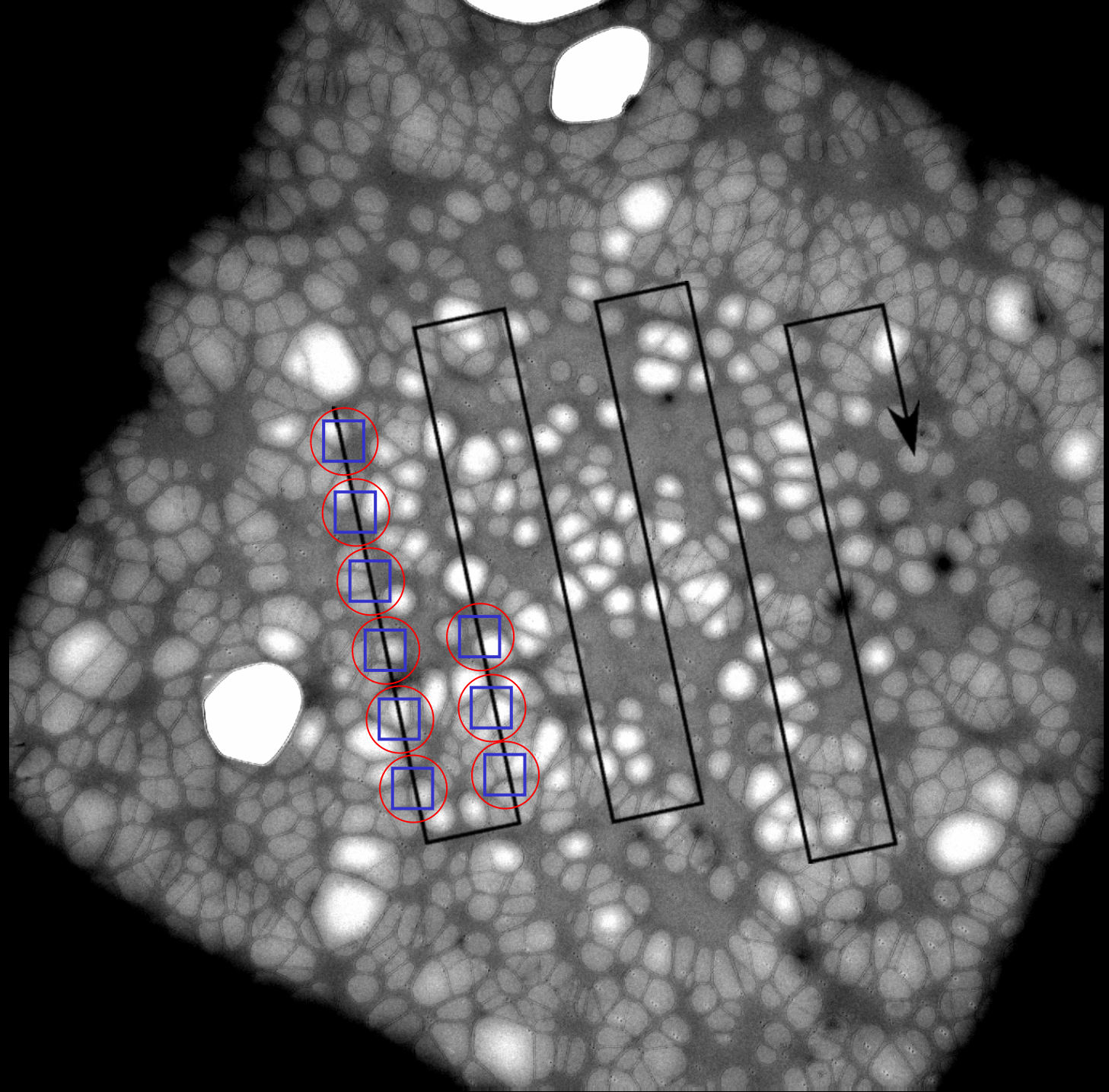


Nickell et al., FEBS Letters (2007)



Grid scan: zoom





Particle Picking

The screenshot displays the `tom_av2_particlepickergui` software interface. The main window shows a cryo-EM image of a sample with several particles highlighted by green arrows. The interface is divided into several panels:

- Filebrowser:** Shows the current file path: `/fs/sally07/lv01/pool/pool-nickell/26S/em/data/071018/high/au_bsa_2nm_003.dat`. It includes a `Reload` button, a `Goto last picked` field with the filename, and a `Resample:` field set to `3`.
- Image Selection Tool:** Features a `good` (green) and `bad` (red) button, and a counter showing `144` good and `0` bad particles. `Load` and `Save` buttons are also present.
- Histogram:** Displays a histogram of particle sizes. The x-axis ranges from 8000 to 11000, and the y-axis from 0 to 2. It includes `Set` and `Reset` buttons, and a `Set Manually` button. Below the histogram, there are `Pick Limit` fields with values `7788.46` and `11909.3`.
- Powerspectrum:** Includes a `Show Powerspectrum` checkbox, an `Average` field set to `1`, and an `Open in new window` button.
- Bandpass Filter (pixel):** Has an `Enable bandpass filter` checkbox and `low` (`0`) and `high` (`2048`) fields.
- Particles:** Contains `Pick`, `Delete`, `Delete All`, and `Refine` buttons. It also has a `radius` field set to `128` and a `Show numbers` checkbox checked.
- Alignment:** Includes a `Perform alignment` checkbox checked, and a `Translation (x,y):` field set to `20`. `Load Ref...` and `Save Ref...` buttons are also present.
- Classes:** Shows a `Class` dropdown set to `default`, with `Add...`, `Delete`, and `Save Stack...` buttons.
- Alignment Comparison:** Shows four small images: `particle clicked`, `particle aligned`, `particle average`, and `average filtered`.
- Rotate avg:** Has an `Angle (deg):` field set to `0` and an `Apply` button.
- Shift avg:** Includes a `Center` button.
- Filter avg:** Has an `Enable` checkbox and `low` and `high` input fields.
- Polygon mask:** Includes a `Draw` button.
- Picklist:** Has `Load` and `Save` buttons, and an `Autosave` checkbox.
- Image Info:** Displays metadata: `Image Size: 1365 x 1365`, `Pixel Size: 0.278 nm`, `Voltage: 160 kV`, `Defocus: 2.1 um`, and `Cs: 2 mm`.
- Classes Summary:** Shows `default: 14 particles` in green text.

tom_av2_particlepickergui



2D alignment

2D alignment

Input stack and alignment file

Input stack: important data [Browse...]

Input alignment file: alignment file for important data [Browse...]

Input reference file: choose a beautiful one [Browse...]

Output stack and alignment file

Output stack: sub stacks

Output alignment file: twisted important data [Browse...]

Output reference file: see here how beautiful the data was twisted [Browse...]

Parallel Options

Change... [Change...]

Parallel Mode on

Stack Info

[Browse]

[Make ref]

Reference Info

[Browse]

[Modify]

Filter Selection

Alignment

Classification

Mask Selection

Alignment

Classification 1

Classification 2

CC Rotation

CC Translation

Stack Sample Particle

[Mask]

[Filter]

Reference Sample Particle

[Mask]

[Filter]

Filter

defaults off

times: []

bandpass

low: []

high: []

smooth: []

Kernel

real fourier

quadratic circular

radius: []

Mask

defaults off

sphere

radius: []

sigma: []

center: [] []

rectangle

radius: [] []

sigma: []

center: [] []

Settings

[Load...] [Save...]

Demo Mode

Alignment

Multi Reference

Iterations

Refinement: [5]

Alignment: [5]

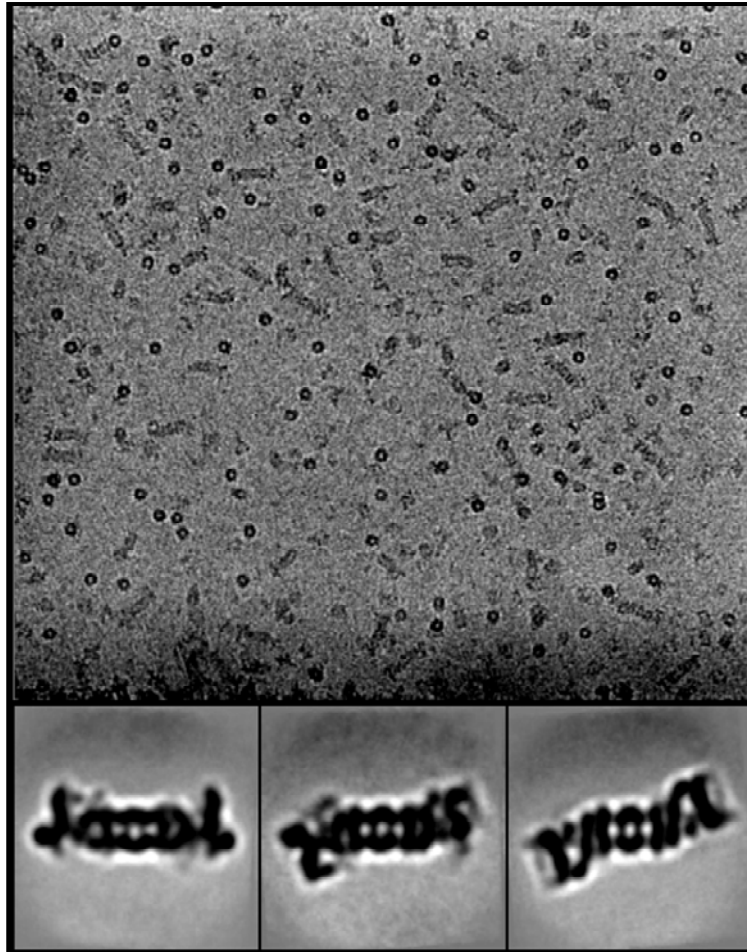
Run alignment

[Run]

tom_av2_alignment

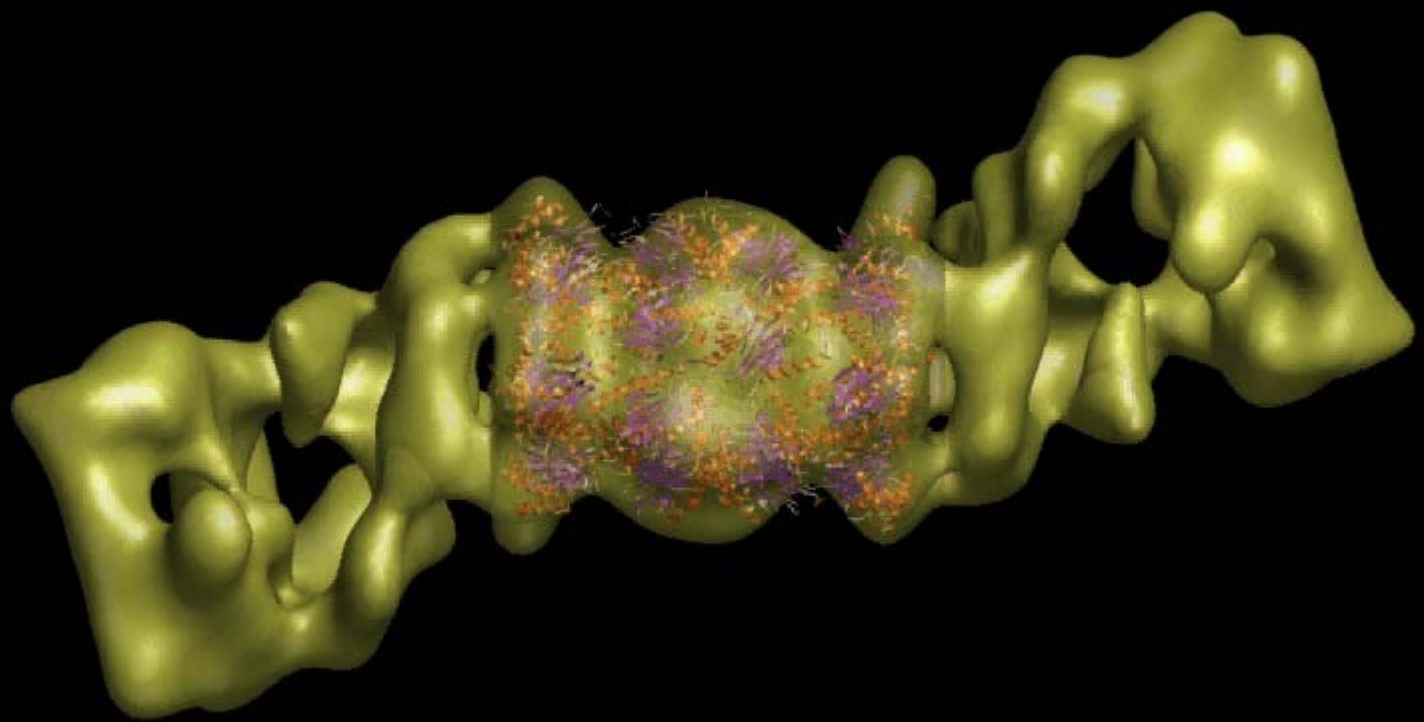


Overview and class averages

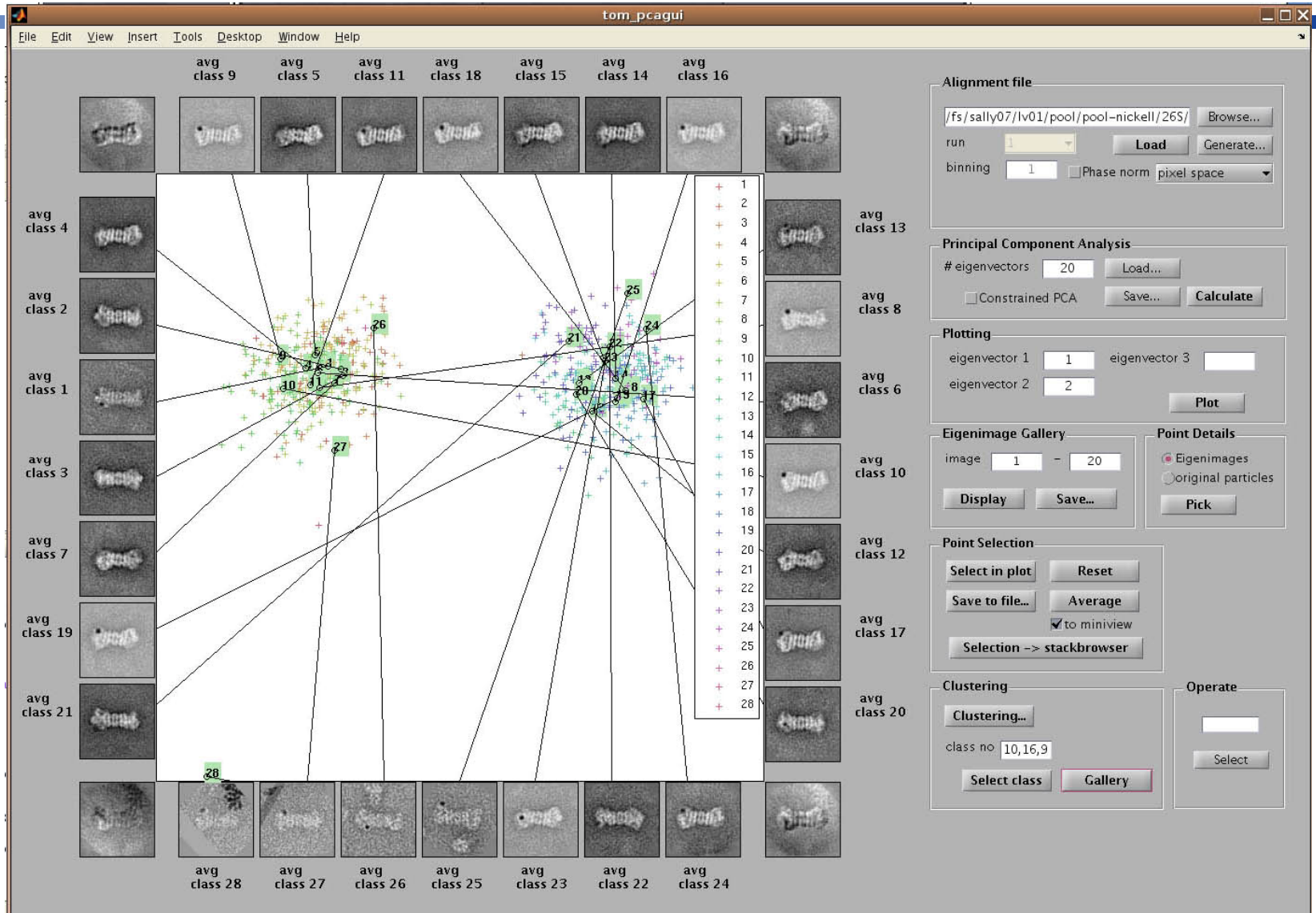


Nickell et al., FEBS Letters
(2007)





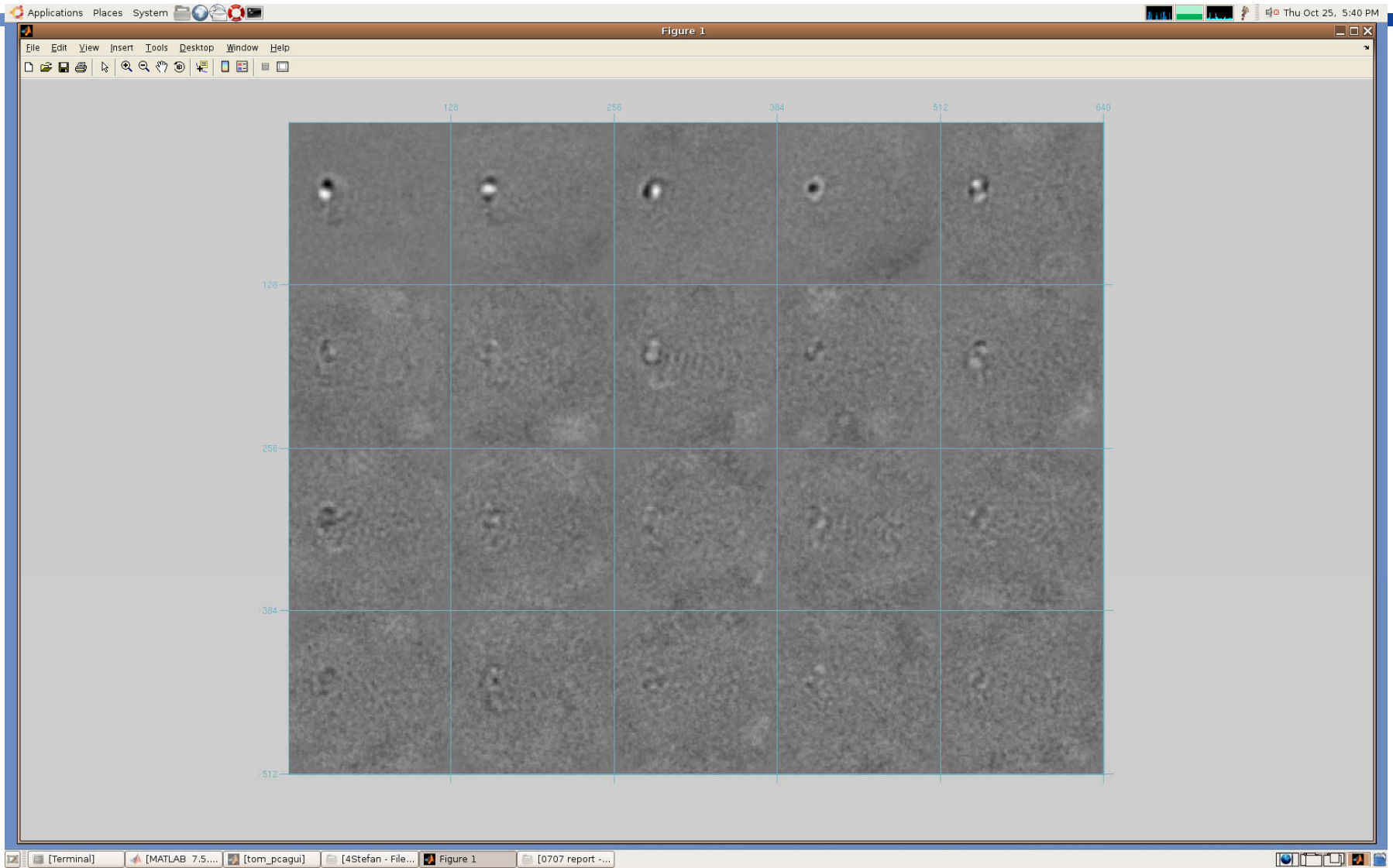
Multiclass Analysis



tom_pcgui



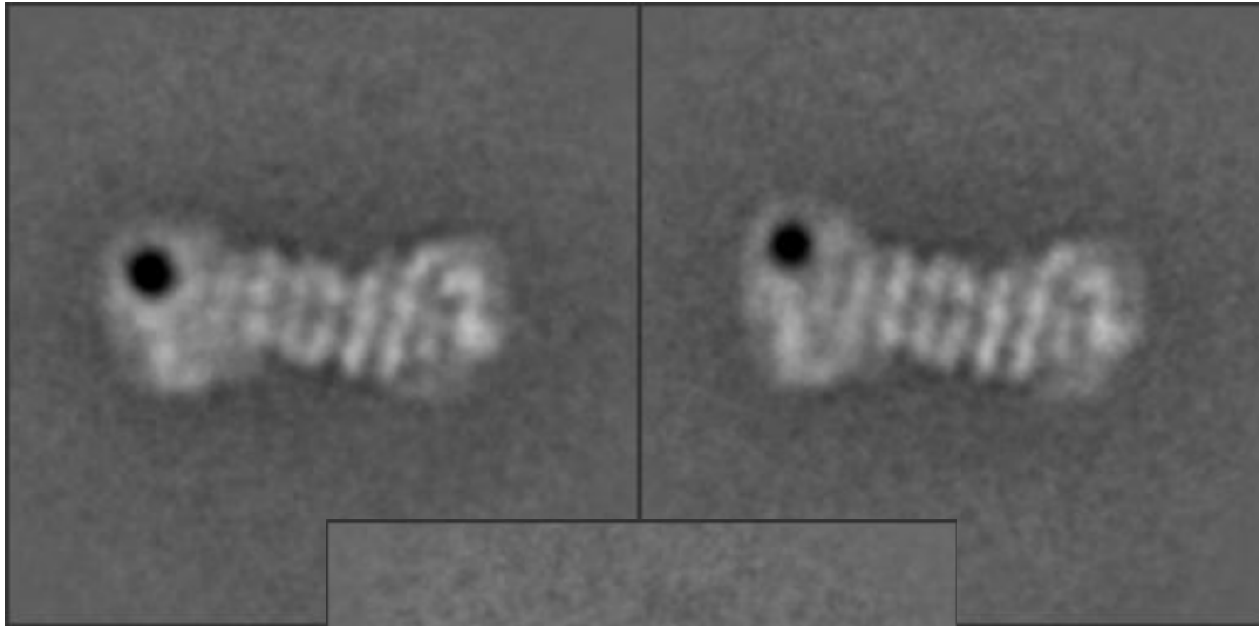
Eigenimage Gallery



tom_pcagui

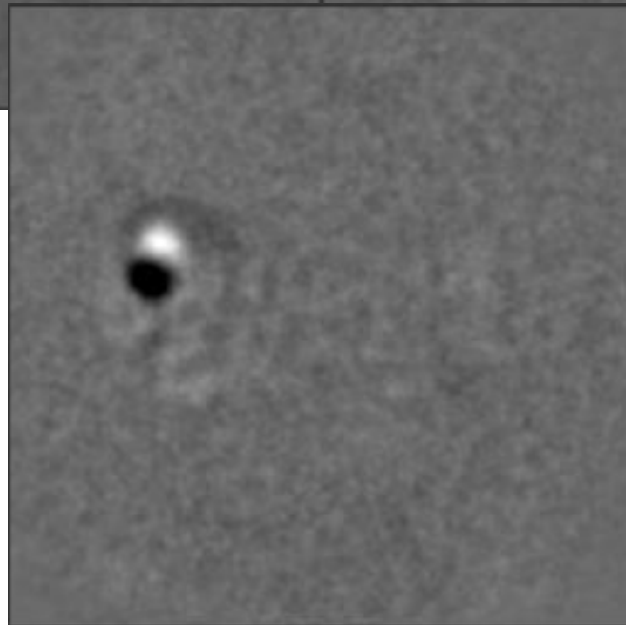


Two class averages of Ub-Gold labelled 26S



143 particles

114 particles



difference

A Titan...



Acknowledgments

26S Proteasome

**Stephan Nickell
Florian Beck
Oana Mihalache
Inga Wolf
Andreas Korinek
Reiner Hegerl
Ohad Medalia**

EM

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Günter Pfeifer**

and Wolfgang Baumeister

