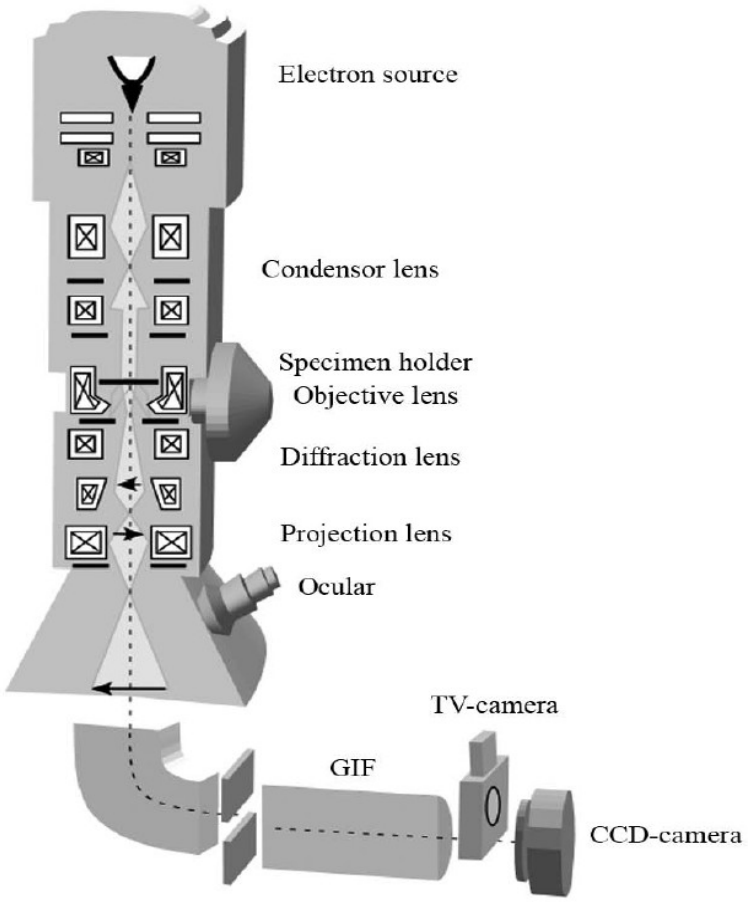
Challenges for Intelligent Image Processing in Electron Microscopy

Christoph Best
Max Planck Institut für Biochemie
Martinsried, Germany

Outline

- Introduction
- Tomography
  - Alignment — belief networks
  - Reconstruction — level sets; Monte Carlo algorithms
  - Denoising/segmentation — anisotropic diffusion; scaling index
- Single-particle analysis — EM algorithm
  - Automated particle picking — supervised learning using SVMs
  - Model-free initial models — unsupervised learning
probabilistic modelling

Electron microscopy



From Schweikert, 2004

(Macro-)Molecular resolution O(5 nm)

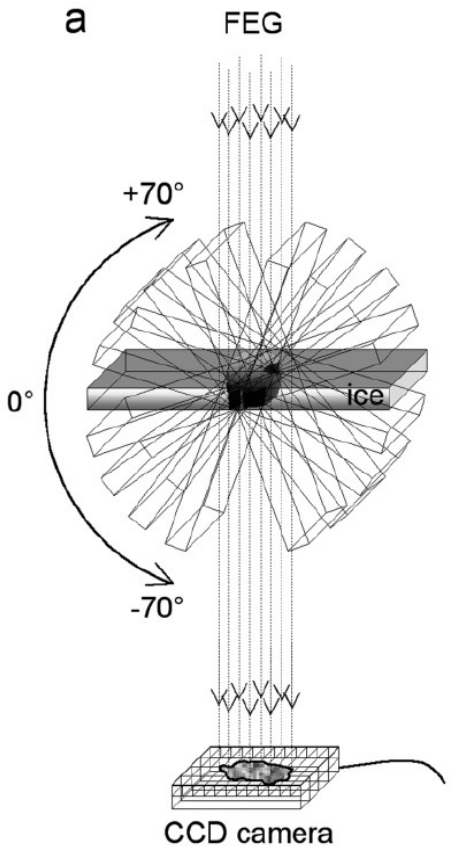
Near-native state:
Cryo-electron microscopy:
Specimen embedded in **vitreous ice**

Problems:

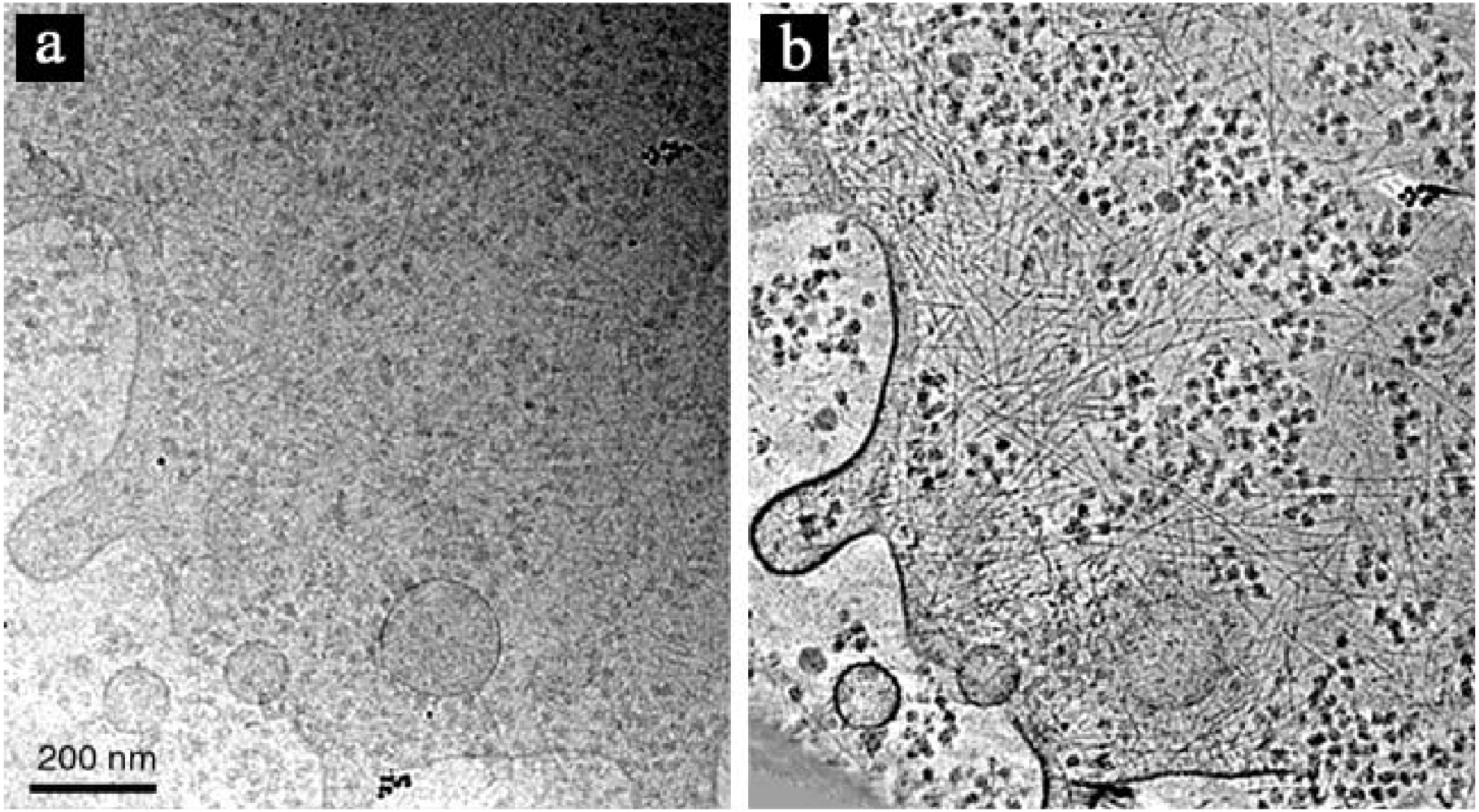
- Beam damage, low dose, high noise
  ➜ **denoising**
- No fluorescent markers
  ➜ **nano-gold markers**
- Low penetration (100nm)
  ➜ **cryosectioning**

Electron tomography

- 3D reconstruction by taking a series of images from different angles
- Difficulty:
  - Nanometer accuracy (basically solved, at least for single axis)
- <u>Problems:</u>
  - Limited tilt range
    ➜ missing wedge
  - Imperfections of the tilt
    ➜ alignment
  - Reconstruction algorithms under high noise



Tomography of eukaryotic cells



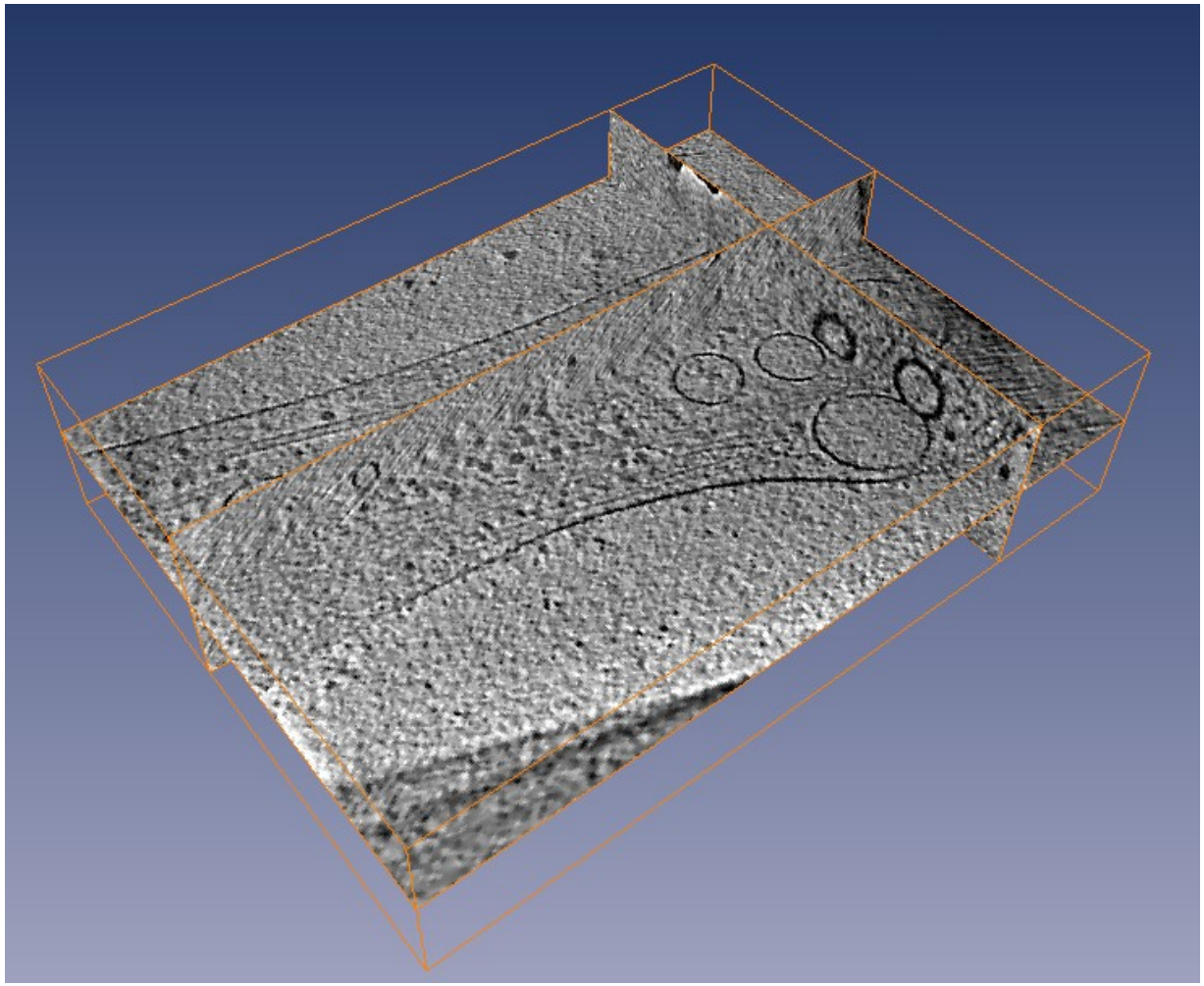
PROJECTION

SLICE

Dictyostelium discoideum

O. Medalia *et al*, *Science*, 2002

3D imaging on a molecular scale



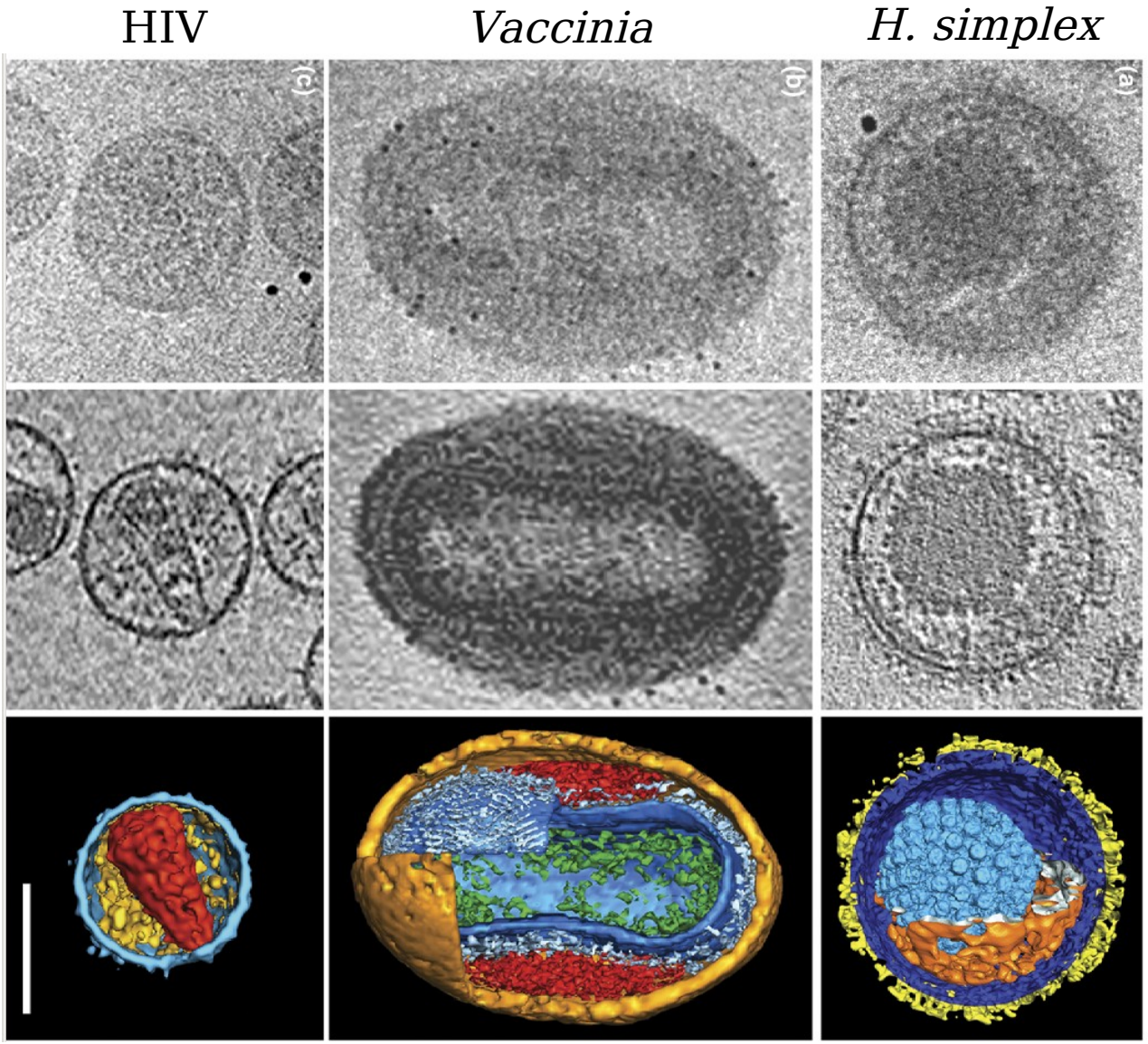
Mouse adeno-carcinoma cell grown on a TEM grid

assembled from 66 images over a 132° range

thickness ≈ 520nm

courtesy of Andrew Leis, Martinsried

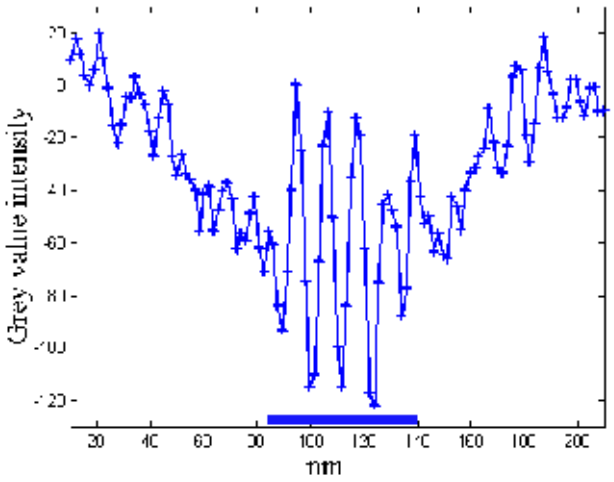
Viruses

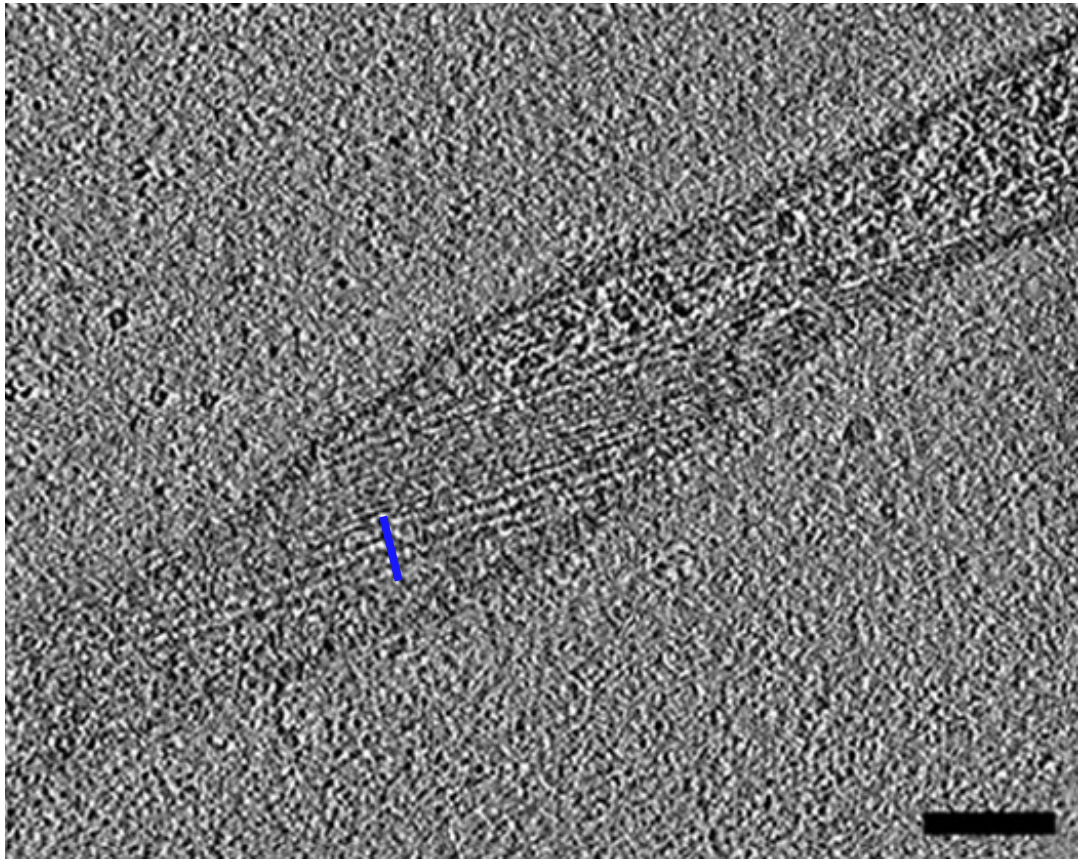


Grünewald & Czyrklaff,
Curr. Opin. Microbiol., 2006

Cytoskeleton

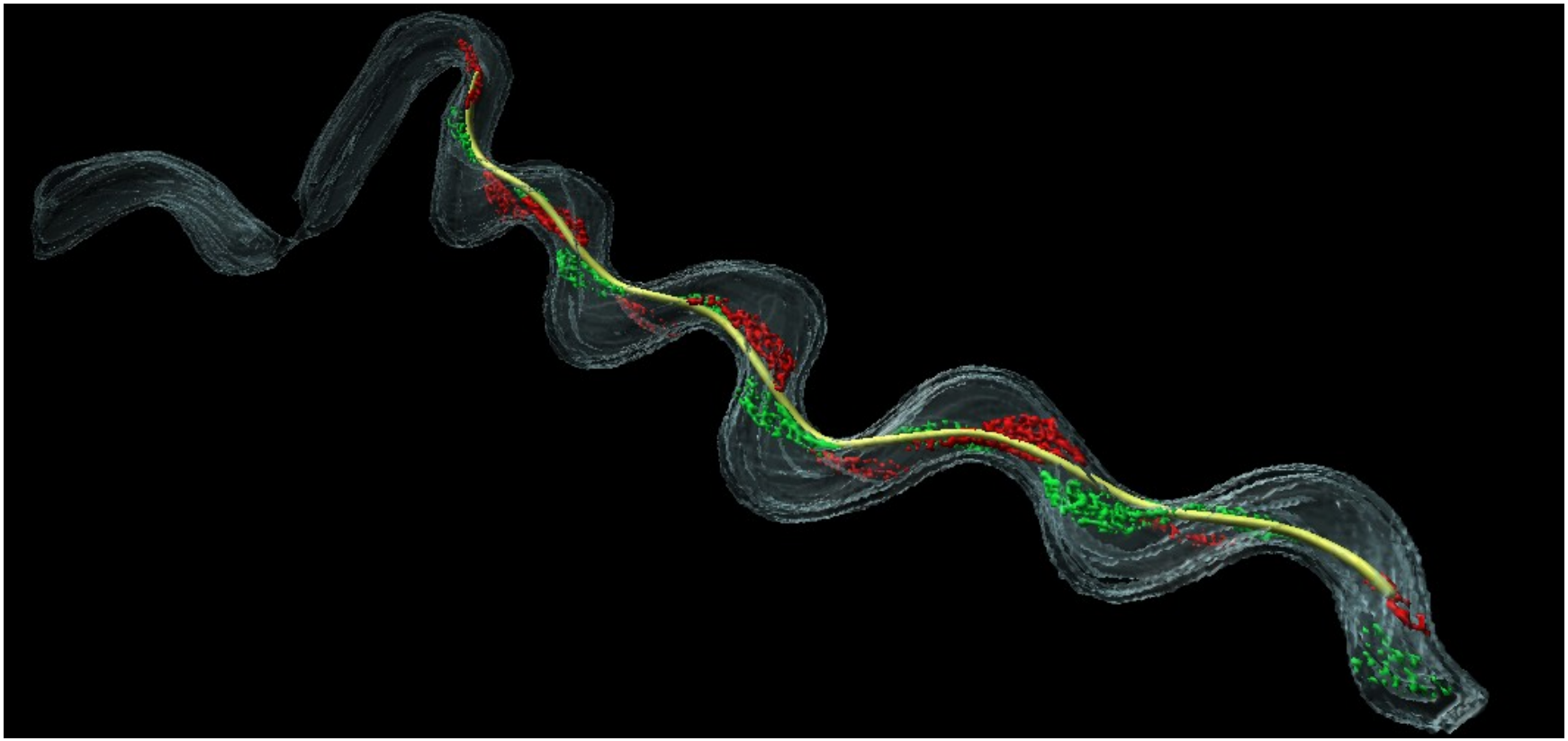
Cytoskeleton of *Spiroplasma melliferum*





J. Kürner *et al.*, *Science*, 2005

The cytoskeleton of *S. melliferum*

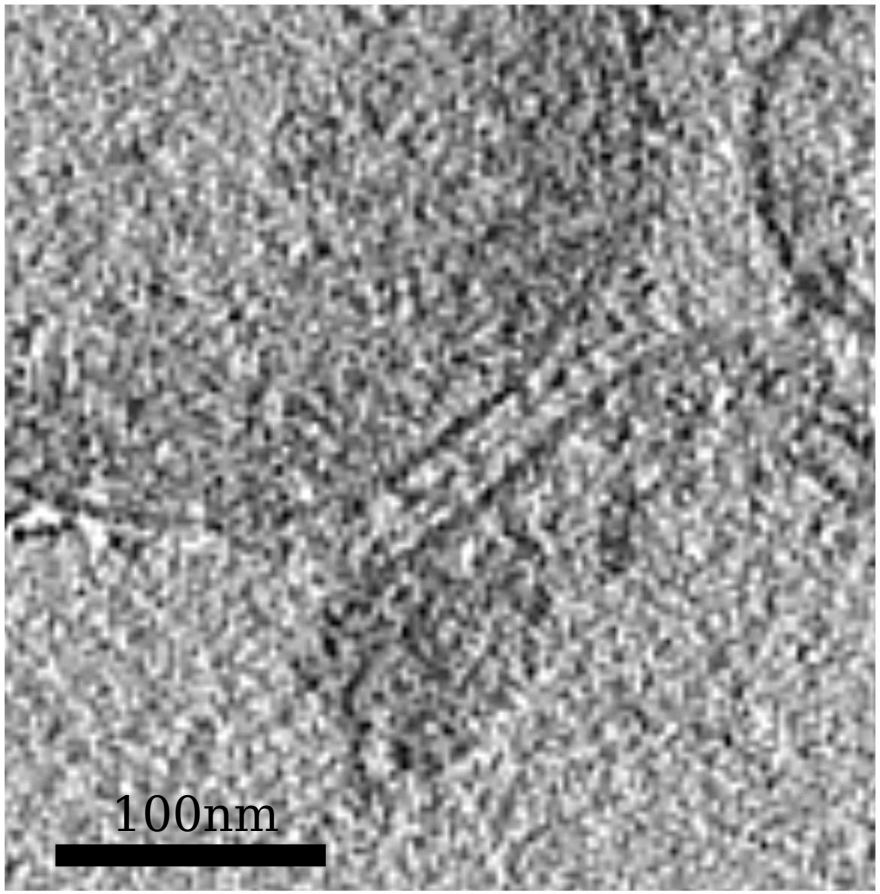


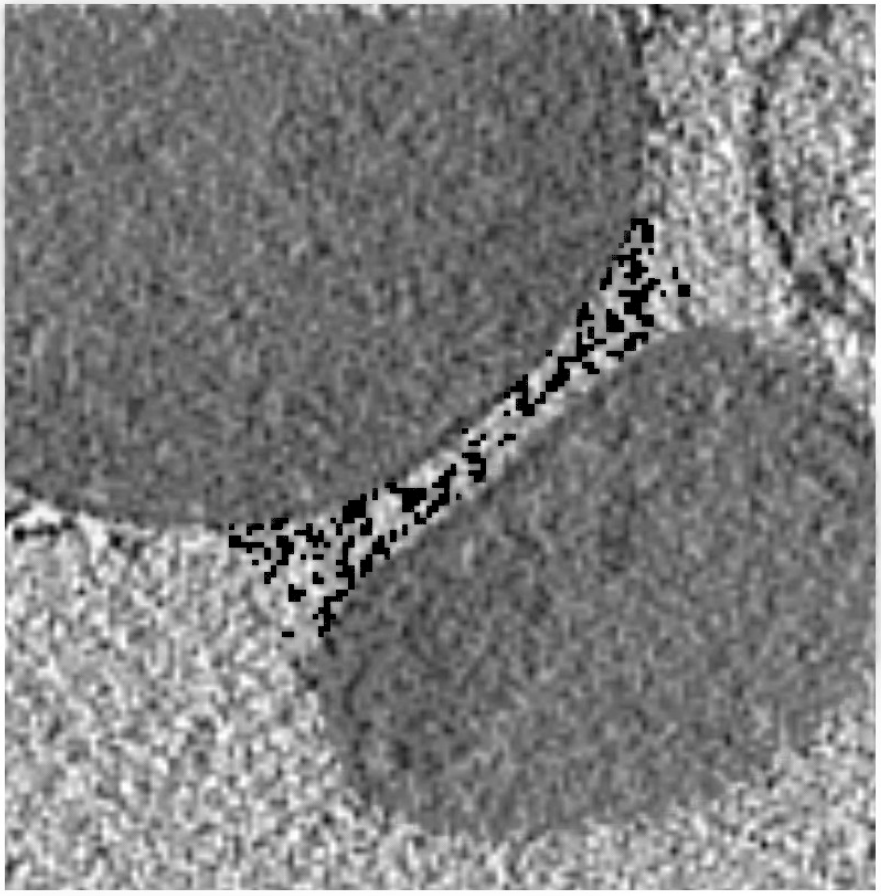
yellow: geodetic line

J. Kürner *et al., Science,* 2005

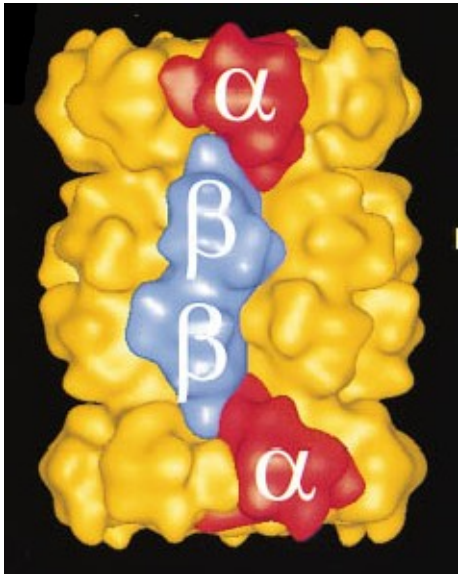
Neurobiology applications

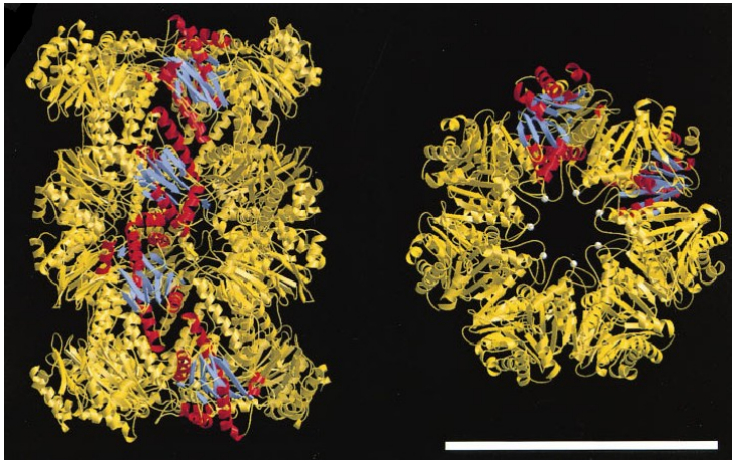
Molecules released in the synaptic cleft of a mammalian synapse.
(courtesy of V. Lucic, Martinsried).

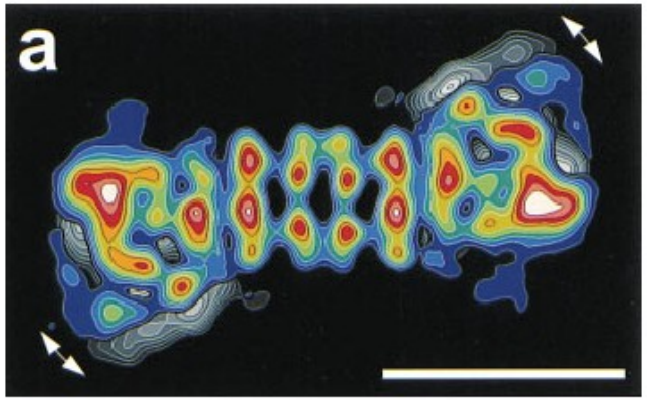


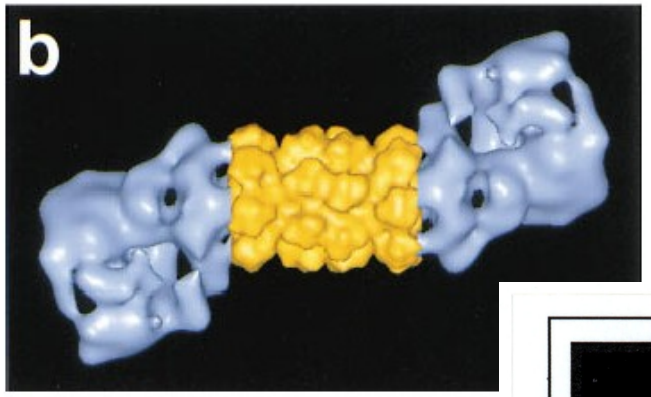


Structural Biology: The Proteasome





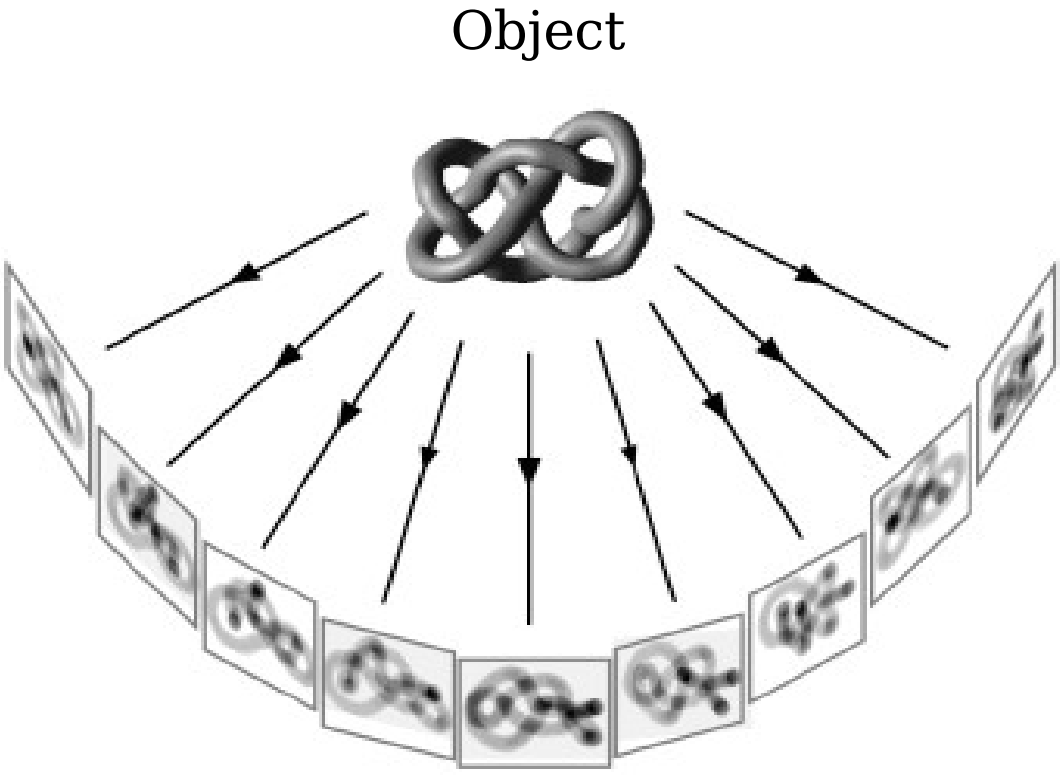




Tomography

Tomographic reconstruction problem:

Find an object consistent with all projections.



Data set size: ≈ 500 MB
Acquisition time: 30 min

Alignment

- Problem:
  Different images must be **shifted/rotated** to correspond to a common reference frame
- Strategies:
  - **Markers**: Add nanogold particles to object, use markers as reference points
    [manual selection]
  - **Features**: Select features that can be identified in several images
  - **Correlation**: for images with overall similarity (based on a Gaussian noise model)

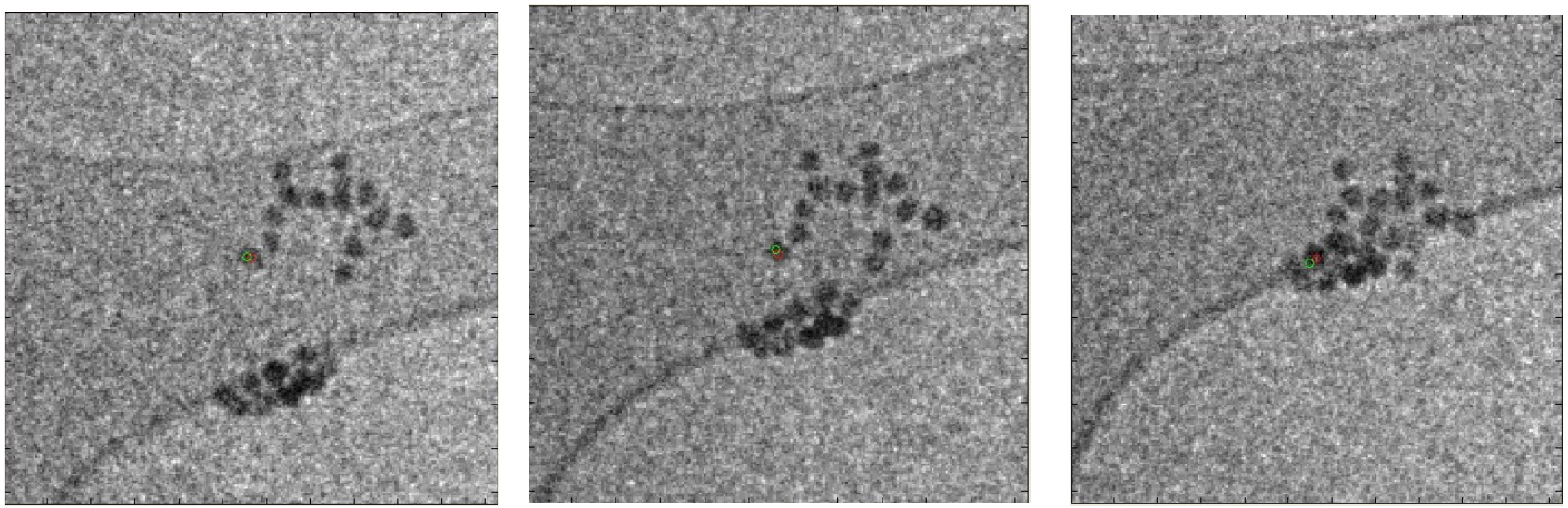
Algorithmic alignment problem

- Given:
  projected positions $x^{(n)}(\phi^{(n)})$ taken at angles $\phi^{(n)}$
- Determine:
  Which marker corresponds to which spots in the different projection images?
- Difficulty: Combinatorial problem
  but there are good heuristics
- Applications:
  - Automatic alignment
  - Multiscopic immunomarker registration

Automated tracking

Fernando Amat, Farshid Moussavi, Mark Horowitz:

“Automatic tracking of fiducial markers across very low SNR images”, 4ICET, San Diego, 2006



Using a Markov Random Field and Loopy Belief Propagation

Tomographic reconstruction

- Classical result: Radon 1917

  n-dimensional functions can be reconstructed from their (n-1)-dimensional integrals

- Special case: tomography:

  integrals (=sums over voxels) along straight rays

- But how?
  - Simplest algorithm: weighted back projection
  - Iterative algorithms: ART, SIRT

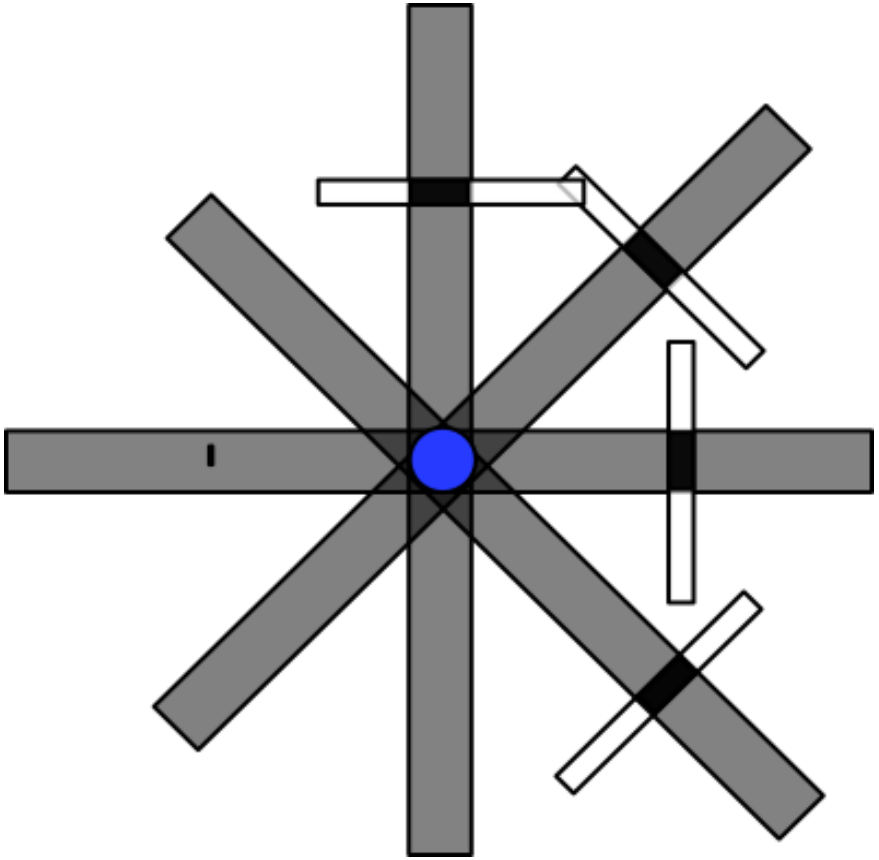
Reconstruction algorithms

- **Reconstruction problem:**

  Given a set of projections and their angles, determine the/a 3D density that generates these projections

- Underdetermined problem (less image pixels than voxels in volume) ➜ needs regularization
- Volumes are large: typically 512x512x256 or larger (several 100 MB to GB)
- Classical algorithms are simple: WBP, ART, SIRT

Weighted back projection

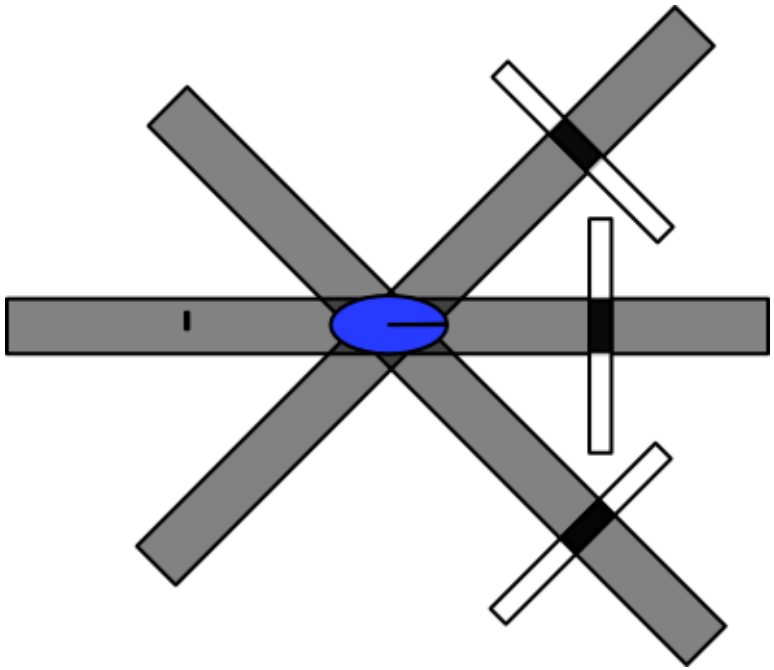


Computationally very simple

First-order approximation to true solution

Good enough when many equally spaced projections are taken

Weighted back projection



Missing wedge ➜ distortion

Iterative methods

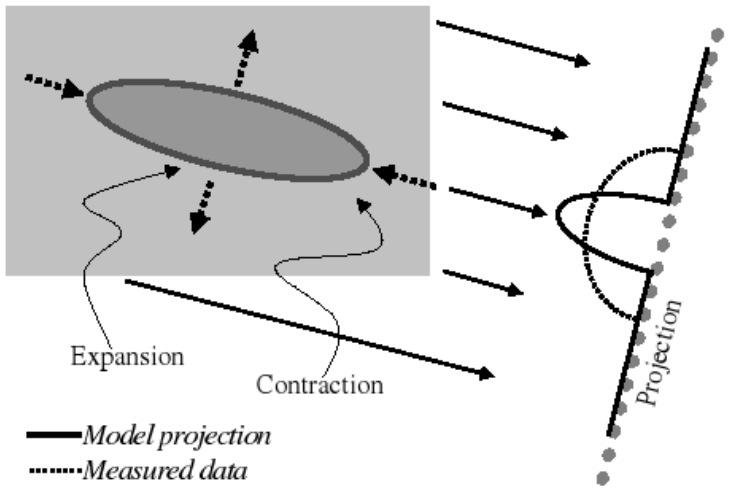
- Iteratively compare observed and calculated projections
- Update 3D density accordingly
- Classical implementations:
  - ART (algebraic reconstruction technique)
    projections views processes sequentially
  - SIRT (simultaneous iterative reconstruction)
    all projections processed in parallel
- Maximum-entropy methods (Skoglund, *JSB*, 1996)

Room for Improvement

- Iterative algorithms:
  - Conjugate-gradient
- Probabilistic methods:
  - Monte Carlo reconstruction
- Long-term goal: Incorporate previous knowledge
  - Discrete tomography
  - Combine denoising & reconstruction
  - Algorithms that require fewer projections

Level set reconstruction

- Goal: reconstruct with a small number of views
- Idea:
  - Represent shape by a level set function
  - Compare projected outlines to views
  - Accumulate force from each view
  - Update level set
  - Combines segmentation with reconstruction

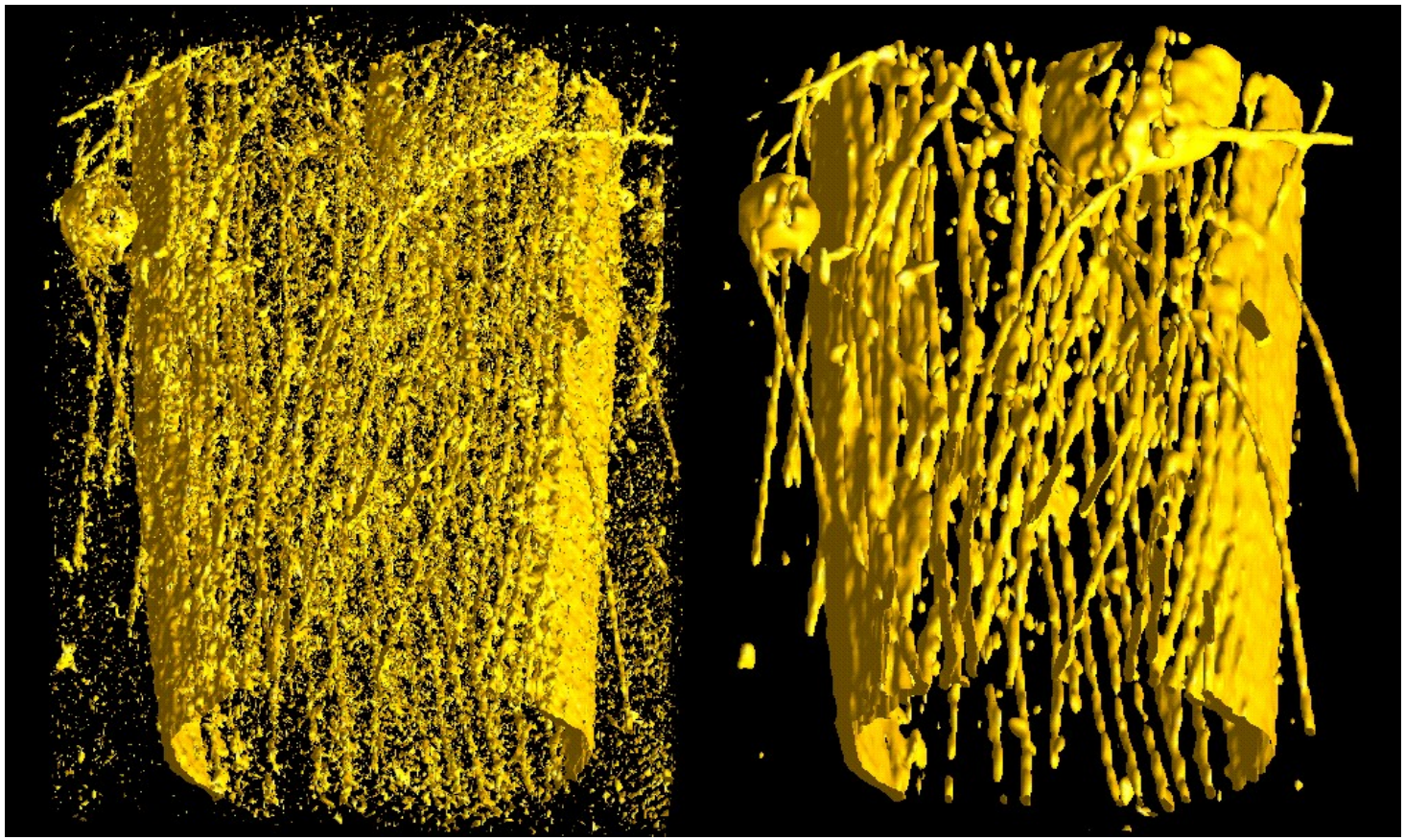


Whitaker, *Med. Image Anal.*, 2002

Anisotropic diffusion

- Why denoising?
  - Not for solving structures!
  - Important for **segmentation**
  - Useful for **visualization**
- Physical approach:
  - Diffusion is smoothing
  - Modify diffusion to respect edges
    ➜ anisotropy and nonlinearity
- Diffusion constant is determined by local gradient

Anisotropic diffusion

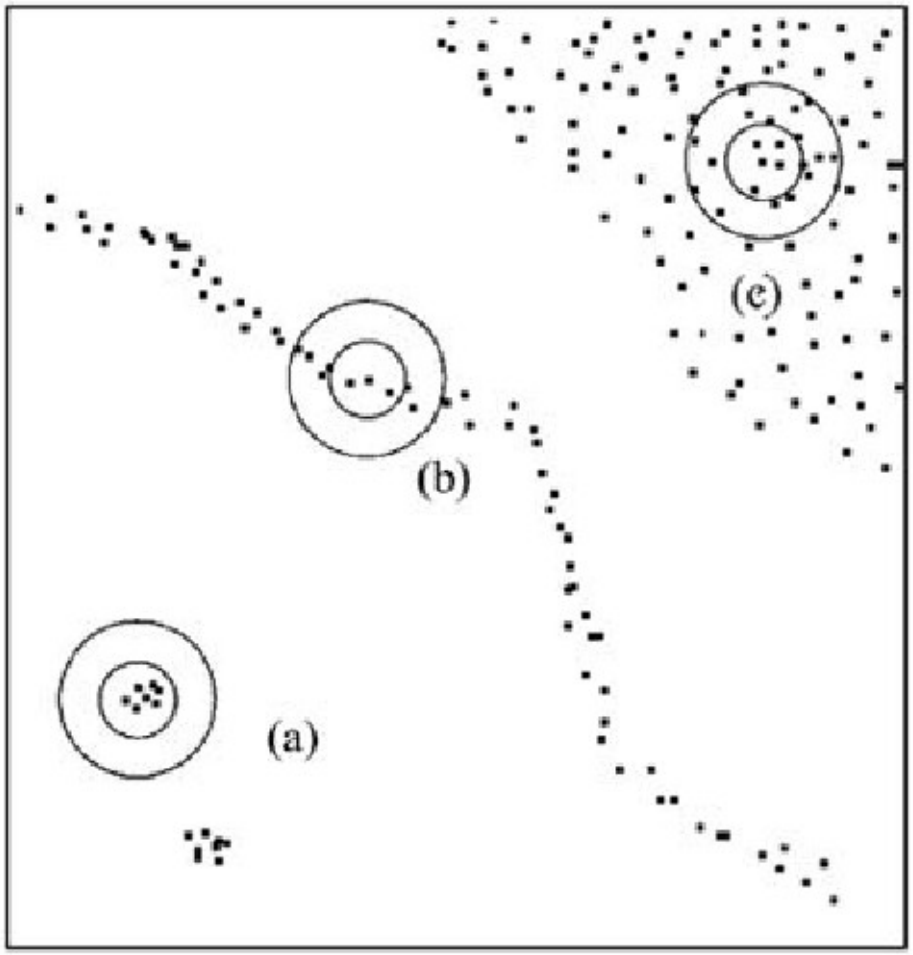


original denoised

Electron tomographic reconstruction of a DMPC vesicle with actin filaments

R. Grimm, M. Bärmann, W. Häckl, D. Typke, E. Sackmann, W. Baumeister: Biophys. J. 72 (1997) 482-489

Scaling-index method



# of points in a sphere that belong to the object

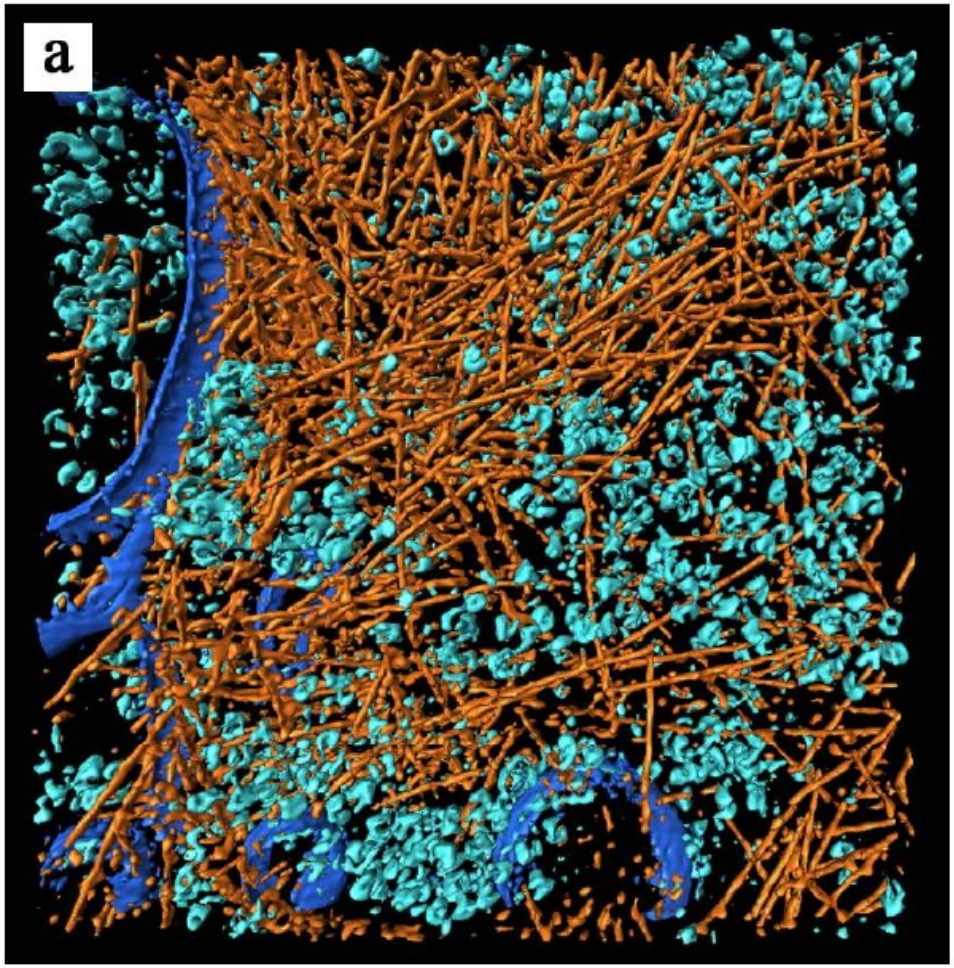
characterizes the dimensionality of the object

$$N(p_i, r) \propto r^{\alpha}$$

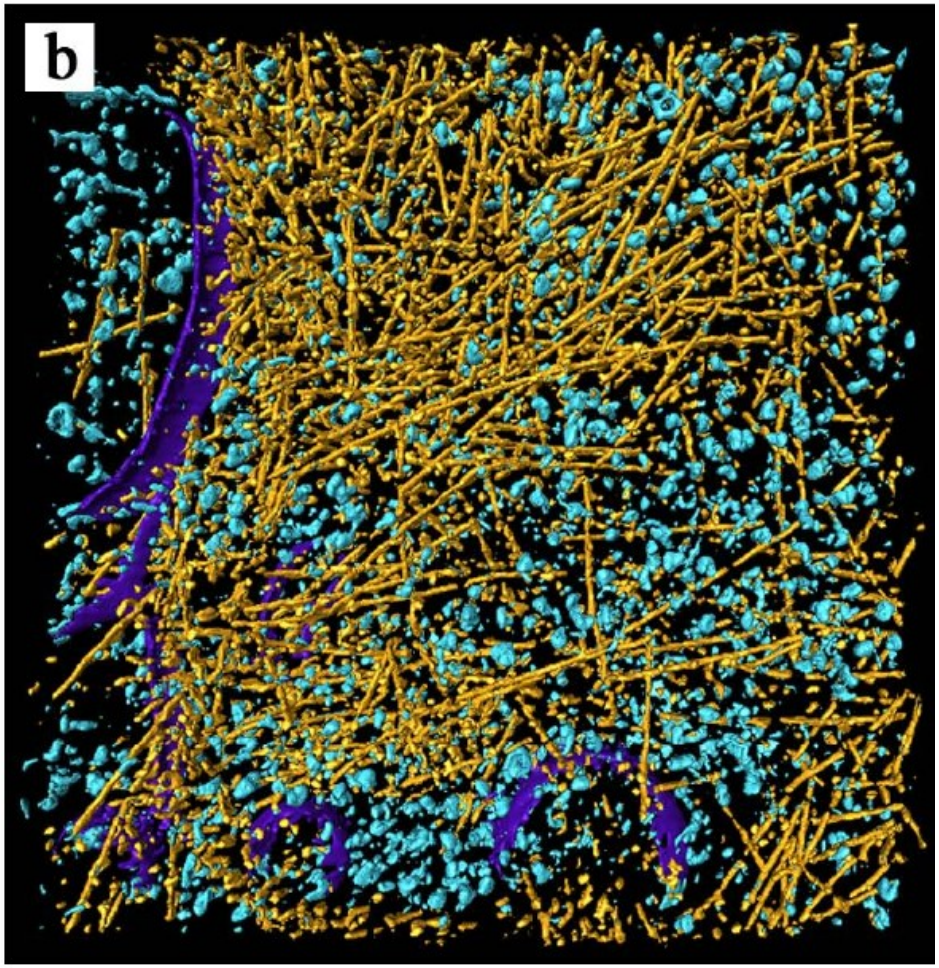
Jamitzky et al, *Ultramicroscopy, 2001*

Scaling-index segmentation

A. Linaroudis, Ph.D. Thesis, 2006

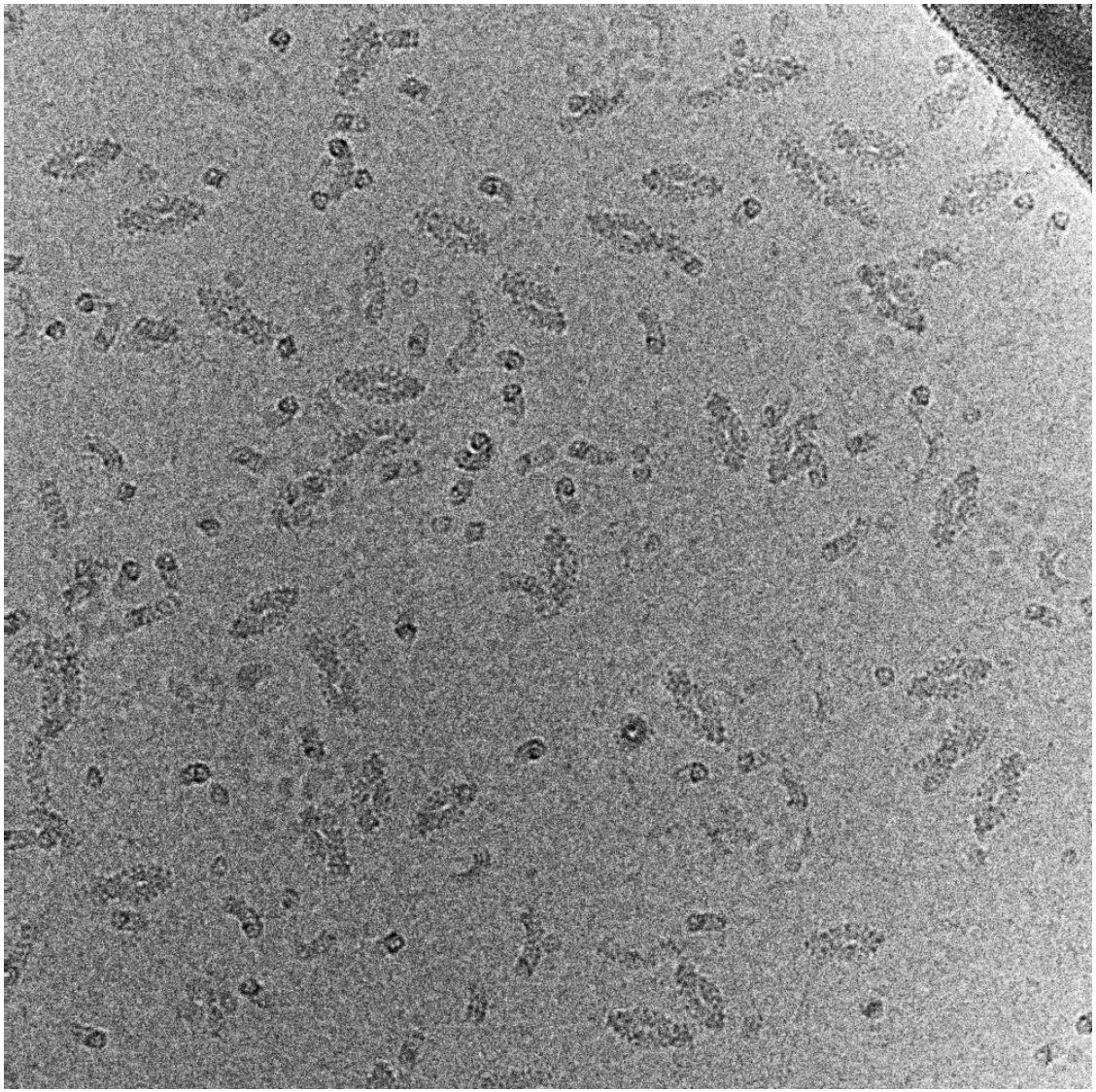


Manual



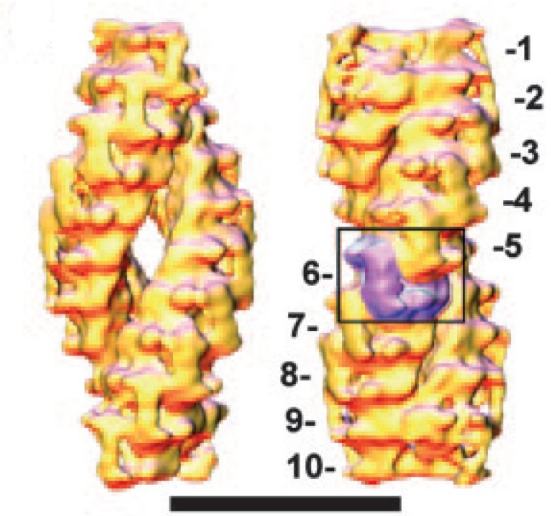
Automatic

Single-particle method



Tripeptidyl-peptidase II
(TPP II)

courtesy of B. Rockel, Martinsried



Single-particle analysis

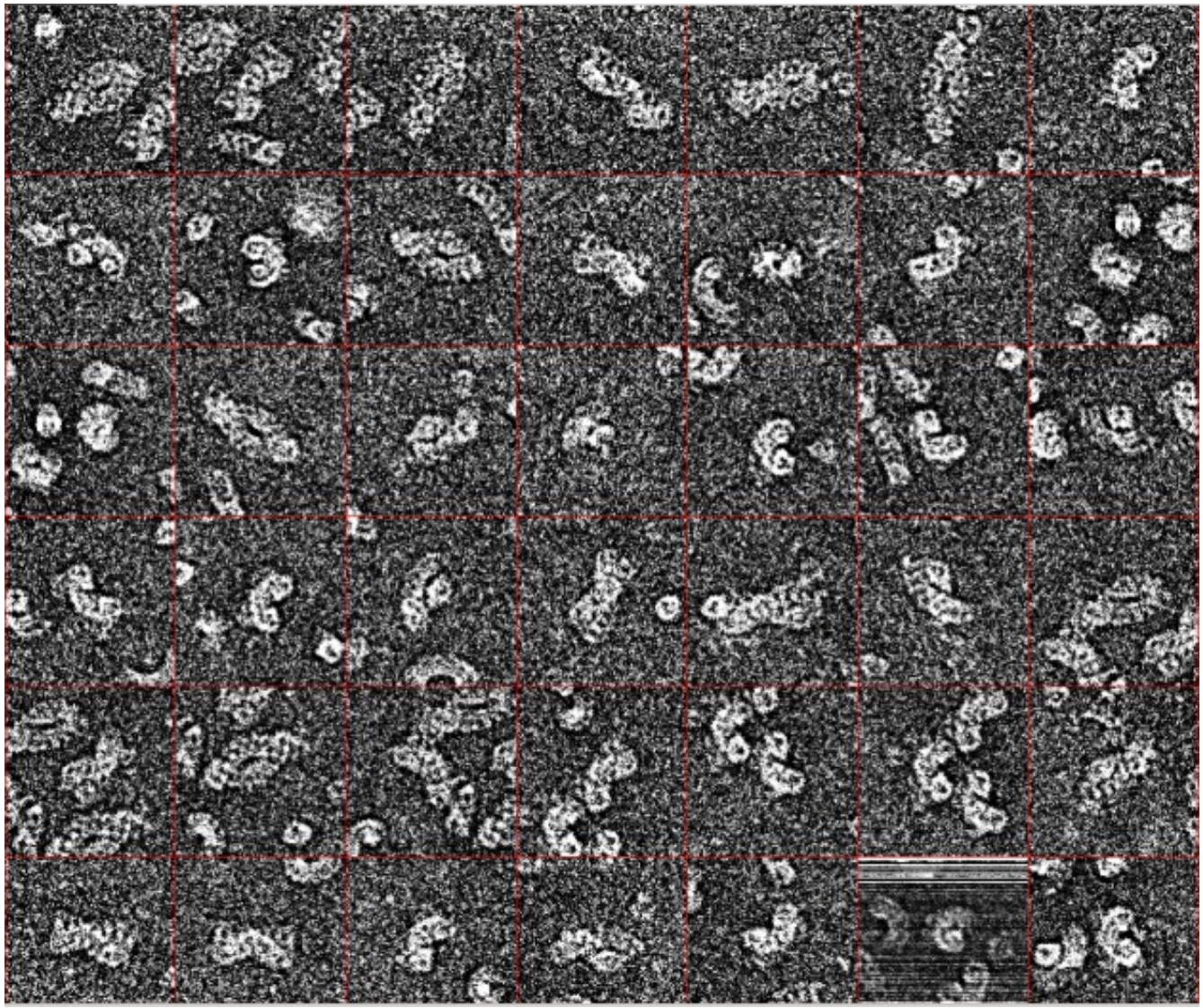
- Obtain high resolution by computational averaging of images
- Assemble 2D projection views into a consistent 3D model
- Problem:

  Orientations of views are unknown
  ➜ hidden variables problem
  EM algorithm

- Current solution:

  ad hoc algorithm (EMAN)

Single particle stack

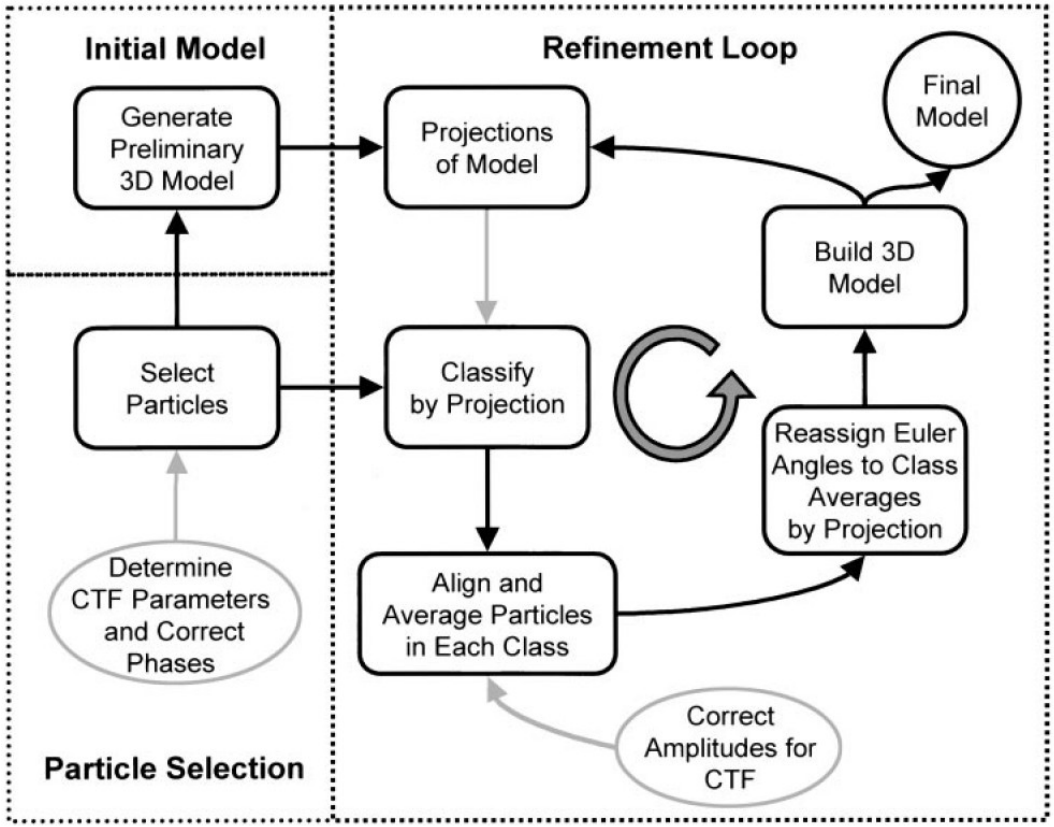


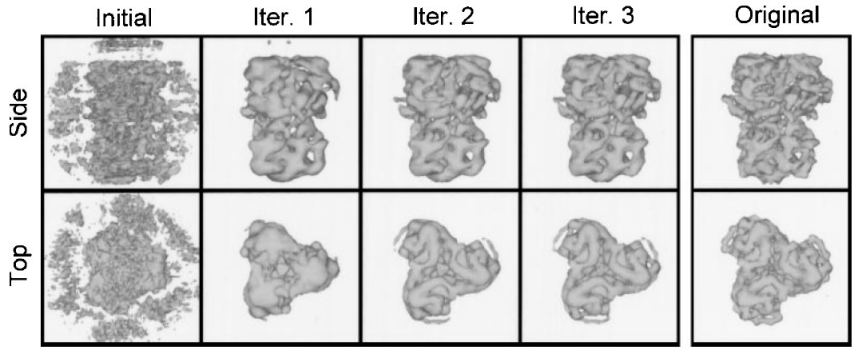
Typically several 1000 to 50000 individual images

Problems:

- Automatic selection of particles
- Alignment
- Classification
- Angular assignment
- 3D reconstruction

EMAN algorithm





EMAN single-particle reconstruction code

Steve Ludtke, Baylor College, Houston, TX

Ad hoc algorithm:
- No proof of convergence
- Local optima?

from: S. Ludtke, *J. Struct. Biol.*, 1999

EM algorithm

Parameter estimation with hidden variables:

Joint probability distribution:

normal distribution

$$p(I, \phi | V) = \mathcal{N}(I - P_\phi V)$$

One parameter (to be estimated):

V = 3D volumetric density

Two random variables:

I = Set of projection images (observed)

ϕ = Set of projection angles (hidden)

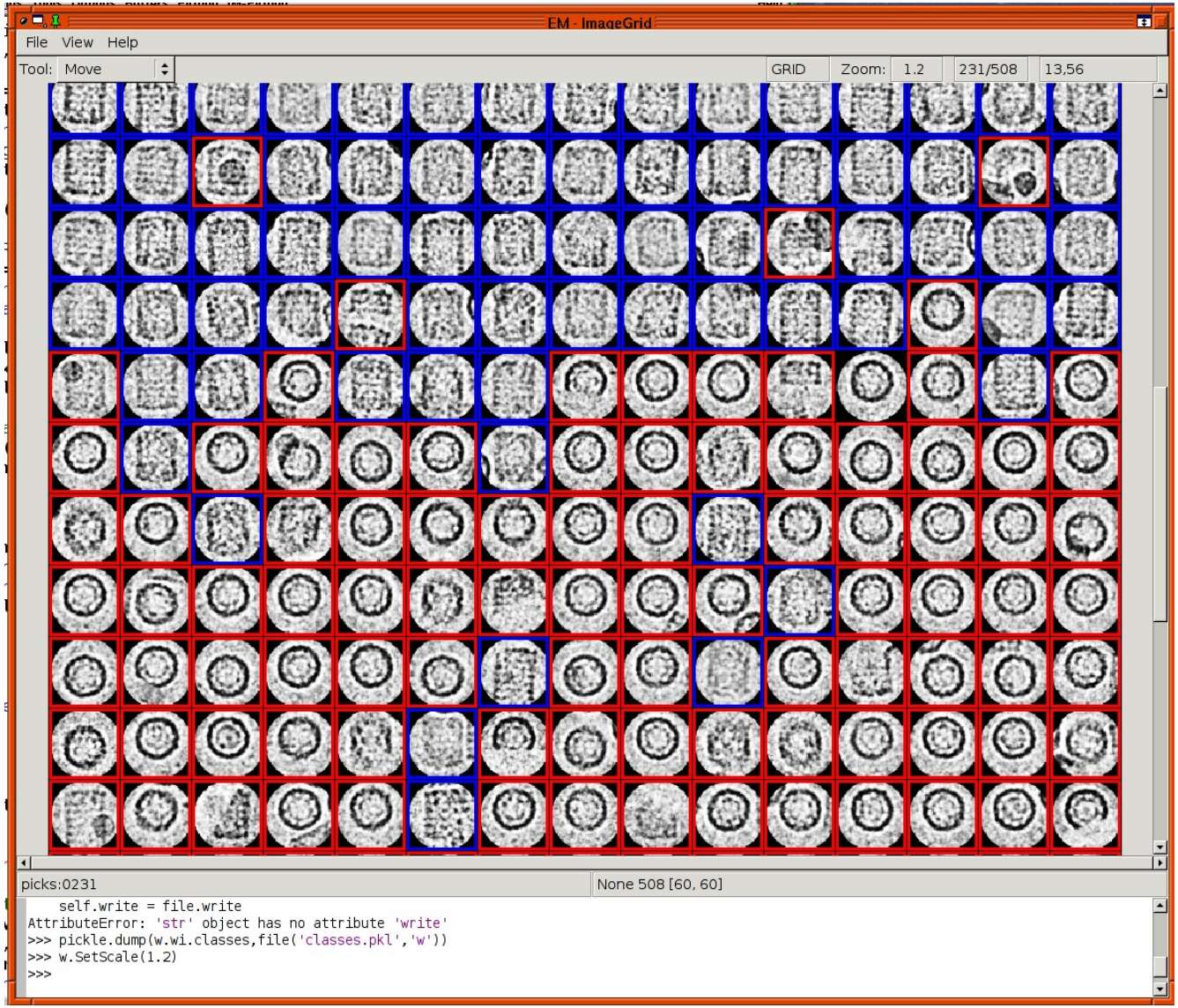
Particle picking

- Problem:
  - Views are randomly distributed on images
  - Must pick regions with particles from image
- Difficulty: high noise ➜ simple template matching does not work
- Approach:

Initial picks by linear correlation

Use a Support Vector Machine (SVM) to select for correct particles according to a manually chosen data set

Picking by template matching



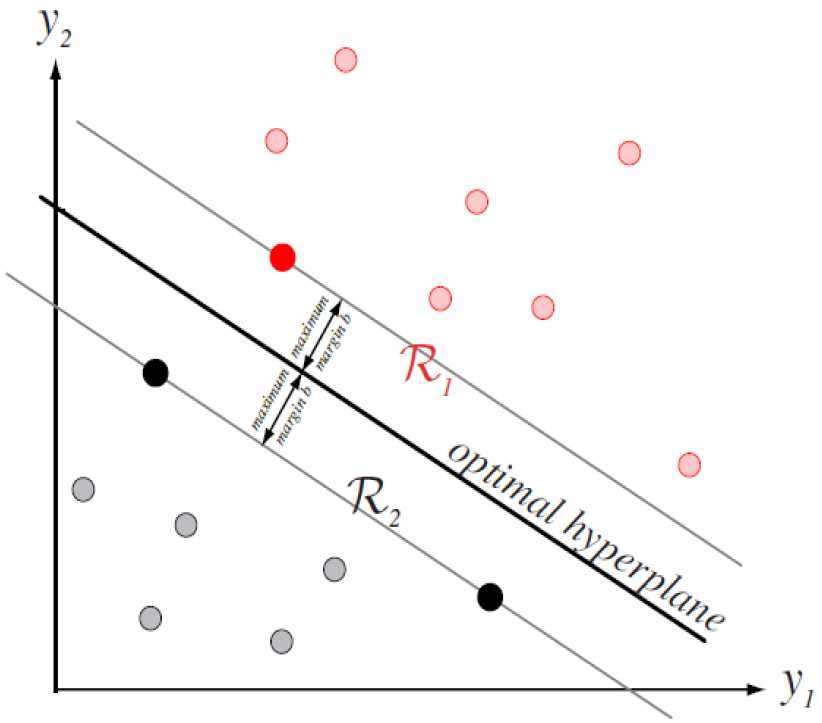
Picking by
linear correlation

many
mis-picks

Apply SVM
to pixel vector
(reduced) of
the images

Coloring: training data set

Support Vector Machines



From Duda *et al.*, *Pattern Classification*

Machine Learning:

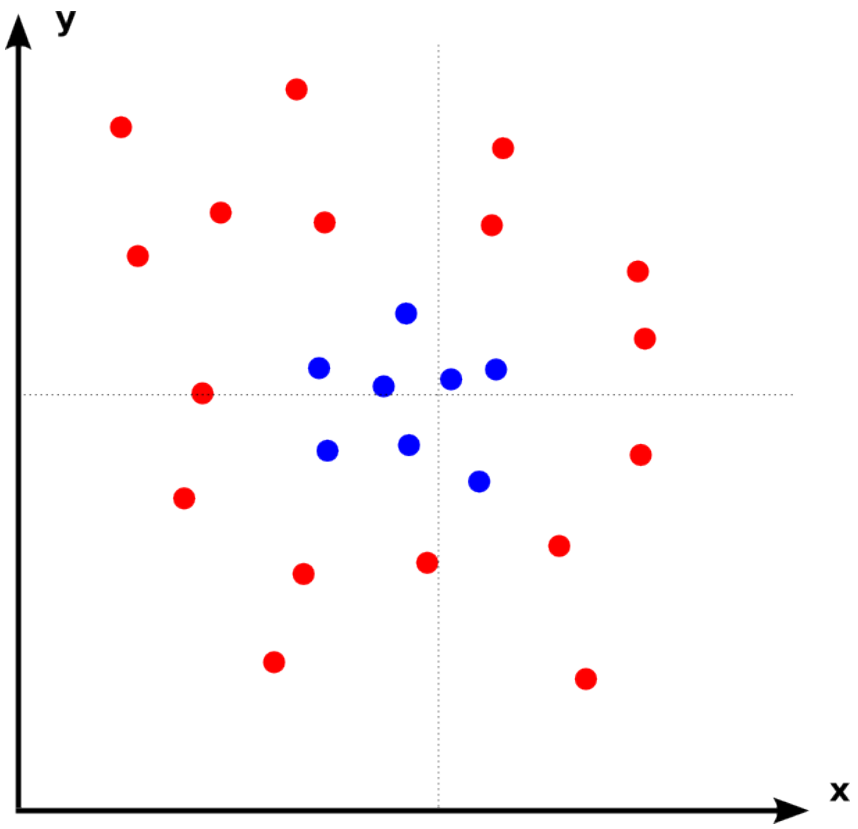
- Training (vs rules)

Support Vector Machine:

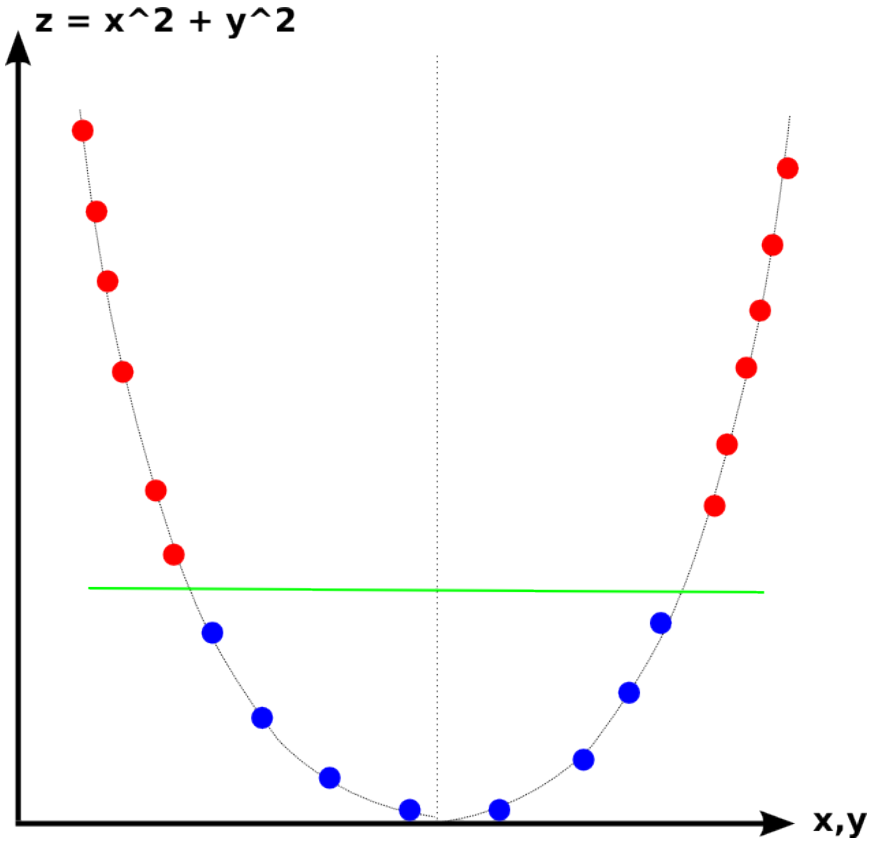
- Linear classifier
- Extended to higher polynomials
- Efficient calculation of the separating hyperplane by duality transform

Non-linearity

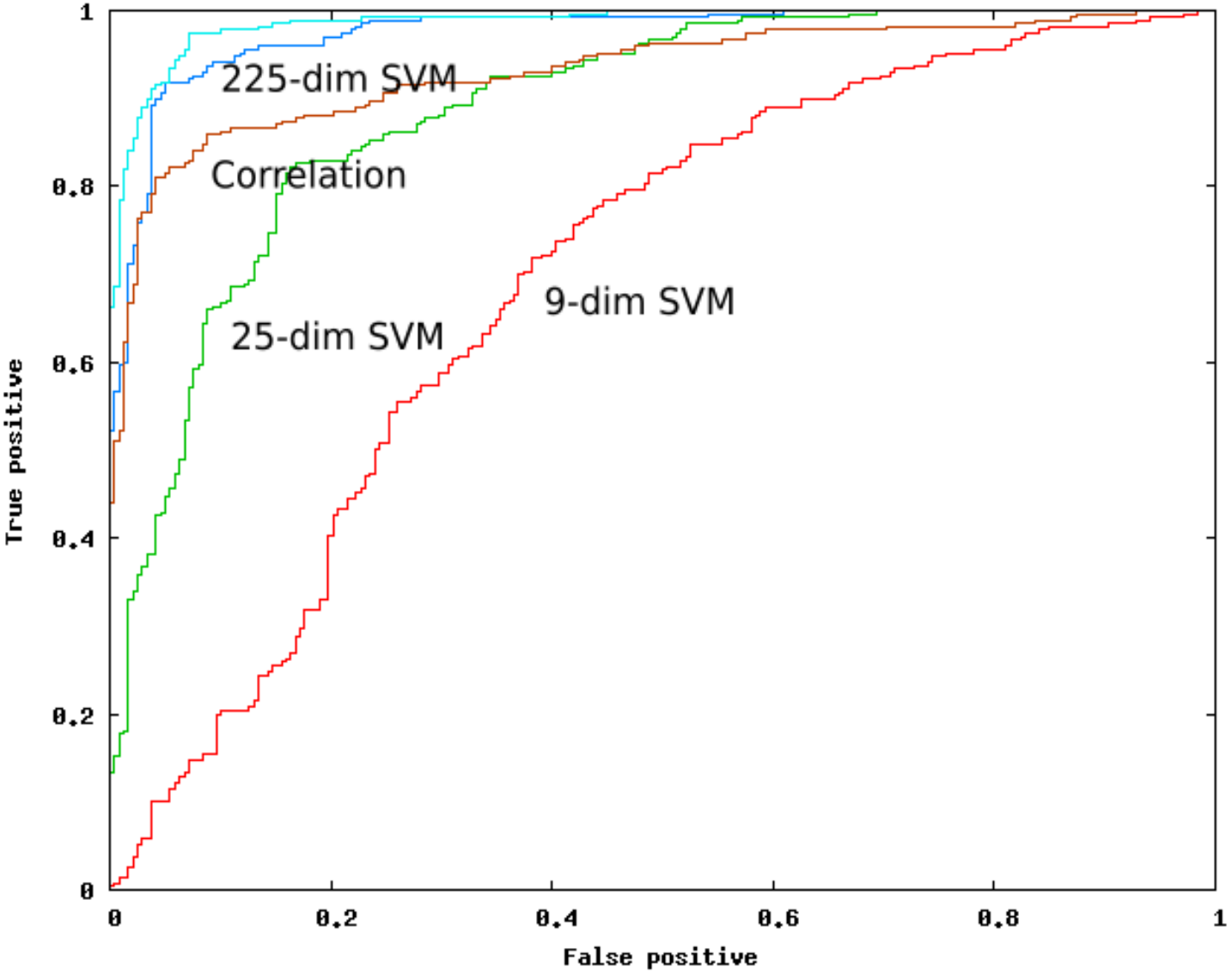
Linearly inseperable



Linearly separable
after introduction of pseudo-variable



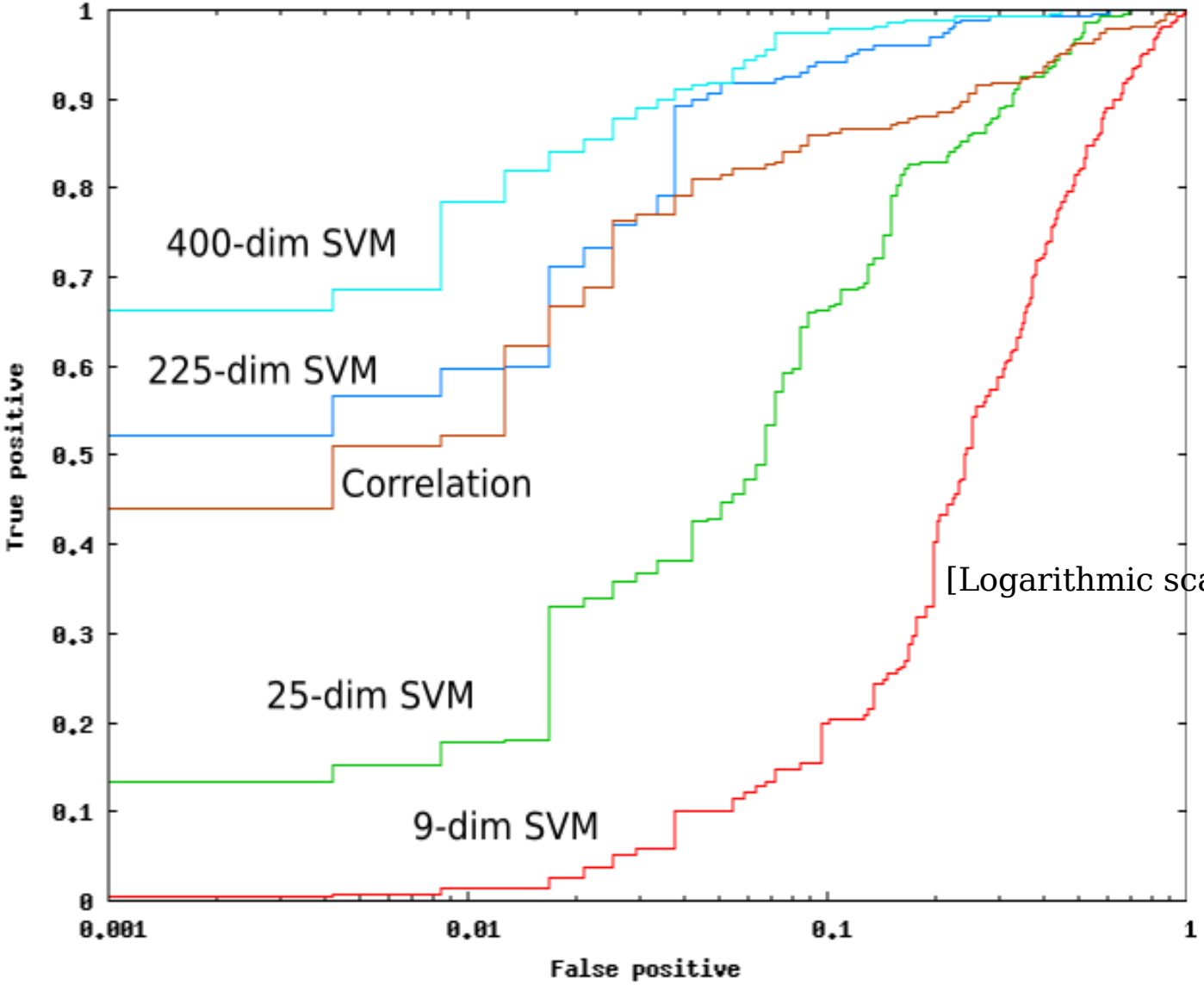
Improving picking using SVMs



Receiver operating characteristics for different feature set sizes

M. Tacke, C. Best 2006

Improving picking using SVMs

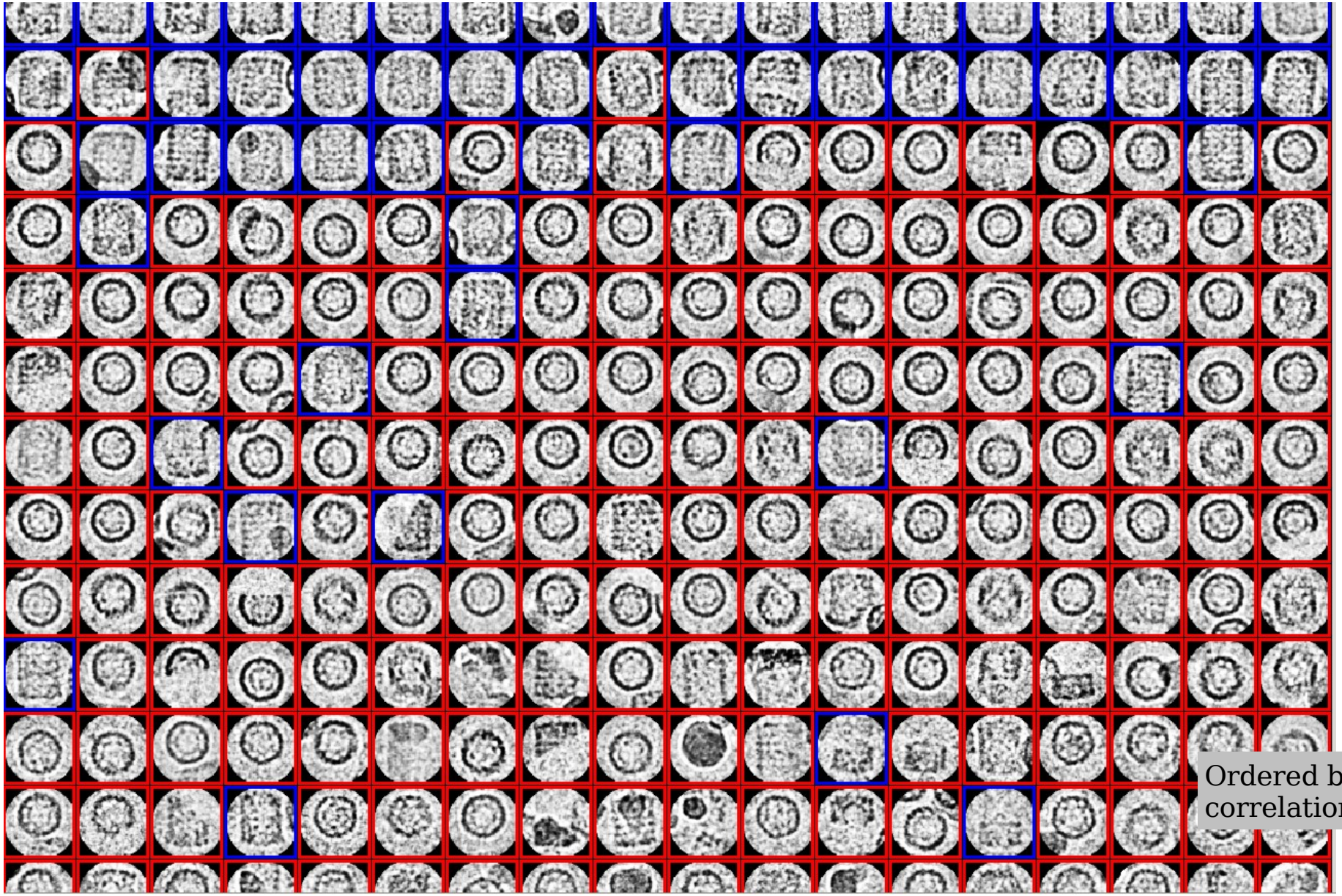


tics
t

[Logarithmic scale]

Best

Picking result



Model-free particle classification

- How can we sort the views of a particle according to the viewing angle (elevation, azimuth) ?
- Answer: Similar angles ➜ similar images
- Probabilistic model:

$$P(M|\phi; M_0, \phi_0) = \left(\frac{1}{2\pi\kappa(|\phi - \phi_0|)}\right)^{D/2} \exp\left(-\frac{|M - M_0|^2}{2\kappa(|\phi - \phi_0|)^2}\right)$$

Angular distance-to-similarity kernel

- Probability for an image M given an assigned angle ϕ, a reference image $M^{(0)}$, and a reference angle $\phi^{(0)}$:

Gaussian with a width that gets wider when the images are farther apart.

Self-organizing point map

- Joint probability distribution:

$$P(\{M^{(n)}\}|\{\phi^{(n)}\}) = \prod_{i=1}^{N} P(M^{(i)}|\{M^{(i')}, \phi^{(i')}\})$$

- Maximum-likelihood principle ➜ Hamiltonian:

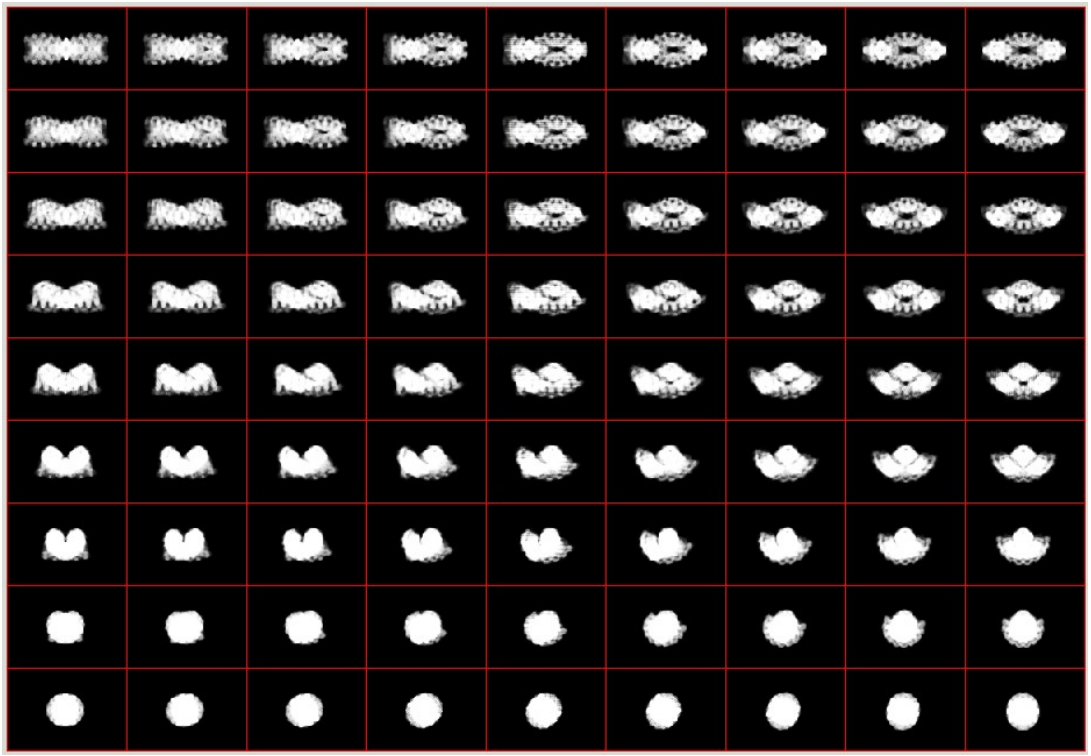
$$-\ln L(\phi) = \sum_{n,m} \left(\frac{D}{2} \ln 2\pi\kappa(|\phi^{(n)} - \phi^{(m)}|) + \frac{|M^{(n)} - M^{(m)}|^2}{2\kappa(|\phi^{(n)} - \phi^{(m)}|)^2} \right)$$

Attractive force Repulsive force

Point-to-point potential ➜ multidimensional scaling

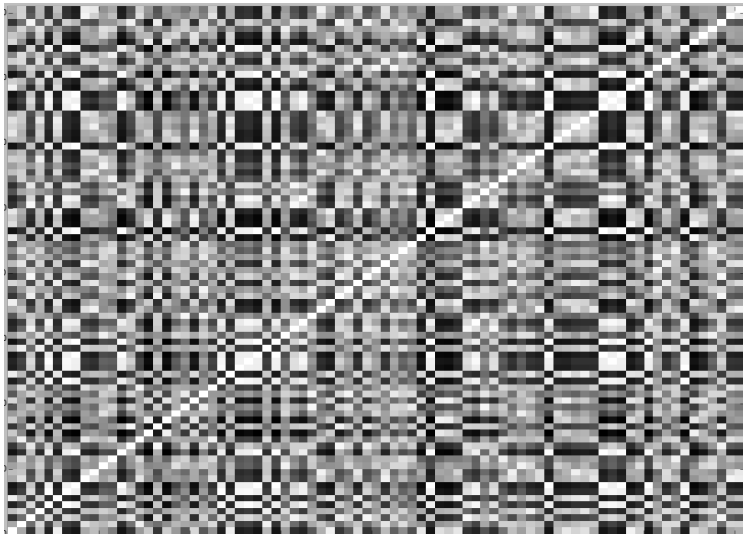
- Gradient descent solution

Similarity matrix



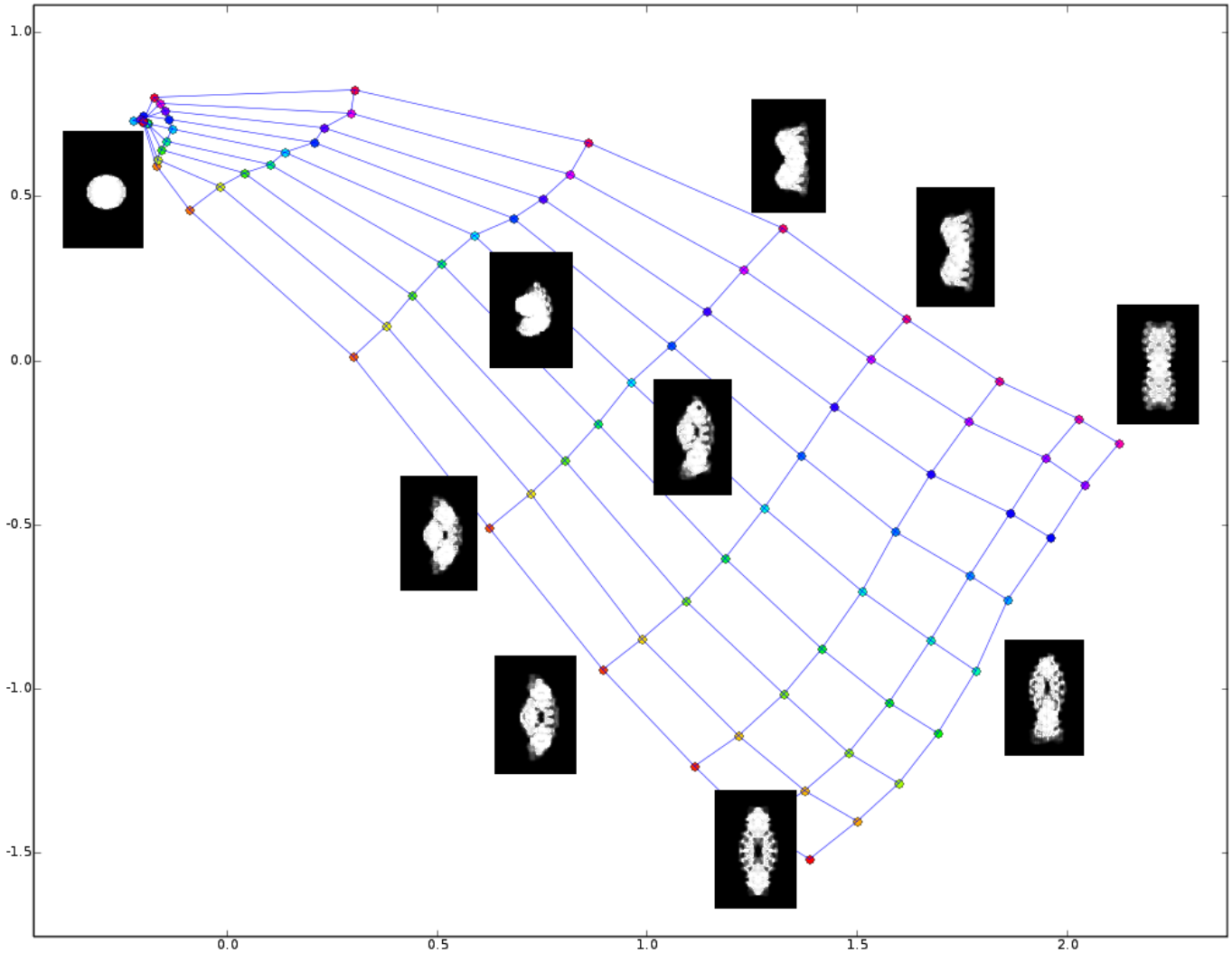
9x9 projections
of TPP2

Correlation matrix:



Pairwise correlation
max. over translations and
rotations

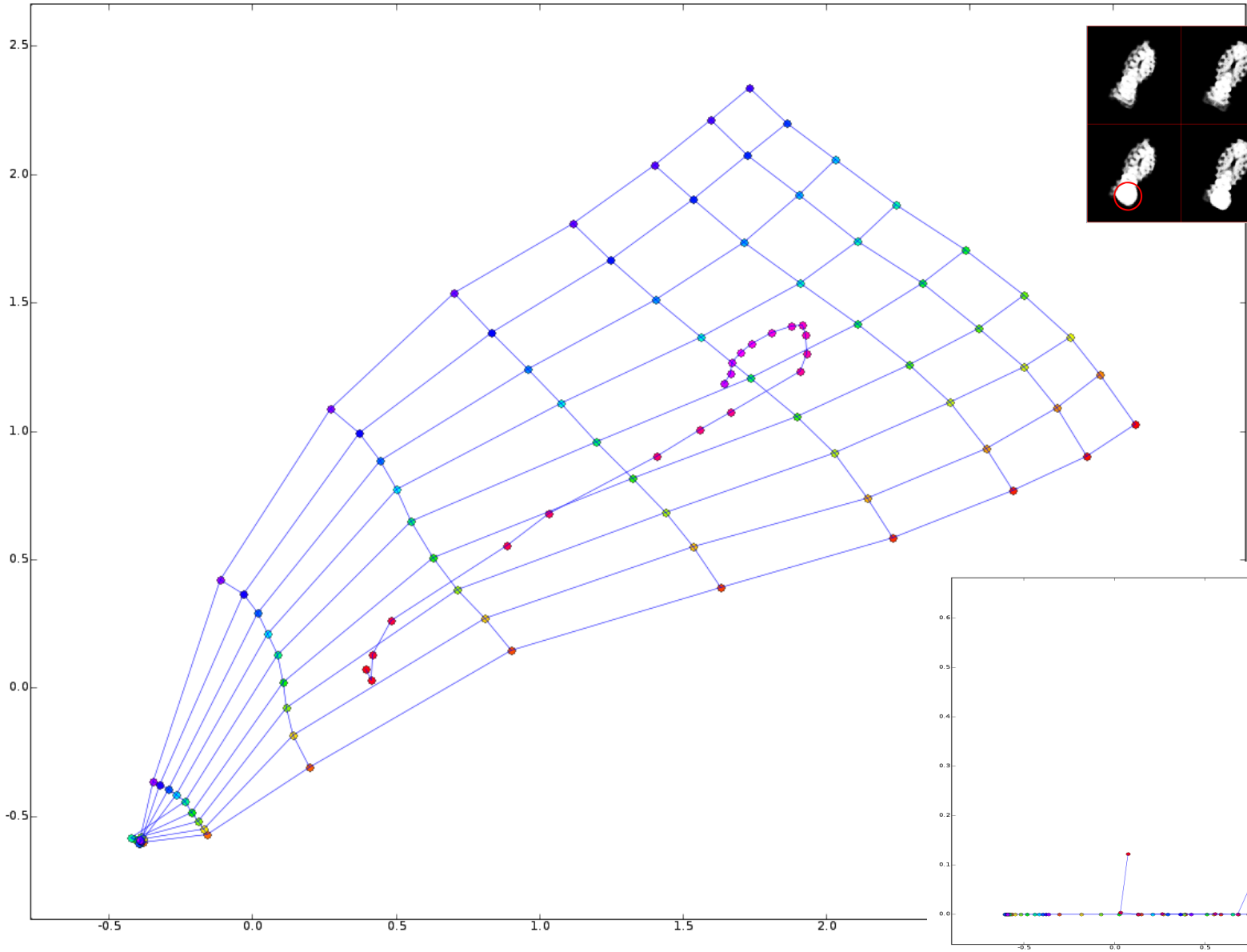
Result

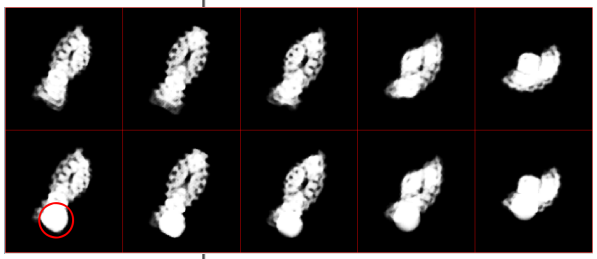


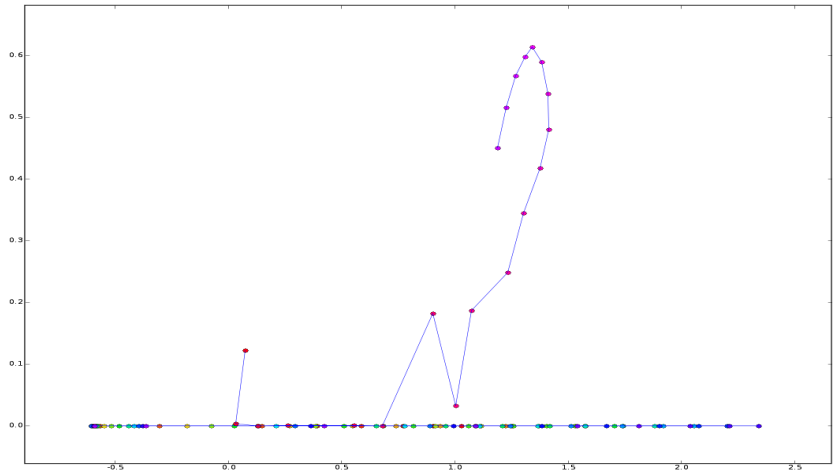
Good representation of original distribution of viewing angles

Good as an initial model for iterative refinement

Tomographic classification

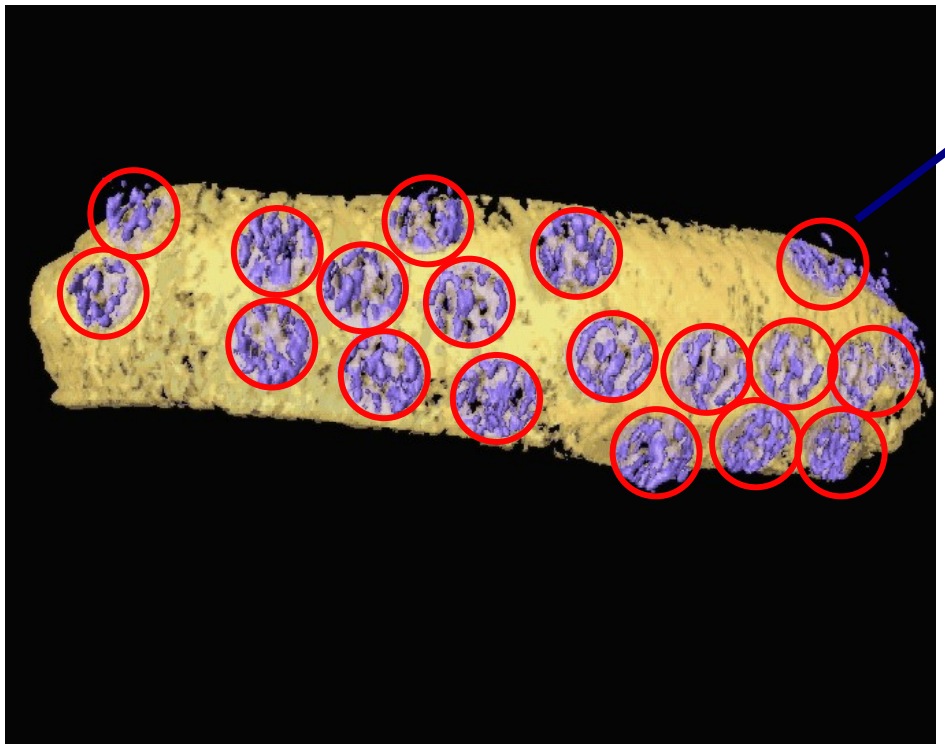






3D averaging

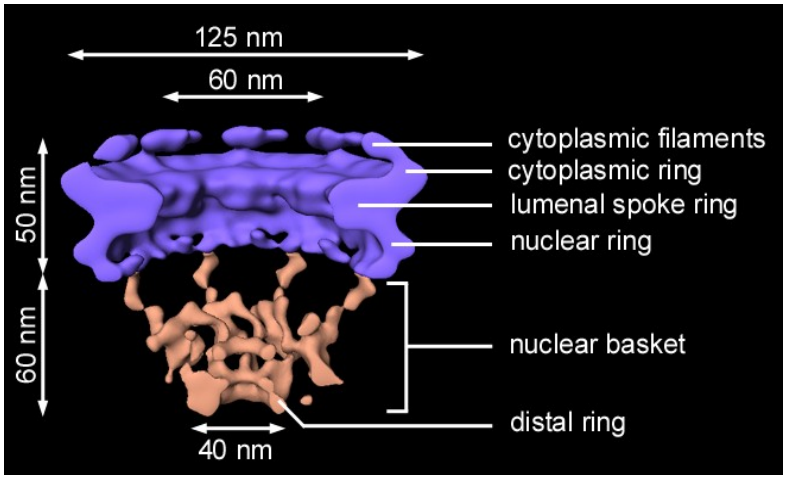
Nuclear pore complex



Matching

Aligning

Averaging

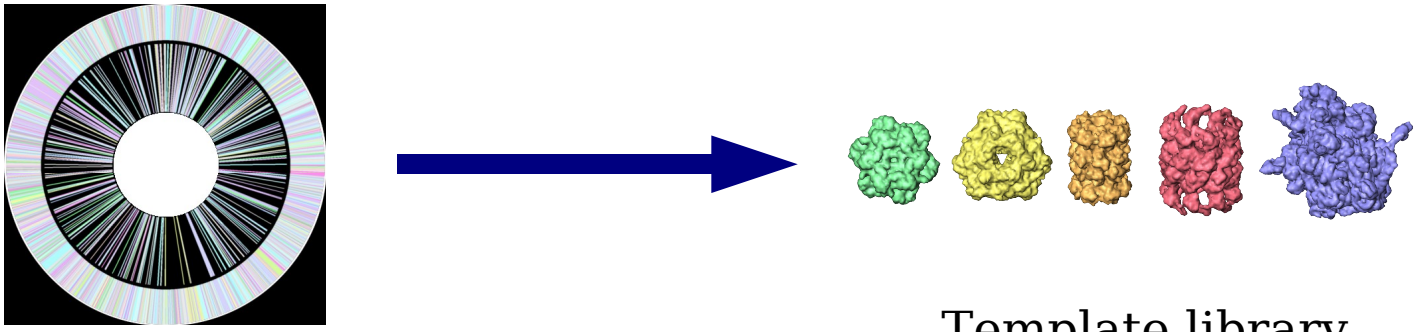


3D reconstruction

Beck *et al.*, *Science*, 2004

“Visual proteomics”

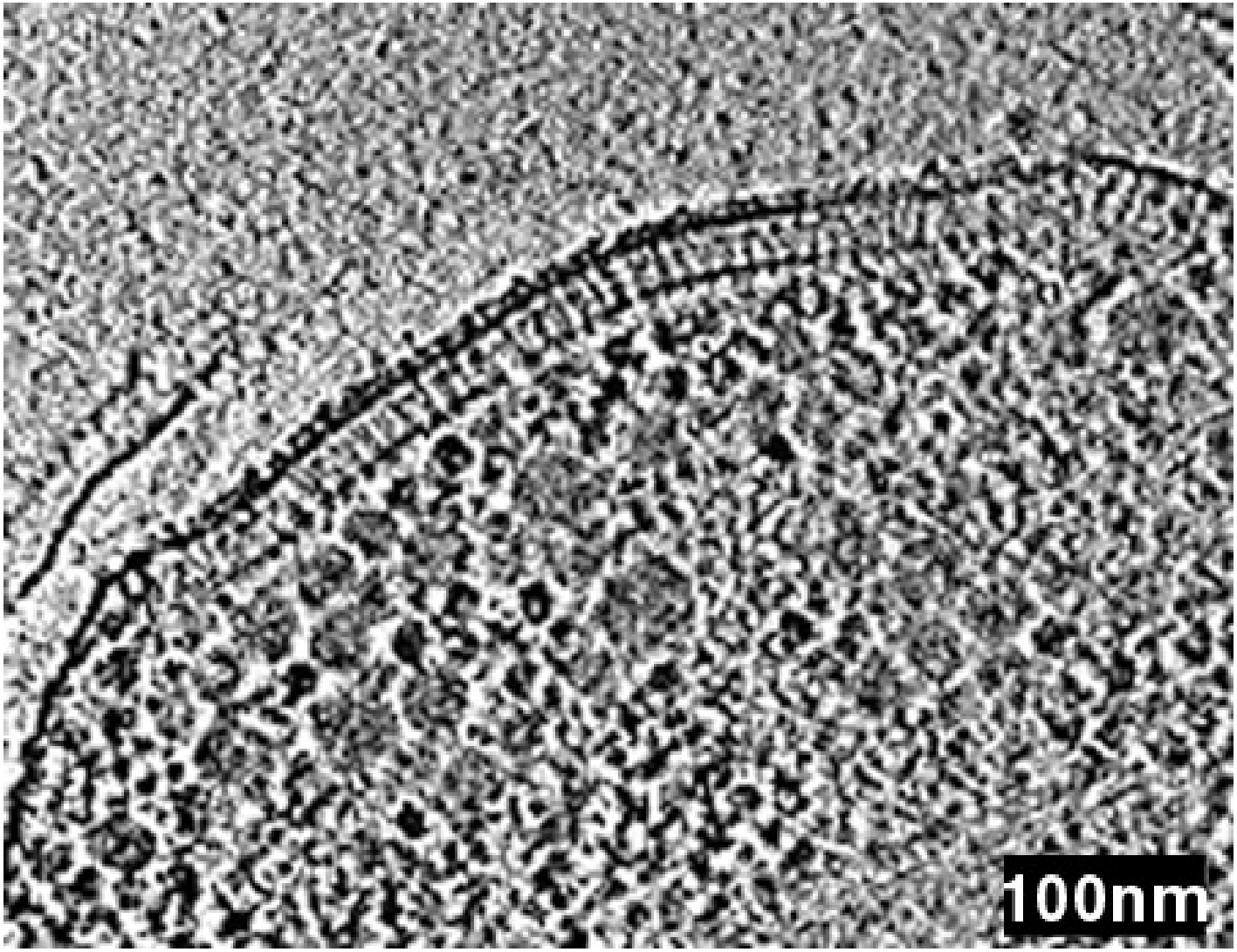
- Identify proteins in cryo-electron tomograms of intact cells or cryo-sections
- Pattern matching against a template library
- Map protein interaction landscape in cells
- High-throughput pipeline



Genome / proteome

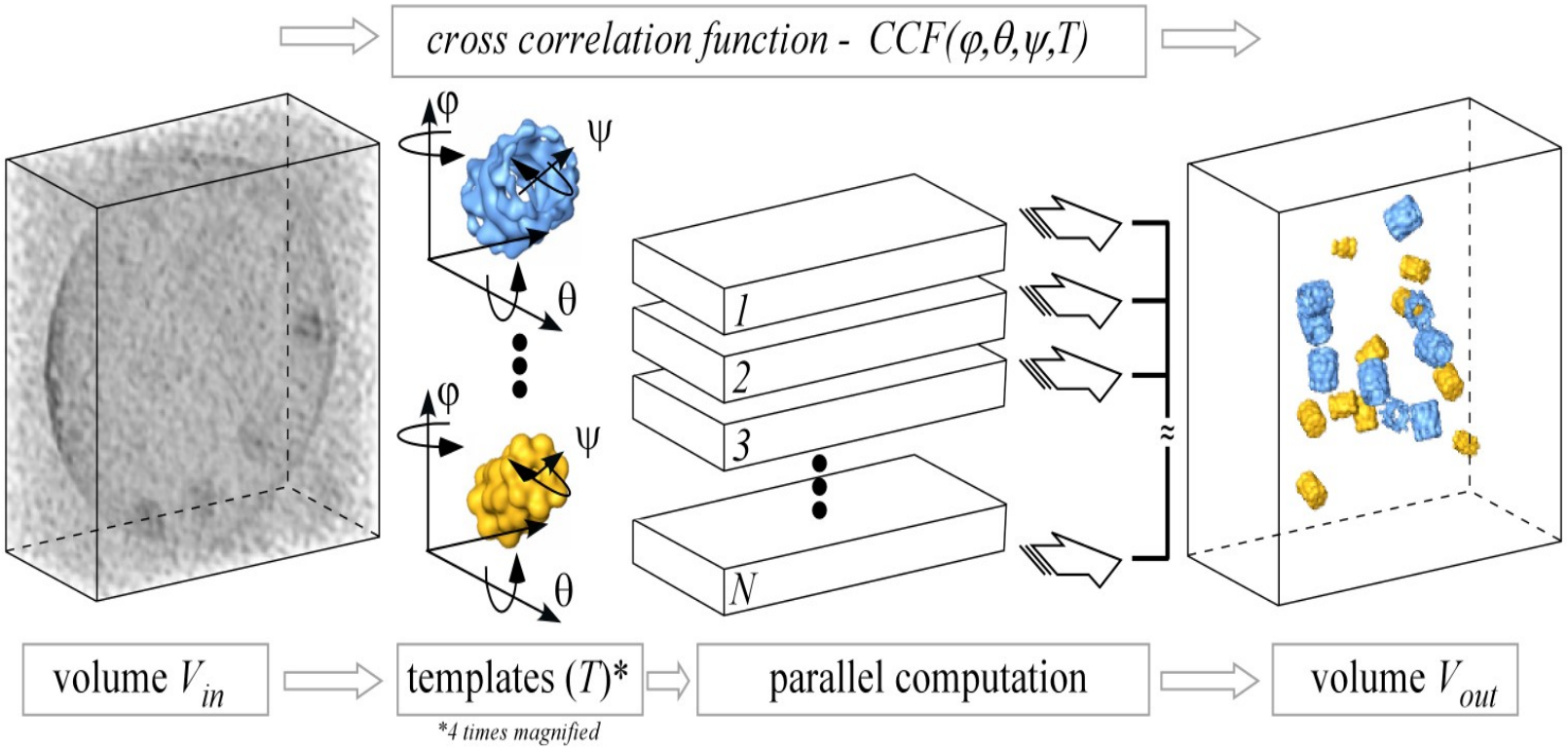
Template library

Macromolecular crowding

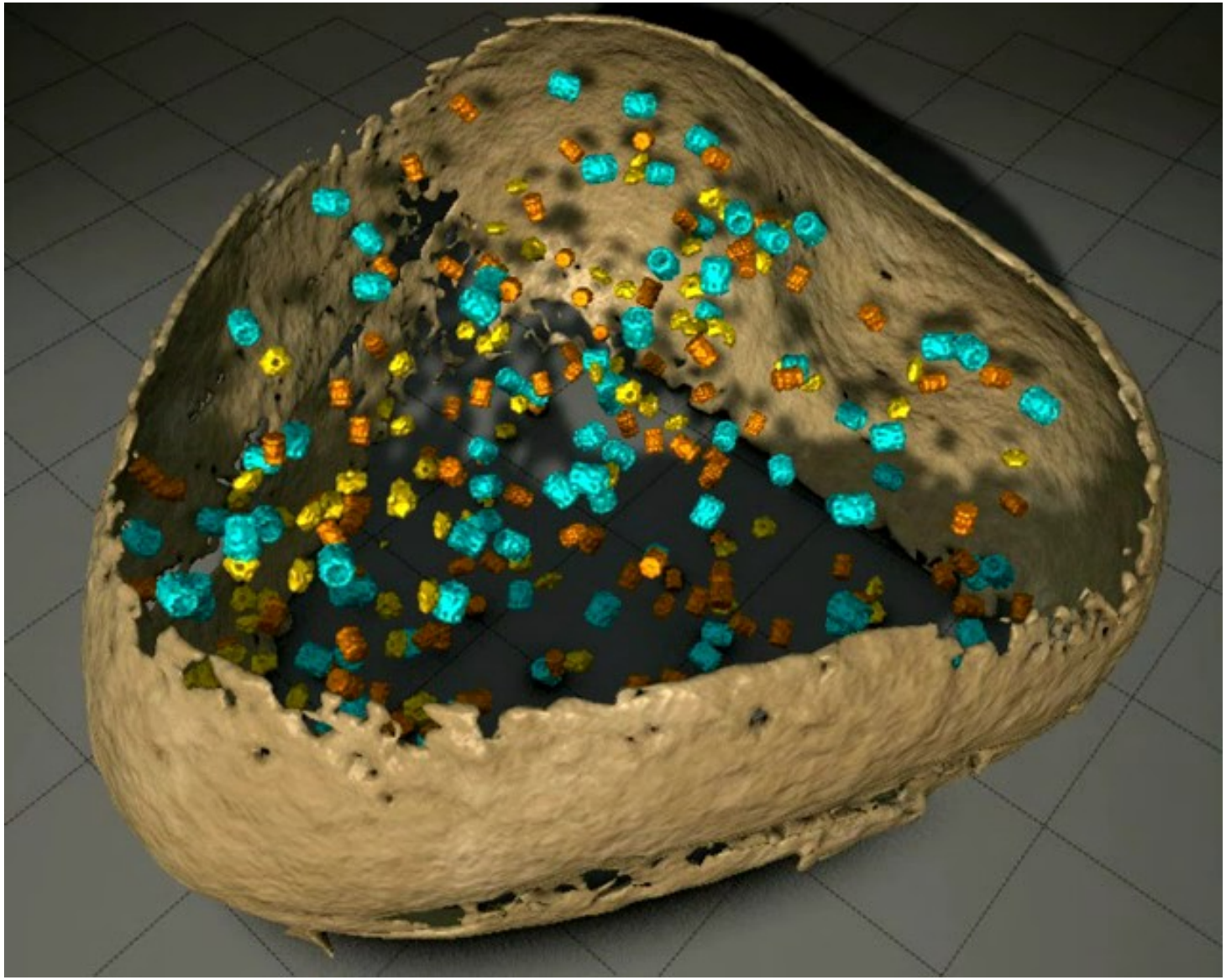


Visual proteomics

Computational pipeline:



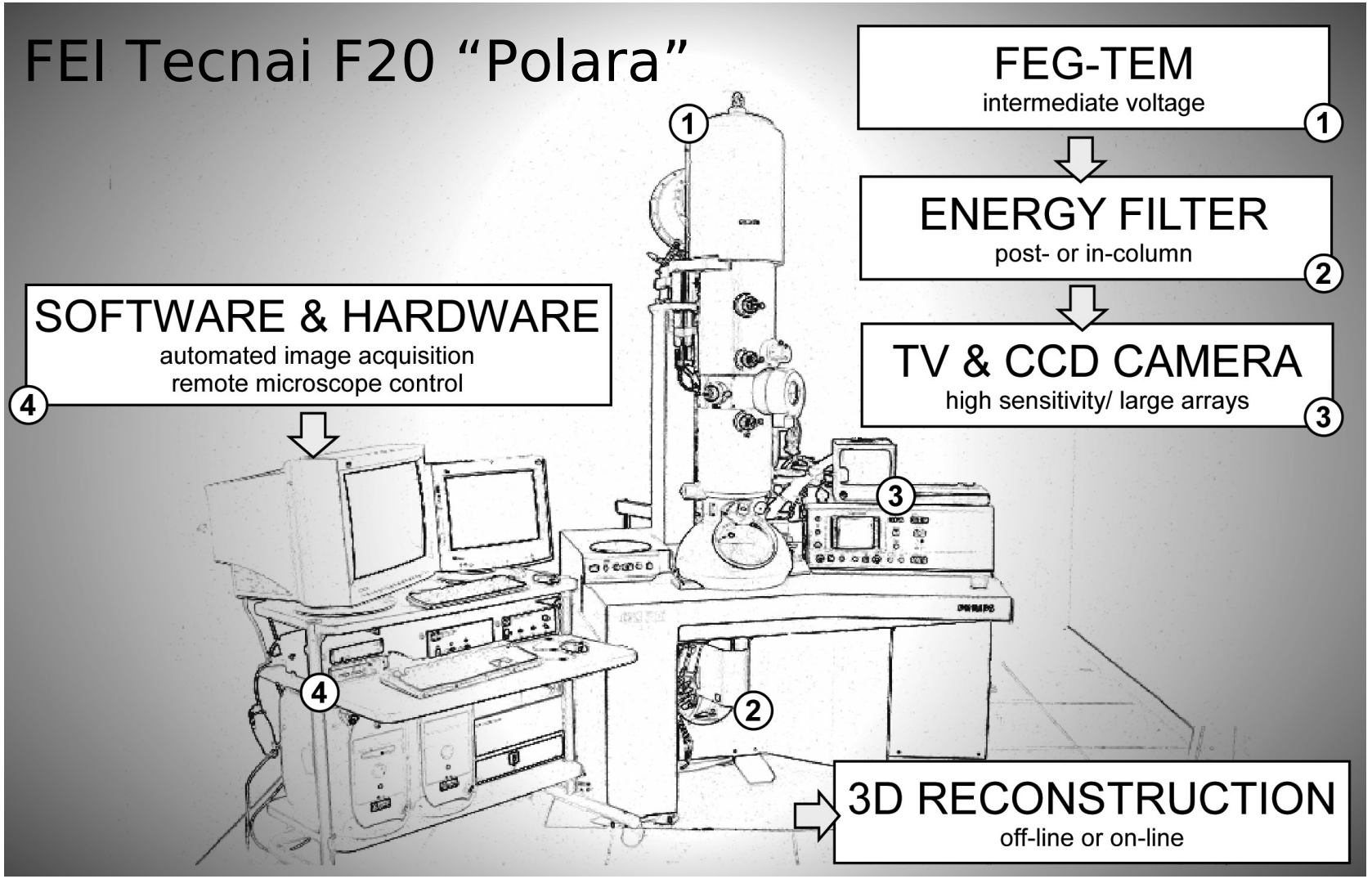
“Visual proteomics”



Conclusions

- Cryo-electron tomography allows **molecular resolution** imaging of cells in **near live** conditions
- Depends heavily on computational methods for **reconstruction**, **denoising**, and **segmentation.**
- Single-particle averaging methods (both 2D and 3D) requires sophisticated **pattern recognition**, **classification**, and **clustering methods**
- Future influences:
  - Probabilistic modelling
  - Monte Carlo algorithms
  - Machine learning

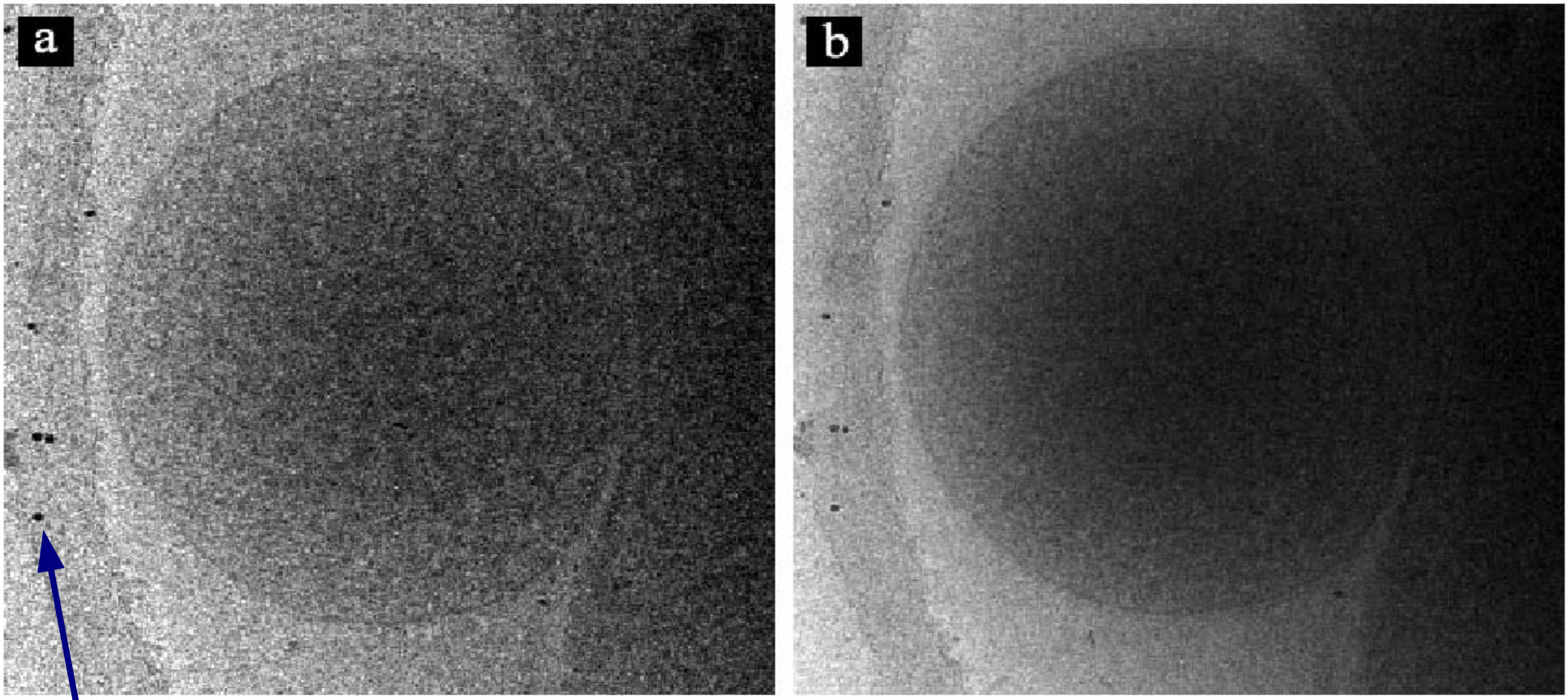
Electron microscope



Price tag: around $1.5M

Electron micrographs

Thermoplasma acidophilum



No energy filtering

With energy filtering

Gold markers for aligment

Image: C. Kofler, Martinsried

Molecular scale imaging

- Light microscopy: limited by wavelength O(100nm)
- Alternative probes:
  - X-rays
    - Short wavelength ➜ atomic resolution (< 0.1nm)
    - Low cross-section, difficult optics ➜ need crystals
  - Electrons:
    - Particle-wave duality, de Broglie wavelength
    - Charged massive particles ➜ high cross section
    - But high damage to specimen ➜ low dose
    - **High noise**

EM algorithm

Estimation step:

$$Q(V|V_0) = \sum_{\phi} p(\phi|I,V) \log p(I,\phi|V)$$

Minimization step:

$$V^{(n+1)} = \max_{V} Q(V|V^{(n)})$$

Differences to ad hoc algorithm:

- $p(\phi|I,V)$ is unity (for assigned angle) or zero (otherwise)