SPHIRE

SParx for HIgh Resolution Electron Microscopy

Initial model strategies

UTMB Workshop

May 7, 2019

Outline

- 3D reconstruction
  - Goal
  - Requirements
  - Reference-based alignment vs. *ab initio* reconstruction
- Different methods
  - Common lines/angular reconstitution
  - Random conical tilt
  - VIPER

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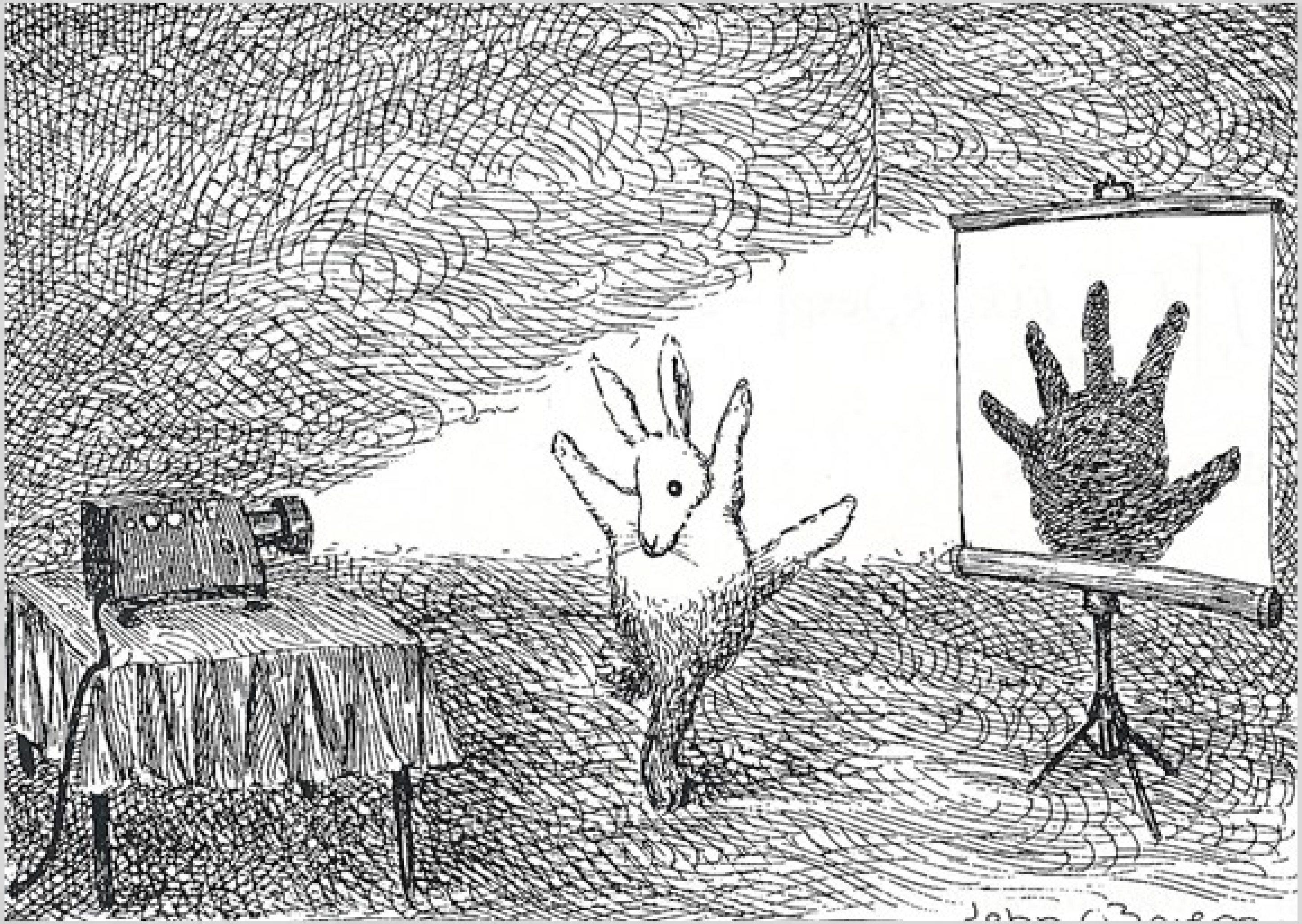
What information do we need for a 3D reconstruction?

1. different orientations
2. known orientations
3. many particles

You can be fooled if you don't have different orientations



John O'Brien, 1991, *The New Yorker*

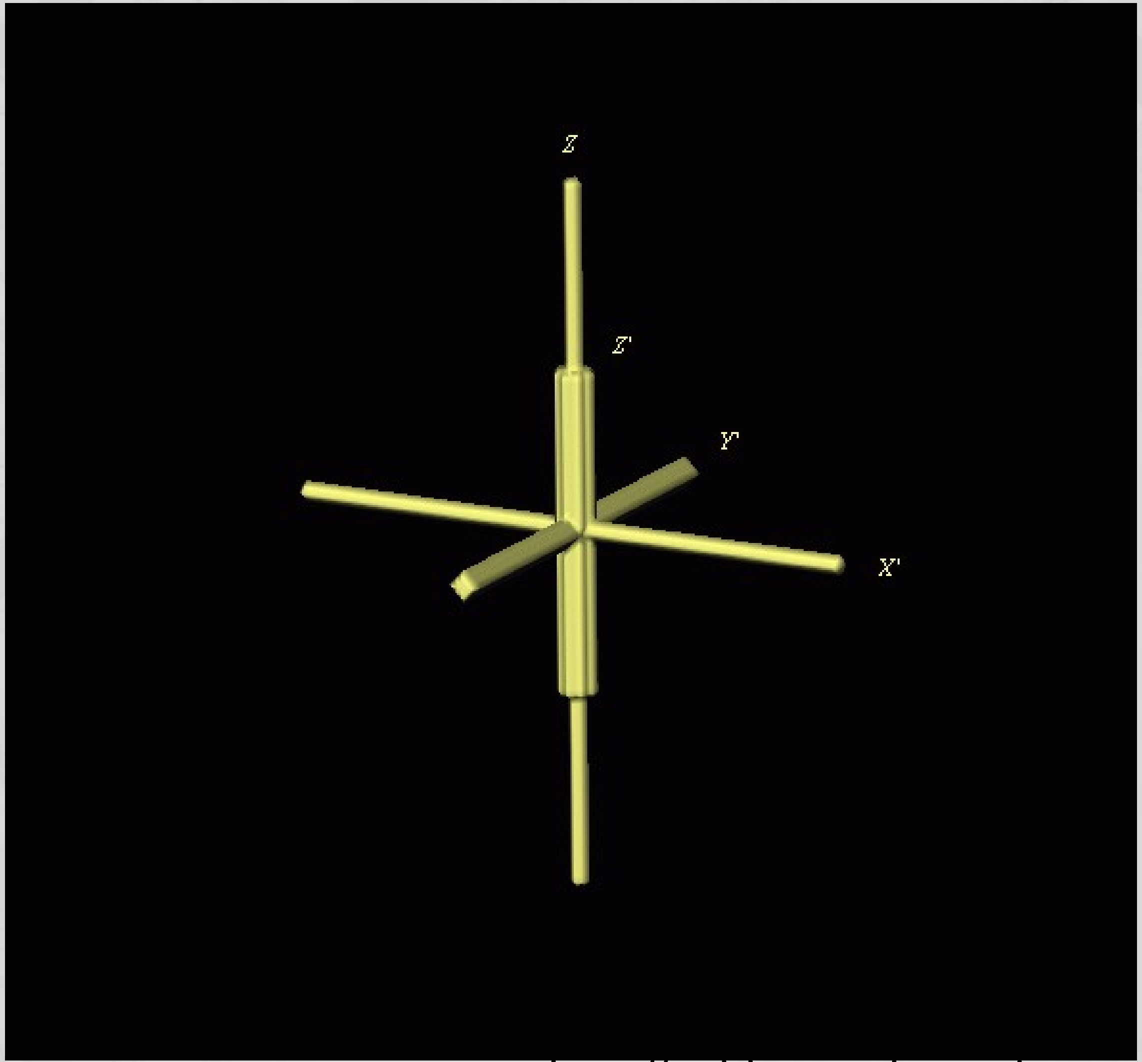
3D reconstruction: Parameters required

Two translational:

- Δx
- Δy

Three orientational (Euler angles):

- ϕ (about z axis)
- θ (about y)
- ψ (about new z)



http://spider.wadsworth.org

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Reference-based alignment

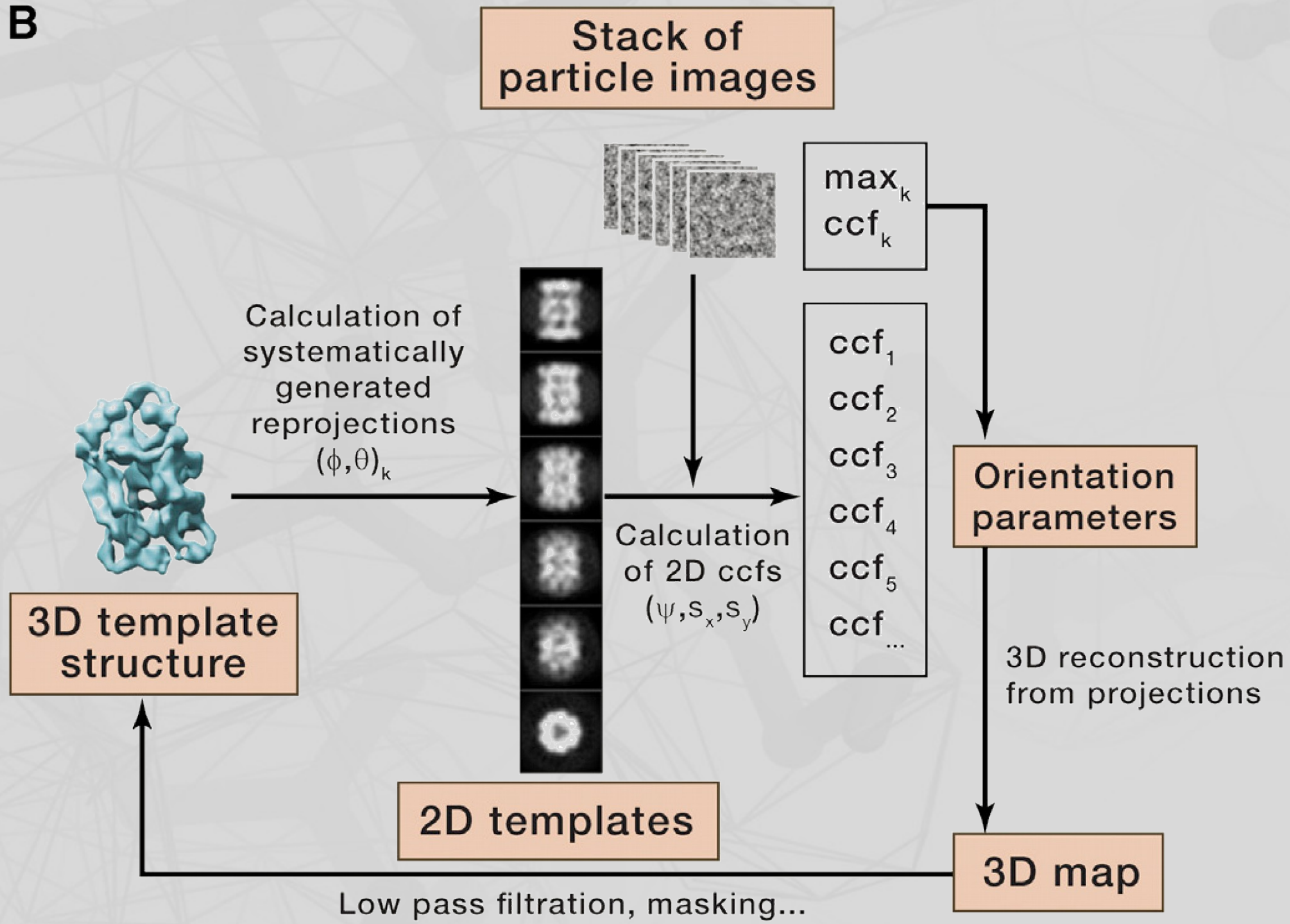
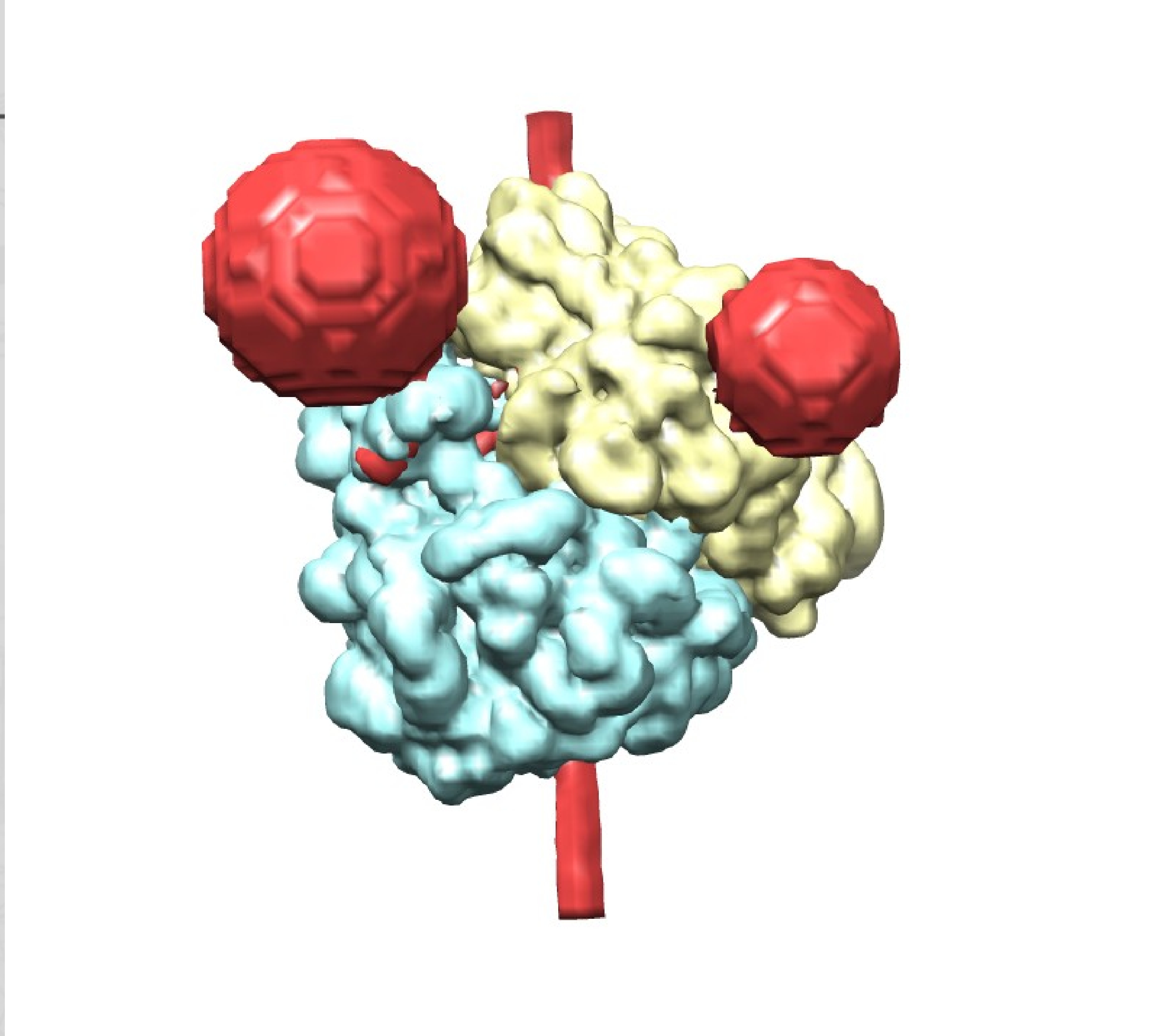
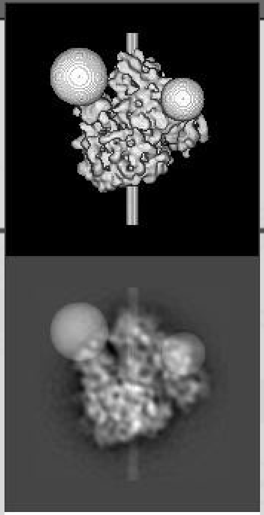


Image from Cheng *et al.* (2015) *Cell* **161:** 438 – 449

The model



(The extra features helped determine handedness in noisy reconstructions.)



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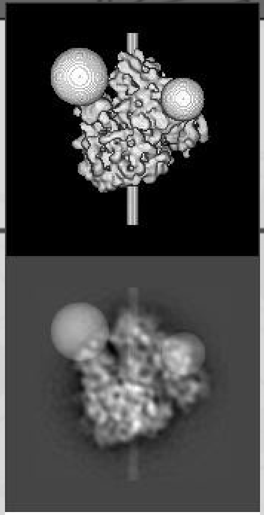

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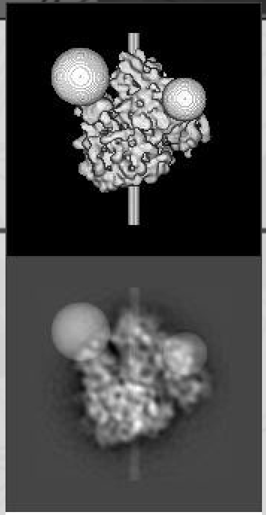

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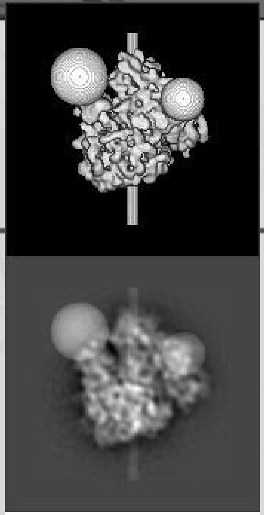

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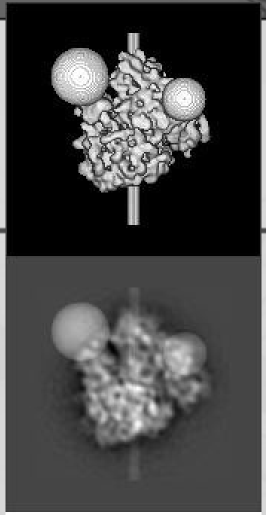

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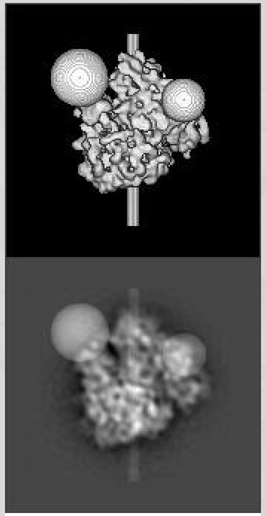

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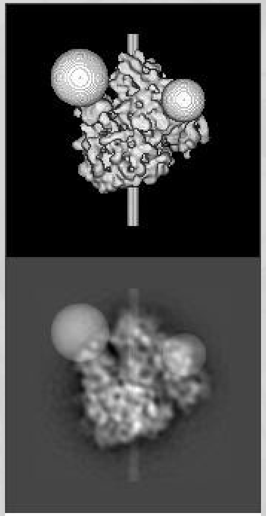

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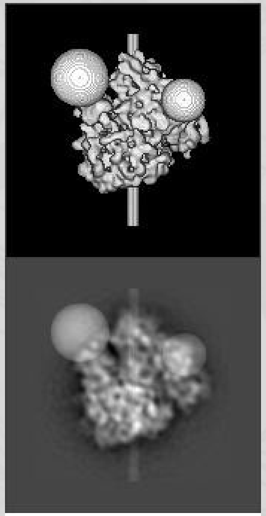

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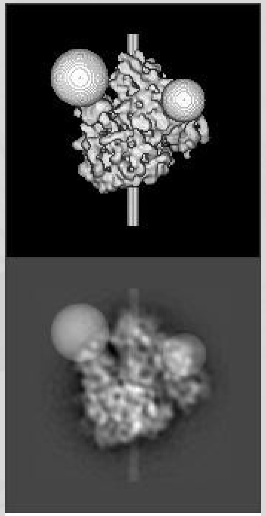

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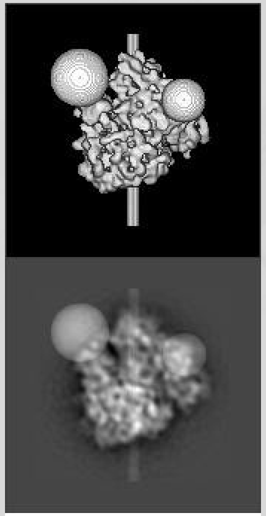

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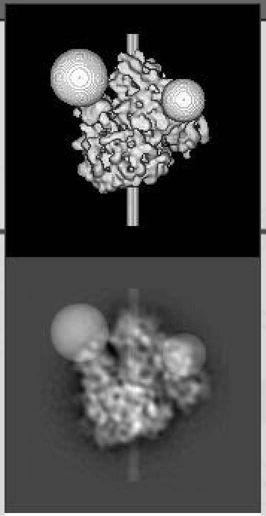

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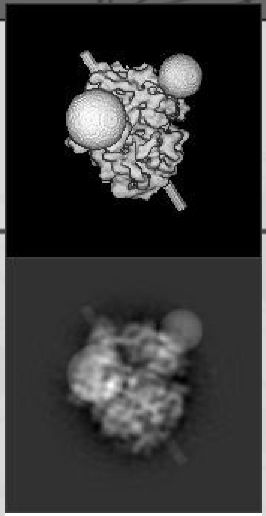

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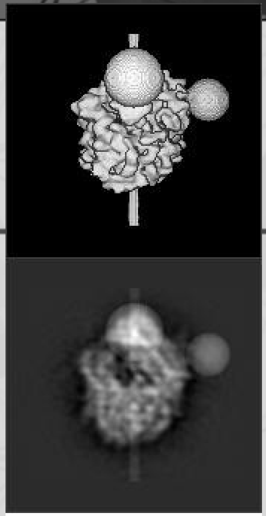

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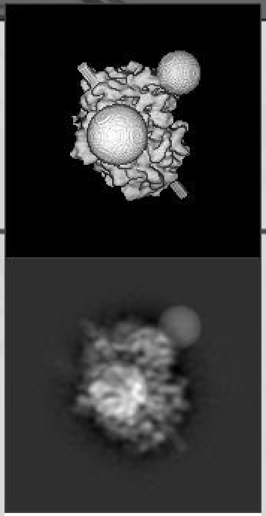

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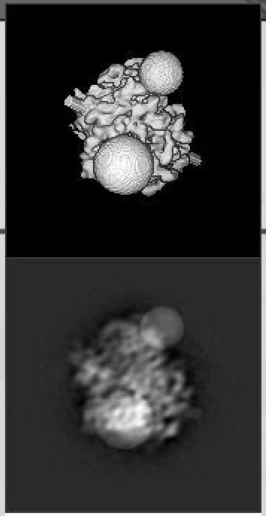

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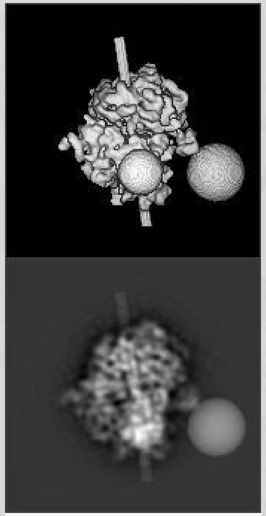

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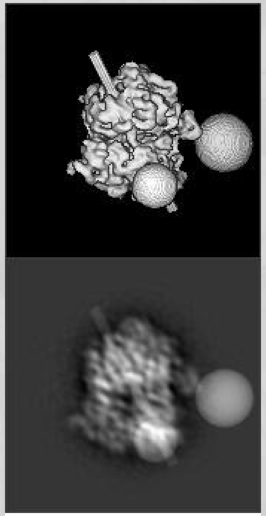

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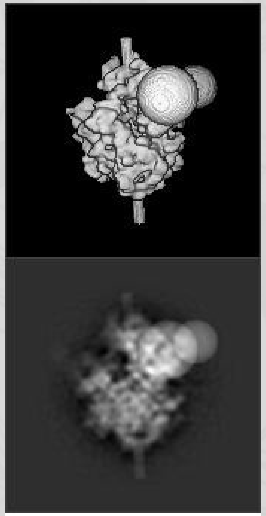
theta=045
psi=000



phi=016
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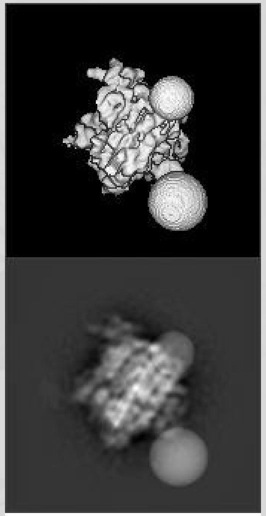

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theta=075
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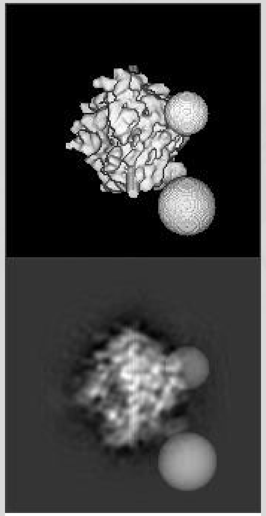

phi=131
theta=090
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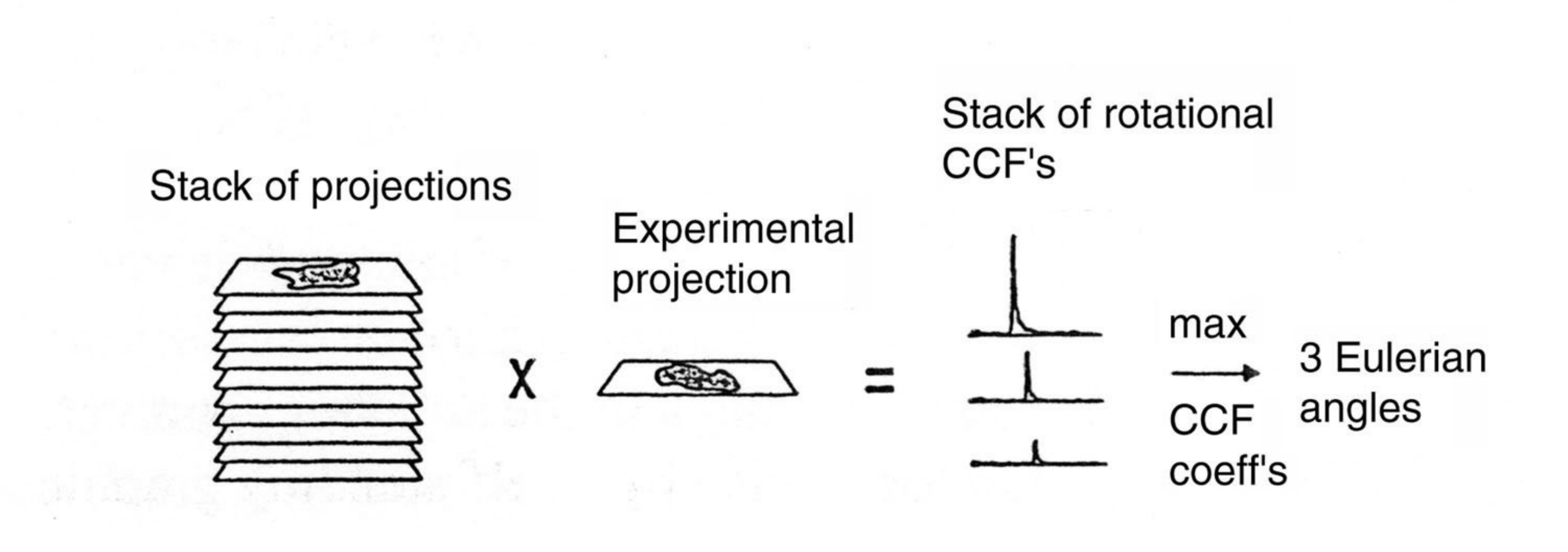
Reference-based alignment



From Penczek *et al.* (1994), *Ultramicroscopy* **53**: 251-70.

Steps:

1. Compare the experimental image to all of the reference projections.
2. Find the reference projection with which the experimental image matches best.
3. Assign the Euler angles of that reference projection to the experimental image.

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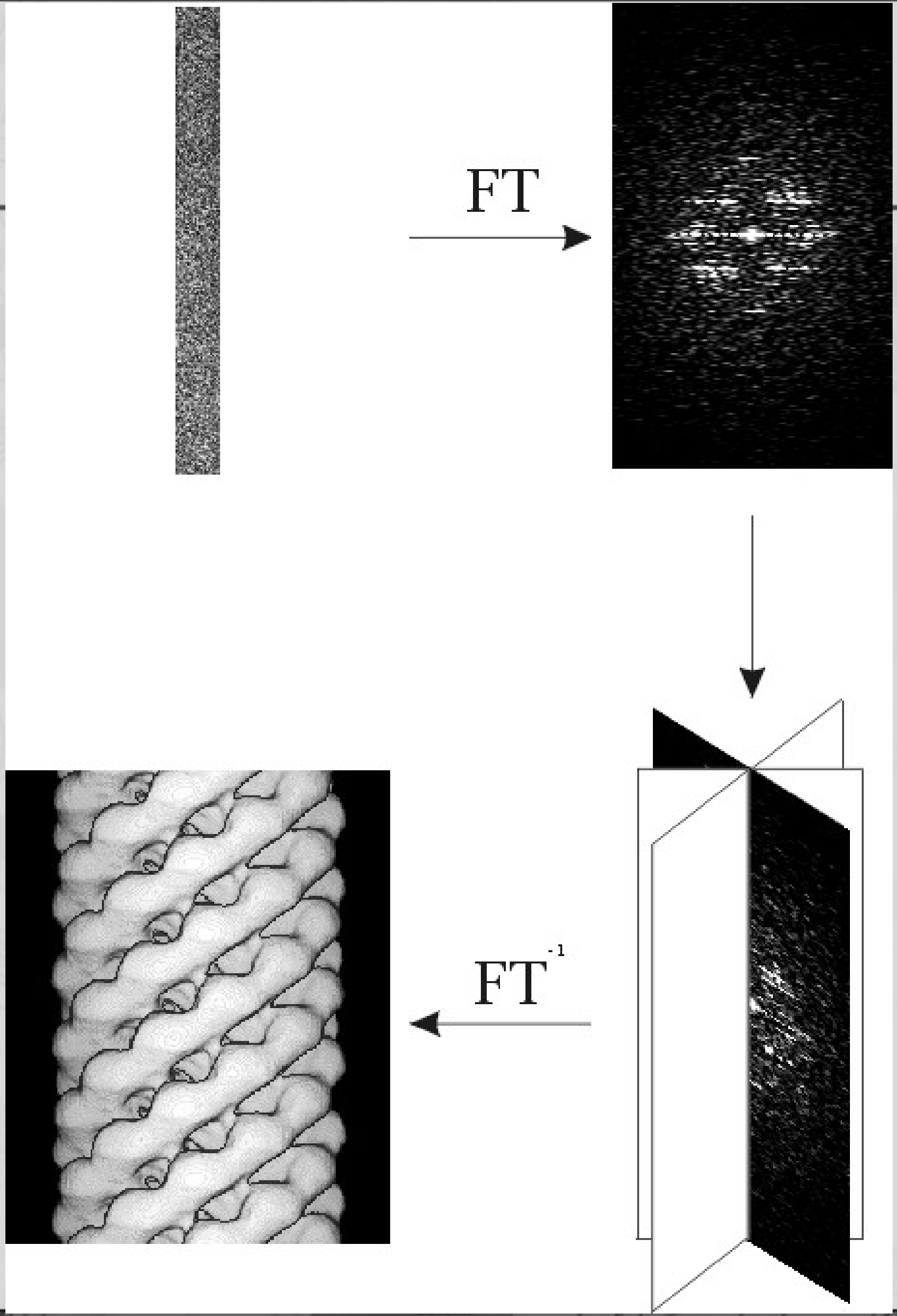
Projection theorem:
(or Central Section Theorem)

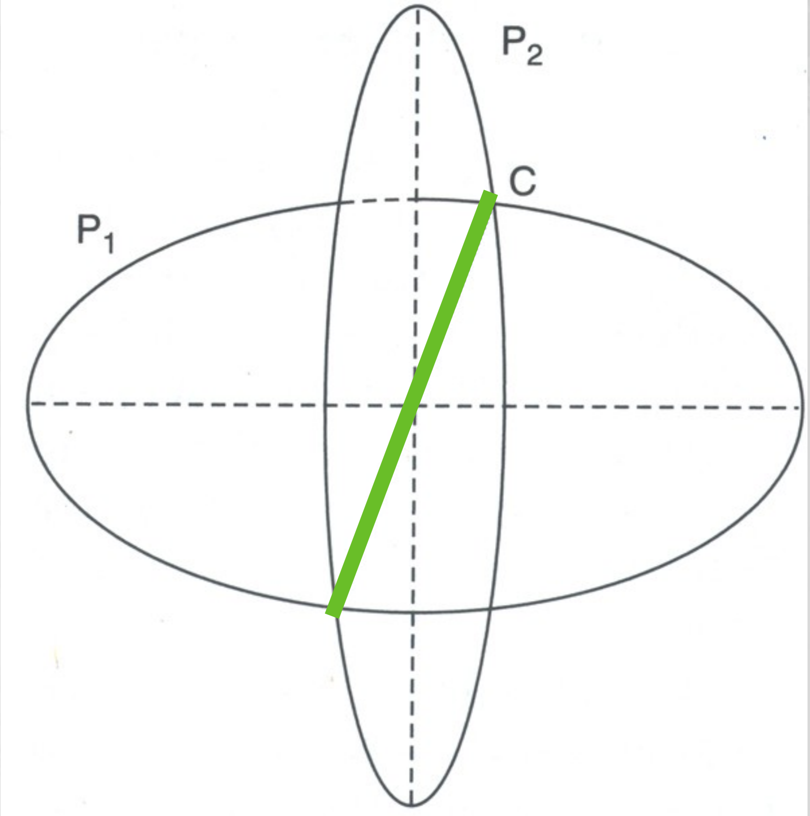
- The 2D Fourier transform of a projection image represents one section through the 3D Fourier transform of a volume.
- Each 2D FT goes through the origin in the 3D FT.
- If you can populate the 3D FT with enough 2D sections, you can reconstruct the 3D volume.
- Task: to find the relative orientations of each of the 2D sections (Δx, Δy, ϕ, θ, ψ)



Common lines (or angular reconstitution)

Summary:

- A central section through the 3D Fourier transform is the Fourier transform of the projection in that direction
- Two central sections will intersect along a line through the origin of the 3D Fourier transform
- With two central sections, there is still one degree of freedom to relate the orientations, but a third projection (i.e., central section) will fix the relative orientations of all three.



Frank, J. (2006) 3D Electron Microscopy of Macromolecular Assemblies

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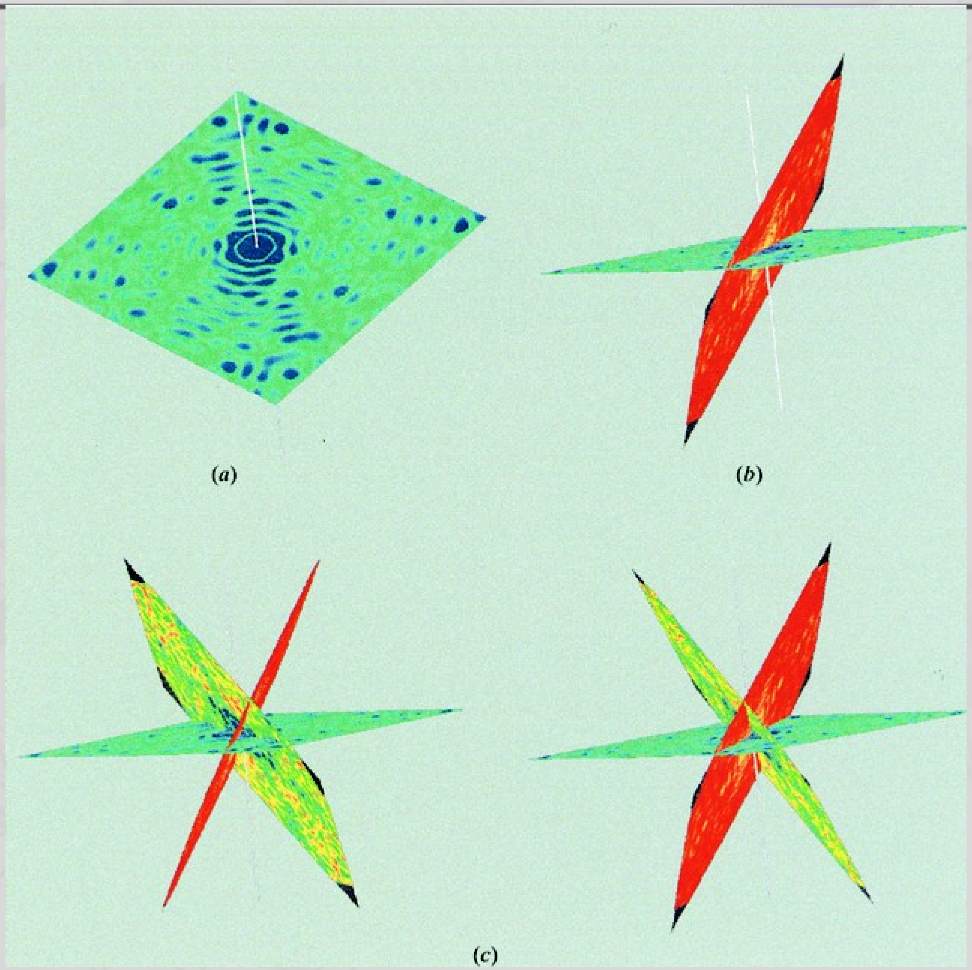

From Steve Fuller

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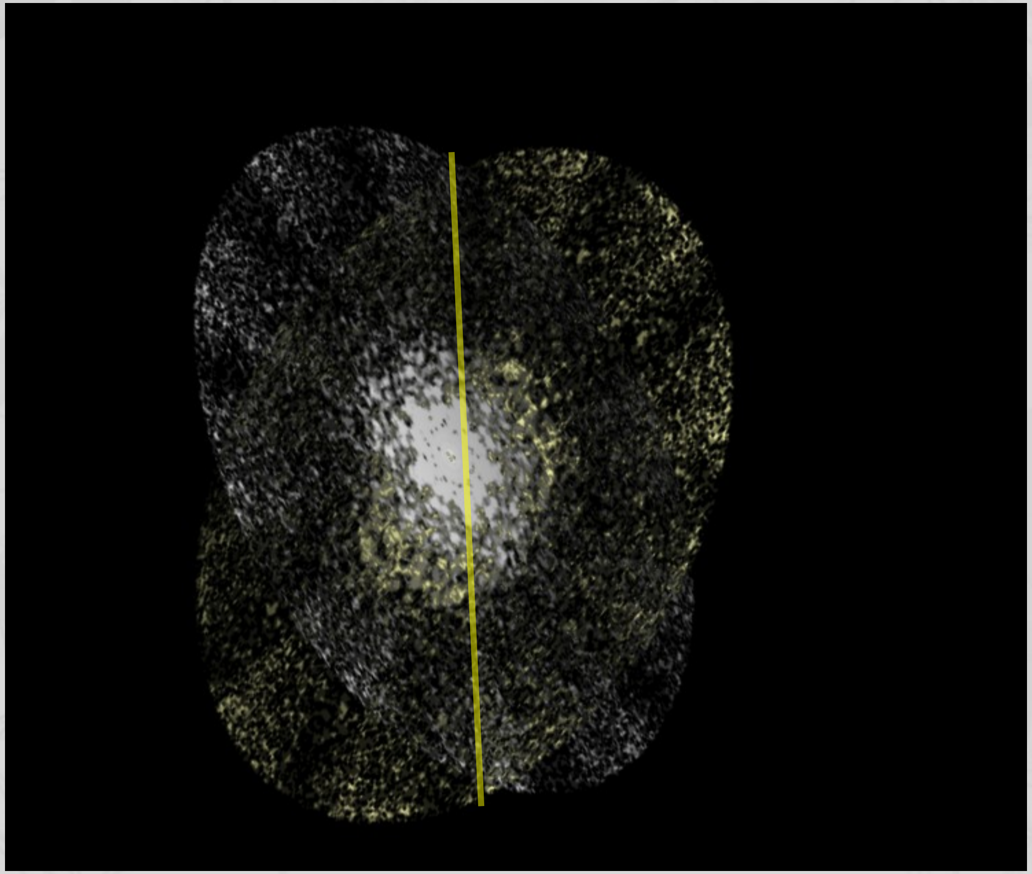

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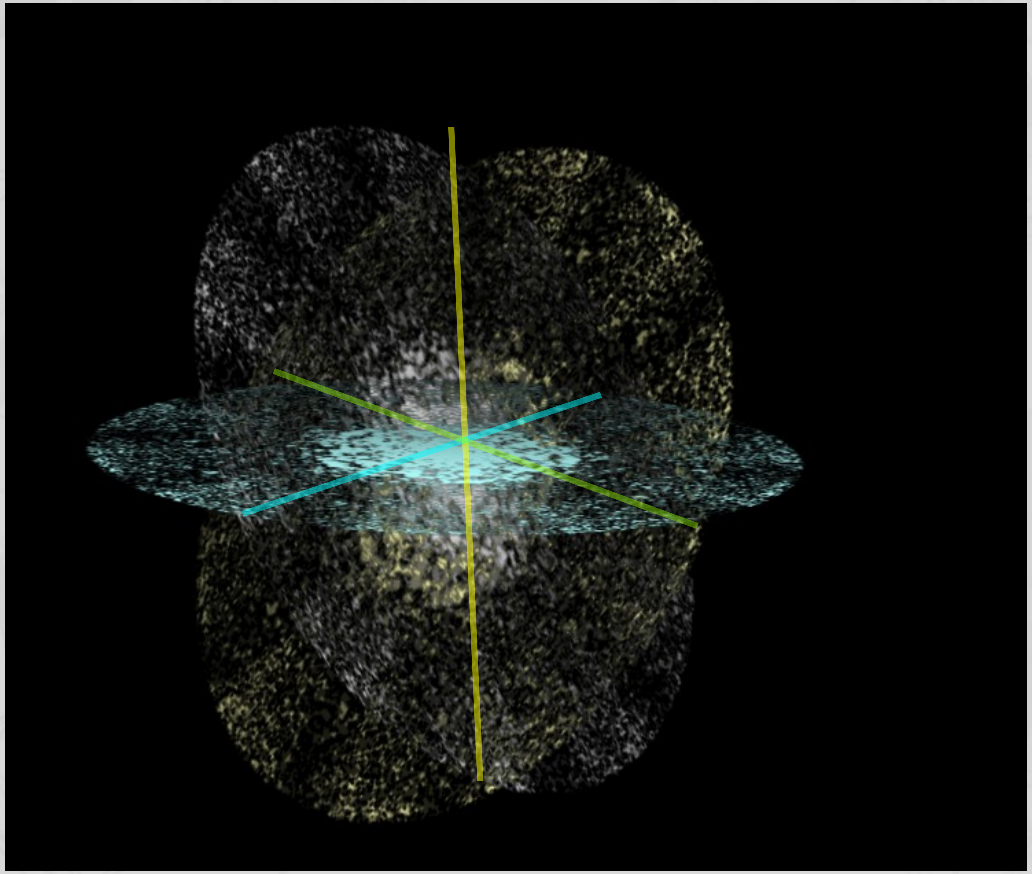

SPHERE

Common lines: Problems

- Noise can lead to incorrect angles
  - Symmetry helps
- Handedness cannot be determined without additional information
  - Tilting
  - Secondary structure
  - Metal shadowing
- Assumes conformational homogeneity

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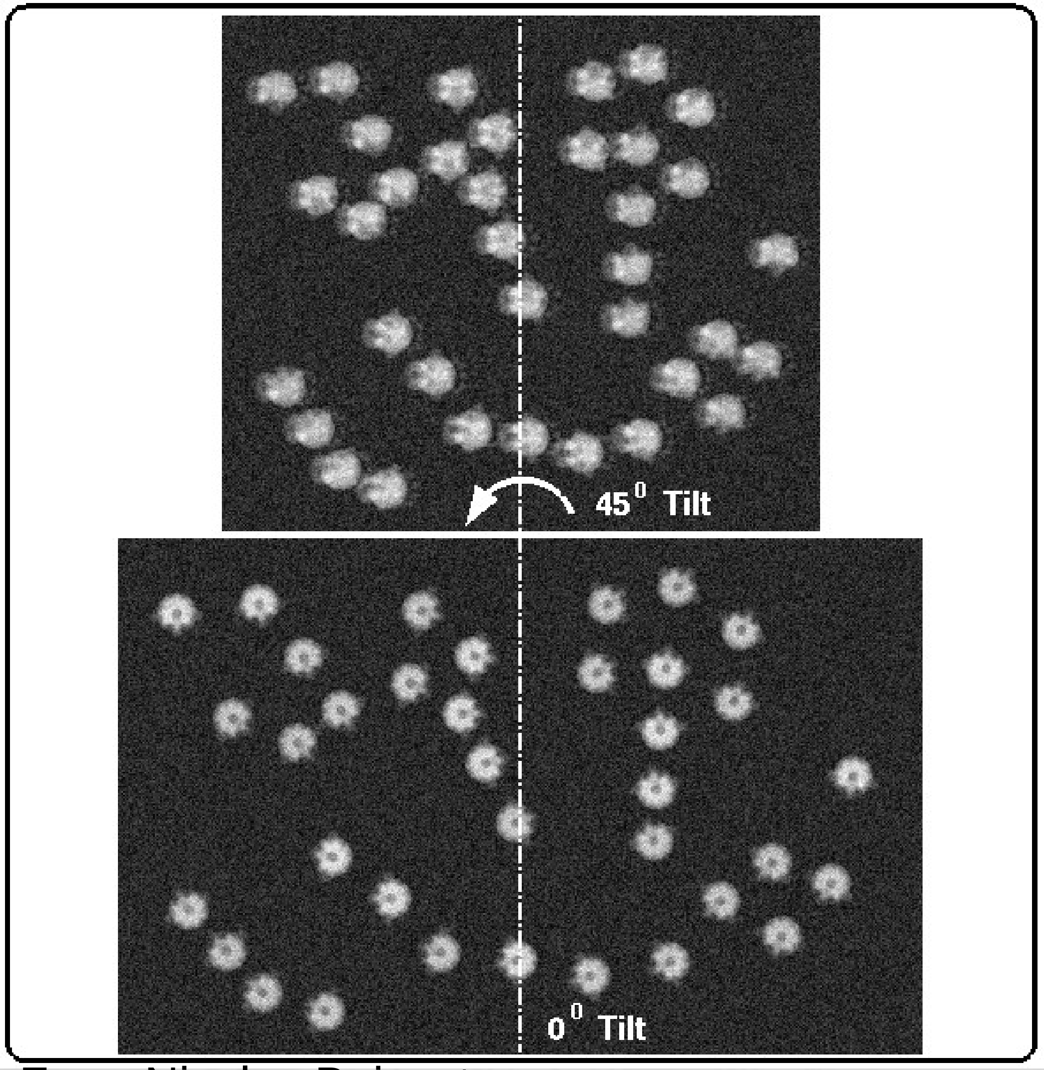
Random conical tilt: Determination of Euler angles

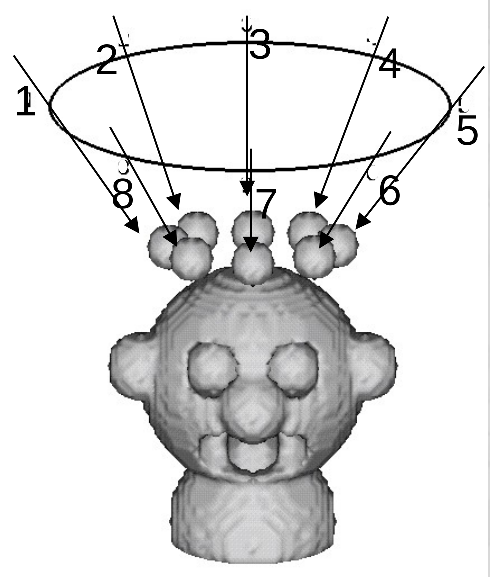


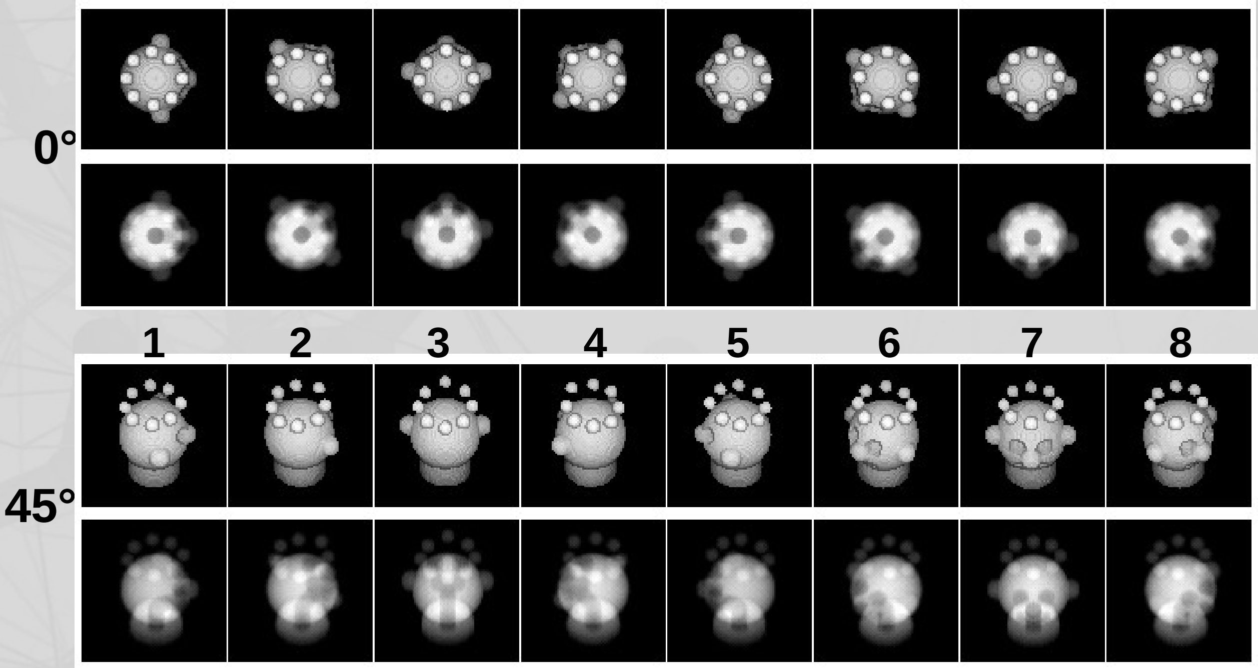
From Nicolas Boisset

This scenario describes a worst case, when there is exactly one orientation in the 0º image. Since the in-plane angle varies, in the tilted image, we have different views available.

Random conical tilt: Geometry

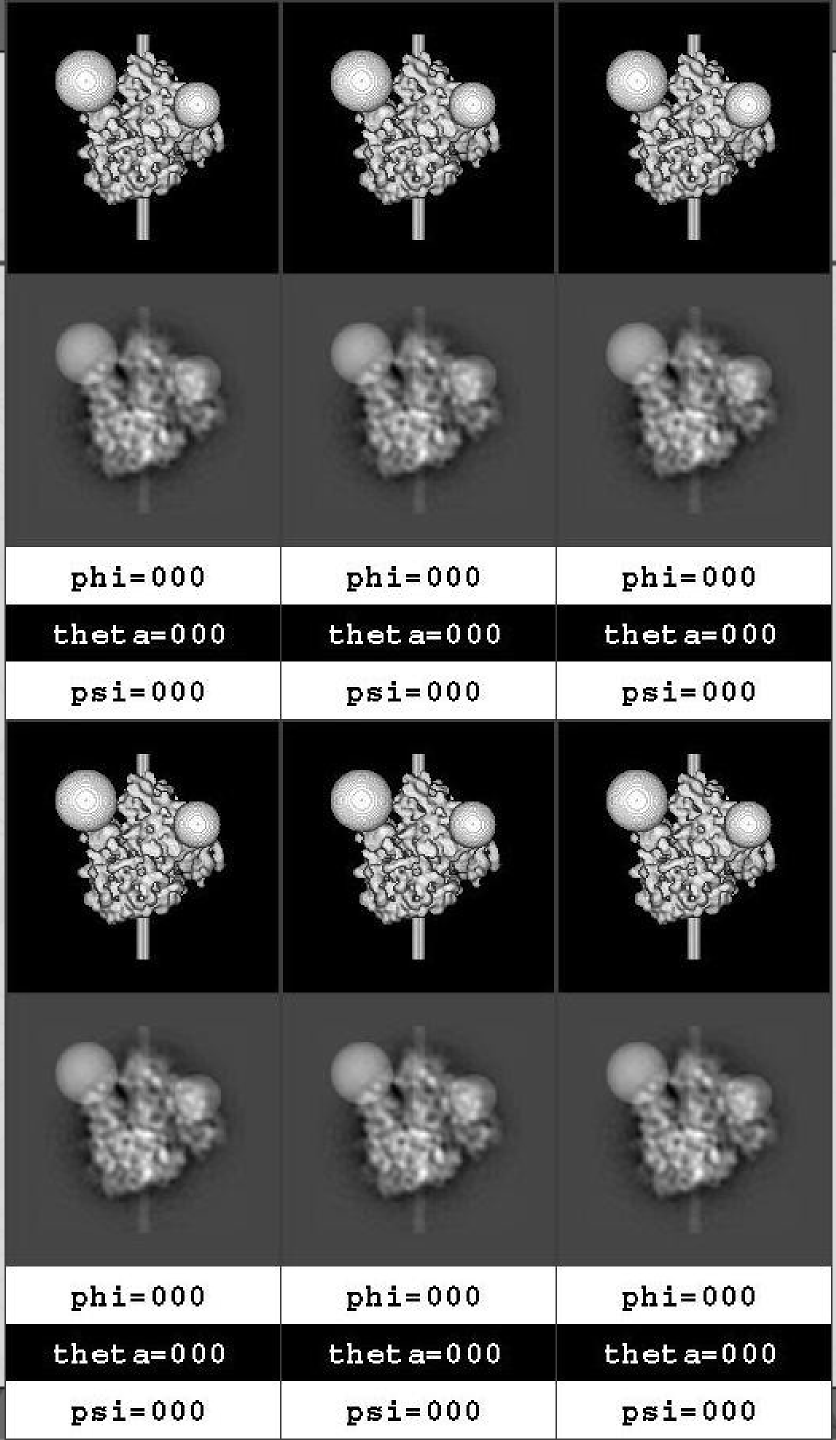
Two images are taken: one at 0° and one tilted at an angle of 45°.





Radermacher, M., Wagenknecht, T., Verschoor, A. & Frank, J. Three-dimensional reconstruction from a single-exposure, random conical tilt series applied to the 50S ribosomal subunit of *Escherichia coli*. *J Microsc* **146**, 113-36 (1987).

From Nicolas Boisset



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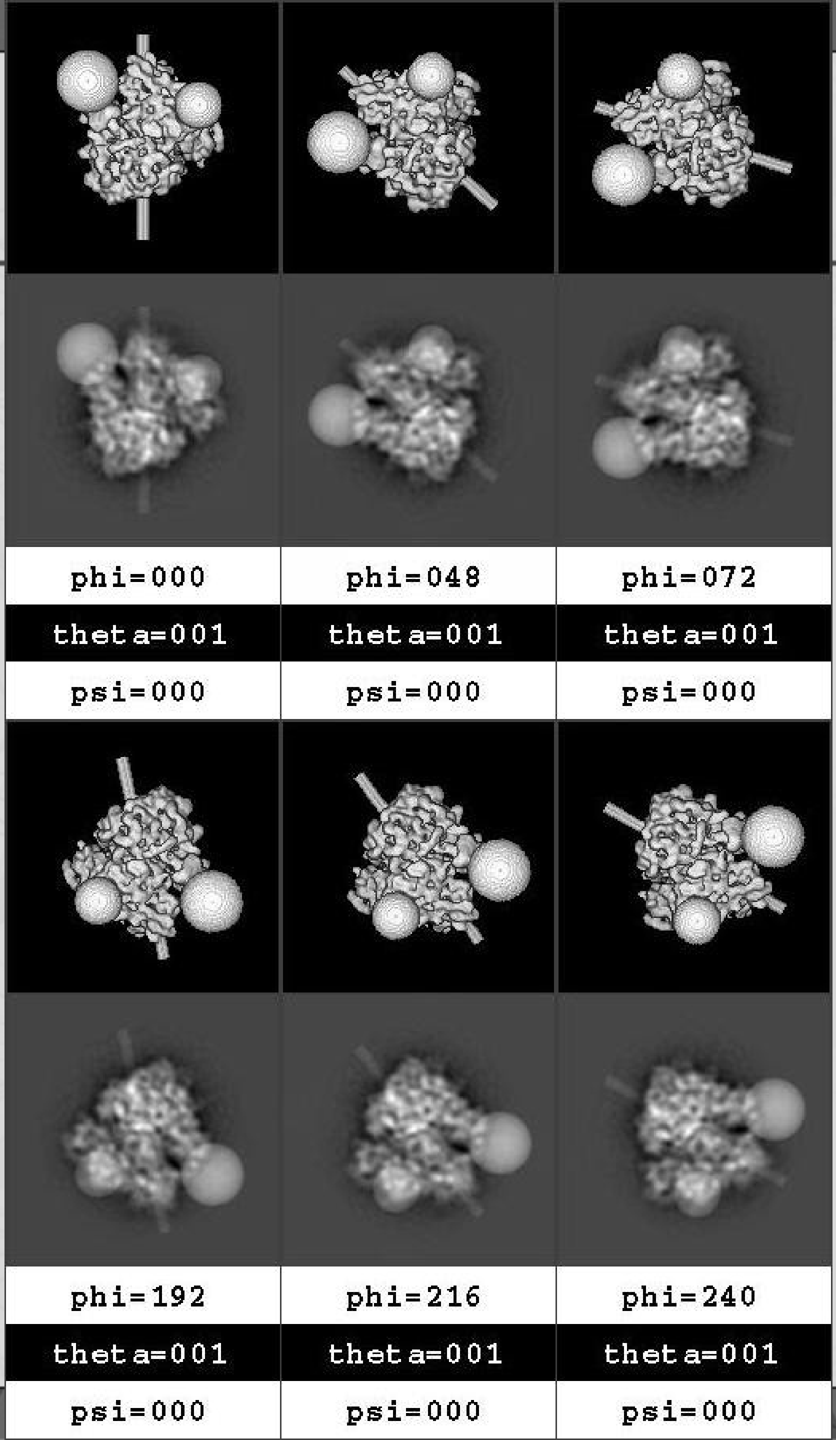
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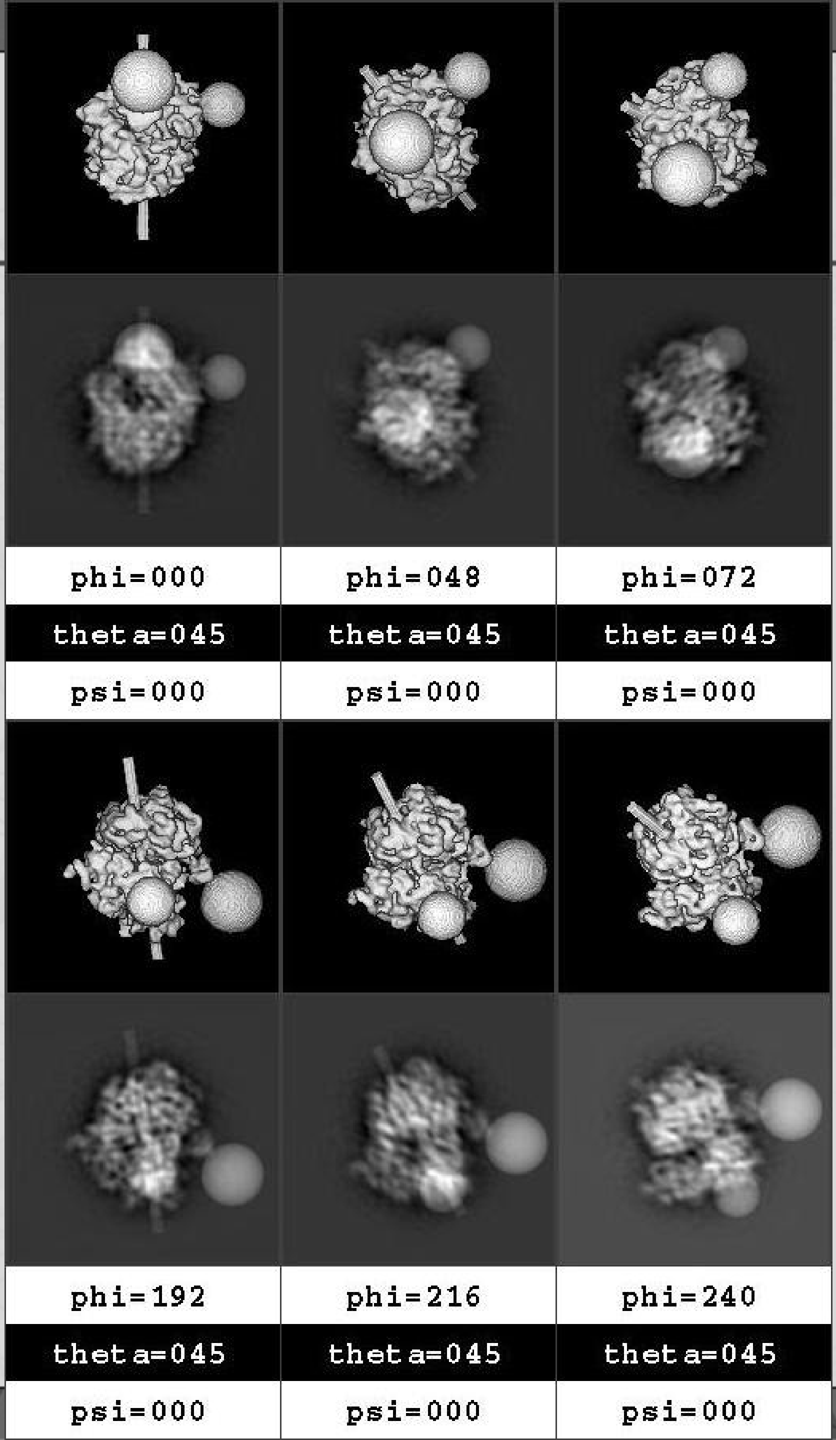
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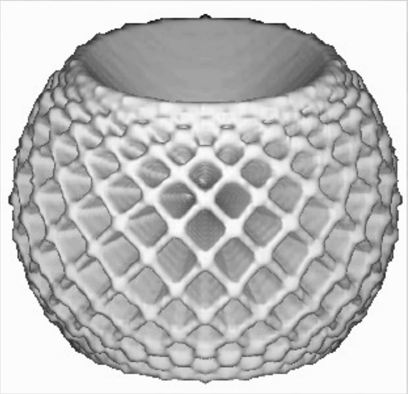
One problem though

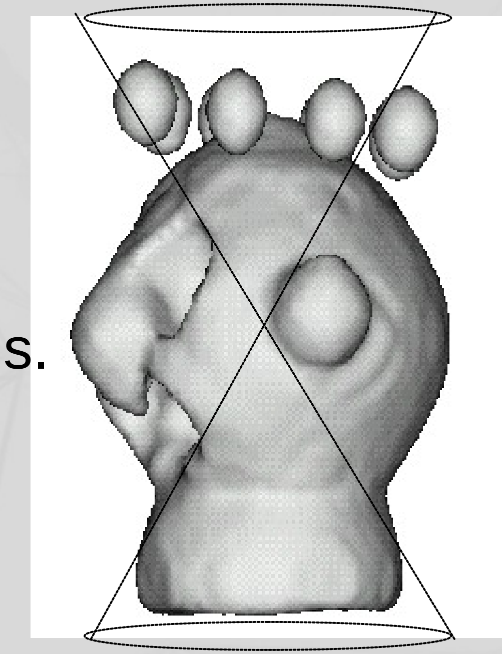
We can't tilt the stage all the way to 90 degrees.

Random conical tilt: The missing cone

Representation of the distribution of views, if we display a plane perpendicular to each projection direction



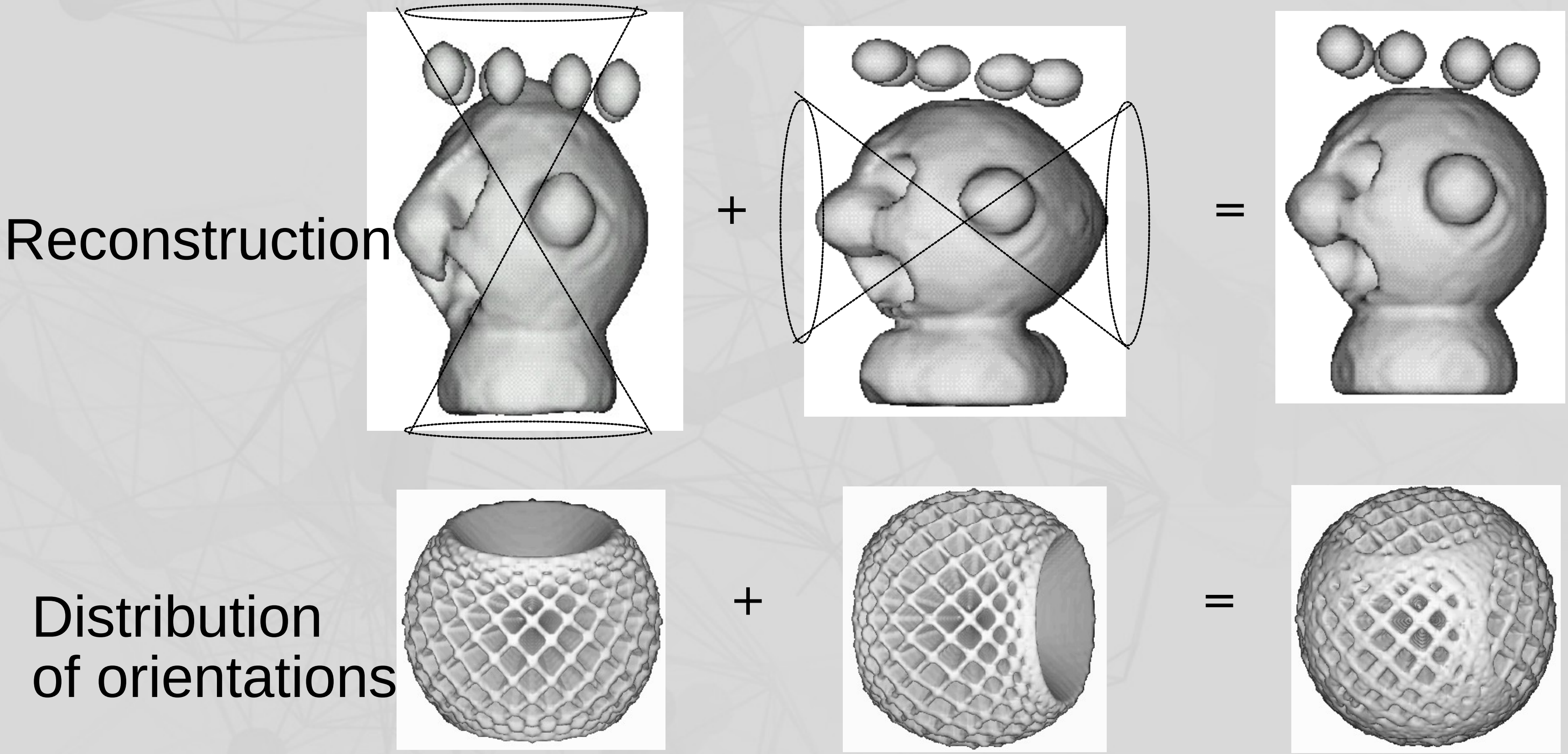
The missing information, in the shape of a cone, elongates features in the direction of the cone's axis.



From Nicolas Boisset

Random conical tilt: Filling the missing cone

If there are multiple preferred orientations, or if there is symmetry that fills the missing cone, you can cover all orientations.



From Nicolas Boisset

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Another method

How is a structure obtained from images of its 2D projections?

This need to be initialized somehow

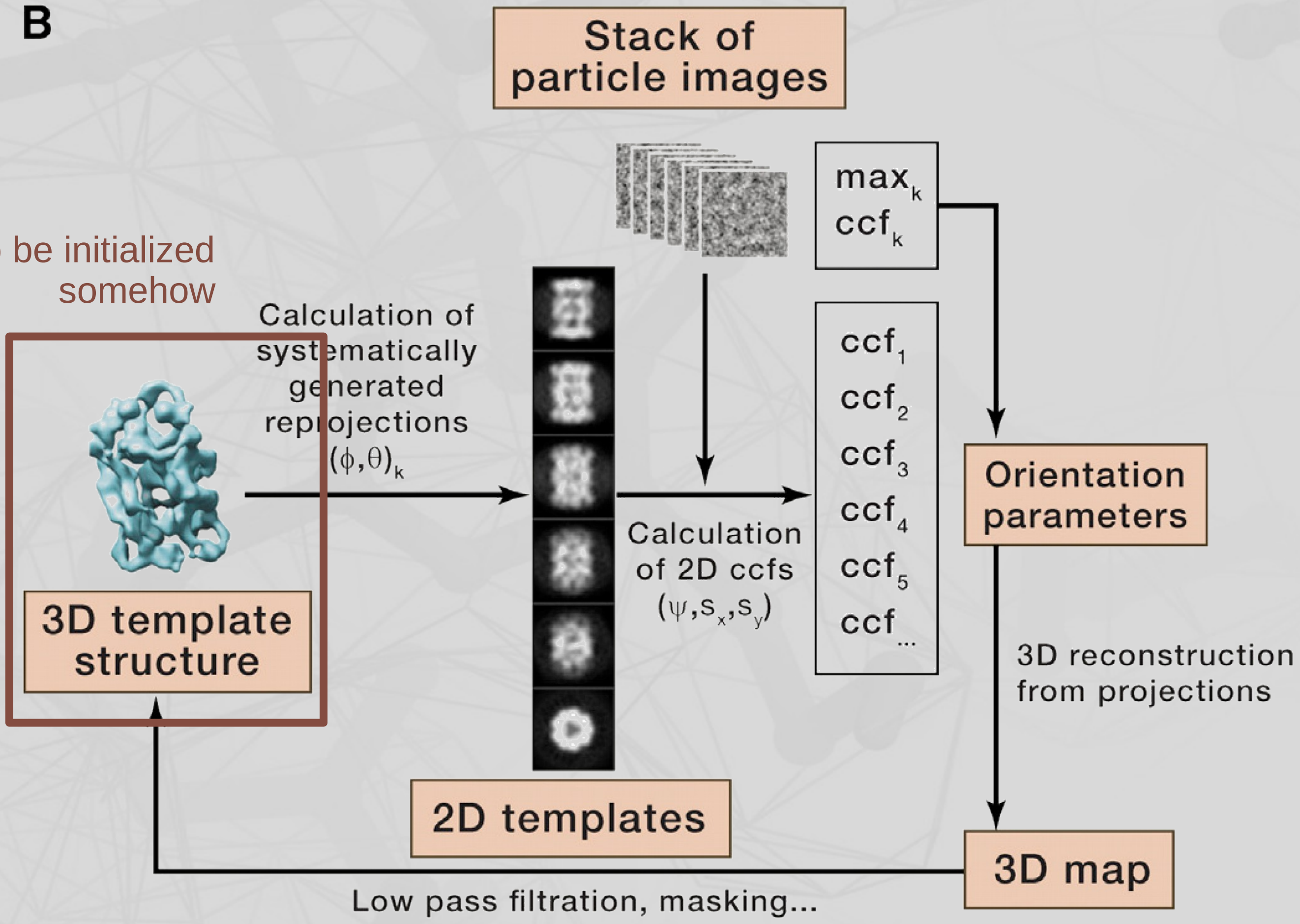


Image from Cheng *et al.* (2015) *Cell* **161:** 438 – 449

How to overcome a greedy algorithm: Stochastic Hill climbing

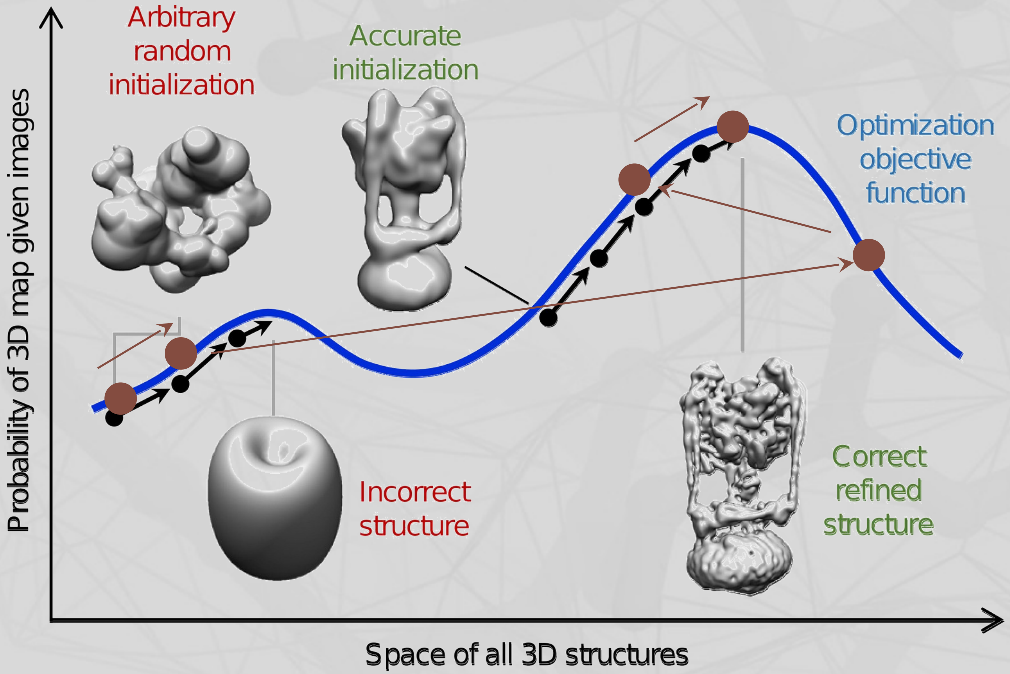


Image modified from Punjani *et al.* (2013) *Nature Methods* **14:**290 – 296

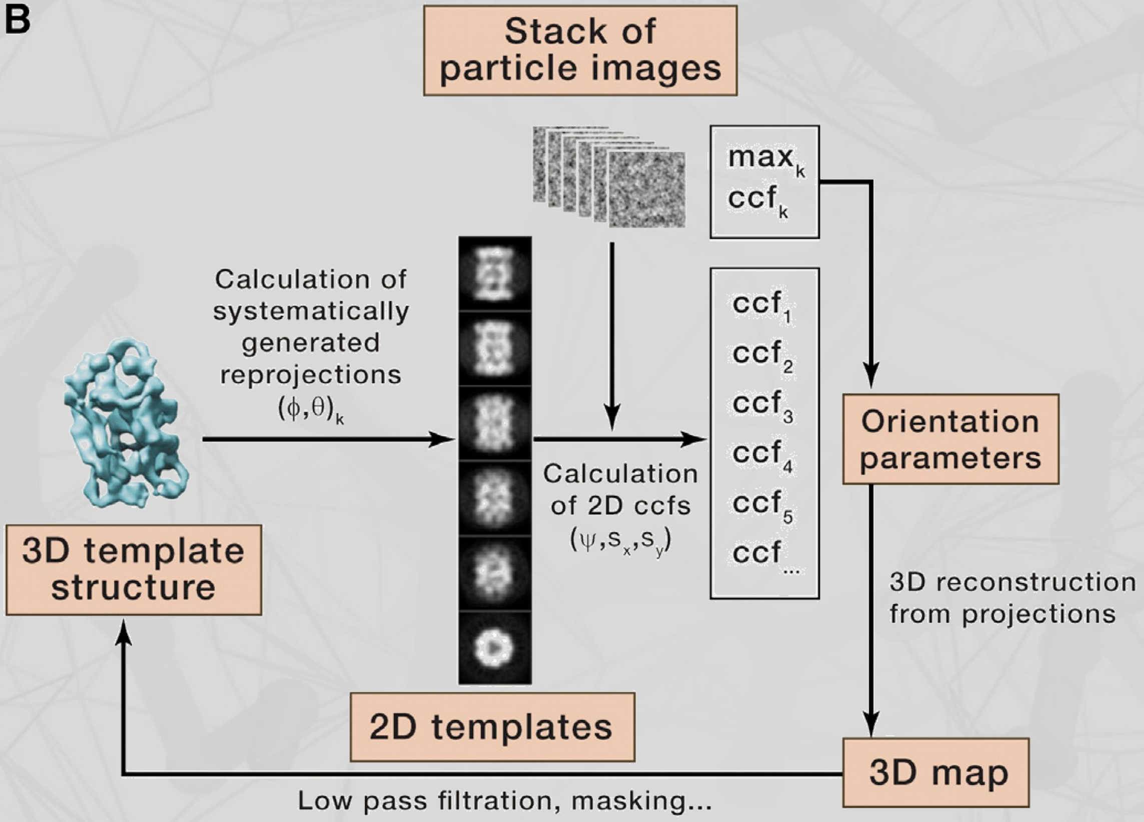
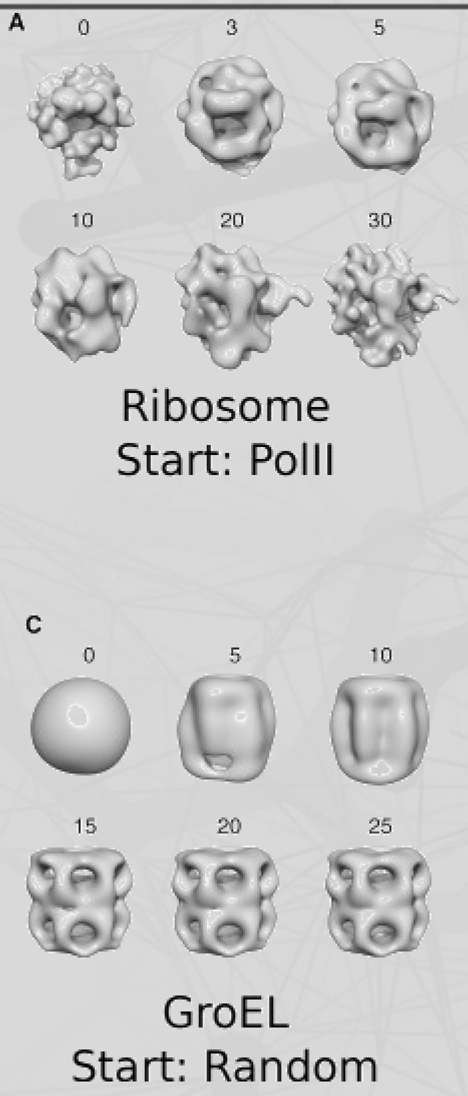
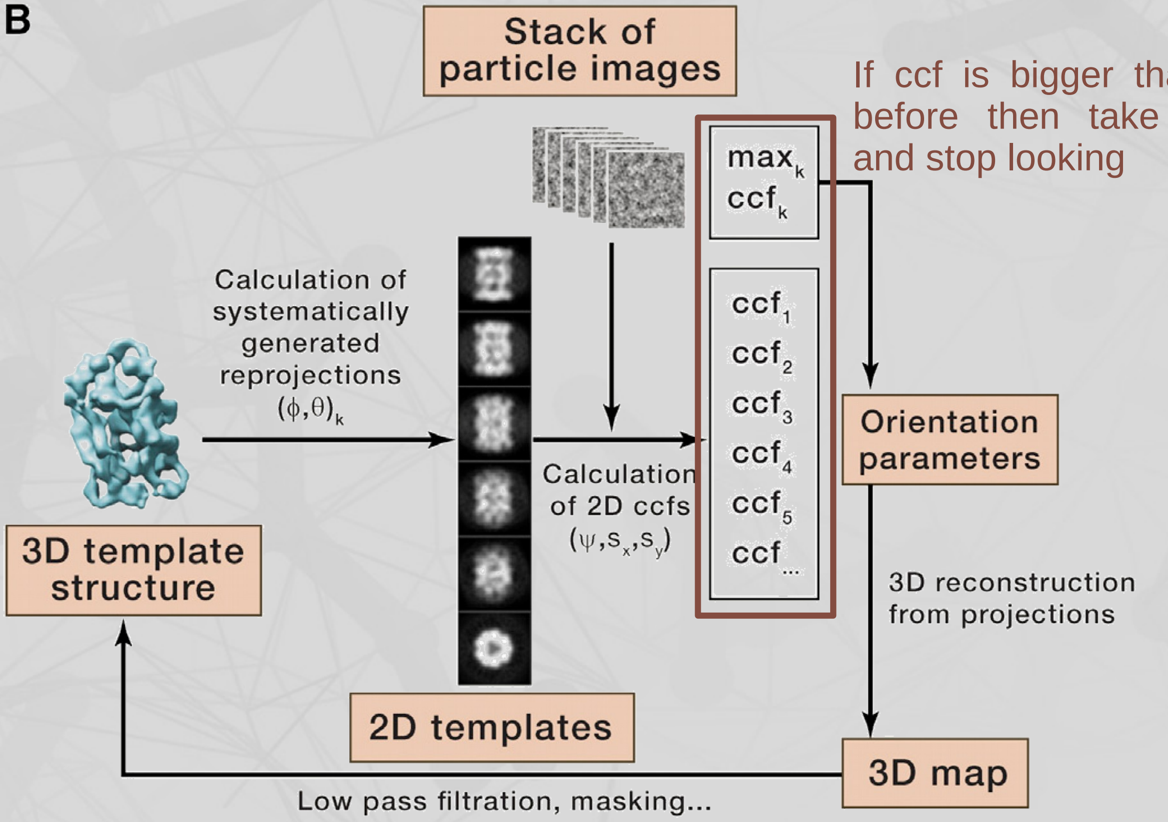


Image from Cheng *et al.* (2015) *Cell* **161:** 438 – 449

Original concept: PRIME



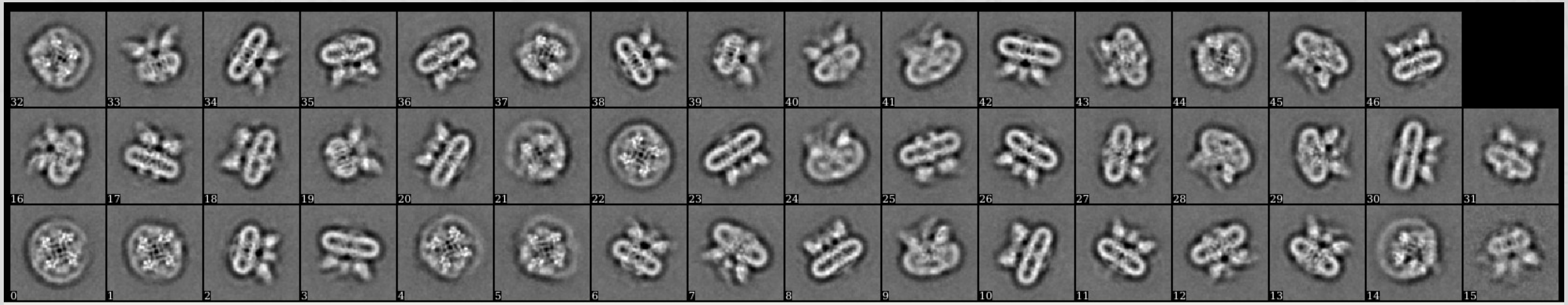
Elmlund *et al.* (2013) *Structure* **21:**1299 – 1306

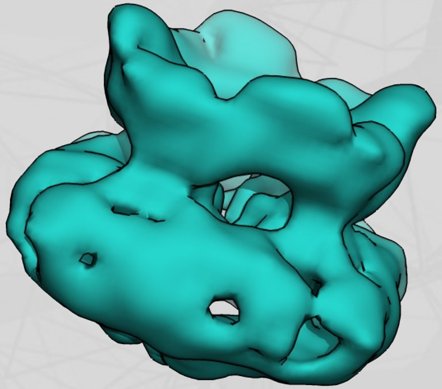


If ccf is bigger than before then take it and stop looking

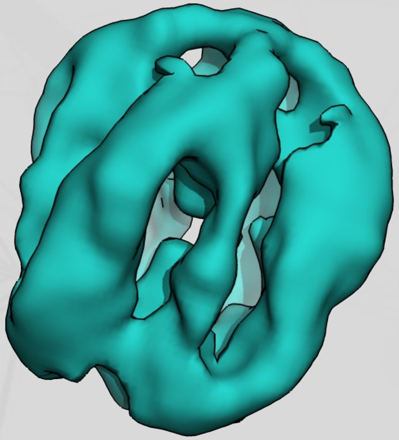
Image from Cheng *et al.* (2015) *Cell* **161:** 438 – 449

Stochastic hill climbing: When things go awry





In some nice cases

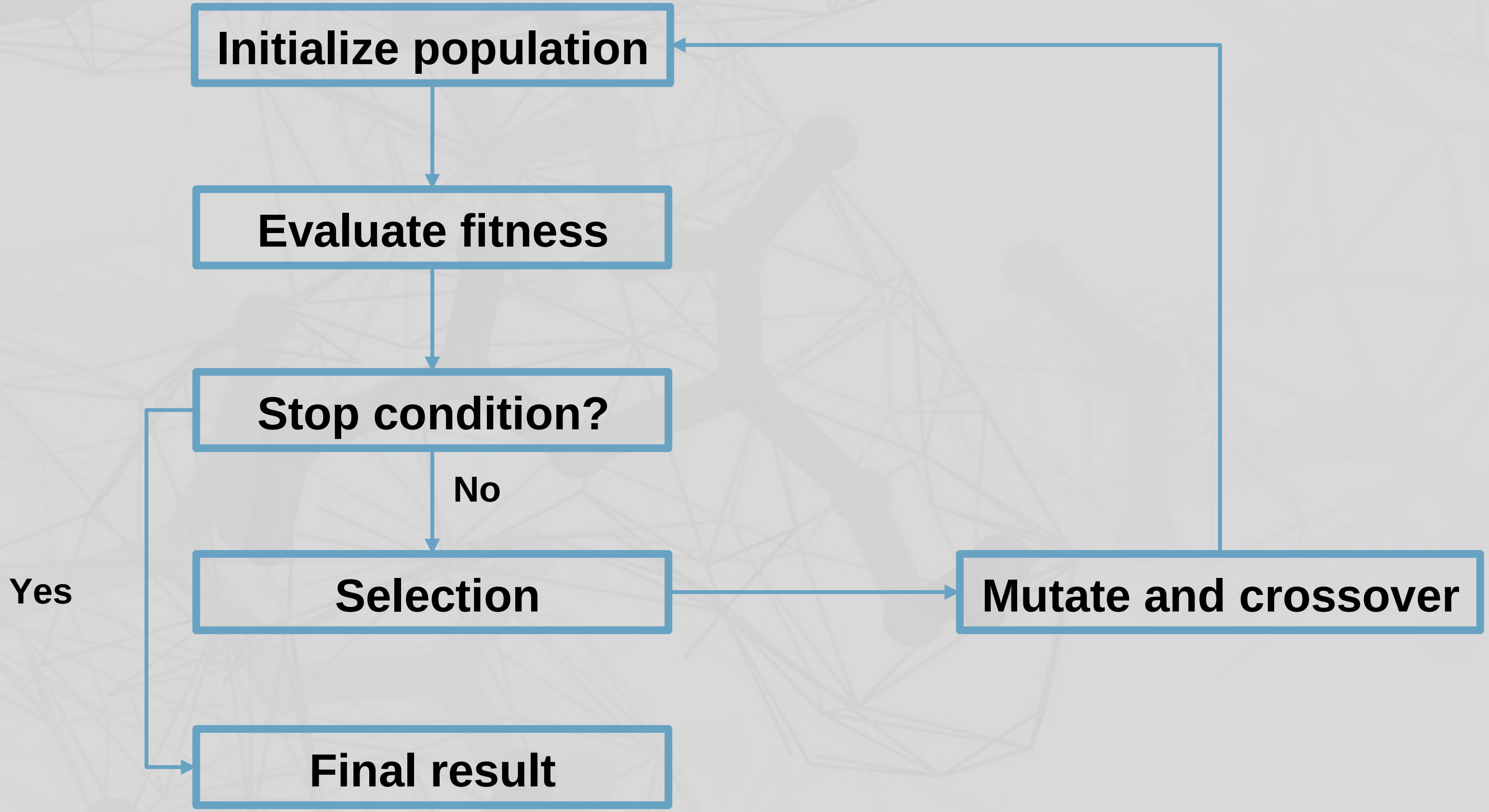


But sometimes...

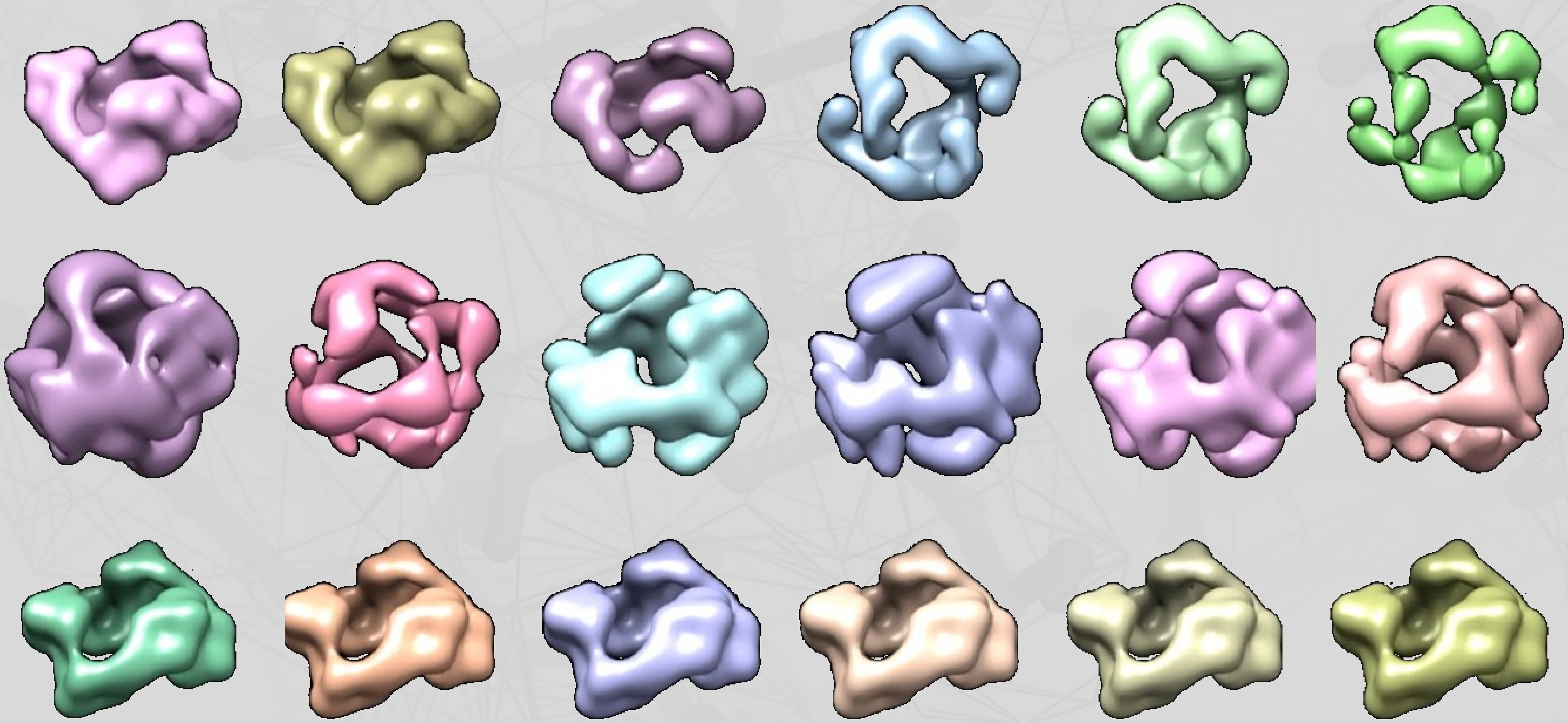
Stochastic hill climbing meets genetic algorithm

What‘s a genetic algorithm?

An optimization technique inspired in natural selection.



How is this implemented?
The idea behind VIPER



Generation 1
Six independent stochastic hill climbing runs

Start for generation 2
(Here's the genetic algorithm's turn)

Generation 2

Slide from Pawel Penczek

SPHERE

Other methods

- Subtomogram averaging
- Orthogonal tilt reconstruction
  - Stage is tilted to -45 and +45 degrees
  - No missing cone
  - Can work if even distribution of orientations
- Other software packages
  - RANSAC from XMIPP

Suggestions

- Try different methods, different software packages
- VALIDATE!