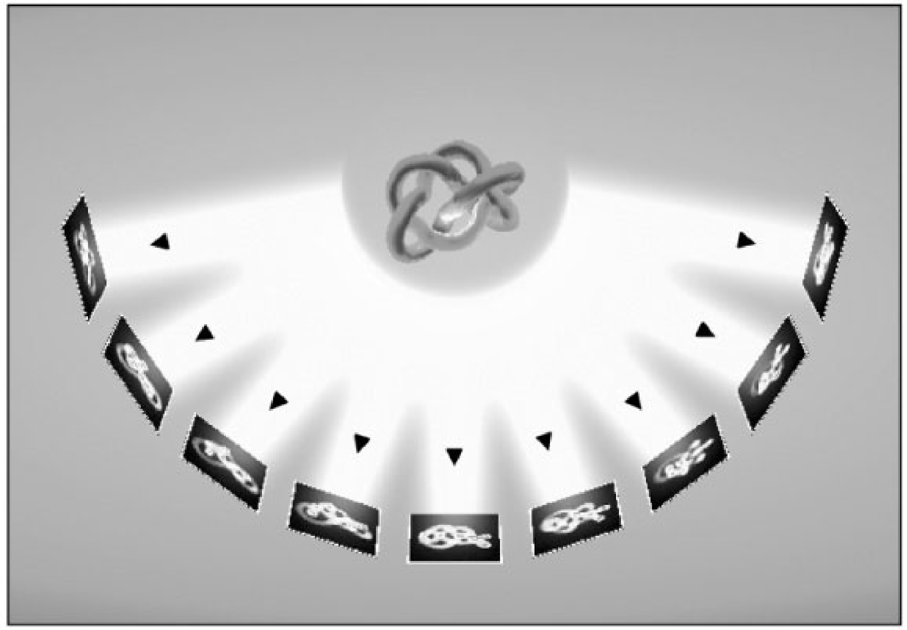
Random Conical tilt 3D reconstruction

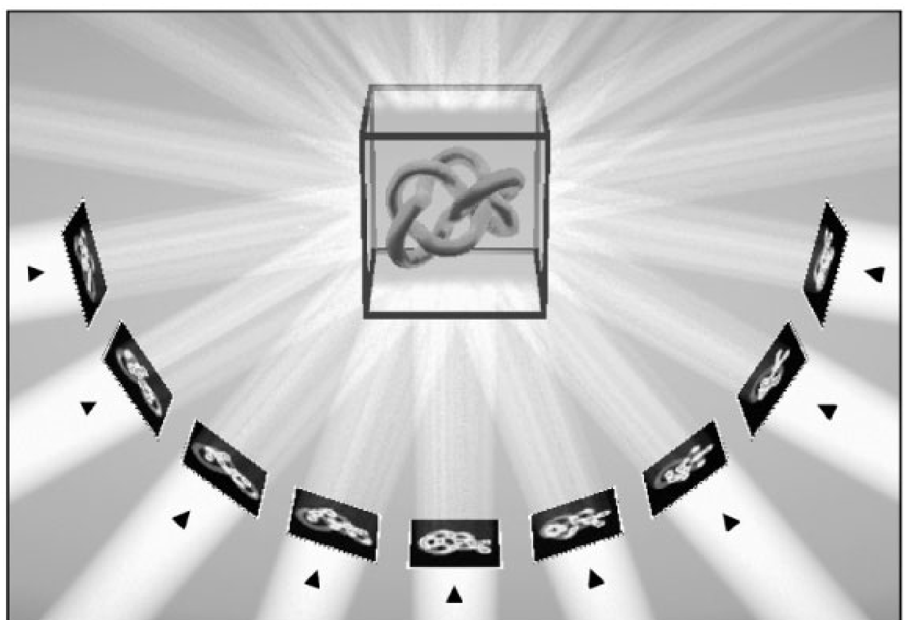
- Central section theorem
- Euler angles
- Principle of conical tilt series
- Missing cone artefact
- Multivariate statistical analysis
- Early 3D studies and negative staining problems
- Perspectives and new trends

Nicolas Boisset CNRS
Département de Biologie Structurale
Institut de Minéralogie et de Physique des Milieux Condensés
I.M.P.M.C. UMR 7590 CNRS, Université Pierre & Marie Curie
140 Rue de Lourmel, 75015 Paris
email: nicolas.boisset@impmc.jussieu.fr

Sali A, Glaeser R, Earnest T, Baumeister W. (2003) From Words to literature in structural proteomics. *Nature* **422**(6928): 216-225.

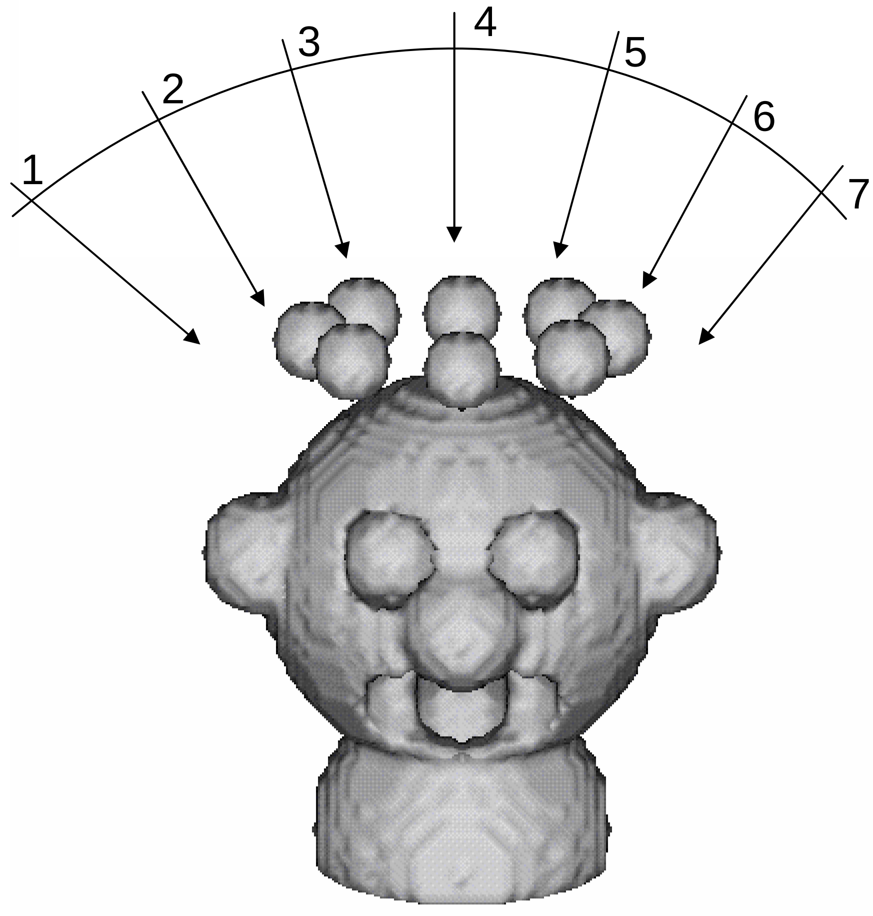


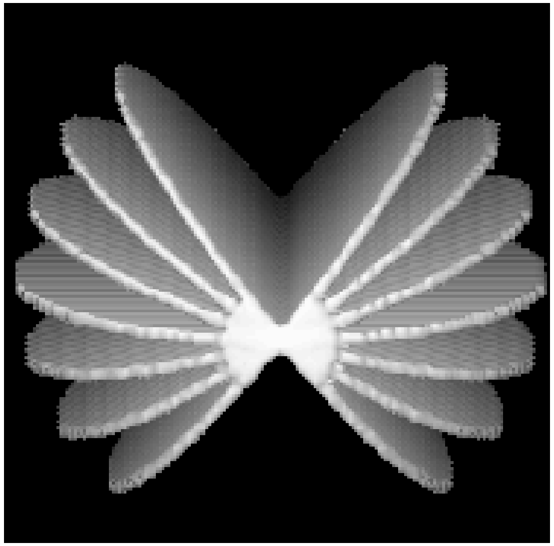
Tilt series

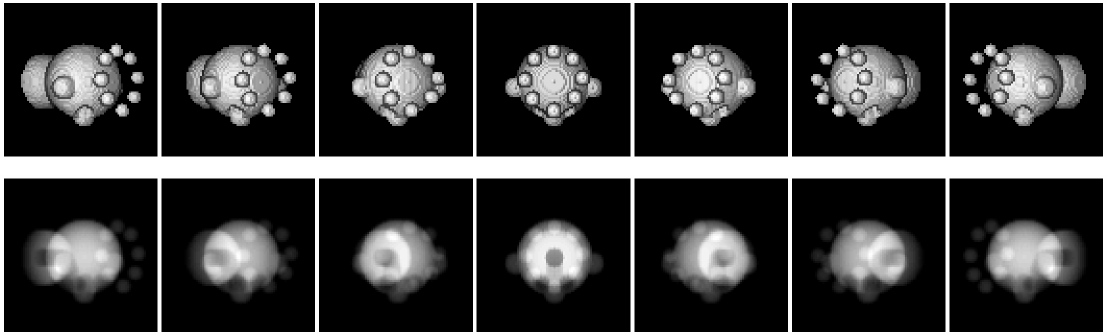


Back-projection &
3D reconstruction

Central projection theorem







In reciprocal space, every 2D projection of a 3D object corresponds to a central section in the 3D Fourier transform of the object. Each central section is orthogonal to the direction of of projection.

Constraints of cryoEM on biological objects :

Work with low electron dose (~$10e^{-}/Å^{2}$)

=> the less exposures, the better.

Images have a low signal-to-noise ratio

Compromise defocus level with resolution (CTF)

Computing 2D or 3D numeric averages

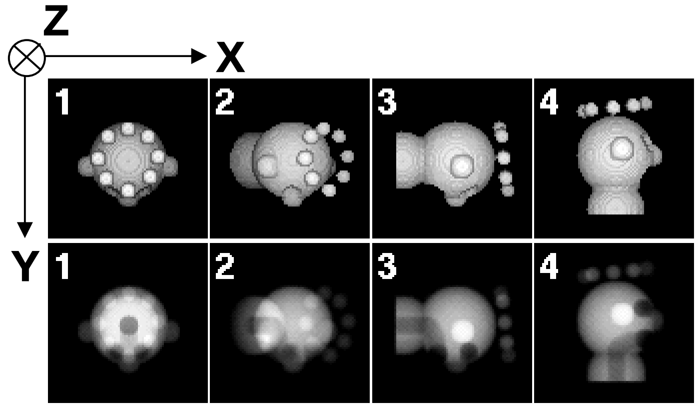
→ (only one conformation assumed in the sample)

Use internal symmetries of the objects :

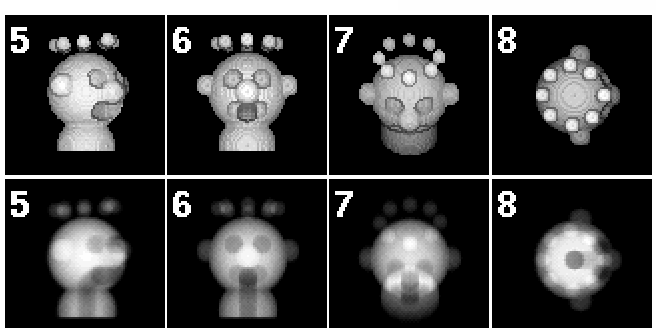
helicoïdal symmetry, icosaedral symmetry,

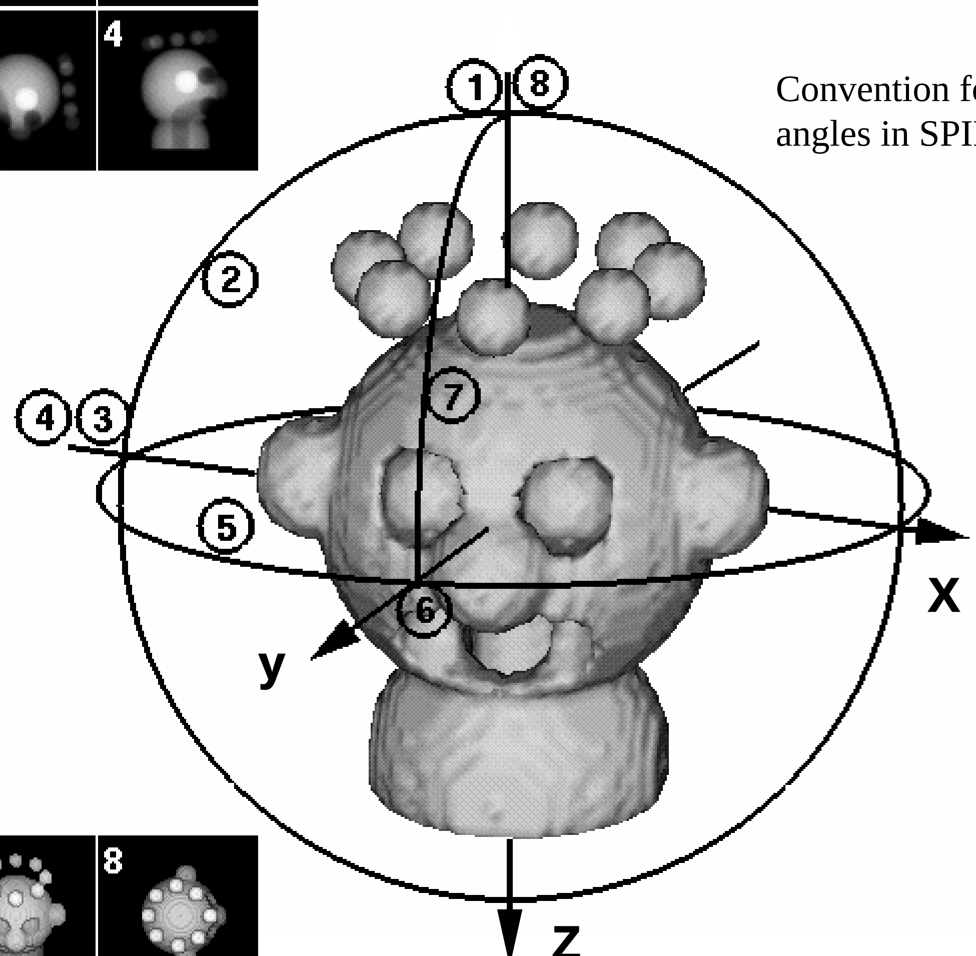
2D crystals, or no symmetry at all…

Euler angles



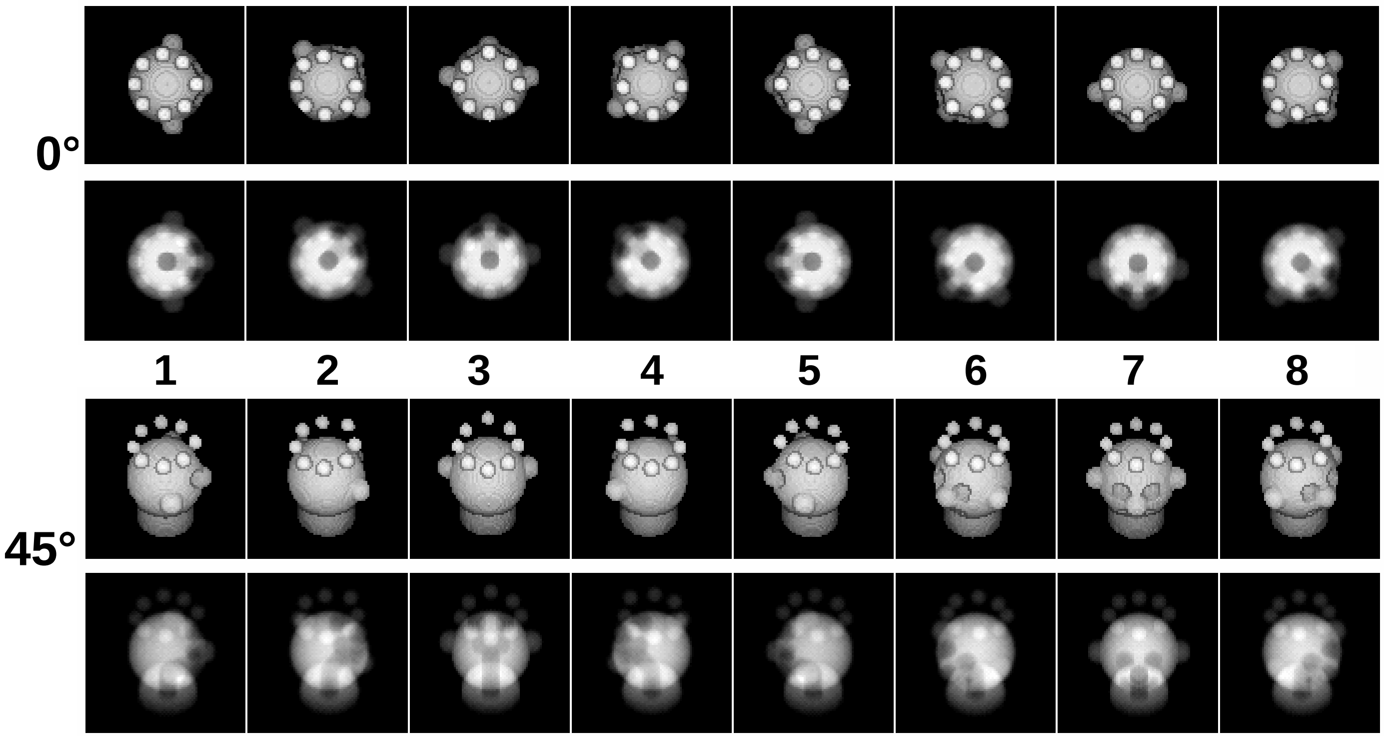
	Phi φ	Theta θ	Psi ψ
1:	0	0	0
2:	0	45	0
3:	0	90	0
4:	0	90	90
5:	–45	90	90
6:	–90	90	90
7:	–90	45	90
8:	0	0	90

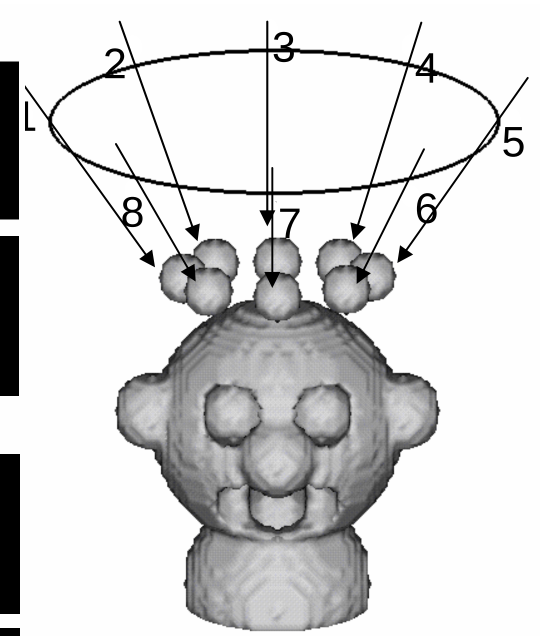




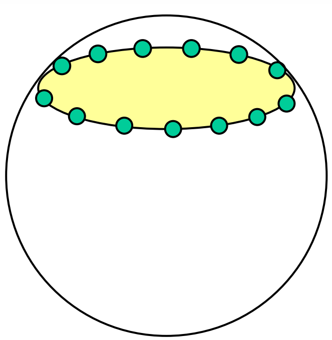
Convention for Euler angles in SPIDER

With only two exposures a conical tilt series can be generated

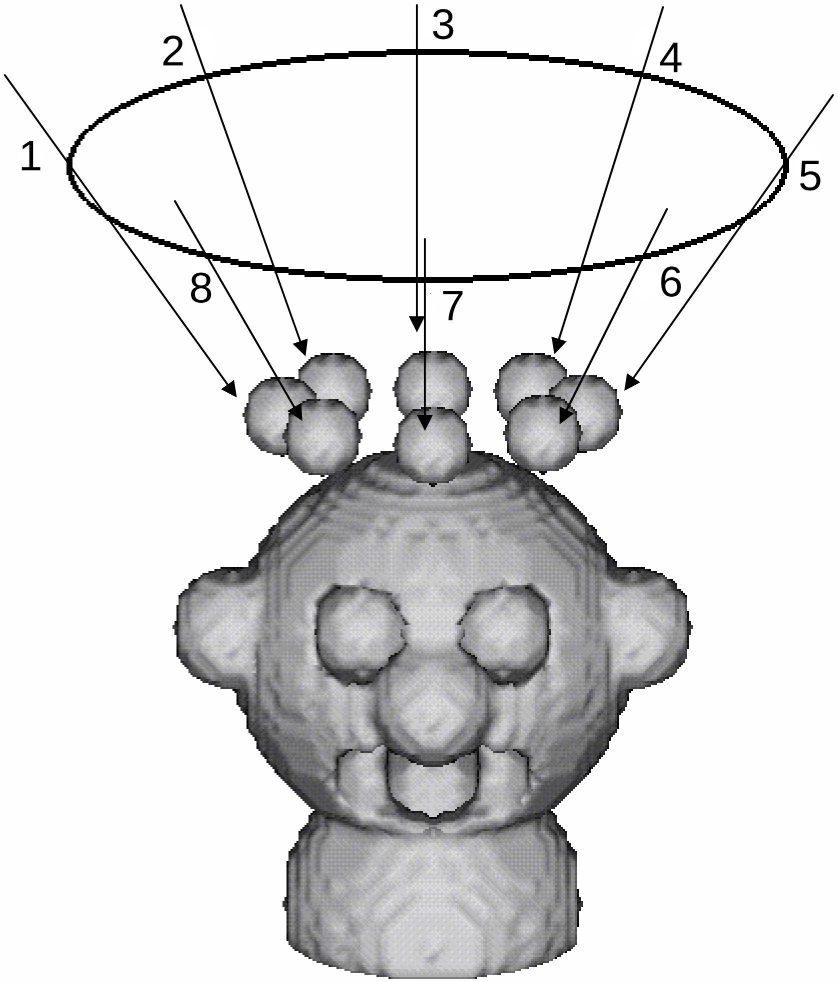




Radermacher, M., Wagenknecht, T., Verschoor, A. & Frank, J. Three-dimensional reconstruction from a single-exposure, random conical tilt series applied to the 50S ribosomal subunit of *Escherichia coli*. *J Microsc* **146**, 113-36 (1987).

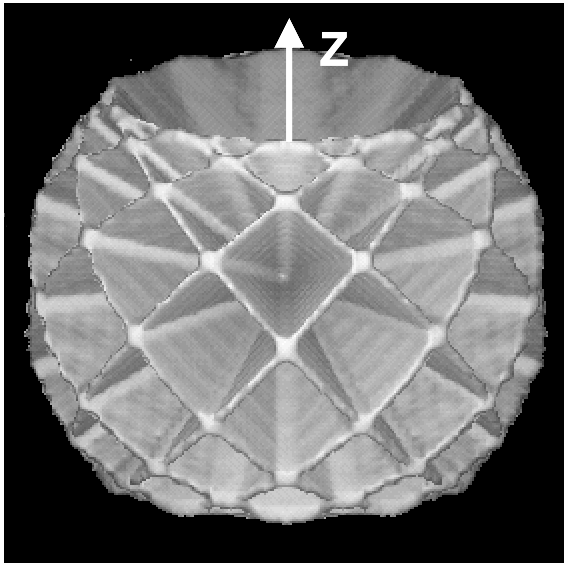


Angular distribution represented on a spherical angular map



Principle of random conical tilt series

You just need to determine de Euler angles specific to each tilted-specimen image.

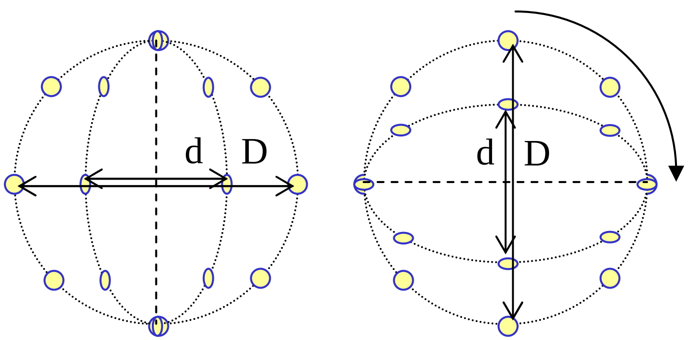


Reciprocal space

Radermacher, M. (1988) Three-dimensional reconstruction of single particles from random and nonrandom tilt series. *J Electr.Microsc. Tech.* **9(4):** 359-394.

Interactive particle selection. Picking of image paires (45° & 0°) provides a mean to compute the :

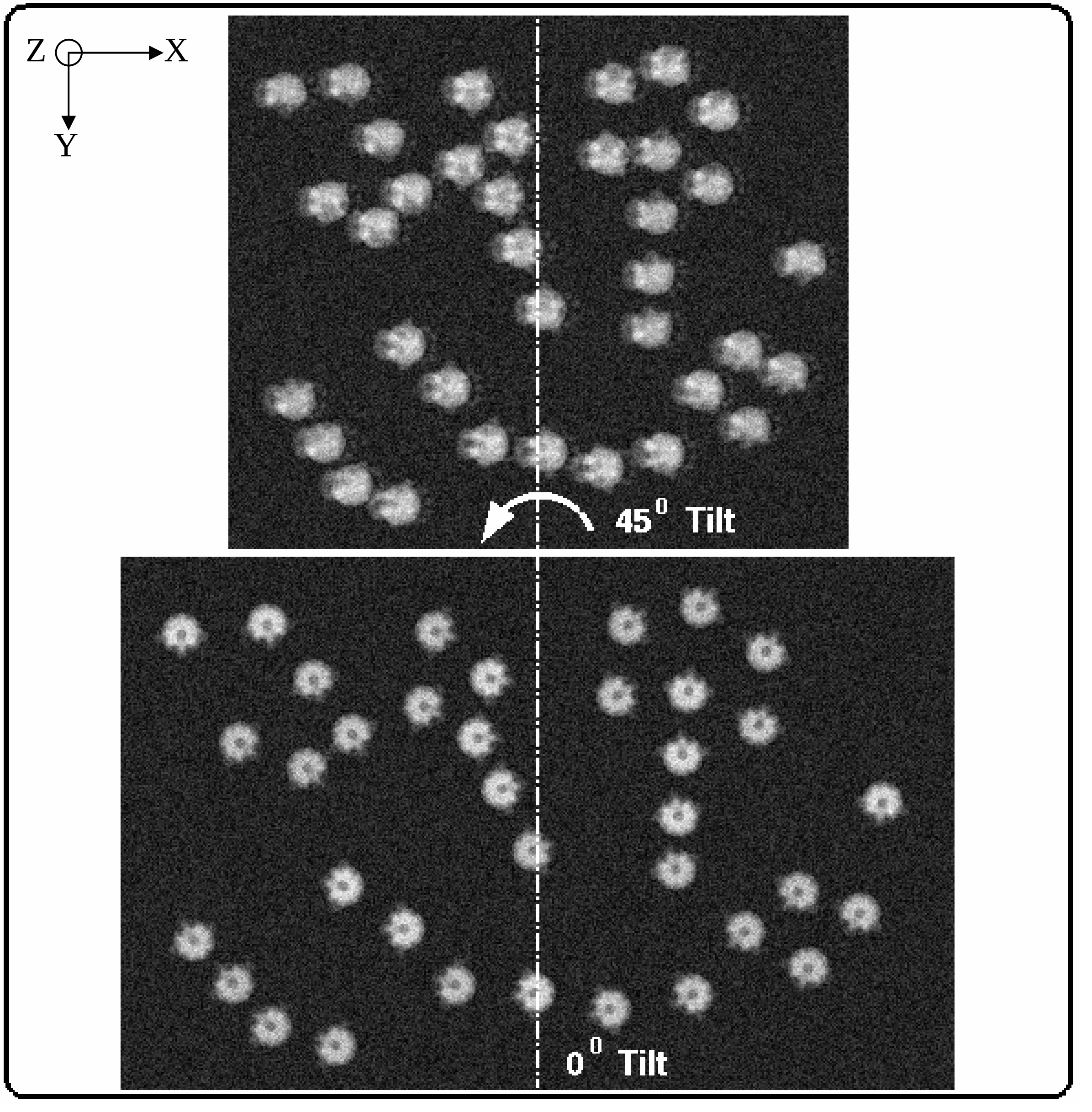
- direction of tilt axis (ψ)
- and the tilt angle (θ).



Ψ = 0°   Ψ = 90°

Ψ = in-plane direction of tilt axis
If Ψ parallel to axis Y, then Ψ = 0°

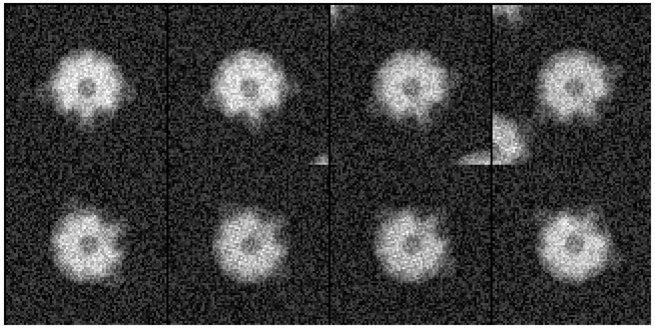
θ= Tilt angle => COS (θ) = d / D
(but you don't know if it is + or – θ)



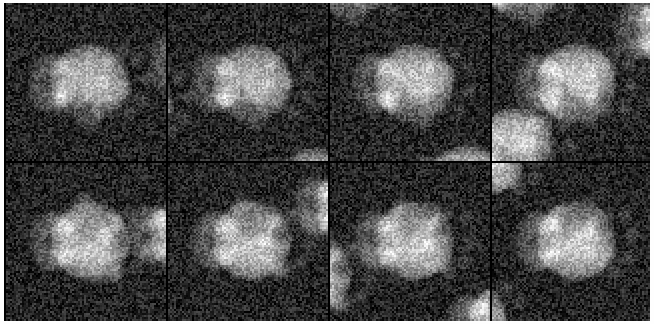
2D projections are identical, except for an in plane rotation corresponding to Euler angle ϕ.

2D projections are not identical due to the tilt. Moreover, neighboring particles start to overlap

Interactive windowing at 0° and 45° tilt



0°

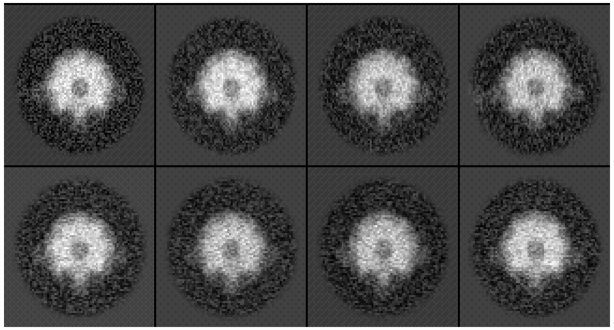


45°

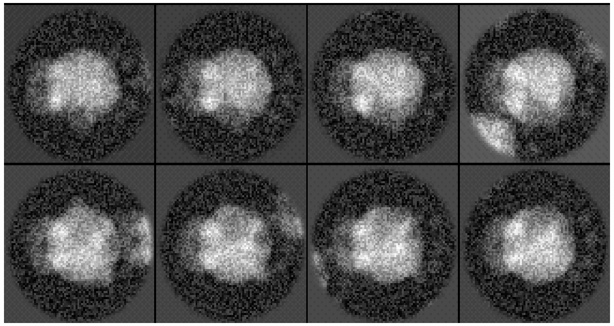
Rotation of each 0° projection by its -ϕ angle

A circular mask hides (up to a certain point) the neighboring particles.

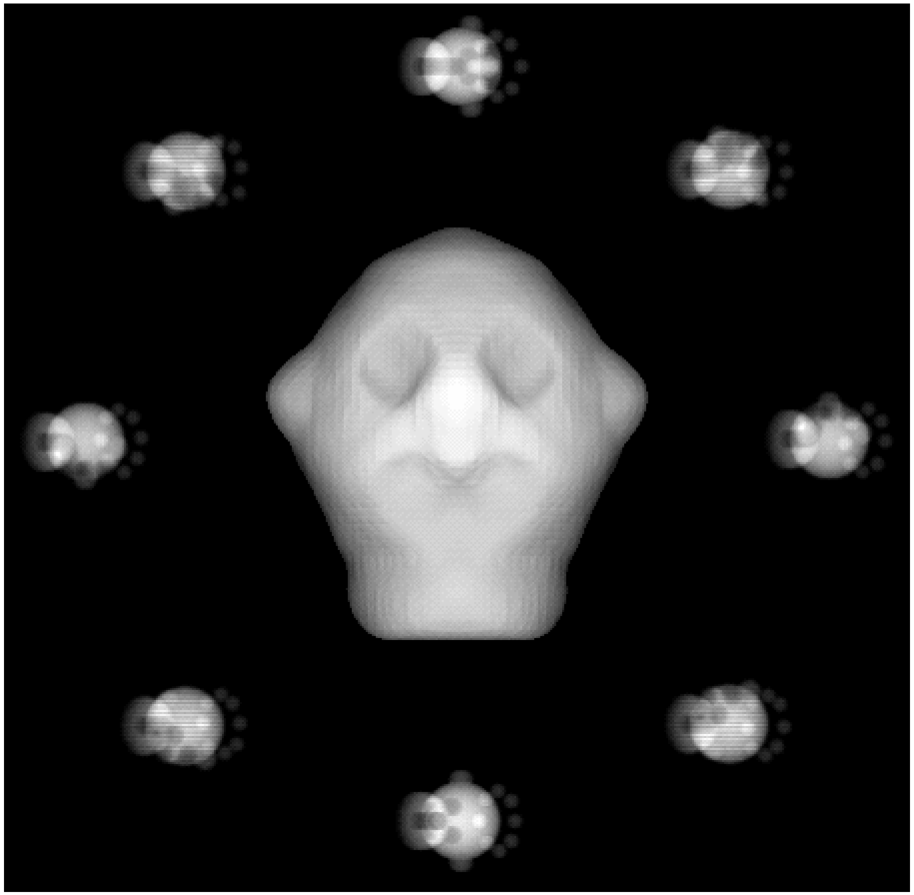
2D alignment of untilted-specimen images and computation of angle ϕ



Centering and masking of tilted-specimen images

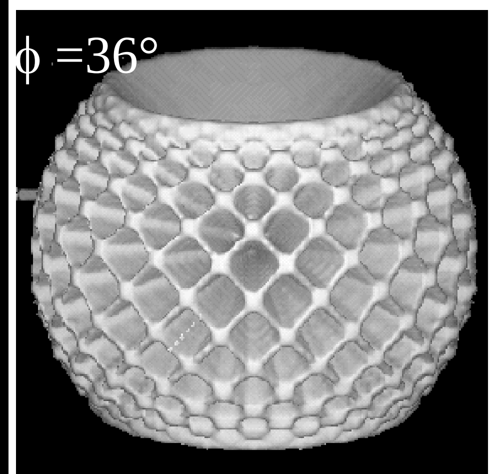


Simple back-projection

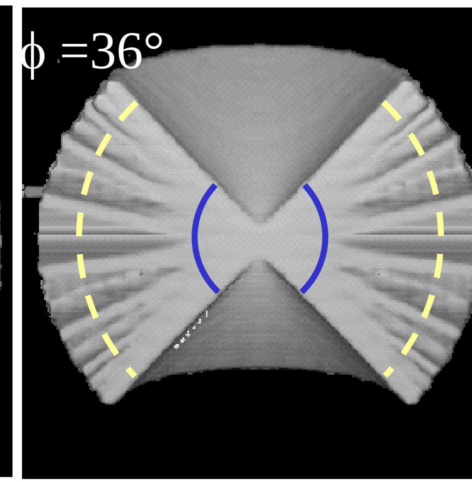


Why does it look so bad?

Reciprocal space



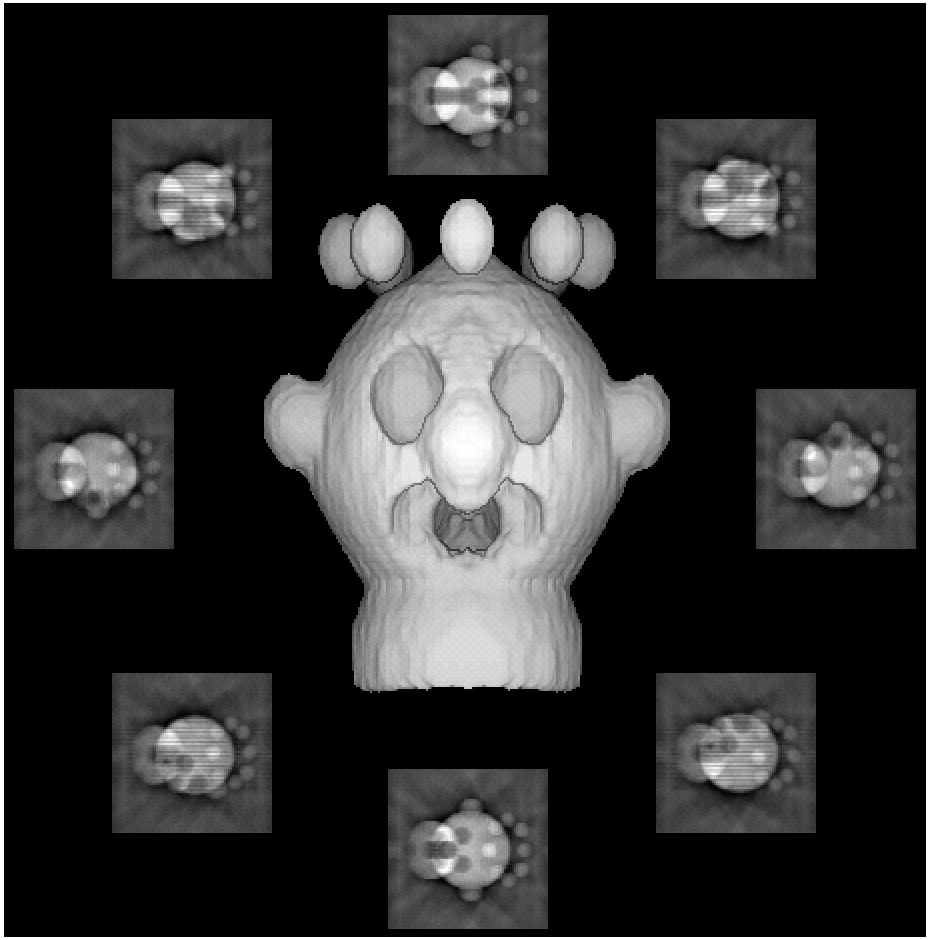
half-volume



Uneven distribution of the signal

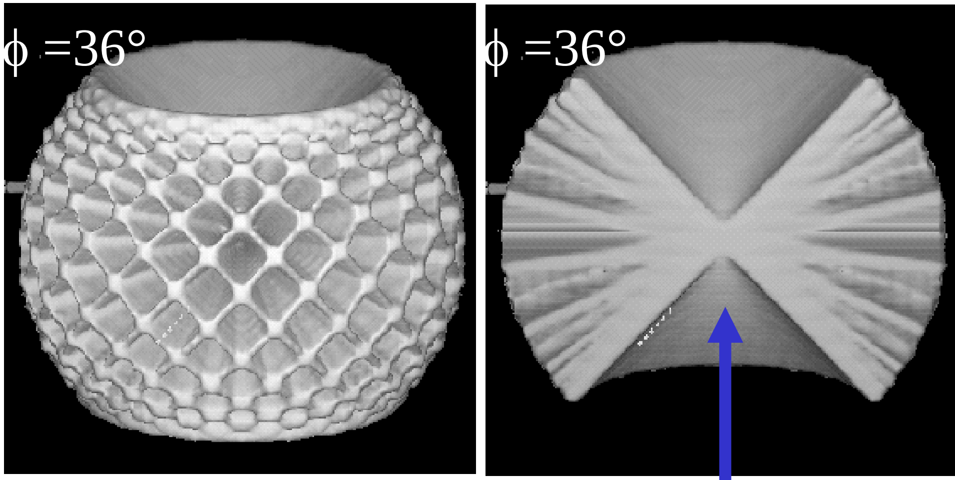
Once the 3 Euler angles are determined, the 3D reconstruction can be performed from the tilted-specimen projections. The simple back-projection is nothing more than adding in reciprocal space the FT of the 2D projections in their relative orientations (waffle-like distribution of central sections), followed by Fourier transform of this 3D distribution to come back in real space.

Weigthed back-projection



It is better, but we have a non-isotropic reconstruction. Why ?

Reciprocal space half-volume

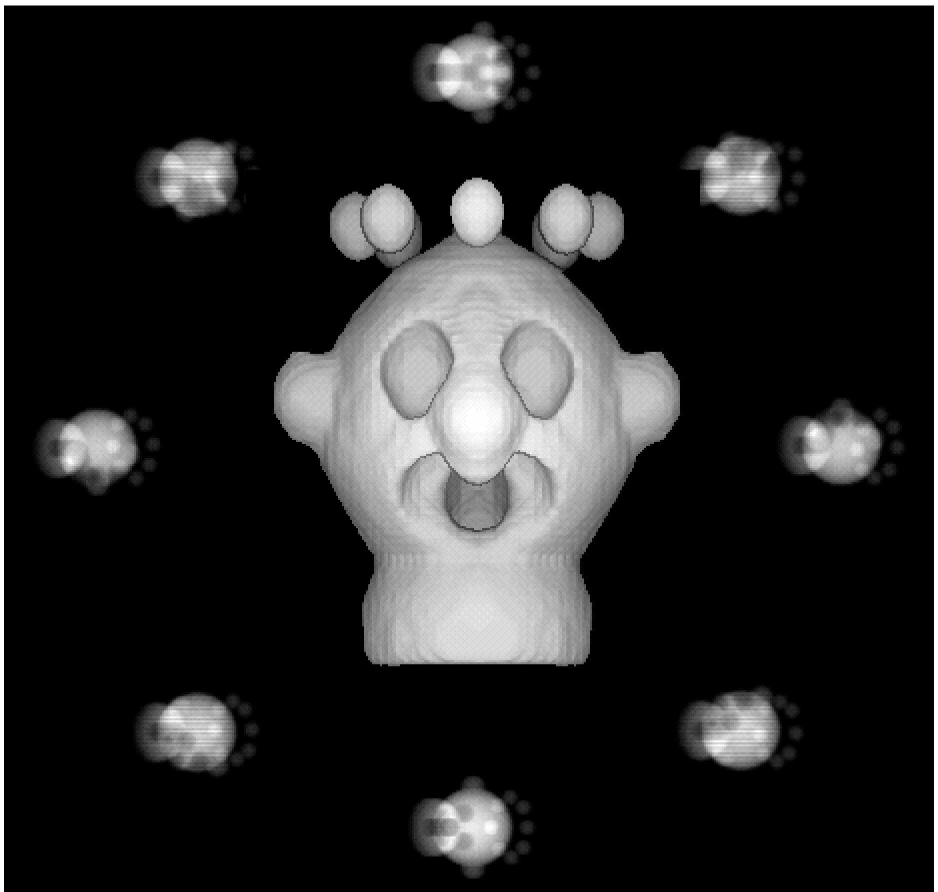


Missing cône

Similar as previously, but after applying a band-pass filtering or **R* weighting** of the signal (lowering contribution in low spatial frequencies).

Simultaneous Iterative reconstruction techniques (SIRT)

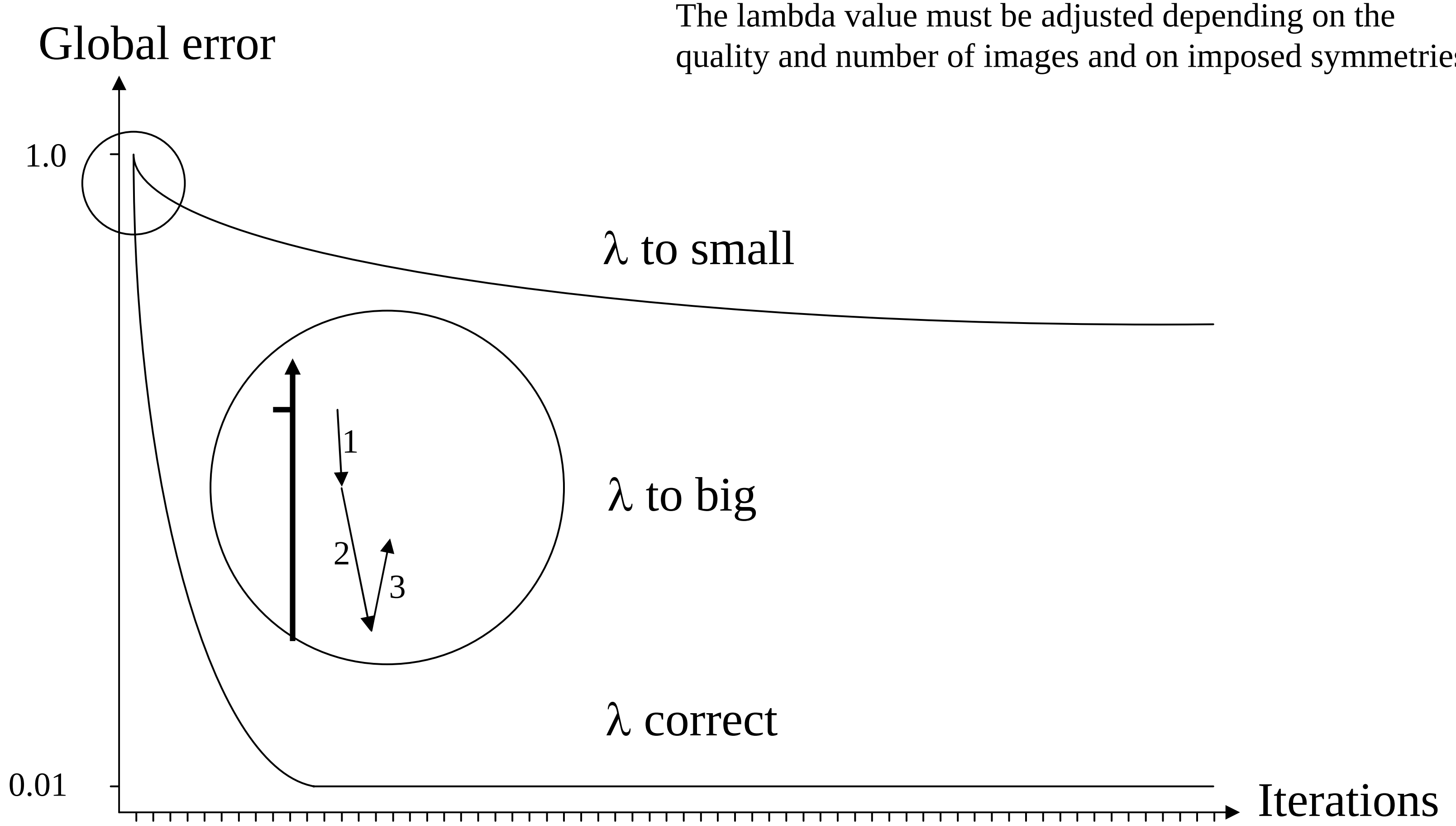
Real space & iterative:



In real space with iterative methods, a starting volume is computed by simple back-projection. Then, the volume is re-projected in its original directions and 2D projection maps are compared with the experimental EM images. The difference maps [(EM) minus (2D projection of the volume)] are computed and back-projected to correct the 3D reconstruction volume. To avoid "over-correcting" the structure, the 2D difference maps are multiplied by an attenuation factor λ, (with λ ~ 0.5·E-04 to 0.1.E-06). This process is iterated and at each step the "global error" between EM images and the computed volume is measured to check improvement.

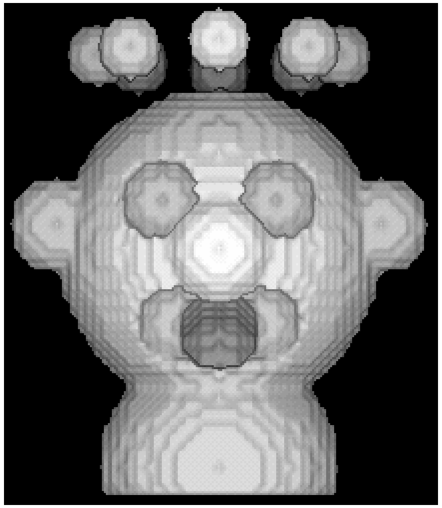
Correct λ value ?

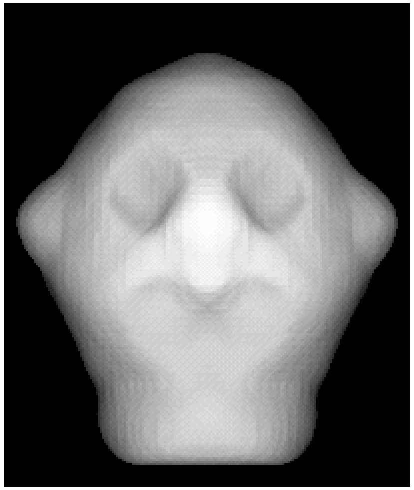
The lambda value must be adjusted depending on the quality and number of images and on imposed symmetries



Comparing 3D reconstruction techniques

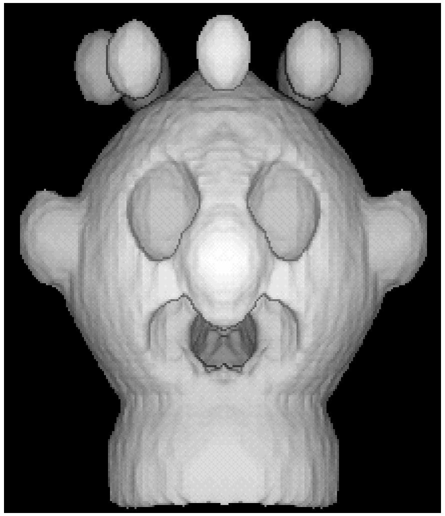
Original object

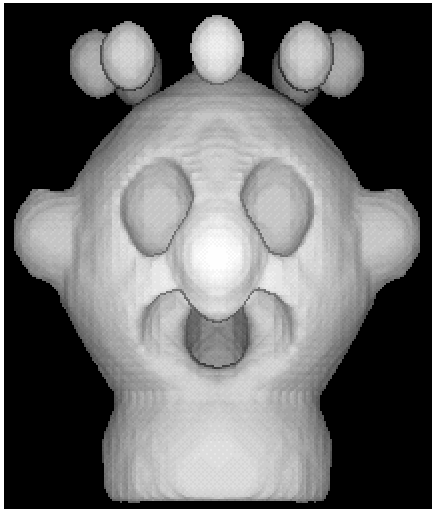




Simple back-projection

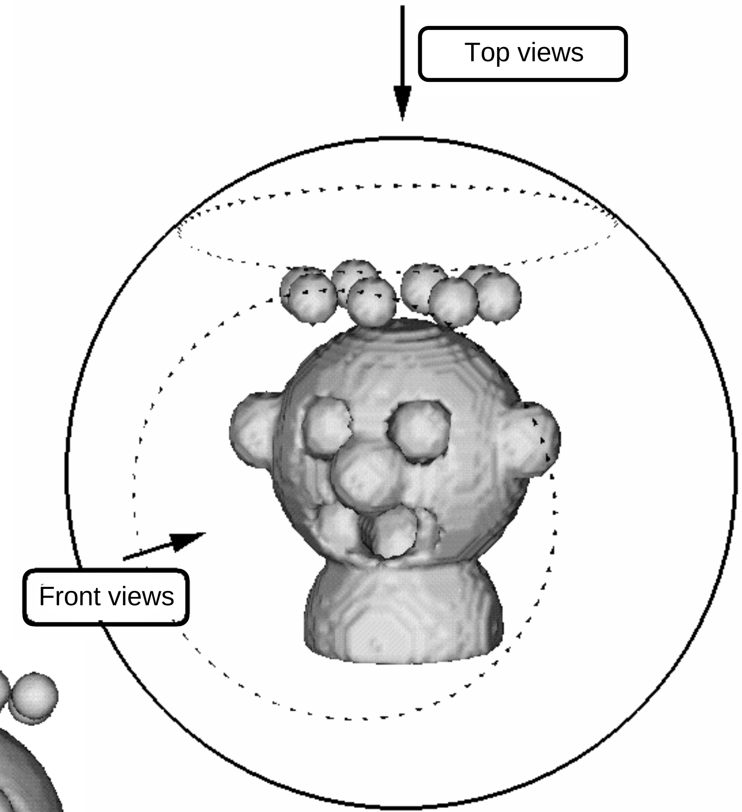
Weigthed back-projection



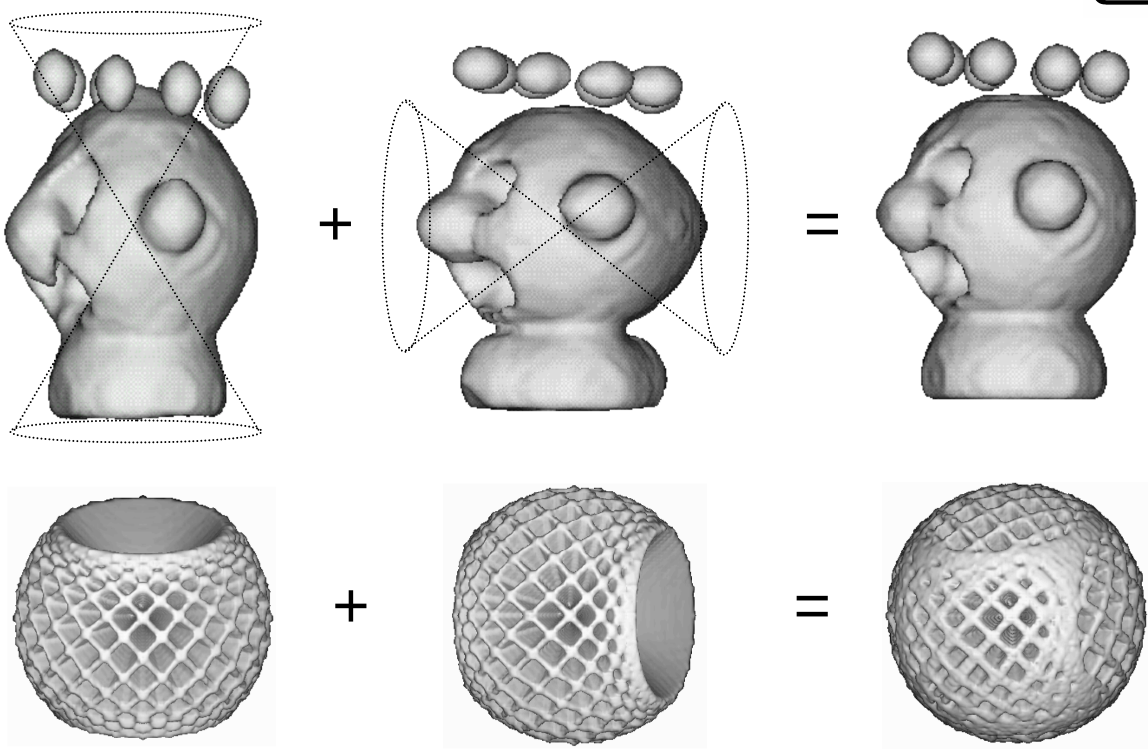


SIRT

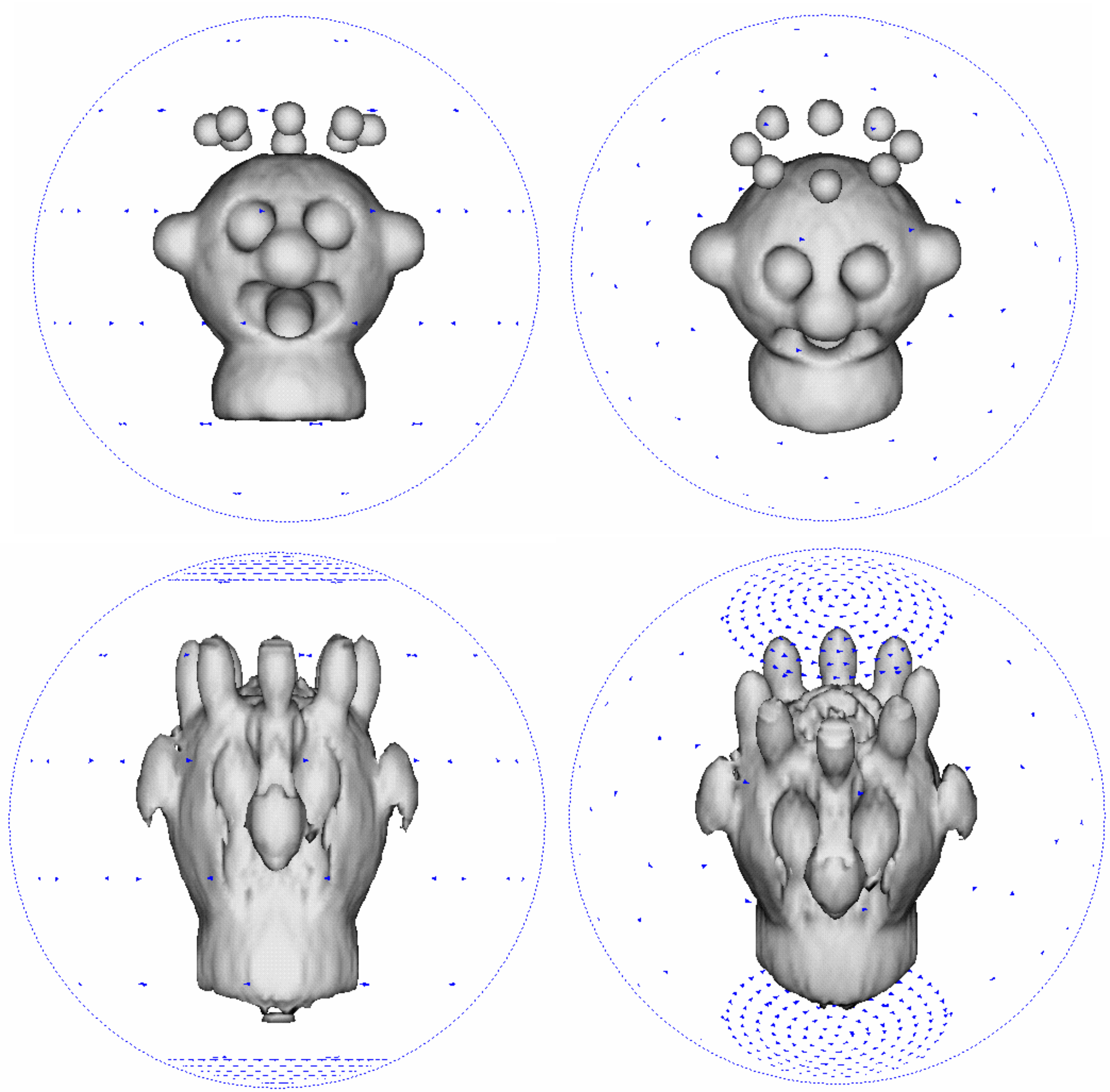
The missing cone artifact



Top views **Front views**



In rare occasions, a single overabundant preferential orientation can distort your structure when using SIRT

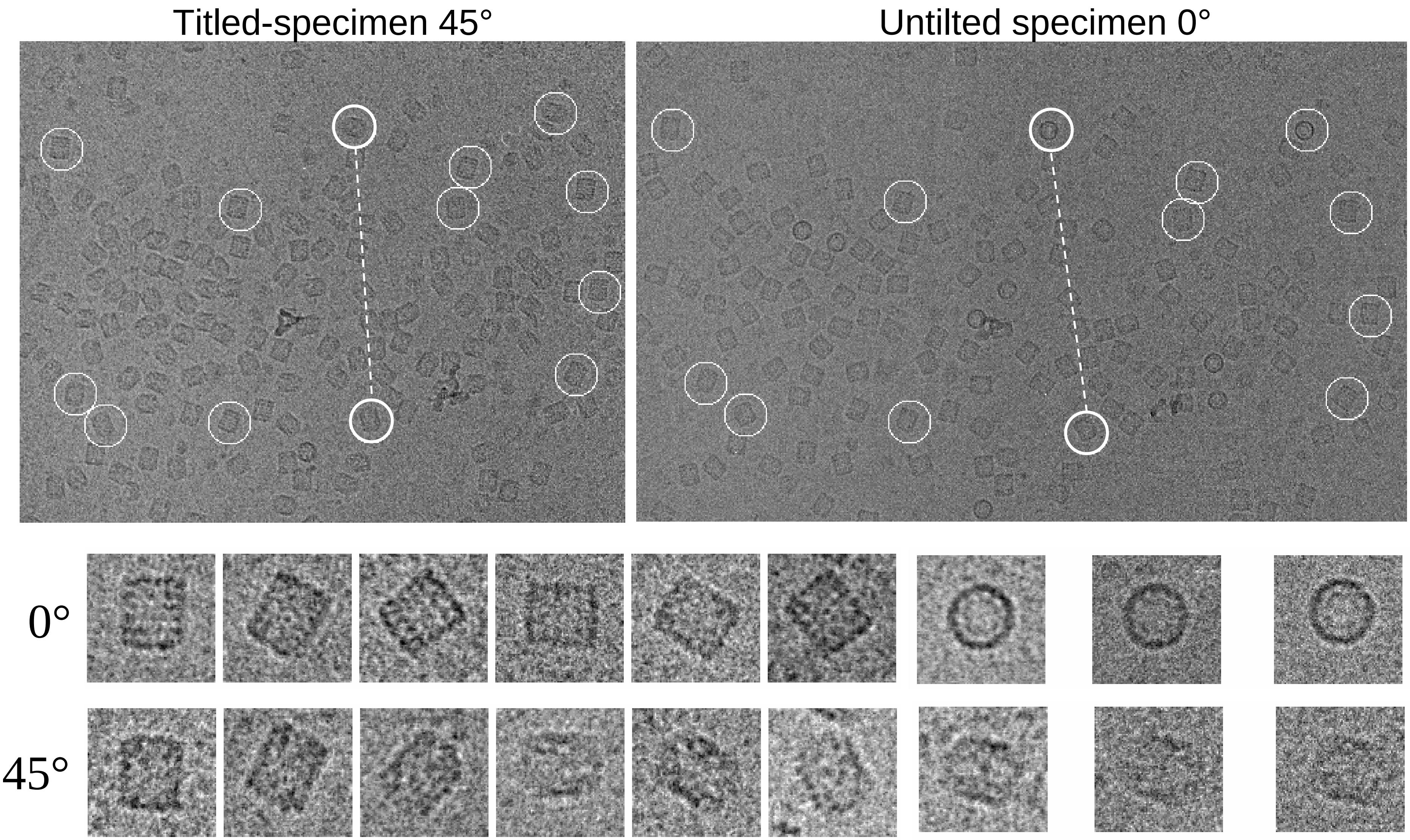


even angular distribution

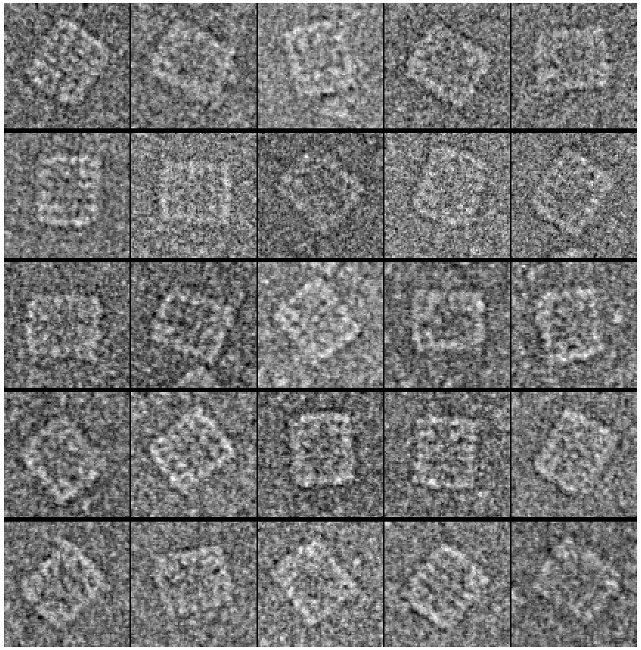
uneven angular distributions

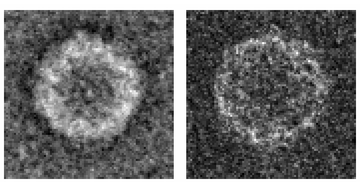
Boisset N., Penczek P., Taveau J.C., You V., de Haas F., Lamy J. (1998) Overabundant single-particle electron microscope views induce a three-dimentional reconstruction artifact. *Ultramicroscopy*, **74**: 201-207.

Interactive particle picking with determination of tilt axis direction (ψ) and tilt angle (θ)



Original side views

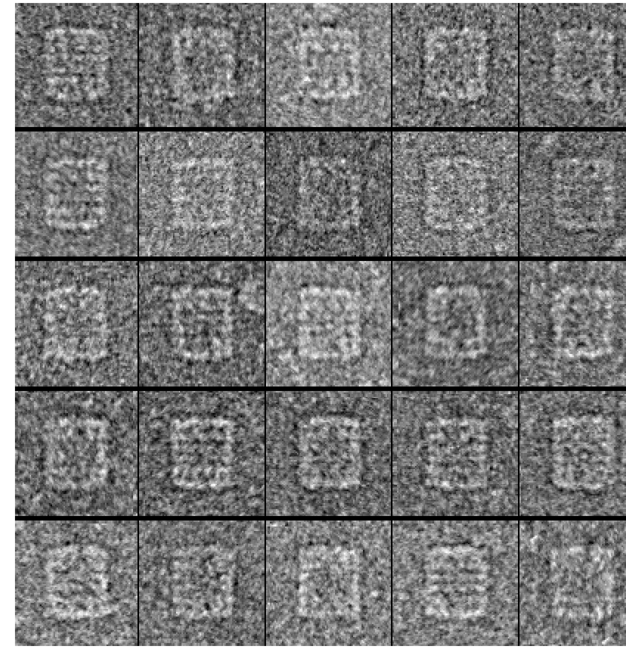


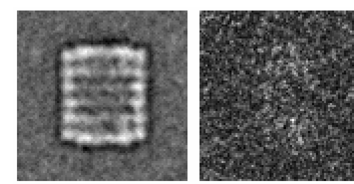


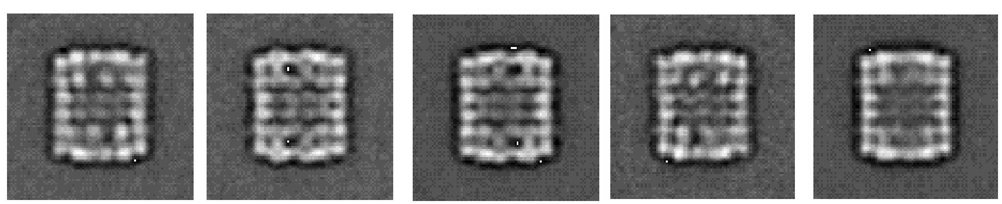
Alignment

Determination of angle (ϕ)

Aligned side views



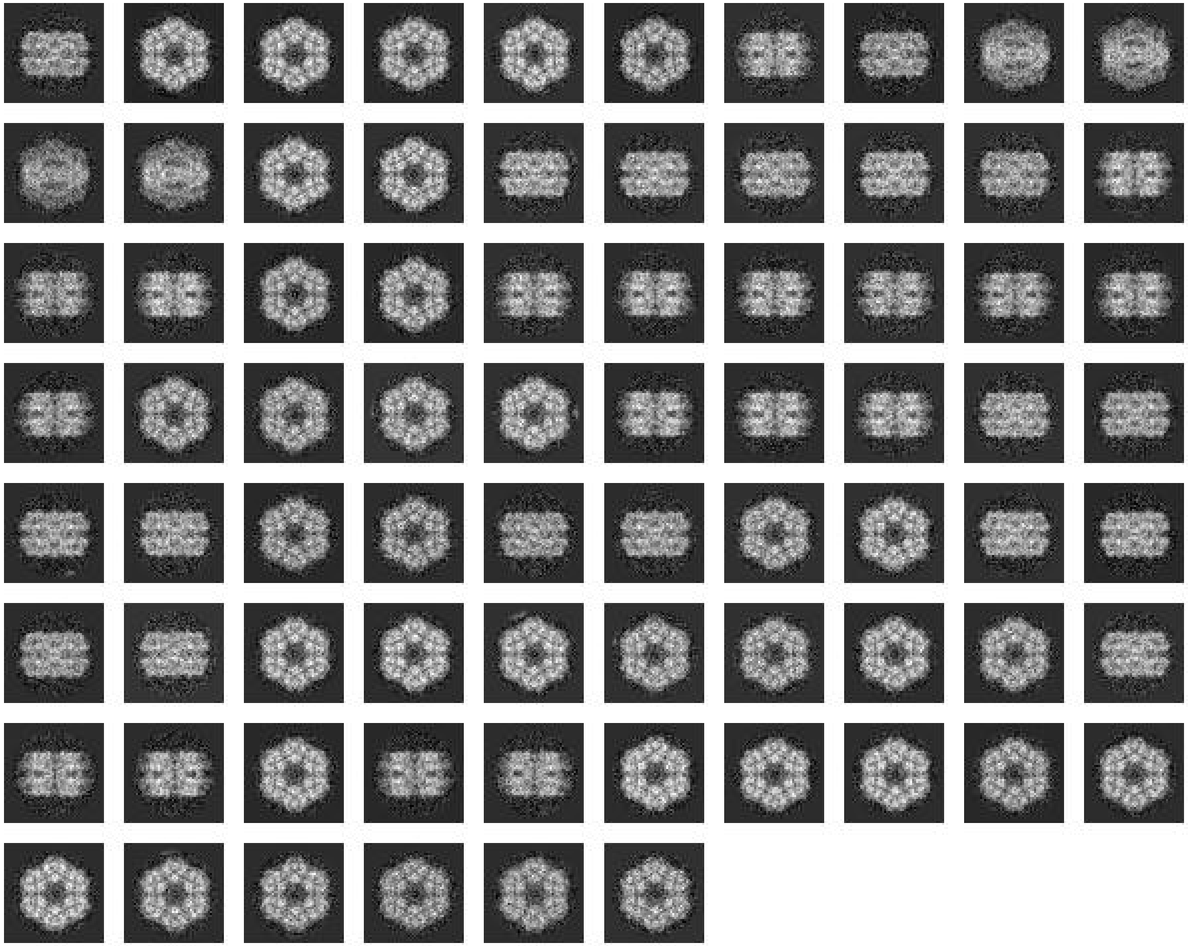




MSA and clustering → 5 views

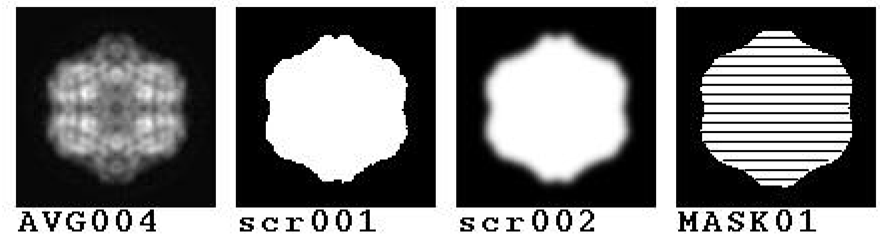
A simplified and therefore mathematically incorrect description of Correspondence analysis (CORAN) To get the "flavour" of this method.

You have normalized, aligned a set of noisy images and you want to sort them automatically. *(For correspondence analysis no negative density is tolerated, while for principal component analysis (PCA) you don't care).*



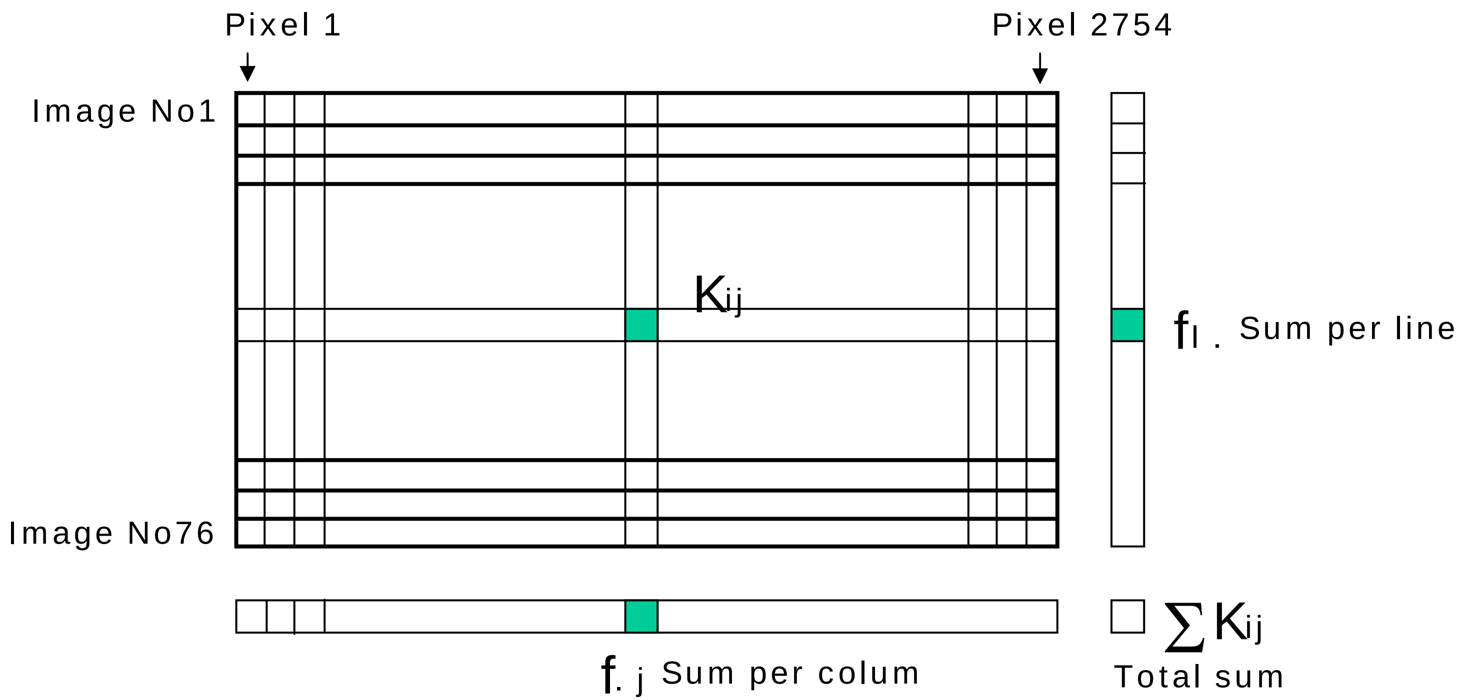
Latin is like mathematics an integrated language. For example in latin “*Intelligenti pauca*” can be translated by “ intelligent people understand each other with a few words ! ”

1- Create a mask following the shape of the total average



2- For each image, extract all densities from the pixels falling within the mask and re-dispose then into a line.

3- Place theses lines of densities into a table also called “TABLE DE CONTIGENCE”



4- An other way to consider the data is to say that these densities are coordinates in a multidimensional space.
5- Hence in this example, each image having 2754 pixels under le mask, our data set corresponds to 76 images, that we can consider as 76 dots in a space of 2754 dimensions.

Intuitively one can guess that two identical images will have similar coordinates in the multi-dimensional space. Therefore in the multidimensional space they correspond to two dots located near each other. Conversely, two dissimilar images will correspond to two dots located far away from each other.

Multi-dimensional statistical analysis (MSA), reinforces this idea of "similarity = proximity" but it changes the coordinate system of our data set in order to reduce the number of dimensions to a number a few meaningful axes. These axes or "eigen vectors" correspond to main "trends" or "variations" within our population of images.

1. Absolute values → frequencies

$Kij \rightarrow Kij / \Sigma kij = fij$

2. Euclidian distance → χ^2 distance

$fij \rightarrow fij / fi.\sqrt{f.j}$

3. Image mass i = fi.

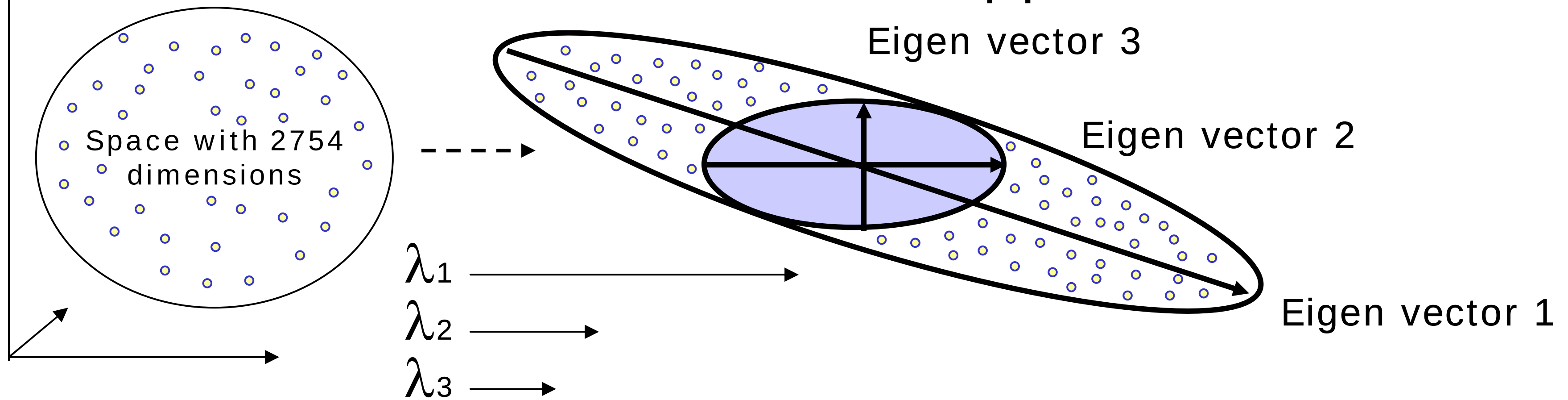
Origine changed to the center of gravity of the table = -f.j

4. Diagonalization of the covariance matrix

$Xij = (fij - fi.\ f.j) / \sqrt{fi.\ f.j}$

equivalent to a least square fit to define new factorial axes (eigen vectors) and the coordinates of each image on these axes.

The ALMOND approach



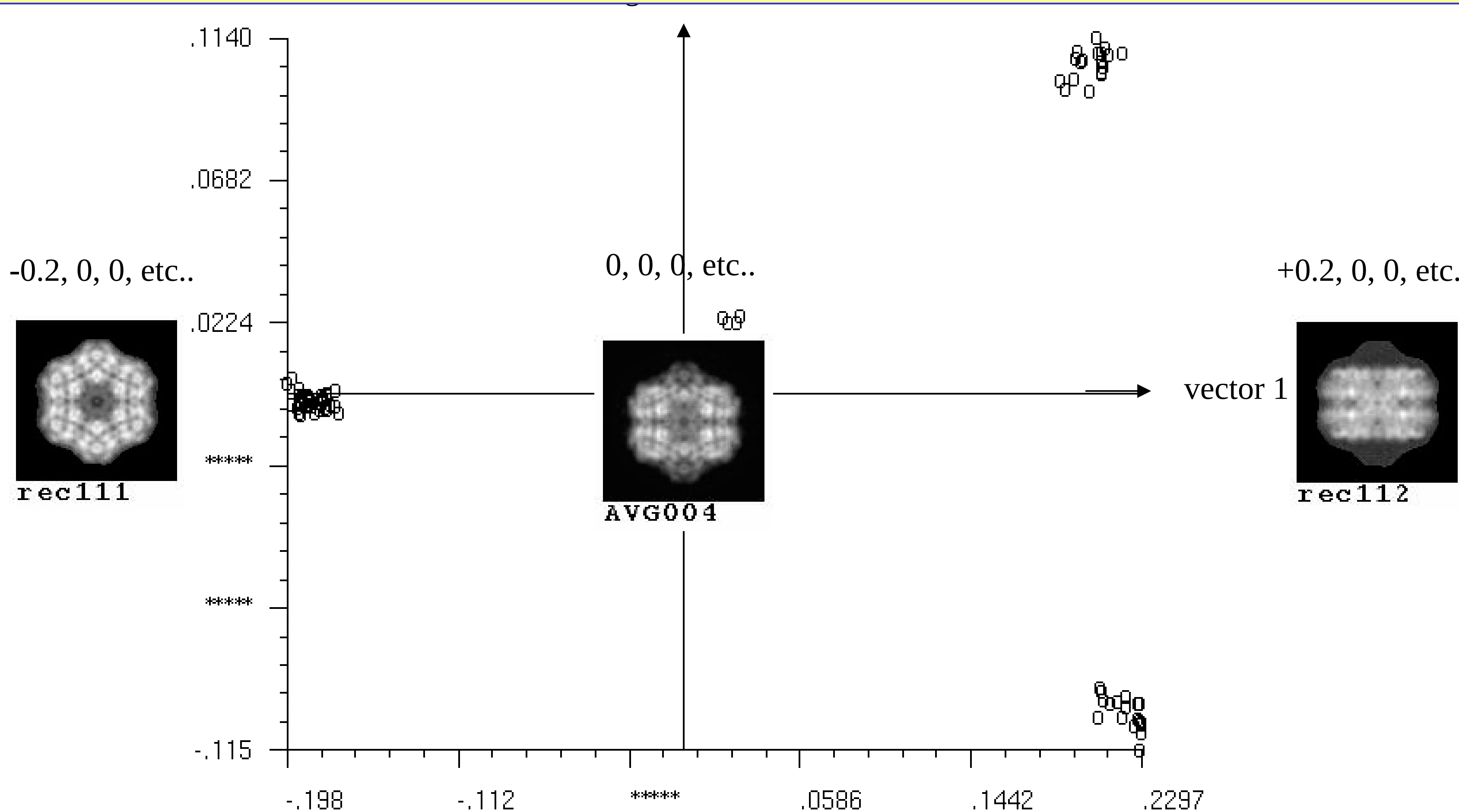
One method of diagonalization of the co-variance matrix (T = X' X), called "la méthode de la dragée" or the "Almond method" illustrates what happens at this stage.

The original multi-dimensional space has been distorted by the chi square matrix to express the variations among the images. Schematically one can say that the cloud of 76 dots (representing our 76 images) which was originally contained in a multi-dimensional "sphere" is now contained within a multi-dimensional "almond".

0. The origin of the new coordinate system is set to the center of gravity of the almond.
1. The **longest dimension of the almond** corresponds to the major "trend" or variation among the image set and is defined as the first eigen vector. Its amplitude (length) corresponds to the first eigen value λ1. Coordinates of our 76 dots along this new axis are calculated.
2. Then, the **second longest dimension** of the almond, **orthogonal to the first eigen vector** is determined (width of the almond). This second direction corresponds to eigen vector number two and corresponds to the second variation among the images. The amplitude of this second vector is the second eigen value λ2. Coordinates of our 76 dots along this new axis are calculated.
3. Then the **third longest dimension** of the almond, **orthogonal to the first and second eigen vectors** is determined (thickness of the almond). This third direction corresponds to eigen vector number three and corresponds to the third variation among the images. The amplitude of this third vector is the third eigen value λ3. Coordinates of our 76 dots along this new axis are calculated.

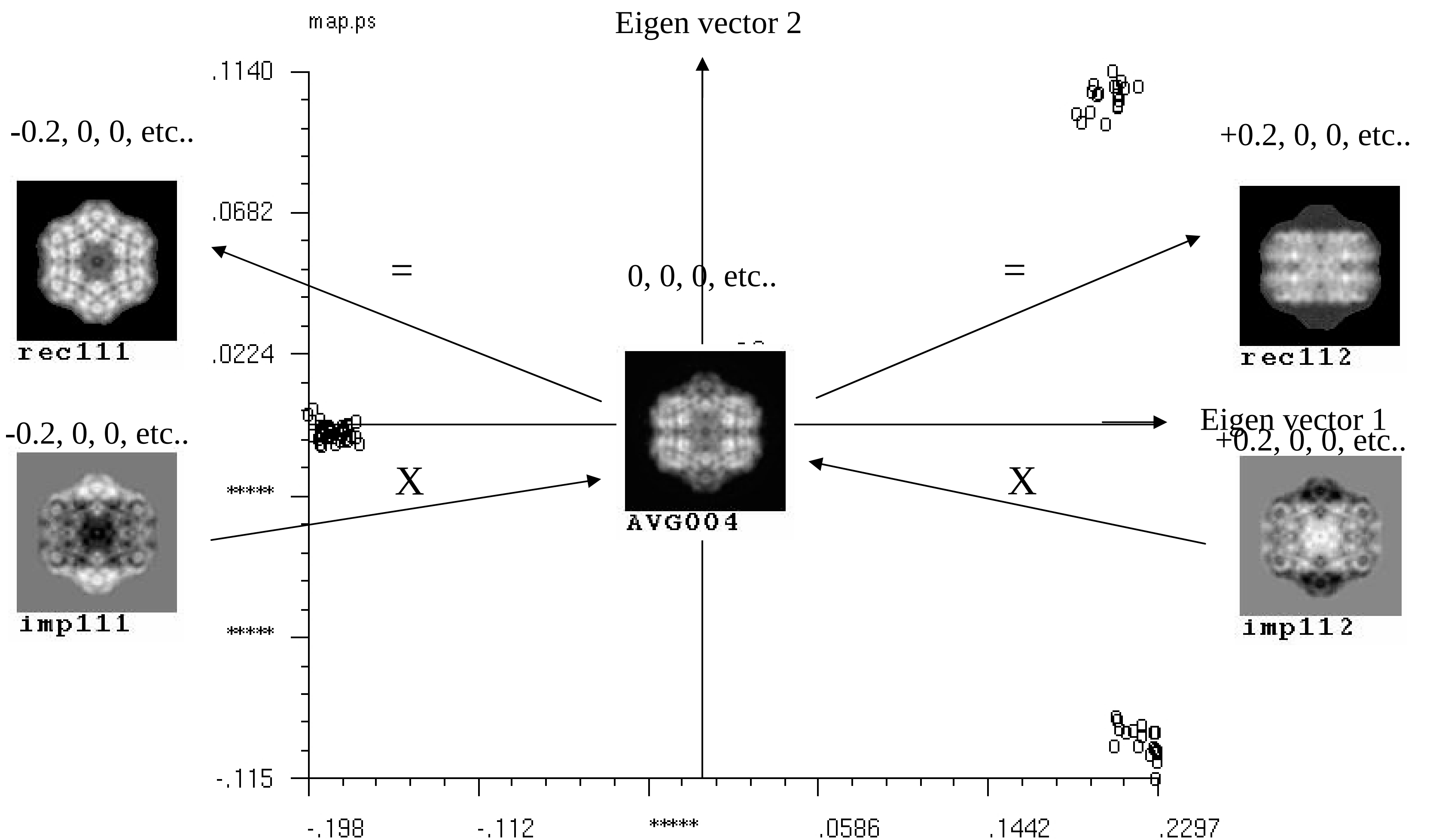
etc…. The same process can continue until up to 75 eigen vectors are determined.

The 76 dots can be projected on planes defines by two selected eigen vectors. Here again the “proximity = similarity” rule applies, and we can identify four types of images in the example set of images. In fact, the information contained in our data is so much compressed that a set of coordinates on the eigen vectors can characterize a given image. Hence with a set of coordinates on the eigen vectors on can go all the way back to an imaginary line in the TABLE DE CONTINGENCE which can be “RECONSTITUTED” into a 2D image. For example, how would look like an imaginary image having for coordinates 0,0,0,0, etc on all eigen vectors of the analysis ?



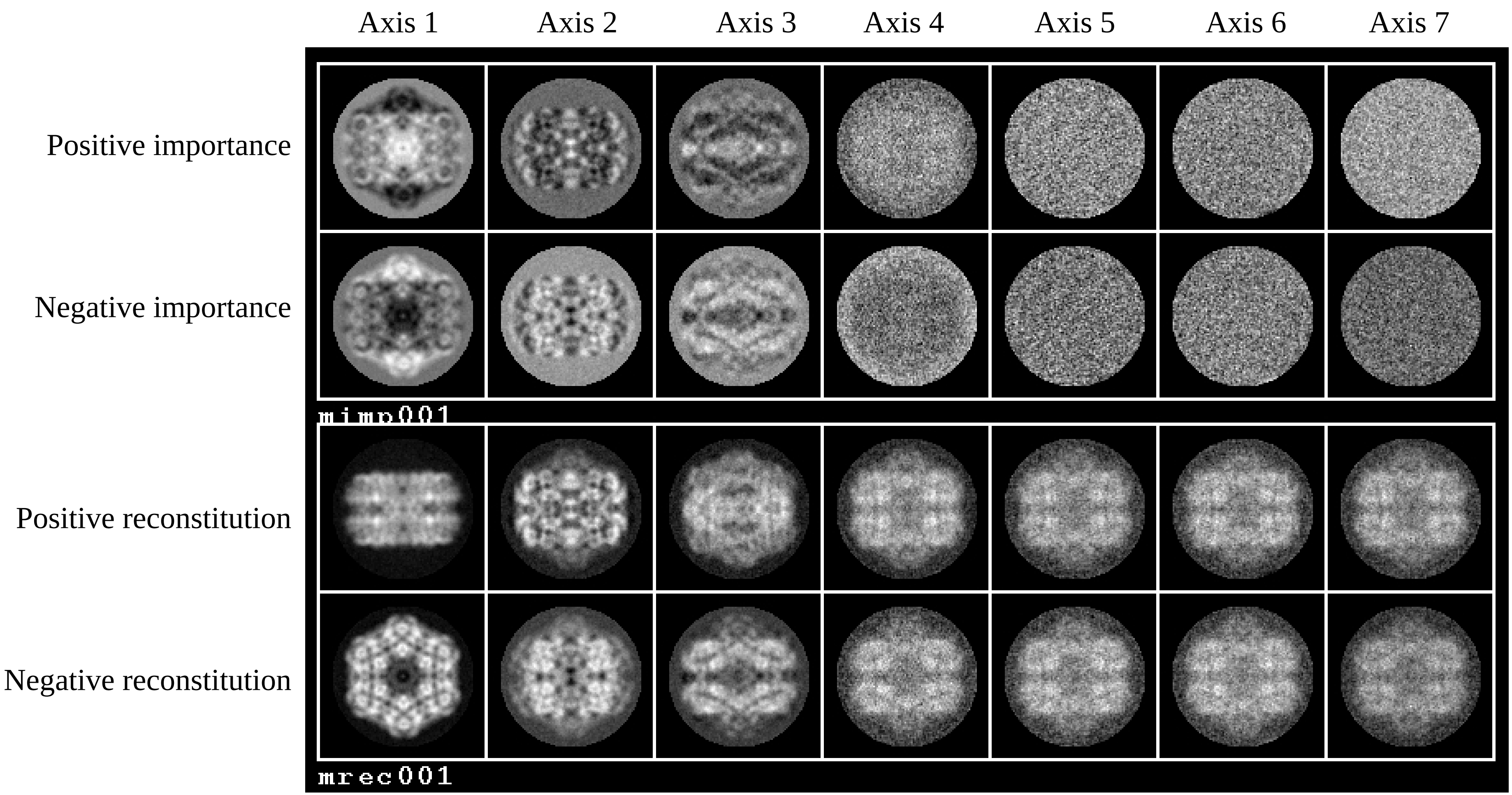
This reconstituted image is equivalent to the global average of all images in the “Table de contingence”. On more practical terms on can explore the significance of a selected eigen vector, by reconstituting two images having coordinates 0 on all other eigen vectors, and extreme positive and negative values on the selected eigen vector. Hence, these paires of images describe the variation expressed by the selected Eigen vector. Here vector 1 is separates top ans side views.

Jean-Pierre Brétaudière and Joachim Frank designed **“reconstitution and importance images”** to express this relationship and to explore the variation related to each eigen vectors, and Marine van heel created the **“Eigen images”** to explore the contribution of each pixel to the eigen vectors. For example, the importance images related to the significance of eigen vector 1 are called here IMP111 and IMP112.

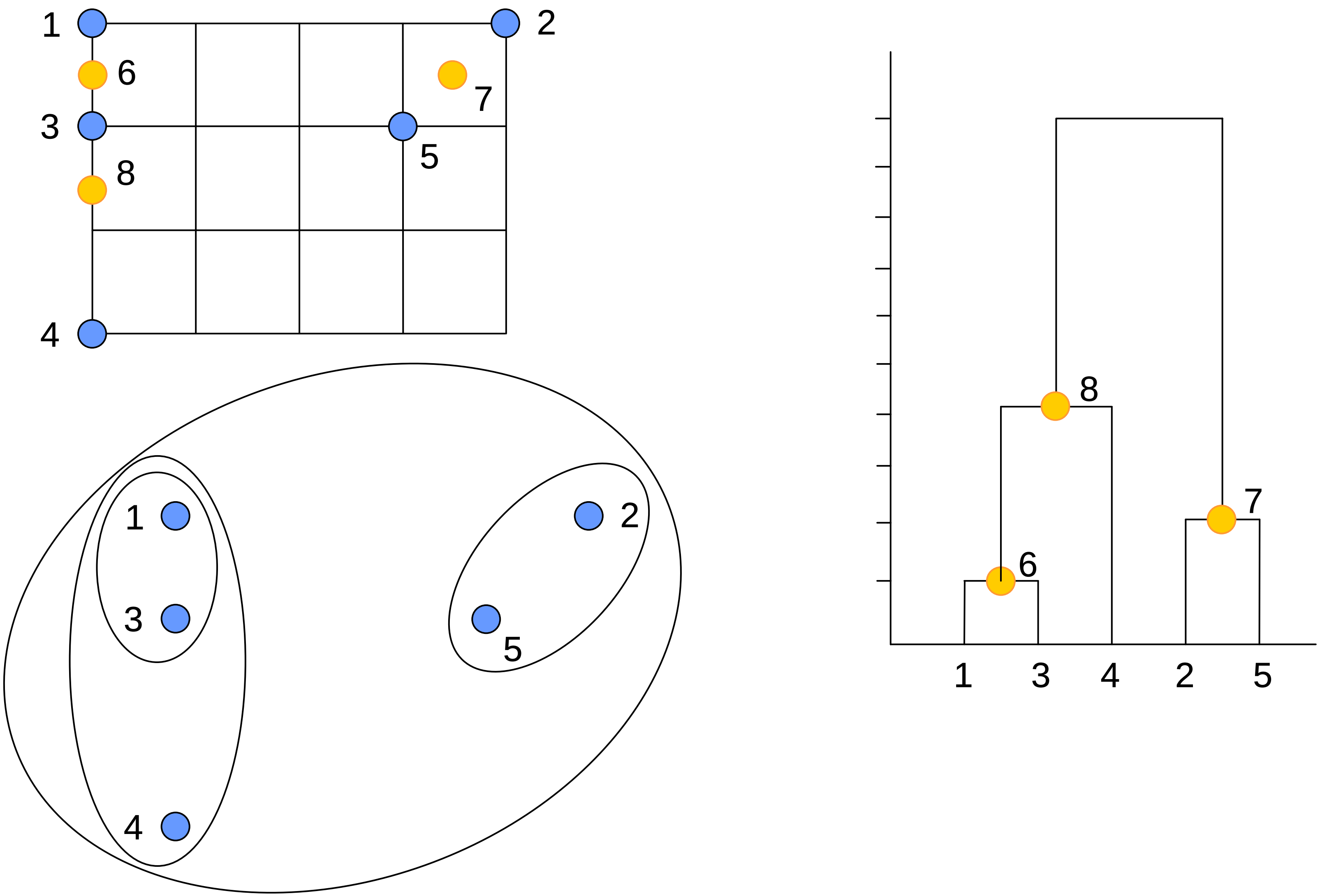


In reality it is more complicated than that, but t simplify, you can imagine that:
If you multiply the global average AVG004 by the importance image IMP111, you obtain the reconstitution REC111.
If you multiply the global average AVG004 by the importance image IMP112, you obtain the reconstitution REC112

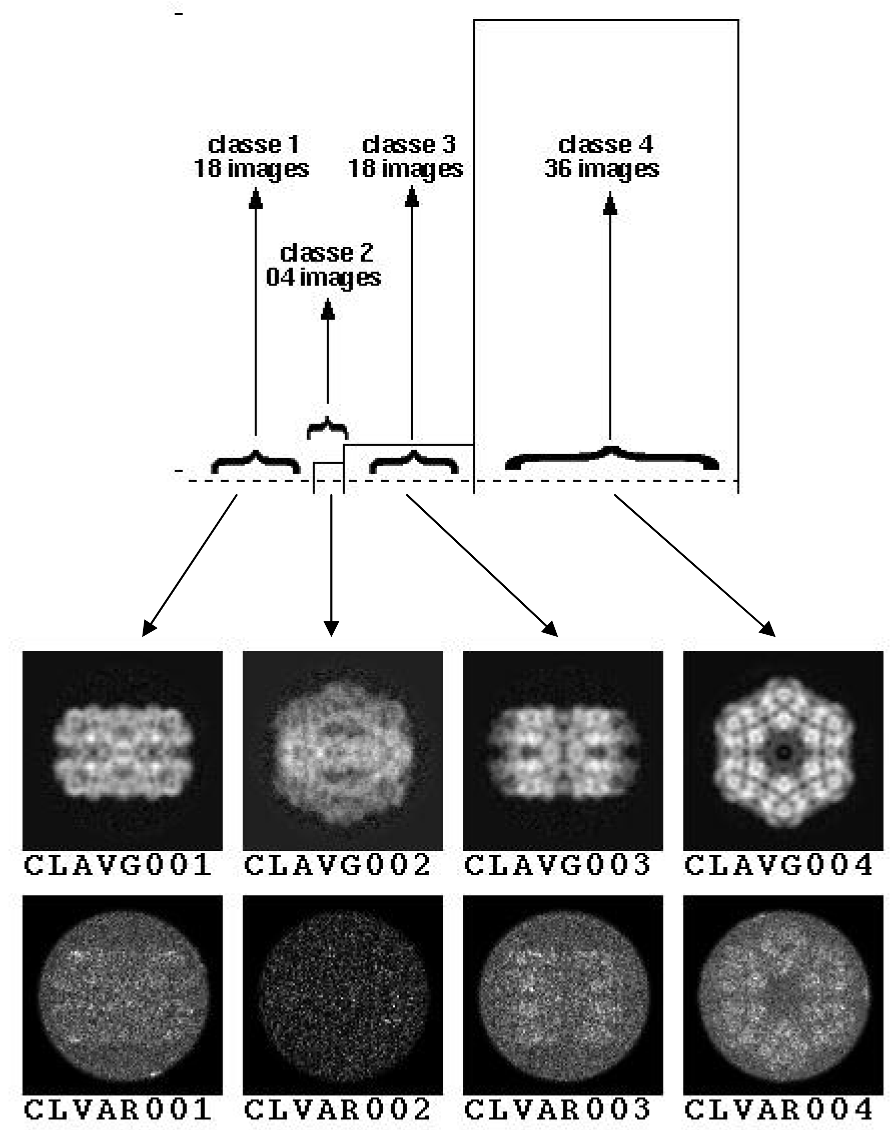
Brétaudière JP and Frank J (1986) Reconstitution of molecule images analyzed by correspondence analysis: A tool for structural interpretation. *J. Microsc.* **144**, 1-14.

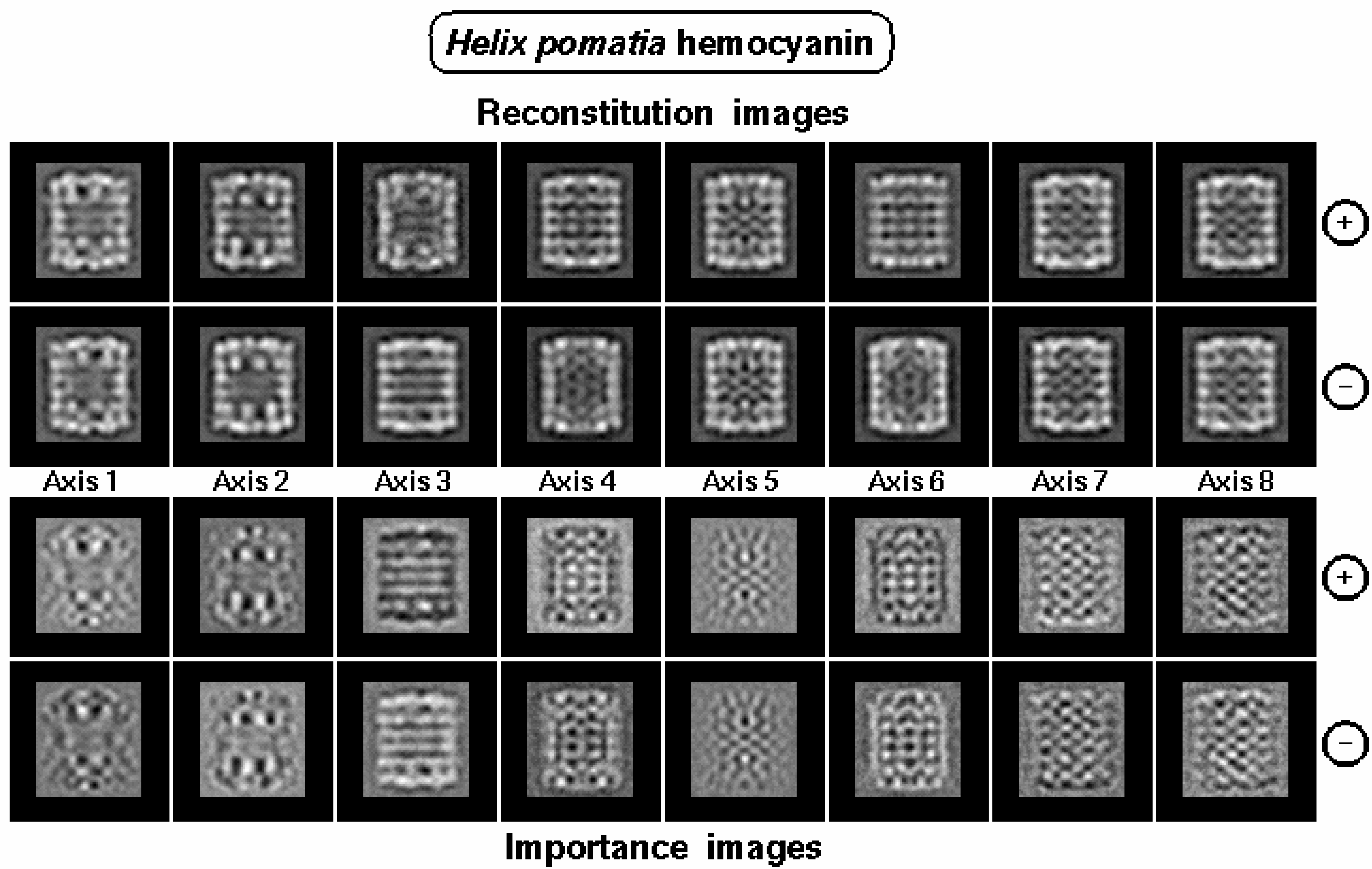


Classification Ascendante Hiérarchique

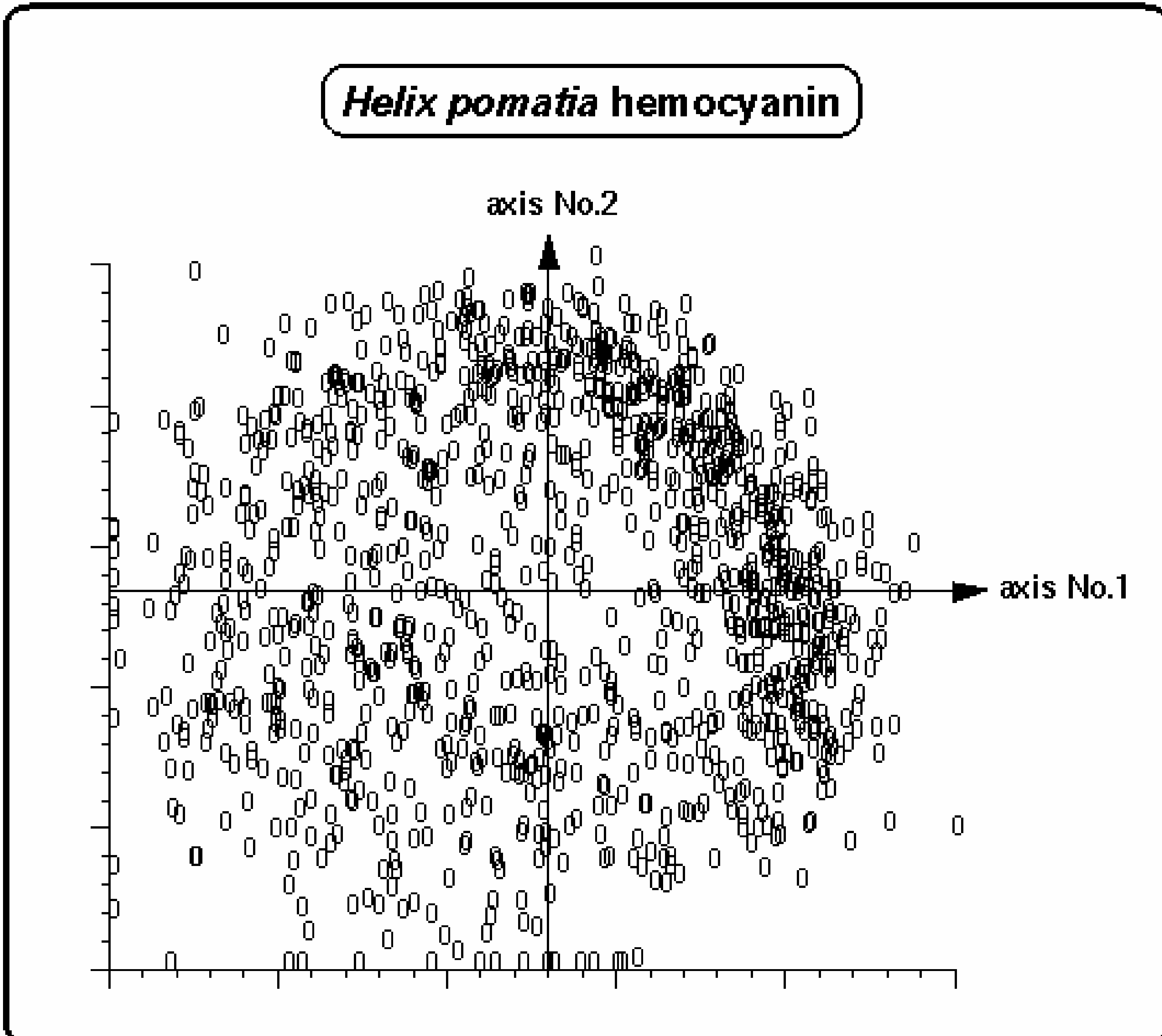


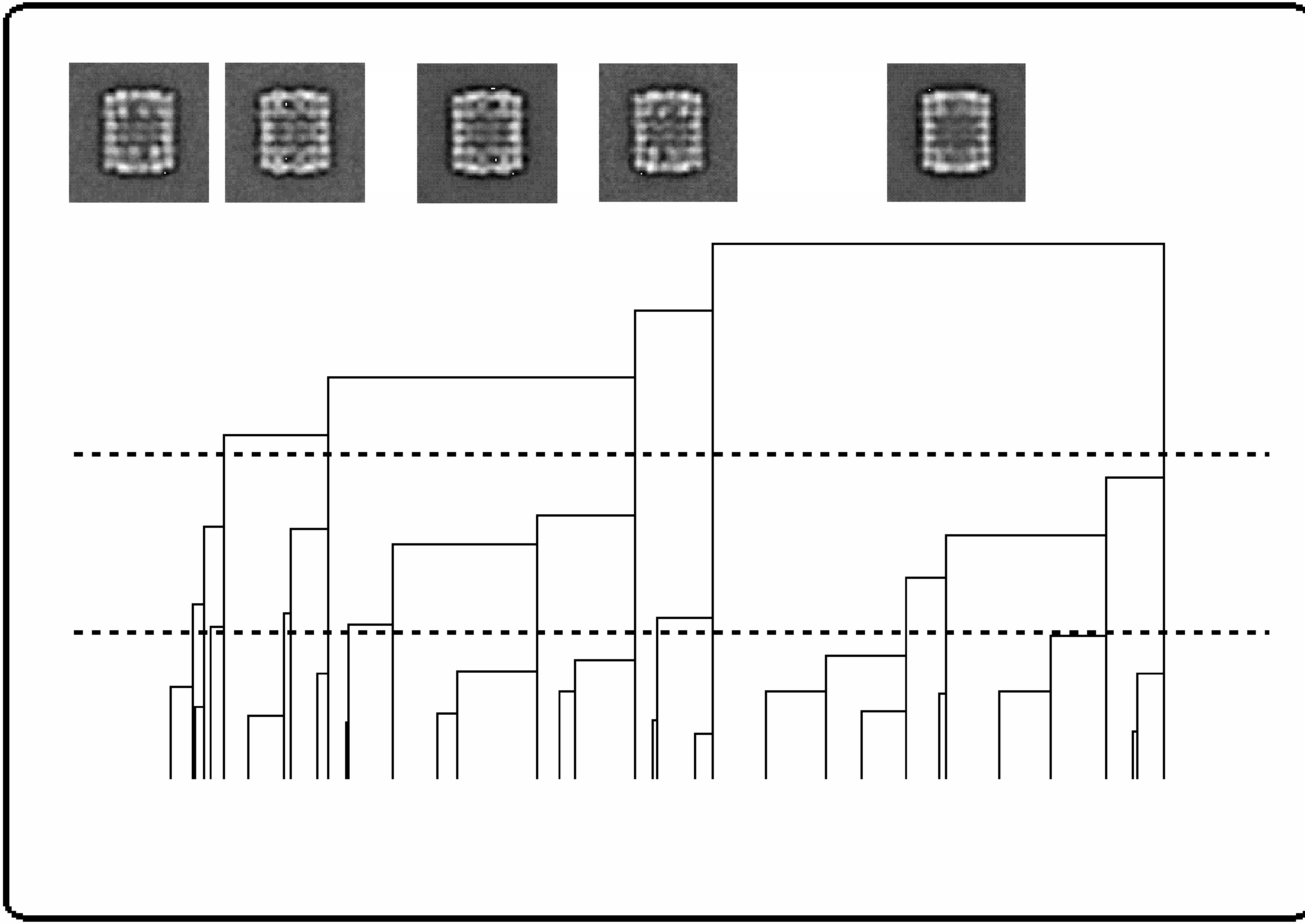
Hierarchical ascendant classification

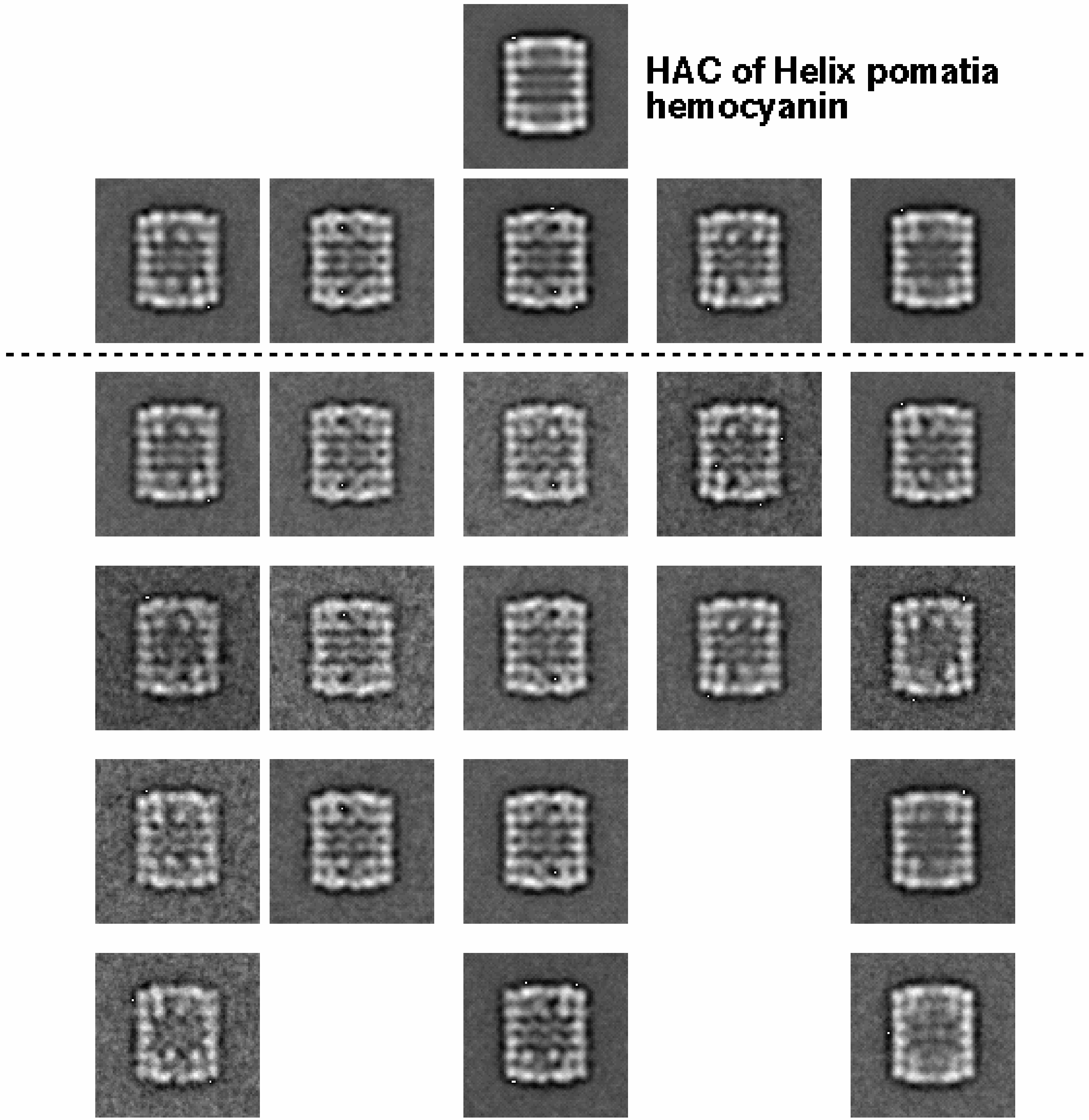




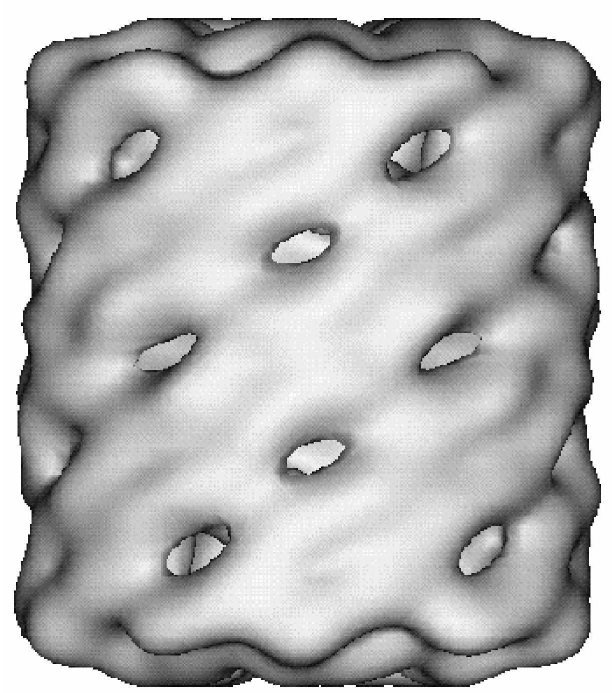
Helix pomatia hemocyanin
Reconstitution images
+
−
Axis 1
Axis 2
Axis 3
Axis 4
Axis 5
Axis 6
Axis 7
Axis 8
+
−
Importance images


Helix pomatia hemocyanin
axis No.2
axis No.1

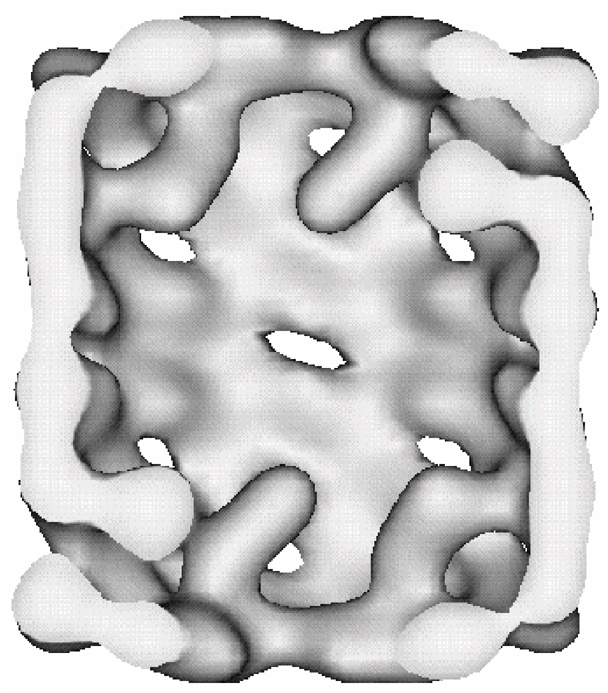



HAC of Helix pomatia hemocyanin

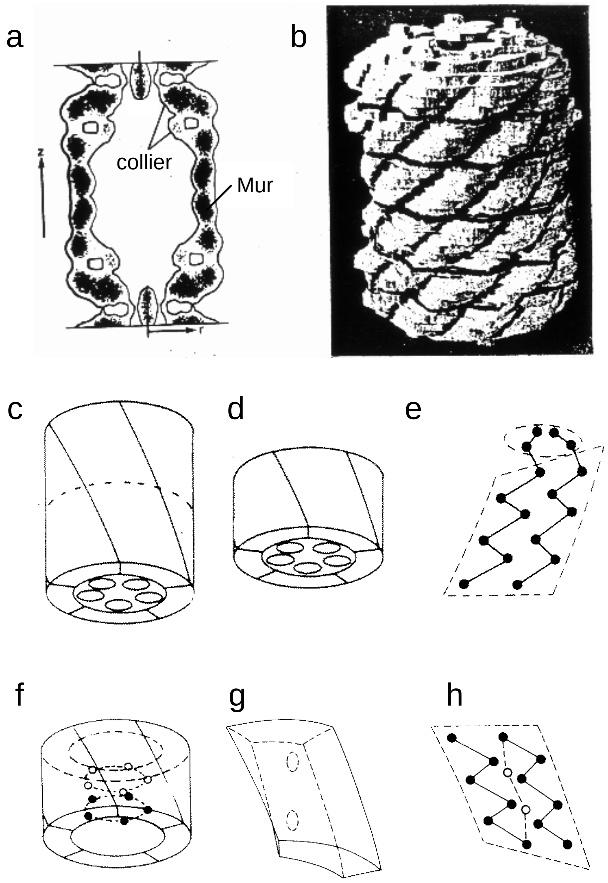
Hémocyanine of *Helix pomatia*



External surface rendering



Central cavity

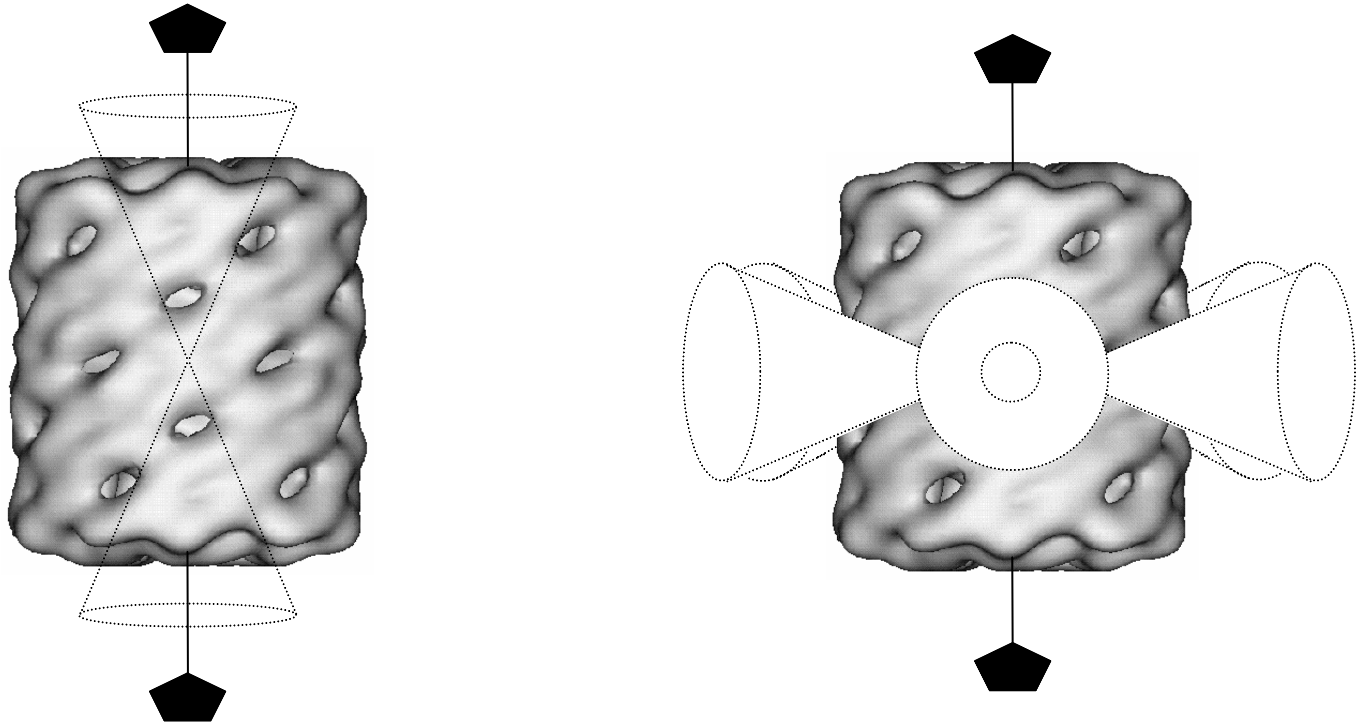


Model of Mellema & Klug (1972)

Question : Which EM views would you use to suppress the missing cone while enforcing a D_5 symmetry ?

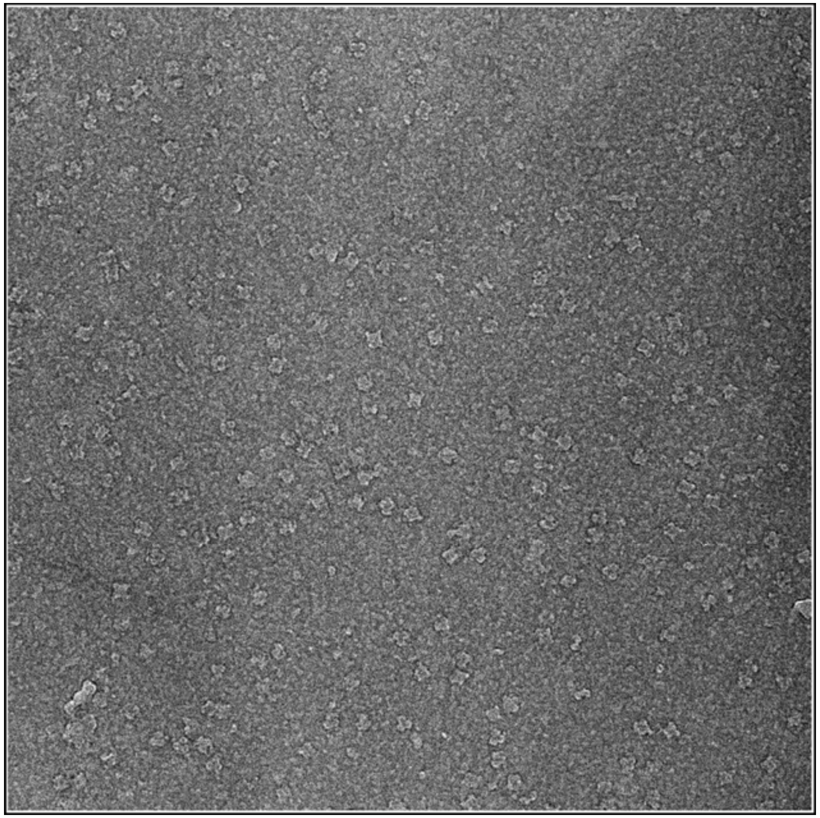
If 0° tilt images = TOP views, the missing cone axis is parallel to the five-fold axis of the cylinder.

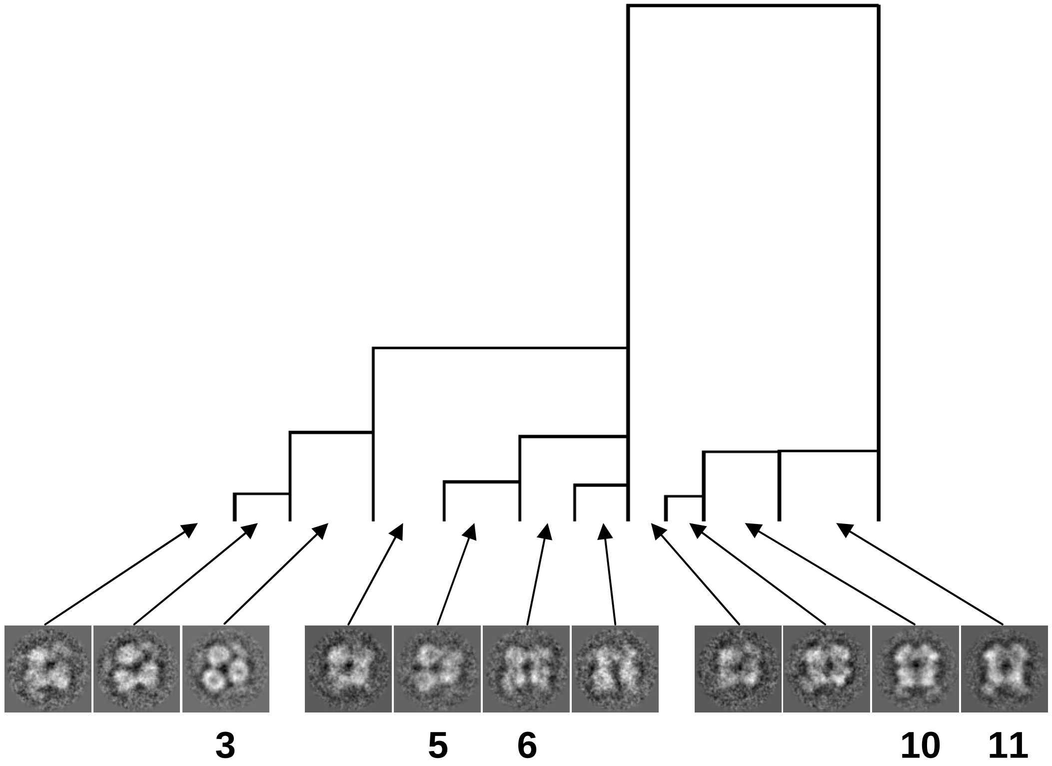
If 0° tilt images = SIDE views the missing cone axis is orthogonal to the five-fold axis of the cylinder.



When enforcing symmetry D5 or C5, you will fill up the missing cone of the SIDE views, but the missing cone of the TOP views will always superpose to itself and remain empty.

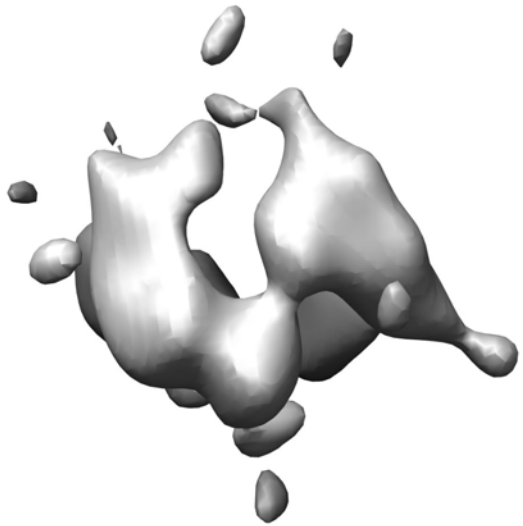
How to merge volumes when you don’t know if your structure has symmetries ?



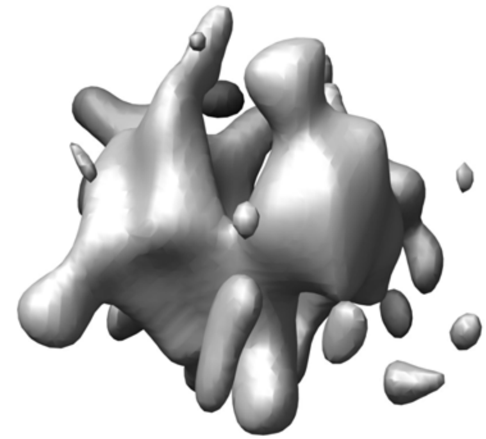


Poster of Magali Cottevieille on the Glutamate synthase complexe.
magali.cottevieille@impmc.jussieu.fr

Aligning the first volumes with large missing cone artifact can be challenging if you don’t enforce any symmetry.

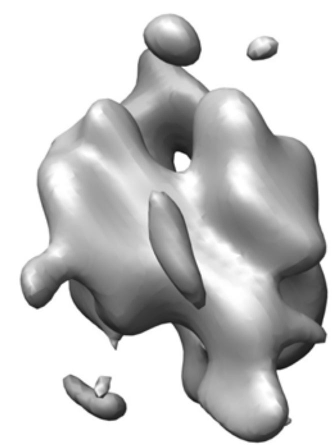


VCLA005 (48 images)



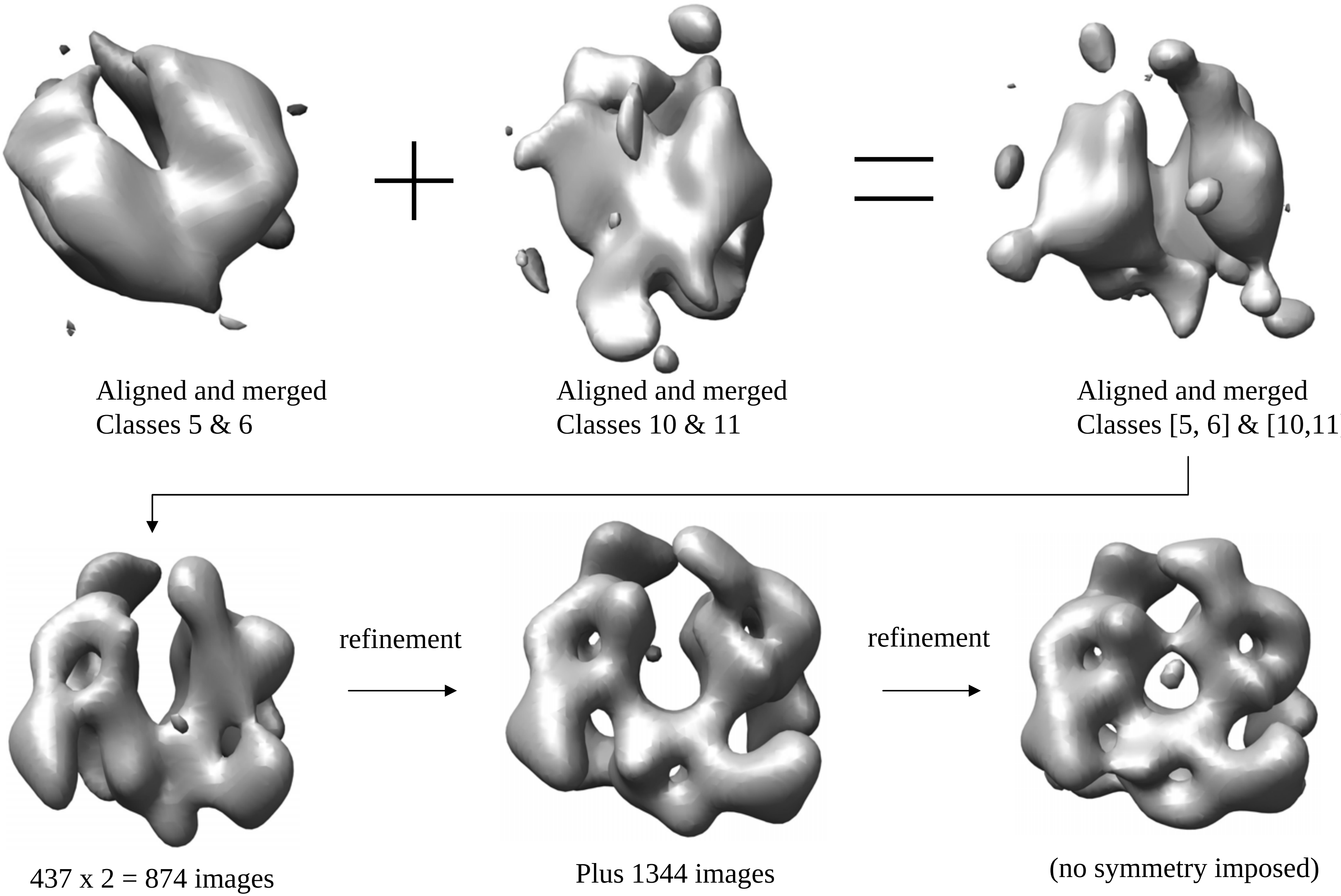
VCLA006 (35 images)

VCLA010 (48 images)

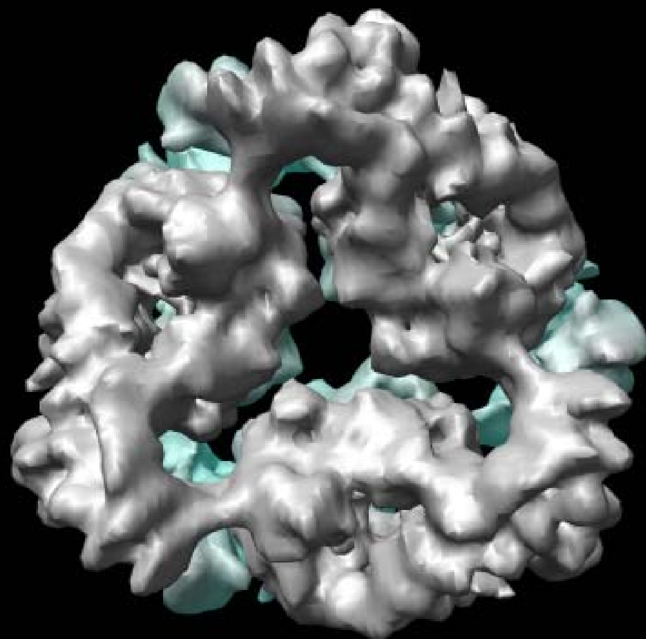


VCLA011 (63 images)

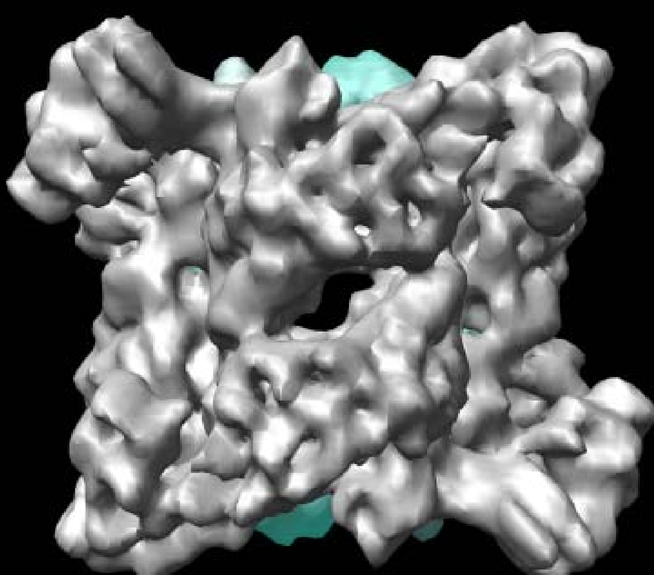
Successive merging of volumes by pairs of closely related EM views. The resulting volume was then used as a reference to align all available images (tilted and untilted-specimen images 437 x2). At last additional untilted-specimen images are added to the refinement process (1344 images).



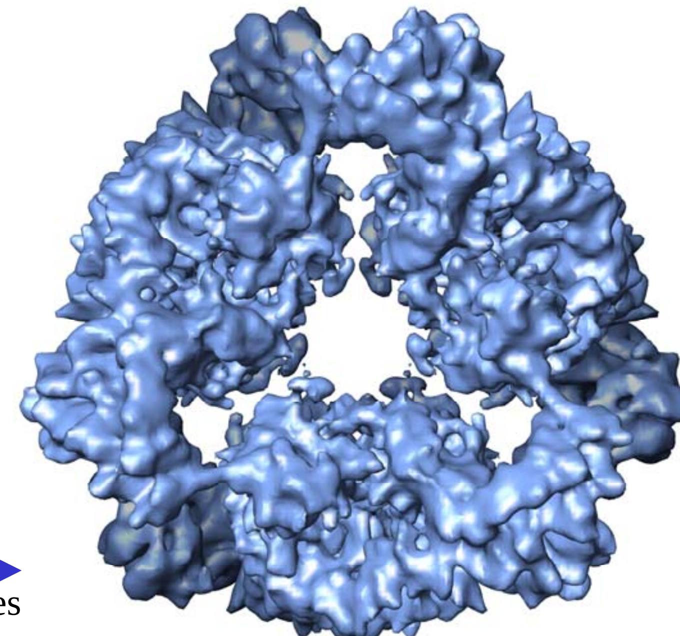
9.5 Å resolution volume

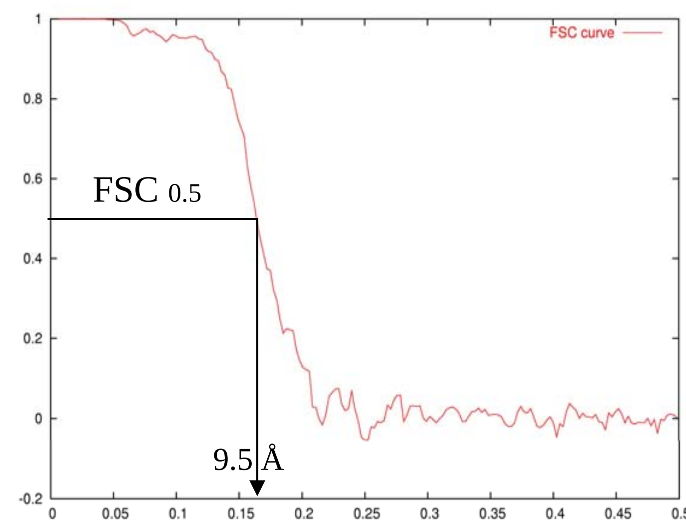


Amplitude corrected with SAXS data



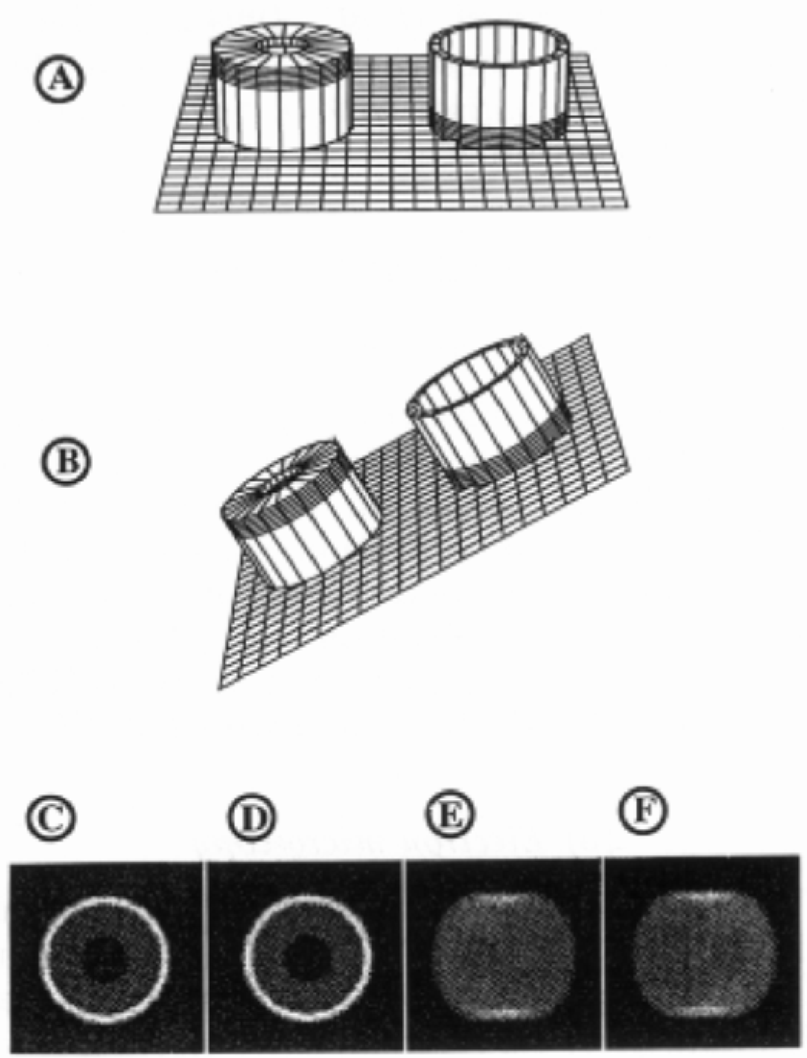
re images
efinement

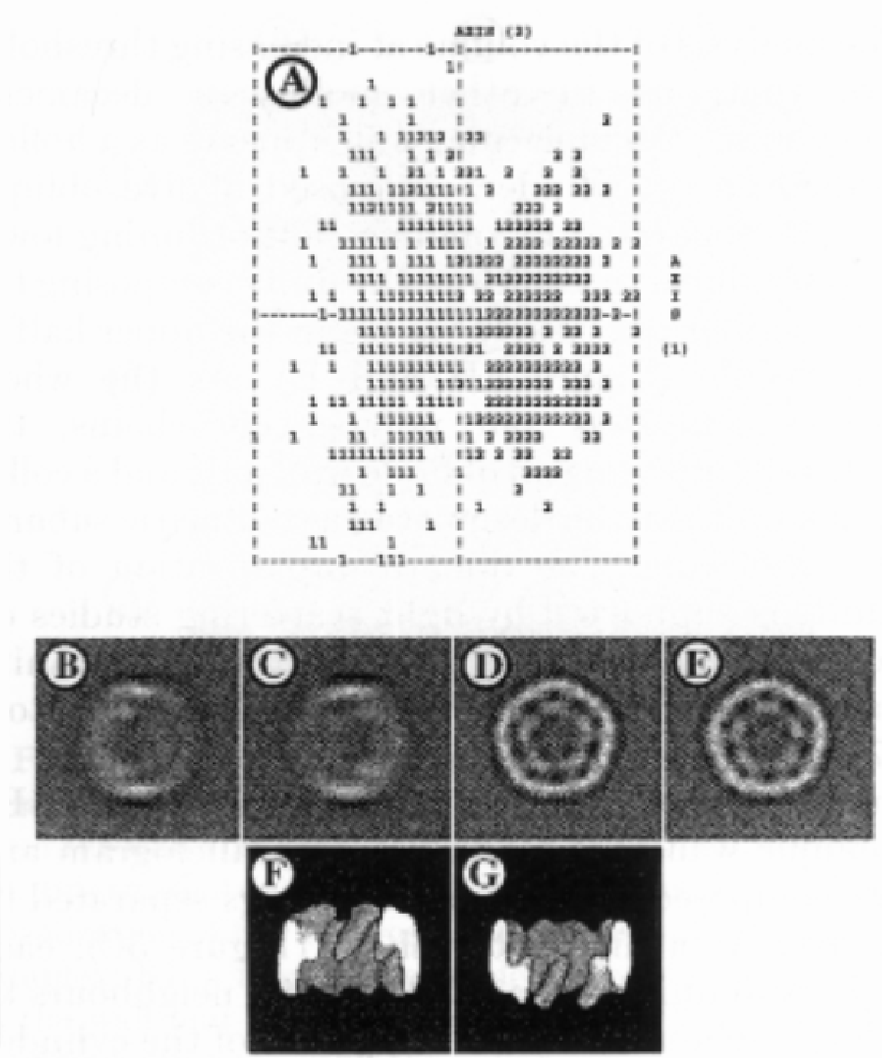




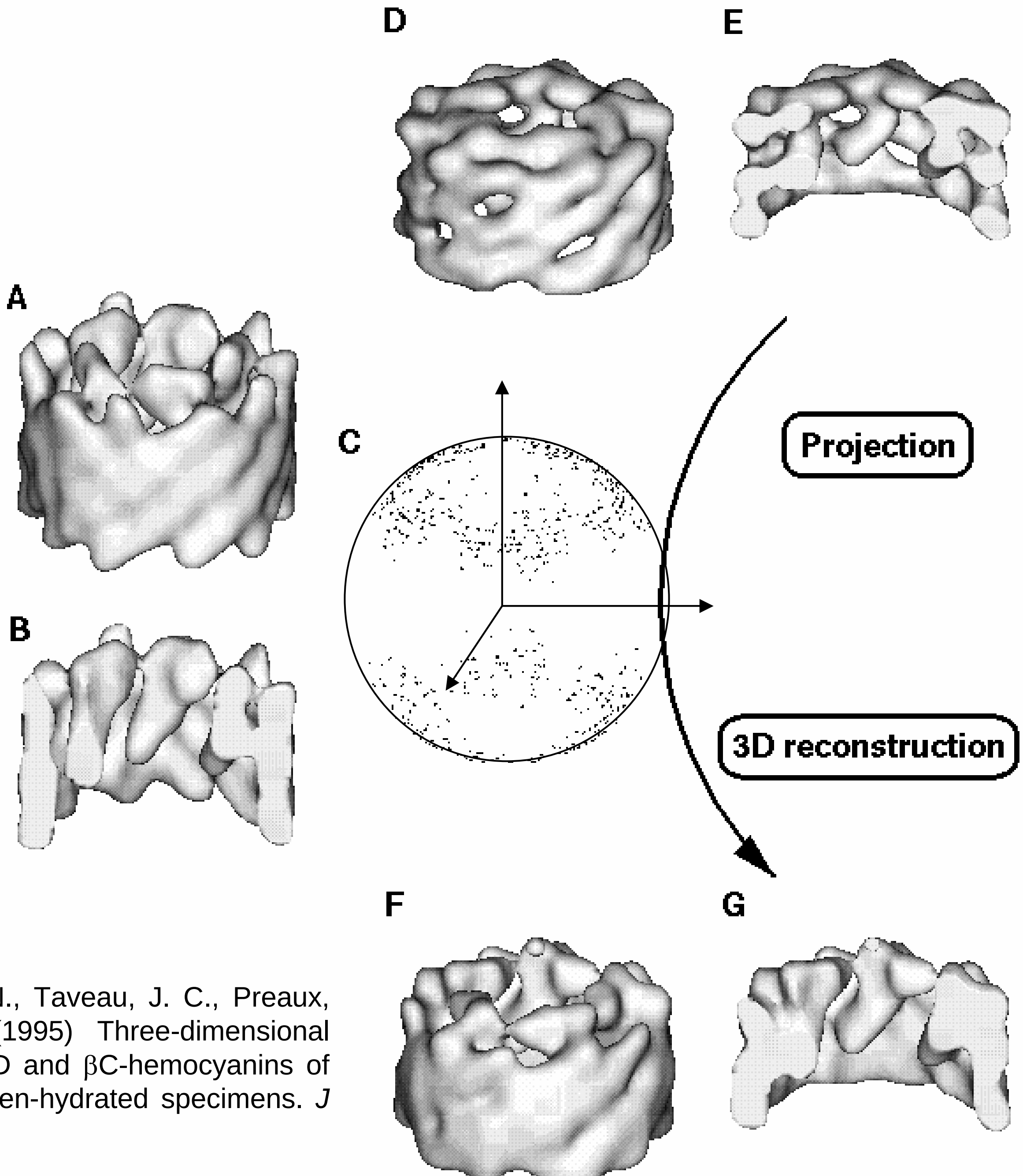
5 nm

How to sort cylindrical particles with Cn symmetry ?





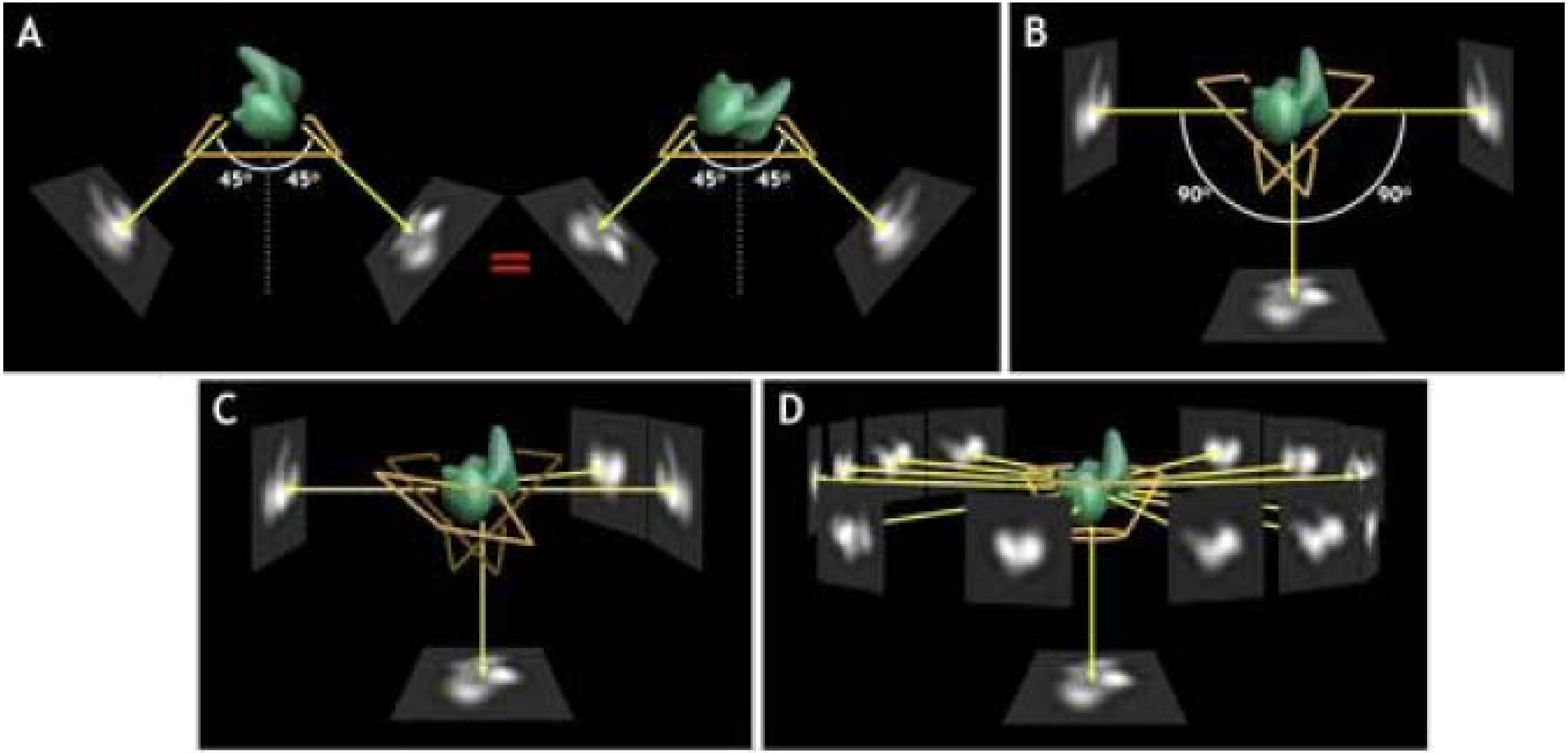
Lambert, O., Boisset, N., Taveau, J. C. & Lamy, J. N. (1994) Three-dimensional reconstruction from a frozen-hydrated specimen of the chiton *Lepidochiton sp*. hemocyanin. *J Mol Biol* **244**, 640-7.



Lambert, O., Boisset, N., Taveau, J. C., Preaux, G. & Lamy, J. N. (1995) Three-dimensional reconstruction of the αD and βC-hemocyanins of *Helix pomatia* from frozen-hydrated specimens. *J Mol Biol* **248**, 431-48.

The Orthogonal tilt reconstruction method
Andres E. Leschziner & Eva Nogales

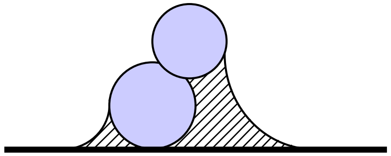
Two images are recorded with specimen tilts of -45° and +45°



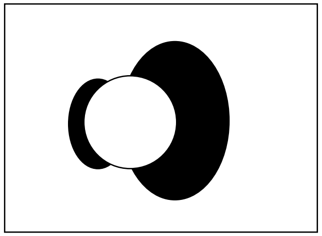
In this case you don’t get a conical tilt but the equivalent of a 360° tomogram.

Why not use negatively stained images ?

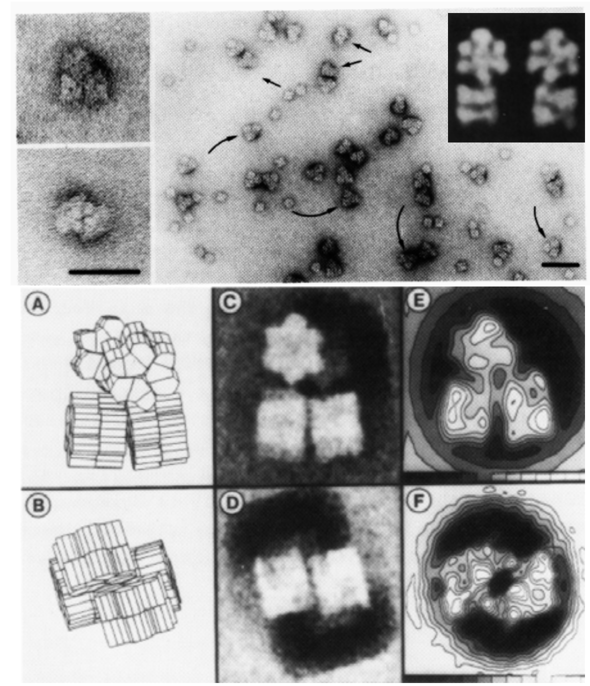
Single layer negative staining technique



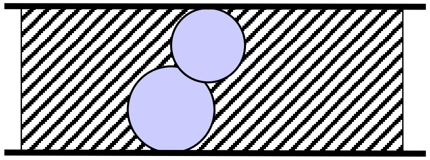
Small particles
less than 400 kDa
or hardly visible
in cryoMET

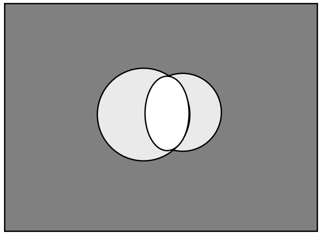


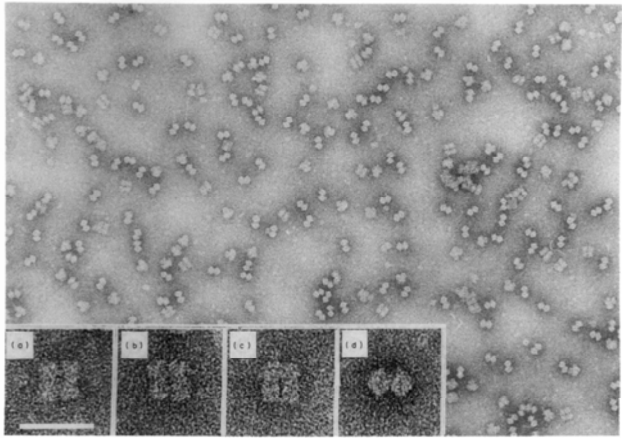
Avoid thin stain !

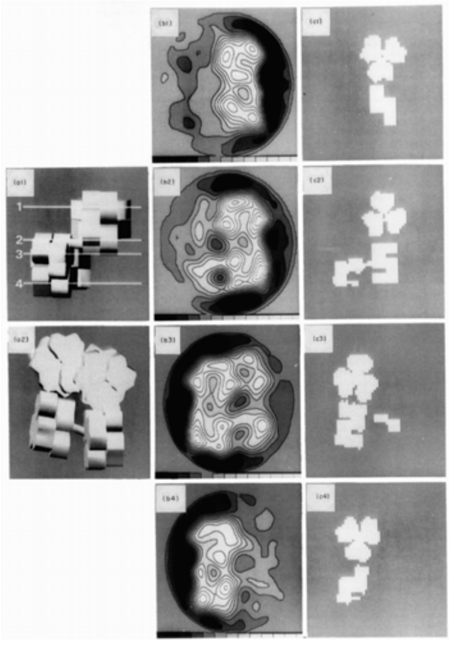


**Use thick staining or
double-layer negative staining
or « sandwidch » technique**

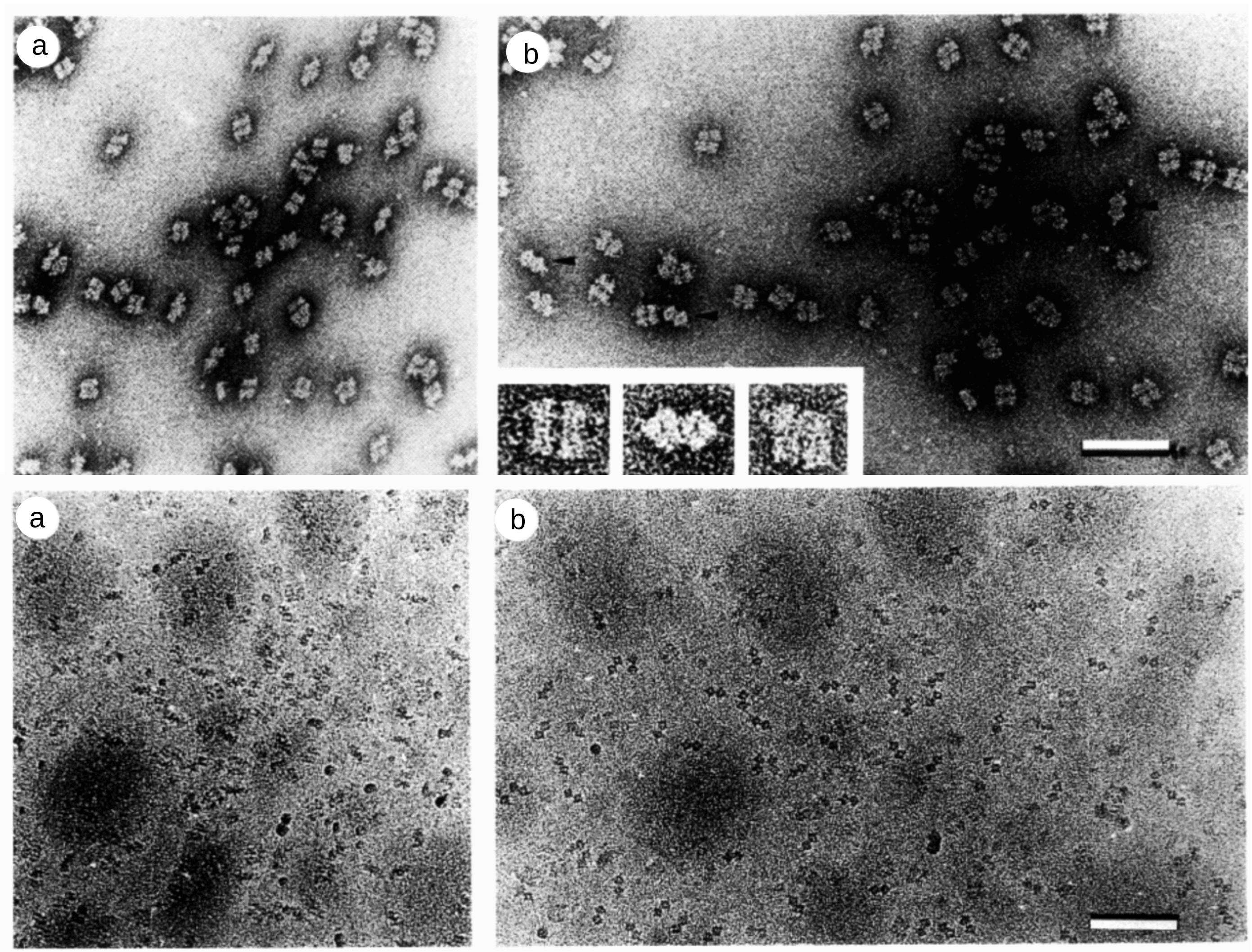


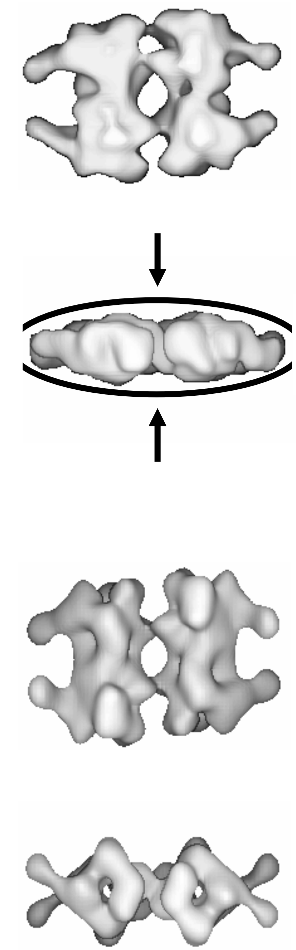






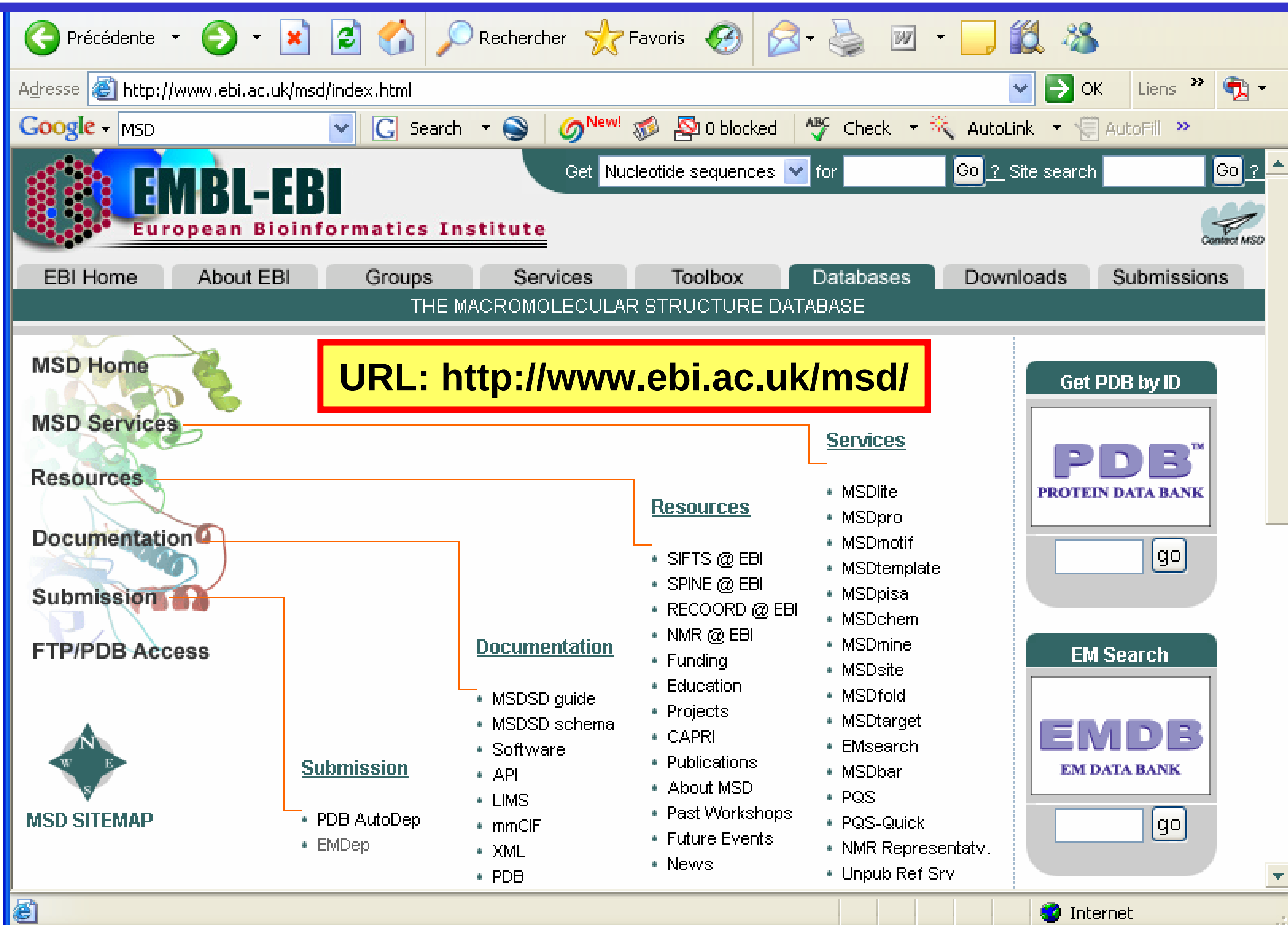
Double-layer negative staining technique





Frozen-hydrated sample

Integration of 3D Cryo-EM in MSD & other data banks



EMBL-EBI

Macromolecular Structure Database

http://www.ebi.ac.uk - addFeatures - Microsoft Internet Explorer

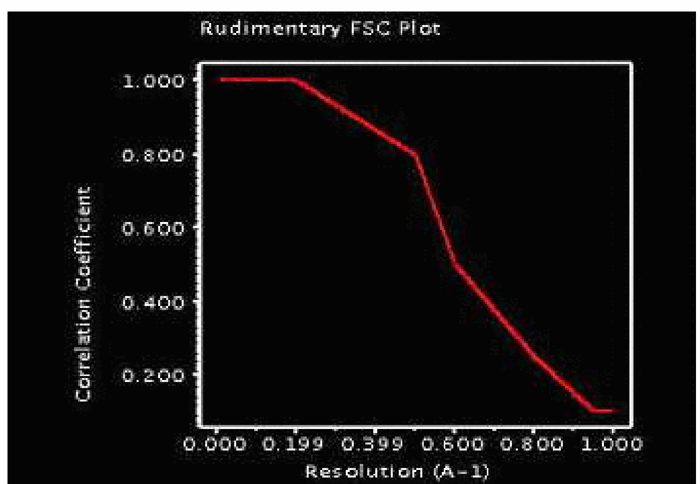
3) Uploading of Fourier Shell Correlation (FSC) data xml

FSC plots can now be uploaded as an xml format which must conform to the **EMD FSC schema**. A visual representation of this can be seen at: http://www.ebi.ac.uk/msd/iims/visualFscSchema.html or alternatively be downloaded from: ftp://ftp.ebi.ac.uk/pub/databases/emdb/doc/XML-schema/emd_v1_0_fsc.xsd.

An example FSC data plot which conforms to the EMD FSC schema may look like :

```
<?xml version="1.0" encoding="UTF-8"?>
<fsc title="Rudimentary FSC Plot"
                xaxis="Resolution (A-1)" yaxis="Correlation Coefficient">
 <coordinate>
  <x>0.01</x>
  <y>1.0</y>
 </coordinate>
 <coordinate>
  <x>0.2</x>
  <y>1.0</y>
 </coordinate>
.....
</fsc>
```

Internet



references:

-Random conical tilt series (RCT) :

Radermacher, M. (1988) Three-dimensional reconstruction of single particles from random and nonrandom tilt series. *J Electr.Microsc. Tech.* 9(4): 359-394.

1. Check the Spider web site where tutorials are available,
2. or contact Michael Radermacher at University of Vermont (Burlington) .

- SIRT and alignment of 3D volumes :

Penczek, P.A., Grassucci, R.A., Frank, J. (1994) The ribosome at improved resolution: new techniques for merging and orientation refinement in 3D cryo-electron microscopy of biological particles. Ultramicroscopy **53:** 251–270.

- Review :

Sali A, Glaeser R, Earnest T, Baumeister W. (2003) From Words to literature in structural proteomics. *Nature* **422**(6928): 216-225.

- Orthogonal tilt reconstruction (OTR) :

Andres E. Leschziner & Eva Nogales (2005) The Orthogonal tilt reconstruction method: an approach to generating single-class volumes with no missing cone for ab initio reconstruction of asymmetric particles. *J. Struct. Biol* (in press).

Oxford University Press: Search Results - Microsoft Internet Explorer

Fichier Edition Affichage Favoris Outils ?

Précédente Rechercher Favoris

Adresse http://www.oup.com/us/catalog/general/?queryField=author&query=Joachim+Frank&view=usa&viewVeritySearchResults=true OK Liens

UNIVERSITY PRESS | The World's Largest University Press: Excellence, Tradition, Innovation

SEARCH Keyword Search! Advanced Search»

Enter Promo Code GO
What are sales promo codes?

You are here: OUP USA Home > U.S. General Catalog

Browse Subjects Series

U.S. General Catalog
- Agriculture
- Anthropology
- Archaeology
- Art & Architecture
- Astronomy
- Atmospheric Science
- Bibles
- Biography, Letters, & Memoirs
- Business
- Chemistry
- Children's Books
- Classical Studies
- Communication
- Computer Science
- Cultural Studies
- Earth Sciences
- Economics
- Education
- Engineering & Technology
- Film, Media, & Performing Arts
- Finance
- Food & Wine
- General Science
- Geography

Search Results

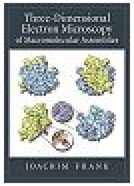
We found 136 items matching "Joachim Frank"

1 2 3 4 5 6 7 8 9 10 | Next»

Never miss an Oxford Sale!
Your Email
More mailing lists » Subscribe

Sorted by: Relevancy

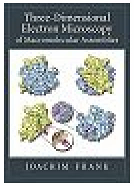
Results per page: 10 Display book covers: Yes No Sort



Three-Dimensional Electron Microscopy of Macromolecular Assemblies
with CD-ROM
Joachim Frank

95,00 $ (06), Not Yet Published
0195182189 , Paper, Nov 2005

Add to Cart



Three-Dimensional Electron Microscopy of Macromolecular Assemblies
with CD-ROM
Joachim Frank

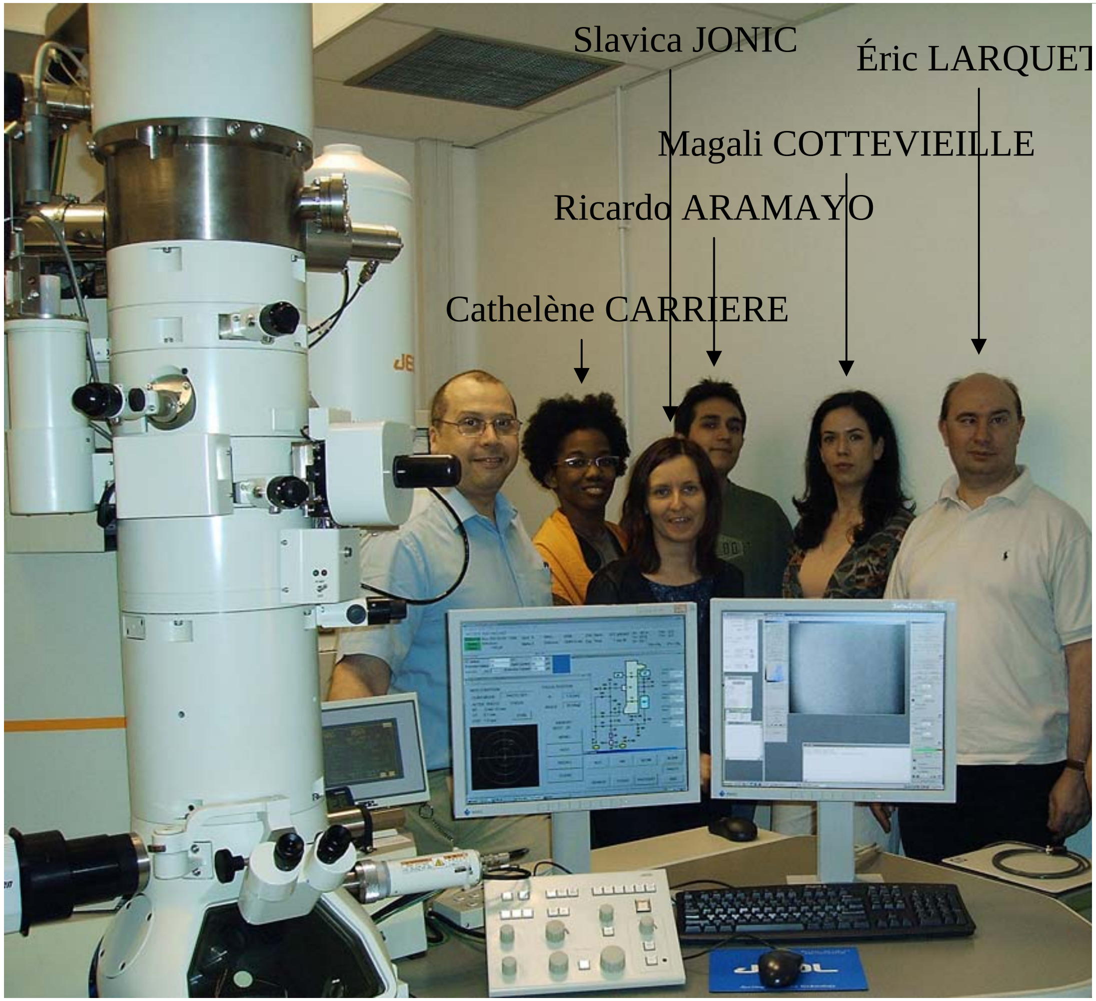
175,00 $ (06), Not Yet Published
0195150961 , Hardback, Nov 2005

Add to Cart

Internet

Acknowledgements:

Thank you for your attention !



Institut de Minéralogie et de Physique des Milieux Condensés,
UMR7590 CNRS Université Pierre et Marie Curie,
140 Rue de Lourmel, 75015 Paris, France.