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

Constraints of cryoEM on biological objects:

Work with low electron dose (~10^-10/e/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:
helicoidal symmetry, icosaedral symmetry,
2D crystals, or no symmetry at all…

With only two exposures a conical tilt series can be generated

Tilt series

Back-projection & 3D reconstruction

Principle of random conical tilt series

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

Reciprocal space


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

• direction of tilt axis (\(\phi\))
• and the tilt angle (\(\theta\)).

\[ \text{Tilt angle} = \cos(\theta) = \frac{d}{D} \] (but you don’t know if it is + or –)

Interactive windowing at 0° and 45° tilt

2D projections are identical, except for an in-plane rotation corresponding to Euler angle \(\phi\).

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

2D alignment of untilted-specimen images and computation of angle \(\phi\)

Centering and masking of tilted-specimen images

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

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.

Weighted back-projection

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

Reciprocal space half-volume

Missing cone

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 \( \lambda \) (with \( \lambda \approx 0.5 \times 10^{-4} \) to \( 0.1 \times 10^{-6} \)). This process is iterated and at each step the “global error” between EM images and the computed volume is measured to check improvement.

Correct \( \lambda \) 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

Weighted back-projection

SIRT

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

Interactive particle picking with determination of tilt axis direction (\( \psi \)) and tilt angle (\( \theta \))

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).

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 them into a line.
3. Place these lines of densities into a table
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 the mask, our data set corresponds to 76 images, that we can consider as 76 dots in a space of 2754 dimensions.

"Intelligent people understand each other with a few words ! …

Intuitively one can guess that two identical images will have similar coordinates in the multi-dimensional space. Therefore in the multi-dimensional 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 $\rightarrow$ frequencies $K_{ij} \rightarrow K_{ij} / \sum K_{ij} = f_{ij}$
2. Euclidian distance $\rightarrow \gamma^2$ distance $f_{ij} \rightarrow f_{ij} / f_{ii} f_{jj}$
3. Image mass $i = f_{ii}$
   Origine changed to the center of gravity of the table $= -f_{ij}$
4. Diagonalization of the covariance matrix $X_{ij} = (f_{ij} - f_{ii} f_{jj}) / f_{ii} f_{jj}$
   Equivalent to a least square fit to define new factorial axes (eigen vectors) and the coordinates of each image on these axes.

The 76 dots can be projected on planes defined 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. Jean-Pierre Brétaudière and Joachim Frank designed "reconstitution and importance images" to express this relationship and to explore the variation related to each eigen vector.

For example, according to you, how looks an image having for coordinates zero on all eigen vectors ?

Classification Ascendante Hiérarchique

Hierarchical ascendant classification

Helix pomatia hemocyanin

Reconstitution images

Importance images
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 D₅ 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 complex.

magali.cottevieille@impmc.jussieu.fr

VCLA005 (48 images)
VCLA006 (35 images)
VCLA010 (48 images)
VCLA011 (63 images)

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

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 untiiled specimen images 457 x 2). At last additional untilted specimen images are added to the refinement process (1344 images).

VCLA005 + VCLA006 = VCLA05 & 6
VCLA010 + VCLA011 = VCLA10 & 11

Align aligned and merged Classes 5 & 6
Align aligned and merged Classes 10 & 11
Align aligned and merged Classes [5, 6] & [10, 11]

457 x 2 + 874 images

Refinement

Plus 1344 images

(refine without symmetry imposed)
Amplitude corrected with SAXS data.

9.5 Å resolution volume

How to sort cylindrical particles with Cn symmetry?

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?

Double-layer negative staining technique

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

URL: http://www.ebi.ac.uk/msd/

references:

- Random conical tilt series (RCT):
  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:

- Review:

- Orthogonal tilt reconstruction (OTR):

A BIG THANK YOU

to all the people involved in the organization and running of the course, and especially to

Bridget, Clint, and Ron.