3D TRANSMISSION ELECTRON MICROSCOPY OF BIOLOGICAL NANOMACHINES

Slavica Jonić[1], **Carlos Oscar Sanchez Sorzano**[2], **Nicolas Boisset**[1]

[1]Université Pierre et Marie Curie, IMPMC-UMR7590, Paris, F-75005 France; CNRS, IMPMC-UMR7590, Paris, F-75005 France; Université Paris 7; IPGP
[2]Bioengineering Laboratory Escuela Politécnica Superior. Univ. San Pablo CEU Campus Urb. Montepríncipe s/n. 28668 Boadilla del Monte, Madrid, Spain

1. INTRODUCTION

Transmission electron microscopy (TEM) is a powerful technique for studying the three-dimensional (3D) structure of a wide range of biological specimens. Knowledge of this structure is crucial for a full understanding of complex relationships among macromolecular complexes and organelles in living cells. Here, we present principles and main application domains of 3D-TEM in structural biology.

2. DATA ACQUISITION

To select an appropriate technique for data acquisition, one should consider the nature of the specimen, data collection geometry, and possibilities for automated data collection.

Nature of the specimen: Depending on the nature of each sample, different strategies must be chosen. Thus, if the sample contains a single object, electron tomography should be chosen. This is the case for most cellular organelles (mitochondria, Golgi, etc.). Electron tomography allows 3D reconstruction of a single object after acquisition of tilt series, corresponding to a set of 2D projections of this object, with the sample gradually rotated along an axis defined as the tilt axis. Conversely, when the sample is composed of multiple copies of the same object, such as proteins and macromolecular complexes, the strategy of data collection and 3D reconstruction is different and aims at the computation of a 3D average map. Here again, different approaches can be taken, depending on internal symmetries of the particles and on the regular organization of the particles (low point-group symmetries, 2D crystals, icosahedral, or helical). For example, when the particles lack any particular symmetry (as is the case of the ribosome), or show only low point-group symmetries (GroEL), an approach named *single-particle analysis* (SPA) can be applied, either using tilted pairs of images [1] or using 0°-tilt images and relying on sinograms [2].

Data collection geometry: Ideally, we should acquire image projections of an object from all directions to determine its 3D structure. In practice, however, we use a finite set of projections. Due to this constraint and to the high noise level, we can only compute an approximate 3D structure, containing some artifacts and noise. If the object is unique by

nature (as is the case in electron tomography), different views are acquired by tilting the specimen grid in the microscope around a fixed axis (this way of collecting data is called single-axis tilt geometry). The more the specimen is tilted, the thicker it is. Hence, a practical tilting limit is reached around 70º.

The central-slice theorem (CST) relates the 2D Fourier transform of each projection image to the 3D Fourier transform of the object [3]. This theorem states that "*the Fourier transform of a 2D projection of any 3D object is equal to a central slice of the 3D Fourier transform of this object, the slice having the same orientation as the projection"*. Therefore, as the maximum tilt of the specimen is limited, a region of Fourier space will always remain empty, inducing anisotropy in the 3D reconstruction volume. Such problem is called the *missing wedge artefact*. There are collection geometries that aim at reducing this missing wedge by using two or multiple tilt axes.

In the case of multiple copies of differently oriented objects (as single particles, tubular assemblies with helical symmetry or icosahedral viruses), angular sampling is theoretically unlimited since the projection directions are generated without tilting the specimen grid. However, in practice, there might be missing projection directions because the particles take preferred orientations in vitreous ice.

Automated data collection: Electron microscopy of biological materials has recently experienced (and is still experiencing) a huge transformation of several steps of image acquisition. Automation of electron microscopes started in the early 1990s. Nowadays, robots automatically loading samples in the microscope have been developed. Microscopes can be automatically driven to find sample grids, select areas with proper vitreous ice thickness, autofocus, and record images [4]. Specimen tilt can also be automatically monitored [5]. In the same line, one can detect cryo-EM images presenting defects (charging, drift, astigmatism) or simply a lack of detectable signal at high resolution (undetectable Thon rings even when using *enhanced power spectrum* algorithm) [6]. In SPA, automatic procedures can select and extract particles from micrographs [7]. The combination of all these automatic procedures aims at computing a 3D map at sub-nanometer resolution within 24h after inserting the specimen grid in the microscope.

3. 3D RECONSTRUCTION

The reconstruction of an object from its 2D projections acquired in the electron microscope is usually performed in two steps: image alignment and tomographic reconstruction. Many algorithms have been developed for image alignment that is specific to the nature of the data [8-14]. 3D reconstruction can be accomplished in real space or in reciprocal space. Three families of reconstruction algorithms can be distinguished according to the reconstruction principle: Fourier direct methods, backprojection methods, and series expansion methods such as ART (Algebraic Reconstruction Technique) and SIRT (Simultaneous Iterative Reconstruction Technique). These and similar algorithms are available in several software packages for electron microscopy [15-21].

If microscopes were totally devoid of defects, their resolution would only be limited by the wavelength of the beam, therefore even at 80kV, the theoretical resolution limit

that could be reached by an electron microscope should be close to 1/0.15 $Å^{-1}$ [22]. However, this high resolution is never achieved, especially with biological samples that impose additional limiting factors: radiation damage, sample heterogeneity, microscope aberrations, additive noise, and missing orientations. Several measures of resolution of the reconstructed volumes have been developed [23-26].

4. PERSPECTIVES : COMBINATION WITH OTHER STRUCTURAL DATA

The routine combination of EM data with other structural resources is a topic that will be presumably very active in the near future. Fitting of high resolution structures into low/medium resolution EM maps is becoming more and more popular. The fitting may be rigid or it may take into account the flexibility of proteins [27]. This approach is also complemented with the prediction of structural folding [28]. However, fitting high resolution structures into low/medium resolution volumes is still quite human-dependent and fully automatic and reliable methods are still under development.

Finally, the information provided by EM should be integrated into larger structures. As is already the case of molecular structures fitted in electron tomograms [29], electron tomograms should be integrated into even larger volumes obtained by X-ray tomography [30] or even optical microscopy. This combination of information is giving raise to new fields such as correlative microscopy [31] and visual proteomics [32].

5. REFERENCES

[1] M. Radermacher, J Electron Microsc Tech **9** (1988), 359-394
[2] M. van Heel, Ultramicroscopy **21** (1987), 111-123
[3] A. C. Kak, M. Slaney, Principles of Computerized Tomographic Imaging, IEEE Press, New York, 1988
[4] C. Suloway, J. Pulokas, D. Fellmann, A. Cheng, F. Guerra, J. Quispe, S. Stagg, C. S. Potter, B. Carragher, J. Struct Biol **151** (2005), 41-60
[5] U. Ziese, W. J. Geerts, T. P. V. D. Krift, A. J. Verkleij, A. J. Koster, J Microscopy **211** (2003), 179-185
[6] S. Jonic, C. O. Sorzano, M. Cottevieille, E. Larquet, N. Boisset, J Struct Biol **157** (2007) 156-167
[7] Y. Zhu, B. Carragher, R. M. Glaeser, D. Fellmann, C. Bajaj, M. Bern, F. Mouche, F. de Haas, R. J. Hall, D. J. Kriegman, S. J. Ludtke, S. P. Mallick, P. A. Penczek, A. M. Roseman, F. J. Sigworth, N. Volkmann, C. S. Potter, J Struct Biol **145** (2004), 3-14
[8] N. Grigorieff, J Struct Biol **157** (2007), 117-125
[9] S. Jonic, C. O. Sorzano, P. Thevenaz, C. El-Bez, S. De Carlo, M. Unser, Ultramicroscopy **103** (2005), 303-317
[10] P. A. Penczek, R. A. Grassucci, J. Frank, Ultramicroscopy **53** (1994), 251-270
[11] C. O. S. Sorzano, S. Jonic, C. El-Bez, J. M. Carazo, S. De Carlo, P. Thevenaz, M. Unser, J Struct Biol **146** (2004), 381-392
[12] M. Radermacher, V. Rao, R. Grassucci, J. Frank, A. P. Timerman, S. Fleischer, T. A. Wagenknecht, J Comput Biol **127** (1994), 411-423
[13] S. Brandt, J. Heikkonen, P. Engehardt, J Struct Biol **133** (2001), 10-22

[14] H. Winkler, K. Taylor, Ultramicroscopy **106** (2006), 240-254
[15] S. Nickell, F. Forster, A. Linaroudis, W. D. Net, F. Beck, R. Hegerl, W. Baumeister, J. M. Plitzko, J Struct Biol **149** (2005), 227-234
[16] C. Messaoudi, T. Boudier, C. O. S. Sorzano, S. Marco, BMC Bioinformatics (submitted) (2007)
[17] J. Frank, M. Radermacher, P. Penczek, J. Zhu, Y. Li, M. Ladjadj, A. Leith, J Struct Biol **116** (1996), 190-199
[18] C. O. S. Sorzano, R. Marabini, J. Velazquez-Muriel, J. R. Bilbao-Castro, S. H. Scheres, J. M. Carazo, A. Pascual-Montano, J Struct Biol **148** (2004), 194-204
[19] S. J. Ludtke, P. R. Baldwin, W. Chiu, J Struct Biol **128** (1999), 82-97
[20] M. van Heel, G. Harauz, E. V. Orlova, R. Schmidt, M. Schatz, J Struct Biol **116** (1996), 17-24
[21] R. A. Crowther, R. Henderson, J. M. Smith, J Struct Biol **116** (1996), 9-16
[22] P. D. Nellist, M. F. Chisholm, N. Dellby, O. L. Krivanek, M. F. Murfitt, Z. S. Szilagyi, A. R. Lupini, A. Borisevich, W. Jr Sides , S. J. Pennycook, Science **305** (2004), 1741
[23] P. A. Penczek, J Struct Biol **138** (2002), 34-46
[24] M. Unser, C. O. Sorzano, P. Thevenaz, S. Jonic, C. El-Bez, S. De Carlo, J. F. Conway, B. L. Trus, J Struct Biol **149** (2005), 243-255
[25] M. van Heel, M. Schatz, J Struct Biol **151** (2005), 250-262
[26] D. Sousa, N. Grigorieff, J Struct Biol **157** (2007), 201-210
[27] F. Tama, O. Miyashita, C. L. Brooks, 3rd, J Struct Biol **147** (2004), 315-326
[28] D. Baker, A. Sali, Science **294** (2001), 93-96
[29] A. S. Frangakis, J. Bohm, F. Forster, S. Nickell, D. Nicastro, D. Typke, R. Hegerl, W. Baumeister, Proc Natl Acad Sci U S A **99** (2002), 14153-14158
[30] M. A. Le Gros, G. McDermott, C. A. Larabell, Curr Opin Struct Biol **15** (2005), 593-600
[31] R. D. Leapman, Curr Opin Neurobiol **14** (2004), 591-598
[32] S. Nickell, C. Kofler, A. P. Leis, W. Baumeister, Nat Rev Mol Cell Biol **7** (2006), 225-230

6. ACKNOWLEDGEMENTS

We are grateful to European Commission for NoE "3D-EM" contract No. LSHG-CT-2004-502828, Region Ile-de-France for convention SESAME 2000 E 1435, program C'Nano Ile-de-France for supporting 3D cryoEM at IMPMC.