QUANTITATIVE COMPARISON OF 3D RECONSTRUCTION ALGORITHMS UNDER CONDITIONS OF UNEVEN ANGULAR DISTRIBUTION IN ELECTRON MICROSCOPY.

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Abstract

The full understanding of the way proteins and other macromolecules work in living beings is highly demanded in the design of selective drugs and development of illness treatments. In this way, the macromolecular structure determination is a key problem in biology in order to understand deeper the functionality of a given complex and the way it interacts with other particles. There are several methods of collecting such structural information. One among them is the 3D reconstruction from projections obtained by means of an electron microscope. Recently, several concerns in the field have been raised about the 3D reconstruction algorithms performance when the angular distribution of the projections is highly uneven, which can be the case due to the differential macromolecular interactions with the supporting film that results in having more projections in some "preferred" directions. In this work a quantitative comparison among Weighted Back Projection (the standard reconstruction method in the field), SIRT and ART is done. At the end we will show that under this uneven distribution ART outperforms by far SIRT, and behaves slightly better than WBP.

Introduction to Transmission Electron Microscopy (TEM)[1]

Electron microscopy is one of the most versatile and direct techniques used to obtain information about three-dimensional structures. It can provide structural information on biological molecules at atomic resolution. X-ray crystallography and nuclear magnetic resonance (NMR) can also be used for this purpose but are restricted either to crystals with sufficiently large dimensions and good crystal order or to molecules with a maximum weight of 30k/da. The main advantage of Cryo-TEM is to preserve the biological samples in their native environment and to provide images closely related to true 2D projections of the observed particles

In a transmission electron microscope the magnitude and phase of the electron beam emitted by the filament are modified as they go through the specimen being visualized. The resolution of the obtained images is limited (in an ideal microscope) by the electron wavelength and the angular aperture of the objective lens which is, under the standard working conditions) about 0.2 nm. Unfortunately, this resolution is

never reached due to substantial instrumental aberrations, intrinsic limits in the specimen preparative steps, low contrast, and radiation damage of the sample. Other points to have into account are the Contrast Transfer Function of the microscope (strongly attenuating some frequencies, and even forcing a phase change in the image); the extremely low Signal to Noise Ratio of the images (in the order of 0.55); and the lack of proper control in the data collection strategy, that may lead to problems such as uneven distribution of projection directions, including substantial gaps among the experimentally achieved direction of projection.

3D reconstruction algorithms in Electron Microscopy

Among all the possible 3D reconstruction from projections algorithms, the one used the most in the field of electron tomography is Weighted Back Projection [2] (WBP). Another family of algorithms try to iteratively reconstruct the specimen working all time in real space. A volume can be regarded as the sum of a set of basis functions placed on a given 3D grid and multiplied by the right coefficients. The problem of 3D reconstruction, then, can be expressed as the iterative solution of a set of equations in which the unknowns are the coefficients multiplying the basis. SIRT (Simultaneous Iterative Reconstruction Technique) and ART (Algebraic Reconstruction Technique) by blocks belong to this family. The difference between both is that ART updates the volume each time a projection is presented to the algorithm, while SIRT updates the volume each time the whole set of projections is presented. The SIRT[3] and ART[4] compared in this work are implemented using blobs[5] distributed in a Body Centered Cubic grid [4], while WBP uses voxels in a simple Cubic grid (the implementation of SIRT with blobs on a BCC grid is new and has been specially developed for allowing more proper comparisons between ART and SIRT).

Problem definition

As it has been already pointed out, the position of the macromolecular assembly on the supporting film, which holds it inside the electron microscope, is not random: there are preferential biochemical interactions resulting in preferential views of the complex. This is translated into a larger number of projections from one direction than from the rest, and consequently in an anisotropic distribution of information within the reconstructed volume. However, there are algorithms, like WBP and SIRT, which seem to fail when dealing with these uneven angular distribution, creating an artefactual elongation along the direction which has been overloaded. This problem was initially described by Boisset et al in [6] on an implementation of SIRT without blobs. Then, we provided the first qualitative comparison of ART, SIRT, and WBP in [7], where we have shown that our implementation of ART does not suffer from this artefactual elongation. In this communication we will expand into this study, introducing some quantitative results.

Phantoms and Figures of Merit

As a quantitative measure of performance is pursued in this work, some figures of merit (FOM) have been defined to assess a numerical comparison. We will

make use of some of the defined in Marabini et al. [4], concretely fFOM (a training FOM to optimize algorithm parameters) and vrFOM (vertical resolution FOM to measure elongations). These FOMs are designed for the special case in which we have a phantom compound of a set of F pairs of cylinders, which are supposed to be embedded in a sphere where there's nothing else but background and the feature itself ([4]).

In the following formulas, *range* stands for the range of the phantom (this way the measure is independent from the signal power), F is the number of features (in this case ellipsoids) present, P refers to the phantom, and R to the reconstruction.

Feature	$fFOM = 1 - \frac{1}{F}\sum_{f=1}^{F} e_f \,/\, range^2$	e_f is the mean squared error over the feature
Vertical Resolution	$vrFOM = \frac{\frac{m_{1R} + m_{2R} - 2m_{3R}}{\sqrt{v_{1R} + v_{2R} + v_{3R}}}}{\frac{m_{1P} + m_{2P} - 2m_{3P}}{\sqrt{v_{1P} + v_{2P} + v_{3P}}}}$	m_i refers to the mean and v_i to the variance within the plane i. Planes 1 and 2 are central to the couple of cylinders while 3 is in between them.

Experiment design

Analysis of the algorithms indicates that they treat all directions in the same way. The observed elongation comes from the uneven distribution of projections. In our experiments we selected the preferred direction to be Z. First, we optimize each algorithm parameter to be sure that the comparison is fair. We can benefit from the results in [4]. To perform the optimization, 6 random phantoms with 7 features (couples of cyilinders) have been used to determine every single measure. The relaxation parameter λhas been optimized according to the method in [4], for the case of ART with two iterations and SIRT with 5 iterations (this compromise of running SIRT more than twice the iterations than ART has been taken since SIRT results got better much slower than ART, but at the same time we wanted to keep within the same range of computational time). A total of 869 unevenly distributed projections were considered, realistic levels of Gaussian noise were added to the pixel values (μ=0 and σ=16), tilt angles (μ=0 and σ= 4.16º), rotational angles (μ=0 and σ=1.15º), and center of the images (μ=0 and σ=0.3 pixels), for details of the meaning of this terms see [4].

Results

For the statistical comparison, phantoms with the same characteristics as in the training set have been reconstructed from 869 projections, 720 of them concentrated within 15º around the Z axis. The following table shows the vertical resolution FOMs for the three methods under this uneven distribution of projection directions. Note that ART is very significantly (0.005 level) better than WBP, and WBP is significantly (0.05 level) better than SIRT with respect to elongations along the preferred (Z) direction.

	ART	WBP	SIRT
vrFOM	0.645±0.107	0.38040±0.0601	0.2480±0.0491

In addition, we have investigated the algorithms for a phantom described at atomic level and composed by 12 spheres. Its projection images have been generated using a very realistic electron-atom interaction model. The results are shown in fig. 1

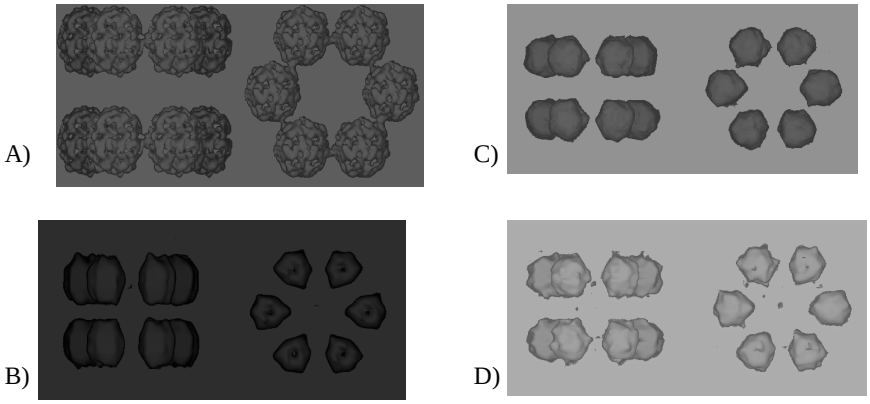

Fig. 1. A) Phantom constructed by pieces of actin at atomic resolution. B) ART reconstruction C) SIRT reconstruction D) WBP reconstruction

Discussion

We conclude that when considering the case of an uneven distribution of projections, ART proves to be better than WPB, while the implementation of SIRT considered in this work (as well as others used in the field of 3D electron microscopy, data not shown) performs much worse. Detailed mathematical explanation of the difference between the behavior of SIRT and ART remains to be found. At the intuitive level we can say that while SIRT and WBP gives more importance to the information available in the projections near the preferred directions than to the rest, this is not the case for ART, since once a certain view in the volume is sufficiently matched, the volume is no longer updated with the same information time and time again.

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References

[1] R. Marabini, C. San Martin, J.M. Carazo, Contemporary perspectives in Three-Dimensional Biomedical Imaging ed. by C.Roux and J.L.Coatrieux. IOS Press. 1997.
[2] M. Radermacher. Electron Tomography, ed. by J.Frank, Plenum Press, 1992.
[3] P. Penzeck, M. Radermacher, J. Frank.. Ultramicroscopy 40, 1992, 33-53.
[4] R. Marabini, G.T. Herman, J.M. Carazo. Ultramicroscopy 72, 1998, 53-65.
[5] S. Matej, R.M. Lewitt. IEEE Trans. on Medical Imaging 15, 1996
[6] N.Boisset, P.Penzeck, J.C.Taveau, V.You, F. Haas, J.Lamy. Ultramicroscopy 74, 1998, 201-207.
[7] C.O.S. Sorzano, R. Marabini, J.M. Carazo, E. Rietzel, R. Schroeder, G.T. Herman, N. Boisset. Proc. ICEM 14, 1998. Vol I, 771-772.

[8] R. Marabini, E. Rietzel, R. Schroeder, G.T. Herman, J.M.Carazo. Journal of structural biology 120, 1997, 363-371.