



Phan3D: design of biological phantoms in 3D electron microscopy

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ABSTRACT

Summary: Three-Dimensional Electron Microscopy (3DEM) has turned out to be a major player in structural proteomics. In 3DEM, tens of thousands of EM images from a macromolecule at different views are combined to derive its structure by means of a 3D reconstruction algorithm. However, for an optimal reconstruction, the most suitable algorithm has to be used and its parameters have to be tuned for the macromolecule under study and the experimental conditions found. The use of phantoms is central to objective comparison of reconstruction algorithms and optimization of their parameters. Phan3D is a tool intended to provide a fully visual and interactive environment that facilitates the design of phantoms resembling biological specimens in 3DEM. Phan3D has been developed as an alternative to the tedious, error-prone and old-fashioned phantom design that is based on hand-written text description files.

Availability: Phan3D has been integrated into Xmipp: <http://biocomp.cnb.uam.es/Biocomp/public/Software/>

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INTRODUCTION

Three-Dimensional Electron Microscopy (3DEM) has consolidated its role as a major player in structural biology (Sali *et al.*, 2003). 3DEM allows structure determination of macromolecules at medium/high resolution, typically in the range of 6–20 Å. The combination of 3DEM with electron tomography of cellular components has the potential to bridge the gap between cellular and molecular biology and allow a comprehensive description of the molecular interactions underlying the cellular function.

3DEM involves combining tens of thousands of EM images taken from the specimen at very different orientations to derive structural information. There exist different families of algorithms for 3D reconstruction from projection images

(Frank, 1996). Objective comparison of the reconstruction methods is essential to claim the superiority of one method over others and, furthermore, to determine the optimal parameters of a reconstruction method for the specimen under study. However, for this objective assessment, knowledge of the exact 3D reconstruction is required.

The current methodologies for objective evaluation and comparison of reconstruction algorithms are based on synthetic volumes (also known as phantoms) that resemble the biological specimens under study (Frank, 1996; Marabini *et al.*, 1998). Projection images are then generated from the phantoms by simulating the image formation process of the microscope. 3D reconstructions are then computed from these projections using the methods to be evaluated. Finally, the quality of the reconstructed volumes is determined by means of metrics that measure the resemblance with the phantom.

Generation of phantoms is therefore a key stage in the methodology for objective comparison and optimal parameter tuning in 3DEM. The design of phantoms that represent biological macromolecules is very common in 3DEM for several reasons. First, there exists an enormous variety of biological specimens whose sizes, shapes and structural features are completely different. Second, they can be studied at a wide range of resolutions. Therefore, the design of a new phantom is then required to tune the parameters of the algorithms for the specimen and resolution under consideration, or to accomplish objective comparison among different methods (Marabini *et al.*, 1998).

CREATION OF PHANTOMS

Phantoms or virtual models of biological specimens are usually created using 3D primitives like spheres, 3D ellipses, cubes, cylinders, cones, etc. To date there has been no graphical software tool that allowed interactive creation of 3D phantoms that, afterwards, could be used by standard packages in 3DEM. SPIDER (Frank *et al.*, 1996) and

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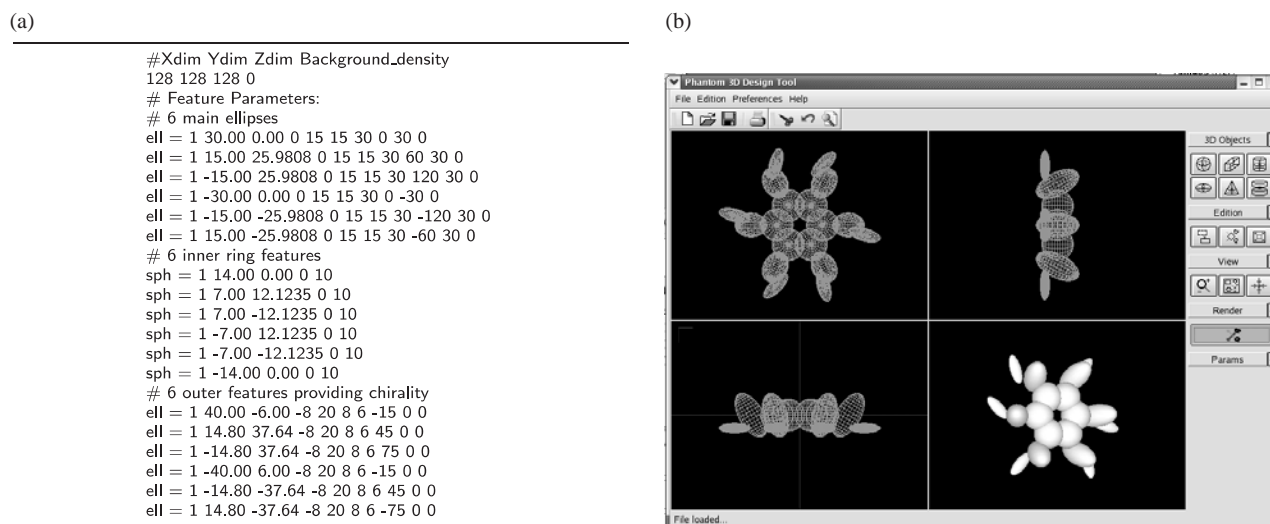


Fig. 1. (a) Example of a Xmipp phantom description file. (b) Perspectives of the model as viewed in Phan3D.

Xmipp (Marabini *et al.*, 1996), very well known packages in structural biology, allow the generation of 3D phantoms by interacting with the package via batch-files or text files, in a non-interactive way. Those description files contain the representation of the 3D model resembling the biological specimen in terms of basic primitives, and are made by hand. Designing a 3D model in this way proves to be a hard, tedious and error-prone process.

Figure 1a shows an example of description file in Xmipp. The primitives and their parameters shown there define a phantom resembling the polyhead of the T4 bacteriophage (Steven *et al.*, 1976). This phantom is made up of 18 features: 12 3D ellipses and 6 spheres. The phantom is embedded into a volume of $128 \times 128 \times 128$ voxels. The parameters associated to the 3D ellipses are the density of the feature, the coordinates (x, y, z) of its center, the three radii, and finally three euler angles to set the orientation of the ellipse in the 3D space. The parameters associated to a sphere are the density, the coordinates of its center and the radius.

A simple inspection of this description file allows one to imagine how difficult is creation of 3D phantoms by hand. The process of defining the features and its location in the 3D space is cumbersome. Furthermore, the lack of interaction with the model in the 3D scene precludes getting a clear idea of what the phantom looks like.

Phan3D: GRAPHICAL PHANTOM DESIGN

Phan3D was developed as an interactive graphical tool intended to provide a friendly environment for designing phantoms of biological specimens for their use in 3DEM. Phan3D allows interactive creation of 3D models, working in the 3D scene, and interacting with the model continuously. The user can see—from the very beginning—what the model looks

like from any view in the 3D world. The user can thus figure out which structural features should be included or removed to refine the model, and apply the proper modifications so that it better resembles the biological specimen under consideration.

Phan3D allows creation of 3D phantoms using basic primitives: spheres, cubes, cylinders, double cylinders, 3D ellipses and cones. For all of them, parameters like geometry, size, colour, density, rotations and others can be defined at insertion time. All those parameters can be modified at any time. An option allows the visualization of the scene applying a blending effect proportional to the density of each object.

The tool has four views of the model being created (Fig. 1b). Three of them are orthogonal 2D perspective views and allows edition of the model. The other one is a rendered 3D view and can be rotated so that the scene is viewed from any point of the virtual space. Any of the views can be zoomed in and out. Axes can be shown in each view and a grid can be plotted to have a reference of the scene size. The background, axes and grid colours can be modified by the user and all these parameters will be stored in a configuration file.

Phan3D is Xmipp-compliant in the sense that the mathematical description of phantoms can be loaded/stored in the Xmipp phantom description standard. Consequently, all the Xmipp programs related to phantom operations, determination of optimal parameters, objective comparison of 3D reconstruction algorithms, etc. can be directly used on the phantom generated with Phan3D. A text editor has been included in Phan3D to allow manual edition of the description files, if wanted. The user can thus comment, modify and save multiple different description files for a single scene.

Phan3D includes an extensive user's manual. A help browser has been included which reads information from

HTML files. This makes the process of creating help files for the program a straightforward task and allows language translation in an easy way.

Figure 1b shows a screenshot of Phan3D in the process of creation of the T4 polyhead phantom whose text description was shown in Figure 1a. Different perspectives of the model are shown in the four main windows. The user can interact with the model in any of the windows. The window at the bottom right is called the 'Render window' and is basically intended for visualization, where no edition is possible and the user can only change the view (orbit around the object, zoom in and out, move right, left, up and down). On the right of the screen there is a control panel where all the drawing functions are located. In this panel the user can select the primitive to insert, edit or delete and establish parameters like density, colour, etc. In the same panel, there are tools to move through the virtual world such as zoom or rotation and to reset the view.

Phan3D has been developed following an object-oriented approach, using C++ as the programming language, Qt (Dalheimer, 2002) as the graphic user interface (GUI) design library and OpenGL (Woo *et al.*, 1999) as the 3D graphics library. This makes Phan3D highly portable to most operating systems where those libraries are available, in particular those Windows-based and Unix-based. Phan3D binaries have been successfully built and tested for Linux and Windows systems. Phan3D has been integrated into Xmipp, and it is available from Xmipp's site. In this web, a full example containing all the stages in a typical 3DEM study can be found. In particular, the steps related to design of a phantom, determination of optimal parameters

for reconstruction algorithms, and objective comparison are described.

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