Volumetric Feature Extraction and Visualization of Tomographic Molecular Imaging

Zeyun Yu and **Chandrajit Bajaj** (CCV), **and Manfred Auer** (NYU, Rockefeller)

Introduction

Most if not all proteins in a cell are organized into cellular machines that are built from up to several dozens of individual proteins. For such multi-protein complexes, electron tomographic imaging provides the only foreseeable way to obtain 3D structural information. All other structural techniques such as spectroscopic, diffraction or single-particle analysis cryo-electron microscopic techniques rely implicitly or explicitly on averaging of a large number of identical particles. Electron tomography, in contrast, can provide 3D structural information of such unique volumes as whole cells. Although cellular tomographic imaging is no means a new technique, only recently it has received more attention. While recording devices (CCDs) are becoming larger, and data collection becomes faster, the bottleneck in this emerging field lies more and more on the visualization and interpretation of the tomograms. So why are tomograms so much harder to study and interpret? The answer may lie in the following co- mingled reasons: First, most tomograms exhibit a very low signal-to-noise ratio. Second, the cellular machine does not reside in isolation but are embedded in their cellular context, and densely surrounded by other proteins that may or may not directly interact with the cellular machine. Third, we don't know the exact composition and conformation of cellular machines at the time of investigation.

The poor signal-to-noise ratio usually observed in tomograms complicates the visualization of the volume as well as the automated feature extraction. Hence, noise reduction is always in demand as a pre-processing step to improve the signal-to-noise ratio. Segmentation is often necessary to obtain an unobstructed view into the machinery's architectural organization, and to reduce the complexity of the scenery to allow for biological interpretation. Feature extraction is particularly challenging if cellular machine of interest is in close contact to its cellular surrounding, and if there is no preconception of its 3D structure. In such cases, manual segmentation approaches appear somewhat subjective and become less feasible even with the help of 3D data re-slicing along non-orthogonal angles to obtain a more favorable view, and sophisticated graphics tools. Moreover, they are unlikely to keep up with the amount of data that can be generated by modern-day electron microscope data collection schemes. The complexity of cellular 3D volumes requires some form of data reduction and simplification. Skeletonization may be a way to simplify 3D data sets while retaining their characteristics, which is also important in comparing two complexes that are similar but not identical. Skeletons will be helpful in comparing two such cellular machines and describing their similarities and discrepancies.

Visualization Tool

The volume-rendering client can act as a 3D roving microscope, allowing users to visualize data that is too large to fit on a single machine. The graphical user interface allows for interactive visual selection of transfer function and isosurface. The user interface also allows the user to move and resize the sub-volume window. The data within the sub-window is then transmitted by the server to the client computer, and displayed interactively using fast texture based volume rendering that can be combined with rendered geometry. The rover connects to a data server that contains large datasets. The server can extract and resample sub-volumes of different sizes, which are then transmitted to the client for visualization.

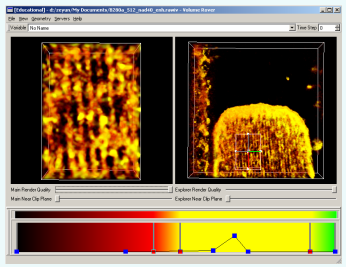

Fig. 1 Example of Volume Rover

Anisotropic Filtering

Our approach to three dimensional nonlinear noise reduction filters, such as bilateral pre-filtering coupled with an evolution driven anisotropic geometric diffusion PDE (partial differential equation), have shown significant results in enhancing the visualization of macromolecular tomographic imaging. The PDE model is :

$$\partial_t \phi - \|\nabla \phi\| div(D^\sigma \frac{\nabla \phi}{\|\nabla \phi\|}) = 0$$

The efficacy of our method is based on a careful selection of the anisotropic diffusion tensor based on estimates of the normal and two principal curvatures and curvature directions of a feature isosurface in three dimensions.

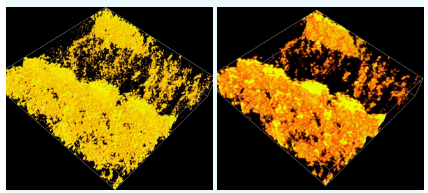

Fig. 2 A sub-volume of dataset comprising extracellular links between a stereocilium and the kinociliar bulb in a bullfrog macular sensory epithelia hair bundle.

3D Gradient Vector Diffusion

We propose a new method for gradient vector diffusion, based on anisotropic PDE-based diffusion:

$$\begin{cases} \frac{du}{dt} = \mu\nabla(g(\alpha)\nabla u) - (u - f_x)(f_x^2 + f_y^2 + f_z^2) \\ \frac{dv}{dt} = \mu\nabla(g(\alpha)\nabla v) - (v - f_y)(f_x^2 + f_y^2 + f_z^2) \\ \frac{dw}{dt} = \mu\nabla(g(\alpha)\nabla w) - (w - f_z)(f_x^2 + f_y^2 + f_z^2) \end{cases}$$

where $g(\alpha)$ is a decreasing function and α is the angle between the central vector and the surrounding vectors. For faster implementation, the calculation of the angle between two vectors is usually approximated by the inner-product of two vectors divided by their magnitudes.

3D Segmentation

Our segmentation algorithm is based on the fast marching method and includes the following steps: First, we compute and diffuse the gradient vector field of the original density map. Then the critical points (local maxima and local minima) are calculated and used as the seeds of the marching contours. The critical points, and accordingly the marching contours, are classified into several groups. Two marching contours merge into a single one if they come from the same group. Otherwise, they automatically stop on their common boundaries.

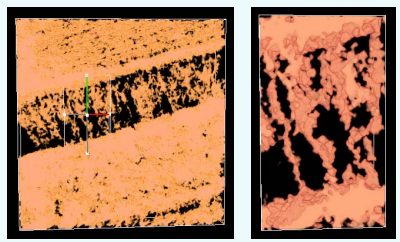

Fig. 3 Segmentation of densely grouped extracellular fibrillar proteins

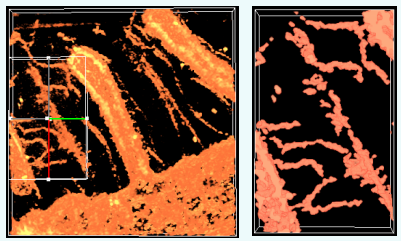

Fig. 4 Segmentation of extracellular filaments being secreted from frog saccular sensory epithelium supporting cells

3D Skeletonization

Our skeletonization algorithm is based on the anisotropic vector diffusion. We first compute and diffuse the gradient vector field, and then calculate a map, called *skeleton magnitude map*, by summing up all the outgoing vectors minus all the incoming vectors, at each voxel. The obtained map gives a magnitude (or, possibility) for each voxel being on the skeletons. From the skeleton magnitude map, we then trace the skeletons by, for example, Canny's edge tracing algorithm or other ridge tracing methods. The problem to be solved is the proof of the connectivity, the thickness, and the topology-preservation of the obtained skeletons.

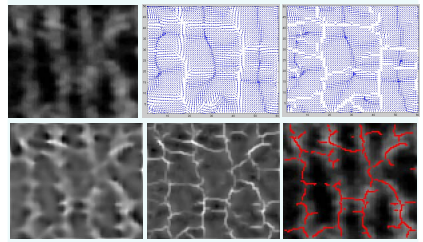

Fig. 5 Top: original density map; gradient vector fields (iso- & aniso-)
Bottom: skeleton magnitude maps (iso- & aniso-); skeleton tracing

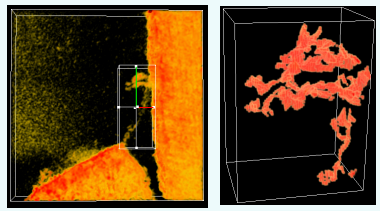

Fig. 6 The skeletons of the bullfrog hair bundle tip link.
Left: overall dataset. Right: skeletons of a sub-volume

Acknowledgments

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Contact information

Chandrajit Bajaj: bajaj@cs.utexas.edu
Zeyun Yu: zeyun@cs.utexas.edu
Manfred Auer: auerm@mail.rockefeller.edu