Precise Termination of Actin Filaments in Hair Cell Stereocilia

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October 31, 2018

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Electron cryotomography (Cryo-ET) is an imaging technique used to produce 3-dimensional (3D) images of large biological molecular complexes and cells (Tocheva et. al, 2010). The cryo-ET technique is important, as it is a biophysical technique with the potential to derive atomic structures of large molecular complexes at cellular scale. In 2017, the Nobel Prize in chemistry was given to three scientists with pioneering work in cryo-electron microscopy, a sister technology of cryo-ET but with smaller scale. As more 3D images are being produced using cryo-ET technique, computational methods are needed to analyze the images. An example of a cryo-ET 3D image of stereocilia is shown in Figure 1 (Fig. 1 – Left). Using BundleTrac, a method developed at Dr. He's group, actin filaments (Fig. 1 - Right Purple) of hair cell stereocilia can be detected in the corresponding 3D cryo-ET density maps (Sazzed et. al, 2018). Using another method developed at Dr. He's group, membrane (Fig. 1 – Right Blue) can be detected as well. Although with the latest version of BundleTrac position and direction of filaments can be detected, a crucial step is missing with our current method of filament detection. At times filaments are produced longer than expected (Fig. 1 – Right White Arrow). Precise termination is needed to perform accurate length detection of the filaments.

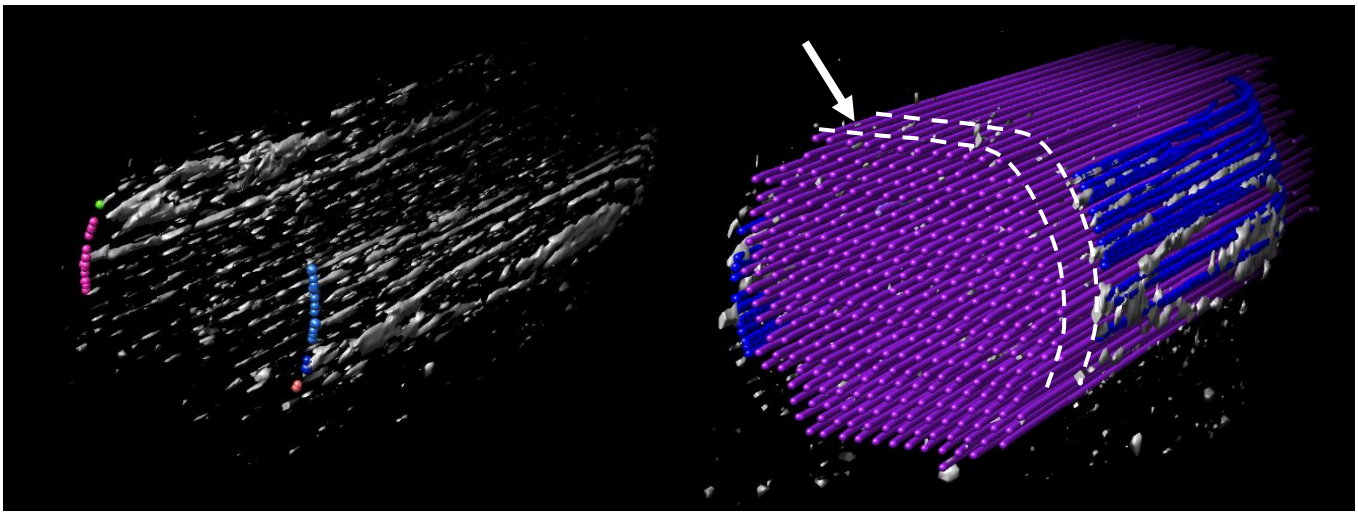

Figure 1. Visualization of the cryo-ET 3D Image of a portion of Stereocilium and Detected Actin Filaments and Membrane. (Left) : Surface representation of the cryo-ET density map. Seed/Initial Points of membrane are represented using 4 unique clusters (Colors). (Right) : Traced actin filaments (purple) and membrane (blue). (Right) : Arrow points to a section of filaments that are over-labeled.

Project Description and Significance

With the current version of BundleTrac, actin filaments are extended further than the length of the membrane, which should serve as a maximum length for those of the filaments (Fig. 1 – Right Arrow Indicator). Moreover, the filaments are not precisely terminated, with many being over-labeled, or terminated past the desired point. It is laborious and cumbersome for one to manually trim hundreds of the over-labeled filaments, as they are not all necessarily of the same length, and the trimming would thus have to be performed on an individual basis. With this research project I will rectify this and develop a computational method to determine the precise termination point for each filament. This method will be integrated into BundleTrac. With the addition of the termination step, BundleTrac can be a practical tool for tracing filaments in a bundle in cryo-ET 3D images.

This interdisciplinary project serves for me not only as an effective form of training, but also as a suitable introduction to bioinformatics research, as it grants the opportunity for me to learn biological content, techniques, and computational methods used often in this field.

Proposed Methodology and Preliminary Work

For the project I will design an algorithm to be implemented in the C++ programming language so that I can expand upon the work already done with BundleTrac. In order to trim the over-labeled filaments, I will use the membrane as a reference for termination and monitor the overall density change along each individual filament. There will be a sliding “window” (subsection of a filament) that contains a collection of points along the filament. I will calculate the average density of a window. Repetition of this process for every window will allow me to monitor the average density of a filament, which will in turn allow me to determine where the filament should terminate, or more specifically, where the density drops below a certain threshold.

PhD Student Salim Sazzed wrote the C++ code for BundleTrac (Sazzed et. al, 2018). The program was further converted to a software plugin by Devin and Claus who are MS students (Haslam et. al, 2018). My work will naturally expand upon and follow their preliminary work closely, and there is further explanation of this in the later “Project Independence” section.

Outcomes

The outcomes of this project include an enhanced version of the BundleTrac program. The enhanced BundleTrac will detect precisely-terminated filaments. The updated BundleTrac application will be inserted as a plugin into the Chimera visualization tool, as is currently done for the BundleTrac and MemTracing applications (Haslam et. al, 2018). Through the improvements to BundleTrac I will have produced a practical tool for biologists. Dr. He has been collaborating closely with Dr. Auer of the Lawrence Berkeley National Laboratory in California (Sazzed et. al, Haslam et. al, 2018). The enhanced BundleTrac may benefit Dr. Auer's research and any other when filaments are needed to be detected from the 3D image.

Budget

Funds equaling $2000 will be paid as a scholarship to the student directly. I plan to continue my research with Dr. He beyond the spring 2019 semester, and I have even spoken with Professor Brunelle, the Chief Departmental Advisor for the computer science department at ODU, about future application to the Linked BSCS/MS program in computer science, which would allow me to join the graduate program at ODU.

Independence of the Project

In this project, I am expected to learn independently about multiple topics such as the Cryo-ET biophysical technique of deriving atomic structures, the handling of 3-dimensional models in Chimera software, and the accessing of 3D image data. Whilst I am building upon previous work in BundleTrac, the work I do is more than the simple usage of existing tools. The work I will do in this project is programming intensive. I will be developing the algorithm and code for increased BundleTrac accuracy under the guidance of Dr. He and with partial assistance of lab mates.

References

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(LNCS), volume 10847, pp 233-238, International Symposium on Bioinformatics Research and Applications (ISBRA), 2018.