Manfred Auer, Ph.D.   Principal Investigator & Staff Scientist

phone: 510-486-7702 ; facsimile: 510-486-6488 ; email: mauer@lbl.gov

Manfred Auer, Ph.D.
Department of Structural Biology and GTL, Life Sciences Division
Lawrence Berkeley National Laboratory

Re : Our manuscript entitled **From Voxels to Knowledge: A Practical Guide to the Segmentation of Complex Electron Microscopy 3D Data**

September 29, 2013

Dear Madam or Sir,
We are submitting this original work solely to *Journal of Visualized Experiments* for consideration for publication. We would like to request Dr. Zhao Chen as editor, as we had been in contact with her to discuss the scope of the manuscript

In this manuscript, we address one of the fundamental bottlenecks in 3D Electron Microscopy imaging of cells and tissues, namely how to efficiently extract the features of interest for further 3D visualization and quantitative analysis. Features of interest are typically surrounded by a wealth of other biological material obstructing their direct visualization and analysis. Segmentation allows the feature of interest to be isolated from its context and analyzed. While segmentation methods have been around for decades, they were mostly developed to solve a particular visualization problem and are rarely general enough to guide a non-expert through the feature extraction process. While some segmentation tools are automated, others require extensive user interaction or completely manual. To our knowledge, there hasn't been a practical assessment of the different segmentation approaches, that would guide a non-expert in this crucial image analysis step. We have therefore undertaken a systematic study, where we subject a total of 6 data sets to four different segmentation strategies and compare the results for each approach. We noted that different approaches work well for certain data sets but not for others, and have begun to compile a list of objective (density map quality-based) and subjective (other important considerations) criteria that characterize the different data sets and the characteristics of feature presentation. We then go on to utilize this list as a triaging tool/decision making tree in order to recommend certain segmentation approaches for different data types.

Be believe that this manuscript will be very helpful to a non-expert in the field, who is contemplating image analysis of complex cellular 3D volumes, and thus is ideally suited for JoVE with its mission. We further think that the images are visually stunning and will be of significant interest to a broad audience. Given that to our knowledge no such systematic comparison and triage recommendation exists in the literature, we would expect this to become a landmark paper with a high citation rate.

Thank you for considering our work for publication!
Yours most sincerely,
 .

Sincerely,

Manfred Auer, Ph.D.
Staff Scientist and Principal Investigator, Lawrence Berkeley Lab
Director of Physical Characterization, Joint BioEnergy Institute