

Reviewer Report

Title: Qiber3D - an open-source software package for the quantitative analysis of networks from 3D image stacks

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Reviewer name: Chris Armit

Reviewer Comments to Author:

This interesting Technical Note describes the Qiber3D software tool, which is used for image analysis of 3D image stacks. There is a need to identify contiguous morphological components, such as alveolar airspaces and vascular networks, in 3D confocal image data and the Qiber3D toolkit was developed to address this issue. Importantly, the authors provide illustrative use case scenarios that include: 1) a synthetic example network; 2) a 3D reconstruction of the skeleton of a microvascular network from a confocal image stack of in vitro cultured mouse vascular cells, and; 3) a 3D reconstruction of a marsupial gigantopyramidal neuron. The software is publicly available from GitHub (<https://github.com/theia-dev/Qiber3D>) where it has been ascribed an Open Source Initiative-approved MIT license. In addition, test confocal image data of a 1GB vascular network used by Qiber3D is available on FigShare (<https://doi.org/10.6084/m9.figshare.13655606.v1>). These image data are of high quality and the Nikon ND2 files can be read using Fiji / ImageJ with the Bio-Formats plugin. Movies of the 3D reconstructions can be viewed using private sharing links.

Major comment

Can the authors please describe the morphological operations used by Qiber3D? Specifically, I wish to know which morphological operations (erosion, dilation, etc.) were used by Qiber3D to improve segmentation of the contiguous vascular networks in the image stack of in vitro microvasculature that is shown in Figure 6 and Supplementary Figure S1.

Minor comment

I request that the movies of the 3D reconstructions are made publicly available on FigShare. The DOIs for the four movies should be included in the revised GigaScience manuscript.

Level of Interest

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Quality of Written English

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I work for GigaScience where I perform checks on the quality of image data and the image analysis approach. My funding is not dependent on the outcome of this review, and I declare that I have no competing interests.

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