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Applications Note

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OpenSegSPIM: a user-friendly segmentation tool for SPIM data

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Abstract

Summary: OpenSegSPIM is an open access and user friendly 3D automatic quantitative analysis tool for Single Plane Illumination Microscopy data. The software is designed to extract, in a user-friendly way, quantitative relevant information from SPIM image stacks, such as the number of nuclei or cells. It provides quantitative measurement (volume, sphericity, distance, intensity) on Light Sheet Fluorescent Microscopy images.

Availability and implementation: freely available from http://www.opensegspim.weebly.com. Source code and binaries under BSD License.

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Supplementary information: Supplementary data are available at *Bioinformatics* online.

1 Introduction

Single Plane Illumination Microscopy (SPIM) was developed in 2004 by Stelzer and colleagues (Huisken *et al.*, 2004) to image 4D live developing embryos. Further improvements have been made by (Keller *et al.*, 2008) and more recently (Chhetri *et al.*, 2015). Intrinsic to the concept of SPIM was low phototoxicity and photodamage. Development on SPIM technology over the last 10 years has led to the more general term of Light Sheet Fluorescent Microscopy (LSFM) to describe this imaging technique. The advent of OpenSPIM (Pitrone *et al.*, 2013) and OpenSPIN (Gualda *et al.*, 2013) has opened a new era in LSFM. These two projects provide detailed information and resources to allow any scientist to build and start LSFM. Importantly, these Open projects, are associated with websites and the ability to interact with the developers.

Here, we provide details of an image processing tool, OpenSegSPIM, which is made available on the same basis as OpenSPIN and OpenSPIM. The combination of OpenSPIN/OpenSPIM and OpenSegSPIM allows image generation to be connected to image processing, allowing the automated extraction of quantitative information from LSFM images. LSFM generates large numbers of raw image stacks (Gigabytes, going to Terabytes in a time series) that require initial processing steps such as registration of multiple view and deconvolution to reconstruct the data (Preibisch *et al.*, 2010). Acquisition, visualization and quantitative analysis are the 3 milestones to go from a biological sample to quantitative results. We propose OpenSegSPIM here as a user-friendly tool to perform the quantitative analysis step. To obtain the information on a cell-by-cell basis, the objects in the 3D stacks should be separated from each other, i.e. segmented, before further quantitative analysis.

A variety of algorithms (Eliceiri *et al.*, 2012; Jug *et al.*, 2014; Yu *et al.*, 2009) are available for segmentation of nuclei, including: watershed, iterative voting methods, level set approach based on gradient flow and flexible contour model. However the implementation of these algorithms to segment LSFM data is not straightforward, due to complex installation procedures, or difficulty in tuning the parameters for individual biological samples. Moreover, a good segmentation result relies not only on an efficient segmentation algorithm but also on a sequence of steps preparing the image so that the

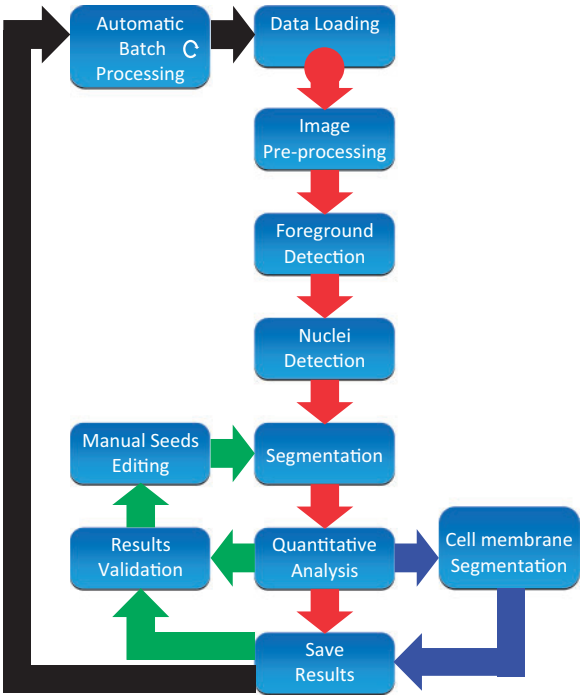

Fig. 1. OpenSegSPIM flowchart. (Red arrow) Main process: load a 3D image and process it semi-automatically. (Black arrow) Automatic batch process of a list of images. (Blue arrow) Load and process cell channel after nuclei analysis. (Green arrow) Reload a processed image and manually correct detection errors

segmentation algorithm can perform efficiently. Therefore, putting all these steps together requires a systematic design of the pipeline and the interface.

Currently, few graphical user interfaces are specifically designed for nuclei segmentation in 3D especially cell aggregates. Modular Interactive Nuclei Segmentation was developed as a user interface to segment early mouse embryo (Lou *et al.*, 2014) but it is only available for Matlab License owner. Dedicated effort is urgently needed to build user friendly interface for LSFM users.

2 OpenSegSPIM

OpenSegSPIM is a user-friendly interface dedicated to segmentation and analysis of LSFM data that assumes no prior knowledge of image processing or programming. The OpenSegSPIM workflow (Fig. 1) consists of a succession of simple steps to segment and analyse nuclei (loading, enhance, foreground, detection, segmentation and analysis).

In general, the quality of segmentation depends greatly on the accuracy of the algorithm input parameters. Therefore the number of input parameters as well as the complexity to find the right value is critical for an accurate quantitative analysis.

In OpenSegSPIM, the number of parameters is reduced to only a few essential ones. Only a few manual nuclei diameter measurements and an intuitive interactive intensity adjustment on the image are required from the user to fine tune the necessary parameters. The software provides an interactive interface and a direct visual feedback for each step. Other features of OpenSegSPIM include, (i) a simple manual nuclei editing tool, (ii) an automatic batch process of time series facilitating the further use of tracking algorithms and (iii) a cell membrane segmentation and analyses tool.

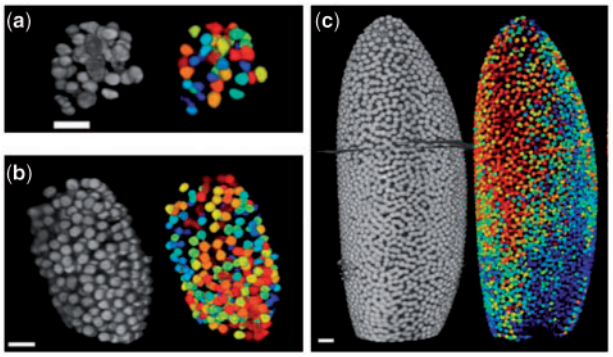

Fig. 2. Segmentation by OpenSegSPIM. **(a)** Neurosphere, **(b)** *C. elegans* embryo, **(c)** *Drosophila* embryo. 3D view of original image (grayscale) and segmented result (color coded, blue to red correspond at small to large volume). Scale bars 30 mm

Table 1. Accuracy of OpenSegSPIM semi-automated segmentation before any manual editing for the sample displayed in Figure 2

Samples Segmentation	Neurosphere	C. elegans embryo	Drosophila embryo
Ground truth	52	339	2818
Detected	**51**	**327**	**2884**
True positives	51	326	2806
False positives	0	1	78
False negatives	1	13	12
Precision %	**100**	**99.7**	**97**
Recall	**0.98**	**0.96**	**1**
Computation time	2 min	7 min	25 min

We evaluated the segmentation accuracy of OpenSegSPIM on LSFM data by visually comparing raw and segmented data and manually counting all differences.

The number of nuclei in the original image ($N_{Groundtruth}$), number of segmented nuclei ($N_{detected}$), number of true & false segmentation ($N_{truepositive}$ and $N_{falsepositive}$) and number of missing nuclei ($N_{falsenegative}$) allow us to measure the precision ($P = N_{truepositive}/(N_{truepositive}+N_{falsepositive})$ and recall ($R = N_{truepositive}/N_{Groundtruth}$) of OpenSegSPIM. The software successfully segment Neurospheres, *Caenorhabditis elegans* and *Drosophila* embryo with minimal error (Accuracy > 92% before manual editing) (Fig. 2 and Table 1).

We did a comparative study of OpenSegSPIM performance against other existing segmentation tools showing OpenSegSPIM to have better or equal segmentation accuracy (see Supplementary Materials).

In line with OpenSPIN and OpenSPIM we include a website to promote easy access and use of the software. We provide a complete user guide and tutorial to guide the user through the segmentation of their data and provide detailed explanation of the algorithms used for each steps. Software executable and source code are freely available at (http://opensegspim.weebly.com).

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Conflict of Interest: none declared.

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