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Probability Map Viewer: near real-time probability map generator of serial block electron microscopy collections

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Abstract

Summary: To expedite the review of semi-automated probability maps of organelles and other features from 3D electron microscopy data we have developed Probability Map Viewer, a Java-based web application that enables the computation and visualization of probability map generation results in near real-time as the data are being collected from the microscope. Probability Map Viewer allows the user to select one or more voxel classifiers, apply them on a sub-region of an active collection, and visualize the results as overlays on the raw data via any web browser using a personal computer or mobile device. Thus, Probability Map Viewer accelerates and informs the image analysis workflow by providing a tool for experimenting with and optimizing dataset-specific segmentation strategies during imaging.

Availability and implementation: <https://github.com/crbs/probabilitymapviewer>.

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

1 Introduction

Automated 3D electron microscopy (EM) platforms, such as serial block-face scanning EM (SBEM) (Denk and Horstmann, 2004), are capable of producing expansive volumes of data at resolutions sufficient for visualization of organelles and certain macromolecular complexes. In the neurosciences, such data are being leveraged to map neuronal wiring diagrams (Kasthuri *et al.*, 2015) and quantify the morphology, distribution and state of organelles (Perez *et al.*, 2014). Such analyses, however, are critically dependent upon the accurate delineation of structures of interest via robust segmentation routines. Since the man-hour cost of manual segmentation often scales in a linear manner with dataset size, it does not represent a sustainable solution for annotating the teravoxels of image data now being produced daily by automated 3D EM platforms.

To address this bottleneck, a number of approaches for automated segmentation have been developed (Peddie and Collinson,

2014). Supervised algorithms, which train voxel classifiers (VCs) by learning features based on a supplied set of training data, have proven optimal for tackling the challenges posed by densely stained EM images (Liu *et al.*, 2014). Though the time required to generate such training sets pales in comparison to that of manually segmenting the entire volume, it is not insignificant; some use cases require days of manual effort to produce training sets without guarantee that the resultant VCs will prove viable. Furthermore, under the current paradigm, segmentation decisions are typically made only after the full dataset has been acquired, resulting in a significant delay between data collection and the quantitative analysis of biological data.

To address these limitations, we developed Probability Map Viewer, a web-based visualization and processing tool that computes and displays automated VC probability map results in near real-time (NRT) during active SBEM collections. Probability Map Viewer allows users to select one or more VCs, apply a subset of

them to collected data, and assess their performances on the fly, long before the acquisition is complete.

2 Implementation

Probability Map Viewer is a Java web application with a back-end processing engine that allows a caller to perform rapid, image probability map generation from one or more VCs on an arbitrarily large image. The web application utilizes Leaflet (<http://leafletjs.com/>), an open-source JavaScript library and Jetty (<http://www.eclipse.org/jetty>), an embedded webserver to display SBEM images and VC results in NRT on a web browser. The web-based front-end was chosen because of its ubiquity and as well as ease of setup as a display kiosk. Although other microscopy web viewers exist, see (Bajcsy et al., 2016) and (Winter et al. 2015), they do not appear to be built for the usage scenario described below. Probability Map Viewer is available for download from <https://github.com/crbs/probabilitymapviewer> and is free for non-commercial use.

2.1 Hardware and data transfer

Our SBEM imaging system consists of a Gatan 3View (Gatan, Inc.) incorporated into a Zeiss Merlin scanning electron microscope (Zeiss). A data acquisition computer running Windows 7 and the DigitalMicrograph (DM) software (Gatan, Inc.) controls the acquisition and stores all output images in the DM (.dm4) format. To prevent disruption of a live imaging run, newly collected data are rapidly transferred from the acquisition computer to a Windows 7 data server via a 10G Ethernet connection (Supplementary Fig. S1). Both the acquisition computer and data server use RAID 5 storage with an SSD cache for increased performance and redundancy. The transfer of newly collected images to the data server is facilitated by the Syncovary file synchronization software (Super Flexible Software Ltd. & Co. Münster, Germany), which runs at the lowest priority on the acquisition computer and checks for new image files in a collection-specific path every five seconds. The most recently synced data are stored in the SSD cache of the data server to ensure rapid accession.

2.2 Visualization and NRT probability map generation

The Probability Map Viewer instance is initialized from the command line of a local Linux machine. Probability Map Viewer automatically monitors the data server's collection path, and, with the help of IMOD command line tools (Kremer et al., 1996), converts each full SBEM raster into a set of square tiles in the PNG image format. Once complete, the full image is displayed seamlessly

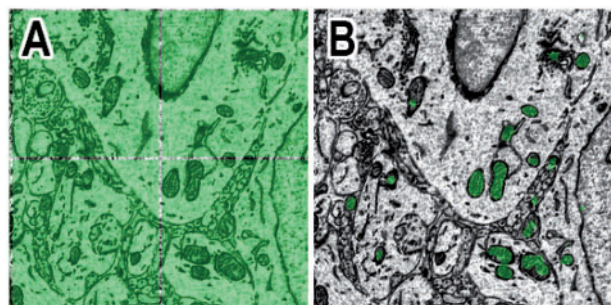


Fig. 1. Probability Map Viewer's graphical interface. (a) Colored, semi-transparent overlays indicate tiles that have been submitted for analysis. (b) As results become available, the full tile overlays are replaced by overlays of VC results, in this case for mitochondria classified by CHM

in the Probability Map Viewer web application via an embedded Jetty web server that is run from the local machine (Supplementary Figs S2 and S3). Probability Map Viewer provides an interactive interface through which the image can be explored using Google Maps-like controls, such as panning.

Probability Map Viewer supports VC probability map generation via external command line scripts. For each enabled VC, colored, semi-transparent overlays are rendered over each of the currently displayed tiles, indicating that they have been sent to the back-end engine for probability map generation (Fig. 1a). The results of multiple VCs are rendered in different user-selected colored overlays. As the user pans around the image, the list of tiles to be analyzed is automatically updated to include all newly displayed tiles. The back-end engine passes these tiles individually to the VCs for probability map generation. This approach enables fast parallel operation but increases risk of edge artifacts. Following successful analysis of each tile, the web application automatically refreshes the image and replaces the tile overlays with VC results, allowing the user to visually track and assess the VC as it completes tiles across the display (Fig. 1b). For Figure 1b probability maps are generated via cascaded hierarchical model (CHM; Seyedhosseini et al., 2013).

3 Conclusion

Probability Map Viewer provides users with the unique ability to generate probability map results on SBEM data during an active acquisition using one or many pre-trained VCs. Results are visualized in NRT via Probability Map Viewer's web app, allowing users to rapidly make informed decisions at the instrument level to achieve better analytical results. Since Probability Map Generator VCs are external results from other machine learning or computer vision algorithms can easily be incorporated and displayed using Probability Map Viewer's front-end. To further improve utility, support will be added for zooming and image stack scrolling. For more information please visit <https://github.com/crbs/probabilitymapviewer>.

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Conflict of Interest: none declared.

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