Automatic Neural Reconstruction from Petavoxel of Electron Microscopy Data

Adi Suissa-Peleg[1], Daniel Haehn[1], Seymour Knowles-Barley[1,2], Verena Kaynig[1], Thouis R. Jones[1,2], Alyssa Wilson[3], Richard Schalek[2], Jeffery W. Lichtman[2] and Hanspeter Pfister[1].

[1.] School of Engineering and Applied Sciences, Harvard University, Cambridge, MA, USA.
[2.] Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA, USA.
[3.] Department of Physics, Harvard University, Cambridge, MA, USA.

Connectomics is the study of the dense structure of the neurons in the brain and their synapses, providing new insights into the relation between brain's structure and its function. Recent advances in *Electron Microscopy* enable high-resolution imaging (4nm per pixel) of neural tissue at a rate of roughly 10 terapixels in a single day, allowing neuroscientists to capture large blocks of neural tissue in a reasonable amount of time. The large amounts of data require novel computer vision based algorithms and scalable software frameworks to process this data. We describe RhoANA [1], our dense Automatic Neural Annotation framework, which we have developed in order to automatically align, segment and reconstruct a 1mm^3 brain tissue (~2 peta-pixels).

Our workflow is depicted in Fig. 1. A Zeiss MultiSEM 505 (Fig 1-a) captures the images (Fig 1-b) of a neural tissue block that is sliced into thin, 30nm thick, sections using an automatic microtome. The images of each section are stitched together and the entire volume is 3D aligned using our stitching and registration tool (Fig 1-c). Each cell membrane is detected using a membrane classification algorithm, and the cells are reconstructed into 3D objects (Fig 1-d, 1-e). Finally, we use our web-based tool to manually proofread the output, and ensure reconstruction correctness (Fig 1-f). The rest of the paper details the software tools that are used after the image acquisition by the microscope.

The Zeiss MultiSEM 505 simultaneously captures 61 image tiles (multi-beam field-of-view, or mFoV) at a time in a hexagonal shape (Fig 2). Multiple mFoVs are captured to cover the targeted region in each section (Fig 2). To give immediate feedback, we have developed a web-based application that allows viewing a section after it is acquired by the microscope. Our application can also overlay additional information on each mFoV (e.g., image quality score), and perform basic image processing operations.

The first stage of the pipeline performs 2D stitching and 3D registration. When collecting multiple mFoVs at the nanometer resolution, the microscope estimation of image location is imprecise. The stitching process extracts and matches features from the boundaries of each pair of adjacent tiles, and finds a per-tile rigid transformation that minimizes the distances of these features. 3D registration finds a per-section elastic (non-affine) transformation that compensates for non-linear distortions, and is based on TrakEM2's alignment tool [2]. The registration overlays a grid of points on each section that need to be matched to the adjacent section. A small image patch is taken around each of the points, and a corresponding patch is searched for in a restricted area of the adjacent section. Finally, an optimization process minimizes the deformation by simulating an elastic system of springs connected between the grid points, and outputs a per-tile transformation that can be used to render the tile. To overcome artifacts that deform parts of the sections (e.g., dirt) and make the alignment more robust, the registration process also compares sections that are 1 or 2 sections apart.

Once the sections are aligned, the second stage of our pipeline performs membrane classification and 2D segmentation [2]. The membrane classification algorithm uses state-of-the-art deep learning techniques in order to generate membrane probability maps. The trained network is applied to small patches on each tile and returns the probability that the center pixel should be classified as a membrane. The classification process is followed by a 2D segmentation stage that creates multiple candidate segmentations using a watershed algorithm on the membrane probability maps.

3D segmentation starts by dicing the volume into small sub-volumes for computational feasibility. For each sub-volume an Integer Linear Program is solved to match the segments from each pair of adjacent sections. As is done in the 3D registration process, the segmentation also attempts to overcome artifacts and 2D segmentation errors by comparing sections that are 1 or 2 sections apart. Finally, adjacent segmented sub-volumes are stitched together to create a single coherent segmented volume.

Any remaining segmentation errors must be fixed in proofreading. To this end, we have developed Dojo [4], a web-based application that enables concurrent users to view the segmented volume, and to fix the segmentation errors by merging segments that were erroneously separated, and by splitting segments that were merged.

References:

[1] http://www.rhoana.org/
[2] S. Saalfeld, *et al.* Nature Methods, **9(7)** (2012), p. 717.
[3] V. Kaynig, *et al.* Medical Image Analysis, **22(1)** (2015), p. 77.
[4] D. Haehn, *et al.* IEEE Transactions on Visualization and Computer Graphics, **20(12)** (2014), p. 2466.
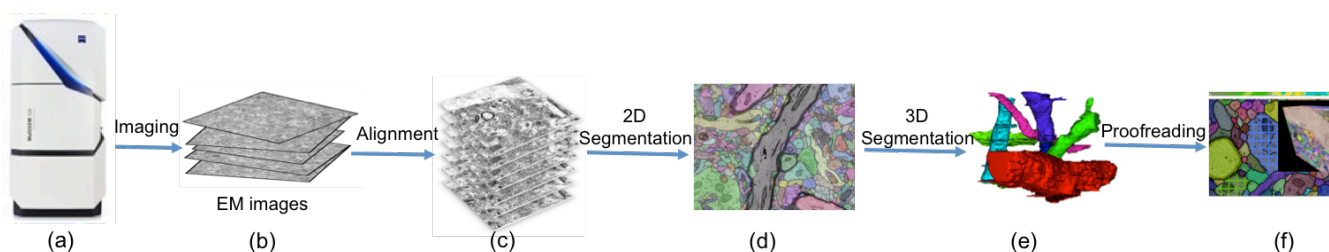
Figure 1. The *RhoANA* pipeline: (a) Imaging using Zeiss MultiSEM 505; (b) Viewing the images; (c) Stitching and aligning the images; (d) Classifying membranes and segmenting objects in 2D; (e) Creating a single segmented 3D volume; (f) Manually proofreading the automatic pipeline's result.
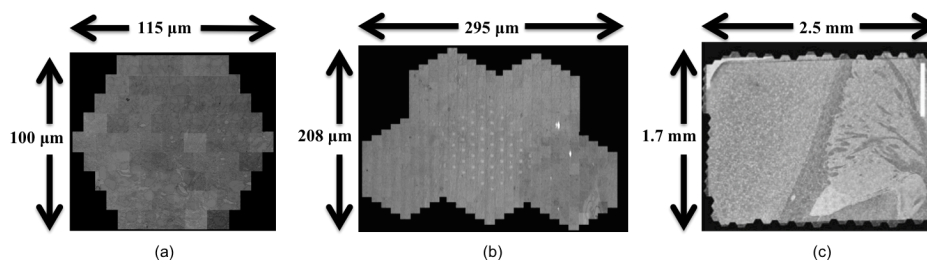
Figure 2. Images acquired by the MultiSEM microscope: (a) A single mFoV – 61 tiles; (b) Five mFoVs; (c) More than 500 mFoVs.