Automated Reconstruction of a Serial-Section EM *Drosophila* Brain with Flood-Filling Networks and Local Realignment

Peter H. Li[1*], Larry F. Lindsey[2], Michał Januszewski[3], Mike Tyka[2], Jeremy Maitin-Shepard[1], Tim Blakely[2], and Viren Jain[1]

[1.] Google AI, Mountain View, CA, USA.
[2.] Google AI, Seattle, WA, USA.
[3.] Google AI, Zürich, Switzerland.
* Corresponding author: phli@google.com

Large-scale connectomic reconstruction of neural circuitry at single-synapse resolution is an attractive target for improving understanding of the nervous system in health and disease. However, challenges in image acquisition and analysis have limited most connectomic studies to image volumes that span at most millions of cubic microns (~100 billion voxels), and that encompass only limited subregions of brain circuitry. Further, in many cases only a fraction of the imaged data have been reconstructed and analyzed [1].

A recent milestone delivers significantly increased imaging scale: a forty trillion voxel serial-section electron microscopy volume of a complete adult *Drosophila* brain [2] (Fig. 1). Two challenges in analyzing any large serial-section dataset are the difficulty of aligning millions of potentially distorted image tiles into a single coherent three-dimensional volume [3], and the handling of inevitable data irregularities such as tears, folds, cracks, and contaminant particles [2,4]. Naive tracing over such irregularities and misalignments introduces significant errors for both automated algorithms as well as trained human annotators [5]. But at the scale of the complete adult fly brain and beyond, fully coherent global alignment and correction of irregularities may be intractable.

We therefore integrated two new compensatory procedures into the recently introduced flood-filling network (FFN) segmentation method [6]. In "local realignment" (LR), we exploit the divide-and-conquer nature of FFN pipelines, which typically operate on local subvolumes before building up a whole volume segmentation. LR attempts to refine the alignment of each local subvolume provided to the FFN, compensating on-the-fly for any errors in the global alignment of the input volume (Fig. 2). In "irregular section substitution" (ISS), we automatically detect data irregularities that remain after LR and, where possible, replace affected regions with data from neighboring sections (Fig. 3). After applying LR and ISS, we make a final recheck of subvolume alignment quality, so that the FFN can be prevented from tracing over any remaining poorly aligned or irregular regions.

Integrated with an established FFN segmentation pipeline [6], LR and ISS allowed dense segmentation of the entire adult fly brain dataset (Fig. 1), with a very low rate of merge errors (processes erroneously connected to one another), and with segment lengths sufficient to assist many common tracing workflows and analyses. LR with recheck reduced merge errors by an order of magnitude, while ISS particularly reduced split errors (processes erroneously disconnected from one another), thus tripling the "expected run length" of resulting segments [6]. The forty trillion voxel segmentation will be publicly released to support further efforts in *Drosophila* circuit understanding and connectomic algorithm development.

References:

[1] J Kornfeld and W Denk, Curr Opin Neurobiol **50** (2018), p. 261.
[2] Z Zheng et al., Cell **174** (2018), p. 730.
[3] S Saalfeld et al., Nat Methods **9** (2012), p. 717.
[4] AW Wetzel et al., AIPR (2016) p. 1.
[5] CM Schneider-Mizell et al., eLife **5** (2016).
[6] M Januszewski et al., Nat Methods **15** (2018), p. 605.
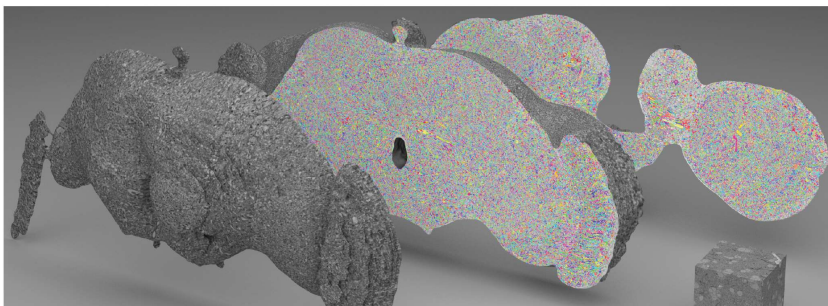[7] SY Takemura et al., PNAS **112** (2015), p. 13711.

Figure 1. Forty trillion voxel complete adult fly brain volume [2], with dense segmentation visible across arbitrary sectioning planes. Blocks at lower right show two other recent dense segmentation results [6, 7]. Scale bar 200 μm.

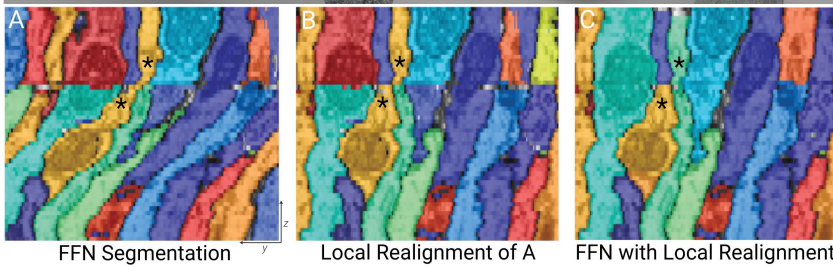FFN Segmentation   Local Realignment of A   FFN with Local Realignment

Figure 2. After initial FFN segmentation (A), local realignment (B) reveals a merge error (stars). Segmenting only after realignment (C) corrects the merger, and fixes many splits.

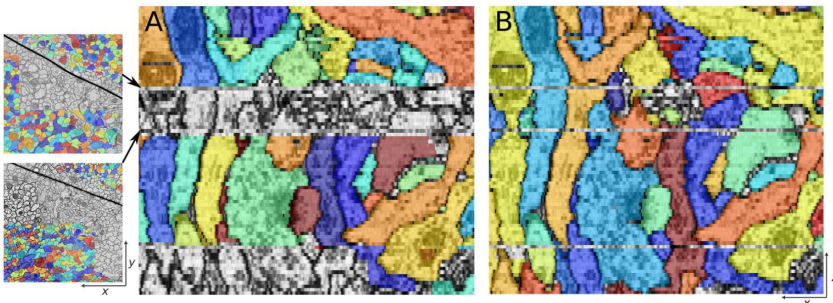**Figure 3.** FFN initially blocked by damaged sections (A) can segment through smoothly after irregular section substitution (B).