

Supplementary Material

Feasibility of 3D Reconstruction of Neural Morphology using Expansion Microscopy and Barcode-Guided Agglomeration

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- **1** Supplementary Data
- 1.1 Supplementary Figures

Supplementary Material



2

Supplementary Figure 1. Simulation procedure. (a) Ground truth input dataset obtained from a fully reconstructed electron microscopic volume. (b) Fluorophores were randomly assigned. (c) Local clusters of fluorophores, or puncta, were created by convolving each fluorophore with a random size Gaussian kernel to reflect local clusters of multiple fluorophores on antibodies bound to protein targets. (d) The synthetic volume was projected to a 3-D image stack by convolving the volume with the 3-D point-spread-function (PSF) of a confocal microscope. (e) Poisson noise and read-out noise were added to the image.



Supplementary Figure 2. 3-D surface renderings of the simulation input data sets. (a-c) 3-D renderings of the ground truth input data sets used for the simulation of ExM images for training the segmentation algorithm. (d) 3-D rendering of the ground truth input data set used for the simulation of an ExM image for testing the segmentation algorithm.

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Supplementary Figure 3. Boundary probability map before and after post-processing. (a) Boundary probability map straight out of a ConvNet. (b) The boundary probability map was post-processed by taking the median value of three ConvNet outputs and applying a 3-D median filter.