Supplementary Information: Flexible Learning-Free Segmentation and Reconstruction for Sparse Neuronal Circuit Tracing

Ali Shahbazi[1,+], **Jeffery Kinnison**[1,2,+], **Rafael Vescovi**[2,3], **Ming Du**[4], **Robert Hill**[5], **Maximilian Jösch**[6], **Marc Takeno**[7], **Hongkui Zeng**[7], **Nuno Maçarico da Costa**[7], **Jaime Grutzendler**[5], **Narayanan Kasthuri**[2,3], **and Walter J. Scheirer**[1,*]

[1]Department of Computer Science and Engineering, University of Notre Dame, Notre Dame, IN, USA
[2]Center for Nanoscale Materials, Argonne National Laboratory, Lemont, IL, USA
[3]Department of Neurobiology, University of Chicago, Chicago, IL, USA
[4]Department of Materials Science and Engineering, Northwestern University, Evanston, IL, USA
[5]Department of Neurology, Yale University, New Haven, CT, USA
[6]Neuroethology Group, IST Austria, Klosterneuburg, Austria
[7]Allen Institute for Brain Science, Seattle, WA, USA
[*]walter.scheirer@nd.edu
[+]These authors contributed equally to this work.

ABSTRACT

This document contains all supplemental figures and methods descriptions.

Supplementary Methods: Evaluation Metrics

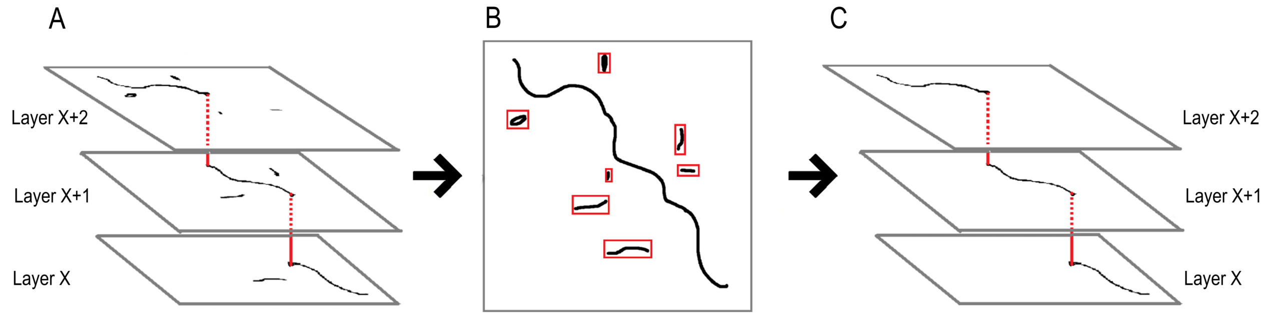
In our experiments we compared our EM reconstructions and one of our μCT X-ray stacks against expert annotated ground-truth. Since 3D reconstruction is the goal of the work, we chose Hausdorff distance as an evaluation tool. It is a well-known evaluation method for 3D meshes and reconstructions[1]. Instead of one-by-one matching as is done when calculating variation of information, which checks the location of each point on the first model with a similar location on second model, Hausdorff distance considers many-to-many correspondence. Consider a 3D reconstruction A and corresponding 3D ground-truth B: $A = \{a_1, a_2, ..., a_n\}$ and $B = \{b_1, b_2, ..., b_n\}$. The Hausdorff distance from A to B will be $\tilde{\delta_H}(A,B) = max_{a \in A} min_{b \in B} \|a - b\|$. Throughout this article, we use the convention A is in X/Y of the distance of B. Thus when we say A is in 1/1000 of the distance of B, this means the average distance between A and B is less than 0.001.

Supplementary Methods: 3D U-Net Training

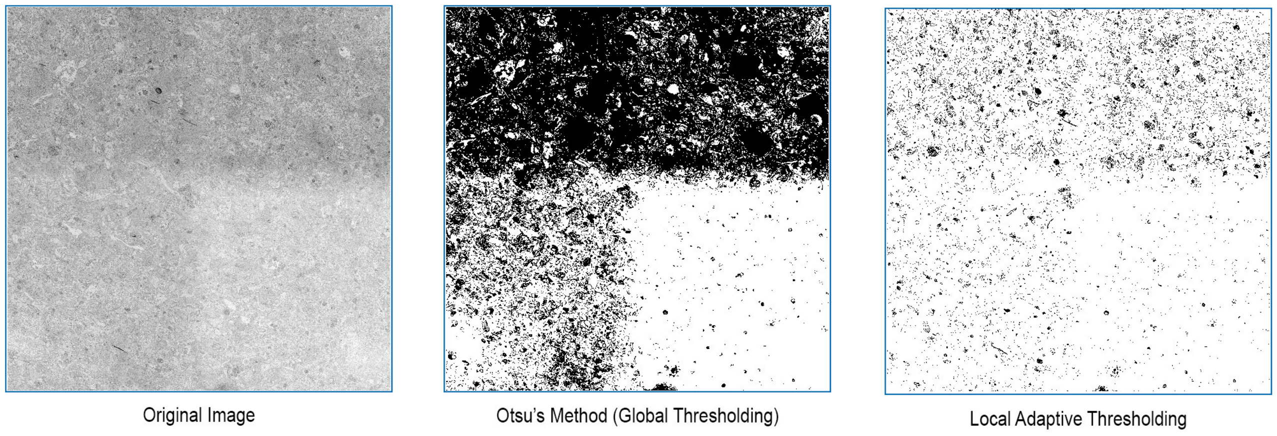
For μCT X-ray segmentation, a standard 3D U-Net model[2] was trained on the manual segmentation of the SRB dataset[3], which consists of a $300 \times 300 \times 100$ voxel volume. Due to memory constraints, each training batch consisted of a single $256 \times 256 \times 19$ block augmented by randomized rotation, transpose, and grayscale intensity scaling. The model was trained for 60 epochs using weighted mean-squared error loss and the Adam optimizer[4] with a learning rate of 1×10^{-4}. Training for sSEM APEX2-positive SAC segmentation proceeded using the same protocol, however the model was trained on manual segmentations created by Jösch *et al.*[5].

Supplementary Table S 1. Running time comparison to the previous version of the pipeline[5].

Method	Running time
Jösch et al. [5]	136 min
2D FLoRIN	24.82 min
3D U-Net	3559.3 min



Supplementary Figure S 1. The 2D Identification process in FLoRIN first registers all connected components discovered in the Identification stage into a single plane. Connections are preserved by connecting components that overlap with one another in 2D, and unconnected components are discarded. The remaining components are then placed into a 3D volume.


Original Image
Otsu's Method (Global Thresholding)
Local Adaptive Thresholding

Supplementary Figure S 2. A comparison of the performance of global versus local thresholding methods on a noisy image. (Left) Neural images are often subject to large shifts in the grayscale distribution that can hamper reconstruction efforts. (Center) Otsu's method[6] binarizes images using a global threshold value, however due to grayscale shifts the binarization includes large portions of the image background. (Right) Our LAT algorithm operates by observing a local neighborhood around each voxel to reduce the impact of distant noise. In this case, LAT captures more of the features of interest despite large grayscale shifts.

References

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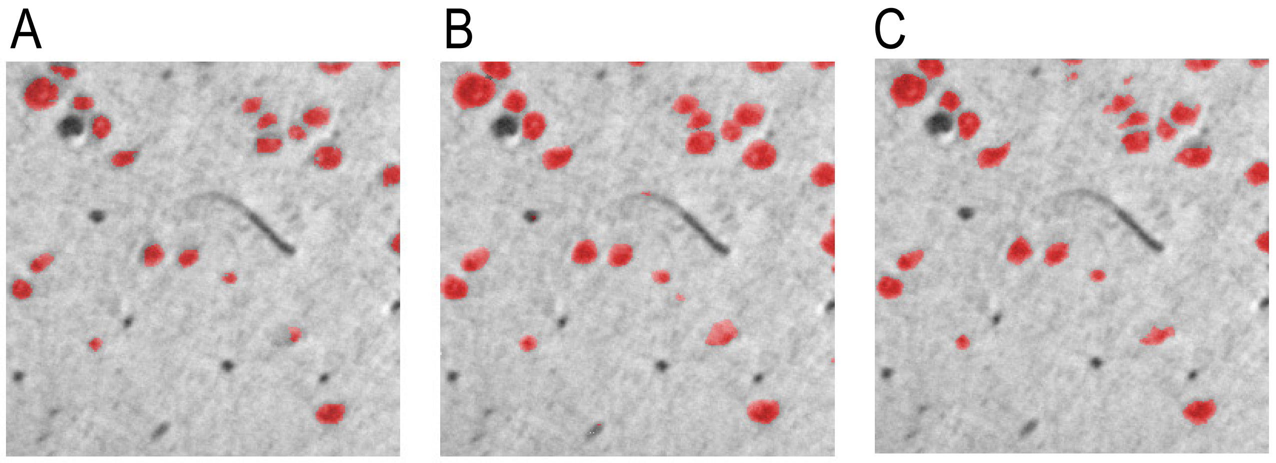

Supplementary Figure S 3. A comparison of SRB (A) manual annotations, (B) 3D U-Net cell segmentation, and (C) 3D FLoRIN cell segmentation. In general, 3D U-Net tends to over-segment the cells, incurring merge errors with nearby cells and on correctly identifying a vasculature segment as a cell. 3D FLoRIN, on the other hand, clearly separates grouped cells and does not misclassify vasculature.

```
Data: img: an n-dimensional image to threshold
Data: n: the dimensionality of img
Data: d: an n-tuple containing the size of each dimension of img
Data: s: an n-tuple containing the dimensions of the box around each pixel
Data: t: the threshold value to use, number in range $[0, 1]$
Result: binarization of img
let out be an array the same size as img;
intImg = img;
for i in 1..n do
    // Compute the cumulative summation over dimension i
    intImg = cumulativeSummation(intImg, i);
end
let indices be the set of all binary strings length n;
let low, hi, vertex be length n arrays filled with zeros;
parity = n mod 2;
foreach element e in intImg do
    x = index(intImg, e);
    for i in 1..n do
        low[i] = x[i] - s[i] / 2;
        hi[i] = x[i] - s[i] / 2;
        if low[i] $<$ 1 then
            low[i] = 0;
        end
        if hi[i] $>$ d[i] then
            hi[i] = d[i];
        end
    end
    count = $\prod_{i=1}^{n} hi[i] - low[i]$;
    sum = 0;
    foreach idx in indices do
        p = 0;
        for i in 1..n do
            p = p + idx[i];
            if idx[i] = 1 then
                vertex[i] = hi[i];
            else
                vertex[i] = low[i];
            end
        end
        p = p mod 2;
        if p = parity then
            sum = sum + intImg[vertex];
        else
            sum = sum - intImg[vertex];
        end
    end
    if img[x] $\times$ count $\leq$ sum $\times$ (1.0 - t) then
        out[x] = 0;
    else
        out[x] = 1;
    end
end
return out
```

Algorithm 1: Local Adaptive Thresholding