Online Multi-spectral Neuron Tracing
Supplementary Material

Sample Slices of Used Datasets

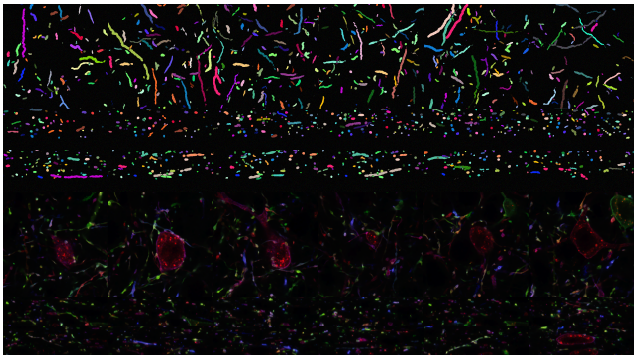

Figure 1. **Sample slices on simulated (top 3 rows-Z, Y, X planes) and real (bottom 3 rows-Z, Y, X planes) multi-spectral datasets with high labeling density**. All datasets are denoised using BM4D [6] as data preprocessing.

Sample Tracing Video

We attach a video – `sample_tracing.mp4` to demonstrate our tracing method. The top right boxes in the video are detected bifurcation candidates. You might need a media player (H.264 Codec enabled) to play the video.

Evaluation Metrics Details

(**i**) VSA score measures the agreement between the reconstructed trace and gold-standard (GS) trace. The well-adopted DIADEM [4] metric in neuron reconstruction is not suitable here since DIADEM focuses more on single neuron quantification, and it puts heavy penalties for incomplete traces, i.e., a low DIADEM score does not necessarily mean a bad reconstruction. Specifically, we first count the pairs of agreement between the reconstructed trace and GS trace under a given threshold. By meaning variation, we sum up the ratio of pairs of agreement to the GS trace (a2g) and the ratio of pairs of agreement to the reconstructed trace (a2r). This final score is calculated as VSA $= (1-\beta)\cdot$a2g$+\beta\cdot$a2r, where β is set to 0.1. The range of the VSA score is [0, 1], where 0 means no pair of agreement, and 1 means perfect reconstruction. Our VSA gives a reasonable score even for partial trace. The reason why we use variations (i.e. a2r and a2g) is that we consider the penalty of over-reconstruction here. For example, suppose we have two different reconstructions but with the same alignment pairs to GS trace. In this case, a2g scores are the same, but the a2r score of the over-reconstructed one should be lower for fair comparison, where 0.1 is set to balance the penalty and score, empirically. (**ii**) MFD: we first identify the nearest point in the GS trace for each point of the reconstruction, and we take an average over all such minimum distances. The least MFD is 0 (perfect match) and with no upper bound. (**iii**) BRR is only calculated when the GS trace has more than one branch. It is the ratio of bifurcations that are currently retrieved to the GS trace except the main branch.

Implementation Details

For xBTracer, we use Colornames [8] and gray-scale of image features to learn the correlation filter and RGB color histogram with 32 bins per channel to learn the global color model. Color histogram adaptation rate is set to η_g=0.01, retention rate to η_r=0.2, filter learning rate to η_w=0.01, numbers of history to estimate mean mask and estimate tracing direction are both set to 50, standard deviations for estimating cross-section plane and bifurcation probability are set to σ_c=0.4, σ_b=0.2, neuron presence uncertainty threshold τ_u=3, neuron presence history is set to 20, cut-off intensity τ_c=30 and dim-aware counter to 3, bifurcation probability threshold τ_b=0.9, bifurcation detection threshold τ_d=5. All other parameters for channel reliability, alpha channel estimation, ADMM optimization, and scale estimation are set to values commonly used in studies [1, 2, 5].

Besides, we also compare our method with state-of-the-art deep learning neuron tracing methods. We first skeletonize their predicted segmentation using the MATLAB code from [3] and use that as their tracing results.

Quantification for Applying to fMOST

Dataset	VSA	MFD
fMOST	0.92	5.4

Table 1. **Our performance on fMOST imaging modality.**

Our proposed method consists of alpha channel enhanced DCF and neuron-dedicated models that can be generalized to other neuron imaging modalities. Here, for ex-

ample, we test our method in fMOST data, which is a single-channel image modality. We compare our reconstructed traces to the human gold standard traces qualitatively as in Table 1.

Parameters Sensitivity Analysis

metric \ η_r	0.1	0.2	0.3	0.4	0.5
VSA	0.78	**0.82**	0.78	0.77	0.75
MFD	24.49	**23.71**	23.91	24.08	24.02
BRR	0.16	**0.18**	0.16	0.17	0.17
Precision	0.85	**0.87**	0.84	0.82	0.82
Recall	0.62	**0.65**	0.61	0.59	0.62

Table 2. **Parameters sensitivity study on sim. dataset.**

We divide all parameters into two groups – intensity-related and probability-based. Among the two groups, intensity-related parameters (e.g., cut-off) are non-sensitive. Setting the cut-off intensity from 30 to 25 makes little difference so we don't focus on those parameters. For probability-related parameters, the main goal is to convert the probability into the range $[0, 1]$ where the standard deviations control the slopes of the exponential function and only need to be tuned once for a new dataset for better performance where we are using the same parameters throughout our experiments. The results are shown above for different retention rates η_r which we find most critical since it determines when to stop.

Quantification of Saving Human-effort

Method	1st bbox	n-th bbox	tracing direction	VSA
nTracer	0.1s	n×0.5s	0	1
xBTracer	0.1s	0	0.5s	0.89

Table 3. **Quantification of human time on nTracer [7] (semi-manual) and xBTracer (ours).** For selecting the n-th bbox, the human tracer has to eye-track the neuron and click on the next position. This time is usually longer.

We quantify the human time spent for a semi-manual tracer (nTracer) with our method (xBTracer). For using nTracer, the human-tracer has to click the first bounding box (bbox) and the second bbox, when the path-finding algorithm (i.e., $A^\star$) finds the path linking the two bboxes. After that, the human tracer has to select the next bbox and use the previous second bbox as the first bbox. To summarize, the whole process of nTracer tracing is to select bbox repeatedly and manually. Typically, for the sake of fast computation of pathfinding algorithm so that the human-tracer would not have to wait, the distance between two bboxes should not exceed 100 voxels (during our experiments). For our algorithm, we only need to input the starting bbox and tracing direction. Besides, our algorithm can be easily paralleled, compared to the semi-manual method, as we can input all starting bboxes and tracing directions into the algorithm and paralleled them as no human interventions need during tracing. For a fair comparison, we only compare the human time spent on selecting boxes and tracing directions, where the waiting time for algorithm computation is not considered. We ask 10 trained human tracers to test both methods where we record the time cost. The quantification is shown in the Table 3, where all values are averaged out.

Overall, compared to the semi-manual method, our method can save approximately $(n-1)\times$time selecting the next bboxes, where n depends on how many bboxes the human tracer selects for the traced neuron.

Additional Proof

Theorem. The lower bound of the path cost is 0, and the upper bound is c, where c is the channel number of the image patch used to find the path.

Proof. The distance between two points is minimally 0 since we used Euclidean distance. It is easy to show that the low bound is obtained when there is no color difference among the paths. For the upper bound, every time, if the maximum distance is c, after the summation and division over the entire path length, the cost is still c.

References

[1] Qifeng Chen, Dingzeyu Li, and Chi-Keung Tang. Knn matting. *TPAMI*, 35(9):2175–2188, 2013.

[2] Martin Danelljan, Gustav Häger, Fahad Shahbaz Khan, and Michael Felsberg. Discriminative scale space tracking. *TPAMI*, 39(8):1561–1575, 2016.

[3] Bin Duan, Logan A Walker, Douglas H Roossien, Fred Y Shen, Dawen Cai, and Yan Yan. Unsupervised neural tracing in densely labeled multispectral brainbow images. In *ISBI*, 2021.

[4] Todd A Gillette, Kerry M Brown, and Giorgio A Ascoli. The diadem metric: comparing multiple reconstructions of the same neuron. *Neuroinformatics*, 9(2-3):233, 2011.

[5] Alan Lukežič, Luka Čehovin Zajc, Tomáš Vojíř, Jiří Matas, and Matej Kristan. Fucolot–a fully-correlational long-term tracker. In *ACCV*, 2018.

[6] Matteo Maggioni, Vladimir Katkovnik, Karen Egiazarian, and Alessandro Foi. Nonlocal transform-domain filter for volumetric data denoising and reconstruction. *TIP*, 22(1):119–133, 2013.

[7] Douglas H Roossien, Benjamin V Sadis, Yan Yan, John M Webb, Lia Y Min, Aslan S Dizaji, Luke J Bogart, Cristina Mazuski, Robert S Huth, Johanna S Stecher, et al. Multispectral tracing in densely labeled mouse brain with ntracer. *Bioinformatics*, 35(18):3544–3546, 2019.

[8] Joost Van De Weijer, Cordelia Schmid, Jakob Verbeek, and Diane Larlus. Learning color names for real-world applications. *TIP*, 18(7):1512–1523, 2009.