Automated reconstruction of vasculature in cleared, intact brain specimens

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Introduction

3DISCO, uDISCO, CLARITY, and other related tissue clearing agents have opened the possibility for unprecedented studies of neuroanatomy and cytoarchitecture by removing the necessity of tissue sectioning. As imaging methodologies evolve for these intact specimens it is also evident that specialized quantitative tools are needed.

Intact cleared specimens are often multi-millimeter thick which require sophisticated and expensive microscopy equipment (e.g., light-sheet microscopy and super long working distance -objective lenses) and long duration acquisition times. The resulting image data can be very large (>0.5 TB). Further, the focus resolution limitations due to the objectives generally result in voxel dimensions that are challenging for automated reconstruction. As such, quantitative software that can analyze cleared image data faces special challenges.

In the current study, we demonstrate the utility of image data from cleared specimens with the functionality of specialized quantitative software for reconstruction of brain microvessels and neurons with standard computer equipment.

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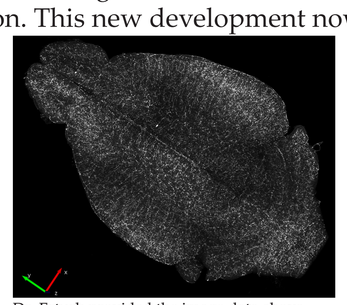
Challenges with Image Data from Intact Specimens

Tissue cleared with CLARITY, 3DISCO, or similar methods offer the opportunity to investigate the critical role of microvasculature in the plasticity of the brain in detail. By perfusing the vasculature with fluroescently-conjugated lectin, the microvessels are specifically labeled, however, large caliber vessels can appear hollow.

The novel automated methods presented are computationally efficient and therefore greatly reduce the time needed for analysis. Image management is a central concern, because there is a simultaneous need to have the ability to interact with the entire image volume and with a small subset that represents the current region of interest. Vesselucida addresses this need by using an interactive 3D visualization environment that enables multi-resolution image handling. Real-time volume rendering of large amounts of data using non-specialized computer hardware is then used to elucidate spatial orientation. Automated methods for reconstructing vascular features across multiple fields of view further streamline the data acquisition. This new development now makes it possible to perform automated quantitative analyses at the maximal resolution of the image volume while having the morphological and organizational context of the intact tissue specimen.

Computer Workstation Configuration

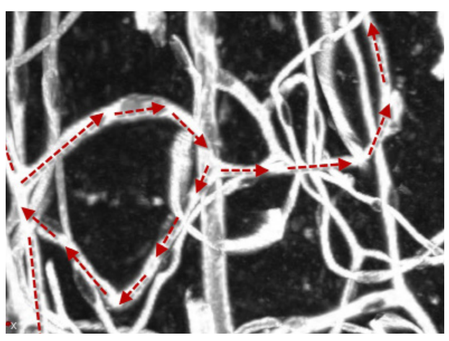
Dell Precision T5810 Desktop Intel Xeon Processor 3.1 GHz, with 64 GB RAM
Microsoft Windows 7 64-bit
Vesselucida (prototype software)
AMD Radeon R9 200 graphics card with 4 GB RAM



Dr. Erturk provided the image data above from an experiment performed in accordance with guidelines for the care and use of laboratory animals.

Data Model

Vesselucida prototype software provides tools to automatically, interactively, or manually reconstruct blood vessels as a network structure.



Accurate modeling of microvessel networks cannot be done using the tools currently used for reconstructing neuronal structures because looping structures (left) are not permitted in a tree model. To overcome this, a new data model was implemented that accurately accommodates loops and abruptly changing segment diameters.

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Automatic reconstruction

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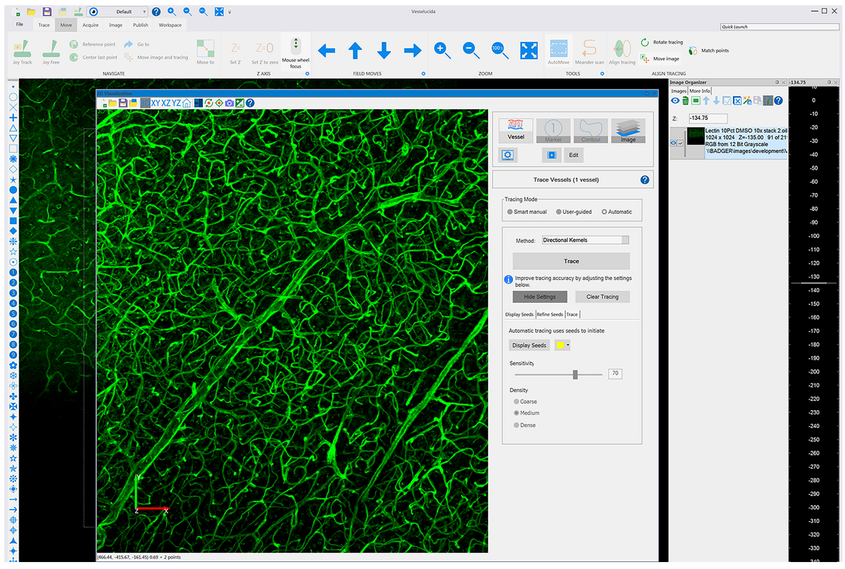
The protoype Vesselucida software has an intuitive user interface, including ribbon tool bars, and workflows.

The vasculature can be traced automatically using a choice of 3 algorithms, each designed for different image characteristics.

In user guided mode, the user can switch between the three algorithms to account for variability within the image volume.

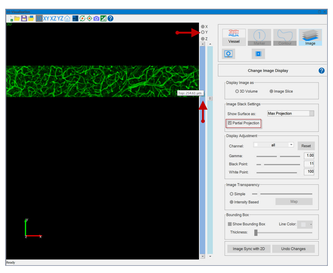
Smart manual tracing and a full complement of editing tools are provided so that a complete and accurate representation of the network can be created.



Dr. Watson provided the image data above from an experiment performed in accordance with guidelines for the care and use of laboratory animals.

Visualization in Dense Networks

A



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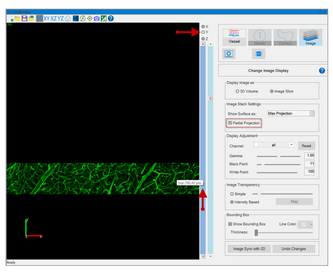

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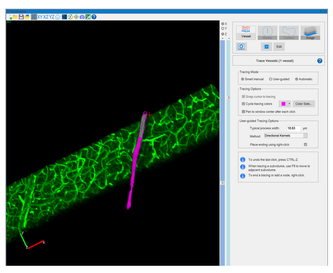


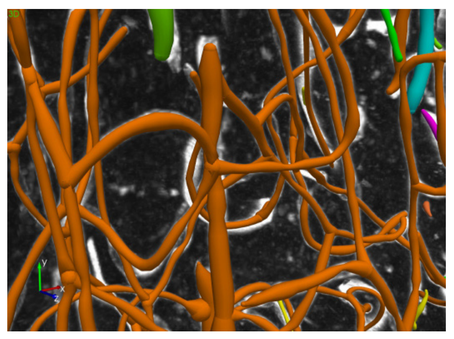
Displaying the image volume as a partial projection addresses the problem of context in large or complicated image scenes.

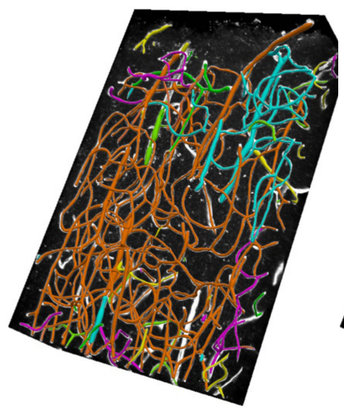
The partial projection can be used to limit the amount of image data displayed so that it is easier to navigate and work with. The resulting subvolume can be modified in the X, Y, and Z dimensions. The size and height of the image volume displayed is adjusted using a slider. Tracing can continue in the partial projection without reverting to the original display by adjusting the sliding scale.

Due to the complexity of vascular networks in intact specimens, it is essential to restrict the image display to manageable portions of the image for situations which require user input, including user guided tracing and editing.

Network Metrics

Accurate automatic morphologic reconstruction of the vasculature as a network is available for the first time. Analysis of total length, branching patterns, and organization can be quantitatively measured.





All Graph Totals

	Isolated Elements	Total Capillaries	Total Nodes	Branching Nodes	Open Nodes	Total Length (µm)	Total Volume (µm³)	Loop Count
1	1	668	624	341	253	82284.680	4347840.500	47

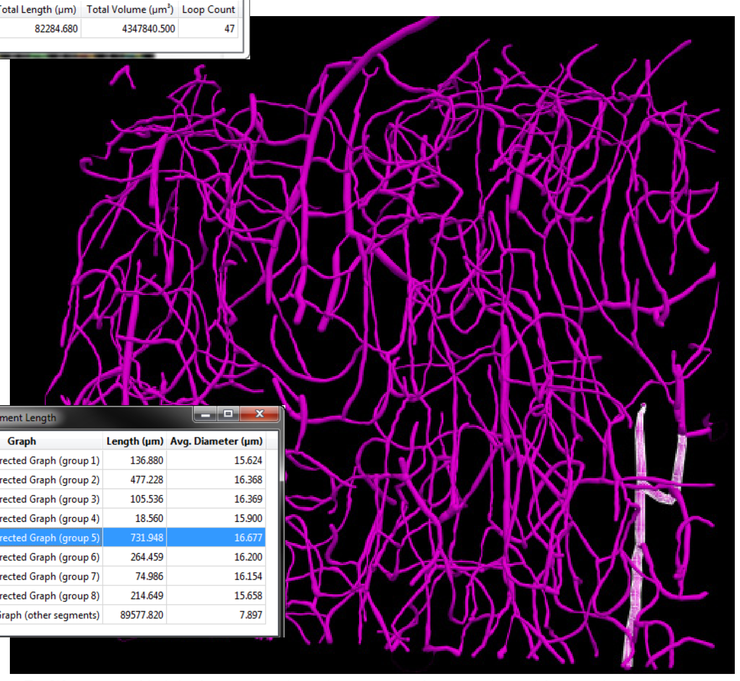
The reconstructed microvessel network can be analyzed to explore a number of metrics including the total length, total volume, connections (collaterals) and loops.

Segment analysis provides information on each segment of the network. This includes segment length and diameter. Continuous segments can be grouped based on average diameter, which can be used to identify larger caliber vessels within the network.

Modeling additional features of the vessel, including inner and outer diameter, and pericyte modeling are planned.

Graph - Segment Length

	Graph	Length (µm)	Avg. Diameter (µm)
1	Undirected Graph (group 1)	136.880	15.624
2	Undirected Graph (group 2)	477.228	16.368
3	Undirected Graph (group 3)	105.536	16.369
4	Undirected Graph (group 4)	18.560	15.900
5	Undirected Graph (group 5)	731.948	16.677
6	Undirected Graph (group 6)	264.459	16.200
7	Undirected Graph (group 7)	74.986	16.154
8	Undirected Graph (group 8)	214.649	15.658
9	Undirected Graph (other segments)	89577.820	7.897



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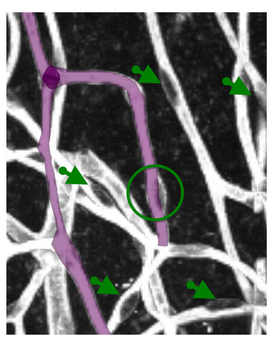
Conclusions and Future Directions

Vesselucida provides an immersive, dynamic environment for reconstruction of vascular features. Reconstruction of vasculature requires a network model capable of permitting recurrent connections. Vesselucida includes this network model and is ideally suited to work well with multi-scalar images of cleared tissue imaged with light sheet microscopes.

To improve the performance of Vesselucida, further development will include:

- Refining algorithms for greater speed and robustness
- Addition of vessel types for classification, including penetrating vessel, capillary, artery, etc.
- Algorithm development to model pericytes
- Algorithm performance improvements for large, hollow vessels
- Rendering improvements to represent network structures more reliably, e.g., branch points.
- Additional analyses for microvessel networks, including segment angles, branching metrics, and fractal analysis.



We thank Stanley Watson and Brian Martin, at the University of Michigan, for the CLARITY labeled image data.