An Open Source Tool to Aid in the Analysis of 2D Vascular Networks

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Introduction

Angiogenesis and vasculogenesis are fundamental biological processes. Computational models of these processes contribute to our understanding of this, but ultimately the behavior of the model must be compared to measures of extant vascular network morphology. This project created the Vascular Network Toolkit (VNT), an open source tool for understanding vascular networks in this way.

VNT uses ImageJ to take a digital image of a vascular network to make biologically meaningful measurements of the network. These measurements aid in comparing networks. In addition to network measurements that are common in the literature (*e.g.*, vascular area, thickness, length), the toolkit creates a shape and graph theoretic description of the network from which more topological and spectral information can be calculated. Biological significance is demonstrated using a time series of images.

Segmentation

- Computerize segmentation of an image is difficult
- VNT can be customized to identify the vascular network in an image, orsegmentation can be done by hand
- the binary representation of the network is automatically cleaned of spurrious information

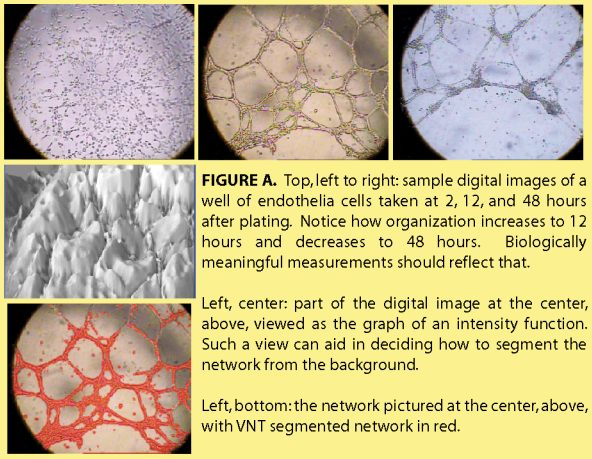

FIGURE A. Top, left to right: sample digital images of a well of endothelia cells taken at 2, 12, and 48 hours after plating. Notice how organization increases to 12 hours and decreases to 48 hours. Biologically meaningful measurements should reflect that.

Left, center: part of the digital image at the center, above, viewed as the graph of an intensity function. Such a view can aid in deciding how to segment the network from the background.

Left, bottom: the network pictured at the center, above, with VNT segmented network in red.

Shape Description

- ImageJ's Euclidean Distance Map (EDM) is used to create a Blum skeleton of segmented vasculature
- After pruning, the skeleton is used to create a set of nodes and edges for a graphical representation
- EDM at nodes used to simplify graph in biologically meaningful way
- Tool outputs adjacency information for post-processing

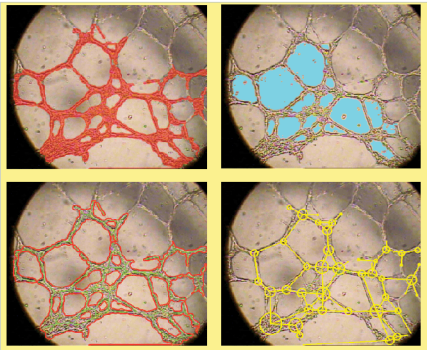

FIGURE B. Images (from upper left, clockwise) of the segmented and pruned network, the enclosed meshes, the graphical representation of the network (from a simplified skeleton model), and the boundary (red) and Blum skeleton (green) of the vascular network. All images are output from the ImageJ VNT toolkit.

Measurement & Visualization

- Pruned segmentation mask gives area of vasculature, original segmentation mask gives total cellular area
- Blum skeleton gives network length, with EDM information gives thickness
- Adjacency list gives connectivity information,
- Numerical output alone makes validation difficult
- For each input, the VNT Toolkit creates 10 digital images that allow a technician to verify measurement accuracy
- Segmentation output can be corrected"by hand" and used modification as part of VNT Toolkit workflow
- Value of measurements confirmed against time series images of human umbilical vein endothelia cell (HUVEC) networks

Plots of parameters support biological significance

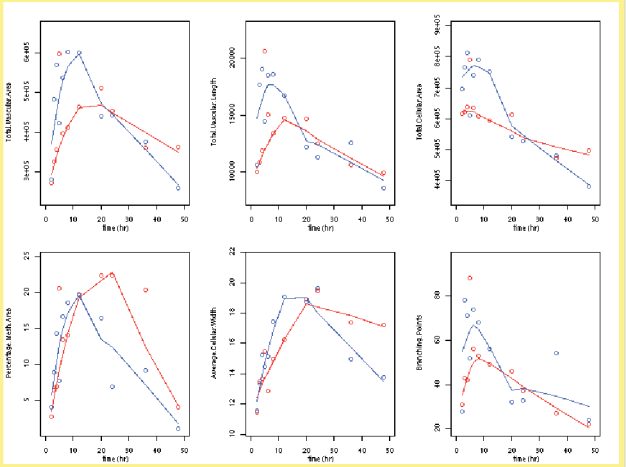

FIGURE C. Plots of measurements against time show that the VNT Tooklit produces biologically meaningful information. Pictured are curves for measurements of two networks.

Validation

- Trends in measurments corrolate to biological expectations (e.g., most highly organized at 12 hours, degrades afterward) and results in the scientific literature
- Results are repeatable
- Algorithms for the extraction of morphological and other descriptive data are robust to noise, rotation, blurring, and tolerance intervals are known.

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