mvn-analysis

Software to Characterize the Structure and Fluid Flow Forces within Engineered Microvascular Networks

Senior Honors Thesis
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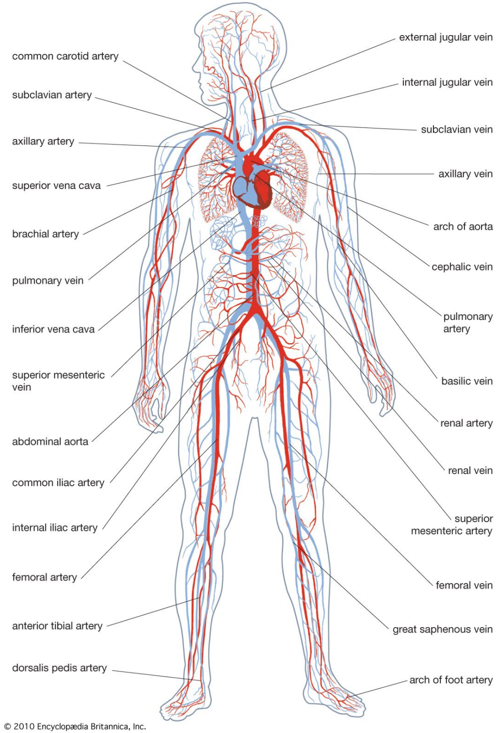
Microvasculature

The vascular system serves as the body's scale linking mechanism.

- Connects respiratory, digestive, endocrine, and excretory system to individual cells by transporting gases, nutrients, enzymes and heat.
- Majority of the transfer occurs at the **microvascular** scale
    - Blood vessels < 150 microns in diameter.
- These nutrients only diffuse a few hundred microns within tissue.



Therefore, viable, metabolically active cells must have nearby access to microvascular blood vessels.

Image reproduced from the Encyclopedia Britannica: https://www.britannica.com/science/circulatory-system

Angiogenesis and Fluid Flow

Creation of new blood vessels from existing blood vessels.

Angiogenesis is known to be driven by chemical gradients:

- Example: FGF and VEGF promote angiogenesis
- Hypoxic cells upregulate FGF receptors so they stimulate angiogenesis.
- Molecules that bind to VEGF (cancer drug Avastin) inhibit angiogenesis.
- **Stimulation of angiogenesis could be therapeutic to:**
    - Ischemic heart disease, peripheral arterial disease, and wound healing
- **Inhibition of angiogenesis could limit:**
    - Cancer metastasis, ophthalmic conditions, and rheumatoid arthritis.

In addition to chemical gradients, hemodynamics such as **wall shear stress** have also been shown to impact angiogenesis, but are less understood.

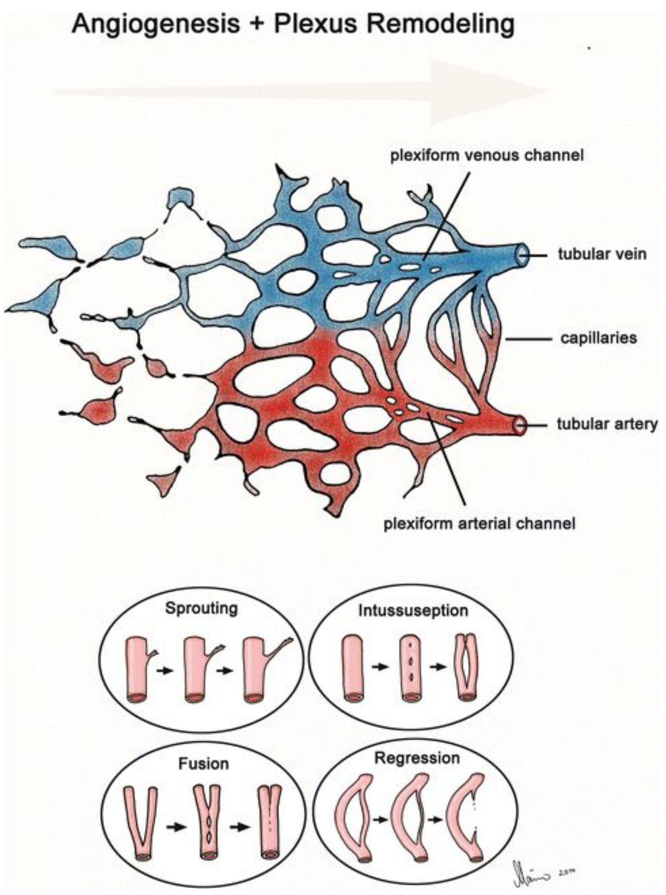
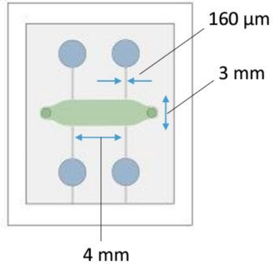
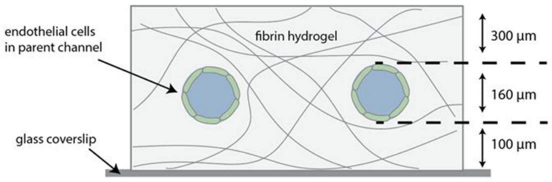


Image reproduced from: Pardanaud *et al.* 1989

Polacheck Microfluidic Devices

Novel platform capable of growing three dimensional microvascular networks within a physiological ECM.



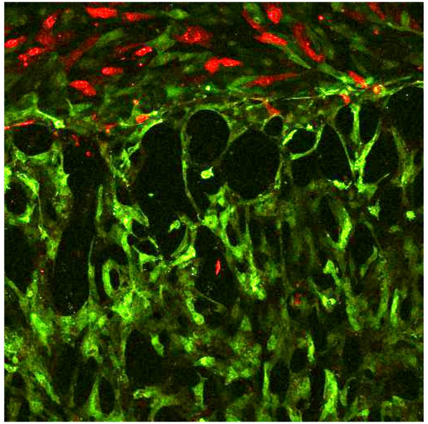


Development

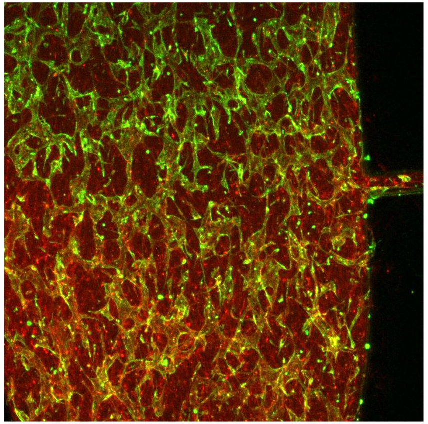
- The devices are still undergoing development.
- Need a robust method of comparing changes in manufacturing to changes in the MVN.

Experimentation

- Fluid flow forces cannot be measured directly, requiring computational modeling.



(a) 10× magnification.



(b) 4× magnification.

Diagram by Crescentia Cho. Images by Joanna McDonald

Data, Methods, and Motivations

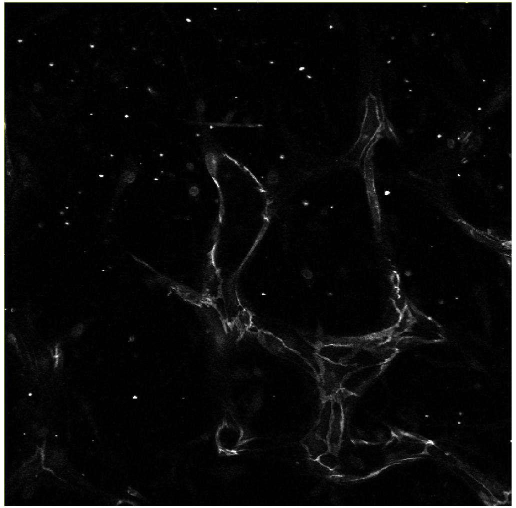
Goals

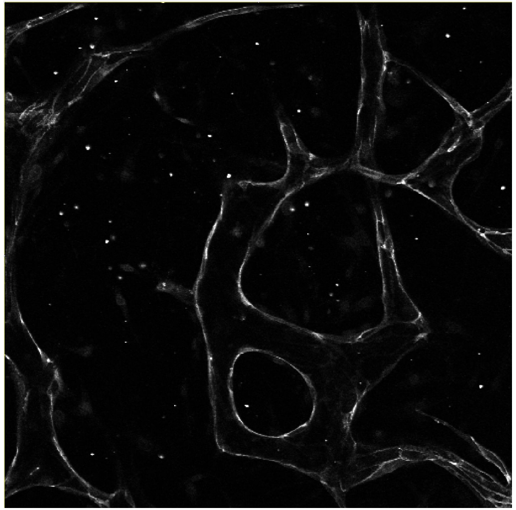
1. Ultimately generate a volume mesh of the vessel lumens that can be used in flow simulations

2. Collect as much data along the way that can be used to characterize the networks.

Input: Confocal TIF Stack

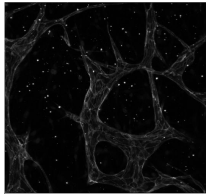
- Discrete z-step (often +2X the *xy* resolution)
- Fluorescence from GFP expressing HUVECS
    - Not uniform, not in lumen
- **Input: images of the lumen surface that are discontinuous in all dimensions and of varying intensity.**

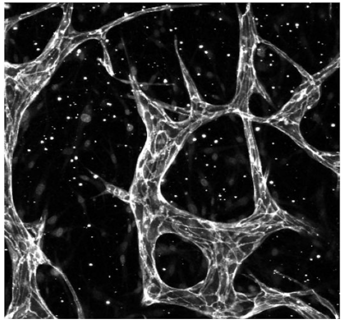




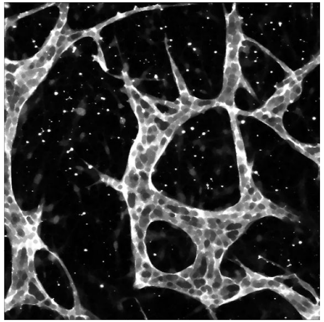
Characterizing Structure

2D Segmentation (Major Steps)

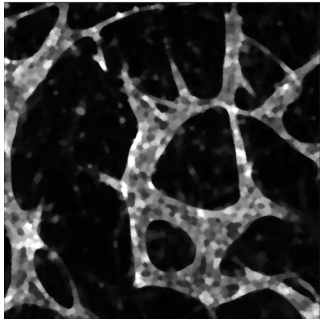




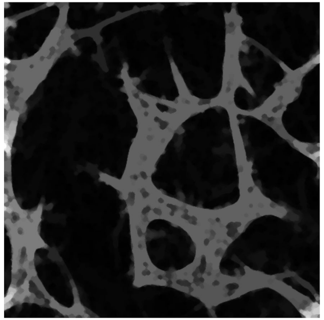
Enhance Contrast
(local or global)



Fill Surface Holes



Remove artefacts



Reconstruct via Dilation

Threshold
(Minimum Cross-Entropy or Random Walker)

Clean Binary Image

Identify Connected Regions

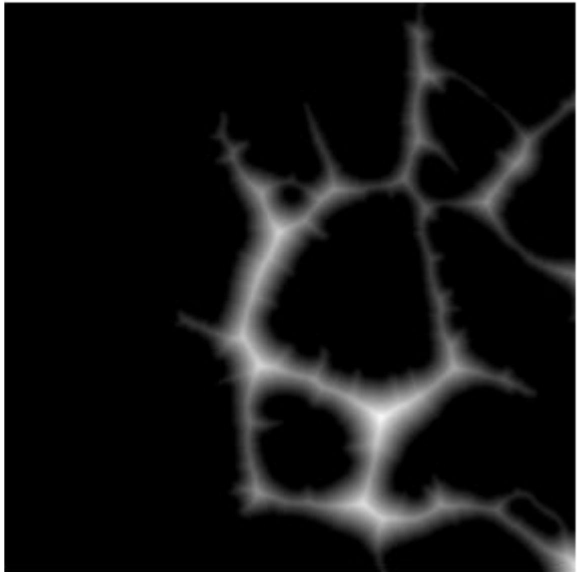
Retain largest connected region (optional)

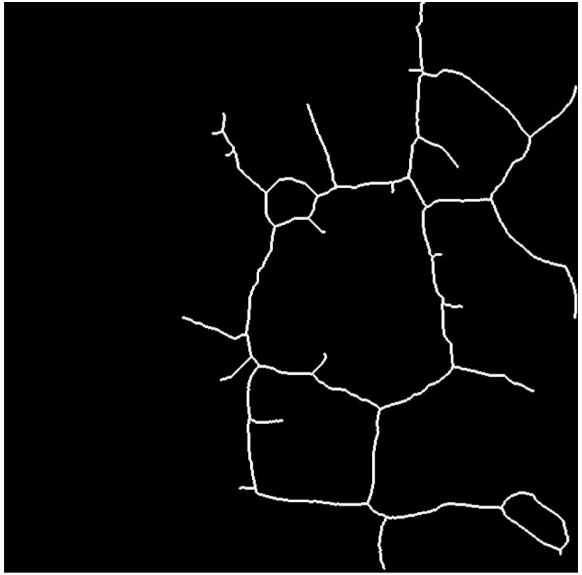
2D Segmentation (Transformations)

Scaling

The resulting binary mask is scaled in the *xy* plane such that each pixel physically represents a determined global unit.

$$\text{Global Unit} = \left\lceil \frac{\text{maxPhysicalDimension}}{750\mu\text{m}} \right\rceil$$





Scaling

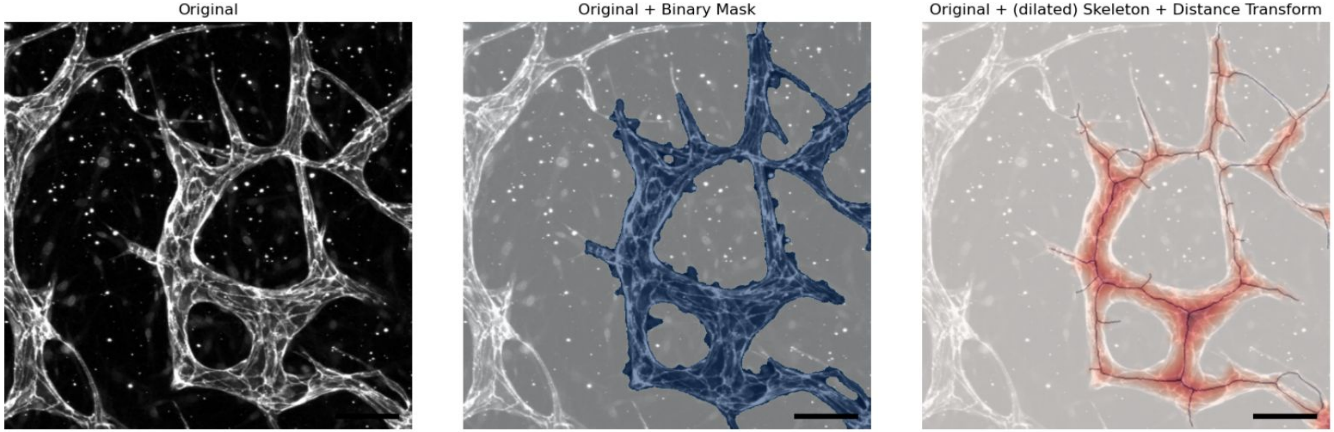
The Global Unit is used to ensure the later processes do not require > ~7 GB of memory

Euclidean Distance Transform

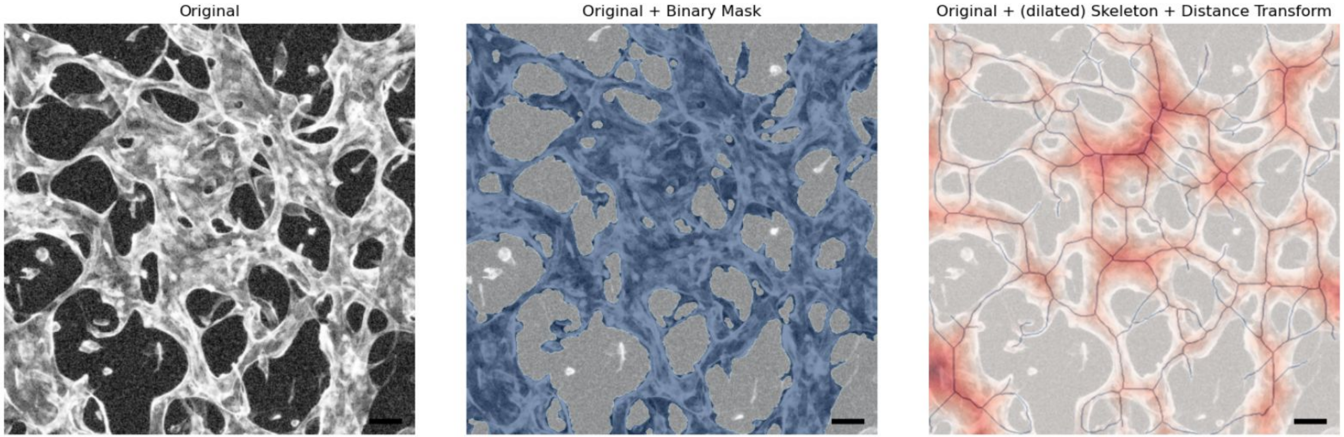
Grayscale image where pixel intensity equals the minimum distance from lumen to the background

Skeletonization

Binary image representing the centerlines of the lumens



(a) Sample A: Scale 100 μm



(b) Sample B: Scale 100 μm

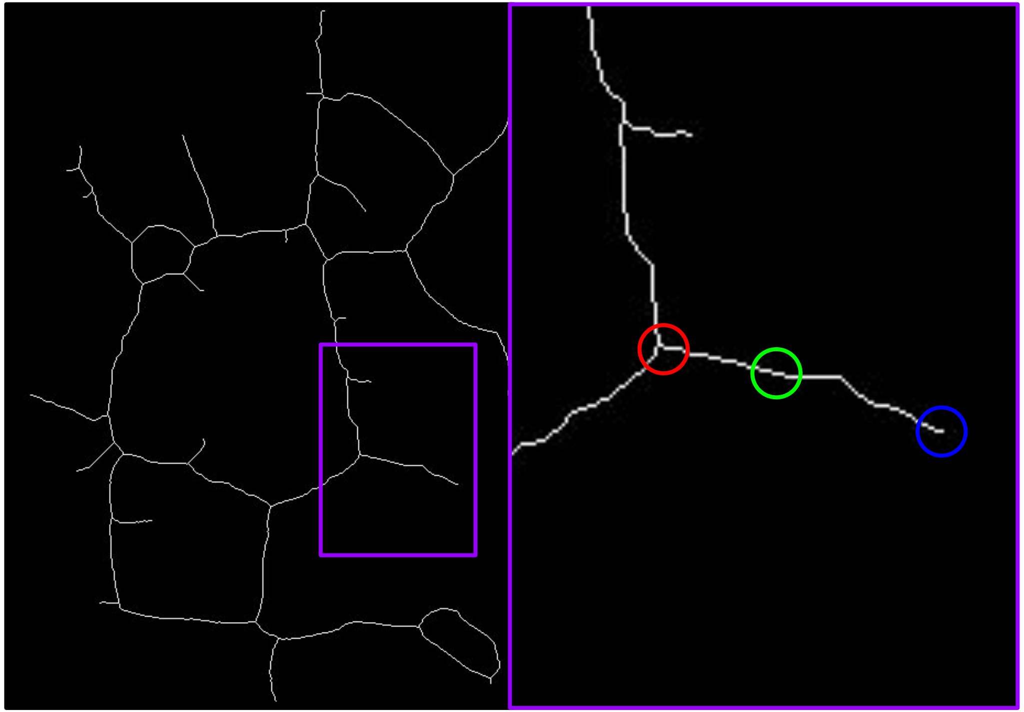
2D Weighted Graph

- A graph is a collection of nodes and edges.

- In our case, nodes are vessel branch and terminus locations.

- Edges are the lumens

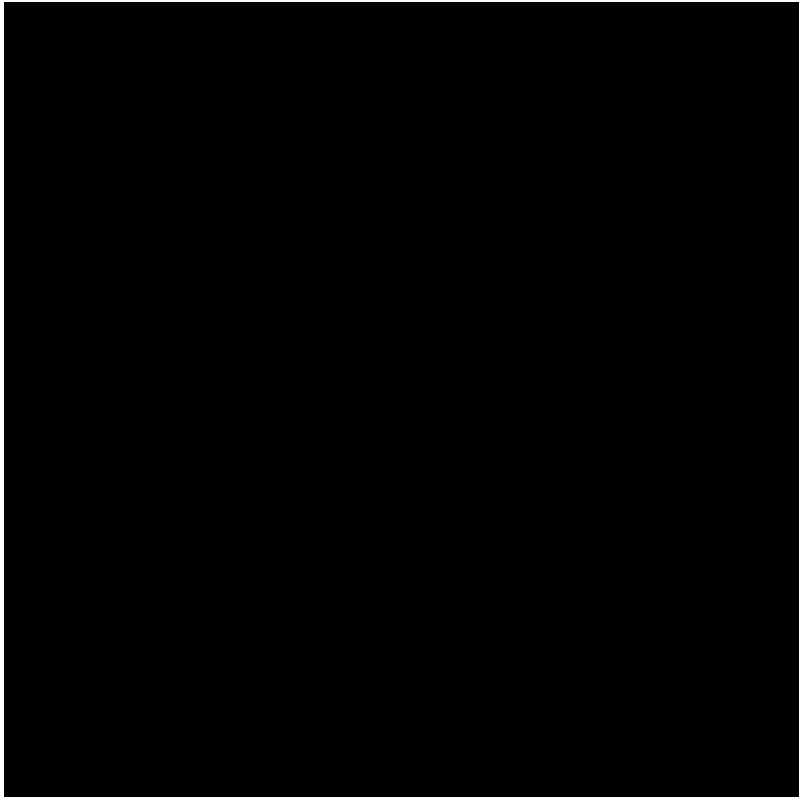
- Effective method for extracting information from the skeletonization and distance transforms



Determining node locations.

$$\left\{ \begin{array}{l} \#\text{Neighbors} = 1 \rightarrow \text{End} \\ \#\text{Neighbors} = 2 \rightarrow \text{Segment} \\ \#\text{Neighbors} > 2 \rightarrow \text{Branch} \end{array} \right\}$$

Generating The Graph Edges



Procedure 1 SkeletonWalker

Input: skeleton, distTransform, originNode, totLength, totVolume, totSurfaceArea

Input: The first call of SkeletonWalker from any origin node sets all parameter totals to 0

erodedSkeleton = skeleton − current pixel location

neighbors = non zero elements in surrounding 3x3 area

for all neighbors **do**

  move to neighbor

  d = distance to neighbor

  r = value of distTransform at current location

  totLength = totalLength + d

  totVolume = $d * \pi r^2$

  totSurfaceArea = $d * 2\pi r$

  Call SkeltonWalker with updated location and weights

end for

if there are no neighbors **then**

  Determine what destination node has been reached by searching ends and branches

  Create NetworkX edge between originNode and currently reached node with the current SkeletonWalker weights

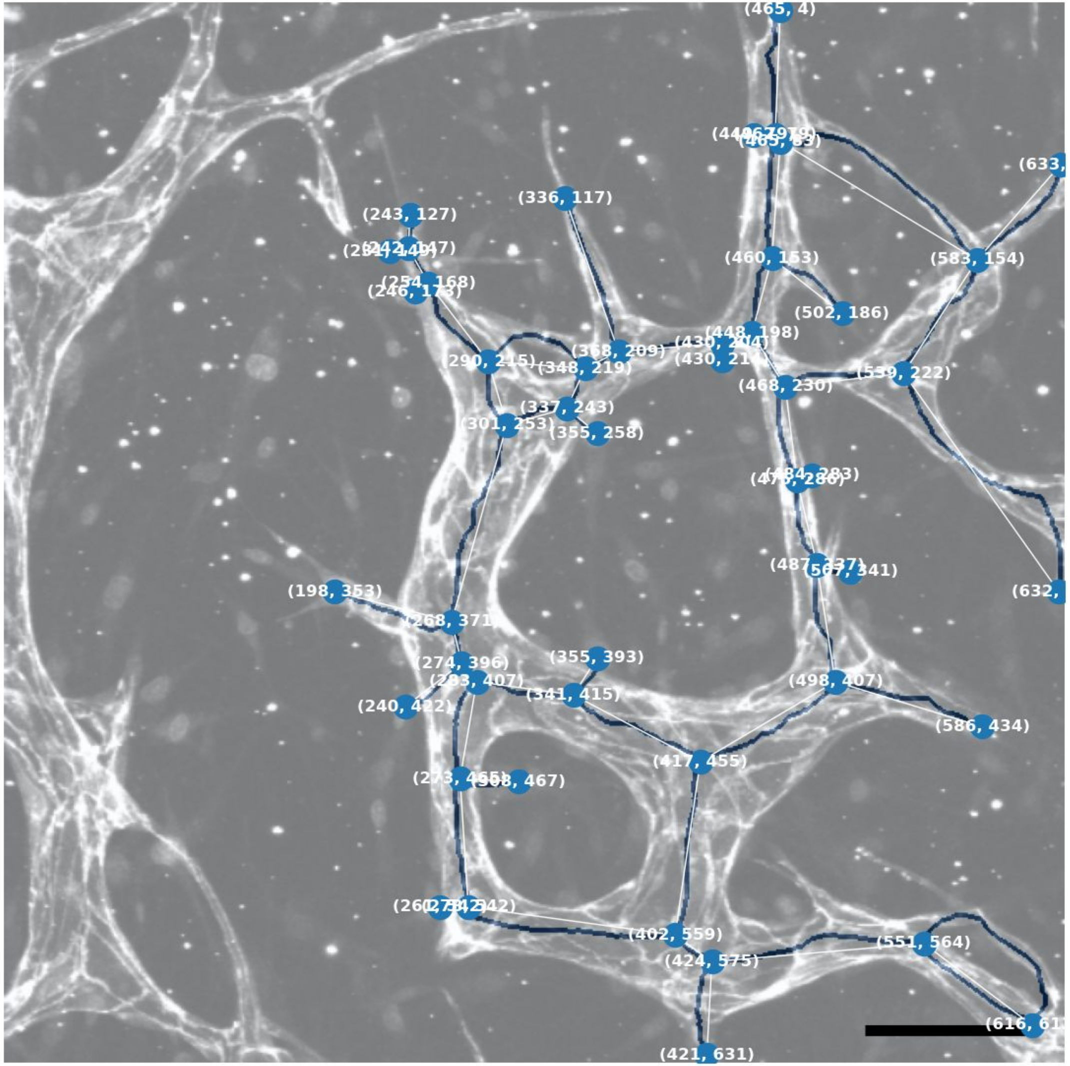
end if

Cleaning the Graph

To meaningfully compare MVNs using the Graphs, they must have similar (or reduced) noise.

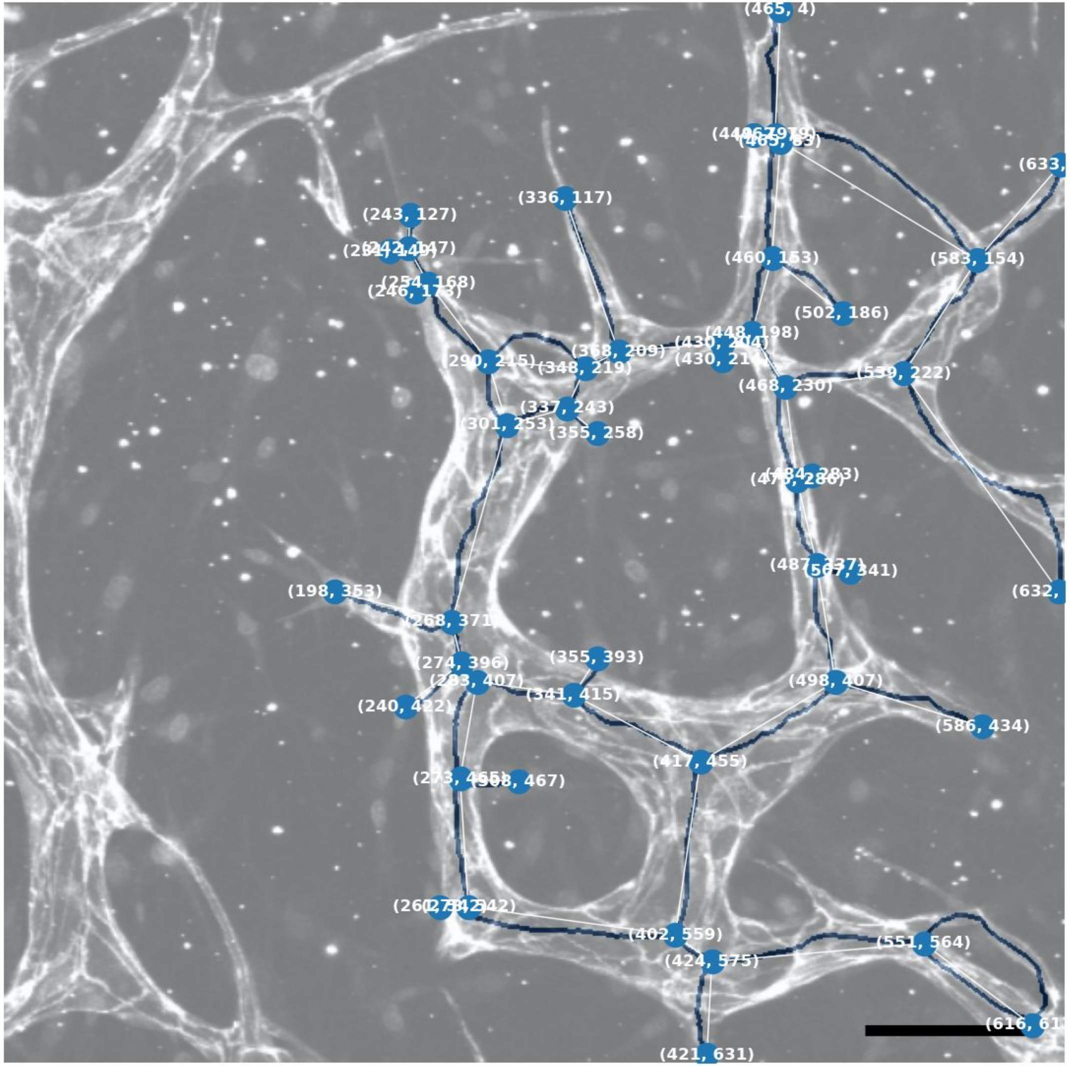
Graph cleaning:

1. Combines near nodes (within a tolerance)
2. Removes short edges (below a tolerage)
3. Removes subgraphs that do not meet a minimum node tolerance.



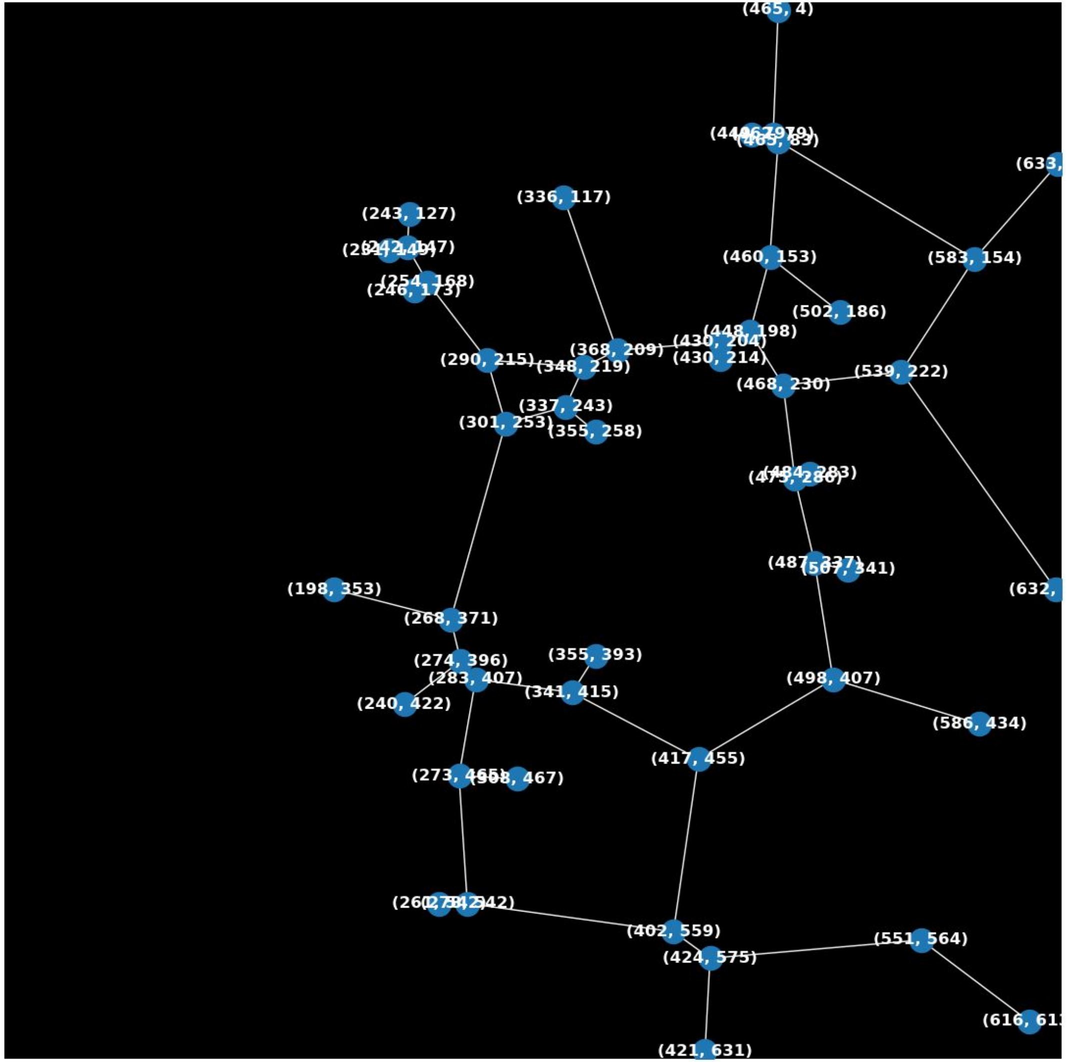
CONNECTED_NETWORK=1
LENGTH_TOL=3
MIN_NODE_COUNT=3

NEAR_NODE_TOL= 5



CONNECTED_NETWORK=1
LENGTH_TOL=3
MIN_NODE_COUNT=3

NEAR_NODE_TOL= 5



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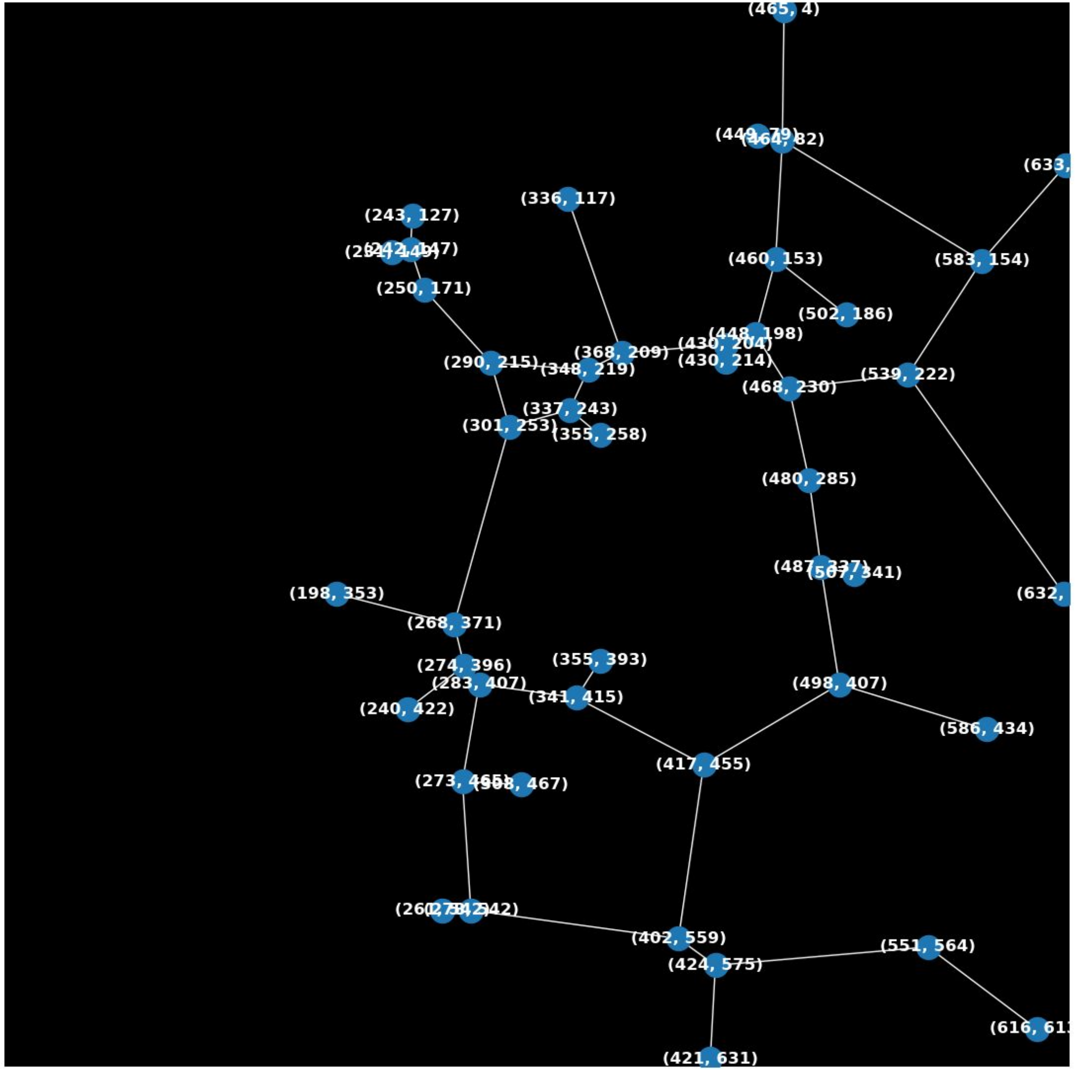
MIN_NODE_COUNT=3

NEAR_NODE_TOL= 10



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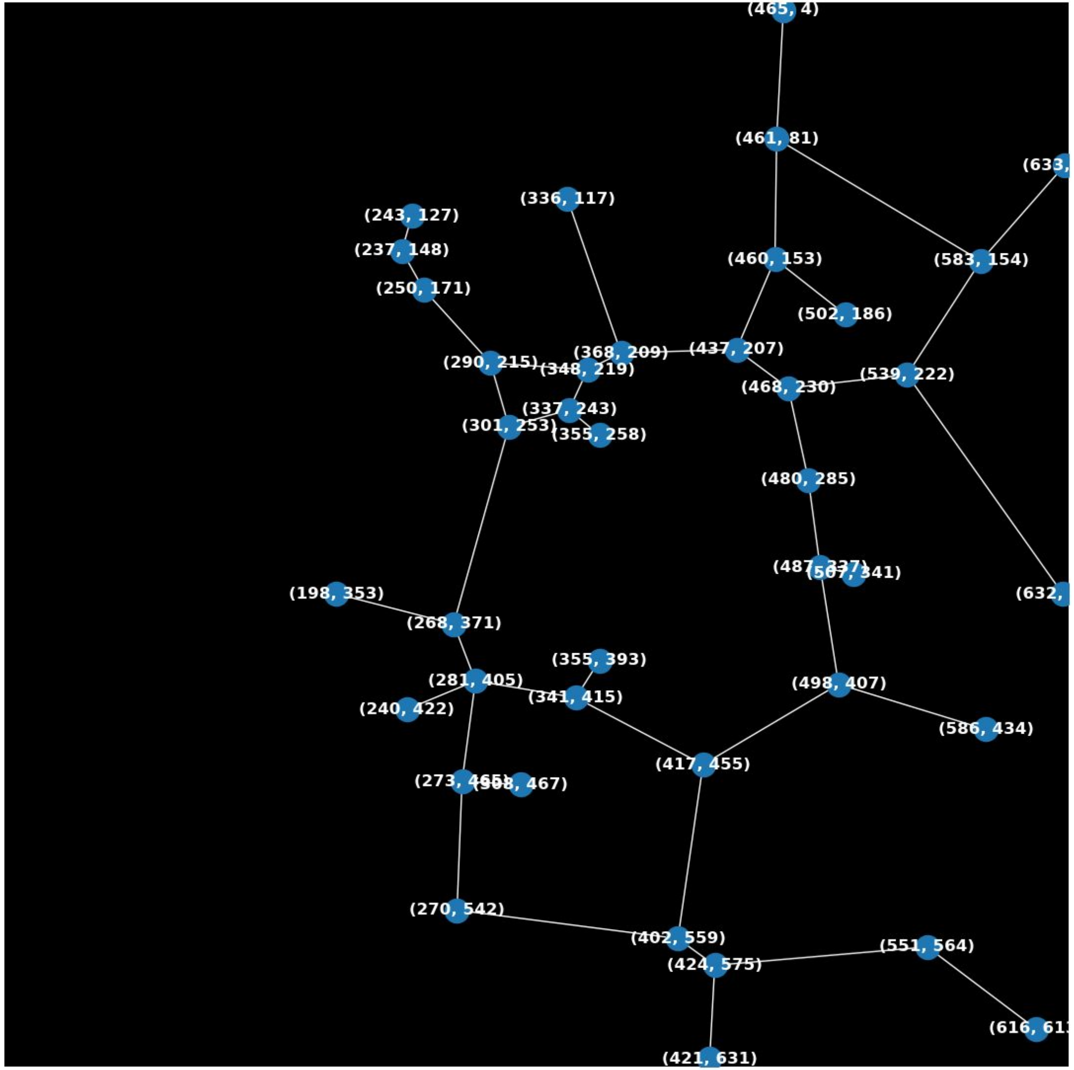
MIN_NODE_COUNT=3

NEAR_NODE_TOL= 20



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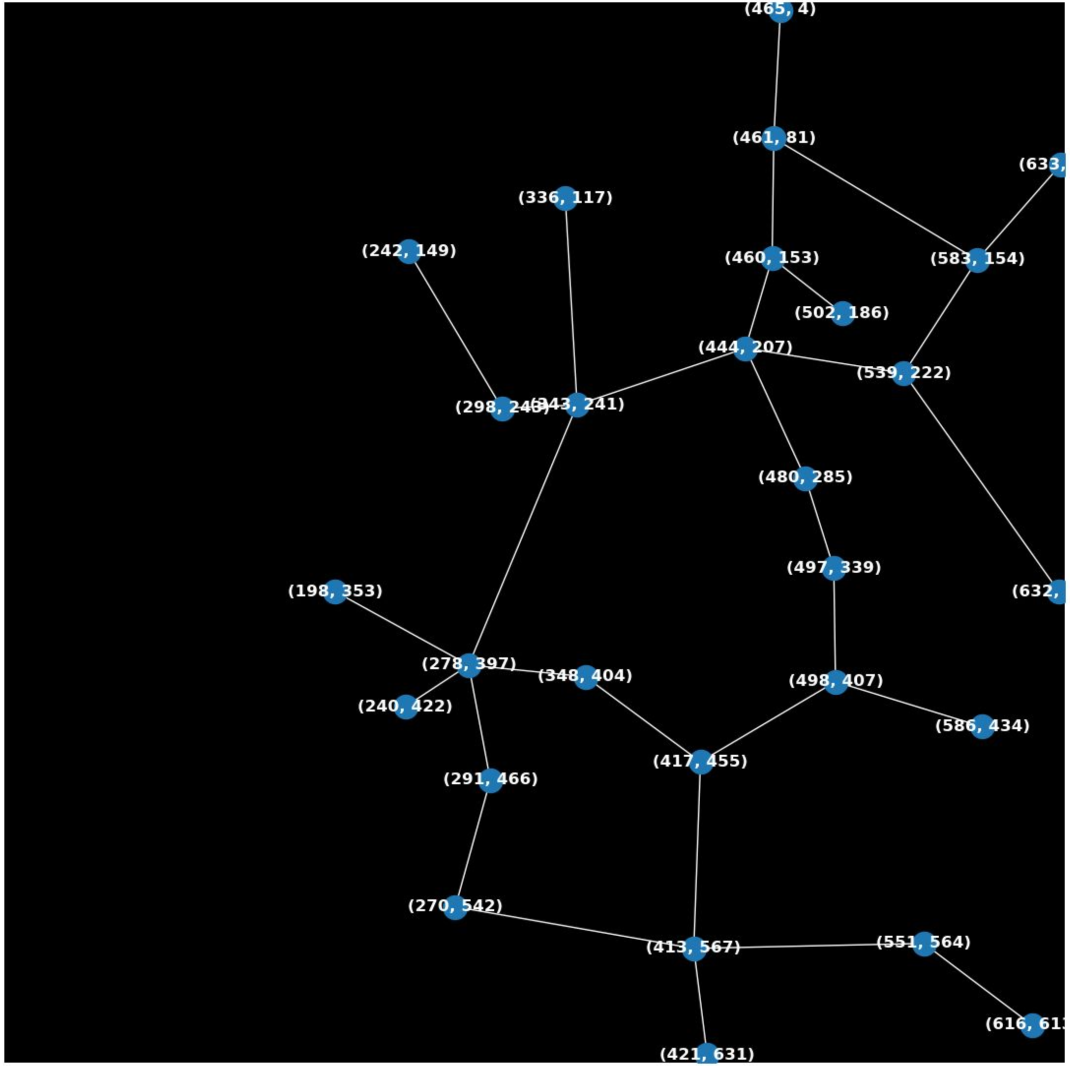
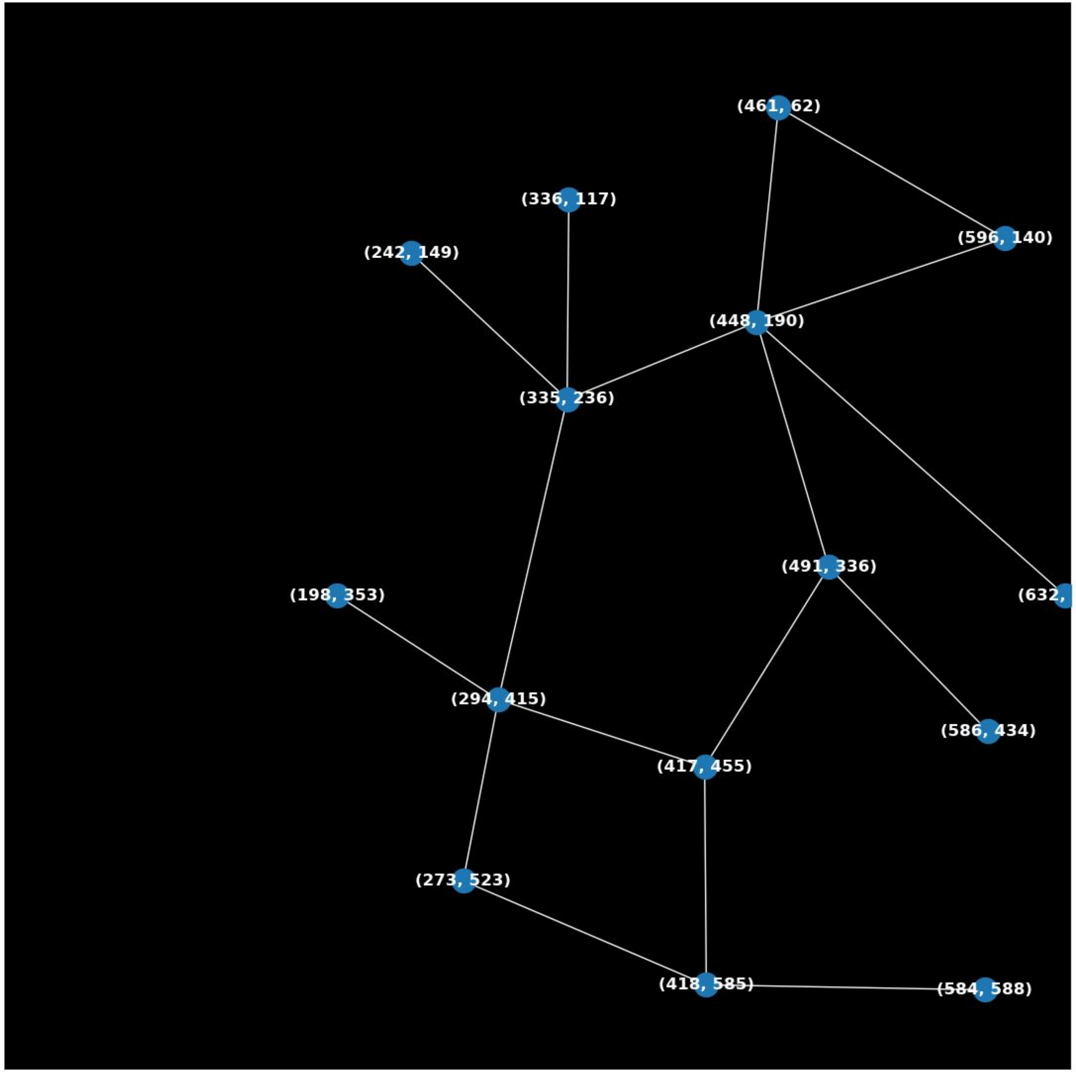
LENGTH_TOL=3
MIN_NODE_COUNT=3

NEAR_NODE_TOL= 40



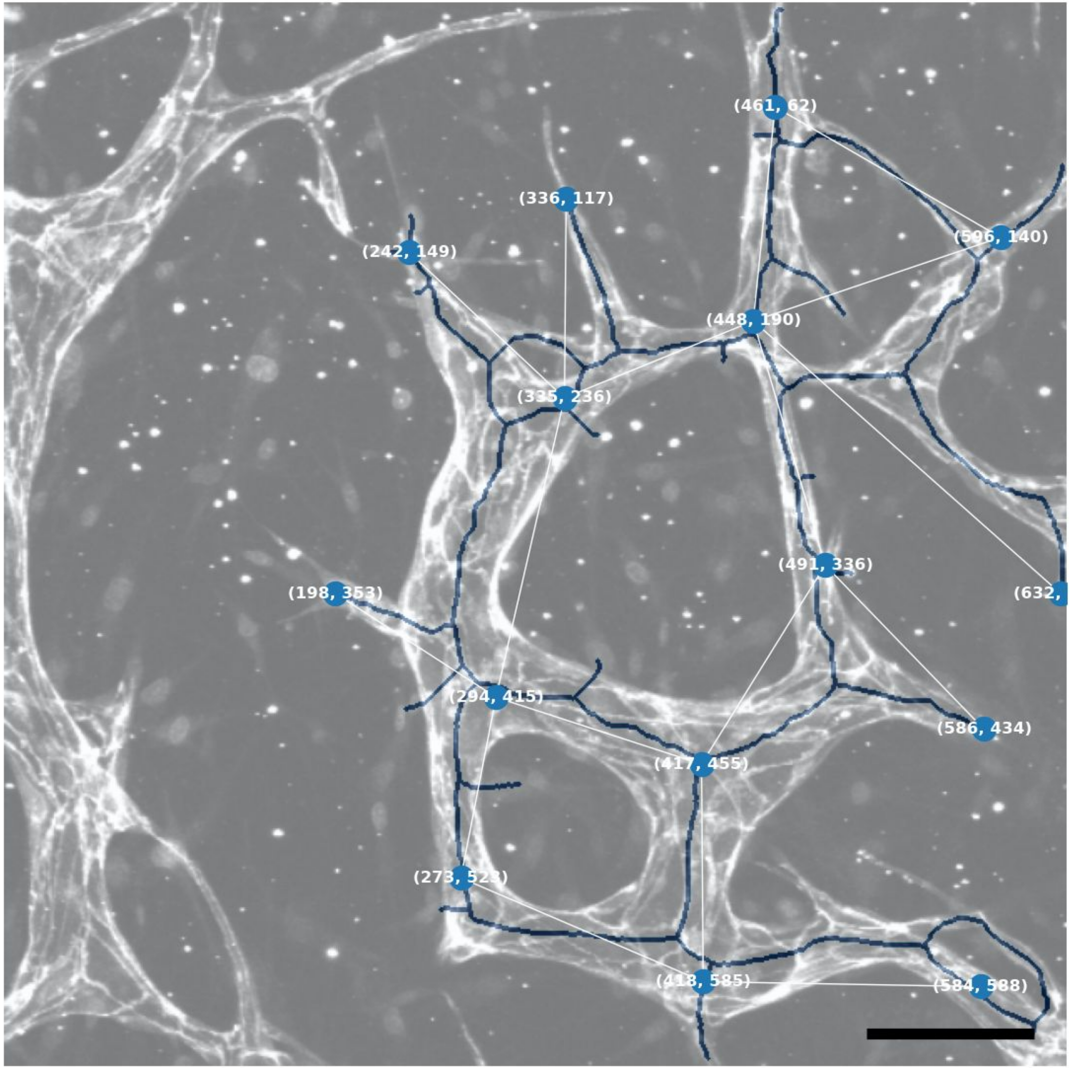
CONNECTED_NETWORK=1
LENGTH_TOL=3
MIN_NODE_COUNT=3

NEAR_NODE_TOL= 80



CONNECTED_NETWORK=1
LENGTH_TOL=3
MIN_NODE_COUNT=3

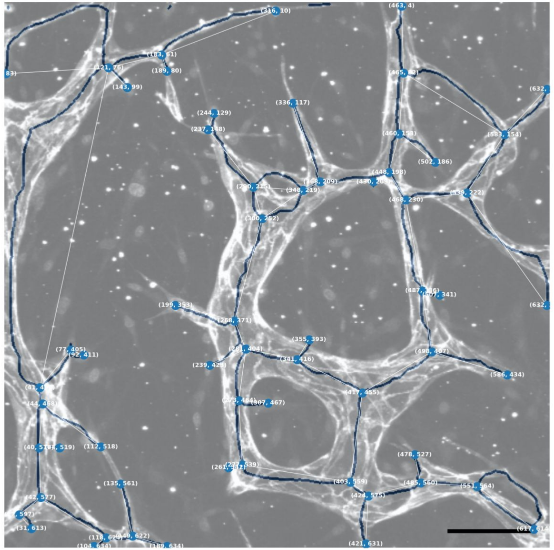
NEAR_NODE_TOL= 80



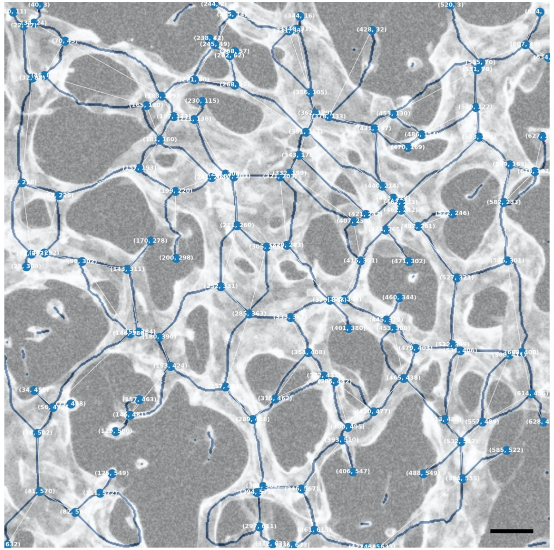
Numerical Summary

Graph Characteristic	Sample A	Sample B
Number of branch points	34	103
Number of end points	27	33
Total length μm	4783.2	18804.1
Total surface area μm^2 (assumes circular vessels)	477455.3	4450488.0
Total volume μm^3 (assumes circular vessels)	5057376.9	122078184.2
Average branch length μm	75.9	108.7
Average branch surface area μm^2	7578.7	25725.4
Average branch volume μm^3	80275.8	705654.2
Average branch radius μm	17.3	45.0
Average fractal dimension	1.023	1.0213
Average contraction factor	0.9106	0.9144
Average node connectivity	1.1949	1.6339

$$\text{contraction factor} = \frac{\text{displacement}}{\text{length}} \qquad \text{fractal dimension} = \frac{\ln(\text{length})}{\ln(\text{displacement})}$$



(a) Sample A: Scale 100 μm



(b) Sample B: Scale 100 μm

NetworkX Algorithms

Average node connectivity	1.1949	1.6339

Node Connectivity

- The minimum number of nodes that must be removed (along with their incident edges) to disconnect to non-adjacent nodes

- For averaging, we do not consider the trivial value of 0 (nodes already disconnected)

`closeness_vitality`(*G, node=None, weight=None, wiener_index=None*) [source]

Returns the closeness vitality for nodes in the graph.

The *closeness vitality* of a node, defined in Section 3.6.2 of [1], is the change in the sum of distances between all node pairs when excluding that node.

`edge_betweenness_centrality`(*G, k=None, normalized=True, weight=None, seed=None*) [source]

Compute betweenness centrality for edges.

Betweenness centrality of an edge e is the sum of the fraction of all-pairs shortest paths that pass through e

$$c_B(e) = \sum_{s,t \in V} \frac{\sigma(s,t|e)}{\sigma(s,t)}$$

where V is the set of nodes, $\sigma(s,t)$ is the number of shortest (s,t)-paths, and $\sigma(s,t|e)$ is the number of those paths passing through edge e [2].

Extendability

- NetworkX algorithms will “just work.”
- Currently writing an interface to visualize the outputs

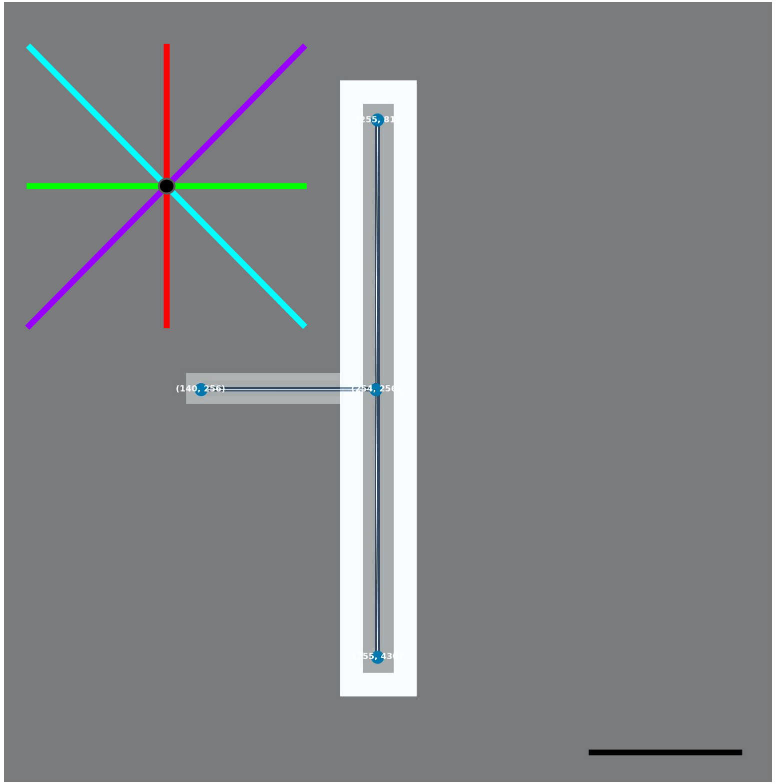
https://networkx.github.io/documentation/stable/reference/algorithms/index.html

Calculating Anisotropy

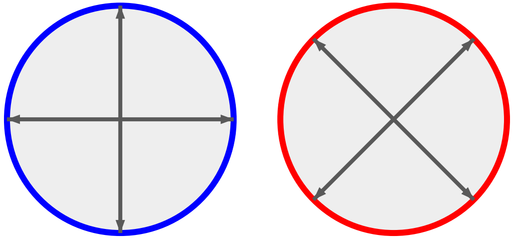
While generating graph edges, the SkeletonWalker code can only step along 1 of 4 axes.

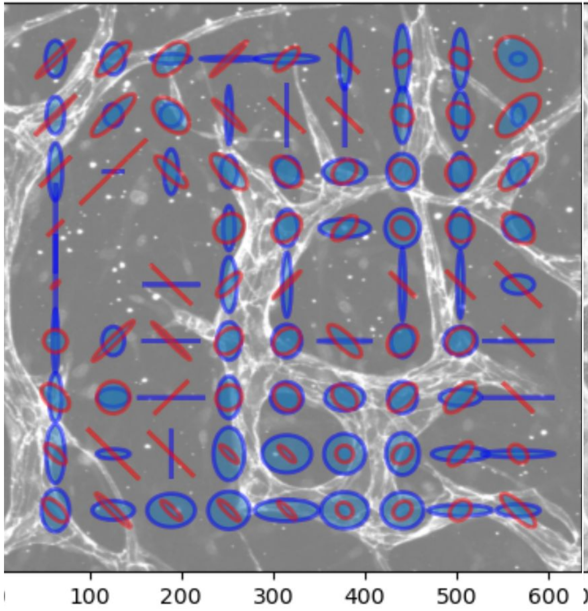
Tracks count of each type of (scaled) step.
Weights based on vessel radius.

Direction	Unweighted	Weighted
Percent y-axis	0.7487	0.8649
Percent x-axis	0.2452	0.1277
Percent y=-x axis	0.0031	0.0037
Percent y=x axis	0.0031	0.0037
Max	0.7487	0.8649

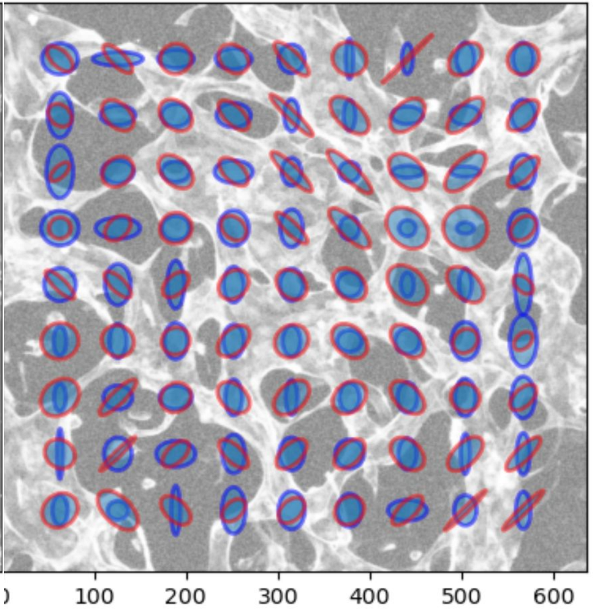


Visualizing Anisotropy





(a) Sample A



(b) Sample B

Direction	Sample A Unweighted	Sample A Weighted
Percent y-axis	0.3188	0.3334
Percent x-axis	0.2514	0.2421
Percent y=-x axis	0.234	0.2052
Percent y=x axis	0.1958	0.2192

Direction	Sample B Unweighted	Sample B Weighted
Percent y-axis	0.2716	0.2701
Percent x-axis	0.2067	0.2025
Percent y=-x axis	0.269	0.2873
Percent y=x axis	0.2527	0.2400

2.5D Mesh Representation

What and why?

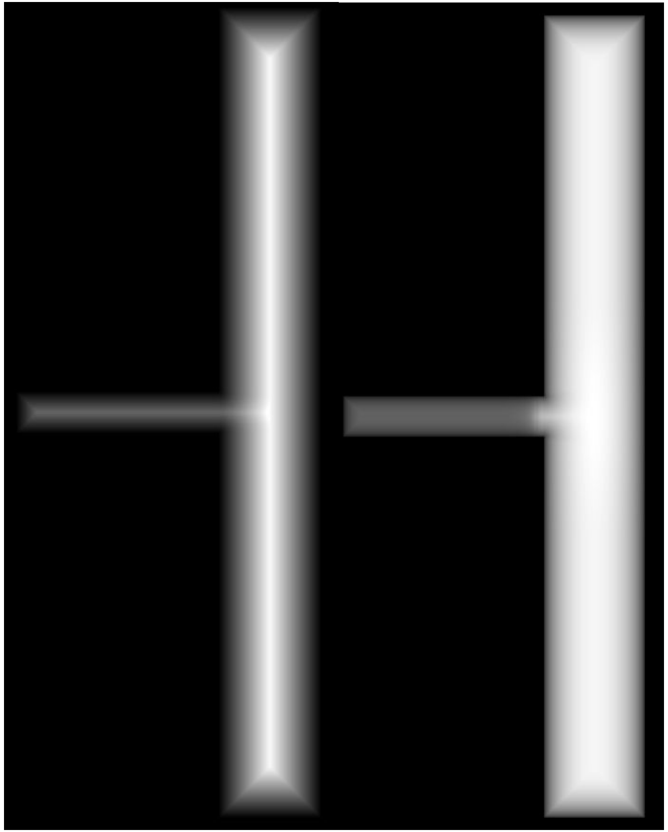
- 3D representation generated from a 2D representation
- Lightweight, robust, and provides some 3D data

Distance Transform + Skeleton → Z-Depth
i.e. linear → quadratic

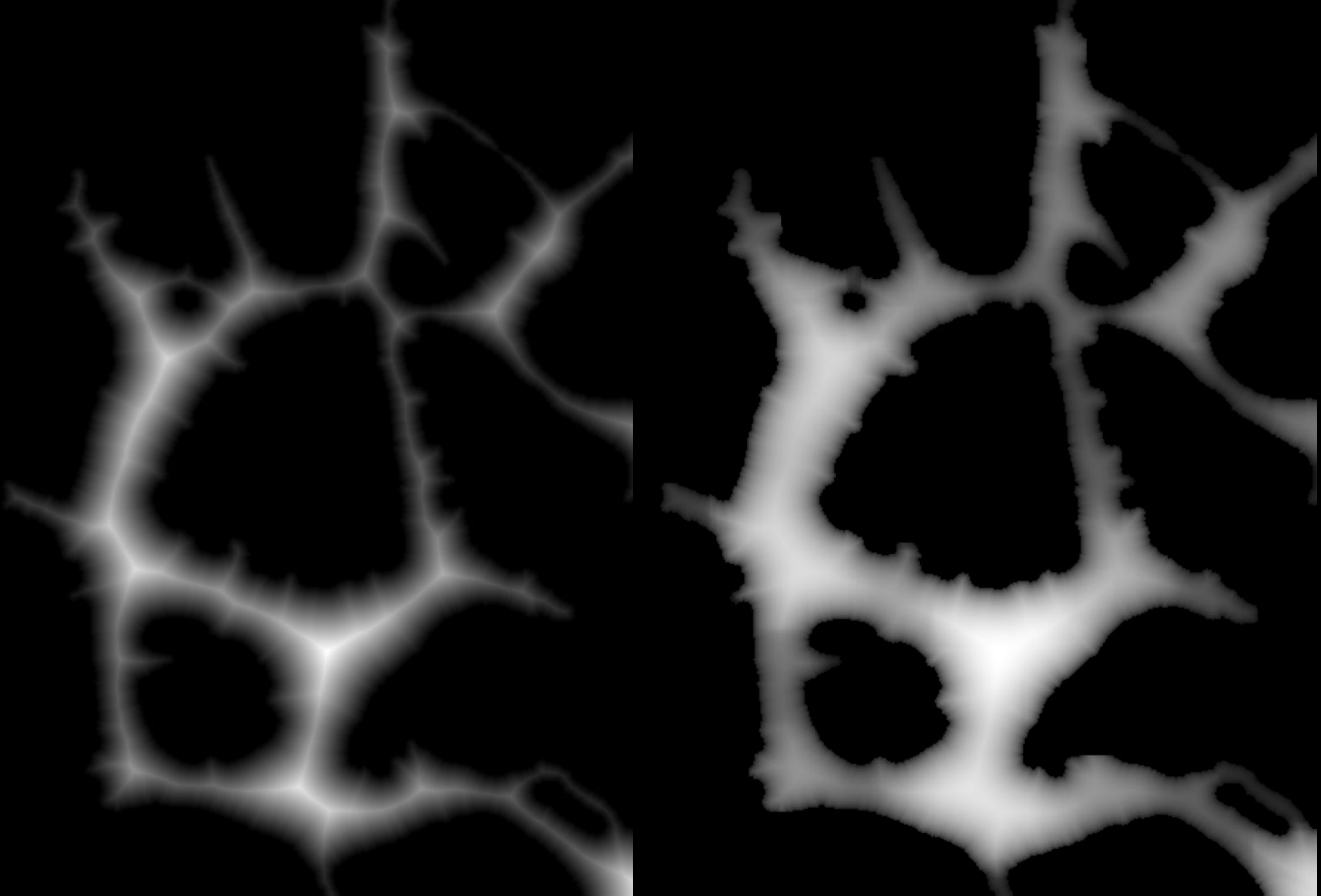
$$\text{distVal} = \text{distance transfrom} \times \text{skeleton}$$

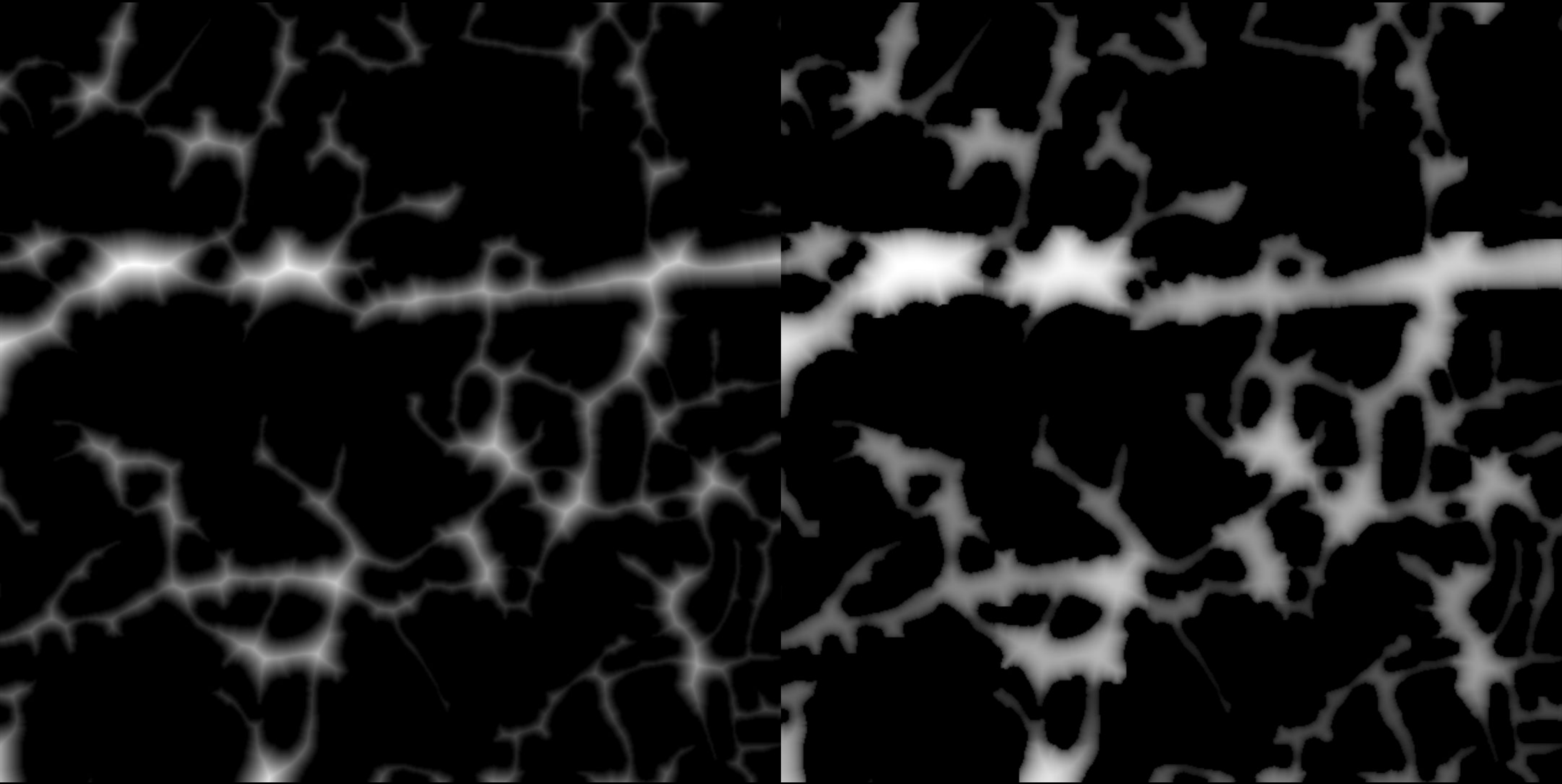
$$\text{distVal} \times \text{distVal neighborhood}$$

$$\text{scaled} = \sqrt{(\max - \min)^2 - (\max - \text{original})^2} + \min$$



Left: Euclidean distance transform
Right: Mapped z projection depths





Projecting Into 3-Space and Meshing

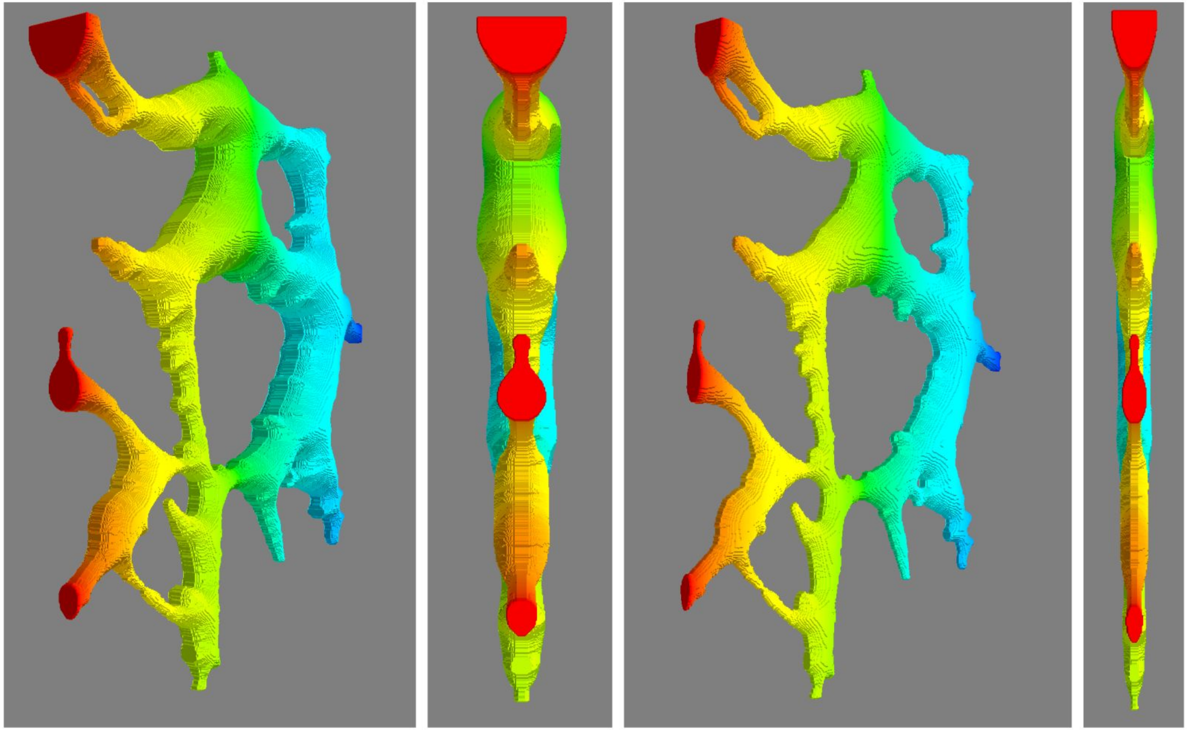
Projecting

For each pixel in the z-depth filtered image, the *n* voxels above and below the image plane are included within the lumen

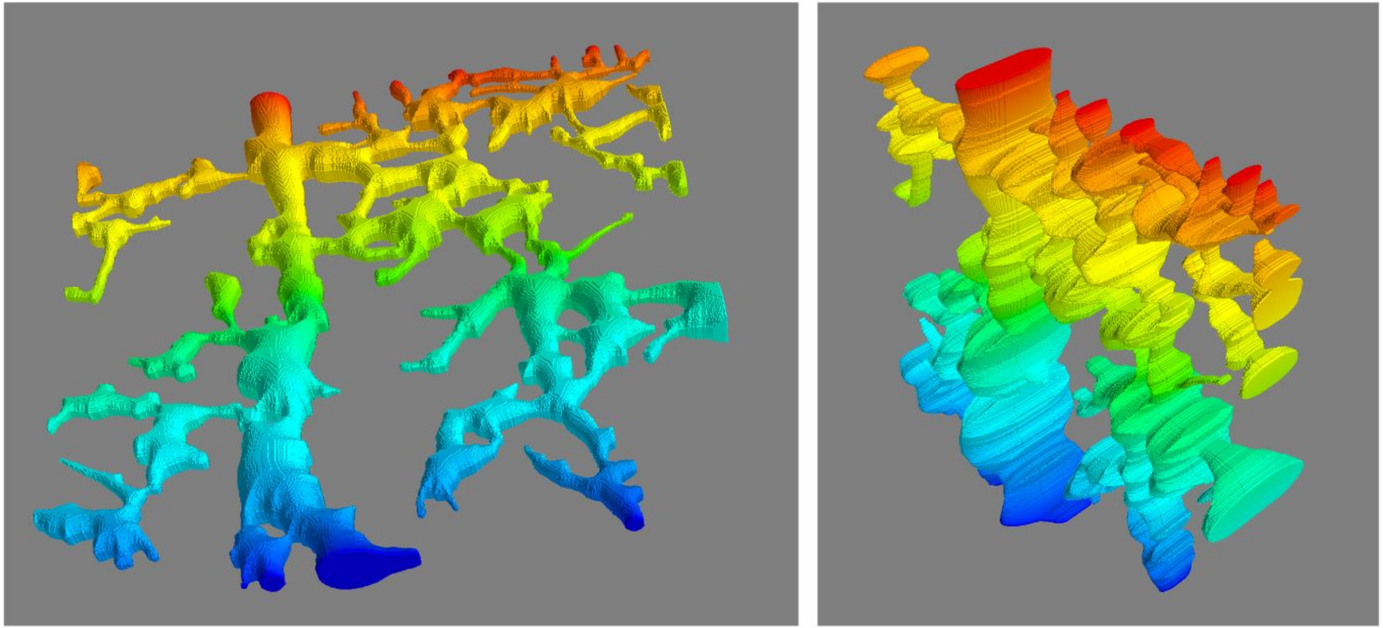
n = the pixel intensity

Optional

A scalar can be premultiplied to the original distance transform to change the lumen cross section from circular to ellipsoidal.



Limitations



- Poor representation of highly three-dimensional or irregular networks
- Overconnects vessels (appear to intersect but rather cross in separate z-planes)

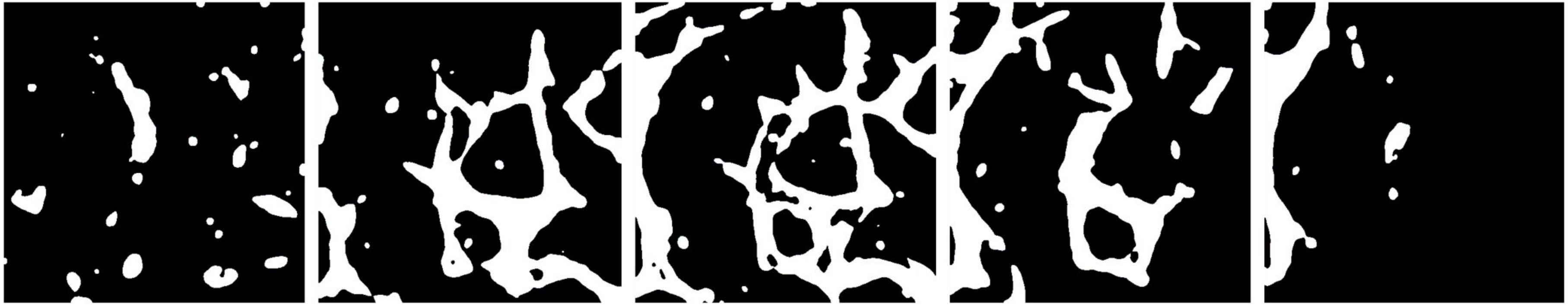
3D Segmentation

Goal

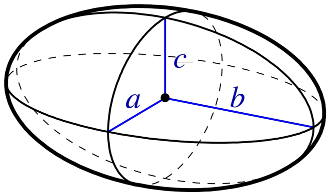
- Segment each plane individually and interpolate between planes.

Major Implementation

- 2D Segmentation steps + a 3D local thresholding prior (to utilize information from other z planes)

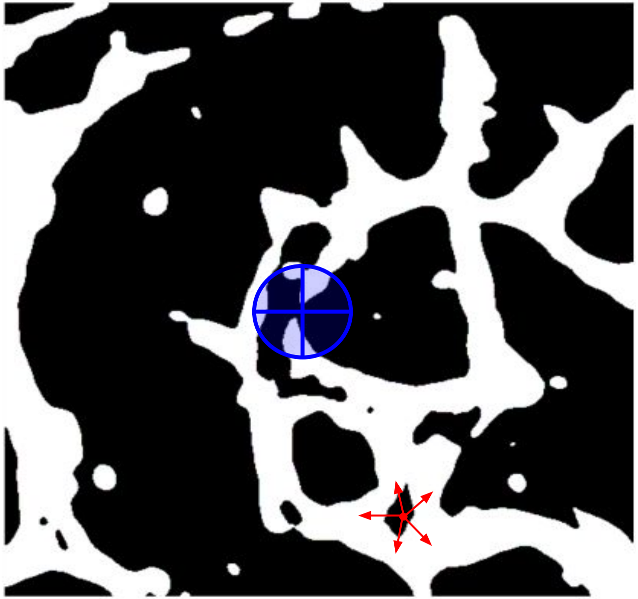


Lumen Filling:



Ellipsoid Octants

- Generate ellipsoidal structuring filter and subdivide it into 8 regions.
- From within potential lumen holes, determine percentage of voxels in each octant that are bright.
- Decrease the size of the ellipsoid and re-filter.
- **Finite range action. Good for sealing wall breaks.**



Ray Casting

- From within potential lumen holes, cast out rays in three dimensions.
- Continue the ray propagation until it encounters a bright voxel (part of the lumen) or the image wall (ray escaped).
- Threshold the voxel based on percentage of rays that escape.

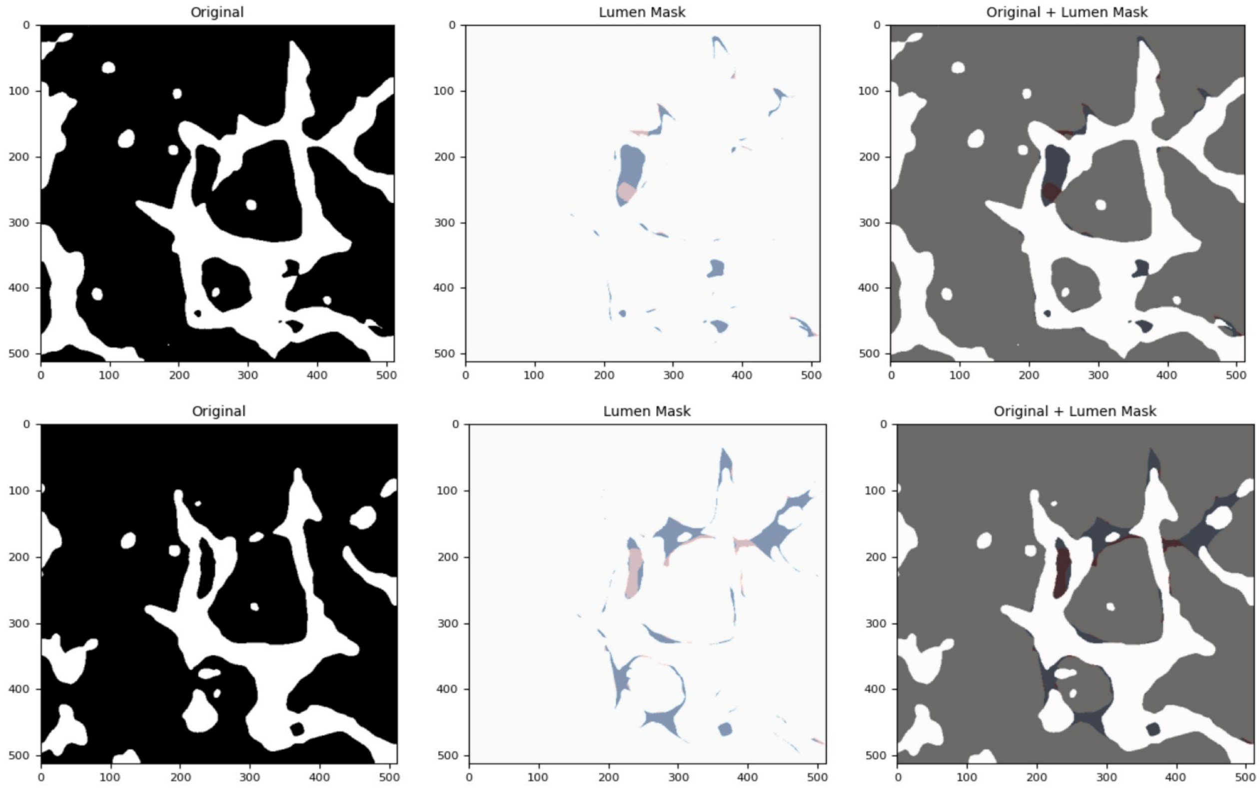
- **Infinite range action. Good for filling large, mostly sealed holes.**

Ellipsoid Octants and Ray Casting Effects

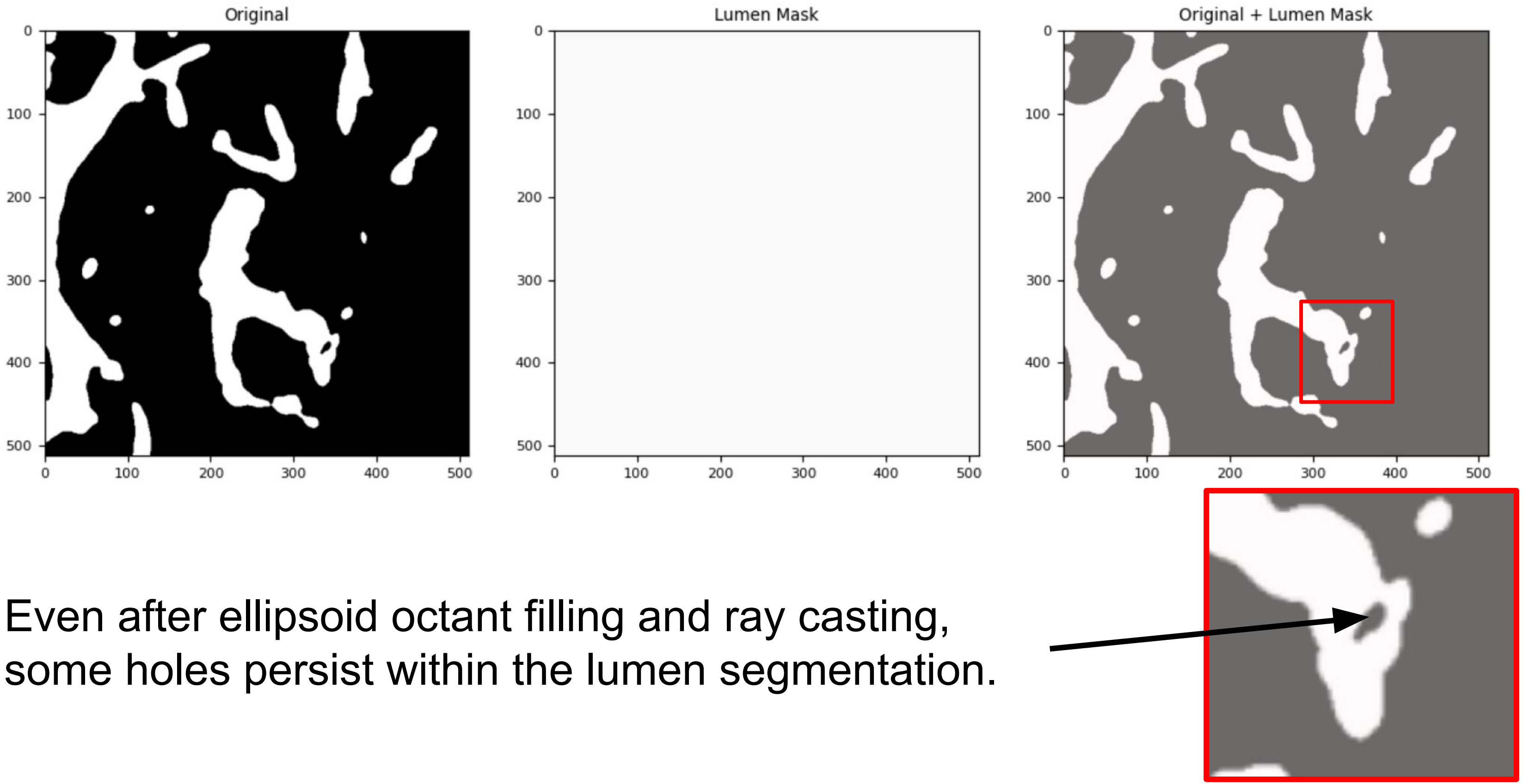
First: Ellipsoid Octant filling
blue

Second: Ray casting filling
red

Prior: Binary Lumen Mask



Lumen Filling: Not Complete



Even after ellipsoid octant filling and ray casting, some holes persist within the lumen segmentation.

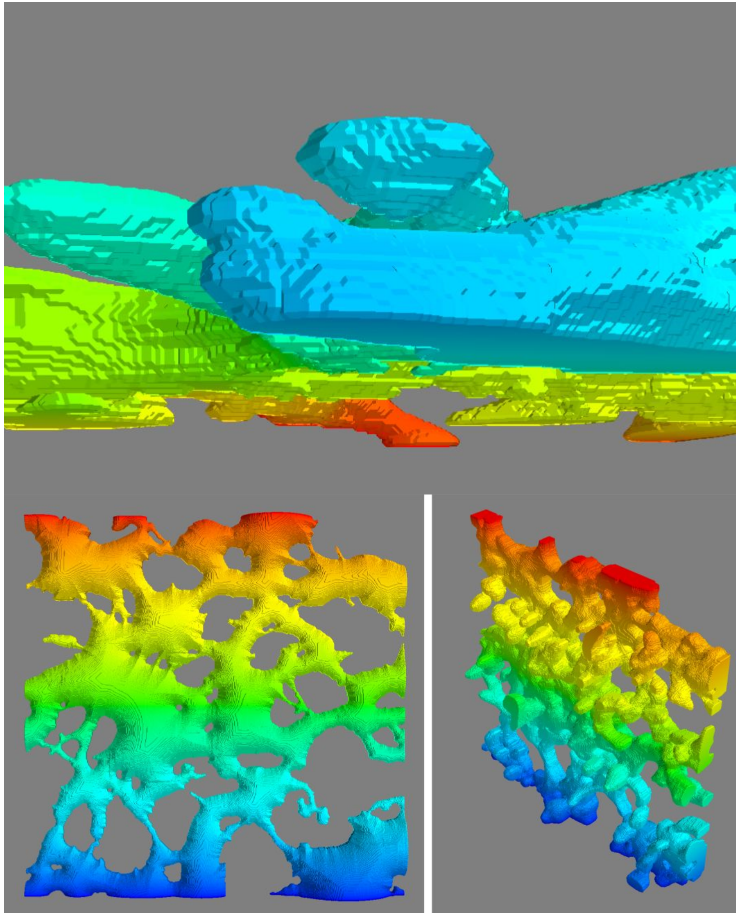
Final Steps

Lumen Filling: Iteratively Meshing

- The meshing procedure (marching cubes) identifies iso-surfaces within a 3D volume.
- Lumen holes are dark voxels surrounded by bright voxels and therefore can be represented as a surface.
- Mesh the 3D image → Identify sub-surfaces → Fill the 3D image at the hole location → Repeat

Interpolation and Scaling

- The resultant 3D image spreads its planes with respect to the global unit.
- An interpolation algorithm then generates intermediate slices that need to be filled (slices skipped during the discrete z-stepping of the microscope)
- *xy* planes are then rescaled to respect the global unit

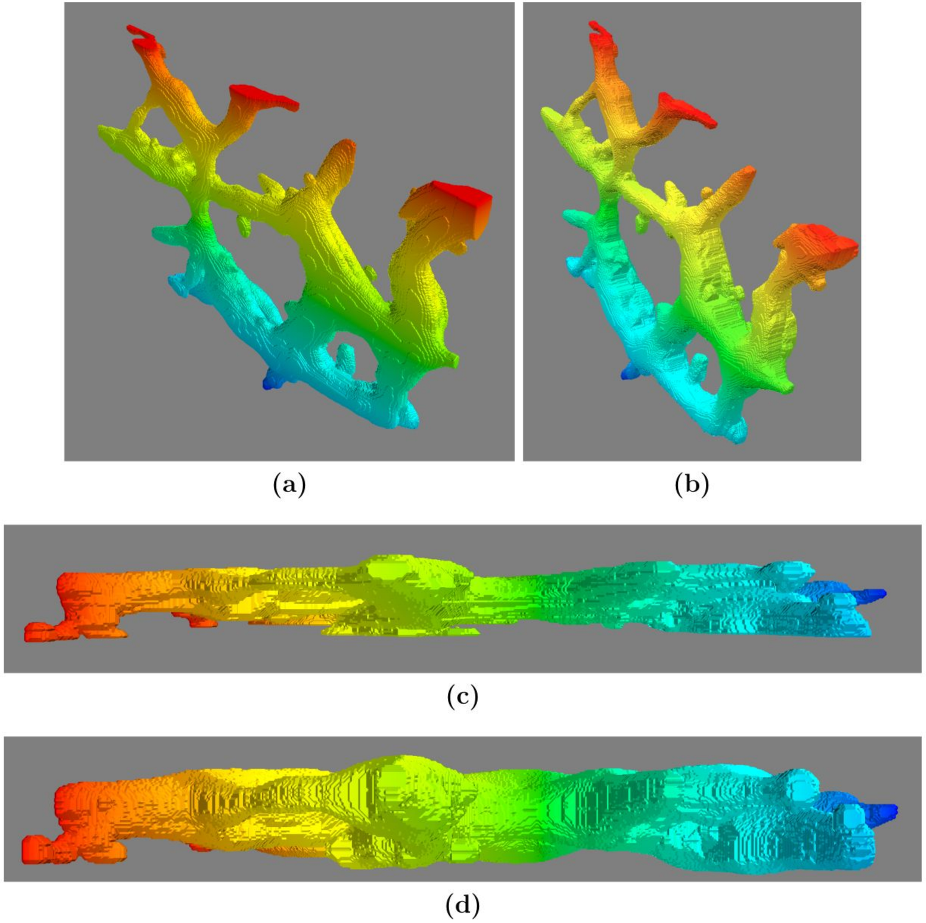


Comparison between 2.5D and 3D meshing

Final Steps

Repairing Top and Bottom Planes

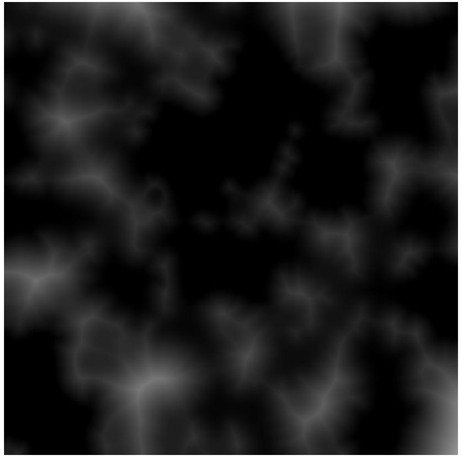
- The top and bottom planes of the segmentation are often flat (failure to threshold the thin sections)
- Option to use the 2.5D projection algorithm to repair these surfaces.

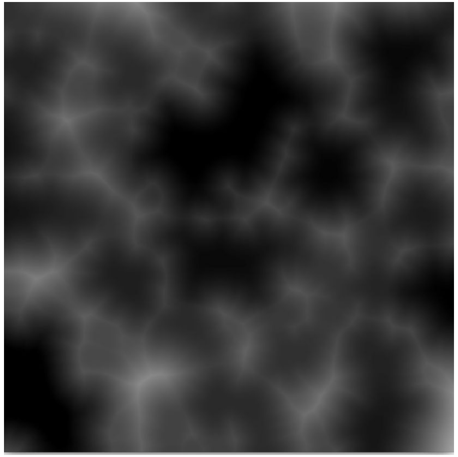


Shows optional top and bottom plane repair

Volume Data

Source	25D	Honest 3D	Rounded 3D prefactor-0.5
Connected	True	True	True
Bounding box (z, x, y) (μm)	(238, 1270, 1270)	(232, 1270, 1270)	(302, 1270, 1270)
Total volume μm^3	91363744	101779440	109411016
Vascular density ($\frac{VesselVolume}{TotalVolume}$)	0.2380	0.2651	0.2246
Avg dist from vessel wall μm	45.7	46.4	48.2
Max dist from vessel wall μm	183.0	216.8	230.5
Avg max dist from vessel wall per z-plane μu	162.0	166.3	172.5





Example slices from the 3D distance transform of the non-vascularized region of Sample B.

Validation

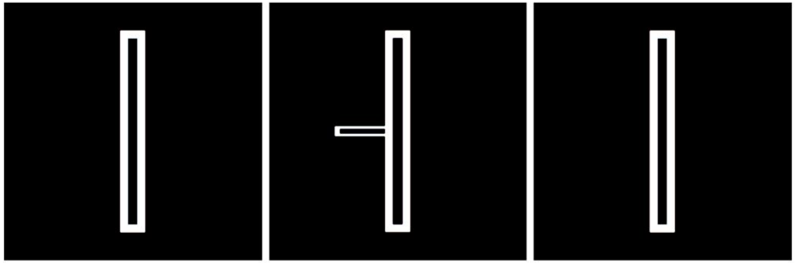
Synthetic Data

- A structure of known volume was created and represented as hollow slices at every over z-plane.
- The segmentation reconstructed the geometry and calculated a volume that was **2 percent** below the known.

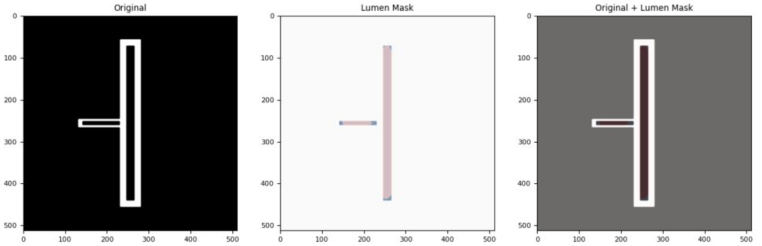
Cross-Validation of Sample B

- Standard deviation ~10.5 % of the mean

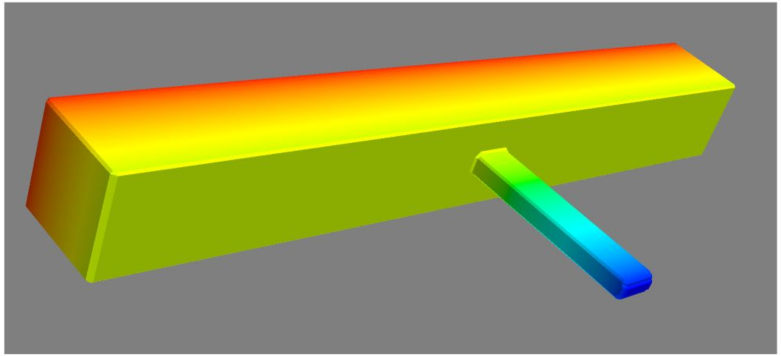
Source	2.5D	Honest 3D	Rounded 3D	Graph
Volume	91363744	101779440	109411016	122078184
Mean	106158096		**Std.**	11203528



(a) Slice 5 (b) Slice 25 (c) Slice 45



(d) Lumen filling



(e) Resulting 3D Segmentation

Characterizing Fluid Flow Forces

Stokes Flow

$\rho =$ fluid density

$\mu =$ fluid viscosity

$u =$ fluid velocity

$D =$ vessel diameter

$\mathbf{f} =$ external force

Reynolds Number, $\mathrm{Re} = \dfrac{\rho u D}{\mu}$

- Ratio of inertial flow forces and viscous flow forces.
- High Reynolds number flow are expected to be turbulent.
- Low Reynolds number flows are expected to be laminar.
- Transition occurs near $\mathrm{Re} = 2300$.
- Flow within the MVNs is expected to be laminar.

$\max(u) \approx 2.2\ \mathrm{mm/s}$	Literature
$\max(D) < 300\mu\mathrm{m}$	Observed
$\min(\mu) \approx 0.65 \times 10^{-3}\,\frac{\mathrm{kg}}{\mathrm{ms}}$	Water
$\max(\rho) \approx 1100\,\frac{\mathrm{kg}}{\mathrm{m\hat{}3}}$	Blood

$\boxed{\max(\mathrm{Re}) \sim 1 \times 10^{-4}}$

Stokes Flow (Incompressible)

- For extremely low Reynolds number flows, the inertial forces are negligible compared to the viscous forces.
- Navier Stokes equations reduce to the Stokes equations (linear in pressure and velocity).

$$\mu \nabla^2 \mathbf{u} - \nabla p + \mathbf{f} = 0$$

$$\nabla \cdot \mathbf{u} = 0$$

Max flow velocity from: KP Ivanov, MK Kalinina, and Yu I Levkovich. “Microcirculation velocity changes under hypoxia in brain, muscles, liver, and their physiological significance”. In: Microvascular research 30.1 (1985), pp. 10–18

BeatIt

“C++ code for heart biomechanics and more” - Dr. Simone Rossi

github.com/rossisimone/beatit

Stoke Flow Solver

Finite element solution to the Stokes equations.

Input: Tetrahedral Volume Mesh

- Pressure Boundary Conditions
- No-Slip Boundary Conditions
- Zero Traction Boundary Conditions

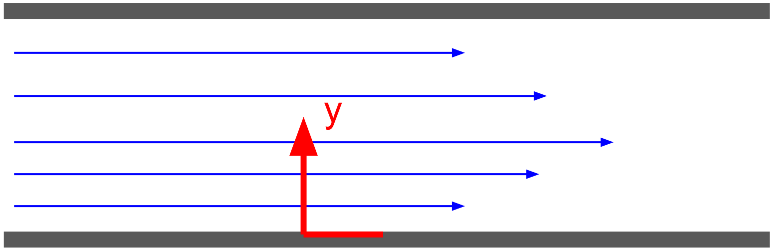
Outputs: Pressure and Velocity Fields

Calculating Wall Shear Stress

Wall shear stress is the shear stress in the layer of fluid next to the wall of a pipe (or in our case, a vessel).

$$\tau_w = \mu\left(\frac{\partial u}{\partial y}\right)_{y=0}$$

ParaView software be used to extract **velocity gradients** and **surface normls.**



Viscous stress tensor

$$\mathcal{T} = \mu(\nabla \mathbf{u} + (\nabla \mathbf{u})^T) \cdot \mathbf{n}$$

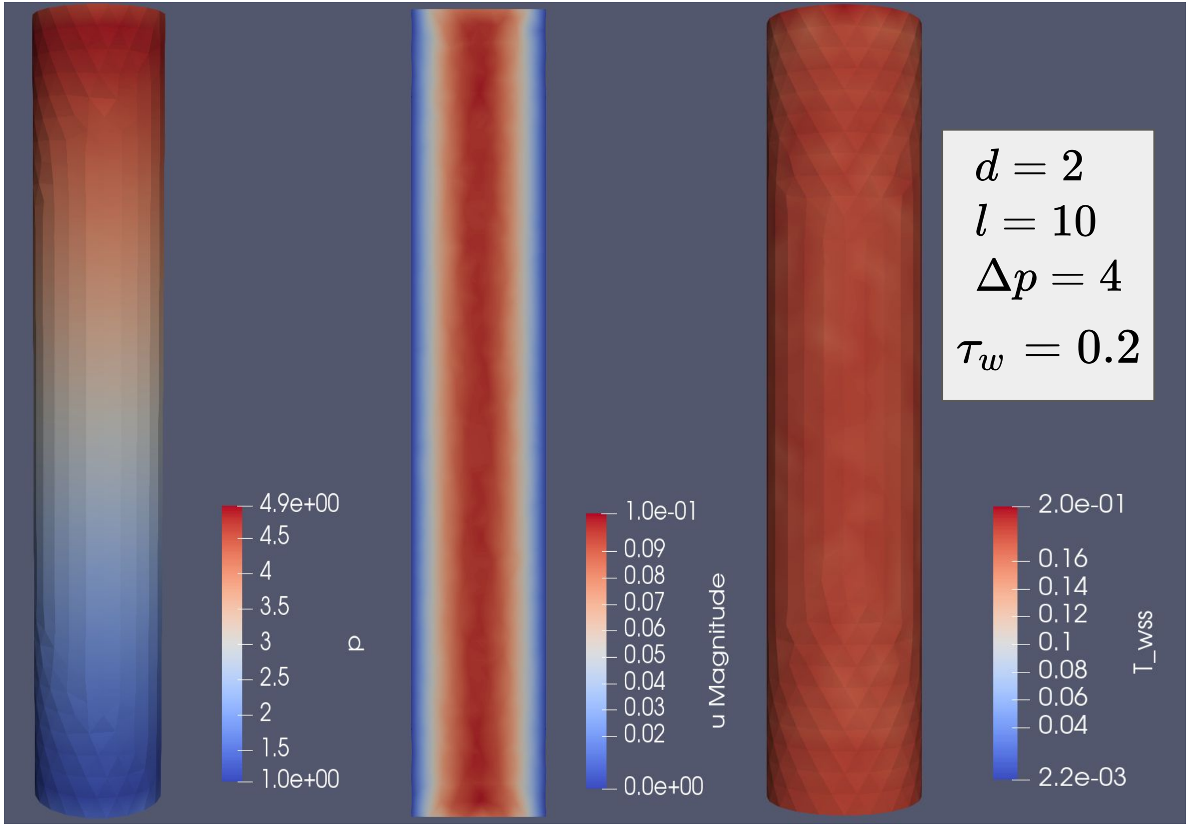
$$\tau_w = \mathcal{T} - (\mathcal{T} \cdot \mathbf{n})\mathbf{n}$$

Validating WSS Calculations

Pressure → Pressure

For a cylindrical pipe under laminar flow, WSS can be directly calculated.

$$\tau_w = \frac{d\Delta p}{4l}$$

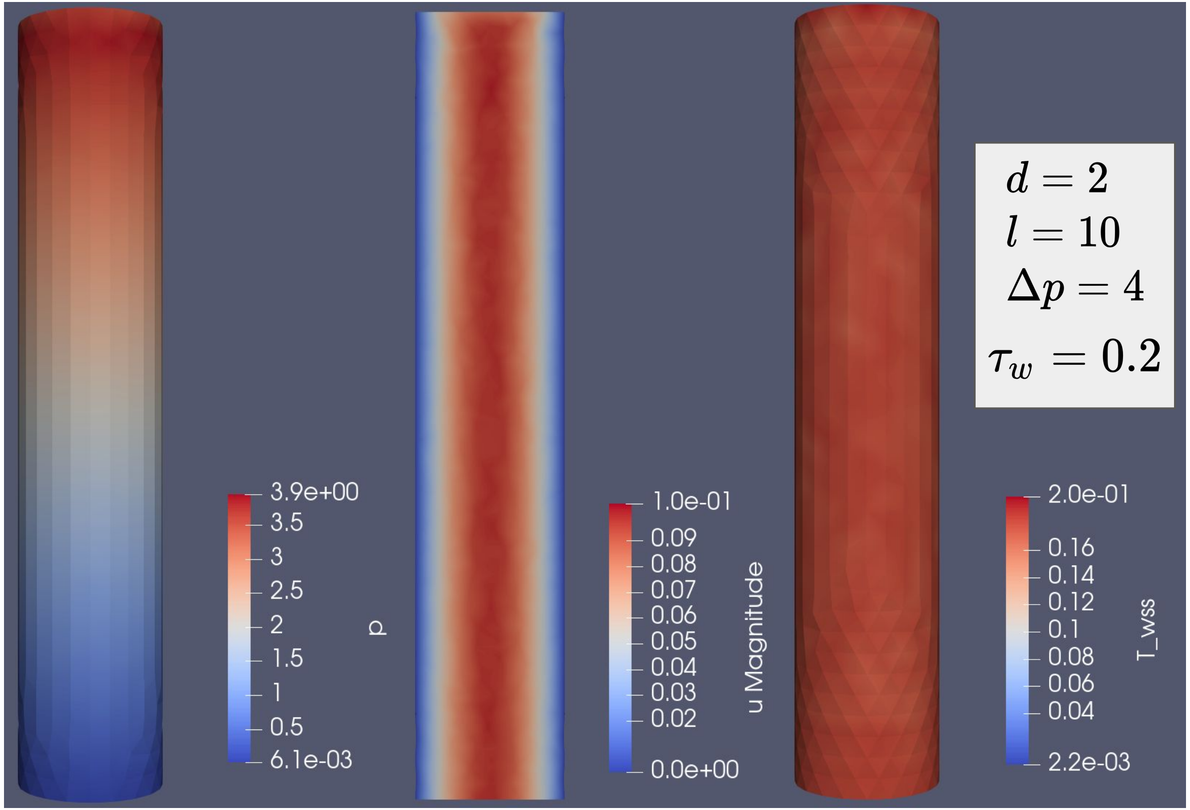


Validating WSS Calculations

Pressure → Zero Traction

For a cylindrical pipe under laminar flow, WSS can be directly calculated.

$$\tau_w = \frac{d\Delta p}{4l}$$



MVN Test

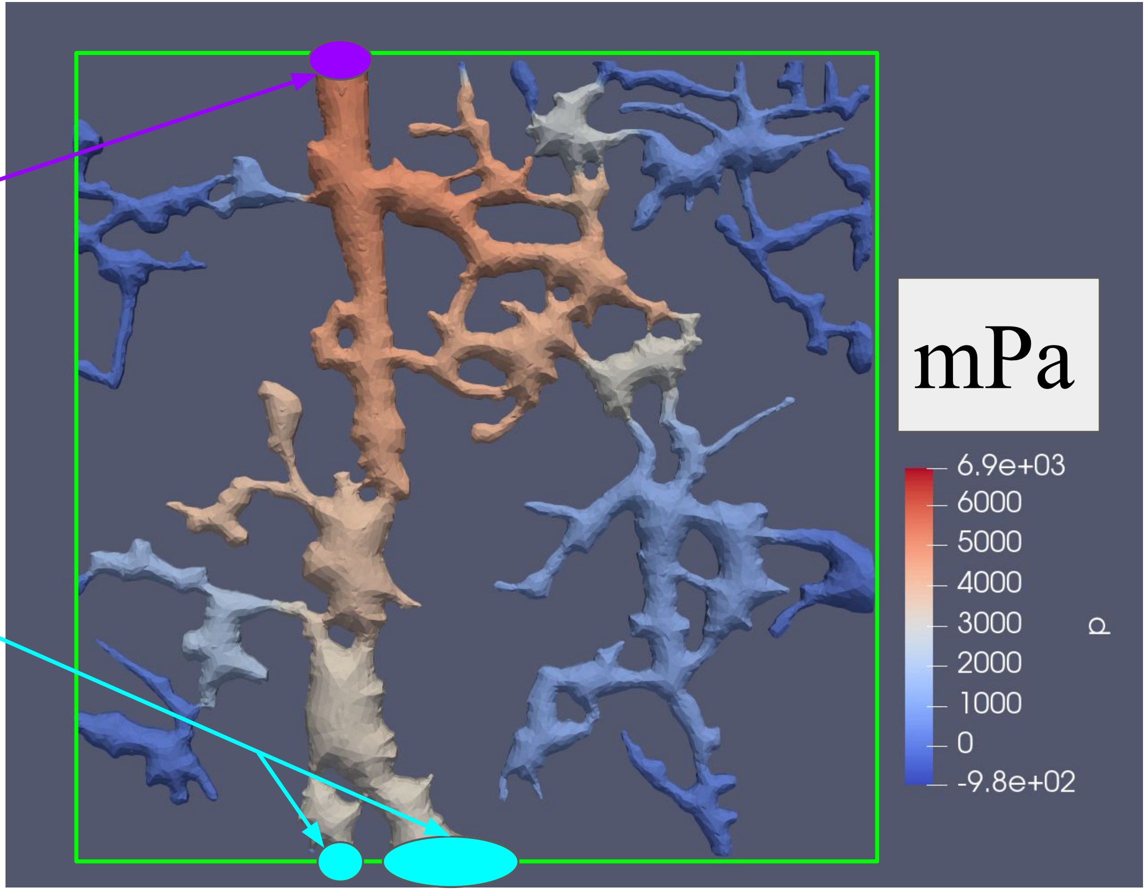
Pressure Inputs

5.0 Pa

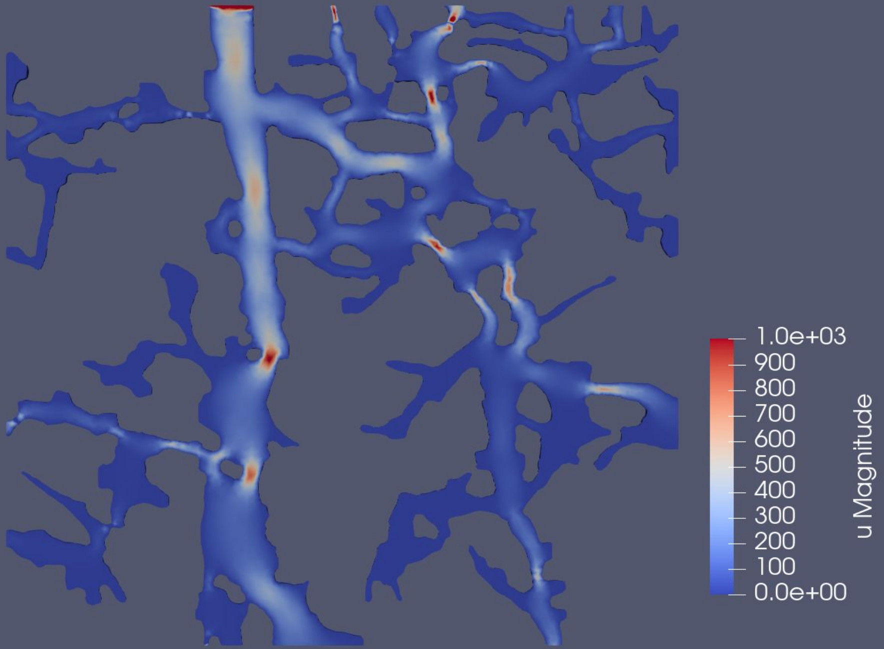
3.0 Pa

Zero Traction

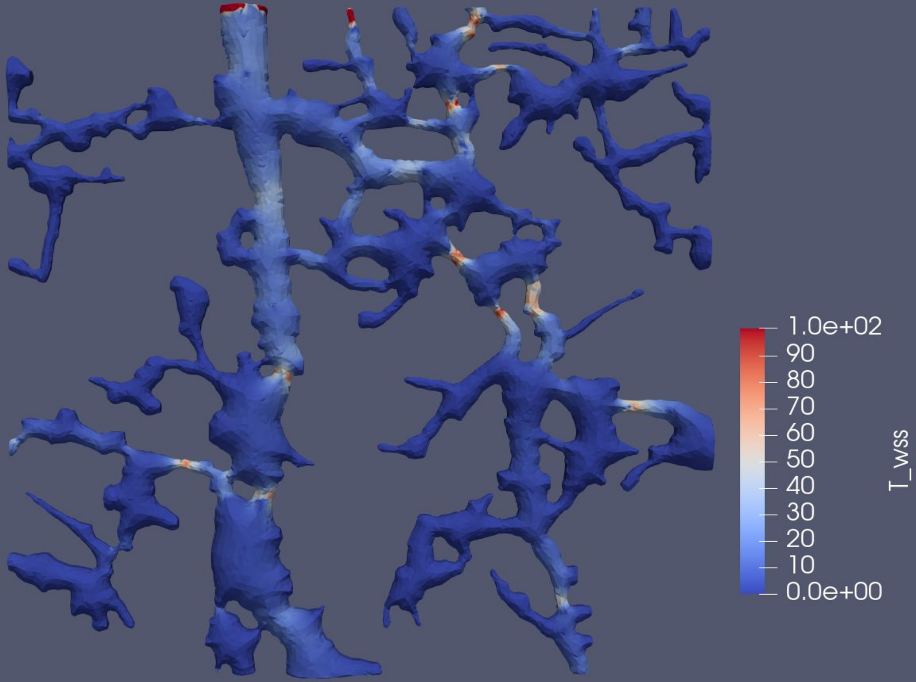
No-Slip Boundary



Velocity and WSS Outputs



Velocity Profile $\mu m/s$



Wall Shear Stress mPa

Using the Developed Methods

Preliminary Data Investigation

Nutrient Concentration

Testing if low nutrient conditions influence vascular network morphology.

- “Regular” Media: EGM2 media from Promocell with added nutrients and growth factors.
- “Basal” Media: No additions nutrients / growth factors
  - 50.0% serum depleted media – cells cultured in 50.0% regular media + 50.0% basal media
  - 25.0% serum depleted media – cells cultured in 25.0% regular media + 75.0% basal media
  - 12.5% serum depleted media – cells cultured in 12.5% regular media + 87.5% basal media

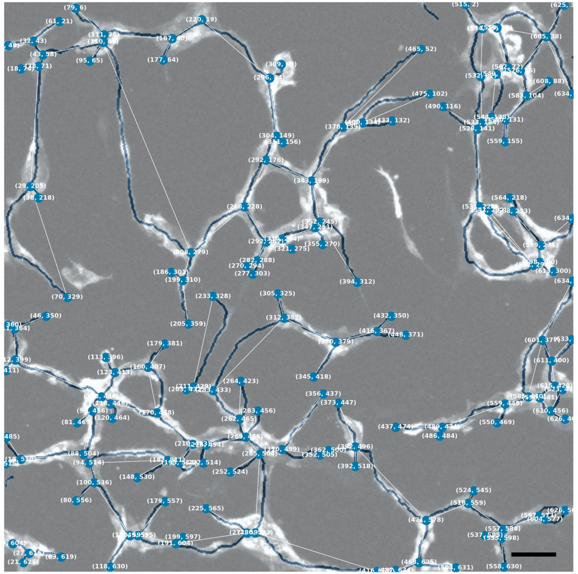
Static Growth

Testing if dynamic vs. static storage conditions influence vascular network morphology.

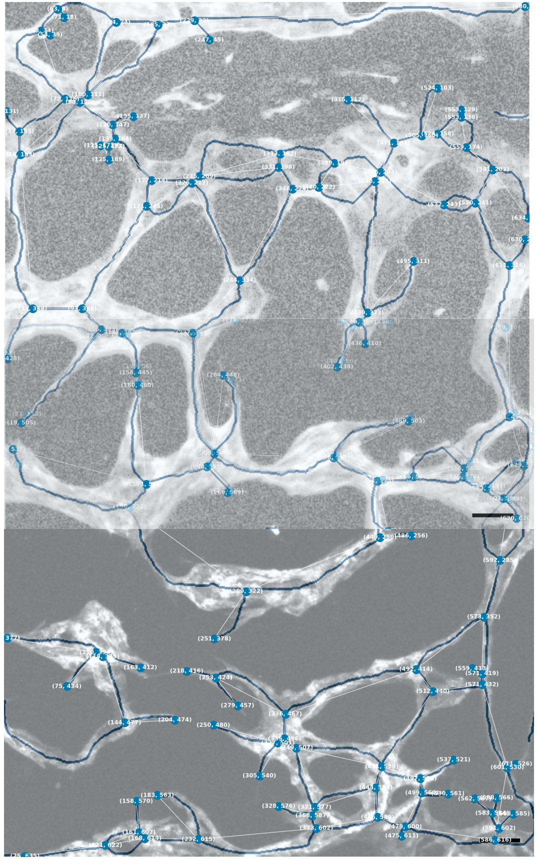
- Control: Devices were maintained on a rocker
- Static: Devices were not maintained on a rocker.

These networks were grown and imaged by Crescentia Cho.

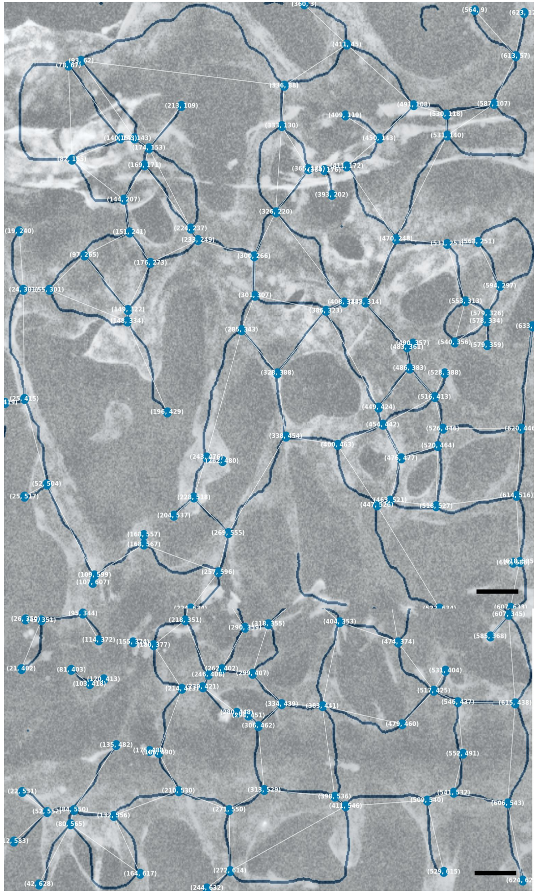
Nutrient Concentration



12.5% Serum



25% Serum



50% Serum

Increasing levels of nutrients

CONNECTED_NETWORK=0; MIN_NODE_COUNT=3;
NEAR_NODE_TOL=15; LENGTH_TOL=3

Nutrient Concentration Graph Data

The segment characteristic averages for the resultant graphs were fit linearly on the range

[12.5%, 50%]

using the SciPy stats package.

$|R| \approx 0$

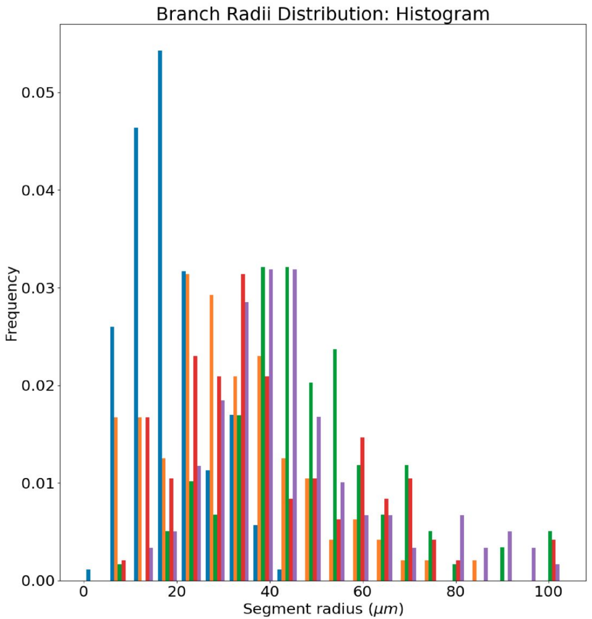
$|R| > 0.50$

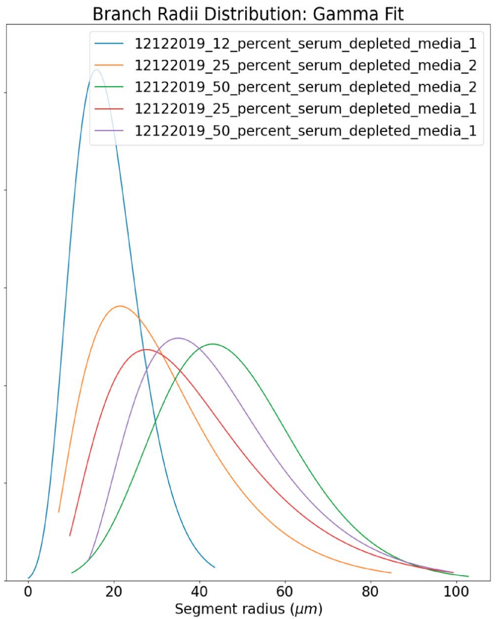
$|R| > 0.75$

$|R| > 0.90$

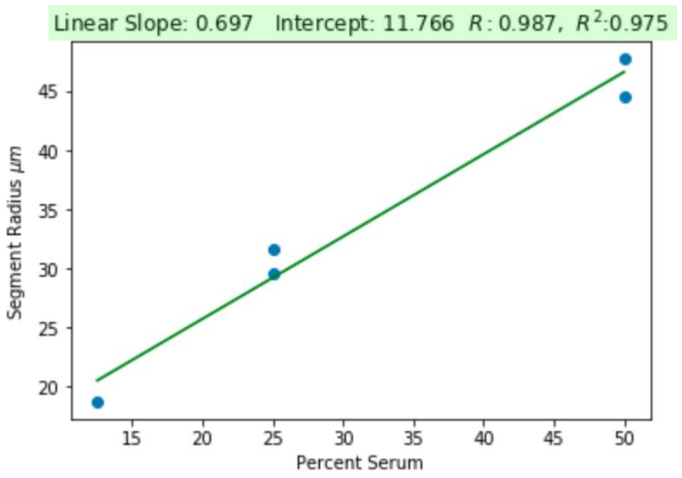
Characteristic	**12.5% Serum**	**25% Serum**	**25% Serum**	**50% Serum**	**50% Serum**	R	R^2
$\uparrow$ **Length**	91.0	145.0	135.6	138.8	133.7	0.583	0.340
$\uparrow$ **Surface Area**	9055	31035	23433	37302	33122	0.862	0.743
$\uparrow$ **Volume**	961223	739003	456894	984040	846587	0.892	0.796
$\uparrow$ **Radius**	18.7	29.5	31.6	47.8	44.6	0.978	0.975
$\downarrow$ **Fractal Dimension**	1.0251	1.0222	1.0203	1.0191	1.0205	-0.819	0.671
$\uparrow$ **Contraction Factor**	0.9042	0.9025	0.9128	0.9158	0.9124	0.753	0.568
$\uparrow$ **Connectivity**	1.1350	1.3002	1.0838	1.6582	1.6383	0.936	0.877
Unweighted Directionaliy	0.2667	0.2721	0.3504	0.2764	0.2895	-0.041	0.002
Weighted Directionality	0.2619	0.2888	0.366	0.2713	0.3004	-0.054	0.003

Nutrient Levels and Segment Radii





A cursory glance at the distributions of segment radii indicate the positive correlations is primarily due to a shifting of the distributions (rather than a re-shaping)



The high R^2 value indicates this linear fit may possibly be useful for producing networks with a specific desired average segment radius.

*Note: The histograms and distribution fits are automatically generated when mvn-analysis is run in batch mode.

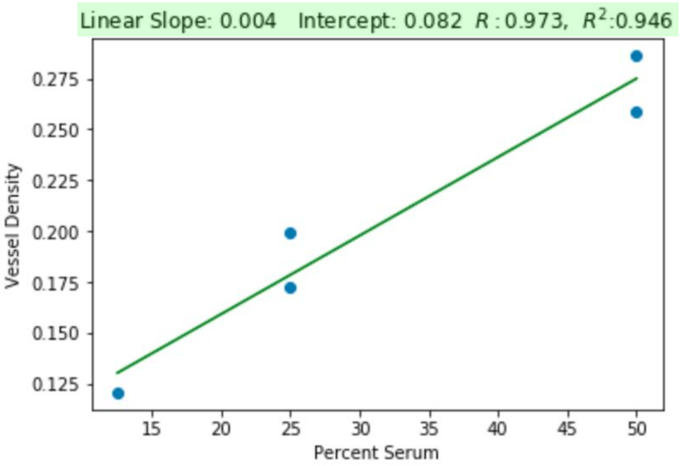
Nutrient Concentration Volume Data (2.5D Segmentation)

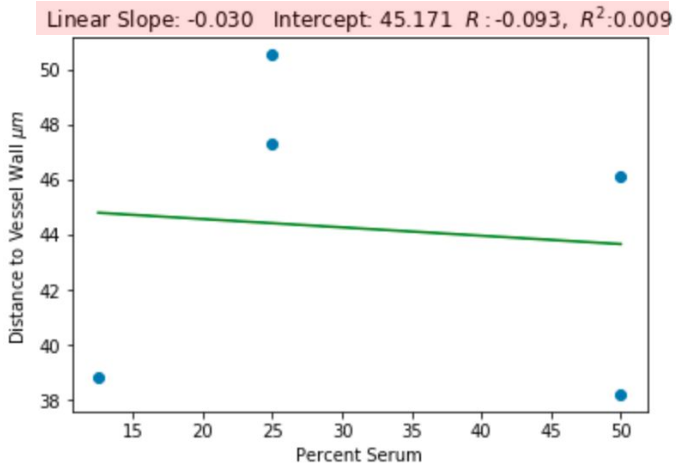
Avg. Volume Characteristic	12.5% Serum	25% Serum	25% Serum	50% Serum	50% Serum	R	R^2
Vessel Density	0.1207	0.1994	0.1723	0.2862	0.2589	0.973	0.946
Avg. Distance from a Vessel Wall	38.8	50.5	47.3	38.2	46.1	-0.093	0.009

- We can conclude with high certainty that vessel density (volume of vessels / vascularized volume) is **positively correlated** with percent serum (nutrient concentration) .

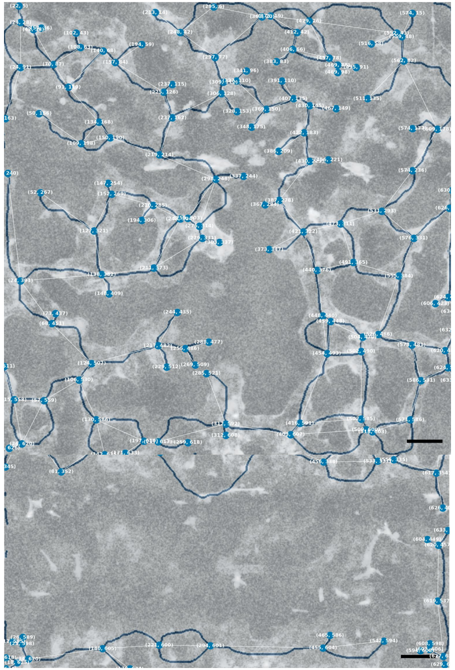
- However, we conclude **no significant correlation** between **average distance from a vessel wall** and percent serum (nutrient concentration).

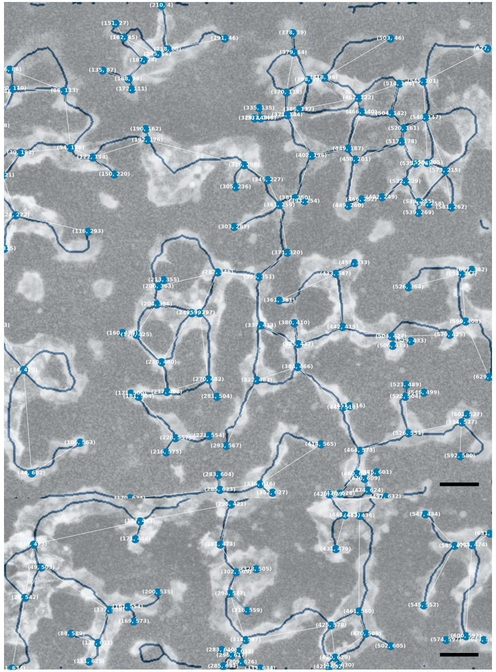
Together, this shows an **increased vascular "efficiency"** for nutrient deprived networks.

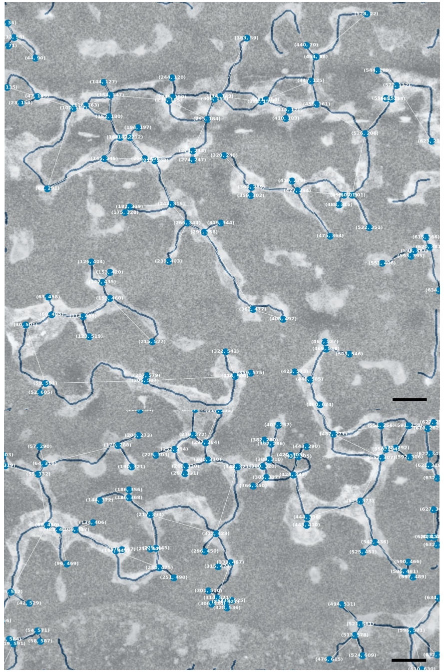




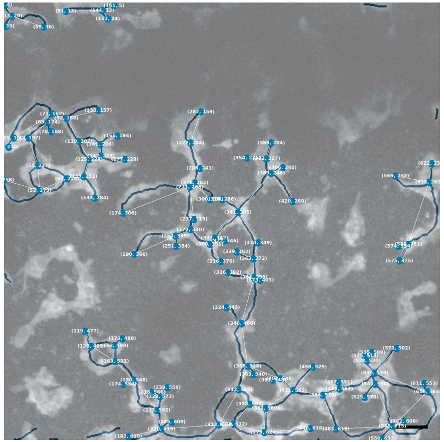
Static Storage







CONNECTED_NETWORK=0
MIN_NODE_COUNT=3
NEAR_NODE_TOL=15
LENGTH_TOL=3



Control

Static Conditions

Static Storage Graph Data

$H_n : \mu_c = \mu_s$	$n_c = 2$
$H_a : \mu_c \neq \mu_s$	$n_s = 5$

Two-sample Z-tests were performed on the averages of the control and static characteristics

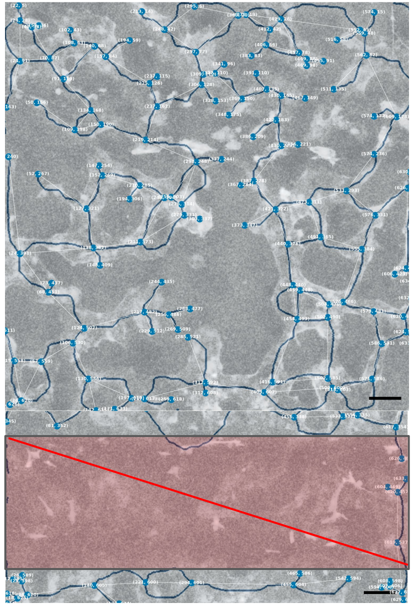
$$Z = \frac{\bar{x}_c - \bar{x}_s}{\sqrt{\frac{\sigma_c^2}{n_c} + \frac{\sigma_s^2}{n_s}}}$$

$P < 0.05$

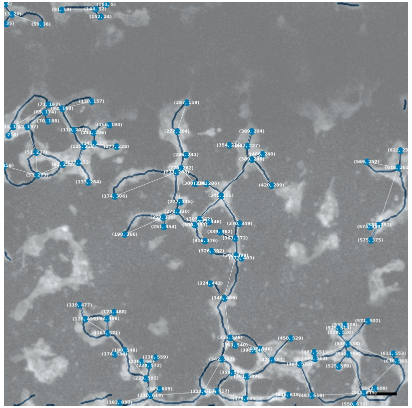
$P < 0.10$

Average Branch Characteristic	Control	Static	Z	P
Length	116.6 $\uparrow$	101.0 $\downarrow$	1.656	0.0977
Surface Area	24218 $\uparrow$	15856 $\downarrow$	1.939	0.0525
Volume	543415 $\uparrow$	252100 $\downarrow$	1.856	0.0634
Radius	36.5 $\uparrow$	26.8 $\downarrow$	3.141	0.0017
Fractal Dimension	1.0229	1.0229	0.000	1.0000
Contraction Factor	0.9037 $\uparrow$	0.8872 $\downarrow$	2.3172	0.0205
Connectivity	1.2502 $\uparrow$	1.0404 $\downarrow$	2.5972	0.0094
Unweighted Directionality (Max)	0.29175	0.27862	0.8751	0.3815
Weighted Directionality (Max)	0.30085	0.27622	0.9731	0.3305

Static Storage Volume Data (2.5D Segmentation)



Volume of the parent channel excluded from density calculations



Static dataset outlier

Avg. Volume Characteristic	Control	All Static	Z	P
Vessel Density	0.2431	0.1449	6.3175	< 0.00001
Avg. Distance from a Vessel Wall	38.9	47.38	0.9260	0.3544

Avg. Volume Characteristic	Control	Removed Outlier Static	Z	P
Vessel Density	0.2431	0.1596 ↓	12.762	< 0.00001
Avg. Distance from a Vessel Wall	38.9	39.956 ↑	0.1976	0.8434

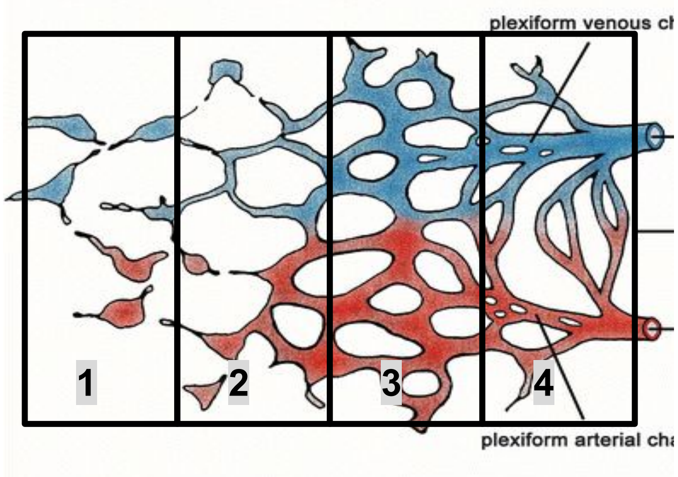
- We can conclude with high certainty that **static storage decreases vessel density** (volume of vessels / vascularized volume).

- However, we conclude **no significant change in average distance from a vessel wall**.

Together, this shows an **increased vascular "efficiency"** for statically stored devices.

Preliminary Data Investigation Summary

Both low-nutrient and static networks displayed **decreased vessel density** with **maintained average distance to to a vessel wall**.



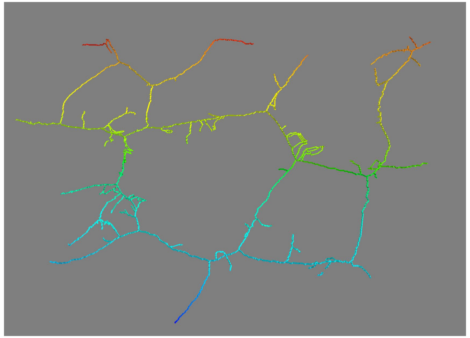
Additionally, for both low-nutrient and static networks,

- The percent decrease in segment length was less than the percent change in segment radius (perceived segment *elongation*)
- Segment contraction factor decreased (*increased curvature*)
- Connectivity decreased (*rough increase in hierarchy*)

Hypothesis 1: These networks are advanced	**Hypothesis 2: These networks are delayed**
Low-nutrient and static networks are in region 2 or 4.	Low-nutrient and static networks are in region 2.
High-nutrient and control networks are in region 1 or 3 (respectively).	High-nutrient and control networks are in region 3.

**Probably neither: I should leave the hypothesizing to the cell biologists.

Current and Future Development Work



3D NetworkX Representation

- Create weighted graph representation using the 3D segmentation.

Full validation

- Create more realistic synthetic data (currently only tested on the rectangular T-Junction).
- Value comparisons to similar published work.

Investigate and eliminate systematic error

- How does error propagate from one filter/process to another.
    - Example: For a given network, does average connectivity increases when imaged at a lower magnification image?

Scalability: Run faster, waste less memory, accept larger images.

Future Experimental Work

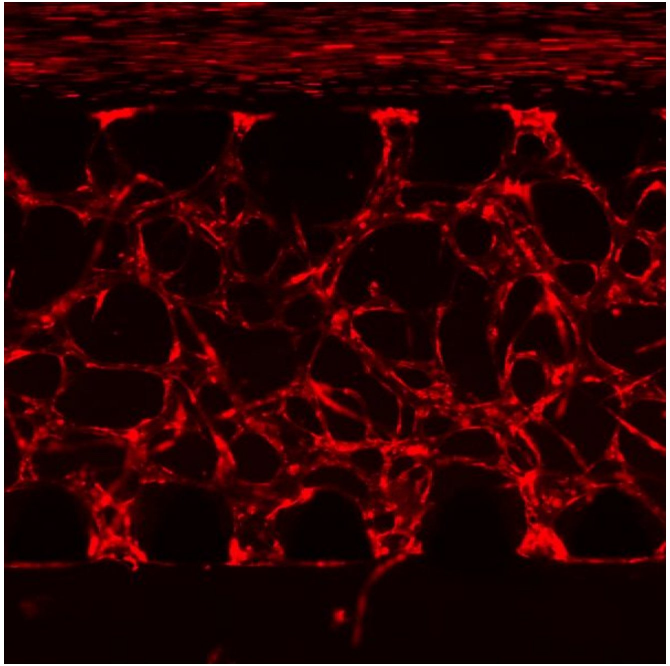
Device Parameter Characterizations

- Similar to the preliminary data exploration presented, only with other network parameters.

Flow Tests

- Perfuse the networks, measure boundary conditions, and run simulations to characterize pressure and WSS distributions.
    - Initially flow cell media culture, then flow blood.

Pet Project: Investigate the and adjust for the impact of Red Blood Cells and non-Newtonian dynamics within the microvasculature.



GIF Reproduced from AIM Biotechnologies
https://www.aimbiotech.com/vasculogenesis.html

Summary (and abbreviated demo)

mvn-analysis **outputs from an input** .tif

- 2D Segmentation
  - Binary lumen mask
  - Vessel centerlines (skeleton)
  - Euclidean distance transform
- 2D Graph
  - MVN segment characteristics, distributions, and comparisons
  - MVN full-network characteristics
  - Anisotropy calculations
  - mvn-analysis and NetworkX objects for user directed statistics
- 2.5D Projection
  - Watertight surface and volume representation of the lumens (symmetric across the *xy* plane)
- 3D Segmentations
  - Volume and surfaces meshes generated utilizing each image plane (with optional smoothing and reconstruction)
- Volume Based Data
  - Total lumen volume and vascular density
  - Distances of non-vascularized regions to vessel walls
- Geometries
  - Surface OBJs
  - Volume .mss, ExodusII

BeatIt **outputs from annotated ExodusII**

- Stokes flow pressure and velocity fields

ParaView **Post-Processing**

- Wall shear stress values and visualizations

Acknowledgments

The work presented would not have been possible without the generosity and support of the following wonderful people.

Dr. Boyce Griffith and Dr. Bill Polacheck: Co-advisors.

Dr. Simone Rossi: Development, training, and support for *BeatIt* Stokes flow solver + advice for the ParaView post processinging of the simulations.

Crescentia Cho: Primary development and imaging of the MVNs.

Joanna McDonald: Supplementary MVN development and imaging.

Margaret Anne Smith: Trelis software training.

Questions?