Fungal Network Tracing

Fiji (ImageJ) was used to trace and analyze fungal network patterns. Simple Neurite Tracer (SNT), a Fiji plugin designed to analyze filamentous neuronal structures, was repurposed to trace and fit fungal hyphal filaments. Fungal microscopy data (in TIF format) was imported into Fiji as a virtual stack. The diameter of the largest hypha was measured (in pixels) and recorded for later radial measurement calibrations. The Neuroanatomy SNT plugin was then used to trace over the various paths in the fungal network. SNT's fitting operation is then used to estimate the thickness of traced paths. The recorded maximum radius is used as a width constraint for the fitted paths, and the path node coordinates are refined by snapping them to the centroid of the fitted cross-section. This trace and fitting data was saved as a .traces file for later analysis using Python.

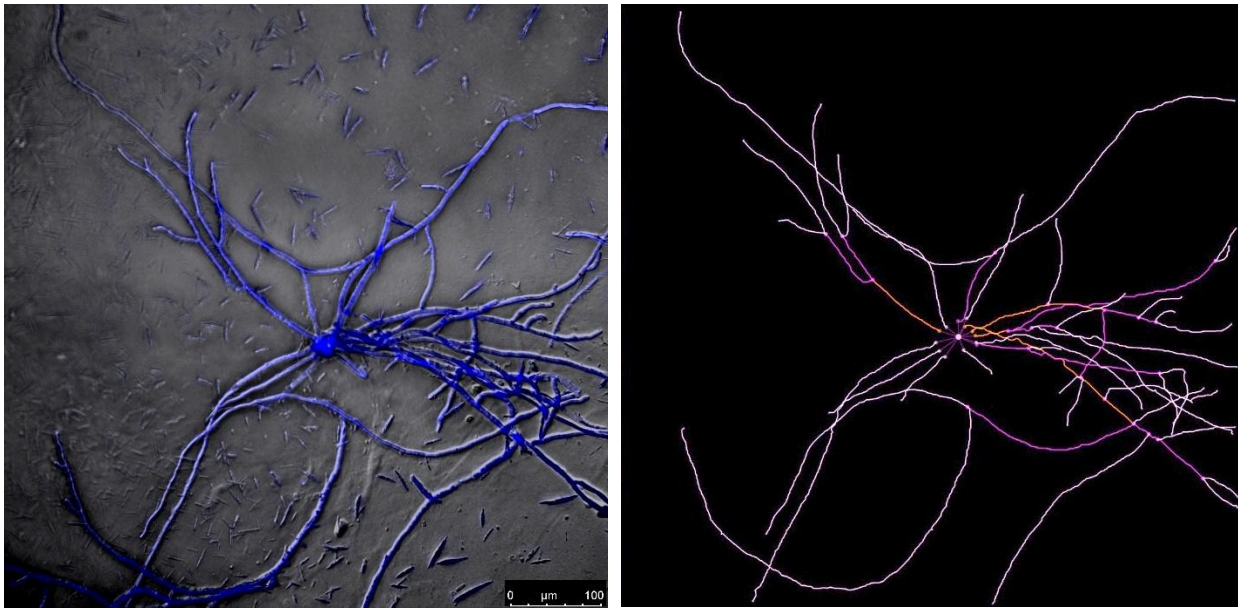

Figure 1: ImageJ SNT allows for semi-automation of path tracing for filamentous structures and generation of .traces files for analyses by Python.

Path Data Analysis

Python was used to decode and parse through the traces file and create an Excel file with node coordinates and radii for each path in the network. Python was then used to create line plots and histograms of radii along the nodes of each path. Additionally, Python was used to reconstruct the fungal network as an DXF file using the node coordinates and radii. A separate layer, *tip_domains*, was created in the DXF file containing the 15 µm long regions from the ends of each hyphal filament. This DXF file was imported into COMSOL MultiPhysics software to simulate fluid transport inside fungal networks.

COMSOL MultiPhysics

The two-dimensional fungal geometry was imported into COMSOL using the DXF file generated

Figure 2: Python was used to generate a DXF file of hyphal filaments and tip regions for COMSOL geometry imports.

by Python code. This prevented complicated geometries caused by manually creating the network geometry using interpolation curves, thus making it easier for COMSOL to create meshes for the network. A circle was sketched at the center of the network cutting through the inner tips of the fungal paths and then converted to a solid. An additional concentric smaller circle was sketched, converted to a solid, and then subtracted from the bigger circle. This served as inlet boundaries for fluid flow, in order to simulate fluid flow from the basal center to the apical ends of the fungal network.

Fluid flow in fungal networks is directed towards the tips due to the concentration gradient caused by uptake of ions at the fungal tips. A time-dependent study and two physics were added to model this – Laminar Flow (*spf*) and Transport of Diluted Species (*tds*). A custom material was added to the component with a density of 1000 kg/m^3 and a viscosity of 2 cP to mimic cytoplasmic flow.

The laminar flow physics was set up with open boundaries at all the tips. A velocity of 10 μm/s was set up at the inlets. Additionally, a volume force dependent on the concentration, temperature and position, was added to direct flow towards the tips. The *tds* physics was set up with a single

solute with default diffusion properties. Given that the purpose of this simulation was to model mass flow from the base to the tips, exact biological solute concentrations were not included. This model instead operated on the difference in solute concentration between the network base and edge. Ergo, the initial value of internal network solute concentration was set at 0 mol/m^3. Boundary conditions of 200 mol/m^3 were set at each hypha tip. Finally, a solute consumption reaction was imposed across the network to simulate the organism's ability to maintain a constant internal solute concentration (i.e. via diffusion, passive transport, or active transport across the cell membrane).

Three 2D Plot Groups were used to analyze the velocity, pressure and concentration profiles in the fungal network after the time-dependent study was computed.

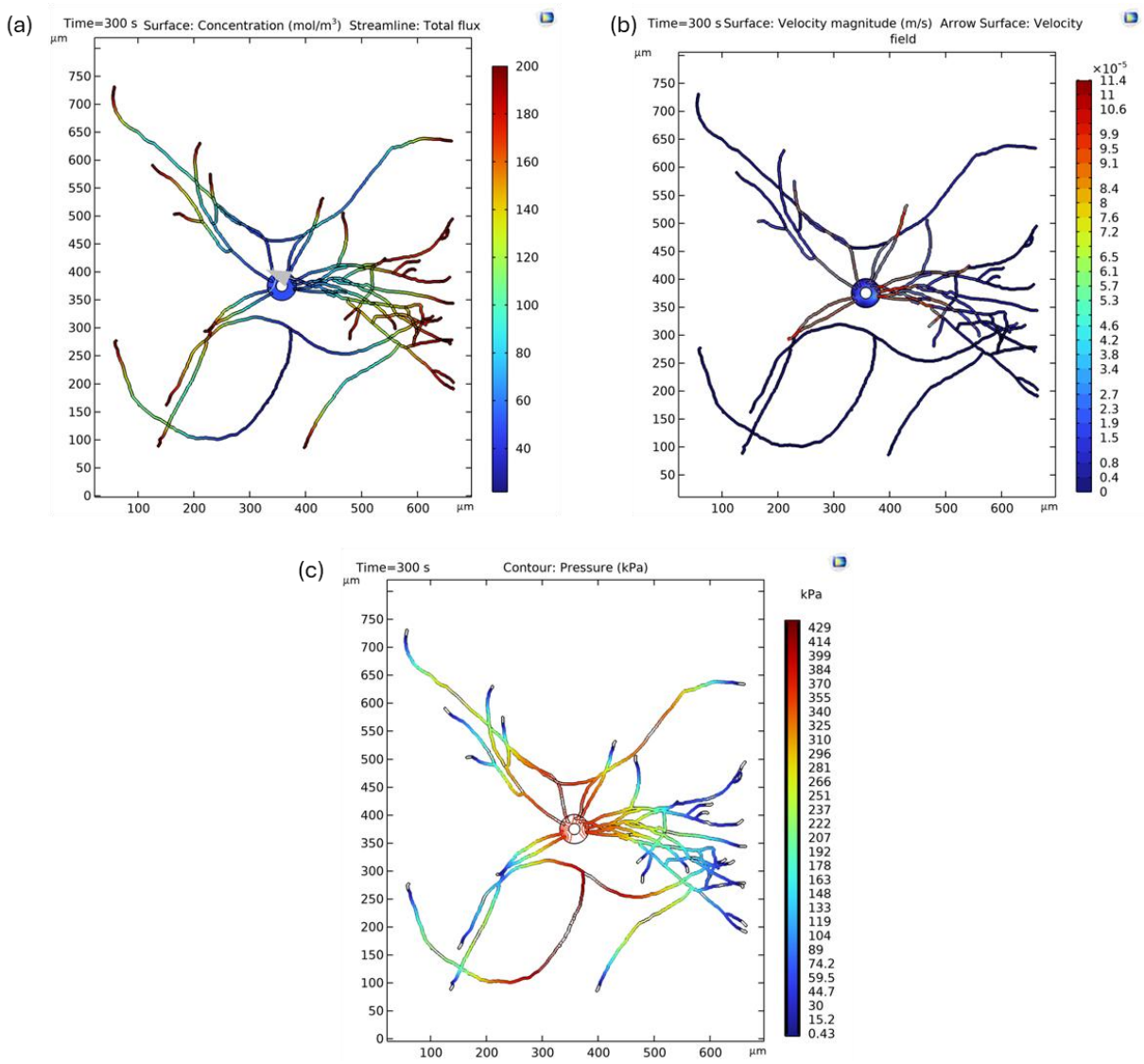

Figure 3: COMSOL MultiPhysics was used to generate (a) concentration, (b) velocity and (c) pressure profiles of cytoplasmic fluid throughout the network.

Fungal Network Complexity and Flow Patterns

With an initial model set up, the first goal was to analyze the effects of different domain selections on the flow patterns in the network. The hyphal paths were separated into three separate domain selections – Outer, Middle and Deep, based on numerical measures of path branching complexities generated by ImageJ SNT Horton-Strahler analyses.

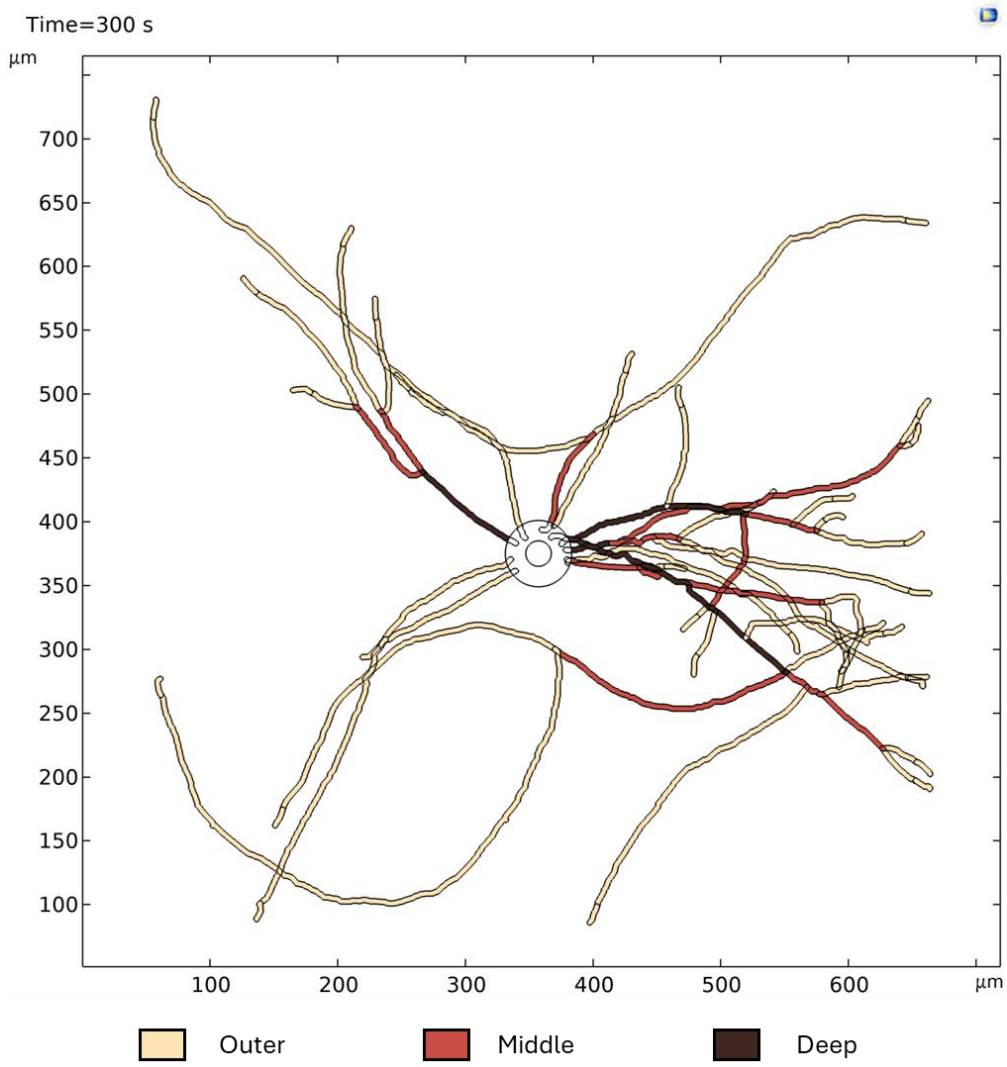

Figure 4: Hyphal paths were separated into three domains based on branching complexities – Outer, Middle and Deep.

Specific parts of a hyphae could be removed from the domains of interest in the *tds* (Transport of Diluted Species) and *spf* (Laminar Flow) physics to mimic a dead hypha with no cytoplasmic flow. Individual hyphal sections in all three domain selections were removed from the physics and then the number of dead tips (hyphal tips with no fluid flow) were counted. It was observed that removing sections from the Outer domain did not significantly affect the organism. While removing sections in Deep domain region had a greater impact, however, fluid flow could be

redirected due to greater connectivity in this region. Removing sections from the Middle domain regions had the highest impact with the largest number of dead hyphal tips, which could be because this region contains critical links between the base and exploratory tips.

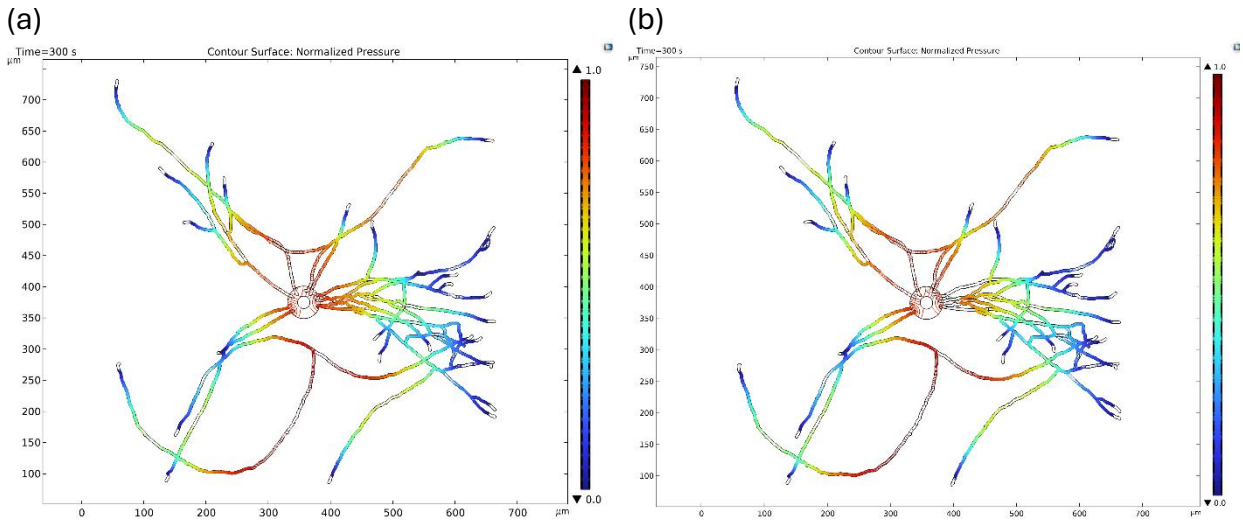

Figure 5: Normalized pressure plots in (a) a complete fungal network compared to (b) a network with dead hyphal sections

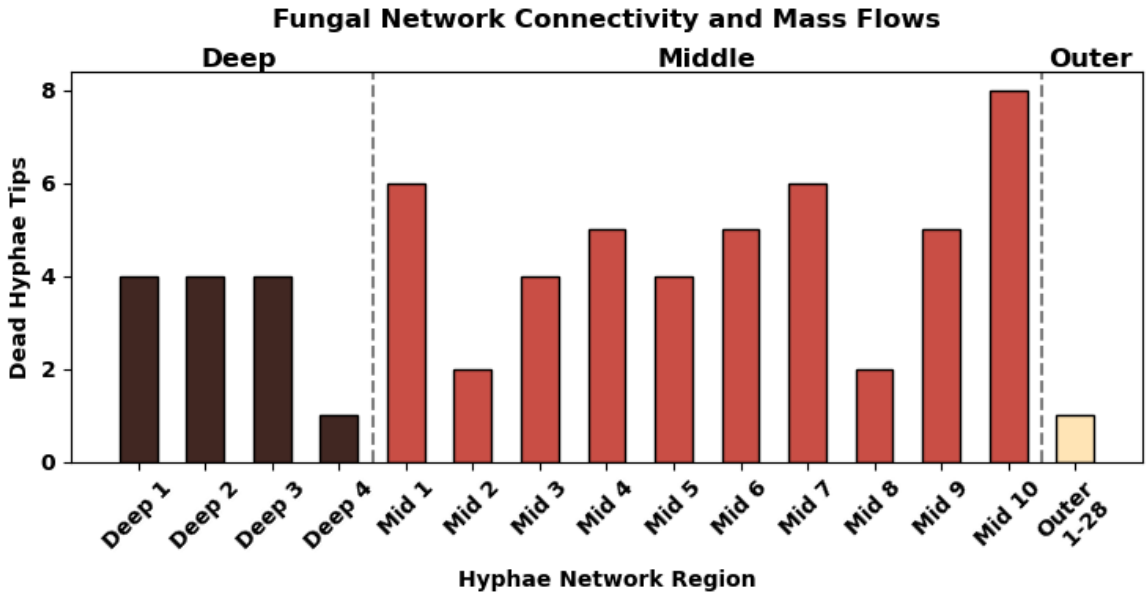

Figure 6: Effects of hyphal section eliminations in different domain regions of a given fungal network