Comprehensive Quantitative Analysis of Mycelium's Heterogeneous Network

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Introduction

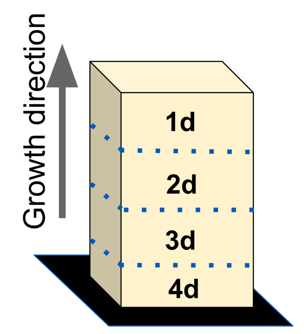
- Mycelium, the root structure of mushrooms, is a heterogeneous material formed of a tunable fibrous network.
- Ecovative Design uses mycelium in the development of biodegradable alternatives to plastics and textiles.
- An understanding of mycelium's microstructure is required to construct the process-structure-property relationships needed for various products.

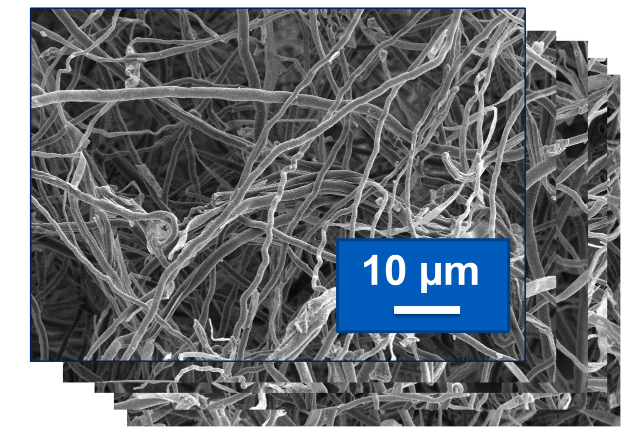
Aim

- In this project, a combination of image-processing software [1, 2] and data clustering methods were used to quantitatively characterize features of mycelium's microstructure.
- The spatial heterogeneity of mycelium was studied as well and how this heterogeneity varies as a function of growth direction.

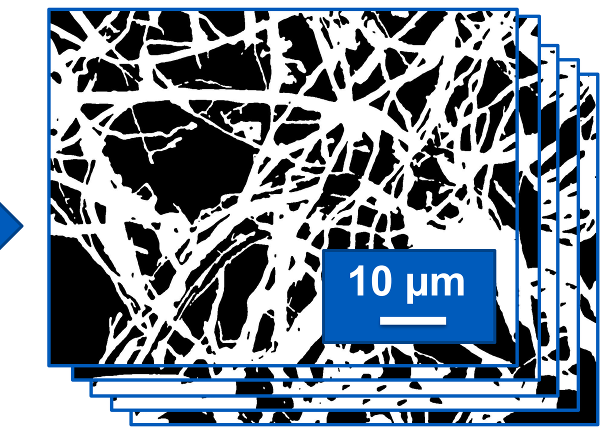
G. Lucidum (Reishi)

Mycelium on Ecovative growth substrate





3K magnification SEM images of mycelium acquired at different depths. 20 images are used in total.



Segmented SEM image

Challenges:

- **Information misreading**
- **Information loss**
- **Information aggregation**

Building Process-Structure-Property correlations to advance the product design of mycelium-based materials.

Results

Different challenges in recognizing and quantifying the below features in SEM images of mycelium were identified. Each challenge required specific modifications in the development of our analysis.

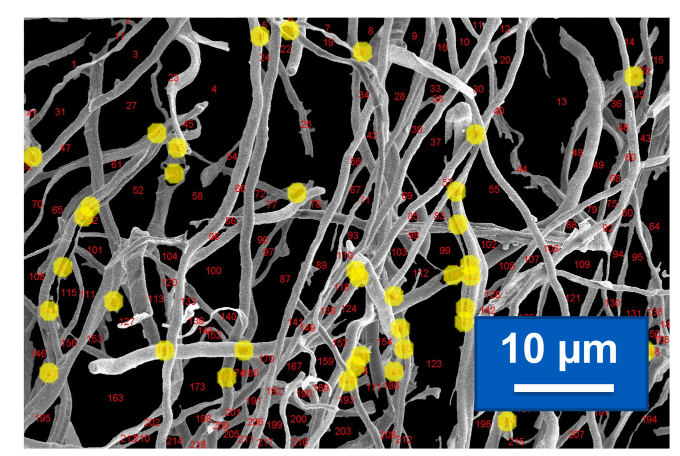
1. Pore Area

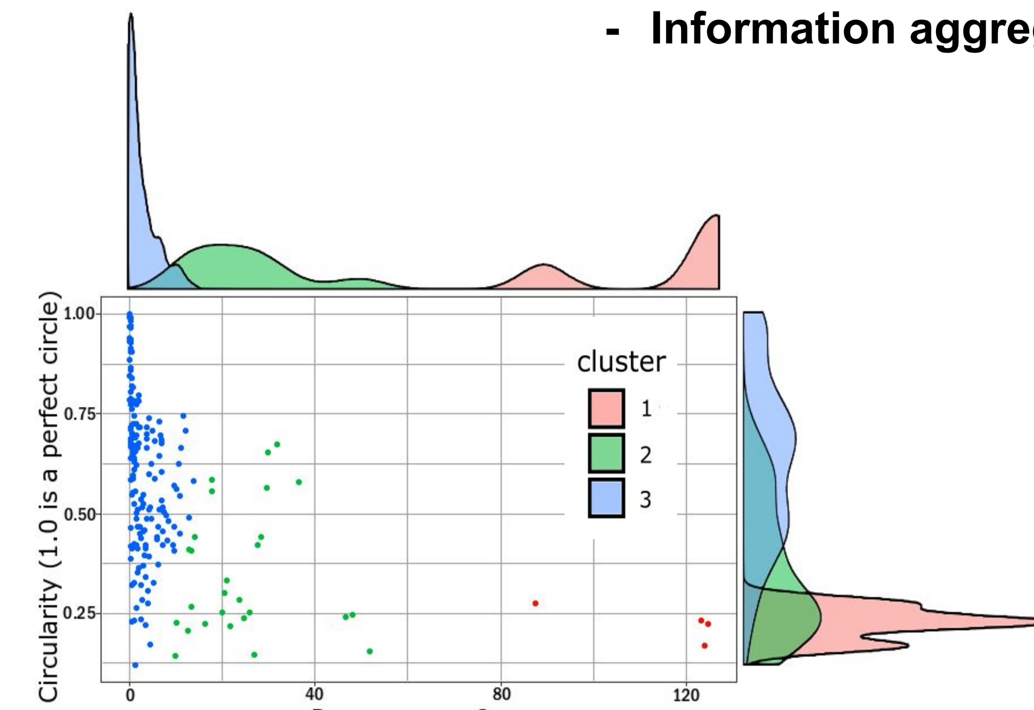
a. Challenge*: Shading and the overlap of fibers create fake pores in segmented images (**information misreading**)*

b. Solution: Features of fake pores (e.g., area and circularity) are used to cluster pores into groups containing 'real' and 'fake' pores.

c. Details: This grouping of pores employed the unsupervised clustering method k-means (k=3).

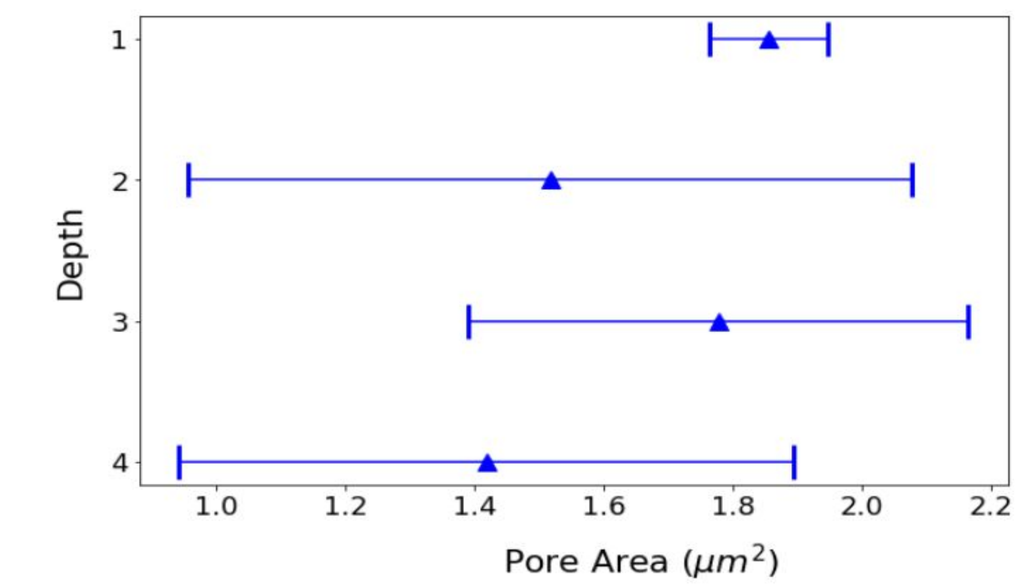
d. Results: *Pore area does not change significantly with the growth direction.*





(a) Manually annotated falsely identified pores

(b) Histogram and scatterplots of pore area and circularity with respective densities per cluster
Cluster 1: Largest and least circular pores
Cluster 2: Representative pores for analysis
Cluster 3: Smallest and most circular pores



The average pore area is fairly insensitive to the growth direction.

2. Intersections

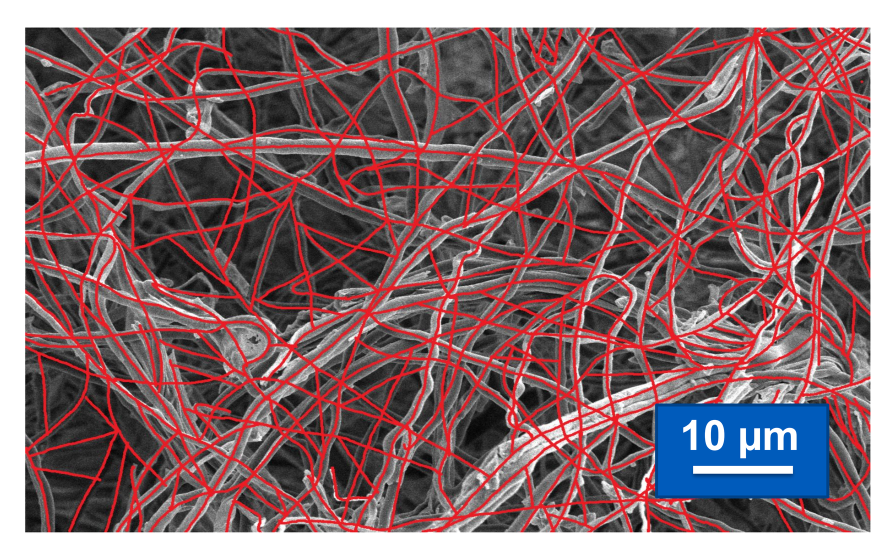
a. Challenge*: Bundles of fibers are aggregated during the automated segmentation and skeletonization of our images leading to a **loss of information** about these intersections.*

b. Solution: SEM images of the fiber network are manually skeletonized, which are then coupled with the automated detection of intersections.

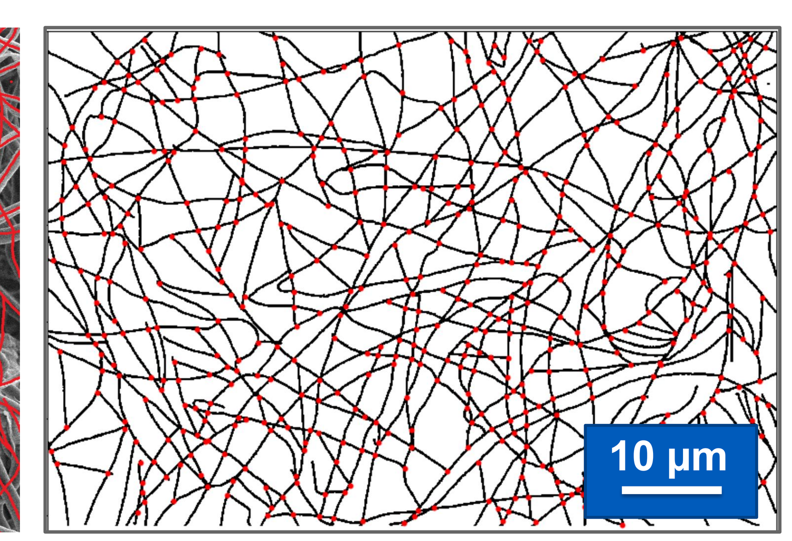
c. Details: Intersections are clustered using k-nearest neighbors (k = 17)

d. Result: T*he number of intersections increases with depth.*

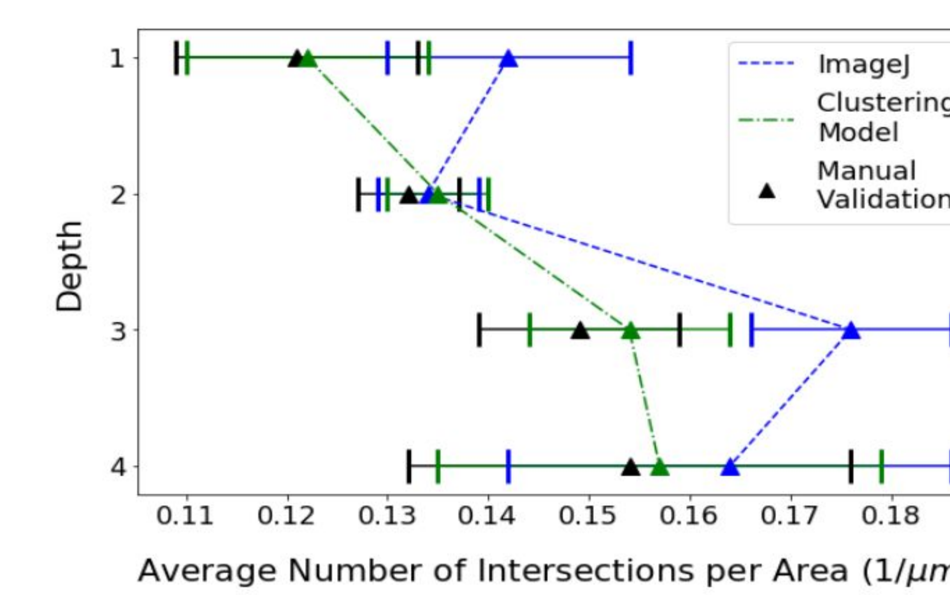
i. Our results are more accurate than results from automated analysis



Manual Skeletonized Network



Manually skeletonized network with intersections detected by clustering



Segmentation is important for the calculation of intersections (comparison between our approach and ImageJ algorithm).

3. Fiber Radii

a. Challenge*: **Information aggregation** at low SEM magnifications.*

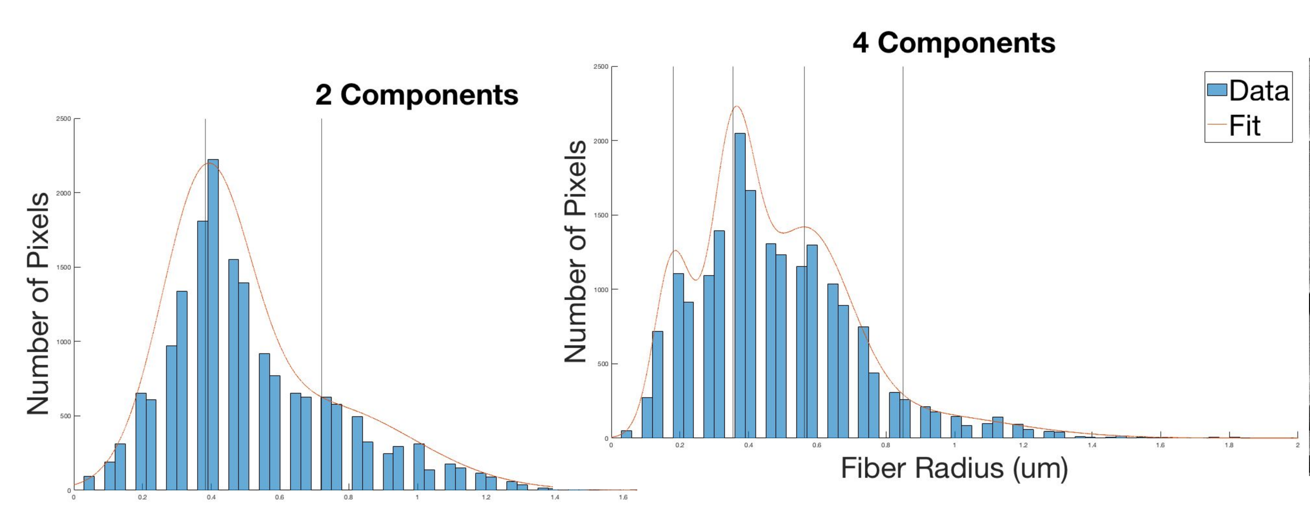
i. Diameters of different fibers are difficult to extract as a single parameter from a single radii distribution

b. Solution: The histogram of fiber radii is fit using Gaussian Mixture Models (GMM).

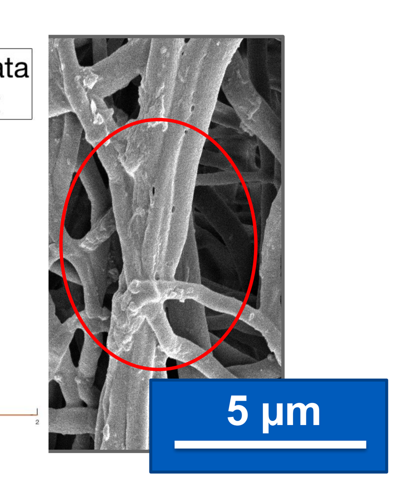
c. Results: Four types of fibers are identified. Older layers (4d) are characterized with more types of fibers than younger layers (1d).

i. We confirmed that type 4 fibers with radii of 1.2 um correspond to bundled fibers.

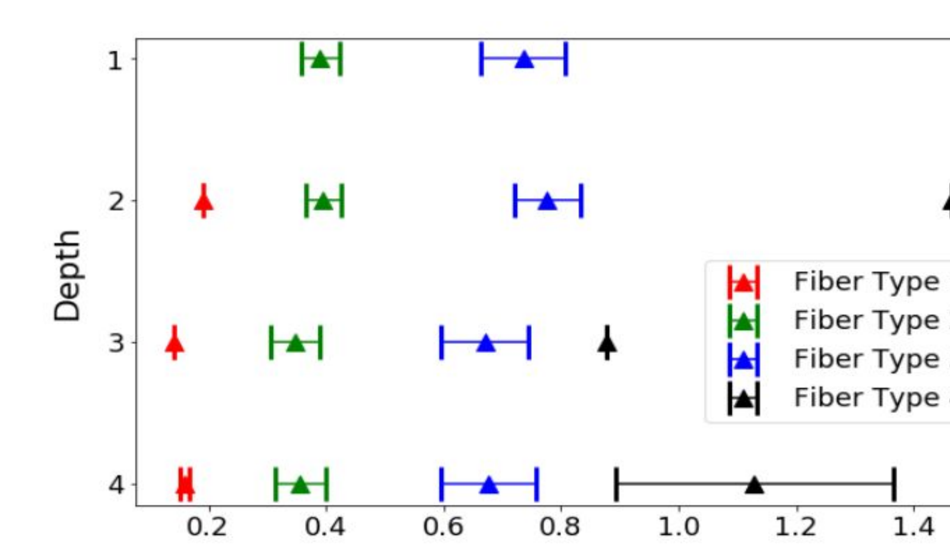
ii. . The number of distinct fiber types increases with growth direction.



Radii histograms with GMM fit for the youngest (left: depth 1) and the oldest samples (right: depth 4).



Bundling of fibers with radii ~1.2 um (Fiber type 4)



GMM is used to detect four different types of fibers. The number of types vary with the growth direction. Younger mycelium has only two types of fibers, while older samples develop additional components.

References

[1] Rueden, C. T.; *et al.* Image J2: ImageJ for the next generation of scientific image data. *BMC Bioinformatics 2017 (18) 529.*

[2] Hotaling N. A. *et* al. DiameterJ: A validated open source nanofiber diameter measurement tool. *Biomaterials* 2015 (61) 327.