Comprehensive Quantitative Analysis of Mycelium's Heterogeneous Network

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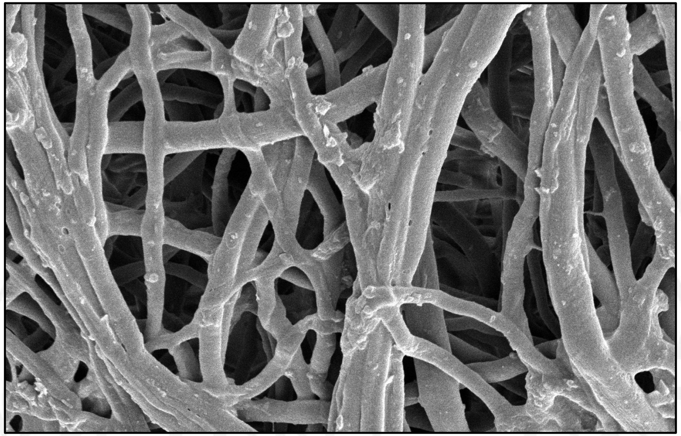
Introduction

G. Resinaceum/Lucidum (Reishi)

Mycelium on growth substrate



SEM image of mycelium network.

Ecovative LLC is a sustainable manufacturing company that employs the root structure of mushrooms - **mycelium** - to design products such as packaging material, food, and textiles.

Mycelium is a tunable biological network formed from filaments called **hyphae**.

ecovative

Ecovative Design, LLC and their products

Hyphae Growth

Mycelium grows by **extension**, **bifurcation** or **branching**.

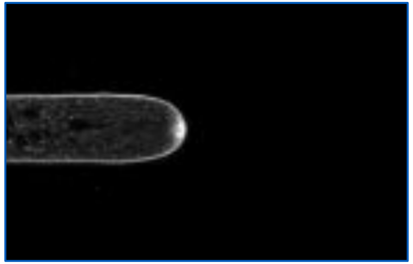
Fibers can additionally **fuse** together.

Mycelium can consist of different types of hyphae, such as:

a) **Generative** - thin walls, contain septa, branch.
b) **Skeletal** - thick walls, doesn't contain septa, long

Network growth

Hyphal extension



Bifurcation

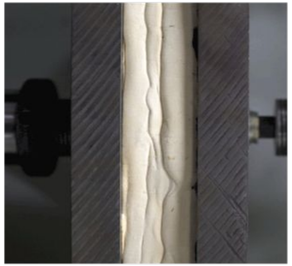
Branching

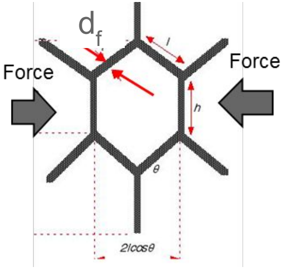
Septate formation

Patrick C. et al. Fungal Genetics and Biology, 2002.

Motivation

Structure – Mechanical Correlations



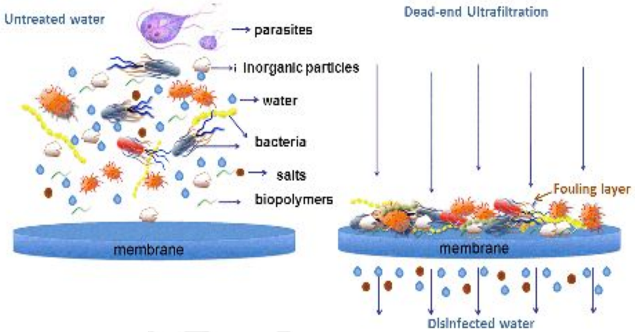


- Mycelium is an open form structure
- Young's modulus of such a structure depends on the cell wall thickness d_f :

$$\frac{E_1^*}{E_s} = \left(\frac{d_f}{l}\right)^3 \frac{\cos\theta}{\left(\frac{h}{l} + \sin\theta\right)\sin^2\theta}$$

Young's Modulus of honeycomb structure (measure of the stiffness of a structure)

Structure – Transport Correlations



The filtration efficiency of a membrane depends on the diameter of fibers (d_f):

$$E_t = 1 - \exp\left[\frac{-4cEh}{\pi(1-c)d_f}\right]$$

For homogenous networks

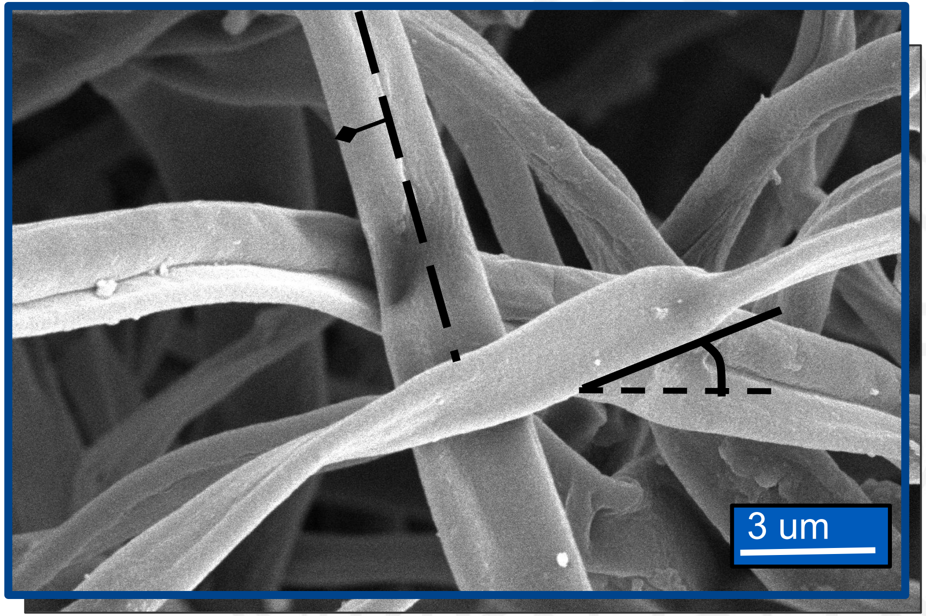
E filtration efficiency of a single fiber
c fiber volume fraction
h thickness of filter.

Properties of mycelium are dependent on the microstructure such as hyphae diameter

Project Scope

- **Scanning electron microscope (SEM)** images of mycelium's network were used to quantify the material's microstructure **Fiber diameter**.
- To understand the effect of magnification of imaging on fiber diameter quantification.
- To understand how the growth conditions (as function of time) effect the hyphae diameter distribution.

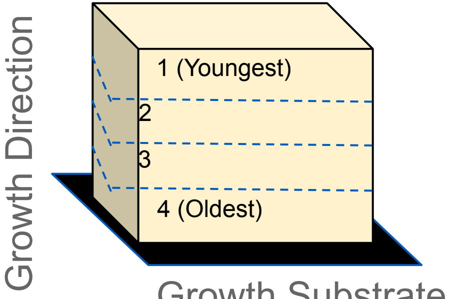
<u>This project aligns with MDI project scope</u>
- Using database of images for image processing (80 images)
- Quantifying structure for the design and development of biofilters



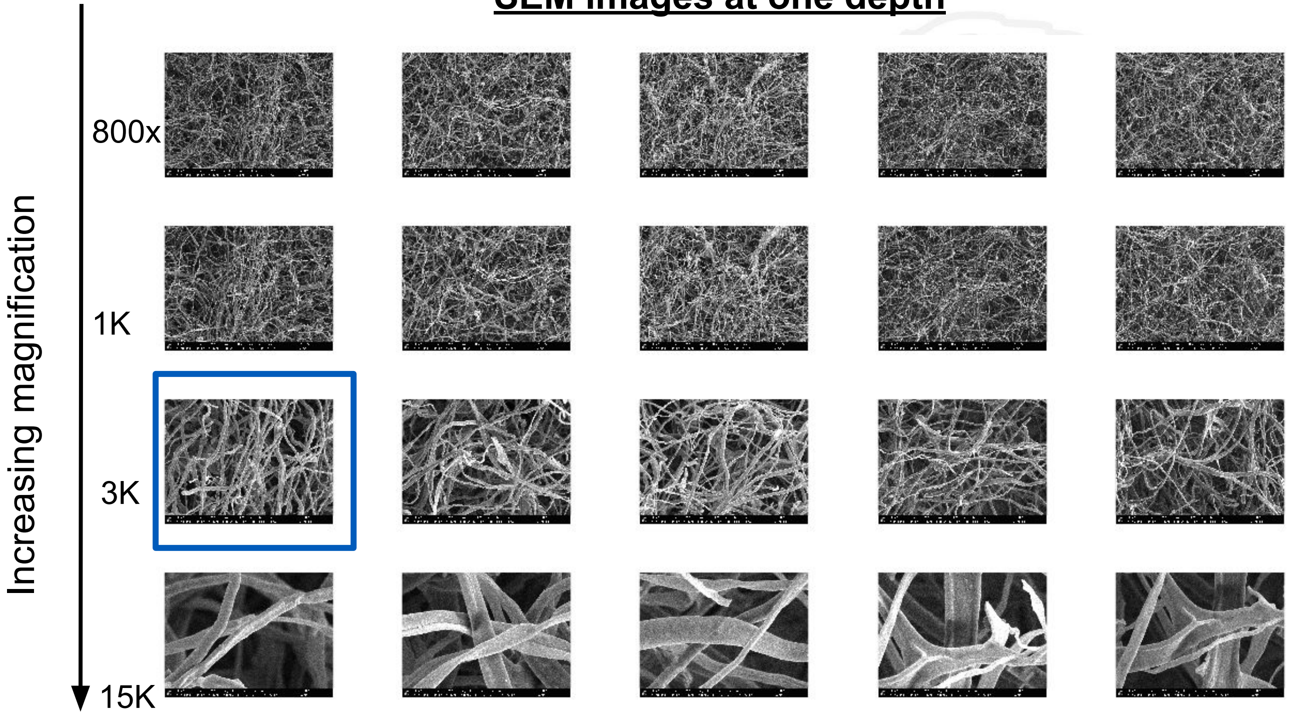
SEM images of mycelium samples at 15K magnification

Data Set obtained from SEM

Mycelium sample on growth substrate

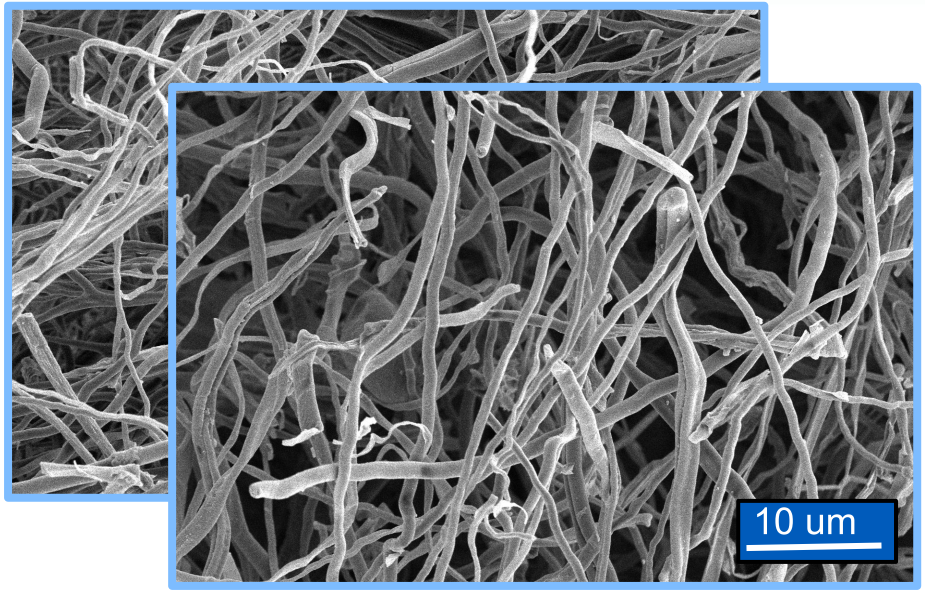


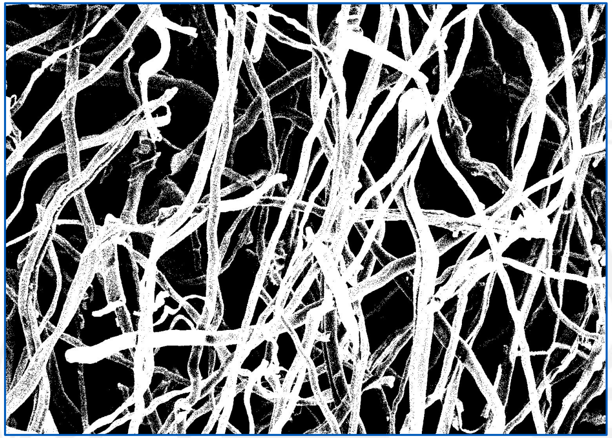
SEM images at one depth

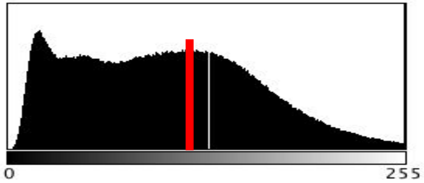


Five images per depth for a total of 80 images.

Image processing to extract fiber radius





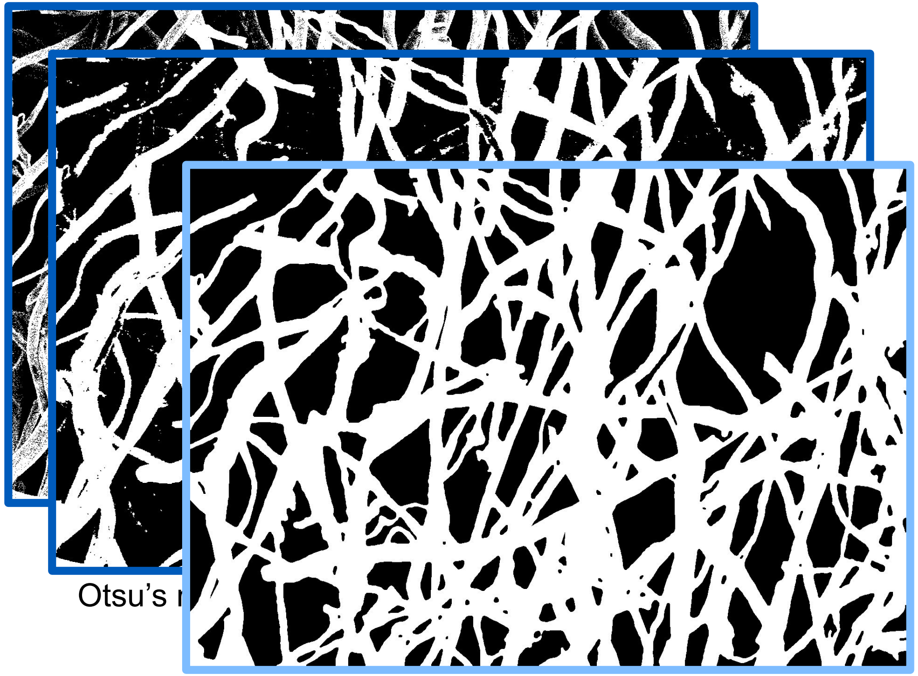


In studying hyphae diameter, we wish to:

1. Segment the image's pixels to differentiate fiber from pore.
2. Measure the distance between a pore and the centerline of a fiber.
3. Determine unique fibers from data.

Pixel intensity can be used to segment the image, a process known as **thresholding.**

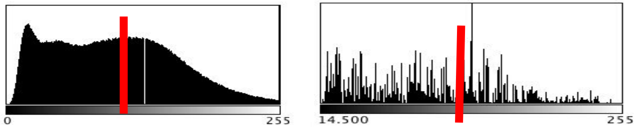
Thresholding with SRM



Otsu’s r

Segmented Image after noise reduction

Threshold: 106 Threshold: 105.98



Histogram with threshold for raw image
Histogram with SRM (right)

Otsu’s Thresholding Method
Calculate all possible thresholds and the spread of pixels on each side. Minimize the sum of the spreads.

Statistical Region Merging
Combines neighboring pixels into regions of a similar intensity.

Noise Reduction
- Median filtering – despeckling
- Erosion/Dilation – smooth edges

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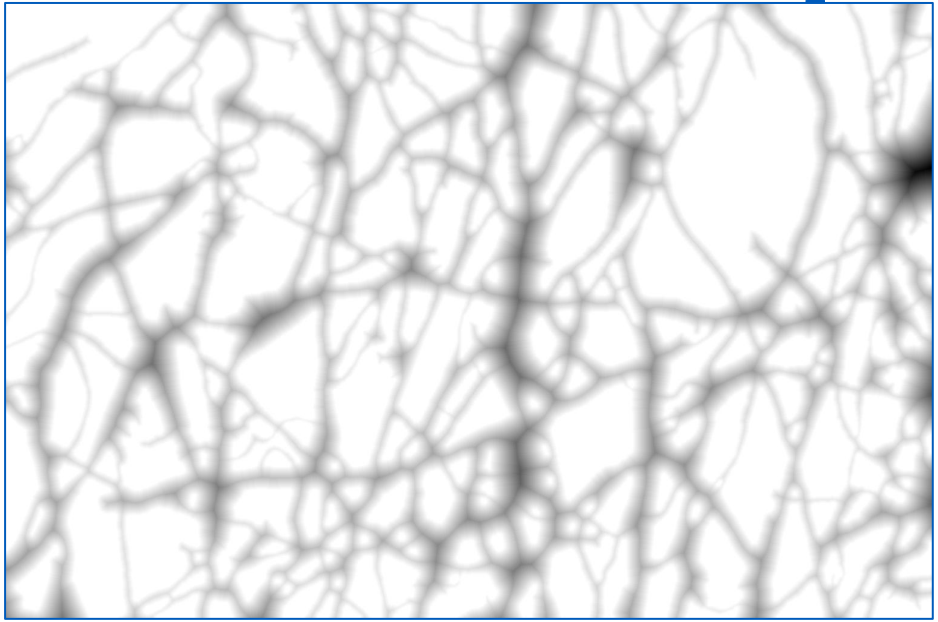
Extracting Radii from segmented images

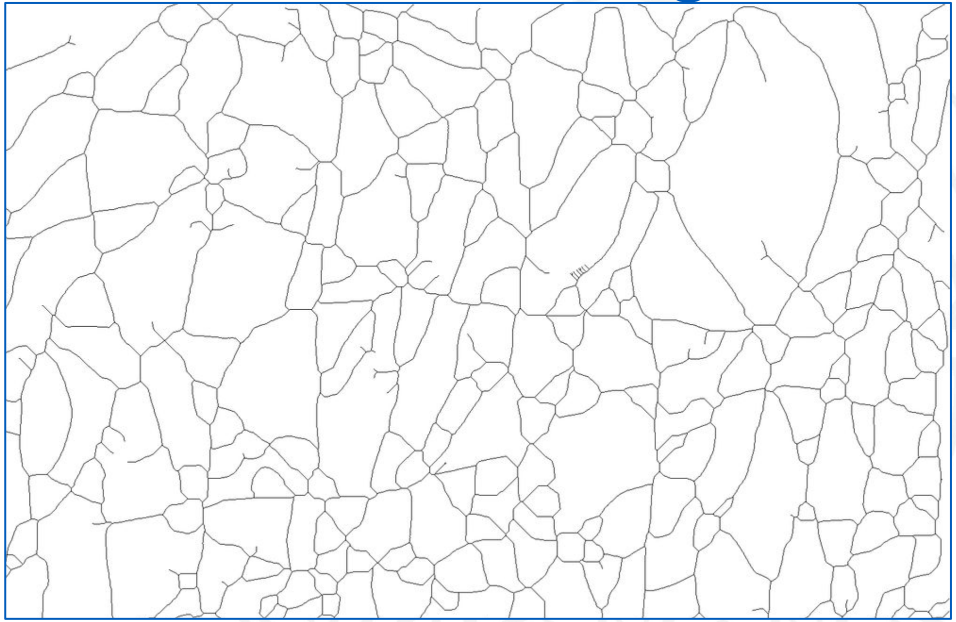
Euclidean Distance Map



Euclidean Distance Map of segmented mycelium image

The number of pixels between a fiber pixel and the nearest orthogonal pore is calculated. The pixel then takes a grayscale value equal to this distance.

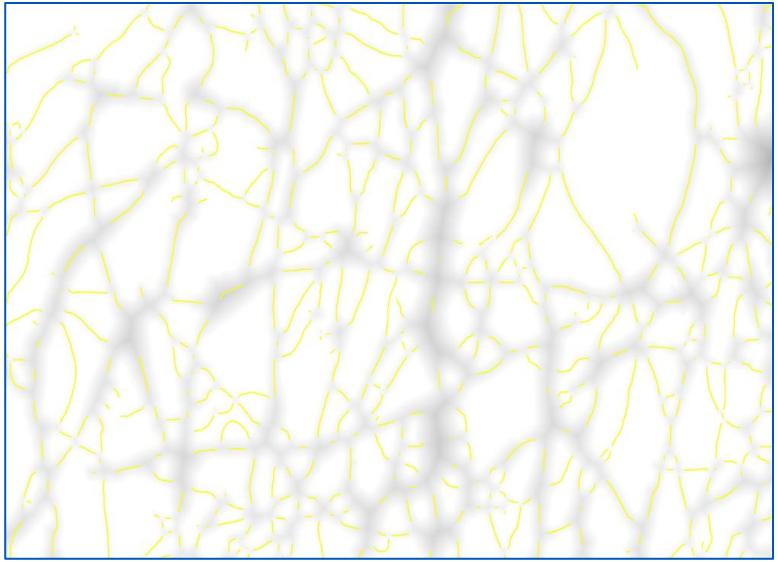
Axial Thinning

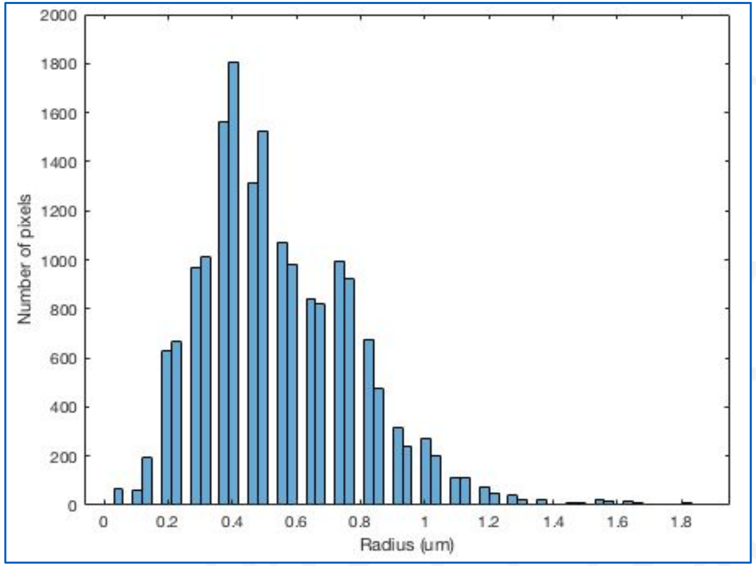


Axially thinned mycelium network

Pixels at the edge of a fiber are removed until a common center point is reached.

Extracting Radii from segmented images

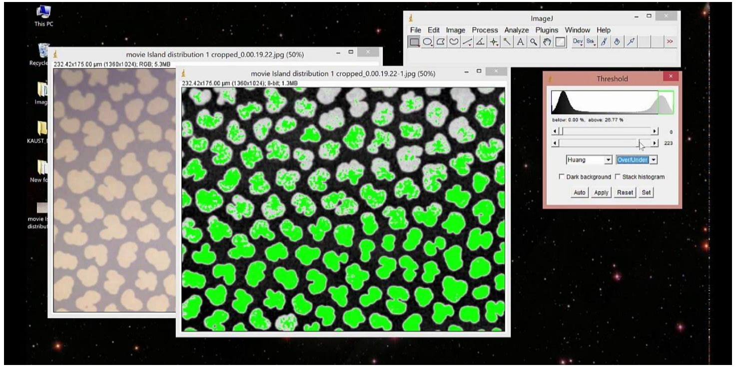

Overlay of EDM and skeleton (yellow)



Histogram of fiber radii

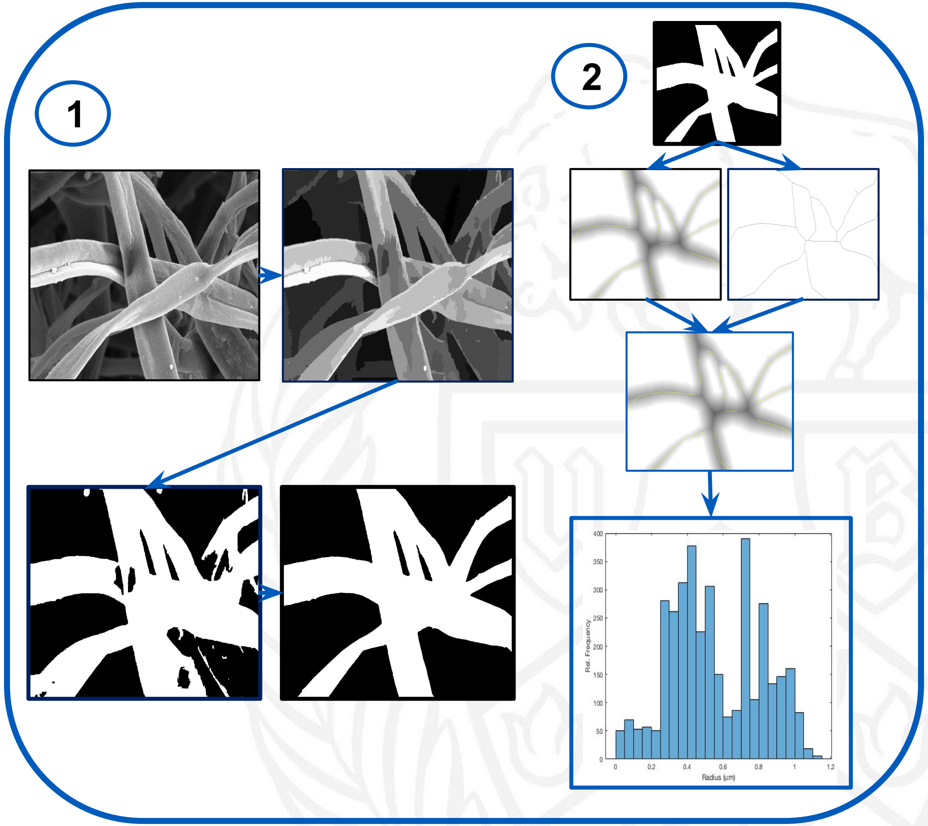
The images produced by the Euclidean distance transformation and axial thinning are overlaid. Each centerline intensity is measured and a histogram of the fiber radii is formed.

Software



Screenshot of Fiji display/Fiji logo

These processes are automated by the image processing software ImageJ/FIJI and the DiamterJ plugin.



Schneider, Nature method, 2012

Fiber analysis workflow

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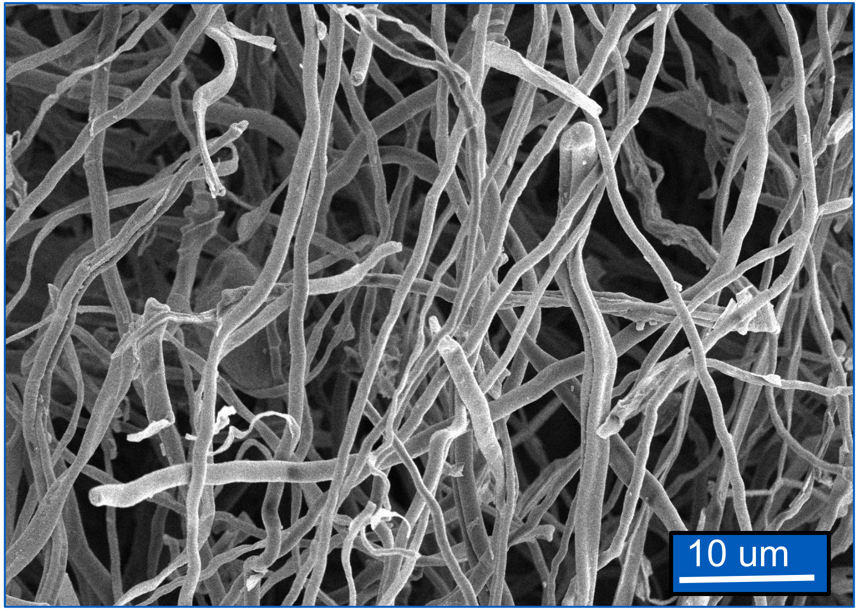
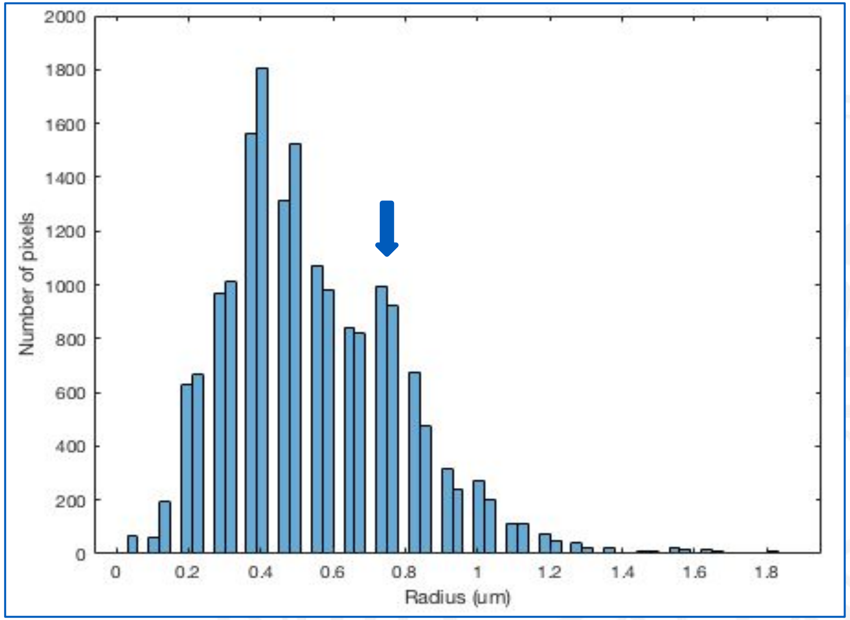
Fitting Radius Distribution



Mycelium image at 3K-1d

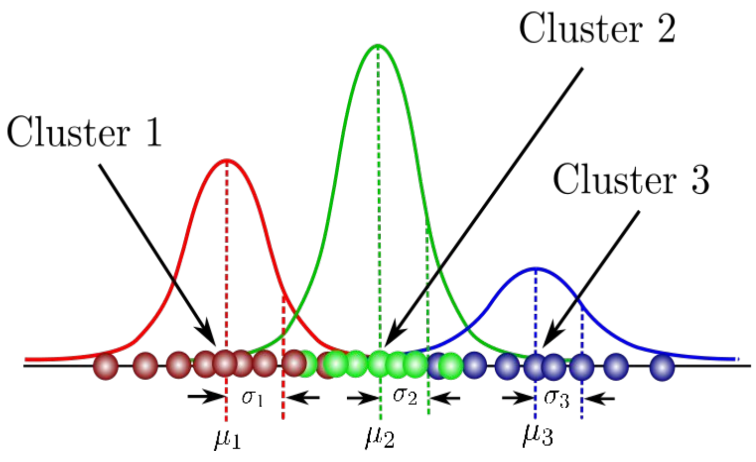


Radius Histogram

Histogram has the shape of a skewed Gaussian.

Gaussian Mixture Model

Assuming that fiber radii are normally distributed, the probability density function will consist of the sum of individual Gaussians with distinct parameters **Gaussian mixture model**.



Gaussian Mixture Model; k=3

$$p(x) = \sum_{i=1}^{K} \phi_i \mathcal{N}(x \mid \mu_i, \sigma_i)$$

$$\mathcal{N}(x \mid \mu_i, \sigma_i) = \frac{1}{\sigma_i\sqrt{2\pi}} \exp\left(-\frac{(x-\mu_i)^2}{2\sigma_i^2}\right)$$

$$\sum_{i=1}^{K} \phi_i = 1$$

A **Gaussian Mixture Model (GMM)** can be fit using Expectation Maximization.

The output parameters are mean, variance, **mixing factor,** and the **number of components (k).**

Gaussian Mixture Model

<u>**Determining the number components to fit the distribution:**</u>

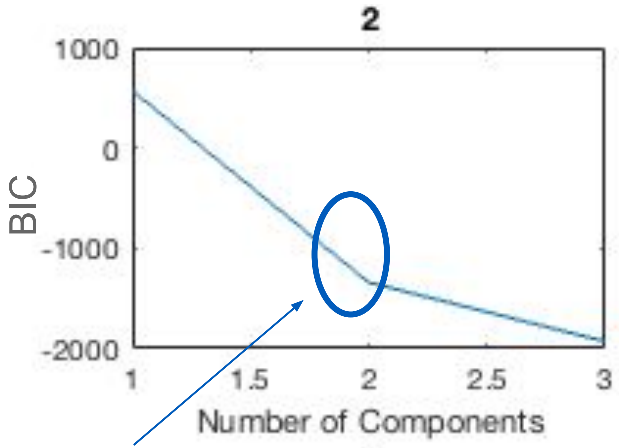
Bayesian Information Criteria (BIC):

The "ideal" number of components will minimize the BIC when compared to other models.

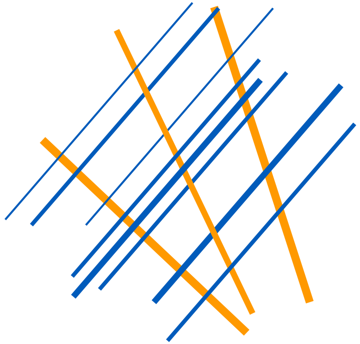
$$BIC = -2\ln(L) + C\ln(k)$$

L = Maximum value of the likelihood function for a model

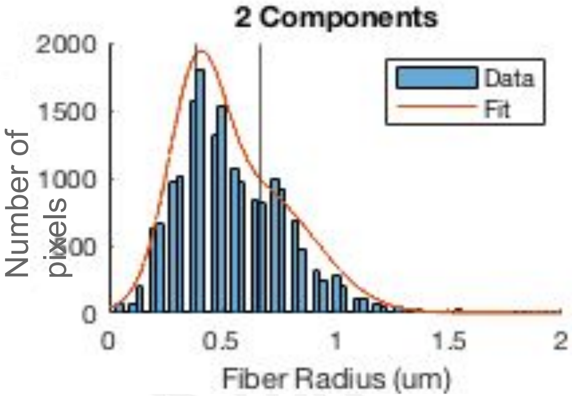
k = Number of components



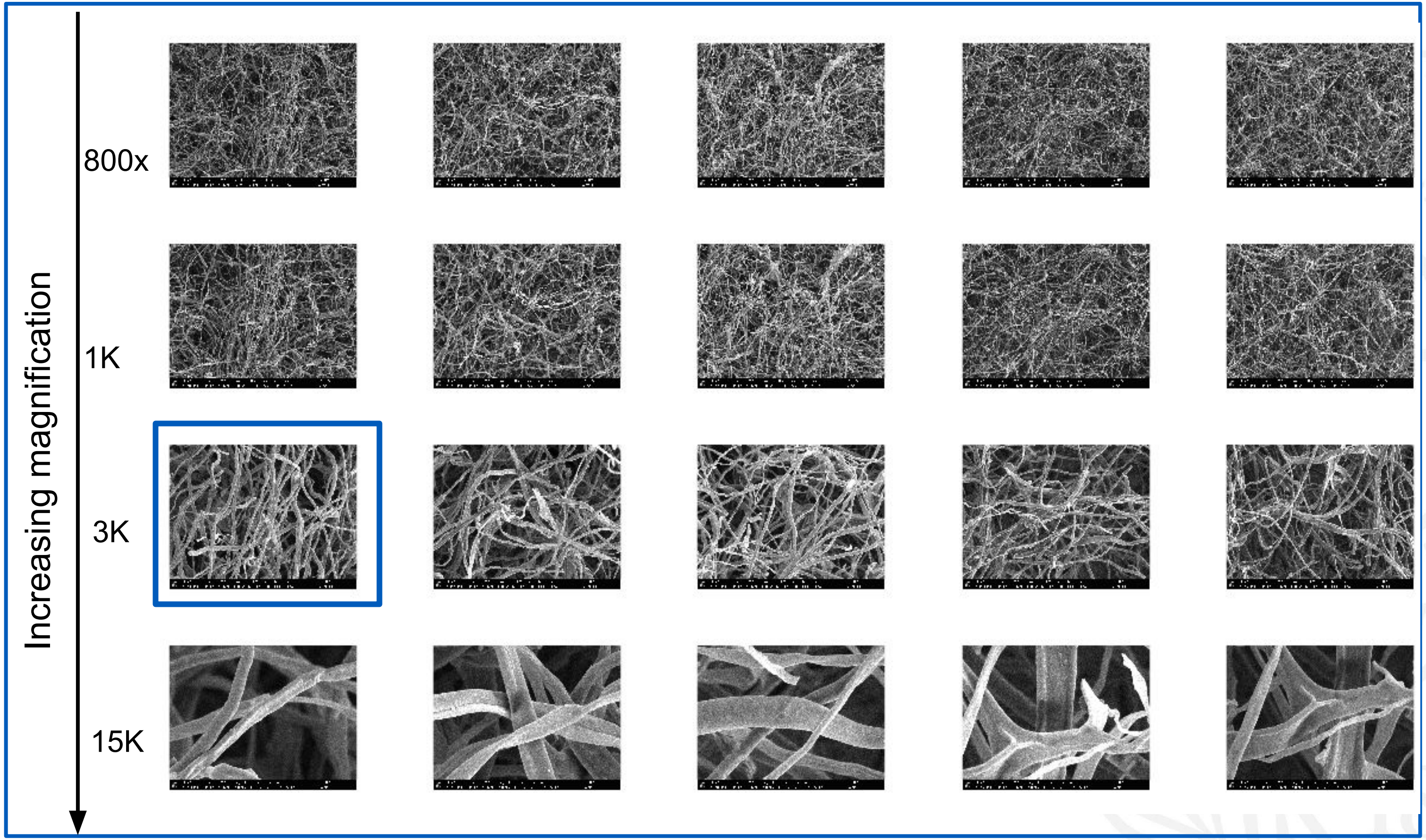
"Elbow rule" – optimized components



Two Gaussians centered around 0.4 and 0.6 um appears to be a reasonable fit.



Impact of image magnification on fiber radius



Impact of image magnification on fiber radius

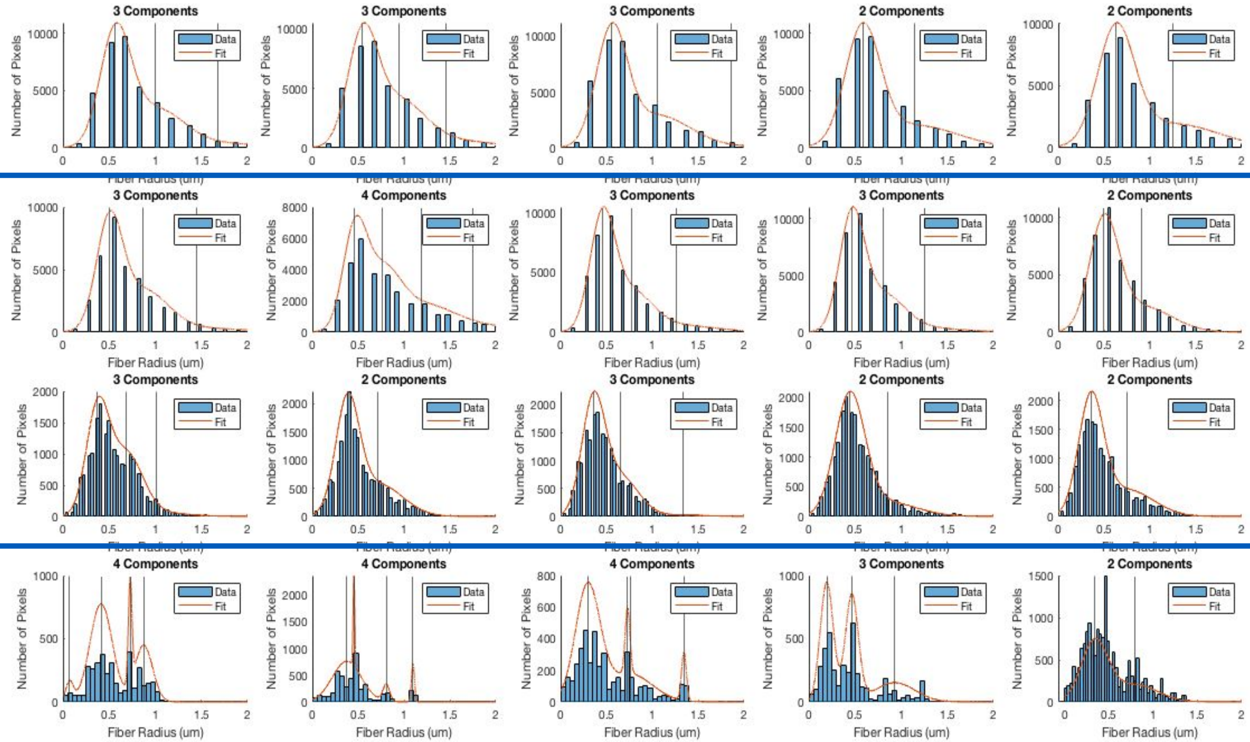
Increasing magnification

800x

1K

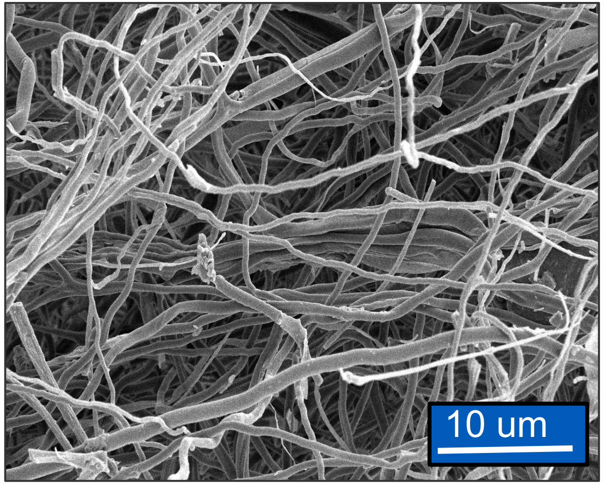
3K

15K

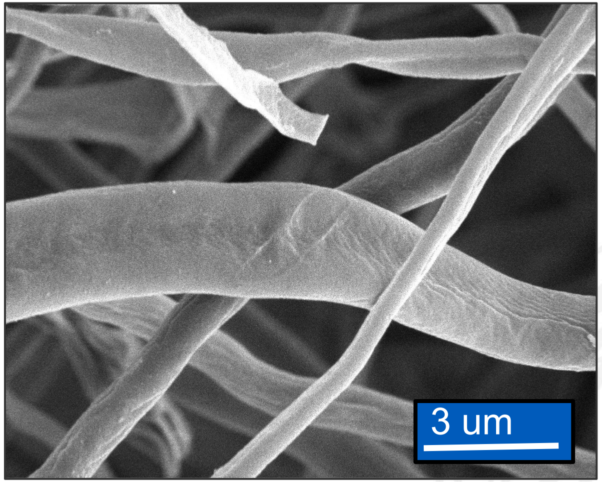


- Mean fiber radii are found around 0.4 and 0.8 um at all magnifications.
- Additional fiber radii are found at 0.2 and 1.2 um especially at higher magnifications

Impact of image magnification on fiber radius



3K magnification of mycelium



15K magnification image of mycelium

The smallest detected fiber radii is ~0.2 um.

At 800x, 1 pixel = 0.16 um

At 1K, 1 pixel = 0.14 um

- 800x and 1K data satisfies the resolution limit for fibers of 0.2 um, but have large bin sizes.
- 15K data contains usually three to four fibers per image □ prevents generalizations of networks

3K images are chosen as optimum representative image for further analysis

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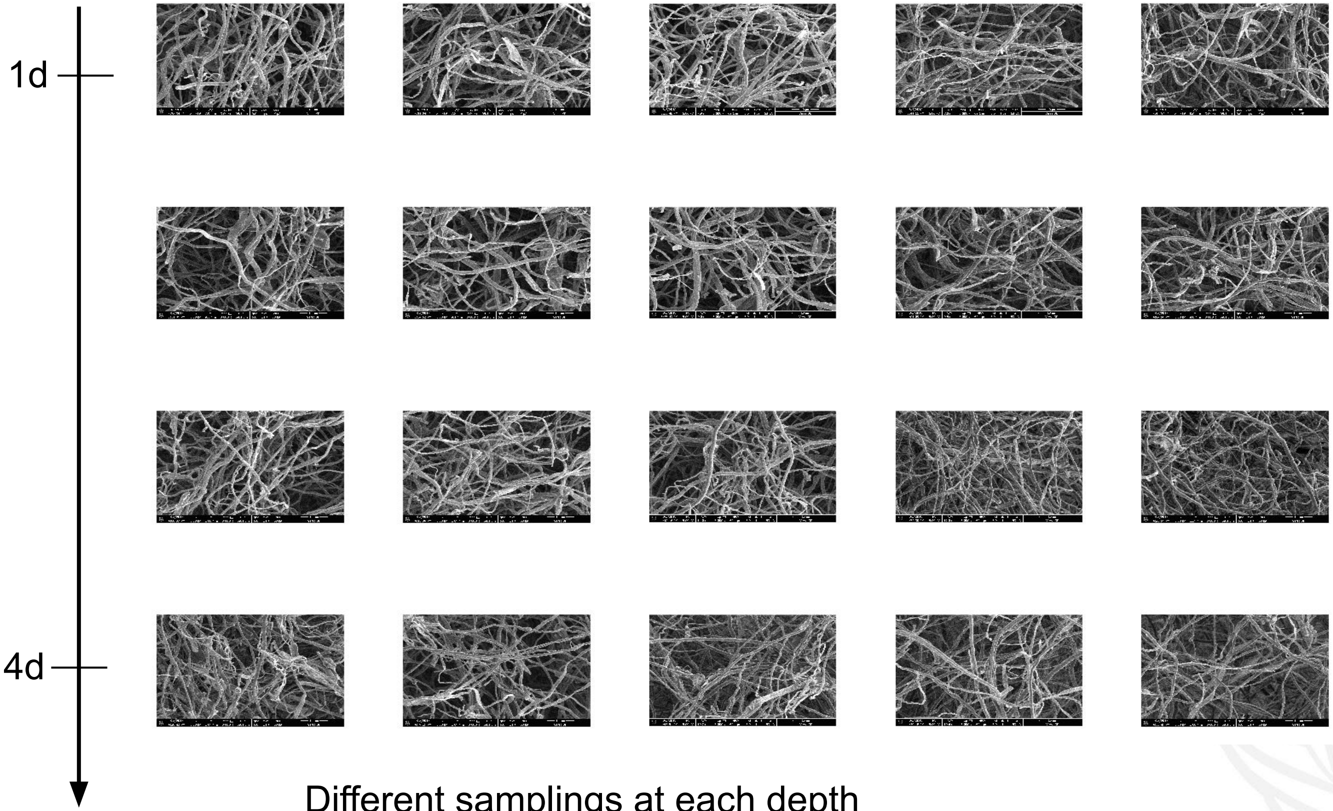
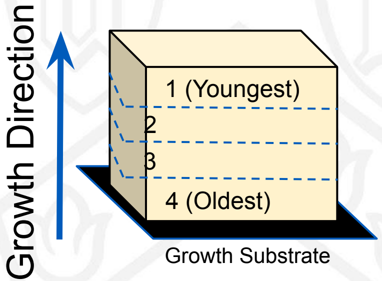
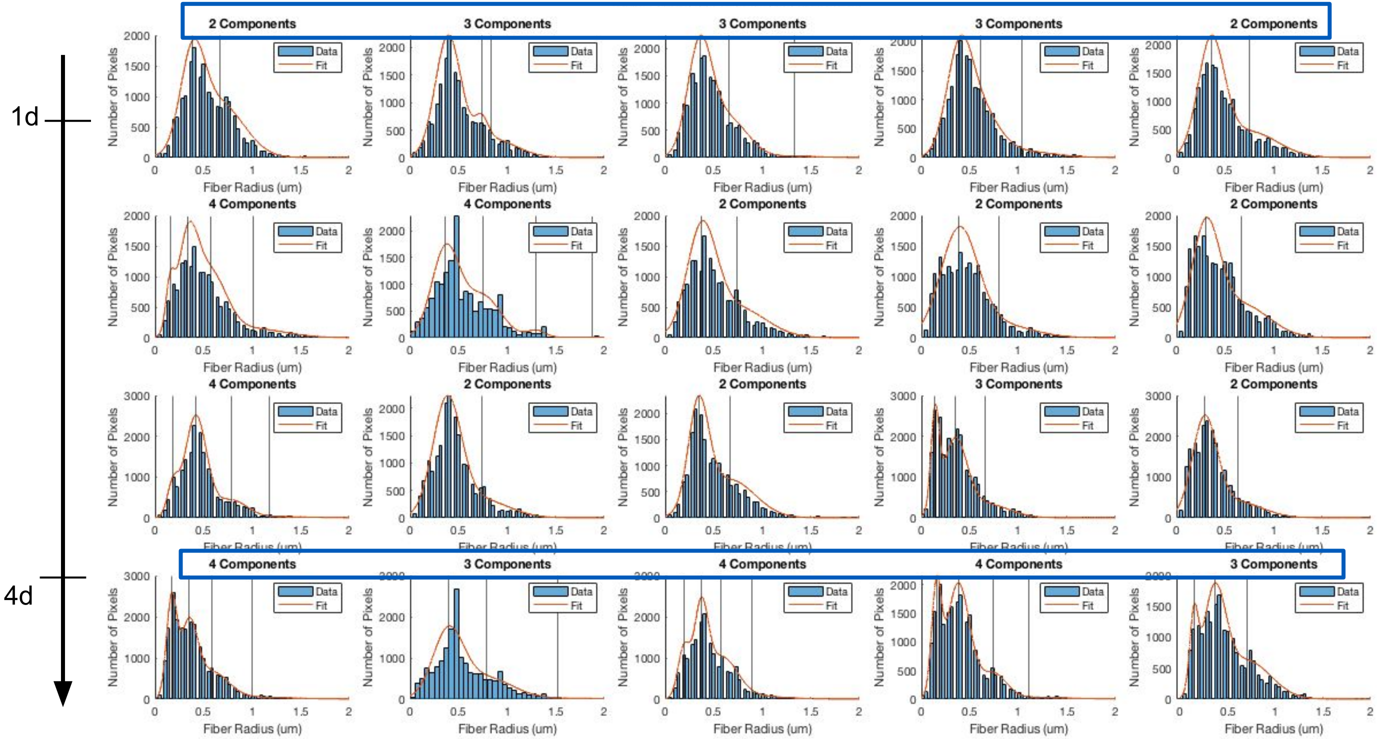
Depth dependence (fiber growth) on Fiber Radius



Different samplings at each depth

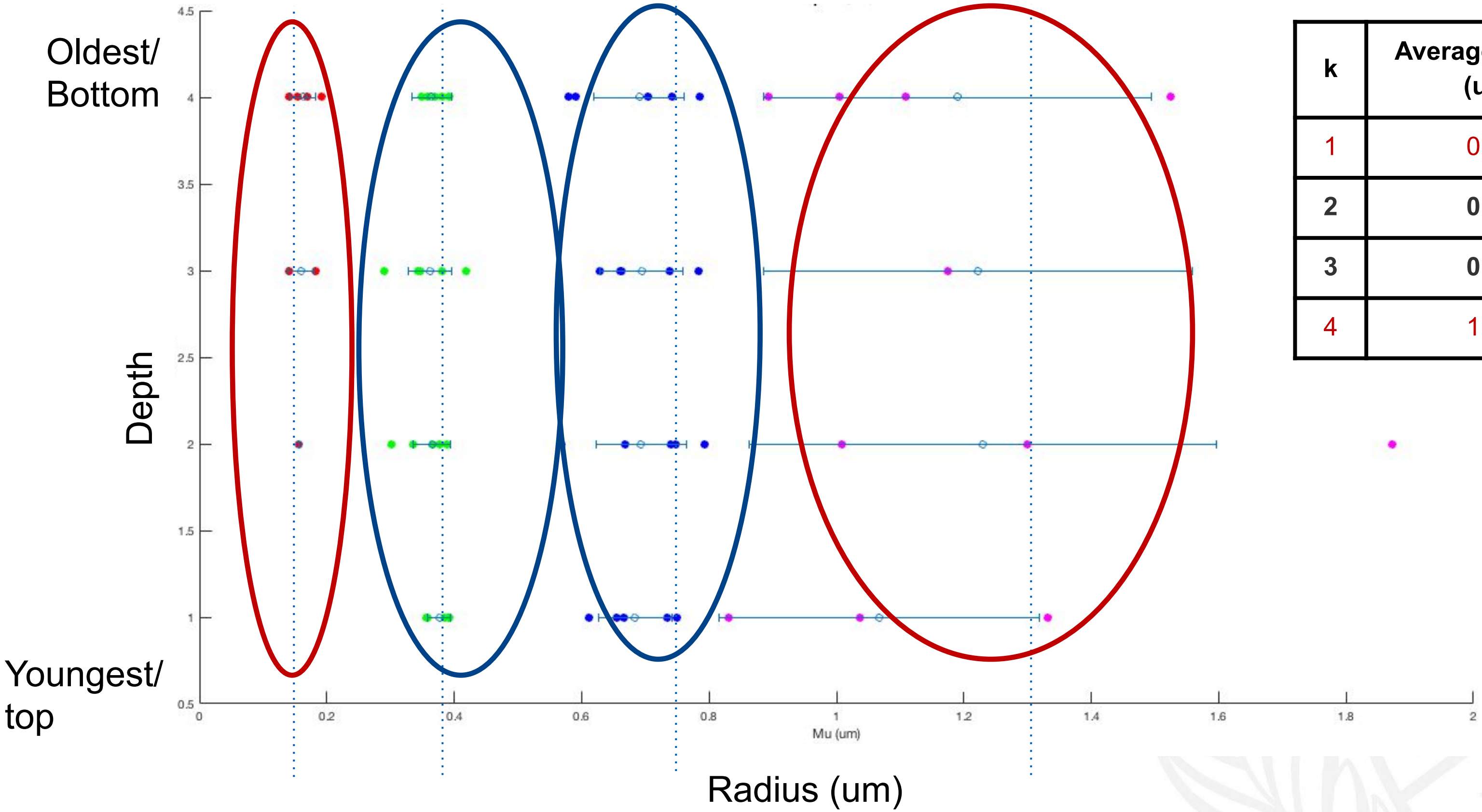


Depth dependence (fiber growth) on Fiber Radius



- With increase in depth (older fibers) an increase in the number of components are observed ~ 0.2 and 1.2 um.

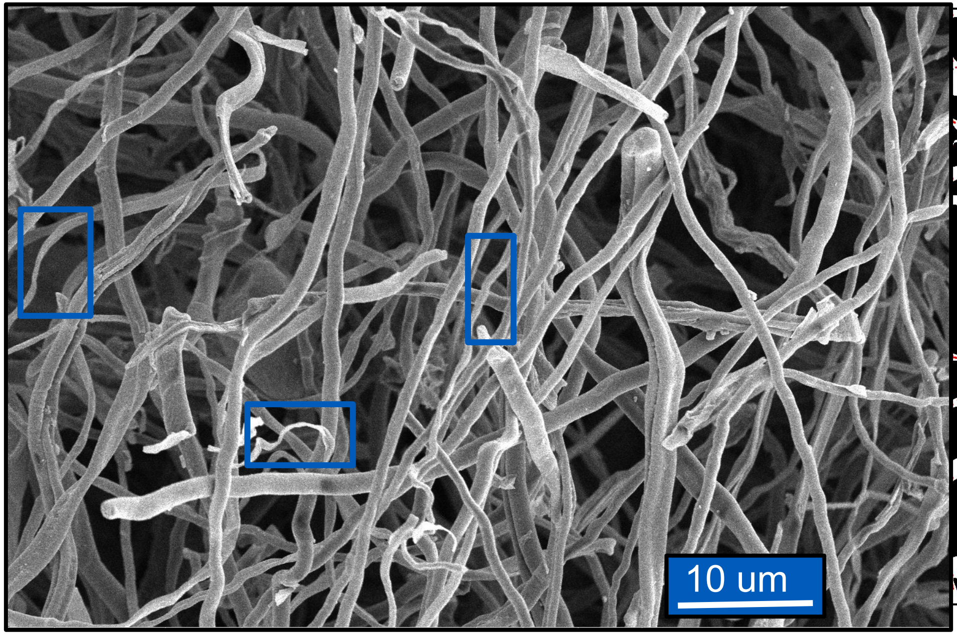
Depth dependence (fiber growth) on Fiber Radius

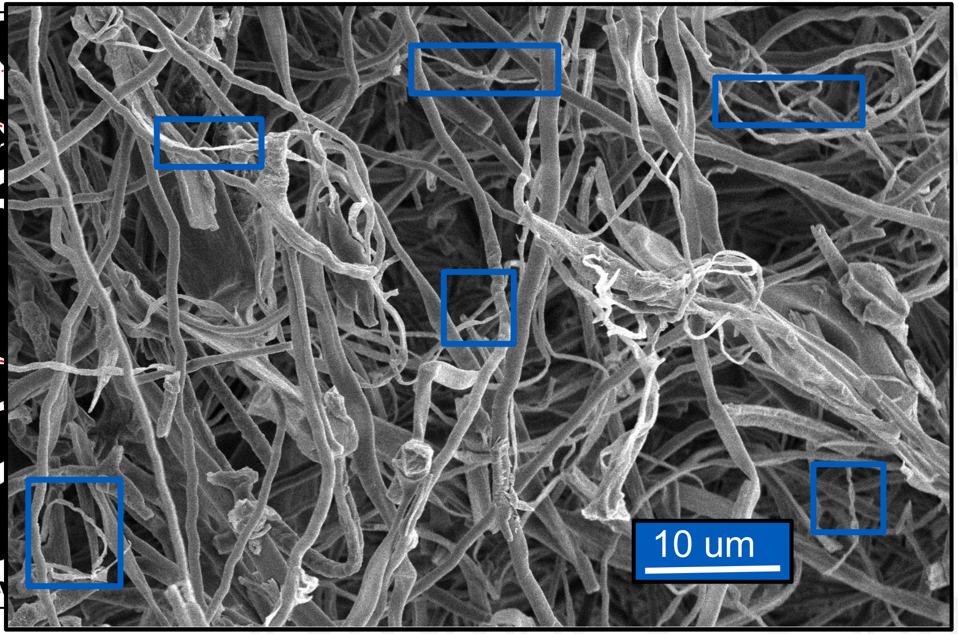


k	Average Radius (um)	Stdev
1	0.17	0.0268
2	0.38	0.0358
3	0.72	0.0873
4	1.23	0.2229

Representation of identified fibers

~ 0.15 - 0.2 um

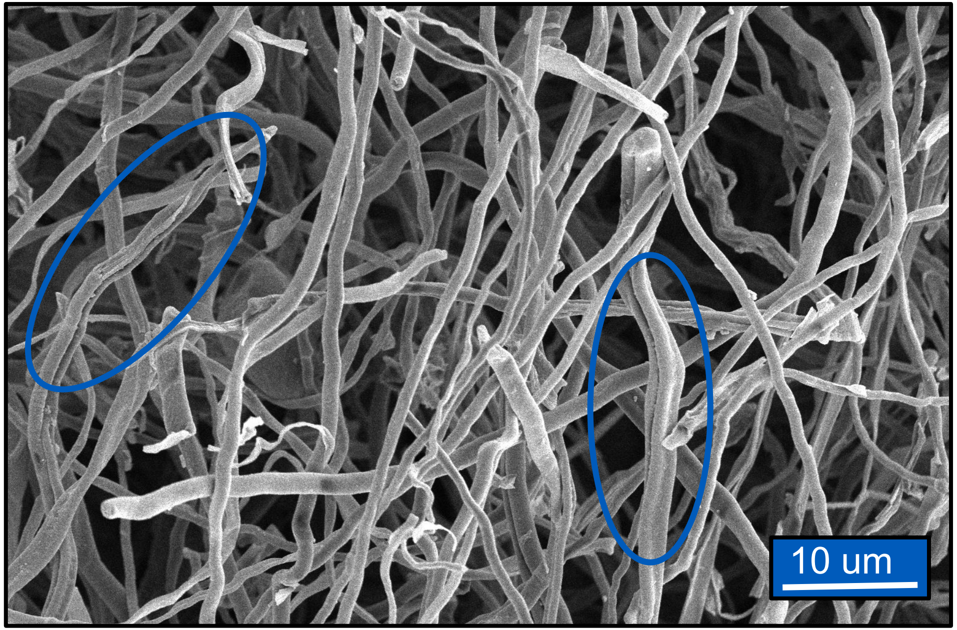




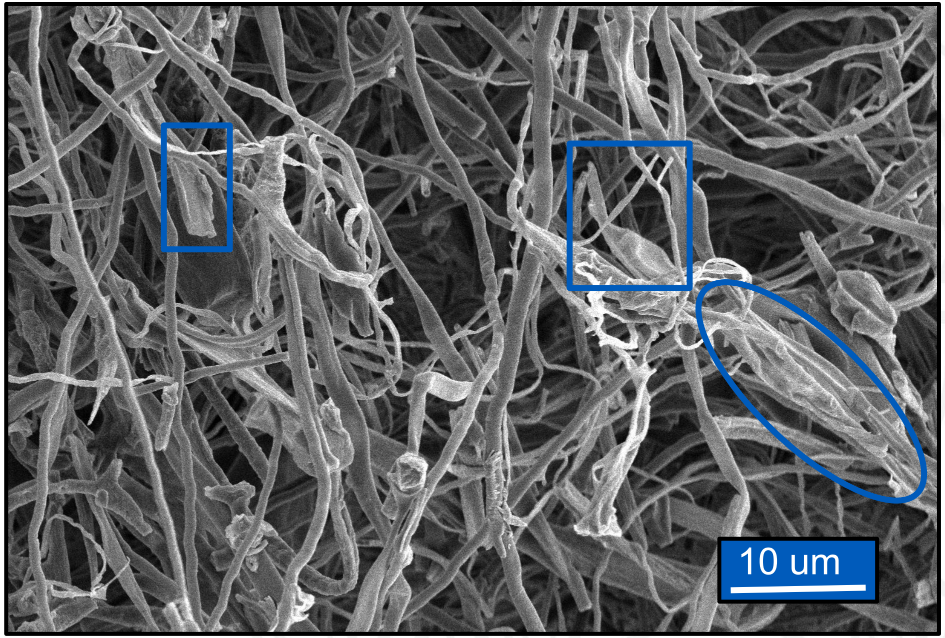
Fewer fibers with radii varying between **~ 0.15 - 0.2 um were observed in depth 1 (younger) than depth 4 (Older)**

Representation of identified fibers

0.94 - 1.46 um



1d



4d

Conclusions

Developed a Methodology for the analysis heterogeneous biological structures under any growth condition.

Above: light fixtures using mycelium

Quantified Fiber Radii in the network:

- Identified key fibers existing at 0.4 and 0.8 um.
- 0.2 and 1.2 um clusters were observed in older samples.

Quantified the increased structural complexity with growth

As the sample gets older, there is an increase in the number of components – branching modes seem to vary with growth.

Product Design Recommendation

Homogenous structure needs younger growth conditions.

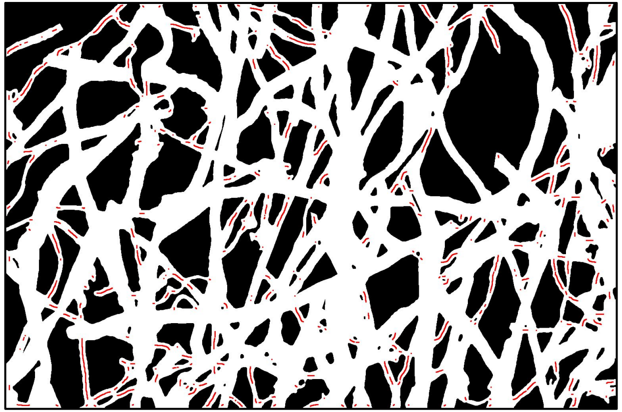
Future Work:

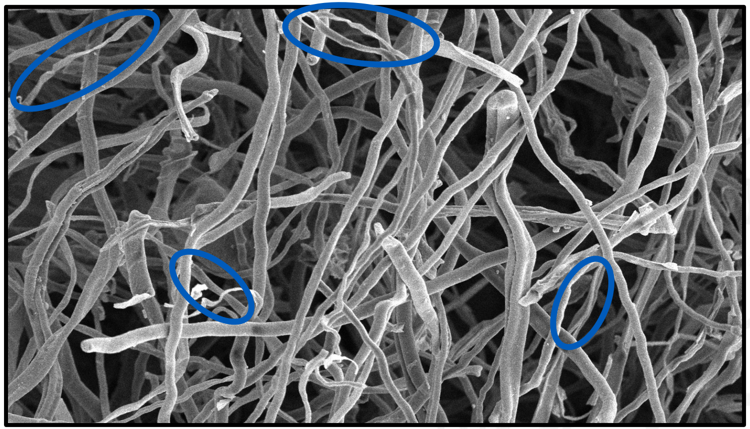
- Employing Deep Learning methods to improve fiber classification
- Quantify relative fiber frequency (mixing factor)
- Quantify pore area, fiber orientation, intersection density
- Building structure-property correlations by measuring filtration measurements.

Thank you

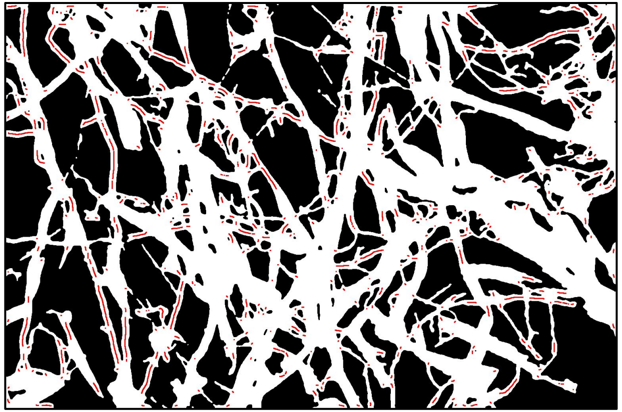
0.4 um

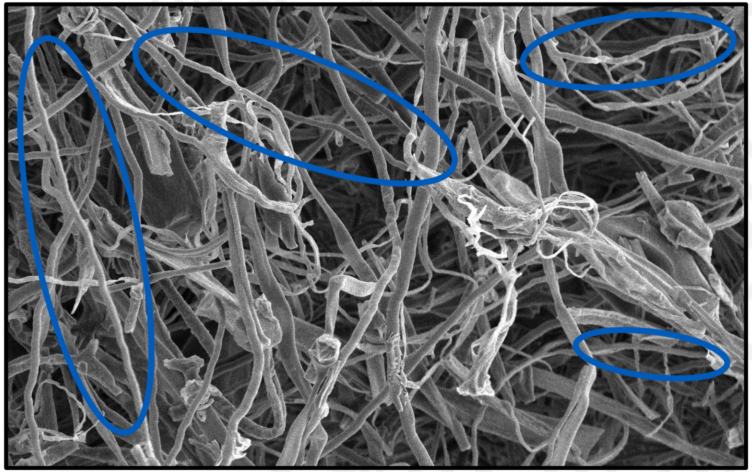
1d





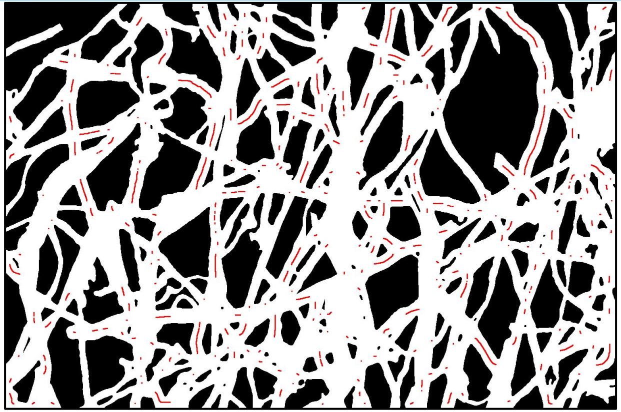
4d

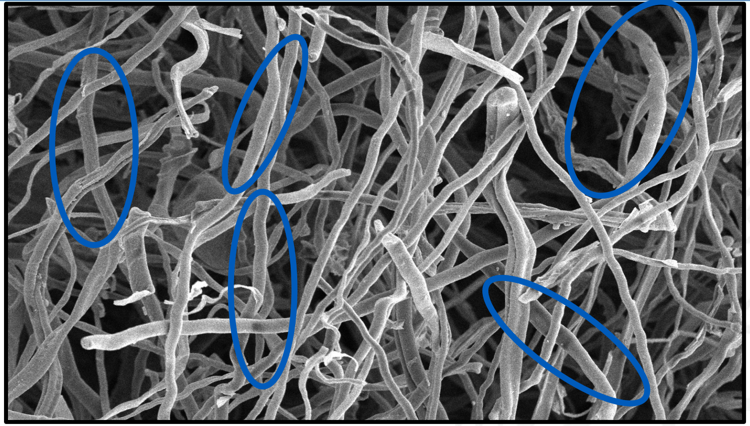




0.8 um

1d





4d