

"FiBar": a Tool for Automated Analysis of Complex Biomaterials from Microscopy Images

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Introduction

Complex biomaterials contain fibers as well as some other compound, like bacterial cells (refer to Fig 1 for an example). In order to assess the quality of the material, researchers often measure fiber diameters as well as the viability of bacterial cells [1]. This being said, both approaches are often done manually which is rather low-throughput and biased [2]. The image processing tool "FiBar" aimed to automate both techniques completely with very little input from the user.

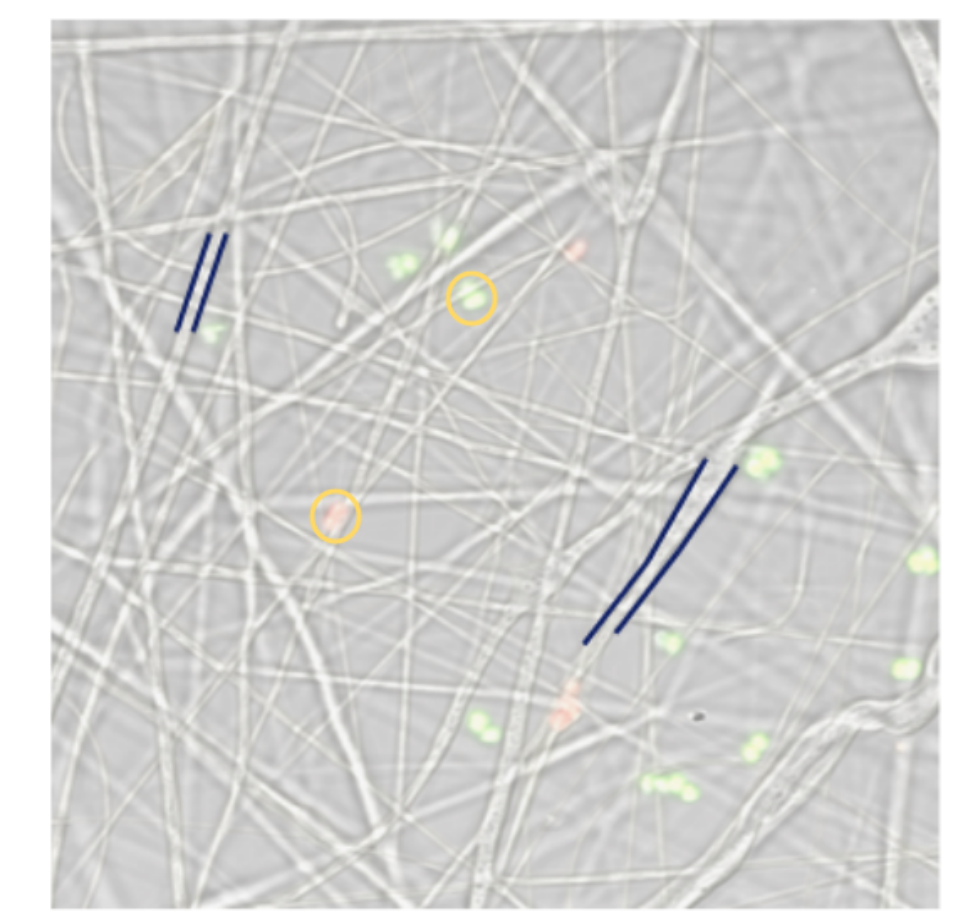
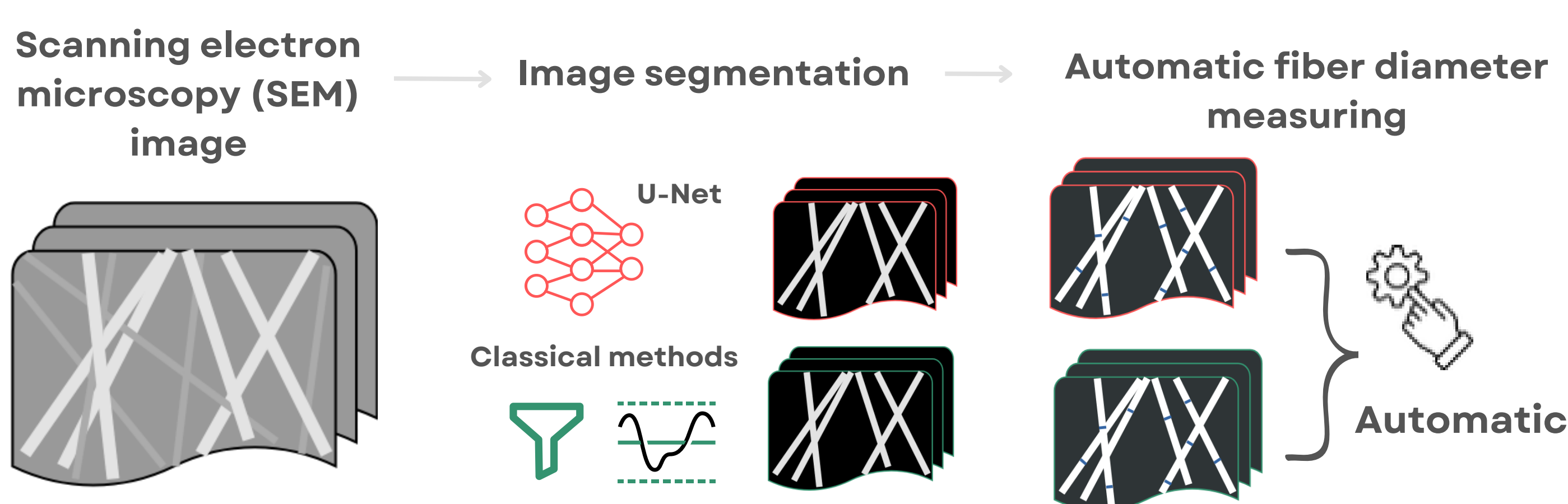


Fig 1. Example of a complex biomaterial with fibers (outlined with dark blue lines) and fluorescent bacteria (golden circles).

Fiber analysis pipeline



U-Net model approach: original network trained with 90 SEM subimages.
Classical approach: Kuwahara filter (3x3 window), Huang thresholding and median filter (15x15 window).

Automatic fiber diameter measuring: random start point selection, diameter line drawing and pixel-to-nm conversion using Pytesseract's OCR Engine.

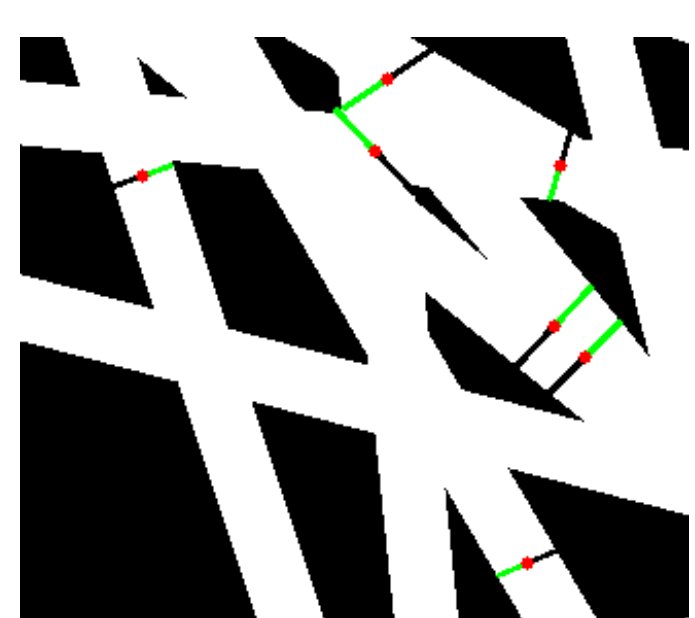


Fig 2. Automatic diameter measurements visualised - black and green segments show the diameters' first and second half, red is the midpoint of the diameter.

Images of simulated fibers of different diameters (10-30 pixels) and fiber styles

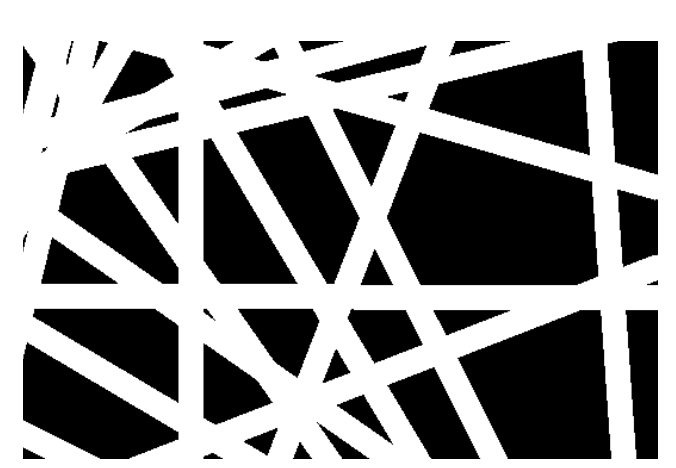


Fig 5. Straight fibers example.

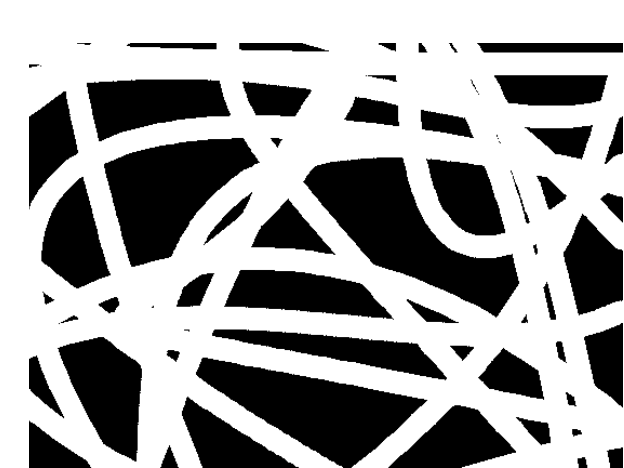


Fig 6. Curved fibers example.

Real scanning electron microscopy (SEM) images

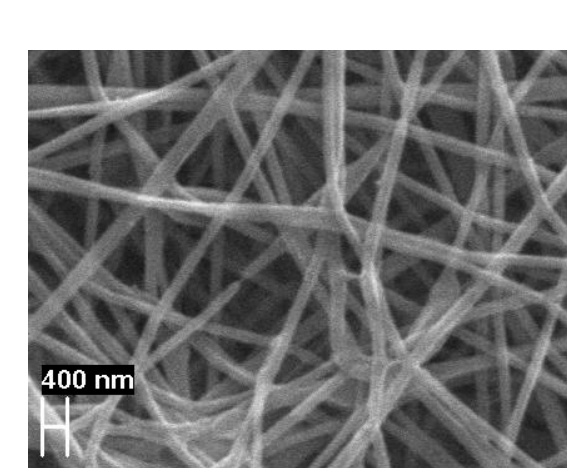


Fig 7. SEM image example.

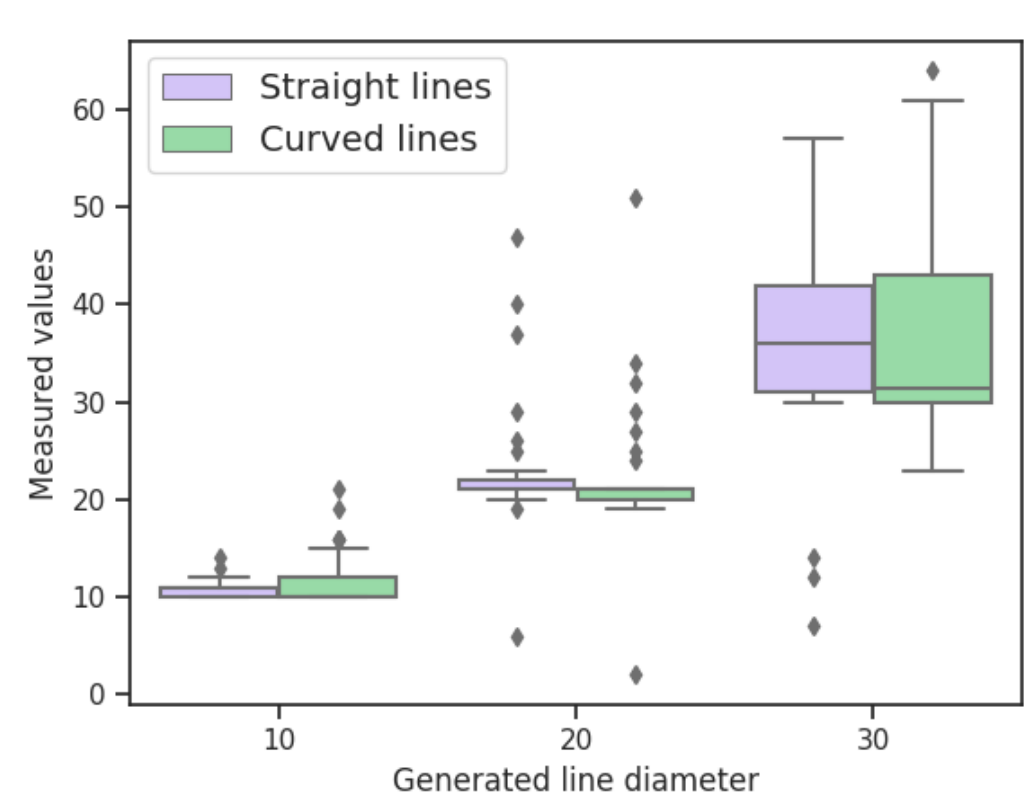


Fig 10. Automatically measured diameters (px) compared to the generated line diameters (10,20,30 px).

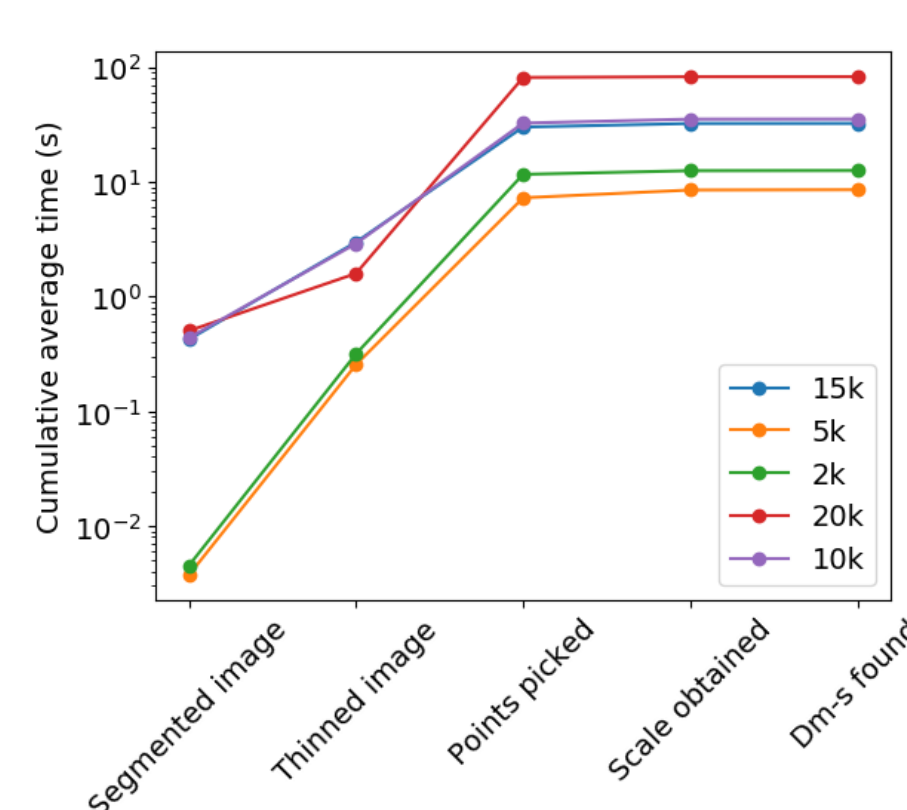


Fig 11. Time performance of every action in the fiber analysis pipeline (classical approach).

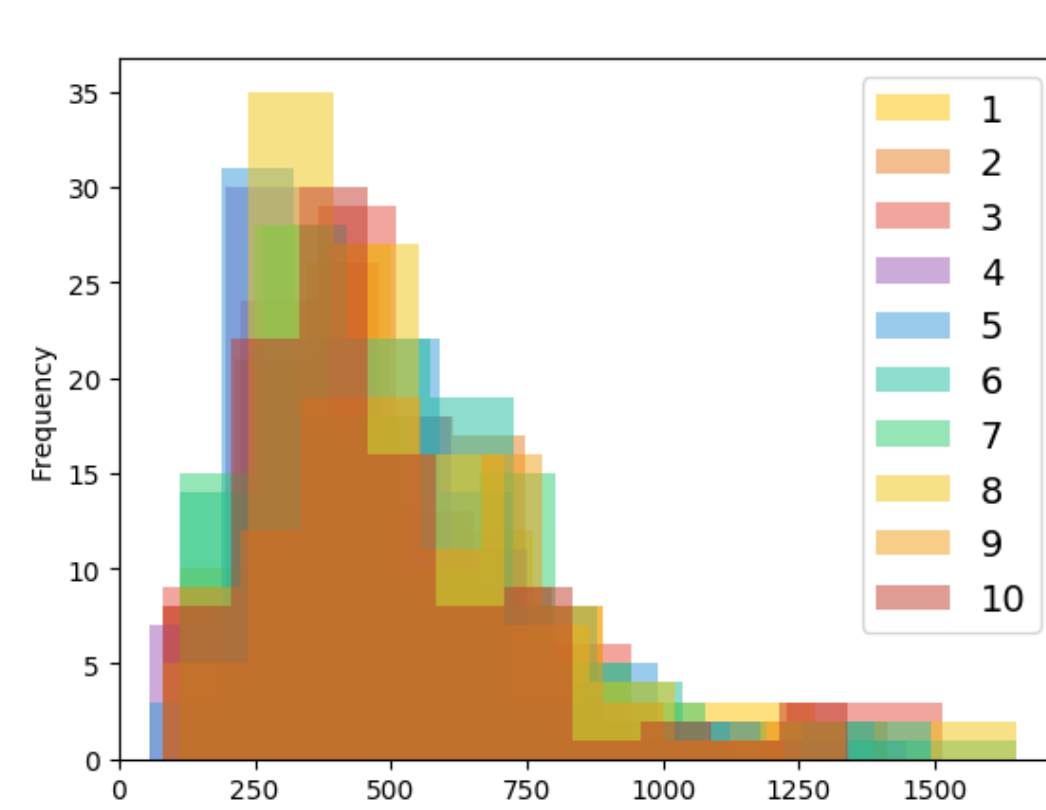
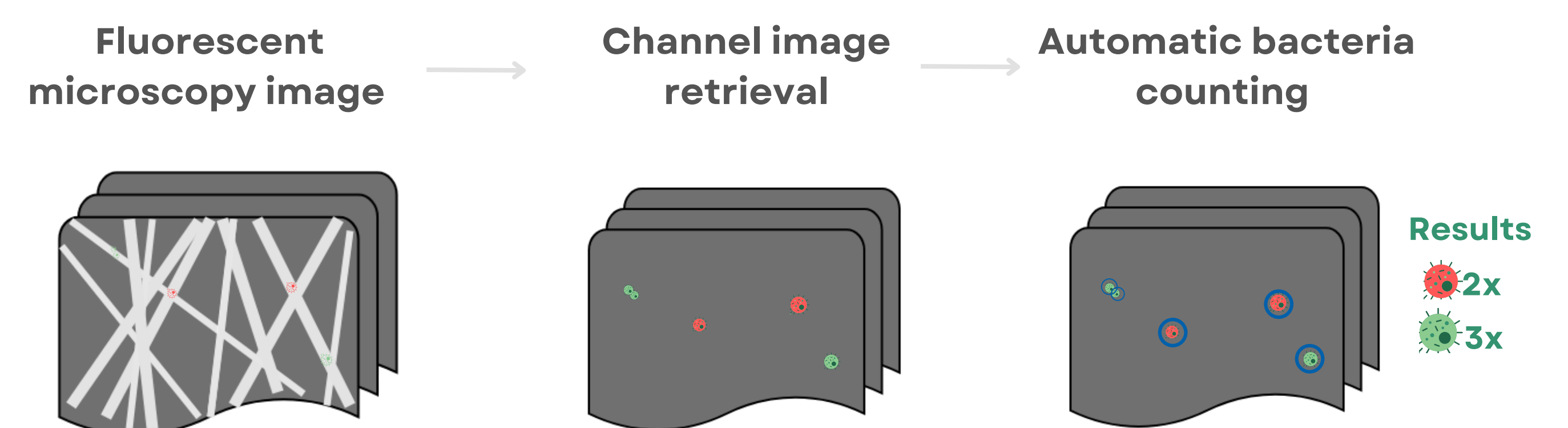


Fig 12. 10 iterations of 100 diameter measurements of a 10k SEM image.

Bacteria analysis pipeline



Red and green channel image retrieval: CZI files converted to image arrays using the AICSImageIO, czifile Python libraries and metadata info from the CZI file format design specifications.

Automatic bacteria counting: finding detected bacteria peaks (each peak is one cell) from the red and green channel images using image processing methods.



Fig 3. Red channel image of bacteria.



Fig 4. Detected bacteria peaks.

METHODS

Images with generated red and green bacteria (circle, ellipse shapes)

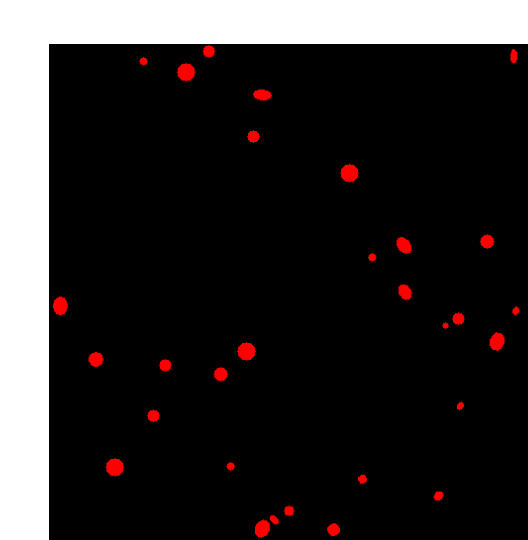


Fig 8. Simulated red bacteria.

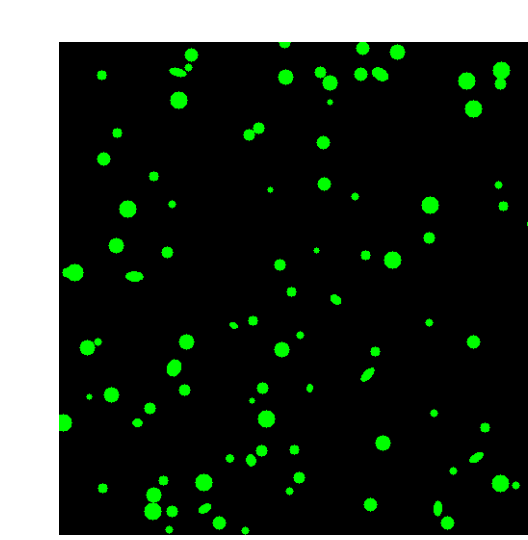


Fig 9. Simulated green bacteria.

CZI microscopy files with green and red bacteria provided by the Pharmaceutical R&D lab at the Institute of Pharmacy.

DATA

RESULTS

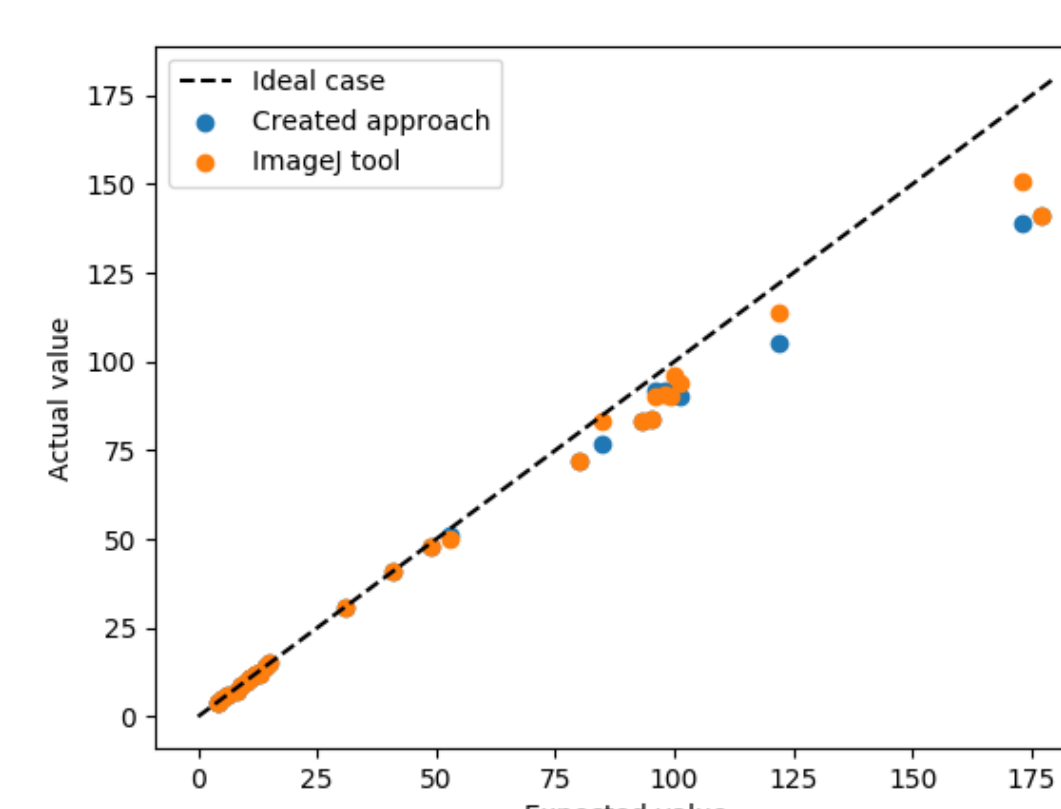


Fig 13. Automatic bacteria counting performance compared to the ImageJ Particle Analyzer tool.

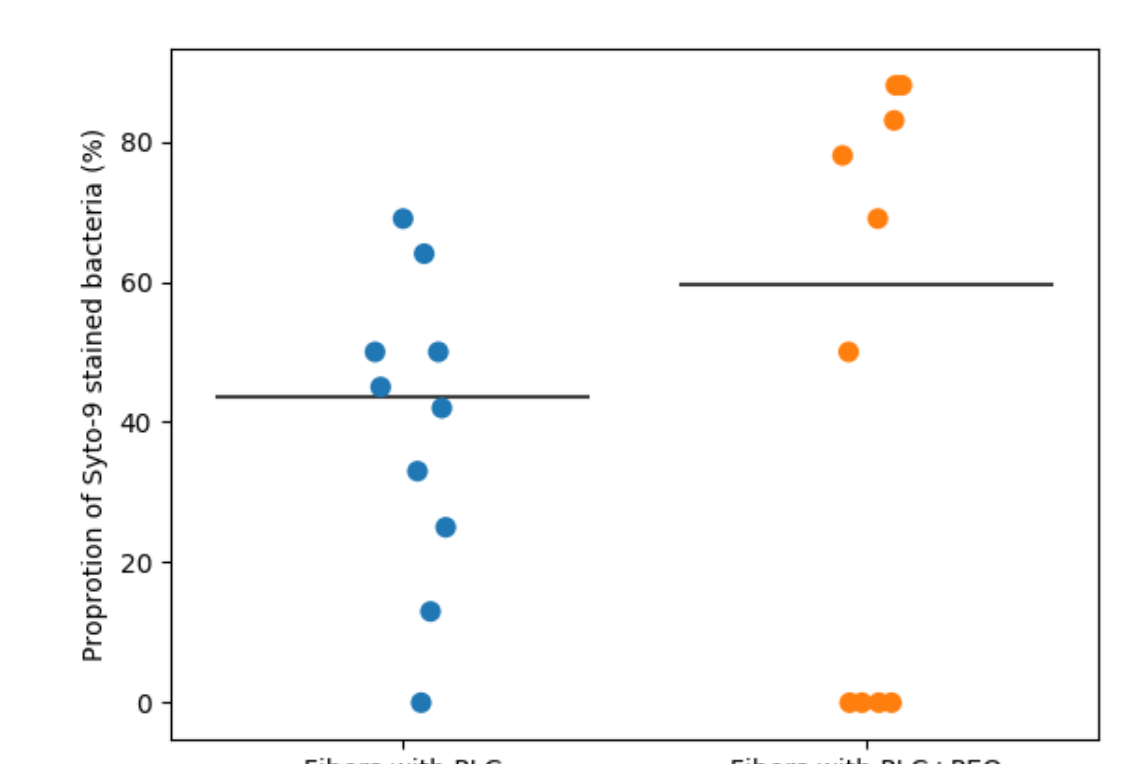


Fig 14. Bacteria analysis pipeline applied in a biological case study - effect of PEO on SYTO-9 (green) bacteria.

"FiBar" is easy-to-use:



References

- [1] Lanno et al., 2020, Antibacterial porous electrospun fibers as skin scaffolds for wound healing applications. ACS Omega, 5(46):30011-30022.
[2] Götz et al., 2020, Computed fiber evaluation of SEM images using DiameterJ. Current Directions in Biomedical Engineering, 6(3):438-441.

