Supplementary Information for

Modular microscopy (ModMicro) solution for volumetric epifluorescence microscopic imaging

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Before beginning, note that this research can be reproduced combining the resources of
(1) the research article "*Modular microscopy (ModMicro) solution for volumetric epifluorescence microscopic imaging*",
(2) this supplementary file,
(3) Phase 2 files from https://github.com/AkhilKallepalli/ModMicroUofG, and
(4) ModLight light source as made available through "*Modular light sources for microscopy and beyond (ModLight)*" (1).

1. Hardware

The hardware in the modular microscopy (ModMicro) epifluorescence system adopts the OpenFlexure main body as the starting point. This fundamental choice is made based on the accessibility of the platform and the flexibility to make necessary changes in this study. Being the most up-to-date version of the OpenFlexure apparatus, this is the most appropriate model to use but the optical components of the epifluorescence microscope are interchangeable with the OpenFlexure Delta stage microscope with a customised base. In the following sections, we will detail the build and assembly instructions of the microscope system.

A. The Build.

1. To minimise delays, we suggest 3D printing all the necessary components whilst awaiting the delivery of parts listed in the **Bill of Materials**. We will divide the build process into two sub-builds:
    1. OpenFlexure (2, 3) components and software
    2. ModMicro assembly and ModLight (1)

A.1. OpenFlexure components and software.

(a) Assembling the OpenFlexure parts using the OpenFlexure instructions and treating "**Base1**" as an OpenFlexure base is the most effective way to build this microscope. By selecting the "Build a Microscope" instructions and following the "Build the high-resolution microscope" subsections:
    1. Prepare the main body
    2. Assemble the actuators
    3. Assemble the motors
    4. Attach the sample clips
    5. Mount the optics and the microscope (Step 2: Mount the microscope only)
    6. Assemble the illumination (following step 1 and step 2 with "illumination_dovetail" and "condenser", respectively).

(b) Installing the software for your Raspberry Pi and Computer should again be done through OpenFlexure. The Software section contains "Raspbian-OpenFlexure" and "OpenFlexure Connect".
    1. Raspbian-OpenFlexure should be installed on the SD card for the Raspberry Pi 3B+.
    2. OpenFlexure Connect should be installed on the users' computers to control the stage and camera.

(c) The Motor Controller Board/ Sangaboard assembly instructions can be found in the "Build a Microscope" instructions and follow the "Build the high-resolution microscope" subsection "Complete the wiring".

A.2. ModMicro assembly and ModLight. This section of the instructions focuses on the assembly and integration of ModMicro into the OpenFlexure microscope, as well as the integration of ModLight.

(a) Once the OpenFlexure components are secured onto "**Base1**". Place and secure "**Base1**" to "**Slab_Lasercut2**" with four M6 screws and hold this platform 125mm above the breadboard. To hold the platform at 125mm, use four "**pedestal_posts**" that will be secured to "**Slab_lasercut2**" with four M6 screws.

(b) Assembling the components in the optical path, First secure magnets, with glue, to the top of "**Cube1**" and the top and bottom of "**Cube2**", making sure the magnetic poles are aligned before securing them in place.

(c) Secure magnets to "**Tube_lens_holder**", again making sure the magnetic poles are aligned with the top of "**Cube2**", and insert 125mm Tube lens.

(d) Attach Raspberry Pi Camera to "**Cube1**" with two No 2 6.5mm self-tapping screws and "**pi_camera_cover**". Detailed instructions in "Build a Microscope" instructions and the following "Build the high-resolution microscope" subsection "Assemble the high-resolution optics module" will provide the necessary details to do this.

(e) Insert the Mirror into "**Cube1_mirror_holder**" and slide into the bottom of "**Cube1**".

(f) Insert Dichroic mirror and emission filter into "**Dichroic_filter_holder**" and "**Excitation_filter_holder**", respectively. Then slide "**Dichroic_filter_holder**" into "**Cube2**" at 45 degrees and "**Excitation_filter_holder**" horizontally.

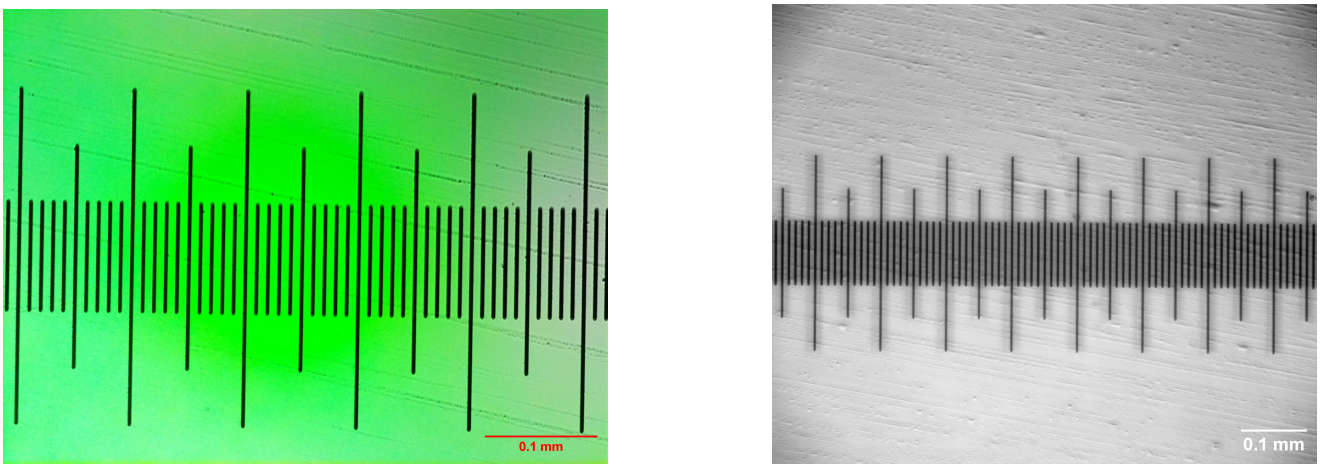

Fig. S1. A micron ruler imaged to determine the field of view of ModMicro, shown on the left and the Nikon Ti Eclipse, as shown on the right. ModMicro is in a transmission set-up with an emission filter and dichroic mirror in the optical path. The bottom right of the images has a scale bar of 0.1 mm.

(g) Screw in your objective to the "**Objective_attachment**" following "Build the high-resolution microscope" subsection "Assemble the high-resolution optics module" step 5 and step 6 and attach to the "**Main-body**" as detailed in "Build a Microscope" instructions and following "Build the high-resolution microscope" subsection "Mount the optics and the microscope" step 1.

(h) Magnetically connect "**Cube1**" and "**Cube2**" and align directly underneath the "**Objective_attachment**".

(i) Following the detailed assembly instructions for ModLight, your light source should be ready for use.

(j) Align ModLight to the middle of the dichroic mirror inserted into "**Cube2**". To do this attach the fibre to "**illumination_modified**" which you can mount to "**ModMicroTubeLens**" with two M3 x 10 mm screws. This can be held by one "**Slip Ring**" attached to "**Optical Post**" and "**Pedestal Post Holder**". Follow the OpenFlexure "Assemble the illumination" (following step 2) to insert the "**Condenser Lens**.

(k) Attach the Motor controller board to Raspberry Pi with MicroUSB and attach the monitor, keyboard and mouse to Raspberry Pi or attach an ethernet cable to the laptop and Raspberry Pi and power up. Again following OpenFlexure.

(l) Refer to HardwareX paper for ModLight.

B. Standard operating procedure.

1. It is important to maintain up-to-date software. Regularly update OpenFlexure Connect and Raspberry Pi packages.

2. Operating the system with OpenFlexure connect is explained clearly by OpenFlexure, specifically following the "Microscope Use" section.

3. Taking z-stack images, set x and y step values to 1 and z-steps no smaller than 50. The autofocus should not be used when taking a z-stack.

4. Before using the autofocus control, for regular image taking, manually find the optimal focus of the objective. Do this by having the whole system aligned and switched on, with an M2.5 screwdriver placed into the M2.5 screw attaching the "**objective_attachment**" to the "**main-body**", manually raise or lower this screw to find the optimal focus, secure in place making sure not to secure the "**objective_attachment**" too tight so the objective remains vertical in the optical path.

5. The system is easier to align and optimise when in a transmission microscope set-up and can easily be switched to epifluorescence by moving ModLight.

C. ModMicro testing and field of view determination.

1. In order to obtain the field of view for ModMicro and the Nikon Ti Eclipse, a micron ruler slide was used.

    1. We inserted ModLight into the transmission set-up for ModMicro and placed the micron ruler slide onto the stage and secured it with the sample clips.

    2. The transmission set-up of ModMicro uses "**condenser**" that inserts into "**illumination_dovetail**". Follow OpenFlexure instructions "Build a Microscope" and "Build the high-resolution microscope" subsection "Assemble the illumination" for more detail.

3. An image was then taken and processed with ImageJ (4) to measure the number of pixels across a known length.

4. Using a straight line tool a line was drawn between two markings on the micron ruler which has a known distance of 0.1 mm. The "Set scale" tool was used to calculate the number of pixels between this known length, which can then be used to calculate the full field of view of the system.

5. The process was repeated with the Nikon Ti Eclipse microscope and processed the same way with ImageJ. Figure S1 shows the micron ruler imaged by both microscopes in a transmission set-up.

2. Imaging results and discussion (Zones 1-3)

1. The line intensity profiles of Zone 1 were imaged with ModMicro, ModMicro with a sharpness enhancement algorithm and a Nikon Ti Eclipse.

2. The line intensity profiles of Zone 2 were imaged with ModMicro, ModMicro with a sharpness enhancement algorithm and a Nikon Ti Eclipse.

3. The line intensity profiles of Zone 3 were imaged with ModMicro, ModMicro with a sharpness enhancement algorithm and a Nikon Ti Eclipse.

4. The line intensity profiles plotted position (mm) against normalised intensity.

5. Sharpness plot of each Zone with a sigmoidal function fitted.

References

1. GM Gibson, R Archibald, M Main, A Kallepalli, Modular light sources for microscopy and beyond (modlight). *HardwareX* p. e00385 (2022).
2. JT Collins, et al., Robotic microscopy for everyone: the openflexure microscope. *Biomed. Opt. Express* **11**, 2447–2460 (2020).
3. S McDermott, et al., Multi-modal microscopy imaging with the openflexure delta stage. *Opt. Express* **30**, 26377–26395 (2022).
4. J Schindelin, et al., Fiji: an open-source platform for biological-image analysis. *Nat. methods* **9**, 676–682 (2012).

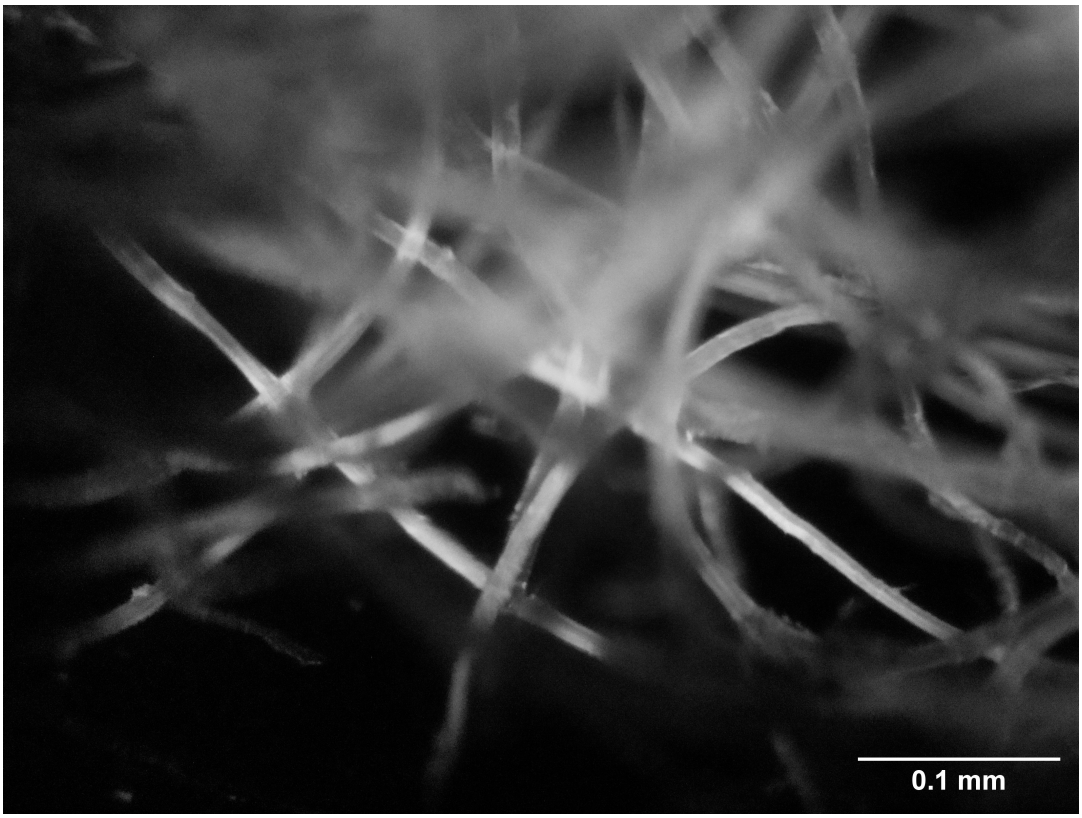

Fig. S2. Tissue paper dyed in fluorescence imaged with the ModMicro epi-fluorescence microscope and a 10x, 0.25 NA infinity corrected objective.

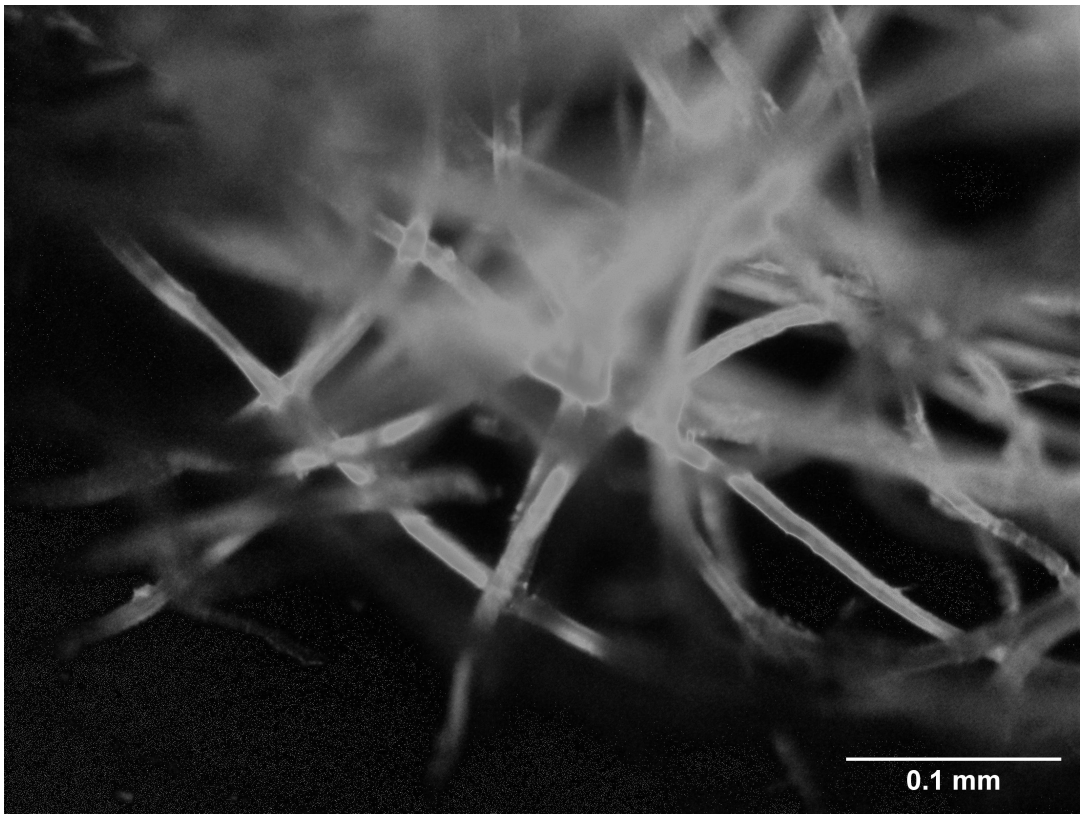

Fig. S3. Tissue paper dyed in fluorescence imaging with the ModMicro epi-fluorescence microscope and a 10x, 0.25 NA infinity corrected objective. A novel sharpness enhancement algorithm was applied to the images.

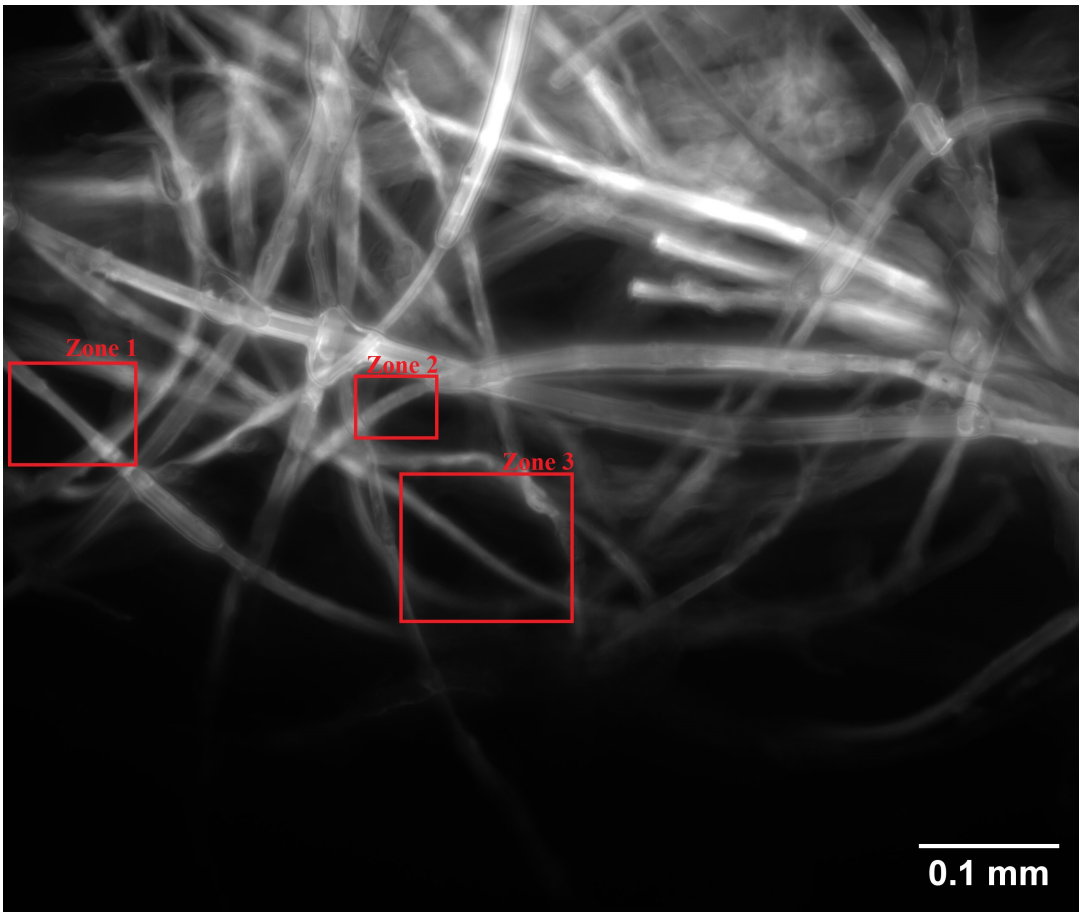

Fig. S4. Tissue paper dyed in fluorescein imaged with a Nikon Ti Eclipse and Zyla 5.5 sCMOS detector. The red boxes indicate where the intensity line profiles were taken of the sample both under the Nikon Ti Eclipse and the ModMicro.

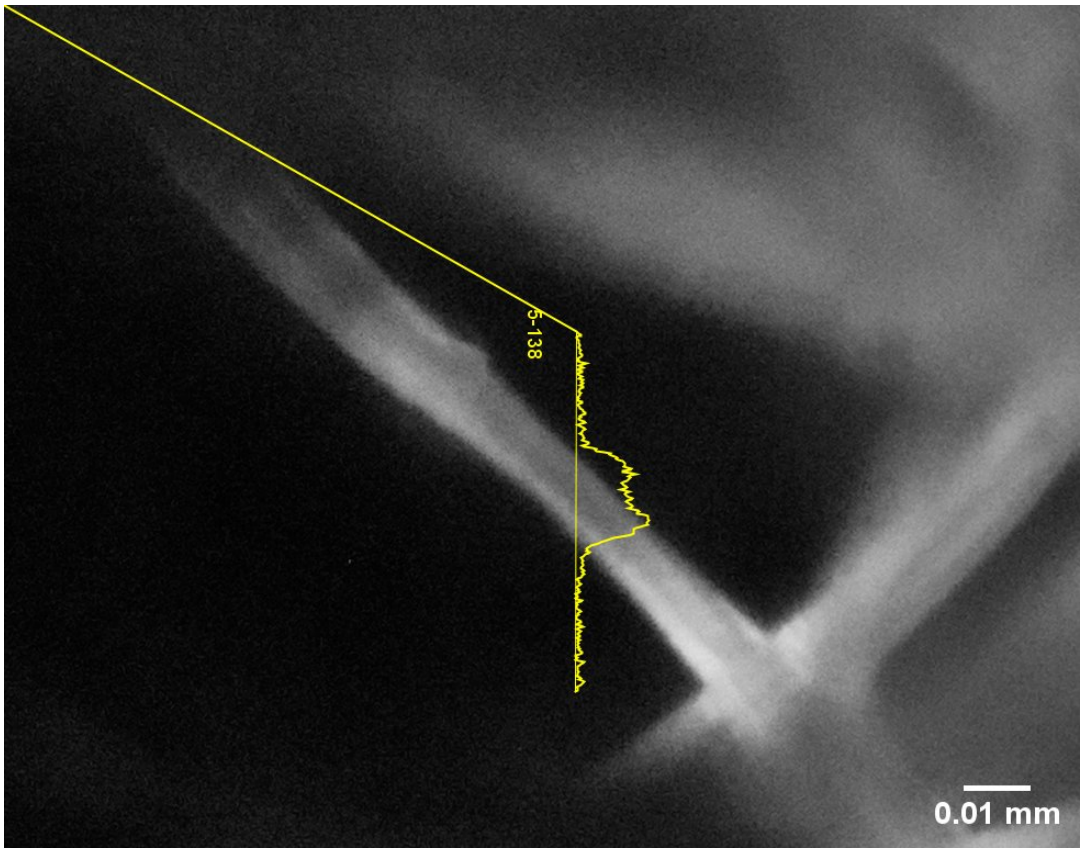

Fig. S5. Tissue paper dyed in fluorescein imaged with the ModMicro epi-fluorescence microscope and a 10x, 0.25 NA infinity corrected objective. An intensity line profile was taken in ImageJ of Zone 1.

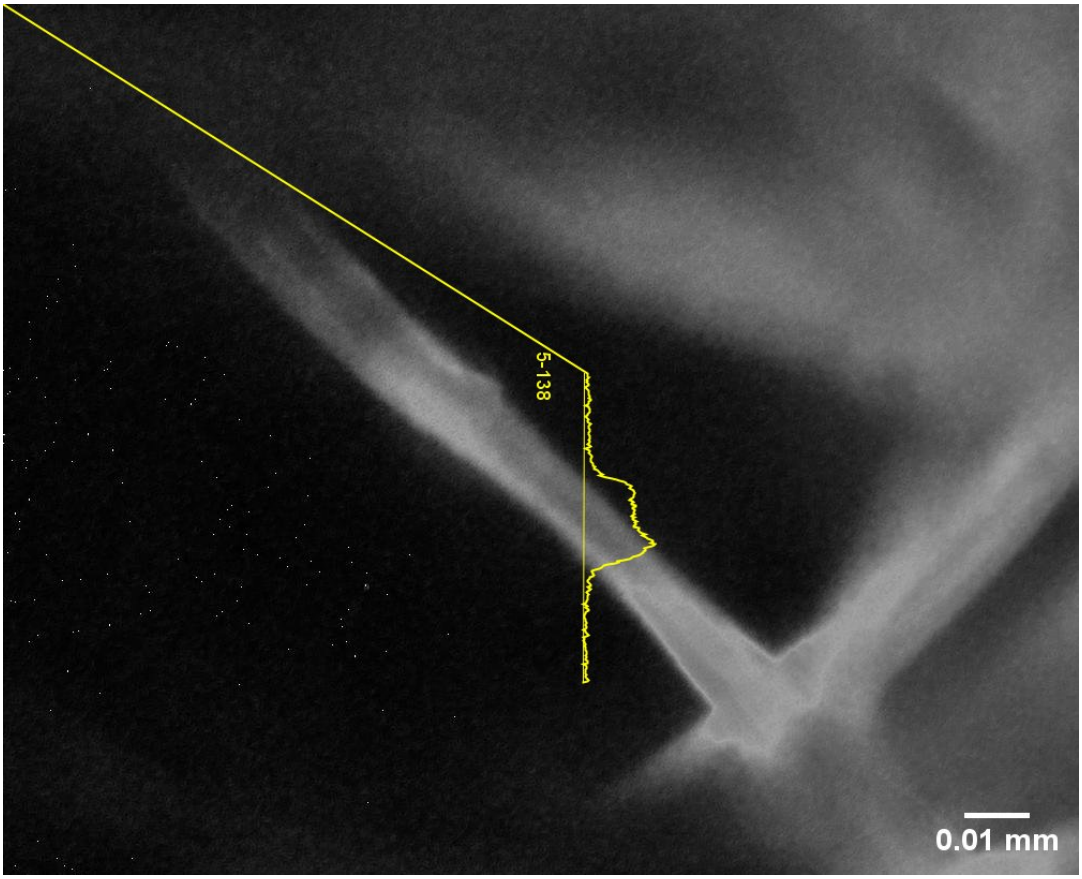

Fig. S6. Tissue paper dyed in fluorescence imaged with the ModMicro epi-fluorescence microscope and a 10x, 0.25 NA infinity corrected objective. A novel sharpness enhancement algorithm was applied to the images and an intensity line profile was taken in ImageJ of Zone 1.

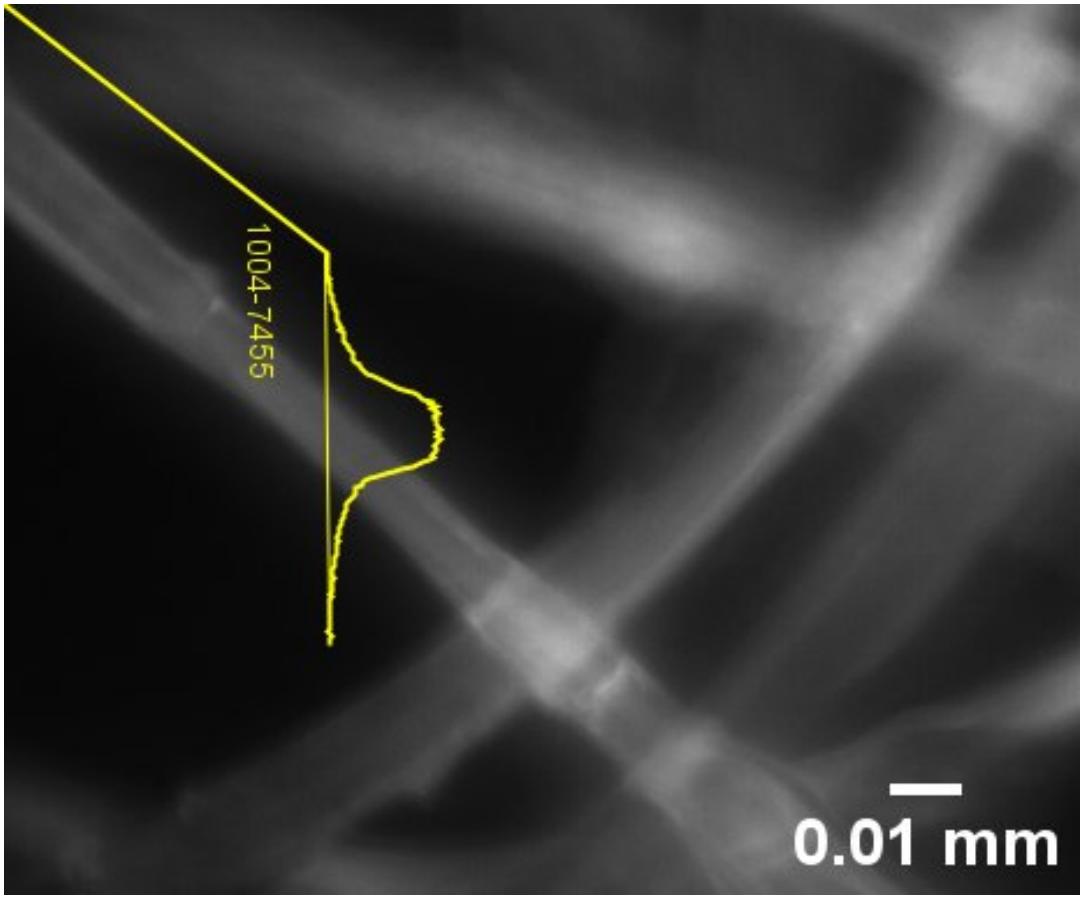

Fig. S7. Tissue paper dyed with fluorescein imaged with a Nikon Ti Eclipse and Zyla 5.5 sCMOS detector. An intensity line profile was taken in ImageJ of Zone 1.

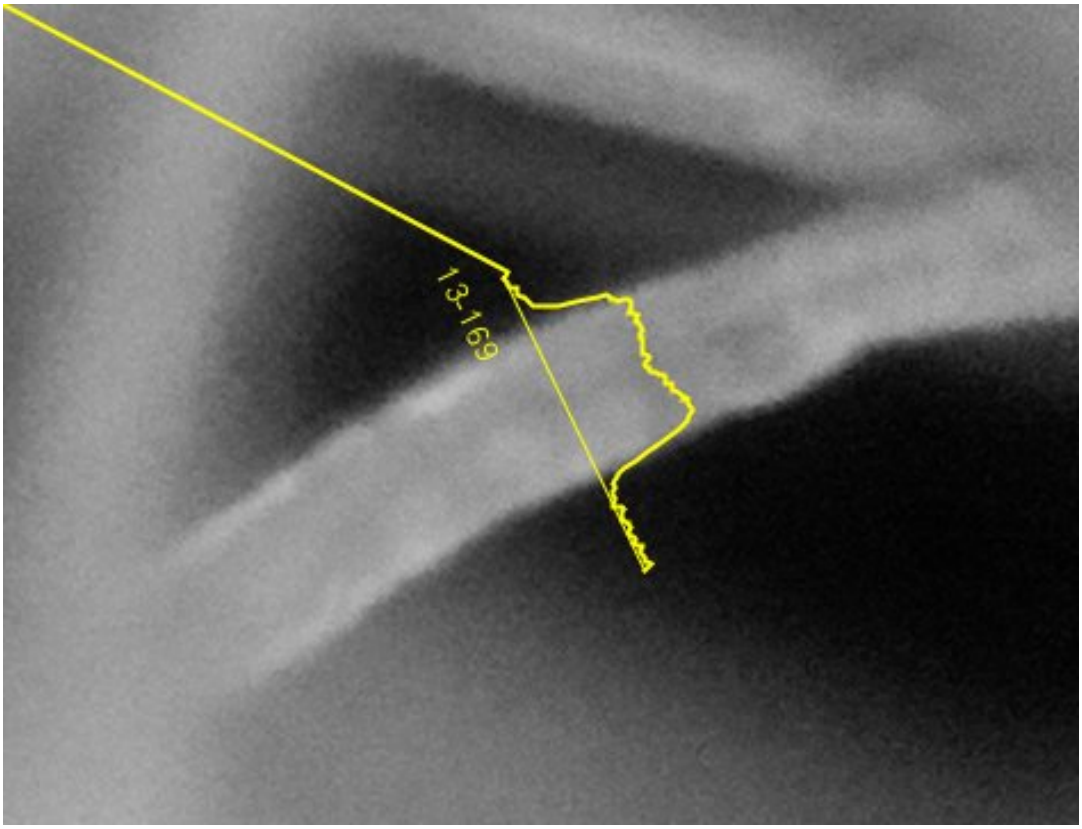

Fig. S8. Tissue paper dyed with fluorescein imaged with the ModMicro epi-fluorescence microscope and a 10x, 0.25 NA infinity corrected objective. An intensity line profile was taken in ImageJ of Zone 2.

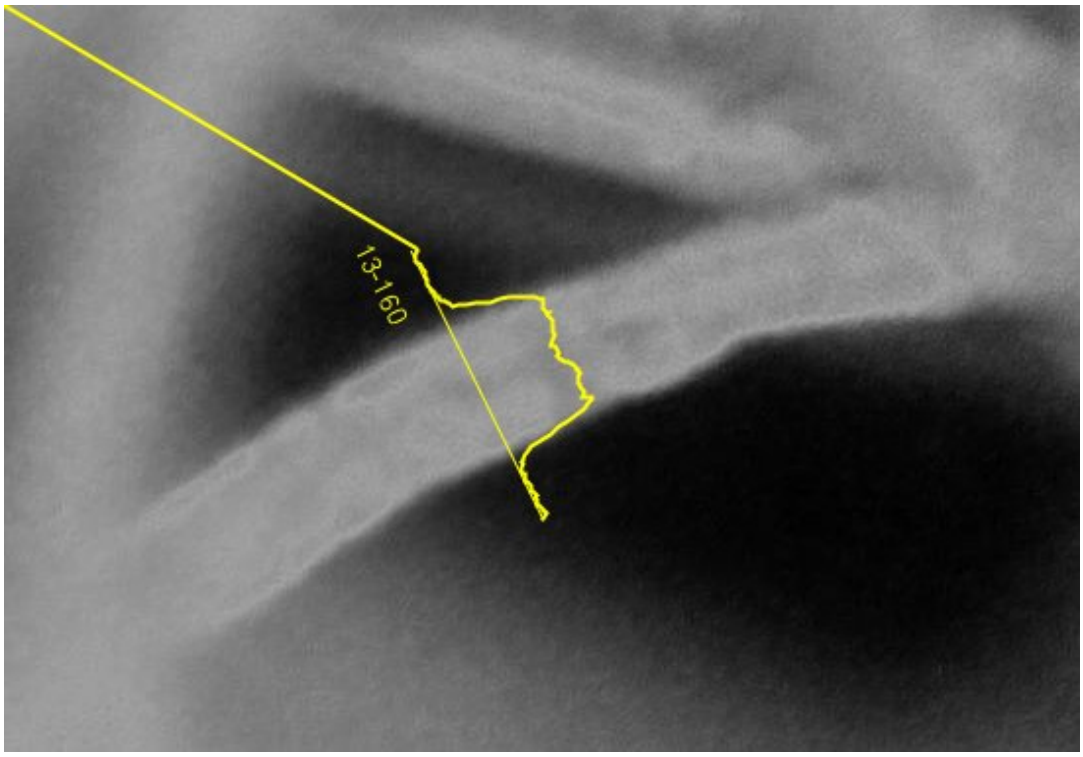

Fig. S9. Tissue paper dyed with fluorescein is imaged with the ModMicro epi-fluorescence microscope and a 10x, 0.25 NA infinity corrected objective. A novel sharpness enhancement algorithm was applied to the images and an intensity line profile was taken in ImageJ of Zone 2.

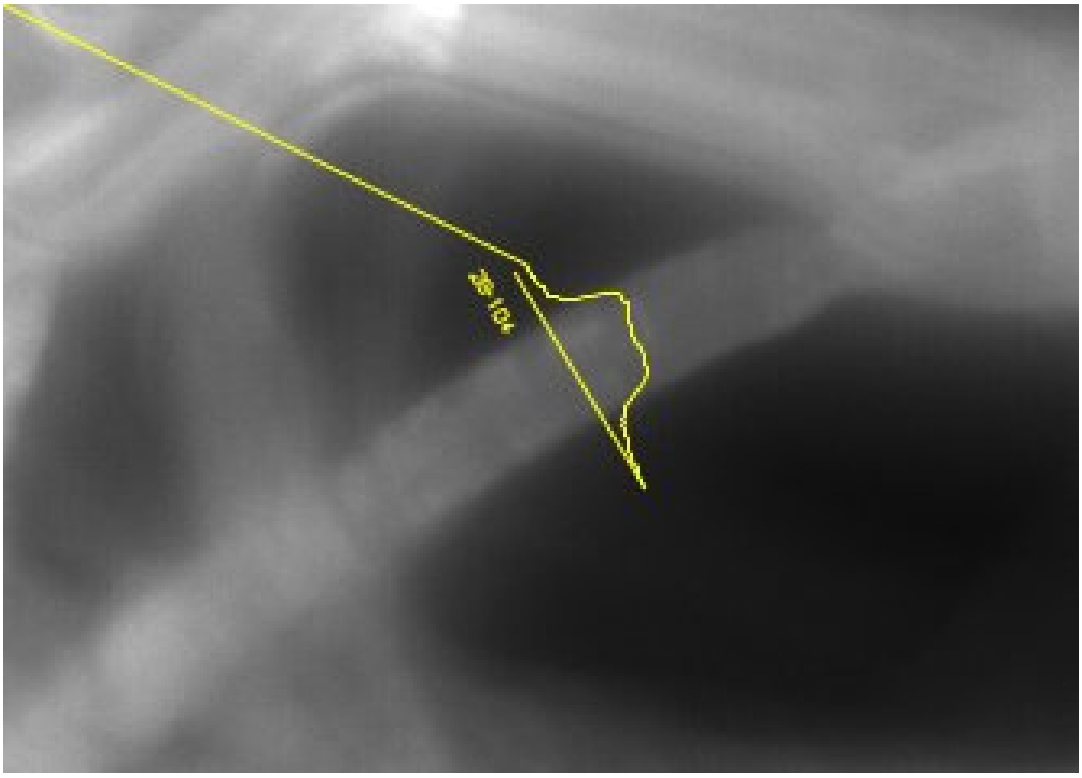

Fig. S10. Tissue paper dyed with fluorescein is imaged with a Nikon Ti Eclipse and Zyla 5.5 sCMOS detector. An intensity line profile was taken in ImageJ of Zone 2.

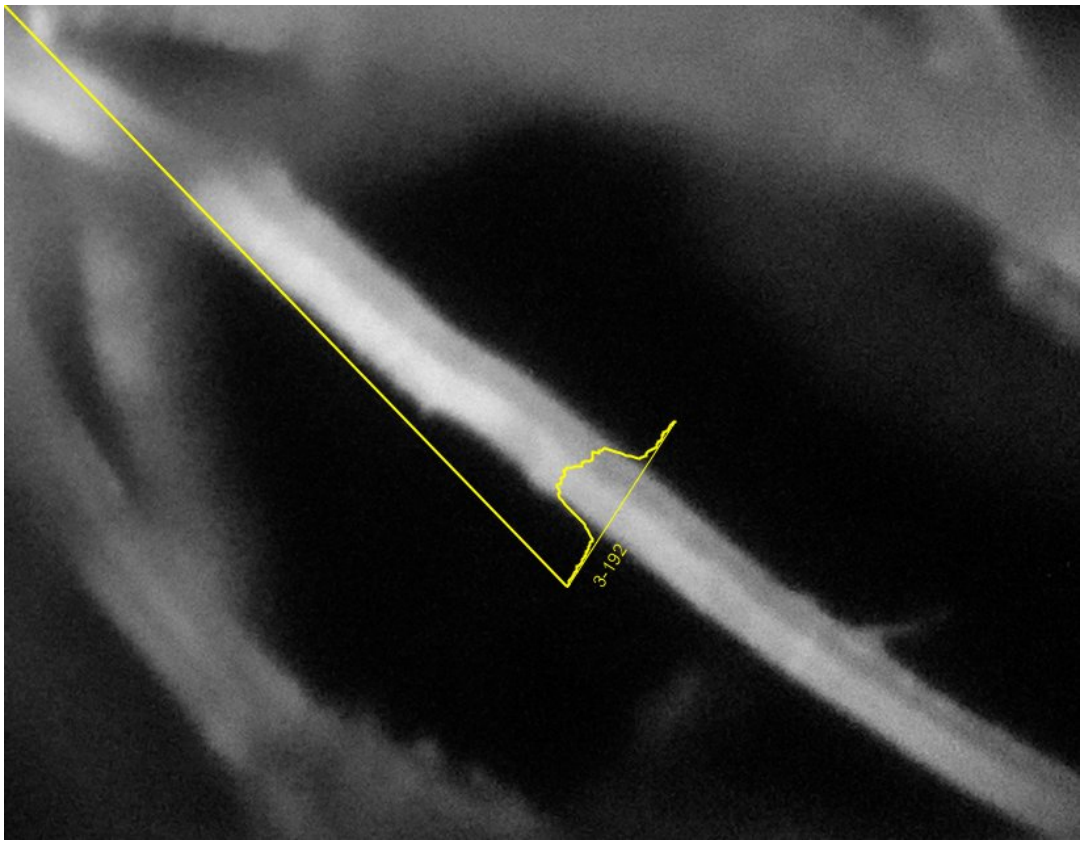

Fig. S11. Tissue paper dyed with fluorescein is imaged with the ModMicro epi-fluorescence microscope and a 10x, 0.25 NA infinity corrected objective. An intensity line profile was taken in ImageJ of Zone 3.

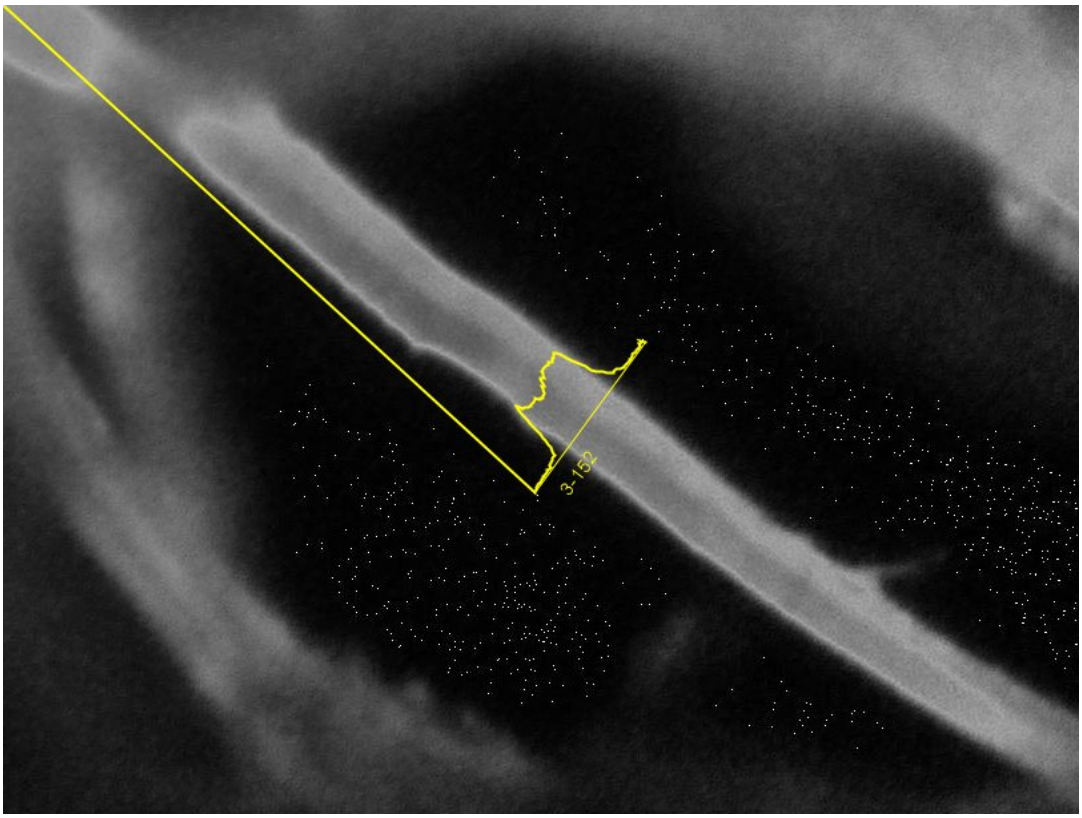

Fig. S12. Tissue paper dyed with fluorescein is imaged with the ModMicro epi-fluorescence microscope and a 10x, 0.25 NA infinity corrected objective. A novel sharpness enhancement algorithm was applied to the images and an intensity line profile was taken in ImageJ of Zone 3.

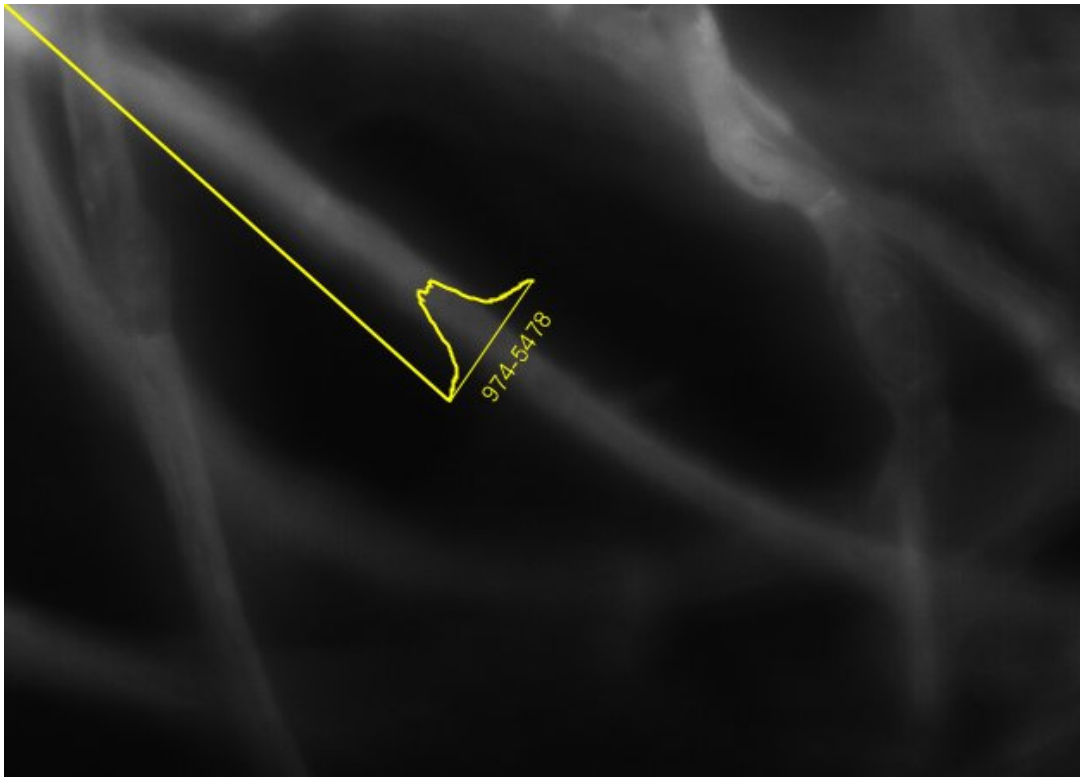

Fig. S13. Tissue paper dyed with fluorescein is imaged with a Nikon Ti Eclipse and Zyla 5.5 sCMOS detector. An intensity line profile was taken in ImageJ of Zone 3.

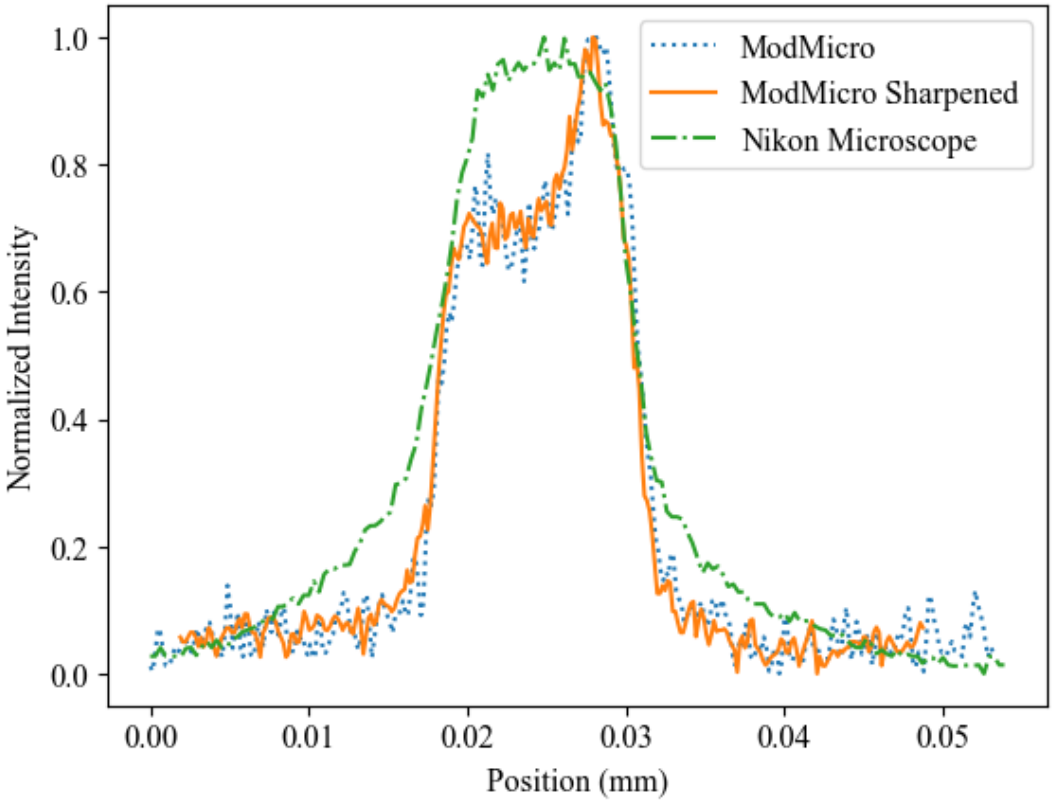

Fig. S14. Plotted line intensity profile of ModMicro, ModMicro with a sharpness enhancement algorithm and a Nikon Ti Eclipse at zone 1.

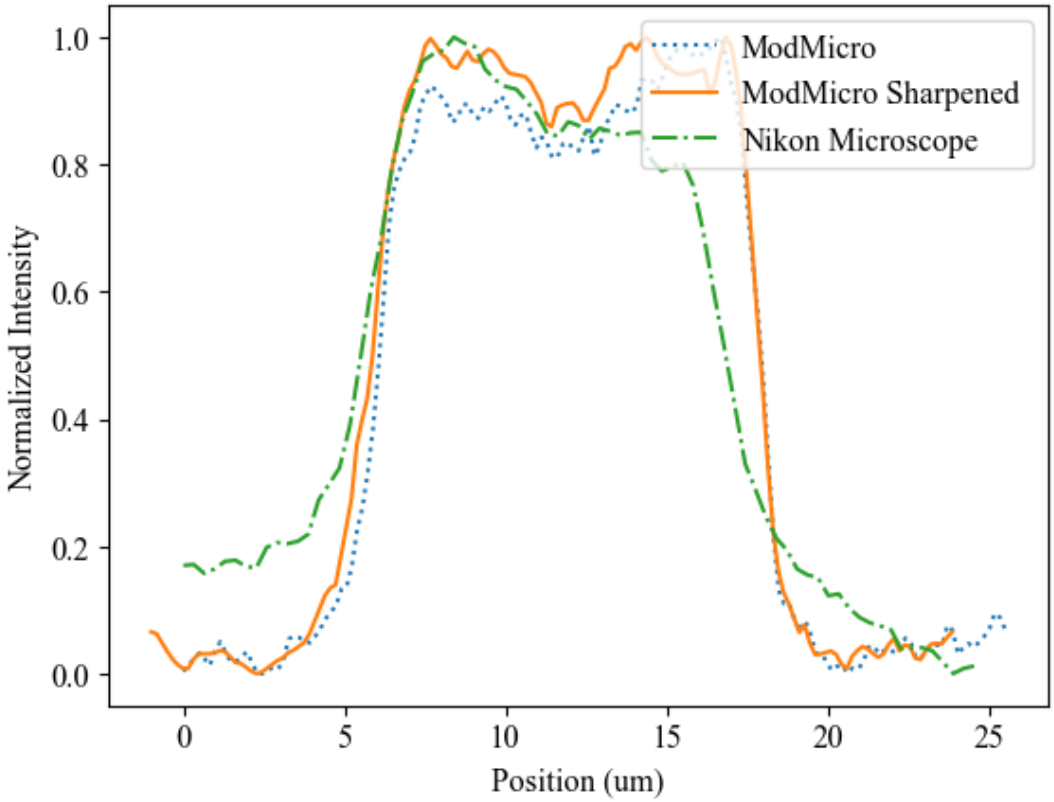

Fig. S15. Plotted line intensity profile of ModMicro, ModMicro with a sharpness enhancement algorithm and a Nikon Ti Eclipse at zone 2.

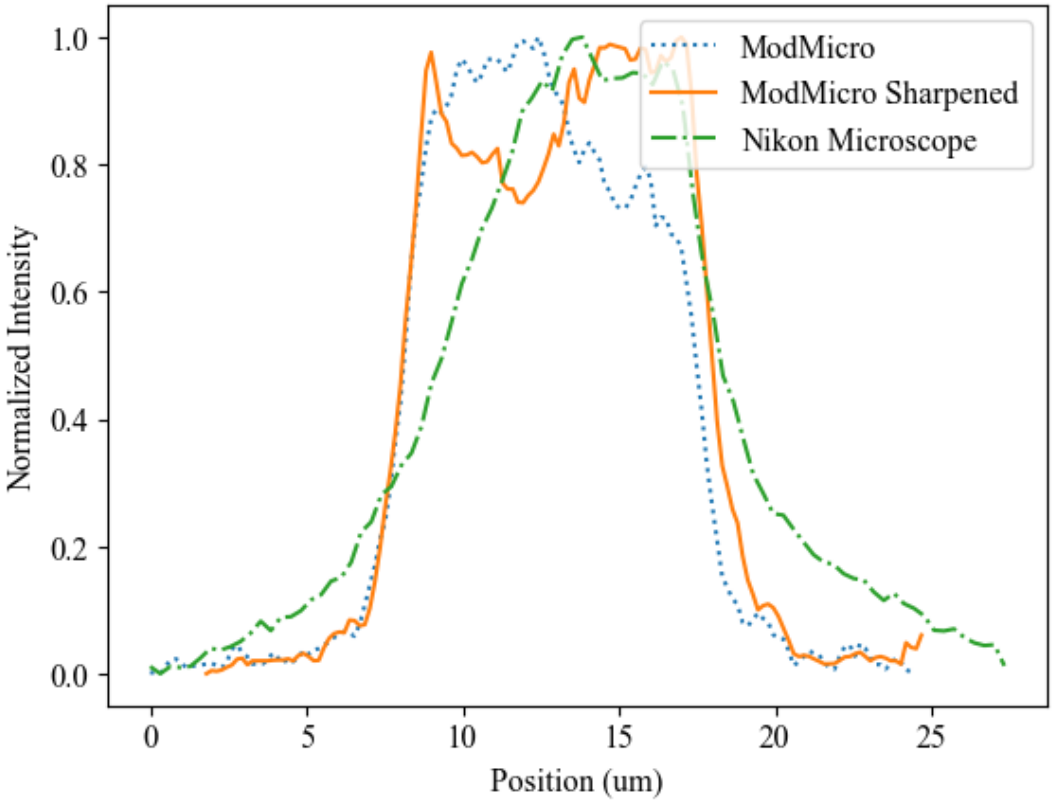

Fig. S16. Plotted line intensity profile of ModMicro, ModMicro with a sharpness enhancement algorithm and a Nikon Ti Eclipse at zone 3.

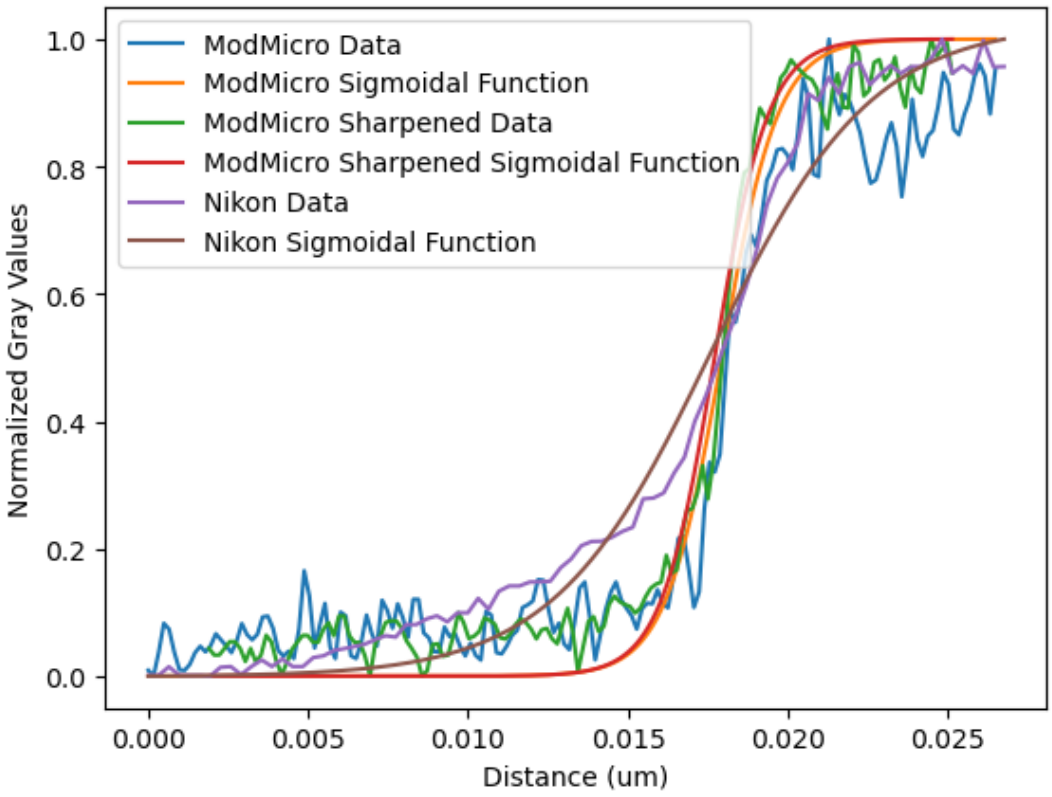

Fig. S17. Plotted line intensity profile of ModMicro, ModMicro with a sharpness enhancement algorithm and a Nikon Ti Eclipse at zone 1. Taking the first half of the data and fitting a sigmoidal function to find the sharpness values.

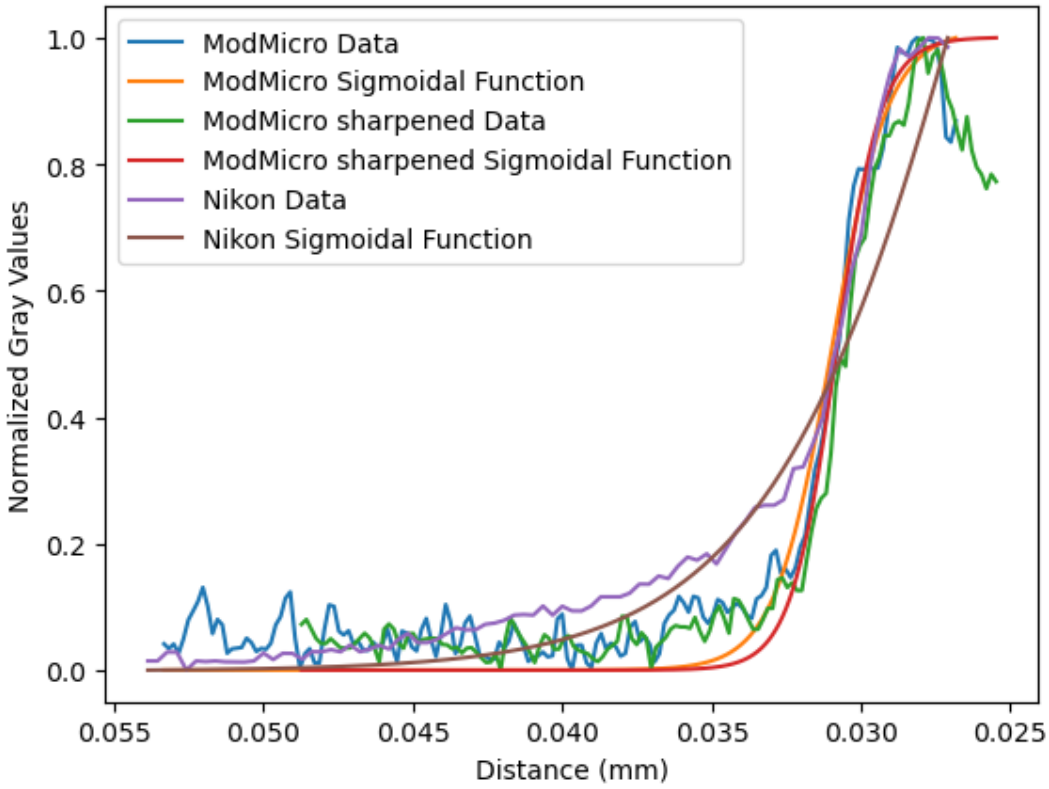

Fig. S18. Plotted line intensity profile of ModMicro, ModMicro with a sharpness enhancement algorithm and a Nikon Ti Eclipse at zone 1. Taking the second half of the data and fitting a sigmoidal function to find the sharpness values.

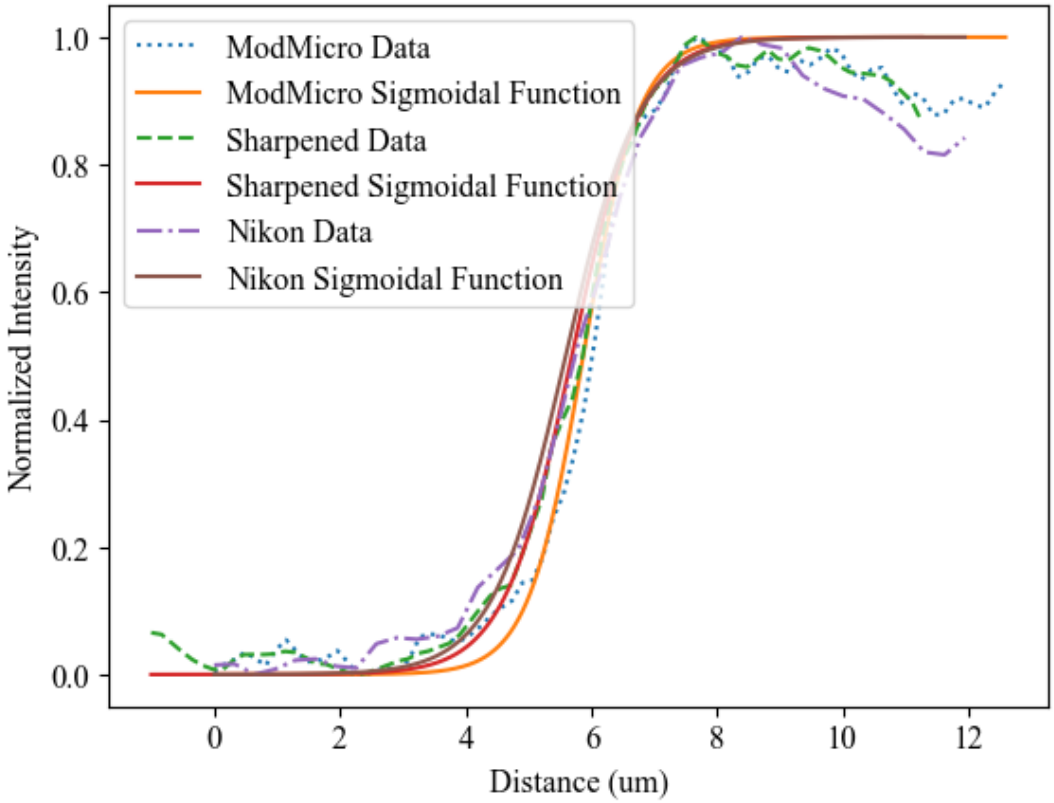

Fig. S19. Plotted line intensity profile of ModMicro, ModMicro with a sharpness enhancement algorithm and a Nikon Ti Eclipse at zone 2. Taking the first half of the data and fitting a sigmoidal function to find the sharpness values.

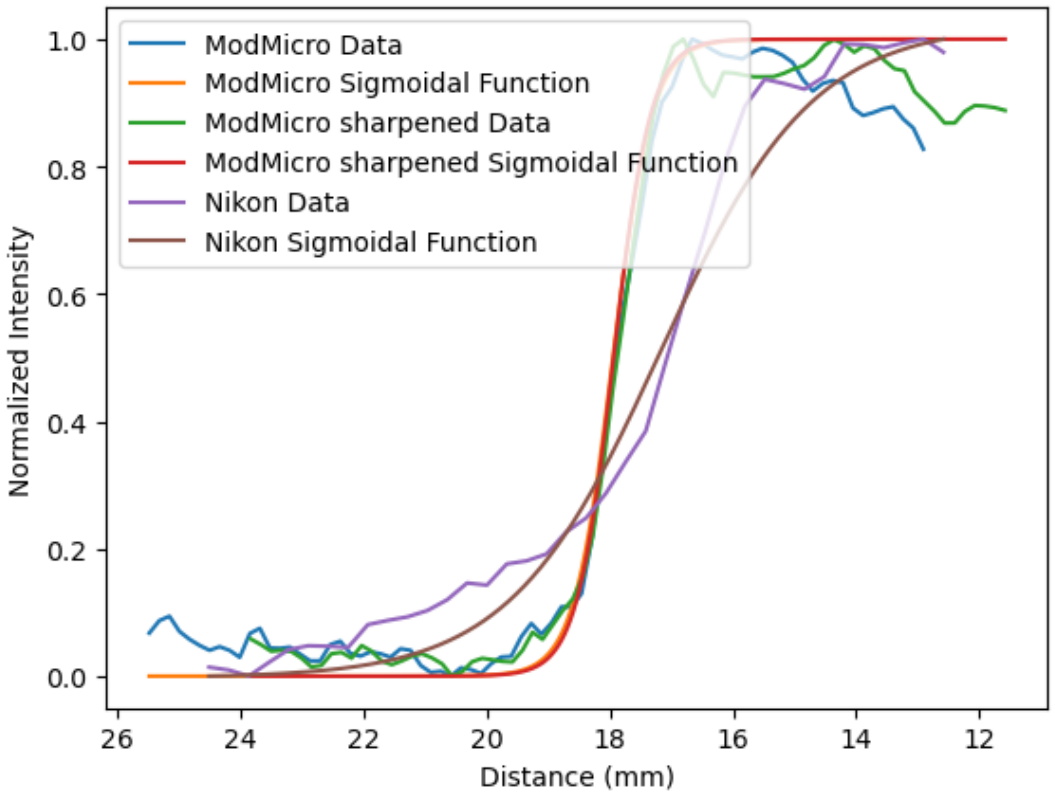

Fig. S20. Plotted line intensity profile of ModMicro, ModMicro with a sharpness enhancement algorithm and a Nikon Ti Eclipse at zone 2. Taking the second half of the data and fitting a sigmoidal function to find the sharpness values.

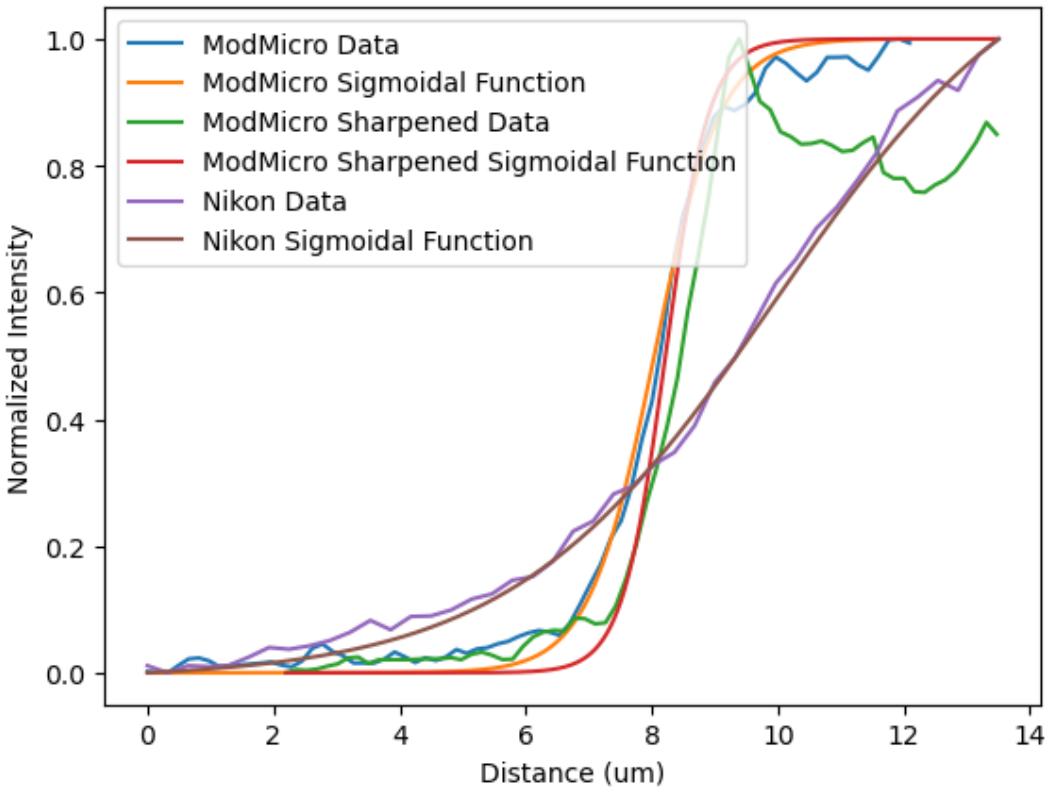

Fig. S21. Plotted line intensity profile of ModMicro, ModMicro with a sharpness enhancement algorithm and a Nikon Ti Eclipse at zone 3. Taking the first half of the data and fitting a sigmoidal function to find the sharpness values.

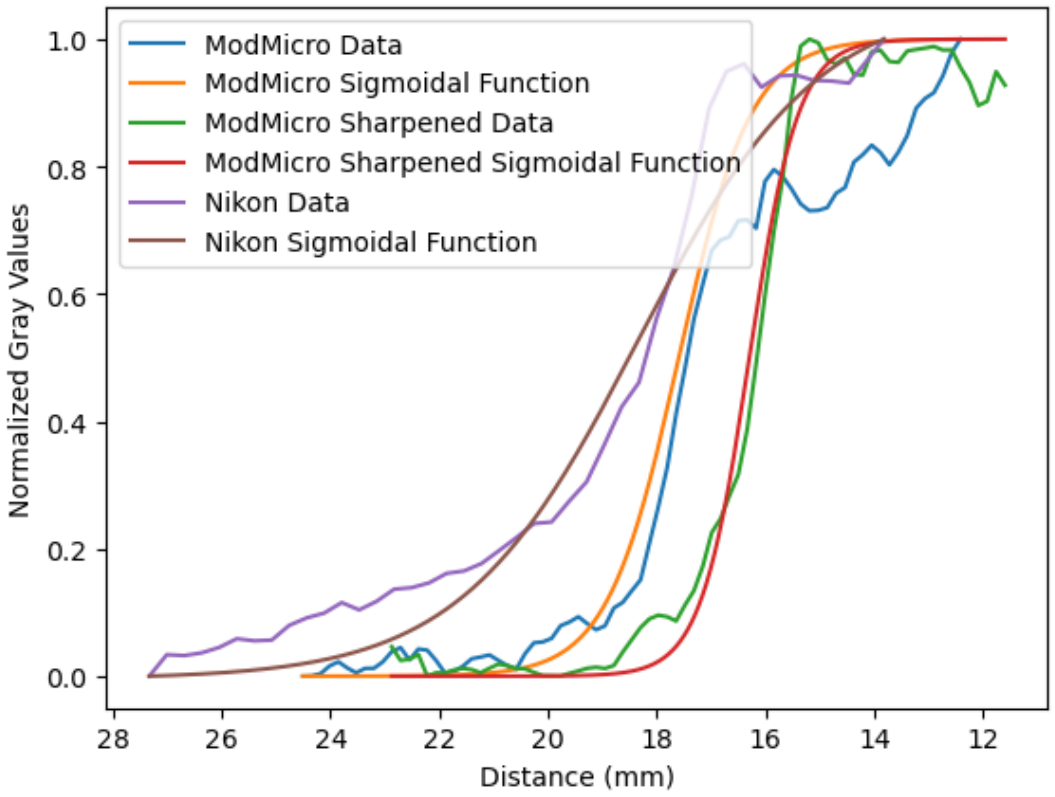

Fig. S22. Plotted line intensity profile of ModMicro, ModMicro with a sharpness enhancement algorithm and a Nikon Ti Eclipse at zone 3. Taking the second half of the data and fitting a sigmoidal function to find the sharpness values.