Label-free prediction of fluorescently labeled fibrin networks Supplementary Materials

Sarah Eldeen[1*†], Andres Felipe Guerrero Ramirez[1,2†], Bora Keresteci[3], Peter Chang[2,*], and Elliot Botvinick[3,4,5,6,*]

[1]Department of Mathematical, Computational, and Systems Biology, University of California, Irvine, USA.

[2]Department of Radiological Sciences and Computer Sciences, University of California, Irvine, USA.

[3]Department of Biomedical Engineering, University of California, Irvine, USA.

[4]Beckman Laser Institute and Medical Clinic, University of California at Irvine, Irvine, USA

[5]Edwards Lifesciences Foundation Cardiovascular Innovation and Research Center, University of California, Irvine, Irvine, USA

[6]Department of Surgery, University of California, Irvine, Irvine, USA

[*]Address correspondence to: ebotvini@uci.edu, changp6@hs.uci.edu

[†]These authors contributed equally to this work.

Statistical Metrics

- Mean Square Error (MSE): We normalize y and $\hat{y}$ to be within a 0 to 1 domain, and implement the MSE calculation as it is used in the `skimage.metrics` sublibrary. With i representing the i^{th} pixel of any given image and N representing the total number of pixels, equation is as follows:

$$MSE(y, \hat{y}) = \frac{1}{N}\sum_{i=0}^{N}(y_i - \hat{y}_i)^2 \tag{3}$$

- Structural similarity index measure (SSIM): Given a ground truth S_y image and its corresponding predicted version S_x, we implement the SSIM calculation as built-in in the TensorFlow library, which in turn is based on the definition of Zhou Wang et. al. [47]:

$$SSIM(S_x, S_y) = \frac{(2\mu_x\mu_y + C_1)(2\sigma_{xy} + C_2)}{(\mu_x^2 + \mu_y^2 + C_1)(\sigma_x^2 + \sigma_y^2 + C_2)} \tag{4}$$

  where $\{\mu_x, \mu_y\}$ and $\{\sigma_x, \sigma_y\}$ are the means and variances of S_x and S_y, respectively. σ_{xy} is the covariance of S_x and S_y. The two constants C_1 and C_2 are defined as $C_1 = (k_1L)^2$ and $C_2 = (k_2L)^2$. We use the built-in default values for $k_1 = 0.01$, and $k_2 = 0.03$, and $L = 1$.

- Peak Signal-to-Noise Ratio (PSNR): The Peak signal-to-noise ratio (PSNR) is the ratio between the maximum possible power of an image S_y and the power of corrupting noise that affects the quality of its representation S_x. The PSNR is defined as the ratio of the square of the largest possible pixel value $L = 1$ and the root mean square error $MSE(S_x, S_y)$

$$PSNR = 10 \log_{10} \left(\frac{L^2}{MSE(S_x, S_y)} \right) \tag{5}$$

- Spearman Correlation: The Spearman correlation assesses how well the relationship between two variables can be described using a monotonic function. We used the `scipy` sublibrary `stats` built-in function `stats.spearmanr`. The Spearman correlation coefficient ρ can be defined as:

$$\rho = 1 - 6 \frac{\sum_{i=1}^{N} \left(R(S_{x_i}) - R(S_{y_i}) \right)^2}{N(N^2 - 1)} \tag{6}$$

Where $R(S_{x_i})$ denotes the rank of the i^{th} element in S_x, and N is the total number of pixels.

Mathematical Definitions

- ReLU (Rectified Linear Unit): The ReLU function is defined as:

$$ReLU(x) = \max(0, x) \tag{7}$$

with x being the input to the ReLU function. The function outputs x if x is positive, and 0 if x is negative or zero.

- Batch Normalization Layer (BatchNorm): The Batch Normalization operation normalizes the output of a layer by adjusting and scaling the activations. For a mini-batch of activations $\mathbf{x} = \{x_1, x_2, \ldots, x_m\}$, the batch normalization is computed as follows [48]:

  1. Calculate the mean and variance

$$\mu_{\text{batch}} = \frac{1}{m} \sum_{i=1}^{m} x_i \tag{8}$$

$$\sigma^2_{\text{batch}} = \frac{1}{m} \sum_{i=1}^{m} (x_i - \mu_{\text{batch}})^2 \tag{9}$$

  2. Normalize the batch through Z-score

$$\hat{x}_i = \frac{x_i - \mu_{\text{batch}}}{\sqrt{\sigma^2_{\text{batch}} + \epsilon}} \tag{10}$$

  where ϵ is a small constant added for numerical stability.

  3. Scale and shift

$$y_i = \gamma \hat{x}_i + \beta \tag{11}$$

  where γ is the scale parameter, β is the shift parameter, and y_i is the final output after batch normalization.

- Conv3D (3D Convolution): Conv3D is a convolution operation that works on 3D spatial data. The mathematical definition of a 3D convolution is given by:

$$y[i,j,k] = \sum_{d=0}^{D-1} \sum_{h=0}^{H-1} \sum_{w=0}^{W-1} x[i+d, j+h, k+w] \cdot w[d,h,w] + b \tag{12}$$

where: x is the input 3D volume of size (I_x, I_y, I_z), w are the learnable parameters of the 3D kernel (filter) of size (D, H, W)., b is the bias term, $y[i,j,k]$ is the output at position (i,j,k), and D, H, and W describe the size in terms of depth, height, and width of the kernel, respectively.

- Average Pooling: Average Pooling is a down-sampling operation that effectively reduces spatial dimensions by averaging values within a given spatial vicinity (pool window). The mathematical definition for a 2D average pooling operation, applied to each channel independently, is given by:

$$y[i,j] = \frac{1}{K_h \times K_w} \sum_{m=0}^{K_h-1} \sum_{n=0}^{K_w-1} x[i+m, j+n] \tag{13}$$

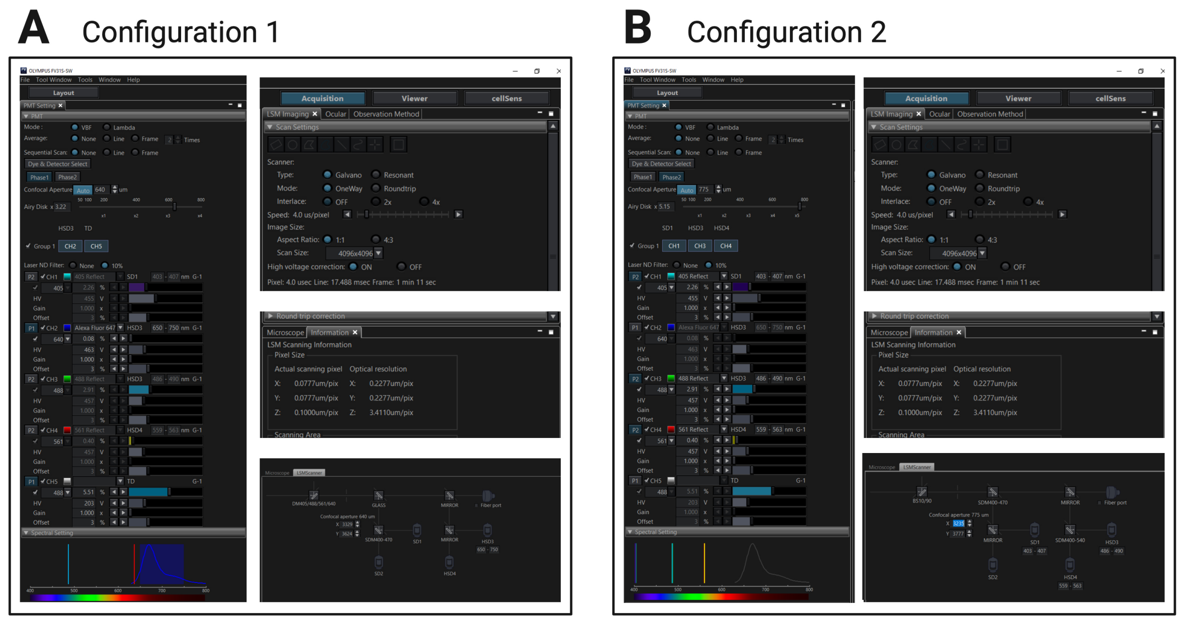
  where: $x[i,j]$ is the input feature map, K_h and K_w are the height and width of the pooling window, respectively, and $y[i,j]$ is the output after pooling. For 3D data, such as volumetric data, the equation extends similarly with an additional dimension.

- Conv3D Transpose (Transposed 3D Convolution): Conv3D Transpose, also known as deconvolution, is a convolution operation that increases the spatial dimensions of the input. The mathematical operation can be understood as the reverse of Conv3D:

$$y[i,j,k] = \sum_{d=0}^{D-1} \sum_{h=0}^{H-1} \sum_{w=0}^{W-1} x'[i-d, j-h, k-w] \cdot w[d,h,w] + b \tag{14}$$

  where: x' is the input feature map, w is the 3D kernel (filter), b is the bias term, and $y[i,j,k]$ is the output at position (i,j,k).

Figures

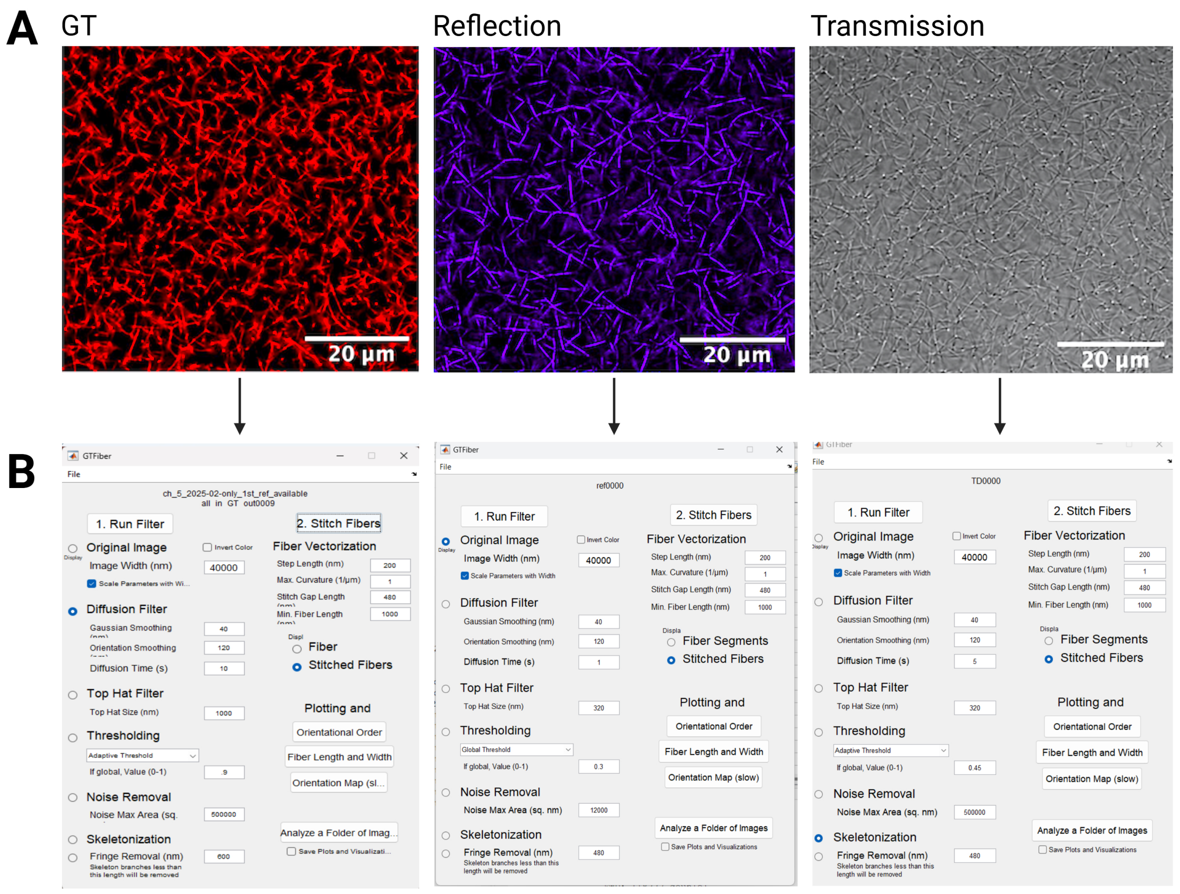

A Configuration 1

```
OLYMPUS FV31S-SW
PMT Setting
PMT
Mode:             [✓ VBF]  Lambda
Average:          [✓ None]  Line  Frame  2 Times
Sequential Scan:  [✓ None]  Line  Frame
Dye & Detector Select
[✓ Phase1]  Phase2
Confocal Aperture  Auto  640 um
Airy Disk x 3.22
HSD3  TD
✓ Group 1  CH2  CH5
Laser ND Filter:  None  [✓ 10%]

P2 ✓CH1  405 Reflect       SD1   403 - 407 nm G-1
   ✓ 405   2.26 %   HV 455 V   Gain 1.000 x   Offset 3 %
P1 ✓CH2  Alexa Fluor 647   HSD3  650 - 750 nm G-1
   ✓ 640   0.08 %   HV 463 V   Gain 1.000 x   Offset 3 %
P2 ✓CH3  488 Reflect       HSD3  486 - 490 nm G-1
   ✓ 488   2.91 %   HV 457 V   Gain 1.000 x   Offset 3 %
P2 ✓CH4  561 Reflect       HSD4  559 - 563 nm G-1
   ✓ 561   0.40 %   HV 457 V   Gain 1.000 x   Offset 3 %
P1 ✓CH5                    TD              G-1
   ✓ 488   5.51 %   HV 203 V   Gain 1.000 x   Offset 3 %

LSM Imaging
Scan Settings
Scanner:
  Type:       [✓ Galvano]  Resonant
  Mode:       [✓ OneWay]  Roundtrip
  Interlace:  [✓ OFF]  2x  4x
Speed: 4.0 us/pixel
Image Size:
  Aspect Ratio:  [✓ 1:1]  4:3
  Scan Size:     4096x4096
High voltage correction:  [✓ ON]  OFF
Pixel: 4.0 usec Line: 17.488 msec Frame: 1 min 11 sec

LSM Scanning Information
Pixel Size
Actual scanning pixel     Optical resolution
X: 0.0777um/pix           X: 0.2277um/pix
Y: 0.0777um/pix           Y: 0.2277um/pix
Z: 0.1000um/pix           Z: 3.4110um/pix

LSMScanner
Confocal aperture 640 um  X 3329  Y 3624
```

B Configuration 2

```
OLYMPUS FV31S-SW
PMT Setting
PMT
Mode:             [✓ VBF]  Lambda
Average:          [✓ None]  Line  Frame  2 Times
Sequential Scan:  [✓ None]  Line  Frame
Dye & Detector Select
Phase1  [✓ Phase2]
Confocal Aperture  Auto  775 um
Airy Disk x 5.15
SD1  HSD3  HSD4
✓ Group 1  CH1  CH3  CH4
Laser ND Filter:  None  [✓ 10%]

P2 ✓CH1  405 Reflect       SD1   403 - 407 nm G-1
   ✓ 405   2.26 %   HV 455 V   Gain 1.000 x   Offset 3 %
P1 ✓CH2  Alexa Fluor 647   HSD3  650 - 750 nm G-1
   ✓ 640   0.08 %   HV 463 V   Gain 1.000 x   Offset 3 %
P2 ✓CH3  488 Reflect       HSD3  486 - 490 nm G-1
   ✓ 488   2.91 %   HV 457 V   Gain 1.000 x   Offset 3 %
P2 ✓CH4  561 Reflect       HSD4  559 - 563 nm G-1
   ✓ 561   0.40 %   HV 457 V   Gain 1.000 x   Offset 3 %
P1 ✓CH5                    TD              G-1
   ✓ 488   5.51 %   HV 203 V   Gain 1.000 x   Offset 3 %

LSM Imaging
Scan Settings
Scanner:
  Type:       [✓ Galvano]  Resonant
  Mode:       [✓ OneWay]  Roundtrip
  Interlace:  [✓ OFF]  2x  4x
Speed: 4.0 us/pixel
Image Size:
  Aspect Ratio:  [✓ 1:1]  4:3
  Scan Size:     4096x4096
High voltage correction:  [✓ ON]  OFF
Pixel: 4.0 usec Line: 17.488 msec Frame: 1 min 11 sec

LSM Scanning Information
Pixel Size
Actual scanning pixel     Optical resolution
X: 0.0777um/pix           X: 0.2277um/pix
Y: 0.0777um/pix           Y: 0.2277um/pix
Z: 0.1000um/pix           Z: 3.4110um/pix

LSMScanner
Confocal aperture 775 um  X 3235  Y 3777
```

Figure S1: Fluoview Settings: (A): Screenshots of fluoview software showing settings for configuration 1 (labeled as Phase 1 in the software). (B): Screenshots of fluoview software of configuration 2 (Phase 2).



GTFiber
File

ch_5_2025-02-only_1st_ref_available
all in GT out0009

1. Run Filter | 2. Stitch Fibers

○ Original Image	☐ Invert Color
Image Width (nm)	40000
☑ Scale Parameters with Wi...	
● Diffusion Filter	
Gaussian Smoothing (nm)	40
Orientation Smoothing (nm)	120
Diffusion Time (s)	10
○ Top Hat Filter	
Top Hat Size (nm)	1000
○ Thresholding	Adaptive Threshold
If global, Value (0-1)	.9
○ Noise Removal	
Noise Max Area (sq.	500000
○ Skeletonization	
○ Fringe Removal (nm) Skeleton branches less than this length will be removed	600
Fiber Vectorization	
Step Length (nm)	200
Max. Curvature (1/µm)	1
Stitch Gap Length (nm)	480
Min. Fiber Length (nm)	1000
Displ: ○ Fiber	
● Stitched Fibers	

Plotting and: Orientational Order | Fiber Length and Width | Orientation Map (sl...
Analyze a Folder of Imag... ☐ Save Plots and Visualizati...

GTFiber
File

ref0000

1. Run Filter | 2. Stitch Fibers

● Original Image	☐ Invert Color
Image Width (nm)	40000
☑ Scale Parameters with Width	
○ Diffusion Filter	
Gaussian Smoothing (nm)	40
Orientation Smoothing (nm)	120
Diffusion Time (s)	1
○ Top Hat Filter	
Top Hat Size (nm)	320
○ Thresholding	Global Threshold
If global, Value (0-1)	0.3
○ Noise Removal	
Noise Max Area (sq. nm)	12000
○ Skeletonization	
○ Fringe Removal (nm) Skeleton branches less than this length will be removed	480
Fiber Vectorization	
Step Length (nm)	200
Max. Curvature (1/µm)	1
Stitch Gap Length (nm)	480
Min. Fiber Length (nm)	1000
Displa: ○ Fiber Segments	
● Stitched Fibers	

Plotting and: Orientational Order | Fiber Length and Width | Orientation Map (slow)
Analyze a Folder of Images ☐ Save Plots and Visualizations

GTFiber
File

TD0000

1. Run Filter | 2. Stitch Fibers

○ Original Image	☐ Invert Color
Image Width (nm)	40000
☑ Scale Parameters with Width	
○ Diffusion Filter	
Gaussian Smoothing (nm)	40
Orientation Smoothing (nm)	120
Diffusion Time (s)	5
○ Top Hat Filter	
Top Hat Size (nm)	320
○ Thresholding	Adaptive Threshold
If global, Value (0-1)	0.45
○ Noise Removal	
Noise Max Area (sq. nm)	500000
● Skeletonization	
○ Fringe Removal (nm) Skeleton branches less than this length will be removed	480
Fiber Vectorization	
Step Length (nm)	200
Max. Curvature (1/µm)	1
Stitch Gap Length (nm)	480
Min. Fiber Length (nm)	1000
Displa: ○ Fiber Segments	
● Stitched Fibers	

Plotting and: Orientational Order | Fiber Length and Width | Orientation Map (slow)
Analyze a Folder of Images ☐ Save Plots and Visualizations

Figure S2: Comparison of GT to denoised GT: (A): GT and denoised GT images acquired approximately 10μm above the glass bottom of the Petri dish. Intensity profiles along the dashed white line on the images. (B): GT and Denoised GT images acquired at a depth of approximately 50μm above the glass bottom of the Petri dish.

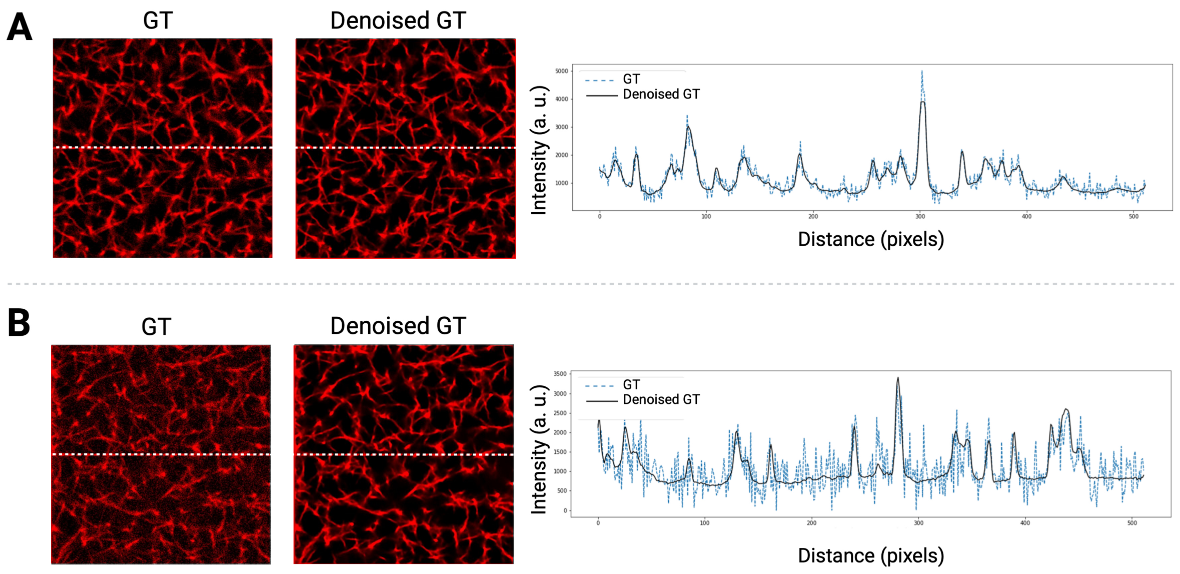


Figure S3: GTFiber Settings: (A): GT, RCM (at 405 nm), and transmission images of the same focal plane within a fibrin scaffold. (B): GTFiber screenshots showing settings for each image type.

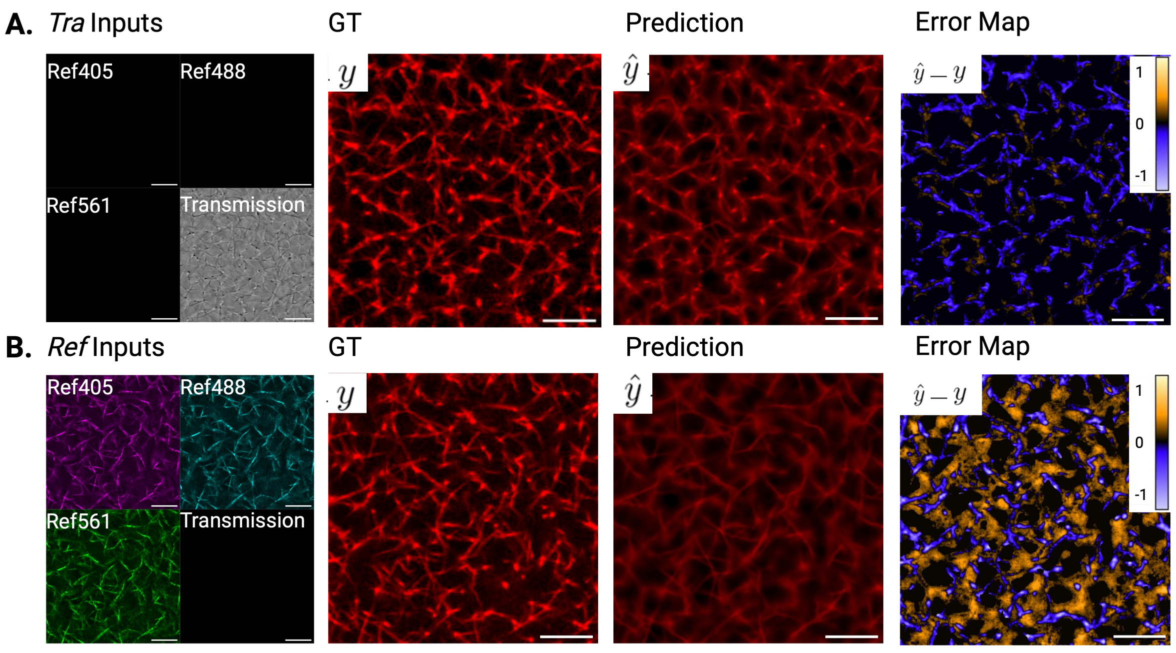


Figure S4: Performance of Tra (A), and Ref (B). The error map image ($\hat{y} - y$) is computed from GT (y) and prediction ($\hat{y}$)

.

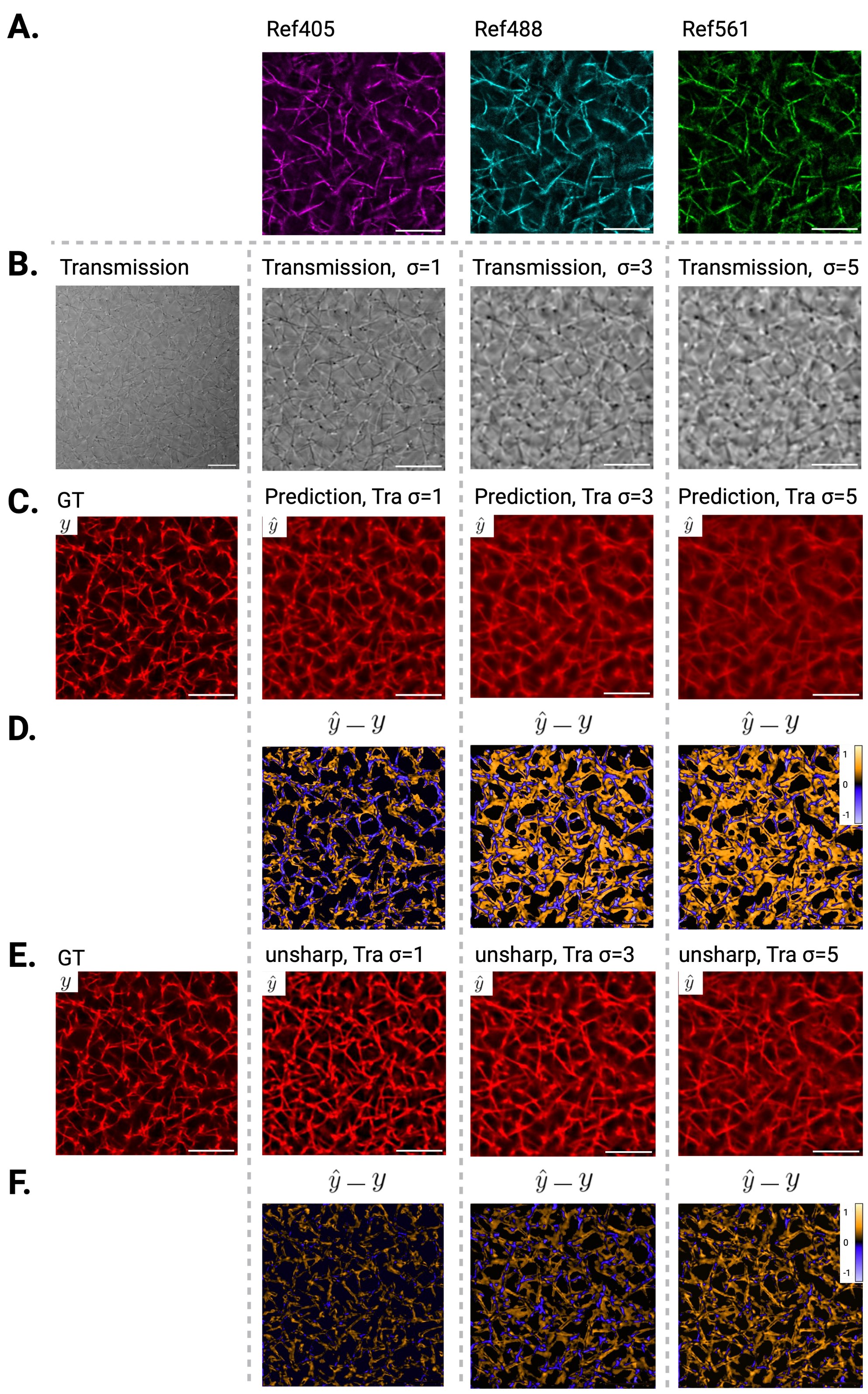


Figure S5: Effect of a blurred transmission channel: (A): RCM inputs for a specific ROI. (B): Transmission image of the same ROI blurred using a Gaussian filter of $\sigma = 1$, 3, or 5.(C): GT along with predictions trained and tested using the different blurred transmission models. (D) Error maps of each model. (E): Prediction in (C) with an applied sharpening mask. (F): Error maps of predictions presented in (E).

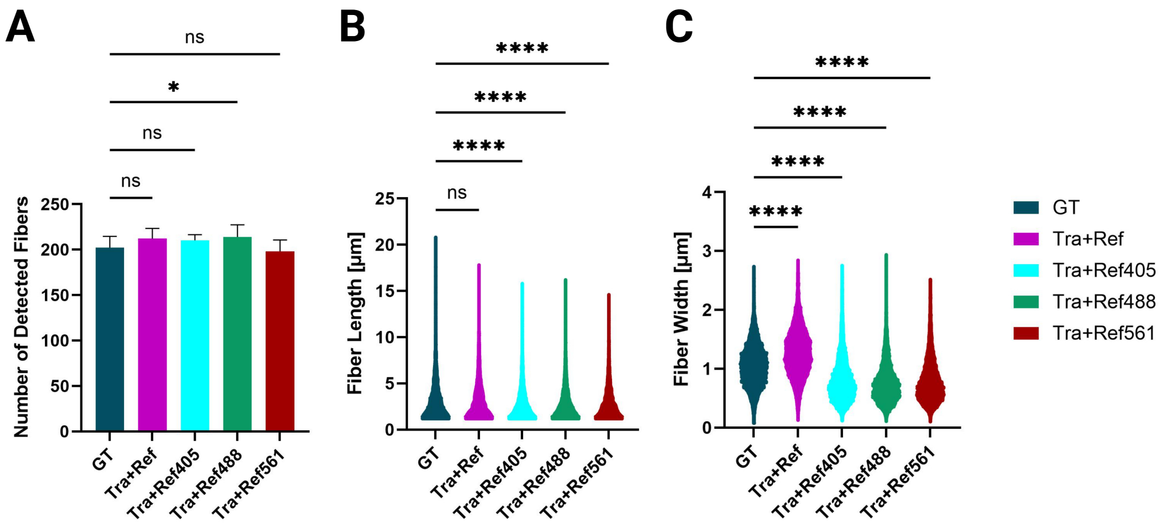


Figure S6: RCM wavelength Contributions: Distributions per slice of number of detected fibers (A), fiber length (B), and fiber width (C) for GT, $Tra + Ref$, $Tra + Ref405$, $Tra + Ref488$ and $Tra + Ref561$ predictions.

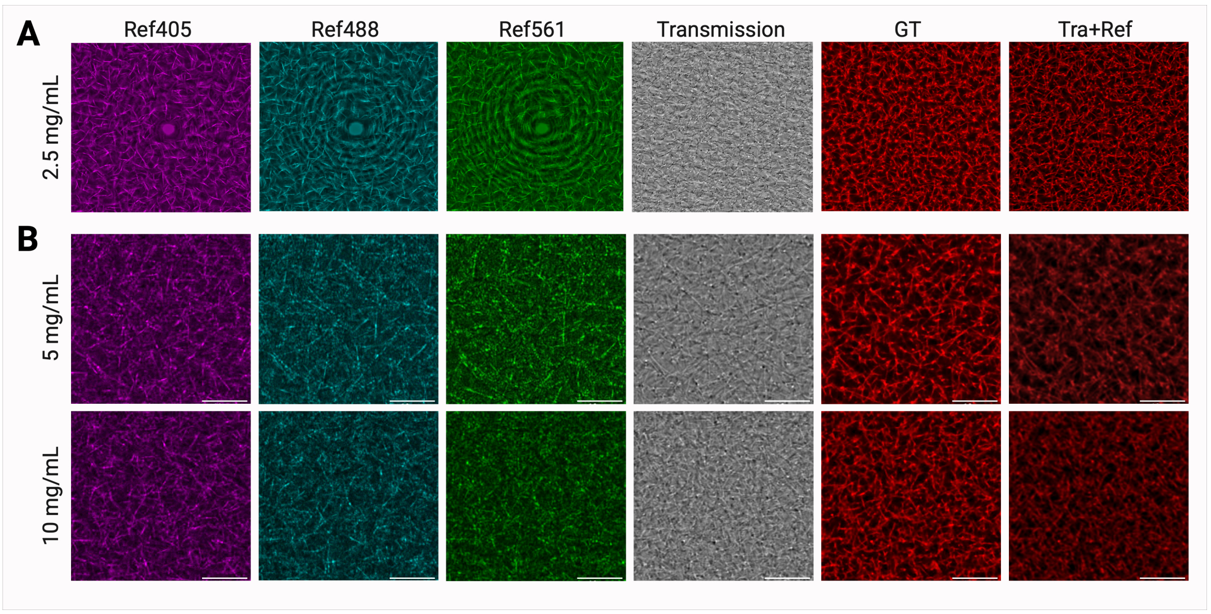


Figure S7: Generalizability of $Tra + Ref$: (A) Example of inputs, RCM (405/488/561) nm and transmission, along with GT and prediction of a 2.5 mg/mL fibrin scaffold around RCM interference ring artifacts. (B) Examples of inputs, RCM (405/488/561) nm and transmission, along with GT and prediction of higher density samples at 5 mg/mL and 10 mg/mL. Scale bars: 10μm.