Figure S1 Serially-sectioned unstained and PSR-stained human pancreatic cancer tissues visualized with and without linear polarizers. Minimal birefringence is observed in the unstained tissue under polarized light. When PSR-stained tissue is visualized in the same manner, birefringence is greatly enhanced as expected. To compare the signals between unstained and stained tissues under polarized light, exposure time was kept constant during imaging.

Figure S2 Representative human breast tissue visualized with PSR-POL and SHG. Tumor-associated collagen signatures (TACS) have previously been shown in breast cancer using SHG to correlate with patient survival. Scale bars = 100 µm.

Figure S3 Representative human prostate tissue visualized with PSR-POL and SHG. Different organizations of collagen are observed between normal prostate and cancer. Scale bars = 100 µm.

Figure S4 A representative section of human pancreatic cancer stained with PSR and visualized with PSR-POL, forward-detected SHG, and backward-detected SHG. All images are converted to 8-bit grayscale before CT-FIRE processing. The right column depicts individual fiber segmentation overlays by CT-FIRE. Scale bars = 100 µm.

Figure S5 Left column: Quantification of different collagen fiber metrics in PDAC images acquired using forward-detected and backward-detected SHG. Right column: Pearson correlation analysis between metrics generated by forward-detected and backward-detected SHG. Data represents 15 ROIs.

Figure S6 Quantification of different collagen fiber metrics (alignment, length, straightness, width) in images acquired from prostate tissues using SHG and PSR-POL. Data represents 20 ROIs from 10 patients.

Figure S1

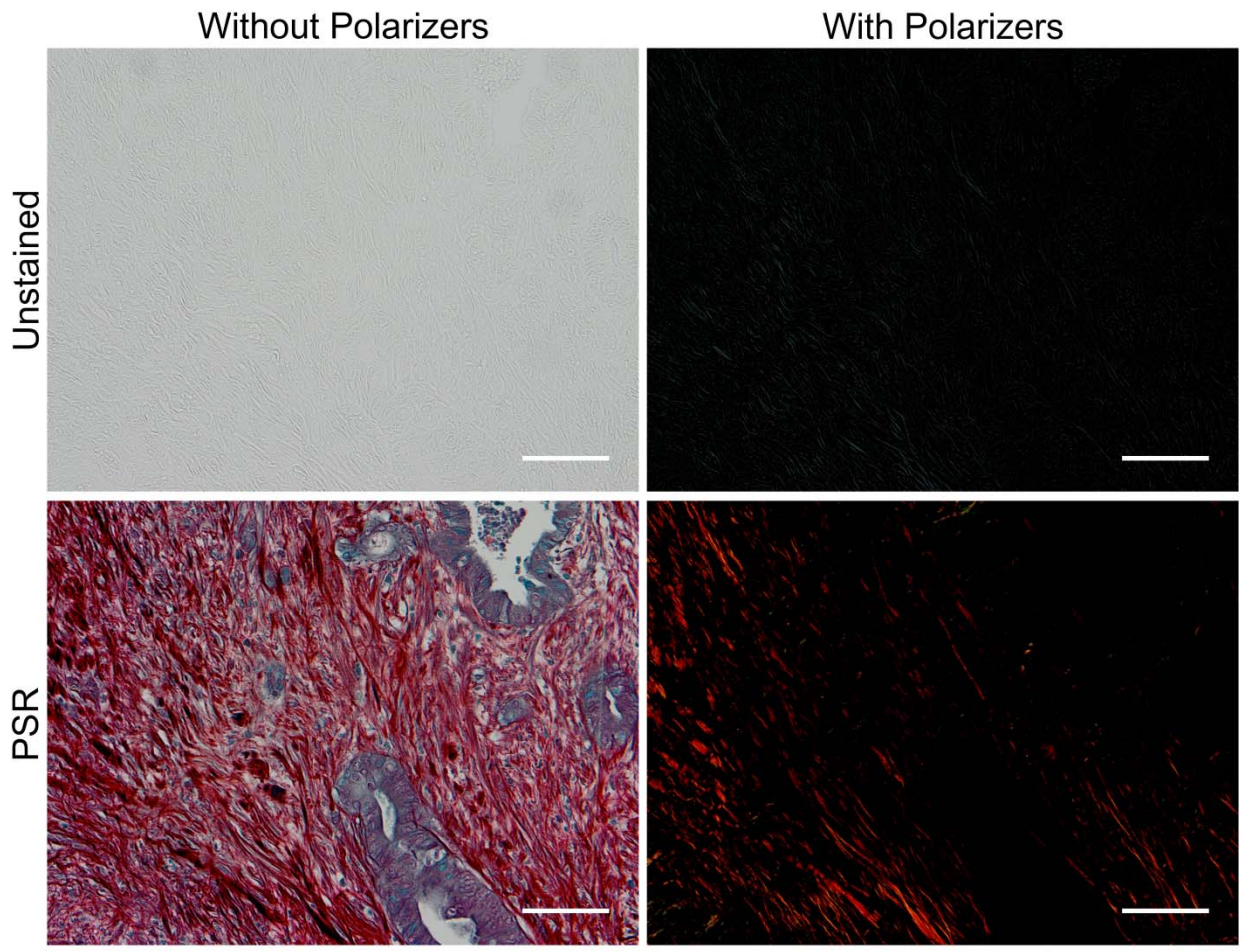


Figure S2

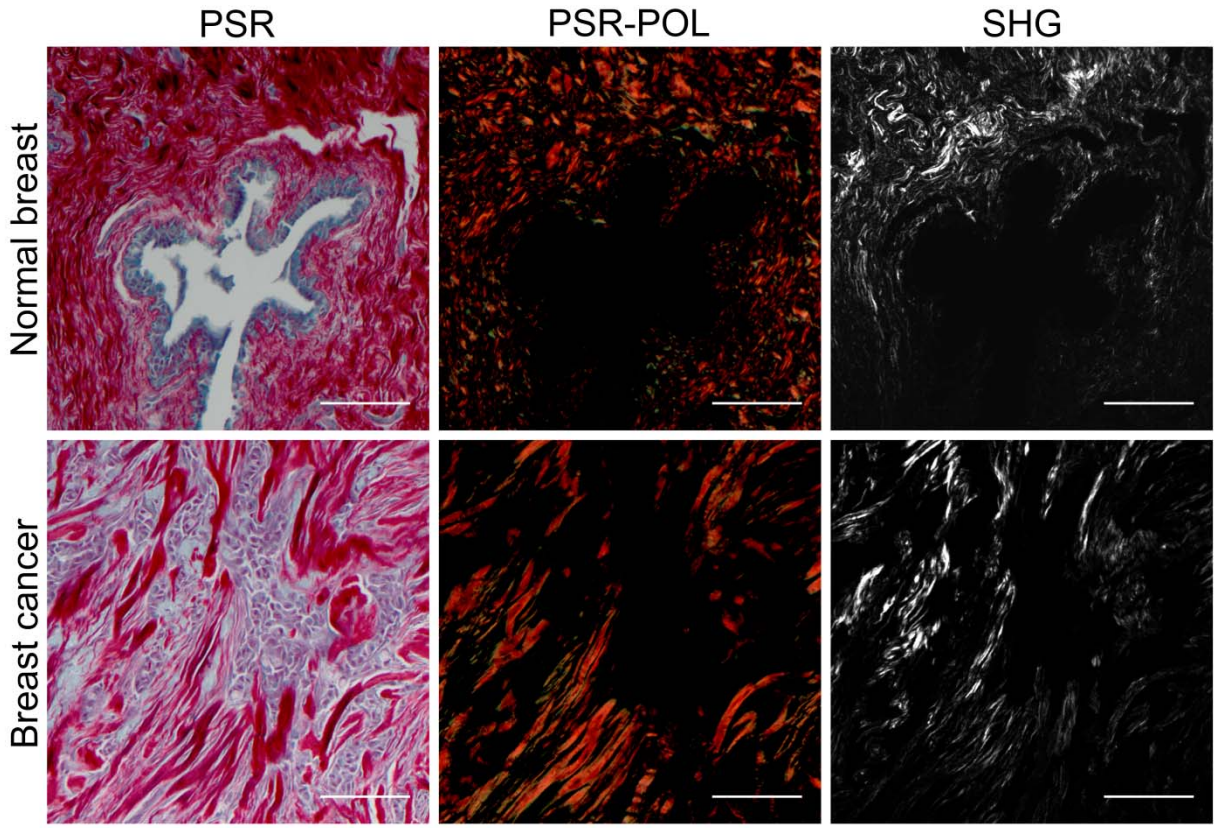


Figure S3

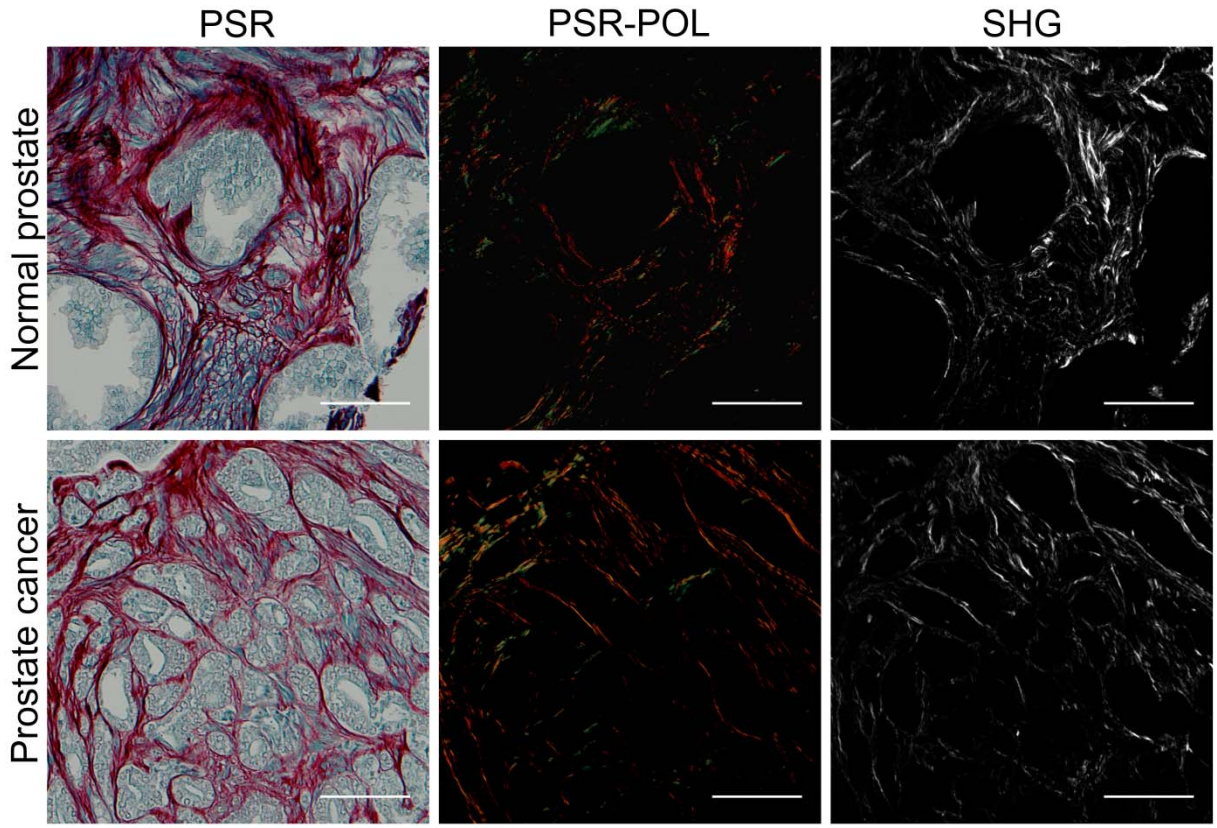


Figure S4

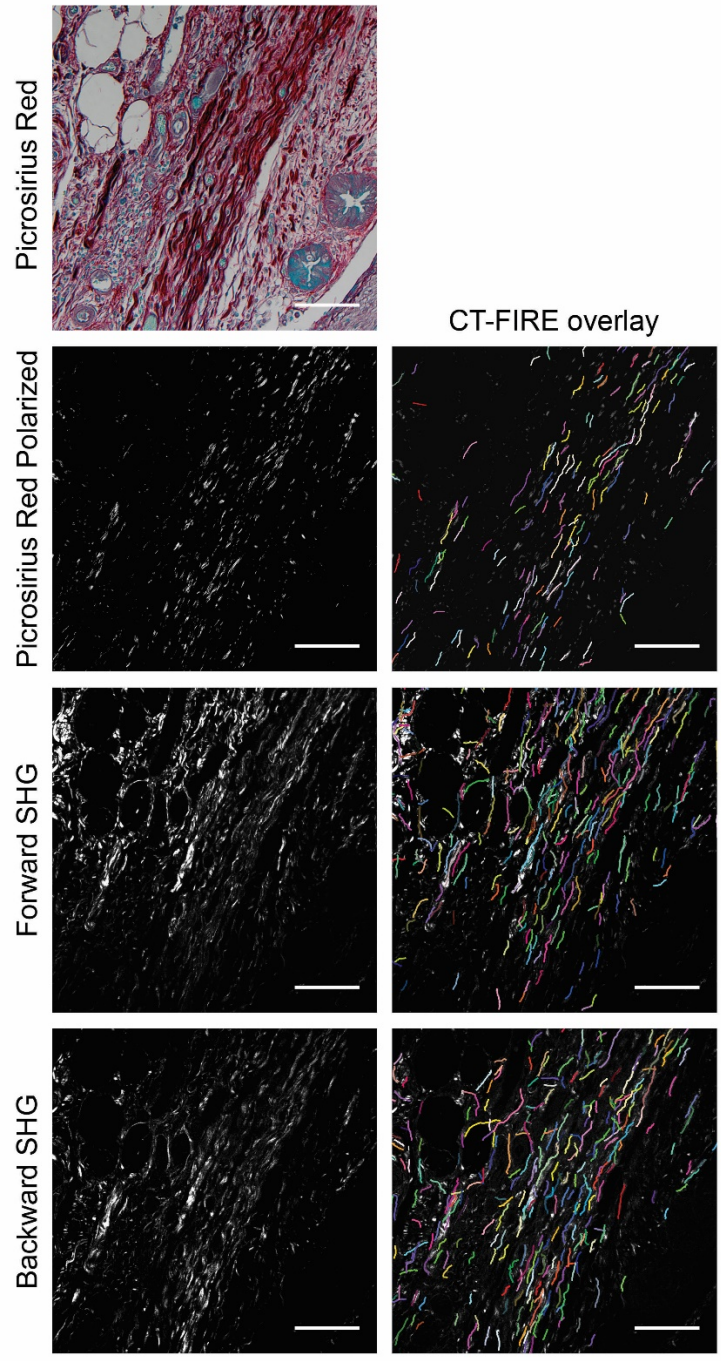

Picrosirius Red
CT-FIRE overlay
Picrosirius Red Polarized
Forward SHG
Backward SHG

Figure S5

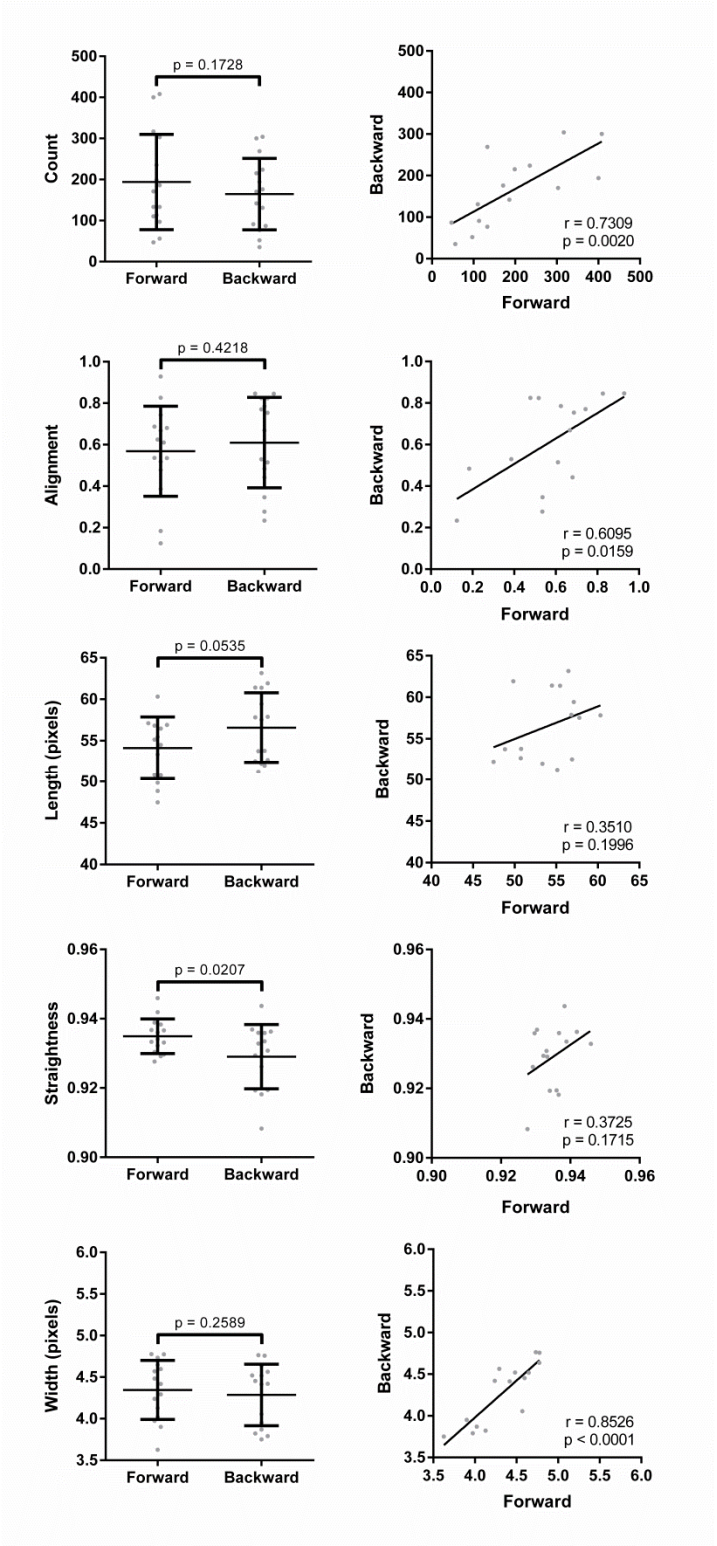
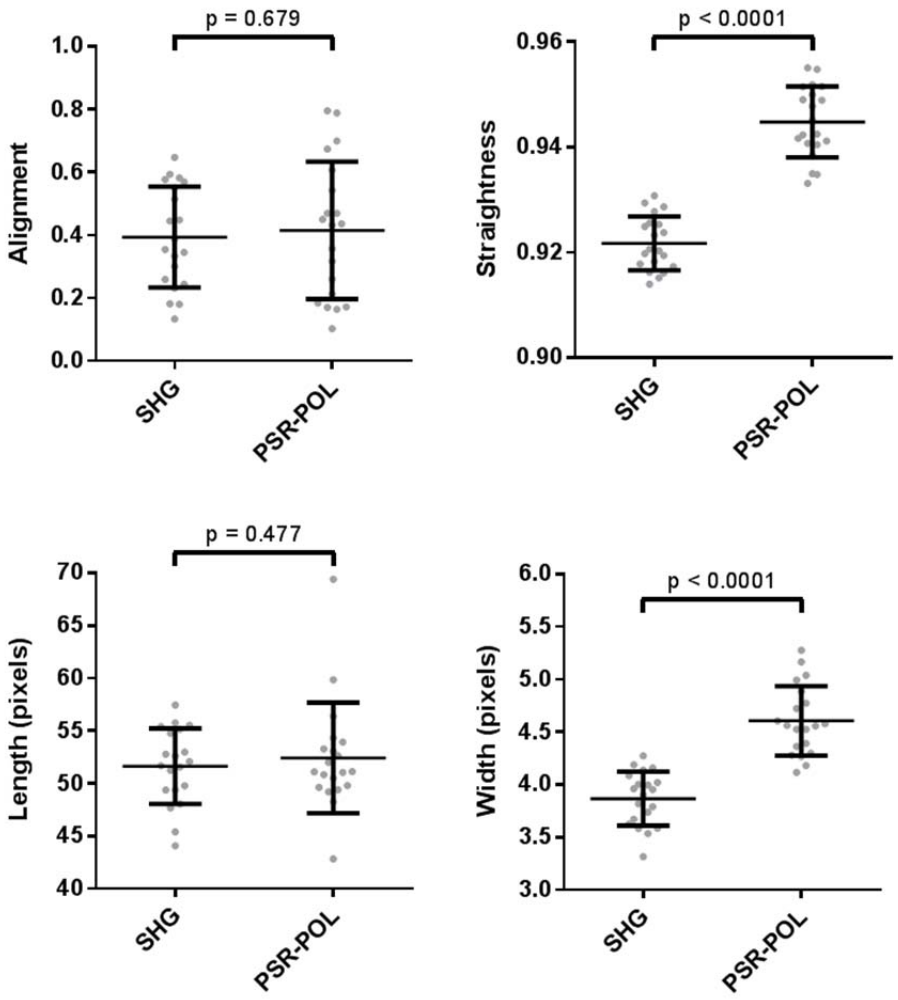

p = 0.1728
Count
Forward
Backward
Backward
r = 0.7309
p = 0.0020
Forward
p = 0.4218
Alignment
Forward
Backward
Backward
r = 0.6095
p = 0.0159
Forward
p = 0.0535
Length (pixels)
Forward
Backward
Backward
r = 0.3510
p = 0.1996
Forward
p = 0.0207
Straightness
Forward
Backward
Backward
r = 0.3725
p = 0.1715
Forward
p = 0.2589
Width (pixels)
Forward
Backward
Backward
r = 0.8526
p < 0.0001
Forward

Figure S6


p = 0.679
Alignment
1.0
0.8
0.6
0.4
0.2
0.0
SHG
PSR-POL
p < 0.0001
Straightness
0.96
0.94
0.92
0.90
SHG
PSR-POL
p = 0.477
Length (pixels)
70
65
60
55
50
45
40
SHG
PSR-POL
p < 0.0001
Width (pixels)
6.0
5.5
5.0
4.5
4.0
3.5
3.0
SHG
PSR-POL