

## **SUPPORTING INFORMATION**

### **Supplemental Methods S1**

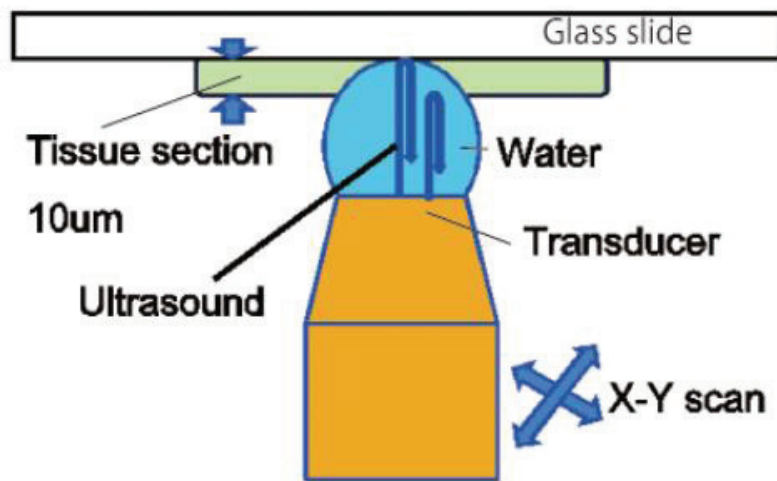
#### **Observation by scanning acoustic microscopy**

The scanning acoustic microscopy (SAM) used a single pulsed wave to image objects. SAM functions by directing focused sound from a transducer to a small area of the target object on a glass slide (Supplemental Figure 1). The sound emitted by an acoustic transducer hits or penetrates the tissue and is reflected onto the surface of the tissue or glass. It is then returned to the detector, which is coincident with the transducer. The speed of sound (SOS) through the tissue is calculated by comparing the “time of flight” of the pulse from the surfaces of both tissue and glass. Each specimen was scanned in a frame of  $300 \times 300$  pixels, and an area  $1200 \times 1200 \mu\text{m}^2$  was scanned to obtain each image within 3 min. The two-dimensional distribution of the echo intensity of sound, SOS, attenuation of sound (AOS) through the tissues, and the thickness of the tissue was obtained and plotted (Supplemental Figure 2). The penetration depth was up to a maximum of  $20 \mu\text{m}$ . The observation range within which the values were plotted could be adjusted.

## Supplemental Figure 1

### Principle of a scanning acoustic microscope

The ultrasound emitted by an acoustic transducer hits or penetrates the tissue and is reflected onto the surface of the tissue or glass slide. It is then returned to the detector. The speed of sound through the tissue is calculated by comparing the “time of flight” of the pulse from the surfaces of both the tissue and glass.

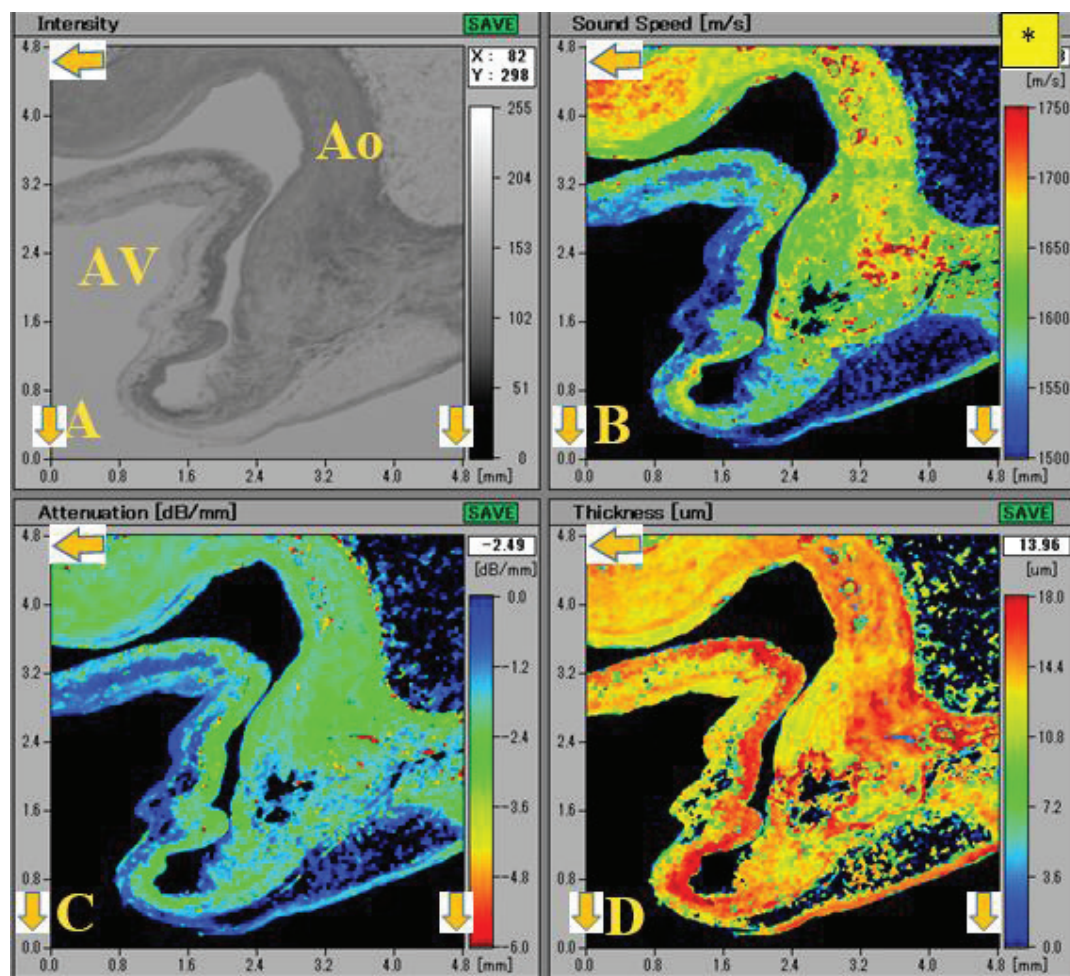


## Supplemental Figure 2

**SAM images of intensity (A), sound speed (B), attenuation (C), and thickness (D) of the tissue**

On the screen, the vertical bar at the left side and the horizontal bar at the bottom of each figure indicate the distance (mm) on the slide (arrows).

The vertical colour column at the right side of the “Sound-Speed” figure (upper right image indicated by \*) indicates the average SOS of each square area on the section. Similarly, the vertical colour columns in “Attenuation” (lower left image) and “Thickness” (lower right image) indicate the attenuated intensity-of-sound per mm (dB/mm) and the thickness of each square area ( $\mu$  m), respectively.



## Supplementary Table S1

Analysis of variance table (AV thickness, Fibrosa thickness, SOS through fibrosa)

### AV thickness

Source	SS	dF	MS	F-statistic	P-value	F (0.95)	F (0.99)
Total variation	6.32	86					
Between group	0.53	2	0.265	3.84	0.0254	3.105	4.867
Within group	5.79	84	0.069				

### Fibrosa thickness

Source	SS	dF	MS	F-statistic	P-value	F (0.95)	F (0.99)
Total variation	6.32	86					
Between group	0.53	2	0.265	3.84	0.0254	3.105	4.867
Within group	5.79	84	0.069				

### SOS through fibrosa

Source	SS	dF	MS	F-statistic	P-value	F (0.95)	F (0.99)
Total variation	637580	777					
Between group	5	2	422842.6	59.26	1.13E-24	3.007	4.633
Within group	845685	775	7135.6				

SS, sum of squares; dF, degrees of freedom in the source; MS, mean sum of squares due to the source.

## Supplementary Table S2

## SOS after protease digestion

### SOS after collagenase (AS cases)

AS collagenase	n	Mean (m/s)	U	SD	SE
0 h	30	1701.0	10408.7	102.0	18.6
1 h	30	1679.7	5949.7	77.1	14.1
3 h	30	1608.5	7015.6	83.8	15.3

### Analysis of variance table (ANOVA Table) (AS cases)

Source	SS	dF	MS	F-statistic	P-value	F (0.95)	F (0.99)
Total variation	818755.5	89					
Between group	140909.6	2	70454.8	9.043	0.00027	3.10	4.86
Within group	677845.9	87	7791.3				

### SOS after collagenase (AR cases)

AR collagenase	n	mean(m/s)	U	SD	SE
0 h	30	1727.0	10228.6	101.14	18.5
1 h	30	1695.6	7396.4	86.00	15.7
3 h	31	1669.9	7748.5	88.03	15.8

### ANOVA Table (AR cases)

Source	SS	dF	MS	F-statistic	P-value	F (0.95)	F (0.99)
Total variation	793345.1	90					
Between group	49765.01	2	24882.5	2.94	0.058	3.10	4.85
Within group	743580.1	88	8449.8				

### SOS after pepsin (AS cases)

AS Pepsin	n	mean(m/s)	U	SD	SE
0 h	50	1709.4	3350.7	57.9	8.19
1 h	51	1660.0	4048.2	63.6	8.91
3 h	51	1614.1	2999.6	54.8	7.67

### ANOVA Table (AS cases)

Source	SS	dF	MS	F- statistic	P- value	F (0.95)	F (0.99)
Total variation	745815.7	151					
Between group	229241	2	114620.5	33.1	1.31E-12	3.06	4.75
Within group	516574.6	149	3466.944				

SOS after pepsin (AR cases)

AR pepsin	n	mean(m/s)	U	SD	SE
0 h	71	1745.0	8769.1	93.6	11.1
1 h	70	1722.1	7573.6	87.0	10.4
3 h	72	1706.7	8324.5	91.2	10.8

ANOVA Table (AR cases)

Source	SS	dF	MS	F- statistic	P- value	F (0.95)	F(0.99)
Total variation	1780673	212					
Between group	53223.52	2	26611.8	3.24	0.041	3.04	4.71
Within group	1727450	210	8226.0				

U, unbiased estimate of population variance; SS, sum of squares; dF, degrees of freedom in

the source; MS, mean sum of squares due to the source.