

1 **Histological imaging of gastric tumors by scanning acoustic microscope**

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9

10 **Abstract**

11 Background

12 Human body tissues have their own speed-of-sound (SOS) by which sound travels through them. Harder
13 materials exhibit greater SOS; thus, SOS through each tissue can provide information regarding its
14 elasticity. For tumor discrimination in clinical medicine, palpation provides important information.
15 However, manual palpation is subjective, whereas SOS data are objective and can be directly used to
16 compare lesions.

17 Methods

18 A scanning acoustic microscope (SAM) uses ultrasound to image an object from SOS through tissues. We
19 investigated the utility of SAM in imaging gastrointestinal lesions.

20 Results

21 We visualized the digestive tract as a layered structure and discriminated tumors to a degree comparable
22 with that observed by light microscopy. Areas with greater SOS corresponded to those with higher
23 collagen or muscle fiber concentrations. Cell-poor areas or regions with degeneration demonstrated less
24 SOS than surrounding tissues. Gastric tumors displayed appropriate SOS similar to their original tissues
25 and showed significant differences in SOS between scirrhous carcinomas and well-differentiated
26 medullary carcinomas, scirrhous carcinomas and malignant lymphomas, and leiomyomas and
27 leiomyosarcomas.

28 Conclusions

29 SAM offered the following benefits over LM: (1) images are acquired in a few minutes without special
30 staining; (2) repeated observations of the same section are possible, ~~even after staining~~; (3)
31 high-resolution digital imaging from SOS is comparable to LM; (4) SAM analysis could be helpful in
32 understanding endoscopic ultrasonography imaging; and (5) digitized SOS data could be statistically
33 compared among different stomach lesions.

34

35 Key words: scanning acoustic microscope, tissue elasticity, cancer imaging, gastric tumor, fibrosis

36

37 **Introduction**

38 Humans possess two major sensory organs, the eyes and ears, which facilitate vision and hearing,
39 respectively. Material discrimination by light microscopy (LM) has been useful in elucidating various cell
40 systems, although sound has been rarely used in medical science. Some mammals, such as bats and
41 dolphins, use high-frequency (>20 KHz) ultrasound (US) for chasing game.

42

43 Human body tissues have their own speed-of-sound (SOS) by which sound travels through them. Harder
44 materials exhibit greater SOS; thus, SOS through each tissue can provide information on its elasticity[1].
45 For tumor discrimination in clinical medicine, palpation provides important information: most sarcomas
46 are softer than carcinomas, and scirrhous carcinomas are harder than medullary carcinomas. However,
47 manual palpation is subjective and depends on experience, whereas SOS data is objective and can be
48 directly used to compare lesions.

49

50 Endoscopic ultrasonography (EUS) has recently become a popular option for observing deep submucosal
51 or deeper lesions, such as cancer invasion, submucosal tumors, and lymph node metastasis in the
52 gastrointestinal (GI) tract[2, 3]. However, EUS is macroscopic and cannot be used to predict histology.

53 A scanning acoustic microscope (SAM) is a device that uses US frequency (>80 MHz) to microscopically
54 image an object[4]. We recently reported that SAM could be used to generate useful information about

55 lung[5] and lymph node[6] lesions.

56

57 Can SAM visualize GI normal structures? How are lesions detected by SAM? Does SOS really

58 correspond to tissue elasticity? Is SAM superior to LM?

59

60 Here, we show representative SAM gastric images and discuss its utility for clinical diagnosis.

61

62 **Materials and Methods**

63

64 **Materials**

65 All materials from anonymous donors were prepared from the Hamamatsu University Hospital archives.

66 The research protocol using stored samples without link to patient identity was approved by the research

67 ethics committee of Hamamatsu University School of Medicine. Formalin-fixed, paraffin-embedded

68 (FFPE) blocks were flat-sectioned in 10- μ m thick sections. Because deparaffinized sections repelled

69 water due to surface hydrophobicity and dried out easily, it was necessary to soak sections enough

70 in distilled water to reduce hydrophobicity. So deparaffinized, unstained sections were soaked in

71 distilled water for at least 3 h before observation. Continuous sections were stained with hematoxylin &

72 eosin (H&E) for comparison of SAM images with corresponding light microscopy (LM) images.

73

74 **Tissue samples**

75 We examined and selected normal and neoplastic stomach lesions, including adenocarcinomas (well or
76 poorly differentiated), carcinoid tumors, GI stromal tumors (GISTs), leiomyomas, malignant lymphomas,
77 leiomyosarcomas, and schwannomas.

78

79 **SAM observation**

80 The SAM was supplied by Honda Electronics (Toyohashi, Japan) and was equipped with a 120-MHz
81 transducer, which has a resolution of approximately 13 μm (Fig. 1). SAM functions by directing focused
82 sound from a transducer to a small area of the target object on a glass slide. The sound emitted by an
83 acoustic transducer hits or penetrates the tissue and is reflected on the surface of the tissue or glass. It is
84 then returned to the receiver, which is coincident with the transducer. The SOS through the tissue is
85 automatically calculated by comparing the time-of-flight of the pulse from the surfaces of both tissue and
86 glass.

87

88 To perform SAM imaging, the slide sections were placed upside-down on the stage above the transducer,
89 and distilled water was applied between the transducer and the section as a coupling fluid; this was done

90 because air interferes with sound transmission. After mechanical X–Y scanning, SOS from each point on
91 the section was calculated and plotted on the screen to create two-dimensional, color-coded images (Fig.
92 2A). The vertical bar on the left and the horizontal bar at the bottom of each figure indicate the distance
93 (mm) on the slide. The vertical colored column on the right side of the figure indicates the average SOS
94 of each square area on the section. The region of interest (ROI: 1.2, 2.4, or 4.8 mm²) for acoustic
95 microscopy was determined from the LM images. SOS values at 300 × 300 points were calculated and
96 plotted on the screen to create the images, and sound data from 64 cross points on the lattice screen were
97 used for statistical analysis.

98

99 Other data such as thickness of the section and sound attenuation were also available from each point and
100 were shown on the screen (data not shown).

101

102 **Statistical analysis**

103 SOS data from each tissue element are presented as mean ± standard deviation (SD; m/s). Student's *t*-tests
104 were used to determine statistically significant differences among the specimens. The level of statistical
105 significance was set at $P < 0.01$.

106

107 **Results**

108

109 Normal gastric structure

110 From the inner to outer surfaces, normal gastric layers consist of mucosa, lamina muscularis mucosae

111 (MM), submucosa (SM), muscularis externa (ME), subserosa, and serosa. The SAM system can

112 effectively discriminate among these gastric layer structures (Fig. 2A). In the mucosa, foveolar and

113 glandular structures were well visualized by SAM (Fig. 2B).

114 Cell-rich areas displayed greater SOS than cell-deficient areas. MM displayed linear band structure with

115 greater SOS and showed a clear boundary with SM. Smooth muscle layers, including MM and ME,

116 exhibited greater SOS than the mucosal layer (Table 1, Fig. 3).

117

118 Carcinomas of the stomach

119 Well and poorly differentiated adenocarcinomas arising from the mucosa formed tubular structures and

120 signet-ring cells, respectively (Figs. 4A, 4B). The SOS of adenocarcinomas was low, which was identical

121 to that of normal mucosa. No significant difference was observed between well and poorly differentiated

122 carcinomas in the mucosa (Table 1, Fig. 3).

123 At sumucosal invasive sites, well-differentiated adenocarcinoma with medullary growth exhibited SOS

124 which were the same as that of the original mucosa (Fig. 4C), while poorly differentiated scirrhou

125 carcinoma exhibited greater SOS than normal mucosa (Figs. 4D and 4E). The SOS values of scirrhou

126 carcinomas were significantly higher than well-differentiated carcinoma with medullary growth (Fig. 3,
127 Table 1).

128

129 Carcinoid tumors of the stomach

130 Carcinoid tumors consisting of trabecular structure with scarce stroma showed less SOS than the
131 surrounding mucosa (Fig. 5).

132

133 Malignant lymphomas

134 Malignant lymphoma displayed the least SOS of all assessed tumors (Fig. 6A and 6B, Table 1). The SOS
135 values of the lymphomas were significantly lower than those measured in scirrhous carcinoma samples
136 (Fig. 3).

137

138 Leiomyomas and leiomyosarcomas

139 Leiomyomas appeared as sharp nodular structures and demonstrated SOS values as high as those of
140 smooth muscles of MM, ME, and blood vessels (Figs. 7A and 7B, Table 1). Thick muscle bundles
141 exhibited the greatest SOS values (Fig. 7C).

142 Leiomyosarcoma with great SOS area showed geographic myxoid degeneration with less SOS area (Fig.
143 8A), and their transitions were abrupt and irregular. Multinodular leiomyomas with high cellularity

144 displayed less SOS compared with septal fibrous bands (Fig. 8B). The SOS values of leiomyosarcomas
145 were significantly lower than those of leiomyomas (Fig. 3).

146

147 GIST

148 GISTs of the stomach formed large submucosal nodules, and their SOS values were almost identical to
149 those measured in the mucosa (Figs. 9A and 9B, Table 1). Focal areas with myxoid change exhibited
150 lower SOS values than cell-rich areas.

151

152 Schwannomas

153 Schwannomas included both cell-rich and cell-deficient areas, which exhibited large and small SOS
154 values, respectively (Fig. 10).

155

156 **Discussion**

157 The biomedical application of SAM was first described by Lemons and Ouate in 1975[7]. SAM is a
158 confocal imaging device in which thin specimens are scanned point-by-point using very strongly focused
159 beams[4]. The attenuation of US in tissues is much greater than that in water and increases with elevation
160 in the frequency.

161

162 The data on the SOS values in biological tissues in various organs can be found in reviews[8, 9]. Studies
163 of the acoustic properties of tissues at low frequency suggest that SOS values in soft tissues differ only
164 slightly from those in water and are virtually independent of frequency. Significant differences between
165 SOS in water and objects were only observed for solid biological tissues, such as bones and tissues that
166 contain large concentrations of fibrillar proteins[9]. Because the stomach has muscle layers and exhibits
167 reactive fibrosis, data from high-frequency SAM may provide useful information to complement
168 low-frequency EUS imaging.

169

170 The abovementioned SAM results show both normal and abnormal gastric images. Normal layer structure
171 was destroyed by various causes, including neoplastic and inflammatory lesions. Each layer or lesion is
172 characterized by their own SOS, which is useful for discriminating lesions. On EUS imaging, five distinct
173 layers are seen[2, 3], which correspond to the following: layer 1, interface echo between the superficial
174 mucosa and the acoustic coupling medium; layer 2, deep mucosa; layer 3, SM plus the acoustic interface
175 between SM and ME; layer 4, ME without the acoustic interface between SM and ME; and layer 5, serosa
176 and subserosal fat. In SAM, layer 1 corresponded to surface mucous contents; layer 2 was equivalent to
177 MM; and layers 3 and 4 almost corresponded to SM and ME, respectively, although their interface was
178 sometimes obscured owing to the presence of loose muscle fibers.

179 In a normal stomach, muscle layers such as MM and ME were harder on palpation than mucosa or SM,

199 Tubular structure architecture was irregular or lost in well and poorly differentiated adenocarcinomas,
200 respectively. At the cancer invasive site through MM, the smooth muscle layer appeared split or
201 penetrated by cancer cells and exhibited a lower SOS value compared with the surrounding intact MM.

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213 In general, carcinomas are harder than sarcomas, and this was definitely supported by the SAM data.

215 induced stromal desmoplastic reactions in invasion sites with increased collagen fibers, which resulted in

216 increased SOS. Scirrhou carcinomas exhibited significantly greater SOS values than non-invasive
217 carcinomas or non-scirrhou carcinomas.

218

219 Scirrhou carcinomas also demonstrated significantly higher SOS values than other sarcomas, such as
220 lymphomas and GISTs, which could be useful supplementary data for making histological tumor
221 diagnoses.

222

223 Compared with benign leiomyomas that are conspicuously hard, leiomyosarcomas are usually softer upon
224 palpation. The SAM data proposed significantly less SOS of leiomyosarcomas than that of leiomyomas,
225 which confirms is in accordance with the aforementioned findings.

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233 We used FFPE sections instead of fresh-frozen tissue. In the literature, low-frequency studies (1–7 MHz)

234 of acoustic parameters, including SOS, have demonstrated that fixation in 4% formalin solution changed
235 these parameters only slightly[10]. FFPE kidney tissue showed no significant differences from
236 fresh-frozen kidney tissue[11]. Moreover, we assessed the effect of fixation effect on SAM observations
237 before performing this study. We prepared fresh-frozen sections and soaked them in 10% formalin to
238 observe changes in SAM findings according to the fixation duration. Because mucosal imaging was
239 unstable before 1-h fixation (Supplemental Fig. 1), we used paraffin sections that had been fixed for more
240 than 1 h in this study. Other merits of using FFPE included the availability of archive blocks and easy
241 preparation of 10- μ m flat sections compared with fresh-frozen sections.

242

243 Malignant tumors are, in general, harder than surrounding normal tissues. Manual palpation has been used
244 to assist in diagnosis, but it is subjective and restricted to large and more superficial structures. In the
245 upper GI tract, EUS has recently been used to diagnose subepithelial lesions, estimate the invasive depth
246 of early GI cancers, and evaluate of esophageal varices[12]. For definite diagnosis of subepithelial lesions,
247 particularly deep SM and ME layers, histological access, such as that achieved with EUS-guided fine
248 needle aspiration, is usually needed[3]. SAM results indicated that SM non-epithelial tumors
249 demonstrated similar SOS values. Irregular margins and geographic myxoid changes may be suggestive
250 of malignancy, and biopsy specimens should be obtained from these sites.

251

252 An ultrasonic imaging system typically needs to be able to resolve structures of ~1 mm size at depths of
253 up to ~150 mm in humans. US waves travel ~1500 m/s through water. The wavelength, which is one of
254 the factors that determines spatial resolution, is optimal for abdominal scanning, for example, 0.5 mm at 3
255 MHz[4]. Objects smaller than the wavelength do not reflect US. The penetration length through tissues
256 decreases proportional to the wavelength. For histological imaging, we employed a 120-MHz transducer
257 that had about 13- μ m spatial resolution, which barely allows it to detect single cells.

258

259 We previously reported SAM imaging of lungs[5] and lymph nodes[6]. In the latter, statistically
260 significant differences in SOS values were observed among scirrhous carcinomas, lymphomas, and
261 medullary carcinomas. The SOS values of these tumors correlated with the elasticity on manual palpation.
262 These results were also applicable to gastric lesions. In the lung, the degree of pulmonary fibrosis was
263 reflected with SAM and was statistically comparable between lesions. In gastric lesions, tumor
264 invasion-induced stromal fibrosis was detected by SAM, and the SOS data revealed a statistical difference
265 between non-invasive and invasive carcinomas.

266

267 In conclusion, based on our experiences and the present observation, SAM offers the following benefits
268 over LM: (1) images are acquired in only a few minutes without the need for special stains; (2) repeated
269 observations of the same section are possible even after staining; (3) digital SOS imaging achieves high

270 resolution, approaching that achieved with LM; (4) SAM analysis could be helpful to understand
271 ultrasonic endoscopic images; and (5) digitized SOS data can be used to statistically compare different
272 stomach lesions.
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274

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282

283 **Competing interests**

284 The authors declare that they have no competing financial interests.

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287 **References**

288

- 289 1. Wells PNT. Velocity, absorption and attenuation in biological materials. In
290 *Biomedical ultrasonics*. Academic Press: London, 1977; 110-137.
- 291 2. Hwang JH, Rulyak SD, Kimmey MB. American Gastroenterological Association
292 Institute technical review on the management of gastric subepithelial masses.
293 Gastroenterology 2006;130:2217-2228.
- 294 3. Sakamoto H, Kitano M, Kudo M. Diagnosis of subepithelial tumors in the upper
295 gastrointestinal tract by endoscopic ultrasonography. World J Radiol
296 2010;2:289-297.
- 297 4. Wells PN. Advances in ultrasound: from microscanning to telerobotics. Br J Radiol
298 2000;73:1138-1147.
- 299 5. Miura K, Yamamoto S. Pulmonary imaging with a scanning acoustic microscope
300 discriminates speed-of-sound and shows structural characteristics of disease. Lab
301 Invest 2012;92:1760-1765.
- 302 6. Miura K, Nasu H, Yamamoto S. Scanning acoustic microscopy for characterization of
303 neoplastic and inflammatory lesions of lymph nodes. Sci Rep 2013;3:1255; DOI
304 10.1038/srep01255.
- 305 7. Lemons RA, Quate CF. Acoustic microscopy: biomedical applications. Science
306 1975;188:905-911.
- 307 8. Chivers RC, Parry RJ. Ultrasonic velocity and attenuation in mammalian tissues. J
308 Acoust Soc Am 1978;63:940-953.
- 309 9. Maev R. Acoustic properties of biological tissues and their effect on the image
310 contrast. In *Acoustic microscopy, Fundamentals and application*. Wiley-VCH:
311 Weinheim, Germany, 2008; 212-213.
- 312 10. Bamber JC, Hill CR, King JA, Dunn F. Ultrasonic propagation through fixed and
313 unfixed tissues. Ultrasound Med Biol 1979;5:159-165.
- 314 11. Sasaki H, Saijo Y, Tanaka M, Okawai H, Terasawa Y, Yambe T et al. Influence of
315 tissue preparation on the high-frequency acoustic properties of normal kidney tissue.
316 Ultrasound in Med & Biol 1996;22:1261-1265.
- 317 12. Kim EY. Endoscopic ultrasound, where are we now in 2012? Clin Endosc
318 2012;45:321-323.

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321 Table 1 The mean speed-of-sound through gastric location (m/s), SD and reference range

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323 Foveolar muc, foveolar mucosa; Gland muc, glandular mucosa; MM, muscularis mucosae; ME,
324 muscularis externa; AD-Well(muc-sm), well-differentiated adenocarcinoma (mucosa, submucosa);
325 AD-Por(muc), poorly differentiated adenocarcinoma (mucosa); Sci ca (sm), scirrhous carcinoma
326 (submucosal invasion); GIST, gastrointestinal stromal tumor; RR lower, lower reference range; RR upper,
327 upper reference range.
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329

330 **Figure Legends**

331 Figure 1. Principles of scanning acoustic microscopy (SAM)

332 Ultrasonic waves from the transducer reflect off both the glass slide and the section and return to the
333 transducer. These waves pass through 10- μ m sample sections with different ultrasonic properties. The
334 transducer automatically scans the section to calculate the speed-of-sound through each area. The section
335 is placed upside down on the transducer, and distilled water is applied between the transducer and the
336 section as a coupling fluid. The control speed-of-sound (SOS) through water is 1500 m/s.

337

338 Figure 2A. Scanning acoustic microscopy imaging of normal stomach tissue

339 From top to bottom, the gastric wall consists of the mucosa, the lamina muscularis mucosae (MM), the
340 submucosa (SM), the muscularis externa (ME), and the serosa. The acoustic image (right) clearly
341 discriminates these layer structures comparable with its corresponding H&E-stained light microscopy
342 image (left). The speed-of-sound values of the thick ME is highest among these layers.

343

344 Figure 2B. The acoustic image (right) of normal gastric mucosa and submucosa and its corresponding
345 light microscopic image with H&E staining (left)

346 Foveolar and glandular structures are clearly visualized by scanning acoustic microscopy. The cell-rich
347 area shows higher speed-of-sound (SOS) than the cell-deficient area. The muscularis mucosae exhibit a

348 linear, thin-banded structure with higher SOS. In the submucosa, blood and lymph vessels show narrow
349 tubular structures with greater SOS.

350

351 Figure 3. The mean and SD (m/s) of speed-of-sound through gastric location are shown

352 Foveolar muc, foveolar mucosa; Gland muc, glandular mucosa; MM, muscularis mucosae; ME,

353 muscularis externa; AD-Well(muc-sm), well-differentiated adenocarcinoma (mucosa, submucosa);

354 AD-Por(muc), poorly differentiated adenocarcinoma (mucosa); Sci ca (sm), scirrhous carcinoma

355 (submucosa); GIST, gastrointestinal stromal tumor, ** $P < 0.01$

356

357 Figure 4A. A well-differentiated adenocarcinoma with mucosal spread

358 Irregular tubular structures with fused glands are shown among normal foveolar tubules from the right

359 upper to the left lower areas in the figure. Smooth muscles in the muscularis mucosae separate the

360 clusters of cancer cells with conspicuous speed-of-sound values.

361

362 Figure 4B. A poorly differentiated adenocarcinoma spreading through the mucosa

363 The normal foveolar and glandular structures have disappeared and are replaced by signet ring cells that

364 are represented as scattered dots with increased speed-of-sound values (right).

365

366 Figure 4C. A well to moderately differentiated adenocarcinoma invading from the mucosa to the
367 submucosa with medullary growth
368 The normal tubular architecture is replaced by cancer cells. The smooth muscle layer of muscularis
369 mucosae is split (arrow) by invading cancer.

370

371 Figure 4D. A poorly differentiated adenocarcinoma with scirrhous submucosal invasion
372 Many signet ring cells spread in the mucosa and infiltrate beyond the muscularis mucosae into the
373 submucosa. In the submucosa, desmoplastic reactions with collagen fiber proliferation are seen, and
374 spicular structures exhibit greater speed-of-sound.

375

376 Figure 4E. A poorly differentiated adenocarcinoma with scirrhous spread
377 Many signet ring cells infiltrate from the mucosa to the submucosa with desmoplastic reactions, which
378 demonstrate speed-of-sound similar to that observed in muscularis mucosae or perivascular smooth
379 muscle. An arrow indicates split smooth muscles of the muscularis mucosae.

380

381 Figure 5. A small gastric carcinoid tumor in the mucosa
382 The carcinoid portion consisting of trabecular structure with scant fibrous components exhibits lower
383 speed-of-sound than the surrounding mucosa (right).

384

385 Figure 6A. A gastric malignant lymphoma infiltrating from the mucosa to the submucosa

386 The lymphoma shows reduced speed-of-sound and spreads among mucosal tubules (right).

387

388 Figure 6B. Higher magnification of the gastric lymphoma shown in figure 6A

389 The lymphomatous area exhibits the least speed-of-sound (SOS) among the mucosal components. The

390 dilated lymph vessels and the congested veins in the lymphoma show lower and higher SOS, respectively.

391

392 Figure 7A. A gastric leiomyoma in the ME

393 An elliptical leiomyoma with clear margins shows speed-of-sound (SOS) similar to that of the smooth

394 muscles of muscularis mucosae, ME, and blood vessel walls. The surrounding ME displays higher SOS

395 due to compression artifact.

396

397 Figure 7B. Higher magnification of the leiomyoma shown in figure 7A

398 The higher speed-of-sound areas in the right scanning acoustic microscopy image correspond to dense

399 fascicles of smooth muscle and interstitial blood vessels.

400

401 Figure 7C. A leiomyoma with dense muscle bundles

402 The highest speed-of-sound values (right) were observed from the mucosa to the muscularis externa.

403

404 Figure 8A. A leiomyosarcoma with myxoid areas

405 The hyalinized area (H), tumor cell-rich area (R), and myxoid transition area (M) display the highest,

406 middle, and lowest speed-of-sound values, respectively. The hot spots in the cell-rich area correspond to

407 blood vessels.

408

409 Figure 8B. A gastric leiomyosarcoma comprising multinodular structures with irregular outer contours

410 The nodules are made up of tumor cells that are separated by a fibrous band and exhibit lower

411 speed-of-sound than the fibrous band.

412

413 Figure 9A. A gastrointestinal stromal tumor in the submucosa

414 The tumor margin has numerous blood vessels and shows higher speed-of-sound (SOS), whereas the

415 tumor center displays almost the same SOS as that of the foveolar epithelium. The mucosal surface shows

416 greater SOS, which corresponds to rich mucousal content.

417

418 Figure 9B. A gastrointestinal stromal tumor (GIST) protruding from the muscularis externa into the serosa.

419 The GIST shows variable speed-of-sound (SOS) among different areas. The cell-rich serosal surface

420 displays higher SOS and the cell-deficient central area with myxoid change (asterisk) exhibits reduced
421 SOS. The spotty areas with increased SOS correspond to blood vessels.

422

423 Figure 10. A gastric schwannoma in the submucosa

424 The schwannoma mixed with cell-rich and cell-deficient areas demonstrate higher and lower
425 speed-of-sound, respectively.

426

427

428 Supplemental Figure 1

429 A fresh-frozen section of mouse small intestine before fixation (a) and at 3 min (b), 1 h (c), 12 h (d), 24 h

430 (e) after 10% formalin fixation, and their corresponding H&E section (f). The mucosa of unfixed section

431 shows almost the same SOS as that of water and internal structures are invisible. At 3 min, mucosal

432 cytoskeleton appears as axes of villi structures. After 1 h and over, mucosal cytoplasm displays almost

433 uniform SOS and internal structures become unclear.