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Three-dimensional evaluation of the relationship between speed of sound and scattering characteristics of lymph nodes in tumor-bearing mice

マウス担がんリンパ節における音速と散乱特性の関係性の三次元評価

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1. Introduction

In order to minimize the invasion of intraoperative lymph node dissection and improve the treatment effect, highly accurate screening for cancer metastasis is required. In our previous study, the cancer metastasis in excised human lymph nodes could be evaluated with a positive diagnosis rate of more than 90% by analyzing the scattering characteristics with high-frequency ultrasound[1]. More accurate assessment and *in vivo* application of various tumor properties will be enabled when the relationship between the microscopic acoustic properties and tissue structure of lymph nodes in three-dimensional (3D) space is understanding.

In this study, we evaluated the scattering properties of lymphadenopathy model mice inoculated with cancer tumors by using echo data acquired *in vivo* and assessed the speed of sound (SoS) by a bioacoustic microscopy in three-dimensions (3D) after excision. The possibility of the evaluation of the macroscopic properties of *in vivo* tissues from microscopic acoustic properties was examined by linking the two acoustical characteristics.

2. Materials and Methods

2.1 Target materials and data sets

A mouse sub-iliac lymph node was inoculated with KM-Luc/GFP mouse malignant fibrous histiocyte-like cells as tumor cells[2]. Ten days after inoculation, the mouse was placed under anesthesia, and ultrasound echo signals from the lymph node were acquired *in vivo*. After that, the lymph node was removed, formalin-fixed, paraffin-embedded, and serially sliced at 7 μm in thickness. 50 consecutive thin-sliced samples were evaluated by ultrasonic microscopy and pathological imaging.

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2.2 Statistical amplitude envelope analysis

To measure lymph node, a mechanical scan probe (RMV710, VisualSonics) with a center frequency of 25 MHz was scanned by a motor stage on a veterinary ultrasound system (Vevo770, VisualSonics), and 3D RF data were acquired. The RF data were recorded by the A/D board (M4i.44xx-x8, Spectrum) with a sampling frequency of 250 MHz and 14-bit quantization. The amplitude envelopes of the acquired RF data were analyzed for their scattering characteristics using the Nakagami model[3] as shown as

$$p(x) = \frac{2\mu^{\mu}x^{2\mu-1}}{\Gamma(\mu)\omega^{\mu}} exp\left\{-\frac{\mu}{\omega}x^2\right\} \qquad (1)$$

where x is the amplitude envelope, Γ is the gamma function, and μ and ω are parameters related to the scatterer density and the power of the echo signal, respectively. The case of μ<1 is classified as pre-Rayleigh (low density or non-uniform scatterer structure), μ=1 as Rayleigh, and μ>1 as post-Rayleigh (high density and uniform). In this study, the parameter μ was derived by the maximum likelihood estimation method. The analysis window was set to 0.5 mm cube, and only the parenchymas of the lymph node was analyzed.

2.3 SoS analysis

A ZnO transducer (HT-400C, Honda Electronics) with a center frequency of 300 MHz was excited by a pulser (GZ1120ME-03 GEOZONDAS) to acqumulate 3D RF data by scanning the transducer in two-dimensions with a scan interval of 4 μm. RF data were recorded by an A/D board (ATS9373, AlazarTech) with a sampling frequency of 4 GHz and 12-bit quantization. The echo signal of the glass section was used as the reference signal, and the SoS analysis was performed with echos form surface and bottom of each sliced sample that separated using an eighth-order autoregressive model[4]. Each sliced sample was HE-stained after ultrasound observationto acquire digital pathology

images (1 μm resolution) with a virtual slide scanner (NanoZoomer s60, Hamamatsu Photonics) to observe the tissue structure.

2.4 Registration of SoS map and histological image

Using the SoS map obtained in section 2.3 as a reference, registration with the histological image of the same sliced sample was performed, and the relationship between the acoustic characteristics and the tissue structure was confirmed. As a procedure, a histological image was gray-scaled and performed by Otsu's binarization to obtain shape information. Using the shape information, the histological image was affine transformed, and the Jaccard index in the SoS map and the transformed histological image was maximized[5]. The same registration process was performed on the data of all the consecutive thin slice samples, then the 3D maps of SoS and histology images were generated by stacking the data[6].

3. Result and discussion

Figures 1(a) and **1(b)** show the C-section of the central lymph node and the 3D image of the entire lymph node with the Nakagami-μ superimposed. It was confirmed that the low values of μ were highly distributed in the parenchyma of a lymph node. It suggests that there are many areas with low scatterer density or non-uniform scatterer distribution in the lymph node parenchyma.

Figures 2(a-1) and **2(b-1)** show the overall SoS map and the histological image in the same sliced sample. **Figs. 2(a-2)** and **2(b-2)** show the 3D images of the SoS and histology of a region containing luminal structures in the lymph node (2 mm × 2 mm × 0.35 mm). **Fig. 2** shows that the luminal structures in the lymph node are distributed in many places in each direction, and the SoS tends to be low around the luminal structures. It suggests that the luminal structure and the acoustic properties evaluated by ultra-high frequency ultrasound (300 MHz) affect the value of the Nakagami parameter μ estimated by high-frequency ultrasound (25 MHz).

4. Conclusion

It was confirmed that the 3D tissue structure and acoustic properties evaluate at the micro size affect the amplitude envelope characteristics estimates at the macro size.

In future studies, the acoustic impedance of raw specimens will also evaluate in various state lymph nodes to understand the more detail between physical tissue characteristics and acoustic properties and scattering properties.

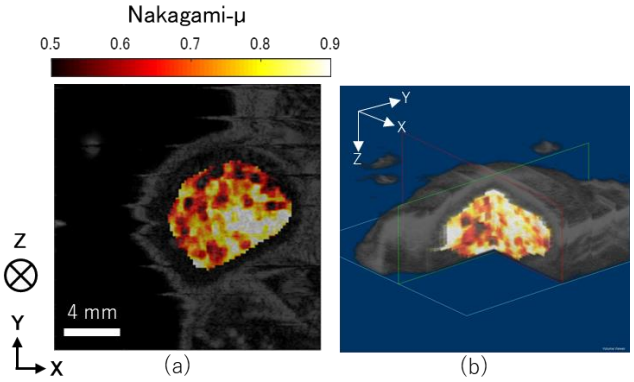

Fig.1 Cross-sectional (s) and 3D (b) images of Nakagami-μ of mouse lymph node.

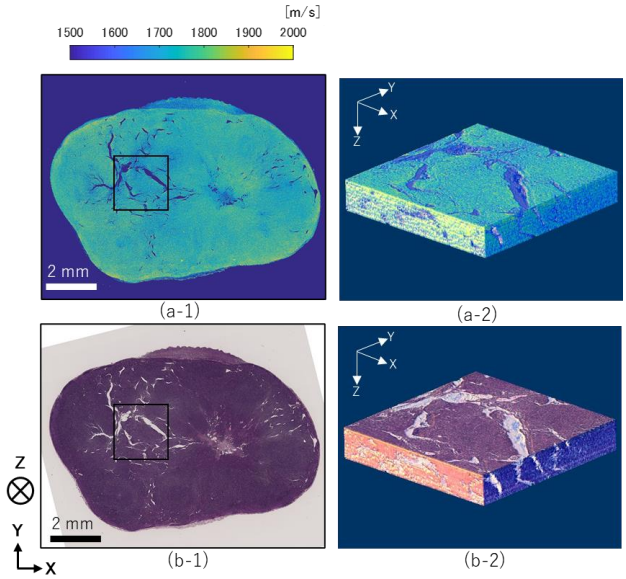

Fig.2 SoS map (a) and pathological image (b) of mouse lymph node. (*-1) overall, (*-2) 3D image of region containing luminal structure.

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