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Application of high-resolution ultrasound / photoacoustic imaging for medicine and biology

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1. Introduction

One of the characteristics of ultrasound imaging is multi-scale and functional. Because the wavelength and beam width are inversely proportional to the frequency of ultrasound, higher frequency ultrasound provides higher resolution imaging. We have been developing scanning acoustic microscopy (SAM) for medicine and biology since 1985. Quantitative SAM has clarifeid biomechanical properties of tissues and cells because the square of sound speed is proportional to elasticity and attenuation is related to viscosity.

Sub-micron resolution imaging by SAM was difficult because the highest frequency availabe in the liquid was limited to 1.3 GHz. Our first motivation of introducing laser to SAM; in other words, development of photoacoustic microscopy (PAM), was to simply improve the resolution. Of course, PAM also provides higher contrast imaging of vasculature because the red blood cells produce strong PA signal. Here developments of SAM and PAM for medicine and biology are introduced.

2. Development of acoustic microscope systems

2.1 Original SAM system (1985-2000)[1-3]

The motivation of our first SAM was to assess the origin of the echo signal in clinical ultrasound images. The system used a planar ZnO transducer with a sapphire acoustic lens to focus the ultrasonic beam. The central frequency of the transducer was 170 MHz and the burst waves varying 100-200 MHz were generated. The transducer was mechanically scanned by a flat plate spring with a vibration frequency of 60 Hz. Thus, one single frequency image consisted of 480 x 480 pixels was obtained in 8 sec. The most significant feature of this SAM was to obtain quantitative parameters such as attenuation or sound speed. For these measurements, precise measurement of the thickness of thinly sliced biological sample was necessary. Because optical method was not available at that period, we proposed acoustic measurement by analyzing frequency dependent characteristics of amplitude and phase of the received signal which was the sum of reflections from the surface of the tissue and the interface between the tissue and slide glass.

2.2 Sound speed / impedance acoustic microscope systems (2001-present)[4]

Thanks to the introduction of “plug and play” concept by Windows 95, the researchers made the measurement and scanning systems easily configurable. High frequency digitizer which was developing at the same era was installed into a conventional personal computer to obtain a single pulse. New concept of SAM; instead of changing the frequency of the burst wave signal, the frequency characteristics was analyzed by FFT of a single ultrasonic pulse. A concave PVDF-TrFE transducer with the central frequencies of 80-120 MHz was used. The transducer was mechanically scanned beneath the tissue or cell. The only disadvantage of these new SAMs compared with the original SAM is the scanning speed driven by a linear or servo motor. It takes approximately 1 min for acquisition of a single image.

2.3 Optical / acoustic hybrid microscope for observation of cells (2014-present)[5]

For simultaneous observation of morphological and biomechanical properties, optical / acoustic hybrid microscopy was developed. Optical modules mainly consisted of inverted optical microscope and CMOS camera. In this new system, a thermo-control system was installed for observation of living cells. The temperature of the medium was kept between 35 and 37°C during measurement.

Ultrasonic unit mainly consisted of ultrasonic transducer, pulser/receiver, A/D converter, and automatic stage. The transducer was made of thin film ZnO attached with sapphire lens. The central frequency of the transducer was 250 MHz, focal length was 0.5 mm, and aperture length was 0.45 mm. The pulser/receiver had the operating frequency of 450 MHz and amplification of 23 dB. High speed digitizer was used to digitize the ultrasonic signal with the sampling rate of 8 GS/s and resolution of 8 bit. The piezo actuator automatic stage was used for two-dimensional mechanical scan with the precision of 1 nm. All the instruments were controlled by a LabVIEW program on the PC. The scan area was 80 x 80 micron with up to 200 x 200 sampling points and the resolution was enough to visualize a single cell.

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3. Development of photoacoustic microscope systems

3.1 Acoustic resolution photoacoustic microscopy (AR-PAM)[6]

Laser pulses were generated by a semiconductor laser with the repetition rate of 50 Hz. PA signal was received by a 50 MHz concave ultrasound transducer with a hole in the central part to get through a light fiber in order to align light illumination and signal reception concentrically. The prototype of the AR-PAM visualized morphology and microvasculature of a human nail bed.

The AR-PAM system has been improved in collaboration with Advantest Corporation. HADATOMO Z^{TM} features dual wavelength laser with 532/556 nm to obtain oxygen saturation. Further development of the system has realized a long depth of field (DOF) imaging by using an annular array transducer.

3.2 Optical resolution photoacoustic microscopy (OR-PAM)[7]

In the transmission mode OR-PAM, 532 nm diode-pumped solid-state (DPSS) pulsed laser (6 ns pulse width; 2 nJ/pulse laser energy; 10 kHz pulse repetition rate) was used as the light source. The focused laser beam was irradiated into the objects and the generated PA waves were received by a concave single-element ultrasonic transducer (50 MHz center frequency; 3.2 mm focal length; 2.4 mm aperture diameter) aligned coaxially and confocally with the laser beam. The optical part was placed underneath the OR-PAM, which employed a 1/1.8" CMOS camera and a white light source (400-800 nm wavelength). The objects were placed on a glass-bottom dish with a thickness of about 100 μm where optical aberrations were negligible. The whole dish was scanned by a piezo stage with the minimum scanning step of 1 nm and the maximum scanning width of 200 μm.

In bovine red blood cell imaging, the biconcave shape peculiar to red blood cell was clearly visualized by the hybrid microscopy. The overlay image could complement the external shape (morphological information) of the red blood cell and the internal hemoglobin distribution (functional information) at the same position. The OR-PAM system also visualized a cell phagocytosis of gold nanorod (AuNR). The macrophage or monocyte with AuNR may accumulate at inflammation or atherosclerosis spots.

3.3 Realtime photoacoustic tomography with a hemispherical array transducer[8]

A spherical-curvature array transducer consisting of 256 elements made of 1-3-composite material. The transducer geometric had a focal depth of 30 mm and a 10.4-mm hole in the center through which to irradiate targets with a laser. The bandwidth was 10-23 MHz (-6 dB range), and the center frequency was 16.5 MHz. The short-pulse wavelength-tunable OPO laser was used for generating the PA signals. The PA signals were acquired using a programmable acquisition system (256 Tx/Rx channels) with a sampling frequency of 62.5 MHz. The acquisition system was connected to pulse generator to synchronize it with the laser irradiation. The synchronization permitted 3D PA imaging in real time at 20 vols per second (vps). The PA images were reconstructed by applying the delay-and-sum beamforming method to the acquired signals. In this method, the signals received by each ultrasonic detector array element are summed after compensating for the different travelling times from the elements. The system enabled a real-time imaging with the frame rate of 20 Hz to observe a single red cell movement in a micro vessel of a human palm.

4. Summary

SAM and PAM systems for application of medicine and biology are introduced. These systems were created through all-Japan joint research projects. Further developments for stable, compact and inexpensive systems are desired for clinical application.

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