"Sonocytometry" – Novel Diagnostic Method of Ultrasonic Differentiation of Cells in Blood Flow

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***Abstract*—Novel diagnostic method named "sonocytometry", in which streaming blood cell is diagnosed by the reflection of high frequency ultrasound from the cell, is proposed. In the present study, the differentiation of the particle size is performed as a basic study on sonocytometry. Ultrasonic backscatter signal from either 80 or 100 μm diameter polystyrene particles was measured by an ultrasonic transducer with the central frequency of 30 MHz. The spectrum of the reflected signal showed different characteristics according to the particle diameter. Theoretical value of backscatter was calculated by Faran-Hickling model and the correlation coefficient of measured and theoretical value by varying the spherical diameter showed the local maximum value at either 80 or 100 μm diameter. The principle was also validated on the streaming particles in a flow channel. The method successfully classified the particle size. Sonocytometry would be clinically applied for diagnosis of malaria or leukemia.**

I. Introduction

Flow cytometry is widely used to classify individual cells optically but blood sampling is still required for conventional blood cell measurements. If the principle of the cytometry is applicable directly *in vivo*, differentiation of the white blood cell in leukemia or detection of infected red blood cell in malaria, would become faster and easier by non-invasive manner. Some optical methods have been applied for *in vivo* blood cell imaging [1, 2]. However, optical observation of the cell in blood flow of vessels beneath the skin is nearly impossible because absorption and scattering of the light is large in skin. In the present study, a novel diagnostic technique called "sonocytometry" is proposed in which blood cells are non-invasively differentiated by the reflection of high frequency ultrasound.

Individual red blood cells can be visualized with high frequency ultrasound close to 1 GHz but direct observation of the red blood cell *in vivo* is difficult because the penetration depth is limited. For application of *in vivo* measurement, frequency spectral analysis of the ultrasonic backscatter from a cell with several tens of MHz range ultrasound, which has sufficient penetration depth, is tested. We measure the difference of the scattering caused by the mechanical characteristics and the size of red blood cells, instead of direct visualization of a single cell. In this study, particles with a similar size of the wavelength are used as red blood cell model. We also investigate to show the possibility of measurement in a flow channel and differentiation of the particle size theoretically and experimentally as a basic study of sonocytometry.

II. Methods

A. Ultrasonic Scattering from Spheres

Size parameter was introduced to the theory of ultrasonic scattering from particles. Size parameter ka is described as the product of the wave number k and the particle radius a. When $ka>>1$, the reflection is considered as planar reflection. When $ka<<1$, Rayleigh scattering is applied. In the present study, the radius of the particle is 80 - 100 μm and the wavelength of the 30 MHz ultrasound is approximately 50 μm. When $1<ka<30$, the scattering follows Faran-Hickling model described below [3-6].

$$P_s(t,k_3,\theta)=\frac{P_i a}{2r}\left\{\frac{2}{x_3}\sum_{n=0}^{\infty}(2n+1)(-i)^n \sin\eta_n\, e^{-\eta_n}\, P_n(\cos\theta)\right\}e^{-ik_3(c_3t-r)}$$

where P_i is the incident wave amplitude, a is the radius of the spherical scatterer, P_n is the n-th order Legendre polynomial of argument $\cos\theta$. The distance to the observation point r and the scattering angle θ are defined in Figure 1. $x_3=k_3a$, $k_3=2\pi f v/c_3$. c_3 is the longitudinal sound speed of surrounding medium.

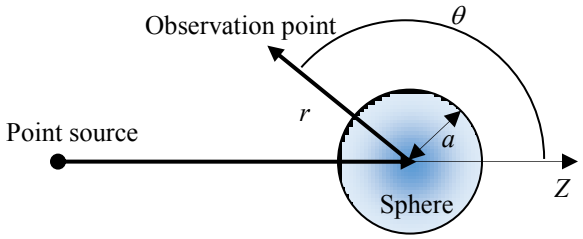

Figure 1. Faran solution coordinate system for a sphere. The origin is at the center of the scatterer

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B. Backscatter Transfer Function

The absolute value of the Backscatter Transfer Function (BSTF) was defined as the following equation.

$$BSTF = \left| \frac{R(f,r)}{R_{ref}(f,r)} \right|^2 = \left| \frac{A_{\exp} H_{bs} + N(f,r)]}{R_{coef} A_{ref} + N_{ref}(f,r)} \right|^2$$

C. Experimental Setup at Static Condition

A PVDF ultrasonic transducer with the diameter of 6 mm, the focal point of 12 mm and the central frequency of 30 MHz (Special order, Honda Electronics, Toyohashi, Japan) was driven by an originally developed pulser-receiver with the maximum output voltage of 110 Vp-p and the repetition frequency of 8 kHz. The reflected ultrasound was digitized by a digitizer card (DP1400, Acqiris, Geneva, Switzerland). The frequency characteristic of the transducer is shown as below. In the frequency domain analysis, 27 - 37 MHz range was used.

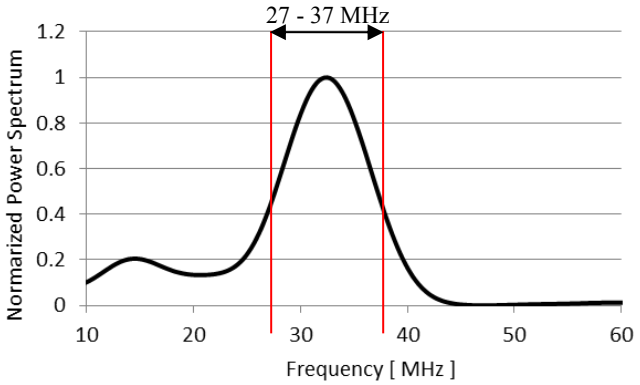

Figure 2. Frequency characteristic of the transducer

Polystyrene microspheres with the diameter of 80 and 100 μm (NIST Traceable, Thermo Fisher Scientific, Waltham, MA, USA) were used as the model of cells. The particles were placed on the agar phantom to reduce the unwanted reflection from the interface.

Obtaining the reflected wave from the plane in the same depth as the scatter, the frequency spectrum of the backscattered signal was normalized by the frequency spectrum of the reflected signal.

D. Flow Channel

The flow channel was made of PVA (Polyvinyl alcohol) with the lumen diameter of 1.0 mm. 15 wt% of PVA (SV: 98-99%, Japan Vam & Poval, Osaka, Japan) was dissolved in 80 wt% of DMSO (Dimethyl-sulfoxide) at 373.15 K and frozen at 253.15 K. An aluminum wire with the diameter of 1.0 mm was placed in the PVA just before freezing and pulled out at the room temperature to remain the lumen.

Figure 3 shows the schematic illustration of the flow channel. Two syringe pumps (LEGATO200, KD Scientific, Holliston, MA, USA) were connected to the side branch of the flow channel so as to center the polystyrene particles to the center of the flow channel. BSTF of a particle moving in the flow channel was observed in the model.

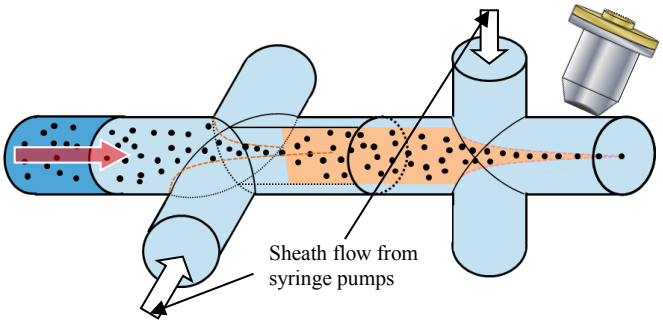

Figure 3. Schematic illustration of the flow channel

III. Results

A. Ultrasonic Reflection and Frequency Characteristics at Static Condition

Figure 4(a) shows the ultrasonic reflection from 80 μm polystyrene particle placed in the agar gel. Figure 4(b) shows the frequency characteristics of the theoretical (blue) and experimental (red) values of BSTF.

Figure 5(a) and (b) shows the same results using 100 μm polystyrene particle.

The frequency characteristics at 27 - 37 MHz showed similar pattern and the maximum peak and trough fit each other in the theoretical and experimental curves.

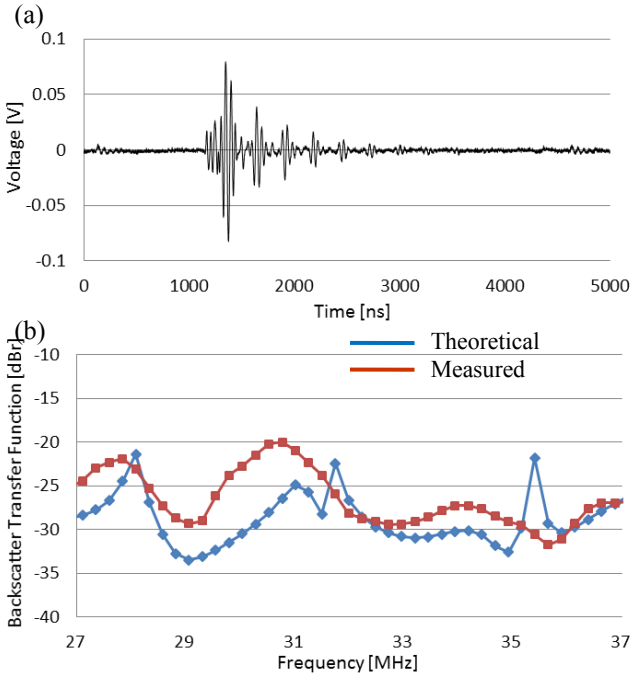

Figure 4 (a): Ultrasonic reflection from 80 μm polystyrene sphere. (b): frequency characteristics of the theoretical and measured backscatter

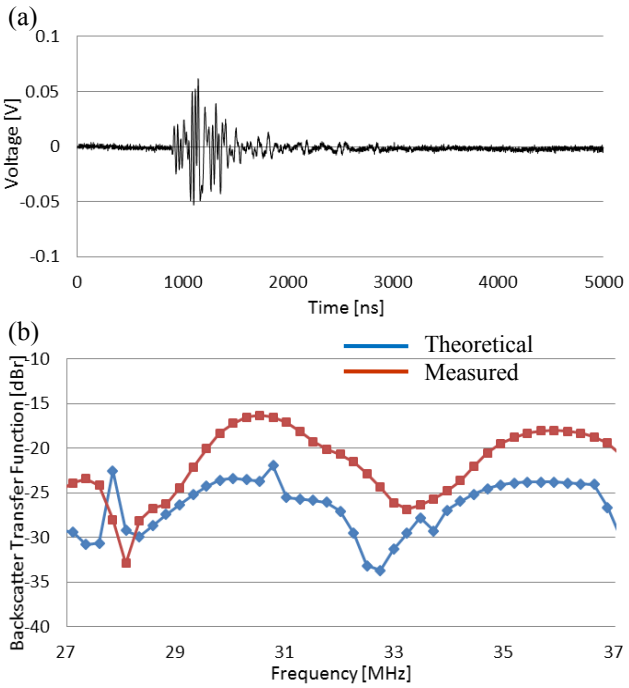

Figure 5 (a): Ultrasonic reflection from 100 μm polystyrene sphere. (b): frequency characteristics of the theoretical and measured backscatter

B. *Ultrasonic Reflection and Frequency Characteristics at Streaming Condition*

Figure 6 shows the RF signal obtained from flow channel. The echo signals from the anterior interface and the posterior interface of the flow channel were obtained.

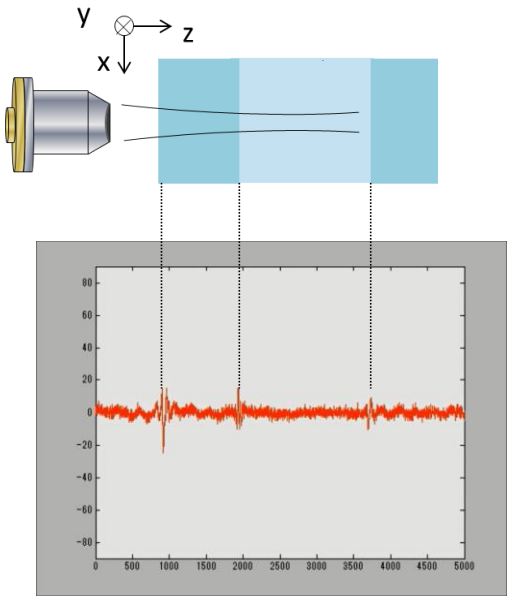

Figure 6. RF signal obtained from the flow channel without a particle

Figure 7 shows the RF signal obtained from flow channel and the streaming polystyrene sphere. The echo signal from the particle was successfully obtained.

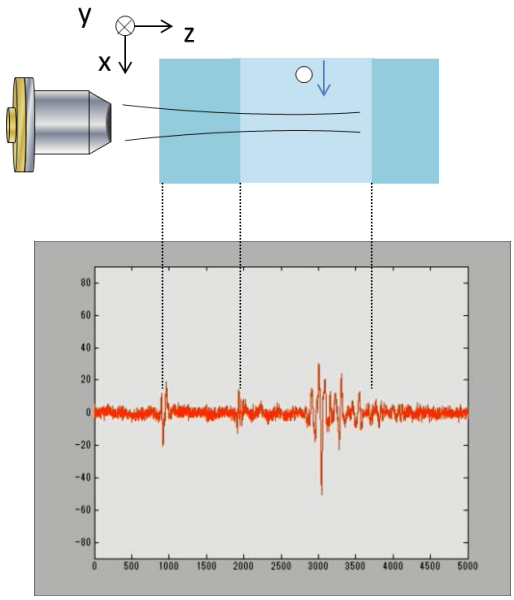

Figure 7. RF signal obtained from the particle in the flow channel

Figure 8(a) shows the frequency characteristics of the 80 μm particle obtained at the static condition (blue) and flowing condition (green). The flow rate was 1 cm/s. Figure 8(b) shows those of the 100 μm particle.

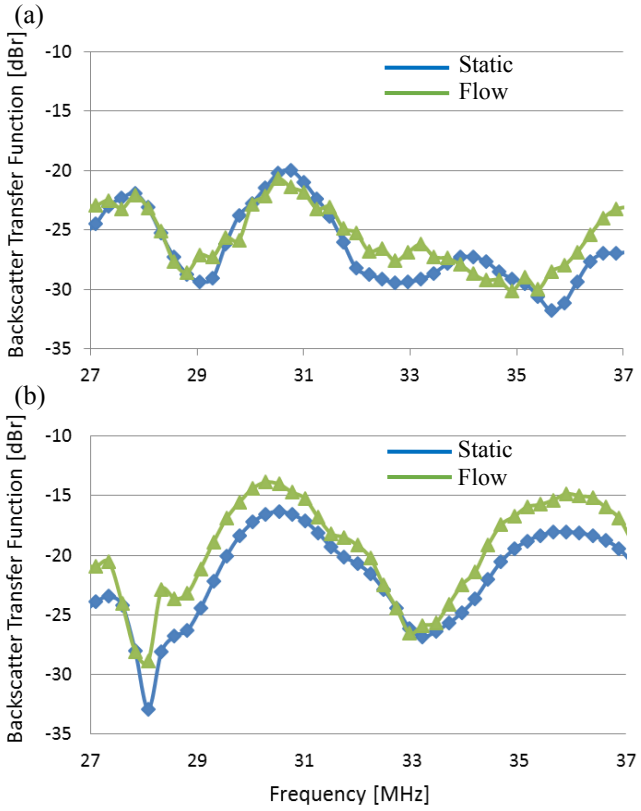

Figure 8(a). Frequency characteristics of the 80 μm particle (blue: static, green: streaming) (b) those of the 100 μm particle.

Figure 9 shows the Pearson's product-moment correlation coefficient of the experimental value to the theoretical value. In this case, the maximum value was obtained at approximately 100 μm and the particle size was estimated as 100 μm.

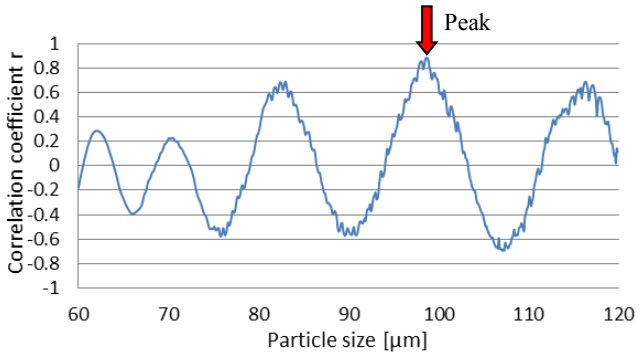

Figure 9. Pearson's product-moment correlation coefficient

Figure 10 shows the differentiation of the particle size of 80 and 100 μm. Each diameter of 10 particles were tested by the method. 8 from 10 particles with the diameter of 100 μm and 9 from 10 particles with the diameter of 80 μm were successfully differentiated.

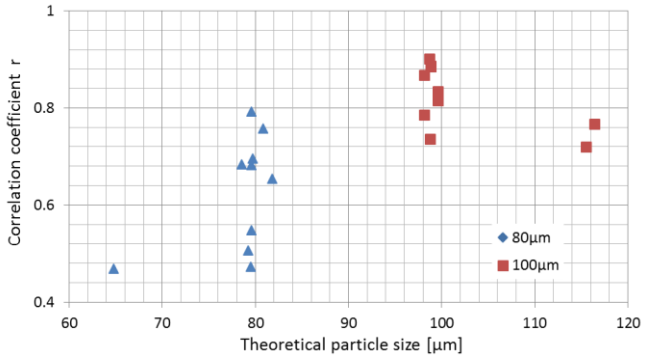

Figure 10. Differentiation of the particle size

IV. Discussion

For the particles at the static condition, the spectrum of the ultrasonic backscatter signal showed a characteristic form depending on each diameter. Experimental BSTF showed similar pattern with that of the theoretical BSTF, but the both BSTF were not matched completely. The error may be caused from that the density and sound speed of the polystyrene particles used in the experiment were slightly different from pure polystyrene. The values in the scientific table were used whereas Poisson's ratio of the polystyrene was 0.34. However, the Poisson's ratio was calculated as 0.353 when it was calculated from the longitudinal and shear velocities in the same scientific table.

The experimental model of the blood cells was simplified to include two different sized polystyrene particles. It was possible to differentiate the particle diameter by correlation coefficient of the measured BSTF to the theoretical value. Although real situation of the blood cells including flat red blood cell and spherical white blood cell is more complex, this study has clearly shown the potential of sonocytometry. In clinical settings, microvessels close to the skin surface would be used as the in vivo flow channel. This study was performed in the frequency range of 30 MHz, and the particle size was 80 - 100 μm. The particle size of the cells in the blood is 6 - 10 μm and higher frequency ultrasound is needed for the application of this method. 120 MHz ultrasound imaging has been established and it would be applied for in vivo sonocytometry.

V. Conclusion

The differentiation of the particle size is performed as a basic study on sonocytometry. Theoretical value of backscatter was calculated by Faran-Hickling model and the correlation coefficient of measured and theoretical value was analyzed. The method successfully classified the particle size. Sonocytometry would be clinically applied for diagnosis of malaria or leukemia.

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