A LASER SCATTERING METHOD FOR CHARACTERIZATION OF THROMBOEMBOLI IN WHOLE BLOOD

Jungkuk Kim*, James C. Lin**, and Larry O. Reynolds+

*Bioengineering Program and ** Electrical Engineering and Computer Science Department.
+ Deceased. University of Illinois at Chicago, 851 S. Morgan St.
Chicago, IL. 60607-7053

***Abstract*— An optical scattering method, which measures the size, number, and type of microemboli located in flowing whole blood, is described. A perturbation solution to the one speed radiative transport equation is used to model the scattering geometry. The approximate two-parameter phase function is used to investigate scattering characteristics of microemboli. The nature of microemboli is determined by comparing the scattered intensity ratio in two forward directions, while the size and number are determined by considering intensities scattered perpendicularly to the incident direction. This method has been tested with several sizes of polystyrene spheres, air bubbles, and blood clots. The results showed excellent agreement with calculations.**

INTRODUCTION

The problem of optically characterizing inhomogeneities in material media composed of a dense distribution of scatterers is theoretically difficult. The characterization of microemboli generated as a result of blood material interaction is one such problem. Research conducted to date have included the measurement of size and refractive index of red blood cells and marine microorganisms by flow cytometric light scattering method [1,2,3]. Also, the detection of buried dielectric targets has been explored using T-matrix method [4]. These works are based on single scattering of individual particles. The size and number of microemboli in whole blood have been determined by applying a perturbation solution to the radiative transport equation, assuming the nature of the inhomogeneous thromboemboli is known [5,6,7]. In this case, microemboli are considered as secondary sources by assuming only one microembolus exists at any given measurement moment, while the multiple scattering red blood cells are represented as a conventional scattering term. By monitoring and comparing the change of intensities scattered perpendicularly to the incident direction, microemboli are detected and sized in flowing whole blood. In this paper, we describe a method that identify the nature of microemboli in whole blood along with their size and number.

METHODS

The transport equation for unpolarized light is given by [8]

$$\left(\frac{\partial}{\partial s}+\Sigma_t\right)\varphi(\bar{r},\hat{\Omega})=\int\Sigma_s(\bar{r},\hat{\Omega}'\cdot\hat{\Omega})\varphi(\bar{r},\hat{\Omega}')d\hat{\Omega}'+S(\bar{r},\hat{\Omega}) = Q(\bar{r},\hat{\Omega}) \quad (1)$$

where the angular flux $\varphi(\bar{r},\hat{\Omega})$ propagating through a multiple scattering medium is described at the position $\bar{r}$ and direction $\hat{\Omega}$, with the total macroscopic extinction cross section Σ_t, the source of light $S(\bar{r},\hat{\Omega})$ within a scattering volume V, and the differential scattering function of the continuum $\Sigma_s(\bar{r},\hat{\Omega}'\cdot\hat{\Omega})$ describing the probability of light scattered into the direction $\hat{\Omega}$ from incident direction $\hat{\Omega}'$.

A general perturbation solution $\varphi(\bar{r},\hat{\Omega})$ is obtained from Eq. (1) with the assumption that the light scattered from the continuum scatterers (erythrocytes) can be decomposed into two components, one component entirely in the direction of incidence and the other component isotropically scattered into whole space, $\Sigma^I_s(\bar{r},\hat{\Omega}'\cdot\hat{\Omega})$. It is also required and has been verified [9] that the isotropic component be significantly less than the forward component.

The perturbation solution obtained is

$$\varphi(\bar{r},\hat{\Omega})=\int_0^{Sb}\{\int\Sigma_s^I(\bar{r}-s\hat{\Omega},\hat{\Omega}')e^{-\bar{\tau}_s}\varphi_i\delta_2(\hat{\Omega}\cdot\hat{\Omega}_0)d\hat{\Omega}' + S(\bar{r}-s\hat{\Omega},\hat{\Omega})\}\, e^{-\tau_s}ds+e^{-\bar{\tau}_s}\varphi_i\delta_2(\hat{\Omega}\cdot\hat{\Omega}_0) \quad (2)$$

where Σ_{tr} is transport cross section ($\Sigma_t - \bar{\mu}\Sigma_s$), $\bar{\mu}$ is the average scattering cosine, $\tau_s = \Sigma_{tr}S$,and $\bar{\tau}_s = \Sigma_{tr}S_i$.

In Eq. (2), the scattered radiation from microemboli is represented as a source term, $S(\bar{r},\hat{\Omega})$. Because the source is a secondary radiation by light incidence on the microemboli, it can be written as

$$S(\bar{r},\hat{\Omega}) = \varphi_i\Sigma_{ss}f_s(\hat{\Omega}_0\cdot\hat{\Omega})e^{-\bar{\tau}_s} \quad (3)$$

where Σ_{ss} is the scattering cross section of a microembolus and $f_s(\hat{\Omega}_0\cdot\hat{\Omega})$ is the phase function of the microembolus. Note that the incident higher order scattering intensities from surrounding red blood cells are considered as perturbations. For convenience, we restrict our scope of microemboli types to platelet aggregates, erythrocyte thrombin, and air bubbles of 30, 60, 90, and 120 µm in diameter. Their phase functions are calculated by using the approximate two-parameter phase function [10]. From the calculated phase function, a pair of detector positions in the forward direction are chosen at θ1=5° and θ2=20° because the intensity ratios at the two scattering angles for each type of microemboli are all different and easily distinguishable.

The devised microembolus characterizing geometry is shown in Figure 1. A monochromatic collimated light beam illuminates a

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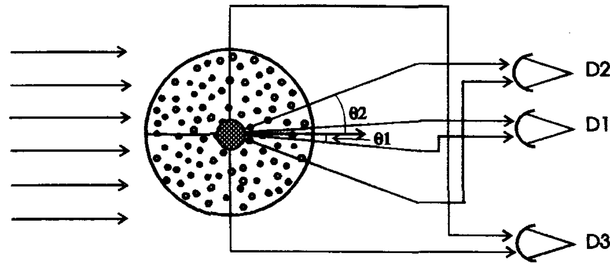

Fig. 1. The light scattering geometry of microemboli characterization.

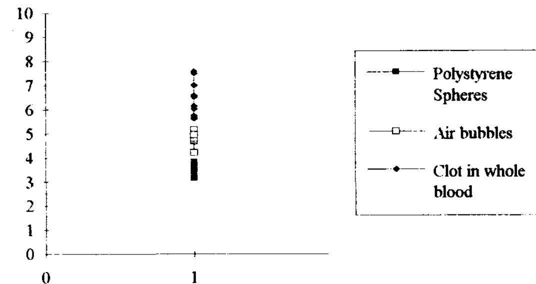

Fig. 2. Ratio distribution of polystyrene spheres, air bubbles, and clots in whole blood. The vertical axis represents the scattered intensity ratio obtained as (intensity at D1)/ (intensity at D2).

flowing cylindrical whole blood medium. A microembolus is located close to the center of the blood medium, and the three detectors, D1, D2, and D3, are positioned as shown. The detector, D3, is positioned at 90° and is used to detect the size of microemboli by monitoring the scattered intensity as prior microemboli detection systems [5,6].

The intensities arriving at the detectors are the sums of the intensities from red blood cells and microembolus as shown in Eq. (2). To obtain the isotropic diffuse scattering term of red blood cells, the intensities at detectors, D1, D2, and D3, can be measured in the absence of inhomogeneous microemboli. The scattered intensities from microemboli are obtained by subtracting the premeasured intensities from total intensities. Finally, the ratios are calculated and used to identify the nature of the microemboli.

RESULTS

An *in vitro* measurement system was constructed with the scattering geometry as shown in Figure 1. Blood flowing inside silastic tubing was illuminated by a He-Ne laser beam (λ=0.639μm). The size of the silastic tubing at the measurement site was 2.216 mm (outer diameter) and 1.016 mm (inner diameter). The distance between the center of the tubing and detectors was 10 mm and the angle of acceptance of the detectors was 3.2° in our geometry. Each measurement was repeated three times with bovine blood drawn from three different animals. Bovine blood (20 ml) was drawn from the aorta and 10 % of anticoagulation agent was added to prevent clotting or thromboembolization. One type of inhomogeneous particles was added to the blood at a time, and the blood was circulated at a speed of 60 ml/minute using a roller pump.

The measured intensity ratios at 5° and 20° for polystyrene spheres, air bubbles, and clots generated inside the *in vitro* loop (without adding anti-coagulating agent) are shown in Figure 2. The ratios of the same type of inhomogeneous particles are clustered together and are well separated from other types. Also, the calculated ratios for polystyrene sphere and air bubbles (43.8, 90, and 202 μm) show good agreement with the measurement.

CONCLUSIONS

In this study, a three detectors measurement method has been developed to determine the size, number, and nature of microemboli located in whole blood medium. A pair of forward scattering detectors are added to the 90° cuvette, that has been used to detect size and number of microemboli. The angles of the new detectors have been chosen to produce maximum ratio differences among different type of microemboli. The method was tested using polystyrene spheres, air bubbles, and clots located in whole bovine blood. The results showed an excellent agreement between theoretical calculations and measurements, and the type of inhomogeneities in whole blood can be readily identified using this method.

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