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**Review Paper**

# Ultrasonic Visualization of Dynamic Behavior of Red Blood Cells in Flowing Blood

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***Abstract*** : It is well known that the scatter of ultrasound by blood is mainly attributed to red blood cells (RBCs) and RBC aggregation. In the present review, researches of hemodynamic influence on RBC aggregation and ultrasound backscatter from blood were overviewed. A mock flow loop and a cylindrical chamber were employed to produce various blood flows, such as pulsatile, oscillatory, and rotational flow. The "black hole" (BLH), a dark hole at the tube center surrounded by bright zone in the cross sectional B-mode image and "bright collapsing ring" (BRCR) phenomena, appearance of bright ring at the periphery and collapse of it at the center during a pulsatile cycle, were observed under pulsatile flow. The combined effects of shear rate and flow acceleration on RBC aggregation were suggested as a possible mechanism for these phenomena. The stroke volume-dependence of the "bright ring" phenomenon under oscillatory flow could also be explained by flow acceleration. The enveloped echo images from rotational flow in a compact blood chamber showed the spatial and temporal variations of RBC aggregation, which varied with the mammalian species. In the stenotic model, it was found that the echogenic variation increased locally at a distance of three tube diameters downstream from the stenosis during decelerating period, which was proposed to be mainly due to flow turbulence. The similar 'bright ring' was also observed from *in vivo* human carotid artery in harmonic imaging.

***Keywords*** : Blood echogenicity, Flow turbulence, Flow acceleration, Hemodynamics, Red blood cell aggregation, Shear rate, Ultrasonic visualization.

## 1. Introduction

Ultrasound imaging has been extensively used as a diagnostic tool to obtain the anatomical and pathological information of internal organs or tissues because it has the major advantages of noninvasive and real time measurements *in vivo*. In addition, conventional color Doppler and power Doppler imaging visualize blood flow in vessels and provide hemodynamic information *in vivo*. The former gives the information about flow speed and direction with limited sensitivity, whereas the latter has higher sensitivity visualizing very small vessels without flow speed and directional information. B-mode imaging is not usually used for blood flow visualization since the dynamic range between tissue and blood is too big, so that blood in tissues looks dark in *in vivo* images. As frequency is increasing for better resolution in commercial ultrasound systems, echo from blood is getting larger, decreasing the dynamic range. Moreover, recent transducer techniques provide better sensitivity. Therefore echo from blood in tissues *in vivo* is sometimes visible in ultrasonic scan

imaging, especially in veins where blood flow speed is lower. Up to now, B-mode imaging is seldom used for blood visualization, and this paper reviews the physical and physiological aspect of blood backscattering and blood echogenicity in B-mode imaging, focusing *in vitro* studies with a potential of *in vivo* applications of blood visualization.

Sigel et al. (1983) first showed that ultrasound backscattering was dependent on shear rate, suggesting that RBC aggregation at lower shear rate was a major cause of the increased backscattering from blood. Since then, ultrasonic investigation of RBC aggregation in blood has attracted considerable interest. The ultrasound backscattering from blood has been estimated by the measurement of backscattering coefficient (Yuan and Shung, 1989; Wang and Shung, 1997; Yu and Cloutier, 2007), backscattering power (Qin et al., 1998b; Cao et al., 2001; Cloutier et al., 2004; Nam et al., 2006, 2009), and Doppler power (Shung et al., 1992; Cloutier and Shung, 1993; Paeng et al., 2001, Paeng and Shung, 2003). However, in real time *in vivo* scanning, the backscatter in commercial ultrasonic scanners is usually shown as echogenicity in B-mode imaging. The echogenicity in B-mode is calculated by several nonlinear processings such as filters, time gain compensator, demodulator, logarithmic compressor, and scan converter for better image quality after collection of the backscattered rf signals. Therefore the echogenicity cannot be directly thought as backscattering coefficient without compensation of those nonlinear processings, and it is almost impossible to quantify echogenicity through compensation only. If all the control setting in an ultrasonic system is fixed and echogenicity is calibrated with flow speed under steady flow, then nonlinearity of echogenicity can be minimized and echogenicity could be correlated with backscattering from RBCs and RBC aggregation in flowing blood.

Previous studies performed by the authors (Cao et al., 2001; Paeng et al., 2001, 2004a, 2004b; Paeng and Shung, 2003; Nam et al., 2006, 2008, 2009) and others (Yuan and Shung, 1989; Qin et al., 1998a, Nguyen et al., 2008) have produced interesting, and even surprising, findings about the backscattering variation from flowing blood. The BLH phenomenon, a central hypo-echoic hole with a surrounding hyper-echoic ring in cross-sectional B-mode images of whole blood, was first reported under steady flow in a tube (Yuan and Shung, 1989). Further studies were followed by many researchers to investigate the mechanism for this phenomenon (Mo et al., 1991; Shehada et al., 1994; Qin et al., 1998a). They suggested that the BLH arose from disaggregated RBCs that had insufficient shear rate to reaggregate when they reached a steady state at the center of the tube. The authors (Cao et al., 2001; Paeng et al., 2004a) observed it under pulsatile flow and extended the study to pulsatile flow condition. The BLH was more pronounced at higher beat rates when the peak velocity was constant. The BRCR phenomenon, a bright echogenic ring converging from the periphery to the center of the tube wall and eventually collapsing during a pulsatile cycle in cross-sectional B-mode images, was observed under various flow speeds and stroke rates. The BRCR was stronger as the peak speed was increased at certain speed ranges. These observations showing high echogenicity during systolic phase were not explained by a shear-rate analysis alone. Therefore, it was proposed that flow acceleration might be another important factor to contribute to the formation of the BRCR under pulsatile flow. Further studies under oscillatory flow supported the combined effects of shear rate and acceleration on RBC aggregation (Paeng et al., 2004b).

Earlier reports (Shung et al., 1984, 1992; Yuan and Shung, 1988a) showed that flow turbulence increased ultrasound backscattering. The rationale for the phenomenon has been well analyzed and explained by the theoretical models (Lucas and Twersky, 1987; Mo and Cobbold, 1992; Bascom and Cobbold, 1995), which show that the scattering from a packed distribution of small scatterers is related to fluctuation of the number and size of the scatterers in a volume rather than the number itself. Experimental reports from RBC suspension by Cloutier et al. (1995, 1996, 2000) showed that flow turbulence downstream from a stenosis resulted in increased Doppler power. Bascom et al. (1997) also examined the flow turbulence and the nature of the flow field downstream from an asymmetric stenosis using a photochromic dye technique. Later, Nam et al. (2008) used the B-mode imaging to examine the echogenic variation in the post-stenotic region and verified the flow turbulence downstream in whole blood that allows RBC aggregation under pulsatile flow.

The aim of the present review concentrates on how to visualize blood in terms of echogenicity mainly in B-mode imaging and how to examine the effects of hemodynamic parameters, such as shear rate, flow acceleration, and turbulence on RBC aggregation under various flow conditions.

# 2. Background

### *RBC aggregation*

RBCs in plasma aggregate to form rouleaux and rouleaux networks under normal physiological conditions as a reversible process. This phenomenon continues to be of interest in the field of hemorheology since flow dynamics and flow resistance of blood are influenced by RBC aggregation. However, its pathophysiological impacts have not been fully resolved yet. The mechanisms of rouleaux formation and dissociation are very complex and not well understood. Two models have been proposed to explain the aggregation of RBCs, the bridging model and the depletion model (Rampling et al., 2004). In the bridging model, the formation of RBC aggregates results from the adsorption of plasmatic macromolecules at the surface of the RBC (Chien, 1975; Brooks, 1988). In contrast, the depletion model suggests that RBC aggregation results from a lower localized macromolecule concentration near the cell membrane as compared with the suspending medium (Van Oss et al., 1990; Evans et al., 1991). The exclusion of macromolecules near the RBC surface generates a reduction of the osmotic pressure in the gap between two nearby RBCs, creating an attractive force between them. These two models are in conflict and mutually exclusive. However, later publications including the studies of RBC electrophoretic mobility have provided theoretical support for the depletion model (Donath et al., 1993; Armstrong et al., 1999; Bäumler et al., 1996, 1999). More recent studies have shown that RBC cellular properties, such as RBC deformability, morphology, and surface charge, also play a very important role in the aggregation process (Rampling et al., 2004). Although the morphology and the physiological functions of RBC are similar in all mammalian species, each species has its own tendency of RBC aggregation, resulting in great difference among them (Ohta et al., 1992; Weng et al., 1996; Baskurt et al., 1997; Windberger et al., 2003). The comparative study of RBC aggregation in different mammals may provide valuable information, such as its formation mechanism and pathophysiological roles.

RBC aggregation can be considered as a result of a balance between aggregating and disaggregating forces that are affected by shear rate. Previous studies have suggested that the aggregation process involves three steps, that is, the formation of short linear rouleaux which is composed of several RBCs, following the development of long linear rouleaux, and finally, the formation of complex 3-dimensional structures of branched rouleaux, whose occurrence is decreased with increasing shear rate (Shiga et al., 1983). Recently, it was suggested that blood flow acceleration is another factor that enhances the aggregation by increasing the probabilities of RBC collisions due to different inertia and compressional forces (Cao et al., 2001; Paeng et al., 2004a). The relationship between hematocrit and RBC aggregation has been reported in the several publications (Donner et al., 1988; Kim et al., 1989; Shung et al., 1992; Deng et al., 1994), proving that hematocrit plays an important role in RBC aggregation. The results, however, varied with the measurement method and flow condition. In recent studies, it was suggested that the extent and the rate of aggregate formation increase in proportion to hematocrit at certain shear rates but in inverse proportion to hematocrit at other conditions (Paeng et al., 2004a; Nam et al., 2009).

### *Scattering of ultrasound by blood*

RBCs are the dominant ultrasonic scatterers in blood, because they constitute a great portion of the cellular components of blood and their acoustic impedance is different from water impedance. Because the dimension of RBC is much smaller than the ultrasonic wavelength for frequency ranges of conventional diagnostic imaging devices, an RBC can be considered as a Rayleigh scatterer for frequencies up to as high as 30 MHz (Kuo and Shung, 1994; Cloutier et al., 2004). However, RBCs are packed in blood, so the backscattering is not linearly proportionate to number of RBCs or hematocrit. Therefore, the backscattering from RBC suspension is a function of backscattering cross-section of an RBC, hematocrit, and a packing factor which is nonlinear function of hematocrit. The blood scattering models typically can be divided into two categories of particle model and continuum approach. In the particle model, the total scattering wave from an interrogated scattering medium is assumed to be the summation of all contributed scattering waves from each scatterer (Twersky, 1988). The continuum model recognizes that the scattering wave is determined by the density and compressibility fluctuation of the interrogated scattering medium as a source term in an inhomogeneous wave equation (Angelsen, 1980). The particle model may give an explanation of the

backscatter by RBC suspension at low hematocrit. However, the scattering by blood is a complex phenomenon because of the high density of RBCs in blood. For a dense suspension of scatterers, uncorrelated positions of scatterers can no longer be assumed. Even under nonaggregating conditions, their positions are significantly correlated. Under this condition, the backscattered power is a function of the spatial arrangement of RBCs and is not simply proportional to the number of RBC. The particle approach with the concept of the packing factor was introduced to account for the effect of interaction among scatters (Twersky, 1988; Berger et al., 1991), where the packing factor is affected by the hematocrit, flow turbulence, and RBC aggregation (Shung et al., 1984; Yuan and Shung, 1988b). A nonlinear relationship between hematocrit and ultrasonic backscatter of RBC suspension was experimentally found and the results were in excellent agreement with the theoretical models which predict a scattering maximum peak at 13-20 % hematocrit (Shung et al. 1976; Yuan and Shung 1988a, 1988b). Later, the packing theory was extended to the continuum model and the particle and continuum models were unified as a hybrid model (Mo and Cobbold, 1992). According to the theory, it was postulated that ultrasound backscatter should be sensitive even to a small degree of RBC aggregation.

Although the experimental results and the theoretical models were well corresponded to each other, it is still unclear if the packing factor approximation is fully valid in the presence of RBC aggregates because the distance of correlation among the positions of the scatters can increase significantly in case of the aggregation. In addition, blood during the process of RBC aggregation involves the formation of short and long straight chains of RBCs and their complex 3-dimensional networks. Therefore, it is challenging to estimate the rate and amount of RBC formation, and size of RBC aggregates quantitatively, especially in flowing blood *in vivo*.

# 3. Discussion

The data in this paper were selected from the recently published results by the authors that were related to ultrasonic visualization of flowing blood mainly in B-mode or M-mode imaging (Paeng et al., 2003, 2004a, 2004b, 2004c, 2009; Nam et al., 2006, 2008).

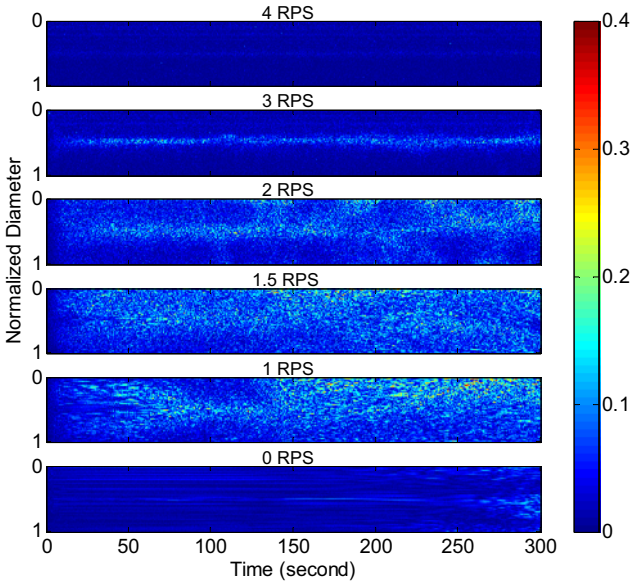


Fig. 1. Ultrasonic visualization of RBC aggregation by the Hilbert transformed echo envelope from horse blood across the diameter of a cylindrical chamber as a function of time under rotational flow at various stirring rates. Zero time indicates the swiching point to the corresponding stirring rates or flow stoppage from 4 rps (Nam et al., 2006).

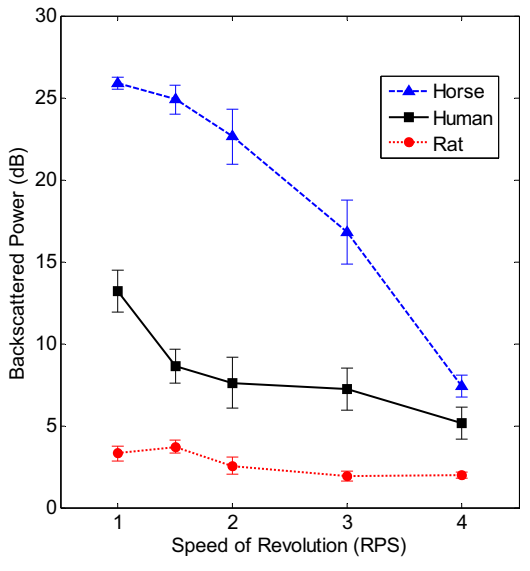


Fig. 2. Ultrasound backscattered power from human, horse, and rat blood as a function of stirring rate. Marks and error bars are the mean and the standard deviation of 6 blood samples, respectively (Nam et al., 2006).

In order to visualize RBC aggregation level in various mammalian bloods in flowing conditions using ultrasound, a compact cylindrical chamber, where flow was controlled by a stirring magnet, was employed. A 5 MHz focused transducer in a pulse-echo setup was used. Figure 1 (Nam et al., 2006) shows typical echo images from horse blood across the chamber diameter as a function of time, where the echo signals were enveloped by the Hilbert transform. The y-axes are the normalized diameter of the blood chamber. The x-axes represent the time after a stop or a corresponding stirring rate from 4 rps (revolutions per second) at zero time. These results visually manifested the spatial and temporal variations of RBC aggregation at different stirring rates. The bright zone along the tube center at 3 rps could be explained by the distribution of RBC rouleaux caused by radial variation of shear rate; the low shear rate around the tube center enhanced higher RBC aggregation. The bright zone was expanded toward the tube wall as the stirring rate was lowered except stasis, where RBCs aggregated slowly. Figure 2 (Nam et al., 2006) shows the backscattered power in human, horse, and rat blood as a function of stirring rate. The backscattered power was calculated from the midpoint between the tube center and the tube wall at steady state. The results presented the different levels of RBC aggregation among the species with the order of horse > human > rat, which is in agreement with the previous reports measured by optical methods such as Myrenne aggregometer (Weng et al., 1996; Baskurt et al., 1997; Windberger et al., 2003). Since rat RBCs did not aggregate, there was minimal change of backscattered power with stirring rate. Ultrasonic measurement and visualization might have an advantage in comparison with photometric measurement, because the application of photometry is restricted to tiny tubes or vessels in laboratory and *in vivo* due to the limitation of light opacity in blood.

All experiments from this point were performed using a LOGIQ 700 expert system with an M12L linear transducer (13 MHz) from porcine blood in a mock flow loop and human carotid arteries. Previous report showed that the Doppler power from whole blood decreased as flow speed increased, whereas RBC suspensions remained unchanged (Paeng et al., 2001). Therefore it was investigated that blood echogenicity of a LOGIQ 700 expert ultrasonic scanner is also mainly from RBC aggregation, even though the echogenicity of a commercial scanner includes nonlinearity from the electronic system and image processing. The echogenic variations under steady flow in Fig. 3 (Nam et al., 2008) are in good agreement with the results from Doppler power by authors and backscattering coefficient of other report (Yuan and Shung, 1988a). The increase of echogenicity at lower blood flow speed in a tube was caused by the shear rate-dependent RBC aggregation in plasma, but not in RBC suspension. The echogenic increase at the higher flow velocity in RBC suspension is thought to be mainly attributed to the flow turbulence even though other nonlinear speckle patterns might be involved.

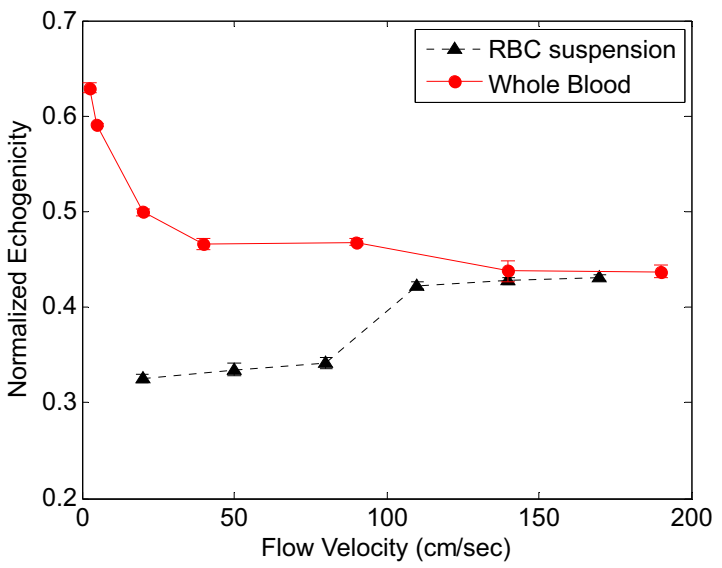


Fig. 3. Ultrasonic echogenicty from whole blood and RBC suspension as a function of flow velocity at the tube center (Nam et al., 2008).

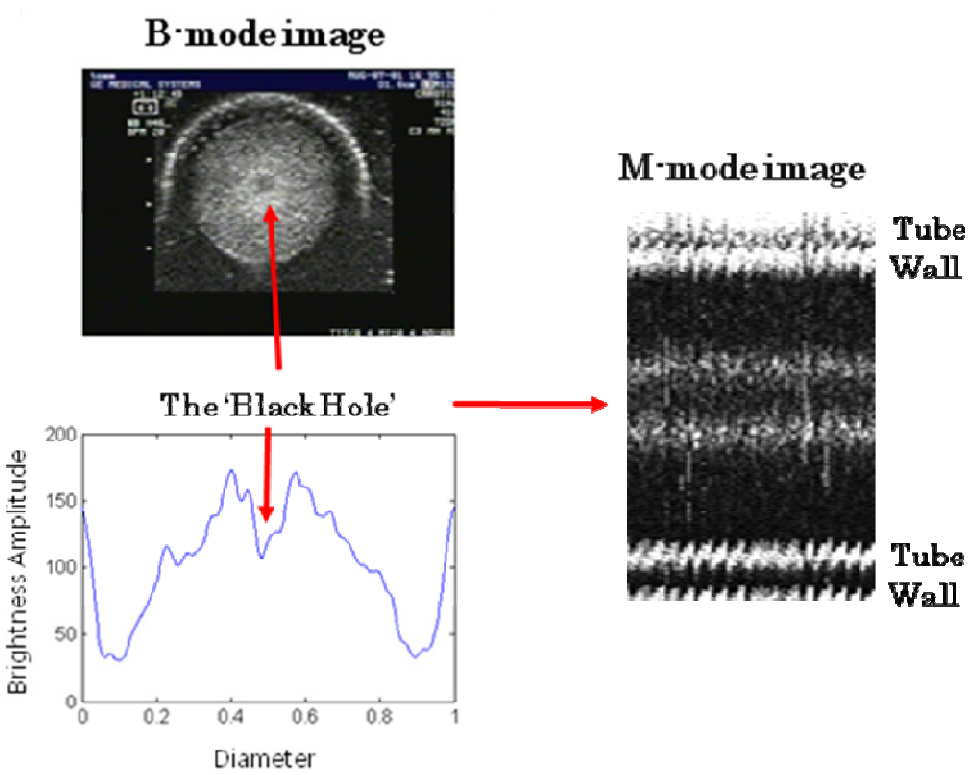


Fig. 4. The BLH phenomenon observed in B-mode cross sectional imaging (left top panel) and M-mode imaging (right panel, Cao et al., 2001) of porcine blood in a tube and its line plot of blood echogenicity across the tube center (left bottom panel) (Paeng et al., 2004c)

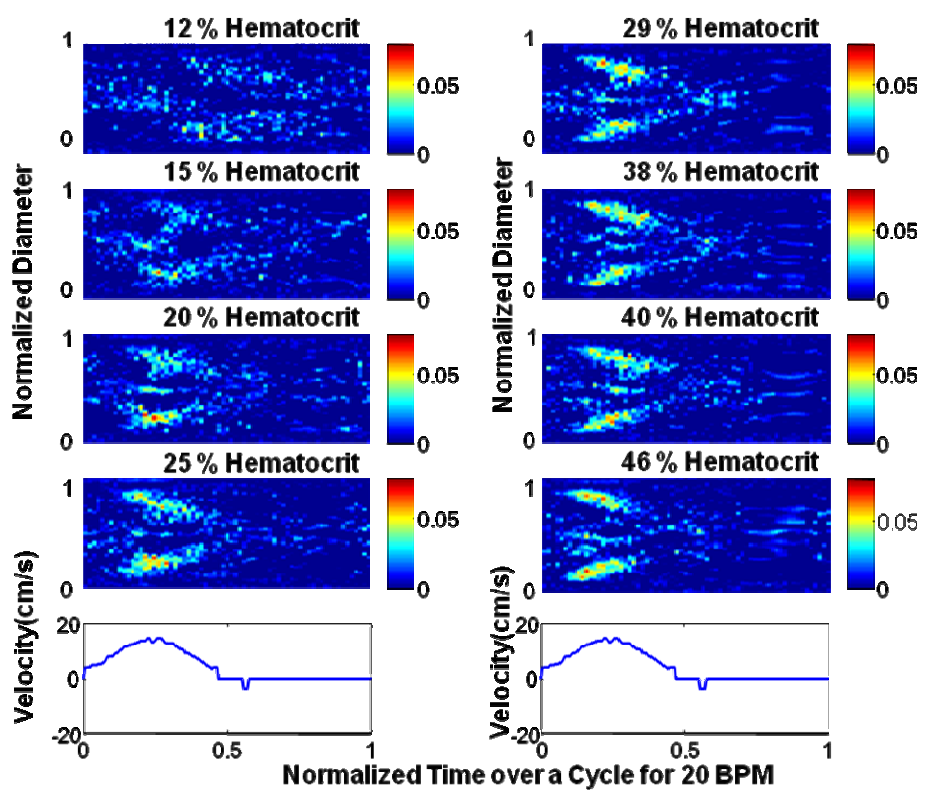


Fig. 5. The BRCR phenomenon under pulsatile flow at various hematocrit levels. Echogenicity variation subtracted by mean echogenicity over a pulsatile cycle was calculated as a function of time and radial position across the diameter of a tube in a mock flow loop (Paeng et al., 2004a).

The temporal and radial variations of the echogenicity from porcine blood were observed in a mock flow loop under pulsatile and oscillatory flow. Cross-sectional B-mode images were obtained and digitized at 30 frames/s. Figure 4 (Paeng et al., 2004c) shows a typical B-mode image taken at the peak accelerating period under pulsatile flow. The BLH phenomenon was clearly shown in the B-mode cross sectional image and in the corresponding line plot across the horizontal tube diameter. In longitudinal B-mode or M-mode image, the BLH is visualized as dark zone at the center of the tube surrounded by bright zone as shown in the right panel of Fig. 4.

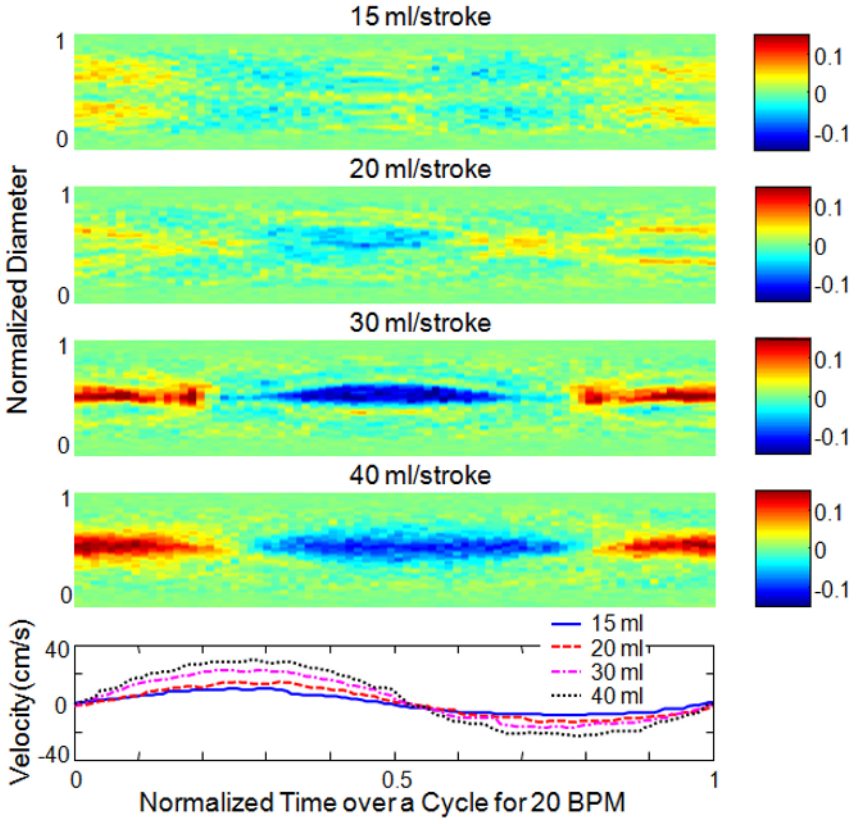


Fig. 6. The “bright ring” phenomenon under oscillatory flow for various stroke volumes (Paeng et al., 2004b).

In Fig. 5 (Paeng et al., 2004a), the cyclic variation deviated from temporal mean echogenicity was shown at various hematocrits. To see the echogenic variation during a flow cycle more clearly, the temporal mean echogenicity over a flow cycle was subtracted from instantaneous echogenicity across the horizontal line of cross-sectional B-mode images. At higher hematocrit, the cyclic variation during systole was more obvious and the BRCR phenomenon was also obviously visualized. Strong bright echogenicity was observed at the periphery during accelerating period and converged to the tube center at early diastole. The BLH became weaker at higher speeds, whereas the BRCR was more apparent with increased speed. In the analysis to see the hematocrit dependence at different radial positions, the temporal mean echogenicity at the periphery where shear rate is higher showed that the echogenicity nonlinearly increased with hematocrit from 12 to 20 %, which could be explained by the packing factor theory (Twersky, 1988; Berger et al., 1991). Figure 6 (Paeng et al., 2004b) shows the cyclic variation under oscillatory flow. At higher stroke volume, strong cyclic variation was observed. No BLH was observed under oscillatory flow. During acceleration period, the echogenicity showed positive variation, but negative at deceleration period. In cross-sectional images, the negative variation at the tube center appeared as a “bright ring”. This phenomenon suggests that RBC rouleaux during acceleration period may have certain alignment along the flow direction because the all images were acquired with oblique angle between transducer and tube axis to avoid the multiple reflections at the perpendicular angle. The rouleaux orientation in flowing blood was suggested by Allard et al. (1996) and Qin et al. (1998), and the result by the authors (Paeng et al.,

2004b) confirmed the angular dependence of transducer beam for various angles under oscillatory flow. Because the shear rate increases during acceleration period, the BLH under pulsatile flow and the positive variation of echogenicity under oscillatory flow cannot be explained by shear rate alone. As a possible mechanism, it was suggested that flow acceleration might increase the collision rate of RBCs, which may enhance the RBC rouleaux formation. Therefore, the complicated patterns of the echogenic variation should be explained by the RBC aggregation due to the combined effects of flow acceleration and shear rate.

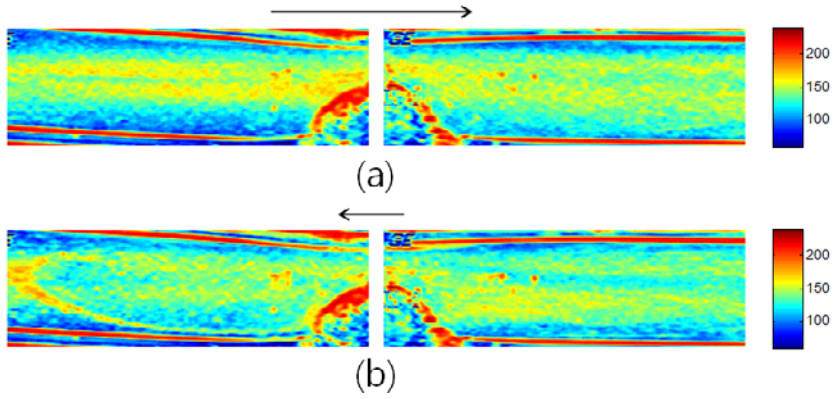


Fig. 7. Snap shots of B-mode images from porcine whole blood extracted at systole (a) and diastole (b) under pulsatile flow (20 bpm). The arrows show blood flow direction. The small arrow in (b) indicates weak backward flow at diastolic phase. The hyper-echoic hemisphere at the center is the artificial stenosis (modified from Nam et al., 2008 and Paeng et al., 2009).

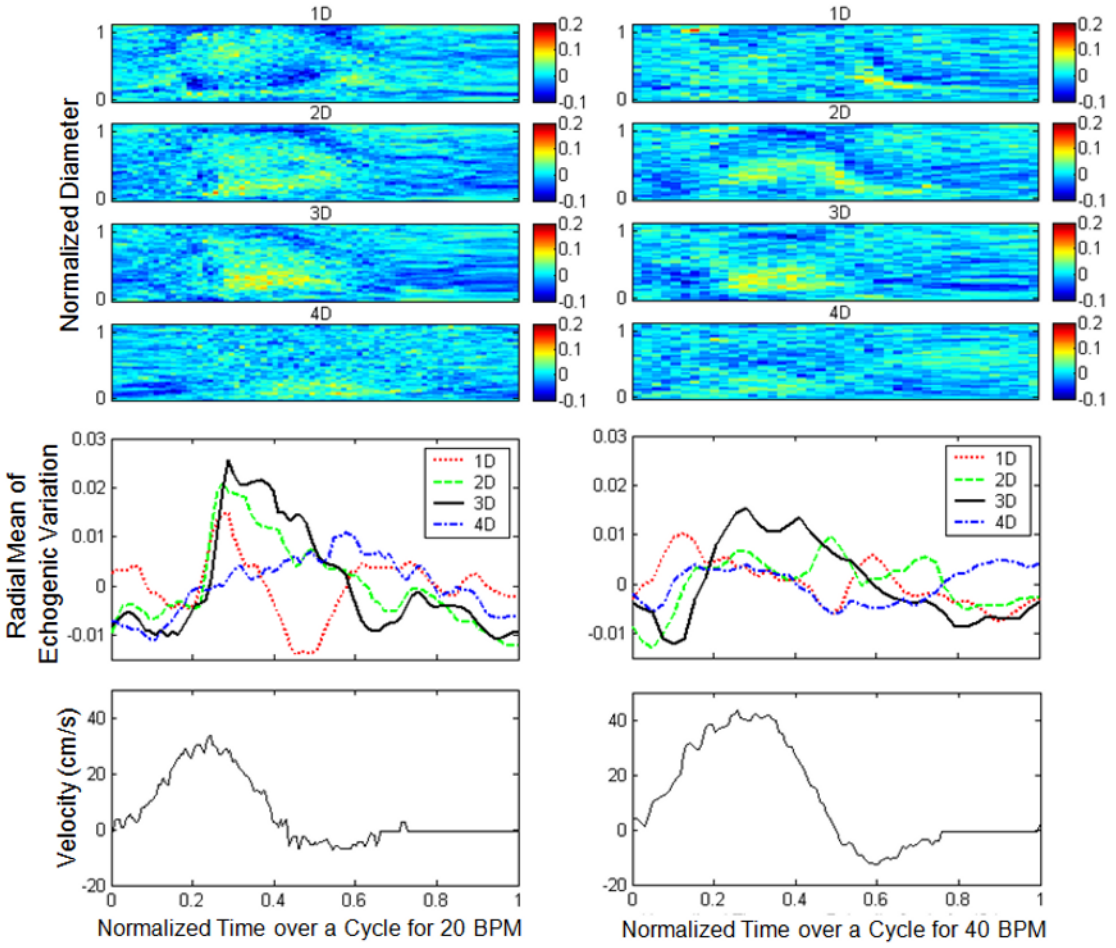


Fig. 8. The cyclic and regional variation of echogenicity downstream of a stenosis under pulsatile flow (Nam et al., 2008).

Many studies have been reported that flow turbulence increased ultrasound backscatter from blood (Shung et al., 1984, 1992; Yuan and Shung, 1988a; Cloutier et al., 1995, 1996, 2000; Bascom et al., 1997). However, most of the results were performed in RBC suspension rather than whole blood. RBC suspension cannot form rouleaux, so it does not represent the physiological blood of human or porcine in the vessel *in vivo* in terms of RBC aggregation. To examine the effect of flow turbulence on echogenic variation from whole blood, a mock flow loop with an eccentric stenosis was employed. Longitudinal B-mode images were obtained with Doppler spectrograms at pre- and post-stenotic region. Figure 7 (modified from Nam et al., 2008 and Paeng et al., 2009) displays typical B-mode images upstream and downstream of a stenosis. The hyper-echoic parabolic or eddy-like profiles were visually identified upstream of the stenosis during diastole (left panel of Fig. 7 b), which might arise from the blood flow disturbance caused by reversed blood flow. In spite of the flow disturbance during diastole, the BLH phenomenon was seen at the center of the tube along the flow direction in the left panel of Fig. 7 a. The echogenic variation at post-stenotic region was analyzed by the subtraction of the temporal mean echogenicity as shown in Fig. 8 (Nam et al., 2008), showing the cyclic and local variation of echogenicity from 1D (1 diameter of the tube) to 4D downstream. The upper four panels display the ecogenicity deviation from temporal mean echogenicity. The middle panels show the radial mean calculated from the upper four panels. At both 20 and 40 bpm (beats per minute), the radial variation was at its maximum 3D downstream. The sudden increase of the variation at 3D could be explained by a computational simulation of turbulent flow in artery (Mittal et al., 2001; Mallinger and Drikakis, 2002) and the experimental reports (Cloutier et al., 1996; Bascom et al, 1997; Ahmed, 1998), where they suggested that the flow is laminar or transitional near downstream, but fully turbulent at the area far from the stenosis and weaker further downstream. The higher cyclic variation was observed at 1D-3D during the decelerating period of systolic phase. This phenomenon is in good agreement with Yellin's report (1966) that flow during acceleration may be laminar while deceleration may produce flow disturbance. It was analyzed that the bright tail-like profiles in 1D and 2D at 40 bpm during 0.5-0.7 normalized time over a cycle were caused by the flow disturbance during diastolic period.

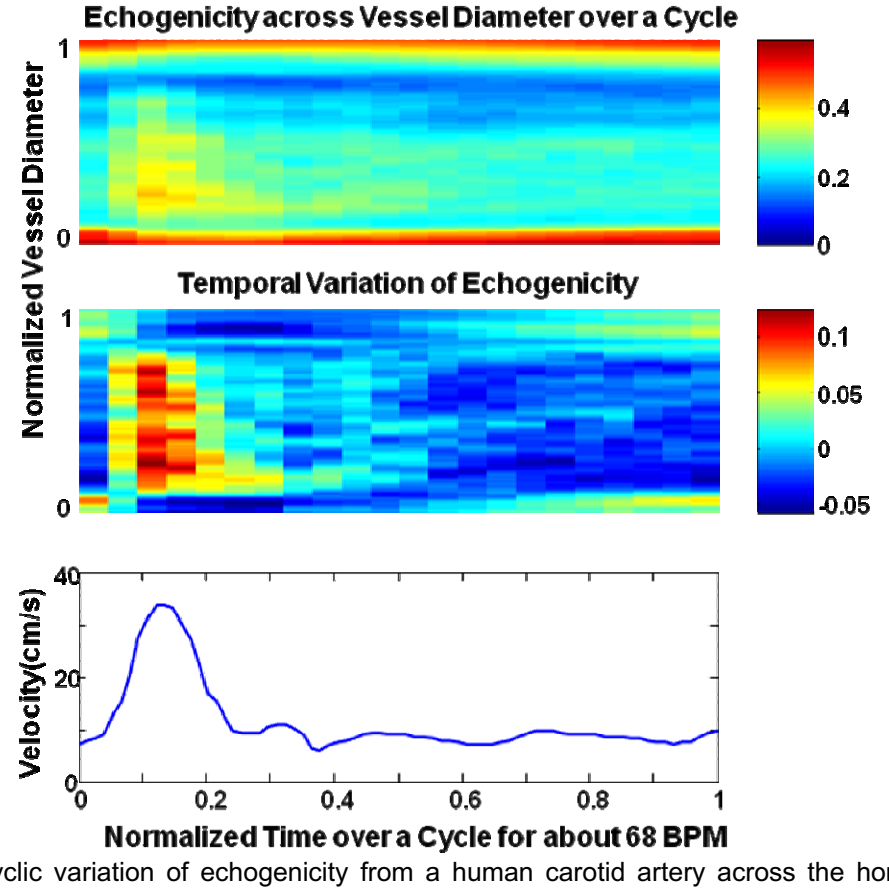


Fig. 9. The cyclic variation of echogenicity from a human carotid artery across the horizontal vessel diameter synchronized with the speed profile over a heart cycle (Paeng et al., 2003)

A preliminary *in vivo* visualization of cyclic variation in the human carotid artery was carried out (Paeng et al., 2003). Even though several imaging methods such as B-mode, B-flow, power Doppler, and color Doppler mode were collected to investigate, the 'bright ring' was strongly observed during systolic phase only in the harmonic imaging technique (Fig. 9). The dynamic range of the echos was too big to observe the cyclic variation of echogenicity from vessel lumen lying under the tissue from B-mode images, but harmonic imaging technique of this GE LOGIQ 700 system reduced this dynamic range. Although the visualization of the dynamic behavior of RBCs in pulsatile flow in real artery has some limitations to attain at present because of the high dynamic range between tissue and blood, it has important implication in investigation of real time *in vivo* status of RBC aggregation and diagnostic potentials of various blood related diseases such as atherosclerosis and thrombosis. For more clear visualization of the blood echo in the vessel excluding the strong tissue signals in an ultrasound scanner, the development of the high sensitive image modality at higher frequency or quantified Doppler power mode is required.

# 4. Conclusion

An excessive level of RBC aggregation and flow turbulence in blood vessel are associated with various cardiovascular diseases and other pathologies. Characterization of the phenomena has been studied using ultrasound backscatter measurement. Through the brief summary of the ultrasonic visualization of blood in various *in vitro* flow conditions, it was demonstrated that ultrasound is a good modality to visualize the dynamic variation of RBC aggregation. The temporal variation during a pulsatile cycle and the spatial variation within the vessel could be explaned by the combined effects of shear rate and flow acceleration. Further efforts are needed to attain the real time ultrasonic visualization of RBC behavior in *in vivo* application, which would be possible together with the development of ultrasound instrumentation and understanding of physical and physiological aspects of RBC aggregation and its ultrasonic scattering mechanism.

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