THE LEADING EDGE IN DIAGNOSTIC ULTRASOUND

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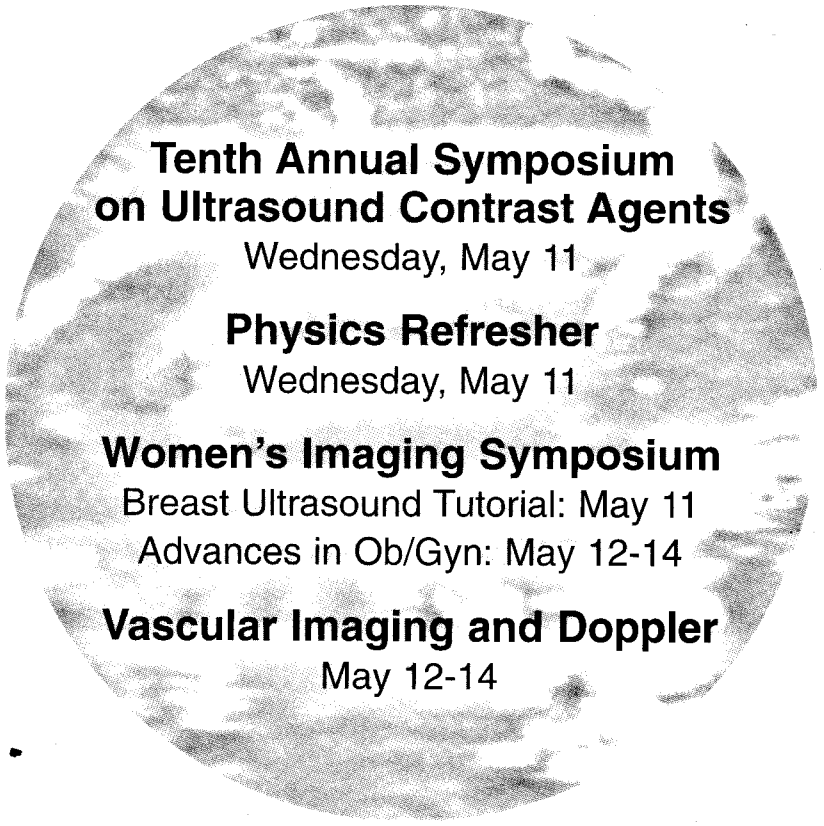

Tenth Annual Symposium on Ultrasound Contrast Agents
Wednesday, May 11

Physics Refresher
Wednesday, May 11

Women's Imaging Symposium
Breast Ultrasound Tutorial: May 11
Advances in Ob/Gyn: May 12-14

Vascular Imaging and Doppler
May 12-14

Program Director
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Sponsored By
The Division of Diagnostic Ultrasound,
Department of Radiology, Thomas Jefferson University Hospital
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Conference Location
The Borgata Hotel Casino and Spa
Atlantic City, New Jersey

Tumor perfusion by multigated contrast-enhanced Doppler imaging

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Tumors begin as a vascular aggregates of cells that stimulate the growth of microvessels for their blood supply during the development process. The formation of these vessels, termed angiogenesis, is correlated with rapid tumor growth and metastasis. A method to enhance the echo intensity of blood is needed in order to visualize the extent of these blood vessels and the blood flow though them by ultrasound,. Many laboratories around the world are developing methods that use microbubble-based agents to meet this need.

One of the problems with using ultrasound contrast agents is that the microbubbles are destroyed by the ultrasound pulses during the passage of the agent through the image plane. That is, the change in bubble concentration used to measure flow is artificially enhanced due to bubble destruction. While considerable progress has been made in the formulation and use of contrast agents, the effect of bubble destruction on flow-related measurements has not yet been fully resolved. This effect can be significant when the flow is slow and the contrast agent spends a long time in the image field of view. In this study, we propose an approach that uses the gating of the ultrasound scanner at multiple frequencies to vary the destruction of microbubbles. The rate of bubble destruction, instead of the concentration of microbubbles, is used to evaluate flow. The hypothesis underlying this approach is that the number of bubbles destroyed during the passage of the contrast agent through a fixed image field of view varies in proportion to the flow rate. The implication is that the rate of microbubble destruction can be used to evaluate flow.

Through mathematical modeling it can be shown that power Doppler signal from contrast agent following bolus injection is modulated as the gating frequency of the ultrasound scanner is varied. The rate of change in enhancement (S) with gating frequency is inversely related to mean flow (F) through the image plane.

Two sets of experiments were performed to demonstrate the feasibility of our approach.

The first set of experiments was conducted using a flow phantom with variable flow rates. 0.1 ml of contrast agent (Optison, Mallinckrodt, WA) was injected into a mixing chamber containing 400 ml of 10% aqueous solution. The ultrasound scanner was gated at 0.5, 2, and 4 Hz, while maintaining an MI of 0.9. Each scan or trigger rate was maintained for a 20s. The cycle of 0.5, 2, and 4 Hz was repeated until the microbubbles could no longer be visualized on the power Doppler image. After imaging, the tube leading to the mixing chamber was disconnected to measure flow by collecting a fluid sample in a graduated cylinder. The true flow of contrast agent was determined by multiplying the volume of fluid pumped per minute by the concentration of contrast solution. The above process of contrast injection and imaging was repeated at flow rates from 8.5×10^{-4} to 8.0×10^{-3} ml of contrast agent per minute.

The images on the VHS tape were digitized at a frame rate of 5 frames/s. A fixed region of interest representing the lumen of the flow tubing was superimposed on each image, and color level within this region was analyzed using computer software designed for this purpose. The average color level over all the pixels within the region of interest was determined for each image and used to construct dilution curves for multigated imaging. The data was parsed manually into three subgroups, with each group corresponding to one of the three specific gating rates (0.5 Hz, 2 Hz, and 4 Hz). A plot of each data subset generated a dilution curve, and the area under the curve was measured. Area versus gating rate data was fitted to a linear model by the least squares method to determine rate of change (slope) and amount of contrast flow (intercept). Contrast flow index (CFI) was then obtained by dividing the intercept by the slope.

Figure 1 shows the results of this study as a plot of CFI measured at different flow rates of the contrast agent. An increase in CFI is observed with increasing flow rate. The solid line in the figure represents the least square fit of data to the linear model y=mx. A strong correlation described by R^2=0.87 was observed between the two variables. A one-tailed t test shows the correlation to be significant to $p \leq 0.05$. This demonstrates that the method appropriately accounts for the bubble destruction by the ultrasound pulses and can be used to assess flow.

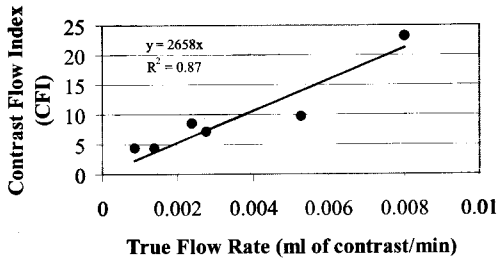
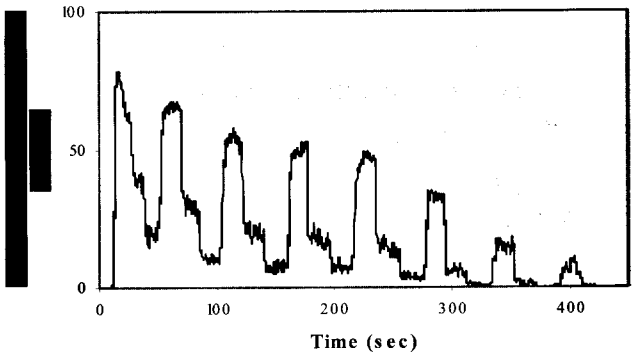


Figure 1: A plot of the CFI measured at different flow rates of the contrast agent.

The second set of experiments was conducted to demonstrate the feasibility of the approach in mouse tumors. K1735 VEGF-3 murine melanoma cells (10^6 cells/100 microliter) were injected s.c. into the lower flanks of female mice (C3H/HeN). When tumors appeared approximately 2 weeks after cell injection and their size became reliably measurable (1-2 mm diameter), the mice were anesthetized with ketamine and xylazine and imaged by power Doppler ultrasound. The skin overlying the tumor was shaved and the imaging transducer was aligned along the long axis of the tumor. A specially designed holder was used to hold both mice and transducer fixed in place during contrast injection.0.1 ml of microbubble contrast agent (Optison, Mallinckrodt) was injected intravenously through the tail vein. Imaging was performed using ATL 5000 (Philips Ultrasound, Bothell, WA) with a

Figure 2: The quantitative changes in color level, $\overline{\Delta CL}$, with time during multigated imaging of the tumor.



12-5 MHz broadband transducer. The scanner was gated externally as described above in a sequence of 0.5 Hz, 2 Hz, and 4 Hz.

The quantitative changes in color level, $\overline{\Delta CL}$, with time during multigated imaging of the tumor is shown in Figure 2. A recurrent cyclic change in $\overline{\Delta CL}$ similar to phantom studies is observed. Each successive cycle is reduced in amplitude due to the dilution of contrast concentration. During each cycle the $\overline{\Delta CL}$ decreases with the increase in gating frequency from 0.5 to 4 Hz.

Microbubbles of contrast agent are known to produce variable enhancement. One potential issue is how reproducible CFI measurements are for the proposed technique. To address this issue, three repeated injections were made on the same mouse while keeping the image plane fixed. Table 1 lists the measurements in all mice. Fairly reproducible measurements of CFI were obtained. Mouse A showed higher variation compared to the other mice. The reason for this difference is not known and is perhaps part of normal variations in experimental parameters.

Table 1: Values of repeat measurements of contrast flow index in mice.

	Injection No.	CFI	Mean±Std
Mouse A	1	4.35	
	2	5.54	
	3	3.91	4.60±0.84
Mouse B	1	4.43	
	2	5.09	
	3	4.47	4.66±0.37
Mouse C	1	4.27	
	2	4.98	
	3	4.30	4.52±0.40

The proposed method yielded reproducible CFI for mice tumors and demonstrates that multigated contrast-enhanced power Doppler imaging may be a reliable means of non-invasive measurement of tumor perfusion in mice.

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