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● *Original Contribution*

INVESTIGATION OF THE RELATIONSHIP OF NONLINEAR BACKSCATTERED ULTRASOUND INTENSITY WITH MICROBUBBLE CONCENTRATION AT LOW MI

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Abstract—The aim of this study was to measure the relationship of image intensity with contrast agent concentration. *In vitro* experiments were performed with a flow phantom and a sulphur hexafluoride filled microbubble contrast agent (SonoVue) at different concentrations (0.004‰ to 4‰) covering the range commonly encountered in clinical practice. The concentration of microbubbles in the contrast agent solutions was confirmed optically. Images were collected with a diagnostic ultrasound system (iU22, Phillips Medical Systems, Bothell, WA, USA) and with a nonlinear imaging technique (power modulation) at low mechanical index (MI = 0.05) to avoid bubble destruction. The mean intensity within a region of interest was measured to produce time-intensity curves from linearized (absolute scale) data. The relationship of linearized image intensity to contrast agent concentration was found to be linear up to 1‰ and reached a plateau at approximately 2‰. To operate in the linear range of the intensity-concentration relationship the contrast agent dose should be adjusted to avoid those high values *in vivo* and the highest dynamic range of the ultrasound system should be used to avoid unnecessary signal saturation.
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Key Words: **Ultrasound contrast agents, Concentration, Dynamic range, Linearized image intensity.**

INTRODUCTION

One of the most important developments in oncology in recent years is the introduction of antiangiogenic agents. Antiangiogenic therapies do not simply target the tumor cells but instead the new vessels being created within the tumor (Folkman 2007; Browder et al. 2000; Kerbel 2006). For the assessment of therapy the need to image and quantify blood perfusion within the tumor and the surrounding normal parenchyma arises.

To image blood flow at the microvascular level with diagnostic ultrasound, contrast agents in the form of encapsulated microbubbles are used. The images produced can be used to quantify blood flow using the image intensity of a region-of-interest (ROI) as a function of time. Quantification of tissue perfusion and tumor angiogenesis with ultrasound and microbubble contrast agents is currently a very active field of research (Lassau et al. 2007; Arditi et al. 2006; Ferrara et al. 2000; Wei et al. 1998; Lucidarme et al. 2003). The introduction of contrast agents in the body is done either as a bolus injection or a constant infusion spanning several minutes. With a constant infusion protocol a high mechanical index flash is used to destroy the microbubbles in the image plane; the subsequent replenishment of the region with microbubbles is measured to extract blood flow parameters (Wei et al. 1998). With a bolus injection protocol time-intensity curves for a region-of-interest are acquired and analyzed to extract local hemodynamic parameters (Averkiou et al. 2009; Lassau et al. 2007). The underlying assumption in all research for perfusion quantification is that the relationship of the image intensity (in linear units, *i.e.*, not logarithmically compressed) and microbubble concentration is linear.

Tiemann et al. (2000) in a series of experiments performed to evaluate the behavior of microbubbles in the myocardium, investigated the relationship of microbubble concentration with the area under the curve of time-intensity curves. They varied the amount of contrast agent injected (Levovist; Schering, Berlin, Germany) as a bolus in a flow phantom and formed time-intensity curves with uncompressed image data. They also calculated the difference of the mean intensity between two regions-of-interest (proximal and distal) within the same tube to investigate acoustic shadowing. The area under the curve of time

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intensity curves was found to be linearly related with contrast agent concentration up to a certain amount injected (<420 mg). This work was done using harmonic power Doppler imaging on an HDI-5000 (H-PDI), a technique that uses a high mechanical index (MI) with intermittent scanning at 1 Hz. The units shown for area under the curve were dB×s so the linear relationship that is shown is actually logarithmic. Schlosser (Schlosser et al. 2003) has shown a linear relationship between the concentration of microbubbles (Levovist) and H-PDI intensity but it is not clear if in that work the signal intensity was linearized (logarithmic compression removed).

Sboros et al. (2002) have shown the relationship of ''average pixel intensity'' with microbubble concentration, for a range of concentrations (approximately 1–13 bubbles/μL) and with varying peak negative pressures (0.269–1.515 MPa). At 3 MHz center frequency, the resulting MI was relatively high (0.15–0.85). This work did not take into account the nonlinear response of the bubbles since the scanner used had only conventional (fundamental) imaging mode available. The contrast agents used were Definity (Bristol-Myers Squibb Inc., Waltham, MA, USA) and Quantison (Quadrant Healthcare, Nottingham, UK).

Goddi et al. (2004) have produced a relationship between relative echogenicity and contrast agent (SonoVue) concentration. Relative echogenicity was obtained by comparing the backscattered intensity from contrast agent with that from a linear target in a phantom. Imaging was performed at low MI and logarithmically compressed image data were collected.

Before any attempt of perfusion quantification using image intensity from contrast enhanced ultrasound images, it is necessary to know the relationship of backscattered intensity and microbubble concentration. Thus, the aim of this study is to investigate this relationship for low MI contrast imaging and nonlinear pulsing schemes (power modulation) used today in modern diagnostic ultrasound systems (iU22; Philips Medical Systems, Bothell, WA, USA). Linearized values for the mean intensity of a region in a flow phantom (where the logarithmic compression was removed) were extracted. SonoVue (Bracco S.P.A., Milan, Italy), a clinically approved agent in Europe and Asia was used. The contrast agent concentration was carefully controlled and confirmed using a light microscope.

MATERIALS AND METHODS

Flow phantom set-up

A solution of deionized water and contrast agent (SonoVue) was pumped with a peristaltic pump (Masterflex; Cole-Palmer, Vernon Hills, IL, USA) into a tissue mimicking flow phantom (Model 523A; ATS Laboratories Inc., Bridgeport, CT, USA) with attenuation coefficient

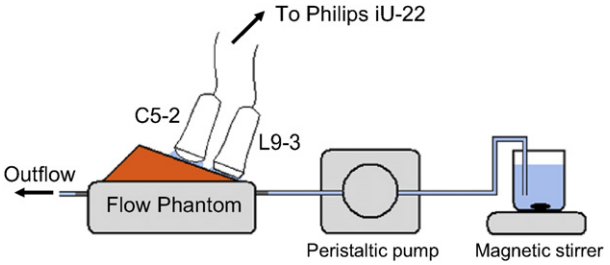

Fig. 1. Flow phantom set-up.

0.5 dB/cm/MHz and then discarded as shown in Figure 1. The solution was drawn from a beaker that was continually stirred by a magnetic stirrer. Two transducers (a curvelinear array C5-2 and a linear array L9-3) were placed in line able to image the same tube without requiring repositioning. The two transducers were placed so as to image the 8 mm flow channel close to the depth they are normally used in clinical practice (approximately 12 cm for C5-2 and 3.5 cm for the L9-3).

Before each solution was imaged, the pump was activated so as to push the previously imaged solution or the water in the phantom out of the image plane and bring in fresh bubbles. By pumping approximately 50 to 100 mL of the solution, new bubbles were brought in since the total volume of liquid in the set-up (all tubing plus the phantom) was approximately 30 to 50 mL. Fresh microbubbles were also introduced between imaging with the C5-2 and L9-3 probes so as to avoid bubble floatation and remove any large bubbles. During data acquisition there was no flow within the phantom to mimic blood flow in the microcirculation where the flow is close to zero.

Contrast agent solutions

SonoVue was reconstituted as advised by the product manufacturer. We measured contrast agent concentration by comparison with a reference dilution named ''A''. The dilution ''A'' is the highest concentration we used

Table 1. List of contrast agent concentrations used

Relative concentration	Contrast agent concentration (‰)	Number of bubbles per unit volume/Standard deviation (bubbles/μL)
A	4	785/126.71
A/2	2	318/91.9
A/4	1	195.6/17.1
A/10	0.4	66.7/15.03
A/20	0.2	16.7/5.56
A/40	0.1	15.9/5.59
A/100	0.04	3.7/0.64
A/200	0.02	1.5/1.69
A/400	0.01	-
A/1000	0.004	-

The concentration of each solution is given in milliliters of contrast agent per liter of deionized water (‰) and in terms of bubbles per microliter measured with a microscope and counting chamber.

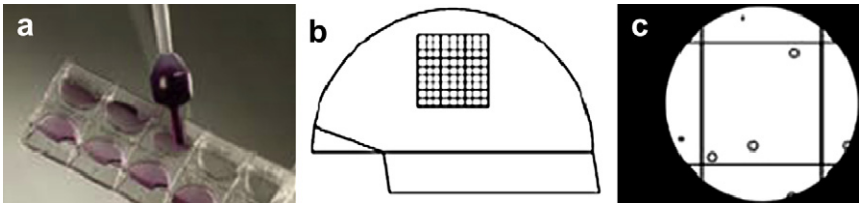

Fig. 2. Images depicting the counting chambers used. (a) Kova slide, (b) Kov slide chamber and (c) graphical representation of a microscope image of a square within the grid.

at 1 mL of contrast agent in 250 mL of water (deionized water, resistivity 15 MΩ). The contrast agent concentrations used in these experiments are shown in Table 1.

To minimize variation in the contrast agent solutions, we performed all of each set of measurements from the same vial of agent. We performed the experiment as fast as possible so as to minimize possible microbubble changes. The duration for the completion of the measurements required (one loop from each transducer for each concentration) was less than 1 h.

We have also confirmed with a light microscope (Model DC3-163; National Optical and Scientific Instruments Inc., San Antonio, TX, USA) and Kova slides (HYCOR Biomedical Inc., Garden Grove, CA, USA) that the concentration of contrast agent solutions was proportional to the concentration of microbubbles. To measure the concentration of microbubbles, a sample from the solution containing microbubbles was taken and placed in a chamber of a Kova slide. The samples were taken just before the solution was pumped into the flow phantom to perform the acoustic measurements. The solution is placed in the chamber (Fig. 2a) that has a grid pattern as depicted in Figure 2b. By counting the number of microbubbles in a number of squares (Fig. 2c), the concentration of microbubbles can be determined. The chamber volume was 6.6 μL and the depth was 100 μm, which is significantly larger than microbubbles. For greater accuracy this was repeated three times for each sample. The $\times$10 (0.25 numerical aperture) objective lens were used and the overall magnification of the microscope was $\times$ 100. The resolution of the microscope is approximately 2 μm. Each measurement required approximately 1 min. Thus, for each concentration the optical measurements required 3 min. No changes to the bubble population were observed during optical measurements.

Imaging protocol and intensity measurement

We used ''contrast side/side'' imaging which is a mode that displays the contrast and tissue images in a side-by-side fashion. The frame rate was set to 2 Hz by using a triggered mode (500 ms trigger interval for image acquisition) to limit the amount of collected data. The nonlinear pulsing scheme was power modulation. The transmit center frequency was 1.7 MHz for the

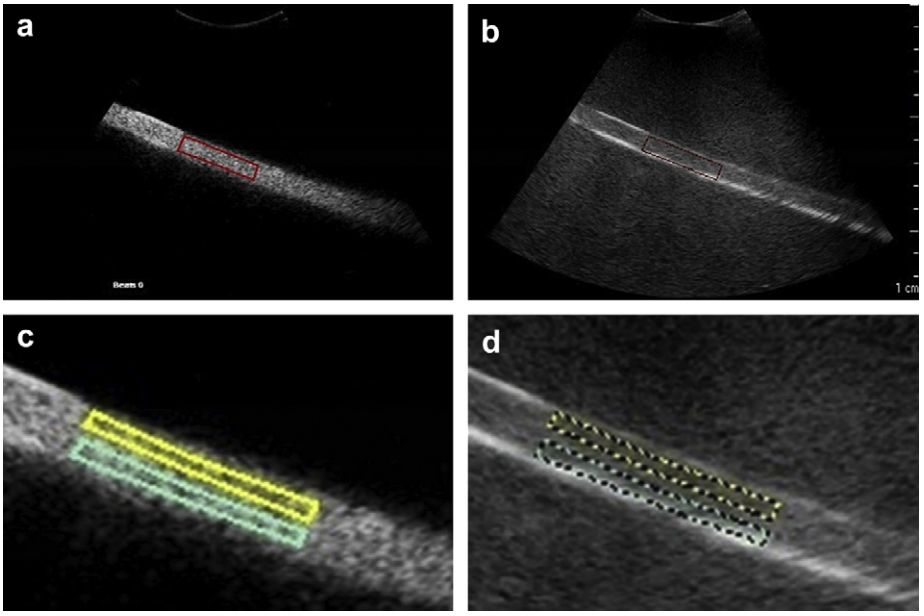

Fig. 3. Examples of region-of-interest (ROI) selection. The images in the left (a) and (c) show the contrast specific image (power modulation) whereas the images in the right (b) and (d) show the tissue (fundamental) image. The images in (a) and (b) show the ROI that includes the whole flow channel, whereas (c) and (d) show the smaller ROIs at the top and bottom of the channel.

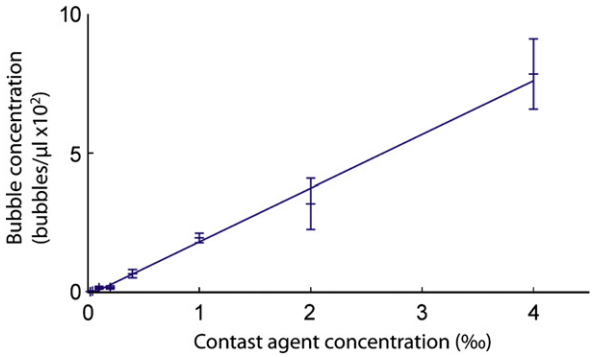

Fig. 4. Bubble number per unit volume vs. contrast agent concentration.

C5-2 transducer and 3.1 MHz for the L9-3 transducer. We acquired image loops of 15 s (30 frames). The compression was set at the maximum that was available on the scanner (50 dB) to best accommodate a large range of signals and avoid saturation. The specific scanner used in this work displays a compression value that it is higher than the actual dynamic range and the justification according to the manufacturer is that the overall image esthetics are equivalent to the noted compression value. Thus, 50 dB on the scanner corresponds to a ''true'' dynamic range of about 36 dB. The two-dimensional (2-D) gain was set at 77%. The MI used was 0.05 for the contrast side and 0.04 for the tissue side for both probes, with a focus at 12 cm for the C5-2 and 3.5 cm for the L9-3. The persistence was turned off to avoid averaging of the image data between consecutive frames.

Time-intensity curves were formed from the image loops with QLAB software (Philips Healthcare, Andover, MA, USA) for the different solutions of contrast agents. QLAB allows for selection of a ROI on the image (Fig. 3a and b) and produces time-intensity curves. The intensity is obtained from uncompressed enveloped detected data squared and then averaged from all pixel values in the ROI. The ROIs were selected manually for each image loop and an effort was made to place the ROI in the same spot in every loop. To repeatedly select the same region, the ROI shape was always a polygon with right angles and with two points being set at the same depth for all image loops. The ROIs were 7 mm wide by 35 mm long (approx. 250 mm^2).

To evaluate the attenuation over the width of the channel, two additional ROIs (2.25 mm wide by 35 mm long) were placed at two different locations in the channel. One was placed at the top of the flow channel and the other at the bottom as can be seen in Figure 3c and d.

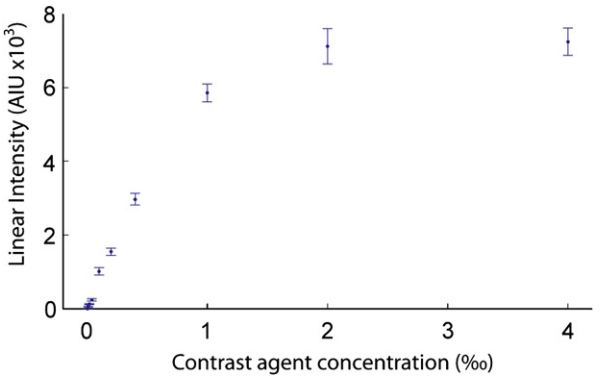

Fig. 5. Backscattered intensity vs. contrast agent concentration (scanning is performed with the curve-linear C5-2 array).

RESULTS

The measured number of bubbles per unit volume as a function of contrast agent concentration is presented in Figure 4. A line was fitted through the data, $y = 1.93 \times 10^5 x - 1.192 \times 10^4$, with $R^2 = 0.992$. For the case in which no contrast agent is present in the solution, the bubble concentration is extrapolated to a small negative value. This is probably due to the inability of the microscope to detect bubbles smaller than 2 μm diameter. According to Gorce et al. (2000), the range of diameters of microbubbles in the solutions prepared is 0.7 to 10 μm. Since the microscope resolution is approximately 2 μm, the smaller bubbles are not detected. In general, the results in Figure 4 confirmed that as the contrast agent concentration increased, the measured number of bubbles linearly increased as expected.

In Figure 5 the relationship of intensity [intensity is given in arbitrary intensity units (AIU)] with contrast agent concentration when scanning with the C5-2 probe is shown. Similar results were obtained for the L9-3 probe but not shown here. For concentrations of contrast agent in the range of 0‰ to 1‰, there is a linear increase of backscattered linearized intensity with bubble concentration. Above 1‰ and up to 4‰, relationship is not linear, where a plateau is reached at the concentration of 2‰.

The data presented in Figure 5 (taken with the C5-2 probe) and also data taken with the L9-3 probe were fitted with a straight line up to the concentration of 1‰ and are shown in Figures 6 and 7, respectively. The results of these fits are $y = 5199x + 167.8$, and $y = 82.5x + 1.01$, for C5-2 ($R^2 = 0.984$) and L9-3 ($R^2 = 0.986$), respectively. A similar relationship is found for both transducers (Figures 6 and 7), with the overall trend being the same but the actual values of backscattered intensity for L9-3 being much lower due to the higher frequency used. The higher frequency results in (1) higher attenuation and (2) smaller number of bubbles at resonance both factors attributing toward a lower image signal intensity.

In Figure 8 the effects of acoustic shadowing over the length of the flow channel are demonstrated. The three

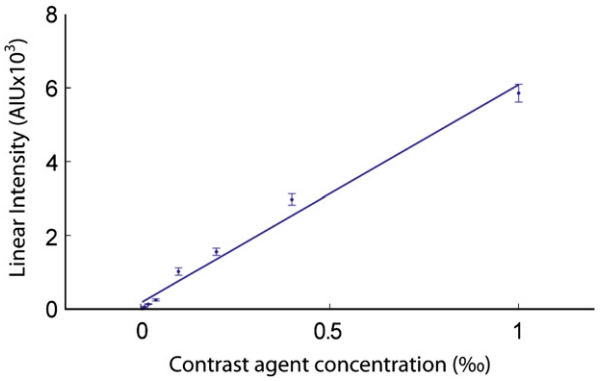

Fig. 6. Backscattered intensity vs. contrast agent concentration fitted to a straight line for concentrations up to 1‰ (scanning is performed with the curve-linear C5-2 array).

data sets shown are the intensity as a function of bubble concentration curves for a ROI at the top part of the flow channel (×), at the bottom (+) and the entire width of the channel (●) as shown in Figure 3. The ROI in lower part of the channel had consistently lower intensity than that of the top part as a result of shadowing. As expected, the data for the ROI that spans the whole flow channel width lies between those for the top and bottom ROIs. The attenuation experienced by the acoustic pulses is dependent on the path length through the bubble cloud. The difference between the curves for the top and bottom ROIs indicates the effect of shadowing (8–10 dB for the highest concentration). When shadowing is less pronounced as is the case for the top ROI, the plateau in the intensity vs. concentration relationship is further delayed. In addition, the departure from linear relationship between intensity and concentration for the top ROI is probably due to machine (scanner) specific factors like compression, digital and analog gain and other receive signal path parameters.

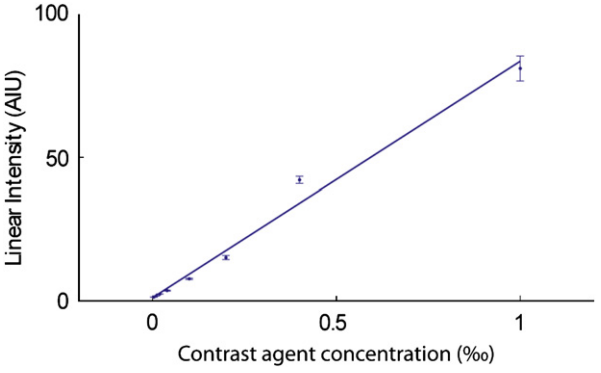

Fig. 7. Backscattered intensity vs. contrast agent concentration fitted to a straight line for concentrations up to 1‰ (scanning is performed with the linear L9-3 array).

DISCUSSION

Based on our results, linearized image intensity and microbubble concentration are linearly related for a range of concentrations. This result is consistent with acoustic scattering theory for microbubbles. We can assume microbubbles to be Rayleigh scatterers since the radius of microbubbles (approx. 2–10 μm) is much smaller than the wavelength of the incident ultrasound wave (approx. 0.5–1 mm) therefore, we expect the intensity of backscattered signal would be linearly related to the number of microbubbles in the region (Forsberg et al. 2001). Backscattered intensity and attenuation are related and are both dependent on the concentration of microbubbles. At higher concentrations the effect of attenuation due to scattering becomes more dominant eventually causing the received signal to reach the saturation level (Forsberg et al. 2001). The attenuation due to scattering increases exponentially whereas backscatter intensity increases linearly with increased bubble concentration (Marsh et al. 1998). The intensity vs. concentration relationship should be linear at lower concentrations and reach a plateau at higher concentrations. At very high concentrations, the intensity should start to decrease although this has not been observed in our experiments. The concentration that the plateau is reached is dependent on the path length (in our case the placement of the ROI in the flow channel), the attenuation coefficient for the specific type of contrast agent and the frequency of the transducer.

It has been shown by numerical investigation (Allen et al. 2003) that the response of microbubbles in proximity with other microbubbles (of different size) is affected by varying the separation distance. A decrease of 10 dB in integrated scattered power has been observed at 10 μm compared with 500 μm separation distance. It is reasonable to assume that with increased microbubble concentration, the average separation distance will be reduced and there is a very disperse size distribution in most clinical contrast agents and hence a decrease of signal may be due to this effect.

To bring the contrast concentrations into clinical perspective, we take the case of the right ventricle, which receives the total amount of the bolus in a few seconds after injection. It has a volume of about 200 mL and we estimate that the concentration there with a 2.4 mL bolus injection would be more than 10‰. In the case of tissue perfusion (such as liver, kidney etc.), the bolus is more diluted (spread-out) and the partial volume of blood in the imaged region would be further reduced. For tissue perfusion cases it is expected that the microbubble concentrations will be in the linear region of Figure 5. Finally, when a constant infusion is used, the maximum concentration is probably less than 1‰ with a single vial of SonoVue (complete mixing of 4.8 mL of contrast

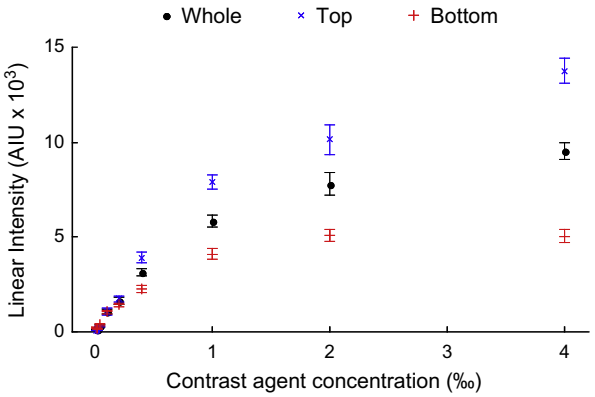

Fig. 8. Backscattered intensity vs. contrast agent concentration. The • represents the average intensity over the entire flow channel, the × represents the average intensity of the region-of-interest (ROI) at the top part of the channel and the + represents the average intensity of the ROI at the bottom part of the channel (as shown in Fig. 3).

agent in 5 L of blood) even without taking into account the decay of the agent in the circulation.

The saturation of the backscattered intensity and contrast concentration relationship can also be attributed to the limited dynamic range of the ultrasound system. The dynamic range of the systems is limited first by the gain of the analog amplifier usually around 50 dB and second, by the logarithmic compression applied to the radio frequency data. The compression is set by the operator on most diagnostic scanners between about 20 and 40 dB. Since the actual backscattered signals from microbubbles are very low in real-time nondestructive imaging, diagnostic systems use low dynamic range to better present the data. However, if we plan to quantify the image data, care must be taken to use the highest possible dynamic range to accommodate a larger range of signals.

Another observation that requires attention is that the intensity decreases with time during data acquisition as seen in Figure 9. It is suggested that there is no destruction of SonoVue microbubbles at MI = 0.04 to 0.05 (Tang et al. 2005; Averkiou et al. 2003). To separate the effects of ultrasound-induced bubble destruction and ''natural'' (not induced) bubble deterioration, we have devised the following experiment. We collected 15 s image loops and measured the slope of the time intensity curves for MI in the range of 0.02 to 0.21 with two different frame rates. If bubble destruction occurred due to the applied sound field, the time intensity curve with the higher frame rate would exhibit a higher slope (more negative) compared with the one with a lower frame rate. If no or little destruction occurred the slope of the two curves would be approximately the same. On Figure 10 the slope of backscattered intensity is approximately the same for MI in the range of 0.02 to 0.06 and differs significantly at higher MI. The mechanical index (MI = 0.05) used for our experiments is in the range were it is believed that no ultrasound induced microbubble destruction occurs. Thus, the gradient of time intensity curve in Figure 9 is not due to ultrasound induced bubble destruction but due to ''natural'' bubble deterioration or floatation of the microbubbles toward the proximal wall. For MI = 0.1 and 0.21, there are significant differences between the slopes with the two different frame rates due to ultrasound induced bubble destruction (the higher frame rate destroys bubbles faster).

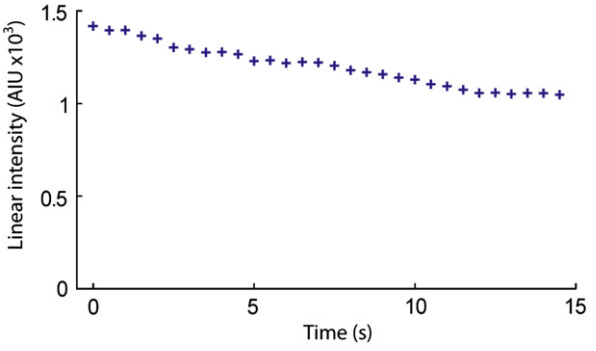

Fig. 9. Example of a time-intensity curve at concentration A/40 acquired using the C5-2 transducer showing a small reduction of signal with time.

The experiments were aimed at mimicking a clinical scenario of blood flow in the microvasculature. It must be noted that since we are using microbubbles in an *in vitro* set-up the population of microbubbles is not the same as it would have been in an *in vivo* environment. Large microbubbles of more than 7 to 10 μm are filtered out after passage though the lungs. As mentioned earlier, the contrast agent solution was not flowing during our measurements. In a typical image of an organ, stationary or very slow moving bubbles in the microcirculation coexist with faster moving bubbles in larger vessels. This may affect the intensity vs. concentration relationship (Bruce et al. 2004) and it remains a subject to be investigated. It has been shown that the natural frequency of microbubbles is altered when their oscillation is restricted by narrow vessels such as capillaries (Qin and Ferrara 2007). In our set-up, microbubble were essentially free to oscillate without any obstacles other than the channel walls and off curse other bubbles may cause results to differ from those obtained by microbubbles in a perfusion network.

Finally, considering that the source of nonlinear signals in the various other pulsing schemes commercially available today (pulse inversion, and power modulated pulse inversion) is the same as power modulation, which we have used in our work, our findings and conclusions should also apply to those pulsing schemes as well.

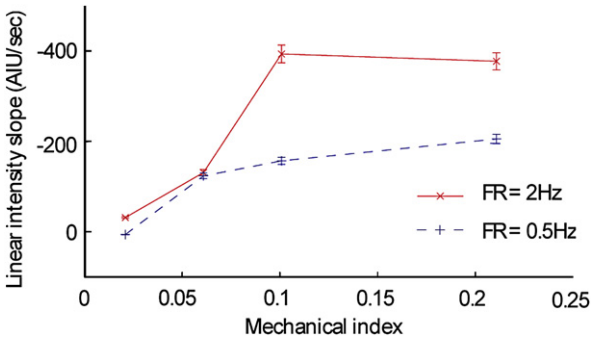

Fig. 10. The slope of the time-intensity curve with respect to MI for two different frame rates of 0.5 Hz and 2 Hz. Notice the y-axis is inverted. Negative slope is related to bubble destruction.

CONCLUSION

We have found that the relationship between backscattered intensity and contrast agent concentration is linear up to concentrations of 1‰ and reaches a plateau around 2‰. This saturation is due to (1) increased attenuation due to shadowing caused by the large bubble population and (2) machine settings such as analog gain and logarithmic compression that limit the overall signal. It is suggested that contrast doses are limited to avoid shadowing and that high dynamic range (compression) is selected on the scanner to avoid reaching the saturation zone.

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