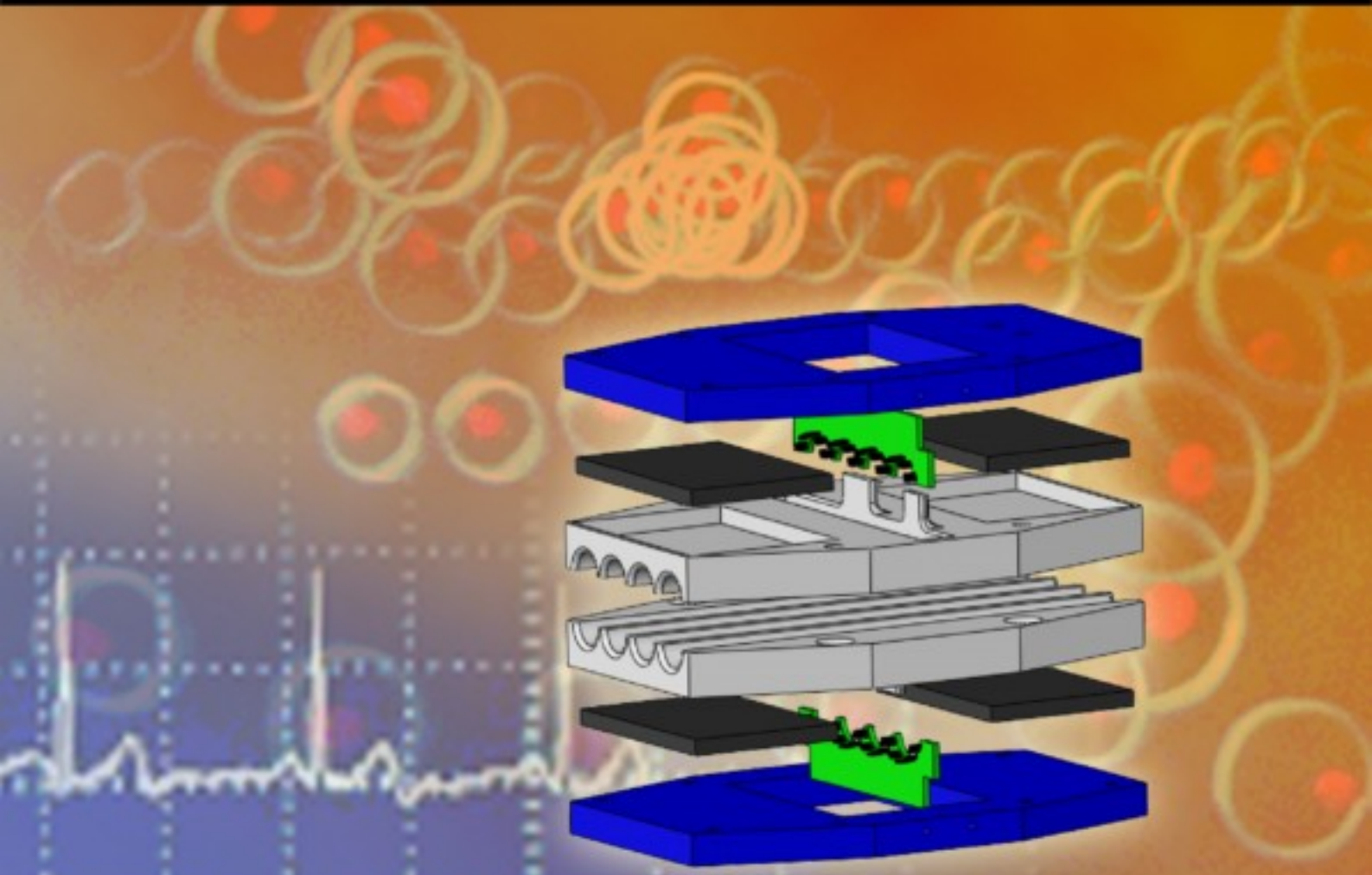


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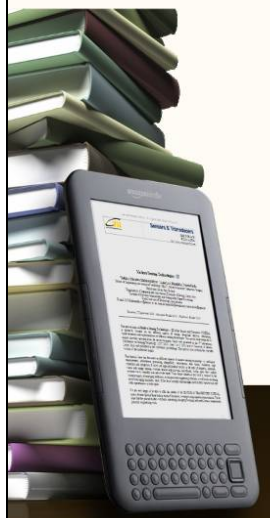
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- Chemoinformatics
- Bioimaging
- Neuroinformatics

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- Biocomputing
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- Microbiology

**C. Biotechnologies and biomanufacturing**

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- Biodevices
- Biomedical technologies
- Biological technologies
- Biomanufacturing

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Submission (full paper)	January 10, 2011
Notification	February 20, 2011
Registration	March 5, 2011
Camera ready	March 20, 2011

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- GRIDNS: Grid Networks and Services
- EDNA: Emergency Services and Disaster Recovery of Networks and Applications
- IPv6DFI: Deploying the Future Infrastructure
- IPDy: Internet Packet Dynamics
- GOBS: GRID over Optical Burst Switching Networks



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- Safety in industrial systems
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## Ultrasound Signal Analysis Applied to Determine the Optimal Contrast Dose for Echographic Examinations

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**Abstract:** In recent years the understanding of the behaviour of currently available ultrasound contrast agents (UCAs), in the form of gas-filled microbubbles encapsulated in elastic shells, has significantly improved thanks to “ad hoc” designed “in vitro” studies. However, in several studies there has been a tendency to use high UCA concentrations, potentially reducing the safety of microbubbles in clinical applications. In this study we investigated a possible strategy to improve microbubble safety by reducing the injection dose and employing low ultrasound intensities. We measured the achievable contrast enhancement insonifying microbubbles at different low concentrations (range 0.01-0.10  $\mu\text{L}/\text{mL}$ ) using a very low mechanical index ( $\text{MI}=0.08$ ). Our results, based on the use of advanced techniques for signal processing and spectrum analysis, showed that UCA backscatter strongly depends on microbubble concentration also in the considered low range, providing useful indications towards the definition of an optimal low contrast dose, effectively employable at low MIs.  
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**Keywords:** Ultrasound signal analysis, Ultrasound contrast agent, Echographic imaging, Radiofrequency spectrum analysis, Microbubble acoustic behaviour.

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### 1. Introduction

Quantitative evaluation of contrast enhancement in specific human body districts is one of the main aims of ultrasound (US) contrast imaging. In order to optimize these imaging modalities, a full

knowledge of the behaviour of contrast microbubbles (MBs) when placed inside an US field is needed. Therefore, “in vitro” experiments are required to better understand the interactions existing between microbubbles and ultrasound waves and to determine the factors that affect contrast enhancement on echographic images.

Many discussions have been recently raised concerning safety in the use of MB contrast agents [1-7]. A common literature-reported practice is to consider the minimum MB concentration capable of causing acoustic shadowing as a superior limit to contrast doses effectively employable “in vitro”. However, this is not enough, since the actually adopted concentrations are relatively high and they might be not completely safe if transferred to a clinical context. Furthermore, these “high” concentrations might affect acoustic measurement results in a complex manner. Multiple bubble scattering, for instance, has been usually assumed to be negligible but it has been demonstrated that sometimes it becomes dominant [8-10]. Another important consideration concerns bubble-bubble interactions. Theoretically, at high concentration levels, bubbles are close enough to affect each other’s oscillatory motion, and consequently display a different acoustical behaviour than that encountered when the bubbles are isolated. The implications of such interactions on the behaviour of individual bubbles have not been investigated in contrast bubble clouds, and therefore their significance is unknown. This adds a further dimension to the problem of characterizing ultrasound scattering from a population of bubbles.

In this study we showed the differences in contrast enhancement by varying concentration conditions of the microbubbles of a last generation experimental phospholipid ultrasound contrast agent (supplied by Bracco Research SA, Geneva, Switzerland) at low frequency and low mechanical index (MI) in order to optimize the injection dose.

We used a custom-designed “in vitro” system that can be developed and modulated in order to be able to study the ultrasonic signal behaviour in almost all kind of human tissues, being able to cover all vessel sizes and to reproduce the different vascular system conditions in terms of spatial and geometrical configurations and flow velocity ranges of human body. The last technological innovations in the field of signal processing and radiofrequency spectrum analysis, then, enable to bring together multiple efforts to solve a common safety problem.

This approach could provide the experimental bases for developing new analytical and numerical models for flowing microbubble behaviour, having many parameters under a possible easy control, such as tissue attenuation, flow velocity, flow pressure, MB concentration, temperature, US pulse parameters, spatial distribution of MB, interaction of MB with vessel wall surfaces, etc.

## **2. Materials and Methods**

### **2.1. Ultrasound Contrast Agent**

The last generation ultrasound contrast agent (UCA) used in this experiment consists of perfluorobutane gas microbubbles encapsulated in phospholipidic shells.

The adopted UCA concentrations were obtained by diluting the native contrast agent suspension with different volumes of saline solution. Initially, 1.3  $\mu\text{L}$  of microbubble suspension were introduced in a beaker containing 100 mL of 0.9 % NaCl saline solution and mixed with a magnetic stirrer operating at low velocity, so obtaining a contrast solution at the concentration of 0.013  $\mu\text{L}/\text{mL}$ . In the same way we prepared the other solutions employed in this study, having the following concentration levels: 0.025  $\mu\text{L}/\text{mL}$ , 0.033  $\mu\text{L}/\text{mL}$ , 0.050  $\mu\text{L}/\text{mL}$  and 0.10  $\mu\text{L}/\text{mL}$ . The employed concentration range was chosen taking as a reference a recent experimental study involving the same contrast agent [11].

## **2.1. Tissue-Mimicking Flow Phantom**

The experimental measurements described in this paper were performed on a custom-designed tissue-mimicking phantom, based on an hydrogel having a sound propagation velocity close to that of the human liver (1560 m/s) [12]. Within this tissue-mimicking matrix we obtained two vessels, whose walls were made of a different stiffer hydrogel. The final employed phantom was 6 cm deep, 5 cm long, 8 cm wide and had two 1-mm diameter vessels, both placed at 2 cm from the upper surface. The described phantom was inserted in a flow circuit containing a peristaltic pump (Peri-Star Model 500304, WPI Inc., FL, USA) that pumped the contrast solution into the phantom vessels at a constant flow rate (8 mL/min), at room temperature (25 °C).

## **2.2. Ultrasound Signal Acquisition**

The phantom was insonified with 0.08-MI US pulses produced by a linear transducer (LA532, Esaote Spa, Florence, Italy) with a nominal central frequency of 3.15 MHz and 3-dB bandwidth of 0.75 MHz. The transducer was positioned on the top of the phantom, so that the image plane resulted perpendicular to the vessels, and it was fixed using a shaped Plexiglas<sup>®</sup> connector as reference and coupling gel. The transducer was linked to a digital ecograph (Megas GPX, Esaote Spa, Florence, Italy) which was connected through an optical fibre to a prototype for radiofrequency (RF) signal acquisition and analysis (FEMMINA, developed by Florence University), able to get the full raw signal of the probe with no hardware nor software filtering of the ecograph itself [13].

RF signals were sampled at 40 MHz (16 bits) and the number of data points was 3200 for each echographic track, which is equivalent to 6 cm depth. This information was acquired for 180 tracks in each frame. A sequence of at least 180 frames was acquired for each tested UCA concentration, digitizing one frame per second and storing it in FEMMINA hard-disk. The whole described experiment was repeated three times.

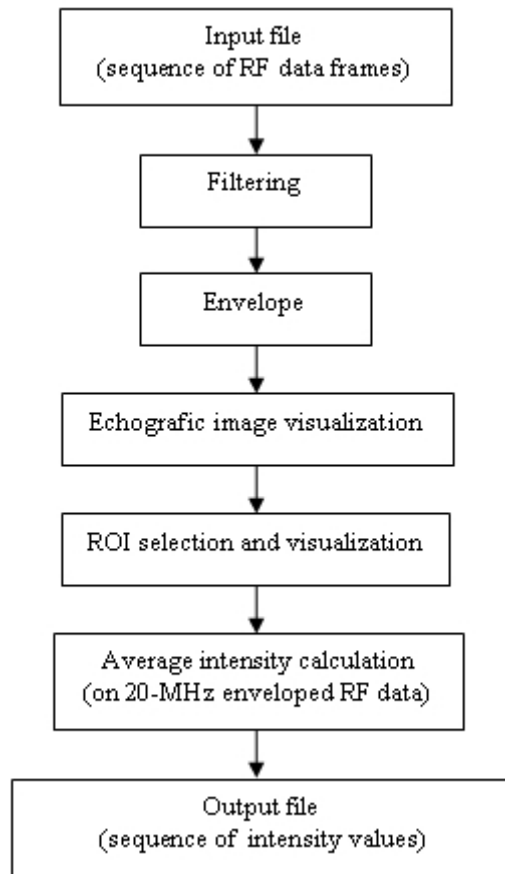
The prototype software (Fortezza, supplied by Florence University) was used to reconstruct the reference echographic images and to perform the off-line analyses described in the next paragraph.

## **2.3. Data Analysis**

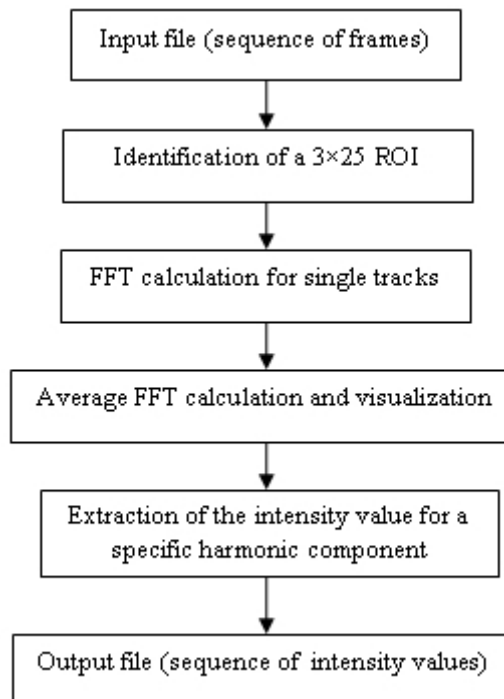
Quantitative off-line analyses were performed using custom-developed Fortezza algorithms whose flowcharts are illustrated in Fig. 1 and Fig. 2.

Referring to Fig. 1, selection of the region of interest (ROI), for each frame, was gained through visualization of the whole echographic image, reconstructed by high-pass filtering and low-pass enveloping the corresponding RF signals. In this way we were able to precisely localize the vessel cavities, avoiding reflections and artifacts due to vessel walls. In addition, the appearance and real time behaviour of echoes present during pure saline solution flow were also used as guidance for correct ROI selection. Each selected ROI covered exclusively the microbubble flow and was composed of 3 tracks and 25 data points per track, which is approximately equivalent to a square of less than 0.5 mm in side.

Once ROI coordinates had been chosen, the filtering step (Fig. 1) was removed together with the low-pass filter of the enveloping step, so the raw data were no more actually filtered, but only enveloped with the highest possible cut frequency (20 MHz), in order to obtain the absolute value of the signal.



**Fig. 1.** Flowchart of Fortezza algorithm used to select the ROI and to calculate the corresponding average intensity for each acquired frame.



**Fig. 2:** Flowchart of Fortezza algorithm used to calculate the average FFT of the ROI for each acquired frame and to extract the values of specific harmonic components.

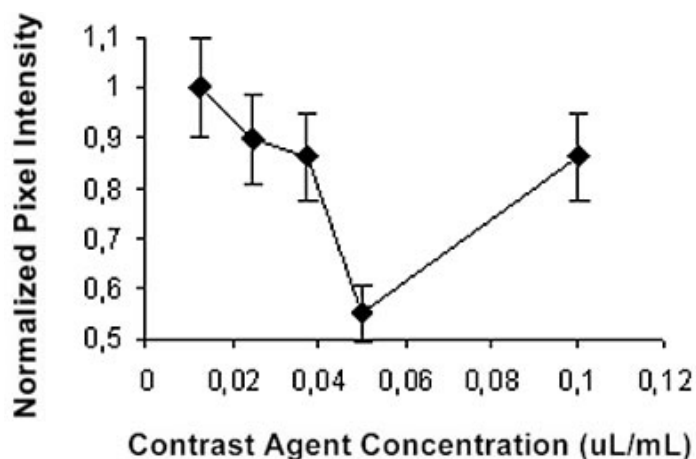
The mean backscatter intensity of the “absolute valued” raw data in the defined ROI was then calculated for each acquired frame and recorded in a Fortezza proprietary format file, then converted in XLS format by an “ad hoc” implemented MATLAB<sup>®</sup> program (The Mathworks, Inc., Natick, MA). Data were analyzed with a significance level of  $p < 0.05$ . For each sequence of significant data, the mean values and the corresponding standard deviations were calculated by Origin<sup>®</sup> software (OriginLab Corporation, Northampton, MA) and plotted against contrast agent concentration. Plotted values were normalized with respect to the highest obtained intensity, in order to emphasize the enhancement reductions produced by the employment of different concentration values.

For each considered ROI, we also calculated the average Fast Fourier Transform (FFT) curve and extracted from it the values of specific harmonic components through another custom-developed Fortezza algorithm, whose flowchart is reported in Fig. 2.

Before FFT calculation, the raw data corresponding to the identified ROI were selected by means of a 25-point “Rect” window and zero-padded to 4096 points in order to increase the frequency resolution of the corresponding spectra. FFT was calculated in this way for all the three tracks of the selected ROI and the resulting curves were averaged to obtain the FFT representative of the ROI for the specific considered frame. Subharmonic, fundamental and second harmonic backscatter values were extracted from mean FFT curves and averaged over the corresponding frame sequence. These values were plotted against contrast agent concentration.

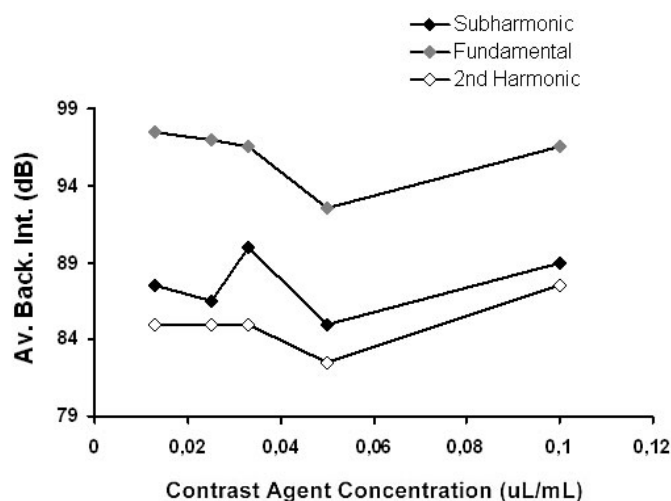
### 3. Results

Fig. 3 shows the relationship between normalized pixel intensity and contrast agent concentration in the considered ROI. The highest intensity was observed for the lowest tested microbubble concentration (0.013  $\mu\text{L/mL}$ ). Then image intensity decreases linearly ( $r = 0.995$ ) in the range 0.013-0.033  $\mu\text{L/mL}$  and reaches a minimum at 0.050  $\mu\text{L/mL}$ . Finally, signal intensity rises again when contrast concentration is increased to 0.100  $\mu\text{L/mL}$ .



**Fig. 3.** Plot of normalized ROI pixel intensity against contrast agent concentration.

This trend was at least qualitatively confirmed by subharmonic, fundamental and second harmonic FFT component curves reported in Fig. 4.



**Fig. 4.** Values of subharmonic, fundamental and second harmonic FFT components against contrast agent concentration.

The highest value of signal intensity was found at 3.15 MHz component (fundamental harmonic) for all the concentrations. We can also notice that the highest value of the first harmonic is at 0.013  $\mu\text{L}/\text{mL}$ , then it decreases till 0.033  $\mu\text{L}/\text{mL}$  and finally rises again at 0.10  $\mu\text{L}/\text{mL}$ . The subharmonic (1.575 MHz) and second harmonic (6.30 MHz) exhibit analogous behaviours, but showing their maximum values at 0.033  $\mu\text{L}/\text{mL}$  and 0.100  $\mu\text{L}/\text{mL}$ , respectively.

Therefore, the fundamental component trend confirms what observed in Fig. 3 and it seems to be the component that mainly influences the intensity values.

#### 4. Discussion

The studied phospholipidic contrast agent showed the highest echographic image intensity in correspondence of the lowest considered microbubble concentration. Then intensity value gradually decreased till reaching a minimum and finally raised again up to about the 85% of its initial value at a concentration that was almost 8 times higher than the initial one (Fig. 3). In particular, average signal intensity presented a strong linear correlation ( $r = 0.995$ ) with contrast agent concentration in the range of 0.013-0.033  $\mu\text{L}/\text{mL}$ , while for higher microbubble concentrations this linear relationship disappeared.

The described intensity trend was confirmed also by the values of main FFT components. Fig. 4 shows that the component that gives the greatest contribution to the signal intensity is the fundamental component.

Obtained results demonstrate that microbubble signal intensity is not linearly dependent on concentration and it is not possible to precisely predict the behaviour of a given microbubble concentration.

It is known [14-15] that US signal intensity of multiple scatterers within a volume increases with incident frequency to the fourth power and with scatterer size to the sixth power. Backscattering and attenuation are interrelated and both of them depend on UCA concentration, but this dependence is by no means linear: scattering increases with low concentration, while attenuation caused by multiple scattering dominates when the concentration is high [16-18]. This poses a limit concentration beyond which the UCA cannot be effectively used.

In our case, this is valid in the range 0.013-0.050  $\mu\text{L}/\text{mL}$  and it might be due to increasing destructive signal interference effects [19-21]. Finally, signal intensity rises again, probably because concentration becomes so high that microbubbles tend to flow in agglomerates rather than as single bubbles.

## 5. Conclusions

We have investigated a wide range of microbubble concentrations in order to evaluate the US backscatter intensity for a constant incident frequency and a very low mechanical index. The highest backscatter intensity has been found in correspondence of the lowest considered concentration. In fact, backscatter intensity decreases with arising UCA concentration until reaching a minimum and then it rises again. This indicates that there is an optimized concentration that allows the achievement of the best contrast enhancement: 0.013  $\mu\text{L}/\text{mL}$ . For this reason the injection dose might be reduced, at least when using the low frequency of 3.15 MHz and the very low MI=0.08.

Further investigations and measurements are needed in order to obtain the complete behaviour of microbubble at concentrations lower than those employed in this study, in order to cover all possible concentration ranges and to fully explore the yet unknown possibilities offered by last generation US contrast media.

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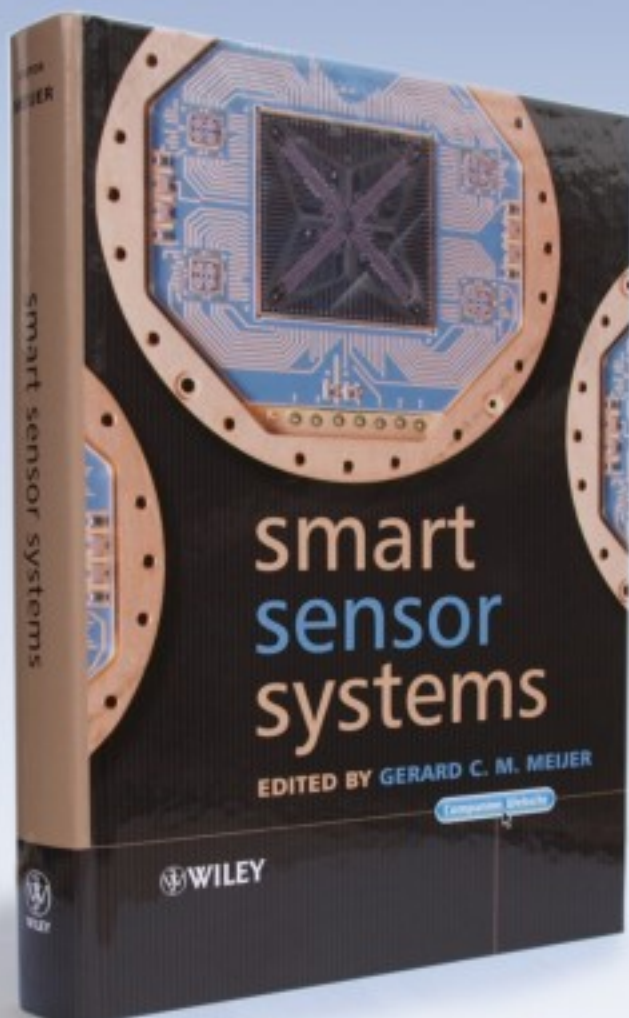
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