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● *Original Contribution*

REGULARIZED ESTIMATION OF CONTRAST AGENT ATTENUATION TO IMPROVE THE IMAGING OF MICROBUBBLES IN SMALL ANIMAL STUDIES

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Abstract—Quantitative analysis of tissue perfusion using contrast-enhanced ultrasound is still limited by shadowing, which is caused by inadequate compensation for microbubble contrast agent attenuation. Many previous methods have been developed for attenuation correction in soft tissues. However, no method has been proposed to correct for microbubble attenuation *in vivo*. In this article, a model to estimate microbubble attenuation is presented, using the time-intensity variation in a highly echogenic distal area without contrast uptake. This model is based on the assumption that a linear relationship holds between local microbubble attenuation and local backscatter. The model was applied to 12 murine renal perfusion studies. Parametric images of microbubble attenuation were generated, corresponding to dynamic contrast agent-specific sequences without shadowing. Contrast uptake kinetics consistent with the physiology were retrieved in all perfused areas. This method therefore proved to be of potential interest in the quantification of tissue perfusion in small animal studies. (E-mail: sebastien.mule@imed.jussieu.fr)   © 2008 World Federation for Ultrasound in Medicine & Biology.

Key Words: Contrast-enhanced ultrasound, Attenuation correction, *in vivo*, Microbubbles, Perfusion, Small animal study.

INTRODUCTION

Contrast-enhanced ultrasound imaging is a modality of growing interest in small animal studies. It enables the assessment of tissue perfusion in various organs such as the brain (Seidel et al. 2001), liver (Li et al. 2005) and kidneys (Lucidarme et al. 2006; Potdevin et al. 2006; Schlosser et al. 2001). Vascularization of experimental melanomas (Schroeder et al. 2001) can also be visualized. Nevertheless, the quantification of contrast uptake kinetics from ultrasound images is still affected by several artefacts because of the complex response of both anatomical structures and microbubble contrast agents to an ultrasound beam. One of these artefacts is shadowing (Bos et al. 1996; de Jong and Hoff 1993), which results from an inaccurate correction of both tissue and microbubble attenuation.

Clinical scanners display the magnitude of the backscattered signal at the transducer over time, which is then interpreted as depth, assuming a constant speed of sound. The magnitude of this signal is affected by the attenuation caused by soft tissues and microbubbles between the transducer and the depth reported and by the backscatter at this depth. Reduction in amplitude because of tissue attenuation is typically compensated for by applying a time-gain compensation (TGC). Nevertheless, variation in attenuation across the image at a given depth is not accounted for by TGC, which is based on the assumption that attenuation is a function only of depth.

Several methods for automatic compensation of tissue attenuation have been developed (Knipp et al. 1997; Pye et al. 1992; Valckx et al. 2000; Walach et al. 1989). However, the reliability of such methods depends on a relatively high level of homogeneity in the processed regions, which is not necessarily true in real data. Rather than assuming homogeneity in the images, Hughes and Duck (1997) developed a method based on the assump-

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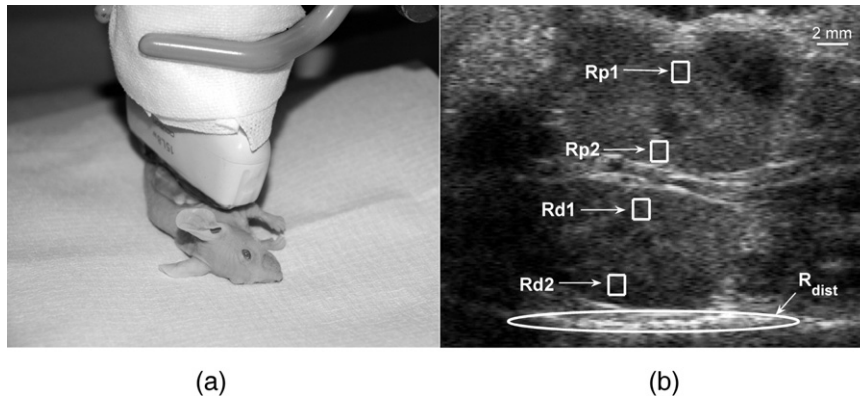
Fig. 1. Visualization of the R_{dist} area in the sequence acquired in fundamental mode and definitions of the four 10 × 10 pixel ROIs *Rp1*, *Rp3*, *Rd1* and *Rd2* for assessing the quality of the estimated attenuation coefficients.

tion that there is a specific relationship between local acoustic pressure, local attenuation and local backscatter. More recently, the approach of estimating attenuation from the radio-frequency spectrum has been investigated by Treece et al. (2005) using *in-vitro* data.

Although these methods provide a useful correction for soft tissue attenuation, microbubbles attenuate ultrasound in a very different way, and none of the methods described is adapted for the correction of microbubble attenuation. In contrast with local tissue attenuation, local microbubble attenuation varies with time. A dynamic approach to the correction of microbubble attenuation is thus needed. As microbubbles produce larger variations in signal amplitude than soft tissues, there is a crucial need to remove shadowing artefacts caused by microbubbles to allow the accurate quantification of microbubble concentration and tissue perfusion.

Microbubble attenuation has been investigated widely *in vitro* and many measurements have shown its concentration dependency (Herman et al. 2000), combined frequency and concentration dependency (Chatterjee et al. 2005; de Jong and Hoff 1993; Marsh et al. 1998; Zhang et al. 2000) and combined frequency and acoustic pressure dependency (Chen et al. 2002; Frinking and de Jong 1998; Sboros et al. 2002; Tang and Eckersley 2007; Tang et al. 2005). The studies above provide a better understanding of ultrasound attenuation caused by microbubbles. However, no method has been developed to correct for the attenuation caused by microbubble contrast agents *in vivo*.

In this article, we propose a numerical model to estimate microbubble attenuation *in vivo* from conventional B-mode images, using *in-vitro* results obtained in the previously cited studies (Tang and Eckersley 2007; Tang et al. 2005). Moreover, the time-intensity variation in a highly echogenic distal area without contrast uptake, which provides the cumulative microbubble attenuation

in the whole field-of-view, is introduced as a boundary condition to the propagation equations. Using reasonable linear assumptions, we derived an original mathematical solution to estimate dynamic attenuation sequences. The comparison of these sequences with native images shows a large recovery of the signal in the nonsuperficial regions.

MATERIALS AND METHODS

Data acquisition

Experiments were performed as part of a study on the quantitative assessment of Wilms' tumor microcirculation in a murine model. The experimental protocol was approved by the ethics committee of the CNRS (Centre National de la Recherche Scientifique). Twelve renal perfusion studies of female NMRI nude mice (Elevage Janvier, Le Genest-St. Isle, France) were used. Mice were anesthetized by intraperitoneal injection (5 mL/kg) of a solution of ketamine (100 mg/kg) and acepromazine (10 mg/kg) and placed on the right flank against a heating pad (Fig. 1a).

A 3-s-long bolus injection of 0.1 mL undiluted SonoVue (Bracco SpA, Milan, Italy) was manually performed *via* the periorbital route using a 26*g* syringe. The bolus arrival time was in the order of 1 s. As soon as the UCA was administered, contrast ultrasound sequences were acquired at 25 frames/s during 10 s at a low mechanical index of 0.09 using an Acuson Sequoia 512 device (Siemens Medical Solutions, Mountain View, CA). The linear-array transducer (15L8w, Siemens Medical Solutions, Mountain View, CA, USA), with a central frequency of 7 MHz, was mechanically fixed above the mouse, allowing it to breathe freely. The depth of the field-of-view was adjusted to include both kidneys and the metallic surface of the heater positioned behind the mouse (Fig. 1b).