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A review of Single Microbubble Acoustics

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ABSTRACT

Ultrasound microbubble (MB)-enhanced imaging is currently applied in the clinic for heart and liver diagnosis. The potential use of quantifying microvascular flow has been researched for over 20 years. The necessity for investigating the acoustics of single MBs stems from the lack of a single or a predictable distribution of their acoustic responses. In other words investigations of MB clouds are limited in providing information on the individual scatter components, thus making difficult the comparison of experimental and theoretical data, but also the assessment of the performance of signal processing algorithms. Single MB acoustics measurements have provided high quality data that may advance MB theory and signal processing research. With the help of accurate calibration of MB scatter it is possible to observe and study physical phenomena such as resonance, the onset of transient cavitation, MB cracking, the different contributions of the shell, gas and environment including narrow tubing, and the various decay mechanisms. It is possible to capture large sample sizes of signal distributions and enable thorough signal processing analysis without the prerequisite of a model for MB behaviour.

INTRODUCTION

Sub-capillary sized gas microbubbles (MBs), encapsulated in a thin shell, and have been introduced in recent years to improve the visualisation of the vascular bed under the modality of contrast enhanced ultrasound (CEUS) [1,2]. Normally in the form of an injectable they are stable and have similar rheological properties to red blood cells [3]. Image contrast enhancement is available for several seconds, and at most up to a few minutes. The goal of the modality is to assess blood flow at microvascular level. The most successful areas of CEUS to date include cardiology and liver radiology [4,5].

The potential of molecular imaging applications is explored with the development of site-targeted MBs that may attach to specific markers of disease [6]. Further, MB may also address localised drug and gene delivery [6], which promises to combine therapy with simultaneous pathology monitoring, recently termed “theranostics”. Considering the low cost, portability, good spatial and excellent temporal resolution of ultrasound imaging and the fact that it is one of the most widely available diagnostic imaging modalities, research into the above areas may provide a high pay-off.

The choice of ultrasound contrast materials in the form MBs is obvious as a gas bubble in a liquid interface would provide maximum Rayleigh scatter [7]. However, it is shown that MBs do not remain linear scatterers in the presence of ultrasound unlike most imaging modalities’ contrast media, including amongst others X-ray, magnetic resonance and radionuclide imaging technologies. It is this complex nonlinear interaction of MBs with ultrasound that stimulated a very active research field that is yet to realise its full potential.

EARLY EXPERIMENTAL PROCEDURES

The understanding that bubbles oscillate nonlinearly and resonate in the presence of an acoustic field historically precedes encapsulated MBs [8]. The nonlinear nature of MB scatter spectra was demonstrated early on with signals produced using narrow band transmitted pulses [9-11]. Experimental investigations in these early days employed protocols that were inherited by linear scatterer acoustics. The frequency response of MBs was originally measured using tone bursts [12-24], and was later criticised as attenuation spectra from broadband transmission are strongly dependent on the centre frequency of the transducers and differ significantly to narrow band transmission data [25].

Another common approach utilised MB populations [26,27]. The use of a linear scatterer suspension to calibrate the receiver was proposed as an alternative to the perfect reflector [28-29], which was commonly used.

A more important problem however is attached to acoustic signals that are generated from MB populations. For the MB Quantison™ it was shown that there are two subpopulations of scatter, a weak and strong one [30], and it was proposed that such backscatter measurements are of limited value if a single distribution of scatter is assumed [31], a premise that underlies studies that used MB populations. In general, a successful MB model is not available. Thus, fitting acoustic data to a theoretical distribution of responses is prohibitive.

Finally, another aspect of acoustic measurements with MB populations is the transmit field. The position of the scatter response can only be assessed axially. This means that MB responses cannot be discriminated across the width of the beam. Thus MBs are exposed to a range of acoustic pressures from the peak, which is in the centre, to zero. When the acoustic pressure is not very low, then nonlinear propagation makes this problem more complex and the spectral signature of the transmit beam across its width changes dramatically. Even if attenuation is negligible, the recorded signal is the accumulation of nonlinearly responding MBs to this field parameter range. It therefore becomes obvious that it is ideal to study MBs in isolation at well calibrated location for both the transmit and receive beams.

FEASIBILITY OF SINGLE MICROBUBBLE MEASUREMENTS

It is possible to detect single MB echoes, count them and measure their scatter [32-35]. This initial work confirmed that the number of scatter events from Definity® (Lantheus Medical Imaging, N. Billerica, MA, USA) MBs is almost identical to the estimated number of MBs in the region of interest [33]. This improved the physical understanding of the scatter mechanism of the rigid-shelled Quantison™. The number of counted scatterers correlated with acoustic pressure, inferring a leaking or cracking process that releases gas from the shell that otherwise seemed intact after ultrasonic exposure [21,33]. Another important finding was that the scatter from lipid shelled Definity® was similar to Quantison™ free gas bubbles above 1MPa, confirming MB destruction. At acoustic pressures lower than 0.7 MPa Definity® provided larger scatter than Quantison™ which was attributed to the soft lipid shell.

The above work led to the construction of an acoustic setup for the acquisition of single MB scatter [36], and subsequently to the need of a precise calibration approach. The transmit calibration is straight forward with the use of a hydrophone. The experimental procedure requires only a good alignment method with the peak amplitude axis of the beam in order to ensure that the calibrated peak pressure and spectral content is used for MB exposure. The receiver calibration is more challenging and is best calibrated using the scatter of a small metal sphere at the intended location of the MB [36]. The use of a theoretical derivation of the scatter from copper spheres enabled a precise calibration of RF data with 18% uncertainty. Apart from the fundamental frequency, the field may be calibrated at the 2nd harmonic generated by the scatter from the nonlinearly propagated field.

The experimental setup should address some other important aspects. As MBs are required to be in a narrow stream, the use of narrow tubing is required. However, the static pressures applied onto the MBs should be kept to a minimum. Thus a gravity-fed MB suspension is optimal. The confirmation of the measurement of single MB scatter events is also important in these measurements. Ensuring that MBs are sparsely flowing minimises the chance that they will be close to each other when insonated. This also ensures that MBs are exposed to the ultrasound beam for the first time at the calibrated field location, after travelling from a sonically shielded environment. Visual inspection under the microscope of MB suspensions to ensure that there is no affinity between MBs, that may lead to double MBs or clusters, is recommended.

MICROBUBBLE BEHAVIOUR

A calibrated acoustic system can provide a statistically adequate population of MB echoes that facilitates the understanding of their distribution. Within such a population a distribution of spectral and intensity behaviours may exist as well as further information on echo duration and decay [37]. The first data on single biSphere™ MBs (Point Biomedical, San Carlos, CA, USA) showed that short echo durations are more likely at lower frequencies. It has been shown optically that a number of MBs perform a small oscillation, which leads to cracking and subsequent release of gas [38]. This ejection of free gas mechanism correlated with increased MB echo disappearance in subsequent pulses [37]. The possibility of MB dissolution during an ultrasonic pulse was also proposed.

BiSphere™ is a rigid shelled agent that encapsulates the water-soluble nitrogen, while Definity® is a soft/lipid shelled MB encapsulating a non-soluble perfluorocarbon. Monitoring the recurrence of microbubble echoes from consecutive exposures to ultrasound pulses and analysing their spectral content, apart from an understanding the natural of ultrasound induced decay mechanisms also offers an assessment on the state of cavitation. Definity® provides increased survival rates compared to biSphere™ at 500 kPa peak negative pressures owing to its lower gas solubility [37]. The increase of acoustic pressure provided an increased decay of Definity® echoes. Ultra- and subharmonic signatures appear in echoes at 800 kPa and become dominant above 1200 kPa. Above these pressures the proportion of echoes that reappear increases, which is a confirmation of inertial cavitation.

The identification of resonance was an important finding. For the first time primary resonant lipid MB scatterers were identified as having maximum response at the fundamental frequency and, in agreement with theory, presented a bell-shaped envelope [39]. At 1.6 MHz and 550 kPa peak negative pressure 22% of the total number of detected MB echoes were identified as resonant, providing 70%, 15% and 51% of the total fundamental, second harmonic and third harmonic energy of scatter from the MB distribution respectively. The rest of the scatter was provided by MBs below resonant sizes. In these sizes the second harmonic is the dominant spectral component as a secondary resonance takes place [39].

In an attempt to simulate in vivo conditions single MB acoustic measurements may be performed in narrow tubing similar to arterioles and capillaries, or attached to an interface which may simulate molecular targeting conditions. Both these experimental systems stem from the previous with slight modifications. BiSphere™ MBs showed no significant differences in scatter response in the two environments, but the attached MBs showed decreased decay which is open to interpretation [40]. Increased damping and/or decreased cracking due to the presence of the wall may be possible. The same MB showed a slightly decreased scatter and an increased decay in a 200μm tube [41]. Tubes that are smaller (50μm) provide significantly increased 2nd harmonic MB signatures [42]. This is an important finding and it would be interesting to await future work in vivo.

SIGNAL PROCESSING

The pulsing regimes available in medical ultrasound equipment detect non-linear microbubble signals, make use of microbubble destruction [43,44] or reject tissue linear signals [45,46], and are mainly signal processing achievements. Tissue cancellation has been more successful than MB echo enhancement because of the better physical understanding of the ultrasound scattering properties of tissue [45,46]. The pulse sequence performance relies in principle on the nonlinear property of MBs and already has delivered improved contrast for ultrasound imaging. MB acoustic experiments showed that an amplitude modulated sequence (AM) may not generate a response from a large number of scatterers in the first transmitted pulse [47]. The amount of non-responding scatterers rises from 30% at 200 kPa peak negative pressure (of the full amplitude pulse) to 90% at 550 kPa. The lack of MB response has been shown for rigid MBs like biSphere™ and Quantison, but also for lipid-shelled ones like Definity® and SonoVue™ [47]. For lipid bubbles a number of MBs provide echoes that are below the noise of the receiver, and therefore are not recorded. Subsequently the echoes in response to the full pulse are above noise. Resonant MBs are more likely to be detected in the half pulse. Also increasing amplitude from the half pulse to the full pulse may enable a MB, of size slightly below resonance in the half pulse, to become resonant due to spectral broadening of resonance in the full pulse. Destruction and decay adds further complication. From a signal processing point of view resonating and non-resonating MBs provide different performances for the AM sequence. In the light of this the AM should be subject to further optimisation and in general such data demonstrate that the design of pulse sequences should be guided by single MB echo data. The absence of a successful model of MB behaviour in the complex environment of the in vivo microvascular space beckons for such an approach.

DISCUSSION - APPLICATIONS

Fast acquisition optical microscopy has offered a breadth of information previously not available. A range of behaviours and phenomena have been demonstrated, which provided significant advances to the field of contrast ultrasound imaging. Apart from the high cost, however, there are some limitations: Most of the MB data are collected in challenging experimental setups from an ultrasound viewpoint. It is difficult to calibrate the beam in the location of the MB and the majority of data are collected in the vicinity of a tube wall, although feasible otherwise with optical tweezers [48]. In addition the spatial and temporal resolution of optical microscopy are limiting factors in converting the optically observed MB oscillation into the scattered wave, which is important in the development of MB specific signal processing algorithms.

Several researchers have measured the scattering properties of single MBs. Other approaches to the one presented here used direct scatter measurement [49-52], scatter signal decorrelation [53], scatter in conjunction with radiation pressure [54], and monitoring with high frequency ultrasound [55]. The complexity of all such measurements requires a meticulous scrutiny upon testing the operation of these setups. However, it has been shown that a well calibrated single MB acoustic setup provides highly accurate measurements and can detect small differences in scatter that occur between populations of free flowing and MBs that are in a 200μm tube [41]. Unlike the free flowing single MB measurements the experiments that measure attached MB echoes are more time consuming as the confirmation of a single MB measurement is required to be done under the microscope. This, however, gives the added advantage of sizing, which is not available in single MB acoustic setups and may be an important addition.

Theoretical work on MB is not yet conclusive. Due to limitations in the available experimental tools modelling investigations resort to fitting experimental data to an assumed behaviour, particularly for the shell property. A number of theoretical models have been proposed to explain the behaviour of lipid MBs and they appear to converge in lower acoustic pressures if an appropriate set of parameters is fitted to the data [56]. At higher acoustic pressures a number of instabilities appear to disturb the spherical oscillation as well as the lifetime of MBs. Using single MB acoustic data it is possible to simulate the gas leaking mechanism of rigid-shelled biSphere™ that occurs in acoustic pressures of the destructive range [57]. A qualitative comparison with theory provided the identification of resonance [39], and showed that the fitting of parameters may be improved by a more systematic comparison between theory and experiment. Scattered amplitude or energy was used alongside the envelope of the fundamental component of the signal [39]. The degrees of freedom may increase by using the expansion to compression ratio, phase information, and a wide range of transmit pulse parameters such as frequency, acoustic pressure and duration. More experiments in a range of environmental conditions such as temperature, ambient pressure, viscosity and in the presence of a wall or a tube may add valuable data in such an effort. In addition, the decay may also be vital in the comparison between theory and experiment, as decaying MBs change in size.

It is important to note that current spectral analysis techniques are dominated by non-parametric Fourier based algorithms which do not assume any prior knowledge of the signal structure. Attempting to represent short scatter pulses of only a small number of samples by a large number of basis functions is a wasteful approach. White the Fourier transform can be used to provide an accurate measurement of dominant spectral maxima in the absence of noise, in the presence of a large amount of noise this basic approach it is limited and provides spurious bandwidth information [58]. The nature of the MB signals requires a more sophisticated statistical signal processing technique. Applying the principles of Bayesian inference into the spectral analysis improves spectral resolution compared to the conventional Fourier power spectrum or the periodogram [58]. In addition this detection is fully automatic incorporating the detection of the signal's temporal boundaries [59] or pulse duration, which has been confirmed for multiple MB signals [60]. Although this parametric model-based technique is computationally expensive and would be useful for offline analysis at present, it offers optimal spectral analysis that may prove useful in an adaptive signal processing framework for future ultrasound contrast imaging.

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