Figure 1 shows the experimental setup to take the shadowgraph images. A spherical transducer was placed in a water tank and driven at a frequency of 1.14 MHz. An expanded and collimated green laser passed through the ultrasound field. The shadowgraph images were taken with a CCD camera which focused on a holographic diffuser. The optical phase modulation due to the acoustic pressure was considered for the optical measurement. Furthermore, neglecting the effect of the optical diffraction and assuming the optical deflection angle due to the ultrasound pressure distribution is sufficiently small, the integration of the acoustic pressure along the optical propagation axis was calculated from shadowgraph images with and without ultrasound exposure. The pressure field was reconstructed from the projected acoustic fields by CT algorithm.

Results/Discussion

The optical reconstruction well reproduced the pressure field measured with a hydrophone when the pressure amplitude at the focal point was less than 1 MPa. Increasing the acoustic pressure over 2 MPa, the reconstruction underestimated the pressure. Since the distance between the ultrasound propagation region and the optical focal plane corresponding to the position of the diffuser was measured easily and accurately, the focused ultrasound pressure field at pressure amplitude less than 1 MPa was successfully reconstructed without calibration. It would make it possible to optically measure the pressure field at larger pressure amplitude if the effect of the optical diffraction was considered by iterative calculation of the optical intensity modulation at the position of the diffuser owing to the optical phase modulation.
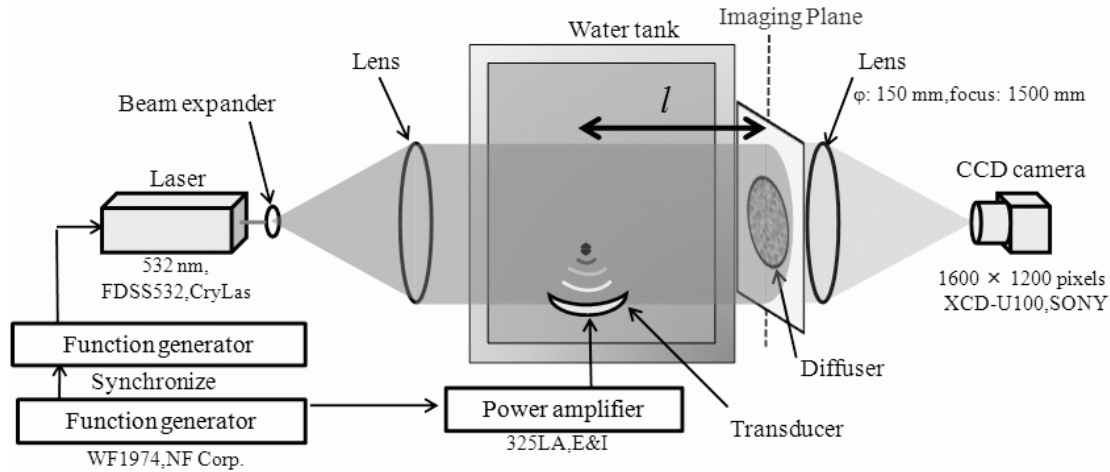

Figure 1 Experimental setup

Improved Finite Amplitude Insertion Substitution technique for acoustic nonlinearity parameter measurement

Bajram Zeqiri[1], Lise Retat[2], Pierre Gelat[1], Gail ter Haar[2]; *[1]Acoustics and Ionising Radiation Division, National Physical Laboratory, Teddington, Middlesex, United Kingdom, [2]Therapeutic Ultrasound Team, Institute of Cancer Research, Sutton, Surrey, United Kingdom*

Background, Motivation and Objective

The acoustic non-linearity parameter, commonly referred to as B/A, is a crucial parameter which dictates the way in which a finite amplitude acoustic wave distorts during propagation within any medium. At elevated acoustic pressure amplitudes, as distortion leads to the generation of higher harmonics of the fundamental frequency that are subject to greater absorption, B/A also plays a key role in terms of influencing thermal effects within biological media. Accurate knowledge of this parameter is therefore required to provide confidence in applied theoretical models that predict heat deposition for clinical procedures such as High Intensity Focused Ultrasound.

Statement of Contribution/Methods

The Finite Amplitude Insertion Substitution (FAIS) has been developed as a method which can be practically applied for B/A determination of tissues(1). The technique involves determining, for very slightly acoustically distorted waveforms, the degree of second harmonic generated during propagation through any test sample, relative to level generated through a reference medium (water) whose B/A value has been well characterized. Since the original paper, a number of variants of the technique have been implemented primarily to overcome the effects of acoustic diffraction. However, all of these methods ignore finite amplitude distortion of the acoustic wave which emerges from the test sample, and subsequently propagates to the device used to record the acoustic wave, either a second transducer or a hydrophone.This paper describes a refinement of the FAIS technique which includes modeling of the impact of finite amplitude distortion from the rear of the sample on B/A measurements.

Results/Discussion

Involving the application of a 2 MHz acoustic field, the study has demonstrated that, under certain conditions, ignoring this contribution can lead to an error in the measured B/A value in excess of 10%. The actual magnitude of the error depends on the acoustic properties of the material under test, particularly the frequency dependent attenuation coefficient. Another novel feature of the work presented is the use of a large area receiver in the form of a broadband membrane hydrophone of active element diameter 30 mm. This has enabled the effects of acoustic diffraction to be minimized, allowing measurements to be performed with the receiver positioned within the transducer near-field. B/A measurements are presented on two fluids commonly employed as reference materials within the technical literature: corn oil and ethylene glycol; as well as samples of an agar-based tissue-mimicking material. A systematic measurement uncertainty assessment will be presented alongside recommendations for optimized nonlinearity parameter measurement protocols.

(1) Xui-Feng Gong, Zhe-ming Zhu, Tao Shi and Jian-hong Huang, "Determination of the acoustic nonlinearity parameter in biological media using FAIS and ITD, J. Acoust. Soc. Am., 86, 1, 1-5, 1989.

Real-Time Imaging of Plasma Membrane Dynamics in Sonoporation: From Perforation to Recovery

Yaxin Hu[1], Jennifer M. F. Wan[2], Alfred C. H. Yu[1]; *[1]Medical Engineering Program, The University of Hong Kong, Pokfulam, Hong Kong, [2]School of Biological Sciences, The University of Hong Kong, Pokfulam, Hong Kong*

Background, Motivation and Objective

To properly harness sonoporation for therapeutic applications, it is unarguably vital to characterize the fundamental biophysical processes involved. Of particular relevance are two membrane-level processes that epitomize the notion of sonoporation: 1) how membrane perforation is induced by ultrasound-microbubble interactions, and 2) how the membrane remodels itself following an episode of sonoporation. Acquiring direct observations of these processes is however not a straightforward task (ironically, these membrane-level events have yet to be convincingly demonstrated in-situ). In this investigation, our aim is to acquire the first series of direct evidence on the time course of membrane perforation and recovery in sonoporation. In particular, we seek to unravel the time-varying surface topography of sonoporated cell membrane in-situ.

Statement of Contribution/Methods

A real-time imaging platform for monitoring of cell-microbubble interactions was first developed, and it was a composite system that coupled a 1 MHz ultrasound module to a laser scanning confocal microscope. A nose-cone shaped waveguide (1" diameter, 7.5 cm height) was devised to align the ultrasound beam focus to the microscope's imaging plane. This waveguide was angled at 45 deg. with respect to the imaging plane normal. A custom-made cell chamber was mounted onto the imaging plane, and it housed a 20x28 mm observation window with acoustically thin top and bottom layers (<0.16 mm thick). MRC5 fetal fibroblasts were seeded onto the bottom layer of the observation window, and their plasma membrane was fluorescently labeled using the CellMask Orange dye at 2.5 ug/ml concentration. Lipid-shelled microbubbles (Targeson) were then introduced on a 1:1 cell/bubble ratio, and they were allowed to passively settle onto the fibroblast monolayer. After that, a single ultrasound pulse (1 MHz frequency, 10 cycles, 0.75 MPa in situ peak negative pressure) was applied to instigate microbubble pulsation and collapse. Over this process, the surface topography of fibroblast membrane at positions with microbubbles was imaged in real-time using the confocal microscope. Pore size and recovery time were quantified from the acquired cineloops.

Results/Discussion

Localized perforation of cell membrane was synchronized with the time course of microbubble collapse. The pore size was highly time-dependent: it expanded for a limited time after microbubble collapse (up to 7 um diameter), after which resealing started to take place. The pore size was generally greater than the microbubble (mean diameter: 2.2 um). Membrane striation was also evident away from the perforation site: this signifies an increased membrane tension. During recovery, the perforation site exhibited a contractile ring morphology, and the endoplasmic reticulum was found to be actively participating in the resealing process. These findings demonstrate that membrane-level processes in sonoporation are highly dynamic.

IUS1-K3-5 _____

Relation between cell membrane tension and repair of membrane damaged during sonoporation

Yuto TANAKA[1], Nobuki KUDO[1]; [1]*Graduate School of Information Science and Technology, Hokkaido University, Sapporo, Hokkaido, Japan*

Background, Motivation and Objective

Repair of a cell membrane damaged during sonoporation depends on membrane tension, and a difference in membrane tension has a great effect on sonoporation efficiencies in *in vitro* and *in vivo* conditions. In this study, cell membrane tension was controlled by changing osmotic pressure of the culture medium, and the effect of cell membrane tension was investigated by fluorescence microscopic observations of cells during sonoporation.

Statement of Contribution/Methods

Human prostate cancer cells (PC-3) were cultured on a coverslip, and the coverslip with cells facing down was attached to an observation chamber created in the bottom of a water bath (Fig. 1a). The observation chamber was filled with an isotonic or non-isotonic solution supplemented with 5 µg/ml propidium iodide (PI), which permeates only through damaged cell membranes and produces red fluorescence. Hanks' balanced salt solution was used as the isotonic solution. A hypertonic solution was prepared by addition of 0.2 g NaCl to a 50-ml isotonic solution, and a hypotonic solution was prepared by mixing the same volumes of 1.3 mM $CaCl_2$ solution and the isotonic solution. The solutions were also supplemented with the ultrasound contrast agent Levovist. After sufficient time to allow bubbles to come into contact with cells (30 min), the cells were exposed to a single shot of 10-cycle ultrasound pulse of 1 MHz in center frequency and 1.3 MPa in peak-negative pressure. Occurrence of cell membrane damage was discerned by increase in PI fluorescence inside the cells, and success or failure in membrane repair was discerned by a transient or persistent increase in fluorescence intensity. The repair rate was calculated as the number of repaired cells divided by the number of damaged cells.

Results/Discussion

Fig. 1b shows the repair rates of cells treated under the three conditions of osmolality. The repair rate under a hypertonia condition was significantly higher than the rates under the other two conditions. These results indicate that a decrease in cell membrane tension promotes repair of membranes damaged during sonoporation. However, there was no significant difference between iso- and hypotonic conditions, suggesting that decreased Mg^{2+} concentration in a hypotonic solution has a positive effect on membrane repair (R.A. Steinhardt, Ann. N. Y. Acad. Sci. 1066(2005) 152-165).
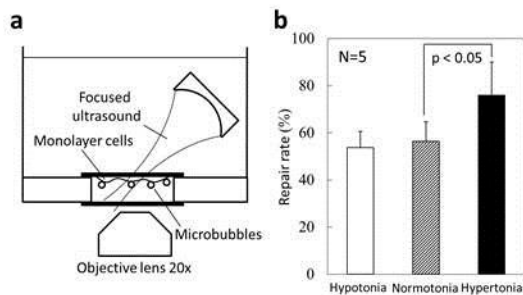

Fig. 1. (a) Experimental setup of the observation (b) Repair rates of cells treated under various conditions of osmolality. Error bar represents SD.

IUS1-K3-6 _____

Sonoporation-Induced Endoplasmic Reticulum Stress: Signaling Pathway Analysis

Wenjing Zhong[1], Xian Chen[1], Pingping Jiang[2], Jennifer M. F. Wan[2], Peng Qin[3], **Alfred C. H. Yu[1]**; [1]*Medical Engineering Program, The University of Hong Kong, Pokfulam, Hong Kong*, [2]*School of Biological Sciences, The University of Hong Kong, Pokfulam, Hong Kong*, [3]*Department of Instrumentation Science and Engineering, Shanghai Jiaotong University, Shanghai, China, People's Republic of*

Background, Motivation and Objective