1. Introduction

In fundamental studies on biological effects of ultrasound, a petri dish is frequently used as an exposure chamber, and cells cultured on the dish are irradiated by ultrasound generated by a transducer placed beneath the bottom of the dish. In this situation, acoustic fields generated inside the dish are disturbed for two major reasons: one is the presence of a dish wall inside which ultrasound propagates, and the other is reflection of ultrasound at the water surface (boundary between air and water inside the dish) that causes generation of a standing wave field inside the water. Furthermore, since the acoustic radiation force of ultrasound with intensities used for safety studies often disturbs the water surface, the ultrasound field inside the dish becomes more complex. The purpose of the present study was to visualize ultrasound fields inside small containers that mimic a petri dish [1] using focused shadowgraphy [2,3] and to investigate the effects of wall propagation and water surface reflection.

2. Materials and methods

Figure 1 shows the focused shadowgraphy system used for the present study. Short pulsed light (wavelength: 850 nm, pulse width: 5 ns, peak optical power: 1 W) emitted by a laser diode is collimated by a convex lens to illuminate an ultrasound field from a direction perpendicular to that of the ultrasound propagation. A CCD camera (BU-51LN, Bitran) placed just behind a container captures the light transmitted through the ultrasound field.

A laboratory-assembled disk-shaped transducer of 1 MHz in resonant frequency and 40 mm in diameter was driven by 1- or 50-cycle burst pulses to generate ultrasound pulses of 0.39 MPa in peak-to-peak pressure at a pulse repetition frequency (PRF) of 1 kHz. The camera shutter was kept open for 1 s, and light pulses were generated at the same PRF with a time delay that determines the position of an instantaneous ultrasound field captured using a stroboscopic imaging technique. Two images were taken in the presence and absence of ultrasound exposure, and sensitive detection of light deflected by the ultrasound field was realized by image subtraction.

Figure 2 shows three types of containers and arrangements of the transducer. Container 1 of 14 × 10 × 15 mm³ (W×D×H) in inner dimension was constructed using an acrylic plate of 2 mm in thickness. Container 2 and Container 3 were made using a plate of polyolefin resin form (Koyo soft board, Koyo Sangyo) of 10 mm in thickness. The resin form was cut into the shapes shown in Figs. 2(b) and 2(c), and the shape shown in Fig. 2(c) was determined to mimic disturbed water surface. The incident and exiting surfaces of the illuminating light were covered by 2-mm-thick acrylic plates. Containers 1 and 2 were used for visualization of ultrasound fields with and without wall propagation, and Containers 2 and 3 were used for visualization of the fields without and with disturbance of the water surface.
3. Results and discussion

Figure 3 shows shadowgrams taken using Containers 1 and 2. Both images were taken with a delay of 10 µs after insonation, at which time the generated ultrasound has not reached the water surface. The transducer was driven by the 1-cycle pulse, and higher brightness regions have higher pressure amplitudes.

Figure 3(a) shows a shadowgram taken in the presence of ultrasound propagation inside the acrylic wall (Container 1). Two types of a plane wave propagating in different directions, one is a vertical direction and the other in an oblique direction, were confirmed. A leaky wave of ultrasound propagating inside the wall produces the oblique wave, and the oblique angle is determined by the ratio of sound speeds inside the wall and water (2.7 × 10³ m/s and 1.5 × 10³ m/s), which can be calculated to be ≈ 60 deg.

Figure 3(b) shows a shadowgram taken in the absence of ultrasound propagating inside the wall (Container 2). Also in this case, two types of waves were confirmed; however, not the oblique plane waves but spherical waves with their centers at the corner of the container bottom were visualized. These waves were generated by the edge effect of an ultrasound irradiating surface (bottom of the container) and reflected by water-acrylic or acrylic-air interfaces.

In a case that the transducer was driven by a longer duration pulse, the ultrasound fields generated inside the container were greatly disturbed after ultrasound sound reflection several times between the bottom and surface, resulting in generation of a complicated honeycomb-shaped ultrasound field (images not shown).

Figure 4 shows shadowgrams of standing wave fields generated inside the containers: (a) without surface disturbance (Container 2) and (b) with surface disturbance (Container 3). The transducer was driven by 50-cycle burst pulses, and the images were captured 25 µs after insonation, indicating that the wavefront of the ultrasound pulse experienced 1 lap between the transducer and water surface. Figures 4(c) and 4(d) show maximum intensity projection (MIP) images created using ten shadowgrams captured with time delays ranging from 25 to 26 µs by 0.1-µs steps, showing anti-node and node lines (bright and dark lines) of the standing wave fields. In the case without the disturbance, the dark line appears at the water surface, and in the case with the disturbance, high-pressure bright spots appear at the peaks of the water surface. These results suggest that the ultrasound field in the petri dish is significantly different from the standing wave field produced by simple plane waves.

4. Conclusion

To achieve correct dosimetry of ultrasound exposure on cells cultured using a petri dish, ultrasound fields inside a small container that mimics a petri dish were visualized using the focused shadowgraphy technique. The results revealed that wall propagation and water surface reflection of ultrasound make a complex field, which is very different from a standing wave field predicted by a simple model of water surface reflection. It was also shown that the disturbance of the water surface produces high-pressure spots at the peaks of the water surface, suggesting a possible mechanism of ultrasonic atomization.

References