

## Sonochemical and high-speed photographic investigation on efficiency of reactive oxygen generation by high-intensity focused ultrasound for sonodynamic therapy

音響化学反応と高速度撮影を用いた音響力学治療用集束超音波の活性酸素生成効率の検討

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### 1. Introduction

Sonodynamic therapy (SDT) is a non-invasive cancer treatment using high-intensity focused ultrasound (HIFU) and reactive oxygen species (ROS) inducing cytotoxicity. ROS can be generated from the collapse of cavitation bubbles produced by HIFU, at which the gas inside the bubble is compressed nearly adiabatically producing high temperature and pressure.<sup>1)</sup> At this high temperature, water molecules can be decomposed to OH radicals. A focal spot for focused ultrasound treatment is generally chosen smaller than the subject to be treated for geometric selectivity, resulting in a disadvantage of long treatment time. An ultrasound exposure method improving the efficiency to generate ROS must be studied. In previous experiments, it was found that the ROS generation efficiency changed depending on scanning of HIFU focus.<sup>2)</sup> In this study, two dimensional focal scanning on the efficiency was investigated.

The “Trigger HIFU” sequence, consisting of a highly intense short pulse called “trigger pulse” followed by a moderate intense long burst called “sustaining burst”, in which cavitation bubbles are generated by the pulse and utilized by the burst, has been proven to be effective for cavitation-enhanced HIFU heating. The trigger pulse can create a cavitation cloud upstream of the HIFU focus typically through a shock scattering mechanism. The sustaining burst can oscillate the generated bubbles and also induce the repeated collapse and regeneration. Therefore, the sequence should be effective also for SDT.

### 2. Materials and Methods

Experiments were performed in a water tank containing degassed water at room temperature, in which a HIFU transducer and luminol-soaked polyacrylamide gel were submerged, as shown in Fig. 1, and used for a target to visualize the area where ROS was generated. The gel was degassed in 0.7

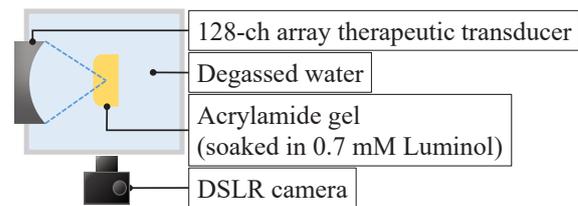


Fig. 1. Schematic of experimental setup.

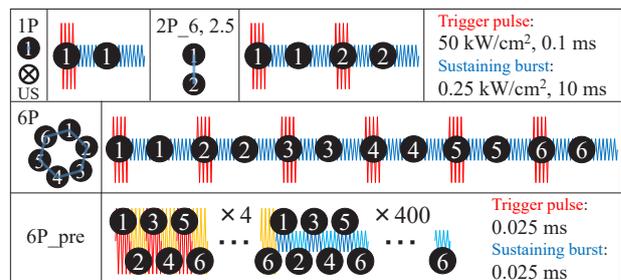


Fig. 2. Exposure patterns.

mM luminol for 6 hours. When the luminol molecule reacts with OH<sup>-</sup>, it is excited and emits blue light as an energy to return to the ground state.<sup>3)</sup> Although it may not be exactly the same ROS mediating SDT, luminol emission in a gel was chosen because of its spatial sensibility. The 128-channel 2D-array therapeutic transducer (Japan probe) with a focal length of 120 mm and a diameter of 147.8 mm, which was driven by a staircase driving system (Asahi) at 1 MHz. In this study, the five exposure sequences were tested as shown in Fig. 2. The 1P sequence was the standard trigger HIFU sequence consisting of a trigger pulse of 0.1 ms immediately followed by a sustaining burst of 10 ms. In the 2P\_6 and 2P\_2.5 sequences, the focal points of both the trigger pulse and sustaining burst were set sequentially at two laterally separated points. The lateral distance between the two points was 6 or 2.5 mm respectively, and the upper focal point was exposed first. In the 6P\_pre<sup>4)</sup> and 6P sequences, ultrasound was irradiated to each corner of a regular hexagon 2.5 mm each side. The 6P\_pre was the sequence developed for HIFU heating treatment. In the 6P\_pre sequence, first, the trigger pulse was sequentially irradiated to 6 points with the duration of 0.025 ms per each point, and this cycle was

repeated 4 times. After that, the sustaining burst was irradiated as same as the trigger pulse, but the cycle was repeated 400 times. The resulting total irradiation time of the trigger pulse and sustaining burst was 0.1 and 10 ms per each point, respectively. In the 6P sequence, the 1P sequence was irradiated at each corner. All sequences were continued for 30 s at a pulse repetition frequency of 3 Hz. The trigger pulse and sustaining burst were at an intensity of 50 and 0.25 kW/cm<sup>2</sup>, respectively. A digital single lens reflex camera (Nikon) was used to observe the sonochemiluminescence induced by each sequence during ultrasonic irradiation for 30 s in a darkroom.

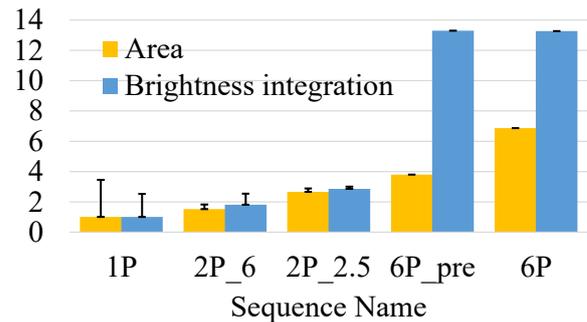
### 3. Results and Discussion

**Fig. 3** shows the light emission area and integrated brightness of each sequence with the 1P sequence as a reference. The 2P and 6P sequences had 2 and 6 times energy than the 1P sequence respectively. **Fig. 4** shows the raw pictures of the 1P and 6P sequence. **Fig. 5** shows the phenomenon that the remaining bubbles around the first focal point also were oscillated by ultrasound focused to the second focal point with the inter-focal distance of 2.5 mm. In contrast, with the distance of 6.0 mm, the bubble oscillation was not observed. This is considered to be the reason why ROS was generated by the 2P\_2.5 sequence more than the 2P\_6 sequence and even more than twice as much as the 1P sequence. In the 6P\_pre and 6P sequences, the area of ROS generation was about 3.8 or 6.8 times larger than that of the 1P sequence, respectively. Since overlapping perpendicular to the pictures is not considered in **Fig. 3**, the ratio in the three-dimensional ROS generation volume could have been larger than the ratio in **Fig. 3**.

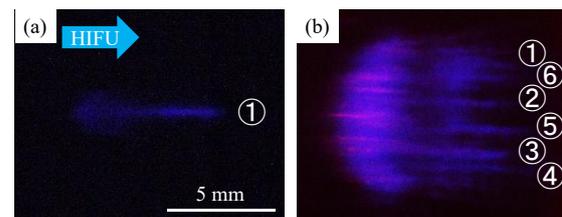
Assuming that the amount of ROS generation is proportional to the brightness, it could be said that the ROS generation efficiency was greatly improved by the 6P\_pre and 6P sequences. The 6P sequence showed higher efficiency than the 6P\_pre sequence in this study, but they should be three-dimensionally compared in the future.

### 4. Conclusion

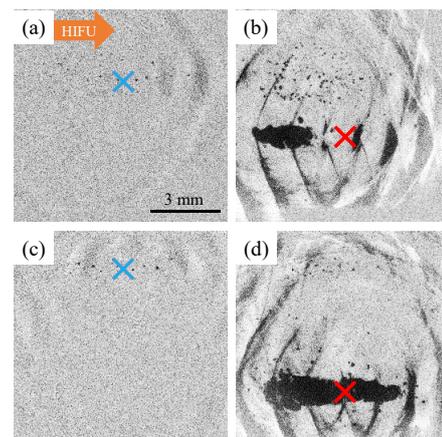
For SDT in which the improvement in treatment efficiency is a problem, two-dimensional scanning of the focal point with proper separation is suggested. The ROS generation efficiency could be thereby improved without sacrificing the ultrasonic energy efficiency.



**Fig. 3.** Comparison of ROS generated area and brightness integration.



**Fig. 4.** Raw pictures of (a) 1P and (b) 6P sequence captured by DSLR camera. ①–⑥ represent the order of the focus to be exposed.



**Fig. 5.** Pictures captured by high-speed camera when (a), (c) the sustaining burst is irradiated to the 1st focal point, (b), (d) the trigger pulse is irradiated to the 2nd focal point. The inter-focal distance are (a), (b) 2.5 mm, (c), (d) 6.0 mm.

### References

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