An *In Vivo* System for the Determination of the Effect of Temperature on Backscattered Ultrasound Energy in Ultrasonic Images

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Abstract

In the past our group has shown that the ultrasound power backscattered from subvolumes of *in vitro* tissue preparations is dependent on the temperature of the tissue. The backscattered power tends to monotonically increase or decrease depending on the dominant type of scatterer within the subvolume. We have extended this research to an *in vivo* system to test this hypothesis in living perfused tissue. The animal system consisted of nude mice with implanted tumors (HT29 colon cancer line) on their hind quarters. The mice were anesthetized with Ketamine Xylazine prior to being secured to a platform. The platform and lower section of the mouse were submerged in a temperature controlled water bath filled with degassed water. The temperature of the mouse was measured with an implanted thermistor in a contralateral limb which had a similar implanted tumor and was similarly submerged in the water bath. The transducer from a commercial laptop-based ultrasound system (Terason 2000, Teratech Corporation) was coupled to the target tumor through the water bath and images of the heated tumor were acquired at 0.5 ºC increments from 37.0 to 45.0 ºC. These data are being analyzed in a manner similar to that used for data acquired from *in vitro* samples. From these experiments we expect to learn the effects of perfusion and other physiological effects on our measure of the temperature dependence of backscattered ultrasound. Thus far we have completed the experimental design and have run experiments on a sacrificed mouse and on a living mouse. We will report on the suitability of the experimental setup as well as analysis of the backscattered ultrasound.

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Goals

ï Find an experimental set up that will allow the measurement of the change in backscattered ultrasound energy in an *In Vivo* model.

ïDevelop and evaluate algorithms for tracking tissue regions and determining the change in backscattered energy for an *In Vivo* system.

Experimental Requirements

ïStability ñ No motion other than the natural physiologic motion of the animal preparation.

ïEasy set up to minimize time animal anesthetized.

ïConsistent experimental set up with *In Vitro* specimen experiments for translation of earlier experiments and analysis to the *In Vivo system.*

ïUniform temperature distribution necessary for analysis at this time.

Experimental Setup Figures

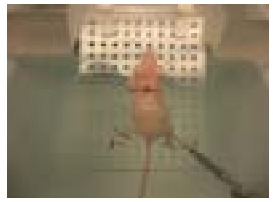


Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

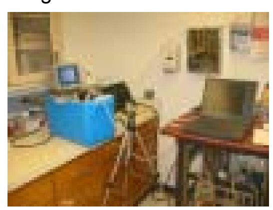


Fig. 6

Fig. 7

Current Experimental Setup

ï Nude Mice (bilaterally implanted HT29 colon cancer tumors) attached to angled tray to allow for partial submersion. The entire tumor on both thighs is completely immersed in water. **(Fig. 1)**

ï Degassed water is heated with a circulating heater (Thermo HaakeÆ) in a thermally insulated tank. **(Figs 2,5,6)**

ï 7 MHz 128 element linear array ultrasound transducer is positioned to image one of the tumors. The transducer is held in place with a tripod. **(Figs. 3,4,5)**

ï Contralateral tumor temperature is measured using an interstitial (RTD thermistor) temperature probe. The temperature of the water bath is controlled based on this temperature reading. **(Figs. 1,2)**

ï The water bath is used to heat the tumor from 37.0 to 45.0 ºC in 0.5 ºC increments.

ï A MatlabÆ routine controls the circulating heater via RS232 communication and starts an AutoItÆ routine that saves the ultrasound image produced by the TerasonÆ software. All of these routines run on a single laptop. **(Fig. 6)**

ï The entire experiment lasts approximately 0.5 hours.

ï The mouse is euthanized without recovery immediately after the experiment.

ï The saved ultrasound images and RF data are processed so that they can be analyzed using MatlabÆroutines. **(Fig. 7)**

ï The data from these live animal models will be analyzed in a manner similar to that for our previous *In Vitro* experiments.

Discussion and Conclusions:

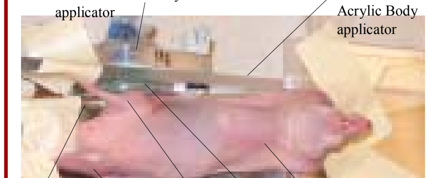
This experimental setup has proven to be stable and reproducible enough to generate stable (in terms of motion) data that can be analyzed in the same manner as our previous *In Vitro* studies. To date we have run experiments on three mice. One of them was a skid mouse and we found that hair was a confounding factor. We have decided to continue with the nude mouse model for this reason and the fact that shaving the mouse uses valuable time while the mouse is anesthetized. From these experiments we expect to learn the effects of perfusion and other physiological effects on our measure of the temperature dependence of backscattered ultrasound.

Future Directions:

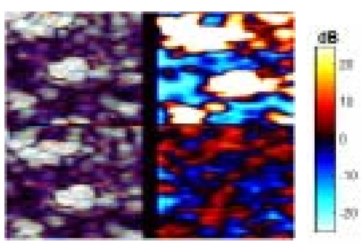
ï The stability of this set up using the tripod needs to be improved.

ï We plan to analyze the ultrasound imaging data in the near future and confirm the same temperature effect on the backscattered energy that we see in the *In Vitro* case.

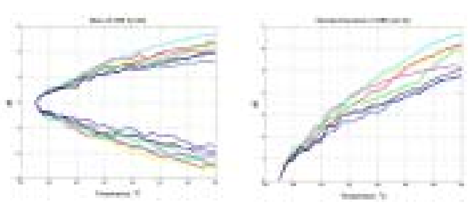
ï We have available systems designed to heat mouse tumors (Small Animal Heating Ultrasound System - SAHUS) using ultrasound. We will take advantage of these systems in the future. These systems will also challenge our model and analysis for *In Vivo* systems with inhomogeneous heating.



Previous ***In Vitro*** **Results**



RF images (left) of the segmented region uncompensated (top) and compensated (bottom) for motion. The top right is the corresponding backscattered energy color map at 50 ºC, and the bottom right shows relative backscattered energy at 50 ºC normalized to the reference image at 37 ºC. The color map was chosen to show positive and negative excursions in the relative backscattered energy.



The mean (left plot) of the positive and negative excursion regions for the relative change in backscatter energy for the regions of interest shown in the ultrasound image (fig 6) and the standard deviation (right plot) of the change in backscatter energy for these regions as the temperature rises from 37 to 50 ºC.