The effect of centre frequency on the resolvability of sub-diffraction limit *in-vitro* features resolved by microbubble localization imaging.

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Background, Motivation and Objective 542

Ultrasound localization microscopy has demonstrated its capability to map microvasculature at sub-diffraction limit resolution. However, the relationship between center frequency and resolvability of sub-diffraction features has not been experimentally investigated; for instance, when a low frequency pulse is transmitted to achieve greater penetration depth, microvessel resolvability at those frequencies is unknown. In this work, we aim to fill this knowledge gap by characterizing the resolvability of ultrasound localization microscopy under different imaging frequencies through a series of *in vitro* experiments.

Statement of Contribution/Methods 871

Two polyimide tubes (ID: 124.5 µm, Cole-Parmer) were affixed to a 3D printed tubing mount with a separation of 280 µm apart. 25 gauge needle tips were secured to the inlets for both tubes to perfuse microbubbles (MB). The setup was then submerged in degassed water. In-house MB were diluted and fed into the setup at 0.17 uL/s using a syringe pump. Imaging was done using a SonixTouch scanner for transmit frequencies of 5, 10, 14 MHz using a L14-5 array, and 15 & 20 MHz with a L26-12 of unsteered plane waves (3-cycle pulse; 1 kHz PRF; 10 sec acquisition). Images were beamformed with a 10 µm pixel resolution. Moving MB were identified by first applying a highpass filter along slow-time and followed by a cross-correlation algorithm. Localization of MB was done by deconvolving the image with the Gaussian template acquired for each frequency from a 25 µm tungsten wire. The microvessel was finally mapped by accumulating the localized MB. The resolvability was evaluated as the microvessel size measured as full-width at half maximum (FWHM).

Results/Discussion 515

Fig. A-D show the mapped microvessels for centre frequencies of 5,10, 15, 20 MHz. It can be seen that microvessels were clearly highlighted by the localization method and the measured diameters were close to the tube size (124.5 µm). Fig. E and F plots the measured microvessel diameter for different center frequencies; the measured diameter differed slightly from the expected value dependent on the centre frequency. Our results show that the resolution of ultrasound localization microscopy is likely dependent of center frequencies that were investigated in this study.