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Contrast-Enhanced Photoacoustic Imaging of Low-boiling-point Phase-Change Nanodroplets

Ge Zhang[1], Bingxue Wang[1], Anant Shah[2], Jeffrey Bamber[2], and Meng-Xing Tang[1]
[1]Department of Bioengineering, Imperial College London, London, United Kingdom
[2]Joint Department of Physics and CRUK Cancer Imaging Centre, The Institute of Cancer Research and The Royal Marsden NHS Foundation Trust, London, United Kingdom
Email: Mengxing.Tang@Imperial.ac.uk

***Abstract*— Contrast-enhanced photoacoustic imaging has shown a great potential in various medical imaging applications. Although different types of customized contrast agents were developed over the decade, there is no commercial FDA-approved photoacoustic imaging contrast agents. This study shows the in vivo photoacoustic imaging of low-boiling-point phase-change nanodroplets, made using ingredients from an existing commercial microbubble ultrasound contrast agents without any dye coated, in a mouse to demonstrate that commercial ultrasound contrast agents may have the potential to be used to facilitate the clinical translation of photoacoustic imaging. Decafluorobutane-core lipid-shell nanodroplets were manufactured. The homemade decafluorobutane droplet solution has the same core composition as the commercial MicroMarker© (FUJIFILM, Visualsonics) and Sonazoid© (GE Healthcare) contrast microbubbles. The results show that, after activation, signals from the spleen region have been significantly enhanced. As the droplets do not have any dye coating on the surface, one potential reason for the photo-activation of the droplets could be that the blood cells nearby absorb energy from the light. As the ingredients of the droplets are the same as some existing commercial microbubble contrast agents, this study demonstrates that droplets made from condensed commercial bubbles may have the potential to be used to facilitate the clinical translation of contrast-enhanced photoacoustic imaging.**

Keywords*— *Photoacousitc Imaging, Decafluorobutane Nanodroplets, High Frame Rate, Flow Independent, Microbubbles

I. Introduction

Photoacoustic imaging is an emerging technique which can provide high-resolution, multi-contrast images of biological structures [1]. Absorption of light by endogenous biomolecules or exogenous photoacoustic contrast agents induces thermoelastic expansion and acoustic waves, the detection of which is used to form an image [2].

Exogenous photoacoustic contrast agents have shown great potential in medical contrast-enhanced photoacoustic imaging applications. Different forms of photoacoustic contrast agents have been studied for different imaging applications. Optically activatable indocyanine green (ICG) and Cyanine 7.5 coated nanodroplets were used to generate both ultrasound and photoacoustic contrast enhancements [3, 4]. Gold nanoparticles were widely investigated in photoacoustic molecular imaging applications [5, 6]. Polyethylene microspheres [7, 8] were investigated as a photoacoustic super-resolution imaging contrast agents.

Over the last decade, photoacoustic super-resolution imaging has begun to be investigated. A non-contrast optical wavefront shaping approach was first studied to allow resolution to be optically determined rather than acoustically determined [9]. Inspired by super-resolution optical fluctuation imaging (SOFI) [10], non-contrast statistical approaches apply high order statistics of deliberately varied illumination speckle to achieve photoacoustic super-resolution [11, 12]. Recently, contrast particle-localization based super-resolution photoacoustic imaging was first demonstrated *in vitro* using flowing optically absorbing polyethylene microspheres [7, 8].

Although different types of customized contrast agents were developed over the decade, there is no commercial FDA-approved photoacoustic imaging contrast agents. This study shows the *in vivo* photoacoustic imaging of low-boiling-point phase change nanodroplets, made using ingredients from an existing commercial microbubble ultrasound contrast agents without any dye coated, in a mouse to demonstrate that commercial ultrasound contrast agents may have the potential to be used to facilitate the clinical translation of photoacoustic imaging.

II. Methods and Experiment

A. Nanodroplet Preparation

The preparation of the nanodroplet solution has been adapted from previously described methods [13, 14]. The lipid shell was generated by dissolving 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] (DSPE-PEG-2000) into a solution of phosphate-buffered saline (PBS), propylene glycol, and glycerol. All the lipids described above were purchased from Avanti Polar Lipids, Inc., USA. The lipid solution was added to a glass vial and the headspace of vial was exchanged with octafluoropropane gas (Fluoromed, USA) via an inlet needle along with a vent needle. Mechanical agitation was sufficient to produce the formation of lipid-shell

octafluoropropane-core microbubbles. In order to condense microbubbles into nanodroplets, the headspace of the vial was pressurised while the vial immersed in the ice-water bath according to the previously described method [15].

B. Experimental Setup

A female CrTac:NCr-Fox1nu athymic nude mouse was used for the in vivo imaging. After injecting 200 µL of a nanodroplet solution into the tail vein, laser activation of nanodroplets and whole-body cross-sectional photoacoustic imaging was performed at the abdominal level. 2000 images were acquired at 10 Hz over a period of 200 s.

C. Image Acquisition

The images were acquired using a multispectral optoacoustic tomography (MSOT) system (inVision 256TF, iThera Medical GmbH, Munich). Photoacoustic imaging was performed at a single optical wavelength of 788 nm (peak absorption of Cyanine 7.5) with a fluence per light-pulse estimated at the surface of the mouse body to be 20 mJ/cm2, based on manufacture-nominal total laser energy per pulse and the approximate surface area of a mouse illuminated. Each pulse (pulse duration 10 ns, pulse repetition frequency 10 Hz) generated a complete image.

The raw radiofrequency (RF) data were beamformed by a customized back-projection algorithm to obtain the image data. Briefly, a temporal delay for each channel was applied according to their different positions along the 270° concave transducer array. Then the information from each channel was summed over all the 256 channels in order to form the final image. The geometrical parameters were applied according to the MSOT transducer design described by [15].

D. Image Analysis

Singular value decomposition (SVD) processing was used to obtain the changing contrast signals. The SVD thresholds were automatically determined from the location of the largest gradient on the energy versus singular value order curve. The application of SVD for contrast-enhanced ultrasound was detailed in [16]. Briefly, the spatial and temporal information was combined by sorting the data into a Casorati matrix. Each frame was vectorized and added as a column. SVD factorizes this Casorati matrix to:

$$Signal = [U][S][V]^*$$

where columns of U represent spatial singular vectors and corresponding columns of V represent the associated temporal vectors. S is a diagonal matrix, where the elements are the singular values. A low singular value threshold is set so that any signals below this threshold are discarded as unwanted tissue signals.

III. Reasults and Discussions

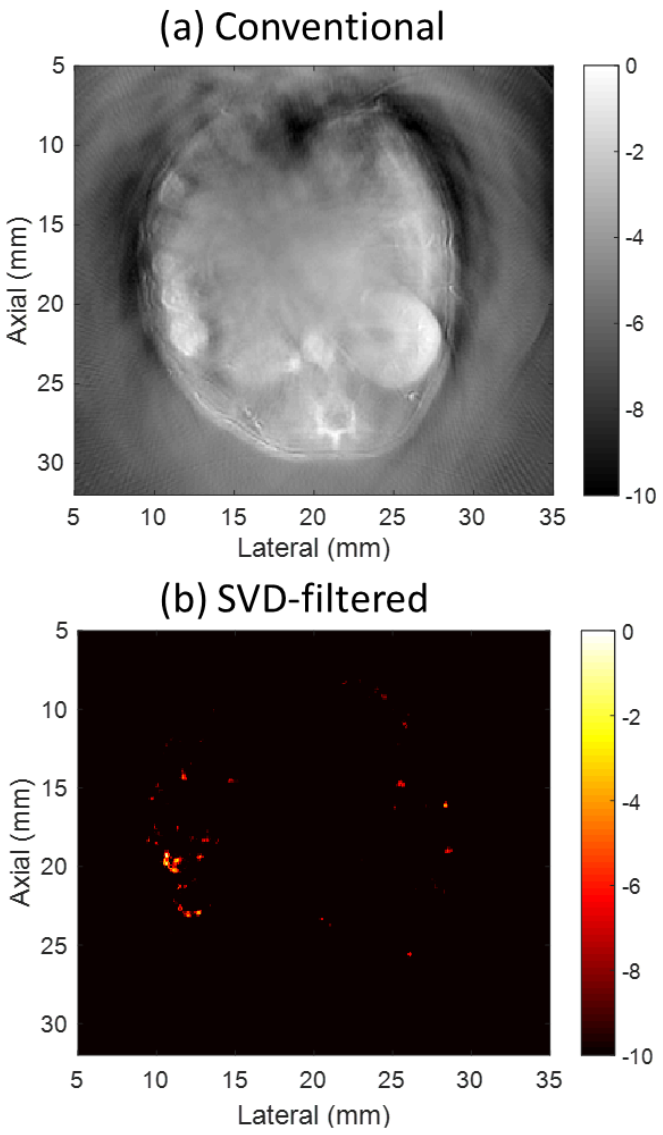

Fig. 1. Summation of (a) conventional and (b) SVD-filtered images of mouse cross-section before the injection of nanodroplet contrast agents.

Fig 1 shows the summation of conventional and SVD-filtered images of mouse cross-section before the injection of nanodroplet contrast agents respectively. As can be seen from Fig 1(b) that, the contrast signal can hardly be seen. Some small visible signals on Fig 1(b) may be due to the motion of heart and breathing.

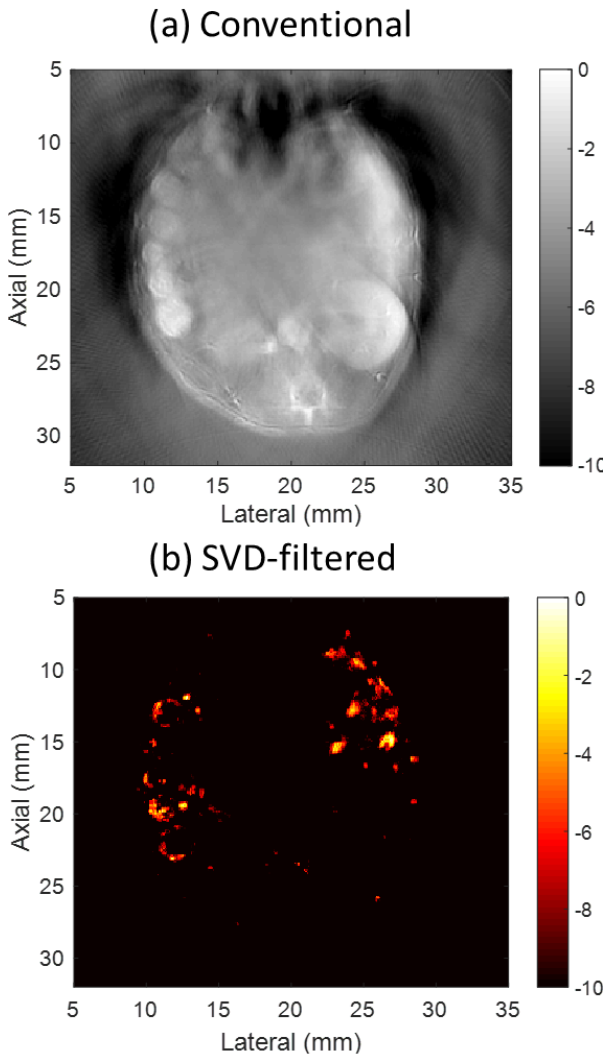

Fig. 2. Summation of (a) conventional and (b) SVD-filtered images of mouse cross-section after the injection of nanodroplet contrast agents.

Fig 2 shows the summation of conventional and SVD-filtered images of mouse cross-section after the injection of nanodroplet contrast agents respectively. As can be seen from Fig 2(b) that, more contrast signals can be visualized, especially in the spleen (top right) and intestines (top left) regions compared to the SVD-filtered image before the injection.

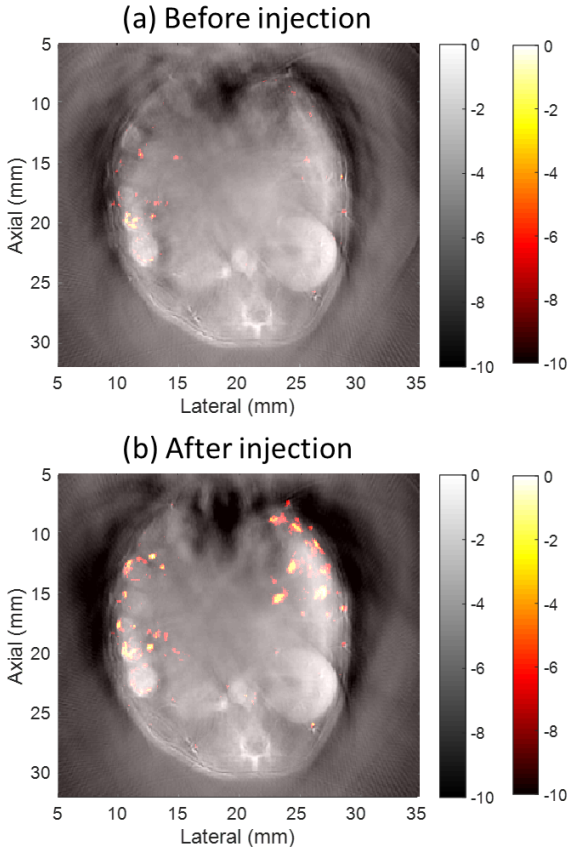

Fig. 3. Superposition of conventional and SVD-filtered images of mouse cross-section (a) before and (b) after the injection of nanodroplet contrast agents.

Fig 3 shows the superposition of conventional and SVD-filtered images of mouse cross-section before and after the injection of nanodroplet contrast agents respectively. The results show that, after activation, the signals from the spleen region have been significantly enhanced. As the droplets do not have any dye coating on the surface, one potential reason for the photo-activation of the droplets could be that blood cells nearby generate a temperature increase by absorbing light energy.

IV. Conclusion

In summary, this study demonstrates that droplets made by condensing commercial microbubbles may have potential to be used may have the potential to be used to facilitate the clinical translation of contrast-enhanced photoacoustic imaging, as the ingredients of the droplets are the same as some existing commercial microbubble contrast agents.

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