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● *Original Contribution*

A TEMPORAL AND SPATIAL ANALYSIS APPROACH TO AUTOMATED SEGMENTATION OF MICROBUBBLE SIGNALS IN CONTRAST-ENHANCED ULTRASOUND IMAGES: APPLICATION TO QUANTIFICATION OF ACTIVE VASCULAR DENSITY IN HUMAN LOWER LIMBS

WING KEUNG CHEUNG,* KATHERINE J. WILLIAMS,† KIRSTEN CHRISTENSEN-JEFFRIES,‡ BRAHMAN DHARMARAJAH,† ROBERT J. ECKERSLEY,‡ ALUN H. DAVIES,† and MENG-XING TANG*

*Department of Bioengineering, Imperial College, London, UK; †Section of Surgery, Imperial College, Charing Cross Hospital, London, UK; and ‡Division of Imaging Sciences & Biomedical Engineering, King's College London, London, UK

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Abstract—Contrast-enhanced ultrasound (CEUS) using microbubble contrast agents has shown great promise in visualising and quantifying active vascular density. Most existing approaches for vascular density quantification using CEUS are calculated based on image intensity and are susceptible to confounding factors and imaging artefact. Poor reproducibility is a key challenge to clinical translation. In this study, a new automated temporal and spatial signal analysis approach is developed for reproducible microbubble segmentation and quantification of contrast enhancement in human lower limbs. The approach is evaluated *in vitro* on phantoms and *in vivo* in lower limbs of healthy volunteers before and after physical exercise. In this approach, vascular density is quantified based on the relative areas microbubbles occupy instead of their image intensity. Temporal features of the CEUS image sequences are used to identify pixels that contain microbubble signals. A microbubble track density (MTD) measure, the ratio of the segmented microbubble area to the whole tissue area, is calculated as a surrogate for active capillary density. *In vitro* results reveal a good correlation ($r^2 = 0.89$) between the calculated MTD measure and the known bubble concentration. For *in vivo* results, a significant increase (129% in average) in the MTD measure is found in lower limbs of healthy volunteers after exercise, with excellent repeatability over a series of days (intra-class correlation coefficient = 0.96). This compares to the existing state-of-the-art approach of destruction and replenishment analysis on the same patients (intra-class correlation coefficient ≤0.78). The proposed new approach shows great potential as an accurate and highly reproducible clinical tool for quantification of active vascular density. (E-mail: mengxing.tang@imperial.ac.uk) **© 2017 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Contrast-enhanced ultrasound, Lower limb, Vascular density quantification, Image segmentation, Temporal analysis, Reproducibility, Peripheral arterial disease.**

INTRODUCTION

Ultrasound is a non-ionizing, affordable and accessible front-line clinical imaging modality, characterised by real-time image display. Recent advances in contrast-enhanced ultrasound (CEUS) imaging provide the possibility of specifically imaging blood vessels with high sensitivity and resolution. Microbubbles move through the body while being confined to blood vessels, distinguishing them as an excellent intravascular contrast medium. They vibrate under ultrasound in a non-linear fashion, generating specific harmonic signatures that allow them to be distinguished from background tissue signals with a high sensitivity.

Contrast-enhanced ultrasound is ideally suited for measurements of flow and vascular density, as bubbles move within the blood vessels at speeds comparative to those of blood cells. A destruction–replenishment (DR) approach has been used in many *in vitro* and *in vivo* trials with success. High-amplitude ultrasound is used to destroy microbubbles within the imaging plane, then the replenishment of the region is observed over time. To quantify vascular density, time–intensity curve (TIC) analysis is conducted to extract physiologic parameters such as peak intensity and flow rate. This method estimates parameters related to vascular characteristics of

Address correspondence to: Meng-Xing Tang, Department of Bioengineering, Imperial College London, SW7 2 AZ, London, UK. E-mail: mengxing.tang@imperial.ac.uk

the tissue and has been applied to the study of liver (Claudon et al. 2013) and heart (Senior et al. 2013; Wei et al. 1998). Recent studies have exhibited particularly great promise in evaluating neovascularisation in atherosclerotic plaques (Hellings et al. 2010; Huang et al. 2008; Xiong et al. 2009), the myocardial microcirculation (Senior et al. 2013; Wei et al. 1998) and the musculoskeletal microcirculation of the lower limbs (Amarteifio et al. 2011, 2013; Duerschmied et al. 2009; Krix et al. 2009, 2011; Lindner et al. 2008; Mitchell et al. 2013; Song et al. 2014).

However, the quantification of vascular density using CEUS is affected by many confounding factors (Tang et al. 2011). In particular, most existing analysis is image intensity based, and such an approach is vulnerable to problems such as signal attenuation, and non-linear imaging artefacts (Cheung et al. 2015; Yildiz et al. 2015). An alternative approach to individual bubble tracking and quantification within the image has been reported, particularly in peripheral imaging applications where relatively high frequencies are commonly used (4–15 MHz). Although imaging with such frequencies reduces sensitivity in bubble detection (Tang and Eckersley 2007) and only the brightest bubbles show up in the CEUS images, the improved spatial resolution associated with such high frequency could facilitate the tracking of individual bubbles. Hoogi et al. (2012) proposed a method for segmenting the contrast spots within atherosclerotic plaques in individual images by tracking individual microbubbles. The main advantage of this approach is that the temporal behaviour of bubble flow can be observed. This makes it robust to noise and allows differentiation between blood vessels and artefacts.

Recently, several groups have developed various methods for single-bubble detection and tracking by taking advantage of some temporal information. Viessmann et al. (2013) and Christensen-Jeffries et al. (2015) used rolling background subtraction to remove unwanted background signals from static structures such as the echo from the tube wall. Ackermann and Schmitz (2016) adopted a temporal median filtering and foreground/background subtraction to detect and track multiple microbubbles in ultrasound B-mode images. Errico et al. (2015) developed an ultrafast ultrasound localization technique for deep super-resolution vascular imaging by exploiting the coherence of backscattered signals; the spatiotemporal filtering approach discriminates slowly moving bubbles of sub-wavelength size (low spatial coherence) from slow motion tissue signals whose temporal variations affect many neighbouring pixels the same way (high spatial coherence). Gessner et al. (2013) developed acoustic angiography to visualise microvascular architecture without significant contribution from background tissues by using super-harmonics and a customised dual-frequency probe. Mischi et al. (2012) used spatiotemporal analysis of ultrasound contrast agent dispersion kinetics to image angiogenesis. In this study, we propose a different method which examines frequency features in the temporal domain in CEUS sequences and apply it to a clinical application of quantifying active vascular density in human lower limbs.

In CEUS image sequences, we hypothesised that the temporal profile of each pixel can be used to detect microbubbles passing the pixel. The relative area of these "bubble pixels" can provide an area-based vascular density measure that may be more robust than existing image intensity-based approaches. Furthermore, the pixel-based temporal analysis can be reduced to an automated algorithmic process, yielding advantages in terms of user interface, output speed and interpretation over existing approaches.

The objective of this study was to develop a robust and automated quantification tool for microbubble activity in CEUS image sequences using an algorithm based on pixel-level temporal and spatial analysis. This technique is illustrated with a flow phantom and then, as an initial clinical demonstration, applied to the quantification of *in vivo* musculoskeletal microcirculation in lower limb vascular density of healthy human patients.

METHODS

Microbubble detection algorithm

The proposed algorithm works at a pixel level to detect microbubble signals. The image contains primarily three components: tissue artefact, noise and microbubble signals. Initially, the average image intensity and coefficient of variation are used to remove tissue signals, and then microbubbles are distinguished from noise by examining the frequency composition of the pixel's temporal signal. The temporal signal of a pixel within a vessel with bubble(s) passing through has a frequency composition very different from that with noise only (Fig. 1). The microbubble detection algorithm consists of two specific steps.

Detecting tissue-only regions. Given the signal, $I(t)$, the coefficient of variation (COV) is calculated as

$$\mathrm{COV} = \frac{\sqrt{\left\langle \left(I(t) - \langle I(t) \rangle\right)^2 \right\rangle}}{I(t)} \qquad (1)$$

where $<I(t)>$ is the temporal average intensity. If we assume tissue signals to be higher in amplitude than noise background and not to change significantly over time, the combination of COV and average intensity can be used to identify tissue signal. If a signal's COV is smaller than a threshold T_{COV} and its average intensity is larger than a threshold T_{AI}, this signal is classified as tissue