In vivo imaging of the microcirculation of the volar forearm using correlation mapping optical coherence tomography (cmOCT)

Joey Enfield,1 Enock Jonathan,1 and Martin Leahy1,2

1Tissue Optics and Microcirculation Imaging Facility, National Biophotonics and Imaging Platform, Limerick, Ireland
2Royal College of Surgeons (RCSI), Dublin, Ireland.
*enock.jonathan@ul.ie

Abstract: Correlation mapping optical coherence tomography (cmOCT) is a recently proposed technique that extends the capabilities of OCT to enable mapping of vasculature networks. The technique is achieved as a processing step on OCT intensity images that does not require any modification to existing OCT hardware. In this paper we apply the cmOCT processing technique to in vivo human imaging of the volar forearm. We illustrate that cmOCT can produce maps of the microcirculation that clearly follow the accepted anatomical structure. We demonstrate that the technique can extract parameters such as capillary density and vessel diameter. These parameters are key clinical markers for the early changes associated with microvascular diseases. Overall the presented results show that cmOCT is a powerful new tool that generates microcirculation maps in a safe non-invasive, non-contact technique which has clear clinical applications.

©2011 Optical Society of America

OCIS codes: (170.4500) Optical coherence tomography; (170.3880) Medical and biological imaging

References and links

1. E. M. Kohner, “Dynamic changes in the microcirculation of diabetics as related to diabetic microangiopathy,” Acta Med. Scand. Suppl. 578, 41–47 (1975).
2. M. M. Smith, P. C. Chen, C. S. Li, S. Ramanujam, and A. T. Cheung, “Whole blood viscosity and microvascular abnormalities in Alzheimer’s Disease,” Clin. Hemorheol. Microcirc. 41(4), 229–239 (2009).
3. J. Folkman, “Proceedings: Tumor angiogenesis factor,” Cancer Res. 34(8), 2109–2113 (1974).
4. M. CutoLO, W. Grassi, and M. Matucci Cerinic, “Raynaud’s phenomenon and the role of capillaroscopy,” Arthritis Rheum. 48(11), 3023–3030 (2003).
5. R. H. Bull, D. O. Bates, and P. S. Mortimer, “Intravital video-capillaroscopy for the study of the microcirculation in psoriasis,” Br. J. Dermatol. 126(5), 436–445 (1992).
6. K. Weidlich, J. Kroth, C. Nussbaum, S. Hiedl, A. Bauer, F. Christ, and O. Genzel-Boroviczeny, “Changes in microcirculation as early markers for infection in preterm infants—an observational prospective study,” PaeDIat. Res. 66(4), 461–465 (2009).
7. Y. Kabasakal, D. M. Elvins, E. F. Ring, and N. J. McHugh, “Quantitative nailfold capillaroscopy findings in a population with connective tissue disease and in normal healthy controls,” Ann. Rheum. Dis. 55(8), 507–512 (1996).
8. C. C. Roberts, A. W. Stanton, J. Pullen, R. H. Bull, J. R. Levick, and P. S. Mortimer, “Skin microvascular architecture and perfusion studied in human postmastectomy oedema by intravital video-capillaroscopy,” Int. J. Microcirc. Clin. Exp. 14(6), 327–334 (1994).
9. J. O’Doherty, J. Henricson, C. Anderson, M. J. Leahy, G. E. Nilsson, and F. Sjöberg, “Sub-epidermal imaging using polarized light spectroscopy for assessment of skin microcirculation,” Skin Res. Technol. 13(4), 472–484 (2007).
10. J. O’Doherty, P. McNamara, N. T. Clancy, J. G. Enfield, and M. J. Leahy, “Comparison of instruments for investigation of microcirculatory blood flow and red blood cell concentration,” J. Biomed. Opt. 14(3), 034025 (2009).
11. M. J. Leahy, J. G. Enfield, N. T. Clancy, J. O’Doherty, P. McNamara, and G. E. Nilsson, “Biophotonic methods in microcirculation imaging,” Med. Laser Appl. 22(2), 105–126 (2007).
12. P. M. McNamara, J. O’Doherty, M. L. O’Connell, B. W. Fitzgerald, C. D. Anderson, G. E. Nilsson, R. Toll, and M. J. Leahy, “Tissue viability (TIvi) imaging: temporal effects of local occlusion studies in the volar forearm,” J Biophotonics 3(1-2), 66–74 (2010).
13. J. T. Oh, M. L. Li, H. F. Zhang, K. Maslov, G. Stoicu, and L. V. Wang, “Three-dimensional imaging of skin melanoma in vivo by dual-wavelength photoacoustic microscopy,” J. Biomed. Opt. 11(3), 034032 (2006).
The microcirculation is a term used to describe the small vessels in the vasculature network which are responsible for the distribution of blood and nutrients through the body; as opposed to larger vessels in the macrocirculation which transport the blood to and from the organs. The microcirculation serves several key functions within the body including regulation of blood pressure, body temperature, blood flow within tissues and delivery of nutrients and removal of metabolic waste products.

Structural and functional changes within the microcirculation have previously been associated with various pathological conditions including diabetes [1], Alzheimer’s [2], cancer [3], Raynaud’s disease [4] and psoriasis [5]. Weidlicy et al. has recently demonstrated that monitoring changes within the microcirculation can provide an early indication of disease prior to clinical suspicion [6]. Thus the clinical diagnostic value of techniques that enable imaging of the microcirculation is of key importance as detecting early changes within the microcirculation provides early indication of disease.

A key issue with microcirculation imaging however is the limited non-invasive techniques available. To date extensive study of the microcirculation has been achieved under various pathological conditions using capillaroscopy based techniques [4,5,7,8]. These techniques use a microscope with a magnification > 100x to enable capillary imaging. These techniques are limited to imaging vessels close to the surface of the skin, thus have been limited to regions such as the nail fold where the vessels can be clearly demonstrated that monitoring changes within the microcirculation can provide an early indication of disease prior to clinical suspicion [6]. Thus the clinical diagnostic value of techniques that enable imaging of the microcirculation is of key importance as detecting early changes within the microcirculation provides early indication of disease.

1. Introduction

The microcirculation is a term used to describe the small vessels in the vasculature network which are responsible for the distribution of blood and nutrients through the body; as opposed to larger vessels in the macrocirculation which transport the blood to and from the organs. The microcirculation serves several key functions within the body including regulation of blood pressure, body temperature, blood flow within tissues and delivery of nutrients and removal of metabolic waste products.

Structural and functional changes within the microcirculation have previously been associated with various pathological conditions including diabetes [1], Alzheimer’s [2], cancer [3], Raynaud’s disease [4] and psoriasis [5]. Weidlicy et al. has recently demonstrated that monitoring changes within the microcirculation can provide an early indication of disease prior to clinical suspicion [6]. Thus the clinical diagnostic value of techniques that enable imaging of the microcirculation is of key importance as detecting early changes within the microcirculation provides early indication of disease.

A key issue with microcirculation imaging however is the limited non-invasive techniques available. To date extensive study of the microcirculation has been achieved under various pathological conditions using capillaroscopy based techniques [4,5,7,8]. These techniques use a microscope with a magnification > 100x to enable capillary imaging. These techniques are limited to imaging vessels close to the surface of the skin, thus have been limited to regions such as the nail fold where the vessels can be clearly demonstrated that monitoring changes within the microcirculation can provide an early indication of disease prior to clinical suspicion [6]. Thus the clinical diagnostic value of techniques that enable imaging of the microcirculation is of key importance as detecting early changes within the microcirculation provides early indication of disease.

1. Introduction

The microcirculation is a term used to describe the small vessels in the vasculature network which are responsible for the distribution of blood and nutrients through the body; as opposed to larger vessels in the macrocirculation which transport the blood to and from the organs. The microcirculation serves several key functions within the body including regulation of blood pressure, body temperature, blood flow within tissues and delivery of nutrients and removal of metabolic waste products.

Structural and functional changes within the microcirculation have previously been associated with various pathological conditions including diabetes [1], Alzheimer’s [2], cancer [3], Raynaud’s disease [4] and psoriasis [5]. Weidlicy et al. has recently demonstrated that monitoring changes within the microcirculation can provide an early indication of disease prior to clinical suspicion [6]. Thus the clinical diagnostic value of techniques that enable imaging of the microcirculation is of key importance as detecting early changes within the microcirculation provides early indication of disease.

A key issue with microcirculation imaging however is the limited non-invasive techniques available. To date extensive study of the microcirculation has been achieved under various pathological conditions using capillaroscopy based techniques [4,5,7,8]. These techniques use a microscope with a magnification > 100x to enable capillary imaging. These techniques are limited to imaging vessels close to the surface of the skin, thus have been limited to regions such as the nail fold where the vessels can be clearly demonstrated that monitoring changes within the microcirculation can provide an early indication of disease prior to clinical suspicion [6]. Thus the clinical diagnostic value of techniques that enable imaging of the microcirculation is of key importance as detecting early changes within the microcirculation provides early indication of disease.

1. Introduction

The microcirculation is a term used to describe the small vessels in the vasculature network which are responsible for the distribution of blood and nutrients through the body; as opposed to larger vessels in the macrocirculation which transport the blood to and from the organs. The microcirculation serves several key functions within the body including regulation of blood pressure, body temperature, blood flow within tissues and delivery of nutrients and removal of metabolic waste products.

Structural and functional changes within the microcirculation have previously been associated with various pathological conditions including diabetes [1], Alzheimer’s [2], cancer [3], Raynaud’s disease [4] and psoriasis [5]. Weidlicy et al. has recently demonstrated that monitoring changes within the microcirculation can provide an early indication of disease prior to clinical suspicion [6]. Thus the clinical diagnostic value of techniques that enable imaging of the microcirculation is of key importance as detecting early changes within the microcirculation provides early indication of disease.

A key issue with microcirculation imaging however is the limited non-invasive techniques available. To date extensive study of the microcirculation has been achieved under various pathological conditions using capillaroscopy based techniques [4,5,7,8]. These techniques use a microscope with a magnification > 100x to enable capillary imaging. These techniques are limited to imaging vessels close to the surface of the skin, thus have been limited to regions such as the nail fold where the vessels can be clearly demonstrated that monitoring changes within the microcirculation can provide an early indication of disease prior to clinical suspicion [6]. Thus the clinical diagnostic value of techniques that enable imaging of the microcirculation is of key importance as detecting early changes within the microcirculation provides early indication of disease.

1. Introduction

The microcirculation is a term used to describe the small vessels in the vasculature network which are responsible for the distribution of blood and nutrients through the body; as opposed to larger vessels in the macrocirculation which transport the blood to and from the organs. The microcirculation serves several key functions within the body including regulation of blood pressure, body temperature, blood flow within tissues and delivery of nutrients and removal of metabolic waste products.

Structural and functional changes within the microcirculation have previously been associated with various pathological conditions including diabetes [1], Alzheimer’s [2], cancer [3], Raynaud’s disease [4] and psoriasis [5]. Weidlicy et al. has recently demonstrated that monitoring changes within the microcirculation can provide an early indication of disease prior to clinical suspicion [6]. Thus the clinical diagnostic value of techniques that enable imaging of the microcirculation is of key importance as detecting early changes within the microcirculation provides early indication of disease.

A key issue with microcirculation imaging however is the limited non-invasive techniques available. To date extensive study of the microcirculation has been achieved under various pathological conditions using capillaroscopy based techniques [4,5,7,8]. These techniques use a microscope with a magnification > 100x to enable capillary imaging. These techniques are limited to imaging vessels close to the surface of the skin, thus have been limited to regions such as the nail fold where the vessels can be clearly demonstrated that monitoring changes within the microcirculation can provide an early indication of disease prior to clinical suspicion [6]. Thus the clinical diagnostic value of techniques that enable imaging of the microcirculation is of key importance as detecting early changes within the microcirculation provides early indication of disease.

A key issue with microcirculation imaging however is the limited non-invasive techniques available. To date extensive study of the microcirculation has been achieved under various pathological conditions using capillaroscopy based techniques [4,5,7,8]. These techniques use a microscope with a magnification > 100x to enable capillary imaging. These techniques are limited to imaging vessels close to the surface of the skin, thus have been limited to regions such as the nail fold where the vessels can be clearly demonstrated that monitoring changes within the microcirculation can provide an early indication of disease prior to clinical suspicion [6]. Thus the clinical diagnostic value of techniques that enable imaging of the microcirculation is of key importance as detecting early changes within the microcirculation provides early indication of disease.

A key issue with microcirculation imaging however is the limited non-invasive techniques available. To date extensive study of the microcirculation has been achieved under various pathological conditions using capillaroscopy based techniques [4,5,7,8]. These techniques use a microscope with a magnification > 100x to enable capillary imaging. These techniques are limited to imaging vessels close to the surface of the skin, thus have been limited to regions such as the nail fold where the vessels can be clearly demonstrated that monitoring changes within the microcirculation can provide an early indication of disease prior to clinical suspicion [6]. Thus the clinical diagnostic value of techniques that enable imaging of the microcirculation is of key importance as detecting early changes within the microcirculation provides early indication of disease.

A key issue with microcirculation imaging however is the limited non-invasive techniques available. To date extensive study of the microcirculation has been achieved under various pathological conditions using capillaroscopy based techniques [4,5,7,8]. These techniques use a microscope with a magnification > 100x to enable capillary imaging. These techniques are limited to imaging vessels close to the surface of the skin, thus have been limited to regions such as the nail fold where the vessels can be clearly demonstrated that monitoring changes within the microcirculation can provide an early indication of disease prior to clinical suspicion [6]. Thus the clinical diagnostic value of techniques that enable imaging of the microcirculation is of key importance as detecting early changes within the microcirculation provides early indication of disease.

A key issue with microcirculation imaging however is the limited non-invasive techniques available. To date extensive study of the microcirculation has been achieved under various pathological conditions using capillaroscopy based techniques [4,5,7,8]. These techniques use a microscope with a magnification > 100x to enable capillary imaging. These techniques are limited to imaging vessels close to the surface of the skin, thus have been limited to regions such as the nail fold where the vessels can be clearly demonstrated that monitoring changes within the microcirculation can provide an early indication of disease prior to clinical suspicion [6]. Thus the clinical diagnostic value of techniques that enable imaging of the microcirculation is of key importance as detecting early changes within the microcirculation provides early indication of disease.

A key issue with microcirculation imaging however is the limited non-invasive techniques available. To date extensive study of the microcirculation has been achieved under various pathological conditions using capillaroscopy based techniques [4,5,7,8]. These techniques use a microscope with a magnification > 100x to enable capillary imaging. These techniques are limited to imaging vessels close to the surface of the skin, thus have been limited to regions such as the nail fold where the vessels can be clearly demonstrated that monitoring changes within the microcirculation can provide an early indication of disease prior to clinical suspicion [6]. Thus the clinical diagnostic value of techniques that enable imaging of the microcirculation is of key importance as detecting early changes within the microcirculation provides early indication of disease.

A key issue with microcirculation imaging however is the limited non-invasive techniques available. To date extensive study of the microcirculation has been achieved under various pathological conditions using capillaroscopy based techniques [4,5,7,8]. These techniques use a microscope with a magnification > 100x to enable capillary imaging. These techniques are limited to imaging vessels close to the surface of the skin, thus have been limited to regions such as the nail fold where the vessels can be clearly demonstrated that monitoring changes within the microcirculation can provide an early indication of disease prior to clinical suspicion [6]. Thus the clinical diagnostic value of techniques that enable imaging of the microcirculation is of key importance as detecting early changes within the microcirculation provides early indication of disease.
visualized. Other techniques have been combined to enhance imaging capabilities. Techniques based on orthogonal polarization spectral imaging has demonstrated to enhance visualization depth [9] while optical Doppler imaging and speckle variance techniques have enabled quantification of blood flow within the microcirculation [10–12]. One issue with all these methods is the limit of depth ambiguity. It is therefore difficult to assess which vessels are being examined. This has led to a recent drive towards the development of tools that enable 3D imaging of the microcirculation.

In recent years photoacoustic imaging has served as a powerful tool in 3D microcirculation imaging. The principle behind the technique is that as light energy is absorbed by a chromophore within the tissue, a resulting acoustic wave is generated. This can be detected to generate 3D maps of the desired chromophore. Through the selection of suitable wavelengths of light various chromophores such as blood, melanin [13] or other contrast agents [14] can be mapped. The technique, however, is limited by the spatial resolution of ultrasound detection. The use of focused ultrasonic transducers in photoacoustic microscopy (PAM) enhances spatial resolution to ~45 µm [15]. Further enhancements in the resolution are achievable through the use of highly focused light in optical resolution photoacoustic microscopy (OR-PAM) achieving a resolution of ~5 µm [16]. This technique has demonstrated high resolution capillary-level imaging of the microcirculation. However, the resulting depth of focus is greatly reduced and the structural imaging of the surrounding tissue layers is still limited by the acoustic resolution. High resolution structural imaging combined with microcirculation imaging can provide additional clinical information thus the low acoustic resolution can be an issue. Another limitation of photoacoustic techniques is that a coupling medium is required to provide direct contact with the tissue under investigation. This coupling can interact with the tissue function and affect the microcirculation.

Optical coherence tomography (OCT) addresses many of the limitations of photoacoustic imaging providing non-contact structural imaging of the tissue with micron resolution. OCT itself does not directly produce microcirculation imaging; however several technologies have been developed to extend the capabilities of OCT to visualize the microcirculation. The original technique demonstrated was Doppler OCT (DOCT) [17]. The technique operates on the Doppler shift caused by moving scatters within the tissue. DOCT has enabled quantification of blood flow dynamics with high spatial resolution [18] and has been demonstrated in various in vivo applications [19,20]. The technique suffers from an angular dependence and is unable to detect flow perpendicular to the scanning beam. This is a key limitation for microcirculation imaging as blood vessels within the tissue are orientated at varying angles and can be tortuous in shape. Thus DOCT would produce incomplete maps of the microcirculation. Another technique that has been demonstrated is termed speckle variance OCT (svOCT) [21]. This technique is based on the change in speckle pattern caused by moving scattering particles from the structural OCT signal. The svOCT signal is determined by calculation of the variance of the signal intensity using either a spatial window [21] or temporal window [22]. The key advantage of svOCT is that it does not suffer from angular dependence like DOCT. One issue with svOCT is in interpreting the variance results. The calculated variance is in the range of ±∞ and depends on the chosen window size. Thus the variance values itself does not directly indicate flow and a prior knowledge of the structure is required to separate regions with and without flow. Another technique that has shown very promising results is optical microangiography (OMAG) that has emerged to enable microcirculation imaging [23]. The original OMAG technique did not provide flow velocity information, but a hybrid method termed Doppler OMAG (DOMAG) has enabled velocity measurements [24] which offers higher signal to noise ratio compared to standard DOCT. The technique was originally demonstrated in small animal cerebral blood flow imaging, but has since been demonstrated to image the microcirculation at various anatomical locations such as retinal and choroid [25], sentinel lymph node [26] and the cochlea [27]. The flow sensitivity has recently been enhanced using a new processing technique termed ultra-high sensitive OMAG (UHS-OMAG) which has enabled vivo imaging of the microcirculation for human skin [28]. However, the OMAG technique requires extensive
post processing on data which is reported to require up to 35 min to process a single volume [23] which can limit clinical suitability.

To overcome the limitations associated with existing technologies we has recently developed a new technique termed correlation mapping OCT (cmOCT) [29]. In this paper we will demonstrate the suitability of cmOCT techniques as a tool that enables non-invasive, non-contact mapping of the microcirculation. We apply the cmOCT technique to in vivo imaging of the human volar forearm and demonstrate the vascular maps that can be produced.

2. Processing Principles of cmOCT

2.1. OCT Imaging System

All OCT imaging in this work was performed using an unmodified commercial OCT system (OCM1300SS, Thorlabs Inc., Newton, NJ, USA). The system is a FD-OCT system using a swept source laser (SL1325-P16, Thorlabs Inc.). The laser has a center wavelength of 1325 nm and operates at a scanning rate of 16 kHz providing an axial resolution of \(~12 \mu m\). The sample arm is focused with a LSM003 (Thorlabs Inc.) scanning lens which provided a lateral resolution of 25 \(\mu m\) and XZ imaging range up to 11x11 mm. The system is capable of acquiring and saving 3D OCT volumes consisting of 1024x1024 A-scans in approximately 70 s.

2.2. cmOCT Processing Technique

The cmOCT algorithm is achieved purely through processing on the OCT signal intensity and does not require phase information. The first stage in the cmOCT algorithm is determination of the correlation between two OCT frames or B-scans (XY slices) captured at the same location. This is calculated by cross correlating a grid from frame A \((I_a)\) to the same grid from frame B \((I_b)\) using Eq. (1).

\[
\text{cmOCT}(x, y) = \sum_{p=0}^{M} \sum_{q=0}^{N} \frac{I_a(x+p, y+q) - \bar{I}_a(x,y)}{\sqrt{(I_a(x+p, y+q) - \bar{I}_a(x,y))^2 + (I_b(x+p, y+q) - \bar{I}_b(x,y))^2}}
\]

where \(M\) and \(N\) is the grid size and \(\bar{I}\) is the grids mean value. This grid is then shifted across the entire XY image and a 2D correlation map is generated. The resulting correlation map contains values on the range of \(0 \pm 1\) indicating weak correlation and strong correlation respectively.

The kernel size used in this work was arbitrarily chosen for optimal image quality with the trade-off of processing time, larger kernels require greater processing times. The size of the chosen kernel should not have an effect on cmOCT as regions of static tissue will still have a high correlation while regions containing flow will still have a low correlation. If too large a kernel is chosen, a “blurring” and loss of smaller vessels can occur. If a very small kernel is used such as 2x2, the resulting data set can be quite noisy as small changes in the structural signal can result in decorrelation.

As the background noise has weak correlation it must be suppressed. This is achieved by masking the correlation image with a “structural mask”. The structural mask represents regions that contain structural information in the OCT frame. This mask is generated by performing a kernel blur followed by a binary threshold on the source OCT structural image. The threshold value is currently chosen to be above the mean background value. The implications and optimal selection of this threshold correlation value is still under investigation.

This binary mask can then be applied to the correlation map to reveal the cmOCT image. These processing steps are illustrated in Fig. 1.

The resulting cmOCT image contains correlation values on the range of \(0 \pm 1\). This allows for immediate determination of flow regions as higher correlation represents static regions of the sample and low correlation represents regions of flow within the sample.
Fig. 1. Processing steps for cmOCT algorithm. Higher correlation is shown as darker color while lower correlation is shown as brighter color. The figure shows a 200 µm capillary tube containing intralipid solution moving under Brownian motion embedded in excised porcine tissue.

In this work we assume that static features have a correlation value > 0.6 and thus color mapping is applied below this range.

To generate 3D cmOCT volumes, two scanning techniques can be used. The first method requires repeated B-scans to be acquired at the same location. The cmOCT processing is then performed on the two frames and the scan location is then moved. This can be repeated to generate a 3D cmOCT volume. However, this was not possible using the commercial OCT system that was utilized for this work as direct control over the scan pattern was not available. Thus a second method to generate a cmOCT volume was required. The system enabled 3D volumes to be captured over a selected area. The area was chosen such that there was dense sampling between adjacent B-scans resulting in a frame spatial separation below the lateral resolution of the OCT system. This ensured that there was a strong correlation for static regions of the sample, but 3D data could be still acquired. The cmOCT processing could then be applied between adjacent B-scans within the volume and the 3D cmOCT volume could be generated. The temporal separation between frames should also affect the resulting correlation values. If too low a temporal separation is used, low flow velocities might not be detected. The limitations of the spatial and temporal frame separation are currently being investigated.

The software implementation of the cmOCT processing technique has been implemented using in-house developed code written using the Java programming language. The software can process and render a cmOCT volume consisting of 1024x1024 A-scans, each of 512 pixels deep within 28 s using a 3x3 grid and 6 sec using 512x512 A-scans. A kernel choice of 7x7 can be processed within 27 sec for a volume consisting of 512x512 A-scans and within 11.2 s for 1024x1024 A-scans. This illustrates the speed that is achievable and could be further advanced using optimized code or GPU based techniques.

3. Results

3.1. Phantom Imaging

To demonstrate the imaging capabilities of cmOCT at detecting low flow velocities, a multiple layer capillary tube phantom was fabricated. The phantom consisted of 3 layers of capillary tubes embedded within a scattering epoxy resin matrix (3M DP-100NS). The capillary tubes were orientated such that each layer was crossing the previous layer. The capillaries were filled with a 3% intralipid solution which was to move under Brownian
motion. A 3x3x3 mm region was imaged using the OCT system with voxel size of 1024x1024x512 (ZXY). The acquisition time for a volume of this size was 70 s. The volume was processed using the previously outlined cmOCT processing algorithm with a 5x5 grid in 59 s (see Fig. 2).

![Fig. 2. Sample cmOCT processing for a scattering epoxy resin phantom with three layers of capillary tubes containing an intralipid solution moving under Brownian motion. (A) 3D rendering of the structural OCT data set. (B) 3D rendering of resulting cmOCT processed volume. A movie showing a 3D rotation of the merged data sets is also provided (Media 1). MIP projections through the cmOCT volume are also shown illustrating the 3 detected capillary layers; Top layer (C), Middle layer (D) and Bottom layer (E).](image)

The results illustrate the sensitivity of cmOCT at detecting low flow velocities. The 3D structural OCT data set is rendered in Fig. 2 (A). This was then processed using the previously outlined cmOCT technique and the resulting cmOCT is rendered in Fig. 2 (B). A supporting movie is also provided which shows the rotating structure and cmOCT volume (Media 1). One result that must be noted is that the three capillary layers of the phantom can each be clearly identified. This is illustrated in Fig. 2 (C,D,E). The frames are maximum intensity projections (MIP) through several XZ slices of the cmOCT data set at varying depths within the volume. A distortion in the cmOCT projections for the lower capillary layers can also be seen in (D,E). This is caused by the refraction of the probing beam as it travels through the higher layers of the phantom. This is a limitation of OCT which could be corrected with knowledge of the refractive index [30].

These phantom results demonstrate that flow in all capillary tubes is detected even though the intralipid is undergoing purely Brownian motion. This demonstrates that very low flow velocities can be identified using cmOCT which would be far below the sensitivity of DOCT. This sensitivity to low flow velocity is key when attempting to image the microcirculation of the skin.

### 3.2. In vivo Imaging

To illustrate the suitability of cmOCT for in vivo mapping of the microcirculation, imaging was performed on a health male aged 26 years with informed consent. The subject had no history or existing conditions that would affect the microcirculation. All imaging was performed on the volar forearm region of the subject. Prior to imaging the subject was placed in an upright sitting position and allowed to acclimatize to the laboratory for 5 min. After this acclimatization the subjects forearm was placed under the OCT probe and a soft cushion was placed underneath the forearm. A 3D OCT scan of the
forearm was acquired over an area of 2.5x2.5 mm with using 1024x1024 A-scans. The resulting volume was then processed using the outlined cmOCT technique with a 7x7 kernel in 116 s. The results of this are illustrated in Fig. 3.

![Fig. 3. cmOCT of the volar forearm for a 2.5x2.5x3 mm region. (A) OCT B-scan image of the forearm, (B) cmOCT image generated using A. (C) 3D rendering showing a cutaway structural OCT volume revealing the cmOCT generated volume. A supporting movie is also provided (Media 2) (D) Maximum intensity projection through the cmOCT volume (scale bar 500 µm). (The blue line represents the location of the slice in A,B).](image)

The figure illustrates a sample network of vessels that has been identified from within the forearm using the cmOCT technique. Figure 3 (A) shows a sample OCT B-scan of the forearm where the dermal and epidermal layers can be clearly identified. The corresponding cmOCT for the same location showing regions of detected flow is shown in Fig. 3 (B). The figure shows that the location of vessels that can be seen in the structural OCT image, which show up as dark regions, corresponds to the detected regions of flow in the cmOCT image which are marked with the red arrow in both frames. Figure 3 (C) shows a 3D rendering of the cmOCT volume combined with the structural OCT imaging. The figure shows clearly the 3D network structure that has been detected using the cmOCT processing. A supporting movie has also been provided showing a rotation of the structure (Media 2). Figure 3 (D) shows a MIP projection through the full cmOCT volume. The figure better illustrates the structure of the vessel network. The blue horizontal line indicated in the figure identifies the location of frames from (A) and (B). The advantage of performing the MIP is that measurements of the vessel can be performed. Several vessels are marked in the image and their diameters have been calculated as the average of several measurements. The vessels (1,2,3), have an average diameter of 33 µm, 69 µm and 72 µm respectively. These results clearly show that cmOCT is capable imaging an underlying network within the tissue. However, the exact location of these vessels within the tissue is not entirely clear.
To allow better assessment of the location of the vessels, a series of MIP projections were generated at various depths within the cmOCT volume. Due to the natural curvature and orientation of the skin it was not sufficient to perform the MIP projection directly over XZ slices. To enable accurate depth projections, the surface of the skin was first estimated using the structural OCT volume. Once the location of surface was estimated the projections were calculated at depths determined from this surface. The result of this processing is shown in Fig. 4.

![Fig. 4. Location of detected vessels within the forearm (A) Structural OCT image of volar forearm (XY) with the estimated surface shown (red line). (B) A series of MIP projections (XZ) at varying depths through the sample as measured from the estimated surface. (1) 20-70 µm (2) 130-180 µm (3) 310-360 µm. An enface flythrough movie of the cmOCT data is also provided (Media 3).](image_url)

The figure illustrates the multiple layers of the microcirculation that have been detected. An accompanying movie (Media 3) shows a enface depth fly through of the cmOCT volume to illustrated the vessels that have been detected. Figure 4(A) shows a structural OCT scan through the volar forearm. The red line indicates the estimated surface of the tissue which was used when performing the MIP projections. Figure 4 (B) shows the MIP projections for 3 different depths within the tissue.

Figure 4(B1) shows a projection over a depth range of 20-70 µm into the skin. The slice does not show any detected vessels. This is as would be expected as the region is located within the epidermis and is a non-vascular region of the skin. Figure 4 (B2) is a projection over the range 130-180 µm. At this depth a series of vessels can be clearly identified. These vessels appear vertically orientated and are seen to be rising up from the underlying vessel network. This location is around the dermal-epidermal junction which would follow the accepted anatomical description of the microcirculation for these regions indicating that these are the capillary loops feeding the epidermis. Figure 4 (B3) shows the vessels over the range of 310-360 µm. These vessels appear to form an interconnecting network of vessels that feed the capillary loops which indicates this network is the dermal plexus. This follows the anatomically accepted structure of the microcirculation and shows the technique can extract the microcirculation structure.

It must be noted that additional information can be extracted using Fig. 4 (B2). It is also possible to calculate the capillary loop density for the volar forearm by counting the number of detected vessels. Following this the capillary density of the volar forearm has
been calculated to be ~32 loops/mm², this value is around the previously reported range from 14 to 30 loops/mm² [31].

The result indicate that the spatial separation is a key parameter in that using the current commercial system, when larger areas are required multiple smaller regions should be scanned with a low spatial separation. The resulting data sets should then have sufficiently high correlation for the static structures and could be merged to generate larger mapping of the microcirculation. An example of this is demonstrated in Fig. 5 for the volar forearm showing a region of 8x7 mm for the marked region.

Fig. 5. cmOCT of an 8 mm x 7 mm region of the vascular network in the volar forearm. The figure is made up of 6 x 5 individual 2.5 x 2.5 mm OCT scans that have been manually aligned (scale bar 1 mm).

The figure was generated by scanning a series of 30 individual 2.5x2.5 mm scans with an overlap of approximately 1.5 mm between adjacent scans. The large overlap was included for ease of alignment for the resulting data. The data set was then processed using the cmOCT algorithm and MIP’s were generated using a 7x7 kernel. The resulting 30 MIP images were then manually aligned to generate the large map of the vascular network which can be seen in Fig. 5 (B). The map clearly shows that cmOCT is capable of mapping the microcirculation reproducibly over a large area. This could have applications in searching for microcirculation abnormalities. The horizontal lines that are scattered throughout the image are the result of subject movement during the imaging process. Provided that the movement is within the plane of the B-scan these could be suppressed using suitable image alignment between adjacent B-scans prior to cmOCT processing which has not been implemented for the current figure. If however, the movement is out of the imaging plane this could not be removed using such techniques.

4. Summary

In this paper we have presented correlation mapping OCT as a new powerful tool for extraction of flow information from OCT data sets. The technique is capable of extracting flow information using purely the OCT signal intensity without the requirement of phase information.

Using a capillary tube model we have demonstrated that cmOCT has a high sensitivity to flow. The technique was capable of detecting Brownian movement in intralipid filled capillary tubes while suppressing the static structure. The multiple layers of the phantom could be clearly identified which is key when attempting to image the microcirculation.
We have demonstrated this new technology through in vivo human imaging of the microcirculation in the volar forearm regions. The results have illustrated that the technique is capable of generating 3D maps of the microcirculation structure. The detected microcirculation has been shown to follow the accepted anatomical structure. We have also been able to extract parameters such as capillary density and vessel diameter. These are key clinical parameters as density variations of the capillary network are important in assessing the severity of arterial and venous diseases [32].

The results have also demonstrated that image quality and imaging speed could be enhanced using different OCT hardware. Overall we have shown that cmOCT offers the possibility of a clinical tool that provides 3D structural imaging of the tissue combined with vascular maps in a completely non-contact and non-invasive manner. The results would indicate that cmOCT could provide a powerful new tool with clear clinical applications.

**Acknowledgments**

This research was supported by the National Biophotonics Imaging Platform (NBIP) Ireland funded under the Higher Education Authority PRTLI Cycle 4, co-funded by the Irish Government and the European Union - Investing in your future.