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Adrian Podoleanu
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The CID number appears on each page of the manuscript. The complete citation is used on the first page, and an abbreviated version on subsequent pages. Numbers in the index correspond to the last two digits of the six-digit CID number.

Contents

ix	Conference Committees
xi	Sponsors
xiii	Introduction
xv	<p><i>Can normal lymph node architecture be characterised by optical coherence tomography? (Abstract Only)</i></p> <p>R. A. McLaughlin, L. Scolaro, B. R. Klyen, Univ. of Western Australia (Australia); S. Hamza, Sir Charles Gairdner Hospital (Australia); P. Robbins, PathWest QEII Medical Ctr. (Australia); C. Saunders, Sir Charles Gairdner Hospital (Australia) and Univ. of Western Australia (Australia); D. D. Sampson, Univ. of Western Australia (Australia)</p>
xix	<p><i>A first demonstration of audio-frequency optical coherence elastography of tissue (Abstract Only)</i></p> <p>S. G. Adie, S. A. Alexandrov, J. J. Armstrong, B. F. Kennedy, D. D. Sampson, Univ. of Western Australia (Australia)</p>

OPTICAL SOURCES

7139 02	<p>Femtosecond lasers for optical coherence tomography (Invited Paper) [7139-01]</p> <p>H. M. Crespo, C. C. Rosa, Univ. of Porto (Portugal)</p>
7139 03	<p>Optical fiber sources for measurement and imaging (Invited Paper) [7139-02]</p> <p>A. B. Lobo Ribeiro, M. Melo, J. R. Salcedo, Multiwave Photonics, S.A. (Portugal)</p>
7139 04	<p>Towards 1.0 W CW reliable SLD at 840 nm [7139-03]</p> <p>Yu. O. Kostin, P. I. Lapin, V. V. Prokhorov, V. R. Shidlovsky, S. D. Yakubovich, Superlum Diodes Ltd. (Russian Federation)</p>
7139 05	<p>Towards 100 nm Band NIR SLDs [7139-04]</p> <p>Yu. O. Kostin, P. I. Lapin, V. V. Prokhorov, V. R. Shidlovsky, S. D. Yakubovich, Superlum Diodes Ltd. (Russian Federation)</p>
7139 06	<p>Development of fibre optic broadband sources at 1 μm region for optical coherence tomography [7139-05]</p> <p>I. Trifanov, M. O. Berendt, J. R. Salcedo, Multiwave Photonics S.A. (Portugal); A. G. Podoleanu, Univ. of Kent (United Kingdom); A. B. Lobo Ribeiro, Multiwave Photonics S.A. (Portugal)</p>
7139 07	<p>Improvement of the mode quality in large mode area (LMA) fibres [7139-06]</p> <p>S. Grünsteidl, Multiwave Photonics S.A. (Portugal) and National Univ. of Ireland (Ireland); J. M. Sousa, Multiwave Photonics S.A. (Portugal); G. O'Connor, T. Glynn, National Univ. of Ireland (Ireland)</p>

OCT TECHNOLOGY

- 7139 08 **Theory and applications of multi-beam OCT** [7139-07]
J. Holmes, Michelson Diagnostics Ltd. (United Kingdom)
- 7139 09 **Multi-channel time domain spectroscopic optical coherence tomography system** [7139-08]
A. Meadway, S. H. H. Darbrazi, R. Cernat, G. Dobre, A. G. Podoleanu, Univ. of Kent (United Kingdom); R. B. Rosen, The New York Eye and Ear Infirmary (United States)
- 7139 0A **Multiple delay lines full-field optical coherence tomography** [7139-09]
J. Wang, Univ. of Kent (United Kingdom); C. Dainty, National Univ. of Ireland (Ireland); A. G. Podoleanu, Univ. of Kent (United Kingdom)
- 7139 0B **En face OCT system at 1060 nm** [7139-10]
L. Neagu, Univ. of Kent (United Kingdom); A. B. Lobo Ribeiro, Multiwave Photonics S.A. (Portugal); R. G. Cucu, A. Bradu, L. Ma, A. G. Podoleanu, Univ. of Kent (United Kingdom)
- 7139 0C **Application of optical coherence tomography for imaging of scaffold structure and micro-flows characterization** [7139-11]
B. Veksler, E. Kobzev, M. Bonesi, I. Meglinski, Cranfield Univ. (United Kingdom)
- 7139 0D **Theoretical approach on a galvanometric scanner with an enhanced duty cycle** [7139-12]
V.-F. Duma, Aurel Vlaicu Univ. of Arad (Romania); A. G. Podoleanu, Univ. of Kent (United Kingdom)
- 7139 0E **Denoising based on noise parameter estimation in speckled OCT images using neural network** [7139-13]
M. R. N. Avanaki, P. P. Laissue, A. G. Podoleanu, A. Hojjat, Univ. of Kent (United Kingdom)

OCT MICROSCOPY

- 7139 0F **Gabor domain optical coherence microscopy (Invited Paper)** [7139-14]
J. P. Rolland, P. Meemon, S. Murali, A. Jain, N. Papp, College of Optics & Photonics, Univ. of Central Florida (United States); K. P. Thompson, Optical Research Associates (United States); K. Lee, College of Optics & Photonics, Univ. of Central Florida (United States)
- 7139 0G **Using en face optical coherence tomography to analyse gene function in *Drosophila melanogaster* larval heart** [7139-15]
A. Bradu, L. Ma, J. Bloor, A. Podoleanu, Univ. of Kent (United Kingdom)
- 7139 0H **In vivo imaging of adult zebrafish using optical coherence tomography** [7139-16]
Y. Verma, K. Divakar Rao, P. K. Gupta, Raja Ramanna Ctr. for Advanced Technology (India)

OCT IN THE CLINIC

- 7139 0I **Doppler optical coherence tomography in cardiovascular physiology** [7139-17]
M. Bonesi, Univ. of Sheffield (United Kingdom); I. Meglinski, Cranfield Univ. (United Kingdom); S. Matcher, Univ. of Sheffield (United Kingdom)

- 7139 OJ **Imaging of basal cell carcinoma tissue using en face OCT** [7139-18]
B. R. Penmetša, Univ. of Kent (United Kingdom); M. Khandwala, Maidstone and Tunbridge Wells NHS Trust (United Kingdom); A. Bradu, M. Hughes, Univ. of Kent (United Kingdom); C. A. Jones, J. Schofield, Maidstone and Tunbridge Wells NHS Trust (United Kingdom); A. G. Podoleanu, Univ. of Kent (United Kingdom)
- 7139 OL **Structural characterization of hair fiber by optical coherence tomography (OCT)** [7139-20]
A. Z. Freitas, Instituto de Pesquisas Energéticas e Nucleares (Brazil); M. V. Robes Velasco, Univ. de São Paulo (Brazil); M. P. Raele, Instituto de Pesquisas Energéticas e Nucleares (Brazil); T. M. Kaneko, Univ. de São Paulo (Brazil); N. Dias Vieira, Jr., Instituto de Pesquisas Energéticas e Nucleares (Brazil); A. R. Baby, Univ. de São Paulo (Brazil)
- 7139 OM **En-face OCT microleakage investigation after laser-assisted dental hard tissue treatment** [7139-21]
C. Todea, C. Balabuc, C. Sinescu, M. Negruțiu, L. Filip, Victor Babeș University of Medicine and Pharmacy, Timișoara (Romania); A. Bradu, A. G. Podoleanu, Univ. of Kent (United Kingdom)
- 7139 ON **Optical coherence tomography and confocal microscopy investigations of dental prostheses** [7139-22]
M. L. Negruțiu, C. Sinescu, Victor Babeș University of Medicine and Pharmacy, Timișoara (Romania); M. Hughes, A. Bradu, Univ. of Kent (United Kingdom); M. Rominu, C. Todea, Victor Babeș University of Medicine and Pharmacy, Timișoara (Romania); G. Dobre, A. G. Podoleanu, Univ. of Kent (United Kingdom)
- 7139 OO **An innovative approach for investigating the ceramic bracket-enamel interface: optical coherence tomography and confocal microscopy** [7139-23]
R. O. Romînu, C. Sinescu, M. Romînu, M. Negruțiu, Victor Babeș University of Medicine and Pharmacy, Timișoara (Romania); P. Laissue, Univ. of Kent (United Kingdom); S. Mihali, Victor Babeș University of Medicine and Pharmacy, Timișoara (Romania); L. Cuc, Aurel Vlaicu Univ. (Romania); M. Hughes, A. Bradu, A. G. Podoleanu, Univ. of Kent (United Kingdom)

IMAGING THE EYE

- 7139 OP **Simultaneous SLO/OCT imaging of the human retina in vivo with high speed axial eye motion correction (Invited Paper)** [7139-24]
M. Pircher, E. Götzinger, B. Baumann, H. Sattmann, C. K. Hitzenberger, Medical Univ. of Vienna (Austria)
- 7139 OQ **Revealing fine microstructural morphology in the living human retina using optical coherence tomography with pancorrection (Invited Paper)** [7139-25]
C. Torti, B. Považay, B. Hofer, A. Unterhuber, B. Hermann, W. Drexler, Cardiff Univ. (United Kingdom)
- 7139 OR **High-speed high-resolution optical coherence tomography at 800 and 1060 nm** [7139-26]
B. Považay, B. Hofer, B. Hermann, C. Torti, V. Kajić, A. Unterhuber, W. Drexler, Cardiff Univ. (United Kingdom)

MODULATION OF OPTICAL REFLECTIVITY

- 7139 OS **Retinal intrinsic optical signal and optical coherence tomography (Invited Paper)** [7139-27]
A. R. Tumlinson, B. Hermann, T. H. Margrain, B. Hofer, B. Považay, W. Drexler, Cardiff Univ. (United Kingdom)
- 7139 OT **En face differential absorption optical coherence tomography with gold nanorods as the contrast agent** [7139-28]
M. Leitner, Univ. of Porto (Portugal), INESC Porto (Portugal), and Univ. of Kent (United Kingdom); A. Henkel, C. Sönnichsen, Univ. of Mainz (Germany); C. C. Rosa, Univ. do Porto (Portugal) and INESC Porto (Portugal); A. G. Podoleanu, Univ. of Kent (United Kingdom)
- 7139 OV **Nanoparticles for enhanced contrast optical coherence tomography** [7139-30]
C. D. Maule, Univ. of Porto (Portugal) and INESC Porto (Portugal); P. Quaresma, Univ. of Porto (Portugal) and Univ. Nova de Lisboa (Portugal); P. A. Carvalho, Instituto Superior Técnico (Portugal); P. Jorge, INESC Porto (Portugal); E. Pereira, C. C. Rosa, Univ. of Porto (Portugal)
- 7139 OW **Ex vivo and in vivo OCT image contrast** [7139-31]
N. Krstajić, Univ. of Sheffield (United Kingdom); J. Jacobs, M. Bonesi, L. E. Smith, P. Deshpande, S. MacNeil, Kroto Research Institute (United Kingdom); R. Smallwood, Univ. of Sheffield (United Kingdom); S. J. Matcher, Kroto Research Institute (United Kingdom)

ADAPTIVE OPTICS

- 7139 OX **Challenges and possibilities for developing adaptive optics: ultra-high resolution optical coherence tomography for clinical in vivo retinal imaging (Invited Paper)** [7139-32]
R. J. Zawadzki, S. S. Choi, J. W. Evans, J. S. Werner, Univ. of California, Davis (United States)
- 7139 OY **Optical coherence tomography for the investigation of posterior and anterior eye segments (Invited Paper)** [7139-33]
Y. Yasuno, Univ. of Tsukuba (Japan) and Tokyo Medical Univ. (Japan)
- 7139 OZ **Does transverse chromatic aberration limit performance of AO-OCT retinal imaging?** [7139-34]
D. T. Miller, B. Cense, E. Koperda, R. S. Jonnal, W. Gao, Indiana Univ. (United States)
- 7139 IO **Adaptive optics loop for en face optical coherence tomography and laser scanning confocal microscopy** [7139-35]
S. Tuohy, A. Bradu, Univ. of Kent (United Kingdom); F. Harms, N. Chateau, Imagine Eyes (France); A. G. Podoleanu, Univ. of Kent (United Kingdom)
- 7139 II **Performance assessment of a pupil tracking system for adaptive optics retinal imaging** [7139-36]
B. Sahin, Imagine Eyes (France) and National Univ. of Ireland, Galway (Ireland); F. Harms, B. Lamory, Imagine Eyes (France)
- 7139 IJ **Optimization of the temporal performance of a deformable mirror for use in ophthalmic applications** [7139-37]
E. Odlund, Univ. of Kent (United Kingdom) and Imagine Eyes (France); M. Navarro, Imagine Eyes (France); E. Lavergne, Imagine Optics (France); F. Martins, X. Levecq, Imagine Eyes (France); A. Dubra, Univ. of Rochester (United States)

- 7139 13 **First steps toward 3D high resolution imaging using adaptive optics and full-field optical coherence tomography** [7139-38]
L. Blanco, LESIA, Observatoire de Paris, CNRS, UPMC, Univ. Paris Diderot (France); M. Blavier, LESIA, Observatoire de Paris, CNRS, UPMC, Univ. Paris Diderot (France) and Ctr. d'Investigations Cliniques du CHNO des Quinze-Vingts, Univ. Paris VI (France); M. Glanc, LESIA, Observatoire de Paris, CNRS, UPMC, Univ. Paris Diderot (France) and Groupement d'Intérêt Scientifique PHASE, ONERA, Observatoire de Paris, CNRS, and Univ. Paris Diderot (France); F. Pouplard, S. Tick, Ctr. d'Investigations Cliniques du CHNO des Quinze-Vingts, Univ. Paris VI (France); I. Maksimovic, LESIA, Observatoire de Paris, CNRS, UPMC, Univ. Paris Diderot (France); G. Chenegros, L. Mugnier, DOTA ONERA (France) and Groupement d'Intérêt Scientifique PHASE, ONERA, Observatoire de Paris, CNRS, and Univ. Paris Diderot (France); F. Lacombe, LESIA, Observatoire de Paris, CNRS, UPMC, Univ. Paris Diderot (France) and Mauna Kea Technologies (France); G. Rousset, LESIA, Observatoire de Paris, CNRS, UPMC, Univ. Paris Diderot (France) and Groupement d'Intérêt Scientifique PHASE, ONERA, Observatoire de Paris, CNRS, and Univ. Paris Diderot (France); M. Pâques, J. Le Gargasson, J. Sahel, Ctr. d'Investigations Cliniques du CHNO des Quinze-Vingts, Univ. Paris VI (France)
- 7139 14 **Full-range, high-speed, high-resolution 1 μm spectral-domain optical coherence tomography with BM-scan method for the human posterior eye imaging** [7139-39]
S. Makita, Univ. of Tsukuba (Japan) and Computational Optics and Ophthalmology Group (Japan); T. Fabritius, Univ. of Tsukuba (Japan) and Univ. of Oulu (Finland); M. Miura, Tokyo Medical Univ. (Japan) and Computational Optics and Ophthalmology Group (Japan); Y. Yasuno, Univ. of Tsukuba (Japan) and Computational Optics and Ophthalmology Group (Japan)

OCT FOR ART

- 7139 15 **Optical coherence tomography in archaeological and conservation science: a new emerging field (Invited Paper)** [7139-40]
H. Liang, B. Peric, Nottingham Trent Univ. (United Kingdom); M. Hughes, A. Podoleanu, Univ. of Kent (United Kingdom); M. Spring, The National Gallery (United Kingdom); S. Roehrs, The British Museum (United Kingdom)
- 7139 16 **Optical coherence tomography for non-destructive investigations of structure of easel paintings** [7139-41]
E. Kwiatkowska, M. Sylwestrzak, B. J. Rouba, L. Tyimińska-Widmer, M. Iwanicka, P. Targowski, Nicolaus Copernicus Univ. (Poland)
- 7139 17 **A swept source OCT at 1300 nm with angular compounding for art and archaeological conservation** [7139-42]
M. Hughes, D. A. Jackson, A. G. Podoleanu, Univ. of Kent (United Kingdom)

Author Index

Conference Committees

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- 4 Optical Sources I
Antonio B. Lobo Ribeiro, Multiwave Photonics S.A. (Portugal)
- 5 Optical Sources II
Thomas Glynn, National University of Ireland, Galway (Ireland)
- 6 Modulation of Optical Reflectivity
Yoshiaki Yasuno, University of Tsukuba (Japan)
- 7 OCT for Art
Michael Pircher, Medizinische Universität Wien (Austria)
- 8 General Skills
Adrian Podoleanu, University of Kent (United Kingdom)
- 9 Adaptive Optics I
Robert Zawadzki, University of California, Davis (United States)
- 10 Imaging the Eye I
Christopher Dainty, National University of Ireland, Galway (Ireland)
- 11 Adaptive Optics II,
Donald T. Miller, Indiana University (United States)
- 12 Imaging the Eye II
Wolfgang Drexler, Cardiff University (United Kingdom)

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Introduction

The 1st Canterbury Workshop on Optical Coherence Tomography and Adaptive Optics has been initiated as a reporting meeting of the Marie Curie Training Site (MCTS) for early stage training (EST) researchers, MEST-2005-020353 supported by the European Commission (EC) with participants from National University of Ireland, Galway, University of Porto, Imagine Eyes, Multiwave Photonics, and coordinated by the University of Kent. The reporting meeting has the generous support of the EC to gather over 20 supervisors and EST researchers to review the achievements in research of the Marie Curie Training Site.

In opening participation to this meeting to the outside world we aimed not only for a wider scientific communication exercise, but also to add education to our EST researchers by involving them in an international exchange of ideas with other specialists in their fields: optical coherence tomography (OCT) and adaptive optics (AO). At the same time, we hope that other researchers and specialists in these fields take advantage of the educational component of this meeting.

We are grateful to the sponsors who agreed to offer their generous support to transform the Marie Curie meeting into a larger size workshop with wide international participation. These industrial sponsors are innovative companies active in the field of systems and devices for OCT and AO: Imagine Eyes (France), Michelson Diagnostics (United Kingdom), Multiwave Photonics (Portugal), Thorlabs (United Kingdom), Santec (Japan), and Superlum (Russia).

We also appreciate the continuing support given by the Ratiu Family Charitable Foundation to the University of Kent in the form of bursaries enabling Romanian students to further their education in the field of biomedical imaging and we thank the Foundation for co-sponsoring this workshop.

The site of the event is the University of Kent where research in OCT dates back to 1991, when some of the precursors of spectral domain OCT, Talbot bands, and channelled spectrum low coherence interferometry for sensing were investigated.

The field of non-invasive high resolution imaging has progressed considerably in the last three years. Among the participants to the workshop we were fortunate to have leaders in the field, who have been pushing the limits of acquisition speed. We will all be interested in finding out where those limits stand today. In the last few years, spectral domain OCT led to a significant increase in the number of Mega-voxels acquired from the tissue volume, 100 times more than in time domain OCT. Using swept sources, we expect this limit to exceed 350 Mega-voxels, while good

images of the retina at 122 Mega-voxels have been demonstrated so far¹. The time to generate an en-face image in spectral domain OCT has also decreased, but we have not achieved as yet the capability of resonant scanning time domain OCT. A steady flow of research is manifest in improved resolution: in depth by OCT, and lateral by AO. In a B-scan generated by spectral OCT, we have recently seen the first demonstration of AO correction on shrinking the depth profile of the confocal microscope in the retina, however we are still far from achieving an ultimate resolution of 50 microns in the in vivo retina.

How will the two technologies, OCT and AO, look when this limit is finally achieved? How will they be best combined? These are stimulating challenges for our continuing research in pushing the limits of our capabilities.

We hope that the 1st Canterbury Workshop in Optical Coherence Tomography and Adaptive Optics will contribute towards advancing the frontier of knowledge in OCT and AO beyond the 'natural' limits which appear to be imposed by current technology.

Adrian Podoleanu

Conference Chair and Marie Curie Coordinator

¹ A. Gh. Podoleanu, R. B. Rosen, "[Combinations of techniques in imaging the retina with high resolution.](#)" *Progress in Retinal and Eye Research*, Vol. 27, No. 4, 464-499 (2008).

Can normal lymph node architecture be characterised by optical coherence tomography?

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ABSTRACT

Assessment of lymph node involvement is a key prognostic marker in early breast cancer. This paper demonstrates the ability of optical coherence tomography (OCT) to characterise the micro-architecture of healthy, non-cancerous lymph nodes. OCT is shown to differentiate stroma, cortex and adipose tissue. Characteristic patterns are also identified for germinal centres and blood vessels within the node. Results are correlated against a histopathological gold standard.

Keywords: breast cancer, lymph node, optical coherence tomography, OCT.

1. INTRODUCTION

Breast cancer is the most commonly diagnosed cancer in women in the USA, with an incidence rate of 117.7 per 100,000 women [1]. In England, over 38,000 cases are diagnosed each year [2]. A critical stage in the management of cancer is to assess the spread (metastasis) of the malignancy to the lymphatic system. The lymphatic system is a network of vessels and lymph nodes throughout the body which forms a key part of the immune system.

A common surgical option to evaluate lymph node involvement is axillary clearance. This involves removal of some or all of the 25-30 lymph nodes from the axilla (armpit). The excised lymph nodes are examined using histopathological techniques to identify the presence of malignant cells. However, this results in the excision of many healthy (non-cancerous) lymph nodes and approximately 26% of patients will suffer lymphoedema [3]. Lymphoedema is caused by an accumulation of lymph (fluid) in the tissue, and manifests as a chronic swelling of the arm, breast or chest wall.

Optical coherence tomography is an optical imaging modality, using coherence-gated light in the near infrared spectrum. It has been used to obtain high-resolution images of the structural changes caused by cancer in the gastrointestinal tract [4][5], breast [6], biliary system [7] and cervix [8]. Recently, Luo et al. [9] proposed using OCT in the assessment of axillary lymph nodes. OCT provides the possibility of an imaging-based optical biopsy method for determining lymph node involvement. If performed in vivo, such a tool could potentially avoid the unnecessary excision of healthy lymph nodes; thus, reducing the incidence of complications such as lymphoedema.

When cancer metastasises to a lymph node, it disrupts the node's normal micro-architecture. In this paper, we describe the use of OCT to characterise the micro-architecture of excised normal (non-cancerous) lymph nodes. This work is an important preliminary step in establishing the feasibility of using OCT to optically identify cancerous lymph nodes.

2. EXPERIMENT

For this study, 18 lymph nodes were obtained from 13 patients undergoing either axillary clearance or sentinel lymph node biopsy. Within 10 minutes of excision, the fresh (unfixed) tissue was dissected into 2mm slices. Each slice was imaged using a time-domain OCT system with a source centre wavelength of 1320nm and full-width-half-maximum bandwidth of 101nm. Axial and lateral resolutions of the system were ~11µm. After scanning, each sample was immediately fixed in formalin and underwent histological assessment using Haematoxylin and Eosin (H&E) staining.

Image registration was then performed between the 3D OCT and 2D histology. Custom visualisation software was implemented in C++ on a Windows platform to enable the arbitrary rotation, translation and zooming of the 3D OCT data. This enabled the H&E image to be matched against the optimal 2D plane extracted from the 3D OCT data.

Figure 1: (Left) OCT image of lymph node. (Right) H&E histology: showing lymph cortex, adipose and stroma.

3. RESULTS AND DISCUSSION

Figure 1 demonstrates the ability of OCT to differentiate the primary tissue types found in lymph nodes: stroma, cortex and adipose (fat). The stroma consists of fibrous tissue forming the lymph node capsule, and also the delicate trabeculae which extend into the node parenchyma. The cortex is the outer portion of the lymph node. The dark staining visible in the H&E stained image is due to the high concentration of lymphocytes found in the cortex. Lymphocytes are a type of white blood cell produced in the lymph node and play a critical role in the body's immune system. The adipose tissue appears as a white area with a characteristic honeycomb structure, which is also evident in the histological images.

Figure 2 shows a cross-sectional image through two node-related blood vessels. The lymph node contains a network of lymphovascular spaces that allow the various cellular components of the immune system to enter and depart the node. Blood vessels are characterised by a dark ring surrounding an area of low back-reflection.

A non-cancerous reactive lymph node is shown in Figure 3, with prominent 'secondary' lymphoid follicles containing germinal centres within the cortex. Broadly speaking, secondary 'active' lymphoid follicles form in the cortex as part of the B-cell immune response to antigens. Under H&E staining, they are characterised by a pale germinal centre, encircled by a darker mantle zone of lymphocytes. Under OCT scanning, the circular structure of the germinal centre can be seen.

Several artefacts were observed during imaging. Adipose tissue often resulted in an increased back-reflection signal from deeper tissue. This was evidenced as dark spots (high reflection) further into the A-scan. This artefact can also be seen in the images of [9]. The mechanism for this effect has not yet been verified and will require further investigation. Additionally, the image quality was noted to deteriorate deeper in the tissue due to the cumulative effects of scattering, absorption and beam spread.

Figure 2: (left) OCT and (right) H&E histology showing blood vessels.

Figure 3: (left) OCT and (right) H&E histology showing lymph germinal centres and mantles.

4. CONCLUSION

In this paper, we have demonstrated the ability of OCT to image the micro-architecture of normal lymph nodes, with correlation against a histological gold standard. OCT was seen to differentiate tissue types such as cortex, stroma and adipose. In addition, characteristic patterns have been identified for microvasculature and germinal centres. Future work will focus on characterising changes in the micro-architecture due to the presence of cancer.

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A first demonstration of audio-frequency optical coherence elastography of tissue

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ABSTRACT

Optical elastography is aimed at using the visco-elastic properties of soft tissue as a contrast mechanism, and could be particularly suitable for high-resolution differentiation of tumour from surrounding normal tissue. We present a new approach to measure the effect of an applied stimulus in the kilohertz frequency range that is based on optical coherence tomography. We describe the approach and present the first *in vivo* optical coherence elastography measurements in human skin at audio excitation frequencies.

Keywords: Elastography, optical coherence tomography, tissue mechanical properties

1. Introduction

Optical coherence elastography (OCE) reported to date has been based on speckle-tracking techniques, and has employed predominantly quasi-static mechanical loading of tissue to quantitatively assess local tissue motion [1-4]. In this paper, we present a new approach to OCE suitable for the quantitative measurement of tissue mechanical properties in the hundred Hertz to kilohertz frequency range.

2. Theory and experimental method

Consider an interferometric signal generated by a reference light beam combining with light backscattered from particles undergoing harmonic displacement along the optical (z) axis. At frequencies up to several kHz (with corresponding sound wavelength $\Lambda \sim 1\text{m}$), particles in a medium (of typical thickness $\sim 1\text{mm}$) can be expected to move in phase with each other. The dynamic interferometric signal amplitude of interest depends not only on the scatterer's vibration amplitude but also on the quasi-static phase of the interferometer, which in turn is governed by the precise differential axial position of the particle relative to the reference path in the interferometer. This unwanted dependence of the dynamic displacement on the quasi-static displacement is generally known as interferometric signal fading [5,6]. It can be overcome by various means, e.g., by polarization-based optical quadrature detection [7].

A schematic of the experimental fibre-based, time-domain OCT system utilizing balanced optical quadrature detection is presented in Fig. 1. A broadband source with a near-Gaussian spectrum centred at 1334nm with 3dB bandwidth of 42nm was employed, and the sample arm utilized a triplet lens ($f = 30\text{mm}$) to focus the beam through a glass window that provided a rigid platform upon which the samples were placed. Dynamic compression was applied to the sample, as indicated in Fig. 1 (inset), with a PZT actuator. The frequency-domain optical delay line (FD-ODL) was operated off-pivot to acquire conventional OCT images using a carrier frequency of 1150 Hz [8] and on-pivot when the vibration of sample scatterers generated the carrier frequency.

Figure 1: Schematic of the fibre-based OCT system employing balanced optical quadrature detection and the experimental geometry.

The detected photocurrents in the orthogonal polarization channels (see Fig. 1), denoted by the subscripts 1 and 2 can be expanded as a series of Bessel functions [6], resulting in the following equation:

$$i_1(z, t) = 2\rho\sqrt{I_R I_S} \left\{ \cos \phi_{DC} \left[J_0(\phi_s) + 2 \sum_{n=1}^{\infty} J_{2n}(\phi_s) \cos(2n\Omega t) \right] + \sin \phi_{DC} \left[2 \sum_{n=0}^{\infty} J_{2n+1}(\phi_s) \sin((2n+1)\Omega t) \right] \right\}, \quad (1)$$

$$i_2(z, t) = 2\rho\sqrt{I_R I_S} \left\{ \sin \phi_{DC} \left[J_0(\phi_s) + 2 \sum_{n=1}^{\infty} J_{2n}(\phi_s) \cos(2n\Omega t) \right] + \cos \phi_{DC} \left[2 \sum_{n=0}^{\infty} J_{2n+1}(\phi_s) \sin((2n+1)\Omega t) \right] \right\}, \quad (2)$$

where ρ is the detector responsivity, I_S and I_R are the sample and reference optical intensities, respectively, ϕ_{DC} is the quasi-static interferometric phase (modulo 2π) governed by the axial position of the particle, $\phi_s = \frac{4\pi}{\lambda} d(z, \Omega)$, where λ is the mean optical wavelength in the medium, d is the local, generally frequency-dependent, amplitude of vibration, Ω is the angular vibration frequency and J_n is the n^{th} order Bessel function of the 1st kind corresponding to the n^{th} order harmonic of the excitation frequency. It is readily seen from Eqs. (1) and (2) that the value of ϕ_{DC} alters the detected signal and has the potential to produce distortions in the dynamic signal. In channel 1 (Eq. (1)), the even harmonics of the signal fade when $\phi_{DC} = \pi/2$; conversely, in channel 2, the odd harmonics of the signal fade when $\phi_{DC} = \pi/2$.

The objective of the OCE scheme presented here is to deduce the absolute vibration amplitude (i.e., dynamic displacement) of the scatterer through an interferometric measurement of ϕ_s . In our approach, the theoretical values of J_3/J_1 are used to map the experimentally measured J_3/J_1 ratios to vibration amplitude, as described previously [6]. Furthermore, we use the ratio of J_4/J_2 to extend the unambiguous range of operation to beyond approximately $\lambda/3$; the first calibration range given by $J_4/J_2 < -4.696\text{dB}$ and the second by $J_4/J_2 \geq -4.696\text{dB}$. At an operating mean wavelength of $\lambda = 1300\text{ nm}$, this permits measurement of absolute vibration amplitudes of up to about $0.6\mu\text{m}$ (optical depth).

Analysis of the full-fringed digitized data was carried out in post-processing, separately for each channel. We calculated intensity images for four separate harmonic frequencies by applying bandpass filters centred at Ω , 2Ω , 3Ω and 4Ω (producing in total eight frames from the two detection channels). These frames were then separately demodulated utilizing the Hilbert transform and the resulting orthogonal envelopes at each vibration harmonic were incoherently combined. The resulting four frames, immune to signal fading, were used to produce a frame containing local vibration amplitude using the ratio J_3/J_1 as described above.

3. Results and discussion

Figure 2 presents results of *in vivo* measurements of human thick skin (on the palm) obtained by compressing the “webbing” between the thumb and index finger between the PZT rod and the sample-arm window. The OCT image (without applied vibration) consists of 32 A-scans taken over a lateral scan range of 0.8 mm . The lateral spacing was relatively large compared to the lateral resolution in order to obtain an adequate field of view with the relatively slow A-scan rate. The vibration amplitude measurement consisted of 8 A-scans centred at the same lateral position, but over a 0.4-mm range, again to avoid boundary effects. A relatively uniform signal from the skin surface to $z = 0.2\text{ mm}$ optical depth can be distinguished in both the OCT image (Figure 2a) and the average A-scan (Figure 2b), which corresponds to a layer with low vibration amplitude in Figure 2c. This layer is attributed to the stratum corneum, which displays negligible vibration amplitude due to its tight coupling to the rigid glass window and its low compressibility.

Below the stratum corneum, the OCT image does not show any clear boundary between skin layers until a depth of approximately 0.85 mm . In contrast, the vibration amplitude traces show a distinct difference in slope at about 0.65 mm that is replicated over the range of PZT drive voltages. The change in slope of the vibration amplitude traces indicates two layers of different compressibility, with the deeper layer, with greater slope, being relatively more compressible. We attribute these layers to the epidermis and dermis respectively, which are known to have distinct mechanical properties. Beyond a depth of $z = 0.85\text{ mm}$, the signal is most likely due to the multiple scattering background. The discontinuity in

the vibration traces of Figure 2d is a stitching artefact occurring at the intersection of the calibration curves (when the vibration amplitude is approximately $\lambda/3$). The dynamic OCE method put forward here naturally detects the axial motion of scatterers. Accordingly, our experimental geometry was designed so that, in each case, the PZT transducer applied even pressure to the sample coaxially with the optical beam. Further work is required to establish the feasibility of accurately detecting vibration amplitude in the presence of lateral scatterer motion, such as when shear waves are excited.

Figure 2: OCT A-scan and OCE-derived vibration amplitude versus optical depth for human skin *in vivo*: (a) OCT image without applied vibration; (b) Average A-scan; (c) Vibration amplitude image for PZT drive voltage of 2.4Vpp; and (d) Average vibration amplitude for various PZT drive voltages.

4. Conclusions

In conclusion, we have presented a new dynamic OCE method suited to quantitative displacement assessment at frequencies in the hundreds of Hertz to kilohertz regime. The method was able to distinguish the layers in a 3-layer phantom with different elasticity based on differences in the measured vibration amplitude in each layer. The first quantitative *in vivo* OCE measurements of human skin clearly distinguish layers based upon their mechanical response. In these measurements, increased driving amplitude produced a higher gradient of vibration amplitude vs. depth, indicating greater compression of compliant layers. The technique can be adapted to perform high-spatial resolution vibration-based spectroscopy, where the excitation frequency is tuned over the 100 Hz to 10 kHz range. Such spectroscopic measurements based on the frequency-dependent mechanical response of tissue could potentially enhance the capability of OCE in diagnosis of a range of medical conditions, including tumours and arterial plaques.

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