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Dynamic Light Scattering in Biomedical Applications: feature issue introduction

IGOR MEGLINSKI,[1,6] ANDREW DUNN,[2,7] TURGUT DURDURAN,[3,8] DMITRY POSTNOV,[4,9] AND DAN ZHU[5,10]

[1] *College of Engineering and Physical Science, Aston University, Birmingham, B4 7ET, United Kingdom*
[2] *Department of Biomedical Engineering, The University of Texas at Austin, Austin, Texas 78712, USA*
[3] *ICFO-Institut de Ciències Fotòniques, The Barcelona Institute of Science and Technology, Castelldefels (Barcelona), Spain*
[4] *Department of Clinical Medicine, Aarhus University, Universitetsbyen 3, 8000 Aarhus, Denmark*
[5] *Britton Chance Center for Biomedical Photonics – MoE Key Laboratory for Biomedical Photonics, Wuhan National Laboratory for Optoelectronics – Advanced Biomedical Imaging Facility, Huazhong University of Science and Technology, 430074 Wuhan, Hubei, China*
[6] *i.meglinski@aston.ac.uk*
[7] *adunn@utexas.edu*
[8] *turgut.durduran@icfo.eu*
[9] *dpostnov@sund.ku.dk*
[10] *dawnzh@mail.hust.edu.cn*

Abstract: The feature Issue on "Dynamic Light Scattering in Biomedical Applications" presents a compilation of research breakthroughs and technological advancements that have shaped the field of biophotonics, particularly in the non-invasive exploration of biological tissues. Highlighting the significance of dynamic light scattering (DLS) alongside techniques like laser Doppler flowmetry (LDF), diffusing wave spectroscopy (DWS), and laser speckle contrast imaging (LSCI), this issue underscores the versatile applications of these methods in capturing the intricate dynamics of microcirculatory blood flow across various tissues. Contributions explore developments in fluorescence tomography, the integration of machine learning for data processing, enhancements in microscopy for cancer detection, and novel approaches in optical biophysics, among others. Innovations featured include a high-resolution speckle contrast tomography system for deep blood flow imaging, a rapid estimation technique for real-time tissue perfusion imaging, and the use of convolutional neural networks for efficient blood flow mapping. Additionally, studies delve into the impact of skin strain on spectral reflectance, the sensitivity of cerebral blood flow measurement techniques, and the potential of photobiomodulation for enhancing brain function. This issue not only showcases the latest theoretical and experimental strides in DLS-based imaging but also anticipates the continued evolution of these modalities for groundbreaking applications in disease detection, diagnosis, and monitoring, marking a pivotal contribution to the field of biomedical optics.

The Biomedical Optics Express journal covers a broad range of topics, including basic research, technological advancements, biomedical investigations, and clinical uses associated with optics, photonics, and optical imaging within the biomedical field. Within this expansive domain, Dynamic Light Scattering (DLS) stands out as an experimental approach uniquely tailored for characterizing small particles in motion within turbid media [1,2]. DLS has become instrumental in the non-invasive examination of biological tissues, leveraging coherent laser light scattering caused by particle movement to provide deep insights into microcirculatory blood flow [3]. This allows researchers to delve into the intricate dynamics of blood within the brain, skin, muscles, and other biological tissues. The evolution of DLS has brought about the integration of Laser Doppler Flowmetry (LDF), Diffusing Wave Spectroscopy (DWS), and Laser Speckle Contrast Imaging

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(LSCI), offering a comprehensive non-invasive imaging of blood flow in the brain, skin, muscles, and other biological tissues [4]. Subsequent developments in the Diffusing Wave Spectroscopy (DWS) methodology, which employs near-infrared (NIR) light for non-invasive measurements aimed specifically at the direct evaluation of local microvascular cerebral blood flow (CBF), have led to these techniques being collectively known as Diffuse Correlation Spectroscopy (DCS) [5–7].

The ability of DLS-based technologies to illuminate the complexities of blood flow has paved the way for groundbreaking research in neurology, dermatology, and tissue engineering. These methods have not only enhanced our understanding of fundamental biological mechanisms but also fostered the development of novel diagnostic tools and therapeutic strategies. From monitoring cerebral blood flow to assessing tissue perfusion and characterizing microcirculation, DLS-based imaging technologies have demonstrated unparalleled potential in both clinical and preclinical research settings [8].

This feature issue aims to highlight the latest developments and applications of DLS in biomedical research. By bringing together cutting-edge studies and innovative methodologies, we seek to showcase the versatility and impact of DLS techniques in advancing biomedical optics and improving patient care. Through this collection, we invite readers to explore the current frontiers of DLS applications, reflecting on the challenges overcome and the opportunities that lie ahead in harnessing light to decipher the complexities of living systems. This feature issue comprises 20 papers (from a total of 28 submissions), predominantly focusing on the latest theoretical and experimental advancements in DLS-based imaging modalities within various biomedical studies, including clinical and preclinical applications. Nevertheless, editors anticipate a significant increase in publications dedicated to the translational clinical applications of DLS-based imaging modalities, driven by their ongoing advancement, in the years ahead. The subjects of the current feature issue are listed below, and include:

- Diffuse and Fluorescence Tomography
- Machine Learning and Processing
- Microscopy
- Optical Biophysics and Photobiology
- Optical Biosensors
- Optical Coherence Tomography
- Optical Therapies
- Tissue Optics and Spectroscopy

We provide a short introduction for each paper and classify them into several categories as follows:

Diffuse and fluorescence tomography

A novel high-resolution computational speckle contrast tomography system for deep blood flow imaging – “SpeckleCam” is introduced by Maity et al. [9]. By leveraging a camera-based approach and Monte Carlo simulations-based convolutional forward models, SpeckleCam overcomes the limitations of existing methods, such as poor spatial resolution and intensive computational demands. It achieves detailed 3D imaging of blood flow up to 6 mm deep in tissue, demonstrating its efficacy through human experiments on vascular occlusion. This advancement enhances the capability for non-invasive deep tissue blood flow analysis, offering potential for improved diagnostic and monitoring applications in vascular and neurological conditions.

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Machine learning and processing

Rivera and Schaffer present a novel approach for real-time multi-exposure speckle imaging of tissue perfusion, termed Rapid Estimation of Multi-exposure Imaging (REMI), which provides a quasi-analytic solution for fitting multi-exposure speckle imaging (MESI) data [10]. The proposed REMI technique significantly accelerates the processing of full-frame MESI images, achieving speeds of up to 8 Hz with minimal errors compared to traditional, time-intensive least-squares methods. This advancement enables real-time quantitative measurements of perfusion changes using simple optical systems, a significant leap forward from previous methods that were limited by lengthy data processing times and were thus unsuitable for real-time clinical applications. The paper validates the accuracy of REMI through simulated data and real-world experiments, including a mouse model of photothrombotic stroke, demonstrating its efficacy in capturing dynamic perfusion changes efficiently. This work promises to enhance the application of laser speckle contrast imaging in both research and clinical settings by providing a faster, equally accurate alternative to existing MESI data analysis methods.

Yu et al. introduces and validates a convolutional neural network (CNN) approach for fast, model-free blood flow imaging utilizing multiple exposure speckle imaging (MESI) [11]. The study evaluates the effectiveness of a machine learning model, specifically a CNN, as a superior alternative to the traditional, computationally intensive non-linear fitting approach for calculating blood flow maps from speckle contrasts. The CNN, trained with annotated speckle contrast data from microfluidic experiments, demonstrates its potential by providing real-time, accurate blood flow maps, contrasting the limitations of previous methods that were not universally applicable due to their dependence on specific mathematical models for different types of blood vessels. The research showcases the application of the CNN to both in vitro and in vivo data, revealing its ability to outperform the non-linear fit approach in terms of efficiency and applicability across various experimental settings. This advancement marks a significant step forward in the real-time analysis of blood flow, offering a practical solution for rapid assessment in biomedical research and clinical diagnostics.

Microscopy

Lin at al. presents a breakthrough approach by integrating DLS microscopy with deep learning to identify triple-negative breast cancer (TNBC) cells without the need for labels [12]. Leveraging the sensitivity of DLS microscopy to intracellular movements, especially mitochondrial dynamics, the research unveils how mitochondrial behavior impacts DLS imaging patterns in TNBC cells. A two-stream deep learning model is developed, utilizing DLS imaging data to differentiate between TNBC and HER2-positive breast cancer subtypes with a high classification accuracy of 0.89. This innovative method showcases the potential of combining optical imaging and machine learning for non-invasive cancer diagnosis, marking a significant step forward in label-free cellular analysis.

Mikkelsen et al. introduces a method for monitoring perfusion in the mouse hippocampus using LSCI and Dynamic Light Scattering Imaging (DLSI) through a chronic optically transparent window [13]. The study highlights the distinct optical properties of the hippocampus compared to the cortex, particularly noting that hippocampal blood vessels appear more out-of-focus due to the inverse vascular topology and an increased contribution of multiply-scattered photons. DLSI data supported this observation, revealing a significant increase in the contribution of multiple-scattering unordered dynamics in the hippocampus. This research underscores the importance of understanding the unique hemodynamic characteristics of the hippocampus to better study its role in learning, memory, and conditions like dementia and cognitive impairment. The findings also suggest that while LSCI is effective for 2D imaging of perfusion dynamics, its application in the hippocampus necessitates additional considerations due to the complexity of light scattering in this region.

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Optical biophysics and photobiology

Huo et al. represents a dynamic skin spectral reflectance model (DSSR) to investigate how skin strain influences spectral reflectance *in vivo*, considering strain-induced alterations in skin thickness and surface roughness [14]. The study integrates the hyper-elastic model with the Kubelka-Munk theory to correlate spectral variations to skin strain. By conducting *in vivo* experiments using Hyperspectral Imaging (HSI), the model captures changes in epidermal and dermal thickness across different strain states, demonstrating that stretching increases spectral reflectance while compression decreases it. This finding suggests potential applications for non-contact strain measurement and health monitoring using HSI. The research highlights the DSSR model's capability to accurately represent spectral variations in response to skin strain, offering insights into the optical behavior of skin under mechanical stress and its implications for healthcare and dermatology.

Optical biosensors

Mahler et al. explores the depth sensitivity of laser interferometry speckle visibility spectroscopy (iSVS) in measuring cerebral blood flow (CBF) by varying the source-to-detector distance [15]. This technique, applied to both human and rabbit subjects, identifies the transition point where CBF detection starts, offering a promising approach for non-invasively monitoring cerebral blood flow. The study underscores the challenge of isolating CBF signals from superficial layers like the scalp and skull. By adjusting the source-to-detector distance, researchers were able to pinpoint when deeper brain blood flow becomes detectable. Results showed a correlation between the increased source-to-detector distance and the ability to detect CBF, providing insights into depth sensitivity that align with MRI and X-ray findings for humans and rabbits, respectively. This advancement in iSVS technology highlights its potential for enhancing non-invasive brain studies, particularly in understanding the dynamic changes in cerebral blood flow with implications for diagnosing and monitoring neurological conditions.

Park et al. developed and validated a multi-channel multimode-fiber diffuse speckle contrast analysis (MMF-DSCA) system for deep tissue flowmetry, specifically targeting acupuncture points within traditional Chinese medicine (TCM) [16]. Utilizing this system, researchers conducted an acupuncture study focusing on the large intestine meridian, examining deep tissue blood flow at four different acupuncture points (LI1, LI5, LI10, and ST25) under pressure stimulus applied on LI4 (hegu or hapgok). Despite no significant changes in the blood flow index (BFI) and blood volume (BV) post-stimulus, an increase in the amplitude and complexity of low-frequency oscillations (LFOs) in microcirculation was observed, indicating potential alterations in microcirculatory function. The study highlights the system's capability for simultaneous blood flow measurements at multiple body locations, contributing to the understanding of acupuncture's impact on microcirculation and potentially offering insights into the physiological mechanisms underlying acupuncture effects.

Zora-Guzman and Guzman-Sepulveda demonstrate that coherence-gated DLS (CG-DLS) can effectively measure the size distribution of native aerosols from electronic cigarettes (ECs) without requiring sample processing [17]. It marks the first application of CG-DLS in aerial media. Using two common EC moisturizers, propylene glycol and glycerol, the experiments showed that glycerol-based aerosols become more polydisperse and contain larger particles as the burning power increases. Conversely, propylene glycol aerosols exhibited negligible changes in particle size distribution relative to burning power. This passive optical characterization of native aerosols offers critical insights for dosimetry and potential respiratory system impact, avoiding the drawbacks of aerosol dilution which can alter native compositions. The findings emphasize CG-DLS's potential for broader applications in studying optically dense aerosols.

Optical coherence tomography

Cheishvili et al. quantifies the precision and bias in DLS optical coherence tomography (DLS-OCT) measurements of diffusion coefficients and flow speeds [18]. It examines the impact

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of correlations between errors in normalized autocovariance functions on measurement accuracy and introduces a method for reducing these correlations by mixing statistically independent autocovariance functions at every time delay. This approach significantly improves precision without affecting the standard error of the mean. The paper also explores the precision of DLS-OCT using different averaging techniques and demonstrates that, while precision is identical across these techniques, averaging measured correlation functions before fitting model parameters yields the lowest bias. The findings enable a more accurate quantification of DLS-OCT measurements and validate the approach against the Cramer-Rao bound, providing insights into achieving optimal measurement precision and accuracy in biomedical imaging applications.

Liu et al. presents a study on dynamic optical coherence microscopy (dOCM) as a label-free method for assessing cell viability in murine syngeneic tumors and primary mouse liver tissue [19]. The study demonstrates dOCM's capability to distinguish live from dead cells based on intracellular dynamics, offering a novel approach to assess the effects of various therapies on tumor tissues. By comparing dOCM live contrast to viability dyes and correlating it with the optical redox ratio from metabolic imaging, the researchers establish dOCM's effectiveness in providing reliable contrast for cell viability. The technique's ability to highlight live cells in 3D volumes without the need for labels opens new avenues for investigating the physiological and pathological states of tissues, particularly in the context of immuno-oncology, targeted therapies, chemotherapy, and cell therapies.

Optical therapies

Semyachkina-Glushkovskaya et al. explores the use of photobiomodulation (PBM) during sleep to enhance the brain's drainage system, potentially improving learning and memory in male mice [20]. Utilizing confocal imaging to observe dye spreading in the brain and its accumulation in peripheral lymphatics, the research demonstrates stronger effects of PBM on the brain's drainage system in sleeping animals compared to those awake. The study employs behavioral tests, such as the Pavlovian instrumental transfer probe and the 2-objects-location test, to ascertain that a 10-day course of PBM during sleep promotes significant improvements in learning and spatial memory. This pioneering research introduces a technology for PBM under electroencephalographic (EEG) control, utilizing modern optoelectronics and biopotential detection, which could be constructed with relatively inexpensive and commercially available components. These findings suggest a novel approach in the development of phototherapy technologies for brain diseases, emphasizing the potential benefits of applying PBM during sleep.

Tissue optics and spectroscopy

Akther et al. examines the impact of light polarization on dynamic light scattering in LSCI, focusing on brain perfusion imaging [21]. By comparing no polarizer, parallel polarizer, and cross polarizer configurations in mice, Akther et al. finds that cross-polarization significantly affects the dynamic scattering parameters, including the decorrelation time, making it the preferred option for deep tissue imaging. Cross-polarization enhances coherence and sensitivity to deeper perfusion, suggesting its advantage for trans-cranial studies and emphasizing the need for careful consideration of polarization effects in LSCI data interpretation.

Liu et al. offers a comprehensive review and theoretical elaboration of DLS and LSCI, two closely related techniques exploiting speckle pattern statistics to measure CBF [22]. Addressing the need for a detailed guide due to rapid advancements in CMOS detection technology, the paper provides a thorough derivation of DLS and LSCI models, considering aspects such as non-ergodicity, laser coherence, and scatterer motion types. It emphasizes the importance of choosing appropriate models for analyzing spatial and temporal speckle statistics *in vivo*. The paper highlights how theoretical models have often been misapplied due to a lack of comprehensive summaries that fully characterize the measurements for different motion types and coherence properties. Through detailed derivations and in vivo mouse brain measurements using high frame rate CMOS cameras, this work clarifies the differences between spatial and

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temporal averaging, contributing significantly to the accuracy of blood flow measurements and the understanding of speckle dynamics in biomedical applications.

Kobayashi Frisk et al. presents a comprehensive workflow for simulating diffuse speckle statistics for optical blood flow measurements, specifically focusing on speckle contrast optical spectroscopy and tomography (SCOS and SCOT) [23]. It details a method for simulating speckle contrast signals including the effects of detector noise, validated experimentally. By examining the influence of various physical and experimental parameters, the study reveals how detector noise decreases the accuracy and precision of speckle contrast (κ) in low signal regimes, providing guidelines for SCOS and SCOT instrument design and usage. This research fills a gap in understanding the impact of experimental parameters on the accuracy and precision of speckle contrast values, particularly under low light levels, offering a valuable resource for designing and optimizing speckle-based blood flow imaging systems.

Fang et al. introduces a method to overcome camera frame rate limitations in measuring the intensity autocorrelation function $g_2(\tau)$, critical in various optical sensing applications from astrophysics to biomedical sciences [24]. The technique, called 2-pulse within-exposure modulation, allows for quasi $g_2(\tau)$ mapping in a wide field using ordinary-frame-rate cameras. By modulating the illumination within the camera's exposure using two pulses with varied temporal separation, the method enables characterization of rapid intensity fluctuations that traditional cameras' frame rates cannot capture. This approach not only simplifies the acquisition process but also significantly reduces the equipment cost compared to high-speed cameras, without sacrificing field of view or spatial resolution. The study validates this method against traditional single-point photodiode measurements, showcasing its effectiveness in both in vitro and in vivo settings, including blood flow imaging. This advancement holds promise for a wide range of applications, facilitating easier and more accessible measurement of $g_2(\tau)$ at short time lags, independent of camera frame rates.

Yi et al. enhances and validates the spatiotemporal speckle correlation model for rolling shutter speckle imaging (RSSI), a technique that captures the temporal dynamics of scattering media in a single shot using rolling shutter CMOS sensors [25]. The improved model accurately describes the row-by-row correlation of dynamic speckles, including the effects of finite exposure times, which enhances measurement accuracy and the range of speckle decorrelation time and dynamic scattering fraction. Through simulations and phantom experiments, the study establishes RSSI's parameter design and dynamic measurement capabilities, demonstrating its effectiveness in various imaging conditions. This advancement positions RSSI as a robust, cost-effective technique for quantifying deep blood flow and resolving static scattering challenges in biomedical applications.

Zavriyev et al. explores measuring pulsatile cortical blood flow and volume during carotid endarterectomy (CEA) using a combination of near-infrared spectroscopy and diffuse correlation spectroscopy [26]. In 12 patients undergoing CEA, the study observed alterations in pulsatile amplitude, pulse transit time, and beat morphology ipsilateral to the surgery side. These pulsatile hemodynamic signals offer potential for discovering non-invasive biomarkers related to cortical perfusion. The study aims to enhance clinical management of cerebral blood flow during CEA by focusing not only on average cerebral perfusion but also on cerebral hemodynamic pulsatility, providing insights into the efficacy of cerebral and systemic microvascular blood flow and clinical perfusion management strategies.

Otic et al. presents a novel standalone multi-wavelength multi-distance diffuse correlation spectroscopy (MW-MD-DCS) system for non-invasively monitoring cerebral hemodynamics in premature infants [27]. It overcomes the limitations of existing technologies by using time-multiplexed long-coherence lasers at multiple near-infrared wavelengths to simultaneously measure cerebral blood flow index (CBFi) and hemoglobin oxygen saturation (SO2). Validated through liquid phantom experiments and clinical trials on healthy adults and preterm infants, this

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system demonstrates accurate, continuous monitoring capabilities. Its development represents a significant advancement in neonatal care, potentially improving the detection of physiological instabilities and enhancing neurodevelopmental outcomes for premature infants.

Mazumder et al. utilizes interferometric near-infrared spectroscopy (iNIRS) to show that the Blood Flow Index (BFI) in the human forearm varies with wavelength, increasing between 773 nm and 855 nm [28]. This variation, not seen in a dynamic scattering phantom, suggests the wavelength dependence stems from dynamic scattering probabilities in biological tissues. Additionally, it finds that the complexity of Red Blood Cells' (RBCs) movement decreases with wavelength. Employing wavelength-division multiplexing for precise measurements, the research enhances our understanding of tissue perfusion dynamics and the interpretation of blood flow indices across wavelengths.

We hope that this feature issue will encourage the scientific community to contribute more actively to this exciting emerging research field of dynamic light scattering in biomedical applications. By showcasing the latest advancements and innovative methodologies, we aim to inspire further exploration, development, and application of these technologies. The diverse studies presented here underline the potential of DLS and related techniques to revolutionize our understanding of biological processes, improve diagnostic capabilities, and enhance patient care. As this field continues to evolve, we look forward to witnessing new discoveries, the development of novel diagnostic tools, and the translation of these technologies into clinical practice. We believe that the collaborative efforts of researchers, clinicians, and technologists will play a crucial role in advancing this domain, paving the way for significant impacts on healthcare and biomedicine.

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