Title : Multiscale imaging of skin blood flow combining Optical Coherent Tomography and Multiple Exposure Laser Speckle Imaging

Supervision : Co-supervision Laboratoire Charles Fabry , IOGS Université Paris Saclay (Arnaud Dubois, Frédéric Pain) , DAMAE medical (Jonas Ogien) and University of Latvia (Inga Saknite)

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Interdisciplinary, international, intersectoral dimension of the PhD project

The PhD project is highly interdisciplinary with innovative developments in optics, instrumentation, microfluidics, image analysis and clinical applications. It gathers an international team of three partners gathering two academic laboratories and an industrial partner (Laboratoire Charles Fabry (Palaiseau), DAMAE Medical (Paris) and University of Latvia (Riga). The co-supervisors (Inga Saknite (PhD) (University of Latvia), Jonas Ogien (PhD) (DAMAE medical), Arnaud Dubois (PhD, HDR) and Frédéric Pain (PhD, HDR) (Laboratoire Charles Fabry) will share the supervision of the student during the whole PhD project and the specific tasks in a detailed workplan.

Context and Scientific Aims

Line-field confocal OCT (LC-OCT)[1], developed by the company DAMAE Medical is one of the most efficient variation of OCT for high resolution imaging of skin, *in vivo*. LC-OCT combines low coherence interferometry and confocal filtering to provide images with high spatial resolution (~ 1 µm) and appropriate penetration (~ 1 mm) in skin tissues. LC-OCT generates morphological images of skin lesions. It produces vertical and horizontal section images of skin tissues in real time, at a rate of ~ 10 frames per seconds. The combination of cellular resolution, real-time and *in vivo* imaging, offers tremendous capability for the studying of the dynamics of *in vivo* skin at the cellular level. In its current implementation LC-OCT imaging of the skin relies mostly on morphological imaging. Methods based on laser speckle analysis, in particular Multiple Exposure laser Speckle Imaging (MESI), have proven to yield clinically relevant metrics regarding the blood flow dynamics in the skin vessels over large filed of views. Nevertheless, MESI cannot provide depth resolved measurement, and its resolution does not allow to visualize the smallest skin capillaries. Adding functional information to the anatomical LC-OCT images, in particular depth-resolved dynamic metrics regarding the blood flow in skin vessels and capillaries to complement the information yielded by techniques such as MESI, is of great interest for a vast range of clinical conditions.

The aim of the project is to extend the field of LC-OCT to functional imaging methods with metrics, with a focus on **blood flow imaging and quantitation**. Our central hypothesis is that combining LC-OCT with multiple exposure speckle imaging (MESI) will allow an unprecedented multiscale characterization of *in vivo* human tissues, providing new insights into the characterization of the macro and micro environments of skin tumors, inflammation, and the initiation and progression of chronic wounds, where microcirculation impairment is crucial[2–4]. **This multiscale *in vivo* functional characterization, unattainable by any other current technique, will provide a better understanding and subtyping of lesions, an earlier diagnosis and an eased monitoring of the treatments efficacy** eventually leading to personalized therapy based on *the in vivo* measurement of these parameters.

Preliminary results obtained at LCF on microfluidic flow phantoms have established the feasibility of tracking red blood cells with LC-OCT, in a simple way directly implementable on the commercial system developed by DAMAE Medical. Velocity calculation on individual particles was achieved and presented the typical parabolic profile of velocities for a viscous fluid in a cylindrical channel. This approach was further demonstrated in a small-scale study on skin vasculature, on 5 healthy human subjects, demonstrating the feasibility of absolute quantitation of skin blood flow *in vivo*.[16] Yet, no comparison to a gold standard was performed, and the validation of the approach based on well calibrated models still needs to be done, in particular to find the adequate LC-OCT frame rate for optimal measurements,

and possibly fine-tune the axial resolution of LC-OCT. Furthermore, the quantification was only performed by manually locating cells ins subsequent frames, making it unsuitable for real-time quantification of blood flow. **The PhD project will aim at addressing these methodological and instrumental issues.**

Methods

Original dynamic metrics will be developed, based on image analysis carried out and evaluated with scripts developed in Python and ImageJ macro language. An original imaging device will be built combining LC-OCT and MESI for multiscale imaging of microcirculation. This will involve instrumental work in optics and opto-mechanics, control of scanners and associated cameras. The characterization of the device will be carried out on original phantoms, based on microfluidic channels inserted in materials with known optical properties, mimicking biological tissues. MESI data will be integrated in the framework of DAMAE medical software (C++), and co-localization algorithms between LC-OCT and MESI data will be developed and integrated in this framework. The work will therefore be divided between instrumental work on an optical table, development of computer codes for data analysis and experimental validation on phantoms or healthy volunteers of the methods developed. Clinical work will be carried out in clinics already equipped with LC-OCT, using the system developed during the thesis, and as a backup solution the commercial LC-OCT system already present. MESI will possibly be brought to the clinics for co-localized acquisitions.

Candidate profile

The PhD candidate has a background in physics or engineering and will earn his/her master's degree in 2025. She/he is strongly interested in the fields of instrumentation, imaging methodologies and data analysis. Skills in experimental optics, Python, ImageJ, and previous interactions with biologists and clinicians would be a plus but are not a prerequisite. We are looking for a student that is ready to tackle the technical and methodological challenges of this interdisciplinary project. Curiosity and intellectual openness are essential for integrating concepts from multiple disciplines, along with a strong motivation and commitment to the project. As the project is co-supervised by three different partners in two different countries (Laboratoire Charles Fabry, DAMAE Medical, University of Latvia), the student should be able to adapt quickly to different working and cultural contexts and a good proficiency in English is required. The candidate should possess strong communication skills and the ability to collaborate effectively with diverse groups. She/he should be highly organized, capable of managing time and tasks independently, and resilient in handling the challenges of working across different environments. **It is crucial that the candidate get in touch with the supervisors as soon as possible to discuss the PhD aims and expectations.**

References

1.Dubois, A. *et al.* Line-field confocal time-domain optical coherence tomography with dynamic focusing. *Opt Express* **26**, 33534–33542 (2018).

2.Ma, K. F. *et al.* A systematic review of diagnostic techniques to determine tissue perfusion in patients with peripheral arterial disease. *Expert Review of Medical Devices* **16**, 697–710 (2019).

3.Conte, M. S. *et al.* Global vascular guidelines on the management of chronic limb-threatening ischemia. *Eur J Vasc Endovasc Surg* **58**, S1-S109.e33 (2019).

4.Huiming, G. *et al.* Study on the characteristics of microcirculation in the site of pressure ulcer in patients with spinal cord injury. *Sci Prog* **104**, 00368504211028726 (2021).

5.Mennes, O. A., van Netten, J. J., van Baal, J. G. & Steenbergen, W. Assessment of microcirculation in the diabetic foot with laser speckle contrast imaging. *Physiol Meas* **40**, 065002 (2019).

6.Sahu, A. *et al.* In vivo tumor immune microenvironment phenotypes correlate with inflammation and vasculature to predict immunotherapy response. *Nat Commun* **13**, 5312 (2022).

7.Vaz, P. G., Humeau-Heurtier, A., Figueiras, E., Correia, C. & Cardoso, J. Laser Speckle Imaging to Monitor Microvascular Blood Flow: A Review. *IEEE Rev Biomed Eng* **9**, 106–120 (2016).

8.Heeman, W., Steenbergen, W., van Dam, G. M. & Boerma, E. C. Clinical applications of laser speckle contrast imaging: a review. *J. Biomed. Opt.* **24**, 1 (2019).

9.Soleimanzad, H. *et al.* Multiple speckle exposure imaging for the study of blood flow changes induced by functional activation of barrel cortex and olfactory bulb in mice. *Neurophotonics* **6**, 015008 (2019).

10.Soleimanzad, H. *et al.* Obesity in Midlife Hampers Resting and Sensory-Evoked Cerebral Blood Flow in Mice. *Obesity (Silver Spring)* (2020) doi:10.1002/oby.23051.

11.Yu, C.-Y., Chammas, M., Gurden, H., Lin, H.-H. & Pain, F. Design and validation of a convolutional neural network for fast, model-free blood flow imaging with multiple exposure speckle imaging. *Biomed. Opt. Express, BOE* **14**, 4439–4454 (2023).

12.Schuh, S. *et al.* Imaging Blood Vessel Morphology in Skin: Dynamic Optical Coherence Tomography as a Novel Potential Diagnostic Tool in Dermatology. *Dermatol Ther (Heidelb)* **7**, 187–202 (2017).

13.Kim, T. N. *et al.* Line-Scanning Particle Image Velocimetry: An Optical Approach for Quantifying a Wide Range of Blood Flow Speeds in Live Animals. *PLOS ONE* **7**, e38590 (2012).

14.Wu, C.-H. *et al.* Imaging Cytometry of Human Leukocytes with Third Harmonic Generation Microscopy. *Sci Rep* **6**, 37210 (2016).

15.Ye, F., Yin, S., Li, M., Li, Y. & Zhong, J. In-vivo full-field measurement of microcirculatory blood flow velocity based on intelligent object identification. *Journal of Biomedical Optics* **25**, 016003 (2020).

16. Ogien, J., Fuller, J., Dubois, A., Tkaczyk, E. R. & Saknite, I. Blood Flow Velocity in Skin Microvasculature by Line-Field Confocal Optical Coherence Tomography (LC-OCT) at Two Different Acquisition Speeds. in *Optica Biophotonics Congress: Biomedical Optics 2024 (Translational, Microscopy, OCT, OTS, BRAIN) (2024), paper MS5A.2* MS5A.2 (Optica Publishing Group, 2024). doi:10.1364/MICROSCOPY.2024.MS5A.2.