Development of LASCA perfusion measurement system and investigation of the importance of certain processes affecting the data evaluation

Summary of the PhD Thesis

Kondász Bence

Supervisor:
Dr. Smausz Kolumbán Tamás
egyetemi adjunktus

Doctoral School of Physics
Department of Optics and Quantum Electronics
University of Szeged
Faculty of Science and Informatics

Szeged
2023

1. Introduction

When a rough surface is illuminated by coherent light and the scattered light is projected on a screen or onto the image sensor of a camera, a grainy interference pattern is formed, known as laser speckle. This effect is used in a wide range of everyday applications, from optical mouse to 3D imaging.

A blood flow measurement method based on laser speckle contrast analysis (LASCA) was first proposed in the early 1980s[1] as a simple and cheap alternative to the rather expensive laser Doppler measurement systems, that could monitor blood flow over large areas in near real time. Over the years, the method has evolved considerably, both in terms of technical implementations and theoretical models used in data evaluation, but it still has its limitations. While, for example, evaluation of the measurements performs on the fundus of the eye and the surface of the brain do not pose any particular difficulties, in case of the skin the presence of static scattering centers close to the surface can cause serious measurement and evaluation problems. In the latter case, the obtained perfusion results are strongly influenced by the evaluation model used and the way the measurement system is calibrated.

My work focused on the further development of LASCA measurement system available in our laboratory and to study some phenomena, which were mostly omitted in the scientific literature, while these could have an influence on the results of the data evaluation. One of my main tasks was to create small size and lightweight arm-mounted illuminator-camera system. The main challenge was the large mass of the temperature-stabilized laser source, which led me to design an easy to handle optical fiber arrangement. The multimode optical fiber allows for easier coupling of the light with higher power output, but its granular output intensity distribution limited its use in blood flow

measurement. As a possible solution to this problem, I investigated the applicability of a beam homogenizing diffuser.

I present the steps of the development of the LASCA system used in my measurements, including the tuning of components and the rationale for the choice of instrumentation. I also describe the improvements I made to the control software to make the measurements more efficient.

The contrast models also described in the theoretical overview of dissertation are important in correcting for the significant contribution of static scattering, which as a simplification, ignore the importance of so-called mixed scattering, where a given photon scatters on both moving and stationary particles. However, it arises the question of how accurately the models compute the correlation time of intensity fluctuations for different static-dynamic scattering ratios that affect the probability of mixed scattering.

Measurements on tissue models showed that when purely dynamic emulsions were covered with a static scattering layer, not only the infinitely long exposure time contrast $K(\infty)$ increased due to the static scattering. However, the motion insensitive $K(0)$ contrast, corresponding to infinitely short exposure time, also changed, which indicate an alteration in the optical properties of the sample. Based on this observation, my aim was to perform a systematic study to understand the effect of an induced change in the perfusion state of the tissue on the $K(0)$ and $K(\infty)$ values of the same area. The results will help to optimize the measurements and to (partially) calibrate the measurement system.

I believe, that the technical development of the LASCA blood flow measurement system and the performed studies will contribute the evolution of the method and increase the reliability of obtained data. In the longer term, this could lead to a

broadening of the range of applications of the method in both the research and clinical diagnostics.

2. Objectives

The detailed aims related to the further development of the LASCA system and investigation of measurement related phenomena are as follows.

As a first step I planned to study the light scattering patterns of homogenizing diffusers having different projection angles (scattering angles) in terms of their applicability in LASCA systems and machine vision. The primary focus of investigation was the influence of diffusers' illumination with different light intensity distributions on the large-scale homogeneity of the projected spot and the parameters of forming objective speckles.

The next goal in the development of the measurement system was the design and construction of a wavelength-stabilized laser-based coaxial illumination-imaging system.

Earlier results have shown that increasing the probability of static scattering leads to lower calculated flow rates. My aim is to investigate whether this discrepancy is due to errors in the computational models or to the so-called mixed scattering phenomenon (where a given photon scatters on both stationary particles and red blood cells). In the experiments, I planned to investigate the effect of changes in static/dynamic scattering probabilities on the calculated correlation times corresponding to Brownian motion in a classical tissue phantom (microsphere emulsion covered by static scattering layer) and skin perfusion. The results for two different contrast evaluation models were compared. At the same time, for comparative measurements, I aimed to create an experimental model that would allow the

generation of speckle images free of the effect of mixed scattering and to test the computational models in this arrangement.

The accuracy of LASCA measurement systems is highly dependent on the calibration of the measurement system and the monitored tissue. SESI measurement systems using a single exposure time require pre-calibration. Depending on the type of evaluation, MESI measurement systems based on multiple exposure times may require pre-calibration or even be able to re-calibrate the fitting parameters according to the time-varying optical properties. The disadvantage of MESI systems is that their temporal resolution is generally lower than that of SESI methods. For these reasons, I wanted to investigate the extent to which stimuli with large changes in perfusion in skin can alter the fitting parameters of contrast-exposure time curves, since a preliminary system calibration without re-adjustment may lead to erroneous correlation time results. A further aim was to find a combined evaluation method that could provide a measurement reliability similar to the MESI method with increased temporal resolution even in the presence of large perfusion changes.

3. New scientific results

Even though the LASCA technique has been in use for decades, reliable quantitative measurements are often difficult to obtain. The contrast and the derived correlation times are affected by a number of factors including changes in scattering of the tissue, the way the system is calibrated and possible tremor of the tissue under test, therefore further improvements are needed to increase the accuracy of the method and to make it more widely available. The work presented in my PhD dissertation addressed three major issues related to the LASCA measurement technique using multiple exposure times: the development of illumination system, the problem of mixed scattering phenomena, the variation

of tissue optical properties during measurements, and the applicability of new measurement methods. In order to prove my hypotheses and to verify my conclusions, I have carried out experiments in several stages, comparing the new results obtained in each of these areas with the literature. The results are summarized in more details in the following thesis points.

3.1. Thesis points

[T1] Investigating the applicability of beam homogenizing diffusers

To achieve uniform illumination, various manufacturers recommend so-called beam homogenizing diffusers, applicable even for laser light sources. According to the catalogues, such diffusers do indeed produce macroscopically homogeneous illumination, but little information is available on the inhomogeneity created by the objective speckle phenomenon at high angular resolution.

[T1/A] By illuminating the investigated diffusers with light collimated directly from a laser diode or from a MM optical fiber, I showed that the projected grainy image exhibits the characteristics of the objective speckle. The size of the speckles slightly exceeding the value given by a theoretical model of homogeneously illuminated randomly scattering surfaces.

[T1/B] I investigated the conditions under which homogeneous illumination suitable for megapixel machine vision, or LASCA, can be achieved. This requires that the speckle size

is much smaller than the field of view corresponding to single pixel of the sensor. By simulation, I have shown that the ratio of the two sizes must be at least 1:10 in order to achieve a Michelson contrast no larger than a compromise value of 0.3. The highest effective resolution was obtained using laser light collimated from MM fiber: using a 660 nm laser and fully illuminated 24

mm diameter diffusers with projection angles of 20° and 50°, I obtained projected diameters corresponding to 750 and 1900 effective pixels, respectively, under the above specified conditions. **[S1][S9]**

[T2] Improving the optics of the LASCA system

I developed a flexible arm-mounted optical unit with the following main features. A coaxial illumination-imaging system was built in order to eliminate the shadow effect due to oblique illumination and to allow measurements in difficult to access places (e.g. through a narrow slit). The scheme was implemented using a polarizing beamsplitter plate, thus avoiding the multiple reflections typical of beamsplitter cubes. I increased the reliability of the LASCA system by using a VHG wavelength-stabilized laser with light coupled into a single-mode polarizing maintaining optical fiber and guided to the optical unit. The VHG laser more suitable for the applied pulsed mode illumination due to its improved wavelength stability (mode hopping-free operation, high coherence length) as compared to the previously used classical Fabry-Perot laser **[S2,S3][S6-S8].**

[T3] Demonstration the significance of mixed scattering

Models used to calculate the correlation times from the image contrast are based on the simplification that speckle images are formed by photons which scatter exclusively either on the surface immobile particles or in the lower volume containing red blood cells. Earlier measurements on classical tissue phantoms have shown that if the microsphere emulsions are covered with static scattering layer (e.g. Teflon), the correlation times calculated in this way show a slightly higher value as compared to the uncovered emulsion. Since scattering is a probabilistic process there must be photons that undergo mixed scattering, i.e. scattering in both the surface static and in depth moving scattering centers.

[T3/A] In a systematic series of measurements, tissue phantoms consisting of microsphere emulsion covered with static scattering layers and skin surfaces covered with scattering layers were used to investigate the dependence of contrast on exposure time. I have shown that the increase in the relative contribution of static scattering resulted in narrowing of the dynamic range of the contrast curve, while the correlation times obtained from both applied evaluation models shifted towards higher values.

[T3/B] To study the contribution of mixed scattering, I designed a new tissue phantom. By applying a beamsplitter cube, I was able to physically separate the dynamic (microspherical emulsion) and static (scattering layer) scattering and overlapped the scattered photons on the camera sensor, thus creating interference images free of mixed scattering. By comparing these measurements with the results of the classical tissue phantom, I found that both evaluation models can handle static scattering if the probability of mixed scattering is negligible. This means that the aforementioned correlation time shift can be attributed to the averaging effect caused by mixed scattering. In order to increase the accuracy of the measurement, it is necessary to introduce a correction to determine the correlation time for the flowing particles, independent of the surface scattering layer covering them. **[S2][S7]**

[T4] Limitations of the pre-calibration of the LASCA system: dependence of skin scattering properties on perfusion

In LASCA measurements, the applications of models used to calculate correlation times from contrast values involve a (preliminary) calibration of the system, which is influenced by the characteristics of the measurement system in combination with the optical (scattering, absorption) properties of the sample. The precalibration assumes that the scattering properties do not change during a measurement process. Multi-exposure speckle

imaging (MESI) systems are suitable for determining asymptotes of the contrast-exposure time curve (K(T)), which can be used to calibrate the optical properties of the system.

[T4/A] According to the literature, the optical properties of tissues depend on the current perfusion state. In order to investigate the effect of this on K(T) curves, I performed a systematic series of measurements on the inner forearm skin by creating largely different perfusion states using the following stimuli: upper arm occlusion, treatment with capsaicin cream and local cooling. I found that the asymptotes of the contrast curve at short and long exposure times (K(0) and K(∞)) can vary together or separately during the measurements. The largest variation was observed for the cases of occlusion and capsaicin. For arterial occlusion, a small increase (~3%) in K(0) was accompanied by an increase in K(∞) of about 15%, indicating an growth in the contribution of static scattering. When applying capsaicin, which caused a strong increase in RBC concentration, K(0) decreased by about 15% along with a small increase (<5%) in K(∞). These changes suggest that alteration of the optical properties of the skin may invalidate the previous calibrations. Therefore, measurements involving large changes in perfusion may require continuous re-calibration of the system to increase the reliability of the data, e.g. by using MESI systems with the condition that the fitting parameters characterizing the optical properties are free variables.

[T4/B] As the temporal resolution of MESI methods is typically lower than that of single exposure time (SESI) methods, I tested the applicability of different approaches that can combine the advantages of both methods with some trade-offs. The results of 4 computational methods are compared: (i) a continuous MESI (1, 3, 9, 27, 81, 162 ms exposure times), against which I compared the results of the other methods, (ii) a SESI using an exposure

time (9 ms), which falls on the steepest part of the K(T) curve following a pre-calibration by MESI, (iii) fixing K(0) and using long exposure times (81, 162 ms), and (iv) fixing K(∞) and using short exposure times (1, 3 and 9 ms) in evaluation. The results of these calculations show that the largest deviation from the continuous MESI, exceeding 50%, was observed for the pre-calibrated SESI (ii), which would provide the best temporal resolution. The best agreement was obtained in case (iv), where the deviations were within the statistical standard deviation. Using the latter method at least doubles the temporal resolution compared to MESI, but with further optimization, using only two exposure times (e.g., 0.1 and 9 ms) may be sufficient. **[S3]**

Publication list

Publications related to the thesis points

[S1] B. Kondász, B. Hopp, and T. Smausz, "Homogenization with coherent light illuminated beam shaping diffusers for vision applications: spatial resolution limited by speckle pattern," J. Eur. Opt. Soc. Publ. 14, 27 (2018). MTMT identifier: **30386979**

[S2] B. Kondász, B. Hopp, and T. Smausz, "Mixed scattering as a problem in laser speckle contrast analysis," Appl. Opt. 60, 6593 (2021). MTMT identifier: **32337027**

[S3] T. K. Smausz and B. Kondasz, "Multi-exposure laser speckle contrast analysis system calibration limited by perfusion dependent scattering on the skin,". J. Biomed. Opt. 28(9), 096006 (2023). MTMT identifier: **34144534**

Other publications

[S4] S. Tomi, G. Kecskeméti, B. Kondász, G. Papp, Z. Bengery, K. Judit, and H. Béla, "Nanoparticle Generation From Nitinol

Target Using Pulsed Laser Ablation," J. Laser Micro/Nanoengineering 10, 171–174 (2015). MTMT identifier: **2948864**

[S5] T. Smausz, B. Kondász, T. Gera, T. Ajtai, N. Utry, M. Pintér, G. Kiss-Albert, J. Budai, Z. Bozóki, G. Szabó, and B. Hopp, "Determination of UV–visible–NIR absorption coefficient of graphite bulk using direct and indirect methods," Appl. Phys. A 123, 633 (2017). MTMT identifier: **3320425**

Conference papers

[S6] B. Kondász, B. Hopp, and T. Smausz Kolumbán, "A vegyes szórás mint probléma LASCA perfúzios mérések esetén," in Kvantumelektronika 2021 (Szegedi Tudományegyetem Természettudományi és Informatikai Kar Fizikai Intézet, 2021), pp. 108–111. MTMT identifier: **32063359**

[S7] T. Smausz and B. Kondasz, "Perfusion Measurement on Skin Model with Lasca Affected by Static and Mixed Scattering," in 2019 Conference on Lasers and Electro-Optics Europe & European Quantum Electronics Conference (CLEO/Europe-EQEC) (IEEE, 2019), pp. 1–1. MTMT identifier: **34103109**

[S8] B. Kondasz, B. Hopp, and T. Smausz, "Lasca Perfusion Histogram on Tissue Phantoms Composed of Bimodal Speed Distribution Scattering Centers," in 2019 Conference on Lasers and Electro-Optics Europe & European Quantum Electronics Conference (CLEO/Europe-EQEC) (IEEE, 2019), pp. 1–1. MTMT identifier: **31170437**

[S9] B. Kondász, B. Hopp, and T. Smausz Kolumbán, "Homogenizaló diffúzorok alkalmazása lézeres

kivilágításra: lehetőségek és korlátok," (2018). MTMT identifier: **2814971**

[S10] T. Smausz Kolumbán, G. Kecskeméti, B. Kondász, P. Gergely, B. Zsolt, J. Heszlerné Kopniczky, and B. Hopp, "Nanoparticle Generation From Nitinol Target Using Pulsed Laser Ablation," in The 15th International Symposium on Laser Precision Microfabrication (2014), p. 44. MTMT identifier: **2814971**

[S11] B. Kondász, G. Kecskeméti, T. Smausz Kolumbán, C. Tápai, J. Heszlerné Kopniczky, and B. Hopp, "Nitinol nanorészecskék előállítása lézeres besugárzással," in VII. Szimpózium a Hazai Kvantumelektronikai Kutatások Eredményeiről (2014). MTMT identifier: **2949403**

References

1. A. F. Fercher and J. D. Briers, “Flow visualization by means of single-exposure speckle photography,” Opt. Commun. **37**(5), 326–330 (1981) [doi:10.1016/0030-4018(81)90428-4].