A new way to monitor eye microcirculation

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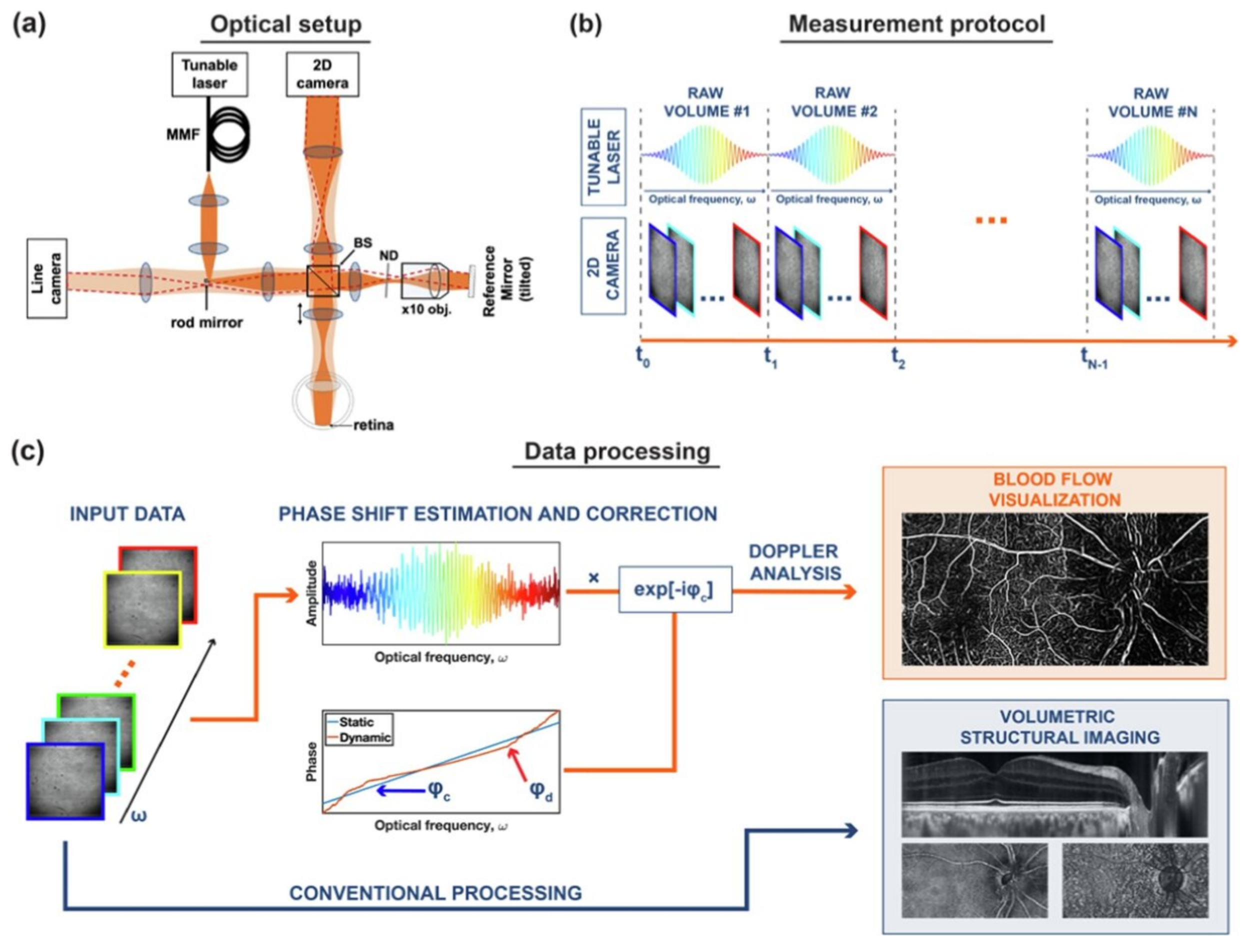


Diagrams of the optical setup, acquisition, and conceptual illustration of data processing. Credit: *Biocybernetics and Biomedical Engineering* (2024). DOI: 10.1016/j.bbe.2024.03.002

For the eyes to function properly, they must be adequately supplied with blood, and abnormalities in the microcirculation may indicate dysfunctions in other arteries, which are difficult to examine. For the first time, scientists from the International Centre from Translational Eye Research (ICTER), operating within the Institute of Physical Chemistry of the Polish Academy of Sciences, used multiwavelength laser Doppler holography to assess blood flow in various layers of the human retina in vivo, which may impact the diagnosis of circulatory disorders.

Spatio-temporal optical coherence tomography (STOC-T) is a novel method for fast and aberration-free three-dimensional retinal imaging in vivo. In previous research, ICTER scientists used a multimode optical fiber, i.e. one that at its end emits several hundred non-repeating spatial patterns in the cross-section of the beam (so-called transverse modes) to obtain hundreds of OCT images which, when added together, reduce undesirable effects, including: speckle noise.

It turns out that the data set obtained during the STOC-T study can be processed in such a way as to reveal blood flow in the human retina. Classically, visualization of blood vessels requires at least two volumes. Subtracting them from each other allows you to determine voxels whose intensity changed during the measurement. From there images of blood vessels are generated. However, this approach requires very fast repetition times, which are not available in STOC-T.

To solve this problem, ICTER scientists have developed a new method, called multiwavelength laser Doppler holography (MLDH), which allows the generation flow images from one volume, which may revolutionize the way of monitoring not only the microcirculation of the eye but also the condition of the entire body.

The research was carried out by Dawid Borycki, Egidijus Auksorius, Piotr Węgrzyn, Kamil Liżewski, Sławomir Tomczewski, Karol

Karnowski and Maciej Wojtkowski from ICTER, and the results were published in the journal *Biocybernetics and Biomedical Engineering* in a paper titled "Multiwavelength laser Doppler holography (MLDH) in spatiotemporal optical coherence tomography (STOC-T)".

What is microcirculation?

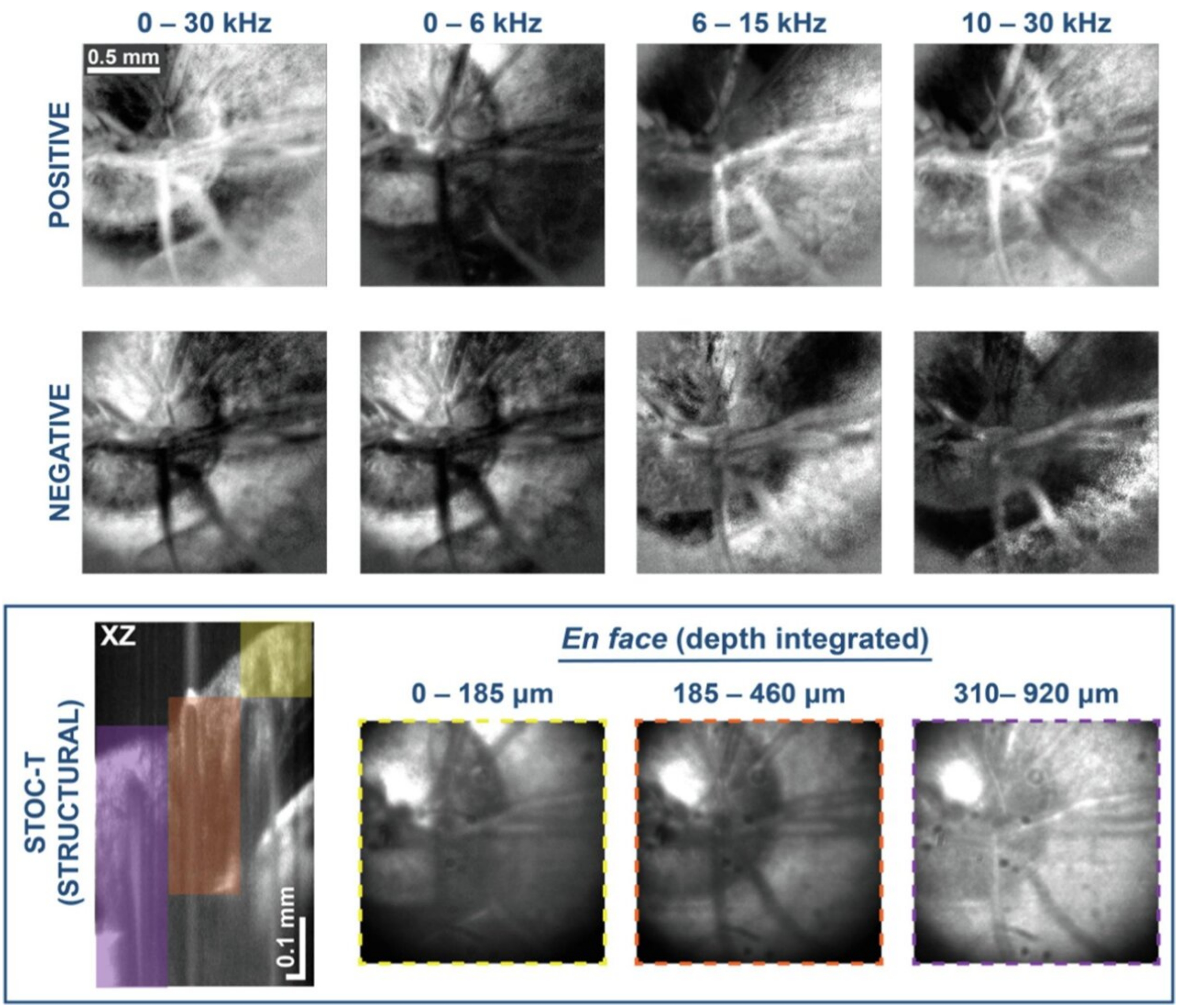
Microcirculation is the part of the cardiovascular system located between the arterial and venous systems. Microcirculation consists of vessels with a diameter of less than 150 μm, called capillaries. Arterial and venous elements are connected by "bridges" called metarterioles, from which some of the capillaries branch off. They contain the so-called precapillary sphincters, which regulate blood flow through the capillaries. The task of microcirculation is to deliver nutrients, exchange gases and metabolites, as well as regulate thermal and humoral processes.

Due to their unique accessibility, retinal arteries enable easy assessment of early vascular changes in vivo. Changes in retinal microcirculation mean global changes in the circulatory system, and therefore potential cardiac disorders. Additionally, pathological changes detected during the assessment of retinal microcirculation are one of the first signs of organ damage, which may precede, for example, proteinuria.

The retina is vascularized by two vascular systems: the choroid, which primarily supplies cones and rods; and the central retinal artery, mainly feeding the nervous tissue in the inner layers. The two systems differ in the amount of blood flow, which is much higher in the choroid than in the retinal vessels. Moreover, in the choroid, there are also significantly lower differences in blood oxygenation between arterial and venous vessels. When assessing retinal microcirculation, it is very important to precisely determine the measurement site.

Since the invention of the first ophthalmoscope in 1851 by Helmholtz, the fundus of the eye has been assessed. Even though this test was not very accurate, it allowed a small extent to assess the damage to the retinal microcirculation in the course of various diseases. In 1939, a 4-stage classification of hypertensive angiopathy and the relationship between subsequent stages of retinal vessels and an increased risk of a cardiovascular event were presented.

The study of retinal vessels has undergone a huge revolution, especially noticeable in the last 30 years. Currently, there are many tools available to assess the diameter of the vessel, the thickness of its wall, or the speed of blood flow based on the assessment of flowing erythrocytes or leukocytes. Another one just appeared.



Imaging of the optic disk with MLDH is compared to STOC-T. Credit: *Biocybernetics and Biomedical Engineering* (2024). DOI: 10.1016/j.bbe.2024.03.002

Laser Doppler flowmetry and its modifications

One of the first non-invasive methods for assessing retinal microcirculation was laser Doppler flowmetry (LDF). In the early 1980s, it began to be more widely used in the study of flows in tissues and organs. This method uses a helium-neon laser with a wavelength of 632,8 nm.

Light is reflected from red blood cells moving in the vessels and from the solid, motionless surface of the skin. LDF results are presented as erythrocyte flow values expressed in arbitrary perfusion units (PU), as it is not possible to calibrate the measurement to physiological units. This is not an ideal method because it assumes that the examined area should remain completely still, otherwise, artifacts will be created that affect the result.

An extension of LDF is scanning laser Doppler flowmetry (SLDF), which allows not only the assessment of retinal microcirculation parameters but also the morphology of the arterioles themselves. In turn, bidirectional laser Doppler flowmetry (BLDV) involves a complete assessment of the flow velocity of erythrocytes in the retina.

The Doppler spectrum of the laser can be decomposed to obtain the velocity distribution of moving cells. Recently, a similar approach was used to visualize in vivo velocity-resolved images of human retinal blood flow. For this purpose, laser Doppler holography (LDH) was introduced and used, in which the shifted Doppler optical field, backscattered from the retina, is detected using a holographic or interferometric full-field optical system.

A new technique for imaging eye microcirculation

Both LDF and LDH use light with a fixed wavelength. For this reason, both techniques in their original implementation do not provide detailed information about blood flow encoded in the optical field, which changes over time due to movement. A very interesting approach is the combination of dual-beam Doppler with optical tomography (OCT), which enables imaging and assessment of retinal layers. This, in turn, allows for simultaneous assessment of blood velocity and blood flow in the retinal vessels.

ICTER scientists recently demonstrated that by spatially modulating the phase of incident light, the laser's spatial coherence can be reduced. Using a technique called spatio-temporal optical coherence tomography (STOC-T), it is possible to obtain many different OCT images, which, when averaged, allow for the removal of noise and distortions. This approach allows for in vivo imaging of the choroid with high spatial resolution.

It turns out that the same dataset can also be used to extract dynamic images of blood flow in the human retina. Individual two-dimensional STOC-T images, after appropriate digital correction, can be used to increase time resolution and obtain flow images.

Now, a team led by Dr. Borycki has developed and tested an innovative method using STOC-T tomography to improve the visualization of blood flow in the human retina in vivo using the so-called multiwavelength laser Doppler holography (MLDH). It combines laser flowmetry with holographic multiwavelength detection, allowing non-invasive visualization and quantification of blood flow in various layers of the retina. This is possible at high blood cell flow rates and with high resolution. This combined approach enables effective assessment of eye microcirculation and, ultimately, extrapolation of the obtained results to the entire circulatory system.

"Our method enables the acquisition of two-dimensional images of blood flow en face from a stack of interferometric images with different wavelengths recorded in ~8.5 ms. This time is comparable to the time needed in the case of conventional optical OCT (assuming a scanning frequency of 100 kHz) to register a pair of repeated cross-sectional scans, from which a one-dimensional image of blood flow can be obtained," says Dr. Borycki from ICTER, one of the authors of the newly published work.

It is worth adding that the implementation of MLDH does not require any modification of the standard STOC-T tomography protocol because this method uses blood flow information from the same data set. Therefore, MLDH can be considered a valuable extension of STOC-T tomography, which gives a complete picture of what is happening in our retina.

More information: Dawid Borycki et al, Multiwavelength laser doppler holography (MLDH) in spatiotemporal optical coherence tomography (STOC-T), *Biocybernetics and Biomedical Engineering* (2024). DOI: 10.1016/j.bbe.2024.03.002

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