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Title of the PhD Project:

Multispectral Preclinical Cardiovascular Diagnostics

Private and confidential material

Brief introduction to my project from my web profile: http://www.phoqus.eu/projects_esr12.shtml

Cardiovascular Disease (CVD) represents the main cause of deaths worldwide. Traditional risk factors such as hypertension, high cholesterol, age, sex, smoking, etc. are not able to fully explain the incidence of the disease. For this reason cardiovascular research is focusing on the exploration of novel markers (e. g. inflammation and oxidative stress) for a better assessment of CVD risk. Oxidative stress and inflammation cause endothelial dysfunction in the peripheral circulatory system before any clinical manifestation. In addition several studies report that patients with impaired coronary vascular function also have evidence of peripheral microvascular dysfunction, suggesting a generalized disorder in the regulation of the microvasculature. Thus the evaluation of vascular function and stress markers in microvasculature represents a potential tool for preclinical assessment of CVD risk. Skin is a suitable model to study *in vivo* these putative novel surrogate markers, due to the abundance of peripheral small blood vessels in the dermal layer and also for the easy external accessibility to perform the analyses. The non-invasive laser-based *Laser-Doppler*, *Tissues Reflectance Oximetry*, and *Pulse Oximetry* methods allow *in vivo* study of skin microvascular function, and the *Laser Fluorescent Diagnostic* technique the investigation of skin fluorescent biomarkers related to oxidative stress (e. g. NADH, Flavins) through the stimulation by external laser irradiation of the natural endogenous tissue fluorescence. Therefore the aim of this research is to explore and combine such methods to obtain reproducible measurements from the skin and establish novel early surrogate markers for the preclinical assessment of CVD risk. The technique will be optimized in mouse models and then translated to humans.

LAKK-M device

The non-invasive multifunctional laser-based *LAKK-M* device (**Figure 1**) is a potential tool for the evaluation of both of skin microvascular function and **endogenous autofluorescent biomarkers**. This system combines *Laser-Doppler Flowmetry* (LDF), *Tissues Reflectance Oximetry* (TRO), and *Pulse Oximetry* methods to study *in vivo* simultaneously different microcirculatory parameters in the skin, such as index of perfusion (I_m) which defines dynamic characteristics of blood microcirculation, oxygen saturation (SO_2) which defines the delivery and consumption of oxygen in the tissue, and the relative erythrocyte fraction volume (V_r) in the analyzed area (**4-5 mm^3 of tissue**). The device allows also the real time study of **skin fluorescent biomarkers** using the ***Laser Fluorescent Diagnostic* (LFD)** method which induces endogenous fluorescence of natural tissue biomarkers (e. g. NADH, Flavins, etc.) by external laser irradiation.

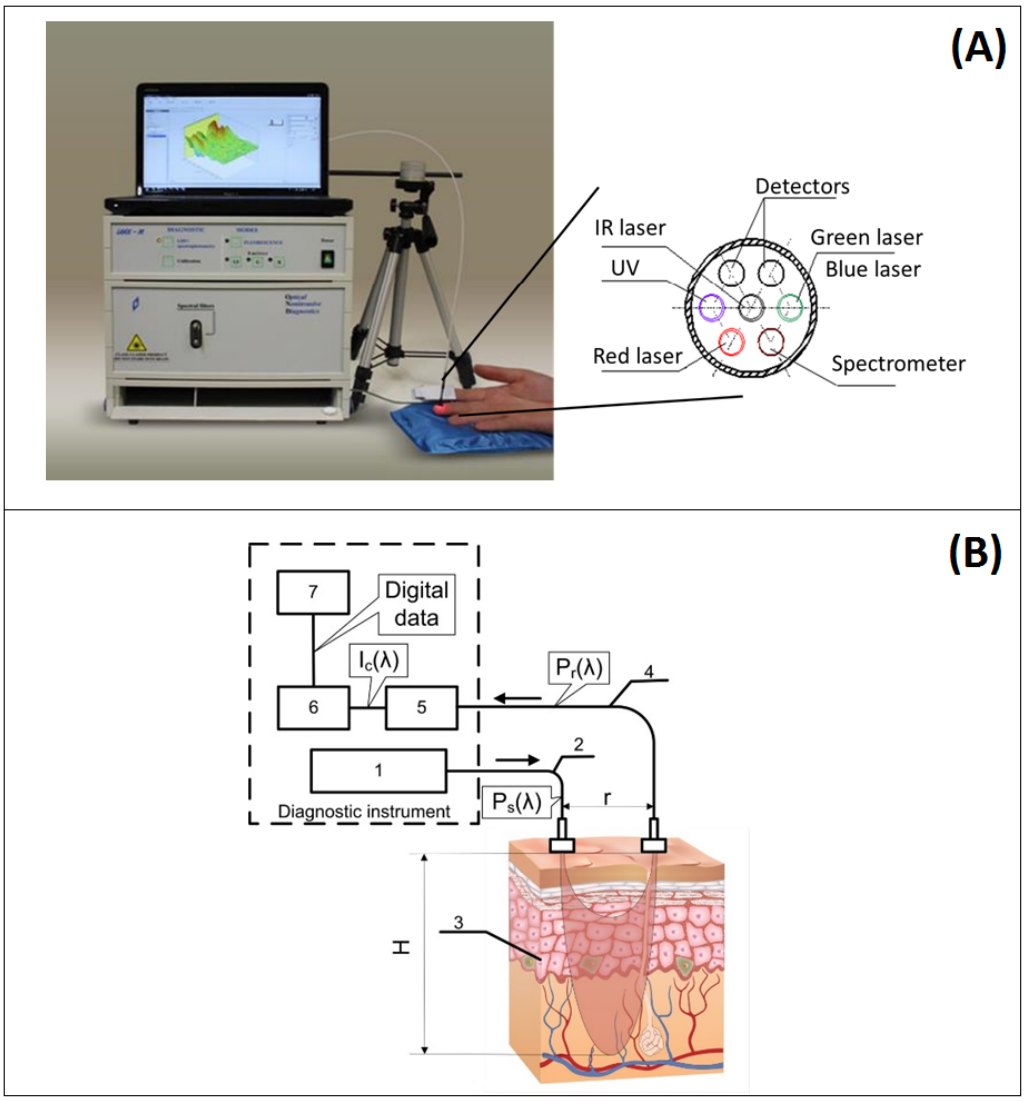

Figure 1 ***LAKK-M device***

(A) The system is provided with a multi-probe composed by 7 different sensors: spectrometer, infra-red, UV, blue, green and red lasers, and two detectors. The advantage is the possibility to apply a multi-parametric approach, measuring simultaneously different parameters, through the placement of the probe at direct contact with a specific site in the skin surface. (B) The general operation scheme of the device is the following: (1) The system is provided of an optical radiation source with adjustable wavelength λ and power $P_s(\lambda)$. (2) An optical fibre allows to transfer the radiation from the light source to the biological sample. (3) The radiation is scattered and partly absorbed by the biological sample. (4) A fraction of the scattered light constitutes the backscattering radiation. A fraction of the emitted backscattering radiation (with $P_r(\lambda)$ power < $P_s(\lambda)$ power) is transmitted to a detector by an optical system. (5) The detector converts the $P_r(\lambda)$ power into voltage $I_c(\lambda)$. (6) The electric signal $I_c(\lambda)$ is filtered and digitized. (7) The digital data is applied to a computer for mathematical processing.

The laser beams are delivered through an optical multi-fiber which includes a probe at the extremity. The probe contains all of the sensors for the delivery of the different beams, and the detectors for the collection of the backscattered light and the fluorescence becoming from the tissue (**Figure 1A**). The distance between the irradiating sensor and the detector in the probe is around 1mm. The optical fiber is fixed in a tripod in order to ensure that the probe is perpendicular to the surface of the analysed skin. The probe has to be placed at direct contact with the skin before the delivery of the beam and the analysis are performed.

Fluorescence measurements by LAKK-M

Fluorescence data are collected from the right middle finger (palmar side) or the forearm using UV (365nm), blue (430nm), green (532nm) and red (635nm) radiation wavelengths. The switching between different fluorescence spectral channels is made in manual mode using different optical filters specific for each laser (UV, blue, green and red). The **Figure 2** shows examples of the output complex emission spectra obtained collecting the fluorescence measurements from human skin.

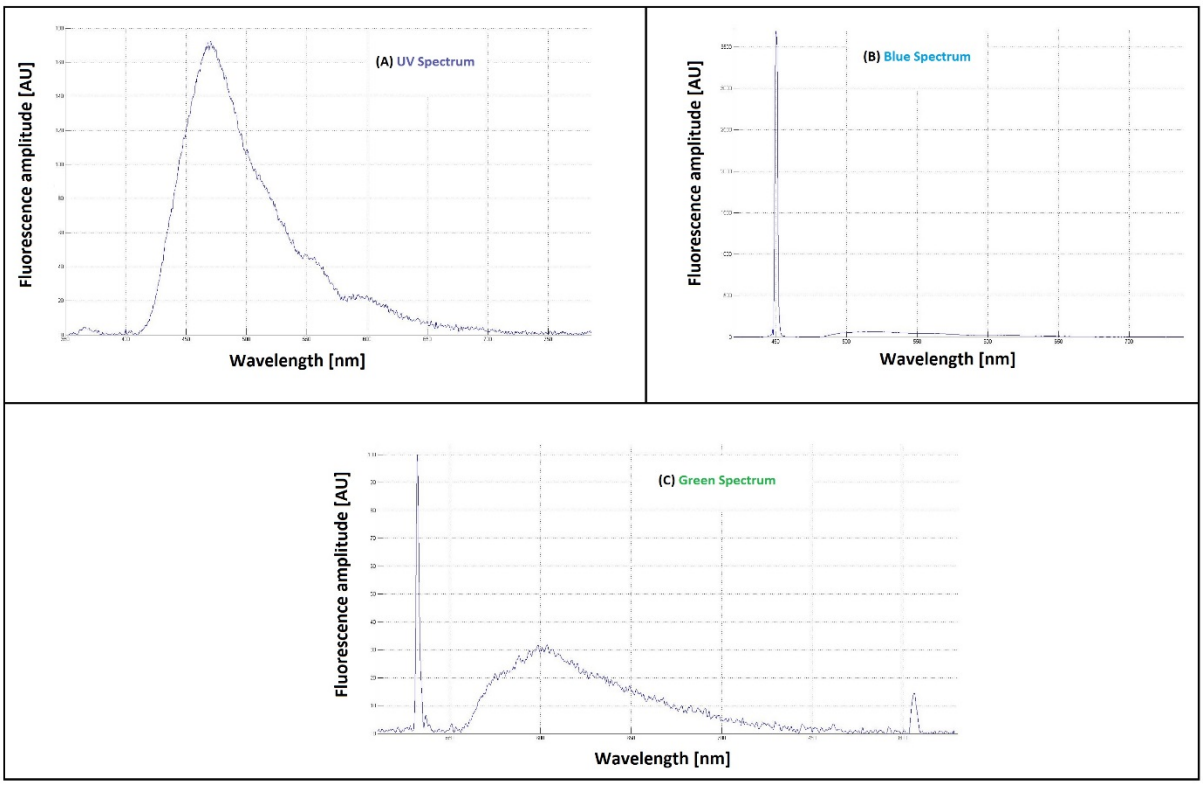

Figure 2 *LAKK-M fluorescence emission spectra*

Examples of fluorescence emission spectra obtained after the irradiation of the human right middle finger at 365nm (A), 450nm (B) and 532nm (C).

The fluorescence peaks of the investigated natural biomarkers contributing to the spectra are deduced in relation to their theoretical emission wavelength (according to the literature and to the manufacturer of the device):

- **Fluorophores excited by UV light.** Collagen 420nm, Elastin 450nm, NADH 490nm, Piridoxyne 525nm, Flavins 550nm, Lipofuscin 570nm, Beta-Carotene 608nm.
- **Fluorophores excited by blue light.** Flavins 510nm emission wavelength.
- **Fluorophores excited by green light.** Porphyrins 1 640nm, Porphyrins 2 680nm, Beta-Carotene 608nm, Lipofuscin 570nm.
- **Fluorophores excited by red light.** Porphyrins 710nm, Keratin 670nm.

What we need to figure out?

The rationale of performing a 3D modelling analysis is related to the need of getting a better understanding of what we are exactly measuring using the LAKK-M device. This is an important point to clarify before testing the machine for biomedical applications in the cardiovascular field. The challenge is to understand the following points:

- How much of the fluorescent signal is due to the contribution of the investigated fluorophores?
- How much is the overlapping of the signals from different fluorophores (**Figure 3**)?
- How much of the signal is masked and affected by the absorption of natural absorbers such as haemoglobin and melanin?
- Do the dynamic processes in the tissue (e. g. changes in blood volume, blood flow and oxygen saturation) affect the signal and how much?
- What other factors in the skin tissue are affecting the fluorescent signal and how much?
- From which layers of the skin the fluorescent signal arises?
- How the spatial variability due to the heterogeneous anatomy of the skin affects the signal of multiple different measurements collected from the same or different subjects?
- How the detected signal changes from different tested areas such as finger or forearm?
- How the skin colour affects the signal?

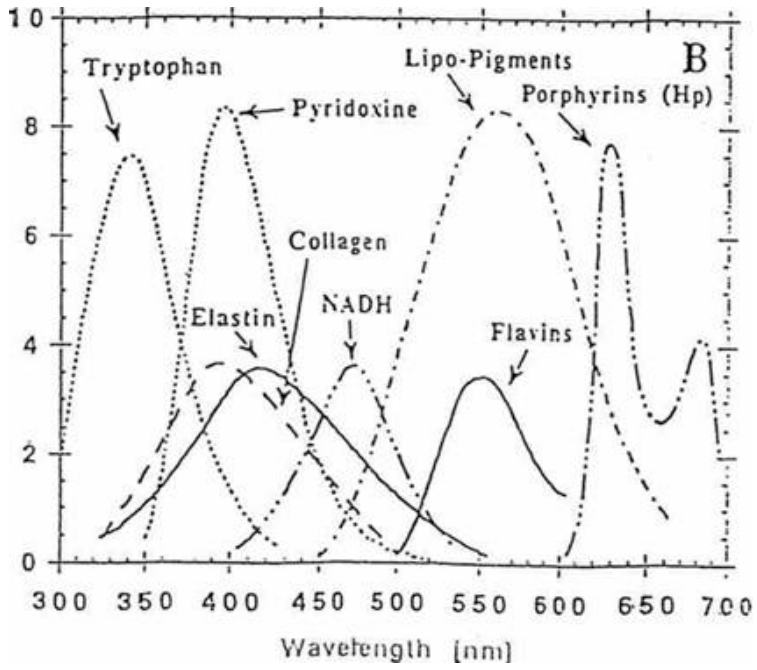

Figure 3 *Overlapping of tissue biomarkers fluorescence spectra*