Response to Reviewer 1 Comments

The authors present a compact hybrid device combining hyperspectral and thermal imaging for functional characterization of human skin in vivo. The occlusion tests have been used to validate system performance. The paper adequately describes the development and testing of the device. However, it leave a space for the improvements and corrections.

Dear reviewer. Thank You for valuable comments. We take in account all of your suggestions and we made improvements of the paper. In a response to Your comments here are answers to questions.

1. Please specify what is the irradiance level on the skin surface during the measurements?

Measured irradiance in the skin plane is 1 mW/cm².

2. Specify the field of view diameter and the spatial resolution of the system in both cases for hyperspectral and thermal imaging.

Hyperspectral subsystem: field of view diameter = 50 mm, spatial resolution = 0.18 mm;

Thermal subsystem: field of view = 150x112 mm (640x480pix.), spatial resolution = 0.23 mm

3. What are the data acquisition time and data processing time in both cases?

Hyperspectral subsystem: Data acquisition time is 80 ms (12.5 fps). Data processing time for each saturation map is 1 second.

For thermal subsystem, data acquisition time is 40 ms (25 fps).

4. Please explain in more details what is the meaning of A matrix in the eq. 1.

The matrix A in eq. 1, is a tabulated Ximea sensor sensitivity data for 16 spectral channels (data rows) in wavelength interval 400-1000nm (data columns). Sensor sensitivity data are provided by Ximea manufacturer page. The matrix A is used in eq. 1 to provide spectral unmixing of sensor raw data.

5. Same for w_i in the e.q 7.

The parameter w_i is a weight factor for each cluster of saturation map, where i=1:K, K is a number of clusters. The weight w_i depends on relative count of pixels included in corresponding cluster, and can be found by following formula:
$w_i = A_i / A_{RoI}$, where $A_i$ is an area of $i$-th cluster (count of pixels), $A_{RoI}$ is an area of all visible region of interest (count of pixels inside the disk area which is enclosed by rectangular RoI, see Fig. 2b).

6. The authors have reported that the measured blood oxygenation in the base line is close to 100%. This is valid for the arterial blood, however, the skin contains venous blood as well with the oxygenation of 80-70%. I suppose that you measure the averaged value of the oxygenation of the mixed blood. Did you apply any calibration/correction?

Dear reviewer. We understand your concerns regarding skin hemoglobin saturation with oxygen, as present literature report very controversial baseline values, ranging from 40-98% [1]–[7] when registering from the skin at different body sites.

We think these controversies could be partly explained by measurement methodology (used optical model and its parameters, illumination, etc.), skin temperature which influences perfusion and to the large extend measurement site.

At the adequate perfusion, in normal physiological conditions arterial blood saturation (SaO2), is usually in the range of 95-100%. However tissue capillary SaO2 largely depends on arterIALIZATION of blood, corresponding to a certain venous admixture, which depends on the arterio-venous difference [8]. As the skin metabolism and oxygen extraction rate is low, present A-Vdiff is rather small.

The structure of non-glabrous palmar skin is different from that in the rest of the body. It exhibits much higher capillary density, and larger count of arteriovenous anastomoses which insures low arterio-venous difference and high blood arterialization, while skin metabolism is rather low.

Moreover the cutaneous dermal layer comprises mixture of microcirculatory vessels, predominantly capillary loops, but also arterioles and venules, which all influence SaO2 in integral manner.

Speaking technically-oxygen rich arterial blood (SaO2 close to 100%) is directly drained to veins via arterio-venous anastomoses resulting in high saturation of venous blood and consequently nearly arterial values of integral baseline SaO2.

It is also supported by our previous study assessing saturation of skin capillary blood from the non-glabrous skin of the fingers utilizing whole blood analyzer as a referent method [9], and our unpublished pilot study assessing non-glabrous skin capillary blood oxygenation of palm (present HSI recording site) using medical grade whole blood analyser (Avoximeter 1000E, Accriva Diagnostics) obtaining SaO2 values ranging from 93-99%, which correspond to arterial blood.

Taking into consideration saturation measurement error of whole blood analyser (±2%) results are reliable and similar to our present HSI findings. The baseline group mean SaO2 values reported in present study is 99.8±0.7%, which correspond to arterial, seems logical, as our measurement site has largely arterialized capillary blood, and subjects had adequate blood perfusion (31–33 C, as indicated by thermal imaging).We believe that high SaO2 baseline values are exclusively attributable to arterio-venous anastomoses rich sites in the non-glabrous skin.


7. Correct the table 2 so that the values are placed within one line. Currently, it is quite hard to read.

The table 2 is corrected for better view.

8. What are the star-like or branch-like structures in fig 4? Unfortunately, I cannot distinguish them.

In the thermal image, acquired in first minutes of hyperemia stage there are not distinguishable star-like spots, but there are visible small areas with higher temperature (“hot-spots”). Unfortunately, we are not able to distinguish star-like structures using Xenics thermal camera used in our device. Previously, in the laboratory, we measured spot phenomena by more advanced FLIR camera, and we were able to identify star and branch-like structures during the first minutes of hyperemia.

Here we emphasized some of thermal spots, arising at hyperemia stage, by white circles (fig 4c).

9. On page 9, the authors report about the scattering parameter that has been measured. What it actually is?

The scattering parameters $\mu_s$ (500nm) (reduced scattering at 500 nm), $f_{Ray}$ (Rayleigh scattering) and $b_{Mie}$ (the scattering power for Mie scattering) were used in model calculations. These parameters were not measured in our experiments.
10. Add scale bars to fig 3 and fig 4.

Scale bars was added in figures 1, 3 and 4.

11. On page 9, the authors discuss about the comparison of the developed system to more expensive spectral imaging systems. Could you please estimate the cost of your system? Please provide also the links to the systems you are talking about.

The cost of our system includes the following costs: HSI system 15KEur, TG system 12.5 KEur, CPU 1KEur, other components 1 KEur. The total cost of our system is 29 500 EUR.

In the reference section, there are links provided to commercial systems [14,15] and prototype devices which are studied in other works [16-24].

12. Please perform a professional language check as the current manuscript contains some stylistic issues, grammar mistakes and typos.

The manuscript was checked by English native speaker and corrections were done.