**SCIENTIFIC REPORT**

**on the 3rd period results of the NRP SOPHIS, project # 3**

**„Biophotonics: Imaging, Diagnostics and Monitoring”**

(01.04.2016.-31.12.2016)

Goal of the project: to develop innovative technologies for obtaining and processing of the bio-object images.

Research tasks for the 3rd period:

1. To take laboratory and clinical measurements for approbation of the novel imaging technologies:

1.1. obtaining several monochromatic spectral images from the data of a single digital colour image;

1.2. non-contact monitoring of cardiovascular parameters at the near infrared spectral range;

1.3. Tissue imaging at the near infrared spectral range (1-2 microns).

2. To validate clinically the improved skin multimodal imaging prototype device „SkImager”

**Introduction**

The main attention during the project 3rd period was drawn to practical validation of the developed technologies and data processing tools in laboratory and clinical conditions. All clinical measuments were taken under permission of the Ethics Committe, University of Latvia, in the presence of certified medical doctors and with written consent of the patients to participate in validation of the novel technologies. The clinical measurements took place at the Clinics of Laser Plastics, Riga Hospital of Traumatology and Orthopaedics and Prof. J.Kisis Clinics of Aestetic Dermatology.

* 1. **Laboratory and clinical measurements for obtaining several monochromatic imagaes from the data of a single colour image.**

The three spectral line laser illuminator elaborated at the initial phase of this project together with a Nexus5 smartphone was approbated on three kinds of skin pathologies – nevi, keratoses and hemangiomas, alltogether 32 pathologies. The first two of them are pigmented pathologies with increased melanin content, and the third is vascular pathology with incresed concentration of blood haemoglobin.

Results are illustrated on the figures 1-3. 5 typical malformations are selected from each pathology group. On the left (A) a colour photo taken under illumination by 3 spectral lines (448nm, 532nm un 569nm) is presented, followed by the calculated distribution maps of oxyhaemoglobin (B), deoxy-haemoglobin (C) and melanin (D) relatively to the healthy skin.

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| dz6_att | dz6_ox | dz6_deox | dz6_mel |
| dz29_att | dz29_ox | dz29_deox | dz29_mel |
| Nevus | Oxy-haemoglobin map | Deoxy-haemoglobin map | Melanin map |

Fig.1. Relative changes of the chromophore concentrations in skin pigmented pathologies – nevi. The colour scale is graduated in milimoles.

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| --- | --- | --- | --- |
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| dz22_att | dz22_ox | dz22_deox | dz22_mel |
| dz8_att | dz8_ox | dz8_deox | dz8_mel |
| dz3_att | dz3_ox | dz3_deox | dz3_mel |
| **Seborrheic keratosis** | Oxy-haemoglobin map | Deoxy-haemoglobin map | Melanin map |

Fig.2. Relative changes of the chromophore concentrations in skin pigmented pathologies **– seborrheic keratosis.** The colour scale is graduated in milimoles.

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| dz14_att | dz14_ox | | dz14_deox | | dz14_mel |
| dz16_att | | dz16_ox | | dz16_deox | dz16_mel |
| Hemangioma | | Oxy-haemoglobin map | | Deoxy-haemoglobin map | Melanin map |

Fig.3. Relative changes of the chromophore concentrations in skin vascular pathologies **–** hemangiomas. The colour scale is graduated in milimoles.

The image processing requires knowledge of of the photon absorption pathlength in skin, which was estimated by means of Monte Carlo simulations in collaboration with the University of Oulu in Finland (Dr. A.Bykov). Results of the simulations are presented on Fig.4 and Table 1.

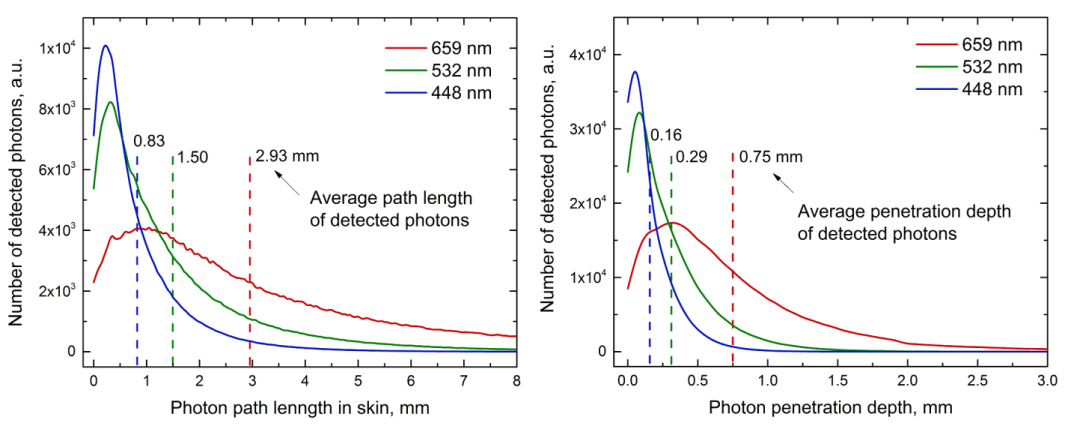
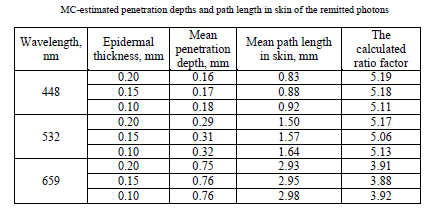


Fig.4. Results of Monte Carlo simulations for photon propagation in skin at the exploited wavelengths.

Table 1

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**The main conclusions**:

* The developed prototype device and algorithm provides the clinical data that are in qualitative agreement with the physiologically expected ones:
  + Increased epidermal melanin content in the pigmented pathologies (nevi, keratoses),
  + Increased oxy-haemoglobin and decereased deoxy-haemoglobin content in the vascular pathologies (hemangiomas) .
* In most cases insignificant or no changes of haemoglobin was observed in the maps of pigmented pathologies, and insignificant or no changes of melanin observed in the maps of vascular pathologies
* Grainy structure of images due to laser specles was observed
* Calibration of the colour scales in milimoles opens possibilities for quantitative diagnostics, i.e. to estimate increase or decrease of the chromophore concentration
* The data related to the healthy skin (c = 0) are limited - it is impossible to determine the relative change of chromophore concentration in the pathologies
* Use of the Beer’s absorption law in image processing requires knowledge of the mean path of the remitted photons in skin; the Monte Carlo data have to be validated experimentally, e.g. by the time-of-flight methods.
* Besides the three regarded chromophores, also bilirubin, beta-carotine, lipids and water can contribute in skin absorption. To map distribution of these chromophores, the doulbe-snapshot technology at two combinations of the illumination spectral lines has to be developed.

In addition to the clinical measurements, during the 3rd period the previously patented approach for obtaining n>3 monochromatic images (LV 15106) was implemented in an experimental device comprising four laser switchable illuminator and a smartphone. Laser beams of different wavelengths from 4 modules were directed to a diffusive reflector vibrating with ~80 Hz frequency. 8 vibrations during the 100 ms exposure time had “smeared” the laser speckles so improving the quality of images. Two modules could be manually switched, so two sets of 3-wavelengths illumination was obtained proving the possibility to map 4 skin chromophores. Design scheme of the new device is presented on Fig.5.

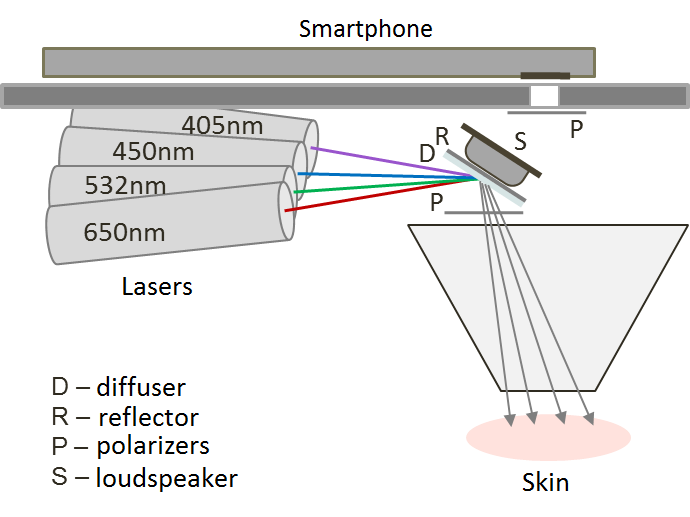
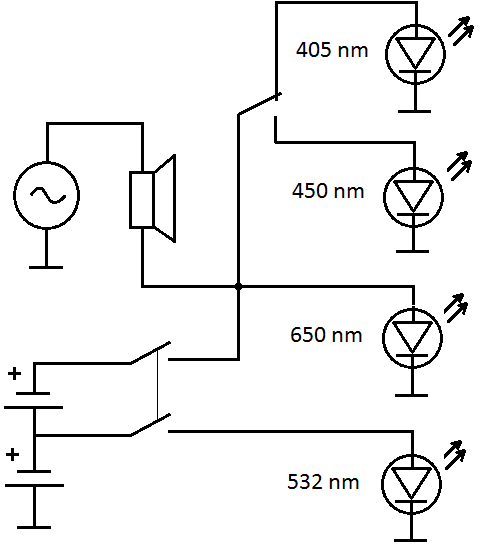


Fig.5. Design scheme of the four laser illuminator.

The prototype device (Fig.6) contains 4 laser modules emitting at 405, 450, 532 and 650nm. Four rechargeable AA-type batteries are used as power supply providing voltages 3V and 6V. Alminium foil reflector R covered by a diffuser D is fixed on a smal-size speaker S that generates ~80 Hz vibrations. Electonic circuit is presented on Fig.6, b.

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a b

Fig.6. Prototype device for 4-wavelength illumination (a) and its electronic circuit scheme (b).

**1.2. Laboratory and clinical measurements for the contactless monitoring of cardiac and circulatory parameters in the near infrared spectral range**

During the project, the laboratory tests and clinical validation of the newly developed prototype device were performed. In the preceding period of the project we were developed two prototype devices for contactless heart and circulatory monitoring using near-infrared radiation. The aim of our work was to test the possibilities of these devices for measurement of human skin microcirculation amplitude under different hemodynamic load conditions. It was carried out by an external provocation (pressure cuff or regional anesthesia), causing direct circulatory changes that can be measured by developed devices. Below is the detailed measurement and description of the results.

***1.2.1. Laboratory measurements performed by prototype camera and high-resolution camera for contactless subcutaneous photoplethysmography measurements in the near IR range***

For study of contactless skin circulatory processes there was used newly developed device, which includes an IR illuminator and a compact video camera (Ximea, CMOS 8-bit 480x640 pix. resolution). In order to objectively assess the camera's sensitivity of blood circulation determination, the measurements were performed simultaneously by professional scientific camera (ANDOR, cooled sCMOS matrix, high-dynamic range16-bit 2048x2048 pix. resolution). The aim of tests was to make sure about the usefulness of the developed prototype device for skin blood flow measurements, and to what extent it is "lagging behind" the professional scientific hardware.

In laboratory experiments 11 healthy people were participated (10 successful measurements obtained from 3 men and 7 women). Each person was situated on supine position on couch, right hand was fixed by vacuum pillow but fingertips were fixed by adhesive tape to prevent any movements that could cause artifacts in the PPG signal. Above the wrist there was placed a device (at distance 10-15 cm) that includes a plate of the IR emitters, the prototype camera, professional camera and control unit (see. Fig 7a).

For obtaining high variability of the PPG signal we used a human-friendly mechanical provocation, when the measurements of skin were performed at a "normal blood flow" conditions and "without blood". For the changing of microcirculation of palm, the compression pressure cuff (220 mmHg) was applied to the upper arm. The measurement was carried out in 3 phases: 1) normal condition - 2 min., 2) cuff compression (occlusion) - 3 min., 3) compression release (hyperemia) - 5 min.

During the measurements the video recording of outer palm skin surface was performed simultaneously by both cameras (Ximea - 640x480 pix., 50 frames/ sec, Andora - 512x120 pix., 50 frames/ sec). The intensity and spatial distribution of IR radiance were adjusted with regards to obtain the maximum possible video image intensity and to reach flat-field light distribution of the surveyed skin area.

Later the video signals were analyzed by the PPG analysis software (previously developed in IAPS UL). Each video file was analyzed and the PPG signal was calculated from chosen area of skin (Figure 6 b). Pulsatile signal component (cardiac pulse in the range 42-120 beats/min) and the amplitude of each cardiac cycle was calculated from whole PPG signal. The median average values of PPG signal amplitudes were calculated for each subject in two loads: 1) normal condition in the time interval 30-120 sec and 2) occlusion in the interval 150-300 sec. It was assumed that the PPG signal does not depend on the blood flow during the occlusion period, but it is affected only by camera noise. Thus, the PPG signal was normalized to this "zero" level for every measurement.

Figure 8 and 9 shows the amplitude dynamics during the three loads and the average levels of normal condition and occlusion. The PPG signal amplitude varies for every subject, that can be explained by individual differences in microcirculation. The results showed that regardless of the subject's microcirculation differences, the circulation intensity in palm skin can be measured by both prototype camera and high-resolution Andor camera. The results showed that the sensitivity of the Andor camera exceeds sensitivity of prototype camera more than 2.75 times in determination of microcirculation amplitude in wrist flow measurements.





a b

Figure 7. The experimental setup (a) and video frame of palm skin under IR illumination (b).



Figure 8. The graphs of dynamics of skin microcirculation PPG amplitude under three loads: 1) normal condition, 2) occlusion, 3) hyperemia. The red lines show the borders between the loads; black lines shows median values of amplitudes obtained in these loads.

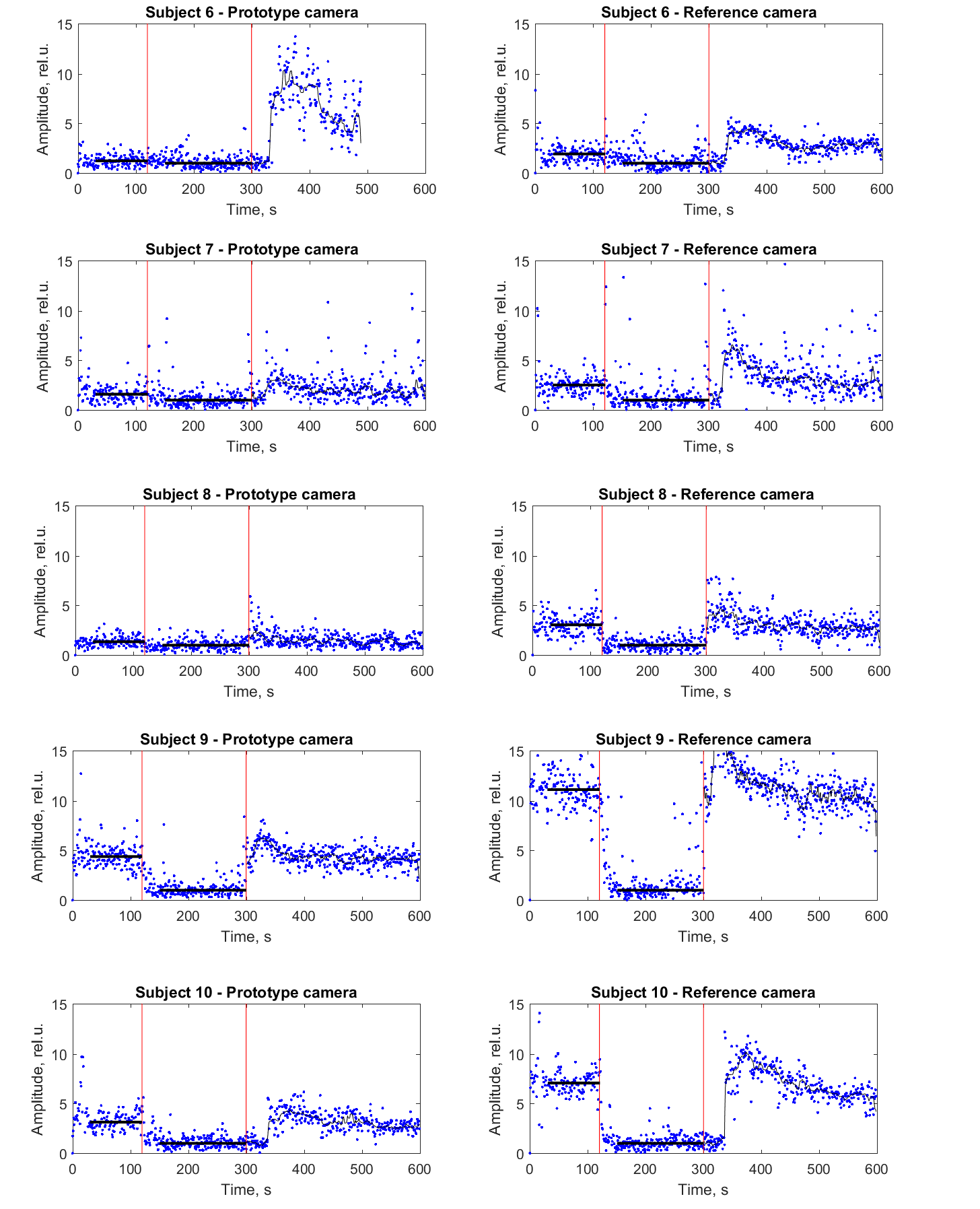


Figure 8. The graphs of dynamics of skin microcirculation PPG amplitude under three loads: 1) normal condition, 2) occlusion, 3) hyperemia. The red lines show the borders between the loads; black lines shows median values of amplitudes obtained in these loads.

Figure 10. Correlation between the averaged PPG amplitudes in normal condition obtained from 10 patients, using prototype camera and ANDOR camera.

***1.2.2. Clinical measurements performed by remote photoplethymography prototype device for assessing of regional anesthesia.***

In the preceding period of the project a miniature prototype device for contactless monitoring of skin blood flow was developed. The prototype was tested in the IAPS UL laboratory and later it was validated in a clinical environment (see. Fig.11). This device has been connected to a computer, and it works together with remote PPG analysis software. The system was approbated in the Hospital of Traumatology and Orthopedics for patients with wrist injury undergoing regional anesthesia (RA) procedure before the surgery. RA is a standard procedure for excluding of pain sense during the surgery. The doctor-anesthetist performs the administering of the local anesthetic in the area of armpit (or neck), and 5-15 minutes later the palm (arm) skin becomes insensitive. Local anesthetic affects the sympathetic innervation of circulation system that leads to an increase in regional blood flow intensity. Thus, a successive effect of RA could be determinated by detection of increasing of microcirculation intensity in palm skin.

Figure 11. The validation of remote PPG prototype device in the Clinics of Traumathology and Ortophedics

During the project, our developed remote PPG system has been successfully applied to the RA monitoring. The study involved six persons aged 18 to 40 years. During the procedures while measurements, a subject was in lying position. Palm was fixed by a custom made foam polystyrene support, which was fixed by arm support, for any possible movement exclusion during the RA procedure. A remote PPG device was mounted by the flexible stand at the distance of ~15 cm to the palm. Furthermore, the set of thermography images of palm were recorded by compact thermal camera (FLIR-C2). Duration of each measurement lasted 15 minutes, recording of video was performed 1 minute before the RA procedure and continue to record the signal the rest of the time. RA procedure was performed with a local anesthetic (Sol.Lidocaini 2% - 10 ml and Sol.Bupivacaini 0.5% - 10 ml) administration in armpit level, the procedure was monitored by ultrasonic device. Usually the RA procedure takes about 5 minutes. The efficacy of anesthetic for each entity was controlled by touching the skin with ice cubes.

During the measurements were obtained a high-resolution skin microcirculation amplitude maps, which make easy to determinate palm skin areas where blood flow increases. This greatly facilitated for the doctor-anesthetist to conclude on the effectiveness of the RA in the corresponding skin areas. Measurements were analyzed, by analyzing of impact of parameter value to the sensitivity of microcirculation map, calculated by processing algorithm. The PPG maps were compared with the thermal images.

In the first study we analyzed how the spatial processing parameter (Gaussian pyramid level) affects the quality of PPG microcirculation map. In this case, the spatial decomposition of video gives improvement of sensitivity and spatial resolution of skin microcirculation maps. However, this method has its drawbacks. Too high value of decomposition level *L* increases the PPG signal sensitivity (reducing binary noise of camera), but also reduces the spatial resolution, and vice versa, at small *L* values map sensitivity decreases rapidly (see. Figure 12). During the analysis it was concluded that starting from *L=3* there are seen the areas with microcirculation changes, but *L>5* practically does not influence the quality of PPG maps.

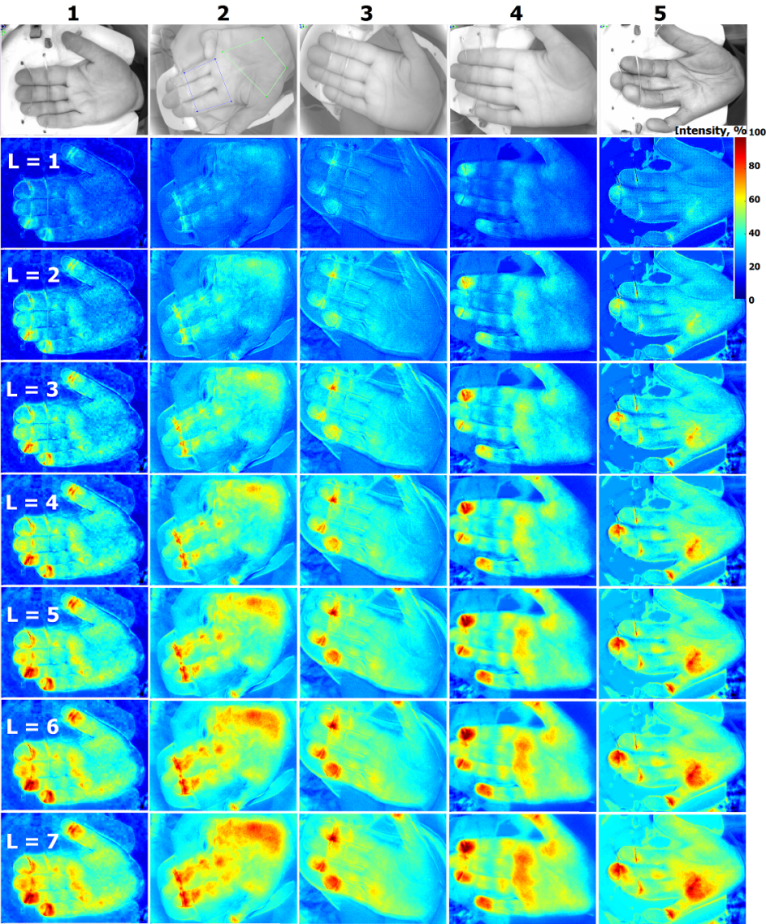


Figure 12. The dynamics of remote PPG map intensity after the local anesthetic administration for 5 subjects. Maps are calculated at different pyramid levels *L*.

In the second study we analyzed how the different spatial frequency components affects the quality of microcirculation map. During the study it was discovered that the low spatial frequency components more affects the sensitivity of PPG map than the high frequency components. During the pyramid reconstruction procedure the superposition of different spatial frequency components with normalization constants were calculated. The ratios between the normalization constants are determined by parameter *p*, from which depends the map sensitivity and spatial resolution. Similarly, as in the previous case, in order to achieve high sensitivity and high spatial resolution map, choose the optimal *p*-value is important.

Fig. 13 shows thermal images (top row) and microcirculation maps at different *p* values obtained at five time moments: 1) before the RA procedure, 2) 2 min. after the completion of the RA procedure, 3), 3 min, 4) 5 min. and 5) 9 min. later. Thermal imaging can be used as standard method to determine the effectiveness of the RA, however, it is still not being used in Latvia. In this study we compared thermography method with a PPG mapping. The results obtained by with both methods showed practically same skin areas, which have been selectively innervate by the RA procedure (lower part of palm). Map calculations showed that increasing of *p*-parameter value leads to increasing of map sensitivity and reducing its spatial resolution.

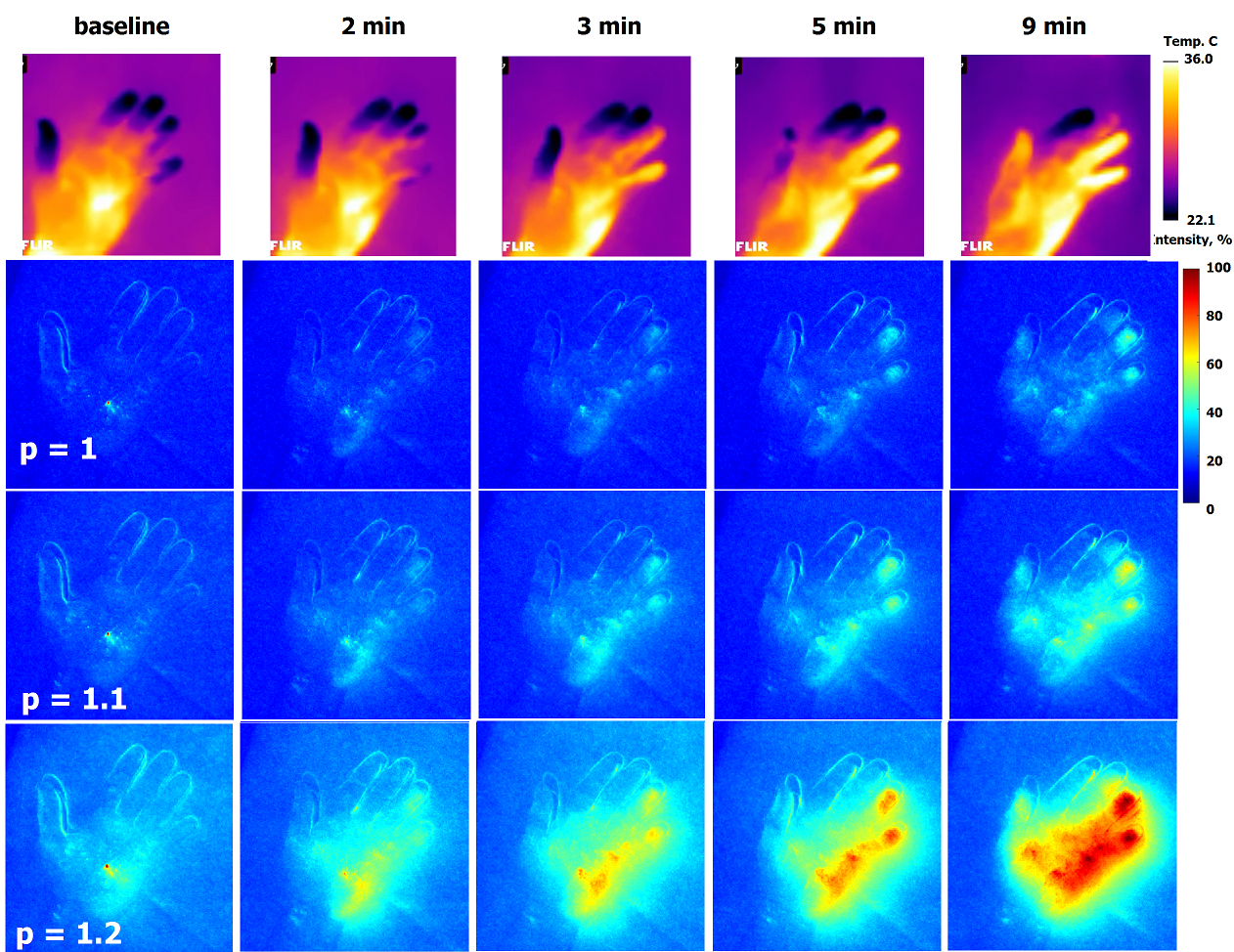


Figure 13. Thermal images (1st row) and PPG maps (next rows) before and after the regional anesthesia procedure. Maps ar different reconstruction *p*-parameter values.

**1.3. Clinical measurements and data analysis for tissue imaging in the near-infrared spectral range**

The developed prototype device for estimation of skin hydration by near-infrared reflectance spectroscopy was validated during clinical measurements of 39 volunteers. The goal of this study was to determine the long-term effect of a specific moisturizing cream X and serum Y on skin hydration. Participants of this study were using a moisturizing cream X on the proximal part of volar aspect of left arm (closer to the elbow), moisturizing serum Y on the dorsal part of the volar aspect of left arm (closer to the palm), while the volar aspect of the right arm was control – volunteers were asked not to use any moisturizing cream on the right arm (Fig. 14).

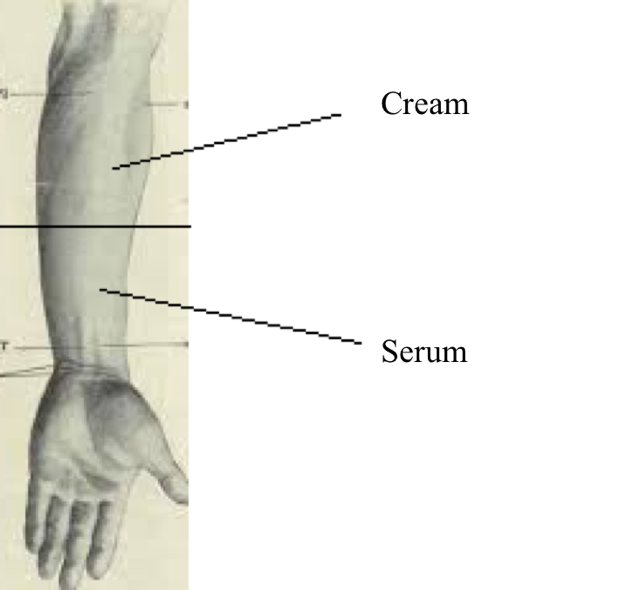


Fig. 14. Volar aspect of the left arm: cream was applied on the proximal part (closer to the elbow), and serum was applied on the dorsal part (closer to the palm).

Three measurements at different spots on skin were performed for each region of interest (dorsal part of left arm, proximal part of left arm, and right arm), and the average value was calculated at each region of interest. Measurements were performed in the beginning of January and in February – one month later, to compare the effect of cream and serum on skin after 1 month. Afterwards, average values at each region of interest were compared between the beginning and one month after.

All measurements were performed by two different devices - the developed device for estimation of skin moisture level by near-infrared spectroscopy, and a commercial device *DermaLab* (by *Cortex Technology*) which estimates skin hydration based on changes in skin conductance (with increased water volume fraction in skin or skin hydration, there is an increase in electrical conductance of skin). Measurements were performed in the same conditions for all 39 participants.

Absorption spectra in the near-infrared spectral range of 900-1700 nm of skin were acquired by the developed prototype device. However, only absorption (Optical Density) value at 1450 nm was used for further analysis, as during laboratory measurements it was concluded that absorption at this wavelength offers the best sensitivity for estimation of change in skin hydration. Optical Density values at 1450 nm were on average in the range of 1.5 to 1.8 (relative units). For easier data analysis and comparison of results to those of the other device, an empirical parameter for estimation of skin hydration was chosen, calculated as 10OD. Thus, relative skin hydration values for all 39 volunteers were in the range of 30 – 70. For comparison, *DermaLab* results for all 39 volunteers were in the range of 30 to 300. This shows that for small skin hydration changes, *DermaLab* shows a larger change in skin hydration parameter than the developed prototype device.

In the beginning of analysis, *Lilliefors* statistics test was performed on results of all 39 volunteers to test whether all values of the same skin region conform to normal distribution. Afterwards, a *Two sample T Test* was performed to see whether there is a statistically significant change between results of all 39 volunteers in the beginning of the study compared to 1 month after. This was done for each skin region of interest separately: dorsal part of the left hand, proximal part of the left hand, and right hand. It was expected that there would be no statistically significant change for the control group (right hand). However, there should be a statistically significant difference for the results of the left hand if there is any long-term effect of the cream or the serum.

Results are represented in 5 parts for each device (in total, 10 parts); each part shows the average skin hydration value of all 39 volunteers:

* Right arm (control) group in the beginning;
* Right arm (control) group 1 month after;
* Left hand group in the beginning;
* Left hand cream group 1 month after;
* Left hand serum group 1 month after.

In order to perform the *Two sample T Test*, the *Lilliefors* test was performed to see whether all data in the same group are normally distributed. It was concluded that 8 out of 10 groups conform to normal distribution, and 2 do not – *DermaLab* results of left arm cream group 1 month after, and *DermaLab* results of right arm (control) group 1 month after. For comparison, Figure 15 shows histograms of results by both devices for right arm (control) group 1 month after. However, the *Two sample T Test* was performed on all 10 groups even though for the 2 abovementioned groups the results can be less reliable.

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Fig. 15. Histograms of skin hydration parameter values for right arm (control) group 1 month after by both devices – the developed NIR prototype device (on the left) and *DermaLab* (on the right).

For result analysis, the following parameters were calculated:

* Average value of all volunteers in the beginning, subtracted from the average value of all volunteers 1 month after: ;
* Standard deviation (distribution of results inside one group): ;
* Standard error , where is the count of volunteers.

The *Two sample T Test* showed the following results:

1. By the developed NIR prototype device:
   * Comparison of the right arm (control) group in the beginning and 1 month after showed **no statistically significant** **change** () between both groups;   
     .
   * Comparison of the left arm **cream** group in the beginning and 1 month after showed **no statistically significant change** () between both groups;  
     .
   * Comparison of the left arm **serum** group in the beginning and 1 month after showed a **statistically significant change** () between both groups;  
     .
2. By the commercial device *DermaLab*:
   * Comparison of the right arm (control) group in the beginning and 1 month after showed **a statistically significant** **change** () between both groups:  
     .
   * Comparison of the left arm **cream** group in the beginning and 1 month after showed **a statistically significant change** () between both groups:  
     .
   * Comparison of the left arm **serum** group in the beginning and 1 month after showed a **statistically significant change** () between both groups:  
     .

It was expected that there should be no statistically significant change between results of the right arm (control) group in the beginning and 1 month after, and it is shown by the results of the developed NIR prototype device. By subtracting the average value in the beginning of all volunteers (38) from the value 1 month after, the result was 0. However, the standard deviation of these results is relatively large: 4. However, *DermaLab* results showed a statistically significant change between results of the control group, which was not expected.

When comparing cream and serum groups, the results acquired by the developed NIR prototype device showed no statistically significant change for the cream group, but a statistically significant change for the serum group (). However, results acquired by *DermaLab* showed a statistically significant difference for both cream and serum; in addition, the increase in skin hydration parameter value was almost twice as large in case of the cream group (), compared to the serum group ().

In conclusion, the results show that the commercial device *DermaLab* is much more sensitive to small changes in skin hydration, compared to the developed NIR prototype device. *DermaLab* allowed the comparison between cream and serum groups, while the NIR prototype device did not. However, *DermaLab* results did not come out as expected either, at least not for the control group. It is possible that some volunteers were using moisturizing cream on the right arm as well, or there were other factors affecting skin moisture for the right arm (control) group, not related to use of a moisturizing cream. Such factors could be outside or room temperature, physiological condition etc.

***1.3.1. Monte Carlo simulations for the analysis of effects caused by optical properties in the near-infrared spectral range***

A *Matlab* based graphical user interface was created for Monte Carlo simulation input creation and output reading to simplify the whole process for research purposes. A short description of the created program:

* The user has an option to choose the wavelength range between 250-1700 nm and the spectral resolution;
* Choose the number of photons that will propagate in the tissue model;
* Select the number of layers of the tissue model and specify optical parameters for each layer – refractive index, scattering coefficient, anisotropy factor and layer thickness. Option to choose which chromophores are included in each layer and specify the volume fraction of each chromophore. Select different scattering coefficient calculation modes or leave it constant;
* Specify the model grid sizes in each direction in 3D;
* Specify the refractive index above and below the tissue model;
* An option to include different simulation programs if the program uses similar kind of input (i.e. *GPUmcml*);
* When all input parameters are specified, Monte Carlo simulations begin and output can be read in different modes – as a data array, table and graph, which displays values like absorption, specular reflection, diffuse reflection and transmittance as a function of wavelength.

Calculations.

Monte Carlo simulations can be used to approximate different optical parameters of human tissue in the near infrared spectral range of 900 – 1300 nm (Volume fractions of chromophores, scattering coefficient, refractive index, etc.).

Figure 16. Absorbing chromophore absorption coefficients in the 900-1300 nm spectral range as a function of wavelength.

Optical parameters are changed in the model to create an understanding of how each of the parameters affects the resulting diffuse reflectance spectra. The information gathered is then used for further spectral analysis. An issue regarding spectral overlapping when looking at spectra with different optical parameters is identified and several solutions to this problem are presented. The accuracy of the inverse Monte Carlo method is tested with experimental spectra of a material with different volume fractions of water.

Possible comparisons between experimental and simulated spectra – exact spectral comparison (Figure 17), area analysis under absorption maxima, chromophore absorption spectra (Figure 16) fitting to experimental spectra.

Table 1 Tissue model for Monte Carlo simulations. – Volume fractions of water, lipids, collagen, elastin; – layer thickness; – anisotropy factor; – refractive index.

|  |  |  |  |
| --- | --- | --- | --- |
| Parameter | *Stratum Corneum* | Epidermis | Dermis |
|  | 0.30 | 0.65 | 0.65 |
|  | 0.05 | 0.05 | 0.10 |
|  | 0.40 | 0.20 | 0.15 |
|  | 0.25 | 0.10 | 0.10 |
|  | 20 | 80 | 2000 |
|  | 0.86 | 0.80 | 0.90 |
|  | 1.50 | 1.35 | 1.40 |

Figure 17. Spectral overlapping at different optical parameters of the tissue model. The straight line represents a tissue model with volume fraction of water at 50% in the epidermis. The data points represent a tissue model with the epidermis layer thickness changed to 60 . The secondary axis shows residual values.

**2. Clinical validation of the skin multimodal imaging prototype device „SkImager”.**

RGB images were taken from 50 patients with rosacea at different stages by SkImager. Rosacea is a chronic inflammatory skin disease that involves flushing, transient or persistent erythema, visible blood vessels, as well as papules and pustules. Physicians classify disease by skin visual assessment using Clinician’s Erythema Assessment (CEA) grading scale that ranges from 0 (clear skin, no erythema) to 4 (severe erythema).

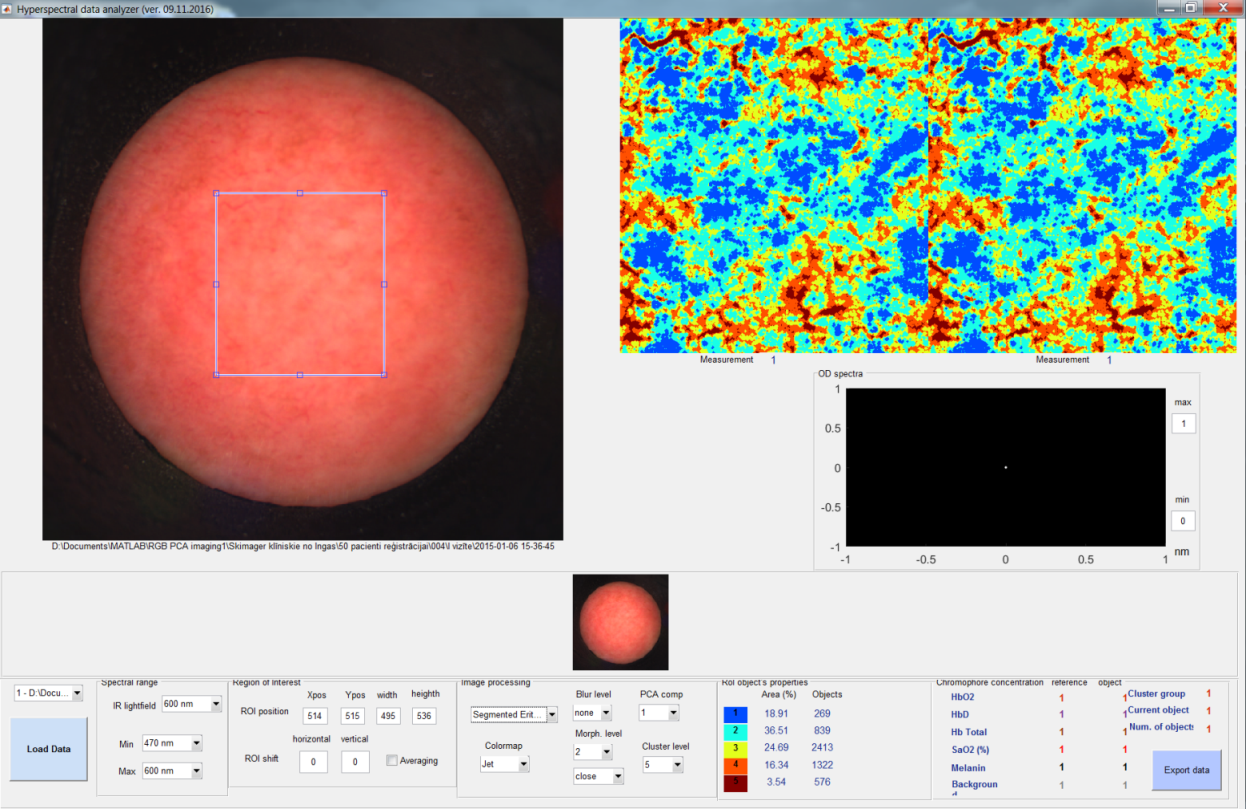
Images were taken from five parts of facial skin for each patient (central forehead, right cheek, left cheek, right part of nose and left part of nose).

Images of 13 patients with different diagnosed CEA index - CEA=1 (3 patients), CEA=2 (5 patients), CEA=3 (5 patients) - were selected for further analysis. Images of the lesion taken in the first visit to the doctor (before lesion treatment) from central forehead and both cheeks were analyzed by software that performs segmentation of erythema index (EI) and principal component analysis (PCA) maps.

Software allows to download R, G and B images (fig.18) and to select the region of interest on the RGB image where segmentation maps are calculated. Software calculates EI and PCA maps (by selecting the appropriate option), divide calculated values in five clusters and calculate the percentage of the share of each cluster. Fifth cluster corresponds (dark brown color) to higher erythema indices, first cluster (blue color) to the lowest. Maps of erythema index were calculated by dividing red image by green: EI=R/G.

Segmentation maps

Region of interest



A percentage of area and number of objects for each segment

Map type menu

Fig.18. Image processing software

The maps of erythema index and three principal components (PCA1 – first component, PCA2 – second component, third component) are compared in figure 19. The figure shows, that the EI and PCA1 segmentation maps outline blood vessels, the PCA2 map doesn’t give any information about the areas of blood vessels, while more segments similar to blood vessels are seen in the PCA3 map and some of them match the blood vessels in the RGB image.

Figure 20 shows the influence of dark pigments on segmentation. If selected region contains very dark nevi or pigmentation then the algorithm outlines them in separate clusters (fourth, fifth cluster) that influences the segmentation accuracy of surrounding blood vessels.

Figure 21 shows examples of segmentation maps of three different rosacea stages on right cheek. EI segmentation selects blood vessels more precisely than PCA1, that allows to define the areas of blood vessels as well as to track changes more accurately.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| RGB image | EI map | PCA1 map | PCA2 map | PCA3 map |
|  | EI segmentation | PCA1 segmentation | PCA2 segmentation | PCA3 segmentation |

Fig.19. Comparison of erythema index (EI) un PCA components for one particular case (CEA=3, right cheek).

|  |  |  |  |
| --- | --- | --- | --- |
| RGB image | Region of interest | EI segmentation | PCA1 segmentation |
| RGB image | Region of interest without pigmentation | EI segmentation without pigmentation | PCA1 segmentation without pigmentation |

Fig.20. The influence of dark pigmentation on segmentation (CEA=3, left cheek).

|  |  |  |
| --- | --- | --- |
| RGB image (CEA=1, Pat.1) | EI segmentation | PCA1 segmentation |
| RGB image (CEA=2, Pat.7) | EI segmentation | PCA1 segmentation |
| RGB image (CEA=3, Pat.3) | EI segmentation | PCA1 segmentation |

Fig.21 Segmentation maps for three different stages of rosacea (CEA=1, CEA=2, CEA=3, right cheek).

**Conclusions:**

1. EI segmentation selects an area of blood vessels more precisely than the first component of PCA.
2. Segmentation maps of PCA first component shows visual blood vessels, but these maps are dependent on inhomogeneity of lighting field, shadows on curvature and light pigmentation (impossible to distinguish blood vessels and redness from pigmentation and shadows – all are segmented in one cluster).
3. Second component of PCA gives no information about distribution of blood vessels and redness.
4. Third component of PCA showed similarity with the visible blood vessels in RGB images in some cases, but further analysis is required, because there were cases when blood vessels were not seen in RGB images, but PCA maps showed the areas similar to blood vessels.

**Further tasks:**

1. Clinical measurements of rosacea patients.
2. Mapping and analysis of skin blood vessels before and after treatment.

**Publicity of the 3rd project period**

**Submitted publication, SNIP>1**

J.Spigulis, I.Oshina, A.Berzina, A.Bykov, “Smartphone snapshot mapping of skin chromophores under triple-wavelength laser illumination”, *J.Biomed.Opt*., submitted 2016. **(SNIP=1.181).**

**Publications cited in SCOPUS or WoS (3)**

1. J. Spigulis, I. Oshina, Z. Rupenheits, "Smartphone single-snapshot mapping of skin chromophores," in *Biomedical Optics 2016*, OSA Technical Digest (online), JTu3A.46, <https://www.osapublishing.org/abstract.cfm?uri=Cancer-2016-JTu3A.46>.

2. E.Kviesis-Kipge, U.Rubīns. „Portable remote photoplethysmography device for monitoring of blood volume changes with high temporal resolution” BEC-2016, Proc. 15th Biennial Conference on Electronics and Embedded Systems. pp. 55-58 (2016); *IEEE Explore*, DOI: [10.1109/BEC.2016.7743727](http://dx.doi.org/10.1109/BEC.2016.7743727).

3. U.Rubins, J.Spigulis, A.Miscuks, “Photoplethysmography imaging algorithm for continuous monitoring of regional anesthesia”, ESTIMedia'16, Proc.14th ACM/IEEE Symp. on Embedded Systems for Real-Time Multimedia, pp. 67-71 (2016), <http://dl.acm.org/citation.cfm?id=2994308&CFID=863483070&CFTOKEN=69466985> *(IEEE Explore).*

**Conference reports (6)**

1. I.Saknite, A.Zavorins, I.Zablocka, J.Spigulis, J.Kisis, "Comparison of Optical and Conductance Methods for Estimation of Skin Hydration", Norwegian Electro-Optics Meeting 2016, Voss, Norway, April 13-15, 2016.
2. J.Spigulis, I.Oshina, Z.Rupenheits, "Smartphone single-snapshot mapping of skin chromophores", OSA Biomedical Optics Congress, Fort Lauderdale, USA,  25 - 28 April 2016.
3. I.Saknite, A.Zavorins, I.Zablocka, J.Spigulis, J.Kisis,"Near-Infrared Reflectance Spectroscopy System for Noninvasive Estimation of Skin Hydration", The 2nd Biomedical Imaging and Sensing Conference, May 17-20, 2016, Yokohama, Japan.
4. J.Spigulis ”Multi-laser illumination designs for skin chromophore mapping”, Int. Conf. "Advanced Laser Technologies" (ALT16), 12-16 September 2016, Galway, Ireland.
5. E. Kviesis-Kipge, U.Rubīns. „Portable remote photoplethysmography device for monitoring of blood volume changes with high temporal resolution”,  BEC2016, 15th Bien. Conf. on Electronics and Embedded Systems, October 3-5, 2016 Tallinn, Estonia.
6. U.Rubins, J.Spigulis, A.Miscuks, “Photoplethysmography imaging algorithm for continuous monitoring of regional anesthesia”, ESTIMedia'16, 14th ACM/IEEE Symp. on Embedded Systems for Real-Time Multimedia, 6-7 October 2016, Pittsburgh, USA.*(IEEE Explore)*

**Public outreach**

1. The elaborated methods and devices were presented to general public (>800 attendees) during the European Researcher’s Night on 30.09.2016. at the Institute of Atomic Physics and Spectroscopy (Riga, Skunu Str. No.4).
2. The novel technology “Non-invasive skin assessment with a smartphone” was presented at the international exhibition RIGA COMM 2016 (October, 2016).
3. Related Internet publication (in Portugese): <http://www.ifsc.usp.br/index.php?option=com_content&view=article&id=3956:docente-da-university-of-latvia-visita-grupo-de-optica&catid=3:ifsc-hoje&Itemid=281>
4. Weekly journal article (in Latvian): žurnāls IR,#51/51, pp. 24-25. “Kas nodarbina pētnieku prātus? Jānis Spīgulis: Tehnoloģija operatīvam ādas stāvokļa novērtējumam ar viedtālruni”.

**Defended Master Theses:**

Reinis Janovskis, “Infrared spectroscopy and imaging for evaluation of skin moisture”, supervisors: Prof. Jānis Spīgulis, Dr. Phys. Inga Saknīte.

PI of the 3rd project,VPP SOPHIS: J.Spigulis