Contract #10-5/82, 26.11.2018
Poject # lzp-2018/2-0006

Final Scientific Report

Project title: **Advanced spectral imaging technology for skin diagnostics**

1. Scientific excellence

This project was aimed at further developments of the originally proposed snapshot multi-spectral-line imaging (SMSLI) technology for distant mapping of four main skin chromophores, combined with skin fluorescence imaging.

New design solutions and know-how have been created for future implementations of SMSLI technology for skin diagnostics. In particular:

- Two novel design concepts of four spectral line imaging devices have been elaborated – double-camera design and single-camera design;
- Both concepts have been implemented in two functioning prototype devices, successfully tested in laboratory and clinical measurements;
- One design solution – exploitation of side-emitting optical fibre loops for uniform multi-laser illumination – has been patented;
- Results of the research in frame of this project have been published in six SCOPUS-cited papers (one of them in Q1-level journal) and reported at seven international conferences.

Publications:

1. J.Spigulis, Z.Rupenheits, U.Rubins, M.Mileiko, I.Oshina, "Spectral line reflectance and fluorescence imaging device for skin diagnostics", *Appl. Sci.* **10**, 7472 (2020); doi:10.3390/app10217472. https://www.mdpi.com/2076-3417/10/21/7472/htm.

2. J. Spigulis, Z. Rupenheits, M. Matulenko, I. Oshina, U. Rubins "A snapshot multi-wavelengths imaging device for in-vivo skin diagnostics", *Proc. SPIE*, **11232**, 112320I-1 (2020). https://www.spiedigitallibrary.org/conference-proceedings-of-spie/11232/112320I/A-snapshot-multi-wavelengths-imaging-device-for-in-vivo-skin/10.1117/12.2547286.short?webSyncID=e46e9e6e-c7a4-9dab-6a0c-bad059329ad8&sessionGUID=83c9d902-bc99-93ce-d268-bead49a28531&SSO=1

3. J.Spigulis, V.Lukinsone, I.Oshina, E.Kviesis-Kipge, M.Tamosiunas, A.Lihachev, "Recent Results of Riga Group on Laser Applications for Skin Diagnostics", *J.Phys.:Conf. Series* (2020, accepted). https://www.lu.lv/fileadmin/user_upload/LU.LV/www.lu.lv/Zinatne/Programmas_un_projekti/Spigulis-ICSQE.PDF.

4. J.Spigulis, I.Kuzmina, I.Lihacova, V.Lukinsone, B.Cugmas, A.Grabovskis, E.Kviesis-Kipge, A.Lihachev, "Biophotonics research in Riga: recent projects and results", *Proc.SPIE* **11585,** 1158502 (2020). https://www.spiedigitallibrary.org/conference-proceedings-of-spie/11585/1158502/Biophotonics-research-in-Riga-recent-projects-and-results/10.1117/12.2581799.short?SSO=1

5. J.Spigulis, I.Oshina, M.Matulenko, "Laser illumination designs for snapshot multi-spectral-line imaging", *IEEE Xplore*, https://ieeexplore.ieee.org/document/8872998 (2019).

6. J.Spigulis, I.Oshina, P.Potapovs, K. Lauberts, "Snapshot multi-spectral-line imaging for applications in dermatology and forensics", *Proc.SPIE*, **10881**, 1088114 (2019). https://ebooks.spiedigitallibrary.org/conference-proceedings-of-spie/10881/1088114/Snapshot-multi-spectral-line-imaging-for-applications-in-dermatology-and/10.1117/12.2508204.short?SSO=1

Conferences with oral presentations (J.Spigulis):

1. *SPIE Photonics West / BIOS'2019,* San Francisco, USA, 2-7/02/2019.
2. *CLEO-Europe,* Munich, DE, 23-27/06/2019.
3. *COST Multiforesee 2019,* Catania, IT, 16-18/09/2019.
4. *Advanced Laser Technologies 2019,* Prague, CZ, 15-20/09/2019 (invited).
5. *SPIE Photonics West / BIOS'2020,* San Francisco, USA, 1-6/02/2020.
6. *Biophotonics – Riga 2020*, Riga, LV, 24-25/08/2020 (remote).
7. *Int. Conference and School on Quantum Electronics, ICSQE-21*, Varna, BG, 21-24/09/2020 (invited, remote).

Patent:

LV 15491 B, 2020: "Device for uniform surface illumination simultaneously by several laser spectral lines" (J.Spigulis, I.Oshina, Z.Rupenheits, M.Matulenko).

2. Impact

2.1. Scientific results of the project

Table 1

Impact indicator of the results	Number	
	Planned	Achieved
1. Scientific publications		
1.1. scientific papers (*SCOPUS*)	3	6
1.2. other peer-reviewed papers	-	-
1.3. peer-reviewed monographs	-	-
2. IPR (patents).	1	1
3. International scientific conferences where reports were planned	5	7

The **main scientific results** of this project are:

- Clinically validated **innovative method** for distant diagnostics of skin pathologies;
- **New clinical data sets** on optical (spectral and fluorescent) properties of widespread skin malformations, obtained in a patient friendly non-contact way;
- **Advanced algorithms** and computer programmes for processing of clinical images in order to identify the pathology and map distributions of skin chromophores;
- New **technological knowledge** (know-how) obtained by designing and optimizing two original SMSLI-based prototype devices with technology readiness level TRL = 5;

2.2. Prospects of research development.

This was the first attempt to implement four spectral line snapshot imaging method in a clinically user-friendly device. Results of the project confirmed validity of the SMSLI concept in the case of four spectral line imaging and its applicability for clinical diagnostics in dermatology. The second (single-camera) proof-of-concept prototype design is more appropriate for further developments and eventual commercialization. There are several issues to be solved in future, e.g. related to internal cooling of the device, removal of image artefacts caused by the camera filter created interference fringes, improved communication between the mini-computer and display, software inability to exploitat all 4-channel camera image pixels, proper calibration of the device and some more. Less expensive components should be used to make the device affordable for GPs and small clinics.

We have submitted a FLPP project proposal on continuing activities; unfortunatelly, it was not supported so we have to find other options for implementation of project results, eventually in collaboration with some industrial partner. The next step may include also extended clinical measurements with improved spectral image processing technology. In meantime, we will continue collaboration with hospitals and clinics in Latvia (Table 2). At the current stage, also due to increased Covid-19 restrictions, the project results are temporarily freezed. The developed prototypes will be further used to collect clinical data on four spectral line images of various skin malformations in order to extend the existing data base aiming at standartization the proposed methodology for routine clinical use. We also plan to investigate eventual forensic applications of this approach, e.g. for determination of bruise ages by bilirubin content analysis and for identification of counterfeit documents and banknotes by comparing selected spectral line images.

Table 2

No.	Collaborating institution, country	Kind of collaboration	Time period
1.	Oncology Center of Latvia (Dr. A.Deryabo)	Clinical validation of diagnostic prototypes on pigmented skin malformations	2021 - 2023
2.	Laser Plastic Clinics, Latvia (Dr. A.Berzina)	Clinical validation of diagnostic prototypes on vascular skin malformations	2021 - 2023

2.3. The socio-economical impact of results.

After complementary applied research, results of this project would be exploited in clinical medicine (dermatology and oncology - quantitative imaging and identification of skin malformations) and medical engineering (optimal design and manufacturing of diagnostic devices, based on spectral imaging). The main **target groups** of this project are medical professionals (dermatologists, cosmetologists, oncologists, plastic surgeons, GPs, nurses), their patients, medical equipment manufacturers and distributors, as well as researchers and engineers dealing with development of new diagnostic methods and devices. Besides, they were acquainted with both developed prototype devices in the "Biophotonics – Riga 2020" conference exhibition (Fig. 1).

Further knowledge transfer is planned in collaboration with our partners in Slovenia (Jozef Štefan Institute, group of Dr. Boris Majaron) to investigate the skin bilirubin distribution maps in bruises and find correlations with numerical models. This, as well as counterfeit detection by four spectral line image comparative analysis, may find forensic applications so we also look forward to collaborate also with Forensic Research Group at Amsterdam University headed by Prof. Maurice Aalders.

Table 3.

No.	Collaborating institution, country	Kind of collaboration and description	Time period
1.	Jozef Štefan Institute, Slovenia	Clinical measurements of bilirubin distribution in bruises in collabolation with Dr. Majaron's group	2021 - 2023
2.	Amsterdam University, the Netherlands	Optical non-contact examination of skin bruises and coloured counterfeits in collaboration with Prof. Maurice Aalders group	2021 - 2023

2.4. Publicity and communications

A number of project publicity events have been organized, including the 3rd International Conference ***"Biophotonics – Riga 2020"*** in August, 2020 with published papers in a special issue of *SPIE Proceedings*, USA (https://www.spiedigitallibrary.org/conference-proceedings-of-spie/11585.toc.). Conference attendees and University students and employees were acquainted with the developed prototype devices in frame of the conference exhibition (Fig.1).

Fig. 1. Demonstration of the developed prototypes at the exhibition of conference "Biophotonics – Riga 2020".

Table 4.

No.	Activity	Description - target group, website, etc.	Time period
1.	Kick-off meeting	https://www.lu.lv/fileadmin/Progresiva_spektralas_attelosanas_tehnologija_adas_diagnostikai__Kick-off_.pdf	07.12.2018.
2.	Media release about the project	https://www.lu.lv/par-mums/lu-mediji/zinas/zina/t/46264/	10.12.2018.
3.	Researcher's Nigth - 2019	Poster on project and demonstration of the 1st prototype	25.09.2019.
4.	Project midterm seminar	Presentation and demonstration of the 1st prototype	12.12.2019.
5.	*Biophotonics-Riga 2020*	Organization of the conference, the opening presentation with published paper, participation at conference exhibition, editing of the conference proceedings book (Proc.SPIE vol.11585, 2020). www.bpr20.lu.lv	24-25.08.2020
6.	UL Technology Day	Presentation and demonstration of the 2nd prototype. https://www.youtube.com/watch?v=WRVzKWIR_98&list=PLxc2e81TLgVR5yIkyjZo1ZtHpNT3l5Esw&index=26	25.09.2020.
7.	Project conclusion seminar	Presentations and demonstrations of both prototypes; full record: https://failiem.lv/u/22858emqv	26.11.2020.
8.	Regularly updated three project websites	www.lu.lv/zinatne/programmas-un-projekti/nacionalas-programmas-un-projekti/progresiva-spektralas-attelosanas-tehnologija-adas-diagnostikai-1/ https://www.asi.lu.lv/programmas-un-projekti/nacionalas-programmas-un-projekti/aktivi-flpp/progresiva-spektralas-attelosanas-tehnologija-adas-diagnostikai/ https://www.researchgate.net/project/Advanced-spectral-imaging-technology-for-skin-diagnotics	01.12.2018. – 30.11.2020.

2.5. Contribution to capacity building of project research staff (including students) and improvement of the study environment

Reserch in frame of this project has enhanced capacity building of all team members and also has contributed to the study environment, including three final thesis of students as stated below:

Table 5

PhD and Bachelor theses supervised or consulted by project PI or the main executers				
No.	Author	Title	Supervisor/consultant	Defence
1.	Margarita Matulenko	BSc Theses (RTU) "Influence of homogeneity of laser diode beam on the quality of skin phantom chromophore maps"	I.Oshina / J.Spigulis	May 2019
2.	Margarita Matulenko	Engineer project (RTU) "Illumination device of different laser wavelengths comprising side-emitting fiber for skin diagnostics"	I.Oshina / J.Spigulis	February 2020
3.	Ilze Oshina	PhD Theses (UL) "Acquisition and applications of monochromatic spectral images"	J.Spigulis	Expected in year 2021

3. Implementation

A number of UL staff members have contributed to implementation of this project, in particular:

1. Janis Spigulis, Lead Researcher and PI (0.5 FTE) – 01.12.2018.-30.11.2020.
2. Zigmars Rupenheits (student), Engineer (0.5-0.7-1.0 FTE) – 01.12.2018.-30.11.2020.
3. Margarita Matulenko (student), Engineer and Research Assistant (0.5-0.7 FTE) – 07.12.2018.- 21.02.2020.
3. Ilze Oshina (student), Researcher (0.5 FTE) – 01.06.2019.-30.11.2020.
4. Uldis Rubins, Lead Researcher (0.15-0.75 FTE) – 01.09.2019.- 30.11.2020.
5. Alexander Derjabo, Researcher (0.1-0.35 FTE) – 01.10.2019.- 30.11.2020.
7. Anna Berzina, Research Assistant (0.2 FTE) – 01.10.2019.-30.11.2020.
8. Egita Lakstigala, Engineer (0.5 FTE) – 01.12.2018.-26.07.2019.
9. Ilona Laganovska, Engineer (0.2 FTE) – 01.11.2019.-30.04.2020.
10. Liga Zeltina, Engineer (0.2 FTE) – 01.05.2020.-30.11.2020.
11. Rihards Skuja (student), Engineer (0.3 FTE) – 01.12.2019.-30.06.2020.
12. Toms Kusins, Engineer (0.5 FTE) – 02.03.2020. -29.05.2020.
13. Madars Mileiko, Engineer (1.0 FTE) – 05.05.2020.-30.11.2020.

The project team members had regular meetings on a weekly basis over the whole project period. Five specific work packages WP1-WP5 were completed, along with the management/dissemination/commercialization work package WP6. Some modification of specific tasks and deadlines took place during the implementation period at various progress stages of the project. Details on the performed activities and obtained results are presented below.

WP1 ***Design solution of the new prototype, elaboration of software algorithms and programmes, purchase of materials/components*** (planned: M1-M4, in reality up to M8).

Personnel mainly involved: Z.Rupenheits**,** M.Matulenko**,** J.Spigulis.

General design of the new prototype was elaborated and agreed, specifications for material and component purchase prepared and purchase tender announced. Component purchases were performed in accordance to the regulations of Latvia. It was discovered that price of the 4-band camera exceeded all component/material budget of this project so a less expensive double-camera concept was discussed and accepted. In parallel, software algorithms and programmes for operation of the device and its wireless communications with the external computer were developed. The design concepts were reported at *SPIE-BIOS* conference in San Francisco (February 2019).

WP2 ***Assembling and laboratory tests of the new prototype*** (M5-M12).

Personnel mainly involved: Z.Rupenheits, M.Matulenko, I.Oshina, J.Spigulis.

The double-camera prototype device was assembled and laboratory-tested. Patent application was submitted. The illumination concept and its implementation results were reported at the *CLEO-Europe* conference in Munich (June 2019). The assembled prototype was demonstrated at the project mid-term seminar. A brief **description of the double-camera prototype** is given below.

The portable proof-of-concept prototype device with **double-camera** image recording system comprises optical unit that ensures even illumination of the skin target area simultaneously at four laser wavelengths - 450 nm, 523 nm, 638 nm and 850 nm, micro-computer managed operation system and a touch-screen display for image control and displaying the concentration distribution maps of four skin chromophores.

Functional scheme of the device is presented on Fig.2. Two laser modules – RGB fiber coupled module (*Elite Optoelectronics*, CN) emitting ~20 mW at each of the three spectral lines (450 nm, 523 nm, 638 nm) and 850 nm / 40 mW module (RLDH850-40-3, *Roithner*, AT) – are used for 4-wavelengths illumination of skin via the light diffusingsystem and visible-NIR polarizer. Two cameras (RGB and NIR, MQ022CG-CM and MQ022RG-CM respectively, *Ximea, DE*) equipped with 425 nm and 800 nm long-pass filters (mod. #84-742 and #66-235, *Edmund Optics*, GB, respectively), and orthogonally oriented polarizers (not shown in the scheme) are capturing simultaneously images of the same skin area with subsequent extraction of four spectral line images for further calculation of four chromophore distribution maps using the previously developed methodology. Within a second, the laser modules are switched off and skin autofluorescence (AF) image at the G-channel of RGB camera is captured under illumination by four 405 nm, 40mW laser diodes (DL-5146-101S, *Roithner*, AT), in order to discriminate skin melanoma from seborrheic keratosis.

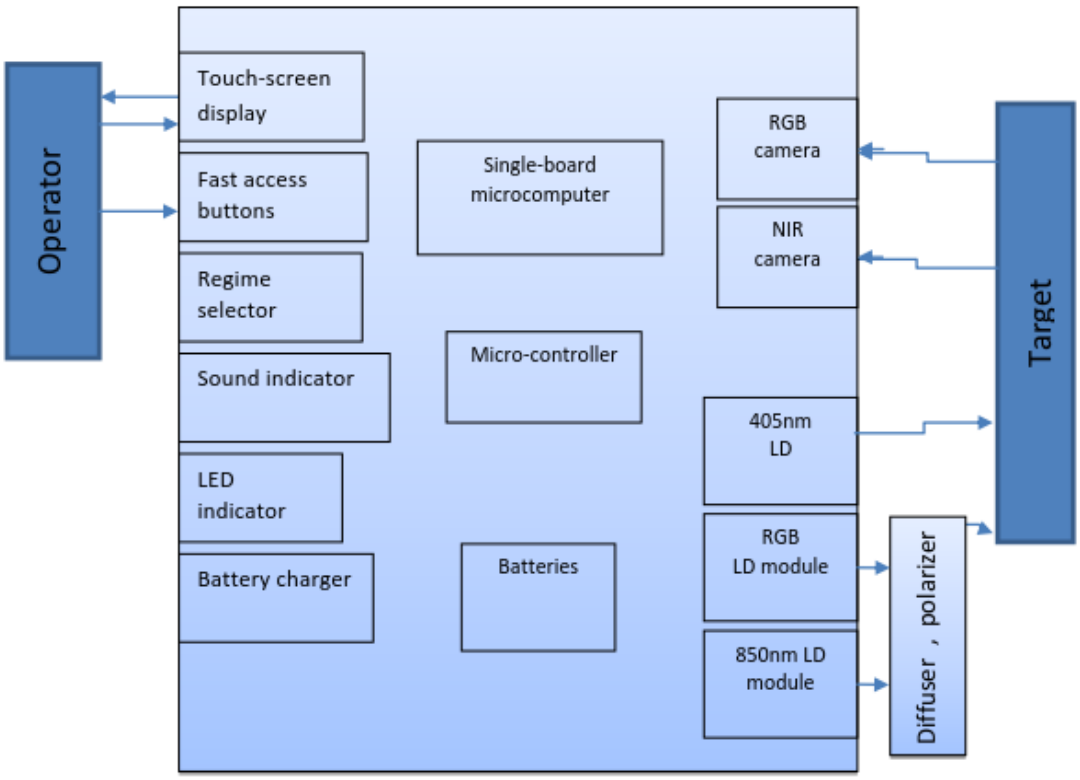


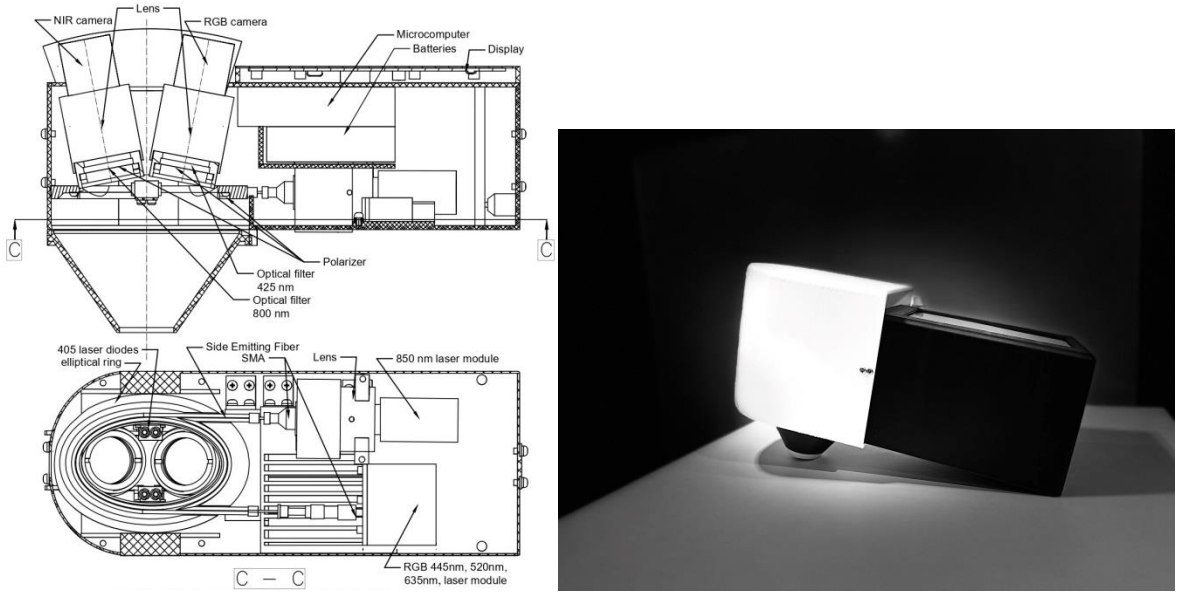
Fig.2. Functional scheme of the double-camera device.

The device is initiated by operator pushing the START button and selecting the appropriate operation regime (SMSLI only, AF image only, combined mode) and exposure times for the RGB and IR cameras. Then both cameras and RGB lasers are switched on and the device is properly placed on the skin target area (monitored on the display). After pressing the SHOT button, micro-controller (STM32G071, *STMicroelectronics*, CH) synchronizes proper illumination of the target to capture the image (or set of images) that is/are read by the single-board-computer (SBC, Rock960, *Vamrs*, CN). The recorded images can be seen on the display (5.5inch HDMI AMOLED, *Waveshare*, CN) or transmitted via SBC's *wi-fi* to the remote computer for calculation of chromophore maps or performing other tasks. The device is fully self-sustained by using rechargeable Li-ion batteries (INR18650-25R, *Samsung*, KR) as the power supply.

Design scheme of the double-camera device is presented on Fig.3. Both cameras with lenses are tilted to capture images of the same round skin area of diameter 30 mm. Several semi-elliptical loops of side-emitting 400 micron silica core optical fiber (*Light Guide Optics Ltd*., LV) are exploited as the light source for even multi-laser illumination of the target area. The fiber is SMA-terminated at both ends; one of them is used for the RGB laser input and the other one – for the 850 nm laser input. In result, the examined skin area is uniformly illuminated by the four above-mentioned laser spectral lines. Four 405 nm laser diodes for autofluorescence excitation are square-placed in the middle zone of illuminator.

The developed user's interface (Fig.4) allows adjusting on the touch-screen the proper exposure times for both cameras and entering specific information about the patient and examined skin malformation, as well as restoring the previously stored images listed on the right bottom corner. After completing the working cycle, the color (RGB) image, 850 nm line (IR) spectral image and autofluorescence (AF) image of the malformation are visualized at the upper part of screen. The operator can monitor each measurement also in offline mode. The three spectral line images are extracted from the RGB image data by a corresponding algorithm on the remote computer, along with the 850 nm image captured by the NIR camera and the autofluorescence image captured at the G-band of RGB camera at 405 nm laser excitation.

Results on development and tests of the double-camera prototype were reported at the SPIE/BIOS conference "Multimodal Biomedical Imaging" in San Francisco, USA (February 2020).



a b

Fig. 3. Design scheme (a) and outlook (b) of the double-camera prototype device.

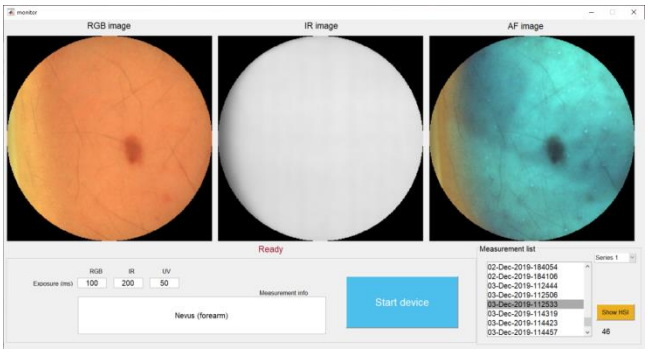


Fig. 4. The user's interface for double-camera prototype.

WP3 ***Preparatory measures for clinical measurements*** (M11-M12).
Personnel mainly involved: A.Berzina**,** A.Derjabo, I.Oshina.

Application to the local Ethics Committee was prepared/submitted and ethics approval received; patient groups were generally selected, written consent forms prepared and signed.

WP4 ***Clinical measurements with the prototype device*** (M13-M20).
Personnel mainly involved: A.Derjabo, A.Berzina, I.Oshina, U.Rubins.

Clinical validation was performed from January to November 2020 with assistance of two doctors-dermatologists: Anna Bērziņa and Aleksandrs Derjabo. Overall, **133 volunteers** have been involved. Volunteers with skin photo-types I or II (Fitzpatrick classification), aged between 12 and 88 (Fig.5), were examined with their written consent under permission of the local Ethics Committee. The images of **245 different skin neoplasms** have been captured. Highest percentage ration was for combined nevus (28%), dermal nevus (18%) and seborrheic keratosis (17%). Junctional nevi were measured in 9% of measurements and hemangioma in 8 %. Table 6 provides detailed description of the measured skin neoplasms.

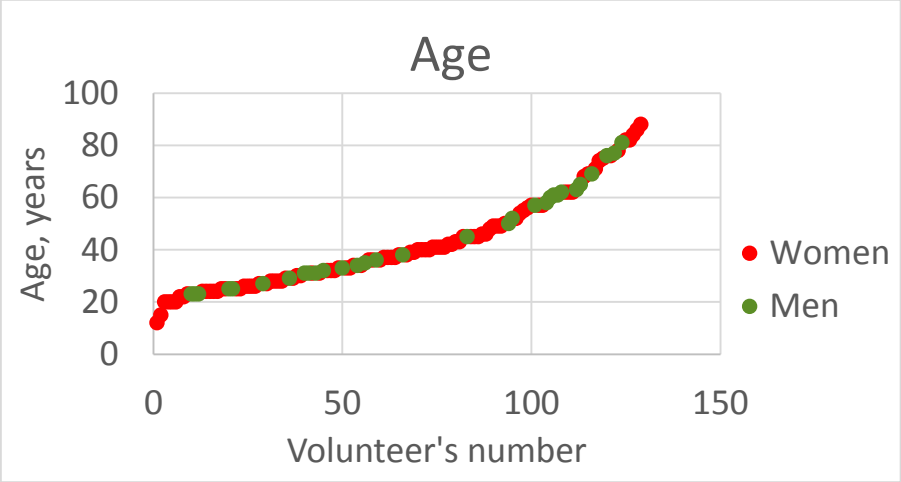


Fig. 5. Volunteer's age distribution.

Table 6. Statistics of the measured skin neoplasms from January to November 2020

Diagnosis	Number		
	First prototype	Second prototype	Both prototypes
Dermal nevus	41	21	45
Combined nevus	64	26	68
Junctional nevus	22	3	22
Dermatofibroma	2	2	3
Halo nevus	-	1	1
Papilloma	-	2	2
D22	3	33	34
Seborrheic keratosis	35	14	41
Hyperkeratosis	-	1	1
Actinic keratosis	-	1	1
Hemangioma	18	7	20
Cavernous hemangioma	-	1	1
Skin vascular formation	1	1	2
Scar	-	2	2
Basal Cell Carcinoma	1	2	2
Total	**187**	**117**	**245**

The general procedure for clinical measurements

1. A doctor explains to a patient the measurement procedure, an impact of the diagnostic device, as well as information in the patient consent form and patient rights.
2. The volunteer signs the patient consent form if he/she agrees to the procedure.
3. The doctor captures images of neoplasm(s) by a dermatoscope and writes down the clinical diagnoses to each lesion.
4. Switch on the device.
5. Fill patient's ID code and diagnoses.
6. Locate the neoplasm and place the device in the best capture position.
7. Capture the neoplasm under illumination at the following wavelengths: 450nm, 523nm, 638nm and 850nm.
8. Switch second mode with 405nm laser diodes and capture another image.

After images have been captured for one lesion, the next neoplasm(s) are imaged.

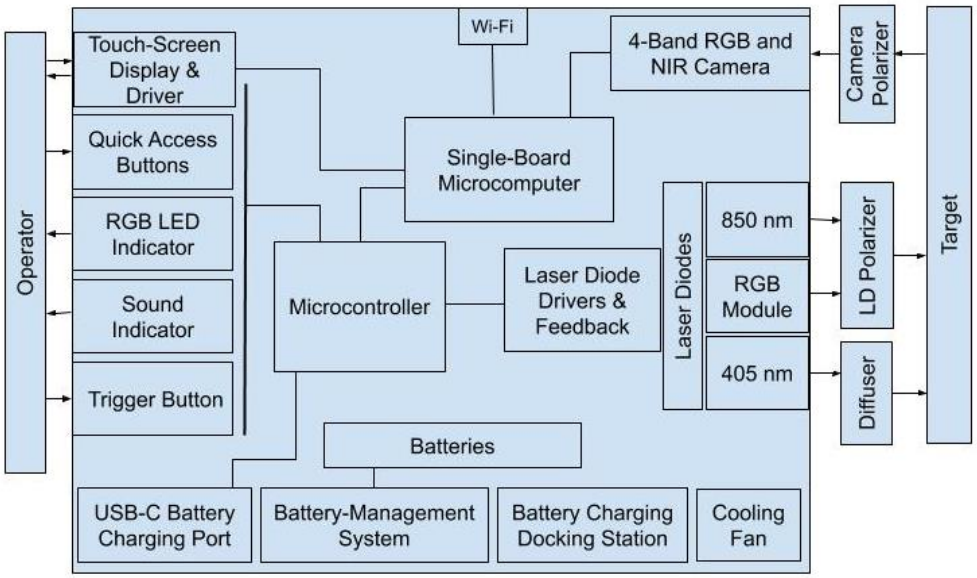


Fig. 6. Block-diagram of the single-camera device.

9. Switch off the device.
10. During the procedure, the patient is questioned if everything is all right and whether the patient feels comfortable.
11. After the measurement, the patient gets dressed, and, if patient has any additional questions, they are addressed accordingly.
12. The measurement is finished. The device is being cleaned with a disinfectant wipe.

Although formally the double-camera design has met all the project conditions, its clinical validation showed several drawbacks limiting its further clinical implementation. It was too robust and inconvenient for patient measurements and the images taken by both cameras not always overlapped as the skin surface under examination could be dome-shaped due to pressure caused by the light-screening nozzle. Therefore we took decision to develop a handier prototype design using a single four-band camera for spectral line image acquisition. This activity was performed in synergy with another local project of Institute of Atomic Physics and Spectroscopy.

A brief description of the initially unplanned second – **single-camera prototype device** is presented below.

The illumination system of the **single-camera device** (Figs. 6-8) is similar to that of the first prototype, only the side-emitting fiber loop is flat spiral-shaped and the target area is round with diameter of 10 mm, 20 mm or 30 mm, depending on the used changeable conical nozzle (inside-covered by a black coating film - Spectral Black, *Actar*, IL). Four 405 nm, 40 mW laser diodes (DL-5146-101S, *Roithner*, AT) are square-placed inside the fiber spiral to enable excitation of skin autofluorescence. Device is fully self-sustained using four rechargeable Li-ion batteries (INR18650-35E, *Samsung*, KR) for power supply. Four band RGB-NIR camera (MSC-RGBN-1-A, *Spectral Devices Inc*., CA – Fig.7) covered by a 420 nm long-pass filter, equipped with objective lens (25mm #67-715, Edmund Optics, US) and top-mounted orthogonally oriented VIS-NIR polarizer, is capturing image of the targeted skin area with subsequent extraction of the four spectral line images. They are further stored in the embedded single-board computer(SBC, Rock960, *Vamrs*, CN) and wi-fi transmitted to external computer for calculation of four chromophore distribution maps using the previously developed algorithm. Within a second, the laser modules are switched off, violet lasers switched on and autofluorescence image at the G-channel of camera captured. This image is also transmitted to the external computer in order to discriminate seborrheic keratosis from skin melanoma and other pigmented lesions. The second imaging step can be skipped or used alone, if necessary.

The device is initiated by operator pushing the START button and selecting the appropriate operation mode (SMSLI only, AF image only, combined mode) and exposure time for the RGB-NIR camera. Then the camera and lasers are switched on and the device is properly placed on the skin target area (monitored on the round display). After pressing the SHOT button, micro-controller (STM32G071, *STMicroelectronics*, CH) synchronizes proper illumination of the target to capture the image (or set of images) which is/are read by the single-board-computer. The recorded

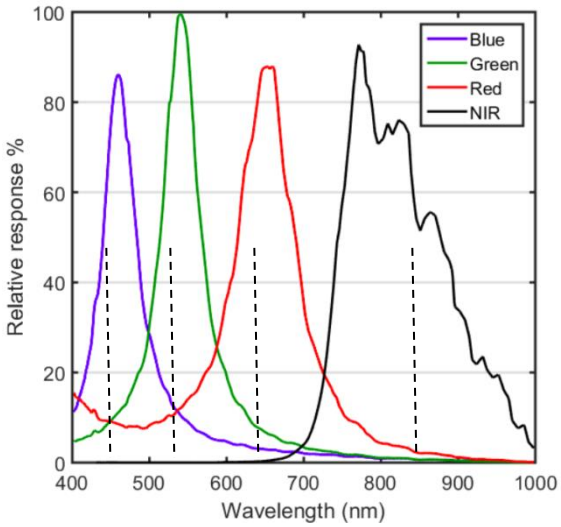


Fig.7. Spectral sensitivities of the used 4-band image sensor; dotted lines represent the working wavelengths.

images can be seen on the display and/or transmitted via SBC's *wi-fi* to the remote computer for calculation of chromophore maps or performing other tasks.

The recorded RAW images (2048×2048 pixels) are stored on the computer hard disk. In order to get correct multispectral reflectance images, first the RAW data are pre-processed. RAW images are converted into R-G-B-IR spectral line images (512×512 pix.) using the *SpectralDevices debayering* algorithm and the available spectral sensitivity curves of four detection bands (Fig. 6). Then the spectral un-mixing is done using linear operation:

$$I = A * I_s^{-1} \quad (1)$$

where $I_s(x,y,\lambda)$ is a matrix consisting of intensity values of spectral image at *xy* pixel and the given wavelength λ, and $I(x,y,\lambda)$ is spectrally-unmixed image; $A(\lambda)$ is correction matrix calculated from the camera photo-sensitivity curves. Next, flat-field correction for each spectral image is performed, using special image filtering technique. The idea is to compensate possible uneven illumination caused by deformed skin surface caused by the device's cap pressure. To find the non-evenness function of surface illumination, 2D median filter is applied (kernel size 128x128 pixels), and then spectral image is divided by this function. The normalized reflectance image is calculated as:

$$R = (I - I_d) / (I_f - I_d) \quad (2)$$

where $R(x,y)$ is a reflectance at each *xy* pixel of the spectral image, I_d is an image measured in dark conditions (the image values depends of camera gain, exposure and temperature), and $If(x,y)$ is the filtered image. The I_d spectral images are acquired before or after each measurement. Finally, the image outside the region of interest is masked with dark pixels to highlight circular image area on the monitor screen. The processing of MS data is done by custom designed Matlab software. The software performs off-line processing of RAW image data using the algorithms described above.

The user's interface (Fig.9) is designed for live showing of skin target and the recorded/stored skin images. The device can be managed by means of the touchscreen display. The software allows manual adjustment of device parameters such as intensity of light sources, camera gain and exposure. Before each measurement, specific patient data and identification of the examined skin malformation are stored. The two image sets (VIS/NIR and autofluorescence images) are captured within two seconds. The recorded four spectral line images and the autofluorescence (AF) image are transmitted to the monitor screen. The measurement data also can be transferred to the remote server-computer for more detailed analysis.

The single-camera prototype in operation is presented on Fig.10.

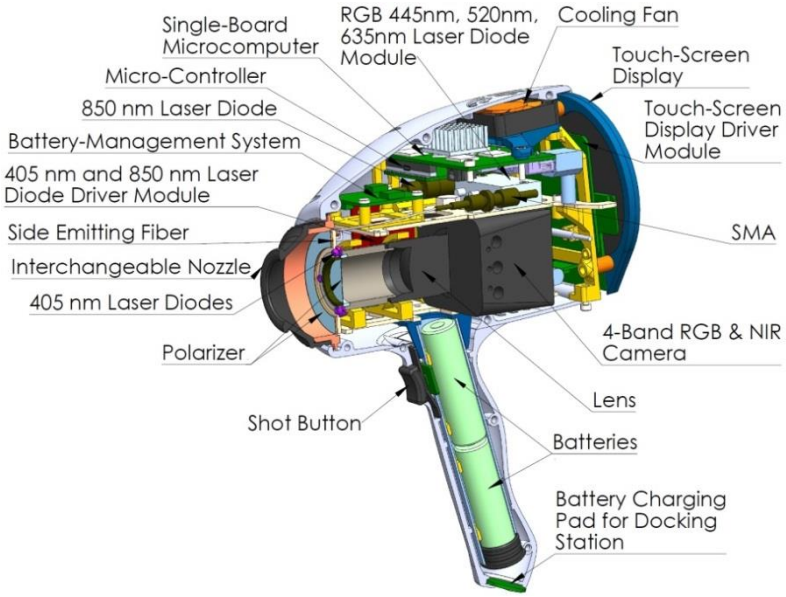


Fig. 8. Design scheme of the single-camera prototype device.

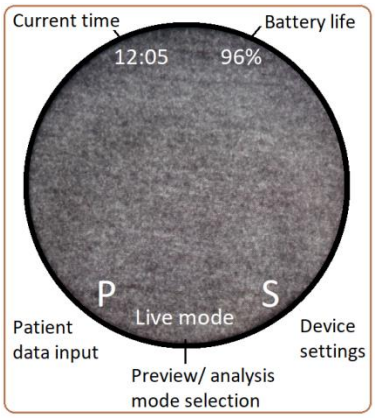


Fig. 9. The user's interface for single-camera device.

Fig. 10. The single-camera prototype device in operation.

WP5 ***Analysis of the clinical data and optimization of the prototype design*** (M17-M22).

Personnel mainly involved: M.Mileiko, U.Rubins, Z.Rupenheits, I.Oshina, J.Spigulis.

Development and implementation of the optimized single camera design took several months and the second prototype was assembled by M22. Then it was tested in laboratory and clinically on limited number of volunteers; clinical measurements became problematic due to the restrictions caused by Covid-19 pandemic. Design and test results of the single-camera prototype were described in a paper published in Q1-journal *Applied Sciences* [1].

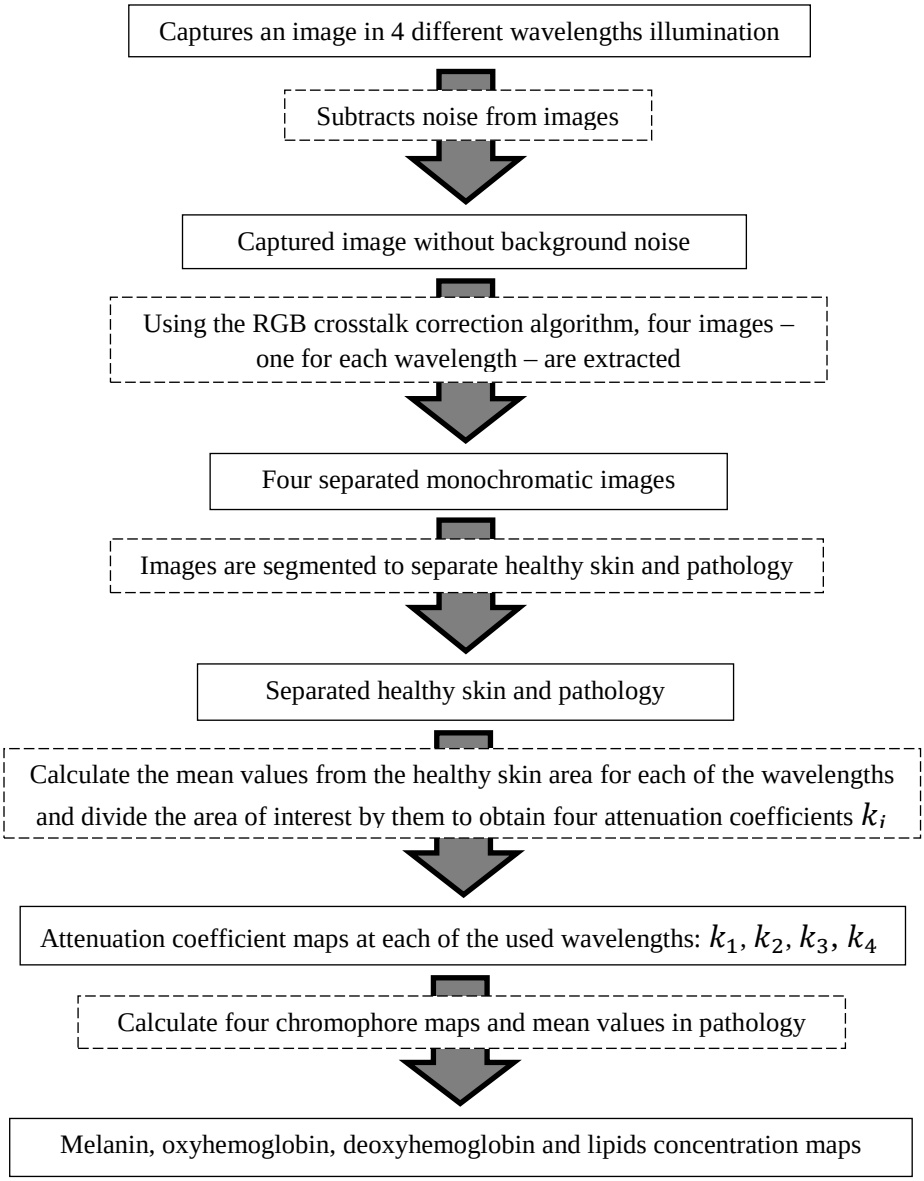


Fig. 11. The chromophore-mapping algorithm scheme.

For image processing, the Beer-Lambert-Bouguer law adapted for skin reflectance was used:

$$I = I_0 e^{-\mu_a \cdot l} \quad (3),$$

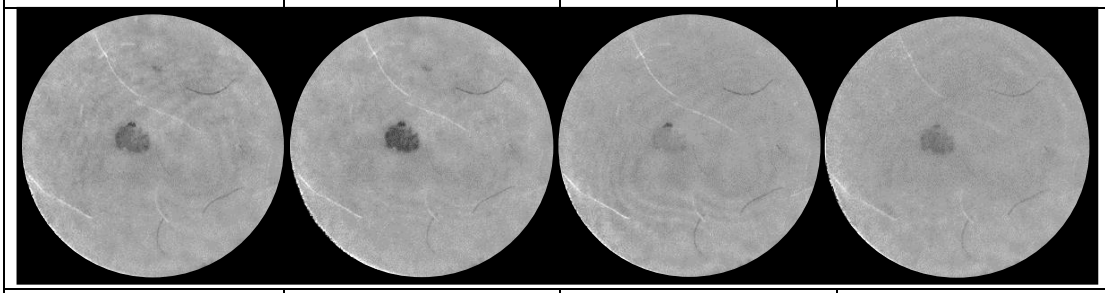
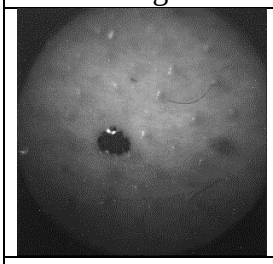
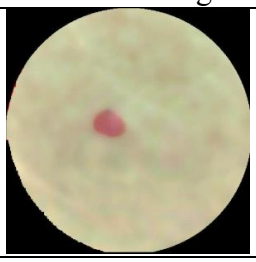
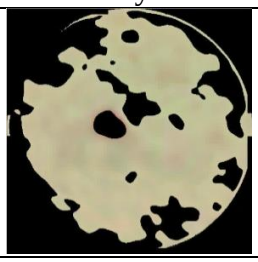
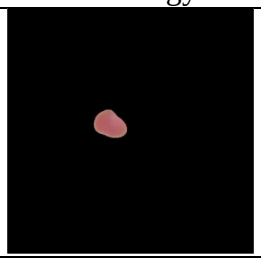
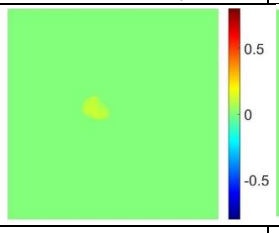
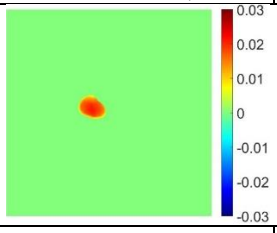
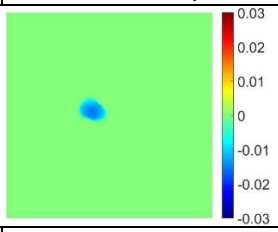
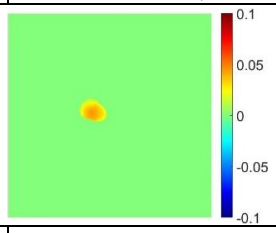
where μ_a – absorption coefficient, I – intensity of diffused reflected light from the skin pathology, I_0 – intensity of diffused reflected light from the healthy skin, l – photon mean path length in the skin. This can be expressed for the four exploited wavelengths and the four considered chromophores (melanin, oxy-hemoglobin, deoxy-hemoglobin and lipids) as follows:

$$\begin{cases} c_{\text{Mel}} \cdot \varepsilon_{\text{Mel}}(\lambda_1) + c_{\text{Ox}} \cdot \varepsilon_{\text{Ox}}(\lambda_1) + c_{\text{Deox}} \cdot \varepsilon_{\text{Deox}}(\lambda_1) + c_{Lip} \cdot \varepsilon_{\text{Lip}}(\lambda_1) = \frac{ln\frac{I_0(\lambda_1)}{I(\lambda_1)}}{2{,}303 \cdot l(\lambda_1)} \\ c_{\text{Mel}} \cdot \varepsilon_{\text{Mel}}(\lambda_2) + c_{\text{Ox}} \cdot \varepsilon_{\text{Ox}}(\lambda_2) + c_{\text{Deox}} \cdot \varepsilon_{\text{Deox}}(\lambda_2) + c_{Lip} \cdot \varepsilon_{\text{Lip}}(\lambda_2) = \frac{ln\frac{I_0(\lambda_2)}{I(\lambda_2)}}{2{,}303 \cdot l(\lambda_2)} \\ c_{\text{Mel}} \cdot \varepsilon_{\text{Mel}}(\lambda_3) + c_{\text{Ox}} \cdot \varepsilon_{\text{Ox}}(\lambda_3) + c_{\text{Deox}} \cdot \varepsilon_{\text{Deox}}(\lambda_3) + c_{Lip} \cdot \varepsilon_{\text{Lip}}(\lambda_3) = \frac{ln\frac{I_0(\lambda_3)}{I(\lambda_3)}}{2{,}303 \cdot l(\lambda_3)} \\ c_{\text{Mel}} \cdot \varepsilon_{\text{Mel}}(\lambda_4) + c_{\text{Ox}} \cdot \varepsilon_{\text{Ox}}(\lambda_4) + c_{\text{Deox}} \cdot \varepsilon_{\text{Deox}}(\lambda_4) + c_{Lip} \cdot \varepsilon_{\text{Lip}}(\lambda_4) = \frac{ln\frac{I_0(\lambda_4)}{I(\lambda_4)}}{2{,}303 \cdot l(\lambda_4)} \end{cases} \quad (4),$$

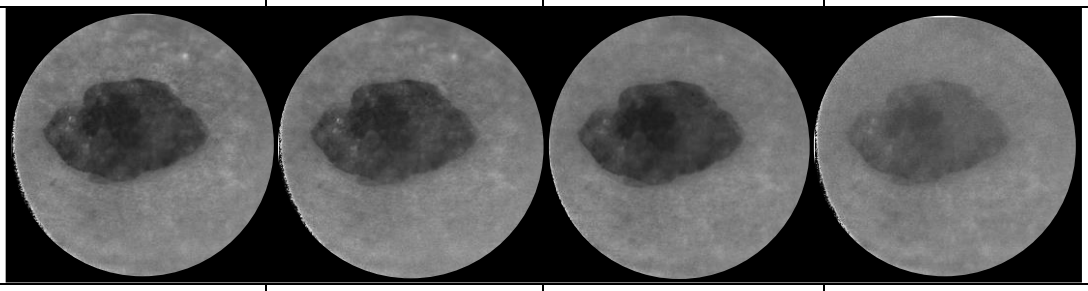
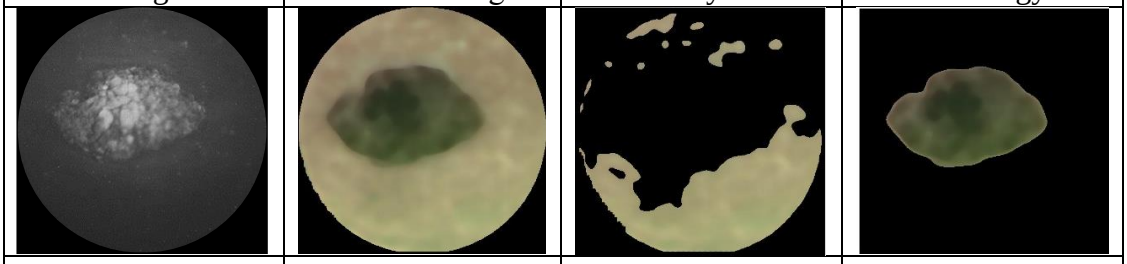
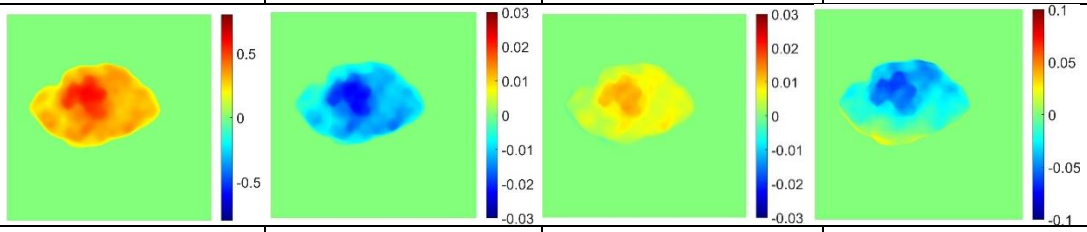
where Mel – melanin, Ox – oxyhemoglobin, $Deox$ – deoxyhemoglobin, Lip – lipids, ε – extinction coefficient, c – chromophore concentration.

Two examples of captured and processed clinical images are presented below.

Validation of the algorithm - spectral images, fluorescence image, “white” image, segmented images and the related chromophore maps for a **hemangioma**:

450 nm	523 nm	638 nm	850 nm
			
Fluorescence image	“White” image	Healthy skin	Pathology
			
Melanin concentration, mM	Oxy-hemoglobin concentration, mM	Deoxy-hemoglobin concentration, mM	Lipids concentration, mM
			
$\overline{c_{Mel}} = 0{,}096 \pm 0{,}020\ mM$	$\overline{c_{Ox}} = 0{,}016 \pm 0{,}004\ mM$	$\overline{c_{Deox}} = -0{,}011 \pm 0{,}003\ mM$	$\overline{c_{Lip}} = 0{,}031 \pm 0{,}009\ mM$

Validation of the algorithm - spectral images, fluorescence image, “white” image, segmented images and the related chromophore maps for a **seborrheic keratosis**:

450 nm	523 nm	638 nm	850 nm
			
Fluorescence image	“White” image	Healthy skin	Pathology
			
Melanin concentration, mM	Oxy-hemoglobin concentration, mM	Deoxy-hemoglobin concentration, mM	Lipids concentration, mM
			
$\overline{c_{Mel}}$ $= 0{,}334$ $\pm 0{,}100\ mM$	$\overline{c_{Ox}}$ $= -0{,}011$ $\pm 0{,}005\ mM$	$\overline{c_{Deox}}$ $= 0{,}006$ $\pm 0{,}003\ mM$	$\overline{c_{Lip}}$ $= -0{,}025$ $\pm 0{,}019\ mM$

Based on the measurement results, statistically mean values of concentration changes of specific skin chromophores in the examined malformations (if compared to the surrounding healthy skin) have been calculated (Fig.12).

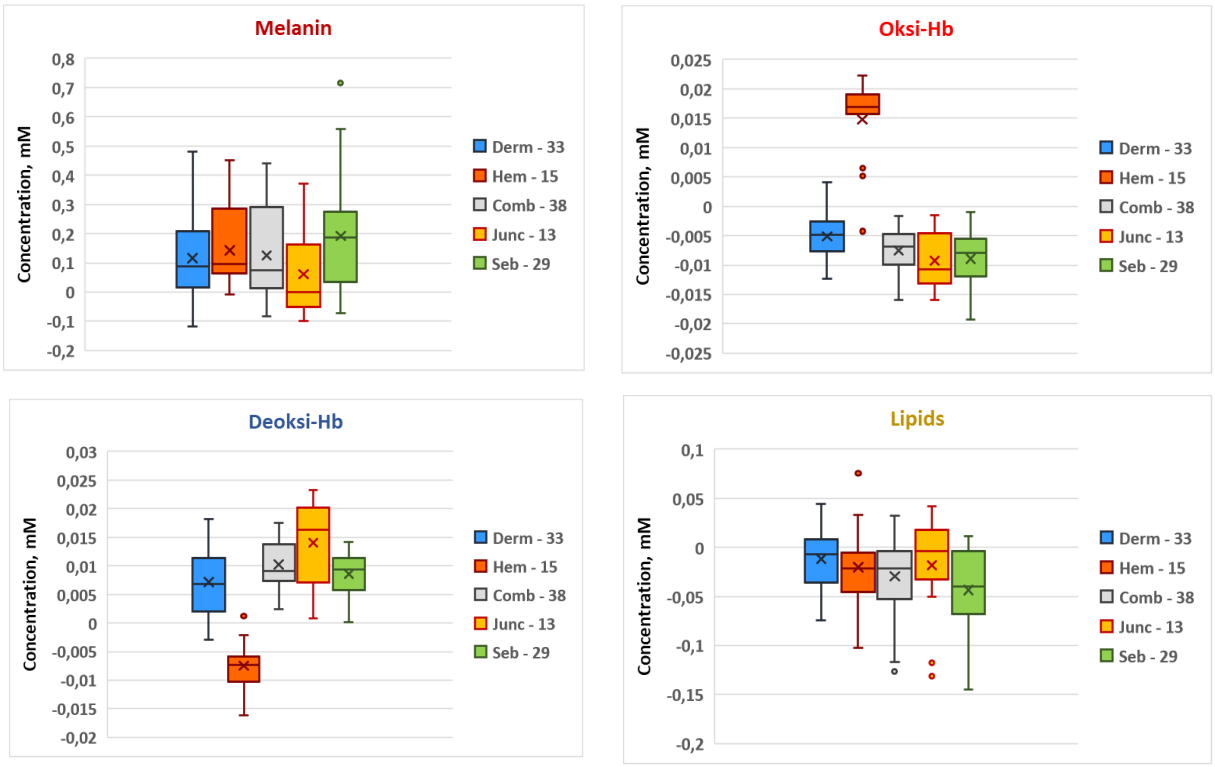


Fig. 12. Concentration differences (mean values) of melanin, oxy- and deoxy-hemoglobin and lipids for dermal (Derm) nevi, hemangioma (Hem), combined (Comb) nevi, junctional (Junc) nevi and seborrheic keratosis (Seb). Concentration increase/decrease values are compared in malformation and in healthy skin.

WP6 ***Project management, dissemination of results and technology transfer*** (M1-M24).
Personnel mainly involved: J.Spigulis, L.Zeltina**,** I.Oshina.

Project management was performed accordingly to the proposal; in order to increase efficiency of work, the project group meetings took place on a weekly basis, instead of monthly meetings as initially proposed. All meetings were documented. Dissemination and managerial activities were carried out over the whole project period as initially proposed (see Table 4). The main dissemination activity – conference "Biophotonics – Riga 2020" – was influenced by the Covid-restrictions and took place in a combined mode – all reports were remote while the catering, exhibition and social event took place on-site. Due to additional workload related to development and tests of the second prototype, as well as the unforeseen shifts in the time schedule and Covid-delayed clinical measurements, all technology transfer activities could not be completed within the timeframe of this project as initially planned and will be performed after the project and funded by the grant recipient.

Summary of the project

To summarize, this project has resulted with two functioning diagnostic prototype devices of different designs, implementing the novel four spectral line imaging concept along with fluorescence image capturing of the same skin malformation. Both devices have passed laboratory tests on colour standards and tissue phantoms and also clinical validation tests on 245 skin malformations. Valuable experience on prototype development for quantitative non-contact skin assessment has been gained and a rich clinical material for further studies collected. The obtained results were published in six SCOPUS-cited papers (planned 3) and presented in seven conference reports (planned 5); one technological solution was patented. Project results were disseminated also in other ways (Table 4), including organization of the 3rd International Conference *"Biophotonics – Riga 2020"* with book of proceedings, recently published in USA (https://www.spiedigitallibrary.org/conference-proceedings-of-spie/11585.toc). Due to extreme working conditions and restrictions caused by the Covid-19 pandemic, technology and knowledge transfer activities were not performed as initially planned and will be carried out on own resources when the situation will stabilize.