RECENT RESULTS OF RIGA GROUP ON LASER APPLICATIONS FOR SKIN DIAGNOSTICS

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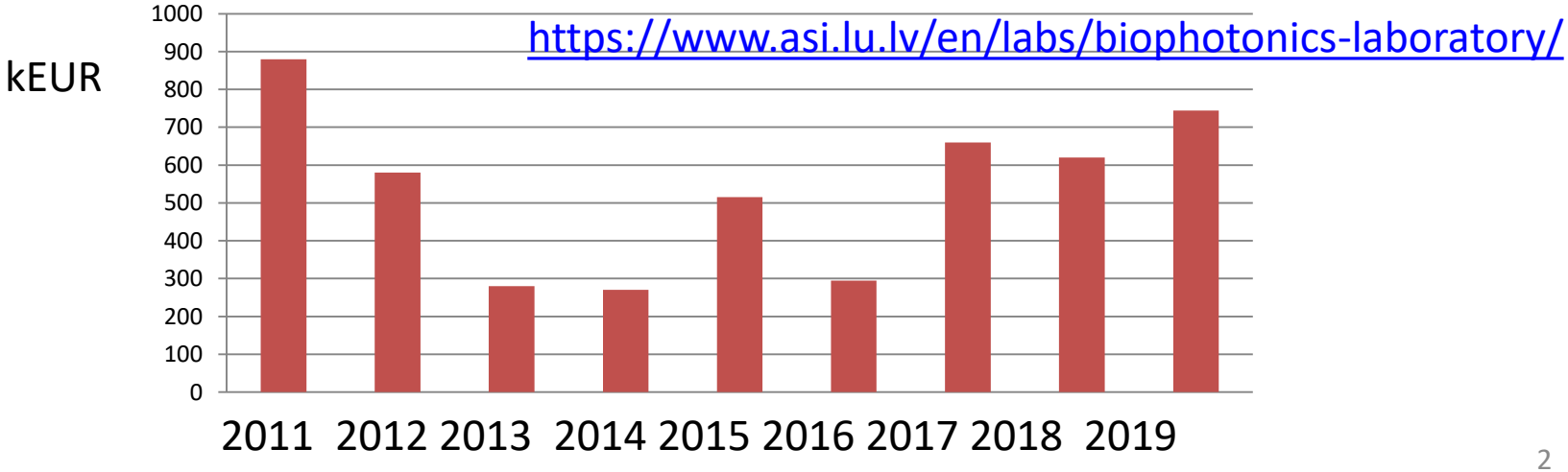
ICSQE, Varna (BG), 21/09/2020

Biophotonics Laboratory at IAPS UL

- Established in 1997 as Bio-optics and fiberoptics group
- Main research direction: optical assessment of *in-vivo* skin
- ~25-30 staff members involved in projects, incl. 10 PhDs
- 2019: 12 research projects, incoming budget ~765 kEUR



https://www.asi.lu.lv/en/labs/biophotonics-laboratory/

Running projects on laser applications

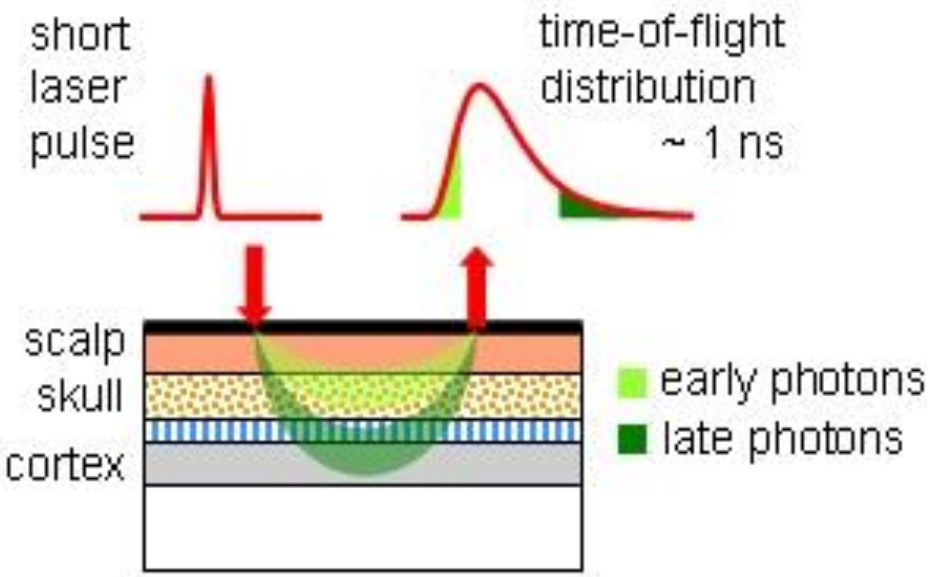
- **European Regional Development Fund (ERDF) projects:**
  - *Multimodal imaging technology for in-vivo diagnostics of skin malformations* (**Janis Spigulis**).
  - *Time-resolved autofluorescence methodology for non-invasive diagnostics of skin cancer* (**Alexey Lihachev**, postdoc).
  - *Development of prototype devices for nonivasive assessment of skin condition*, (**Edgars Kviesis-Kipge**, postdoc).
- **Latvian Council of Science (LCS) project:**
  - *Advanced spectral imaging technology for skin diagnostics* (**Janis Spigulis**).

FLPP
FUNDAMENTAL AND APPLIED RESEARCH PROJECTS

Today's topics

1. Picosecond lasers for determination of remitted photon path lengths in skin
2. Laser multi-line illumination prototypes for mapping of skin chromophores and fluorescence imaging
3. First results on skin Raman spectroscopy and imaging at 785 nm laser excitation
4. Experiment on laser-excited cell autofluorescence photo-bleaching

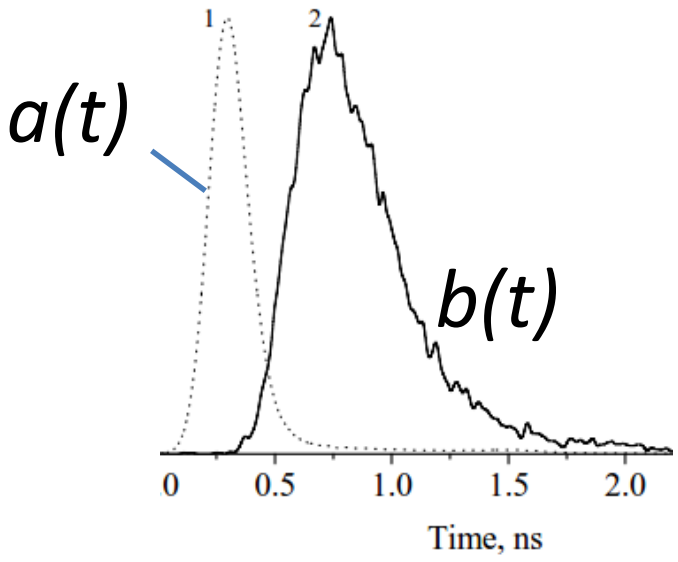
Time-of-flight (TOF) spectroscopy

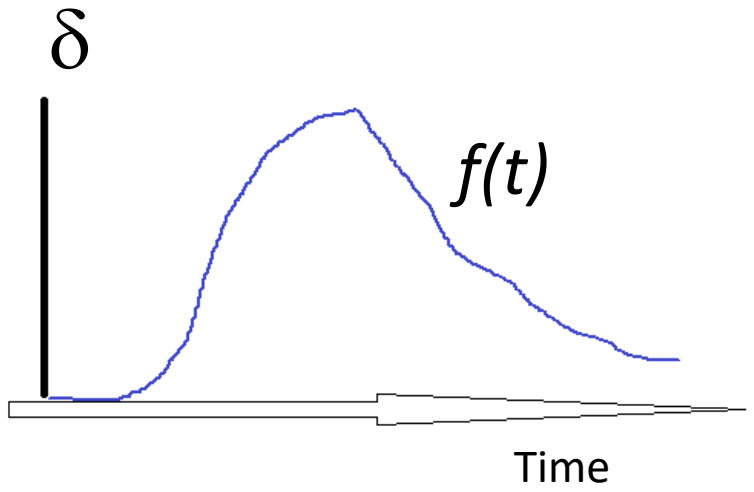


Time-of-flight (TOF) spectroscopy is a tool for characterization and analysis of highly scattering (turbid) materials, such as biological tissue, powders, and pharmaceutical samples. The main principle is to deliver a very short laser pulse into the material, and to analyze the resulting pulse at some distance.

Online: https://www.ptb.de/cms/en/ptb/fachabteilungen/abt8/fb-83/ag-831/kopf.html

The main idea

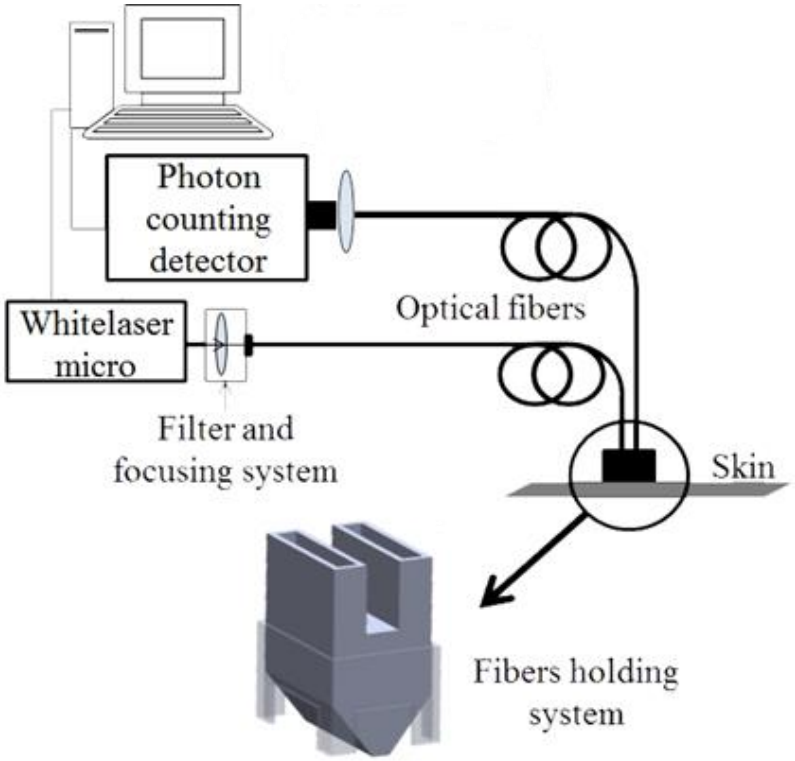


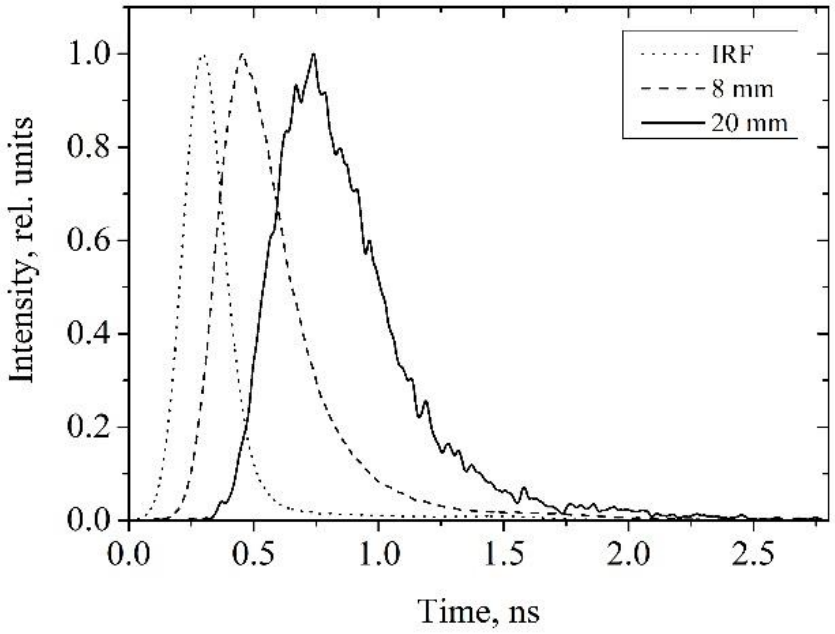


$$b(t) = \int_0^t a(t-\tau) f(\tau) d\tau$$

$f(s) = f(t) \cdot c/n$

1. Remitted photon path lengths in skin: time-resolved measurements (V.Lukinsone & Co.)

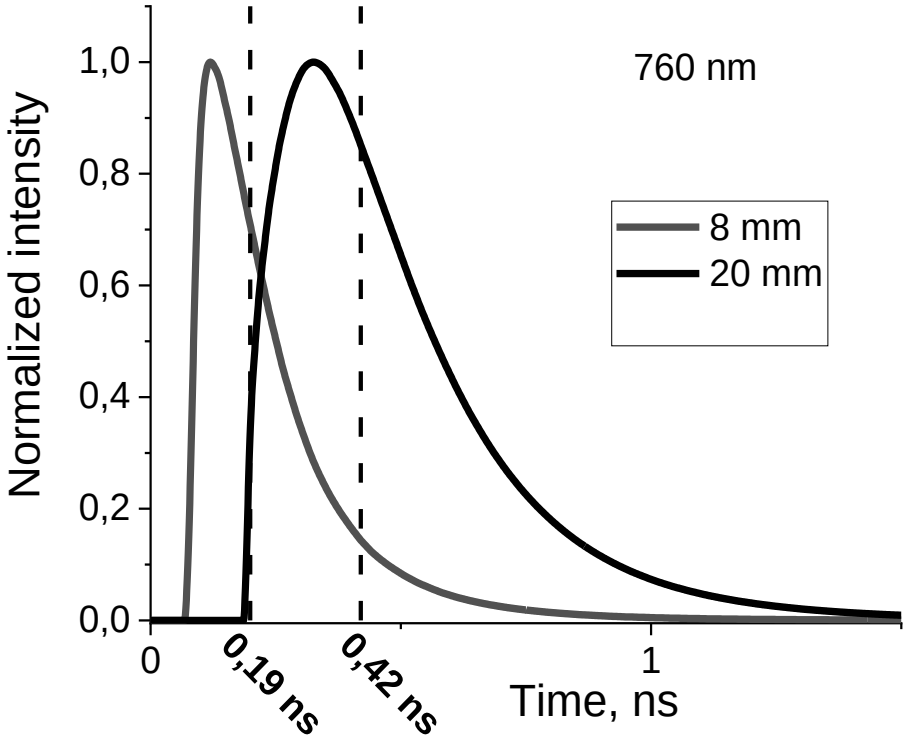




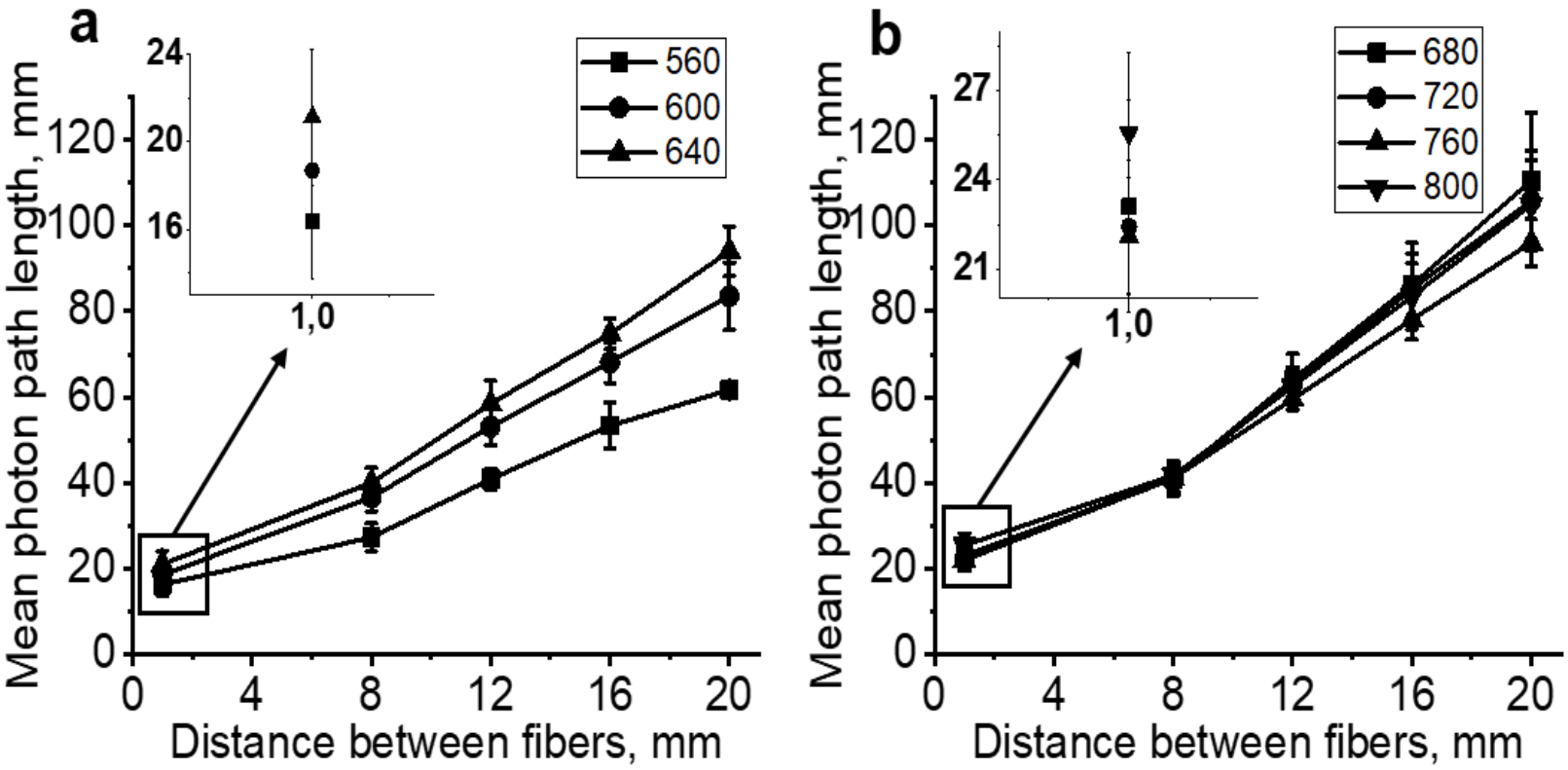
White laser FWHM 6 ps, 10 nm interference filters for 7 spectral bands 560-800 nm, 5 inter-fiber distances 1 ... 20 mm, 35 spectral-spatial combinations.
Results: MPL ~ 16 ... 105 mm, longer than modelled by MC; minimum at 760nm (Hb?)

Deconvolution: $b(t) = \int_0^t a(t-\tau)f(\tau)d\tau$

The smoothed skin-remitted photon arrival time distributions *f(t)* and their mean values for the 760 nm band at two inter-fiber distances (a single volunteer).

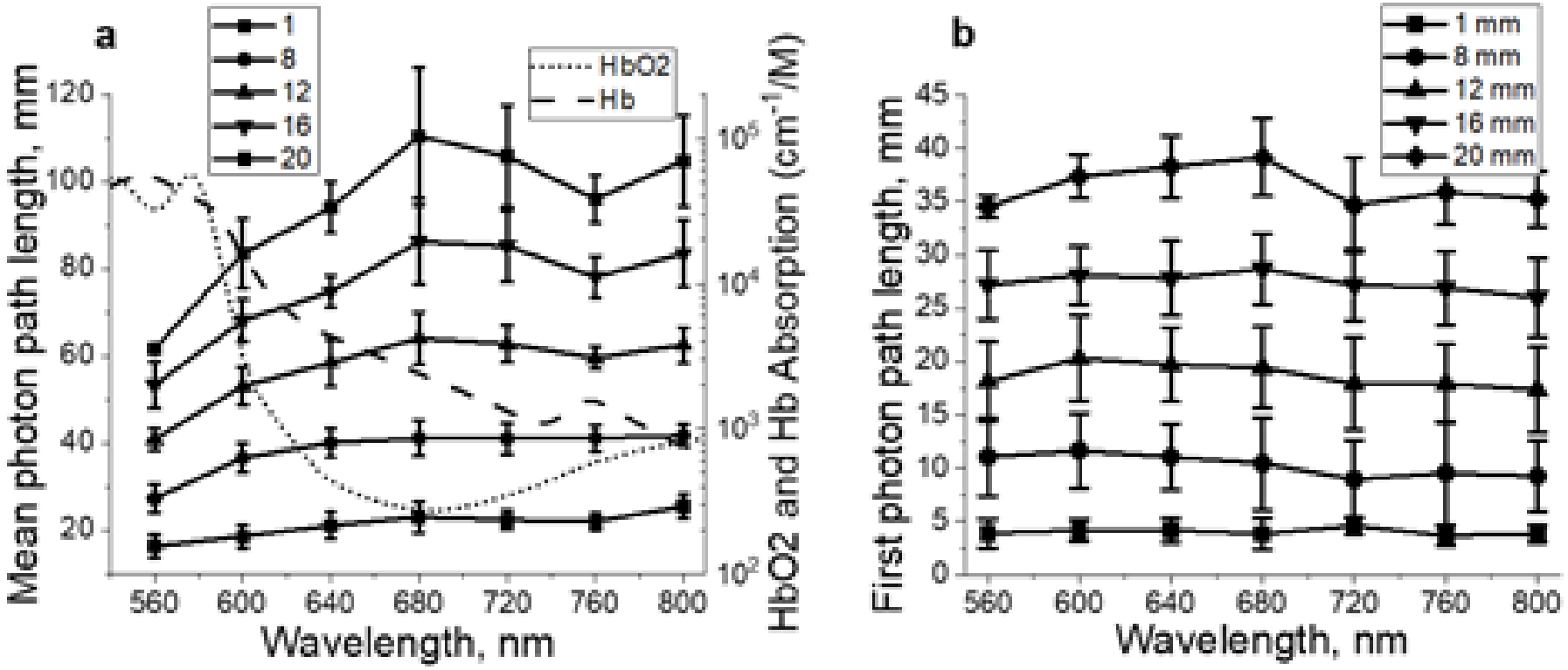


Spatial dependences of the mean path lengths of skin-remitted photons



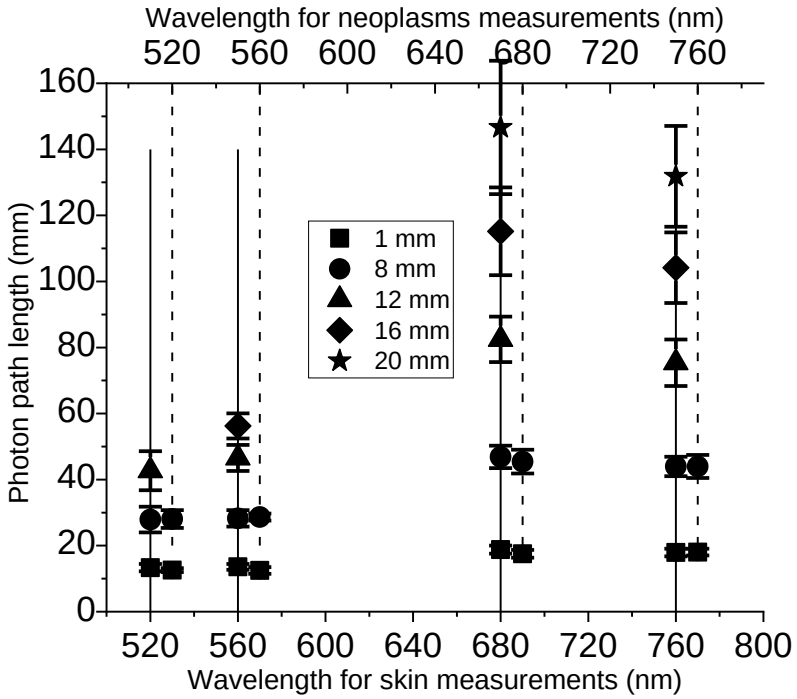
V.Lukinsone et al., “Remitted photon path lengths in human skin: in-vivo measurement data”, ***Biomed.Opt.Expr***. 11(5), 2866-2873 (2020).

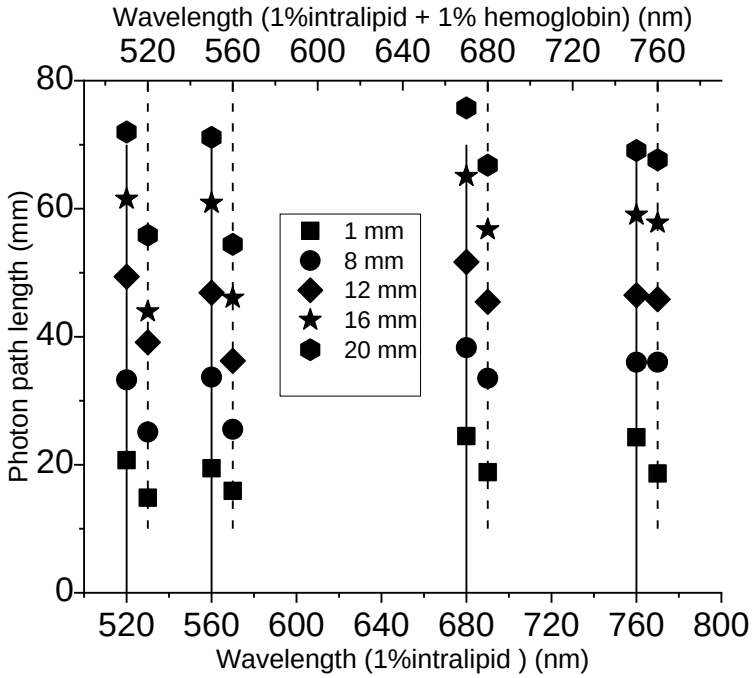
Spectral dependeces of the mean path length of skin-remitted photons



V.Lukinsone et al., "Remitted photon path lengths in human skin: in-vivo measurement data", ***Biomed.Opt.Expr***. 11(5), 2866-2873 (2020).

Skin neoplasms and phantoms





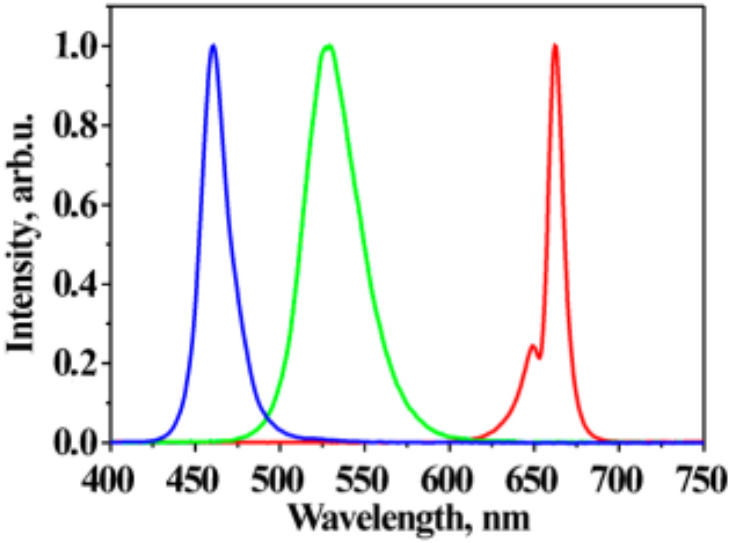
The mean remitted photon path lengths in skin (–) and neoplasms (--)

The data for phantoms with 1% of intralipid concentration (–) and with 1% of intralipid and 1% of hemoglobin concentration (--)

Lukinsone V. et al., "Remitted photon path length in human skin, skin phantoms and cell cultures", *Proc. SPIE* **11363**, 1136320 (2020)

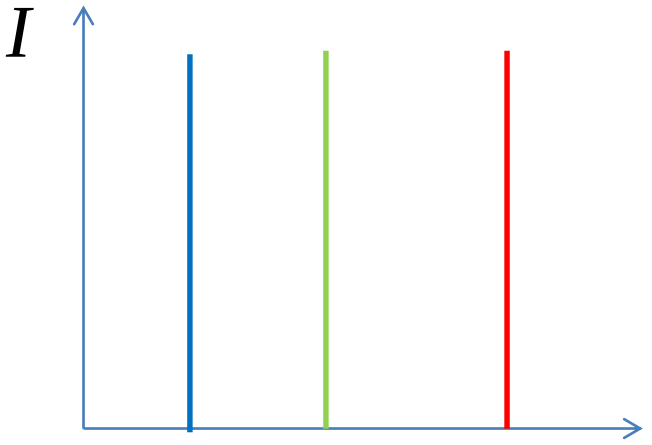
2. Multi-spectral-line imaging for skin chromophore mapping (J.Spigulis & Co.)

Conventional:
Spectral **band** images



Sequential (t>>0)

Novel:
Spectral **line** images

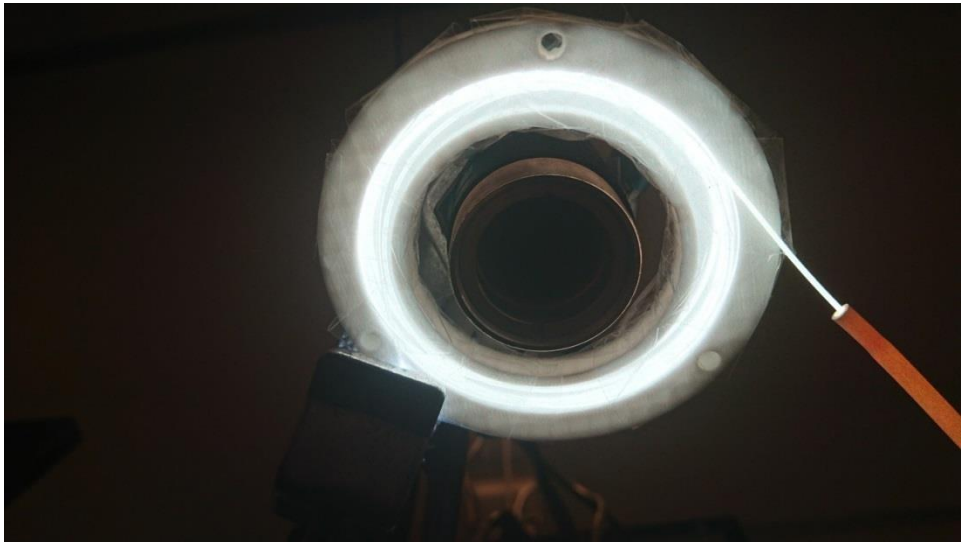


Single snapshot (t→0)

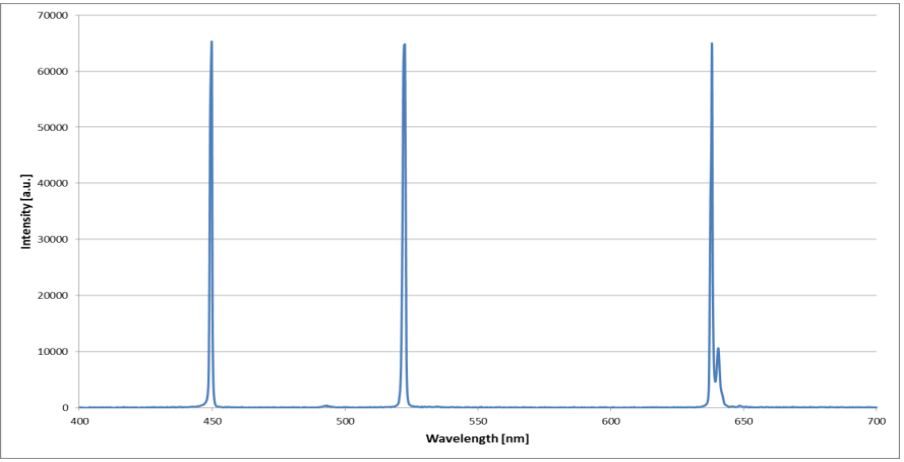
n =3 → n > 3

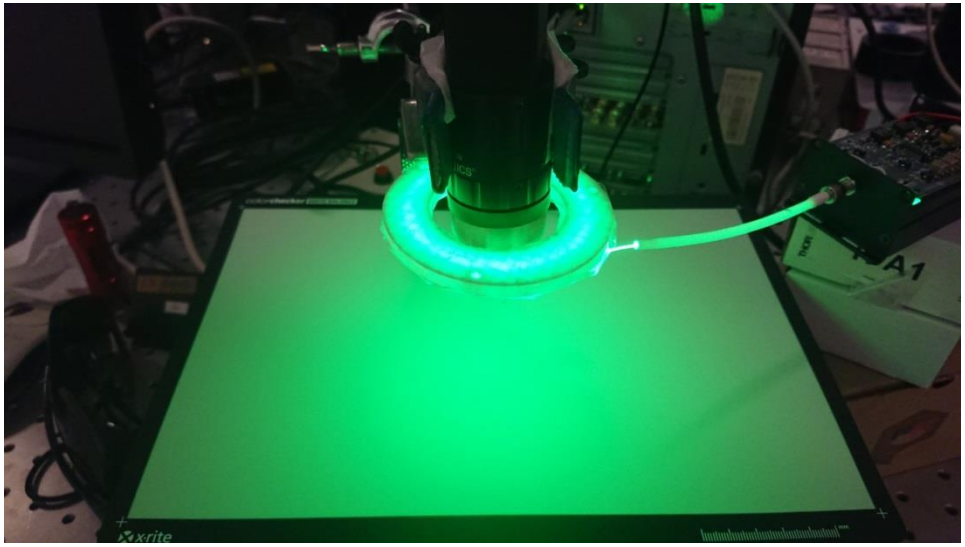
Benefits:
- Increased (ultimate) spectral selectivity, <0.01 nm
- Improved imaging quality (snapshot → avoided motion artefacts)
- Simpler/faster image processing (numbers instead of integrals over wavelength bands

Novelty: uniform four laser line illumination by a side-emitting optical fiber loop



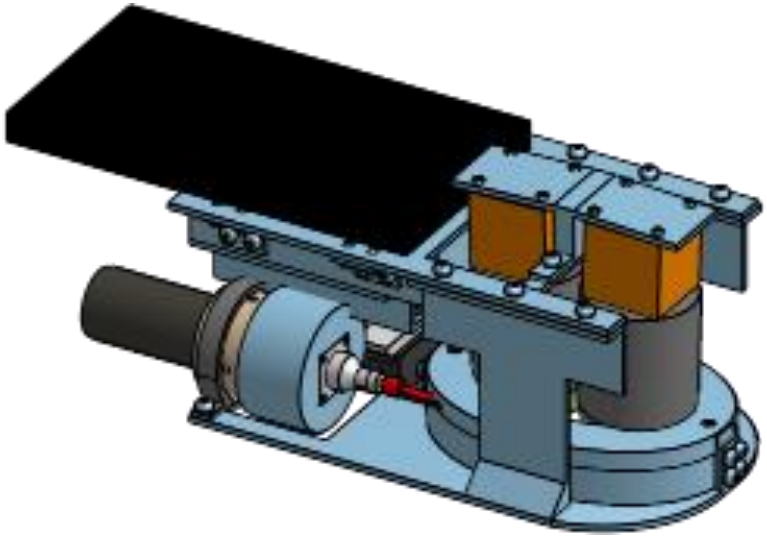
450/523/638 nm + 850 nm





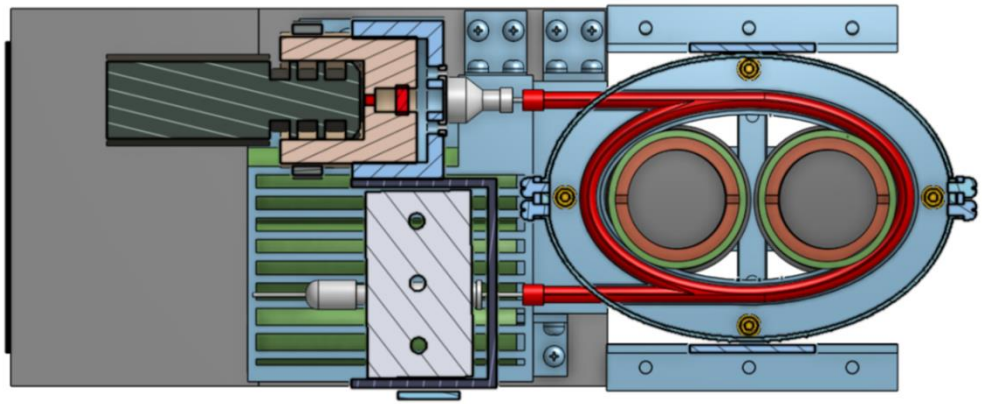
LV 11644 B, 1995. Side-emitting optical fiber (D. Pfafrods, M. Stafeckis, J. Spigulis, D. Boucher); LV 15491 B, 2020 (J.Spigulis, I.Oshina, Z.Rupenheits, M.Matulenko)

The (4+1) wavelength prototype: design concept



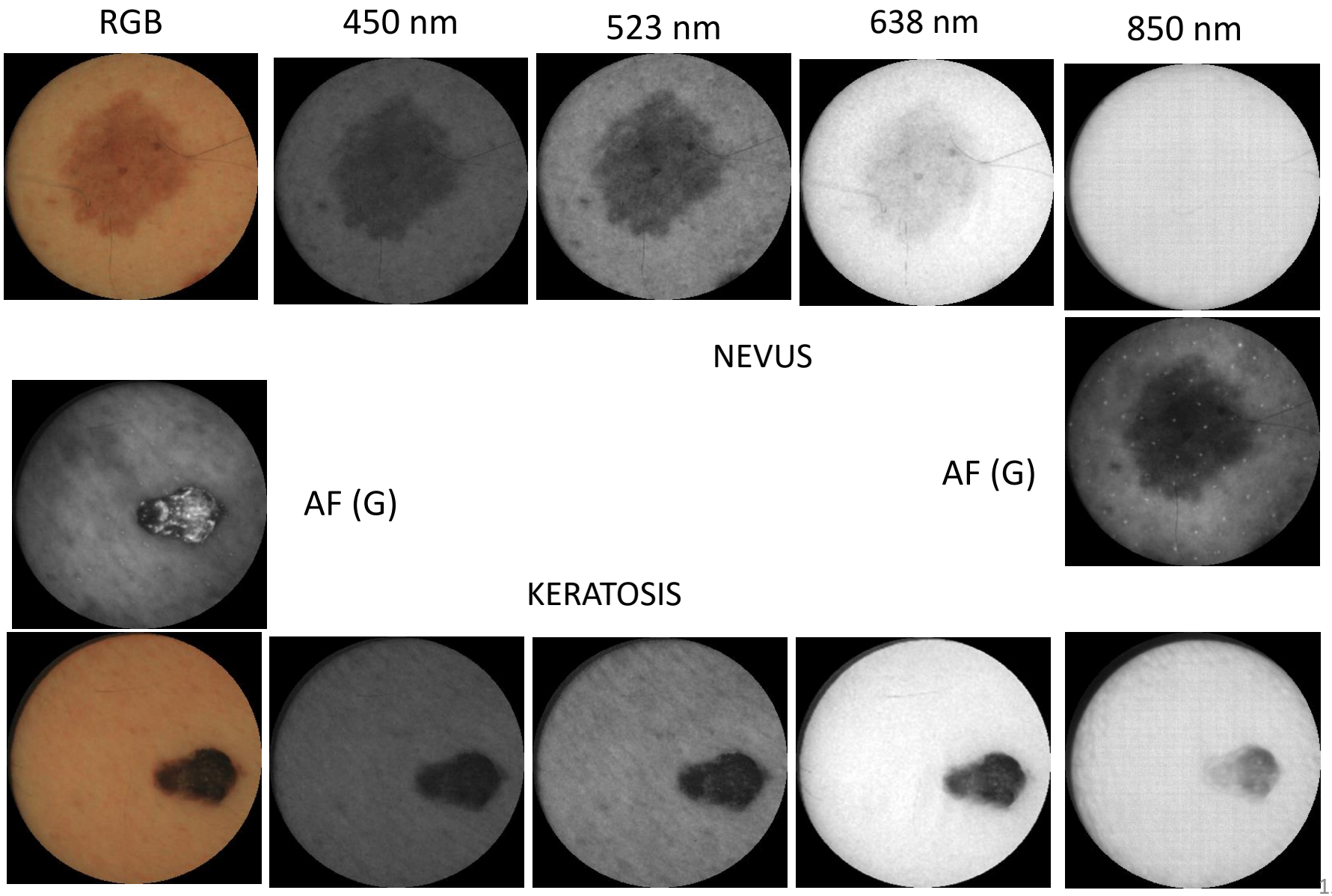
Step 1 - 450/523/638/850 nm illumination for snapshot mapping of 4 skin chromophores (HbO, Hb, Mel, Blr) and calculation of the MM criterion;

Step 2 – 405nm excitation for skin fluorescence imaging (MM – SK differentiation)



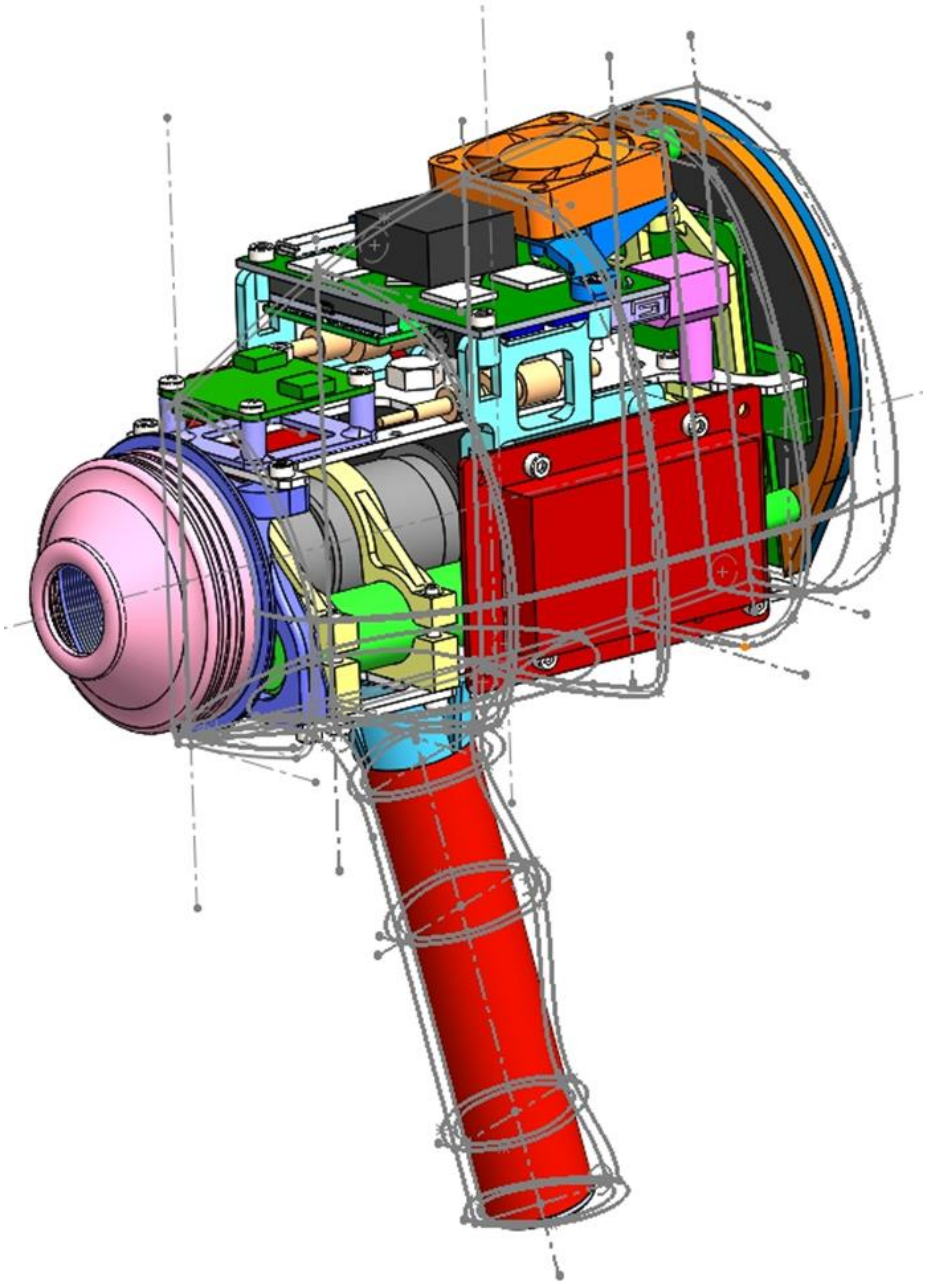
J.Spigulis et al., “A snapshot multi-wavelengths imaging device for in-vivo skin diagnostics”, Proc.SPIE 11232, 112320I-1 (2020).

Combined nevus *vs* seborrheic keratosis

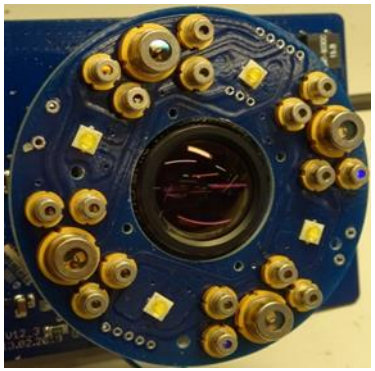


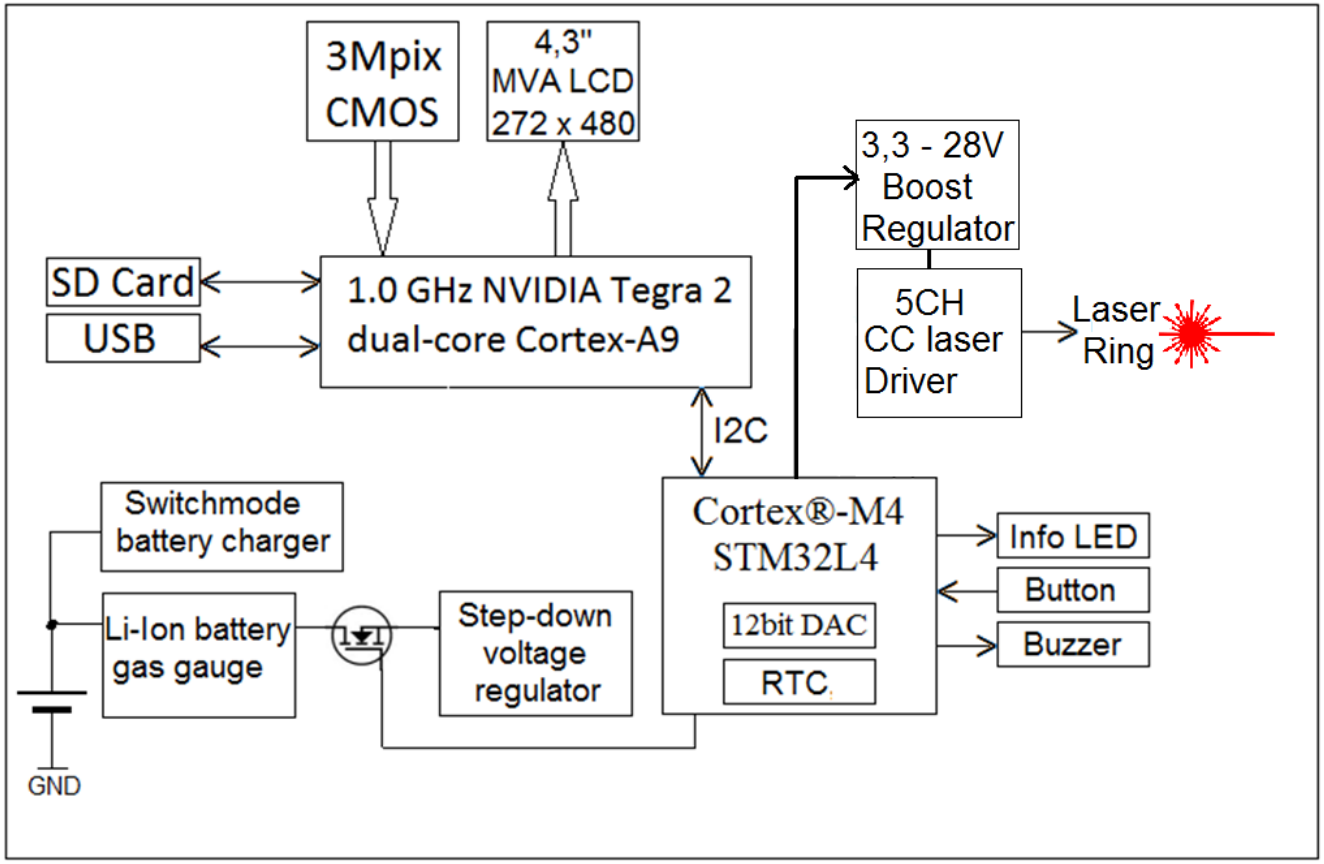
The four-band camera prototype

(405+450/523/638/850 nm, under development)



Five laser line device with LD ring source (E.Kviesis-Kipge)



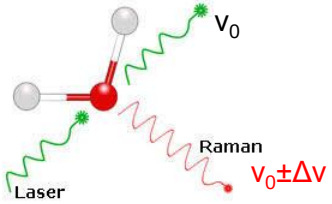


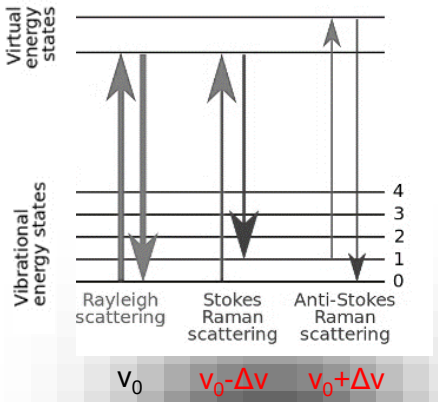
Laser diodes (x4): **405nm**-20mW, **450nm**-80mW, **525nm**-50mW, **655nm**-15mW, **845nm**-50mW

E. Kviesis-Kipge, "Development of skin chromophore mapping device using five spectral line illumination", OSA Technical Digest (2019), ITh4B.3 (2019).

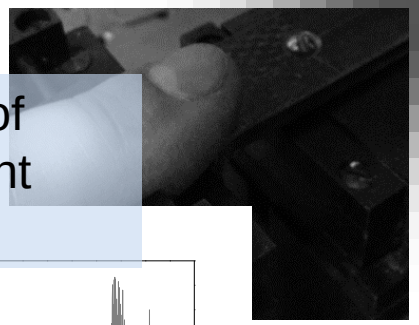
3. Raman principle and biomedical applications

It is a light scattering technique, where a photon interacts with a sample molecule to produce different wavelengths of scattered radiation

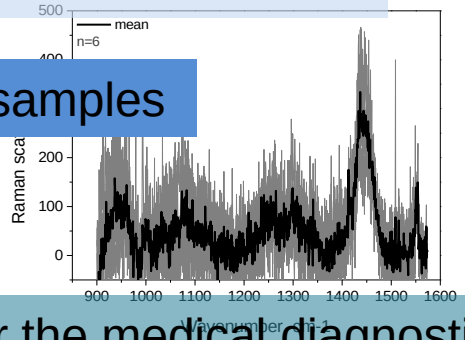




Useful for chemical identification, characterization of molecular structures effects of bonding, environment and stress on a sample



Useful for characterization of *ex vivo* biopsy samples

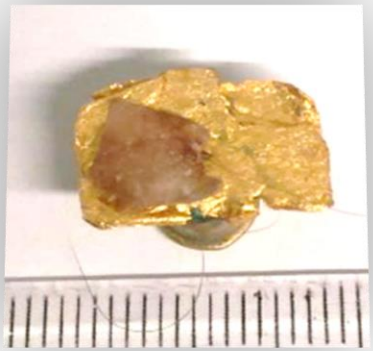


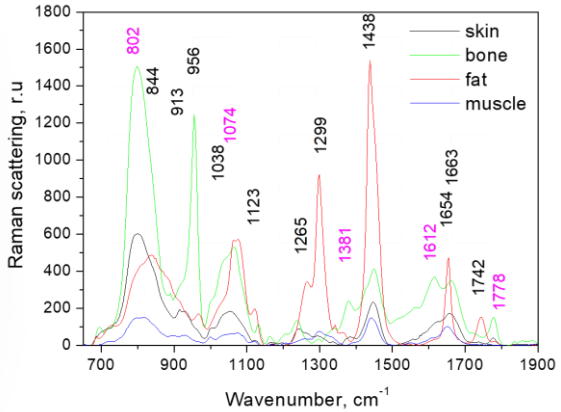
Useful for intraoperative assistance and for the medical diagnostics on a variety of diseases and tissue types including **skin cancer**

3. How to measure the Raman spectrum (M.Tamosiunas)

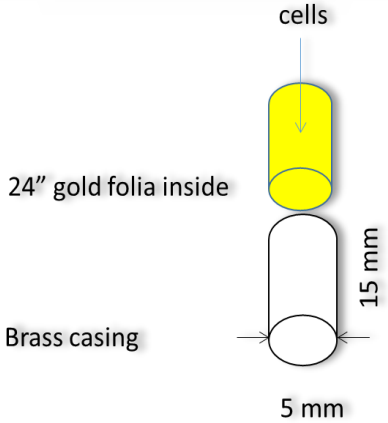
Ex. – NIR laser (785 nm; 500 mW/cm2 max.); L1 – fiber coupling lens; L2, L3 – collimating lenses; CF – laser clean-up filter; LP – long pass filter; DM – dichroic mirror; Fb. – fiber bundle for Raman spectra collection; TS – tissue/cell sample; xy – translational stage.

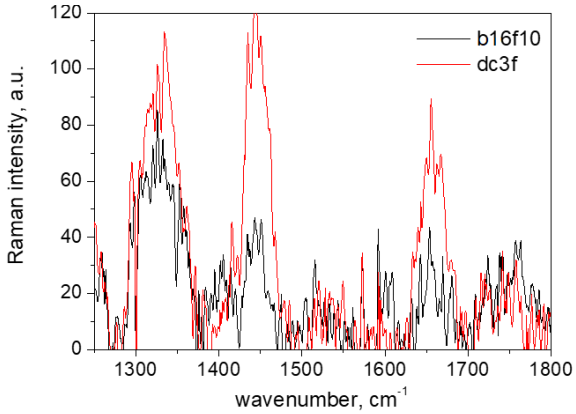
Sample holder ex vivo

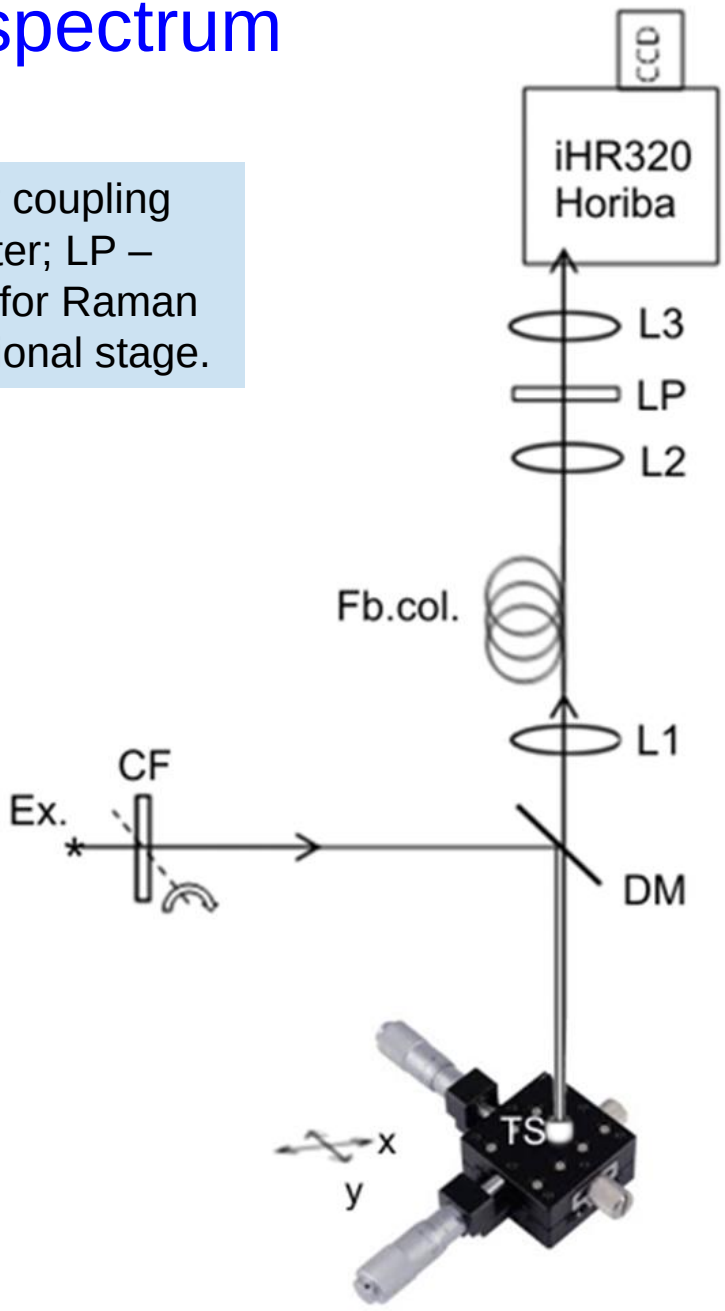




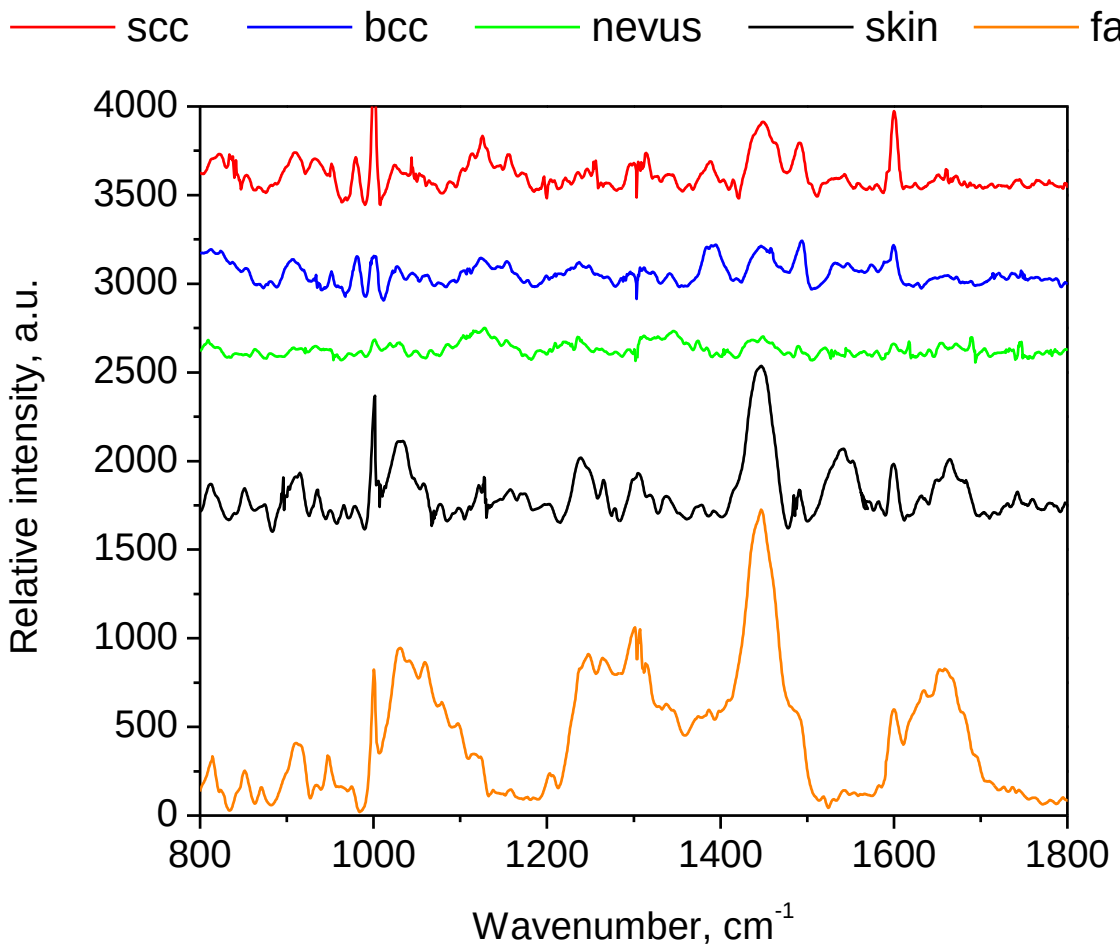
Cell sample compartment



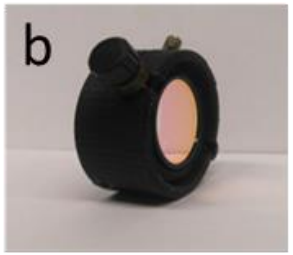


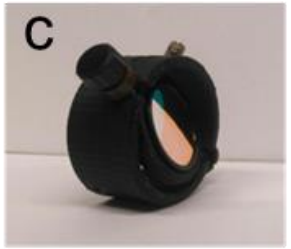


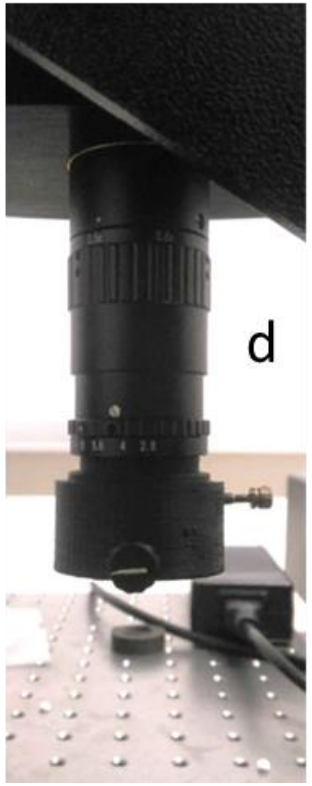
Measured mean Raman spectra of skin, subcutaneous fat tissue, nevus and skin cancer (SCC and BCC)

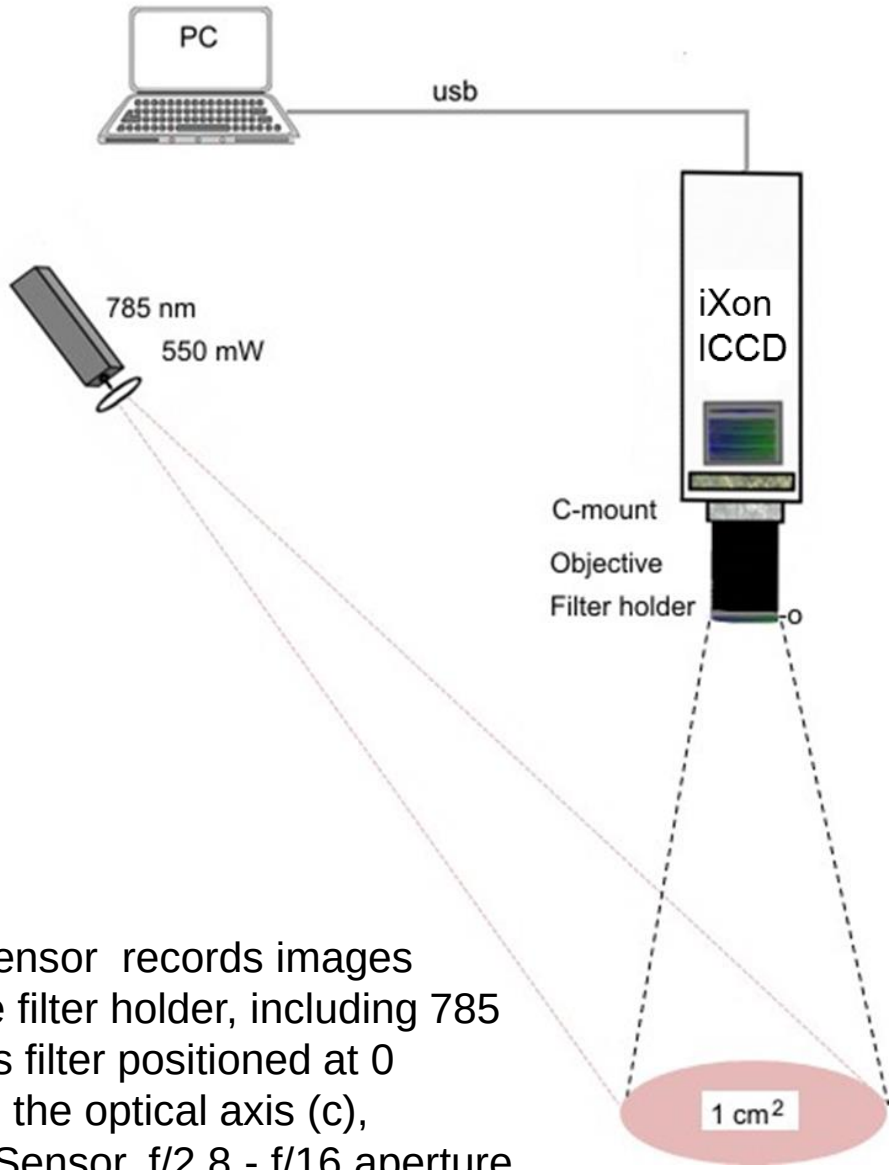


Raman band imaging schematics:





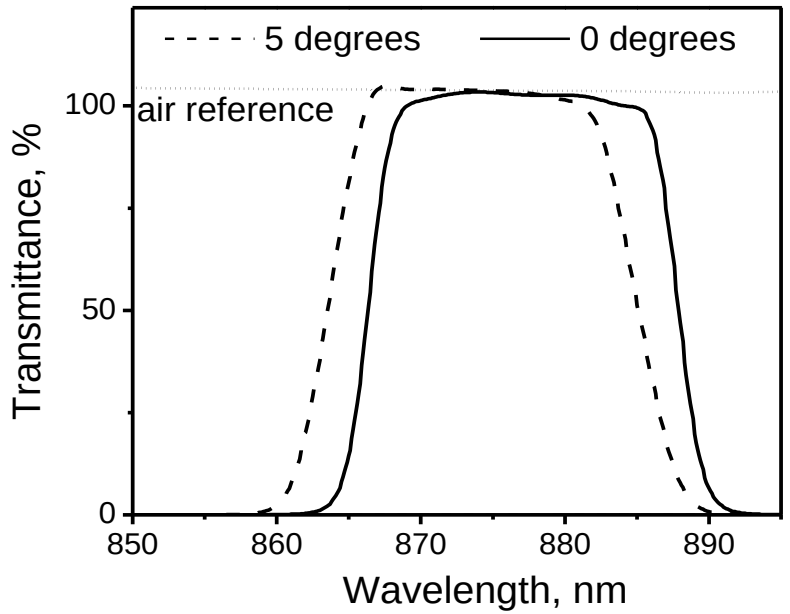




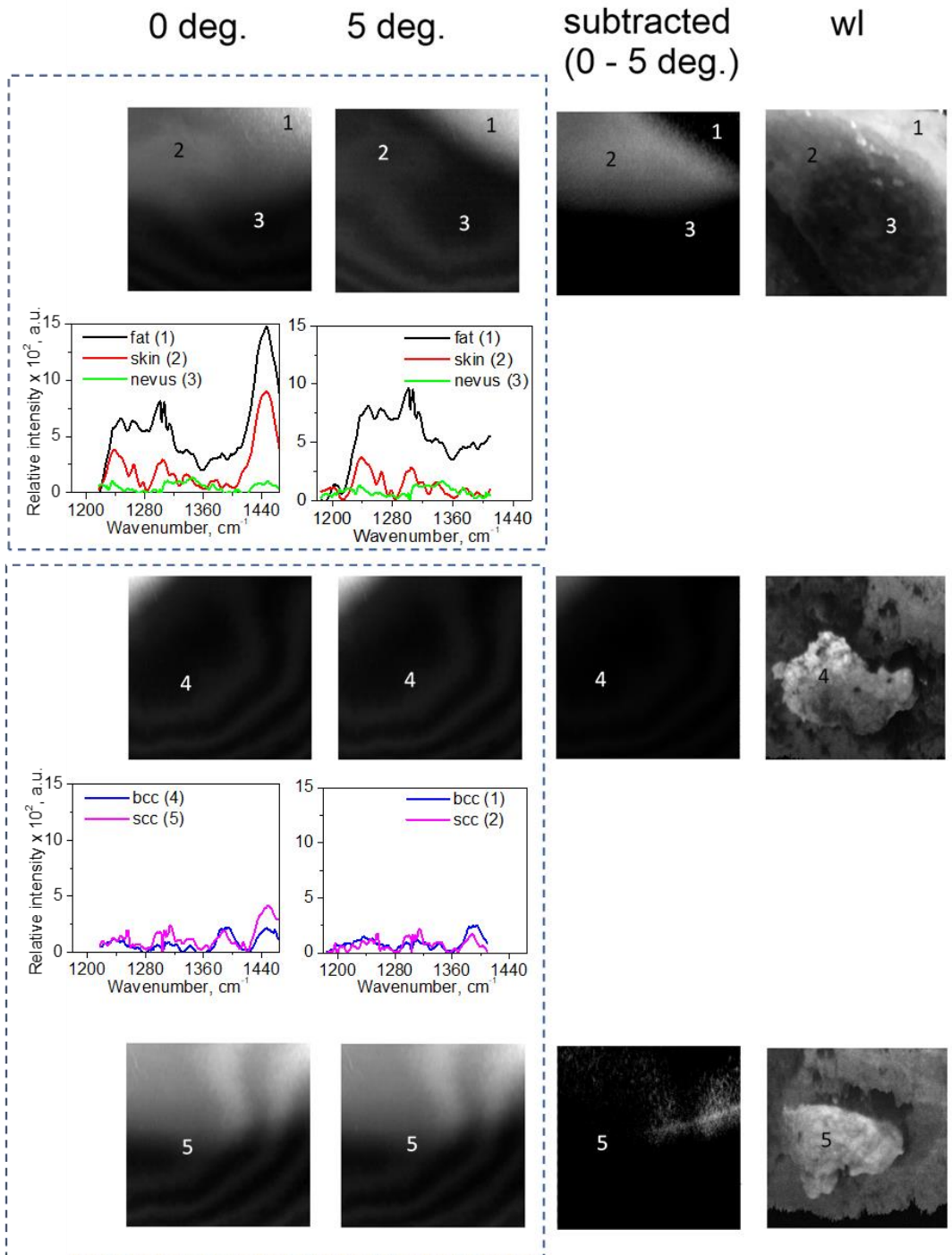
Intensified CCD camera (iXon Ultra 888, Andor) sensor records images through the set of optical filters embedded into the filter holder, including 785 nm laser rejection filter (a) and rotated band pass filter positioned at 0 degrees (b) or at 5 to 10 degrees perpendicular to the optical axis (c), mounted on Macro lens 35mm Focal Length,1.1" Sensor, f/2.8 - f/16 aperture objective (d).

Raman imaging filter specification:

Angular adjustment of narrow-band filter produced ~3 nm Anti-Stokes shift at 90% of transmittance level.



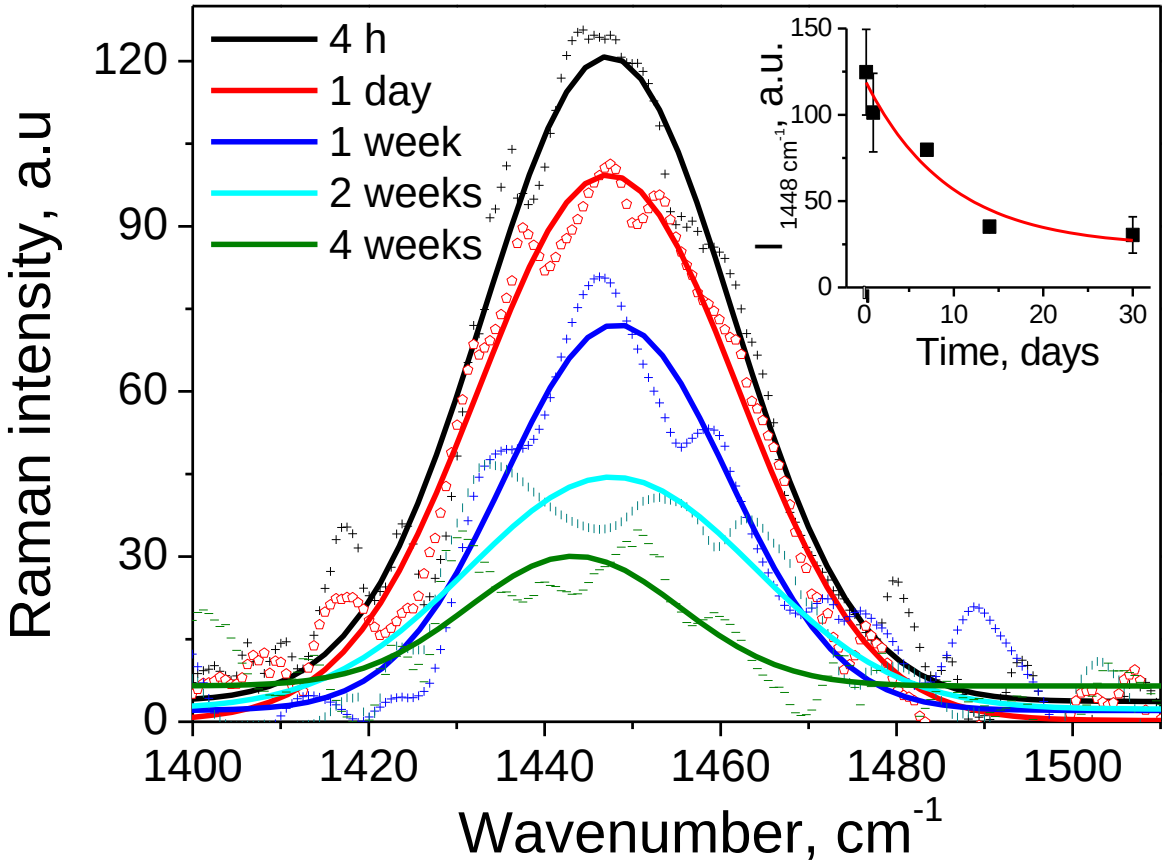
Filter name	Transmission band > 90% (nm)	Center wavelength (nm)	Transmission band **at 0 degrees,** (cm^{-1})	Transmission band **at 5 degrees** (cm^{-1})
FF01-880/11-25	874.5 – 885.5	880	1217 – 1456	1185 - 1409



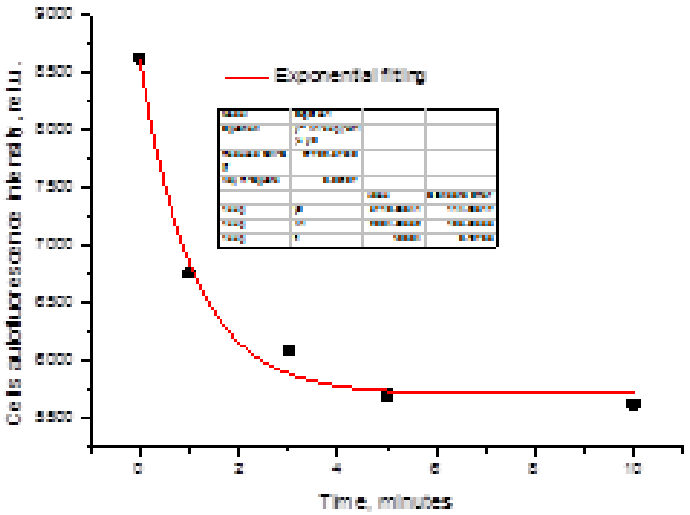
Top view of Raman macro images at 0 degree and 5 degree filter turning and white light images, *ex vivo*.

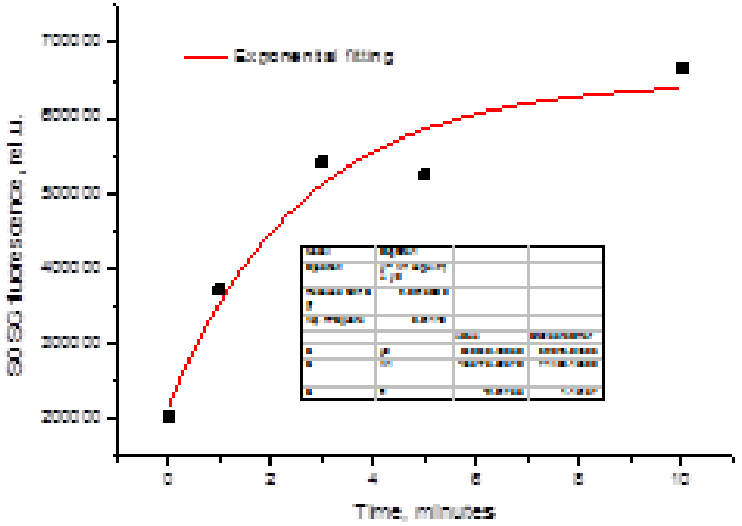
Numbers on imaging data denote tissue morphological localization: 1 – subcutaneous fat; 2 – skin; 3 – nevus; 4 – BCC; 5 - SCC.

The differences in Raman band intensity at 1448 cm^{-1} of live dc3-f cells and dead cells stored at 4°C in 1x PBS for 1 day, 1 week, 2 weeks and 4 weeks.



4. Photobleaching of cell autofluorescence: correlation with singlet oxygen production (A.Lihachev)





Left – melanoma cell autofluorescence intensity decrease during 10 minutes under 405nm continuous excitation measured at 480 nm band. τ_1 ~ 1.1 minute +/-18%.

Right – singlet oxygen fluorescence measured at 520 nm band under 473 nm excitation in the same experiment. τ_2 ~ 2.6 minutes +/-44%.

Conclusion: AF is quenched by singlet oxygen and other radicals emerged by irradiation.

SUMMARY

- Three aspects of laser applications for skin assessment have been studied – pulsed scattering for skin-remitted photon path length estimation, multi-spectral-line imaging for skin chromophore mapping and camera-based Raman imaging for analysis of skin malformations.
- Experimentally determined mean values of skin-remitted photon path lengths were notably higher than those obtained by MC-simulations.
- Three design options for skin imaging with essentially higher spectral selectivity using multi-laser illumination have been implemented in prototype devices
- A new research line – Raman skin spectroscopy and imaging – has been initiated. Advantages of Raman macro-imaging - ability to provide a wide field of view (~ cm^2), fast image acquisition (10 seconds) and skin-safe laser power density.
- Further studies are in progress

Acknowledgements for project support

- **European Regional Development Fund**: #1.1.1.1/18/A/132, #1.1.1.2/VIAA/1/16/014, #1.1.1.2/VIAA/1/16/070
- **Latvian Council of Science:** # lzp-2018/2-0006

Thank You!

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