Combined multispectral, fluorescence lifetime and Raman imaging for skin diagnostics

Janis Spigulis,
I.Kuzmina, V.Lukinsone, M.Tamosiunas, I.Oshina, L.Dambite, A.Maslobojeva, M.Kuzminskis, O.Cizevskis

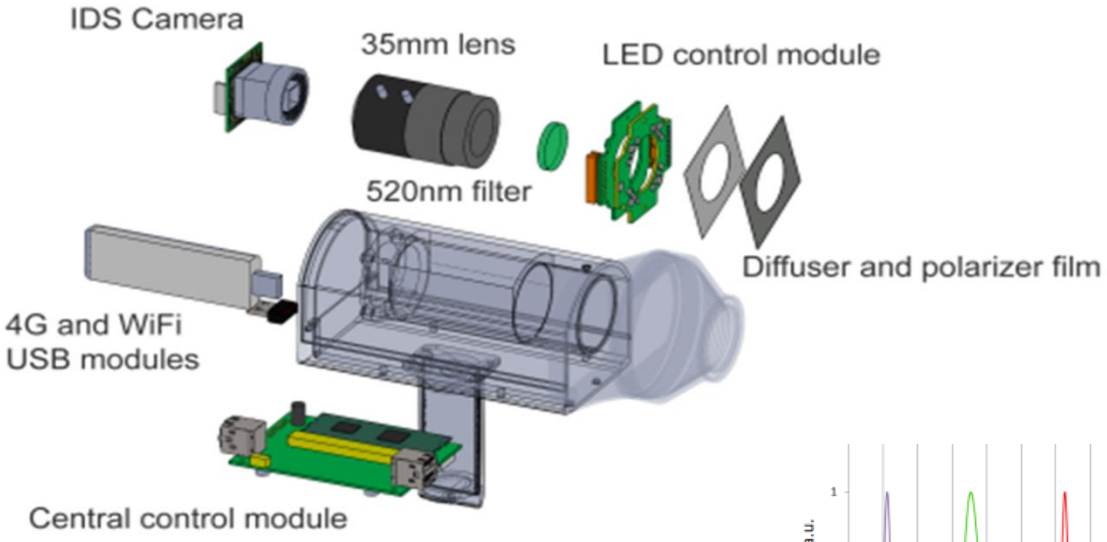
Biophotonics Laboratory
Institute of Atomic Physics and Spectroscopy (ASI)
University of Latvia, Riga

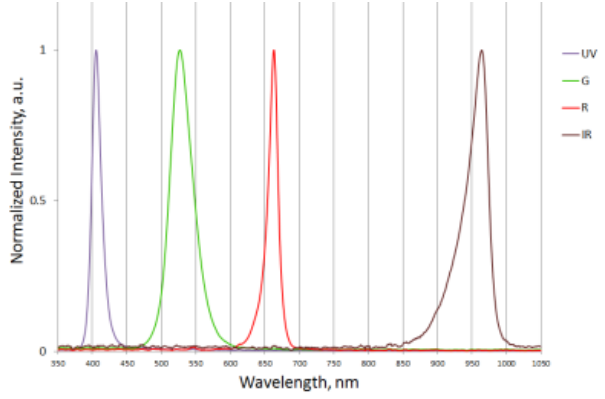
Oporto Biophotonics (PT), 22/04/2022

Introduction

- Optical skin diagnostics offers several advantages - noninvasive/noncontact and fast procedure which results with quantitave data (objective), documentable post-treatment monitoring, … → potentially powerful tool for dermatologists
- Availability of advanced cameras → imaging (no-touch) technologies
- Various camera-based imaging modalities exist – RGB colour imaging (reflectance, fluorescence), multispectral, hyperspectral, spatial frequency domain, etc. → enable parametric mapping of malformation, facilitate objective diagnostics
- Sensitivity & specificity of a single modality: can reach/exceed 90%
- Further improvements may be achieved by combining several imaging modalities; as example - bi-modal imaging

Bi-modal imaging example: skin melanoma checker



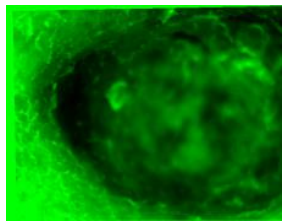


4 prototypes assembled, 1500+ clinical tests in LV, HU, BG; sensitivity ~85%, specificity ~95%

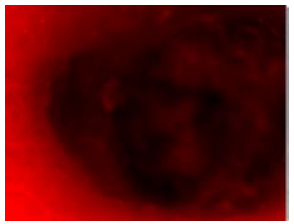
V.Lukinsone et al., “Multispectral and autofluorescence RGB imaging for skin cancer diagnostics”, *Proc.SPIE* **11065**, 110650A (2019).

Parametric map for melanoma (left) and for non-melanoma skin malformation (right)

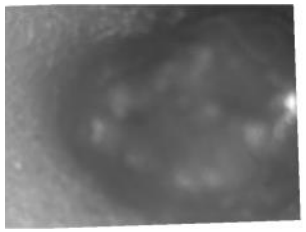
λ=526nm

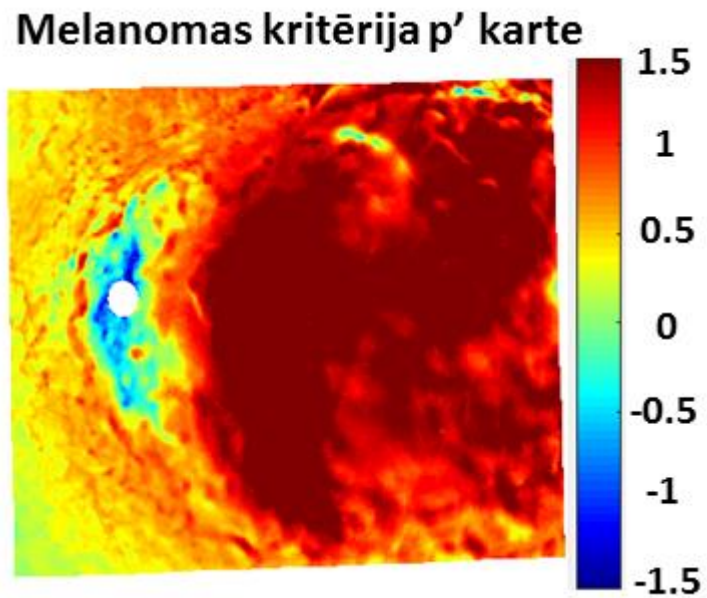


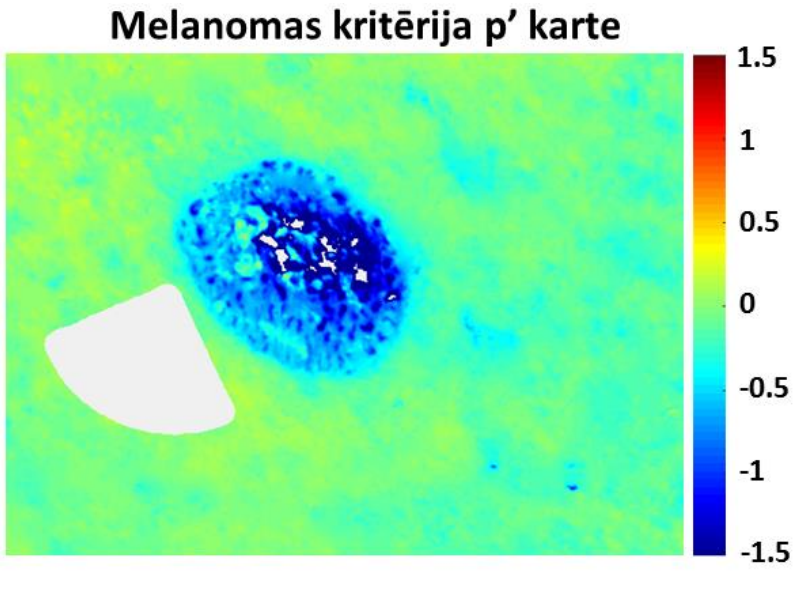
λ=663nm



λ=964nm







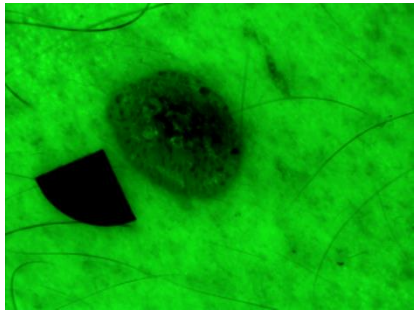
Melanoma: $p' > 1$

$$p' = lg\left(\frac{I(526)\cdot I_{skin}(663)\cdot I_{skin}(964)}{I_{skin}(526)\cdot I(663)\cdot I(964)}\right)$$

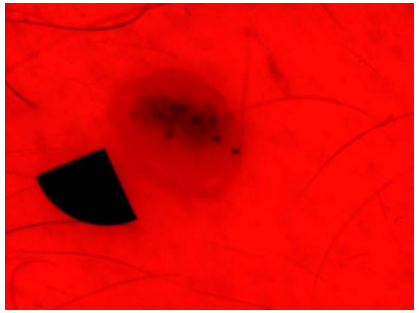
Multispectral & autofluorescence imaging (bi-modal): *seborrheic keratosis*

RGB image at white illumination

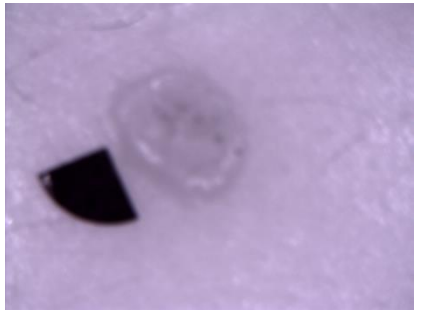
λ=526nm



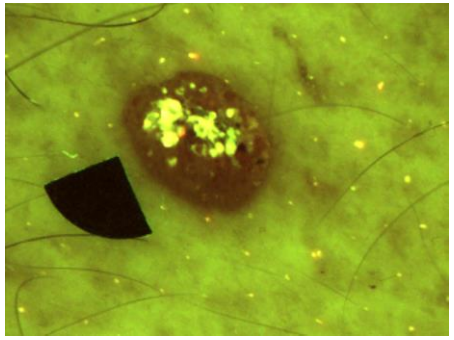
λ=663nm



λ=964nm



Autofluorescence image at 405nm excitation

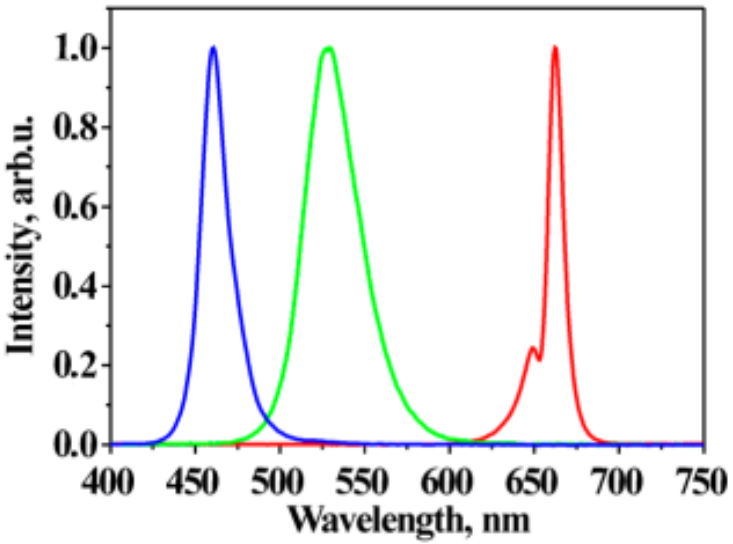


The pilot project idea and timing

- Combination of three imaging modalities to better diagnose the same skin malformation:
  - (1) RGB triple spectral line imaging (TSLI) → 3 chromophore maps
  - (2) autofluorescence lifetime imaging (AFLTI) → 2 fluorophore maps
  - (3) specific Raman band imaging (RBI) → 2 maps of Raman band intensity distribution
- First stage – advancing each of 3 imaging modalities
- Second stage – combining 2 of them (e.g. TSLI-AFLTI, AFLTI-RBI)
- Third stage – tri-modal imaging & data processing for diagnostics

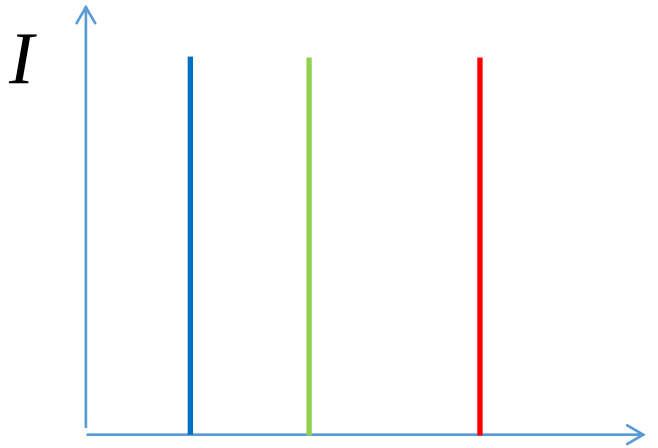
(1) Spectral band imaging → spectral line imaging

Conventional:
Spectral **band** images



Sequential (t>>0)

Improved:
Spectral **line** images



Single snapshot (t→0)

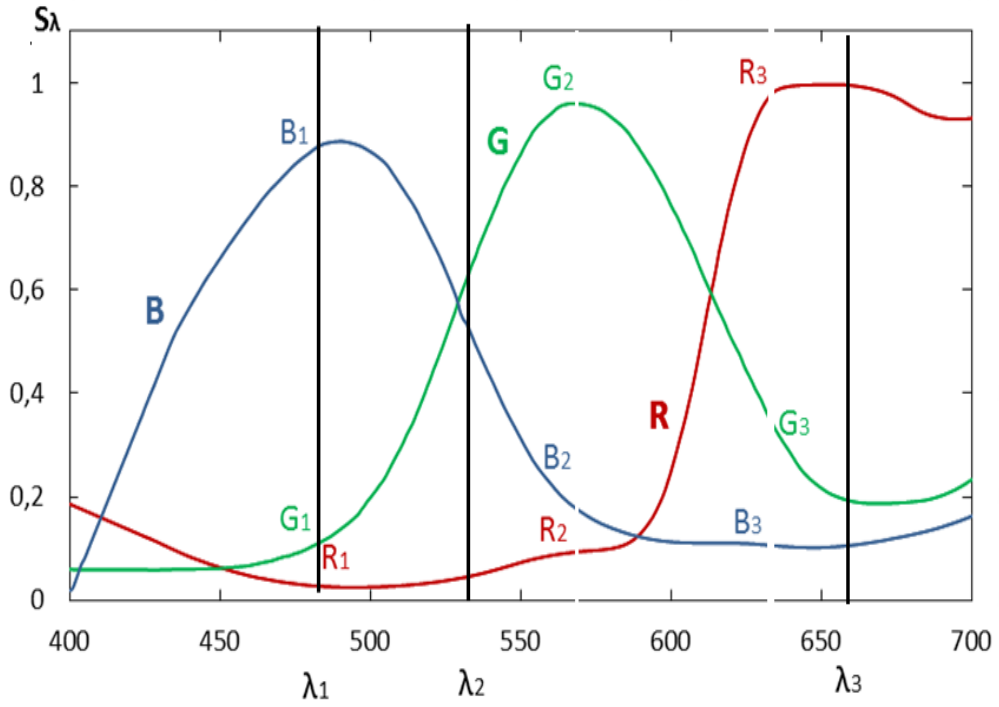
n =3 → n > 3

Benefits:
- Notably increased spectral selectivity, <0.1 nm
- Improved imaging quality (snapshot) → avoided motion artefacts
- Simpler/faster image processing (numbers instead of integrals over wavelength bands)

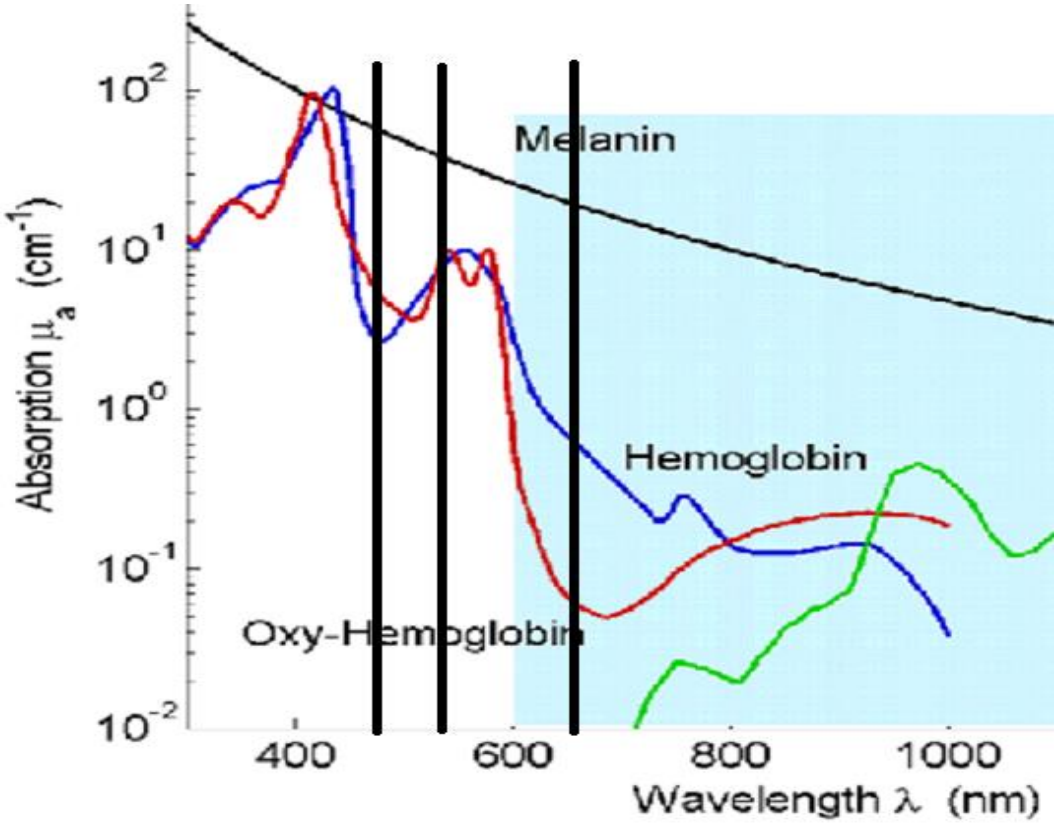
WO2013135311 (A1), 2012. Method and device for imaging of spectral reflectance at several wavelength bands (J.Spigulis. L.Elste).

Triple spectral line imaging for chromophore mapping

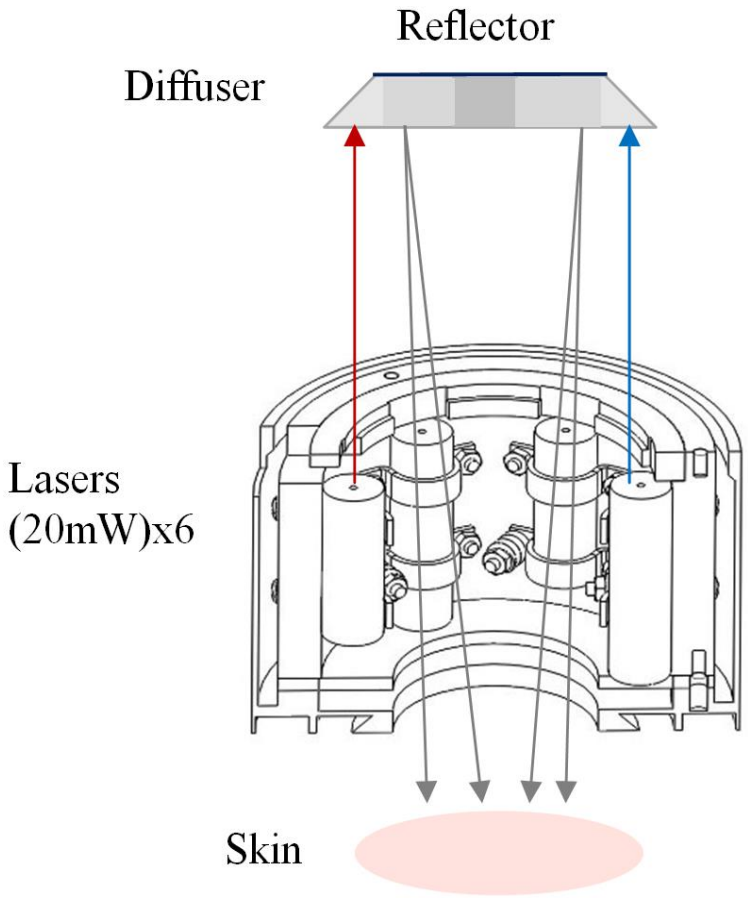
Spectral sensitivity bands of RGB image sensor and 3 spectral lines of illumination

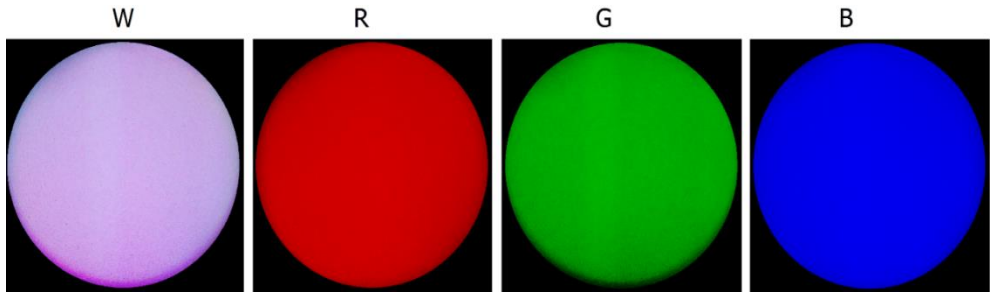


Contributions of 3 main skin chromophore absorption at the 3 selected wavelengths



Triple wavelength laser add-on to a smartphone

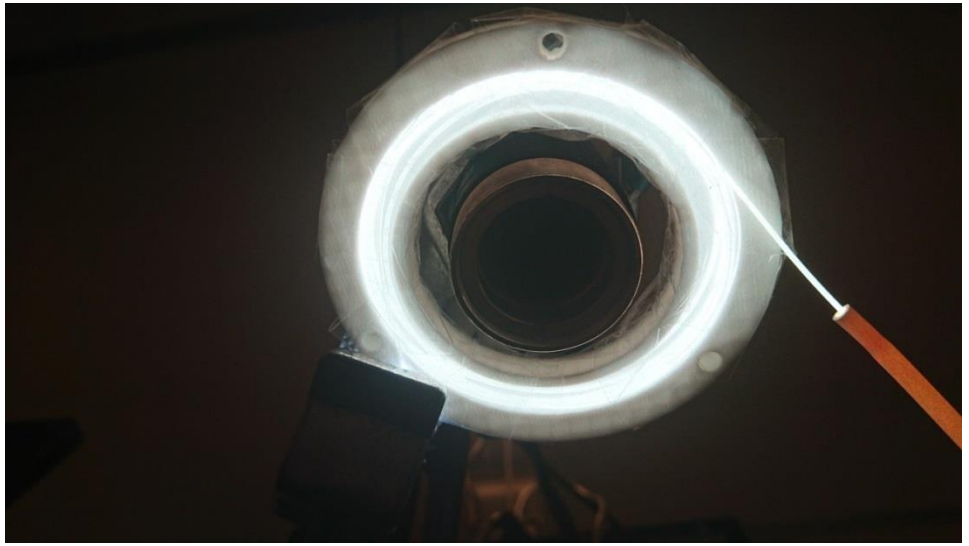




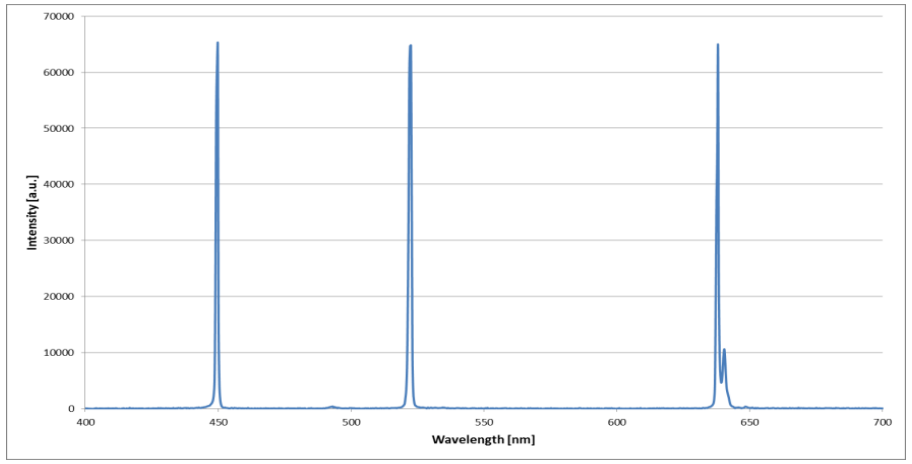
659 nm 532 nm 448 nm

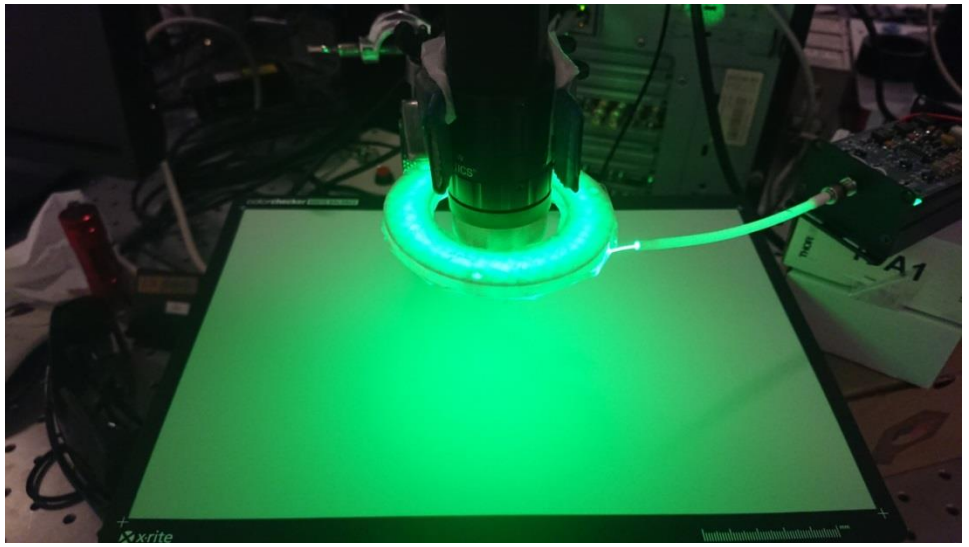
J.Spigulis, et al., “Smartphone snapshot mapping of skin chromophores under triple-wavelength laser illumination”, *J.Biomed.Opt.*, **22**(9), 091508 (2017).

Uniform triple spectral line illumination: by RGB-laser coupled side-emitting optical fiber loop



450/523/638 nm





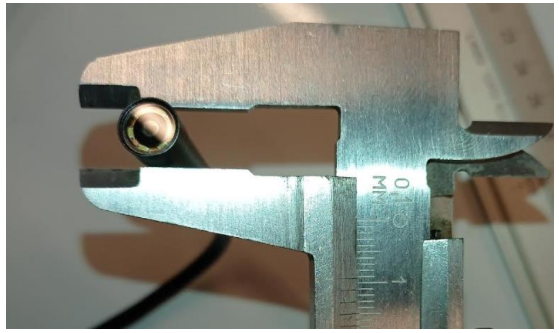
LV 11644 B, 1995. Side-emitting optical fiber (D. Pfafrods, M. Stafeckis, J. Spigulis, D. Boucher);
LV 15491 B, 2020. Device for uniform surface illumination simultaneously by several spectral lines (J.Spigulis, I.Oshina, Z.Rupenheits, M/Matulenko)

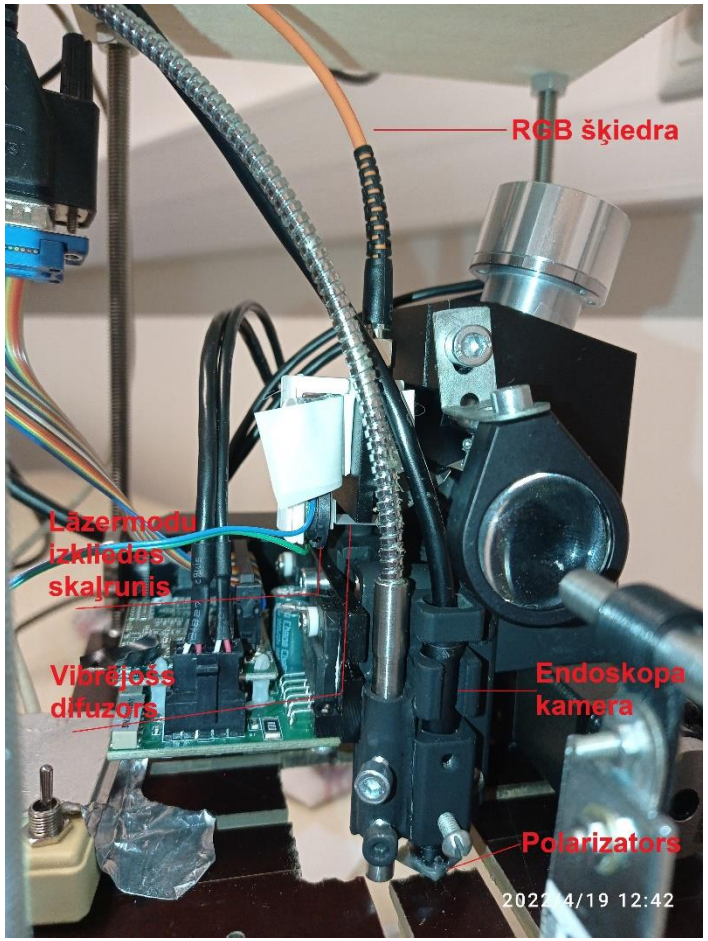
This work:
illumination from RGB-laser coupled transmission fiber output + imaging by the endoscope RGB camera

450nm, 520nm, 635nm

RGB-90 SMA laser module
(*Elite Optoelectronics, CN*)

SMA





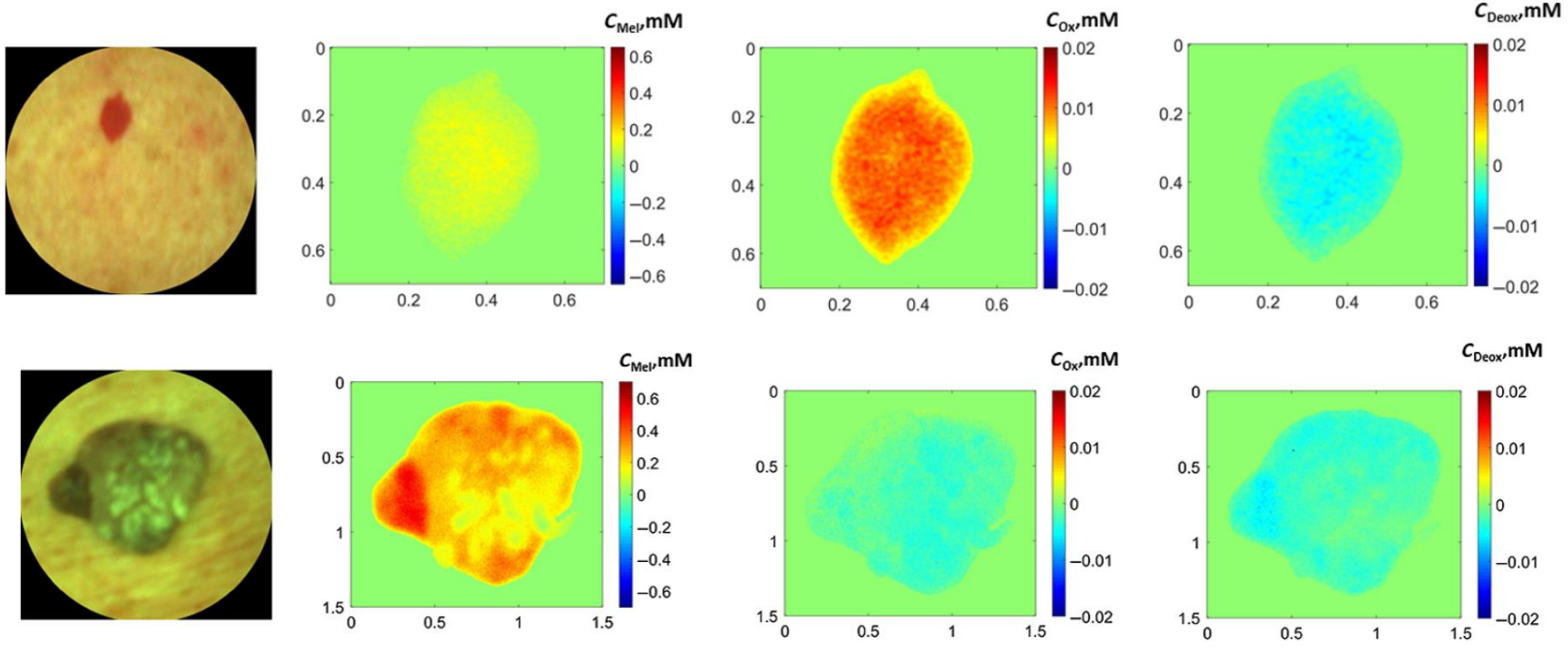
Clinical validation: the tested skin malformations

Diagnosis	Number
Junctional nevus	19
Combined nevus	19
Dermal nevus	23
Seborrheic keratosis	23
Hemangioma	21
Total	105

Chromophore variation (increase/decrease) maps obtained from single snapshot three spectral line images

Hemangioma

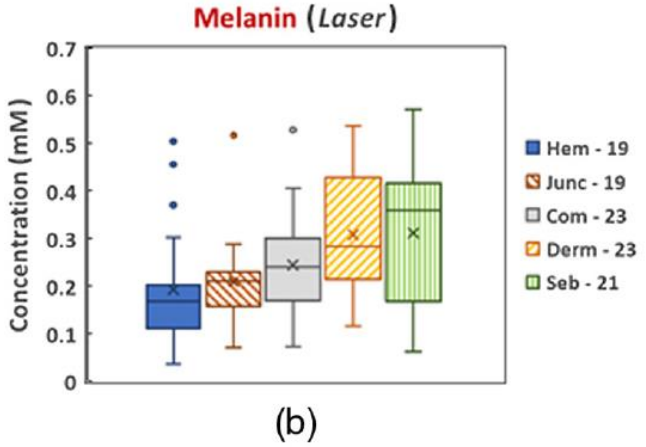
Seborrheic keratosis



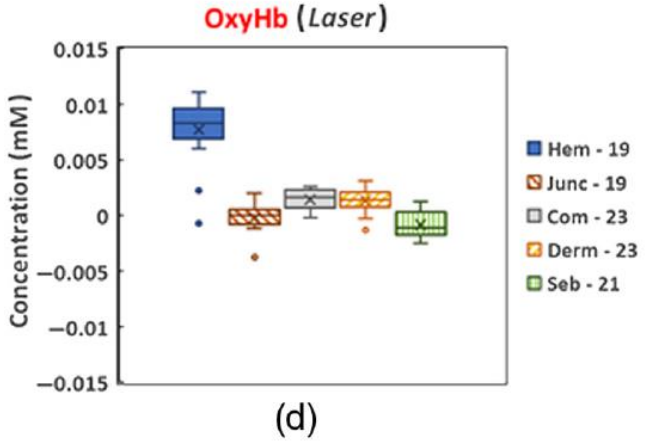
RGB Melanin Oxy-hemoglobin Dexy-hemoglobin

I.Kuzmina, et al. “Skin chromophore mapping by smartphone RGB camera under spectral band and spectral line illumination”, *J.Biomed.Opt.* **27**(2), 026004 (2022).

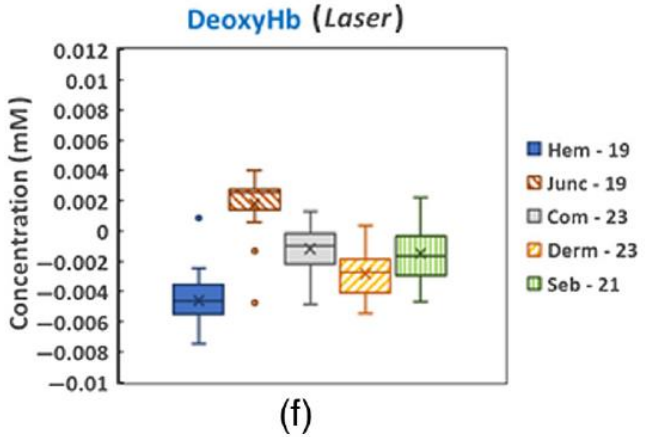
Chromophore concentration increase/decrease in the examined skin malformations



(b)



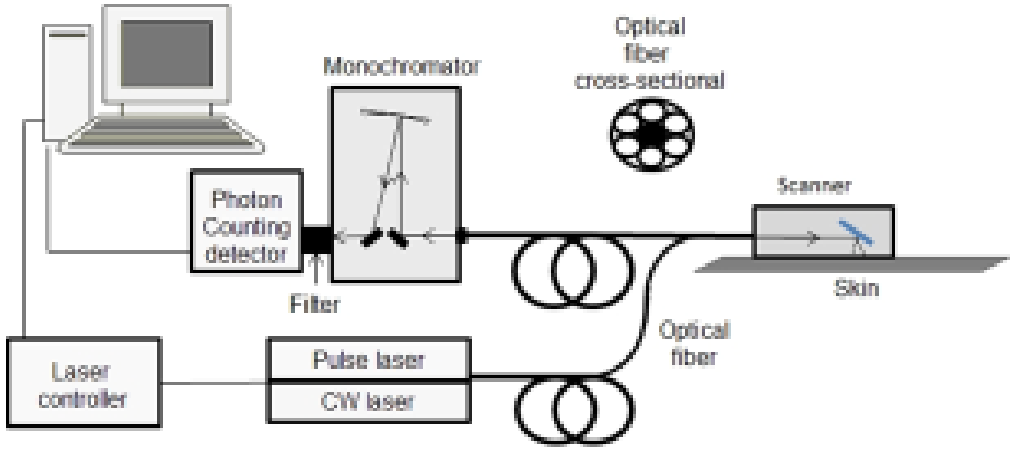
(d)



(f)

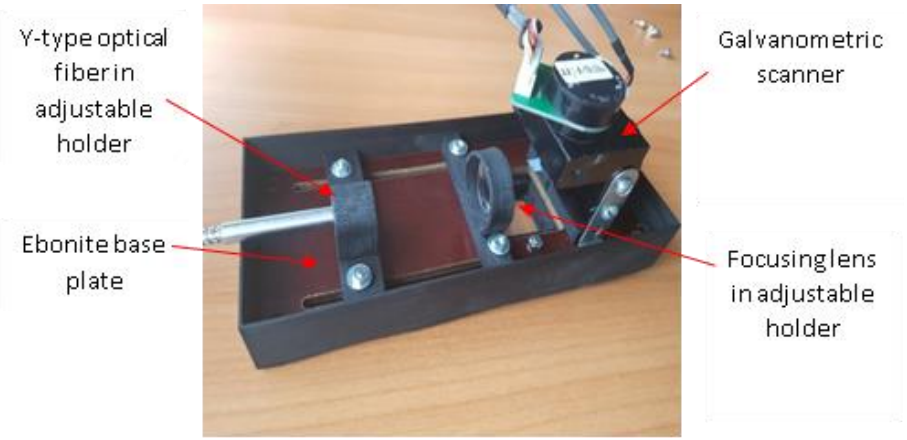
I.Kuzmina, et al. “Skin chromophore mapping by smartphone RGB camera under spectral band and spectral line illumination”, *J.Biomed.Opt.* **27**(2), 026004 (2022).

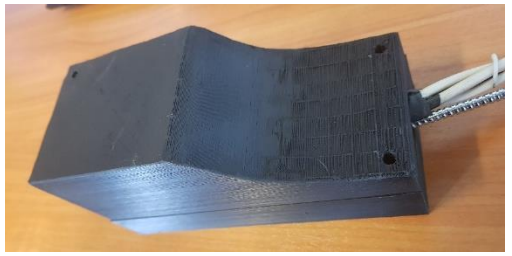
(2) Fluorophores involved?
Fluorescence lifetime imaging (FLIM)



- **Lasers**: PicoQuant 405/470/510 nm (mod. LDH-D-C-405/407/510)
- **Pulse half-width**: 59/73/107 ps
- **Repetition rate**: 20 MHz
- **Photon counting detector**: Becker&Hickl, PMC-100-4.
- **Data processing card**: Becker&Hickl, TCSPC, mod. SPC-150.
- **Fiber optics**: 200-μm silica core Y-type optical fiber bundle with SMA-connector.
- Diameter of the irradiated skin spot ~3 mm.

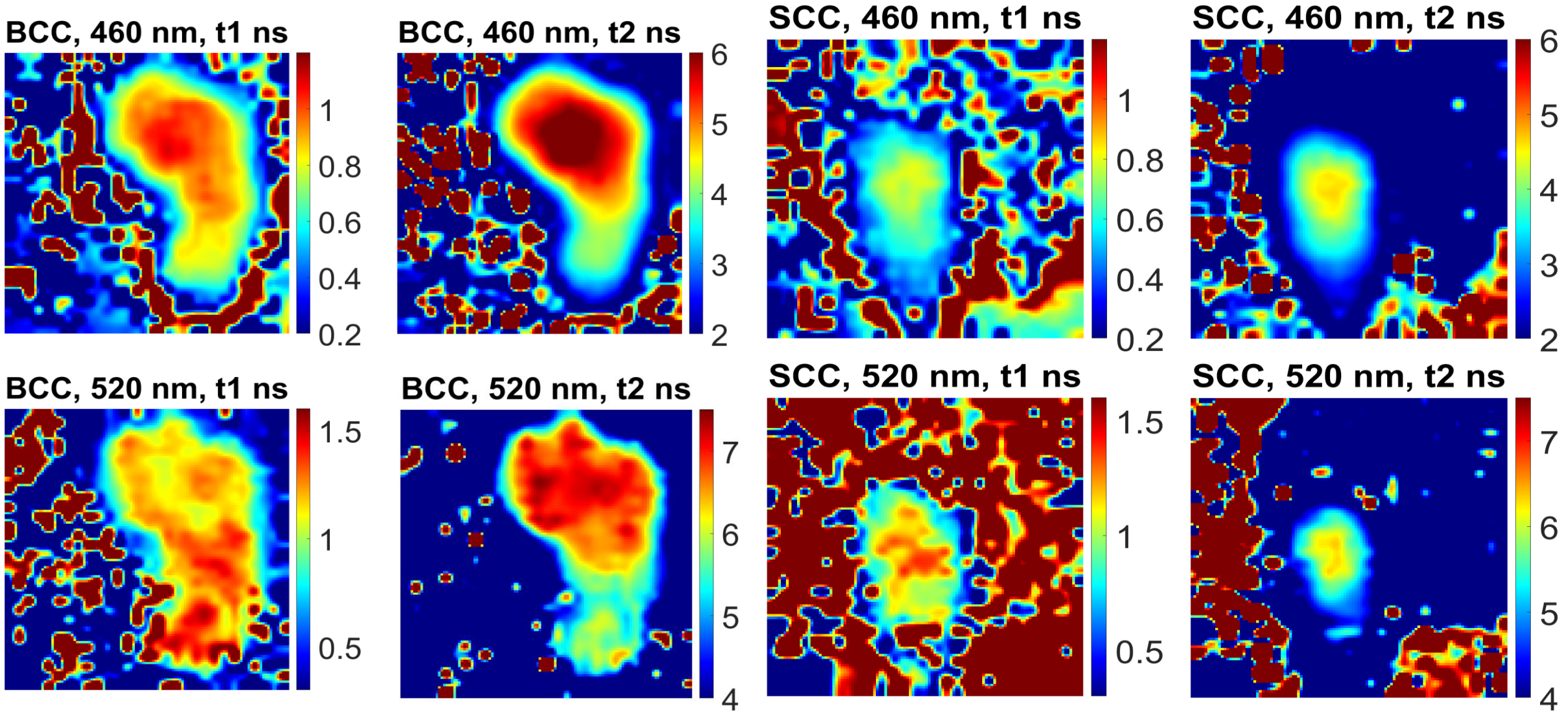
29



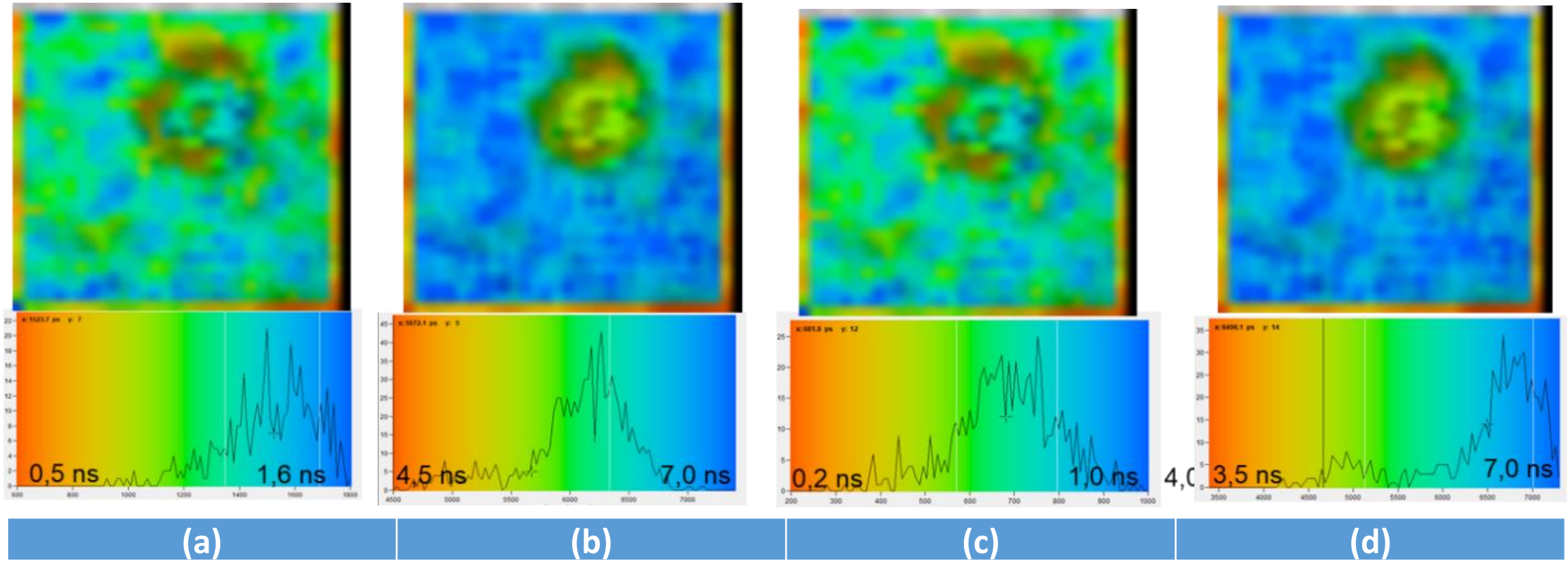


Filters in front of the photo-detector:
~460 nm (NADH) and ~520nm (FAD)

Examples of AF lifetime images: *ex-vivo* BCC and SCC



Fluorescence lifetime images of a dermal nevus: (a) τ_1 - 460nm, (b) τ_2 - 460nm, (c) τ_1 - 520nm, (d) τ_2 - 520nm

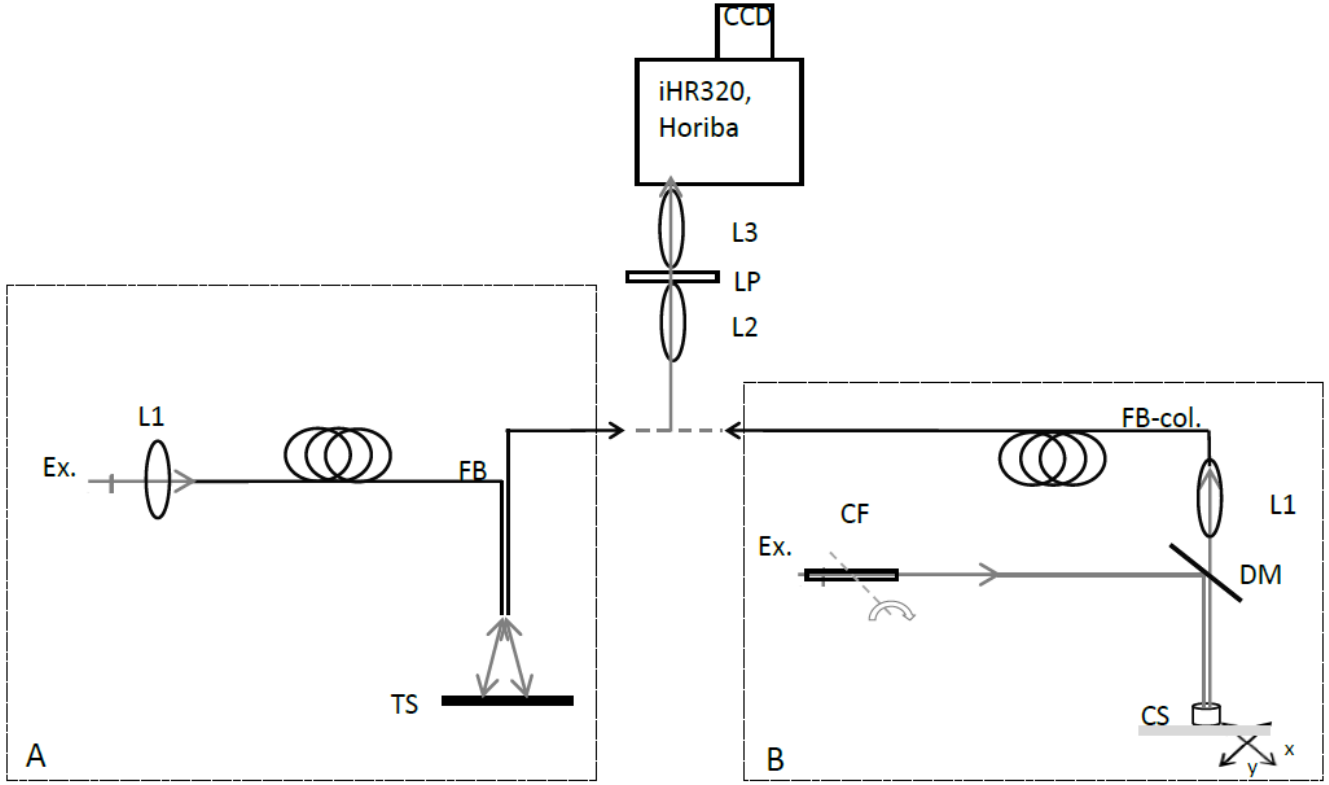


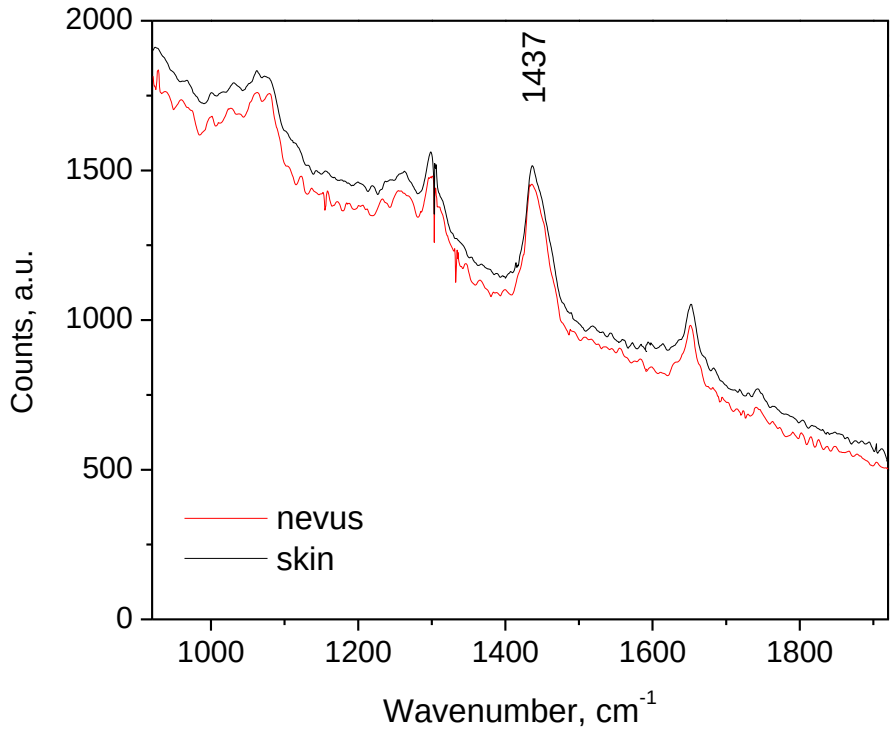
(3) Raman band imaging (RBI): the most challenging modality

Starting with single-point measurements, three RBI options examined:

- By dual-mirror laser beam scanner → filtered PMT output (2 sets of IF)
- By a filtered camera under uniform 785nm illumination (2 different *Andor* cameras, 2 sets of IF)
- By automatic mechanical x-y scanning of dual-fiber Raman probe → output of a filtered PMT (2 sets of IF)

Raman point spectrometry measurements

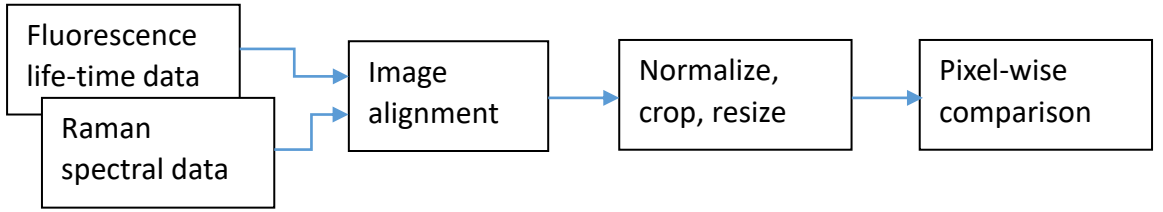


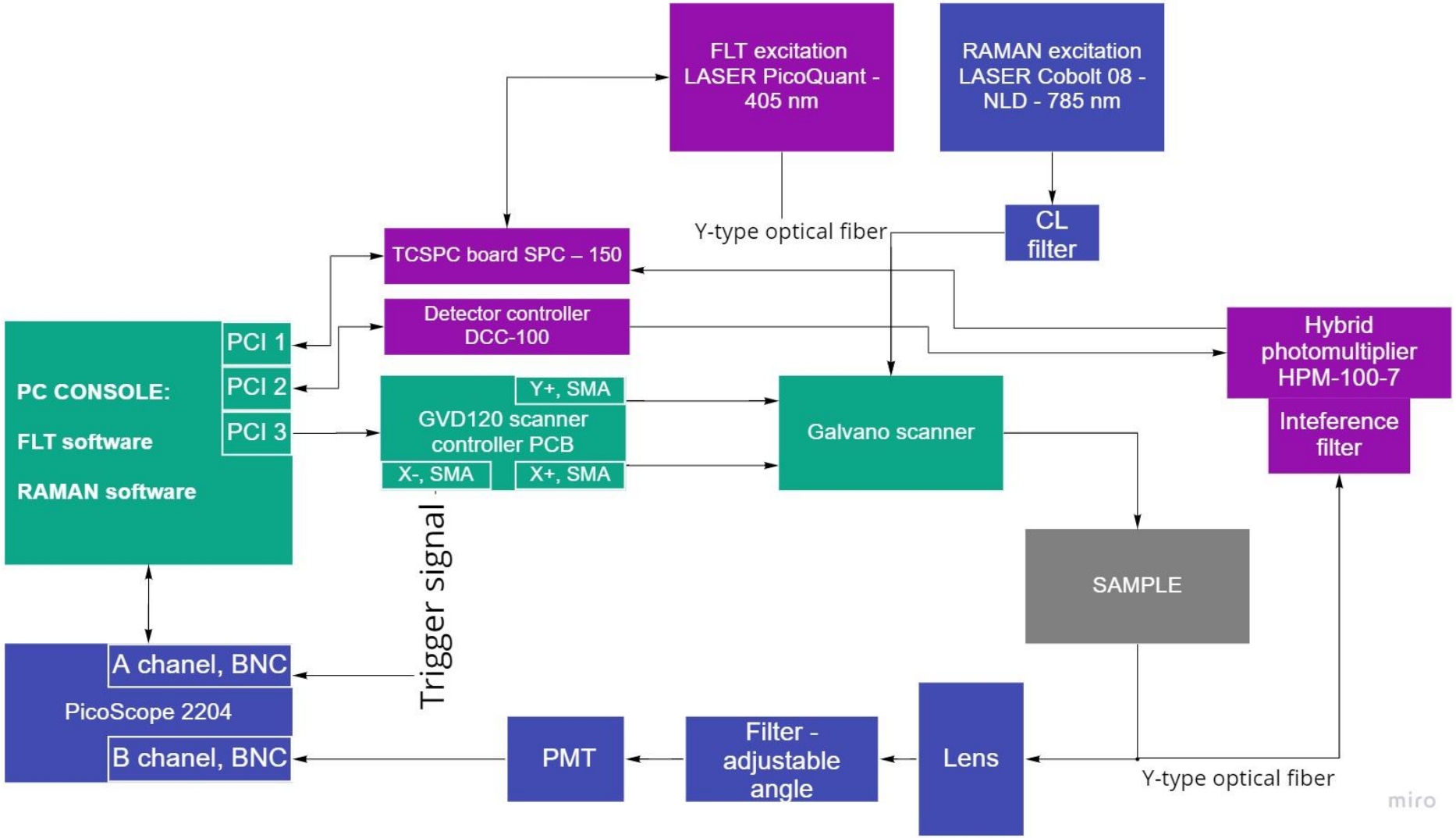


2 target bands: - **1448 cm^{-1}** (CH_2/CH_3 bands; C-H bending, stretching, scissoring, asymmetric deformations, lipids and proteins);

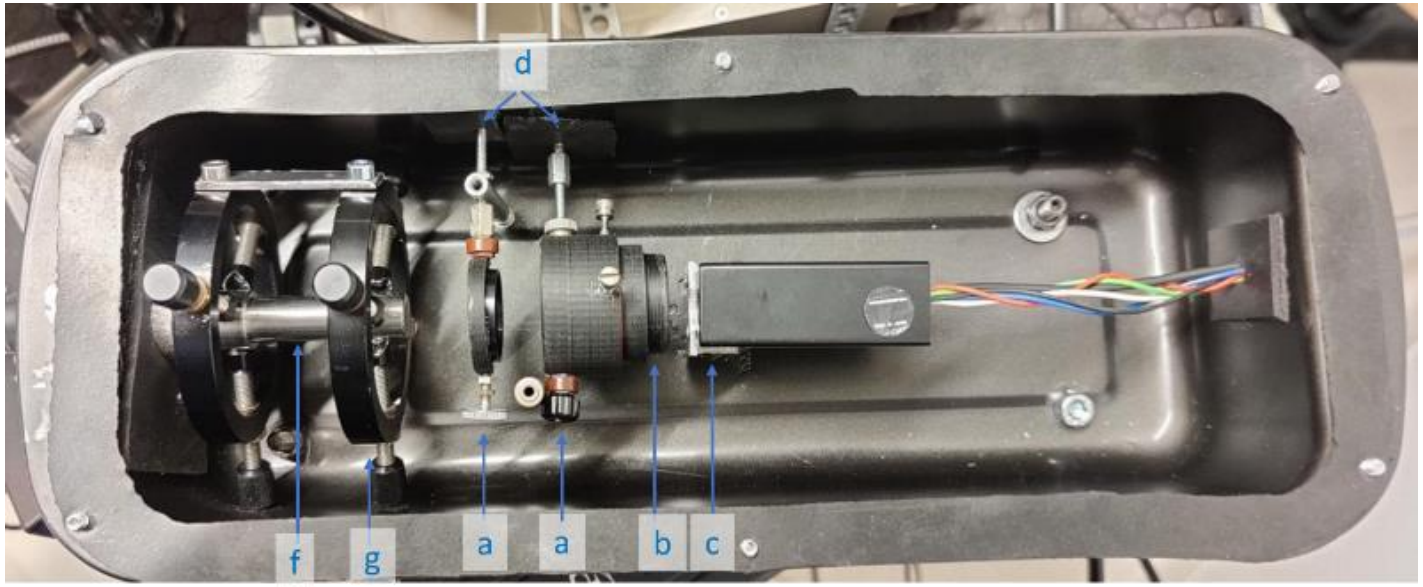
- **1647/54 cm^{-1}** (Amide I, C=C lipid stretch);
  & **1659/61 cm^{-1}** (C=O stretching of amide I, C=C alkyl stretching of lipids, C=C stretching of squalene, nucleic acids; bending of H_2O)

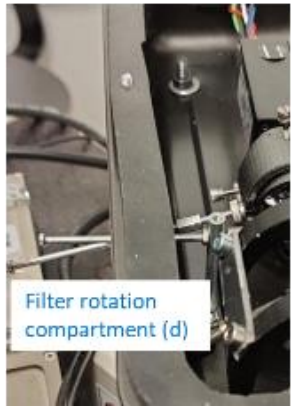
Option 1: scanned 785nm laser beam → bi-modal Raman-AFLT imaging





The filtered PMT (*Hamamatsu H10722-20*) housing

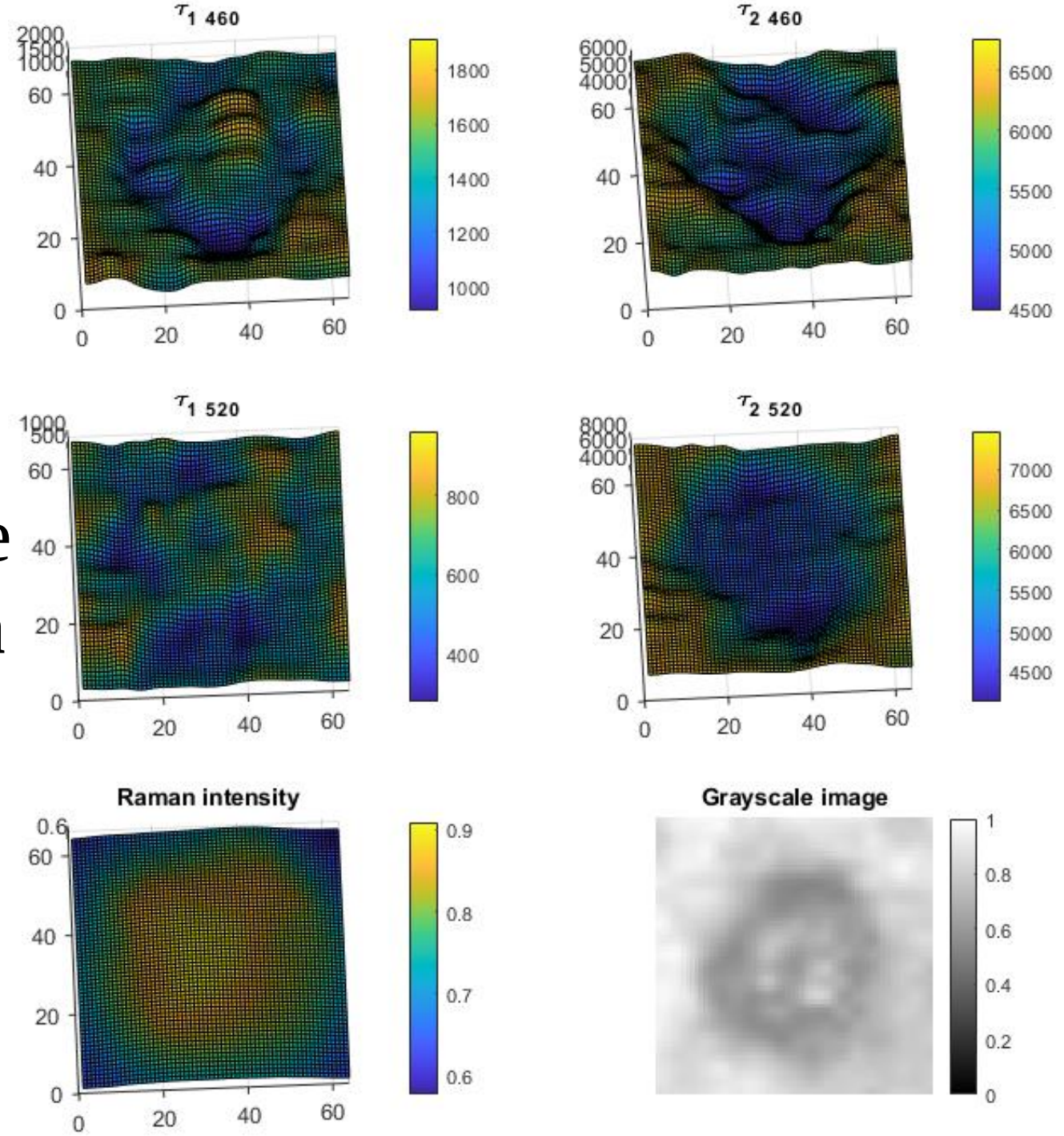





Focussing lens (b)

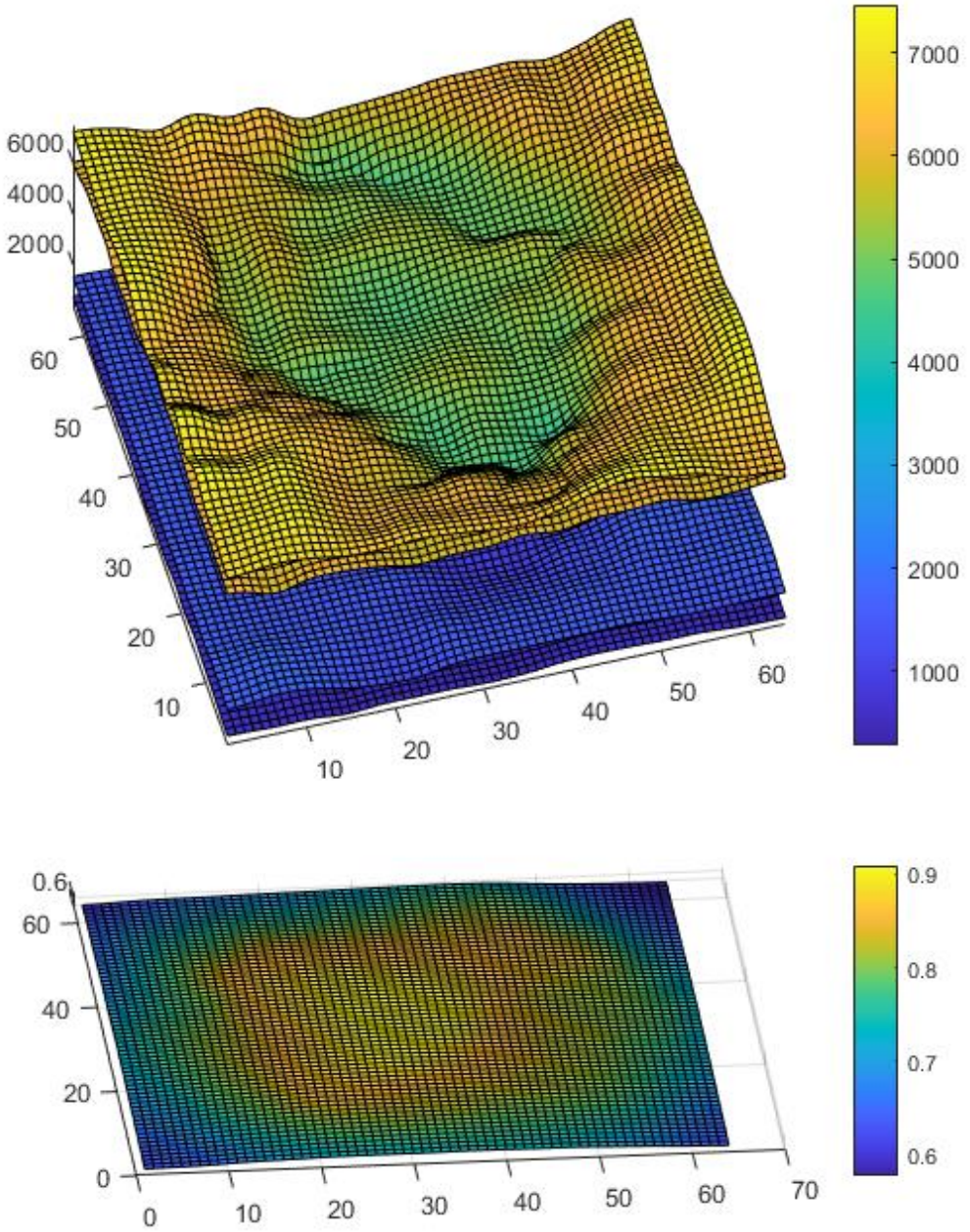

Raman bandpass
filter compartment (a)

3D representation of τ_{1_460} , τ_{2_460} , τ_{1_520} , τ_{2_520} , Raman intensity (1437 cm^{-1}) and grayscale images of an ***ex-vivo*** **skin nevus sample**

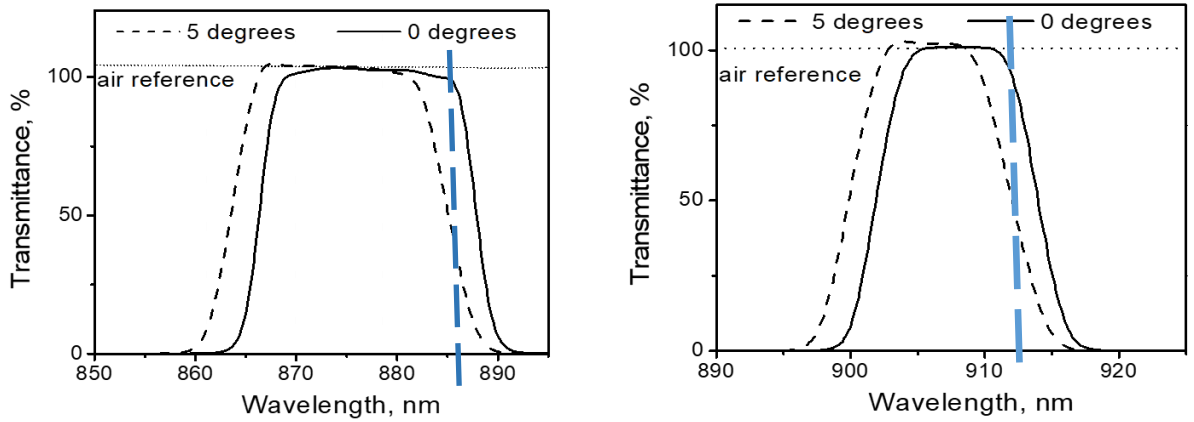


3D-representation of Raman intensity image at 1437 cm^{-1} (I_{1437}) and autofluorescence image with τ_{1_520} , τ_{1_460} , τ_{2_460} , τ_{2_520} , integrated into a single image content.

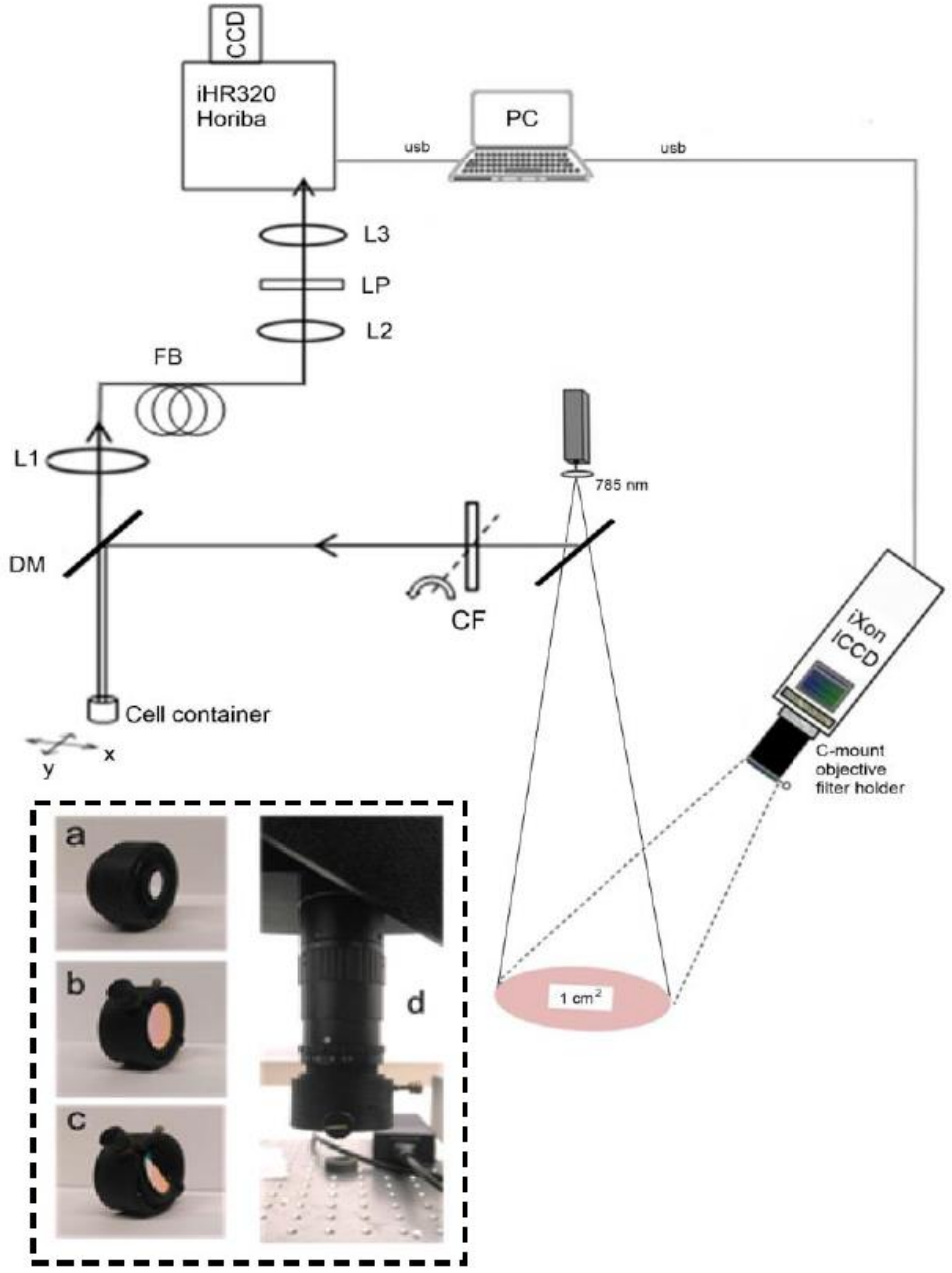
<u>A problem</u>: image acquisition time ~10 min. for both AFLT images and ~15 min. for a single Raman band image → affordable only for *ex-vivo* samples



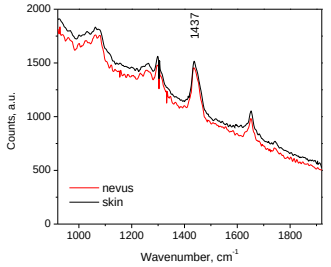
Option 2: filtered camera imaging of two selected Raman bands

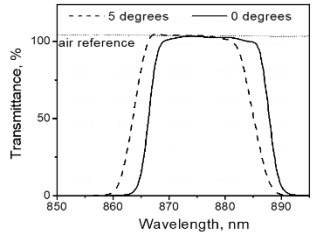


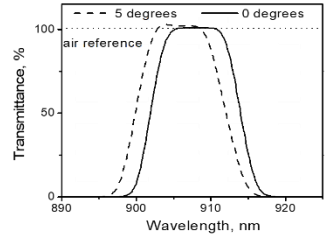
#	Filter name	Transmittance band > 90% (nm)	Center wavelength (nm)	Transmittance spectral region at 0 degrees,(cm^{-1})	Transmittance spectral region at 5 degrees (cm^{-1})
1.	FF01-880/11-25	874.5 – 885.5	880	1217 – 1456	1185 – 1409
2.	FF01-910/5-25	903.8 – 912.1	908	1674 – 1775	1651 – 1747

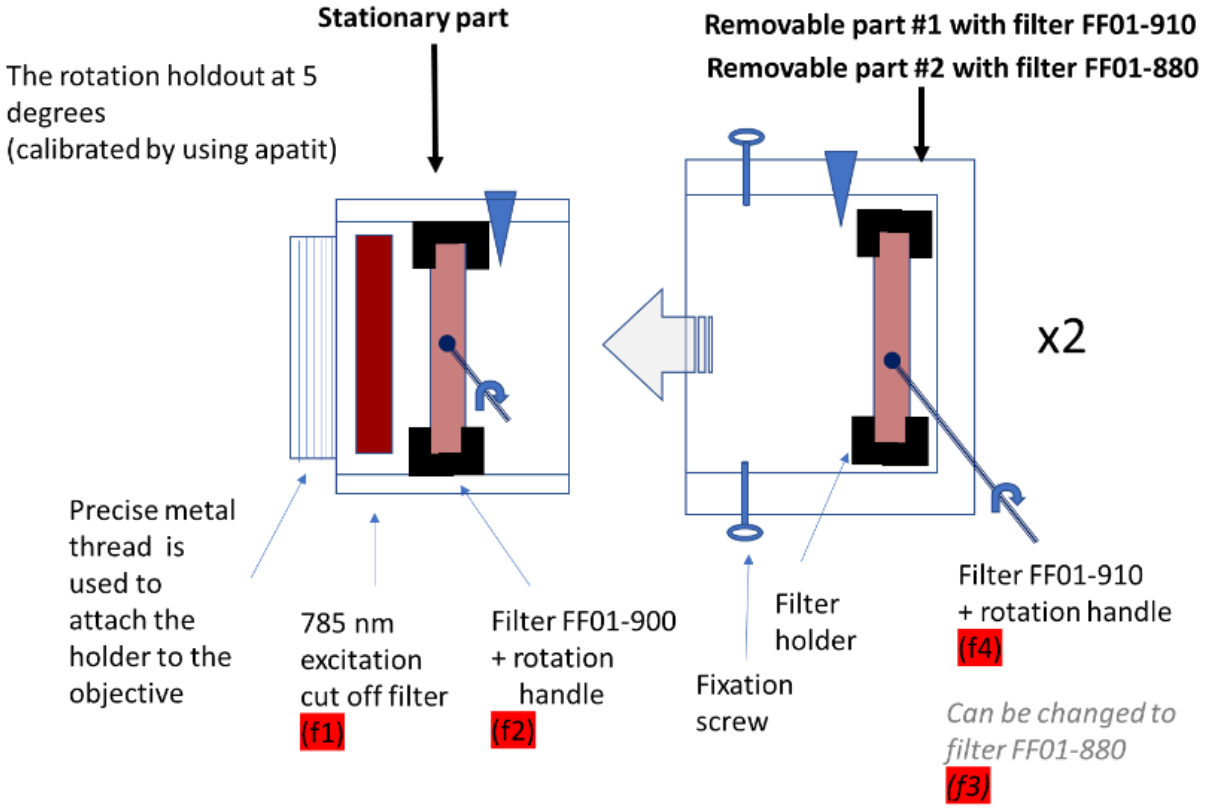


Triple-filter compartment

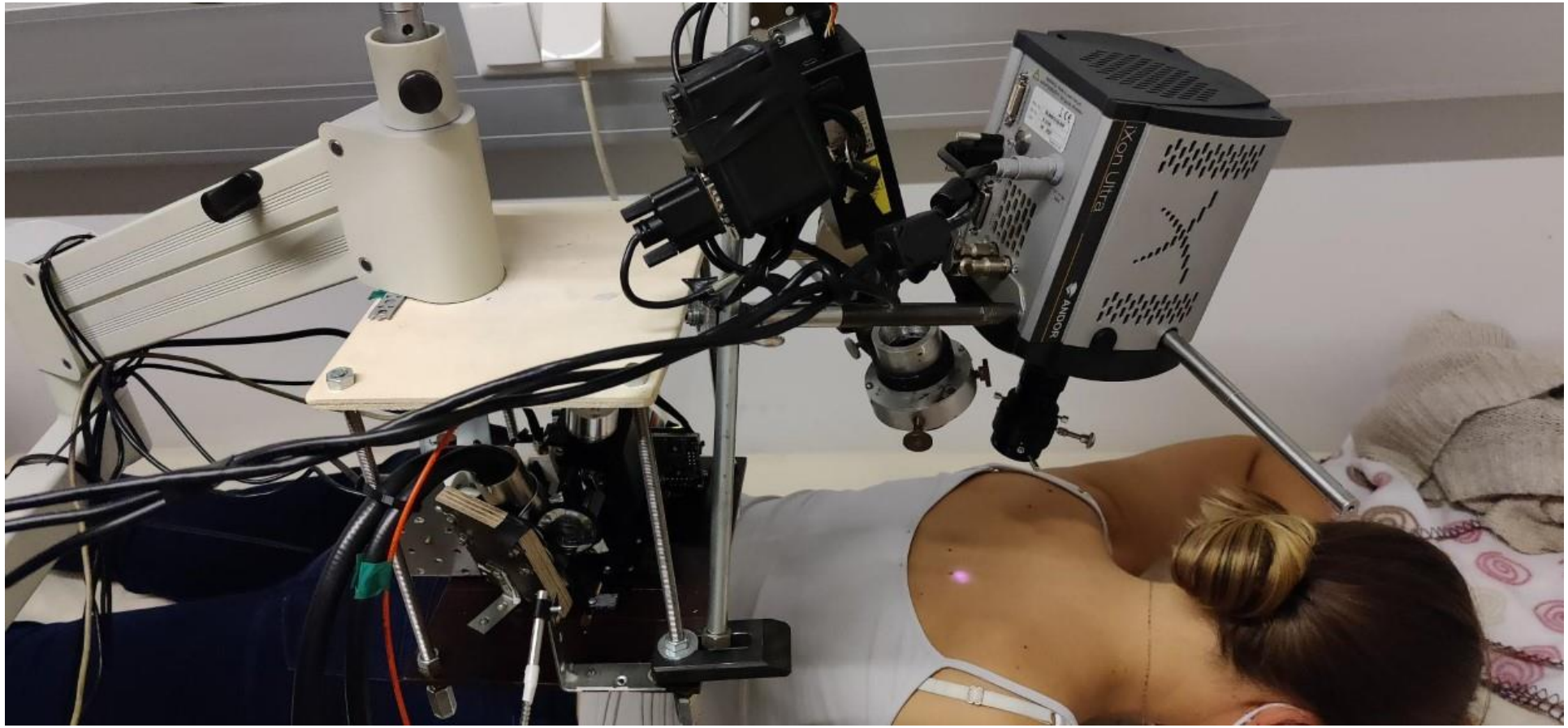








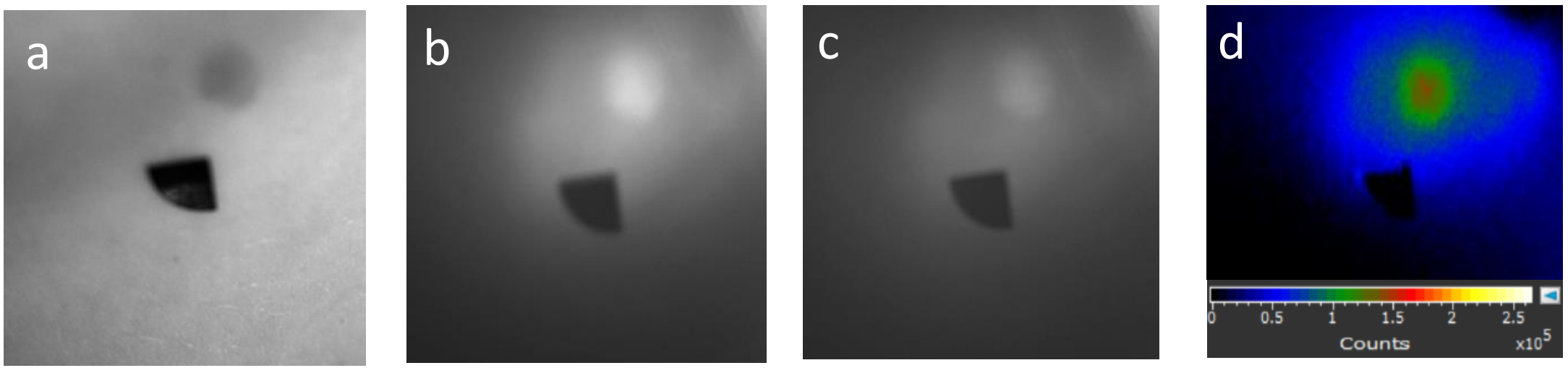
Camera-based Raman band imaging of a skin lesion



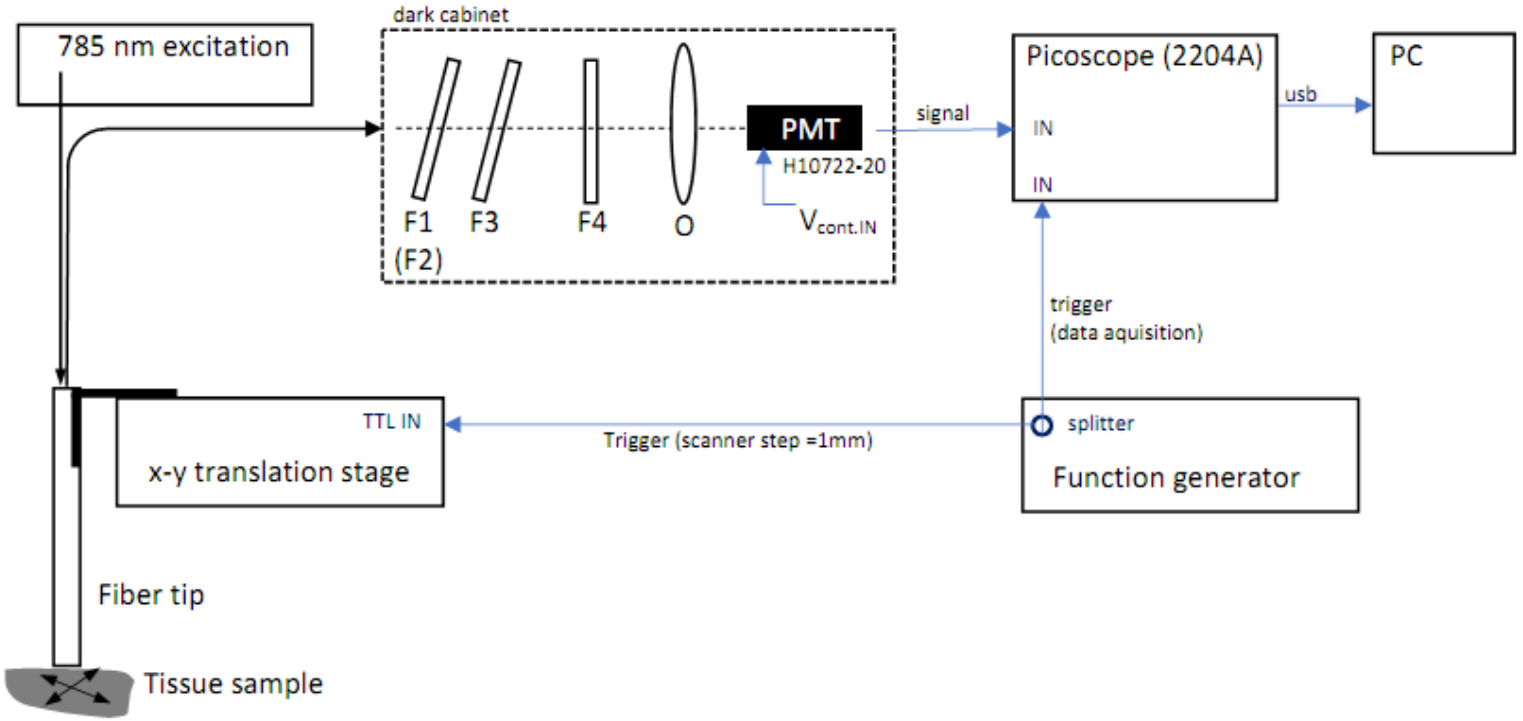
Camera DU-888U3-CS0-BVF (*Andor*)

Raman images of human dermal nevus (10 seconds acquisition time):

a) white light image;
b) Raman+autofluorescence image with parallel filters;
c) Raman+autofluorescence image at calibrated F900 tilting;
d) extracted Raman image for 1437 cm^{-1} band.

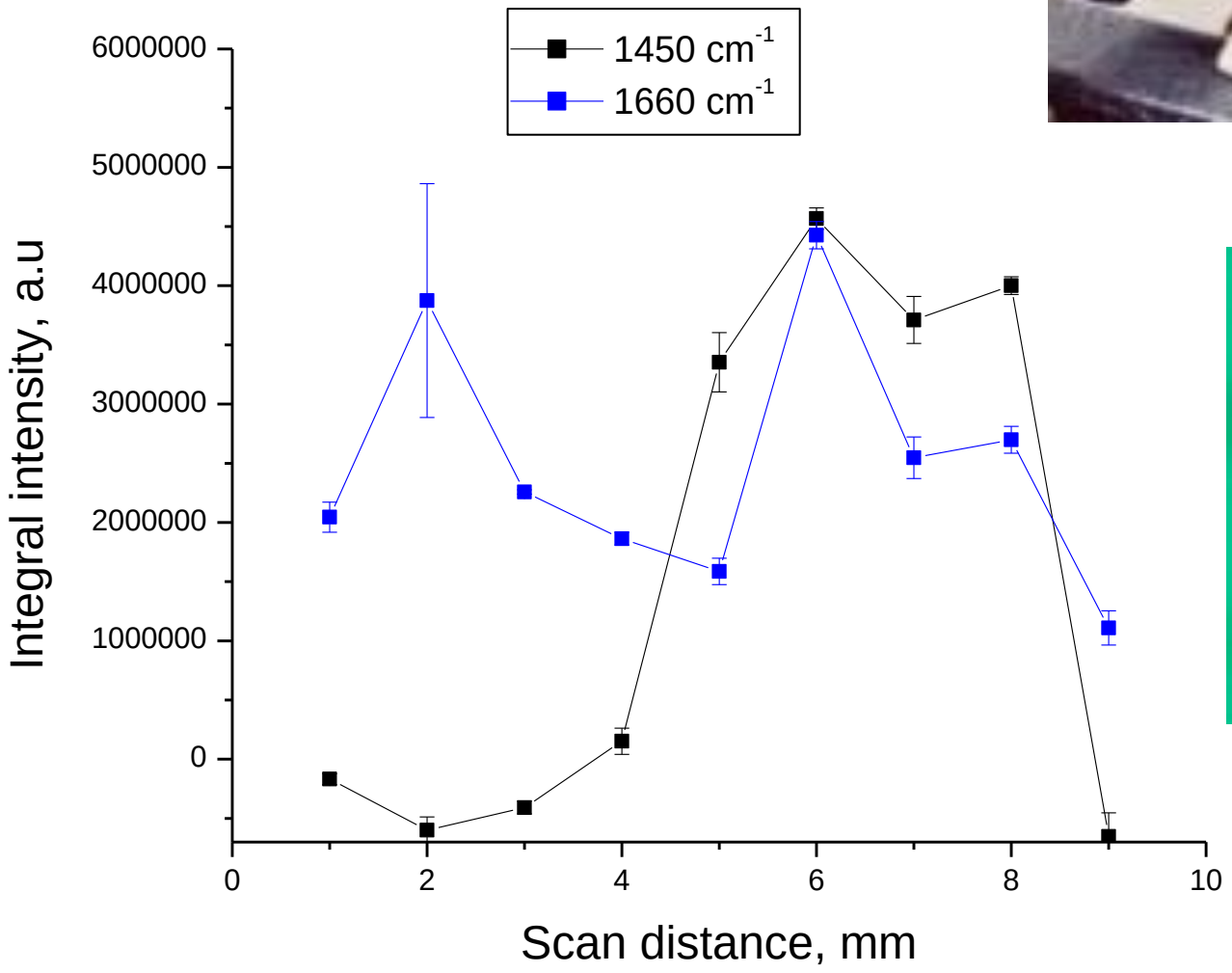


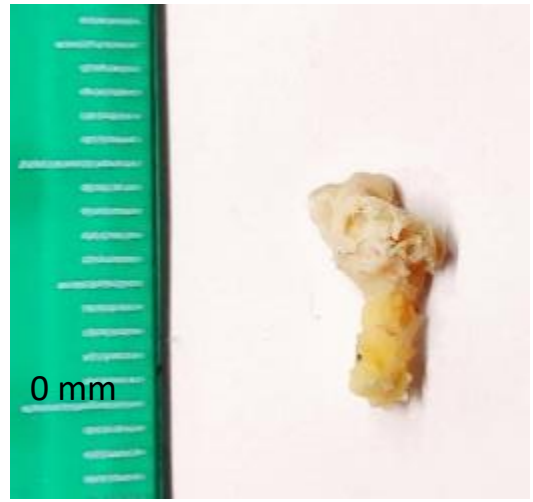
Option 3: Raman spectral band imaging by x-y translation of a double-fiber probe



Filters (Semrock USA): F1 (FF01-880/11-25), F2 (F910/5-25), F3 (FF01-900/11-25), F4 (BLP01-785R-25)
O – N-BK7 bi/cx lens, D=25.4 mm, F=25 mm;
PMT (Hamamatsu Photonics, Japan): photosensor module: H10722-20;
Laser (Hubner Photonics, Sweeden): Cobolt 08-NLDM, < 500 mW output; 785 nm, < 70 pm bandwidth;
x-y translation stage (Standa, Lithuania): 8MT173-25XY
Function generator: TG 4001; Thurlby-Thandar Instruments, UK

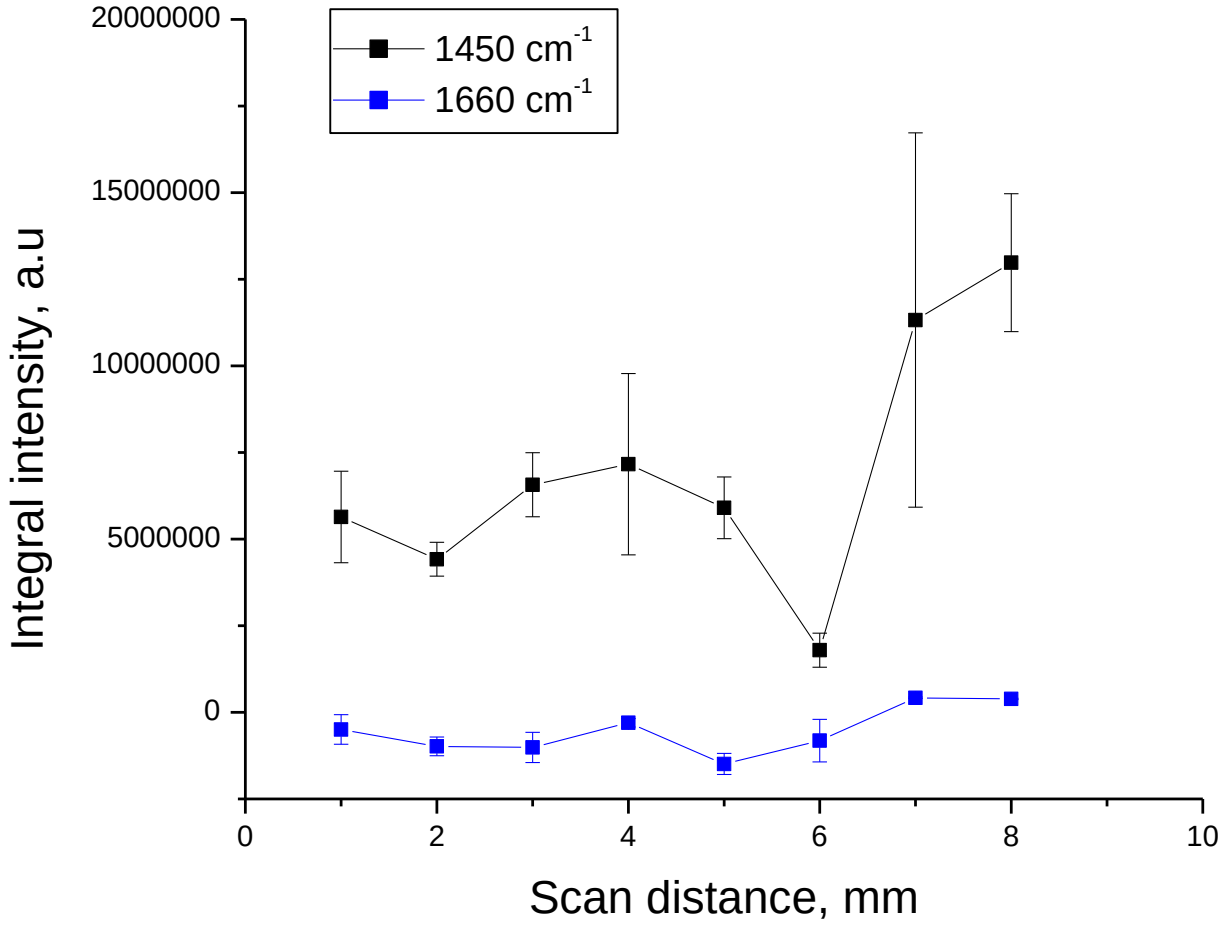
First results of line scanning: *ex-vivo* BCC (20 sec scanning time)





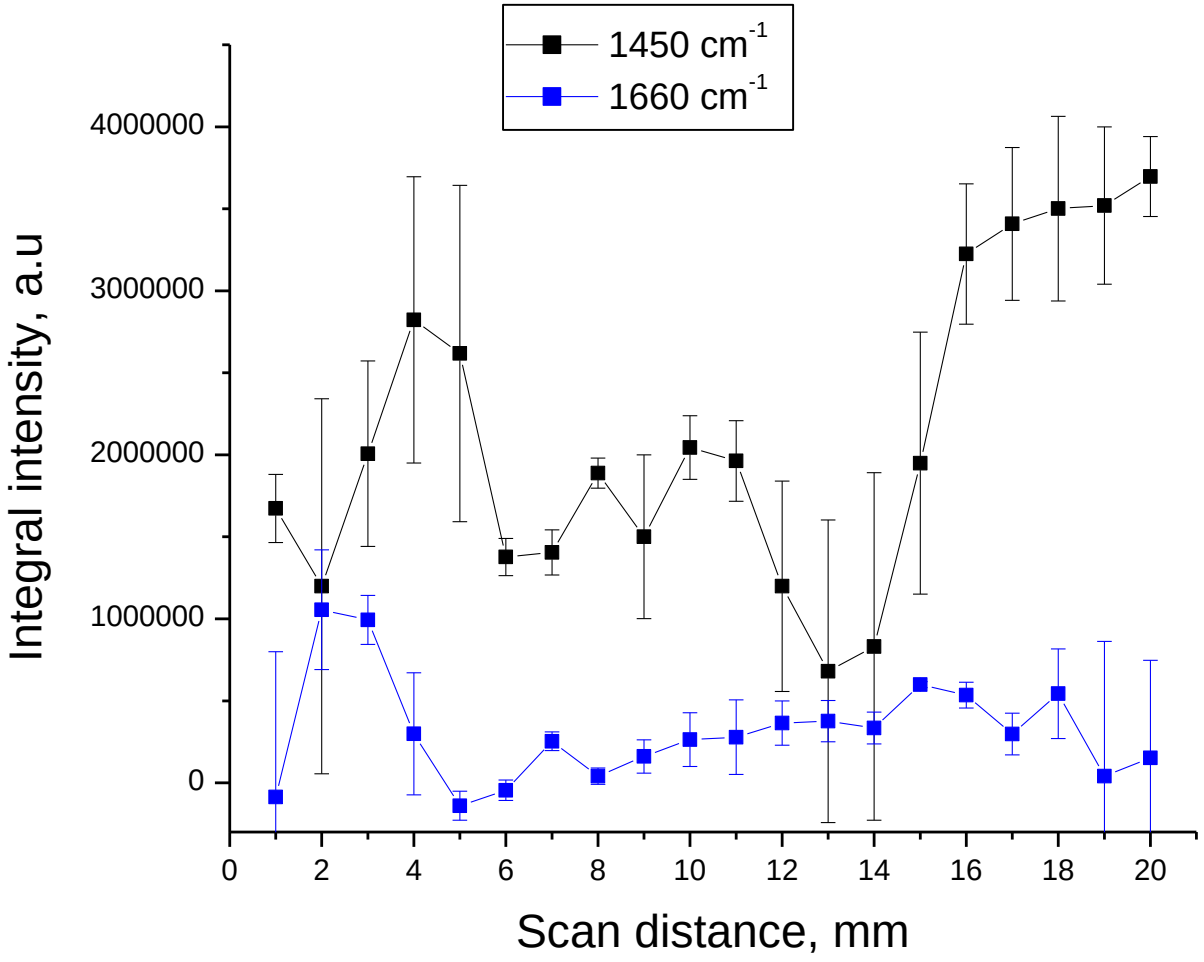
Scan direction

Results: SCC

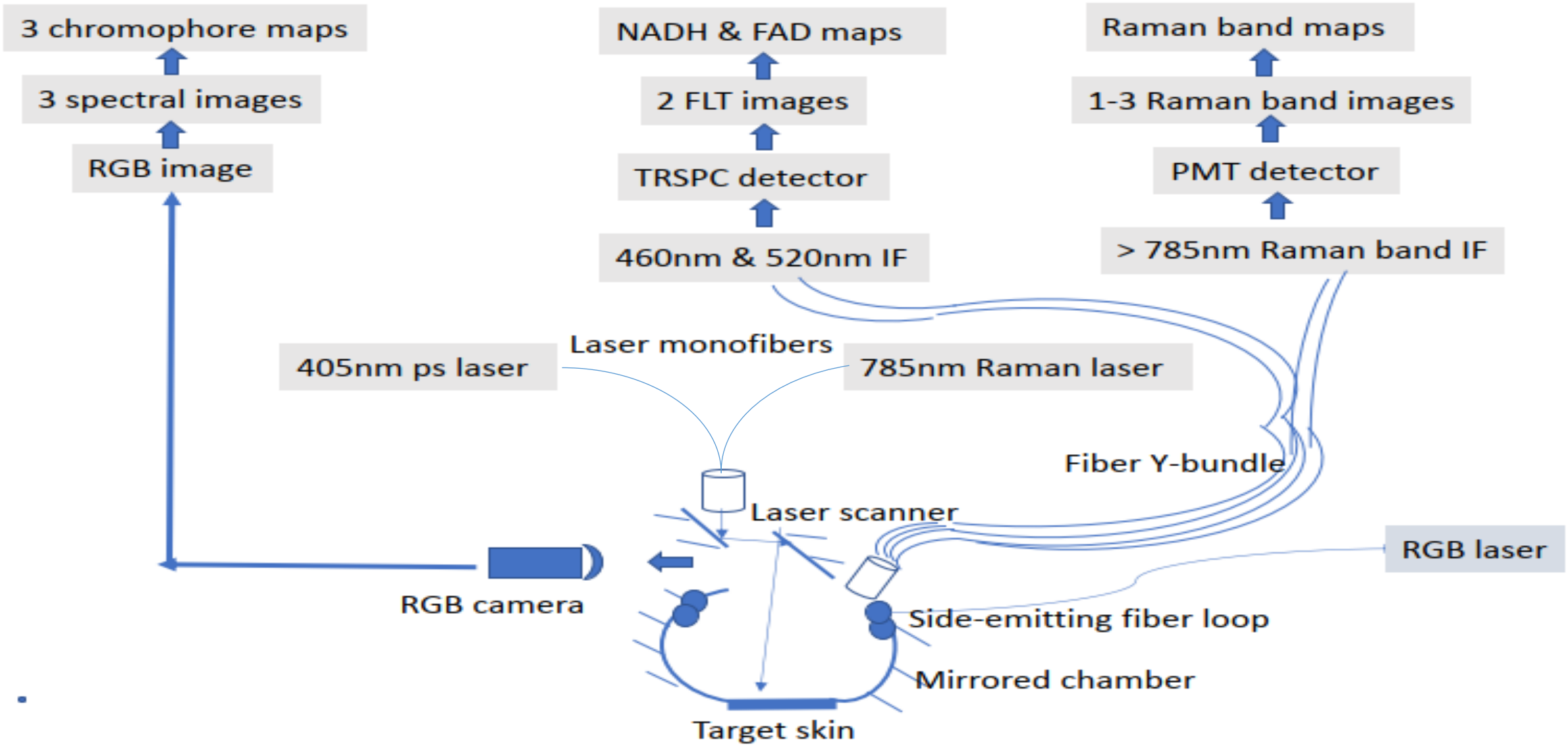


Scan direction

Results: nevus with adjacent skin

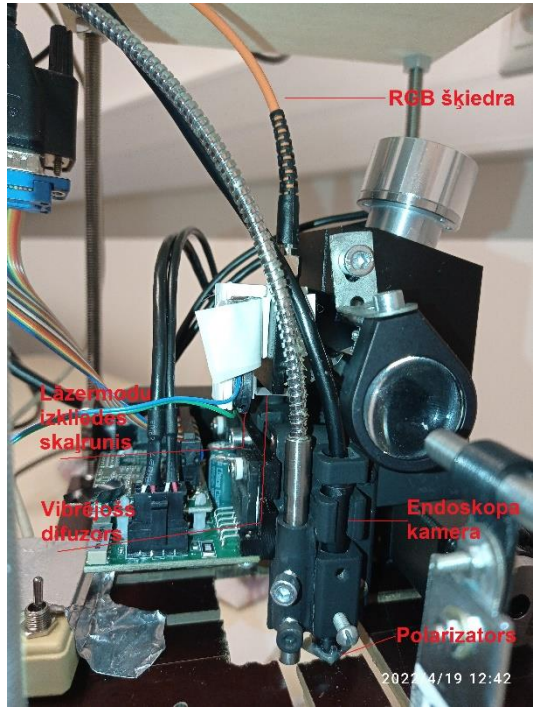


Tri-modal skin imaging: initial concept-scheme



How the tri-modal imaging system looks like now:

2 measurement positions – one for bi-modal multispectral-AFLT imaging (*in-vivo*) and one for Raman Y-probe scanning by an automatic x-y translation stage (mainly *ex-vivo*).



SUMMARY

- Proof-of-concept study of trimodal skin imaging (by MSI, AFLTI, RBI) is underways
- Technologies exploited:
  - Triple spectral line imaging under RGB laser-fiber illumination
  - Autofluorescence lifetime imaging by picosecond 405nm laser scanning
  - Raman band imaging by (i) 785nm laser beam scanning, (ii) specifically filtered high-sensitive cameras, (iii) mechanical x-y scanning of Raman Y-shaped fiber probe
- Five types of skin malformations (>100 in total) examined *in-vivo* to obtain:
  - maps of melanin, oxy-hemoglobin and deoxy-hemoglobin content changes
  - distributions of two lifetime components at two emission bands (related to NAHD and FAD)
  - filtered camera images of two Raman band (~1437 cm^{-1}, ~1660 cm^{-1}) intensity distributions
- Conclusions on clinical applicability:
  - snapshot triple spectral line imaging works well
  - autofluorescence lifetime imaging works but takes minutes (a problem *in-vivo*)
  - Raman band imaging acceptable only by narrowband filtered high-performance cameras
- Potential S/N increase by shortening the Raman laser wavelength (e.g. ~600 nm → higher PMT/camera sensitivities)
- Positive outcome - lots of experience gained ☺

Acknowledgement

This work was supported by the European Regional Development Fund project #1.1.1.1/18/A/132 “Multimodal imaging technology for in-vivo diagnostics of skin malformations”.

Thank You!

- janispi@latnet.lv
- http://home.lu.lv/~spigulis