Lightnovo

APPLICATION NOTE

In-vivo skin measurements

INTRODUCTION

Raman spectroscopy has shown to be a promising technique for different skin diagnostics such as cancer and atopic dermatitis. This technique is non-invasive and can provide several information regarding the molecular composition of the surface of the skin and up to several hundred micrometers in depth.
However *in-vivo* skin measurements are typically associated with complex Raman instrumentation that requires a deep cooling sensor due to the low Raman cross section of skin, especially at a depth of more than 100µm[1,2]. Additionally, *in-vivo* skin measurements require the development of an immersion probe with high numerical aperture NA that can provide a small laser spot size in depth of tissue.

TECHNOLOGY

Raman spectroscopy provides a unique opportunity to study the chemical composition of materials at the microscale.
Such capabilities come at the cost of extremely high requirements for instrumentation: lasers with stabilization of wavelength and power, low noise spectroscopic sensors, and a large clear aperture of spectrometer's optics. Therefore, demanding Raman spectroscopy and microscopy applications usually require high-end, bulky, and costly Raman instrumentation.
Lightnovo ApS found possible solutions to the most critical Raman miniaturization challenges: need for laser temperature and power stabilization, reduction of sensor dark noise, compensation on pixel-to-pixel quantum efficiency (QE) variation, laser optical isolation and achieving high spectral resolution.

As result a novel optics miniaturization strategy allows us to create **compact Raman spectrometers and microscopes** based on non-stabilized laser diodes, densely-packed optics, and non-cooled small pixel size sensors.
Lightnovo ApS proposed miniaturization concept based on real-time calibration of Raman shift and Raman intensity using an in-built reference channel that collects the Raman spectrum of polystyrene located in the spectrometer. We have demonstrated the miniaturization of the whole device dimensions down to several centimeters and achieved excellent sensitivity, low power consumption, perfect wavenumber and intensity calibration combined with high spectral resolution of around 7 cm^{-1} within the spectral range of 400-4000 cm^{-1}.

Details for miniRaman MRs patent

MATERIALS, SAMPLE PREPARATION AND MEASUREMENTS

Anti-sun cream Sollotion SPF30 produced by DermaPharm A/S was used in this study. Cream was applied on human palm that was previously cleaned with water-soup solution to avoid skin surface contamination by dust.

RESULTS

A small laser spot size will improve the ratio between Raman and fluorescence signal. This happens due to a non-linear saturation of the fluorescence signal and a linear growth of the Raman signal when the laser power is increased[3]. Moreover, it is preferable to produce the last optical element of the probe from fused silica. This will generate optimized conditions for laser/Raman beam propagation in/out of the stratum corneum skin layer and provide a matching of the reflection index between the last optical surface of the probe and the skin media[1]. An extremely compact version of a skin probe is shown in Figure 1a-1b. Our probes can be optimized for skin measurements at different depths; between 0 to 150μm. When our miniaturized Raman spectrometer is equipped with this probe, we were able to collect Raman spectra of skin at a depth of 10-20 μm with SNR better than 500:1 (1sec exposure time, 5 repetitions). Typical application examples on studying anti-sun cream penetration and water content are shown in Figure 2 and 3b, 3d, 3f respectively. Figures 3a-f demonstrate Raman spectra obtained from two lasers (785 and 675nm) at different skin areas (finger, hand and cheek). Water content difference at different skin areas could be clearly seen by intensity ratio of CH (2800-3000cm^{-1}) and OH (3100-3500cm^{-1}) bands. Compared to similar previous experiments[1,2] our *in-vivo* Raman measurements of skin seem to have unprecedented high SNR (500:1, 1 sec exposure time, 5 repetitions).

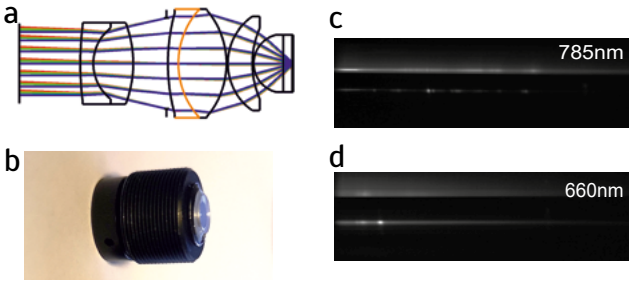


Figure 1.
a) optical schema of the Raman probe with high NA (0.95) developed for in-vivo skin measurements,
b) photograph of "skin probe",
c), d) CMOS image of the measurement process of skin in-vivo, demonstrating sharp focusing of the Raman signal in the vertical dimension of the sensor under laser excitation wavelength of 785nm (c) and 660nm (d).

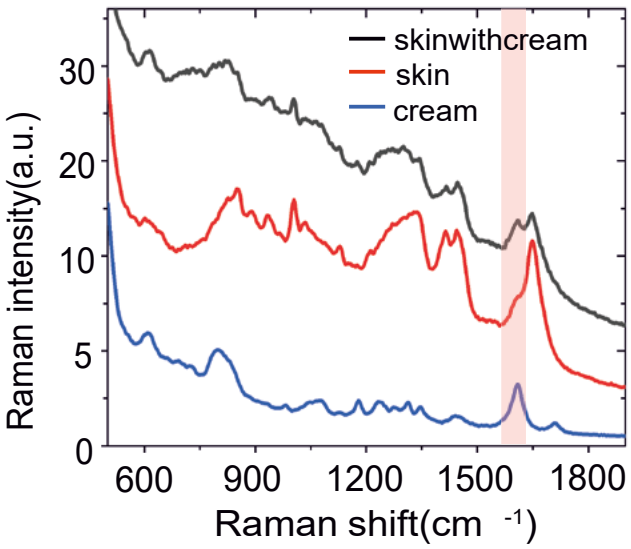


Figure 2.
Raman spectra
of cream penetration.

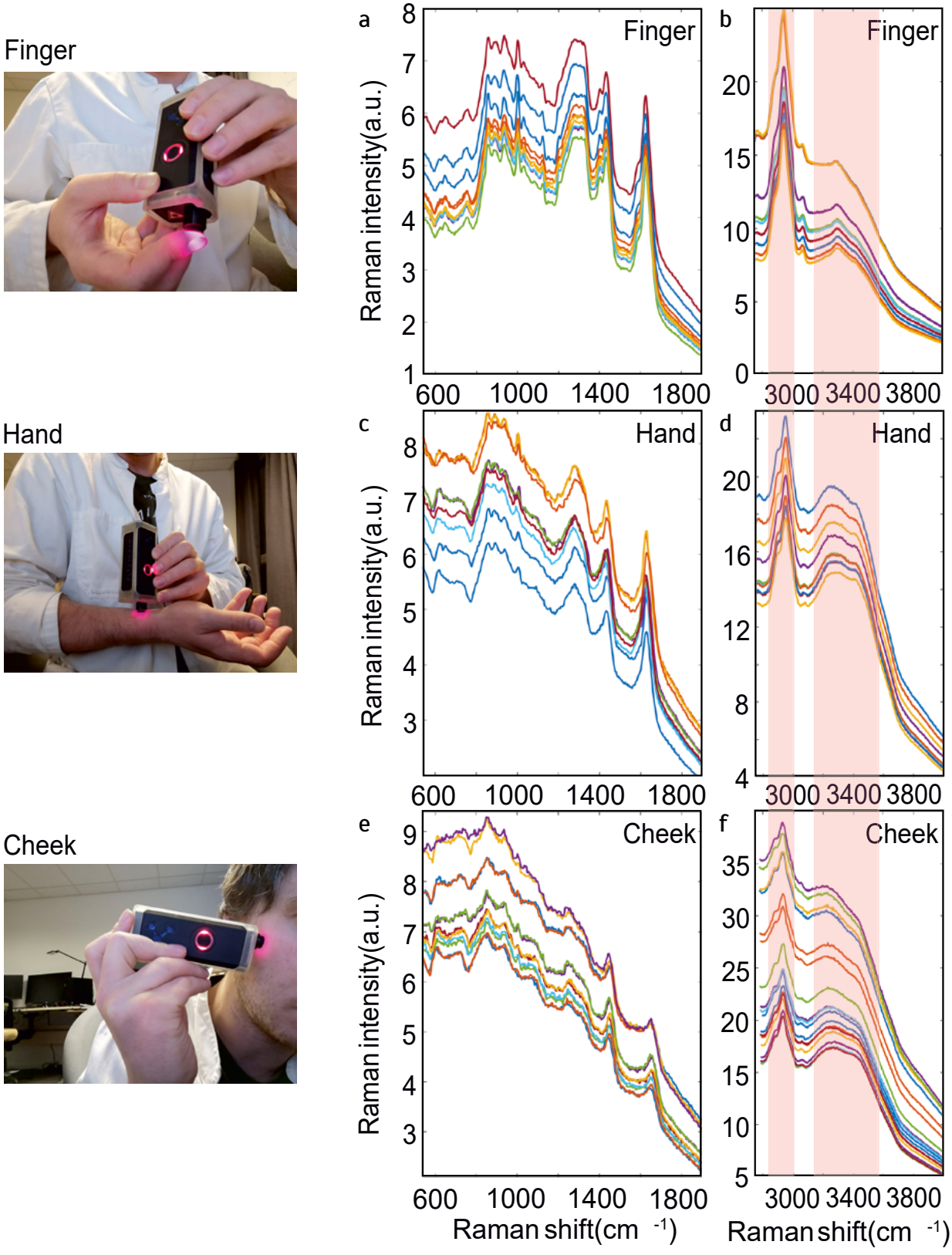


Figure 3.

a-f) Raman spectra of normal skin collected at the depth of 10-20μm under a laser excitation wavelength of 785nm (a,c,e) and 675nm (b,d,f);
pink bands indicate spectral regions of CH and OH peaks;
Raman spectra were collected on finger (a,b), hand (c,d) and cheek (e,f);
spectrum color represents different probe locations over the skin area around 1cm^2.

CONCLUSION

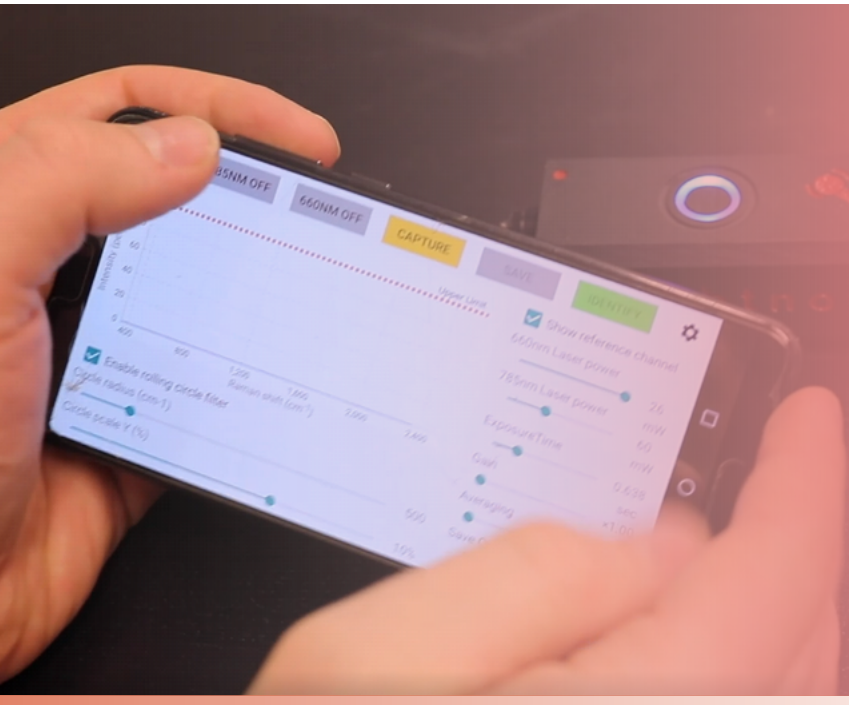
Lightnovo ApS demonstrated technology for both miniaturizing and democratizing Raman spectrometers, making Raman spectroscopy more accessible to researchers as well as consumers. We believe that the presented instrumentation could be applied for numerous *in-vivo* applications in the future including skin disease diagnostics, skin aging, determination of molecular concentration profiles from the skin surface into the dermis, measuring of the distribution of intrinsic skin constituents (amino acids, sweat constituents, lipids, proteins, water), skin penetration and permeation of topical formulations, distinguishing of the difference between volar forearm skin, cheek, forehead, scalp, axilla, and other.

LITERATURE

1 Caspers PJ, Bruining HA, Puppels GJ, Lucassen GW, Carter EA. In Vivo Confocal Raman Microspectroscopy of the Skin: Noninvasive Determination of Molecular Concentration Profiles. *J Invest Dermatol* 2001; **116**: 434–442.

2 Nakagawa N, Matsumoto M, Sakai S. In vivo measurement of the water content in the dermis by confocal Raman spectroscopy. *Ski Res Technol* 2010; **16**: 137–141.

3 Slipets R, Ilchenko O, Mazzoni C, Tentor F, Nielsen LH, Boisen A. Volumetric Raman chemical imaging of drug delivery systems. *J Raman Spectrosc* 2020; **51**: 1153–1159.



Harness the power of Raman spectroscopy and make it widely accessible for the benefit of mankind.

- Lightnovo's mission

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