

Dual vibration resonance frequency CARS microscopy imaging of basal cell carcinoma to achieve stain free histopathology

Norbert Kiss ^{1, 2}, Ádám Krolopp³, Kende Lőrincz¹, Róbert Szipőcs^{2, 3} and
Norbert Wikonkál¹

1 Department of Dermatology, Semmelweis University, Budapest, Hungary

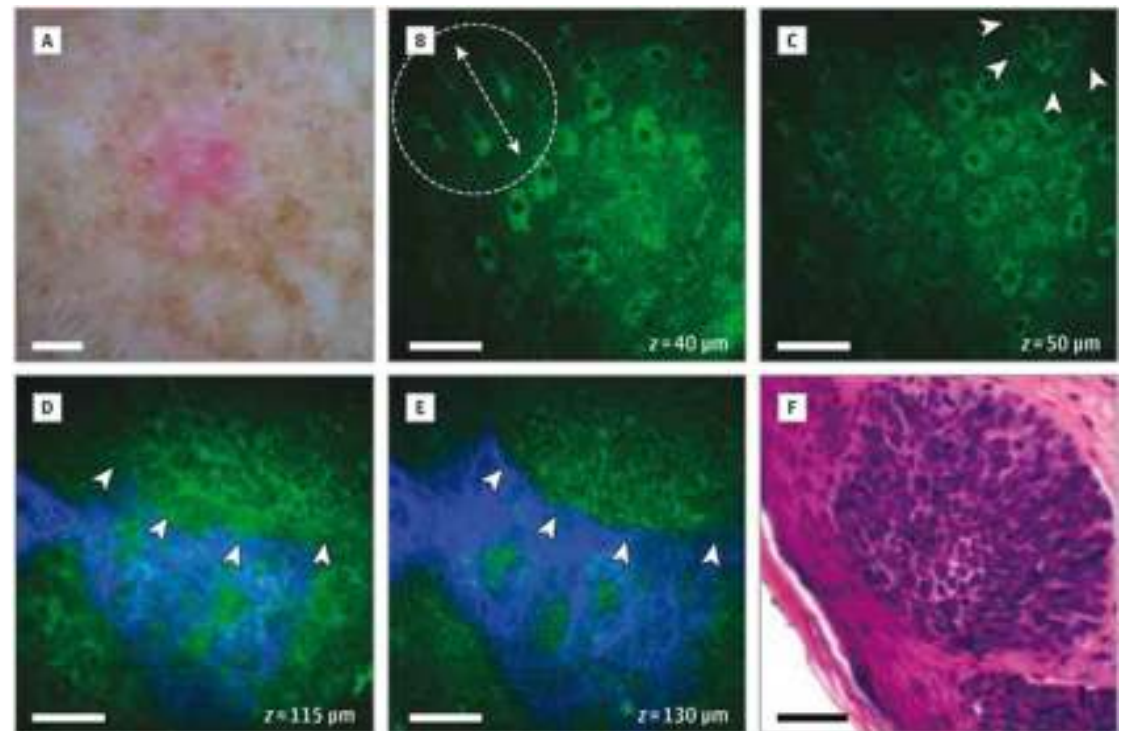
2 Wigner RCP, Institute for Solid State Physics and Optics, Budapest, Hungary

3 R&D Ultrafast Lasers Ltd, Budapest, Hungary



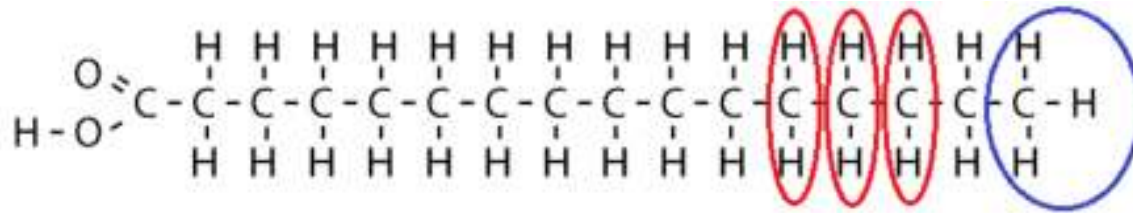
Introduction

- Basal cell carcinoma (BCC): most common malignancy in Caucasians
- Gold standard treatment: surgical excision
- Histopathological evaluation: haematoxylin and eosin (H&E) stain
- Incomplete resection -> recurrence -> **estimation of tumor borders** is needed
- Nonlinear microscopy has been previously utilized for the stain-free imaging of BCC
- The acquired images did not correlate with the standard H&E staining

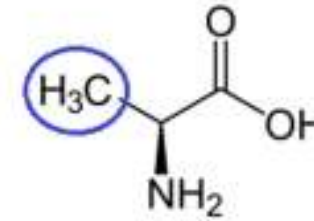


Balu et al. JAMA Dermatology 2015

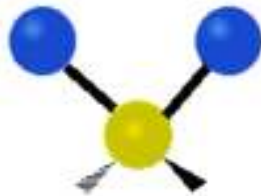
Vibrational spectra of lipids, protein and water is different



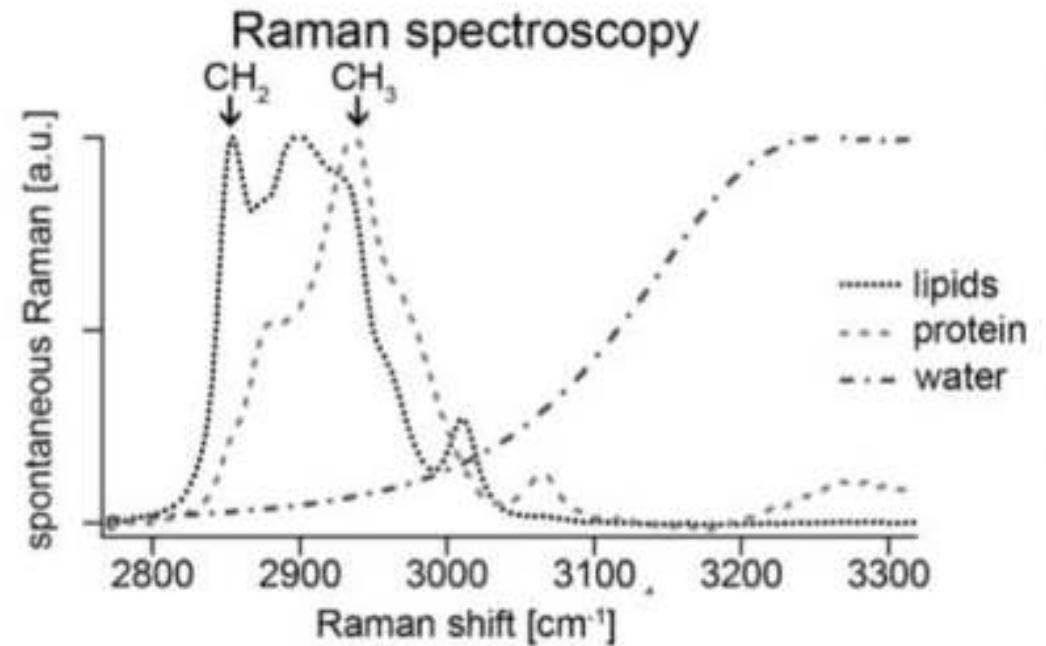
Saturated fatty acid



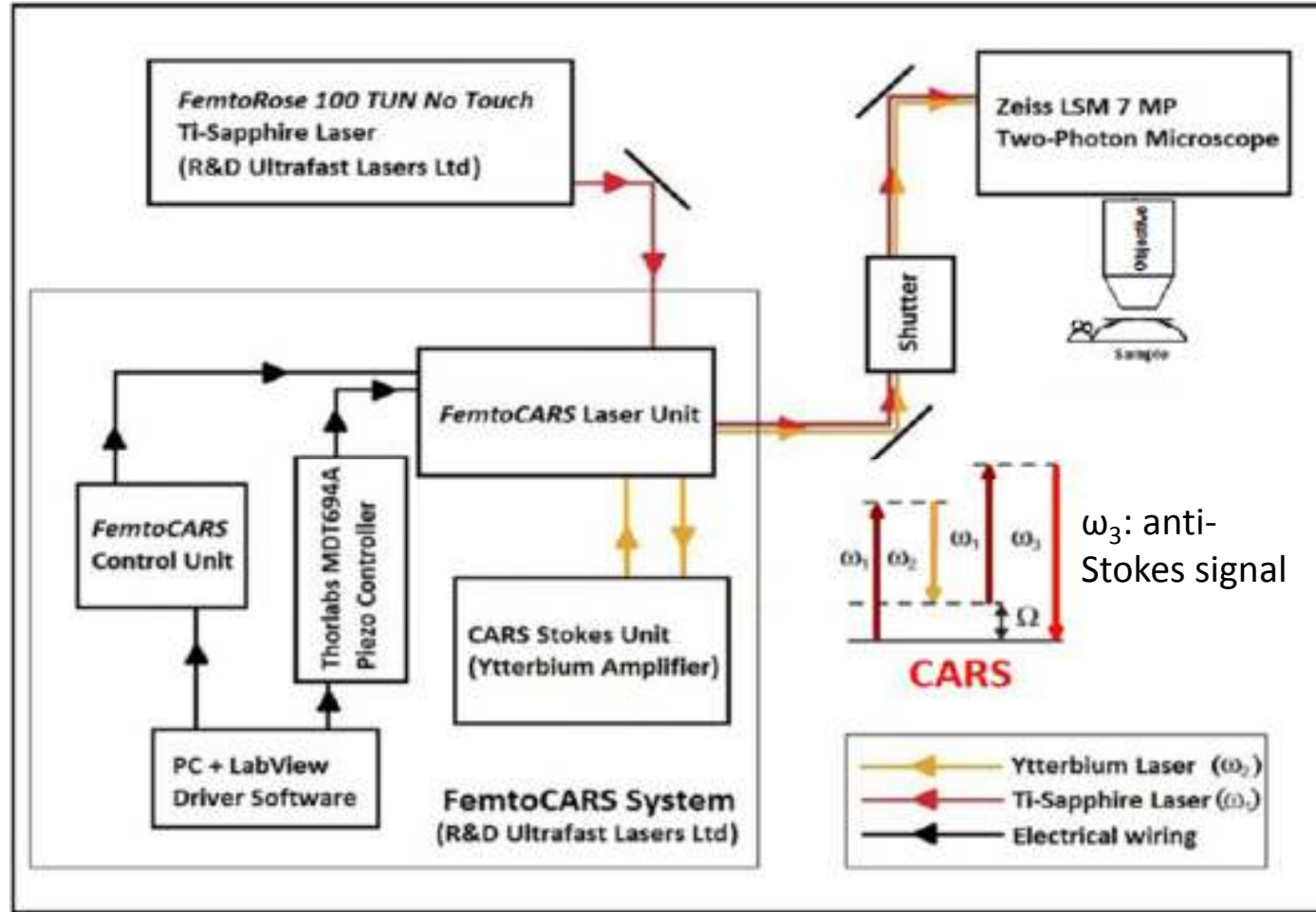
Alanine



Symmetric stretching of the CH₂ groups



Layout of our experimental setup used for dual vibration resonance frequency (DVRF) Coherent anti-Stokes Raman scattering (CARS) imaging



Previously, we utilized CARS microscopy to visualize lipids in the adipocytes of murine skin

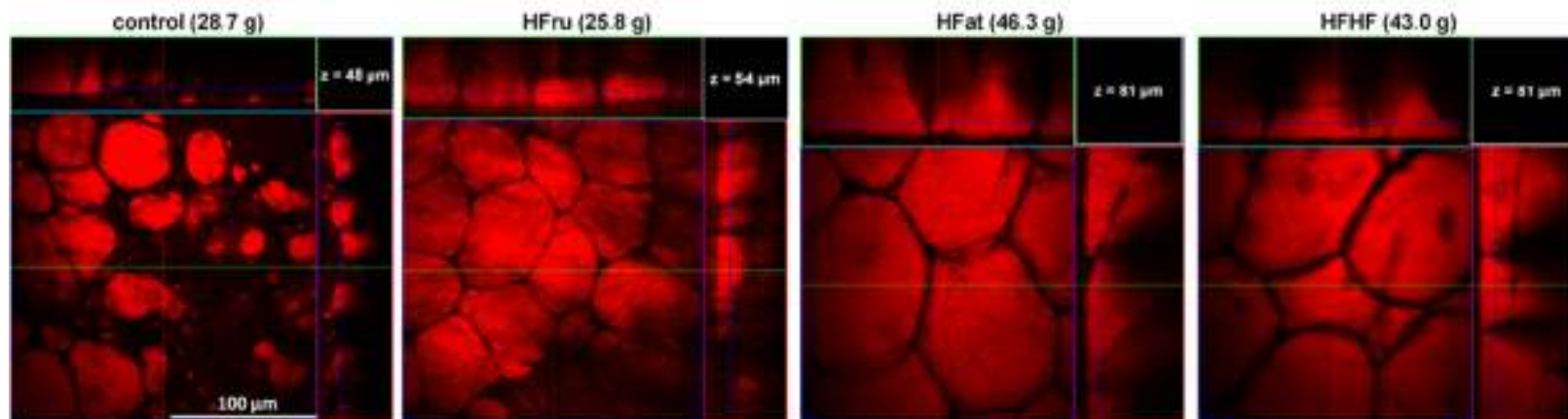


Biomed. Opt. Express, 2016 Nov 1, 7(11): 4480–4489.
Published online 2016 Oct 7, doi: [10.1364/BOE.7.004480](https://doi.org/10.1364/BOE.7.004480)

PMCID: PMC5119589

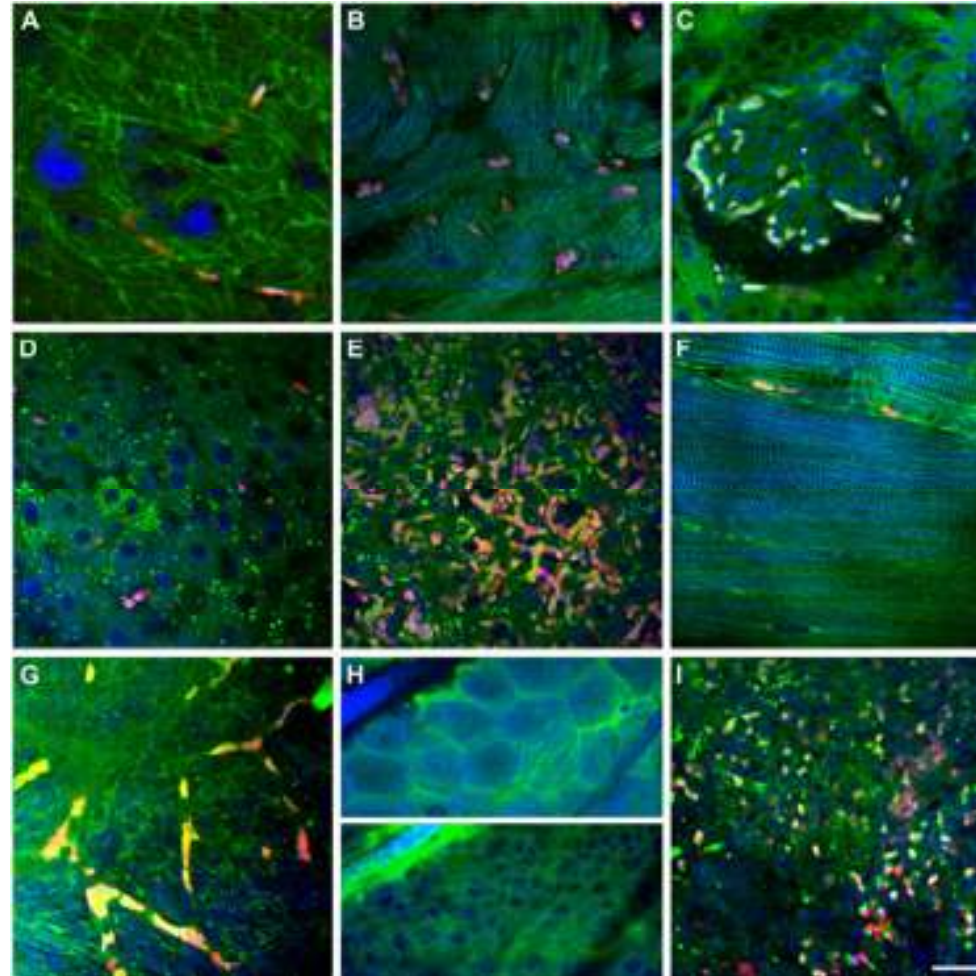
Diet-induced obesity skin changes monitored by *in vivo* SHG and *ex vivo* CARS microscopy

[Dóra Halaszka](#)^{1,2} [Kende Lőrincz](#)¹ [Norbert Kiss](#)^{1,2} [Róbert Szipócs](#)^{2,3} [Enikő Kuroli](#)¹ [Nóra Gyöngyösi](#)¹ and [Norbert M. Wikonka](#)^{1,*}



Multicolor stain-free CARS images of mouse organs

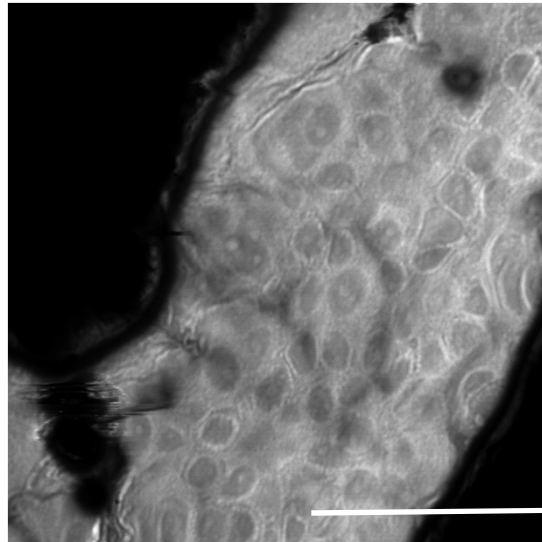
CH_2
 $\text{CH}_3\text{-CH}_2$



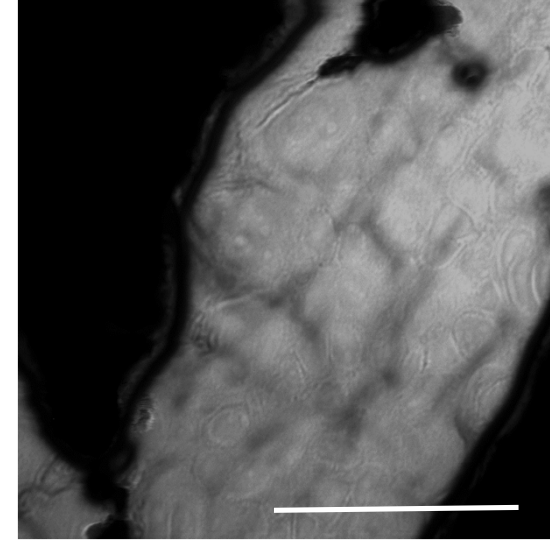
Freudiger et al. Lab Invest. 2012

CARS images of mouse epidermis

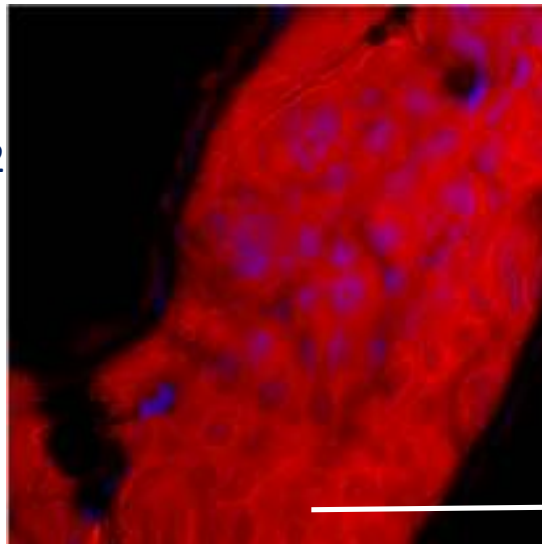
CH_2



CH_3

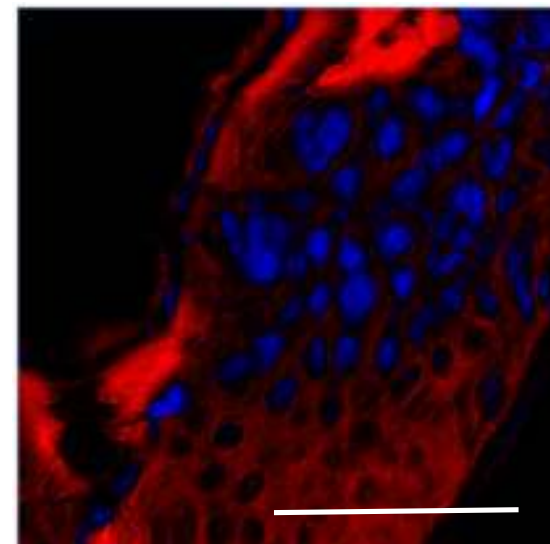


CH_2
 $\text{CH}_3\text{-CH}_2$



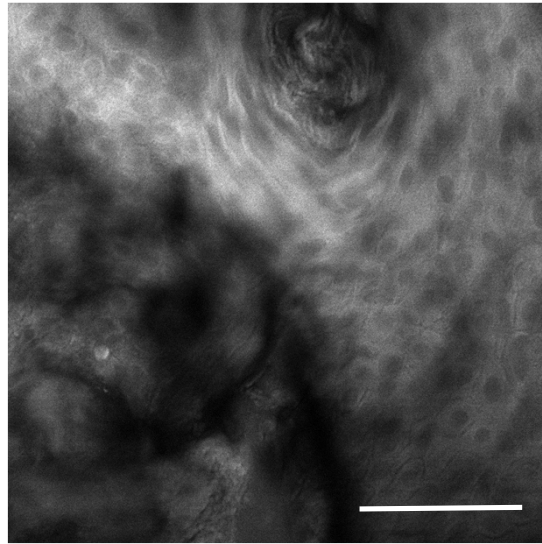
Scale bar:
50 μm

$\text{CH}_2\text{-CH}_3$
 $\text{CH}_3\text{-CH}_2$

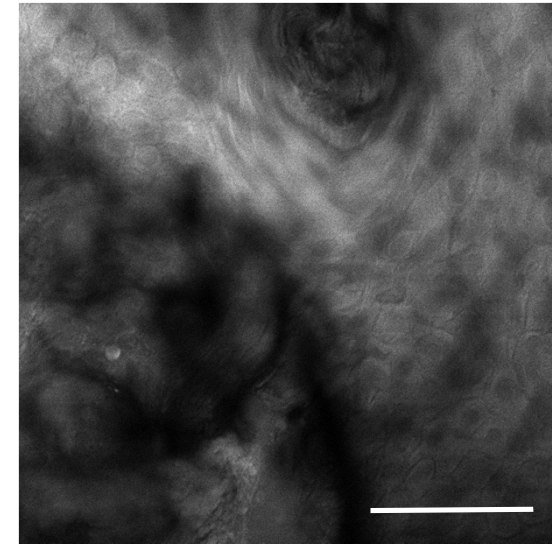


CARS images of human epidermis

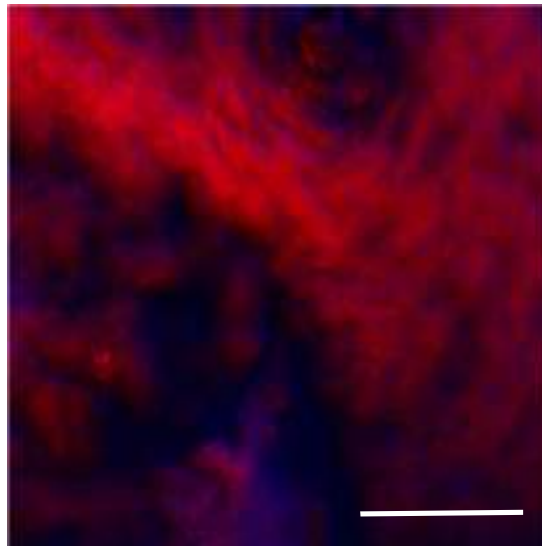
CH_2



CH_3

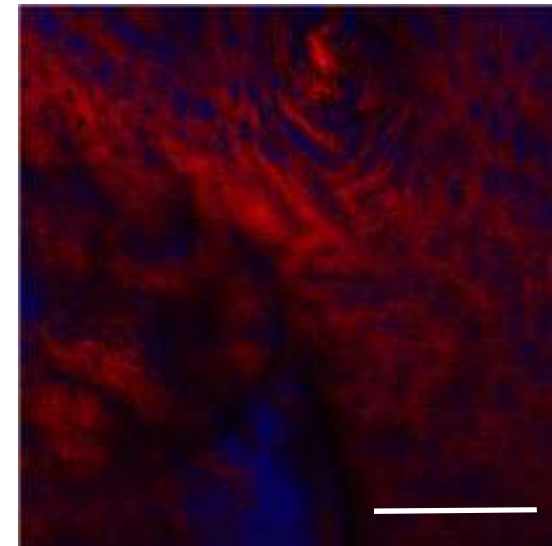


CH_2
 $\text{CH}_3\text{-CH}_2$



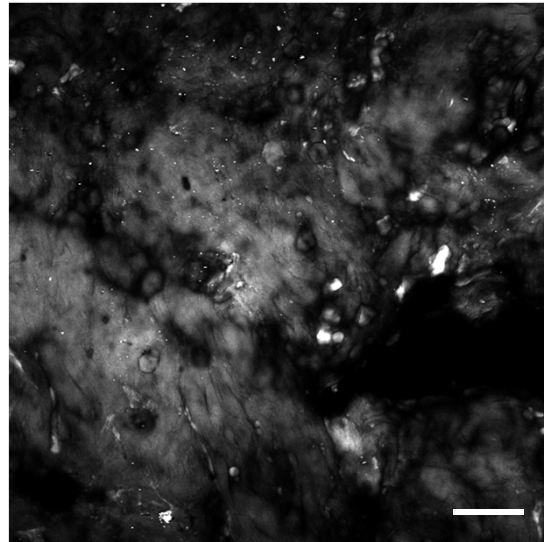
Scale bar:
50 μm

$\text{CH}_2\text{-CH}_3$
 $\text{CH}_3\text{-CH}_2$

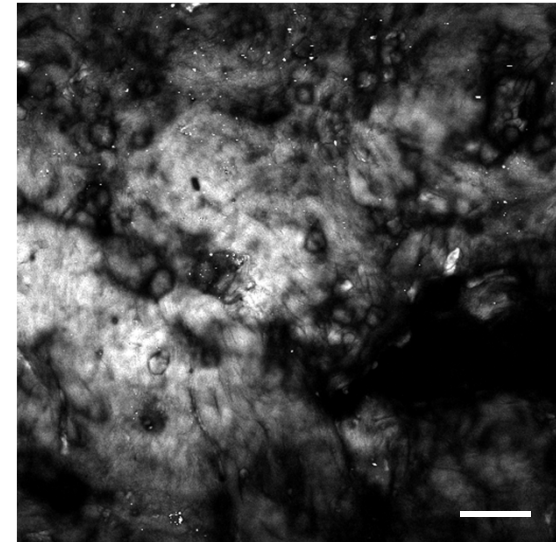


CARS images of healthy human dermis

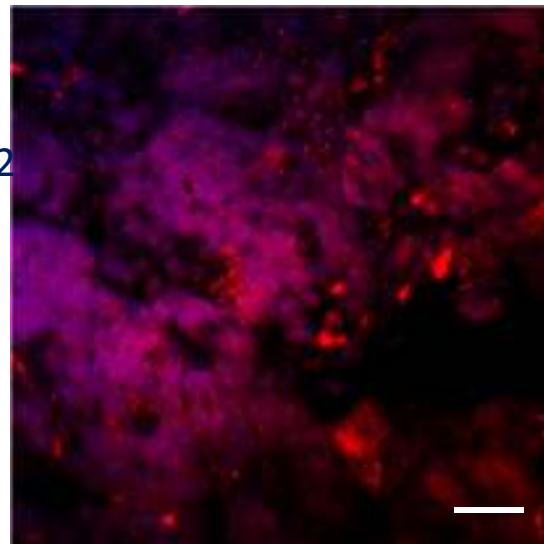
CH_2



CH_3

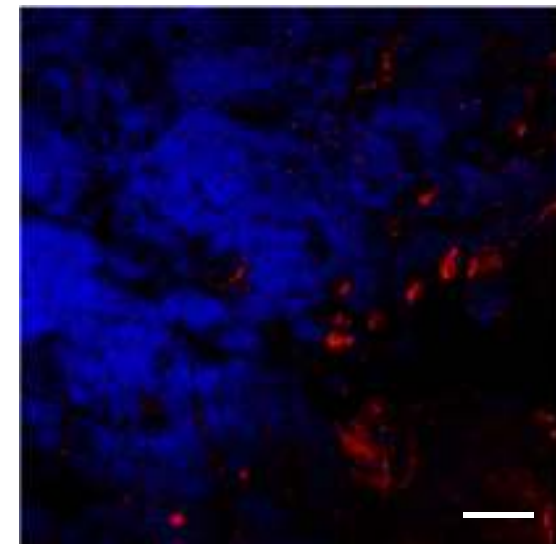


CH_2
 $\text{CH}_3\text{-CH}_2$



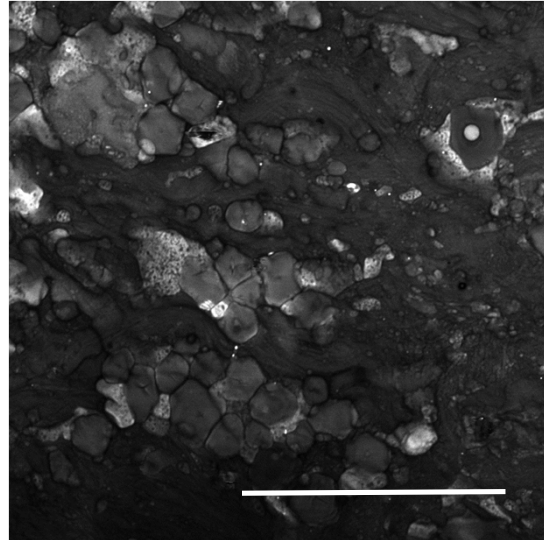
Scale bar:
50 μm

$\text{CH}_2\text{-CH}_3$
 $\text{CH}_3\text{-CH}_2$

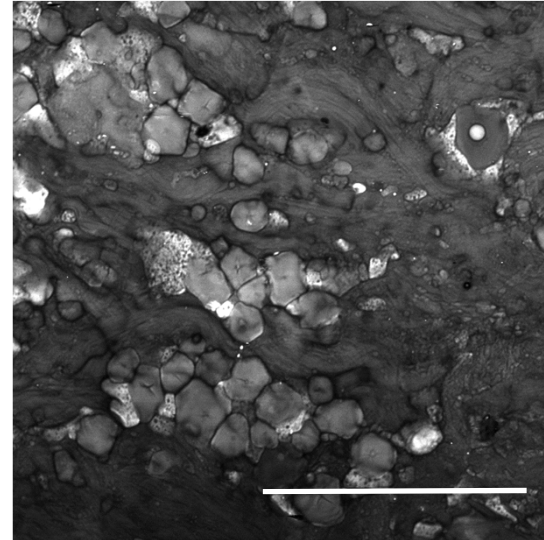


CARS images of human basal cell carcinoma

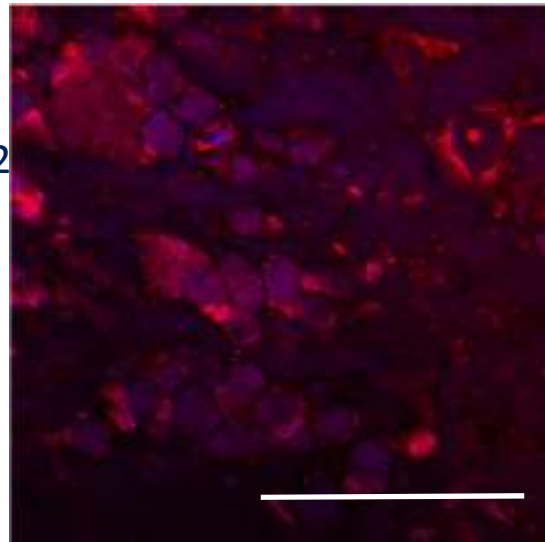
CH_2



CH_3

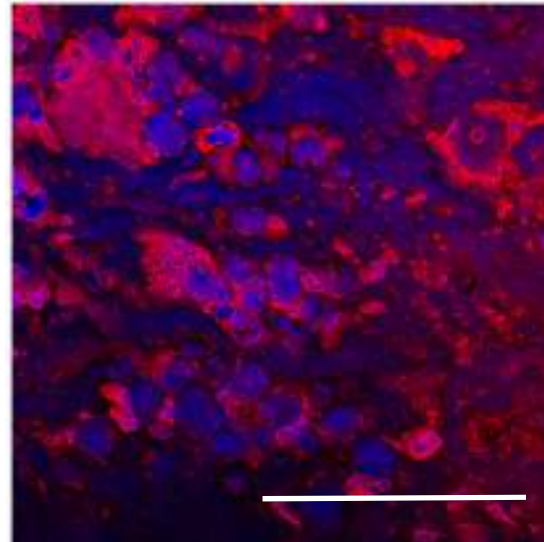


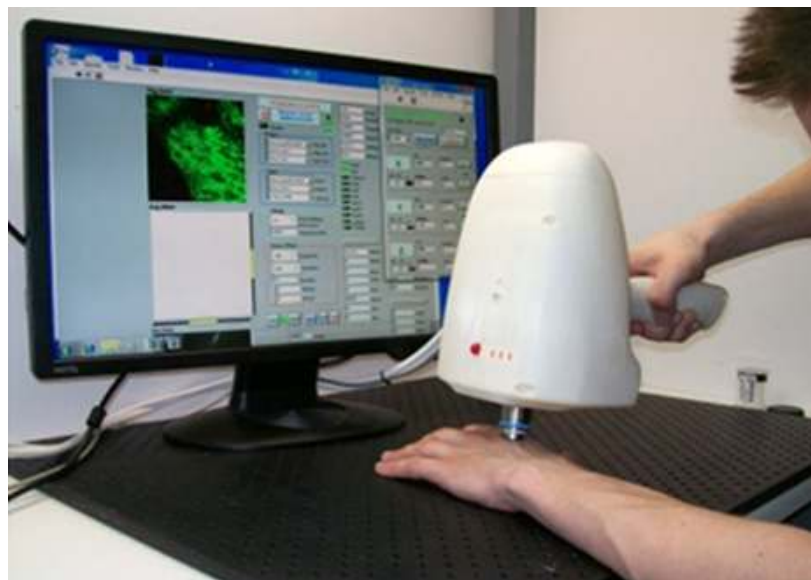
CH_2
 $\text{CH}_3\text{-CH}_2$



Scale bar:
50 μm

$\text{CH}_2\text{-CH}_3$
 $\text{CH}_3\text{-CH}_2$





Krolopp et al. Biomed Opt Express. 2016

- We have performed *ex vivo*, proof of concept experiments on a table top, laboratory setup with an overall system cost of c.a. 200-300 kEUR
- A similar imaging setting can be used for stain-free histopathological evaluation of *ex vivo* skin samples
- We plan to adapt our fiber laser based, handheld nonlinear microscope system for DVRF CARS measurements, that could considerably reduce the imaging system cost down to the 70 kEUR/per system range.

Conclusion

- We have introduced a novel image processing method of DVRF CARS images, which provides an improved contrast and sharpness
- Our method is capable of generating pseudo H&E images of BCC
- Nonlinear microscopy systems upgraded for real time DVRF CARS imaging can be suitable for *in vivo* stain free assessment of BCC in the future -> dermatosurgeons can plan the optimal safety margin before surgery -> minimized recurrence rate