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

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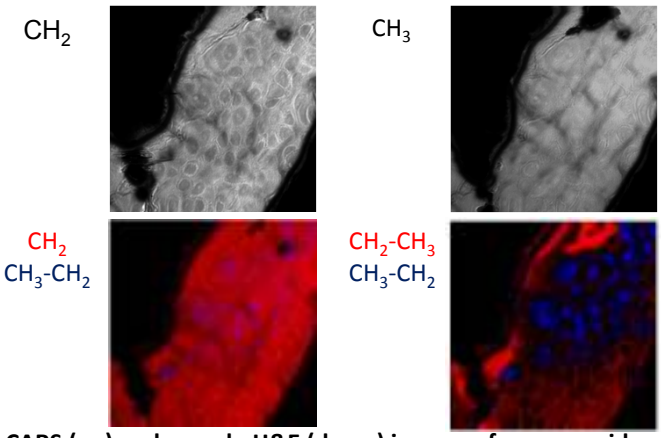
Basal cell carcinoma (BCC) is the most common malignancy in Caucasians and its incidence is increasing [1].

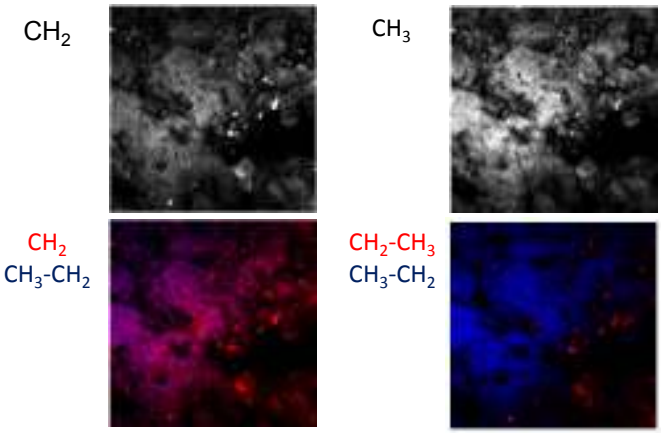
Nonlinear microscopy has been previously utilized for the imaging of BCC in different experimental setups. To date, these experiments were carried out solely on frozen sections, and the captured images do not correlate with the standad rhaematoxylin and eosin (H&E) staining [2]. Recently, Freudiger et al. introduced a novel method to visualize tissue morphology analogous to H&E staining, using coherent anti-Stokes Raman scattering (CARS) technique [3].

In our present work, we introduce a novel algorithm to post-process images obtained from dual vibration resonance frequency (DVRF) CARS measurements to acquire high-quality pseudo H&E images of ex vivo BCC samples. We adapted our CARS setup to utilize the distinct vibrational properties of CH_3 (mainly in proteins) and CH_2 bonds (primarily in lipids). In a narrowband setup, the central wavelength of the pump laser is set to 791 nm and 796 nm to obtain optimal excitation. Due to the partial overlap of the excitation spectra and the 5-10 nm FWHM spectral bandwidth of our lasers, we set the wavelengths to 790 nm (for proteins) and 800 nm (for lipids). Nonresonant background from water molecules also reduces the chemical selectivity which can be significantly improved if we subtract the DVRF images from each other. As a result, we acquired two images: one for “lipids” and one for ”proteins” when we properly set a multiplication factor to minimize the non-specific background. By merging these images, we obtained high contrast H&E “stained” images of BBC’s.

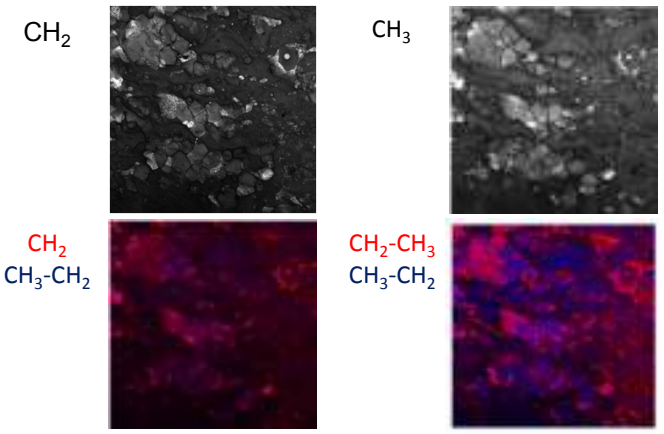
Nonlinear microscope systems upgraded for real time DVRF CARS measurements, providing pseudo H&E images can be suitable for in vivo assessment of BCC in the future.



CARS (up) and pseudo H&E (down) images of mouse epidermis



CARS (up) and pseudo H&E (down) images of human dermis



CARS (up) and pseudo H&E (down) images of human BCC

References

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3. Freudiger CW, Pfannl R, Orringer DA, Saar BG, Ji M, Zeng Q, Ottoboni L, Wei Y, Waeber C, Sims JR, De Jager PL, Sagher O, Philbert MA, Xu X, Kesari S, Xie XS, Young GS (2012) Multicolored stain-free histopathology with coherent Raman imaging. Laboratory investigation 92 (10):1492-1502.