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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 the Caucasian race, and its incidence is increasing. 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 standard H&E staining. Recently, Freudiger et al. introduced a novel method to visualize normal tissue morphology analogous to H&E staining, using coherent anti-Stokes Raman scattering (CARS) technique. In our present work, we propose a novel algorithm to post-process images obtained from dual vibration resonance frequency (DVRF) CARS measurements to increase chemical selectivity of the imaging and to acquire high quality pseudo H&E images of ex vivo healthy and BCC skin samples. We adapted our CARS setup to utilize the distinct vibrational properties of CH_3 (found mainly in proteins) and CH_2 bonds (primarily in lipids). *In a narrowband* setup, the centra*l wavelength of the pump laser* is set to 791 nm and 796 *nm* to obtain optimal excitation of the CH_3 and CH_2 *bonds.* Due to the partial overlap of the excitation spectra of proteins and lipids, and the 5-10 nm FWHM spectral bandwidth of our lasers, we set the wavelengths to 790 nm (for excitation of proteins) and 800 nm (for lipids). In addition to the spectral overlap, nonresonant background signal 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 (typically in the 0.9-1.1 range) to minimize the non-specific CARS (and autofluorescence) background. By merging these images, we obtained high contrast H&E “painted” ex vivo microscope images of BBC. Nonlinear microscope systems upgraded for real time DVRF CARS measurements providing pseudo H&E images can be suitable for *in vivo* assesment of BCC in the future.