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SHORT COMMUNICATION

Stain-free Histopathology of Basal Cell Carcinoma by Dual Vibration Resonance Frequency CARS Microscopy

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Abstract Basal cell carcinoma (BCC) is the most common malignancy in Caucasians. Nonlinear microscopy has been previously utilized for the imaging of BCC, but the captured images do not correlate with H&E staining. 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. 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 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 (proteins) and 800 nm (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.

Keywords Basal cell carcinoma · Nonlinear microscopy · Coherent anti-stokes Raman scattering · Pseudo HE images · Cancer detection

Introduction

Each year, more than 2 million non-melanoma skin cancers are diagnosed in the USA, of which approximately 4 in 5 cases are basal cell carcinomas (BCC). In fact, BCC is the most common type of cancer in Caucasian individuals, and is showing a worldwide increase in incidence. The most prevalent risk factor for BCC is exposure to UV light from the sun or indoor tanning beds. Other risk factors include immunosuppression and ionizing radiation. Although BCC metastasizes extremely rarely, it can be highly invasive locally, causing disfigurement as facial localization occurs frequently. Early diagnosis and careful management is essential to prevent tissue destruction and the progression of the disease [1].

An initial diagnosis of BCC can be established based on the clinical appearance of the skin lesion, but a skin biopsy is required for a definitive diagnosis. In the management of BCC, a wide range of treatment modalities are available. Nevertheless, the gold standard treatment is surgical excision, followed by a histopathological confirmation of clearance, largely based on haematoxylin and eosin (H&E) staining [2]. In order to achieve aesthetically acceptable post-surgical results, there is a high demand for novel, non-invasive imaging methods, which could be utilized by physicians to determine tumor-free margins.

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