Discriminating vital tumor from necrotic tissue in human glioblastoma samples by Raman microspectroscopy

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Brain tumour malignancy designation is performed according to the presence of histological parameters such as endothelial proliferation and necrosis, which are often not evenly distributed throughout the sample [1]. Furthermore, the grading of tumours as glioblastoma (grade IV, the highest malignancy classification of gliomas) is often performed on samples obtained from stereotactic surgery. This procedure, while suitable for sampling biopsies from regions of the brain which are otherwise not accessible, suffers from the disadvantage that the samples are small and subject to sampling error; resulting in the under-estimation of the malignancy grade. Although necrotic tissue is important for grading, a diagnosis cannot be made when only necrotic tissue is present in the sample [2]. In recent years, significant progress has been made in the application of Raman spectroscopy for ex vivo and in vivo tissue characterization [3]. Vital and necrotic glioblastoma tissues were studied by Raman microspectroscopy in order to identify possibilities for the development of an in vivo Raman method for real-time intra-operative brain biopsy guidance.

Raman microspectroscopic mapping studies were performed on unfixed thin cryo-sections of human glioblastoma samples obtained from 20 patients. Adjacent thin sections stained with hematoxilin and eosin (H&E) served to guide the localization of regions of interest on the tissue samples mapped. Raman spectroscopic mapping was performed using a near-infrared multichannel Raman microspectrometer built in-house and consisted of a Leica DM-RXE microscope coupled to a Renishaw System 100 Raman spectrometer with laser light at 847 nm focused on the samples. Using a computer-controlled xyz-stage that allowed scanning during measurements, consecutive Raman spectra were collected from the tissue. The calibrated spectra were used to construct maps using multivariate statistical techniques. Following measurements, the sections were stained with H&E for histological confirmation.

K-means cluster analysis of the spectra resulted in groups of similar spectra that could be assigned colours to generate pseudo-colour Raman maps of the tissue sections (Fig. 1). Comparing these maps with the H&E staining revealed that Raman spectra of vital tumour tissue differ significantly from spectra of necrotic tissue (Fig. 2). Taking a difference of the averaged spectra from each of these two regions revealed that necrotic tissue was found to consistently contain higher levels of cholesterol and cholesterol-esters. Further studies involving the development of a classification model for non-
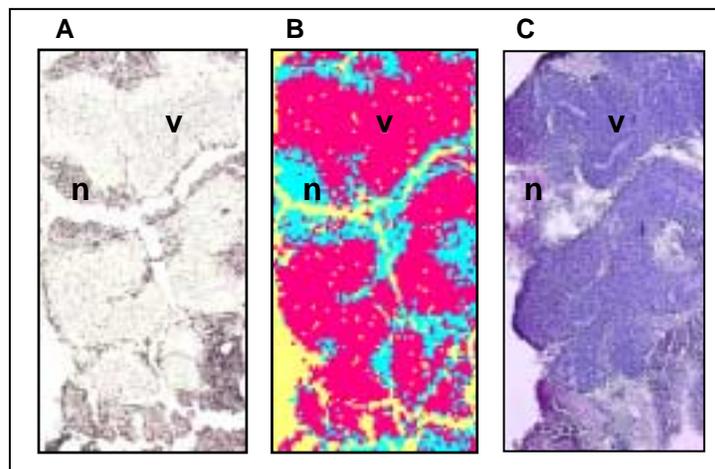
FIG. 1. A: Photomicrograph of unstained (25 μm thick) human glioblastoma cryo-section used in Raman mapping experiment. B: Corresponding psuedo-colour Raman map based on K-means cluster analysis and LDA-model prediction of Raman spectra. Dark-grey pixels denote vital tumour, light-grey pixels denote necrotic tissue. C: Photomicrograph of the same tissue section after H&E staining following Raman measurements. (vital tissue designated with **v** and necrotic tissue with **n**)
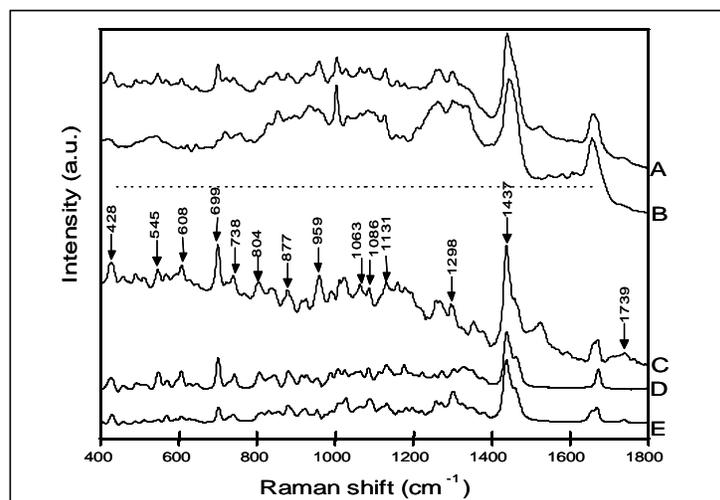
FIG. 2. Averaged Raman spectra from necrotic (A) and vital glioblastoma tissue (B). The difference spectrum (C is A minus B; intensity multiplied by factor of 2) is compared with the Raman spectra of pure cholesterol (D) and cholesterol oleate (E). (a.u., arbitrary units)

subjective discrimination between vital and necrotic tissue based on linear discriminant analysis (LDA) were also performed. Testing the model on 9 independent tissue sections yielded 100 % accuracy rates. This in vitro result indicates that Raman spectra contain biochemical information that can be used to distinguish vital glioblastoma from necrosis suggesting that Raman spectroscopy is a powerful candidate for guidance of stereotactic brain biopsy.

References
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