
10th conference
SPEC
2018
10–15 June 2018
University of Strathclyde
Technology and Innovation Centre
Glasgow
Abstract Book
www.spec2018.com

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FTIR microspectroscopy discriminating skin cancer using tissue sections on glass

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Introduction:

Vibrational spectroscopic methods have shown great promise as diagnostic tools to discriminate cancerous from healthy tissue in a non-subjective manner. However, translation into clinical practice has been relatively slow, mainly due to the expensive and fragile infrared substrates required to perform the measurements. Thus, this study aims to demonstrate the ability of FTIR microspectroscopy discriminating healthy skin from hyperplastic, papilloma and squamous cell carcinoma using standard H&E stained samples placed on glass slides.

Methods:

Cutaneous neoplastic lesions were chemically-induced in Swiss mice using a well-stablished two-stage carcinogenesis protocol. Healthy tissue was collected from animals non-exposed to chemicals and different diseased stages (hyperplastic epidermis, papillomatous lesions and squamous cell carcinoma (SCC)) were obtained by varying the exposure time of the animals to carcinogenenic factors.Tissue sections of 5 µm thickness were obtained from formalin-fixed paraffin-embedded (FFPE), hematoxylin & eosin stained and placed on glass slides. FTIR images were acquired in transmission mode over the spectral range 3000-3800 cm^{-1} with a pixel size of 6.25 × 6.25 µm at a spectral resolution of 4 cm^{-1}. Spectral data were vector normalised and subjected to smoothing using Savitzky–Golay filtering with a polynomial of second order in an eleven point window. Principal components analysis (PCA) was applied and the PC scores were used as input data for linear discriminant analysis (PC-LDA) in a binary classification test. The groups were pairwise compared and the method was validated by leave-one-out cross-validation (LOOCV). All pre-processing and spectral analysis were performed on Matlab® R2017 (MathWorks, Natick, MA, USA).

Results and Discussion:

Figure 1A shows the averaged spectra collected from healthy, hyperplastic epithelium, papilloma and SCC at 3000-3700 cm^{-1} spectral region, which depict bands associated to N–H, O–H and C–H stretching vibrational modes.

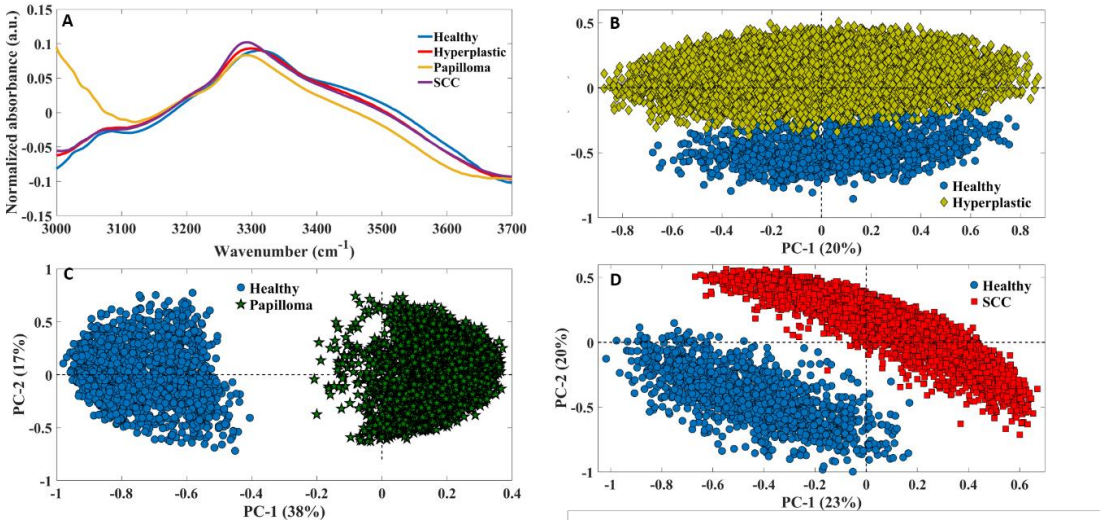

Figure 2

Figures 1B-D display PCA score plots for the pairwise comparisons of heatlhy tissue against hyperplastic epithelium, papilloma and SCC. Data for pairwise comparisons between diseased tissue are not shown.

Sensitivity and specificity values obtained for PC-LDA classification method are shown in Table 1.
Table 1. Statistical measurements obtained for PC-LDA as method for binary classification

	Sensitivity (%)	Specificity (%)
Healthy × Hyperplastic	98	98
Healthy × Papilloma	100	100
Healthy × SCC	99	100
Hyperplastic × Papilloma	100	100
Hyperplastic × SCC	72.5	93.1
Papilloma × SCC	100	100

Despite the low sensitivity value for the pairwise comparison between Hyperplastic × SCC spectral data, excellent classification values and good discrimination were achieved using PC-LDA as a classification method (>90%). Thus, although the "fingerprint" region of the IR (900-1800 cm^{-1}) spectrum is not accessible using glass slides as substrates, we demonstrate that the information contained in the high wavenumber range is sufficient to discriminate normal and malignant tissue using H&E stained samples as received from the pathologist.