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## Methodological development for the improvement of IR spectral histopathology applied to human colon cancer samples

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### 1 Introduction

Developed from the combination of infrared (IR) spectral imaging and chemometrics tools, spectral histopathology has been successfully applied to identify the histological structures of samples and to make an automatic diagnosis. However, sample storage constraints imply the embedding of the samples into different substances, such as paraffin or OCT. It is usual to acquire IR images including areas free of tissue but full of these embedding media.

For proper application of further processing tools such as regression or classification, it is thus necessary to first detect and remove these areas composed of outlier spectra. The spectral variability of these compounds prevents the direct application of data analysis tools to easily retrieve the tissue structures. Several methods have been proposed in the literature to achieve this task, such as spectral quality test [1-2], numerical deparaffinization applied on formalin-fixed paraffin-embedded biopsies from colon lesions [3-4] but all these methods are based on subjective threshold choices or on univariate analysis.

### 2 Material and methods

A total of 14 formalin-fixed, paraffin-embedded tissues sections and 7 unfixed cryosections were obtained from human colon cancer biopsies and xenografted colon carcinoma C57BL mice.

IR spectral images of the tissue sections were recorded using a Perkin Elmer Spectrum Spotlight 300 FTIR Imaging System, on the spectral range  $750\text{ cm}^{-1}$  to  $4000\text{ cm}^{-1}$ , with a spatial resolution of  $6.25\text{ }\mu\text{m}$ , a spectral resolution of  $4\text{ cm}^{-1}$  and averaged over 16 scans per pixel.

We propose an automatic and multivariate analysis method to eliminate completely the contaminated signal with known spectral effects (paraffin or OCT) from colon cancer tissue. Our methodology is based on the pre-processing of the spectral images using Extended Multiplicative Signal Correction (EMSC) incorporating an interference model of paraffin or OCT. Then, the resulting EMSC fitting coefficients from the reference spectrum, from the interference model and from the model error are simultaneously analysed by the KMeans clustering algorithm in order to partition the data into two groups, one for the embedding medium and one for the tissue. Our proposed method has been compared with other existing outlier detection techniques such as spectral band ratio and histogram analysis of the EMSC fitting coefficients and evaluated in terms of the efficiency of paraffin removal and its ability to extract relevant information from the tissue.

### 3 Results and discussion

Compared to spectral band ratio and histogram analysis of the EMSC fitting coefficients methods, our proposed method allows reproducible and automatic selection of the outlier paraffin pixels in the case of a formalin-fixed paraffin-embedded tissue section (Fig.1) and of OCT pixels in the case of a frozen tissue section (Fig.2).

### 4 Conclusion

We propose a new multivariate method to remove automatically outlier spectra of embedding medium (paraffin or OCT) from infrared images of human and mice tissue sections. We have proved that this method is flexible; it can be applied to different tissue preparations. It is conceptually and computationally simpler and quicker than usual used methods. Further analysis tools such as regression or classification can thus now be applied properly on the remaining tissue spectra in order to characterize the tissue histological structures.

### 5 References

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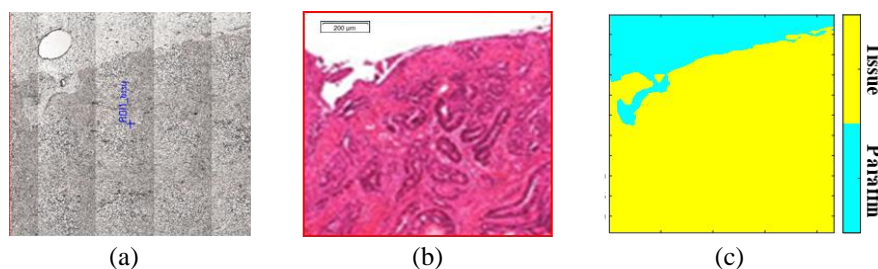


Figure 1 – Outliers removal by our proposed multivariate approach for a formalin-fixed, paraffin-embedded tissue section: (a) unstained tissue section, (b) HE stained image of an adjacent tissue section, (c) Result obtained by the proposed multivariate approach. Scale bars indicate 125  $\mu\text{m}$ .

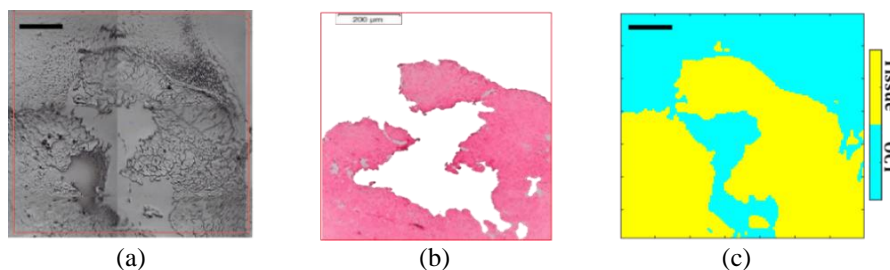


Figure 2 – Outliers removal by our proposed multivariate approach for an OCT-embedded frozen tissue section: (a) unstained tissue section, (b) HE stained image of an adjacent tissue section, (c) Result obtained by the proposed multivariate approach. Scale bars indicate 125  $\mu\text{m}$ .