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Increasing the speed of tumour diagnosis during surgery with selective scanning Raman microscopy

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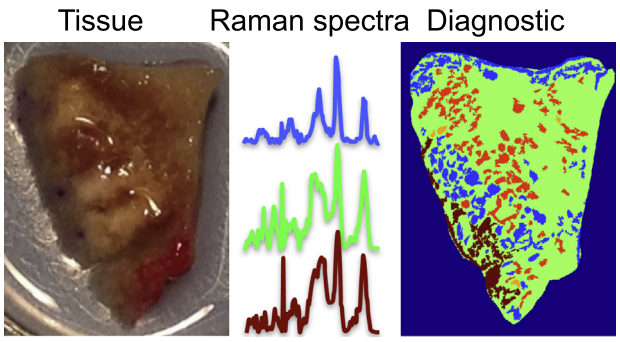
HIGHLIGHTS

- Raman microscopy is an accurate technique for diagnosis of cancer.
- Selective-sampling generates sampling points based on spatial features of sample.
- Selective-sampling reduces the acquisition times compared to raster-scanning.
- Diagnosis of large tissue samples can be completed within 20–30 min.

GRAPHICAL ABSTRACT



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ABSTRACT

One of the main challenges in cancer surgery is ensuring that all tumour cells are removed during surgery, while sparing as much healthy tissue as possible. Histopathology, the gold-standard technique for cancer diagnosis, is often impractical for intra-operative use because of the time-consuming tissue preparation procedures (sectioning and staining). Raman micro-spectroscopy is a powerful technique that can discriminate between tumours and healthy tissues with high accuracy, based entirely on intrinsic chemical differences. However, raster-scanning Raman micro-spectroscopy is a slow imaging technique that typically requires data acquisition times as long as several days for typical tissue samples obtained during surgery (1×1 cm^2) – in particular when high signal-to-noise ratio spectra are required to ensure accurate diagnosis. In this paper we present two techniques based on selective sampling Raman micro-spectroscopy that can overcome these limitations. In selective sampling, information regarding the spatial features of the tissue, either measured by an alternative optical technique or estimated in real-time from the Raman spectra, can be used to drastically reduce the number of Raman spectra required for diagnosis. These sampling strategies allowed diagnosis of basal cell carcinoma in skin tissue samples excised during Mohs micrographic surgery faster than frozen section histopathology, and two orders of magnitude faster than previous techniques based on raster-scanning Raman microscopy. Further development of these techniques may help during cancer surgery by providing a fast and objective way for surgeons to ensure the complete removal of tumour cells while sparing as much healthy tissue as possible.

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Introduction

Raman micro-spectroscopy (RMS) is a powerful technique for measuring the chemical properties of complex biological samples, such as cells and tissues. One application in which this technique has great potential is cancer diagnosis, in particular intra-operative detection and imaging of tumour margins. The availability of fast and objective diagnosis during surgery would spare healthy tissue and reduce unnecessary surgery by checking if all tumour cells have been removed. Histopathology remains the gold-standard for the diagnosis of human cancers; however, this technique has changed little during the last century: it still requires fixation, sectioning and staining of tissues, as well as subjective interpretation of images. These time-consuming and costly procedures make histopathology impractical for use during surgery of most cancer types. Although many optical techniques have been investigated, intra-operative diagnosis of tumors raises huge challenges to any imaging technique: (i) it requires the analysis of wide tissue areas (1–2 cm); (ii) requires high spatial resolution (10–20 μm); (iii) diagnosis times shorter than ~10 min; (iv) should not rely on time consuming tissue preparation stages, such as tissue sectioning and staining and (v) high diagnosis accuracy without requiring subjective interpretation.

Compared to other optical techniques, RMS has significant advantages and meets all the above criteria except speed. RMS has high chemical specificity and multivariate classification models based on these techniques have been developed and used for objective diagnosis of independent tissue samples obtained from new patients. Diagnosis models with simultaneous sensitivity and specificity higher than 90% have been demonstrated for many cancer types, including skin [1–4], breast [5], oesophagus [6], prostate [7–9], lung [10,11] or brain [12]. In addition, the diagnosis can be obtained without any tissue preparation, such as tissue sectioning and staining [e.g. 2, 4, 5].

Nevertheless, achieving objective diagnosis images with high spatial resolution needed long data acquisition and analysis times (~5 h per 1 mm^2) and required tissue sectioning [3,13,14]. Because the tissue layers removed during cancer surgery can be as large as few centimetres square, RMS diagnosis by raster-scanning is impractical for intra-operative use. Several methods have been proposed for increasing the speed of raster-scanning Raman imaging, such as line-scanning and wide-field imaging using selected Raman bands [15]. Line-scanning increases the speed by spreading the laser spot into a line and collect simultaneously full Raman spectra corresponding to the points along the area illuminated. However, the area illuminated is limited by the power of the laser. Increasing the laser spot decreases the power density, thus decreasing the signal to noise ratio of the Raman spectra. Recent studies have demonstrated that an increase of a factor of 10 can realistically be achieved for imaging tissues, compared to point-by-point scanning [16]. However, this factor is still not sufficient for intra-operative use. In wide-field Raman imaging, a large area of the sample is illuminated by the laser and a CCD is used to provide images corresponding to selected discrete Raman bands (selected by suitable filters). This method provides high speed imaging for samples exhibiting intense and isolated Raman bands and low background. However, in the case of tumour diagnosis, this is not the case. The Raman spectra of tissues consist of highly

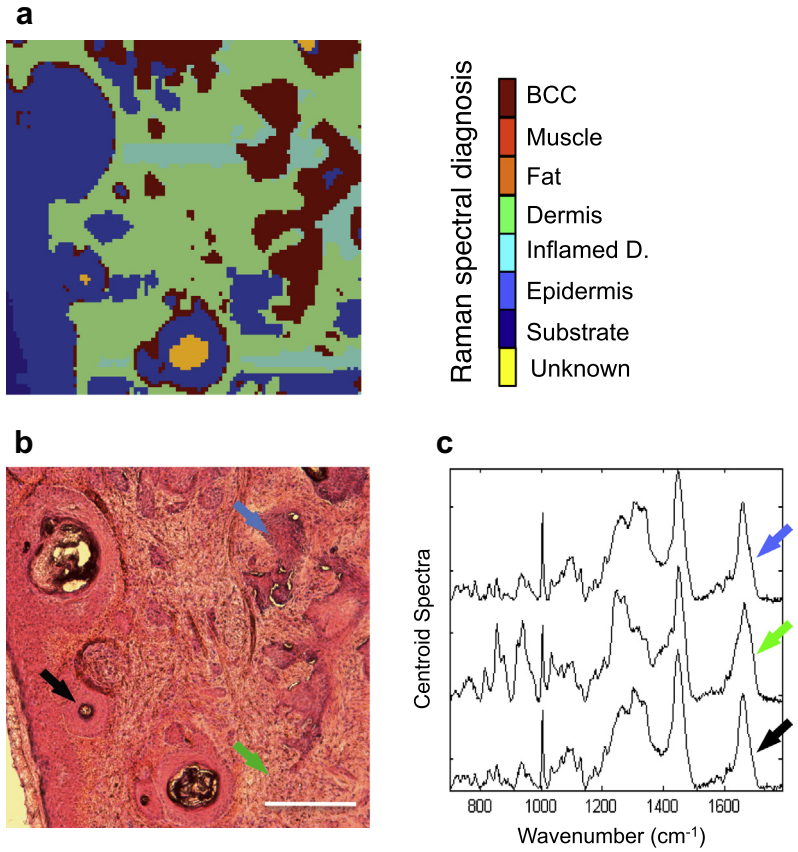

Fig. 1. Diagnosis of basal cell carcinoma (BCC) by raster-scanning Raman spectroscopy. (a) Diagnosis of a skin tissue sample containing BCC, epidermis, hair folicles and dermis (classification model described in [2]). (b) H&E histopathology diagnosis. (c) Examples of centroid Raman spectra of BCC and tissue structures: BCC (blue arrows), dermis (green arrow), hair follicle (black arrow). Scale bar: 250 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)