Raman Spectroscopy for Tumor Diagnosis in Mammary Tissue

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Abstract: This paper demonstrates the potential of Raman spectroscopy in the *ex-vivo* analysis of resected normal and tumor mammary mouse glands, using a commercial confocal Raman microscope. The Raman spectra were acquired with a 785 nm excitation laser at 40 mW and using an integration time of 2 s. The Raman spectra for normal and tumor mouse mammary glands are presented and compared with literature results. The Raman spectrum of normal mammary mouse gland is dominated by lipid signatures. On the other hand, the Raman spectrum of tumor mammary gland is dominated by DNA and protein signatures. The molecular information obtained by using Raman spectroscopy can be fundamental for a more precise and complete diagnosis of tumors, for intraoperative assessment of tumors margins during surgeries and for tumors grade classification.

1 INTRODUCTION

Globally, cancer is a leading cause of death, being the breast cancer one of the most common causes of cancer death, accounting 627 000 cases in 2018 (WHO, 2018). Cancer early diagnosis decreases healthcare costs and increases the probability to save the patient life (WHO, 2017).

Raman spectroscopy is a non-invasive and powerful tool for molecular analysis of disease. This optical technique is based on inelastic scattering of light by molecules. The shift in energy of the scattered light is specific to the vibrational modes of the molecules from a tissue. Thus, the molecular information obtained by using Raman scattering can be used to discriminate diseased and healthy tissues (Stevens, Petterson, Day, & Stone, 2016).

The key idea behind Raman spectroscopy is that a disease causes changes in the molecular composition of a tissue and these changes can be reflected in the tissue Raman spectrum (Choo-Smith *et al.*, 2002). The changes are usually related with variations in the relative concentrations of lipids, proteins, nucleic acids, etc. (Stevens et al., 2016). The Raman spectrum is a molecular fingerprint of the tissue (Kong, Kendall, Stone, & Notingher, 2015).

Majumder *et al.* (Majumder, Keller, Boulos, Kelley, & Mahadevan-Jansen, 2008) performed a study comparing the capabilities of fluorescence, diffuse reflectance and Raman spectroscopy for discrimination of different human breast tissues. Raman spectroscopy showed a superior performance. The Raman spectrum is a set of narrow bands derived from molecular vibrations of a large number of biochemicals presented on the tissues. Thus, with Raman is possible to acquire molecular information with superior detail, comparing with fluorescence or

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diffuse reflectance (Patil, Bosschaart, Keller, Leeuwen, & Mahadevan-Jansen, 2008).

Raman spectroscopy is a non-destructive and chemical-specific technique, being ideal for the detection of microcalcifications in breast tissue during biopsies, for example. Raman spectroscopy can be executed with fiberoptic probes compatible with biopsy needles (Barman *et al.*, 2013).

Frank *et al.* (Frank, Redd, Gansler, & McCreery, 1994) performed one of the first studies about breast cancer diagnosis using Raman spectroscopy. The Raman spectra of normal and cancerous human biopsy samples demonstrated Raman differential features of lipids and carotenoids. In a second work (Frankt & McCreey, 1995), the authors also studied normal and diseased human breast tissues and reported a weaker lipid band and a more pronounced collagen band in diseased samples. Haka *et al.* (Haka *et al.*, 2005) reported the use of Raman spectroscopy to diagnose benign and malignant lesions in *ex-vivo* samples from human breast tissue. They developed a diagnostic algorithm for distinguish cancerous tissues from normal and benign tissues, with 94% sensitivity and 96% specificity. In a second study (Haka *et al.*, 2006), the authors reported the *in-vivo* collection of Raman spectra during partial mastectomy, suggesting the potential of Raman spectroscopy for intraoperative margin assessment (Cui, Zhang, & Yue, 2018).

For breast tissues analysis is also reported the use of an excitation laser of 785 nm and a maximum power of 300 mW (Horsnell *et al.*, 2010; Stevens *et al.*, 2016).

Along this paper, the potential of Raman spectroscopy is shown with the *ex-vivo* analysis of resected normal and tumor mammary mouse glands, using a commercial equipment. Final considerations were also discussed related to the potential of Raman spectroscopy in routine clinical practice, associated with intraoperative assessment of tumors margins during surgeries and also with tumors grade classification.

2 RAMAN SPECTROSCOPY OF BIOLOGICAL TISSUE

2.1 Optical Considerations for Raman Spectroscopy

The Raman system used in this study was the WiTec Alpha300 R confocal Raman microscope, from Witec Ulm Germany. This system combines a highly sensitive confocal microscope with an ultrahigh-throughput spectroscopy system. A high spatial and spectral resolution are achieved by combining several optical components such as filters, lenses, objectives and a sensitive detector.

"Confocal" means "having the same focus" and defines an optical microscope in which the sample is illuminated with a point source. The "image" of this point is detected using a pinhole in the optical fiber that drives the signal to the spectrometer and then to the detector. Thus, only the light originated from the focal plane is detected and contribute to the "image" (Giridhar, Manepalli, & Apparao, 2017).

The WiTec Alpha300 R confocal Raman microscope has a lateral resolution of 200-300 nm, a depth resolution of 500 nm, a spectral resolution of approximately 1 cm^{-1} and a spectrometer with a variety of focal lengths.

The system allows different types of measurements, but it was used for single spectrum acquisition with a 785 nm excitation laser. Figure 1 represents a schematic of the WiTec Alpha300 R confocal Raman microscope system.

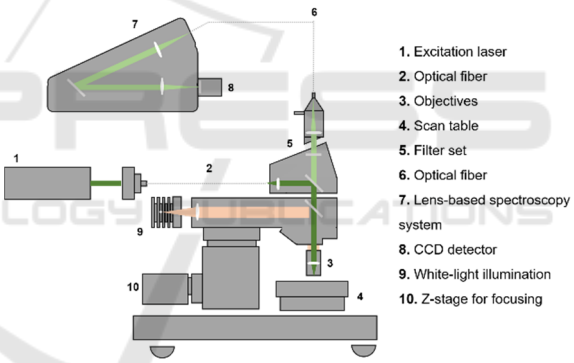

Figure 1: Schematic of the WiTec Alpha300 R confocal Raman microscope, from Witec Ulm Germany.

2.2 Raman Spectroscopy with WiTec Alpha300 R

Raman spectra with WiTec Alpha300 R system were collected from resected mammary mouse glands. The main objective is to analyze the power of Raman scattering to differentiate a normal mammary mouse gland from a tumor mammary mouse gland. The frozen mammary mouse glands (normal and tumor) were sliced and put on a glass slide for the Raman analysis.

The spectra were acquired with the Witec control software, using a 785 nm excitation laser at 40 mW, 2 s of integration time and 100 accumulations. After acquisition, the spectra were baseline corrected

considering a 4th order polynomial fitting and filtered with a filter size of 3th order and a dynamic factor of 1. The baseline correction and filtering were also performed at the Witec control software.

Figure 2 shows the Raman spectra from literature of an *ex-vivo* human breast sample, acquired with a 785 nm diode laser, a long-pass filter to reduce Rayleigh scattering, a CCD camera and other optical components (Patil, Bosschaart, Keller, Leeuwen, & Mahadevan-Jansen, 2008).

Figure 3 shows the acquired Raman spectra, using the WiTec Alpha300 R confocal microscope, for normal and tumor mammary mouse glands. The Raman spectra from the normal and tumor mammary mouse glands are clearly different.

The obtained experimental results (Figure 3) from normal and tumor mammary mouse glands, as expected, presents the signature of lipids and proteins, in the range of 800 to 1800 cm^{-1}, and are very similar to the literature spectra (Figure 2).

Moreover, the experimental spectra (Figure 3) reveal clear differences between the normal and tumor tissue. From Figure 3, it is seen that the normal spectrum is dominated by lipid signatures, namely peaks at approximately 1070, 1300 and 1440 cm^{-1}. On the other hand, the tumor spectrum is dominated by protein (peaks at approximately 1450 and 1660 cm^{-1}) and by DNA (peak at 1336 cm^{-1}) signatures, usually indicative of malignancy (Movasaghi, Rehman, & Rehman, 2007; Patil *et al.*, 2008; Stone, Kendall, Smith, Crowa, & Barra, 2004).

Additionally, it is also seen on Figure 3: i) a strongly decrease in the intensity together with broadening of the modes in the tumor gland; ii) a strongly decrease in intensity of the peaks at approximately 1100 cm^{-1} and the doublet at 1300 cm^{-1} for the tumor tissue; and iii) the peak at 1750 cm^{-1} is absent in the tumor gland. All these effects can be indicative of malignancy.

Finally, comparing Figure 2 and Figure 3 for normal tissues, it can be seen that several ratios between peaks are very similar, for example: between 1440 and 1300 cm^{-1} (ratio of approximately 1.8); and between 1440 and 1650 cm^{-1} (ratio of approximately 2). In the case of the tumor tissues, the ratios between the peaks are not very similar, which can be related with the degree of malignancy of each sample (for Figure 2 and Figure 3). Despite this differences, the most important feature is the clearly difference between the Raman spectra of the normal and tumor tissues, for both figures.

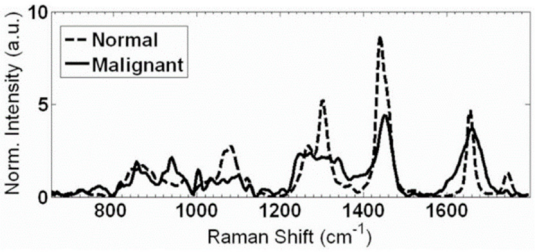

Figure 2: Raman spectra of an *ex-vivo* human breast sample, considering a 785 nm excitation diode laser (Patil et al., 2008).

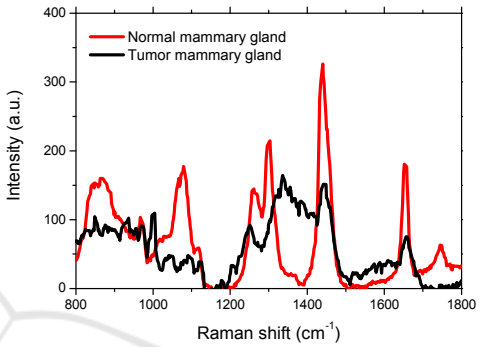

Figure 3: Raman spectra of resected normal mammary gland and tumor mammary gland of a mouse. The spectra were acquired with the Witec Alpha300 R system, with an integration time of 2 s and 100 accumulations.

3 CONCLUSIONS AND FUTURE GUIDELINES

The *ex-vivo* analysis of biological tissues using a commercial Raman equipment was presented. The results proved the potential of Raman spectroscopy in differentiate the normal and tumor tissues, using a 785 nm laser with 40 mW total power, an integration time of 2 s and 100 accumulations.

In Raman spectroscopy is crucial to take into account the maximum permissible light exposure and avoid the tissue temperature increase, relevant to minimize tissue damage. This parameter is even more important during *in-vivo* spectra acquisition, to ensure patient comfort.

These preliminary tests with *ex-vivo* analysis of resected normal and tumor mammary mouse glands, prove the potential of the used Raman microscope to differentiate normal and tumor tissues: the normal mammary gland spectrum is dominated by lipid signatures and the tumor mammary gland spectrum is dominated by DNA and protein signatures. These promising results open the future possibility to use

this commercial equipment to other type of analysis, for example, the analysis and differentiation of normal and malignant human tissues, for a variety of applications.

A great area of expansion of the Raman spectroscopy technique is the use of the high Raman molecular specificity for intraoperative assessment of tumors margins during surgeries, in order to reduce the re-operation procedures. If the patient re-operation is avoided, the healthcare costs and patient anxiety are also reduced. For this application, it is required the implementation of a portable Raman system to be use in the operation rooms.

Finally, the application of Raman spectroscopy in routine clinical practice can also have a huge impact in grade classification, essential for diagnosis and prognosis of diseases, i. e., to predict how quickly a tumor will grow and spread. This knowledge is crucial for planning the best treatment for the patient, increasing the probability of a success treatment and cure. Again, for this last described application, it is desirable the implementation of a portable Raman system or the adaptation of a Raman probe to the existing methods of diseases diagnosis in routine clinical practice.

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