Prospects for the diagnosis of breast cancer using transmission Raman spectroscopy

P. Matousek
Central Laser Facility, STFC, Rutherford Appleton Laboratory, Harwell Science and Innovation Campus, Didcot, Oxfordshire, OX11 0QX, UK

N. Stone
Biophotonics Research Group, Gloucestershire Royal Hospital, Great Western Road, Gloucester, GL1 3NN, UK

Main contact email address **P.Matousek@rl.ac.uk**

Introduction

The presence of calcifications on mammography is a feature of particular diagnostic significance, as sometimes this may be the only marker of a malignant breast lesion. Mammography can detect microcalcifications not detectable by physical examination but it has no definitive criteria for classifying benign and malignant calcifications. It has in fact been found that 70-90% of mammographically detected lesions are found to be benign upon needle biopsy [1].

Microcalcifications can be divided into two types; type I, which consist of calcium oxalate dihydrate (cod), and type II deposits, which are composed of calcium phosphates, mainly calcium hydroxyapatite (hap). The types are thought to correlate with disease [2]. Calcium oxalate crystals are mainly found in benign ductal cysts and rarely found in carcinoma [3], whereas calcium hydroxyapatite deposits are found both in carcinoma and in benign breast tissue [2]. Furthermore, it has been shown that type II calcifications found in benign ducts appear spectroscopically distinct from type II calcifications found in malignant lesions. The benign hydroxyapatite calcifications have been shown to contain larger concentrations of calcium carbonate [2]. As there are differences in chemistry between these two types of calcification, significant insight may be gained by using vibrational spectroscopy to probe their biochemical composition.

Raman spectroscopy holds a particular promise for detecting these non-invasively for its high chemical specificity and compatibility with water containing samples. However, until recently, it could be applied to probing tissue in depths of only several hundred micrometers due to the diffusely scattering nature of tissue. Here we demonstrate how a transmission Raman geometry [4,5,6] can enable detailed spectroscopic information relating to the interior of the sample to be obtained (see figure 1).

Our earlier research has demonstrated the ability of the Raman Kerr gating approach to recover the Raman spectra of the calcified materials non-invasively from behind 0.9 mm of chicken breast tissue [7]. This depth was subsequently increased to 8.7 mm using Spatially Offset Raman Spectroscopy (SORS) [8]. Here we report the reaching of a new milestone, the recovery of chemically specific information on calcified material through a 16 mm thick slab of breast tissue using transmission Raman. The experiments were performed on chicken breast tissue phantoms chosen to permit direct comparison with our earlier measurements. The full account of this work is given in Ref. [9].

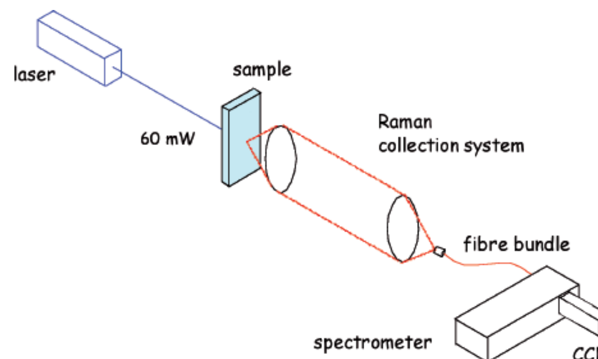

Figure 1. Schematic of the experimental setup.

Experimental section

The Raman spectra in this work were obtained using a Raman apparatus as follows. The probe beam was generated using a temperature stabilised diode laser for Raman spectroscopy and operating at 827 nm (60 mW). The laser spot diameter before the sample was ~4 mm. The beam was spectrally purified by removing any residual amplified spontaneous emission components from its spectrum using two 830 nm bandpass filters (Semrock).

The Raman light was collected using a standard 50 mm diameter uncoated fused silica lens with a focal length of 60 mm. The second lens, identical to the first, was then used to image, with magnification 1:1, the sample interaction zone onto the front face of a fibre probe. The fibre probe comprised of 7 fibres tightly packed at the centre of the probe and 26 fibres distributed on a ring of 3 mm radius. The fibres were made of silica with a core diameter of 200 mm and a numerical aperture of 0.37. The bundle was custom made by C Technologies Inc. The Raman light was propagated through the fibre systems of length ~1 m to the linear fibre end oriented vertically and placed in the input image plane of a Kaiser $f^{\#} = 1.4$ NIR spectrograph. The Raman spectra were collected using a NIR back-illuminated deep-depletion CCD camera (Andor Technology, DU420A-BR-DD, 1024 × 256 pixels). The acquisition time was 100 s for each spectrum.

Results and discussion

Figure 2 illustrates the experimental result of probing a 16 mm slab of chicken tissue with a thin layer (100-300 µm) calcified material smeared in the middle of the slab. Despite the large thickness of tissue the identifiable Raman spectra of individual calcified compounds have been recovered by subtracting signal with and without calcifications. The

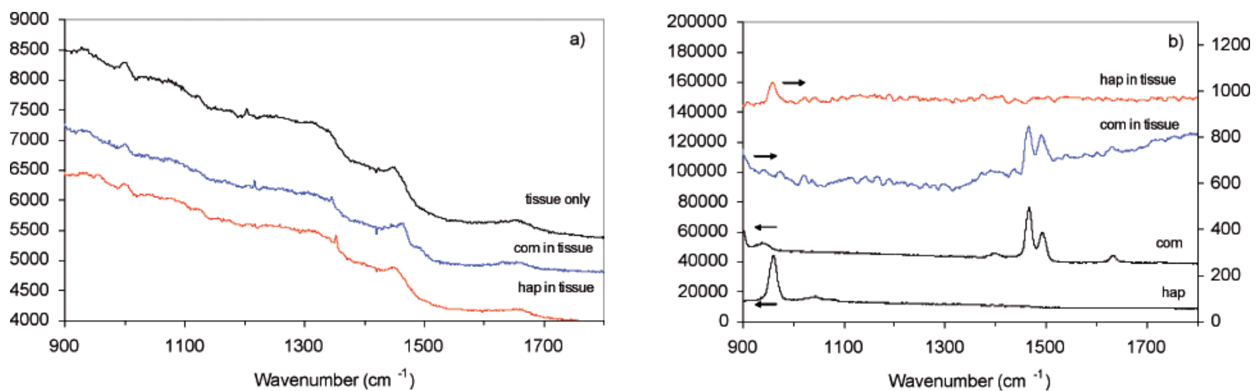

Figure 2: a) Raman spectra of calcified material contained within a 100-300 µm thick powder layer smeared in the middle of the tissue slab of an overall tissue thickness of 16 mm ('hap in tissue', 'com in tissue') along with the spectra of tissue only measured from the same slab in the absence of the powder layer ('tissue only'), b) The Raman spectra of calcified materials obtained by subtracting raw Raman spectra of tissue only from those of tissue containing calcified material along with the pure Raman spectra of individual calcified materials. The acquisition time was 100 s for each spectrum. The spectra are offset for clarity.

attained depth of 16 mm is approaching the lower range of the clinically relevant depths required for the deployment of the technique in clinical environment for probing human breast tissue *in vivo*. In terms of sensitivity to the clinically relevant concentration of calcifications we estimate that the sensitivity of this measurement was around two orders of magnitude below the clinically relevant level [9]. Consequently, two orders higher signal-to-noise ratio would be required to detect typical calcification cluster and identify its chemical composition.

Such large improvement could be potentially attained by enhancing the illumination area and boosting laser power. An additional improvement is possible by further optimising the collection system to collect Raman signals effectively from larger areas. This can be achieved using wider spectrograph slits enabling the coupling of a larger number of fibres into the detection system while maintaining the spectral resolution. This can be accomplished, for example, by using larger dispersion gratings in the spectrograph and using a consequently narrower spectral region centred only on the spectral region of interest and, or in conjunction with, recently developed coded aperture spectroscopy [10] permitting operations with wider spectrograph slits while maintaining the original spectral resolution. These large technological advancements obviously need further experimental validations and this will be the focus of our future studies.

Conclusions

We have demonstrated the ability of detecting and identifying the chemical composition of calcified material within a depth of 16 mm thick chicken tissue using transmission Raman spectroscopy. This is the highest depth reported so far and demonstrates the potential of the concept for reaching, with further improvements, the clinically relevant range required for potential application *in vivo*. The technique if validated could be ultimately used in conjunction with the existing mammography or ultrasound techniques to enhance their diagnostic potential.

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References

1. A. S. Haka, K. E. Shafer-Peltier, M. Fitzmaurice, J. Crowe, R .R. Dasari and M. S. Feld, *Proc. Natl. Acad. Sci. USA* **102**, 12371-12376 (2005).
2. A. S. Haka, K. E. Shafer-Peltier, M. Fitzmaurice, J. Crowe, R. R. Dasari and M. S. Feld, *Cancer Res.* **62**, 5375-5380 (2002).
3. Lenkinski, R.E., Ahmed, M., Zaheer, A., Frangioni, J.V., Goldberg, S.N., *Academic Radiology,* **10**, 1159-1164 (2003).
4. M.L. Myrick, S.M. Angel and R. Desiderio, *Appl. Optics* **29**, 1333-1344 (1990).
5. P. Matousek and A.W. Parker, *J. Raman Spectrosc.* **38**, 563 (2007).
6. P. Matousek and A.W. Parker, *Appl. Spectrosc.* **60**, 1353 (2006).
7. R. Baker, P. Matousek, K. Ronayne, A. W. Parker, K. Rogers and N. Stone, *Analyst* **132**, 48 (2007).
8. N. Stone, R. Baker, K. Rogers, A. W. Parker and P. Matousek, "Future possibilities in the diagnosis of breast cancer by subsurface probing of calcifications with surface offset Raman spectroscopy (SORS)," *Analyst*, submitted, 2006.
9. P. Matousek and N. Stone, *J. Biomed. Optics* **12**, 024008 (2007).
10. S. T. McCain, M. E. Gehm, Y. Wang, N. P. Pitsianis and D. J. Brady, *Appl. Spectrosc.* **60**, 663-671 (2006).