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This is a published version of a paper published in *The Analyst*.

Citation for the published paper:

Nyberg, M., Ramser, K., Lindahl, O. (2013)

"Optical fibre probe NIR Raman measurements in ambient light and in combination with a tactile resonance sensor for possible cancer detection"

The Analyst, 138(14): 4029-4034

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Optical fibre probe NIR Raman measurements in ambient light and in combination with a tactile resonance sensor for possible cancer detection

Cite this: *Analyst*, 2013, **138**, 4029

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Received 31st January 2013

Accepted 8th May 2013

DOI: 10.1039/c3an00243h

www.rsc.org/analyst

First measurements on a combined instrument with a thin fibre optic Raman probe mounted inside a hollow tactile resonance sensor have been performed in ambient light on porcine tissue. The ambient fluorescent light was removed successfully from the spectra. The stiffness and the biomolecular composition of the tissue were analysed.

Introduction

Prostate cancer (PCa) is a high prevalence disease among men. It was estimated that 89 300 men died from PCa in Europe in 2008 and 382 300 men were diagnosed with PCa.¹ In 2010, the expected number of PCa diagnoses in the US was 217 730 cases with 32 050 lethal outcomes.² Radical prostatectomy, *i.e.* surgical removal of the prostate, is a common choice of PCa treatment.³ A positive surgical margin, *i.e.* tumour cells at the surgical surface, indicates an elevated risk of PCa recurrence.⁴ The methods used presently, such as online frozen section histopathology, lack in sensitivity.⁵ Permanent section analysis is too labour intensive and time consuming to be used during surgery and hence, complementary methods that prevent positive surgical margins directly would be valuable.^{4,5}

Raman spectroscopy and the tactile resonance method (TRM) have each shown to be able to discern and localise PCa in tissue.^{6,7} Both modalities have their own benefits and drawbacks. Our idea is to reduce the drawbacks and keep the benefits of each method by combining the two modalities into one integrated probe. Previous studies have shown that the datasets of Raman spectroscopy and TRM can be combined and that they complement each other.^{8,9}

Raman spectroscopy provides detailed information of the biochemical tissue content and can be used to identify and grade cancerous prostate cells.^{6,10–12} Raman scattering is a weak optical phenomenon; the acquired tissue spectrum might easily be drowned by ambient light and is therefore usually

performed in the dark. In a situation such as the surgical room, ambient light and short measurement times are desired prerequisites. Another drawback is that to investigate the complete surgical margin many measurements are required as the measurement area for optical fibre Raman spectroscopy is small, *i.e.* around $<1\text{ mm}^2$.^{8,13} As a further consequence, the sample will be irradiated with laser light for long times and there is a risk of creating photo-induced effects or drying out of the sample.

The TRM provides non-invasive and fast information on tissue stiffness and has been used for numerous medical applications.¹⁴ The principle of the TRM has been described in previous studies.^{14,15} In brief, the tissue stiffness is proportional to the TRM parameter $dF/d\Delta f$.¹⁶ For instance, PCa tumours are often felt as harder lumps in the prostate and stiffer tissue indicates malignancy. However, the TRM cannot discern between malignant stiff tissue and benign stiff tissue such as prostate stones and stroma.

By combining the two, detailed Raman spectroscopy can be performed at points of interest, where the TRM has detected a suspicious area. The result is likely to be a fast, reliable, and minimally invasive method for detection of prostate cancer.

Here we present the first prototype of a combined probe, where a thin Raman probe was integrated inside a hollow tactile resonance element. The aim of this study was to develop and evaluate methods for performing Raman spectroscopy in ambient light and to investigate the functionality of the prototype probe for combined Raman and tactile resonance measurements.

Material and methods

Combined Raman spectroscopy and tactile probe

The TRM sensor consisted of a cylindrical ceramic PZT piezoelectric element (5 mm outer diameter, 3 mm inner diameter, 15 mm length), a hemispherical plastic tip (2.5 mm radius) covered by a thin hemispherical glass window (microscope

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cover slip Nr 1, Menzel-Gläser, Portsmouth, NH, USA), pick-up and driving electronics (P&M Co., Ltd., Aizuwakamatsu, Fukushima, Japan), and a strain gauge force transducer. The unloaded TRM sensor frequency was 95.58 kHz.

A 0.8 mm optical fibre NIR Raman probe (Machida Endoscope Co., Tokyo, Japan) was affixed inside the hollow TRM probe. The optical fibres of the probe consist of pure fused silica.¹³ The laser light is delivered by one central fibre, and the scattered laser light is collected by eight surrounding fibres.¹³ The RS probe ends with a 10 cm long steel pipe, with a lens and a low pass filter for the collection fibres and a band pass filter for the excitation fibre as described by Komachi *et al.*¹³ The Raman probe was connected to a RXN1 spectroscope equipped with a 785 nm cw Invictus laser diode (Kaiser Optical Systems, Ann Arbor, MI, USA).

The combined probe was mounted on a XYZ-translation stage (NRT150P1, three NRT100, BSC103, Thorlabs, Newton, NJ USA) together with a CMOS camera (SPC230NC, Philips, Amsterdam, Netherlands) (Fig. 1). The experimental setup was controlled by in-house developed software (LabVIEW™ 8.6, National Instruments, Austin, TX, USA).

The ambient light was provided by two fluorescent tubes (LUMILUX FH 28W/830, OSRAM GmbH, Munich, Germany) reflected by the white inner ceiling. The intensity of the ambient light was measured close to the sensor probe and also beside

the experimental setup using a digital light meter (DVM1300 – Luxmeter, Velleman, Gavere, Belgium).

Silicone and porcine samples

Silicone samples were used for calibration and to evaluate the reproducibility of the TRM part of the combined sensor. They have previously been used to study the reproducibility of tactile sensors.^{7,17} Six silicone samples (Wacker SilGel 612, Wacker-Chemie, Munich, Germany) with penetration values (DIN ISO 2137, hollow cone 150 g) ranging from $51 \text{ mm} \times 10^{-1}$ to $235 \text{ mm} \times 10^{-1}$ had been prepared in Petri dishes (87 mm diameter, 13 mm height).⁷ The stiffest silicone sample had been prepared in a smaller Petri dish (54 mm diameter, 13 mm height). The Petri dishes were completely filled with silicone.

Porcine abdominal tissue was chosen as model tissue in this study as it consists of visible discernible regions of fat and muscle tissue. Porcine abdominal fat and muscle tissue were expected to be discernible with both TRM and Raman spectroscopy.⁸ Five porcine samples were prepared from one single package of porcine abdominal tissue acquired from the local grocery store. A flat, rectangular sample (approximately $30 \times 40 \times 14 \text{ mm}$) consisting of both fat and muscle tissue was cut before each measurement series. The cut porcine sample was brushed with saline solution (E404 PBS, Amresco, OH, USA) approximately every fifth minute during the time of measurement to prevent dehydration.

Measurement procedure on silicone samples

Six TRM measurements were performed in the centre of each Petri dish to investigate the reproducibility of the TRM sensor. The tactile sensor impression depth was 1 mm and the impression speed was 0.2 mm per second. The tactile sensor tip was raised an additional distance between impressions and a recovery period of one minute was kept between consecutive measurements.

Measurement procedure on porcine samples

The intensity of the ambient light was measured before the start of each measurement series. Furthermore, two sets of reference Raman spectra were acquired without the presence of porcine sample. The Raman probe tip was positioned 10 cm above a black non-reflective paper. The first set of spectra consisted of three Raman spectra of the ambient light and the glass window of the tactile sensor tip. The second set consisted of three Raman spectra of the tactile sensor tip without the presence of ambient light. The Raman spectra were acquired during 10 s integration time and with a laser output power of $61.7 \pm 5.6 \text{ mW}$, as measured at the tactile sensor tip.

A 2×3 measurement grid was chosen for each porcine sample to contain an equal number of fat and muscle points. Three TRM measurements and three Raman spectra were acquired at each point before the probe was moved to the next point in the grid. The TRM measurements were acquired with the same parameter choices and refractory period as for the silicone samples. The Raman spectra were acquired immediately after the last TRM measurement, with the same settings as

Fig. 1 Combined probe setup: (a) CMOS camera, (b) strain gauge force transducer, (c) optical fibre of Raman probe, and (d) TRM sensor tip. (Inset) Raman probe tip and TRM tip, (e) Raman probe, (f) plastic, (g) hemispherical glass, and (h) maximum tissue level at 1 mm impression depth.

the reference Raman spectra. The tactile sensor tip was kept impressed 1 mm into the tissue during Raman spectroscopy. The tissue type of each measurement point was determined by visual inspection to fat or muscle.

Processing of TRM data

The TRM data consisted of recorded impression force and frequency of the tactile element. The force signal was calibrated against an electronic balance (HT-600H, Elastocon, Borås, Sweden). The tactile frequency shift Δf was calculated as the difference between the frequency of the unloaded tactile sensor and the frequency during impression. The stiffness parameter $dF/d\Delta f$ was calculated by a linear regression analysis of ten data points close to the maximum impression depth.

Processing of Raman spectra

The Raman spectra was analysed in the 900–1785 cm^{-1} wavenumber region. Average spectra ($n = 3$) were calculated for each measurement situation.

The spectral locations of the peaks induced by the ambient light were detected. This was done by first subtracting the Raman spectra of the tactile sensor tip obtained in the dark from the one obtained in ambient light. The high peaks were then detected by a threshold, arbitrarily set higher than the base line. The ambient light peaks were then cut out of all Raman spectra by setting their intensities to the nearest neighbour's value.

The remaining spectral contribution of the ambient light and the tactile sensor tip was then subtracted from the Raman spectra using the SDVM-algorithm suggested by Loethen *et al.*¹⁸

The spectra were then lightly smoothed using Eilers' algorithm for Whittaker smoothing ($d = 2$, $L = 10$),¹⁹ followed by a polynomial base line reduction.²⁰ The final Raman spectra were vector normalised.

Statistical methods

The average stiffness and standard deviation of the TRM measurements were calculated within each group, as grouped by the PCA and clustering of the Raman spectra. The non-parametric Kruskal–Wallis test was used to test the difference in stiffness between two groups. Statistical test results with $p < 0.05$ were considered statistically significant.

The processed Raman spectra were analysed using PCA. The spectra were divided into 2 to 10 groups according to their PC scores by a clustering algorithm using Ward's linkage based on the Euclidian distances. Average spectra within the resulting groups were then calculated. The final spectra corresponding to each group were assigned to molecular vibrations.^{11,21}

Results

TRM silicone sample measurements

The TRM sensor gave similar results during repeated measurements on silicone samples (Fig. 2). Stiffness values corresponding to the sample penetration values are given in Table 1.

Fig. 2 TRM force and frequency shift readings of a silicone sample ($n = 6$). The stiffness value was evaluated at the cross-marked part.

Table 1 Silicone sample's average TRM stiffness values and standard deviations ($n = 6$). The silicone with penetration value $51 \text{ mm} \times 10^{-1}$ is the hardest

Penetration value ($\text{mm} \times 10^{-1}$)	Stiffness ($10^{-5} \text{ N Hz}^{-1}$)
51	9.23 ± 0.31
84	2.05 ± 0.09
117	8.75 ± 1.03
168	1.93 ± 0.37
192	0.69 ± 0.19
235	0.30 ± 0.06

Raman and TRM porcine sample measurements

Both Raman spectroscopy and TRM measurements were performed at every measurement grid point within 90 min from start of each measurement series. One measurement was excluded from further analysis as the TRM failed to obtain a reliable reading at that single measurement point.

Three high intensity peaks induced by the ambient fluorescent light were located in the analysed wavenumber regions 985–993 cm^{-1} , 1343–1353 cm^{-1} , and 1537–1547 cm^{-1} (Fig. 3a). These peaks could be automatically detected and replaced by neighbouring values. This allowed the SDVM-algorithm to remove the remaining unwanted contributions.

PCA of the processed Raman spectra revealed that the first PC explained 76% of the total variance whereas the first ten PCs explained 99%. Clustering of the PC scores in increasing number of groups indicated that one group of spectra was closely related within the group. The other Raman spectra showed similarities in peaks but differences in regions without peaks and were divided accordingly (Fig. 3a). The spectra were finally divided into two groups on the basis of peak differences. Average spectra of the two categories (Fig. 3b) showed marked differences in the following peaks:

(1) Spectrum A had notable higher intensities at 1062 cm^{-1} , 1297 cm^{-1} , and 1440 cm^{-1} . These peaks were assigned to: skeletal C–C stretch lipids/ n (C–C) trans; CH_2 deformation (lipid)/palmitic acid; and fatty acid band. Spectrum A also had higher peaks at 1127 cm^{-1} , 1655 cm^{-1} , and 1735 cm^{-1} assigned

Fig. 3 (a) Processed Raman spectra ($n = 30$) of groups A and B. Down scaled raw spectrum, black, shown for comparison. Dotted lines mark the location of ambient light peaks. (b) Average spectra of group A ($n = 16$) and B ($n = 14$). The inset is the difference spectrum.

to: C–C stretching mode of lipids; amide I (C=O stretching mode of proteins)/C=C lipid stretch; and esters/lipids.

(2) Spectrum B had higher intensities in 1002 cm^{-1} , 1154 cm^{-1} , and 1327 cm^{-1} . These peaks were assigned to: phenylalanine; C–C (and C–N) stretching of proteins/glycogen; and collagen/nucleic acid/phospholipids, respectively.

The result from the TRM measurements showed that group A was significantly stiffer than group B ($p < 0.000011$). The

Fig. 4 Group A ($n = 15$) and B ($n = 14$) average stiffness and standard deviations from the TRM measurements.

stiffness values of the points classified as group B had less variation, $(1.65 \pm 0.70) \times 10^{-5} \text{ N Hz}^{-1}$, than the stiffness values of the points in group A, $(9.74 \pm 7.2) \times 10^{-5} \text{ N Hz}^{-1}$ (Fig. 4).

Discussion

Previous studies have shown that the datasets from Raman spectroscopy and TRM give complementary information for tissue characterisation.^{8,9} We have taken a new step forward by integrating both methods into one probe. We have also developed and evaluated a method to perform Raman spectroscopy in ambient light.

TRM measurements

The stiffness parameter was calculated near the maximum impression depth to ensure that the stiffness was evaluated at the same depth for all the TRM measurements. The stiffness can be evaluated at an arbitrary impression depth, but fluctuations in the force readings are more pronounced at small impressions.

The TRM measurements on silicone samples indicated that this particular TRM sensor had less resolution for soft samples. This may explain the lower variation of the stiffness measurements within the softer group. The overall repeatability of the TRM sensor was good. The standard deviations of the calculated stiffness for the silicone samples were low (Table 1), except for the sample with penetration value $117 \text{ mm} \times 10^{-1}$ for which the stiffness value also seemed out of place. The frequency shift for this sample was not completely continuous.

The TRM sensor tip was partly covered by a thin glass sheet. The glass had been heated and shaped into a hemisphere. The impression depth was chosen to be 1 mm to allow the sensor tip to maximise the contact area while avoiding pressing the sensor tip deep enough for the sample to reach the glass edge (Fig. 1).

Although the range and resolution of this particular TRM sensor could be improved by changing its dimensions, it is evident that the hollow shape used here allowed for a fibre optical Raman spectroscopy probe to be integrated inside of the TRM sensor. For comparison with our previous study,⁸ an arbitrary threshold was set to divide the two groups according to their stiffness values. By doing so, all but two measurement points were classified correctly as fat or muscle.

Raman spectroscopy

While clustering the processed Raman spectra according to their PC scores in increasing number of groups it became evident that one set of spectra quickly became, and stayed, separated in one group. This indicated that the biochemical content of the measured points could be classified to mainly belong to two different categories (Fig. 3a). The two categories aligned with the tissue types are classified by visual inspection. Group A and B corresponded to fat and muscle, respectively, in all but one of the measured points. The major spectral peaks of group A were assigned to lipids and fatty acids, which indicates fat. Correspondingly, the spectral peaks of group B are assigned to constituents present in porcine muscle tissue. The Raman

spectra assigned as fat showed less variance than the spectra of muscle, which is not surprising since lipids are strong Raman scatterers.

The high-intensity peaks induced by the fluorescent ambient light were suppressed by identifying their locations with a threshold value and by replacing the intensity value with the neighbouring values. This enabled application of the SDVM-algorithm for further treatment of the spectra. The removal of the peaks had the drawback of removing biochemical information on the tissue in these regions. Stone *et al.* have performed Raman spectroscopy of normal and pathological prostate tissue. In the regions that were cut off here it is only the CH₃CH₂-wagging mode of collagen at 1335–1345 cm⁻¹ that overlaps with Stone *et al.*'s analysis on prostate tissue.¹¹ Hence, we conclude that the analysis did not influence the information excessively. However, the Raman spectra of muscle were noticeably influenced by fluctuations in the wavenumber regions 1322–1413 cm⁻¹ and 1531–1614 cm⁻¹. It can be noted that these wavenumber regions were in the region where the intensity peaks induced by the ambient peaks had been cut off (Fig. 3a). Perhaps these variations are remnants from the removed peaks. However, excluding these regions from the PCA and clustering analysis did not influence the result of the analysis on the model system used here. We recommend being observant about information from these wavenumber regions when using the proposed method in clinical studies. Changing the ambient light might be an option if such a particular region happens to be considered important for classification. It has previously been shown that the impacts on spectral features caused by the ambient light are different between different types of light sources.²² Incandescent light, *e.g.* gives a smooth and continuous spectral contribution without the sharp peaks induced by fluorescent light. It contributes continuously over all frequencies and easily floods the Raman spectrometer to such an extent that it becomes oversaturated. Although the fluorescent light gives rise to sharp peaks with high intensities, it is preferred as the intensities are much lower between the peaks.

The integration of the Raman probe inside the TRM sensor also had the benefit of keeping the Raman probe tip at a constant distance from the sample surface, which is good as the fibre optic probe has an optimal focal distance, which affects the overall spectral intensity.

The results of this study indicate that Raman spectroscopy and the TRM method could be combined into one single probe. The benefits of each modality, *e.g.* the specificity of the Raman spectroscopy and the speed of the TRM sensor, are preserved. The disadvantages, *e.g.* the comparably long measurement time of the Raman spectroscopy and the non-specificity of the TRM method, are minimised. The methods presented in this study for performing Raman spectroscopy in ambient light indicate that the combined probe could be used in a clinical situation *e.g.* a surgical room during radical prostatectomy.

Conclusions

Here we present for the first time successful experiments with a prototype probe where Raman spectroscopy has been combined

with the TRM. Both Raman spectroscopy and TRM measurements could be performed using an integrated probe and gave good results using a simple model system, *i.e.* fat and muscle tissue from porcine abdominal tissue could be distinguished.

Raman measurements in an environment with the presence of ambient light could be performed by acquiring reference spectra and removing the spectral contribution of the ambient light. Although care has to be taken in some few regions, depending on the spectral features of the ambient light.

The results indicate that an integrated probe combining Raman spectroscopy and TRM could be used for tissue characterisation, *e.g.* during radical prostatectomy. First clinical tests on prostate tissue will be performed soon.

List of abbreviation

Δf	Resonance frequency shift
F	Impression force
NIR	Near infrared
PC	Principal component
PCA	Principal component analysis
PCa	Prostate cancer
TRM	Tactile resonance method.

Acknowledgements

This work was supported by the EU Structural fund, Objective 2, Norra Norrland, and the Kempe foundation.

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