Clinical superficial Raman probe aimed for epithelial tumor detection: Phantom model results

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Abstract: A novel clinical Raman probe for sampling superficial tissue to improve in vivo detection of epithelial malignancies is compared to a non-superficial probe regarding depth response function and signal-to-noise ratio. Depth response measurements were performed in a phantom tissue model consisting of a polyethylene terephthalate disc in an 20%-Intralipid® solution. Sampling ranges of 0-200 and 0-300 μm were obtained for the superficial and non-superficial probe, respectively. The mean signal-to-noise ratio of the superficial probe increased by a factor of 2 compared with the non-superficial probe. This newly developed superficial Raman probe is expected to improve epithelial cancer detection in vivo.

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References and links

1. M. S. Bergholt, W. Zheng, K. Lin, K. Y. Ho, M. Teh, K. G. Yeoh, J. B. Yan So, and Z. Huang, “In vivo diagnosis of gastric cancer using Raman endoscopy and ant colony optimization techniques,” Int. J. Cancer 128(11), 2673–2680 (2011).
2. P. Crow, J. S. Uff, J. A. Farmer, M. P. Wright, and N. Stone, “The use of Raman spectroscopy to identify and characterize transitional cell carcinoma in vitro,” BJU Int. 93(9), 1232–1236 (2004).
3. R. O. Draga, M. C. Grimbergen, P. L. Vijverberg, C. F. van Swol, T. G. Jonges, J. A. Kummer, and J. L. Ruud Bosch, “In vivo bladder cancer diagnosis by high-volume Raman spectroscopy,” Anal. Chem. 82(14), 5993–5999 (2010).
4. E. M. Kanter, E. Vargis, S. Majumder, M. D. Keller, E. Woeste, G. G. Rao, and A. Mahadevan-Jansen, “Application of Raman spectroscopy for cervical dysplasia diagnosis,” J. Biophotonics 2(1-2), 81–90 (2009).
5. A. Molekovskey, L. M. Song, M. G. Shim, N. E. Marcon, and B. C. Wilson, “Diagnostic potential of near-infrared Raman spectroscopy in the colon: differentiating adenomatous from hyperplastic polyps,” Gastrointest. Endosc. 57(3), 396–402 (2003).
6. G. Shetty, C. Kendall, N. Shepherd, N. Stone, and H. Barr, “Raman spectroscopy: elucidation of biochemical changes in carcinogenesis of oesophagus,” Br. J. Cancer 94(10), 1460–1464 (2006).
7. J. C. Day, R. Bennett, B. Smith, C. Kendall, J. Hutchings, G. M. Meaden, C. Born, S. Yu, and N. Stone, “A miniature confocal Raman probe for endoscopic use,” Phys. Med. Biol. 54(23), 7077–7087 (2009).
8. Y. Hattori, Y. Komachi, T. Asakura, T. Shimosegawa, G. Kanai, H. Tashiro, and H. Sato, “In vivo Raman study of the living rat esophagus and stomach using a micro-Raman probe under an endoscope,” Appl. Spectrosc. 61(6), 579–584 (2007).
9. Z. Huang, S. K. Teh, W. Zheng, J. Mo, K. Lin, X. Shao, K. Y. Ho, M. Teh, and K. G. Yeoh, “Integrated Raman spectroscopy and trinodal wide-field imaging techniques for real-time in vivo tissue Raman measurements at endoscopy,” Opt. Lett. 34(6), 758–760 (2009).
10. A. Mahadevan-Jansen, M. F. Mitchell, N. Ramanujam, U. Utzinger, and R. Richards-Kortum, “Development of a fiber optic probe to measure NIR Raman spectra of cervical tissue in vivo,” Photochem. Photobiol. 68(3), 427–431 (1998).
11. J. Mo, W. Zheng, J. J. Low, J. Ng, A. Ilancheran, and Z. Huang, “High wavenumber Raman spectroscopy for in vivo detection of cervical dysplasia,” Anal. Chem. 81(21), 8908–8915 (2009).
1. Introduction

Raman spectroscopy is a highly specific optical technique that has been proven to non-invasively detect epithelial tissue malignancies. It uses molecular-specific inelastic scattering of photons to assess the molecular contents of biological tissue. Photons of monochromatic light (usually from a laser) interact with molecular bonds of biological tissue, which results in an energy shift of the incident photons. Different molecular bonds create different energy shifts that can be visualized as intensity peaks at specific wavenumbers. This allows to obtain information on the molecular composition of the studied tissue and may enable differential diagnosis of (pre-) malignancies in tissues [1–5].

To use Raman spectroscopy for cancer diagnosis it must be incorporated in the current diagnostic approach for suspicious lesions. Our group has special interest in urothelial cell carcinoma diagnosis which is currently based on visual inspection during white light endoscopy. After cystoscopic detection of a suspicious lesion in the outpatient clinic that lesion is endoscopically resected in the operating theater. The resected tissue is then analyzed by the pathologist to obtain the diagnosis. To use Raman spectroscopy in a clinical situation and improve the diagnostic accuracy of urothelial carcinoma, an endoscopic approach is required. Therefore, the Raman probe should have a sufficiently small diameter to allow passage through the endoscopic channel. Furthermore, as part of the clinical Raman system the Raman probe should also meet the requirements of the Medical Device Directive (MDD) as well as other clinical functional requirements. Industrial Raman probes with excellent specifications in the Raman domain are available; however, these are usually not suitable for clinical application because of their limiting factors e.g. the spot-size, sampling volume, acquisition time and the MDD requirements such as the biocompatibility.

The biocompatibility demanded by the MDD entails the use of biocompatible materials and application of strict hospital hygiene requirements including (re)sterilization without damaging the Raman probe.

According to the clinical and functional requirements the probe, should be suitable for endoscopic use, as well as providing good signal quality, i.e. have a high signal-to-noise ratio (SNR). However, clinically, a collection time of longer than 5 s is not feasible as probe displacement during a measurement results in meaningless data. Furthermore, some materials used in probe designs (e.g. sapphire) might interfere more than others with the tissue Raman
signal; use of such materials should (ideally) be avoided in a clinical probe design. Finally, the probe optics should be optimized to obtain an adequate clinical sampling range because epithelial carcinoma originate superficially (100-200 μm below the surface) on top of the underlying stromal tissue [6].

During the last 20 years, several groups have constructed probes that suffice with respect to most of the criteria [7–13]. Nevertheless, to our knowledge, a superficial Raman probe that complies with both the MDD and the clinical/functional requirements is not yet available.

Using gathered information from our previous clinical study with a non-superficial Raman probe [3], a more superficial probe has been designed to meet these requirements. This new probe was designed for a clinical trial in which our group investigates in vivo Raman spectroscopy for the diagnosis of bladder cancer. The probe is constructed of biocompatible materials that result in minimal interference with tissue Raman signals; it can be sterilized and its physical shape allows endoscopic application. At the same time it is designed to provide an ample SNR at clinically acceptable laser exposure and collection times.

The aim of the present study is to compare the sampling range and SNR of the superficial Raman probe with those of a non-superficial probe [3] in a phantom tissue model.

2. Materials and methods

2.1 Raman system

For all measurements a portable Raman spectroscopy system was used as described by Draga et al. [3] The system consists of a 785 nm diode laser (DFB-0785-1000, Sacher Lasertechnik, Marburg, Germany), a Raman probe (EmVision LLC, Loxahatchee, Florida, USA), a spectrograph (HoloSpec Imaging Spectrograph f/1.8i (HSG-785-LF), Kaiser Optical Systems, Ecully, France), a charge-coupled device camera (PIXIS 256 BRDD, Princeton Instruments, Trenton, New Jersey, USA) and a personal computer. The system has been approved for clinical research by the Dutch Health Care Inspectorate (IGZ) allowing a multicenter trial within the European Union.

2.2 Probe designs

For the present study, the newly developed clinical superficial Raman probe was compared with the non-superficial Raman probe which was used in an earlier clinical study by our group [3,13]. Both probes enable efficient excitation and collection of Raman excited light from a 785 nm laser; they are described below and illustrated in Fig. 1.

2.2.1 Non-superficial Raman probe

The non-superficial Raman probe is a standard bundle-style probe. It contains seven low hydroxyl (OH) content Raman collection fibers (300 μm core/0.22 NA) which are incorporated surrounding a central excitation fiber (400 μm core/0.22 NA). A small band pass filter is positioned in front of the delivery fiber and a donut-shaped long pass filter is positioned in front of the seven collection fibers, which rejects the laser light and passes the Raman signal from the sample. All parts of the probe are bound together with an epoxy-based glue and placed inside a stainless steel needle tube, with an outer diameter of 2.1 mm. This allows easy passage through the channel of urological endoluminal scopes.

2.2.2 Superficial Raman probe

The design of the superficial Raman probe (EmVision LLC, Loxahatchee, USA) is uses the same external dimensions as the above-mentioned non-superficial Raman probe; however, the seven collection fibers surround a stainless steel tube which (on the inside) has a central excitation fiber of a smaller diameter (200 μm core/0.22 NA). To enable sampling of superficial tissue layers, an overlap of the focus of the excitation beam and the collection region is created by incorporation of a unique two-component converging lens. This lens
consists of a distal element (2-mm diameter) which is a 1-mm thick flat window of fused silica and a proximal element of a plano-convex sapphire lens (Fig. 1(B)). The high refractive index of the lens bends the light sharply. This configuration allows overlap of the excitation and collection light at the sample without interference from the sapphire Raman signal and an approximate 0.5 mm surface diameter of the region sampled. The superficial Raman probe design is described in detail in its patent [14].

Fig. 1. Top left (A) and right (B) are exploded views of the distal probe tip. The Raman laser excitation region and the direction of the Raman collection cone(s) create an overlap with the laser cone at the surface of the lens and is illustrated for the non-superficial Raman probe (A) and the superficial Raman probe (B) (1 = 7 times collection fibers, 2 = excitation fiber, 3 = Raman laser cone, 4 = Raman collection cone, 5 = two component front lens). Bottom left (C) and right (D) are Zemax ray traces of the non-superficial and the superficial Raman probe, respectively, using the refractive index of water, which is comparable to the 20%-Intralipid® that was used in the phantom tissue model. The Raman excitation light and only one collection fiber cone is illustrated; however, all the collection light is altered in the same way as this one.

2.3 Phantom tissue model and measurements

The phantom tissue model consists of two layers in order to determine the depth response function of the probes, referred to here as ‘sampling depth’ (Fig. 2). The top layer, with an adjustable thickness, consists of a 20%-Intralipid® solution that is widely used to simulate scattering of the 785 nm light of epithelial tissue [15–18]. A polyethylene terephthalate (PET) slide, 170-μm thick, was used as a sub layer to mimic the stromal tissue as this material has specific Raman peaks (e.g. 732.5 cm⁻¹). Accordingly, each Raman spectrum from the phantom tissue model contains signal from both layers (the top layer and the sub layer). Aluminum foil was used as shielding material to prevent the Raman signal from being acquired from the cup itself (Fig. 2(A)).
In the model, by increasing the top layer thickness, the signal intensity of the sub layer decreases as the probe moves relatively further away from the sub layer (Fig. 2(B)). The sampling depth is defined as: the top layer thickness where the normalized signal intensity of the top layer meets the normalized signal intensity of the sub layer.

To determine the specific top layer and sub layer Raman signal contributions in each spectrum, top layer-only and sub layer-only spectra were obtained, respectively, at a top layer thickness of 1.5 cm and directly on the sub layer with no top layer in between (without 20% Intralipid®). From these spectra a specific top layer peak (1439.5 cm\(^{-1}\)) with minimal signal interference of the sub layer material and a specific sub layer peak (732.5 cm\(^{-1}\)) with minimal signal interference of top layer material were determined.

Sets of 10 Raman spectra (acquisition time of 500 ms) were taken at increasing top layer thicknesses from 0 to 1,500 μm, with incremental steps of 50 μm.

Spectral calibration was performed for each probe using a neon-argon lamp to calibrate the spectral dispersion of the detection system and acetaminophen to standardize the Raman shift axis. The spectra were processed for background fluorescence subtraction using a modified polyfit algorithm based on an iterative fitting procedure that converges the spectrum to its baseline [19]. Furthermore, each spectrum was normalized towards its mean spectral intensity to account for variations in absolute spectral intensity.

For all Raman spectra sets at each top layer thickness, the mean intensity of the top layer (1,439.5 cm\(^{-1}\)) and sub layer (732.5 cm\(^{-1}\)) peaks was obtained. Eventually, these mean top layer and sub layer peak intensities were normalized to their maximal intensity, to account for the difference in absolute top layer and sub layer peak intensity. The sampling depth was determined regarding at which top layer thickness the intensities of both peaks were equal.
Fig. 2. Top (A) shows a schematic depiction of the phantom tissue model based on two layers i.e. 20%-Intralipid® (top layer) and PET (sub layer). The Raman probe is vertically fixed perpendicular to the PET slide (1 = Raman probe, 2 = 20%-Intralipid®, 3 = PET slide, 4 = 30 cc cup, 5 = Aluminum foil, 6 = Hollow tube). Bottom (B) shows a schematic depiction of the probe volume in the phantom tissue model that mimics epithelial tissue (top layer) and its stromal tissue (sub layer) below. The top layer is variable in thickness as opposed to the sub layer which has a constant thickness of 170 μm. By increasing the top layer thickness, the specific top layer and sub layer contributions can be compared and the sample depth is determined at which top layer thickness both contributions are equal.
2.4 Signal-to-noise ratio comparison

To compare the spectral quality of the two probes one measurement set from the above sampling depth experiment of both probes was used, i.e. 10 spectra of each probe at a top layer thickness of 200 μm, which is similar to the urothelial tissue thickness.

The mean SNR over the spectral range (SNR_{msr}) is used to assess the quality of the entire spectrum for both probes. The SNR_{msr} was determined by using the mean of two consecutive spectra and the standard deviation (SD) of the difference between those two consecutive spectra within the same set [20]. To identify the possible difference of Raman activity for both substances used in the sampling depth experiment, the SNR at the specific sub layer peak (732.5 wavenumbers) and at the top layer peak (1,439.5) was also determined from that SNR_{msr} spectrum.

3. Results

3.1 General performance

In Fig. 3 the mean raw spectra of both probes without correction are depicted.

![Graph of mean raw Raman spectra](image)

Fig. 3. Mean raw Raman spectra of all measurements per probe, before correction.

Figure 4 shows the mean spectra of both probes after fluorescence subtraction to the baseline using the modified polyfit method [19].
3.2 Specific sub layer and top layer intensities

Mean spectra of the top layer-only and sub layer-only measurements show unique spectral peaks at 1,439.5 cm$^{-1}$ (top layer peak) and 732.5 cm$^{-1}$ (sub layer peak). This is depicted in Fig. 5.
Fig. 5. Mean Raman spectra after normalization to their mean intensity of top layer-only and sub layer-only measurements. The main peak of the top layer (light blue) was found at a wavenumber shift of 1,439.5 cm$^{-1}$ and the main peak of the sub layer (dark blue) was found at 732.5 cm$^{-1}$.

3.3 Sampling depth measurements

The mean peak intensities of the top layer (1,439.5 cm$^{-1}$) and sub layer (732.5 cm$^{-1}$) after correction for excitation intensity were calculated at each incremental top layer thickness. To correct for the absolute intensity difference between both peaks, these intensities were normalized to their maximal intensity per probe (Fig. 6). The maximal intensity for the top layer and sub layer peaks were found at a 1,500 μm and a 0 μm top layer thickness, respectively. When the top layer thickness was increased, the top layer and sub layer peak intensities showed an increase and decrease, respectively. In Fig. 6, the intersection of these normalized top layer and sub layer peak intensities determines the sampling depth for each probe. The sampling depth of the non-superficial Raman probe was approximately 300 μm and that of the superficial Raman probe approximately 200 μm.
Fig. 6. Raman peak intensities at 1,439.5 cm$^{-1}$ and 732.5 cm$^{-1}$ and (top layer and sub layer peak) as a function of top layer thickness in the phantom tissue model for the non-superficial and the superficial Raman probe with their standard deviation. The intersections of the non-superficial Raman probe and the superficial Raman probe at approximately 300 μm and 200 μm, respectively, indicate that the sampling depth is closer to the distal probe tip for the superficial Raman probe.

3.4 Signal-to-noise ratio comparison

Table 1 presents the SNR of both probes in the phantom tissue model with a top layer of 200 μm. The SNR of the superficial Raman probe has increased by a factor of 2 compared with the non-superficial Raman probe. Similarly, at the specific sub and top layer peaks the SNR was also increased by a factor 2 for the superficial probe compared to the non-superficial probe.

Table 1. Signal-to-noise ratio over the mean spectral range (SNR) measured in the phantom tissue model at 200 μm probe distance from the sub layer and specific SNR of the sub layer and top layer peaks.

|                  | Non-superficial Raman probe | Superficial Raman probe | Factor |
|------------------|-----------------------------|-------------------------|--------|
| SNR (sub layer peak 732.5 cm$^{-1}$) | 315.1                      | 596.7                   | 1.9    |
| SNR (top layer peak 1439.5 cm$^{-1}$) | 77.9                       | 166.5                   | 2.1    |

4. Discussion

A clinical superficial Raman probe that meets the MDD and clinical/functional requirements is presented. The superficial Raman probe is constructed of biocompatible materials. Moreover, it has shown to withstand repeated (>15 for multiple probes) plasma (STERRAD®) sterilization (results not shown). Furthermore, the short collection time of 5 s (10 x 500 ms) minimizes probe movement during the measurement, while the probe’s diameter of 2.1 mm allows endoscopic use as required for urothelial tissue diagnosis.
In this study, the sampling depth of the superficial Raman probe was compared with the non-superficial Raman probe in a phantom tissue model. In this model the intersection of the sub layer and top layer peak intensities indicate a sampling depth which allows comparison of the two probes. The optimal sampling range of the superficial probe is 0-200 μm and for the non-superficial probe 0-300 μm. The depth setting error was maximal 5 μm and the SD of the intensity at the intersection was 0.02 and 0.08 for the non-superficial and the superficial probe, respectively. The superficial probe more closely approximates the sampling range of the origination of urothelial carcinoma (100-200 μm from the surface). Accordingly, this probe should enable better urothelial cancer diagnosis because the Raman signal is mostly obtained from the urothelial cell layer and is less clouded by signal from its underlying stromal layer (Fig. 7). However, because this test was performed to compare the probes, these results cannot be used to indicate the exact sampling range in tissue because the model is a simplification of tissue composition. As opposed to tissue, the model contains materials with uniform consistency, and has a straight transition of materials and constant thicknesses. It also has different optical properties.

To determine the SNR, raw spectra without background subtraction and noise smoothing were used. The measurement sets with a top layer thickness of 200 μm for both probes were chosen for SNR determination, because this thickness is similar to the normal urothelial thickness overlying its stromal sub layer. Therefore, these measurement sets are most similar to the in vivo situation.

In general, the SNR_{m} of the superficial probe is twice as high compared with the previously used non-superficial probe in the phantom tissue model with a top layer thickness of 200 μm. This is also the case for the specific top layer and sub layer peaks. Accordingly, to obtain the same signal quality, a clinical Raman measurement of 5 s with the new superficial probe would take up to 20 s with the old non-superficial probe, which is a clinical challenge. Furthermore, as the SNR does not linearly increase with longer integration times, an even longer integration time than 20 s might be required to obtain the same signal quality.

These results allow to conclude that the SNR of the superficial probe is improved compared to the non-superficial probe. However, the absolute SNR_{m} outcomes only allow comparison of the two probes; these data are not suitable for comparison with other SNR experiments because of the specific Raman contributions of the phantom tissue model substances (as described above).
Since the mid-1990s several Raman probe designs have been developed for cancer diagnosis (Table 2). Mahadevan-Janssen et al. were the first to construct a clinical probe in 1998; they also attempted to miniaturize this design to enable clinical endoscopic use [10]. Shim et al. constructed a miniature Raman probe which focuses on superficial layers using a beam steering technique with beveling of the collection fibers [13]. Their sampling range was 0-600 μm with a 10° beveled probe; however, this probe was not qualified for clinical use with respect to biocompatibility as required by the MDD. Furthermore in their probe design, the filters are placed approximately 3 cm from the distal end of the needle tube which limits flexible endoscopic application. Although, Hattori et al. produced an endoscopic probe, the integration time of 50 s would not have been practical as probe movement might occur during urological endoscopy. Furthermore, with a focal depth of 10 mm the contribution of the stromal tissue signal might obscure the Raman signal of the thin urothelial cell layer where urothelial cancer originates [8].

Several groups have constructed superficial Raman probes using ball lenses [7,11,12,21,22]. The sampling range of these probes is minimized to 40-300 μm and focuses on the urothelial cancer origin. Unfortunately, ball lenses are generally composed of sapphire which generates high fluorescence background signals that might interfere with the urothelial tissue Raman signal. Consequently, we aimed to design a probe using silica, which has a lower fluorescence background compared with sapphire [13,23].
Table 2. Information on the Raman probe developed since the 1990s

| Authors                  | Year | Probe diameter (mm) | Sampling range | Acquisition time (sec) | Biocompatible | Sterilizable | Design                                                                 |
|-------------------------|------|---------------------|----------------|------------------------|---------------|--------------|------------------------------------------------------------------------|
| Mahadevan-Jansen et al. [10] | 1998 | 12                  | 900 μm Ø      | 90                     | NA            | NA           | Beams steering beveled probes in 0°, 5°and 10°                          |
| Shim et al. [13]         | 1999 | 1.5                 | 0-600 μm (10°) | 1                      | NA            | NA           | Sapphire                                                              |
| Motz et al. [12]         | 2004 | 3                   | 500 μm        | 10                     | NA            | NA           | Microscopic system with grin lens                                      |
| Hattori et al. [8]       | 2007 | 2.5                 | 10 mm focal depth | 50                    | NA            | NA           | Sapphire                                                              |
| Day et al. [7]           | 2009 | 2.8                 | 147 μm        | 2 and 10               | Yes           | Yes          | Sapphire ball lens                                                    |
| Mo et al. [11]           | 2009 | 8                   | 700 μm max depth | 1                     | Yes           | NA           | Different refractive indexes Ball lens Sapphire                         |
| Mo et al. [21]           | 2010 | 3                   | 40-300 μm     | NA                     | Yes           | NA           |                                                                       |
| Draga et al. [3]         | 2010 | 2.1                 | 0-300 μm      | 0.5x10                 | Yes           | Yes          |                                                                       |
| Wang et al. [22]         | 2013 | 1.8                 | NA            | Yes                    | NA            | NA           | Ball lens Refractive index 1.76                                       |
| Agenant et al.           | 2013 | 2.1                 | 0-200 μm      | 0.5x10                 | Yes           | Yes          |                                                                       |

NA = not applicable

In conclusion, to our knowledge the superficial Raman probe presented here is the first clinically applicable urological endoscopic probe with a shallow sampling range and with minimal signal interference from the materials used. Although, it has a sampling range of 0-200 μm, this absolute determination of sampling range only allows comparison of the two probes described here. No comparison can be made with the probes described in Table 2 as these research groups might have used different definitions of sampling range and measuring methods.

The sampling range and SNR results of this new superficial Raman probe are improved compared with the non-superficial probe in the phantom tissue model. Nevertheless, the clinical diagnostic sensitivity, specificity and accuracy of this new probe needs to be examined in a clinical situation, because the materials used in the phantom model are a simplification of real tissue. Our group is currently investigating this in an ongoing clinical trial on in vivo Raman spectroscopy for bladder cancer diagnosis. To date, monitoring of the probe performance (80 patients) has shown acceptable variations in the constancy test and
mechanical integrity during each new procedure after sterilization. For commercial acceptability, additional sterilization contamination tests are required.

5. Conclusion

Since the 1990s, substantial research led to the design and development of several Raman probes for real-time clinical cancer diagnosis. However, a probe meeting MDD and functional requirements for clinical urothelial carcinoma diagnosis has not yet been developed.

A superficial clinical Raman probe design, approved for investigative use, is presented. It has a superficial sampling range of 0-200 μm which should improve the diagnosis of (superficial) urothelial carcinoma, and has an improved SNR compared with the non-superficial probe used in our earlier clinical feasibility study. The ultimate goal is to detect urothelial (pre) carcinoma with high accuracy in real-time using an optical non-invasive Raman measurement in the outpatient clinic, rather than an invasive biopsy in the operating room.