LETTER

Side-viewing handheld confocal Raman probe coupled with an off-axis parabolic mirror for superficial epithelial Raman measurements of luminal organs

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Abstract
We report on the development of a side-viewing handheld confocal Raman probe coupled with an off-axis parabolic (OAP) mirror for superficial epithelial Raman measurements of luminal organs. The OAP mirror realizes an effective numerical aperture of 0.36, a measured Raman excitation spot diameter of 130 μm and a depth of focus of 128 μm, allowing real time Raman spectra acquisition. The OAP mirror tilts the Raman optical path by 90°, making it well suited for superficial epithelium Raman interrogation of luminal organs. In addition, the probe results in no Raman interferences that may arise from transmission optics. The superficial epithelium Raman interrogation capability of the probe developed is validated through a two-layer tissue phantom experiment. This work suggests the potential of the side-viewing handheld confocal Raman probe coupled with an OAP mirror for enhancing the diagnosis of epithelial precancer and cancer of luminal organs.

KEYWORDS
handheld probe, off-axis parabolic mirror, Raman spectroscopy, side-viewing

1 | INTRODUCTION

Neoplasm initiated from the epithelia, the tissue type that lines all body cavities, account for as many as 90% of human cancers [1]. The epithelial precancer starts from the superficial epithelium, and gradually invaded into the underlying stroma accompanying the increased severity of the epithelial neoplasm. If the epithelium precancer can be detected early, the patient survival rate could be improved significantly. Among the various optical techniques (ie, Raman spectroscopy [2], optical coherence tomography [3], fluorescence spectroscopy [4], and diffuse reflectance spectroscopy [5]) developed for epithelium precancer detection, Raman spectroscopy, a unique vibrational technique working on the principle of inelastic Raman scattering [6], reveals chemical composition and structure of epithelial tissues. Raman spectroscopy is therefore capable of probing biomolecular structures and compositions of tissue associated with epithelial neoplasm, and has been demonstrated for excelled epithelial tissue diagnosis in both non-tubular (ie, cervix [7], breast [8], brain [9], stomach [10], etc) and tubular (ie, esophagus [11], colon [12], lung [13], etc) organs.

Abbreviations: DM, dichroic mirror; DOF, depth of focus; NA, numerical aperture; OAP, off-axis parabolic.
A key component pushing the frontier of biomedical Raman spectroscopy is the compact (handheld [14], endoscopic [15] or needle [16]) Raman probes developed [14], making it possible to acquire Raman spectra from the previously inaccessible organs sites. Side-viewing Raman probes offering angular Raman measurements are needed and in general preferred for luminal organs (eg, rectum and vagina, etc), allowing consistent positioning of the Raman probes against the tubular tissue under investigation. In particular, side-viewing handheld Raman probes satisfying the following two requirements are preferred. First, biomedical Raman probes favor confocal designs, offering the strongest depth selectivity and minimizing the Raman interrogation regions and avoiding interference from the surrounding tissues [14]. Second, Raman probes with reflective distal optics is highly desired to enable background-free Raman spectroscopy. This is because even the Raman background arising from the delivery fibers could be minimized through external or in-line filters, the distal optics attached at the Raman probe tip were, nevertheless, found accompanied with interfering background (eg, at Raman peaking positions of 3240 cm⁻¹ [17] attributed to the distal sapphire ball lens), requiring special attention when selecting transmissive distal optics (eg, sapphire ball lens). Therefore, in this study, using an off axis parabolic (OAP) mirror, we designed and constructed a side-viewing handheld confocal Raman probe free of Raman interferences from transmissive distal optics. The probe developed was able to facilitate superficial Raman measurements, which is critical for enhancing the diagnosis of epithelial precancer and cancer of luminal organs.

2 | EXPERIMENTAL METHODS

Figure 1A shows the schematics of the OAP mirror coupled side-viewing handheld confocal Raman probe developed for superficial epithelial Raman measurements. The probe consisted of Raman excitation and collection paths, which were co-aligned by a dichroic mirror (DM, Di02-R785-25x36, Semrock, Rochester, NY). First, the Raman excitation portion consisted of an excitation fiber (FG105LCA, Thorlabs, NJ; Core diameter: 105 μm, numerical aperture: 0.22), a fiber collimator, a bandpass filter, a reflection mirror, and an OAP mirror (#37-282, Edmund Optics, NJ). Raman excitation launched from a 785 nm diode laser (Real Light, Beijing, China) was delivered by the 2-m-long excitation fiber, collimated by the fiber collimator, and then filtered by a bandpass filter (LL01-785-12.5, Semrock, Rochester, NY) to remove the interfering fluorescence and Raman spectra generated by the excitation fiber itself. The band-pass filtered Raman excitation was tilted 90° by the 45° positioned reflection mirror, further reflected by the dichroic mirror (DM), and then focused onto the epithelial tissue by the OAP mirror. The backscattered Raman signal arising from the epithelial tissue was collected and collimated by the same OAP mirror, passed through the DM, filtered by a long pass filter (BLP01-808R-25, Semrock, Rochester, NY) to remove Rayleigh backscattered Raman excitation laser, passed through the other 2-m-long Raman collection fiber (FG105LCA, Thorlabs, NJ; Core diameter: 105 μm, and numerical aperture: 0.22) before being detected by a preconfigured Raman spectrometer (QE Pro-Raman, Ocean Insight, FL). Figure 1B shows the photograph of the assembled probe operated in a handheld manner.

The distal tip of the probe (Figure 1C) was housed inside a silica glass tube (inner diameter of 8 mm and outer diameter of 10 mm). Since the working distance of the probe is 1.35 mm, the probe works in non-contact mode. Due to the confocality, the tissue Raman signal will be highly dependent on the distance to the tissue. To use the probe clinically, either a spacer made of medical grade steel can be developed and added to the probe tip, or an OAP mirror of shorter focal length matching the outer diameter (ie, 10.3 mm) of the probe tip could be explored, ensuring the probe developed works in contact with the tissue under interrogation. The overall length of the probe was ~200 mm, dimension sufficient to interrogate luminal organs. Tissue Raman spectra could be acquired within 1 second by using the probe developed with the Raman spectrometer. Slits of different widths (ie, 50, 100 and 200 μm) were experimentally tested in combination with a collection fiber with core size of...
3 | RESULTS AND DISCUSSION

With the assembled probe (Figure 1) and the Raman acquisition platform, we investigated how the probe developed performs to eliminate interfering Raman spectra that may arise when using transmission optics [17]. Raw Raman spectra were recorded when using an aluminum reflection mirror (PF10-03-G01, Thorlabs, NJ) as sample (Figure 2A), showing smooth background without any Raman peaks in both the fingerprint (800-1800 cm$^{-1}$) and high-wavenumber (2800-3600 cm$^{-1}$) regions, confirming the capability of probe developed to eliminate the interfering Raman peaks. One notes that the probe background spectra was found with discernible Raman peaks at 264 and 417 cm$^{-1}$ attributed to the silica materials of the collection fiber [18], under incident power of 100 mW onto the reflection mirror. We however, noticed that with a clinically acceptable power (eg, of $\sim$12 mW [7, 11, 12]), the amplitude of the Raman peaks at 264 and 417 cm$^{-1}$ was low, imposing limited interferences on the Raman spectra from the tissue of interest. One also finds a Raman peak at 0 cm$^{-1}$ (Figure 2A) that can be attributed to the Rayleigh scattered Raman excitation at 785 nm, as consistent with previous reports [19, 20]. This peak was caused by the imperfect transmission performance of the dichroic mirror and the long pass filter incorporated into the probe. Besides, the peak amplitude increased with increasing Raman excitation power. The results in Figure 2A demonstrate the probe developed suppresses and minimizes the interfering Raman spectra background.

We further quantitatively characterized the optical performance of the probe developed, through measurement of the probe NA, focal spot dimension and the depth of focus (DOF). The 785 nm Raman excitation beam incident onto the OAP was measured 4.46 mm ($1/e^2$ diameter of the far field profile, BGS-USB-SP620, Ophir Photonics, UT), resulting in a theoretical NA of 0.575, a focused spot diameter of $\sim$0.83 μm (ie, 0.61$\lambda$/NA) and DOF of 3 μm (ie, $\lambda\sqrt{n^2-NA^2}$/NA$^3$ [21], $\lambda$ = 785 nm, $n$ = 1.4). However, when using the probe for real applications, the performance of the OAP mirror was sensitive to aberrations arising from the misalignment of the collimated Raman excitation beam and the OAP mirror optics axis (Figure 2B). For instance, angular displacement of the incident beam from the OAP mirror optical axis will lead to comatic aberration. Besides, the multi-mode nature and large core size of the Raman excitation and collections fibers (Figure 1A) lead to deviation of the practical probe focus dimension and DOF [22]. We have therefore experimentally measured the probe NA, focus spot diameter and DOF. The probe NA was determined through the measurement of the $1/e^2$ radius of the far field profile as a function of distance from OAP mirror.

![Figure 2](image-url) (A) Recorded probe background corresponding to different integration time (10 ms to 1 second) with incident power of 100 mW onto an aluminum reflection mirror (PF10-03-G01, Thorlabs, NJ). (B) Schematics illustration of the misalignment between OAP mirror optics axis (black dotted line) and the collimated incident beam (red solid lines). (C) Far field beam radius changes as a function of distance from the focus of the probe developed. The linear fit determined the probe NA $\sim$0.36 at 785 nm. (D) Knife edge test showing the detected intensity changes vs the knife edge positions, with a determined OAP mirror focus spot diameter $\sim$130 μm. (E) Probe DOF (128 μm at full width half maximum) measured on probe silica background when using mirror as sample.

0.2 mm (FG200LEA, Thorlabs, NJ). The spectral resolutions were found $\sim$0.8, 1.2 and 2 nm, corresponding to 9.8, 14.7 and 24.6 cm$^{-1}$, respectively at 1655 cm$^{-1}$ (amide I ν[C=O] of proteins) [15]. To balance the spectral resolution, spectra acquisition speed and probe confocality (Figure 2E), a slit with width of 100 μm and a collection fiber with core size of 105 μm (FG105LCA, Thorlabs, NJ) were employed for the Raman spectra as reported in this paper. One notes that slits of different widths and collection fiber of different core sizes can be readily replaced depending on whether higher spectral resolution or faster spectra acquisition speed is prioritized.
focus (black markers in Figure 2C) [23]. The 1/e² radius of the far field profiles were determined using the beam profiler, and the distance from OAP mirror focus was adjusted from 20 to 60 mm with a manual translation stage (LJ1710, LBTEK, Shenzhen, China). We found the beam radius increased with enlarged distance from the OAP mirror focus. To quantify the probe NA, linear fit was conducted, giving a NA valued 0.36, which deviates from the theoretical NA of 0.575, but was still considered reasonably good as supported by the probe focus beam spot diameter and DOF (as will be shown below). We next determined the probe focus beam spot diameter associated with the probe NA of 0.36 through a knife edge test, showing a probe focus spot diameter ~130μm (Figure 2D). The tight focusing capability enabled by the OAP mirror allows efficient Raman excitation and a resultant real-time (<1 second) Raman spectra acquisition with a Raman spectrometer.

We then determined the probe DOF by measuring the intensity of the inherent silica Raman peak (417 cm⁻¹) at various probe working distances [24]. The silica Raman peak intensity changes vs distance from focus was included in Figure 2E. The DOF determined by the FWHM value was 128μm, deviating from the micrometer-scale DOF of confocal Raman microscopes employing dedicated microscope objective [22]. The deviation was likely caused by the lacking of objectives which are too bulky to be integrated into the probe developed. Further, we found the DOF of 128μm was close to those achieved with compact fiber optic Raman probes [16, 24, 25]. Besides, the confocal gating of the probe developed was constituted by the excitation and collection fibers (as in Figure 1A), and can be tuned through implementation of fibers with larger or smaller core sizes.

With the quantified optical performance of the probe developed (Figure 2), we further evaluated how the newly designed probe facilitates superficial epithelial Raman measurements through Raman measurements conducted on a two-layer tissue phantom with an incident power of 12 mW [15]. The two-layer tissue phantom consisted of a 0.3 mm-thick chicken muscle tissue as first layer on top of a 2 mm-thick chicken fat tissue as bottom layer. Figure 3 shows the representative Raman spectra of chicken muscle and fat layers. As shown, distinct Raman peaks assignable to protein and/or lipid were found associated with the two different tissue types. For instance, the Raman spectrum of chicken fat tissue was characteristic of five prominent Raman lipid peaks at 972 cm⁻¹ (calcium-phosphate stretching of cholesterol/lipids), 1078 cm⁻¹ (C=C/O stretching of phospholipids), 1301 cm⁻¹ (CH₂ twisting and wagging modes of lipids, triglycerides), 1445 cm⁻¹ (CH₂ bending of lipids and proteins) and 1745 cm⁻¹ (C=O stretching of ester [phospholipids]) [15, 26]. While six distinct Raman protein peaks were found with chicken muscle tissue at 936 cm⁻¹ (C=C stretching of proteins), 1004 cm⁻¹ (C=C stretching of phenylalanine), 1209 cm⁻¹ (C₆H₅ stretching of tryptophan and phenylalanine), 1335 cm⁻¹ (CH₂CH₂ wagging of collagen), 1445 cm⁻¹ (CH₂ bending of lipids and proteins) and 1655 cm⁻¹ (amide I ν[C=O] of proteins) [15, 26].

The measured phantom Raman spectra was also included in Figure 3, which showed prominent Raman peaks and overall resembles that of the top chicken muscle tissue, implying the superficial Raman spectra acquisition using the probe developed. Also, we noticed the typical Raman peak of chicken fat at 1745 cm⁻¹ was not present in the measured phantom Raman spectra, confirming the superficial interrogation capability of the probe developed. Besides, typical Raman peak of proteins (ie, 1655 cm⁻¹ (amide I ν[C=O] of proteins)) was found associated with chicken fat (Figure 3), observation consistent with previous Raman studies showing amide I Raman peak of pure fatty acids Raman spectrum [27, 28]. Similarly, Raman peak of lipid (ie, 1078 cm⁻¹ (C=C/O stretching of phospholipids)) was found within the Raman spectra of chicken muscle, but with much reduced peak amplitude compared to that of the chicken fat (ie, 2.1E–3 vs 1.2E–2), reflecting the small amount of lipid content embedded within the chicken muscle tissue. Further quantitative analysis using the least-squares regression on the Raman spectra measured from the two-layer tissue phantom confirmed that the OAP mirror

![Figure 3](image-url)
coupled side-viewing handheld confocal Raman probe collects ~88% of the total Raman signal attributed to the top layer (chicken muscle tissue), reconfirming the ability of the probe developed for improving superficial tissue Raman measurements in the two-layer tissue model.

Compared with our previously developed side-viewing handheld Raman probe [29], the reported probe increased the epithelium Raman signal proportion from 80% to 88%, and decreased the Raman excitation beam spot diameter from 360 μm to 130 μm, making it better suited for targeted epithelium Raman interrogation. Besides, unlike previous studies that utilize OAP mirrors for either enhanced Raman signal collection at long working distance [30], augmented polarization preservation for polarized Raman spectroscopy [31], or suppressed Raman spectral background interference for on-site detection of weak Raman signals (eg, from uranium materials) [32], the reported work demonstrated the capability of OAP mirror for removing interference from transmission optics and improving superficial epithelium Raman signal collection, which are critical for the clinical translation of OAP mirror coupled side-viewing handheld confocal Raman probe. In addition, the current 90° OAP mirror can be readily replaced with OAP mirror of obtuse offset angles (eg, 160°) to reach different anatomical locations requiring specific angles (eg, 20°) rather than 90°. One notes that the currently developed probe was relatively bulky (ie, with a probe tip outer diameter of 10 mm), making it suitable for large luminal organ (eg, rectum and vagina) interrogation but unsuitable for other luminal organs (eg, blood vessels [33] and/or lung [34]) of smaller dimensions. Nevertheless, one notes that with an OAP mirror of smaller diameter (eg, 1 mm by asphericon GmbH, Jena, Germany) fabricated, and the commercially available compact GRIN (GRINTech, Jena, Germany) lens as collimator, the probe developed herein is modifiable and scalable into an endoscope probe or catheter with an overall diameter <1.5 mm, providing angle-resolved Raman spectra with distal scanning, thereby potentially benefiting the cardiovascular [33] and/or bronchoscopy [34] applications of Raman spectroscopy, and the beyond.

CONCLUSION

In summary, we have developed a side-viewing handheld confocal Raman probe coupled with an OAP mirror for superficial epithelial Raman measurements of luminal organs. We have demonstrated that the Raman probe developed favors superficial epithelium Raman signal collection, and is free of Raman interferences that may arise from transmission optics. The probe developed is of great potential for epithelial precancer detection of lumina organs (eg, rectum and vagina).

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CONFLICT OF INTEREST

The authors declare no financial or commercial conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available upon reasonable request.

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