Wavefront correction enables vibrational imaging of bacteria with multimode fibre probes

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Abstract

Raman spectroscopy is a valuable tool for non-invasive and label-free identification of sample chemical composition. Recently a few miniaturized optical probes emerged driven by the need to address areas of difficult access, such as in endoscopy. However, imaging modality is still out of reach for most of them. Separately, recent advances in wavefront shaping enabled different microscopies to be applied in various complex media including multimode fibers. Here we present the first and thinnest to date Raman fiber imaging probe based on wavefront shaping through a single multimode fiber without use of any additional optics. We image agglomerates of bacteria and pharmaceuticals to demonstrate the capability of our method. This work paves the way towards compact and flexible Raman endoscopy.

Keywords: wavefront correction, adaptive optics, Raman spectroscopy, Raman imaging, optical trapping.

I Introduction

Raman scattering is a prominent spectroscopy tool originating from the specificity of inelastic scattering of light towards chemical composition of a given sample. It is particularly useful for applications where otherwise similar compounds or objects should be told apart, such as genuine and counterfeit drugs, or healthy and cancerous tissues. For the biomedical and clinical applications, the need to access restricted areas and internal organs has triggered miniaturization of Raman probes and development of new fiber-based Raman devices[1]. However, the limited design options for sub-mm sized probes mostly restrict their use to single-pixel bulk acquisition.

At the same time, methods of optical wavefront correction have enabled several microscopies to be implemented with a multimode fiber[2,3]. Raman imaging, however, has not benefited from the wavefront shaping technique to date. In this work we implement wavefront correction to enable lensless imaging with a single multimode fiber, which represents the smallest Raman imaging probe to date (125 μm outer diameter of the fiber). We demonstrate the capabilities of our method by differentiating pharmaceuticals on a glass slide, as well as by imaging clusters of *M. smegmatis* bacteria. Given the previously reported results[2], our probe is also compatible with simultaneous 2D optical trapping.

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II Materials and Methods

1. Experimental setup

The experimental setup functionally represents a Raman microscope with an added liquid crystal spatial light modulator (SLM) for wavefront shaping, and a multimode fiber (MMF) acting as a microscope objective.

Upon expanding, a 532 nm beam from Verdi Coherent V5 is sent on a Hamamatsu X10468 SLM, which shapes the phase of the incoming light. A linear grating is applied to the SLM mask to divert the phase-modulated beam into the first diffraction order, which is picked up by a pinhole. The pinhole plane is imaged onto the input facet of a 14cm long MMF (Thorlabs AFS50/125Y). The SLM masks create focused spots on the sample plane behind the output facet of the MMF. Light scattered from the sample is collected via the same MMF, and then put through a dichroic mirror and a notch filter to remove the 532 nm contribution. The filtered light is then analyzed by a fiber-coupled spectrometer.

2. Wavefront correction

The wavefront correction with an SLM was performed using transmission matrix (TM) method[4], which requires a preliminary calibration step. Upon TM acquisition, raster scanning of the focused spot can be performed by changing the SLM mask.

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3. Spectral imaging

The excitation beam was digitally scanned across the image-forming pixel grid, and a spectrum acquired for each pixel. This formed a measurement matrix N x P, where N is the total number of pixels, and the P is the length of a single spectrum. To construct images based on the acquired spectra, a non-negative matrix factorization[5] was performed on the matrix, to decompose it into individual *spectral components* corresponding to chemical compounds, and their corresponding *weights* which define the intensity of image pixels.

III Results and Discussion

Raman spectroscopy is widely used for drug analysis and quality control[6], and we therefore use drug identification as a demonstration for our fiber imaging system. Figure 1 shows Raman spectra (a) and the Raman image (b) with paracetamol and ibuprofen intensities encoded in red and green channels of an RBG image, respectively. Due to geometrical reasons, a FOV of arbitrary size can in principle be obtained at the expense of the image resolution and collection efficiency. Here we show a FOV of 200 μm diameter, which significantly exceeds the fiber diameter, while preserving image features with a reasonable SNR (~20). For the spectra identification, we choose the so-called 'lipid region' of vibrational spectrum which spans 2800-3300 cm^{-1}. The main reason for this is that the fingerprint region 200-1600 cm^{-1} in our fiber is contaminated by strong background Raman signal from the silica[7] the fiber is made of.

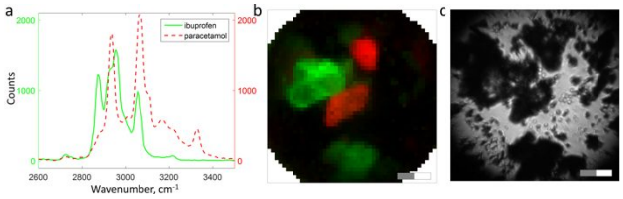

Figure 1. Raman imaging of pharmaceutical compounds within a mixture: a) Raman spectra; b) Raman image of paracetamol / ibuprofen with a ⌀200 μm FOV; c) bright field image of drug clusters. Scale bars are 40 μm full, 20 μm white.

We also apply our fiber for imaging clusters of bacterium *M. smegmatis*[8], which Raman spectrum and Raman image are shown on the Figure 2. While the clusters can clearly be visible near the edge of the FOV, the sensitivity of the system is not sufficient to detect individual bacteria, as can be seen by comparing 2b with the bright field image 2c.

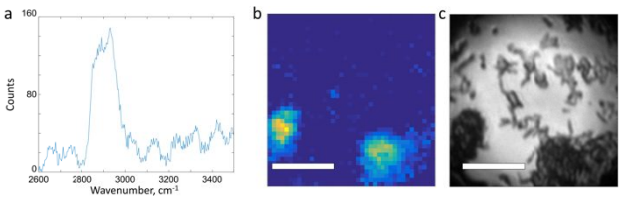

Figure 2. (a) Raman spectra of *M. smegmatis* bacteria; (b) Raman image of bacterial clusters; (c) Bright field image of the same area as in (b). Scale bars are 20 μm white.

4. Conclusion

We report the thinnest fiber Raman probe with imaging capability to date, which represents a single multimode optical fiber with no additional focusing optics and filters integrated. Our results show multicolor imaging and chemical discrimination, FOV exceeding the dimensions of the probe itself, and application to biological samples, all this with a flexible probe compatible with endoscopic settings. We therefore believe that our work paves the way towards new opportunities for Raman spectroscopy in clinical and biomedical studies with restricted access.

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