Serial Raman spectroscopy of particles trapped on a waveguide

Pål Løvhaugen,[1] Balpreet Singh Ahluwalia,[1] Thomas R. Huser,[2] and Olav Gaute Hellesø[1,*]

[1]*Department of Physics and Technology, University of Tromsø, 9037 Tromsø, Norway*
[2]*Department of Physics, University of Bielefeld, Bielefeld, Germany*
[*]*olav.gaute.helleso@uit.no*

Abstract: We demonstrate that Raman spectroscopy can be used to characterize and identify particles that are trapped and propelled along optical waveguides. To accomplish this, microscopic particles on a waveguide are moved along the waveguide and then individually addressed by a focused laser beam to obtain their characteristic Raman signature within 1 second acquisition time. The spectrum is used to distinguish between glass and polystyrene particles. After the characterization, the particles continue to be propelled along the straight waveguide. Alternatively, a waveguide loop with a gap is also investigated, and in this case particles are held in the gap for characterization before they are released.

OCIS codes: (130.3120) Integrated optics devices; (350.4855) Optical tweezers or optical manipulation; (170.5660) Raman spectroscopy.

References and links

1. S. Kawata and T. Tani, "Optically driven Mie particles in an evanescent field along a channeled waveguide," Opt. Lett. **21**(21), 1768–1770 (1996).
2. T. Tanaka and S. Yamamoto, "Optically induced propulsion of small particles in an evenescent field of higher propagation mode in a multimode, channeled waveguide," Appl. Phys. Lett. **77**(20), 3131–3133 (2000).
3. K. Grujic, O. G. Hellesø, J. S. Wilkinson, and J. P. Hole, "Optical propulsion of microspheres along a channel waveguide produced by Cs^+ ion-exchange in glass," Opt. Commun. **239**(4-6), 227–235 (2004).
4. B. S. Ahluwalia, P. McCourt, T. Huser, and O. G. Hellesø, "Optical trapping and propulsion of red blood cells on waveguide surfaces," Opt. Express **18**(20), 21053–21061 (2010).
5. K. Grujic, O. G. Hellesø, J. Hole, and J. Wilkinson, "Sorting of polystyrene microspheres using a Y-branched optical waveguide," Opt. Express **13**(1), 1–7 (2005).
6. B. S. Schmidt, A. H. Yang, D. Erickson, and M. Lipson, "Optofluidic trapping and transport on solid core waveguides within a microfluidic device," Opt. Express **15**(22), 14322–14334 (2007).
7. M. Lankers, J. Popp, and W. Kiefer, "Raman and Fluorescence Spectra of Single Optically Trapped Microdroplets in Emulsions," Appl. Spectrosc. **48**(9), 1166–1168 (1994).
8. K. Ajito and K. Torimitsu, "Near-infrared Raman spectroscopy of single particles," TrAC Trends in Analytical Chemistry **20**(5), 255–262 (2001).
9. C. Xie, M. A. Dinno, and Y. Q. Li, "Near-infrared Raman spectroscopy of single optically trapped biological cells," Opt. Lett. **27**(4), 249–251 (2002).
10. H. Tang, H. Yao, G. Wang, Y. Wang, Y. Q. Li, and M. Feng, "NIR Raman spectroscopic investigation of single mitochondria trapped by optical tweezers," Opt. Express **15**(20), 12708–12716 (2007).
11. D. V. Petrov, "Raman spectroscopy of optically trapped particles," J. Opt. A, Pure Appl. Opt. **9**(8), S139–S156 (2007).
12. A. Y. Lau, L. P. Lee, and J. W. Chan, "An integrated optofluidic platform for Raman-activated cell sorting," Lab Chip **8**(7), 1116–1120 (2008).
13. H. C. Hunt and J. S. Wilkinson, "Optofluidic integration for microanalysis," Microfluid. Nanofluid. **4**(1-2), 53–79 (2008).
14. C. Lim, J. Hong, B. G. Chung, A. J. deMello, and J. Choo, "Optofluidic platforms based on surface-enhanced Raman scattering," Analyst (Lond.) **135**(5), 837–844 (2010).
15. O. G. Hellesø, P. Løvhaugen, A. Z. Subramanian, J. S. Wilkinson, and B. S. Ahluwalia, "Surface transport and stable trapping of particles and cells by an optical waveguide loop," Lab Chip **12**(18), 3436–3440 (2012).

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16. B. S. Ahluwalia, A. Z. Subramanian, O. G. Hellesø, N. M. B. Perney, N. P. Sessions, and J. S. Wilkinson, "Fabrication of submicrometer high refractive index Tantalum Pentoxide waveguides for optical propulsion of microparticles," IEEE Photon. Technol. Lett. **21**(19), 1408–1410 (2009).

1. Introduction

Optical trapping on waveguides has since the mid-1990's been shown to be a high-precision, low-velocity transportation method for cells and microparticles [1–4]. The technique uses the evanescent field generated above the waveguide to propel the objects along the waveguide path and thus provides excellent control over the translational movement of particles without exposure to high optical intensities. Sorting and assembly line-like transportation have been demonstrated, either using Y-branched waveguides or combined with microfluidics [5, 6]. However, information on the trapped particles, besides size or refractive index estimates, is not provided by the waveguide trapping technique itself.

Raman spectroscopy provides detailed information on the identity and structure of objects under study. Systems combining optical tweezers and Raman spectroscopy have been used to characterize microdroplets, microparticles, cells, mitochondria, etc [7–11]. Raman spectroscopy and optical tweezers have also been combined with microfluidics for delivery, identification, and simultaneous sorting of individual cells [12]. Furthermore, a number of optical techniques have been combined with microfluidics for chemical and biochemical analysis, including surface enhanced Raman scattering (SERS) [13, 14].

Whereas microfluidics can transport a large number of particles fast, waveguide trapping provides higher precision over movement and is ideal for moving and studying single particles. Waveguide trapping still takes the conveyor-belt approach of microfluidics, which is not the case for optical tweezers. By combining Raman spectroscopy with waveguide trapping, a tool can be made for the specific sorting of particles on waveguide structures, for providing information on how trapped microspheres or cells are affected by evanescent fields, or for studying near-surface interactions. The low velocity of waveguide trapping is compatible with the long exposure times necessary to acquire weak Raman spectra. A single particle can thus be analyzed while the previous particle is moved away and the next particle arrives along the waveguide.

Here we report the integration of waveguide trapping with Raman spectroscopy. Raman spectra are acquired from particles propelled along the waveguide. Our focus was on investigating how fast particles can be identified, rather than acquiring highest-quality spectra. Recently, a gap in a waveguide loop was presented for holding particles at a fixed location [15]. Whereas waveguides propel particles forward, the gap is ideal for stopping particles for characterization. To compare this behavior with the direct characterization of particles above waveguides, we also acquired Raman spectra of particles held within the gap.

2. Experimental setup and procedure

The setup for our single particle characterization experiments is shown in Fig. 1. The setup uses laser source L_1 to couple light into the waveguide, and another laser source L_2 to excite Raman scattering. The use of two lasers provides independent control of microparticle propulsion and Raman excitation. Particularly, an independent laser for excitation controls excitation power and acquisition position, and in addition allows free-space optical trapping of particles. In total the setup consists of three parts, a waveguide section, a microscope section, and a spectrometer.

The waveguide section controls the propulsion of microparticles. It consists of a laser and three translation stages as can be seen in the bottom part of Fig. 1. The first translation stage is piezo-electrically controlled to optimize light coupling into the waveguide. The second stage holds the waveguide assembly and sample chamber, stably positioned by a vacuum suction cup. The third stage holds the objective lens that collects the output light for transmission to a photodetector. The beam from laser L_1, a high-power 1070 nm fiber laser,

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passes a beam expander to fit the entrance aperture of an IR coated objective lens (Nachet 80X NA0.9). This lens couples the laser beam into the waveguide. The evanescent field of the light interacts with microparticles in the sample chamber.

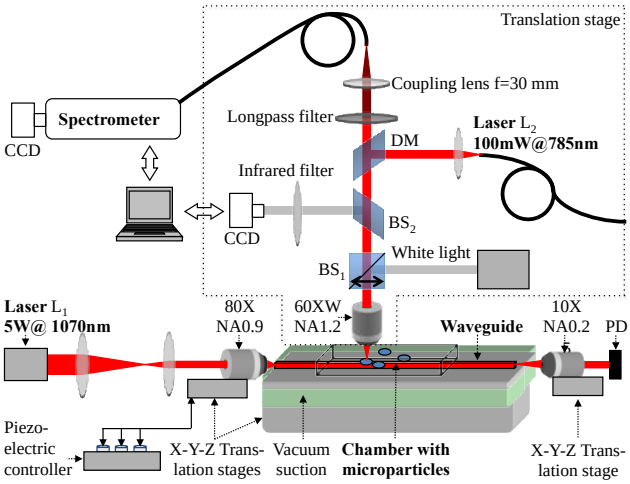

Fig. 1. Setup for waveguide propulsion and Raman spectroscopy. Laser L_1 (up to 5W at 1070 nm) is coupled into a waveguide with an IR objective lens (NA0.9). The waveguide is imaged with a modified microscope (inside the dotted line). Laser L_2 (100 mW at 785.8 nm) is focused with an objective lens (NA1.2) to excite scattering from particles on the surface. Scattered light is collected by the microscope, filtered, and sent to the spectrometer for analysis. A separate white light source and CCD camera is used to image the waveguide.

Light from the exit face of the waveguide is collected by an objective lens (Leitz, 10X NA0.25) for output power measurements. The input power was 700mW.

The microscope section controls sample imaging, Raman excitation and collection. The section is outlined by the dotted line in Fig. 1, and consists of a laser L_2, a white light source, a CCD camera and optical elements. It is based on an upright Olympus microscope, modified by standard and custom-made parts. The entire microscope is placed on a computer controlled translation stage. The beam from laser L_2, a fiber-coupled 785.8 nm solid state laser, is collimated onto a dichroic mirror DM. The mirror reflects the laser beam through beamsplitter BS_2 (pellicle 92/8) and beamsplitter cube BS_1. During excitation and collection, beamsplitter BS_1 is moved out of the beam path. The beam is focused onto the sample with an objective lens that also collects scattered light (Olympus water immersion 60X NA1.2). Scattered and Stokes-shifted light passes through the pellicle beamsplitter, the dichroic mirror (17 dB@785 nm) and a long pass filter (60 dB>792.9 nm), before a lens (NIR-coated, f = 30 mm) is used to couple it into a multimode fiber (Thorlabs, NA = 0.275). The fiber guides the Stokes-shifted light to the spectrometer.

For imaging of the sample, a white light source is used for sample illumination. Light enters the sample through a removable beamsplitter cube (BS_1). Beamsplitter BS_2 reflects collected light to a CCD camera connected to a computer.

The spectrometer is a Triax320 imaging spectrometer from Jobin Yvon. The spectrometer uses a 900 lines/mm grating with 850 nm blaze angle, giving 2 cm^{-1} resolution at 800 nm with a water-cooled (to –85°C), high-resolution (2048 × 512) CCD camera from Andor (Newton DU940N-UV) with quantum efficiency between 0.2 and 0.5. The spectral data are smoothed with a 5-point median filter and a 3-point average filter.

The waveguides used for particle propulsion were manufactured with a core of tantalum pentoxide on a silica-clad substrate. Rectangular strip waveguides were used, 7 μm wide and 200 nm thick. The waveguides were produced with ion-beam milling as described by Ahluwalia et al. [16]. The microspheres were polystyrene particles (n = 1.59) bought from

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Duke Scientific and Bangs Laboratories, and borosilicate glass particles (n = 1.56) bought from Duke Scientific. A dilute solution of microspheres in DI water was injected into a well (GeneFrame from ABGene, 25 µL, 250 µm thick). The wells were sealed with a glass cover slip.

In addition to straight waveguides, a waveguide loop with a gap was also used to stop the particles for characterization. The waveguide loop with an intentional gap at the center is shown schematically in Fig. 2(a). Particles are propelled along the branches of the loop and are delivered to the gap as shown in Fig. 2(c,d). The counter propagating light from the two opposite sides of the gap holds the particle stationary in the gap. A Raman spectrum of the trapped sphere is acquired by switching on the Raman laser (L_2) as shown in Fig. 2(e). Trapping by a gap has recently been studied experimentally and numerically [15]. For small gap dimensions, e.g. 2 µm, the interference between the counter-propagating beams can be used to trap a single particle with a diameter of 1-3 µm. For larger gap sizes, e.g. 10-30 µm, several particles can be trapped in the gap. In this case interference is less dominant and the trapping is weaker. For Raman-spectroscopy, a loop diameter of 100 µm and gap lengths of 2 and 10 µm were used. The waveguide branches of the loop were 1 µm wide. An input power of 500 mW is used in the experiment.

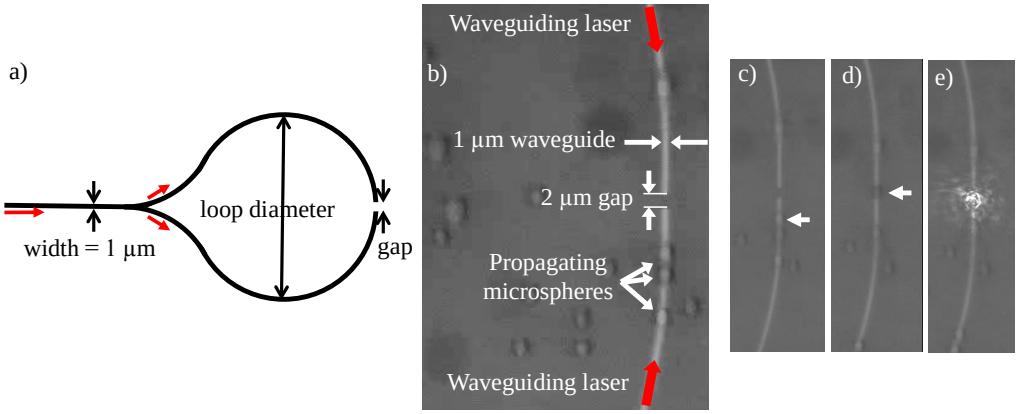

Fig. 2. (a, b) Illustration of gap design. c, d) A 3 µm polystyrene sphere is trapped and delivered to a 2 µm wide gap, e) Raman laser is switched on to excite Raman spectra. The diameter of waveguide loop is 100 µm. Online supplementary Media 1.

3. Results

Polystyrene and glass spheres with diameters of 7 and 8 µm, respectively, were propelled along a straight waveguide, as shown in Fig. 3. Light from laser L_2 was focused to a tight spot at a location of a few micrometers above the waveguide surface by a high NA water immersion objective lens. The optical forces imparted on the particle by the focused Raman beam were stronger than the radiation forces of the evanescent field from the waveguide. Consequently, when a sphere propelling along the waveguide reached the focus position (of Laser L_2), it was optically trapped and held in place by the focus. Scattering from the sphere in the strong focus produced strong Raman scattering, which was collected by the same high NA lens. After Raman signal acquisition, laser L_2 was blocked to release the microsphere, which continued along the waveguide.

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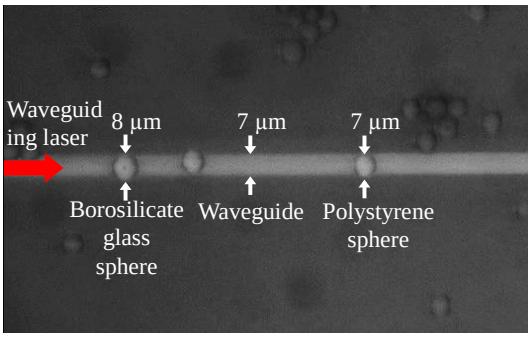

Fig. 3. Trapping and Raman spectroscopy of particles on a straight waveguide. Microspheres are trapped and propelled along the waveguide. Imaging with 60X objective lens. Online supplementary Media 2.

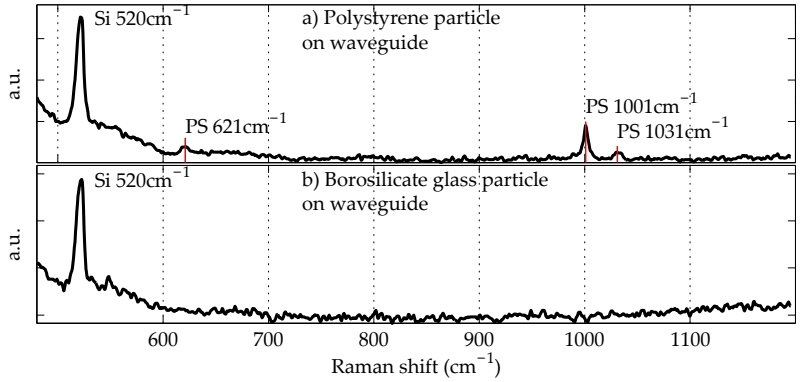

Fig. 4. Raman spectra acquired within 1 second exposure time from a) a 7 µm diameter polystyrene microsphere and b) an 8 µm diameter borosilicate glass microsphere. Three distinct polystyrene Raman peaks are shown in a).

Figure 4 shows spectra acquired in 1 second from a 7 µm diameter polystyrene sphere (Fig. 4(a)) and an 8 µm diameter borosilicate glass sphere (Fig. 4(b)). Three distinct Raman peaks of polystyrene can be seen in the polystyrene sphere spectrum; 621 cm^{-1} (benzene ring deformation mode), 1001 cm^{-1} (benzene ring breathing mode), and 1031 cm^{-1} (CH in-plane bending mode). The strong peak in the spectra at 520 cm^{-1} is attributed` to silicon, the substrate base material. Other spectral background contributions originate in the waveguide substrate, vitreous silica (SiO_2), and the waveguide material, tantalum pentoxide (Ta_2O_5).

The spectrum of the borosilicate glass sphere shows no peaks above the background signal, and is clearly distinguishable from the polystyrene sphere spectrum. The slightly smaller polystyrene sphere propagates faster along the waveguide than the borosilicate glass sphere. The observed size and propagation velocity differences (supplementary Media 2 attached with Fig. 3) between the spheres confirm the spectral differences.

In a separate experiment, a 2 µm diameter polystyrene sphere was propelled along a branch of the loop waveguide and into the gap in the waveguide. Laser L_2 was then focused at the trapped sphere. Scattered light was collected by the microscope for 2 seconds acquisition time.

After the Raman spectrum was acquired, both the lasers L_1 and L_2 were blocked, letting the microsphere diffuse away. When laser L_1 was turned on again and light was guided in the waveguide, a new microsphere was propelled into the gap as shown in the supplementary Media 1 attached with Fig. 2.

Figure 5 shows a spectrum acquired from a 2 µm diameter polystyrene particle trapped in a 2 µm gap. Figure 5(a) shows the spectrum at full scale, while Fig. 5(b) shows the same

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spectrum with the same axes as in Fig. 4(a). The dotted lines are set at equal heights in a) and b) so the signal levels can be compared.

The Raman signal is weaker from the 2 μm sphere compared with the signal from the 7 μm sphere on a straight waveguide. The increase in acquisition time from 1 to 2 seconds and the smaller sphere size lead to stronger background levels in the spectra.

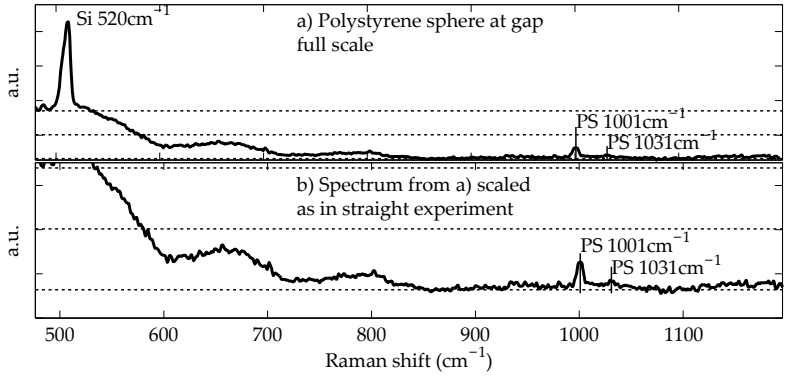

Fig. 5. Raman spectra acquired in 2 seconds from a 2 μm diameter polystyrene microsphere, trapped in a waveguide gap. a) shows the spectrum at full scale. b) shows the same spectrum with similar axes as in Fig. 5(a). The three dotted lines in each Fig. are inserted for comparison of signal levels in a) and b).

4. Conclusions and future work

We proposed a set-up that combines optical waveguide trapping and Raman spectroscopy. Using acquisition times of 1 second, Raman signatures from polystyrene microspheres propelled along the waveguide were clearly distinguishable from Raman signatures from borosilicate glass microspheres. Raman signatures from polystyrene microspheres propelled into a gap on a waveguide loop structure were acquired with 2 seconds acquisition times. With this, it has been demonstrated that the combination of Raman spectroscopy within waveguide propulsion is a useful technique for the characterization of microparticles trapped on and propelling along an optical waveguide. Waveguide trapping and Raman spectroscopy work well in combination, as both techniques are highly specific, handling the microparticles one at a time. Also, the long acquisition times of Raman scattering match the low velocity of the particles on the waveguide, meaning that none of the techniques slow down the characterization process more than the other. The ability to characterize microparticles propelled along the waveguide without interrupting the flow of particles could become useful for automated sorting and characterization applications, in particular for non-adherent cells. To ensure single particle characterization in a conveyor belt-like fashion, propulsion speed needs to be carefully matched to the spectrum acquisition time by considering laser power, waveguide width and particle concentration.

A waveguide loop was used for trapping and a focused beam from above was used to excite Raman-scattering. The evanescent field from the waveguide propels and delivers the particle towards the gap. As an extension of this method, the diverging light in the gap could be used both for trapping and exciting Raman spectra. This would simplify the set-up, requiring only one laser source, but would require a higher power at the gap. In this work, the optical waveguides were designed and optimized for 1070 nm and had relatively high losses at 785 nm. By reducing the losses, e.g. by increasing the waveguide width, the guided power can be increased and the gap can be used for both trapping and excitation of Raman spectra. We have done preliminary experiments with the existing waveguides and used the gap to excite Raman-scattering in ethanol by integrating for a longer period.

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Exciting and collecting spectra close to the waveguide surface increases the background levels and reduces the signal-to-noise ratio of the desired Raman signal. However, the waveguide, the waveguide substrate and the waveguide cover materials all have distinct Raman signatures. By acquiring background spectra, many of the spectral contributions from these regions can be subtracted from the microparticle spectra. As the scattering properties change when a particle is introduced, it is necessary to normalize the background before subtraction. Efficient normalization would be based on a unique Raman peak in the background spectrum to make the subtraction process independent of the scattering from the spheres.

Finally, deposition of metal colloids or a short metallic layer on the waveguide or in the waveguide gap for surface-enhanced Raman spectroscopy has the potential to significantly increase the signal.

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