Investigation of the Background Signal from 532 nm Excitation for Waveguide-based Raman Spectroscopy and Microscopy

(Student paper)

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ABSTRACT

The simple integrated waveguide has found many new applications recently, including waveguide enhanced Raman spectroscopy and chip based super-resolution microscopy. Chip based microscopy relies exclusively on visible light excitation, whereas Raman spectroscopy is done at a range of wavelengths. Here, we investigate two popular waveguide platform, Si_3N_4 and Ta_2O_5, for use at 532 nm excitation. We also present spontaneous Raman spectroscopy of methanol and iso-propanol using Ta_2O_5 strip waveguides and 532 nm excitation light.

Keywords: Waveguide, Raman spectroscopy, super-resolution imaging

1. INTRODUCTION

Integrated photonics is an important part of lab-on-a-chip technology and has already seen many applications. There are several potential advantages to using integrated photonics for e.g. biosensors, ranging from cost effectiveness and size reduction to increased sensitivity. Today, integrated photonics offers a vast number of different components, but a lot can be achieved with only the simplest component: the waveguide. Here, we focus on two recent developments in integrated photonics: waveguide based Raman spectroscopy and chip based super-resolution microscopy[1]. In both applications, the primary contribution of the waveguide is sample excitation. For fluorescence imaging applications, the excitation light will be between 405 nm and 660 nm. Raman spectroscopy, on the other hand, can be performed at both visible and near-infrared wavelengths. Lower wavelengths will increase the amount of Raman scattering and is thus favourable. Additionally, several interesting samples experience resonance Raman effects at lower wavelengths, giving a massive increase in Raman signal strength. Haemoglobin, the major component in blood, has resonance effects at around 532 nm excitation. In this paper, we discuss the challenges for both Raman spectroscopy and microscopy when moving towards visible wavelengths.

Whenever light is guided within a waveguide, a portion of the light will be guided in an evanescent field outside of the physical waveguide structure. Any sample that is in contact with the waveguide will thus interact with the evanescent field. The evanescent field intensity can be increased through careful choice of waveguide materials and design of the waveguide geometry. As the evanescent field decays over only a few hundred nanometers, it is ideal for e.g. monolayer sensing. In the field of super-resolution imaging, the high optical sectioning of the excitation is highly attractive, as it can reduce noise and yield better images by avoiding signal beyond the focal plane.

Recently, several different materials have been investigated for waveguide Raman spectroscopy. High refractive index contrast materiels (HIC) are preferred, not only due to the increased intensity of the field, but also because it offers a higher field confinement. The increased confinement allows for sharp bends and thus the waveguide can be structured in e.g. tight spiral structures. Si_3N_4 is today a well-established platform that has seen many application in the recent years, including waveguide based Raman spectroscopy. Dhakal et al. have demonstrated waveguide enhanced Raman spectroscopy using 785 nm excitation and Si_3N_4 waveguides[2]. Another material that has been investigated is TiO_2 as it has a higher refractive index[3]. A third option, is Ta2O5, has been used for Raman spectroscopy with 637 nm excitation light. Since most of the light is guided inside the waveguide structure, the excitation light will interact with the waveguide material. A Raman and auto-fluorescence signal can be generated from the waveguide material itself, which can be challenging for spectroscopic and imaging applications. We have investigated Si_3N_4 and Ta_2O_5 as possible platform going to visible wavelengths,

specifically 532 nm excitation. We also present spontaneous Raman spectroscopy of methanol and iso-propanol results using a Ta_2O_5 platform using 532 nm excitation.

2. Set-up and Background Measurements

The excitation light was coupled into the waveguides by end fire coupling through a 0.5 NA aspheric lens. All experiments were performed with a 532 nm laser (Verdi V10). Small PDMS frames were used to contain the sample to the region of interest on the waveguides and the frames were sealed off with a cover glass. Collection was done from the top through and a 60x/1.2 NA WI objective lens for the background measurements and a 40x/0.75 NA air objective lens for the hemoglobin measurements. Collection for spectroscopic measurements were performed using an Andor Holospec-F/1.8-VIS with a 532 nm edge filter (Semrock RazorEdge). For imaging, we used a Hamamatsu Orca Flash 4.0 v.2 CMOS camera. The system is sketched in Figure 1.

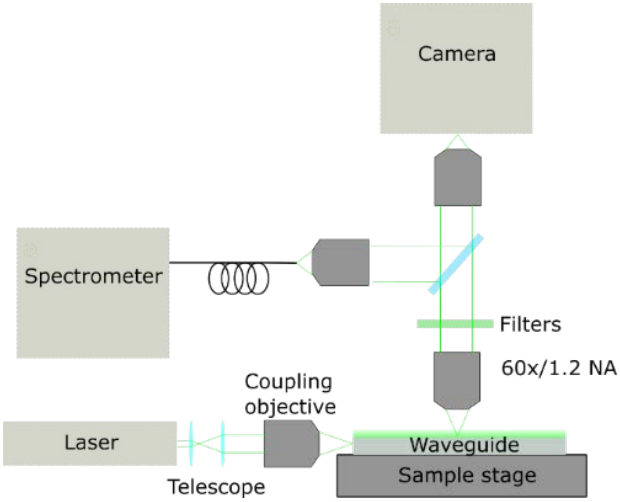

Figure 1. A 532 nm laser was coupled into the waveguide by end fire coupling through a 0.5 NA aspheric lens. A telescope was used to expand the beam prior to coupling. The waveguide was held by a vacuum chuck and placed on a three stage. The sample was placed in a PDMS chamber on the waveguide and collection performed from the top. A flip mirror was used to switch between imaging and spectroscopy.

High intensity in the evanescent field of the waveguide is desirable. Here, we employed 150 nm thick strip waveguides. The waveguides were 70 µm wide. Si_3N_4 waveguides were fabricated by first thermally growing a 2 µm SiO_2 layer in a silicon chip. Si_3N_4 was then deposited using low-pressure chemical vapour deposition at 800 °C[4]. The waveguide geometries were made by standard photolithography followed by reactive ion etching. Finally, the remaining photoresist was rinsed off. Ta_2O_5 waveguides were fabricated by first sputtering the Ta_2O_5 material, followed by conventional photolithography and finally ion beam milling was used to define the waveguides[5]. Post-fabrication annealing of Ta_2O_5 was performed at 600° C for 3 hours to further reduce the loss.

We observed a background signal from the Si_3N_4 at 532 nm wavelength, as seen in the image of the background in figure 2b. The Ta_2O_5 does not have any similar signal (figure 2a). Fluorescent beads were used to compare the background intensity with the bead intensities. Super-resolution optical microscopy through single molecule localisation requires fitting of fluorescent emitters and any background present may reduce the localisation precision. This can in turn lead to a loss in resolution. The background spectra of the two waveguides were collected to further investigate the background, without any beads present. The Ta_2O_5 background agrees very well with literature, with a main peak at approximately 680 cm^{-1} and very little background above 1000 cm^{-1}. The Si_3N_4 waveguide we used, on the other hand, has a broad and much stronger background. Based on the background results, we only used Ta_2O_5 for Raman measurements.

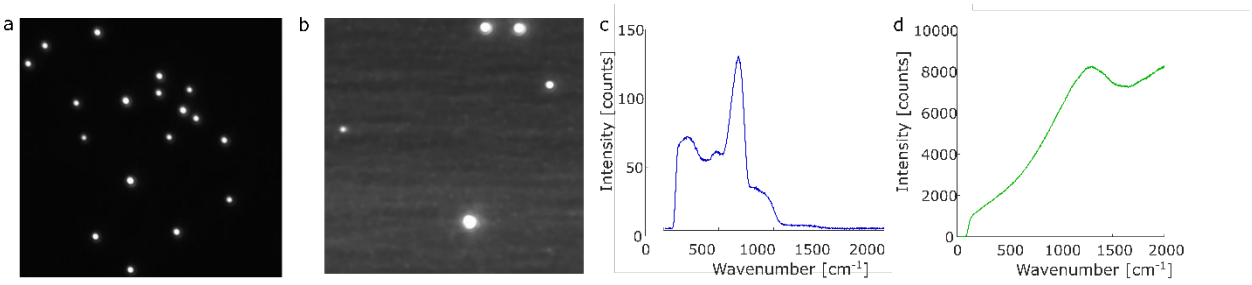

Figure 2. (a-b) Images of fluorescent beads on a 70 um wide strip waveguide of Ta_2O_5 and Si_3N_4, respectively. The contrast has been adjusted to make the bead intensities comparable. Both mages were taken through a 561 nm long pass filter. The multi-moded background pattern is clearly visible for the Si_3N_4 waveguide used. (c-d) Background spectra collected for Si_3N_4 and Si_3N_4, respectively.

3. Raman Spectroscopy

Using a Ta_2O_5 strip waveguide we measured spontaneous Raman scattering from methanol and iso-propanol samples with 532 nm excitation. 395 mW was launched through the coupling lens. The sample was filled the PDMS chamber and a cover glass was used to ceil it off. Collection was performed from the top and the resulting spectra are presented in figure 3. The two samples are easily distinguished by e.g. the peaks at 822 cm^{-1} (methanol) and 890 cm^{-1} (iso-propanol).

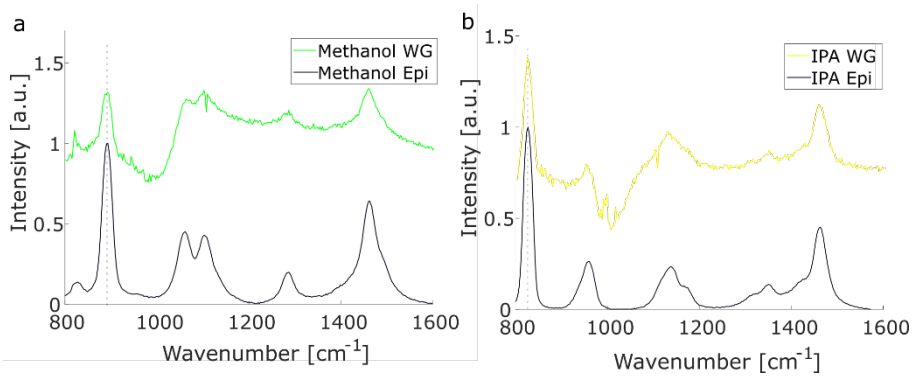

Figure 3. (a) Raman spectrum of methanol captured with waveguide excitation, compared to the spectrum collected using epi-illumination. (b) Raman spectrum of iso-propanol (IPA), with a comparison to epi-illumiation.

4. CONCLUSIONS

We have investigated Ta_2O_5 and Si_3N_4 as potential platforms for chip-based Raman spectroscopy and super-resolution imaging at visible wavelengths. Minimizing the background signal is preferential in both applications and, using the given fabrication procedures, Ta_2O_5 offers a significantly lower background signal. Using a Ta_2O_5 platform we have demonstrated spontaneous Raman using 532 nm excitation. The next step is to investigate the source of the background, as well as quantify the effect it might have on super-resolution imaging. It might be possible to reduce the background signal of Si_3N_4 waveguide platform for visible wavelengths by optimization of the fabrication process[6], which will be carried out in future work.

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