Detection of nanoplastic by surface-enhanced Raman microscopy

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Abstract

Currently the extent of nanoplastic in the environment can only be estimated by extrapolation from the plastic waste that can be detected. To be able to quantify the whole extent of the problem, detection methods have to be developed that can also identify particles that are smaller than 1 µm. Here we employ surface-enhanced Raman scattering (SERS) to image and identify single nanoplastic particles down to 100 nm in size. We obtain an experimental enhancement factor of more than three orders of magnitude measured on a single plastic particle instead of averaging over a concentration. Our results contribute to the better understanding and employment of SERS for nanoparticle detection and present an important step for the development of future sensors.

The global increase in plastic production and disposal has resulted in large amounts of plastic that end up in our environment. This was even further intensified by the recent COVID19 pandemic which required the widespread increased use of single-use-plastics for testing and personal protection [1]. Fragmentation of this plastic waste in the environment leads to micro- and nanoplastics which are dispersed even more easily and are harder to contain. Nanoplastics in particular are very difficult to detect because of their small size but can cause great harm as these particles are able to cross the blood-brain barrier in living organisms and even enter the placenta [2]. Harmful effects on human health that arise due to continued exposure to micro- and especially nanoplastic are therefore an active field of research [3]. As a first step in tackling the nanoplastic problem, suitable detection methods are required that can visualize the problem at hand. Thus far, the slightly larger microplastic is mostly detected by visual inspection, Fourier transform infrared (FTIR) spectroscopy [4] and Raman microscopy [5]. While visual inspection [6] is fastest and most widely used, it depends heavily on the interpretation of the observer and also lacks chemical information. Further, it cannot be applied for nanoparticles. FTIR and Raman spectroscopy are able to provide information on the vibrational structure of the detected molecules but still have some disadvantages. FTIR is restricted to larger particle sizes on the order of 20 µm and suffers from strong background signals from water which requires samples to be carefully dried. Raman microscopy works for smaller particles and can be used for spectroscopy in water but Raman scattering is an inherently weak process, with only about one in 10⁸ photons of the excitation beam contributing to the Raman signal [7]. Other methods such as thermal analysis yields chemical information but is destructive and information about number and size is not provided [8] and scanning electron microscopy (SEM) can provide information on the geometry of small particles.

Figure 1: Sketch of SERS substrate with polystyrene beads (left) and scanning electron microscope image of SERS substrate used for the detection of nanoplastic (right).
Figure 2: a.) and b.) show confocal images of 800 nm polystyrene particles on glass. In a.) the scattered Raman signal is detected whereas in b.) the backscattered laser light is collected. c.) shows the characteristic Raman spectrum measured on a single polystyrene nanoparticle (circled in the above image) and the zoom in shows the same spectrum taken with a bandpass filter to collect only the most significant Raman peak.

but requires careful sample preparation and makes it difficult to obtain chemical information. For these reasons, methods are required that offer good signal levels to ensure fast detection times, while still yielding information on the type of the analyte.

Here, we show that by employing surface-enhanced Raman scattering (SERS), the Raman signals can be vastly enhanced by more than three orders of magnitude and by combining SERS with confocal microscopy, we are able to image and identify single plastic particles down to sizes of 100 nm. While surface-enhanced Raman spectroscopy has shown its benefits in the detection of small molecular coverages [9, 10], it is only very recently that it has been applied for the detection of nanoparticles and aerosols [11, 12, 13, 14] and in particular nanoplastics [15, 16, 17, 18, 19, 20, 21, 22, 23, 24]. With our system we are able to image and identify single nanoparticles and can obtain the enhancement factor without the need to average over different concentrations. Hence, our results aid in advancing the understanding and employment of SERS for nanoparticle detection.

SERS enhancement is generally implemented in two different ways: either the analyte is mixed with a solution of plasmonic nanoparticles and then deposited on a non-SERS substrate [25, 21, 17, 19, 23] or a plasmonic substrate is manufactured beforehand and the analyte is then deposited on it [22, 24, 18, 20, 15]. The latter case has the advantage that in principle a higher reproducibility of the results should be possible. We therefore follow the latter method by employing a nano-patterned gold substrate purchased from Pico Foundry [26] which consists of 3D crossed gold nanowires that form a square grid with a side length of 100 nm (Fig. 1). In our case, the enhancement of the Raman signal is due to electromagnetic enhancement. This means that as gold has a plasmon resonance in the visible, localized surface plasmons get excited and thus light gets tightly confined by the gold nanostructure forming “hot spots”. The scattering of particles that sample these “hot spots” then also gets intensified by the plasmonic enhancement.

1 Results

Nanoplastics are commonly defined as particles with one dimension being smaller than 1 µm [27] and are present in the environment in a range of sizes. For our study we have taken measurements
Figure 3: a.) Raman spectra obtained from a single nanoparticle on a SERS substrate (top) and on a glass substrate (bottom). Two unbroadened Raman modes that are visible in both spectra are indicated via dashed lines. The inset shows the Raman spectrum on the SERS substrate with the bandpass filter to select the brightest ν(C-C) vibrational mode. b.) Agglomerates of and single 300 nm PS particles on the SERS substrate visualised via Raman imaging (left) and via recording the backscattered laser beam (right).

of polystyrene (PS) beads with diameters of 800 nm, 300 nm and 100 nm. We dilute the PS beads in ultrapure water (1 µl of solution with 4 ml of water) and deposit a drop on the SERS substrate (Fig. 1)). As a reference measurement, we also deposit beads on glass substrates. We illuminate particles with a 638 nm laser and collect both the backscattered laser light and the scattered Raman light simultaneously on two different single photon counting modules (spcm). The backscattered light field consists of a contribution which is reflected by the substrate and a contribution that is affected by any particle present on the substrate by being either scattered or absorbed. When this light field is imaged as a function of position via the confocal microscope, the particles thus appear as shadows. An example is displayed in Fig. 2 a.), where polystyrene beads with a diameter of 800 nm are imaged. Simultaneously we also collect the Raman scattered field by filtering out the excitation wavelength and sending it either to a spectrometer or another spcm. When sending the light at the locations of the particles onto the spectrometer, we then detect the known Raman spectrum of polystyrene (Fig. 2 c.)). To be able to take fast Raman images using the spcm instead of employing Raman mapping and maximising the contrast of the obtained images, we not only filter out the excitation laser by two longpass filters but also employ a bandpass filter that is centered on the brightest Raman line at 1003 cm$^{-1}$, corresponding to the ν(C-C) vibrational mode of polystyrene$^{[28, 29]}$. This enhances the ratio of Raman intensity to background signal. The spectrum with the bandpass filter introduced is displayed in the inset of Fig. 2 c.). When these

Figure 4: Comparison of confocal images taken of 800 nm and 300 nm polystyrene particles on a glass and a SERS substrate.
particles are placed on the SERS target some Raman modes get broadened (Fig. 3 a.) but the \( \nu(\text{C-C}) \) mode remains unaffected and can still be used for identifying the particles as polystyrene. In Fig. 3 b.) a 40 \times 40 \mu m \) surface-enhanced Raman image is displayed which shows agglomerates and individual 300 nm PS particles. Here, a log scale is applied for the colorbar to best visualise single particles as well as the brighter agglomerate. This stresses the importance of being able to image individual nanoparticles to make sure the detection method is really suitable for that particular size range. To compare these results to the Raman images obtained on a glass substrate, Fig. 4, shows confocal images taken of 800 nm and 300 nm polystyrene particles on a glass and on a SERS substrate. For the 300 nm particles, the conventional Raman signal is already very small and thus the particles are barely visible, whereas on the SERS substrate they can be clearly distinguished from the background (Fig. 4).

By directly using appropriate filters instead of employing Raman mapping, these Raman images are also obtained at a much greater speed. Fig. 5 shows an image of 800 nm PS spheres on the glass and the SERS substrate with an integration time of 10 ms/pixel. While on the glass, the particles in the Raman image are barely visible, the high Raman scattering count rate on the SERS substrate ensures a strong contrast between the Raman signal and the background. For the SERS substrate in principle an integration time of even < 1ms is feasible considering the scattering rate. In our case we are only limited by the way the piezo is implemented in our experimental control program. To evaluate the experimental enhancement factor of the SERS substrate and obtain the improvement of the images in terms of contrast, we fit the images to the point spread function of the scatterers (Fig. 6), which is well approximated by a two-dimensional Gaussian [30]. This yields the peak intensity of the Raman emission and we calculate the experimental enhancement factor.
Figure 7: Spectrum and surface-enhanced Raman image of a 100 nm PS particle.

The enhancement factor is defined as:

$$\text{EF} = \frac{I_{\text{SERS}}}{I_{\text{glass}}}$$  \hspace{1cm} (1)

where $I_{\text{SERS}}$ is the peak intensity of the particles on the SERS substrate and $I_{\text{glass}}$ is the peak intensity on the glass substrate for the same Raman band. In both cases the background has been subtracted. We find that the SERS substrate enhances the Raman signal by more than 3 orders of magnitude (Table 1). To our knowledge this is the highest enhancement factor yet that has been measured on single particles [22]. Although the photoluminescence of the SERS target is also higher than that of glass, we nearly gain one order of magnitude in the signal to background ratio. This is further visualised in Fig. 6, where a slice through an image is taken, depicting the particles as peaks in this 2D plot. The values for the enhancement factor are given in Table 1 for a laser power of $23.32 \pm 0.85$ mW at the target, which was the highest power used and the best in terms of contrast for the glass substrate. While we are only able to see Raman images of the plastic particles on glass for excitation laser powers of $>4$ mW, for the SERS substrate powers as low as $150 \, \mu$W show a clear signal. For lower powers the experimentally evaluated enhancement factor therefore represents a lower limit. In Table 1, the signal to background ratio is given for different particle sizes. There is a tendency that even for the SERS substrate this ratio decreases as the size of the particles decreases. This is not surprising as the size of the particles gets smaller and thus the fraction of light from our diffraction limited excitation laser beam of about $1.45 \, \mu$m that illuminates the particles with respect to the illuminated area of the background, decreases as well. However, due to the significant enhancement in Raman signal obtained from the SERS substrate we are still able to detect and image individual particles with a diameter of only 100 nm, which is far below the diffraction limited spot of our laser beam. A SERS image of a 100 nm particle and the corresponding spectrum with bandpass filter can be seen in Fig. 7.

| particle size | S/B glass | S/B SERS substrate | EF      |
|--------------|-----------|---------------------|---------|
| 800          | 1.849 +/- 0.098 | 6.425 +/- 0.839     | 979 +/- 189 |
| 300          | 1.116 +/- 0.033  | 3.743 +/- 1.165     | 2219 +/- 1109 |
| 100          | -         | 2.205 +/- 0.327     | -       |

Table 1: Experimental enhancement factor and the signal to background ratio (S/B) for single nanoparticles of different sizes on a glass or SERS substrate.

2 Conclusion

In summary, we have demonstrated the imaging and identification of nanoplastic particles down to sizes of 100 nm via surface-enhanced Raman microscopy. For particles of 300 nm and 800 nm we compare our measurements to Raman microscopy on a non-SERS substrate and find enhancement factors of more than three orders of magnitude. To our knowledge, these are the largest enhancement factors seen so far for individually imaged particles [22].

Employing SERS for nanoparticles is a very new field that has only recently shown very promising results [25, 21, 17, 19, 23, 22, 24, 18, 20, 15]. Previously, enhancement factors for SERS have
been determined for different concentrations of the analyte without any spatial information, which leaves a large uncertainty on the number of particles that actually contribute to the signal [17] and often the advantage of SERS is illustrated by stating a minimum detectable concentration of nanoparticles [20, 19, 23], which is still larger than the concentrations that are expected in the environment and often lacks spatial information. Being able to image single particles is therefore an important step towards developing precise sensors in the future and for understanding and optimizing SERS microscopy for nanoparticles. Instead of using Raman mapping we employ filtering of the brightest Raman band of the analyte to obtain direct Raman images using our confocal microscope and simultaneously image the particles by recording the backscattered laser light. Compared to Raman mapping which usually takes 1-10 s integration time per pixel, this is a vast improvement [15] in acquisition time as integration times of less than 10 ms/pixel are possible for an image of individual nanoparticles. Our way of imaging can be even further improved by employing more narrowband filters [31] possibly also for different Raman bands. In addition, if one wanted to record the whole spectrum for a wide wavelength range, spectra only need to be taken for those coordinates where bright particles are visible in the confocal images. Further, simultaneous imaging of the backscattered laser light lets us image particles down to sizes of 300 nm. In principle this resolution can be further improved by better control over the phase of the laser beam [32]. While not being particle specific, this way of imaging also allows a quick overview of interesting parts of the sample with minimal laser powers.

In a time where not much is known yet about the dispersion of these nanoparticles in the environment, our results not only aid in understanding and employing the SERS enhancement for nanoparticle detection in general but are also an important step towards developing a suitable detector to monitor nanoplastics in the environment in the future.

3 Methods

For all measurements of the PS nanoparticles (purchased from Sigma Aldrich), a 500 mW 638 nm laser is connected to our homebuilt confocal microscope setup via a fiber. The excitation beam is then focussed via a Mitutoyo 50x objective with an NA of 0.55 onto the sample, which can be scanned by a stepper motor and a piezo in closed-loop operation. The maximum power at the sample is about 24 mW. The signal is then collected by the same objective and transmitted through a dichroic mirror and 75 µm pinhole. It is split into two beams by a 50/50 beamsplitter, which are filtered and then coupled into two optical fibers which lead to two single photon counting modules. One of the beams is attenuated by neutral density filters before entering the detector, while for the other beam, we filter out the excitation beam completely by two longpass filters (LP 650 nm) and pick out the brightest part of the Raman band of polystyrene by a bandpass filter (BP 680 nm). The maximum scan range of the motor is 25 x 25 mm and that of the piezo is 100 x 100 µm. Typical pixel sizes of a piezo scan are between 100-300 nm. The typical time taken for closed-loop operation is 0.2 s per pixel, where 0.1 s integration time is used and the rest of the time is taken by the program to stabilize the piezo at the required position. To get an overview of a big area, the pixel size can be increased and the piezo can be operated without feedback with a lower integration time of down to 10 ms/pixel. Concerning our observed Raman scattering rate of the particles on the SERS substrate, integration times of less than 1 ms/pixel are possible. At the moment we are only limited by the way our piezo stage is currently implemented in our experimental control.

To obtain a wide Raman spectrum from about 290-4000 cm⁻¹, the Raman emission can also be sent to a spectrometer (Shamrock SR-303i, Andor Technology) instead of the single photon counting module and the bandpass filter is removed. We have used two different gratings of 300 l/mm and 600 l/mm and typical integration times are between 1-10 s.

4 Data availability

The data that support the findings of this study are available from the corresponding author upon request.

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