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IMPROVEMENT OF SERS SIGNAL MEASURED BY PORTABLE RAMAN INSTRUMENT USING RANDOM SAMPLING TECHNIQUE

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Abstract. In recent years, portable Raman spectrometers and commercialized surface-enhanced Raman scattering (SERS) substrates have become increasingly popular. They have turned out to be great tools for both substance detection, identification, and on-site analysis. SERS is one of the methods of resolving the bottleneck issue of Raman spectroscopy due to its inherent low signal level of the inelastic scattering of photons on molecules. The localized electromagnetic field at the hot spots on the SERS substrates helps to boost up the Raman signal many orders of magnitude. However, there is difficulty in the collecting Raman spectra using SERS substrates caused by the distribution of the hot spots and/or investigated molecules at their proximity and photodegradation processes. This work addresses the technique to collect proper Raman spectra using SERS substrates and portable Raman spectrometers. We propose a random sampling technique that gives representative and high-quality spectra with high intensity and good resolution. This technique was tested on a home-built portable Raman spectrometer and SERS substrates based on metal film over nano-spheres (MFON) structure. The experimental results showed that the peaks of the Raman spectrum collected using random sampling technique are significantly narrower than those of spectra measured by conventional method, and can prevent samples and SERS substrates from photo-induced degradation. Potentially, this method can promote quantitative SERS and chemical trace analysis using portable Raman spectrometers.

Keywords: surface-enhanced raman scattering, metal film on microspheres, raman sampling

Classification numbers: 2.1.1, 2.1.2, 2.5.1, 2.10.3

1. INTRODUCTION

Belonging to molecular spectroscopic techniques that can provide specificity and identification of the molecules through the frequencies of their vibrational modes, Raman spectroscopy has been widely used in laboratories for qualitative and quantitative analysis of substances [1]. In recent years, with the development of compact narrow-line diode lasers, miniaturized spectrometers, high-quality laser rejection filters, and powerful microprocessors, novel classes of handheld and portable Raman spectrometers have appeared on the scientific instrument market. These apparatuses allow the lab-grade measurements to be performed on-site instead of taking samples to the laboratories [2]. Moreover, portable Raman spectrometers can be integrated into the production lines to help control the manufacture of biological and chemical materials and optimize the production processes [3]. Accompanying the Internet of Things (IoT) platforms, portable Raman spectrometers are also expected to revolutionize smart sensors for process analytical technology (PAT) [4].

An inherent disadvantage of Raman spectroscopy is the weakness of the Raman signal [5]. Various techniques such as stimulated [6], coherent anti-Stoke [7], and surface-enhanced Raman scattering (SERS) [8] have been developed to overcome this drawback. Among the techniques mentioned, SERS does not require a complex ultra-shortlaser system. More importantly, SERS substrates can be designed and used with conventional Raman spectrometers. In general, SERS substrates can significantly enhance the Raman signals with an enhancement factor (EF) of 10^8 or even higher, enabling the detection of a single molecule [9, 10]. These advantages of SERS substrates complement portable Raman spectrometers making field chemical trace analysis possible. Many works on combining SERS substrates with portable Raman spectrometers have been reported in the last few years. Not surprisingly, their uses for medical studies [11 - 13] and food safety [14, 15] have been demonstrated.

Compared with the laboratory benchtop Raman spectrometers, portable devices are often equipped with lower-quality, uncooled photodetector arrays. Therefore, a higher laser intensity is required to compensate for signal quality. Unfortunately, this sometimes damages the samples, especially for dark materials and delicate SERS substrates due to the heat accumulation at the laser focus or other photo-induced sample degradation processes [16]. Another less serious undesirable effect is the broadening of Raman peaksand the background of the Raman spectra [16 - 18], which makes the automatic substance identification and component analysis functions based on calibrated spectral libraries of the portable/handheld Raman spectrometer less accurate or even failed. Designs of the portable Raman spectrometers are geared towards non-scientist operators such as customs and law enforcement officers whose works strongly rely on the automated functions of the devices. Therefore, the spectral distortion issues need to be addressed. Orbital raster scanning technique has been introduced for new types of portable Raman spectrometers to eliminate these undesirable effects [19, 20]. However, this technique is difficult to apply to conventional Raman spectrometers in general and especially to old portable/handheld Raman spectrometers since a laser beam scanning element must be added into the beam path of the spectrometers.

In this paper, we propose the use of a miniaturized XY scanning stage for random sampling SERS signal. On the one hand, this technique allows us to measure a spatially averaged spectrum over a relatively large area of the samples. On the other hand, it helps to prevent samples and SERS substrates from laser-induced damages. The experimental data also showed that this technique allows tens of milliwatts of the laser beam to be used to excite the SERS samples while keeping the resolution. It is worth mentioning that this miniature XY scanning stage can be easily integrated into most Raman spectrometers and helps to exploit the full potential of portable/handheld spectrometers and SERS substrates.

2. EXPERIMENT

SERS substrates based on the metal film over nano-sphere (MFON) structures were used throughout this work. The fabrication and optimization of SERS substrates by tuning polystyrene (PS) bead sizes and metal film thickness for the 638 nm excitation laser have been reported in other works [21 - 23]. In short, a close-packed monolayer of the 477 ± 7 nm PS beads was self-assembled on the water/air interface then transferred onto clean silicon substrates. Finally, a 200 nm silver film was deposited on top of PS beads by an e-beam evaporator (Leybold Univex 400). Figure 1b shows the top-down and cross-sectional SEM images of the SERS substrate fabricated and used in this work. The quartz crystal microbalance integrated into the vacuum chamber was used to monitor the thickness of the silver film. The silver film thickness of 200 nm was chosen to guarantee suppression of the background due to underlayer PS beads while remaining high SERS enhancement of the MFON structure.

Raman spectra were recorded by a home-built portable micro-Raman spectrometer (Figure 1a). A 638 nm diode laser (Ondax Surelock round laser module) was used to excite the Raman scattering spectra of the studied samples via a 10x/0.25 microscope objective. Raman signals were collected in the back-scattering configuration by the same objective. After passing through a long-pass filter to block the parasite laser radiation, signals were focused on the round end of a round-to-linear fiber bundle (7 × Φ105 μm core fibers) while the linear end of the fiber bundle was aligned parallel to the 50 μm wide entrance slit of the AvaSpec-ULS2048 miniaturized spectrometer. The spectral resolution of the Raman spectrometer is 15 cm^{-1}.

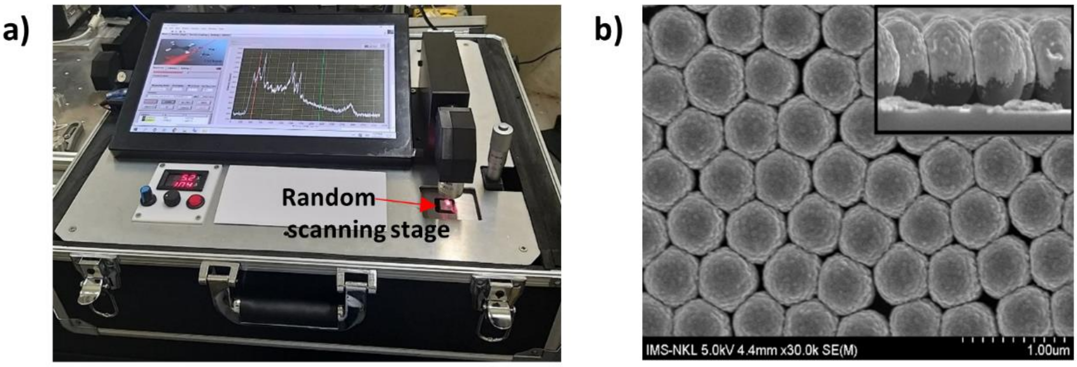

Figure 1. a) Home-built portable Raman spectrometer equipped with a random scanning stage; b) Top-down view and cross-sectional SEM images of the SERS chip made of silver film over 477 ± 7 nm polystyrene nano-spheres (Ag-MFON).

Rhodamine 6G molecules were used to investigate the influence of the sampling modes (single spot versus random sampling) on the quality of the collected surface-enhanced Raman spectra. Equal volumes (5 μL) of the 10^{-6} M rhodamine 6G solution were dropped onto the SERS chip and left to dry under ambient conditions. After that, the samples were placed on a 2-axis motorized stage. In the single spot sampling mode, the stage is not moving. The Raman spectrum is collected from one spot during the whole spectrum-integration time. In contrast, in the random sampling mode, during the spectrum-integration time, the sample was continuously moved so that the laser spot randomly scans an area of 1×1 mm^2 on the surface of the samples with a speed of 1.5 mm/sec. In general, this technique allows us to measure spatially averaged Raman spectrum over a relatively large sample area with the largest scan region of 3×3 mm^2. Besides, it helps prevent the sample from damages due to laser-induced heating or photodegradation. The baselines of the Raman spectra were automatically removed by a self-developed Labview routine implementing an adaptive polynomial fitting algorithm [24]. The spectrum-integration time of one second was chosen to ensure that the signal-to-noise ratio is high at low laser power excitation, and the luminescence background does not saturate the detector when the laser power is high.

3. RESULTS AND DISCUSSION

As shown in Figure 1b, the fabricated SERS substrates based on the MFON structure possess high uniformity. Their SERS active hot spots, where the localized electromagnetic field is concentrated, mainly appear in the adjacent area between neighboring metal-capped spheres [22, 25]. Therefore, the density of the hot spots is well defined, making the Raman spectrum measurements with this type of SERS substrates more reproducible and repeatable. The 477 nm size of the PS beads was chosen to match the 638 nm excitation laser [23]. Theoretically, the 10x/0.25 microscope objective produces a laser spot with a diameter of 3 µm on the sample placed at the focus. This laser spot covers about three circles of PS beads, with nearly 90 hot spots being exposed at the same time. In fact, the laser spot size is bigger, around 10 µm. Thus, the Raman signal from a large number of hot spots can be collected simultaneously, expectedly giving a decent spatially averaged spectrum.

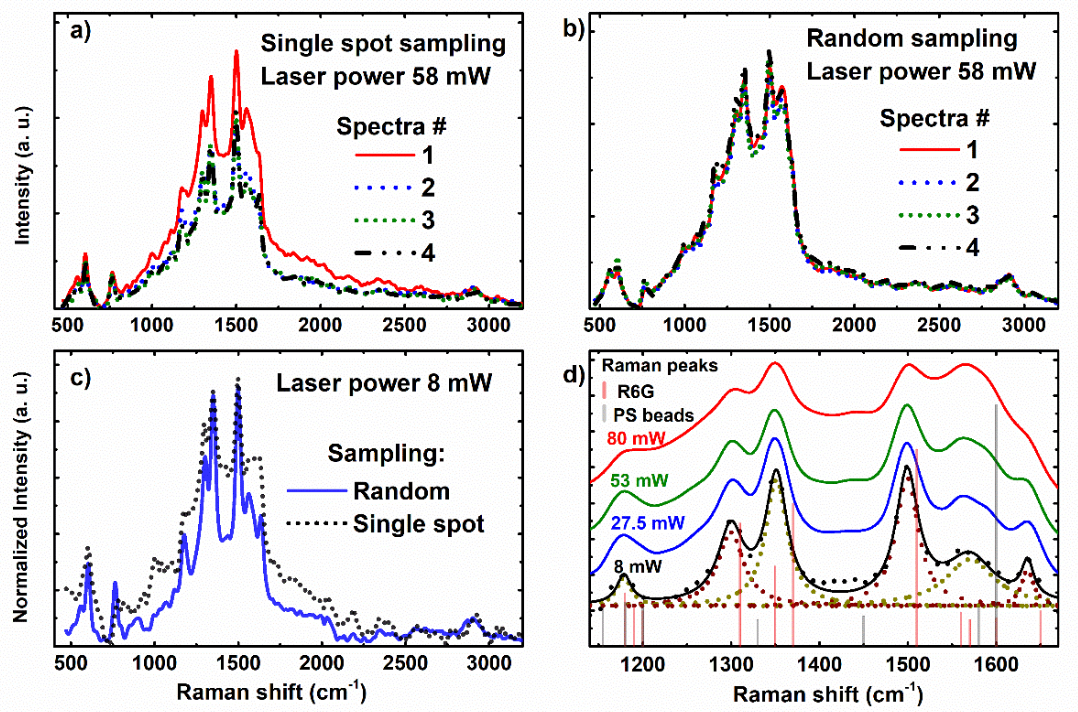

Figure 2.a,b) Raman spectra of rhodamine 6G measured with Ag-MFON SERS substrates and portable Raman spectrometer under single and random sampling modes, respectively. c) When excited by the same laser power, peaks of Raman spectrum collected using random sampling technique are significantly narrower than those of spectra measured in single spot sampling mode. d) Raman spectra under different excitation laser power. Raman peaks broaden as the laser power increases. The solid curves are experimental data, and the dotted curves are individual and accumulated Lorentzian fitted peaks. Vertical lines are tabulated Raman peaks of the PS beads and rhodamine 6G [26, 27].

Figure 2a shows Raman spectra of the rhodamine 6G on the SERS substrates. Spectra were collected from the same position on the sample (in the single spot sampling mode) and taken automatically in turn, with one second spectrum integration time. The laser intensity was set to 58 mW, measured at the sample's position. This laser intensity is about 70 % of its maximum power. As aforementioned, Ag-MFON SERS substrate has high uniformity, and the laser spot covers many hot spots; one can expect the stability of the signal. However, the spectra show significant fluctuations with a tendency to decrease, implying that the laser has hastened the degradation of the sample and/or SERS substrates.

Figure 2b presents the Raman spectra of the same sample under similar experimental conditions, except that the XY stage was randomly moving (in the random sampling mode). In contrast to Figure 2a, now the spectra are almost overlapping. The movement of the stage guarantees that the signals from the fresh areas are collected and prevents the samples from laser-induced damages. As demonstrated, random sampling mode significantly improves the stability and reproducibility of the Raman spectra. This high stability of the signal also suggests the possibility of using the random sampling technique to improve quantitative SERS analysisusing portable Raman spectrometers [28, 29].

Alternatively, lowering the laser power to prevent the samples from being damaged is also a common practice. Figure 2c shows the Raman spectra collected when the laser power was set to the minimum of 8 mW. The solid and dotted lines present the spectra recorded using a random and single spot, respectively. It is clear that the peaks of the Raman spectrum collected using the random sampling technique are significantly narrower than those measured in the single spot sampling mode, resolving the main peaks.

Further, the stability of the Raman signals, thanks to using random sampling mode, allows us to study the influence of the laser power spanning from 8 to 80 mW on the shapes of the spectra. Figure 2d shows some representative spectra collected at different laser powers to demonstrate the effect. Indeed, the spectra were collected at the finner laser power step. The analysis data of the complete spectrum set will be presented below. Plotted vertical lines present Raman spectral positions of the rhodamine 6G and polystyrene beads. Our portable Raman spectrometer does not have enough resolution power to resolve all spectral lines. However, collected spectra agree quite well with the spectrum of the rhodamine 6G [26]. No characteristic

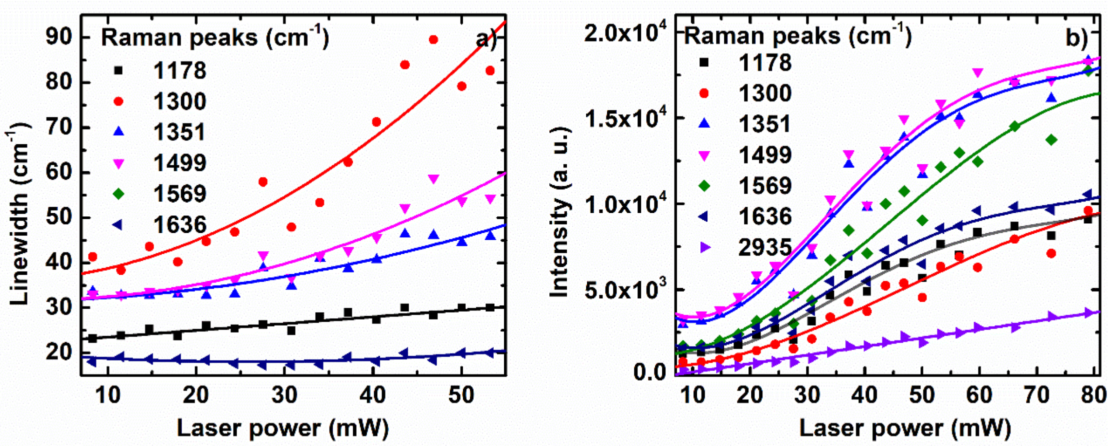

Figure 3. a) The linewidths of the Raman peaks accessed from Lorentzian fitted data and b) the amplitudes of these peaks at different laser excitation powers, respectively. The spectral linewidthsgrow monotonically while the peak intensities are saturated as the laser power increases.

PS beads peaks were observed, indicating that the silver film completely covers the PS beads layer. As the laser power increases, the line width of the rhodamine 6G spectra broaden noticeably. The Raman peaks intensities also increase accordingly but at different rates. At low laser power, small peaks at 1178 and 1636 cm^{-1} appear clearly. However, at a high limit, these peaks merge into the shoulders of the larger peaks and are barely noticeable.

In order to get more insight into spectral line broadening, laser power-dependent spectra set was thoroughly analyzed. Each spectrum in the range from 1140 to 1660 cm^{-1} was fitted by a sum of six Lorentzian peak functions [30]:

$$y = y_0 + \sum_{i=1}^{6} \frac{2A_i}{\pi} \frac{w_i}{4(\lambda - \lambda_{ci})^2 + w_i^2}$$

where y_0 is the baseline, A_i, w_i, and λ_i are amplitude, peak width, and central wavelength of the ith peak, respectively. The black dotted curve is the accumulation of the peaks (Figure 2d). It fits pretty well with the experimental data with a coefficient of determination R^2 of more than 0.95. However, the fitting diverges at a laser power of more than 55 mW. The fitted linewidths w_i are plotted against laser power in Figure 3a. In contrast, figure 3b shows the dependence of the intensities of the peaks taken from the spectra. The SERS Raman line broadening mechanism can be due to the electron transfer from the Fermi level of silver to the molecule’s excited states [16] or because of a raised temperature at the laser exposure spots [17]. The spectral resolution of the portable Raman spectrometer is quite low to reveal the physics behind it. However, the effect of the laser power is noticeable. It is evident that the spectral linewidths monotonically grow but the SERS Raman peaks’ intensities are saturated. Therefore a good practice is to work with as low as possible laser power.

4. CONCLUSION

This work demonstrated the advantages of using the random sampling technique for collecting Raman spectra using portable Raman spectrometers and SERS substrates. Experiments were conducted with a home-built portable Raman spectrometer and MFON SERS substrates collecting Raman spectra of the rhodamine 6G in a single shot and random sampling modes. In addition to facilitating the collection of signals from large interrogation areas of the sample, random sampling delivers higher intensity spectra while maintaining the resolution compared to conventional point and shot practice. Since the prototype miniaturized XY random scanning stage can be easily integrated with any portable/handheld spectrometers, we believe that this technique will capacitate portable Raman spectrometers and SERS substrates for on-field qualitative and quantitative chemical trace analysis, further promote their uses for real-life applications outside of the labs.

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CRediT authorship contribution statement. N.V.T.: Methodology, Investigation, N.T:N.: Formal analysis. N.T.H.L: Formal analysis, Supervision. V.D.: Formal analysis, Visualization. D.Q.H.: Review and Editing., D.C.D.: Investigation, P.N.N.: Review and Editing, N.M.H.: Funding acquisition, Supervision, Writing—original draft preparation.

Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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