Investigation of the reproducibility and repeatability of commercial SERS substrates

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Abstract

Surface-enhanced Raman spectroscopy (SERS) is a powerful tool for the observation, the detection and the identification of chemical or biological species at low concentrations due to it's high sensitivity, specific fingerprinting spectra and real-time detection. However, an important task is to define a suitable and reliabale protocol to ensure the reproducibility and repeatability of the SERS measurements in teh framework of SERS sensors.

In this paper , a special protocol of measurement have been applied to a commercial SERS Hamamatsu substrate from Hamamatsu Photonics by using diluted solution of 4-mercaptobenzoic acid (MBA) at excitation wavelengths 633nm. This protocol consists in recording 25 SERS maps equally distributed on the whole surface substrate. For each map, 16 spectra have been measure to calculate an average SERS signal of the map. A large set of recorded data for a total of 400 spectra have been collected and analyzed by using the integrated intensities of MBA bands to determine the reproducibility and repeatability of the substrate. This protocol could be applied to other substrates and to precisely evaluate their sensing performances.

1. Introduction

Surface-enhanced Raman spectroscopy (SERS) has been largely used for decades in various application fields such as food quality control[1],[2], environment control[3],[4], biomolecule analysis[5],[6] or medical detection[7],[8],[9] due to its high sensitivity and specific fingerprinting for various molecules[10],[11]. Compared to normal Raman spectroscopy, this phenomenon can induce a huge signal amplification of the Raman signal with enhancement factor as high as 10^{10} [12] and as a consequence allows the identification and the detection of various analytes[13],[14] at very low concentrations. Therefore the SERS substrates play an important role for SERS detection and the development of miniaturised sensors paving the way of several applications as on site detection or point of care[15]. However, the SERS is strongly dependent upon the employed metallic nanostructures: surface substrates or colloidal nanoparticles[16],[17],[18] as the enhancement of the Raman signal of the analyte molecules are strongly related with the localized surface plasmon resonance[19],[20],[21],[22],[23] of the excited nanostructures [21],[24].

Various production techniques of SERS substrates have been carried out to control the size of the metal nanostructures, the optical and near-field properties of plasmonic nanostructures and the surface chemistry. Such methods can be electron-beam lithography, focused-ion lithography, scanning probe lithography, laser interference lithography, nanosphere lithography or nanoimprint lithography[25],[26],[27],[28],[29],[30],[31]. Even with all these techniques, they are still produced at the laboratory experiment scales and are often cost effective. The commercial SERS substrates could be an alternative to overcome the large-scale and high-throughput issues [32],[33]. In recent years, several companies proposed some commercial SERS substrates. A non-exhaustive list is summarised in the table 1.

Company	Products
Ocean Optics	Paper-based gold (Au) or silver (Ag) nanoparticles substrates and the glass-based gold/silver film
Enspectr	Green&Red Substrates and Blue&Green Substrates
The Q-SERS™	Nanova
Hamamatsu	Commercialization of SERS substrates fabricated by the nanoimprint technology and dedicated to point-of-care analyze

From all the commercial substrates, Liu et al. [34] have found that the Enspectrc, Q-SERS™ and Hamamatsu substrates are the more reliable ones, enabling their use as SERS platforms for detection issues. They performed SERS mapping with three probes molecules: 4-mercaptobenzoic acid (4-MBA), 1, 2-bis (4-pyridyl) ethylene (BPE) and methylene blue (MB). They found that the Hamamatsu samples proposed the best reproducibility (with a SERS signal deviation close to 5% around the average SERS signal). This study have been extended by Azziz et al[35]. They have chosen three commercialized substrates: RAM-SERS-SP from Ocean Optics, Q-SERS from Nanova Inc. and Hamamatsu from Hamamatsu Photonics. They deposited increasing concentrations (from 1nM up to 100 μM) of one probe molecule (the 4-mercaptobenzoic acid, MBA) and used two different excitation wavelengths (633 and 785nm). They performed 4 SERS maps of 25 points (square map of 5x5 points) randomly distributed at the SERS surface. They demonstrated that the best SERS sensing performances (limit of detection, limit of quantification and sensitivity) were reached by the Hamamatsu substrate for an excitation wavelength of 633 nm[36], However, they measured a SERS signal deviation larger than the one observed by Liu et al.[34] Recently, a new study of commecial SERS substrates were published by Rahmani et al.[37] They study Hamamatsu, SERSitive (Premium Ag–Au supports) and Ocean Insight (RAM–SERS–Au) substrates. They found some limits of detection in the nM range for crystal violet and thiophenol probe molecules. The study of the performances of SERS commercial substrates is then an active field and is of first importance to be able to use them in SERS applications as sensors.

In this paper we have directly studied the Hamamatsu SERS substrate due to its sensing performances determined by the former studies. Our objectives were to determine the reproducibility as well as the repeatability of the substrates on a wide surface. Indeed, the total surface of the Hamamatsu substrate is 2.7x2.7 mm^2. For the other studies, the investigated surfaces were limited to specific zones and were not representative of the whole surface. We then proposed a specific methodology to extract the relevant SERS performances of the substrate and we were able to explain the apparent contradiction between the Liu et al.[34] and Azziz et al.[35] papers. For this investigation, we have chosen the 4-mercaptobenzoic acid (MBA) as probe molecule. It was deposited on the surface with a concentration of 10^{-4} M to saturate the surface. The MBA is well known to provide high SERS signal and to define a specific protocol to provide a representative view of the whole surface. To do this, we carried out 25 SERS maps equally distributed on the whole surface. For each map, we measured 16 SERS spectra to calculate an average SERS signal of the map. We then recorded a total of 400 spectra and 25 representative localisations on the substrate surface. The integrated intensity has been analyzed to determine the reproducibility of the substrate. The proposed protocol can be applied to other large SERS substrates and for the evaluation of their sensing performances.

2. Materials and methods

2.1. Chemical and materials

The Hamamatsu substrates were purchased from Hamamatsu photonics K. K. (Hamamatsu City, Japan). The 4-mercaptobenzoic acid (MBA) was purchased from Sigma Aldrich (Darmstadt, Germany, purity: 90%)

2.2. Samples preparation

A solution of 1mM of MBA was obtained by dissolving 3mg of MBA powder in 180 mL of ethanol.The MBA solution with a concentration of 10^{-4} M was obtained by a dilution of the raw MBA solution with 1:10 (volume) as a dilution factor.

The SERS substrates were totally immersed into 300μL of MBA solution for overnight at room temperature. They were washed thoroughly with deionized water to remove the unbound molecules, and then dried at room temperature for 2 hours.

2.3. SERS measurements

The SERS spectra were collected by using a Raman spectrometer (WITec alpha 300 R) equipped with a confocal microscope. The SERS spectra were recorded with a x100 magnification objective (Numerical aperture = 0.9, Zeiss) in backscattering configuration. One excitation wavelength (λexc) was used: 633nm (HeNe laser, Research Electro-Optics) with a spectral resolution of 1.77cm^{-1}. We reduced the laser power used on the Hamamatsu substrate to avoid molecular degradation induced by photochemical or thermal effects. The laser power and the acquisition time were fixed at 0.3mW and 10s, respectively.

We performed 25 SERS maps equally distributed on the whole surface of the substrate (Fig. 1). The mapping areas were 8 × 8 μm2 with step size of 2 μm (4 × 4 points for each map). It takes around 3 minutes to record each map.

The intensity of the two main SERS bands of the MBA (1075 cm^{-1} and 1585 cm^{-1}) were measured by fitting the bands with a Lorentzian profile using the Origin software. To be able to compare the SERS signal from the different plasmonic substrates, the intensity of all spectra was divided by the acquisition time and the laser power.

3. Results and discussions

The whole Hamamatsu surface were imaged with an optical objective X10 (WITec alpha 300 R) (Fig. 1). The substrate formed an homogenous surface of gold nanostructures. The chip size is 4000x4000 μm^2 on which the SERS active area measures 2700x2700 μm^2 and 25 points have been selected on the active aera (blue crosses on the Fig. 1). The gap between each point is fixed to 500 µm. This point distribution allows to cover nearly the whole surface and extract the SERS signal from a number of localisations sufficiently representative.

The SERS maps on the 25 positions were first carried out without MBA to record a reference spectrum of the substrate (Fig. 2a). Then the substrate was incubated in a solution of MBA (300µL with a concentration of 10^{-4} M) for an overnight and thoroughly washed with deionized water. To determine the reproducibility of the SERS measurements on this substrate, we performed 25 maps of 16 spectra (400 spectra in total) for each measurement. To determine the repeatability, the SERS measurements were done twice, meaning that we redo a second time the SERS measurement using the same protocol (25 maps of 16 spectra) on the same substrate. Subsequently, what we will call a SERS measurement will then correspond to collection of the 25 maps. The localisation of the 25 maps of the second SERS measurement could be slightly shifted compared the first SERS measurement as the positioning of the SERS substrate under the microscope could not be exactly reproduced. The average spectra of each SERS measurement are presented on the Fig. 2. They were calculated from the 400 Raman spectra of each SERS measurements. Both average SERS spectra are dominated by two strong SERS peaks at 1075 cm^{-1} and 1586 cm^{-1} which are both assigned to the ν_{C-C} ring-breathing mode [38]. It can be noticed that the two spectra are similar with the same position and intensity for the spectral bands. To confirm this, we compared the intensity of both bands for the two SERS measurements (Fig. 2b). The average integrated intensities of the 1075 cm^{-1} and 1586 cm^{-1} peaks have been calculated from the 400 spectra of each measurement. As shown in Fig. 2, the SERS intensities are similar in both cases and the differences are lower than 4.4%.Thus the average SERS spectra recorded on the whole substrate are very reproducible even if the 400 SERS spectra are not located at the same position.

In a second step, for each of the 25 mappings, we calculated the average intensity (I_{ave}) of the 1075 cm^{-1} band as well as the relative standard deviation (RSD) from the 16 spectra obtained from each SERS mapping. The RSD were then used to calculate the uncertainties, σ, and the error bars assuming a confidence constant of 1 ($RSD = \sigma$ and $I_{SERS} = I_{ave} \pm \sigma$). [34]. The result is shown on the Fig. 3. The 25 maps have various integrated intensities from 500 up to 6000. There is a strong variation of the SERS signal at the surface of the sample. However, the relative standard deviation is limited for the majority of the positions and is lower than 20%. Only few positions (from 20 to 24 positions) exhibit large standard deviations (larger than 20%). Thus the Hamamatsu substrate present some high reproducibility on small surface (lower than 10x10 μm^2) as observed by Liu et al.[34] but it loses its reproducibility when the complete surface is considered as observed by Azziz et al.[35] This can be due to the inhomogeneity of substrate surface and can explain the apparent contradiction observed between Liu et al. and Azziz et al. on the standard deviation of the SERS signal.

In a third step, we studied the repeatability of the measurements. For this, we recorded the SERS measurement two times and we compared the integrated intensities of the 1075 cm^{-1} and 1586 cm^{-1} bands calculated from the 16 spectra obtained for the 25 maps (Fig. 4) for both SERS measureaments. For both bands and both SERS measurements, we get a large variation of the SERS signal on the whole surface. Moreover, the 25 average intensities measured for the second SERS measurement are different from the first one whereas the localisation of the 25 maps are slightly shifted. This confirms the limited reproducibility of the substrate on large surface.

Thus in summary, the Hamamatsu substrate exhibits a high reproducibility at the scale of few micrometers but a low reproducibility at the scale of the whole surface. However, even if we observed some large variation of the SERS signal at different locations on the surface, it is remarkable that the average signal on the whole surface is highly reproducible with few deviation between two sets of measurements. This is of high importance for potential application of such substrate for SERS sensor. Indeed, the detection of one specific molecule can be reproducible and the molecular concentration quantified if the whole surface of the substrate is scanned as proposed by our measurement protocol.

Conclusions

In this paper, we studied the reproducibility and the repeatability of commercial Hamamatsu SERS substrates from Hamamatsu Photonics. By using diluted solution of 4-mercaptobenzoic acid (MBA) and an excitation wavelength at 633 nm, we propose a specific protocol to measure the SERS signal on the whole surface of the substrate splitted in 25 SERS maps of 16 spectra. With such a protocol, we can measure the local variations of the SERS signal at the micrometer scale as well as its variation at the scale of the whole surface. We were then able to demonstrate that the SERS signal is highly reproducible (signal deviation lower than 10%) at the local scale (lower than 10 μm^2) but not at the large scale (substrate scale) with signal deviation higher than 50%. We also made two SERS measurements on the surface and we determine that the SERS signal is not repeatable at the local scale but it can be repeatable at the whole substrate scale as the average SERS intensity is identical for both SERS measurements. Therefore, to have an accurate result and the best performances of the Hamamatsu substrate, the average SERS signal on the whole surface has to be considered.

Declarations

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Disclosures. The authors declare no conflicts of interest.

Data availability. Data underlying the results presented in this paper are not publicly available at this time but may be obtained from the authors upon reasonable request.

Author contributions. QL and AA made the SERS experiments; QL, AA, CA, MM, FA, ME and ML prepared the SERS experiment methodology; QL, AA, CA, MM, YX, WF, FA, ME and ML analysed the results; QL and AA have written the original draft; YX, WF and ML get the fundings; all the authors have reviewed the paper.

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Figures

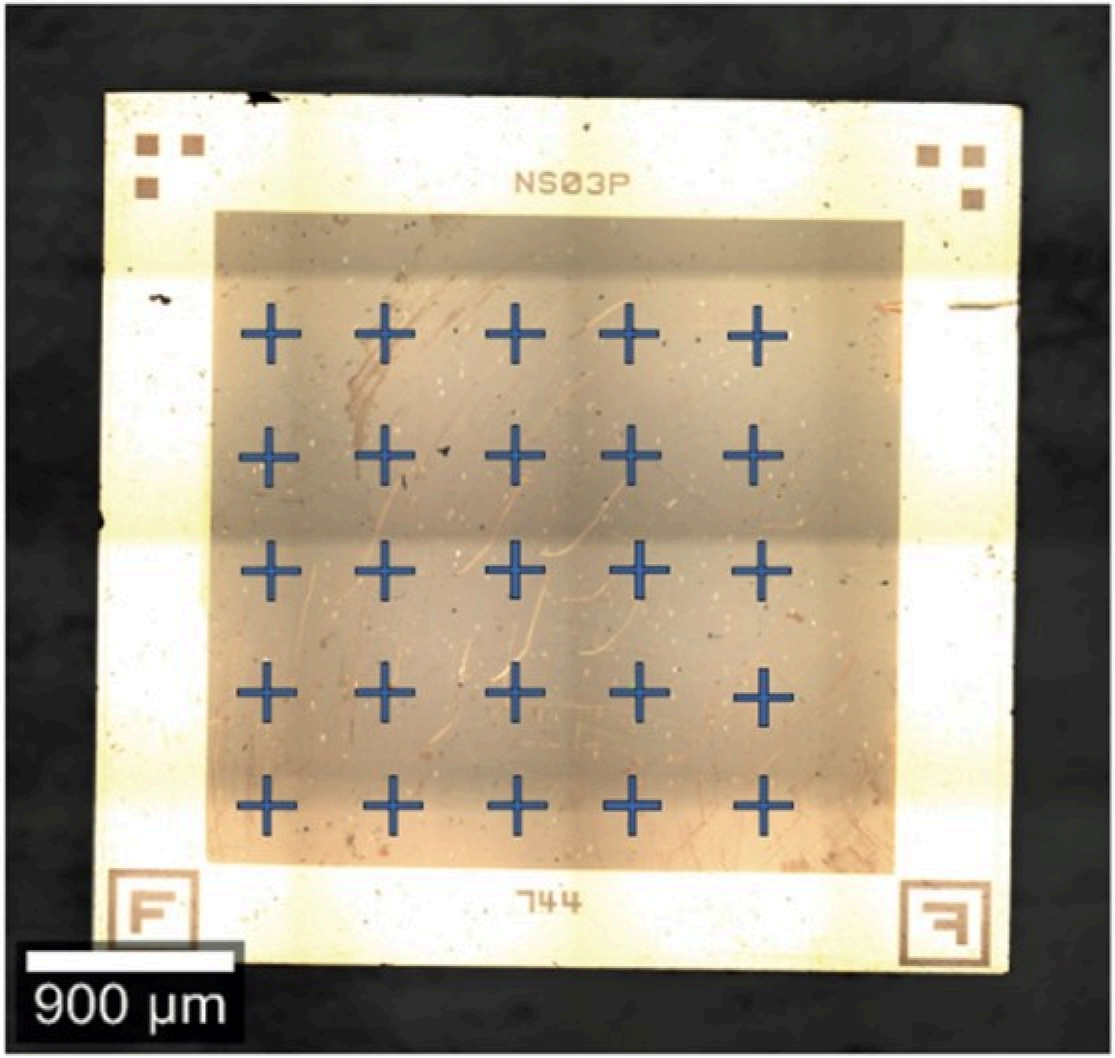

Figure 1

Localisation of the 25 SERS maps performed on the Hamamatsu SERS substrate (blue crosses)

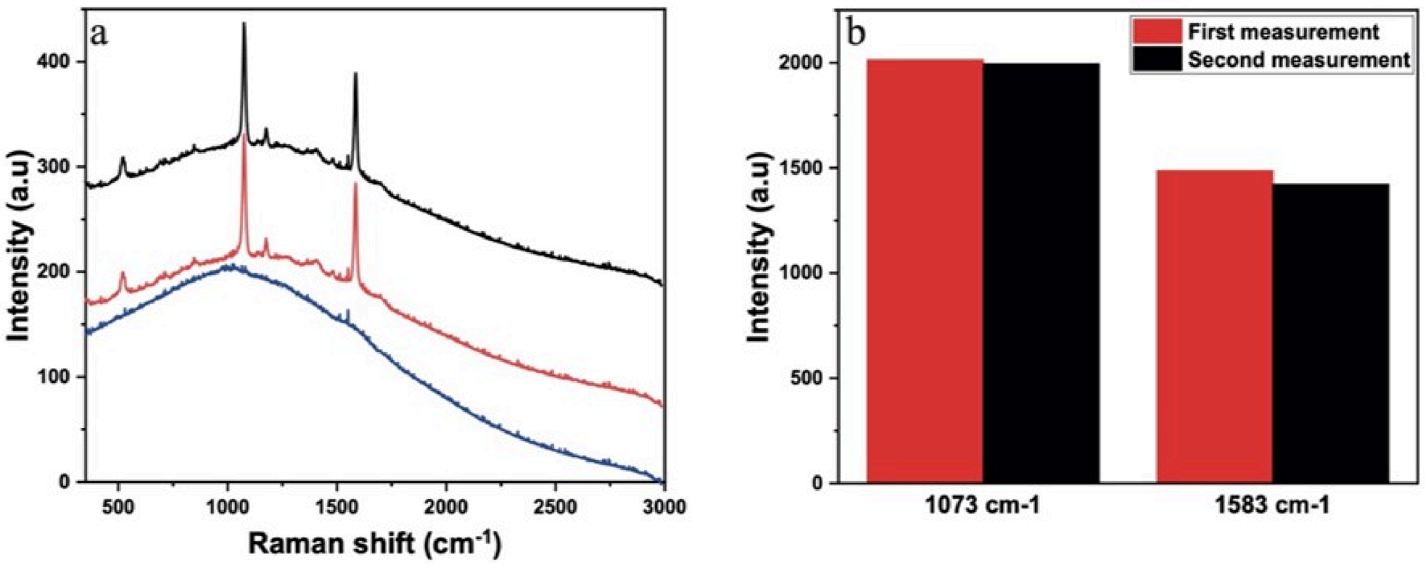

Figure 2

a) Average SERS spectra calculated from the 25 maps (400 spectra) on the Hamamatsu substrate with (black and red spectra) and without MBA(blue spectrum). b) Integrated intensities of the 1075 cm^{-1} and 1586 cm^{-1} bands for the 2 SERS measurements (red boxes from the red spectrum of the fig 2a and black boxes for the black spectrum of the fig 2a).

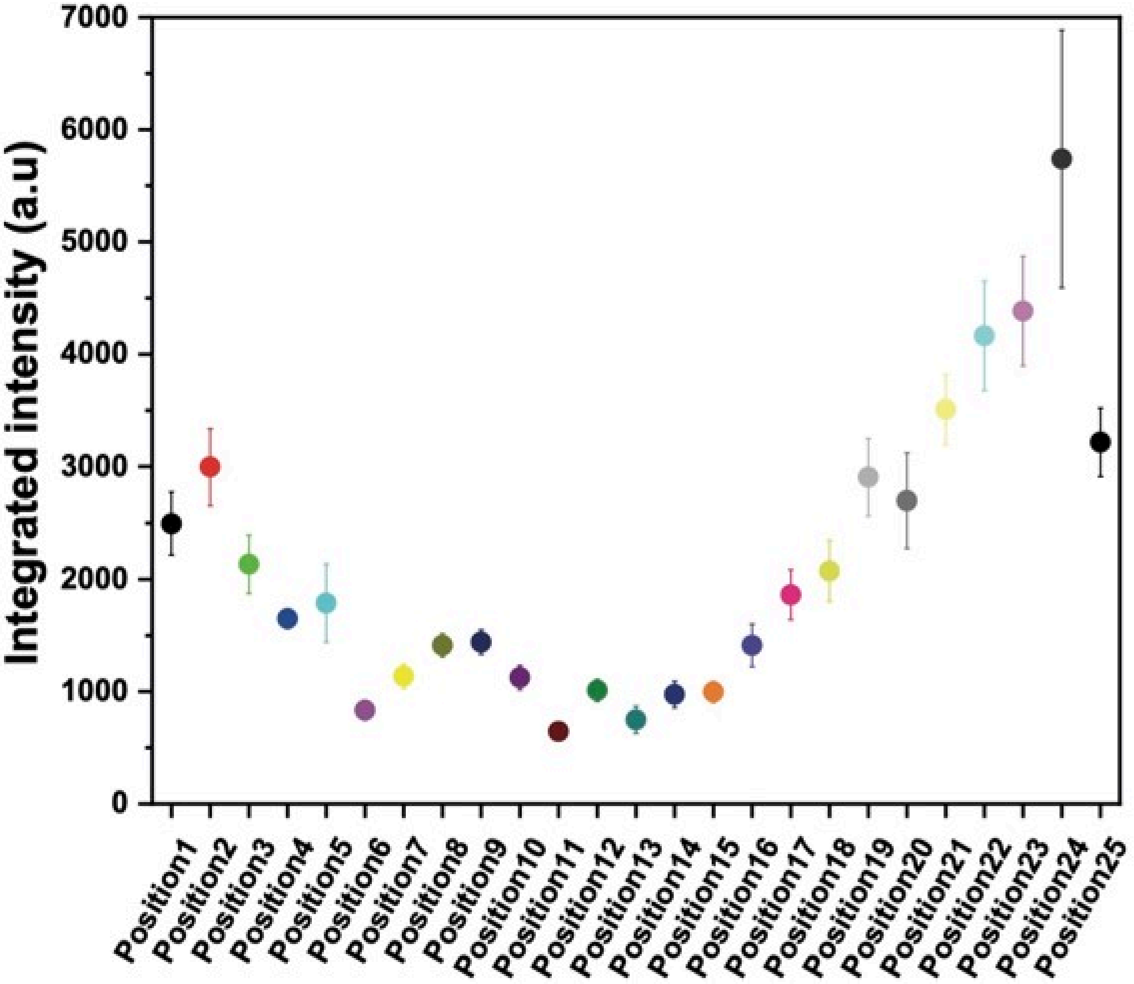

Figure 3

Average intensities measured foreach SERS maps of 16 spectra.

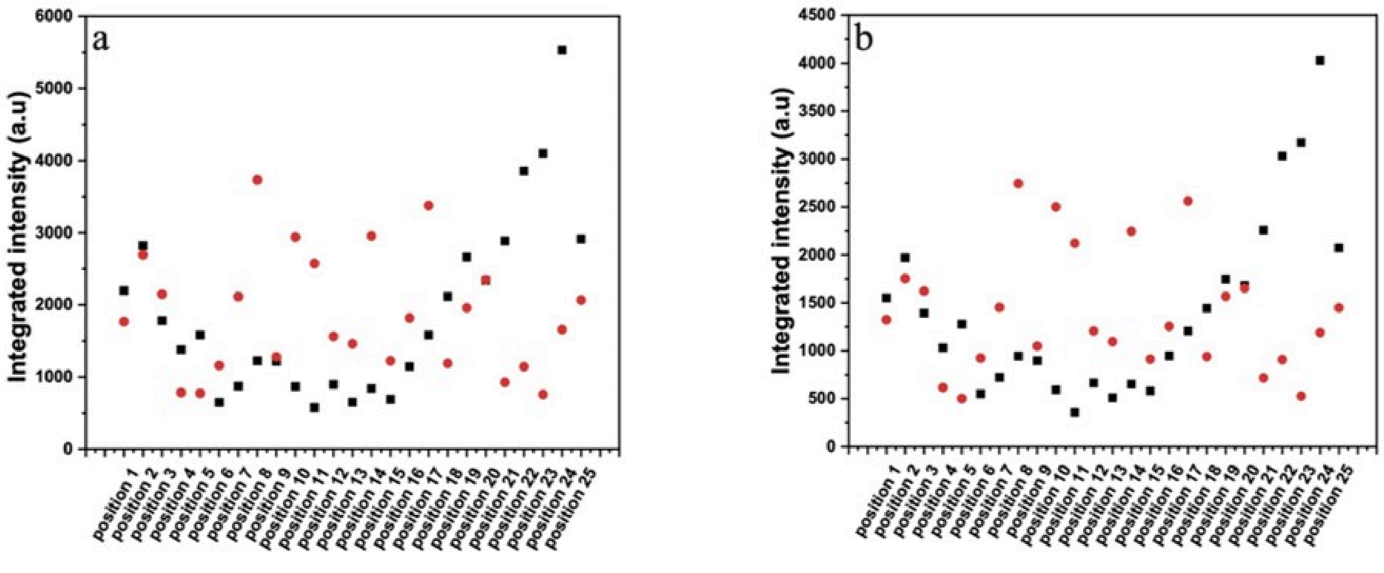

Figure 4

Average intensities measured for each SERS maps of 16 spectra for the 1075 cm^{-1} band (a) and 1586 cm^{-1} band (b). The black squares correspond to the first SERS measurement and the red dots to the second SERS measurement.