Comparison study of Surface-enhanced Raman spectroscopy substrates

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Abstract. Different parameters are used when comparing SERS substrates in the research of most scientific groups. These parameters are more often aimed at comparing theory and experiment. We described experimental models for comparison and explain their applicability. In this work, a reaction mixture volume fixing technique was chosen when comparing different substrates. The intensity distribution maps with an area of 2025 μm² (9X9 points) were recorded and described. We successfully used the parameters average intensity of intensity distribution maps and percentage gate of intensity distribution maps for comparison of enhancing substrate. We have observed uniform enhancing across the entire surface of self-assembled plasmonic film. A comparative study has shown that the self-assembled plasmonic film sample has high enhancing factors and good reproducibility of factors over the studying sample surface. Average intensity of dropcasted colloidal gold nanoparticles (Au-NPs) sample is equal to 1860 counts and percentage gate of intensity data equals 125%. Average intensity of self-assembling plasmonic film is equal to 112000 counts and percentage gate equals 1.67%.

1. Introduction
Surface-enhanced Raman scattering (SERS) is a commonly used sensing technique in which inelastic light scattering by molecules is enhanced by factors up to 10⁵ or even larger [1]. SERS has two main types of enhancing media. They are colloidal suspensions of plasmonic nanoparticles and solid SERS substrates with immobilized plasmonic nanoparticles on solid surface. Colloidal suspensions of plasmonic nanoparticles have high enhancing factors. However, colloidal suspensions have time dependences of enhancing factors. The use of solid substrates solves this problem, but most solid substrates have irreproducible surface distribution of enhancing factors [2]. Regularly patterned surfaces obtained via lithographic and other [3] techniques can solve irreproducible surface distribution problem, but they are expensive to synthesize.

Raman spectroscopy allows quickly obtaining information about the substance structure by compiling spectral maps from several areas of the object surface. The comparison of the spectral maps of the enhancing gold colloid dropcasted (CGD) to the substrate and self-assembled plasmonic film (SPF) makes it possible to obtain the dependencies of the enhancing factors (EFs) on the ordering of the film structure. The evaluation of the brightness of an SERS media relies on measuring the SERS EF. The EF is a relative...
measurement of the effect of the plasmonic nanoparticles on the Raman scattering intensity of analyte EF \[4\] was calculated by formula 1.

\[
EF = \frac{I_{SERS}c_{SERS}}{I_R c_R}
\]  

(1)

where \(I_{SERS}\) and \(I_R\) are the intensities of the SERS and Raman signals, respectively, and \(c_{SERS}\) and \(c_R\) are the concentrations of the analyte in the SERS and control Raman experiments, respectively.

Definition of EF depends on the calculation way chosen by the researchers. The development of the field of single-molecule SERS allowed access to information about the EF distribution beyond average signals and contributed to a better understanding of the important concept of a hot spot. The hype of enormous SERS EFs has slowly died down, and most overestimations of the EFs can be tracked to improper definitions or other, sometimes more subtle, sources of errors \[4\].

The field enhancement distribution at the surface of a plasmonic substrate is highly inhomogeneous and mainly localized in very small spatial regions called “hot spots”. From a structural point of view, these hot spots are often identified as very sharp tips or as nanogaps between few nanoparticles or between a nanoparticle and a surface \[5\]. These areas of plasmon structures are remarkably more efficient in enhancing the optical signals than the single plasmonic particle. The ability to carry out routine quantitative SERS is dependent on having stable, affordable, and reproducible enhancing substrates available. Self-assembled plasmonic films (SPF) have been studied as potential SERS active substrates.

Self-assembled plasmonic films are metal films at the interface between an aqueous gold colloid and a solution of a modifier in an organic solvent. Main types of modifier are transition metal complexs, thiols, quaternary ammonium and arsonium salts crown ethers etc. SPF is formed when an organic solution of modifier is shaken vigorously with a portion of aqueous gold colloid as soon as the agitation stopped. This assembly is associated with adsorption of the hydrophobic molecules at the plasmonic nanoparticle surface, which increased their hydrophobicity and caused them to migrate to the liquid–liquid interface \[6\].

In this paper, we tried to develop the comparison model of different SERS substrates. The intensity distribution map (IDM) of the dropcasted colloidal Au-NPs (DGC) and SPF will be recorded and compared for this. In addition, we tried to describe the EFs and reproducibility using average intensity of IDM and IDM gate values.

2. Experimental

2.1. Materials.
Chloroauric acid (99.995%), trisodium citrate, tetraoctylammonium bromide (TOABr), Pseudoisocyanine iodide (97%) were purchased from Aldrich. Toluene, KOH, HCl, HNO\(_3\) were purchased from Vekton, Russia. Chemicals were used without additional purification. Ultrapure water (Milli-Q) was used throughout the experiments.

2.2. Characterization.
All spectroscopic measurements were carried out in triplicate. Absorption (ABS) spectra were obtained with spectrophotometer UV-3600 (Shimadzu). Scanning electron microscopy (SEM) images were collected on a Merlin instrument (Zeiss, Oberkochen, Germany). Raman spectra were obtained by InVia (Renishaw) with 514 nm excitation. A Solver Pro-M (NT-MDT, Moscow, Russia) atomic-force microscope (AFM) was used to study the morphology and thickness of thin films.

3. Results

3.1. Preparation of Gold Colloid (GC).
The aqueous colloid of gold nanoparticles was prepared by the Lee and Meisel \[7\] method that is reduction of chloroauric acid using trisodium citrate. The glassware was processed from organic and inorganic contaminants before the GC synthesis. All glassware was washed in ethanolic potassium hydroxide to remove any organic impurities and then cleaned with aqua regia. Finally, glass was thoroughly rinsed (twenty times) with deionized water. The colloid had \(\lambda_{max} = 530\)nm. The average Au-
NPs size is approximately 20 nm (Fig. 1.a). The size of gold nanoparticles was calculated using electron microscopy. Aggregates of Au-NPs with a diameter of 20 nm were observed in the GC (Fig. 1.b). The resulting film can contain similar aggregates and be heterogeneous in thickness. The thickness of the synthesized film was studied by atomic force microscopy (AFM).

![Figure 1 (a, b). (a) SEM images of gold colloid nanoparticles; (b) SEM images of gold colloid nanoparticle aggregates.](image)

### 3.2. Preparation of SPF.

The SPF were prepared in a polypropylene test tube (4 ml volume). 2 cm³ of GC was vigorously shaken for 1 min with 1 cm³ of organic solution of TOABr [2]. Organic solvent was toluene. SPF formed spontaneously within a few seconds after shaking. The synthesis of SPF is shown on Fig.2.a. The contents of the test tube were poured into microscope slides. SPF reformed spontaneously on a microscope slides surface.

### 3.3. Preparation of samples.

Different ways to compare SERS substrates exist. The main ways of comparing SERS substrates are shown on Fig.2. Fixed parameters were allocated for the quantitative comparison of SERS substrates. These parameters were the equal number of Au-NPs and the same study areas. Equal number of Au-NPs is achieved by taking equal volumes of gold colloid at this stage of study. The problem of the same study areas was solved several ways. Equal area of sample is reached in different ways. For instance, this is achieved by fixing the area to which the reaction mixture is applied or fixing of reaction mixture. The area fixation method seemed the most promising at the initial stage of the study. The first method is the Equal Petri Dish (EPD). EPD is shown on Fig.2 (b). Dropcasted colloidal Au-NPs (DGC) was a control sample of SERS substrate. DGC was obtained similarly without adding the solution of TOABr in toluene. DGC and SPF samples were poured into the petri dishes. Then the petri dishes with them were covered with foil to prevent exposure to light and left for 48 hours to evaporate the solvents.

Second method of area fixation was named Bounding Walls (BW). The DGC and SPF samples are not poured into a petri dish, but into a container with removable walls in this method. Further manipulations were similar to the EPD method. BW is shown on Fig.2 (c) The main disadvantage in the practical implementation of the EPD and BW methods turned out to be that Au-NPs are concentrated on the vertical surface, which limits the surface area occupied by the reaction mixture.

The next developed method fixes not the area occupied by the SERS substrate, but the reaction mixture volume, which was dropcasted to the microscope slides. It was named Equal Volume of Reaction Mixture (EVRM) and shown on Fig.2 (d). The main idea of this comparison method is that equal volumes of the
same solvent on the same surface occupy equal areas. Three samples were prepared on a glass substrate for the comparison of enhancement substrates study. They were dye solution, DGC sample with applied dye solution and SPF sample with dye solution.

3.4. Coating a layer of dye.
There are not only several models for preparing substrates, but also several methods of applying the analyte. First way is dropcasting of a very dilute dye solution. Second way is spin-coating. The technique involves dropping the analyte solution onto a spinning sample of the enhancing substrate. In this case, a thin layer of the dye solution was obtained on the surface. During the application, the solvent evaporated, then Raman maps were obtained from the surface of the samples.
3.5. Comparison of samples.

The intensity distribution maps of the dye, dropcasted colloidal Au-NPs and SPF samples were recorded for different applying of dye solvent. The first sample was the dye spin-coated to glass. The second sample was dropcasted colloidal Au-NPs (DGC) spin-coated with a thin layer of dye. The third was self-assembled plasmonic film (SPF) on glass spin-coated with a dye layer. The intensity distribution map of samples with an area of 2025 μm² was obtained on a Renishaw spectrometer. The concentration 7.50·10⁻⁶ M of pseudoisocyanine iodide was chosen to the comparison of enhancement substrates study. It was necessary to reduce the concentration of the dye by a factor of 10 to study the SPF sample, since the spectrum had very high intensity and the spectrometer could not register it. An intense 1364 cm⁻¹ band was selected for the study. It is described in papers [8, 9]. The IDMs of the 1364 cm⁻¹ band for the dye and 1365 cm⁻¹ for the enhancement substrates is shown in Figure 3. Green color indicates the intensity values on the dye's sample (without enhancing), red - DGC as an enhancing substrate, blue - intensity distribution map over the surface of the studied SPF.

The parameters average intensity of IDM (AIM) and IDM percentage gate (%MG) were very useful in the SPF optimization study. AIM is the average intensity value over 81 spectra of the IDM and proportional to the EF. The %MG was calculated by formula 2:

$$%MG = \frac{MG}{AIM} \cdot 100\%$$

where MG is the map gate. It is the span of IDM data excluding 10% of the outliers. AIM is average intensity of all IDM data.

![Intensity distribution maps](image)
The IDM percentage gate shows the reproducibility of the enhancing factor over the studying samples area. Smaller value of %MG equals better reproducibility. The concentration of the dye for the SPF sample was reduced by a factor of 10. For an adequate comparison of values EF (formula 1) in terms of AIM, the average intensity of IDM of the SPF sample must be multiplied by 10. AIM of dye IDM is equal to 991 counts and %MG equals 87.8%. That means that dye sample has a low enhancing factor and low reproducibility of the enhancing factor. In addition, Fig. 3 (b) shows that the dye is unevenly distributed over the glass, which is possibly due to unevenness on the glass, which is invisible to the naked eye. AIM of DGC IDM is equal to 4200 counts and %MG equals 24.5%. AIM of SPF IDM is equal to 96500 counts and %MG equals 1.70%. It is obvious that the spin-coating technology does not provide a uniform application of the dye layer. It can also be seen from the figure that more dye accumulates on the rough surface of the reinforcing substrate than on glass; therefore, it is difficult to say that the intensity of the 1364 cm\(^{-1}\) line increases due to plasmon resonance or an increase in the thickness of the dye.

Let us compare the enhancing substrate presented in terms of the same parameters described earlier. AIM of DGC IDM is equal to 1860 counts and %MG equals 125%. AIM of SPF IDM is equal to 112000 counts and %MG equals 1.67%. Upon careful examination of Fig. 4 (a, b, c), it is obvious that only one hot spot was formed on the DGC sample, which is comparable in magnification to the SPF sample. This can also be seen when analyzing the values AIM and %MG. Thus, the last presented method is the most indicative when comparing SERS substrates.
4. Conclusions
Comparison of the self-assembled plasmonic film and dropcasted colloidal Au-NPs samples showed that an increase in the intensity of the bands of the analyte (Pseudoisocyanine iodide) Raman spectrum occurs over the entire area of the SPF, where the Au-NPs are located. In contrast, single hot spots are formed on the surface of the DGC sample.
Two parameters were chosen for the comparison study, showing different qualities of the enhancing substrates. The average intensity of IDM (intensity distribution map) is proportional to the analytical enhancing factor. However, the correction of average intensity of IDM, if the analyte concentration for SERS substrates is different, is necessary. The IDM percentage gate (%MG) shows the reproducibility of the enhancing factor over the studying samples area. %MG could be more than 100% for irreproducible SERS substrates. %MG of SPF samples is equal to about 2%.

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