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Detection of DNA molecules by SERS spectroscopy with silvered porous silicon as an active substrate

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Here we report the SERS spectroscopy study of the herring sperm DNA adsorbed on the silvered porous silicon. Porous silicon has been fabricated by an electrochemical anodic etching of a highly doped n-type silicon wafer. It has been shown that the following silver immersion deposition on porous silicon lead to the formation of a layer of silver nano- and microparticles assembled in a quasi-ordered array. Reflectance spectroscopy has revealed that the silver layer demonstrates the surface plasmon resonance band expanded to near-IR range. Preliminary SERS measurements with rhodamine 6G have showed that the silvered porous silicon is characterized by a very good reproducibility of the SERS signal and one-year shelf life. It has been found that the silvered porous silicon is SERS-active in relation to the herring sperm DNA under the excitation at 473, 633 and 785 nm. Collection of the SERS spectra of the DNA molecules in the random points of the silvered porous silicon has resulted in their weak reproducibility typical for the solid SERS substrates. However, the SERS mapping has helped to find the classical DNA spectra. In addition, the herring sperm DNA at an extremely low concentration of 1 mg mL^{-1} has been detected with the SERS substrate based on the silvered porous silicon.

1 Introduction Detection of DNA molecules today is widely used in many areas of human life such as medical diagnostics, gene therapy, forensic science, etc. [1, 2]. For many years, the polymerase chain reaction and fluorescent spectroscopy have been the most popular methods used in practice to study DNA [3]. Such methods are proven and reliable but often require expensive chemicals and take a long time to get results. Surface enhanced Raman scattering (SERS) spectroscopy is an alternative attractive way to study DNA with its remarkable ability of single molecule detection [4]. The study of the DNA components by the SERS spectroscopy was first reported in 1980 [5]. The authors showed the SERS spectra of the 10^{-3} M adenine, adenosine, and adenosine-5′-monophosphate at the electrochemically roughened silver electrode. Later the detection limit of the DNA components was improved to 10^{-6} M with SERS-active silver colloids [6]. Further progress in an engineering of the SERS substrates has driven a tremendous interest to study DNA by the SERS spectroscopy [7–9]. However, the practical application of this method is still in its infancy comparing to the traditional techniques of the DNA detection. It is mostly caused by severe dependence of the spectral quality and reproducibility on variations of the DNA conformation and/or packing density on the SERS substrates. For example, it was shown that the SERS spectrum of λ-DNA in the silver colloid is slightly shifted with respect to the conventional Raman spectrum [7]. The other paper reported on a weak reproducibility of signal-to-noise ratios in the SERS spectra of the DNA molecules adsorbed on the solid SERS substrate made of gold nanoshells [8]. In addition, small amount of organic compound can

degrade under the laser excitation [10]. It is especially typical for blue (near-UV) irradiation favorable for an activation of the silver-based SERS substrates. In assessing the potential of SERS spectroscopy for the DNA study, it is, therefore, important to adjust to the measurements with the selected SERS substrates, i.e., to find correlation between the measuring regimes, the substrate type, and the resulting spectra.

It has been previously shown that the SERS substrates based on a silvered porous silicon (PS) give rise to a strong enhancement of the signal from a rhodamine 6G [11] and a cationic Cu(II)-tetrakis(4-N-methylpyridyl) porphyrin resulting in an extremely high sensitivity [12]. In this article, we investigate the effect of such substrates on the spectra of the herring sperm DNA to choose optimal conditions of the SERS measurements resulting in a reliable DNA study.

2 Experimental

2.1 Sample preparation Monocrystalline n^+-type Si wafers were used as initial substrates. Prior to PS formation Si wafer was cleaned in a solution of NH_4OH, H_2O_2, and H_2O mixed in a volume ratio of 1:1:4 according to the procedure described elsewhere [13]. The native SiO_2 was removed from the Si wafer in diluted HF (4.5%). The front face of the Si wafer was then rendered porous to a depth of 5 μm by an electrochemical anodic etching in a mixture of HF, H_2O, and C_3H_7OH at a current density of 100 mA cm^{-2}. The sample was rinsed with deionized water and spun dry.

The Si wafer with porous front face was cut into a number of 5×7 mm samples. Each sample was immersed in a water-ethanol solution of 3 mM $AgNO_3$ for 70 min resulting in a deposition of silver nanoparticles on PS. The silvered PS samples were rinsed with deionized water and then air-dried.

An adsorption of analyte molecules was realized by a drop deposition of R6G water solution or herring sperm DNA in 0.01 M NaCl water solution on the silvered PS.

The shelf life of the SERS substrates was assessed by the SERS measurements after 1, 6, and 12 months of their keeping in a zipped plastic bag. Prior to the analyte deposition, the substrate was refreshed in diluted HCl for 30 s.

2.2 Measurements Electrochemical process was carried out with the potentiostat/galvanostat AUTOLAB PGSTAT302n. A scanning electron microscope (SEM) Hitachi S-4800 was used to study the morphology of the silvered PS. Reflectance spectra of the samples were recorded in the range from 200 to 1100 nm with MC 122 Proscan spectrometer. The Raman and SERS spectra were measured with a 3D scanning confocal microscope Confotec NR500. Laser wavelengths of 473, 633, and 785 nm were used to excite the samples. Laser powers were 1.45, 0.68, and 0.86 mW, respectively. During SERS measurements, the power of the blue laser was reduced by two orders of magnitude while the power of the red laser was reduced by one order of magnitude. Laser spot diameters were about 300 nm (for the wavelength of 473 nm), 400 nm (for the wavelength of 633 nm), and 500 nm (for the wavelength of 785 nm).

3 Characterization of the SERS substrates The regimes and solutions for the silvered PS were chosen based on the results reported elsewhere [12]. Such conditions were shown to provide the greatest intensity of the SERS signal. In this work, the morphology of the most active sample was accurately studied. The quantitative analysis of Fig. 1 revealed that the silver deposit is composed of the particles of two size ranges. The sizes of the particles in the first group are varied from 10 to 150 nm while the second group has larger particles of 150–700 nm in diameter. The content of the small particles is about 83%. Namely such nanoparticles are favorable for the SERS effect. The distance between many nanoparticles is no longer than 10 nm promising so-called "hot spots" where a huge SERS intensity takes place.

The optimal excitation wavelength must match the surface plasmon resonance (SPR) band of the SERS substrate. Here the SPR of the silvered PS was found from its reflectance spectrum. Figure 2 shows that the absorbance band related to the SPR is atypically wide for the silver nanoparticles and reaches near-IR region. This effect is observed due to different plasmon oscillation modes in the small silver particles of a non-uniform size distribution.

The SERS activity of the silvered PS was verified by the detection of 10^{-6} M R6G. The spot-to-spot and sample-to-sample deviation of the SERS signal varied from 5 to 7%. In addition, the SERS substrates demonstrated an extremely long shelf life up to 1 year.

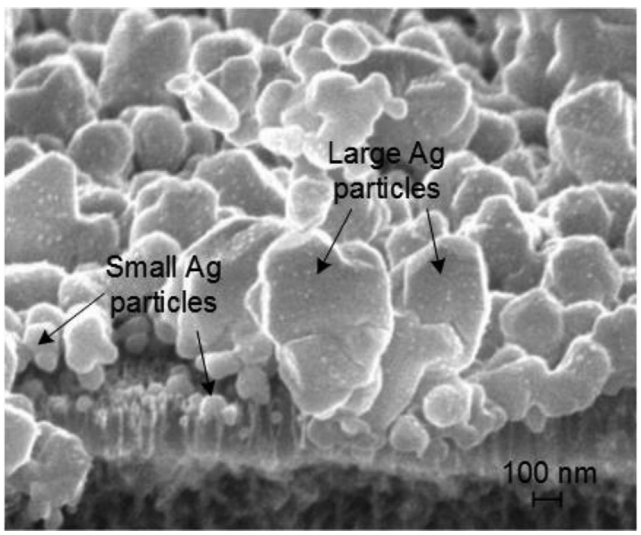

Figure 1 SEM top views of the PS samples after the immersion into 3 mM $AgNO_3$ solution for 70 min.

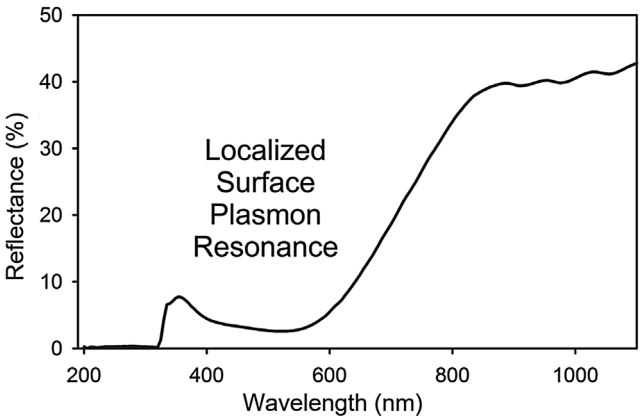

Figure 2 Reflectance spectrum of the PS samples after the immersion into 3 mM $AgNO_3$ solution for 70 min.

4 SERS study of DNA The Raman and SERS measurements of the herring sperm DNA were carried out under different laser wavelengths ranging from blue to near-IR regions (Fig. 3).

The peaks observed in the Raman spectra around 730 cm^{-1} (adenine), 787 cm^{-1} (thymine, cytosine), 1104 cm^{-1} (ν(C–O), deoxyribose-phosphate), 1242 cm^{-1} (cytosine, adenine), 1381 cm^{-1} (thymine, guanine, adenine), 1490 cm^{-1} (guanine, adenine), and 1581 cm^{-1} (guanine, adenine) are the characteristic Raman bands of the herring sperm DNA molecules [10]. SERS spectra were collected in 10 random points of the silvered PS. Remarkably, the silvered PS demonstrates SERS activity for all lasers proving the suggestion proposed in the previous section. However, the SERS spectra of DNA recorded in different points of the silvered PS samples show weak reproducibility which is in a good accordance with the data reported elsewhere [8]. Despite some bands of the DNA bases can be recognized, their intensity and position are not stable enough. The variation of the DNA SERS spectra mainly appear due to changes in molecules conformation after their deposition and drying on the silvered PS as well as an increase of randomness under laser irradiation. The intensity and the band position also depend on wavelength and power of the laser and accumulation time. For example, long UV radiation can destroy the DNA molecular group and bonds. Thus power of 473 nm laser was reduced about two orders of magnitude. Pronounced dominance of the adenine band is caused by its greater Raman cross section compared with that of guanine, cytosine, and thymine [8]. An increased background hiding the DNA bands from 1300 to 1600 cm^{-1} in the spectra collected under blue excitation can be explained by the decomposition of the DNA bases in the presence of noble metal and severe irradiation [7].

The following SERS mapping of the substrates allowed to find identifiable the SERS spectra of the herring sperm DNA collected under each laser excitation (Fig. 4).

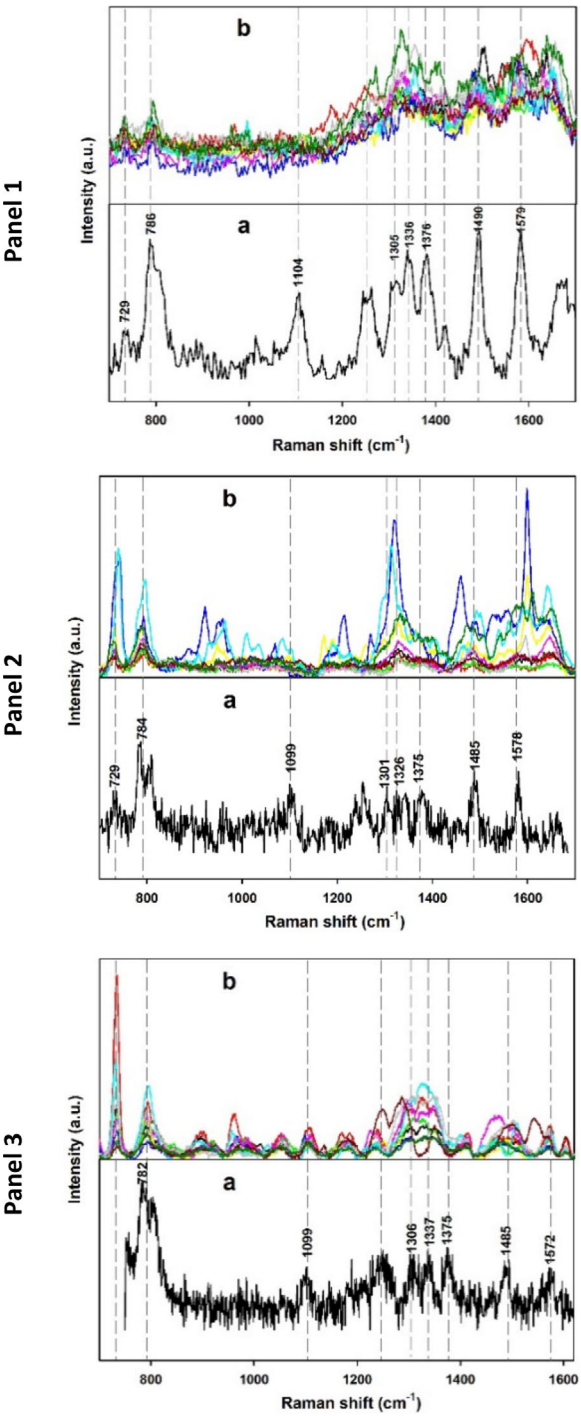

Figure 3 Raman (a) and SERS (b) spectra of the 10^{-8} M herring sperm DNA collected under the 473 (panel 1), 633 (panel 2), and 785 (panel 3) nm excitation wavelengths.

The silver nanoparticles of the same sizes were organized in the monolayer in some places on PS (see Fig. 1). We suppose this provided the DNA molecule alignment and a stable surface enhancement along the molecule resulting in the possibility to see the classical

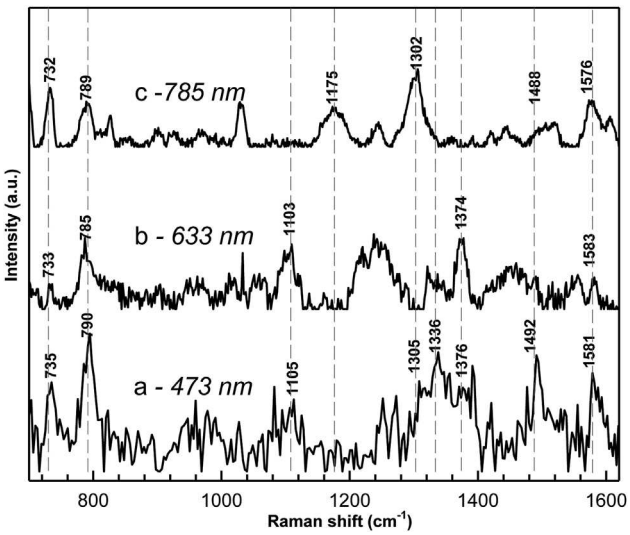

Figure 4 SERS spectra of the 10^{-8} M herring sperm DNA adsorbed on the surface of the silvered PS. The spectra were collected under the 473, 633, and 785 nm excitation wavelengths.

DNA spectrum. As shown in Fig. 2, the SPR maximum is about 500–520 nm and the SERS activity is expected to be stronger at the excitation of 473 nm than 633 and 785 nm. However, comparing SERS intensities in Fig. 4 shows that the situation is opposite proving the assumption on the destruction of the DNA molecules under excitation with wavelength close to the UV.

Finally, the SERS mapping was used to detect the herring sperm DNA at an extremely low concentration of 10^{-10} M. In Fig. 5, comparison of two SERS spectra of the DNA molecules at different concentrations is presented. The SERS spectra were recorded under the 473 nm excitation wavelength as both red and near-IR lasers showed no results for the lowest concentration. In contrast to the regime applied in case of Fig. 4, accumulation time was increased from 1 to 5 s. In spite of the shift of some bands and new bands arising it can be argued that the DNA molecules at the 0.01 mg mL^{-1} concentration can be detected.

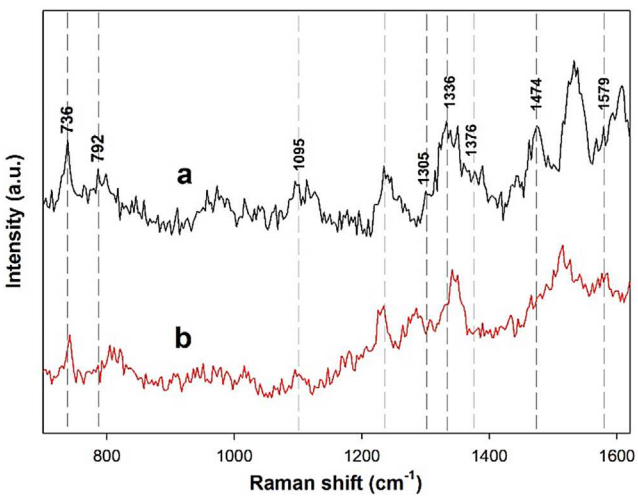

Figure 5 The SERS spectra of the 10^{-8} M (a) and 10^{-10} M (b) herring sperm DNA adsorbed on the surface of the silvered PS. The spectra were collected under the 473 nm excitation wavelength.

5 Conclusions The results on the DNA study by the SERS spectroscopy with the silvered PS presented here were partially similar to those with solid SERS substrates that gave surface enhancement from DNA but weak reproducibility of the spectra. This was typical for the measurements in random points on the SERS substrate. However, from the results of this article it is likely that the classical spectra of the DNA molecules can be found by the SERS substrate mapping. Moreover, the prospects for the DNA detection by the SERS spectroscopy with lasers of 473, 633, and 785 nm wavelengths are very encouraging. The most promising result is in the detection of the DNA molecules at very low concentration (10^{-10} M) with the silvered PS. According to our knowledge, detection of such a small amount of DNA has not been reported elsewhere. It shows an advantage of the developed silvered PS compared to other solid SERS-active substrates. Although in a competitive field, the SERS substrate based on PS is a very unusual material that can help to overcome some of the existing problems in the DNA study by SERS spectroscopy.

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