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

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Received 19 June 2016, revised 13 September 2016, accepted 6 October 2016
Published online 1 November 2016

Keywords DNA, porous silicon, silver, surface enhanced Raman scattering

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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.

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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

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deteriorate under the laser excitation [10]. It is especially
1 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 NH₄OH,
H₂O₂, and H₂O mixed in a volume ratio of 1:1:4 according
to the procedure described elsewhere [13]. The native
SiO₂ 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, H₂O, and C₃H₇OH 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 AgNO₃ 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](image.png)

**Figure 1** SEM top views of the PS samples after the immersion into 3 mM AgNO₃ solution for 70 min.
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\(^{-1}\) (v(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).

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
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.

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.

Acknowledgements The authors would like to thank very much Dr. Andrei Panarin and Dr. Sergei Terekhov for the fruitful discussions on the SERS spectroscopy of the DNA molecules as well as Dr. Vitaly Bondarenko for the useful consultation on formation and properties of porous silicon. This work has been supported in parts by the Belarusian State Research Program “Photonics, opto- and micro-electronics” (task no. 1.4.01), the Belarusian Republican Foundation for Fundamental Research (grant no. T16-099), and JINR (theme no. 04-4-1111).

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