Conference materials
UDC 539.234
DOI: https://doi.org/10.18721/JPM.153.120

Fabrication of SERS-sensitive nanopipette with silver nanoparticles obtained by vacuum thermal evaporation

A. D. Overchenko [1] ✉, S. V. Dubkov [1], D. V. Novikov [1], V. S. Kolmogorov [2]
L. D. Volkova [3], T. S. Grishin [3], P. A. Edelbekova [3]

[1] National Research University of Electronic Technology, Moscow, Russia;
[2] National University of Science and Technology "MISIS" (MISIS), Moscow, Russia;
[3] Institute of Nanotechnology of Microelectronics RAS, Moscow, Russia
✉ alexsey7840@mail.ru

Abstract: This work is concerned with developing an approach to producing an array of plasmonic Ag nanoparticles on the nanopipette surface. The vacuum thermal evaporation method followed by annealing was used to form the nanoparticle array. The surface morphology of the modified pipettes was investigated by scanning electron microscopy. Based on the SEM images obtained, the most efficient method for particle deposition on the pipette was selected. It was found that two-stage depositions on the horizontally mounted pipette formed an array of silver nanoparticles with a size of about 16 nm. The obtained modified nanopipettes were investigated by Raman spectroscopy. A laser with a wavelength of 532 nm was used to obtain the spectra. Rhodamine in the R6G modification was used as an analytical substance. The enhance factor of the modified pipette was calculated by comparing it with pure glass at the same power values of the laser and concentration of the analytical substance, rhodamine R6G. The developed approach to modifying the surface of nanopipettes allows fabricating SERS pipettes for monitoring various intracellular biomarkers.

Keywords: nanopipette, SERS, Ag-particles, Raman spectroscopy

Funding: The work was supported by the Russian Science Foundation (project № 21-19-00761).

Citation: Overchenko A. D., Dubkov S. V., Novikov D. V., Kolmogorov V. S., Volkova L. D., Grishin T. S., Edelbekova P. A., Fabrication of SERS-sensitive nanopipette with silver nanoparticles obtained by vacuum thermal evaporation, St. Petersburg State Polytechnical University Journal. Physics and Mathematics. 15 (3.1) (2022) 119–124. DOI: https://doi.org/10.18721/JPM.153.120

Материалы конференции
УДК 539.234
DOI: https://doi.org/10.18721/JPM.153.120

Изготовление SERS-чувствительного нанокапилляра с серебряными частицами с помощью метода вакуум термического испарения

А. Д. Оверченко [1] ✉, С. В. Дубков [1], Д. В. Новиков [1], В. С. Колмогоров [2]
Л. С. Волкова [3], Т. С. Гришин [3], П. А. Едельбекова [3]

[1] Национальный исследовательский университет «МИЭТ», г. Москва, Россия;
[2] Национальный Исследовательский Технологический Университет МИСиС, г. Москва, Россия;
[3] Институт нанотехнологий микроэлектроники РАН, г. Москва, Россия
✉ alexsey7840@mail.ru

Аннотация. Данная работа посвящена разработке подхода к формированию массива

плазмонных наночастиц Ag на поверхности нанопипетки. Для формирования массива наночастиц использовался метод вакуум термического испарения с последующим отжигом. Морфология поверхности модифицированных пипеток была исследована с помощью растрового электронной микроскопии. На основе полученных РЭМ изображений была выбрана наиболее эффективная методика осаждения частиц на пипетку. Установлено, что при двух стадийном нанесении на горизонтально закреплённую пипетку формируется массив наночастиц серебра с размером порядка 16 нм. Полученные модифицированные нанопипетки исследовались с помощью рамановской спектроскопии. В ходе получения спектров использовался лазер с длинной волны 532 нм. В качестве аналитического вещества использовался родамин в модификации R6G. Был проведен расчёт коэффициента усиления модифицированной пипетки путём сравнения с чистым стеклом при одинаковых значения мощности лазера и концентрации аналитического вещества родамин R6G. Разработанный подход к модифицированию поверхности нанопипеток возможен для изготовления SERS-пипеток для мониторинга различных внутриклеточных биомаркеров.

Ключевые слова: нанопипетка, SERS, Ag-частицы, рамановская спектроскопия

Финансирование: Исследование выполнено за счет гранта Российского научного фонда (проект № 21-19-00761).

Ссылка при цитировании: Оверченко А. Д., Дубков С. В., Новиков Д. В., Колмогоров В. С., Волкова Л. С., Гришин Т. С., Едельбекова П. А. Изготовление SERS-чувствительного нанокапилляра с серебряными частицами с помощью метода вакуум термического испарения // Научно-технические ведомости СПбГПУ. Физико-математические науки. 2022. Т. 15. № 3.1. С. 119–124. DOI: https://doi.org/10.18721/ JPM.153.120

Introduction

Substance diagnostics is an indispensable part of many fields. It is especially important in the medical industry, where biomarkers are identified to detect various diseases [1]. Detection of biomarkers from a single cell represents an important task today. The low concentration of biomarkers and the dynamic nature of living cells make it challenging to use traditional methods for analysis of intracellular contents of single cells [2, 3]. One example of such methods is atomic force microscopy (AFM). It is difficult to use classical AFM to visualize the dynamics of living biological objects due to the time required to obtain an image of surface morphology. In addition, as the tip always exerts a mechanical load on the sample, it can be damaged, therefore, it is difficult to image soft biomaterials with the AFM [4]. Tip-enhanced Raman spectroscopy (TERS) can be used to obtain the most detailed and accurate information about the studied object. TERS is a well-known method that combines scanning probe microscopy and Raman spectroscopy [5], allowing to carry out effective investigations into the objects of interest [6]. One of the disadvantages of TERS is that it works with only one type of molecule at a time, which limits its deposition [7]. Raman enhancement with TERS is associated with strong fields, which can destroy molecules, leaving only carbon residues [7]. Although the above methods have achieved some success for the study of biochemical processes within cells or the interaction of a cell with its environment, they are still complicated to apply to observation of individual living cells.

Raman spectroscopy has been increasingly used with modified pipettes for analysis of biomaterial because it has a minimal impact on the cell considered during its introduction to the cell due to the nanoscale tip of the pipette (10–100 nm) [8]. The pipette modified with plasmonic metals can be used to regulate the delivery of molecules/ions and perform in situ measurements of the effects of delivered molecules/ions on a living cell via Raman spectroscopy. The main advantage of using nanopipettes is the simplicity and low cost of the manufacturing process, so such pipettes will allow rapid intracellular studies to obtain accurate information about the single cell structure without the need for complex techniques. However, the nanopipette tip

surface has a complicated topology, which makes the formation of plasmonic particles a non-trivial task. Current methods of pipettes modification have a number of limitations. Ho and colleagues [9] developed a modified pipette by holding in a solution of Ag particles synthesized by reduction from $AgNO_3$ with ethanol. During the modification, the authors used a large number of deposition stages, preparation of several solutions and a large amount of time to successfully precipitate the nanoparticles. It was established in [10] that a pipette could be modified with gold for highly sensitive detection of DNA damage in living cells using electrodeposition from $HAuCl_4$ solution was shown. For successful deposition, the pipette was treated with carbon from the inside via butane pyrolysis, which greatly complicates the modification process. The given methods of pipette modification are cheap to use, but they, as well as a number of others, don't have the reproducibility [11,12]. An alternative method of forming arrays of nanoparticles on the pipette surface is the method of vacuum-thermal evaporation followed by annealing. This method has high reproducibility, controllability of the process to control the size of nanoparticles [13, 14]. It is worth noting that the obtained nanoparticle arrays or thin films have a distinct interfacial boundary, which is important for surface plasmon resonance.

This work is dedicated to developing techniques for forming Ag nanoparticles on the nanopipette surface. In the course of the experiments, the morphology of the pipette surface was studied using scanning electron microscopy. The optimal parameters of Raman studies of the modified nanopipette were revealed and the enhance factor was calculated for the obtained SERS (surface enhanced Raman spectroscopy) active structure.

Materials and Methods

We conducted a series of experiments with borosilicate glass pipettes prepared from a CO_2-laser-based glass pipette (P-2000, SutterInstrument Co.). The length of the fabricated pipettes was about 45 mm, the size of the exit hole was about 50–70 nm.

Before the nanoparticles deposition process, the pipettes were washed as usual to remove the impurities. Cleaning was performed in peroxide-ammonia solution (PAS) and deionized water at ~50 °C followed by drying in isopropyl alcohol vapor.

The formation of metallic nanoparticles was performed using vacuum thermal evaporation followed by heat treatment. As the evaporation material for every deposition was used silver with a mass of 3 mg. The working pressure in the chamber was about $3 \cdot 10^{-5}$ Torr. The samples were annealed in vacuum at a pressure of $3 \cdot 10^{-5}$ Torr at 230 °C for 30 minutes.

Two techniques were used to form an array of silver nanoparticles on the pipette: one deposition on the vertically fixed pipette; two consecutive depositions on the two sides of the pipette. A schematic representation of the methods of nanoparticles formation on the pipette is shown in Fig. 1. Depending on the variant of the technique and deposition, the pipette was attached to the substrate holder in different ways and the number of particle deposition processes varied.

A Helios C4 GX scanning electron microscope was used to study the morphology of the obtained arrays of silver nanoparticles. A Raman spectrometer based on an inVia confocal microscope (Renishaw) was used to obtain Raman spectra. A laser with a wavelength of 532 nm and a power of about 100 mW according to the documentation, 44 mW according to the lens exit

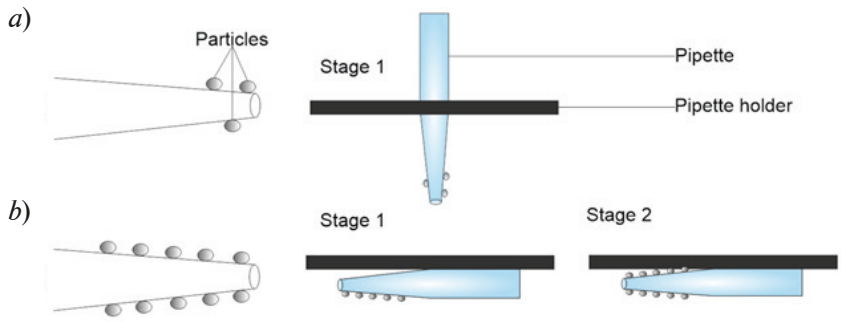

Fig. 1. Illustration of techniques for forming nanoparticles on a pipette: one deposition on a vertically attached sample (*a*); two consecutive depositions on a horizontally attached pipette (*b*)

measurement and a spot size of 2 μm were used. Rhodamine R6G with a concentration of 1 mM was used as the analyte. The analyte was applied by dipping in the appropriate analyte solution for 5 seconds followed by drying for 10 minutes.

Results and Discussion

Analysis of the pipette surface morphology with arrays of silver nanoparticles is shown in Fig. 2. Notably, nanoparticles were not present on the pipette surface when the first technique was used. With the second technique, the average particle size was ~ 16 nm and the distance between them was ~ 14 nm, as shown in Figure 2, *a*.

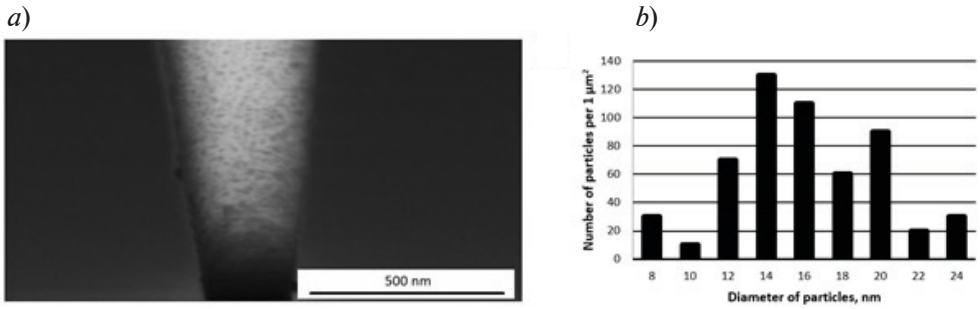

Fig. 2. SEM images of the Ag nanoparticle array for the sample with two horizontal depositions (*a*) and histogram of nanoparticle size distribution per 1 μm^2 (*b*)

Figure 3 shows the results of a Raman spectroscopy study using a pipette with two consecutive depositions at a wavelength of 532 nm, the spot power density was on the order of 0.007 and 0.14 mW/μm^2. Fig. 3 shows that the spectrum obtained at 0.007 mW/μm^2 shows clearly distinguishable peaks corresponding to R6G [15]. When the laser power was increased

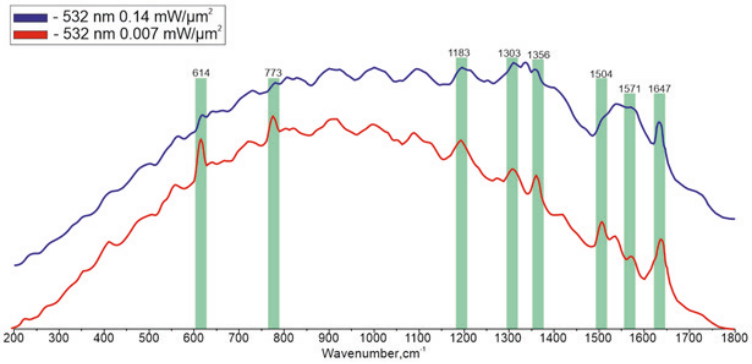

Fig. 3. Raman spectra for a pipette with two consecutive depositions at 532 nm and different laser powers during the study of R6G concentration of 1 mM

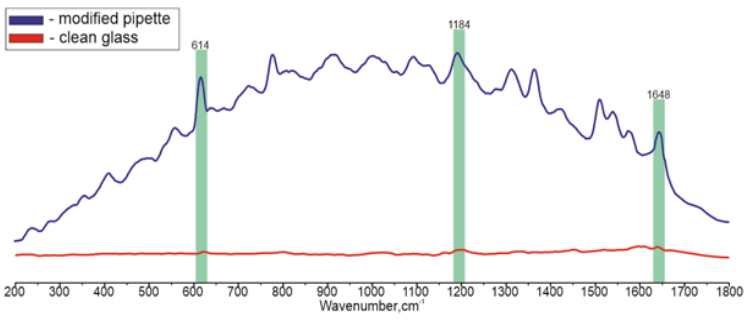

Fig. 4. Raman spectra obtained from pipettes with two consecutive depositions and clean glass at 532 nm of R6G analyte concentration 1 mM

to ~ 0.14 mW/μm^2, individual R6G modes were observed as well as characteristic peaks of amorphous carbon at 1536 cm^{-1} and in the 1500–1600 cm^{-1} region [16]. The presence of characteristic peaks of amorphous carbon is associated with the burning of the analytical substance [17]. Further Raman studies of modified Ag pipettes were performed at ~ 0.007 mW/μm^2.

Figure 4 shows the results of Raman spectroscopy of a pipette with two consecutive depositions and pure glass with the analyte R6G 1 mM at a wavelength of 532 nm with a laser power of 0.007 mW/μm^2. Based on these spectra, the enhance factor of the SERS pipette was calculated [18]. The calculated enhance factor of the modified pipette was ~ 10^3. The lines in (Fig. 4) mark the R6G characteristic peaks, which were used to calculate the enhance factor of the SERS-active pipette.

Conclusion

This paper has outlined an approach to forming the SERS active layer based on the array of silver nanoparticles on the pipette surface by vacuum thermal evaporation method followed by annealing. Morphological study of the modified pipette surface by scanning electron microscopy showed that an array of silver nanoparticles with a size of about 16 nm was formed at two stages of deposition on the horizontally mounted pipette. It was found that the optimal laser power for Raman studies is 0.007 mW/μm, as there are no amorphous carbon peaks at this value. When this laser power is used, the R6G modes are present in the Raman spectrum. The calculated enhance factor of the modified pipette was ~10^3.

REFERENCES

1. **Maruvada P., Wang W., Wagner P.D., Srivastava S.,** Biomarkers in molecular medicine: cancer detection and diagnosis, BioTechniques. 4 (38) (2005) 9–15.

2. **Dittrich P., Jakubowski N.,** Current trends in single cell analysis, Analytical and Bioanalytical Chemistry. 27 (406) (2014) 6957–6961.

3. **Yuan G., Cai L., Elowitz M., Enver T., Fan G., Guo G., Irizarry R., Kharchenko P., Kim J., Orkim S., Quackenbush J., Saadatpour A., Schroeder T., Shvidasani R., Tirosh I.,** Challenges and emerging directions in single-cell analysis, Genome biology 18 (84) (2017) 1–8.

4. **Bandyopadhyay A., Bose S.,** Characterization of biomaterials, Vol. 2, Introduction to Biomaterials: Basic Theory with Engineering Applications, Cambridge University Press, Cambridge, 2013.

5. **Cao Y., Sun M.,** Tip-enhanced Raman spectroscopy, Reviews in Physics. 8 (2022) 100067.

6. **Lin W., Xu X., Quan J., Sun M.,** Propagating surface plasmon polaritons for remote excitation surface-enhanced Raman scattering spectroscopy, Applied Spectroscopy Reviews. 10 (53) (2018) 771–782.

7. **Haldavnekar R., Venkatakrishnan K., Tan B.,** Next generation SERS-atomic scale platform for molecular level detection, Applied Materials Today. 1 (18) (2020) 100529.

8. Bulbul G., Chaves G., Olivier J., Ozel R. E., Pourmand N., Nanopipettes as monitoring probes for the single living cell: state of the art and future directions in molecular biology, Cells 6 (7) (2018) 55.

9. **Ho V. T. T. X., Park H., An S., Kim G., Ly N., Lee S. Y., Choo J., Jung H. S., Joo S. W.,** Coumarin–lipoic acid conjugates on silver nanoparticle-supported nanopipettes for in situ dual-mode monitoring of intracellular Cu (II) and potential chemodynamic therapy applications, Sensors and Actuators B: Chemical. 344 (2021) 130271.

10. **Zhou J., Yang D., Liu G., Li S., Feng W., Yang G., He J., Shan Y.,** Highly sensitive detection of DNA damage in living cells by SERS and electrochemical measurements using a flexible gold nanoelectrode, Analyst. 7 (146) (2021) 2321–2329.

11. **Yang D., Liu G., Li H., Liu A., Guo J., Shan Y., Wang Z., He J.,** The fabrication of a gold nanoelectrode–nanopore nanopipette for dopamine enrichment and multimode detection, Analyst 3 (145) (2020): 1047–1055.

12. **Hong-Na L., Dan Y., Ao-Xue L., Guo-Hui L., Yu-Ping S., Guo-Cheng Y., Jin H.,** Facile fabrication of gold functionalized nanopipette for nanoscale electrochemistry and surface enhanced Raman spectroscopy, Chinese Journal of Analytical Chemistry 8 (47) (2019) 19104–19112.

13. **Gromov, D. G., Dubkov S. V., Savitskiy A. I., Shaman Y. P., Polokhin A. A., Belogorokhov I. A., Trifonov A. Y.,** Optimization of nanostructures based on Au, Ag, AuAg nanoparticles formed by thermal evaporation in vacuum for SERS applications, Applied Surface Science 489 (2019) 701–707.

14. **Dubkov S. V., Savitskiy A. I., Trifonov A. Y., Yeritsyan G. S., Shaman Y. P., Kitsyuk E. P., Tarasov A., Shtyka O., Ciesielski R., Gromov D.G.,** SERS in red spectrum region through array of Ag-Cu composite nanoparticles formed by vacuum-thermal evaporation, Optical Materials. 7 (2020) 100055.

15. **He X. N., Gao Y., Mahjouri-Samani M., Black P. N., Allen J., Mitchell M., Xiong W., Zhou Y. S., Jiang L., Lu Y. F.,** Surface-enhanced Raman spectroscopy using gold-coated horizontally aligned carbon nanotubes, Nanotechnology. 20 (23) (2012) 205702.

16. **Dychalska A., Popielarski P., Frankyw W., Fabisiak K., Paprocki K., Szybowicz M.,** Study of CVD diamond layers with amorphous carbon admixture by Raman scattering spectroscopy, Materials Science-Poland. 4 (33) (2015) 799–805.

17. **Ho M., Lau A.,** Amorphous carbon nanocomposites, Material Science. (2015) 309–328.

18. **Kosuda K. M., Bingham J. M., Wustholz K. L., Van Duyne R. P.,** Nanostructures and surfaceenhanced Raman spectroscopy, Handbook of Nanoscale Optics and Electronics. 309 (2010).

THE AUTHORS

OVERCHENKO Aleksei D.
alexsey7840@mail.ru
ORCID: 0000-0003-1313-2128

VOLKOVA Lidiya D.
Ilidiya.volkova.96@mail.ru
ORCID: 0000-0003-4860-0585

DUBKOV Sergey V.
sv.dubkov@gmail.com
ORCID: 0000-0003-1507-8807

GRISHIN Timofey S.
grishin.t@outlook.com
ORCID: 0000-0001-6261-5316

NOVIKOV Denis V.
tororo@bk.ru
ORCID: 0000-0002-9518-1208

EDELBEKOVA Polina A.
polinaedel51@gmail.com
ORCID: 0000-0002-8422-9798

KOLMOGOROV Vasilii S.
vskolmogorov@gmail.com
ORCID: 0000-0002-7135-8910

Received 21.05.2022. Approved after reviewing 25.07.2022. Accepted 26.07.2022.