Surface enhanced IR absorption by cortisol molecules

I. A. Milekhin\textsuperscript{1,2}, S. A. Kuznetsov\textsuperscript{1,3}, A. G. Milekhin\textsuperscript{1,2}, T. A. Duda\textsuperscript{1}, O. P. Cherkasova\textsuperscript{4}, E. E. Rodyakina\textsuperscript{1,2}, A. V. Latyshev\textsuperscript{1,2}

\textsuperscript{1}Novosibirsk State University, Pirogov 2, 630090 Novosibirsk, Russia
\textsuperscript{2}Rzhanov Institute of Semiconductor Physics, Lavrentiev Ave. 13, 630090 Novosibirsk, Russia
\textsuperscript{3}Rzhanov Institute of Semiconductor Physics, Novosibirsk Branch “TDIAM”, Lavrentiev Ave. 2/1, Novosibirsk 630090, Russia
\textsuperscript{4}Institute of Laser Physics, Novosibirsk, Russia

E–mail of the corresponding author: mia2994@gmail.com

Abstract. We report on the study of surface enhanced infrared absorption (SEIRA) by steroid hormone cortisol molecules with different concentrations deposited on linear Au nanoantenna arrays. Localized surface plasmon resonances (LSPRs) arise in the nanoantennas under the influence of external electromagnetic radiation. LSPR frequency depends mainly on the nanoantenna length and can vary from the visible to the terahertz range. We establish the ratio between the structural parameters of nanoantenna arrays and LSPR frequencies based on the results of 3D electrodynamic simulations. Using nanolithography we fabricate nanoantenna arrays having LSPR frequencies close to the frequencies of the most intense absorption modes of steroid hormone cortisol. We deposit cortisol molecules onto the surface of nanoantenna arrays by drop–coating. SEIRA spectra of the nanoantenna arrays make it possible the determination of the presence of cortisol and establishing the sensitivity limit of this method. Thus, we show possibility of SEIRA application for cortisol concentration analysis.

1. Introduction

Among the variety of organic compounds, steroid hormones are of great importance, since they affect many physiological processes in humans and animals. Cortisol is the main glucocorticoid hormone produced both in the adrenal gland and in some other types of tissue, to regulate blood glucose level. Violation in the cortisol production leads to the development of diabetes, Cushing's syndrome and other pathologies [1]. Therefore, the determination of cortisol concentrations in blood and tissue is an important and urgent task.

The typical IR spectroscopy has a relatively low sensitivity for detecting the vibrational modes in organic or inorganic molecules which is however not sufficient for biological sensors applications. The idea of SEIRA is based on specially designed Au nanoantenna arrays providing high electromagnetic field in the immediate vicinity of the plasmon nanostructure under resonance excitation when the frequency of the incident electromagnetic radiation coincides with the eigenfrequency of the electronic oscillations in the nanoantennas and the LSPR arises [2]. As a result, the local electromagnetic field near the edges of the nanoantennas is enhanced, which makes it possible to detect a small amount of substances [3] placed in the local field [4–6]. The linear nanoantenna array consists of metal rods (for example, Au, Al, Ag) with a width of about 100 nm, the...
characteristic length which can vary in the range from tens of nanometers to several micrometers. As a rule, nanoantenna arrays are fabricated on dielectric (SiO$_2$, CaF$_2$, Al$_2$O$_3$) or semiconductor (Si) substrates using nanolithography. In our work, we successfully employed SEIRA for detection of cortisol with a low concentration.

2. Experimental

The optimum structure values were determined by electrodynamic simulations using 3D full wave simulations in the software ANSYS Electromagnetics Suite 18 [8] to set up the plasmon resonance to a specific wavelength in the mid infrared spectral range.

Homogeneous arrays of linear Au nanoantennas 3×3 mm$^2$ in size with structural parameters determined from the calculations were formed on pure Si (001) substrates by focused ion beam as described earlier [7]. The width (height) was chosen to be 60 nm (50 nm), and the antenna length was varied within 2–15 μm. Figure 1 demonstrates a typical scanning electron microscopy (SEM) image of nanoantenna array using the same Raith-150 system at 10 kV acceleration voltage, 30 μm aperture, and 6 mm working distance. Structural parameters determined from the SEM image coincide with the calculated ones with the experimental accuracy of about 10 nm.

Cortisol was extracted from human blood plasma according to the method [9] and extract was dissolved in 24 μl of a mixture of ethanol–water (80:20 v/v) and dropped onto the substrate surface from solutions. The drop volume was 2 μl.

IR transmission spectra of Au nanoantenna arrays with deposited cortisol molecules were measured in the spectral range from 600 to 5000 cm$^{-1}$ using a Bruker Vertex 80v Fourier spectrometer. For further estimations, the ratio of the IR transmittance spectra corresponding to light polarization along the axis of the nanoantenna arrays and perpendicular to it was calculated and analyzed.

3. Results and discussions

The experimentally determined LSPR wavelength depends linearly on the antenna length in a wide spectral range (Fig. 2).

![Figure 1](https://example.com/fig1.png)  
**Figure 1.** Representative SEM image of a linear Au nanoantenna array.

![Figure 2](https://example.com/fig2.png)  
**Figure 2.** LSPR energy in nanoantenna arrays fabricated on bare Si surfaces as a function of the nanoantenna length.

Cortisol molecule has two carbonyl groups, which are responsible for spectral features in the range of 1600–1700 cm$^{-1}$ [11]. These modes have a strong intensity in IR absorption and were used for further enhancement by SEIRA technique. SEIRA spectra of cortisol covered by nanoantennas with specific lengths providing the LSPR mode (deep minima near 1500 cm$^{-1}$) with the frequency close to that IR absorption bands of cortisol molecules to achieve the maximum absorption enhancement are shown in Figure 3 [10].
Figure 3. IR spectra of nanoantenna arrays after dropping 8 μl of blood plasma extract with different quantities of cortisol: 1 – low concentration of cortisol (2 μg/μl); 2 – high concentration of cortisol (11 μg/μl); 3 – without cortisol. Inset to the figure shows the fragments of the same spectra with subtracted background.

As you can see from the figure nanoantennas covered by cortisol with different concentration reveal similar IR response that indicates exceeding measurement limit. With decreasing the concentration of cortisol allows to determine the sensitivity limit of cortisol detection which amounts to 10 ng/μl. Thus, a possibility of application of SEIRA for analysis of steroid hormone cortisol in blood plasma was shown.

4. Conclusion
We employed the SEIRA detection of cortisol molecules extracted from human blood and deposited on linear Au nanoantenna arrays. Nanoantennas with optimal structural parameters providing the LSPR frequencies overlapping the characteristic vibrational modes of cortisol were fabricated. We measured IR response by cortisol with different concentration deposited on Au antenna arrays and determined the detection limit of 10 ng/μl.

5. Acknowledgment
This work has been supported by Russian Foundation for Basic Research (project 18–32–00551 mol –a).

References
[1] Cherkasova O P., Activity of 11β– hydroxysteroid dehydrogenase of rat kidney and liver in inherited stress– induced arterial hypertension, Biochemistry (Moscow) 2007;1:172–175. doi: 10.1134/S1990750807020126.
[2] Schatz, G. C.; Young, M. A.; Van Duyne, R. P. Electromagnetic Mechanism of SERS. In Surface–Enhanced Raman Scattering: Physics and Applications; Kneipp, K.; Moskovits, M.; Kneipp, H., Eds.; Topics in Applied Physics, Vol. 103; Springer: Berlin, Germany, 2006; pp 19–45.
[3] Nanoantenna: Plasmon–Enhanced Spectroscopies for Biotechnological Applications, ed. Chapelle, M. L. de la and Pucci, A. CRC Press.Taylor & Francis Group: Boca Ratn, 2013. 405 p.
[4] Hartstein, A.; Kirtley, J. R.; Tsang, J. C. Phys. Rev. Lett. 1980, 45, 201–204.
[5] Osawa, M. Surface–Enhanced Infrared Absorption, Kawata S. (Ed.): Near– Field Optics and Surface Plasmon Polaritons, Topics Appl. Phys. 2001, 81, 163–187, Springer– Verlag Berlin Heidelberg 2001.
[6] Orendorff, C. J.; Gole, A.; Sau, T. K.; Murphy, C. J. Anal. Chem. 2005, 77 (10), 3261–3266.
[7] Yeryukov, N.A.; Milekhin, A.G.; Sveshnikova, L.L.; Duda, T.A.; Rodyakina, E.E.; Sheremet,
E.S.; Ludemann, M.; Latyshev, A.V.; Zahn, D.R.T. *Thin Solid Films* 2013, 543, 35–40.

[8] ANSYS, Inc.; url: https://www.ansys.com/products/electronics.

[9] Cherkasova O. P., Biochemistry (Moscow), 2007, Vol. 1, No. 2, pp. 172–175.

[10] Milekhin, A.G., Cherkasova, O.P., Kuznetsov, S.A., et al, Beilstein Journal of Nanotechnology, 2017, 8 (1), p. 975–981.

[11] Minaeva V. A., Minaev B. F., Baryshnikov G. V., et al. Study of IR and Raman spectra of steroid hormones cortisone, cortisone acetate and cortisol by quantum–chemical method of densityfunctional theory // Cherkassy University Bulletin:Chemical sciences, 2011, vol. 195, part II, 60–83.