Surface Enhanced Spectroscopy for biomolecules and cells study



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Suface Enhnced Spectroscopy

- 1)Surface Enhanced Infrared Absorption (SEIRA)
- Surface Enhanced Raman Scattering (SERS)
- Surface Enhanced Fluorescence (SEF)

Method SEIRA, SERS, SEF could be efficiently used:

- for determination of type of molecules and impurity in the probe of extremely small amount
- for analysis of conformation forms of biological macromolecules and their interactions
- for enhanced and contrast imaging of cells in vivo and in vitro





Earning on human health...



Introduction in spectroscopy

frequency ranges of electromagnetic waves





Dispersive spectrometer (monochromator)





History of the Surface Enhanced Spectroscopy

(Surface Enhanced Infra Red Absorption-SEIRA)

- 1980 p. The first registration of SEIRA effect by Harstain (Harstain A., Kirtley J., Tsang J. // Phys Rev Lett. – Vol.45. – 1980. – P.201-209).
- 1983 p. Theoretical explanation the surface enhanced optical signals and physical process near rough metal surface ,(Kosobukin V.A.. // Surface: physics, chemistry, mechanics. – Vol. 12.- 1983.-P.5-20).
- 1991 p. The effect was named as SEIRA by Osawa and Ikeda (M. Osawa, M. Ikeda // J. Phys. Chem.- Vol. 95.-1991. -P. 9914-9919).
- 2001 p. the first our department publication on application of the SEIRA for study of DNA from tumor cells (G.I..Dovbeshko, O.P.Gnatyuk, V.I.Chegel, Yu.M.Shirshov, // Biophysical bulletin.-Vol.528, №2(9).-2001. - C.93-99).
- 2003 Ataka K., Heberle J. Electrochemically induced surfaceenhanced infrared difference absorption (SEIDA) spectroscopy of protein monolayer// J Am Chem Soc. 125.
- 2005 p. –review on SEIRA by Ricardo Aroca and Concha Domingo with 5 citations of our publications . (R. Aroca, C. Domingo. Surfaceenhanced Infrared Spectroscopy // Focal point. – Vol.58. – 2005.-P.324-338).

History of the Surface Enhanced Spectroscopy

(SERS- Surface Enhanced Raman Scattering)

- Fleischmann, M.; PJ Hendra and AJ McQuillan (15 May 1974). "Raman Spectra of Pyridine Adsorbed at a Silver Electrode". <u>Chemical Physics</u> <u>Letters</u> 26 (2): 163–166.
- Jeanmaire, David L.; Richard P. van Duyne (1977). "Surface Raman Electrochemistry Part I. Heterocyclic, Aromatic and Aliphatic Amines Adsorbed on the Anodized Silver Electrode". <u>Journal of Electroanalytical</u> <u>Chemistry</u> 84: 1–20.
- Emelyanov V.I. Koroteev N.I. Effect of giant Raman Scattering by molecules adsorbed at the metal surface // Uspehi fizycheskih nauk. – 1981. – V.135, №2. – P.345-361.
- 4. Cheng P., Furtaka T. Giant Raman Scattering M.: Mir, 1984. 408p.
- Kneipp K., Kneipp H., Itzkan I., Dasari R., Feil M. Surface-enhanced Raman scattering and biophysics // J. Phys.: Condensed Matter. – 2002. – Vol.14. – P.R597-R624.
- Galina Dovbeshko, Olena Fesenko, Konstyantyn Yakovkin, Sirena Bertrione, Alessandro Damin, Domenica Scarano, Adriano Zecchina and Elena Obraztsova The poly-A interaction and interfaces with carbon nanotubes. // Molecular Crystals Liquid Crystals. – 2008. – Vol 496, №345331. – pp. 170-185.

History of the Surface Enhanced Spectroscopy (Surface Enhanced Fluorescence - SEF)

- Lakowicz J.R., Shen B., Gryczynski Z., D'Auria S, Gryczynski I. Intrinsic fluorescence from DNA can be enhanced by metallic particles, Biochemical and Biophysical Research Communications. 286 (2001) 875-879
- G. Dovbeshko, O. Goncharuk, O. Pavlovich, O. Fesenko, O. Gnatiuk, V. Gorchev, S.Karachin, New strategy to study of embryonic cells on the basis of effect of plasmonic enhancement, Nanobiophysics: fundamental and applied aspects" 5-8 October, 2009, Kharkov, Ukraine. P.91.
- G.I. Dovbeshko, O.M.Fesenko, V.V. Boyko, V.F. Gorchev, S.O. Karakhin, N.Ya. Gridina, V.S. Gorelik, V.N. Moiseenko, Novel photoluminescence enhancing substrates for image formation of biological objects, Ukr.Phys.Zurn,v.57,N.7, 2012, 732-738





The theoretical basis of the effect of IR enhancement

The theoretical interpretation of the effect is connected with enhancement of external electric field due to excitation of local plasmon vibrations at essential curvature of rough surface or on metal particles. In such systems, according to Kosobukin, the electric field in the point of space r could be presented as :

 $E(r,t) = exp(-i\omega t)[E0(\omega) + E1(r,\omega)]$, where ω - frequency, t - time. (1)

The additional term $E_1(r,\omega)$ is connected with excitation of local (surface) plasmon vibrations. The enhancement of the external field can be presented as:

$$\boldsymbol{E}_{\alpha}(\boldsymbol{r},\boldsymbol{\omega}) = \boldsymbol{g}_{\alpha\beta}(\boldsymbol{r},\boldsymbol{\omega})\boldsymbol{E}_{\boldsymbol{\theta}\boldsymbol{\beta}}(\boldsymbol{\omega}) , (2)$$

where $g_{\alpha\beta}(\mathbf{r}, \omega)$) is coefficient of the enhancement.

The effective cross-section of the process of light interaction with a molecule adsorbed on metal surface could be expressed as:

 $\sigma_{\alpha}(r,\omega) = \int |\langle f| H(r,\omega) |i\rangle |^{2} \delta \left[h(\omega-\omega_{fi}) \right] \sim \sigma_{\alpha}(0) |g(r,\omega)|^{2} |h(r)|^{2},$

where $\sigma_{\alpha}(0)$ - cross-section in the absence of metal $(d = d_0, E = E_0)$; $i \rightarrow f$ – optical transition from i (with the energy ε_i) to f state; $cE_0^2/8\pi$ - density of an incident energy flux, c – light velocity; $\langle f/H(r,\omega)/i \rangle$ - matrix elements of Hamiltonian of molecule interaction with a field; h(r) – enhancement coefficient of dipole moment of adsorbed molecule.

SEIRA mechanism



- The explanation of the effect includes several mechanisms :
- i)the increase of the electromagnetic field near rough metal surface or island metal films,
- ii)the increase of the dipole transition moment of the adsorbed molecules, etc.

Dovbeshko G.I., Fesenko O.M., Chegel V.I., Shirshov Y.M. "Enhancement of optical transition near rough metal surface", Semiconductor physics, quantum electronics and optoelectronics, v.7, №4, 2004, p.215-225).

The effective cross-section of the process of light interaction with a molecule adsorbed on metal surface could be expressed:

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 $\sigma_{\alpha}(0)$ - cross-section for free molecule (E = E₀); $g(r,\omega)$ -coefficient of the enhancement for electric field; h(r) - coefficient of the enhancement for dipole moment of adsorbed molecule

• Calculated enhancement factor:

$$g_{po3} = \mathcal{E}_{M}'(\omega) / \mathcal{E}_{M}''(\omega)$$

• Experimental enhancement factor:

$$g_{e\kappa c} = I_{M}(\omega) / I_{CaF_{2}}(\omega)$$

Factor of enhancement in surface enhanced spectroscopy

- Surface enhanced infrared absorption (SEIRA)(g=10-500)
- Surface enhanced Raman spectroscopy (SERS) (g=10-1000000)
- Metal enhanced fluorescence (MEF) (g=10-100)
- Optical imaging with better contrast and resolution



Factors which influences the enhancement in SEIRA effect

Type and morphology of metal or dielectric substrate, position of plasmon or phonon resonances

Geometry of the experiment

Type of interaction between molecules and substrates, composition of the adsorbed molecules



Images of gold home-made SEIRA substrates





AFM - images of gold surface of home-made SEIRA substrates



AFM images of gold films with 250 (a) and 350 Å (b) thickness and 30 A roughness on SiO2 substrate



Enhancement of optical signal of molecules on rough metal surface



Geometry of experiment

G.I. Dovbeshko, V.I. Chegel, N.Y. Gridina, O.P. Repnytska, Y.M. Shirsho Tryndiak, I.M. Todor, G.I. Solyanik, Surface enhanced infrared absorptic nucleic acids from tumour cells: an FTIR reflectance study, Biospectro

The advantages of the SEIRA in reflectance mode

- We used home-made rough Au metal substrates produced by thermal vacuum evaporation Au onto glass supports. Numerous experiments on SEIRA of p-NBA in transmittance and ATR modes were reported in the literature, and one of the distinctive features of the present work is the fact that we used a reflectance mode with an incident beam of 16.5° close to normal to the surface. This angle, which must be less than 20°, was chosen to forbid the beam to enter the glass support, thus enhancing the possibility and sensitivity of registering SEIRA effects (the same holds also for the surfaceenhanced Raman spectroscopy experiments).
- Another point is the use of home-made rough gold substrates which give possibility to register larger number of the bands in comparison with other SEIRA experiments due to surface morphology.

Jaroslaw J. Panek, Aneta Jezierska, Aleksander Koll, **Galina Dovbeshko, Olena Fesenko,** p-Nitrobenzoic acid adsorption on nanostructured Au surface investigated by combined experimental and computational approaches, ChemPhysChem // 12, p.1 – 12.

Dependence of enhancement factor on the type of metal





Dependence of enhancement factor on the distance



The advantages of the SEIRA

- Enhancement of absorption by 3-200 times
- The reflected light is preferentially polarised in the plane of incidence due to geometry of experiment and thin film
- It is possible to determine molecular orientation in oriented samples.

$$I \sim \left|\frac{d\vec{\mu}}{dQ}\right|^2 \cdot \left|\vec{E}\right|^2 \cos^2 \alpha$$



Selected publications on spectroscopy of biological polymers

- The SEIRA spectroscopy data of nucleic acids and phospholipids from sensitive- and drugresistant rat tumours / Chekhun V. F., Solyanik G. I., Kulik G.I., Tryndiak V.P., Todor I.N., Dovbeshko G.I., Repnytska O.P. // Journal of Experimental Clinical Cancer Research. – 2002. – Vol.21, №4. – P.599-607.
- DNA interaction with single-walled carbon nanotubes: a SEIRA study / Dovbeshko G.I., Repnytska O.P., Obraztsova E.D., Shtogun Y.V. // Chemical Physics Letters. – 2003. – Vol. 372. – P.432 – 437.
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- G. Dovbeshko, O. Fesenko, K. Yakovkin, S. Bertrione, A. Damin, D. Scarano, A. Zecchina, E. Obraztsova. The poly-A interaction and interfaces with carbon nanotubes. // Molecular Crystals Liquid Crystals. 2008. Vol 496, №345331. pp. 170-185.
- G. Dovbeshko, O. Fesenko, O. Gnatyk, Ya. Shtogun, L. Woods, S. Bertarione, A. Damin, D. Scarano, A. Zecchina. Nucleic acid interaction and interfaces with single-walled carbon nanotubes. In Book "Carbon Nanotubes" / Edited by Jose M/Marulanda, 2010, In-Teh. P. 697-720.
- H.Arakawa, G.Dovbeshko S.Diamantoglou, Heidar-Ali Tajmir Riahi. Oxovanadium Ions Bind Transfer RNA at Multiple Sites, DNA and cell biology. – Vol. 29, №8, 2010.

The main absorption bands of proteins, DNA, lipids and characteristic frequencies of different molecular groups



Secondary Structure Determination in proteins

Amid I : C=O Stretching vibration Η Amid I - band reveals secondary structureinformation



ISS Bukovel 2012 Conformation analysis of proteins



Conformation of polypeptides chain:

(a) - α-helix; (b) - parallel β-form;
(c) - antyparallel β-form.

Secondary	Amid I, cm ⁻¹		
structure	Mean value	Maximum	
α-helix	1654	1648-1657	
<i>Q</i> form	1633	1623-1641	
<i>p</i> -10111	1684	1674-1695	
Turns	1672	1664-1686	
Disorder	1654	1642-1657	

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 Goormaghtigh E. Determination of soluble and membrane protein structure by Fourier transform infrared spectroscopy / Goormaghtigh E., Cabiaux V., Ruysschaert J.-M. // Subcell. Biochem. – 1994. – Vol. 23. – P. 405–450.

Different DNA conformations and their structure



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DNA interaction with carbon nanotubes probed by SEIRA



G.I. Dovbeshko, O.P. Repnytska, E.D. Obraztsova, Y.V. Shtogun. DNA interaction with single-walled carbon nanotubes: a SEIRA study. Chemical Physics Letters 2003; 372: 432-437

Carbon nanotubes enhance the vibration modes of sugars



g=4-8



G.I. Dovbeshko, O.P. Repnytska, E.D. Obraztsova, Y.V. Shtogun. DNA interaction with single-walled carbon nanotubes: a SEIRA study Chemical Physics Letters 2003; 372: 432-437

Principal component analysis for determination DNA conformation



Principal component analysis of free DNA in A and B forms; A and B DNA on gold and DNA with SWCNT on gold, each SEIRA spectr presented by 1 point at the PC plane

FTIR spectra of free film consisting from DNA adsorbed on the carbon nanotubes



g=8-10

SEIRA spectra of SPEV cells



In the process of cell deposition from the suspension on the gold support, the cells did not cover the substrate by monolayer, as usually they form thick layer. In this case the spectrum from the thick layer is not good due to IR saturation for separated bands. As it was seen on top curve, the more intensive spectra are registered after double washing. In last case the cells are placed separately one from another and it was confirmed by confocal microscopy.





SERS method



Schematic diagrams of SERS





Probing Molecules with Photonic Crystals:SERS and SEIRA



Figure I:

a) Schematic of a gold-coated photonic crystal SERS substrate. (b) SEM images of two photonic crystals with different voids shapes and dimensions.

Caterina Netti, John R.Lincoln, Microscopy and Analysis, 19 (6):17-19 (EU), 2005



Old CD-disks could be used as SEIRA and SERS substrate





Klarite Surface Enhanced Raman







Klarite support



Substructure of Klarite substrate







CM-AFM images of PolyA/SWCNT on Klarite





Plasmon vibrations in Au and Au-SWCNT





Poly-A enhanced by single walled carbon nanorubes

Single strand of Poly A ^{ISS Bukovel 2012} (left) and double strand of Poly A (right)







Nanotubes in solution of poly-A before and after centrifugation





Poly-A with nanotubes after centrifugation





SERS of poly-A with carbon nanotube



g=10⁵



SERS of poly-A with carbon nanotube



Diameter of nanotubes derived from SERS of individual nanotubes

<i>V</i> , <i>cm</i> ⁻¹	264	4	238	220	210	162	158	
D, nm	0,9	4	1,04	1,13	1,18	1,53	1,57	
	$v(cm^{-1}) = \frac{248}{D_6(nm)};$							

Diameter of nanotubes derived from RS of the bungle

<i>V</i> , cm⁻¹	177	169	162	156	148	136
D, nm	1,26	1,32	1,38	1,43	1,51	1,65
$v(cm^{-1}) = \frac{223.75}{D_1(nm)};$						

Surface enhanced fluorescence



Basis of the enhancement for optical

The basis is an enhancement of optical signals from cells adsorbed on the nanostructured metal surface or incubated with nanoparticles. The first conditions for optical signal enhancement on the border of 2 medium (1 - cell, 2 - nanoparticle) is the followings:

$$\varepsilon_{2}'(\varpi) < 0, \varepsilon_{1}'(\varpi) < 0 \quad \text{for} \quad \varpi_{1} < \varpi < \varpi_{2}, \quad \frac{\left|\varepsilon_{2}'(\varpi)\right|}{\varepsilon_{2}''(\varpi)} >> 1$$

The second condition is en increase the polarizability of the molecules (cell components) induced by nanoparticles.

- G.I. Dovbeshko, O.M. Fesenko, Yu.M. Shirshov, V.I. Chegel. The enhancement of optical processes near rough surface of metals. Semiconductor Physics, Quantum Electronics & Optoelectronics. 2004. V. 7, N 4. P. 411-424.
- Dovbeshko G.I., Chegel V.I., Paschuk O.P., Shirshov Yu.M, Nazarova A., Kosenkov D., Fesenko O. Biological Molecule Conformations Probed and Enhanced by Metal and Carbon Nanostructures // Fronties of Multifunctional Integrated Nanosystems, Ed.: E. Buzaneva, P. Scharff, Kluwer Academic Publishers. – 2004. – P.447-466
- 3. Dovbeshko G., Fesenko O., Nazarova A. Effect of nanostructured metal surface on SEIRA spectra of albumin and nucleic acids // Journal of Physical Studies.-2006.-Vol.10, №2. P.127-134.

Metal-enhanced fluorescence



Schematic diagram of fluorescence.



Metallic surface, islands or colloids

Predicted distance dependences for a metal surface on the transition of fluorophore. The metallic surface can cause Forsterlike quenching with a rate, km, can concentrate the incident field, Em, and can increase the radiative decay rate, *Гm*.





T₀

No metal

High photonic mode density Low photonic mode desity

r

High and low photonic mode densities in the presence and absence of metal, respectively



Metal-enhanced fluorescence



Scheme of classical Jablonski diagram for the PL of the molecule in free space and the modified form in the presence of metallic surface: E - incident field, Em concentrated by metal the incident field, Γ – radiative or emission decay rate, Γm – radiative decay rate in the presence of metal, knr and *km*- nonradiative decay rate the other represents all rate processes returning the molecule the ground state without to emission without and with metal, accordingly.

- 1. Lakowicz, Analist. 2008. 133. 1308-1346
- G.I. Dovbeshko, O.M. Fesenko, Yu.M. Shirshov, V.I. Chegel. The enhancement of optical processes near rough surface of metals. Semiconductor Physics, Quantum Electronics & Optoelectronics. 2004. V. 7, N 4. P. 411-424.

Metal-enhanced fluorescence

The quantum yield, Q_0 , of a fluorophore shows a competition between radiative decay and nonradiative processes:

The fluorescence lifetime, or decay time, is the average time for an ensemble of fluorescent molecules to remain in the S_1 state:

When fluorophores are placed at suitable distances from metallic particles or surface, fluorophores can undergo modifications of their radiative decay rates, Γ_m , where an increase in Γ_m results in an increase in fluorescence intensity, Q_m , and reduction in lifetime τ_m , which is converse to the free-space condition in which both change in unison





$$\begin{split} \mathcal{Q}_m = & \frac{\Gamma + \Gamma_m}{\Gamma + \Gamma_m + k_{nr} + k_q} \,, \\ \tau_m = & \frac{1}{\Gamma + \Gamma_m + k_{nr} + k} \,. \end{split}$$

Confocal microscopy image of cells enhanced by nanostructured gold surface



Images of SPEV cells excited by three lasers via long pass filters LP 420, LP 505 and LP 650.



Images of SPEV cells excited by three lasers and registered via band pass filters of BP 420-480, BP 505-530 and LP 650.

Confocal microscopy image of fibroblasts and SPEV cells on home-made gold support



Image of fibroblasts on gold support excited by UV lamp



Image of the SPEV cells cultivated on gold support excited by UV lamp

Imaging of DNA on nanostructured gold support with confocal microscopy



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Photonic Crystals

Microstructure of synthetic opal (SEM images in SEI mode)



V. Boyko, G. Dovbeshko, O. Fesenko, V. Gorelik, V. Moiseyenko, V. Romanyuk, T. Shvetsc, P. Vodolazkyy. New Optical Properties of Synthetic Opals Infiltrated by DNA // Mol. Cryst. Liq. Cryst., Vol. 535: pp. 30–41, 2011

Optical imaging of photonic crystal and DNA at the surface of photonic crystal



Photonic crystal via confocal microscope



DNA at the surface of photonic crystal via confocal microscope



Optical imaging of blood cells on the surface of PC



erythrocytes

blood cells

G.I. Dovbeshko, O.M.Fesenko, V.V. Boyko, V.F. Gorchev, S.O. Karakhin, N.Ya. Gridina, V.S. Gorelik, V. Moiseenko, Novel photoluminescence enhancing substrates for image formation of biological objects, Ukr.Phys.Zurn,v.57,N.7, 2012, 732-738



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Thanks for your attention

All propositions, please, send to e-mail: gd@iop.kiev.ua

