Report on the concluding project and theme: 04-4-1133-2018-2020

“Modern Trends and Developments in Raman Microspectroscopy and Photoluminescence for Condensed Matter Studies”

and

proposal for its extension and opening of a new project “Biophotonics” for the period of 2021–2023

Theme leaders:

Grigory Arzumanyan and Norbert Kučerka

52nd meeting of the PAC for Condensed Matter Physics

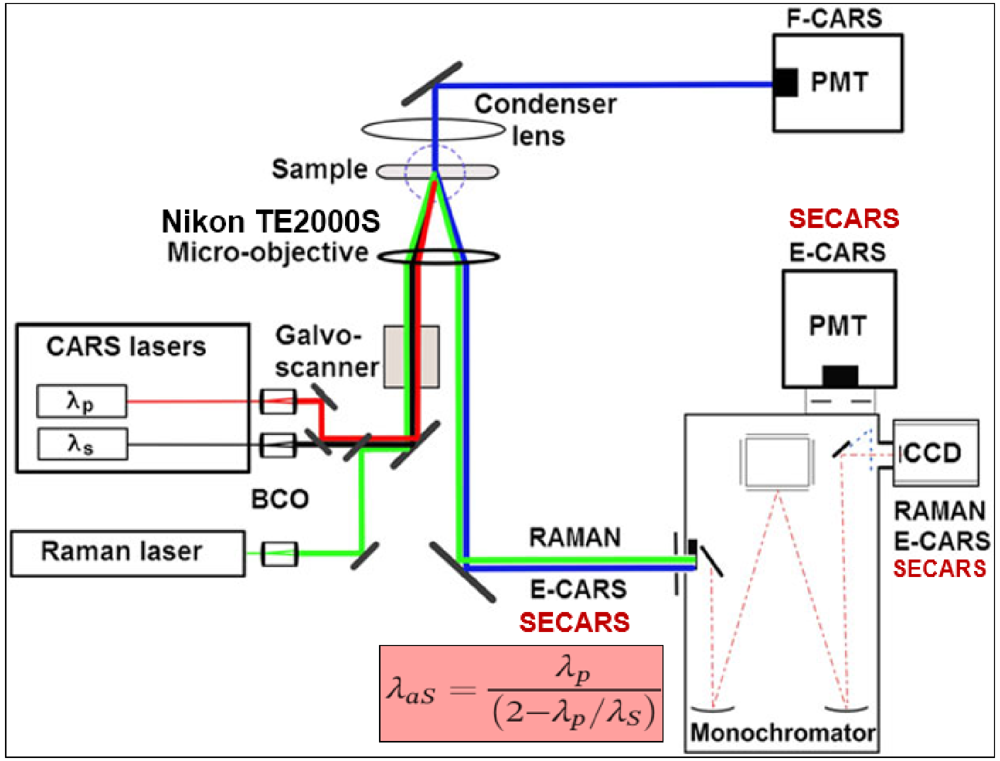
2-3 July, 2020

Expected main results by the theme/project completion:

- **Upgraded optical platform for ultrasensitive surface-enhanced CARS spectroscopy (SERS + CARS = SECARS) .**
- **Systematic experiments on SECARS spectroscopy and intensity mapping with picosecond laser pulses.**
- **Selection of the most optimal and effective SERS-active substrates for a SERS and SECARS.**
- **Detection of extremely low concentrations of organic molecules with the use of SERS.**
- **Synthesis and study of the spectral and structural characteristics of “core-shell” upconversion phosphors with various rare earth elements located in their core and tests in biomedical application (in particular, PDT) .**
- **Realization of a contrast and selective imaging of model samples by Raman microscopy and up-conversion luminescence.**

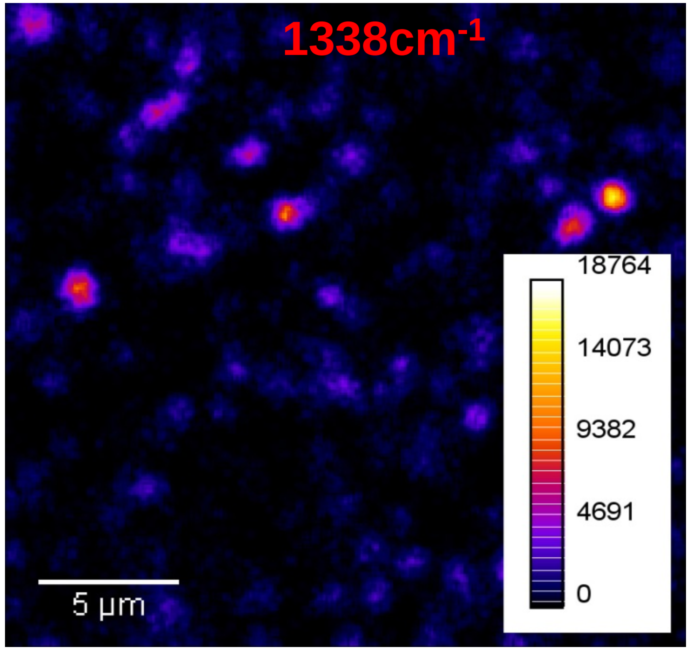
1. SECARS – modern Raman modality

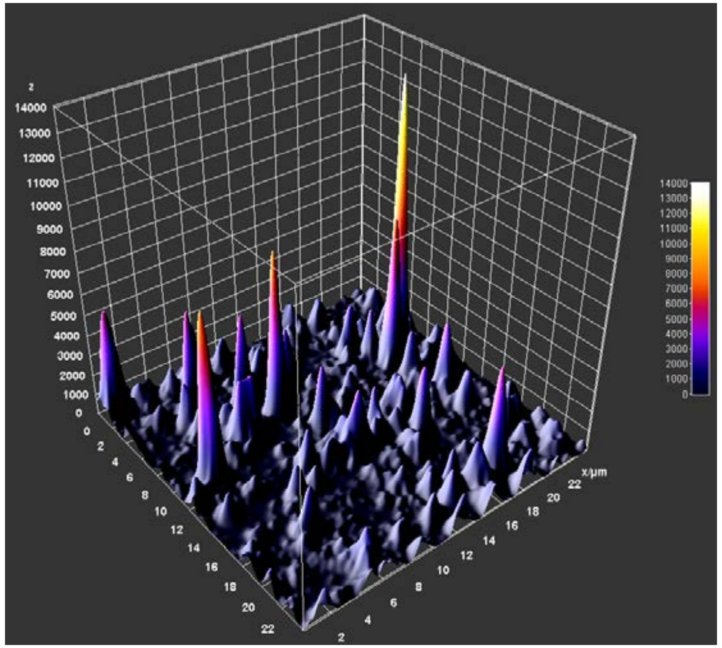
Highly-contrast SECARS resonant micro-image of a sample surface area modified with TNB/Au-NPs conjugates



$$\lambda_{as} = \frac{\lambda_p}{(2-\lambda_p/\lambda_S)}$$

Upgraded optical layout for SECARS experiments



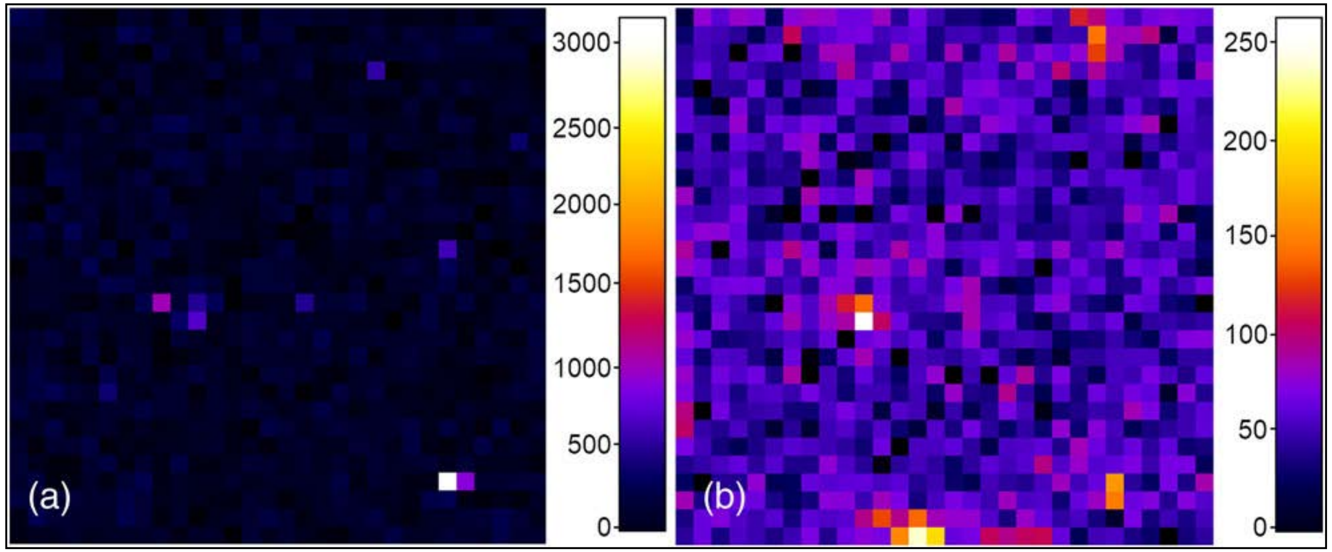


Scan area: 24 x 24 µm, resolution: 251 x 251 pl
λ_p = 932 nm (≈300 µW), λ_S = 1,064 nm (≈1,000 µW), λ_{aS} = 828 nm
fast-mapping, step 0.1 µm, 1s/image
Background signal ~ 45 counts

Contrast of the SECARS image of TNB/Au-NPs ~ 400

Comparison/correlation of SECARS и SERS signals distribution from mPBA analyte molecules

(a) SECARS, 1571 cm^{-1}



(b) SERS, 1571 cm^{-1}

Imaging contrasts ~ 50 (SECARS) и 7 (SERS)

The very first and so far the only experiments on SECARS have been done in Russia, FLNP, JINR.

G. Arzumanyan, K. Mamatkulov, V. Fabelinsky, et al., JRS, 2018, 49

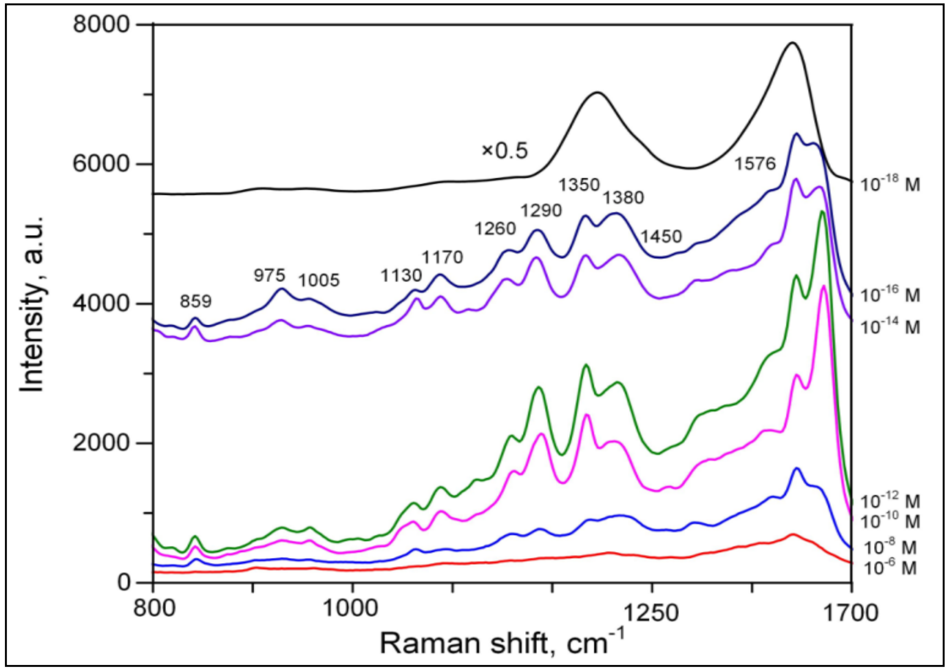
V. Fabelinsky, V. Smirnov, G. Arzumanyan et al., JRS, 2019, 50

G. Arzumanyan, invited report at the jubilee Raman conference, Novosibirsk, 2018

2. Detection of extremely low concentrations of organic molecules with the use of SERS.

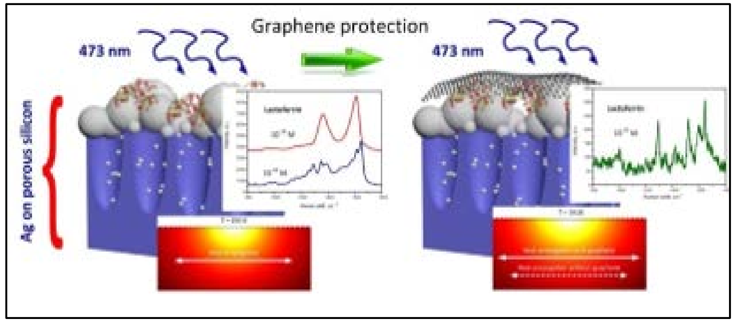
Ultrasensitive detection of lactoferrin molecules covered by graphene at attomolar concentration by Raman spectroscopy

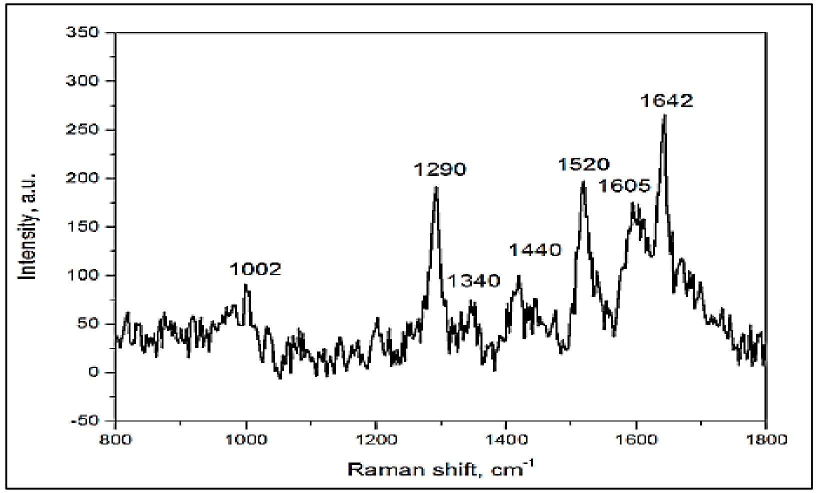
Lactoferrin mostly exists in tetramer and monomer forms determined by its concentration.



SERS spectra of lactoferrin at 10^{-6} to 10^{-18} M concentrations obtained by the collection of the SERS spectra maps (10 x 10 μm)

Overcoming the limitation

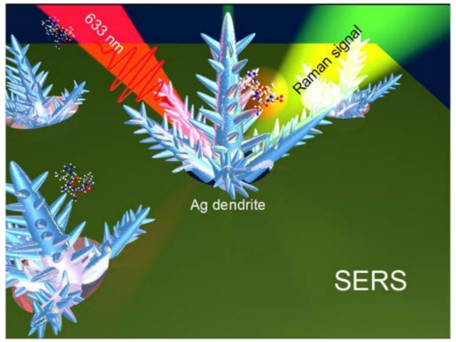




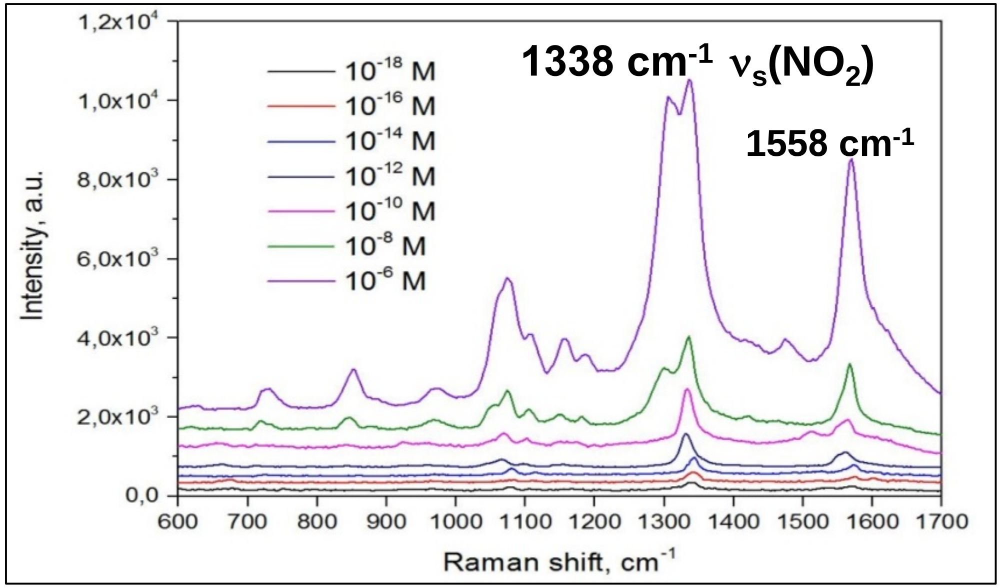
Most of the lactoferrin Raman bands are presented in the spectrum at the 10^{-18} M concentration: 1002 cm^{-1} (Phe), 1290 cm^{-1} (Amide III), 1340 cm^{-1} (Trp), 1440 cm^{-1} (CH^2), 1605 cm^{-}1 (Tyr) and 1642 cm^{-1} (Amide I).

Biosensors, 2019, 9, 34, pp. 1-19.

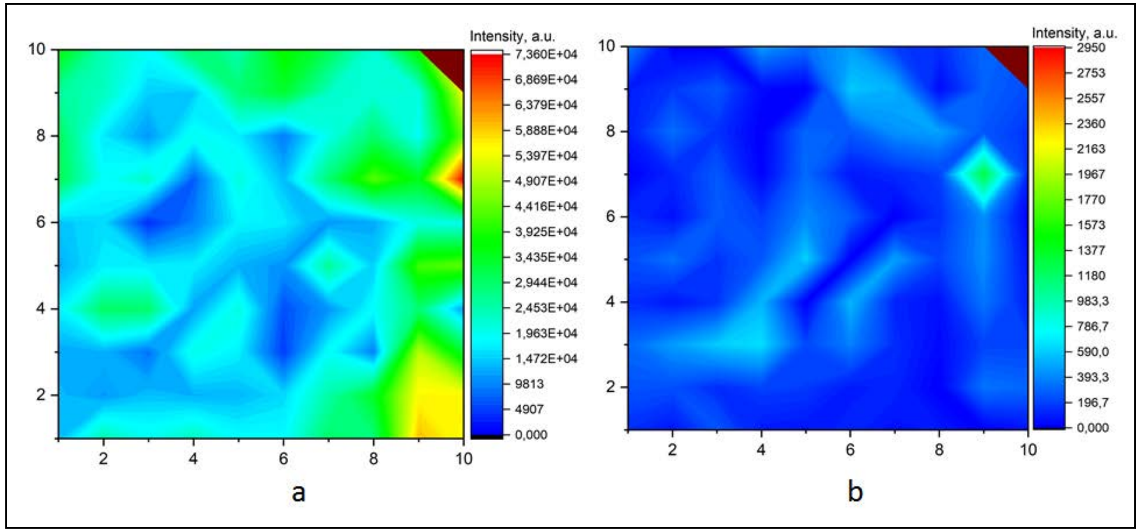
Silver 3D dendrites for attomolar limit of analyte molecules detection by SERS



Detection limit of DTNB analyte/reporter molecules adsorbed at the silver dendrites on macro-PS



Average SERS spectra for three maps of the Ag dendrites kept in the DTNB solutions in the range of 10^{-6} – 10^{-18} M concentrations.



Distribution maps of 1338 cm^{-1} band intensity in the SERS spectra of DTNB molecules from solutions at concentrations: (a) 10^{-6} M ; (b) 10^{-18} M

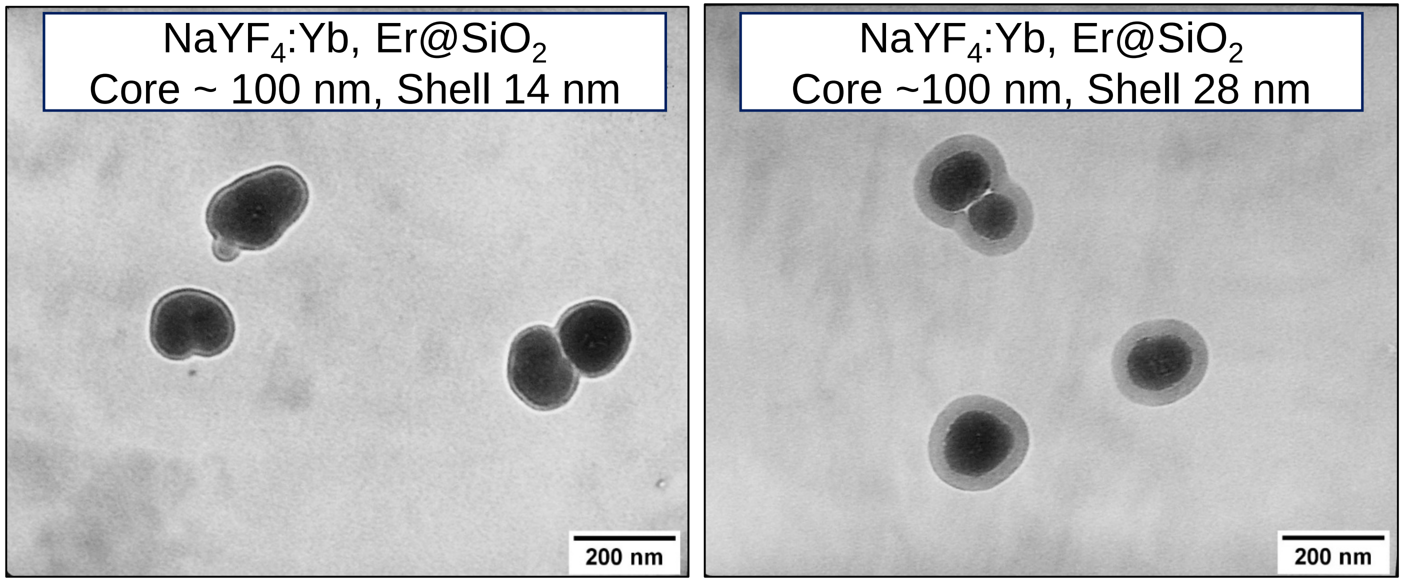
1) *J. Appl. Phys, 2019, 126*
2) *Submitted to Biosensors, 2020*

3. Synthesis of “core-shell” $NaYF_4$:Yb:Ln^{3+}@SiO_2 luminescence nanoparticles for biomedical studies in PDT

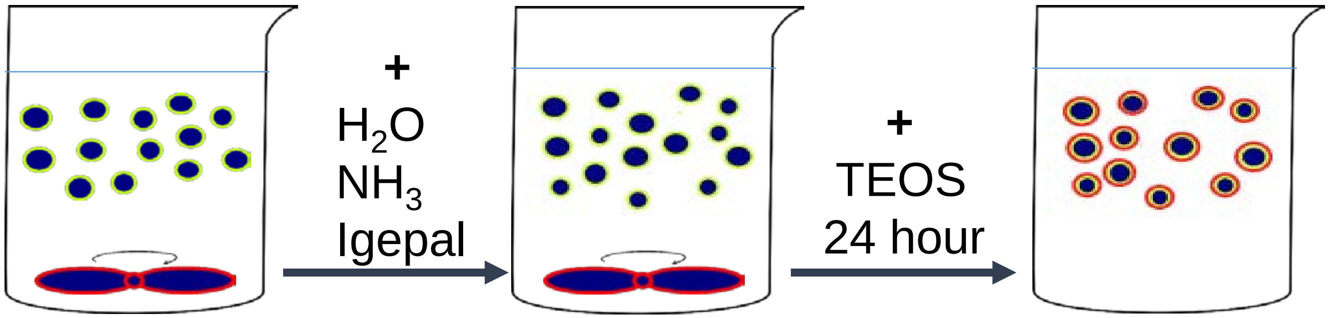
CORE-SHELL formation

Advantages of SiO_2-shell creation :

- chemical nonreactive
- increase of core stability
- reduce toxicity of core



TEM pictures of the obtained core-shell NPs: Controlling the content of TEOS enables to product different shell thickness

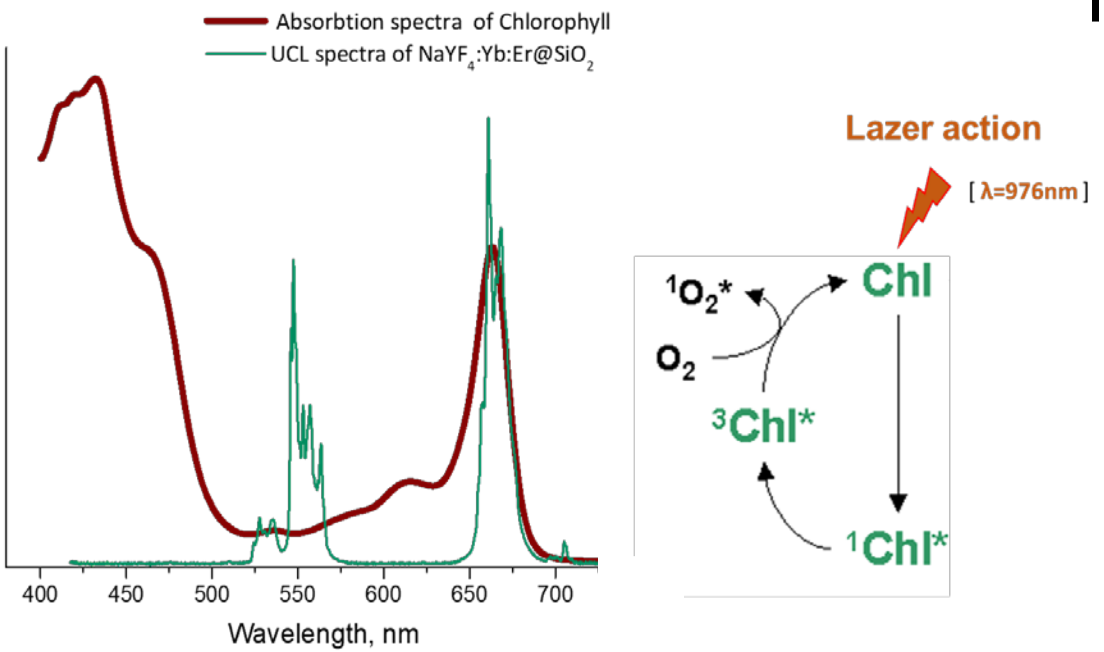


IGEPAL – surface-active substance
TEOS – tetraethoxysilane ether

Core-shell luminescent NPs in Photodynamic therapy (PDT)

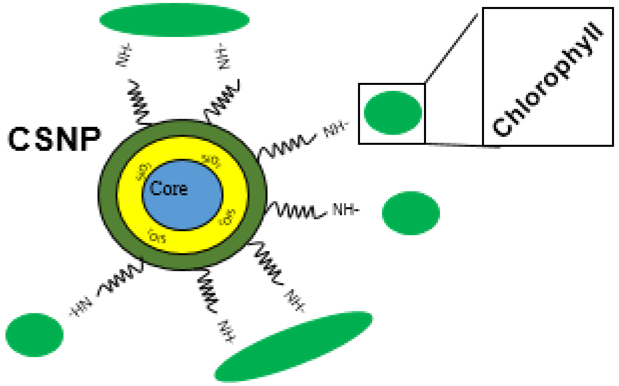
Photodynamic therapy (PDT) involves the combination of a ***photosensitizer*** (PS), ***light***, and ***ROS***.

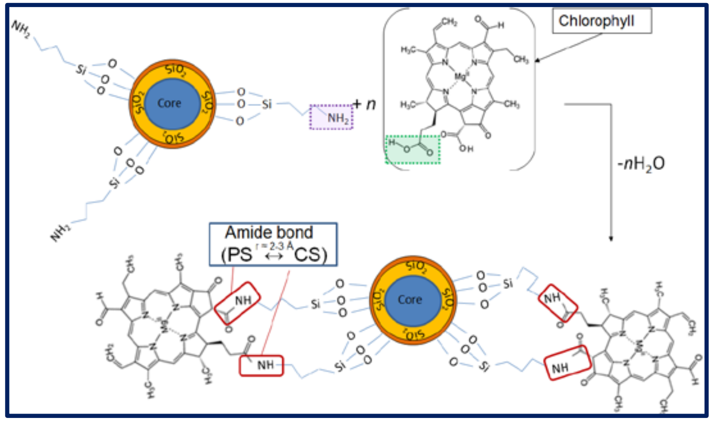
Chlorophyll – a proper photosensitizer
for $NaYF_4$:Yb:Er@SiO_2

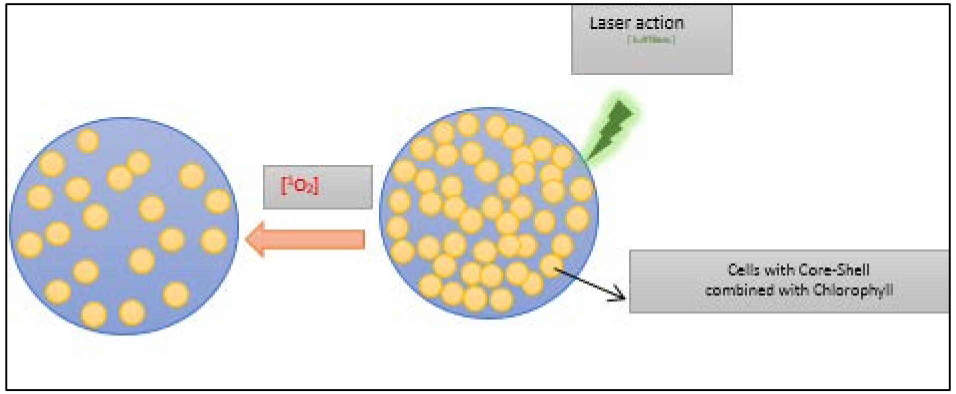


Core-Shell nanoparticles (CSNP) – benefits for PDT – have the ability to activate photosensitizer agents to produce singlet oxygen.

Immobilization of chlorophyll onto a SiO_2 shell using APTMS







Primary results on PDT test with fibroblast cells

Quantitative assessments of cytotoxicity

Staining with trypan dye assay			
№	Sample name	Negative control (without laser radiation)	Positive control (with laser radiation)
1.	Cells (fibroblasts)	92 ± 3 %	90 ± 5 %
2.	Cells + CS NPs (C=200ppm)	90 ± 4 %	87 ± 4 %
3.	Cells + CS NPs (C=200ppm) combined with Chlorophyll	**94 ± 3 %**	**69 ± 5 %**

Identified *25%* of cell death under the light exposure to UCNPs attached with chlorophyll.

Arzumanyan G.M., Linnik D.S.,et al., K. Mamatkulov, J. Biomed. Sc. & Eng., 2019, 3

STUDENT PROGRAMME

1) 2018 – 2020: 3 students defended their master's theses

2) 2018 – 2019: 2 students defended their bachelor's theses

3) 2018 – 2019: 2 students, JINR UC Summer School, Serbia and Egypt

4) 2018 – 2020: 6 students, Student summer practice program

List of main publications

Arzumanyan G.M., Mamatkulov K.Z., Fabelinsky V.I., et al., “Surface-enhanced micro-CARS mapping of a nanostructured cerium dioxide/aluminum film surface with gold nanoparticle-bound organic molecules”, JRS, 49, **2018**, 7(2), pp. 1145-1154.

Conferences with our participation

Арзуманян Г.М., 28 мая – 01 июня 2018 г., г. Новосибирск, Россия. **Keynote report**

Arzumanyan G.M., “Polarization-sensitive CARS imaging and surface-enhanced micro-CARS of organic molecules”, BIT’s 2nd International Biotechnology Congress-2018, October 14-16, 2018, Fukuoka, Japan.

Publications, conferences, grants & programs, JINR prizes

- ***15 publications (2-3 more are expected by the end of 2020)***
- ***21 conferences (4 keynote presentations, 12 oral, 5 posters)***
- ***Grants & programs of Plenipotentiaries: Bulgaria, Poland, Romania and Slovak Republic***
- ***FLNP first prize for “Experimental research”, 2019***

International collaboration: 9 countries (8 – JINR Member States)

Armenia, Belarus, Bulgaria, Egypt, Poland, Romania, Russia, Slovak Republic, Ukraine.

Randomly -Nanostructured SERS-Active Surfaces”, Proceedings of the 18-th European conference on non-linear optical spectroscopy (ECONOS), **2019**, April 7-10, Rouen, France, p.74, (Microspectrometer “CARS”).

Yakimchuk D.V., Kaniukov E.Yu., Arzumanyan G.M., et al., "Self-organized spatially separated silver 3D dendrites as efficient plasmonic nanostructures for Surface-enhanced Raman spectroscopy applications", J. Appl. Phys, **2019**, 126

Arzumanyan G.M., Gur'ev A.S., Mamatkulov K.Z., et al. "Micro Raman spectroscopy for NETosis detection”, JRS, **2020,** 1-10

Oral report

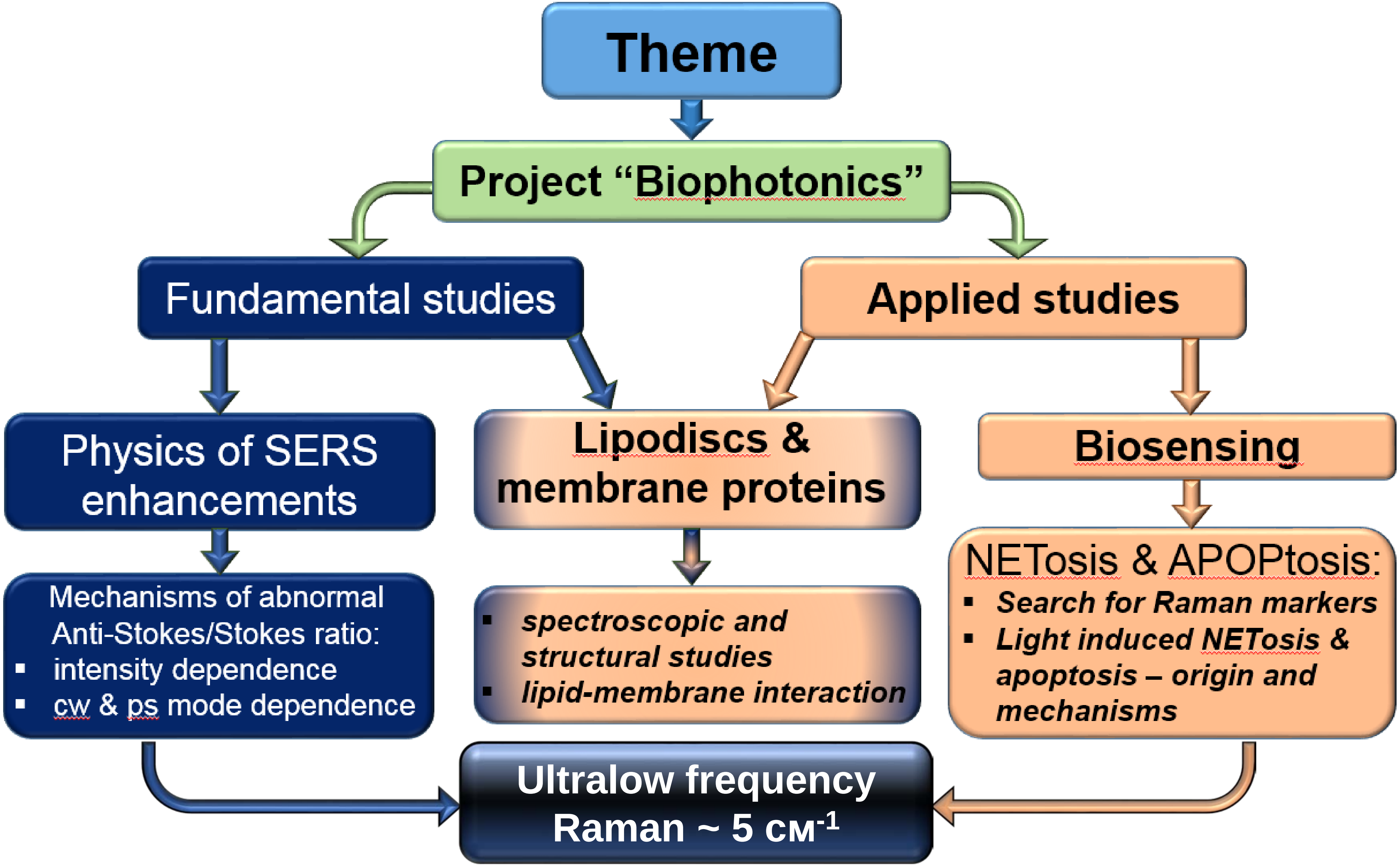
Marchenko A.S., Vereshchagin K.A., Volkov A.Yu., Mamatkulov K.Zh., Arzumanyan G.M., International Conference on Radiation Applications (RAP 2019), September 16-19, 2019, Serbia, Belgrade. Poster

Vorobeva M.Yu., Mamatkulov K.Zh., Bandarenka H.V., Arzumanyan G.M., EuroSciCon Conference on Nanotechnology and Smart Materials, 2019, 08-10 July, Prague, Czech Republic, Oral report

Arzumanyan G.M., Bandarenko A.V., Mamatkulov K.Zh., 2020, Feb. 21-22, EuroSciCon-2020, Amsterdam, Netherlands, **Keynote report**

Proposal on the theme extension and opening of a new project “Biophotonics” for the years of 2021-2023.

Team and project leaders:
G.M. Arzumanyan and N. Kučerka


Theme
Project "Biophotonics"
Fundamental studies
Applied studies
Physics of SERS enhancements
Lipodiscs & membrane proteins
Biosensing
Mechanisms of abnormal Anti-Stokes/Stokes ratio:
intensity dependence
cw & ps mode dependence
spectroscopic and structural studies
lipid-membrane interaction
NETosis & APOPtosis:
Search for Raman markers
Light induced NETosis & apoptosis – origin and mechanisms
Ultralow frequency Raman ~ 5 см-1

Physics of SERS enhancement: abnormal aS / S ratio in SERS

Motivation dates back to 1996:
first time vibrational (Raman) pumping was reported in SERS

VOLUME 76, NUMBER 14 PHYSICAL REVIEW LETTERS 1 APRIL 1996

Population Pumping of Excited Vibrational States by Spontaneous Surface-Enhanced Raman Scattering

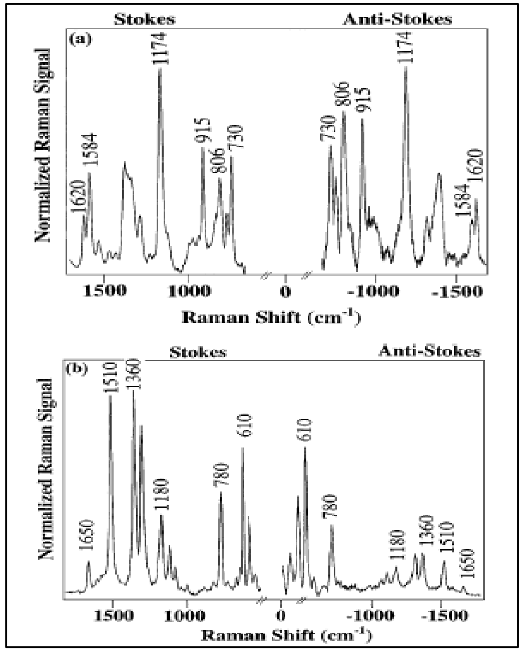
Katrin Kneipp, Yang Wang, Harald Kneipp,* Irving Itzkan, Ramachandra R. Dasari, and Michael S. Feld
George R. Harrison Spectroscopy Laboratory, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, Massachusetts 02139
(Received 4 August 1995)

Authors suggests a significant transfer of ground state population to the first excited vibrational state by spontaneous SERS.

Evidence for this pumping includes:

(i) anti-Stokes to Stokes ratios which exceed those expected from a Boltzmann distribution,

(ii) a quadratic dependence of the aS signal on the excitation intensity.

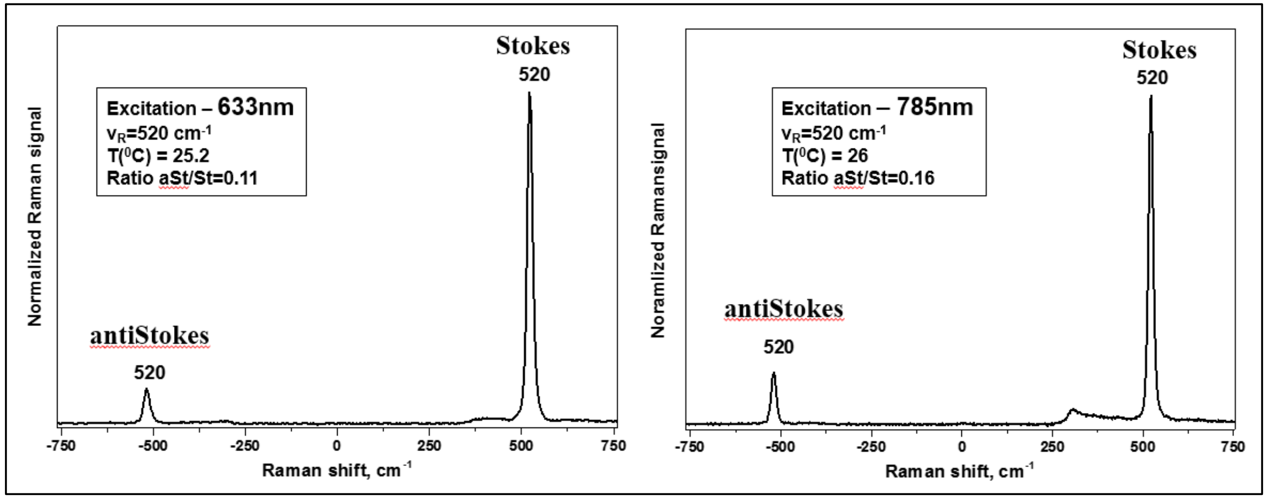


SERS Stokes and anti-Stokes spectrum of (a) crystal violet and (b) rhodamine 6G.

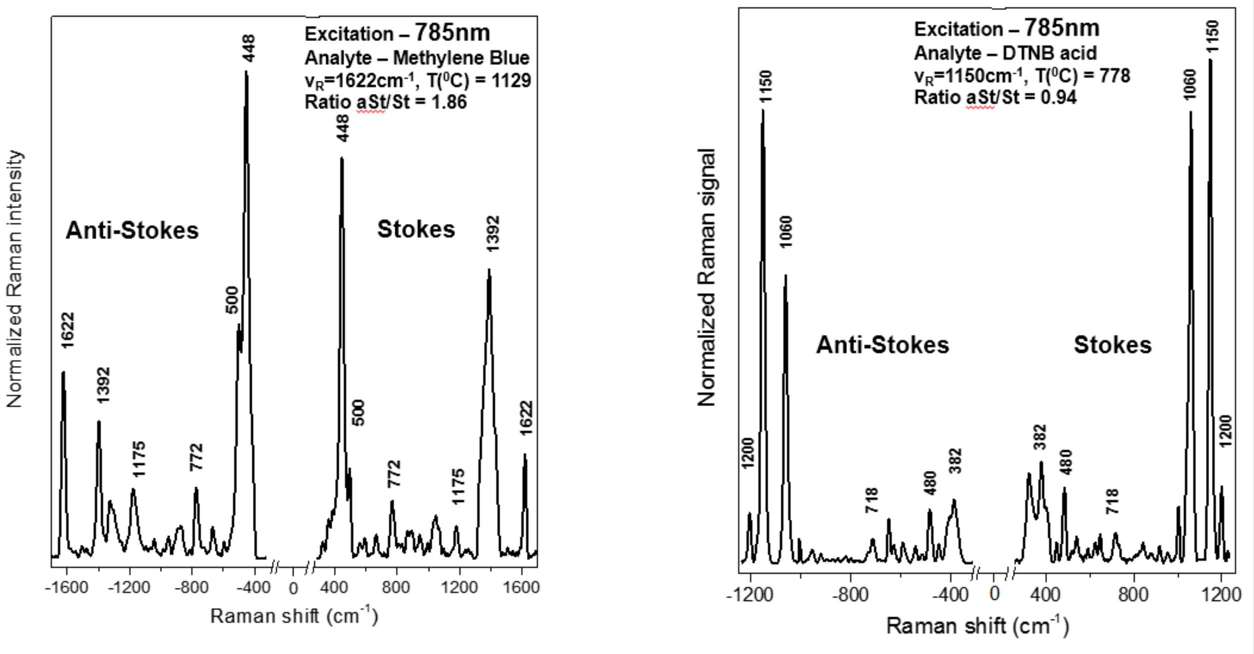
However, this issue is still widely debated in the Raman community with many authors denying the existence of Raman pumping attributing the effect to laser heating, resonance effects, or combinations thereof.

Our results

antiStokes / Stokes Raman spectra of Si plate



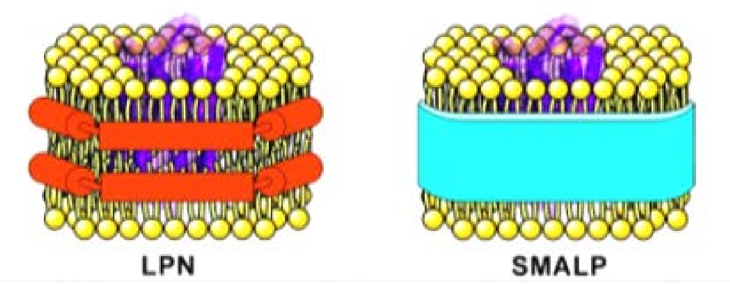
antiStokes / Stokes SERS spectra of MB and DTNB



Lipodiscs with embedded membrane proteins: spectroscopic and structural studies

LPN (Lipid-Protein Nanodisk) – represent the membrane mimicking medium consisting of discoid patches of the lipid bilayer stabilized in solution by an amphiphilic belt of membrane scaffold protein (MSP).

LIPODISCS: SMALP vs LPN



SMALP – Styrene Maleic Acid Lipid Particle: a new and promising tool in membrane research – detergent-free solubilization of membrane proteins by styrene–maleic acid copolymers (SMAs).

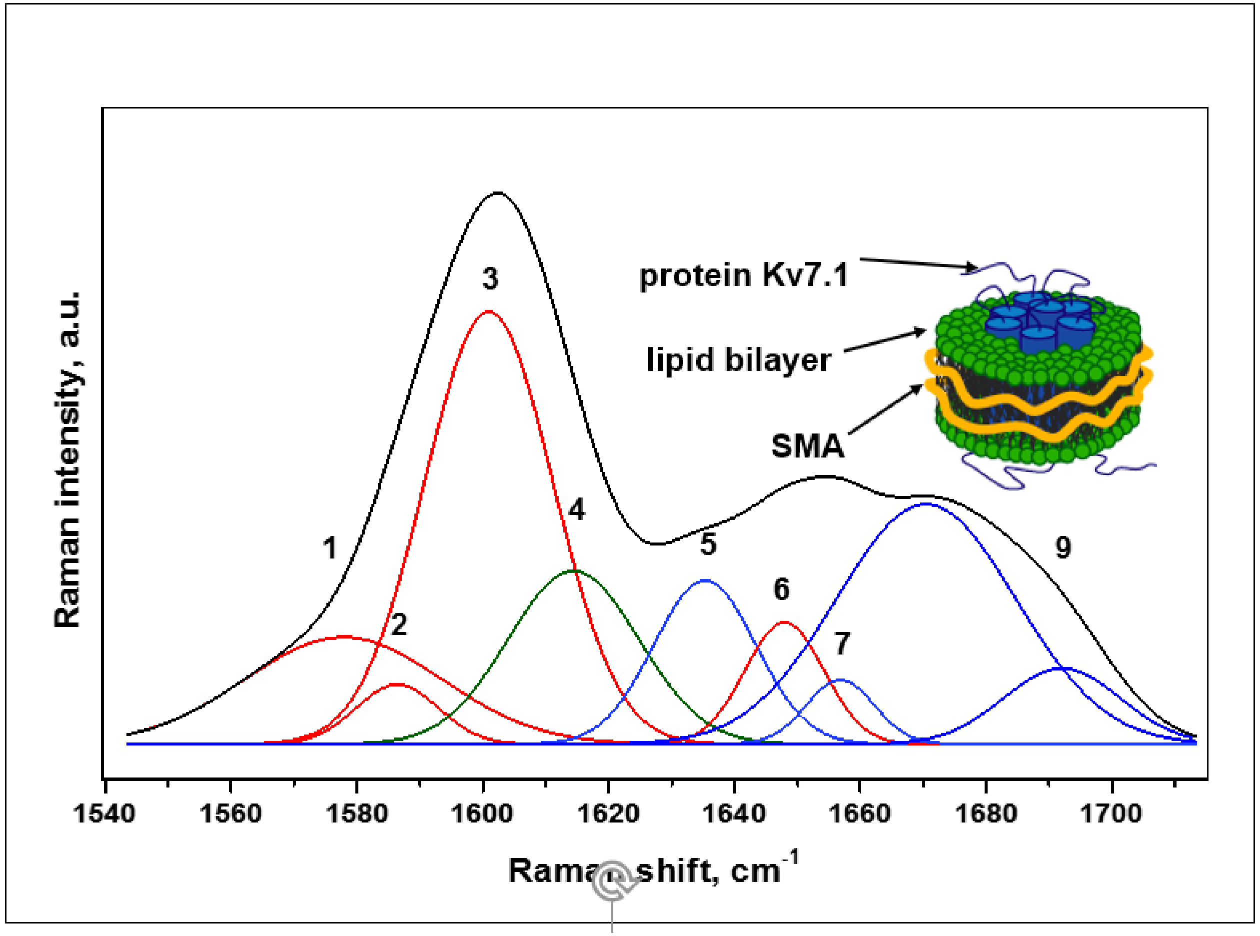
Advantages of SMALP configuration:

- **conformational stability of membrane proteins**
- **detergent free system**
- **no need for crystallization processes**

Main partners:

- **Moscow State University, Faculty of Bioengineering, Russia**
- **Pavol Jozef Šafárik University in Košice, Slovakia**
- **Institute of Solid State Physics BAS, Sofia, Bulgaria**

First test measurements at FLNP - 2020



Deconvulated of Raman spectrum of Kv7.1 protein embedded into the lipodisc

№	Raman shift, cm^{-1}	Assignment
1	1578	SMA
2	1586	SMA
3	1601	SMA
4	1614	Tyr, Trp, Phe
5	1635	amide I: unordered
6	1648	SMA
7	1657	amide I: α-helix
8	1670	amide I: β-sheet
9	1692	amide I: β-turn

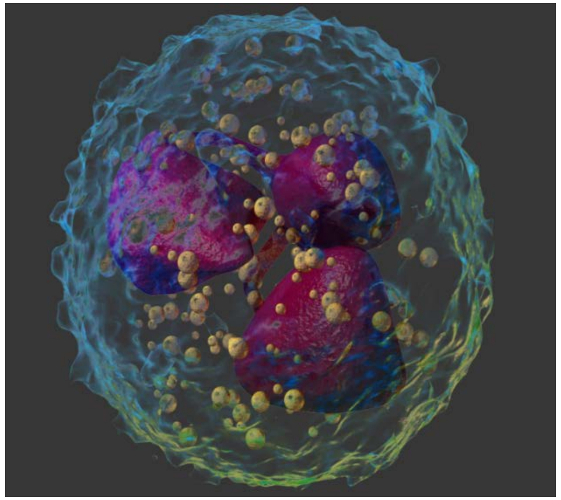
Region: 1500-1800 cm^{-1}

Raman spectroscopy as an early diagnostic tool

Neutrophils and NETosis

NETs – Neutrophil Extracellular Traps

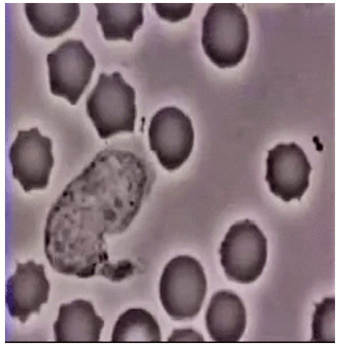
Neutrophils are the immune system's *first line of defense* against bacterial, fungal and in viral infections

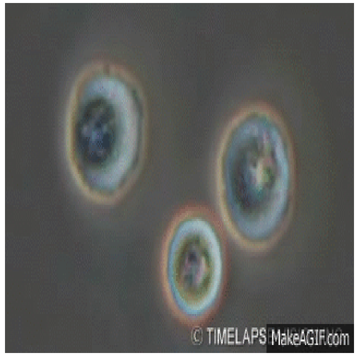


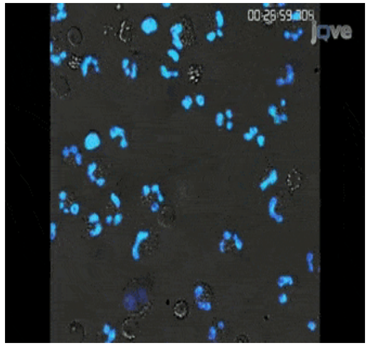
In the cytoplasm of neutrophil cells there are granules with antimicrobial agents

Protective capabilities of neutrophils

- Phagocytosis
- Chromatine decondensation
- Formation of NETs







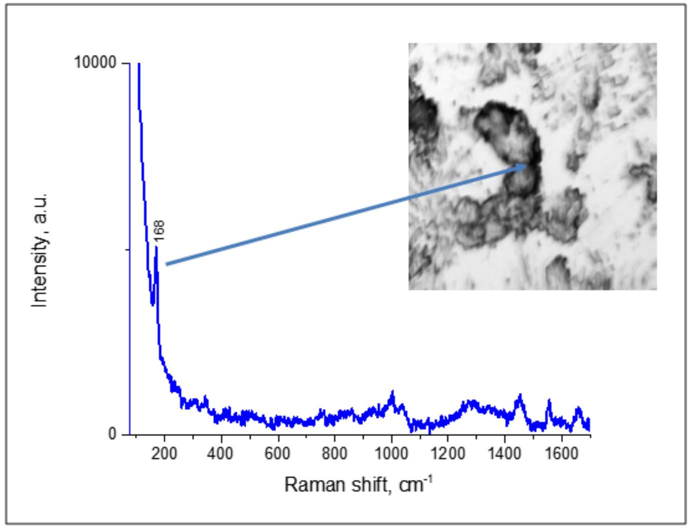
Klebsiella pneumoniae in NET

NET has an electrostatic charge which holds pathogens

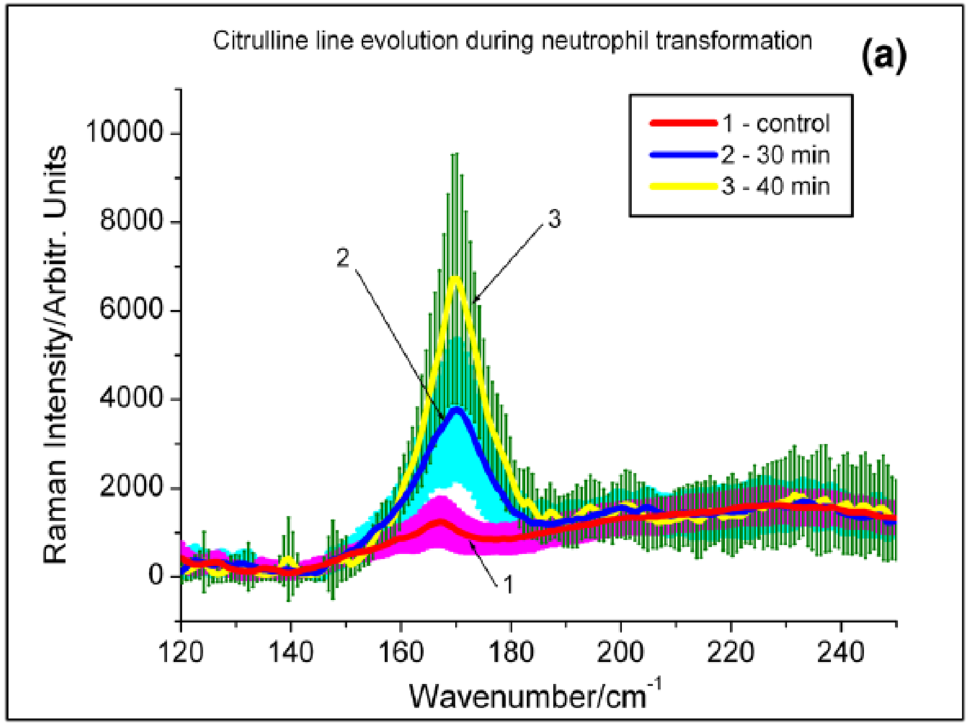
In cooperation with MONIKI - Moscow Regional Research Clinical Institute

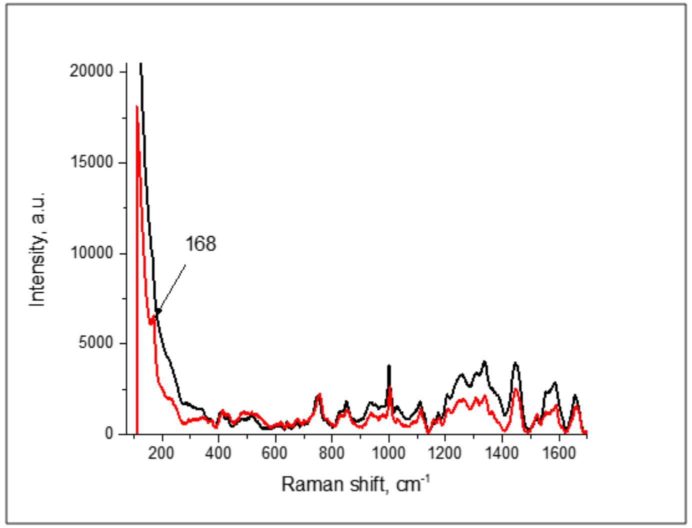
Raman Marker of NETosis

NETs preformation: citrullination of histones (nuclear proteins)



The averaged Raman spectrum of neutrophils chemically activated (30 min)





The averaged Raman spectrum of neutrophils activated with bacteria (40 min)

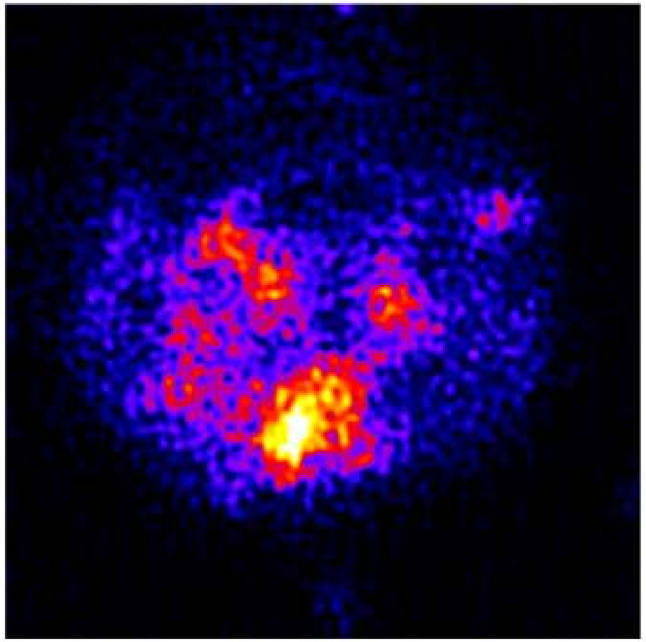
Raman spectrum: low-frequency range of citrulline line evolution indicating the pre-activation of NETosis

Since NETosis is a process of programmed neutrophil cell death, the growth of the "citrulline" peak in Raman spectrum indicates that cell death will occur soon.

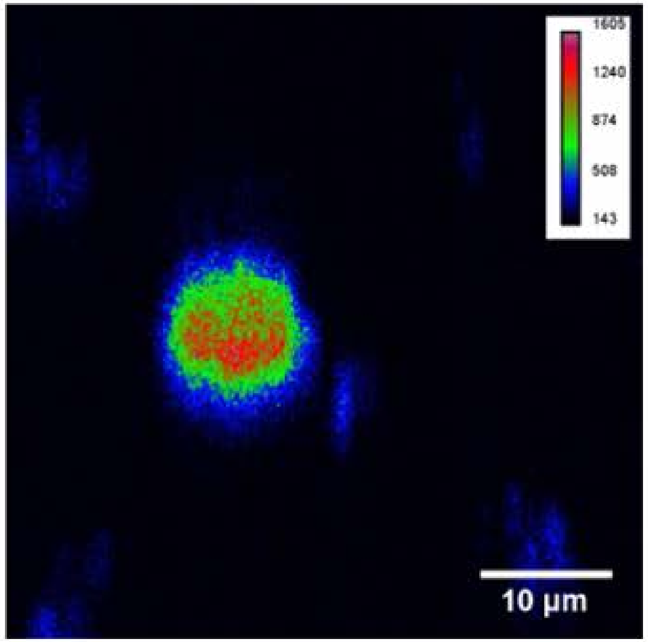
Arzumanyan G.M., Mamatkolov K.Z., Volkov A.Yu., et al., J. of Raman spectroscopy, 2020, https://doi.org/10.1002/jrs.5844

Spectral and AFM imaging of neutrophil cell

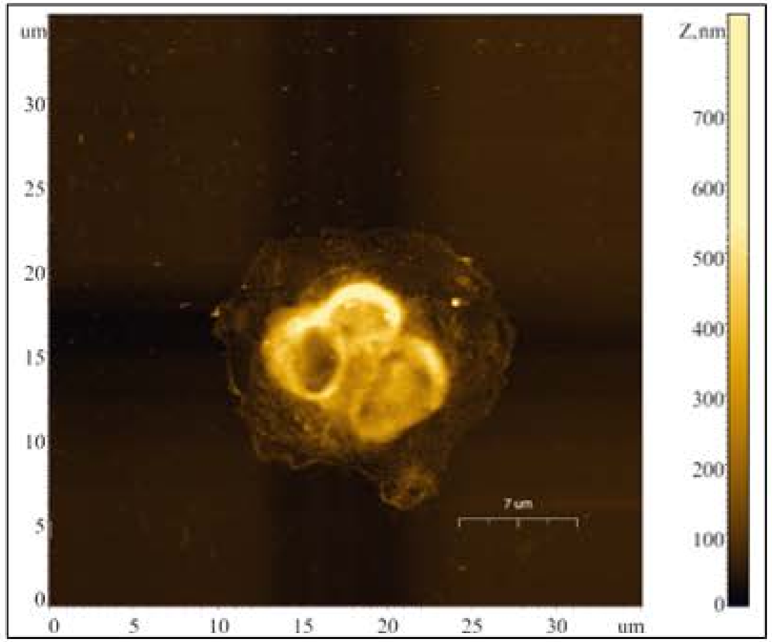
CARS



UC luminescence



AFM



List of activities 2021-2023

- **Study of the features of the Stokes and anti-Stokes components of the SERS spectra from analyte molecules in order to understand the processes of enhancement in SERS spectroscopy.**
- **Testing of SERS active substrates with organic / bio molecules in order to determine the range of pump intensity for recording reproducible aSt / St spectra.**
- **Investigation of a possible nonlinear dependence of the SERS spectrum on the intensity and the pump mode used.**
- **Testing the technique for obtaining Raman spectra of lipodisks with and without membrane.**
- **Study of the influence of the lipid environment on the structure of the membrane protein.**
- **Search for spectral / Raman markers of NETosis.**
- **Reveal the mechanisms of sterile activation of NETosis under UV radiation.**
- **Ultra-Low frequency Raman spectroscopy ~ (5-10) cm^{-1} – upgrade of the optical platform.**

Expected main results upon the completion of activities

- A comparative analysis of the ratio of the intensities of the SERS lines in aSt / St spectral regions depending on the pump radiation power.
- Determination of the characteristics of the intensity ratio aSt / St depending on the excitation wavelength of analyte molecules.
- Identification, comparison and characterization of the mechanisms of formation of aSt / St components of SERS spectra in continuous and pulsed modes.
- Detailed analysis and interpretation of the Raman spectra of lipodiscs with various membrane proteins.
- Confirmation of the incorporation of membrane protein into lipodisc and determination of the features of its structure.
- Obtaining new information about the structure of lipodiscs with membrane proteins and "empty" lipodisc.
- Identification of Raman markers of NETosis in various regions of the Raman spectrum.
- Determination of the mechanisms of the formation of NETosis under the influence of UV radiation.
- Gaining experience in operating with ultra-low frequency Raman spectrometer ~ (5-10) cm^{-1}.

Schedule proposal for the years 2021-2023

Activities		Plans per year		
		2021	2022	2023
1.	Study of the features of the ratio of intensities aSt / St in SERS spectra depending on the power and pump wavelength.			
2.	Identification of mechanisms of formation of aSt / St spectra in continuous wave and pulsed modes.			
3.	Systematic experiments aimed at formulating the conditions for recording reproducible SERS spectra for biosensorics.			
4.	Development of a technique for recording and analyzing Raman spectra of lipodiscs without and with embedded membrane proteins.			
5.	Study of the influence of the lipid environment on the structure of the membrane protein.			
6.	Obtaining new spectroscopic information on the structure of lipodiscs with membrane protein.			
7.	Search for spectral / Raman markers of NETosis.			
8.	Investigation of the mechanisms of sterile activation of NETosis under UV radiation.			
9.	Ultra-Low Frequency Raman spectroscopy ~ (5-10) cm^{-1}			

Budget and terms of the theme/project implementation: 2021 – 2023

Structure and staff

Sector of Raman spectroscopy, FLNP

Staff number – 11, among them young researchers – **7 (< 35)**

Including staff from JINR Member States – 2

Total estimated cost of the project implementation according to JINR's Seven-Year Plan:

YEAR	k$
2021	127
2022	122
2023	116

Thank You!