Приложение 2

**Questionnaire**

for the extraordinary session of the PAC for Condensed Matter Physics for the assessment of related JINR projects

***Project:*** ***Raman microspectroscopy in biomedical study (“Biophotonics”), 2021-2023***

***Project leaders: Grigory Arzumanyan, Norbert Kučerka***

**PART A: Achievements**

1.   Contributions of the JINR group:

-List the contributions of the JINR group in hardware (including use of JINR computing resources for the project), software development and physics analyses.

The Sector of Raman spectroscopy was established as a self-acting structural unit of the Frank Laboratory of Neutron Physics (FLNP) in 2015. The basic spectroscopic facility here is a unique“Confotec CARS” multi-channel 3D scanning laser microscope-spectrometer (SOL Instruments, Belarus).

Over the past few years, the optical platform of the CARS microscope has been significantly extended and supplemented with new functionalities by the FLNP team of Raman spectroscopy making it truly multimodal optical platform. Among them:

* for spontaneous Raman scattering signal generation, along with the 633nm excitation, an additional optical system (lasers and corresponding optics) was installed at the CARS microscope platform to excite the Raman signal at 532 nm and 785 nm;
* to achieve an ultrahigh sensitivity in Raman-signal detection on the level of a few/single molecules Surface-Enhanced Raman Spectroscopy (SERS) was adapted to the optical platform using various wavelengths of laser excitation;
* polarization sensitive CARS has been developed to suppress a non-resonant background of CARS signal;
* as a complementary option to CARS imaging, second harmonic imaging was also developed and installed on the platform for visualization of biological objects which is known as Second Order Nonlinear Imaging of Chiral Crystals (SONICC);
* for the first time in Russia, combination of two Raman enhanced modalities - SERS with CARS (SECARS) was developed and realized;
* starting from 2020 the CARS microspectrometer is a new stage of hardware upgrade aimed at the home-made option of ultra-low frequency Raman (~ 10 cm-1) operating both in Stokes and anti-Stokes regions at the 633 nm excitation. This is of a high importance in SERS and biomedical studies;
* up-conversion luminescence spectroscopy and imaging based on the phosphors activated with various rare-earth elements were developed using NIR excitation at 976 nm (for biomedical studies core-shell phosphors were developed as well);
* for the phospholipid dynamics characterization by Raman spectroscopy our group uses the following LIT computing resources via Heterogeneous platform “HybriLIT”:
* GROMACS – a molecular dynamics package mainly designed for simulations of proteins, lipids, and nucleic acids.
* Amber - a set of programs for biomolecular simulation and analysis.

These MD simulations are implemented in parallel with the neutron scattering studies (leader Norbert Kučerka**)**, in particular by SANS, for the same task and samples.

* as for the physics analyses of the obtained results the team of the Raman spectroscopy uses a number of programs like: hierarchical cluster analysis (HCA), principal component analysis (PCA), NANO SP – initially developed by the “SOL Instruments” and further several times upgraded with the participation of Raman spectroscopy team, etc.

***The above-mentioned points of the hardware developments were done on the full initiative of the FLNPs’ Raman spectroscopy group in just three years.***

-List the responsibilities of JINR group members within the management structure of the collaboration, if any, giving the name of the JINR member, the managerial role and the appointment period.

The Sector of Raman spectroscopy constantly expands its international cooperation, first of all, with the JINR Member countries. Over the past three years, this division has become particularly attractive to Slovak Republic, Poland, Romania, Bulgaria. This interest is reflected, among the others, in the support of grants and cooperation programs from the Plenipotentiary Representatives of these Member-State countries. Also, very large cooperation has been established with a number of research organizations in Belarus Republic. Starting this year, closer cooperation is also planned with Armenia and Uzbekistan as well.

Co-leaders of the project “Biophotonics” – Grigory Arzumanyan and Norbert Kučerka are playing the key managerial roles in these cooperation activities.

2.   Publications:

-List the papers published in the refereed literature (no conference proceedings) in which the JINR group had a major contribution (e.g. author of the analysis, promoter of the experiment, corresponding author, realization of a key equipment etc.). Give title of paper, reference and describe in 1-2 sentences the JINR contribution. Only papers published since the last approval of the project should be listed.

Since the new project "Biophotonics" was implemented only three months ago (starting from January 2021), the publications in the refereed literature and invited talks are listed mainly for the years 2019-2020 (+2 more from 2018), project “Nanobiophotonics”, 2018-2020.

1. “Surface-enhanced micro-CARS mapping of a nanostructured cerium dioxide/aluminum film surface with gold nanoparticle-bound organic molecules”, J. Raman. Spectroscopy (JRS), 49, 2018, 7(2), 1145-1154, doi: 10.1002/jrs.5333.
2. “Laser intensity limits in surface‐enhanced linear and nonlinear Raman micro‐spectroscopy of organic molecule/Au‐nanoparticle conjugates”, JRS, 2019, (50), 1311–1320, doi: 10.1002/jrs.5645.
3. “Hexakis(dimethylsulfoxide-O)-cobalt(II) hexatungstate, [Co(C2H6OS)6][W6O19]: synthesis from aqueous di ethylsulfoxide solution, crystal structure determination, FT-IR and Raman spectroscopy analysis, and surface micromorphology”, J. Coord. Chem., 71(3), 2018, 1-19, doi: 10.1080/00958972.2018.1440394.
4. “Synthesis of NaYF4:Yb,Er@SiO2@Ag core-shell nanoparticles for plasmon-enhanced upconversion luminescence in bio-applications”, Annals of Biomedical Science and Engineering, 2019, 3, 013-019, doi: 10.29328/journal.abse.1001006.
5. “Surface Enhanced Raman Spectroscopy of Lactoferrin Adsorbed on Silvered Porous Silicon Covered with Graphene”, Biosensors, 2019, 9, 34, 1-19, doi: 10.3390/bios9010034.
6. "Self-organized spatially separated silver 3D dendrites as efficient plasmonic nanostructures for Surface-enhanced Raman spectroscopy applications", J. Appl. Phys, 2019, 126, doi: 10.1063/1.5129207.
7. “Micro Raman spectroscopy for NETosis detection”, J. Raman Spectr., 2020, 51,
1960-1969, doi: 10.1002/jrs.5844.
8. “Morphology and Microstructure Evolution of Gold Nanostructures in the Limited Volume Porous Matrices”, SENSORS, 2020, 20, 4397, doi: 10.3390/s20164397.
9. “Modulation of cytotoxicity by consecutive adsorption of tannic acid and pesticide on surfactant functionalized zeolites”, 2020, Environmental Science: Processes & Impacts, doi: 10.1039/D0EM00251H.
10. "3D silver dendrites for single-molecule imaging by surface-enhanced Raman spectroscopy", ChemNanoMat, 2020, doi: 10.1002/cnma.202000521.
11. “Thermally expanded graphite from graphite nitrate cointercalated with ethyl formate and acetic acid: morphology and physicochemical properties”, 2020, J. of Physics: Conference Series, doi:10.1088/1742-6596/1658/1/012004.
12. “Anisotropic Photoluminescence of Poly(3-hexyl thiophene) and Their Composites with Single-Walled Carbon Nanotubes Highly Separated in Metallic and Semiconducting Tubes”. Molecules 2021, 26, 294, doi: 10.3390/molecules26020294.
13. “Investigating the competitive effects of cholesterol and melatonin in model lipid membranes”, 2021, (submitted to BBA, three referees’ remarks are mostly positive).

JINR's contribution to the majority of the above works is decisive and is expressed in the following: in 10 out of 13 works, the entire experimental part was carried out at FLNP JINR, and the Raman spectroscopy group members were promoters and/or corresponding authors of the papers.

3.   PhD theses:

-List the PhD theses completed within the last 3 years, or expected to be completed within 2021, by JINR students within the project, giving the student name, thesis title and graduation year.

 As it was mentioned above the Sector of Raman spectroscopy is relatively new structural unit at FLNP. The majority of the staff members are young scientists, engineers and technicians. Naturally, the student program is in the tight focus of our educational and research activities. During the years of 2018-2020 five master’s theses were defended:

2018 – Victoria Shatilova and Kristina Demeshenkova (mark – Excellent, both)

2019 – Anastasia Marchenko (mark – Excellent)

2020 – Sergey Rudnikh and Sanal Marmakov (mark – Excellent, both)

4.   Talks:

- List the invited plenary talks given by members of the JINR group at international conferences, workshops… since the last approval of the project: give name and date of the conference, title of talk and speaker name.

1. First International Conference on Molecular Modeling and Spectroscopy, 19-22 February, 2019, Egypt, “Polarization-sensitive CARS imaging and surface-enhanced micro-CARS of organic molecules”, Arzumanyan G.M., **Keynote report**
2. EuroSciCon Conference on Nanotechnology and Smart Materials, 08-10 July ,2019, Prague, Czech Republic. “Coherent surface-enhanced raman scattering: chemical imaging and intensity limits”, Arzumanyan G.M., **Keynote report**
3. International Conference on Nanomaterials and Quantum Plasma Science, 21-22 February, 2020, Amsterdam, Netherlands. “Ultrasensitive detection of analyte molecules at attomolar concentration by Raman spectroscopy”, Arzumanyan G.M., **Keynote report**
4. 21st Intern. School on Condensed Matter Physics (online) – Prospe cts and Perspectives in Functional Materials, 2020, 31st August – 04 September, Varna, Bulgaria. "Surface-Enhanced Raman Spectroscopy and Biosensing", Arzumanyan G.M., **Invited talk.**
5. Second International Online Conference on Molecular Modeling and Spectroscopy, 2020, 23 -24 September, Cairo, Egypt. “Single-molecule imaging by Surface-Enhanced Raman Spectroscopy”, Arzumanyan G.M., **Keynote report**

**-** Give a similar list for parallel talks.

1. BIT’s 2nd International Biotechnology Congress-2018, October 14-16, 2018, Fukuoka, Japan, “Polarization-sensitive CARS imaging”, Arzumanyan G.M., Oral talk.
2. International Conference on Analytical and Nanoanalytical Methods for Biomedical and Environmental Sciences: “IC-ANMBES 2018”, 23-25 May, 2018, Brasov, Romania. “Detection of DNA molecules by SERS spectroscopy with silver porous silicon as an active substrate”, Doroshkevich N.V., Section talk.
3. Int. conf. SPIE-2018, 19-23 August, 2018, San Diego, California, United States, “Optical properties of mesoscopic, multiscale silver films: surface plasmon localization and giant SERS”, Sarychev A.K., Oral talk.
4. VIII Int. conference on Solid State Physics-2018, 24-28 September, 2018, Minsk, Belarus. «Photoluminescence and structural characteristics of glass-ceramics composed of zinc oxide nanocrystals and europium ions”. Mudrii A.V., Oral talk.
5. Advanced Photonics Congress, 02 – 05 July, 2018, [ETH Zurich, Zürich, Switzerland](http://maps.google.com/?q=ETH+Zurich%2c++R%c3%a4mistrasse+101%2c+Z%c3%bcrich%2c+++Switzerland&lightbox%5bwidth%5d=610&lightbox%5bheight%5d=360), “Comparison of SERS-activity of Silver Dendrites and Nanoparticles on Structured Silicon”, Bandarenka H.V., Oral talk.
6. International School of Nuclear Physics «JINR Days in Bulgaria», 2019, 13 -17 May, Borovets, Bulgaria. “Modern Trends in Raman Microspectroscopy”, Arzumanyan G.M., Oral talk.
7. 4th International Conference on Nanotechnologies and Biomedical Engineering, ICNBME-2019, September 18-21, 2019, Chisinau, Moldova. “Surface Enhanced Raman Spectroscopy of Organic Molecules Adsorbed on Silvered Porous Silicon Covered with Graphene”, Mamatkulov K.Z., Oral report
8. 18-th European conference on non-linear optical spectroscopy (ECONOS), 2019, 7-10 April, Rouen, France. “Peculiarities of Micro Raman Spectra of Blood Neutrophils Transformed during NETosis as a Possible Marker of Sepsis Mortality”, Vereshchagin K.A., Oral report
9. Openbio-2019, Novosibirsk, Russia, 22-25 October, 2019. “On structural peculiarities and vibrational spectroscopy of phospholipids”, Rudnikh S.K., Section talk.
10. VI International Caparica Conference on Analytical Proteomics 2019, 08 – 11 July 2019, Caparica, Portugal. “Study of proteomic analytes by surface enhanced Raman spectroscopy”, Bandarenka H.V., Oral talk.
11. International Conference on Radiation Applications (RAP 2019), September 16-19, 2019, Serbia, Belgrade. “Raman spectroscopy of NETosis: search for spectral biomarkers”, Marchenko A.S., poster.
12. EuroSciCon Conference on Nanotechnology and Smart Materials, 2019, 08-10 July, Prague, Czech Republic, “Highly-sensitive Surface-enhanced Raman scattering based on silver dendritic nanostructures”, Vorobeva M.Yu. poster.
13. XXIV International Scientific Conference of Young Scientists and Specialists (AYSS-2020), April 2020, Dubna University, Russia, “Core-shell nanostructures: synthesis and possible applications”, Rudnikh S.K., Oral talk

**PART B: Plans and requests**

5.   Plans

-Describe the plans of the JINR group within the project, in physics analysis, data taking, software development. detector R&D, detector operation and maintenance, upgrade activities… for the period of time of the requested extension.

***Project: Raman microspectroscopy in biomedical study (“Biophotonics”), 2021-2023***

1. ***General Information***

The project “Biophotonics” comprises fundamental and applied parts. As for the basic research, the activities will be aimed at identifying and understanding the mechanisms of the anomalous ratio of intensities of the antiStokes/Stokes components in the spectrum of surface-enhanced Raman scattering. This will allow to formulate the conditions for obtaining reproducible SERS spectra during the development of biosensors. Applied tasks are related to (i) spectroscopic and immunofluorescence studies of the programmed cell death NETosis, in particular, to search for Raman markers of this phenomenon, as well as to determine the mechanisms triggering the sterile activation of NETosis under the influence of UV radiation, and, (ii) lipid-protein interaction using modern membrane mimetic – lipodiscs, nanodiscs, and liposomes.

To implement the proposed project, a multimodal optical platform based on the CARS microscope, atomic force microscopy (AFM), dynamic light scattering (DLS), electron microscopy (SEM, TEM), small-angle neutron scattering (SANS) and other instrumentations will be employed. Also, ultra-low frequency (~ 10 cm-1) Raman spectroscopy will become one of the key instruments for the project realization.

1. ***Project description in more details***

***Anomalousness in the Stokes to anti-Stokes ratio in the SERS spectrum***

Accuracy and reliability are important biosensing requirements to minimize the risk of false results. In this regard, Raman spectroscopy, as one of the most informative, sensitive and non-invasive methods of light analysis of the molecular structures of condensed matter, is considered as a very effective tool for such purposes. For a deeper physical understanding of the approaches in the development of biosensors, it is necessary, inter alia, to conduct studies in the anti-Stokes part of the Raman spectrum, where in some cases the ratios of intensities of the anti-Stokes and Stokes components of the SERS spectrum are different from the expected equilibrium (Boltzmann) values. The nature of these phenomena is not yet completely clear. Therefore, the goal of the upcoming experiments within the project will be the identification of physical mechanisms leading to the observed anomalies in the SERS spectra. This is especially true for achieving the level of reproducible registration of SERS spectra when creating highly sensitive biosensors.

A number of studies have been devoted to solving this problem, but the bulk of the experiments were carried out using cw lasers. The use of picosecond lasers is very promising for better understanding of this problem, since this duration corresponds to the scale of the times of flows and energy redistribution in nanostructured materials with analyte molecules adsorbed on them. **According to our information, based on scientific literature, experimental work in this direction is not yet conducted in Russia.**

To achieve qualitative results on this problem, it is extremely important to have a highly sensitive Raman spectrometer that records the spectrum both in the Stokes and anti-Stokes regions, including the low-frequency region of the vibrational spectrum. At the FLNP Raman Spectroscopy Sector, the task of low-frequency Raman spectrometer has already been partially solved for excitation with a red laser at a wavelength of 633 nm. The next step is to achieve the same results for the cw and ps 785 nm excitations which is favorable for biological samples. The development and construction of this optical scheme (which is different and more complicated in comparison to 633nm) is currently underway in the frame of the project.

***Expected main results:***

* 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.

***Membrane proteins and lipodiscs***

The study of membrane proteins (MPs) is one of the major challenges in the current research of molecular life sciences. It is known that membrane proteins make up 20-30% of the human proteome. Knowledge of their structure helps the development of medicine and pharmacology - among the proteins targeted by drugs, the proportion of membrane proteins is known to be up to 50%. At the same time, membrane proteins practically do not lend themselves to crystallization, and therefore, to study their structure with the traditional method of x-ray structural analysis is almost inapplicable. This project is devoted to the development of a method for stabilizing membrane proteins using nanodiscs and more preferably lipodisсs - fragments of a lipid membrane (membrane mimetic) limited to amphiphilic polymers and studying their chemical structure by Raman spectroscopy. There are relatively few studies on lipodisсs in particular, as opposed to lipid-protein nanodisсs stabilized in solution with apolipoprotein or a special amphiphilic protein MSP (membrane Scaffold Proteins), although these objects are of considerable interest from the point of view of structural biology and experiments with few/single molecules or their complexes. The goal of this project is to improve existing procedures for isolating and studying the structure of membrane proteins using Raman spectroscopy, to obtain new information on the structure of lipodiscs with membrane proteins and compare it with empty lipodiscs.

It is important to note, that before starting these experiments, we plan to preface them with studies of conformational changes in phospholipids with the addition of cholesterol and melatonin. ***This work has already launched and is in a good progress being implemented in close cooperation with the neutron scattering experiments of the FLNP initiated by the project co-leader Norbert Kučerka.*** *The main goal here is to study the competitive effects of cholesterol and melatonin in model lipid membranes.*

**The project provides for the solution of the following tasks in this area:**

1. To study the conformational changes in phospholipids in presence of cholesterol, melatonin and their mixture;
2. To reveal the competitive effects of cholesterol and melatonin in model lipid membranes.
3. ***To demonstrate the complementarity between SANS and Raman***.
4. To develop a technique for obtaining Raman spectra and maps from samples of lipodiscs/liposomes with membrane proteins and “empty” lipodiscs.
5. Highlight fingerprint lines in Raman spectra that are associated with the presence of each of the three components — lipids, proteins, and copolymer.
6. To study the composition of proteins and lipids contained in lipodiscs.

***UV-activated NETosis***

We consider this task as an ambitious for the following reasons:

* unlike the well-known and investigated two other types of programmed cell death - apoptosis and necrosis, netosis is currently poorly studied and understood, although it is the first line of the body's immune defense against pathogens in the locus of inflammation;
* this is especially true for UV (sterile)-activated netosis (a few publications);
* and to an even greater extent this relates to the study of netosis by Raman spectroscopy;
* presently, to our knowledge, there are no publications also on neutron-activated netosis.

 The team of the Raman spectroscopy sector already has a certain experience in using Raman spectroscopy to identify spectral markers of netosis by chemicals (PMA) or bacteria (see publication # 7 in the above list). Based on the results of this work, it was revealed that a low-frequency Raman is also needed here.

 ***Plan on UV-induced NETosis for the years of 2021-2023***

* study of UV-induced netosis in a dose-dependent manner
* study of UV-induced netosis in an excitation wavelength-dependent manner
* search for Raman markers of netosis, including citrulline, free DNA, myeloperoxidase (MPO), and others
* complementary analysis by immunofluorescence microscopy (2022)
* tests on the possible neutron-activated netosis (2023)

6.   Group size, composition and budget

-List the JINR personnel involved in the project, including name, status (e.g. PI, researcher, post-doc, student, engineer, technician…) and FTE. Mention the total number of people in the collaboration.

The composition of the Raman Spectroscopy Sector consists of one group of “Nonlinear microspectroscopy” (6 employees). Presently all the 11 staff members of the Sector are engaged in the activities of the project “Biophotonics”:

1. Arzumanyan Grigory – project leader, FTE = 1,0
2. Kučerka Norbert – project co-leader, FTE = 0,5
3. Mamatkulov Kahramon – project deputy leader, PI, head of the group, FTE = 1,0
4. Vorobyeva Maria – researcher, FTE = 1,0
5. Arynbek Ersultan – researcher, FTE = 1.0
6. Morkovnikov Ivan – program engineer, FTE = 0,3
7. Korobchenko Mikhail – engineer, FTE = 0,5
8. Melkova Irina – engineer, FTE = 0,5
9. Damir Ayjan – technician, FTE = 1,0
10. Denisova Lubov, technician, FTE = 1,0
11. Zakritnaya Darya, technician, FTE = 1,0

-Present the JINR group budget for the period of time of the requested extension, specifying the main budget items (equipment, computing, salaries, common funds, travel…)

The PAC for CMP in its regular 52nd meeting (July 2020) supported opening the “Biophotonics” project for implementation in 2021–2023 with the budget highlighted at that meeting by the project leader (see table below).

**Estimated expenditures for the Project “Biophotonics”**

|  |  |  |  |
| --- | --- | --- | --- |
| **#** | **Expenditure items** | **Total cost,****k$** | **Expenditures per year (k$)** |
| **2021** | **2022** | **2023** |
| 5. | Materials | 50 | 10 | 20 | 20 |
| 6. | Equipment | 220 |  85 |  70 | 65 |
| 9. | Payments for agreement-based research | 26 | 9 | 9 | 8 |
| 10. | Travel allowance, including: а) non-rouble zone countries b) rouble zone countries c) protocol-based | 69 | 23176- | 23176- | 23176- |
|  | **Total direct costs:** | **365** | **127** | **122** | **116**  |

Salary of the Raman spectroscopy group is about 88,0 k$ per year.

-Indicate the use or needs of JINR computing resources for the group and for the project if any.

During the project implementation the Raman spectroscopy group intend to continue the use of JINR’s LIT computing resources via Heterogeneous platform “HybriLIT”.

**PROJECT LEADERS:**

**GRIGORY ARZUMANYAN and NORBERT KUČERKA**