Appendix 1

Form No. 24

Ultrasensitive SECARS microspectroscopy and luminescent core-shell nanostructures

CODE NAME OF PROJECT OR COLLABORATION: **Nanobiophotonics**

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DATE OF SUBMISSION OF PROPOSAL OF PROJECT TO SOD _________

DATE OF THE LABORATORY STC **20.04.2017** DOCUMENT NUMBER **37**

STARTING DATE OF PROJECT 01.01.2018_________

Appendix 2

Form No. 25

PROJECT ENDORSEMENT LIST

Ultrasensitive SECARS microspectroscopy and luminescent core-shell nanostructures

CODE NAME OF PROJECT OR COLLABORATION: **Nanobiophotonics**

NAME OF PROJECT LEADERS: **Grigory Arzumanyan and Norbert Kučerka**

APPROVED BY JINR DIRECTOR	SIGNATURE	DATE
ENDORSED BY		
JINR VICE-DIRECTOR	SIGNATURE	DATE
CHIEF SCIENTIFIC SECRETARY	SIGNATURE	DATE
CHIEF ENGINEER	SIGNATURE	DATE
HEAD OF SCIENCE ORGANIZATION DEPARTMENT	SIGNATURE	DATE
LABORATORY DIRECTOR	SIGNATURE	DATE
LABORATORY CHIEF ENGINEER	SIGNATURE	DATE
PROJECT LEADERS	SIGNATURE	DATE
PROJECT DEPUTY LEADERS	SIGNATURE	DATE
ENDORSED		
RESPECTIVE PAC	SIGNATURE	DATE

Ultrasensitive SECARS microspectroscopy and luminescent core-shell nanostructures

Content

Abstract

The main goal of the project is aimed at the development of the modern methods for highly sensitive (single/units of molecules), selective nonlinear laser Raman micro-spectroscopic ways of detection - primarily, Coherent Anti-Stokes Raman Scattering (CARS) spectroscopy of organic molecules, which are adsorbed on nanostructured surfaces, which provide Surface-Enhanced Raman Scattering (SERS), employing ultra-short (picosecond) pulses of laser radiation. When a molecule is in close proximity (at a nanoscale) from the metal nanostructure and is irradiated with two pump frequencies needed for specific molecule, then CARS polarization can be induced by locally amplified fields. ***This phenomenon is known and called as "Surface-Enhanced Coherent anti-Stokes Raman Scattering – SECARS".***

Another important component of the project is highly-contrast and spectral-selective imaging of samples (mainly of a biological nature) both by nonlinear Raman microscopy methods (CARS, SECARS) and **by upconversion luminescence using promising core-shell nanostructures.** These structures are known for their multifunctionality and diversity of their chemical composition, which, recently, allows them to be successfully applied in various applied tasks, in particular, in photodynamic therapy of cancer (PDT), contrast visualization of fibers, etc.

To realize the above mentioned project tasks, we will employ the multimodal optical platform based on the "CARS" microscope, which, after a relatively recent modernization (2015), rightly claims to be a unique in Russia in its functional characteristics and quite competitive at the world level as well.

The project will involve a qualified and experienced team of the Raman Spectroscopy Sector of the FLNP JINR in close cooperation with interested partners from various countries and organizations. Financing of the project is requested at the level of 150k$ annually.

Introduction

The project is aimed at the following:
- development and application of ultrasensitive and selective modification of Raman scattering, known as SECARS, for the purpose of stable and reproducible single (units) molecule detection of Raman scattering from analyte molecules;
- creation of a unified optical platform for complementary microscopy (imaging) using SERS, CARS, SECARS and upconversion luminescence (UCL) methods using advanced core-shell nanostructures. Since the end of the second year of the implementation of this project, the application of such luminescent biomarkers in test experiments on photodynamic therapy of oncological diseases is planned.

Raman spectroscopy is well known in the world as one of the most informative methods of light analysis of molecular structures of condensed matter. It is used in a wide range of applications: in physics, chemistry, materials science, pharmaceutics, criminalistics, in the study of polymers, thin films, semiconductors, in the analysis of fullerene and carbon nanotube structures, etc. And the role of Raman spectroscopy and microscopy in biomedical studies (Life Science) can't be overestimated, as the progress of cell biology and medicine is associated with methods and equipment that allow non-contact and non-invasive structural analysis of biological samples and processes in them with high resolution and in real time.

However, with all its advantages, the spontaneous Raman signal is weak enough and is accompanied by intense autofluorescent background, which sometimes makes it difficult to detect. That is why modern methods of Raman spectroscopy and microscopy are aimed not only at the development, but also at the permanent improvement of the enhanced modifications of this highly informative spectroscopy and microscopy. Priority methods for enhancement of Raman scattering signal are CARS, SERS, and SECARS is the most promising one, though not very simple for implementation.

The topicality of the formulated problem from the point of view of physics is caused by the following: the ability of highly sensitive, selective and reproducible detection and identification of ultra small amounts of organic molecules and compounds is very important for solving both fundamental biochemical and medical-biological problems, and modern practical tasks of express diagnostics in medicine, biology and pharmacology, safety, environmental protection, food control, etc. as well. It is assumed, the samples of interest will be both: model and many basic biologically-significant molecules like peptides and proteins, lipids and fatty acids, nitrogenous bases, DNA and RNA, etc.

As for luminescent biomarkers based on core-shell nanostructures, their main advantage is the polyfunctionality realized by separating the functions of the core and the shell, as well as the possibility of optimizing the target physico-chemical properties of the core material. The creation of core-shell particles can be caused by the need to isolate the core from the effects of the environment, fixing specific groups and reagents on specific layers for specific biomedical tasks (target drug delivery, PDT, etc.), intensifying the absorption and emission of energy by luminescent particles (nanoplasmonics) and etc.

All of the above mentioned is in mainstream of the world trends in the development of Raman spectroscopy and microscopy, as well as in the upconversion luminescence, and, thereby, determines the modern innovative nature of the proposed project.

State of the art of the science case proposed

The aim of the proposed project is to develop modern methods of highly sensitive, selective and reproducible laser detection of single (units) analyte molecules by nonlinear micro-raman spectroscopy. The analyte molecules will be adsorbed and localized on nanostructured surfaces that provide giant Raman scattering known as Surface-Enhanced Raman Scattering (SERS) using ultrashort (picosecond) pulses of laser radiation. First of all, we are considering Surface-Enhanced CARS micro-spectroscopy – SECARS.

The enhancement of CARS signal generated by the nanosecond laser pulses at the silver surface was investigated for the first time in 1979 and 1994 [1, 2]. Many years later, the opportunity of the surface-enhanced CARS spectroscopy was demonstrated with ultrashort laser pulses. Thus, in studies [3, 4] the applicability of combining SERS with CARS for a single-molecule detection was reported. In [5] the subwavelength imaging of a two attached gold nanoparticles using a multi-focus CARS microscope was obtained. In [6] the surface-enhanced CARS method for visualization of biological objects was used. Recently, in 2014-2016, authors used the surface-enhanced CARS technique with ultrashort laser pulses to detect a single molecule vibration [7,8]. The experimental studies were followed by the theoretical researches [9-11].

In recent years, the novel laser sources and the highly sensitive photodetectors were developed, as well as the nanostructured materials, which provide the huge enhancement of the local electromagnetic field and of the effect of the strong light interaction with molecules immobilized at the surface. For instance, the very weak Raman scattering of light increases many orders of magnitude, is known as SERS effect. The investigations of the SERS, which was discovered in mid-seventies, has boomed in last ten years. As a result, new opportunities are opened for linear, but also for nonlinear-optical detection and identification of ultra-small quantities of various molecular substances. The nonlinear surface-enhanced spectroscopy is expected to become universal and more sensitive than already "traditional" SERS technique.

- **It is important to note that despite the opening prospects, <u>only about 20 original works have been published worldwide in this field</u>, and it is still considered as an ambitious task.**

- **As far as the authors of the proposed project on publications are aware, there are no any research works in this field in Russia.**

<u>The scientific novelty of the SECARS project.</u>

First, fundamental properties and features of multi-photon nonlinear interactions of laser fields with single molecules (1-100 species) deposited on nanostructured SERS-active substrates are not thoroughly investigated. The detailed theoretical description of this interaction is absent as well. Besides this, despite all the remarkable progress in employing SERS, which has single-molecule sensitivity in some specific cases, the general approach for reproducible and robust probing the wide range of biologically relevant molecules is still absent.

Second, for practical application, the implementation of the combination of nonlinear laser microspectroscopy with SERS-active substrates should significantly enlarge the scope of organic molecules that are available for reproducible and rapid detection in ultra-small amounts using optical techniques and not requiring neither high-vacuum equipment, nor special sample and complex preparation procedures, nor expensive reagents.

The project implementation implies a complex approach and consists in:

- modification and testing of the broad wavelength range picosecond laser CARS/Raman microspectrometer, which provides local nonlinear optical excitation of a surface with a sub-micrometer resolution, using a number of model SERS-active substrates and reporter molecules;
- comparative experimental investigations of the peculiarities of nonlinear interaction of the pump laser light (in particular CARS) with different types of nanostructured gold- or silver-containing surfaces and adsorbed reporter molecules with ultra-low surface concentration;
- evaluation of SECARS spectroscopy capabilities to reproducibly attain single-molecule detection sensitivity level;
- theoretical description/model of the SECARS-process.

The main world scientific competitors

1. Professor Richard P. Van Duyne Research group, Northwestern University, Department of Chemistry, Evanston, Illinois 60208, USA.
2. Professor Eric Olaf Potma, Potma Labs, Department of Chemistry, Natural Sciences II, University of California, Irvine, CA 92697, USA.
3. Professor Marlan O. Scully Research Group, Texas A&M University, College Station, TX 77843, USA.

Luminescent core-shell nanostructures

Nanoparticles have several exciting applications in different areas and biomedial field is not an exception of that because of their exciting performance in bioimaging, targeted drug and gene delivery, sensors, and so on. The early diagnosis of various diseases such as cancer, diabetes, stroke, Alzheimer's disease, and others, today are the main focuses of biomedical field to save human life.

In the recent years it has been found that among several classes of nanoparticles core/shell is most promising for different biomedical applications because of several advantages over simple nanoparticles such as less cytotoxicity, increase in dispersibility, bio- and cyto-compatibility, better conjugation with other bioactive molecules, increased thermal and chemical stability and so on [12,13].

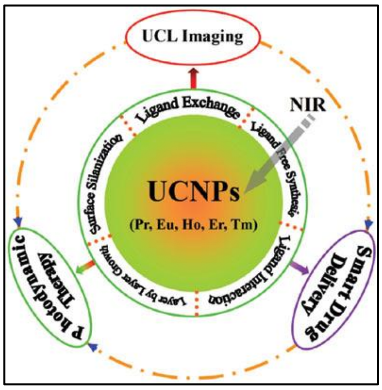
In connection with the proposed project, we note that recent studies have shown that core-shell nanostructures are successfully used in the upconversion luminescence for visualization, as well as for biomedical purposes [14-20]. Relying on this, and also taking into account the experience accumulated by the authors of the project in the field of upconversion luminescence (UCL) in the past several years, it is proposed to move from the works with the up-conversion phosphors based on glass-ceramic matrices activated by rare earth elements (REE) to the UCL studies based on more promising and modern core-shell nanostructures.

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

- To implement enhanced modification of the UCL based on core-shell nanostructures using REE as a core and Ag@SiO2 as a shell (analogous to SERS-active structures).

- To create a unified optical platform for contrast and selective bioimaging based on two methods - nonlinear Raman microscopy of CARS and SECARS and upconversion luminescence.

- At the final stage of the project, it is planned, in close cooperation with partners in the field of biomedicine, to test the luminescent nanostructure "core-shell" with a special modification of porphyrin as a membrane (biomarker), in photodynamic therapy of cancer.



Photodynamic therapy (PDT) is one of the non-surgical methods of treatment of oncological diseases based on the use of special markers-photosensitizers selectively accumulating in tumor cells and significantly increasing its sensitivity to light. Under the influence of light waves of a certain length, these substances cause a chemical reaction and, as a result, lead to the formation of active forms of oxygen (including singlet oxygen), which oxidizes proteins and other biomolecules and, thereby, destroys (necrosis and / or apoptosis) internal structure of the tumor cell. Then the dead cells are absorbed by phagocytes. In addition to directly killing cancer cells, PDT appears to damage blood vessels reaching the tumor, which disrupts the delivery of nutrients and oxygen, and also contributes to its death.

Most often, PDT is used to treat skin and mucosal cancers. The method is simply indispensable in cases where the tumor is located in "uncomfortable" places: on the lip, in the mouth, on the eyelid, on the auricles. Surgery in such cases leads to serious cosmetic defects and functional limitations.

In photodynamic therapy, mainly photosensitizers from the group of porphyrins are used. There is some data that porphyrins bind to serum proteins, including low-density lipoproteins. And tumor cells contain a large number of special receptors to which lipoproteins can be attached. Therefore, photosensitizers complex with lipoproteins accumulate on cytoplasmic membranes of the cell and membranes of intracellular organelles: mitochondria, lysosomes, nuclei.

Among the porphyrins used in PDT, cationic porphyrins (Fig.1) take a special place, due to their high selectivity of accumulation in the malignant cell [21]. Our partners from the Institute of Biochemistry of the NAS of Armenia have accumulated rich experience in synthesizing a large class of new cationic water-soluble porphyrins and metalloporphyrins with various central metal atoms (Zn, Ag, Co, Fe, Mn, Cu, etc.) and various peripheral functional groups – in total more than 100 compounds [22,23].

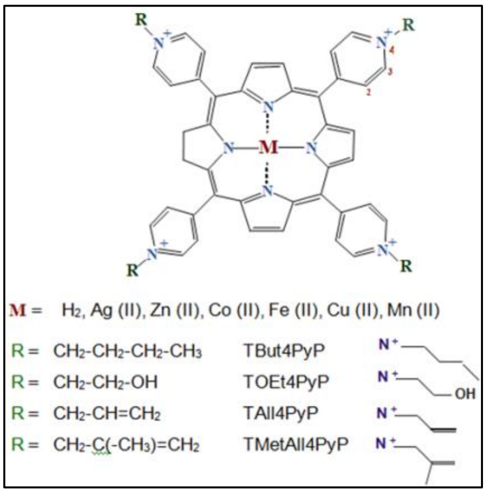


Fig.1 Structure of cationic porphyrins. M - central metal atoms (Zn, Ag, Co, Fe, Mn, Cu, etc.), R - peripheral groups

One of the most important criteria for the effectiveness of photosensitizers is the quantum yield of generation of singlet oxygen (γ_Δ) [24]. Joint research of Armenian and Belarus scientists has shown that γ_Δ of new cationic porphyrins reach a very high level (0.77-0.79 and 0.85-0.97, in porphyrins and Zn-metalloporphyrins, respectively) [13].

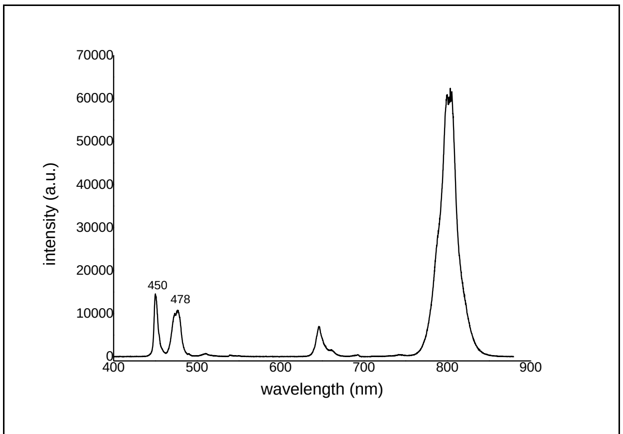


Fig.2 Upconversion luminescence spectrum of $NaYF_4$:Yb^{3+},Tm^{3+} at 976nm excitation

It is with this factor in mind that the synthesis of core-shell nanostructures is proposed, where the role of the shell will be played by a new class of cationic / metalloporphyrins, and the role of the "nucleus" of $NaYF_4$: Yb^{3+}, Tm^{3+} nanocrystals, which have two intense bands of UCL (Fig.2) in the blue region of spectrum (450nm and 475nm), the first of which coincides with the absorption band of the porphyrins we are considering.

It is expected that the proposed core-shell structure (to our knowledge previously unstudied - the novelty of the project!) should work in PDT in terms of increasing the efficiency of tumor cells destruction.

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Description of the proposed research

Focus of the research

Development of new highly sensitive and selective methods for optical detection and identification of ultra-small amounts of organic compounds adsorbed on nanostructured substrates, and the creation of a unified optical platform for imaging/bioimaging using nonlinear Raman spectroscopy and upconversion luminescence options.

Assumptions and hypotheses

The attractiveness of SECARS spectroscopy of organic molecules on SERS-active surfaces is that if the enhancement factor of the local-field surface is g, then the intensity of the SERS increases as g^4, and the intensity of SECARS as g^8 (Fig. 1), and the theoretically attainable surface-enhanced gain of the intensity of the scattered light is about 10^{10}-10^{11} for the Raman scattering [1], and 10^{20}-10^{22} for CARS [2].

What might be even more important, since in case of SECARS the frequency of the anti-Stokes scattered light is blue-shifted relative to that of the pump light, the luminescence background, originating from the sample surface and being the main obstacle for SERS analysis in the Stokes region, is absent or drastically suppressed, - contrary to the case of SERS. Assuming so high scattered light intensity enhancement factors and signal detection in spectral region free from background luminescence, one could expect that single-molecule sensitivity in the case of SECARS spectroscopy would be attainable for a much larger number of substances than that comprising molecules already available for detection in "classical" SERS experiments [3-5].

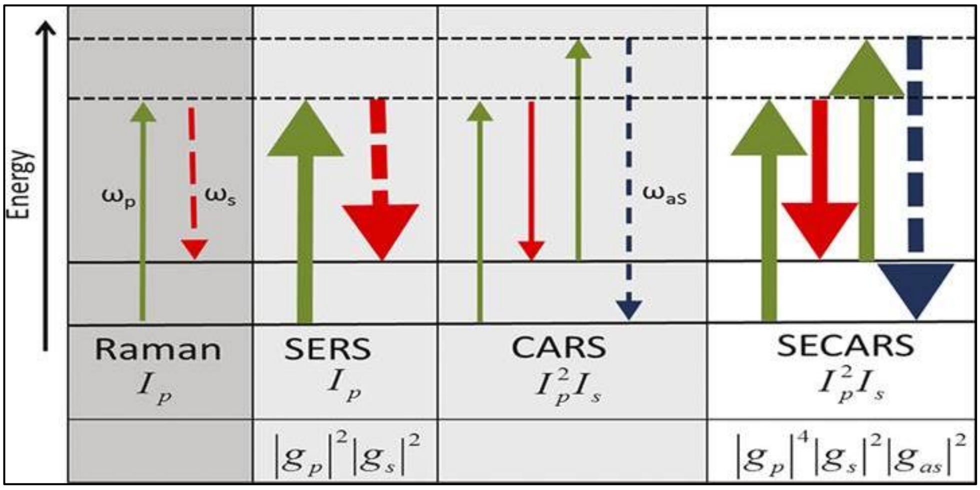

Fig.1 Energy diagram and enhancement factors for Raman, SERS, CARS and SECARS

However, first experiments [6,7] revealed that the experimentally observed surface enhancement of CARS intensity is much less than that theoretically predicted; the dipole and quadrupole noble metal nanostructures delivering the highest possible gain values are easily destroyed by pump laser fields; the structure of the points of the highest scattered light intensity ("hot-spots") is very fragile and poorly reproducible.

These experiments demonstrated that in order to realize the potential of SECARS spectroscopy - both for carrying out fundamental research and for highly sensitive, reproducible diagnostics of molecules - it is necessary to continue and expand the work on the development of the method.

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Research methods and experimental facilities

In order to advance picosecond nonlinear Raman micro-spectroscopy towards reproducible selective high-sensitivity way of detection of organic reporter molecules deposited on the nano-structured SERS-active surfaces the following methods are planned to be applied:

1. For each system comprising sample + SERS-active surface a set of nonlinear spectroscopic experiments (mainly SECARS) aimed to clarifying the nature of "hot spots", the origin of nonresonant background and the ways to suppress it. We plan to perform the measurements of coherence effects and the effects of interference of coherent and non-coherent contributions to the scattered signal intensity. These experiments will be supplemented by regular spontaneous SERS experiments in order to compare image contrasts and background levels. For all the samples are also planned to obtain the optical and SEM images. For these experiments a CARS/Raman microspectrometer with picoseconds pump lasers is planned to be used. In order to solve the problems formulated in this project, technical requirements for modification of the existing device will be worked out and implemented together with the system manufacturer.

2. For the processing and analysis of spectral information, both existing algorithms and programs, as well as modified and specially developed software and algorithms (NanoSP, ImageSP, etc.) will be used.

3. As for samples with a SERS-active surface, first of all, substrates with silver nanoparticles localized on a mesoporous silicon surface developed at BSUIR (Minsk) will be used. In addition, it is planned to test nanostructured substrates with gold nanoparticles of various configurations, developed at the Institute of Semiconductor Physics, Novosibirsk. Some commercially available SERS substrates like ATO-ID, etc. will also be used in proposed experiments.

4. Model (dithionitrobenzoic acid DTNB) and a number of biologically significant molecules/compounds are planned to be used as localized organic analyte molecules. While preparing samples and immobilizing molecules on gold/silver nanoparticles and SERS-active surfaces, methods and technologies developed by our partners from the Moscow State University and the Institute of Biochemical Physics of the Russian Academy of Sciences will be used.

5. For the processing and analysis of experimental results using modern analytical and numerical methods of theoretical physics, it is planned to develop theoretical models for the interaction of laser radiation with the surfaces under study and molecules immobilized on them.

6. In studies on up-conversion luminescence based on core-shell nanostructures, it is proposed to purchase relatively inexpensive $NaYF_4$:Yb^{3+}, Er^{3+}, and NaYF4:Yb^{3+}, Tm^{3+} nanoparticles ("core") as one of the most effective upconversion nanocrystals. As the "shells" will be used biocompatible SiO_2 film and silver nanoparticles for plasmon-enhanced UCL and various porphyrins for PDT.

7. Considering that UCL is the same anti-Stokes process as CARS, in experiments with luminescent biomarkers almost all the same optics and registration system will be used, which is installed on an optical platform for Raman spectroscopy.

Multimodal optical platform – microscope “CARS”

The laser scanning confocal microscope “CARS” is a laser-optical-mechanical complex-optical platform (Fig.2), developed in a compact configuration and mounted on a vibration-resistant desk SDANDA (Lithuania).

Fig.2 General view of the microscope “CARS”

In 2015, the optical platform was modernized, as a result of which, along with spontaneous Raman scattering and ordinary CARS, several new options were developed and installed: polarized CARS microscopy (P-CARS), nonlinear imaging based on second-harmonic generation (SHG/SONICC), SERS spectroscopy, spontaneous Raman scattering at 532nm and 785 nm excitations. Fig. 3 shows the modernized optical scheme of the device.

Thus, at present, for spontaneous Raman scattering and SERS, three laser sources with wavelengths of 532nm, 633nm, and 785nm are used, depending on the task and the sample. To generate CARS process on the platform a picosecond tunable Nd:YVO_4 laser (model PT257-SOPO, EKSPLA, Lithuania) is installed. The pulse repetition rate is 85 MHz, pulse duration is ~ (5-6) ps, mean power (50-200)mW.

It’s well-known that the pulse duration of several picoseconds is a proper compromise between high intensity and narrow spectral bandwidth necessary for the CARS microscopy. Besides, its intensity is sufficient also for detection of other nonlinear processes, in particular the second and sum harmonic generation. Only a small portion of biologically tolerable laser power is used for CARS and SHG imaging.

The basic laser beam at 1.06μ serves as the source of the Stokes wave (ω_S) in CARS and is simultaneously used to synchronously pump an intracavity-doubled crystal optical parametric oscillator (SOPO). Thereby, the SOPO coherent device provides temporal synchronization with the Nd:YVO_4 and serves as a source of the pump beam (ω_p) which is tunable in the range between (690-990) nm with a maximum output power of 200mW and linewidth ~(5-7)cm^{-1}. Both excitation picosecond pulse trains are made coincident in time and in space utilizing an computer-controlled optical delay line and a series of dichroic mirrors. For CARS microscopy, we use a water-immersion objective lens with a high numerical aperture (NA=1.2, UPLANAPO-60x, Olympus) to focus the beams tightly. With the tight foci, the phase-matching conditions are relaxed because of the large cone of wave vectors of the excitation beams and the short interaction length.

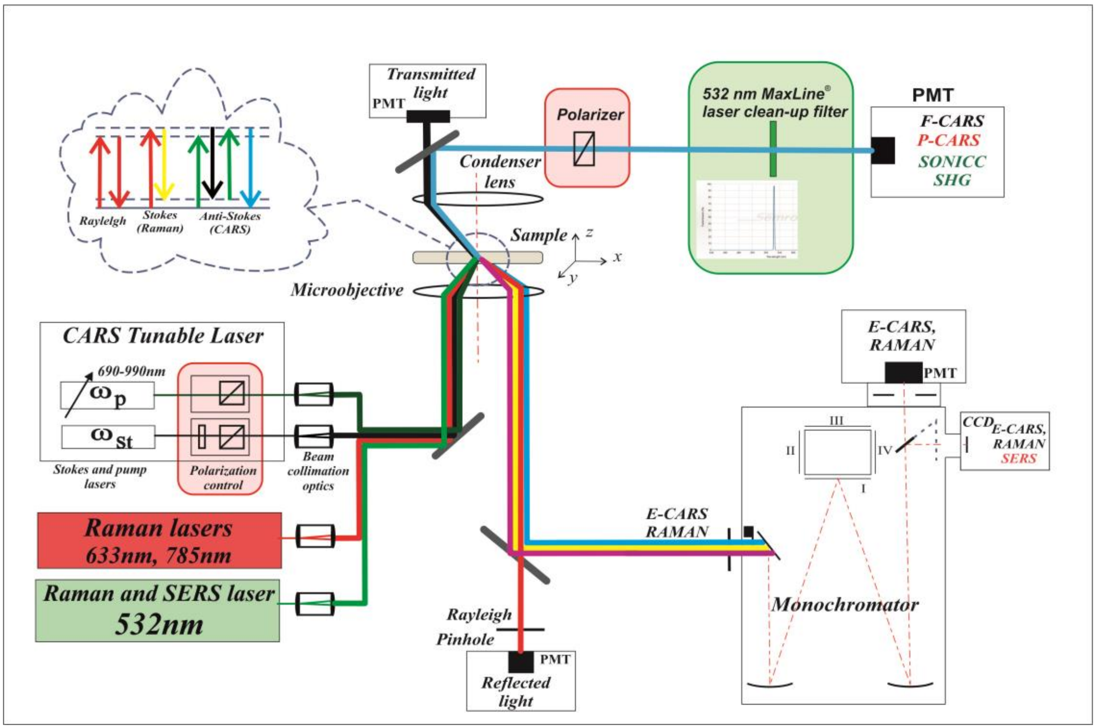


Fig.3 The layout of the platform after its modification in 2015

Using our optical platform, a sample can be analyzed and imaged by utilizing vibration frequencies in the spectral range of (1000−3580) cm^{-1}, which covers all most important vibrational modes of bio-molecules. Five detection channels allow two forward- and three backward- propagated signals to be recorded. The polarization control is adjustable with a half-wave plate in the Stokes beam and the Glan-Taylor polarizer installed on a rotation mount directed to the photomultiplier (Hamamatsu, H6780-01) in the forward direction.

A computer-controlled XY galvanometer scanner (GSI-Lumonics VM1000) provided a fast scan of the sample in the lateral focal plane of the objective. In the fast mapping option the signal integration time is 3 μs/pixel which results in a total acquisition time of about 3s. The microobjective is mounted on a computer-controlled Z-axis translation piezostage for scanning through the microscope's optical axis with a minimal scan step of 0.1μm (3D-mapping).

As for the SHG imaging we exploit the same PMT for detection of P-CARS signals (see Fig.2). The Stokes wave serves for second harmonic (532 nm) generation and a narrow bandpass filter (Semrock, FF01-532/3-25) for a SHG signal filtration and further detection on the PMT

The main project stages and expected results.

- Development and formulation of scientific-technical requirements for the picosecond CARS/Raman microspectrometer hardware and software modifications based on the results of the preliminary experiments.

- Resonant and nonresonant SECARS signal detection in the "hot spots"; SECARS mapping of different SERS-active surfaces with different reporter molecules; comparative SERS measurements at the same surface areas.

- Achievement of a single (units) molecule reproducible detection level by means of the SECARS modality.

- Theoretical support and modeling of the SECARS process.

- Verification of tumor and stem cells by Raman and CARS spectroscopy and microscopy.

- Studies of structural (SEM, TEM, AFM, SANS) and spectroscopic (including luminescent) properties of core-shell nanostructures: $NaYF_4:Yb^{3+},Er^{3+},Tm^{3+}@SiO_2$.

- Creation of a unified and complementary optical platform for a contrast and selective imaging/bioimaging of samples by the methods of nonlinear Raman microscopy and upconversion luminescence.

- Test application in the photodynamic therapy of cancer of the method of upconversion luminescence with "core-shell" nanostructures using porphyrins as sensitizers.

Present experimental background of the proposed project

In 2017, trial work was started to develop and obtain the first test results for SECARS. As for the analyte molecules, trinitrobenzene (TNB) was selected, which was immobilized to Au nanoparticles on the SERS-active substrate.

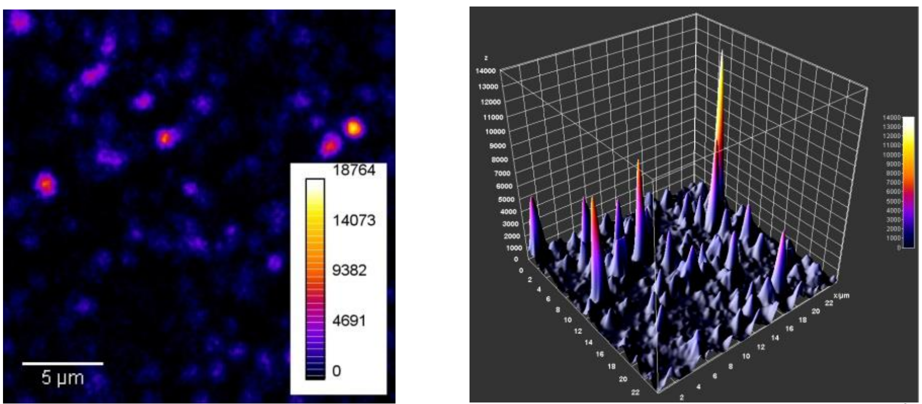


Fig.4 SECARS intensity mapping of TNB molecules at 1338cm^{-1}

The first micro-images of SECARS intensity on the characteristic Raman line 1338 cm^{-1} of the TNB spectrum were obtained at picosecond excitation of molecules in the NIR spectral region with a contrast at the level of 1.5×10^4 (Fig.4). This is a very encouraging test result for SECARS-microscopy on our optical platform, besides this is the first SECARS result in Russia. In April 2017 this study was presented at the international conference in Germany ECONOS-2017 [link 18 in the list of conferences].

In the field of luminescent research, two important results have been obtained in terms of future implementing of the proposed new project:

1) plasmon-enhanced upconversion luminescence (UCL) based on $NaYF_4$:Yb^{3+},Tm^{3+} nanocrystals with an enhancement factor of ~ 18 at the "hot" points (Fig. 5).

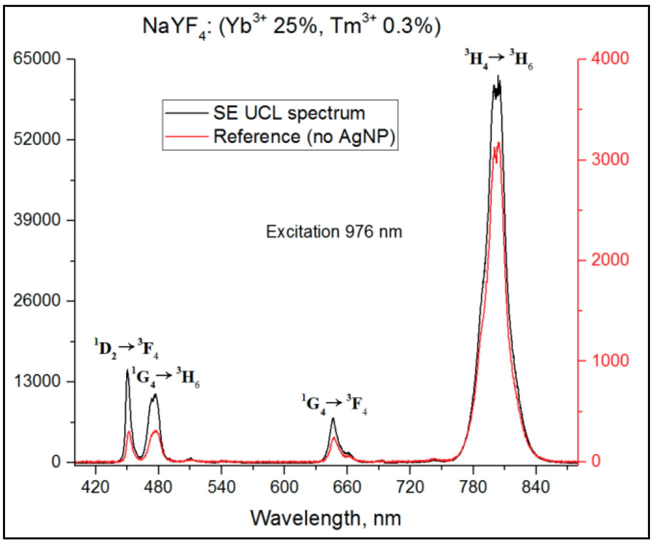


Fig.5. Comparative spectra of conventional UCL with NaYF4:Yb^{3+}, Tm^{3+} nanoparticles (red) and plasmon-enhanced one (black). The gain factor is ~ 18 at the "hot" spots.

2) imaging of cotton fibers with a diameters in the range of several to tens of microns by means of upconversion luminescence (Fig. 6).

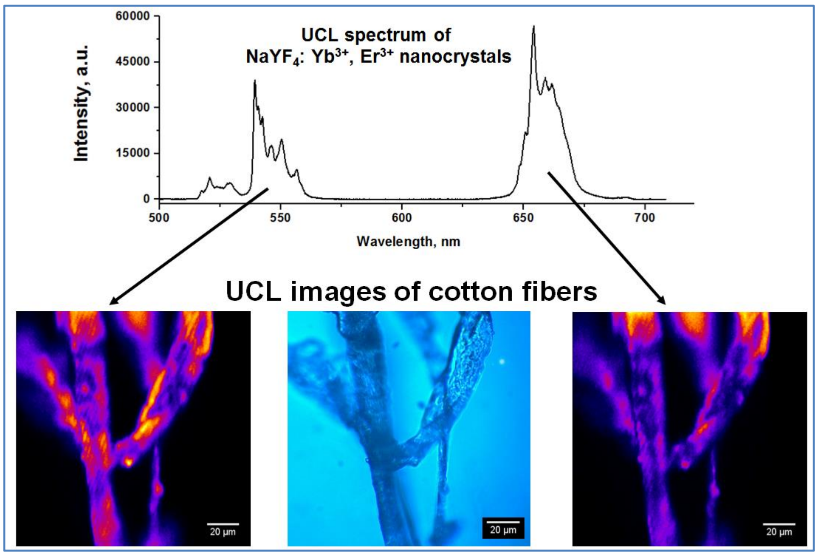


Рис.6 UCL spectrum of $NaYF_4$:Yb^{3+},Er^{3+} nanocrystals (top) and cotton fiber images with various diameters from few to tens of mkm

The obtained two preliminary results undoubtedly indicate on the possibility of obtaining contrast imaging of samples of various nature by the UCL method, as well as on plasmonic enhancement of the UCL intensity.

Almost all spectroscopy and microscopy for the proposed project will be performed on the “CARS” microscope at FLNP. Over the past 3-4 years, the staff of the Raman spectroscopy sector has acquired sufficient experience and skills to successfully cope with the implementation of the new project. This is also confirmed by a number of publications in high-ranking journals and participation in large international conferences for the past reporting period (the corresponding lists are attached). The role of the partners of the project also can not be underestimated both in terms of the delivery of various samples, and in terms of analysis and discussion of the results. Particular attention will be paid to choosing a partner for theoretical support of the project.

In the Raman spectroscopy sector there are currently 12 employees (5 of them with part-time work). Seven out of twelve are young scientists and engineers under the age of 35. The sector consists of two groups - a group of nonlinear Raman spectroscopy, and a group of applied research. The whole staff members of the Sector will be involved in the implementation of the proposed new topic and project.

Thus, the optical platform described above, with its modern characteristics for Raman microspectroscopy and luminescence studies, is potentially ready for a new project implementation in the frame of the new theme in the both modalities: SECARS microspectroscopy and up-conversion luminescence based on core-shell nanostructures.

Appendix 3

Form No. 26

Schedule proposal and resources required for the implementation of the Project

“Ultrasensitive SECARS microspectroscopy and luminescent core-shell nanostructures”

<table>
<tr><td colspan="3" rowspan="2">Expenditures, resources, financing sources</td><td rowspan="2">Costs (k$)
Resource requirements</td><td colspan="3">Proposals of the Laboratory on the distribution of finances and resources</td></tr>
<tr><td>1st year</td><td>2nd year</td><td>3rd year</td></tr>
<tr><td colspan="2" rowspan="2">Expenditures</td><td>Main units of equipment, work towards its upgrade, adjustment etc. (microobjectives, measuring equipment, spectrophotometer, etc.)</td><td>62</td><td>20</td><td>21</td><td>21</td></tr>
<tr><td>Materials</td><td>38</td><td>13</td><td>12</td><td>13</td></tr>
<tr><td rowspan="2">Financing sources</td><td>Budgetary resources</td><td>Budget expenditures including foreign-currency resources.</td><td>100</td><td>33</td><td>33</td><td>34</td></tr>
<tr><td>External resources</td><td>Contributions by collaborators.
Grants.
Contributions by sponsors.
Contracts.
Other financial resources, etc.</td><td>-</td><td>-</td><td>-</td><td>-</td></tr>
</table>

PROJECT LEADERS G. Arzumanyan

N. Kučerka

Schedule proposal for the years 2018-2020 гг.

	Activities	Plans per year		
		2018	**2019**	**2020**
1.	Development and formulation of scientific-technical requirements for the modification of the CARS/Raman-microspectrometer under epy SECARS modality.			
2.	Resonant and nonresonant SECARS signal detection in the "hot spots"; SECARS mapping of different SERS-active surfaces with various reporter molecules.			
3.	Achievement of a single (units) molecule reproducible detection level by means of the SECARS modality.			
4.	Theoretical support and modeling of the SECARS process.			
5.	Verification of tumor and stem cells by Raman and CARS spectroscopy and microscopy.			
6.	Studies of structural (SEM, TEM, AFM, SANS) and spectroscopic (including luminescent) properties of core-shell nanostructures: $NaYF_4:Yb^{3+},Er^{3+},Tm^{3+}@SiO_2$.			
7.	Creation of a unified and complementary optical platform for a contrast and selective imaging/bioimaging of samples by the methods of nonlinear Raman microscopy and upconversion luminescence.			
8.	Test application in the photodynamic therapy of cancer of the method of upconversion luminescence with "core-shell" nanostructures using porphyrins as sensitizers.			

PROJECT LEADERS G. Arzumanyan

N. Kučerka

Appendix 4

Form No. 29

Estimated expenditures for the Project “Ultrasensitive SECARS microspectroscopy and luminescent core-shell nanostructures”

#	Expenditure items	Full cost, k$	Costs by years		
			1st year	2nd year	3rd year
5.	Materials	38	13	12	13
6.	Equipment	62	20	21	21
9.	Payments for agreement-based research	26	8	9	9
10.	Travel allowance, including:	54	18	18	18
	a) non-rouble zone countries		14	14	14
	b) rouble zone countries		4	4	4
	c) protocol-based		-	-	-
	Total direct expenses**:**	**180**	**59**	**60**	**61**

PROJECT LEADERS G.M. Arzumanyan

N. Kučerka

LABORATORY DIRECTOR V.N. Shvetsov

LABORATORY CHIEF ENGINEER-ECONOMIST L.S. Ovsjannikova

Main publications and patent

1. Arzumanyan G.M., Doroshkevich N.V., Mamatkulov K.Z., Gordeliy V.I., et al., “Highly Sensitive Coherent anti-Stokes Raman Scattering Imaging of Protein Crystals”, JACS, 2016, Volume 138(41), pp. 13457-13460

2. Arzumanyan G.M., Kuznetsov E.A., Zhilin A.A., et al., “Photoluminescence of transparent glass-ceramics based on ZnO nanocrystals and co-doped with Eu3+, Yb3+ ions”, J. Optical Materials, 62, 2016, 666-672

3. Girel K., Yantcevich E., Arzumanyan G, et al., “Detection of DNA molecules by SERS spectroscopy with silvered porous silicon as an active substrate”, Phys. Status Solidi A, 1–5 (2016) / DOI 10.1002/pssa.201600432

4. Othman H.A., Arzumanyan G.M., Moncke D., “The influence of different alkaline earth oxides on the structural and optical properties of undoped, Ce-doped, Sm-doped, and Sm/Ce co-doped lithium alumino-phosphate glasses”, J. Optical Materials, 62, 2016, 689-696

5. Arzumanyan G.M., Mamatkulov K.Z., Fabelinsky V.I., et al., “Surface Enhanced CARS from Gold Nanoparticle-Immobilized Molecules at Cerium Dioxide/Aluminium Film”, Technical Digest (Nanophotonics and Plasmonics) of Int. Conf. ICONO/LAT 2016, p. 88-89, Minsk, Belarus, September 26–30, 2016.

6. Kaniukov E.Yu., Belonogov E.K., Arzumanyan G.M., et al., “Features of forming of copper deposit in pores of silicon oxides”, Proceedings of the NAS of Belarus, Physico-Technical series 2016, # 3, pp 11-15

7. O.Yu. Poimanova, A.O. Medvid, I.S. Kolomets, G.M. Arzumanyan, “Synthesis of barium isopolytungstates from aqueous dimethylformamide solutions”, Nauka Kubani, 2016, #4, 4-11

8. Arzumanyan G.M., Kulchitsky V.A., Dosina M.O., Mamatkulov K.Z., et al., JSCRT, Vol. 1(1), Dec. 2016

9. P.A. Loiko, G.E. Rachkovskaya, G.M. Arzumanyan, et al., “Mechanisms of upconversion luminescence in glass-ceramics containing Er:PbF_2 nanocrystals”, J. Appl. Spectr., 2017, 84(1)

10. Arzumanyan G.M., Mamatkulov K.Z., Fabelinsky V.I., et al., “Surface Enhanced micro-CARS from Gold Nanoparticle-Immobilized Organic Molecules”, Int. conf. ECONOS, 2-5 April, 2017, Jena, Germany, Technical Digest, pp.73-74

11. V. Polovinkin, D. Willbold, G. Arzumanyan, J.-L. Popot, V. Gordeliy, et al., “Nanoparticle Surface-Enhanced Raman Scattering of Bacteriorhodopsin Stabilized by Amphipol A8-35”, J. Membrane Biol (2014), 247, 971–980

12. Patent of the Russian Federation, “Luminescent glass-ceramics”, # 2579056, 02.03.2016

Conferences

1. 2nd International conference on NT and Biomedical Engineering, April 18-20, 2013, Chisinau, Rep. of Moldova
2. International conference on RARE EARTH MATERIALS, REMAT-2013, Wroclaw, Poland, 26-28 April
3. XVII scientific conference of young researchers and specialists, 08-12 April 2013 г., JINR, Dubna
4. Int. conferenceICNRP-2013, 24-27 September 2013, Kazakhstan
5. 27th International Congress “Laser Florence 2013”, Nov 9-10, 2013, Italy
6. 41st Annual Meeting of the European Radiation Research Society-ERR-2014, September 14-19, 2014., Greece
7. Nano-2014 Belarus-Russia-Ukraine, 7-10 October, 2014, Minsk, Belarus
8. 2nd International Summer School and Workshop, 29 Sept.-3 Oct., 2014, Dubna,
9. Third International Conference on Radiation and Applications in Various Fields of Research – RAD 2015, Montenegro
10. III International Conference on Small Angle Neutron Scattering dedicated to the 80th anniversary of Yu.M. Ostanevich, 6-9 June, 2016, JINR, Dubna
11. Int. conference on Coherent and Nonlinear Optics (ICONO)&Conference on Lasers, Applications, and Technologies (LAT) - ICONO / LAT 2016, Minsk, Belarus, September 26–30, 2016.
12. The 7th International Conference on Optical Spectroscopy, Laser and their Applications, 18-20 October 2016, NRC, Cairo, Egypt
13. BIT’s 6th Annual World Congress of Nano Science and Technology-2016 (Nano S&T-2016), Singapore, October 26-28, 2016
14. Nano-2016 Belarus-Russia-Ukraine, 22-25 November, 2016, Minsk, Belarus
15. Int. conference FTT-2016, 22-25 November, 2016, Minsk, Belarus
16. Int. conference PIERS 2017, 22 - 25 May, 2017, St Petersburg.
17. Nanomeeting-2017, 30 May – 02 June, 2017, Minsk, Belarus
18. Int. conference ECONOS-2017, April, 2-5, 2017, Abbe Center of Photonics, Jena, Germany
19. BIT's 1st Int. Biotechnology Congress (IBC-2017), Xi'an, China, April 25-27, 2017.

ОБЪЕДИНЕННЫЙ ИНСТИТУТ ЯДЕРНЫХ ИССЛЕДОВАНИЙ

ВЫПИСКА
из Протокола №37 Научно-технического совета
Лаборатории нейтронной физики им. И. М. Франка

20.04.2017 г.

Численный состав НТС: 35 чел.

Присутствовало на заседании: 24 чел.

5.1. Слушали: Г.М. Арзуманяна с отчетом по теме ПТП ОИЯИ 04-4-1111-2013/2017 «Мультимодальная платформа Рамановской и нелинейной оптической микроскопии и микроспектроскопии для исследования конденсированных сред».

В обсуждении принимали участие: С.А. Куликов, А.И. Куклин. А.И. Франк.

Постановили: по результатам открытого голосования (24 – за, против – нет, воздержавшихся – нет): Отчет по завершению темы ПТП ОИЯИ 04-4-1111-2013/2017 «Мультимодальная платформа Рамановской и нелинейной оптической микроскопии и микроспектроскопии для исследования конденсированных сред» принять.

Председатель НТС ЛНФ — А.И. Франк

Секретарь НТС ЛНФ — Т. В. Тропин

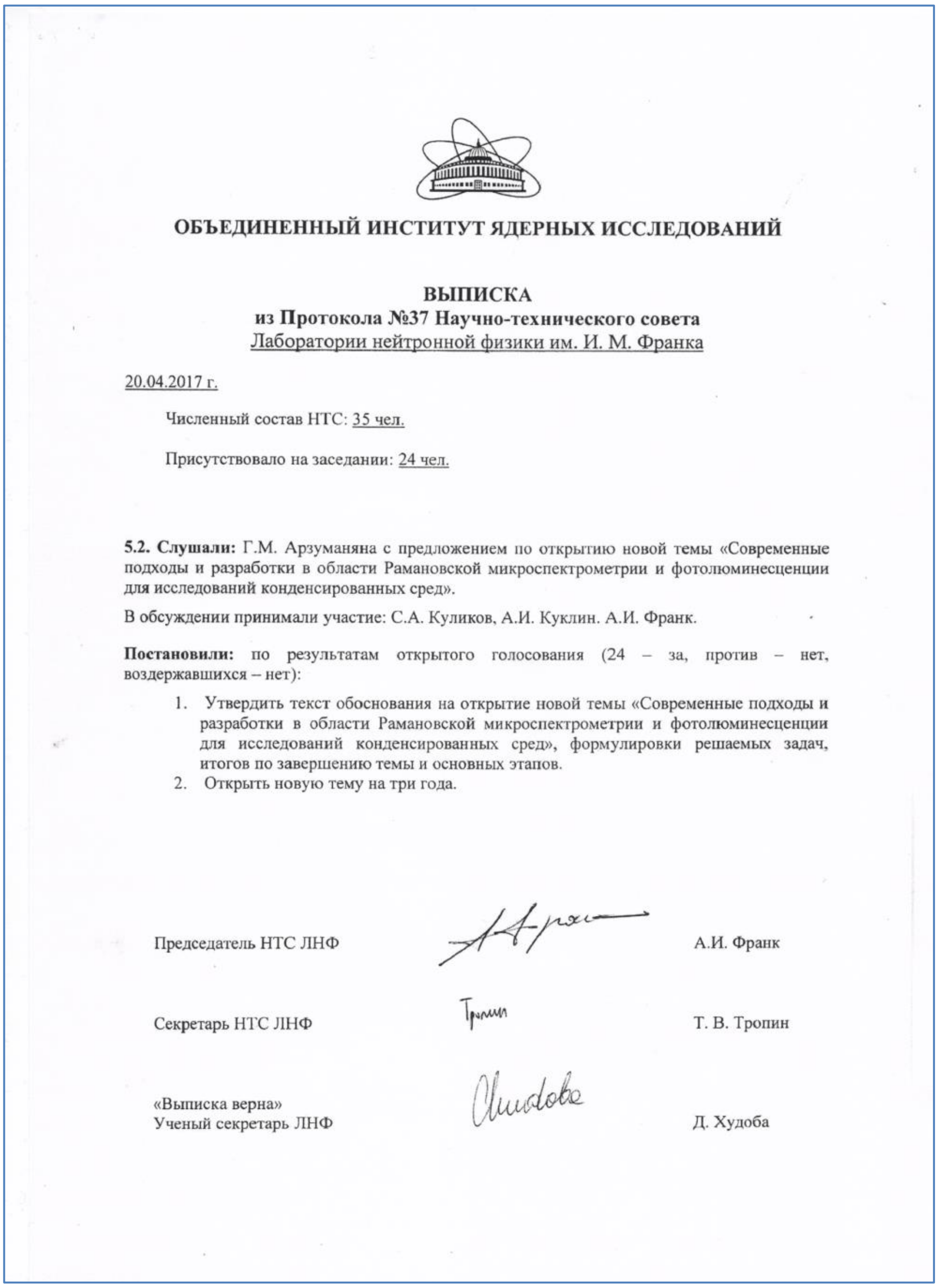
«Выписка верна»
Ученый секретарь ЛНФ — Д. Худоба

EXTRACT from the FLNP's STC PROTOCOL # 37

20.04.2017

5.1 Speaker: G.M. Arzumanyan, report on the theme 04-4-1111-2013/2017 "Multimodal platform for Raman and nonlinear optical microscopy and microspectroscopy for condensed matter studies".

Results of the voting: 24 – for, against – no, abstain – no



ОБЪЕДИНЕННЫЙ ИНСТИТУТ ЯДЕРНЫХ ИССЛЕДОВАНИЙ

ВЫПИСКА
из Протокола №37 Научно-технического совета
Лаборатории нейтронной физики им. И. М. Франка

20.04.2017 г.

Численный состав НТС: 35 чел.

Присутствовало на заседании: 24 чел.

5.2. Слушали: Г.М. Арзуманяна с предложением по открытию новой темы «Современные подходы и разработки в области Рамановской микроспектрометрии и фотолюминесценции для исследований конденсированных сред».

В обсуждении принимали участие: С.А. Куликов, А.И. Куклин. А.И. Франк.

Постановили: по результатам открытого голосования (24 – за, против – нет, воздержавшихся – нет):

1. Утвердить текст обоснования на открытие новой темы «Современные подходы и разработки в области Рамановской микроспектрометрии и фотолюминесценции для исследований конденсированных сред», формулировки решаемых задач, итогов по завершению темы и основных этапов.
2. Открыть новую тему на три года.

Председатель НТС ЛНФ — А.И. Франк

Секретарь НТС ЛНФ — Т. В. Тропин

«Выписка верна»
Ученый секретарь ЛНФ — Д. Худоба

EXTRACT from the FLNP's STC PROTOCOL # 37

20.04.2017

5.1 Speaker: G.M. Arzumanyan, proposal on the opening new theme "Modern Trends and Developments in Raman Microspectroscopy and Photoluminescence for Condensed Matter Studies"

Results of the voting: 24 – for, against – no, abstain – no

ОБЪЕДИНЕННЫЙ ИНСТИТУТ ЯДЕРНЫХ ИССЛЕДОВАНИЙ

ВЫПИСКА
из Протокола №37 Научно-технического совета
Лаборатории нейтронной физики им. И. М. Франка

20.04.2017 г.

Численный состав НТС: 35 чел.

Присутствовало на заседании: 24 чел.

5.3. Слушали: Г.М. Арзуманяна с представлением проекта «Ультрачувствительная микроспектроскопия SECARS и люминесцентные биомаркеры на основе наноструктур ядро-оболочка» в рамках темы «Современные подходы и разработки в области Рамановской микроспектрометрии и фотолюминесценции для исследований конденсированных сред».

Постановили: по результатам открытого голосования (24 – за, против – нет, воздержавшихся – нет) одобрить проект «Ультрачувствительная микроспектроскопия SECARS и люминесцентные биомаркеры на основе наноструктур ядро-оболочка» на 2018-2020 гг., рекомендовать представить проект на 46-й сессии ПКК ОИЯИ по КС и открыть проект с первым приоритетом.

Руководитель проекта: Г.М. Арзуманян.

Председатель НТС ЛНФ — А.И. Франк

Секретарь НТС ЛНФ — Т. В. Тропин

«Выписка верна»
Ученый секретарь ЛНФ — Д. Худоба

EXTRACT from the FLNP's STC PROTOCOL # 37

20.04.2017

5.1 Speaker: G.M. Arzumanyan, proposal on the new project "Ultrasensitive SECARS microspectroscopy and luminescence biolabeling with core-shell nanostuctures".

Results of the voting: 24 – for, against – no, abstain – no

Review of the proposal to launch a new research theme
"Modern Trends and Developments in Raman Microspectroscopy and Photoluminescence for Condensed Matter Studies"
and the project
"Ultrasensitive SECARS microspectroscopy and luminescence biolabeling with core-shell nanostructures".

The proposed new research theme is a harmonic and natural continuation of theme No. 04-4-1111-2013/2017 "Multimodal platform for Raman and nonlinear microscopy and microspectroscopy for condensed matter studies", which is successfully coming to an end.

I would like to begin my assessment with a very important point that the Frank Laboratory of Neutron Physics, JINR, has Russia's only and world's unique functioning setup, namely, the picosecond microspectrometer of coherent anti-Stokes Raman scattering, or CARS microscope. Though Raman spectroscopy is a rather new field at JINR, where this research started in 2013, the indisputable advantage of the highly skilled team engaged in this field is their continuous aspiration to keep up with the dominant world trends of research in Raman spectroscopy and microscopy. Since the Raman spectrum is very informative while its intensity is extremely low, various schemes and methods are under development in the world for considerably increasing scattering efficiency of the light that carries information on the structure of the medium and physical and chemical processes occurring in it. Among these methods, SERS (surface enhanced Raman scattering), CARS (coherent anti-Stokes Raman scattering), and TERS (tip enhanced Raman scattering) are of particular interest. These trends are reflected in the titles of the new theme and project on research and development in highly-contrast ultrasensitive Raman microspectroscopy.

The results of three years' activities on mastering quite a complicated CARS method that underlie the design of the setup and developing the SERS method with application to this setup on the initiative of the theme leader have allowed the scientists of the Raman Spectroscopy Sector to come to grips with the problem of combining these two mutually enhancing methods. Microscopy of surface enhanced coherent anti-Stokes Raman scattering (SECARS) by molecules localized on SERS-active surfaces is attractive because the theoretically achievable scattered light intensity gain for SECARS is orders of magnitude higher than for SERS. In addition, being higher than the pump light frequencies, the scattered anti-Stokes light frequency allows, unlike the case of ordinary SERS, radically tuning from overshadowing Stokes luminescence typical of most interesting samples for biological, chemical, and material science research. This holds out hope of considerably extending, due to nonlinear optics, the range of objects detected by optical methods and achieving sensitivity at the level of single-few molecules in the region under investigation. **It is also worth noting that there are only few single works on this topic in the world literature.**

The undoubted merit of the project is also the proposal to create and develop a single complementary optical platform for the biovisualization of objects using Raman microscopy and luminescence methods based on core-shell nanostructures.

Summing up, I note that the project "Ultrasensitive SECARS microspectroscopy and luminescence biolabeling with core-shell nanostructures" proposed within the new theme is of high current importance and scientific significance and deserves support for its implementation at FLNP, JINR, in the coming three years.

V. V. Smirnov
Professor, Head of the Optical Spectroscopy Department
Prokhorov General Physics Institute, Russian Academy of Sciences

Moscow, 3 May 2017

Referee report on the opening of new theme and project "Ultrasensitive SECARS microspectroscopy and luminescence biolabeling with core-shell nanostructures"

The project under review is aimed at solving modern fundamental and practical problems in the field of photo and upconversion luminescence within the new JINR theme put forward for consideration.

As we know from the published works, the multimodal optical platform based on the CARS microscope was successfully used in the past years not only for investigations in Raman and nonlinear spectroscopy and microscopy but also, in view of unique specific features of this device, for luminescence investigations of various optical matrices doped with rare-earth elements. Uniqueness of the approach is that in addition to measurement of photoluminescence at high-energy optical excitation, the optical platform allows excitation of a sample by near-IR radiation (multiphoton absorption) and detection of luminescent signals in the anti-Stokes spectral region, as in CARS spectroscopy. This phenomenon, known as upconversion luminescence (UCL), has a few interesting applications. In particular, UCL-featuring nanoparticles are more and more often viewed as an effective alternative to traditional fluorescent markers for visualization of biological objects because they are free of some limitations that are typical, for example, of dyes, such as optical instability, photo fading, etc.

Recently many world's leading research centers active in this field have been using promising nanostructures known as core-shell nanoparticles. They are attractive by the diversity of their chemical composition and structure, which makes them multifunctional and ensures their high-intensity luminescence. Luminescent characteristics are most brightly manifested in synthesis of composite shells, including those consisting of silver or gold nanoparticles. It is investigations of these nanostructures that are to be carried out in the scope of the project under review. Scientifically and practically, the project under review is undoubtedly a promising direction in physics similar to the observed SERS phenomenon.

As an applied research after achieving high-intensity luminescence of the core-shell nanoparticles, the authors of the project intend to test them in photodynamic cancer treatment investigations using $NaYF_4$: Yb,Tm nanoparticles as cores and porphyrins as shells. This demonstrates not only the importance of the work but also some novelty of the research within the given area.

In conclusion, a few important points must be noted:

1. Investigations with two complementary methods, Raman scattering and luminescence, are a productive approach. Implementation of the project under review within the framework of international collaboration, including Belarussian scientists, can complement the Raman scattering project in terms of applications, e.g., high-contrast optical visualization of biological objects, diagnostics of oncological diseases and diseases of a bacterial nature, etc.

2. The choice of the core-shell structures with cores of $NaYF_4$: Yb,Tm nanoparticles and shells of silver or gold nanoparticles for enhancement of luminescence signals via plasmon interaction is important for creation of competitive phosphors, being in line, in terms of the underlying scientific ideology, with current research, development, and applications in this field.

Based on the aforesaid, we recommend with confidence the implementation of this project on the multimodal optical platform at the Frank Laboratory of Neutron Physics within the proposed new theme in 2018–2020.

V. M. Fedosyuk,
Corresponding-member of NAS of Belarus
Director General
State Scientific Production Association
“Scientific and Practical Center for Material Science,
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