JOINT INSTITUTE FOR NUCLEAR RESEARCH
Frank laboratory of Neutron Physics
Department of Raman Spectroscopy
(Centre “Nanobiofhotonics”)

**FINAL REPORT ON THE
SUMMER STUDENT PROGRAM**

Studies on Raman Scattering and Upconversion Luminescence at the “CARS” Microscope

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Introduction

Raman spectroscopy is an important part of optical spectroscopy studying the interaction of monochromatic radiation, usually from a laser source, with matter. It is accompanying by a change in the energy of the scattered radiation compared to the energy of the incident excitation radiation. Raman scattering is due to inelastic photon collisions with molecules in which they exchange energy. Frequency of photons is shifted up or down, that contains the information about vibrational, rotational and other low transitions modes in molecules. Accordingly, Raman spectroscopy can be used to study solid, liquid and gaseous samples.

Up-conversion is a type of anti-stokes radiation. The term up-conversion refers to the process of converting low energy incident radiation into higher energy output radiation. Actually interest to the phenomenon of up-conversion has received a new impulse due to the synthesis of modern matrix elements that contain various nanoparticles or/and nanocomposites.

Short Review of Literature

Raman spectroscopy

Phenomenon of inelastic scattering was predicted in 20-s last century, and later proved independently by two scientists group: Raman C. V., Krishnan K. S. [1] and Landsberg G.S., Mandelstam L.I. It is one of the most important analytical and research methods of structural studies up to the present time. It has a lot of applications in many areas: physics, biology, medicine, chemistry, pharmaceutics, criminalistics, etc. This type of analysis is incredibly informative for chemical identification and molecular structure research.

Raman spectra are very sensitive to the nature of chemical relations - in organic molecules and polymeric materials, as well as in inorganic lattices. Because of that each substance and material has its own individual spectrum (fingerprint). Spectra mainly consists of lines corresponding to the deformation and the stretching vibrations of the chemical bonds of carbon (C) with, as usually, nitrogen (N), oxygen (O) and hydrogen (H) and some others. These lines appear in the range from 600 cm^{-1} (stretching vibrations of single C-C bonds) to 3600^{-1} (vibrations of hydroxyl -OH group) [2].

Using Raman spectroscopy the interaction of lysozyme and trehalose was studied in [3]. Almost all phospholipids, including DPPC, are detailed studied by means of Raman spectroscopy [4]. Traditional or spontaneous Raman

spectroscopy, however, is not so effective tool for bioanalyte detection, especially at trace concentrations, because a relatively low number of photons are Raman, or inelastically, scattered as compared to the number of photons that are Rayleigh, or elastically, scattered. To overcome this problem, the use of surface-enhanced Raman spectroscopy (SERS) has been instituted. At present, SERS is very promising analytical tool for many researches for biological structure studies. Hope T. Beier and Christopher B. Cowan in their work had showed suitability of SERS for detection of amyloid beta and they produced SERS spectra that indicate the feasibility of detecting Aβ oligomers into the picomolar range [5]. SERS method, for instance, was also used on colloidal silver clusters as an excellent technique for single molecule detection that is applicable for a broad range of molecules including ‘‘colorless’’ biomolecules, for example nucleotides in DNA sequencing [6].

Up-conversion luminescence

The optical phenomenon of up-conversion luminescence has researched since 60’s last century. First efforts were made by famous physicists Bloembergen, Auzel, Ovsyankin and Feofilov. Their works have been dedicated to study up-conversion in rare earth-doped bulk phosphors [7]. In subsequent years, many scientists have studied this phenomenon that have received number of applications such as short-wavelength lasers [8], solar cells, temperature sensors, three dimensional displays [9], etc.

There exist a large number of materials in which up-conversion has been observed, with very large differences in the actual up-conversion efficiently. Up-conversion materials are generally comprised of an inert host material doped with optically active sensitizer and activator ions. Host materials are low-phonon energy, chemically stable, mechanically durable and thermally stable. Fluorides, oxides, bromides and other materials are popular choice as host materials. Dopant materials must be with clearly defined energy levels and two or metastable states. Therefore, up-conversion is most efficiently in doping host materials with lanthanide ions.

Lanthanide-doped upconversion nanoparticles are capable of emitting in the visible region of spectrum upon excitation with a near-IR light source. A lot of works are dedicated to up-conversion of such type of specimen. UCL efficiency depends on both concentration of doping elements [10], and properties of substrate/specimen. In their work Zhengquan Li and Yong Zhang has suggested method for hexagonal-phase NaYF4:Yb, Er/Tm nanocrystals creation as more

thermodynamically stable than cubic-phase crystals. As a result, they received quite strong fluorescence at low laser power [11]. In another work Peiyan Yuan,ab Yih Hong Lee and etc. Has created NaYF4:Yb,Er@SiO2@Ag nanocrystals. Researches shown that saturation of specimen with silver ions has usefull application in biological imaging agents [12]. Also, up-conversion on plasmonic architecture of Au-Ag nanocages and gain conditions was studied. This has the potential in different bio-applications, photonic and photoelectric sensing devices [13].

Facility specification

A scanning confocal laser Raman microspectrometer "Confotec CARS" coupled with a NIKON TE2000-E inverted microscope and employing two microobjectives 40x-0.6NA and 60x-1.2NA are used to acquire all of the Raman and up-conversion luminescence spectra Fig.1. The 520 cm^{-1} band of a silicon wafer was used for frequency calibration. Three color lasers are installed at the optical platform for Raman scattering studies. The excitations at 633 and 532 nm were provided by a He-Ne laser (Melles Griot 05-LHP-991) and a diode laser with an adjustable output power (model SLM-417-20) respectively. The He-Ne laser has an output power 10 mW and beam divergence is less than 1 mrad. The excitation at 785nm was provided by 85 MHz passively mode-locked picosecond laser (model EKSPLA, PT257-SOPO) tunable in the range of 690-990nm. A selection of dichroic mirrors, clean-up, and edge filters from Semrock were used in association with 532nm, 633nm and 785nm excitation wavelengths.

For up-conversion luminescence investigations, we used mainly laser excitation at 976nm which is very proper wavelength for many phosphors co-doped with Yb^{3+} ions.

Figure 1. General view of the "Confotec CARS" microscope.

The average laser power on the sample is controlled by a variable neutral filter with the 0–3 optical density which allows to range the power from few μW to tens of mW. The excitation light was focused on the sample in ~ 1 μm laser spot (40x) or ~ 650 nm laser spot in the case of water emersion objective with high NA=1.2.

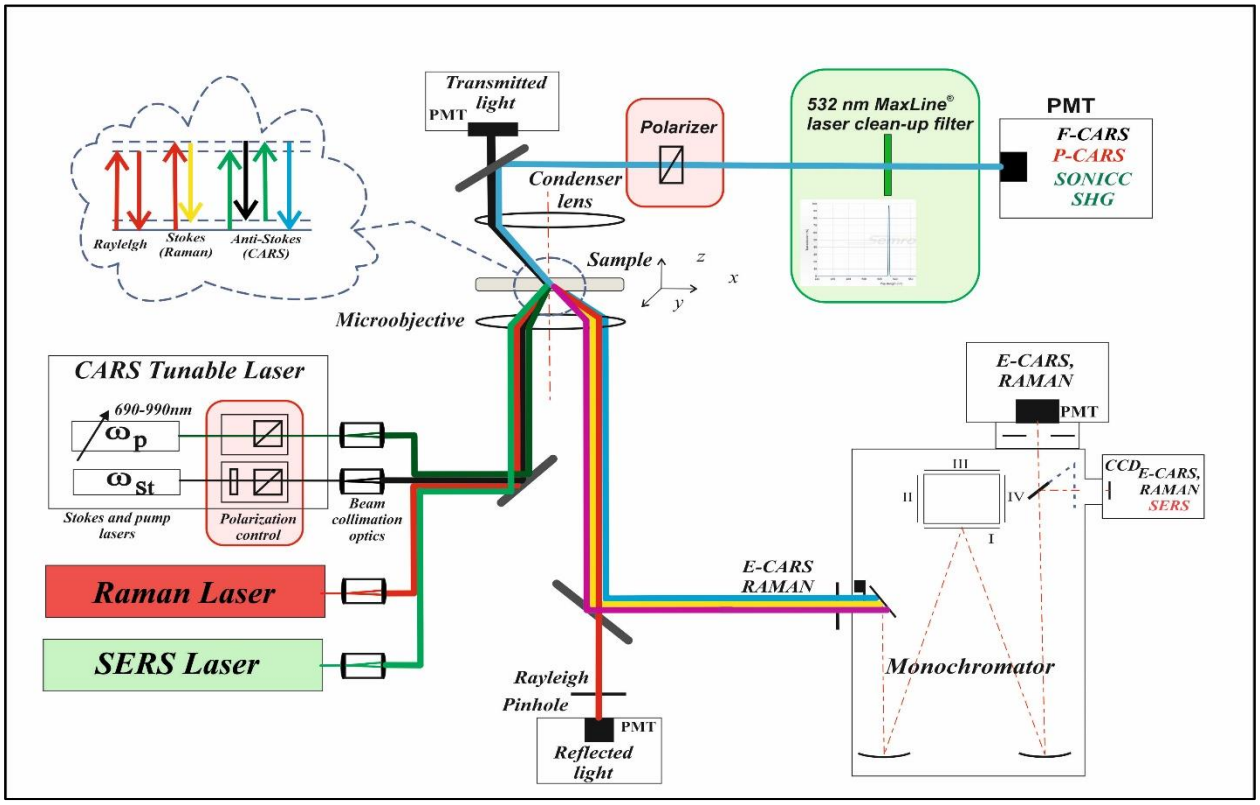

Figure 2. Layout of the "Confotec CARS" microscope.

The Raman and upconversion luminescence signals from the analytes and phosphors, located at the motorized sample position adjustment stage (Prior Scientific, H117TE), are collected in epi-direction, dispersed by monochromator-spectrograph MS520 with a 600 grooves/mm grating and detected with a Peltier-cooled CCD camera (ProScan HS−101 H) cooled to −20 °C. Usually spectra are collected at different localizations of the samples with an integration time of (1-30)s with a spectral resolution of 2.0cm^{-1}. A screen image recorder camera attached to the microscope enabled the acquisition of the white-light micrographs of the area under the investigation.

Results and Discussion

Raman spectroscopy

During the Summer Student Program a lot of Raman spectra were measured and studied at a confocal CARS microscope. For researches we choose following lyophilized powders with 90-99% purity made by Sigma Aldrich (Germany):

1. **Albumin from bovine serum (BSA);**
2. **L-α-Phosphatidylcholine, dimyristoyl (DMPC);**
3. **Myelin basic protein bovine;**
4. **Ribonucleic acid from Torula yeast (RNA);**
5. **Deoxyribonucleic acid sodium salt from herring testes (DNA);**
6. **1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC);**
7. **1,2-dioleoyl-*sn*-glycero-3-phoshocholine (DOPC);**
8. **Cholesterol;**
9. **Melatonin;**
10. **Amyloid-β-protein;**
11. **Lysozyme;**
12. **Stearic acid.**

We prepared solutions of these substances for obtaining Raman spectra. Proportion of 1 mg of each powder and 100 μl of solvent was used. As for solvent were used: for DNA, RNA and myelin - distilled water; for BSA, lysozyme and stearic acid — hexane of 99% purity made by Sigma Aldrich (Germany); for DPPC, DOPC, DMPC, cholesterol, melatonin and β-amyloid – chloroform 99% purity made by Acros Organics (USA). Obtained solutions were thoroughly mixed in a centrifuge-mixer CM-70M-09 (Elmi Ltd. laboratory equipment) for 5 minutes. After that using batcher each substance drop of 1 μl was applied to a sodium-calcium substrate and allowed to dry at room temperature for one day. Dried specimens were used for the measurements upon the excitation of 633nm He-Ne laser through a microobjective 40x-0.6NA, and exposition time of 10 s.

Figures 3-14 shows all recorded Raman spectra for the above listed bio-samples which are in excellent agreement with literature data.

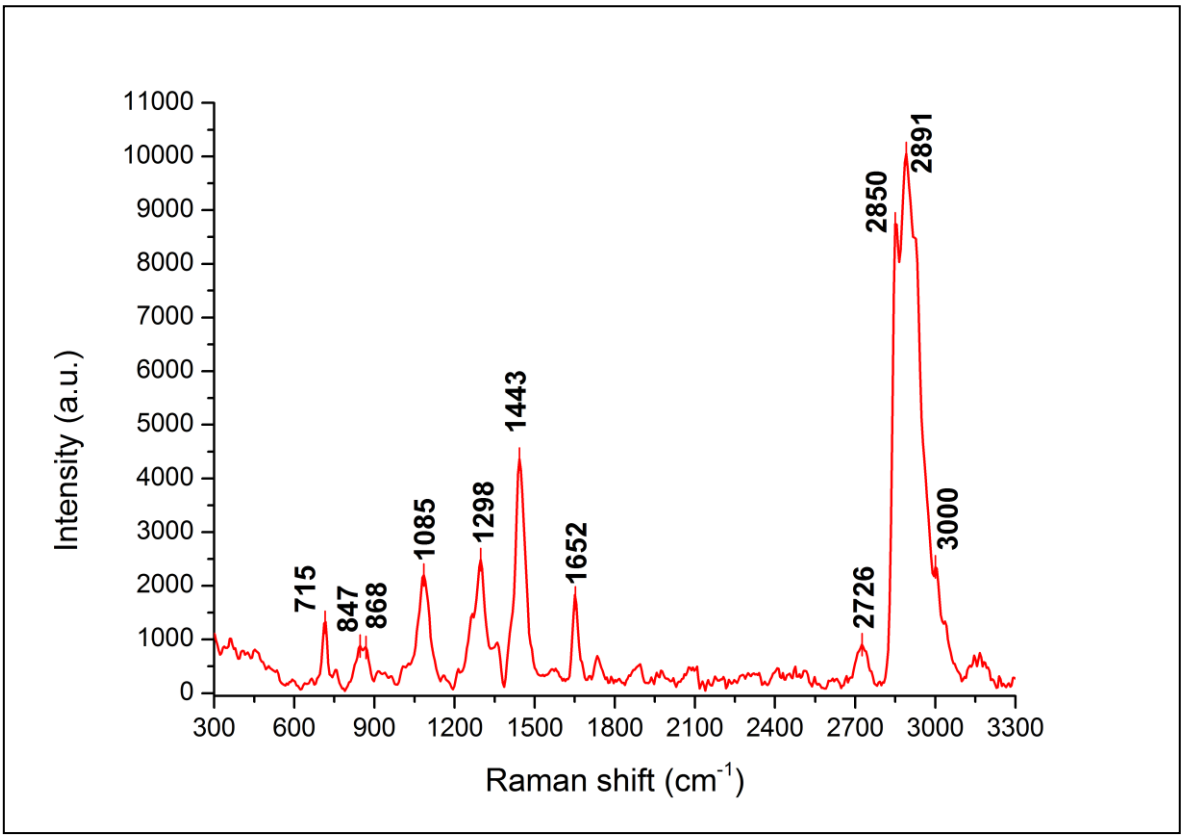

Figure 3. Raman spectra of DOPC.

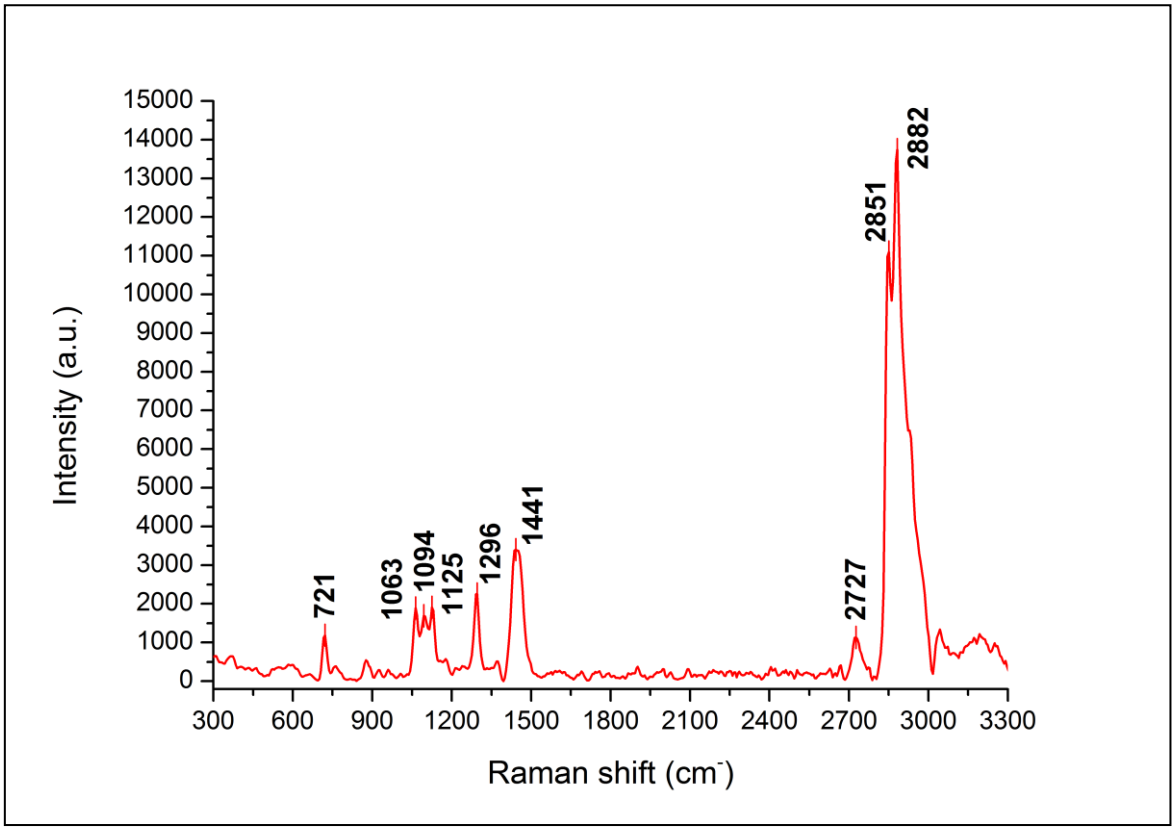

Figure 4. Raman spectra of DPPC.

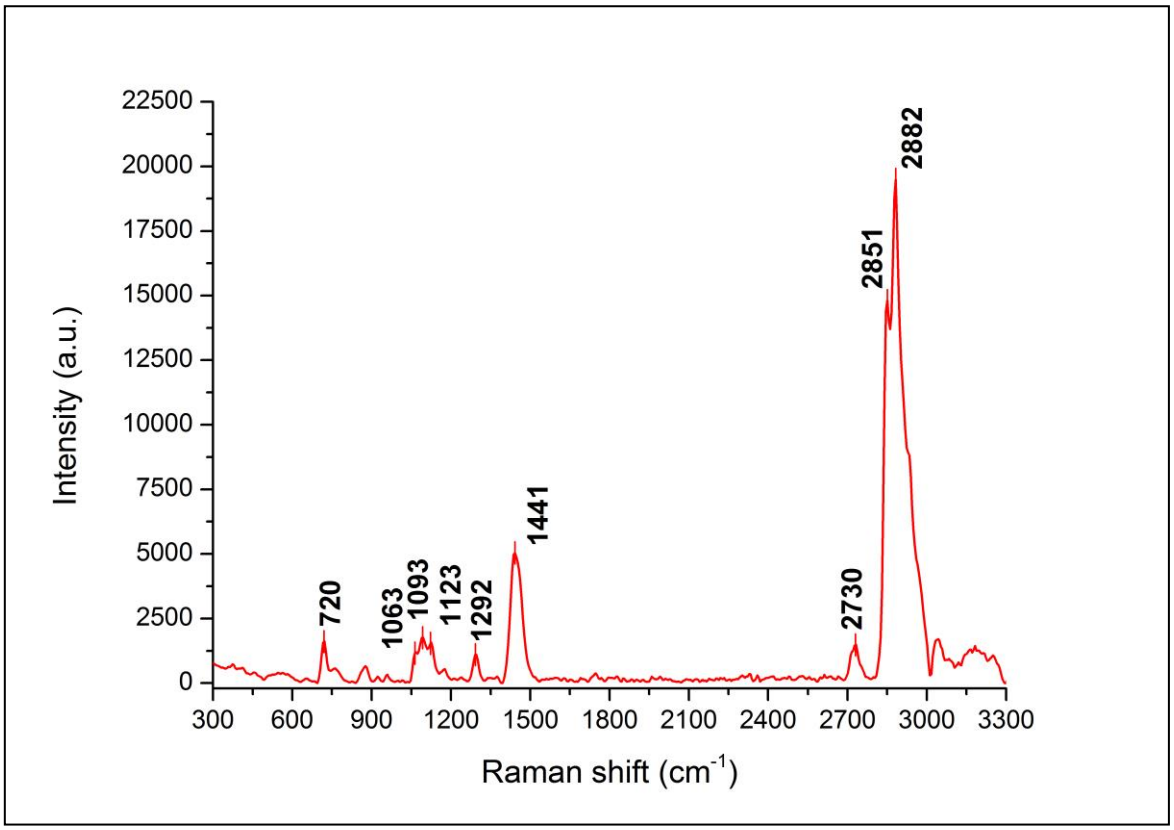

Figure 5. Raman spectra of DMPC.

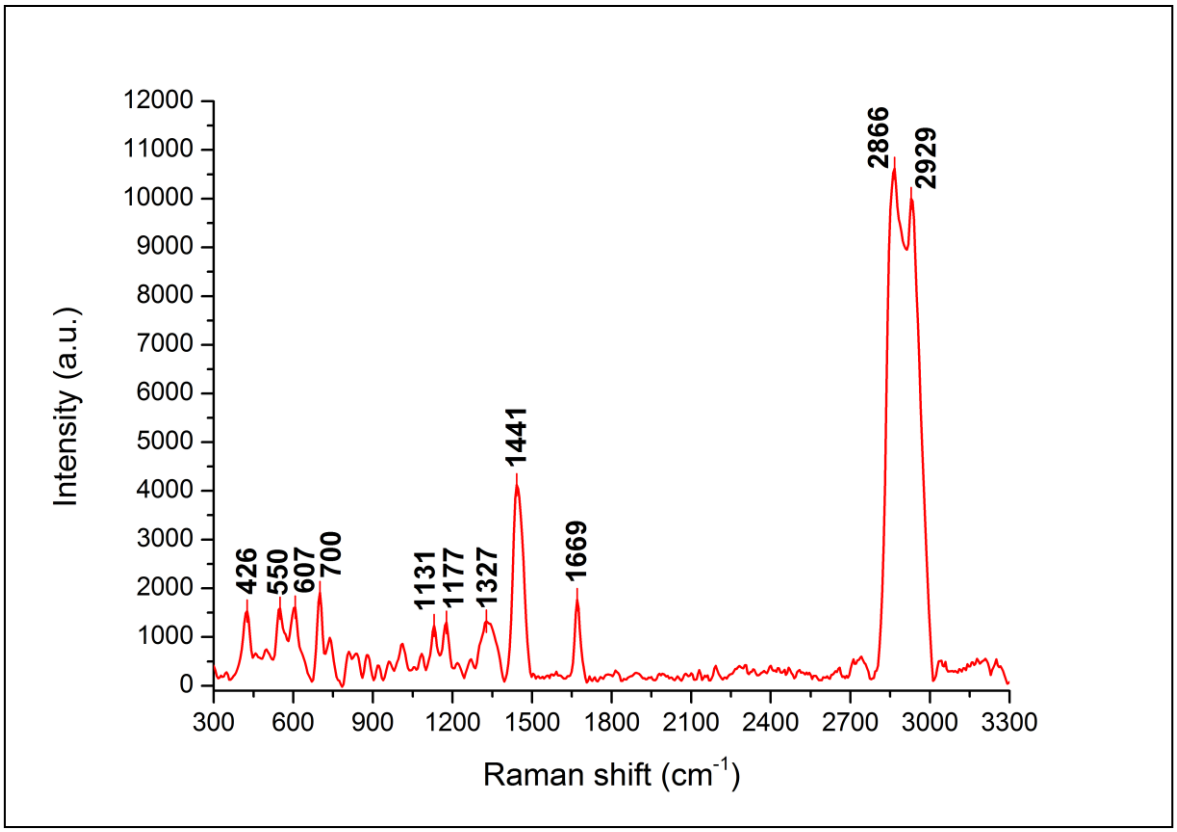

Figure 6. Raman spectra of cholesterol.

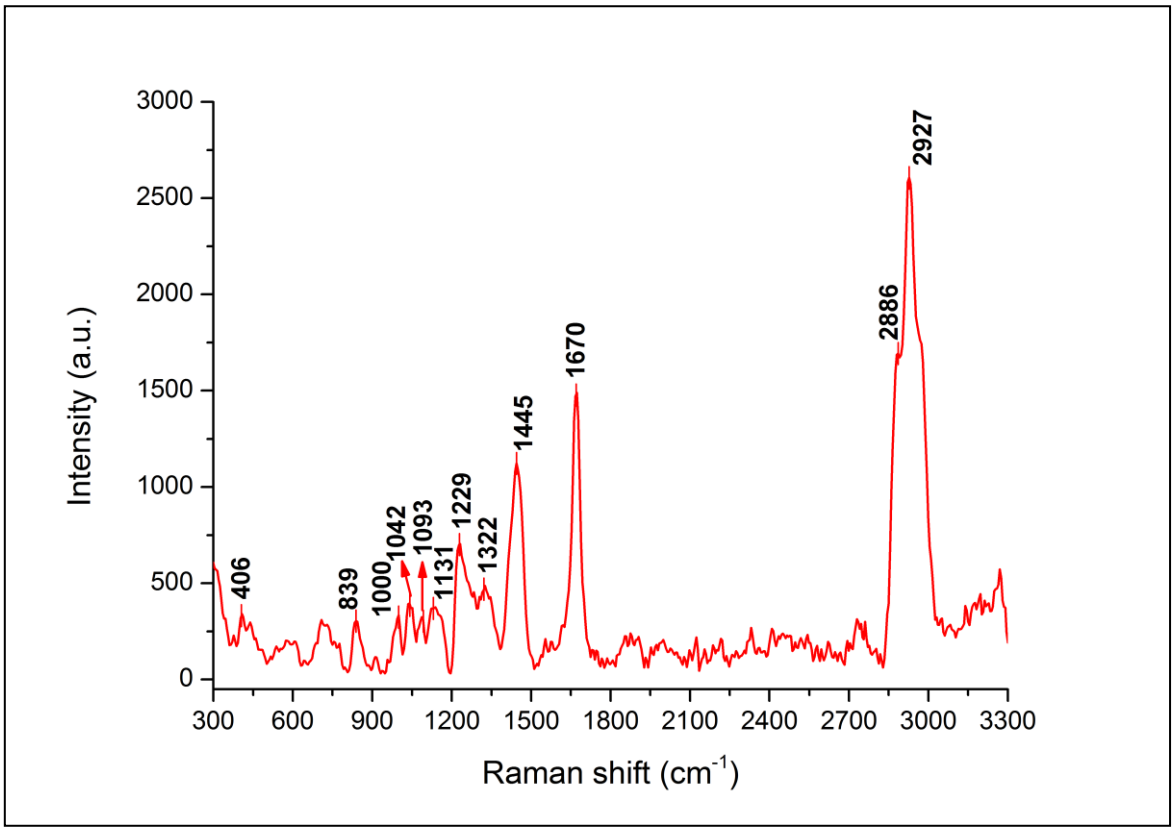

Figure 7. Raman spectra of amyloid-β-protein.

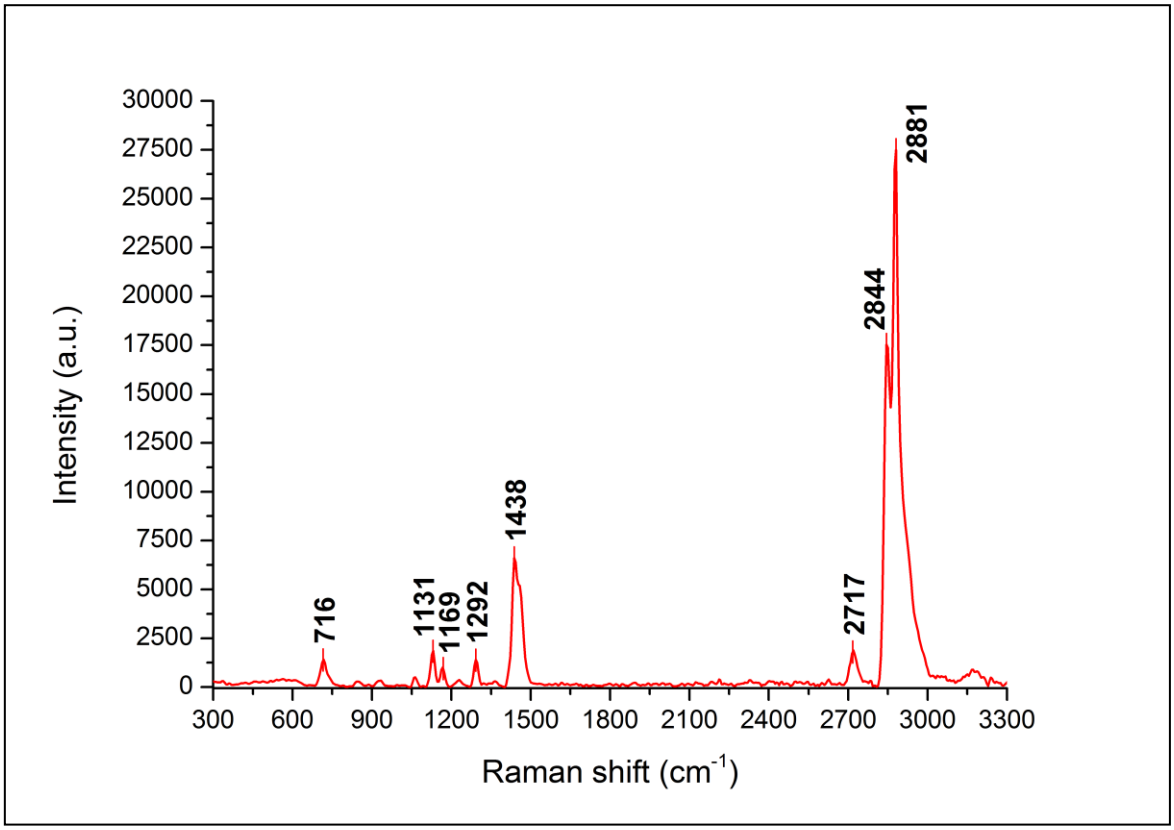

Figure 8. Raman spectra of albumin.

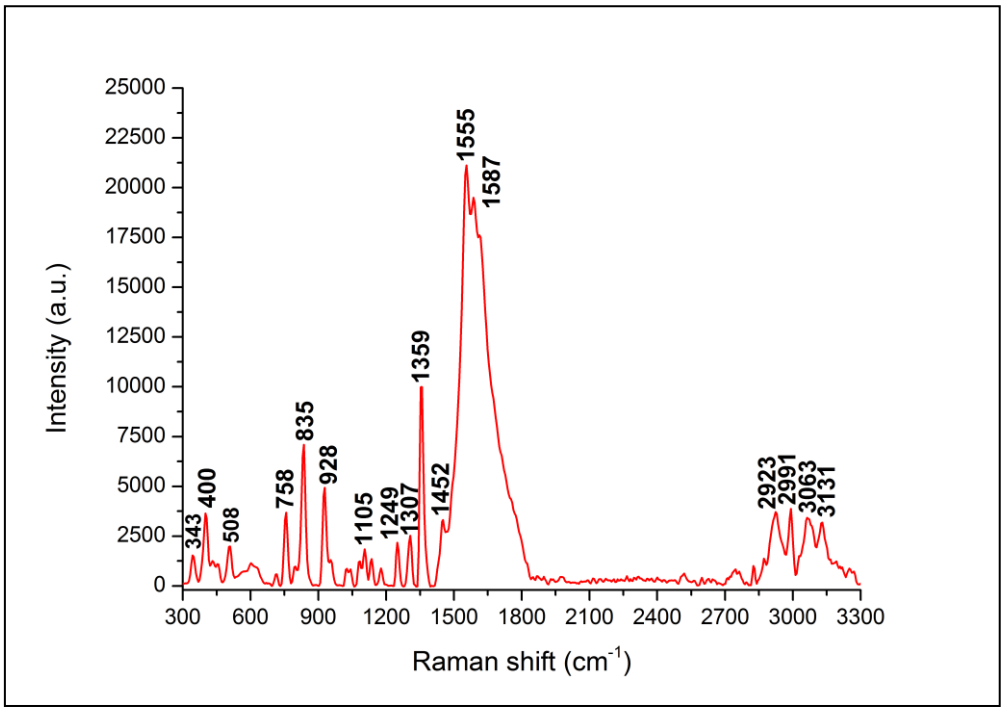

Figure 9. Raman spectra of melatonin

In the case when the Raman signal was very weak, we employed an accumulation option integrated in the soft of the microscope program. This allows to take Raman spectra with an exposition time of 30 sec or even more, which is not possible in the normal option due to saturation of the intensity accompanying the useful Raman signal. For example, DNA, RNA, myelin, stearic acid and lysozyme were measured employing the accumulation option.

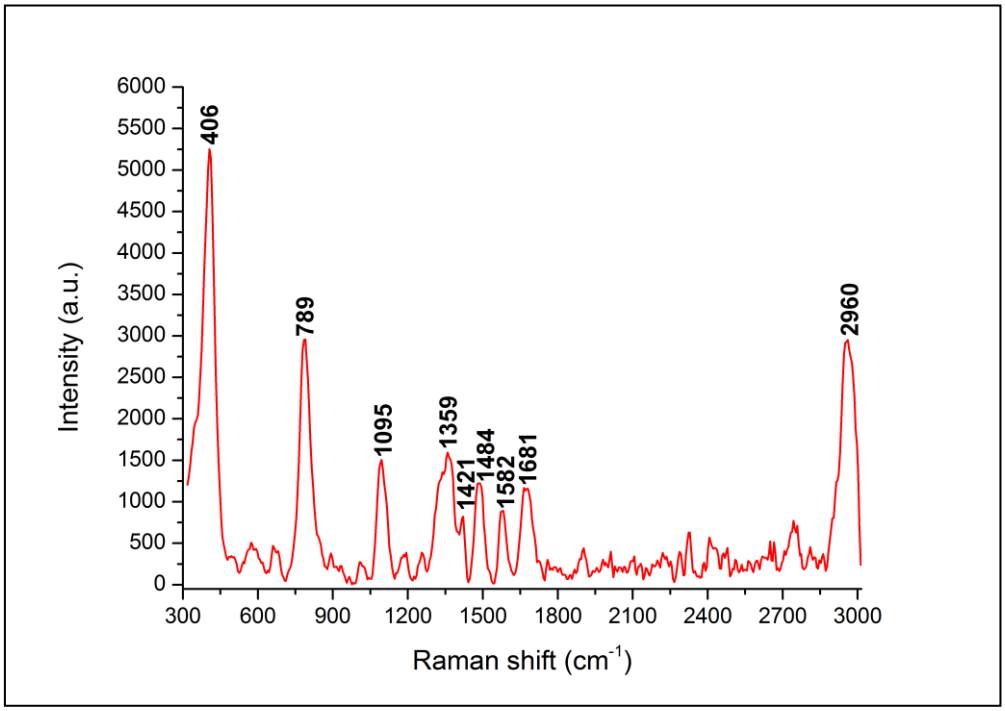

Figure 10. Raman spectra of DNA.

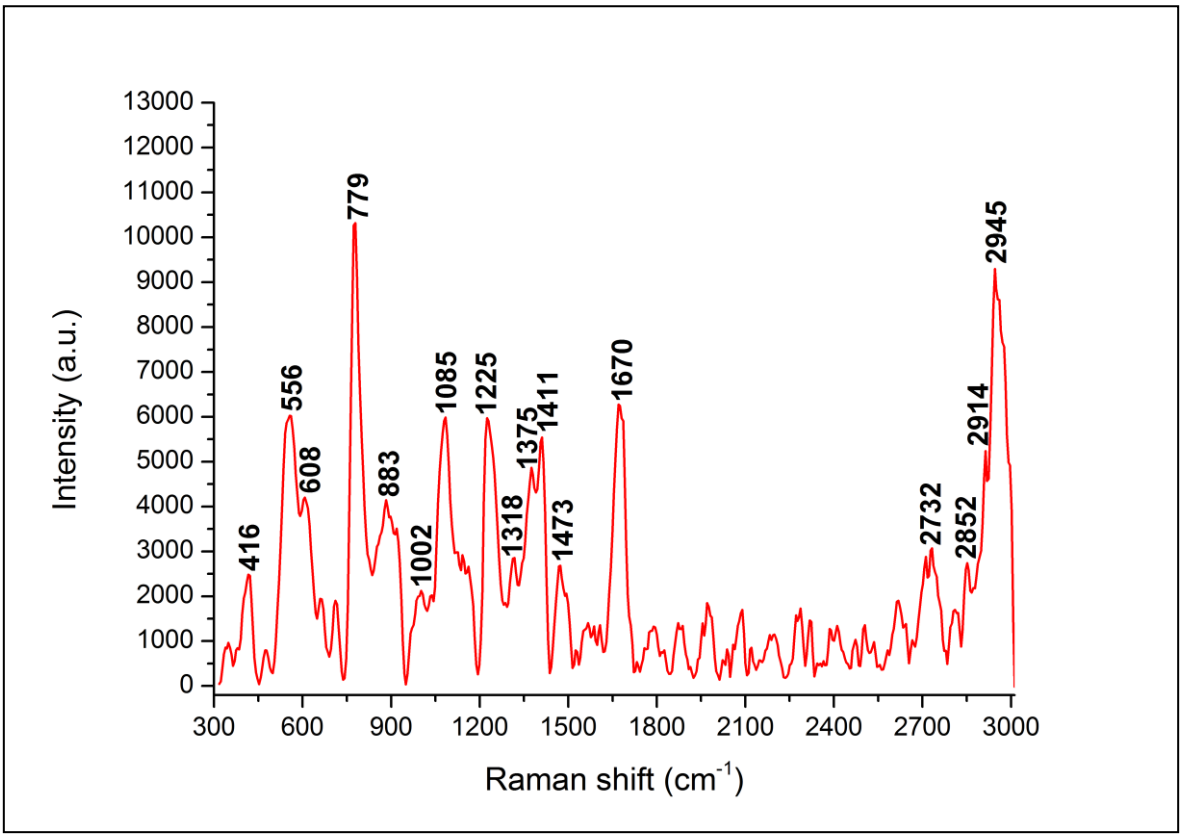

Figure 11. Raman spectra of RNA.

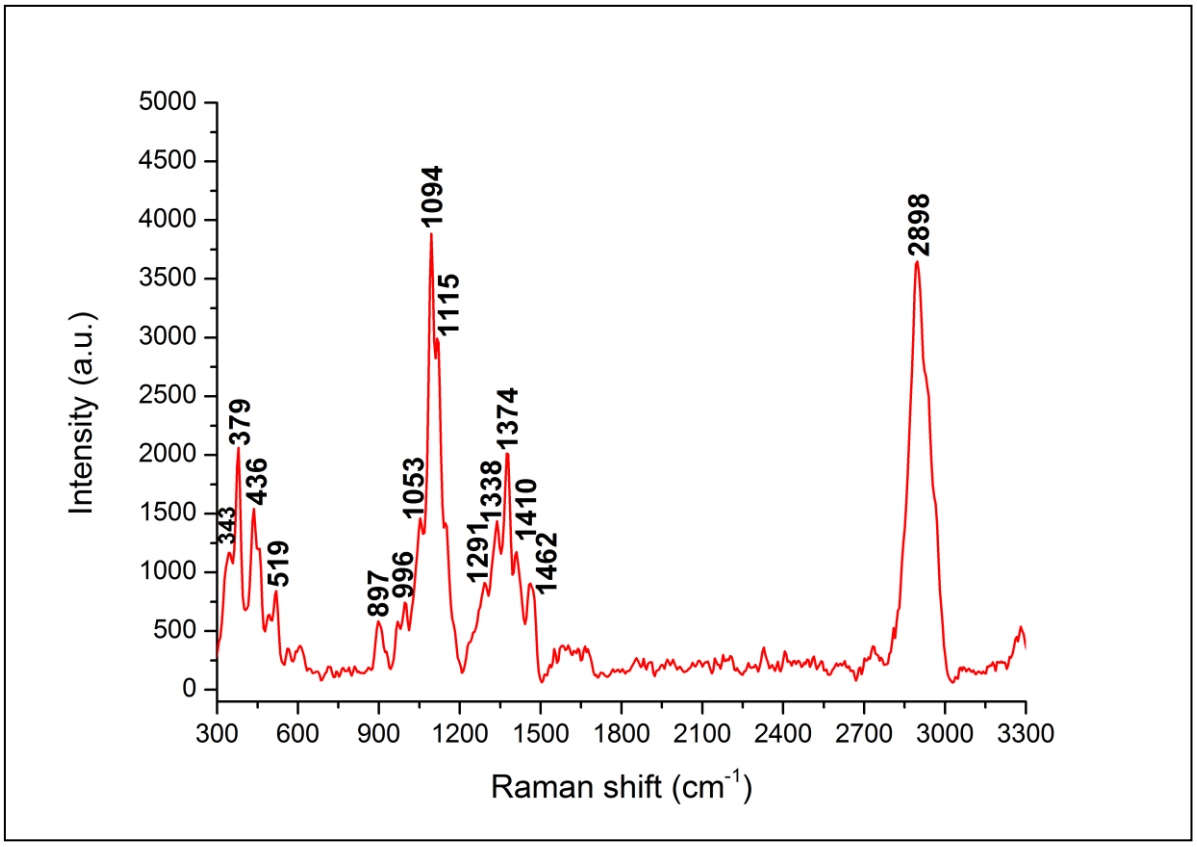

Figure 12. Raman spectra of myelin.

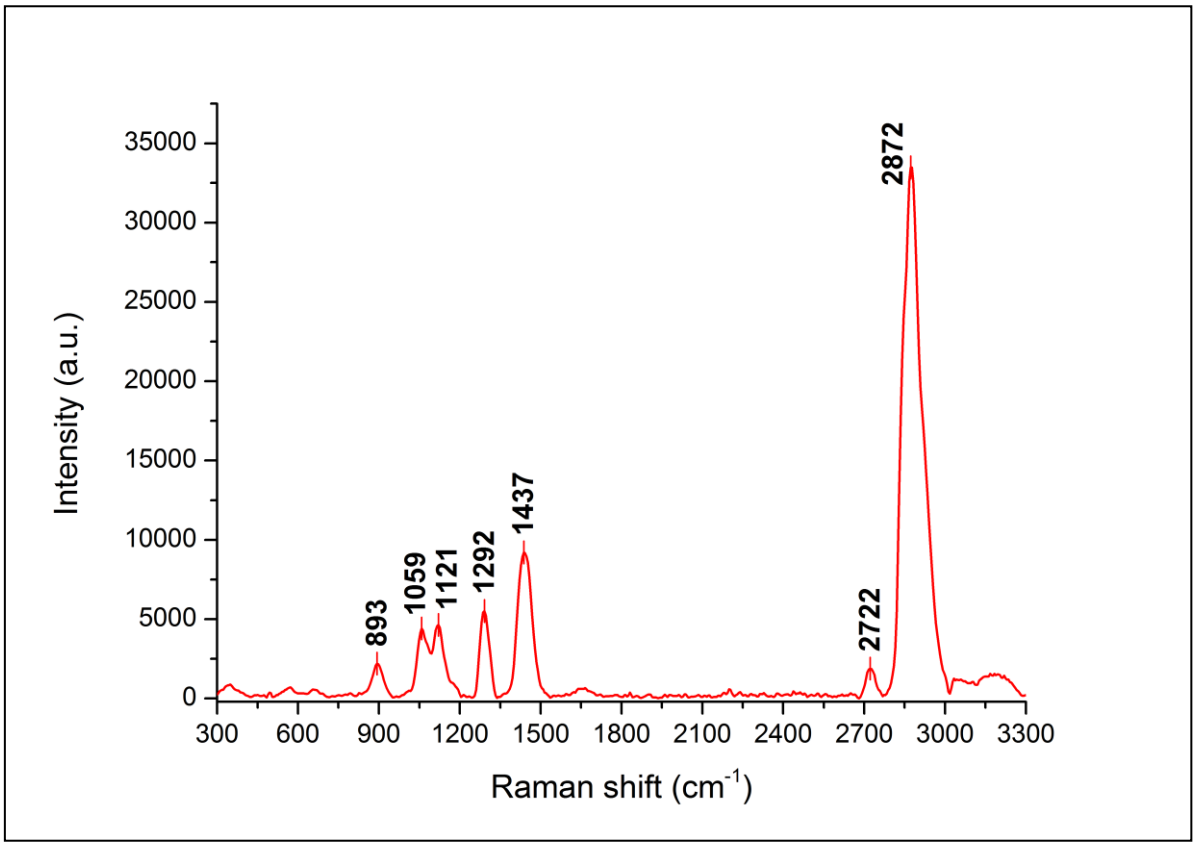

Figure 13. Raman spectra of stearic acid.

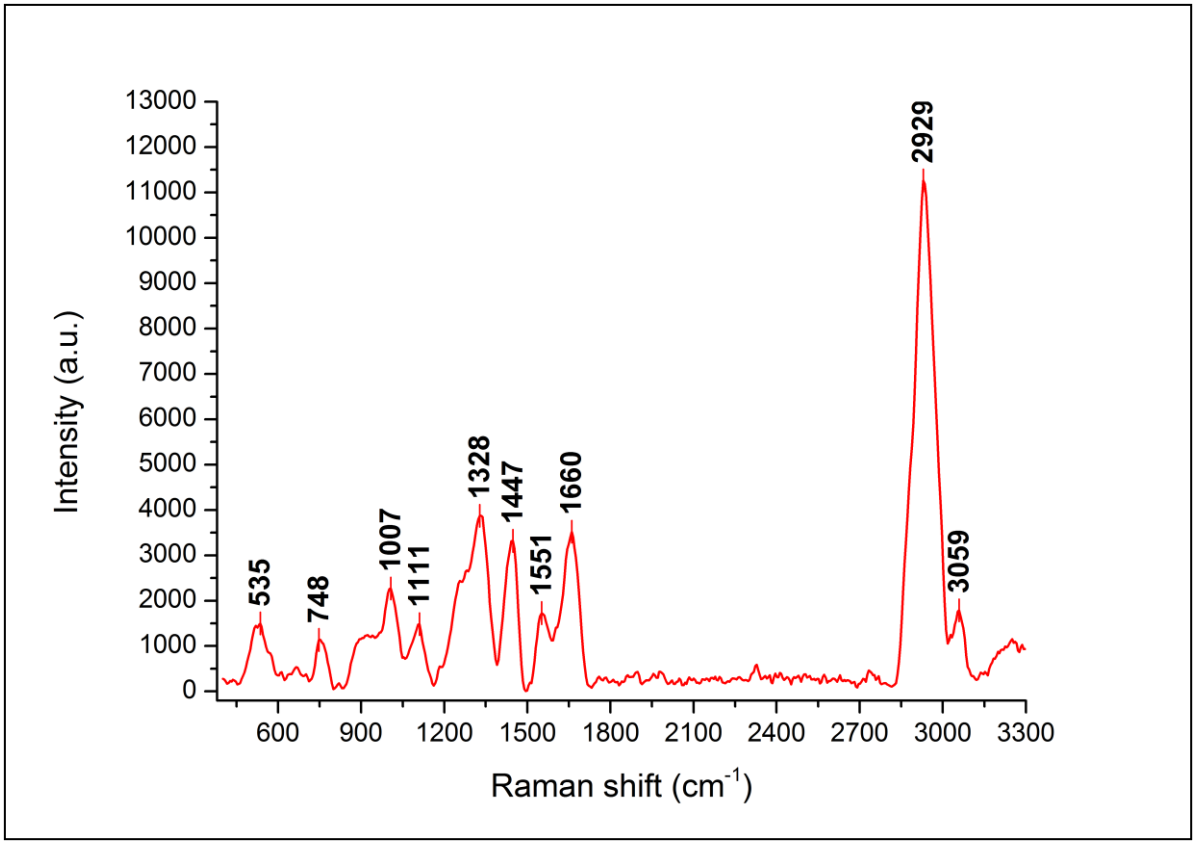

Figure 14. Raman spectra of lysozyme.

Up-conversion luminescence

In the present work the phosphor consisting of $NaYF_4:Yb^{3+}:Er^{3+}$ (18 mol%, 2 mol%) and $NaYF_4:Yb^{3+}:Tm^{3+}$ (25 mol%, 0.3 mol%) suspension dissolved in cyclohexane and being in tight contact with Ag nanoparticles island films deposited on the porous silicon plate were chosen for studies of UCL. For the excitation we employed 976nm laser radiation. The obtained results on up-conversion luminescence (UCL) spectra are demonstrated in the Figures 15 and 16.

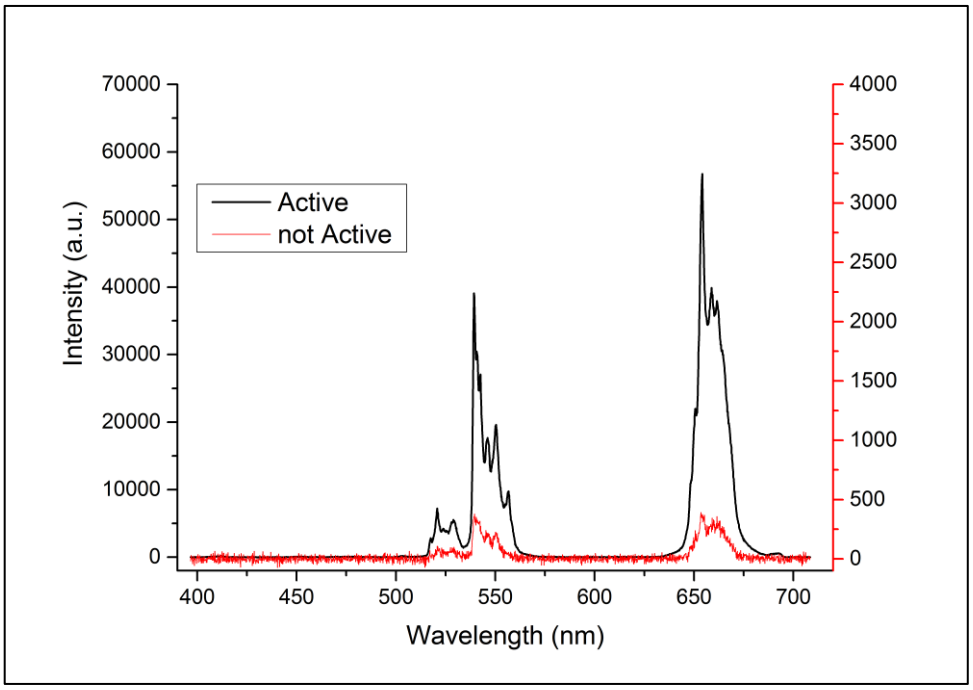

Figure 15. UCL spectra of $NaYF_4:Yb^{3+}:Er^{3+}$.

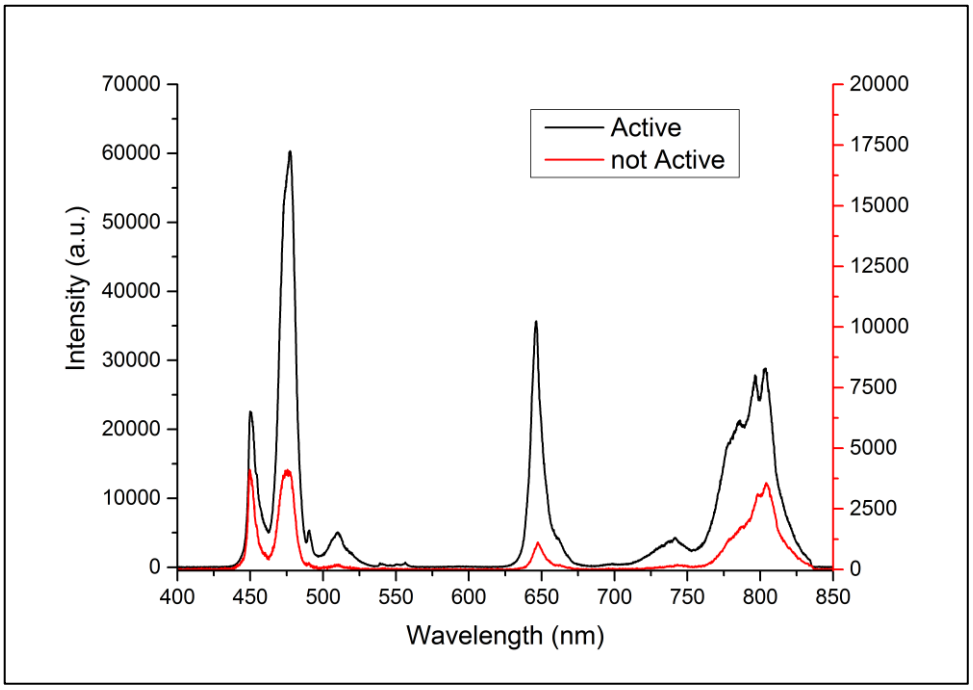

Figure 16. UCL spectra of $NaYF_4:Yb^{3+}:Tm^{3+}$.

As it was expected we observed a strong enhancement of the UCL spectra intensity taken from the Ag-NPs active-area in comparison with that of pure silicon plate locations (not-active area). This is due to the plasmon enhancement effect well known for the Raman spectroscopy enhanced option, namely, SERS – surface enhanced Raman spectroscopy. The same physics mechanisms take place in the case of luminescence/upconversion luminescence emission from the plasmon active area, though the later one is much less investigated for the present time.

Conclusion

During the summer student program at the Sector of Raman Spectroscopy, LNP, JINR I got a very nice experience working at the multimodal optical platform equipped with Raman micro-spectrometer, several laser sources and detection systems. During only one month of my practice period we had a very saturated program resulted in a large amount of Raman spectra measurements for different bio-species. Along with the Raman spectroscopy I got acquainted with antiStokes type of luminescence emission – so-called up-conversion luminescence when two or more infrared photons are absorbed by a phosphor doped with rare earth elements and emit one visible photon. We observed a notable result on the luminescence signal enhancement when the phosphor was deposited on the nanostructured surface of noble metal (Ag, Au) nanoparticles. This phenomenon is known as plasmon enhancement of luminescence.

Acknowledgements

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