Stimulated low-frequency Raman scattering in biological nanoparticles suspensions

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Abstract. The interaction of electromagnetic radiation with nanoscale particles systems (including suspension of metallic dielectric and semiconductor nanoparticles, biological nanostructures, etc.) can lead to various non-linear effects, in particular, to the stimulated low-frequency Raman scattering (SLFRS). It can provide important information about investigated system elastic properties. In the present study low-frequency vibrational modes in different biological nanoparticles systems were investigated, such as tobacco mosaic viruses (TMV), two types of potato viruses (PVX and PVA), cauliflower mosaic virus (CaMV), human and bovine serum albumin (HSA and BSA) in Tris-HCl pH7.5 buffer and in water. 20 ns ruby laser pulses were used for excitation. SLFRS frequency shifts, corresponding to acoustic eigenfrequencies of the samples were registered by Fabri-Perot interferometers. Conversion efficiency and threshold were also measured for the first time. SLFRS can be applied for nanoobjects identification and effective impact on biological nanoparticles systems.

1. Introduction

Biological nanoparticles are the subject of intense experimental and theoretical investigations now. It is important to develop new methods of the biological nanoparticles identification and impact on them. Some methods of laser optics have been suggested for study of nanoparticles, in particular, biological nanosized objects [1, 2]. Low-frequency Raman scattering (LFRS), a result of light interaction with nanoparticles vibrations, was shown to be effective and convenient tool for the investigation of nanoparticles [3-5]. It can be used for study of various biological objects with size from several nanometers to micrometer [6, 7]. This type of scattering is manifested by the appearance in the scattering spectrum of additional components with the frequency shifts corresponding to the eigenfrequencies of nanoparticles vibrations [6,7]. Each nanoparticle has a set of acoustic eigenfrequencies, some of them are Raman active. Many biological objects of interest have eigenfrequencies lying in gigahertz range (for example viruses and proteins). While the eigenfrequency of the nanoparticle is determined by its morphology, such important characteristics as the size and sound velocity can be determined from the LFRS spectra. Stimulated analog of LFRS – stimulated low-frequency Raman scattering (SLFRS) gives possibility to increase conversion efficiency of the scattering and to decrease its threshold. We registered
SLFRS in different nanosized systems, consisting of metal, dielectric and semiconductor nanoparticles, both high-ordered and random materials [8-10]. In the present work we show that SLFRS can be used for biological nanoobjects investigations.

Viruses consist of genetic material (RNA or DNA), surrounded by protein shell (capsid). The dimensions of the most viruses’ capsids are in the range from 10nm to 100nm. Some biological nanoparticles have spherical or cylindrical form and change size or shape in response to various changes in external conditions [11]. Such systems are widely used in various fields, including optoelectronics and nanotechnology [12, 13]. Their structures have been well studied by X-ray crystallography [14] in contrast to their dynamic properties. But due to the fact that the morphology of such nanoparticles significantly depends on environment conditions, the study of dynamic properties is an important task. In a number of works acoustic vibrations of some viruses have been investigated using LFRS methods [7, 15, 16].

There are two theoretical approaches to calculate the discrete spectrum of the acoustic eigenfrequencies of spherical biological objects: an elastic sphere model [17] and a liquid drop model [18,19]. In the elastic sphere model, the frequency of the normal mode:

\[ \omega = \frac{V}{r} \]  

where \( V \) is the sound velocity, \( r \) is the radius of a virus particle, and \( \xi \) is a dimensionless parameter depending on the relation between the longitudinal and transverse sound velocities. In the case of a liquid drop model, the frequency of the lowest vibrational mode:

\[ \omega = \left( \frac{\gamma}{\rho r^3} \right)^{\frac{1}{2}} \]  

where \( \gamma \) is the surface tension, \( \rho \) is the material density, and \( r \) is the radius of the biological nanoparticle.

In [18] Ford made calculations for both models of a spherical virus particle and concluded that the elastic sphere model better describes a virus particle. In any case, the question of choosing the model remains open.

Balandin and Fonoberov [7] for the first time calculated the lowest vibrational modes of tubular viruses (M13 bacteriophage and tobacco mosaic virus TMV) in water and air. The observed Raman mode has been shown to belong to one of the Raman-active axial torsion modes of the M13 phage protein coat. It was also theoretically and experimentally shown that only the axial modes can be excited in the Raman experiment, whereas the radial modes are strongly damped due to the radiation of the acoustic energy into the environment. Murray and Saviot [20, 21] showed the influence of viscosity of water on the oscillation frequency of viruses in water.

One of the proposed methods of influence on viruses is microwave absorption [22]. However, its main disadvantage is the fact that water absorbs in this range. Thus, it is very difficult to transfer microwave excitation energy to the vibrational energy of microorganisms. In order to avoid this shortcoming, one could transfer the excitation sources from the microwave range to visible range.

In [23] authors used near-infrared subpicosecond laser pulses for selective inactivation of viruses (tobacco mosaic virus, human papilloma virus, bacteriophage M13 and HIV) without harming healthy cells.

Another alternative way of the effective impact on viruses is to use biharmonic pumping, when two incident waves in the visible range with a frequency difference corresponding to the eigenfrequencies of the system are used. In the process of ponderomotive interaction, oscillation amplitude increases, which can lead to destruction of the object under study.

Thus, as it was shown in the works mentioned above, for nanoscale viruses both the frequency and damping of the vibrational modes are significantly affected by the properties of surrounding medium. So, considering that the spontaneous scattering mode is characterized by low scattered radiation intensity, the use of its stimulated analog (SLFRS) seems more promising for vibrational dynamic study. Stimulated scattering, excited with short and powerful laser pulses, is characterized by high conversion efficiency. It can also be applied as a source of biharmonic pumping with the difference frequency tuning range from a few gigahertzes to terahertz. As a result, we have the vibrational modes resonance excitation that can be used as an effective method of influencing biological nanoparticles system.
2. Samples
In this work we registered SLFRS in four types of plant viruses (tobacco mosaic viruses, A and X potato viruses, cauliflower mosaic viruses) and two types of simple proteins (human and bovine serum albumin) suspensions in buffer Tris-HCl pH7.5 (C₄H₁₂ClNO₃) and in water.

Tobacco mosaic virus (TMV) has 18 nm diameter and 300 nm modal length; it consists of 2130 identical 17.5 kDa protein subunits helically arranged into a rigid tube. The protein subunits form a tight helical array with 1613 units per turn, and the RNA is packed between the turns at a radius of about 4 nm from the helix axis [24]. Potato viruses are thinner and longer than TMV and less rigid. PVA virions are filamentous, usually flexuous, 730 nm long and 15 nm in diameter. Symmetry is helical with a pitch of 3.4 nm. Virions are composed of 5% nucleic acid and 95% protein. Genome RNA is single-stranded and contains 9700 nucleotides. Coat protein (CP) - 29.8 kDa. PVX virions are also filamentous and flexuous, 500 nm long and 13 nm in diameter. Symmetry is helical, with pitch of 3.6 nm. Virions composed of 6% nucleic acid and 94% protein (6400 nucleotides and CP - 25 kDa). TMV strain U1 was isolated from systemically infected Nicotiana tabacum L. cv. Samsun plants [25]. Virions concentration in the test sample was 50 µg/ml, the number of particles in the sample was analyzed by Nanoparticle tracking analysis (NTA) according to [26, 27] and was 0.5 x 10¹² particles/cm³. CaMV virions have icosahedrons shape and a characteristic size of 35 nm. Virions concentration in the sample under study was 50 µg/ml, the number of particles in the sample was analyzed using the “Nanoparticle tracking (NTA)” analysis and was 0.5 x 10¹² particles/cm³.

Albumin is a polypeptide chain forming a globule with a size of ~8 nm. HSA and BSA are homologous and differ only in some amino acid residues. They play an important role in blood plasma, definable by a wide variety of functions of these proteins. Both albumins are nowadays well studied, and it is well known that they may form associates with size of hundreds nanometers. Because of the widespread use of albumins, the task arises of measuring the parameters of monomers and formed aggregates. In our experiment, we used albumin water solutions with the pH value equal to 7. The mass concentration of serum albumin in the solution was 10%. Radius distribution of HSA aggregates was obtained using dynamic light scattering. Besides monomer with size 8 nm, aggregates with radius 50 nm were present in solution.

3. Experimental
SLFRS was excited by single pulses of ruby laser (λ = 694.3 nm, τ = 20 ns, Eₘₐₓ = 0.3 J, Δν = 0.015 cm⁻¹, divergence 3.5 x 10⁻⁴ rad). Laser light was focused at the center of the 1 cm quartz cell with sample by the lens with focal length 5 cm. SLFRS spectra have been registered with Fabri-Perot interferometers with different ranges of dispersion from 0.3 cm⁻¹ to 8.3 cm⁻¹ (9-250 GHz). Experimental setup for SLFRS investigations in TMV suspensions is shown in the figure 1.
Figures 1. Experimental setup. 1 – ruby laser, 2 – glass plates, 3 – system for laser pulse characteristics measurement, 4 – lens, 5 – mirror, 6 – quartz cell with a sample, 7 – Fabry-Perot interferometers, 8 – CCD matrices for registering SLFRS spectra.

Spectra of the light, scattered by the nanoparticles suspensions, have been registered simultaneously in forward and backward direction. SLFRS Fabry-Perot interferograms and frequency shifts in a number of investigated samples in Tris-HCl pH7.5 buffer and in water for different exciting energy are presented in the figure 2. At the small pumping light intensity only one rings system was registered which corresponded to the exciting light frequency (figure 2a). At the intensity exceeding some definite threshold additional lines appeared in the spectra both in forward and in backward direction, which corresponded to the SLFRS components.

a) Ruby laser  

b) TMV in Tris-HCl, \( \Delta \nu = 60 \text{ GHz} \)  
c) CaMV in water, \( \Delta \nu = 58 \text{ GHz} \)  
d) PVA in water, \( \Delta \nu = 9 \text{ GHz}; 18 \text{ GHz} \)  
e) PVX in Tris-HCl, \( \Delta \nu = 6 \text{ GHz} \)  
f) CaMV in Tris-HCl, \( \Delta \nu = 6 \text{ GHz} \)  
g) HSA in Tris-HCl, \( \Delta \nu = 2 \text{ GHz} \)  
h) BSA in Tris-HCl, \( \Delta \nu = 9 \text{ GHz} \)

Figure 2. Fabri-Perot interferograms and SLFRS frequency shifts corresponding to the scattered radiation in b) TMV in Tris-HCl, c) CaMV in water, d) PVA in water, e) PVX in Tris-HCl, f) CaMV in Tris-HCl, g) HSA in Tris-HCl, h) BSA in Tris-HCl.

For the used intensity levels of the exciting radiation, the scattering excitation threshold in BSA at room temperature was not reached. For BSA, the threshold was reached only at liquid nitrogen temperature (77 K).
It is necessary to note that at the same experimental conditions SLFRS in the cell filled only with Tris-HCl pH7.5 buffer was not registered.

SLFRS characteristics: conversion efficiency (\(\eta\)), threshold (P), Stokes components frequency shifts (\(\Delta \nu\)), size of the viruses (D – diameter, L – length), range of dispersion (\(\Delta \nu_0\)) are shown in the Table 1.

| Virus type | \(\eta\) % | P (GW/cm\(^2\)) | \(\Delta \nu\) (GHz) | DxL (nm) | \(\Delta \nu_0\) (GHz) |
|------------|------------|-----------------|---------------------|---------|---------------------|
| TMV        | 5          | 0.07            | 60                  | 18x300  | 75.0                |
| TMV        | 5          | 0.07            | 9; 13.5             | 18x300  | 21.42               |
| PVA        | 10         | 0.03            | 9; 18               | 15x730  | 37.5                |
| PVX        | 10         | 0.035           | 6; 12               | 13.5x715| 30.0                |
| CaMV       | 20         | 0.10            | 58                  | 35      | 75.0                |
| CaMV       | 20         | 0.10            | 6                   | 35      | 25.0                |
| HSA        | 55         | 0.10            | 6; 10; 15.6         | 50      | 9-249               |
| BSA        | 25         | 0.10            | 8.7; 16.5           | 46      | 9-249               |

Frequency values are just slightly different for HSA and BSA, which is connected with their similar structure. Based on the fact that these proteins have rather complex morphology, there is no physical model that could describe their oscillatory dynamics. But taking into account equation (1), and assuming the speed of sound in a protein as 1550 m/s, we came to the conclusion that the resulting frequencies are related with the aggregates with average size of hundreds nanometers, which are presented in solution. Each SLFRS spectral line corresponds to the different types of the acoustic eigenvibrations of aggregates constituting this system. For rather rigid viruses of the simple form it is possible to estimate vibration eigenfrequencies. For TMV, which is rigid cylinder, LFRS radial breathing mode frequency was calculated in [7]. According to these calculations it is 2.1 cm\(^{-1}\) (63 GHz), which is near to our experimental value 60 GHz.

4. Conclusions
Analysis of the low frequency spectrum of the inelastically scattered light in rigid biological structures like in any nanoparticles system can give very important information about their mechanical properties and can be used for their identification. Any spectral component of SLFRS corresponds to the vibrational mode of the biological nanoparticle. To estimate the theoretical value of the vibrational frequency it is necessary to use a proper theoretical model (for spherical virus one can use a liquid drop model or an elastic sphere model). In the simplest case of the spherical particle it is necessary to divide the sound speed by its diameter. But in the case of nonspherical shape or undefined elastic constants the exact calculation of vibrational frequencies becomes rather complicated.

The knowledge of this value is very important for realization of the resonant impact on nanoobject, which can even lead to its destruction. This very important application can be realized in the case of exact coincidence frequency of external influence with the particle eigenfrequency. Ultrasound or electromagnetic radiation of the proper frequency can be used as the external source of excitation. These methods are not very effective because of damping in the surrounding material. Another way for effective impact realization is biharmonic pumping – electromagnetic radiation containing two spectral components separated by the frequency corresponding to the eigenfrequency of the biological nanoparticle. SLFRS can be very effective source for the biharmonic pumping creation. There are two waves similar by the wavelength and intensity in the scattered light and the frequency shift coincide exactly with the eigenfrequency of the virus. It is well known that albumin’s conformation as well as viral capsids is highly dependent on various external conditions, as pH, temperature, and others. So, changing these external parameters, we can vary the frequency shift between laser excitation and Stokes component. If one sends this radiation to the object under study, in the case of exact coincidence of the frequency shift with the object eigenfrequency, the resonant impact on the system can be realized.
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