Stimulated low-frequency Raman scattering in plant virus suspensions

E K Donchenko¹, O V Karpova¹, A D Kudryavtseva², S M Pershin³, V I Savichev²,⁴, M A Strokov², N V Tcherniega²,⁴ and K I Zemskov²

¹M.V. Lomonosov Moscow State University, Vorobevy Gory, 1, Moscow, 119991, Russia
²P.N. Lebedev Physical Institute of the RAS, Leninskii pr, 53, Moscow, 119991, Russia
³A.M. Prokhorov General Physics Institute of the RAS, Vavilova, 38, Moscow, 19991, Russia
⁴Bauman Moscow State Technical University, ul. 2 Baumanskaya, 5, Moscow, 105005, Russia

Abstract. The study deals with laser pulse interaction with plant viruses: we investigated tobacco mosaic virus (TMV) and two types of potato viruses (PVX and PVA) in Tris-HCl pH7.5 buffer and in water. We used 20 ns ruby laser pulses for excitation. We employed Fabry–Pérot interferometers to record spectra of the light passing through the sample and reflected from it. For TMV and PVX in Tris-HCl pH7.5 buffer, same as for PVA in water, we observed additional spectral lines corresponding to the stimulated low-frequency Raman scattering (SLFRS). We believe we were the first to measure SLFRS frequency shifts, conversion efficiency and threshold. High conversion efficiency of the scattered light is evidence of laser pulses efficiently exciting gigahertz vibrations in viruses. SLFRS can be used to identify and affect biological nanoparticles.

1. Introduction

Biological nanoparticles are now the subject of intense experimental and theoretical investigations [1]. It is important to develop new methods of identifying and affecting biological nanoparticles. One of the most effective ways to affect a virus system (same as any other biological system) is to stimulate its vibrational modes by resonant excitation. Vibration eigenfrequencies in viruses lie in the gigahertz range, which means that it is possible to excite resonance using microwave electromagnetic radiation, hyperson sound or pulsed optical electromagnetic radiation. The first two approaches are difficult to implement in real-world biological systems due to strong absorption of microwave radiation by the water contained in all biosystems and a short hyperson sound propagation distance, which is on the order of several hundred nanometres in the systems under consideration. Using pulsed electromagnetic radiation in the optical range ensures the maximum possible penetration depth into the system under
study and, consequently, the highest effect. As shown in [2, 3], short laser pulses can provide a powerful impact on a virus system by means of the impulsive stimulated Raman scattering mechanism [4, 5]. Another approach is to use a stimulated analogue of low frequency Raman scattering (LFRS) [6, 7], that is, stimulated low frequency Raman scattering (SLFRS) [8]. LFRS is the inelastic scattering of light by localized acoustic vibrations of nanoparticles. This type of scattering manifests itself by additional spectral components appearing in the spectrum of scattered light, these components displaying frequency shifts corresponding to nanoparticle vibration eigenfrequencies in the gigahertz or terahertz ranges. Each nanoparticle has a set of natural acoustic frequencies which are determined by its morphology. It is possible to calculate nanoparticle eigenfrequencies with the help of Lamb's theory, which deals with free vibrations of a spherically shaped homogeneous elastic body under stress-free boundary conditions [9, 10].

LFRS was first experimentally observed in nucleated cordierite glass [6], to be recorded later in many nanoparticle systems. Currently LFRS is commonly seen in systems containing metallic, insulator, or semiconductor nanoparticles. Analysing the LFRS spectrum provides a unique possibility to obtain information about morphological properties of a nanoparticle system.

Under certain conditions it is possible to excite SLFRS in systems of nanosized or submicron particles. SLFRS was observed in different materials: highly ordered samples such as opal matrices and nanocomposites based on them, nanostructured thin films, and disordered materials such as suspensions of nanoparticles (metal, semiconductor and dielectric) [11-13]. Frequency shifts of the SLFRS components are in the terahertz or gigahertz range and are determined by the shape, size and elastic properties of nanoparticles. Since SLFRS frequency shifts are small, to excite SLFRS efficiently, it is necessary to use a laser source with narrow spectral linewidth. Another important factor affecting the efficiency of SLFRS excitation is the monodispersity of the systems under study.

Cylindrical or spheroidal viruses vibrating at their eigenfrequencies are a good example of a highly monodisperse (uniform) nanoparticle system. SLFRS aids in studying such systems, having several substantial advantages as compared to spontaneous scattering. One of the difficulties in exciting spontaneous scattering is damping of particle vibrations due to the energy radiating into the environment. Stimulated scattering, excited by short and powerful laser pulses, solves this problem. The possibility to control the frequency shift in the giga- and terahertz range along with high conversion efficiency not only make SLFRS an efficient biharmonic excitation source for nanosized and submicron system spectroscopy, but also allow this phenomenon to induce a powerful resonance in such systems. The purpose of this article was to implement SLFRS excitation in certain types of plant viruses and to determine its spectral and energetic characteristics.

2. Samples
We recorded SLFRS in plant viruses for the first time. In [14] we presented our first experimental results of recording SLFRS in tobacco mosaic virus (TMV) in Tris-HCl pH7.5 buffer (C₃H₇ClNO₃). In this work we present results of investigating SLFRS in TMV and two types of potato viruses: PVA and PVX. Figure 1 shows a typical transmission electron microscopy image of a TMV sample.

![Figure 1](image_url)

**Figure 1.** TMV sample in Tris-HCl buffer; picture obtained with a transmission electron microscope.
Tobacco mosaic virus (TMV) is 18 nm in diameter and 300 nm in modal length; it consists of 2130 identical 17.5 kDa protein molecules assembled into a rigid rod-like helical structure. The viral RNA is intercalated between the protein turns [15]. 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, featuring helical symmetry with a pitch of 3.4 nm. Virions consist of 5% nucleic acid and 95% protein. Their genome RNA is single-stranded and contains 9700 nucleotides. The c oat protein (CP) has a molecular weight of 29.8 kDa. PVX virions are also filamentous and flexuous, 500 nm long and 13 nm in diameter. Their symmetry is helical, with a pitch of 3.6 nm. Virions consist of 6% nucleic acid and 94% protein (6400 nucleotides, and CP with a molecular weight of 25 kDa). We isolated the TMV U1 strain from systemically infected Nicotiana tabacum L. cv. Samsun plants [16]. The virion concentration in the test sample was 50 µg/ml; we used Nanoparticle Tracking Analysis (NTA) according to [17, 18] to determine the number of particles in the sample, which turned out to be 0.5 x 10^12 particles/cm^3.

3. Experimental
SLFRS was excited by single pulses of ruby laser (λ = 694.3 nm, τ = 20 ns, E_{max} = 0.3 J, Δν = 0.015 cm^{-1}, divergence 3.5x10^{-4} rad). We used a lens with a 5 cm focal length to focus the laser light at the centre of a 1 cm quartz cuvette containing our sample. We recorded SLFRS spectra by means of Fabry–Pérot interferometers with different baselines (and, correspondingly, different dispersion ranges). We used the same quartz cuvette filled only with Tris-HCl pH7.5 buffer or water as a reference sample for comparison. Figure 2 shows the experimental setup for SLFRS investigation in virus suspensions.

We recorded the spectra of the light scattered by the virus suspension simultaneously in forward and backward directions. Figure 3 presents SLFRS interferograms in TMV in Tris-HCl pH7.5 buffer for various excitation energy values. A small excitation light intensity caused only a single ring system to be recorded, the system corresponding to the excitation light frequency (Fig. 3a). When the intensity exceeded a certain threshold, additional lines appeared in the spectra in both forward and backward directions. These lines corresponded to the SLFRS components (Fig.3b).
Figure 3. Fabri-Perot interferograms corresponding to forward-scattered radiation in TMV suspension in Tris-HCl pH7.5 buffer for laser intensities of a) 0.02 GW/cm$^2$, b) 0.08 GW/cm$^2$.

Figure 4 presents the SLFRS spectrum corresponding to the interferogram 3b.

Figure 4. Spectrum of the SLFRS in TMV suspension in Tris-HCl pH7.5 buffer for a laser intensity of 0.08 GW/cm$^2$.

It is necessary to note that the same experimental conditions did not lead to recording SLFRS in the cell filled only with Tris-HCl pH7.5 buffer.

Figure 5 presents the interferograms and spectra of the SLFRS in potato viruses.

Figure 5. Interferograms and spectra of the SLFRS in potato virus suspension: a) PVX in buffer, b) PVA in water.

The SLFRS line width and divergence are close to the corresponding values of the excitation light. Table 1 lists the following SLFRS characteristics: conversion efficiency ($\eta$), threshold ($P$), Stokes shifts ($\Delta \nu$), virus size ($D$ – diameter, $L$ – length), range of dispersion ($\Delta \nu_0$).

| Virus type | $\eta$% | $P$ (GW/cm$^2$) | $\Delta \nu$ (GHz) | $D\times L$ (nm) | $\Delta \nu_0$ (cm$^{-1}$) |
|------------|--------|----------------|----------------------|-------------------|-----------------------|
| TMV        | 5      | 0.07           | 60                   | 18x300            | 2.50                  |
| PVA        | 10     | 0.03           | 9, 18                | 15x730            | 1.25                  |
| PVX        | 10     | 0.035          | 6, 12                | 13.5x715          | 1.00                  |

For rather rigid viruses of a simple shape it is possible to estimate vibration eigenfrequencies. For TMV, which is a rigid cylinder, the LFRS radial breathing mode frequency was calculated in [7]. According to these calculations, it equals 2.1 cm$^{-1}$ (63 GHz), which is close to our experimental value of 60 GHz. The main difficulty concerning calculating eigenfrequencies of biological nanoobjects is
that their complex composition prevents knowing the exact values of their acoustic characteristics (for instance, speed of sound). We assumed the speed of sound to match that of the main constituent protein. As for potato viruses, they are much more flexuous than tobacco mosaic virus; therefore it is difficult to calculate their vibrations.

4. Conclusions
Analysing the low frequency spectrum of inelastically scattered light in rigid biological structures, same as in any nanoparticle system, can provide very important information about their mechanical properties and can be used for their identification. Any spectral component of the SRWS corresponds to a vibrational mode of the virus. To estimate the theoretical value of a vibrational frequency, it is necessary to use a proper theoretical model (for a spherical virus one can use a liquid drop model or an elastic sphere model). In the simplest case of a spherical virus it is necessary to divide the sonic speed by the diameter of the virus. But in the case of a non-spherical shape and (or) poorly defined elastic constants, calculating the vibrational frequency exactly becomes rather complicated, and it becomes easier to obtain vibrational frequency information from experimental data. It is very important to know this value so as to be able to affect the virus through resonance, which can even lead to virus destruction. Matching the external source frequency to the virus eigenfrequency exactly makes this very important application possible. The external source may be ultrasound or electromagnetic radiation of a suitable frequency. These methods are not very effective because of environmental damping. Another way to implement this effect efficiently is biharmonic excitation, that is, electromagnetic radiation containing two spectral components separated by the frequency corresponding to the eigenfrequency of the virus vibration. SLFRS can be a very effective biharmonic excitation source. The scattered light contains two waves with close wavelengths and intensities, and the frequency shift matches the virus vibration eigenfrequency exactly.

References
[1] Tsen K, Dykeman E, Sankey O et al 2006 Virology Journal. 3 79
[2] Tsen K-T, Tsen S W D, Chang C L, Hung C F, Wu T C and Kiang J G 2007 Inactivation of viruses by coherent excitations with a low power visible femtosecond laser Virol. J. 4 50
[3] Tsen K-T, Tsen S-W D, Chang C-L, Hung C-F, Wu T-C and Kiang J G 2007 Inactivation of viruses with a very low power visible femtosecond laser J. Phys.: Condens. Matter 19 322102
[4] Yan Y X, Gambel E B Jr and Nelson K A 1985 Impulsive stimulated scattering: general importance in femtosecond laser pulse interactions with matter, and spectroscopic applications J. Chem. Phys. 83 5391–9
[5] Tsen K-T, Tsen S-W D, Sankey O F and Kiang J G 2007 Selective inactivation of microorganisms with near-infrared femtosecond laser J. Phys.: Condens. Matter 19 472201
[6] Duval E, Bukenzer A and Champagnon B 1986 Phys. Rev. Lett. 56, 2052
[7] Balandin A and Fonoberov V 2005 Journal of Biomedical Nanotechnology. 1 90
[8] Tcherniega N and Kudryavtseva V 2009 Nonlinear-optical properties of photonic crystals. Journal of Surface Investigation: X-ray, Synchrotron and Neutron Techniques. 3 513
[9] Lamb H 1882 Proc. London Math. Soc. 13 189
[10] Saviot L, Murray D, Mermet E and Duval E 2004 Phys. Rev. E 69 023901
[11] Tcherniega N, Samoylovich M, Kudryavtseva A, Belyanin A, Pashchenko P and Dzbanovski N 2010 Optics Letters 35 300
[12] Kudryavtseva A, Tcherniega, Samoylovich M and Shevchuk A 2012 International Journal of Thermophysics 33 2194
[13] Tcherniega N, Zemskov K, Savranskii V, Kudryavtseva A, Olenin A, and Lisichkin G 2013 Optics Letters 38 824
[14] Karpova O V, Kudryavtseva A D, Lednev V N, Oshurko V B, Pershin S M, Petrova E K, Tcherniega N V and Zemskov K I 2016 Stimulated low-frequency Raman scattering in
tobacco mosaic virus suspension Laser Phys. Lett. 13, 085701

[15] Klug A 1999 The tobacco mosaic virus particle: structure and assembly Philos Trans R Soc Lond B Biol Sci. 354 531–535

[16] Karpova O, Nikitin N, Chirkov S, Trifonova E, Sheveleva A, Lazareva E and Atabekov J 2012 Immunogenic compositions assembled from tobacco mosaic virus-generated spherical particle platforms and foreign antigens Journal of General Virology 93 400–407

[17] Nikitin N, Trifonova E, Karpova O and Atabekov J 2013 Examination of Biologically Active Nanocomplexes by Nanoparticle Tracking Analysis Microscopy and Microanalysis 19 808-813.

[18] Petrova E, Nikitin N, Trifonova E, Protopopova A, Karpova O and Atabekov J 2015 The 5'-proximal region of Potato virus X RNA involves the potential cap-dependent “conformational element” for encapsidation Biochimie 115 116-119