

## Low-frequency stimulated light scattering in virus suspensions under picosecond excitation

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When excited by a powerful picosecond pulse of the second harmonic of a YAP:Nd laser, inelastic scattering lines with frequency detunings in the range of  $\sim 10\text{--}130$  GHz relative to the laser line were detected in suspensions of morphologically similar AltMV and PVX viruses. This scattering has a threshold nature, and the sets of its spectral lines are different for different virus samples.

**Keywords:** picosecond pulse, light scattering, excitation threshold, virus suspension.

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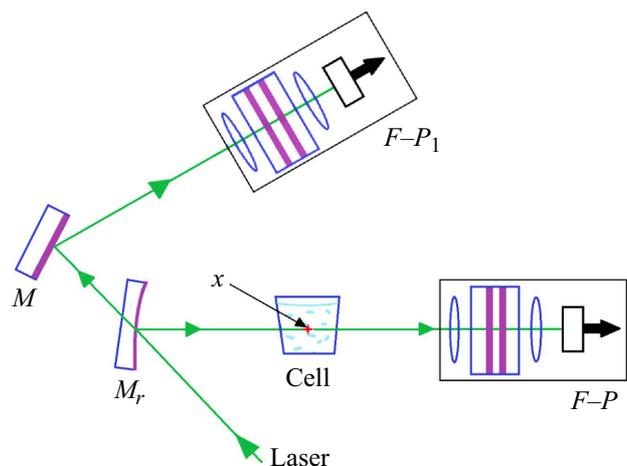
### Introduction

In recent studies devoted to the investigation of nonlinear optical properties of nanoparticles, a powerful nanosecond laser pulse with a fairly narrow spectral line of emission was used as a source of excitation of the medium [1–3]. As a result, the researchers have succeeded to register a number of features of the light scattering in heterogeneous media: both in nanoparticle suspensions themselves and in virus suspensions, which may be of interest for virology [4,5]. In particular, it was found that in suspensions of morphologically similar viruses, the spectra of stimulated low-frequency scattering of laser radiation, located within the frequency detuning range of  $\sim 3\text{--}60$  GHz, differ significantly, which makes it possible to use this fact to identify viral nanoparticles in liquid suspensions, i.e. in an environment close to the native one. One of the causes for the differences in the spectra in this case may be the presence of regulatory functional groups as well as structural functional groups of the virion (for example, capsomeres [6]), differing in their mechanical properties in the proposed model of a heterogeneous elastic rod [4,5].

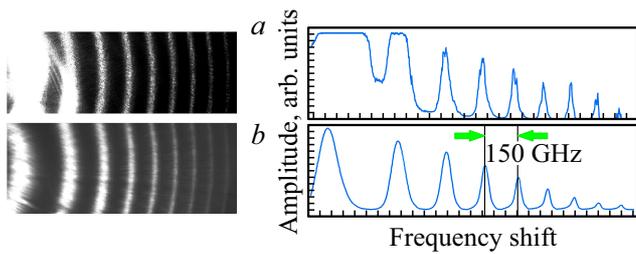
### Experiment

Previously studied suspensions of viruses were used as experimental samples: Alternanthera mosaic virus (AltMV, virion length is  $\sim 570$  nm, diameter is  $\sim 15$  nm) in a tris-aminomethane buffer solution ( $(\text{HOCH}_2)_3\text{CNH}_2 \cdot \text{HCl}$ , abbreviated notation: Tris-HCl) and a virus with a helical structure of *Potexvirus* genus (*Alphaflexiviridae* family) – Potato Virus X (PVX, virion length is  $\sim 515$  nm, diameter is  $\sim 13$  nm) also in Tris-HCl. These viruses belong to the *Potexvirus* genus and the *Alphaflexiviridae* family and are flexible thread-like particles with a helical structure [4,7,8]. Suspensions of viruses with concentrations

of  $C_a = 8.8 \cdot 10^{13} \text{ cm}^{-3}$  (AltMV) and  $C_x = 6.6 \cdot 10^{13} \text{ cm}^{-3}$  (PVX) were poured into identical quartz Cells with a working length of 40 mm. The cells were placed one by one on the setup (Fig. 1). A high-power laser with a pulse duration of 50 ps was used as a radiation source. Single pulses of the second harmonic radiation of a picosecond YAP:Nd laser mode-locking operated at a wavelength of  $\lambda = 540$  nm, with a linewidth of  $\delta\nu \sim 1.1 \text{ cm}^{-1}$ , a pulse duration of  $t_p \sim 50$  ps, a pulse energy of  $E_p$  to 3.0 mJ were focused by a spherical mirror  $M_r$  with a focal length of  $f \sim 12.5$  cm in the middle of the Cell. The intensity of laser radiation in the caustic region in a pure liquid was as high as  $\sim 3 \cdot 10^{10} \text{ W/cm}^2$ , and no breakdown was observed. The forward scattering signal excited in the cell and the



**Figure 1.** Schematic diagram of the measurements. Laser — direction of picosecond laser radiation; Cell — cell with the test substance;  $F-P$ ,  $F-P_1$  — Fabry-Perot interferometers with a registration system;  $M$  — totally reflecting plane mirror;  $M_r$  — spherical mirror ( $f \sim 12.5$  cm, reflectance is  $\sim 80\%$ )  $x$  — location of the focused laser beam waist in the middle of the cell.



**Figure 2.** Interference patterns of the „forward“ scattered radiation obtained from the  $F-P$  interferometer (with buffer solution in the Cell —  $a$ ) and the  $F-P_1$  interferometer (laser emission spectrum  $b$ ). The dispersion regions of the interferometers are the same and equal to 150 GHz. Hereinafter: the bright spot in the interference pattern in Fig. 2,  $a$ , 3 and 4 is caused by illumination from the SRS component excited in the buffer liquid.

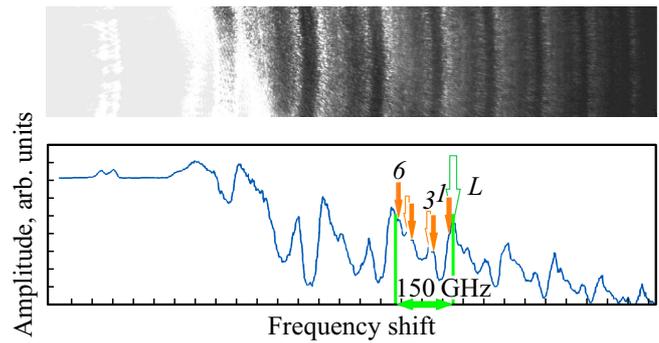
laser radiation were fed to a Fabry-Perot interferometer ( $F-P$ ). Part of the radiation (about 20%) was diverted by the mirror  $M$  to the reference interferometer  $F-P_1$ , the dispersion region of both interferometers was  $5\text{ cm}^{-1}$ , or 150 GHz. Downstream of the interferometers, the optical signals were directed to CMOS-cameras and processed on a computer in the LABVIEW software environment. The laser pulse energy was monitored using an *Ophyr Vega ROAS* device (not shown in the diagram in Fig. 1), which received 4% of the laser radiation reflected from the input window of the Cell. The laser pulse energy instability was 1.8–3.0 mJ. The measurements were carried out at room temperature.

### Results and discussion

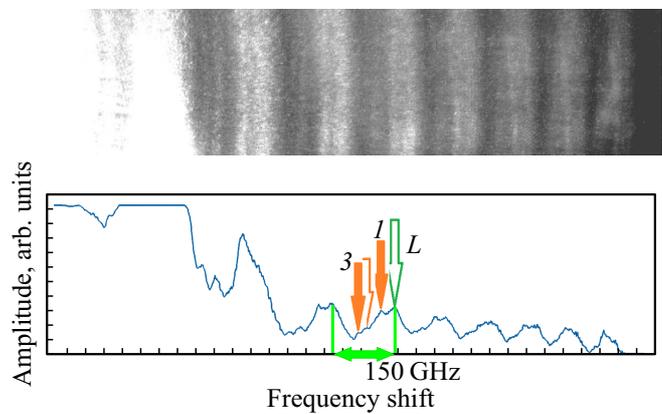
Significant differences in the spectra of low-frequency stimulated scattering of nanoparticles of Alternanthera mosaic viruses and Potato Virus X in a liquid suspension were discovered when excited by high-power picosecond laser pulses in a frequency detuning range of  $\sim 10\text{--}130\text{ GHz}$ , and the registered natural frequencies of virions differ from the previously recorded frequencies of the same virions when the spectra are excited by nanosecond laser pulses [4,5]. The difference in system responses to the excitation of inelastic light scattering in virus suspensions for laser pulse durations of  $\sim 10\text{ ns}$  and  $\sim 50\text{ ps}$  can be explained by the excitation of natural vibrational modes of the virion as a whole or its individual parts.

The spectrum of scattered radiation of the buffer solution (Fig. 2,  $a$  and  $b$ ) had no pronounced features, however, in the spectra of inelastic scattering of virus suspensions additional lines appeared upon achievement of an energy of  $\sim 1.6\text{--}2.4\text{ mJ}$  (Fig. 3, 4). The differences in the scattering spectra of different samples are shown in the table.

In our opinion, the spectra (Fig. 3, 4) are indicative of a complex „mechanical“ structure of virions associated with a heterogeneous distribution of density both along the nucleic acid (where the difference in nucleotide masses can be up



**Figure 3.** Interference pattern of the „forward“ scattered radiation obtained using the  $F-P$  interferometer (with the AltMV virus suspension in the cell). The dispersion region of the interferometer is 150 GHz. Hereinafter:  $L$  is laser emission line,  $1-6$  is numbering of Stokes lines (see the table for frequency detuning values).



**Figure 4.** Interference pattern of the „forward“ scattered radiation obtained using the  $F-P$  interferometer (with the PVX virus suspension in the cell). The dispersion region of the interferometer is 150 GHz. Here  $1-3$  is numbering of Stokes lines.

Values of scattering frequency detuning in the studied virus suspensions (laser exciting pulse duration is  $\sim 50\text{ ps}$ , laser line width is  $\delta\nu \sim 1.1\text{ cm}^{-1}$ )

Numbering of spectral lines Fig. 3, 4	Scattering frequency shifts, GHz	
	AltMV, GHz	PVX, GHz
1	11.0	33.7
2	54.7	70.1
3	60.8	87.5
4	96.1	—
5	117.7	—
6	131.7	—

to 30%) and over the volume of the virion itself, associated with the presence of regulatory and structural functional groups unique to each virus. Based on a comparison of

the above-listed results and the results of [4], an assumption can be made that there are two different mechanisms for the excitation of inelastic scattering in the studied virus suspensions. In [4,5], the spectra were due to stimulated low-frequency scattering because of the phased movement of virions in the buffer liquid, whereas in this case, with picosecond excitation, the obtained frequencies belong to phased vibrations of individual functional groups of the virion.

The spectra of low-frequency stimulated scattering, excited by laser pulses of nanosecond and/or picosecond duration (table [4,5]), can be used in developing the corresponding database, for example, to compile a „frequency portrait“ of virions for the purpose of their subsequent identification.

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### Conflict of interest

The authors declare that they have no conflict of interest.

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