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# Optical Express Methods of Monitoring of Pathogens in Drinking Water and Water-Based Solutions

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Additional information is available at the end of the chapter

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## Abstract

Hygienic standards to the quality of potable water require continuous monitoring of the absence of pathogenic microorganisms directly in water flow. Despite a great number of laboratory devices for checking the quality of potable water, there are no express analyzers for monitoring of pathogenic organisms, which could be embedded directly into the automatic checkout systems. The reasons of it are low concentration of pollutions and the presence of additional effects, which impede automatic data processing. The new method, the express analysis of pathogens in water, was developed. It shall be mentioned that the proposed method of express diagnostics allows detection of infectious agents in the water in minutes based on nonlinear effects. This research has, hopefully, laid the foundation for development of a prototype for determination of the content of the genetically modified soy in meat products. The inventive methods can be recommended for DNA diagnostics in medicine, veterinary sciences and insanitation. The main advantage of this method is that there is no need for DNA isolation. It is sufficient to create a suspension of the product by centrifugation.

**Keywords:** express diagnostics, viruses, pathogens, laser monitoring, colloidal solution

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## 1. Introduction

Hygienic standards to the quality of potable water require continuous monitoring of the absence of pathogenic microorganisms directly in water flow. Despite a great number of laboratory devices for checking the quality of potable water, there are no express analyzers for monitoring of pathogenic organisms, which could be embedded directly into the automatic checkout systems. The reasons of it are low concentration of pollutions and the presence of additional effects, which impede automatic data processing. At present, monitoring

of microbiological parameters of the composition of drinking water is of great importance. Water supply systems are the key to ensuring the livelihoods of cities, in connection with which the issue of realizing the control of drinking water parameters directly in the pipeline flow in real time is becoming increasingly acute. To solve this problem, it is necessary to implement continuous monitoring, including super-toxicants in drinking water. At the same time, much attention is paid to the development of fundamentally new and highly effective detection and control systems for the spread of pathogenic microorganisms. The problem is complicated by the fact that the maximum permissible concentration of pathogenic microorganisms can be within even a few molecules.

To date, the control is performed by the laboratory methods. That process takes from several hours to several days, depending on the method. In addition, these tests must be conducted in laboratory conditions by highly qualified personnel. That necessitates the development of a device that can be integrated into an automated line for monitoring drinking water. For example, the PCR method is an excellent diagnostic method, but it requires preliminary DNA isolation. The method of luminescent analysis using fluorescent markers requires very specialized sample preparation.

Thus, the problem of real-time assessing quality of drinking water to guarantee the absence of hazardous biological and chemical substances, even in small and ultrasmall concentrations, is becoming increasingly important. The content of pathogenic microorganisms in water can vary from  $10^{-8}$  up to  $10^5$  mg/m<sup>3</sup>. Although there are a great number of laboratory devices for checking the quality of potable water, there are no express analyzers for monitoring of pathogenic microorganisms that could be embedded directly into the automatic checkout systems. The optical phenomena of stimulated scattering in water solutions, which contain DNA, are investigated insufficiently.

Yet such research would be of great interest for the development of automatic control systems of potable water. Laser methods are widely applied to the analysis of structures of multi-component liquids and are thus useful as a tool for the determination of low concentrations. In this chapter we describe new methods of express monitoring of viruses and pathogenic organisms in water and aqueous solutions based on nonlinear effects. We also describe a modified PCR method of diagnosis of genetically modified foods. The proposed methods can be recommended for the diagnosis of DNA, in the field, in medicine and veterinary sciences, in sanitary epidemiological studies to detect agents of dangerous infections in the event of potential bioterrorist attacks

### **1.1. Aim and objectives of our studies**

The purpose and objectives of our research are to determine in spectra what kind the optical parameters allows us to diagnostic pathogens with a probability of not less than 95%. To do this, no less than 1500 spectra were obtained for each of the pathogens studied. A large amount of experimental material allowed to create software that automatically determines the type of pathogen and signals its presence.

## 2. Methods and findings

As a preliminary to a detailed description, a comparative analysis of the methods is given in **Table 1**. This comparative analysis is shown to highlight the benefits of our method, which will allow automated monitoring of pathogens. It should be noted that the certification of the method was carried out on the laboratory basis of the State Research Center for Applied Microbiology and Biotechnology (Obolensk, Moscow Region) and at the All-Russian Research Institute of Veterinary Sanitation, Hygiene and Ecology of the Russian Academy of Agricultural Sciences. Approbation of the device was carried out by the double-blind method. The double-blind method is an experimental procedure in which neither the representatives of the testing organization nor the researchers know what is in the tested samples. The double-blind method is used to avoid the appearance of the effects of the experimenter's prejudice relative to the studied characteristic (the Rosenthal effect) and to eliminate the possibility of distorting the results of the experiment of the knowledge effect of what is required of the subject.

In **Table 2**, results of comparison of tests by a double-blind method against a standard test method are shown. It should be noted also that we studied transmission IR spectra of a number of pathogen (salmonella, viruses of herpes genitaler, hepatitis A and C, grippe A and B) solutions and luminescence of nanomarkers. In our experiments, the laser radiation passed through a quartz cell with water solutions of the pathogens, nanosilver,

Method	Need for sample preparation	Special laboratory conditions	Use of reagents	Duration of the measurement	Duration of result processing
1. Agglutination method	Yes	Yes	Yes	1 h	1 day
2. Immune deposition	Yes	Yes	Yes	20–60 min	1 day
3. Immunobloom	Yes	Yes	Yes	2–4 h	1 day
4. Method of a fixation of the complement	Yes	Yes	Yes	1 h	1 day
5. Phagocytic activity test	Yes	Yes	Yes	24 h	2 days
6. Immune-enzyme analysis	Yes	Yes	Yes	2–4 h	1 day
7. Neutralization method	Yes	Yes	Yes	4–7 days	5–8 days
8. Method of the luminescence	No	Yes	No	15 min	30 min
9. The method of stimulated luminescence and SBS	No	No	No	2 min	1 min

**Table 1.** Comparison of methods by duration and sophistication.

Pathogen/the method and result of research	Test/ positive	Device/ positive	Test/ negative	Device /negative duration of the measurement	Test/false negative	Device/false negative	Test/false positive	Device/false positives
<i>E. coli</i> 12571 agglutination method	20 yes	20 yes	20 yes	1 h	0	0	0	0
<i>Enterococcus faecalis</i>	20 yes	20 yes	20 yes	20–60 min	0	0	0	01
<i>Shigella flex</i> 3 immunobloom	10 yes	10 yes	10 yes	2–4 h	0	0	0	01
Method of a fixation of the complement	10 yes	10 yes	10 yes	1 h	0	0	0	01
Kolifag MS-2 phagocytic activity test	5yes	5yes	10yes	24 h	0	0	0	01
<i>Staphylococcus aureus</i> by immune-enzyme analysis	5 yes	5 yes	5 yes	2–4 h	0	0	0	02

**Table 2.** Comparison of results by methods used by duration and false positive.

or nanomarkers. We found that exciting radiations with wavelengths 1017 and 810 nm induce the stimulated Brillouin scattering (SBS) in spectra of the water containing DNA. We believe that the power threshold for the onset of this effect is achieved by adding a forced radiation luminescence and laser radiation. A comparative analysis of the methods is given in **Table 1**. Comparative analysis shows that our method (9) is promising for automated monitoring of pathogens.

**2.1. Basic concepts of methods for monitoring pathogens in water**

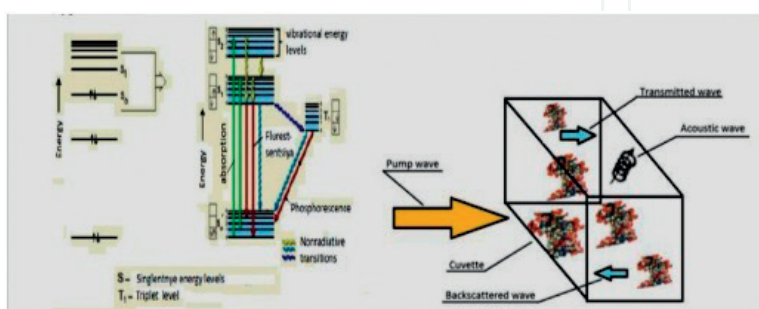
*2.1.1. Overview of bioluminescence*

Bioluminescence is a form of chemiluminescence where light energy is released by a chemical reaction. The reaction can occur either inside or outside the cell. Chemiluminescence occurs like many chemical reactions, for example, by recombination of free radicals or the oxidation reaction. In this case, as in the bioluminescence reaction, released energy is not dissipated as heat, as is the case during most of the exothermic chemical reactions, and consumed in the formation of one of the reaction products in an excited electronic state. Emitting light during the chemiluminescent reaction is necessary to satisfy at least two conditions: Firstly, the energy released during the reaction must exceed about 41–71.5 kcal/mol, and, secondly, the energy difference between the ground and excited states that the reaction product should be less than the enthalpy of the chemical reaction. Under these conditions, it can be formed with a sufficiently high transition of oxidized luciferin in the excited state and the subsequent transition of it to the ground state with the emission of photons

The ratio of the number of emitted photons to the total number of elementary events is called the quantum yield of the reaction. The quantum yields of bioluminescence, unlike most chemiluminescent reactions, are very high, reaching values of 0.1–1. Chemiluminescence is rarely carried out for the reaction processes in aqueous solutions at neutral pH at such a quantum yield. Bioluminescent process power radiation depends on the difference in energy between the oxidized forms of the ground and excited states of luciferin. This power is related to the radiation frequency by the relation  $\Delta E = h\nu$ ; On half the width of the radiation band, which has a width usually about 50 nm (**Figure 1a**) [1].

### 2.1.2. Overview on stimulated Brillouin scattering

The stimulated Brillouin scattering (SBS) is a nonlinear process that can occur in liquid media at large intensity. The mechanism of the appearance of Brillouin radiation arises from the photon-phonon interaction. The basic mechanism of SBS phenomenon is illustrated in **Figure 1b**. In scattering effects, energy gets transferred from one light wave to another wave at a longer wavelength or lower energy. The lost energy is absorbed by the molecular vibrations, or phonons, in the medium. Stimulated scattering is affected by the threshold level. The SRS is a nonlinear parametric interaction between light and molecular vibrations. Optical phonon participates in SRS, but acoustic photon participates in SBS. Due to SRS power transferred from shorter wavelength channels to the longer wavelength channels. SRS occurs in both directions, either forward or backward direction. The input signal from the laser source is the pump signal and generates a new wave due to scattering in the medium. This wave is called the Stokes wave. SBS occurs only in the backward direction; for example, when input power exceeds threshold power, Stokes power shifted to the backward direction. Pump wave losses power, while Stokes wave gains power. The pump wave creates acoustic wave in transmission medium through a process called electrostriction. The interaction between pump wave and acoustic wave creates the generation of back propagating optical wave which is called Stokes wave. When acoustic waves travel through the transparent liquid media, they induce spatially periodic local compressions and expansions which in turn cause local increases and decreases in the refractive index. This phenomenon is known as photoelastic effect altered to a degree such that a significant portion of the optical signal is back-scattered. So, we can say that the acoustic wave alters the optical properties of the fiber, including the refractive index. This fluctuation of refractive index scatters the incident wave and creates Stokes wave which



**Figure 1.** Comparison of the phenomenon of luminescence (a) and stimulated Brillouin scattering (b).

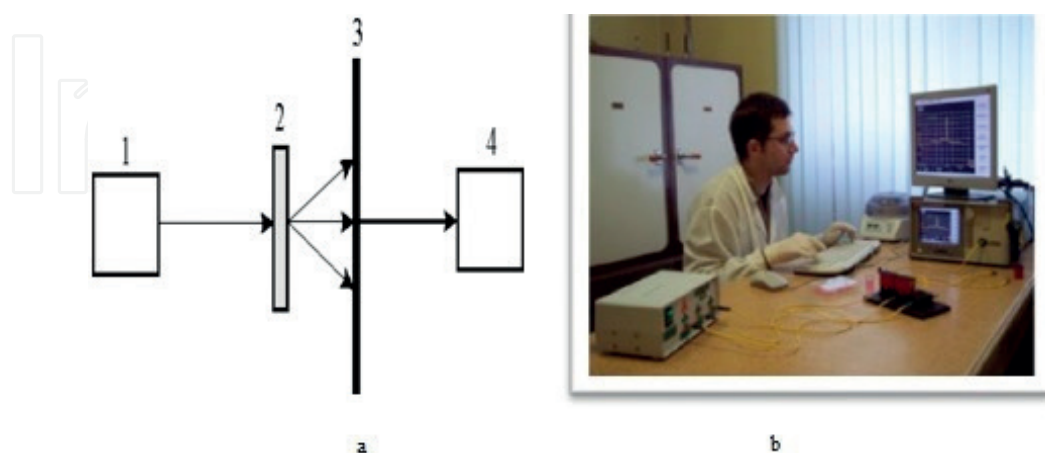


propagates in the opposite direction. The magnitude of the photoelastic effect increases with increasing input optical power. If the input power reaches the threshold level of SBS and the refractive index is variable, then an acoustic wave appears. Modern research shows that water has a quasi-crystalline structure. Similar effects were observed in the liquid and solid [2].

Our investigations showed the occurrence of luminescence mainly in solutions containing DNA or fluorescent markers. Brillouin scattering was observed in solutions containing low concentrations of particles, including nanoscale. Based on these investigations, we developed the method of monitoring nanoparticle sizes, composition, chemical reactions, and transition processes occurring in solutions. At present we are creating series of devices for monitoring MPC (maximum permissible concentration) in tap water by using the proposed method.

## 2.2. Description of the monitoring of pathogens in water by resonance laser spectroscopy techniques

We used a specially designed testing bench and the scheme of experimental setup shown in **Figure 1**. We analyzed about 700 spectra for each of the pathogens. Pathogens that we studied are shown in **Table 1**. We excited viruses and bacteria suspensions with a laser beam having wavelengths of 810 and 1017 nm and then evaluated the forward-scattering spectra. The testing bench consists of the following components: (1) the block of the three of semiconductor laser sources with wavelengths  $\lambda_1 = 1017$  nm,  $\lambda_2 = 810$  nm, and  $\lambda_3 = 670$  nm; (2) the quartz cuvette; (3) spectrum analyzer “Agilent” with the spectral resolution 0.05 nm, equipped with the microcomputer for processing spectra; (4) and (5) waveguides for the radiation input-output ; and (6) the computer equipped with original software for processing spectra (**Figure 1a, b**). The laser radiation passed through a waveguide into the cuvette with the sample. Sometimes, we used an additional laser with a wavelength  $\lambda_4 = 532$  nm. That source enhanced the intensity of luminescence. The radiation, which passed through the cuvette and the output waveguide, was analyzed by a spectrum analyzer (**Figure 2**).



**Figure 2.** (a) The experimental setup: (1) the block of the three of the semiconductor laser sources with exciting wavelengths  $\lambda_1 = 1017$  nm,  $\lambda_2 = 810$  nm, and  $\lambda_3 = 670$  nm; (2) the quartz cuvette; (3) spectrum analyzer “Agilent”; and (4) computer equipped with original software for processing spectra.; (b) photo.

This radiation was registered in short-range IR. Experiments were repeated, at least, 40 times for statistical significance. Spectral characteristics were measured for a range of concentrations of pathogens, from  $10$  to  $10^9$  (cells/ml). The data were placed into a spectra database. We developed the coherent spectroscopic method for the monitoring of the pathogenic organisms directly in water pipeline, genetically modified products, and nanostructured materials in colloidal solution. The method is based on an analysis of spectral characteristics of stimulated radiation, passed through the solution. Spectra were analyzed by the spectrum analyzer "Agilent" (USA) with spectral resolution  $0.5$  nm or by the spectrum analyzer "AQ6370C," which provides spectral resolution,  $\pm 0.01$  nm and  $0.02$  nm; maximum input power,  $+20$  dBm; and a sensitivity level of input power,  $-90$  dBm. The input-output waveguides bring the exciting and passing radiations from laser to the cell and from cell to the analyzer, respectively.

We found that exciting radiations with wavelengths  $1017$  and  $810$  nm induce the stimulated Brillouin scattering in spectra of the water containing pathogen DNAs. We revealed that peak positions and widths of "fingerprints" for pathogens under study and optical densities of these bands were proportional pathogen content, if their content was less 15%. Thus, Stokes and anti-Stokes bands of the stimulated Brillouin scattering can be used to recognize the pathogens. Nonlinear effects like SBS arise due to the density of radiation in this solution [3]. The similar density of radiation is reached due to a combination of the luminescence fields and laser radiation [2] giving the characteristic peak. The modal frequency of this peak depends on the type of DNA accordingly. The strains of viruses were obtained from the Museum of the State Scientific Center. We investigated the inelastic scattering spectra from water solutions of many pathogens (**Table 3**). Traditional methods for detection of microorganisms are based on the enumeration of bacterial cells after their cultivation on a nutrient media. This method is sensitive, inexpensive, and simple, but it takes at least several days for completion. Two known methods for reducing the detection time—immunoassay and polymerase chain reaction—are complicated. At the present time, the problem of automatic monitoring of characteristics of drinking water directly within a water stream is particularly acute. Two resonance control techniques could be particularly instrumental in finding solution of that problem—spectroscopy of Raman scattering and stimulated Brillouin scattering. The utilization of Raman scattering spectroscopy technique requires transferring an enormous amount of energy for extracting an informative signal, which is considerably lower than the noise [4]. At present laser-based technology requires no consumables, or reagents are currently being developed [2]. A new method for detection of live or dead pathogens in water is described below. It is based on the diagnostics of nonlinear effects, which comprises two phenomena: an induced luminescence of DNA under the influence of laser radiation and a stimulated Brillouin scattering (SBS) [5]. The following model parameters were selected for the identification: of the peaks positions of the spectral line, corresponding to a pathogen and the difference between two wavelengths corresponding, respectively, to the laser mode maximum and of the peak of a spectral line of Stokes. Previously, we studied the spectra of virus [6]. The present work is focused on studying spectra of bacteria. The induced luminescence of DNA under the influence of laser radiation differs from ordinary luminescence in their characteristics [1]. Luminescence phenomenon was observed if the laser power is below the threshold of the Brillouin scattering effect.



Viruses		Bacteria	
1	Herpes simplex virus	17	<i>Mycobacterium tuberculosis avium</i>
2	Herpes zoster virus	18	<i>Mycobacterium tuberculosis bovis</i>
3	Herpes genitaler virus	19	<i>Mycoplasma hominis</i>
4	Epstein-Barr virus	20	<i>Neisseria gonorrhoeae</i>
5	Cytomegalovirus	21	<i>Neisseria meningitidis</i>
6	Hepatitis A virus	22	<i>Peptostreptococcus anaerobius</i>
7	Hepatitis B virus	23	<i>Proteus mirabilis</i>
8	Hepatitis C virus (genotype 1B)	24	<i>Staphylococcus aureus</i>
9	Hepatitis D virus	25	<i>Streptococcus β-hemolytischer</i>
10	AIDS virus	26	<i>Streptococcus pneumoniae</i>
<b>Bacteria</b>		27	<i>Streptococcus viridans</i>
11	<i>Compylobacter jejuni</i>	<b>Fungus and parasites</b>	
12	<i>Chlamydia psittaci</i>	28	<i>Candida albicans</i>
13	<i>Chlamydia trachomatis</i>	29	<i>Gardia lamblia intestinalis</i>
14	<i>Enterococcus</i>	30	<i>Plasmodium malariae</i>
15	<i>Helicobacter pylori</i>	31	<i>Trichomonas vaginalis</i>
16	<i>Mycobacterium tuberculosis hominis</i>		

Table 3. Pathogens included in the experiment.

## 2.3. Investigation of luminescence in the water solution

### 2.3.1. Description of the experiment

We studied the luminescence of the hepatitis C virus. For this we used a source with a power below the threshold. The virus was diluted in the different media (physiology liquor, water, alcohol) in proportions of the greatest possible ( $10^{10}$  cells/ml) concentration, intermediate ( $10^5$  cells/ml) concentration, and minimum concentration ( $10^1$  cells/ml) of a virus in liquor. We used a strain of HCV genotype 1b isolated from a patient with hepatitis C. Then we cleaned and Concentration of the hepatitis virus in solution . At the initial stage of the purification of the culture medium containing the virus was removed from the cell pellet using low-speed centrifugation. To reduce the loss of infectivity and minimize proteolysis of proteins, centrifugation was performed at 4 C. The supernatant was completely removed, and the precipitate was resuspended by adding the virus into each centrifuge cup 1 ml NTE buffer pH 7.4. Then, the contents were combined, and the resulting cup material was further resuspended in a Dounce homogenizer (Dounce Tissue Homogenizers). Then, for destruction of virus aggregates, resulting suspension was sonicated for 1 min at setting Soniprep 150 (Sanyo).

To obtain a viral suspension of high purity, zonal ultracentrifugation method was used in a sucrose density gradient. Two solutions were prepared by sucrose (Sigma-Aldrich, USA) in NTE buffer at pH 7.4 at a concentration of 10 and 50%. For this we used three basic solutions Sucrose with a concentration of 20%, 30, 40%.

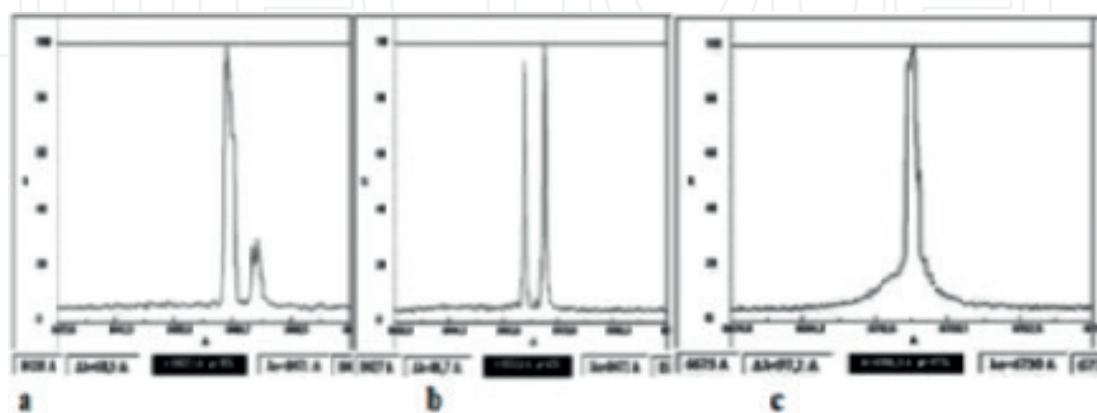
The gradient was prepared by laminating successive to each other 50, 40, 30, 20, and 10% sucrose solution in amounts of 6 ml each tube to nitrocellulose followed by diffusion at 4 °C for 12 h. The solution containing the virus was layered gently onto the surface gradient in the volume of 1 ml in each tube. We carried out the study of the dynamics of change in the intensity of luminescence. To do this, we utilized the laser, with the power being two times lower than the SBS phenomena threshold.

We continuously measured the spectral characteristics by the analyzer. The reference time started after the cuvette with a given concentration of the bacteria was placed in the optical path. The saved spectra retained their timing marks. We found that frequency of mode has a time drift (**Figure 3**). We observed two peaks in 1–2 s; then both peaks merged into one, and after 3–4 s, the luminescence disappeared.

### 2.3.2. Spectra of viruses

We investigated scattering, arising at the passage of laser radiation through colloidal solution (water, alcohol, physical solution) and fine-dispersed phase. We studied transmission spectra of a substance containing DNA: solutions of viruses and microorganisms.

Two shifted and one unshifted frequency components relative to laser frequency were detected in these spectra. The center of our attention was placed on the shifted component study, due to the fact that the analysis of processes in the fine-dispersed phase information is simple. We observed that the shifted components occur as in the Stokes and anti-Stokes ranges. On our opinion spectra of some samples under study are the spectra of Brillouin scattering as the distance between the central and the shift components does not depend on the excitation source wavelength. In other spectra we observed the spectral distributions of induced super fluorescence in liquids. In this case, the intensity of the shifted spectral distribution was higher than the intensity of the spectral distribution of laser radiation by several orders of magnitude, and the width of the spectral peak was decreased sharply. In both cases the width of the Lorentzian component, selected from such shifted spectral distribution, is proportional to the size of the particles, even if the solution contains impurities mainly proteinaceous.

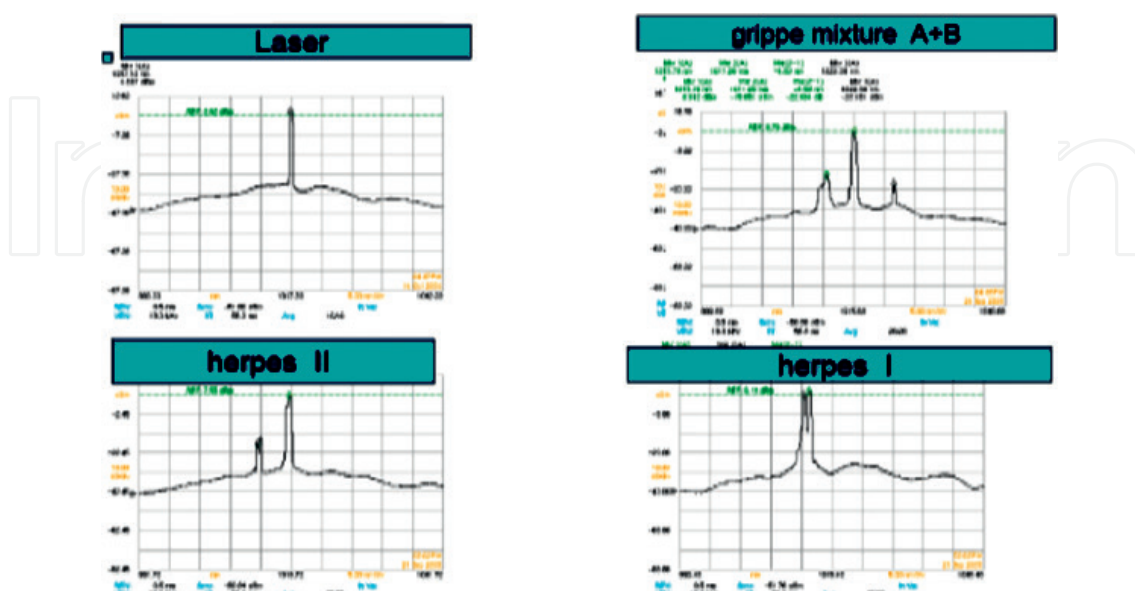


**Figure 3.** Intensity of stimulated luminescence of hepatitis C in the physiological solution from time (a) 30 s, (b) 1 min, and (c) 2 min.

We find out that the peak intensity of shifted distribution does not always have the logarithmic dependence on composition (**Figure 4**). We created autocorrelation functions of such dependencies. Our investigations showed the occurrence of super luminescence mainly in solutions containing DNA or fluorescent markers. Brillouin scattering was observed in solutions containing low concentrations of particles, including nanoscale.

### 2.3.3. Spectra of bacteria

We studied the inelastic scattering spectra from water solutions, containing *Escherichia coli* with concentration from 10 cells/ml to  $10^9$  cells/ml, as well as from a mix of *E. coli*, *B. subtilis var. niger*, *Shigella flexneri*, and the latex beads. The strains of *E. coli*, *B. subtilis var. niger*, and *Sh. flexneri* were obtained from the Museum of the State Scientific Center of Applied Microbiology and Biotechnology. The bacterial cultures were grown at 37°C for 48 h under aerobic conditions on a solid nutrient media (agar Hottinger, pH 7.2). Suspensions with bacterial cells ( $10^9$  cells/ml) were prepared in 2 ml of 0.9% NaCl solution by using industry standard samples with 10-unit turbidity. Then, suspensions were diluted with distilled water to final concentrations ranging from  $1 \times 10^8$  to  $1 \times 10^2$  cells/ml. Every sample was tested by placing 0.1 ml of bacterial suspension into the appropriate solid nutrient medium. And then, the number of colonies was calculated after 48 h incubation period at  $37 \pm 1^\circ\text{C}$ . At least 30–50 colonies must be growing on the agar medium. The suspensions of bacterial cell (*B. subtilis var. niger* and *Shigella flexneri*) were prepared for the analysis as 2 ml of 0.9% NaCl samples with the concentrations of  $10^5$  and  $10^3$  cells/ml. For assessing the sensitivity, we used bacterial suspensions of live microorganisms with seven concentrations —  $1 \times 10^2$ ,  $1 \times 10^3$ ,  $1 \times 10^4$ ,  $1 \times 10^5$ ,  $1 \times 10^6$ ,  $1 \times 10^7$ ,  $1 \times 10^8$  cells/ml—and the suspensions of *E. coli* microorganisms with the same concentrations but inactivated for 30 min at 1000C. For assessing the specificity, we used the mixes of *E. coli* and *Sh. flexneri* microorganisms with the concentration of  $1 \times 10^4$  cells/ml and with the ratios,



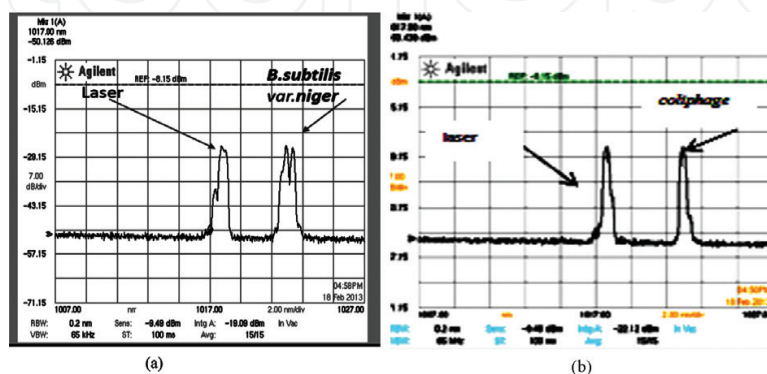
**Figure 4.** Example of distributions of the logarithm of the spectral intensity (dBm) of different viruses: (a) laser, (b) mixture grippe A+B, (c) herpes 1, and (d) herpes II.

respectively, of 1:3, 1:1, and 3:1. We also used mixes of *E. coli* and latex particles with the same concentrations and the same respective ratio concentration.

The spectra of bacteria, unlike viruses [4], often have several maxima (**Figure 4**). However, as a rule, only one peak has an intensity maximum at the same frequency in different solutions, such as water or physiological solution. And, the position of only one peak depends on the concentration.

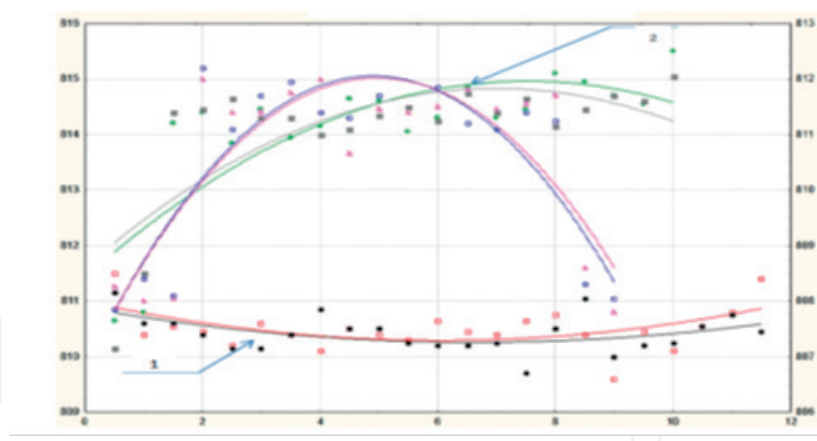
The logarithm of the intensity at that frequency is a linear function of the concentration up to a level of  $10^5$  of cells/ml. However, the intensity is not an informative parameter, because it will depend on a power of IR source (**Figure 5**). At the same time, for the developed method, the difference between the frequencies (wavelengths) of the maximum of the Lorenz component of a laser mode and the maximum of the Lorenz component of a Stokes component was found to be an informative parameter [1]. However, the Stokes component exists no more than 20 min in the spectrum. Obviously, pathogen DNA will be destroyed during this time. For a practical application, we can use the difference between the frequency maximum of the laser mode and the frequency maximum of the fundamental mode of the bacteria (**Figure 6**) exceeded  $10^2$  cells/ml; its respective peak appeared in the anti-Stokes region. We examined the forward-scattering spectra for the mixes containing two or more pathogens (**Figure 6**). The average difference between wavelength of the peak maximum of the laser mode and the peak of Stokes components was equal to  $1 \pm 0.03$  nm, which considerably exceeded the spectral resolvability of the device. The signal from pathogen organism was observed in the limited time interval.

To do this, we utilized the laser, with the power being two times lower than the SBS phenomena threshold. We continuously measured the spectral characteristics by the analyzer. The reference time started after the cuvette with a given concentration of the bacteria was placed in the optical path. The saved spectra retained their timing marks. We found that frequency of mode has a time drift. This process was continued until the intensity of the Stokes component had been reduced to the level of noise. Obviously, this effect was caused by DNA damage. It has been found that Stokes component appeared much earlier, within 25–30 seconds after the start of irradiation of the object, at moderate concentrations of  $10^4$  to  $10^6$  cells/ml. In the case of low solutions with concentrations, up to  $10^6$ , the signal existed longer than in the case of higher concentrations. We believe that at low concentrations the scattering of a signal delay is caused by a



**Figure 5.** Example of distributions of the logarithm of the spectral intensity (dBm) for *B. subtilis var. niger*: (a) concentration of  $10^3$  cells/ml and (b) bacteriophage (coliphage) concentration of  $10^3$  cells/ml.





**Figure 6.** The logarithm of the intensity of the laser mode (1) and Stokes component (2) vs. laser exposure time for the living *E. coli*: laser with  $\lambda_2 = 810$  nm; log of intensity Stokes component (2); — concentration  $10^3$  cells/ml; — concentration live  $10^5$  cells/ml; — concentration live  $10^7$  cells/ml; log of intensity laser mode (1) in the presence of *E. coli*; — concentration live  $10^3$  cells/ml; — concentration live  $10^7$  cells/ml.

nonuniform distribution of contaminated DNA in the volume of a tested specimen. The rapid decay indicates that fewer cells are rapidly destroyed by the action of coherent radiation. In the case of extremely high concentrations, we believe that the increase in the threshold in a signal generation was due to an increase in the required power of the exciting radiation of the pump. Most likely, that was because the destroyed cells were also absorbing the stimulating radiation, but they were not contributing to the formation of resonance scattering. For more details, please refer to [7].

Spectra from suspensions of the mix of pathogens with the latex beads have two Stokes components. One of them appears without delay in time, and its intensity was independent of the concentration of the latex beads. The intensity of bead component has several orders of magnitude lower than one of the pathogens. However, we did not detect the anti-Stokes component of the peaks corresponding to the dispersion of latex beads. In this case, we did not observe any change in the magnitude of this component in time.

To identify the main informative parameters of the method, the experimental samples were statistically processed. Initial comparative analysis was based on Student's t-test [6].

We developed a proprietary software for the diagnostics of the pathogens [8]. The informative parameters for diagnostics were (a) the reference spectra previously registered in the database and preprocessed based on statistical analysis of the spectral distribution of the pathogen; (b) the difference between the lengths of the laser mode high waves and Stokes (anti-Stokes component); and (c) the number of peaks in the distribution of the Stokes spectrum. Statistical analysis indicated that the above algorithm of recognition is capable of detecting the presence of bacteria spectrum with 0.95 probability [6, 9]. The pathogen monitoring method has developed. We obtained a large amount of experimental data and conducted statistical analysis aiming to determine the probability of detection of spectra by Student's t-test. We have studied the dynamics of the spectra for a number of direct dispersion of bacteria. In addition, we have studied the dynamics of changes in the



spectra for solutions containing live bacteria and dead cells. The analysis of these dynamics demonstrated that dead cells do not produce luminescence. The peak of a Stokes component of dead cell is formed by the energy transfer from the laser mode. Central peak frequency of a Stokes component at a concentrations above 102 cells/ml is displaced into the anti-Stokes region (1010 nm), which utilized laser with  $\lambda_1 = 1017$  nm. On the basis of these results, the method and the corresponding device have been developed. That device was tested on a water supply pipeline.

## 2.4. Detection of live and dead bacteria

Accurate determination of live and dead bacteria is important in many microbiology applications. Traditionally, viability in bacteria is synonymous with the ability to form colonies on solid growth medium. Alternative methods, such as fluorescent in situ hybridization (FISH); nucleic acid amplification techniques such as real-time quantitative PCR (RT-qPCR or qPCR), reverse transcriptase PCR (RT-PCR), and propidium monoazide-PCR cannot be used for continuous monitoring, as they require complex sample preparation [9]. These traditional methods are time-consuming, and they do not provide real-time results or timely information required in the industrial applications. We have developed a method of detection of live and dead cells of pathogenic organisms. Separately, we have studied the luminescence of live and dead cells. To do this, we took the spectral distribution at a pump power slightly below the threshold. Linear relationship between intensity and the concentration for luminescence was not observed.

We conducted studies that determine the difference in spectra between live and dead pathogens, and detoxification of organism suspensions of microorganisms was disinfected by boiling for 30 min. We have found that the dead cells do not produce luminescence. We researched the difference in time dynamics of appearing spectral line for live and dead pathogens. In the first 2 or 3 min, we did not observe Stokes component for dead cells (**Figure 9**). Over time, the peak in the Stokes region appeared, and the intensity of the laser mode decreased (**Figures 8 and 10**). The peak of the Stokes component is a result of energy transfer from the laser mode. The fall of the laser intensity modes was observed in lasers with both employed wavelengths:  $\lambda_2 = 840$  nm and  $\lambda_1 = 1017$  nm. However, in the second case, the drop in intensity was stronger, and the frequency maximum of the second mode was observed in anti-Stokes region (1010 nm). Due to this study, it was possible to identify live and dead *E. coli* cells in the solutions with previously undetected pathogenic components.

## 2.5. Spectroscopic methods for monitoring genetically modified products

At the present time, the areas under the transgenic crops are being increased all over the world. Thus, 60% of all the areas allocated for cultivation of soy are occupied by the plants containing the transgenic lines of this product. Genetically modified organisms are the live organisms, which due to introduction of alien genes acquired new phenotypic properties. Usage of living modified organisms (viable crops) in the territory of the Russian Federation is forbidden by the law, and only import of the vegetative raw materials processed in appropriate way is permitted

Today, the most effective control method is the method of PCR (polymerase chain reaction), allowing not only to detect the presence of GMO in products but also to determine their quantity. Medical practice widely employs invasive methods of infectious disease detection based on the analysis of blood samples with the help of polymerase chain reaction and immune-enzyme analysis (IEA). The use of these methods for express diagnostics in mass screening implies a number of problems: the need of a special laboratory equipment according to sanitary and epidemiological standards, expensive test systems, and time that is necessary to get results (from several hours to several days). These factors hamper rapid epidemiological interventions in case of spreading infections as viral hepatitis and immunodeficiency virus and call for updating of the present and development of new diagnostic methods. Improvement of express diagnostics includes possibility to use the method in field conditions, its economical efficiency, high capacity, and quick results.

Unfortunately, the PCR method of analysis has its drawbacks. It requires difficult, long preparation of tests, expensive equipment, and specially equipped premises. All this does not allow us to use the PCR method for a mass express screening of products.

#### *2.5.1. Overview on PCR method*

Polymerase chain reaction (PCR) is an experimental method of the molecular biology, allowing us to achieve a substantial growth of the small concentrations of certain fragments of nucleic acids (DNA) in a biological material (test). The method relies on thermal cycling, consisting of cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA. Primers (short DNA fragments) containing sequences complementary to the target region along with a DNA polymerase, which the method is named after, are key components to enable selective and repeated amplification. As PCR progresses, the DNA generated is itself used as a template for replication, setting in motion a chain reaction in which the DNA template is exponentially amplified. PCR can be extensively modified to perform a wide array of genetic manipulations. PCR is not generally considered to be a recombinant DNA method, as it does not involve cutting and pasting DNA, only amplification of existing sequences.

In the first step, the two strands of the DNA double helix are physically separated at a high temperature in a process called DNA melting. In the second step, the temperature is lowered, and the two DNA strands become templates for DNA polymerase to selectively amplify the target DNA. The selectivity of PCR results from the use of primers that are complementary to the DNA region targeted for amplification under specific thermal cycling conditions. PCR amplifies a specific region of a DNA strand (the DNA target). The PCR was carried out in an amplifier, a device, which ensures a periodic cooling and heating of the test tubes, usually with the accuracy not less than 0.1°C. Modern amplifiers allow us to set complex programs, including with a possibility of “a hot start.” For real-time PCR, special devices are produced equipped with a fluorescent detector.

When chains are disengaged, the temperature is lowered, so that the primers could contact a one-chained matrix. This stage is called hybridization. The temperature of the hybridization

depends on the composition of the primers and is usually 4–5°C below the temperature of their fusion. Duration of the stage is 0.5–2 min. A wrong choice of a temperature results either in a bad linkage of the primers with a matrix (if the temperature is too high) or in a linkage in a wrong place and appearance of nonspecific products (if the temperature is too low).

By using a primer, the DNA polymerase makes a copy of a matrix chain. This is a stage of elongation. The polymerase begins a synthesis of the second chain from the primer's 3'-end, which has bonded a matrix, and moves along the matrix in the direction from 3' to 5'. The temperature of the elongation depends on a polymerase. The frequently used polymerases Taq and Pfu are most active at 72°C. The period of elongation depends on both the kind of DNA polymerase and on the length of an amplified fragment. Usually, the period of elongation is accepted as equal to 1 min per each thousand of base pairs. When all the cycles are finished, an additional stage of the final elongation is often done to complete the construction of all the one-chained fragments. This stage lasts 7–10 min. The quantity of the specific product of the reaction (limited by primers) theoretically increases proportionally to  $2^n$ , where  $n$  is the number of reaction cycles.

DNA markers are based on PCR primers. For real-time analysis, special DNA amplifiers with an optical unit are used to detect fluorescence within the reaction tube during the reaction. For real-time analysis, special DNA amplifiers with an optical unit are used to detect fluorescence within the reaction tube during the reaction [7]. These amplifiers are known as markers.

Now, carrying out of an express analysis is essentially impossible, because a preliminary extraction of a DNA of an investigated product is necessary. This can be done only in the conditions of a special laboratory. This is due to the fact that, if the lab ware or reactants are polluted by the products of the other reactions or other protein molecules, the results will be incorrect. Special premises are necessary for cleaning of the nucleic acids and for the analysis of the products of amplification. Considerable time is also necessary for a periodic heating of the tests. If the latter problem is solved, the division of DNA and cleaning of the nucleic acids essentially do not allow us to develop an express analyzer working directly with a product.

### *2.5.2. Description of the experiment*

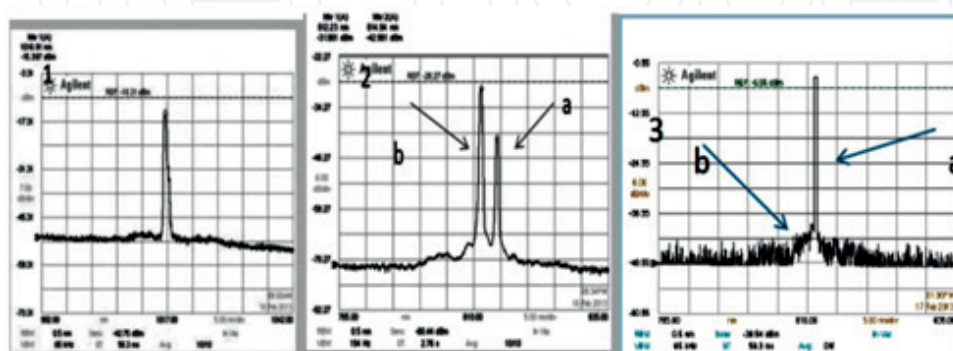
This experiment was conducted in order to develop a prototype of an express analyzer of a genetically modified soy. To do so, a certain number of problems are needed to be solved such as ensuring optical control of the parameters of soy in a multicomponent environment containing parasitic parameters of DNA, optimizing heat exchange system in a real-time mode, and developing an optical scheme for reading of the informative parameters and a signal processing system with account of the errors. With the above aims in mind, we undertook a research of the markers applied for carrying out of the reaction. In order to obtain a stimulated luminescence, the light-emitting diode pumping was replaced with a laser pumping, which resulted in an increase of the marker's own luminescence by more than an order. We replaced the lamp in the optical unit with a laser in order to reduce noise. The equipment for removing and analyzing the spectral characteristics and spectra of the inelastic scattering of marker are shown in **Figure 7**. We modified the experimental setup described in Part 2 a little.

We used an additional laser with a wavelength of 532 nm to enhance luminescence of the marker and achieve the SBS threshold.

As our previous research has shown, there is a protein luminescence (meat, milk) in the visible spectral range. The protein luminescence is absent in the near-IR range radiations. Also, the power of a luminescent marker is insignificant, and it is necessary to carry out rather many PCR reactions. The luminescence of an extraneous protein does not allow us to carry out diagnostics because of an inadmissible signal/noise ratio. Thus, if we analyze the spectrum of the investigated object in the near infrared region, we can confine ourselves to centrifuging the sample for uniform distribution in solution.

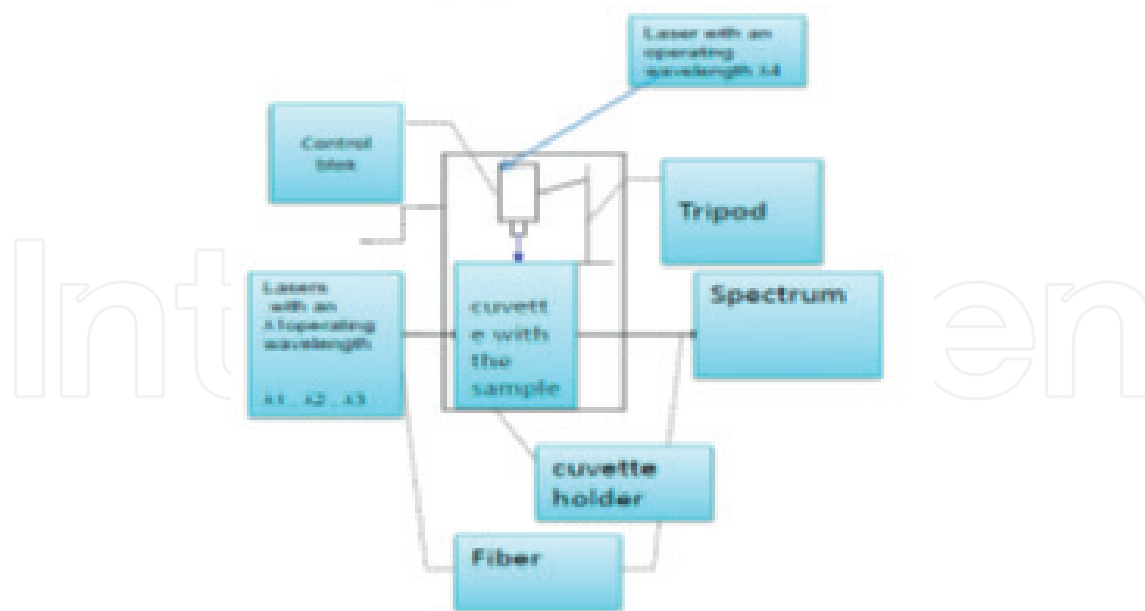
Also, spectral distributions during excitation of the markers by two types of lasers, with the wavelength corresponding to the wavelength exciting a marker and the wavelength lying within the range of the registration of the radiation, are obtained. An example of the of spectral intensity distribution (dBm) marker HEX-1030 with laser pumping with an operating wavelength  $\lambda_3 = 1016\text{nm}$  and laser pumping with an operating wavelengths  $\lambda_3 = 1016\text{nm}$  and  $\lambda_4 = 532\text{ nm}$  is shown in **Figure 8a** and **b** accordingly. The research also demonstrated that in the frequency range of about 1.01 microns, a peak of the inelastic scattering appears, proportional to keeping of a marker in a solution. Since luminescence in this area should fall, the reasons for occurrence of this peak are most likely connected with SBS effect. The maximum peak power increases by more than 1 dBm, if we used laser pumping of two lasers. We assumed that the difference in the position of the laser maximum and Stokes intensities in the spectra of the inelastic light scattering in the natural and modified soy is connected with the change of the molecular structure of the genetically modified samples, which in turn leads to a change of the electronic power levels of the biological objects (**Figure 9**).

Thus, the registration of the position of the maximum and the intensity of the inelastic diffrused light in the field of inelastic scattering of light in the field of 850–1100 nm can be used as informative parameters for determination of the genetically modified soy. The spectral distributions of the inelastic scattering can serve as informative parameters for determination of the presence of a genetically modified product.



**Figure 7.** An example of the spectral intensity distributions (dBm); a(2) live *E. coli*; b(3) dead *E. coli*. Arrow “a” shows the peak corresponding to indicate *E. coli* laser mode; arrow “b.”

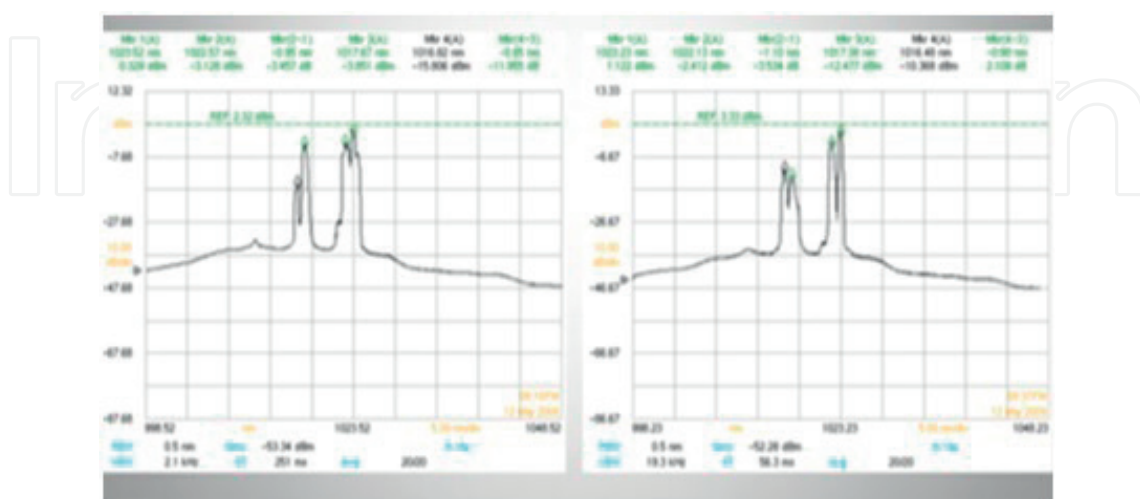




**Figure 8.** The equipment analyzing the spectral characteristics  $\lambda_4 = 532$  nm or 488 nm, 633 nm  $\lambda_1 = 674$  nm,  $\lambda_2 = 810$  nm, and  $\lambda_3 = 1010$  nm HEX-1030 laser with an operating wavelength  $\lambda_3 = 1016$  nm.

The maximum peak power increases by more than 1 dBm, if we use laser pumping of two lasers. However, for practice, only one laser can be used. The spectrum analyzer can be replaced with a photodetector, if you control only one component. This allows you to develop cheap devices. We developed an experimental sample of a device using one laser in the near-field region (**Figure 10**).

The spectrometer was replaced by a receiver. We did not isolate soy DNA. We received a soy-bean mixture with water and centrifuged. Intensity of the inelastic scattering of the natural and genetically modified soy in absolute and relative figures is shown in **Figure 9a, b**. So, we



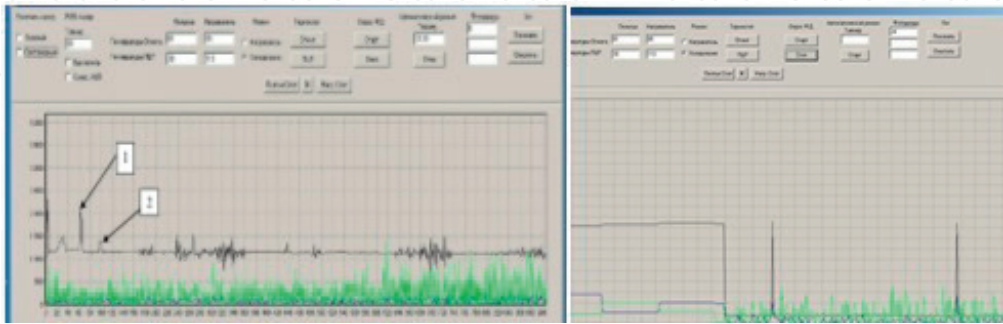
**Figure 9.** Marker HEX-1030 (a) laser pumping with an operating wavelength  $\lambda_3 = 1016$  nm; and (b) laser pumping with an operating wavelengths  $\lambda_3 = 1016$  nm and  $\lambda_4 = 532$  nm.



have also established the new method for rapid analysis of genetically modified soy in meat products detected by a modified PCR fluorescent method.

2.5.3. Comparison of standard PCR method and a modified PCR method

**Table 4** compares the two methods. Analysis shows that our method can be the basis of devices working outside the laboratory, directly in shops and cafes.



**Figure 10.** Distribution of signals in the presence of genetically modified soybean: (1) laser signal and (2) marker signal.

Standard PCR	Modified PCR method
The need for DNA extraction	Diagnosis is made immediately in the sample
The need to organize specialized laboratories	The need to organize specialized laboratories
Reaction time (1 h)	Reaction time (1 min)

**Table 4.** Comparison of standard and modified PCR methods.

3. Discussions

Traditional methods for detection of microorganisms are based on the enumeration of bacterial cells after their cultivation on a nutrient media. This method is sensitive, inexpensive, and simple, but it takes at least several days for completion.

Two known methods for reducing the detection time—immune assay and polymerase chain reaction—are complicated. In addition to the fact that this method requires the isolation of DNA, it also uses various sets of reagents to identify and differentiate the tested pathogen. For example, there are the set of reagents for the detection and differentiation of DNA from bacteria of the genus *Shigella* spp. and enteroinvasive *E. coli* (EIEC), *Salmonella* (*Salmonella* spp.), and thermophilic *Campylobacter* spp., modified with specific primers to *B. subtilis* var. *nigger*). In environmental objects and clinical material by polymerase chain reaction (PCR) with hybridization-fluorescent detection, “AmpliSens® *Shigella* spp. and EIEC/*Salmonella* spp./*Campylobacter* spp.-FL” and “AmpliSens® *Bacillus anthracis*-FRT” are often used in practice.

The PCR Stafipol-RV kit is designed to quantitatively detect the in vitro DNA of the causative agent of *Staphylococcus aureus* in a biological material by polymerase chain reaction with a fluorescent detection of the result in real time.

Another set of reagents is used to quantify the hepatitis C virus (HCV) RNA by polymerase chain reaction (PCR) with hybridization-fluorescent detection in real-time mode "AmpliSens HCV-Monitor-FL." The use of various consumables for different pathogens makes this method very expensive. Therefore, at the present time, the problem of automatic monitoring of characteristics of drinking water directly within a water stream is particularly acute.

Two resonance control techniques could be particularly instrumental in finding solution of that problem—spectroscopy of Raman scattering and stimulated Brillouin scattering. The utilization of Raman scattering spectroscopy technique requires transferring an enormous amount of energy for extracting an informative signal, which is considerably lower than the noise [3].

Laser-based technology requires no consumables, or reagents are currently being developed [10]. A new method for detection of pathogens in water and water-based solutions is described below. It is based on the diagnostics of nonlinear effects, which comprises two phenomena: an induced luminescence of DNA under the influence of laser radiation and a stimulated Brillouin scattering (SBS) [11]. The following model parameters were selected for the identification of the peak positions of a spectral line corresponding to a pathogen and the difference between two wavelengths corresponding, respectively, to a laser mode maximum and to a peak of a spectral line of a pathogen.

We studied transmission IR spectra of a number of pathogen (salmonella, viruses of herpes genitaler, hepatitis A and C, grippe A and B) solutions and luminescence of nanomarkers. In our experiments, the laser radiation passed through a quartz cell with water solutions of the pathogens, nanosilver, or nanomarkers. We found that exciting radiations with wavelengths 1017 and 810 nm induce the stimulated Brillouin scattering (SBS) in spectra of the water containing DNA. We believe that the power threshold for the onset of this effect is achieved by adding a forced radiation luminescence and laser radiation.

## 4. Conclusions

The new method, the express analysis of pathogens in water, was developed. It shall be mentioned that the proposed method of express diagnostics allows detection of infectious agents in the water in minutes based on nonlinear effects. This research has, hopefully, laid the foundation for development of a prototype for determination of the content of the genetically modified soy in meat products. The inventive methods can be recommended for DNA diagnostics in medicine, veterinary sciences and in sanitation. The main advantage of this method is that there is no need for DNA isolation. It is sufficient to create a suspension of the product by centrifugation.

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