REAL-TIME MONITORING OF CHANGES OF THE PATHOGENS CONCENTRATION IN WATER UNDER INFLUENCE OF NANOSILVER PARTICLES BY RESONANCE LASER SPECTROSCOPY TECHNIQUES

T.Yu. Moguilnaya[1], P.V. Bobkov[1], V. I. Tomilin[1], and A. B. Kononenko[2]

1 - JSC "SERVET-M", 115404, Russia, Moscow, ul. 8th Radial,

2 - Veterinary Sanitation, Hygiene and Ecology Institute 123022, Russia, Moscow, Zvenigorodskoe Highway 5

E-mail: mogilnay@mail.ru

Abstract We investigated the nonlinear scattering in solutions of water containing E. coli and the mixture of E. coli and silver nanoparticles. As a result of these investigations, we developed a method of controlling the concentration of pathogenic organisms in the water and the concentration of the nanoparticles influences these organisms. For the study we selected pathogens - goldish staphylococcus, E-coli and coliphage MS-2. Standard biological method is qualitatively in agreement with our results.

1 INTRODUCTION

The optical phenomena of the stimulated scattering in water solutions, which contain DNA, are investigated insufficiently. However, these researches are great interest for the development of automatic control systems of potable water. Content of pathogenic microorganisms in water can be varied from 10^{-8} to 10^{5} mg / m^3. Hygienic standards of quality of potable water require a continuous of monitoring absence pathogenic microorganisms directly in a water flow.

Despite of a great number of laboratory devices for potable water's quality, there is no express analyzer for monitoring of pathogenic organisms, which could be directly embedded into the automatic checkout systems. The reasons are low concentrations of pollutions and additional effects (superluminescence, stimulated Brillouin scattering (SBS), which impede automatic data-processing.

Laser methods are widely applied analysis of structure of multicomponent liquids and the unique express tool for low concentration's determination.

In our previous papers, we showed different pathogens [1-3], that stimulated radiations (superluminescence in water solutions, which contain DNA) can appear at enough low power of

Content from this work may be used under the terms of the Creative Commons Attribution 3.0 licence. Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI.
Published under licence by IOP Publishing Ltd

exciting radiation [1]. Frequencies of these stimulated radiations depend on a DNA type and, accordingly, on a type of pathogenic organism. [3]. The radiation density in this solution allows nonlinear phenomena, such as stimulated Brillouin scattering (SBS)- [1]. This density is reached due to combining of the superluminescence fields and laser radiation [2]. In result, characteristic peak occurs. Similar peaks are well interpreted by methods of the theory of pattern recognition. The purpose of our research is showing that the residual impurity in potable water don't give superluminescence and don't capable to form significant peak and thus to complicate automatic recognition. In addition, we are interested in the opportunity of optical controlling method of destruction pathogenic organisms in the stream on the same hardware. We are developed a method for verifying the effectiveness silver's influence to pathogens. We investigated the possibility of realizing this method Also, we investigated the possibility of destruction pathogenic organisms by adding a solution of silver nanoparticles.

2 EXPERIMENTAL SETUP

We use a made-self stand in our experiments. The Fig. 1 shows the scheme of the experimental stand. The device contains the block with sources of radiation in optical IR-range. The receiver laser radiation with a high accuracy of spectral resolvability ($\Delta<0.02$ nm) is chosen. As a result the developed experimental stand included the following elements (fig.1):

The input waveguide brings one of the exciting radiations (with wavelength $\lambda1=1017$ nm, $\lambda2=810$ nm $\lambda= 670$ nm) to the cuvette, containing the sample. The radiation, which passed through the cuvette and output waveguide, was analyzed by spectrum analyzer "Agilent"

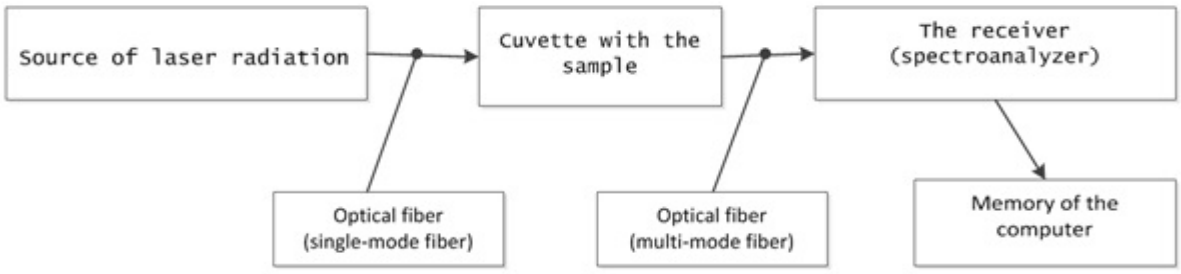

Fig.1. Scheme of the experimental stand

3 THE STUDY OF WATER

In order to reveal effects, which can impede automatic data-processing, we examined probes of potable water (without E- coli), which was given from five different areas of Russia. The creation of express analyzers, allowing full automatic signal processing, requires a study of additional signal characteristics apart from analysis of main spectral characteristics (peak position, intensity and shape of spectral bands) of radiation. For instance, a choice recognition algorithm depends on strong

additional signal's characteristics, such as a signal/noise ratio, an averaging, stability of exciting and emitted radiations, absence of overlapping spectral bands from analyzed substance (E-coli) and other substances (water and impurities).

To study stability of exciting radiations from our laser sources, we study spectra of their radiations, passing through the empty cuvette. The fig. 2 shows the spectra of radiations from the laser sources with wavelengths 1017, 810, respectively. The laser sources with wavelengths 1017, 810 nm demonstrated unimode laser radiations with deviation of wave about 3 nm. However, we observed multimode laser radiation from the laser source with $\lambda 3 = 670$ nm (fig.4). The visible spectral region is inconvenient for automatic recognition [3]. For this reason, we didn't use laser source with $\lambda 3 = 670$ nm our further experiments. Spectra of the laser sources which have passed through object of research have qualitatively identical appearance. Therefore we give selective results for one lasers wavelength. However, we used all results for developing the software. It is made to provide signal recognition with probability 0.95.

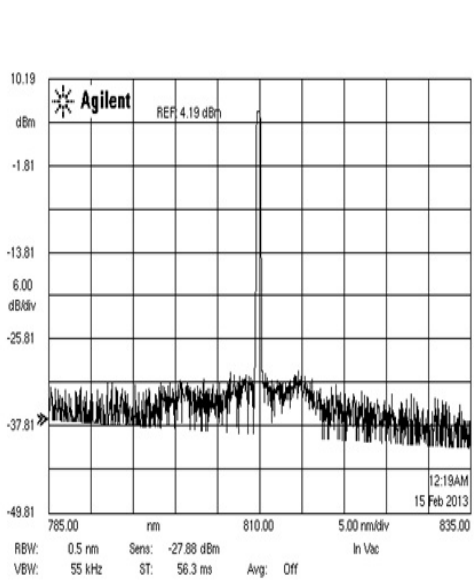

Fig.2. The spectrum of laser radiation with wavelength λ2 = 810 nmpassing through the empty cuvette with waterat averaging degrees 10

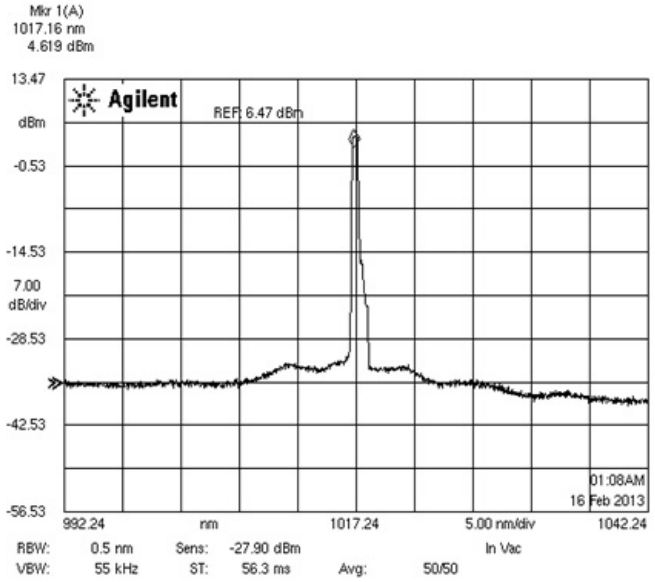

Fig.3. The spectrum of laser radiation with wavelength λ1 = 1017 nm, passing through the empty cuvette with waterat averaging degrees 10

Also we determined optimal averaging degrees of signal characteristics. The fig. 3 shows spectra of laser radiation with wavelength $\lambda 1 = 1017$ nm. We found out, that the increase of averaging degrees from 10 to 50 did not change signal/noise ratio. Thus, we preferred averaging degree 10. We received the same results for the laser with a wavelength 810 nm.

The analysis of spectra of water from the different regions revealed, that additional spectral bands from water impurities doesn't appear in wavelength regions from 750 to 1500 nm, which we selected for diagnostic of E-coli. However, we observed deviations in intensity and in wavelength of

exciting radiations. We investigated water for Mosrentgen's region and the region Pechatniki, water from the well, and water from the Moskva-river. Fig. 4 shows the results of statistical analysis of the intensity from exciting radiations, passing through the samples of water from these regions.

Variable	Descriptive Statistics (OSA in Mosrentgen.stw)				
	Valid N	Mean	Minimum	Maximum	Std.Dev.
Amplitude	60	5,242	3,536	7,695	1,110093
WaveLength	60	16,933	1015,850	1017,650	0,362410

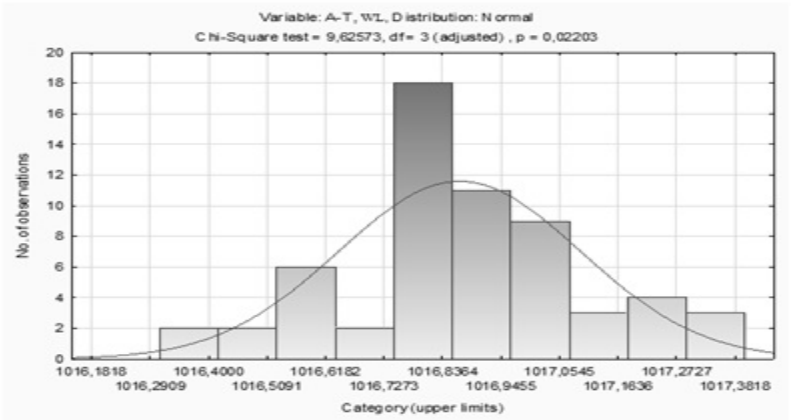

Fig.4. Results of statistical processing statistics (above) and distribution of deviation of parameters of the laser peaks (below) for various, impurities of water

Fig. 5 represents an example of statistical processing of intensity of laser radiation for different samples from one region. Figure 6 shows to intensity of peaks laser and impurity from frequency for various samples from different regions.

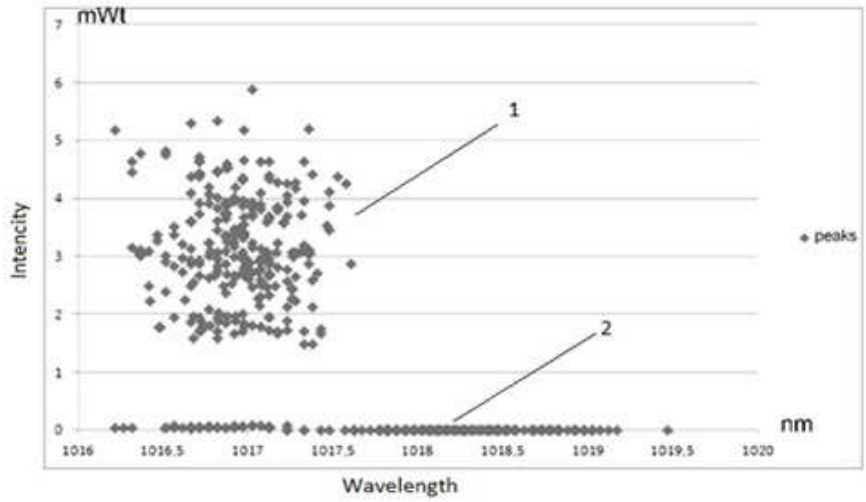

Fig.5. The distributions of the peak position: 1 - distribution of the maximum of the spectrum of the laser radiation passing through the water, taken in different regions 2- distribution of maxima of the spectra of impurities.

The statistical processing of the portable water spectra from different region shows, that the standard deviations for the characteristics of the exciting radiations doesn't exceed 20% for intensity and 0,03% for wavelength. The analysis of water spectra from different regions has shown, that in the chosen range of frequencies there are no parasitic peaks of a luminescence of water impurity. The mistake caused by own noise, does not exceed 0.001%. Therefore, the frequency maximum of intensity peak can serve as informative parameter at recognition of an image.

4 THE STUDY OF WATER SOLUTION OF E-COLI

We researched water solution of E- coli (strain K12) with concentrations 10, 10^2, 10^3, 10^4, 10^5, 10^6, 10^7, 10^8, 10^9 cells/ml (lg tds50).. The water solutions of E- coli (strain K12) were prepared by the following procedure. The culture was grown on the meat-peptonic agar during 24 hours, after produced washing off sterile physiological solution, set on the standard absorbance concentration 10^9 microbial mews/ml. The other solutions were prepared by the tenfold breeding prepared suspensions containing from 10^8 to 10 microbial/ml).

We investigated E-coli solutions of that what to receive informative parameters for automatic recognition of existence it in water. We analyzed about 700 spectra of the E- coli for exciting wavelength 810 nm, about 100 spectra of the E- coli for wavelengths 670 and 1017 nm, and 1400 spectra of the water (without E- coli) for each the areas.

The fig. 6 shows the example of spectra for two concentration 10 and 10^3 cells/ml. We found, that if concentration of E-coli is less than 10^3 cells\ml, we could see the peak in Stokes area. And if concentration of E-coli changes from 10^3 to 10^7 cells\ml, we could see the peak is in anti-Stokes area. The peak is in Stokes area if concentration of E-coli exceeds 10^8. The fig. 7 shows dependence of the logarithm of intensity on concentration E-coli at averaging 10.We found out that the logarithm of intensity depend on concentration same at environment excitement by the laser with a length of wave of 1017 nm.

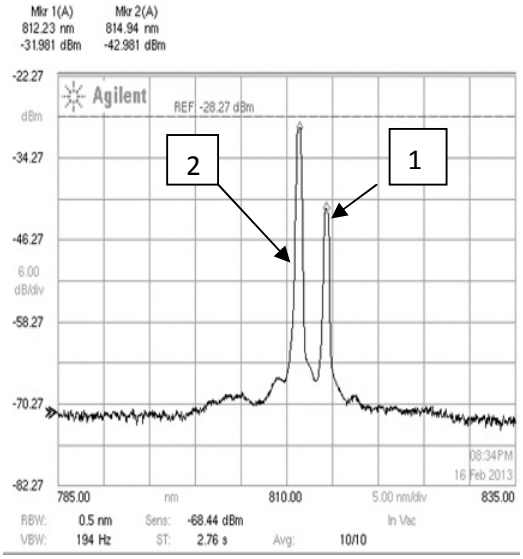

Fig.6. Example of spectra of radiation with concentration E.coli 10^3 o/l: 1 –laser; 2 E – coli

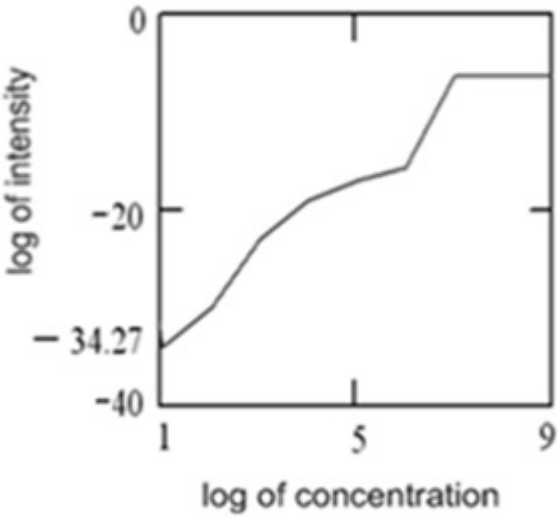

Fig.7. The dependence of the logarithm of intensity on concentration E.coli at averaging 10

We studied change peak dynamics of the pathogens. Dynamics change of intensity distribution of E-coli differs from laser radiation dynamics. The peak arises after some delay. Time of the delay varies from shares of seconds up to ten seconds. The E-coli peak grows, and the laser peak decreases. The laser's peak becomes less than the luminescence peak, if E-coli concentration of more than, 10^3. Both radiations are polarized and coherence [1]. The fig. 7 shows the dependence of the logarithm on intensity concentration E-coli (dBm) at time (μs) at virus concentration 10^2 cells\ml.

We found out, that exciting radiations with wavelengths 1017 and 810 nm induce the stimulated Brillouin scattering in spectra of the water, containing pathogen DNAs. Threshold power level for appearance the stimulated Brillouin scattering was achieved, by electromagnetic fields from the exciting radiation and the stimulated fluorescence. We found out, that peak positions and widths are "fingerprints" for pathogens under study, and optical densities of these bands is proportional pathogen content, if this content is less 15%. Thus, Stokes and anti-Stockes bands of the stimulated Brillouin scattering can be used to recognize the pathogens under studies.

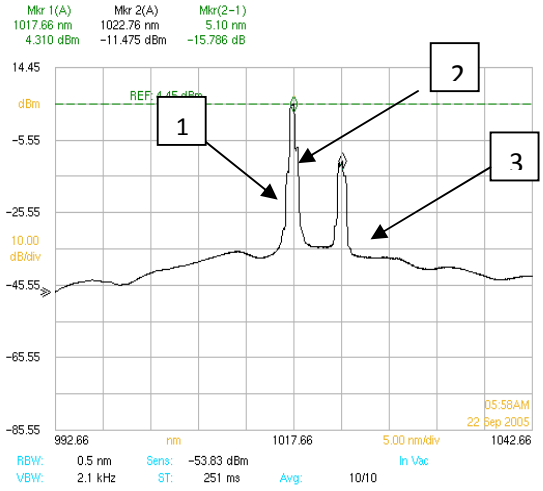

Fig. 8. The emission spectra: 1 – of the laser and 2-silver in the water content of0.0001mg silver\cell water excitation wavelength λ2.=1017nm; 3 – of the pathogen7lgn tcd50

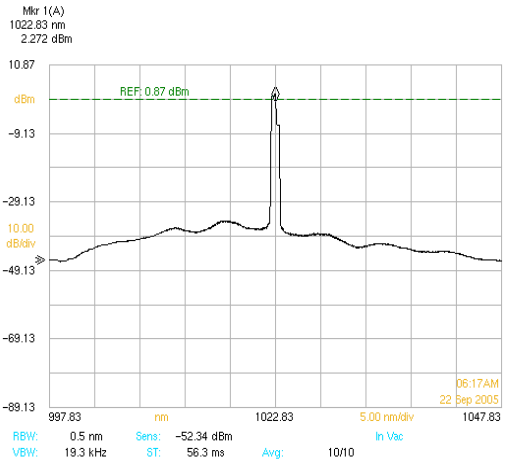

Fig. 9. The emission spectra: – of the laser and the silver content of silver in water0.001mg\lcell water excitation wavelength λ2.=1017nm; 2 – of the pathogen content7lgn tcd50

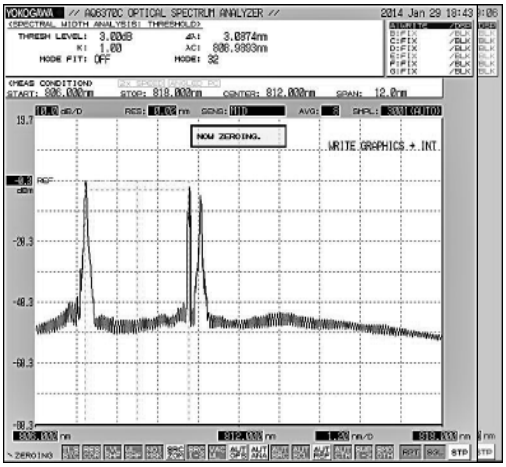

Fig. 10. *The emission spectra: 1 – of the laser and silver in the water content of 0.0001mg silver\cell water excitation wavelength λ2.=1017nm; 2 – of the pathogen (goldish staphilococcus) 7lgn tcd50*

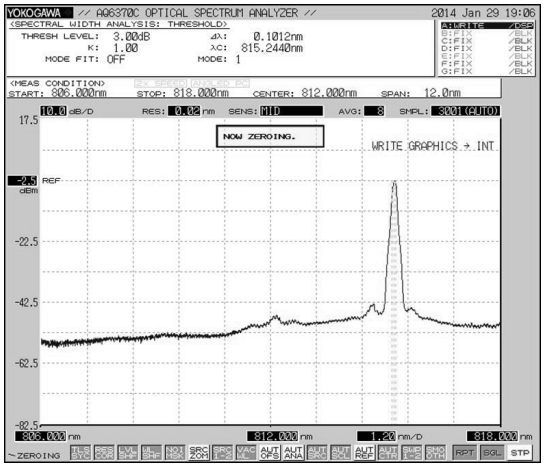

Fig. 11. *The emission spectra: 1 – of the laser and the silver content of silver in water0.001mg\lcell water excitation wavelength λ2.=1017nm; 2 – of the pathogen content (goldish staphilococcus)7lgn tcd50*

Based on these studies, we have developed a working model of the device for the automated control of pathogens in the water directly.

5 THE STUDY OF WATER SOLUTION OF NANSILVER AND PATHOGENS

We studied the influence of nanosilver on changes on the concentration of the live pathogens. For the study we selected E-coli, Salmonella and goldish staphilococcus.

We added to solutions containing pathogens, solutions of silver nanoparticles in water. Fig 8 and 9 show typical spectra of the E-coli solutions. Figure 10 and 11 shows the emission

spectra of the laser and silver in the water content of 0.001 mg silver \ l cell water excitation wavelength λ2. = 1017 nm goldish staphilococcus 7 lgn tcd 50 (10^7 cells\ ml) a (Fig 9). The emission spectra of the laser and the silver content silver in water 0.0001 mg o\ ml cell water excitation wavelength λ2. = 1017 nm patogens content lgn tcd 7 50 (10^7 cells \ ml). The intensity of this peak decreases with the concentration of the Bouguer's law. Comparative analysis of the spectral distribution of the solution in water, silver and silver virus at a wavelength of 1017 nm showed (Figure 8 9-), that at silver concentrations up to 0.01 mg \ L, reaction occurs protein with silver. That leads to the emission peak of silver at a wavelength of 1022. Peaks, corresponding to E-coli disappears completely. Whereby, this emission spectrum has a width of distribution lower than the spectral width of the laser in the air. The intensity of these peaks is depend on the content of silver in the solution, indicating complete separation of the protein from the nucleic acid in solution and complete destroying of the virus. Other pathogens studies showed similar results.

5 CONCLUSION

We developed a new method that combines the method of forced luminescence and stimulated Brillouin scattering. We carried out the statistical spectral analysis of water from different regions in order to determine the statistical errors of the method.

The analysis of spectra of water from different regions showed, that there are not parasitic peaks of a luminescence of water impurity in the chosen range of wavelength (750-1400 nm). The error of signal caused by own noise, doesn't exceed the 0.01%. Therefore the frequency of maximum of peak of intensity can serve as informative parameter at recognition of an image.

We researched spectral characteristics of E-coli in water an amount necessary for the initial identification with 95% probability.

We found out that exciting radiations with wavelengths 1017 and 810 nm induces the stimulated Brillouin scattering in spectra of the water, containing pathogen DNAs. Threshold power level for appearance the stimulated Brillouin scattering was achieved, by electromagnetic fields from the exciting radiation and the stimulated fluorescence. We found out, that peak positions and widths of are "fingerprints" for pathogens under study, and optical densities of these bands is proportional pathogen content, if this content is less 15%.

Thus, Stokes and anti-Stockes bands of the stimulated Brillouin scattering can be used to recognize the pathogens under studies.

Based on these studies, we have developed a working model of the device for the automated control of pathogens in the water directly.

We studied the nanosilver influence on the pathogens concentration. For the study we selected pathogens - goldish staphylococcus, E-coli and coliphage MS-2. Standard biological method is qualitatively in agreement with our results.

REFERENCES

[1] T.Yu. Moguilnaya., Botikov .A. G., Saguitova EA Influence of instability of laser radiation on accuracy of record and reading of the information of diagnostic complex " Intest1" " ; Proceeding of Spie 31 .07 -4.07 2005, USA Volume 5908-41; 12 cnh.;

[2] T.Yu. Moguilnaya E.A. Saguitova, K.A. Prokhorov, , E.I. Grebenuk, N.A. Grebenuk Laser instrumentation for express diagnostic of toxin in liquid biological systems Fall Meeting 2000: Optical Technologies in Physics and Medicine II, Proceeding of SPIE, Vol.4241 (2001), p.504-506 –

[3] T.Yu. Moguilnaya A. Saguitova, K.A. Prokhorov, E.I. Grebenuk, N.A. Grebenuk Laser instrumentation for express diagnostics of impurities and toxins in liquid food Photonic Detection and Intervention Technologies for Safe Food, Proceeding of SPIE, Vol. 4206 (2001), p. 256-265