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Review Article

Method and Device for Raman Fluorescence Diagnostics of the State of Human Tissues in Health and Pathology

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Abstract

In the presented work, the substantiation of the development of an innovative, universal, digital diagnostic complex of express Raman fluorescence diagnostics is given. The range and algorithm of its clinical application are presented, which provides a sanogenic and pathogenetic approach to the objectivity and effectiveness of the assessment of human tissues and organs in normal and pathological conditions: diseases and processes of microbial and neoplastic nature (in clinical microbiology, dentistry, obstetrics and gynecology, The solution of these issues is based on the methodology of laser digital photometry and the basic requirements and principles of modern science and practice that provide controlled treatment of a specific disease in a particular patient.

Keywords: Laser Radiation; Laser Photometry; Mechanism of Biological Action of Laser Radiation; Raman Fluorescence Express Diagnostics; Hardware and Software Complex; Sulfur Substrates; Application Algorithm; Range of Clinical Application; Norm; Pathology; Diseases and Processes of Microbial and Nonplastic Nature; Clinical Microbiology; Dentistry; Obstetrics and Gynecology; Oncology; Gastroenterology; Pharmacology; Express Diagnostics "on Site"; Feedback; Prospects for Application

Introduction

At present, both experimental studies and clinical observations are being carried out to study the possibilities of using quantum electronics as sources of optical radiation (including low-intensity laser radiation) for the purposes of diagnosis, prevention and treatment of diseases. In general, the biological effect and clinical effect of low-intensity laser radiation depends on the optical characteristics of tissues (reflection, transmittance, absorption coefficients), electrical, acoustic, biochemical, physicochemical, physico-mechanical properties of tissues (heat capacity, thermal conductivity, specific gravity, etc.), the functional state of the biological object (norm/pathology). As a result of the interaction of the above factors (after absorption In the irradiated tissues, the following physicochemical changes occur primarily: the emergence of excited states of molecules, the formation of free radicals, stereochemical rearrangement of molecules, coagulation and elastic oscillations of protein structures, changes in the electric field of the cell, changes in the chemism of the cell, etc. Some of them are associated with the activation of enzyme systems, in particular, succinate dehydrogenase, NAD-H2, NADP-H2, LPO, etc.

Laser radiation directly and mainly affects cell membranes, enzyme systems and the receptor apparatus of the body at various levels of its organization. Another part of the secondary effects due to the conversion of laser radiation in a biological object (BO) leads to the formation, for example, of acoustic and ultrasonic oscillations, soft ultraviolet and X-ray radiation, fluorescence, Rayleigh and Raman scattering, which leads, in turn, to an even greater increase in secondary (local and general, specific and non-specific) biological effects and an increase in their diversity. In addition, both laser radiation itself and products resulting from primary and secondary effects have a pronounced effect on nerve endings and

indirectly on the nervous system as a whole. In addition to primary and secondary effects, nervous reflex and neurohumoral responses occur in the body: the sympathoadrenal and immune systems (local and general, specific and non-specific factors) are activated, the concentration of adaptive hormones increases, thus a complex of adaptive and compensatory reactions arises in the whole organism, which are not aimed at restoring its homeostasis [1].

As a result, under the influence of laser irradiation, changes occur that are registered at all levels of the organization of living matter:

- Subcellular (absorption of the quantum of light by the pigment BO, the emergence of excited states of molecules, the formation of free radicals, stereochemical rearrangement of molecules, coagulation of protein structures, etc., which, in case of positive destruction, leads to an increase in the rate of synthesis of protein, RNA, DNA, acceleration of maturation of collagen and its precursors, etc.);
- Cellular (change in the charge of the electric field of the cell, change in the membrane potential of the cell and its permeability, increase in metabolic and, in particular, synthetic activity, etc.);
- Tissue (change in the chemism and pH of the intercellular fluid, change in microcirculation, etc., change in oxygen balance and activation of redox processes);
- Organ (stimulation or inhibition of the function of an organ);
- Systemic (the occurrence of adaptive nervous reflex and neurohumoral reactions with activation of the sympathoadrenal and immune systems);
- Restoration of metabolic and structural-functional homeostasis of CO at all levels of its organization.

Depending on the specific combination of the influencing factors of laser exposure (irradiation parameters, metabolic and functional state of the irradiated tissue and individual characteristics of the body), the resulting response of the whole organism can be expressed in the activation of its functions (with an adequate magnitude of exposure) or their inhibition (with an inappropriately large magnitude of exposure), as well as in the absence of any significant changes (with an inappropriately small magnitude of exposure) local and general, specific and non-specific responses of a biological object depending on the level of its organization and its functional state (homeostasis).

At the same time, there is an objective possibility of registering the above-mentioned optical, metabolic and bioenergetic processes, MORPHOMETRIC AND FUNCTIONAL CHARACTERISTICS OF BIO as a means of diagnosis, which provides feedback in the treatment of the patient on the principles of real time.

The use of laser radiation is especially relevant in modern clinical practice, when it is necessary to carry out treatment and assess its effectiveness according to the modern principles of "diagnosis at the point of treatment", which should objectively ensure both therapeutic and diagnostic use of laser radiation affecting a biological object. This is necessary in order to identify positive (negative) physiological and clinical effects, to ensure timely correction of negative effects of laser exposure on a living organism or the absence thereof. This methodology determines the necessity and validity of considering the general biological concept of interaction of laser radiation (LI) with biological warfare as a therapeutic and diagnostic one.

Both primary and secondary effects, as well as functional changes in biological warfare caused by them and the pathological process, can be registered by modern technical means and used for the purposes of optometry of the course and assessment of the effectiveness of treatment using exposed laser radiation and laser medical equipment and medical technology [1,7,11,12].

For example, microbiological monitoring of the state of the body using a laser-fluorescence complex of the "Spectrolux-MB" type seems to be a technological and intellectual means of indirect diagnosis of tuberculosis, i.e. without determining the species specificity of the pathogen, prompt mass screening of the population and dynamic monitoring of the correction process and selection of an effective drug treatment strategy based on an indirect sign - a change in the amplitude-spectral characteristics of plasma blood [2].

The development of LASER diagnostic technologies is raman fluorescence diagnostics.

The advantages of this new and promising technology for many branches of medicine are the compactness and portability of the equipment used, high resolution of about 1Å, sensitivity and reproducibility of measurement methods, small measurement error, the possibility of using microvolumes of the studied material, the

absence of distortion of the captured signal and the influence of background light on the measurement results, the ability to normalize the signal in real time. An important feature of this method is the ability to suppress the Rayleigh scattering signal by cutting it with an Edge filter [9].

Raman fluorescence spectroscopy methods and their technological and hardware implementation are playing an increasingly important role in biophysics, microbiology and medicine. At the same time, the tasks of identification and structural characterization of organic molecules, including monitoring of their structural changes, measurement of the concentrations of substances included in the sample, come to the fore. Laser spectroscopy is widely used to control processes in chemical industries, to analyze product quality in pharmacology and food industry, to detect counterfeits, to detect narcotic and potent substances, and to analyze water pollution. Raman spectroscopy, which allows you to unambiguously recognize organic molecules by the spectra of inelastic light scattering, due to the excitation of a large number of different specific vibrational and rotational modes, is the best suited for these purposes. Raman spectroscopy is one of the most accurate methods for analyzing organic substances [10,13,14].

Fluorescence and Raman radiation are used to diagnose the state of tissues and organs of biological organisms in normal and pathological conditions, namely in diseases and processes of microbial and neoplastic nature, as well as in other types of their pathology [3-7].

An analogue of the claimed device is the "Raman spectrometer", patent: US 7403281 B2. The patent describes a system, method and device for obtaining the Raman scattering spectrum of a sample. An integrated Raman spectrometer is available in one version. In another embodiment, a portable Raman scattering spectrometer is provided. There is a variant of a Raman scattering spectrometer comprising a collimating ray tube to transmit excitation radiation to external optical systems such as a microscope, telescope, or cameras. In the following embodiment, a method is used to correct the Raman scattering spectrum by subtracting the background interference spectrum. In another embodiment of the spectrometer, a method is used to subtract the fluorescence spectrum from the Raman scattering spectrum.

The disadvantages of the device are the lack of a fluorescence signal in the scattered light spectrum, which carries part of the information about the data obtained; large dimensions; impossibility of use for medical purposes.

The prototype of the claimed device is "Raman and photoluminescence spectroscopy", patent: US 7362426 B1. The patent describes systems and methods for simultaneous Raman and photoluminescence spectroscopy. In the scattered light, the components of the Raman signal and the photoluminescence signal are isolated and separated. The first detector allows you to obtain a component of Raman scattering, and the second detector - photoluminescence.

The disadvantages of the device are its large dimensions and the impossibility of using it for medical purposes.

The medical and technical result achieved by using the described device is the creation of a medical and biological diagnostic complex of a wide range of applications based on a highly sensitive, small-sized, portable raman fluorescence spectrometer with the possibility of its autonomous power supply and containing special nozzles for the study of extra- and intracorporeal objects in liquid, solid and bulk states, as well as providing the possibility of research tissues and smear-prints by optical method with registration of their raman-fluorescence spectral characteristics using SERS substrates that amplify the signal of at least 10 6-1010 and a device in the form of a specialized camera that provides image visualization and a picture of the distribution of fluorescence over the area of the object. In this case, a single hardware and software technical solution is used.

The result is an increase in the sensitivity, specificity and accuracy of the diagnostic complex, an expansion of the range of its medical application, miniaturization of the complex, its use as a portable and/or autonomous field hardware and software device. The complex provides the possibility of using it for the study of tissues and organs in normal and pathological conditions, both *in vivo* and *in vitro*, both locally, i.e. pointwise, and in terms of the area of the object of study. These capabilities are determined, in general, by spectral and energy parameters and adequate dose-dependent biological effects under the influence of laser radiation on the object under study, implemented at various levels of its organization.

This result is achieved by the fact that the described raman-fluorescence complex for diagnosing the state of human biological tissues in health and *in vitro* pathology (Figure 1) includes: a laser with a laser filter (1); a system of mirrors and lenses (2); a system that collects signals emanating from the object of study and the object of normalization, and a cutting filter (3); a spectrometer with a CCD camera, which provides feedback to the control of the laser and Raman scattering and fluorescence recording (4); a personal computer (5) on which special software is installed that implements algorithms for monitoring, diagnosing and correcting the state of the subject and/or biological tissues of a person in health and pathology. Software processing, calibration, normalization and scaling of the data obtained are carried out. Various types of attachments and modifications of the device are used. *For in vivo* analysis, a fiber-optic cable is used, *for in vitro* analysis, other different types of nozzles are used, and a microscope is used for image imaging. For *in vitro* studies of microdoses of biological fluids and tissue smears, special silver nanostructured SERS substrates are used, on which the liquid phase of the biological object under study is applied using a micropipette. visualization of the image and the fluorescence distribution pattern, a specialized camera is used.

The declared medical complex for raman fluorescence diagnostics of the state of human tissues in normal and pathological conditions consists of two components that are structurally, functionally combined and containing a single program, namely the complex of *in vivo* raman-fluorescence diagnostics for biomedical research and the complex of *in vitro* raman fluorescence diagnostics for medical and biological research, whereby for the preliminary extra- and intracorporeal search for a pathological focus, infection, tumor or other pathology and the subsequent collection of material from it using traditional methods, *in vivo* diagnostic components are used, and *in vitro* studies – *in vitro* components Diagnostics. At the same time, the results obtained complement each other and increase the sensitivity of the hardware and software diagnostic solution. This is ensured, in particular, by the fact that the indicators of *in vitro studies* on SERS substrates increase the sensitivity of diagnostics by 106 times or more, which makes it possible to detect the initial manifestations of pathology that are not available for *in vivo* studies - that is why we consider the proposed hardware-software complex of raman-fluorescence diagnostics in the hardware-structural and software unity of the utility model.

Here are possible examples and options for the use of raman-fluorescence diagnostic complexes in biomedical research, both *in vivo* and *in vitro*: For identification, indication and differentiation of microorganisms, as well as for determining the sensitivity of microbes to antimicrobial drugs and for monitoring blood plasma. These studies are carried out *in vitro* using SERS substrates on a raman fluorescence diagnostic complex in combination with a vertical nozzle or microscope, as well as scanning on the surface of the sample under study during the measurement. Giant Raman scattering method on SERS substrates increase the detection sensitivity and specificity of identification of certain microorganisms, such as microorganisms that contain pigments or have Raman-active waste products, compared to other existing express methods (Figure 1, 8, 9).

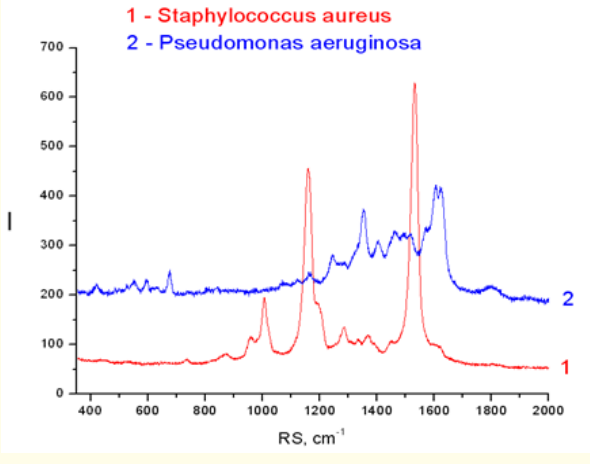

Figure 1: Comparison of spectra of *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

These studies are carried out both *in vitro* and *in vivo* on the complex of raman fluorescence diagnostics. It is possible to combine the RAMAN-fluorescence diagnostics complex with any of the included nozzles and with a microscope. *In vivo* research, a spectrometer with a fiber-optic cable is used when working with the object of study (Figure 2, 3).

For the diagnosis and study of tumor-like formations, both benign and malignant, and healthy tissues *in vivo* and *in vitro*. These

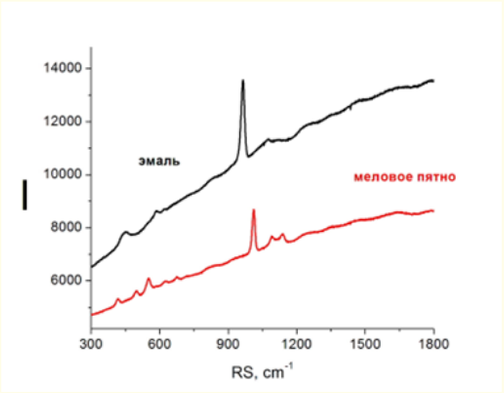

Figure 2: Finding of Raman lines of hydroxyapatite, calcium apatite and fluorapatites in the chalk spot (this spot occurs with fluorosis) and in the enamel.

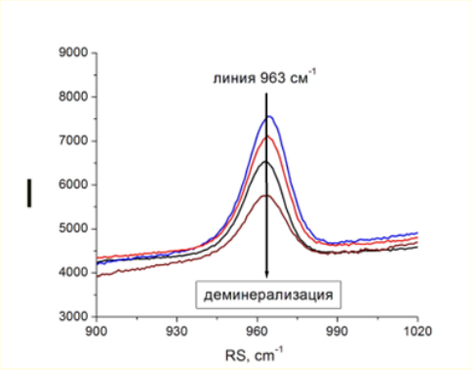

Figure 3: Decrease in the intensity of the Raman line of hydroxyapatites (963 cm^{-1}) with the development of caries due to a decrease in the degree of tooth demineralization.

studies are carried out both *in vitro* and *in vivo* on the complex of raman-fluorescence diagnostics. It is possible to combine the Raman Fluorescence Diagnostic Complex with any of the included attachments and with a microscope. By simultaneously measuring Raman scattering and fluorescence signal on a bulk piece of tissue under study and on a thin layer of the liquid phase of the tissue smear, the spectra of benign, malignant and healthy tissues are recorded on SERS substrates, the analysis of which reveals a number of characteristic differences. *In vivo* research, a spectrometer with a fiber-optic cable is used when working with the object of study (Figure 4.5).

For the diagnosis of drugs (Figure 6, 7).

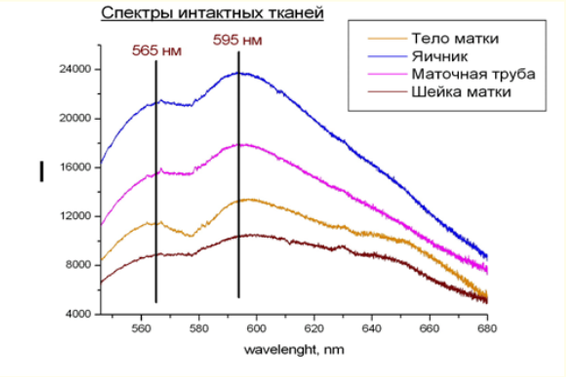

Figure 4: Spectra of intact tissues.

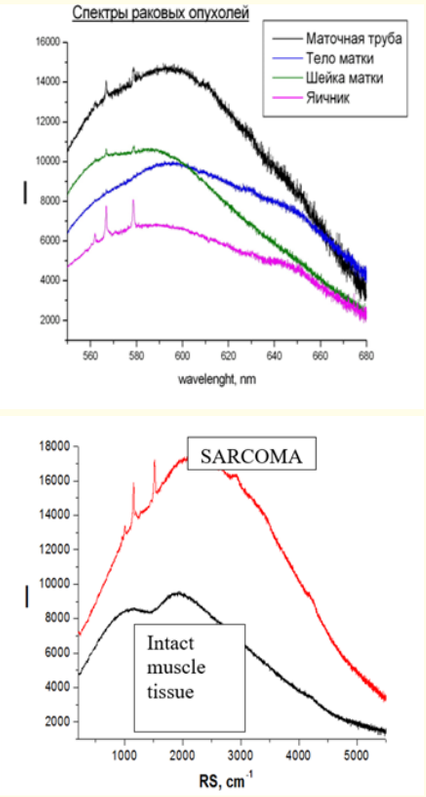

Figure 5: Spectra of cancer tissue.

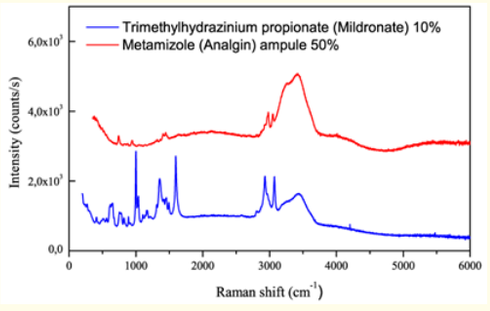

Figure 6: Upper spectrum (red) – metamizole spectrum; lower (blue spectrum) – spectrum of Mildronate.

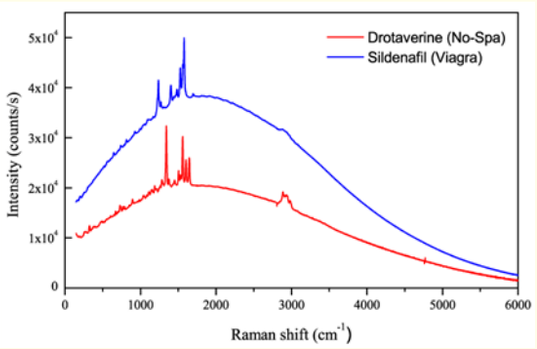

Figure 7: Upper spectrum (blue) - Viagra spectrum, Lower spectrum (red) - No-Shpa spectrum.

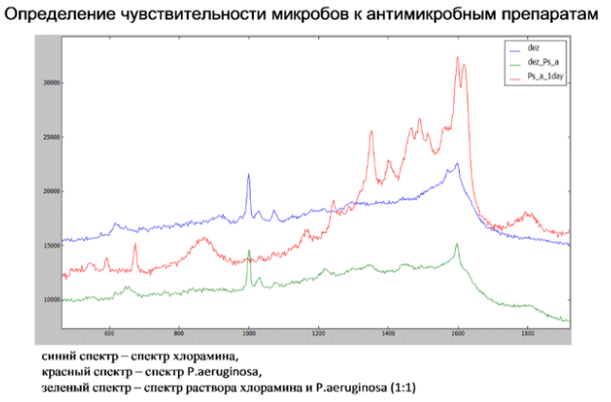

Figure 8: Arrest of the microbe spectrum under the influence of an antimicrobial drug.

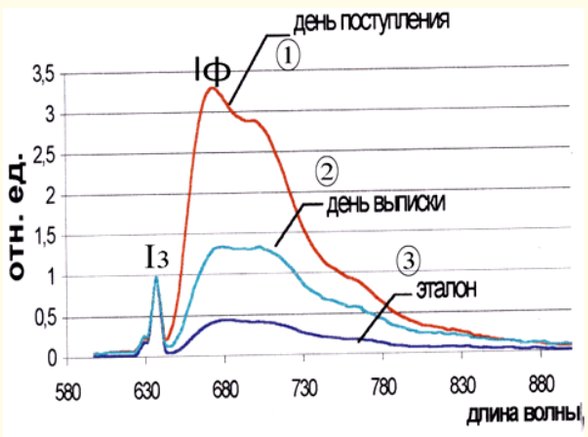

Figure 9: Decrease in the intensity of fluorescence of the gastrointestinal substrate against the background of taking antibiotics (dibacteriosis).

These advantages are distinctive for a similar type of installations manufactured both in Russia and abroad, which makes the declared hardware and software complex of raman fluorescence *in vivo* and *in vitro* diagnostics a priority for today and in the coming years.

The availability and high sensitivity, small size and autonomy of the raman fluorescence diagnostics complex allows it to be used in wide clinical practice.

To date, the basic elements of portable raman fluorescence complexes have been developed: low-noise CCD cameras operating at room temperature, high-power compact semiconductor and solid-state lasers, ultra-cut-edge Raman filters that provide transmission of scattered laser radiation at the level of 10^{-6} at a shift of 100 cm^{-1} from the laser line.

The complete set of portable raman fluorescence complexes with SERS-active substrates allows you to increase the amplitude of scattered light, and proportionally reduce the volume of analytes. Portable raman fluorescent complexes can already be used to identify microdoses (up to several dozen molecules) of organic substances. It is assumed that cheap SERS active substrates made on the basis of the new technology, in combination with a miniature and cheap Raman spectrometer, will soon be widely used in many areas of science and human life:

- In medicine, for the diagnosis of infectious and non-infectious diseases in its various sections and branches - oncology, dentistry, gynecology, dermatology, immunology. For the study of various biological fluids of the body
- In biology and microbiology for the study of cultures of microorganisms, cells and tissues
- In physics, to study the basics of spectral analysis on the example of Raman scattering and fluorescence, as well as absorption spectra. Use of optical methods for studying physical objects
- In nanotechnology for the study of all types of nanostructures
- In organic and inorganic chemistry, the study of the mechanisms of reactions and characterization of synthesis products
- In materials science in the study of all types of inorganic and organic materials, including semiconductor elements
- In mineralogy in the study of precious stones and minerals
- In the course of forensic and customs examinations
- In the pharmaceutical industry, in the development and control of the production of tablet forms and creams

- In industry for quality control of food, animal feed and crop products
- For environmental assessment of the environment, soils and water resources.

This makes it possible to objectively assess the patient's rehabilitation process, the effectiveness of the choice of the preferred antimicrobial drug and its individual clinical efficacy almost in real time on the principle of feedback ("bedside diagnostics" - diagnosis at the place of treatment).

The use of raman fluorescence diagnostic complexes has significant advantages, is clinically expedient and can be recommended for the assessment of both pathogenetic and sanogenetic processes.

Thus, the proposed complex of raman-fluorescence diagnostics is designed to measure, record and interpret Raman scattering spectra and/or photoluminescence spectra of biological fluids, skin, mucous membranes and tissues of the body (*in vivo* and *in vitro*), to diagnose and treat a wide range of diseases and functional disorders of the human and animal body, as well as to analyze the contents of tablets. capsules, powders and liquids. In addition to the applications described above, this complex can be used to assess the human environment.

In general, the proposed complex of raman-fluorescence diagnostics consists of a spectrometer with a diffraction grating, which has no moving parts, and a laser assembly rigidly connected to it. The spectral range of the complex covers the region of molecular vibrations of organic and inorganic substances, which makes it possible to measure the Raman and/or fluorescence spectrum of the object under study within a few seconds, to determine the spectral position and relative intensity of Raman and fluorescence spectral lines — a kind of "fingerprints" of the object under study, to search for and compare these "fingerprints" with the spectral database of known objects.

Express analysis does not require preliminary preparation or processing of the objects under study. Their identification can be made directly in closed containers, vessels, bottles, flasks and ampoules with transparent or translucent walls.

The use of the raman fluorescence diagnostics complex does not require a special room, so the device can be used to control organic and inorganic substances both at the initial and final stages of production, and during transportation. The analysis results are processed using a convenient user program interface.

The operation of the raman fluorescence diagnostics complex is carried out using a personal computer (PC) via a USB port. The software of this device allows for qualitative and quantitative analysis of the obtained spectra.

The device consists of a laser radiation source, a spectrometer of the Czerny-Turner type [15], a system for collecting, filtering and analyzing scattered radiation. registration of amplitude and spectral characteristics of scattered radiation. Access to the hardware of the raman fluorescence diagnostics complex and the data obtained is carried out through a built-in microcontroller with a USB interface. The software allows not only to obtain the raman fluorescence spectra of various objects, but also to recognize them by comparing them with reference ones.

The following options for working with the raman-fluorescence diagnostics complex are possible (samples of installations are shown in photos 1a, b).

Without the use of SERS substrates

- For *in vivo* research, a complex of raman-fluorescence diagnostics with a fiber optic cable termination is used.
- For *in vitro research,* a complex of raman-fluorescence diagnostics is used with any types of nozzles and in combination with a microscope with a device for mounting a spectrometer.

Using SERS substrates

For the analysis of microdoses of the substances under study, specialized nano-structured SERS substrates on which micropipettes a drop of the test substance is applied or a smear of biological warfare is made.

In combination with a nozzle for vertical mounting of the spectrometer.

In combination with a device for vertical mounting of the spectrometer. For this complex, in combination with a microscope, the optical scheme consists of a microscope module and a spectrum analyzer module, articulated using a beamsplitter module. For visual examination of the object, the standard mode of operation of the microscope using eyepieces and/or a video camera is used. The configuration of the device allows the use of any optical illumination scheme of the object both "for reflection" and "for transmission".

For some tasks, for example, when searching for cancer cells in a biological substrate or when analyzing blood plasma for the presence of microbes and viruses in it, it is planned to use scanning xyz in the complex of raman fluorescence diagnostics - a movement that provides the recording of spectra from the studied sample along a given trajectory with a given step. The spectra of inelastic light scattering and fluorescence signal can be measured with a spatial resolution of 3 μm. It is in this size that it is possible to focus the laser beam on the object. Block diagram and algorithm of the study on the hardware and software complex of Raman-fluorescence diagnostics is shown in Figure 11 and photos 1a, b.

СРАВНИТЕЛЬНАЯ ОЦЕНКА ДИАГНОСТИЧЕСКОЙ ЭФФЕКТИВНОСТИ РАМАН-ФЛЮОРЕСЦЕНТНЫХ МЕДИЦИНСКИХ ТЕХНОЛОГИЙ

- Высокая точность диагностики: 90-95%
- Высокая скорость проведения диагностики: в 10-1000 раз быстрее существующих методов
- Средняя ценовая категория диагностического оборудования: от 500 тыс. до 1,5 млн. рублей
- Простота в использовании: не требует дополнительного специального образования
- Низкая себестоимость 1 исследования: в 10-100 раз дешевле, т.к. Практически не используются реагенты
- Компактность и мобильность комплексов

Метод	Стоимость одного исследования, руб.	ДЛИТЕЛЬНОСТЬ ИССЛЕДОВАНИЯ	Экспрессный и Интегральный характер оценки эффективности лечения и его коррекции	Возможность исследования большого количества проб (in situ)	Определение эффективности препарата (антибиотик И антисептик,онко препараты,лучевая терапия) в экспресс режиме
Бактериологический	200-1000	7-10 дней	-	-	-
Газожидкостная хроматография	200-680	30-100 мин.	-	-	-
Масспектроскопия	200-600	40-60 мин.	-	-	-
Раман-флюоресцентная диагностика	100-500	1-2 мин.	+	+	+
Полимеразная цепная реакция	ОТ-1000	Несколько часов	-	-	-
Реакция иммунофлюоресценции	500-1000	40-60 мин.	-	-	-
Высоковольтный электрофорез	-	60 мин.	-	-	-

Figure 10: Expressiveness and efficiency of Raman-fluorescent technologies.

Thus, the proposed hardware and software complex for raman fluorescence diagnostics will technically, technologically and methodologically allow (Figure 9, Photo 1b). With a high degree of sensitivity embedded in it with equipment and software, expressively, online, objectively and reliably carry out the most important stage of the examination of patients with diseases and processes of microbial and neoplastic nature - diagnosis of the disease, monitoring of its course and the rehabilitation process as a whole. In addition, the proposed medical technology makes it possible to identify drug support products and assess their effectiveness.

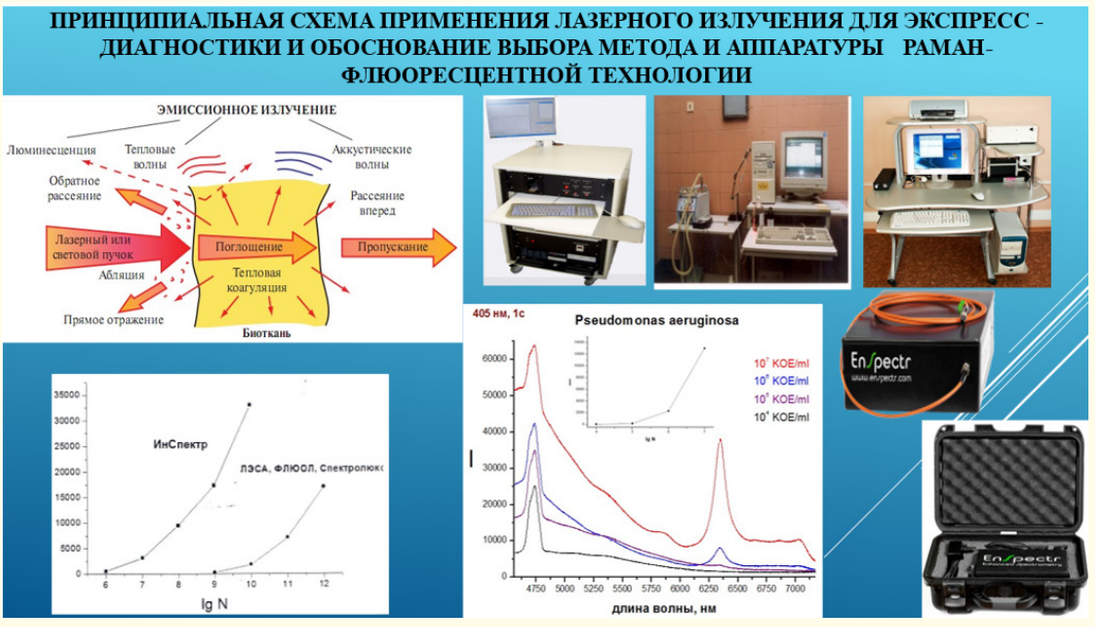

Photo 1a: Samples of laser medical devices for medical purposes and recorded spectra.

Photo 1b: Prospects for the application of Raman-fluorescence technologies.

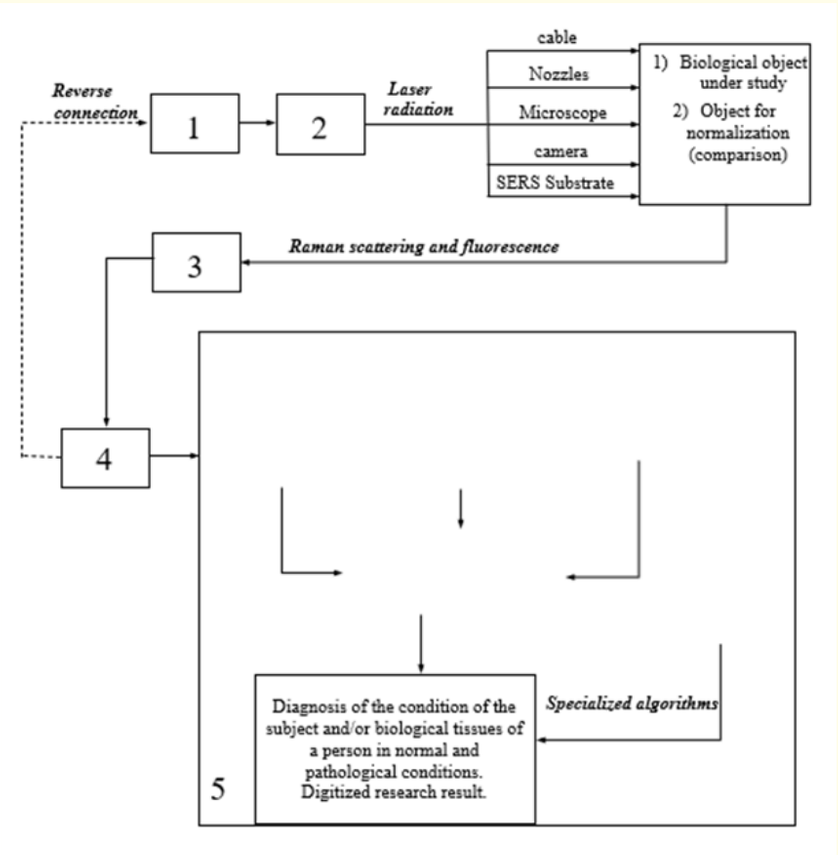

Figure 11: Structural diagram of the ramane-fluorescence complex for multifactorial monitoring and correction of human condition.

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