sciendo RIGA TECHNICAL UNIVERSITY
Electrical, Control and Communication Engineering
ISSN 2255-9159 (online)
ISSN 2255-9140 (print)
2019, vol. 15, no. 1, pp. 39–46
doi: 10.2478/ecce-2019-0006
https://content.sciendo.com

Raman Spectroscopy Principles for *in vivo* Diagnostic by Ellipsoidal Reflectors

Mikhail Bezuglyi* (*Igor Sikorsky Kyiv Polytechnic Institute, Kiew, Ukraine*),
Natalia Bezuglaya (*Igor Sikorsky Kyiv Polytechnic Institute, Kiew, Ukraine*)

***Abstract* – The paper presents the analysis of the functional abilities of measurement tools with ellipsoidal reflectors for Raman spectroscopy. The investigated structural scheme of the setup is intended for use in Raman spectroscopy *in vivo* by ellipsoidal reflectors. The setup can be used as a prototype for development of a device for non-invasive control of sugar level. Additionally, the investigation demonstrates the efficiency of ellipsoidal photometry method for registration of Raman scattering signal on test-solutions. The testing was performed for different sugar concentrations with the laser radiation wavelength 980 nm. In addition, the selecting principles of laser radiation source parameters (including beam diameter and power) were investigated. During the research, the data about spatial distribution of the backscattered light in human shoulder and finger tissues during photometry by ellipsoidal reflectors were received. The procedure involves application of Monte Carlo simulation. The dependency of the external and middle ring illuminance of photometric images on the diameter and power of the laser beam is represented based on the zone analysis.**

***Keywords* – Biomedical electronics; Biomedical optical imaging; Biophotonics; In vivo; Mirrors.**

I. Introduction

The investigation of biological media on the molecular level is based on the inelastic scattering, which occurs during energy exchange between a photon and a molecule. Such investigations cover structure of the molecules and the environment around them, as well as intermolecular bonds. Such scattering can have fluorescent or Raman (combined) nature. Methods, based on the fluorescent scattering (like optical fluorescent microscopy), have high sensitivity. However, biological media (BM) samples are under impact of perturbation by intrusive labelling with fluorescent probes and antibodies. Additionally, various destructive processes occur, such as cell fixation or lasing, and in most cases research is performed under *in vitro* experimental conditions. The measurements *in vivo* conditions (based on Raman spectroscopy) allow investigating both atomic and molecular structure of BM. During such measurements, results of non-invasive investigations with stable chemical specificity are used [1]. The following methods (based on the Raman scattering) were used for analysis of BM molecules: near-infrared (NIR) dispersive Raman [2], Fourier transform Raman (FT-Raman) [2], surface-enhanced Raman (SERS) [3] and ultraviolet resonance Raman (UVR) spectroscopy [4]. Additionally, modern methods based on the stimulation Raman, tip-enhanced Raman and coherent anti-Stokes Raman scattering were applied [5]. However, not all methods of Raman spectroscopy are suitable for non-invasive research. Such effect refers to:

- long collection times (for FT-Raman);
- complicated introduction and tracking of the traced amounts of functionalized nanoparticles in the human body (SERS);
- appearance of photolysis of the BM sample and its further destruction;
- weakening of the resulting signal by fluorescence emission;
- mutagenicity of UV radiation.

Application of non-linear processes for description and increasing of the signal level can have significant limitations, associated with the necessity to use complex ultrafast laser systems. Such limitations negatively affect the expedience of clinical researches *in vivo* in the real time [1]. Due to the mentioned reasons, the most suitable method for non-invasive research is near-infrared (NIR) dispersive Raman. Additional significant advantage of NIR Raman spectroscopy is absence of fluorescent scattering inside tissues. The effect can be reached due to higher intensity and overlapping of the combination scattering during use of NIR range. As it was discovered during literature survey [2], the optimal wavelength should be more than 800 nm. Reduction of efficiency of operation of silicon CCD detectors on the wavelengths 1000–1100 nm, which corresponds to the Raman fingerprint excitation region for 850 nm, is a limitation.

The main task of NIR Raman spectroscopy is increase of the sensitivity of receiving channel for signal registration and treatment methods (statistical, chemical, and morphological methods). The reason is the fact that only 1 in 10^8 photons can be shifted by Raman and increase of the quantity of photons can lead to destructive changes in the molecules [1]–[4].

NIR Raman spectroscopy has become a potentially powerful tool for determining the optical properties and biochemical composition of human tissue [3]. The reason is that the illness progress is accompanied by chemical changes. It can be used for minimum invasion diagnostic of tissues *in vivo* in real time in cases when biopsy is impossible. Such cases include coronary arteries illnesses and Alzheimer's disease, or the cases where the high frequency of fault positive screening tests leads to unnecessary biopsy procedures, like in cases of breast cancer [1].

In modern clinical research, Raman spectroscopy *in vivo* is used in various areas, namely, monitoring of cataract formation [6], molecular diagnosis of atherosclerotic lesions in coronary

* Corresponding author.
E-mail: m.bezuglyi@kpi.ua

arteries [7], diagnosis of diseases in human tissues [8], diagnosis of skin cancer [9] and examination of hollow organs using endoscopic equipment (cervix, oesophagus, rectum, and other) [10], monitoring of peripheral blood gases and analytes [11].

Investigation and control of glucose level in blood is one of the most common applications of *in vivo* Raman spectroscopy. According to the data of the Ministry of Health of Ukraine, as of 2016, 1 200 000 citizens had diabetes. 78 000 of them were insulin dependent patients, which requires daily running control of blood sugar level. The number of patients in the European countries equals to 58 million among adult people aged between 20 and 79 years, according to the data of the International Diabetes Federation as of 2017. The need for creation of non-invasive optical glucometer (OG) is obvious. The task is important and topical due to its value in control of glucose in organism for both kinds of patients: insulin dependent and with diabetes of Type 1.

The most widely used optical methods for non-invasive diagnostic of sugar level in blood include near infrared and mid-infrared spectroscopy, Raman spectroscopy, photoacoustic spectroscopy, optical coherence tomography, as well as glucose-induced polarization changes, the arrays of photonic crystal, and fluorescence technology [12]. According to the authors' observations, the methods and measuring devices, which operate on Raman spectroscopy, demonstrate the highest efficiency. Raman spectroscopy can be used for qualitative and quantitative analysis of a wide spectrum of glucose containing biological tissues due to high chemical stability, depth of penetration, and number of implemented methods for quantitative data analysis. The change of wavelength in the part of radiation, which interacts with the medium, is fundamental for optical control. Effects of light scattering and absorption appear during registration of optical light emitting for both forward and back scattering. The absorption during this process predominantly depends on structural water content, haemoglobin, protein, scattering centers in skin layers, and other factors. Scattering and absorption are specific for glucose molecules, and these effects can be used during their detection in the multilayer object volume. Thus, the investigation of Raman spectrum during transcutaneous measurements, which contains information about molecular energy level change under the influence of laser radiation, and further separation of scattered light allows conducting photometry [1]. It is important to consider that wavelength difference depends on the specific energy of chemical bonds, which determines characteristic peaks in the spectrum [13], and molecular specificity and minimal influence of water in blood spectrum allow forecasting the combination of glucose spectrum during non-invasive spectroscopy [14].

However, several unsolved issues remain, such as biophysical model of optical glucose metering and constructional optimization of separate blocks of non-invasive glucometers during solution of the tasks on registration, transformation, and treatment of data for improvement of blood sugar level determination accuracy.

The application of photometric systems with ellipsoidal reflectors (ER) for investigation of optical properties of biological media has proved its adequacy and authenticity [15], [16]. The existing prototypes of optical glucometers are generalized relatively to Raman spectrums, and allow evaluating physical and mathematical models of biophotonics. Thus, application of new methods and registration systems, such as photometry by ellipsoidal reflectors, allows solving the tasks of optimization of technical solutions of OG.

Therefore, the purpose of this work is development of preconditions for creation of the tools with ellipsoidal reflectors for *in vivo* Raman spectroscopy and prototyping of optical non-invasive glucometer on its basis.

II. General Principles of Raman Spectroscopy by Ellipsoidal Reflectors

For the development of structural and fundamental scheme of the measurement unit by Raman spectroscopy (Fig.1) the typology of measuring devices with ellipsoidal reflectors [16] was considered, which was previously tested in numerous experimental researches [15]–[18].

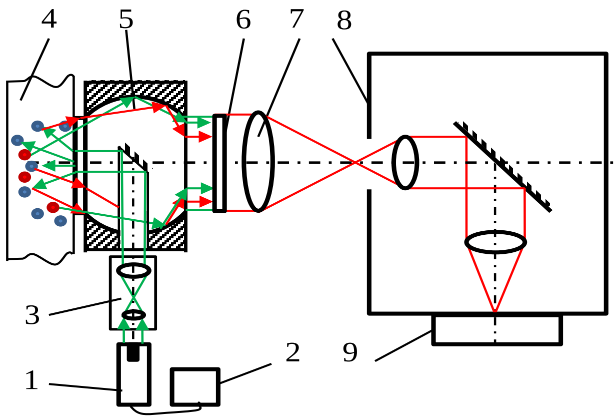


Fig.1. Structural and fundamental scheme of the setup for *in vivo* Raman spectroscopy by ellipsoidal reflectors: green colour – incident light; red colour – Raman scattering; 1 – laser light source; 2 – power regulator; 3 – optical collimator; 4 – biological object; 5 – optical block with ER; 6 – filter; 7 – lens; 8 – spectrograph; 9 – CCD camera.

The practical measurements are supported by directing of the laser source radiation of necessary power and diameter. This radiation is regulated by optical collimator into the cavity of the optical block with ellipsoidal reflector. In the geometric center of the reflector cavity plane mirror is situated under angle 45° relatively to large and small semi-axes of ellipsoid of revolution. The position allows redirecting of optical axis of light by 90° toward the investigated section of a biological object. The interaction of optical radiation on the atomic and molecular level leads to photo-excitation of molecules in specific components and is represented as the backscattered Raman signal. In most cases the ellipsoidal reflector is installed closely to the surface of the investigated object by the area, which contains its first focus [16], [18]. Due to that fact, and correct selection of reflector parameters and incident radiation diameter, capturing of almost all radiation leaving the biological object due to back scattering is performed. Optical properties of the ellipsoid of revolution with internal mirror surface allow transmitting the scattering spot from the BM plane to the plane of the second focus of the ellipsoid with further adjustment of the optics directing it into the spectrograph inlet aperture. The optical block outlet filter can cut off (edge filter) or select (liner and notch filter) the necessary component of Raman spectrum for qualitative and/or quantitative identification of the diagnosed substance. CCD camera is used for convenient fixation of shifting spectrum and further regressive analysis.

III. Non-Invasive Optical Glucometer With Ellipsoidal Reflectors

A. Technical Preconditions

Based on the developed and tested functionalities of the photometers with ER, a schematically technical solution of non-invasive OG with ellipsoidal reflectors is proposed (Fig. 2). The operation of the device is based on the extension of infrared radiation from laser diode 1 to the necessary diameter using telescoping system 2. Then the radiation through the inlet window 3 is delivered to prism 4, which contains mirror plane, and after deviation of the optical axis by 90° is directed into the investigating object (human finger), which is situated on slide glass 5. The interaction between laser radiation and the finger leads to the appearance of optical radiation from its both parts in the direction of optical radiation propagation. Then the back-scattered light is collected by bottom ellipsoidal reflector and projected in the second focal plane of ER 6, in which the edge filter 7 for Raman spectroscopy analysis is placed immediately or at a small distance.

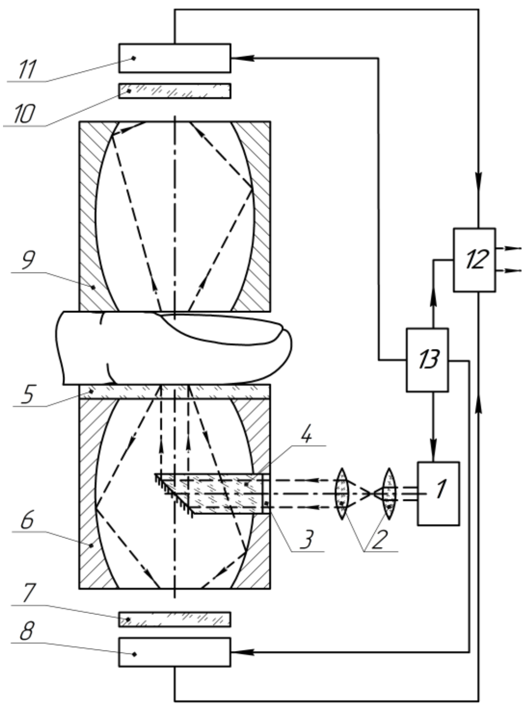


Fig. 2. The scheme of the optical glucometer with ellipsoidal reflectors: 1 – laser; 2 – telescoping system; 3 – inlet window; 4 – prism; 5 – slide glass; 6 – bottom ellipsoid; 7, 10 – edge filters; 8, 11 – photo diodes; 9 – top ellipsoid; 12 – processing system; 13 – power supply.

The separated signal then is delivered into the photo receiver 8 and further into the processing system. The top ER 9 is intended for collecting the light, which is transmitted through the finger and scattered forward. Edge filter 10 and photo detector 11 have similar functional purposes as in the case of registration of backscattered light for bottom ER. It is important to mention that radiation system at the current stage envisages both simultaneous use (not using) of two edge filters and their alternate entering into the beam path. The model for signal separation, which corresponds to different concentrations of glucose level in the investigated object, is not finally substantiated, thus such variability of use of the reference beam (without edge filter) at the prototyping stage in top and/or bottom channels of OG is possible and expedient.

B. Elementary Base of OG

Let's investigate preconditions, which became the basis for the selection of elementary base for OG with ER. It is important to note that recently a significant progress in investigation of glucose spectrum in near ultraviolet and visible light has been made [11]. However, considering the results of successful research of the glucose spectrum in infrared and mid-infrared spectral ranges [19], it analyses spectrum of absorption of glucose solutions with concentrations 3 mmol/l, 16 mmol/l, and 550 mmol/l, considering that the last one is the standard for the mentioned test solutions. Paper [17] showed that peak values can be observed on the wavelengths 960–990 nm, which can be used for determination of glucose level, considering the absorption and scattering spectrum of water, melanin, and deoxyhemoglobin. Considering that, the laser diode LJ980LD100N4T was selected as the radiation source, the nominal power of which during investigations was decreased 20–50 times. The constructive analog of FD-344-01 was selected as the photodetector. Except for energetic and spectral characteristics, the geometrical sizes of working window of mirror ellipsoids of revolution were considered. From the other side, the ER geometry was defined considering the anatomic properties of the investigated object and set by the focal parameter 7.5 mm. The production of ER was performed using the trajectory copying method, however, the authors additionally investigated other methods of forming of internal reflection ellipsoidal surface, including metallization of plastic detail, which was produced by 3D printer.

The details of the body and fastening elements of the printed circuit boards, laser diode and prism were produced by 3D printing from coPET plastic. Projecting of one-layered circuit boards without using of solder mask was performed using DipTrace. Circuit boards were manufactured using photo resisting technology of obtaining the scheme on the foiled textolite with the thickness of copper foil layer 36 µm. The scheme of the circuit board was transferred on the textolite with the help of the ultraviolet lamp with exposure time 10 minutes and further manifestation after rinsing in the solution of soda ash NaOH. After that, circuit board was immersed into the solution of bleached iron $FeCl_3$ and remained there during 15 minutes to receive the scheme of current conduction traces.

C. Prototyping of OG With ER

The prototype of the non-invasive optical glucometer with ellipsoidal reflectors has modular construction and consists of the laser diode power module, photo diodes power module, control block module, and optical module. All control elements and power module elements are arranged in one electronic block (Fig. 3). The optical-electronic block of OG contains an optical module, which consists of two ER, prism, edge filter (the prototype represented in Fig. 3 ensures registration of Raman spectrum only in the backscattered light), and laser diode and photodiodes. The 0.96 Inch OLED I2C Display is situated on the cap of the optical-electronic block.

Fig. 3. The non-invasive optical glucometer prototype with the ellipsoidal reflector.

To simplify prototype development AVR microcontroller of type Atmega 8 was used. DC-DC Step-Up modules were used as the power modules, which operate in microcircuits XL60, and set up for output voltage 5 V and 20 V. A 5 V line supplies power to the microcontroller and transistor key, which is based on the n-p-n transistor 2N2222. A 20 V line feeds photodiode control plate. The information from photodiodes is delivered to analog ports of microcontroller and then transformed into digital form by ADC of microcontroller.

The modes, in which the measurement of full-scattered flux or signal of Raman scattering in non-invasive OG with ER can be performed, ensure registration of light in reflected and/or transmitted light. There can be various combinations and alternations of application of certain types of radiation sources with various spectral and energetic characteristics and edge filters. Applying the proposed working wavelength 980 nm, the specificities of parallel application of selected photodiodes for providing of OG operation in any of the possible modes are considered.

Unlike the analogs, photodiodes FD-344-01 are not typical optical-electronic devices, which are applied in biomedical sphere, thus they can have larger differences in output characteristics in various specimens. The first reason is complicated technological process of their production, thus during the registration of the light flux in several photometric channels (the presence of which is stipulated by the one incident flux), the corresponding setting of measuring system was performed. The measurement of outlet voltage in both photodiodes with parallel connection was performed under varying power of laser radiation and different modes of radiation incidence on the photo-sensitive elements, considering the control of dark current. The received values of outlet voltage during all investigated combinations of signal measurements from the first photodiode proved its constant dependency on the working voltage and integral sensitivity to current. However, for the second photodiode such dependency was not confirmed, which points at the necessity of load resistor recalculation. Repeated measurements with new values of resistance of series resistor (replacement of 1 kΩ resistor by 910 Ω one) demonstrated the dependency similar to that of the first photodiode with slightly lower amplitude, and that was considered using software during further projecting of OG. Such setting also allows receiving adequate results during photodiode switching in turn. The received outlet parameters allow using two photodiodes on transmission and reflection without impact of their position inside the device; and the result of research will be correct, which is the purpose of the setting.

Three solutions with different glucose concentrations – 3 mmol/l, 7.5 mmol/l, and 33 mmol/l – were used for device calibration. Minimal glucose value was selected for calibration, which corresponds to minimal norm prior to taking food, average value is a usual norm after food intake, and maximal concentration determines one of the most critical conditions – ketoacidotic coma. The solutions were placed inside the quartz cuvette with width 1 cm, and aligned in the ray flux instead of the biological object (human finger in Fig. 1). Research was performed under different values of laser radiation power using the edge filter in case of construction of OG with ellipsoidal reflectors, and also without them with conservation of all constructive and optical features of the functional scheme (Fig. 1). The results of the measurements are represented in Fig. 4–5.

As it can be observed in Fig. 4 and Fig. 5, the signal of Raman scattering is significantly higher in the backscattered light compared to the forward scattered light. Thus, an assumption can be made that further optimization of parameters and functioning of OG can be performed only in the reflected light. At the same time, in the transmitted light the dependency of Raman scattering light on the optical power during the use of ER is more linear, which can also significantly simplify the development of the mathematical model of OG functioning. The voltage of photodiodes during their use in the measuring channels of ER for both cases of transmitted and reflected light has more absolute values. The relative difference between signal levels with and without ER use is insignificant. The authors assume that such insignificant tendency for weakly scattering biological media, which are represented by glucose solutions, will have more important quantitative expression in case of the experiment is conducted on the real object, which contains multiple layers with different optical properties and is considered as optically turbid media [16], [18].

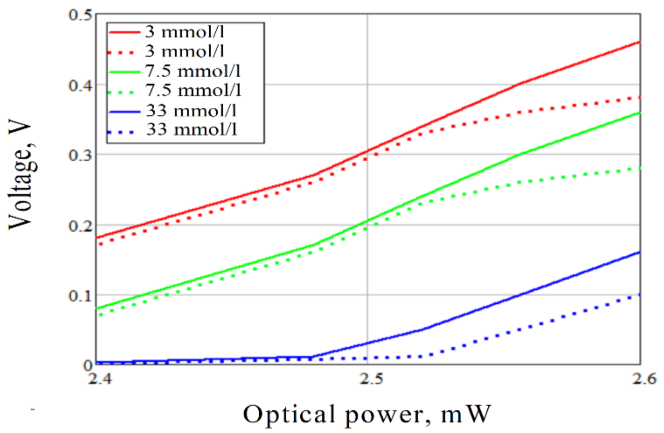


Fig. 4. Dependency of outlet voltage of photodiodes on the power of incident radiation in the system of the optical glucometer with (uniform line) / and without (dotted line) of ellipsoidal reflectors in the forward scattered light.

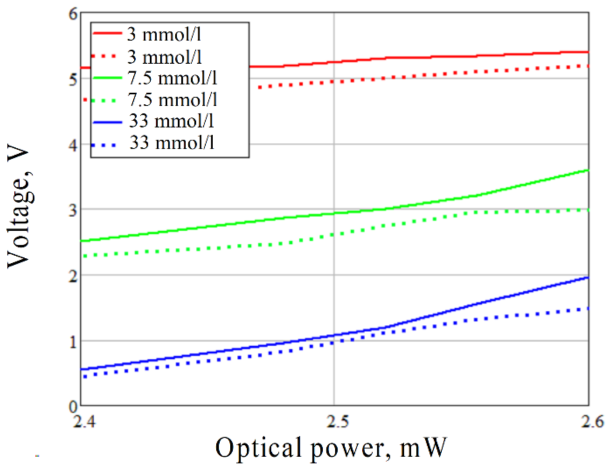


Fig. 5. Dependency of outlet voltage of photodiodes on the power of incident radiation in the system of the optical glucometer with (uniform line) / and without (dotted line) of ellipsoidal reflectors in the backscattered light.

Practical linearity of the obtained results can be explained by conducted measurements on standard solutions, which are devoid of dynamic influence of scattering spectrums of living biologic object and possible fluctuations, which are caused by oxygenation level and blood saturation inside the investigated area. Clinical research on the real biologic objects may require application of other measuring modes and even the change of radiation parameters, however, the expediency of application of ellipsoidal reflectors for increasing of specific weight of the registered light is obvious for the authors of the current research.

IV. Influence of Laser Beam Parameters on Raman Spectroscopy by Ellipsoidal Reflectors

Let's consider the critical values of the geometric size of inlet aperture (through which the laser radiation enters the ER cavity), and functional dependence of Raman signal on the power and width of the incident beam [1] during photometry by ellipsoidal reflectors in the reflected light. The device for glucose level control (Fig. 2 and Fig. 3) in the tissues of human shoulder and finger is the reference. To perform this, estimation of spatial distribution of the scattered light in the second focal plane of ER was performed, which can be analysed integrally by spectrograph (Fig. 1), or differentially by placing of CCD camera after filter [15], [16].

Optical properties like refraction index *n*, absorption μ_a and scattering μ_s coefficients, scattering anisotropy factor *g*, and geometric sizes of human skin layers and muscle tissues are presented in Table I. They illustrate the relative variation of multi-layered biological tissue properties on the wavelength 980 nm. Considering the modelled experiment, they determine the main principles of selection of radiation source parameters for ensuring effective Raman spectroscopy by ellipsoidal reflector.

TABLE I

Optical Properties of Human Skin Layers [18], [20]

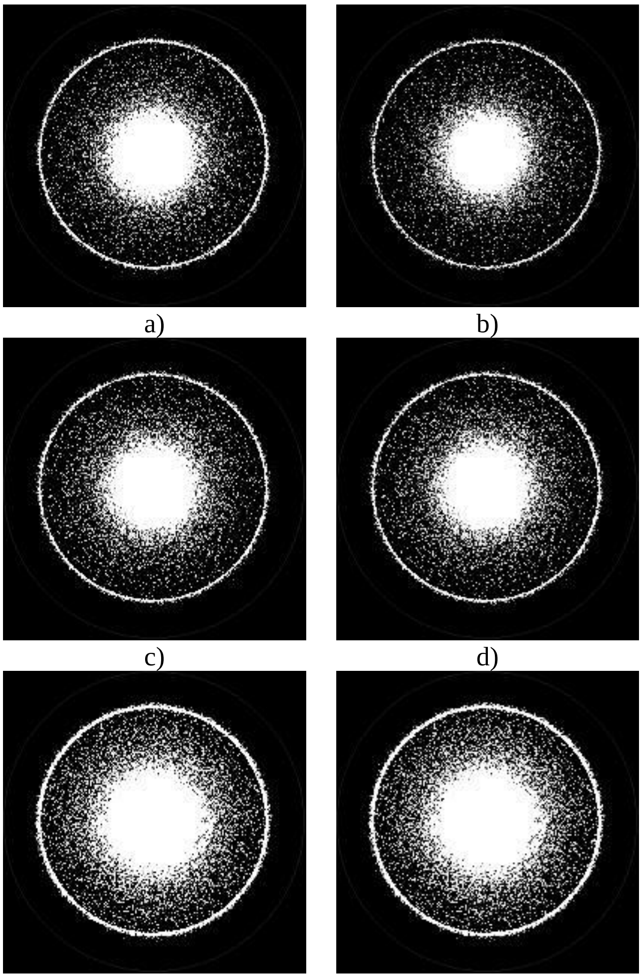
Skin layer	n	μ_a, cm^{-1}	μ_s, cm^{-1}	g	Layer thickness, cm	
					Shoulder	Finger
Corneous layer	1.5	0.1	100	0.8	0.0035	0.039
Epidermis	1.34	0.15	45	0.8	0.0107	0.0473
Derma	1.39	0.073	20	0.76	0.246	0.128
Adipose tissue	1.44	0.068	15	0.8	0.072	0.181
Muscle tissue	1.37	2	215	0.9	1	1

It is important to note that human skin layers on various body sections have constant optical properties, but different thickness. The selection of muscle tissue thickness is justified by creation of semi-infinite BM modelling, and thus the non-invasive nature of the experiment.

The illuminance dependency in photo-receiving section of the photometer with ER from cross-section profile of the central mode laser beam TEM_{00} for different human skin layers in the scattered light [18] is of tendentious and monosemantic nature. It has exponential nature depending on the human skin layer thickness, and the illuminance level of different zones during the aperture CCD photometry increases during the change of the laser beam cross-section profile from semi-infinite to uniform. The first one is typical of many practical realizations of Monte Carlo simulation of optical radiation distribution in biological tissues, while the second one is often used in real measuring devices due to the influence of transmitting optical elements. That is the reason why in the current investigation the uniform profile of incident beam was applied during simulation. Such conditions are close to real experiment conditions, and considering the results of [18] it can be easily extrapolated to other laser beam profiles.

The ellipsoidal reflector parameters are as follows: focal parameter 7.5 mm and eccentricity 0.66. Studying reflectors with the same focal parameters and different eccentricities using the mechanisms of ray-tracing in ellipsoid of revolution [16], it was observed that the illuminance of the middle ring of a photometric image decreases with an increase of the eccentricity to 0.8 in both the forward and back scattered light. At the same time, the illuminance of the external ring decreases until the value of eccentricity 0.6 is reached. The smallest illuminance of the corresponding area of photometric image shows the minimal effect of constructive parameters of reflectors on the ray deviation due to aberrations at the scattering spot projection from the first focal plane to the second. Considering the technological features of ellipsoidal reflectors production, the optimal eccentricity value is 0.66. Thus, the light scattering by human skin at this eccentricity value will be more concentrated in the areas of the middle and external rings of the photometric image.

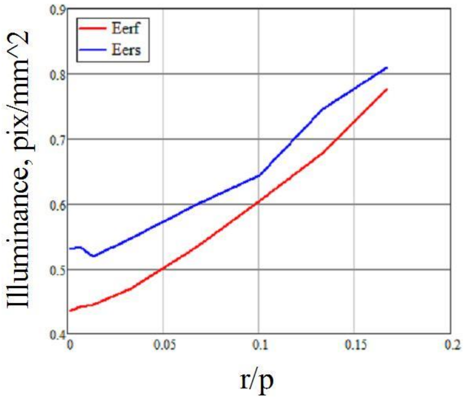
Fig. 6 illustrates the distribution of the scattered radiation on the photo receiver plane during the work with the ellipsoidal reflector in the reflected light, depending on the diameter of the incident beam.



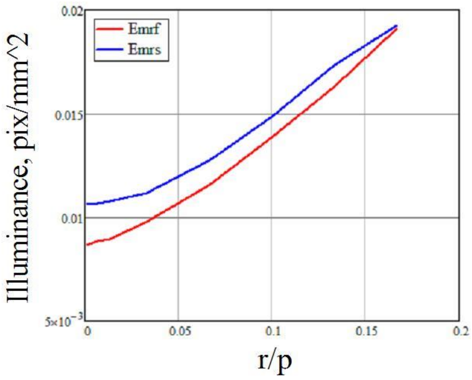
a) b) c) d) e) f)

Fig. 6. The distribution of the scattered light in the second focal plane of the ellipsoidal reflector in the reflected light for multi-layered model of human shoulder tissue (a, c, and e) and finger (b, d, and f) for the incident laser beam diameter 0.01 mm (a, b), 0.5 mm (c, d), and 1.25 mm (e, f).

As it may be seen from Fig. 6, there are differences in photometric images for backscattered light by shoulder and finger tissues, which have reduced visibility during increasing of incident beam diameter. At the same time, the influence of diameter value on the laser beam diameter, which tends to conditions of being infinitely small, is more noticeable. This calls for more precise analysis of spatial distribution of the scattered light, which can be captured by CCD camera. Fig. 7 represents graphs of dependency of relative illuminance (depending on the quantity of incident photons) of different zones of the second focal plane of ellipsoidal reflector on the diameter of the incident laser beam. The x-axis in Fig. 7 represents the ratio of the incident light beam diameter to the diameter of the operating orifice (double focal parameter) of ER.



a)

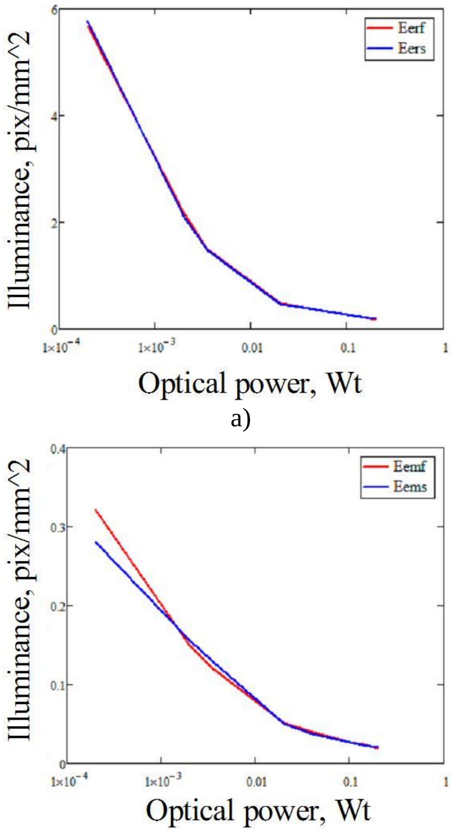


b)

Fig. 7. Relative illuminance of external (a) and middle (b) ring of photometric images in the backscattered light for human shoulder (blue line) and finger (red line) tissue depending on the laser beam diameter.

The illuminance of both external and middle rings of photometric images increases linearly depending on the diameter of the incident light. As it may be seen from Fig. 7, for small values of laser beam diameter such dependency is non-linear. However, it must be noted that it is complicated to reach such small diameters (less than 0.1 mm) in practice. For practical implementation, it is possible to consider the linear approximation in the range of important values for the real experiment. The quantity of light, which is captured in the external ring zone (Fig. 7a), is significantly larger than radiation concentrated in the middle ring. This indicates the increasing quantity of photons, influenced by the multiple reflections from the internal surface of ER [16] and demonstrates efficiency of using reflector for capturing of the reflected radiation. Multiple reflections from internal surface of ER can be distinguished by the part of the reflector side, which is more involved in the process of transferring radiation from the first focal plane to the second. In this case, if the lower part of ER [16] is involved, it can be asserted that double reflection is more typical of the rays with exit coordinates from the BM more distant from the revolution axis of the ellipsoid. If the upper part of ER is used [16], then the higher order reflections predominate in the nature of the rays, which are located near the boundaries of the aperture of the second focal plane, independent on their coordinates as regards the exit from the BM.

Let’s consider the influence of Raman signal power in the diffused reflected light (captured by ER) and the results of Monte Carlo simulation of optical radiation propagation in the system “BM + ER + edge filter” on the spatial distribution of the scattered light in the photo receiving plane of CCD camera. The Raman signal part changes in the range 10^3–10^6 photons, which corresponds to the incident laser beam power 0.2 mW to 0.2 W. Laser operates in the continuous mode at the wavelength 980 nm, and the capturing time in the CCD camera cell equals to 0.1 ms. Fig. 8 illustrates the dependency of the illuminance of different zones of the photometric images on the power of the incident laser beam on the surface of the human shoulder and finger.



a)

b)

Fig. 8. Relative illuminance of the photometric image in the scattered light for human shoulder tissues (blue line) and finger (red line), depending on the power of incident light power.

As it may be seen from Fig. 8, the relation of illuminance for both external and middle rings of photometric images with the power of the incident laser source almost coincide for the tissues of human shoulder and finger, and decreases exponentially. Due to the low illuminance, which is created by the Raman signal passing through the edge filter, the ellipsoidal reflector photometry on signals 0.2–0.02 mW is inexpedient. At the same time, the increasing of the incident power will lead to the over illuminance of all area of photometric image, and this also made application of aperture CCD camera ineffective.

In such case, during the selection of the laser source parameters (the beam diameter and power) to increase efficiency of the Raman spectroscopy by ellipsoidal reflectors the parameters of the optical power should be primarily considered, as the dependency on the incident beam diameter is linear.

V. Conclusions

The possibility and expediency of the application of the principles of photometry by ellipsoidal reflectors and fundamentals of Raman spectroscopy during the construction of the informational and measuring devices for control of sugar level in blood have been demonstrated. The received dependencies of illuminance of various zones of photometric images in the back scattered light for human shoulder and finger tissues on the diameter and power of laser beams reveal the specificities of the execution of Raman spectroscopy by ellipsoidal reflectors. Based on the mentioned information, in case corresponding experiments and clinical investigations are conducted, the authors consider it possible to create an adequate physical-mathematic model, which would connect the sugar level in blood during conduction of non-invasive measurement and calibration of device for the specific patient.

References

[1] A. M.-Jansen, Ch. A. Patil, and I. J. Pence, “Raman spectroscopy: from benchtop to bedside”, in Biomedical Photonics Handbook vol. II: Biomedical Diagnostics, T. Vo-Dinh Duke University Durham, North Carolina, USA: CRC Press, Taylor & Francis Group, 2015, pp. 759–803.

[2] D. Naumann, “FT-infared and FT-Raman spectroscopy in biomedical research”, *Applied spectroscopy reviews*, vol. 36, no. 2&3, pp. 239–298, 2001. https://doi.org/10.1081/ASR-100106157

[3] D. Cialla, A. März, R. Böhme, F. Theil, K. Weber, M. Schmitt, and J. Popp, “Surface-enhanced Raman spectroscopy (SERS): Progress and trends”, *Analytical and Bioanalytical Chemistry*, vol. 403, no. 1, pp. 27–54, 2012. https://doi.org/10.1007/s00216-011-5631-x

[4] K. T. Schomacker, J. K. Frisoli, C. C. Compton, Th. J. Flotte, J. M. Richter, N. S. Nishioka, and Th. F. Deutsch, “Ultraviolet laser-induced fluorescence of colonic tissue: basic biology and diagnostic potential”, *Lasers in surgery and medicine*, vol, 12, no. 1, pp. 63–78, 1992. https://doi.org/10.1002/lsm.1900120111

[5] O. Masihzadeh, D. A. Ammar, M. Y. Kahook, and T. C. Lei, “Coherent Anti-Stokes Raman scattering (CARS) microscopy: A novel technique for imaging the retina”, *Investigative Ophthalmology & Visual Science*, 2013, vol. 54, no. 5, pp. 3094–3101. https://doi.org/10.1167/iovs.13-11642

[6] Ch.-Ch. Lin, M.-T. Kuo, and H.-Ch. Chang, “Review: Raman spectroscopy – A novel tool for noninvasive analysis of ocular surface fluid”, *Journal of Medical and Biological Engineering*, vol. 30, no. 6, pp. 343–354, 2010.

[7] A. Mahadevan-Jansen, M. G. Sowa, G. J. Puppels, Z. Gryczynski, T. Vo-Dinh, and J. R. Lakowicz, “Diagnosis of atherosclerosis in human carotid artery by FT-Raman spectroscopy: Principal Components Analysis algorithm”, *Proceedings of SPIE The International Society for Optical Engineering*, 2004, vol. 5321, pp. 205–211.

[8] P. Chen, A. Shen, X. Zhou, and J. Hu, “Bio-Raman spectroscopy: a potential clinical analytical method assisting in disease diagnosis”, *Anal. Methods*, no. 3, pp. 1257–1269, 2011. https://doi.org/10.1039/c1ay05039g

[9] K. Kong, C. A. Kendall, N. Stone, and I. Notingher, “Raman spectroscopy for medical diagnostics — From in-vitro biofluid assays to in-vivo cancer detection”, *Advanced Drug Delivery Reviews*, vol. 89, pp. 121–134, 2015. https://doi.org/10.1016/j.addr.2015.03.009

[10] S. Jeong, et al., “Fluorescence-Raman dual modal endoscopic system for multiplexed molecular diagnostics”, *Scientific Reports*, 2015, vol. 5, article 9455, pp. 1–9. https://doi.org/10.1038/srep09455

[11] A. M. K. Enejder, T.-W. Koo, J. Oh, M. Hunter, S. Sasic, and M. S. Feld, “Blood analysis by Raman spectroscopy”, *Optics Letters*, 2002, vol. 27, no. 22, pp. 204–206. https://doi.org/10.1364/OL.27.002004

[12] Sh. N. M. M. Nezhad, Z. Hozhabri, A. Haghparast, G. Karami, and P. Hejazi, "Optimization of parameters in 16-slice CT-scan protocols for Reduction of the Absorbed Dose", *Iran J Med Phys*, 2014, vol. 11, no. 2 & 3, pp. 270–275.

[13] M. S. Wróbel, "Non-invasive blood glucose monitoring with Raman spectroscopy: prospects for device miniaturization", *IOP Conference Series: Materials Science and Engineering 012036*, 2016, vol. 104. https://doi.org/10.1088/1757-899X/104/1/012036

[14] R. Pandey, S. K. Paidi, T. A.Valdez, et al., "Noninvasive Monitoring of Blood Glucose with Raman spectroscopy" *Acc. Chem. Res*., 2017, vol. 50, no. 2, pp. 264–272. https://doi.org/10.1021/acs.accounts.6b00472

[15] M. Bezuglyi, N. Bezuglaya, and A. Viruchenko, "On the possibility of ellipsoidal photometry and Monte Carlo simulation to spatial analysis of biological media", Electronics and Nanotechnology, *2017 IEEE 37th International Conference on Electronics and Nanotechnology* (ELNANO), pp. 321–324, 2017. https://doi.org/10.1109/ELNANO.2017.7939771

[16] M. A. Bezuglyi, N. V. Bezuglaya, and I. V. Helich, "Ray tracing in ellipsoidal reflectors for optical biometry of media," *Appl. Opt*., 2017, vol. 56, no. 30, pp. 8520–8526. https://doi.org/10.1364/AO.56.008520

[17] M. Bezuglyi, N. Bezuglaya, O. Kuprii, and I. Yakovenko, "The non-invasive optical glucometer prototype with ellipsoidal reflectors," *2018 IEEE 59th International Scientific Conference on Power and Electrical Engineering of Riga Technical University* (RTUCON), 2018, pp. 1–4. https://doi.org/10.1109/RTUCON.2018.8659864

[18] M. Bezuglyi, N. Bezuglaya, and S. Kostuk, "Influence of laser beam profile on light scattering by human skin during photometry by ellipsoidal reflectors", *Devices and Methods of Measurements*, 2018, vol. 9, no. 1, pp. 56–65. https://doi.org/10.21122/2220-9506-2018-9-1-56-65

[19] P. Bhandare, Y. Mendelson, R. A. Peura, et al., "Multivariate determination of glucose in whole blood using partial least-squares and artificial neural networks based on mid-infrared spectroscopy," *Appl. Spectroscopy*, 1993, vol. 47, no. 8, pp. 1214–1221. https://doi.org/10.1366/0003702934067928

[20] I. V. Meglinski, A. N. Bashkatov, E. A. Genina, D. Yu. Churmacov, and V. V. Tuchin, "Study of the possibility of increasing the probing depth by the method of reflection confocal microscopy upon immersion clearing of near-surface human skin layers", *Quantum Electronics*, 2002, vol. 32, no. 10, pp. 875–882. https://doi.org/10.1070/QE2002v032n10ABEH002309

Mikhail Bezuglyi received the B. sc. and M. sc. degrees in laser and optoelectronic technic from National Technical University of Ukraine "Kyiv Institute", Kiew, Ukraine, in 2000 and 2002, respectively. He received his Doctoral degree in biological and medical devices and systems from the National Technical University of Ukraine "Kyiv Polytechnic Institute", Kiew, Ukraine, in 2008.

He is currently an associated professor of the Instrumentation Design and Engineering Department, Igor Sikorsky Kyiv Polytechnic Institute. He has authored more than 30 published papers on biophotonics and biomedical optics. His research interests include design, production and control of optical and optoelectronic systems for biomedical area.

Address: National Technical University of Ukraine "Igor Sikorsky Kyiv Polytechnic Institute", Instrumentation Design and Engineering Department, Peremohy Ave. 37, off. 285-1, Kiew, Ukraine.
E-mail: m.bezuglyi@kpi.ua
ORCID iD: https://orcid.org/0000-0003-0624-0585

Natalia Bezuglaya received the B. sc. and M. sc. degrees in laser and optoelectronic technic from the National Technical University of Ukraine "Kyiv Polytechnic Institute", Kiew, Ukraine, in 2005 and 2007, respectively. She received her Doctoral degree in biological and medical devices and systems from the National Technical University of Ukraine "Kyiv Polytechnic Institute", Kiew, Ukraine, in 2016.

She is currently an associated professor of the Instrumentation Design and Engineering Department, Igor Sikorsky Kyiv Polytechnic Institute. She has authored more than 20 published papers on biophotonics and biomedical optics. Her research interests include methods and systems for spatial analysis of biological media.

Address: National Technical University of Ukraine "Igor Sikorsky Kyiv Polytechnic Institute", Instrumentation Design and Engineering Department, Peremohy Ave. 37, off. 285-1, Kiew, Ukraine.
E-mail: n.bezugla@kpi.ua
ORCID iD: https://orcid.org/0000-0002-4321-2068