Optical Investigation of Hematocrit Level in Human Blood

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In this paper use of selected optical methods of a hematocrit measurement has been presented. Elaborated methods have numerous advantages: relatively simple configurations, potentially low cost and high resolution. Investigation confirmed their ability to determine the hematocrit value with appropriate measurement accuracy. Furthermore, simultaneous use of complementary optical methods can substantially increase measurement reliability, because low-coherence interferometric measurement is based on physical (mainly optical) properties of the investigated object, while Raman spectroscopy is based on study of its molecular composition.

1. Introduction

Blood analysis is frequently performed for medical diagnosis. One of the important analytes is the blood hematocrit (HCT), defined as the ratio of packed red blood cells volume to whole blood volume. Measurement of blood hematocrit (HCT) is essential since it provides information on the total oxygen-carrying ability of patient. The normal ranges of the hematocrit are: 39–50% for male, 35–45% for female, and 30–40% for small children and babies, respectively.

The main reason of increased level of the hematocrit can be dehydration, burns, diarrhea, postpartum eclampsia, polycythemia vera. The high HCT level is also indicated as risk factors for heart and cerebral infraction because of hemoconcentration. Furthermore, the increased hematocrit is a factor of thrombus formation and increases a risk for thrombosis. This high HCT level is especially dangerous for patients with artificial heart, in dialysis, and during open-heart surgery. On the other hand, when the level of hematocrit is reduced, symptoms of anemia and bleeding are usually suspected, as well as diseases of the bone marrow, leukemia, malnutrition, and overhydration. Furthermore, each change of hematocrit value affects the safe control of blood pump.

Therefore, the hematocrit value as well as blood pressure should be controlled during the daily life as the indices of various physiological conditions in order to reduce the cardiovascular disease risk factor. It should be noted that the continuous monitoring of the HCT is also needed to perform appropriate dialysis and blood infusion.

The blood hematocrit is routinely determined in the clinic by analysis of blood samples. There are several

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methods of measurement of the HCT. Unfortunately, all of them require either blood sampling or catheterization. Repeatable blood sampling during continuous monitoring of the hematocrit value is associated with an increasing risk of infection (e.g. HIV or hepatitis). Moreover, such way of examination and loss of even small volume of blood required for the HCT measurement can be harmful to the patients (especially for neonates, small children and old people). Therefore, there is a need of non-invasive method of the hematocrit measurement, because recently used methods are based on the *in vitro* measurement.

There is a great interest in optical measurement that would permit simultaneous analysis of multiple components (analytes) in whole blood without the need for conventional sample processing, such as centrifuging and adding reagents. There are few optical methods of the hematocrit measurement. Schmitt et al. [1] uses the dual-wavelength near IR-photoplethysmography. Intensity sensitivity detector working with dual wavelength has been described by Oshima and Sanakai [2]. Xu and Chen applied optical coherence tomography for investigating the HCT value [3]. Enejder et al. [4] used the Raman spectroscopy and partial least squares (PLS) data analysis for simultaneous measurement of concentrations of multiple analytes in whole blood, including the hematocrit and hemoglobin. There are also works in which authors tried to find correlation between oxygen saturation of whole blood and hematocrit value [5]. However, all of them investigated the HCT value *in vitro* by using sample of blood. Recently, only one method of non--invasive hematocrit measurement by the use of spectral domain low coherence interferometry has been demonstrated by Iftimia et al. [6]. Unfortunately, until known it is possible to make such a measurement only insight the eye, which is difficult because of the eye movement and uncomfortable for the patient. The light beam in-

terrogating the eye must be stabilized on a fixed location on a specific retinal vessel to collect reproducible depth--reflectivity profiles in the blood. This is possible only by employing the eye motion stabilization technique, which generally is accomplished invasively. Therefore, at the present state of art *in vivo* measurement of the HCT is more complex and expensive than laboratory analysis of collected blood samples.

Until recently, reported optical hematocrit measurement methods have showed around 5 Hct% accuracy even when they were performed *in vitro* [2, 7]. Therefore, the purposes of presented preliminary stage of study are analysis of application problems of selected complementary optical methods of the HCT measurements (the Raman spectroscopy and low-coherence interferometry) and development of accurate and stable optical hematocrit measurement methods. In frame of the research sufficient systems for the HCT determination are to be set up and *in vitro* blood investigation is to be carried out in order to confirm efficiency of the proposed methods.

2. Measurement methods

2.1. Raman spectroscopy

The Raman spectroscopy is powerful and versatile technique that enables investigation of molecular composition of human tissues, including measurement of blood parameters [4, 8, 9]. This method is based on the recording and analysis of light scattered inelastically by the investigated object. It can be observed as a result of interaction between monochromatic light and dipoles induced in an oscillating molecule, when the molecule is excited to a virtual state, which does not have to correspond to a real energy level of the molecule. The molecule may return to the energy level different from the initial one. Photons observed in scattered light may have energy lower than in an incident beam, which is called the Raman scattering in the Stokes band. The photon energy greater than in excitation light is related to the anti--Stokes band. The difference between wavenumbers of photons in incident $(1/\lambda_0)$ and scattered light is known as a Raman shift. It is related to characteristic oscillation frequencies of a molecule. They may be the oscillations of a simple band, as well as of a larger fragment of a material lattice. Therefore, the Raman spectrum can be considered as "fingerprints" of investigated materials. A particular vibration is Raman active if it is connected with the change in the polarizability tensor of molecule. For the specified excitation wavelength λ_0, the Raman intensity can be expressed as

$$I_{\mathrm{R}} = (I_{\mathrm{L}}\sigma K)PC \,, \tag{1}$$

where I_{R} — measured Raman intensity [photons per second], I_{L} — laser excitation intensity [photons per second], σ — absolute Raman cross-section [cm^2 per molecule], K — a constant accounting for measurement parameters, i.e. optical collection efficiency, optical transmission of the Raman spectrometer, etc., P — sample path length [cm], C — concentration [molecules per cm^3]; the influence of parameter K may be eliminated by comparative measurements.

The analysis of vibrational spectra of human tissues is difficult because of their complex molecular composition. Characteristic Raman shifts may depend on neighbourhood of the group of atoms and their position in molecule. Also peaks assigned to coupling and resonance between single oscillations may appear in the spectrum. Dependence of the Raman signal intensity I_{R} on particular substance concentration C shown in Eq. (1) can be base for quantitative analysis, e.g. determination of particular component content, although sufficient calibration and classification algorithms must be developed.

The Raman investigation of blood carried out in *in vitro* mode is based on investigation of collected blood. There are also reports about trials of *in vivo* determination of selected blood parameters (e.g. glucose level) based on acquisition and spectral analysis of the light scattered on skin and sub-skin tissues [9]. Main application problems of conventional Raman spectroscopy in blood diagnostics are as follows: (1) low level of useful Raman signal and strong background stimulates use of high laser power and long exposure time (up to several ten minutes) which can be painful and harmful for examined patients; (2) requirement of adequate predictive ability — it is important for the classification model developed for the individual patients to be transferable by a larger population.

Problem of low level of useful Raman signal can be solved by the use of method that enables enhancement Raman signal (e.g. surface enhanced Raman spectroscopy — SERS) and by selection of the optimal excitation laser wavelength λ_0. As the level of the Raman signal is inversely proportional to λ_0^4, application of VIS or UV laser as the excitation source should be more effective than an IR one. However, the influence of fluorescence induced by a laser beam, which is usually the strongest for the excitation wavelength range from 270 to 700 nm, must also be taken to account. Commonly used 830 nm laser [4, 9], although is good solution when need of reduction of the fluorescence is considered, has also serious drawbacks. Respective Raman signal is generated in a spectral range where sensitivity of CCD arrays is poor as well as absorption of the radiation in water can be serious problem. Therefore, such excitation wavelength stimulates use of high laser power and long exposure time, which is not sufficient for examined patients. Therefore, more efficient excitation wavelengths λ_0 have to be found. For numerous materials, such a wavelength is selected to be related to difference between real energy levels in the investigated materials thus creating significant amplification of the signal by the Raman resonance effect. Such enhancement can be observed for hemoglobine when 532 nm laser is used [7].

All the approaches, including those presented above, requires efficient classification and calibration algorithms to extract information from recorded Raman spectra.

Use of such as PLS regression analysis or support vector machine (SVM) for data analysis can be efficient classification tool [4, 9].

2.2. Low-coherence measurement method

The low-coherence interferometric measurement system consists of a broadband source, a sensing interferometer and an optical processor. The light from the broadband source is transmitted to the sensing interferometer, where the amplitude of light is divided into two components and an optical path difference (OPD) is introduced between them. The sensing interferometer is designed in such a way that a defined relationship exists between the optical path difference and the measurand (mainly its dimension or refractive index). The signal from the sensing interferometer is transmitted back to the optical processor. The optical processor consists of a second optical system, the output of which is a function of OPD generated at the sensing interferometer. The optical processor is either a second interferometer (when the phase processing of the measured signal is used) or a spectrometer (when the spectral processing of the measured signal is used) [10]. The measurement system with the phase processing of the measured signal possesses very high measurement sensitivity and resolution, much higher than that of the system with spectral processing. However, low-coherence measurement system based on the spectral signal processing has decisive advantages — there is no need of precise mechanical scanning and no need of use of movable adjusting components. Moreover, the system using the spectral signal processing and the Fabry–Perot sensing interferometer is not sensitive for any change of a transmission of the optical system. This is possible because in the system information about the measurand is encoded in the spectra of the measured signal [11].

Optical intensity at the output of such an interferometer can be expressed as [12]:

$$I_{\text{out}} = \langle EE^* \rangle, \tag{2}$$

where $E = E_1 + E_2$, E_1 and E_2 — amplitudes of the electric vector of the light wave reflected from the first and the second reflective surfaces inside the sensing interferometer respectively, brackets $\langle \rangle$ denote time averages; asterisk $*$ denotes the complex conjugation.

When the spectral signal processing is used, the recorded signal can be expressed as [10]:

$$I_{\text{out}}(\nu) = S(\nu)[1 + V_0 \cos(\Delta\phi(\nu))], \tag{3}$$

where $S(\nu)$ — spectral distribution of the light source; V_0 — visibility of the measured signal, $\Delta\phi(\nu)$ — the phase difference between interfering beams.

The phase difference between interfering beams can be calculated from a following equation [11]:

$$\phi(\nu) = \frac{2\pi\nu\delta}{c}. \tag{4}$$

δ — optical path difference, c — velocity of the light in vacuum.

If the light source exhibits a Gaussian spectrum, the normalized spectra pattern is predicted to be a cosine function modified by the Gaussian visibility profile. In the spectral domain signal processing the modulation frequency of the measurement signal gives information about the measurand (Eq. (3)). It can be noted that for $\Delta\phi = 0$ there is no spectral modulation. If the phase difference between the interfering beams varies from zero, the function takes the form of the cosine curve. The spacing of adjacent transmission peaks is proportional to the inverse of the optical path difference $(1/\delta)$ [12].

Because of its decisive advantages the low-coherence measurement system based on the spectral signal processing is the most convenient for the low-coherence hematocrit measurement.

3. Experimental

3.1. Object of investigation

In order to find out whether proposed method has sufficient accuracy to be base of system for *in vivo* monitoring of the hematocrit, series of *in vitro* measurements was carried out. During experimental work authors used the whole human blood for tests. Such approach has significant advantage, because we were able to use wide representative group of volunteers. It should be noted that samples were get from rather healthy volunteers and therefore our measurement range of the hematocrit measurement was limited to the value of 30 to 50%. This range was wide enough to find out if resolution of the measurement is sufficient. At this stage we were not measuring blood samples having very extreme HCT values which refer to very sick patients. Moreover, the hematocrit level of each blood sample was obtained by clinical diagnostics as reference measurements, as well.

3.2. Raman measurement setup

Preliminary Raman investigation was carried out with excitation wavelength $\lambda_0 = 785$ nm. As intensity of the spontaneous Raman signal was not satisfying, the Raman spectra were intensified with use of SERS method. The measurement setup consisted of diode laser, fiber-optic focusing probe, SERS substrates and Ocean Optics QE65000 spectrometer coupled with TE-cooled (minus 15 centigrade) CCD array. The single acquisition time was equal to 7 min.

3.3. Low-coherence measurement setup

The developed low-coherence fiber-optic setup for hematocrit measurement consists of low-coherence source, an optical processor and sensing interferometer. As a low-coherence source the superluminescent diode with Gaussian spectral intensity distribution (Superlum Broadlighter S1300-G-I-20 with following optical parameters: $\lambda = 1290$ nm, $\Delta\lambda = 50$ nm). As an optical processor the optical spectrum analyzer Anritsu MS9740A was

implemented. As a sensing interferometer authors implemented a low-finesse fiber-optic Fabry–Perot interferometer. The Fabry–Perot interferometer was built using the standard single mode telecommunication optical fibres, a fibre coupler, the measurand field and a mirror. The interferometer consists of optic-fiber with uncoated end, which has a reflectance of 0.04. The second reflectance surface is made by the silver mirror with reflectance of 0.99. This configuration enabled high visibility value of measured signal.

If the measurand field is filled by the investigated sample, the reflective surfaces of the Fabry–Perot are made by two boundaries: fibre/blood and blood/mirror, respectively. Each change of hematocrit level caused by change of erythrocytes number in the whole blood volume, effects in change of the refractive index of the blood. In the low-coherence measurement setup each change of refractive index of the investigated blood results in the change of the optical path difference of interfering beams and the phase difference of those beams due to Eqs. (3) and (4).

4. Results and discussion

4.1. Raman spectroscopy

Comparison between the Raman spectra of two blood samples having different HCT level is shown in Fig. 1. Significant difference between these two samples is that the Raman range below 1000 cm^{-1} (e.g. bands at 411 cm^{-1} and 863 cm^{-1}) can be base for determination of the hematocrit level. However, high noise level and influence on many factors on the Raman spectra of blood stimulate creation of more advanced calibration models (e.g. by means of PLS or SVM) to make accurate Raman measurements.
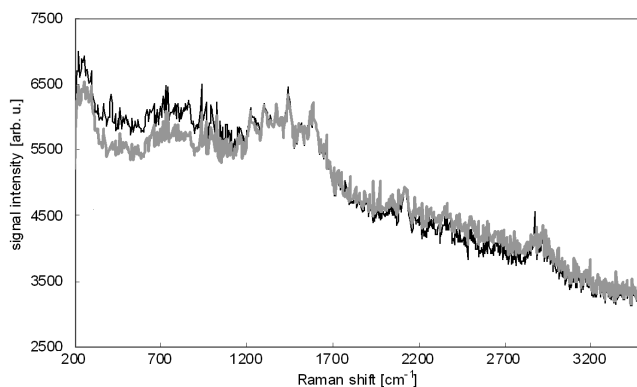

Fig. 1. Raman spectra of blood samples having different hematocrit level: 46.7 — grey curve and 40.3 — black curve; time of acquisition — 7 min.

4.2. Low-coherence interferometry

With the use of elaborated low-coherence system with the Fabry–Perot interferometer, the hematocrit value of numerous blood samples was measured. Experimental investigation gave a series of recorded spectra. In Fig. 2 measured reference signal from the sensor without any liquid is presented. Measured signals of the blood sample with HCT = 35.2% and with HCT = 49.2% are shown in Fig. 3a and b, respectively.
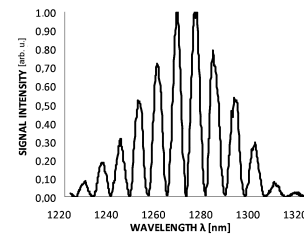

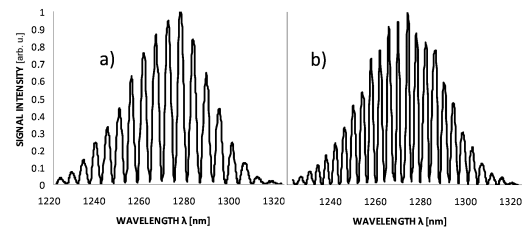Fig. 2. The reference signal from the sensor without any liquid.

Fig. 3. Measured signal of the blood sample with (a) HCT = 35.2%, (b) HCT = 49.2%.

It should be noted (Fig. 2, Fig. 3) that by the use of a dedicated sensing interferometer, designed in our laboratory, it was possible to get visibility of the measured signal $V = 0.98$, which is really hard to achieve in really optoelectronic low-coherence system.

As it is shown in Fig. 3a and b, the change of the hematocrit value changes the refractive index of the blood sample and optical path difference between interfering beams as well. It occurs in phase changes (Eqs. (3) and (4)) and can be detected by the analysis of the measured signal modulation. Mathematical processing of the measured signals provided information about measurand from the spectrum of signal, which is shown in Fig. 4.

The result of experimental works show (Fig. 4) that low-coherence method of hematocrit measurement with Fabry–Perot interferometer configuration has proper accuracy to measure the hematocrit value. The output signal was analysed by measurement the change of fringe numbers of the spectra pattern. The change of the number of fringes of investigated spectral pattern equal to 8 was achieved in investigated range, thus obtained sensitivity of the hematocrit measurement can be estimated as 0.4 [a.u./%]. Time required for single measurement was in range of 0.8–1.2 s. Experimental configuration had the very high value of visibility of the measured signal (usually 0.98), which always leads to decrease of the value of signal-to-noise ratio of the measured signal. Fur-
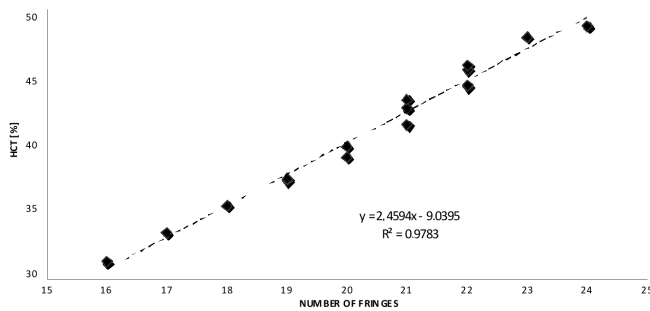
Fig. 4. The change of number of signal spectra fringes vs. hematocrit value: dots — measured value, dashed line — regression line (R^2 — determination coefficient of measured value).

thermore, it had very high determination coefficient of measured value ($R^2 = 0.978$) as well.

5. Conclusions

In this paper successful use of two optical methods of the *in vitro* hematocrit measurement and their application problems have been presented. There were the Raman spectroscopy and low-coherence method with spectral signal processing, respectively. Simultaneous use of these two complementary optical methods can substantially increase measurement reliability, because low-coherence interferometric measurement is based on physical (mainly optical) properties of the investigated object, while the Raman spectroscopy is based on study of its molecular composition.

The investigation of this method confirms its ability for the hematocrit control in appropriate measurement range with sufficient accuracy. The presented preliminary results can be the base for building sensor ready for practical applications and in the opinion of authors it will be possible to build the low-coherence optical *in vivo* hematocrit sensor. In the further stage of the research, developed system will be applied for the *in vivo* measurements carried out through the skin. The best place on the human body for measurement as well as method of preparation patient skin surface will be determined. During the *in vivo* low-coherence measurements, results would be probably influenced not only by refractive index of the blood but also by change of the Fabry–Perot cavity dimensions caused by a human pulse. Also the Raman measurements will be influenced by optical background. Therefore, we predict that phase-sensitive detection synchronised with the human pulse should be applied. Moreover, it was confirmed that enhancement of the Raman signal is required, so conditions for resonance amplification of signal assigned to the hematocrit will be searched during next stage of research in order to make the Raman *in vivo* investigation of blood possible.

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