Optical Transmission of Blood: Effect of Erythrocyte Aggregation

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***Abstract*—The influence of red blood cell (RBC) aggregation on transparency of blood in the red-near infrared spectral range is investigated. We argue that for relatively thin blood layers the light diffraction on aggregates becomes the dominant phenomenon. The nature of pulsatile changes of blood transparency is explained by pulsations of RBC aggregate size. For another case of over-systolic vessel occlusion the following time evolution of blood transparency strongly depends on light wavelength. This dependence may serve as a basis for an alternative approach to noninvasive blood tests: occlusion spectroscopy. Theoretical results well correspond to both *in vivo* and *in vitro* measurements reproducing pulsatile blood flow and long occlusion as well.**

***Index Terms*—Light scattering, noninvasive blood test, noninvasive glucose (hematocrit) measurement, pulse oximetry, RBC aggregation, whole blood.**

I. Introduction

TO MAKE all kinds of blood tests noninvasive is no doubts one of the exciting challenges in medical physics of nowadays. The most accepted tool for this is red-near infrared (RNIR) spectroscopy. All the major existing noninvasive techniques of blood parameter measurements are connected with processing of time dependent optical transmission data in the RNIR spectral range. The most popular example of RNIR spectroscopy application is a pulse oximeter that is a generally accepted standard of everyday clinical practice.

The operation of pulse oximeters is well studied practically and may be reduced to the following. RNIR light passing through the patient's finger comes out modulated by the waveform of his heartbeats. The arterial hemoglobin oxygen saturation (SaO_2) is received from the analysis of magnitudes of this modulation for two basic wavelengths (e.g., 670 and 960 nm). In this paper, we target the clarification of physical mechanism of this modulation. To clarify, this mechanism is absolutely necessary if we want to expand the limits of noninvasive measurements from SaO_2 to glucose, hemoglobin and other blood constituents.

The most widely recognized nowadays physical picture may be roughly reduced to obvious mechanism, called "volumetric." According to this mechanism, the blood amount in a body portion irradiated by RNIR light changes with heartbeats. For the wavelengths used in pulse oximetry, the attenuation of RNIR light by tissue is determined mainly by blood. So, this is caused by heart beats periodic fluctuations of blood amount in the irradiated portion of body that result finally in the appearance of ac component of out-coming signal.

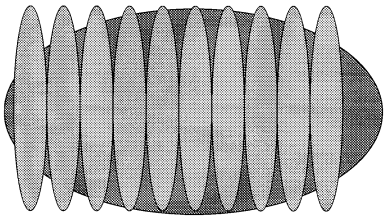

Fig. 1. The model of 1-D RBC aggregates: Two equal axes of the effective spheroid are equal to large axis of erythrocyte a and the third axis of the spheroid c grows with time in the process of aggregation.

In this paper, we present a theoretical analysis and an experimental confirmation of the existence of alternative mechanism of optical pulsations. This mechanism is associated with periodic changes of light scattering in whole blood.

In whole blood the light scattering in RNIR spectral range occurs mainly on erythrocytes [1]–[7]. The refraction index of erythrocytes is around 1.4 and differs from the one of blood plasma (around 1.33). Erythrocytes form rouleaux, i.e., aggregates of various shape and size (Fig. 1). The geometry of red blood cell (RBC) aggregates is affected by shear forces and, thus, depends strongly on the parameters of the blood flow [8], [9], so that in real vessels it changes with the heartbeats. The size of erythrocyte aggregates varies from comparable to the wavelength of RNIR light to that exceeding it substantially. Thus, the light scattering is strong [1]–[18] and varies in time following the variations in RBC aggregation. For instance, in [6] the light scattering spectroscopy has been used as a tool to study aggregational ability and deformability of RBC. The process of aggregation/disaggregation in its turn is fast enough to result in optical transparency pulsations at the heart frequency.

In other words, we argue that in real *in vivo* situation, e.g., in the case of pulse oximeter, the finger transparency would fluctuate with heartbeats even if the amount of blood remains constant, i.e., without volumetric changes at all. The blood transparency can oscillate as a result of light scattering changes caused by the process of RBC aggregation-disaggregation.

In Section II, we present the main physical assumptions, choose the model of light transmission most appropriate for the real parameters and simulate time-dependent optical transmission for the most important cases of periodic blood flow and its sudden stop. In Section III, we compare theoretical results with the experiment. Section III is divided into two subsections dealing with the pulsatile flow experiments and

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long over-systolic occlusion consistently. (By "over-systolic occlusion" we mean vessel occlusion caused by application of over-systolic pressure.) Both *in vivo* and *in vitro* sets of measurements are presented. In our *in vitro* experiments, we also consider separately another alternative mechanism of optical pulsations, i.e., the periodic changes of RBC orientation in the pulsatile flow. All the major conclusions are summarized in Section IV.

II. Basic Model Assumptions

Following the physical picture presented earlier [4], [5] we work in the following basic assumptions:

First, we consider the optical transparency of the medium consisting of blood solely. It consists of blood serum and RBC aggregates inside. This simplified object is suggested instead of a very complicated real media containing also tissue, skin, bones, etc. Time evolution of optical transmission is to be simulated and all the time variations are attributed to blood. Second, the thickness "d" of the blood layer is fixed. In each case, it may consist one or a few millimeters, but it is given and does not vary in time. Thus, the volumetric mechanism discussed in the introduction stays out of the frame of our consideration. The only justification for not considering the volumetric mechanism here is the methodological clearness, i.e., the analysis of main physical consequences of scattering-related mechanism solely is targeted.

The blood as a medium may be characterized by average absorption coefficient μ_{a}, which is the linear combination of the ones of Hb, $\mathrm{HbO_2}$ and water, taken proportional to their portion. The spectral behavior of the absorption coefficient for all the constituents is taken standard [3] and the $\mathrm{SaO_2}$ value (parameter s) is thought to be 98% (unless otherwise is declared)

$$\mu_{\mathrm{a}} = \left(\mu_{\mathrm{a_{HbO_2}}}\mathrm{s} + \mu_{\mathrm{a_{Hb}}}(1-\mathrm{s}) - \mu_{\mathrm{a_{H_2O}}}\right)\mathrm{H} + \mu_{\mathrm{a}}\mathrm{H_2O}. \quad (1)$$

The hematocrit "H," $0 < \mathrm{H} < 1$, is the fraction of volume occupied by erythrocytes. Normally, the hematocrit of a healthy person is $\mathrm{H} = 0.4 \div 0.5$. All the parameters in (1) do not depend on time even when the blood flow is time-variant. But the resulting transparency of blood in this case may vary in time because of scattering.

The only scatterers under consideration are single erythrocytes and their aggregates. As known [1]–[4], the average size of these scatterers in blood has an order of magnitude of a few microns and, therefore, is larger than the wavelength λ for RNIR light. Besides, the relative index of refraction of scatterers does not differ much from unity (for serum $\mathrm{n_{H2O}} = 1.33$ is assumed and for RBC $\mathrm{n_{Hb}} = 1.404$) and this difference is a good theoretical small parameter. Known that for such a combination of the parameters in a single scattering act the forward scattering strongly dominates [10]–[13]. Therefore, we treat here the integrated transmission of blood as a solution of diffusion-like equation in the assumption [11], [12] of small angle scattering, so that one gets the following expression for integrated transmission [12]:

$$\mathrm{T} \propto \left(\frac{1}{\cosh\left(\left(\mu_{\mathrm{a}}\mu_{\mathrm{tr}}\right)^{1/2}\mathrm{d}\right)}\right)\mathrm{e}^{-\mu_{\mathrm{a}}\,\mathrm{d}}. \quad (2)$$

Here, μ_{a} is blood absorption for a given wavelength defined in (1), while for the reduced scattering coefficient μ_{tr} one gets [3], [13]

$$\mu_{\mathrm{tr}} = \mu_{\mathrm{S}}(1-\mathrm{g}) = \pi\left(\mathrm{a}^2 + \mathrm{c}^2\Xi\right)\frac{\mathrm{H}(1-\mathrm{H})(1-\mathrm{g})\mathrm{K}(\rho)}{\mathrm{V_0}} \quad (3)$$

where μ_{s} is the scattering coefficient and ρ is an effective phase shift

$$\rho = 2\pi\mathrm{c}\Xi\frac{(\mathrm{n_{HB}} - \mathrm{n_{H2O}})}{\lambda}. \quad (4)$$

The aggregate shape is approximated by spheroid (with axes a and c), $\mathrm{V_0} = 4\pi\mathrm{a}^2\mathrm{c}/3$ is the spheroid volume, while ρ is related to the averaged angle of scattering. Dimensionless parameter Ξ is also connected with shape of aggregate and explained later on in (6a) and (6b).

Everywhere below we took the same factor of scattering anisotropy $\mathrm{g} = 0.995$, therefore the reduced scattering coefficient μ_{tr} is much lower than the scattering coefficient μ_{s}, as it is always for the RNIR light scattering in blood and tissue [7].

Function $\mathrm{K}(\rho)$ reflects the interference nature of each single act of scattering (see Fig. 2)

$$\mathrm{K}(\rho) = 1 - \left(\frac{\sin(2\rho)}{\rho}\right) + \left(\frac{\sin(\rho)}{\rho}\right)^2. \quad (5)$$

It oscillates as a function of an effective phase shift ρ, maxima and minima of these oscillations correspond to constructive and distractive interference of free coming wave and the wave passing the scattering center.

For time-variant blood flow, shear forces affect the process of aggregation, so that in the case of pulsatile flow the average size of aggregates also pulsates, while the blood flow cessation favors the monotonous growth of aggregates far above their normal size.

Time variations of optical transparency result from these changes of aggregate geometry caused by the process of aggregation/disaggregation. In our model, the RBC aggregation is thought to be one-dimensional (1-D) [4], [5], i.e., erythrocytes aggregate side by side. The aggregate shape is approximated by spheroid. Two equal axes of the spheroid are equal to large axis of erythrocyte a and the third axis of the spheroid c grows with time in the process of aggregation (Fig. 1). Further on, we denote $\mathrm{x} = \mathrm{c}/\mathrm{a}$ and imply $\mathrm{x} = \mathrm{x}(\mathrm{t})$.

For the pulsatile flow, $\mathrm{x}(\mathrm{t})$ is a periodic function of time. In this case, $\mu_{\mathrm{tr}}(\mathrm{t})$ (3) and, consequently, the total optical transparency $\mathrm{T}(\mathrm{t})$ (2) also oscillate with time.

For the case of sudden cessation of blood flow and the following growth of aggregate size the dependence of $\mathrm{T}(\mathrm{t})$ is less straightforward. To understand the time evolution of optical transmission following the over-systolic occlusion, one has to analyze (2)–(5) when $\mathrm{x}(\mathrm{t}) = \mathrm{c}(\mathrm{t})/\mathrm{a}$ grows with time.

In the starting stages of aggregation for $\mathrm{c} < \mathrm{a}$ dimensionless parameter Ξ, that enters (4) for a phase shift, is equal

$$\Xi = \left(\frac{1}{2\varepsilon}\right)\mathrm{Log}\left[\frac{(1+\varepsilon)}{(1-\varepsilon)}\right] \quad (6a)$$

where $\varepsilon = (1 - (\mathrm{c}/\mathrm{a})^2)^{1/2} = (1 - \mathrm{x}^2)^{1/2}$. This parameter reflects the degree of anisotropy of the scatterer and implements

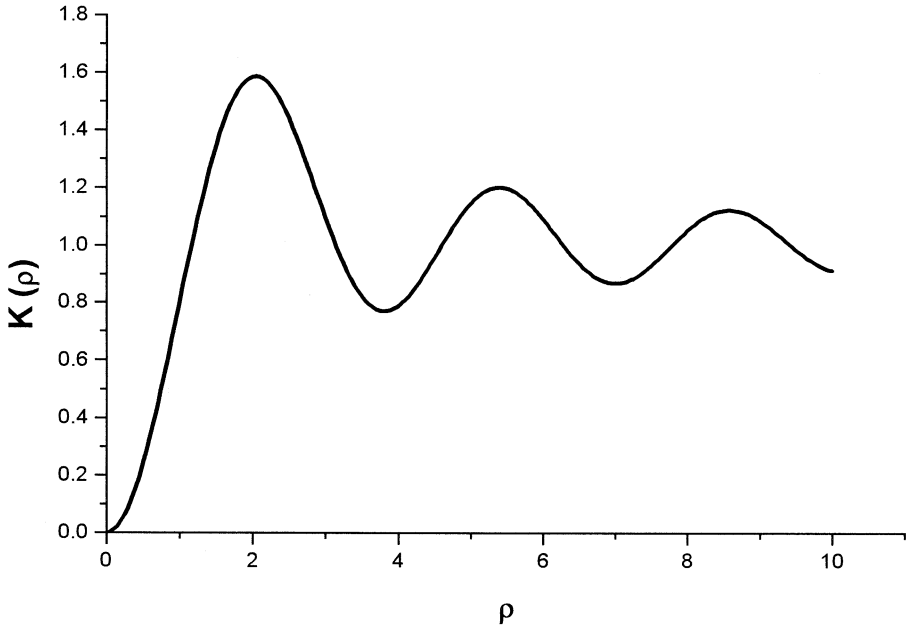

Fig. 2. Function $\mathrm{K}(\rho)$.

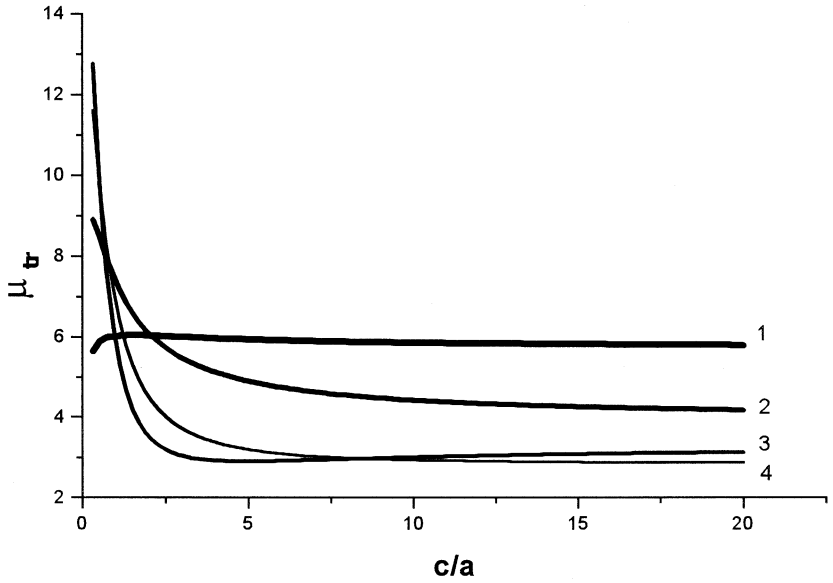

Fig. 3. Dependence of μ_{tr} (mm^{-1})) on c/a ratio. Curve #1—1300 nm, #2—760 nm, #3—670 nm, and #4—960 nm.

the averaging over all possible aggregate orientations [3]. For larger aggregates ($\mathrm{c} > \mathrm{a}$, or $\mathrm{x} > 1$), Ξ has to be continued in a complex plane

$$\Xi = \left(\frac{1}{\mathrm{z}}\right) \mathrm{arctg}\, \mathrm{z} \tag{6b}$$

where $z = ((\mathrm{c}/\mathrm{a})^2 - 1)^{1/2} = (\mathrm{x}^2 - 1)^{1/2}$.

In general, the growth of RBC aggregate size causes the decrease of μ_{tr} and the corresponding growth of transmission, but because of oscillating character of $\mathrm{K}(\rho)$ (Fig. 2) this growth may become nonmonotonous for certain wavelengths. Equations (1)–(6b) describe the time evolution of the optical transmission of the whole blood for any concrete function $\mathrm{x} = \mathrm{x}(\mathrm{t})$. Fig. 3 demonstrates the dependence of $\mu_{\mathrm{tr}}(\mathrm{x})$ for several wavelengths of very high actuality for *in vivo* RNIR spectroscopy. One can see that the time evolution of optical transmission caused by RBC aggregation has different limits for various wavelengths (We took $\mathrm{a} = 4\ \mu\mathrm{m}$, $\Delta\mathrm{n} = \mathrm{n}_{\mathrm{Hb}} - \mathrm{n}_{\mathrm{serum}} = 0.074$). For wavelengths lower than 760 nm this dependence is not monotonous. For 1300 nm, all the range of changes of $\mu_{\mathrm{tr}}(\mathrm{x})$ is rather small. The physical reason for these phenomena is quite transparent. For various wavelengths, the ranges of change ρ in (3)–(6b) are limited and different and, therefore, favors mainly either constructive or destructive interference. For 1300 nm, all this range is concentrated around $\rho = 2$, i.e., the point of maximum of the function $\mathrm{K}(\rho)$ (Fig. 2), so small changes in $\mathrm{K}(\rho)$ result in small changes of μ_{tr}. For lower wavelength ($\lambda \sim 950\ \mathrm{nm}$), the range of effective change of ρ for all possible x grows as $1/\lambda$ [see

(4)–(6b)] and covers nearly all the $\mathrm{K}(\rho)$ branch $2.2 < \rho < 3.7$. It results in much larger change in $\mathrm{K}(\rho)$, $\mu_{\mathrm{tr}}(\mathrm{x})$ and $\mathrm{T}(\mathrm{x})$. For $\lambda < 760\ \mathrm{nm}$ when x grows, ρ reaches and passes 3.8 the minimum of $\mathrm{K}(\rho)$, so $\mathrm{T}(\mathrm{x})$ becomes nonmonotonous. Contrary to the previous case of oscillating transmission, such dependence cannot be explained within the volumetric mechanism. Nevertheless, as we see below, it is observed experimentally and may be used in the design of new kinds of noninvasive measurements. The challenge is to find a proper parameter accumulating an information on main blood characteristics, i.e., hematocrit, glucose, etc.

For practical purposes, when the actual value of the large axis c of aggregate is a result of averaging over certain distribution of sizes varying from case to case, the exact location of maximum of $\mathrm{T}(\mathrm{t})$ hardly can be accepted as an established experimental parameter. However, for a very long over-systolic occlusion, the asymptotic behavior of $\mathrm{T}(\mathrm{t}, \lambda)$ for long t and correspondingly high c/a ratios is expected to be stable.

To clarify theoretically this asymptotic behavior, let us consider the expansion of (1)–(6b) over small $1/\mathrm{x}$. One easily gets

$$\frac{\mathrm{dT}}{\mathrm{dc}} = \left(\frac{\mathrm{dT}}{\mathrm{d}\mu_{\mathrm{tr}}}\right)\left(\frac{\mathrm{d}\mu_{\mathrm{tr}}}{\mathrm{dc}}\right). \tag{7}$$

The first derivative in the right-hand side of (7) does not demonstrate peculiar behavior for any model and has to be taken for $\mu_{\mathrm{tr}} = \mu_{\mathrm{tras}}$, while the asymptotic behavior of transmission is defined by the asymptotic behavior of dependence $\mu_{\mathrm{tr}}(\mathrm{c})$, when $\mathrm{c}/\mathrm{a} \to \infty$. (Further on, by subscript "$_{\mathrm{as}}$" we denote any variable for $\mathrm{c}/\mathrm{a} \to \infty$, e.g., μ_{tras}, ρ_{as}, etc.).

Thus, from (3)–(6b)

$$\rho_{\mathrm{as}} = \left(\frac{\pi^2 \mathrm{a}\,(\mathrm{n_{Hb}} - \mathrm{n_{serum}})}{\lambda}\right) \tag{8}$$

and for large values c/a one gets: $\rho = \rho_{\mathrm{as}}(1-(2\mathrm{a})/(\pi\mathrm{c}))$. Thus keeping the terms of second-order on a/c for the case $\mathrm{c}/\mathrm{a} \to \infty$

$$\begin{aligned}\mu_{\mathrm{tr}} = \mu_{\mathrm{tras}} &- \frac{3\pi}{8}\left(\frac{1}{\mathrm{a}}\right)\mathrm{H}(1-\mathrm{H})(1-\mathrm{g}) \\ &\cdot \mathrm{K}'(\rho_{\mathrm{as}})\left(\frac{2\pi\mathrm{a}(\mathrm{n_{Hb}} - \mathrm{n_{serum}})}{\lambda}\right)\left(\frac{\mathrm{a}}{\mathrm{c}}\right) \\ &+ \frac{3\pi}{16}\left(\frac{1}{\mathrm{a}}\right)\mathrm{H}(1-\mathrm{H})(1-\mathrm{g}) \\ &\cdot \left[\mathrm{K}(\rho_{\mathrm{as}}) + \mathrm{K}''(\rho_{\mathrm{as}})\left(2\pi\mathrm{a}\left(\frac{\mathrm{n_{Hb}} - \mathrm{n_{serum}}}{\lambda}\right)\right)^2\right]\left(\frac{\mathrm{a}}{\mathrm{c}}\right)^2.\end{aligned} \tag{9}$$

The first two terms in the right-hand side (9) are responsible for asymptotic behavior of transmission for large 1-D aggregate sizes. This behavior (growth or decline) depends on the sign of the first derivative $\mathrm{K}'(\rho_{\mathrm{as}})$. For $\lambda = 960\ \mathrm{nm}$, $\mathrm{K}'(\rho_{\mathrm{as}}) < 0$ (see Fig. 2), so that $\mu_{\mathrm{tr}}(x)$ asymptotically declines and transmission grows. For $\lambda = 670\ \mathrm{nm}$, $\mathrm{K}'(\rho_{\mathrm{as}}) > 0$ and, on the contrary, $\mu_{\mathrm{tr}}(\mathrm{x})$ asymptotically grows, while transmission declines. The transition from the first kind of behavior (i.e., monotonic) to the nonmonotonic one obviously happens on wavelengths λ_{cr} satisfying the equation: $\rho_{\mathrm{as}} = \rho_{\mathrm{min,max}}$, where the symbol $\rho_{\mathrm{min,max}}$ corresponds to the extremal points of function $\mathrm{K}(\rho)$ (Fig. 2). At these wavelengths the first-order term in (9) disappears and the resulting dependence $\mu_{\mathrm{tr}}(\mathrm{x})$ starts from the second-order terms, i.e., becomes very "flat". For the standard set of RBC parameters: $\mathrm{a} \sim 4\ \mu\mathrm{m}$, $\Delta\mathrm{n} \sim 0.07 \div 0.074$, one can expect λ_{cr} corresponding to the minimum of $\mathrm{K}(\rho)$ to be around 725–767 nm. As it is shown experimentally below, this is very close to what is observed. According to (8), ρ_{as} depends only on small size "a" of aggregate (Fig. 1). So, one can hope that the asymptotic behavior of transmission is quite a stable characteristic even in real *in vivo* measurements. We have realized this approach in the new method called the occlusion spectroscopy, when certain blood parameters are extracted from the time evolution $\mathrm{T}(\mathrm{t})$ following the over-systolic occlusion.

The principal possibility of realization of occlusion spectroscopy is based on the fact that model expressions (1)–(6b) contain in principle the information on all the substantial parameters considered here, i.e.,: oxygen saturation ($\mathrm{SaO_2}$), hemoglobin (H), glucose (G). Nevertheless, there is still a possibility to measure these parameters separately from the same physical characteristics (2). This possibility is based on the fact that oxygen saturation ($\mathrm{SaO_2}$), hemoglobin (H) and glucose (G) enter equations differently. Oxygen saturation enters through μ_{a} only. Hemoglobin enters both through μ_{a} (linearly) (1) and through μ_{tr} as $\mathrm{H}(1-\mathrm{H})$ (3). Dependence on glucose concentration is reduced entirely to the scattering term μ_{tr} (3) through the corresponding change of refraction index mismatch $\Delta\mathrm{n} = \mathrm{n_{Hb}} - \mathrm{n_{serum}}$.

To conclude, the physical mechanism of nonmonotonous RNIR $\mathrm{T}(\mathrm{t})$ dependence for the range of wavelengths below 760 nm is the light diffraction on RBC aggregates. In other words, the assumption of 1-D aggregate growth results in a strong influence of destructive interference on a forward scattered light starting from a certain length of rouleaux. Experimental verifications of these dependencies presented below prove that our assumption of 1-D character of aggregates is reasonable.

Another important feature derived from the Fig. 3 is that the ratio $\mathrm{d} \gg (\mu_{\mathrm{a}}\mu_{\mathrm{tr}})^{-1/2}$ is valid only in a certain range of parameters λ and x. For example, for advanced stages of aggregation ($\mathrm{x} \gg 1$) one usually gets $(\mu_{\mathrm{a}}\mu_{\mathrm{tr}})^{1/2}\mathrm{d} \sim 1$ (especially for a red light) and so the deviation of dependence (2) from the pure exponential one becomes important.

In conclusion, we notice that for the case of pulsatile blood flow qualitative predictions of volumetric model and the present model nearly coincide: both mechanisms lead to pulsations of optical transparency. They cannot be differentiated qualitatively even by the phases of pulsations. Indeed for volumetric mechanism the systolic part is associated with the increase of the effective thickness of blood layer and with the transmission drop. For scattering assisted mechanism, the transmission also falls down in the systolic phase, because high shear forces of strong blood flow break a large amount of roulauex.

Nevertheless, long occlusion easily differentiates between two mechanisms even qualitatively. The change of time-evolution of optical transmission with light wavelength from nonmonotonic to monotonic (see Fig. 4) definitely cannot happen within volumetric model. On the other hand, for interference of scattered light this behavior is very much typical and well known in optics.

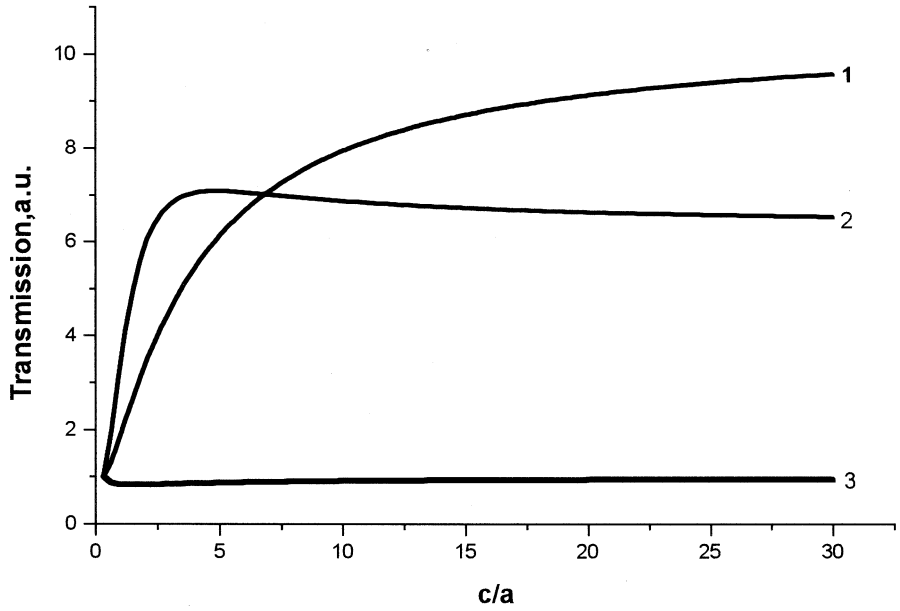

Fig. 4. Calculated dependencies $T(c/a)$. Curve #1—960 nm, #2 —670 nm, and #3—1300 nm.

III. Experimental Results and Discussion

In this section, we target the following: 1) to present an experimental proof of the existence of scattering-caused mechanism of optical pulsations for *in vitro* model system; 2) to verify the interconnection of the shape of optical signal and geometry of RBC aggregates; and, finally, 3) to check the time-evolution of optical transmission for long over-systolic occlusion for both *in vitro* model system and *in vivo* measurements.

A. Pulsatile Flow

Direct reproducible *in vitro* measurements are difficult to carry out in whole blood due to variability in plasma viscosity, concentration of the plasma, etc. Furthermore, the RBC aggregation, which is a key factor in phenomena under study here, cannot be readily controlled in whole blood. So, we designed the model solution for *in vitro* measurements. It is based on RBC suspensions in saline. Main optical properties of such a substitute are nearly identical to that of whole blood. The only substantial difference is the absence of RBC aggregation. In saline solutions, erythrocytes are single and do not form rouleaux [8], [9], [19], [20], because of the absence of long organic molecules that normally trigger the aggregation. Within such a system, aggregation accompanied fluctuations of scattering do not exist and cannot be observed.

To enable the RBC aggregation we added high molecular dextran to the suspension. In the presence of dextran, the RBC aggregation occurs the way resembling the rouleaux formation in the whole blood. Thus, we have the blood substitute with complete control on aggregation, i.e., it may be switched "on" and "off" by adding dextran [19], [20]. Then the optical transmission of model RBC suspensions was measured on specially designed glass-made rigid cuvette of 1.5-mm thickness. The pulsatile flow was arranged by peristaltic pump operated at 1.5 Hz. Optical transmission was measured at the 660-, 685-, 810-, 880-, 920-, 940-, 1200-, and 1300-nm wavelengths. To emphasize the strong similarity between our model system and the real blood let us mention that all the experiments of "Aggregation on" kind described below we also performed with the fresh blood and got very much the same results.

The results of *in vitro* measurements are summarized in Fig. 5. The curve D demonstrates the absence of pulsations of optical transmission when the peristaltic pump is in operation but the suspension is of "Aggregation off" kind. Very weak perturbations of the signal on the frequency of peristaltic pump result from a slight shaking of the cuvette walls that cannot be eliminated completely. Curve C in Fig. 5 demonstrates very strong well-pronounced optical pulsations measured on the RBC suspension of the "Aggregation on" kind. Microscopic observations in this case confirm the presence of RBC aggregates.

Rigid character of cuvette walls dictates the absence of absorption related mechanism of transparency pulsations. Thus, the appearance of a pulsatile optical signal results entirely from scattering fluctuations. The scattering fluctuations in pulsatile flow could be associated with the change of erythrocytes orientation [14]. Following this logic we have investigated the alternative option, i.e., that dextran changes the resulting viscosity of the system. The arising change of share forces might result in pulsatile variation of geometric alignment (orientation) of single erythrocytes. With such a mechanism transparency of the system pulsates without the assumption of RBC aggregation. To check this hypothesis, we prepared a different model solution.

Solutions of comparable viscosities were obtained by addition of 0.5% to 2% of high molecular weight dextran ($\mathrm{MW} = 148\,000$) and 0.5% to 4% low molecular weight dextran ($\mathrm{MW} = 9500$). The high-molecular dextran is well known to promote rouleaux formation, whereas the low-molecular dextran has no

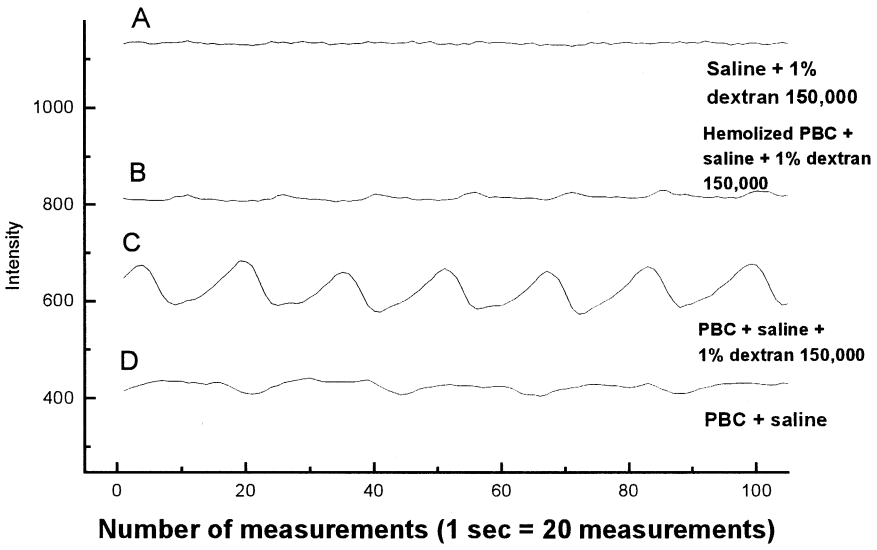

Fig. 5. The results of *in vitro* experiments with peristaltic pump.

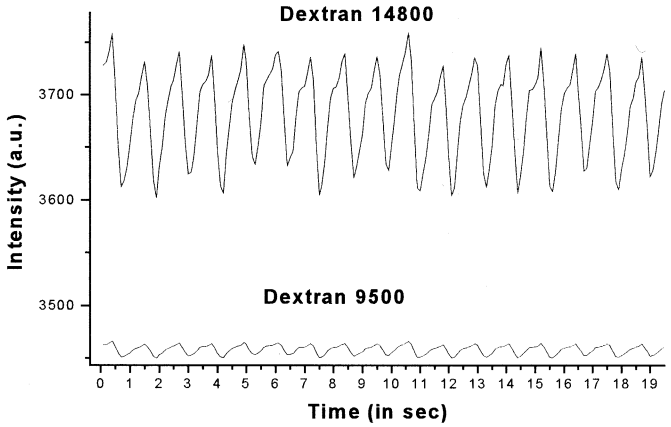

Fig. 6. Pulsatile signals obtained *in vitro*. For high-molecular dextran, the pulsations are much larger.

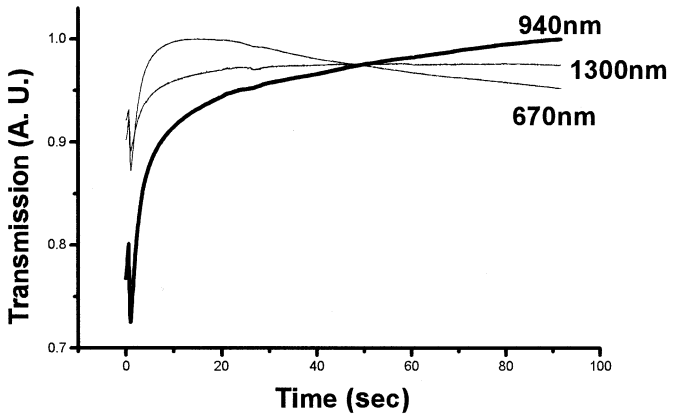

Fig. 7. Time evolution of light transmission. *In vivo* measurements.

influence as such. On the other hand, because of almost identical viscosities one does not expect substantial differences in pulsations of geometrical alignment.

The typical results are shown in Fig. 6, for 660 nm. It is clearly shown, that the amplitude of pulsations of intensity of the optical signal is significantly higher in presence of dextran MW148 000 than in the presence of dextran MW9500. Thus, the change in intensity observed in the presence of high molecular weight dextran, is mainly due to the formation of rouleau. Changes due to modification in the viscosities of the solutions appear to play only a very minor role.

We want to underline that in a rigid cuvette there is no room for volumetric mechanism. So, the major correlation between flow pulsations and optical pulsations comes from scattering. Thus, RBC aggregation -assisted scattering and the nature of pulsatile signal have been proven. Such a direct observation also shows that for realistic hematocrit values ($H \sim 0.4-0.5$) the process of aggregation is fast enough in the typical time scales of the heartbeats. It well corresponds to the fact that such high values of H favor strongly nonlinear regime of aggregation [8], [9], [18].

B. Long Occlusion: In Vivo Versus in Vitro. Results and Discussion

The system of LEDs operating and data acquisition was adopted for *in vivo* experiments. The probe for *in vivo* experiments comprised a unit that was built in a form of a clip supporting illumination-detection assembly. The whole unit was adopted for the transmission measurement on the fingertip (like in pulse oximetry case). In addition, there was a pressurizing cuff for applying the over-systolic pressure. The cuff, which was built in a ring-like form, was mounted on the patients finger. A processing unit was designed to control the level of pressure in a finger cuff. The over-systolic pressure being applied on the cuff causes the sudden cessation of blood flow to the extreme of a finger [15]–[17].

The series of 500 measurements have been carried out in total on more than 200 patients. The over-systolic pressure was applied on the patient finger for 80 s. The most typical resulting dependencies are presented in Fig. 7. The picture strongly resembles our theoretical predictions presented earlier. The over-systolic occlusion results in a cessation of transmission pulsations and in a following growth of transmission. Further on, for

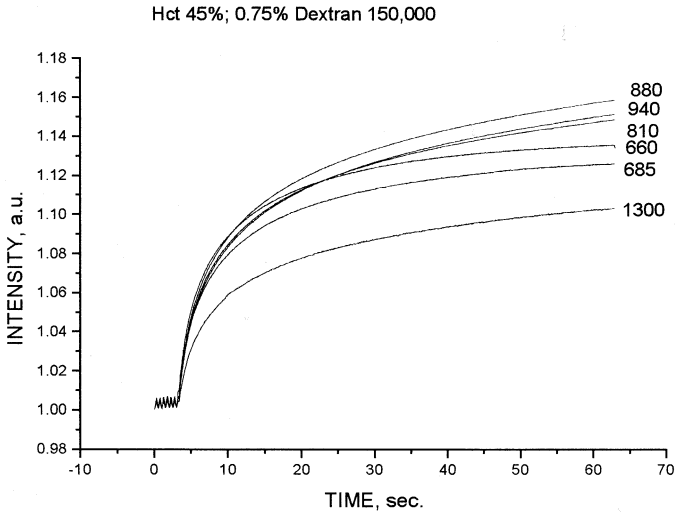

Fig. 8. *In vitro* results of spectro-kinetic behavior during the three-dimentional growths of RBC aggregates.

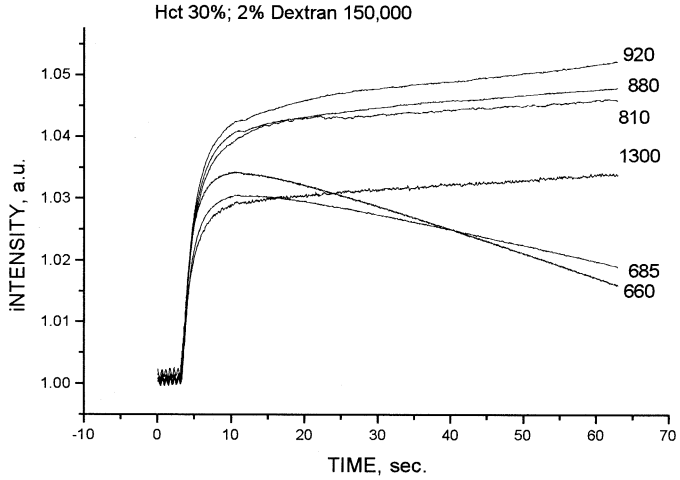

Fig. 9. *In vitro* results of spectro-kinetic behavior during the 1-D growth of RBC aggregates.

wavelengths lower than 760 nm this growth becomes nonmonotonic. The statistical analysis of the list of critical wavelengths of transition from nonmonotonic to monotonic behavior extracted from our measurements gives an average value $\lambda_{\rm cr} = 724$ nm and a standard deviation 25 nm. This result is in excellent correspondence with theoretical predictions for 1-D RBC aggregation, if the most typical values for erythrocytes refraction index (around $\rm n = 1.4$) and RBC's large semi-axis ($\rm a = 4\ \mu m$) are adopted. We interpret that as a result of strong dominance of signal coming from narrow vessels, where mainly 1-D roulaex are formed. (We have also considered an alternative hypothesis, i.e., blood de-oxygenation. It would correspond to expected value of critical wavelength 810 nm. This value is much higher than the experimental one and therefore the "de-oxygenation" hypothesis has to be rejected statistically.)

To reproduce this result *in vitro* we had to modify the cuvette design. We actually performed two series of *in vitro* measurements with the model suspension described above. In the both,the over-systolic occlusion has been simulated by the sudden cessation of the flow through cuvette while operating the peristaltic pump. Figs. 8 and 9 present the most typical experimental data. In accordance with theoretical predictions, the occlusion is followed by substantial growth of transmission. This picture is observed only for solution containing high-molecular dextran. In other words, the growth of transmission results from RBC aggregation. Nevertheless, contrary to the theoretical predictions in Fig. 8 after decay of pulsations the transmission grows for all the wavelengths. The absence of nonmonotonic behavior for lower wavelength we attribute to the non-1-D growth of RBC aggregates. To overcome this obstacle, we filled the cuvette with optical fibers in order to favor the 1-D aggregation. The details of the cuvette design are the following: Tightly packed glass optic fibers of 50-mum diameter have been used to fill the channel. The fibers have been oriented originally parallel to the blood flow and perpendicular to the light, though the pressure applied in the process of cuvette preparation caused a certain randomization of the orientation of the fibers. Results in Fig. 9 clearly show the expected asymptotic decline for the wavelengths lower than 760 nm. This value rather well corresponds to the theoretical predictions resulting from (8)–(9) and to *in vivo* results as well (Fig. 5). To our mind, this agreement proves the validity of our theoretical results as follows.

1) Pulsatile changes of blood transparency may result from periodic changes of RBC aggregate geometry and corresponding scattering changes.
2) The assumption of 1-D RBC aggregation is reasonable. In spectral range below 760-nm, time dependence of transmission following the long over-systolic occlusion exhibits an asymptotic decline. This results from the destructive interference of light diffracted on long RBC roulauex.

It is logical to note here that the magnitude of the optical signal change resulting from the occlusion far exceeds that of regular heart beats pulsations. This factor gives a substantial advantage to occlusion spectroscopy from the point of view of high signal to noise ratio. We have made a series of attempts to utilize this advantage for direct noninvasive measurements of key blood parameters, e.g., oxygen saturation, hematocrit and blood glucose by occlusion spectroscopy [4], [5], [15]–[17]. The detailed analysis of these encouraging results we postpone for future publications.

IV. Conclusion

First, let us concentrate on the limits of validity of theoretical considerations presented in the Section II. As mentioned above, a single act of scattering is considered within the semi-classical approximation. It is reasonable because of $\rm a, c \gg \lambda$ and $\Delta \rm n \ll 1$. The validity of (2) is based on the explicit use of small angle approximation and therefore is limited to relatively thin blood layer. The thickness of blood layer has to be comparable with $\mu_{\rm tr}^{-1}$. In real *in vivo* measurements, this assumption may be valid or nearly valid. In the last case, the direct usage of (2) might be problematic, but qualitatively the predictions will not change much.

Therefore, it is important not to confuse the presented mechanism with another one that is also scattering assisted. For the case of very thick blood layers $d \gg (\mu_a \mu_{tr})^{-1/2}$, the process of aggregation also favors the transparency growth. But in this case the physical reason is different. Contrary to our consideration, the total amount of single scattering acts becomes so high that all the interference features of single scattering are going to be smeared. Thus, the dominant effect of aggregation for thick layers is a statistical decrease in the amount of scattering acts.

The major conclusions of the paper are summarized as follows.

1) For thin layers of whole blood, diffraction of light on RBC aggregates is among the key factors governing the blood transparency in RNIR spectral range.
2) In the case of pulsatile blood flow, the periodic changes of RBC aggregate geometry results in oscillations of μ_{tr} and finally in pulsations of optical transparency. This theoretical prediction is proved by direct *in vitro* experiments showing identical behavior for the cases of real blood and model dextran containing solution. Thus, it might happen that aggregation/disaggregation mechanism is at least of the same importance as the volumetric one for real *in vivo* oximetry-like measurements.
3) For the case of long over-systolic occlusion, the process of RBC aggregation is nearly 1-D. As a consequence, the time dependence of optical transmission exhibits different asymptotic behavior in various spectral ranges: the asymptotic decline for $\lambda < \lambda_{cr}$ and the asymptotic growth for higher wavelengths. The experimental values of λ_{cr} for both *in vivo* and *in vitro* measurements well correspond to theoretical simulations of light diffraction on RBC roulauex with the most typical set of parameters.
4) *In vivo* over-systolic occlusion results in large ac signal containing information on various blood parameters. Thus, the occlusion spectroscopy arises as a new paradigm of noninvasive blood tests with a considerable promise.

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