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Abstract. We introduce a sensitive method that allows one to distinguish positive and negative agglutination reactions used for blood typing and determination of Rh affinity with a high precision. The method is based on the unique properties of photonic crystal waveguides, i.e., microstructured waveguides (MSWs). The transmission spectrum of an MSW smart cuvette filled by a specific or nonspecific agglutinating serum depends on the scattering, refractive, and absorptive properties of the blood probe. This concept was proven in the course of a laboratory clinical study. The obtained ratio of the spectral-based discrimination parameter for positive and negative reactions (I + /I−) was found to be 16 for standard analysis and around 2 for used sera with a weak activity. [DOI: 10.1117/1.JBO.20.4.040503]

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Hollow core microstructured waveguides (MSWs) are novel unique materials that can be successfully used as highly sensitive sensors for biomedical applications.[1–5] The basic advantages include a high sensitivity, short response time, and small size. The MSW is a special type of photonic crystal waveguide. Its hollow core diameter is a few times larger than the lattice period of the surrounding photonic crystal structures.[4] The cross section of the MSW used in our work is shown in Fig. 1. It was designed and manufactured by SPE "Nanostructured Glass Technology" (Saratov, Russia). The structure of the MSW consists of five functional concentric layers of capillaries and an external buffer layer that provided structural totality in the drawing process. The MSW possesses a few narrow and smooth transmission bands in the visible/NIR wavelength range (Fig. 1). The location and number of transmission bands depend on the diameter of the MSW's core. The spectral properties of MSW were discussed in detail in Refs. 2–4. In this study, we used short pieces of MSW with a diameter of the hollow core of 150 μm and the outer diameter around 1 mm made from flint glass as smart cuvettes. Such a microvolume cuvette serves as a basic replaceable element of a biosensor for blood typing using a standard agglutinating serum technique. The protocol of the MSW technique for blood typing is present in Fig. 2.

A blood type (or group) is one of the most important characteristics of blood evaluated by the presence or absence of agglutinogens (antigens) on the surface of red blood cells (RBCs) and antibodies in the blood plasma. The ABO, based on A and B antigens, and the Rh, based on D antigen, systems are the most significant blood-group systems used in human-blood transfusion.[6] Most methods of blood typing are performed by identification of the specific agglutinogens on the RBCs by using the agglutination sera. In the case of a positive agglutination reaction, antibodies in the serum induce RBC aggregation. There are two basic manual methods that are widely used in hospitals and clinics because of low cost: slide and tube (Fig. 2). The slide technique has several disadvantages: drying up of the reaction mixture can cause aggregation of cells, giving false-positive results; weaker reactions are difficult to interpret.[7] The tube technique is more sensitive, but it requires the use centrifuge and more reagents (not only agglutination sera but also reagent RBC suspensions (Ac, Bc, and Cc).[7] There are some more sophisticated techniques of blood typing that require specific equipment which is not available in every hospital, such as microplates and gel tests, and a solid-phase method.[8]

The risk of blood testing errors occurs for any of the techniques and has the potential for morbidity and mortality upon transfusion.[9] Mistakes of a blood group determination typically happen because of incorrect analysis or in the case of a hardly determinable blood group. An incorrect interpretation of positive and negative agglutination reactions in the classical method of blood typing can be caused by using standard serum that has a low activity. In the case of a positive agglutination reaction, complexes of RBCs are too small to be seen with the naked eye (see Fig. 2). In accordance with publicly available reports[10] in 2007 on the primary testing for ABO-groups, the blood type was determined wrongly in more than 1% of the cases. The hardest blood type to determine is the AB type. The percentage of mistakes rises to 2.76%.

The new technique of blood typing based on hollow core MSW potentially allows one to decrease the percentage of mistakes. This novel method combines the simplicity of a standard technique and the unique features of MSW providing a high sensitivity to alterations in scattering, absorption, and refractivity of solutions filling up the MSW's core and channels in cladding.[2] After mixing the serum with a drop of blood, in the case of a positive agglutination reaction, the majority of RBCs are clumped together. Thus, the number of RBCs suspended in the solution is reduced, which results in the reduction of the scattering coefficient of the solution and leads to transformation of its transmittance signature in the visible/NIR.[11]

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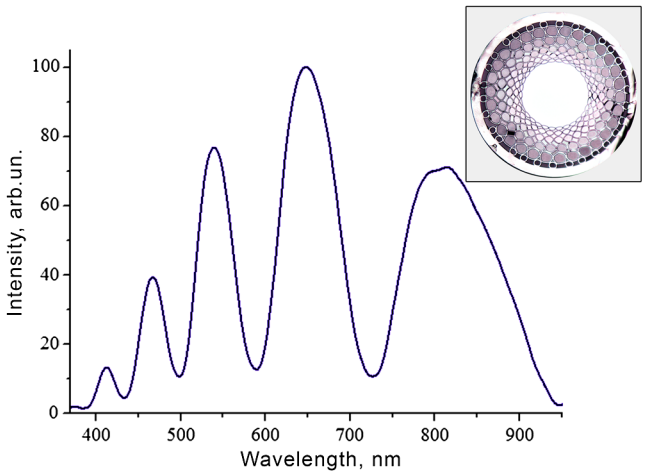

Fig. 1 The transmission spectrum of a microstructured waveguide (MSW) (hollow core diameter is 150 μm). The top right insertion is the electronic micrograph of the MSW cross section.

The following materials and methods were used in this work. The 6-cm MSWs with a hollow core diameter of 150 μm were utilized as a microvolume smart cuvette. Transmission spectra of MSW were measured using a setup containing a broadband light source (halogen lamp), fiber-optical elements for launching and collecting light, adjustable optomechanics providing a positional resolution of 1.25 μm, a spectrometer, Ocean Optics HR4000, operating in the visible/NIR range, and a PC (schematic diagram of the setup is present in Fig. 2).

Laboratory clinical studies for nine volunteers with different blood types (O-blood—two volunteers; A-blood—three volunteers; B-blood—two volunteers; and AB-blood—two volunteers) have shown the effectiveness of the developed method. A BD Vacutainer® (Becton Dickinson) blood collection tube with spray-coated K_2EDTA was used for blood draws and storage. The protocol (Fig. 2) prescribes the separate mixing of the 10 μl drops of each blood sample with 90 μl of antisera anti-A and anti-B in separate test tubes. After 2 min elapsed, each solution was 100-fold diluted by the isotonic sodium chloride solution in order to get an optical signal with sufficient intensity. Then an MSW smart cuvette is filled up by the analyte under study and the transmission spectrum of the MSW is recorded. It should be noted that the MSW filling procedure and follow up spectral measurement take only 30 s, whereas the RBC sedimentation is noticeable only after 3 min.[12] Therefore, blood precipitation cannot dramatically change the light transmission and adversely affect the results.

The main experimental results are given in Fig. 3, where the transmission spectra of the identical 60-mm-long MSWs filled up by solutions containing products of positive agglutinating reaction (A-blood with anti-A serum, B-blood with anti-B serum and AB-blood with anti-A and anti-B sera; totally—9 samples) and with products of negative agglutinating reaction (A-blood with anti-B serum, B-blood with anti-A serum and

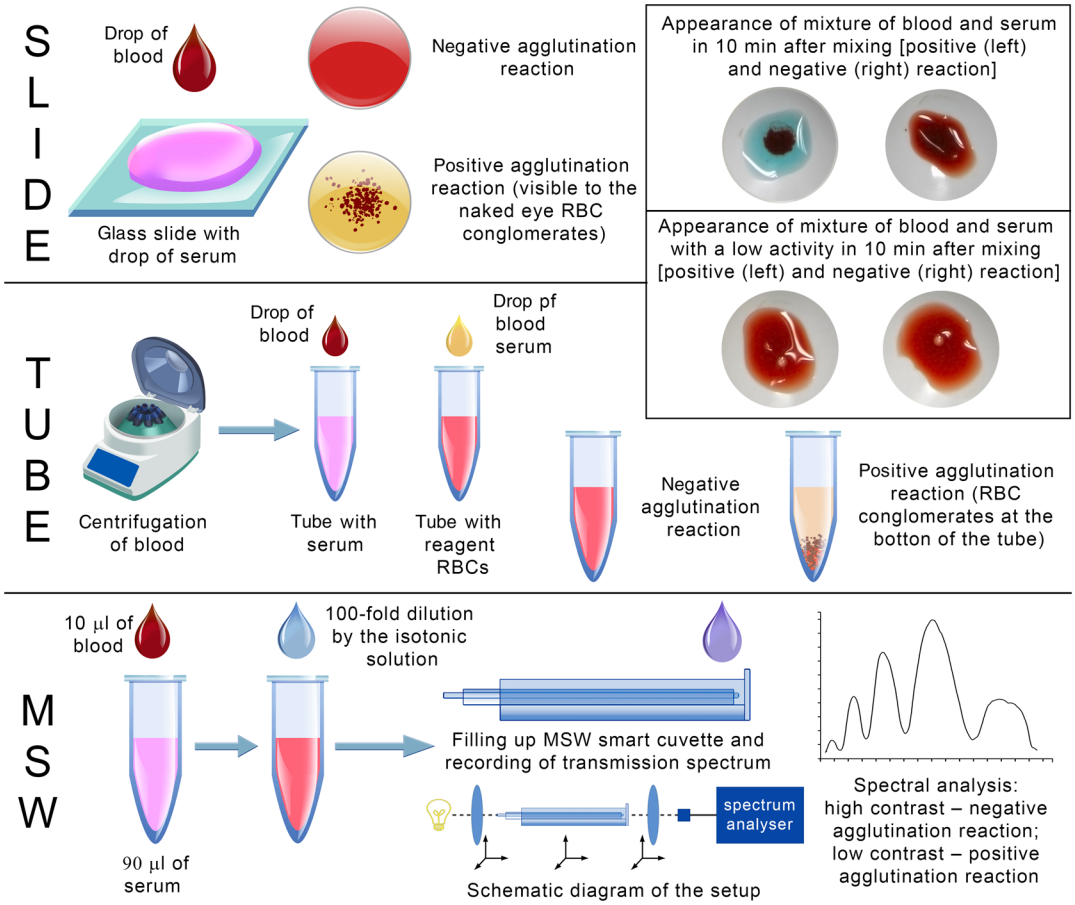

Fig. 2 Slide, tube, and MSW methods for blood typing. Top right: photographs of mixtures of whole blood and standard sera with normal and low activities.

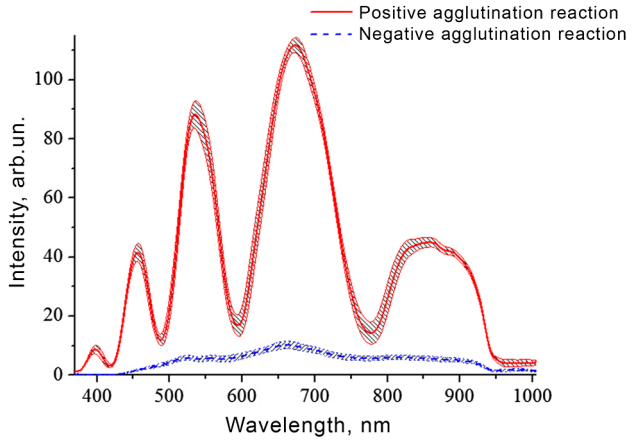

Fig. 3 Transmission spectra of identical 60-mm-long MSW smart cuvettes filled by the products of positive (+) and negative (–) reactions of blood. Spectra were averaged over nine independent measurements with hatched areas showing the absolute errors of each measurement.

O-blood with anti-A and anti-B sera; totally—9 samples) are presented.

To discuss the ability of the proposed method to distinguish positive and negative agglutination reactions we have to compare the intensities of the recorded spectra. From the data presented in Fig. 3, it is seen that in the case of a positive agglutination reaction, the maximal transmittance at the wavelength 540 nm reaches 86.5 ± 5.4 units (I+), while in the case of a negative reaction it is only 5.4 ± 0.9 units (I–). Thus, the ratio of the transmission intensities for different reactions (I + /I–) is equal to 16.

To avoid some experimental errors associated with intensity measurements, a few other parameters can be used to evaluate the agglutination ability. For example, a spectral area integrated over a range of 485–585 nm [ratio $(\mathrm{I}+/\mathrm{I}-)_{485-585} = 10.3$] or contrast ratio $\mathrm{C}_+/\mathrm{C}_- = 6.5$, where $\mathrm{C} = (\mathrm{I}_{670\ \mathrm{nm}} - \mathrm{I}_{580\ \mathrm{nm}})/\mathrm{I}_{580\ \mathrm{nm}}$ can be used.

In the case of using serum with a low activity, the ratio of transmission intensities for different reactions is still well detected as $(\mathrm{I}+/\mathrm{I}-) = 1.82$, and the ratio of contrasts is also high, $(\mathrm{C}_+/\mathrm{C}_-) = 2.14$, despite the fact that one cannot differentiate the type of reaction with the naked eye.

Therefore, from the spectral transmission measurements using an MSW smart microcuvette, we can exactly distinguish positive and negative agglutination reactions.

In this study, we proposed a new optical technique for blood typing. We demonstrated that smart microcuvettes made from microstructured waveguides are prospects for biosensing, including such an important area of application as distinguishing the positive/negative agglutination reactions of RBCs. This method requires only a 10 μl of blood for one analysis. It is fast and simple enough to be used as a point-of-care modality. We have experimentally proven that the magnitude of the MSW transmitted intensity in the case of a positive agglutination reaction is at least 10 times larger than that for a negative one. Moreover, MSW technology allows one to differentiate the type of reaction even when the agglutinating serum has a low activity. More sophisticated signal processing of MSW transmission spectra is possible in order to further reduce the risk of blood testing errors and gain an improvement in biosensing in general.

Acknowledgments

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