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**Article type:** Letter

## Impact of temperature on optical sensing in biology based on investigation of SARS-CoV-2

Małgorzata Szczerska<sup>1\*</sup>, Paweł Wityk<sup>2</sup>, Paulina Listewnik<sup>1</sup>

Listewnik Paulina (Orcid ID: 0000-0002-4857-1019)  
Szczerska Malgorzata (Orcid ID: 0000-0003-4628-6158)

<sup>1</sup> Department of Metrology and Optoelectronics, Faculty of Electronics, Telecommunications and Informatics, Gdańsk University of Technology, Narutowicza Street 11/12, 80-233 Gdańsk, Poland

<sup>2</sup> Department of Biopharmaceutics and Pharmacodynamics, Medical University of Gdańsk, Al. Gen. J. Hallera 107, 80-416 Gdańsk, Poland

\*Correspondence

Małgorzata Szczerska, Department of Metrology and Optoelectronics, Faculty of Electronics, Telecommunications and Informatics, Gdańsk University of Technology, Narutowicza Street 11/12, 80-233 Gdańsk, Poland

Email: [malszcze@pg.edu.pl](mailto:malszcze@pg.edu.pl)

### Abstract

In this paper, we present an investigation of the influence of the temperature on the sensing of biological samples. We used biofunctionalized microsphere-based fiber- optic sensor to detect IgG attached to the sensor head at temperatures relevant in biological research: 5°C, 25°C and 55°C. The construction of the sensor allowed us to perform measurements in the small amount of solution. The results of our experiment confirm substantial changes in the measured reflected optical power, indicating the need to control the temperature during such measurements. The sensitivity of the sensor used in this research is 8.82 nW/°C. Coefficient R was also calculated and it equals 0.998, which shows good fit between theoretical linear fit and obtained measured data.

### Keywords:

biosensing; microsphere; photonic sensor; temperature

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**Abbreviations:** IgG, Immunoglobulin G; IgM, Immunoglobulin M

## 1 INTRODUCTION

Temperature is one of the most important physical quantities. Temperature measurements are used in every field of life. However, in this paper we want to focus on its impact on optical sensing in biology.

We can discern two ways in which the temperature influences measurements of biological samples using optical methods. Firstly, from the biological point of view, the nature of this phenomena relays in structure of surrounding matter - molecules. Each molecule can be characterized by their physical and chemical properties. For example, the length of the bond between atoms strictly depends upon the temperature changes, the solubility of salts in water is also temperature dependent as well as the rate of the reactions, such as fluorescence, luminescence or electron transfer [1,2]. Thus, we can conclude that if the temperature defines the geometrical, chemical and physical parameters of small molecules and their interactions, it has to change the properties of bigger molecules like e.g., proteins or DNA [3].

From the optical perspective, the changes in the temperature during the measurements effect optical properties of the sample, especially their refractive index. With increasing temperature, the density of liquids, including biological samples lowers, allowing the light to travel faster through a measured medium. Because of the decreased ratio of the light in both media, the value of the refractive index also decreases. Dependence between the temperature and the refractive index of various aqueous solutions can be found in literature [4,5].

There are vast number of techniques already available for researchers in order to study the temperature dependence of various processes. The most direct ones are differential scanning calorimetry (DSC), isothermal titration calorimetry (ITC) - allowing for measuring the stability of proteins or DNA or their complexes [6]. The latter one even allows for measuring affinity and stoichiometry of biological systems. Additionally, researchers are investigating the temperature dependence in almost all the experiments because temperature have to be strictly controlled. The rate of biochemical reaction e.g., bond forming reaction according to the laws of thermodynamics is temperature dependent and proportional according to the formula (1):

$$k(T) = Ae^{\left(-\frac{E_a}{RT}\right)} \quad (1)$$

where:  $E_a$  – activation energy,  $R$  – gas constant and  $T$  – temperature.

Moreover, the rate of the electron transfer reaction is also temperature dependent according to the Marcus electron transfer theory. What is more, the absorbance, especially fluorescence, is

strictly temperature dependent process, which is always taken into consideration during conducting research [7].

Currently the development of new techniques for temperature monitoring allows to detect even the smallest changes in energy flow (temperature). The sensitivity of temperature changes in order to meet the demands of the micro and nano scale biological system should be still increasing [8]. The typical volume of biological sample after purification is less than 10  $\mu\text{L}$ , the direct measurements in body e.g., to detect the places where the metabolism is higher (cancer tumors) we should consider the volume in nanometers. The interaction between modified surface of the sensor with detected IgG/IgM in the solution are strong (as almost all interaction between antibody and antigen), nevertheless the strength of those interaction decreases with the temperature. The increasing (with temperature increase) vibrational modes of atoms involved, weakens interaction between sensor and detected antibody. Thus, measurements should be performed in strictly monitored environment. The measurements with regular interferometric setup are difficult to perform in constant temperature in small volumes (less  $< 5\mu\text{L}$ ) due to surface tension of water/biological fluids.

Although aforementioned methods have good metrological parameters and are popular in the laboratory usage, there should be noted that their use always required the application of complex equipment operated by trained professional. There is a niche for sensors, which metrological parameters can be worse than those achieved by the laboratory equipment, however their price and simplicity of application could make attractive alternation for the first one. Taking into consideration many factors as development of fiber optic technology and its prevalence as well as availability of fiber optic items in reasonable prices, it seems that fiber optic sensors are the best candidates. There is a very broad range of fiber optic temperature sensors, however their construction addresses the need of industry, and they are not appropriate for application in the biomedicine. There are temperature sensors constructed with materials which parameters changes [9] (e.g., refractive index) due to the temperature changes [10–12]. Despite many changes of such sensors, their probes are based on the materials which make them toxic for biological tissue, their dimensions are too big to measure small samples [13–15] or the needed power for the proper operation can destroy the biological samples [16–18].

Taking it under consideration, there is understandable need for monitoring and controlling temperature when performing optical investigation of biological samples. And while there are many methods and devices to measure temperature, we propose to use microsphere-based fiber-optic sensor. The innovation of this solution lies in possibility of controlling point-wise real

time, on demand temperature of small amount of biological tissue with constant control of the sensor technical condition.

- (1) Due to the fact our measurement head is the size of less than 250  $\mu\text{m}$  we are able to make point wise measurement without disturbing the measurement environment which is unsolved problem when using other measurement method.
- (2) Furthermore, by the use of the fiber optic construction our probe does not influence the biology of the sample and investigated sample are not able to destroy the probe, so it makes possible to reuse the sensor for a long time.
- (3) Because we used single-mode fiber, we delivered to the sample so small amount of optical power as it does not influence the behavior of biological sample.
- (4) The use of telecommunication optic fiber gives the possibility to make remote and real time control with the use of telecommunication network. Furthermore, the data can be easily delivered in many points of control of the same time.
- (5) The application of microsphere, which introduced the additional modulation of measured signal, can the real time update the status of the sensor technical condition because with any damage of the sensor or it's coating the modulation could be seen in the spectra.

## 2 EXPERIMENTAL SECTION

The microsphere-based sensor immersed in the antibody solution of concentration 10  $\mu\text{g/mL}$  was placed in the thermocycler (PT200 Bio-Rad, USA) to investigate the influence of the temperature on the measured samples. The container with the sample was placed in the thermocycler and the temperature was stabilized and kept at 5°C, 25°C and 55°C throughout the entirety of measurements, therefore ensuring controlled environment. While typical temperature range for incubation and testing of the proteins is between 4°C-37°C, we decided to extend it up to 55°C because in the measurement environment within a temperature of more than 25°C we can expect the elimination of the binding of structures similar to the antibody or antigen [19–21]. This gives us the possibility to elevate the specificity of the sensor. The temperature stability of the proteins mainly depends on the proteins' structure and chemical and physical properties of the buffer solution. As presented in the literature the protein degradation temperature can be modified up to 120°C [22], or less than 0°C [23–25] according to the experimental condition. The measurements were executed using a broadband light source with a wavelength of  $1310 \pm 10 \text{ nm}$  (SLD-1310-18-W, FiberLabs Inc., Japan), which produces an

optical signal. It goes then through an optical coupler (50/50%, 2:1, G657A, CELLCO, Poland) to the designed sensor and the data is collected and analyzed using Optical Spectrum Analyzer (OSA, Ando AQ6319, Japan). We have constructed biosensor which composes of quartz fiber optic ended with microsphere head. The head of the fiber optic was at first chemically modified with APTES solution to introduce reactive centers onto the surface of the microsphere. Then with use of chemical reaction the biotin molecule was attached. After the chemical modification with biotin, we have immobilized S1-Avidin protein to the surface of chemically modified microsphere by using the Biotin/Avidin interaction. At this stage the sensor can be used to detect the S1-anti-antibodies. Fig. 1 represents the layers attached to the head of the microsphere – fully functional biosensor.

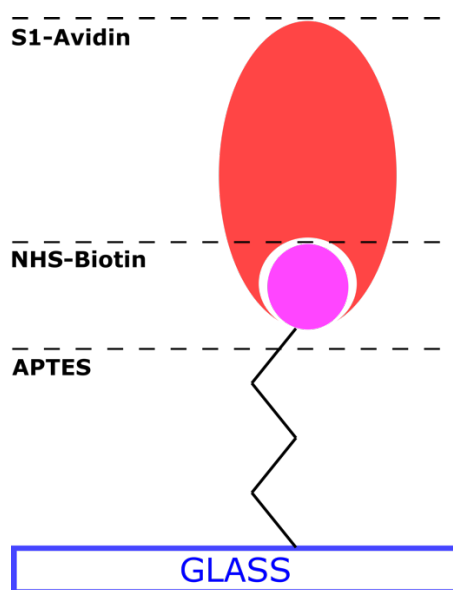


Figure 1. The schematic representation of the surface preparation. The fiber optic microsphere made from quartz glass is covered by biochemical layer. The first step is to attached APTES to the glass, after that NHS-Biotin coating is made, lastly the S1-Avidin interface is produced. During measurement the IgG antibodies are attached by this layer.

The measurement with biosensor (see Fig. 1) was performed in 2 stages: (i) – the immersion of the probe in blank solution as reference (without antibodies), (ii) – immersion of the sensor in the antibody solution. The immersion of the sensor in antibody solution results in bond formation between antibody and sensor head. Indeed, the binding of antibodies to the surface of the optical fiber was recorded by the optical power changes of reflected signal. The measurements were performed with a probe in 1xPBS as a reference. Next, the probe (microsphere with immobilized S1 protein – see) was immersed in the pure 1xPBS solution and then transferred to the dilution of anti-S antibody at 10<sup>-6</sup> g/mL The sensor was submerged in the anti-S antibody solution and the measurement was done. The figure has been added to the

manuscript (Figure 1). As indicated above the measurement should be temperature depended, due to the fact that the structure of water, ions and proteins are highly temperature dependent. Thus, when temperature increases the energy absorption by those molecules and ions should decrease because they will dissociate for the proximity of the sensor head. Looking at this from the optical viewpoint, the increase in the intensity of the light reflected on the cladding/investigated medium is caused by changes in interactions between antigen and antibody, which is temperature-dependent. On the other hand, the changes in the structure of the fiber optic sensor can be calculated based on the phase difference. The phase difference  $\Delta\phi$  and subsequently changes in the measured response of the optical power  $P$  can be calculated from the formulas (2) and (3):

$$\Delta\phi = \frac{4\pi n L}{\lambda_0} \left[ n \frac{\partial L}{\partial T} + L \frac{\partial n}{\partial T} \right] \Delta T \quad (2)$$

where:  $n$  – refractive index,  $L$  – cavity length,  $T$  – temperature.

$$P = S(\nu) \cos(\Delta\phi) \quad (3)$$

where:  $S(\nu)$  – spectral distribution of the light source.

The thermal coefficient of pure silica ( $4.1 * 10^{-7}/^{\circ}\text{C}$  at  $20^{\circ}\text{C}$ ) is not enough to have impact on the structure of the uncoated sensor, therefore ensuring that any changes observed during resented investigation are the result of the biological and chemical interaction.

When the microsphere head of the sensor is fabricated, two microspheres are made, one inside another, the smaller one is made of an optical fiber core, and the bigger one form the fiber cladding. The schematic, showing how the sensor works is included in Figure 2.

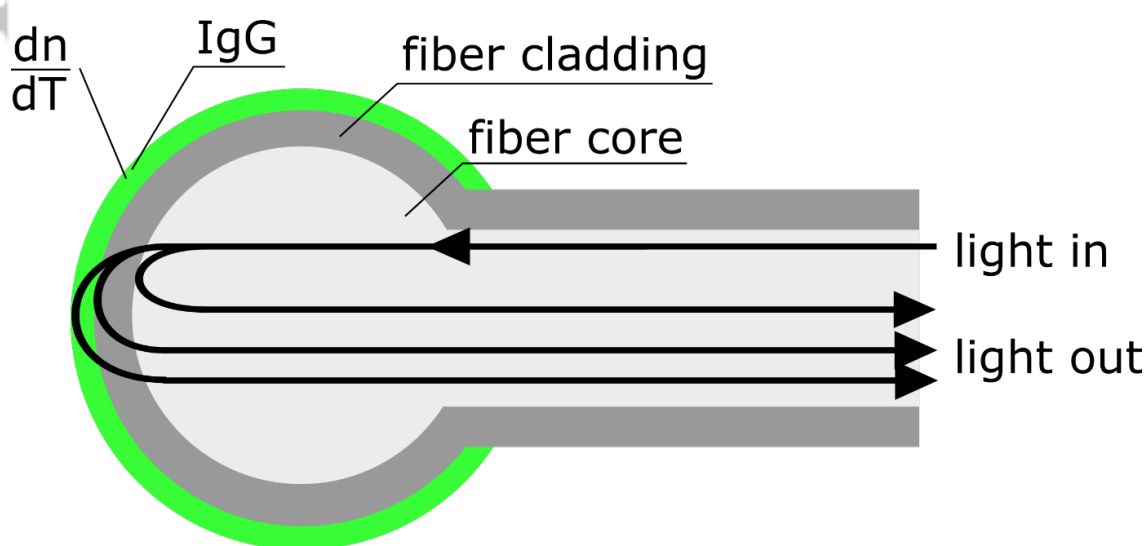


Figure 2. Schematic of the sensor.

Interference forms when the wave is reflected from the core/cladding boundary between spheres and then from the cladding/measured medium boundary. And while the first wave reflected at the first interface stays the same, the second one is dependent on the changes to the surrounding medium (i.e., temperature, refractive index).

### 3 MEASUREMENT AND RESULTS

Firstly, the behavior of the sensor response on each subsequent stage of the probe preparation has been tested. The results are shown in the Figure 3.

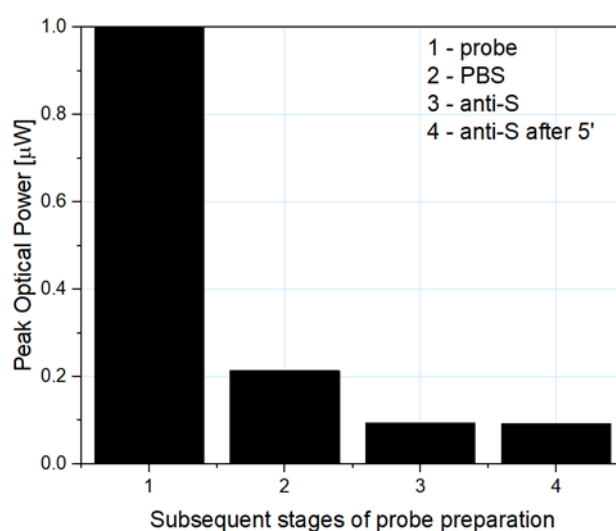


Figure 3. Subsequent stages of the probe preparation, where: 1 – probe covered in biofilm, 2 – probe immersed in the PBS, 3 – probe immersed in the anti-S solution, 4 – probe immersed in the anti-S solution after 5 minutes.

As can be seen, the optical power of the reflected signal is changing according to the subsequent stages of probe preparation. Starting with uncoated microsphere-based probe, which was assumed as a reference level. Next stage was to immerse the probe in the PBS to test its operation. Lastly, the probe was put into the container, which held the anti-S solution. Presented graph confirms proper operation of the sensor. Moreover, it is known from the literature that the immobilized proteins are stable in the surface of optical fiber and the antigens can be presented for antibody interaction [26–28].

In the following steps, the measurement has been performed in the range of temperature from 5°C to 55°C. This range has been chosen as the most important in biology. For each temperature we collected the spectra of light reflected of biolayer covered the fiber optic microsphere. Each



temperature has been investigated over a time of 15 minutes to ensure the stability of measurement condition. In figure 4. the representative measurement signal is presented, which confirmed that the measurement system was able to ensure the stable environment of investigation.

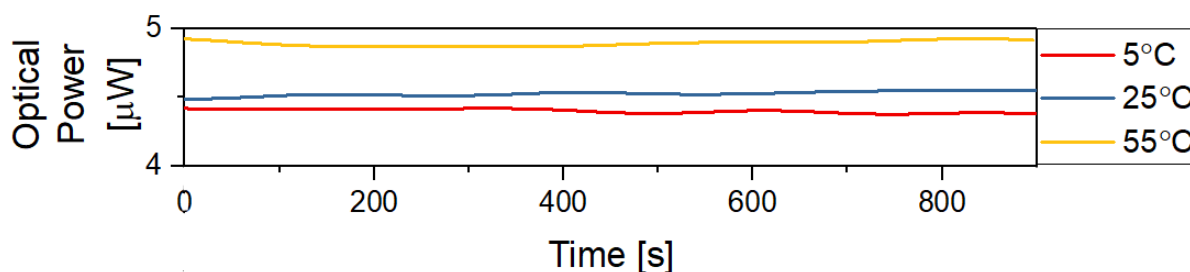


Fig.4. Representative reflected signal response for stabilized temperature measurements at 5°C, 25°C and 55°C over a period of 15 minutes

Whereas in the figure 5 the average spectra for three representative temperatures: 5°C, 25°C and 55°C has been presented. It can be noted that the modulation of the spectra has not changed in each series, however we can observe different level of the optical power.

Please note that the temperature dependence is almost linear in 5°C to 55°C range which is relevant in biological systems. The measured response of the sensor for each temperature can be observed in Figure 4. Taking into consideration those observation it was possible to define the relationship between the optical signal reflected from the bilayer and the temperature, which is shown in fig.5.

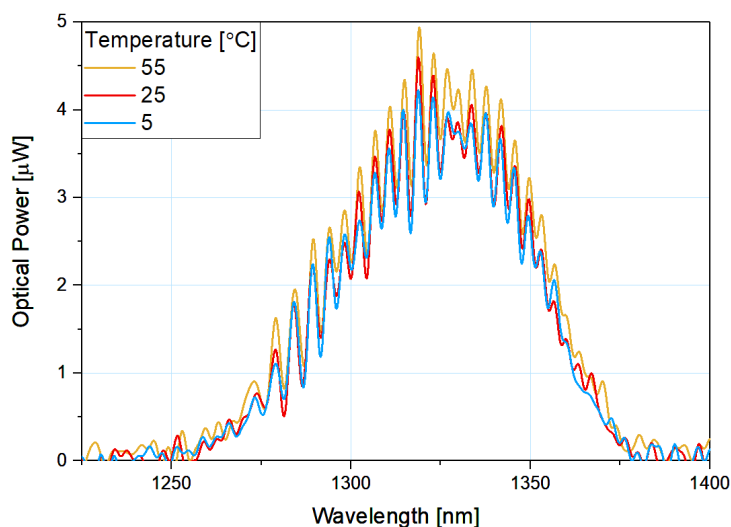


Figure 5. Measured response of the sensor at 5°C, 25°C and 55°C.



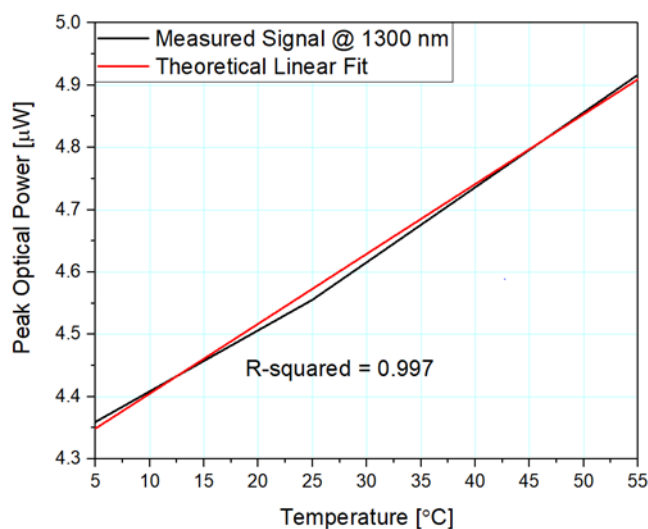


Figure 6. Changes in the peak optical power of the reflected signal during alteration to the sample temperature.

As presented in Figure 6, the peak optical power of the reflected signal increases due to the increase of temperature of the biological sample from 4.35  $\mu W$  for 5°C to almost 4.9  $\mu W$  for 55°C. The obtained results are consistent with the known phenomenon explained in [29]. It is a consequence of decrease in the antibody's bindings, which are dependent on the temperature. However, we are able to observe the phenomenon, from the optical point of view. The theoretical linear fitting of that relationship has been performed and added as a black line the figure. The coefficient of determination ( $R^2$ ) obtains the value of 0.997, which ensure us that the theoretical, statistical model can be used to predict the future changes in the biolayer due to the temperature changes. Whereas the Pearson correlation coefficient value is 0.998 close to 1, what statistically confirms the linear relationship between power of reflected optical signal from biolayer and temperature.

#### 4 DISCUSSION AND CONCLUSION

As it has been showed, the biological system, as biolayer, can change its properties and could not preserve the same specific interaction (protein changes their structure) between antibody and antigen in different temperature. In that way, our observation confirms the phenomena of temperature dependence of protein interaction reported by F. Schwesinger et al. [30]. The stable frequency of modulation into measured spectra introduced by the signal reflected by the internal mirror into microsphere, ensure us that the changes due to thermal expansion of the fiber material does not exist in our set-up. Therefore, any changes in optical power can be directly indicating as the changes coming from interaction of antibody with antigen.

In this paper, we investigated the impact of the temperature on the optical sensing in biology. The investigation was carried on the biological samples with temperature maintained at 5, 25 and 55°C. The results show the dependence of the biological the temperature during sensing of biological samples. It can be observed, the optical power of the reflected signal is significantly higher at 55°C than at 5°C. The increase of the signal is linear according to the temperature rise. It is a consequence of the changing properties of protein and difference in the interaction between antigen and antibody. Presented results confirm the necessity of keeping control over temperature while performing examination of biological samples. What more, the proposed microsphere-based fiber-optic sensor is an excellent tool for such measurements, because of its advantages over other sensors, especially its ability of operation in small solution volumes.

#### **AUTHOR CONTRIBUTIONS**

Conceptualization M.S, P.W, P.L; Data curation P.L; Formal Analysis M.S, P.W, P.L; Funding acquisition M.S; Investigation M.S, P.W, P.L; Methodology M.S, P.W; Project administration M.S; Resources M.S, P.W, P.L; Supervision M.S; Validation M.S; Visualization P.L; Writing – original draft M.S, P.W, P.L; Writing – review & editing M.S.

#### **FINANCIAL DISCLOSURE**

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#### **CONFLICT OF INTEREST**

The authors declare no financial or commercial conflict of interest.

#### **DATA AVAILABILITY STATEMENT**

Data available on request from the authors

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