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OPTICAL AND PHOTOELECTRIC PROPERTIES OF DRY BACTERIORHODOPSIN SENSORS

Examiners: Prof. Lasse Lensu

Assoc. Prof. Erik Vartiainen

Abstract

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In this thesis, bacteriorhodopsin (BR) photosensor's optical and electrical properties were studied. The BR sensor consisted of a dry film with BR in polyvinyl alcohol and covered with transparent conductors. In the experiments the BR photocycle was started with two lasers. The characteristics of the BR sensor were measured in two ways. The first approach was theoretical and it required knowing the laser parameters. The second way required assembling a measurement setup for the optical response measurements. However, no measurable results were obtained due to low laser power. The photoelectric response was measured in the experiments with two laser systems and the amplifier was tested. In the experiment with a Cavitar laser, the photoelectric response was obtained. In the experiment with FemtoFiber Pro laser, the photoelectric response was not measured. The expected amplitude of the response was obtained. The experimental data was analyzed and possible solutions for reducing the interference were given.

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I would like to thank Prof. Lasse Lensu who helped, supported me and explained the most difficult questions. Without him all the work will be impossible. I thank Juha Parvianen with whom we made all the laser setups. Due to his experience we could launch the tunable laser system, the pulse picker device and make all the experiment setups. I thank Joonas Talvitie for his electronic device construction. I thank Erik Vartianen for his help to find, configure and launch the laboratory equipment. I also thank him for his support in all the calculations and data processing.

Contents

Abbreviations and symbols

BR – Bacteriorhodopsin

CMOS - complementary metal-oxide-semiconductor

LB Langmuir-Blodgett

PM – purple membrane

PVA – polyvinyl alcohol

1. Introduction

Photoactive molecules

In modern data processing devices electronic circuits are commonly based on metal-oxide semiconductors. Devices should require two contradictory user demands: On one side the devices should work faster and have more functions, which require more semiconductors in the construction. On the other side the devices should be portable, have compact size and little weight. This two reasons lead to the process called downscaling. In accordance with Moore's law every 1.5 years the number of transistors in a semiconductor is two times bigger than previous year. [17] However, element size restrictions exist in complementary metal-oxide-semiconductor (CMOS) fabrication technology. Even if the size limit is not reached, it is possible in the near future. One of the alternative progress ways is using of molecular components in electronic devices. Molecular electronic devices can convert, process and store the input information [29]. Light-to-electricity conversion devices are of interest due to high frequency capabilities and other important application, such as imaging [1]. That explains the interest in the study of photoactive molecules.

Photoactive molecules widely exist in the nature. According to their chemical composition, they are retinal proteins [1, 2]. This molecules generate a response under light influence. In animal species, this response is a neuron signal containing information about any visible object [2]. Photoactive molecules were found in Archaeans too [1]. This discovery aroused great interest in modern science because of the possibility to use photoactive molecules in electronic devices. It was found, that photoactive fragments can be extracted, and the electrochemical energy, caused by light might be converted to electromotive force. The particles, that exhibit such properties, are called bacteriorhodopsins (BR) [2].

Bacteriorhodopsin is a retinal protein in an archaean's purple membrane which are the light-sensitive properties [1, 2]. When exposed to light, biomolecules called bacteriorhodopsin generate a proton gradient which is a part of the chemical energy conversion [1]. One of the most interesting effects is that bacteriorhodopsin acts as a proton pump. Potential difference appears as a result of this reaction. Due to this property it is possible to integrate it in an electronic device system.

Using of biomolecules

An electric response, named photocurrent can be obtained from such molecules in special conditions [2]. Bacteriorhodopsin in a polymer forms as a thick layer which is placed between two transparent electrodes and one of the electrode is a conductive glass plate. The bacteriorhodopsin layer surface is the input for the light signal and the electrodes are the output ports for the electric response signal. One of the possible applications of a molecular light sensor is a camera [1]. An imaging device consists of three types of elements with different absorption characteristics. Photosensors form a 2-D array. The imaging array device is color sensitive and acts like an RGB camera [1].

Bacteriorhodopsin plays the main role in archaean energy conversion process [1]. Without oxygen BR transfers a proton through the membrane. It grabs a proton from the intracellular space of the membrane and then it starts the chain of reactions. As a result the proton is discharged to the extracellular space. This process contributes to the adenosine diphosphate (ADP) to adenosine triphosphate (ATP) conversion [3].

The BR molecule contains a protein chain and a photo-sensitive retinal. The series of sequential changes in the BR is called the photocycle [1]. The photocycle consists of several intermediate states; every state has its own absorption spectrum and duration times [2]. The duration times are relatively short which emphasizes the importance of using ultra short light pulses to research the BR properties. If the light influences the BR in one of the states it can return to the ground state bypassing the following states.

BR can be represented as a water solution or dry film. In this work dry films will be researched. The preparation methods will also be considered. The output characteristics of the photosensor depend on the thickness impedance and capacitance of the film [7].

Measurements

The measurement system contains a light source which is a 570nm laser connected with a picosecond pulse device in one experiment and 690 nm laser in another experiment. The measurement consists of the BR sensor, the amplifier and the oscilloscope. The BR sensor photo-voltage is amplified and then represented on the digital oscilloscope. The measurement system registers a photo-voltage which is the response on light excitation. In the third experiment the system contains an absorption measurement device to control the transmitted light through the BR sensor to find out how long it takes BR sample to complete the photocycle.

Objectives

The main objective of this thesis is to study the photoelectric properties of dry Bacteriorhodopsin films by electronic and optical measurements. The second objective is to obtain the photoelectric response of the BR sensor. Also the number of molecules in the BR film and the number of the molecules which start the photocycle will be calculated. The measurement setup for the optical response measurements will be built and described. The experiment with a picosecond laser will be made to study whether the BR film response can be characterized better when compared to preceding measurements with slower light sources.

2. Materials and methods

Bacteriorhodopsin is a biomolecule suitable for bioelectronics with unique capabilities. Its functions are connected with light excited energy conversion. Bacteriorhodopsin was found in purple membranes in *HalobacteriumSalinarium*[6].

BR differs from known natural and synthetic photochromes by its unmatched cyclicity of operation (not less than 10^6 cycles) and sensitivity [3]. Materials based on BR have exceptional stability. Proteins are very resistant to external environment. This is because of extreme conditions of living bacteria in nature (high salt concentration, high temperature and intensive solar radiation). The efficiency of functional structures based on BR persists for 15 years and not less than 10^4 hours under the influence of laser radiation. [3]

Bacteriorhodopsin

BR is produced by halobacteria which are archaea. Archaea are special evolution branch; it is different from eubacteria and eukarya [1]. A halobacteria membrane consists of the lipid bilayer with special areas named the purple membrane (PM) which contains hundreds of thousands of BR molecules. The molecules are arranged in crystal hexagonal structures (see Figure 1). The purple membranes contain 75% proteins and 25% lipids. Strictly oriented protein molecules permeate the membrane. The membrane thickness is equal to 5 nm. The side length of the lattice triangle is 6.2 ± 0.2 nm [1].

Figure 1. Purple membrane: structure of BR molecules [6].

Sodium chloride concentration reduction to 2 M leads to the disintegration and death of halobacterium as a result of the osmotic shock. This phenomenon is usually used for BR separation. During this process the purple membrane keeps its structure. The purple membrane is a crystalline structure that can keep its properties as dry and polymer films during several years. [6]

By its chemical nature BR is a transmembrane molecule that consists of a protein and a retinal chromophore. The protein is formed by a chain of 248 amino acids, and it is folded into seven α-helices denoted by the letters A through to G. The molar mass of BR is 26785 g/mol and the size of a molecule is about 5 x 5 x 5 nm^3 [1, 6]. The α-helices are located into the membrane and non-spiral *cis* parts are located on the both sides of the membrane (see Figure 2).

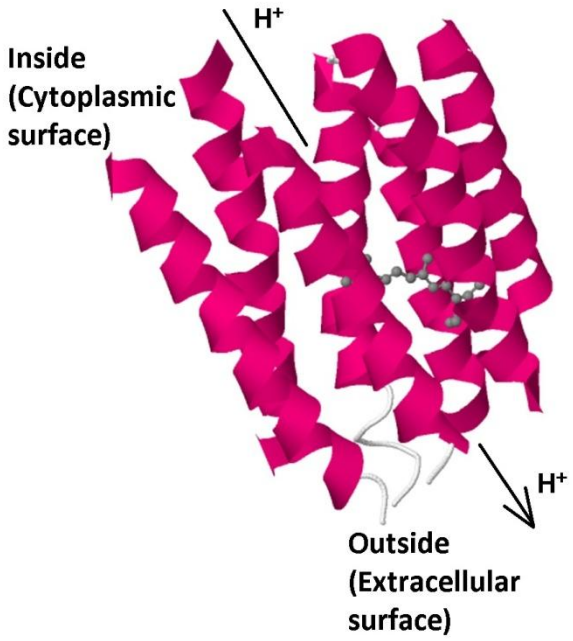

Figure 2. Molecular structure of bacteriorhodopsin containing the seven α-helices surrounding the retinal-controlled proton pumps [27].

One of the sides of the molecule faces the external environment and another faces inside the cell interior. The chromophore is a light-sensitive retinal, an aldehyde of vitamin A. It is bound via the Schiff base to Lys-216 of the protein [1].

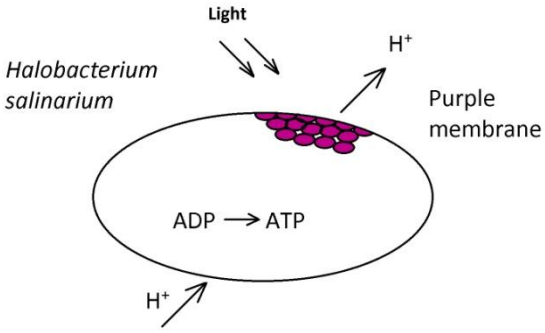

Figure 3. *Halobacterium salinarium* under the influence of light (reproduced from [18]).

Photocycle

Implementation of the photochemical cycle is a fundamental property of BR. In the absorption of a photon, the BR molecule passes through a sequence of the following states. BR returns to the ground state either by the thermal transitions (black line) or by colored light transitions (solid line in Figure 4).

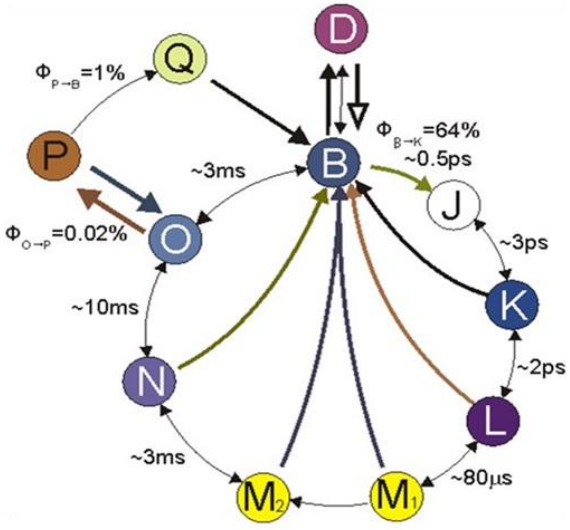

Figure 4. Scheme of the BR photocycle. D and B letters stand for dark-adapted (66% of retinal in 13-cis conformation, 34% are all-trans) and light-adapted (98% of retinal in all-trans conformation) ground states. The color of a circle represents the approximate color of molecules in that intermediate state. Thin arrows stand for thermal transitions and thick ones denote photochemical transition induced by colored light [1]. The color of a state represents the approximate color of light, corresponding to the absorption maximum in that intermediate state.

Arrows denote photochemical transitions induced by colored light. The transition times are approximate values of neutral aqueous solution at ambient temperature [1].

The BR photocycle has several intermediate states, however, due to absorption maximum of light (see Figure 5) in the M state. That state can be easily distinguished from the others so in this thesis we will operate with 2 states B and M.

Changing the state of the BR molecule results in a change in the optical characteristics: the refractive index and absorption. Every intermediate state has a corresponding absorption spectrum. The thermal relaxation times of the intermediate states are in the range from 0.5 ps to 10 ms [1].

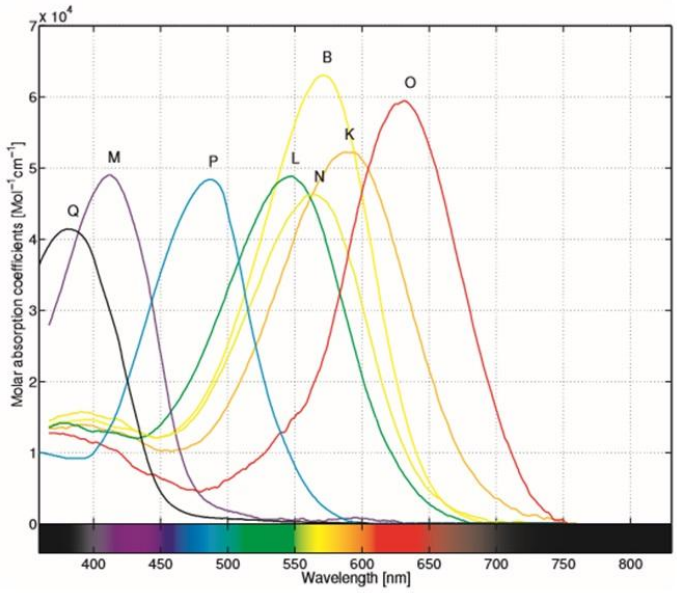

Figure 5. Spectra of the bacteriorhodopsin intermediate states [1, 13, 14, 15].

The main BR function in purple membrane is light-induced proton transfer through the membrane. This process results in hydrogen electrochemical potential. The cell consumes the energy. H^+ is ejected on the external side of the cell membrane, and H^+ is taken from the internal side [3].

Spectral sensitivity of BR is in the visible region of the spectrum. The absorption maximum in the ground state B equals to the wavelength of 570 nm. The main intermediate state M has 412 nm wavelength of maximum absorbance. The M state has high absorption in the purple region of the spectrum and low in yellow. The absorption of the photon at the 570 nm wavelength and transition of the molecule to the M state results in decreasing absorption in the yellow range and increasing absorption in the purple range. Light at 412 nm causes a fast light-induced transition from the M state to the B state.

Preparation method

There are two methods for obtaining BR: artificial cultivation of microorganisms of the genus halobacteria and isolation of protein from halobacteria cells [3].

Cultivation of bacteria producing Bacteriorhodopsin

Culturing of *Halobacterium Salinarium* requires the following conditions: The temperature in the culture medium is 38 to 39 ^{0}C and pH=7.2. Air aeration and fluorescent lighting are also required. The composition of the culture medium consists of sodium chloride, magnesium sulfate, potassium chloride, and yeast extract peptone. Depending on the particular strain and the culturing methods the duration of cultivation is of 50 to 100 hours. The cell mass is precipitated in a centrifuge (8000-15000 Rpm, 20-40 min). The cell output is from 3 to 10 g/l, and the BR output is 0.3-1.0% of the cell amount [3].

Isolation and purification of bacteriorhodopsin

BR isolation technology consists of three phases:

1. Disintegration of the body cells. The procedure is usually performed by an osmotic shock and lyses of the cell wall of DNase. It results in allocation purple membrane fragments, which contain BR.
2. Precipitation in a centrifuge.
3. Cleaning by multiple precipitation in a centrifuge [1, 3]

BR dry films

Dry purple membrane films are the most convenient form for researching the different external effects on Bacteriorhodopsin such as low temperatures, humidity, electric and magnetic fields. [5] A BR sensor can have the following construction. The BR film is placed between two transparent electrodes. The dry BR film is located on the surface of a substrate, between the substrate and the conducting layer. This construction is like a “sandwich” with the BR dry film in the middle. The substrate can be transparent, conductive, and solid, so it also has a protective role. This form is suitable to mount it in a measurement system. This construction allows studying external influence factors and registering optical and electrical changes in the purple membrane.

The easiest method to prepare a dry BR film is to apply water solution on the substrate and let it dry [1]. The advantage of this method is relatively easy and quick obtaining of big amount of BR sensor without special equipment. Orientation of the PM patches is random, so some samples have high quality and some samples are defective. PM patches are disoriented; the

concentration varies at various points. More complex methods require the orientation of PM patches. Several methods exist and can be used. [4, 5]. The first one is Langmuir-Blodgett (LB) method. An LB film contains one or more monolayers. All the bio molecules are strictly oriented. The quality of this film is very high; it is well suited for studying the BR properties. The disadvantage of this method is high complexity of preparation process and a large amount of time required. [4]

The next technique is electrophoretic deposition technique (EPD). This method applies to produce oriented films. PM solution is located in the electric field of two electrodes. An anode attracts PM particles and the solution dries. The films produced this way are highly oriented, but the number of layers is unknown [4, 5].

Using polymers in according to EPD can increase the strength of the film [4]. It should be taken into account that additives in the BR film can change BR properties. If the preparation is carried out not for research but for practical application, then the change in the properties is not being so important [4]. In some cases, the property we are using in our application might even get improved when the environment of BR is modified. [5]

In our studies, BR molecules were mixed with polyvinyl alcohol. The preparation method consists of the interfusion of PM particles with polyvinyl alcohol then spreading the solution on the conductive glass and letting it dry.

Bacteriorhodopsin sensor

The conductive glass acts as the first electrode in the “sandwich” when it is covered with SnO_2 coating. Another electrode is formed by a thin transparent layer of gold. The gold was chosen from other conductors due to its high conductivity [4]. As a result, the output photo-voltage is not decreased by high resistivity of the electrode. The structure of a simple BR sensor is presented in Figure 6.

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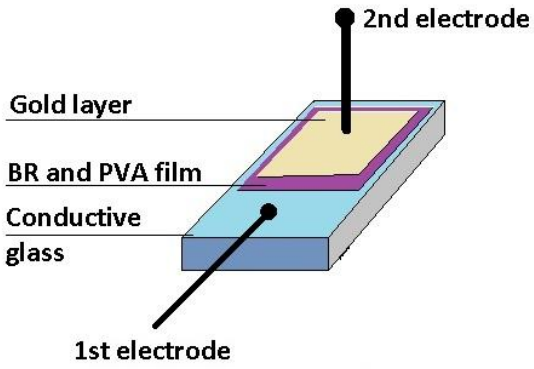


Figure 6.The structure of simple bacteriorhodopsin sensor.

Under light illumination BR produces a photocurrent. It is also called the photoelectric response. The photoelectric response is a result of light-induced chemical transitions in the BR sensor. The photocycle includes different intermediate states. All the intermediate states have their own lifetimes, which range the values from 0.5 ps to 10 ms [1]. According to the output signal, the intermediate lifetime constants can be estimated (see Figure 7).

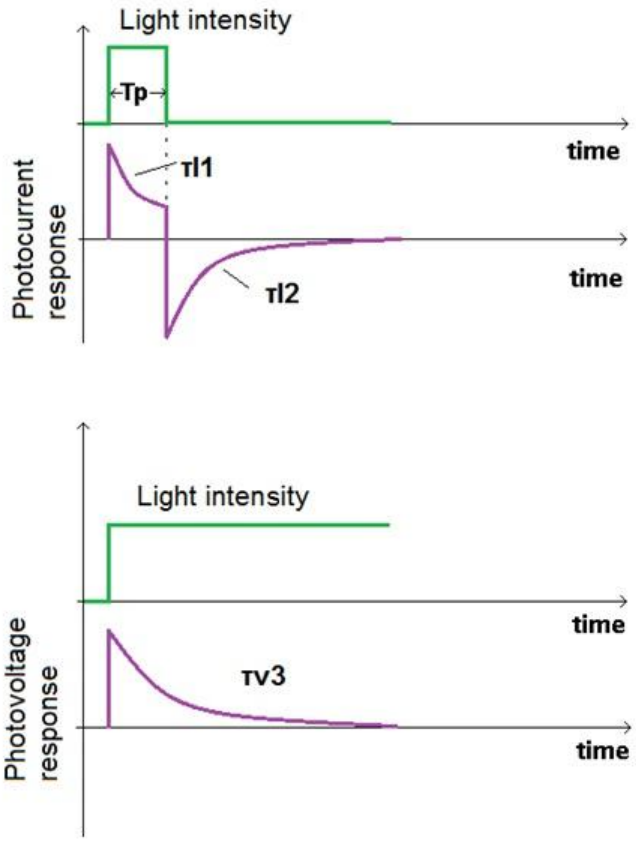


Figure 7.Photo-voltage and photocurrent response diagram of the BR sensor.τI1, τI2 – time constants of the photocurrent response. τV3 – time constant of the photo-voltage response. (Reproduced from [10])

Estimating the number of excited BR molecules in dry film by transmission spectrum measurements

Let us consider the BR sensor, where the photocycle is started by a very short pulse from a laser. At first the BR molecules are in the ground state (B state in Figure 4). The maximum absorbance wavelength in that state is ~560 nm. So the laser produces a very short pulse at the maximum absorbance wavelength and starts the photocycle. The number of photons does not reach or exceed the number of molecules in the BR film. However a number of the molecules starts the photocycle. At the same time a very weak constant illumination from another source influences on the BR at the wavelength of ~420 nm, which is the maximum absorbance of the M state (see Figure 7). The constant illumination causes very few molecules to start a new photocycle due to its low energy and the significant separation from the maximum absorption at the B state (ground state). However when the BR reaches the M state the transmitted illumination is absorbed stronger in the sensor (in Figure 8) and the change in the transmitted light can be measured.

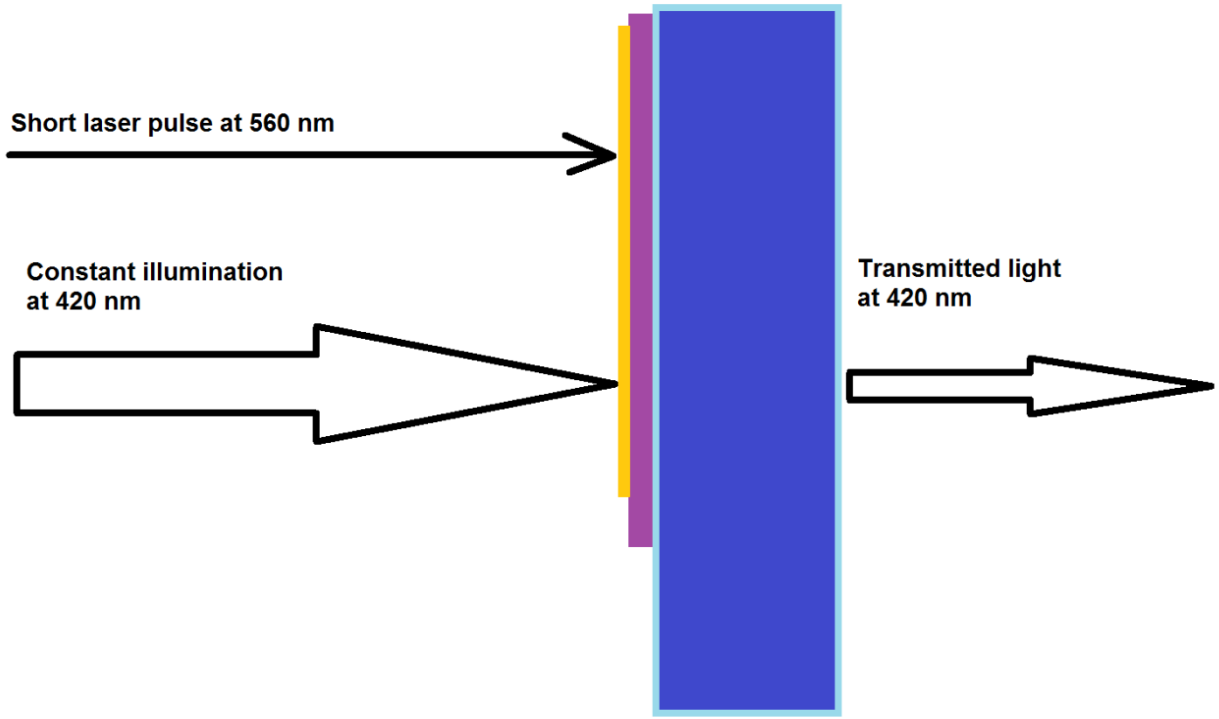


Figure 8. BR absorption measurement setup.

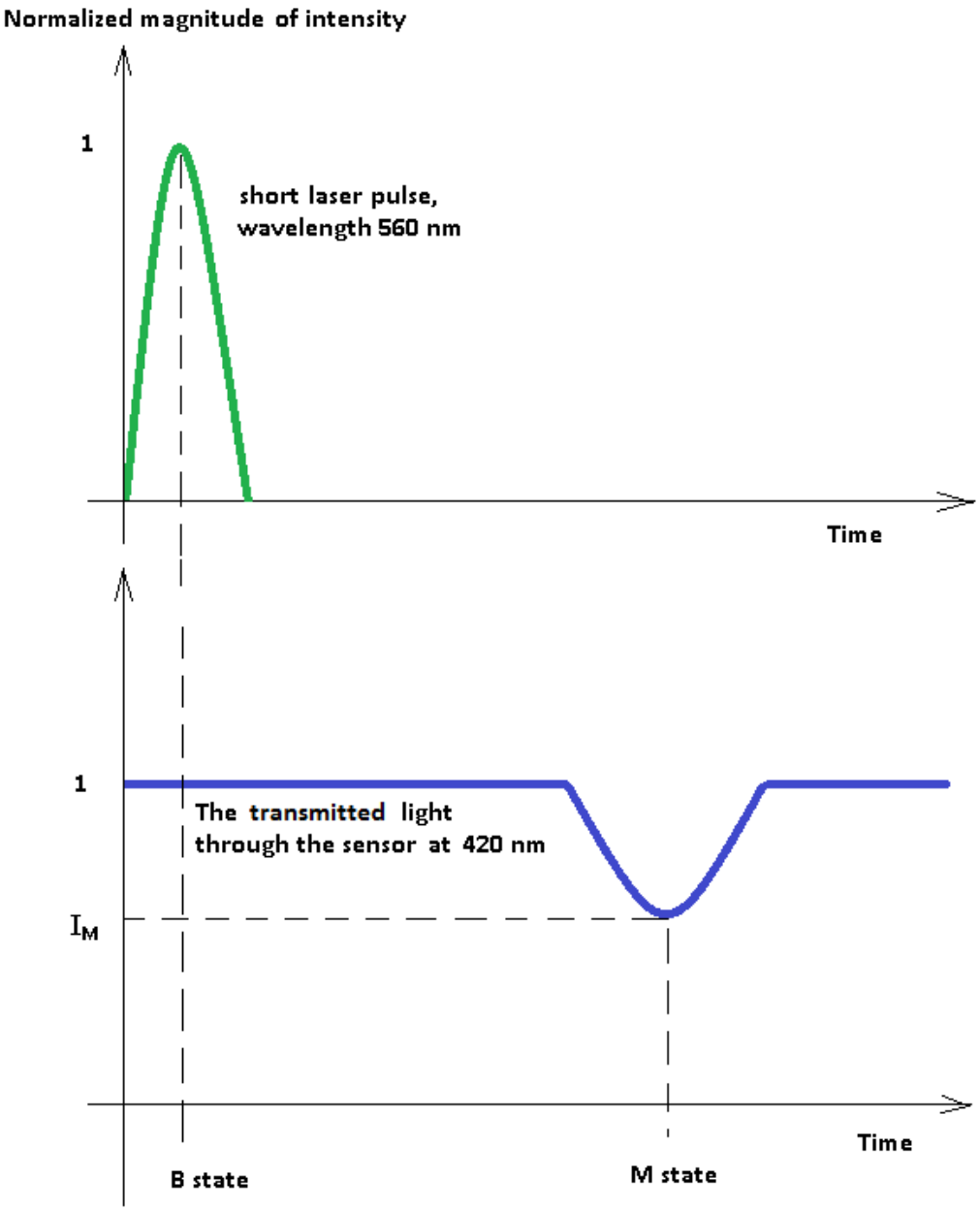


Figure 9. Schematic diagram of the transmitted light intensity. In the M state, the intensity of light is lower at 420 nm.

Using the data from these measurements, we can obtain the number of excited BR molecules which started the photocycle. The background of this estimation is the Beer–Lambert law, which relates the attenuation of light to the properties of the material through which the light is transmitted. [11].

$$T = \frac{I_t}{I_r} = 10^{-A} \qquad (1)$$

$$A = -\log_{10} T$$

where T – transmittance, is the ratio of the transmitted intensity I_t to that incident on the sample I_r [28], In our case the decrease of the intensity of the transmitted light in the M state is of interest. A is absorbance, which is the logarithm to the base 10 of the ratio of the radiant power of the incident radiation (P0) to the radiant power of the transmitted radiation (P). In solution, the absorbance is the logarithm to the base 10 of the radiant power transmitted through the reference sample to that of the light transmitted through the solution.

According to the Beer-Lambert Law, the number of molecules in the light pass have an influence on the absorption of the sample. Using the absorbance diagram (Figure 5), BR film transmission spectrum (Figure 17), and Beer-Lambert Law, it is possible to calculate the number of BR molecules. I_r is measured as the light transmitted through the conductive glass without BR film. I_t is measured as the light transmitted through the conductive glass with BR film on it. Absorbance is estimated as follows:

$$A = \varepsilon c l \qquad (2)$$

where ε is the molar attenuation coefficient. c is the molar concentration of the attenuating species in the material, and l is the path length (equal to the thickness of the BR film). To estimate the number of molecules which start the photocycle (B to J state) the molar attenuation coefficient should be ε = 63000 $N^{-1}cm^{-1}$ [12], To estimate how the transmission is changed at the M to B state transition with light at 420 nm the molar attenuation coefficient should be 63000 $N^{-1}cm^{-1}$. Matching (1) and (2) we can obtain the molar concentration value

$$c = \frac{A}{\varepsilon l} = \frac{-\log_{10} T}{\varepsilon l} \qquad (3)$$

Molar concentration is the number of molecules per volume. The number of molecules in the sample is

$$N = cVN_A. \qquad (4)$$

where N is the number of molecules in the sample, V is the volume of the sample, which can be calculated as the thickness multiplied by the square of the sample and N_A *is the* Avogadro constant.

Let as consider the case where the incident light is either absorbed or transmitted and no light is reflected. The number of excited molecules is proportional to the absorbed light. In the extreme case where the transmitted light is equal to the incident light, the absorbance is equal to zero (1). It means that no light is absorbed and none of the molecules reach the excited state. However as lower is the transmitted light, the bigger the absorbance and the bigger the number of excited molecules. The same time the number of excited molecules $N_{\text{excitedBRmolecules}}$ is proportional to the number of incident photons, and the quantum efficiency naturally affects this.

$$N_{\text{excitedBRmolecules}} \sim NA \quad (5)$$

where N is the number of molecules in the sample, and A is absorbance, which is calculated as

$$A = -log_{10}\frac{I_M}{I_{const}}$$

where I_M is the transmitted light intensity in the M state in Figure 9 and I_{const} is constant light illumination in Figure 9. In case that we cannot avoid reflections and other material absorption we can approximate the number of the absorbed photons using the laser beam data:

$$N_{\text{absorbed photons}} < N_{\text{PH}} \quad (6)$$

$$N_{\text{excitedBRmolecules}} = N_{\text{absorbed photons}}\, Q$$

where N_{PH} is the number of photons in the laser beam, and Q = 64% is the quantum efficiency related to the absorption. This is the quantum efficiency of BR molecule to reach the J intermediate state after absorbing the photon [1]. The probability of the molecule to reach any further state can be lower, because there is a chance to return to the ground state by light induced transitions [1]. If the BR molecules will be excite with a very short laser pulse and there will not be any extra light the BR molecule will reach further states and complete the photocycle. The probability of the molecule to reach the M state will be less than 64%. Assuming Equations (5) and (6) we obtain that

$$N_{\text{excitedBRmolecules}} \leq N_{\text{PH}}\, Q \quad (7)$$

An interesting question is that how strong photoresponse can be obtained from N excited BR molecules. Different equivalent circuits of BR sensors have been proposed in the literature [7, 19-22]. Common to all those models is that the photo-electromotive force is connected to the measurement instrumentation through a series capacitance [7]. If the BR sensor acts like a capacitor, BR molecules are dielectrics. So we cannot consider them like voltage sources connected in series (see Figure 10).

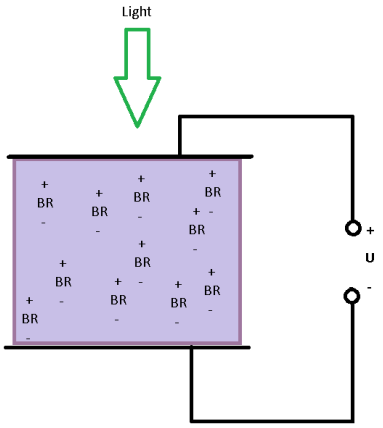


Figure 10. BR thick film schematic diagram.

There are 2 hypotheses about BR photo-voltage properties

Hypothesis #1

Unlike most of the bioelectric signals generated by ionic diffusion, the fast photosignals from dried BR film are referred to as displacement photocurrent (24). According to the Ramo–Shockley theorem [25, 26], the motion of a single charge can induce instantaneous current in a neighboring electrode. The current may be detected through the external circuit during the time interval that the charge approaches the electrode due to the instantaneous change of electrostatic flux lines. [23]

The resulting voltage stems from BR molecules which are located close to an electrode. The BR molecules which are excited far away from an electrode do not effect on total output voltage. The BR molecules do not act like serially connected current sources, even in the case of strictly oriented molecules. If the molecules are located randomly, they mutually counteract and finally decrease charges of each other.

Hypothesis #2

A BR molecule is able to pump the proton at some finite length, and its influence exponentially decreases by analogy with the Beer–Lambert law. $T = 10^{-Kl}$ where K is the coefficient, and l is the length that proton passes through, and T is the force of the proton-molecule interaction. [11]

During the further experiment, we will study the BR behavior from the standpoint of the both hypotheses. Also it will be interesting to compare the measured voltage with theoretical voltage.

Estimating the number of photons

As it was mentioned in Chapter 2.3, estimation of the number of excited BR molecules is of interest. According to (6), it depends on the number of the photons in the laser beam, which start the photocycle [9]

$$E_{1\text{pulse}} = \frac{P_{ave}}{F_{\text{rep.rate}}} \tag{7}$$

where the energy of a single photon is equal to $E = \hbar\nu = \frac{\hbar c}{\lambda}$. In our case, λ = 570 nm. The energy of a photon

$$E_{1\text{ph}} = \frac{\hbar c}{\lambda} \tag{8}$$

Using (3) and (4) we obtain (5):

$$N = \frac{E_{1\text{pulse}}}{E_{1\text{ph}}} \tag{9}$$

where N is the number of photons emitted in a pulse.

3. Experiments and results

Estimation the output power of the laser system with a pulse picker

In the experiments we used FemtoFiber pro_02243 laser. It is a visible spectrum tunable laser, so we can obtain the output wavelength which is in the maximum absorption region of the B state. ($\lambda \sim 568\, nm$ [1]). The laser was tuned at λ = 570 nm. The repetition rate of the laser is equal to $F_{\text{rep.rate}} = 80.04\, MHz$. [8]

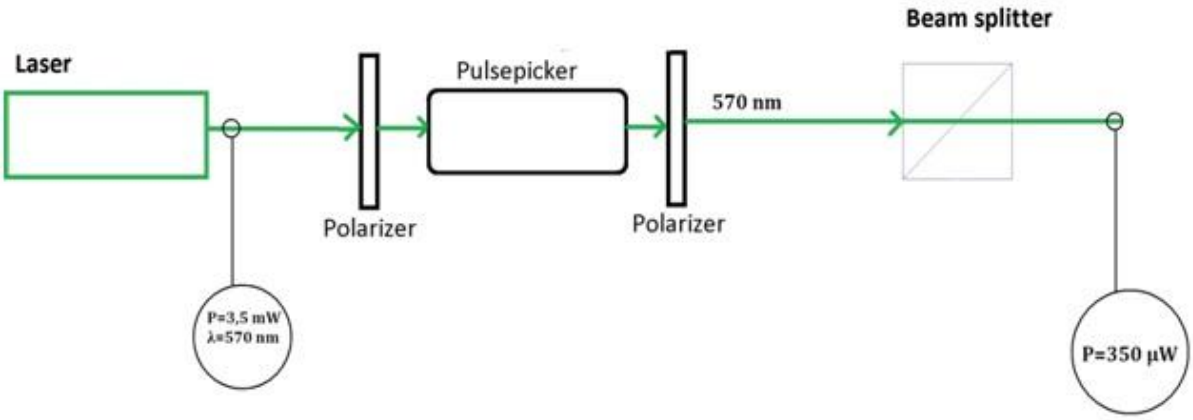


Figure 15. Estimation the average power of the laser signal setup.

The average power of the laser beam was measured with the intensity meter Coherent LaserMate-Q. The laser output intensity was 3.5 mW. The wavelength was 570 nm. The average power of the laser signal was 350 µW.

The pulse picker device was used with the laser to produce short pulses. The pulse picker worked correctly. The photomultiplier started to show the signal when we combined it with a pre-amplifier. The pulse picker works with a pair of polarizers. The system contains also a beam splitter because it is needed in further experiments. So the power of the laser signal transmitted through the pulse picker, two polarizers and beam splitter is of interest.

Table 1. The laser signal parameters

Wavelength, nm	Average power, μW	Repetition rate, MHz	Pulse width, ps
570	350	80.04	0.4

The wavelength of 570 nm was considered as the absorption maximum of BR in the ground state. The repetition rate and the pulse width were set as the maximum values for the pulse picker device (Lasermetrics, model 5046ER-VC). Shorter pulse width or repetition rate would give lower average power.

Estimating the number of photons

Using data from Table 1 and (7) we can obtain the power and the energy of one pulse.

$P_{\text{ave}} = 350$ μJ

$$E_{1\text{pulse}} = \frac{P_{\text{ave}}}{F_{\text{rep.rate}}} = \frac{350}{80.04\,MHz} = 4.37\,nJ$$

After that using (8) we can obtain the energy of one photon.

$$E_{1\text{ph}} = \frac{\hbar c}{\lambda} = \frac{1.54 \cdot 10^{-34} \cdot 3 \cdot 10^{8}}{570 \cdot 10^{-9}} = 5.54 \cdot 10^{-20} J$$

Finally we can calculate the amount of the photons in one pulse using (9).

$$N = \frac{E_{1\text{pulse}}}{E_{1\text{ph}}} = 7.8 \cdot 10^{10}$$

Transmission spectrum measurements

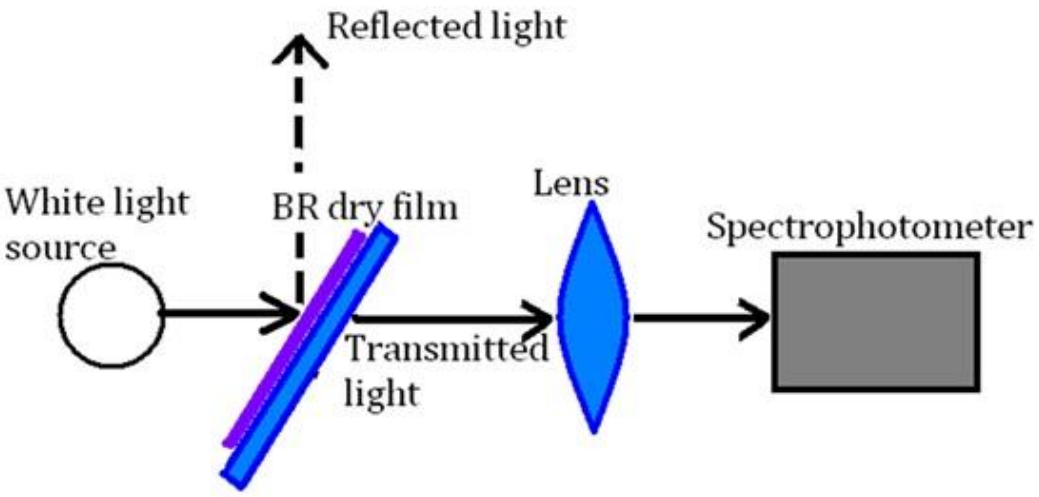


Figure 16. Transmitted spectrum measuring setup.

The setup for the transmission spectrum measurements consists of a white light source, dry BR film, lens and spectrophotometer, which measures the spectrum of light, transmitted through the BR film. The surfaces of the BR film and glass substrate generate unwanted reflections. Reflections do not fall onto spectrophotometer. To decrease the influence of reflections the BR film is rotated to an angle of 45^0. The spectrum was measured for two kinds of samples: dry films on conductive glass substrate and a liquid sample in transparent quartz bulb. For the dry films (3 samples) the measuring method contains the clear substrate measurements (10 times) and BR film (on the substrate) measurements (10 times each of 3 samples). Then the average was calculated, and the subtraction of BR film spectrum and conductive glass spectrum is presented in Figure 17 (full line). Some reflections influence the result, so the dotted line represents a polynomial approximation of the trend. Gretagmacbeth Eye One Pro spectrophotometer was used to measure the transmission spectrum.

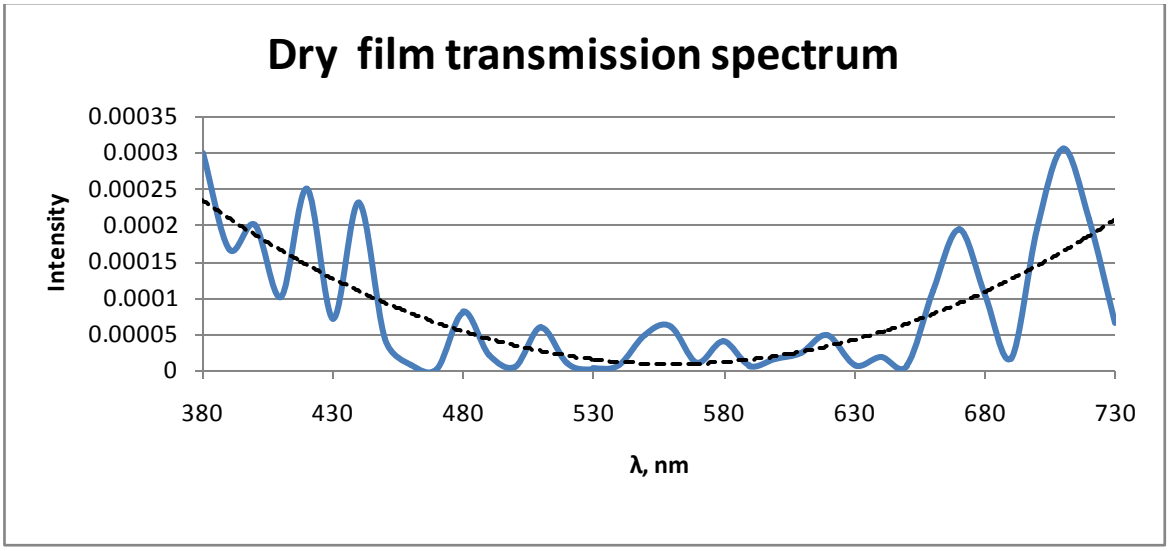


Figure 17. Dry BR film transmission spectrum.

The transmitted spectrum for liquid sample was measured in the same way. However, the liquid sample in a transparent quartz bulb does not generate reflections. So the result is closer to the expected one [1].

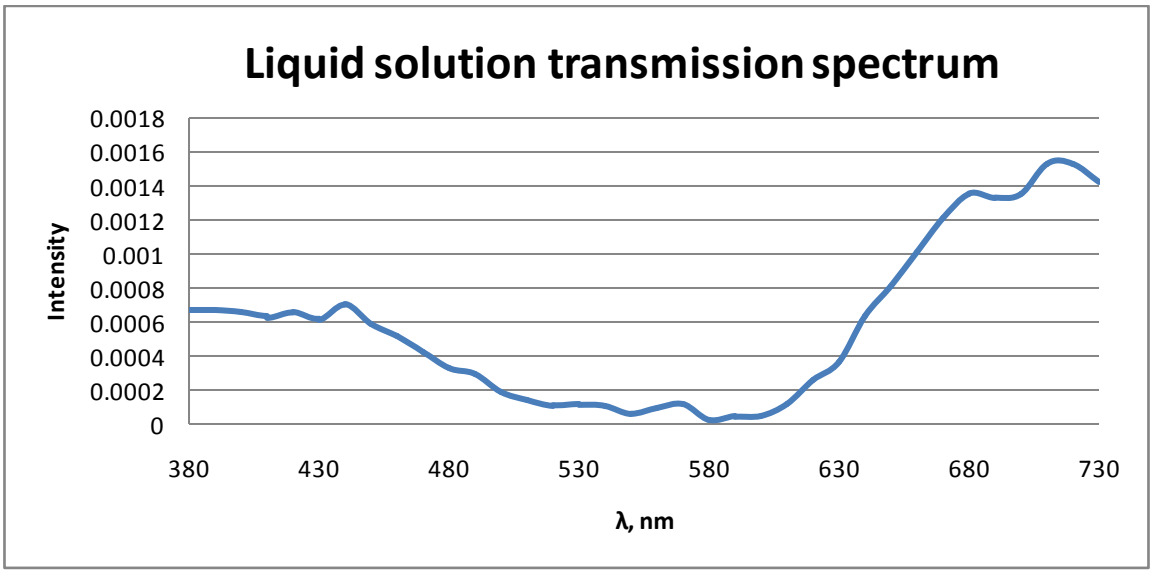


Figure 18. Liquid sample transmission spectrum.

Estimating the BR molecule concentration in the dry film

Matching Equations (8, 9) and the experimental data we can obtain the BR molecules concentration. The transmittance was measured by the spectrophotometer (see Figure 17-18). Thickness l was measured with the micrometer. The average thickness of the dry BR samples used in this thesis was 123 μm. T is taken from the experimental data at the wavelength of 570

nm, as the absorption maximum of BR. Matching Eq. (8) with the data, we obtain the value of molar concentration c

$$c = \frac{A}{\varepsilon l} = \frac{-\log_{10} T}{\varepsilon l} = \frac{1{,}48}{490 \cdot 27 \cdot 10^{-6}} = 24.81 \frac{mol}{m^{-3}}.$$

Molar concentration is the number of molecules per volume. The number of molecules in the sample is

$$N = c \cdot V \cdot N_A = 7{,}6 \cdot 10^{17} (molecules). \qquad (10)$$

Estimating the number of excited BR molecules

Using Equation (6), data from Figure 5 and the amount of the photons in a single laser pulse from Section 3.1 we obtain:

$$N_{\text{excitedBRmolecules}} = N_{\text{PH}} \cdot 64\% \cdot \frac{\varepsilon_{M_{state}}}{\varepsilon_{B_{state}}} = 4 \cdot 10^{10} (molecules) \qquad (11)$$

This number shows how many molecules start the photocycle after one laser pulse of the fiber laser operating with a pulse picker. Comparing Equations (10) and (11) we can see that the ratio of BR molecules that started the photocycle to all the molecules in the sample is equal to $\sim 5 \cdot 10^{-8}\%$. It can explain why the experiment 3.4 and 3.5 did not give any result. To find more lasers parameters and its influence on the response see the Discussion chapter.

Photoelectric response with Cavitar Laser

The aim of the first BR measurement is to check the efficiency of the new electronic device, made by Joonas Talvitie, which contains a protective screen for the BR sensor, and the amplifier for the BR signal measurements.

The measurement setup for the first BR measurement repeated the setup from [16], however, the amplifier was new. The measurement setup contains: the laser system, which effects the BR sensor. The BR photoelectric response is amplified and then registered by the oscilloscope (see Figure 11).

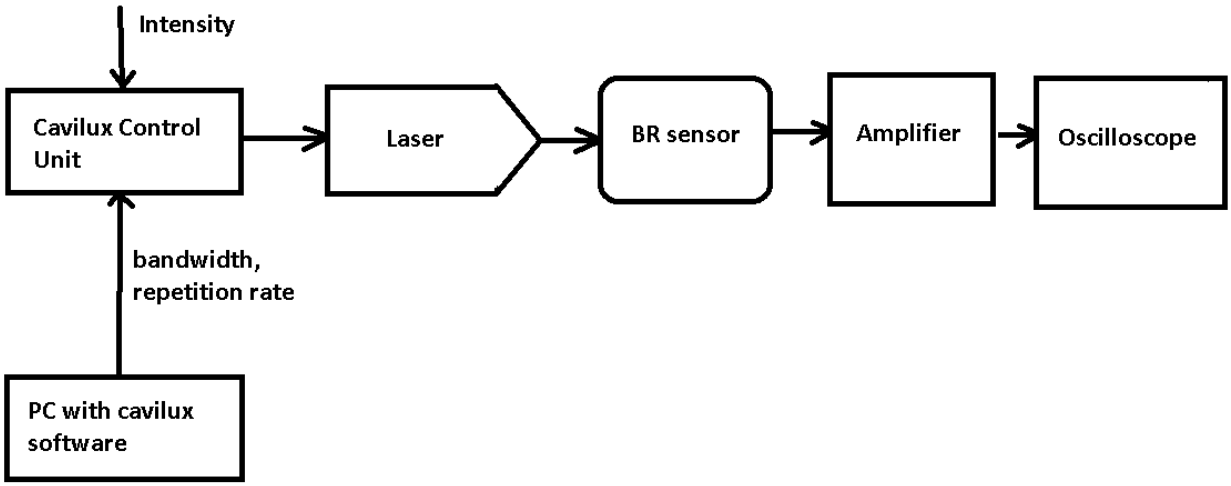


Figure 11.The BR measurement system.

The 630 nm laser radiation parameters are controlled by the Cavilux PC program. The pulse length and the repetition rate are set by this program. The intensity is controlled by the Cavilux control unit. The laser beam with specified parameters excites the BR sensor and starts the photoelectric response. The weak BR signal is amplified and represented on the oscilloscope screen.

The BR sensor is a thick film on the transparent substrate, covered with a transparent gold conductor. Both electrodes of the sensor are connected with the amplifier system. The device is constructed in a conductive box to avoid electromagnetic interference. The opening for the light excitation exists in the box's side and covered by the piece of conductive glass (see Figure 12).

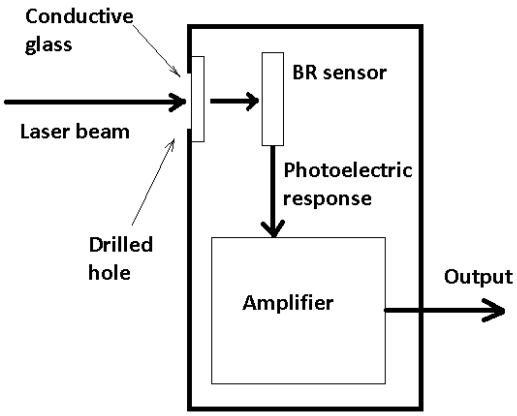


Figure12. Electronic device schematic diagram.

The obtained results: The photoelectric response signal has a form as in Figure 13. These experiment showed that the BR sensor works and produces the photoelectric response. The new amplifier works correct too, in case of strong light excitation the photoelectric response waveform is close to expected. In Figure 17 the photoelectric response and the laser signal is represented. The photocycle starts with the laser signal and then stops after 5 µs, due to the light-induced transitions.

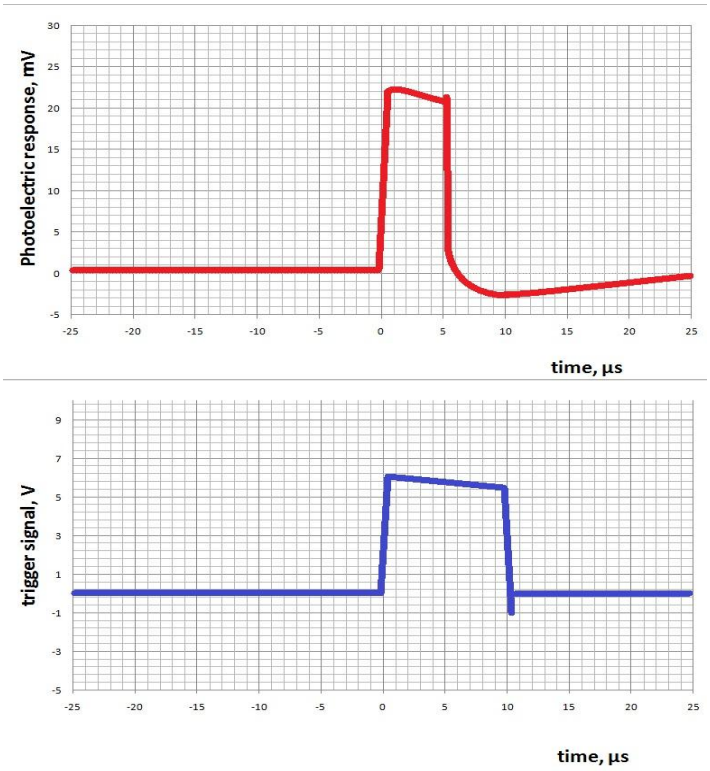


Figure13. The photoelectric response obtained from the BR sensor. The blue line represents the trigger signal (the laser signal). The length of the laser pulse is 10 µs. The shortest pulselength, which gives the signal distinguishable from noise is 50 ns. The repetition rate was 23 Hz.

The red line represents the photoelectric response. The maximum value is ~22,4 mV. The length of the response is 5 µs. It is shorter than the trigger signal because of two reasons: all the BR molecules finish the photocycle and need time to start new photocycle; when the first photons start the photocycle additional photons return the BR molecule to the ground state as light-induced transitions (see Figure 4).

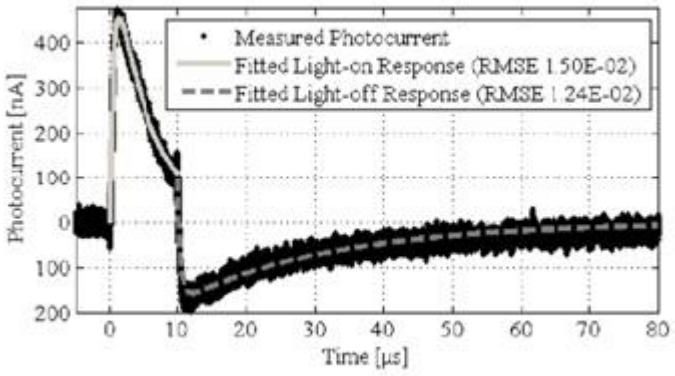


Figure 14. The photoelectric response from [16] – the same experiment with old amplifier.

Comparing the signals in Figures 13 and 14, we can see that the waveforms are alike; however, the signal is not as sharp. The waveform depends on the amplifier parameters. The new amplifier has higher gain and higher inertness. We can see that the length of the signal is not the same in both responses. The response length in the system with old amplifier is 10 µs and the length of the response in the system with the new amplifier is 5 µs. The amplitude decreases more in the system with the old amplifier from 0 to 10 µs. Then it increases sharper after 10 µs than the new amplifier response after 5 µs.

Photoelectric response of BR sensor in the setup with pulse picker and fiber laser

The experiment is very similar to the experiment which is described in 3.1, however the fiber laser system was used. FemtoFiber pro_02243 is a laser with tunable wavelength, so it was possible to adjust the wavelength to 570 nm, which is close to the maximum absorbed wavelength of the B state. In principle this wavelength can start the photocycle easily when compared to the Cavitar laser, the absorption of the B state at 630 nm is very low (see Figure 5). In addition, the laser was used with the pulse picker device (Lasermetrics, model 5046ER-VC). The system allows obtaining the 1.2 µs pulses, which is shorter than the 10 µs of the Cavitar laser with the repetition rate of 80.04 MHz (25 Hz for the Cavitar Laser). However the output power of the new laser id 3.5 mW. The setup requires reflectors and lenses so after all the losses the power of the signal, which reaching the sample is 350 µW.

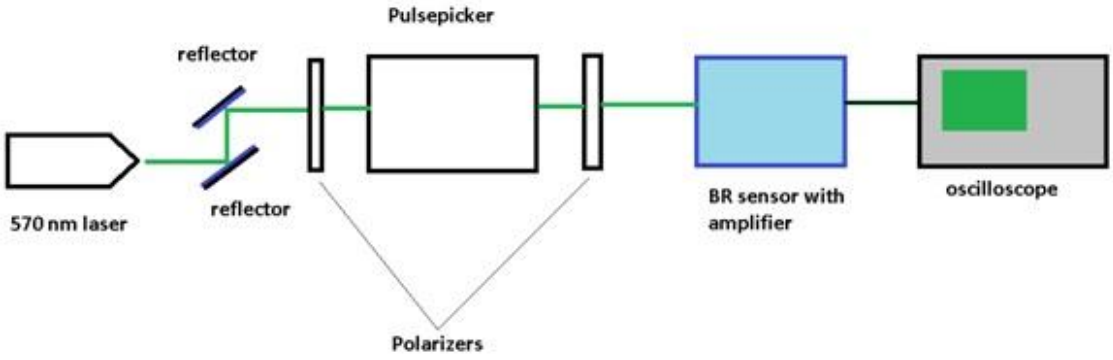


Figure 19.Photoelectric response measurement setup.

Even if the wavelength of the fiber laser is more suitable to start the photocycle, the output power is too low. The measured result is shown in Figure 20. No observable response could be seen on the oscilloscope screen.

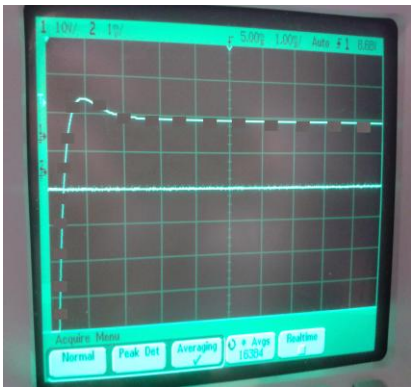


Figure 20.Photoelectric response results.

Optical response measuring transmission

The experiment is based on Equation (5) and the theoretical background described in section 2.4. The number of excited BR molecules is calculated using the transmission intensity data. The decrease in the transmitted light intensity is proportional to the number of excited molecules in the film.

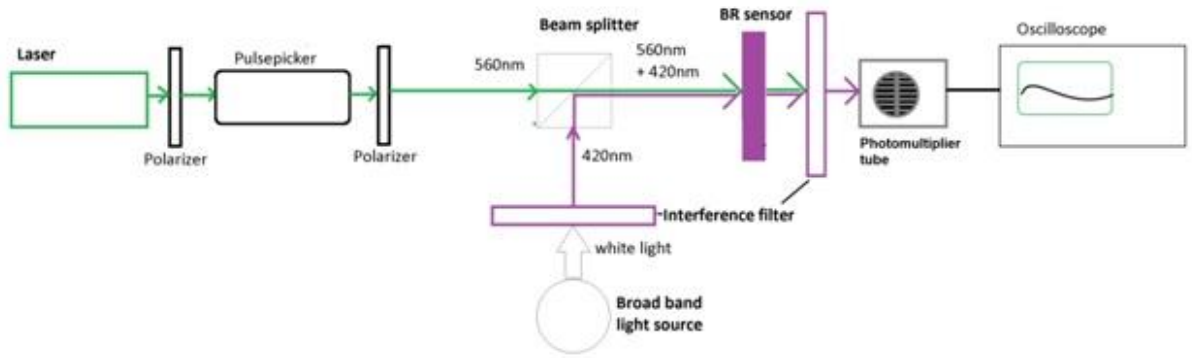


Figure 21.The measuring setup for optical response measurements.

The measurement setup includes a tunable laser, for which the output wavelength was 560 nm. The pulse width was 0.4 ps and repetition rate was 25 Hz. The laser signal form obtained with the pulse picker device is presented in Figure 22.

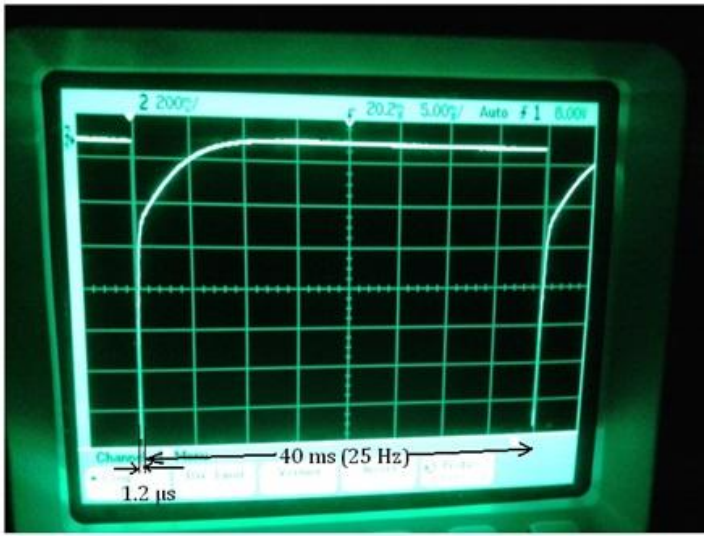


Figure 22. The laser beam signal obtained with the pulse picker device. Repetition rate frequency is 80.04MHz.Pulse width was 0.4 ps on the pulse picker device. The signal was measured by using a photomultiplier tube.

Another light beam with at the wavelength of 470 nm was obtained by using a broad band light source with an interference filter. These two beams were combined with the beam splitter and illuminate BR sensor. The laser pulse starts the photocycle in the BR sensor. There will be drop in transmitted intensity at 470 nm when the BR molecules will go to the M state. This intensity was measured by the oscilloscope.

Liquid sample

At first the transmitted light was measured through the BR liquid sample in a transparent quartz bulb. The expected time between starting the photocycle and the M state is 82.0035µs [1]. However, two drops in the transmitted intensity were observed, one after ~1 ms and another after ~15 ms. The second reduction is stronger as can be seen in Figure 23.

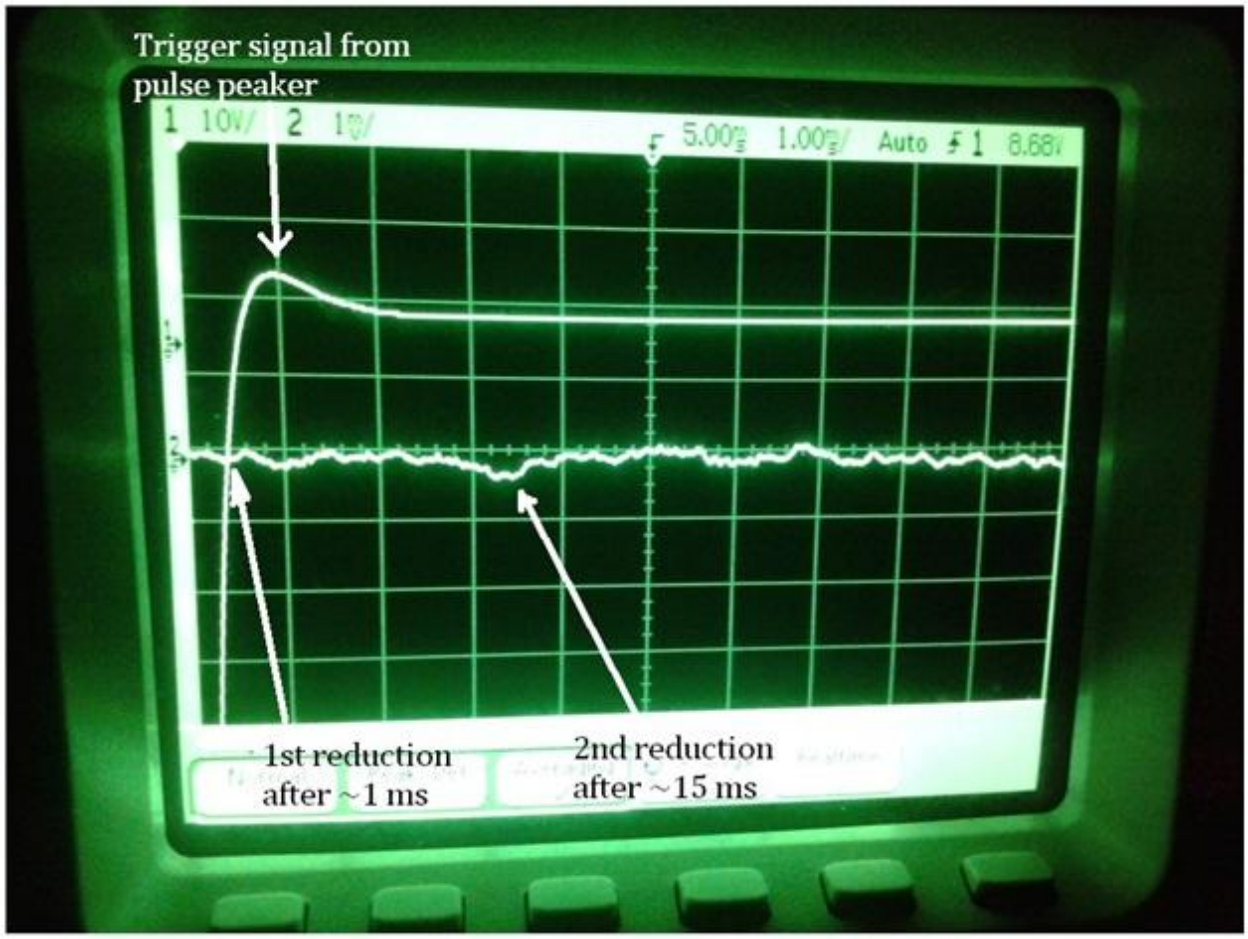


Figure 23. The liquid sample transsion at 420 nm measured with a photomultiplier tube in according with the scheme presented in Figure 21. The signal is obtained by averaging 16000 samples to reduce the noise.

Dry film

Then the dry film light transmission was measured. To increase the light influence three samples were stacked. However the signal was weaker in comparison with the liquid sample.

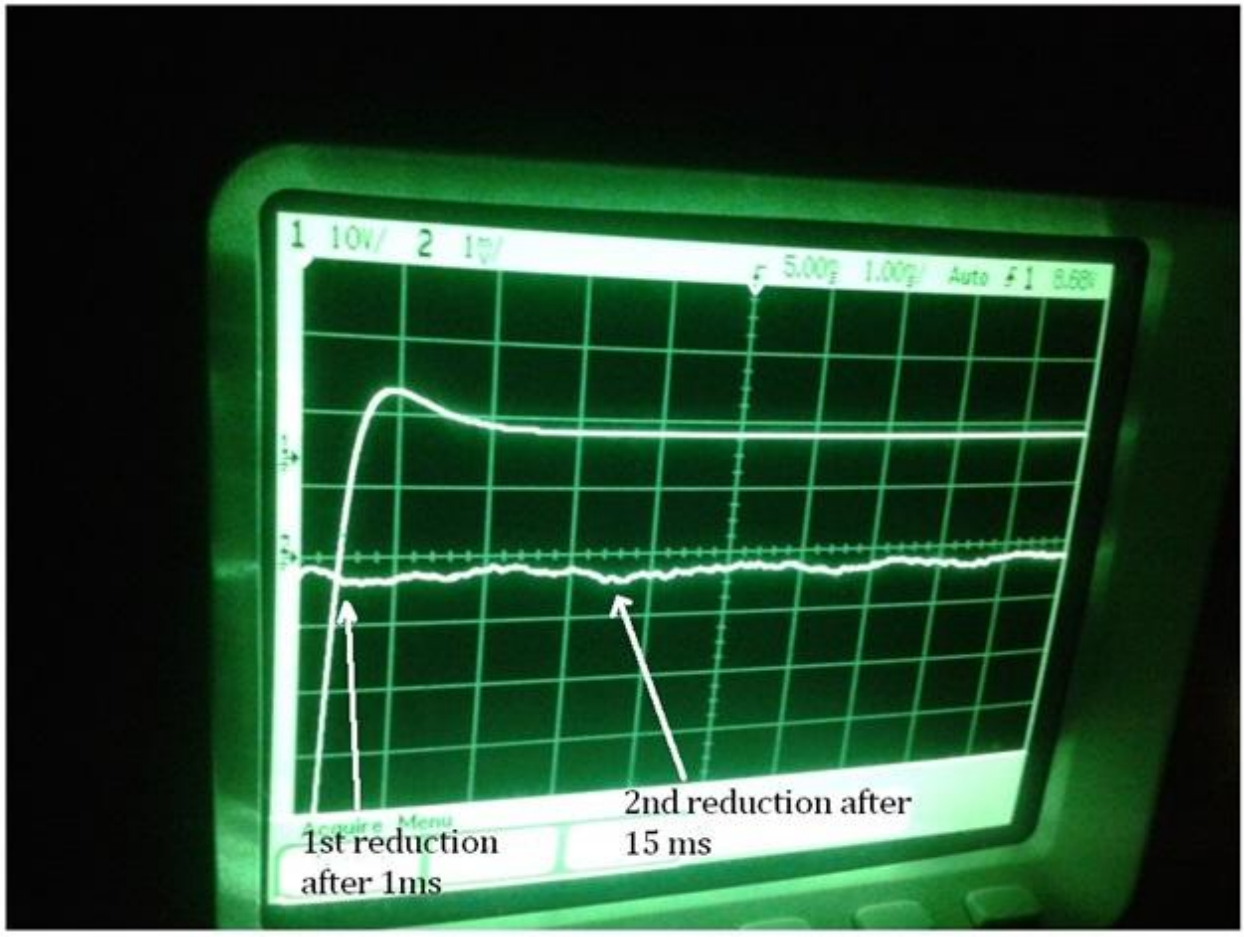


Figure 24. The dry sample transmission at 420 nm measured with a photomultiplier tube with the setup presented in Figure21. The signal is obtained by averaging 16000 samples to reduce the noise.

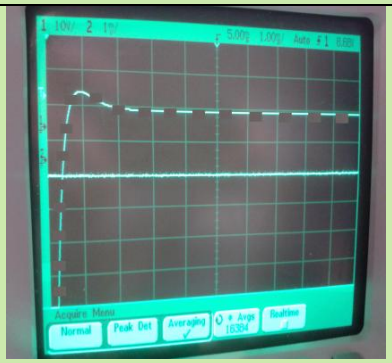
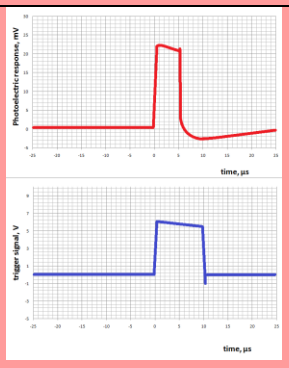
4. Discussion and future work

Experiments 3.4 and 3.5 should provide us information about photoelectric properties of the BR sensors. However, experiments with tunable fiber laser showed no measurable results. Possible reasons for this are analyzed below by using the data from Experiments 3.1, 3.2 and 3.3.

Comparison of the old and new laser parameters

Let us compare the characteristics of the two lasers using experimental data from 3.1.

Table 2.Old and new laser comparison.

	New laser	Old laser
Name	FemtoFiber pro_02243	Cavitar CAVILUX Smart laser
Color	Tunable	red
Wavelength	480-640 nm	690 nm
Average power	350 µW (after pulse picker)	400 W
Power of 1 pulse	4.37 nJ	16 J
Number of photons in a pulse	7.8*10^10	3.5*[10]^20
Photoelectric response _ _ _ _ Trigger signal _____ Response		
Maximum value of photoelectric response	0 V	25 mV
Expected response	25 nV	25mV-

Possible reasons of negative results of the experiments

According to Equations (10) and (11), only $\sim 5 \cdot 10^{-8}\%$ of the BR molecules start the photocycle under the influence of the fiber laser. It is too small proportion of the molecules to measure any response, electrical or optical with our measurement setup. At the same time Experiment 3.3 showes that BR sensors work correctly with the Cavitar laser and it means that the expected processes should happen with the other laser, too. In Figure 20, the photoelectric response is denoted with a full line. No observable signal was obtained in this experiment.

Future work

Make a new amplifier with higher gain. The photoelectric response is approximately 10^{12} times weaker with the tunable fiber laser than with the Cavitar laser. So the estimated amplitude of the photoelectric response is 25 nV.

Decrease light losses in the system, place the components on a single optical axis to move out reflectors or using optical fiber. As it was shown in Experiment 3.1, the losses in the optical system were 90% of the initial laser energy. Most of the energy is lost because of the pulse picker device. However, two reflectors and one lens cause energy loss, too.

Use a more sensitive oscilloscope. Some processes should happen even with the small energy of laser beam, so there must be a way to measure weak signals.

The photocycle time period obtained with this experiment (see Figure 23-24) was far from the expected values (10 ms instead of 80 µs [1]). The experimental data is 15 ms. It is probably the interference of the pulse picker high voltage signal of 1000 V. Possible solution is to protect the high voltage cables of the pulse picker with aluminum foil and protect the cables which connect the BR sensor with oscilloscope with the foil screen.

5. Conclusion

Optical and electrical properties of dryBR films were experimentally studied with Cavitar Laser, FemtoFiber Pro laser and Eye One Pro spectrophotometer. The new amplifier was tested to show that the BR sensor works and produces the response. The BR sensor photoelectric response in the experiment with the Cavitar laser was obtained. However no observable photoelectric response was obtained in the experiment with FemtoFiber Pro laser and pulse picker device. At least one of the reasons was the low power of the laser. The explanation was obtained by analyzing the output power of the laser. The number of photons was not enough to obtain the response. The number of molecules in the sensor was estimated. Also the number of excited molecules was estimated. The measurement setup for the optical response was built and described. However, no measurable response was obtained due to the low power of the laser. The BR sensor properties could not be characterized better with ultrafast pulse devices.

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