Measuring C-Reactive Protein Using Microring Resonators

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***Abstract*—A rapidly developing application of microring resonators is highly specific biosensing. By covalently binding antibodies to a microring chip, interaction with its corresponding protein will effect a change in mass concentration, which can be read out as an optical wavelength shift. Using low-cost setups for both microfluidic and optical input/output components, we have successfully performed concentration measurements of C-Reactive Protein in order to establish the output sensitivity in the linear regime. We show that the measurement response is highly specific for C-Reactive Protein. Through the use of these commercially available components, we underline the effectiveness and accessibility that microring resonators offer as biosensors.**

***Keywords*: Microring Resonator, Point of Care, Diagnostics, C-Reactive Protein, Biosensor**

I. Introduction

Microring resonators (MRRs) are optical sensors that are able to measure changes in the refractive index of fluid samples accurately. This is done by coupling laser light into a waveguide, from where its evanescent field reaches an adjacent microring [1] [2]. Due to the evanescent field of the microring itself, the total optical path length of the ring also depends on the fluid that runs on top of the ring. By sweeping the wavelength of the laser light, the relative effective refractive index n_{eff} of a ring of length L, can be determined from resonant wavelength(s) λ_m

$$\lambda_m = \frac{L \cdot n_{eff}}{m}, \tag{1}$$

corresponding to resonance mode m = 1,2,3.., . Covalently binding antibodies to the microring allows this process to be used for biosensing, allowing only a specific protein to bind and enact such a change in relative refractive index [3]. By performing these measurements at different protein concentrations, the sensitivity *s* of the sensor can be determined, which is defined as the change in relative refractive index per unit of protein concentration. The setup required to perform protein measurements with MRRs is dependent on two additional systems: an optical setup to couple laser light into a sensor chip and read out the resonant wavelengths, and a microfluidic setup to flow the protein samples over the sensor chip [4]. This paper discusses the MRR setup using low-cost, readily available components, and presents representative measurements on C-reactive protein [5]. The measurement setup shows a high specificity for C-reactive protein and an improved speed of operation when compared to literature [6]. In Section 2, we give an overview of the developed measurement setup. In Section 3, the concentration measurements of C-reactive protein are discussed, starting with an explanation of the used measurement protocol, followed by the gathered results. Section 4 offers the conclusion that follows from these results and a discussion thereof.

II. Low-Cost Setup

In this section, we discuss the different setup components that have been developed, namely the optical and microfluidic subsystems.

A. Optical Input and Readout

For the optical system, an Agilent 8164A sweeping laser was connected as the input and a PicoScope 2206B computer oscilloscope as the readout device. To control the laser light in the microring resonator, an Agilent 8164A Lightwave Measurement System was used with an Agilent 81640A Tunable Laser as the active module. This system can perform a laser sweep in a wavelength range of 1400-1670 nm, allowing the setup to continuously search for resonant wavelengths in a set range. The different Agilent instruments are synchronized via TTL triggers. The chip holder stage allows for translation and rotation in the horizontal plane, and is used to get a consistent alignment of the sensor chip and makes it possible to couple light into the chip at different points. Note that this subsystem functions as a proof of concept; the concentration measurements in Section 3 are performed using the Delta Diagnostics MRR.

B. Microfluidic Components

For the microfluidic system, a Harvard Apparatus 33 DDS syringe pump and AMF RVMLP rotary valve have been integrated. All of these instruments are controlled through a single Graphical User Interface (GUI) that has been developed in Python, allowing for platform-independent usage. The Harvard Apparatus 33 DDS syringe pump produces a vacuum to move the liquids through the setup by having a stepper motor move the plunger of an inserted syringe. Rather than connecting

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various syringes with different samples in sequence that are pushed through the setup, the choice is made to have the syringe pump create a vacuum and use the syringe as a waste container that the samples are drawn into. This does necessitate the use of a rotary valve in order to selectively draw from the samples. Fluids that are to be used during an experiment are first stored in Eppendorf tubes, which are connected to an AMF RVMLP rotary valve. After passing the rotary valve, a fluid is drawn over the MRR-chip that is sealed of with a flow cell. Before ending up in the syringe, the flow speed of the liquid is measured in an Elveflow MFS4 flow sensor, the results of which can be read out via the accompanying software. The tubing between the Eppendorf tubes and the rotary valve has an inner diameter of 0.2 mm, in order to minimize the amount of reagents that is wasted while purging. The rest of the tubing has an inner diameter of 0.8 mm. A schematic overview of the complete microfluidic setup is shown in Figure 1.

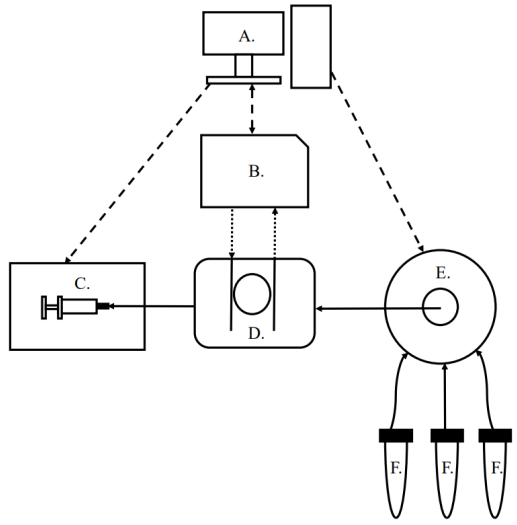

Fig. 1. *Schematic overview of the microfluidic setup, consisting of the following components: A. Measurement PC; B. Delta Diagnostics MRR; C. Harvard 33 DDS syringe pump; D. MRR chip; E. AMF RVMLP rotary valve; F. Eppendorf tubes containing reagents.*

III. C-Reactive Protein

This section shows the application of the developed setup in performing concentration measurements of C-reactive protein.

A. Concentration Measurement Method

The sensor chip that is used contains six microrings, and has been coated with a hydrogel by XanTec. Of these six rings, ring 4 is covered with a silicon-oxide layer, which shields the microring from the reagents and allows it to function as a reference regarding pressure and temperature influences. The hydrogel serves as the base layer upon which the antibodies are placed. The chip is functionalized with mouse-derived CRP-antibodies, acquired from ThermoFisher. Before the actual start of a measurement, the tubing is purged to prevent contamination during the experiment. The CRP has been dissolved in Phosphate-Buffered Saline solution with Tween (PBST). This buffer solution does not interact with the protein, and allows for a stable reference measurement if no CRP is dissolved in it. A concentration measurement starts by regenerating the sensor chip, or removing contaminants, by flowing the buffer solution, followed by a 50 mM glycine solution, and another round of the buffer solution. This is done for 1 minute per reagent, with a flow speed of 100 µl/min. Once the regeneration process is completed, the buffer solution is flowed over the sensor chip with a flow speed of 20 µl/min for 5 minutes to create a stable readout baseline. Then, the CRP sample is introduced, likewise by flowing at 20 µl/min for 5 minutes, followed by the buffer solution. It is during this final step that the response is determined, at a set time after switching to the buffer solution, to ensure that the measured response is a result of protein-antibody interaction rather than electrostatic attraction of the protein. Afterwards, the regeneration process is repeated in order to quickly remove all of the bound protein from the chip.

B. Dose-Response Results

Measurements have been performed with doses of 0.3, 1.0, 3.0 and 10 µg/ml CRP. The measurement of 10 µg/ml is shown in Figure 2 as an example. It can be seen how rings 1, 5, and 6 show a response when the CRP sample is introduced, which slowly moves toward an equilibrium state where the rate of association and dissociation between the CRP and the antibodies are equal. The difference in response between these rings can be explained by the fact that they are placed in a straight line on the sensor chip: the CRP first reaches ring 1, thereby lowering the amount of available molecules in the sample that reaches the later rings. It is unclear why rings 2 and 3 show a lower response.

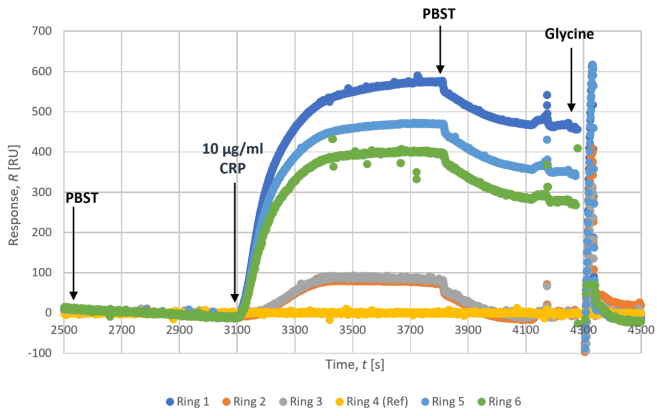

Fig. 2. *Results of concentration measurements of samples with a CRP concentration of 10 µg/ml. Only ring 1, 5 and 6 are functionalized with mouse-derived anti-CRP.*

By repeating this measurement at different concentrations, a dose-response curve can be created, as is shown in Figures 3-5 for the individual rings. The corresponding fit values are included in Table I. The measurement results of ring 1, 5 and

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6 are presented in Figures 3-5. The response of the measured complex (CRP binding with anti-CRP) is plotted against the dose, in which the x-axis is plotted logarithmically. The fit for the dose response curve is obtained by using non-linear regression in Python with the curve fit function from Scipy with the Levenberg-Marquardt algorithm. The resulting fit parameters for ring 1, 5 and 6 are listed in Table I. From these fits, the sensitivity s has been determined, resulting into s_1 = (80±12) RU(µg/ml)$^{-1}$, s_5 = (76±5) RU(µg/ml)$^{-1}$ and s_6= (77±10) RU(µg/ml)$^{-1}$ for rings 1, 5 and 6 respectively.

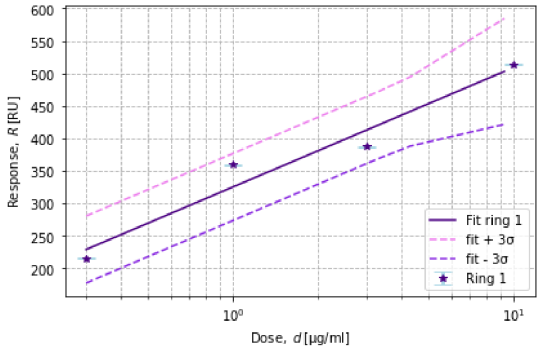

Fig. 3. Dose-response curves for ring 1 of the formed CRP complexes with mouse-derived anti-CRP.

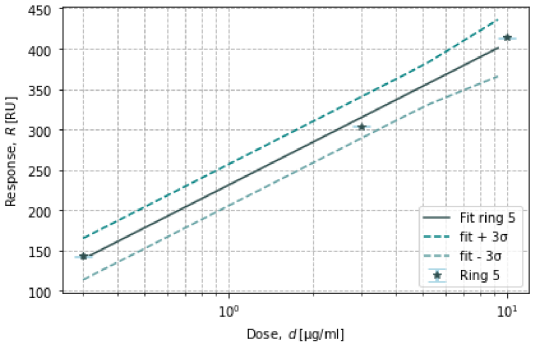

Fig. 4. Dose-response curves for ring 5 of the formed CRP complexes with mouse-derived anti-CRP.

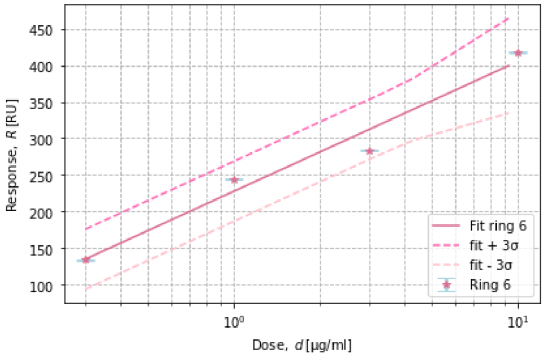

Fig. 5. Dose-response curves for ring 6 of the formed CRP complexes with anti-CRP produced in a mouse from the company ThermoFisher.

TABLE I
MEASUREMENT RESULTS

Ring	Fit Values
1	$R(d) = (80 \pm 12) \cdot \log(d) + (324 \pm 17)$
5	$R(d) = (76 \pm 5) \cdot \log(d) + (231 \pm 9)$
6	$R(d) = (77 \pm 10) \cdot \log(d) + (228 \pm 14)$

IV. CONCLUSION AND DISCUSSION

In this paper, we have outlined a method to create a low-cost optical and microfluidic setup that can be used to perform biosensing experiments with microring resonators. To prove this concept and display the accessibility of this technique, we have successfully performed concentration measurements of C-Reactive Protein. The determined sensitivity s of the setup is (80±12) RU(µg/ml)$^{-1}$, (76±5) RU(µg/ml)$^{-1}$ and (77±10) RU(µg/ml)$^{-1}$ for the three different microrings on the sensor chip. The relatively large uncertainty is caused by the limited amount of different concentrations that have been measured due to time constraints. This implies that the uncertainty can be reduced without further investments. Furthermore, these measurements have only been performed in the linear regime of the dose-response curve. It is not yet clear what the lower limit of detection of the setup is and, while unlikely, whether the setup will influence the sensitivity near the upper limit of its dynamic range.

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