

Development of Plasmonic Biosensor Platform for Multiplex Sensing of miR-21

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Summary:

The long-term aim of this research is to create a highly sensitive, localized surface plasmon resonance-based biosensor enabling the multiplex detection of microRNA-21. This molecule is an important cancer biomarker, and as miRNAs in general, has a low abundance in biological samples, therefore the need for low limit of detection (LOD). In this paper, we present the current state of this research, including the device used for multiplex sensing, experiments regarding the detection of the target molecule and possible ways to improve the LOD.

Keywords: localized surface plasmon resonance (LSPR), microRNA, limit of detection (LOD), microfluidics, hybridization chain reaction.

Background, Motivation and Objective

There is an increasing research and interest in miRNAs, as these small, non-coding RNAs can serve as biomarkers for a wide range of diseases, including various types of cancers, cardiovascular and neurodegenerative diseases [1]. These molecules have the potential to provide a faster diagnosis, and thus treatment, which can decrease mortality. The main obstacles to use them in clinics include low concentration in bodily fluids and the need to measure several different ones for a complete and accurate diagnosis [2].

The goal of this study is to provide a biosensor capable of meeting these requirements. The sensor element consists of gold nanoparticles which exhibit a phenomenon known as localized surface plasmon resonance: when interacting with light at the resonance wavelength, the electrons of the nanoparticles start to oscillate, leading to an absorption peak in the spectrum [3]. The resonance wavelength is affected by the refractive index of the surrounding medium, thus the presence of the analyte (which increases the refractive index) can be measured.

Description of the New Method or System

Usually, plasmonic sensors rely on optical spectroscopes for determining the wavelength shift. The drawback is that measurement can only be conducted in a single spot. Our custom-made device uses LEDs and a camera to enable

multiplex detection, thus several measurements can be carried out parallelly, and in the future, several miRNAs can be detected simultaneously. Fig. 1. an image of the experimental setup.



Fig. 1. LSPR imaging measurement setup.

Glass slides serve as substrates for the nanoparticles and are integrated in a microfluidic system with polydimethyl siloxane cells. Thiolated oligonucleotides are used as receptors.

Results

First, experiments were carried out to immobilize the probe (receptor) molecules on the surface of

the gold nanoparticles, then oligonucleotides modelling miR-21 (same nucleotide sequence) were introduced.

Fig. 2. shows the results of one of these measurements during evaluation. The different colors represent the different areas on the sensor element. The position of the LSPR peak (in nanometers) is displayed in the function of time (in seconds).

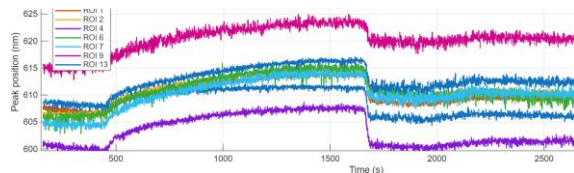


Fig. 2. Evaluation of wavelength peak shift during probe immobilization and target binding.

The first segment, until around 450 seconds, shows the flow of the buffer, then the probe oligonucleotide is introduced, the immobilization leads to a 5-8 nm shift in the spectrum. This is followed by a change of buffers, which causes the drop, then at 2000 seconds, a solution containing the target molecule enters the microfluidic cell. An increase of the peak's wavelength of 1-3 nm is observable.

In order to improve the LOD, several steps can be taken. The signal to noise ratio can be increased during evaluation by using appropriate filters, the buffers and other technical parameters (time, pH, concentration etc.) can be optimized, contributing to a better LOD. An additional approach is to use some kind of signal amplification technique, such as the hybridization chain reaction. Briefly, two hairpin oligonucleotides are added, binding to the target then each

other alternately, increasing the LSPR peak shift [4].

All of the above-mentioned methods are currently being investigated, and the final paper will report the results.

References

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