A New Application of Metasurface Biosensors for Non-Invasive Specimens

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Abstract:

Recently, Dr. Iwanaga's research has focused on exploring the use of all-dielectric metasurface fluorescence (FL) biosensors, as highly effective antibody detectors, at very small concentrations. The goal of this project is to be able to detect small amounts of Immunoglobulin A (IgA) protein in a raw saliva sample, by immobilizing the protein onto a metasurface FL biosensor using the sandwich method and FL sensing. Dr. Iwanaga's lab has designed reusable metasurface FL biosensors, consisting of an all-dielectric metasurface substrate, and a microfluidic chip made of transparent polydimethylsiloxane (PDMS), which I have continued to use this summer. This project will include using a microfluidic protocol to check the sensitivity of these biosensors in salivary IgA detection and using an ELISA kit to collect data to compare the results to.

Step	Reagent	Volume	Flow Rates	Flow Duration
1	PBS Pre-Flow	400 µl	600 pps	4 minutes
2	**Cys-Streptavidin**	100 µl	200 pps	5 minutes
3	PBS Rinse	300 µl	200 pps	2 minutes
			120 pps	10 minutes
			200 pps	5 minutes
4	Background Measurement			
5	**Biotin IgA Antibody**	100 µl	200 pps	5 minutes
6	PBS Rinse	300 µl	200 pps	2 minutes
			120 pps	10 minutes
			200 pps	5 minutes
7	**IgA Protein**	100 µl	200 pps	5 minutes
8	PBS Rinse	300 µl	200 pps	2 minutes
			120 pps	10 minutes
			200 pps	5 minutes
9	**HL-555 IgA Antibody**	100 µl	200 pps	5 minutes
10	PBS-T Rinse	300 µl	200 pps	2 minutes
			120 pps	10 minutes
			200 pps	5 minutes
11	Yellow Fluorescent Measurement			

Figure 1: Protein times to adhere to the surface or to the protein below it.

Summary of Research:

The technique that we used to immobilize IgA on the metasurface long enough to image it is the Sandwich Method. The idea is to sandwich the target protein with a capture antibody, and a detector antibody that is labeled by a fluorophore. The capture antibody is a biotin-tagged IgA antibody, so that we can use Cys-Streptavidin for immobilization onto the surface. And the detector antibody is tagged with HiLyte 555 fluorescent molecules, which emit light after being excited by green incident light, signaling that IgA protein is present.

We developed a microfluidic protocol to help immobilize IgA onto the metasurface, so that we can validate that we can detect IgA using FL sensing. To control and optimize this microfluidic protocol, we used a microfluidic set-up connected to a small motor that we can control flow rates of the liquids with. The flow rates are determined based on the volume of the inlet tubes which is 150 microliters. The protocol follows the pattern of flowing the protein required and then pushing phosphate-buffered saline (PBS), giving the protein time to adhere to the surface or to the protein below it (Figure 1).

Sample	FL Intensity
250 ng/ml	1204.228
50 ng/ml	1129.112
10 ng/ml	1065.444
2 ng/ml	1013.250
0.4 ng/ml	843.978
0 ng/ml	551.116

Figure 2

To quantify and analyze fluorescent images, yellow, fluorescent images of the metasurface substrates were taken using green excitation light at intensity level 3, using a 3 second camera exposure and an electric-signal gain of 10. The FL intensities were quantified around the center of the LED illumination. We set a circular region using the image-analysis software, ImageJ, and output histograms of the FL intensities.

We took the data that we collected from the microfluidic experiment targeting a range of concentrations of IgA protein (Figure 2) and constructed a linear and semi-log graph. The linear graph had an exponential curve, and the semi-log had a linear curve, which is typical for this kind of experiment, so this experiment successfully validated that we could detect IgA at small concentrations using metasurface FL biosensors. The next step was to replace IgA with saliva in the microfluidic protocol to explore if we can target and quantify the concentration of IgA in the sample, and to perform an ELISA kit on IgA Protein to find the actual concentration of IgA in our saliva samples.

An ELISA kit is a highly accurate protein-specific quantification kit, which uses sandwich assay.

Sandwich assay is an enzyme-linked immunosorbent assay that detects and quantifies a target antigen that has been bound between two layers of antibodies, i.e., capture and detection antibodies. The technique is very similar to the Sandwich Method described earlier; the main difference is the detector antibody is linked to a color changing enzyme called HRP instead of a fluorophore. The ELISA kit helps us find the concentration of unknown samples by testing standard solutions of known concentrations of IgA so that we can create a standard curve. We tested IgA solutions of concentrations ranging from 0.78 ng/ml - 50 ng/ml and were able to form a linear curve. By using this linear curve as a guide, we can use a measured optical density and we can determine a correlated concentration. Next, we can multiply that value by our dilution factor and find the concentration of IgA in our original saliva sample.

During the next phase of experiments, we proceeded with the microfluidic protocol, and replaced IgA Protein with raw saliva, to test if we could detect IgA protein in saliva using FL sensing. For the analysis of these results, we used the exponential graph from the previous experiment to form a standard curve using a linear curve fit function in the Igor Pro Software and created an extrapolated graph relating the concentration of IgA with the measured average FL intensity. Similarly to the ELISA kit, we can use this graph as a guide to find the concentrations of IgA in the saliva samples we tested, which you can see visually by the lines drawn on the graph (Figure 3). Mathematically we can use the linear equation of the graph to get the exact concentration measured on the metasurface substrate, and then multiply it by our dilution factor to find the concentration in the original saliva sample.

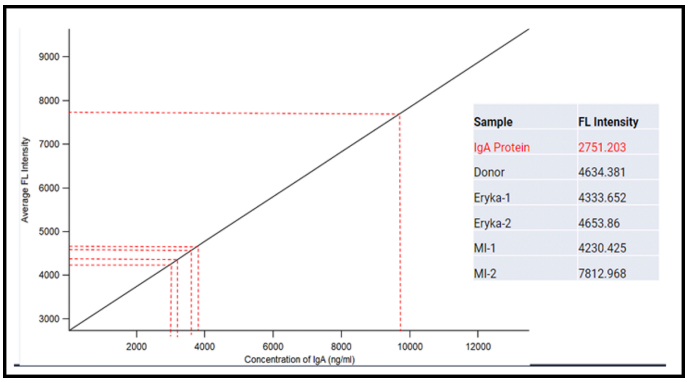

Figure 3

Conclusions and Future Steps:

The final analysis step of this project is to compare the results from the microfluidic protocol targeting IgA protein in raw saliva, and the results from the ELISA protein quantification kit targeting IgA protein in raw saliva (Figure 4). The concentrations of IgA determined using the metasurface FL biosensor, was more than about 10 times higher than the concentrations determined by the ELISA kit. This inconsistency is, inferred to be due to the treatment of the saliva samples; however, this should be determined in a standard manner. Unfortunately, we are not yet sure what the issue is and moving forward we would collect more data from these experiments to draw any conclusions, and to solve the issue.

Acknowledgements:

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References:

[1] Iwanaga M. All-Dielectric Metasurface Fluorescence Biosensors for High-Sensitivity Antibody/Antigen Detection. ACS Nano. 2020 Dec 22;14(12):17458-17467. doi: 10.1021/acsnano.0c07722. Epub 2020 Nov 24. PMID: 33231442.

Sample	Conc. of IgA Measured in ELISA kit (μg/ml)	Conc. of IgA Measured on Metasurface (μg/ml)
Dr. Iwanaga	21.713	293.415
		991.930
Eryka	11.072	313.542
		375.975
Donor	24.178	372.177

Figure 4