Ultrasensitive Exosome Detection on Metasurface Biosensors for Next-Generation Diagnostics

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Program: Cornell/NNCI IREU Program

Funding: NSF OISE-2246252 via International Research Experience for Students Program

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Abstract:

Exosomes are nanoscale, lipid bound extracellular vesicles around 50-250nm in diameter. Secreted by nearly every cell in the human body, exosomes are promising biomarkers for disease diagnostics as their molecular composition reflects the state of the parent cells from which they originated. In this project, we developed a silicon metasurface-based fluorescence biosensor for ultrasensitive exosome detection. Through antibody targeting methodologies, this platform achieved detection of approximately 10^3 particles/mL. This is one of the highest exosome sensitivities reported to date, indicating the potential for next-generation diagnostic applications with these strategies.

Summary of Research:

To detect exosomes sensitively, we used all-dielectric metasurface biosensors made from silicon (Si) nanostructures. With a periodic length of 330nm, and a Si height of 200nm, the nanostructures were optically separated by a SiO_2 layer on the bottom. These metasurfaces were found to have an enhancement factor of up to 941 compared to standard Si substrates, a capability crucial for detecting very small quantities of biomolecules including exosomes. The metasurfaces were temporarily enclosed with a six-channel, transparent poly-dimethylsiloxane (PDMS) microfluidic (MF) chip to construct the complete MF metasurface biosensor, allowing for multiple solution concentrations to be tested simultaneously.

Commercially available exosome standards were utilized for these studies, with a given starting concentration of 10^{10} particles/mL, which were then diluted down for testing. In previous studies, laser scattering microscopy and dynamic light scattering were used to confirm the size and concentration of the standard, concluding that the average diameter of the exosomes was around 163nm with a standard deviation of 93nm.

The surfaces were initially functionalized with a thiolated streptavidin (Cys-SA) binding molecule, followed by the immobilization of biotinylated anti-CD63 antibodies. The exosome standards were then introduced, followed by a fluorescently labeled secondary antibody, which either targeted CD82 or EXOSC5. For imaging of the biosensors before and after flowing of exosomes, a Lumenera Infinity 3 Research Grade CCD Digital Camera and a green, LED excitation light with a 530 nm wavelength was used to capture fluorescent signals. Each channel was individually imaged and analyzed using ImageJ software. These two methods enabled comparisons across different surface marker strategies.

Experiment 1: Targeting CD63 and CD82:

For the first experimental protocol, exosomes were immobilized on the metasurfaces with the use of biotinylated anti-CD63 antibodies on Cys-SA binding molecules. To fluorescently detect the exosomes, CoraLite ® 594 Conjugated Antibody for the CD82 surface protein was flowed and immobilized. Fluorescence intensity appeared to have increased with the increase in concentration of exosome standard, starting at around 10^3 particles/mL, which is indicative of our limit of detection (LOD).

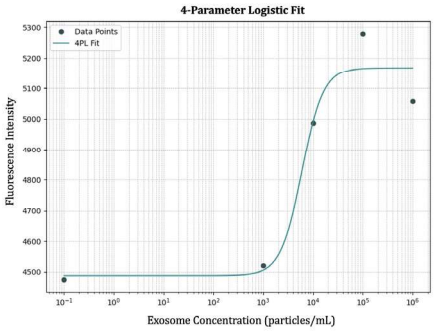

Figure 1: Microfluidic Fluorescent Imaging Results for Experimental Method #1.

As seen in Figure 1, fitting the results to a four-parameter logistic curve indicates the inflection point at approximately 5.98 x 10^3 particles/mL, with a bottom asymptote of 4487.53 and a top asymptote of 5168.16.

Such results suggest that CD82 is a reliable target for confirming exosome presence in combination with the CD63 initial immobilization. The calculated sample fluorescence found for each sample is indicated in Table 1, with each respective exosome concentration for the microfluidic channel and included biosensor.

Experiment 2: Targeting CD63 and EXOSC5:

The second experimental approach followed the same initial surface functionalization and exosome immobilization steps with the anti-CD63 antibodies; however, the final labeling and detection were performed with the anti-EXOSC5 antibody which was fluorescently labeled with NH2-reactive HiLyteTM Fluor 555 molecules. Unlike CD82, a transmembrane protein, EXOSC5 is often found intracellular, inside the exosomes, as the protein is involved in RNA quality control. Similarly to the first experimental method, fluorescence intensity was found to increase with higher exosome concentrations. In Figure 2, the fitted 4-parameter logistic model generated a bottom asymptote of 146.73 and a top asymptote of 385.27. The inflection point occurred at 6.64 x 10^3 particles/mL, similar to the detection limit found in experimental method #1.

Exosome Concentration (particles/mL)	Calculated Sample Fluorescence	Measured Antibody Fluorescence	Background Fluorescence
0.00E+00*	4473.916	10028.499	5554.583
1.00E+03	4520.202	9817.109	5296.907
1.00E+04	4986.197	10879.579	5893.382
1.00E+05	5279.387	10565.437	5286.05
1.00E+06	5057.485	9839.564	4782.079

Table 1: Microfluidic Fluorescent Imaging Fluorescent Values from Experimental Method #1.

Conclusions and Future Steps:

This work has demonstrated that Si metasurface biosensors are capable of detecting exosome concentrations of levels as low as 10^3 particles/mL, which is comparable to, if not more precise than, most detection platforms currently reported. Experimental methods #1 and #2 indicated that both CD82 and EXOSC5 are effective markers for exosomes, when used following CD63 immobilization methods. Exosome concentrations detected by FL ranged from 10^3 to 10^6 exosomes/mL, where 10^3 is equivalent to 1.66 attomolar (molar = mol/L). These results represent one of the most precise detections for exosomes, supporting the applicability of this metasurface platform for various purposes.

With protocol optimization for each use case in future studies, this platform enables diverse applications in exosome informatics, including next-generation diagnostics

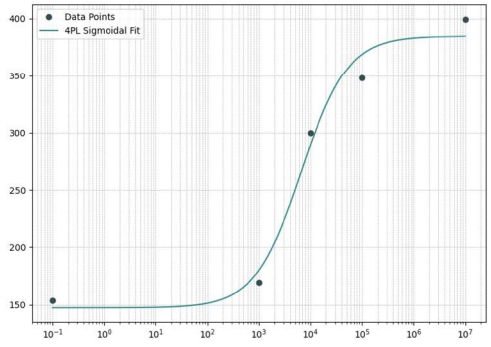

Figure 2: Microfluidic Fluorescent Imaging Results for Experimental Method #2.

Exosome Concentration (particles/mL)	Calculated Sample Fluorescence	Measured Antibody Fluorescence	Background Fluorescence
0.00E+0*	153.648	5090.849	4937.201
1.00E+03	169.228	4740.268	4571.04
1.00E+04	299.983	4758.412	4458.429
1.00E+05	348.281	4815.285	4467.004
1.00E+07	399.173	5012.256	4613.083

Table 2: Microfluidic Fluorescent Imaging Fluorescent Values from Experimental Method #2Experimental Method #2

Acknowledgments:

I would like to thank Dr. Masanobu Iwanaga and the Nanophotonics Group at the National Institute for Materials Science (NIMS) for their mentorship and guidance. Additionally, I am grateful to Dr. Lynn Rathbun and Emma Carlo from the Cornell NanoScale Facility, and Ms. Hiroko Inoue from NIMS, for their unwavering support for this program. Without all this support, my collaboration and involvement on this project would not have been possible.

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