Nanowire assisted fluorescence immunoassay (nFLIA):
Enabling low-cost, high-sensitivity biomarker assays for expanded clinical utility

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INTRODUCTION

The ability to make rapid and early clinical decisions regarding diagnosis and therapy is often highly correlated with the ability to detect one or more specific disease-relevant biomarkers early and at very low concentrations during the onset of a particular illness. High-sensitivity immunoassays employing various methodologies have been instrumental in pushing the limits of detection for immunoassay techniques to lower values for many disease-relevant biomarkers. This in turn has allowed clinicians to make relevant diagnostic and therapeutic decisions earlier than possible when using more conventional immunoassay methodology.

AIM

The unique ability of semiconductor nanowires of specific diameters to enhance and amplify the fluorescence of fluorescent reporter molecules provides a generic way to increase the sensitivity of fluorescent immunoassays (FLIA) and has allowed us to demonstrate a generically applicable mode of FLIA enhancement using semiconductor nanowire arrays. Nanowire enhanced fluorescence thus provides a general way to extend the utility of FLIAs to achieve earlier clinical decision points and improve treatment and clinical outcome.

RESULTS

In general, we were able to demonstrate an enhancement in sensitivity of 50-fold over the same assay conducted on a planar material such as plastic or glass and using the same reagents. Assays for Troponin T and CEA have demonstrated 5 - and 50-fold enhancement over planar assays (data not shown). Results for IL-6 are shown in Fig. 1. For this assay we are beginning to demonstrate reproducible sensitivities at the clinically important medical decision point concentration of 5 pg/mL with an assay format that would be compatible with simple point of care devices.

In addition, we have observed extended dynamic ranges compared to assays run on planar surfaces, often with dynamic ranges of between 4-7 orders of magnitude. Fluorescent intensity measurements of individual nanowires at low concentrations were constant over a range of concentrations while the number of fluorescing nanowires increased with increasing concentrations at these low levels. This suggests single molecule binding events on individual nanowires and thus implies a simple method for digitizing FLIAs using the nanowire arrays.

We are continuing to enhance the capabilities of performing immunoassay on the surface of nanowires by customizing the surface chemistry of the wires to allow for a range of covalent coupling chemistries. This will provide opportunities to develop tailored surfaces suited to individual assays and aid in further increasing sensitivities through retention of capture reagents and the reduction of non-specific adsorption by controlling the ability of surfaces to reject low affinity interactions.

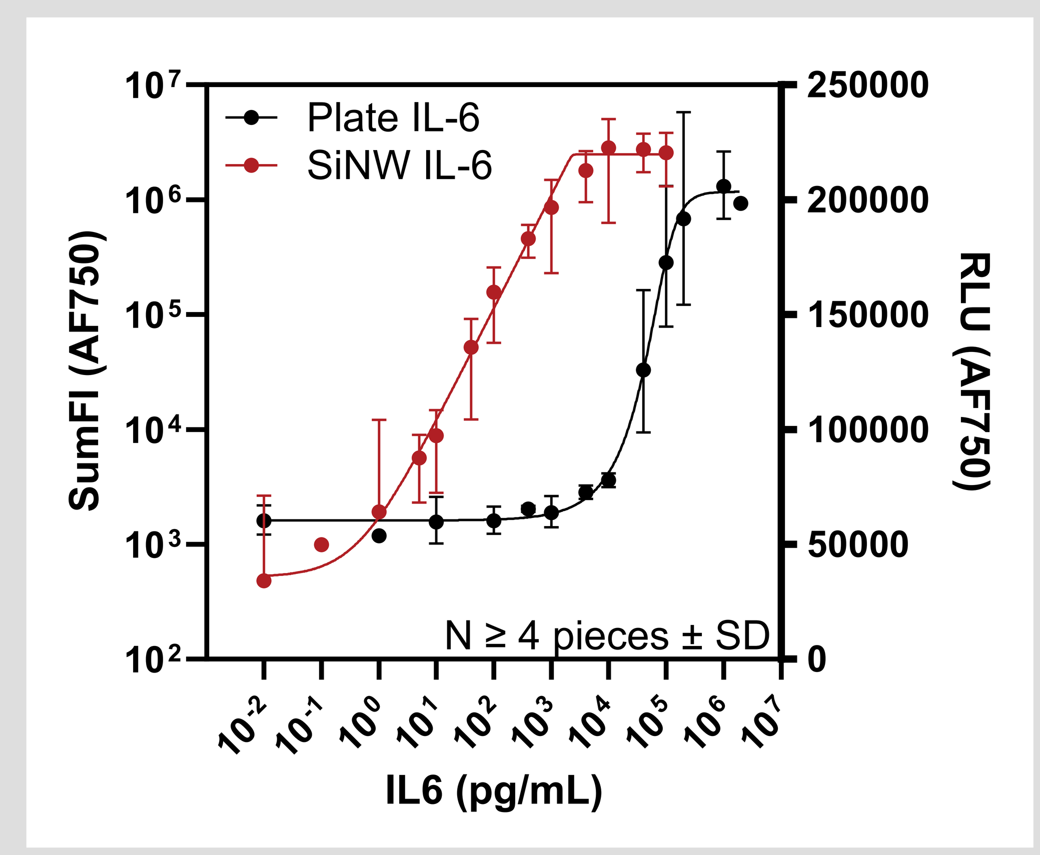
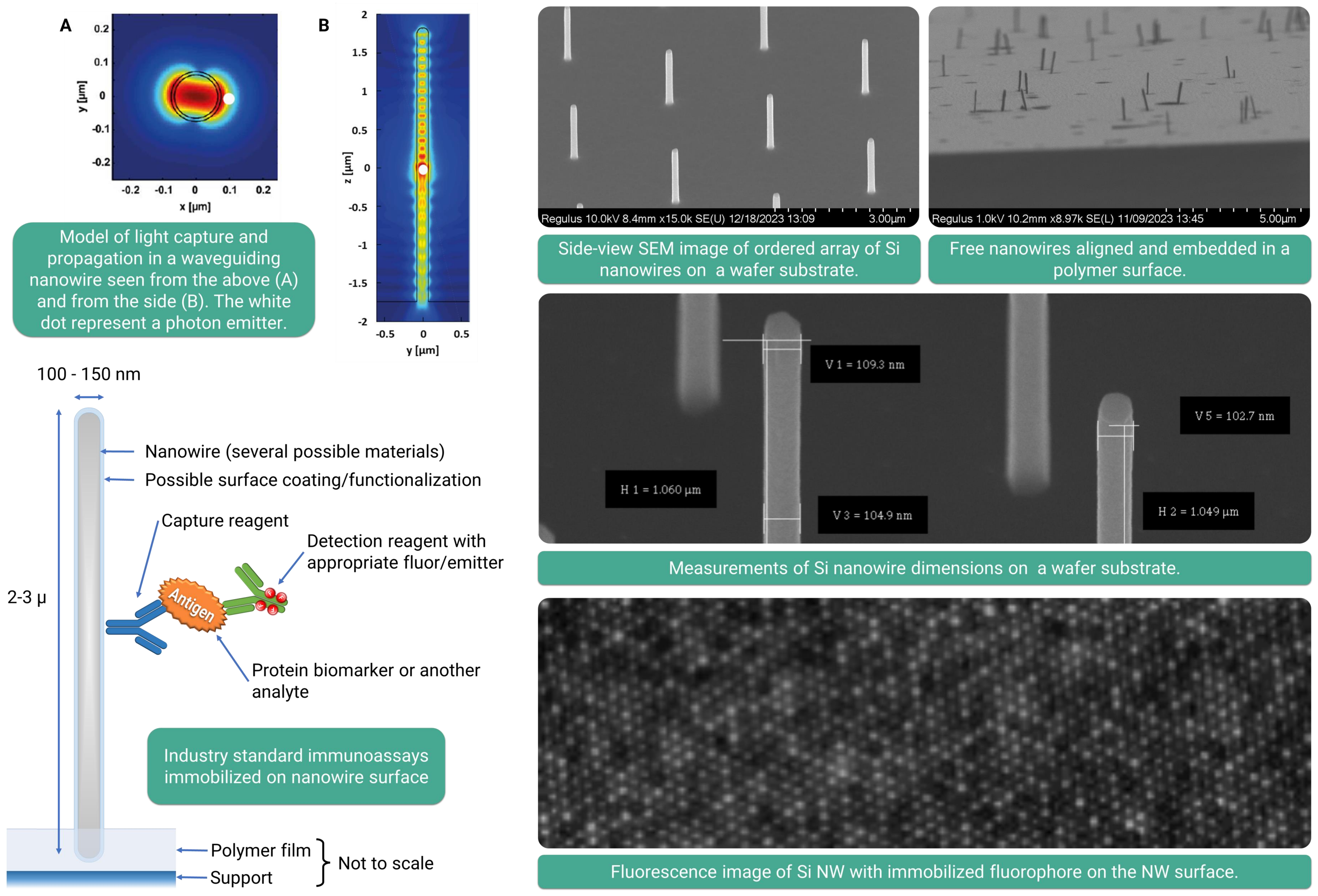


Figure 1: Titration of recombinant human IL-6 (HEK293) using IL-6 antibody clone MAB206 as capture reagent and IL-6 antibody clone IC2061S conjugated with Alexa Fluor™ 750 as detection reagent. The red curve was performed on NW surface and the black curve on Nunc™ 96-well black plate.

METHOD

Nanowires can be configured for many types of immunoassays



Arrays containing silicon nanowires with dimensions designed to interact with specific wavelengths of light were fabricated and used as substrates for performing sandwich type FLIAs for a variety of protein biomarkers. A range of biomarkers including CEA, Troponin and IL-6 were employed to evaluate how general the sensitivity enhancement effects were across different analyte assays. Commercially available antibodies and reagents were used to set up the immunoassays used in these studies. FLIA assays were performed on these nanowire array substrates using standard immunochemical procedures. Following assay for a particular biomarker analyte on the nanowire arrays, the arrays were imaged using low magnification in an inverted fluorescence microscope to record the spatial distribution and intensity of fluorescent signals present on the arrays. These images were further analyzed using a proprietary analysis algorithm to extract values for total fluorescence intensities, and to locate and enumerate the number of fluorescent nanowires and to determine the average fluorescence intensity per nanowire. These values were then used to calculate concentrations of analytes present in calibrator solutions

CONCLUSIONS

The ability of silicon nanowire arrays to enhance the sensitivities of FLIA using low-cost materials and low magnification image analysis has been demonstrated. Nanowire assisted FLIA should be able to supply commercially feasible solutions for establishing high-sensitivity Point of Care biomarker assays that provide unique opportunities for improved diagnosis and therapy across a range of clinical specialties.

REFERENCES

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