Integrated Dielectrophoresis and Fluorescence Enhancement for Detection of Biomarker Molecules

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ABSTRACT

The fluorescence enhancement makes fluorophore molecules a viable option for biomarker detection. In this work, we have used dielectrophoretic force of fluorophore labelled target molecules to place them in plasmonic nano-structures called hotspots and studied the fluorescence enhancement of concentrated molecules. More specifically, we have investigated the fluorescence enhancement of biomarkers molecules labelled with low-quantum yield fluorophore molecules.

KEYWORDS: plasmonic nano-structures, dielectrophoresis and fluorescence enhancement

INTRODUCTION

Fluorophore molecules are commonly used to label biomarker molecules, and their intensity is used to quantify the levels of biomarkers in samples. When biomarker concentrations are low (<1 pM), biomarker molecules cumulatively produce weak fluorescence that is difficult to accurately detect. A potential solution to this issue is to enhance or amplification of fluorescence intensity of molecules. A significant enhancement or amplification of fluorescence signal relative to background requires to achieve superior sensitivity, selectivity, and limit of detection.

Studies have shown that gold or silver nanostructure-based plasmonic nano-structures, called hotspots, could enhance the fluorescence intensity of fluorophore molecules by a number of fundamental near-field light-metal-fluorophore interaction mechanisms [1, 2]. For example, when the wavelength of excitation light is greater than the size of the hotspots, the excitation light scatters and produces locally high electric fields or hotspots. If fluorophore molecules are placed on the hotspots, they can harness the energy from the local high electric fields and enhance their intensity [1, 2]. Toward this end, we have recently demonstrated that dielectrophoretic (DEP) force produced by low frequency AC electric fields (< 10 MHz) applied via electrodes, could be used to place the fluorophore labelled biomarker molecules within the local peripheral hotspots [1]. More specifically, we have studied the fluorescence enhancement of concentrated biomarker molecules labelled with high quantum yield fluorophore (e.g., fluorescein) molecules [1]. In this work, we have investigated the fluorescence enhancement of biomarker molecules labelled with low quantum yield fluorophores. We have used DEP force of fluorophore labelled molecules to concentrate in the hotspots and subsequently studied the fluorescence enhancement. These studies, when combined provide complete picture of the fluorescence enhancement of biomarker molecules labelled with fluorophores molecules.

EXPERIMENTAL

We have used cyanine 3 (Cy3; excitation: 554 nm, emission: 568 nm and quantum yield: .15) fluorophore labelled short single-stranded DNA molecules (21-23 nt long) in the experiments. Short DNA molecules represent biomarker molecules such as circulating cell-free nucleic acid molecules. Figure 1 shows a picture of the device used in the experiments that has T-shape gold interdigitated electrode arrays (TIEs). The hotspots were manufactured in the peripheries of the TIEs for efficient concentration of fluorescence molecules in the hotspots. The size of the hotspots vary from 100-1000 nm. Fabrication of hotspots were discussed elsewhere [1]. During experiments, we first pipetted a 2 µL drop of a fluorophore labeled DNA on the electrode. Second, an AC electric potential (1 Vpp or 10 Vpp) was applied to the electrode for about 15 min to concentrate molecules on the hotspots. Third, fluorophore molecules were excited with appropriate wavelength of the light. Fourth, fluorescence image of the TIEs was captured using 40X magnification and a Motic-C camera. The images were analyzed by ImageJ software to create a histogram representing brightness per pixel. The data was copied into Excel. Two columns, representing the brightness level and corresponding pixel count, were multiplied together. The products were summed to give the total intensity off an image. We then calculated the fluorescence enhancement by taking the difference between

samples produced with DEP force and without DEP force, respectively. Experiments were conducted with the frequencies of 100, 3000 and 10000 kHz. These frequency values were selected to investigate wide range of frequencies that could electrically polarize molecules and produce DEP forces.

RESULTS AND DISCUSSION

Figure 1(b) illustrate the calculated electric fields (COMSOL, Burlington, MA) expect to produce by TIE electrodes for 10 V potential. As expected, large non-uniform electric fields resulting electric field gradients are produced near TIE-electrodes. The electric field calculation show that DEP force, especially attractive DEP force can concentrate fluorophores labelled molecules near metal electrodes. As hotspots are located in the peripheries of electrodes, concentrated molecules can be located in the hotspots. Figure 2 shows the normalized fluorescence intensity values for various AC electric field values. As discussed earlier, AC electric fields (magnitude of the potential and the frequency) dictates the magnitude of the DEP force on molecules. For example, strong DEP force is required to tightly concentrate biomarker molecules in the hotspots. If weak DEP force produced on molecules, concertation of molecules may not be effective. Recently, we have demonstrated that voltage of 10 Vpp and 3 MHz needed to produce large DEP force on short single-stranded nucleic acid molecules. Largest enhancement was recorded at 10 Vpp at 3 MHz. This could be due to concentration of large amount of biomarker molecules in the hotspots and subsequent fluorescence enhancement. Decoupling the effects of molecular concentration by DEP force and the fluorescence enhancement was difficult.

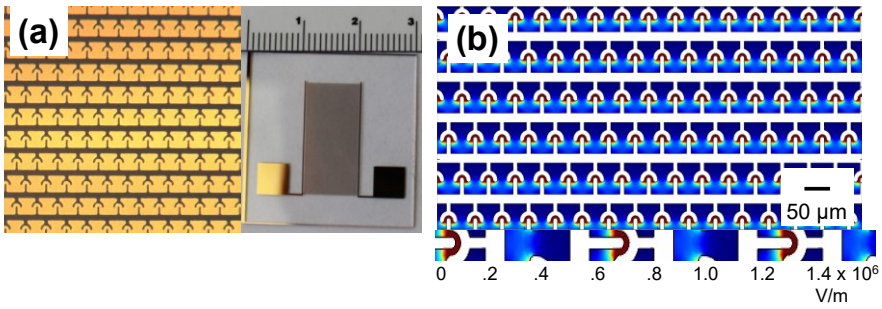

Figure 1: Device and electrodes used for experiments. **(a)** Picture of the TIE-electrodes. Inset shows a picture of the device. TIE-electrodes located in the 1.5x1 cm^2 region. **(b)** Calculated electric fields produced by TIE-electrodes when a 10 V potential was applied.

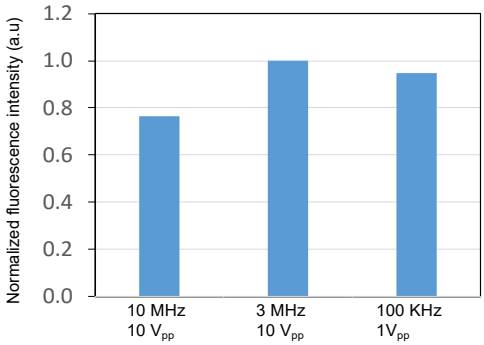

Figure 2: Fluorescence Intensity of DNA labelled with Cyanine 3

CONCLUSION

In conclusion, we have found that DEP force can be used to place short DNA molecules labelled with low quantum yield fluorophore molecules in hotspots. The frequency and potential dependent variation of the fluorescence intensity are due to the level of molecular concentration in hotspots. For example, if there is a higher concertation of molecules by the DEP force produced at a potential and frequency, higher fluorescence intensity is produced.

ACKNOWLEDGEMENTS

This material is based upon work supported by the National Science Foundation under Grant No:1941748.

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