Universal Biosensor for Multiple Biomarker Detection for Medical Applications

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Abstract: We report on the development of universal, disposable biomarker detection platform for accurate detection disease biomarkers within 30 min of assay time. This technology could replace current platform dependent, expensive and less sensitive nucleic acid and antigen detection currently used in screening and diagnosis.

Biomarker molecules that are specific to diseases are analyzed in screening and diagnosis and their up or down regulation is used to measure existence of the disease as wells as to take other clinically important decisions [1]. Protein (e.g., antigens) and nucleic acids (e.g., microRNA or miRNA) are common biomarker molecules that found in the biological fluids such as blood, serum and urine. For example, blood carbohydrate antigen (CA) 19-9 is used as the biomarker for pancreatic cancer. Healthy individuals have expressions in the range from 0-37 U/mL but higher levels could be indicative of pancreatic cancer [1]. The expression profiling of nucleic acid and protein biomarkers are currently done using different assays using instruments that are dedicated to each molecular type. Profiling of nucleic acid is commonly performed using quantitative reverse transcription PCR (RT-qPCR) assay and enzyme-linked immunosorbent assay (ELISA) is the gold-standard laboratory technique for antigen expression profiling [2]. Despite the wide use of these instruments in biomarker analysis, an effort is needed to reduce the instrument and operation cost, increase the molecular analysis throughput and speed, increase the result accuracy, increase the performance, and improve the assay simplicity. To address this issue, we have developed a universal, disposable, and low-cost device that uses an entry-level fluorescence microscope (<$5,000) for accurate and rapid expression profiling (assay time is about 30 min) of low to high (mM to aM) molarities of antigen and miRNA molecules, with an assay cost that is 100-times less expensive than current methods. There are four fundamental steps of the proposed molecular expression profiling. They are:

(1) Conjugate/hybridize target molecules with fluorophore labeled complementary molecules (e.g. antigens are conjugated with complementary fluorophore labeled antibody),
(2) Use molecular-field (electric and temperature) interactions to selectively concentrate fluorophore labeled target molecules in nano-plasmonic structures or hotspots,
(3) Utilize integrated fluorophore-metal-electric field interactions and amplify the weak fluorescence intensity of molecules that are located in hotspots and record a simple fluorescence image, and
(4) Calculate the fluorescence intensity of the image and use fluorescence intensity to calculate the molarity of each molecular target.

Step (1) will be performed in a test tube; steps 2-3 will be performed in the proposed device. When molecular concentrations are low (< 1 μM), a weak fluorescence is produced because each molecule has only one fluorophore molecule. Significant enhancement of fluorescence is therefore required to detect molecules of interest with high sensitivity and selectivity, there is no simple concept that can easily integrate with sensing. In this application, we investigated a novel concept that integrates multiple metal-fluorophore interactions sequentially to achieve fluorescence enhancements up to billion-fold. Moreover, we have utilized low frequency electric fields (<50 MHz) to couple three fluorescence enhancement mechanisms. Details about the fluorescence enhancement is discussed elsewhere [3].

It is required to place fluorophore labeled target molecules in the hotspots prior to fluorescence enhancement. In this application, we have developed a novel concept that uses fundamental molecular-field (electric and temperature) interactions such as thermophoresis and dielectrophoresis that produce thermophoretic and dielectrophoretic diffusion to selectively concentrate fluorophore labeled target molecules (e.g. fluorophore labeled antigen-antibody molecules) in hotspots [2]. Details of the molecular separation was discussed elsewhere [2]. Known molarities of miRNA (let-7a) molecules were spiked to serum samples and measured the total fluorescence intensity values by following the steps discussed above. Figure 1 illustrates the variation of fluorescence intensities of miRNA (let-7b) with their molarities. This is also shows the sensitivity and limit of detection of the detection.

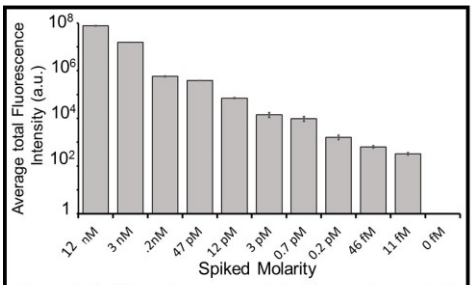

Figure 1: Variation of fluorescence intensity vs. spiked molarity of miRNA let-7b.

Because the molarity values of the target molecules could significantly small (mM - aM), the corresponding fluorescence intensities even with fluorescence enhancement could be difficult to accurately quantify. To further increase the sensitivity and limit of detection, we developed a two-step machine-learning framework for extracting the fluorescence image information. This framework utilized image segmentation and convolutional neural network (CNN) model together [4]. Image segmentation is a 3-step approach. Figure 1(a) is the raw image used for analysis. First, a selective search algorithm was applied on the input image to locate the possible regions of interest (RoIs; figure 1(b)). Second, RoIs are segmented into individual groups based on their region of intersections using intersection over union technique (IOU; figure 1(c)) [4]. Then the outliers in these groups are filtered out using a pre-trained CNN, and thus localizing the cells using machine-learning techniques (figure 1(d)). Finally, the gray-scale mean intensity of each image at each cell are calculated. We have calculated fluorescence intensity of about 22.7×10^6 (a.u.) for 3nM miRNA. In comparison, calculation of fluorescence intensity in Figure 1 produced about 15.4×10^6 (a.u.) for intensity. The difference between the intensities is due to the reduction in background by the conventional approach (without machine learning). Since machine learning approach can learn the background noise effect on the final output, we did not further process the image to reduce background intensity.

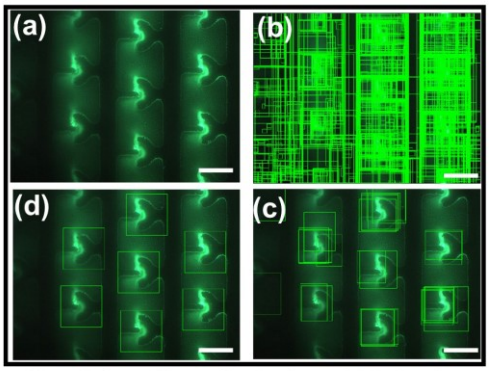

Figure 2: Steps of the machine learning model developed to calculate the molarity. Scale bars indicate 100 µm.

In conclusion, we have presented a universal biomarker detection method. Because of the simplicity of the technique, this method can be employed at point-of-care to perform screening and diagnoses. Additionally, the proposed method could accurately profile low molarity of molecules (< pM), which is difficult to achieve using the current gold standard methods. These advances will positively impact the current capabilities of screening and diagnosis.

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