All-in-one optofluidic platform for differential diagnostics of multiple biomarkers with single molecule sensitivity

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Abstract: Amplification-free and high throughput single nucleic acid detection, with minimal user input, is achieved by integrating optical waveguides with programmable valve array on a single microfluidic platform. Automated preparation and analysis of a dual protein-nucleic acid assay for Zika viral diagnostics is demonstrated.

OCIS codes: (170.4580) Optical diagnostics for medicine; (170.6280) Spectroscopy, fluorescence and luminescence

1. Introduction

Emergence of precision and personalized medicine has led to the need of detecting a multitude of genomic and proteomic biomarkers with high specificity and at ultra-low concentrations for early disease diagnosis [1]. The recent Zika epidemic presents an urgent example for the need for such a device, since viral RNA is usually found in low concentrations and the antibodies exhibit immunological cross-reactivity with other flaviviruses like Dengue. Therefore, standard serological tests may lead to possible false positives, while ZIKV RNAs, though detectable early on (first 1-2 weeks), require concurrent serological tests for diagnosis of acute infection [2]. Further, automation of sample preparation and its integration on the detection platform, minimizes user input and error, and increases accessibility to resource limited settings.

Here, we present an optofluidic platform that integrates waveguide-based single molecule detection with advanced sample preparation on a single chip. A set of in-plane all-PDMS solid-core waveguides are used to generate a femtoliter excitation volume, thus enabling single molecule detection, while a short segment of an orthogonal, leaky-mode liquid-core waveguide couples into another solid-core waveguide to efficiently collect the emitted signal. The time-dependent fluorescence from bioparticles passing this excitation volume can be then used to digitally count individual biomarkers. An upstream array of user-programmable micro-valves automates the constitution of multipart fluorescent assays, before being immediately sent to the optical section for detection. We demonstrate a real world dual protein-nucleic acid assay for ZIKV diagnostics, constituted and detected entirely on this single unified platform and show successful differentiation from Dengue virus.

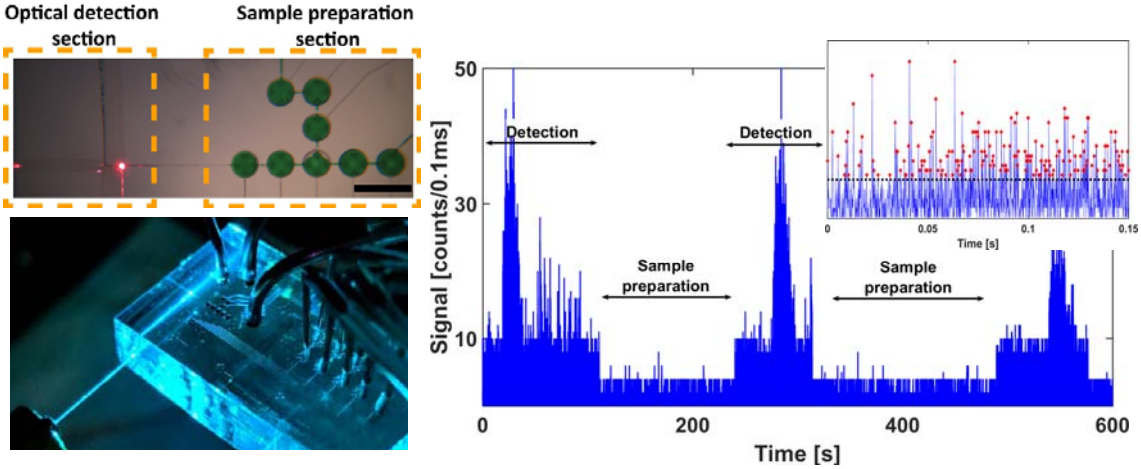

Figure 1: Device design and single molecule detection of λ-DNA molecules: (a) A top-down image of the actual device (Scale bar: 1mm) identifying the optical detection and the sample preparation sections. Bottom: A photograph showing the device coupled to the optical fiber. (b) Fluorescence trace for the detection of λ-DNA molecules fluorescently labelled with SYBR gold. Inset shows a magnified view of the signal observed. All signals above the threshold (dotted line) correspond to individual λ-DNA molecules.

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2. Results and Discussion

The all-PDMS solid-core optical waveguides are constituted by a two-step soft lithography process. The required refractive index contrast for optical propagation in the waveguides is attained by using two different PDMS precursor ratios [4]. This makes the device fabrication process entirely compatible with standard PDMS microfluidic fabrication and can therefore be integrated with a variety of microfluidic designs. For our device, we use an array of 8 individually programmable pneumatic valves for precise fluid control and on-chip sample processing, followed the optical detection section consisting of orthogonal solid-core and liquid –core waveguide (Figure 1(a) and (b)).

The high sensitivity enabled by the integrated optical waveguide architecture and the femtoliter excitation volume allows for detection of individual λ-DNA molecules. We demonstrate this by on-chip labeling of λ-DNA molecules with an intercalating dye SYBR gold, immediately followed by detection (Figure 1(c)). By counting the number of signals above the background threshold, we get a high-throughput digitized count of the number of λ-DNA particles.

For differentiated ZIKV diagnostics, we use the programmable micro-valve array to constitute magnetic bead based sandwich assays specific to both protein and nucleic acid ZIKV targets [3]. Multi-color excitation is used to detect the dual assay simultaneously (Figure 2(a)), where individual peaks in each color channel corresponds to presence of individual protein and nucleic acid assay. Due to the high specificity of the sandwich assay, we observe no signals when non-matching targets are introduced in the sample. Subsequently, we differentiate the presence of ZIKV targets from cross-reacting species like Dengue by the absence of signals in the nucleic acid channel (Figure 2(c)). The presence of a small but detectable signal in the protein channel with Dengue targets indicates its cross reactivity with Zika antibodies and reaffirms the need for a dual protein-nucleic acid assay for differential diagnostics.

Thus, by supplementing the ubiquitous PDMS microfluidics platform with PDMS based planar optical waveguides on the same chip, we have developed a versatile optofluidic sample-to-answer platform for amplification-free detection of multiple biomarkers of interest with minimal user input.

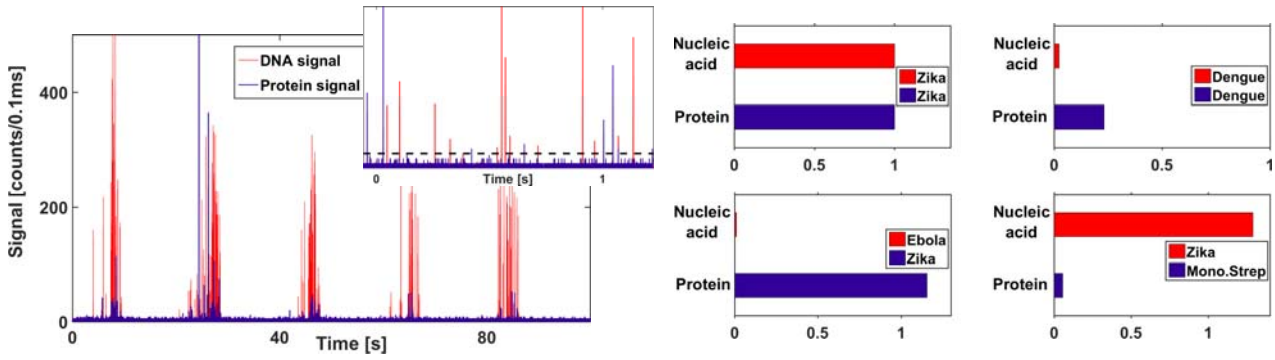

Figure 2: Differential Diagnosis of ZIKV: (a) Fluorescent time domain trace showing peaks observed from nucleic acid magnetic bead complexes (red) and protein magnetic bead complexes (purple) collected in two separate color channels simultaneously. Inset shows a magnified view with individual peaks from both channels (red and purple), clearly distinguishable from the background (dashed line). (b) Bar plots indicating relative number of peaks observed in the nucleic acid (red) and protein (purple) channel in 4 separate tests with different targets. Each bar plot is normalized with respect to the number of peaks observed with Zika nucleic acid and protein targets. Very few peaks are seen with mismatched nucleic acid (Ebola) and protein (Monovalent Streptavidin) targets. However, a significant signal is observed with dengue protein targets due to cross reactivity with Zika NS1 antibodies, but the low signal in the nucleic acid channel confirms the absence of the Zika target.

3. References

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