Microfluidic System for Detection of Viral RNA in Blood using a Barcode Fluorescence Reporter and a Photo-Cleavable Capture Probe

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Abstract

A microfluidic sample preparation multiplexer (SPM) and assay procedure is developed to improve amplification-free detection of Ebola virus RNA from blood. While a previous prototype successfully detected viral RNA following off-chip RNA extraction from infected cells, the new device and protocol can detect Ebola virus in raw blood with clinically relevant sensitivity. The Ebola RNA is hybridized with sequence specific capture and labeling DNA probes in solution and then the complex is pulled down onto capture beads for purification and concentration. After washing, the captured RNA target is released by irradiating the photo-cleavable DNA capture probe with ultraviolet (UV) light. The released, labeled, and purified RNA is detected by a sensitive and compact fluorometer. Exploiting these capabilities, a detection limit of 800 attomolar (aM) is achieved without target amplification. The new SPM can run up to 80 assays in parallel using a pneumatic multiplexing architecture. Importantly, our new protocol does not require time consuming and problematic off-chip probe conjugation and washing. This improved SPM and labeling protocol is an important step toward a useful POC device and assay.

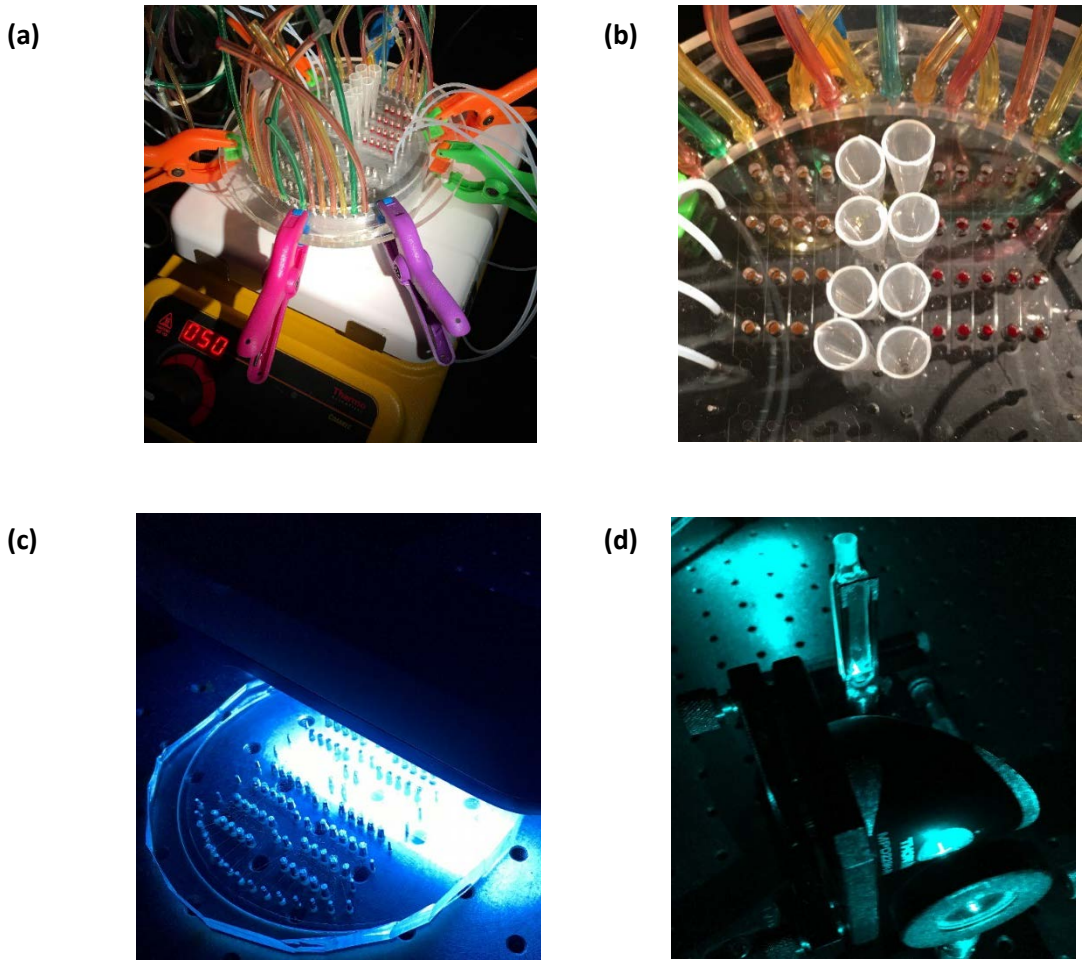
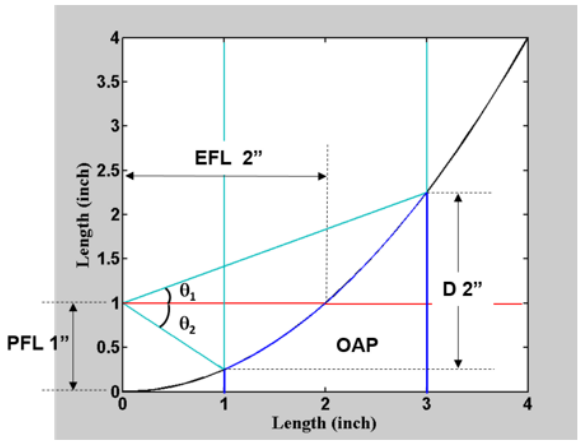


Figure S1. (a) Incubation of reporter tag, probe A, UV-cleavable probe, and Ebola RNA spiked blood on-chip at 50 °C. (b) Adding straptavidin beads into the incubation reservoirs. (c) After washes, Ebola targets released from beads with UVB exposure. (d) Fluorescence measurements.



Effective Focal Length (EFL): 2"

Parent Focal Length (PFL) : 1"

Diameter (D): 2"

θ_1=22.6°, θ_2=36.9°, and average θ=~30°.

Numerical aperture ~0.5

Magnification Ratio ~40X

Figure S2. Schematic for off-axis parabolic (OAP) mirror. High fluorescence collection is achieved by ~0.5 numerical aperture (NA) with 40X magnification ratio.

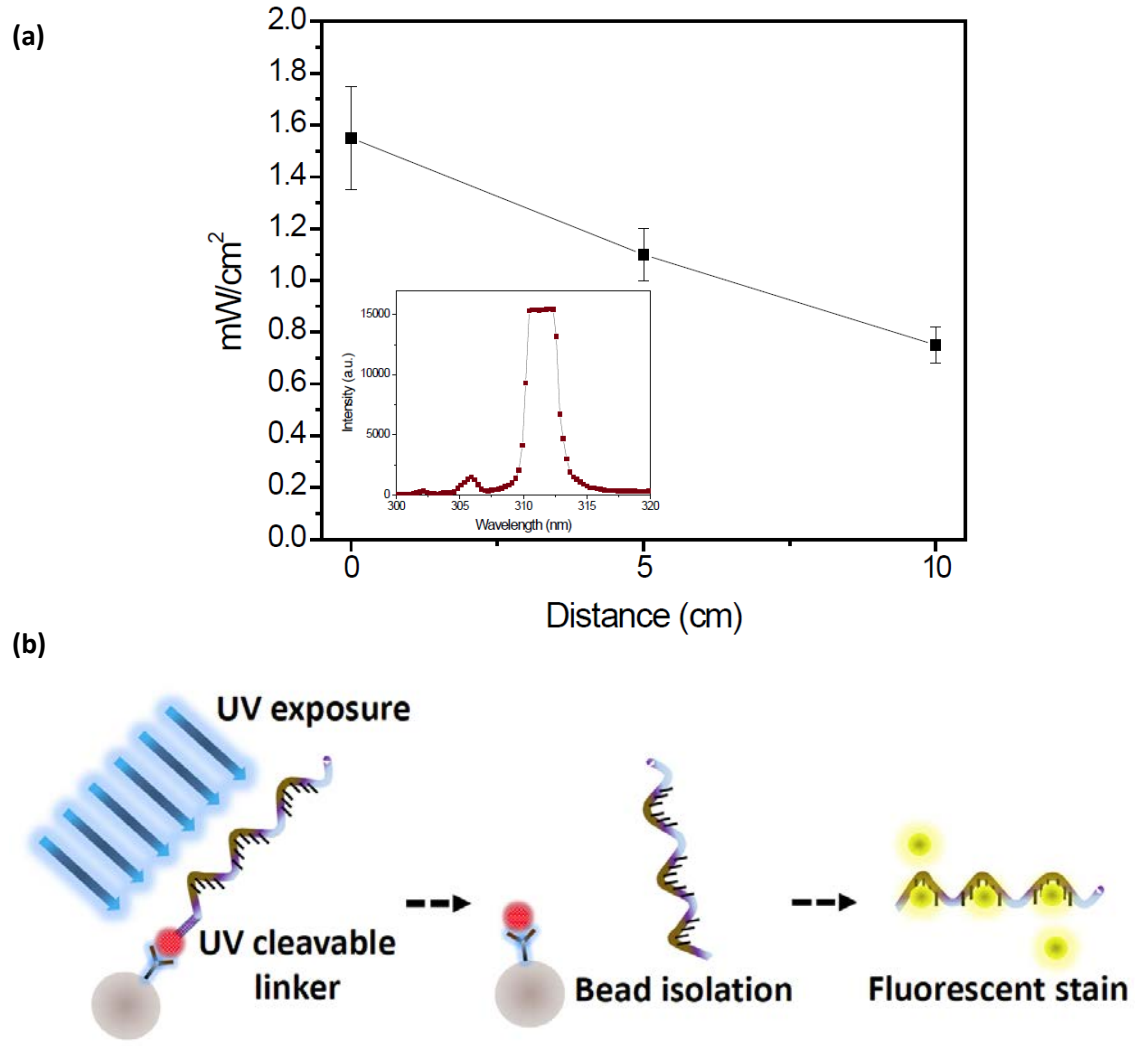


Figure S3. (a) UVB power vs. distance to the SPM. (Inset) The emission peak of the UVB lamp is centered at ~311 nm. (b) Schematic of the characterization procedure for UV release efficiency. After UV exposure, beads were isolated by a magnet and the supernatant was transferred to a new Eppendorf tube. The supernatant was stained with SYBR Gold nucleic acid dye and measured with the fluorometer.

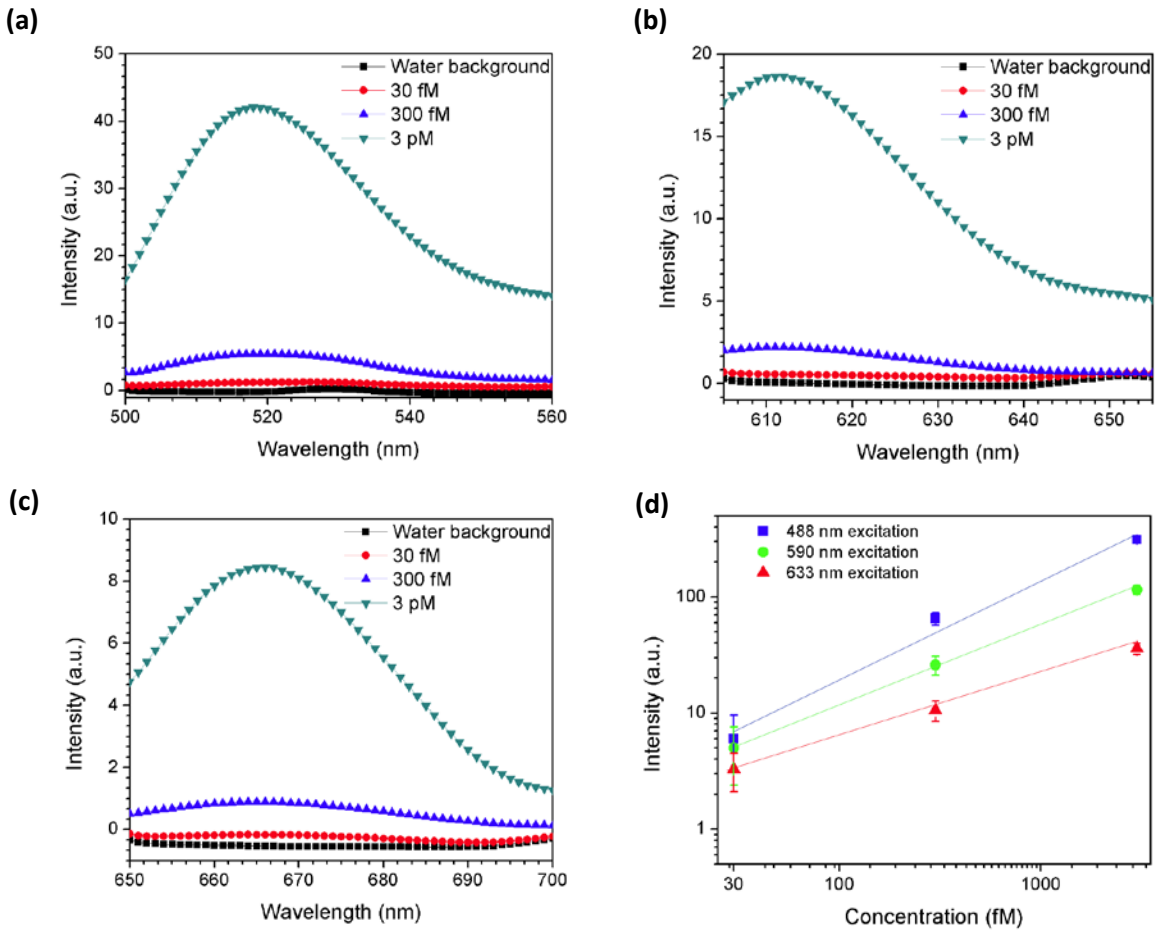


Figure S4. Characterizations of fluorescence reporter with JASCO FP-750 (JASCO) spectrofluorometer. (a) Excited with 488 nm. (b) Excited with 490 nm. (c) Excited with 633 nm. (d) Signal to noise ratio (S/N) of fluorescence signals vs. reporter concentration excited with different wavelengths.

Probe Name	Sequence	Melt Temp (c°)
Probe A	5’/AAATAGCTCACAAAGCTGTTGTGGCGTCACTCCAGCCTTATCAATGTTCCCTCAAGACCTAAGCGACAGCGTGACCTTGTTTCA	62
UV cleavable capture probe	5’/PCBio/GTTCTCTCATTGCTGTTTCACTATGTAGCACAGGATGCCCCCAGTGTTTTTGAATGGA	63.9
Reporter tag	5’/TGAAACAAGGTCACGCTGTCGCTTAGGTCTTGAGG	60
Zaire Ebola RNA (positive)	GeneBank ID: AY354458.1	N/A
Negative control	5’/CTACACCTCAGATATATTTCTTCATGAAGACCTCACAGTAAAAATAGGTGATTTTGGTCTAGCTACAGTGAAATCTCGATGGAGTGGGTCCCATCAGAAT	70.7

Table S1. A list of probes and targets (with melting temperature) used in this study.