Ultrasensitive Ebola virus antigen detection via a nanoantenna-array biosensing platform

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We have developed a plasmonic nanoantenna-array-based biosensor platform for the detection of Ebola virus (EBOV) antigen – soluble glycoprotein (sGP) – that is over 10,000-fold more sensitive than conventional enzyme-linked immunosorbent assays (ELISA). The sensor maximizes the excitation laser absorption efficiency on-chip to 95%, and has demonstrated an analytical sensitivity of 95.8%. These results combined highlight the significant potential of nanostructured biosensors in ultrasensitive detection of pathogens.

EBOV, a Category A bioterrorism agent, poses a significant threat to the public health worldwide. Unfortunately, there is currently no licensed vaccines or treatments against the EBOV infection; therefore, the disease control is relying on early diagnosis and quarantine. Rapid immunoassays targeting at EBOV-specific antigen proteins are suitable for disease screening with the limitation on antigen concentration down to 1ng/mL. Here, we leverage the fluorescence enhancement of a previously developed disk-coupled dots-on-pillar antenna array[1,2], and tuned it for EBOV early diagnosis and single molecule analysis.

The biosensor was nanofabricated through nanoimprint lithography and thin film process (Figure 1a and 1b). The nano-gap between the gold top disk and bottom plane creates localized high electromagnetic field, tremendously enhancing the efficiency of light absorbance at 785nm wavelength (Figure 1c). With a sandwich assay protocol (Figure 2a), the sensor responses to different dilutions of sGP in human plasma samples, which reached a limit of detection (LoD) of 1:12,000 dilution (Figure 2b) compared to a 1:128 dilution in the conventional ELISA (data not shown). The analytical sensitivity of EBOV sGP spiked in human plasma at 2X LoD reached 95.8% (Figure 2c). A pixelated method, counting fluorescence hotspots on the chip, was developed to further lower LoD of sGP in human plasma to 1:1,000,000 dilution (Figure 3).

In summary, we developed an ultra-sensitive Ebola virus antigen test based on nanostructured plasmonic resonance biosensor. This work provides a proof-of-concept for the development of ultra-sensitive tests to diagnose EBOV infection in human plasma samples that could also be used for the detection of wide-range of pathogens early after infection.

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[1] L. Zhou *et al.,* Anal. Chem. 84, 4489−4495 (2012).
[2] W. Zhang *et al.*, Nanotechnology 23, 225301 (2012).

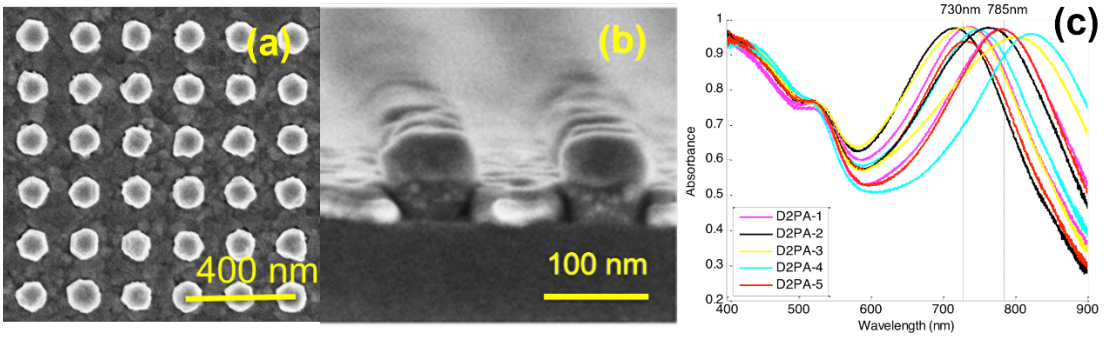

Figure 1. SEM images of D2PA after nanofabrication (a) top view and (b) cross-sectional view. (c) Absorbance of D2PA with normal incident light showing the tuning of D2PA resonance through nanofabrication parameter optimization.

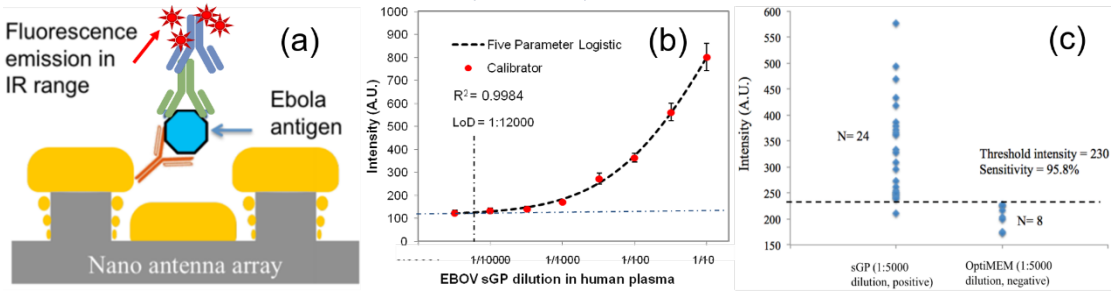

Figure 2. (a) Schematic of D2PA-based Ebola virus antigen detection. (b) Fluorescence intensity responses to different concentrations of EBOV sGP in human plasma. (c) Analytical sensitivity determination of EBOV sGP-spiked human plasma (1:5000 dilution) showing 95.8% sensitivity.

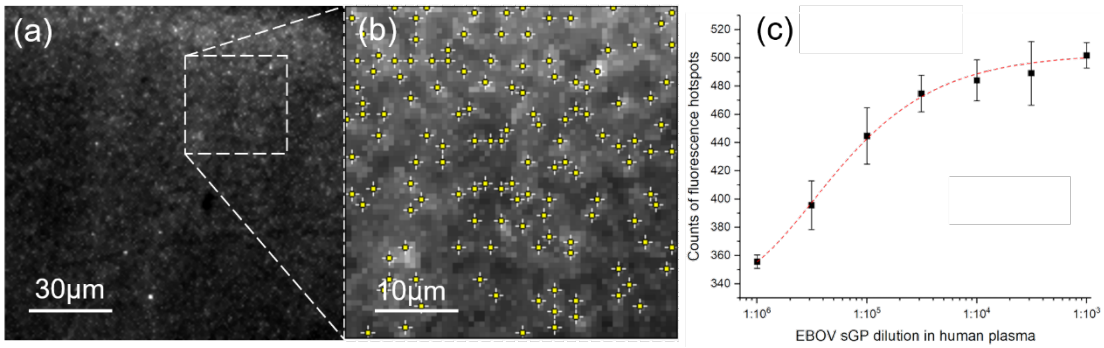

Figure 3. (a) Pixelated method for EBOV sGP single molecule detection on-chip. (b) Exploded view showing fluorophore counting (marked with yellow crosses) through image processing. (c) Correlation of fluorescence hotspot counts with EBOV sGP dilution in human plasma showing enhanced sensitivity.