Fabrication and Demonstration of Ultra-sensitive and Fast Immunoassay Platform With 3D Nanoplasmonic Cavity Antenna and Microfluidics Using Nanoimprint

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Sensitivity and fast testing are two most critical needs for many today's bio/chemical assays. Here we report (a) a new immunoassay platform that increase the detection sensitivity by 10^6 fold using novel plasmonic nanostructures, and reduce the total assay time by 5 times through microfluidic channels; and (b) its fabrication using high-precision and high-throughput nanoimprint.

The new assay platform, microchannel-D2PA, comprises the bottom D2PA sensor layer, middle PDMS channel/sealing layer, and top thin glass cover (Fig. 1a-c). The D2PA (disk-coupled dots-on-pillar nanoantenna-array) is a new nanoplasmonic sensor architecture[1], consisting of a periodic dielectric nanopillar array (200nm pitch and 70nm diameter) with an Au nanodisks on top of each pillar, an Au backplane on the foot, random nanodots (5 nm to 15 nm) on the pillar sidewalls (Fig. 1c) and nanogaps between those metal components. Hence D2PA has dense 3D nano-cavities, and dense nanodots and nanogaps, offering significant high enhancement to fluorescence as well as enhancement uniformity. The structure was optimized to enhance 785 nm fluorescence excitation light absorption, and 800 nm fluorescence radiation.

To fabricate D2PA, the pillars were first patterned on fused silica wafer by nanoimprint and followed reactive ion etching. Then, 40 nm thick gold was deposited in normal direction to form simultaneously the top disk, the backplane and sidewall dots (Fig. 1c-h). Before sealing, a capture agent layer, DSU, (for detection of protein A) was coated on the gold surface of D2PA by self-assembly. In fabrication of the middle and top layers, PDMS was first spin-coated onto a coverslip (the top layer) and imprinted by a Si mold to form microchannels (2 µm deep, 700 µm wide and 1.5 cm long). After curing, the coverslip/PDMS were peeled off from the mold together; and the PDMS surface was activated by oxygen plasma before being bonded to the bottom layer at room temperature through an aligned bonding.

During the fabrication, (a) the uniform nanostructures were selectively patterned with desired size and gap in proper location and aligned along the microchannel (Fig. 2); (b) microchannels made of PDMS instead of glass enabled room temperature bonding, avoiding a high temperature (1000°C) bonding which could melt the nano-metallic structure of D2PA to destroy its enhancement.

In assay testing, IRdye800CW labeled protein A was pumped through the device with a flow rate of 5µL/min and concentration range from 1fM to 100nM (100µL each). The protein A was captured onto D2PA through the coated capture agent, DSU. Unbounded molecules are washed away by washer. By measuring fluorescence intensity vs. solution concentration and fitting data with standard five logistic regression model, we get a limit of detection of 1.1 fM, which is 10^6 fold more sensitive than the reference glass immunoassay (1 nM) (Fig 3). Furthermore, since the microchannel drastically have reduced the travel distances for a molecule being captured by the bottom sensor layer, the incubation time is reduced by 5 times, from 2 hours to 20 mins [2].

In summary, we report a high throughput method to integrate nano-structured plasmonic sensor into microfluidic platform and demonstrated the usage of device through fluorescence immunoassay.

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[1] W. D. Li, et al., Opt. Express., 2011, 19, 3925-3936;
[2] L.C. Zhou, et al., Anal. Chem., 2012, 84, 4489-4495.

Figures:

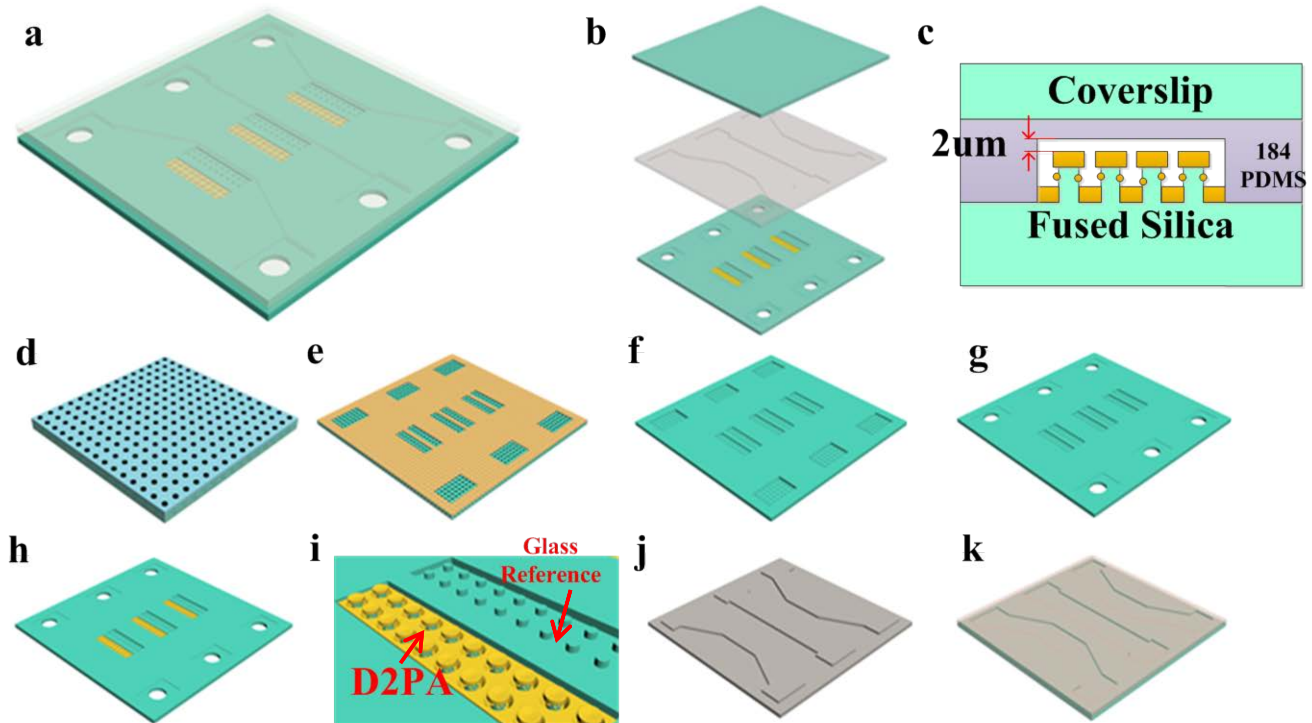

Figure 1. Device Scheme and fabrication process. (a) Device structure; (b) Tri-layer structure; (c) Device cross section; (d) Nanoimprint Cr dots array; (e) Photolithography to pattern sensor region; (f) Etch pillar; (g) Drill through holes for liquid delivery; (h) Gold deposition; (i) Zoom in figure of h; (j) Si channel mold; (k) Coverslip/PDMS hybrid fluidic channel.

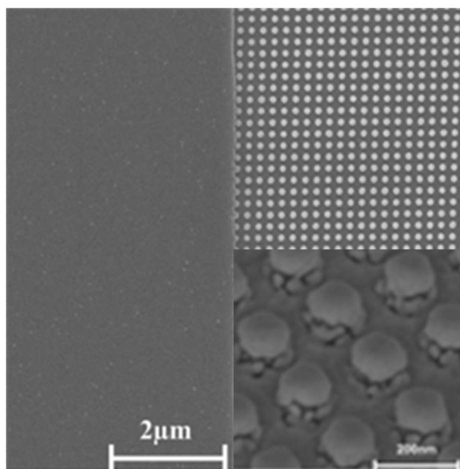

Figure 2 SEM picture. Taken at the edge of D2PA region and flat region. Inset is zoom in figure of D2PA, and scale bar is 200nm.

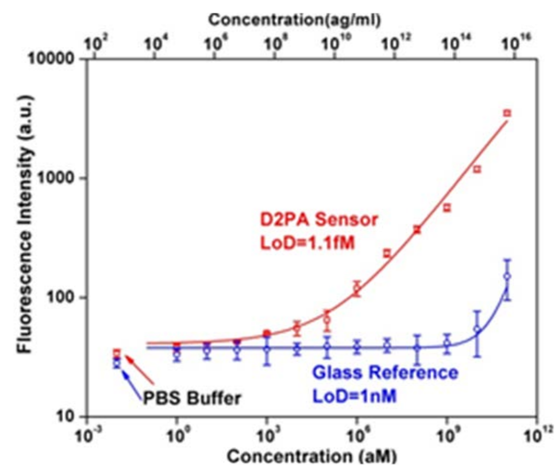

Figure 3. Fluorescence immunoassay results. The limit of detection on D2PA was fitted to be 1.1fM, while 1nM on the glass.

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