# Nanoplasmonic Cavity Enhanced Microfluidic Immunoassay with 5-minutes Incubation Time, 7.8 pM Sensitivity and 100 nL Sample

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Point of care (POC) diagnostics needs to detect targeted analytes sensitively and quickly with small sample volume. Here we report the design, fabrication and performances of a new ultra-sensitive fluorescent immunoassay platform well suited for POC diagnostics—microfluidic D2PA assay (μ-D2PA), which uses (1) a new 3D plasmonic-enhanced fluorescent sensor, D2PA (disk-coupled, dots-on-pillar, plasmonic, antenna array)[1], to enhance the signal, and (2) a capillary microchannel architecture to increase assay speed and reduce sample volume. We have achieved 7.8 pM (2.6 ng/ml) and 0.78 fmole limit of detection (LoD) for human-IgG with only 100 nL sample and 5 minutes incubation time. Compared to previous best reported, our μ-D2PA is ~40-fold more sensitive, 50-fold smaller sample volume, and equally fast incubation time[2].

The μ-D2PA assay comprises two layers bonded together: the bottom layer has the D2PA sensor, and the top layer has a 20 μm deep PDMS microfluidic channel for sample delivering and incubation (Fig.1). The D2PA sensor has a dielectric nanopillar array (200nm pitch, ~100nm diameter and 56 nm height) with an Au nanodisk on top of each pillar, an Au backplane at the foot (Au thickness is 40 nm), random Au nanodots (5 nm to 15 nm diameter) on pillar sidewalls, and nanogaps between the metal components (Fig 1). The D2PA sensor has demonstrated enhance fluorescence signal by three orders of magnitude[3].

In fabrication, the bottom layer was first patterned with D2PA sensors on a fused silica wafer using nanoimprint, reactive ion etching (RIE) and Au evaporation on selected areas. Then a self-assembled monolayer of Dithiobis Succinimidyl Undecanoate (DSU) was coated on the D2PA as the capture agent for the Protein-A molecules. The top microchannel layer was fabricated by casting and curing PDMS on a Si microchannel mold and activated with oxygen plasma to ease liquid wetting. It was then bonded with the bottom layer (Fig. 2). Reference samples without D2PA sensors are also fabricated that uses flat fused silica as the bottom layer.

The μ-D2PA system was tested with a human-IgG three-layer-indirect immunoassay (Fig. 3). The capture agent, Protein-A (220 nL, 5 μM), was first pipetted into the chip and delivered through microchannel into the incubation chamber, being incubated for 5 minutes. A Protein-A layer was bonded to DSU. Then targeted biomarker, human-IgG (100 nL) and blocker (2% BSA in 1x PBS, 100 nL) are pipetted in and incubated for 5 minutes. Finally, detection agent, anti-human-IgG (100 nL) labeled with IRDye800CW was pipetted in and incubated for 5 mins. After each incubation step, the channel was washed by 100 nL washer (PBS+0.5% TWEEN-20) to remove non-specifically bonded molecules. The assay fluorescence signal was then measured (Fig.3). We achieved a LoD of 7.8 pM (2.6ng/ml) for the human-IgG assay on D2PA, which is 0.78 fmole targeted biomarker molecules for a 100 nL sample volume; while on the glass reference, only 500 nM concentration has signal distinguishable from background (Fig 3).

This ultra-sensitive, fast, simple, μ-D2PA immunoassay with low sample volume offers a new path to future POC diagnostics applications.

[1] Li WD, Ding F, Hu J, and Chou SY. *Opt. Exp*.19, 3925-3936, 2011
[2] Kang QS, Shen XF, Hu NN, Hu MJ, Liao H, Wang HZ, He ZK, and Huang WH, *Analyst*, 138, 2613-2619, 2013
[3] Zhang WH, Ding F, Li WD, Wang Y, Hu J, and Chou SY., *Nanotechnology* 23, 225301-225309, 2012

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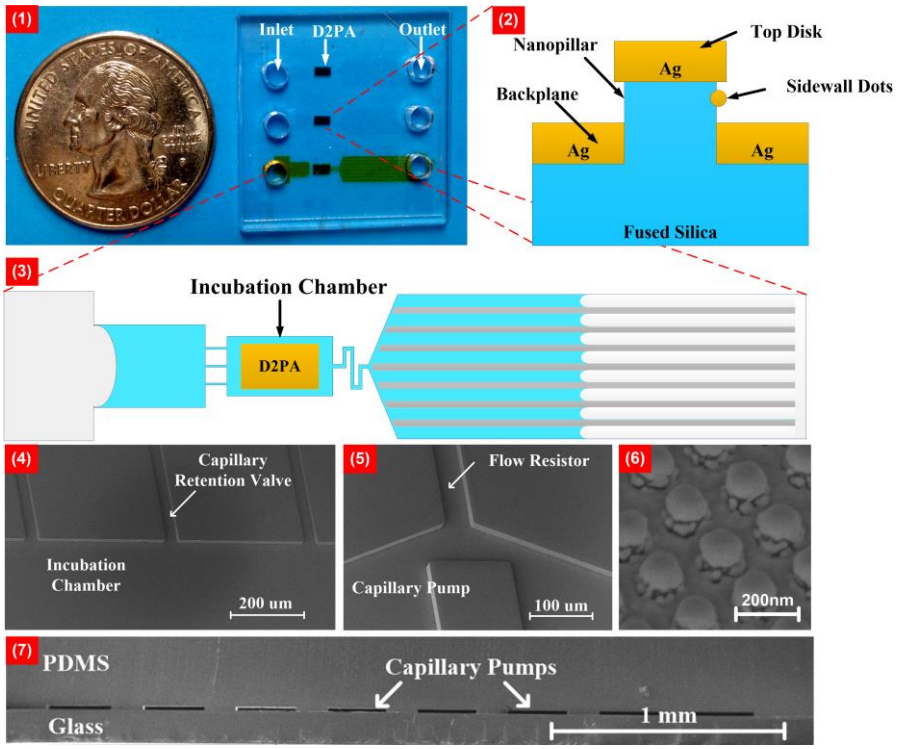


Fig. 1. (1) Photograph of the chip, there are three separate devices on one chip, the bottom one is filled with dye water in order to see the shape clearer; (2) Scheme of D2PA; (3) Structure of the microchannel, the blue color represents the liquid; (4-5) SEM picture of PDMS microchannel; (6) SEM picture of D2PA, top disk, backplane, and sidewall dots are shown; (7) Cross section view of the bonded microfluidic chip.

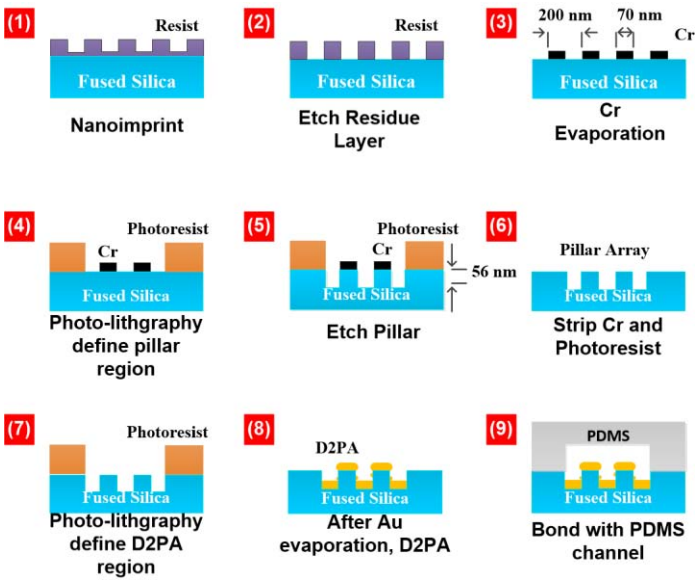


Figure 2. D2PA sensor fabrication process

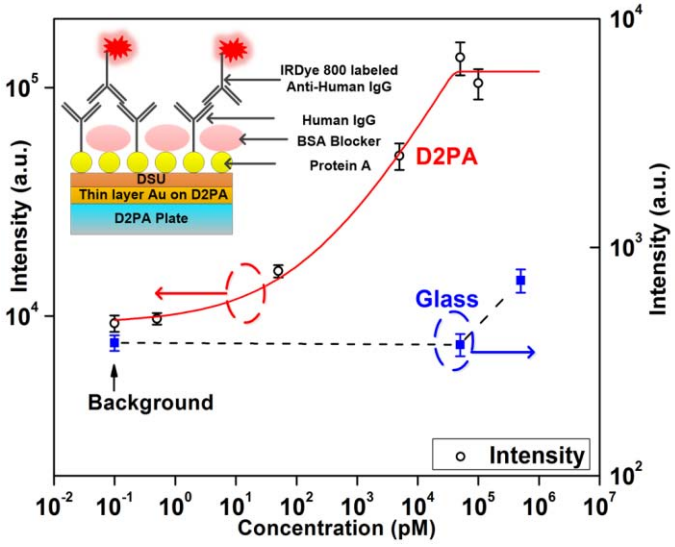


Figure 3. Fluorescence intensity vs. IgG concentration. The limit of detection on D2PA was found to be 7.8 pM, the inset figure is the immunoassay scheme

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