

Support Information

A binary optical encoding strategy for multiplex assay

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Generation of silica PCBs. The silica PCBs were fabricated by the droplet template method. The silica aqueous suspension (15 w/v %) and silicone oil (KF 96-10CSt) were injected into the co-flow microfluidic device, respectively. And then the silica aqueous suspension was broken into monodisperse droplets by the oil flows in the microfluidic channel. The droplets were heated at 70 °C overnight to make the silica nanoparticles self-assembled into ordered lattices. After solidification at 100 °C for 2h, silica PCBs were thoroughly washed with hexane to remove the silicon oil. To improve their mechanical strength and reduce fluorescence background, the silica PCBs were sintered at 700 °C for 3 h.

Preparation of PCBs capture probes and QDs labeled reporter probes. Three kinds of silica PCBs with reflection peak positions at 476, 623 and 650 nm, and two kinds of CdSe/ZnS QDs with fluorescent peak positions at 525 and 605 nm were chosen to demonstrate the reliability of binary optical encoding strategy for multiplex assays. Two kinds of analytes, Mouse IgG and Rabbit IgG, were chosen as the analytes. Detailed experimental processes were as shown in Figure 1b. The antibody functional fragments were modified on silica PCBs and CdSe/ZnS QDs by covalent bonding method. Firstly, silica PCBs were treated with piranha solution (30% hydrogen peroxide and 70% sulfuric acid) for 12 h. After washing thoroughly with water, it was dried under a stream of nitrogen and silylanized with 3-glycidoxypyltrimethoxysilane (GPTMS) by immersing it in toluene solution of 5% GPTMS overnight at room temperature (25 °C). After the coupling reaction, the PCBs were rinsed several times with toluene and ethanol to remove the physically absorbed GPTMS and dried under a stream of nitrogen. Upon this process, many active epoxy groups were exposed on the surface of nanostructure of the PCBs. Then, the PCBs were reacted with capture antibody functional fragments (1mg/mL Goat anti IgG) in PBS buffer solution at 4 °C for 12 h. After that, the silica PCBs were treated with 0.5% BSA (bovine serum albumin) solution for 2h at room temperature (25 °C). The Code-A PCBs modified with capture antibody functional fragments could be regarded as capture probes and multiplex micro-carriers.

Four kinds of QDs-tagged-IgG, including 525 nm QDs-tagged Goat anti Mouse IgG, 525 nm QDs-tagged Goat anti Rabbit IgG, 605 nm QDs-tagged Goat anti Mouse IgG and 605 nm QDs-tagged Goat anti Rabbit IgG were provided by Wuhan Jiayuan Quantum Dots Co., Ltd., China. Both 525 nm QDs-tagged Goat anti Mouse IgG and 605 nm QDs-tagged Goat anti Rabbit IgG were used as reporter probes¹, both and 605 nm QDs-tagged Goat anti Mouse IgG and 525 nm QDs-tagged Goat anti Rabbit IgG were used as reporter probes². These Code-B QDs modified with detection antibody functional fragments could be regarded as reporter probes.

Preparation of QDs-coated Silica PCBs Carriers. We generated QDs-coated silica PCBs carriers by layer by layer assembly approach as the following steps. The first step was the deposition of a primer three layer polyelectrolyte (PE) film, poly (allylamine hydrochloride) (PAH)/poly (sodium 4-styrenesulfonate) (PSS)/PAH, PE₃, onto the silica PCBs. This primer film provided a smooth and uniform, positively charged outer surface to facilitate the adsorption of the QDs. The QDs were immobilized through the electrostatic interaction between carboxyl (–COO–) groups on the surface of QD nanocrystals and charged amino groups (–NH₃⁺) of PAH on the silica PCBs. Each QD layer film was separated with a PE₃ film to regenerate a positively charged surface. Compared with a single interlayer, the deposition of PE₃ layers between nanoparticle layers could result in more uniform nanoparticle coatings. By those steps, we generated the QDs-coated silica PCBs carriers.

Effects of PCBs Micro-carriers and QDs Reporter Probes on Multiplex Assay. Scanning electron microscopy (SEM) images of the silica PCBs were as shown in Figure S3 (a and b). The silica PCBs bead was spherically shaped with a diameter of 200 μm and highly ordered hexagonal symmetric nano-structure, while simultaneously maintains high surface-to-volume ratio (SVR). Ordered hexagonal symmetry structures of a PCB surface could be observed in Figure S3 (b). The silica PCBs had ordered nano-porous structure (as shown in Figure S3 (c)).

The CdSe/ZnS QDs could range in size from 2 nm diameter (480 nm emission) to 8 nm (660 nm emission), whose transmission electron microscope (TEM) image was as shown in Figure S3 (d), and the size of CdSe/ZnS QDs conjugated to detection antibody functional fragments was less than 12 nm. Therefore, the CdSe/ZnS QDs labeled reporter probes could diffuse into the nano-pores of the silica PCBs and immobilized with capture probes, which might lead to a stronger detection signal.

FIGURE CAPTIONS

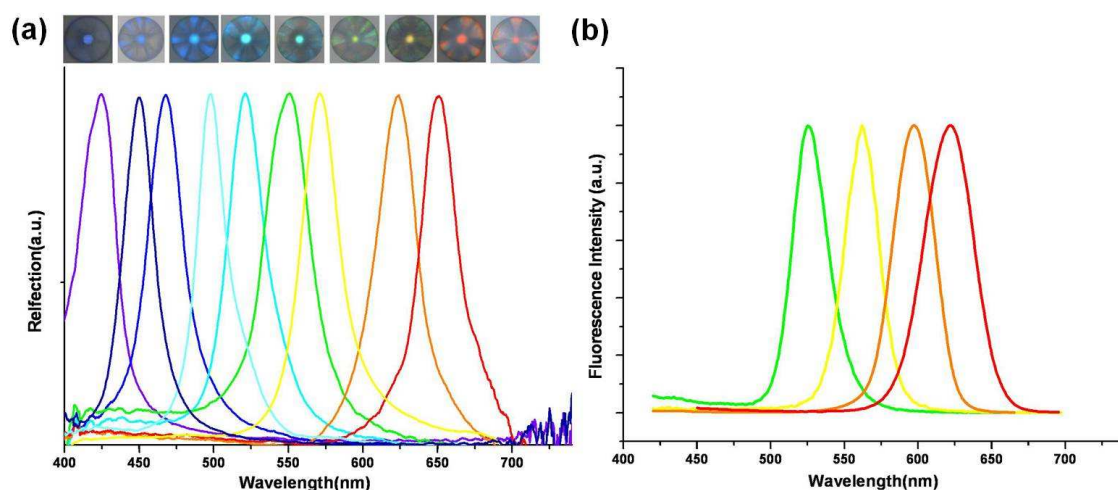


Figure S1. (a) Reflection spectra of nine silica PCBs, and the reflection spectra were 421, 449, 476, 495, 505, 550, 571, 623 and 650 nm from left to right. Measurements were performed when the beads were in solution (Top). Bright-field microscopy images of nine silica PCBs (b) Fluorescence emission spectra of four CdSe/ZnS QDs, and the fluorescence spectra were 525, 565, 605 and 630 nm from left to right.

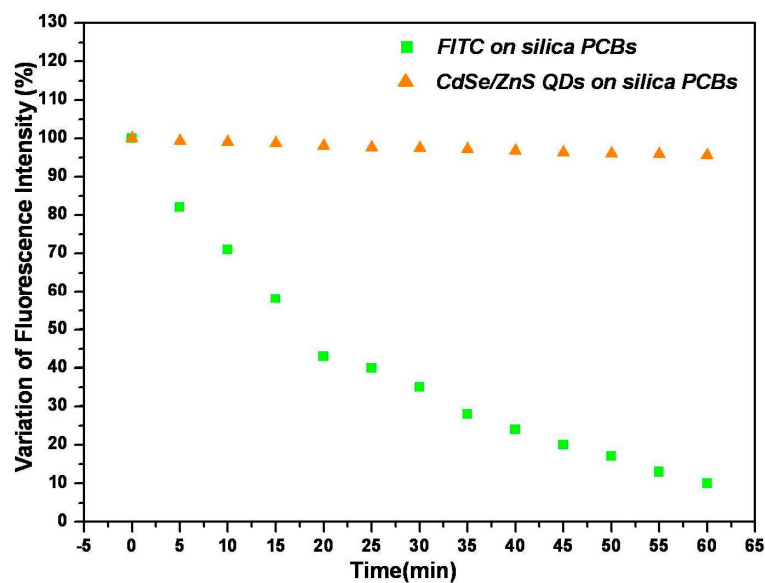


Figure S2. Comparison of photochemically stability between FITC and CdSe/ZnS QDs on silica PCBs, the 523nm FITC-tagged Goat anti Rabbit IgG immobilized on PCBs with reflection peak positions at 650nm and the 605nmQDs-tagged Goat anti Rabbit IgG immobilized on PCBs with reflection peak positions at 650nm were illuminated for an hour under the same intensity laser.

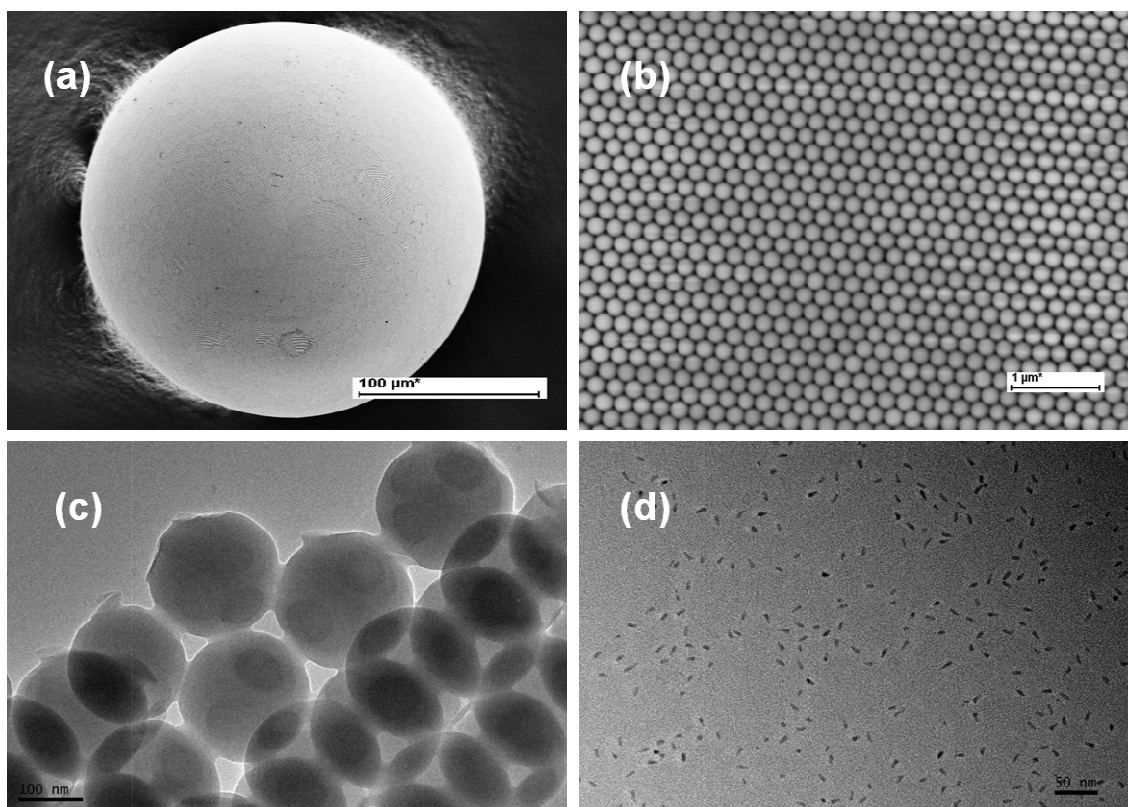


Figure S3. (a)(b) SEM images of silica PCBs: (a) low magnification image of a bead with diameter of 200um, (b) high magnification image of the PCB surface showed the hexagonal alignment of nanoparticles. (c)TEM image showed the internal ordered nanostructure of the PCBs. (d) TEM image of CdSe/ZnS QDs.

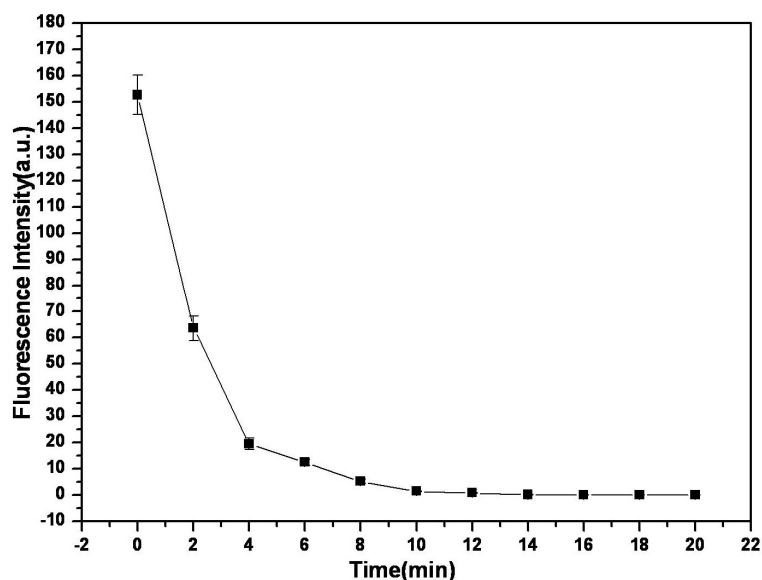


Figure S4. Relationship between the electrophoresis time and the fluorescence intensity of residual non-hybridized QDs labeled reporter probes (80 V, 10mA). The silica PCBs with on probe and 605 nm QDs with Goat anti Mouse IgG was used to detect Mouse IgG. Error bars represent standard deviations.