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Photo-bleaching Immunity Encoded Photonic Suspension Array for Label-Free Multiplex Analysis

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Experimental

Materials and Instruments: LPAN (Mw ≈ 20,000) and HPAN (Mw ≈ 50,000) were gained from Mitsubishi Co., Japan. BBS and Bovine serum albumin (BSA) was received from Sigma Chemicals. Goat-anti-rabbit IgG, goat-anti-mouse IgG, goat-anti-human IgG, mouse IgG, human IgG were purchased from Biodee Biotechnology Co., China. Phosphate buffer saline (PBS, 0.05M, pH 7.4), phosphate buffer saline tween-20 (PBST, 0.05% tween-20 in PBS) were self-prepared. Silica microspheres were synthesized according to a modified Stöber method.[S1] A fiber-optic spectrometer (Ocean Optics, USB2000-FLG) was employed to detect reflection spectra. Fluorescence spectra was recorded by an fluorescence microscope (Olympus IX71) equipped with the fiber-optic spectrometer. SEM images were obtained on a S-3000N operated at 5.0 kV.

Preparation of Stretched BBS-PAN Inverse Opal: First, opal film templates composed of monodisperse silica spheres were deposited on a substrate by vertical deposition.[S2] A N,N-dimethylformamide (DMF) solution (containing 8 wt% PAN and 0.01 wt% BBS) was then infiltrated into the voids of the opal template. After evaporating the solvent and solidifying the film, the silica spheres

were etched with 4% hydrofluoric acid and washed with deionized water to form a free-standing BBS-PAN inverse opal film. Finally, the film was evenly uniaxially stretched at 80^{o}C by a graduated homemade drawing device. The stretch ratio was calculated by the formula: $R_s = \frac{L}{L_0}$, where R_s is stretch ratio, L is the final length of the sample and L_0 is the initial length of the sample.

Stretched BBS-PAN Inverse Opal for Label-free immunoassays: The inverse opal film carriers were firstly washed with PBS buffer solution three times with each for 10 min. Then probe antibodies in PBS buffer solution (1mg/ml) were immobilized on the pore surfaces of the PAN inverse opal film carriers at 4°C for 12 hours. After washed by PBS buffer, BSA (1% in PBS buffer) was subsequently used to block the places without protein adsorption at room temperature for 2 hours. The carriers were washed by PBS buffer again. The probe-modified carriers were then used for analysis by mixing them with an analyte solution containing antigens at 37°C for 2h. After the probe antibodies on the pore surfaces of the carriers specifically bind the antigen, the unbound antigens were washed away with PBST and PBS buffer. Finally, the carriers were sandwich hybridized by antibodies solution at 37°C for 2 hours and washed by PBST and PBS buffer again. The carriers were dried by N_2 and recorded reflection spectra after step of BSA block and sandwich h ybridization.

Spectrums of carriers possessed different polarized luminescent ratio and reflection peak

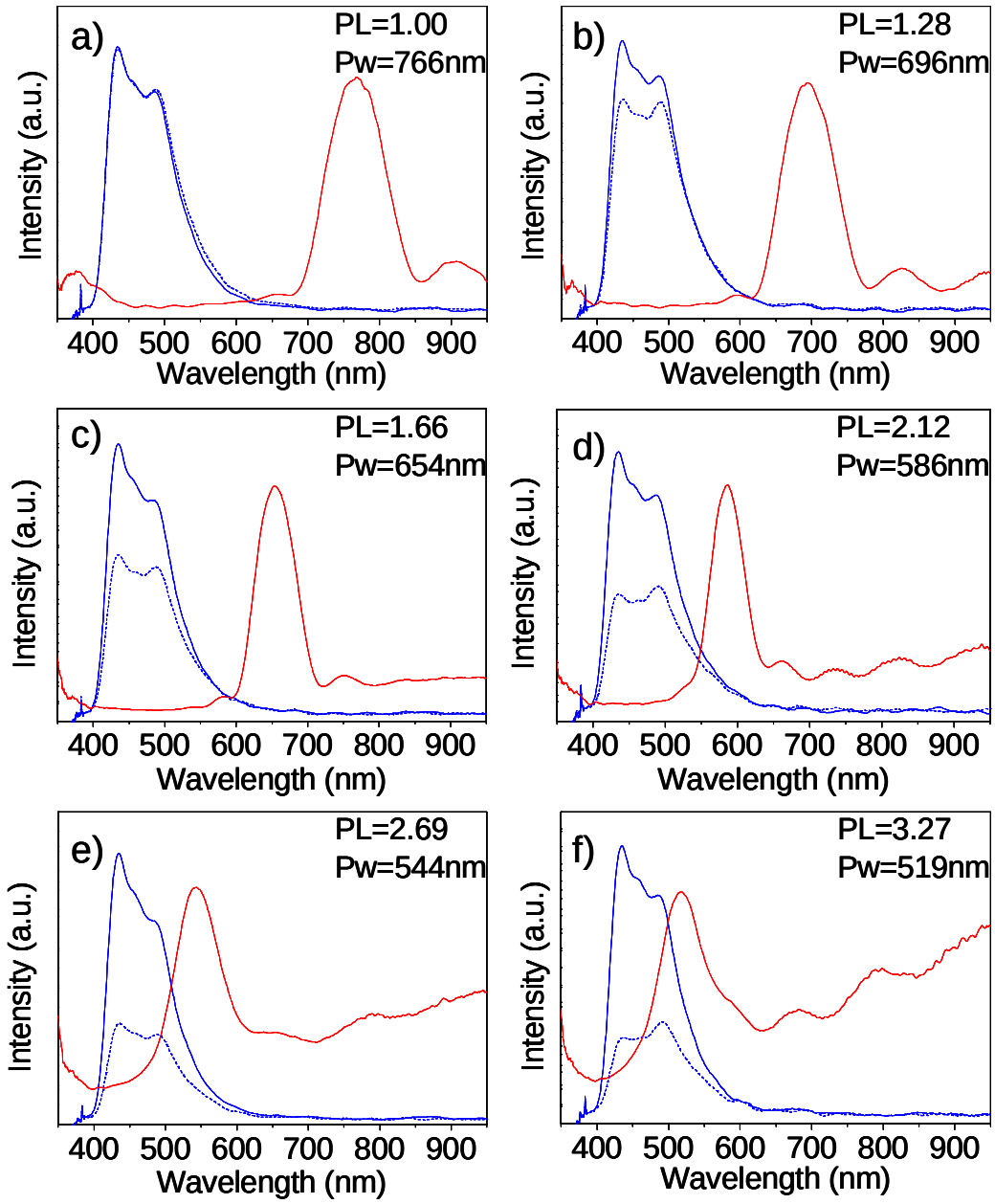


Fig. S1 A series of carriers possessed different characteristic information both polarized luminescent ratio and reflection peak. The solid blue line is polarized photoluminescence spectra with polarization of polarizer parallel to elongation direction, the dashed line is polarized photoluminescence spectra with polarization of polarizer perpendicular to elongation direction, the red line is reflection spectra. The stretch ratio of these carriers is respectively a) 1.0, b) 1.2, c) 1.4, d) 1.8, e) 2.2, f) 2.6.

Schematic illustration of multiplex analysis

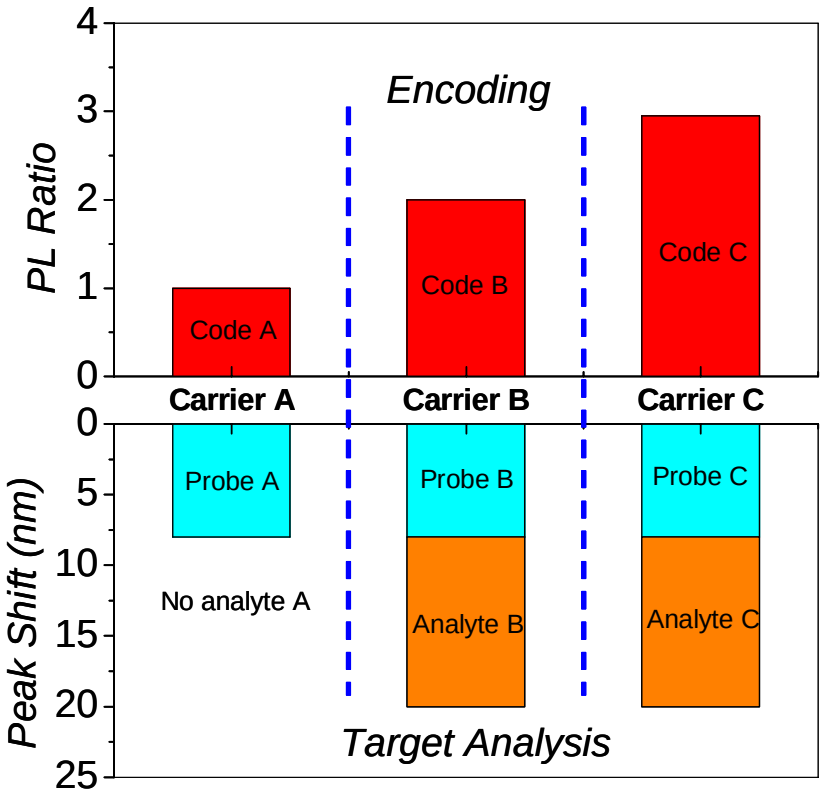
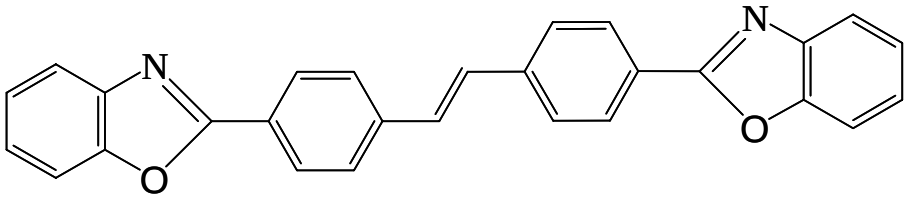


Fig. S2 The schematic illustration of multiplex analysis. Three pieces of carrier A, B and C, which possessed different polarized lumination ratio as code, was chosen to immobilize probe A, probe B and probe C respectively. This would result a red-shift of reflection peak. Then these carriers were mixed in solution containing analyte B and analyte C. After reaction, every carrier's emission spectrums in two polarized directions and reflection spectrum were recorded. The carriers could be judged by polarized luminescent ratio calculated by emission intensity in two polarized directions. And the analyte could be detected by another reflection peak shift of the carrier.

Molecular structures of BBS



4,4’-bis(2-benzoxazolyl)-stilbene (BBS)

Scheme 1. Molecular structures of BBS.

References

S1 Stöber, W.; Fink, A.; Bohn, E. *J. Colloid Interface Sci.* **1968**, *26*, 62.
S2 P. Jiang, J. F. Bertone, K. S. Hwang, V. L. Colvin, *Chem. Mater.* **1999**, *11*, 2132.