Supporting Information

Optically Encoded Semiconducting Polymer Dots with Single-Wavelength Excitation for Barcoding and Tracking of single Cells

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S1. Effective FWHM of blue emission of PFO Pdots

The blue emission of PFO Pdots mainly consisted of two emission peaks (Fig. 1C). Hence an effective full width at half-maximum (FWHM) was adopted, which was derived from the cumulative peak using Gaussian line shape fitting, as shown in Figure S-1.

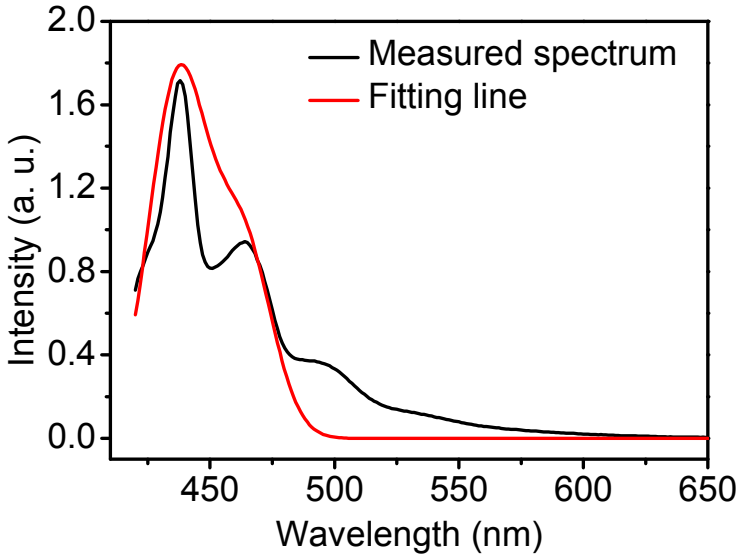


Figure S-1. Emission spectrum of PFO Pots (black line) and Gaussian line shape fitting (red line) of two emission peaks (at 437 and 463 nm). The effective FWHM was determined to be ~ 40 nm.

S-2. Photostability of Pdot barcode

Photostability of fluorescent probes is critically important for many imaging applications, particularly for long-term imaging and monitoring experiments. We examined the photostability of three kinds of representative Pdot barcodes and compared the result with Qdots. The result shows that the fluorescence of Pdots was very stable and comparable in photostability as Qdots under 405-nm irradiation for 2 hrs.

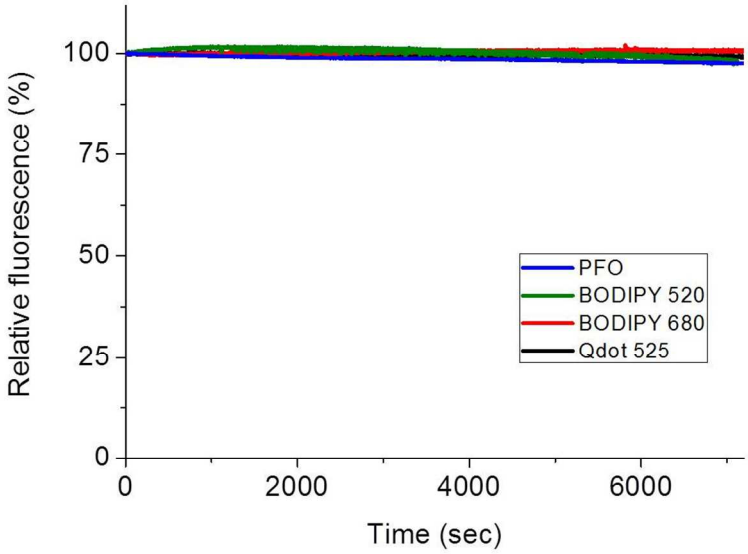


Figure S-2. Photostability test of bulk Pdots formed by PFO, BODIPY 520, and BODIPY 680 polymer. The Pdots were under 405-nm irradiation from a Xe lamp. Like Qdots, all Pdots possessed high photostability after irradiation for 2 hrs.

S3. Tuning Pdot barcodes

There is a linear relationship between the measured fluorescence intensity at each wavelength and the feeding amount of the semiconducting polymer emitting at that wavelength. Although FRET existed between the polymers, this factor was compensated for by tuning the feeding amounts of the different polymers to obtain the desired fluorescence intensity ratios.

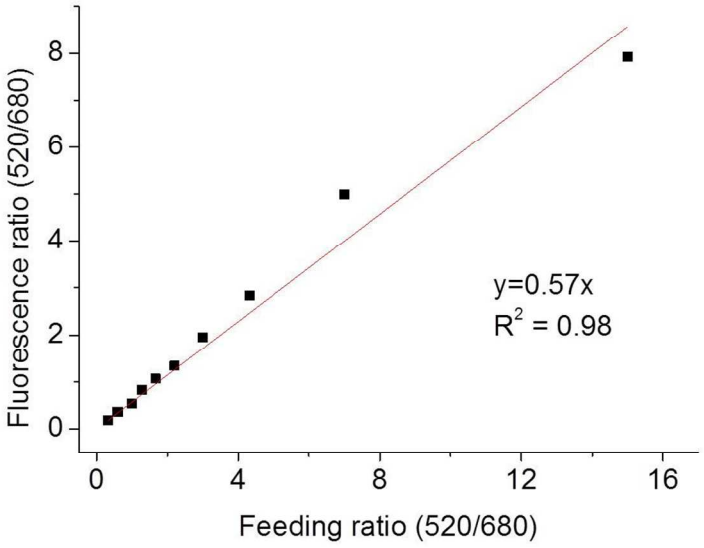


Figure S-3. A linear relationship between the measured fluorescence intensities and the feeding amounts of semiconducting polymer.

S-4. pH insensitivity of Pdot barcodes.

The fluorescence of Pdots was recorded at several pH conditions in 20 mM Hepes buffer. The fluorescence was found to stay steady (variation < 5%) in the range of pH 5.0-9.0. The insensitivity to pH changes is important for biological applications because it rules out the potential interference caused by the pH variations within cells.

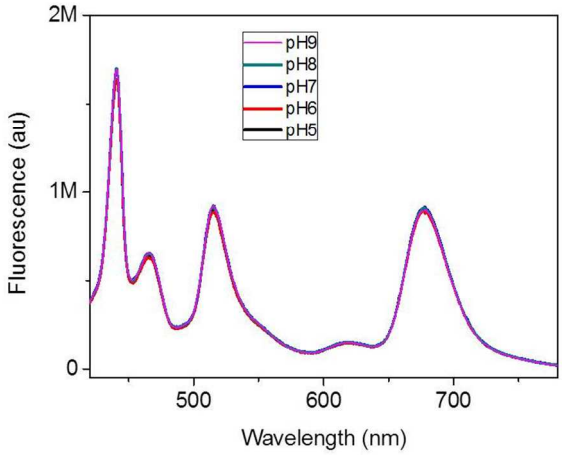


Figure S-4. pH effect on the fluorescence performance of Pdot barcodes. The Pdots (5 ppm) were placed in 20 mM Hepes buffer with pH 5-9. The excitation wavelength was 405 nm from a Xe lamp.

S-5. Pdot barcodes are thermally stable

The fluorescence spectra of Pdots were recorded at 37 °C over 3 days. The fluorescence spectra were found to remain sufficiently stable in this experiment.

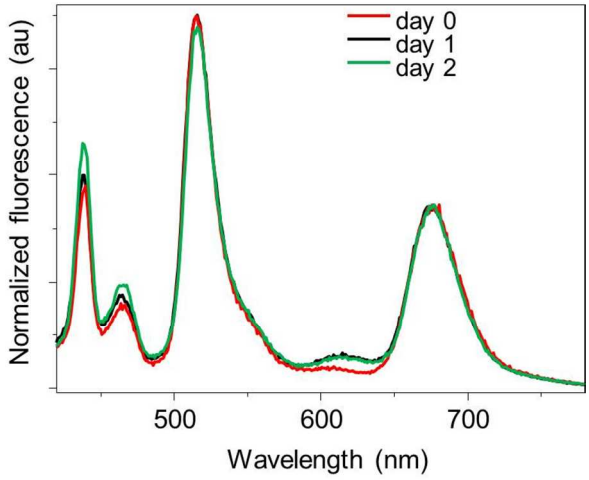


Figure S-5. Thermal stability of Pdot barcodes. Pdots were place in water at 37 ^{o}C, after which their fluorescence spectra were monitored with time. The excitation wavelength was 405 nm from a Xe lamp.

S6. Specific cell labeling by Pdot barcodes

Bioconjugation of Pdots. Bioconjugation was carried out by coupling carboxylate-functionalized Pdots with amine-containing streptavidinased via EDC (1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride)-catalyzed reaction. Typically, for a 4-mL solution of Pdots with the concentration of 50 ppm, 80 μL of polyethylene glycol (5 % w/v PEG, MW 3,350) and 80-μL HEPES buffer (1 M, pH 7.3) were mixed. Then 240 μL of streptavidin (1 mg/mL in 20 mM HEPES buffer) was added to the Pdot

solution and mixed on a vortex. Finally, 80 μL of freshly prepared EDC (10 mg/mL in MilliQ water) was added to the solution. The mixture was stirred for 4 hr at room temperature. After bioconjugation, 80 μL of bovine serum albumin (BSA, 10 wt %) was added to the Pdot solution. The reaction was continued for another 20 min to eliminate the aggregation of Pdots. An 80-μL aliquot of Triton X-100 (2.5 wt % in MilliQ water) was added to the Pdot solution to make the Pdots more stable. The mixture was transferred to a centrifuge ultrafiltration tube (Amicon® Ultra-4, MWCO: 100 kDa, from EMD Millipore, Billerica, MA, USA) and then concentrated to 0.5 mL by centrifugation. Finally, the mixture was purified by gel filtration via Sephacryl HR-300 gel media to obtain streptavidin-functionalized Pdots for cellular labeling. Aliquots of 50 μL BSA (10 wt %) and 5 μL sodium azide were added to the purified Pdot solution for long-term storage.

Labeling Pdots on cell surface via antigen-antibody binding. To harvest the MCF-7 cells, the adherent cells were quickly rinsed with the medium and then incubated in 5-mL trypsin-ethylenediaminetetraacetic (EDTA) solution (0.25 w/v % trypsin, 2.5 g/L EDTA) at 37 ^{o}C for 5-10 min to suspend the cells. The detached cells were collected in a tube and then centrifuged at 2,500 rpm for 10 min to precipitate them to the bottom of the tube. After removing the supernatant, the cells were rinsed and resuspended in 5-mL culture media. The cell concentration was determined under the microscope with a hemocytometer.

For cellular labeling experiments, ~10^6 cells were placed in a Petri dish with medium for at least 5 hr for the cells to re-adhere to the surface. After that, we removed the medium in the Petri dish and incubated the cells with 100 μL of labeling buffer (1× PBS, 2-mM EDTA, 1% BSA) with 0.6 μL of 0.5 mg/mL primary biotinylated anti-human CD326 (EpCAM) antibody purchased from BioLegend (San Diego, CA, USA) on a rotary shaker in the dark and at room temperature for 30 min. This was followed by a washing step using labeling buffer to remove the excess antibody. For the conjugation of cells and Pdots, the biotinylated cells were incubated with ~5.0 nM streptavidin-functionalized Pdots in 0.2-mL BlockAid buffer purchased from Invitrogen (Eugene, OR, USA) for 30 min on a rotary shaker in the

dark and at room temperature, followed by two washing steps with labeling buffer to remove the excess Pdots. The streptavidin-functionalized Pdot solution was sonicated for 3 min before use to disperse any potential aggregates. Cell fixation was performed by immersing the cells in 500 μL of fixing buffer (1× PBS, 2-mM EDTA, 1% BSA, 1% paraformaldehyde) for at least 15 min. The fixation was done prior to the labeling process in order to keep cells from detaching. Finally, the fixed cells were immersed in 500 μL 1× PBS buffer.

Specific cellular labeling with the Pdot-streptavidin probes was confirmed by fluorescence imaging. With observation in 3 different color channels (blue, green, red), the cells labeled with the different Pdot barcodes could be distinguished from one another by their fluorescence emissions. For example, the cells labeled with (333) Pdots exhibited fluorescence at all 3 channels while those labeled with (313) Pdots only emitted blue and red fluorescence.

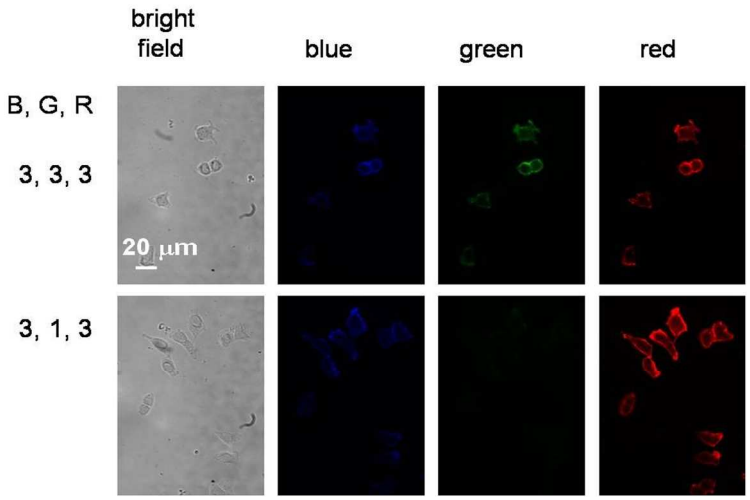


Figure S-6. Fluorescence images of cells labeled with different Pdot barcodes via the biotin/streptavidin binding. The streptavidin-conjugated Pdots were incubated with biotinylated primary antibody-labeled cells. Cells were all excited with a 405-nm laser (~10 mW/cm^2). The blue, green, and red images were obtained by collecting the fluorescence through the band pass of 450/50 nm, 525/30 nm, and 680/40 nm, respectively.

S-7. Flow cytometry measurements of Pdot-barcode labeled cells

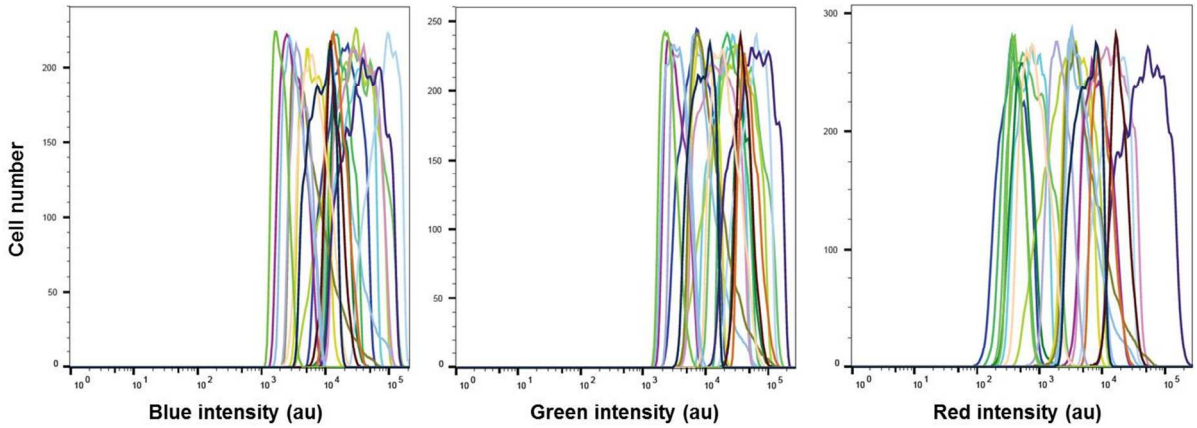


Figure S-7. Fluorescence intensity plots of flow-cytometry measurements from 20 sets of MCF-7 breast cancer cells labeled with Pdot barcodes. When viewing each color channel separately, the fluorescence-intensity based histogram signals appeared to overlap and indistinguishable. However, the 20 different Pdot-barcode labeled cells could be distinguished when looking at the intensity ratios from all 3 color channels (as shown in Figure 4B). The excitation wavelength was at 405 nm. Blue, green, and red fluorescence were collected through the band pass of 450/50 nm, 525/50 nm, and 670/30 nm, respectively.

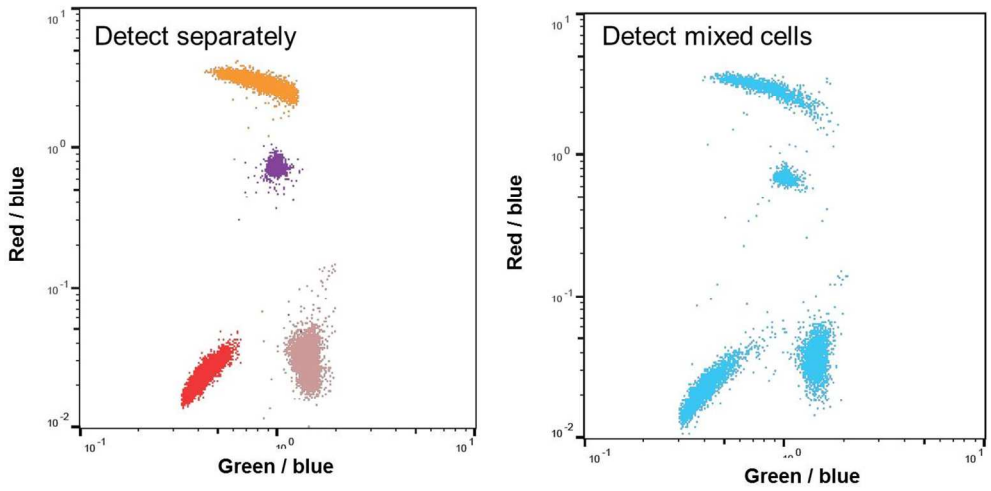
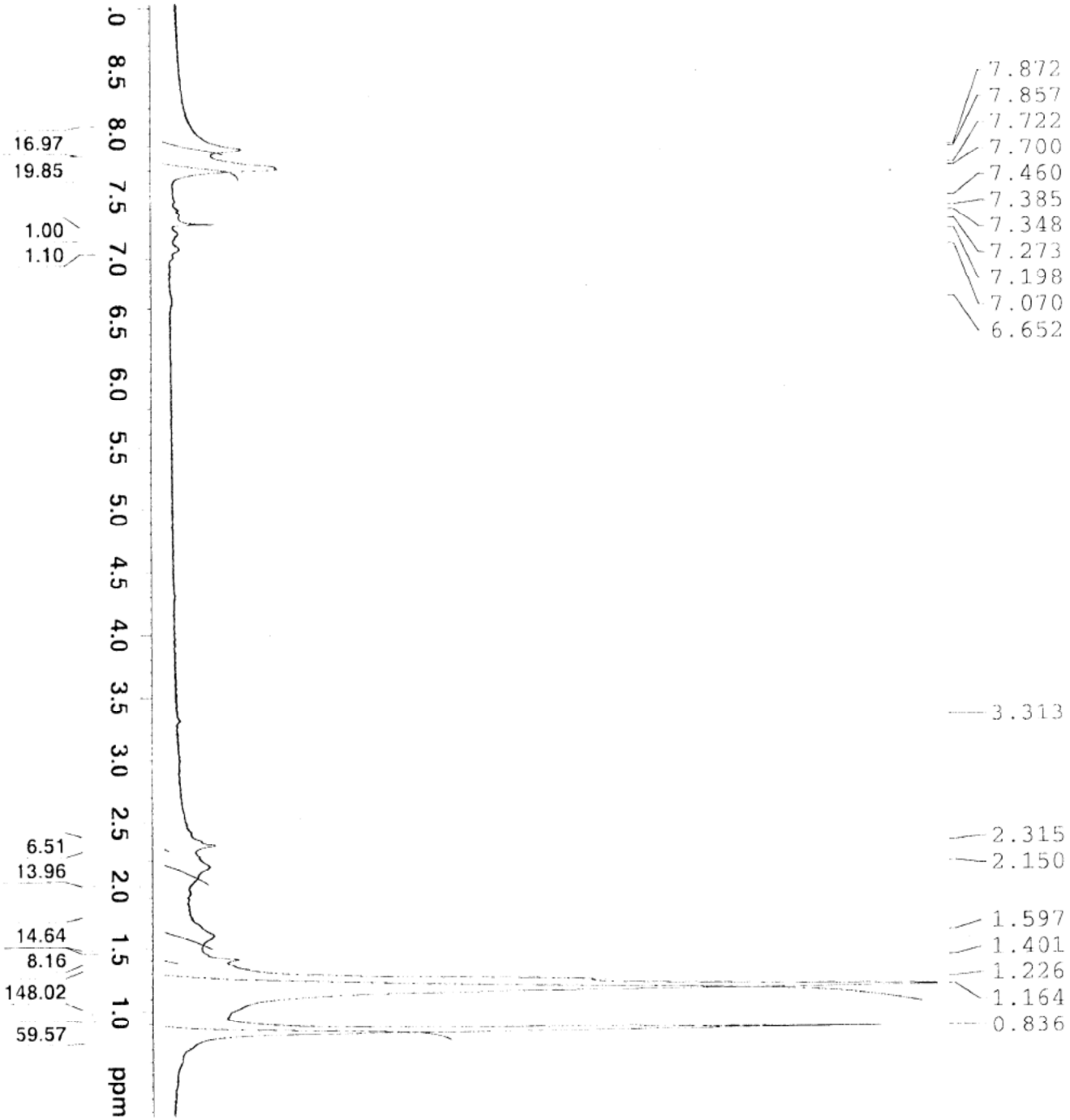


Figure S-8. Separate (left panel) and mixed (right panel) cells labeled with specific Pdot barcodes and detected via flow cytometry. The excitation wavelength was at 405 nm. The blue, green, and red channels collected the fluorescence through the band pass of 450/50 nm, 525/50 nm, and 670/30 nm, respectively.

S-8. NMR spectra of BODIPY 680 polymer.



^{1}H-NMR of BODIPY 680 (5%) polymer

The NMR spectrum of BODIPY 520 (3%) is found in the Supporting Information of *ACS Nano* **2013**, *7*, 376-384.

REFERENCES

1. Y. Rong, C. Wu, J. Yu, X. Zhang, F. Ye, M. Zeigler, M. E. Gallina, I. C. Wu, Y. Zhang, Y. H. Chan, W. Sun, K. Uvdal and D. T. Chiu, *ACS Nano*, **2013**, **7**, 376-384.