Published in final edited form as:

Anal Chem. 2018 September 18; 90(18): 10688–10694. doi:10.1021/acs.analchem.8b03201.

Ratiometric Barcoding for Mass Cytometry

Xu Wu^{1,2,3}, Quinn DeGottardi⁴, I-Che Wu¹, Li Wu¹, Jiangbo Yu¹, William W. Kwok⁴, and Daniel T. Chiu^{1,*}

¹Department of Chemistry, University of Washington, Seattle, WA 98195, USA.

²The Key Laboratory of Carcinogenesis of the Chinese Ministry of Health, Xiangya Hospital, Central South University, Changsha, Hunan 410078, China.

³The Key Laboratory of Carcinogenesis and Cancer Invasion of the Chinese Ministry of Education, Cancer Research Institute, Central South University, Changsha, Hunan 410078, China.

⁴Benaroya Research Institute at Virginia Mason, Seattle, WA 98101, USA.

Abstract

Barcoding is of importance for high-throughput cellular and molecular analysis. A ratiometric barcoding strategy using lanthanides-coordinated polymer dots (Ln-Pdots) was developed for mass cytometric analysis. By using three metal isotopes and four ratio intensity levels, 16 barcodes were generated to code, and later decode, cell samples in mass cytometry. The ratiometric Ln-Pdots barcodes not only provided high mass signal intensities, but also eliminated the bias caused by different concentrations of the labeling reagents/barcodes and run- to-run differences in cell labeling efficiency. The ability to distinguish clearly the 16 sets of labeled MCF-7 cells with mass cytometry demonstrated the excellent resolving power of the ratiometric Ln-Pdots barcodes. Furthermore, the results from barcoding PBMCs samples *via* CD45 specific cellular targeting indicated that the ratiometric Ln-Pdots barcodes could facilitate mass cytometry in high-throughput and multiplexed analysis, especially with precious human samples.

INTRODUCTION

Barcoding has been used for the pooled sample analysis to improve the information throughput and multiplexing ability of the assays.^{1,2} In barcoding assay, a specific identifier, such as fluorophore combinations or mass combinations, is used to uniquely label each sample.^{3–5} Then, all the barcoded samples are pooled together to conduct the subsequent specific staining and analysis. Finally, the information about the individual samples is recovered by the unique barcodes. With a proper barcoding assay, the sample-to-sample variability, bias of the analytical methods, analysis time, and cost can be significantly improved.^{5,6}

These advantages motivated the development of fluorescent-cell barcoding techniques in flow cytometry. A number of fluorescent materials, including organic fluorochromes,⁷

*Corresponding author: chiu@chem.washington.edu.

quantum dots,⁸ and semiconducting polymer dots,⁹ were used to develop different types of fluorescent barcodes. However, flow cytometry has its intrinsic limitations when multiplex detection is needed.¹⁰ Spectral overlap hinders the application of flow cytometry in multiplex analysis. Mass cytometry, a recently developed technique to improve the multiplex analysis capability, employs metal isotope-tags to label cells for single-cell cytometry.^{11,12} The metal isotopes on cells are measured by a time-of-flight mass cytometer instrument (CyTOF).¹³ The number of channels of isotopes that are readily measured in CyTOF is much higher than the number that can be used in most flow cytometers.¹⁴ Furthermore, due to the excellent separation of the mass signals, over 100 isotopes, whose atomic mass unit ranges between 75 and 209, can be potentially used in mass cytometry for multiplex detection without spectral-overlap issues.^{5,15} For instance, Frei et al. developed PLAYR (proximity ligation assay for RNA) coupled with mass cytometry for multiplex detection of more than 40 different mRNA and proteins.¹³ Using mass cytometry, simultaneous detection of up to 50 proteins and their post-modifications in single cells as well as multiplex imaging of more than 32 markers in tissues have been demonstrated.^{16–18}

However, mass cytometry has several limitations, especially when a large number of samples must be measured. First, the cell transmission rate of mass cytometry is only about 30%, which requires a large number of cells per run.¹⁴ Second, throughput of mass cytometry is lower than flow cytometry, on the order of 1000 cells per second rather than tens of thousands of cells per second.¹⁴ The slower analysis speed not only costs time, but also can result in un-predicted variation in signal intensity and can cause bias between different samples.¹⁹ Finally, the cost of metal isotope tags is expensive when a large number of samples are analysed.^{14,20}

Barcoding strategy is an appealing solution to resolve these limitations of mass cytometry.^{21–23} After coding each sample, all the samples can be pooled together into a single tube to be labeled and run in CyTOF. In this case, the barcoding method not only eliminates the sample-to-sample variation and bias of the instrument, but also reduces the consumption of metal-isotope tags and analysis time.^{22–24} For instance, Bodenmiller et al. developed a mass-tag cellular barcoding strategy using maleimido-monoamide-DOTA (m-DOTA), which was used to barcode an entire 96-well plate with seven different metal isotopes-tags.⁵ Recently, Mei et al. described a barcoding approach to identify 20 samples with unique combinations of three different CD45 antibody tags and six metal isotopes-tags.²¹ Newell et al. described a method to prepare 120 codes with 10 different metal isotopes, which they applied for the analysis of antigen-specific T cells and for T-cell epitope mapping.^{15,25} McCarthy et al. also developed a rapid monoisotopic cisplatin based barcoding technique without requiring cell permeabilization to perform multiplex cell analysis in mass cytometry.²⁶ Based on the Pd barcoding strategy, Fluidigm Corporation developed a commercially available Cell-ID™ 20-Plex PD Barcoding Kit, which can be used for coding 20 samples with 6 Pd isotopes. However, these barcoding strategies have some inherent limitations. For example, the barcode signal intensities from these tags can be different if the labeling efficiency or barcodes' concentrations vary in different samples, which can cause false negatives and thus compromise the individual sample recovery rate. Using the ratiometric barcoding strategy described here, we can generate $\sim n^{(m-1)}$ codes with only m isotopes and n intensity levels. This ratiometric approach requires the use of only a few detection channels, thus leaving

more channels for the actual single-cell multiplexed measurements. In addition, this ratiometric barcoding strategy can eliminate potential bias caused by different concentrations of the labeling reagents/barcodes or by different labeling efficiencies between samples.

Recently, we developed a series of lanthanide-coordinated semiconducting polymer dots (Ln-Pdots) for use in dual flow cytometry and mass cytometry applications.²⁷ Our study showed that the Ln-Pdots contained more lanthanides than the commercial mass probe and demonstrated higher signal intensity in mass cytometry compared with the commercial probes. Here, we report the development of a novel ratiometric barcoding strategy for use in mass cytometry using the Ln-Pdots to improve the signal intensity and to generate more barcodes with less occupied isotopes channels.

EXPERIMENTS AND MATERIALS

Preparation of Barcoding Ln-Pdot.

The preparation of the barcoding Ln-Pdots was similar as described in our previous reports with minor modification.^{27,28} Briefly, the carboxyl-functionalized PFBT (synthesized as indicated in our previous report²⁹) was used to coordinate with a mixture of lanthanide ions, including Tb, Ho, and Tm (Sigma-Aldrich, St. Louis, MO) at different ratios in THF for 1 hour. With the addition of PS-PEG-COOH (Polymer Source Inc, Quebec, Canada), the THF solution was quickly injected into water with ultrasonication to form the Ln-Pdots. After removing THF by nitrogen stripping, the Ln-Pdots solution was filtered through a 0.45- μ m cellulose membrane filter (VWR, Radnor, PA). The bioconjugation with streptavidin was performed according to the reported method in our previous works.^{30,31}

Characterization of Ln-Pdots.

Dynamic light scattering (DLS, Malvern Zetasizer Nano ZS) and transmission electron microscopy (TEM, FEI Tecnai F20, 200 kV) were used to analyze the size distribution and morphology of the barcoding Ln-Pdots. We also measured the absorption spectrum by a DU720 scanning spectrophotometer and fluorescence spectrum using Fluorolog-3 (HORIBA Jobin Yvon Inc, Edison, NJ). ICP-MS was used to quantify the lanthanide contents in Ln-Pdots.

Cell Labeling through Endocytosis.

The labeling of the Ln-Pdots were firstly carried by non-specific uptake by cells. MCF-7 cells were seeded in a 6-well plate in EMEM media at 37 °C for 24 h. After washing with PBS, the MCF-7 cells were then incubated with 10 ppm (~ 1.0 nM) Ln-Pdots for another 12 hours in EMEM media (37 °C with 5% CO₂). The excess Ln-Pdots were washed away using cell culture medium at least three times. The Ln-Pdots labeled cells were harvested by treating with 0.5 mL of trypsin-EDTA solution at 37 °C for 10 min. After centrifugation at 2500 rpm for 6 min, the cells were collected and washed with labeling buffer (1× PBS, 2-mM EDTA, 1% BSA) twice, followed by fixation using 0.5-mL fixing buffer (1× PBS, 2-mM EDTA, 3.6 % paraformaldehyde) for at least 60 min. The Ln-Pdots labeled cells were then ready for the following cellular ID intercalator staining and mass cytometric analysis.

Cell Labeling through Specific Antibody-Antigen Binding.

Mobilized human peripheral blood mononuclear cells (PBMCs) were obtained from AllCell (Alameda, CA). After thawing and washing with RPMI-1640 (ATCC, Manassas, VA) with 10% FBS and 1% penicillin-streptomycin, PBMCs were incubated with biotinylated anti-CD45⁺ antibody (Biolegend, San Diego, CA) in labeling buffer at room temperature for 30 min. Then, the excess biotinylated anti-CD45⁺ antibody was removed by washing the PBMCs twice with labeling buffer, followed by incubation with 200 μ L, 2 ppm (~0.2 nM) of Ln-Pdots-SA barcodes for another 30 min at room temperature. In order to label the PBMCs with the commercial available mass tags, anti-CD3-Er¹⁷⁰, anti-CD20-Yb¹⁷¹, anti-CD3-Nd¹⁵⁰, and anti-CD20-Sm¹⁴⁷ (0.5 mg/mL, from Fluidigm, CA) were used to stain the CD3⁺ T cells and CD20⁺ B cells, respectively, in two different samples. Finally, the PBMCs were fixed using 0.5-mL fixing buffer (1 $\times$ PBS, 2-mM EDTA, 3.6 % paraformaldehyde) for at least 60 min, after which the cells were ready for cell ID intercalator staining and mass cytometric analysis.

Mass Cytometry.

Mass cytometric analysis was performed on a partially upgraded CyTOF 1.0 mass cytometer (Fluidigm, CA) at Benaroya Research Institute. Before any measurements, the machine was tuned. CyTOF Instrument Control Software, version 6.1 (Fluidigm) (Finck 2013 Cytometry A) was used and all the events were normalized. For the cell analysis, all the cell samples after the labeling were firstly incubated with Rh or Ir DNA intercalator (Fluidigm, CA) for 15 min. Labeling buffer and water were used to wash the extra intercalator and the cells were finally dispersed into 500 μ L water right before injection into mass cytometer for analysis.

RESULTS AND DISCUSSION

Preparation of Ln-Pdots-based Mass Cytometric Barcodes.

As reported in our previous work,²⁷ lanthanides ions could be easily incorporated into Pdots with high payloads. To construct the barcodes with this Ln-Pdots, different types of lanthanides ions, present at a certain pre-defined ratio, are used to coordinate with the polymer for incorporation into the Pdot. As a proof of concept, we used three lanthanides, Tb, Ho, and Tm, to prepare the barcoding Ln-Pdots (Scheme 1). Similar to other Pdots in our previous works, the barcoding Ln-Pdots could be conjugated to biomolecules through the EDC reaction *via* carboxyl groups on the Pdot surface.^{30,32,33} In this ratiometric barcoding method, we used Tm as an internal reference, and the coding signals were based on the intensity ratios of Tb/Tm and Ho/Tm. Thus, there were $m-1$ “colors” available for barcoding, where m was the number of metal isotopes used. Theoretically, there are $\sim n^{(m-1)}$ codes when n intensity levels are used. In this work, $(m-1)$ was 2 when the internal reference of Tm was subtracted, and 4 intensity levels, including 0.1, 1, 5, and 10 were used to generate 16 codes which we designated as Pdot-(Tb/Tm, Ho/Tm) (Scheme 1).

With this ratiometric barcoding strategy, there are several advantages for identifying samples in mass cytometry. First, the high loading efficiency of Ln in Pdots ensures high signal intensity in the measurements by CyTOF. According to our previous report, the number of lanthanides per Pdot was on the order of 1000–2000, depending on the exact size of the

Pdots,²⁷ which is much higher than the reported m-DOTA based mass-tag cellular barcodes (MCB).⁵ Second, in these ratiometric barcodes based on different intensity levels, only three metal isotopes channels were used to obtain 16 barcodes, which is half that of the reported palladium-based mass tag barcoding technique for achieving 20 codes.²¹ Third, by using Tm intensity as an internal reference, the intensity ratios of Tb/Tm and Ho/Tm will be independent of the concentration of the Pdots on a single cell or potential errors caused by measurement variations. Therefore, this ratiometric strategy will eliminate the bias generated by the cell labeling efficiency and the CyTOF instrument. Finally, the Pdots-based barcodes can be easily taken up by cells through endocytosis, thus simplifies cellular tagging. However, if specific labeling is necessary, the Ln-Pdots can also be readily modified with antibodies for cell labeling for mass cytometry. As a result, we tested the feasibility of both of these two labeling strategies in barcoding samples: nonspecific uptake and specific cellular labeling.

Characterization of the Ln-Pdots-based Barcodes.

By employing similar synthetic strategy as in our previous work, we prepared the Ln-Pdots with 16 different barcodes.²⁷ Figure 1 shows a representative characterization of these 16 Ln-Pdots (Pdots-(5,5)). The Ln-Pdots were around 31.2 ± 6.8 nm in diameter, determined by analyzing more than 100 Pdots in the TEM images. (Figure 1A-B). This size of Ln-Pdots not only provided sufficient Ln loading in the codes, but also ensured the efficient uptake by cells via endocytosis or specific cell labeling via cell-surface biomarkers. Furthermore, we found that the photophysical properties, such as the absorption spectrum (Figure 1C) and fluorescence spectrum (Figure 1D), of Pdots-(5,5)) showed the same profile as that of the single lanthanide coordinated Pdots reported in our previous work.²⁷

Before any cell labeling experiments, we purified Pdots using size exclusion chromatography and confirmed there weren't any free lanthanides left in solution, either from the initial Pdot preparation or from potential subsequent leakage of the lanthanides from the Pdots.²⁷ As the Ln-Pdots barcodes were prepared for labeling cell samples by either endocytosis or specific targeting, their cytotoxicity was also investigated. As shown in Figure 1E, two barcodes, including Pdots-(0.1,0.1) and Pdots-(5,5) were incubated with MCF-7 cells for 24 hours, and the MTT assay were used to test the cell viability. The Ln-Pdots barcodes showed good biocompatibility at the concentration of 50 ppm (~5.0 nM), which is a concentration much higher than that was used to label the cells by both endocytosis (10 ppm, ~1.0 nM) and specific labeling (2 ppm, ~0.2 nM). The results indicate that the Ln-Pdots barcodes will not induce cytotoxicity to the coding samples.

After constructing the 16 barcodes using different combinations of the three lanthanides ions at different concentrations, we tested the resulting Ln-Pdots barcodes with ICP-MS. As shown in Figure 1F, by measuring the average mass intensity ratios of Tb/Tm and Ho/Tm of these 16 Ln-Pdots barcodes obtained from ICP-MS, we could clearly distinguish these barcodes from each other. Therefore, these 16 ratiometric Ln-Pdots barcodes could be used to label and identify cell samples from the mixture.

Cell Labeling with Ln-Pdots-based Barcodes by Endocytosis.

To test the feasibility of the cell labeling of barcodes through endocytosis, we incubated the MCF-7 breast cancer cells with Pdots-(5,5) overnight at a concentration of about 10 ppm (~1.0 nM). The cells were then harvested from the culture plates using trypsin digestion and fixed with 4% paraformaldehyde. Before the analysis with CyTOF, all the cells were stained with Rh-103 cell intercalator ID to identify cell events in mass cytometry. As shown in Figure 2A-C, the average mass intensities of the labeled cells were about 2300, 2350, and 500 in the Tb¹⁵⁹, Ho¹⁶⁵ and Tm¹⁶⁹ channels, respectively. Therefore, the ratios of Tb/Tm and Ho/Tm were calculated to be 4.6 and 4.7, respectively, which were very close to the value we designated for this Pdots-(5,5) barcode. Meanwhile, the strong mass signal intensities demonstrated the effective endocytosis of Pdots in cancer cells with overnight incubation.

To demonstrate the barcoding ability of the Ln-Pdots barcodes, we used the 16 barcodes to label and identify MCF-7 cells through the endocytosis uptake. Sixteen batches of MCF-7 cells were labeled with the Ln-Pdots barcodes separately *via* endocytosis and then analyzed with mass cytometry individually. As shown in Figure 2D, the cells were clearly divided into 16 individual population sets and could be distinguished from each other according to their coordinates in the Tb/Tm-Ho/Tm plot. Furthermore, the cells labeled with barcodes were mixed and analyzed by CyTOF; the results (Figure 2E-F) show both adjacent and distant barcodes could be discriminated from each other in the mixed samples, which validates the concept behind the ratiometric barcoding approach. Although the Ln-Pdots have a relative large standard deviation in size, which translates into variability in the number of Ln atoms loaded into each Pdot, the ratiometric strategy is insensitive to this variability because it is the ratio of intensities, rather than the absolute intensities, which are being measured. Therefore, the distribution in Pdot size did not negatively affect the barcoding performance or the final assay. The results demonstrate that the ratiometric mass encoded Ln-Pdots were able to label and identify cell samples by their unique barcodes through endocytosis.

Barcoding PBMCs with Ratiometric Ln-Pdots in Mass Cytometry

As mass cytometry is often used in immunology studies as a multiplex detection technology, we also tested whether our developed Ln-Pdots barcodes could be used to code the human peripheral blood mononuclear cells (PBMCs) samples through specific cell labeling by CD45 on the cell surfaces. In the developed 16 Ln-Pdots barcodes, we modified the Pdots-(0.1,0.1) and Pdots-(5,5) with streptavidin (SA) through EDC reaction as described in our previous works.²⁷ Then, as shown in Figure 3A, the PBMC-sample 1 was incubated with anti-CD45-biotin and then coded with Pdots-(0.1,0.1)-SA; PBMCs-sample 2 was coded with Pdots-(5,5)-SA under the same condition but in a different tube. Because these two PBMCs samples basically were identical to each other, we stained each sample with different metal-tagged antibodies to test our barcoding strategy. In this case, the PBMCs-sample 1-Pdots-(0.1,0.1) was stained with anti-CD3-Er¹⁷⁰ and anti-CD20-Yb¹⁷¹ in one tube, and the PBMCs-sample 2-Pdots6 (5,5) was stained with anti-CD3-Nd¹⁵⁰ and anti-CD20-Sm¹⁴⁷ in another tube. Then, the samples from these two different tubes were mixed together into a single tube for Cell ID intercalator staining and mass cytometric analysis.

According to this design, if the barcoding strategy worked, sample 1 recovered from Pdts-(0.1,0.1) barcode would only show CD3 staining in Er¹⁷⁰ channel and CD20 staining in Yb¹⁷¹ channel, but the channels for Nd¹⁵⁰ and Sm¹⁴⁷ would be negative. In contrast, sample 2 recovered from Pdts-(5,5) barcode would only have signals in the channels for Nd¹⁵⁰ and Sm¹⁴⁷, but not in the channels of Er¹⁷⁰ and Yb¹⁷¹. As shown in Figure 3B, two clearly distinguished individual population sets were observed in the Tb/Tm-Ho/Tm plot and the coordinates were very close to (0.1,0.1) and (5,5), indicating the successful coding and recovery of the PBMCs samples using CD45 as target for specific labeling. Furthermore, the analyses of the CD3 and CD20 staining in both cell populations were perfectly matched with the experimental design: sample 1 from Pdts-(0.1,0.1) only showed CD3 staining in Er¹⁷⁰ channel and CD20 staining in Yb¹⁷¹ channel; no signals were observed in Nd¹⁵⁰ and Sm¹⁴⁷ channels. Similarly, sample 2 also showed the anticipated results. These demonstrations show ratiometric Ln-Pdts barcodes worked well in the barcoding of PBMCs samples for high-throughput analysis.

CONCLUSIONS

In summary, we developed a ratiometric barcoding strategy for mass cytometry analysis using lanthanides-coordinated Pdts. By employing three metal isotopes and four ratio intensity levels, 16 barcodes were generated to label and identify cell samples in mass cytometry. The ratiometric Ln-Pdts barcodes not only provided high mass signal intensities, but also eliminated any potential bias caused by different concentrations of barcodes used to label cells or by run-to-run differences in cell labeling efficiency and CyTOF measurements. Unlike other barcoding strategies, it is difficult to discriminate cell doublets from singlets using Ln-Pdot barcodes alone, and thus the Cell-ID intercalator-Ir or Rh should be used for cell doublet discrimination. The ability to distinguish clearly the 16 sets of labeled MCF-7 cells with mass cytometry demonstrated the excellent resolving power of the ratiometric Ln-Pdts barcodes. Furthermore, the results from barcoding PBMCs samples *via* CD45 specific cellular targeting indicated that the ratiometric Ln-Pdts barcodes could facilitate mass cytometry in high-throughput and multiplexed analysis, especially with precious human samples.

ACKNOWLEDGMENT

We are grateful to the NIH (R01MH115767 and DK097653), and the National Natural Science Foundation of China (31741049) for the support of this work.

REFERENCES

- (1). Fulton RJ; McDade RL; Smith PL; Kienker LJ; Kettman JR, Jr. Clin. Chem 1997, 43, 1749–1756. [PubMed: 9299971]
- (2). Krutzik PO; Nolan GP Nat. Methods 2006, 3, 361–368. [PubMed: 16628206]
- (3). Han M; Gao X; Su JZ; Nie S Nat. Biotechnol 2001, 19, 631–635. [PubMed: 11433273]
- (4). Sathe TR; Agrawal A; Nie S Anal. Chem 2006, 78, 5627–5632. [PubMed: 16906704]
- (5). Bodenmiller B; Zunder ER; Finck R; Chen TJ; Savig ES; Bruggner RV; Simonds EF; Bendall SC; Sachs K; Krutzik PO; Nolan GP Nat. Biotechnol 2012, 30, 858–867. [PubMed: 22902532]
- (6). Harvey CJ; Wucherpennig KW Nat. Biotechnol 2013, 31, 609–610. [PubMed: 23839145]
- (7). Wang L; Tan W Nano. Lett 2006, 6, 84–88. [PubMed: 16402792]

- (8). Wang G; Leng Y; Dou H; Wang L; Li W; Wang X; Sun K; Shen L; Yuan X; Li J; Sun K; Han J; Xiao H; Li Y ACS nano 2013, 7, 471–481. [PubMed: 23205725]
- (9). Kuo CT; Peng HS; Rong Y; Yu J; Sun W; Fujimoto B; Chiu DT Anal. Chem 2017, 89, 6232–6238. [PubMed: 28499337]
- (10). Perfetto SP; Chattopadhyay PK; Roederer M Nat. Rev. Immunol 2004, 4, 648–655. [PubMed: 15286731]
- (11). Lou X; Zhang G; Herrera I; Kinach R; Ornatsky O; Baranov V; Nitz M; Winnik MA Angew. Chem 2007, 46, 6111–6114. [PubMed: 17533637]
- (12). Spitzer MH; Nolan GP Cell 2016, 165, 780–791. [PubMed: 27153492]
- (13). Frei AP; Bava FA; Zunder ER; Hsieh EW; Chen SY; Nolan GP; Gherardini PF Nat. Methods 2016, 13, 269–275. [PubMed: 26808670]
- (14). Bendall SC; Nolan GP; Roederer M; Chattopadhyay PK Trends. Immunol 2012, 33, 323–332. [PubMed: 22476049]
- (15). Newell EW; Sigal N; Nair N; Kidd BA; Greenberg HB; Davis MM Nat. Biotechnol 2013, 31, 623–629. [PubMed: 23748502]
- (16). Schulz D; Zanutelli VRT; Fischer JR; Schapiro D; Engler S; Lun XK; Jackson HW; Bodenmiller B Cell. Syst 2018, 6, 25–36.e5. [PubMed: 29289569]
- (17). Schapiro D; Jackson HW; Raghuraman S; Fischer JR; Zanutelli VRT; Schulz D; Giesen C; Catena R; Varga Z; Bodenmiller B Nat. Methods 2017, 14, 873–876. [PubMed: 28783155]
- (18). Giesen C; Wang HA; Schapiro D; Zivanovic N; Jacobs A; Hattendorf B; Schuffler PJ; Grolimund D; Buhmann JM; Brandt S; Varga Z; Wild PJ; Gunther D; Bodenmiller B Nat. Methods 2014, 11, 417–422. [PubMed: 24584193]
- (19). Zivanovic N; Jacobs A; Bodenmiller B Curr. Top. Microbiol. Immunol 2014, 377, 95–109. [PubMed: 23918170]
- (20). Di Palma S; Bodenmiller B Curr. Opin. Biotechnol 2015, 31, 122–129. [PubMed: 25123841]
- (21). Mei HE; Leipold MD; Schulz AR; Chester C; Maecker HT J. Immunol 2015, 194, 2022–2031. [PubMed: 25609839]
- (22). Lai L; Ong R; Li J; Albani S Cytometry. A 2015, 87, 369–374. [PubMed: 25645694]
- (23). Zunder ER; Finck R; Behbehani GK; Amir el AD; Krishnaswamy S; Gonzalez VD; Lorang CG; Bjornson Z; Spitzer MH; Bodenmiller B; Fantl WJ; Pe'er D; Nolan GP Nat. Protoc 2015, 10, 316–333. [PubMed: 25612231]
- (24). Behbehani GK; Thom C; Zunder ER; Finck R; Gaudilliere B; Fragiadakis GK; Fantl WJ; Nolan GP Cytometry. A 2014, 85, 1011–1019. [PubMed: 25274027]
- (25). Newell EW; Davis MM Nat. Biotechnol 2014, 32, 149–157. [PubMed: 24441473]
- (26). McCarthy RL; Mak DH; Burks JK; Barton MC Sci. Rep 2017, 7, 3779. [PubMed: 28630464]
- (27). Wu X; DeGottardi Q; Wu IC; Yu J; Wu L; Ye F; Kuo CT; Kwok WW; Chiu DT Angew. Chem 2017, 56, 14908–14912. [PubMed: 28941061]
- (28). Wu X; Wu L; Wu IC; Chiu DT RSC Adv 2016, 6, 103618–103621. [PubMed: 28529727]
- (29). Zhang X; Yu J; Wu C; Jin Y; Rong Y; Ye F; Chiu DT ACS Nano 2012, 6, 5429–5439. [PubMed: 22607220]
- (30). Wu IC; Yu J; Ye F; Rong Y; Gallina ME; Fujimoto BS; Zhang Y; Chan YH; Sun W; Zhou XH; Wu C; Chiu DT J. Am. Chem. Soc 2015, 137, 173–178. [PubMed: 25494172]
- (31). Sun W; Yu J; Deng R; Rong Y; Fujimoto B; Wu C; Zhang H; Chiu DT Angew. Chem 2013, 52, 11294–11297. [PubMed: 24030955]
- (32). Wu C; Chiu DT Angew. Chem 2013, 52, 3086–3109. [PubMed: 23307291]
- (33). Yu J; Rong Y; Kuo CT; Zhou XH; Chiu DT Anal. Chem 2017, 89, 42–56. [PubMed: 28105818]

Figure 1.

(A) Histogram showing the size distribution of Pd dots-(5,5), determined by analyzing >100 particles in TEM images (B). The mean diameter was 31.2 ± 6.8 nm. (B) TEM images of Pd dots-(5,5). The absorption (C) and fluorescence (D) spectra of Pd dots-(5,5). (E) Cell viability of MCF-7 cells after incubation with different concentrations of barcoding Ln-Pdots for 24 hours. (F) The barcodes generated by Ln-Pdots with three lanthanide isotopes and four different intensity ratios; the isotopic intensity ratios were measured with ICP-MS.

Figure 2.

Distribution of 16 sets of MCF-7 cells labeled with different Ln-Pdots barcodes *via* endocytosis and then analyzed by CyTOF. (A-C) Mass cytometry measurements of the mass intensity distributions of MCF-7 cells labeled with Pdot-(5, 5): (A) Tb¹⁵⁹, (B) Ho¹⁶⁵, and (C) Tm¹⁶⁹ channels. (D) Discrimination of 16 sets of MCF-7 cells individually using mass cytometry after coding with 16 Pdot barcodes as described in Figure 1F. The ratios of Tb/Tm and Ho/Tm were plotted for each cell set. (E) Mass cytometric analysis of the mixed samples coded with Pdot-(1, 5), Pdot-(5, 5), Pdot-(10, 5), Pdot-(5, 1), and Pdot-(5, 10). (F) Mass cytometric analysis of the mixed samples coded with Pdot-(0.1, 10), Pdot-(0.1, 5), Pdot-(0.1, 1), Pdot-(0.1, 0.1), Pdot-(1, 0.1), Pdot-(5, 0.1), and Pdot-(10, 0.1).

Figure 3.

(A) Schematic diagram depicting the barcoding of different samples and labeled with different commercially available mass tags. (B) De-barcoding by mass cytometric analysis the mixed samples in (A).

Scheme 1.

Schematic showing the ratiometric barcodes based on Ln-Pdots for multiplex mass cytometric analysis. The Ln-Pdot barcodes can label cells either through endocytosis or specific antigen-antibody binding.