Encoding through the host–guest structure: construction of multiplexed fluorescent beads†

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A new encoding strategy based on the host–guest structure was developed, where encoding is achieved by combinational use of a set of guest particles with different fluorescence intensity levels at various ratios. This system was proven to be well-defined and highly controllable.

Since the advent of suspension arrays, encoding beads have been widely used in the fields of clinical diagnosis, food control, biomolecule screening, and combinatorial chemistry. Encoding beads refer to a set of particles with mutually distinguishable properties, which can be decoded by readout tools such as fluorescence microscopy and flow cytometry. As carriers for capturing and identifying target analytes, encoding beads play a central role in multiplexed assay platforms. Compared with other encoding strategies such as chemical encoding, graphical encoding, and electronic encoding, optical encoding is most commonly used owing to well-developed optical devices and high-throughput decoding processes. Optical encoding is mainly implemented by labelling beads with organic dyes, lanthanide complexes, and quantum dots. With the advantage of cost-effectiveness, high detection sensitivity, and compatibility with a variety of biological chemistries, dye-doped beads have been commercialized and used broadly.

Up to now, there have been three common ways to prepare dye-doped beads: the swelling–shrinking method, copolymerization or co-condensation of dye-conjugated precursors, and layer-by-layer assembly of fluorescently labelled polyelectrolytes. Although high multiplexing capability has been reported, there remain several issues that are difficult to address using these methods, including the reproducibility of production, the compatibility of multiple dyes, the occurrence of fluorescence resonance energy transfer (FRET) when dye molecules are in close proximity, and complicated encoding procedures. All the above-mentioned issues may be attributed to the fact that multiple coding elements are incorporated in a single matrix, which has a limited capacity to accommodate large numbers of dyes in a highly controllable manner.

Herein, we report a new type of optically encoded bead based on the host–guest structure, consisting of two building blocks. Large magnetic beads (5.8 μm) were used as host particles to facilitate separation and automation. Small dye-doped silica nanoparticles (200 nm) were used as guest particles with the advantage of easy preparation and convenient surface modification. As shown in Scheme 1, the host–guest structure was formed by conjugating a great number of carboxylated guest particles onto the surface of amino-functionalized host particles through a carbodiimide-assisted coupling reaction, and encoding was achieved by a combinational use of different kinds of guest particles doped with different fluorescence intensity levels of organic dyes. Different from other encoding systems, the most prominent feature of the present host–guest system was that the...
encoding could be done in separate building blocks before they were assembled, thus circumventing the problem of dye compatibility and the FRET effect in conventional systems. As the apparent fluorescence intensity of the encoded beads depended on both the number of guest particles and their fluorescence intensities, the encoding process could be carried out conveniently by modulating these two parameters. For proof of concept, three guest particles with different intensity levels (referred to as strong fluorescent (SF), weak fluorescent (WF), and non-fluorescent (Blank), respectively) were prepared in this work to generate a total of nine distinguishable encoded beads (Table S2, ESI†).

Successful doping of dye molecules (fluorescein isothiocyanate, FITC) into the guest particles was revealed by fluorescence spectrophotometry (Fig. 1). Strong peaks at around 490 nm (excitation) and 520 nm (emission) for fluorescent guest particles (SF/WF) were observed, matching well with the spectrum of free FITC molecules. Conversely, no apparent excitation/emission peak was observed for blank guest particles (Fig. 1a). Strong linear correlations of the fluorescence intensity and the concentration of guest particles were observed in Fig. 1b, with the slopes of the linear correlations increasing with the relative fluorescence intensity (RFI) of guest particles. The RFI of fluorescent guest particles was approximately 31 times for SF and 5 times for WF when compared to the RFI of blank guest particles. The photo of different guest particle dispersions is presented in the inset (Fig. 1b), clearly showing the difference in the fluorescence intensities of these particles.

Fig. 2 shows TEM and SEM images of the nanoscale guest particles (Fig. 2a) and micron-sized host particles (Fig. 2b) and the encoded beads after their assembly (Fig. 2c and d). Both guest and host particles exhibited uniform morphologies and even size distributions. The average diameters of three guest particles (SF/WF/Blank) were all around 190 nm. After assembly, the guest–host encoded beads also had an excellent uniform morphology, and no aggregation of encoded beads was observed, which was crucial to achieve reproducible detection results. Guest particles were compactly packed on the surface of host particles as a monolayer (Fig. 2d), with a surface coverage of about 0.6 (Fig. S1, ESI†). It should be noted that the variation of coverage in both the same batch and different batches of encoded beads was less than 10%, indicating good controllability and reproducibility of the assembly process.

Spatial distribution of dye molecules on the host–guest encoded beads was examined by confocal laser scanning microscopy. Fig. 3 showing the cross-sectional images of the encoded beads clearly illustrates the presence of a fluorescent ring around the surface of host particles, demonstrating effective anchoring of dye-doped guest particles onto the surface of host particles. As the proportion of SF guest particles decreased, the fluorescence intensity of encoded beads declined. In addition, the fluorescent ring became discontinuous as both fluorescent and non-fluorescent guest particles were evenly distributed on the surface of host particles, suggesting that the self-assembly of guest particles onto host particles was a stochastic process.

To determine the fluorescence properties of the optically encoded bead sets, we mixed together all nine particles with each kind of approximately equal amount and measured the mixture using flow cytometry (Fig. 4). Displayed in the form of a bivariate scatter plot (FSC signal reflecting the size of a single bead versus fluorescence intensity of the bead in the FL1 channel), the mixture of encoded beads was distinguished as nine separate clusters with sizes of different clusters being very comparable (Fig. 4b). Each cluster represented a different kind of encoded bead, corresponding to codes 1–9 in Fig. 4a. The proportion of each bead set in the mixture fell in the range of 8–11% (Table S2, ESI†). No significant overlap was observed in the histogram, demonstrating high bead identification accuracy (Fig. 4c). It was noteworthy that the coefficient of variation (CV) of fluorescence intensities for the same batch of encoded beads was mostly below 20%, attributable to the well-defined assembly process and high uniformity of building blocks, as revealed by the microscopic images in Fig. 2. To guarantee the reproducibility.
of the results, any physically adsorbed guest particles on the host particle surface, which were in the form of agglomerates, should be eliminated, and alkali washing solution was applied in this work to increase electrostatic repulsion between guest particles. In addition, the encoded beads exhibited good fluorescence stability. The fluorescence intensity showed an inter-day CV of 5% during the 7-day storage in water at 37 °C (Table S3, ESI†). Furthermore, the fluorescence properties of the beads were well maintained during bioconjugation and analyte detection, which was critical to guarantee the accuracy of assays.

Two factors were critical in the determination of encoding capacity: (i) the highest fluorescence intensity of encoded beads and (ii) the width of fluorescence distribution for a given batch of encoding beads. For the former, a high packing density of guest particles on host particles was essential, which was achieved by adopting a suitable ionic strength in the assembly process to effectively screen the electrostatic repulsion between guest particles.\textsuperscript{17} For the latter, the encoding strategy itself had the advantage of a narrow fluorescence distribution because both the synthesis of guest particles and the conjugation of host and guest particles were controllable and reproducible, as shown in the above results. In our preliminary work, nine distinguishable encoded beads have been successfully prepared to demonstrate the feasibility of the encoding strategy. The encoding capacity of the present system was similar to the results reported in the literature based on a single dye.\textsuperscript{10} However, inner spaces of host particles have not been utilized yet. Hence we envision that the number of codes would undergo an exponential increase in the future by introducing code elements in both the host particles and the host particles.

Several advantages were introduced by the present encoding strategy compared with previously reported systems: (i) for the swelling–shrinking method, multiple dyes were dissolved in the same organic solvent, which required good compatibility of different dyes. However, in our system, guest particles could be prepared under conditions that are optimal for each dye, thus removing restrictions on dye selection. (ii) Another issue in the conventional system was the change in dye spectra when multiple dyes were incorporated in the bulk matrix, which may be ascribed to FRET.\textsuperscript{14} This issue would be addressed by the present design because multiple dyes were localized in separate guest particles, thus avoiding mutual interference of dyes. (iii) Benefiting from the universality of the assembly process, the encoding process was simplified to a mere change in the ratios of different guest particles, providing that all guest particles had the same surface functional groups.

The feasibility of the encoded beads in biological assays was examined using a model detection system in which immunoglobulin G (IgG) labelled with allophycocyanin (APC) was detected. Good linearity was observed between the mean fluorescence intensity and IgG-APC concentration (0–7000 ng mL\textsuperscript{−1}), demonstrating that the encoded beads can be used as efficient carriers in biosensing (Fig. S2, ESI†). Furthermore, the encoded beads were endowed with excellent magnetic properties by the magnetic host particles, which facilitated separation and showed promise of automation (Fig. S3, ESI†).
In summary, we have successfully developed a new type of optically encoded bead suitable for high-throughput flow cytometry based on the host–guest structure, which in our view may have great potential in multiplexed assays. The power of this encoding strategy lies in an efficient combinational use of a few kinds of dye-doped nanoparticles, through which a large number of distinguishable encoded beads can be achieved. As building blocks, guest particles are prepared separately, which are size-controlled, photostable, reproducible, and have adjustable fluorescence properties. The assembly process is simple and highly controllable to generate narrowly distributed and reproducible fluorescence signals. Future efforts to extend the encoding capacity of the present system will be to take full advantage of the inner space of host particles and introduce multiple dyes into the host–guest structure.

This work is supported by UM-SJTU Collaboration on Biomedical Technologies (to J.F. and H.G., No. 12X120010007), 863 High Tech Program (2013AA032203, 2012AA020103), SJTU funding (YG2013MS29), Shmec Project (14ZZ023), and the US National Science Foundation (ECCS 1231826 and CBET 1263889 to J.F.). We also acknowledge Zhenyuan Qu for helpful discussion, and Ping Xu, Yuan Chen, Pingping Wang, Chang Liu, Tianzhu Xiong and Xiaohe Chen for their experimental help.

Notes and references