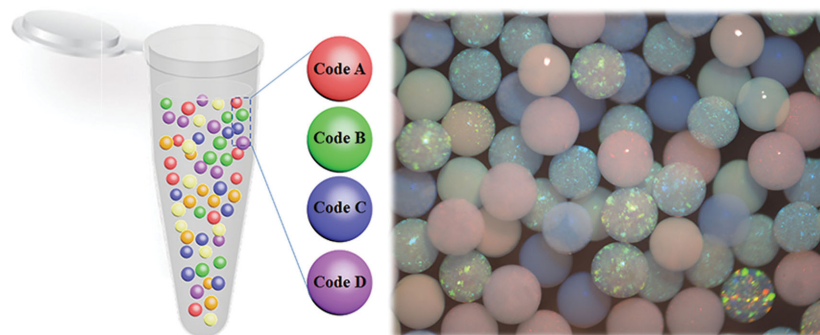


Microfluidic Synthesis of Barcode Particles for Multiplex Assays

Yuanjin Zhao,* Yao Cheng, Luoran Shang, Jie Wang, Zhuoying Xie, and Zhongze Gu*



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The increasing use of high-throughput assays in biomedical applications, including drug discovery and clinical diagnostics, demands effective strategies for multiplexing. One promising strategy is the use of barcode particles that encode information about their specific compositions and enable simple identification. Various encoding mechanisms, including spectroscopic, graphical, electronic, and physical encoding, have been proposed for the provision of sufficient identification codes for the barcode particles. These particles are synthesized in various ways. Microfluidics is an effective approach that has created exciting avenues of scientific research in barcode particle synthesis. The resultant particles have found important application in the detection of multiple biological species as they have properties of high flexibility, fast reaction times, less reagent consumption, and good repeatability. In this paper, research progress in the microfluidic synthesis of barcode particles for multiplex assays is discussed. After introducing the general developing strategies of the barcode particles, the focus is on studies of microfluidics, including their design, fabrication, and application in the generation of barcode particles. Applications of the achieved barcode particles in multiplex assays will be described and emphasized. The prospects for future development of these barcode particles are also presented.

1. Introduction

A multiplex assay is a type of laboratory procedure that simultaneously measures multiple analytes in a single assay. It has attracted considerable interest as it may meet the growing demand in clinical diagnosis, gene expression, drug discovery, and so on.^[1–4] In the past, when multiple analytes had to be analyzed from a single sample, the sample was divided into appropriate aliquots that were individually used for the analysis of a single target. Although this approach is widely used in clinical analysis, it does have several shortcomings, such as large volumes of sample consumed, low throughput, and difficulty in meeting the demand of detecting large numbers of analytes. Today, with the development of scientific instrumentation, the detection of multiple analytes in a single sample has become routine. These detections are usually based on molecular binding or recognition events. To distinguish different binding events in parallel, molecules should be encoded.^[5–8] The most common approach is to use a planar array, such as a nucleic acid and protein microarray, in which the probe molecules are immobilized on a substrate and encoded by the coordinate of their positions, as illustrated in **Figure 1a**.^[9,10] This technique has a major impact on the analysis of ultrahigh-density biomolecules. However, it has some disadvantages, such as a lack of flexibility, reaction speed, repeatability, etc.

In comparison, suspension arrays that use barcode particles as microcarriers for the probes' attachment and reactions (**Figure 1b**) became an attractive alternative as a multiplex assay. They can offer higher flexibility for detecting new analytes and show faster reaction kinetics in solution because of the radial diffusion of analytes or probes.^[5–8] Because of these advantages, the suspension array with fluorescent barcode particles has achieved great success in commercialization. Many other encoding strategies have been suggested for the development of barcode particles and as competition for the fluorescent particles. However, because of the lack of an effective synthesis method, the development process of the barcodes had become very slow.

Recently, microfluidics has created exciting avenues of scientific and engineering research in materials synthesis.^[11–15] It is a multidisciplinary subject involving physics, chemistry, biology, and engineering that deals with the precise control and manipulation of small quantities of fluids constrained in microchannels. Flow in microfluidic devices exhibits many attractive features, such as large specific interface areas, dominant viscous effects, precise control of the flow, and so on. Thus, microfluidics has been applied in various fields including biological research, drug discovery, optical systems development, chemical reactions, and materials synthesis.^[16–21] In particular, as microfluidics was employed for the synthesis of the barcode materials, a series of novel barcodes with distinct features have been created for multiplex assays, and thus the suspension array technology has developed considerably.

In this paper, we present studies dealing with the microfluidic synthesis of barcode particles for multiplex assays. After introducing the general development strategies of barcode particles, we focus on studies of microfluidics, including

their designs, fabrication, and applications in the generation of barcode particles. Examples of typical microfluidic barcode particles are presented. Emphasis is also given to a description of the applications of the achieved particles in multiplex assays. Finally, we present an outlook for the future development of the particle-based suspension arrays.

2. General Encoding Strategies of Barcodes

Barcodes are a part of our everyday life that we see on supermarket commodities. They have become the most popular data-entry method to track the ever-exploding amount of information in the macroscopic world in the past 20 years. In the meantime, this technology has also found important applications in tracking smaller particles for performing high-throughput multiplex assays. For this purpose, many encoding strategies from different areas of science have been proposed for the generation of the barcode microparticles.^[22–48] For example, typical electrically encoded particles have been achieved by enclosing radio frequency (RF) memory tags with dozens-bit identification (ID) codes within glass capsules.^[23–25] Graphically encoded particles with miniaturized supermarket one- and two-dimensional (1D and 2D) barcodes have been realized by a series of methods,^[26–33] such as sequential electrochemical deposition of metals on mesoporous aluminum templates (**Figure 2a**),^[27] fusing and drawing photoluminescent rare earth ions-doped glass blocks into ribbon particles (**Figure 2b,c**),^[29] etching a pattern of holes on an alumina ceramic plate (**Figure 2d**),^[30] and spatially selective photobleaching of stripes on fluorescent microspheres (**Figure 2e**).^[31] Biomolecular encoded microparticles have been demonstrated by using nucleotide sequences as the coding elements (**Figure 3**).^[34–37] However, these particles, as well as some graphically encoded particles, need to be marked with fluorescents during their decoding. Actually, pure fluorescent dyes have already been incorporated into polymer particles for the generation of barcodes, termed an “optical encoding scheme”.^[38,39] These optically encoded particles could have also been achieved by incorporating quantum dots (QDs) and other spectrum tags into the particles (**Figure 2f**)^[40–45] or by constructing the particles with photonic crystal (PhC) structures (**Figure 2g,h**).^[46–48]

A huge number of barcode particles with ID codes could be generated in theory based on these encoding strategies. This would render the barcode particles with a very high

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throughput capability for multiplex assays. However, in practice, the number of distinct barcode particles that can be achieved is limited for a number of reasons, such as the intrinsic defects of the encoding strategies and the lack of effective synthetic methods. Although the above encoding strategies were proposed and experimentally demonstrated nearly 10 years ago, only recently have they been subject to extensive experimental exploration. The reason for the delay is clear: it has been necessary first to develop a reproducible and controllable barcode generation approach. In this regard, ever since microfluidic technologies were employed in the generation of the particle materials, the study of the barcode particle assays has achieved great progress.

Droplet microfluidics and micro-flow lithography are the most studied microfluidic approaches for particle generation.^[49–58] These methods yield advanced particles with various materials, structures, shapes, and functions suitable for various purposes, and in particular, biological barcode applications. The micro-flow lithography gives greater freedom in shaping the particles to obtain desired features. By using appropriate photomasks, 1D and 2D shaping are achievable in a continuous, facile, and reproducible manner. Additionally, 3D-structured particles can also be generated by using a combination of interference and flow lithography.^[12] By contrast, droplet microfluidics generates and manipulates discrete droplets with precisely controlled volume through shearing between immiscible phases in microchannels. Multistage or multicore fluidic channels are also used to produce multicomponent or shell-type particles via an emulsification mechanism. More advanced than the conventional batch process of suspension polymerization, spraying, extrusion, and precipitation, microfluidic emulsification allows the continuous and stable generation of monodisperse barcode particles (with polydispersity below 2–3%) as well as the construction of complex structures.^[57] Therefore, in the following, we focus our attention on the new advances in this field.

3. Barcode Particles from Droplet Microfluidics

Because of its capability of generating monodisperse emulsions and executing precise control over and operations on suspended droplets inside the microchannels, droplet microfluidics has become a powerful tool for fabricating microparticles with desired properties.^[59] Single, Janus, and multiple emulsions generated in microfluidic devices can be composed of a variety of solutions including pre-gels, polymers, and functional nanoparticles. They are ideal microreactors or templates for synthesizing advanced barcode particles by further chemical or physical consolidation processes.

3.1. Fluorescent Particles from Single Emulsions

Synthesis of barcode particles by single-droplet microfluidics begins with the emulsification of a mixture solution of encoding elements and a monomer or a polymer in an immiscible liquid. Emulsification in microfluidic devices can occur



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via different mechanisms that depend on the geometry of the device, the macroscopic properties of the liquids, and the flow rates of the liquids. For the generation of single emulsion droplets, a series of microfluidic devices with the geometries of flow-focusing, T-junction, or co-flow were used. The droplet size can be controlled by varying the ratio of flow rates of the continuous-to-droplet phases, so that for a particular geometry of the microfluidic device and a particular combination of liquids with increasing flow rate ratio, the diameter of the droplets decreases. Several distinct types of emulsion droplets can be prepared using microfluidic emulsification, such as oil-in-water (O/W) and water-in-oil (W/O) emulsions. Based on these emulsion templates, two approaches have been developed to achieve the production of barcode particles: extraction/evaporation-induced solidification and suspension polymerization.

In the approach of extraction/evaporation, barcode elements, such as fluorescent dyes or pigments, are usually dispersed into organic polymer solvents, and then the mixture is emulsified to droplets using microfluidics.^[60,61] After evaporating the solvent of the droplets, it was accessible to obtain composite polymer particles containing the fluorescent elements. Despite having many commercial applications, fluorescent dyes have the problems of photobleaching and red-tailed emission, which could affect the encoded information of the polymer particles. As an alternative, fluorescent QDs have narrow Gaussian emission line shapes, resistance to photobleaching, high quantum yields, single-wavelength excitation, and a large number of codes by adopting different wavelength and intensity QDs. Thus, they are an ideal additive to emulsions for generating barcode particles.^[62–66]

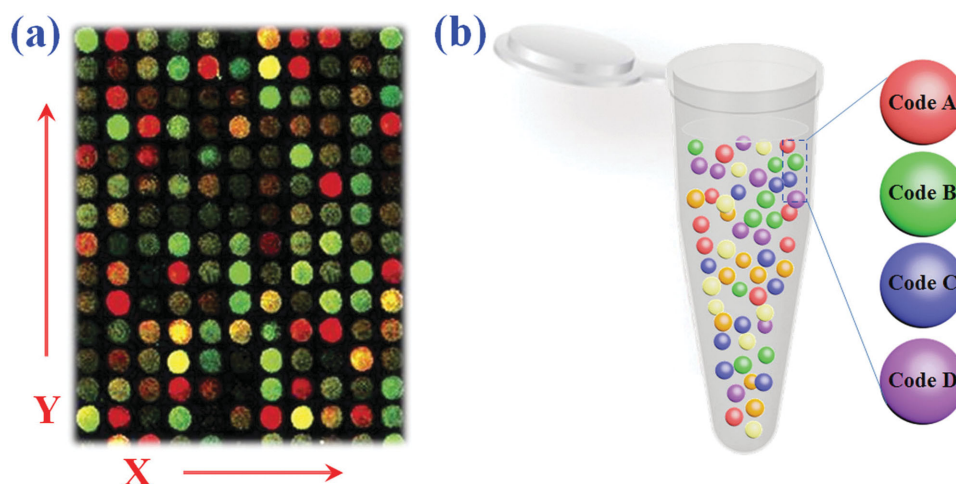


Figure 1. (a) A conventional microarray. (b) Schematic illustration of barcode particle-based suspension arrays.

Fournier-Bidoz et al. mixed polymer and ZnS-capped CdSe QDs into organic solution and emulsified the mixture through a microfluidic device and constructed a device by inserting a nozzle into a pressure-controllable vessel with variable input/output flows in real time, as shown schematically in **Figure 4**.^[67] The nozzle accepted inputs from the organic phase mixture and an aqueous focusing phase that intersected in the nozzle outlet. Control of the flow rates at both inputs resulted in periodic axisymmetric breakup of the QD/polymer-containing fluid stream caused by microfluidic instabilities. Chloroform was used to solvate the QDs and polymer; thus, the emulsion droplets could be casted into solidified, homogeneously dispersed QD barcode polymer particles when drying the chloroform. Poly(styrene-*co*-maleic anhydride) was used as the polymer for particle generation, because it was initially organically soluble and then transitioned into a charged hydrophilic surface after particle formation.

Thus, the resultant particles could prevent aggregation and permit covalent linkage to biorecognition molecules. It was also demonstrated that more than 100 unique barcodes with different ratios, fluorescence wavelengths, and intensity levels of the QDs/polymer particles could be mass produced by using the device in a batch-to-batch manner. This approach was studied by other groups who tried to simplify the microfluidic devices and to use different combinations of biocompatible polymers for the generation of the QD barcode particles.^[68–71]

Despite some success using traditional single-droplet microfluidic methodologies to prepare fluorescent QD-encoded particles, a single synthesis process only produces a single barcode with a constant concentration and ratio of different fluorescent QDs. Thus, every QD barcode requires individual preparation of a precursor solution containing the correct concentration and ratio of different

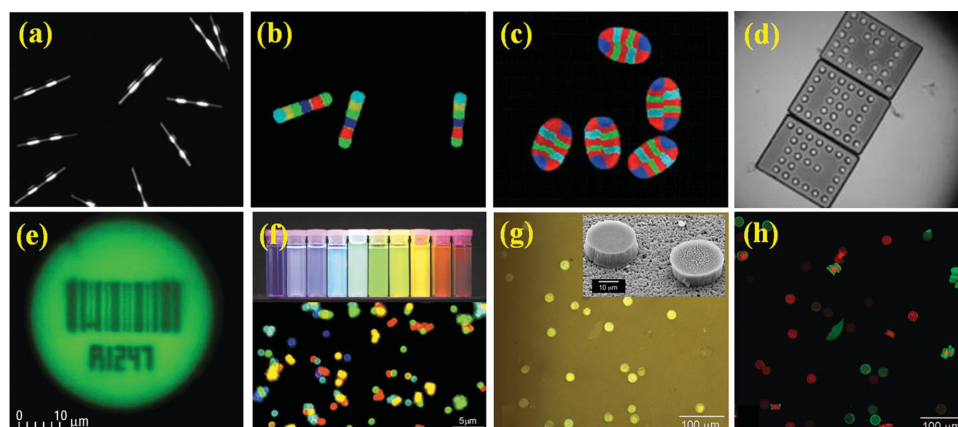


Figure 2. A selection of barcode particles: (a) striped metal nanorods, each of them consists of alternating stripes of gold and silver; (b,c) fluorescence images of rare-earth ions doped glass encoded microparticles; (d) polymer wafers encoded with a pattern of holes; (e) fluorescent microspheres with photobleaching stripe code; (f) microspheres encoded with different colors and ratios of quantum dots; (g,h) microscope images of porous silicon PhC particle ensembles showing results of fluorescence and reflectivity assays, insert is a SEM image of two porous silicon microparticles on a filter support. (a–c) Reproduced with permission.^[27,29] Copyright 2001 and 2003, The American Association for the Advancement of Science (AAAS). (d–f) Reproduced with permission.^[31,40] Copyright 2003 and 2001, Nature Publishing Group. (g,h) Reproduced with permission.^[48] Copyright 2009, The American Chemical Society (ACS).

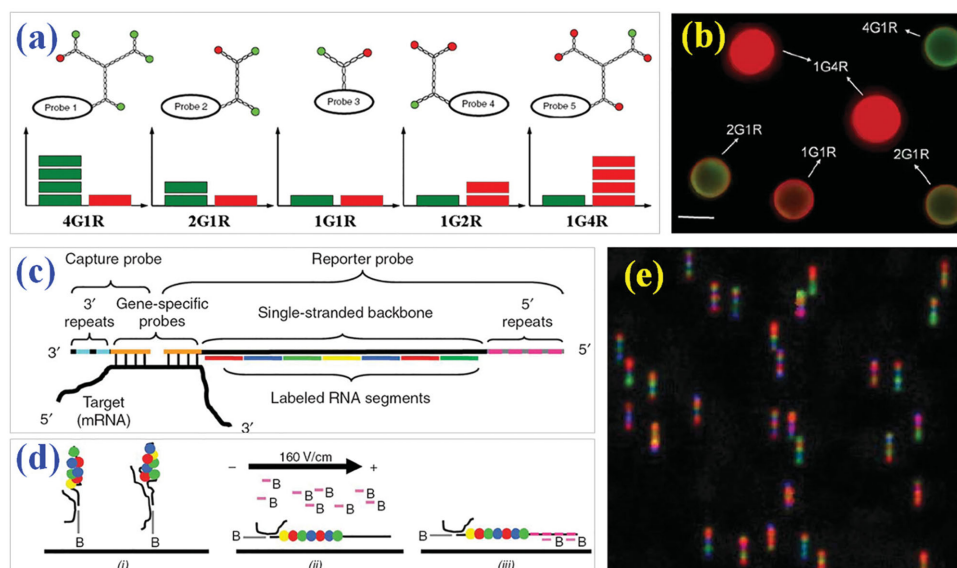


Figure 3. (a) Schematic illustration the DNA dendrimer fluorescent barcode labels by fluorescence intensity ratio; (b) DNA nanobarcode, DNA target and polystyrene microbeads form sandwich structure in multiplex detection, scale bar is 5 μ m; (c–e) Illustration of RNA nanostring encoded assays: (c) complex structures formed after hybridization of a target mRNA, its specific reporter and capture probes; (d) schematic representation of binding, electrophoresis, and immobilization: i) the purified complexes are attached to a streptavidin-coated slide via biotinylated capture probes, ii) voltage is applied to elongate and align the molecules, iii) the stretched reporters are immobilized; (e) the reporter barcodes are imaged with a fluorescence microscope equipped with high resolution and magnification lens. Reproduced with permission.^[34,35] Copyright 2005 and 2008, Nature Publishing Group.

fluorescent QDs. To simplify this procedure, Gerver et al. developed an automated microfluidic device with a staggered herringbone mixer channel for the generation of barcode particles.^[72] Different lanthanide nanophosphors of poly(ethylene glycol) diacrylate solutions flowed into their device and were mixed in the mixer channel. During this process, the relative flow rates from the different nanophosphor inputs determined the relative abundance of each nanophosphor in a barcode. Droplets were generated by letting the mixture flow into an oil stream at a T-junction, and then polymerize into particles through on-chip ultraviolet (UV) illumination. After producing particles with each mixture, the mixing channel was cleared automatically for the next barcode generation. Based on this device, large numbers of barcodes could be achieved in a single synthesis process. Ji et al. developed an integrated parallel microfluidic strategy for simultaneous preparation of QD-encoded particles with controllable barcodes (**Figure 5**).^[73,74] Their method involved the generation of multiple dispersed solutions carrying stepwise concentration gradients of QDs by using a pyramidal microfluidic network, the formation of corresponding droplets in parallel flow-focusing droplet generators, and on-chip solidification of these droplets into particles by chemical polymerization. It was demonstrated that five kinds of QD-encoded alginate hydrogel particles with a five-level stepwise concentration gradient of monochromatic QDs or five controllable ratios of two differently sized QDs could be generated simultaneously by using the microfluidic strategy. Moreover, this microfluidic device was promising as it could be upgraded by increasing or decreasing the inlets and outlets of the pyramidal network, and thus the number of the parallel flow-focusing droplet

generators would increase correspondingly to meet the requirements of particular QD barcodes.

3.2. PhC Barcodes from Single Emulsions

Apart from their use in fluorescent encoding, PhCs could also be incorporated into microfluidic droplets to generate barcode particles. PhCs are a kind of well-known photonic nanomaterial with spatially ordered lattices that exhibit brilliant structural colors.^[75–83] Because of the periodic arrangement of the dielectric materials, an amazing property known as the photonic band gap (PBG) appears. This leads to the light that with certain wavelengths or frequencies located in the PBG being prohibited from propagating through the PhCs. The reflection wavelength is determined by the structural period and system refractive index of the dielectric system according to Bragg's law, $m\lambda = 2nds\sin\theta$, where θ is the peak wavelength of the reflection, d is the distance of the structural period, n is the average refractive index of the constituent photonic materials, and θ is the Bragg angle of incidence of the light falling on the nanostructures. Therefore, by constructing PhCs with different structural periods or different refractive indices, a series of PhC particles with different diffraction peak positions could be obtained for encoding.^[83] Unlike the fluorescence, the spectra of the PhCs originate from the reflection of their physical structures; thus, they are resistant to photobleaching or photoquenching, which makes them a kind of ideal color-encoding element.^[84]

A typical microfluidic PhC particle is based on pearl pigment, and is composed of a plate of mica with a layer of metal oxide film such as titania on its surface.^[85] When the

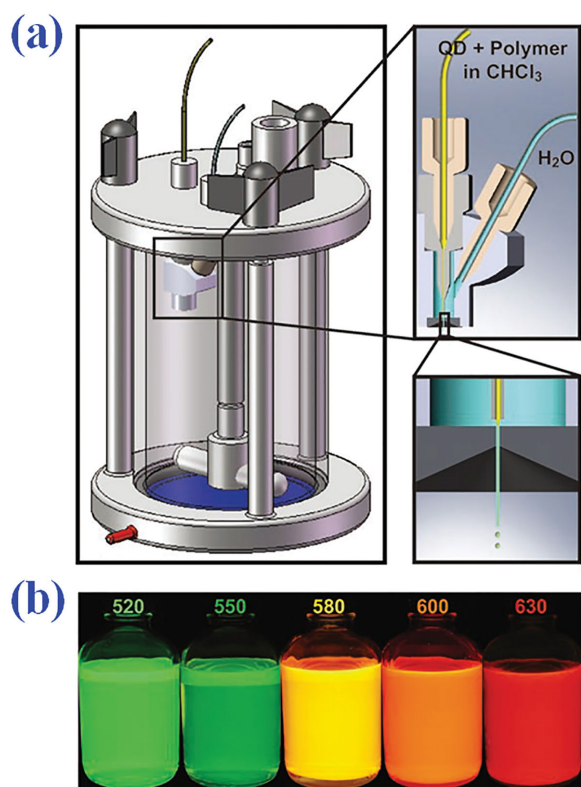


Figure 4. Summary of microfluidic generation of barcode particles presentation: (a) Representation of the particles generation process including the concentration reactor and the production nozzle. The enlargement is shown in the top right corner with a cross-sectional diagram of the working flow-focusing nozzle in which a QDs fluorophore solution in chloroform with dissolved polymer is introduced through the top line (yellow). The flow-focusing fluid (deionized water) is introduced from the right (blue). A close-up view of the QD/polymer solution being focused and “pinched off” into microscopic droplets by the water flow is shown on the bottom right corner. Each small droplet formed will be an active polymer particle with homogeneous quantum dot encoding. (b) UV illuminated picture of the five stock solutions of QDs used to generate a barcode library. The number printed on each bottle represents the fluorescence emission wavelength in nanometers of the respective quantum dots. Reproduced with permission.^[67] Copyright 2008, Wiley-VCH.

pigment is irradiated, light is reflected at the air/metal oxide and metal oxide/mica interfaces. The interference of the reflected light results in a definite color, which depends on the thickness of the metal oxide film. The color of the pearl pigment is easily designed by varying the thickness of the metal oxide layer. Based on the microfluidic single-emulsion templates, monodisperse polystyrene particles with pearl pigment elements can be fabricated for multiplex detection of proteins by the solvent evaporation method.^[86,87] These particles showed a uniform color from any angle and could be identified even with the naked eye if the reflection fell in the visible region. However, the encoded reflectance peaks of the pearl pigment particles were ambiguous and this greatly limits the number of particle codes that can be generated.

As an alternative, a new kind of microparticle with the PhC structure was developed by the evaporation of droplets containing numerous monodisperse nanoparticles (**Figure 6**).^[88–92] During this process, the nanoparticles form

spherical assemblies with a regular arrangement (**Figure 6b**), known as photonic crystal beads (PCBs). PCBs are essentially spherical colloidal crystal clusters. Similar to the colloidal crystals in a plane, the surfaces of the photonic crystal beads lie in the (111) plane of the face-centered cubic (FCC) symmetry. Because of the surface curvature, a close-packed colloidal array has intrinsic defects and grain boundaries. However, when the diameter of the spherical colloidal crystal cluster is much larger than the colloidal size, the effects of curvature on the crystallization become negligible. The isotropic confining effect of the droplet and the heterogeneous crystallization from the interface create onion ring-like structures, with rings composed of a hexagonal array of nanoparticles. Therefore, the PCBs have an isotropic band gap property exhibiting the same reflected color for a fixed incident light source.^[93,94] Based on droplet microfluidics, the size of the PCBs could be controlled well by changing the suspension concentration of the colloidal nanoparticles or the diameter of the droplet templates through adjustment of the flow rates of the water and oil phases. The encoded reflection colors or the diffraction peak positions of the PCBs could be tailored by using colloidal nanoparticles of different sizes as assembly elements (**Figure 6c**). To increase the encoding capacity of the PCBs, semiconductor QDs with different colors and intensities could also be integrated into the PCBs to achieve joint encoding.^[95–97]

Despite its many advantages, the evaporation rate of the approach for PCB generation is usually slow as it takes several hours at room temperature for complete consolidation.^[98] Thus, a major obstacle to the generation of the PCBs in bulk is the lack of simple and reliable methods for consolidating the colloidal particles into crystals in fast time scales. To reduce the evaporation time, Kim et al. introduced microwave irradiation as a heating approach. Microwaves can selectively superheat polar molecules, which are the water molecules in the PCBs' generation, and the emulsion droplets could be consolidated into the PCBs within a very short time and still be of good enough quality to be used as colorful microparticles.^[99]

Recently, optofluidics technology was developed to generate the PCBs by complementary integration of fluidics and photonics.^[100] The dispersed phases in optofluidics were prepared by dispersing monodisperse colloidal nanoparticles in photocurable resin or pre-gel monomers. In relatively high nanoparticle concentrations, significant interparticle repulsion occurs at the average interparticle spacing; and the minimum energy configuration makes the colloidal nanoparticles self-assemble into a non-close-packed body-centered cubic (BCC) or FCC colloidal crystal array (CCA) structure in the resin or monomer solutions.^[101,102] Because of the periodic arrangement of the nanoparticles, the hybrid solutions appear as being the property of the PBG. The solutions could be emulsified and subsequently solidified in a microfluidic device equipped with a UV exposure unit.^[103,104] Based on this method, Kanai et al. have emulsified polystyrene latex-dispersed poly(*N*-isopropylacrylamide) (PNIPAm) pre-gel solution into monodisperse droplets.^[105] After photopolymerizing the droplets, a series of soft hydrogel PCBs with a polystyrene latex CCA nanostructure could be achieved. These

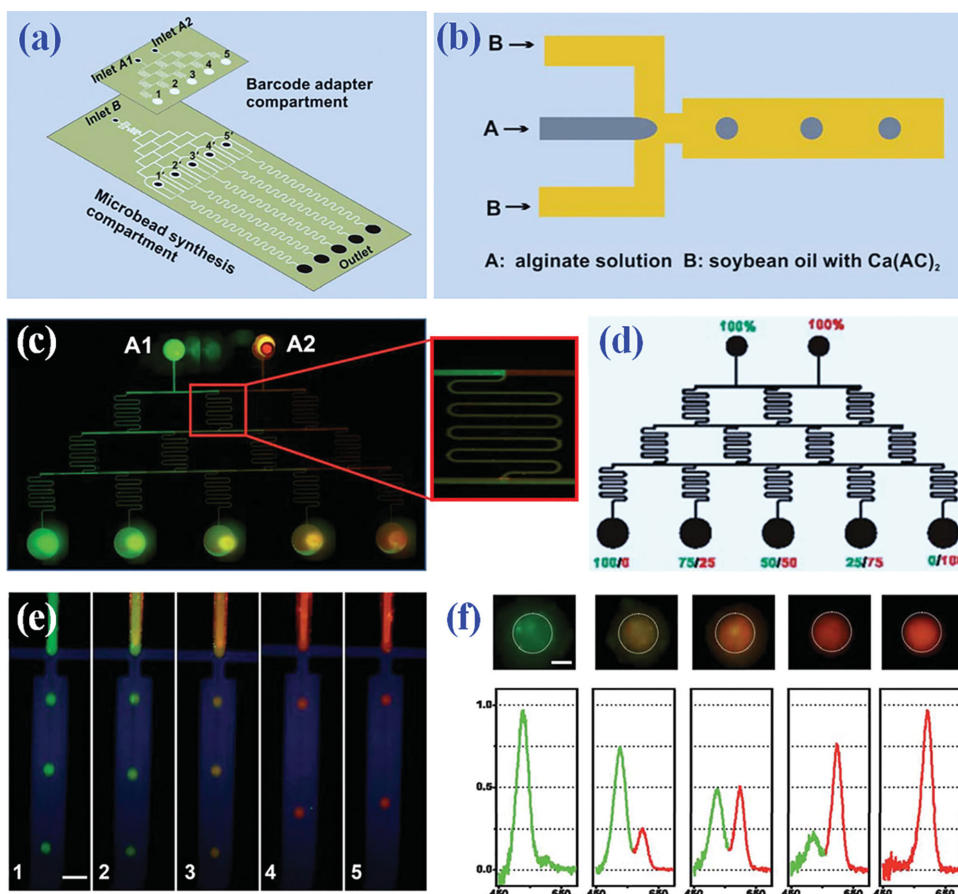


Figure 5. Microfluidic strategy for simultaneous preparation of QDs encoded particles with five controllable barcodes: (a) Schematic diagram of the integrated parallel microfluidic device. The barcode adapter compartment is a pyramidal network with inlets A1 and A2 for dispersed phases and 5-outlets for redistributed dispersed phases (labeled as 1–5). The particles synthesis compartment integrates five parallel flow-focusing droplet generators (labeled as 1'–5') shearing the common inlet B for continuous phase. (b) Schematic diagram of the microfluidic route to produce alginate hydrogel particles by the external gelation strategy. (c) Fluorescence microscope image of the pyramidal network with composite gradients of 533 nm QD (A1) and 600 nm QD (A2) in alginate solutions. (d) The numerically calculated concentrations of 533 nm and 600 nm QDs in isolated alginate solutions. The concentrations of 533 nm QDs in the inlet A1 and 600 nm QDs in the inlet A2 are normalized as 100%, respectively. (e) Fluorescence microscope images of microfluidic emulsifications of five alginate solutions with controllable combination of 533 nm and 600 nm QDs. The scale bar is 150 μm . (f) Fluorescence microscope images of the obtained hydrogel particles with two-colored encoding and their corresponding fluorescent spectra from single-particle. The sum of the two peak intensities in individual spectrum is normalized to 1. The scale bar is 20 μm . Reproduced with permission.^[74] Copyright 2011, The Royal Society of Chemistry (RSC).

PCBs were, with stimuli, responsive to diffraction color and Bragg diffraction wavelengths and thus were potentially useful as biological and chemical sensors for monitoring chemical reactions or changes in the environment.

Yang et al. have fabricated various rigid PCBs by photo-induced consolidation of ethoxylated trimethylolpropane triacrylate (ETPTA) emulsion droplets containing silica nanoparticles (Figure 7).^[106,107] A co-flow microfluidic device composed of two coaxial glass capillaries was used to generate O/W emulsions, which were consolidated by UV downstream of the fluidic channel. The solidified silica-in-ETPTA PCBs have different structural colors depending on the size and concentration of the silica nanoparticles. Because of the strong polarity of the ETPTA matrix, this fabrication approach was quite effective for synthesizing non-close-packed PCBs (Figure 7b). The silica particles in each PCB protruded through the interface and formed a hexagonal array (Figure 7c,d). By selectively decorating the exposed

areas of the silica particles with silver nanoparticles through electroless deposition (Figure 7e), the resulting hierarchically structured PCBs showed high sensitivity and fast binding kinetics in surface enhanced Raman scattering (SERS) detection (Figure 7f,g), because of the dense array of hot spots on each PCB and high mobility of the PCBs, respectively.^[108]

3.3. Janus Barcode Particles from Single Emulsions

Microparticles having a biphasic geometry of distinct compositions and properties are known as “Janus” particles. Barcode particles with these structures can have diverse functions because of their anisotropic nature. Based on single-emulsion templates, Janus particles could be prepared by phase separation in droplets.^[109–111] For example, single aqueous droplets containing monodisperse silica nanoparticles and ultrafine magnetic nanoparticles exhibit phase separation within the

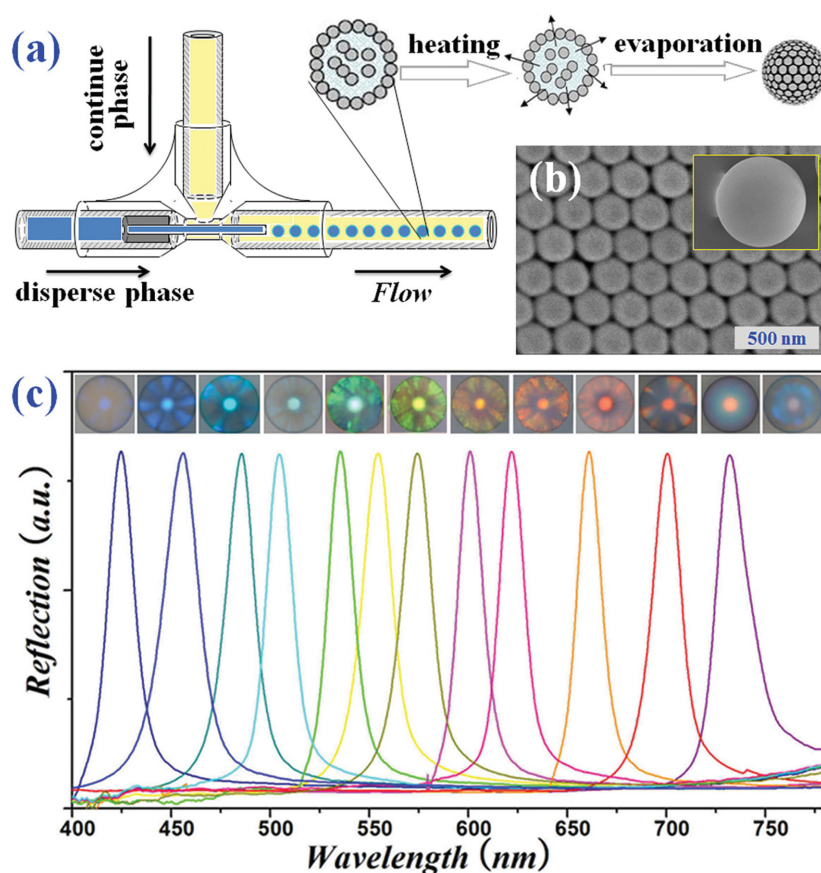


Figure 6. (a) Schematic illustration of a co-flow droplet microfluidics and the PCBs generation by the droplet evaporation; (b) SEM image of the generated PCB surface, insert is the image of a PCB; (c) optical micrographs and reflection spectra of twelve kinds of PCBs. Reproduced with permission.^[90] Copyright 2008, ACS.

droplets under a magnetic field. When these droplets are dried in an oven, the silica nanoparticles in the templates self-assemble into colloidal crystal beads with a close-packed structure to achieve the lowest energy state, and the ultrafine magnetic nanoparticles infiltrate the interstitial sites between the silica nanoparticles in the bottom hemispheres, as schematically described in **Figure 8**. Janus barcode particles with the features of an anisotropic PBG structure and magnetic properties were achieved after these processes (Figure 8d–f).^[111] The resultant particles enabled optical encoding and magnetically controllable motion, making them excellent functional barcode particles for biomedical applications.

The Janus barcode particles could also be achieved by biphasic or multiphasic single-emulsion templates, which consist of two or more separate domains. These emulsion templates were prepared by using microfluidic devices designed to simultaneously inject several distinct fluids in parallel and emulsify these fluids concurrently into single droplets.^[112–115] During this process, the flows were usually laminar as a result of the large viscous forces relative to the inertia. These flows only produced small Reynolds numbers and thus parallel streamlines could be maintained. The convective flow does not affect inter-stream mixing and the streams mix slowly by diffusion alone. Conventional 2D microfluidic devices could provide biphasic or triphasic droplets configured in a side-by-side geometry. Thus, corresponding Janus or triphasic microparticles with

side-by-side geometry could be prepared by injecting two or three distinct flows in parallel into the 2D microfluidic device. However, it was difficult to generate microparticles with more than triphasic geometry with conventional 2D microfluidic devices.

Recently it has been demonstrated that multiphasic particles with a 3D axisymmetric geometry could be prepared using capillary microfluidic devices in which multibarreled capillaries were used to inject distinct fluids in parallel with an angular distribution.^[116,117] In a typical experiment, miscible fluids formed several parallel flows in a microfluidic channel and were emulsified into multiphasic droplet templates at a junction at which a continuous phase exerted a drag force on the parallel streams. Although vigorous rotational flows were generated during this process as a result of external shear forces, the centerline symmetry of the device gave rise to a rotational flow confined within its own hemispherical domain and only produced negligible convective mixing. Thus, the generated droplet templates maintained their compartments for some distance as they flowed through symmetric channels. After the multiphasic droplets form, the monomer inside the droplets should solidify quickly to prevent diffusion and coalescence in different parts of the droplets. Therefore,

the droplets were photopolymerized in the channel to retain the clear boundary and distinct compartments within the microparticles.

To generate the multiphasic barcode particles, silica-in-ETPTA solutions with different structural colors were input into the multibarreled capillary microchannels simultaneously (**Figure 9**).^[118] Monodisperse multiphasic emulsion droplet templates were then produced and stabilized by the strong drag forces of the external fluids with aqueous solution. After polymerization, solidified barcode particles with the corresponding multiphasic geometry were generated. By varying the flow rates of the silica-in-ETPTA solutions, the number and relative sizes of the compartments of the barcode particles could be tuned (Figure 9b–g). Thus, with a single microfluidic device, barcode particles with different kinds of structures could be generated on demand. As the particles had different multicolored hemispheres, the possible numbers they could encode increased. To achieve additional functionality of the barcode particles, magnetic ferric oxide nanoparticles were also dispersed in one part of the multiphasic emulsion droplets before photopolymerization of the ETPTA monomers. In this case, it was noted that silica nanoparticles should also be introduced into the ETPTA simultaneously with the magnetic ferric oxide nanoparticles, which facilitated stable and uniform dispersion of the nanoparticles in ETPTA. Because of the anisotropic distribution

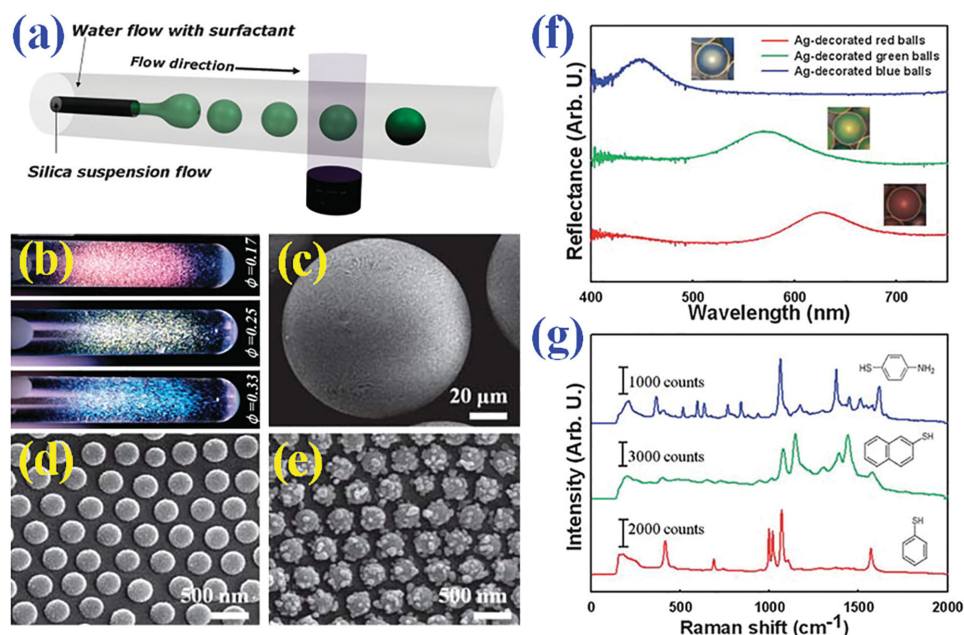


Figure 7. (a) Schematic illustration for the preparation of PCBs by optofluidics: the device was composed of two coaxial glass capillaries, in which the silica-in-ETPTA suspension is fed through the inside microcapillary and the aqueous phase is flowed through the annulus region between the inner and outer capillaries. At the tip of the inner capillary, the flow of aqueous phase sweeps to break the stream of the silica suspension into oil-in-water emulsion droplets, which were consolidated by UV downstream of the fluidic channel. (b) Optical microscopy images of PCBs composed of 165 nm silica particles embedded in ETPTA at three different concentrations. (c, d) SEM images of a PCB and its surfaces with hexagonal arrays silica particles. Reproduced with permission.^[106] Copyright 2008, Wiley-VCH. (e) SEM image of silver-decorated silica arrays on PCB surfaces. (f) Reflectance spectra of silver-decorated photonic microspheres. The insets show optical microscope images of the corresponding microspheres. (g) SERS spectra of 4-ATP (4-aminothiophenol), 2-NT (2-naphthalenethiol), and BT adsorbed on silver-decorated blue, green, and red-colored photonic microspheres, respectively. Reproduced with permission.^[108] Copyright 2011, RSC.

of the magnetic nanoparticles in the barcode particles, the particles could rotate under a rotating magnetic field and be pulled to the side of a vial using a permanent magnet,

which not only increased the sensitivity of the barcode particles, but also enabled their simple capture and enrichment in bioassays.

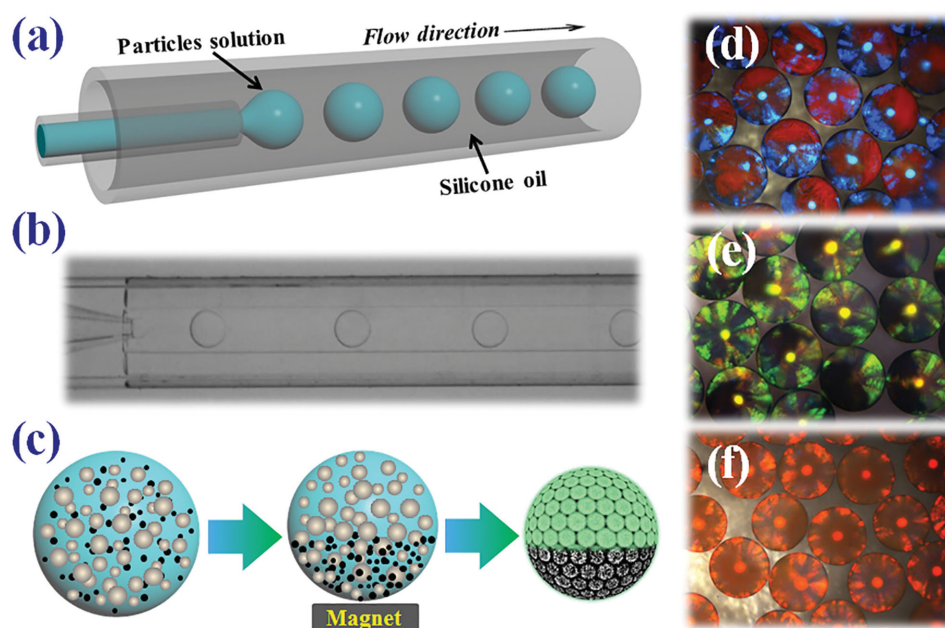


Figure 8. (a, b) Schematic diagram and photograph of the droplet template generation from a co-flow microfluidic device. (c) Schematic diagram of the Janus particle formation process from droplet templates, the Janus particles with anisotropic structure were achieved after separating the magnetic nanoparticles of the droplets by a magnetic field and drying the droplets on an oven. (d–f) Reflection light images of the three kinds of Janus particles by using 220, 260, and 290 nm silica nanoparticles as their elements. Reproduced with permission.^[111] Copyright 2013, RSC.

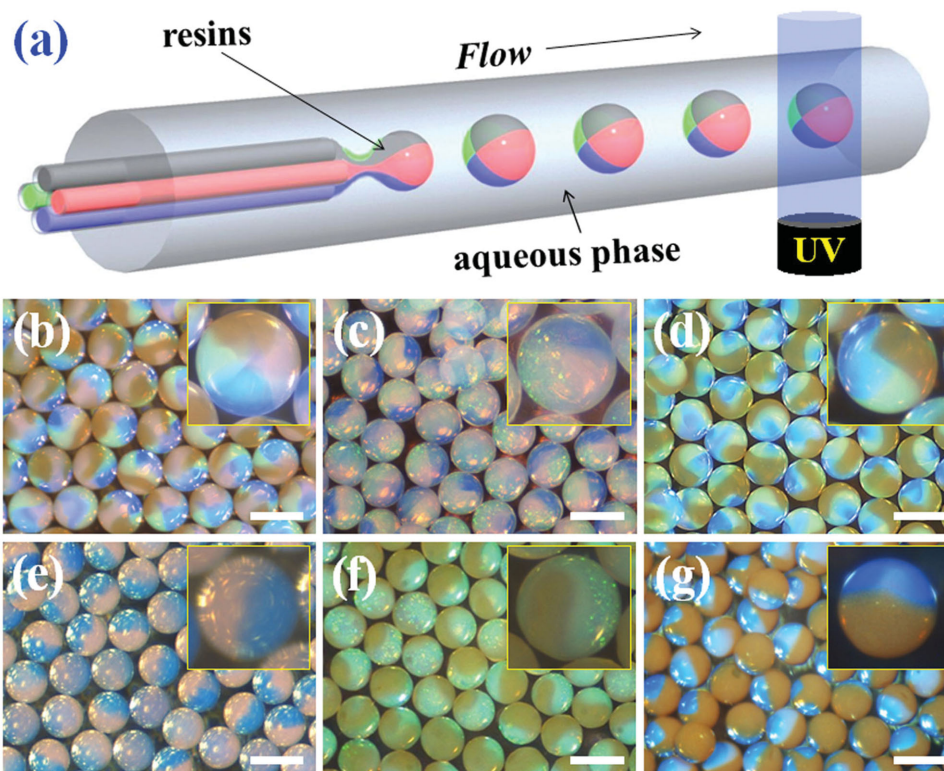


Figure 9. (a) Scheme of a multiple channels injecting microfluidics for the generation of multicomponent emulsions. (b–g) Photographs of multicomponent barcode particles: (b) four compartment barcode particles with red, blue, and green structural colors and a gray magnetic component; (c, d) three-compartment barcode particles; (e–g) Janus barcode particles. Scale bars are 100 μm . Reproduced with permission.^[118] Copyright 2013, ACS.

3.4. Barcode Particles from Multiple Emulsions

Multiple emulsions are hierarchical systems in which small droplets are contained within larger dispersed droplets. The intrinsic complex structural geometry of these multiple emulsions enables the direct construction of barcode particles with many additional features. In this respect, accurate control of the size and structure of the emulsion templates is the primary challenge. Although fabricating such emulsions in a PDMS microfluidic device is desirable, the process requires highly complex and difficult spatial control of the wettability of the PDMS channels. In contrast, glass capillary microfluidic devices, in which the surface wettability can be easily modified and solvent compatibility issues are practically non-existent, can be more easily adapted to fabricate multiple emulsions by one-step or sequential emulsification.

A typical capillary microfluidic device for the one-step emulsification was assembled by coaxially aligning a tapered capillary and a collection capillary inside a square capillary.^[119–122] By combining the co-flow and flow-focusing geometries, the device could be used to generate monodisperse double emulsions. The inner phase was pumped through the tapered capillary, while the immiscible middle phase flowed through the region between the inner round capillary and the outer square capillary in the same direction. The inner droplets form by dripping from the tip of the inner round capillary in the co-flow geometry. The outer phase flows in the opposite direction, through the region between

the collection capillary and the outer square capillary, and hydrodynamically focuses the middle phase containing the innermost droplets; the resultant jet breaks up into monodisperse double-emulsion drops at the orifice of the collection capillary. In this process, two coaxial interfaces were formed in the device, and the streams were simultaneously broken into double-emulsion droplets. This approach obviated the need for sequential emulsification and provided a high degree of control and stability in the generation of double- or multiple-emulsion drops using a simpler device design. However, it is difficult to have precise control over the number of innermost droplets of the double emulsions for the one-step emulsification method.

As an alternative method, sequential emulsification has been developed. Monodisperse single droplets were generated in a first droplet-formation junction, and were injected into a second level of single drops at a second droplet-formation junction.^[123–127] Water-in-oil-in-water (W/O/W) double-emulsion droplets could be prepared using the sequential emulsification by modifying the microfluidic channel surfaces to be hydrophobic in the first junction and hydrophilic in the second junction, to avoid wetting problems. The opposite modification of the microfluidic channel surfaces is required for preparing the inverse oil-in-water-in-oil (O/W/O) double-emulsion droplets. The sequential emulsification method enables independent control over the frequency of drop generation in the first and second junctions, thereby providing for the precise manipulation of the

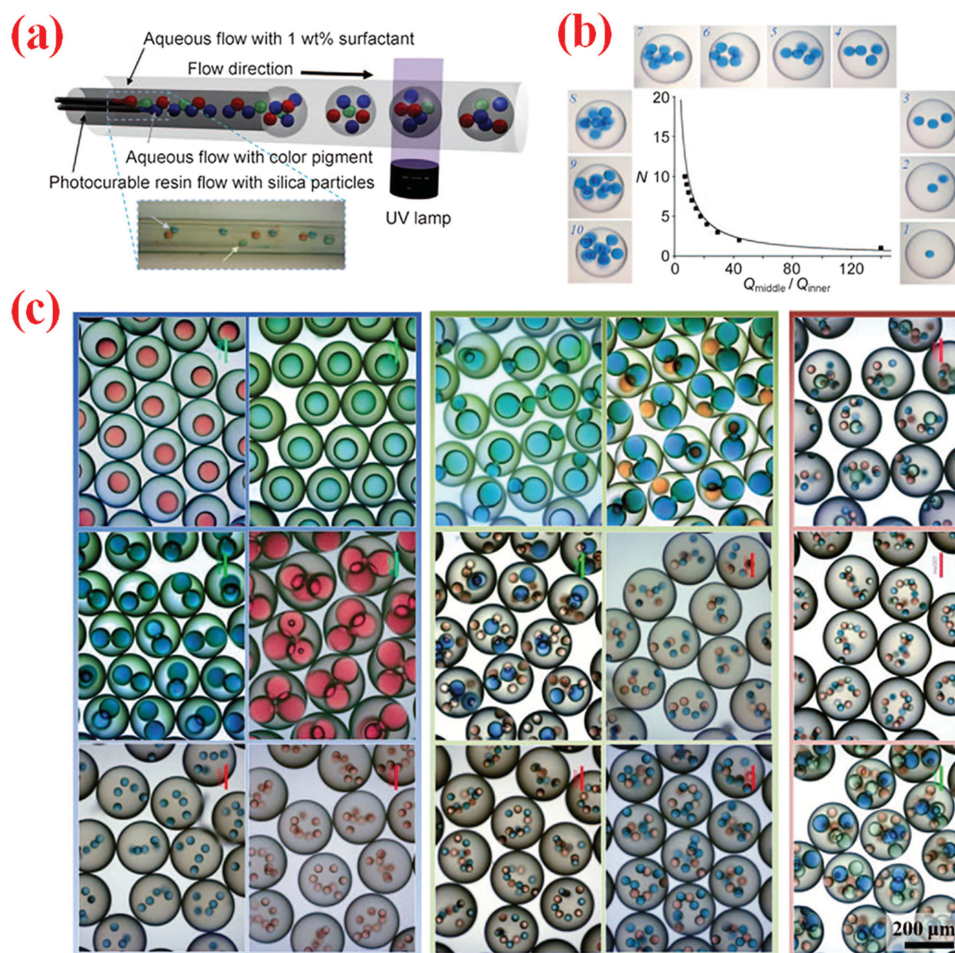


Figure 10. (a) Schematic of the capillary microfluidic device equipped with a UV exposure unit for preparation of photocurable double emulsion droplets containing core droplets of three different colors. The inset shows an optical microscope image of the microfluidic device, where the tips for green and blue core droplets are included within the field of view (denoted with arrows). (b) Control of the number of core droplets with relative volumetric flow rates of the middle to inner streams. Images of double emulsion droplets containing one to ten core droplets are shown. (c) Examples of barcode particles. First two columns, second two columns, and last column show the particles encoded with core droplets of one color, two colors, and three colors, respectively. Reproduced with permission.^[128] Copyright 2011, Wiley-VCH.

number of encapsulated droplets in each double emulsion. Complex emulsion droplets with multiple shells could also be generated by increasing the number of droplet formation junctions in the same manner. Although there are more improvements than the one-step emulsification method, the sequential emulsification devices are complex, and their fabrication procedures are usually in need of spatial control of the wettability of the microfluidic channels. Moreover, full control over the relative diameters of the core and shell of the multiple emulsions is difficult to achieve in the sequential emulsification. Therefore, the one-step emulsification and the sequential emulsification methods are complementary to each other, and the method is selected according to the ultimate purpose.

Based on the microfluidic multiple-emulsion templates, many kinds of elaborated barcode particles can be developed.^[128–131] For example, Kim et al. employed a sequential emulsification glass capillary device with one outer, one middle, and three inner capillaries (**Figure 10a**) for particle generation.^[128] Through the three inner capillaries, aqueous streams with red, green, and blue (RGB) coloring pigments

were forced to flow, while a photocurable silica-in-ETPTA suspension (with low concentration) and an aqueous surfactant solution were introduced in the middle and outer capillaries, respectively. In this way, the RGB core droplets were generated at the tips of the inner capillaries and were subsequently encapsulated into the ETPTA shell droplet at the tip of the middle capillary. Finally, the photocurable shells containing the aqueous RGB cores solidified downstream under UV irradiation. The number of RGB core droplets encapsulated in each particle could be controlled by varying the flow rate and the ratio of the generation frequencies of the cores and shells (**Figure 10b**). These particles had optically and graphically identifiable codes corresponding to their encapsulated colors and numbers of cores (**Figure 10c**). As the ETPTA shells of the particles were transparent, the RGB cores could be identified simply by counting the respective colors and numbers of the core droplets, and the encoding information of the particles could be achieved without any spectral analysis equipment or devices. In addition, the surface of each ETPTA shell was decorated with an array of colloidal silica particles that enabled the formation

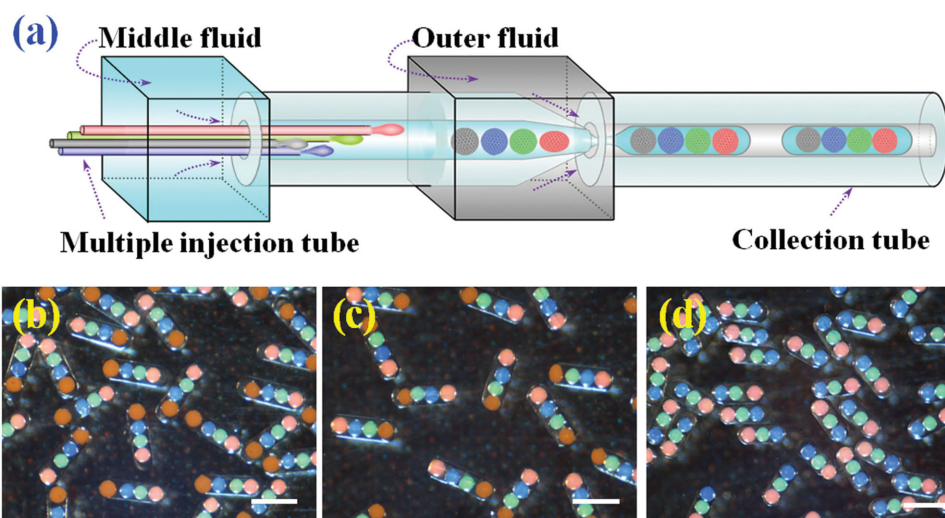


Figure 11. (a) Schematic of the capillary microfluidic device used to generate double emulsions with the rod-like barcodes; (b–d) the polymerized barcodes with the position-indexed PhC encoded cores. Scale bars are 200 μm . Reproduced with permission.^[140] Copyright 2012, NPG.

of functional surface groups for the immobilization and reaction of biomolecules.^[131]

To improve the function of the template-produced multiple-emulsion barcode particles, a different kind of spectrum tags and biocompatible hydrogels were introduced into the templates.^[132–140] In these studies, QD- or CCA-dispersed photocurable resins were used as the inner phases for generating the core droplets, while the aqueous phases of pre-gel flows were used as the middle shell layers of the emulsion templates. After photopolymerizing, barcode particles with QD- or PhC-tagged resin cores and hydrogel shells were generated. These particles have many attributes that make them excellent barcode particles. For example, because of the immobilization and enclosure by the shell layers of the emulsions, leakage of the barcode elements can be prevented. Thus, the barcode particles can be applied to solvents in which their encoded elements are normally unstable. Moreover, the hydrogel shells surrounding the barcodes enable the formation of functional group-enriched 3D scaffolds for bioreactions.

Barcode particles with distinct functions, such as controllable movement and improved sensitivity, could be achieved by encapsulating additional functional elements into the emulsion templates. To fabricate these particles, a capillary microfluidic device that enabled separate injection of multiple inner phases was developed by using a tapered multi-barrel capillary as the inner flow channel for the one-step emulsification.^[138] In this process, the inner phases tend to coalesce and form a single inner-core droplet during the formation of the multiple emulsions. This behavior was ascribed to the hydrodynamically focused inner phases by the co-flowing middle phase and the flow-focused outer phase. Thus, to fabricate the emulsions with more than three kinds of separate inner droplets, an additional immiscible phase (usually the same as the middle phase) was made to flow at the center of the inner phases to prevent their coalescence. Magnetically anisotropic barcode particles were fabricated by flowing ferric-oxide-containing ETPTA in one of the inner channels.

Several kinds of barcode particles with separate inner cores and a single multicomponent core of ETPTA that contains QD, PhC, or magnetic nanoparticles could be generated in the same microfluidic device by tuning the flow rates and the composition of the inner phases. Double emulsions with rod-like morphology could also be achieved more attractively by compressing them into an outer channel with a smaller diameter (**Figure 11a**). The barcode particles derived from these emulsions by in situ photopolymerization were imparted with position-indexed multiple colorful cores (**Figure 11b–d**), which means an increased encoding number can be achieved beyond that of normal spherical core-shell particles.^[139,140] These barcode particles would not only have a substantial number of codes, but also present excellent control over their rotation and aggregation under different magnetic fields, which significantly simplifies the processing of particle-based assays.

Although many novel barcode particles could be achieved, it was more difficult to produce multiple-emulsion templates than the single ones. They are thermodynamically unstable with respect to the coalescence of the inner droplets with the continuous phase, and the emulsions frequently experience structural rupture prior to completing their solidification steps. Thus, careful investigations of the production and stabilization of double-emulsion drops are still desired before the technique could be more widely adopted.

4. Barcode Particles from Flow Lithography

One limitation of droplet-based devices is that most of the produced barcode particles are restricted to a spherical appearance or to shapes that result from the simple geometrical deformations of spheres, such as discoids, rods, or hemispheres, since their morphs strictly depend on the template droplets. However, barcode particles with complex structures might also be desirable in biomedical applications. Microscope projection photolithography techniques have been introduced to fabricate particles with various shapes

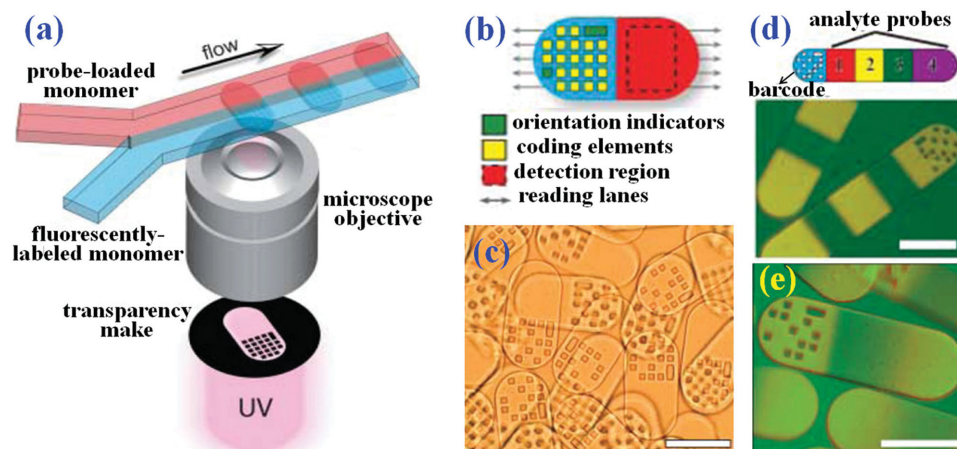


Figure 12. (a) A two laminar flow lithography for manufacturing micro-particles with 2D dotted pattern codes; (b,c) scheme and image of the generated barcode particles; (d,e) scheme and fluorescence image of the barcode particles with multi-probe and gradient probe. Reproduced with permission.^[148] Copyright 2007, AAAS.

using microfluidics and are studied in depth by numerous groups.^[141–161] In contrast to the droplet-based multiphase flow methods, these lithographic techniques rely on transparency masks to provide shape definition. The techniques can be conveniently implemented in microfluidics by using an inverted microscope for projection photolithography. Arrays of the transparency mask-defined polymeric particles are patterned into a UV light-sensitive prepolymer before being streamed out of the microfluidic channels. The ability to create free-standing particles using flow lithography (FL) is based on the inhibition of free radical polymerization reactions at the surface of the PDMS microfluidic channels.^[145] This inhibition is caused by oxygen from the surrounding air diffusing in freely through the porous walls of the PDMS device that form an uncrosslinked “lubrication layer” close to the walls of the PDMS channels for preventing the particles from sticking.

The microfluidic FL was first used for synthesizing barcode particles by Doyle and coworkers.^[146–148] In their system, two monomer laminar streams flowed adjacently through a microfluidic channel, one stream with fluorescent-labeled PEG monomers served to generate the graphical code and the other contained PEG monomers with the oligonucleotide probe that was used to detect the analyte of interest. A key property of these streams was that they could be solidified in a flash of UV light. Thus, as the laminar fluids passed over a microscope objective, they were exposed through a photomask to shuttered UV light that turned the exposed area into solid particles of shapes and dimensions that were defined by the mask (**Figure 12**).^[148] This FL process has led to the generation of functional particles with built-in probes and the corresponding graphical codes of dotted patterns. In this method, the encoding capacity of the barcode particles can be exceedingly large if it is not limited by the size of the encoding section and the resolution of photopolymerization. Moreover, multiple probes or probe gradients can be incorporated into the barcode particles (**Figure 12d, e**), which not only multiplies the encoding number but also allows for comparison of several targets on a single particle or widens the detection range of targets.

As the barcode particles were formed in a flowing stream of monomer, their throughput could not be increased without compromising resolution. This is because the polymerization of the particles occurred within a certain time (0.03 s), and thus the boundaries of the particles would exhibit unacceptable smearing and deformation in high flow rates. When low flow rates were used to increase resolution, particle throughput was adversely affected. To address these deficiencies, stop-flow lithography (SFL) was developed and used to generate barcode particles.^[149] In this system, one can stop the microfluidic flows within a short delay time by using computer-controlled three-way valves, because the Reynolds number and the compressibility of a fluid flowing in a microfluidic channel are very low. Thus, an array of particles was polymerized in a completely stopped flow through a UV exposure with photomask. The formed particles were then flushed out at high flow rates before the cycle of stop-polymerize-flow was repeated. The use of computer-controlled pressure and exposure systems allowed a sequential cycle of these steps, stopping, exposure, and flow processes, to be repeated, as necessary. Thus, the throughput of the qualified barcode particles could be improved up to a thousand times over the continuous FL method. During this process, pattern blurring, which is mainly attributed to motion of the fluids during exposure, was prevented. Thus, the system provided for a much improved resolution (with features down to 1 μm) over particles synthesized in the flow and could produce more delicate structures without any reduction in the production rate. Many kinds of heterogeneous particles have been generated based on SFL, using premixed ingredients.^[150,151] Notably, living cells could be encapsulated within biocompatible hydrogel particles by this method.^[151] The biocompatibility, the high water content, and the free-floating nature of the resulting cell-laden hydrogel particles mean that such systems have great potential in tissue construction.

To get a large number of distinct barcode particles from the FL or SFL systems, a corresponding number of photomasks should be used. At this point, the substitution of the photomasks in the systems with digital micromirror

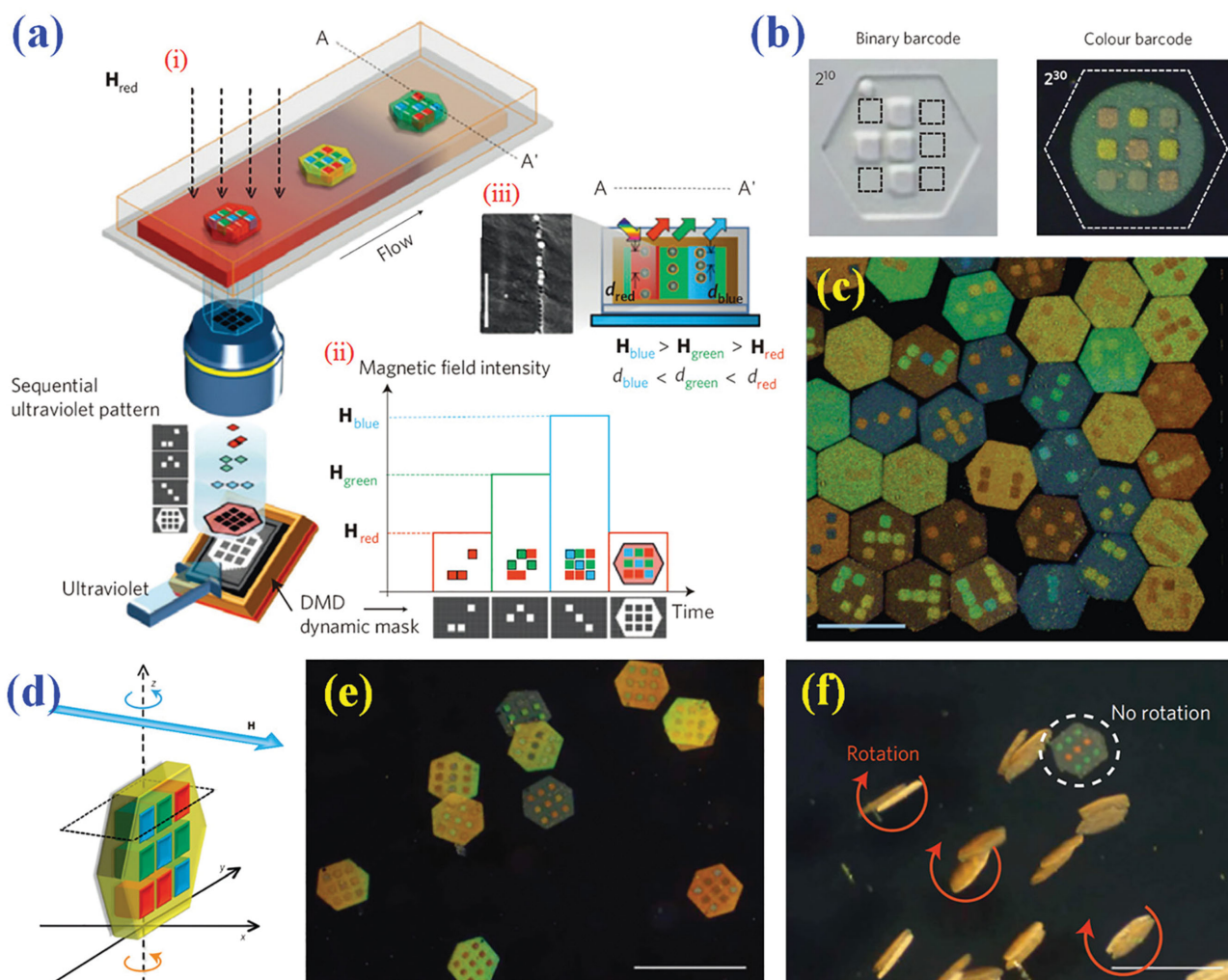


Figure 13. (a) Scheme of the M-Ink color barcode particles generation by the digital micromirror device (DMD)-based flow lithography: (i) the system is composed of sequential processes involving the cooperative action of magnetic-field modulation and spatiotemporally controlled ultraviolet exposure; (ii) time-sequential modulation of the magnetic field according to a specific color and ultraviolet mask pattern for making color-barcoded magnetic microparticles; (iii) cross-section of the PDMS microfluidic channel, by applying the external magnetic field, self-assembly of the magnetic colloids in the form of chain structures was used to create a fully reversible 1D PhC; (b) Coding capacity comparison between a conventional binary barcode and a color barcode; (c) Image of the generated M-Ink color barcode particles; (d–f) scheme and image of the barcode particles under a vertical magnetic-field and a rotating magnetic field, the particle codes are displayed on the 2D surface of the vial under a vertical magnetic-field, and rotated under a rotating magnetic field. The scale bars indicate 500 μm . Reproduced with permission.^[166] Copyright 2010, NPG.

devices (DMDs) was a simple modification that significantly enhanced the flexibility of the systems.^[152–156] The DMDs enable the generation and modification of digital images instantly and freely by using computer processing. Based on this system, Kwon *et al.* have manufactured barcode particles with M-Ink color-encoding patterns (Figure 13).^[156] In this method, the M-Ink color came from the PhC structure of magnetic nanoparticles that were aligned in a magnetic field. As the M-Ink color could be easily tuned by varying the magnetic intensity, the dots in the encoding pattern had different colors, and this enlarged the encoding capacity of the FL particles. In addition, the presence of magnetism in the particles meant that their movement was controllable under magnetic fields (Figure 13d–f); this feature could then be used for active stirring to give improved reaction kinetics in microscale environments.

Sophisticated barcode particles with various spatial morphologies may present greater potentials for a host of applications in biomedicine and engineering. On the basis of FL, many derivative devices, such as stop-flow interference lithography (SFIL),^[157,158] lock release lithography (LRL),^[159,160] and hydrodynamic focusing lithography (HFL),^[161] were developed to meet the desire for fabricating particles with a 3D configuration. In SFIL (Figure 14a),^[157] two masks with distinct functions are used: a transparency mask to define the shape of particles, and a phase mask to induce 3D distribution of light intensity to produce a 3D structure of the particles by selective crosslinking of the oligomer. This technique enables the high-throughput synthesis of 3D patterned polymeric particles defined by a transparency mask with submicrometer feature sizes. The high ratio of surface area-to-volume of the particle structure, which originates

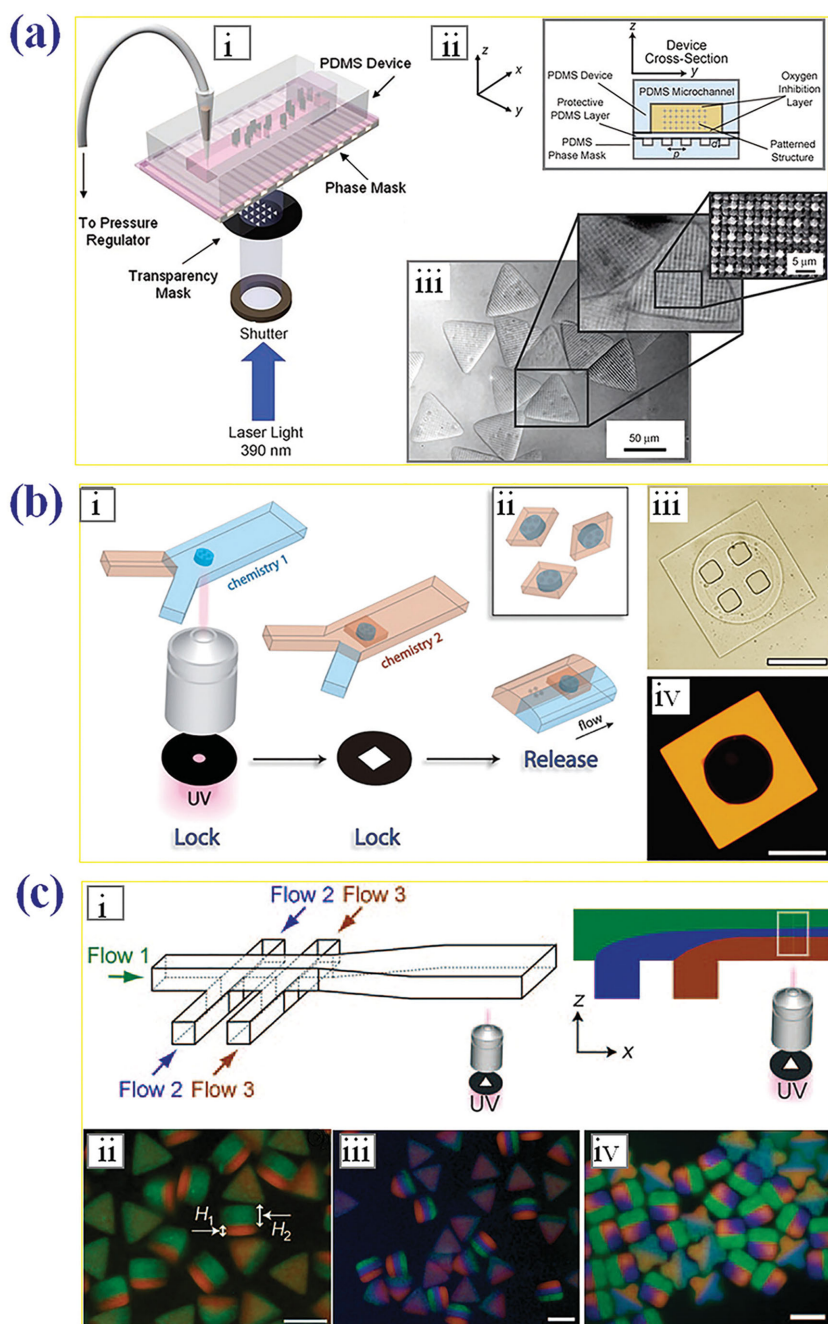


Figure 14. (a) SFIL experimental setup: i) schematic drawing showing the PDMS microfluidic device integrated with a PDMS phase mask; ii) a cross-sectional view of the device; iii) image of the generated triangles after they have been suspended in ethanol. The patterned grid-like structure formed by the phase mask is visible on the surface of the particles and seen more clearly in the inset SEM image. Reproduced with permission.^[157] Copyright 2007, Wiley-VCH. (b) Synthesis of composite particles by LRL method: i,ii) a schematic diagram showing the synthesis of composite particles; iii,iv) differential interference contrast (DIC) and fluorescence microscopy images of the generated particle, two streams containing PEG-DA and PEG-DA with rhodamine-labeled monomer were used to respectively present chemistry 1 and chemistry 2, The scale bars indicate 100 μm . Reproduced with permission.^[159] Copyright 2009, RSC. (c) High-throughput synthesis of multilayered particles: i) schematic drawing showing the synthesis process of trilayered microparticles; ii,iii) fluorescent images of the triangular particles with two layers and three layers; iii) cross shaped particles with red, blue in top, and green layers in bottom; the scale bars indicate 50 μm in (i,ii) and 40 μm in (iii). Reproduced with permission.^[161] Copyright 2010, Wiley-VCH.

from the intricate patterned networks of the particles, may be used to generate high fluorescent signal intensities that could be of benefit to a variety of sensing and diagnostic applications.

The LRL method uses a combination of channel topography, mask design, and pressure-induced channel deformation to form and release particles in a cycled fashion.^[159,160] In this system, 3D PDMS molds containing positive relief structures protruding from the ceiling are used as microfluidic channels to synthesize particles that would normally be “locked” in a flow. These particles are then forced out of the microfluidic channel by using a high pressure pulse that deforms the PDMS device and releases the particles. The overall shapes of the particles are determined by both the photomask design and the dimples in the relief structure in the microfluidic channel. The dimple features can be not only simple shapes such as dots/gratings, but also complicated objects such as crosses and trees. Thus, particles with complex shapes and structures can be achieved by the LRL method. An interesting feature of LRL is the capability of fabricating composite particles (Figure 14b).^[159] This is realized by using several continuous steps, including flowing in one solution, locking a particle in place using one mask, flowing in a second solution and then forming an overlapped region around the first pattern using a different mask. This approach could also be used in the synthesis of multifunctional particles where the distinct sections are not restricted to being parallel stripes but could include more complicated overlaps.

In most FL-based approaches for generating particles with patterned chemistries, the masks need to be aligned precisely at the interfaces of the multiple flows, which is rather time consuming and to some extent limits the throughput. In addition, anisotropic features of the particles are confined to the x, y plane, whereas particles with z direction anisotropy cannot be created using these approaches. To address these shortcomings, the HFL method, which harnesses flow-focusing to create stacked flows in two-layered channels for particle synthesis, was developed.^[161] In this approach, the fluid interface can be perpendicular to the direction of UV light propagation, and therefore precise mask alignment at the interface is no longer needed. This improvement in

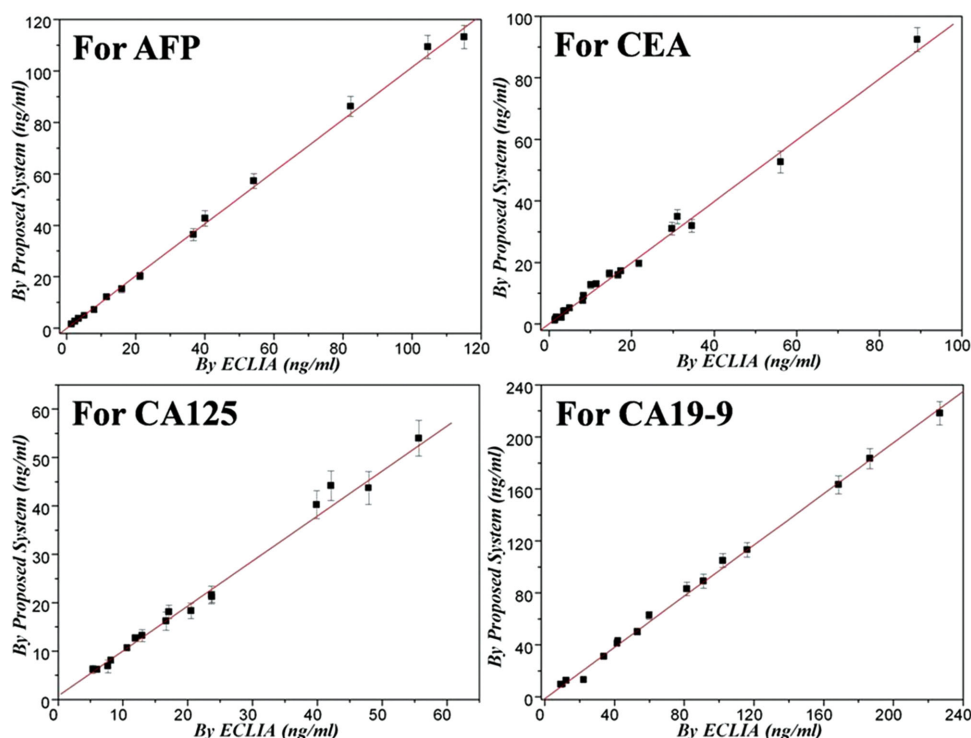


Figure 15. Correlation between the PCBs and the standard electrochemiluminescence immunoassay (ECLIA) method for the four tumor marker measurements of 26 clinical samples. Error bars represent standard deviations. Reproduced with permission.^[162] Copyright 2009, Elsevier.

geometry also allows for polymerization of 2D arrays of particles, which could increase throughput over 200 times over traditional SFL. In addition, multiple monomer streams in HFL can be simultaneously stacked in both the *z* and *y* directions, leading to more complex particles. Figure 14c shows the synthesis process of anisotropic particles composed of multiple layers. In the stacked flows formed in multilayered channels, double- and three-layered triangular particles were synthesized through a mask with triangles. In particular, by combining a 2D flow-focusing geometry, dual-axis particles could also be successfully created. Because the composite microparticles can contain DNA, protein, or cells in different regions, the resultant particles have numerous potential applications in biomedical engineering.

5. Applications of Barcode Particles

5.1. Multiplex Labeling Assays

One of the most promising applications of the microfluidic barcode particles is in particle diagnostics. The barcode particles-based assays are expected to replace traditional plate-based assays for rapid, high-throughput, multiplexed analysis of genomic, proteomic, and metabolomic analytes. Generally, when barcode particles are used for multiplex assays, the detection of targets is performed by measuring the fluorescence of the targets or the target-corresponding probes attached to the surface of the particles by the specifically binding reaction. The intensity of the fluorescence indicates the presence of targets and the interaction strength between

target and probes. Based on this strategy, the commercialized barcode particles have found many practical applications. While most of the studies on the development of barcode particles focus on particle generation, only a few investigate their performance in further applications.

PCBs have been used in clinical diagnosis.^[162–168] The codes of the PCBs are their characteristic reflection peaks originating from the stop-band of colloid crystals. As the peak position is based on its periodical structure, the code is very stable and the fluorescent background is avoided. This solved many problems that existed in the general fluorescence-encoded suspension arrays, such as spectral overlap, quenching of encoded fluorescence signals, and degradation of the particle materials. In addition, as the spherical surface of the PCBs has ordered hexagonal-symmetry nanoparticles, the PCBs can provide not only more surface area for probe immobilization and reaction, but also a nanopatterned platform for highly efficient bioreactions. Because of the reduction in the steric hindrance of the molecules via the artificial separation imposed by the nanopattern, the molecules on this platform are much freer to react with their specific complements and the efficiency of the reaction increases. With a sandwich format, the PCBs are used for multiplex detection of a series of targets, such as tumor protein markers,^[162–166] tumor multidrug-resistance genes,^[163] heart failure and coronary heart disease biomarkers,^[168] environmental toxins,^[169] and so on.^[170,171] The PCBs in these groups all performed satisfactorily. For example, in the detection of multiplex tumor protein markers, the results indicated that the four targets, the α -fetoprotein (AFP), carcinoembryonic antigen (CEA), carcinoma antigen 125 (CA 125), and carcinoma antigen

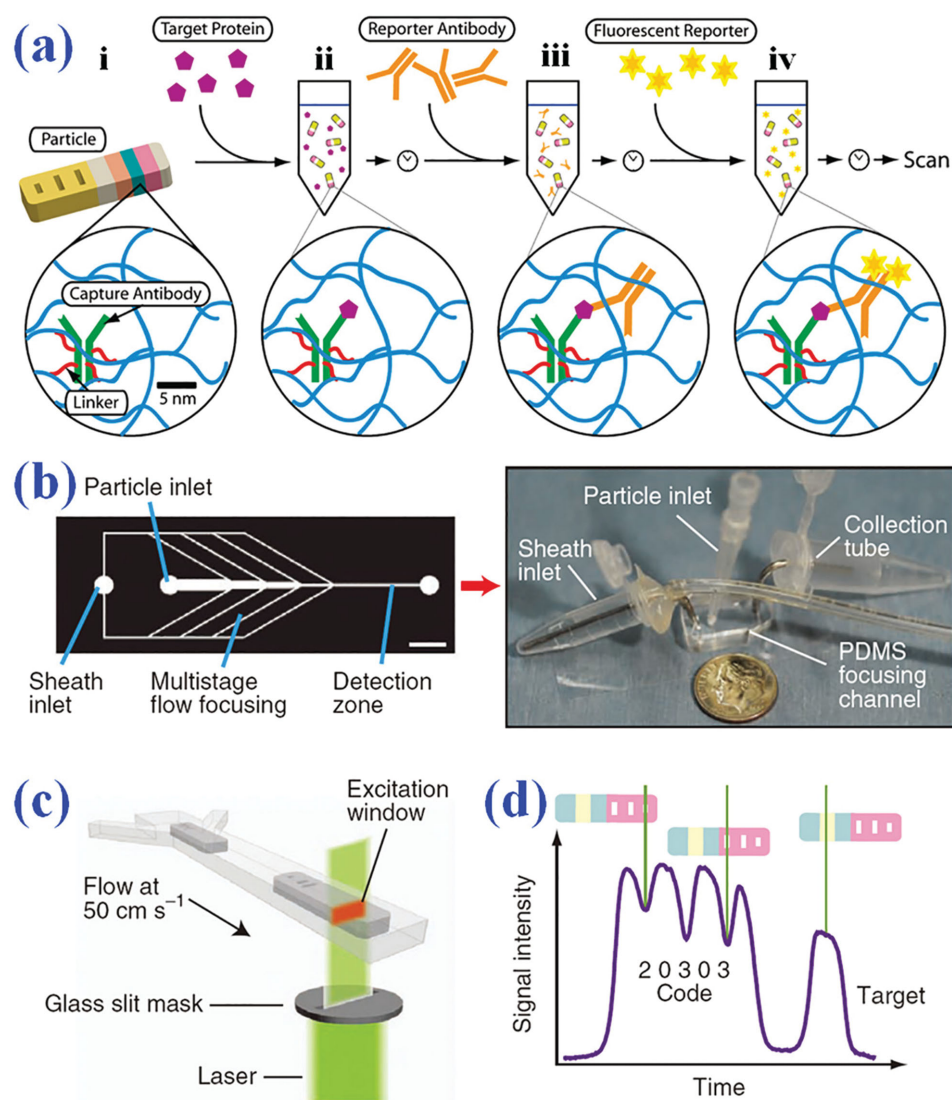


Figure 16. (a) Overview of the protein detection assay, showing an expanded view of the polymer network of the barcode particles. Reproduced with permission.^[172] Copyright 2010, ACS. (b–d) Scanning device and data analysis: (b) Diagram and image of the microfluidic device used for flow alignment of bar-coded particles, along with a photograph of the device with sheath inlet, particle inlet and collection tube attached. The central channel of the device features a series of abrupt contraction points that serve to focus and align the soft gel microparticles for efficient and accurate analysis. Side streams merge with the central channel at these contraction points and provide impinging flows of a sheath fluid for further focusing. The particle detection zone is with the excitation laser and photomultiplier tube (PMT). Scale bar is 1 mm. (c) Schematic of precisely aligned particles passing through the laser excitation window established in the detection zone of the device. A 532-nm laser is used for fluorescent excitation. (d) Actual particle scan illustrating the fluorescence signature of a particle with code 20303 as it passes through the line illumination. Total passage time of the particle is about 500 μ s. Reproduced with permission.^[173] Copyright 2011, NPG.

19–9 (CA 19–9) could be assayed with limits of detection of 0.68 ng/mL, 0.95 ng/mL, 0.99 U/mL, and 2.30 U/mL, respectively. The cut-off values of the four tumor markers in clinical diagnosis are 25 ng/mL, 5 ng/mL, 35 U/mL, and 35 U/mL, respectively. Therefore, the sensitivity and detection ranges of the PCBs were sufficient for practical application. The method also showed acceptable accuracy, detection reproducibility, and storage stability, and the results obtained were in acceptable agreement with those from parallel single-analyte tests of practical clinical sera (Figure 15).^[162]

In contrast to the solid-surface immobilization of probe molecules in conventional planar microarrays and solid barcode particle systems, the hydrogel scaffold offers a

3D, hydrated environment that more closely mimics solution-phase conditions and enables the loading of higher densities of capture species for more sensitive detection (Figure 16a).^[172,173] The biological activity of fragile entities such as cells, enzymes, and antibodies could also be well retained during their immobilization in the hydrogel scaffolds.^[174] As a typical example, graphically encoded PEG particles were generated by the SFL and utilized for DNA, RNA, and protein detection.^[174–179] The PEG-based hydrogels are known to be both nonfouling and biocompatible, and thus the PEG particles could be used for the specific detection of a variety of biological target molecules in complex samples. A multistage flow-focusing microfluidic

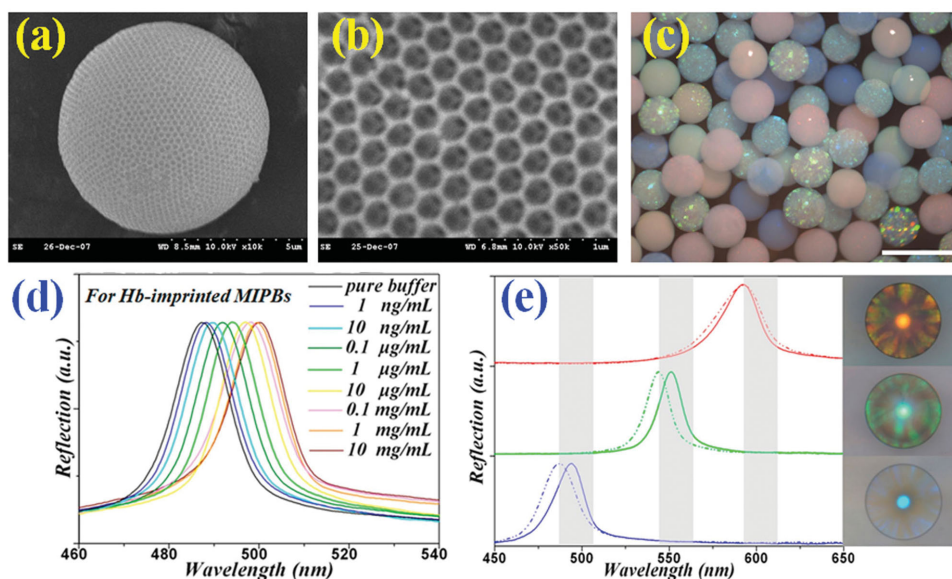


Figure 17. (a,b) SEM images of IPCBs; (c) image of the seven kinds of IPCBs in water, scale bar is 500 μm . (d) Optical response of the protein-imprinted polymer IPCBs to different concentrations of target protein; (e) multiplex label-free bioassay: three kinds of IPCBs with BSA, HRP, and Hb-imprinting, respectively, were mixed and incubated in the mixture of HRP and Hb, the dashed lines and solid lines were the spectra of the IPCBs before and after reaction. It could be observed that the diffraction peak of bovine Hb and HRP imprinted IPCBs shifts to longer wavelengths, while no detectable peak shift occurs on the BSA imprinted IPCBs. These were consistent with the content of sample to which the imprinted IPCBs were exposed. Reproduced with permission.^[200,201] Copyright 2009, Wiley-VCH.

device was also used for decoding and target quantitation of the barcode particles to make the particles align precisely in the central channel and pass through a thin exciting window in the detection zone (Figure 16b–d).^[173] Fluorescence emitted from the code and probe regions of the barcode particle was detected by a flow-through scanner to reveal the code and report the content of the targets. Based on this platform, a 12-plex microRNA expression-profiling study of four human cancer types has achieved limits of detection with two orders of magnitude better than solid particle systems, and with shorter assay times than all other existing quantification platforms.^[176] By assembling an antibody sandwich around the target protein in the hydrogel scaffold of the particles and labeling the sandwich complex with biotin-avidin-aided signal amplification, the assays of the protein target have also achieved high throughput (25 particles/s) and sensitivity (a detection limit of 1 pg/mL).^[172,173]

5.2. Multiplex Label-Free Assays

The ability to measure biomolecular interactions without the use of fluorescent tags or other labels is desirable for practical applications, as the label may alter or inhibit the functionality of the molecule under study.^[180–183] In label-free bioassays, target molecules are not labeled or altered, and are detected in their natural form. These types of biosensors could not only eliminate the time-consuming and expensive labeling step for the target or the reporter molecules, but also allow for quantitative and kinetic measurement of molecular interactions. Multiplex label-free bioassays have been carried

out in planar arrays by taking advantage of techniques such as surface plasmon resonance, microcantilevers, field effect transistors, etc.^[184–187] However, as the barcode particles are small in size and with the form of free diffusion, they are difficult to be incorporated with these label-free sensing technologies. Thus, for the barcode particles to carry out the label-free assays, the interactions of analyte and probe molecules on the particles should result in some detectable physicochemical changes on the particles themselves. At this point, barcode particles with PhC structure would be an ideal candidate because their refractive indices or diffracting plane spacings could be tunable by the bioreactions, and these changes could be detected as the shift in the PBGs of the PhC materials.^[188–199]

To implement the multiplex label-free bioassays in barcode particles, inverse-opaline photonic crystal beads (IPCBs) that are composed of interconnected pores for bimolecular diffusion into their inner structure, are used as encoded microcarriers of the suspension array.^[200–204] The IPCBs are formed by droplet crystallization of a suspension of silica nanoparticles and polystyrene spheres in silicone oil. The polystyrene spheres were calcined, leaving ordered interconnected macropores in the silica beads (Figure 17a,b).^[200] These macropores also form a PhC nanostructure, conferring on the beads a reflection spectrum with a wavelength peak in a certain band gap region. The position of this peak constitutes the identifying code, which can be varied by changing the size of the sacrificed polystyrene spheres (Figure 17c). When used in label-free immunoassays, probe molecules are first immobilized on the pore surfaces of the IPCBs; then, the barcode particles are put into a solution containing analytes and the probe molecules on the pore surfaces of the particles would specifically bind the analytes. This process results in a

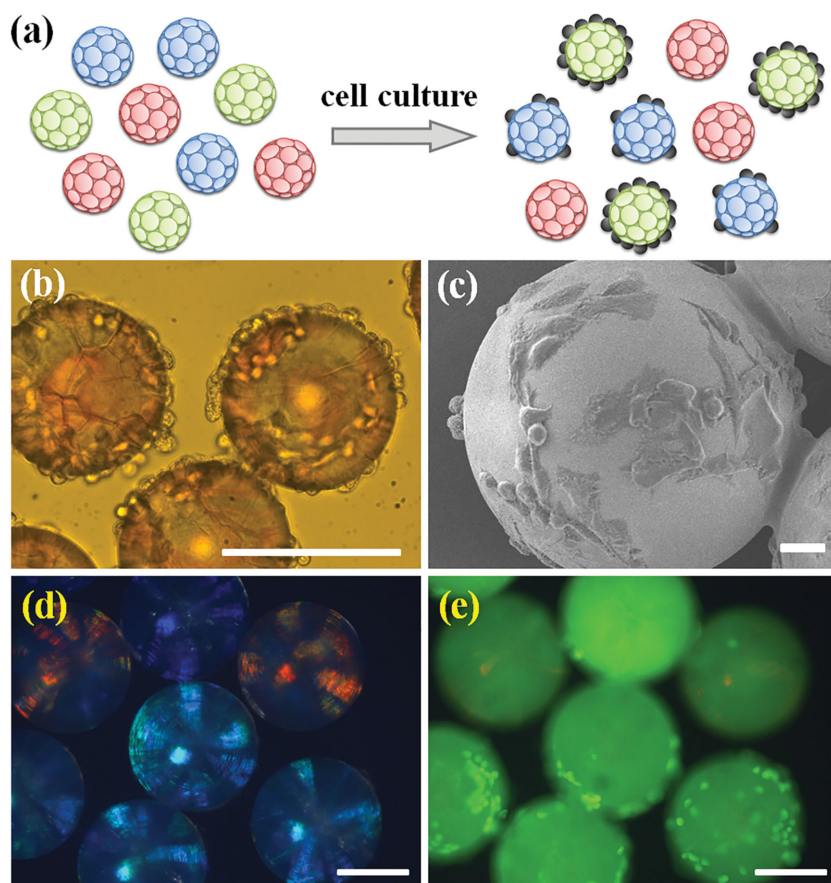


Figure 18. (a) Schematic diagram of the barcode particles for the evaluation of the interactions of HepG2 cells and different materials. The red, green particles were infiltrated with PEG hydrogel and collagen, respectively. The blue particles served as controls with no hydrogel infiltration. (b) Optical microscope image and (c) SEM image of the HepG2 cells on the hydrogel infiltrated barcode particles. Scale bars are 200 μm in (b) and 25 μm in (c). (d,e) optical microscope and the corresponding fluorescence microscope images of the three barcode particles after HepG2 cell culture. Scale bars are 200 μm . Reproduced with permission.^[212] Copyright 2014, Wiley-VCH.

change in the average refractive index of the IPCBs, which is detected as a corresponding red shift in the diffraction peak position. The shift value of the peak position with the target protein binding can be used to quantitatively estimate the amount of the bound analyte.

Further research has found that the sensitivity of the label-free bioassay could be improved by using a bioresponsive hydrogel as the skeleton material of the IPCBs because of the collaborative change of the average refractive index and diffracting plane spacing of the hydrogel scaffolds when the assays are run.^[201–204] For example, by using DNA-responsive hydrogel or protein-imprinted polymer as the skeleton material, the prepared IPCBs could be used for label-free detection of the matched DNA or proteins (Figure 17d). Remarkably, a trace amount of target protein (1 ng/mL) is sufficient to lead to a shift in the diffraction peak. For multiplex label-free bioassays, IPCBs encoded with different spectral ranges and imprinted with different proteins, were mixed in a single tube containing analytes. The analyte proteins could also be detected simultaneously (Figure 17e).^[201] A powerful feature of this method is that both the decoding and

the biomolecule detection are as simple as a one-step measurement of the diffraction peak of the IPCBs, which simplifies the detection instruments and procedures.

5.3. Cell Culture

Microparticles, which are defined as support matrices for the growth of adherent cells, have emerged as biomimic cell culture platforms.^[205–208] They combine the advantages of both adherent and suspension cultures, and are suitable for large-scale cultivation of different cells. However, traditional barcode particles are usually small, several microns in diameter, and thus they cannot be employed for cell culture. Also, the encoded information of the barcode particles would be confusing or incomprehensible if their surfaces were covered by stained cells. These issues, together with debatable biocompatibility, limit the applications of the barcode particles in multiple-cell research.^[209,211] To solve these problems, biocompatible biomaterials and silica hybrid PCBs were suggested for cell culture (Figure 18).^[212] The size of the PCBs can be adjusted by microfluidics to be suitable for cell culture. When cells were cultured on the PCBs, they only interacted with the surface hydrogel and silica, and did not affect the periodic structure and refractive index of the whole microparticle. Thus, the encoded reflection peak positions of the PCBs, which are based on the periodic structure or refractive index of the materials, remain constant during cell adhesion, culture, and staining on their surface.

materials, remain constant during cell adhesion, culture, and staining on their surface.

An interesting feature of the PCBs is that they can be incorporated with different biomaterials and be used for the multiplex bioevaluation of these biomaterials. For this purpose, three kinds of PCBs with RGB structural colors were infiltrated with PEG hydrogel, collagen, and blank, respectively. These barcode particles were mixed together in a single microplate well for the culture of HepG2 cells. Because of the demonstrated promotion effect of the collagen on cell adhesion and growth, it was expected that a large population of HepG2 cells would be observed on the surface of the green PCBs. In contrast, because of the anti-adhesion effect of the PEG hydrogel on cells, it was expected that the surface of the corresponding red PCBs would be difficult for any cell culture. Figure 18d,e presents the results of the cell cultures, which supported the expected situations, indicating the multiplexing capacity of the PCBs in cell research.^[212] We could envision that the high-throughput drug evaluation could also be carried out when different drugs were infiltrated into the PCBs platform.

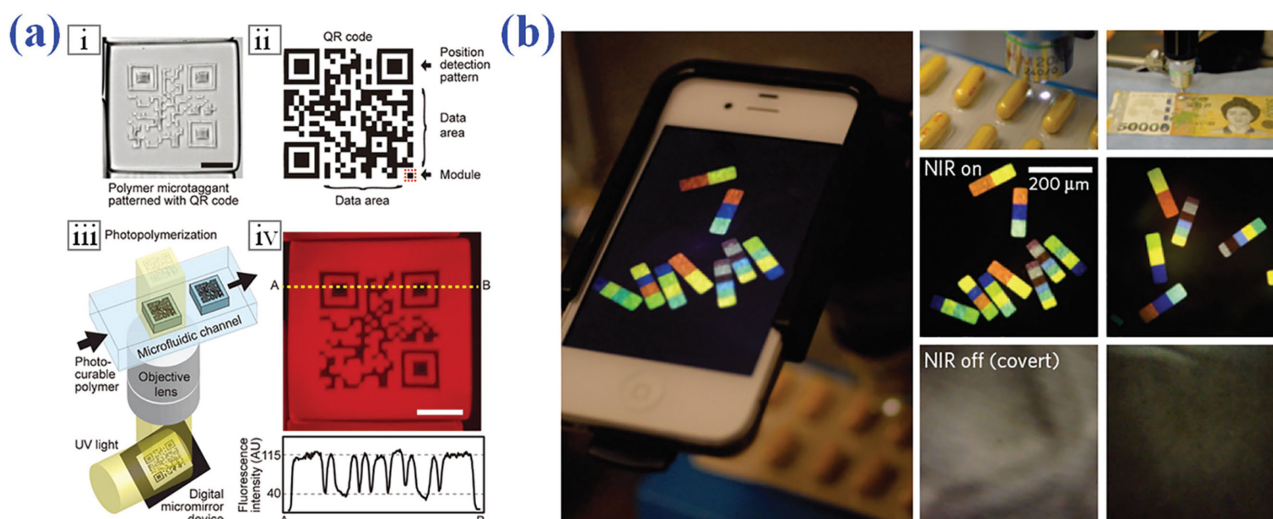


Figure 19. (a) Polymer microtaggant patterned with Quick Response (QR) Code: i) microscope image; ii) structure of QR Code, three square patterns allow the detection of the edge positions of the code, the module indicates the black and white dots composing QR Code; iii) fabrication setup of QR-coded polymer microtaggant using an optofluidic maskless lithography system; iv) fluorescence microscope image of the QR-coded microtaggant and the line profile of the fluorescence intensity. Scale bar is 200 μm . Reproduced with permission.^[215] Copyright 2012, Wiley-VCH. (b) Imaging of spectrally distinct upconversion nanocrystals (UCNs) micropatterned barcode particles with portable decoder in challenging settings: Top: Image acquisition using a portable decoder (Apple iPhone 4S, 20 objective). Middle and bottom: Acquired image on exposure to 1 W 980 nm laser excitation (middle) and in the absence of NIR excitation (bottom), demonstrating covert operation for pharmaceutical blister packs and currency objects. Reproduced with permission.^[216] Copyright 2014, NPG.

5.4. Anti-Counterfeiting Microtaggants

Another main application of the barcode particles is focused on the field of anti-counterfeiting.^[213–216] In this respect, barcode particles are used as microtaggants in manufactured products. Storing information or data on microtaggants could provide the multifunctionality needed for the identification and track-and-trace monitoring of these products. For example, Han and coworkers have developed a functional polymer microtaggant by lithographical patterning of a 2D matrix-type code in a microfluidic channel (**Figure 19a**).^[215] When encapsulated in drug capsules and used for drug anti-counterfeiting, these microtaggants could provide not only authentication but also the possibility of track-and-trace monitoring by allowing encoding of large amounts of drug information. In addition, the partial damage of microtaggants during drug formulation could be tolerated because of their error correction capability. Most importantly, the codes of these polymer microtaggants are read using a simple Quick Response code reader software application that is globally available on smartphones.

Recently, Doyle and coworkers have also used their SFL barcode particles as microtaggants for different anti-counterfeiting applications (**Figure 19b**).^[216] They used this method to synthesize barcode particles by photopolymerizing multiple co-flows of rare-earth upconversion nanocrystal (UCN) dispersed poly(urethane) acrylate (PUA) monomer solutions in a PDMS channel through illumination with photomask-patterned UV light. As different UCNs were dispersed in these monomer solutions, the barcode particles combined the advantages of spatial patterning and UCNs with a single-wavelength near-infrared (NIR) excitation, and could also be read by using a portable

smartphone with an objective. When used as microtaggants, either a representative population of barcode particles was covered on a large portion of the packaged surface, or an individual code consisting of a sequence of multiple particles was placed at a well-defined location. In the latter situation, multiple uniquely encoded PUA particles were suspended in a PUA prepolymer mix and then immobilized within the surface of an object. As the PUA particles and the surrounding polymer have identical refractive indices, the microtaggants were invisible unless illuminated with a proper NIR source. With this strategy, randomly embedding 10 barcode particles from a set of 1000 unique particles could yield an encoding capacity of 10^{30} , which is enough to barcode uniquely every manufactured product on Earth. These features indicate that the polymer barcode particles could be widely used as part of highly powerful, advanced microtaggants for anti-counterfeiting.

6. Summary and Outlook

This review summarizes recent research progress on the microfluidic synthesis of barcode particles and the applications of these particles in multiplex assays. It was demonstrated that both droplet microfluidics and microfluidic lithography are effective approaches to achieve barcode particles. These barcode particles can have distinctive structures and functions, which renders them as an ideal platform for a series of applications, such as labeling or label-free multiplex bioassays, cell culture, anti-counterfeiting microtaggants, and so on.

Despite the many exciting and compelling developments, the majority of the particles-based barcoding technologies still remain in the lab, facing the huge gap between research

results and the requirements of real applications. To overcome this dilemma, three important issues should be considered during technology developments. First, much emphasis is put on the encoding number of the particles, while their applications and concomitant problems are neglected. As described above, millions of barcodes could be achieved by using both droplet microfluidics and microfluidic lithography. However, these numbers mean little in biological applications. The main reason is that the largest multiplicity in protein detection is in the hundreds and this will be further limited by nonspecific reactions because of the cross-reaction of antibodies. Meanwhile, for gene analysis, the production of so many kinds of barcode particles is time consuming, and the size of these microfluidic particles is relatively large, which might require the use of a large number of samples. In addition, it is laborious to detect so many particles by imaging processes or scanning. Second, the convenience of assay operations based on barcode particles should be considered. In practice, a higher throughput of the barcode particle-based assays, which is the benefit of easier operation and higher atomization, is usually preferred to higher-level multiplexing. Therefore, multifunctional barcode particles, for example those with magnetic properties enabling easy separation and mixing, are being extensively explored. Also washing-free or label-free detection will have great prospects on the condition that the sensitivity or accuracy is not strictly required. In addition, regarding the detection systems, a portable instrument that could match the corresponding barcode particles in high-throughput detection and decode the multiplex information would greatly facilitate the assays. On this issue, microfluidic chips could be an optional platform for integrating assay operations, in particular when they have good compatibility with an existing automatization instrument or detection platform. The third issue is about technology promotion. It is important for the technology holders to actively cooperate with the researchers of clinical, medical, food, and environment topics, and get their authority recognized. In other words, the particles-based barcoding technologies have bright market prospects, but the realization of their commercial value still requires the efforts of researchers and entrepreneurs.

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