MINI-REVIEW

Structural color barcodes for biodiagnostics

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Abstract
Suspension array technology has revolutionized the field of biodiagnostics due to its capability of multiplex assays. Construction of the barcodes is essential, where structural color barcode is one of the most satisfying candidates because of its stable encoding and convenient decoding features. Benefiting from microfluidics technique, structural color barcode particles have been synthesized with finely tuned size, configuration, and surface reactive moieties. This allows for the barcode’s interactions with disease-specific biomarkers at multiscale, from the molecular to the tissue/organ level. In this minireview, we present the very latest progress on structural color barcodes for biodiagnostic applications. We discuss in depth through typical examples of how the sensitive and efficient assays of different types of analytes are accomplished concerning the specific features entailed in the barcodes. We also provide critical thinking about the remaining challenges and future development of structural color barcodes from a biodiagnostics perspective.

KEYWORDS
barcodes, biodiagnostics, microfluidics, structural color, suspension array

1 | INTRODUCTION

The development of biodiagnostics has witnessed an explosion of interest in sensitive bioassays. In particular, the requirement for simultaneous detection of large numbers of analytes in small samples has driven the advances of multiplexed assays. One of the most satisfying methods for multiplexed bioanalysis is the suspension array technique.1
Compared with the conventional microarrays, it is flexible, cost-effective, and enables high-throughput identification. The principle of suspension array relies on the barcodes, which are typically micron-size beads encoded with distinguishable information. These barcodes serve as microcarriers for the attachment of bioactive probes and can react with the target molecules in suspension. After the reaction, the probe is identified by decoding the barcodes. Therefore, the key to the suspension array technique is the encoding of the barcodes. Many strategies have been proposed for creating a unique identifier on the barcodes, among which the structural color-based optical encoding method is one of the most likely candidates. Structural color is a kind of coloration widespread in nature creatures. It originates from the light interference with certain micro/nanoscale structure features. Contrary to the coloration from dyes or pigments, structural color is a physically derived phenomenon and is thus more stable against photobleaching or photoquenching. It is thus ideal for color-encoding purposes.

Construction of structural color barcodes involves the synthesis of microparticles with an ordered internal microstructure. One of the most feasible procedure for this is the self-assembly of structural units in emulsion templates as it extends the possibilities of lithography for creating complex nanoscale materials. By microinjection, electrospray, mechanical mixing, or ultrasonic dispersion, droplets of colloidal nanoparticles suspensions can be generated, and particles with an ordered packing architecture can be generated by evaporation-induced colloidal crystallization or in situ polymerization of an ordered colloidal array. Notably, the development of microfluidics technology brings drastic advances to this field. It not only ensures the monodispersity of the droplet templates but also offers a finely controllable route to regulate the morphology and function of the resultant particles. With that, a great variety of structural color barcodes have emerged with distinct shapes, components, and multiple functions. The resultant barcodes find extensive applications in the area of biodiagnostics, expanding from the conventional molecular detection to the construction of the multiplexed cell-culture platform. This enables the implementation of complex cellular and organoids assays systems for the requirement of drug screening, metabolic analysis, organ-on-a-chip engineering, etc.

In this minireview, we present the very latest progress on structural color barcodes for biodiagnostic applications. We first focus on the structural color barcodes with tailoring morphological, component, and functional properties. Then, we introduce the utilization of barcode-based suspension arrays in the analysis of multilevel biomarkers including at the molecular, subcellular, cellular, and organoids level. We discuss in depth through typical examples of how the sensitive and efficient assays of different types of analytes are accomplished concerning the specific features entailed in the barcodes. We do not cover too much about the general mechanisms of structural coloration and the fabrication details of the self-assembled nanostructured materials. Readers interested in these aspects are guided to specified reviews. Finally, a summary and outlook are presented discussing the remaining challenges and future development of structural color barcodes from a biodiagnostics perspective.

2 | TAILORING STRUCTURAL COLOR BARCODES

Microfluidic platform provides a facile way of generating particulate materials through an emulsion template. A liquid droplet can be solidified in situ via physical cross-linking or chemical polymerization. By executing precise control over the fluid flow configurations and the content of the liquids, or executing actuation from external fields, particles with a large diversity of morphologies, components, and functions can be achieved. Accordingly, microfluidics-derived structural color barcodes can either take on a spherical shape or an anisotropic shape. In addition, functional nanoparticles or polymers can be incorporated to form a hybrid system. Moreover, multiple barcodes can be integrated to function as a patterned cluster.

2.1 | Spherical structural color barcodes

2.1.1 | Close-packed colloidal crystals

Close-packed colloidal crystals are typically derived through evaporation-induced nanoparticles crystallization via droplet templates. The colloidal nanoparticles are confined in the droplets and eventually assemble into a close-packed, face-centered cubic structure under the capillary force (Figure 1A). The periodic variation of the refractive index gives rise to a photonic band gap (PBG), and light of a wavelength within the PBG is prohibited from propagating. The resultant colloidal crystal spheres thus exhibit an iridescent structural color, and the reflected wavelength can be estimated from the Bragg-Snell equation, where

\[
\lambda = 2D(n_{\text{eff}}^2 \cos^2 \theta)^{1/2},
\]

where \(n_{\text{eff}}\) is the average refractive index of the constituent materials, \(D\) is the distance of diffracting plane spacing, and \(\theta\) is the incidence angle of the light. The inducing effects of the curved surface and the spherical symmetry property make the reflection spectrum angle independent. Therefore, the colloidal crystals spheres serve as ideal barcodes without error coding problem caused by the alternation of the viewing angle.
2.1.2 | Nonclose-packed colloidal crystals

Colloidal crystal spheres with a nonclose-packed architecture are typically generated by in situ polymerization of a nanocomposite consisting of an ordered colloidal array and a matrix material. The charge-stabilized nanoparticles in the droplet template rely on the electrostatic repulsion to maintain the periodic configuration as a result of the minimization of energy. After polymerization, the periodic structure and the resultant PBG can be fixed by immobilizing the colloidal array in the polymer matrix (Figure 1B). A large number of hydrogels, such as poly (ethylene glycol) diacrylates (PEG-DA) and N-isopropylacrylamide (NIPAm), have been employed for constructing nonclose-packed colloidal crystals, which remain stable in the surrounding environment. Besides, several resin monomers, such as ethoxylated trimethylolpropane triacrylate (ETPTA), have been explored for the dispersing of high-concentration colloidal arrays with the aid of a volatile solvent. After polymerization, an immobilized colloidal crystal sphere could be achieved. Compared with the close-packed colloidal crystals, the nonclose-packed counterparts allow for a tunable lattice constant by varying the nanoparticle volume fraction.

2.1.3 | Inverse opals

Inverse opal refers to a three-dimensional porous scaffold material that contains periodic voids. An inverse opal sphere could be synthesized by negatively replicating the colloidal crystal bead template. The interstitial spaces between the assembled nanoparticles are first solidified by filling of ultrafine nanoparticles, metal oxide precursors, hydrogel monomer, etc. After removing the colloidal nanoparticle units by etching, an inverse replica of the template arrays is obtained. In another method, the colloidal crystal template and the filler can be coassembled via a single evaporation-induced deposition process with a precisely controlled volume ratio, and the voids are created by calcination (Figure 1C). One of the most fascinating features of inverse opals is that the Bragg wavelength can be tuned on-demand if a stimuli-responsive substrate material is used.

2.2 | Anisotropic structural color barcodes

2.2.1 | Core shell

Core-shell structural color barcodes can be generated by replicating and etching the colloidal crystal spheres. As the etching process steps from outside to inside, an inadequate etching would lead to the formation of a close-packed opal PhC core and an inverse opal shell (Figure 1D). The different refractive indexes of the core/shell constituents render two distinct PBGs to the core-shell barcodes. Also, the nonclose-packed core-shell structural color barcodes can be fabricated by using double emulsion droplets as the templates. The colloidal crystal arrays suspension can be encapsulated in the core phase and sheathed with a transparent solid shell without hiding the color. Alternatively, it can serve as the middle phase and form the shell of the capsules after polymerization.

2.2.2 | Janus

“Janus” particles refer to particles of a biphasic geometry with distinct compositions. Janus structural color barcodes can be obtained by inducing phase separation of a disturbing
component during the process of evaporation-mediated colloidal assembling. For example, a spherical Janus barcode with a structural color hemisphere and another magnetoresponsive hemisphere is achieved through a magnetic field-mediated assembling process (Figure 1E). In another attempt, a coassembling process of graphene oxide (GO) and silica nanoparticles results in the formation of Janus barcodes with a flat hemispherical shape. Besides, a nonclose-packed Janus barcode can be achieved by polymerizing biphasic nanoparticles arrays, which is generated by simultaneously emulsifying two parallel flows into droplets consisting of two separate domains.

2.2.3 | Multicompartmental

Multicompartmental structural color barcodes can be obtained directly by coencapsulating multiple colloidal crystals or inverse opal spheres inside a hydrogel microcapsule, in a way that mimics subcellular compartments. By means of microfluidic emulsification, electrospray, or condensing on a superhydrophobic substrate, the number and type of the encapsulated barcodes, as well as their spatial arrangement can be well controlled by changing the operation parameters. Besides, a nonclose-packed multicompartmental barcode can be synthesized in a similar manner as that of the Janus barcodes. Multiple parallel flows of colloidal crystal arrays can be emulsified at the same time through an injection tube consisting of multibarreled capillaries. The resultant droplet could maintain a clear boundary between each phase due to the slow, diffusion-dominated mixing. After polymerization, solidified barcodes with the corresponding ternary or quaternary geometry are generated.

2.2.4 | Others

In addition to the above-described shapes, structural color barcodes with other types of anisotropic morphology have been fabricated in a process mediated by wettability, external fields, geometric confinement, etc. For example, spindle-shaped colloidal crystal beads are generated by evaporation-induced close packing, together with elongation along a hydrophilic fiber substrate. In addition, a fast assembling of the colloidal crystals through rapid solvent extraction results in the formation of a novel stomatocyte-like structural color barcodes. The underlying mechanism is derived as the interface of extraction moving faster than colloidal particles’ diffusion, and the resultant redistribution of the particles led to the stretch and extrusion of the droplets. Besides, a photocurable droplet of a nonclose-packed colloidal crystal array can be extruded in a square capillary tube with different aspect ratios. Accordingly, a variety of rod, disk, or cuboid shaped structural color barcodes could be generated after in situ polymerization (Figure 1F).

2.3 | Hybrid structural color barcodes

2.3.1 | Functional molecules

Functional molecules can be incorporated into the structural color barcodes by surface modification. For example, a polydopamine (PDA)-decorated structural color barcode is achieved by inducing the self-polymerization of dopamine at the surface of a silica colloidal crystal sphere. The presence of PDA offers abundant functional groups for biomolecule immobilization. Besides, a gel-based inverse opal brings new possibilities for surface modification. For instance, an inverse opal with a methacrylate gelatin (GelMA) matrix offers abundant amino groups for functional molecules conjugation such as folic acid. Apart from altering the surface chemistry, the barcodes surface topography can be tuned by decorating a highly branched dendrimer, which helps to create an enhanced nanopatterned surface.

2.3.2 | Functional nanomaterials

A hybrid system of colloidal crystals and nanoparticles renders additional functions to the barcodes. For example, by infiltrating magnetic nanoparticles in the interstitial sites between the colloidal nanoparticle building blocks or by occupying on one compartment of the multiphasic colloidal crystal arrays, the barcodes could be imparted with a magnetically controllable transition and rotation ability. In addition, during the emulsification of the nonclose-packed colloidal crystal array, the nanoparticles in each droplet protrude through the surface and form a hexagonal pattern. After polymerization, the surface area of the barcode can be further decorated with silver nanoparticle through electroless deposition. The resulting hybrid barcodes possess a hierarchical structure, which provides a dense array of hot spots for surface enhanced Raman scattering (SERS). Moreover, graphene oxide (GO) nanosheets could be decorated on the surface of silica colloidal crystal spheres with the aid of PDA as an intermediate adhesive layer. This would largely extend the surface functions of the barcodes.

2.4 | Complex structural color barcodes system

A group of structural color barcodes can be organized into an integrated system with a precisely positioned arrangement of each barcode. This can be achieved through a micromolding method. For example, a dynamic micromold composed
of a positive microcylindrical-array and a complementarily negative microhole-array is used for the stepwise filling of the separate structural color barcodes. Different combinations of the barcodes are achieved through programmatically adjusting the microhole-array position. A photocurable pregel is applied to seal all the barcodes, and a one-dimensional (1D) sequence or a two-dimensional (2D) array of the structural color barcodes is achieved after polymerization (Figure 1K). Similarly, by sequentially loading the colloidal crystal barcodes in a mold consisting of an ordered array of cone microcavities, a microneedle with barcodes encapsulated could be derived (Figure 1L). For large-scale and customizing purposes, the patterning of the structural color barcodes is desired. This can be accomplished through inkjet printing, where the shape and the pattern of the colloidal crystals depend on the printing mode and the predesigned track.

3 | BIODIAGNOSTIC APPLICATIONS OF STRUCTURAL COLOR BARCODES

Structural color barcodes foster the development of biodiagnostics by offering an optically distinguishable encoding-decoding strategy for simultaneous identification and evaluation of multiple biomarkers in a small sample. Over the last decades, structural color barcodes have been extensively applied to molecular diagnostics. The latest researches establish a great leap forward, allowing for multiscale barcoded bioassays including at the cellular and organoids levels. Endeavors are made on tailoring the structural color barcodes for enhancing the detection selectivity and sensitivity for specific analytes.

3.1 | Molecular

3.1.1 | Protein

Abnormally expressed proteins can serve as biomarkers of pathological conditions such as cancer, cardiovascular disease, Down’s syndrome, etc. Multiplexed protein assay can be achieved with structural color barcodes through fluorescence-based immunoassay. For example, Zhao et al. established a silica colloidal crystal barcodes-based suspension array platform for the ultrasensitive detection of four tumor markers, α-fetoprotein (AFP), carcinoembryonic antigen (CEA), carcinoma antigen 125 (CA 125), and carcinoma antigen 19-9 (CA 19-9) in one test tube (Figure 2A). The resultant limits of detection (LOD) are 0.68 ng/mL, 0.95 ng/mL, 0.99 U/mL, and 2.30 U/mL, respectively. The detection accuracy, reproducibility, and stability of this method are acceptable for practical clinical sera detection. Based on this platform, a series of signal amplification strategies have been proposed. For example, a responsive hydrogel inverse opal contributes to the self-amplification of the fluorescent signal by the thermal-triggered shrinking. This enables the sensitive detection of α-fetoprotein (AFP) and carcinoembryonic antigen (CEA). Besides, the unique macropores structure and the stimuli-responsive behavior of the hydrogel-based inverse opal also allow for the label-free detection, where the binding reaction can be characterized without using fluorescent tags or any other labels. Instead, the interaction between the probe and the target results in the detectable shift in the diffraction peak position of the barcodes. For example, the core-shell inverse opals are employed for multiplex quantification of several cardiovascular markers including cardiac troponin I (cTnI), B-type natriuretic peptide (BNP), and myoglobin (Myo).

3.1.2 | Nucleic acid

Nucleic acid (DNA, RNA, miRNA, etc.) assays contribute greatly to the clinical diagnostics including the identification of virus infections, tumor progression and metastasis, Parkinson’s disease, etc. A barcoded multiplex nucleic acid assay can be achieved by applying structural color barcodes through fluorescence-labeled hybridization of the complementary sequences of the probe and target. Alternatively, multiplexed label-free DNA assays could be realized by using inverse opals with a DNA-responsive hydrogel constituent (Figure 2B). To enhance mixing and increase the reaction efficiency, several methods have been proposed to enhance the mobility of the barcodes including magnetic guidance, buoyancy, micromotor actuation, etc. To improve the sensitivity, the barcodes can be integrated with a biological magnification pathway for miRNA quantification. For example, an isothermal, enzyme-based rolling-circle amplification (RCA) approach has been applied where a short primer is amplified into a long strand using a circular template with special polymerases. Alternatively, another type of isothermal, hybridization chain reaction (HCR) procedure ensures enzyme-free amplification with the help of chain initiators and two hairpin probes.

3.2 | Cellular

Cellular or subcellular biomarkers, including exosomes, circulating tumor cells (CTCs) play a vital role in the diagnosis of cancer, neurodegenerative diseases, infectious diseases, obesity, autoimmune diseases, etc. Structural color barcodes can serve as microcarriers for capture, culture, and
analyze the cells or subcellular compartments. The colloidal crystals or inverse opals exhibit a nanoarray surface topography, which offers increased contact area and decreased steric hindrance, thus, feasible for cell attachment. By decorating the surfaces with aptamer probes, binding proteins or adhesive hydrogels, a variety of cell captures have been demonstrated including erythrocytes, platelets, pathogenic bacteria, CTCs, etc. For example, barcodes decorated with a highly branched dendrimer are employed for capturing CTCs with high efficiency (Figure 2C). An aptamer-based inverse opal allows for the identification of *Escherichia coli* at a concentration of 100 CFU/mL within 2.5 h, which is highly competitive to the clinic standard. Besides, the size and configuration of the barcodes can be regulated to adapt to either adherent or suspension cultures. Moreover, the physically originated encoding information would not be largely dimmed even with cell coverage, making it possible for multiplexed cell researches. For example, in a mixed cell culture system with barcodes composed of different hydrogels, the cell growth difference on the surface of each barcode could be distinguished, thereby evaluating the hydrogels’ biocompatibility and cell-materials interactions. Also, by decorating the barcodes with folic acid, three types of different tissue-sourced folate receptor (FR)-positive CTCs, Hela, A02, and Raji could be captured against the FR-negative CTC, A549, and they could be differentiated with reliability through spectral decoding.

### 3.3 Organoids

The rise in organoids researches has ushered in a new era of organoids-based medicine, which is driven by the requirement of generating more complex and functional tissues for modeling diseases, and ultimately, for the diagnostics and therapeutics. Structural color encoded cell microcarriers are important composite materials for real-time monitoring of physiological processes. Therefore, they are starting to play a significant role in metabolic assays within a suspension array bioreactor. The multiplexed analysis capability of structural color barcodes sheds new light on modeling a multiorgan microsystem, in which the tissue- or organ-level communications could be visualized for studying the enzyme cascade reaction activities or drug metabolisms. As an example, a core-shell barcode with a GelMA inverse opal shell and a colloidal crystal core is developed for constructing a spheroids-on-barcodes platform. The GelMA shells create a three-dimensional extracellular matrix (ECM) environment for cell growth, while the cores offer a stable structural color code that differentiates different cell spheroids’ biological response. HepG2, HCT-116, and NIH-3T3 cells are cocultured as liver and tumor cell spheroids on these barcodes. Through this, the enzyme-synthesizing hepatic function is reproduced, which helps to convert the nontoxic prodrug tegafur (TF) to the cytotoxic 5-fluorouracil (5-FU) (Figure 2D). These results indicate the potential value of
4 | SUMMARY AND OUTLOOK

This minireview summarizes recent advances that focus on the generation of structural color barcodes and their applications in biodiagnostics. Multiplex, barcoded suspension array technology has revolutionized the field of biodiagnostics due to its capability of simultaneously detecting large numbers of analytes in a flexible, cost-effective, and high-throughput way. Construction of the barcodes encoded with unique identifiers is essential to the suspension array. Structural coloration is one of the most satisfying coding strategies because of its long-term stability and the convenient coding-decoding procedure. Fabrication of structural color barcodes has been greatly fostered by the emerging microfluidic technique, where a confined assembly of colloidal crystals in the droplet templates gives rise to structural color particles with finely tunable morphologies and components, as well as multiple functions. The resultant particles serve as barcodes for the multiplexed detection, identification, and quantification of molecular biomarkers through specific probe-target reactions. Besides, the size, configuration, and surface architecture of the barcodes can be well controlled adapting to cell capture. This creates a great leap forward as for establishing a barcoded cell assay platform, through which multiplexed cell capture, identification, and release are realized. Moreover, the barcoded microcarriers could accommodate an ECM-mimetic cell-culture environment for spheroids growth. It thus extends the barcoded bioassays to the tissue/organ level, through which a complex metabolic pathway assay is realized.

Despite these exciting and compelling achievements, the challenges of this field remain. From an application-oriented perspective, since the evolution of diseases is a complex process involving cross-scale interactions, the detection of a single type of biomarkers is not sufficient for revealing the mechanisms of the disease occurrence and development. For example, a combinative study of disease-related exosome and the exosomal microRNA can largely reduce the possibility of misdiagnosis that depends solely on the exosome surface markers. Therefore, a systematic bioassays platform that accommodates multilevel analysis of different types of biomarkers is essential for comprehensive interpretation and accurate diagnosis. To achieve this goal, future endeavors can be made on tailoring the structural color barcodes to a larger extent. A lot of functional molecules, nanoparticles, and two-dimensional (2D) materials are promising candidates for generating exquisite, hierarchical structure together with reactive moieties and bioactive groups. Incorporation of these ingredients through coassembly or stepwise assembly could open up new opportunities for enriching special chemical, optical, and electrical properties to the structural color barcodes. However, efforts should be put into the synthesis and stability of these composite barcodes since the doping composition might disturb the ordered structure.

Besides, the future diagnostic tool is to be established that links patient-specific information to clinical drug responsiveness. This calls for the mass production of the structural color barcodes. For this, a robust, scalable droplet generator is anticipated through highly integrated, parallel microfluidics. Alternatively, the advanced printing technology can help in the customized design and the patterning of the structural color barcodes. Last but not least, the development of the instrument is highly desired which allows for automatic sampling, testing, and analysis with easy operations for nonexpert users. This would bring scientists, engineers, and clinicians to work together for the commercialization of this technique. Overall, the structural color barcodes will continue to revolutionize the biodiagnostics field and will provide novel platforms for us to study diseases at multiple levels.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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