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Spectral-optical-tweezer-assisted fluorescence multiplexing system for QDs-encoded bead-array bioassay

Qinghua He[a,b,1], Xuejing Chen[a,b,1], Yonghong He[a,b], Tian Guan[a,c,*], Guangxia Feng[a,c], Bangrong Lu[a], Bei Wang[a], Xuesi Zhou[a], Liangshan Hu[d], Donglin Cao[d,**]

[a] Shenzhen Key Laboratory for Minimal Invasive Medical Technologies, Institute of Optical Imaging and Sensing, Graduate School at Shenzhen, Tsinghua University, Shenzhen 518055, China
[b] Department of Physics, Tsinghua University, Beijing 100084, China
[c] School of Medicine, Tsinghua University, Beijing 100084, China
[d] Department of Laboratory Medicine, Guangdong Second Provincial General Hospital, Guangzhou 510317, China

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ABSTRACT

As an efficient tool in the multiplexed detection of biomolecules, bead-array could achieve separation-free detection to multiple targets, making it suitable to analyze valuable and scarce samples like antigen and antibody from living organism. Herein, we propose a spectral-optical-tweezer-assisted fluorescence multiplexing system to analyze biomolecule-conjugated bead-array. Using optical tweezer, we trapped and locked beads at the focus to accept stimulation, offering a stable and optimized analysis condition. Moving the system focus and scanning the sample slide, we achieved emissions collection to QDs-encoded bead-array after the multiplexed detection. The emission spectra of fluorescence were collected and recorded by the spectrometer. By recognizing locations of decoding peaks and counting the intensities of label signals of emission spectra, we achieved qualitative and quantitative detection to targets. As proof-of-concept studies, we use this system to carry out multiplexed detection to various types of anti-IgG in the single sample and the detection limit reaches 1.52 pM with a linear range from 0.31 to 10 nM. Through further optimization of experimental conditions, we achieved specific detection to target IgG with sandwich method in human serum and the detection limit reaches as low as 0.23 pM with a linear range from 0.88 to 28 pM, validating the practical application of this method in real samples.

1. Introduction

Bead-array bioassay has been becoming an increasingly attractive method for biomolecules detections (Fu et al., 2016; Purohit et al., 2018; Uddin et al., 2017). In current detection modes, microbeads were encoded with optical barcodes to be distinguishable one by one (He et al., 2018; Lu et al., 2018). Then, according to the type of barcodes, specific bioprobes were grafted on the surface of microbeads. Thus, the types of captured biomolecules could be detected by identifying the barcodes of microbeads, realizing the qualitative detection. By counting the numbers of biomolecules labels, usually optical labels, bonded on the microbeads, the quantitative sensing of targets could be achieved (Brenner et al., 2000; He et al., 2016). Compared with traditional biochips, bead-array bioassay owns several distinct superiorities. For instance, bead-array offers a new type of biosensor with high surface-to-volume ratio, leading to a faster binding kinetics and much more abundant binding sites for analytes (Kim et al., 2017; Liu et al., 2017). Also, according to former studies, bead-array detection mode could achieve higher sensitivity and stability by adjusting the ratio of sample volumes and amounts of microbeads (Nakano et al., 2018).

Recently, quantum dots (QDs) have attracted plenty of attentions from bead-array researchers (Liu et al., 2016; Sathe et al., 2006). As a newly developed fluorescence material, QDs own broad excitation band, narrow emission peaks, high luminous efficiency and photobleaching resistance (He et al., 2007; Michalet et al., 2005; Resch-Genger et al., 2008). Most importantly, by changing the structure of QDs nanoparticles, the emission wavelength could be adjusted, which makes them especially suitable for the encoding and labeling of bead-array bioassay (Chen et al., 2016; Liu et al., 2015).

* Corresponding author at: Shenzhen Key Laboratory for Minimal Invasive Medical Technologies, Institute of Optical Imaging and Sensing, Graduate School at Shenzhen, Tsinghua University, Shenzhen 518055, China.
** Corresponding author at: Department of Laboratory Medicine, Guangdong Second Provincial General Hospital, Guangzhou 510317, China.
E-mail addresses: guantian@sz.tsinghua.edu.cn (T. Guan), caodl@gd2h.org.cn (D. Cao).
[1] These authors contributed equally to this work and should be considered co-first authors.

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For acquiring the qualitative and quantitative information of target analytes, an analysis instrument is necessary for manipulating microbeads to decode and count labels. Flow cytometry, as the most common detecting method in the micronbeads-floating liquid environment, can achieve the excitation and collection of fluorescence signal for an individual bead in a short time (Nolan and Sklar, 2002). However, the fast flowing speed and unexpected viscous force in fluid could disturb the transmitting of microbeads, which subsequently causes the instability of optical stimulation conditions, impairing the reliability and accuracy of signals (Li et al., 2017). The other manipulate techniques such as electric, magnetic or acoustic manipulation (Barbee et al., 2009; Barbee and Huang, 2008; Wiklund et al., 2004), more or less have some restrictions like a very low speed with a few minutes (Thompson and Bau, 2010) or deviating from the focus of objective (Wiklund et al., 2004), which restricted the wider application of microbeads-based detection. Therefore, it is necessary to design a simpler, more universal and stable analysis system for microbeads.

Optical tweezers, as a manipulation technology first developed by Ashkin and Dziedzic (1975) and Ashkin et al. (1987, 1986)), can perfectly overcome the shortcomings in the above methods. This technique employs a tightly focused Gaussian laser beam to trap and manipulate the nanoscale and microscale objects, such as gold and silver nanoparticles (Bosanac et al., 2008; Hansen et al., 2005; Jauffred et al., 2015), red blood cells (Mao et al., 2005; Zhong et al., 2013), single proteins (Pang and Gordon, 2012) and so on. Considering the trapping force comes from the photon momentum exchange, optical tweezers could realize the non-touch trapping to objects, which makes it one of the most effective micromanipulation technology in material science and life science and have been progressively used in biomedical research (Galajda and Ormos, 2003; Tanaka et al., 2011; Tanaka and Wakida, 2015). Since the gradient force generated by optical tweezers is sufficient to overcome the fluid viscous force, beads in the flowing liquid system can be quickly trapped in seconds and immobilized to the exact laser focal point (Li et al., 2017). With such property, QDs encoded or labeled microbeads could be put in the optimized location for stimulation, providing accurate and stable signal for target analytes.

Herein, we present a spectral-optical-tweezer-assisted fluorescence multiplexing system and use it to trap and decode QDs-encoded microbeads, realizing multiplexed detection. In our study, QDs with multiple colors and dosages were assembled in polystyrene microbeads by self-healing method to prepare fluorescence encoded microbeads (Song et al., 2014). Then, after surface modification and bioprobe grafting, the microbeads were used in fluorescent immune hybridization to capture the QDs-labeled target biomolecules in liquid samples. The reacted mixture was dropped on the slide of our home-built optical system without separation and purification. The microbeads were trapped at stimulation focus and then decoded to collect the fluorescence spectra. After finishing the analysis to one microbead, the optical tweezer stops working until the trap focus moves an increment which approach to the trapping diameter of optical tweezer field. Through this way, the whole slide could be roughly scanned and the bead-array experienced sufficient data collections.

Spectral-optical-tweezer-assisted fluorescence multiplexing system could be built with simple optical components and the detection is operated in free space, which highly reduce the cost and manipulation difficulty of detection. Further, the optical trap formed by optical tweezer could achieve the trapping of microbeads with different diameters, providing more universality to this multiplexing system. Finally, once microbeads are attracted by the gradient forces from optical trap, they would be dragged to the stimulation focus spontaneously, which provides an optimized stimulation position and stable signal output.

2. Experimental

2.1. The detection principle and optical setup

The illustration of our detection mode and home-built spectral optical tweezer is shown in Fig. 1. First, as shown at the left of the figure, under high temperature and the effect of pore-foaming agent, the porous polystyrene microbeads were swelled and mixed with QDs. With continuous stirring, the QDs penetrated into the microbeads. Then, while the temperature decreasing, the microbeads started the self-healing process and locked the QDs inside themselves. In this

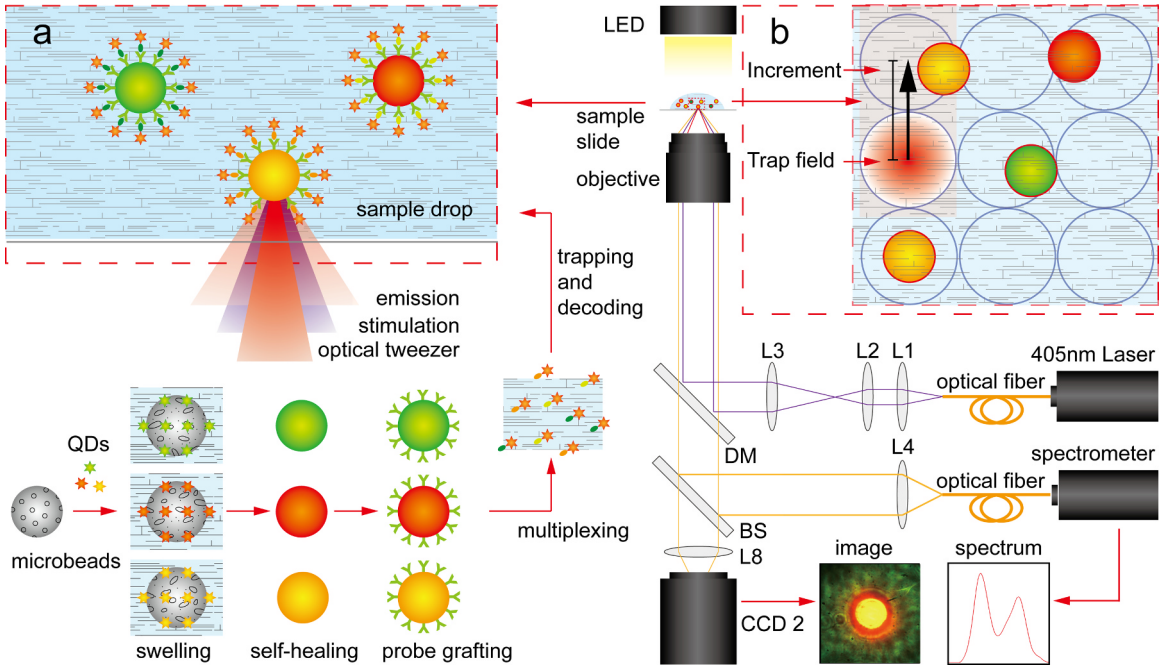

Fig. 1. The schematic illustration of our detection mode with one-step method and the setup of our spectral optical tweezer assisted fluorescence multiplexing system. The purple line represents the optical route of the 405 nm single mode laser beam. The orange line shows the bright-field illumination and the fluorescent signals. The beam expander system of 405 nm laser consists of L2 and L3. L: lens; DM: dichroic mirror; BS: beam splitter. The image represents the monitoring image of the trapped microbead. The spectrum represents the fluorescence spectra, including encoding signal and label signal, of the biomolecule-conjugated microbead. The inset illustration **a** describes the detail in the sample drop. The inset illustration **b** demonstrated the scan mode of the optical system.

procedure, using different proportions of multicolor QDs, we produced multiple types of QDs-encoded microbeads. Afterwards, every single type of encoded microbeads were modified and grafted with bioprobe on the surface. In multiplexed detection, several types of bioprobe-conjugated microbeads were added into the same sample to capture multiple target biomolecules with QDs labels. The biomolecules-conjugated microbeads suspension was dropped onto the sample slide of optical system for next-step detection.

In our home-built fluorescence multiplexing system, optical tweezer was used to trap beads and locked them at stimulation focus point. Then the beads were stimulated and the emissions were collected and analyzed with spectrometer. In detail, 405 nm single mode laser was used as trapping and decoding light source. The laser beam was output from the optical fiber and collimated with L1. Then, the lens group (L2, L3) was used to adjust the diameter of laser beam to make it suitable for the entrance pupil of oil objective lens (Olympus, 100×, NA = 1.30). The laser beam was reflected by DM (Thorlabs, DMLP500) and focused by objective, the focus point was put in the sample on the slide. The 405 nm single mode laser beam could form an optical trap and produce attraction force to the nearby microbeads. Once the microbead was trapped by optical tweezer, it would move to the focus point. Meanwhile, the encoding and label QDs could be stimulated by the 405 nm laser. LED was used as lighting source to offer bright-field illumination to observe the trapping process. The bright-field illumination and fluorescence emission were collected by objective and passed through DM. The BS with a splitting ratio of 1:9 was used to split the fluorescence emission into two beams. The stronger one was reflected and focused by L4 and coupled in self-made spectrometer for spectra analysis. The weaker one passed through the BS and delivered into matrix CCD (ZWO, ASI178MC) by L5 for monitoring. In our experiments, the sample slide was assembled on a three-dimensional translation table. The generated optical trap scanned the focal plane to trap microbeads. Once an encoded microbead was stably trapped, the encoding and label QDs were stimulated. Then the fluorescence emission was collected and analyzed to show the spectra. After that the analyzed microbeads could be released and the optical trap moved an increment and prepared for its next trapping. Every obtained fluorescence spectrum from microbeads consisted of two types of signals, including the decoding signals and label signals. Based on decoding signal, we could recognize the type of microbeads and then realize the qualitative detection to different biomolecules. The label signal reflected the amount of conjugated biomolecules on the microbead, which could linearly indicate the quantitative information of target biomolecules in samples.

2.2. Materials and regents

Monodispersed polystyrene microbeads (PS, diameter = 10 μm and 5 μm, pore diameter ≈ 100 nm) were purchased from Nano-Micro Research Center, Peking University (Beijing, China). CdSe/ZnS QDs (525, 565, 585 and 625 nm) and four types of QDs-labeled Anti-IgGs (525 nm@ goat anti rabbit IgG, 610 nm@ goat anti human IgG, 655 nm@ goat anti mouse IgG, 655 nm@ goat anti human IgG) were obtained from Wuhan Jiayuan Quantum Dot Technological Development Corporation (Wuhan, China). Three types of IgGs (rabbit, human and mouse) and goat-anti-human-IgG were purchased from Bioss Biotechnology Co., Ltd. (Beijing, China). Bull serum albumin and no-protein blocking buffer was supplied by Solarbio Life Sciences. Co. (China). Ethanol, cyclohexane, hexadecane, chloroform, Polyethylenimine (PEI), polysodiumpstyrenesulfonate (PSS) and glutaraldehyde were obtained from Aladdin Industrial Corporation (Shanghai, China). Human serum was supplied by Peking University Shenzhen Hospital (China).

2.3. Preparation of QDs-encoded microbeads

10 mg of PS were dispersed into 4 mL of hexadecane. 100 μL of QDs n-hexane solution (3 mg/mL) were mixed with 3 mL of chloroform. Then, two dispersions were ultrasonically vibrated for 5 min and then poured into a three-necked flask. The three-necked flask was assembled with a condenser pipe and a set of nitrogen protection device. The flask was put into an oil bath pan and the inside dispersion was magnetically stirred. Next, to heat up the dispersion, we raised the temperature of oil bath pan to 180 °C and kept it for 10 min to evaporate the chloroform. After evaporation, the dispersion was transferred into a glass tube and dealt with water-cooling. Finally, the microbeads were collected from the dispersion by centrifugation. After alternate washing for three times with ethanol and cyclohexane, the encoded microbeads were dried at 60 °C in vacuum oven.

2.4. Preparation of immunomicrobeads

10 mg of QDs-encoded microbeads were added into 10 mL PEI solution (4 mg/mL, 0.5 M NaCl) and the mixture was intensely stirred for 1 h. Afterwards, the microbeads were collected and washed with water. The PEI-modified microbeads were added into 10 mL PSS solution (2 mg/mL, 0.5 M NaCl) and stirred for 1 h. After that, another PEI modification was carried out again on encoded microbeads. The modified microbeads were washed and then dispersed into 1 mL of PBS solution (50 mM, pH 7.4). 100 μL of microbeads suspension was took out from the dispersion and put into 1 mL glutaraldehyde solution (5%, PBS). After 3 h of stirring, the microbeads were washed and collected. For the one-step immunoadsorption experiments, the above microbeads were added into 1 mL IgG solution (1 mg/mL, PBS). The dispersion was gently stirred and incubated overnight at 4 °C. Subsequently, IgG as the probe was conjugated to the microbeads, then washed three times with PBS solution and blocked with 1 mL no-protein blocking buffer for 2 h

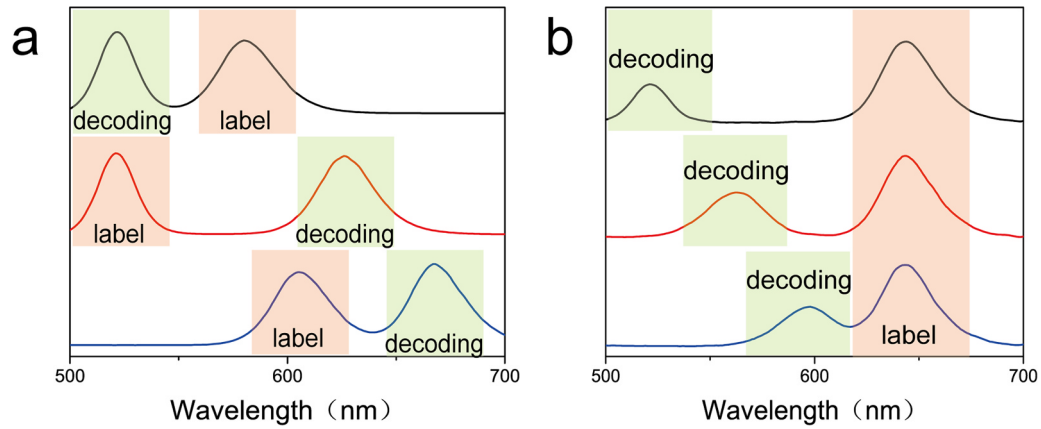

Fig. 2. Principle of optical analysis to the QDs encoded and labeled immunoassay experiments. a. Visual demonstration of multiplexed detection principle by label with different kinds of QDs in one-step immunoadsorption experiments. b. Practical detection model of real sample with sandwich immunoassay method.

at 37 °C. For the sandwich immunoassay experiments, anti-IgG was used as the probe that conjugated to the microbeads.

2.5. Multiplexed and quantitative detection in PBS solution

We carried out one-step immunoadsorption experiments to achieve multiplexed detection to different types of anti-IgGs in the same sample. We selected three types of immunomicrobeads (IM1: 525 nm; IM2: 565 nm; IM3: 625 nm) to perform the multiplexing. The corresponding conjugated-bioprobes are human IgG for IM1, mouse IgG for IM2 and rabbit IgG for IM3. Three types of immunomicrobeads were added into mixed PBS solution of QDs-labeled anti-IgG, including 610 nm QDs-labeled goat-anti-human-IgG, 655 nm QDs-labeled goat-anti-mouse-IgG and 525 nm QDs-labeled goat-anti-rabbit-IgG. The suspension was gently stirred and incubated for 30 min at 37 °C. It would be proved that the qualitative analysis could be successfully achieved if the decoding result match with the type of anti-IgG. Also, as contrast experiment, three types of QDs (525, 565 and 625 nm) encoded microbeads, covered with BSA instead of IgG on the surface, were reacted with a same sample as fake immunomicrobeads (FIM). Further, to verify the concentration response performance of our method, we carried out gradient immunoadsorption experiments to anti-IgG solutions. In detail, anti-IgG in PBS solutions with concentrations of 0.31 nM, 0.62 nM, 1.25 nM, 2.5 nM, 5 nM, 10 nM were tested as samples. Theoretically, the detected fluorescence intensities of label peaks would proportionally change in accordance with gradients of concentration of target molecules solution, providing the signal for quantitative analysis.

2.6. Specificity investigation and quantitative detection in human serum

The sandwich immunoassay method was utilized to carry out the specific and quantitative detection experiments, verifying the application of our method to the unlabeled real samples. In this part, a type of immunomicrobeads (IM4: 565 nm) was conjugated with the goat-anti-human-IgG as bioprobe. To investigate the ability of selective capture, human IgG was dispersed in human serum as the specific sample and pure human serum was considered as the blank sample. The human serum was diluted 10-fold with standard human whole serum. The IM4 suspension was gently stirred and incubated with the specific and blank sample respectively for 1 h at 37 °C. After washing with PBS solution for three times, the above microbeads were dispersed in the separate but equal 655 nm QDs-labeled goat-anti-human-IgG solution, stirred gently and incubated for 45 min at 37 °C. Then, the all reaction was finished and the microbeads were washed with deionized water for five times. Furthermore, to achieve the quantitative analysis of the target analytes, gradient immunoadsorption experiments were carried out. Specifically, human IgG was dispersed in human serum with the concentrations of 0.88 pM, 1.75 pM, 3.5 pM, 7 pM, 14 pM, 28 pM.

3. Results and discussion

3.1. Principle of optical analysis

In this article, encoding and label signal are both provided by QDs. Decoding peaks are used for qualitative analysis, indicating which type

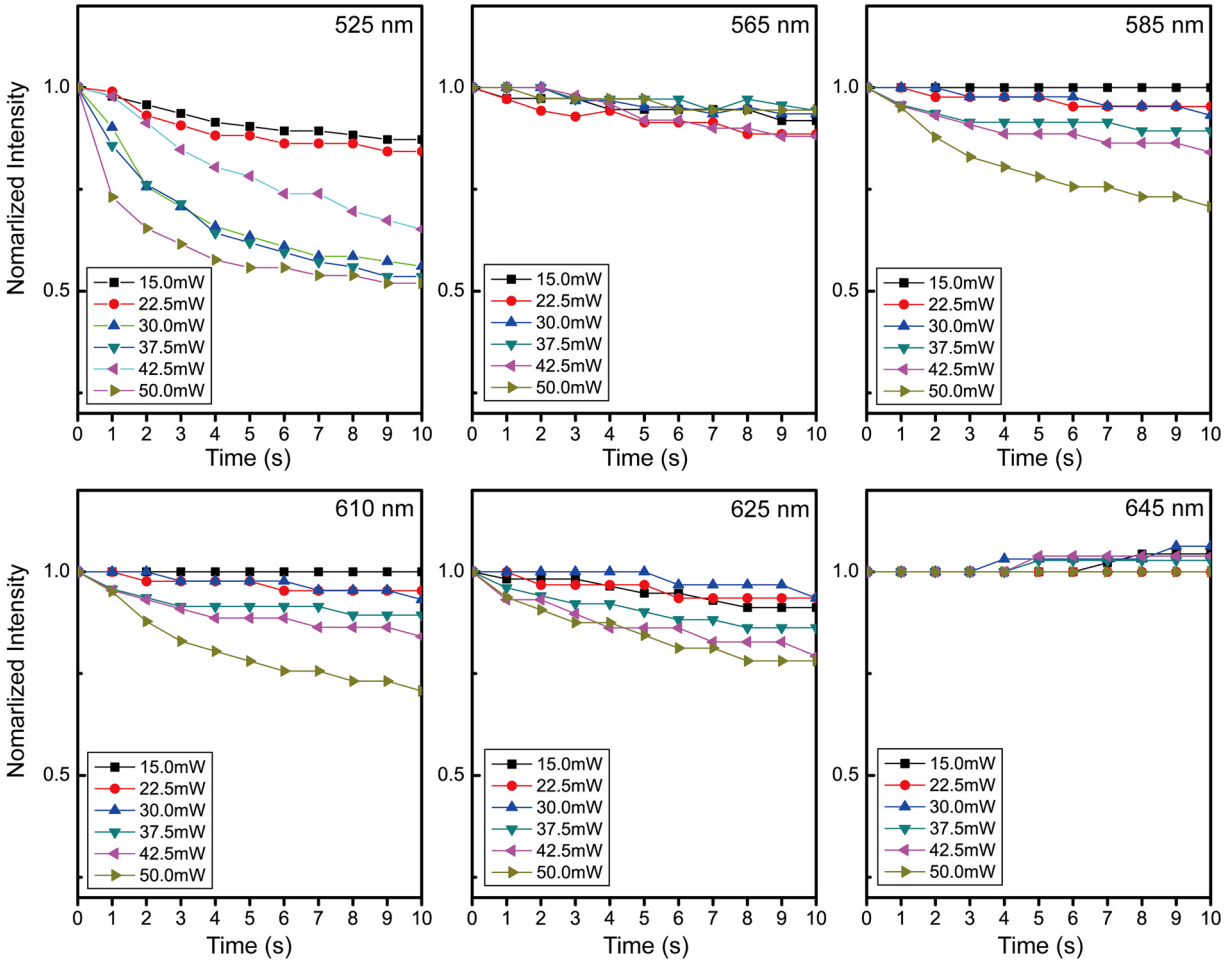

Fig. 3. The fluorescence stability of spectral-optical-tweezer-assisted fluorescence multiplexing system to QDs with different emission wavelengths under different output power. The exposure time was 20 ms.

the analyte is. Label peaks are normally used for the quantification of analytes. The role of each peak is marked with a specific color in all figures where decoding and label peaks are existed in the same spectrum. The multiplexed detection principle by label with different kinds of QDs in one-step immunoadsorption experiments are shown as the Fig. 2a. For making a visual demonstration of multiplexed detection principle, multiple QDs with different emission wavelengths that labeled with different types of anti-IgGs are mixed as the sample in our one-step fluorescence immunoassay multiplexed detection experiment. After capture and stimulation by our spectral-optical-tweezer-assisted fluorescence multiplexing system, the fluorescence spectrum with distinct decoding peaks and label peaks are obtained. Matching the peak positions with emission wavelengths of encoding and label QDs, the detection and analysis of multiple target analytes simultaneously in one sample are achieved. Whereas, for the practical detection model of real sample, sandwich immunoassay method is used. As the Fig. 2b shown, only one type of QDs-encoded anti-IgG are used to quantify the multiple targets.

3.2. Investigation of optical stability and trapping performance

Although QDs materials are well-known for their high photobleaching resistance performance, the fluorescence quenching still happens under strong exposure. To explore the most suitable output power for our optical system, we measured the fluorescence stability to QDs of spectral optical tweezer. In detail, QDs solutions were dropped on the slide to experience the stimulation under different output powers of single mode laser. The fluorescence intensities collected by spectrometer at different times were recorded. The fluorescence quenching curves were shown in Fig. 2. It can be observed that our optical system caused different quenching effect to QDs with different colors in 10 s. However, mostly, the stability of fluorescence could be improved by using lower output powers. Concerning the trapping requirements and fluorescence quenching, we decided to use 22.5 mW as the final output power of single mode laser.

We carried out trapping experiments to microbeads with different diameters to investigate the trapping performance of optical tweezer. Firstly, the hydrophobic monodispersed polystyrene microbeads were added into PEI solution (4 mg/mL, 0.5 M NaCl) to achieve the hydrophilic decoration. Then, they were dispersed into pure water to test the trapping ability of the optical tweezer. As shown in Fig. 3a, the 10 μm microbead was trapped and dragged to the trap focus. The time lines indicated that the microbeads in the trapping field with 10 μm radius could be dragged to the focus in 1 s (see Video 1 in Supplementary Information). In Fig. 3b, the trapped microbead was locked in focus and moved around 36 μm in 1 s (see Video 2 in Supplementary Information). Fig. 3c, d showed the trap and locking performance of 5 μm microbeads. Above results demonstrated that the optical tweezer owns strong trapping ability and locking intensity to microbeads, which guaranteed the trap performance in further steps. Also, to microbeads with different diameters, optical tweezer could be used universally, indicating that this multiplexing system could work with bead-array with different sizes.

Supplementary material related to this article can be found online at doi:10.1016/j.bios.2019.01.004.

3.3. Characterization of QDs-encoded microbeads and decoding performance of optical system

By using high-temperature self-healing method, we prepared eight types of QDs-encoded microbeads. The fluorescence micrographs of eight types of encoded microbeads were shown in the images of Fig. 4a–h. It could be observed that the QDs were well assembled and

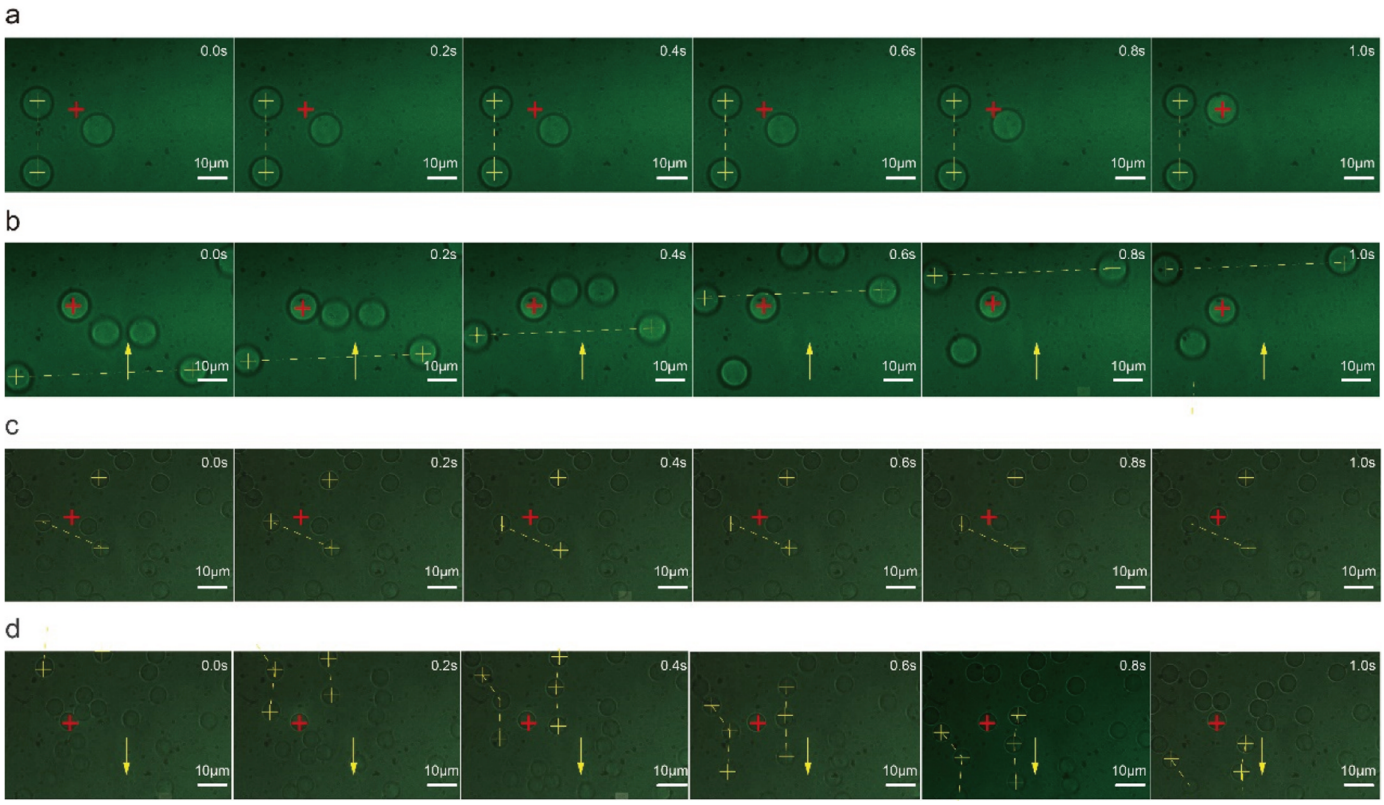

Fig. 4. a. Monitoring images of trapping process to 10 μm microbead. b. Monitoring images of locking and moving process to 10 μm microbead. c. Monitoring images of trapping process to 5 μm microbead. d. Monitoring images of locking and moving process to 5 μm microbead. The red cross markers in all images represent the locations of trapping focus. The yellow arrows represent the relative moving directions of trapping focus. The yellow cross markers and dotted line represent the surrounding references. Scale bars in all images are 10 μm (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

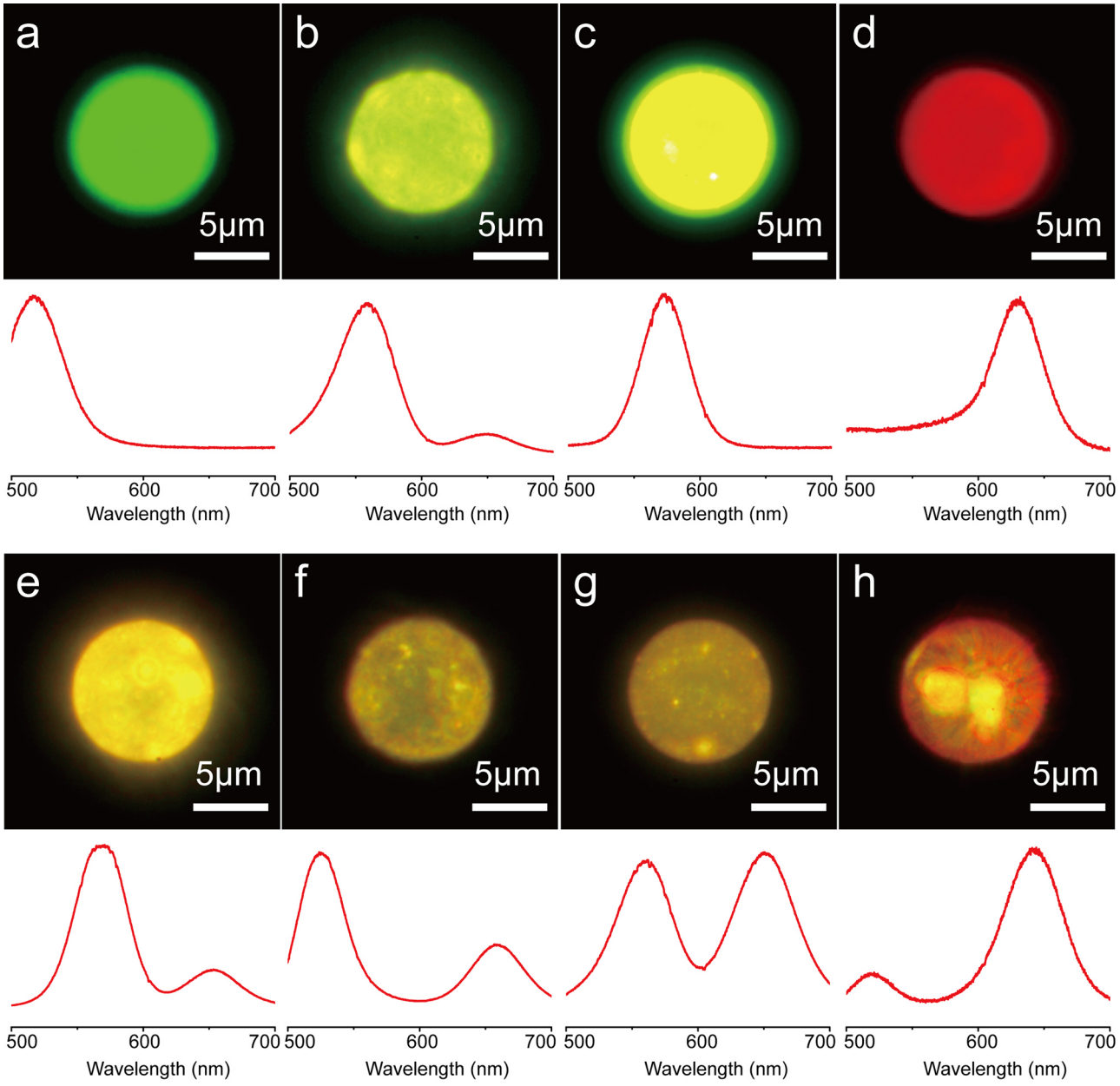

Fig. 5. The fluorescence micrographs and corresponding spectra of synthesized QDs-encoded-microbeads. Scale bars in all images are 5 μm. The microbeads were encoded by a. 525 nm; b. 565 nm; c. 585 nm; d. 625 nm; e. 565 nm: 645 nm = 4:1; f. 525 nm: 645 nm = 5:2; g. 565 nm: 645 nm = 1:1; h. 525 nm: 625 nm = 1:5. All of the spectra were obtained by our home-built system. The exposure time was 20 ms and we sampled 20 microbeads for every gradient.

distributed in the microbeads. Meanwhile, the trapping ability to microbeads is not affect by the assembling of QDs (see Video 3 in Supplementary Information). As shown in the below spectra, the spectra of encoded microbeads were measured with our spectra optical tweezer assisted optical system. As we designed, the fluorescence emissions of microbeads in Fig. 4a–d were encoded with 4 types of monochrome QDs (525, 565, 585 and 625 nm). The microbeads in Fig. 4e–h were encoded with multicolor QDs in different intensity ratios, showing as follow: Fig. 4e: 565 nm: 645 nm = 4:1; Fig. 4f: 525 nm: 645 nm = 5:2; Fig. 4g: 565 nm: 645 nm = 1:1; Fig. 4h: 525 nm :625 nm = 1:5. It is believable that the different types of encoded microbeads had been labeled with their own fluorescence emission spectra. Meanwhile, the decoding results also demonstrated the decoding ability of our optical system.

Supplementary material related to this article can be found online at doi:10.1016/j.bios.2019.01.004.

3.4. Multiplexed detection performance in PBS solution

Using three types of immunomicrobeads, we carried out one-step fluorescence immunoassay multiplexed detection and contrast experiments to explore the multiplexed detection performance of our system. Three types of immunomicrobeads (IM1, 2, 3) were used to detect the sample with QDs-labeled anti-IgGs. After reaction, the anti-IgG-conjugated immunomicrobeads were trapped and stimulated by our system. The stimulated fluorescence micrographs were recorded by the matrix CCD and shown in Fig. 5a, b, c. From the colors of surface, we can determine that the corresponding types of anti-IgGs had been captured by our bead-array. FIM, covered with BSA instead of IgG, acted the bead-array in the contrast group, and the fluorescence micrographs were shown in Fig. 5d, e, f. The comparisons of fluorescence colors directly illustrate the adsorption specificity of our bead-array in multiplexing. The collected spectra, shown in Fig. 5g, h, i, could be

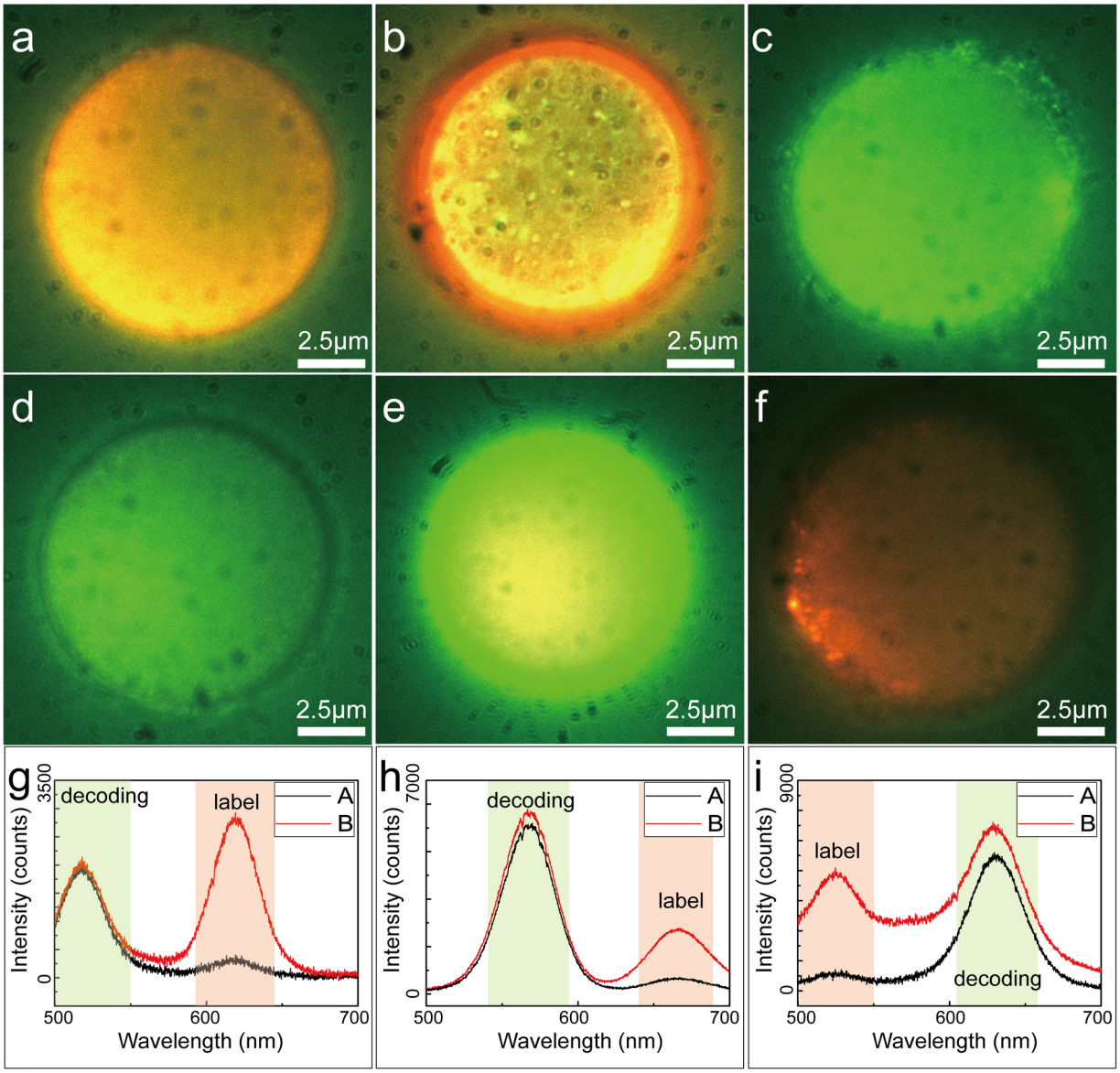

Fig. 6. Multiplexed detection of three types of anti-IgGs in PBS solution. a. Monitoring image of trapped and stimulated IM1 after multiplexed detection. b. Monitoring image of trapped and stimulated IM2 after multiplexed detection. c. Monitoring image of trapped and stimulated IM3 after multiplexed detection. d. Monitoring image of trapped and stimulated FIM1 after multiplexed detection. e. Monitoring image of trapped and stimulated FIM2 after multiplexed detection. f. Monitoring image of trapped and stimulated FIM3 after multiplexed detection. g. Spectral comparison of fluorescence emissions between IM1 (B) and FIM1 (A) after multiplexed detection. h. Spectral comparison of fluorescence emissions between IM2 (B) and FIM2 (A) after multiplexed detection. i. Spectral comparison of fluorescence emissions between IM3 (B) and FIM3 (A) after multiplexed detection. Scale bars are 2.5 μm in monitoring images. All of the spectra were obtained by our home-built system. The exposure time was 20 ms and we sampled 20 microbeads for every gradient.

regarded as more accurate proofs. The red lines in the spectra represent the normal multiplexed detection group and the black ones show the data of contrast group. As we can see, in normal group, all of the emission spectra of immunomicrobeads consisted of distinct decoding peaks and label peaks, and the peak positions match well with colors of encoding and label QDs, which means the bead-array can well and truly recognize their targets in multiplexing. Meanwhile, the label peaks in contrast group were much weaker, verifying the specificity of bead-array in multiplexed detection in PBS solution again.

3.5. Quantitative detection performance in PBS solution

We carried out gradient fluorescence immunoassay experiments to investigate the concentration response ability of our spectral-optical-tweezer-assisted fluorescence multiplexing system. We prepared anti-IgG in PBS solutions at different concentrations, including 0.31 nM, 0.62 nM, 1.25 nM, 2.5 nM, 5 nM, 10 nM. Then we added the corresponding types of immunomicrobeads into the solution to capture anti-IgG molecules. The anti-IgG-conjugated immunomicrobeads suspensions were dropped on the sample slide. As the trap focus moves, different microbeads were trapped and detected, the fluorescence spectra were collected and analyzed. As shown in Fig. 6a, the fluorescence emission spectra of anti-IgG-conjugated IM1 from samples with various concentrations were obtained by our system. Every spectrum was the average value by sampling 20 microbeads in the same concentration. Obviously, the intensities of label peaks show the gradient variation according to concentrations, which were caused by the amount difference of conjugated 610 nm QDs-labeled goat-anti-human-IgG on the surface. Based on these data, we fitted a concentration response curve for IM1 and showed it in Fig. 6b. It turns out we could obtain a linear

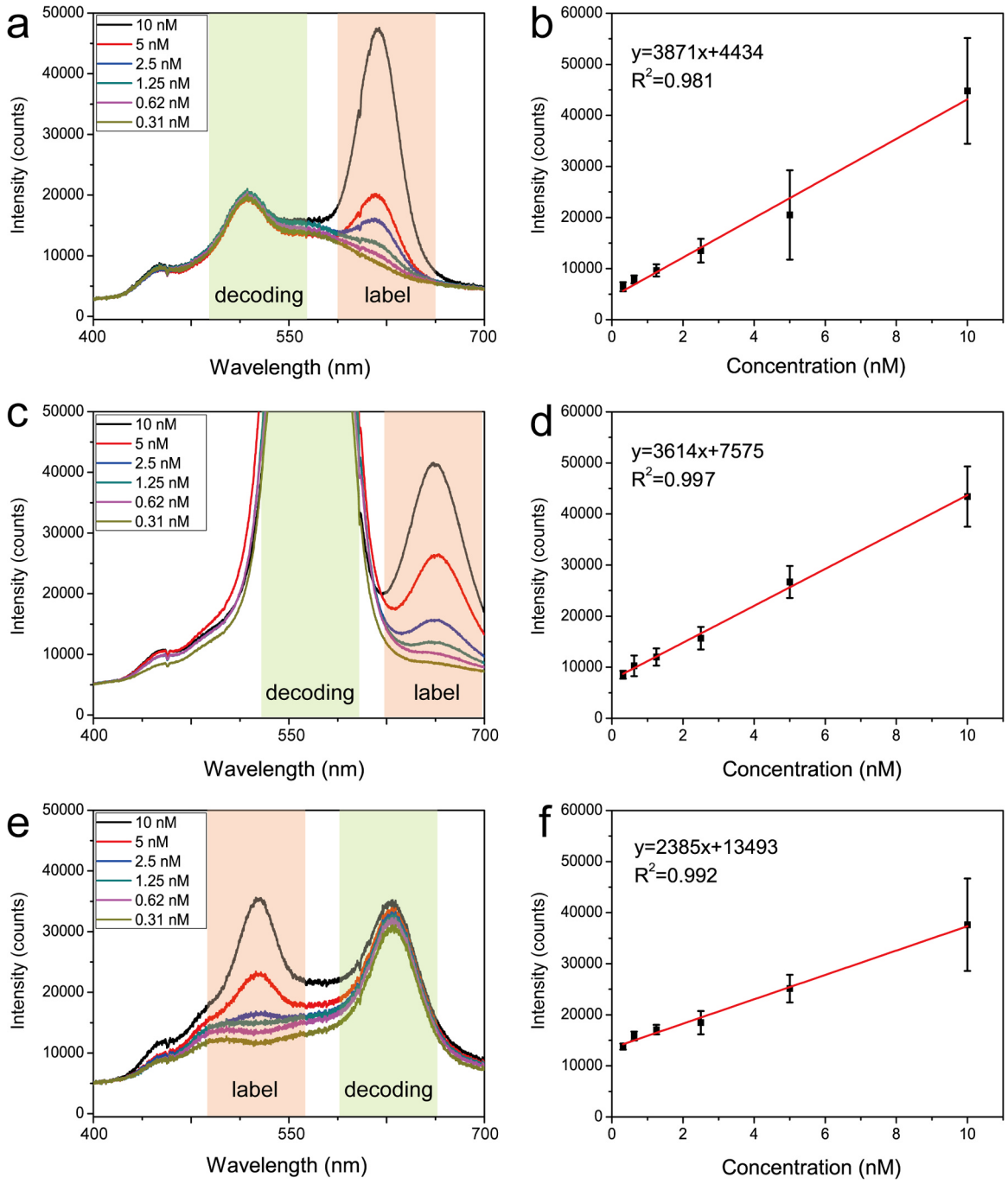

Fig. 7. Quantitative detection of three types of anti-IgGs in PBS solution. a. Fluorescence emission spectra of IM1 in gradient detection from 0.31 to 10 nM. b. The standard response curve of IM1 in gradient detection from 0.31 to 10 nM. c. Fluorescence emission spectra of IM2 in gradient detection from 0.31 to 10 nM. d. The standard response curve of IM2 in gradient detection from 0.31 to 10 nM. e. Fluorescence emission spectra of IM3 in gradient detection from 0.31 to 10 nM. f. The standard response curve of IM3 in gradient detection from 0.31 to 10 nM. All of the spectra were obtained by our home-built system. The exposure time was 20 ms and we sampled 20 microbeads for every gradient.

standard response curve from our detection. We measured the blank signals and their 3-fold the standard deviation (n = 11). The limit of detection (LOD) was calculated to be 1.52 p M. As for IM2 and IM3, the data processing was respectively shown in Fig. 6c–f. The LODs for IM2, 3 are 3.82 pM and 6.45 pM respectively. It is worthy to note that the LOD could be highly optimized by adopting label QDs with higher quantum yields or increasing its binding efficiency with antibody. Furthermore, through attenuating the intensity of the encoding fluorescent signal, background noise can be reduced, which also could contribute to higher sensitivity detection. The multicolor QDs used in our experiments were meant to be illustrations for multiplexing. On the basis of the results of gradient detection, it should be reliable to claim that our spectral-optical tweezer-assisted fluorescence multiplexing system has the quantitative analysis ability (Fig. 7).

3.6. Specificity investigation and quantitative detection in human serum

For confirming the practical application of our method in real sample, the sandwich immunoassay method was used to carry out the specific capture and quantitative detection of un-labeled targets. Since

the practical samples containing many disturbing components, like the other proteins or common ions, the specific ability is critical to detection performance of immunoassay method. To investigate the specificity performance of our method in actual detection environment, all the detection experiments were performed in human serum.

The schematic illustration of sandwich immunoassay method in human serum is shown as Fig. 8a. First, the QDs-encoded microbeads were prepared the same as one-step method, then anti-IgG as the probe was used to capture the target IgG in the human serum, followed by quantification of the target with QDs-labeled anti-IgG. In the specific detection experiment, IM4 were conjugated with the goat-anti-human-IgG, the human IgG dispersed in human serum and pure human serum were utilized as the specific sample and blank sample respectively. As the results show, Fig. 8b, c exhibit the monitoring image of trapped and stimulated IM4 after detection with specific sample and blank sample. The surface color of reacted microbeads in monitoring image indicated that the microbeads we prepared could perform the selectively capture to the specific target with negligible nonspecific adsorption.

Further, the gradient fluorescence immunoassay experiment was carried out for the practical quantitative detection of target. As we mentioned in the previous section, the experiment and detection conditions can be optimized to achieve better performance in LOD. By reducing the fluorescence intensity of decoding signal and adopting label QDs with higher binding efficiency to antibody, a better experimental condition was acquired. Therefore, the human IgG with the concentrations of 0.88 pM, 1.75 pM, 3.5 pM, 7 pM, 14 pM, 28 pM were taken as the sample of IM4. The blank test was conducted in pure human serum. The fluorescence emission spectra of reacted IM4 after gradient detection were obtained by our system, 20 microbeads were sampled to obtain the average value of every spectrum in the same concentration as the results shown in the Fig. 8d. It can be observed that the intensities of label peaks are increased concomitantly with the increasing concentration of the human IgG. For a detailed quantitative investigation, the results were further analyzed by fitting fluorescence intensity with the human IgG concentration and showed in Fig. 8e. With a correlation coefficient (R^2) of 0.997, a linear standard response curve was obtained in our detection range. We measured the blank signal and used their 3-fold standard deviation (n = 11) for the LOD calculation, obtained a value of 0.23 pM. From these results, it can claim that our proposed method of QDs-encoded bead-array combining with the spectral-optical-tweezer-assisted fluorescence multiplexing system has promising potential for specific and quantitative analysis of target molecules in real samples.

3.7. Comparison with other methods for biomolecule detection

Considering the comparison with other methods, ELISA is a simple and relative mature method for quantitative analysis, but needs large

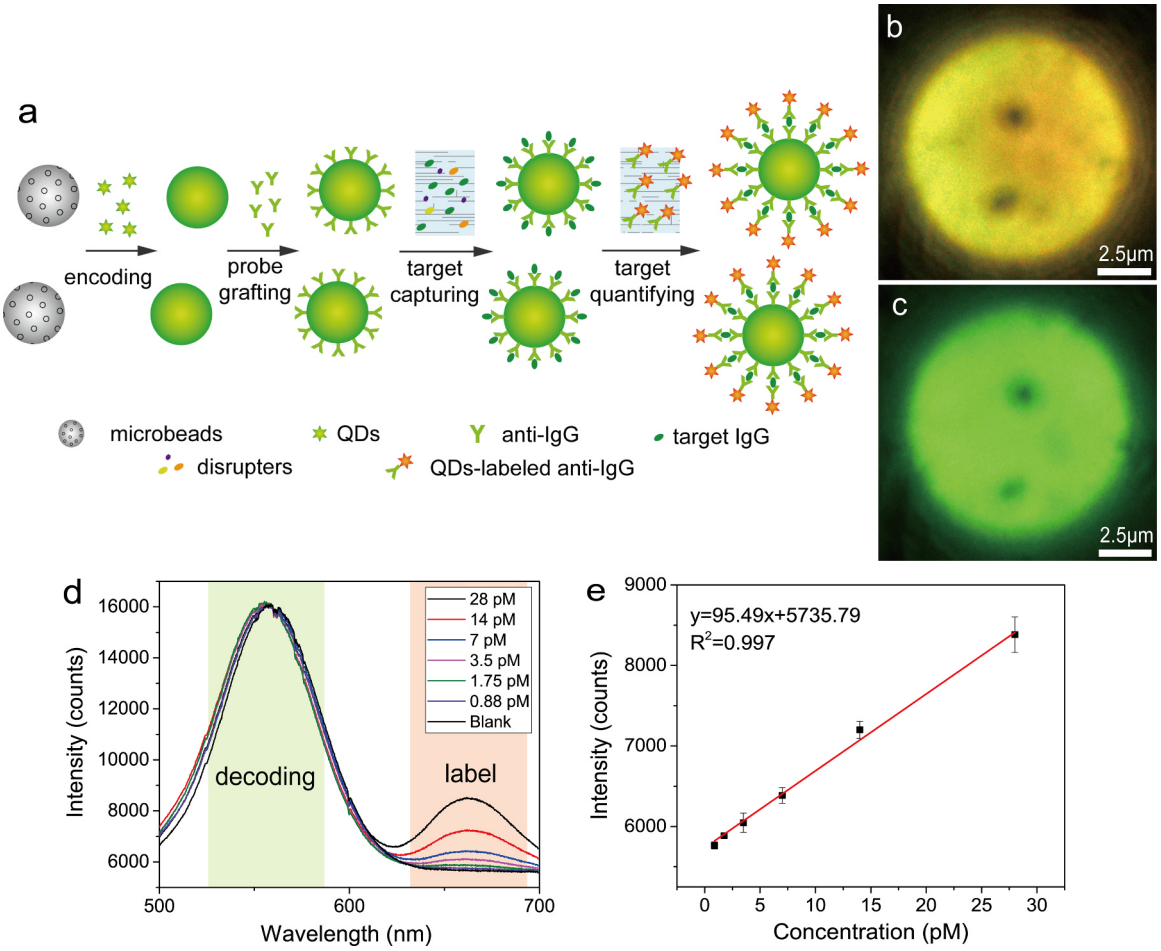

Fig. 8. Specific and quantitative detection of target in human serum a. Schematic illustration of sandwich immunoassay method in human serum. b. Monitoring image of trapped and stimulated IM4 after specific detection of human IgG in human serum (specific sample). c. Monitoring image of trapped and stimulated IM4 after specific detection in pure human serum (blank sample). d. Fluorescence emission spectra of IM4 in gradient detection from 0.88 to 28 pM in human serum. The spectrum of blank sample was obtained by replacing target IgG with pure human serum. e. The standard response curve of IM4 in gradient detection from 0.88 to 28 pM in human serum. Scale bars are 2.5 μm in monitoring images. All of the spectra were obtained by our home-built system. The exposure time was 20 ms and we sampled 20 microbeads for every gradient. The human serum was diluted 10-fold with standard human whole serum.

sample volume and specific equipment, and only one target can be detected at the same time (Krishhan et al., 2009; Spielberg et al., 1989); Western blotting has good sensitivity, however it lies the limitation in inaccuracy results and technical demand (Liu et al., 2013; Mahmood and Yang, 2012); the electrochemiluminescent and electrochemical methods, in benefits of higher sensitivity and wider range in effective detection (Guo et al., 2013; Zhu et al., 2018), whereas, cannot detect multiple targets simultaneously. The equipment of optical tweezer, compared with the method of flow cytometry, has good operability and durability with low cost owing to the use of simple optical component and construction structure. Moreover, once the microbead is captured, it would be dragged to the best stimulation position, providing a stable signal.

For our method, as all the results showed, QDs-encoded bead-array combined with the spectral-optical-tweezer-assisted fluorescence multiplexing system method exhibits good performance in multiplexed immunoassay, and the ability of selective and sensitive capture in human serum has been proved. With the ideal performance in protein detection, we anticipate that the proposed method has powerful application in medical diagnostics by achieving multiplexed detection of biomolecules in the real samples with high sensitivity.

4. Conclusion

In a summary, we proposed spectral-optical-tweezer-assisted fluorescence multiplexing system and used it in the QDs-encoded bead-array bioassay. By trapping and decoding amounts of biomolecules-conjugated immunomicrobeads, we recognized the types of immunomicrobeads and counted the concentrations of targets, thus achieving quantitative and qualitative detection to multiple contents in the same sample. As demonstrations, a series of detection experiments to different types of anti-IgGs in the PBS solution was carried out with our prepared immunomicrobeads and home-built optical system, verifying the multiplexing and quantitative abilities of our method. For confirming the practical application of our method to real samples, specific and quantitative detection experiments were performed in the human serum. The results show high specificity for target and the LOD reaches 0.23 pM. With the above experiments, we demonstrated that our proposed optical system could realize stable and strong trapping to bead-array and provide self-focusing, stable excitation and spectra analysis for it. This open-field detection mode shows high universality to bead-array with different sizes. Also, without highly elaborate components, our method owns superiorities in costs and durability. However, this method still suffers some limitations such as the relatively slow speed, and the future work should be concentrated on increasing the detection efficiency by optically trapping more beads at one time with multiple laser beams. It can be indicated that based on all the abilities and advantages, combined with some improvement, this system can be a powerful tool for further researches in multiplexed detection to biomolecules.

Conflicts of interest

There are no conflicts to declare.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bios.2019.01.004.

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