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Fluorescence Enhancement Utilizing Dielectric Microbeads with Semi-open Microwells

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***Abstract*—Dielectric microbeads can converge light into a narrow beam with high intensity which allows for enhancing the fluorescent signals. However, a critical challenge for the experimental realization is introducing the fluorescent samples into this extremely small beam area. Here, we design and fabricate dielectric microbeads with semi-open microwells in which localized converging light beam of high intensity can be generated. We show that fluorescent microspheres can be efficiently loaded into the semi-open microwells and thus simultaneously illuminated by the converging beam generated by the micro-optical structures without any further manipulations. Pronounced fluorescent enhancement of around 9 folds can be obtained in comparison with the fluorescent microspheres on glass substrates, stemming from the excellent convergent effect of this micro-optical structure.**

I. Introduction

Enhancing the signal of chromophores (i.e., fluorescent molecules or quantum dots) is a highly desirable goal for the practical applications in optical detection and imaging. The common strategy includes the enhancement of the local excitation intensity, the emission rate, or the radiation collection efficiency [1, 2]. These can be generally realized by properly tailoring the electromagnetic environment, via using plasmon coupling structures, photonic crystals, and dielectric microbeads, with demonstrated fluorescent enhancement up to several orders of magnitude [3-9]. Among these approaches, dielectric microbeads are considered as a direct and cost-effective route to enhance the fluorescence signal without requiring expensive nanofabrication facilities or complex near-field configurations. Upon illumination, dielectric microbeads could modulate the wavefront of the incident electromagnetic field, which is converged into a tiny beam of high intensity with subwavelength dimensions along the three directions of space, providing a universal and simple optical structure for fluorescent enhancement applications [10, 11]. However, the converging area is in general generated in an open space with extremely small effective area, making it technically difficult to precisely introduce the fluorescent samples into, hindering their usage in real applications. In this contribution, we fabricate dielectric microbeads with semi-open microwells to efficiently introduce the fluorescent samples into the converging area and simultaneously to enhance their fluorescent signals. The incident light beam can be modulated by this micro-optical structure and is confined in the semi-open microwells with high field intensity that is significantly exceeding the incident light. Fluorescent microspheres can be passively trapped inside the semi-open microwells during the evaporation process and further be enhanced by the high-intensity converging light up to about one order of magnitude.

II. Methods

1. *Fabrication of dielectric microbeads with semi-open microwells*

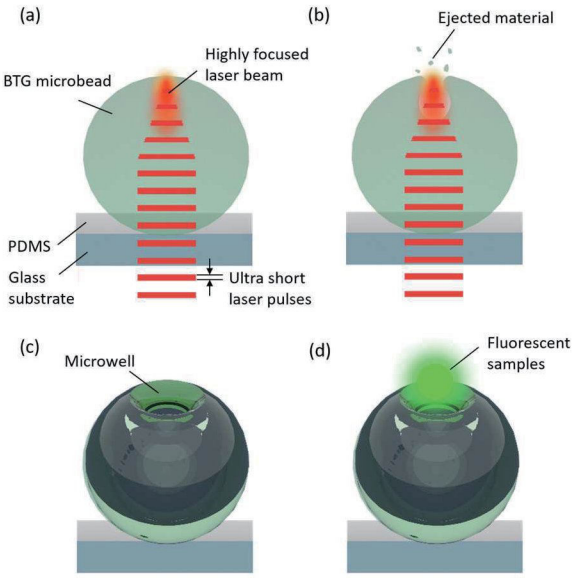

Fig. 1. (a) Barium titanate glass (BTG) microbead is illuminated from the bottom by femtosecond laser and a highly focused laser beam is generated. (b) BTG are melted and materials are removed from the foci. (c) A semi-open microwell is formed. (d) Signals of the fluorescent samples in the microwell can be enhanced.

The fabrication process is schematically illustrated in Fig. 1. Dielectric microbeads composed of barium titanate glass (BTG) with diameter ~80 μm and refractive index of 1.9 are used in our experiment. The BTG microbeads are coated on the glass substrate with a layer of uncured polydimethylsiloxane (PDMS). After the PDMS is cured, unattached BTG microbeads are discarded, leaving a single layer of BTG microbeads on the glass substrate. Femtosecond laser is utilized to fabricate semi-open microwells on the BTG microbeads. Upon exposing to a

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femtosecond laser from the bottom of the glass substrate, a focused high-intensity beam is generated near the top surface inside the BTG microbeads. Due to the heating effect generated by the highly focused laser beam, the illuminated BTG material is melted and ejected from the BTG microbeads, resulting a single semi-open microwell on each BTG microbead.

2. *Introduce of fluorescent microspheres in the microwell by droplet drying self-assembly*

The chip with BTG glass microspheres is cleaned twice with 2-proponal, rinsed with Milli-Q water and dried with nitrogen. Subsequently, it is treated with air plasma for 1 minutes to increase its hydrophilicity. Then 10uL of the fluorescent microspheres was dropped on the chip. Due to the hydrophilicity of the surface, the droplet spreads out over the whole surface area which benefits the evaporation of the liquid. During the droplet drying process, small amounts of the liquid that is trapped in the microwell evaporates slowly, resulting in capillary forces which draw fluorescent microspheres close by into the microwell. Then the chip is completely dried with nitrogen for further characterization.

3. *Fluorescence measurement under light microscope*

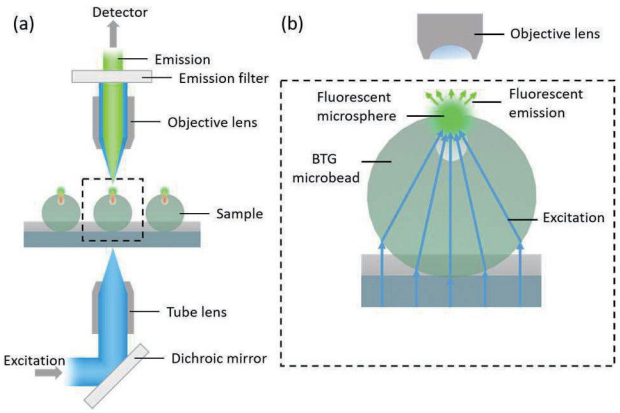

Fig. 2. (a) Optical configuration for recording the signal of the fluorescent microspheres and (b) the enlarged sketch showing the optical path in the black dashed box of (a).

The chip with the fluorescent microspheres is characterized under a light microscope (ZEISS Observer 7) with the light path shown in Fig. 2. The chip is illuminated by a power linearized LED light source (ZEISS Colibri 7) as the fluorescent excitation light. The emission fluorescent signal is collected by a 10× objective with NA of 0.25 (ZEISS Objective N-Achroplan) or a 40× objective with NA of 0.55 (ZEISS Objective LD A-Plan). The objective is focused on the focal plane of the fluorescent microspheres or the outer contour of the BTG glass spheres.

III. Results and Discussion

The prepared BTG microbeads with semi-open microwells are characterized under the light microscope and the scanning electron microscope (SEM, ZEISS Gemini 500), as shown in Fig. 3. The dielectric microspheres with semi-open microwells exhibit a dark circular area on the center of the top, arising from the scattering effect of the walls surrounding the microwell, Fig. 3 (a). The SEM images show the detailed view of the semi-open microwells, Fig. 3 (b) and (c). As a seen from the SEM images, a larger outer contour is on the top of the dielectric microsphere and then the height of the contour gradually decreases and eventually narrows into a semi-open microwell with a diameter of ~6μm. The interior of the semi-open microwell is observed as an ellipsoidal cavity with a maximum cross-sectional profile of ~13 μm and a height of ~20 μm, as illustrated in Fig. 3 (d).

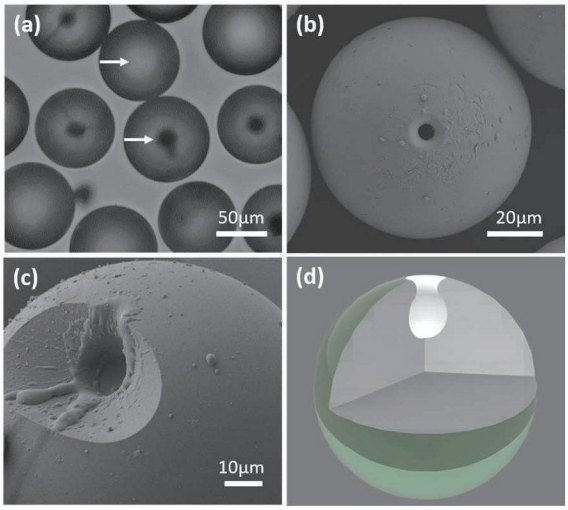

Fig. 3. (a) Optical microscope image and (b) (c) SEM images of the BTG microbeads with semi-open microwell. (d) Sketch illustration of the microbead with semi-open microwell.

Simulation based on finite element method (FEM) shows the ability of this micro-optical structure to converge light inside the semi-open microwell, as presented in Fig. 4. The light intensity inside the semi-open microwell exhibits pronounced enhancement compared with that of the incident light.

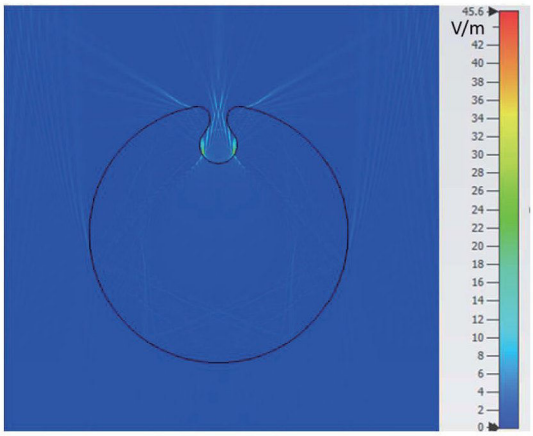

Fig. 4. Simulations based on finite element method (FEM) showing the ability of this micro-optical structure (diameter 80 μm) to converge light inside the microwell.

This micro-optical structure allows to load fluorescent samples directly into the semi-open microwells and to enhance their fluorescent signals. Since for the fluorescent microspheres, the intensity of the emission light has roughly a linear relationship with the intensity of the excitation light, that is, the confined light in the semi-open microwell. Thus the fluorescent microspheres can be utilized as indicators to report the intensity of the confined light in the microwells. It

is hypothesized that the fluorescent microspheres loaded in the microwells are exposed and excited by the confined light generated by the micro-optical structure with high intensity, thus exhibiting a higher intensity on their emission light, in comparison with the fluorescent microspheres outside the microwells, i.e., on the glass substrate. Fluorescent microspheres of different sizes (1μm, 5μm, 10μm and 15μm in diameter, respectively) are loaded into the microwells. Self-assembly is used to load the fluorescent microspheres into the microwells. In the self-assembly process, the geometry of the semi-open microwell benefits a slower evaporation of the suspension containing the fluorescent microspheres, resulting in capillary forces which draw fluorescent microspheres close by into the microwells. It is observed that microwells can be filled at a yield approaching 70% after 5 loading steps (each assembly process is seen as one step) and the loading efficiency depended on the size of the fluorescent microspheres and the number of loading steps. Unfilled microwells can be further eliminated by additional loading steps with a more dilute suspension. However, multiple fluorescent microspheres loaded in one single microwell or located beneath the dielectric microbeads would bring interference on determination of the fluorescent enhancement effect in the following experiments. In order to eliminate this interference, it is necessary to load only one microsphere in each single microwell, which can be achieved by employing a dilute suspension and performing more loading steps. Here, samples with single microsphere loading are utilized in the following experiments.

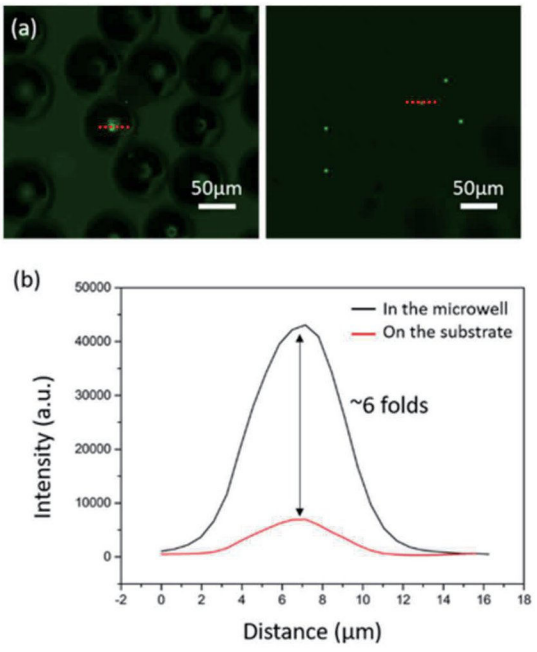

Fig. 5. (a) Optical images of the fluorescent microspheres (diameter 5 μm) in the semi-open microwell (left) and on the substrate (right). (b) The intensity profile of the corresponding fluorescent microspheres.

After loading, the samples are completely dried with nitrogen and their fluorescent signal are collected in the air ambient under the optical configuration as shown in Fig. 2. Their arithmetic mean emission intensity ($I_{emi}{}^{*}$) is the calculated and obtained. As a comparison, the arithmetic mean emission intensity of the microspheres on the glass substrate (I_{emi}) is also collected in the identical parameters (excitation power and the exposure time) under the same optical configuration as the control. It should be noticed that, only the fluorescent microspheres loaded at the expected position are included in our statistics. The electrostatic force may cause some fluorescent microspheres to randomly adhere to the inner surface of the microwell, especially for the spheres of smaller size with relative large spatial freedom (1μm and 5μm). This leaves them at a higher position in the longitudinal direction or an off-center position, which can be distinguished by reading their focal length or measuring their geometric position to the center, and are excluded in our statistics. As expected, microspheres inside the microwells exhibit higher emission intensity than that outside the microwells, Fig. 5. About 6 folds of enhancement can be obtained from the fluorescent microspheres with diameter of 5 μm, Fig. 5(b). The enhancement effect is attributed to the light focusing effect of the microwells due to the generation of confined light with higher intensity that significantly exceeds that of the illuminating light.

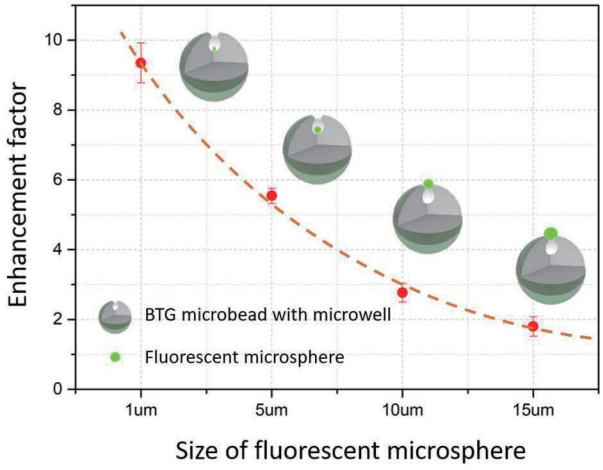

Fig. 6. The enhancement factor v.s. the size of the fluorescent beads used in the experiment. The sketch inside shows the relative position between the fluorescent microspheres and the microwells.

To quantify the enhancement effect of this optical microstructure, we define fluorescence enhancement factor η_F as

$$\eta_F = \frac{I_{emi}{}^{*}}{I_{emi}}$$

where $I_{emi}{}^{*}$ represents the arithmetic mean emission intensity of the fluorescent microspheres in the microwell and I_{emi} represents the arithmetic mean emission intensity of the fluorescent microspheres on the glass substrate. Fluorescence enhancement factor of different sizes are measured respectively. The fluorescence enhancement factor $\eta_F$versus their sizes are shown in Fig. 6. It is shown that, about one order (9.5 folds) of η_F is obtained on the microspheres with diameter of 1μm. with the size of the fluorescent microspheres decreasing, the η_F increases. The relationship between the η_F and size of the fluorescent microspheres arises from the longitudinal position of the fluorescent microspheres in the semi-open microwells.

Due to the ellipsoidal geometry structure of the microwells, these fluorescent microspheres exhibit different

height distributions along the longitudinal direction, inline images in Fig. 6. These semi-open microwells provide decent area for the accommodation of fluorescent samples as well as for enhancing their fluorescent signals. Our study offers a versatile platform to enhance the signal of fluorescent samples via dielectric microbeads, which has great potential in general for biosensing applications.

Ⅳ. Conclusion

In summary, dielectric microbeads with semi-open microwells are fabricated and their ability to enhance the fluorescent signals is demonstrated. Fluorescent microspheres can be passively trapped inside the semi-open microwells, which allows the efficient introducing of the fluorescent microspheres inside the light converging area. The light converging ability of this micro-optical structure is simulated based on finite element method. We show that the incident light can be confined and converged in the semi-open microwells with high field intensity and signals of the fluorescent microspheres inside the semi-open microwells can be enhanced up to one order of magnitude. It is observed that fluorescence enhancement factor is related to the size of the fluorescence microspheres. Fluorescent microspheres of smaller size exhibit higher fluorescence enhancement factor due to the position in the longitudinal direction. This superiorities of the micro-optical structure on the light converging ability possess great potentials in biosensing applications.

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