Label-free super-resolution imaging using patchy microspheres in various mediums

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ABSTRACT

The diffraction limit is a fundamental barrier in optical microscopy, restricting the smallest resolvable feature size of a microscopic imaging system. Microsphere-assisted super-resolution microscopy has emerged as a promising approach for overcoming this limit. This technique offers several advantages, including no need of fluorescent dyes, easy operation under white light illumination, and good compatibility with commercial optical microscopes. Various strategies have been proposed to enhance the imaging performance of microspheres. Recently, patchy microspheres were found to exhibit super-resolution capabilities in air with an enhanced imaging contrast. In this work, we studied the super-resolution imaging performance of patchy microspheres fully immersed in liquid. Furthermore, we demonstrated the formation of photonic hooks from patchy microspheres within a liquid environment. The findings of this study will extent the application of patchy microspheres from air to liquid immersion mode, opening up new possibilities for super-resolution imaging in liquid environment with patchy microspheres.

Keywords: Super-resolution imaging, microsphere, patchy microsphere, photonic nanojet, photonic hook

1. INTRODUCTION

Optical microscopes (OMs) are one of the most widely used tools in scientific research. Various methods have been proposed to improve the performance of conventional OMs.[1] For example, structured illumination microscopy can effectively improve the imaging quality for biological samples stained with fluorescence dyes, polarized microscopy can significantly enhance the contrast of birefringence samples,[2] photoacoustic and multiphoton upconversion super-resolution microscopy are also promising tools for biomedical imaging.[3,4] Recently, computational microscopy has been demonstrated as a promising technique for quantitative phase imaging and full field-of-view high-resolution microscopic imaging.[5–7] However, conventional OMs have a resolution limit due to the Abbe diffraction, which restricts their ability to resolve feature size smaller than $0.5\lambda/\mathrm{NA}$, where λ represents the incident wavelength and NA denotes the numerical aperture of the microscope. Consequently, an OM equipped with a near-unity NA objective and a white light source ($\lambda \sim$ 550-600 nm) typically exhibits a resolution limit of around 300 nm. Within this context, various methods have been proposed to overcome this challenge.

In 2009, Lee et al. successfully used microlenses to resolve feature size beyond the Abbe diffraction limit.[8] In 2011, Wang et al. demonstrated super-resolution imaging of 50 nm feature size using silica microspheres under white light illumination.[9] To further improve the imaging performance of microspheres, various parameters influencing the performance of microspheres have been investigated, including illumination conditions,[10] manipulation methods,[11] the morphology of the microspheres.[12] Patchy particles are anisotropic particles having

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two or more different physical or chemical properties on their surfaces. In 2021, Shang et al. reported the super-resolution imaging with Ag film-coated patchy microspheres,[13] in which they found that patchy microspheres have a much higher imaging contrast in air than conventional microspheres. Simulations based on the Finite-Difference Time-Domain (FDTD) method reveal that patchy microspheres can form photonic hooks under white light illumination.[14] Xu et al. later experimentally demonstrated the generation of photonic hooks by patchy microspheres .[15] In 2024, Zou et al. simplified the structure of patchy microsphere by using Janus microsphere instead.[16] Janus microsphere can be readily fabricated through a one-step glancing angle deposition method. Using Janus microspheres for super-resolution imaging offers a more controlled performance of the microscopic imaging system compared to using patchy microspheres.

In this work, we showed that patchy microspheres fully immersed in liquid also have super-resolution imaging capabilities, and simulation results show the formation of photonic hooks by patchy microspheres in liquid environment. The findings of this work will extent the application of patchy microspheres from air to liquid immersion mode, opening up new possibilities for super-resolution imaging in liquid environment with patchy microspheres.

2. MATERIALS AND METHODS

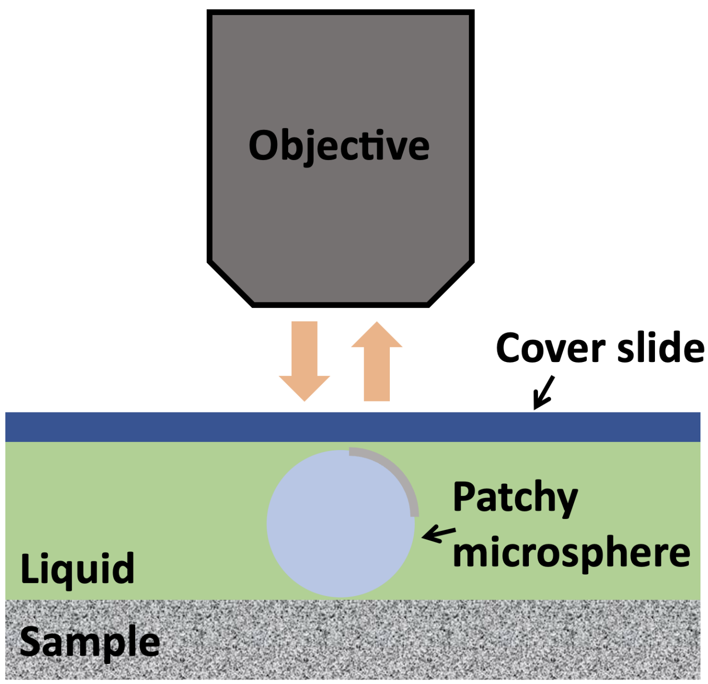

Figure 1. Schematic drawing of the microscopic imaging setup integrated with a patchy microsphere.

In this study, patchy microspheres are fabricated by the glancing angle deposition method.[17] $BaTiO_3$ (BTG) microspheres with a diameter of 27 - 35 μm and a refractive index of 1.9 were arranged into monolayers by drop-casting a small amount of BTG powders on a glass slide. Deionized water was then drop-casted onto the BTG microspheres. The microspheres was compacted together when the water film dried. The BTG microspheres were then deposited with 100 nm-thick Ag films by physical vapor deposition method at an angle (α) of 60°. We call the fabricated patchy BTG microsphere as p-BTG microspheres. After fabrication, the p-BTG microspheres were transferred from a glass slide to an observation sample by a stream of deionized water flow.

Figure 1 shows the schematic drawing of the microscopic imaging system used in this study. A commercially-available light microscope (Axio AX10, Carl Zeiss) was used as the experimental platform. The p-BTG microsphere collects near-field high-frequency optical information from the observation sample and forms a magnified virtual image. The magnified image can then be captured and resolved by the objective lens. The entire imaging process was performed in liquid. A 20 × objective with a numerical aperture (NA) of 0.4 (EC EPIPLAN, Carl Zeiss) was used. The microscope was equipped with a white light source (HAL 100, Carl Zeiss) for illumination. The microscopic images were recorded using a high-speed scientific complementary metal-oxide-semiconductor (CMOS) camera (DFC295, Leica).

2.1 Label-free super-resolution imaging in liquid with patchy microspheres

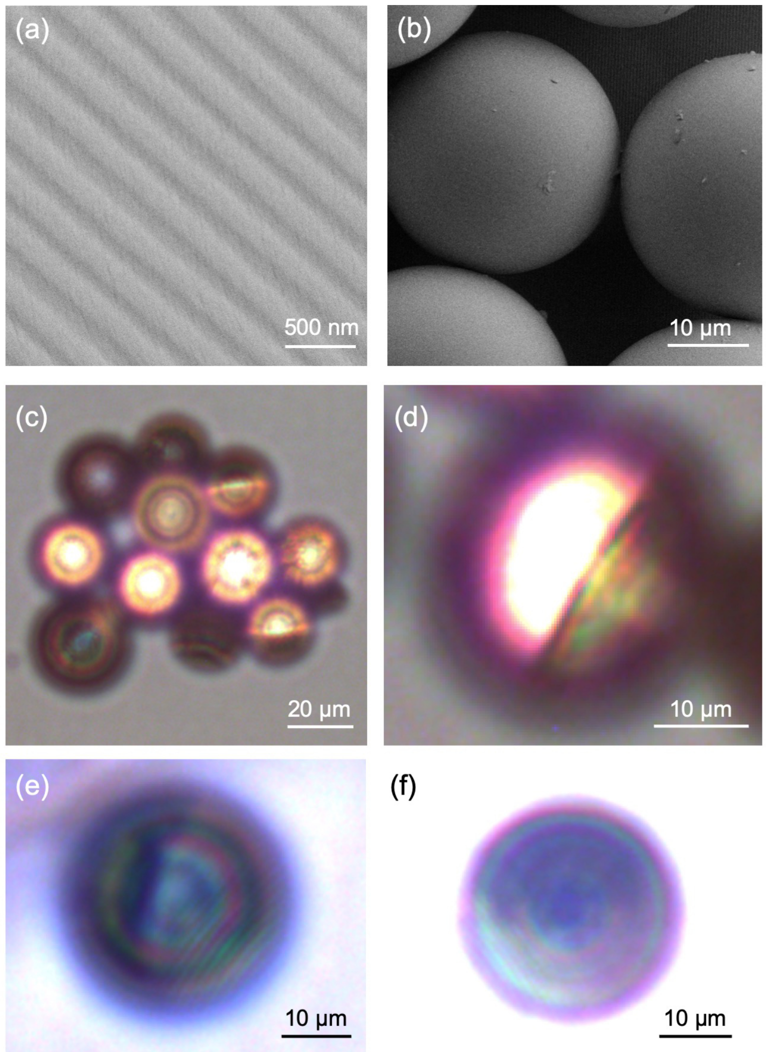

Figure 2. (a) SEM image of a BD substrate; (b) SEM image of BTG microspheres; (c), (d) Optical microscopic images of the p-BTG microspheres; (e), (f) Optical microscopic images of BDs observed through p-BTG microspheres immersed in (e) water and (f) MIO.

Then, we use the p-BTG microsphere to observe the surface of BD substrate. The scanning electron microscopic (SEM) image of the BD substrate is shown in Fig. 2 (a), which shows an uniform periodicity of $\sim$ 300 nm. The SEM image of the BTG microspheres is shown in Fig. 2 (b). As shown in Fig. 2 (c), p-BTG microspheres in water show various appearance due to the different positions of Ag films. Fig. 2 (d) is the OM image of a p-BTG microsphere with Ag films on its side. Figures 2 (e) and (f) are the OM of the BD substrate observed by p-BTG microspheres in water [Fig. 2 (e)] and MIO [Fig. 2 (f)], respectively. We can see that p-BTG microspheres have super-resolution abilities in both liquids. Due to the presence of Ag films, only part of the p-BTG microsphere can image the sample. This result is different than that obtained using p-BTG microsphere in air.[13] The p-BTG microsphere in air shows mirrored shadows due to the total internal reflection phenomenon. We found that the magnification factor of the stripe pattern is higher in water than in MIO, as the patchy microsphere has a shorter focal length in water than that in MIO. The p-BTG microsphere in water and MIO has a magnification factor of 4.17 $\times$ and 3.80 $\times$, respectively.

2.2 Simulations of the light focusing phenomenon of patchy particles

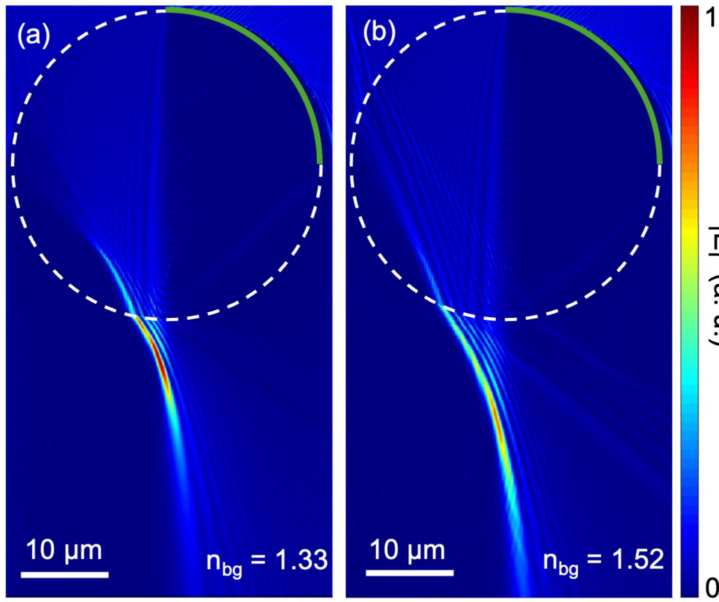

Figure 3. FDTD simulations of the photonic hooks generated by p-BTG microsphere immersed in (a) water and (b) microscope immersion oil.

Then, we study the light focusing phenomenon of patchy microspheres by the FDTD method. As shown in Fig. 3, the patchy microsphere for simulation has a diameter of 35 μm. The surface of the microsphere is partially covered by Ag films with a uniform thickness of 100 nm, as highlighted in green color. The materials of the microsphere and the immersion medium is BTG (n = 1.90), water [n = 1.33, Fig. 3 (a)] and microscope immersion oil [n = 1.52, Fig. 3 (b)], respectively. The patchy microspheres are illuminated by plane waves with a wavelength of 550 nm. We can see that the patchy microspheres can generate photonic hooks in both type of liquids. The focal length of the patchy microsphere is 21.7 μm in water and 28.1 μm in MIO. The focal length in this work is defined as the distance between the maximum intensity spot (I_{max}) of the photonic hook and the center of the microsphere. Photonic hooks have been used to improve the performance of microsphere-based super-resolution imaging systems.[13,16]

3. CONCLUSIONS

In conclusion, we demonstrated in this work the super-resolution imaging capability of patchy microspheres in liquid. We immersed the patchy microspheres coated with 100 nm-thick Ag films in deionized water and MIO, and found that the patchy microsphere has a higher magnification factor in the liquid when the refractive index is small. We also used the FDTD method to study the light focusing properties of patchy microspheres. It is found that the patchy microspheres in liquid generate curved photonic hooks under plane wave illumination. The simulation shows that the focal length of patchy microspheres increases with the refractive index of the immersion liquid. We believe that the findings of this work can further advance the applications of patchy microspheres in the field of super-resolution imaging.

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