Plasmonic dark field microscopy

Houdong Hu, Changbao Ma, and Zhaowei Liu[a)]
Department of Electrical and Computer Engineering, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093-0407, USA

(Received 8 January 2010; accepted 26 February 2010; published online 18 March 2010)

We propose plasmonic dark field microscopy, which utilizes a chip-scale integrated plasmonic multilayered structure to substitute the bulky and expensive conventional condenser optics. Experimental results show that we can get high contrast image using the compact, low-cost, and alignment free plasmonic dark field microscopy. © *2010 American Institute of Physics*. [doi:10.1063/1.3367729]

The rapid progress in nanoscale science and technology demands new microscopy techniques that possess both high resolution and high contrast capabilities. Many techniques have been developed to improve resolution such as near-field scanning optical microscopy,[1–5] stimulated emission depletion microscopy,[6,7] photoactivated localization microscopy,[8,9] stochastic optical reconstruction microscopy,[10] randomly adsorbed molecule microscopy,[11] the superlens,[12,13] and hyperlens,[14,15] structured illumination microscopy,[16,17] etc. Few techniques, however, have been developed for high contrast imaging. Dark field (DF) microscopy is widely used to view the object that has low contrast in bright-field microscopy.

In the conventional DF microscopy, the central part of the illumination light which ordinarily passes through and around the sample is blocked by a light stop, allowing only oblique rays to strike the sample on the microscope slide, as shown in Fig. 1(a). This is of great help when the objects have refractive indices very close to those of their surroundings and are difficult to image in the conventional bright field microscopy. While the conventional DF microscopy can achieve high contrast imaging, its resolution may also be improved using a high numerical aperture (NA) configuration of the condenser/objective pair. However, the NA of the objective cannot be larger than that of the condenser to avoid oblique illuminating rays entering the objective. Also the high NA condensers, such as the cardioid condenser,[18] are very sensitive to alignment and thus must be accurately positioned and aligned to the very sharp cone of illumination, making it very hard to use. In addition, the illumination light in such a high NA arrangement must be very strong due to the sharp illumination cone, which is wasteful of energy. Thus conventional DF microscopy is instrumentally complex, costly, and bulky.

In order to resolve the aforementioned limitations associated with conventional DF microscopy, we propose another DF technology. The proposed technology utilizes a chip-scale integrated plasmonic multilayered structure as the substitute of the bulky and expensive conventional condenser optics. We term this integrated structure as a plasmonic condenser (PC) and this imaging technology as plasmonic dark field (PDF) microscopy. The PDF microscopy is schematically shown in Fig. 1(b).

The PC is the critical element in PDF microscopy, which uses surface plasmons (SPs) to illuminate the sample. The effective NA of the PC is the ratio of the wave vectors (k-vectors) of the SPs to the photons in free space. Because the SPs, which are surface electromagnetic waves formed by collective oscillation of electrons at a metal-dielectric interface, may possess k-vectors much higher than those of the free-space photons at the same frequencies, large effective NAs can be achieved by the PCs. In addition, the SPs are evanescent on the metal-dielectric interface and do not propagate to the far field, so they cannot be detected by an objective lens in the far field. When objects are brought to the vicinity of the metal surface, the SPs can be converted into free-space photons, which can propagate to the far field where they can be detected. Meanwhile the SPs will remain evanescent in the areas without the objects. Therefore, a high contrast DF image of the samples in the far field can be formed. The essential feature of the PC is the integration of a thin layer of metal with a SP excitation mechanism in the very proximity of the metal to excite SPs using the near field coupling. The SP excitation mechanisms can be quantum dots, photoluminescent and electroluminescent materials, etc. In this work, we design and demonstrate the PDF concept using a chip-scale integrated active PC with fluorescent Rhodamine 6G as the active material.

The active PC is schematically shown in Fig. 1(c). It is a three-layer structure, i.e., an active layer of the mixture of polymethyl methacrylate (PMMA) and Rhodamine 6G molecules sandwiched between a glass substrate and a thin layer of silver. When the Rhodamine 6G molecules are illuminated by the incident light from the glass side, the emitted fluorescence will excite the SPs on the silver-air interface by near field coupling. This active PC can be easily fabricated using

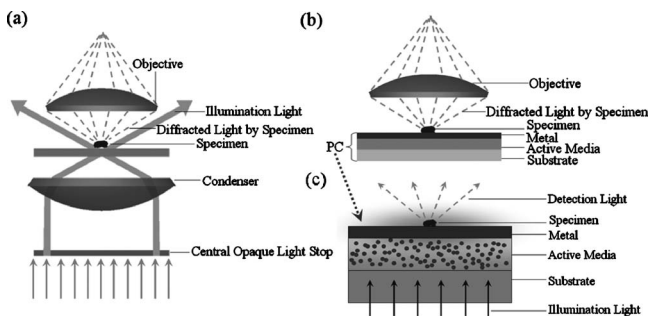

FIG. 1. Schematic configurations of (a) conventional DF microscopy, (b) plasmonic DF microscopy and (c) PC.

[a)]Author to whom correspondence should be addressed. Electronic mail: zhaowei@ece.ucsd.edu.

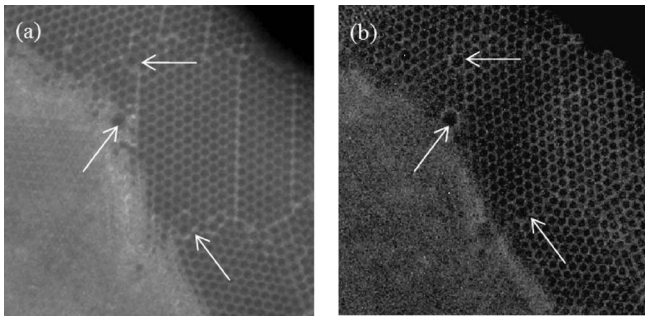

FIG. 2. Images of the monolayer polystyrene bead lattice, obtained using (a) the conventional DF microscopy and (b) the plasmonic DF microscopy. The lattice is composed of self-assembled monolayer of polystyrene beads (diameter ~2 μm).

standard microfabrication techniques. The Rhodamine 6G molecules were first mixed with PMMA at the concentration around 10^{-4} mol/L. Then the mixture was spin coated on a cover glass substrate. After a 2 min soft bake process, a 200 nm thick layer mixture as the active layer was obtained. Finally, a 60 nm thick silver film was deposited on top of the PMMA using the E-beam evaporation method.

To verify the PDF idea, we tested the fabricated fluorescent active PC using aggregations of 2 μm diameter polystyrene beads. A two-dimensional hexagonally close packed lattice of the polystyrene beads was fabricated on top of the silver film using a self-assembly method. The sample was first examined by using a standard optical microscope DF objective (EC Epiplan-Neofluar, 50X, NA=0.8). The reflection mode DF image is shown in Fig. 2(a) with a green filter (560 ± 10 nm, bandpass) added in the light path. As a comparison, the same sample was examined by PDF microscopy and the image is shown in Fig. 2(b). Note that the same objective was used for both techniques, however, in the PDF microscopy, a pair of band pass filters were used for the excitation (530 ± 10 nm, bandpass) and detection light (560 ± 10 nm, bandpass), respectively, based on the properties of the dye. The images obtained using the conventional DF microscopy and the PDF microscopy are shown in Figs. 2(a) and 2(b), respectively. It can be seen from Fig. 2 that the contrast of the PDF microscopy is better than that of the conventional DF microscopy. This is especially true for the contrast between the center parts and the boundaries of the polystyrene beads, as marked by the arrows.

Because the illumination of PDF microscopy only exists at the interface of the PC in the form of SPs, the depth of field, and the sensitivity of the PDF microscopy along the direction normal to the surface of the PC are solely determined by the decay property of the SPs. Due to the differences between the SPs and the conventional illumination light, the image information obtained using PDF microscopy and conventional DF microscopy are significantly different. The sample was examined using another standard optical microscope DF objective (EC Epiplan-Neofluar, 20X, NA =0.22). Figures 3(a) and 3(b) show the images of the sample, which is two layers of 2 μm polystyrene beads, obtained using conventional DF microscopy and PDF microscopy, respectively. It can be seen that conventional DF microscopy mainly provides contrast at the top layer; while PDF image reveals the bottom layer with much improved contrast. Notice the additional layer of particles does not affect the image quality much in PDF microscopy.

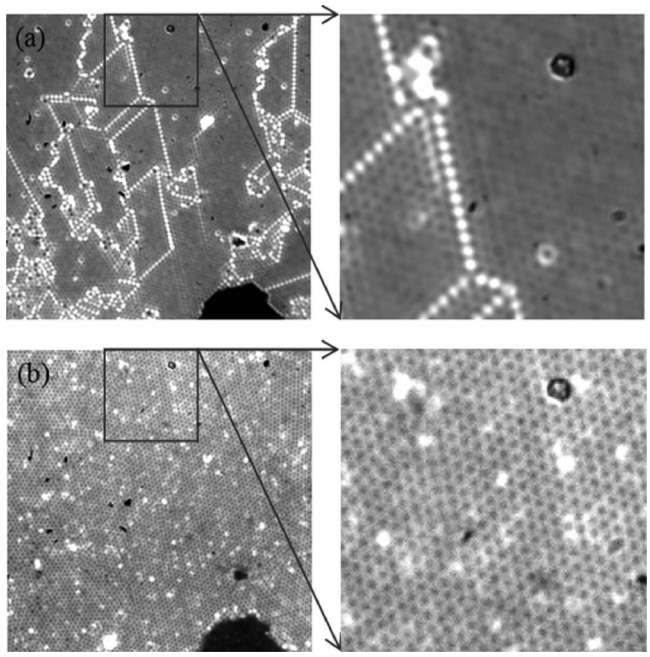

FIG. 3. Images of the dual-layer polystyrene bead lattice (diameter ~2 μm), obtained using (a) the conventional DF microscopy and (b) the plasmonic DF microscopy. The top layer of the beads is clearly imaged by the conventional DF microscopy and shown as white in-line dots in (a). The PDF microscopy clearly resolves the bottom layer of the beads, as shown in (b). Note that the bright areas in (b) are artifacts.

As shown above, we have experimentally demonstrated the PDF concept for high contrast imaging. The image quality of PDF microscopy is strongly related to the near field coupling between the active medium and the SPs on the metal-insulator interfaces in the PC; this has been intensively investigated.[19–21] One of the well-known models is to study the coupling of a dipole in the vicinity of a metallic interface to SPs, as the fluorescent dyes can be modeled as dipoles with isotropic orientations. When the thickness of the silver film is 60 nm, about 40% of the energy can be transferred to the SPs.[20,21] The remaining 60% of the light is attenuated by the 60 nm silver layer, thus resulting in very low transmission. So in the far field only the free-space photons arising from SP scattering by the object can be detected. The surface roughness, in addition to the object, can also scatter the SPs into free space photons, thus contributing to the background. In the demonstrated PC, the surface roughness is less than 2 nm in terms of root mean square. Therefore, the scattered light from the random roughness of such a smooth interface is very weak, resulting in the high contrast as shown in Fig. 2(b).

In conclusion, we have demonstrated the PDF microscopy concept using a chip-scale integrated multilayered fluorescent active PC. It can be easily extended to other types of PCs as mentioned above. The PDF microscopy idea can also be applied to fluorescence microscopy, which is similar to the extension of total internal reflection microscopy to total internal reflection fluorescence microscopy.[22] Because the PCs can have large effective NA, high resolution beyond the diffraction limit may be obtained with the compact and low-cost PDF microscopy. It is worth noting that the field of view of the PDF microscopy may be large, because it is dependent on the planar area of the PC, rather than the relatively short propagation length of the SPs. The proposed PDF technology

shows better contrast imaging capabilities for thin samples than the conventional DF microscopy. Finally, because the PCs are planar, the PDF microscopy does not require very sensitive alignment as in the conventional DF.

[1]E. Synge, Philos. Mag. **6**, 356 (1928).
[2]J. A. O'Keefe, J. Opt. Soc. Am. **46**, 359 (1956).
[3]D. Pohl, W. Denk, and M. Lanz, Appl. Phys. Lett. **44**, 651 (1984).
[4]E. Betzig, J. Trautman, T. Harris, J. Weiner, and R. Kostelak, Science **251**, 1468 (1991).
[5]L. Novotny and B. Hecht, *Principles of Nano-Optics* (Cambridge University Press, Cambridge, 2006).
[6]S. Hell and J. Wichmann, Opt. Lett. **19**, 780 (1994).
[7]T. Klar and S. Hell, Opt. Lett. **24**, 954 (1999).
[8]E. Betzig, G. H. Patterson, R. Sougrat, O. W. Lindwasser, S. Olenych, J. S. Bonifacino, M. W. Davidson, J. Lippincott-Schwartz, and H. F. Hess, Science **313**, 1642 (2006).
[9]S. T. Hess, T. P. K. Girirajan, and M. D. Mason, Biophys. J. **91**, 4258 (2006).
[10]M. J. Rust, M. Bates, and X. Zhuang, Nat. Methods **3**, 793 (2006).
[11]D. Wu, Z. Liu, C. Sun, and X. Zhang, Nano Lett. **8**, 1159 (2008).
[12]N. Fang, H. Lee, C. Sun, and X. Zhang, Science **308**, 534 (2005).
[13]Z. Liu, S. Durant, H. Lee, Y. Pikus, N. Fang, Y. Xiong, C. Sun, and X. Zhang, Nano Lett. **7**, 403 (2007).
[14]Z. Liu, H. Lee, Y. Xiong, C. Sun, and X. Zhang, Science **315**, 1686 (2007).
[15]I. Smolyaninov, Y. Hung, and C. Davis, Science **315**, 1699 (2007).
[16]M. Gustafsson, J. Microsc. **198**, 82 (2000).
[17]M. Gustafsson, Proc. Natl. Acad. Sci. U.S.A. **102**, 13081 (2005).
[18]S. M. Prince and W. G. McGuigan, Proc. SPIE **6676**, 66760K (2007).
[19]R. Chance, A. Prock, and R. Silbey, Adv. Chem. Phys. **37**, 1 (1978).
[20]W. Barnes, J. Mod. Opt. **45**, 661 (1998).
[21]W. Weber and C. Eagen, Opt. Lett. **4**, 236 (1979).
[22]D. Axelrod, T. P. Burghardt, and N. L. Thompson, Annu. Rev. Biophys. Bioeng. **13**, 247 (1984).