Imaging beyond the Resolution Limit with Far-Field Optics via Absorbance Modulation

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Abstract

Historically, improvements in the resolution of imaging have driven the understanding of science and led to the creation of new industries. Imaging with visible light is especially crucial for studying biological entities. Unfortunately, diffraction limits the far-field resolution of conventional optical imaging to about $\lambda/2$. Recently, several techniques, including stimulated-emission depletion (STED) [1], photo-activated localization microscopy (PALM) [2], and stochastic optical-reconstruction microscopy (STORM) [3], have demonstrated far-field fluorescence imaging of structures as small as $\lambda/50$. Because PALM and STORM rely on combining centroid-position maps of multiple single-fluorophore images, they fundamental resolution and throughput tradeoffs [4]. STED works by exciting fluorescent molecules within the focal spot of an initial pulse, followed by a second pulse, having a donut-like focus, which depletes the excited states except in the much smaller center. Significant spatial-resolution improvements via STED require high-intensity pulsed illuminations or special engineering of long-lifetime fluorophores. These limitations could be overcome by using bistable switching states [5].

In this work, we present a STED-like optical-imaging scheme that uses a nearly bistable photochromic layer, the absorbance-modulation layer (AML), which is initially opaque to the wavelength of interest, λ_1 , but upon illumination becomes locally transparent to λ_1 . Illumination at a longer wavelength, λ_2 , converts the AML back to opaque. When the AML is illuminated with a focal spot at λ_1 and a doughnut-shaped spot at λ_2 , the λ_1 light that is transmitted through the AML is spatially compressed, forming a nano-scale probe at the bottom of the AML. The size of this probe depends only on photokinetic parameters of the AML and the intensity ratio of λ_2 to λ_1 , not their absolute intensities [6]. Figure 1 shows a schematic of an absorbance-modulation imaging microscope, with an array of micro-lenses to enable high throughput. Figure 2 shows that absorbance modulation produces images with sharper corners. We have also demonstrated resolution enhancement with absorbance modulation in lithography [7].

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Fig.1(left) Schematic of the absorbance-modulation imaging microscope. The inset on the left shows an array of dichromates to achieve high throughput via parallelism. Each dichromat focuses the λ_1 beam into a round-shaped spot and the λ_2 beam into a doughnut-shaped spot. The scanning stage has a clear aperture, through which the transmitted light is collected. The AML is spin-coated on top of the lithographically patterned chrome-on-glass sample.

Fig.2(right) (a) Scanning-electron micrograph of a chrome-on-glass pattern with 500nm line widths. (b) Optical scanning image using conventional zone pates of 0.83 NA with λ_1 =400nm illumination. (c) Optical scanning images using dichromats of 0.83 NA with only λ_1 illumination and (d) with λ_1 and λ_2 =532nm illumination.

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