Optics Communications xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Optics Communications

journal homepage: www.elsevier.com/locate/optcom

Autofocusing Airy beam STED microscopy with long focal distance

Di Hu[a,b], Yao Liang[a,b], Yin Chen[a,b], Zan Hui Chen[a,b], Xu Guang Huang[a,b,*]

[a] Guangzhou Key Laboratory for Special Fiber Photonic Devices and Applications, South China Normal University, Guangzhou 510006, China
[b] Specially Functional Fiber Engineering Technology Research Center of Guangdong Higher Education Institutes, Guangdong Provincial Engineering Technology Research Center for Microstructured Functional Fibers and Devices, South China Normal University, Guangzhou 510006, China

ARTICLE INFO

Keywords:
Super resolution
Stimulated emission depletion microscope
Diffraction
Airy beams

ABSTRACT

Stimulated emission depletion (STED) is a very important technique in super-resolution microscopy. Until now, while autofocusing Airy beam (AAB) has been an attractive theme for both theoretical and applied researches, there are almost no report on AABs being used in STED microscopy. In this paper, we propose a novel STED microscopy based on AABs. A radially symmetric 3/2 phase plate is involved to simultaneously generate autofocusing excitation- and depletion-Airy beams. Remarkably, the AAB can auto-focus to a wavelength-scale spot with a long focal depth (several millimeters): on the contrary, the working distance of a conventional high numerical aperture (NA) objective is usually very short (about 200 μm). Our calculations indicate that the AAB based STED microscopy can achieve a super-resolution spot with FWHM of 58 nm while the focal length is 4.638 mm. Moreover, with properties of non-diffracting and self-healing, the Airy beam could enable a reduction of the scattering distortion induced by the specimens and has a great potential in imaging thick specimens.

1. Introduction

Since its concept and theory were proposed by Hell and Wichmann in 1994 [1], the stimulated emission depletion (STED) microscopy has drawn much attention in life sciences and in other areas where nanoscale visualization is required [2–5]. As a far-field microscopy, STED microscopy overcomes the diffraction-limitation, offering non-invasive imaging of living specimens in three dimensions [6]. In the STED microscopy, a diffraction limited excitation focus is overlaid with a doughnut-shaped STED focus that can inhibit the fluorescence emission everywhere but at the innermost of the focus by means of stimulated emission. As a result, a diffraction-unlimited resolution can be achieved. Conventionally, to achieve a small focus, a high NA oil objective (NA≈1.4) is used as the focusing element, the working distance of which is typically limited (about two hundred micrometres) [4]. The high quality of imaging at a large penetration depth is so far challenging in STED microscopy, limited by several factors such as the short working distance of its conventional high NA objective, aberrations and scattering distortion caused by specimens. Several strategies have been developed to address this challenge. Bethge et al. [7] combined long-working distance water objective (1.5 mm working distance, NA=1.1) with two-photon excitation in STED microscopy, and successfully obtained a resolution of 89 nm at a depth of 38.5 μm in acute brain slices. The STED microscopy based on the hollow Bessel beam with properties of non-diffraction and self-healing has proposed to achieve a lateral resolution of 113 nm at imaging depth up to 155 μm in solid agarose sample [8].

On the other hand, Airy beams have drawn much attention due to its unique features such as self-accelerating, diffraction-free and self-healing [[9–11]]. These properties make Airy beams very beneficial in many fields, such as particle manipulations [12], plasma-channel generation [13], and optical communications [14]. Furthermore, Airy beams have been successfully applied in light sheet microscopy to obtain increased contrast and field of view (FOV) [15]. The focusing properties of radially polarized circular Airy beam through a hard aperture have been demonstrated [16]. In previous works, Airy beam was generated by a 2 f system [9], where f is the focal length of the Fourier transform lens. Cottrell et al. [17] provided a method to directly generate the Airy beam and to shorten the system length by using a 3/2 phase pattern encoded onto the LCD. Autofocusing Airy beam (AAB) can also be directly generated by a radially symmetric 3/2 phase pattern [18]. This kind of beam is of great interest due to its unique abruptly autofocusing characteristic.

In this paper, we propose, for the first time to our knowledge, the use of AABs in STED microscopy. We find that plane waves with different wavelengths can transform into AABs with an identical accelerating trajectory after passing through the same radially symmetric 3⁄2 phase plate. And the AAB can auto-focus to a wavelength-

* Corresponding author at: Guangzhou Key Laboratory for Special Fiber Photonic Devices and Applications, South China Normal University, Guangzhou 510006, China.
E-mail address: huangxg@scnu.edu.cn (X.G. Huang).

http://dx.doi.org/10.1016/j.optcom.2017.04.036
Received 19 November 2016; Received in revised form 9 April 2017; Accepted 16 April 2017

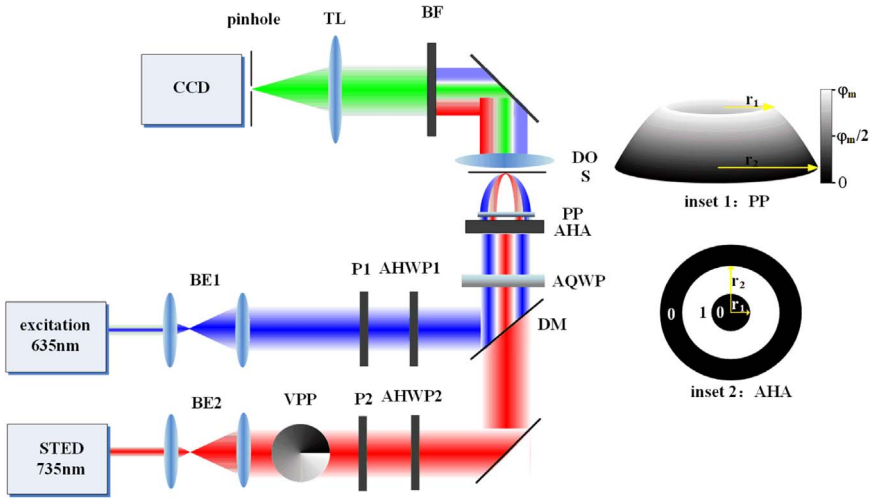

Fig. 1. Schematic of the AAB-STED microscopy setup. The focus of a red-shifted autofocusing Airy (AA) STED beam is superimposed on the AA excitation focus for diffraction-unlimitation. BE1, BE2: beam expander; P1, P2: polarizer; VPP: vortex phase plate; AHWP1, AHWP2: achromatic half-wave plate; DM: dichroic mirror; AQWP: achromatic quarter-wave plate; AHA: annular hard aperture; PP: radially symmetric 3⁄2 phase plate; S: sample; DO: detective objective; BF: bandpass filter; TL: tube lens. Inset 1: radial 3/2 pattern encoded on the phase plate; Inset 2: annular hard aperture used to mask the extra light in the centre and out of the phase plate.

scale spot with a long focal depth (several millimeters). These together provide the possibility of introducing the AABs into STED microscopy as the excitation beam and the red-shift vortex-carrying STED beam. Remarkably, the robust properties of AABs can be very useful in biological imaging. Moreover, our calculations indicate that the AAB-STED microscopy can achieve a super-resolution remaining fluorescence spot with FWHM of 58 nm in the focal plane. With these results, the AAB-STED microscopy is expected a great potential in imaging thick specimens.

2. Theory and setup

To form AAB whose radius (r) decreases with the increase of distance (z) as $r = r_1 - az^2$, a phase pattern given as $\varphi = -8\pi a^{1/2}(r - r_1)^{3/2}/(3\lambda)$ [18,19] is required. A diaphragm is put before the phase plate to block the undesired light in the centre with a radius of r_1 (see Fig. 1, inset 2). The radius of the radial 3/2 phase pattern (see Fig. 1, inset 1) is r_2, and its centre part with radius of r_1 is undefined. To ensure a positive phase, we set the phase pattern as:

$$\varphi_1 = \varphi_m - 8\pi\sqrt{a}\,(r - r_1)^{3/2}/(3\lambda_1) = \frac{8\pi\sqrt{a}}{3\lambda_1}[(r_2 - r_1)^{3/2} - (r - r_1)^{3/2}], \quad (1)$$

where λ_1 is the centre wavelength and $\varphi_m = 8\pi a^{1/2}(r_2 - r_1)^{3/2}/(3\lambda_1)$ is the maximum phase on the plate. The thickness of the 3/2 phase plate can be described by:

$$d = \frac{\varphi_1\lambda_1}{[2\pi(n-1)]} = \frac{4\sqrt{a}\,[(r_2 - r_1)^{3/2} - (r - r_1)^{3/2}]}{3(n-1)}, \quad (2)$$

when $r > r_1$. n is the refractive index of the phase plate (for a phase plate fabricated in the AR-N4400 produced by the German company All Resist, the difference of the refractive index is about 4‰ between the two wavelengths chosen in our paper (635 nm and 735 nm) and can be ignored). For a beam with the wavelength of λ_2, the phase distribution is given by:

$$\varphi_2 = \frac{2\pi d(n-1)}{\lambda_2} = \frac{8\pi\sqrt{a}}{3\lambda_2}[(r_2 - r_1)^{3/2} - (r - r_1)^{3/2}]. \quad (3)$$

Apparently, the same radial 3/2 phase plate has the same effect on the two wavelengths. After passing through the phase plate, the two beams with different wavelengths can transform into AABs with identical accelerating trajectory.

Fig. 1 illustrates the setup of the AAB-STED microscopy. We set the excitation wavelength and STED wavelength to be 635 nm and 735 nm, respectively, according to Ref [20]. Two polarizers are utilized to form linearly polarized beam in each path, while a vortex phase plate (VPP) is used to generate donut-shape beam in the STED path. The phase plate (PP) has an uncoded central area with a radius of r_1 and the outer radius of $r_2 = 2r_1$, as shown in inset 1. The use of PP results in a compact setup with high power efficiency [21], compared with spatial light modulator in generating Airy beams. To have the same effect on the excitation and STED beams, the phase is not wrapped into the range of 0–2π. An annular hard aperture (AHA) (shown in Fig. 1, inset 2) is used to create annular beam that is matched with the PP. The combination of an achromatic half-wave plate (AHWP) and an achromatic quarter-wave plate (AQWP) allows a circular polarization for each beam. In Ref [22]., the best de-excitation happens when the molecules are oriented parallel to the polarization of the laser beams, so as the excitation. Since the orientations of the sample molecules are random, the use of circular polarization beam would increase the resolution to the largest extent. Moreover, the researchers have demonstrated that the circular polarized AAB carrying a vortex or not can maintain its initial polarization in the far field [23]. The fluorescence is collected by a detective objective (DO), filtered with a bandpass filter, focused into a pinhole serving as the confocal detection part to block the background signal. At the premise of ensuring the detection depth and the brightness, we should choose a DO with NA as high as possible and a small size pinhole to ensure the axial resolution. The immersion medium of the DO should also be considered to make sure that it is suitable for the sample. Besides, object scanning should be used in this forward detection system.

In a STED microscopy, the point spread function (PSF) of the remaining fluorescence after depletion is given as follows:

$$h_{\mathrm{fluo}}(r) = h_{\mathrm{exc}}(r)\eta(r), \quad (4)$$

where $h_{\mathrm{exc}}(r)$ is the intensity pattern of the focal spot for excitation beam, and $\eta(r)$ is the remaining factor that describes the fluorescence has not been depleted and still can be detected at position r under the exposure of the STED beam. $\eta(r)$ can usually be well approximated by an exponential [24]:

$$\eta(r) = \exp(-\sigma I_{STED}(r)\tau/(\hbar\omega)), \quad (5)$$

where σ is the cross section for stimulated emission, $I_{\mathrm{STED}}(r)$ is the intensity distribution of the STED beam, τ is the fluorescence lifetime, and $\hbar\omega$ is the photon energy. If we define the effective saturation intensity I_s as the intensity required for the probability of fluorescence