

V-Shape PSF for 3D Wide Field Microscopy

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Abstract: We propose a V-shape PSF generated from double axicon lenses in the collection path of wide-field microscopes for 3D high-resolution imaging with an extended depth of field. © 2022 The Author(s)

1. Introduction

Conventional optical microscopy has a limited depth of field and cannot resolve the three-dimensional sample information. Imaging different depths requires a change of the objective lens' axial position. Point spread function engineering [1] through phase-mask at the Fourier plane enables single-shot 3D imaging. Existing PSFs such as astigmatic PSFs [2], double helix [3], and single helix [4] can achieve high spatial resolution in 3D but typically have a limited depth of field. Light field microscopy [5] places a microlens array at the sample plane for 3D imaging, but it requires extensive calibration and a complex computational algorithm to recover the high-resolution sample information. Here, we propose a new form of PSF which changes its shape but stays spatially confined over a large depth of focus. The PSF appears as a V-shape axially, and could be generated by a tube lens composed of a pair of axicon lenses in wide-field microscopy. With this V-shape PSF, our simulation shows that a $<2\ \mu\text{m}$ lateral resolution and a $<3\ \mu\text{m}$ axial resolution could be achieved over a $>150\ \mu\text{m}$ depth of field in a $10\times$ magnification, 0.5 NA microscopy system at 550 nm light wavelength.

2. V-shape PSF

The V-shape PSF is inspired by the Bessel beam generated by a single axicon lens. Bessel beam could be used to extend the depth of view in a microscope. As there is little change in the lateral PSF shape along the axial direction, it could not resolve individual depth. Here, we propose a new type of PSF formed by two tilted Bessel beams (thus in a V-shape). In the lateral direction, the PSF appears to be two main lobes whose separation distance changes with depth, and both lobes could stay in tight confinement over a large axial distance. Thus, such a V-shape PSF enables high-resolution 3D imaging over an extended depth of view.

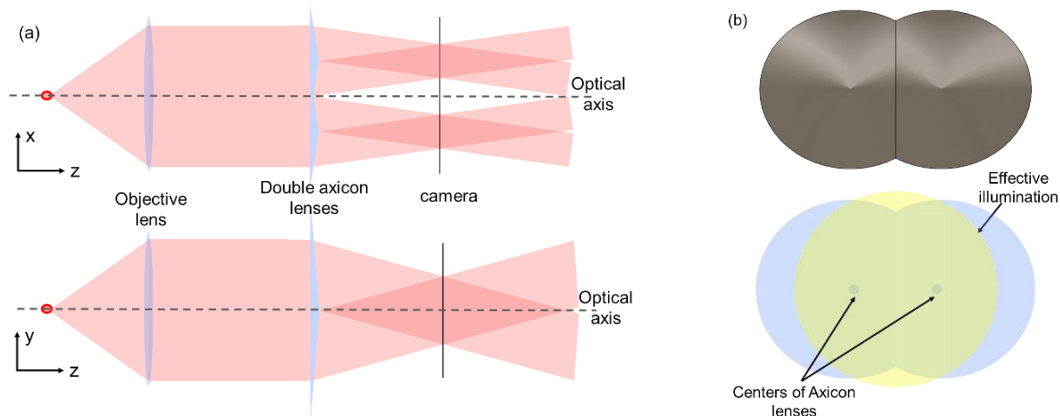


Fig. 1. Double axicon lenses to generate the V-shape PSF. (a) Schematic of the 3D extended depth of view microscope composed of an objective lens and double axicon lenses (drawing not in scale). The double axicon lenses serve as a tube lens. A V-shape PSF is formed where the separation of the two lobes of the PSF changes with respect to object distance. (b) Front view of the double axicon lenses and illumination beam.

The V-shape PSF could be generated by replacing the tube lens in the wide-field microscope with a pair of axicon lenses positioned adjacently (Fig. 1a). The depth of focus of a single axicon lens could be expressed as $R/[(n-1)\alpha]$, where R is the radius of the axicon, n is the refractive index of the lens material, and α is the tilting angle of the conical shape. The nominal focal length of the axicon can be expressed as half of the depth of focus, i.e., $f = R/[2(n-1)\alpha]$, which is set to be the same as that of the tube lens to be replaced. Considering the back aperture size of the objective lens and the circularly symmetric beam, we optimized the diameter and the separation of the two axicon lenses to maximize the light transmission (Fig. 1b). The small overlap of the two axicon lenses creates some asymmetry of the shape of each lobe of the PSF, but it has little impact on the 3D resolving capability and depth of field. As an example, we pair the double axicon lenses with a $10\times$ objective lenses to build an imaging system with $10\times$

magnification and 0.5 NA. The focal length and the radius of the two axicon lenses are set to be 180 mm and 9 mm respectively, and the separation from the center of two axicon lenses is 9 mm.

We investigate the performance of this imaging system through simulation (OpticsStudio). We place an object point source along the optical axis and vary its axial position. The separation $2l$ of two lobes of the PSF changes linearly with respect to the axial position d of the object point source (Fig. 2), in a ratio of $\Delta(2l)/\Delta d = 3.7$. Over an axial distance of 150 μm of the point source, the full-width-at-half-maximum of each lobe of the PSF is $<2 \mu\text{m}$. We can thus deduce the axial resolving power to be $<3 \mu\text{m}$.

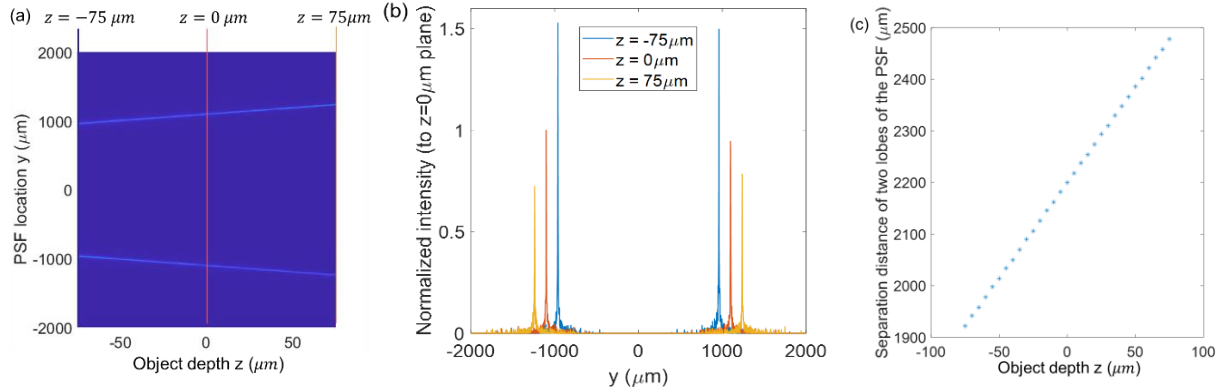


Fig.2. Simulation of the V-shape PSF. (a) Illustration of the two lobes of the PSF imaged by the camera versus the depth of the on-axis object point source. (b) The lateral PSFs at three different object depth, indicated by the vertical lines at (a). The variation of the PSF intensity is due to different effective NA for objects at different depth. (c) Quantitative relationship between the separation of the two lobes versus the depth of the on-axis object point source.

3. Image reconstruction

With the prior knowledge of 3D PSF, objects can be recovered computationally through iterative optimization methods such as Richardson-Lucy (RL) deconvolution or deep learning methods. Using the 3D PSF, we simulated the image of objects distributed in two different depths. We then used RL deconvolution to recover the objects in these two planes (Fig. 3b). The results have a good agreement with the ground truth objects (Fig. 3a).

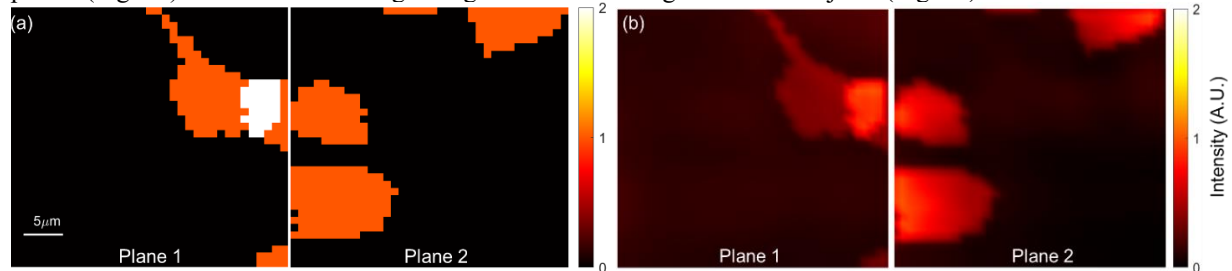


Fig. 3. Reconstruction of two object planes separated by 10 μm in axial direction using RL algorithm. (a) Ground truth objects of two planes, which mimics neuron cells. (b) Reconstruction results after 50 iterations in RL deconvolution.

4. Summary

We propose a new type of PSF for wide-field microscopy with 3D resolving power over an extended depth of field. The PSF has a depth-dependent shape (V-shape), and could be generated by replacing the conventional tube lens with a pair of axicon lenses. We designed and simulated the PSF for a 10x magnification microscope. We reconstructed the images at different depths through Richardson-Lucy deconvolution. The V-shape PSF could be useful for high-resolution 3D imaging over an extended depth of field.

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