Fluorescence saturated super-resolution microscopy in heterochromatic point-spread-function engineering

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1. ABSTRACT

With the advantages of nonlinear photon response [1], the optical resolution of fluorescence microscopy has overcome the diffraction limit. Point spread function (PSF) engineering is one of the super-resolution imaging approaches to encode emitter properties in the shape of the PSF. However, these techniques mostly focus on the single colour emission, restricting the feasible and flexible benefits from multicolour implementation [2]. Herein, we propose a strategy by extending the capabilities of PSF engineering to the spectral regime. Exploiting the distinct nonlinear photon response of each emission band in the multicolour fluorescent probes, we explore an opportunity for a tightly focused doughnut excitation to generate distinct spectral dependent PSFs. With the controllable PSFs from multi-channel emissions, we demonstrate the capacity to achieve super-resolution imaging by a single scan under saturated fluorescence excitation via PSF engineering either in the spatial domain or Fourier domain. We further develop a heterochromatic Fourier spectral fusion algorithm to enlarge the optical system's frequency shifting ability to enhance imaging performance.

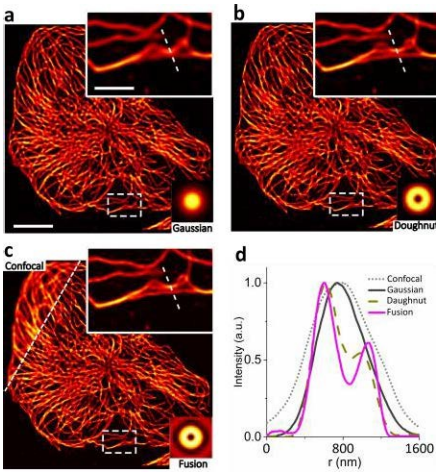

Figure 1. Simulated imaging of the Fusion method to biological application. **a** Image by Gaussian PSF. **b** Image by Doughnut PSF. **c** Image by fusion PSF. **d** Cross line profiles in **a**, **b** and **c**.

Our approach encodes spatial information from the saturated fluorescent into the spectral channels and decodes it by extracting the maximum length of Fourier components for post digital efficient PSF engineering. Due to the multi-photon nonlinear saturation properties, the spectral dependence provides different PSFs for different colours to implement post processing super-resolution imaging. The maximum spatial information corresponding to the Fourier components are fused from each emission bands for post processing optical transfer function to reconstruct the super-resolved object [3]. Finally, the image is reconstructed by summing up the different maximum contributions in Fourier domain using a reconstruction algorithm processes, as shown in Figure 1. The main idea in this work is that nonlinearity in the excitation of the fluorescence results in an equivalent excitation PSF with extended frequency content.

2. REFERENCES

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