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Introduction to the Multicolor Super-resolution Localization Microscopy

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Abstract

Super-resolution localization microscopy is a rapidly emerging new field of microscopy that dramatically improves the spatial resolution of light microscopy by over an order of magnitude (10–20 nm resolution), allowing biological processes to be described at the molecular scale. However the life activities are usually the result of interaction of various proteins, witch usually requires multicolor imaging technology. Several kinds of multi-color imaging technology have been developed. Here, we introduce several working principles of multicolor super-resolution localization microscopy and compares their advantages and disadvantages.

Keywords

Super-resolution localization microscopy, Multicolor imaging.

1. Introduction

Fluorescence microscopy has been central in shaping our understanding of the molecular organization and interactions of biological systems. But the maximum resolution of lens-based microscopy is constrained by the diffraction limit of light. The best lateral resolution attainable using visible light is ~200 nm. Super-resolution microscopy (SRM) techniques manage to surpass the 'classical' diffraction limit of optical resolution of about half the wavelength of the emitted light. These technologies include stimulated-emission depletion (STED) microscopy, structured illumination microscopy (SIM), and super-resolution localization microscopy (SRLM). SRM offers unprecedented gains in resolution, approaching levels formerly within the exclusive realm of electron microscopy. SRLM is the most widely used technology.

SRLM achieves the separation of molecules by stochastically turning on individual molecules within the diffraction-limited volume at different time points, including stochastic optical reconstruction microscopy (STORM) [1] and (fluorescence) photoactivatedlocalization microscopy ((F)PALM) [2,3], and point accumulation for imaging in nanoscale topography (PAINT) [4]. The primary difference between the various SRLM techniques is the choice of fluorophore, and thus the photo-switching mechanism. To image a variety of proteins, multicolor imaging techniques are necessary. Multicolor Super-Resolution Localization Microscopy

2. Multicolor Super-resolution Localization Microscopy

2.1. Activation-dependent Technology

The original STORM method takes an interesting approach whereby pairs of fluorophores, referred to as 'molecular switches' are tagged to the structure of interest. The detected fluorophore is referred to as the 'reporter'; reporter fluorescence is detected and localized. The second fluorophore, referred to as an 'activator', has a blue-shifted emission bandwidth compared to the reporter. Upon absorption the activator facilitates the return of a reporter in close proximity to a fluorescent state in a manner analogous to a FRET donor–acceptor pair. The primary advantage of this technique is that the same reporter can be used to tag multiple

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structures of interest in multi-colour imaging experiments [5]. This is important because of what is still an extremely limited pool of commercially available fluorophores that are suitable for SMLM, the highest performing variants all emit in the far-red spectrum (Cy5, Alexa Fluor 647). The greatest drawback of this approach is the high proportion of cross-talk between imaging channels, typically about 15–25%.

2.2. Excitation-dependent Technology

There are other approaches to multi-colour imaging using synthetic fluorophores. Perhaps the simplest is to forgo implementing molecular switches, and use spectrally distinct reporter probes without an accompanying activator. dSTORM was the first SRLM to utilize unaccompanied synthetic dyes for SRLM [6]. The downside of this approach is that the investigator is required to use non-optimal probes that, though not as powerful as Alexa 647 or Cy5, perform well and on average perform better than fluorescent proteins. Another complication is the need to account for chromatic aberration. Though the use of high-quality apochromatic objectives is typically enough for traditional optical techniques, in the superresolution regime even small degrees of aberration can become strikingly apparent.

2.3. Spectrum-dependent Technology

Another approach is to use spectrally similar probes, with similar buffer and excitation requirements, and spectrally demix their respective signals [7,8]. Cross-talk is still an issue using this approach, but at greatly reduced levels (approximately 10%). In this technology, resorchers can use prism and grating to separate the spectrum. Dy 634 DL 650 CF 660 and CF 680 fluorescent probes were screened to realize four color super-resolution imaging of peroxisome, vimentin, microtubule and mitochondria in cells [7].

2.4. PSF-dependent Technology

Another approach is based on point spread function (PSF) engineering [9]. In this technology, resorchers demonstrate an alternate strategy: directly encoding the spectral information, in addition to three-dimensional position, in the image. By exploiting chromatic dispersion we design a new class of optical phase masks that simultaneously yield controllably different PSFs for different wavelengths, enabling simultaneous multicolor tracking or super-resolution imaging in a single optical path. This method can realize 3D imaging and multicolor imaging simultaneously with low interference between colors, but the number of molecules collected per frame is limited and the collection efficiency is reduced, so it is suitable for imaging of low density objects

3. Conclusion

This paper introduces several working principles of multicolor super-resolution localization imaging and compares their advantages and disadvantages. Activation-dependent technology has the highest proportion of cross-talk between imaging channels. Excitation-dependent technology is easy to implement, and has low interference between colors, but not every channel has a probe suitable for super-resolution imaging. Spectrum-dependent technology has strong color-resolve ability, uniform imaging effect of each channel and low channeling disturbance, but the high cost limits its application. PSF-dependent technology is suitable for imaging of low-density objects.

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