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Optimization of the Excitation Light Sheet in Selective Plane Illumination Microscopy

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Selective plane illumination microscopy (SPIM) is a powerful technique used for 3D live fluorescence imaging in biological research [1-6]. Unlike conventional fluorescence imaging techniques based on the epi-illumination configuration, SPIM uses two objectives with optical axis orthogonal to each other for sample excitation and fluorescence detection separately. By confining the excitation light near the detection focal plane, SPIM allows high speed 3D imaging with high 3D spatial resolution, good optical sectioning capability, and minimal photobleaching and phototoxicity.

In order to maximize the benefit of SPIM, a key problem is how to create a thin and uniform excitation light sheet to cover the region of interest and confine the excitation light near the detection focal plane as much as possible. Different methods have been developed to create the excitation light sheet in SPIM, including the Gaussian light sheet created by cylindrical lenses or scanning Gaussian beams [1-3, 5, 6], the Bessel light sheet created by scanning Bessel beams [4, 7, 8], the Airy light sheet created by scanning Airy beams [9], and the optical lattice light sheet developed more recently created by dithering nondiffracting optical lattice patterns [10]. However, each of these methods has its own strengths and weaknesses, and it is very difficult to obtain a light sheet with thin thickness, large size and tight excitation light confinement at the same time. Therefore, tradeoffs must be made among these factors based on the desired performance and the sample to be imaged no matter what method is to be used to create the excitation light sheet.

The selection of the SPIM excitation light sheet and the optimization of its geometry are complicated. It involves the implementation of the appropriate method to create the light sheet and the tuning of the light sheet geometry based on the application and the specimen to reach the optimal balance between spatial resolution, optical sectioning capability and field of view (FOV). Here, we present a strategy to select, optimize and estimate the linear excitation light sheet in SPIM using the Gaussian light sheet, the Bessel light sheet, and the lattice light sheet as examples.

We show that the spatial resolution of SPIM is determined by both the detection NA and the thickness of the excitation light sheet. However, whether the theoretical resolution can be obtained practically is determined by the optical sectioning capability of SPIM. We also show that the excitation light sheet should always stay in focus in SPIM, and the off focus excitation light should be reduced as much as possible. The alignment of SPIM using higher detection NA and thinner excitation light sheet is more critical, and the optical sectioning capability is worse with higher detection NA than that with lower detection NA, although the spatial resolution is generally higher. By using a detection NA of 1.0 or above, the Gaussian light sheet satisfies general requirements for spatial resolution, FOV and optical sectioning capability when the required SPIM axial resolution is above a micron, and the lattice light sheet should be used when a submicron axial resolution is required because it is thinner than the Gaussian light sheet of the same length and confines excitation light better than the Bessel light sheet of the same thickness. Meanwhile, the thickest and shortest light sheet that is able to give the required axial

resolution and FOV should always be used for all types of the excitation light sheet to minimize the off focus excitation light in SPIM.

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