

DUAL OBJECTIVE MULTIFOCAL PLANE MICROSCOPY FOR SINGLE PARTICLE/MOLECULE IMAGING APPLICATIONS

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In single molecule/particle imaging applications, the number of photons detected from the fluorescent label plays a crucial role in the quantitative analysis of the acquired data. For example, in 2D/3D tracking experiments the localization accuracy of the labeled entity is inversely proportional to the square root of the number of detected photons. Hence achieving high photon collection efficiency is important in such studies. Currently, single molecule/particle imaging experiments are typically carried out on either an inverted or an upright microscope, in which the photons are collected from only one side of the sample (i.e., top side or the bottom side).

Here, we report the development of dual objective multifocal plane microscopy (dMUM) for single particle/molecule studies. The dMUM configuration uses two opposing objective lenses, where one of the objectives is in an inverted position and the other objective is in an upright position. We show that dMUM has higher photon collection efficiency than a regular (inverted/upright) microscope for a given illumination condition. Because of the presence of two objective lenses, dMUM supports simultaneous imaging of different focal planes. This has been shown to be beneficial for high accuracy 3D localization and tracking of single molecules/particles and sub-cellular objects over a wide spatial range in live cells [1-3]. We demonstrate that fluorescent labels can be localized in 2D/3D with better accuracy when imaged through dMUM than when imaged through a regular (inverted/upright) microscope configuration. Analytical tools are introduced to estimate the 2D/3D location from dMUM images and to characterize the accuracy with which they can be determined.

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