

Study on The Relationship of Laser Power Density and Localization Precision in PALM Imaging

Photoactivated localization microscopy (PALM) improves the resolution of microscope to 2 -25 nm with the facility of photoactivated fluorescent proteins(PAFPs). PALM, stochastic optical reconstruction microscopy(STORM) and fluorescence photoactivation localization microscopy (FPALM) are all based on single molecule localization technique. The localization precision of single molecule localization microscopy (SMLM) depends on the photon output of fluorescent probes and background noise. As the optical properties of PAFPs and background noise are related to excitation laser power density, we design a series of experiments to study the relationship between laser power density and localization precision in PALM imaging. We selected and purified most widely used PAFPs(mEos2, mEos3.2, Dronpa, mGeos-M, PAmCherry, PAtagRFP) for further single molecule imaging test. We found the optimal localization precision can be achieved at around 0.5 kw/cm² laser power density for selected red PAFPs. For green PAFPs, mGeos-M delivers the highest localization precision at very low laser power. It shows that mGeos-M is suitable for live cell imaging which is sensitive to photo toxicity. In summary, these results can guide researchers to choose appropriate laser power densities for specific PAFPs in PALM imaging and achieve optimal resolution.