SUPER-RESOLUTION USING NON LINEAR ACTIVATION KINETICS OF PHOTO-ACTIVATABLE FLUORESCENT PROTEINS

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Many genetically-expressible fluorescent proteins (FPs) can be reversibly or permanently activated from a ‘dark’ state to a fluorescent state by irradiation with an appropriate wavelength. These transitions involve a switch from one structural form to another, which is a first-order chemical reaction, and can therefore be described by a single-exponential function. Thus if we convert a spot the profile of the converted volume will not mimic the intensity of the activating PSF, but will come from a much smaller volume with a corresponding increase in resolution. This has led us to model a very simple form of scanning super-resolution microscope using Dronpa \cite{1} as the PAFP. The beam is parked on a spot and the FP is activated by a 405nm laser. Fluorescence is then excited by a 488nm laser, and finally the power is increased to switch the FP back to a dark state. The beam moves to the next spot and the process is repeated. The transition between ‘on’ and ‘off’ in Dronpa is a cis-trans isomerisation of the chromophore \cite{2}, and follows single-exponential kinetics with a rate constant of 6.9 \cite{1}. If the activation wavelength is $\lambda_e$ and the illumination numerical aperture is $n \sin \alpha_e$, the point-spread function (PSF) of the activation path is given by $e(v) = (J_1(v)/v)^2$, where $v = 2\pi(n \sin \alpha_e / \lambda_e) x$ is the normalized radial coordinate. If we assume the photo-activation efficiency to be dependent on the excitation power via a function $N(I)$, the effective PSF of the activation path is given by $N(e(v))$. Figure 1 illustrates the PSF sharpening (b) achieved by assuming the excitation-activation relationship (a) of $N(I) = e^{6.9I}$. This gives an expected lateral resolution of $\sim 78$nm, on a par with commercial STED systems, as well as a substantial boost in axial resolution.

Fig 1: Super-linear dependence of the photo-activation efficiency on the excitation intensity (a) leads to sharper PSF in transverse (b) and axial (c) directions. The solid line is the effective PSF and the dashed line is the airy pattern. FWHM in normalized co-ordinates are, transverse: linear PSF (0.51), non-linear PSF (0.2), axial: linear PSF (3.54), non-linear PSF (1.43).

References
\begin{itemize}
\item \cite{1} S. Habuchi et al., “Reversible single-molecule photoswitching in the GFP-like fluorescent protein Dronpa,” PNAS \textbf{102}, 9511-9516 (2005).
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