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Supplemental Information

Breaking the Diffraction Barrier: Super-Resolution Imaging of Cells

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Fluorescent Probes for Super-Resolution Microscopy by Single-Molecule Switching

Table 1. Fluorescent Probes for Super-Resolution Microscopy Based on Single-Molecule Switching

		Post-activation ex (nm)	Post-activation em (nm)	Notes	References
fluorescent proteins	Photoactivatible – blue				
	bsDronpa	460	504	Reversible photoswitching; Allows simultaneous multi-color imaging with Dronpa	(Stiel et al., 2007)
	Photoactivatible – green	~ 495	~ 515		
	PA-GFP	504	517		(Patterson and Lippincott-Schwartz, 2002)
	PS-CFP2	490	511		(Chudakov et al., 2004)
	Dronpa	503	518	Reversible photoswitching	(Habuchi et al., 2005)
	EYFP	515	527	Reversible photoswitching	(Biteen et al, 2008)
	Photoactivatible – red	~ 560	~ 590		
	PAmCherry	564	595	Allows simultaneous multi-color imaging with PA-GFP	(Subach et al., 2009)
	PAtagRFP	562	595	Allows simultaneous multi-color imaging with PA-GFP	(Subach et al., 2010)
	Photoconvertible green-to-red	550-570	570-590		
	mEos2	573	584	Very low nonspecific activation; Brightest photoactivatible fluorescent protein to date	(McKinney et al., 2009)
	Dendra2	553	573		(Gurskaya et al., 2006)
Organic dyes	Red cyanine dyes	650-750	670-800	Photoswitchable with a thiol buffer; Activation wavelength tunable with attached activator dyes	(Bates et al., 2007)
	Cy5/Alexa 647	647	665	Brightest water-soluble photoswitchable dye to date	(Bates et al., 2005; Bates et al., 2007; Heilemann et al.,

				2005)
Photochromic rhodamines	500-600	540-620	Low nonspecific activation	(Folling et al., 2007)
Caged dyes				
Caged fluorescein	497	516		(Betzig et al., 2006)
Rhodamine and oxazine dyes	490-700	510-800	Photoswitchable with a reductant or reductant/oxidant system	(Heilemann et al., 2009; Vogelsang et al., 2009)
Atto655	663	684	Membrane permeable	(Heilemann et al., 2009; Wombacher et al., 2010)
Dyes with long triplet state life time				(Folling et al., 2008)
Azido push-pull fluorophore	450-570	490-620	Bright, membrane permeable	(Lord et al., 2009)

The red cyanine dyes, Cy5 and Alexa Fluor 647, are excellent probes for STORM/(F)PALM imaging (Bates et al., 2005; Bates et al., 2007; Heilemann et al., 2005; Heilemann et al., 2008; Rust et al., 2006). These fluorophores can photoswitch in thiol-containing buffers compatible with live cell imaging, and their dark state is a thiol adduct of the cyanine dyes (Dempsey et al., 2009). These fluorophores have exceptional brightness (~6000 detected photons per switching cycle), undetectable dark state fluorescence, and low spontaneous activation rates, allowing a theoretical localization precision of a few nanometers. The low but finite activation rate of these dyes by the red imaging laser enables a simple imaging mode, which requires only a single continuous wave (CW) laser without any need for temporal modulation (Egner et al., 2007; Zhuang, 2009). When paired with another fluorescent dye that serves as an “activator”, the activation wavelength of these dyes is easily adjusted according to the absorption spectrum of the activator, allowing multi-color imaging free of chromatic aberration (Bates et al., 2007; Huang et al., 2008).

However, the non-specific activation by the red imaging laser leads to significant color-cross talk between different color channels when imaging samples with a high labeling density. In this respect, the photochromic Rhodamine dyes provide a powerful alternative. Despite a lower photon output of ~ 1000 photons per switching cycle these dyes have a substantially lower non-specific activation rate, which allows a larger number of molecules to be localized within a diffraction-limited volume, and could prove to be advantageous for imaging densely labeled samples (Folling et al., 2007). Photochromic Rhodamine dyes with distinct emission colors also allow multicolor imaging with lower color-cross talk (Bossi et al., 2008). The azido push-pull chromophores represent another family of bright, high-contrast, multicolor, photoactivatable fluorescent dyes for high-quality super-resolution imaging (Lord et al., 2009).

In addition to these photoswitchable dyes, a large number of commonly used dyes have been proven useful for super-resolution imaging due to their spontaneous blinking properties, significantly increasing the spectral range, versatility and ease of the method (Folling et al., 2008; Heilemann et al., 2009; Testa et al., 2010; Vogelsang et al., 2009). As a note of precaution, some of the “blinky” dyes tend to have a high spontaneous activation rate, which leads to a large fraction of the fluorophores being on at the same time. This can substantially limit the number of molecules resolvable within a diffraction limited volume and consequently the final image resolution.

Like fluorescent dyes, a large number of fluorescent proteins are photoswitchable/photoactivatable. Even the commonly used enhanced yellow fluorescent protein (EYFP) photoswitches and can be used for super-resolution imaging (Biteen et al., 2008). Among the photoactivatable fluorescent proteins, Eos (McKinney et al., 2009; Wiedenmann et al., 2004) and Dendra (Gurskaya et al., 2006) are known for their brightness - ~1000 photons can be detected from these proteins before they are photobleached. They also have relatively high-contrast ratio between post- and pre-activation fluorescence and a remarkably low spontaneous activation rate (Betzig et al., 2006), allowing a large number of probes to be localized within a diffraction-limited volume. These properties allow Eos and Dendra to provide the highest image resolution among the photoswitchable fluorescent proteins. However, these fluorescent proteins cover a large spectral range with violet sensitivity for activation, blue-green absorption of the pre-activation state, and yellow-orange absorption of the post-activation form. This broad spectral coverage makes it difficult to perform multi-color imaging with Eos or Dendra FP (Shroff et al, 2007).

Several new fluorescent proteins have been developed to overcome this difficulty. For example, PAmCherry and PAtagRFP are bright photoactivatable red fluorescent proteins that can be paired with PA-GFP for two color imaging (Subach et al., 2009; Subach et al., 2010). Blueshifted Dronpa (bsDronpa) can be paired with Dronpa for two color imaging (Stiel et al., 2007). However, the image resolution of these two-color images does not reach that of the single-color images taken with Eos or Dendra FP due to the relatively low contrast ratio of PA-GFP and the low photon output of Dronpa.

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