dSTORM

Super-Resolution Fluorescence Imaging of Cellular Structures and Dynamics with Conventional Fluorophores

Optical microscopes are subject to the diffraction barrier of light which imposes an optical resolution limit of approximately 200 nm in the imaging plane. dSTORM enables super-resolution imaging with commercially available standard fluorophores and probes at a resolution of ~ 20 nm even in living cells.

Fluorescence microscopy allows the direct observation of cellular processes with molecular specificity and high temporal resolution in three dimensions. Due to the wave nature of light, however, the spatial resolution had been limited to about half of the wavelength of the light in the imaging plane. That is, conventional fluorescence microscopes do not provide insight into the structural organization of vital protein assemblies and machineries with a size of a few tens of nanometers. Only recently methods emerged that enable super-resolution imaging with substantially improved optical resolution.

Single-Molecule Based Localization and Image Reconstruction

Most super-resolution approaches bypass the diffraction limit by controlling the fluorescence emission of fluorophores using either deterministic or stochastic approaches [1]. Among the stochastic approaches, single-molecule based localization is in particular attractive as it is technically easy to accomplish and achieves an impressive resolution of ~ 20 nm in the imaging plane [2-5]. The idea behind is based on the exact position determination (localization) of individual fluorophores that are further apart than the minimal distance resolved by the microscope. Every fluorophore is localized by approximating the fluorescence emission pattern (the point-spread function, PSF) with a two-dimensional Gaussian function. A super-resolved fluorescence image can finally be reconstructed by summing up all localizations (Fig. 1). Ideally, the localization precision depends only on the number of collected photons $n$ and on the standard deviation of the PSF ($\sigma$) and can be approximated by $\sigma/\sqrt{n}$ [6]. For a typical number of ~ 1000 photons detected per fluorophore a localization precision of ~ 10 nm can be expected.
Prerequisite for this strategy, however, is the temporal control of fluorescence emission of individual fluorophores.

This can be achieved using photoactivable or reversibly photoswitchable fluorescent proteins [2, 7] and organic fluorophores [3-5] (Fig. 1).

**The dSTORM Concept**

dSTORM (direct stochastic optical reconstruction microscopy) uses standard organic fluorophores such as Alexa Fluor and ATTO-dyes that can be operated as reversible photoswitches, with representatives covering the whole visible spectrum [4, 5]. Light-induced reversible photoswitching is achieved in the absence of an activator fluorophore in aqueous buffer and in the presence of millimolar concentrations of a reducing thiol compound such as dithiothreitol (DTT), glutathione (GSH), or mercaptoethylamine (MEA) [5, 8]. The reducing thiol compound efficiently quenches the fluorophore's triplet state and generates a stable non-fluorescent reduced state (dark state). The fluorescent state is recovered spontaneously or light-induced through oxidation with molecular oxygen naturally present (~ 250 µM) in aqueous solvents (Fig. 2) [5].

**List of Requirements for dSTORM**

dSTORM relies on the transfer of the majority of fluorophores to a non-fluorescent dark state and precise localization of individual fluorophores. The achievable resolution and herewith the ability to resolve a structural feature depends not only on the brightness of the fluorophores, but also on the labeling density and on the stability or lifetime of the non-fluorescent dark state. In case of a densely labeled structure, the dark state should exhibit a lifetime $\tau_{\text{off}}$ that is substantially longer than the lifetime of the fluorescent state $\tau_{\text{on}}$. Ideally, the lifetime of the on-state $\tau_{\text{on}}$ should be very short, with a high photon yield to allow precise localization [6].
Whereas linear filamentous structures can be resolved easily with low ratios \( r = \frac{k_{\text{off}}}{k_{\text{on}}} = \frac{\tau_{\text{off}}}{\tau_{\text{on}}} \), more complex structures such as adjacent or crossing filaments require \( r > 500 \) (Fig. 3) [9]. With \( \tau_{\text{off}} \) in the range of hundreds of milliseconds to several seconds, the non-fluorescent states of organic fluorophores generated with the dSTORM concept are ideally suited for super-resolution imaging of cellular structures.

**Implementation of dSTORM for Live Cell Imaging**

Because the cysteine containing tripeptide GSH is the most abundant low-molecular weight protectant and antioxidant (reducing agent) in mammalian biology and present in animal cells in the reduced state at millimolar concentrations, dSTORM can be performed also in living cells (Fig. 4) [5]. Importantly, this photo-switching is an inherent property of some organic fluorophores and does not require precise conjugation to a second fluorophore [3]. In combination with chemical tags, dSTORM can provide subdiffraction resolution imaging with significantly improved spatio-temporal resolution. Due to the broad selection of commercially available photoswitchable organic fluorophores, the intriguing simplicity of photoswitching and its straightforward implementation dSTORM volunteers as powerful super-resolution imaging method that can be immediately adopted even by non-experts. Reversibly photo-switchable organic fluorophores have the potential to improve the spatial and temporal resolution of super-resolution imaging because they survive under moderate excitation conditions for prolonged time periods, can emit orders of magnitude more photons than fluorescent proteins, and exhibit tunable photoswitching rates.

**References**


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