Fast, three-dimensional super-resolution imaging of live cells

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We report super-resolution fluorescence imaging of live cells with high spatiotemporal resolution using stochastic optical reconstruction microscopy (STORM). By labeling proteins either directly or via SNAP tags with photoswitchable dyes, we obtained two-dimensional (2D) and 3D super-resolution images of living cells, using clathrin-coated pits and the transferrin cargo as model systems. Bright, fast-switching probes enabled us to achieve 2D imaging at spatial resolutions of ~25 nm and temporal resolutions as fast as 0.5 s. We also demonstrated live-cell 3D super-resolution imaging. We obtained 3D spatial resolution of ~30 nm in the lateral direction and ~50 nm in the axial direction at time resolutions as fast as 1–2 s with several independent snapshots. Using photoswitchable dyes with distinct emission wavelengths, we also demonstrated two-color 3D super-resolution imaging in live cells. These imaging capabilities open a new window for characterizing cellular structures in living cells at the ultrastructural level.

The development of super-resolution fluorescence microscopy has allowed the diffraction-limited resolution to be surpassed[1,2]. This advance typically has been achieved in two ways: (i) spatially modulating the fluorescence emission with patterned illumination as in the cases of stimulated emission depletion microscopy (STED or reversible saturable optical fluorescence transitions (RESOLFT))[1,3] and saturated structured illumination microscopy (SSIM)[4,5], or (ii) stochastically switching individual molecules on at different times as in the case of stochastic optical reconstruction microscopy (STORM), photoactivation localization microscopy (PALM) or fluorescence PALM (FPALM)[6–8]. The latter approach also requires high-precision localization of single molecules[9,10] and photoswitchable probes. These techniques have allowed biological structures to be imaged with resolution as high as ~20 nm. Recent demonstrations of super-resolution imaging in living cells, as exemplified by the video-rate STED imaging of synaptic vesicles in live neurons[11], have enabled the characterization of cellular dynamics with sub-diffraction-limit resolution.

Owing to the intrinsic trade-off between spatial and temporal resolutions, however, the image resolution achieved in live cells is substantially lower than that for fixed samples, for which the imaging speed is not a concern. The spatial resolution reported for the video-rate live-cell STED is ~60 nm in the lateral dimensions, threefold larger than what has been achieved on fixed cells[11]. A recent live-cell STED study reports ~150 nm axial resolution when imaging samples in the *x*-*z* plane[12]. Live-cell SIM has achieved ~10 Hz imaging speed in a wide field with a spatial resolution of ~100 nm in the lateral dimensions[13]. For the single-molecule-based imaging methods, such as PALM, FPALM or STORM, this trade-off arises from the requirement that a sufficiently large number of localizations need to be accumulated for each snapshot in order to define a structure with a desired spatial resolution. This requirement is best characterized by the Nyquist criterion which equates the image resolution to $2/(\text{localization density})^{1/D}$, where $D = 1$, 2, or 3 for one-dimensional (1D), 2D or 3D imaging, respectively[14]. Therefore, although photoactivation-facilitated high-density particle tracking has proven powerful for probing molecular motions in living cells[15–17], the Nyquist criterion has so far limited the spatial resolution of the single-molecule-based imaging methods to 40–70 nm in two dimensions with a 30–60 s time resolution[14,18], when imaging photoactivatable fluorescent proteins in live cells. Two-dimensional super-resolution imaging has also been performed in living cells with photoswitchable dyes[19–22], but the localization density has not been characterized in these cases, and thus the image resolution achieved is unclear. Because more localizations are inherently required to define a structure in three dimensions, it is expected that extending super-resolution imaging to three dimensions will further deteriorate the time resolution. Indeed, 3D super-resolution imaging has not yet been achieved for live cells with the single-molecule-based methods. These limitations have hindered the application of super-resolution fluorescence microscopy to the ultrastructural characterization of living cells.

Here we report 2D and 3D super-resolution imaging of live cells with high spatial and temporal resolutions using photoswitchable dyes. We achieved a Nyquist resolution of ~20 nm with a time resolution as high as 0.5 s for 2D STORM imaging. Moreover, we achieved 3D volumetric super-resolution imaging of live cells

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