polyester films, should lead to significantly improved device stability, which is needed for the introduction of perovskite solar cells into the market. ❐

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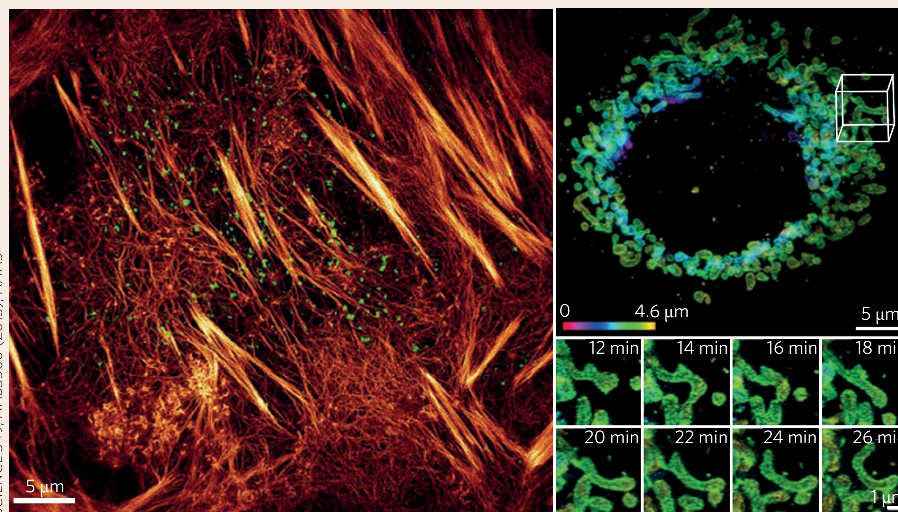
INTRACELLULAR IMAGING

Resolution power

Advances in the understanding of intracellular dynamics are largely dependent on imaging techniques that are capable of tracking the movement of single proteins and molecular complexes, and of visualizing the dynamics of a large number of macromolecular assemblies, such as the actin cytoskeleton. To capture the *in vivo* dynamics of intracellular processes, which typically occur at speeds of tens of nanometres per second, fluorescence microscopy techniques that overcome the diffraction limit are best suited, as they are largely non-invasive and can achieve the necessary spatial resolution (lower than a few hundred nanometres) and acquisition speeds (at least a few frames per second).



Among such super-resolved fluorescence microscopy approaches, probe-based techniques such as photoactivated localization microscopy (PALM), and illumination-based methods such as stimulated emission depletion (STED) microscopy and structured illumination microscopy (SIM), have all greatly contributed to the tracking and imaging of biomolecules and nanoscale features within cells. They all use genetically encoded photoswitchable fluorescent proteins as endogenous labels, and achieve higher resolution images by either rendering composite images from hundreds or thousands of diffraction-limited snapshots — each with a small fraction of the intermittently photoswitched and then photobleached fluorophores (as in PALM) — or by modifying the pattern of excitation light and by quenching fluorescence emission outside the focal spot with a second laser to decrease the size of the spot below the diffraction limit (as in STED and SIM). PALM and STED use excitation intensities at levels (over 10^3 W cm^{-2}) that can cause phototoxic damage to the cell, and typically have slow acquisition speeds (~1 s per frame). Instead, SIM can acquire images at faster speeds (~0.1 s per frame) and at much lower intensities (1–100 W cm^{-2}), yet its spatial resolution has only reached twice that of the diffraction limit (~100 nm with visible light). Hence, except for the tracking of single biomolecules with localization microscopy, which can be done at resolutions below 60 nm, the visualization of intracellular dynamics has been restricted to the regime above the 100-nm resolution limit of conventional SIM.

Eric Betzig and co-workers (*Science* **349,** aab3500; 2015) have now extended SIM to image intracellular dynamics with a 45–84-nm resolution for up to 100 frames while maintaining the speed and low-intensity advantages of the technique. The researchers boosted the resolution to 84 nm (for more than 100 frames) by using a lens with a much higher numerical aperture (which restricts the exposure to a smaller fraction of the sample), and further increased it to 45 nm (for up to about 20 frames, which is a significant reduction despite the modest resolution increase) by overlapping tens of patterns of photoactivation and deactivation of a subset of the fluorescence labels (using a recently developed fluorophore: X. Zhang, *et al. ACS Nano* **9,** 2659–2667; 2015).

The increased spatial resolution and rapid image-acquisition speeds for long durations achieved with SIM by Betzig and colleagues allowed them to image, in living cells and in three dimensions when their extended SIM approach was combined with light sheet microscopy, the remodelling of the actin cytoskeleton (pictured, left), the dynamics of caveolae and of clathrin-mediated endocytosis, vesicle trafficking, and the fusion and fission of mithocondria (pictured, right). They also determined the sizes of individual clathrin-coated pits, and found that the internalization of the pits is often aided by actin filaments. The exceptional visual detail that the extended SIM approaches offer will surely help resolve and quantify plenty of intracellular trafficking events and collective phenomena, including the diffusion and interactions of proteins and protein complexes, signal transduction, raft formation and membrane-protein organization.

PEP PÀMIES

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