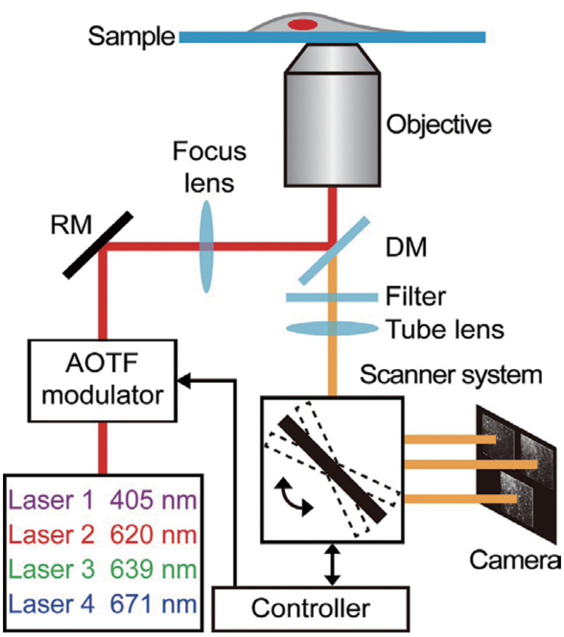
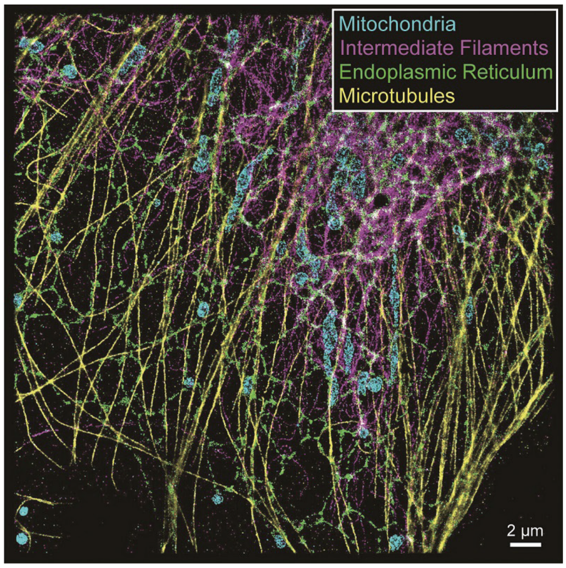
ExR-STORM: A New Multicolor Single-Molecule Localization Microscopy Method for Investigating Nanostructures in Cells

Single-molecule localization microscopy (SMLM) has revolutionized the exploration of nanostructures in cells that were previously hidden by diffraction limits. In addition to spatial resolution, multicolor imaging is crucial for investigating protein colocalizations and organelle interactions in biological research.

In a study, published online in *Light Science & Applications* on January 2, researchers from the Institute of Biophysics (IBP) at the Chinese Academy of Sciences (CAS) have developed a new multicolor SMLM method called excitation-resolved stochastic optical reconstruction microscopy (ExR-STORM).



This figure shows the working principle of ExR-STORM, where three excitation lasers and one activation laser were combined and modulated by an acousto-optic tunable filter (AOTF) prior to illuminating the sample. (Image by IBP)



It depicts tetra-color imaging based on ExR-STORM in a fixed COS-7 cell, where different fluorescent dyes were used to label specific cell structures. (Image by IBP)

This innovative method synchronizes three excitation lasers (620 nm, 639 nm, and 671 nm) with a resonant scanner to capture single molecule images in one frame. The researchers note that the resonant mirror is essential to their technique, enabling fast-switching to record the right photon number distribution.

To verify the capabilities of this method, the researchers labeled microtubules, intermediate filaments, endoplasmic reticulum, and the outer mitochondrial membrane with different fluorescent dyes. They successfully reconstructed tetra-color super-resolution images with negligible cross-talk between dyes.

ExR-STORM provides several advantages, such as negligible chromatic aberrations and the ability to resolve dyes with similar emission spectra. The technique also extends color channels with less than 3% cross-talk and allows for high scanning speeds, enabling the imaging of fast dynamics in STORM imaging.

This research offers a powerful tool for high-throughput investigation of 3D nanostructures in cells, which has great potential for biological and medical research. The researchers believe that ExR-STORM can further advance the understanding of organelle organization and interactions in cell stress responses, migration, division, and differentiation.

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(Source: IBP)

Reference

Wu, W., Luo, S., Fan, C., Yang, T., Zhang, S., Meng, W., . . . Gu, L. (2023). Tetra-color superresolution microscopy based on excitation spectral demixing. *Light Science & Applications, 12*(1), 9. doi:10.1038/s41377-022-01054-6