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Taming the image background beast

Background removal greatly improves localization microscopy performance.

The performance of super-resolution localization microscopy depends on how well individual molecules are localized. It is therefore unsurprising that there has been considerable development of localization algorithms. But the image frames used by these algorithms to reconstruct a final super-resolution image rarely contain nice bright spots on a uniform dark background: real images have background fluorescence that alters and disguises spots. Although many methods of removing background exist, these techniques receive relatively little attention. Marten Postma and colleagues now show that the method used to estimate and remove background from images can profoundly affect the quality of the reconstructed image.

When repeated cycles of fluorophore activation and inactivation are used to generate image frames for fluorophore localization and image reconstruction, specific signals appear and disappear between frames, whereas nonspecific background remains mostly constant or varies over longer timescales. Postma and colleagues applied a temporal filter, specifically a median filter, on each pixel across the image frames. This discarded specific signals and generated an estimate of the background, including slowly varying components, which they subtracted from the image frames before running the localization algorithm of their choice. Using a variety of imaging techniques and samples, they observed that this filtering approach improved the quality of the final image and resulted in more reliable final images. For example, localization algorithms that produced very different reconstructed images on their own generated highly similar images when the temporal median filter was applied first. This filter outperformed both spatial filtering and other temporal filter schemes, although its performance depended on sample and image characteristics.

Temporal filtering isn't new, and even median filtering has been implemented before, but this appears to be the first report to characterize the method's impact. Although it is not a panacea for all localization-based microscopy woes, the authors saw that even applying this filter before analysis with stand-alone localization software improved image quality. This observation comes with caveats, though, and filtering should ideally be integrated into the reconstruction software. But background estimation deserves more attention, and investigators ignore it at their peril.

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RESEARCH PAPERS

Hoogendoorn, E. *et al.* The fidelity of stochastic single-molecule super-resolution reconstructions critically depends upon robust background estimation. *Sci. Reports* **4**, 3854 (2014).

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