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USER-FRIENDLY METHODS TO DETECT ARTIFACTS IN SUPER-RESOLUTION MICROSCOPY

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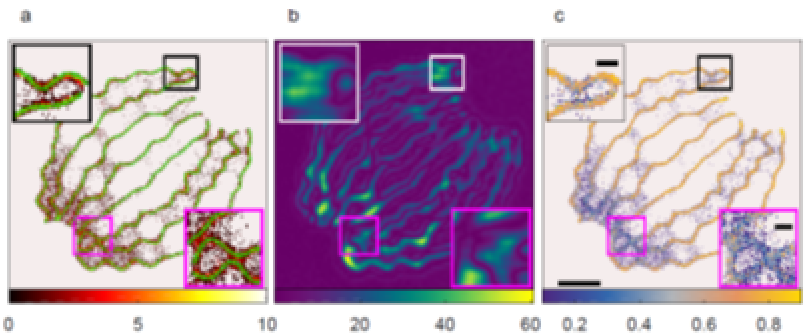
Engineers at Washington University have devised an automated system to enhance super-resolution microscopy images by detecting and quantifying image artifacts using no *a priori* information. This project stems from the advanced imaging research in Prof. Matthew Lew's laboratory that includes optical hardware and computational technologies for super-resolution imaging (detailed below).

Single-molecule localization microscopy (SMLM), a form of super-resolution fluorescence microscopy, is useful for visualizing nanoscale structures in biology and material science. However, SMLM image reconstruction inevitably produces artifacts that must be corrected in order to fully harness the power of SMLM for scientific discovery. Current methods to interpret these artifacts require complicated fabrication of specific calibration samples and/or require *a priori* ground truth information which is not available for experimental datasets. Prof. Lew's new computational technology solves this problem with a broadly applicable method called Wasserstein-induced flux (WIF) that directly measures and robustly quantifies the accuracy of each individual localization in the image. WIF evaluates mathematical models of the imaging system itself (e.g., its PSF) combined with the raw data from the microscope to generate a pixel-by-pixel confidence map of the SMLM image. With these confidences in hand, unreliable localizations may be filtered from SMLM images while retaining sufficient accurate localizations to resolve fine features within the SMLM images. This approach is faster and more robust than relying on expert users to identify correct localizations, and it is more accurate in low signal-to-noise datasets. WIF can be easily implemented in any super-resolution imaging pipeline with only basic knowledge of the imaging system and has the potential to become a valuable tool for SMLM analysis.



WIF Confidence map (C) reveals artifacts in a SMLM image of a tubulin network. (A) SMLM reconstruction (red) from FALCON overlaid with the ground truth (green). (B) Error map of the FALCON image recovered by the SQUIRREL error-estimation algorithm (brighter colors correspond to larger errors).

Stage of Research

The inventors demonstrated that their WIF confidence map outperformed other image-based methods in detecting artifacts in high-density 2D and 3D SMLM using experimentally collected SMLM images of microtubules. They also used WIF to improve reconstruction accuracy and image resolution in super-resolution Transient Amyloid Binding imaging under low signal-to-noise ratio.

Applications

- **Super-resolution microscopy -** SMLM image resolution:
  - quantifying localization accuracy for analysis
  - online parameter tuning during an experiment to optimize results

Key Advantages

- **User-friendly and automated:**
  - produces quantitative, easily-interpreted molecule-by-molecule error maps that directly show which parts are trustworthy and which are not
  - provides consistent measure of accuracy under various imaging conditions without expert knowledge of the sample
  - readily implemented in any super-resolution imaging pipeline (any sample preparation, optical hardware, and image processing software) with only basic knowledge of the imaging system
- **Analysis of experimental images:**
  - no *a priori* or ground truth information needed for the sample being imaged
  - particularly useful in biological samples with high sample-to-sample variation
  - uses model imaging system to judge trustworthiness with no training data needed
- **Nano-scale resolution in quantifying errors:**
  - could detect otherwise hidden properties to discover new biophysical and biochemical phenomena
  - readily extended to 3D or molecular orientation

Related Point Spread Function (PSF) Technologies for Super-Resolution Imaging

Tri-spot point spread function (WUSTL Technology T-017161) – The tri-spot PSF is a new design for an optical imaging system that can be added to existing imaging devices. It produces three spots from a single source of light to provide complete, precise information (all 5 degrees of freedom) about the 3D orientation and the mobility of single molecules inside living cells with maximum signal-to-noise ratio. In addition, this PSF could be used to refocus and digitally sharpen photographs.

Duo-spot point spread function (WUSTL Technology T-019178) – The duo-spot PSF manipulates light to create two spots on the camera per detection channel to more accurately measure the orientation of molecules in low signal-to-noise conditions. This PSF produces brighter images than 3- or 4-spot PSFs, and it can resolve orientations that are ambiguous with conventional single and 2-spot PSFs. This technology could be particularly useful for elucidating nanoscale processes such as protein-protein interactions, protein folding and nanoparticle transport when using weak fluorescent emitters.

Publications – Artifact detection:

- Mazidi, H., Ding, T., Nehorai, A., & Lew, M. D. (2019). Measuring localization confidence for quantifying accuracy and heterogeneity in single-molecule super-resolution microscopy. bioRxiv, 721837.

Publications – Tri-spot PSF:

- H. Mazidi, E. S. King, O. Zhang, A. Nehorai, and M. D. Lew, Dense Super-Resolution Imaging of Molecular Orientation via Joint Sparse Basis Deconvolution and Spatial Pooling, *2019 IEEE 16th International Symposium on Biomedical Imaging (ISBI 2019)*, 325 (2019).
- O. Zhang, J. Lu, T. Ding, and M. D. Lew, Imaging the three-dimensional orientation and rotational mobility of fluorescent emitters using the Tri-spot point spread function, *Appl. Phys. Lett.* **113**, 031103 (2018).
- Zhang, O., Ding, T., Lu, J., Mazidi, H., & Lew, M. D. (2018, February). Measuring 3D molecular orientation and rotational mobility using a Tri-spot point spread function. In Single Molecule Spectroscopy and Superresolution Imaging XI (Vol. 10500, p. 105000B). International Society for Optics and Photonics.

Patents

- Systems and methods for performing optical imaging using a tri-spot point spread function (psf) (U.S. Patent Application Publication No. US20180307132)
- Additional U.S. Patent Application Pending

Website

- Lew Lab