

Engineered point spread function localization using phasor-based single molecule localization microscopy

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Keywords: localization, phasor, saddle-point, double-helix

We have shown recently that phasor-based localization microscopy (pSMLM-3D) is capable of retrieving 2D and astigmatism point spread function (PSF) localizations with good accuracy and with MHz rates on standard, consumer-grade computational processing units (CPUs) [1].

However, astigmatism has a limited axial range ($\sim 1 \mu\text{m}$). Advancements in engineering of PSFs, such as saddle-point and double-helix, realized higher axial range ($> 3 \mu\text{m}$) and better localization precision. Currently the best algorithms designed for localization of these engineered PSFs rely on phase retrieving methods or spline interpolation, which are both computationally expensive and thus require graphical processing units (GPUs) to obtain real-time localization rates.

Via novel adaptations of the phasor-based single molecule localization microscopy (pSMLM-3D) algorithm [1], we are able to obtain very quick retrieval of saddle-point or tetra-pod ($2.5 \cdot 10^5 \text{ loc/s}$) and double-helix ($1.5 \cdot 10^4 \text{ loc/s}$) PSF localizations on a standard CPU. For saddle-point and tetra-pod PSFs, a deconvolution of the spatial plane is achieved by a linear combination in phasor space. This results in a quick and straightforward methodology to obtain the distance between the individual lobes. This distance, after calibration, is a direct measure for the PSFs axial position

- [1] K. J. A. Martens, A. N. Bader, S. Baas, B. Rieger, and J. Hohlbein, "Phasor based single-molecule localization microscopy in 3D (pSMLM-3D): An algorithm for MHz localization rates using standard CPUs," *The Journal of Chemical Physics* **148**(12), 123311 (2018).