

AN OPEN MICROSCOPY FRAMEWORK SUITED FOR TRACKING dCAS9 IN LIVE BACTERIA

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SINGLE PARTICLE TRACKING OF dCAS9

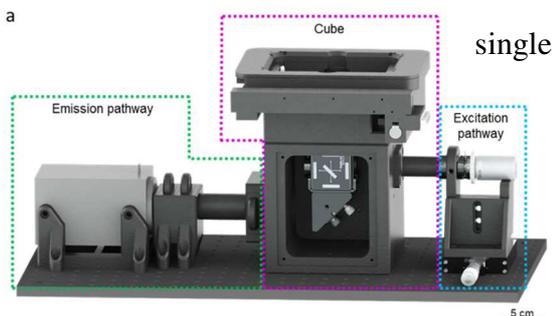
Super-resolution microscopy is frequently employed in the life sciences, but the number of freely accessible microscopy frameworks, especially for single particle tracking photo-activation localization microscopy (sptPALM), remains limited. We designed the miCube: a versatile super-resolution capable fluorescence microscope, which combines high spatiotemporal resolution, good adaptability, and easy installation (preprint [1]). We further achieved fast data analysis via a phasor-based localisation algorithm [2]). The capabilities of the miCube are assessed with a novel sptPALM assay relying on the heterogeneous expression of catalytically inactive Cas9 (dead Cas9) in live *Lactococcus lactis*.

THE MICUBE MICROSCOPY PLATFORM

The miCube microscope (Fig. 1) is an multi-purpose, custom-built and open-access fluorescent microscope capable of high-grade super-resolution experiments. By combining off-the-shelf parts with open-sourced custom-designed aluminium body and 3D printed plastic parts, we designed and built a modular microscope capable of delivering high-grade microscopy measurements. Different imaging modalities can be chosen from during experiments, including regular super-resolution microscopy in both 2D and 3D, total internal reflection fluorescence (TIRF) microscopy, and epifluorescence microscopy.

PHASOR-BASED SINGLE-MOLECULE LOCALISATION MICROSCOPY

We recently developed a fast single-molecule localisation algorithm called “phasor-based molecule localisation microscopy in three dimensions”, or pSMLM-3D [2]. The algorithm is capable of analysing over 3 million localisations per second, which is two orders of magnitude faster than other popular algorithms, while localization accuracies are in line with the most accurate algorithms currently available.



REFERENCES

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