

Microscopy method overcomes the traditional resolution limit for the fast co-tracking of molecules

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Fiona Cole and Jonas Zähringer, joint lead authors of the paper, calibrate a fluorescence microscope. Credit: LMU

Researchers at Ludwig Maximilian University (LMU) have developed an

innovative method to simultaneously track rapid dynamic processes of multiple molecules at the molecular scale.

Processes within our bodies are characterized by the interplay of various biomolecules such as proteins and DNA. These processes occur on a scale often within a range of just a few nanometers. Consequently, they cannot be observed with [fluorescence microscopy](#), which has a resolution limit of about 200 nanometers due to diffraction.

When two dyes marking positions of biomolecules are closer than this optical limit, their fluorescence cannot be distinguished under the microscope. As this fluorescence is used for localizing them, accurately determining their positions becomes impossible.

This resolution limit has traditionally been overcome in super-resolution microscopy methods by making the dyes blink and turning their fluorescence on and off. This temporally separates their fluorescence, making it distinguishable and enabling localizations below the classical resolution limit.

However, for applications involving the study of rapid dynamic processes, this trick has a significant drawback: blinking prevents the simultaneous localization of multiple dyes. This significantly decreases the [temporal resolution](#) when investigating dynamic processes involving multiple biomolecules.

Under the leadership of LMU chemist Professor Philip Tinnefeld and in cooperation with Professor Fernando Stefani (Buenos Aires), researchers at LMU have now developed pMINFLUX multiplexing, an elegant approach to address this problem.

The team has [published a paper](#) on their method in the journal *Nature Photonics*.

MINFLUX is a super-resolution microscopy method, enabling localizations with precisions of just one nanometer. In [contrast](#) to conventional MINFLUX, pMINFLUX registers the [time difference](#) between the excitation of dyes with a laser pulse and the subsequent fluorescence with sub-nanosecond resolution.

In addition to localizing the dyes, this provides insights into another fundamental property of their fluorescence: their fluorescence lifetimes. This describes how long, on average, it takes for a dye molecule to fluoresce after it is excited.

"The fluorescence lifetime depends on the dye used," explains Fiona Cole, co-first author of the publication. "We exploited differences in fluorescence lifetimes when using different dyes to assign the fluorescent photons to the dye that emitted without the need for blinking and the resulting temporal separation."

For this purpose, the researchers adapted the localization algorithm and included a multiexponential fit model to achieve the required separation.

"This allowed us to determine the position of multiple [dyes](#) simultaneously and investigate rapid dynamic processes between multiple molecules with nanometer precision," adds Jonas Zähringer, also co-first author.

The researchers demonstrated their method by accurately tracking two DNA strands as they jumped between different positions on a DNA origami nanostructure, as well as by separating translational and rotational movements of a DNA origami nanostructure and by measuring the distance between antigen-binding sites of antibodies.

"But this is just the beginning," says Philip Tinnefeld. "I am certain that pMINFLUX multiplexing, with its high temporal and [spatial resolution](#),

will provide new insights into protein interactions and other biological phenomena in the future."

More information: Fiona Cole et al, Super-resolved FRET and co-tracking in pMINFLUX, *Nature Photonics* (2024). [DOI: 10.1038/s41566-024-01384-4](https://doi.org/10.1038/s41566-024-01384-4)

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