

## MINFLUX single particle tracking at exceptional spatial and temporal resolution.

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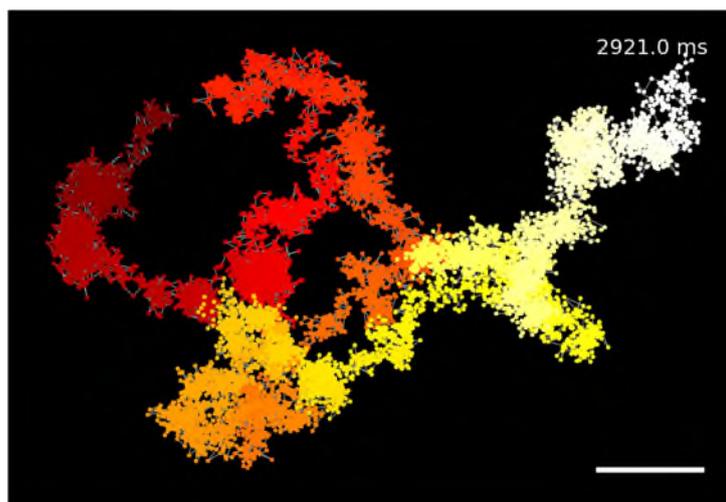
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An instrument capable of localizing single molecules with the highest precision and tracking their positions with a temporal resolution  $> 8$  kHz is desired by many scientists in various fields of research.

MINFLUX can localize single molecules by measuring the fluorescence signal with an excitation beam featuring an intensity zero placed to pre-defined positions in the fluorophore's immediate proximity. Thus, the number of fluorescence photons needed to localize the molecule is minimized. MINFLUX affords fluorophore localization with a very small number of detected photons and consequently within a spatio-temporal regime exceeding that of alternative techniques [1].

In single particle tracking, the limited fluorescence photon budget and emission frequencies constrain the achievable tracking performance using organic fluorophores [2]. MINFLUX overcomes these limitations by using photons more efficiently. We show single fluorophore tracks on supported lipid bilayers recorded with thousands of localizations acquired over several seconds with extraordinary temporal and spatial resolution. The results demonstrate MINFLUX's great potential for future Single Particle Tracking experiments, e. g. tracking of proteins on cell membranes using fluorescent markers or tracking of fluorescent proteins in living cells, keeping the disturbance of the endogenous cellular environment at a minimum.



**Figure 1.** Tracking of a single Atto 647N molecule coupled to a lipid in a supported lipid bilayer.

### References

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- [3] Schmidt R., T. Weihs, C.A. Wurm, I. Jansen, J. Rehman, S. J. Sahl and S. W. Hell (2021) MINFLUX nanometer-scale 3D imaging and microsecond-range tracking on a common fluorescence microscope. *Nature Communications in press*