Turn-key optical tweezers with IRM, TIRF, Widefield and STED: a platform for dynamic single molecule analysis

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Microtubules and actin filaments are dynamic cytoskeletal structures that interact with motor proteins and play a fundamental role in many essential biological processes including cell division, cell migration, and mechanosensing.

Single-molecule analysis played a central role in revealing many aspects of these complex and dynamic interactions. During a typical dynamic single-molecule experiment, cytoskeletal filaments are imaged for an extensive period of time using fluorescence methods. It can be highly desirable to study these individual protein filaments with high contrast and at high temporal resolution, but without the need for fluorescence labeling, to make the assay set-up less laborious, costly and – in some experiments – prevent inducing phototoxicity.

Here we present a easy to use turn-key instrument that includes optical tweezers in combination with Interference Reflection Microscopy (IRM) and Total Internal Reflection Fluorescence (TIRF) Microscopy. Interference Reflection Microscopy is a recently introduced imaging method that allows visualizing biological structures in 3D without the need of fluorescence labeling and with sensitivity exceeding DIC microscopy [1]. Total Internal Reflection Fluorescence microscopy provides high resolution fluorescent imaging of specimen near the working surface with high signal to noise ratio resulting in improved single-molecule surface assays.

In addition, we demonstrate how the combination of optical tweezers with STED microscopy makes it possible to visualize the dynamics of densely packed proteins on DNA.

In this work, we will discuss the experimental design and show an overview of the latest results obtained using this single-molecule approach.

REFERENCES

[1] Simmert, S., Kazem Abdosamadi, M., Hermsdorf, G., & Schäffer, E. (2018). LED-based interference-reflection microscopy combined with optical tweezers for quantitative three-dimensional single microtubule imaging. *Opt. Express*, *26*(11), 14499–14513. http://doi.org/10.1101/277632