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

interference-reflection microscopy combined with optical tweezers for quantitative three-
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