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Capturing Single Molecule Dynamics: An Advanced Microscope Combining Optical Tweezers with Fluorescence Detection Modules

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Complicated yet sophisticated biomolecular networks regulating cellular metabolism are performed by proteins interacting with and processing DNA and RNA amongst other biological molecules. Recent studies have shown insights into these processes, especially kinetics features, providing essential information for understanding the molecular basis of life and the pathological conditions that develop when such processes go awry.
The next scientific breakthrough relies on direct, real-time observations and measurements of the individual mechanisms involved, in order to validate and complete the current biological models. To this end, structural and functional information, which is often indivisible, needs to be studied together in real-time and at the molecular level. In this complex context, single-molecule technologies offer an exciting opportunity to meet these challenges.
Here, we present our efforts to further enable discoveries in cell biology and biophysics using a fully integrated optical tweezers with single-molecule fluorescence microscopy technology. C-Trap™ is correlating Optical Tweezers with Interference Reflection Microscopy (IRM), Total Internal Reflection Fluorescence (TIRF) Microscopy, Confocal Fluorescence Microscopy, and 1D STED. With this powerful technique, manipulation and visualization of single molecules are possible with nanometer spatial resolution and millisecond temporal resolution, while piconewton forces can be measured.

In addition, we demonstrate a user-friendly and high throughput data acquisition method, which is supported by python scripting. The automation of experimental workflows has brought the repeatability and reproducibility of C-Trap™ to the next level.

We demonstrate the latest applications of these technologies that can further our understanding of DNA/RNA structure and their interactions with proteins, as well as molecular motors, protein folding/unfolding, cell membranes, and genome structure and organization. These experiments showcase that the technological advances in combing single-molecule methods can be turned into an easy-to-use, high-throughput, and robust instrument with the ability to open new venues in many research areas.

References

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