Combining scientific techniques that measure different parameters can create powerful and effective experimental methodologies. Indeed, many scientific advances are driven by the success of new technological achievements. But marrying techniques that might have very distinct technical requirements is often extremely challenging. The article by Matthew Lang and colleagues in this issue of the Journal of Biology describes the first successful combination of optical trap and single-molecule fluorescence technologies in a single experiment and on a single molecule (see ‘The bottom line’ box for a summary of the work and the ‘Background’ box for definitions).

These two key technologies, optical traps and single-molecule fluorescence, have dominated the field of single-molecule research ever since they were independently developed over 15 years ago. Optical traps use the radiation pressure from a focused laser beam to trap and move single molecules. They can generate forces in the piconewton range, can measure displacements of nanometers, and have been creatively applied to the study of molecular motors such as kinesins moving along microtubules and to actin-myosin dynamics. Attaching fluorophores to biological macromolecules (protein or nucleic acids) allows investigators to image individual molecules by fluorescence microscopy. Single-molecule fluorescence has provided insights into many biological systems; for example, pioneering studies by Toshio Yanagida and colleagues in Osaka, Japan, investigated the kinetics of myosin binding to ATP using Cy3-labelled fluorescent ATP analogs [2]. Modern single-molecule fluorescence applications include FRET (fluorescence resonance energy transfer), to measure nanometer distances between different fluorescent moieties, and fluorescence quenching.

It has been clear for some time that combining optical trapping and single-molecule fluorescence would offer a powerful approach to monitoring spatial or conformational changes in a temporal manner at the single-molecule level. But several inherent features of the two techniques have prevented this marriage. “The infrared optical trapping light is extremely intense,” explains Steven Block of Stanford University, senior author of the Journal of Biology article. In contrast the single-molecule fluorophores have...
a relatively weak signal. The flux of the high intensity optical traps causes photobleaching of the fluorophores, and any leakage into the light channel drowns out the fluorescent signal. This probably explains why previous attempts to combine optical trap and single-molecule fluorescence failed. Some studies have circumvented these difficulties by sequential use of optical trap and single-molecule fluorescence, or the use of beads or microneedles to spatially separate the trap from the fluorophore [3]. But these solutions create their own constraints on experimental design and interpretation.

Lang et al. managed to overcome these technical problems and apply the two techniques simultaneously to measure the force required to prize apart two strands of DNA. They used complementary DNA strands labeled with rhodamine dye; one of the DNA strands was attached to a polystyrene bead and the other to a glass coverslip. The fluorescence of the dyes was quenched as a result of their proximity. The bead was trapped by the laser beam and the stage moved so as to ‘unzip’ the DNA strands. As the two strands are ripped apart the fluorophores separate, relieving the autoquenching and releasing a short burst of light.

“It’s a technical tour de force”, says Brandeis University’s Jeff Gelles. “This is an important technical development because it integrates two technologies that need to be done in very different conditions.” Although there are lots of groups using optical traps and lots using single-molecule fluorescence, few have the technical know-how to overcome the challenge of combining them. “The Block lab has always been a leader in showing us all how to do these things,” says Gelles. Block explains that his group’s success required bringing together expertise from several fields. He says “It has all been made possible by extremely careful optical design, the use of special filters, a judicious choice of fluorescent dyes and the speed gained by automating many aspects of the experiment.” (See the ‘Behind the scenes’ box for more of the background to the work.)
Now that combined single-molecule fluorescence and optical trapping has been achieved, single-molecule researchers are predicting that it will find applications in a wide range of fields. In fact, it may be useful in any system that explores the conformational changes and biochemical steps involved in macromolecular function. Recent applications of optical trapping include studies of catalytic RNA functions, the chemical properties of DNA and RNA molecules, protein folding and the activities of DNA polymerases. And the combined optical trap and single-molecule fluorescence methodology will enhance the traditional single-molecule study of proteins such as the molecular motors kinesin and myosin. The marriage of optical trapping and single-molecule fluorescence opens up a wealth of new potential applications. Now that the two are finally together, the months ahead should prove to be an exciting honeymoon period for single-molecule research.

References
1. Lang MJ, Fordyce PM, Block SM: Combined optical trapping and single-molecule fluorescence. J Biol 2003, 2:6.
2. Funatsu T, Harada Y, Tokunaga M, Saito K, Yanagida T: Imaging of single fluorescent molecules and individual ATP turnovers by single myosin molecules in aqueous solution. Nature 1995, 374:555-559.
3. Kitamura K, Tokunaga M, Iwane AH, Yanagida T: A single myosin head moves along an actin filament with regular steps of 5.3 nanometres. Nature 1999, 397:129-134.

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