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A marriage of techniques

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Technical wizardry has made possible the combination of two powerful methodologies, optical trapping and single-molecule fluorescence, to measure precisely the force required to unzip DNA.

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* [1] 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

The bottom line

- Lang and colleagues have developed a procedure for combining optical trapping with single-molecule fluorescence on the same macromolecule at the same time.
- Their key achievement was to be able to visualize the fluorescence from a single molecule at the same time and in the same place as the intense optical trapping laser was operating.
- By measuring the force required to separate double-stranded DNA, Lang *et al.* demonstrate a proof-of-principle and open up the potential to apply this approach to study nucleic-acid-processing machines and molecular motors.

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.)

Behind the scenes

Journal of Biology asked Steven Block to comment on the challenges he and his colleagues faced and the potential applications of the new technique.

What was the motivation for figuring out how to combine these techniques?

These two techniques have been around for over a decade but combining them in a single experiment presented a technical challenge. Optical traps (or 'tweezers') offer a wonderful way to grasp and manipulate proteins, but they use lots and lots of photons. In single-molecule fluorescence the signals measured are relatively weak - around ten orders of magnitude weaker than optical traps. That's a fabulous difference and the optical trap obscures the single-molecule fluorescence. People had used the two sequentially or used tweezers in one part of a molecule and looked at fluorescence in another part (over relatively large distances), but no one had shown that they could be used together on the same spot. We knew that others had tried and failed and we thought we understood something about the mechanisms by which it was failing.

How long did it take to do the experiment and what were the steps that ensured success?

It took us about two years to build the equipment. The secret was to find the right combination of dyes, filters and automation. We needed fast dyes and special filters to block out stray light. And it was important to automate extensively so that we could do the measurements really quickly.

What was your initial reaction to the results and how were they received by others?

We were delighted when we saw that it could be done. Two decades ago optical traps sounded like the stuff of science fiction. Now we have demonstrated that we can successfully combine optical trap and single-molecule fluorescence. When we measured the force of ripping apart the DNA we got much the results that we expected. But we can measure these forces with greater precision than they had been measured before. We had to emphasize to colleagues that although optical trap and single-molecule fluorescence have been widely applied, previous experiments that combined them separated the two methods either in time or space.

What are the potential applications of your technique?

The combined technique will be of widespread interest to the single-molecule field and to anyone with a 'motor-like' output. Obvious examples include motors like kinesins and myosin, but also processive nucleic acid enzymes such as polymerases and ribozymes. We can control loads and forces to watch enzyme displacements and measure nanoscale biochemical changes. There are lots of models and hypotheses that can now be tested with the combined technology.

Background

- **Optical traps**, also called 'optical tweezers', use the radiation pressure of single-beam infrared lasers to 'trap' and move molecules.
- **Single-molecule fluorescence** monitors the microscopic location or movement of individual proteins fused to fluorescent dyes.
- **Fluorescence resonance energy transfer** (FRET) measures inter-atomic distances in biological molecules in real time by monitoring the transfer of energy from different donor-acceptor fluorophores.

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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