August 29, 2005 | Vol. 19 | No. 16 | www.the-scientist.com

The Scientist

7 TECHNOLOGIES

THAT ARE TRANSFORMING THE LIFE SCIENCES

THE OPTICAL TRAP

How a laser beam and some clever engineering spawned a biophysics revolution

By Don Monroe

When Art Ashkin, Steve Chu, and their colleagues at Bell Labs in Holmdel, NJ, first invented optical tweezers, they spent their days pushing around tiny, glass spheres. But it wasn't long after their 1986 discovery that they began to think about biology.

"We were trapping submicron particles of Tobacco mosaic virus," Ashkin says. "We left the samples under the microscope for a day or so, and then we discovered strange particles that seemed to be self-propelled." When they looked into the trap with a higher-quality microscope, they confirmed what the mysterious objects [illegible] They discovered bacteria, 350 years too late," jokes Howard [illegible] was then studying bacterial flagella at the Rowland Insti[illegible]bridge, Mass.

[illegible] intense green light quickly killed the bacteria, a [illegible] [illegible]cution." But once the team switched to [illegible] could be kept alive indefinitely, even [illegible] innovation "pointed the way to the [illegible]lly study biological things" using opti[illegible] [illegible], who was then working in Berg's lab. [illegible] were hooked. "We got excited about [illegible] That was on a Tuesday. The following day [illegible] Rowland benefactor Edwin] Land to buy a laser, and by Friday we were up and doing experiments."

They first tethered a flagellum to a substrate, so that its action set the bacteria spinning, like a tail wagging a dog. They then paralyzed the flagellum and used optical tweezers to gently pull the bacteria sideways. By watching it return to position, they measured the elasticity of the specialized protein complex (the "hook") that couples the flagellum to the cell. Thus began the quantitative application of optical tweezers to biological systems.

OPTICAL TRAPPING Optical traps exploit the momentum of laser light to manipulate individual objects—not just beads, but also cells, organelles, and even atoms. After years of work, the Bell Labs group found they could hold a clear bead in place with a single, tightly focused laser beam, a surprisingly simple configuration now known as optical tweezers. If the bead starts to drift away, the laser light is deflected, and the particle is pushed in the opposite direction, back towards the focus.

The result is that the bead is held gently in place, as if by tiny springs. When the experimenter moves the light beam, the bead follows, so optical tweezers can move objects around like their nonoptical namesake. Moreover, unlike alternatives such as glass fibers or micropipettes, optical tweezers can reach into a cell without disrupt-

OPTICAL TRAPPING MILESTONES

1958 — Invention of laser

1970 — Art Ashkin levitates micron-sized particles against gravity

1986 — Demonstration of single-beam, gradient-force optical trap, better known as optical tweezers

1987 — Ashkin manipulates viruses and live bacteria in the optical trap

1989 — Optical trap used to manipulate live sperm

Steven Block and Howard Berg measure stiffness of bacterial flagella

1990 — Steve Chu reports optical manipulation of DNA at the International Quantum Electronics Conference

Block uses trap to study movement of kinesin on microtubules

ing it. They never break, and "cleaning" them is as simple as turning off the light.

Moving beyond traditional tweezers, researchers can also measure and change how hard the trap pulls on the bead. First, they calibrate the trap's "stiffness." Then, by measuring precisely how far off-center the bead is, they can deduce the optical restoring force being applied. The forces, typically on the order of tens of piconewtons, are similar to those exerted by the molecular motors that power muscle and actively transport material within cells. Finally, by incorporating the measurement into a feedback loop, the experimenters can tune the applied force. That makes optical traps particularly useful in molecular motor research, as it becomes possible to ask, how hard must I tug on a protein to make it stop moving? Says Stanford biochemist James Spudich, "Tremendous numbers of things have been learned about the motors using this technology."

MOLECULAR MOTORS When Spudich began using optical tweezers in the early 1990s, he had been engaged for nearly a decade in what he calls a "lively argument" about how myosin moves. Spudich believed this molecular motor took tiny steps, only about 10 nm long. Others favored a step size an order-of-magnitude larger. Both conclusions were based on a system of polystyrene beads coated with purified myosin, which Spudich and Michael Sheetz (now at Columbia University) had developed a decade earlier. The observations were indirect, however, and supported both hypotheses.

Now Spudich's group had a way to answer the question directly. In collaboration with Robert Simmons of King's College London, at Stanford on sabbatical, and Chu, who had arrived from Bell Laboratories in 1987, they built an optical tweezers apparatus that could resolve the steps of a myosin-coated bead to within 10 nm. They applied their beads to actin-coated slides, gently tugged on them with the tweezers, and watched them march along the filament, just as they do in vivo.

"We had to see a small step in a background of Brownian motion noise," Spudich recalls. "If we were right, the step size was going to be buried in that noise." Spudich estimates the team spent about $70,000 assembling the apparatus, but it wouldn't work until graduate student Jeff Finer bought "a few dollars worth of cardboard" at the university art store to block air currents. Finally, they had their answer: the beads moved in discrete steps of about 10 nm. "I don't think there's been any argument since," Spudich adds.

SINGLE-MOLECULE EXPERIMENTS For all their power, optical tweezers are "just one in an arsenal of tools" for single-molecule work, notes Chu. Optical tweezers are most useful for moderate forces, in the range of 1 to 100 piconewtons, says Carlos Bustamante of the University of California, Berkeley. Stronger forces are better studied using atomic-force microscopy or micropipettes, he says, while weaker forces are best studied using magnetic beads in a magnetic field.

There are other tools, too. "With traps you can manipulate things, you can apply forces to them, but you can't actually visualize simultaneously what's happening at the protein level in terms of conformational changes," Block points out. For that, researchers have used tools that can track, with nanometer precision, the movement of fluorophore-tagged protein molecules.

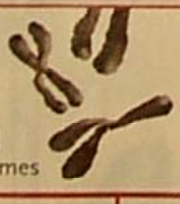
Block's team, and others, have combined fluorescent probes with optical traps — an engineering feat that requires detecting minute flashes of fluorescence in the presence of the intense light used for trapping. "It's now possible to do experiments that were considered pipe-dreams only a decade ago," says Block. Bustamante, who has combined fluorescent probes with magnetic tweezers, says, "This is a very important development."

CONTINUING EVOLUTION Naturally these new systems don't spell the end of the trap's long development. Though a basic commercial optical tweezers set-up can be purchased for around $10,000, a cutting-edge system, capable of applying precise forces to multiple particles and precisely measuring their positions, costs $150,000 or more, just for parts. Perhaps more importantly, the most advanced research still demands expertise in optics and electronics, as well as biology.

For almost a decade, researchers have been building tweezers with two traps, either by using two lasers, or by rapidly switching a single laser between two positions. Recently they have been exploring more complex configurations.

David Grier of New York University, for example, devised a holographic scheme for creating arrays of many traps. Jesper Glückstad of RISO, in Denmark, uses a different concept to form a sort of optical petri dish, which he and his collaborators have used to explore how the propagation of yeast cells changes when they are surrounded by cells of a different species. These multitrap tools could also explore the cell-cell interactions involved in tumor formation or stem cell differentiation.

Other groups have devised ways to apply torque in optical traps, by harnessing light's angular momentum. Traditional optical traps exert linear forces, but not torques. Thus to manipulate topoisomerases and helicases, researchers had to use magnetic beads instead of traps. Using the new schemes, which are still in early development, the torque exerted when light scatters from a rodlike object can be measured and even controlled, just as linear force can.



1991 Michael Sheetz probes motion of glycoproteins in membranes

Karl Greulich and colleagues use optical tweezers to isolate individual chromosomes

1993 J.J. Krol develops multiple-trap system

James Spudich, Chu, and Robert Simmons create a force trap with an active feedback circuit and use it to measure the force produced by a few myosin motors

1994 Spudich (below) measures step size of myosin; Steve Block measures step size for kinesin

1995 Jeff Gelles and Block measure force of an RNA polymerase molecule

1997 Carlos Bustamante and Simmons separately measure the folding and unfolding of titin

1998 Koen Visscher and Block develop molecular force clamp, which can maintain constant force on a single kinesin molecule

2004 Block builds first combination optical trap/fluorescence instrument

Timeline compiled in part by Steve Chu