



# Optical Traps Enter New Era of Nanomanipulation

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The latest positioning architectures enable new applications for optical traps.

In 1969, man landed on the moon, half a million revelers rocked Woodstock and, perhaps most amazingly of all, the New York Mets won the World Series. Amid all this, Arthur Ashkin of AT&T Bell Laboratories began investigating the radiation pressure acting on particles by a laser beam. Besides the axial acceleration of particles that he expected, he noticed that small particles congregated in the center of the beam. He could even steer the particles around by sweeping the beam.

The beam's puzzling tractor effect was soon understood both for very small (sub-wavelength) particles and for larger ones. The radial force that arises from the lens-

ing of light through larger particles — combined with the Gaussian beam profile's significant gradient and the dictates of momentum conservation — pulls toward the center of the beam those particles with a higher refractive index than their suspension medium. A similar net effect arises for smaller particles because the field gradient induces dipole moments in them.

### Trap force

The trap force is readily characterized, easily calibrated and rather strong, meaning that the technique is useful for quantitative studies of natural processes on the nanometer scale as well as for precise non-

contact manipulation of objects as small as 5 nm.<sup>1</sup>

There have been several generations of improvements to the technique, which has come to be known as optical "tweezing." Early on, researchers used it to manipulate a wide variety of particles, including living viruses and cells, and to explore the colloidal physics. These endeavors led Steven Chu of Stanford University in California to his Nobel Prize-winning research into trapping atoms and to the dynamical characterizations of nanoscale molecular motors in living cells made by Stanford's Steven M. Block.

Along the way, three-dimensional traps

were devised, including the single-beam configuration that is the basis of today's most frequently encountered setups. Users have integrated these into commercial microscopes, in which an objective with a high numerical aperture tightly focuses the laser beam, establishing a 3-D gradient that traps particles axially as well as transversely.

Most recently, the novel properties of Bessel-profile beams and holographically generated arrays of beams have enabled precise parallel manipulation of mesoscale arrays of microscopic objects. Also, electronically controlled holographic gratings have emerged for establishing complex and useful configurations of light,<sup>2</sup> and non-Gaussian modal structures and modified phase profiles have proved useful for trapping opaque or reflective particles, for imparting torque and for generating optical vortices. Not a month goes by without the appearance of another ap-

plication that affects the fields of optical physics, materials science or biophysics.

As successive generations of researchers and entrepreneurs build on earlier research, equipment manufacturers have answered the call for increasingly sophisticated building blocks. Most optical trap setups remain homemade, although a few pioneering companies have staked out specific applications to provide preconfigured tools that include optical traps. For example, Cell Robotics International Inc. in Albuquerque, N.M., offers a unit configured for microscopy manipulation, and Chicago-based Arryx Inc. produces a multibeam manipulator based on holographic optics.

Still, researchers generally build upon each other's designs, innovating incrementally. Many setups are based upon high-end commercial microscopes, although some are built from catalog optical components. Lasers are chosen to suit the particles being manipulated, and op-

tical position-detection and imaging hardware are commonly added.

One constant has emerged, however: Relentless demands for increasing performance require precise relative positioning of the beam and the sample.

Some configurations adjust the optical trap by guiding the laser beam as it enters the microscope with steering mirrors or acousto-optic scanners. Positioning also can be achieved through translation of a planoconvex lens<sup>3</sup> in the beam path, which provides axial positioning as well.

However, many advancements have resulted from using nanoscale-precision translation stages to move the trapped object(s) with respect to the microscope structure, which facilitates the dynamic positioning of the trap and the calibration of trap forces.<sup>3</sup> This, in turn, has facilitated insightful applications such as force and step metrology of individual transport and polymerase molecules in

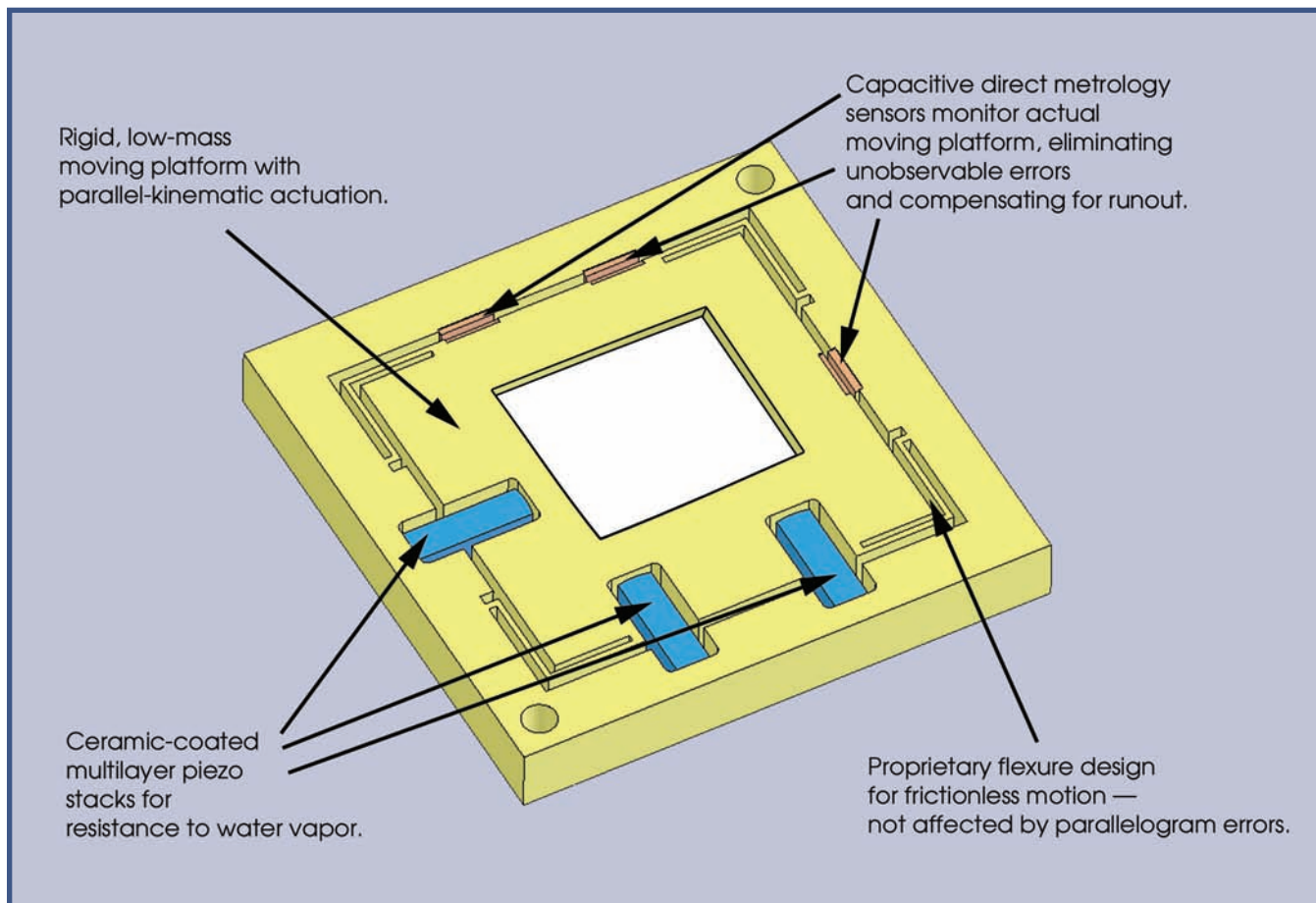


Figure 1. Nanopositioning stages with capacitive sensors for high resolution, bandwidth and stability enable optical trapping applications. Parallel-kinematics/parallel-metrology designs offer superior motion fidelity by positioning and measuring a single platform — using multiple actuators and sensors — against the same stationary reference.

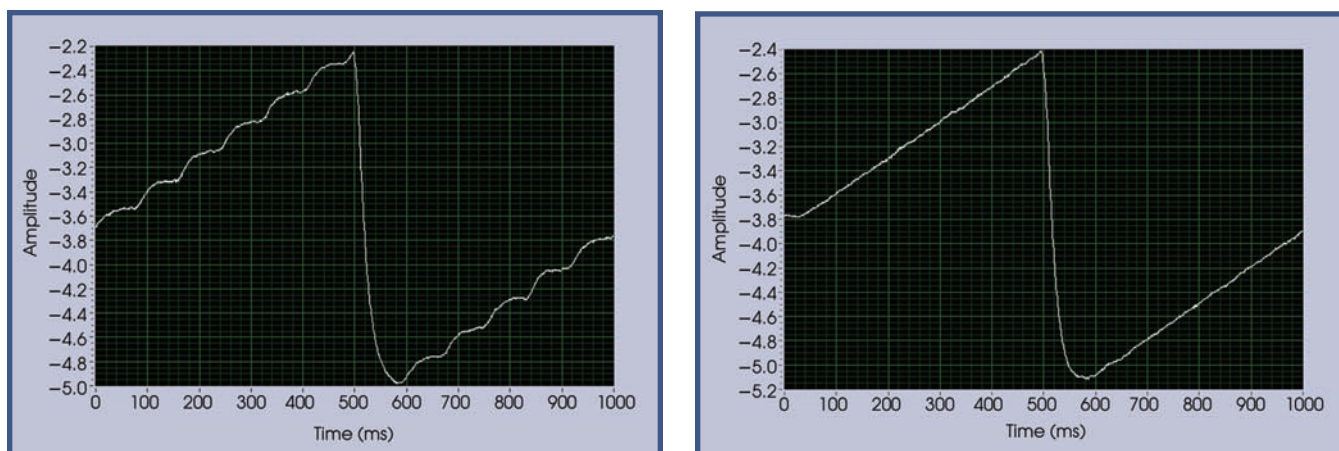


Figure 2. High-resolution automated actuation of a piezo stage is ultimately limited by digital-to-analog converter “bitness.” An interferometric measurement of a sawtooth motion profile — common in scanning applications — shows bit granularity (left). Adding HyperBit to the same hardware and software setup significantly improves the resolution and accuracy with no loss of bandwidth or responsiveness (right).

living cells, illuminating key biophysical processes.

The researcher’s choice of positioning mechanism thus emerges as critical. Resolution, repeatability and stability affect the capabilities of a setup because of the extraordinarily small dimensions and forces being investigated, but dynamic accuracy — the deviation of a rapidly actuated positioning system from the desired position at any instance — has gained importance because speed requirements have ramped up to keep pace with requirements for tracking, scanning and real-time alignment.

For example, Brownian motion is a pervasive issue on the molecular scale, and because this confers random motion on nano- and mesoscale objects of interest, corrective motions may, in some applications, be performed to maintain alignment.

Another example is the motion studies of protein-editing RNA polymerase and transport molecules such as kinesin. The trap must continually be recentered as the tethered molecule methodically moves along.

Other potential bottlenecks can limit a system’s ability to keep pace with rapid application processes. These factors include:

- System bandwidth, the overall responsiveness of a stage/controller combination, which is most often affected by amplifier and processor power.
- Interface throughput, the ability to

transmit commands and receive information such as status information, query responses and motion-complete signals.

- Following error, the system’s ability to be where it is supposed to be as a motion executes.
- Structural resonances, the recoil and ringing that occur in loads and adjacent components when a stage is actuated rapidly.

Fortunately, users benefit from recent technological developments driven not only by increasingly stringent research requirements, but also by industrial demands in arenas such as semiconductors and defense. In a well-designed system, each of these bottlenecks can be addressed.

### Integrated stage position sensors

Position sensors are integral for ensuring repeatability and accuracy in a nanopositioning stage’s movement. Piezoelectric drives, although capable of subatomic resolutions and >1000-g acceleration, exhibit a nonlinear and hysteretic curve of applied voltage vs. position. Generally, a linear relationship enables a repeatably achievable precise positioning. Therefore, stages include position sensors that provide the feedback necessary for an analog or digital servo-controller to eliminate hysteresis and non-linearity.

A variety of sensors have been used over the years. Among the more economical are strain gauges, a class of devices that

includes piezoresistive gauges. Such sensors are flexible films affixed either to the piezo stack or to the flexures of the stage and are capable of superb resolution. However, because they infer the position of the moving platform — the workpiece — indirectly, they are susceptible to losses, deflections and errors downstream from their point of measurement. Thermal instability also is a characteristic of these devices; together these drawbacks serve to limit them to budget mechanisms.

By comparison, capacitive sensors measure a moving platform’s position directly. This allows users to know the position of their load in real time and with sub-nanometer precision.

Capacitive sensors are composed of two plates of exquisite flatness, one affixed to the moving platform and one to the stationary frame of the stage for each axis. As the platform moves, it varies the gap between the plates, which changes the electrical capacitance of the cavity. When excited by a precisely controlled sinusoidal stimulation signal, this provides a sensitive measure of position with inherently superior stability and immunity to electromagnetic noise and ambient variances compared with such techniques as piezoresistive sensors. They have emerged as the premium sensor for the toughest applications because of their unmatched combination of resolution, stability, accuracy, environmental insensitivity and bandwidth.

As Block and his Stanford colleague

Keir C. Neuman noted in a recent review article, piezo-controlled stages permit three-dimensional control of the position of the trap relative to the trapping chamber, which had previously proved difficult or inaccurate.<sup>3</sup> These stages enable such capabilities as accurately calibrated piconewton force metrology and constant-force displacement metrology over their full length of travel, eliminating the working range of the trapping-chamber position detector as a limitation. This has proved to be a key enabler for molecular-force studies and investigation of RNA transcription and editing mechanisms.

Stages using capacitive sensors also are suitable for parallel-kinematics mechanisms, where several actuators move a single rigid workpiece through several degrees of freedom. A good example of this is an X-Y microscopy stage, which could be constructed of two independent, single-axis stages, either stacked or nested. This arrangement, however, results in orthogonality issues, dynamical differences between the axes, extra thickness, reduced rigidity and no way to compensate for orthogonal motion errors such as run-out, or deviation from straight-line travel.

In a superior design using parallel kinematics, a single rigid platform is actuated by multiple actuators simultaneously, with capacitive sensors monitoring the platform's position from several directions. In this way, the sensors compensate for

run-out and other orthogonal errors, and because only the workpiece moves, the moving mass is smaller than that of a stacked or nested configuration. This improves system responsiveness, motion dynamics and package size compared with nested or stacked configurations.

Of particular relevance to optical trap applications are the low-profile, microscope-friendly multi-axis configurations facilitated by this design approach, because even the most advanced nanopositioning technology is useless if it won't physically fit into the optical setup.

### Analog servocontrollers

The stage's servocontroller is another important part of the nanopositioning setup, and it can come in either analog or digital versions.

Analog controllers are capable of high speed and are simple to use. Calibration is performed at the factory and fixed in the system; the electronics and motion device are therefore generally matched. The position command input to the controller can be a voltage or a digital command sent via an RS-232, USB, IEEE-488 or proprietary digital interface.

The best interface is not necessarily the one with the highest data transfer speed, because piezo servocontrollers and motion controllers typically send and receive only a few characters at a time. Exact timing and minimal latency is more im-

portant for most nanopositioning applications, particularly in time-critical tracking and scanning applications.

In the case of an analog servocontroller with an analog voltage command input, the input voltage maps linearly to position. For analog controllers equipped with a digital communications interface, the incoming command is converted to a voltage input to the servo circuit by an internal digital-to-analog converter.

Most commonly, a digital-to-analog converter card installed in the user's PC provides the position command voltage for an analog servocontroller. For example, multifunction cards and applications software written with LabView from National Instruments of Austin, Texas, often are used in optical trapping applications, and the availability of well-designed driver libraries and knowledgeable staff is an important starting point for selecting positioning equipment.

Analog interfacing is fast, with negligible latency, and available tools make it easy to program waveform generation, such as that used to perform force metrology for trap calibration. It is straightforward to program intelligence into the trap-control software, including tightly integrated machine vision for visualization, analysis and position tracking, and for position and force sensing. Synchronization between voltage outputs and data acquisition is easy to arrange, making it

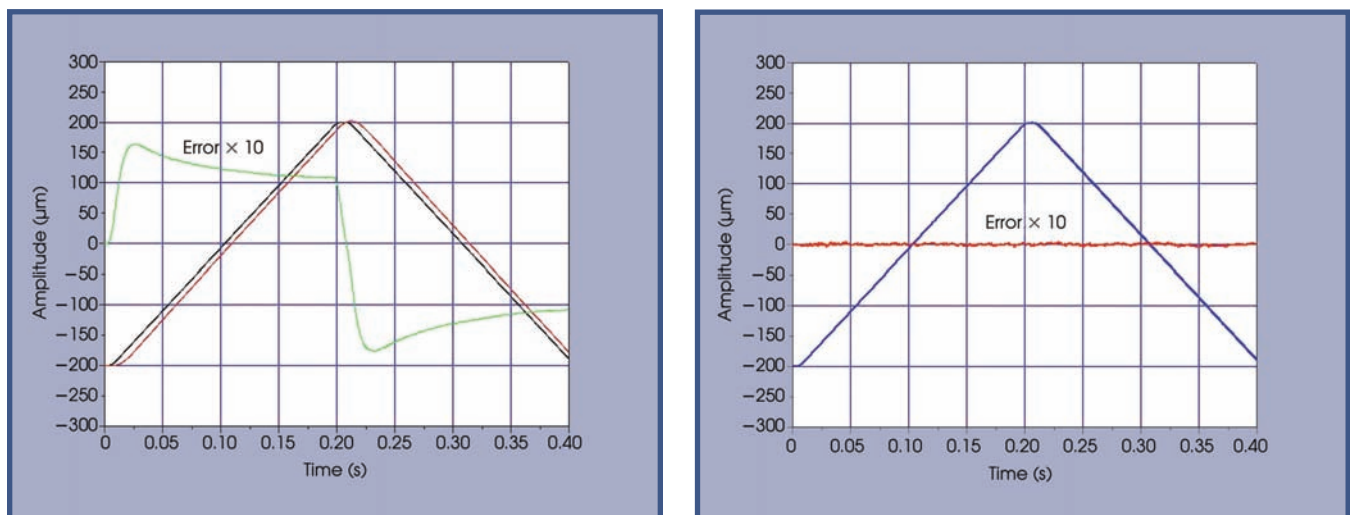


Figure 3. Conventional servos are error-following mechanisms, so the actual position of the stage (red) follows the desired position (blue) by a small amount. The difference is the following error, shown in the green trace (left). Digital dynamic linearization virtually eliminates following error in repetitive motion waveform actuation; the red (actual) and blue (commanded) traces are indistinguishable (right). The following error is reduced to the noise level.

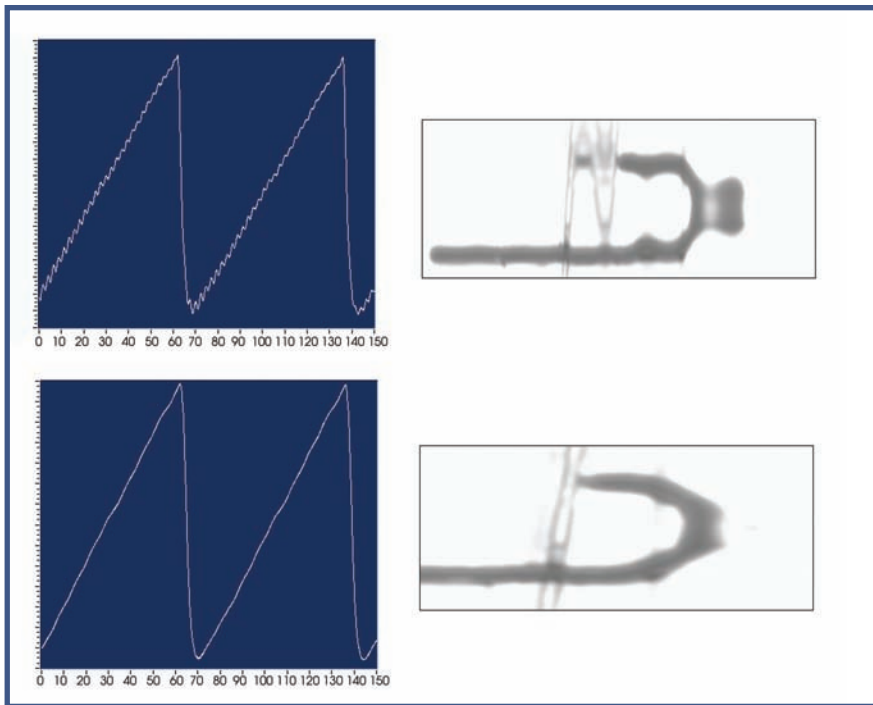


Figure 4. Rapid motions can cause optics and other structures in a microscopy setup to ring. Laser vibrometry data reveals the resonant response of an optic assembly to rapid scanning (top left). This cannot be discerned by the stage controller, so no combination of servo parameters can eliminate it. The ringing causes the scanned image to be smeared (top right). Input Shaping technology eliminates this ringing (bottom left), yielding a higher-accuracy image (bottom right).

simple to tightly couple motion and metrology processes. Such is not always the case with commands sent via such interfaces as RS-232 or USB.

### Internal functionality

On the other hand, servocontrollers with the latest communications interfaces offer internal functionality — such as waveform generation plus synchronization lines — for integration with other instruments so that motion, metrology, video and other processes may be coordinated with good timing accuracy and responsiveness.

Importantly, the resolution of the internal digital-to-analog converters that are integrated into the latest analog servocontrollers can be greater than the resolution of available PC converters and multifunction cards. Twenty-bit internal converters are increasingly common, which gains importance as increasingly longer travel piezo devices are introduced; devices with travel exceeding  $800 \times 800 \mu\text{m}$  are now available. The “bitness” of the converter defines how small

a motion can be commanded by the following formula:

$$\text{Resolution } (\mu\text{m}) = \text{Travel } (\mu\text{m}) / 2^{\text{bits}}$$

Popular digital-to-analog converter and multifunction cards typically top out at 16 bits, or 65,536 possible addressable positions for the piezo nanopositioner. The resulting position resolution would seem to pose significant limitations for applications with nanometer sensitivities such as optical traps when very long travel stages are used. However, HyperBit from PI (Physik Instrumente) LP of Auburn, Mass., provides additional subdivision of the converter resolution to improve positioning resolution by many bits — up to two orders of magnitude — with no loss of bandwidth or accuracy and with ready compatibility with existing programs and most converter hardware. Compatible with popular laboratory automation hardware and software, this patented technology breathes new life into analog-interfacing setups for long-travel applications.

### Digital servocontrollers

Compared with analog servocontrollers, digital ones provide continuous microprocessor or digital signal processor analysis of the position feedback, with continuous updating of the voltage applied to the piezo.

Position sensors are sampled at high speed by an analog-to-digital converter, often with oversampling and statistical manipulation to enhance resolution and reduce noise. This information is compared with the desired instantaneous position in  $N$ -space, where  $N$  is the number of axes under control. In advanced units, when driving a parallel-kinematics mechanism, the axes can be physical or virtual, and complex path planning and coordinate transformation can be handled automatically — for example, to tilt or rotate about the trap’s waist.

The controller’s internal digital-to-analog converters are continuously updated based on the calculations — typically performed by a fast digital signal processor — which can include sophisticated filtering algorithms. Linearization and calibration also are performed digitally; software, therefore, can easily update servo parameters.

High-end digital controllers read calibration information that is stored in the motion device itself, meaning that matching the motion device and controller often is unnecessary.

The processor-based architecture of the controllers facilitates such features as waveform generators and advanced feed-forward techniques that can eliminate following errors in programmatic scanning and patterning operations.

Note that the digital-to-analog converter resides “inside” the servo loop in digital controllers. This is important because the converters can drift, particularly in the case of high-bitness units. Converter drift within a servo loop is automatically compensated by the servo. By comparison, a converter outside the servo loop — as in the case of an analog controller with internal or external converter — will cause unwanted motion when it drifts.

The inherent stability of digital controllers is a great benefit, but only so long as the internal converter is of high resolution. Some units use comparatively low-bitness converters and rely on a slow servo bandwidth to interpolate between adja-

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cent bits, achieving the desired position. The resulting dithering motion and slow responsiveness are unacceptable for optical trapping applications.

Because positional stability is important for most optical trap applications, which can be sensitive well into the sub-nanometer realm, all sources of positional noise and instability should be proactively addressed. Cables and fluid and vacuum piping must be carefully routed and clamped to prevent transmission of vibrations, especially from fan-cooled equipment. The quality and stability of the nanopositioning stage can be no better than the stability of the coarse stage and supporting structure, so these components should not be skimped.

If high-dynamic (rapid and sharp-edged) actuation is an application goal because of tracking or scanning needs, the possibility of motion-driven ringing in the structure, optics and load should be considered, suggesting an analog or digital motion controller with integrated Input Shaping from Convolve Inc. of New York.

If programmatic scans and patterns are part of an application, the consequences of following errors should be explored. A popular trap force calibration procedure is to impart a rapid dithering motion of the trapped particle, such as the dielectric beads used to tether molecules of interest. The viscous fluid medium imparts Stokes drag forces on the trapped particle, which allows calibration of the trap force profile based on the particle's deflection by the scanning. Because the accuracy of this calibration depends on the fidelity of the scanning motion, it can be enhanced by eliminating following error using a digital servocontroller with PI's digital dynamic linearization function.

Electrical noise also can be an issue in sensitive setups. Switcher power supplies — which are used even in very costly lasers — can be troublesome sources of noise, and noise in the servocontroller — particularly in its amplifier — can affect stability.

Interface timing indeterminacy is another important consideration when selecting a controller. RS-232, USB and IEEE-488 allow throughput from the high dozens to low hundreds of commands per second. However, programming technique can have a profound effect on throughput, and timing indeterminacy

resulting from handshaking and background operating system activities can add an unpredictable number of milliseconds to responsiveness.

Proprietary digital interfaces can offer faster and more deterministic communications than general-purpose communications interfaces, often surpassing the motion bandwidth of the system. Specialized interfaces with command cycle times in the microsecond range also are available for some controllers. Analog interfaces, of course, can be rapidly updated independent of PC background processes and merely require adding a digital-to-analog converter card to the user's PC.

Note that a faster interface does not necessarily result in a more responsive system. It is just one potential bottleneck. The resonant frequency of the loaded stage is a fundamental metric of system throughput, as are the controller's amplifier current and slew-rate capabilities.

As Louis Pasteur noted, "Chance favors only the prepared mind." The evolution of the optical trap into an indispensable and diversified tool for manipulating, sorting, characterizing, fabricating and organizing living and inorganic matter without contact and noninvasively is a testament to the ingenuity of three generations of scientists. Supporting their endeavors are an ever-broader wealth of building blocks that are the foundation of tomorrow's applications, promising to affect every facet of life by enabling new materials, addressing environmental concerns, releasing new forms of energy and curing diseases. □

### Meet the author

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