Design Considerations for Micro- and Nanopositioning: Leveraging the Latest for Biophysical Applications

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Abstract: Biophysical applications ranging from fluorescence microassays to single-molecule microscopy are increasingly dependent on automated nanoscale positional control and stability. A whirlwind of motion-industry innovation has resulted in an array of new motion options offering significant improvements in application performance, reproducibility and throughput. The challenge to leverage these developments depends on researchers, engineers and motion vendors acquiring a common language of specifications and a shared understanding of the challenges posed by application needs.

To assist in building this shared understanding, this article reviews today's motion technologies, beginning with a concise review of key principles of motion control focusing on applications. It progresses through illustrations of sensor/encoder technologies and servo techniques. A spectrum of classical and recent motion technologies is explored, from stepper and servo actuation of conventional microscopy stages, to advanced piezo stack nanopositioners capable of picometer precision, to novel ultrasonic resonant piezomotors and piezo-ceramic-based mechanisms capable of high-force positioning over many millimeters while providing resolutions down into the sub-nanometer range.

A special emphasis is placed on the effects of integrating multiple motion technologies into an application, such as stacking a fine nanopositioner atop a long-travel stage. Examples and data are presented to clarify these issues, including important and insightful new stability measurements taken directly from an advanced optical trapping application. The important topics of software and interfacing are also explored from an applications perspective, since design-and-debugging time, synchronization capabilities and overall throughput are heavily dependent on these often-overlooked aspects of motion system design.

The discussion is designed to illuminate specifications-related topics that become increasingly important as precision requirements tighten. Throughout, both traditional and novel techniques and approaches are explored so that readers are left with a solid overview of the state of the art, and an actionable perspective that readies them to discuss and evaluate specifications and vendor capabilities against practical application requirements.

Keywords: Biophysics, microscopy, motion control, piezo, nanopositioner, stability, piezomotor.

SOME ILLUSTRATIVE HISTORY

Around two decades ago, the first motorized positioning stages with linear scale encoders entered the mainstream of motion control. Though today such systems are commonplace, at the time they were a revolutionary and disruptive force in the industry. Their feasibility was driven by (and in turn drove) reductions in sensor costs, plus improvements in reliability arising from advancements in microprocessors, digital signal processing and even the advent of LEDs, which quickly replaced incandescent illuminators in scale readheads [1]. For a while, linear-encoded systems remained costly and exotic, and few controllers of that era had the capabilities to make these stages easy and safe to use. Still, the performance advantages of linear scales were compelling:

- The scales directly encoded the moving platform of the stage rather than the motor's rotation—the drivetrain input—as less-costly rotary encoders do. Backlash, leadscrew inaccuracies, drift and other drivetrain errors could now be automatically compensated.
- Improved bi-directional repeatability was achieved without unreliable and throughput-robbing motion-termination sequences intended to wind up the drivetrain to eliminate backlash.
- New drive technologies such as voice coils, linear motors and resonant piezomotors could now be deployed.

These systems quickly enabled applications ranging from semiconductor lithography to photonics packaging automation to biomedical research. But in parallel came a regrettable era of misleading specsmanship. Consider the situation of a provider of rotary-encoded, leadscrew-driven linear stages: the new linear-encoded stages offered superior performance, but since a motor-mounted rotary encoder's counts-per-rotation were multiplied by the leadscrew and gearbox, the per-count electrical resolution could seemingly surpass that of the best linear encoders. That such a spec was unachievable in terms of motion in any conceivable real-world application was rarely mentioned. (Similarly, the theoretical resolution of a stepper-motor stage driven by a 500X microstepping drive can be impressive, but the open-loop nature of most such systems means such a spec means little in actual usage).

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Today we see many disciplines exploring the world in the nanometer realm. To meet the needs of these applications, a rapid unfolding of technological innovation has taken place among motion vendors. It is commonplace to see nanometer-class specifications on product spec sheets now-- perhaps too common, as there are signs of specs-abuse in the market-place, amplified by the fact that these users are often new to motion control.

THE NEED FOR A COMMON LANGUAGE

Both users and vendors will benefit from reviewing their usage of classical motion specifications and, in those cases where specifications have lost meaning or gained ambiguity, clarifying their discourse with additional terminology and especially data. A good example is the previously-mentioned quantity of "resolution." A bible of mechanical engineering, Slocum's *Precision Machine Design*, states, "Resolution is the larger of the smallest programmable step or the smallest mechanical step the machine can make during point-to-point motion" [2].

Unfortunately, as the example of the rotary-encoder manufacturer cited above illustrates, the marketplace often substitutes "smaller" for "larger" in Slocum's concise definition. This has had the practical effect of rendering the term *resolution* meaningless without clarification, such as a supplementary *minimum incremental motion* specification or statistically relevant data based on measurements of the independently observed motion performance of the system. Similarly, important specifications such as *accuracy* and *repeatability* commonly deviate from their dictionary definitions, requiring the user to look beyond the specs-table to data and discussion with the vendor.

It cannot be overemphasized that specifications must be illuminated by direct metrology of the moving workpiece, ideally by an independent instrument of documentable characteristics such as an interferometer or capacitive gauge. Metrology of the drivetrain input or some intermediate structure generally involves too many leaps of faith to be credible by itself. Similarly, integrated direct-metrology such as a non-contacting linear encoder may or may not play such a role credibly, and specifications based on these elements should be regarded as fodder for commencing discussion with the vendor rather than obviating it. The burden of proof rests on the vendor to support both the rigor underlying their claims and their relevance. Example: a repeatability data-set which contains precisely one reversal is of questionable merit for predicting position reproducibility in real applications. Another example is an accuracy test performed using steps which are an integer multiple of the encoder scale pitch, thereby obscuring cyclic errors in the encoder and its electronics. Unfortunately, both these examples are drawn from incidents one author (Jordan) has witnessed over the course of his career in instrumentation.

Single-molecule biophysical (SMB) applications pose especially daunting challenges since their positioning requirements surpass the measurement capabilities of some of the best conventional instrumentation. A good example is the *stability* of the positioner: SMB researchers investigate positional signatures and trends which are readily corrupted by nanoscale drift processes. But interferometry, the gold-standard for motion metrology, is difficult to stabilize to the necessary degree over the time periods characteristic of SMB applications. Fortunately, as we document below, an advanced SMB platform has illuminated drift behaviors of different motion technologies, providing a relevant comparative measure, significant to the SMB community.

NANOPOSITIONING

Nanopositioning is the science of performing controlled motions over increments down to the sub-nanometer range. Motors are too crude for such positioning. Instead, nanopositioners are based on piezoelectric actuators: exquisite, layered ceramic structures whose dimension changes slightly with applied voltage (Fig (**1**)). This can drive positioning down to picometer levels. Similarly, even the best rolling or sliding bearings have too much friction to guide positioners at this level; flexure guidance is used instead to ensure reliable nano-scale position increments. Piezo actuators provide a maximum of approximately 0.1% of their length in overall travel. Lever mechanisms are commonly used to multiply this, providing overall travel ranges from a dozens to a few thousand μm. Since this range is still too small to span the area of interest in many applications (and since lever amplification comes at the cost of lower stiffness and speed), piezo nanopositioners are often stacked on top of longer-travel motorized or manual positioners.

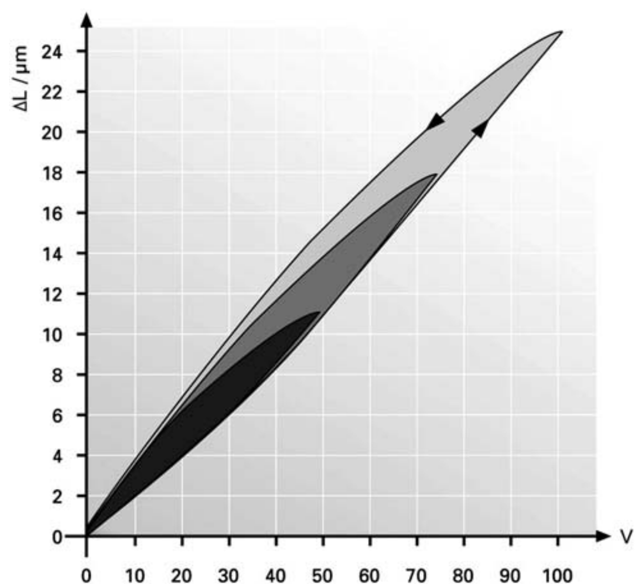

Fig. (1). Typical move-dependent hysteresis and non-linearity of open-loop piezo actuation.
Piezoelectric actuation (vertical axis) is approximately proportional to changes in applied voltage (horizontal axis). The hysteresis and characteristic nonlinearity seen in open-loop actuation is move-dependent (e.g., the plotted white-shaded loop versus the gray- and dark-grey-shaded loops) but can be eliminated with closed-loop actuation (integration of a position sensor and feedback-based controls).

Just as an encoder of some sort is necessary to control a motorized stage's position with accuracy and repeatability, so a position sensor is required for the accurate servo control of nanopositioners. Linear scales of adequate resolution are still too costly and bulky for most nanopositioning applications, so most of today's closed-loop nanopositioners utilize one of two classes of analog position sensor:

1) Strain sensors. These elements are adhesively applied to structural members in the flexure or to the piezo stack itself. Dimensional changes produce an analog signal which can be used for feedback. Bridge configurations can be devised to address the native thermal instabilities of these sensors.

2) Capacitive sensors. Polished metal plates with optical-quality surfaces and exacting configurations of active and passive elements are mounted on the moving and fixed elements of the nanopositioner. As their relative positions change, so does their gap and thereby the capacitance. Electrical excitation and precise frequency measurement yield an analog feedback signal of high temporal and thermal stability, accuracy, bandwidth, electromagnetic interference immunity, and low noise.

There are obvious parallels to the early days of linear encoders in motion control:

- Capacitive sensors, like linear encoders, offer the capability of measuring the actual position of the moving workpiece.
- Strain sensors, like motor-mounted rotary encoders, infer the position of the workpiece from further up the drivetrain but are more cost effective and compact.

Unlike most linear and rotary encoders, these sensors provide absolute position metrology, meaning no homing to a central position switch is necessary to recover the device's coordinate system on power-up. In addition, capacitive sensors can be arrayed around the positioning workpiece, observing it from several directions. This facilitates parallel kinematics, where a single workpiece is controlled simultaneously in several degrees of freedom. By comparison, multi-axis positioning of mechanisms based on strain sensors is achieved by stacking single-axis mechanisms-- typically the lower-cost approach.

USING--AND ABUSING--THE MOTION DEVICE'S BUILT-IN SENSOR FOR METROLOGY

Monitoring a nanopositioning stage's built-in sensor is a valid technique for tuning the servo, measuring step-and-settle, and similar semi-qualitative studies. However, it is sometimes misused, particularly when position-metrology instrumentation resources are unavailable to a vendor.

Importantly, if the sensor does not provide direct motion metrology--measuring the position of the moving platform itself--there's an inherent leap of faith that what it is measuring actually makes it to the moving platform in the form of controlled motion. For dynamic motions down to the sub-nm range, this leap-of-faith is significant.

A particularly misleading test is to dither a strain-sensor-equipped stage sinusoidally at a known frequency and then observe its sensor signal for a signature at that frequency. Now, lock-in techniques of this sort are accepted in fields as diverse as electronics, electrophysiology and optics for separating a signal from the noise spectrum. Using direct motion metrology, one author (Jordan) has utilized this technique to demonstrate enhanced nanopositioning in the chaotic environments of conference rooms and trade-show floors [3]. However, few nanopositioning applications operate in such a modality, so the relevance of such a test is questionable, and the frequency-domain nature of the resulting data can be unintuitive.

Moreover, for mechanisms using indirect metrology such as strain sensors, there is always the question of whether the moving platform actually moves when subjected to a sub-nm dither stimulus. Given the dominance of stiction in this operating regime, observing strain in the flexure is not a guarantee of true system responsiveness in such circumstances— in fact, one could bolt the moving platform to a stationary crossbar to prevent any motion, but the flexures might still flex during actuation of the piezo.

Worse is the temptation to infer some positioning-performance conclusions from the frequency-domain data. In particular, a test of some seconds' length contains very little information relevant to low frequencies, much less about drift or other quasi-monotonic instabilities. An entirely mathematical demonstration illustrates this (e.g., Fig. **2** vs. Fig. **3**).

Another drawback to the representation of positioning performance in the frequency domain is that it is oblique and so conclusions can be hard to draw. Refer to the following illustration of a commanded 2nm amplitude, 17Hz square wave for a model nanopositioner of ~75Hz bandwidth. It is instructive to consider which graph better depicts the crucial point-to-point capabilities of the system (Fig. **4** or Fig. **5**).

Another drawback of a frequency-domain depiction of an indirect sensor's signal is the temptation to conclude that the baseline of the plot says much about the stability of the stage. It is common now to see such plots touted as proving stabilities in the picometer range. Regrettably, as any SMB experimentalist can attest, environments with stabilities to that level are nonexistent. Such plots, then, cannot represent the true noise floor of the positioning platform of the stage. Instead they only represent irrelevant and misleading data which is artificially removed from the real world.

LEVERAGING THE OPTICAL TRAP FOR STABILITY METROLOGY

An example of an advanced SMB experimental setup is the dual-trap optical tweezers configuration based on a modified commercial microscope and incorporating the necessary lasers, optics and positioners to localize, track and manipulate sub-µm fluid-suspended particles such as latex beads [4]. These versatile platforms generally incorporate a coarse/fine stage stack in the image plane, composed of a manual or motorized long-travel substage supporting a piezoelectric stage for fine positioning and high-dynamic operations.

Conventionally, the substage in such an apparatus has been a screw-driven unit actuated by a motor or a micrometer or other manual knob and guided by ball or crossed-roller bearings. The stability of the overall apparatus can be no better than the stability of this substage. Gradual drift behavior, commencing immediately after a motion, is particularly problematic to application performance and throughput. All

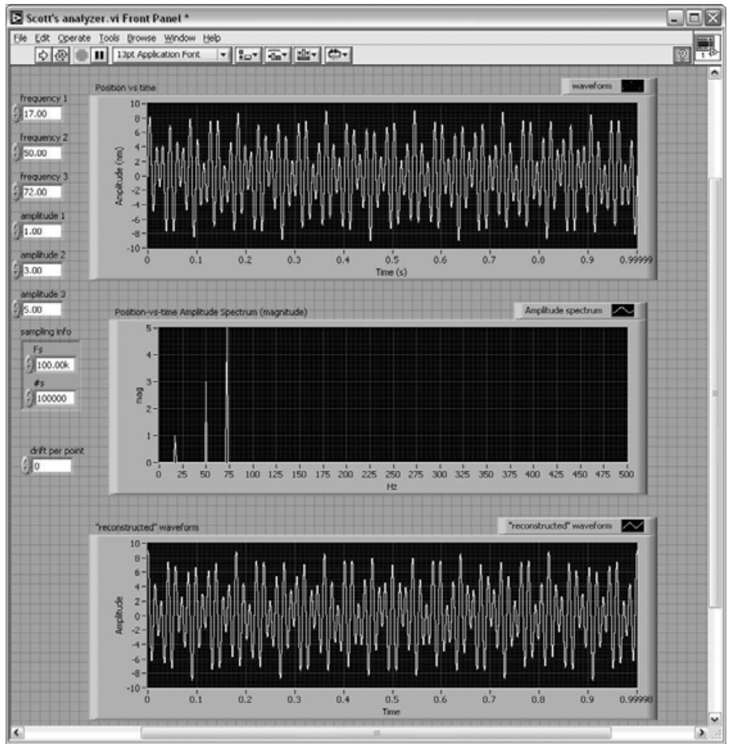

Fig. (2). Simulation illustrating reconstruction of an idealized, driftless position waveform composed of pure sinewaves by Fourier transformation and inverse transformation.

Top graph: Simulated position waveform composed of three sinewaves without drift (vertical axis in simulated µm) versus time (horizontal axis). Fourier transform results in an accurate spectrum composed of the three sinusoidal component frequencies (middle graph; vertical axis units in µm). Accurate reconstruction of the original waveform is achieved by inverse transformation (bottom graph).

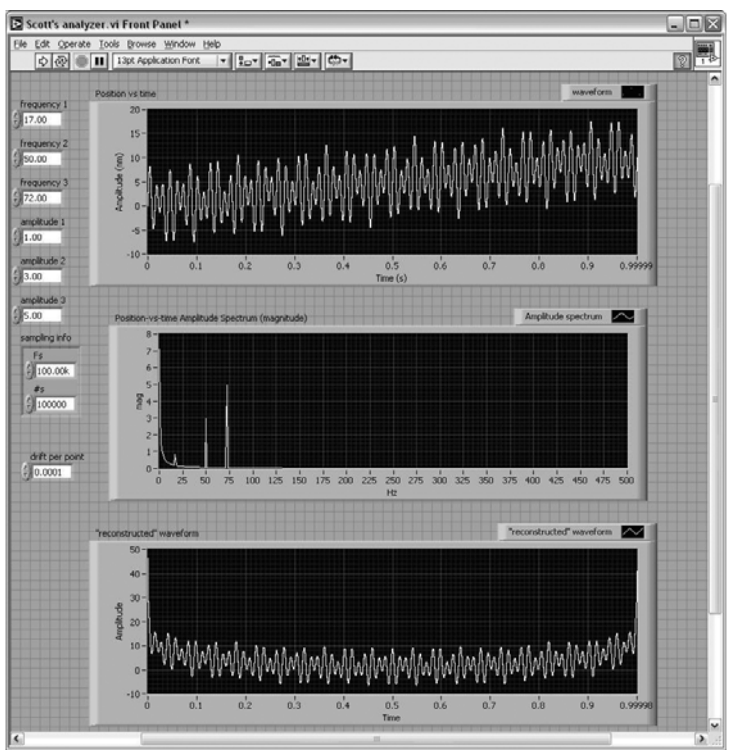

Fig. (3). Simulation illustrating faulty reconstruction of position waveform by Fourier transformation and inverse transformation in the less-idealized presence of drift. Same as Fig. (**2**) plus the addition of a simulated drift mechanism in the waveform. The transform-and-inverse-transform process does not handle the drift well, as can be seen by the nonsensical reconstructed waveform (bottom graph) which differs considerably from the original position waveform.

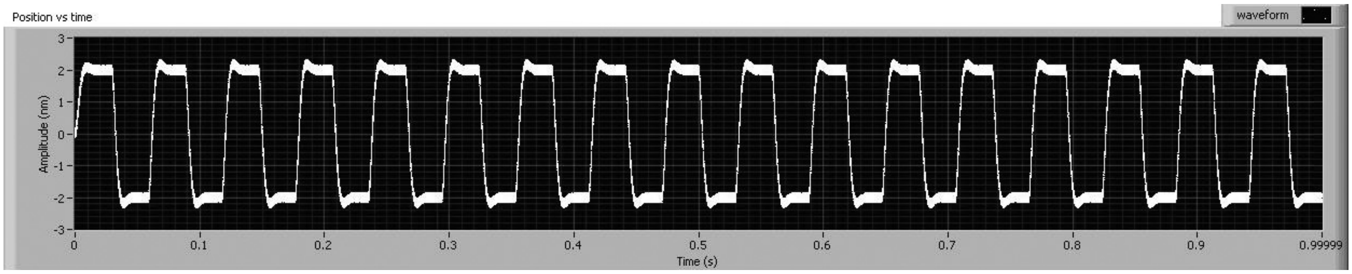

Fig. (4). Model position response of nanopositioner in typical point-to-point actuation.
Position-vs-time metrology of a model nanopositioner. A 2nm amplitude, 17Hz square-wave actuation is simulated here.

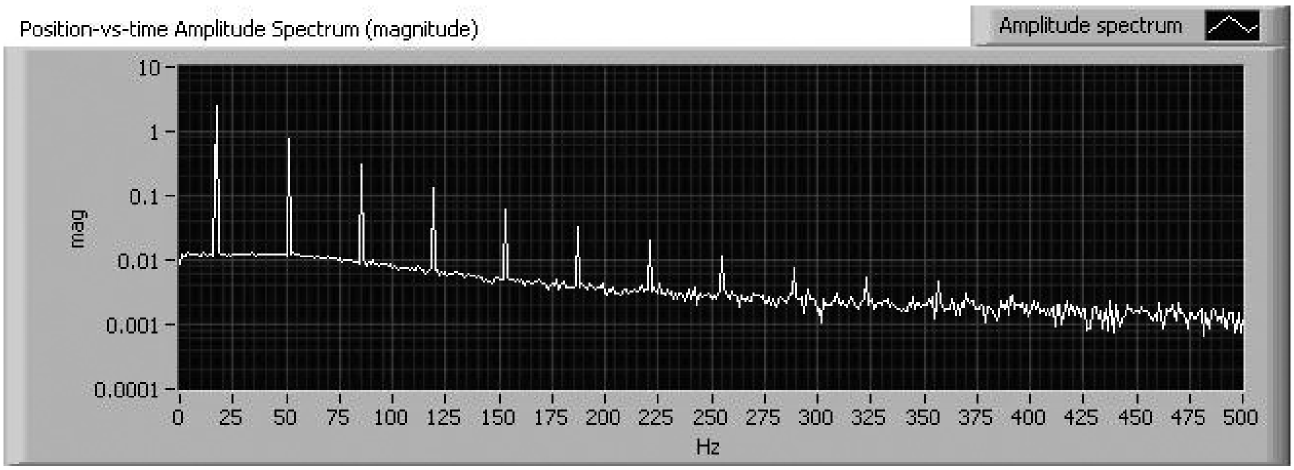

Fig. (5). Spectral analysis of Fig. (**4**)'s point-to-point position waveform via Fourier transform is of questionable utility.
Fourier transform (magnitude in µm, vertical axis) of position-vs-time model data from Fig. (**4**). The point-to-point positioning capabilities of the system which are relevant to most applications are difficult to deduce.

elements of the mechanism are involved in this, but of prime importance is the gradual flow of lubricant in the screw actuation mechanism towards an equilibrium state. The rheology of lubricants such as greases commonly used in screw-actuation mechanisms has been studied extensively, yielding classical models such as the straightforward Bingham Model, supplanted over the years with more elaborate understanding [5] and analyzed experimentally as a function of time in the transition from the dynamic to a quasi-static regime [6].

It follows that eliminating the screw-driven drive in the substage would improve system stability. Applying a stage brake might be another possibility, but these inevitably disturb the position of the mechanism when actuated, and their effectiveness on the nanoscale is unproven. Replacing the screw with a magnetic linear motor or voice coil would be no answer since those mechanisms hold position by consuming current, contributing to thermal drift and "hunting" behavior by the servo.

The class of motors based on ultrasonic actuation of piezoelectric ceramic slabs would seem to offer more promise for building a stable substage. In Physik Instrumente's PIline motors, one of a broad family of unlimited-travel technologies based on piezo ceramics which includes resonant [7] and non-resonant [8] approaches, an oscillatory stimulus drives a nanoscale resonant fluttering of a small ceramic slab; a frictive tip mounted at a node-point is pressed against the stage platen, conferring motion. As with a familiar DC servo motor, the velocity is approximately proportional to the magnitude of the applied stimulus. However, the breakaway stimulus tends to be much higher as a percentage of maximum stimulus. From a microscopic standpoint, the physics of the frictive tip's actuation is similar to that of a pogo-stick, where a certain threshold amount of bounce is necessary before motion commences. This deadband behavior is qualitatively similar to stiction, and in fact stages built on this motor principle are sometimes called stiction stages. A benefit of this behavior is superior in-position stability.

Conventionally, this has been quantified using conventional tools such as interferometers, which present their own unpredictable time-dependent behavior over timescales relevant to SMB applications. Now one author (Anthony) has utilized the optical tweezers instrumentation to quantitatively compare the stabilities of a screw-driven substage and a piezomotor-driven substage (Fig. (**6**)).

The stability of each substage was measured while mounted underneath a Physik Instrumente P-517 XYZ piezoelectric nanopositioning stage on a dual-beam optical trapping microscope. 0.6 µm-diameter polystyrene beads (Bangs Laboratories) were suspended in high-salt buffer and pipetted into a sample cell comprised of a microscope coverslip and slide joined by double-sided tape.

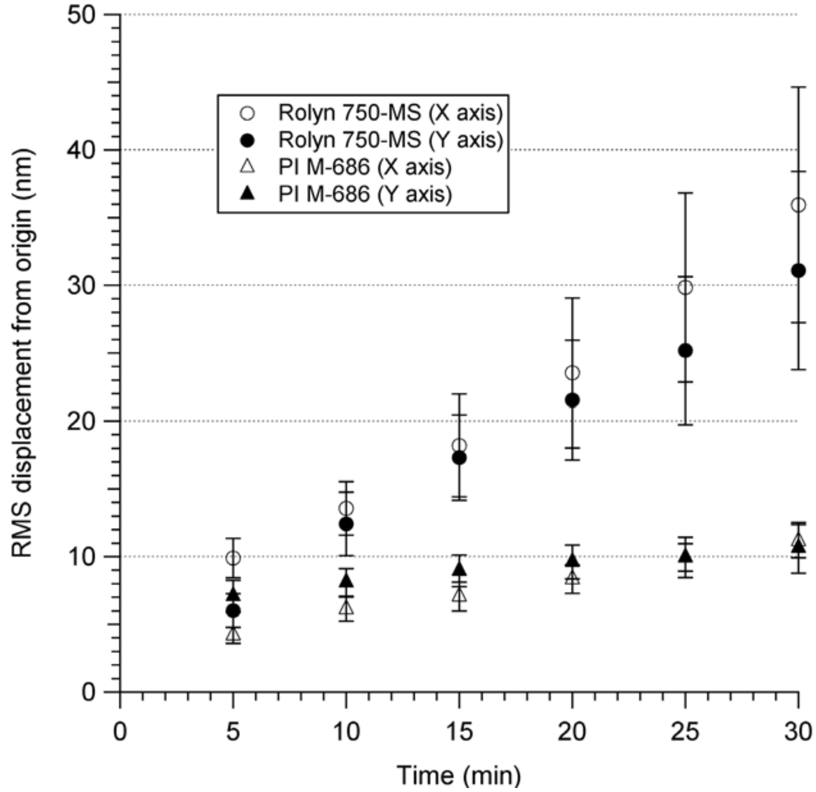

Fig. (6). Drift performance of simple screw-driven microscopy stage versus self-clamping piezomotor stage.
RMS displacements (nm) from the origin of polystyrene beads affixed to the microscope sample coverslip, which was attached to a Physik Instrumente P-517 piezoelectric nanopositioning stage mounted on either a Rolyn 750-MS manual substage (circles, N = 8 measurements) or a Physik Instrumente M-686 PIline substage (triangles, N = 9 measurements). Both stages are of crossed-roller-bearing construction; the screw-driven Rolyn stage has no motors or encoders which might contribute to drift. Displacements were measured by back-focal plane detection (see Methods). Symbols represent mean ± standard error.

Positions of beads stuck to the coverslip were measured by back-focal plane detection [9] using a low-power 632.8nm laser (Uniphase) imaged onto a position-sensitive diode (PSD) (Pacific Silicon Sensor). Once a stuck bead was located, it was placed near the laser focus and "centered" by sweeping the piezoelectric stage over 2μm in each axis while recording the appropriate PSD voltage (differential for X and Y, sum for Z). Each resulting voltage-vs.-position plot was fit by the derivative of a Gaussian, and the bead was moved to the position of the inflection point, which was taken to be the location of the laser focus. Before measurement of stability commenced, the PSD was calibrated for displacements of the bead out of the laser by raster-scanning the piezoelectric stage in the specimen plane in 30nm steps and mapping voltages to bead positions with 5th-order polynomial fitting functions.

The measured position of each bead at time = 0 was taken as the origin of the measurement and was subtracted from the record of position vs. time in order to remove the effects of drift occurring while calibrating the PSD. In order to minimize inaccuracies in the detected positions due to drift in Z, the measurement was paused every five minutes while the bead was recentered. The discontinuity in the record at each recentering event was removed by subtracting the position measured immediately before recentering from that after it, and subtracting the resulting value from the portion of the record subsequent to the recentering. In this manner, the portions of the record between the recentering events were stitched together to represent accumulated displacement from the origin.

CONTROLS CONSIDERATIONS

For piezo nanopositioners, the proportionality between applied voltage illustrated in Fig. (**1**) implies that the minimum incremental motion of the positioner will be limited by the smallest voltage change the controls are capable of producing. This voltage granularity is defined by the bitness of the digital-to-analog converter (DAC) used in the nanopositioner's controls: there are 2^N possible voltage states for an N-bit DAC, equating to 2^N addressable positions. High-bitness DACs have become available in recent years, driven by advancing consumer audio and video applications, but their stabilities have often been problematic and are sometimes left unspecified by their manufacturers. Nanopositioning controls fall into two categories: (1) analog servos driven by an external DAC (either in the controller but external to the servo-loop, or residing in the user's PC) and (2) digital servos in which the DAC resides inside the servo loop. Analog controls have a cost advantage and can be simpler to interface and synchronize with other processes, but they have traditionally been constrained by the granularity of available

PC multifunction boards' DACs. This can be addressed by a novel bitness-enhancing technique [10], but digital servos have an edge in stability since their position feedback mechanisms compensate for any DAC drift, which is not possible with an external DAC.

Both classes of controllers benefit if their manufacturer provides a well-documented and feature-rich developmental software library which supports a spectrum of programming languages, PC operating systems and interfacing techniques. These allow the user to build and maintain complex systems efficiently and become productive quickly, and well-done libraries integrate error-checking formalities which enforce good programming practice. Especially valuable but often overlooked in the purchase decision are synchronization capabilities which allow tight integration of motion with other processes. Several types of standard communications interfaces have grown popular, from venerable RS-232 and IEEE-488 to the newer USB and Ethernet interfaces. In choosing among these, latency is generally more important than bulk throughput since positioning instrumentation commands tend to be terse. Surprisingly, the IEEE-488 interface, promulgated in 1978, remains the lowest-latency standard communications interface [11]. Even lower latencies can be achieved using the proprietary TTL command and signaling interfaces offered by some nanopositioning instruments. The current state of the art leverages the full speed of proprietary, high-speed parallel interfaces available on high-end digital nanopositioning controls together with user-programmable FPGA interfaces in the user's PC. This approach allows time-deterministic command of the nanopositioning controller up to its full servo update rate. It's no surprise that SMB applications are among the first to push this new interfacing technique to its limits [12].

CONCLUSIONS

Single-molecule biophysics and nanopositioning are intertwined in a *pas de deux* of performance and need. Application demands that a few years ago might have been dismissed as impossible are met by innovations which in turn drive new ideas and ways of accomplishing science. It is a measure of these fields' rapid advancement that conventional metrology capabilities and even the language of specifications have been eclipsed. As demonstrated by the comparative stability measurements described above, SMB techniques not only benefit from the new nanoscale motion-control technologies but can validate them.

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ABBREVIATIONS USED

LED	=	Light Emitting Diode
SMB	=	Single-Molecule Biophysics (or Biophysical)
PSD	=	Position-Sensitive Diode
DAC	=	Digital-to-Analog Converter
Hz	=	Hertz
nm	=	10^{-9} m
µm	=	10^{-6} m
RMS	=	Root-mean-square
PC	=	Personal Computer
USB	=	Universal Serial Bus
IEEE	=	"The IEEE name was originally an acronym for the Institute of Electrical and Electronics Engineers, Inc. Today, the organization's scope of interest has expanded into so many related fields, that it is simply referred to by the letters I-E-E-E (pronounced Eye-triple-E)." [http://www.ieee.org/web/aboutus/home/index.html]
TTL	=	Transistor-Transistor Logic
FPGA	=	Field Programmable Gate Array

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