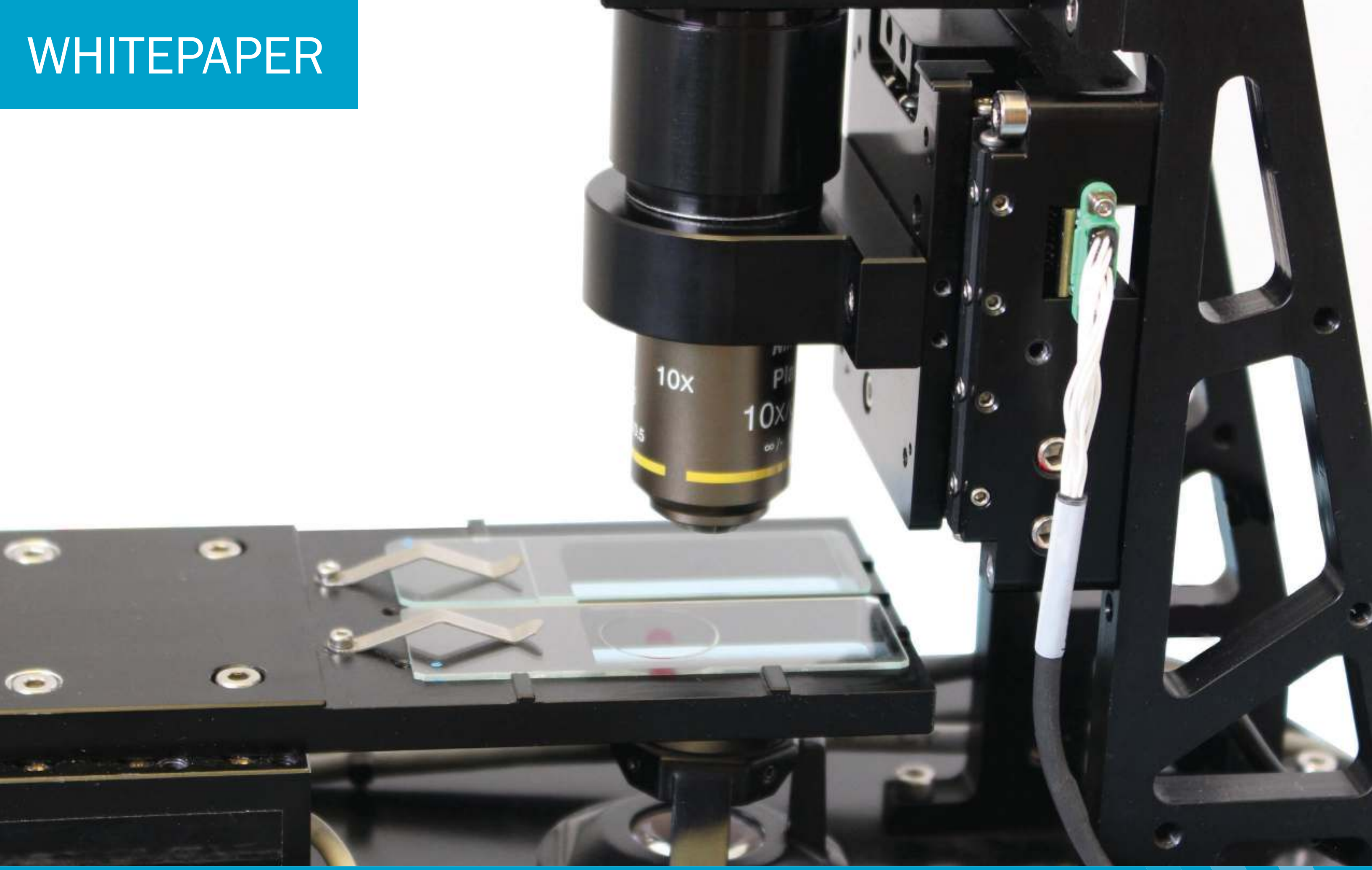
WHITEPAPER



A Better Way to... Focus!

This white paper addresses high performance Z axis focusing for automated microscopy, along with some recent innovations in this space. Historically, piezo driven actuators have been used for these applications, but a Piezo actuator has very distinct limitations.

Direct drive linear motor stages provide many advantages when compared to piezo nanopositioners. This whitepaper discusses both approaches to Z focusing motion.

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A Better Way to… Focus!

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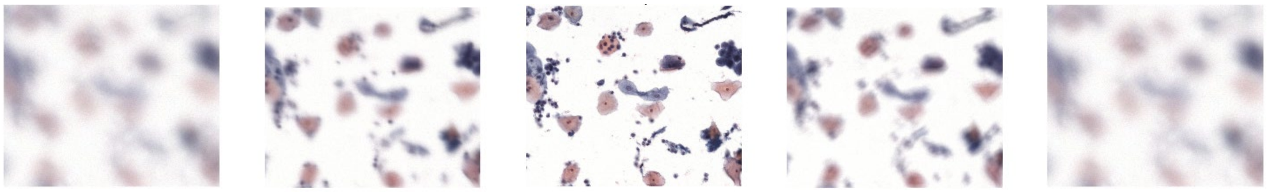


By Kevin McCarthy, Chief Technology Officer

The Long View

For several centuries, the sole sensor used in microscopy was the human eye, looking through an eyepiece (or two eyepieces, in a binocular head). Focusing during this era was purely a manual affair, with focus quality determined by the skill of the microscopist, and the quality of the optics. During the last century, trinocular heads allowed a film camera to be added, with focus remaining the responsibility of the microscopist. More recently, composite analog and subsequently digital image sensors have proliferated, allowing wholly automated microscopy. Today, very high performance CCD and CMOS sensors are widely available, and these have greatly expanded the use of automated digital microscopy. Optical configurations include brightfield, darkfield, epi fluorescence, TIRF, confocal, phase contrast, two-photon, and DIC/Nomarski. More recently, a variety of hyper-resolution techniques have appeared, including structured illumination and light-sheet microscopy. These high-performance advances in microscopy have only increased the applications for very precise focusing.

Automated digital microscopy now supports a wide range of applications, including cellular imaging and diagnostic instruments, automated inspection and metrology, and DNA sequencing. In most of these applications, precision X-Y movement is required, because the sample is considerably larger than the field of view. X-Y motion can either perform sequential field imaging, stepping from field to field, or TDI scanning imaging, in which images are acquired during constant velocity scanning. In all of these applications, precise automated control of the objective's position along the optical axis is also required. This white paper addresses high-performance Z axis focusing, along with some recent innovations in this space. A separate topic, to be covered in an upcoming white paper, is autofocusing. This is the means by which focus quality is automatically determined, generating the commands that drive the Z focusing stage to keep the objective (and hence the sample image) in critical focus at all times.

The Current Art

Historically, automation of focus in microscopy has been performed in one of two ways. In the first method, typically implemented as an add-on to an existing, off-the-shelf microscope, a stepping motor and a rubber belt have been used to drive the fine-focus knob. This is cost-effective, but suffers from a variety of issues. These include intrinsic backlash due to the stepping motor's low angular stiffness, accuracy and repeatability issues, and low bandwidth. This approach is a good match for low-throughput digital imaging using contrast-based autofocus, but is not up to the task for high-throughput and/or high-NA (Numerical Aperture) microscope imaging.

The more common technique for high-performance imaging is to use a dedicated Z axis focusing stage; currently, these are essentially all based on piezo actuators. Despite their current dominance of the microscope focusing market, piezo-driven actuators have a number of very distinct limitations. First and foremost, piezo stages with direct piezo stacks have very limited travel and are excessively long. For these stages, the travel is typically limited to 100 or 200 µm, and the length of the piezo stack is typically 1000x its stroke: 100 mm for a 100 um stroke, and 200 mm for a 200 um stroke. This minute amount of travel drives very tight tolerances into the large number of parts that make up the structural loop between the objective and the sample, and these tolerances alone can eat up much of the already very limited travel. Moreover, these piezo actuators are incapable of moving upward and out of the way to clear proud elements on the sample carrier, or to assist in sample loading and unloading. They are also poorly suited for optically thick subjects, such as tissue samples. The excessive length of direct piezo stacks is also a major drawback. In some systems, the very restricted travel of piezo stages leads designers to implement a "macro/micro" strategy, in which the fine resolution piezo stage rides on an additional coarse resolution, longer travel stage. This approach essentially doubles the number of axes, with hybrid servo controls and increased cost, and is accordingly not recommended.

When operated in open-loop mode, piezo actuators exhibit surprisingly high levels of hysteresis (as much as 15% of the travel), and they also tend to creep off position after a step move. As a result, piezo stages usually incorporate a position feedback device (typically a capacitance gauge). While this allows much more precise closed-loop operation, it also significantly increases the system cost. Piezo control electronics in general tends to be highly custom, produced in low volume, expensive, and bulky.

Several approaches have been developed to remedy the very short travel and excessive length of direct piezo stack actuators. The most common technique involves lever amplification. While this can increase the travel to 400 or 500 µm, and substantially shorten the length of the piezo stack, it does so at the expense of axial stiffness. All piezo systems must deal with an undamped, high-Q resonance at their spring-mass frequency, and the frequency of this resonance decreases by the ratio of mechanical amplification. The result is considerably lower servo bandwidth, and poor step and settle performance.

There are also unlimited travel piezo actuators, in which piezo fingers oscillate in resonance against a ceramic strip. These devices have numerous deficiencies, including a very large transfer function non-linearity, which severely complicates closing a stable servo loop. Their performance and reliability are a function of preload, position, and time, as the high-speed contacting fingers wear and abrade the ceramic strip. Resonant piezo actuators are accordingly not recommended.

Short-stroke piezo actuators of acceptable dimensions typically rely upon flexural guideways to lever amplify the limited travel of a short piezo stack, and these have their own distinct set of limitations. Central among these is inadequate stiffness for both linear and angular out of plane motions. An ideal flexure is extremely compliant in the desired axis of motion, and extremely stiff in the five remaining degrees of motion. No such compact flexure exists. Designers of lever-amplified piezo flexure actuators inevitably have to accept compromises that render the resulting closed loop stage prone to instability due to external vibrations. These vibrations (local accelerations, which produce forces and torques on the customers payload) can originate within the instrument (from fans, pumps, and other components), or from external accelerations (someone walking by, a vortexer or mini-centrifuge on a nearby lab bench, the Really Loud Guy, etc.). We have seen numerous cases where customers were forced to add elaborate and expensive vibration isolation gear to their instrument, merely to keep their objective focusing stage from "lighting off" and breaking into oscillation due to vibration.

Twenty years ago, this author documented the Limitations of Piezos and the Limitations of Flexures in a pair of white papers; no news has turned up in the interim that would lead us to revise those conclusions.

A Question of Balance

While direct-drive actuators utilizing the Lorentz force (linear motors) and optical linear encoders provide the ultimate in high precision positioning, they have not typically been used for automated digital imaging, in large part due to their potential to crash an expensive objective into the sample in the event of power loss. A variety of work-arounds to address this problem have been tried, including high-speed mechanical brakes. These typically cannot react fast enough for the very short working distances in most digital microscopy applications. Next up were a range of counterbalances. Pneumatic counterbalances have been used, but these require clean dry compressed air, and suffer from the same risk of objective crashes, this time when compressed air is lost for any reason. Repulsive magnets are another technique, but their force varies as the inverse cube of travel, restricting them to very short travels. Their highly non-linear force profile also complicates the closing of a high-bandwidth servo loop. A spring can also be used, but its restoring force obeys Hooke's Law, and so varies linearly with travel. While springs are a potential solution, they create a fundamental and low-frequency spring-mass resonance, which can complicate tuning high-bandwidth servo loops, and they have to be fairly long to minimize their variation in force over the desired range of travel. Cables and counterweights have even been tried, but these have a number of drawbacks, including friction, increased inertia, and the need for counterweight guidance.

Direct-drive, linear motor actuators face another challenge when used in vertical applications such as microscope focusing. Lacking any mechanical advantage, they will directly experience, and have to oppose, the gravitational force m * g, where m

represents the mass of the microscope objective, its mounting bracket, and the moving mass of the Z axis focusing stage. The power that the motor will have to dissipate to oppose this entirely constant and anticipable force will equal $(m * g / K_m)^2$, where g is the acceleration of gravity ($9.8\ m/s^2$), and K_m is the linear motor constant, in N/root watt).
For a total moving mass of 1 kg, keeping the motor power dissipation at one watt requires a Km of 9.8 N/root watt, which is a fairly large and massive linear motor.

Direct-drive, linear motor stages do have numerous benefits:

- Very high, nanometer-level resolution
- Very short move and settle times
- Ample travel compared to piezo/flexure stages
- High servo bandwidth, with a critically damped response
- Very high stiffness; no out-of-plane compliance typical in flexure stages
- Extremely long service life, with no need to vary servo tuning

For high-performance focusing applications, the central challenge for direct-drive stages is the force of gravity, which raises the risk of objective crashes, and drives unwanted motor heating. This led Dover Motion to develop novel, very compact, constant force passive magnetic counterbalances. We have scaled these from 1N to 200N in force level, and from 5 mm to 150 mm in stroke. Unlike springs, constant force magnetic counterbalances have no “spring constant”, and hence do not generate any spring-mass resonance. They simply oppose the downward force due to gravity, with a bit of added force to gently bias the objective away from the sample in the event of power loss. This effectively solves the objective crashing issue, and by removing any need for the linear motor to oppose m*g, the linear motor can be much smaller, and still provide high accelerations. The presence of an effective counterbalance also reduces quiescent motor heating to entirely negligible levels (~ 70 mW).

Dover Motion has been shipping standard linear motor stages with passive magnetic counterbalances for some time now, but for the dedicated application of high performance focusing in automated digital microscopes, two remaining parameters remained to be optimized: volumetric efficiency, and cost. Our standard stages also lack a number of specialized features that would be very useful in microscopy applications.

Enter the DOF-5
While a number of off-the-shelf Dover Motion direct-drive stages can be oriented vertically, and equipped with a magnetic counterbalance, their travels are considerably higher than those required for precision focusing, and these products were less than ideal in several other aspects, including moving mass, overall size, feature set, and cost. Given the number of focusing applications that we have been addressing, it seemed worthwhile to start with a clean sheet, and design a dedicated objective focusing stage. This led to develop the “DOF-5” (Dover Objective Focuser, 5 mm travel). The overlap with the acronym DOF as meaning “Depth of Field” was entirely intentional.

A team at Dover Motion invested considerable time in obtaining VOC (Voice of Customer) feedback on key parameters of our proposed dedicated focusing stage. Travel was a key parameter that we tried to nail down early on, and with customer guidance, we concluded that 5 mm would be ample, and a welcome relief from the very restricted travel of piezo stages. We also set out to achieve the following list of goals:

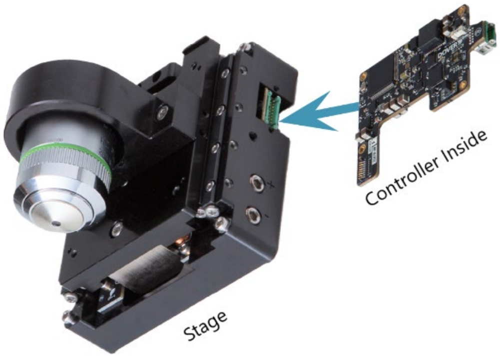
- Internal, high-performance servo drive and control
- Compact mechanical envelope, with convenient mounting and alignment features
- Very low moving mass
- Very stiff guideways, to maximize the first resonance (and hence servo bandwidth)
- Very low jitter and bidirectional repeatability
- Very fast move and settle
- Internal magnetic counterbalance, high resolution linear optical encoder, and linear motor
- Operable with both conventional and inverted objective orientations
- High acceleration capability
- Convenient features, such as finely adjustable hard stops
- Significantly lower cost than other direct drive stages, as well as piezo/flexure positioners

Dover Motion DOF-5

Development of the DOF-5 is now complete, and we are excited to announce that we managed to meet or exceed our goals.

The following is a list of DOF-5 features:

- Internal, high-performance servo controller and drive; no bulky and expensive external electronics



- A single, side-access connector for the DC bus (+12 to +48 VDC), host communication, and autofocus I/O.
- Host communication options include RS-232, RS-485 (half and full duplex), SPI, and CAN, with checksum.
- Motion commands are sent to the DOF-5 via a rich library of C language calls.
- User friendly GUI for servo tuning and initial setup.
- High servo bandwidth, in excess of 225 Hz, providing very fast move and settle performance
- The DOF-5’s high-speed digital I/O signals allow simple integration with laser autofocus systems.
- Nominal travel: 5 mm. Motion between hard stops at full travel: 6 mm
- Overall dimensions: 77 mm high x 82 mm wide x 30 mm deep (excluding objective and objective mount)
- The mounting hole pattern consists of four holes on a 70 x 75 mm spacing, with clearance for front mounting using M3 fasteners.
- These mounting holes are also threaded, permitting mounting from the rear with M4 fasteners.
- Overall mass: 425 grams; moving mass (without an objective): 200 grams (excluding objective and objective bracket).
- Very stiff guideways, with high crossed roller density (hardened steel rollers).
- Exposed crossed roller guide rails allow external pin banking, with highly parallel alignment to the axis of motion.
- Crossed roller guideways feature a black chrome anti-corrosion coating, and special grease.
- Internal, non-contact, precision chrome on glass linear optical encoder with resolution of 1.25 or 5 nanometers.
- Top speed of 32 mm/sec. at 1.25 nm resolution, or 125 mm/sec. at 5 nm resolution.

- Position jitter (at resolution of 1.25 nm): < 5 nm rms; bidirectional repeatability (short-term): < 25 nm (2σ). See plots below.
- Fast move and settle: 100 nm step move with ± 15 nm settling window in under 15 milliseconds (with 250 gram objective). See plots below.
- Built-in passive, centered constant force magnetic counterbalance.
- Counterbalance factory set to provide a gentle (<= 0.5 N) power-off bias force away from the sample, for both conventional and inverted objective configurations.
- Counterbalance force factory adjustable to support objectives from 50 grams to 1,000 grams.
- Optional high-force variant permits large custom objectives, or motorized turret assemblies to be fully supported and precisely focused. Payload masses of up to 1.5 kg are accommodated, with no increase in overall dimensions.
- Built-in half-travel optical vane for unambiguous homing. The vane trip point can be centered or biased toward either end of travel.
- Internal, non-contact, high-force single-phase linear motor; no commutation required.
- High acceleration capability: 1.5 G's (~ 15 m/s^2) with a 250 gram objective and a 50 gram mounting bracket.
- Very low linear motor power during position maintenance: < 70 mW.
- Finely adjustable, side-access hard stop adjustments allow travel to be independently delimited from its nominal ± 2.5 mm travel to as low as ± 0.9 mm.
- With the DOF-5's very fine hard stop adjustment (643 um/turn, so about 25 um resolution), both conventional and inverted objective systems can be protected against inadvertent programming of objective crashes. This hard stop adjustment resolution is a very small fraction of even the shortest objective working distance.
- Three objective mounting locations, at center and each end of the moving carriage.
- The objective mounting surface is recessed within the objective mount, such that the threaded section of the objective is flush with the face of the objective mount. This maximizes the available room to fit optical components within the infinity path between the objective and the tube lens.
- Objective mount banking edge maintains precise parallelism between optical axis and the axis of motion should the objective mount be removed.
- Threaded inserts for the objective mount support all commercial microscope objective thread diameters and pitches.
- The threaded objective insert is bonded into the objective mount in a precision fixture, assuring high parallelism between the optical axis and the axis motion / DOF-5 base

DOF-5 Front View with Objective Installed

Further resources are available on our website; click below to view additional information on these topics:

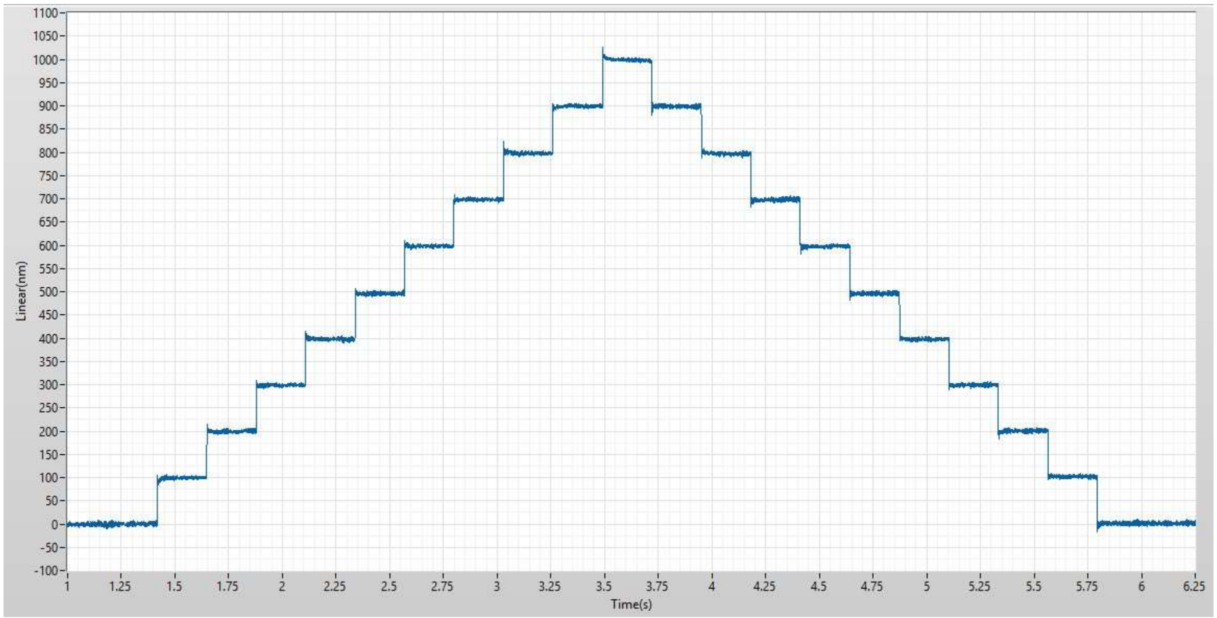
- Automated Imaging
- Focusing
- Article: The X-Y-Z's of Biomedical Imaging
- Article: Limitations of Piezos
- Article: Limitations of Flexures

6 Reasons the DOF-5 is Superior to Piezo Stages for Focusing:

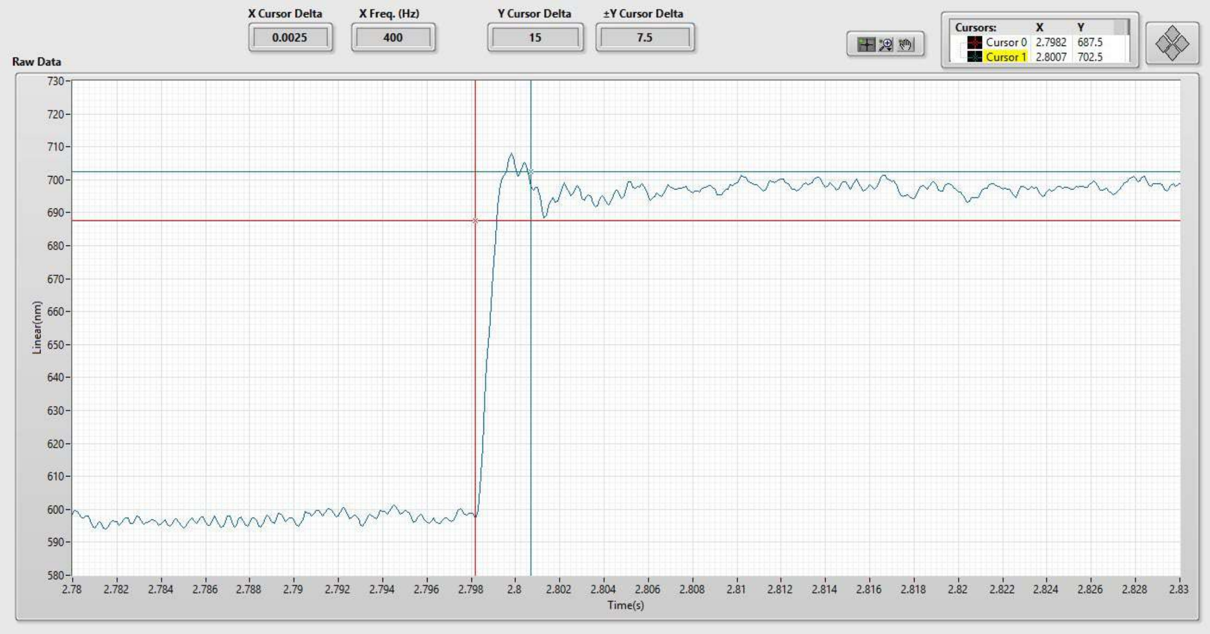
	Piezo Flexure Stages	DOF-5 Objective Focusing Stage
1	Typical cost with controls **~$8,000 – $12,000.**	Single unit price with controls up to **50% less**.
2	**Flexure bearing** results in off-axis motion and position dependent parasitic force.	**Crossed roller bearing** provides higher stiffness for faster moves. This results in increased throughput and longer nanopositioner life.
3	**Stack or oscillating actuation** have a non-linear response and bandwidth decreases as payload mass increases.	**Brushless linear servo motor actuation** provides higher servo bandwidth and a linear response for optical microscopy.
4	Typically **< 300 µm travel** requires precise alignment and an additional coarse axis when more travel is required.	**> 5 mm travel** makes alignment easier. It also helps avoid microscope objective crashes and provides enough travel to clear interferences.
5	Oscillating piezos make a **loud screeching noise**.	A **quiet servo** is valued by lab workers.
6	Off-axis, **complex controls** are typically proprietary which leads to higher costs.	**Onboard controls** result in a lower cost of ownership due to less complexity and fewer cables

DOF-5 Performance Plots

The following plots provide laser interferometer data on DOF-5 performance, with a 175 gram Leica 20X, 0.75 NA objective. The DOF-5 was oriented vertically, with the objective facing downwards. The DOF-5's internal Dover Encoder was set to its maximum resolution (1.25 nanometers). The laser interferometer has a nominal resolution of 0.5 nanometers, and it was set to sample data at 10 kHz. The drive and control electronics were internal to the DOF-5. We set up a simple script to perform ten 100 nanometer moves upward, and then ten 100 nanometer moves downward. We are very happy with the resulting performance, and hope that you will agree.

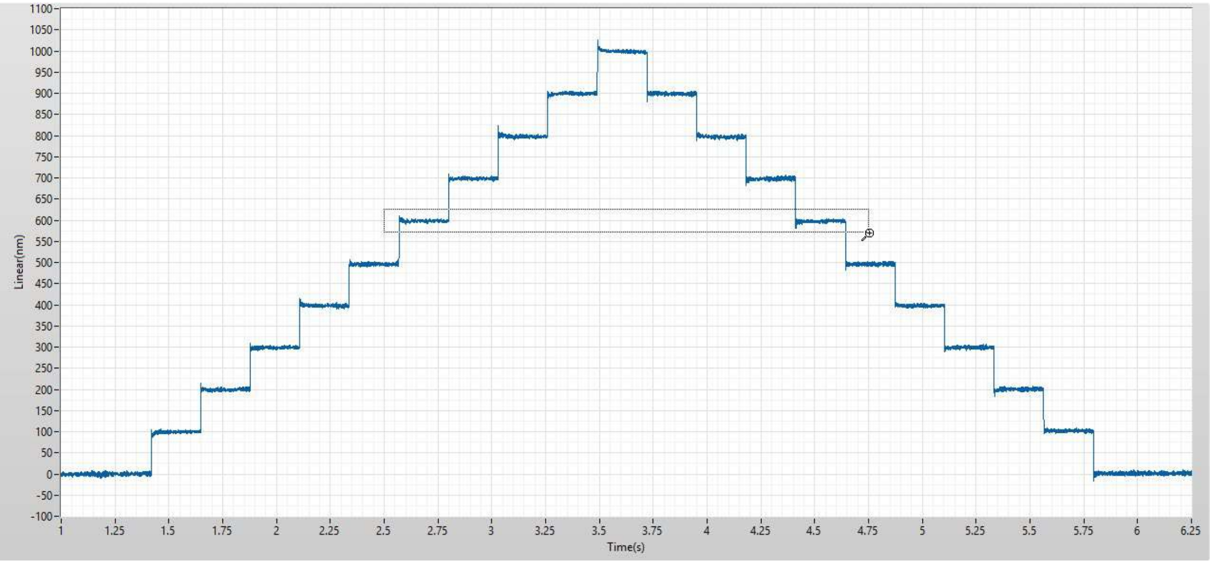


DOF-5 executing a staircase of 100 nanometer moves
Laser interferometer data; sample rate 10 kHz.

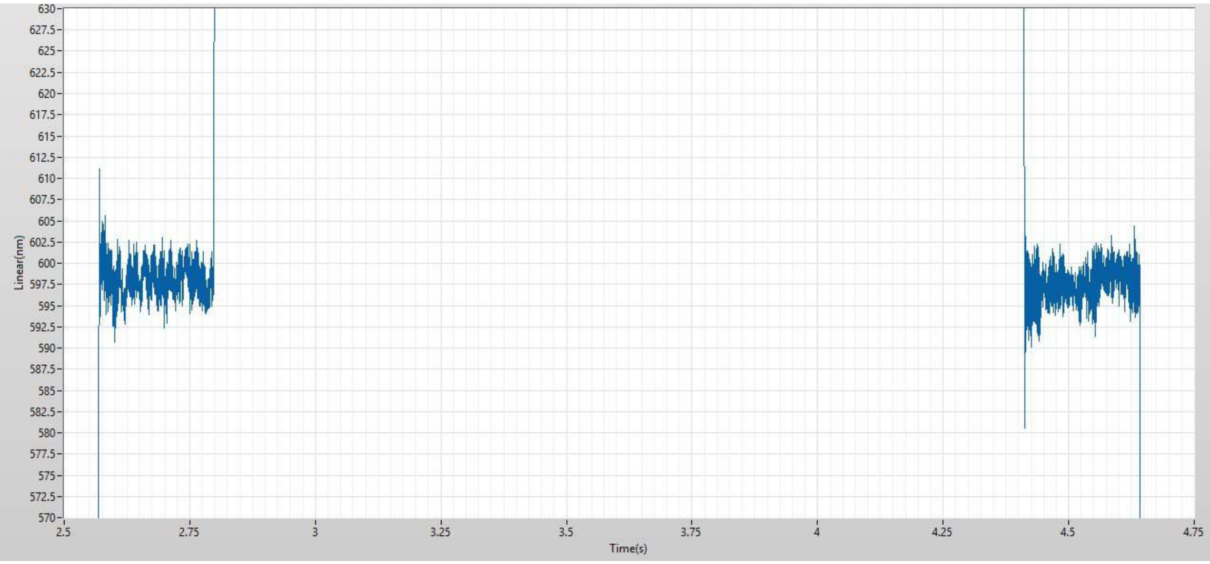

X Cursor Delta 0.0025
X Freq. (Hz) 400
Y Cursor Delta 15
±Y Cursor Delta 7.5

Cursors:	X	Y
Cursor 0	2.7982	687.5
Cursor 1	2.8007	702.5

DOF-5 single 100 nanometer move; vertical cursor separation 2.5 milliseconds
Horizontal cursor separation ± 7.5 nanometers



Zoom box to determine the bidirectional repeatability



Zoomed-in plot with 2.5 nanometers per vertical division,
showing nearly perfect bidirectional repeatability, and position jitter of ~ 3 nanometers rms

ABOUT THE AUTHOR...

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