Piezoelectric Actuation Offers Light Microscopy New Capabilities

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Introduction

The intersection of nanopositioning and light microscopy is expanding and deepening rapidly. Evolving disciplines as diverse as single-molecule biophysics, super-resolution microscopy, and automated microassay scanning share several common themes: higher throughput, reduced resolution tolerances, and dynamic (on-the-fly) techniques. These combine to increase the prevalence of piezo-based positioning systems added to or integrated into microscope setups.

Piezoelectric actuators have traditionally been layered structures of lead zirconate titanate (PZT) ceramic interleaved with electrodes. This ceramic is ferroelectric, and application of a varying voltage produces a nearly proportional change in dimension [1]. The dimensional change can be controlled to minute levels, which is why PZT actuation has been the foundation of atomic force microscopes and other applications requiring nanoscale-controlled motions. PZT actuation is also very quick, so throughput has been another reason for adoption.

Thus, recently developed stage mechanisms present new opportunities for microscopy applications. This article examines how piezo stages can remove previous constraints in ways that advance science.

Focusing Applications

In microscopy, a common application is *Z*-stack data acquisition, in which either the sample or the focusing lens is rapidly stepped or scanned along the optical axis. PZT mechanisms are the choice for this because of their combination of speed and precision. Integration of a frictionless lever amplification element allows design of compact and cost-effective mechanisms with many hundreds of microns of travel. Incorporation of a position sensor such as a strain gauge or capacitive sensor allows the PZT actuation to be made highly linear and repeatable, and the user can thereby acquire position information synchronously with the optical data without stopping, ensuring a reliable and highly deterministic position-domain datum set for their data (Figure 1).

In these applications, a recent trend has been toward longer and longer scan travels, even beyond the several-hundred-µm travels of which conventional lever-amplified PZT mechanisms are capable. Long travel is especially important for microscopy applications with large penetration depth, such as two-photon microscopy. This has necessitated development of novel mechanisms that use revolutionary modalities of piezo actuation. For example, in a novel class of walking-style actuators, the piezo ceramic is configured in a manner that confers longitudinal force on a ceramic actuation rod of arbitrary length. The ceramic elements can be actuated down into the sub-nm realm for small distances, or they may be phased to alternately push and retract, allowing unlimited travel actuation with high axial stiffness and

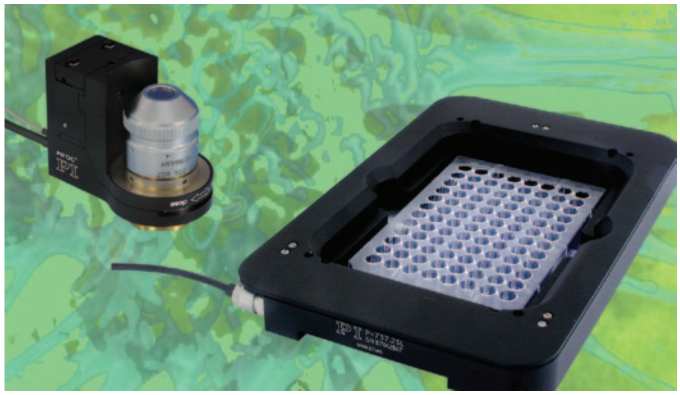

Figure 1: PIFOC® Piezo Lens Positioner and Piezo-Z Stage (PI).

holding force—ideal for focusing mechanisms where stiffness is desirable for stability reasons and to accommodate heavy, high-NA objectives (Figure 2).

Similarly, piezo actuation's high speed and resolution makes it the ideal technology for responsive autofocus implementations in microscopy. The need for focusing mechanisms (either objective positioners or *Z* stages) to instantly acquire focus and keep it locked in despite structural drifts and specimen motions has outstripped the capabilities of previous-generation probe-based sensors that could compensate for only some drift mechanisms. The industry has responded with both image- and sensor-based autofocus approaches that meet these emerging needs and that leverage sophisticated new interfacing capabilities. In particular, a unique laser autofocus sensor coupled with fast nanopositioning controllers can now acquire and lock in perfect focus in milliseconds, even starting from many hundreds of micrometers out of focus. This approach offers many benefits over previous, probe-based platform stabilization, which merely compensated for structural deflections of the microscope platform or other gross elements. Responsiveness is on a millisecond time scale, and all structural and optical contributors to defocusing are

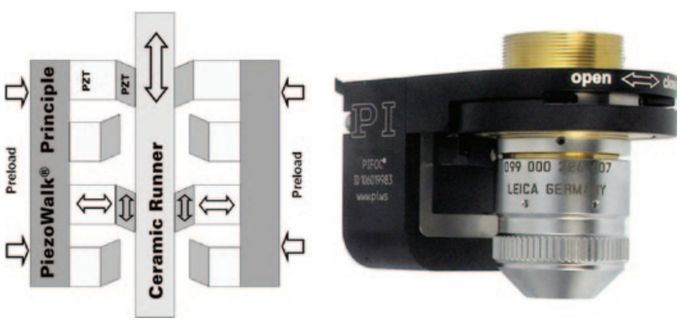

Figure 2: PiezoWalk principle and new 1 mm PIFOC Focusing Drive (PI).

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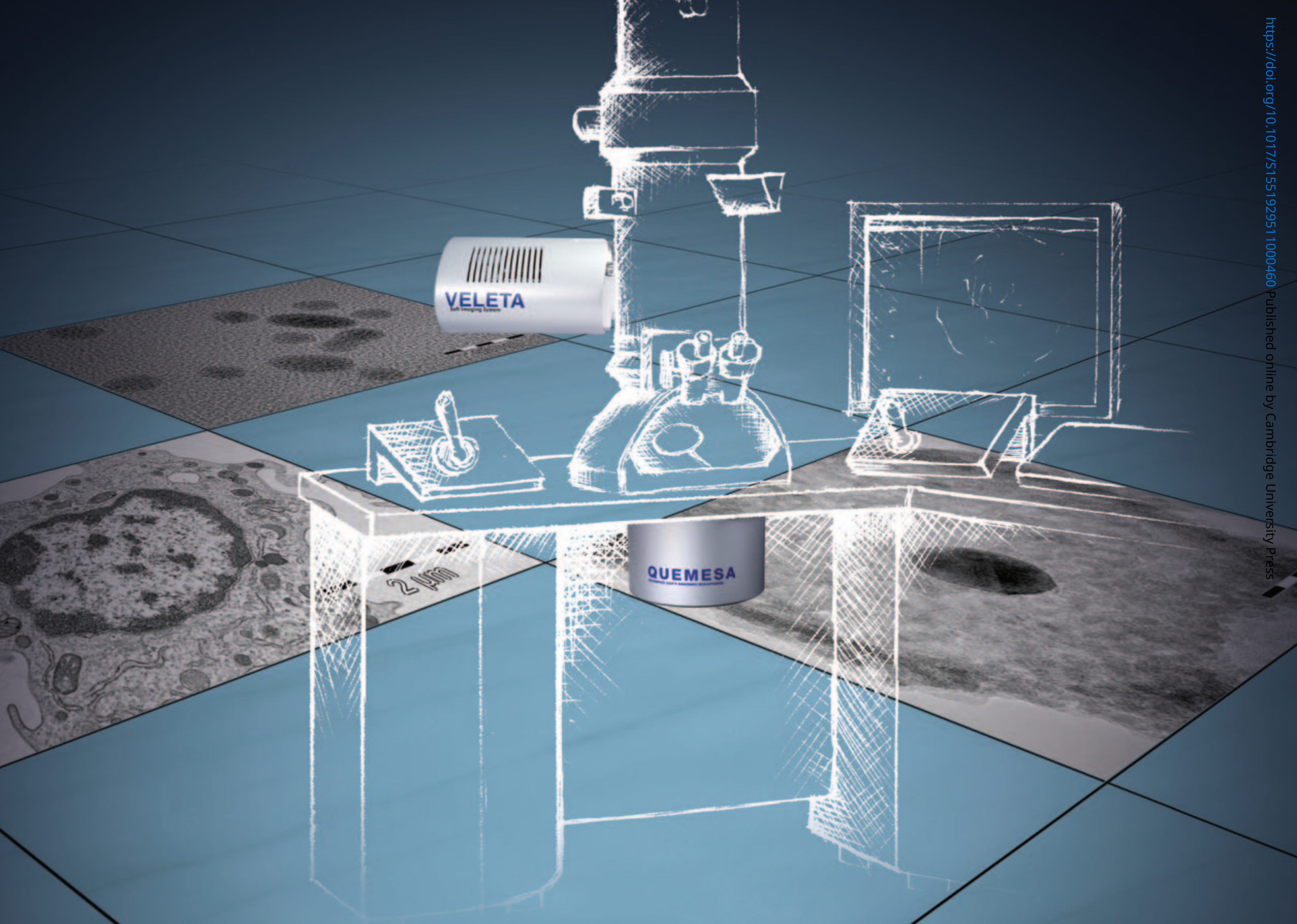

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automatically compensated. In particular, laser autofocus sensors coupled with fast nanopositioning controllers can now acquire and lock in perfect focus in milliseconds, even starting from many hundreds of microns out of focus (Figure 3).

Scanning Applications

Transverse motion of the sample across the image field is another near-universal requirement in microscopy applications. Depending on the application, the required motions may be coarse, over the many mm of a slide or well plate, or they may be fine, with nanoscale precision over just a few dozen µm of range. They can be point-to-point, or they can be linear scans of constant velocity, or even with specific position-versus-time waveforms, as in the case of calibrating optical tweezers. Very often, there is a requirement for both coarse and fine actuation, so that a region of interest can be brought into the field of view and then scanned at high resolution.

Scanning can be accomplished by optical means such as using beam-steering mirrors or acousto-optic deflectors, but moving the sample using a piezo-flexure stage offers benefits for applications requiring the highest high-resolution and flattest scanning and positioning. Piezo-flexure stages can provide up to 2 mm of scanning range, but more typically a less costly piezo stage of ~50–200 µm is stacked on top of a coarse positioning stage. Such coarse/fine applications have seen benefits from piezo-based technical developments in recent months. In particular, coarse-positioning mechanisms using a third type of piezoelectric actuation (besides the layered-stack actuators used in nanoscale positioning, and the walking-type actuators described for long-travel focusing mechanisms above) employ matchbook-sized slabs of piezoelectric ceramic, which are stimulated at their physical resonant frequency, typically in the ultrasonic range above 100 kHz. This stimulation causes a rhythmic fluttering of the slab, and a friction tip placed at the resonance's anti-node performs a quasi-elliptical nanoscale motion. When preloaded against a driven element, this confers a motive force. The stimulus-to-velocity profile of this actuation is similar to that of familiar DC servomotors, but the prominent deadband in the actuation profile means that the device holds the driven element with nanoscale stabilities when quiescent. In addition, lubricant flow mechanisms, which cause long-term drift and settling behaviors in conventional screw-driven coarse-positioner mechanisms, are eliminated. The resulting long-term nanoscale stability and innate position-hold force have improved resolutions and repeatabilities. This development also promises significant benefits in stitching applications, where high-resolution

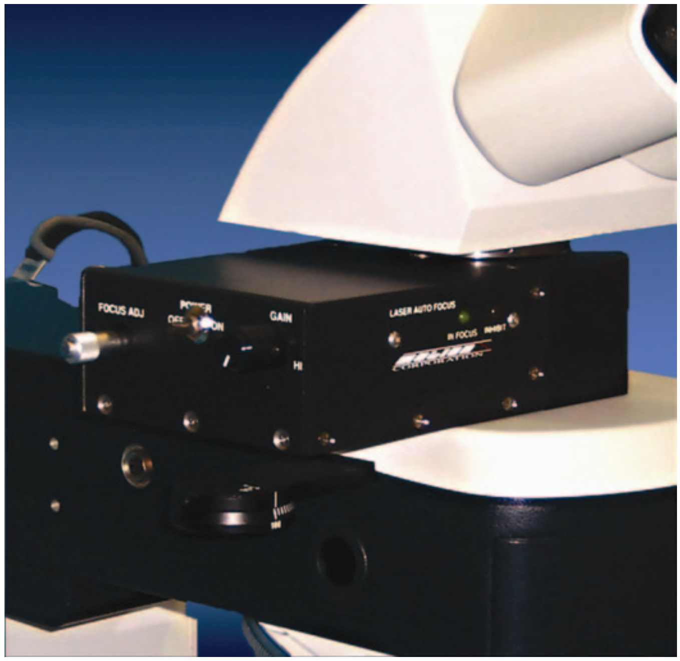

Figure 3: Laser Autofocus Sensor (MotionX Corp.).

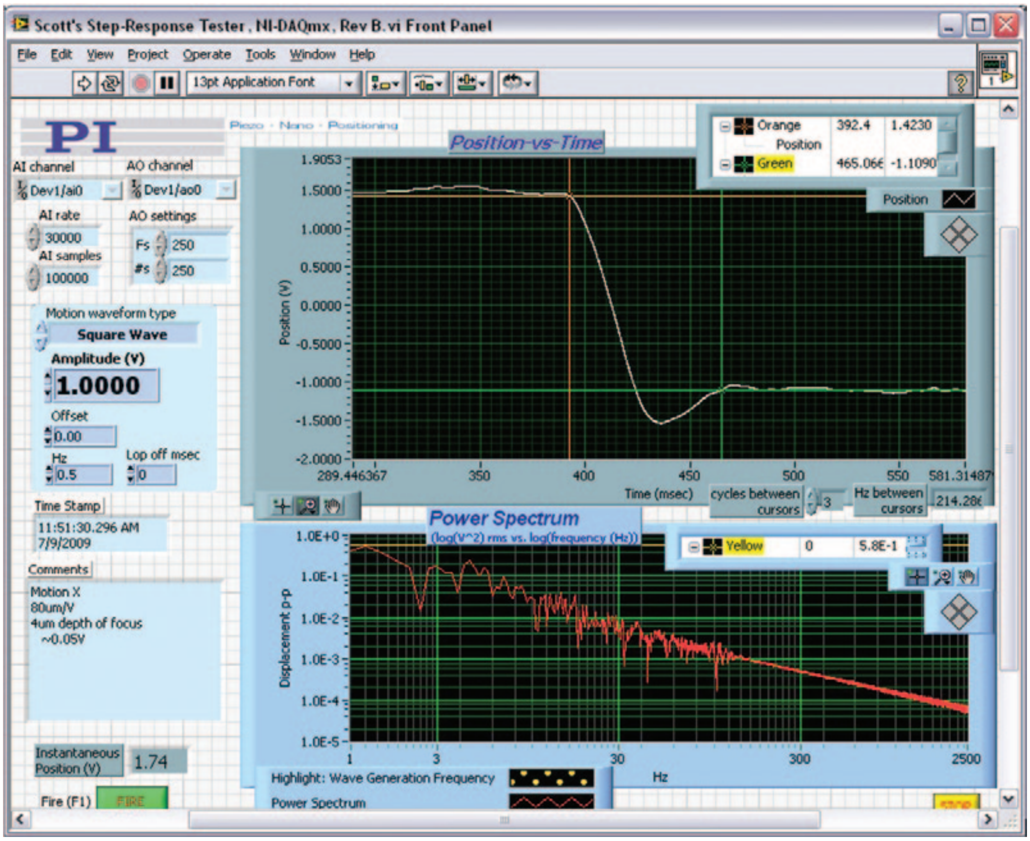

Figure 3a: A 60 msec capture of focus demonstrated by laser vibrometer independently measuring PIFOC objective position versus time.

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imaging is performed in small areas that are subsequently patched together in software.

Ultrasonic piezomotors are also attractive for transverse sample positioning in coarse/fine microscopy applications. They provide the long travel over many millimeters necessary for quickly accessing the full span of a microscope slide or well plate, and they also provide an inherently stiff and stable platform at rest. In fact, data published in a recent peer-reviewed paper [2] reveals nanoscale stabilities over many minutes for a combined coarse/fine stack based on an ultrasonic piezomotor coarse stage and a PZT-stack-based multiaxis nanopositioner. This performance is roughly an order of magnitude more stable than a high-quality screw-driven manual positioning stage.

Sensors

Several types of position sensors are employed in closed-loop nanopositioning mechanisms. The highest-resolution, highest-bandwidth, highest-accuracy, and highest-stability sensor is the two-plate capacitive sensor, in which one highly polished and complexly configured metallic plate is mounted on the moving element of a stage, with a corresponding plate on the fixed structure of the stage. As the stage moves, the actual position of the workpiece is measured in real time. These sensors may be arrayed around the stage's moving platform to monitor its position in multiple degrees of freedom. This facilitates parallel-kinematic configurations, in which a specimen can be actuated simultaneously in several directions. This increases system bandwidths and improves trajectory performance over less-costly stacked multi-axis designs.

For applications where the resolution, accuracy, bandwidth, and stability of capacitive sensors are not needed, various types of strain gauges have been used. Thin-film strain gauge sensors, for example, mount on the piezo stack and measure its expansion and contraction, allowing the servo-controller to linearize its actuation and eliminate hysteresis. These sensors span a significant area, so they are comparatively insensitive to local effects. Silicon piezoresistive sensors, by comparison, are very small, ideal for incorporation into a flexure element of a stage's structure. These are the foundation for a new wave of high-quality, but cost-effective, U.S.-made nanopositioners targeted at research and commercial microscopy applications (Figure 4).

Software and Interfacing Define the Microscope

Imaging software suites that control the microscope, camera, focusing mechanism, and sample positioning stages are gaining in importance. They define the microscope's capabilities and that of the application, and the suites are growing increasingly vertical in their focus. Examples include Metamorph and MetaFluor, Micro-Manager, ScanImage, and Intelligent Imaging Innovations' SlideBook™ suite, all of which support an impressive array of hardware from manufacturers committed to users' productivity. This has increased market awareness of software as not only an enabler for industrial and academic research but also as a pacing item for instrument manufacturers. Generally speaking, microscopy users must prioritize productivity and ease of use, so the majority of today's microscopy applications are well-served by imaging suites that offer plug-and-play support of popular subsystems, and microscope manufacturers and distributors offer configuration services (and sometimes their own proprietary

Figure 4: PInano Microscope Stage family (PI).

platforms) that allow users to devote their attention to their real work. In this way, subsystems have taken on a role reminiscent of common office peripherals like printers and scanners: users expect them to "just work" without a lot of low-level engineering on their part.

Technology Advances Pace the Field

John Hanks, VP for Life Sciences Segment at National Instruments, says "We see the field-programmable gate array (FPGA) and the GPU as hot topics: in the application and integration of lasers, for galvo control, and for inline FPGA processing for optical coherence tomography (OCT); for new medical devices; and for ophthalmic, cardiac, and dental applications. Understanding tradeoffs between FPGAs/GPUs/CPUs is key. FPGAs are good for IO and inline processing, GPUs for rendering and post-processing (large datasets and cache); host computer processing has the advantage of the most mature development environments and ability to manage heterogeneous/parallel processing."

Vijay Iyer, Senior Software Engineer at HHMI/Janelia Farm, states, "Light microscopists in the life sciences are increasingly seeking to interact with entire volumes of tissue—imaging, stimulating, disrupting points throughout those volumes. This demands fast, precise automated motion of the sample or the microscope optics, particularly when interacting with living tissue."

The challenge for subsystem manufacturers is now to support both the plug-and-play applications suites and the scientists immersed in writing their own code. At the same time, subsystem manufacturers must keep pace with the increasing importance of high-speed data acquisition and processing. This has driven a cascade of developments that have included: (a) advanced interface techniques that offer microsecond-scale synchronization between motion and optical processes; (b) novel motion devices with extended travels, resolution capabilities and stabilities, new sensors, and command sets with backward and forward compatibility as new controls are developed; (c) digital nanopositioning controllers of extraordinary capability but that undercut traditional analog controls in price, thanks to semiconductor developments; and (d) innovative control techniques that address fundamental limitations in motion system bandwidths, for more accurate scanning and acquisition.

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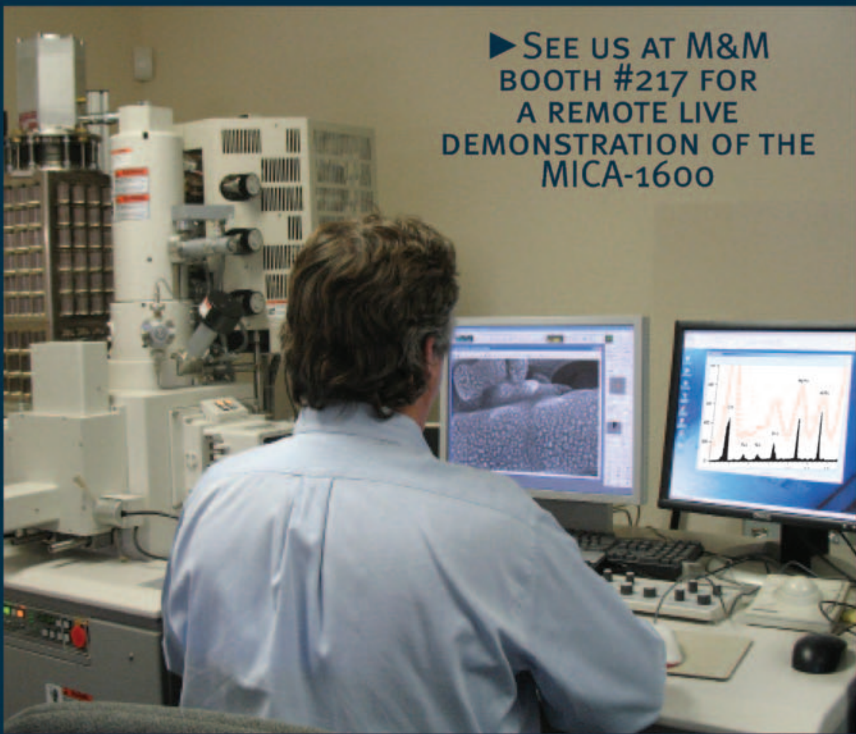

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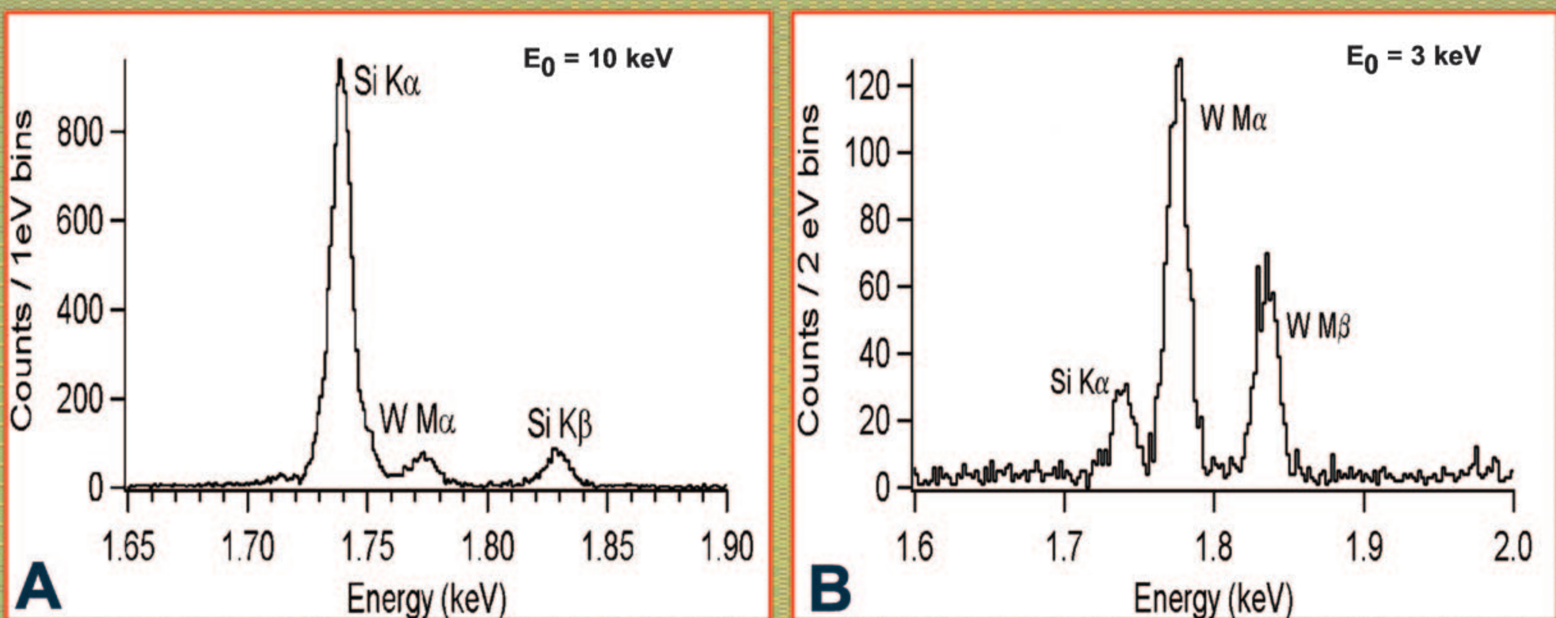

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Figure 5: Active isolation platform for instrumentation such as SEMs and life science microscopes (Technical Manufacturing Corp.).

Vibration Isolation

As image resolution improves and as microscopy techniques emerge from the lab and become incorporated into commercial tools and processes, protection from ambient vibrations becomes critical. Here, too, piezoelectric technology assists in advancing the field. Active vibration control technology has long been a mainstay of semiconductor microlithographic manufacturing and is fundamental to achieving today's rapidly diminishing integrated circuit line widths, often smaller than 32 nm. The leading active vibration control platforms depend on piezoelectric speed, resolution, and reliability to routinely enable such performance on the production floor (Figure 5) [3], and recently these platforms have found their way into the most advanced microscopy applications.

Conclusion

Light microscopy is a broad field and getting broader every week as novel imaging techniques continue to be developed. A common thread is that these techniques are enabled and assisted by application of piezoelectric positioning mechanisms. If there is one thing that is predictable about the future of this field, it is that application needs and the capabilities of piezoelectric actuation will continue to chase each other—to the benefit of science and society.

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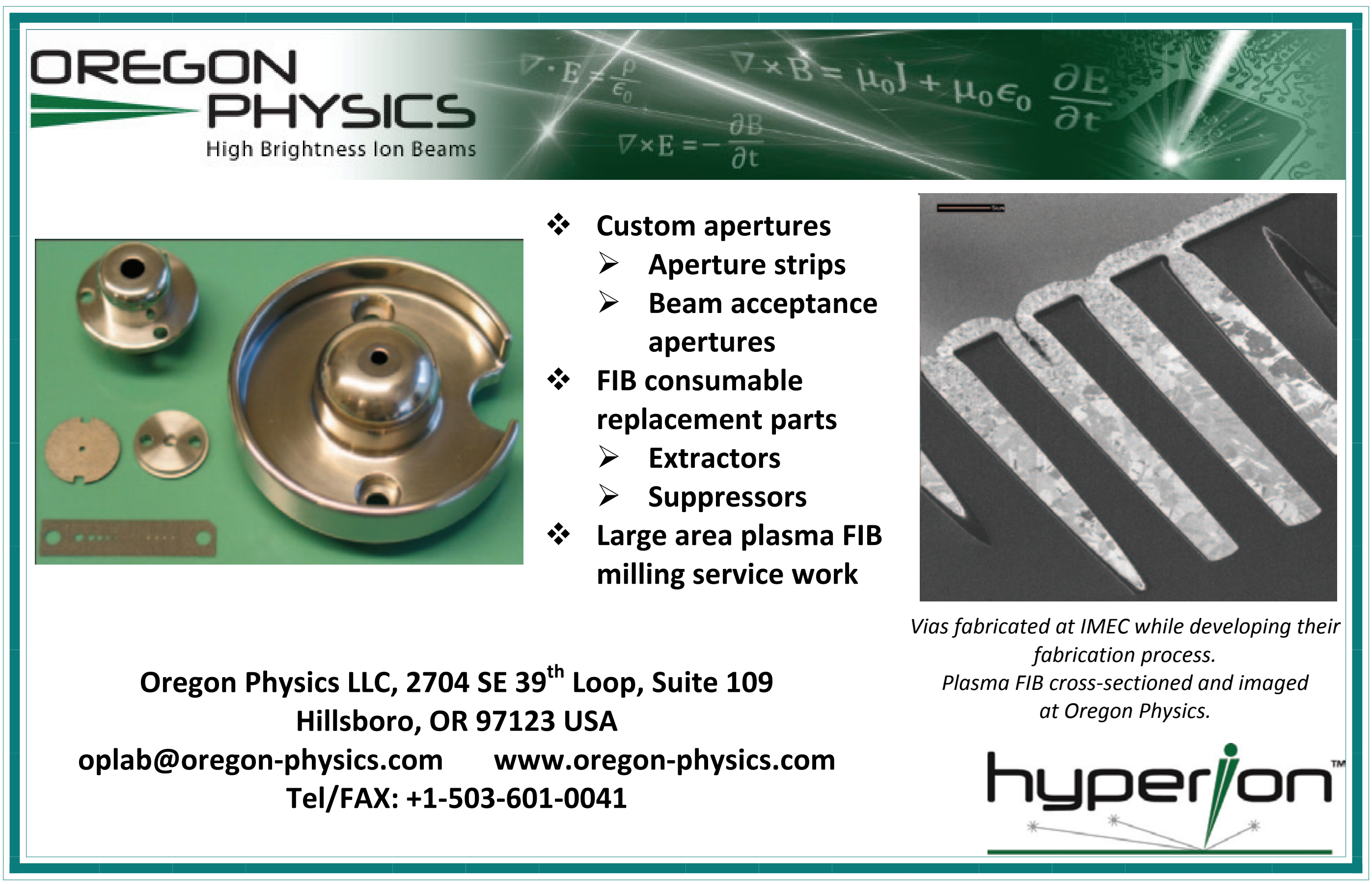

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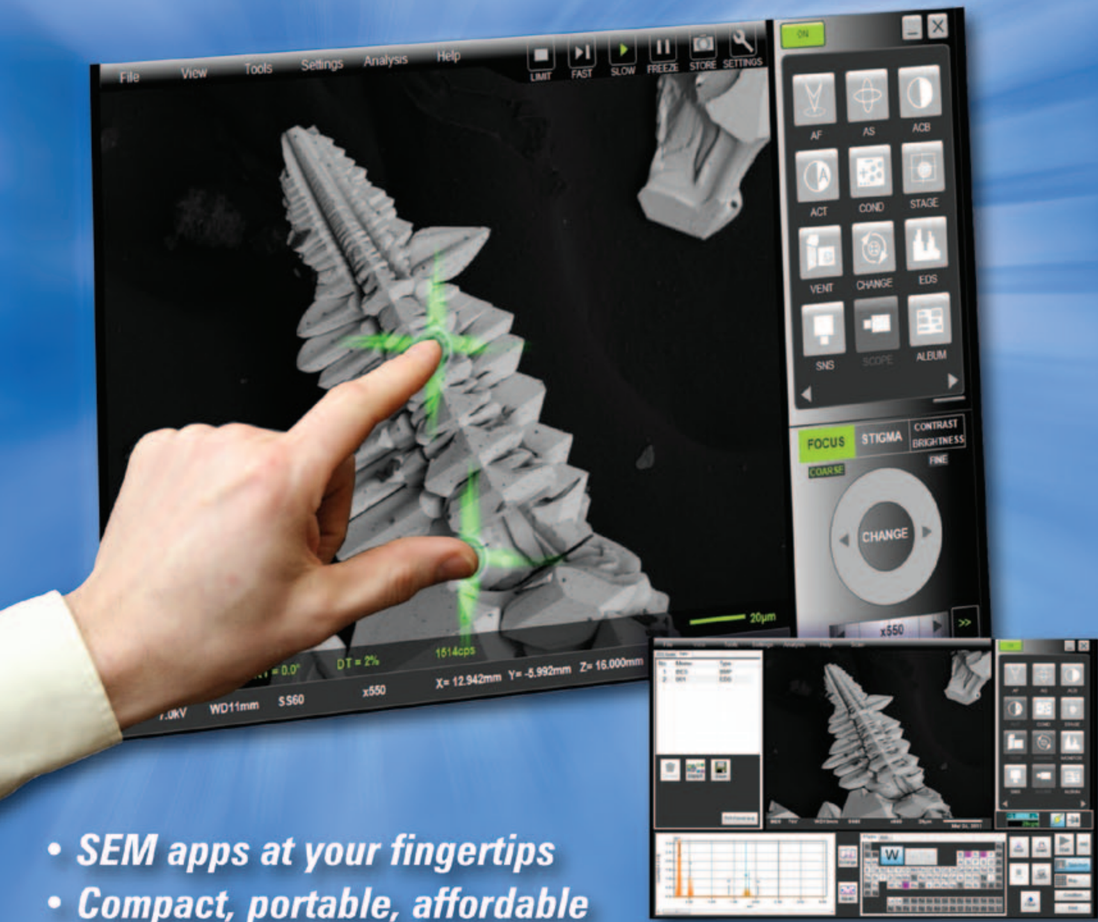

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