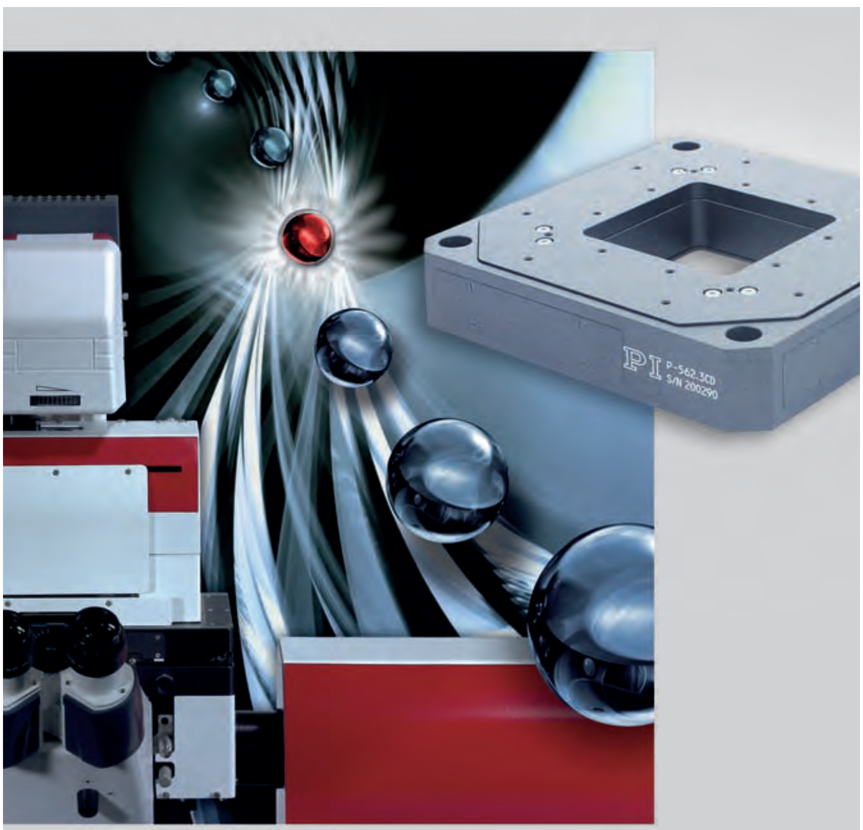
Microscopy news

Issue 42

Piezos in Optical Tweezers

Force Measurement in Nanoscopic Dimensions



Integration of the nanopositioning stage in the NanoTracker (Photo: JPK)

Investigations on interactions and forces at the level of individual molecules assist in gaining a great deal of information on chemical and mechanical properties or biological functions. Special techniques have been developed for this purpose, e.g. atomic force microscopes or so-called optical tweezers. The latter can grip, guide and manipulate miniscule objects by means of a laser beam.

The optical tweez ers consist in principle of a powerful laser and a microscope (Fig. 2). A "trap" which is movable in three dimensions can be generated by suitably split ting the laser beam. This trap makes it possible to virtually capture objects with dimensions in the nanometer or micrometer range, to hold them and to move them in a predefined way with respect to other objects, and all

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this simply by using light. Since small polystyrene beads are easily captured by the beam, these are attached to the samples to be manipulated prior to the experiment. When the laser beam is moved, the bead tries to stay in focus. If it is prevented from doing so by the sample and consequently moves out of focus, one can measure and analyze the forces generated from the resulting deflection of the laser.

Optical Tweezers with Laser: Non-Contact and Three-Dimensional Measurement

Since the technique uses only the radiation pressure of the light, there is no direct contact with the sample. The main advantage of the optical tweezers over atomic force microscopes is that observations are possible not only in the direction of the z-axis, but in all three spatial dimensions and experiments can be carried out interactively.

JPK Instruments, of Berlin, Germany has brought to market optical tweezers ready for series production which provide a time-resolved detection of individual molecules and directly support many of the mathematically complex analytical methods thanks to their extensive software. The NanoTracker (Fig. 1) enables the user to control and manipulate particles measuring from several micrometers down to 30 nm. The corresponding samples, e.g. whole, living cells or also individual molecules, can be observed in real-time with nanometer precision (Fig. 3). The system allows accurate, quantifiable and reproducible measurements of a wide variety of particle or cell interactions.

Nanopositioning System Improves Accuracy and Performance

While measuring the forces, it is basically possible to only acquire

Figure 1: The NanoTracker enables the user to control and manipulate particles measuring several micrometers down to 30 nm. The system allows accurate, quantifiable and reproducible measurements of a wide variety of particle or cell interactions (Photo: JPK)

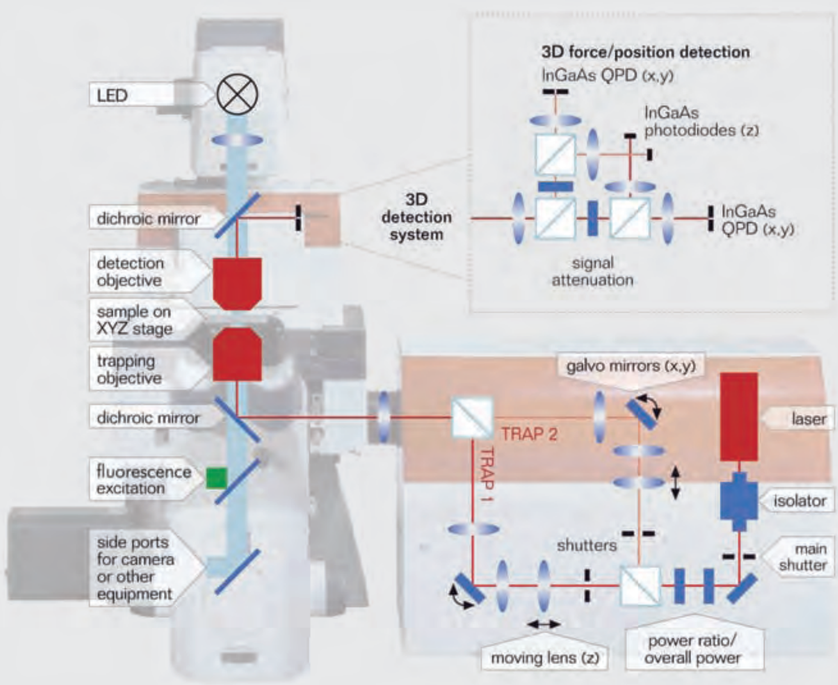


Figure 2: The optical tweezers consists in principle of a powerful laser and an arrangement of optical lenses. By suitably splitting the laser beam, two traps can be generated that are movable in three dimensions (Photo: JPK)

Figure 4: Dr. Joost van Mameren, application scientist at JPK: “We have integrated a piezo-based nanopositioning system into our NanoTracker to increase the accuracy and the experimental flexibility, e.g. to move at constant speed during an analysis.”

data from the trap steering unit, i.e. to evaluate the laser beam deflection. “This is not sufficient for quite a number of applications, however” explains Dr. Joost van Mameren (Fig. 4), an application scientist at JPK. “If an analysis requires motion at constant speed, for example, we integrate a piezo-based three-axis nanopositioning system into our NanoTracker. This will prevent small interfering signals, which could arise with pure trap steering due to the motion caused when the optics are displaced. Furthermore, the positioning system provides the option of shifting the focus in the direction of the Z-axis, if required, in real-time. One should avoid manipulating the optics for this purpose, if at all possible, because measuring errors caused by interfering signals would then be inevitable. Moreover, the nanopositioning system enables the calibration of the optical tweezers to provide a higher positioning accuracy.” JPK chose the P-561.3CD nanopositioning system of the PIMars series from PI. “The parallel kinematics system operates with repeatability in the nanometer range at response times below one millisecond, and the travel range of 100 x 100 x 100 µm matches the requirements of our application perfectly. Moreover, it proves to be very reliable and convinces with its long service life”, Dr. van Mameren continues.

Parallel Kinematics and Direct Metrology

The combined use of parallel kinematics and parallel direct metrology in the PIMars system provides the multi-axis nanopositioning system with simultaneous determination of all degrees of freedom with respect to a common fixed reference. An unintended crosstalk of the motion (e.g. as a result of an external force) into a different axis can thus be detected and actively compensated in real-time. This can keep the deviation from the trajectory down to under a few nanometers, even in dynamic operation. “The capacitive sensors integrated in the nanopositioning system make a decisive contribution to this effect. They provide direct and contact-free measurement of the position. Neither friction nor hysteresis affect the measurement, and this, combined with the position resolution of far below one nanometer, means outstanding values for linearity can be achieved”, explains Dr. van Mameren further.

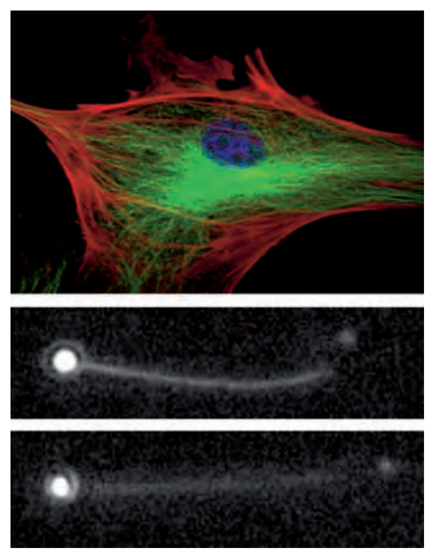
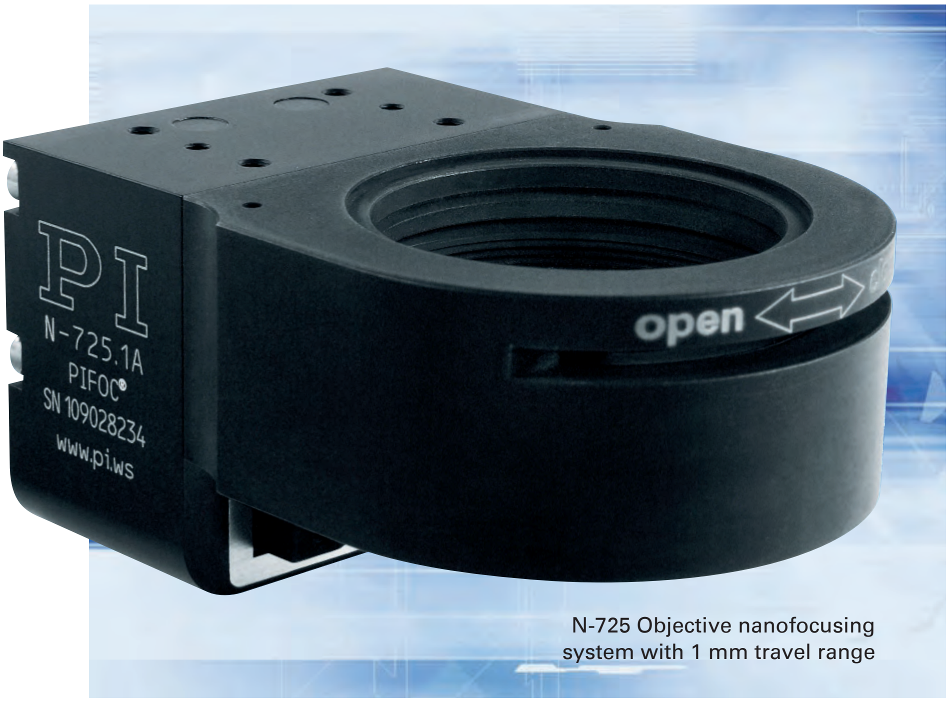


Figure 3: Samples, e.g. whole cells (BPAE Zelle; FluoCells® Slide #2) or even individual molecules (Alexa 555 marked Rad51 protein, image: courtesy of M. Modesti Lab, CNRS Marseilles) can be observed in real-time with nanometer precision. (Photo: JPK)

Something New in Microscopy: 1 mm Objective Stage with Piezo Stepping Drives

Fast Steps with Nanometer Precision over Record Travel Range



N-725 Objective nanofocusing system with 1 mm travel range

Piezo stepping drives in combination with a high-resolution linear encoder offer nanometer accuracy over the complete scanning range – and are now also available for PIFOC® focusing systems for microscope objectives.

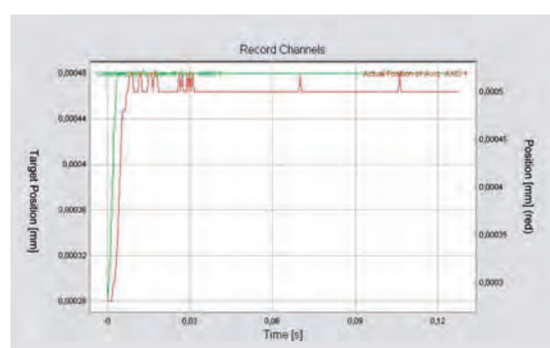
The new N-725 PIFOC ® stage for microscope objectives with NEXACT® piezo stepping drives provides travel ranges of up to one millimeter along the optical axis. At the same time the technology allows rapid positioning: The N-725 focusing system achieves settling times of around 20 milli - seconds for steps of 200 nanometers. It has an extremely compact design and is no larger than conventional, piezo-based PIFOC® objective stages.

The focal plane can easily be changed with a single mec hanical system thanks to the combination of high resolution and long travel ranges. Therefore, there is of ten no need for a combined Z-positioning of the objective, e.g. with stepper motor and additional piezo stages.

With this, the N-725 PIFOC® is optimally suited for microscopic methods which offer a high penetration depth of tissue as does, for example, the two-photon microscopy.

Piezo Stepping Drive – the Multi-Functional Piezo Linear Motor

The NEXACT® piezo stepping drive advantageously combines the best characteristics of piezomotor principles in a compact unit: High resolution, high force and high speed. The feed motion is achieved solely via the nanometer-accurate motion of clamped piezo actuators – non-wearing, high resolution and with controllable speed. The drive is self-locking when it has reached its target and no longer needs to be powered, which is why it does not heat up. The position is stable in the nanometer range so there is no jitter about the target position, as is the case with servo motors and stepper motors.



A step of 200 nanometers is executed within 10 milliseconds. The sensor resolution of the N-725 is 20 nanometers. This equals to the oscillation around the target position. The green curve shows the commanded target position, the red curve shows the actual position.

Microscopy Profits from PI's Piezo Technology

PInano™ Scanning Stages for the Highly Accurate Positioning of Samples

The PInano™ system has been designed for the positioning of samples in high-resolution optical microscopy. The P-545 core component of the PInano™ is an XY or XYZ piezo-driven scanning and positioning stage with a 20 mm height and a large central aperture for transmitted-light microscopy. The large clear aperture accommodates holders for Petri dishes or standard object slides of 25 x 75 mm in size. A holder for additional accessories is also available. The piezo stage provides travel ranges of up to 20 0 µm in two or three axes and reaches a position resolution in the nanometer range. The integrated piezoceramic PICMA® actuators offer maximum reliability and a proven superior lifetime. The PInano™ has its own matched piezo controller, the E-545, which offers 24-bit USB, Ethernet and RS-232 interfaces as well as an analog interface. An extensive software package supports, among others, LabVIEW and Linux. Important for scanning applications are the integrated data recorder, which collects motion parameters as well as system parameters, and the wave generator, which controls the motion of the stage with diverse curves and functions. In addition, the software supports a series of common image acquisition systems, such as µManager and Metamorph. To move the sample along larger travel ranges, the M-545 manual XY microscopy stage can be used to complement the P-545. The XY stage directly fits all common inverted light microscopes and carries the piezo stage. The M-545 XY stage can be equipped additionally with stepper motor drives.

The P-545 PInano™ piezo stage, shown here with clamping adapters for Petri dishes and objective slides

One for All: PI Microscopy Stage Fits all Common Light Microscopes

Manual or Motorized Precision XY Stage

The PInano™ system with its controller (rear), the piezo system attached to the manual XY stage (front left) and on its own (right).

A manual drive with precision micrometer screws? Motorized for operation via a joystick or a host PC? The M-545 XY stage offers both options. It positions samples on an area of 25 x 25 mm and directly fits a number of light microscopes of different makes. Thanks to the adapter plate, a variety of sample holders can directly be attached to it. The P-545 PInano™ piezo scanning stage fits without the use of adapters: With this an additional nanometer-precision positioning of samples over 200 µm in two or three axes is possible. The XY stage can be motorized as an optional extra. A complete package for a comfortable control comprises two stepper-motor actuators with mounting accessories, controllers and a joystick. Alternatively, the linear actuators are delivered with the mounting accessories and can then be controlled with the customer's stepper-motor controllers.

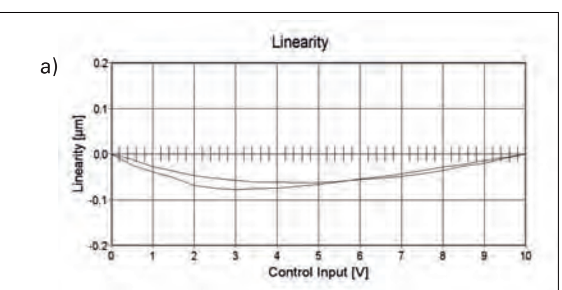
Fast and Accurate in High-Resolution Microscopic Techniques

Piezo Z-Scanner for Object Slides

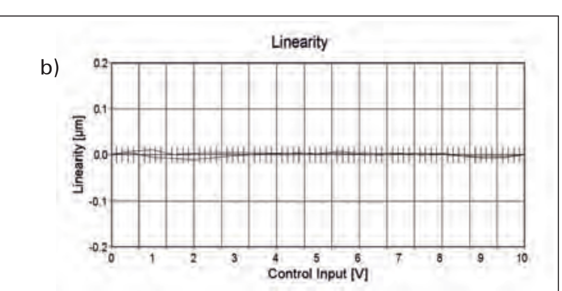
P-736 nanofocusing stage with digital controller

Fast settling times of about 5 milliseconds and a position resolution under one nanometer are the most outstanding characteristics of the P-736 piezo-based focusing system from Physik Instrumente (PI).

a)

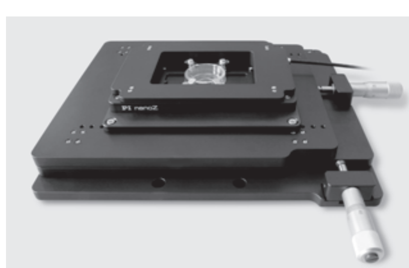


b)



Measurements with a classic analog controller (a) and the digital E-709 (b). Thanks to the digital linearization, the non-linearity with the new E-709 is lower by a factor of up to 10.

The piezo scanner positions object slides up to 200 µm along the optical axis and with that it is ideal for applications in the field of optical imaging and in microscopy. The scanner is 20 mm high and it is compatible to the inverse micro-scopes of Leica, Nikon, Olympus and Zeiss. The stage is delivered as a complete system with a digital controller which has a USB, an RS-232 and an analog interface.



The P-736 fits the M-545 XY stage with a 25 x 25 mm travel range. The M-545 can optionally be motorized.

Digital Single-Channel Motion Controller for Microscopy

The E-709 single-channel piezo controller operates with linearization algorithms which improve the accuracy of piezo systems that employ strain gauge sensors (metal foil or piezo-resistive) by a factor of up to 10 to values of 0.02%. The E-709 can be supplied with commands via the USB and RS-232 interfaces and it allows data to be collected or motion curves to be prescribed. It is easy to work with the digital system, since the setting of the motion and control parameters as well as the programming of the digital I/Os can be made using software. Its compatibility with software platforms such as LabVIEW, µManager, MATLAB or Metamorph and its programmability via DLLs are important for microscopy applications, where the E-709 can operate single-axis piezo systems such as the P-721 objective positioner or the P-736 and P-737 sample scanners.

Holders for object slides and Petri dishes as well as additional accessories are available that fit the large clear aperture.

The digital controller improves the linearity that is achieved and additionally allows the flexible adjustment of the servo control parameters when operating with different loads. The controller has USB, RS-232 and analog interfaces and can therefore be easily integrated into different control systems and is compatible with all common image acquisition systems. PI's PICMA® technology stands for proven, unrivaled reliability and lifetime.

PIFOC® Piezo Drives now with 29 mm clear aperture

More Light for your Microscope

The P-721.SDA PIFOC® (right) for threaded adapters with a larger clear aperture in comparison to the P-721.0LQ

More and more techniques in microscopy, today, require an improved resolution. This often requires the combination of a large numerical aperture of the objective, that is a large clear aperture, and a large working distance along the optical axis. 3D images of living tissue are then possible, for example with the help of two-photon spectroscopy.

For this purpose, PI now offers a PIFOC® piezo Z-drive for objectives with a clear aperture of up to 29 mm. The versions of the proven P-725 and P-721 stage series allow travel ranges up to 400 µm while offering high dynamics and nanometer-level position resolution.

PIFOC® piezo Z-drives are fast positioners for the accurate motion of the objective or the sample in microscopy. For focusing purposes, either the imaging optics or the sample can be moved. PIFOC® piezo Z-drives for the objective can be very compact in size and do not affect the sample while focusing. Their outstanding qualities are:

- Response times of a few milliseconds
- Resolution of a few nanometers
- Simple integration in existing microscopy systems

PIFOC® drives are therefore ideally suited when it comes down to a high throughput and accuracy. Screening tasks in biotechnology, medical diagnostics or pharmacy, for example, require the analysis of up to a few hundreds of samples. The focusing of the individual samples, therefore, has to be fast. In confocal microscopy images of samples are generated in different focal planes and stitched to one 3D image by using software. For this purpose, the position resolution of the piezo Z-drive has to be in the range of 10 nanometers (0.01 µm).

XY Microscope Stages with New Controller

Higher Stability with Ultrasonic Linear Motors

The two-channel C-867.260 controller serves to control XY scanning stages, e.g. the M-686.D64 with 25 x 25 mm

PI's ultrasonic linear motors are self locking at rest and provide superior stability compared to other motor designs, important for long term microscopy experiments and stitching applications. For more information and test data read paper from "Current Pharmaceutical Biotechnology, 2009, 10, 515-521" at http://tinyurl.com/Biophysics-Stability

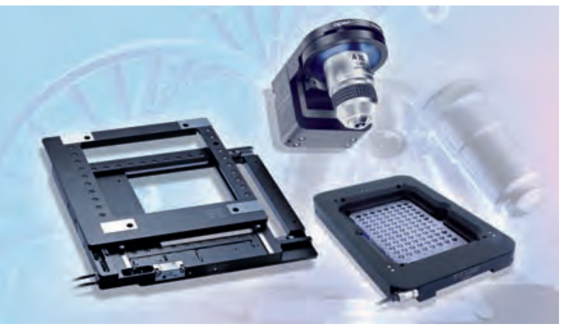
PI manufactures low-profile XY stages with a profile height of less than 30 mm across the whole surface of the stage – there are no lead screw ducts, no flanged motors. A speed of 100 mm/s can be achieved at a resolution of up to 0.1 µm.

Special control algorithms and drive principles are necessary to achieve the high speeds and short positioning times. The C-867.260 motion controller is especially designed for two-axis positioning systems with PILine® motors. It also provides additional performance characteristics for simple integration and rapid operation, such as

- USB, RS-232 and analog interfaces (e.g. for a joystick)
- 4 + 4 programmable TTL I/Os for flexible automation
- Data recorder
- Daisy-chain network of up to 16 units
- High-performance macro programming language, e.g. for stand-alone operation
- Extensive software support, LabVIEW, DLL, ...

Single-Molecule Investigations Demand Accuracy and Stability

Good Reasons for Choosing PI Positioning Technology



PI is one of the supporters of the 2010 "Single Molecule Approaches to Biology" conference in Lucca (Barga), Italy, June 27 to July 2. Organized by the non-profit Gordon Research Conferences, it is the third meeting focused on interdisciplinary exchange of the newest findings in molecular biology and metrology, i.e. instrumentation.

The rapid technical progress, allows groundbreaking research: detecting, identifying, tracing and manipulating even single molecules. This state-of-the-art technology makes it possible to study biomedical reaction sequences in all their intermediate states and in interaction with each other in vivo.

The portfolio of the conference is broad and it deals with topics as diverse as single-molecule sequencing, DNA/RNA/protein interactions, folding machines, cellular biophysics, synthetic biology and bioengineering, force spectroscopy, new frontiers in methods, superresolution and probe development for single-molecule experiments.

PI will present technologies for the extremely accurate and stable positioning of samples or high resolution microscope objectives that satisfy the requirements of the single-molecule analysis methods.

SINGLE MOLECULE APPROACHES TO BIOLOGY
June 27 – July 2, 2010 · Il Ciocco Hotel and Resort, Lucca (Barga), Italy

Chairs:	Nynke Dekker (TU Delft) DNA/RNA/PROTEIN INTERACTIONS William E. Moerner (Stanford University) FOLDING MACHINES
Vice Chairs:	Julio Fernandez (Columbia University) FORCE SPECTROSCOPY Stefan W. Hell (MPI Göttingen) SUPERRESOLUTION

Tradeshows 2010

July, 13 – 15	**Semicon West**	San Francisco (CA), USA	**Booth #6257**
Aug, 3 – 5	**Optics and Photonics**	San Diego (CA), USA	**Booth #702**
Sept, 13 – 16	**MOTEK**	Stuttgart, Germany	**Hall 9, Booth #9007**
Oct, 19 – 21	**Semicon Europe**	Dresden, Germany	**Booth #3.218**

Publisher:
Physik Instrumente (PI) GmbH & Co. KG
Auf der Römerstrasse 1
D-76228 Karlsruhe
Tel. +49 721 4846-0 · Fax +49 721 4846-100
info@pi.ws

Editor: Dr. Karl Spanner
Editorial Staff: Steffen Arnold, Stefan Vorndran, Sandra Ebler
Creation & Design: Regelmann Kommunikation, Pforzheim
Production: Systemedia, Pforzheim

USA

PI (Physik Instrumente) L.P.
16 Albert St.
Auburn, MA 01501
Tel. +1 (508) 832 3456
Fax +1 (508) 832 0506

5420 Trabuco Rd., Suite 100
Irvine, CA 92620
Tel. +1 (949) 679 9191
Fax +1 (949) 679 9292
info@pi-usa.us
www.pi-usa.us

JAPAN

PI Japan Co., Ltd.
Akebono-cho 2-38-5
Tachikawa-shi
J-Tokyo 190
Tel. +81 (42) 526 7300
Fax +81 (42) 526 7301

Hanahara Dai-ni Building, #703
4-11-27 Nishinakajima,
Yodogawa-ku, Osaka-shi
J-Osaka 532
Tel. +81 (6) 6304 5605
Fax +81 (6) 6304 5606
info@pi-japan.jp
www.pi-japan.jp

UK & IRELAND

PI (Physik Instrumente) Ltd.
Trent House, University Way,
Cranfield Technology Park,
Cranfield, Bedford MK43 0AN
Tel. +44 (1234) 756 360
Fax +44 (1234) 756 369
uk@pi.ws
www.physikinstrumente.co.uk

ITALY

Physik Instrumente (PI) S. r. l.
Via G. Marconi, 28
I-20091 Bresso (MI)
Tel. +39 (02) 665 011 01
Fax +39 (02) 610 396 56
info@pionline.it
www.pionline.it

FRANCE

PI France S.A.S.
244 bis, avenue Max Dormoy
92120 Montrouge
Tel. +33 (1) 55 22 60 00
Fax +33 (1) 41 48 56 62
info.france@pi.ws
www.pifrance.fr

CHINA

Physik Instrumente (PI Shanghai) Co., Ltd.
Building No. 7-301
Longdong Avenue 3000
201203 Shanghai, China
Tel. +86 (21) 687 900 08
Fax +86 (21) 687 900 98
info@pi-china.cn
www.pi-china.cn