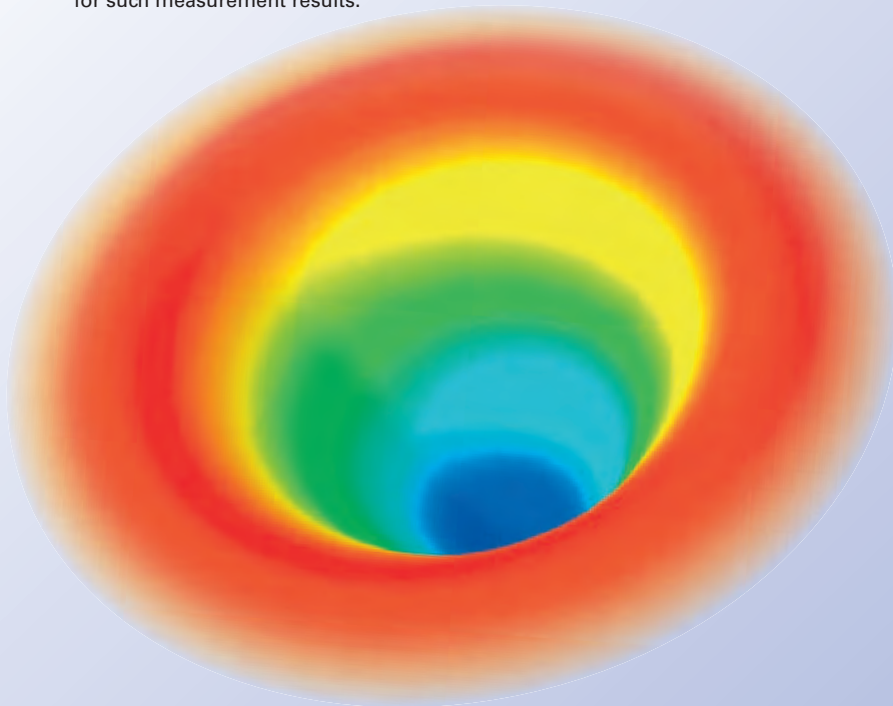


Integrated Piezo Fine Drive for the Complete Revolving Nosepiece of the Objective

Fast, Picometer-precise 3D Surface Inspection

A fast and precise positioning of the objective is an important prerequisite for such measurement results.

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pm



With the BW-D501, Nikon Instech Co.Ltd. has developed a new high-speed measurement device for three-dimensional, non-contact and non-destructive surface control of the most diverse materials with a resolution of one picometer.

The deformation of gel samples or surface coatings when heated can thus be analyzed; a heating table required for this purpose is already integrated in the device. The inspection method is suited for numerous industrial applications since, in contrast to conventional methods, even surfaces with diffuse reflection characteristics are no problem



Fast, high-precision 3D surface inspection done with the BW-D501 from Nikon (Photo: Nikon/PI)

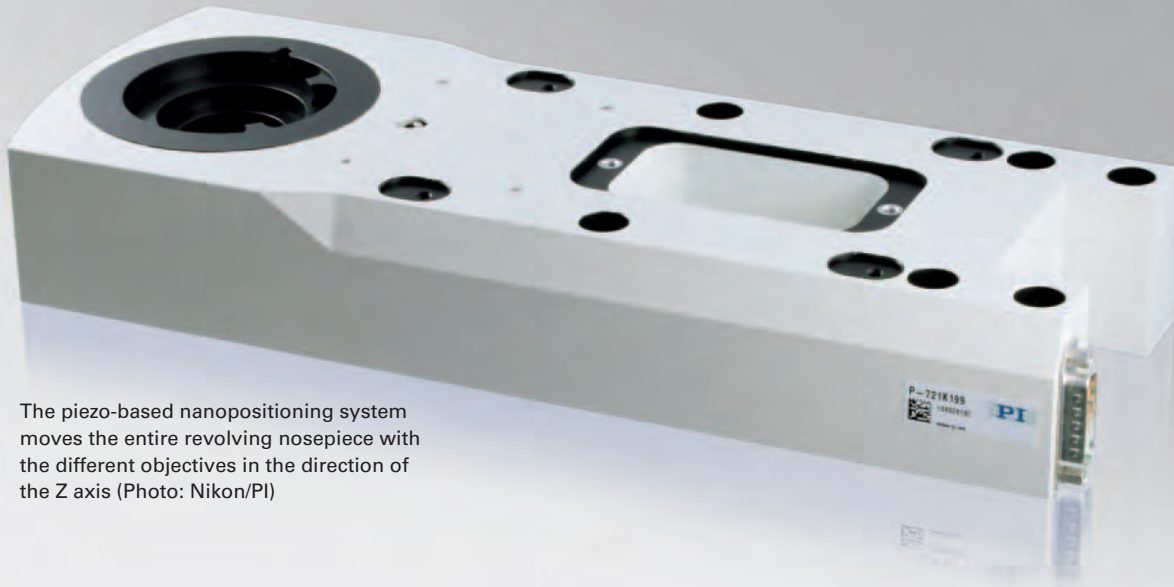
for the measurement device. Surface contours as well as the roughness of different surfaces can now be captured, visualized and analyzed with high resolution.

Capturing Roughness and Contours

The camera system of the measurement device initially examines the surface of the sample with high resolution. To get the depth of field necessary for a three-dimensional analysis, the optics is then moved gradually in the direction of the Z axis.

Continued on page 2

Contents	Page
Integrated Piezo Fine Drive for the Complete Revolving Nosepiece of the Objective	1 – 2
3D Laser Lithography in Biotechnology and Medical Engineering	3
Piezo Drives for Objectives - Precise, Fast and Attractively Priced	4 – 5
PI Software: Easy Operation and Integration	6
Scanning Piezo Stages for High-Precision Sample Positioning	7 – 8



The piezo-based nanopositioning system moves the entire revolving nosepiece with the different objectives in the direction of the Z axis (Photo: Nikon/PI)



The piezo actuators work without wear or friction and with no backlash (Photo: PI)

Depending on the position of the objective, different areas of the sample are put in focus. Afterwards, the individual images can be collated for analysis. Approximately 1000 images are necessary for a detailed, analyzable picture. The system works with considerable speed: Up to 2000 images are taken per second. To be able to display the detailed structure as well as the shape, as for example in an elevation map, the system additionally works with white-light interferometry. The interference pattern provides information on the shape of the surface. The fast and precise positioning of the objectives is an important requirement for the analysis, which is by no means trivial,

and for the "assembly" of the individual measurement results to meaningful pictures. For this purpose, PI has developed a positioning system directly integrated in the microscope that moves the entire revolving nosepiece. With it, all objectives mounted to the nosepiece can be moved with high precision in the direction of the Z axis.

However, the required dynamics can hardly be realized with conventional motor drives. The positioning system is therefore based on piezo actuators that are suitable for the fast settings required for 3D surface measurement in real time as a result of their high stiffness. At the same time, play-free and high-precision flexure guides provide a high focus stability. In this way, travel ranges of up to 100 μm can be achieved in the application described above and all of this practically without lateral runout of the objective. The accuracy of the motion of the kinematics is in the nanometer range.

Capacitive sensors, which come from PI as well, are employed for the precise measurement of the position while in motion. They measure directly and without physical contact the part of the mechanical system that is in motion (direct metrology). Neither friction nor hysteresis interferes with the measurement. Combined with the position resolution in the subnanometer range, very good values in linearity are so achieved. The position of the objective can be matched accurately to the individual image so that the high-precision 3D surface inspection is possible at all.

Nikon Instruments in Brief

The roots of Nikon Corporation, Instruments Company reach as far back as 1917 when three Japanese companies amalgamated to one enterprise which developed and produced high-precision optical glass. In 1925 already, they presented the first microscope which offered a revolving nosepiece where different objectives could be interchanged. In the coming decades, the upcoming enterprise set new standards in microscopy. Today, it is one of the market leaders in digital imaging applications and continually stands

out with innovative products. An example for this is the high-precision and real-time capable surface analysis, including the powerful and easy to use software for the analysis, described in this text. Typical fields of application for high-performance microscopy can be found today in the industry sector as well as in biology, medical engineering and materials research. Nikon has been actively represented on the European market since 1961.

3D Laser Lithography in Biotechnology and Medical Engineering

Cell Cultivation in the Third Dimension

There are limits to classic cell cultivation in a Petri dish. This is because in natural tissue, cells in an organism are normally in an extracellular matrix. A Petri dish cannot simulate these real environmental conditions. A remedy is provided by the described method, which has been utilized by scientists at the Karlsruhe Institute of Technology (KIT). Using 3D laser lithography, freely structured and flexible structures can also be created in a three-dimensional, reproducible way. This means that cells can colonize a specially reproduced spatial matrix.

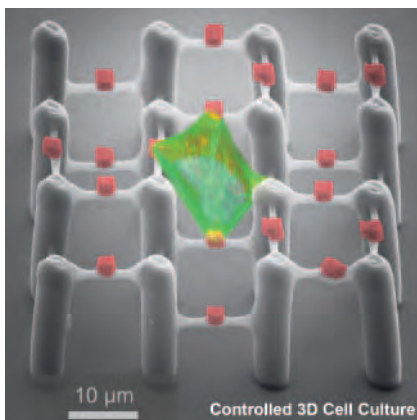
The structure itself consists of 25 µm high posts that are connected with thin rungs at different heights. This basic structure consists of a protein-repellent polymer. In addition, "handholds" or "docking sites" made of a protein-binding photopolymer are located in the middle of the rungs for the cells. Cells can attach here in a defined manner. Such a matrix opens up numerous new possibilities.

In this way, the influence of the physical environment (stiffness and architecture) on stem cell differentiation or cell migration can be investigated. Forces can be measured, and reactions to

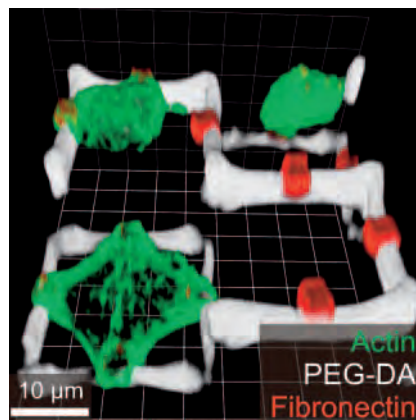
various stimuli can be observed and analyzed. The results include findings on tissue regeneration or the influence of active pharmaceutical ingredients on wound healing, for example.

Nanoscribe GmbH has developed a laser lithography system that can be used to realize complex three-dimensional structures in a fully automatic and reproducible way with a previously unavailable design flexibility on submicrometer scales with structure widths of up to 150 nm and sizes of up to 1 mm. This makes it possible to create microstructures for small pumps and needles or to equip surfaces with particular biometric characteristics. Important keywords in this connection are gecko, lotus or salvinia effect. A typical area of application for 3D laser lithography is also the creation of three-dimensional structures for cell biology.

The driving force of the laser lithography system from Nanoscribe is the P-563 nanopositioning system from PI. This works with travel ranges of up to 300 x 300 x 300 µm³, with repeatability in the nanometer range. Highly linear capacitive sensors integrated in the positioning system provide the precise actual value acquisition that is necessary to move the sample precisely and repeatably in relation to the laser focus. These sensors directly detect the motion and thereby allow a higher phase reliability and bandwidth than indirect systems. A digital controller from PI provides the necessary path control.



Three-dimensional structure: cells dock on the "handholds" (Photo: B. Richter and M. Bastmeyer, Zoological Institute, Karlsruhe Institute of Technology (KIT))



This makes it possible to investigate the influence of the physical environment (stiffness and architecture) on stem cell differentiation or cell migration. (Photo: B. Richter and M. Bastmeyer, Zoological Institute, Karlsruhe Institute of Technology (KIT))



The fine adjustment of the sample is performed by the piezo system that has been integrated in the laser lithography system by Nanoscribe. (Photo: Nanoscribe)

PIFOC®s cover all the demands of modern microscopy

Piezo Drives for Objectives - Precise, Fast and Economical

Autofocus systems, surface analysis, screening tasks, wafer inspection or multiphoton spectroscopy: scans in the Z-direction for three-dimensional tasks have high requirements for precision and velocity.



The PIFOC Z drives can be constructed to be very small and stiff. They react with short response times and position very precisely due to the good guiding, even with relatively long travel ranges of up to 100 or even 400 μm .

Piezo drives are best suited for such tasks for various reasons, regardless of whether the objective, the complete revolving nosepiece of the objective or the sample is to be positioned.

PI was also far ahead of the times in this field, which is so important for microscopy. Precision-guided piezo objective drives were already developed here 2 decades ago and have been continually improved since. The PIFOC® Z drives can be constructed to be very small and stiff. They therefore react with short response times and due to good guiding they position very precisely even with relatively long travel ranges of 100 micrometers to 1 mm and beyond. Fine positioning can be done down to the sub-nanometer range. The very short settling time of less than 0.01 seconds increases the throughput and enables fast focusing or scanning of Z stacks. The latest systems with capacitive position measurement and digital controllers achieve maximum linearities of 0.06 % and a focus stability over several 10's of seconds within a few nanometers.

Easy Integration with Thread Adapter

The PIFOC Z drives are practically integrated in the microscope: the drives can be easily inserted between the objective and the revolving nosepiece with a thread adapter. After the adapter is screwed into the revolving nosepiece, the drive is then fastened in the desired direction. Since the objective positioner does not have to be rotated itself, the cable routing is unproblematic. For applications that require a particularly large clear optic aperture, there is a variant with a 29 mm clear aperture (opening for the light beams) in the threaded insert.

New Package Prices with Digital Controller for the Price of Analog Technology

To reduce costs while simultaneously improving user friendliness, PI now offers especially economical packages, consisting of piezo mechanics and a new, compact digital controller. The user benefits from the higher linearity and the ease of operation resulting from digital control technology.



Compact digital controller that provides digital control possibilities for piezo-driven nanopositioning systems for the price of analog controllers.



Cost-optimized digital piezo controller now also for piezo actuators and nanopositioning stages that work with more attractively priced measurement systems such as strain gauges or piezo-resistive sensors.



The motion parameters can be specifically influenced by algorithms. Furthermore, digital controllers allow servo parameters to be changed immediately, for example whenever the load is changed (i.e. heavier objectives are used). In other words, instead of having to make do with a compromise, the system can always be operated in the optimum window. In addition, the user can choose among different position measuring systems (strain gauges or high-resolution capacitive sensors), depending on the application requirements and budget. The controller can also be used for applications in which analog control signals are available (e.g. as is often the case with autofocus): in addition to a USB and RS-232 interfaces,

a wide-range analog input is included in the standard equipment. The systems can also be controlled with either LABVIEW, μ Manager, MetaMorph or MATLAB. A special F³ operating mode (fast focus & freeze) furthermore allows the controller to switch between an external autofocus signal and the internal position sensor signal quickly and without jumps.

Cost-Optimized Digital Piezo Controller

The cost-effective E-709 digital controller can not only work with capacitive sensors but also with less refined measurement systems such as strain gauges or piezo-resistive sensors; i.e. all of the advantages of digital technol-

ogy can be used even in such applications without incurring higher costs. The limited linearity of these sensors is improved by the digital controller because additional algorithms minimize the deviation between the target and actual position. This improves the linearity by up to three orders of magnitude. In closed-loop operation, if necessary, it is possible to switch between an internal and an external sensor. With the E-609, a "low-cost" version for purely analog control with a digital controller and digital parameter control is also available. In this case, a system component with an analog output signal (e.g. autofocus) can directly generate the position.

Your PIFOC® Selection!

- Very attractive price, accuracy around 10 nanometers, 100 μ m travel range: P-721
- High position stability, accuracy around 1 nanometer and travel ranges up to 400 μ m: P-725
- High position stability, fast settling with heavy objective masses up to 400 g, accuracy of 1 nanometer: P-726
- Travel ranges of 1 mm, accuracy around 5 nanometers, high stability: N-725
- Positioning of the complete revolving nosepiece of the microscope: customized adaptations, e.g. P-721KTPZ
- The P-725 is available in the system with the E-709 digital controller at particularly attractive prices.



Precise and dynamic positioning of the complete revolving nosepiece of the microscope around 80 μ m: the customized P-721KTPZ

PI Software

Easy Operation and Integration

Easy operation even for complex motion sequences with simultaneous easy integration in the control software of the user are important requirements for modern motion controllers. Particularly in microscopy, an additional requirement is compatibility with common software packages from third-party suppliers such as e.g. μ Manager or MetaMorph.

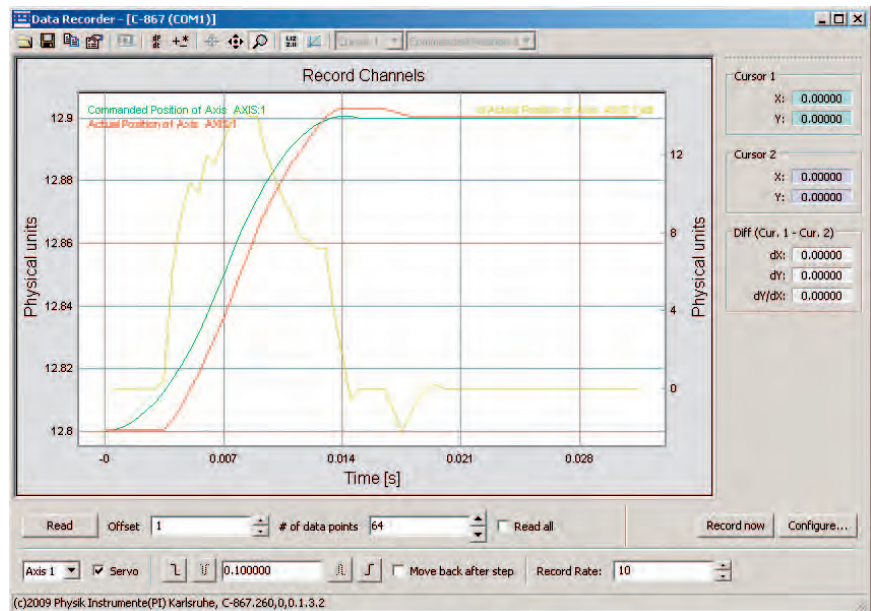
PI controllers fulfill these requirements e.g. with the PIMikroMove[®] user interface, driver components that are compatible across different systems and a uniform command language, the General Command Set (GCS). All necessary components for PI controllers are included in the standard scope of delivery.

PIMikroMove[®] User Interface

The operating software provided by the manufacturer is normally used for typical laboratory tasks. Particularly in microscopy, however, the initial start-up should take place quickly and smoothly; it is often necessary to combine different systems, e.g. XY rough adjustment with a piezo fine focus adjustment in Z.

The free-of-charge PIMikroMove[®] operating software works with the common Windows and Linux operating systems, supports all PI controllers and can operate several different positioning systems at the same time. The automated configuration dialog allows start-up within a few minutes due to pre-configured parameters for each PI stage. Nevertheless, the user can individually change all parameters (e.g. PID, acceleration, velocity). The graphic display of the motion sequence (e.g. settling) facilitates optimization to the customer application.

Simple automation tasks and minor adjustments (e.g. joystick settings for microscope stage control) can be stored on the controllers as a macro with clearly arranged dialogs and then executed independently of the host PC.



The PIMikroMove[®] software makes it possible to record settling processes and to make a direct comparison with the target value.

The Basis:

General Command Set (GCS)

For more complex sequences or integration in systems already present at the customer's, a decisive criterion is how great the expected programming effort for the new subsystem is. This considerably depends on the command set of the controller and how it can be implemented in common programming languages. As a result of the great amount of experience that PI has in this field, there are a large number of tried-and-tested aids available to the user.

- Libraries for Windows and Linux platforms and all common programming languages (e.g. C++, Delphi, VB),
- A comprehensive LabVIEW driver set that is compatible across all PI controllers,
- A large number of documented example programs with open source code significantly simplify integration as well.

Once a PI controller has already been integrated, further PI systems can be integrated with even less effort, since all PI controllers use the same command language, PI General Command Set, and only differ in the range of commands, which is determined by the functionality of the controller.

Third-Party Software Package

Drivers for the PI GCS commands have now been integrated in the most known third-party software packages. It is therefore possible to control PI systems, such as objective scanning for Z stacks or XY scanning with XY microscopy stages, using e.g. MetaMorph, μ Manager, MatLab or ScanImage.

The Plnano® Family Continues to Grow

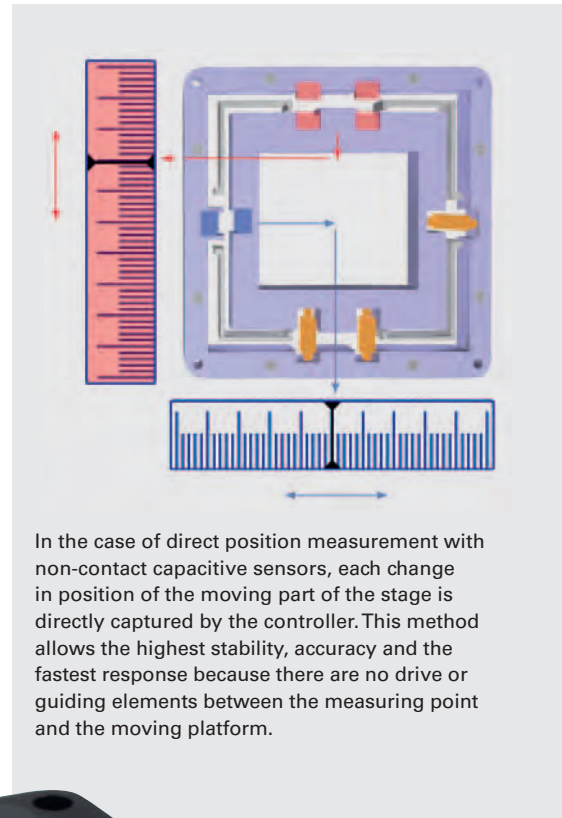
Scanning Piezo Stages for High-Precision Sample Positioning

The Plnano® scanning systems of the P-545 series are specially adapted to the requirements of sample positioning in optical microscopy. They position with subnanometer accuracy, allow travel ranges up to 200 µm on two or three axes and require little space for integration in a microscope due to their low height of only 20 mm.

Transmitted-light applications benefit from the large central aperture. The large aperture accommodates holders for Petri dishes or standard object slides of 25 x 75 mm. A holder for additional accessories is also available. The position of the sample holder in the aperture of the scanning systems makes it possible to turn the revolving nosepiece of the objective without lowering, i.e. it is not necessary to leave the focal position when changing the objective. Another practical addition is an XY stage (M-545) that can be moved either manually or by motor drive and which can be used to (roughly) adjust the samples even over longer paths. It fits directly on all common inverted light microscopes and serves as a kind of adapter for the piezo stage.

Fast, Directly Driven Piezo Stage

The new Plnano®Trak high-speed piezo scanners are optimized for fast response times, which are required in particle tracking, for example. They are offered as two- or three-axis systems with travel ranges of 70 µm x 70 µm in the XY plane and optional 50 µm in the Z. The direct piezo drive leads to response times of less than 5 ms and fast settling at the target position. High-resolution, piezo-resistive strain sensors provide the control. The high sensitivity of this sensor type leads to a high resolution of less than one nanometer.



In the case of direct position measurement with non-contact capacitive sensors, each change in position of the moving part of the stage is directly captured by the controller. This method allows the highest stability, accuracy and the fastest response because there are no drive or guiding elements between the measuring point and the moving platform.



Plnano high-speed piezo scanners with fast response time, as is required e.g. in particle tracking. (Photo: PI)

Continued on page 7



Piezo-based scanning systems that can be easily integrated in the microscopes due to their low height are a good idea for inverted microscopes or applications in which the optical path is not supposed to be changed by the positioning. (Photo: PI)

The Right Solution for Maximum Stability and Linearity

In long-term tests, it is important to prevent any possible drift of the samples. Typical applications are found e.g. in superresolution microscopy, in which image acquisition can take several minutes. Particularly for such applications, two- or three-axis PInano scanning systems are available with travel ranges of up to 200 μm per axis and resolutions in the subnanometer range. Non-contact and directly measuring capacitive sensors ensure improved long-term stability and linearity and replace the standard piezo-resistive sensors that are used. Capacitive sensors have the advantage of higher position stability, which results in less drift behavior. In addition, this sensor type is less sensitive to temperature changes. The linearity is up to $\pm 0.05\%$ for the capacitive sensor in comparison to $\pm 0.2\%$ for piezo-resistive sensors.

PInano®: The System Concept in Focus

Various drive and sensor principles for different requirements in respect to dynamics, stability and precision make the PInano® series a flexible system that is adapted to applied microscopy. A proprietary servo controller significantly improves the motion linearity compared to conventional piezo-resistive sensor controllers. The controller is designed to work with the piezo nanopositioning systems and fulfills all the requirements of high-resolution microscopy. The integrated wave generator with trigger inputs and outputs can save and output periodic trajectories. USB and RS-232 interfaces are available for communication. Numerous accessories round off the offer.

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